WATEREUSE FOUNDATION

Alternative Methods for the Analysis of NDMA and Other Nitrosamines in Water and Wastewater

WateReuse Foundation

Alternative Methods for the Analysis of NDMA and Other Nitrosamines in Water and Wastewater

The mission of the WateReuse Foundation is to conduct and promote applied research on the reclamation, recycling, reuse, and desalination of water. The Foundation's research advances the science of water reuse and supports communities across the United States and abroad in their efforts to create new sources of high quality water through reclamation, recycling, reuse, and desalination while protecting public health and the environment.

The Foundation sponsors research on all aspects of water reuse including emerging chemical contaminants, microbiological agents, treatment technologies, salinity management and desalination, public perception and acceptance, economics, and marketing. The Foundation's research informs the public of the safety of reclaimed water and provides water professionals with the tools and knowledge to meet their commitment of increasing reliability and quality.

The Foundation's funding partners include the U.S. Bureau of Reclamation, the California State Water Resources Control Board, and the Southwest Florida Water Management District. Funding is also provided by the Foundation's Subscribers, water and wastewater agencies, and other interested organizations. The Foundation also conducts research in cooperation with two water research coalitions: the Global Water Research Coalition and the Joint Water Reuse & Desalination Task Force.

Alternative Methods for the Analysis of NDMA and Other Nitrosamines in Water and Wastewater

Prepared by:

Robert C. Cheng and Cynthia Andrews-Tate
Long Beach Water Department

Cordelia J. Hwang, Yingbo "Carrie" Guo, and Salvador J. Pastor
Metropolitan Water District of Southern California

Janel E. Grebel and I.H. "Mel" Suffet
University of California, Los Angeles

Steve Carr and Connie Young
County Sanitation Districts of Los Angeles County

Sponsored and Published by:

WateReuse Foundation
635 Slaters Lane, 3rd Floor
Alexandria, VA 22314

Disclaimer

This report was sponsored by the WateReuse Foundation. The Foundation and its Board of Directors assume no responsibility for the information included in this publication, or for the opinions or statements of facts expressed in the report. The mention of trade names does not represent or imply the approval or endorsement of any commercial products by the WateReuse Foundation. This report is published solely for informational purposes.

For more information, contact:
WateReuse Foundation
1199 North Fairfax Street, Suite 410
Alexandria, VA 22314
www.WateReuse.org/Foundation

ISBN: 0-9747586-4-7
Library of Congress Control Number: 2005935185
WateReuse Foundation Project Number: WRF-01-001
WateReuse Foundation Product Number: 01-001-01
Printed in the United States of America

CONTENTS

TABLES

FIGURES

FOREWORD

The WateReuse Foundation, a nonprofit corporation, sponsors research that advances the science of water reclamation, recycling, reuse, and desalination. The Foundation funds projects that meet the water reuse and desalination research needs of water and wastewater agencies and the public. The goal of the Foundation's research is to ensure that water reuse and desalination projects provide high-quality water, protect public health, and improve the environment.

A Research Plan guides the Foundation's research program. Under the plan, a research agenda of high-priority topics is maintained. The agenda is developed in cooperation with the water reuse and desalination communities including water professionals, academics, and Foundation Subscribers. The Foundation's research focuses on a broad range of water reuse research topics including:

- Defining and addressing emerging contaminants;
- Public perceptions of the benefits and risks of water reuse;
- Management practices related to indirect potable reuse;
- Groundwater recharge and aquifer storage and recovery;
- Evaluating methods for managing salinity and desalination; and
- Economics and marketing of water reuse.

The Research Plan outlines the role of the Foundation's Research Advisory Committee (RAC), Project Advisory Committees (PACs), and Foundation staff. The RAC sets priorities, recommends projects for funding, and provides advice and recommendations on the Foundation's research agenda and other related efforts. PACs are convened for each project and provide technical review and oversight. The Foundation's RAC and PACs consists of experts in their fields and provide the Foundation with an independent review, which ensures the credibility of the Foundation's research results. The Foundation's Project Managers facilitate the efforts of the RAC and PACs and provide overall management of projects.

The Foundation's primary funding partner is the U.S. Bureau of Reclamation. Other funding partners include the California State Water Resources Control Board, the Southwest Florida Water Management District, Foundation Subscribers, water and wastewater agencies, and other interested organizations. The Foundation leverages its financial and intellectual capital through these partnerships and funding relationships. The Foundation is also a member of two water research coalitions: the Global Water Research Coalition and the Joint Water Reuse & Desalination Task Force.

This publication is the result of a study sponsored by the Foundation and is intended to communicate the results of this research project. The goals of this project were to evaluate, refine, and develop multiple methods for NDMA analysis, and where applicable, determine how well these methods were able to detect other nitrosamines.

Ronald E. Young
President
WateReuse Foundation

G. Wade Miller
Executive Director
WateReuse Foundation

ACKNOWLEDGMENTS

The project team would like to acknowledge and thank the many individuals and organizations that contributed to this report. This project was funded by the WateReuse Foundation in cooperation with the U.S. Bureau of Reclamation (USBR) and the California State Water Resources Control Board. This project would not have been possible without the funding agencies' support and the efforts of the project participants. In addition to the names listed below, the authors wish to acknowledge the following individuals from the Metropolitan Water District of Southern California: Mary Ann Viernes, Jacob Nikonchuk, and Rachel M. Kelley for their experimental work and Sylvia E. Barrett for her guidance and technical review. Thanks are also extended to Tai Tseng and Jerry Fields of the Long Beach Water Department, Larry Neisess and Rodger Baird of the Sanitation Districts of Los Angeles County, and Maria Echarte of UCLA for their contributions. Mallinckrodt Baker also donated selected reagents for use during this project.

Report Preparation

Principal Investigator

Robert C. Cheng, Ph.D., P.E., *Long Beach Water Department*

Project Team

Cynthia Andrews-Tate, *Long Beach Water Department*
Janel E. Grebel, *University of California, Los Angeles*
Yingbo "Carrie" Guo, Ph.D., *Metropolitan Water District of Southern California*
Cordelia Hwang, *Metropolitan Water District of Southern California*
Salvador J. Pastor, Ph.D., *Metropolitan Water District of Southern California*
I.H. "Mel" Suffet, Ph.D., *University of California, Los Angeles*

Project Advisory Committee

Harold Bailey, *Padre Dam Municipal Water District*
Andrew Eaton, Ph.D., *MWH Laboratories*
Richard Mills, P.E., *California State Water Resources Control Board*
Andrew Murphy, *U.S. Bureau of Reclamation*
Howard Okamoto, *California Department of Health Services*
Vince Taguchi, Ph.D., *Ontario Ministry of the Environment*

Other Project Participants

Steve Carr, Ph.D., *Sanitation Districts of Los Angeles County*
Steve Fitzsimmons, *Orange County Sanitation District*
David Kimbrough, *Castaic Lake Water District*
Cheryl Ross, *Central and West Basin Municipal Water Districts*
Lee Yoo, Ph.D., *Orange County Sanitation District*
Connie Young, Ph.D., *County Sanitation Districts of Los Angeles County*

WateReuse Foundation

Project Manager: G. Wade Miller

EXECUTIVE SUMMARY

The chemical, N-nitrosodimethylamine (NDMA), has been identified as a probable human carcinogen by the U. S. Environmental Protection Agency (USEPA). Exposure to NDMA at high levels has been demonstrated to cause cancer in laboratory animals. The general population may be exposed to NDMA from various pathways, including inhalation (air) and ingestion (food and beverages). More recently, NDMA has been detected in potable water, recycled water, and wastewater.

The Ontario (Canada) Drinking-Water Quality Standard for NDMA has been set at 9 nanograms per liter (ng/L). The USEPA has set a theoretical 10^{-6} cancer risk for NDMA at 0.7 ng/L, although no maximum contaminant level (MCL) or MCL goal (MCLG) currently exists. The California Department of Health Services (CDHS) established an Action Level (not regulatorily enforceable) of 2 ng/L NDMA in 1998, but due to variabilities in laboratories to detect to such a low level, increased the Action Level to 20 ng/L in 1999. Since 2002, the California Action Level has been maintained at 10 ng/L. Because no approved methods are available for NDMA, relatively few laboratories are currently performing analyses for NDMA and other nitrosamines, thus leading to relatively high analytical costs. Other nitrosamines may be scrutinized more closely by regulators in the future, as evidenced by the CDHS-implemented Action Level for N-nitrosodiethylamine (NDEA) of 10 ng/L on September 30, 2004. On January 1, 2005, the term Action Level was replaced by Notification Level in California.

The goals of this project were to evaluate, refine, and develop multiple methods for NDMA analysis, and where applicable, determine how well these methods were able to detect other nitrosamines. Although low-level detection limits are important considerations for developing these methods, another purpose of this project was to evaluate the performance of multiple methods when applied to a variety of water matrices. Methods achieving the anticipated drinking water detection level requirement (~2 ng/L) in pristine waters may not be capable of detecting to such a low level in other matrices (e.g., wastewater). An additional project goal was to evaluate the capabilities of nitrogen (N)-selective GC (gas chromatography) detectors (i.e., the nitrogen–phosphorous detector (NPD) and nitrogen chemiluminescence detector (NCD)) and compare these detectors to currently used mass spectrometer (MS) detection systems for quantifying nitrosamines. The alternative extraction and detection methods should offer options for laboratories to use for nitrosamine analysis, which should lead to lower costs.

The methods examined in this study are derivations or refinements of existing methods and included liquid–liquid extraction (LLE) and solid–phase extraction (SPE) techniques. Specifically, the methods included a micro LLE (MLLE) method, an Ambersorb SPE (Amb SPE) method, an Ambersorb Envi-carb cartridge SPE (Amb-Envi CSPE) method, and a solid–phase microextraction method (SPME). These methods were tested through an extensive round-robin process, and it was found that the Amb-Envi CSPE, Amb SPE, and the traditional LLE methods were all able to report NDMA to a level of approximately 2 ng/L, and other nitrosamines to levels of between 2 and 4 ng/L across a broad range of sample water matrices. The MLLE technique developed in this study, which uses a sample volume of 100 mL, also appeared to be effective at analyzing a broad range of sample matrices, although the reporting limit for this method was higher than the other methods (7 ng/L for NDMA and comparable levels for other nitrosamines). The SPME method, the least labor intensive of all the methods studied, had the highest detection level of 30 ng/L for NDMA and 60 ng/L for other nitrosamines, except N-nitrosopyrrolidine (NPYR), which was not detected.

Tests performed comparing the alternative detectors to GC/MS quantitation showed that as developed, the GC/MS can currently provide for the lowest detection limits. For example, the method detection limits (MDLs) obtained for a NCD detector combined with the Amb SPE method were 6.5 ng/L for NDMA and 5.7 ng/L to 22.1 ng/L for other nitrosamines. The MDLs obtained for Amb SPE method extracts coupled with NPD quantitation were 10.9 ng/L for NDMA and 13.3 ng/L to 70.1 ng/L for other nitrosamines. GC/MS quantitation with the same extracts resulted in MDLs of 2 ng/L for NDMA and 2 to 4 ng/L for other nitrosamines. However, it is important to qualify these results. Due to equipment availability, the maximum injection volume used with the NCD and NPD (3 µL) was approximately 67% less than

injection volumes used to determine MS detection limits (8 µL). With equipment modifications allowing for comparable injection volumes, NCD and NPD detection limits for NDMA might be decreased to a range of between 2 and 5 ng/L for the NCD and NPD, respectively. As currently developed, quantitation levels for the alternative detectors are sufficient for samples with higher concentrations of nitrosamines.

A total of four different water matrices were analyzed during the round-robin testing. The samples included: (1) chloraminated potable surface water (potable); (2) chlorinated reclaimed effluent treated by reverse osmosis (RO effluent); (3) secondary treated wastewater effluent (secondary effluent); and (4) chlorinated tertiary treated wastewater effluent (tertiary effluent). Although NDMA was the only nitrosamine detected in potable water and RO effluent samples, the secondary and tertiary effluent samples contained detectable concentrations of nitrosodiethylamine (NDEA), nitrosomorpholine (NMOR), nitrosopiperidine (NPIP), nitrosodi-n-butylamine (NDBA), and NPYR. With the exception of NDMA, the other nitrosamines, when detected, were less than 10 ng/L. This is believed to be the first study to document the occurrence of these other nitrosamines with verification by a suite of analytical methods.

Using a per sample analytical time as a rough indicator of analytical cost, the time required for the baseline CLLE method was compared to the time required for the alternative methods. It was determined that the CLLE method would require between approximately 60 to 90 minutes per sample. With the exception of Amb-Envi CSPE method (requiring approximately 40 to 120 minutes), the other methods required equal or less analytical time, with Amb SPE requiring 40 to 80 minutes per sample and MLLE requiring 70 to 80 minutes per sample. These results represent the most conservative estimate because not all participating laboratories had experience with each method prior to the round-robin testing. With additional experience, it is expected that the analytical times required will decrease, and perhaps with an associated decrease in the analytical cost.

While this work has met the goals of the project, more work may be warranted to determine the applicability of these methods to automation (e.g., Amb-Envi CSPE). Further work could also be conducted to better evaluate the applicability of NPD and NCD as a lesser-cost compliance alternative to GC/MS detectors. As more occurrence data and future regulations unfold, these methods may warrant further investigating for more focused optimization of specific nitrosamine species and/or minimum reporting limits.

CHAPTER 1

INTRODUCTION AND OBJECTIVES

Introduction

The chemical, N-nitrosodimethylamine (NDMA), has been identified as a probable human carcinogen by the U.S. Environmental Protection Agency (USEPA). Exposure to NDMA at high levels has been demonstrated to cause cancer in laboratory animals. The general population may be exposed to NDMA from outdoor air; tobacco smoke; diet such as cured meats, fish, and cheese; beverages such as beer and whisky; cosmetics; and rubber products.

More recently, NDMA has been detected in potable water, recycled water, and wastewater. Currently, the Ontario (Canada) Drinking-Water Quality Standard (Ontario 2002) for NDMA is 9 nanograms per liter (ng/L). In the United States, the USEPA has set a theoretical 10^{-6} cancer risk for NDMA at 0.7 ng/L, although no maximum contaminant level (MCL) or MCL goal (MCLG) currently exists.

In 1998 NDMA was found in drinking water wells in California, which led the California Department of Health Services (CDHS) to establish a nonregulatorily-enforceable Action Level of 2 ng/L NDMA, which corresponds to the updated 10^{-6} cancer risk as calculated by the California Office of Environmental Health Hazard Assessment (OEHHA). The Action Level was temporarily increased to 20 ng/L in 1999 (based on variations in laboratories' abilities to detect to 2 ng/L) and currently is at 10 ng/L NDMA (the 5×10^{-6} cancer risk) because of its possible occurrence as a disinfection/treatment byproduct.

It is suspected that the regulators' interest in nitrosamines will increase in the future, as evidenced by the inclusion of monitoring for NDMA in the forthcoming phase 2 of the Unregulated Contaminant Monitoring Rule (UCMR), and the recent implementation of a 10 ng/L Action Level for NDEA by CDHS (September 30, 2004). As of January 1, 2005, the term Action Level has been replaced by Notification Level in California.

Various theories of NDMA formation mechanisms during wastewater and drinking water treatment have been proposed, including the interaction of generally accepted precursors such as nitrite and dimethylamine, as well as the presence of other amine-based polyelectrolytes and fungicides. In some studies, it appears that NDMA formation requires the presence of chlorine; in other studies, chlorine appears to decrease the NDMA concentration. These findings suggest different formation mechanisms exist for the various precursors (Choi and Valentine, 2001).

The formation mechanism that produces NDMA may result in other nitrosamines. Selected data for eight low molecular weight nitrosamines are listed below (Table 1.1), and were investigated as part of this study because of their likely presence in nitrogen-rich waters. Some of these compounds have been detected from natural products (Fiddler et al., 1972).

Because no approved methods are available for NDMA, relatively few laboratories are performing analyses for this contaminant. A survey of the costs and methodologies for NDMA analysis used by five well-known laboratories was conducted in 2002 and the data are presented in Table 1.2. As shown, a wide range of costs and detection limits was observed within the various laboratories. Analyses of other nitrosamines are currently available from a Canadian commercial laboratory at a minimum cost of $530 per sample (NDMA not included).

Table 1.1 Selected characteristics for eight nitrosamines

Nitrosamine		Formula	MW	Carcinogenicity*
N-Nitrosodimethylamine	NDMA	$C_2H_6N_20$	74	Liver, lung, kidney tumors in rats Liver tumors in hamsters
N-Nitrosomethylethylamine	NMEA	$C_3H_8N_20$	88	Liver, lung, esophagus tumors in rats Liver tumors in hamsters
N-Nitrosodiethylamine	NDEA	$C_4H_{10}N_2O$	102	---
N-Nitrosodi-n-propylamine	NDPA	$C_6H_{14}N_2O$	130	---
N-Nitrosomorpholine	NMOR	$C_4H_8N_2O_2$	116	Liver, nasal cavity tumors in rats Liver tumors in mice; trachea tumors in hamsters
1-Nitrosopyrrolidine	NPYR	$C_4H_8N_2O$	100	Liver tumors in rats; lung tumors in mice, trachea tumors in hamsters
N-Nitrosopiperidine	NPIP	$C_5H_{10}N_2O$	114	Liver, esophagus, nasal cavity tumors in rats Liver, forestomach, esophagus tumors in mice; Trachea tumors in hamsters
N-Nitrosodi-n-buytlamine	NDBA	$C_8H_{18}N_2O$	158	---

**Lijinsky, W. 1994.*

Table 1.2 2002 Survey of commercial laboratory capabilities for NDMA analysis

Lab	EPA method	Detection method	MDL (ng/L)	Cost/ sample
1	1625-LLE	SIM	2	$400
2	1625-LLE	SIM	2	$325
3	1625-LLE	SIM	20	$525
4	1625-LLE	SIM	2	$525
5	1625-LLE	HR/MS	2	$350

EPA = U.S. Environmental Protection Agency
SIM = selection ion monitoring

Objectives

The overall objective of this project was to develop alternative accurate and reproducible analytical techniques for measuring NDMA and other nitrosamines in various matrices, including wastewater, recycled water, surface water and groundwater samples. The availability of alternative techniques should help to lower the analytical costs. The specific objectives were to:

1. optimize and refine existing extraction and concentration methods (liquid–liquid extraction (LLE), and solid–phase extraction (SPE)) and investigate *new methods* (solid–phase microextraction (SPME) and cartridge SPE);
2. determine whether a method may be developed to analyze for seven other nitrosamines in addition to NDMA;
3. evaluate the capabilities of nitrogen (N)-selective GC detectors, i.e. the nitrogen–phosphorous detector (NPD) or nitrogen chemiluminescence detector (NCD), for quantitation of nitrosamines at or below the desired method reporting limit (MRL) of 2 ng/L, and compare these detectors to the currently-used gas chromatography/mass spectrometry (GC/MS) detection systems; and
4. determine whether the development of more than one method is required to account for the effect of wastewater matrices as compared to a clean water matrix.

The results from this study were distilled and compiled into a set of standard operating procedures (SOPs) to be used for the analyses of NDMA and other nitrosamines for the water, wastewater, and recycled water industries.

Description of Project Phases

To accomplish the project objectives, the project was divided into six phases.

Phase 1 – optimize and refine existing methods; investigate new methods for sample preparation (extraction and concentration) techniques (e.g., SPE, LLE, CSPE, and SPME).
Phase 2 – optimize analyte separation and measurement and examine various detectors (e.g., NPD, NCD).
Phase 3 – resolve sample collection/holding issues, including disinfectant quenching, preservation, and holding times.
Phase 4 – assimilate data from Phases 1 to 3, and select optimal method(s) for round-robin testing.
Phase 5 – round-robin testing (three to five laboratories per method) of wastewater and clean water to assess the accuracy and repeatability of the methods.
Phase 6 – final report writing, formatted in a standard operating procedure, to be used as the basis for analysis by the water, wastewater, and recycled water industries.

For the purposes of this project, the following definitions are used to describe the various water samples:

Wastewater – water located within any location of a wastewater treatment plant (e.g., influent, primary effluent, secondary effluent). For the purposes of this project, tertiary effluent, or any water which is derived from tertiary effluent, is considered as recycled or reclaimed water.
Potable water - groundwater or surface water that is treated to a quality meeting all USEPA and California standards for human consumption.
Recycled or reclaimed water – a wastewater, which as a result of treatment, is suitable for uses other than potable use (California Health Laws, Title 17).
Clean water matrix – all waters except for wastewater (e.g., potable or recycled water).

Phase 1 focused on the examination of sample extraction and concentration techniques in order to optimize the recovery efficiency while simplifying this process. Two common techniques are currently used for NDMA extraction. The first is a LLE method, using the solvent dichloromethane (DCM), with a 1-L sample volume in either the manual, hand-shaking mode or continuous extraction with evaporative concentration of the extract to 1 mL. The second method is the SPE technique, with Ambersorb® 572 (Ambersorb) as the adsorbing media, a sample volume of 500 mL, followed by physical separation, drying and extraction with 400 μL of DCM.

Both techniques were explored to reduce labor costs (e.g., analyst time), solvent use (minimizing environmental impacts), and sample volume—and also to maximize analyte recovery. Other parameters examined include the effects of shaking modes (various shaking tables), solvent selection, salt addition, pH adjustment, sample size, solvent volume, and extraction time ratios. A factorial design approach for optimizing interactive parameters (e.g., pH and salt addition to maximize analyte recovery with minimum chemicals added) was completed to determine whether a detection level of 1 ng/L or less might be achieved. A determination of the analytical precision and the significant factors was made (Friant and Suffet, 1979).

SPME, a promising new innovative concentration-and-extraction technique, was the third extraction and concentration technique studied. SPME's advantages include no solvents used, sample preparation time minimization, and the possible automation of this technique. Gonzalez et al. (2000) reported the analysis of low levels of disinfection byproducts (DBPs), in the μg/L levels, in drinking water by the SPME technique. SPME has also been applied to the determination of ng/L levels of the taste-and-odor compounds methylisoborneol (MIB) and geosmin (McCallum et al., 1998).

Phase 2 focused on the analysis and quantification of the concentrated extracts. Instrumentation and conditions affecting separation, identification, and quantitation were evaluated to determine the most cost-effective, sensitive, accurate, and reliable system as used at each laboratory. GC was used for separation of target and matrix components, although complete chromatographic resolution was not necessary for the MS techniques to achieve unique identification and quantitation. The instrumentation used by the project teams were all GC with tandem mass spectrometer operated in chemical ionization mode, and will be designated as GC/CI/MS/MS. GC column type (e.g., DB1701, DB5, or MS versions) and film thickness (e.g., 0.5, 1.0 μm) and oven temperature program were examined.

As part of this project, the suitability of two additional detectors, the NCD and NPD, for measuring low levels of NDMA and other nitrosamines were evaluated. After general optimization, these two types of detectors were evaluated for sensitivity, linearity, and reproducibility. The detectors were used in combination with Phase 1 concentration procedures and a variety of matrices to determine the suitability to environmental samples.

In Phase 3, parameters affecting the holding times and storage conditions were examined, including the effect of light, pH, temperature, and the addition of antioxidant quenching agents and biocides. Antioxidants may inhibit nitrosamine formation by stopping the reduction of oxides of nitrogen, and quenching free or combined chlorine residuals. The pH of a sample may also affect nitrosamine formation and stability, and lowered pH can also inhibit biological activity.

Holding studies were conducted in several representative matrices because precursor and reactant types and amounts can vary considerably. It was suspected that sample collection and storage may be a more important issue for wastewater samples (due to the more complex matrix) compared to potable water samples, therefore wastewater as well as reagent and potable water samples were tested. The stability of naturally-occurring NDMA or a 50 ng/L NDMA spike, depending upon the matrix, was studied over a course of 28 days.

After completion of Phases 1 to 3, the final methods were selected and refinements incorporated. To meet the wide range of needs for nitrosamine analysis, four methods were selected in Phase 4 as candidate test methods for the Phase 5 round-robin testing. The methods were divided by calibration ranges (e.g., < 200 ng/L and > 200 ng/L) or sample matrix (e.g., clean water, wastewater). Standard operating procedures (SOPs) were prepared for each method for testing in Phase 5. Utility, government, and commercial laboratories were selected for the round-robin tests for both the clean water method and the wastewater methods. Three matrices—potable water, recycled water, and wastewater—were tested. Not all participants had prior experience in all methods, thus the results generated would provide a conservative test of method performance.

Description of Report Format

This project is divided into nine chapters, and arranged as shown in Table 1.3. The formats of Chapters 3 to 6 are similar, with each chapter describing the experimental design for each extraction method, presenting the results for the optimization studies, and presenting the detection limits achieved with the optimized parameters. The optimized methods were tested through a round-robin process, where three to five laboratories tested each method (except for SPME), and the results are presented in Chapter 8, along with a discussion on each method's accuracy and precision.

The format of Chapter 7 is somewhat different because this chapter describes the development of alternative detectors, not extraction methods, and includes the results of the detector performance with the round-robin samples tested in Chapter 8. Chapter 8 presents the results obtained with each candidate extraction method analyzed under the traditional GC/MS method, and not with the alternative detectors.

Table 1.3 Description of report format

Chapter	Description	Phase(s) addressed
1	Introduction	
2	Sample collection, preservation, and storage	3
3	Liquid–liquid extraction methods	1, 4
4	Ambersorb 572 solid–phase extraction method	1, 4
5	Dual-media cartridge extraction method	1, 4
6	Solid–phase microextraction method	1, 4
7	Alternative detectors (NCD and NPD)	2
8	Comparison of method (round-robin testing)	5
9	Summary, conclusions and recommendations	

Four waters were used during round-robin testing and the analytical results from these waters are presented in Chapters 7 and 8. As shown in Table 1.4, the sources included: (1) chloraminated potable surface water (potable); (2) chlorinated reclaimed effluent treated by reverse osmosis (RO effluent); (3) secondary treated wastewater effluent (secondary effluent); and (4) chlorinated tertiary treated wastewater effluent (tertiary effluent). The NDMA levels in the four source waters were expected to range from less than 10 ng/L to greater than 1,000 ng/L. The concentrations of the other nitrosamines had not been previously reported so no references were available. The potable water (RR-01) and the secondary effluent (RR-04) were selected for nitrosamine spiking, representing one clean matrix and one containing higher background levels of interference, which would allow determinations of accuracy and precision of the various extraction methods to be made.

Table 1.4 Waters used during round-robin testing

Sample ID	Source	Sample type
RR-01	Chloraminated potable water (potable)	Potable
RR-02	Potable water (spiked)	Potable
RR-03	RO effluent	Recycled
RR-04	Secondary wastewater effluent	Wastewater
RR-05	Secondary Effluent (spiked)	Wastewater
RR-06	Tertiary wastewater effluent	Recycled

Common Terms Used

The following is a definition for the common terms used in this report. Other terms are defined in the text as they are used.

Concentration factor (CF) is defined as the original sample volume divided by the final volume of the extract. CF is a measure of the efficiency of the extraction step, and in order to achieve a 1 ng/L method detection level, the CF should be in excess of 1,000.

Method detection limit (MDL) is defined as the lowest analyte concentration that can be detected by an instrument with correction for the effects of sample matrix and method-specific parameters, including sample preparation. MDLs are explicitly determined by the procedure listed in 40 CFR Part 136,

Appendix B (USEPA, 1984), and defined as three times the standard deviation of seven replicate spiked analyses, which represents 99% confidence that the analyte concentration is greater than zero.

Instrument detection limit (IDL) is defined as the lowest analyte concentration that can be detected by an instrument without correcting for the effects of sample matrix or method-specific parameters such as sample preparation. IDLs are explicitly determined and generally defined as three times the standard deviation of the mean noise level. IDLs are determined independent of extraction technique, according to USEPA protocol 40 CFR136 Appendix B (USEPA, 1990). The IDL is generated by multiplying the standard deviation of seven duplicate samples, analyzed over three consecutive days, by the student t number (at 99% confidence).

Lower level of detection (LLD) see definition for **IDL**.

Method reporting limit (MRL) is defined as the lowest analyte concentration that meets the data quality objectives based on the intended use of the method, and is the concentration for which the recovery is predicted to fall between 50 to 150% with 99% confidence. The MRL can be as high as 10 times the MDL, but for the purposes of this report, it is set as three times the MDL.

CHAPTER 2

SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Introduction

The first and arguably most important component in ensuring analytical success is sampling. After ensuring that a sufficient quantity of sample is collected, the sampler must protect the integrity of the constituents by utilizing suitable containers, following appropriate labeling and custody protocols, and controlling the storage environment to prevent sample contamination or degradation. These steps are essential if the results of the analytical process are to be valid. Currently, there are no procedures specifically proscribed for the handling of NDMA or nitrosamines constituents in the low ng/L range.

Method

This study was conducted to determine whether any published sampling protocols, preservation methods, or holding time guidelines were appropriate for NDMA and other nitrosamines. Various preservation schemes and conditions were tested to assess the effect of these additives on NDMA in aqueous matrices. The stability of NDMA to direct sunlight exposure and microbiological degradation was also studied. These efforts should assist in establishing practical guidelines for collection, preservation, and storage of nitrosamine samples and extracts.

Results and Discussions

Sunlight Exposure

Unlike the vast majority of common analytical constituents, nitrosamines have been shown to be susceptible to photo-degradation. The relatively effective photolytic UV destruction of NDMA has been utilized as a clean-up measure to remove NDMA from treated waters. This process has also been observed in samples exposed to natural sunlight. When deionized (DI) water standards or wastewater effluent samples in clear glass containers were exposed to sunlight for two to four hours, greater than 75% of the NDMA constituent was lost (Figure 2.1). This attenuation occurs even when both types of samples are stored in borosilicate or Pyrex containers; this effect was not anticipated. Borosilicate glass has been

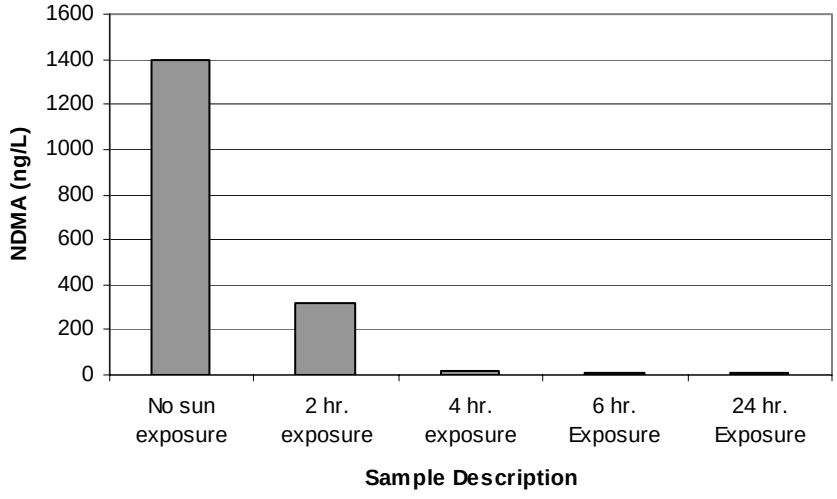


Figure 2.1 Sunlight exposure study at room temperature

shown to have a wavelength cutoff (~354 nm) well above the "active UV adsorption bands" for NDMA. The destruction of NDMA in samples stored in UV_B-opaque containers strongly suggests that there may be other active wavelengths that are important for NDMA photolysis in natural sunlight. For this reason, samples should preferably be collected in amber containers and kept away from direct sunlight.

Biodegradation

NDMA has also been reported to undergo microbial degradation in soil matrices (Figure 2.2). Several studies have indicated that NDMA can be mineralized under both aerobic and anaerobic conditions. Pseudomonas *putida* and *fluorescens* were two microbes that were determined to be involved in this process. This class of microorganism possesses an unusually broad range of metabolic enzymes that are likely involved in the observed destruction. This genus is also known to be ubiquitous in the environment and is therefore likely to be present in nonsterile aqueous samples. It would be prudent to take steps to avoid biodegradation in nondisinfected samples. The matrices utilized in this study were DI water standards that were percolated through soil columns. Refrigeration of samples at 4°C is therefore recommended as a precautionary measure if samples are to be held for a prolonged period before extraction. The following graph demonstrates the NDMA removal efficiency under various conditions. The samples tested were tertiary effluent spiked with a concentration of 500 ng/L NDMA. The samples were filtered through soil columns at room temperature at a flowrate between 1 to 7 mL/min, which resulted in average column contact times between 0.5 to 2.0 hours.

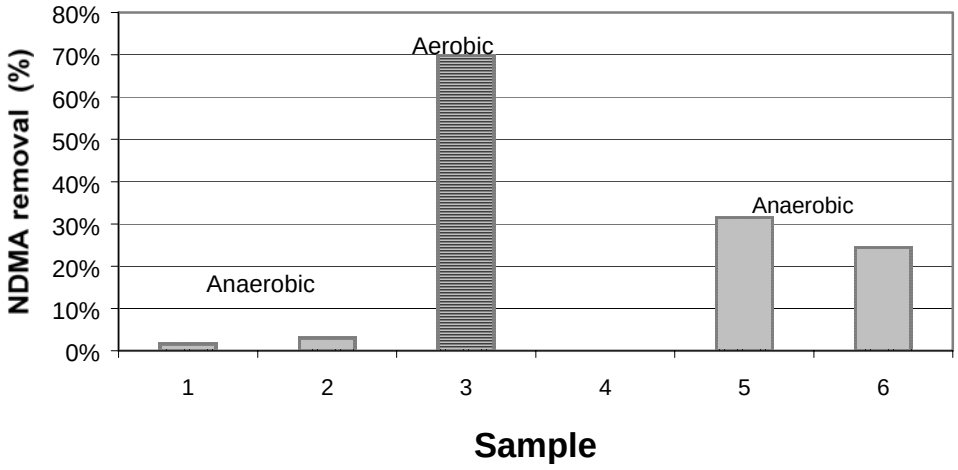


Figure 2.2 Biodegradation of NDMA under aerobic and anaerobic conditions

Sample Preservation

The main goal of this study was to assess the stability of NDMA. Deionized (DI) water standards, potable water, and spiked wastewater effluent samples were preserved using a variety of agents and conditions to determine the effect on NDMA recoveries. To ensure uniformity, 50-L volumes of 50 µg/L nitrosamine standards in DI, potable, and wastewater effluent samples were prepared in stainless steel drums. Four sets of samples were split from these volumes and spiked with 0.25 g/L of the specified agent or adjusted with acid or base to the pH specified in Table 2.1. One set of the spiked samples was extracted immediately (T = 0 days). Replicate sets were then processed after one week (T = 7 days), after two weeks (T = 14 days), and the final set after one month (T = 28 or 33 days).

Table 2.1 Preservation study parameters

Parameter	Variables
Temperature	Room
Preservative	Ascorbic Acid (0.25 g/L) Formaldehyde (0.25 g/L) Gluteraldehyde (0.25 g/L) Chloroform (0.25 g/L) Azide (0.25 g/L)
Sample pH at extraction	<2.0 (HCl) <2.0 (H_2SO_4) <2.0 (H_3PO_4) >12.0 (NaOH)

Reagent Water

The long term stability results suggest that NDMA is conserved during extended storage. Little variability in recoveries was observed with the conditions and preservatives employed. The NDMA concentrations in reagent grade water appear to be insensitive to pH and all of the other agents selected for this study (Figure 2.3).

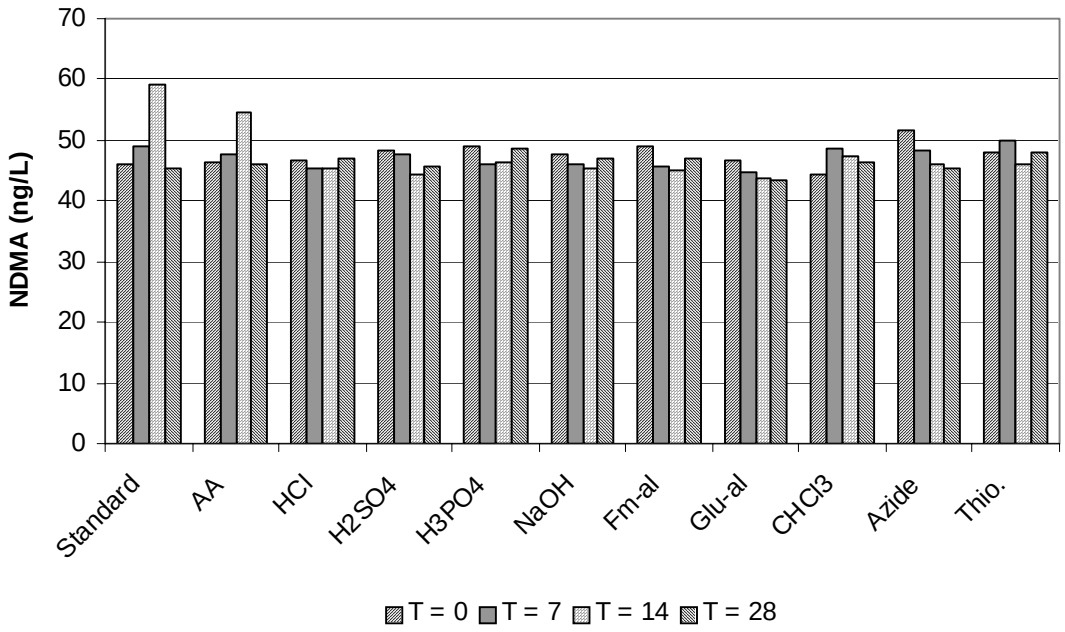


Figure 2.3 Reagent water preservation study up to 28 days (50 ng/L NDMA)

Potable Water Matrix

In general, the influence of preservation agents on potable water matrices appears to mirror the response observed with DI water. The initial NDMA concentrations showed a noticeably positive bias for the acidified samples. The concentrations, however, returned to a "normal" recovery range for the 7-day and 14-day samples, suggesting an analytical rather than a preservation influenced bias. The absence of any clear trends that would indicate either positive or negative recoveries in the 7- and 14-day data sets supports this conclusion. After the initial test, the concentrations appear to fall well within the expected recovery range of the method (±15%). This response could be explained by sample preparation errors or other analytical artifacts. It may be fair to say that preservation does not appear to be a significant factor in the reported recoveries for potable water matrix (Figure 2.4).

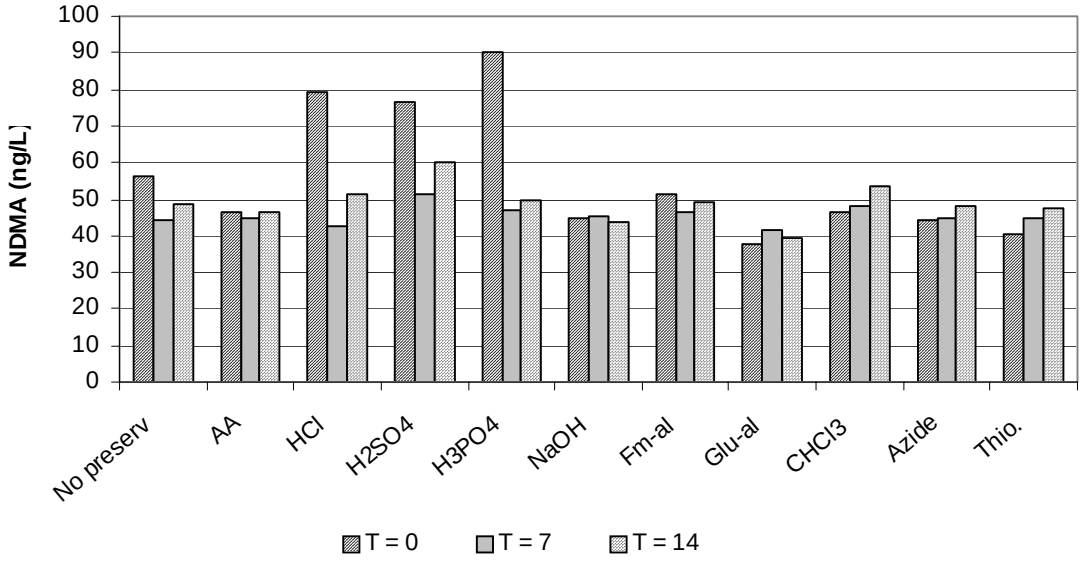


Figure 2.4 Potable water preservation study up to 14 days at room temperature (50 ng/L NDMA)

The stability of all eight nitrosamines in chloraminated potable water was also assessed unpreserved and with ascorbic acid (0.02 g/L) or with 0.1 g/L of sodium sulfite (Figures 2.5 to 2.7). Samples were collected in individual 1-L amber bottles containing the respective preservative, as applicable. A spike equivalent to 51 ng/L of each nitrosamine was then added to each bottle. Samples were stored at 4ºC until analyzed. Duplicate samples were extracted and concentrated by Ambersorb SPE (Taguchi et al., 1994) and analyzed by GC/MS/MS in acetonitrile chemical ionization mode (Chapter 3, Equipment, GC/CI/MS/MS).

NMEA values are not reported for day 7 due to quantitation problems. The unpreserved potable water (initial total chlorine residual as Cl_2 = 2.6 mg/L) showed a slight increase in NDMA concentration (average 63 ng/L) for days 7 through 33. Although the preserved (dechlorinated) samples had a couple of high NDMA levels, there were no consistent trends. The concentration of the other nitrosamines were generally constant within ±20%. NPIP level for the ascorbic acid preserved samples was inexplicably high on day 0 and low on day 7, but was then consistently close to the 51 ng/L spike for measurements on days 14 to 33. This study suggests that quenching of the chloramines residual by ascorbic acid or sodium sulfite aided in the preservation of initial NDMA levels and that neither preservative impacted the other nitrosamines.

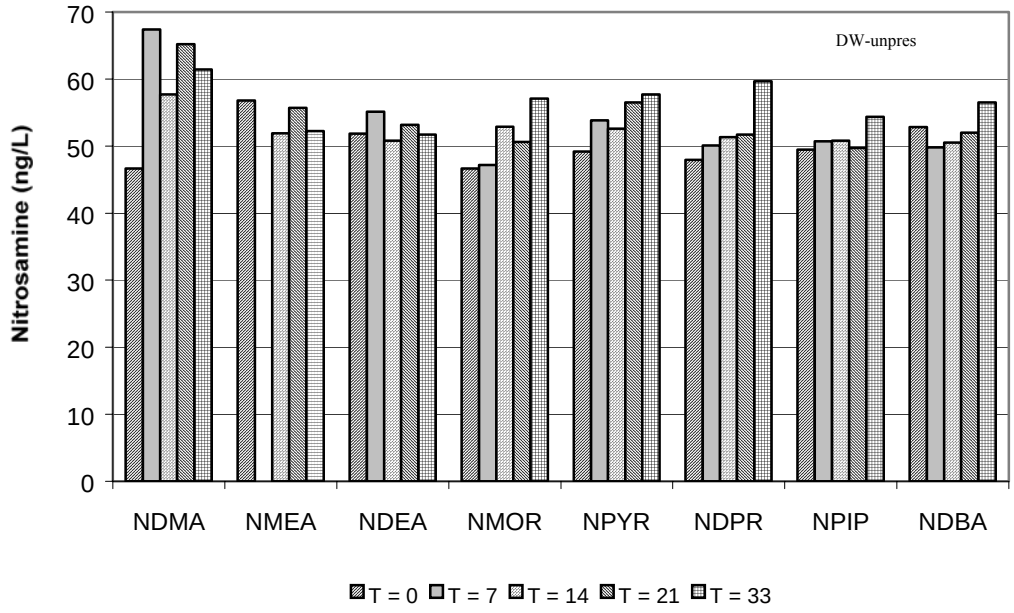


Figure 2.5 **Unpreserved potable water study up to 33 days (51 ng/L spiked of each nitrosamine)**
Data shown represent average of duplicate analyses.

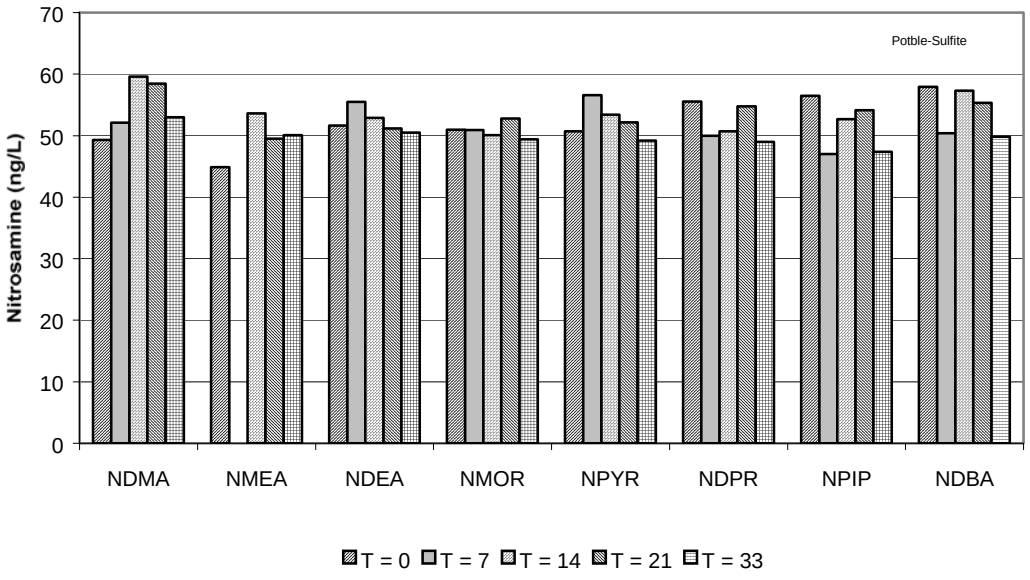


Figure 2.6 **Sodium sulfite preservation study of potable water up to day 33 (51 ng/L spiked of each nitrosamine)**
Data shown represent average of duplicate analyses.

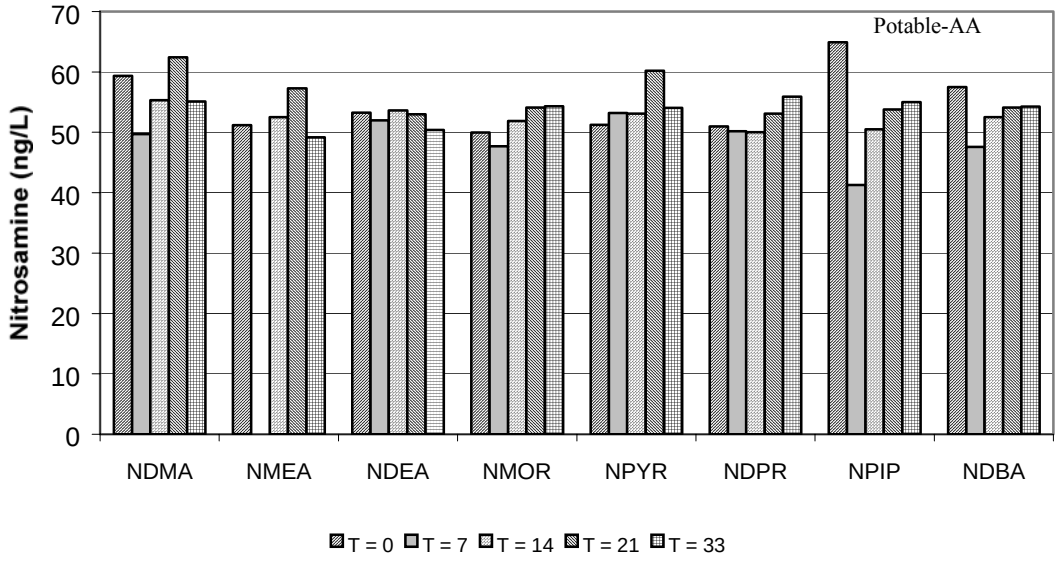


Figure 2.7 **Ascorbic acid preservation study on potable water up to day 33 (51 ng/L spiked of each nitrosamine)**
Data shown represent average of duplicate analyses.

Wastewater Effluent

The effect of the preservation agents and study conditions on effluent waters were clearer (Figure 2.8). There were consistent indications of a link between the preservation agent used and the measured NDMA concentrations. Unlike the recoveries seen earlier for DI and potable waters, the analytical recoveries appear to be significantly influenced by water quality. The elevated recoveries, especially after acidification, for effluent waters may likely be promoted by artifactual matrix influences. Disinfected treated wastewater effluent is known to contain a complex mixture of poorly characterized dissolved organic compounds which include amines, complex humics, polysaccharides and microbiologically derived amino acids. These constituents along with the diverse forms of residual chlorine and chloramine species can serve as a reservoir of nitrosamine forming precursors. The results for DI and potable water, as described earlier, strongly suggested that the stability of NDMA is not directly affected by the agents or conditions employed in this study. The concentration of nitrosamines in clean DI water matrices appears to be quite stable even without refrigeration. The efficacy of the preservation agent in effluent waters therefore may be simply to govern *in situ* NDMA forming reactions and prevents the *in situ* synthesis of nitrosamines rather than to conserve the analyte. NDMA formation in nondechlorinated samples appears to be rapid, with the majority of the formation occurring within the first three hours after collection.

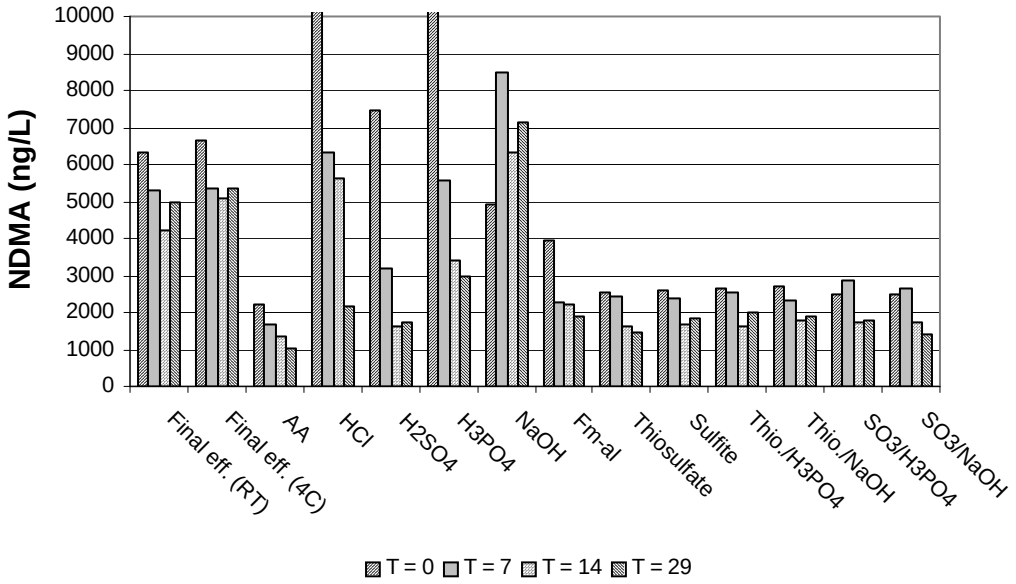


Figure 2.8 Preservation study in tertiary wastewater effluent over 29 days

Extract Storage

Refrigerated NDMA extracted concentrates archived for periods in excess of six months showed minimal NDMA losses, even when storage exceeded one year at freezer temperatures (~–20 ^{o}C) (Figure 2.9). This supports the earlier findings that in the absence of direct photolytic influences, NDMA is a stable constituent that can be safely stored for extended periods.

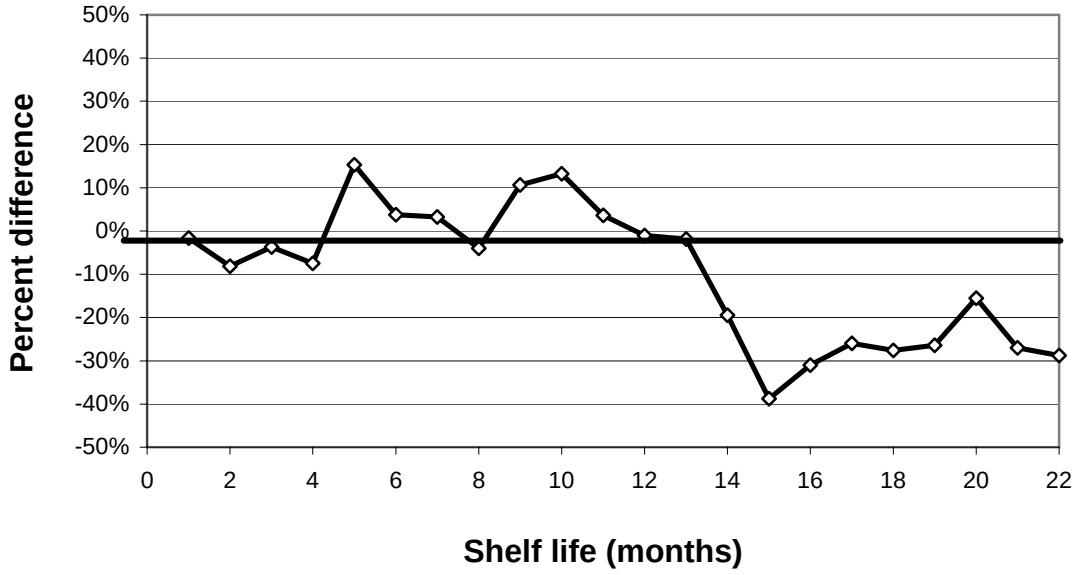


Figure 2.9 Shelf life of archived refrigerated NDMA extracts

Interferences

Sources of nitrosamines include selected treatment chemicals that are used for root control, fungicides, and chemicals used in circuit-board printing shops. In many of these products the active ingredients consist of dithiocarbamates or carbamate analogs, which are known to be contaminated with NDMA. Thiocarbamates have also been reported to produce dimethylamine upon hydrolysis, which is a known NDMA precursor. Contaminants in solvents, reagents, glassware, or other sample processing hardware may also contribute to interferences. Common rubber additives are known to contain compounds that have been identified as NDMA precursors (Fiddler et al., 1972). Matrix interference can also be caused by contaminants that are co-extracted from the sample. The extent of matrix interference will likely vary with sample source. Water softener resins have been reported to be a common source of NDMA (Kimoto et al., 1980). Non-irradiated laboratory DI water should be suspected if a persistent background is evident in blanks. Incorporation of laboratory and trip blanks as part of any sampling SOP is therefore recommended. Analyzing a blank DI water quality control with each batch is also suggested. Finally, extraction solvents should be checked periodically for nitrosamines because reagents have been reported as contamination sources in the past.

Conclusions

Based on the findings of this study, the following recommendations are suggested:

- Keep bottles out of the presence of sunlight during sampling; and after sampling, store in cooler with ice.
- Samples should be collected as representative grabs or composites with a minimum volume of 1-L.
- Use of pre-cleaned, amber glass containers with Teflon-lined caps is recommended.
- The addition of ~0.5 grams of sodium sulfite or sodium thiosulfate for wastewater or 0.04 to 0.1 g of sodium thiosulfate or sodium sulfite (or 0.02 g ascorbic acid) for potable water to the containers should minimize additional nitrosamine formation.
- The dechlorinating agents should be added to the containers prior to filling.
- Avoid storage of samples at low pH conditions because wastewater effluent and potable waters were observed to produce elevated levels of NDMA.
- If samples cannot be analyzed immediately, then refrigerate at 4^{o} C.
- Potable waters may be held up to 30 days, while wastewater effluent samples should be extracted within seven days.

CHAPTER 3

LIQUID–LIQUID EXTRACTION METHODS

Introduction

Liquid–liquid extraction is a classical method for isolation and concentration of organic analytes from water matrices. An advantage of LLE has been its suitability for a wide array of compounds, including pesticides and other natural and synthetic contaminants in the semi-volatile range (Standard Methods, 1995). Extraction efficiencies vary from as low as 30% to close to 100% depending upon various factors, including polarity and solubility. At the start of this project, laboratories commonly analyzed for NDMA by extraction of 1-L samples at neutral or basic pH, either by hand shaking of 2-L separatory funnels or with continuous liquid–liquid extractors (Fitzsimmons et al., 2001). Manual extraction with three aliquots of solvent is labor intensive, and continuous liquid–liquid extractors are costly and require long extraction times (e.g., overnight).

The goal of this project phase was to explore ways of reducing cost as well as maximizing analyte recovery. Cost factors that were considered include labor costs (e.g., analyst time), material cost (e.g., solvent consumption and environmental impact), and shipping and storage costs (e.g., sample size). Quantitation conditions suitable for the mix of nitrosamines studied were determined and are described in the experimental section. Extraction efficiencies were studied as a function of solvent type, sample ionic strength (salting-out phenomena) and pH. *P*-values, the fraction of the total solute that distributes into the nonaqueous phase of an equivolume solvent pair, were determined as a measure for extraction efficiencies and formed the basis of the subsequent optimization of methods (Suffet and Faust, 1972). Optimization focused on two modified extraction methods that reduced sample size and the number of extractions: a simplified liquid–liquid extraction (SLLE) and a micro liquid–liquid extraction (MLLE). The SLLE was selected to develop a method that would be similar to the traditional LLE method. The MLLE method, modeled after the disinfection byproduct (DBP) analysis, was selected as the basis for a method with greater cost saving potential. A detailed procedure for this method is presented in Appendix A.

Experimental

Chemicals

All specifications are suggested. Brand names and/or catalog numbers are included for illustration purposes only.

Reagent water with resistivity of 18.2 MΩ-cm, containing less than one half the MRL of each analyte was obtained from a water purification system (Milli-Q-UV, Millipore Corp, Bedford, MA) just prior to use. All chemicals were American Chemical Society (ACS) reagent grade or better. Sodium chloride (NaCl), sodium sulfate (Na_2SO_4), and sodium bicarbonate ($NaHCO_3$) were used to adjust the ionic strength of samples. Granular anhydrous Na_2SO_4 was also used to dry extracts. Sodium hydroxide and sulfuric acid were used for pH adjustment. In selected experiments, phosphoric acid, monobasic potassium phosphate, and dibasic anhydrous sodium phosphate were used to prepare buffers. Solvents were high-purity, high-resolution gas chromatography grade. Methanol, alkene stabilized DCM, and methyl t-butyl ether (MtBE) were used as extracting and conditioning solvents. Stock standards of the eight target analytes and N-nitrosodiphenylamine at 2000 mg/L each in methanol (Supelco, Bellefonte, PA) and in DCM (Protocol, Middlesex, NJ) were used to prepare working spiking solutions in methanol. *N*-nitrosodimethylamine-d_6 (NDMA-d_6), *N*-nitrosodipropylamine-d_{14} (NDPA-d_{14}) and *N*-nitrosodiethylamine-$^{15}N_2$ (NDEA-$^{15}N_2$) were purchased as individual stock solutions at 1 mg/mL in methylene chloride (DCM-d_6) (Cambridge Isotope Laboratories, Andover, MA, #DLM-2130-S, DLM-2131-S and NLM-3432-S, respectively).

Equipment

Shakers and Concentrators

One or 2 L glass separatory funnels with manual shaking, glass bottles (60 and 125 mL) with use of a reciprocal shaker (Eberbach model 6000) or 1 L glass bottles with use of an orbital shaker (Thermolyne model M73735) were used for the extractions. A TurboVap II concentration workstation (Zymark, Caliper Life Sciences, Mountain View, CA) with 1.0 or 0.5 mL end-point concentrator tubes was used to concentrate solvent extracts under a stream of nitrogen. Volumes of less than 0.5 mL were achieved under manually controlled helium blow-down.

The equipment used for CLLE includes: Continuous liquid–liquid extractor (heavier than water, 1 L capacity), Friedrich condenser, flat bottom boiling flask (250 mL), 340 mm x 22 mm I.D. chromatographic column, Kuderna-Danish concentrator (500 mL flask and 10 mL concentrator tips), Organomation S-evap and N-evap solvent recovery/contentration apparatus (Organomation Associates, Inc., Berlin, MA), heating mantle for 250 mL boiling flask, and boiling chips (Hengar, 16 mesh, solvent rinsed).

GC/CI/MS/MS (including operating and quantification parameters)

A Saturn 2200 ion trap mass spectrometer (MS) (Varian Inc., Walnut Creek, CA) with a Varian 3800 gas chromatograph (with 1079 PTV injector) equipped with liquid chemical ionization (CI) capability was operated in MS/MS mode. A Varian 8400 or a Combipal autosampler (Leap Technologies Carrboro, NC) was used to inject 8 μL of extract onto either a DB1701 (30 m x 0.25 mm x 1 mm) or DB-VRX (60 m x 0.32 mm x 1.8 mm) column, both from Agilent. Acetonitrile was used as the CI reagent. The GC/CI/MS/MS conditions described below were used for the work described in this chapter.

Separation: GC Columns

The baseline GC column for the project has been the DB-VRX column, which chromatographically resolved potential interferences at 81 m/z (NDMA-d_6 $[M+1]^+$ ion) found in some water samples. However, this column does not fully resolve NDPA, NMOR, NPYR and d_{14}-NDPA, so mass spectral separation is required. The DB-1701 column can resolve all eight nitrosamines under the conditions shown in Table 3.1. With the DB-1701 column, the sensitivity and peak shape were also improved because the MS spectral segments could be set up for one or two compounds at a time instead of for the four coeluting compounds. The DB-1701 column, however, exhibits a higher degree of bleeding and is sensitive to degradation by water in the DCM extracts.

Table 3.1 Varian 3800 GC conditions, column information, and nitrosamine retention times

GC conditions		
Column	**DB-VRX**	**DB-1701**
Column length (m)	60	30
Column ID (mm)	0.32	0.25
Column film (μm)	1.8	1
Column flow (mL/min)	1.2	1.5
Detector Type	Varian GC 3800/Saturn 2200, CI(acetonitrile)/MS/MS	
Injector Type	Varian 1079	

Nitrosamine Retention Time In Minutes (grouped by Internal Standard)		
Compound Name		
NDMA-d_6 (Internal Standard)	13.62	10.07
NDMA	13.68	10.09
NDEA-$^{15}N_2$ (IS)	18.68	11.99
NMEA	16.27	11.17
N-Nitrosodiethylamine (NDEA)	18.67	11.99
NDPA-d_{14} (IS)	24.33	13.83
N-Nitrosomorpholine (NMOR)	24.40	14.32
N-Nitrosopyrrolidine (NPYR)	24.44	14.53
N-Nitrosodi-n-propylamine (NDPA)	24.57	13.90
N-Nitrosopiperidine (NPIP)	26.12	14.78
N-Nitrosodi-n-butylamine (NDBA)	30.61	15.90

Injector Program

Temp (^{o}C)	Rate (^{o}C/min)	Hold Time (min)	Total Time (min)	Hold Time (min)	Total Time (min)
35	0	0.8	0.8	0.8	0.8
260	200	2	3.92	2.08	4.00
150	200	31.5	35.97	21.00	25.56

Time (min)	Split State	Split Ratio
Initial	On	5
0.8	Off	Off
2.2	On	100
20	On	30

Table 3.1 continued

Column temperature program: DB-VRX				DB-1701			
Temp. (°C)	Rate (°C/min)	Hold time (min)	Total time (min)	Temp. (°C)	Rate (°C/min)	Hold time (min)	Total time (min)
35	0	4	4.0	35	0	4	4
100	20	2	9.3	200	15	0	15
210	5	0	31.3	240	40	10	26
250	50	5	37.1				

Autosampler conditions, Varian Model 8400	
Solvent plug volume, µL	0.2
Sample volume, µL	8.0
Air plug after sample, µL	1.0
Plunger injection spd, µL/sec	0.2
Post-injection delay, sec	99.9

MS and quantitation conditions

The baseline MS mode for the Saturn system was CI (with acetonitrile as the CI reagent) to produce $[M+H]^+$ ions. This molecular ion was used as the quantitation ion for *p*-value and initial optimization experiments; it was also fragmented by an excitation energy that produces 10 to 25% product ion spectra for confirmation of compound identity. A coeluting compound with the same nominal m/z fragment as the $[M+H]^+$ ion of the analyte can result in a positive interference. Quantitation of a product ion can significantly reduce the occurrence of interference and was used for the later method optimization experiments and for the analysis of analytes in different water matrices. A GC/CI/MS/MS method based on product ion quantitation is given in Table 3.2. The daughter ion spectra were determined empirically. It is beyond the scope of this project to positively identify the daughter ion structures. Some daughter ions are consistent with loss of NO or HNO or an EI fragment +H. The excitation amplitude of fragmentation was optimized to maximize product ion intensity while retaining a precursor ion intensity of 10 to 25% for compound confirmation. No adverse impact on calibration curves, spike recoveries or duplicate differences were observed. Product ion quantitation was used for analysis of natural samples. Calibration curves were based on the isotope dilution technique for NDMA, NDEA and NDPA (i.e., Area/$Area_{isotope}$) and on internal standard area ratios using NDEA-$^{15}N_2$ as internal standard for NMEA and NDPA-d_{14} for the four later-eluting nitrosamines. In summary, quantitation on either $[M+H]^+$ or product ion can be used. Quantitation based on $[M+H]^+$ provides greater sensitivity and may be best suited for clean waters, while quantitation of product ion provides greater specificity and may be needed for wastewater matrices.

Extraction Method

Manual LLE

The sample is placed in a separatory funnel or suitable bottle, adjusted for pH and salt concentration as needed, and spiked with appropriate internal standards. An aliquot of solvent is added and shaken for a specified time. After phases have separated, the solvent layer is removed and dried with prebaked sodium sulfate. Additional solvent aliquots may be used as specified. The combined extracts are concentrated in a water bath under a stream of nitrogen or helium to specified volume (typically 0.5 to 1.0 mL).

CLLE

Although the continuous liquid–liquid extraction technique was not evaluated as part of this project and is not discussed further, the method was used as the reference method for comparison during the round-robin testing and a brief description is presented here. For the CLLE method, 250 mL of DCM and a volume of sample, up to 1 L, was added to each extractor. If the sample volume was less than 1 L, DI water was added to result in a final 1-L volume. NDMA-d_6 (isotope dilution standard) was also added to each extractor to give a concentration of 50 ng/L, and the mixture was extracted for 14 to 16 hours. The extract was passed through a sodium sulfate drying column. The dried extract and rinse were then serially processed through a Kuderna-Danish concentrator/evaporator apparatus to a final volume of 1.0 mL. Immediately prior to loading onto the GC autosampler, 10 µL of 2 µg/mL NDPA-d_{14} internal working standard was added to 200 µL of concentrated sample extract.

Table 3.2 MS/MS conditions for product ion quantitation on DB-1701 column

CI gas: acetonitrile	Eject.amp: 15.0 m/z
CI storage level: 19.0 m/z	Background mass: 40 m/z
Max. ion time: 2000 µsec	Max. reaction.time: 120 millisec
Target TIC 5000 counts	Prescan time: 200 µsec

Segment	Description	Start time	End time	Low mass	High mass	Ioniz. mode	Ion prep
1	Fil/Mul delay	0	7.6	40		CI auto	
2	NDMA	7.6	9.7	40	83	CI auto	MRM
3	NMEA	9.7	11.3	40	91	CI auto	MS/MS
4	NDEA	11.3	15.5	40	107	CI auto	MRM
5	NDPA	15.5	17.7	40	150	CI auto	MRM
6	NMOR,	17.7	18.7	40	125	CI auto	MRM
7	NPYR	18.7	19.1	40	106	CI auto	MS/MS
8	NPIP	19.1	20.0	40	120	CI auto	MS/MS
9	NDBA	20.0	23.5	40	165	CI auto	MS/MS

Segment	Channel	Compound	Parent mass	Isolation window	Quan/Dau. ion	Excit stor level	Excit ampl*
2	1	d_6-NDMA	81	1.5	49	35	0.36
	2	NDMA	75	1.5	44	35	0.34
3	1	NDMA	89	2	61	40	0.31
4	2	$^{15}N_2$-NDEA	105	2	77	40	0.33
	1	NDEA	103	2	75	40	0.34
5	1	NDPA	131	2	89	40	0.34
	2	d_{14}-NDPA	145	2	97	40	0.33
6	1	NMOR	117	2	87	40	0.30
7	1	NPYR	101	2	55	40	0.33
8	1	NPIP	115	2	69	40	0.34
9	1	NDBA	159	2	103	48	0.37

** MS/MS by resonant excitation.*

Results and Discussion

Parametization Experiments and Extraction Efficiency (as p*-value or percent absolute recovery)*

The *p*-value is a way of expressing extraction efficiency and is the fraction of the total solute that distributes into the nonaqueous phase of an equivolume solvent pair. A value to one equals 100% recovery. The *p*-values were used to select the appropriate parameters to use in subsequent method optimization. Values were determined as a function of solvent type, ionic strength, and pH. Conditions are shown in Table 3.3. MtBE is a polar solvent with low toxicity, used for trihalomethane and other DBP analyses. It has a polarity index of ~2.5, slightly lower than the 3.1 of DCM (Snyder, 1974; 1979).

Table 3.3 Optimization parameters for determination of extraction efficiencies

Parameter	Variables
Solvent	MtBE, DCM
Ionic strength	0, 2.0 M sodium sulfate, sodium chloride
Sample pH at extraction	Acidic (pH = 3), neutral (pH = 8), basic (pH = 11)

*Initial nitrosamine concentrations = 10 micrograms per liter. Extracts analyzed without concentration.

Volume changes for water and organic phases were determined by the mixing of known volumes in glass stoppered graduated cylinders that were shaken and then allowed to equilibrate at ambient temperature. Smaller volumes (100 mL) of 0.01 M phosphate buffer spiked with 10 µg/L nitrosamine were extracted with 10:1 sample:solvent ratio. The organic phase was isolated and analyzed by GC/CI/MS/MS without further concentration.

Calculation of *p*-values are presented below (Suffet, 1972):

$$p = \frac{E}{\alpha - E(\alpha - 1)} \tag{3.1}$$

$$E = \frac{A_n V_n}{A_s V_s} \tag{3.2}$$

$$\alpha = \frac{V_n}{V_p} \tag{3.3}$$

p = Fraction of total solute that distributes itself into the nonpolar phase of an equivolume solvent pair.
E = Fractional amount of solute partitioning into nonpolar phase.
α = Volume correction factor for one extraction step of LLE
V_n = Volume of nonpolar phase (solvent phase)
V_p = Volume of polar phase (water phase)
V_s = Original volume of solvent phase
A_n = Amount of analyte in the solvent phase
A_s = Starting amount

Method and extraction efficiencies are also expressed as percent absolute recovery, which is calculated by comparison of detector response for the extract with direct standards. No correction is made for extraction efficiency using internal standards added to the water matrix as would be done to determine accuracy via spike recoveries. Absolute recovery as used in this report should not be confused with spike recovery.

Solvent and Salt Effects

Figure 3.1 shows the *p*-values for both MtBE and DCM for the eight nitrosamines at neutral pH in 0.01 M phosphate buffer, with and without the addition of 2 M Na_2SO_4. Only NDPA and NDBA were extracted efficiently by MtBE without salt. Addition of salt improved the extraction from 18 to 32% increasing the *p*-values for NDEA and NPIP to 0.80. DCM, however, is clearly the more efficient extraction solvent for the nitrosamine family. NDMA is the most difficult nitrosamine to extract.

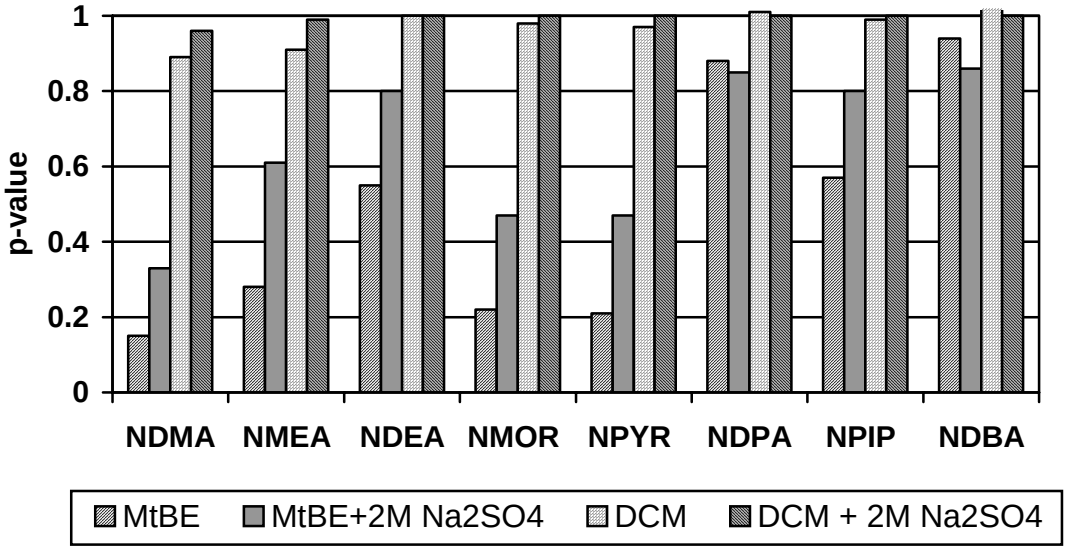


Figure 3.1 *P*-Values for MtBE and DCM extractions, with and without Na_2SO_4

The above experiments showed that increasing the ionic strength of the sample with sodium sulfate increased extraction efficiency. Higher concentrations of Na_2SO_4, however, were difficult to dissolve and manipulate. Therefore, the use of sodium chloride instead Na_2SO_4 was evaluated during optimization of the SLLE method and subsequently in both the SLLE and MLLE methods.

pH Effects

The effects of pH on nitrosamine partitioning were determined for DCM at an intermediate salt concentration of 0.5 M. This level was selected because sodium sulfate precipitated during extraction when the initial concentration was 1.5 to 2 M. The results are given in Figure 3.2. No significant difference in *p*-values was observed for any of the nitrosamines under acid or neutral (ambient) conditions. Extraction, however, was less efficient at pH 11 for all the nitrosamines except NDMA, which was essentially unchanged by pH. Therefore, selection between acidic and ambient extraction pH will be based on other considerations (e.g., ease of set-up, emulsion formation, biocide effect, and holding time).

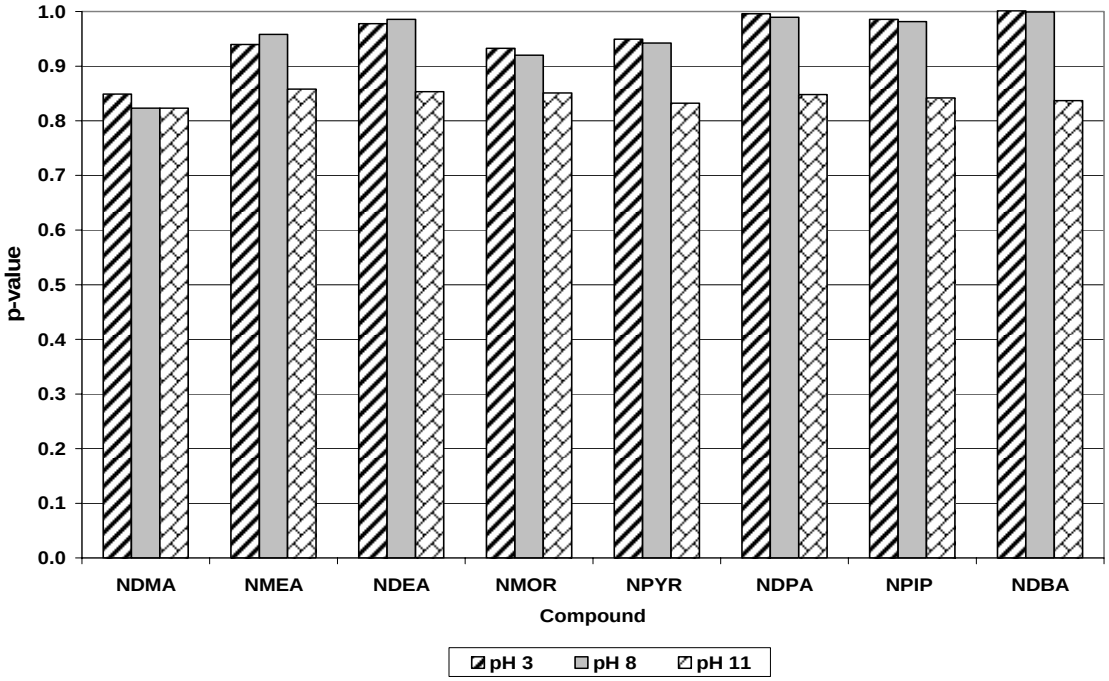


Figure 3.2 Effect of pH on extraction efficiencies expressed as *p*-values

Optimization Experiments

Initial optimization conditions were selected based on the results from *p*-values and from practical considerations. Partition *p*-values indicated:

1. DCM was a more efficient extraction solvent;
2. Samples can be extracted at ambient pH because pH had minimal effect;
3. Salt addition should be included.

Compromises between analyte recovery and practicality (e.g., cost) were necessary in working out the details (e.g., salt concentration, sample size, sample:solvent ratio, etc.) of the two LLE methods. The SLLE method is slightly less expensive compared to the traditional LLE method, and retains a similar high concentration factor. The MLLE method currently represents a greater cost savings at the sacrifice of sensitivity. With some large volume injection systems, the detection limit might be lowered to meet drinking water requirements by increasing the volume of extract injected.

SLLE Method

To reduce the cost of analysis, a single extraction was implemented and the sample size reduced from 1 L to 500 mL. The SLLE optimization examined the variables shown in Table 3.4.

Table 3.4 Optimization parameters for SLLE nitrosamine method

Variable	Range
Salt type	Na_2SO_4, NaCl
Shaking vessel/mode	Bottle/orbital shaker, Separatory funnel/manual
Final extract volume	1.0 mL, 0.5 mL

An initial salt concentration of 1.5 M Na_2SO_4 (100 g/500 mL) was selected to provide some enhancement of extraction efficiency, while minimizing problems due to salt precipitation. To achieve the desired detection of <2 ng/L without increasing injection volume, a sample:solvent ratio of 5:1 was selected which would give approximately 80% recovery for the extraction of NDMA based on the *p*-values. Evaporation of the total solvent extract to a final volume of 1.0 mL would equal a 500 fold concentration factor.

Triplicate 6 ng/L spiked reagent water samples were extracted under the above conditions by shaking in a l L bottle on an orbital shaker at 200 rpm for 30 minutes. The absolute percent recoveries averaged 46% for NDMA, 49% for NMOR, 63% for NPYR, 66% for NDEA and NDPA, 73% for NMEA, and 75% for NDBA. The recovery for NPIP was not reported due to suspected contamination. Separation of the heavier-than-water solvent was difficult without a separatory funnel and sodium sulfate precipitated during solvent separation resulting in time consuming manual manipulation and loss of extract. Good quantitation was achieved using the isotope dilution and internal standard calculations even though the absolute recoveries were less than 80%. Spike recoveries ranged from 83 to 122%, which are within generally accepted control limits of 70 to 130% (CDHS, 2001).

NaCl was investigated as a substitute-salting agent because it dissolved more readily than Na_2SO_4, achieving a higher ionic strength. Reagent water (500 mL) spiked with 200 ng/L nitrosamines with 0, 50, 100, and 150 gm/L pre-baked sodium chloride was extracted with hand shaking in a separatory funnel with 200 mL DCM. The separated organic phase was then dried over Na_2SO_4 and concentrated to 1 mL and spiked with 50 ng $^{15}N_2$-NDEA internal standard. Nitrosamine concentrations were determined by GC/CI/MS/MS. The recovery of NDMA (Figure 3.3) increased from 44% with no NaCl to 74% with excess NaCl. The recoveries shown below include losses caused by incomplete extraction, separation, and concentration of the single extraction**,** which parallels SLLE analytical conditions.

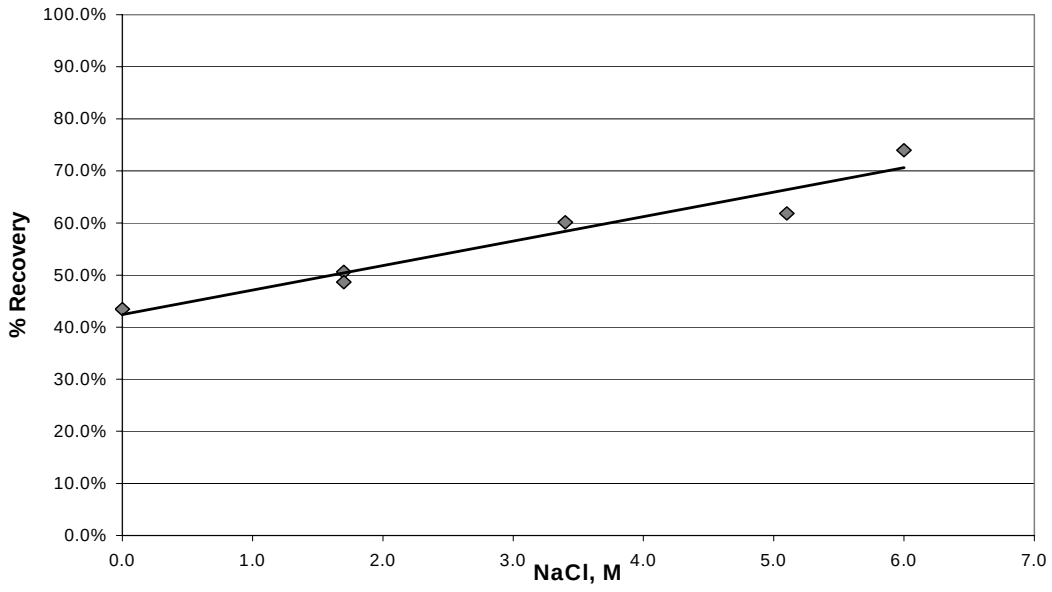


Figure 3.3 Effect of NaCl addition on NDMA recovery at 2.5:1 sample:solvent ratio

The following changes were implemented to "optimize" the SLLE method. The 1.5 M (3.0 N) Na_2SO_4 was replaced by 3.4 M NaCl (100g/500mL) to eliminate salt precipitation after shaking. The sample:solvent ratio was decreased from 5:1 to 2.5:1 by doubling the DCM volume to increase nitrosamine recoveries. Extraction and solvent separation were carried out in a 1 L separatory funnel. The extract was concentrated to a final volume of 0.5 mL to increase the theoretical concentration factor to 1000. The final simplified LLE method is summarized in Table 3.5. Calibration curves from 1 to 200 ng/L were constructed from extracted standards (Figure 3.4). Curves were linear for all compounds with slopes ranging from 0.03 to 0.21 area ratio units per ng/L and r^2 values of 0.99 or better.

Table 3.5 Optimized SLLE method for nitrosamine analysis

Extraction		
Sample: 500 mL, neutral pH	Add 100 gm NaCl, NDMA-d_6, $^{15}N_2$-NDEA, and d_{14}-NDPA, 200 mL DCM	Shake 3 min, separate after 10 min
Concentration		
Dry over Na_2SO_4	Evap. stream of N_2, 38 ^oC bath	Final volume 0.5 mL
Analysis		
Inject 8 µL, 35 ^oC injector with open split	GC/CI/MS/MS, product ion quantitation, precursor, and product ion confirmation	Calibration based on area ratio with labeled surrogates.

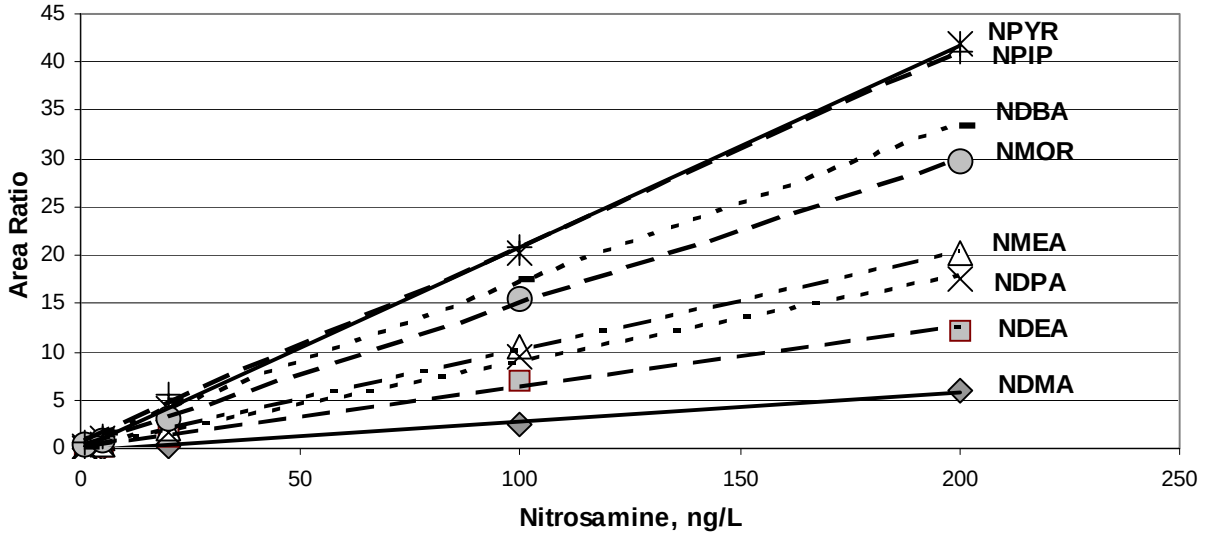


Figure 3.4 Nitrosamine calibration curves for extracted standards (1 to 200 ng/L)

Minimum Detection Limits for SLLE Nitrosamine Method

The detection limits for the SLLE method were determined by analyzing seven aliquots of reagent water spiked at 2 ng/L of each nitrosamine. The MDLs are given in Table 3.6 and ranged from 0.27 ng/L for NDBA to 0.73 ng/L for NDEA. The MDL for NDMA by SLLE was 0.52 ng/L. If MRLs are set at three times the MDL, the MRL for NDMA would be 1.5 ng/L and below the Office of Environmental Health Hazard Assessment (OEHHA) *de minimis* (i.e., 10^{-6}) cancer risk level of 2 ng/L.

Table 3.6 MDLs for SLLE nitrosamines method

Compound	Average (ng/L)	Standard deviation (ng/L)	MDL (ng/L)
NDMA	2.12	0.16	0.52
NMEA	2.21	0.19	0.60
NDEA	2.25	0.23	0.73
NMOR	1.93	0.11	0.33
NPYR	2.12	0.18	0.55
NDPA	2.03	0.19	0.58
NPIP	2.01	0.13	0.42
NDBA	2.01	0.09	0.27

The SLLE method uses the isotopic surrogates internal standards to compensate for incomplete extraction. When switching to the SLLE method from the standard 1-L x 3 extraction LLE procedure, some cost savings may be realized because of reduced analyst time required and fatigue caused by the manual shaking. The method, however, is not a truly low cost method nor is it amenable to automation. Therefore, the SLLE method was not included in the round-robin tests.

MLLE Nitrosamine Method

The MLLE method is modeled after the disinfection byproduct (DBP) analyses that have been used at the Metropolitan Water District of Southern California (similar to USEPA Method 551) and are amenable to automated shaking and large batch extractions with minimum analyst fatigue. Such a method would greatly reduce labor costs, solvent volume, and sample shipping and storage costs. Challenges are presented by the 1 to 2 orders of magnitude higher detection limits for DBPs (25 to 250 ng/L) compared to the target NDMA detection limit of 2 ng/L. NDMA is also more polar than the chlorinated DBPs and less efficiently extracted. Optimization of the nitrosamine MLLE was a compromise between sensitivity and ease/cost of analysis.

The general MLLE procedure was:

1. Transfer sample to glass bottle (60, 125, or 250 mL)
2. Add salt (for 100 mL samples, 30 grams of NaCl) and shake well to dissolve the salt.
3. Add an aliquot of a 0.2 mg/L solution of three internal standards in methanol, e.g., NDMA-d_6, NDPA-d_{14}, and NDEA-$^{15}N_2$, to give a final concentration of each internal standard of 50 ng/L
4. Add DCM
5. Shake the bottle on a reciprocal shaker
6. Let to stand for ~15 minutes for the two layers to separate and separate the DCM layer
7. Dry extract with ~0.4 grams of sodium sulfate, and concentrate with a water bath at 35 °C and stream of nitrogen or helium at 10 psi (such as on a Turbo-vapII concentrator)
8. Transfer to an autosampler vial and analyzed on a Varian Saturn GC/ion trap mass spectrometer using the CI (acetonitrile)/MS/MS mode.

The optimization variables for the MLLE method are listed in Table 3.7.

Table 3.7 Optimization parameters for MLLE nitrosamine method

Variables	Range
Sample volume	40 mL, 100 mL
DCM, volume added	10 mL, 20 mL, 50 mL
Shaking time	5 min, 10 min
Separation setup	Syringe, separatory funnel
Concentration setup	Helium blow-down, TurboVap
Final volume	0.2 mL, 0.5 mL

Optimization experiments focused on NDMA because it is the most difficult nitrosamine to extract. The percent recoveries for the other nitrosamines are all equal to or better than that of NDMA.

Initial sample volume studies using a 40 mL sample required concentration to 200 μL to achieve a theoretical 200 fold concentration factor. This was accomplished with manual concentration. With a sample volume of 100 mL, the final extracts can be concentrated using a Turbo Vap II concentrator to 0.5 mL with the same concentration factor.

Increasing the shaking time from 5 minutes to 10 minutes resulted in an approximately 50% increase in NDMA recovery. Addition of a vigorous 2 minute manual shaking prior to the 10 minute mechanical shaking did not appreciably alter the recoveries.

Increasing the DCM volume from 10 mL to 20 mL doubled the recovery of NDMA. A DCM volume of 50 mL was also tried with slightly increased recoveries. The bigger bottles, however, were too snug in the shaker box, and the small improvement in extraction efficiency did not warrant revamping of the shaker.

Separation of the solvent extract with a syringe was easier, less time consuming, and less costly than using a separatory funnel. The DCM layer was readily separated from the aqueous layer with a 20 mL glass syringe. The syringe needle reaches to the bottom of the bottle, withdrawing approximately 19 mL of solvent extract for standards and clean sample solutions. Less solvent was recovered from samples that formed emulsions; however, the labeled surrogates corrected for this volume difference.

Evaporation under a stream of helium via a needle tip held over the extract was initially used to concentrate the DCM extracts, however, this step required up to an hour to reduce the 20 mL extracts to 0.5 mL, and required careful attention to catch the desired end point. In contrast, the Turbo Vap concentrator required less than 30 minutes to process the same amount of extract. It was operated unattended and stops automatically; therefore, it is the more cost-effective procedure (approximately $7,000 for the concentrator).

Evaporation of the extract to 0.2 mL provided a higher concentration factor than to 0.5 mL, but it required an extra concentration step by the He-blow down after automated Turbo-vap evaporation to 0.5 mL. Concentration to a final volume of 100 to 200 μL is an option that can be incorporated if lower detection limits are critical. It was not included in the MLLE method, and a higher detection limit was adopted for the MLLE method targeting analysis of wastewater and formation potential samples with higher nitrosamine levels.

Optimized MLLE Method

Based on extraction efficiencies and ease of operation, the following parameters were selected: sample volume of 100 mL, shaking time of 10 minutes, 20 mL of DCM extraction solvent, shaking time of 10 minutes on a reciprocal shaker (estimated shaking rate of 150 rpm), syringe used for separation, TurboVap used for concentration, and a final volume of 0.5 mL. The standard operating procedure (SOP) for the MLLE nitrosamine method is given in Appendix A.

Figure 3.5 MLLE Equipment Setup

Recoveries of the eight nitrosamines were obtained by comparison of an extracted standard with the corresponding direct standard based on the concentration factor (100 mL to 0.5 mL = 200). The results are listed in the Table 3.8.

Table 3.8 Absolute recoveries achieved by the MLLE nitrosamine method
(based on direct standards, average of 15 extractions)

	NDMA	NMEA	NDEA	NMOR	NPYR	NDPA	NPIP	NDBA
Recovery	20%	31%	38%	32%	38%	40%	40%	53%

MDLs for MLLE Nitrosamine Method

To calculate the MDL, a set of seven replicate 10 ng/L samples in MilliQ water was extracted and the concentrations of the nitrosamines were obtained. From the concentrations of the seven samples, standard deviations were calculated for each nitrosamine, and the MDL was calculated according to the formula: MDL = 3.14 x standard deviation. The results are shown in the Table 3.9. If the MRL is equivalent to three times the MDL, then the MRL for NDMA is 7 ng/L. This is above the OEHHA *de minimis* (i.e., 10^{-6}) cancer risk level of 2 ng/L, but below the California Action Level of 10 ng/L and Ontario Safe Drinking Water Act of 2002 MAC of 9 ng/L. The MLLE method may be especially suited to measure higher nitrosamine levels as found in some wastewaters and formation potential samples.

Table 3.9 MDLs for MLLE nitrosamine method

Compound	Mean (ng/L)	Standard deviation (ng/L)	MDL (ng/L)
NDMA	11	0.7	2.3
NMEA	12	1.2	3.9
NDEA	10	0.8	2.5
NMOR	10	0.9	2.7
NPYR	10	0.6	1.8
NDPA	12	1.1	3.4
NPIP	9.4	0.7	2.2
NDBA	12	1.2	3.8

MLLE Method Performance

Fourteen surface water and wastewater QA/QC samples, including formation potential test samples, have been analyzed by MLLE for this and other projects. The recoveries and standard deviations for spiked samples and mean relative differences and standard deviations for spiked duplicate samples are listed in Table 3.10. The number of samples (N) ranged from 16 to 19, of which one was spiked at 20 ng/L of each nitrosamine and the remainder evenly split between 50 and 100 ng/L spike amounts. Spike levels were based on the best guess of probable NDMA concentration in the sample. The background levels of NDMA ranged from not detected to 402 ng/L with an average value of 137 ng/L. The average background level for the other nitrosamines were below the reporting limit of 10 ng/L, except for NDBA which was 13 ng/L.

Table 3.10 MLLE precision and accuracy in water and wastewater samples

	Accuracy		Precision	
Compound	Mean rec. (%)	Stdev (%)	Mean rel.diff. (%)	Stdev (%)
NDMA	98	12	10	8.9
NMEA	97	15	12	13
NDEA	93	16	15	15
NMOR	83	19	6.2	3.2
NPYR	92	16	8.3	6.0
NDPA	97	17	11	11
NPIP	87	15	6.2	4.8
NDBA	90	17	13	8.2

Method Comparison Studies

Two reclamation plants were sampled at the influent and reverse osmosis effluent and analyzed by the authors using SLLE, MLLE, and the SPE methods described in Chapters 4 and 5. The MLLE method was also part of an interlaboratory round-robin test conducted in March 2004 in the U.S and Canada. The results of both studies are discussed in Chapter 8.

Summary and Conclusions

Summary

The evaluation of liquid–liquid extraction efficiencies showed:

- Order of extraction efficiency for the target nitrosamines is:
  NDMA < NMEA ≈ NPYR ≈ NMOR < NDEA ≈ NPIP < NDPA ≈ NDBA;
- DCM is a much more efficient solvent compared to MtBE;

- Salting-out greatly increased extraction efficiency especially for NDMA extraction;
- Sodium chloride is more effective and easier to handle than sodium sulfate as a salting agent;
- pH had minimal effect on nitrosamine extraction efficiencies.

SLLE Method

- Sample volume – 500 mL;
- Single 200 mL DCM extraction in separatory funnel;
- Concentration to 0.5 mL, preferably on an automated system;
- MDL for NDMA is 0.5 ng/L and 0.3 to 0.7 ng/L for other nitrosamines.

MLLE Method

- Sample volume is 100 mL;
- Single 20 mL DCM extraction shaken in bottle on mechanical shaker;
- Syringe separation of extract;
- Concentration to 0.5 mL, preferably on an automated system;.
- MDL for NDMA is 2.3 ng/L and for the other nitrosamines from 1.8 to 3.8 ng/L;.
- Accuracy for NDMA is 97.8% ± 12.4% and precision is 10.0% ±8.9%;
- For other nitrosamines, accuracy ranges from 82.8% to 97.2% and precision from 6.2% to 14.6%.

Conclusions

As a results, the following conclusions can be made:

- SLLE and MLLE methods provide alternative non-solid–phase extraction nitrosamine extraction options for those laboratories preferring liquid–liquid extraction techniques;
- SLLE has small cost advantage over traditional LLE method with minimal reduction in recoveries;
- MLLE is much less labor intensive than tradition LLE with corresponding reduction in cost;
- Isotope dilution and procedural standards adequately correct for the low absolute recoveries of the MLLE method;
- MLLE method is suitable when nitrosamine levels are greater than 10 ng/L or when detection limits of 10 ng/L are acceptable.

CHAPTER 4

AMBERSORB 572® SOLID–PHASE EXTRACTION METHOD

Introduction

A solid–phase extraction (SPE) and GC/CI/MS/MS isolation and detection method for nitrosamines in finished drinking water, raw source water, reclaimed water, and wastewater was optimized during this investigation. The SPE method using Ambersorb 572® proposed by Taguchi et al. (1994) to extract NDMA serves as the baseline method that was tested in this chapter. Possible drawbacks of the Ambersorb method are a lower recovery for NDMA than the other nitrosamines and adsorption site competition from other organics. The objective of this project was to examine SPE testing conditions in order to minimize cost, lower detection limits, and optimize the quality of testing for NDMA and other target nitrosamines. The parameters investigated included varying resin mass, extraction times, salt addition, and pH adjustment. A complete procedure is provided in Appendix B.

Experimental

All specifications are suggested. Brand names and/or catalog numbers are included for illustration purposes only.

Chemicals

Reagent water was obtained from a water purification system (Milli-Q-UV, Millipore Corp., Bedford, MA) just prior to use. The adsorbent used was Ambersorb® 572 (Supelco® P/N: 10432-U, Bellefonte, PA), conditioned in a shallow tray at 250 °C for three hours before use, transferred to a capped amber glass bottle and stored in a desiccator. There is evidence that nitrosamines can be transmitted to Ambersorb through the gaseous phase thus proper storage was critical. To ensure a uniform size distribution of beads, it was necessary to sieve with a No. 50 ASTM mesh or allow the finer particles to settle in the storage container taking beads from the upper portion only. The solvents used, methanol (MeOH, B&J Brand®: 230-1) and DCM (EMD™: DX0838-6) were high purity grade. Reagent gases were supplied by Air Source Industries, Inc. and included ultra high purity grade helium (UN 1046, GC carrier gas), nitrogen (UN1066, autosampler pneumatics), and bone dry grade carbon dioxide (UN 1013, GC injector coolant). Chemicals supplied by J.T. Baker® included high purity anhydrous sodium thiosulfate ($Na_2S_2O_3$, 3828-05, dechlorinating agent) and sodium chloride (NaCl, 3628-05). Both chemicals were demonstrated to be free from analytes or interference at levels greater than the lower level of detection (LLD) for each compound of interest. Solution pH was adjusted using sodium hydroxide (NaOH, 4715) and sulfuric acid (H_2SO_4, 4699-01), both of which were also provided by J.T. Baker®.

Target and Surrogate Nitrosamine Standards

Stock standards of the eight target nitrosamines (Supelco, 502138) were obtained from Sigma-Aldrich® at 2000 mg/L each in MeOH. The three isotopically labeled standards (internal standards), NDEA-$^{15}N_2$, NDPA-d_{14}, and NDMA-d_6 were purchased in solutions at 1 mg/mL in DCM from Cambridge Isotope Laboratories Inc. (P/N: NLM-3432-S, DLM-2131-S, DLM-2130-S, Andover, MA).

Equipment

Materials needed for sample preparation included amber glass vials (10 to 20 mL, for standard solution storage) and amber bottles (1-L, for sample extraction) with PTFE-lined screw caps. Additional materials included class A volumetric flasks for preparation of standards and samples, microsyringes of various size and an analytical balance capable of accurately weighing to 0.1 mg. The pH values were measured using a HACH® model Sension 4 meter. A LE2002 heavy-duty Rotator (Environmental Express Inc., Mt.

Pleasant, SC), used for extraction mixing process, was modified to maintain 50 rpm and equipped with a 12 x 1-L bottle capacity. A vacuum filtration apparatus (Nalgene®), equipped with disposable filters (Whatman®, 1001 055) was used to separate the Ambersorb from the extracted samples. Disposable aluminum dishes (VWR™, 25433-008) were used to transfer the dried Ambersorb to 2.0 mL amber glass screw cap auto-sampler vials with PTFE-faced septa. A 3800 GC with a Saturn 2000 ion trap MS (Varian™ , Walnut Creek, CA), was used for the separation and detection of nitrosamines. A Varian 8200cx autosampler was used to introduce the samples to the column. Monitoring and control of the GC/MS workstation parameters were accomplished through the Saturn software package: System Control and Method Builder Version 5.52.

The auto-sampler conditions are listed in Table 4.1. The capillary GC (CP-3800cx) features a split/splitless temperature programmable injector capable of large volume injections. The injector program parameters are shown in Table 4.2. The column, HP-VOC, low polarity (J&W Scientific) was purchased from Agilent Technologies. The column flow rate was 1.2 mL/min and column dimensions were 60-m long x 0.32-mm ID with a 1.8-µm film thickness. The column temperature program is shown in Table 4.3. The MS (Saturn® 2000) is an ultra trace ion trap mass spectrometer, capable of utilizing methanol as a chemical ionization (CI) reagent gas, and performing tandem mass spectrometry (MS/MS). The parameters for CI mode are shown in Table 4.4 and a GC/CI/MS/MS method based on product ion quantitation is given in Table 4.5.

Table 4.1 Varian 8200cx autosample conditions

Parameter	Condition
Syringe wash time, s	40
Solvent plug volume, µL	1.0
Sample volume, µL	8.0
Uptake speed, µL/s	3.0
Injection rate, µL/s	5.0
Needle residence time, min	0.5

Table 4.2 GC (CP-3800cx) injector program

Temp (°C)	Rate (°C/min)	Hold (min)	Total (min)
37	0	0.67	0.7
250	200	27	28.7

Table 4.3 HP-VOC column temperature program

Temp. (°C)	Rate (°C/min)	Hold time (min)	Total time (min)
32	0	1.7	1.7
100	15	2	8.2
190	5	0	26.2
270	50	5	30.0

Table 4.4 MS (Saturn® 2000) ionization mode conditions

CI gas: MeOH	Eject.amp: 17.0 m/z
CI storage level: 19.0 m/z	Background mass: 40 m/z
Max. ion time: 2500 µs	Max. reaction time: 128 ms
Target TIC: 7000 counts	Prescan time: 200 µs

Table 4.5 GC/CI/MS/MS method conditions

Segment	Description	Parent mass	Quant. ion	Start time	End time	Low mass	High mass	Excit. ampl.
	Fil/Mul							
1	delay			0	9.0			
2	NDMA-d_6	81	50	9.0	11.0	40	85	0.62
	NDMA	75	44					0.68
3	NMEA	89	61	11.0	13.5	50	95	0.53
4	NDEA-$^{15}N_2$	105	77	13.5	16.0	65	110	0.59
	NDEA	103	75					0.59
5	None			16.0	19.0			
6	NDPA-d_{14}	145	97	19.0	22.4	50	160	0.72
	NDPA	131	89					0.74
	NMOR	117	86					0.60
	NPYR	101	55					0.60
7	NPIP	115	69	22.4	24.0	50	120	0.49
8	NDBA	159	57	24.0	28.0	50	165	0.60

**Ionization mode: CI auto, Ion prep: MRM, Isolation window: 2, Waveform type: Resonant, Exc. stor. level: 35*

Quantitation

The Saturn View™ portion of the workstation software package was used to calculate nitrosamine concentration in samples by the isotope dilution and internal standard technique. This technique involves:

1. A Saturn 2000 ion trap mass spectrometer (MS) with a Varian 3800 gas chromatograph (GC) equipped with liquid chemical ionization (CI), using methanol and operated in the MS/MS mode. A Varian 8200 auto sampler was used to inject 8 µL of the sample extract onto a HP-VOC column. The MS/MS ion trap detector is capable of selecting excitation energy to produce qualifier ions (molecular/parent ions) for identification (M+1) or product/daughter ions for more analyte specificity, while keeping 10 to 25% of the parent ion. In water matrices that may have other interfering compounds, quantitation on the daughter ion is recommended to minimize false positives.
2. Calibration was performed by extracting procedural standards consisting of at least five calibration points within the range of 1 ng/L to 300 ng/L. The desired amount is added to 500 mL of reagent water, fortified with internal standards (isotope compounds). The selected concentrations should be within the linear range of the instrument. A curve was generated by determining an average relative response factor (RRF) using integrated peak area versus concentrations for nitrosamines of interest.

   The RRF is defined as:

$$RRF = \frac{(A_a)(C_{is})}{(A_{is})(C_a)} \qquad (4.1)$$

   where A_a = Integrated peak area of nitrosamine (analyte) of interest
   C_{is} = Known concentration of internal standard isotope injected
   A_{is} = Integrated peak area of the internal standard isotope
   C_a = Known concentration of nitrosamine (analyte) of interest

   The RRF value must be consistent and the relative standard deviation (RSD) ≤ 20% for integrated peak area over the range of nitrosamine concentrations tested.

3. The concentration of a nitrosamine of interest in a test sample is calculated as:

$$Ca = \frac{(Aa)(Cis)}{(Ais)(RRF)} \quad (4.2)$$

Ambersorb 572® Extraction Conditions

Baseline Extraction Conditions

A 500-mL volume of sample, or sample diluted with reagent water to 500 mL, spiked with internal standards, was extracted via adsorption to 200 mg of Ambersorb for a period of one hour. All extraction conditions such as pH and temperature remained ambient. The resin containing nitrosamines and internal standards from the sample were collected and dried on filter paper and the filtrate was discarded. Nitrosamines were desorbed from the resin with 400 µL of DCM, of which 8 µL was injected into the GC/MS instrument for analysis using the internal standard calculation technique previously described. NDMA-d_6 was used as the internal standard for NDMA and NDEA-$^{15}N_2$ was used as the internal standard for the other nitrosamines. To minimize the use of desorbing solvent to maximize the concentration of potential analyte, 400 µL of solvent was used. The extent to which an analyte is concentrated from the initial sample volume (Sv) to the final desorption volume (Dv) is quantified in terms of concentration factor (CF):

$$CF = \frac{(Sv)}{(Dv)} \quad (4.3)$$

Therefore, the CF under baseline conditions:

$$CF = \frac{(500\,mL)}{(400\,\mu L)} = 1{,}250 \quad (4.4)$$

In general, a CF value greater than 1,000 is required in order to meet detection limits in the ng/L range.

Optimization Experiments

The parameters studied for the development of this SPE method are summarized in the optimization matrix given in Table 4.6. Optimization experiments were conducted using 500-mL volumes of reagent water spiked with 100 ng/L of nitrosamines.

A direct injection standard at a concentration of 100 µg/L in DCM was used to calculate the absolute recovery of the spiked samples during the experimental phase. All extracted experimental samples used reagent water spiked with nitrosamine standards at 100 ng/L prior to extraction. The internal standards (NDMA-d_6, NDPA-d_{14}, and NDEA-$^{15}N_2$) were also added to the resin after the extraction and filtration steps, in the 0.4 mL of desorption solvent, resulting in individual concentrations of 20 ng/L. NDMA-d_6 served as the internal standard for NDMA and NDEA-$^{15}N_2$ was the internal standard for the other nitrosamines. The 100 µg/L direct injection standard signal (DIs) was considered equivalent to a recovered 100 ng/L spike sample signal (Ss) multiplied by the CF.

$$\text{percent absolute recovery} = \frac{(Ss\ RRF * 1000\ ng / 1\ \mu g)}{(DIs\ RRF * CF)} * 100\% \quad (4.5)$$

The percent absolute recovery was used as a means to quantify the efficiency of extraction for method optimization experiments only. Samples analyzed during the detection limit and water matrix studies were treated in the same manner as actual samples, with the internal standards added prior to the extraction step.

Table 4.6 Optimization parameters for SPE nitrosamine method

Parameter	Variables
Solvent	MtBE, DCM
Extraction time (min)	30, 60 120, 240, 1440
Ambersorb mass (mg)	50, 100, 150, 200, 300, 400
Ionic strength	0, 1, 2, 3 M sodium chloride
Sample pH at extraction	Acidic (pH = 3), neutral (pH = 6), basic (pH = 11)

Results and Discussion

Various extraction times, Ambersorb mass, ionic strength, and pH adjustments were tested to examine the kinetics of extraction, Ambersorb extraction capacity, nitrosamine adsorption activity, and extraction environment conditions, respectively. SPE conditions were examined in the order given in Table 4.6 and results of each analysis were taken into consideration for the following tests to improve nitrosamine recoveries. Detection limit studies were performed by first examining the LLD and then estimating its effect combined with that of the extraction process before performing the actual MDL study. The results of optimization and detection limit studies were used to select parameters for the water matrix study, which included accuracy and precision analysis on potable, secondary and tertiary effluent. The results generated from the optimization, detection limit, and water matrix experiments are discussed in detail in the following pages.

Optimization Experiments

Solvent Effects

In addition to DCM, MtBE was also evaluated as a possible desorbing solvent. However, it was found that NDMA recovery was less than 1% with MtBE. The low recovery is due to the lower polarity of MtBE as compared to DCM, which limits the MtBE's ability to desorb NDMA from the resin.

Extraction Time Effects

To examine the kinetics of nitrosamine adsorption by Ambersorb, extraction time was varied from 30 to 1440 minutes while other baseline conditions were maintained. Two sets of extraction experiments were performed. First, the extraction time study was performed on samples spiked with NDMA only (Figure 4.1). These results show a gradual decrease in the rate at which NDMA recovery increases with additional extraction time. The study was repeated on samples spiked with all nitrosamines. Results for nitrosamine under similar conditions are shown in Figure 4.2 and data are presented in Table 4.7.

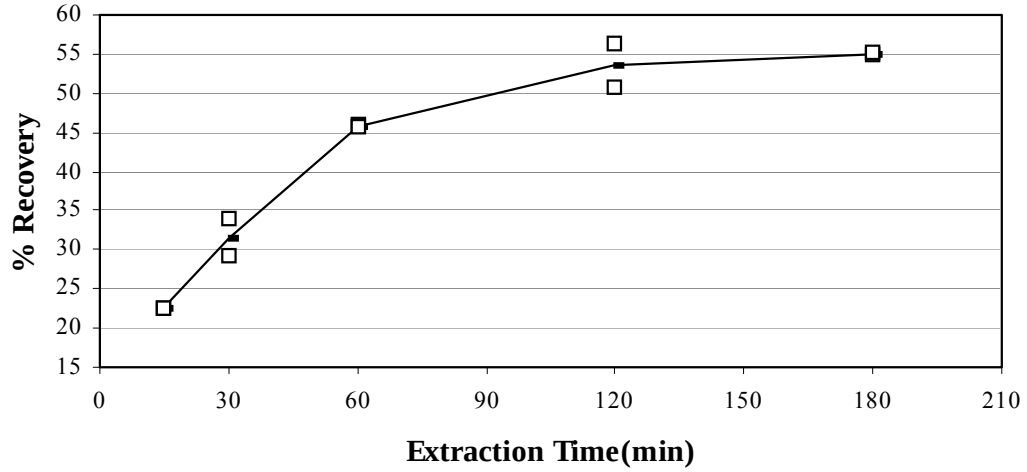


Figure 4.1 Effect of varying extraction time on NDMA recovery (n=2)

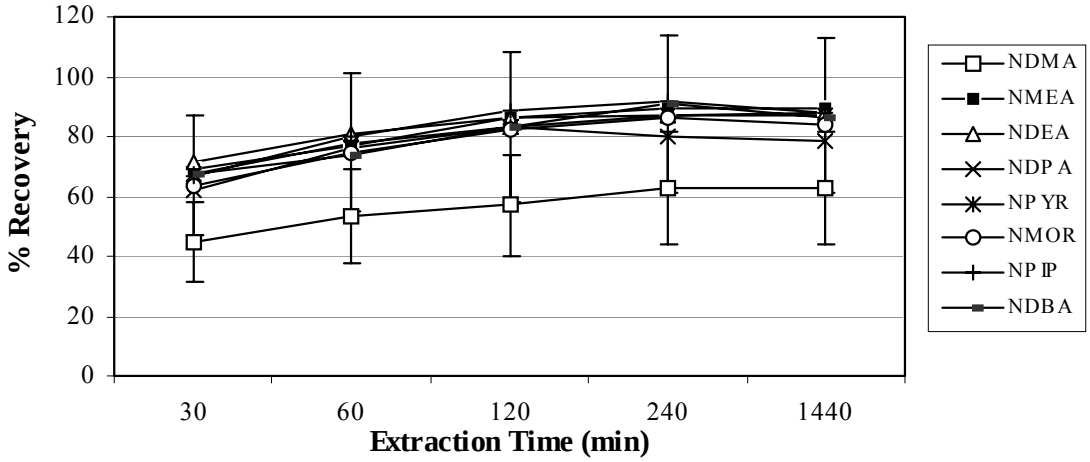


Figure 4.2 Effect of extraction times on nitrosamine recovery (n=3)

Table 4.7 Effect of extraction time on nitrosamine recovery
Average of 3 observations

Nitrosamine	30	60	120	240	1440
NDMA	44 ± 3	53 ± 1	57 ± 3	63 ± 3	63 ± 3
NMEA	69 ± 2	77 ± 1	86 ± 3	89 ± 2	90 ± 4
NDEA	71 ± 7	81 ± 3	86 ± 4	87 ± 5	88 ± 6
NDPA	62 ± 7	76 ± 11	83 ± 4	80 ± 16	79 ± 6
NMOR	67 ± 5	78 ± 3	82 ± 4	86 ± 6	87 ± 7
NPYR	63 ± 1	75 ± 2	82 ± 4	87 ± 5	84 ± 8
NPIP	67 ± 6	80 ± 12	88 ± 7	91 ± 5	88 ± 10
NDBA	68 ± 2	74 ± 6	83 ± 4	91 ± 3	87 ± 6

Baseline conditions, 120 minute extraction time.

With the exception of NDMA, nitrosamine recoveries for samples extracted for at least 120 minutes was greater than 80%. These results confirmed the initial testing results for NDMA only, which indicated that the most extraction was achieved in the first 120 minutes. Extending extraction time beyond 120 minutes

resulted in marginal to no improvement for most nitrosamine species (<5% between 120 and 240 minutes and <1% between 240 and 1440 minutes). NDMA was an exception, showing an increase of 10% between 120 and 240 minutes. Extending the extraction time beyond 240 minutes showed less than 1% improvement for NDMA. Based on these findings, an extraction time of 120 minutes was adopted for the following optimization tests.

Isotherm Variable Mass Study

To examine extraction efficiency as related to the quantity of Ambersorb used, masses were varied from 50 to 400 mg under ambient baseline conditions with 120-minute extraction time. CF values and percent nitrosamine recovery for samples extracted with varying Ambersorb masses are shown in Table 4.8. With the exception of NDMA (58% recovery), all nitrosamine recoveries were greater than 80% with 200 mg of Ambersorb. Above 200 mg of Ambersorb, marginal improvements in recovery are observed for all nitrosamines. In addition, when 300 and 400 mg of Ambersorb were used, larger volumes of DCM were needed for desorption, resulting in CF values below the desired value of 1,000. Thus, 200 mg of Ambersorb was selected as the optimal absorbent mass.

Table 4.8 Effect of Ambersorb mass on nitrosamine recovery

Average of 3 observations

Ambersorb mass	**50 mg**	**100 mg**	**150 mg**	**200 mg**	**300 mg**	**400 mg**
CF	**1250**	**1250**	**1250**	**1250**	**833**	**625**
NDMA	21±2	38 ± 2	42 ± 1	58 ± 3	63 ± 2	66 ± 2
NMEA	52 ± 8	69 ± 9	73 ± 4	83 ± 5	90 ± 1	97 ± 1
NDEA	65 ± 5	80 ± 6	84 ± 7	90 ± 3	95 ± 4	98 ± 1
NDPA	51 ± 5	57 ± 6	63 ± 5	85 ± 10	87 ± 1	84 ± 5
NMOR	56 ± 6	60 ± 7	68 ± 8	83 ± 1	86 ± 1	88 ± 4
NPYR	44 ± 8	52 ± 9	59 ± 1	87 ± 4	86 ± 5	91 ± 1
NPIP	72 ± 10	73 ± 6	82 ± 11	85 ± 5	92 ± 4	88 ± 7
NDBA	67 ± 8	78 ± 3	81 ± 2	83 ± 10	92 ± 3	92 ± 4

Baseline conditions, 120 minute extraction time.

A Freundlich type isotherm plot, which shows the relationship between adsorbate and adsorbent, was used to estimate the Ambersorb mass required for peak extraction capacity for NDMA and other nitrosamines. The Freundlich isotherm equation is as follows:

$$\frac{X}{M} = kCe^{1/n} \qquad (4.6)$$

Where: X/M = amount adsorbed per unit weight of Ambersorb (ng/mg)
k, n = empirical constants (unitless)
Ce = equilibrium concentration of adsorbate in solution after adsorption (ng/mL)

The mass adsorbed per unit weight adsorbent (X/M) was plotted against the equilibrium concentration of nitrosamine left in solution following extraction (Ce), and is shown in Figure 4.3. An isotherm curve was generated for NDMA, however, isotherms were not able to be plotted for the other nitrosamines because of the analytical variability in the data. Using the isotherm result for NDMA, the ideal Ambersorb mass is estimated at a Ce value of 0.1 ng/mL.

NDMA requires the greatest amount of Ambersorb for extraction in comparison to the other nitrosamines, which would be expected because NDMA is the least-easily adsorbed nitrosamine. These results confirm the conclusion that a minimal gain in NDMA recovery is achieved when a mass of Ambersorb in excess of 200 mg is used. It is also important to note that a decrease in CF and consequently signal sensitivity

occur when Ambersorb mass is increased in excess of 200 mg. Based on these results, Ambersorb mass of 200 mg was determined to be optimal and was used for all extractions in this study.

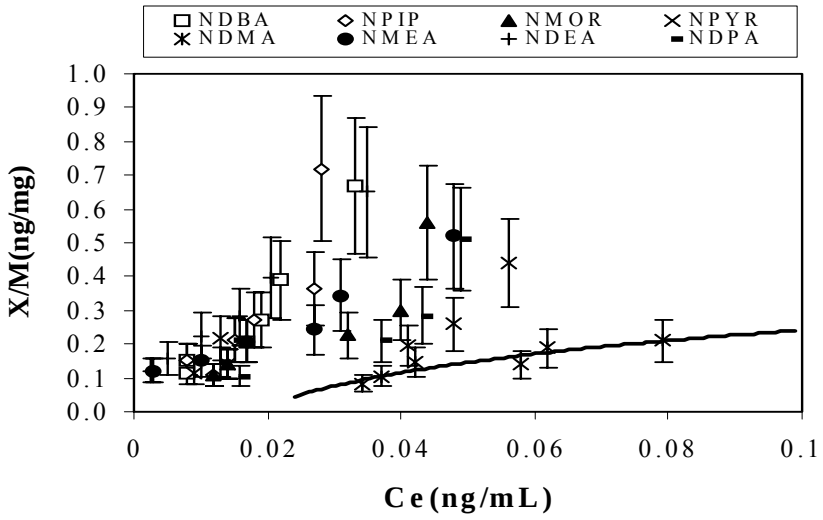


Figure 4.3 Freundlich isotherm plot for nitrosamines using variable Ambersorb mass

Salt Effects

The addition of an inorganic salt has often been used to enhance the extraction efficiency of volatile and semivolatile components in aqueous solutions.To examine the extent of these effects during the extraction process, NaCl was added to samples prior to extraction at concentrations from 0 to 3 M, under baseline conditions with 120-minute extraction time. The percent recoveries for nitrosamine samples with varying salting conditions are shown in Table 4.9. There appears to be a slight increase in recovery for all nitrosamines when salt is added during the extraction process. NDMA recovery increased by 11% with the addition of 1-M salt. However, most of the nitrosamines were recovered greater than 80% with no salt added, which was consistent with the results of initial optimization test. Because of the high recovery achieved without salt addition, salt addition alone may not be necessary. However, it was decided to further evaluate synergistic benefits of salt addition (1 M) to determine whether salt addition would be necessary for the extraction process.

Table 4.9 Effect of salt addition on nitrosamine recovery

Average of 5 observations

[NaCl]	0 M	1 M	2 M	3 M
NDMA	62 ± 4	73 ± 9	74 ± 5	75 ± 5
NMEA	84 ± 2	98 ± 6	90 ± 4	91 ± 2
NDEA	81 ± 3	92 ± 7	89 ± 3	86 ± 3
NDPA	88 ± 10	106 ± 10	95 ± 14	100 ± 12
NMOR	74 ± 2	83 ± 8	76 ± 3	77 ± 4
NPYR	80 ± 3	93 ± 4	89 ± 1	87 ± 3
NPIP	89 ± 3	99 ± 5	94 ± 6	94 ± 3
NDBA	82 ± 1	100 ± 5	91 ± 3	92 ± 3

Baseline conditions, 120 minute extraction time.

pH Effects

To examine the potential effects of variable pH conditions on nitrosamine extraction with Ambersorb, the pH was adjusted prior to extraction. Samples were adjusted to pH 3 (using 0.05 M H_2SO_4) or pH 11 (using 0.02 M NaOH). Synergistic benefits of adding 1 M NaCl were also evaluated. Table 4.10 shows the results of tests performed under these extraction conditions. Adjustments with pH alone during the extraction process appear to have little effect on the recovery of NDMA and the other nitrosamines. When salting and basic pH adjustments were combined, the recovery for NDMA could be as high as 89% (NaCl = 1 M, pH = 11). However, it was decided not to include these two steps in the final method because NDMA recovery without pH adjustment or salt addition was approximately 60% or higher, and as seen later, is sufficient to achieve a detection level of 1 ng/L with GC/CI/MS/MS quantitation. Salt addition in conjunction with base adjustment can be further evaluated if lower detection levels are necessary. Although these additional steps may allow a low detection level for nitrosamines to be reached, isotope dilution is still recommended to correct for recovery and ensure reliable results from the method.

Table 4.10 Effect of pH conditions and salt concentrations on nitrosamine recovery

Average of 4 observations

NaCl	**[0 M]**			**[1 M]**		
pH	**3**	**6**	**11**	**3**	**5**	**11**
NDMA	57 ± 4	57 ± 4	57 ± 3	58 ± 6	68 ± 7	89 ± 5
NMEA	72 ± 4	79 ± 6	66 ± 4	68 ± 6	79 ± 9	75 ± 1
NDEA	85 ± 1	87 ± 4	77 ± 12	84 ± 4	90 ± 8	90 ± 3
NDPA	84 ± 2	85 ± 2	83 ± 9	87 ± 4	87 ± 4	92 ± 7
NMOR	71 ± 3	79 ± 3	65 ± 11	66 ± 5	70 ± 9	70 ± 5
NPYR	70 ± 2	74 ± 4	64 ± 9	69 ± 4	79 ± 6	75 ± 2
NPIP	84 ± 4	89 ± 1	75 ± 10	82 ± 5	85 ± 5	82 ± 3
NDBA	85 ± 5	82 ± 5	81 ± 10	83± 5	90 ± 7	91 ± 1

Baseline conditions, 120 minute extraction time.

Detection Limits

Lower Level of Detection

The LLD (ASTM, 1983) is equivalent to the amount of variance attributed to the instrument used for detection and was determined using seven 1 ng/mL direct injection nitrosamine samples. Table 4.11 shows the LLD values for the nitrosamines of interest. The results of this test were used as justification for starting the MDL study of the nitrosamine extraction process at 1 ng/L. Table 4.12 contains the result of calculations to estimate lowest possible MDLs values that can be obtained under different conditions. Based on these analyses, it appears that an MDL less than 1.0 ng/L can be achieved for all nitrosamines using an extraction time of 120 minutes without pH adjustment or salt addition.

Table 4.11 LLD summary for Amb SPE nitrosamine method

n=7	Mean Area(S/N)	Std dev	Std dev/ (Mean area)*	LLD(µg/L)**
NDMA-d_6	1691	139	0.1	0.3
NDEA-$^{15}N_2$	1365	250	0.2	0.7
NDMA	785	71	0.1	0.4
NMEA	4538	278	0.1	0.2
NDEA	1649	123	0.1	0.3
NDPA-d_{14}	1199	77	0.1	0.3
NDPA	1313	268	0.2	0.8
NMOR	3183	365	0.1	0.5
NPYR	3006	456	0.2	0.6
NPIP	4772	177	0.0	0.1
NDBA	549	82	0.2	0.6

**Normalization to 1 ng/mL.*
***For degrees of freedom=6, 5% probability of false and/or nondetection, student t = (1.9432 * 2)*

Table 4.12 Lowest possible MDL attainable for Amb SPE nitrosamine method

LLD/Recovery, all values reported in ng/L

Method	A	B	C	D	E
Extraction time (min)	120	120	30	60	120
NaCl [M]	[1]	[1]	[0]	[0]	[0]
pH	5	11	6	6	6
NDMA	0.5	0.4	0.8	0.7	0.6
NMEA	0.3	0.3	0.3	0.3	0.3
NDEA	0.3	0.3	0.4	0.4	0.3
NDPA	0.9	0.9	1.3	1.0	0.9
NMOR	0.6	0.6	0.7	0.6	0.5
NPYR	0.8	0.8	0.9	0.8	0.7
NPIP	0.2	0.2	0.2	0.2	0.2
NDBA	0.6	0.6	0.9	0.8	0.7

Method Detection Limit (Practical)

To determine the practical MDL, three nitrosamine samples were prepared, extracted, and analyzed at concentrations of 1.0, 2.0, and 5.0 ng/L on three consecutive days (a total of nine observations at each concentration). Procedure "E" from Table 4.12 was used, which requires the fewest steps of those methods capable of achieving an MDL less than 1 ng/L for all nitrosamines. Table 4.13 shows the MDLs calculated at each concentration. Based on the criteria for the F-test, there is no significant difference between the MDL values obtained at the various concentrations for each nitrosamine. However, as the concentration of nitrosamines increases, the differences in variance between MDL values obtained may become significant. In such a situation, the Hubaux and Vos Method (H/V), (Hubaux and Vos, 1970) is useful in estimating the MDL. The H/V MDL was determined by taking the point where the upper confidence interval band intersects the y-axis horizontally until reaching the linear regression line for observed concentrations, then proceeding vertically to the x-axis, this point corresponds to the MDL. Figure 4.4 demonstrates the determination of the MDL for NDMA by the H/V method. Based on the H/V values shown in Table 4.13, a MDL of 1.1 ng/L can be achieved for all nitrosamines examined.

Table 4.13 MDL values for Amb SPE nitrosamine method at 120 minutes

All values reported in ng/L

Spike (ng/L)	1.0	2.0	5.0	H/V
# Observations	10	9	9	28
NDMA	0.8	1.2	0.8	0.9
NMEA	0.5	1.0	1.4	1.0
NDEA	0.8	1.1	1.8	1.0
NDPA	1.1	1.4	1.6	1.0
NMOR	0.6	1.4	1.4	0.8
NPYR	0.8	0.5	0.8	1.1
NPIP	0.7	0.7	1.4	1.0
NDBA	0.7	0.8	1.6	1.0

For n=9, T=2.821; n=10, T=2.764 at the 99% confidence level.

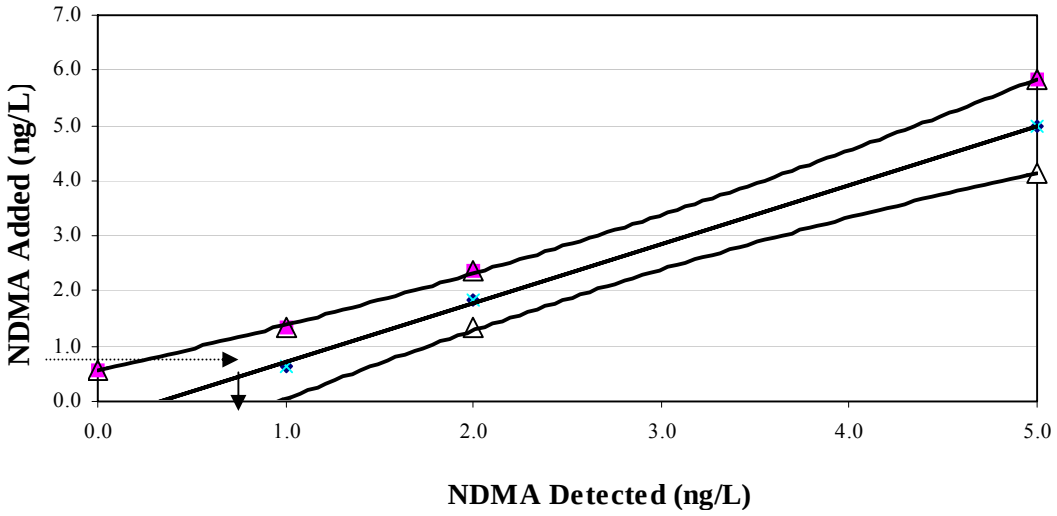


Figure 4.4 Determination of MDL for NDMA by H/V

*Linear regression line with upper and lower 99% confidence interval bands

Table 4.14 shows the degree of variance in values obtained for nitrosamine concentrations that can be attributed to extraction (MDL minus LLD) and detection (LLD) processes individually for samples extracted for 120 minutes under baseline conditions. Analysis of NDMA appears to be equally precise when compared to the other nitrosamines. This observation is based on 0.5 ng/L of the variance in the MDL being attributed to the extraction process as compared to an average of 0.6 ng/L for all nitrosamines. The error that can be attributed to the extraction step is generally slightly higher than in the detection step for all nitrosamines. Thus, future improvements in MDL values may require improvements in instrument detection precision as well as extraction process accuracy, because lower MDLs require improved sensitivity and higher precision

Table 4.14 Variance from extraction and detection process in nitrosamine analysis

Compound	Extraction (MDL – LLD) (ng/L)	Detection (LLD) (ng/L)	Total error (MDL) (ng/L)
NDMA	0.5	0.4	0.9
NMEA	0.8	0.2	1.0
NDEA	0.7	0.3	1.0
NDPA	0.2	0.8	1.0
NMOR	0.3	0.5	0.8
NPYR	0.5	0.6	1.1
NPIP	0.9	0.1	1.0
NDBA	0.4	0.6	1.0
Average	0.6	0.4	1.0

LLD values from Table 4.11
MDL values from H/V in Table 4.13

Water Matrix Study

Based on the results from the MDL studies, the Ambersorb SPE extraction conditions selected are as follows: 120 minutes for extraction time and no pH adjustment or salt addition. These conditions were used for the water matrix testing, and the analyses were conducted by using the internal standard technique previously described. The analytical results for potable, secondary and tertiary effluent samples are shown in Table 4.15. The results show that a concentration near or below 1 ng/L was achievable for all nitrosamines in potable water. The nitrosamine concentrations in secondary and tertiary effluent samples, with the exception of NDMA, were all below 18 ng/L. NDMA concentration of 93 and >1,200 ng/L were obtained in secondary and tertiary effluent samples, respectively.

Table 4.15 Detected nitrosamine concentrations in various water matrices
(values reported in ng/L)

	Potable ($[Cl_2]_{total}$ = 2.68)	Secondary Effluent ($[Cl_2]_{total}$ = 0.04)	Tertiary Effluent ($[Cl_2]_{total}$ = 3.64)
# Observations	**5**	**4**	**6**
NDMA	<1	93	>1200*
NMEA	<1	<4	<4
NDEA	<1	9	<4
NDPA	<1	<4	<4
NMOR	<1	6	11
NPYR	<1	<4	18
NPIP	<1	<4	<4
NDBA	1.1	<4	14

Secondary and tertiary effluent samples were diluted 1:4 with reagent water and values were adjusted to correct for the dilution. Reference method E from Table 4.11 for extraction conditions.
**The values obtained exceeded the range of calibration used (1 to 300 ng/L).*

Method Precision and Accuracy

To determine the quality of analysis in the water matrices, samples were spiked at 20 ng/L concentrations prior to extraction. The criteria for accuracy in terms of acceptable percent spike recovery (± 30%) and

precision in terms of percent RSD (within 20%) were based on commonly used procedures, (Standard Methods, 1995). The results of these tests are shown in Table 4.16. Recovery analyses were not performed for NDMA in tertiary effluent because results exceeded the range of calibration. The data showed acceptable recoveries for all nitrosamines tested, with greater than 80% recovery for most of the nitrosamines, with the exception of NMEA in secondary effluent samples, which was recovered at 71%. The cause of the lower recovery for NMEA in secondary effluent is unclear, but a lower recovery for NMEA was also observed in the tertiary effluent sample, suggesting the presence of interfering compound(s) exists in these waters. The RSD results obtained for all nitrosamines ranged from 0.9 to 6.9%, suggesting the method precision is within acceptable range using environmental samples.

Table 4.16 Single laboratory precision and accuracy data for nitrosamines spiked into various matrices
(values reported in ng/L, based on 4 observations)

	Potable		Secondary Effluent		Tertiary Effluent	
Compound	**% Recovery**	**%RSD**	**% Recovery**	**%RSD**	**% Recovery**	**%RSD**
NDMA	101	2.2	105	0.9	Not Reported*	
NMEA	103	2.5	71	4.2	81	4.1
NDEA	101	1.9	101	3.3	99	5.7
NDPA	101	4.6	106	3.0	105	2.4
NMOR	100	1.1	95	6.6	104	3.4
NPYR	94	3.8	92	6.9	94	4.5
NPIP	97	2.7	102	2.9	97	5.5
NDBA	99	3.6	104	2.1	103	4.0

** Values for NDMA in tertiary effluent samples exceeded the range of calibration.*

Summary and Conclusions

Summary

- An extraction time of 120 minutes was selected for NDMA based on a recovery of 57% and proved sufficient for the other target nitrosamines, with recoveries exceeding 80%.
- Recovery of NDMA increases significantly when Ambersorb is increased from 50 to 200 mg. The percent increase in recovery from 150 to 200 mg of Ambersorb was 38%. The increase in NDMA recovery from 200 to 400 mg of Ambersorb was less significant, less than 14%. This general trend was also observed for the other nitrosamines. NDMA requires the highest amount of Ambersorb for extraction, as compared to other nitrosamines. The most significant NDMA recovery increase of 38% was observed with an increase in mass from 150 to 200 mg of Ambersorb.
- It appears that adding 1 M NaCl during extraction results in an increase in recovery between 12 and 20% for NDMA and the other nitrosamines. Even without salt addition, an average of 60% for NDMA and over 80% for other nitrosamines were achieved, which allowed a detection level of 1 ng/L to be attained.
- Adjustment of pH alone during the extraction process appears to have little effect on the recovery of NDMA and other nitrosamines. NDMA recoveries varied by only 2% between pH 3 and 11. The combination of salt and basic pH adjustments appears to increase the NDMA recovery to 89%. These steps were not incorporated into the final procedure because a detection level of 1 ng/L for NDMA can be achieved.
- A MDL of 1 ng/L can be achieved for all nitrosamines extracted for 120 minutes under baseline conditions. Approximately 60% of the variability in MDL values can be attributed to the extraction process and 40% to the detection process for nitrosamines tested.

- The values for nitrosamine concentrations in potable water samples were less than 2 ng/L for all nitrosamines tested. The nitrosamine concentrations in secondary effluent samples were low (< 10 ng/L), with the exception of NDMA (93 ng/L). The nitrosamine concentrations in tertiary effluent samples were less than 20 ng/L for all nitrosamines tested with the exception of NDMA (> 1,200 ng/L).
- The baseline method for a 120-minute extraction time provided acceptable accuracy for the various water matrices, with the exception of NMEA in secondary effluent samples. The baseline method for a 120-minute nitrosamine extraction was within acceptable precision for all nitrosamines and waters.

Conclusions

A method for extraction and quantitation of NDMA and selected nitrosamines at or below the 1 ng/L level was refined during this testing. A 1 ng/L detection level for all nitrosamines was achieved, and it may be possible to further lower the MDL by adjusting certain extraction parameters, including increasing extraction time, salt addition, and/or pH adjustment to basic conditions. It is recommended, however, to avoid additional extraction steps or parameters that could increase processing time and cost if at all possible. It has also been shown that this method is suitable to test for most nitrosamines in various water matrices. Although it is possible to achieve the 1 ng/L detection limit for nitrosamines with the additional extraction steps, isotope dilution is still recommended to correct for recovery and ensure reliable results when analyzing matrices that may be subject to background interferences, such as wastewater.

Based on the results of MDL and water matrix studies, the conditions defined by method E in Table 4.11 (500 mL of sample spiked with internal standards, 2-hour extraction time, 200 mg Ambersorb, no salt addition or pH adjustment, and desorption with 400 μL DCM) appear to be adequate to attain 1 ng/L for all nitrosamines without compromising accuracy or precision.

CHAPTER 5

DUAL-MEDIA CARTRIDGE EXTRACTION METHOD

Introduction

A cartridge solid–phase extraction (CSPE) method was not included in the original scope of work of the proposal, but CSPE was explored because of the micro liquid–liquid extraction method's inability to achieve an NDMA method detection limit of 2 ng/L. The advantages of CSPE as an analytical technique are that it:

- Uses large sample volumes;
- Achieves large concentration factors for high sensitivity;
- Uses small solvent volumes;
- Is compatible with GC/CI/MS/MS for high accuracy; and
- Runs semi- or fully- automated.

The first task was to determine if a suitable cartridge (i.e., sorbent media) was available for nitrosamine analyses. Once the suitable cartridge was identified, pertinent variables were optimized, including sorption flow rate, cartridge drying time, elution volume, and sorbent amount. The MDLs were established and preliminary method performance examined. A complete list of procedures for this method is provided in Appendix C.

Experimental

All specifications are suggested. Brand names and/or catalog numbers are included for illustration purposes only.

Chemicals

Reagent water with a resistivity of 18.2 MΩ-cm, containing less than one half of the MRL of each analyte was obtained from a water purification system (Milli-Q-UV, Millipore Corp, Bedford, MA) just prior to use. All chemicals were American Chemical Society (ACS) reagent grade or better (J. T. Baker, Phillipsburg, NJ). Sodium bicarbonate ($NaHCO_3$) was used to adjust the pH of samples. Granular anhydrous Na_2SO_4 was also used to dry extracts. Sodium hydroxide and sulfuric acid were used for pH adjustment. Solvents were high-purity, OmniSolv, high-resolution gas chromatography (HR-GC) grade (EMD Chemicals Inc., Gibbstown, NJ). Methanol and alkene stabilized DCM were used as the conditioning and extracting solvents. Stock standards solutions of the eight target analytes and *N*-nitrosodiphenylamine at 2000 µg/mL each in methanol (Supelco, Bellefonte, PA) and in DCM (Protocol, Middlesex, NJ) were used to prepare working spiking solutions in methanol. NDMA-d_6, NDPA-d_{14}, and NDEA-$^{15}N_2$ were purchased as individual stock solutions at 1 mg/mL in DCM (d_6) (Cambridge Isotope Laboratories, Andover, MA, #DLM-2130-S, DLM-2131-S and NLM-3432-S, respectively).

Direct Standards

A set of direct standards, made in DCM, was prepared with each experiment to calculate analyte recovery. Each set contained 5, 25, 50, 100 and 250 µg/L standards, which included all nitrosamines and the three internal standards (NDMA-d_6, NDPA-d_{14}, NDEA -$^{15}N_2$). NDEA -$^{15}N_2$ was used as the internal standard for all the nitrosamines in determining the absolute recoveries for the optimization experiments.

Equipment

SPE extractions were performed on either a vacuum manifold, which is a chemical resistant cartridge holder(s) with individual flow control valves, glass basin, collection rack, large volume samplers, and nonrubber vacuum hose connections (Supelco #57160-U + accessories, Bellefonte, PA) or on a positive pressure automated solid–phase extraction apparatus (Gilson ASPEC XL, Gilson, Inc., Middleton, WI). The following disposable elution cartridges (DEC) evaluated included Oasis® HLB, MCX, and MAX cartridges (Product #WAT094226, 186000254, and 186000368, Waters Corp., Milford, MA), Supelclean ENVI-carb sorbent (Supelco, # 57088, #57092, and 57094) and dual-media cartridges comprised of the ENVI-carb cartridge with an additional 350 mg Ambersorb (Supelco #10432-U) added to the top and held in place with an additional polyethylene frit (Supelco, # 57180-U). Sample containers were amber glass bottles fitted with PTFE-lined screw caps. Collection vials were 3.5 mL clear screw cap septum vials (Pierce, # 13019T) or 10-mL graduated Kuderna-Danish tubes. An inert gas concentrator system with a water bath at 40 ^{o}C, capable of applying a gentle stream of clean, dry, inert gas was used to concentrate the eluate. Various size syringes and volumetric glassware were used in preparation of standards.

A Saturn 2200 ion trap MS (Varian Inc., Walnut Creek, CA) with a Varian 3800 GC (with 1079 PTV injector) equipped with liquid chemical ionization capability was operated in GC/CI/MS/MS mode. Acetonitrile was used as the CI reagent. A Varian 8400 or a Combipal autosampler (Leap Technologies, Chapel Hill, NC) was used to inject 8 µL of extract onto either a DB1701 (30 m x 0.25 mm x 1 mm, from Agilent) column or DB-VRX (60 m x 0.32 mm x 1.8 mm, from Agilent).

The GC/CI/MS/MS conditions described in Chapter 3 were used for the CSPE method development work described in this chapter. In some of the optimization experiments NDEA-$^{15}N_2$ was added to the final concentrated eluate and used as an internal standard for calculation of absolute recoveries. In these cases, NDMA-d_6 was used as the surrogate for NDEA and NMEA for calculating analyte concentration in the water samples.

General CSPE Procedures

Two SPE systems were used in this project, an automated positive pressure system (APPS) and a manually adjusted vacuum manifold. The APPS can maintain set flow rates accurate to 0.6%, however it processes only one sample at a time. The vacuum system can process up to 12 cartridges simultaneously, although six cartridges are more manageable. Flows are adjusted manually giving only estimated and average flow rates (e.g., by counting drops/sec and sample volume/total time for sample transfer).

Most of the optimization experiments used a combination of the two systems. When flow rate was critical, samples were loaded onto the cartridges by the APPS, and then the cartridges were transferred to the vacuum manifold system for drying and elution. Method performance and detection limit studies, as well as routine sample analyses were conducted on the vacuum manifold system alone, and it is the expected apparatus of use for the method.

Automated Positive Pressure System Extraction Procedure

The cartridge was conditioned with 2 mL of methanol, followed by reagent water. The sample was transferred to the cartridge by a low-pressure syringe pump in 20 mL aliquots at selected flow rate. Air was pushed through the cartridges to dry them and the analytes were eluted with 2.0 mL aliquots of DCM at 0.5 mL/min. The final DCM extract was separated from a small amount of transferred water by hand with a Pasteur pipette and the amount of total recoverable DCM was measured (typically 1.6 mL per 2 mL DCM). The eluate was warmed and concentrated under a stream of nitrogen to a final volume of 0.5 mL for analysis. NDEA-$^{15}N_2$ was added to result in an internal standard concentration of 50 ng/mL in DCM.

Vacuum Manifold System Extraction Procedure

The cartridges, typically six per run, were fitted to the top of the vacuum manifold, and a vacuum of –15 in. Hg was maintained during the various steps of the analysis. The cartridges were conditioned with 2 x 2 mL of methanol after which the media was compressed to remove air pockets, and rinsed with 2 x 2 mL of reagent water. The 500 mL sample was then pulled through the cartridge at a flow rate of less than 5 mL/min (~1 – 1.5 drops/sec). The sample transfer lines were removed and the cartridges were air-dried for 1 hour. Glass vials were then placed under each cartridge. The elution procedure typically consisted of applications of 2 mL of DCM to the top of the cartridge, slowly allowing 10 to 20 drops to elute ensuring wetting of the media. Flow was stopped for approximately 5 minutes to maximize media interaction and elution was completed 10 drops at a time, until no solvent remained. The process was then repeated with two additional DCM aliquots. The eluate was warmed and concentrated under a stream of nitrogen to a final volume of 0.5 mL for analysis on a separate system. NDEA-$^{15}N_2$ was added to result in an internal standard concentration of 50 ng/mL in DCM. In some cases, 0.5 mL of eluate was removed before blow down and directly spiked with internal standard and analyzed.

CSPE Optimization Experiments

The parameters studied for the development of this cartridge SPE method are summarized in the optimization matrix given in Table 5.1. Five commercially available sorbents and an augmented cartridge consisting of Ambersorb packed on top of Envi-carb were screened for NDMA and nitrosamine analysis. The samples for the optimization experiments were usually 100 mL of 200 ng/L spiked nitrosamines in reagent water (e.g., 20 ng placed on the column). For the pH study, the nitrosamines solutions (200 ng/L) were prepared in a 0.01 M sodium bicarbonate buffer (adjusted to pH 8.3) and in 0.01 M sodium hydroxide solution (adjusted to pH 12).

Table 5.1 Optimization parameters for CSPE nitrosamine method

Variables	Range
Sorbent	LC-18, Oasis® HLB, MCX and MAX, ENVI-carb, dual-media Amb-Envi.
pH	8.3 (ambient, neutral), 12 (basic)
Mass of Ambersorb® 572 (mg)	0, 50, 150, 250, 350
Loading flow rate (mL/min)	5, 10, 15
Cartridge drying time (min)	15, 30, 45, 60
Elution: No. of 2 mL aliquots	1 – 6
Final volume	Without extract concentration, with concentration

Results and Discussion

Two CSPE systems and different combinations of the systems were used to maximize efficiency and control of critical parameters for each set of optimization experiments. For example, the APPS was used for the flow rate study because it delivers an accurate and constant flow rate, while drying and elution were usually performed on the vacuum system so multiple samples could be run simultaneously. Therefore, results based on absolute recoveries are comparable within a test, but not necessarily across experiments. In contrast, sample analysis and detection limit and performance evaluation experiments were extracted with internal standards (isotopic analogues for three of the nitrosamines) that correct for variation in extraction efficiency. The calibration curves are based on the ratio of the area of analyte to the area of internal standard and were generated from extracted standards.

Cartridge Media Selection

Five commercially-available sorbents commonly used for trace organic analyses were evaluated. NDMA is the smallest and most polar of the nitrosamines and therefore the most difficult to extract. Initially it was tested without the other nitrosamines to avoid competition issues. Both the concentrated DCM eluate and the water sample after extraction were analyzed for NDMA. The percent of NDMA remaining in the water, which represents the unretained NDMA, and that recovered from the cartridge (i.e., in the eluate) are given in Table 5.2. None of the five commercially available cartridges resulted in recoveries greater than 3% for NDMA, although recoveries for the other nitrosamines were much higher. NDEA, NDPA, NDBA, and NPIP had recoveries greater than 50% for the HLB and Envi-carb cartridges (Figure 5.1 and 5.2).

Table 5.2 NDMA recoveries for commercially available 3 mL cartridges

Cartridge type	Sorbent	Residual in water % NDMA	Eluate % NDMA
Oasis® HLB	Copolymer of N-vinylpyrrolidone and divinylbenzene	82	<1
LC18	Reversed-phase C18 sorbent	80	2.2
MCX	Cation exchange and reversed-phase sorbent	76	<1
MAX	Anion-exchange and reversed-phase sorbent	78	<1
Envi-carb	Graphitized nonporous carbon	20	<1

The HLB cartridge was also evaluated under two sample pH conditions to determine if nitrosamine recoveries would be improved. The results are shown in Figure 5.1. Recoveries were greater than 80% for NDEA, NDPA, NPIP, and NDBA, from 18 to 26% for NMEA, NMOR, and NPYR and only 2% for NDMA. The pH (neutral and basic) of the sample at extraction had little effect on the absolute recovery of the nitrosamines, which were the same within experimental error for both pHs.

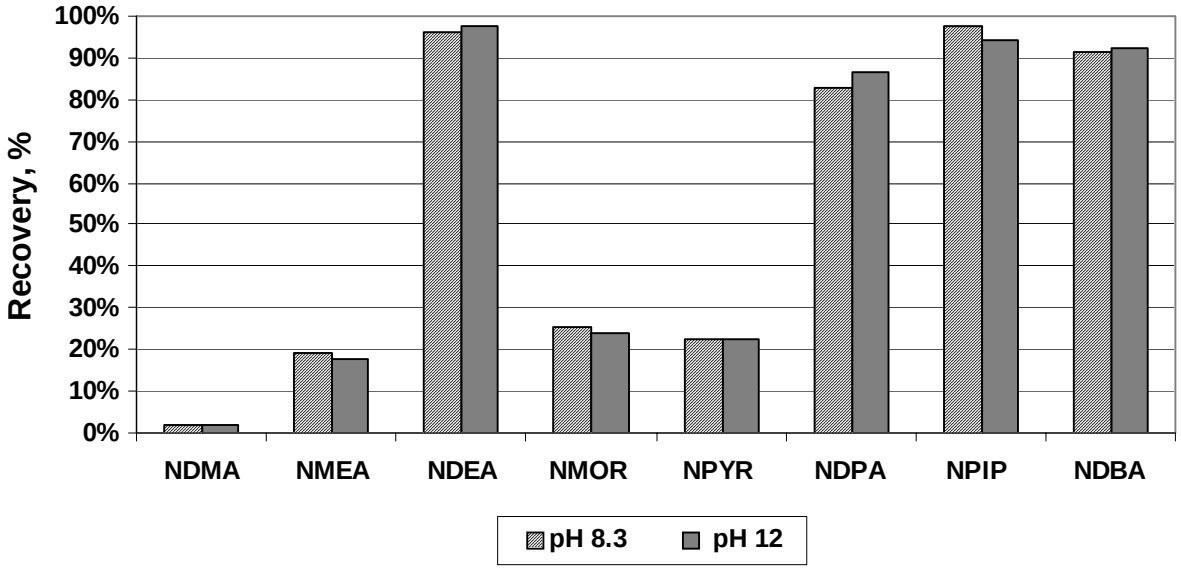


Figure 5.1 Effect of increasing pH on nitrosamine recoveries using 3 mL HLB solid–phase extraction cartridge. (Data shown represent single sample measurement.)

The Envi-carb cartridge was the only media that showed significant retention of NDMA (approximately 80%, Table 5.2). However, the eluate concentration of NDMA was minimal suggesting that most of the NDMA remained on the cartridge. The average recoveries for triplicate analyses of 250 mg and 500 mg, 6 mL Envi-carb cartridges, with one 3 mL DCM elution, for all the nitrosamines is shown in Figure 5.2. NDMA was again essentially not recovered. Other serial elution studies (not shown) indicate that NDMA recovery increases significantly in the second and third elutions in contrast to the NDPA and NDBA, which elute almost totally in the first aliquot. NMEA, NMOR, and NPYR were barely recovered with the 250 mg cartridges, but did show improvement with the 500 mg cartridges. Moderate recovery was observed for NDEA, and NPIP, averaging 57 and 59%, respectively with the 500 mg cartridge, and only low recoveries with the 250 mg cartridge. Recovery was excellent for NDPA and NDBA with both cartridge sizes with recoveries ranging from 81 to 98%. The relative standard deviation for the triplicate runs was 3 to 7% for compounds with good recovery (NDPA and NDBA), and 18 to 25% for moderately recovered compounds. The Envi-carb bed depth is 6 mm for the 250 mg 6 mL cartridge and 13 mm for the 500 mg cartridge. It is suspected that the differences in recovery are due to the difference in contact time resulting from increased bed depth rather than the increased mass of media. This effect is also suggested by results from other flow rate studies.

Initially, poor recoveries for NMEA, NMOR, and NPYR were consistently improved as the amount of Ambersorb increased up to 250 mg. The increased bed depth promoted better interaction that led to higher recoveries. Multiple elutions remove most of the analytes retained on the dual media. A specific study of analyte retention to the media was not conducted for compounds other than NDMA (Table 5.2). It is possible that 15 to 30% of the unrecoverable NMEA, NMOR, and NPYR may be from insignificant interaction with the media as opposed to analyte that cannot be removed from the cartridge.

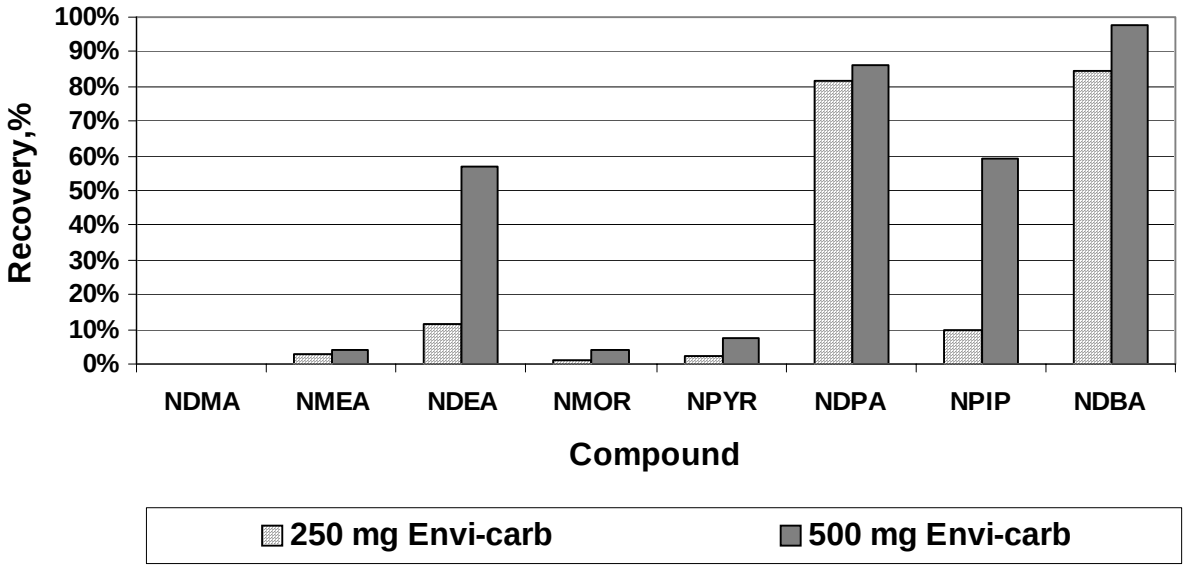


Figure 5.2 **Results of extraction of 20 ng/L nitrosamines standard by 6 mL Envi-Carb cartridges containing two different sorbent capacities (250 mg and 500 mg)**
The average recovery of three trials is shown.

The above work showed very poor recovery of NDMA from all five commercial cartridges, but good retention of NDMA for the Envi-carb cartridge, which is a carbonaceous material. In an attempt to improve the retention/elution of NDMA and the other nitrosamines, a dual-media cartridge was prepared. It was thought that Ambersorb resin, a carbonaceous resin used as free beads in another NDMA method (Taguchi et al., 1994), might be amenable to cartridge SPE. However, Ambersorb resin alone packed in SPE cartridges offers very little resistance and a slow controlled flow rate cannot be maintained. Therefore, a 350 mg layer of Ambersorb resin was packed on top of 250 mg of Envi-carb in commercially

available 3-mL cartridges and topped with a glass frit (Amb-Envi), resulting in a bed depth of 12 mm of Ambersorb and 10 mm of Envi-carb, while leaving sufficient headspace for sample.

Other CSPE methods for nitrosamine analysis have been developed concurrently with this work, one using an Ambersorb-LiChrolut En media (Charrois et al., 2003) and another using a coconut charcoal media (Munch and Bassett, 2003) and are mentioned here as a reference. The main sorbent for nitrosamines, especially NDMA and NMEA, is Ambersorb. The envi-carb guards against breakthrough, however, another support media may be effectively used as reported by Charrois et al. (2003). A purely physical flow controller may further reduce cost and the USEPA method 521 is another alternative. These cartridges are now commercially available through Restek.

Conditions for the preliminary evaluation of the Amb-Envi cartridge were as follows: average loading flow rate of approximately 3.5 mL/min using vacuum manifold, drying time 60 minutes, and two 3 mL DCM elutions. Results for triplicate analyses of 500 mL of 200 ng/L nitrosamine mixed standards by the dual-media Amb-Envi cartridges are shown in Figure 5.3. Average absolute recoveries ranged from 59.4% for NDMA to 94.5% for NDBA. These results suggest that the Amb-Envi cartridge is a viable media for nitrosamine analysis.

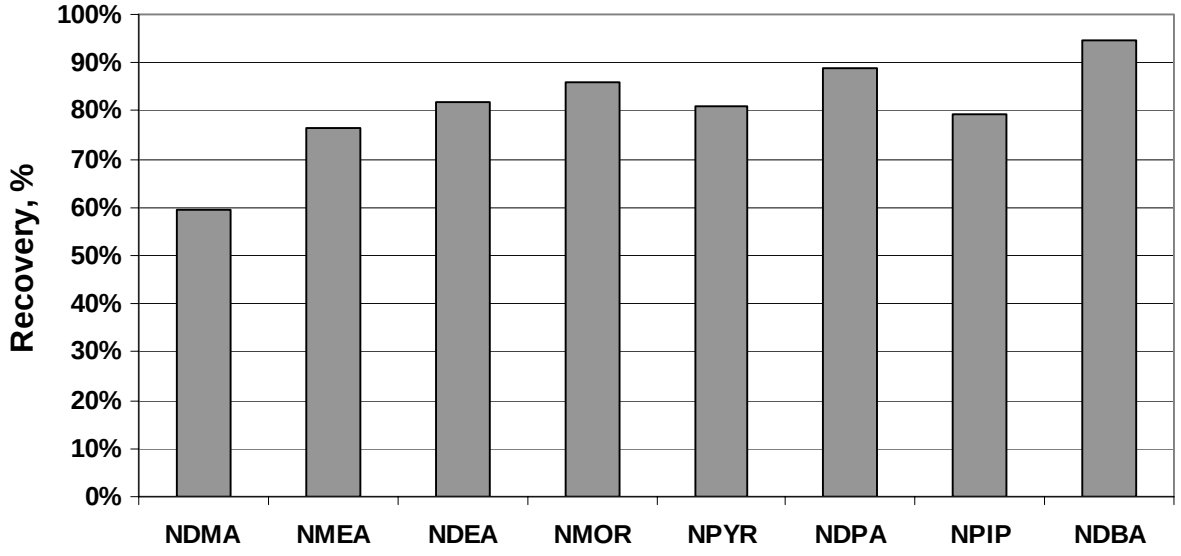


Figure 5.3 Average absolute recoveries of three trials, achieved with Amb-Envi cartridges with two dichloromethane elutions

Ambersorb Mass

The baseline amount of Ambersorb used in the Amb-Envi cartridge was 350 mg. This mass was selected based on the Amb SPE experiment results (Chapter 4, Table 4.8), which indicated that the NDMA recoveries were the highest using 350 to 400 mg of Ambersorb. In the CSPE method, however, bed depth and flow rate in addition to sorbent mass impact recoveries. A series of experiments examined the effect of reducing the amount of Ambersorb, all performed using the traditional vacuum apparatus. The results of replicate analyses, except for 150 mg, are shown in Figure 5.4. Envi-carb with no or 50 mg of Ambersorb resulted in no or very low recoveries. Results for the 150 mg Ambersorb test were greater than 50% for all the nitrosamines except NDMA, which produced questionable results. A duplicate 150 mg run was also unsatisfactory and the 50 mg runs yielded average relative difference for all the nitrosamines of 39%. The average relative difference for the 250 mg and 350 mg tests, on the other hand were within accepted limits, 9 and 13%, respectively. The highest recoveries and best precision for all the

nitrosamines, except NDMA, were observed for the 250 mg Ambersorb cartridges and the NDMA recovery was within experimental error of the 350 mg test. The 350 mg test resulted in the highest recovery for NDMA with a relative difference between runs of 9%.

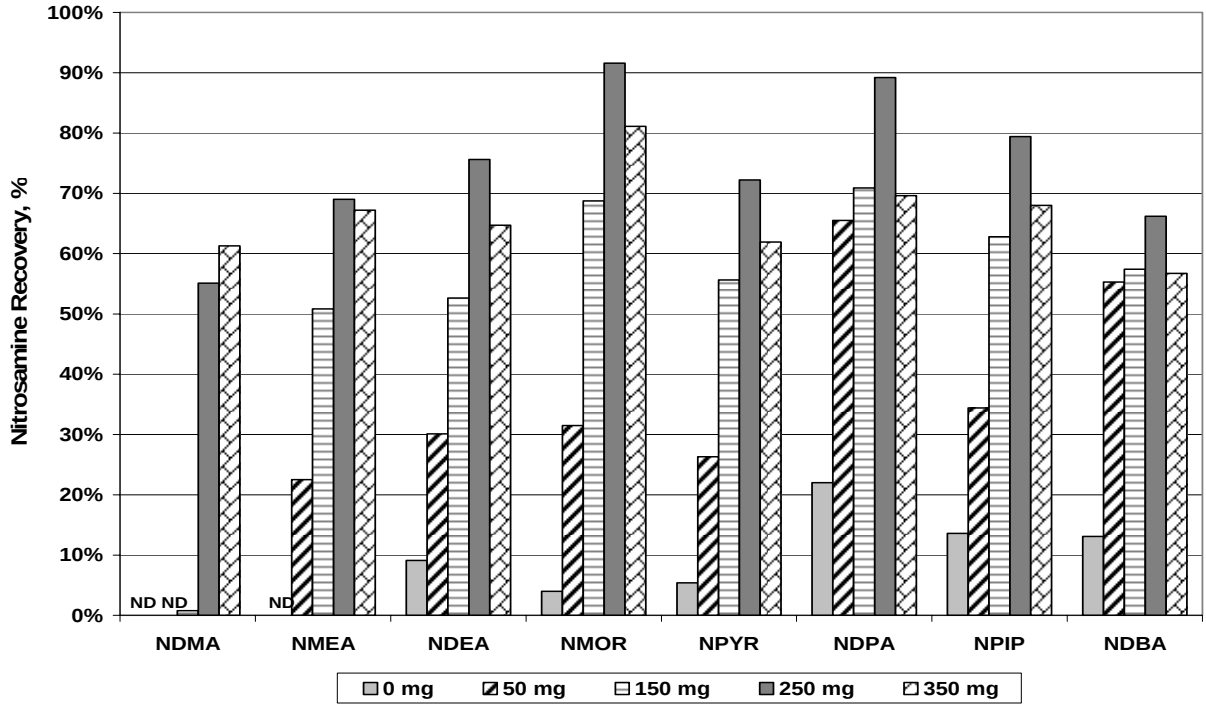


Figure 5.4 **Effect of Ambersorb mass in the Amb-Envi cartridge on nitrosamine recoveries**
Each bar represents average of two sample trials.

Sample Flow Rate

The purpose of this experiment was to determine the optimal flow rate (5 mL/min, 10 mL/min, or 15 mL/min) at which the water transfer could be performed. Experiments were performed on the APPS so that precise flow rates could be achieved. Shorter times (faster flow rates) would decrease the extraction processing time, while longer times (slower flow rates) served to potentially increase analyte recovery by promoting a better interaction between the nitrosamines and the dual-media cartridge (increased mean free path). The effect of increasing sample flow rate on nitrosamine recovery is shown in Figure 5.5. The recoveries of NDMA and NMEA were significantly higher at the lowest flow rate. At a flow rate of 5 mL/min, the percent recovery of NDMA was 76.4 ± 5%, whereas the recovery for 10 mL/min and 15 mL/min were 55.0 ± 10% and 52.0 ± 4%, respectively. Varying the sample flow rates from 5 to 15 mL/min had minimal effect on the recovery of the other six nitrosamines.

Sorbent Drying Time

Before eluting the analytes, the sorbent is dried to remove residual water that can inhibit extraction by DCM. Air was pulled through the cartridges for 15, 30, 45, and 60 minutes. At the 15 and 30 minute drying times, water droplets were observed within the DCM and the recoveries were very variable between replicates, especially for NDMA, where some runs recovered almost no NDMA and less than 50% of the other nitrosamines. The highest recoveries were observed with a 60 minute drying time although the recoveries using a 45 minute drying time were within 10% as shown in Figure 5.6.

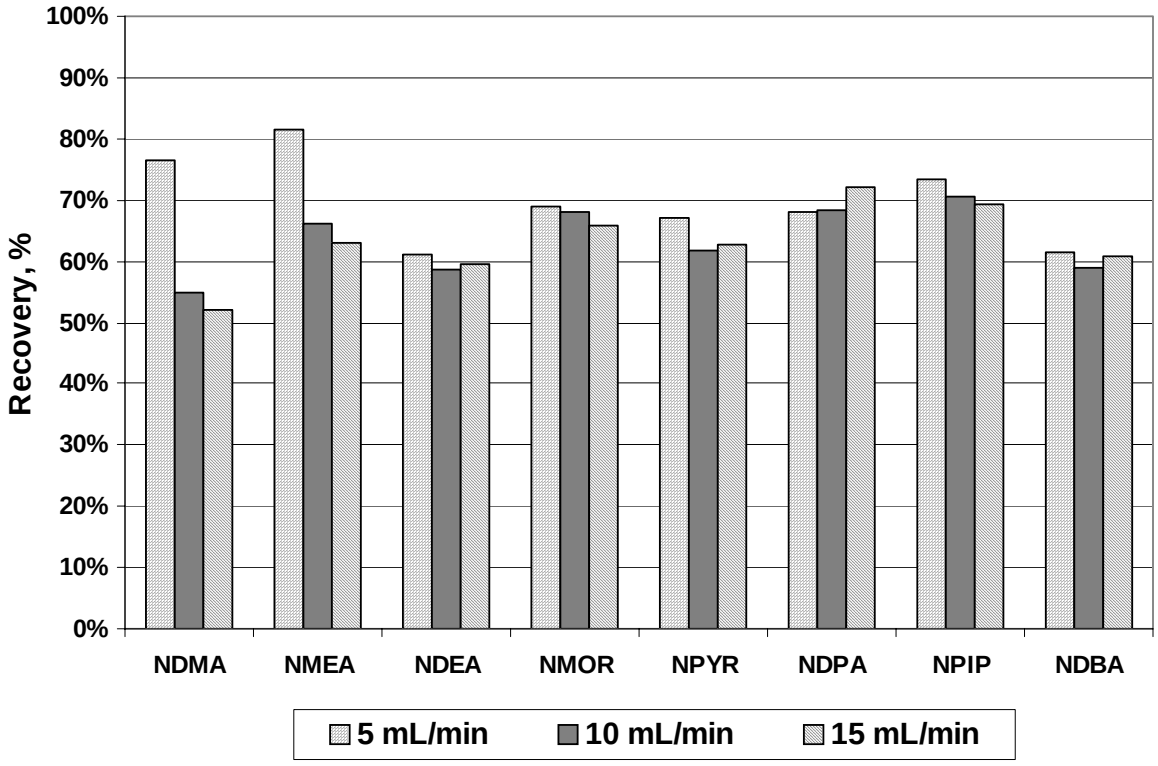


Figure 5.5 **Nitrosamine recoveries with varying flow rates for Amb-Envi CSPE**
Each bar represents average of two sample trials on the APPS instrument.

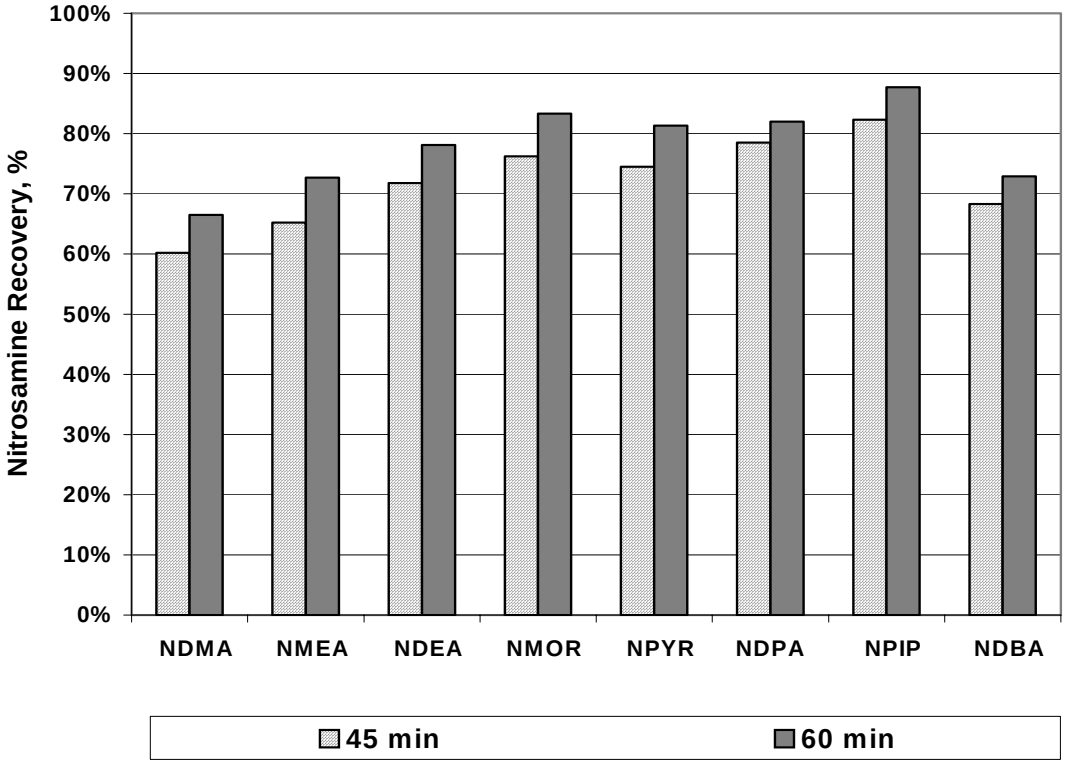


Figure 5.6 **Nitrosamine recoveries with varying drying times for Amb-Envi CSPE**
Each bar represents average of two sample trials.

Number of Elutions

Elution is a critical step in CSPE and was the problem step for the Envi-carb only cartridge. Six consecutive 2 mL DCM extractions were performed on a 200 ng/L nitrosamine mix on the Amb-Envi cartridge (in duplicate). The results are given in Figure 5.7. The first three elutions recovered 96% or more of the amount of recoverable nitrosamines (e.g., the sum of six elutions). The fourth, fifth, and sixth elutions recovered very little if any of the nitrosamines. Therefore, three 2 mL DCM elutions are specified in the optimized CSPE protocol.

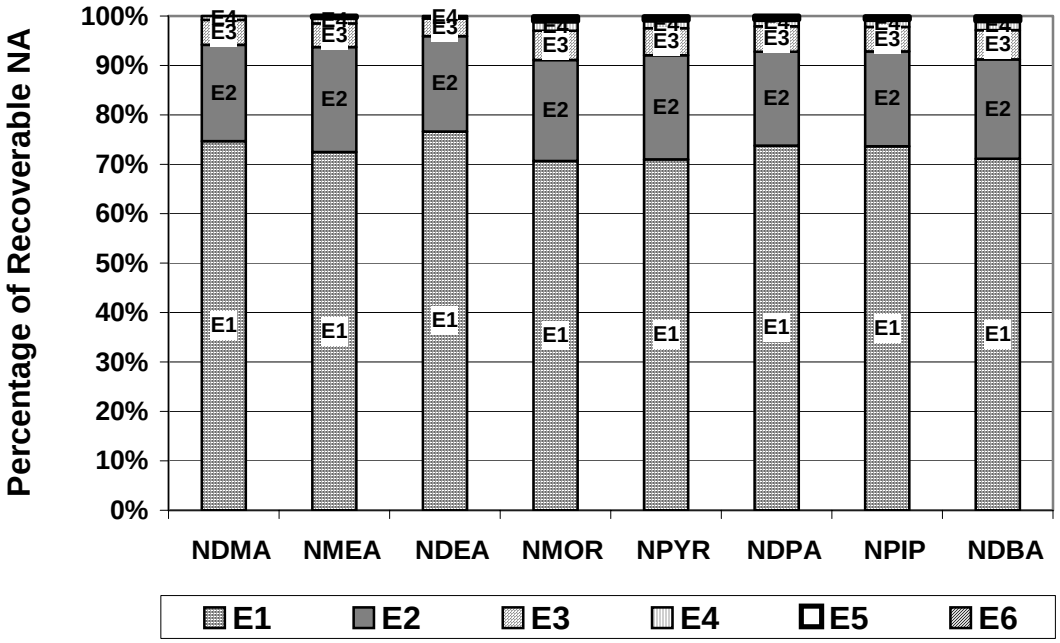


Figure 5.7 **Nitrosamine recoveries with consecutive 2 mL DCM serial elutions for Amb-Envi CSPE**
The average relative recovery of three trials is shown as a percentage of the total amount recovered from six total elutions.

Final Volume

Two options for eluate final volume were evaluated: analysis of the eluate as collected from the cartridge or concentrated to 0.5 mL under a gentle stream of helium. The final volume of eluate collected after three 2 mL DCM extractions was approximately 4 mL so evaporation to 0.5 mL increased the concentration factor 8-fold. Adding the extra evaporation step increased the analysis time, but yielded better results by increasing the area of each nitrosamine peak by a similar factor. The MDLs for the analytically more difficult nitrosamines (e.g., NDMA, NMEA, NDEA, and NPIP) were lowered by a factor of two (Table 5.3).

Method Detection Limits

The MDLs were determined by the extraction of seven replicate aliquots of 5.0 ng/L spiked reagent water and are given in Table 5.3. The MDL for NDMA was 1.7 ng/L without evaporation and 0.7 ng/L when the eluate was further concentrated by evaporation. The MDLs for the other nitrosamines ranged from 0.3 to 1.4 ng/L with concentration and from 0.6 to 3.0 ng/L without concentration of the eluate.

Table 5.3 Method detection limits for nitrosamines by Amb-Envi CSPE method

	With eluate concentration			Without eluate concentration		
Compound	Avg (ng/L)	Stdev (ng/L)	MDL* (ng/L)	Avg (ng/L)	Stdev (ng/L)	MDL* (ng/L)
NDMA	5.7	0.2	0.7	4.8	0.5	1.7
NMEA	5.0	0.4	1.4	4.6	0.7	2.2
NDEA	4.7	0.3	0.8	3.9	0.9	3.0
NMOR	5.2	0.2	0.6	5.5	0.2	0.7
NPYR	5.4	0.2	0.5	4.9	0.2	0.6
NDPA	5.1	0.3	0.8	5.5	0.3	1.0
NPIP	4.9	0.1	0.3	5.2	0.2	0.7
NDBA	6.1	0.2	0.8**	5.1	0.3	0.8

** Method Detection Limits were determined by analyzing seven replicates.*
*** Calculated from five replicates*

Method Performance

Calibration Curves

Sample curves from 2, 10, 50,100, 200, and 500 ng/L extracted standards are shown in Figures 5.8 and 5.9. Extracted standard calibration curves were linear from 2 to 500 ng/L for NMOR, NPYR, NDPA, NPIP, and NDBA with coefficients of determination (r^2) values of 0.997 or better. Calibration curves for NDMA, NMEA, and NDEA were linear from 2 to 200 ng/L and slightly curved (quadratic fit) to 500 ng/L.

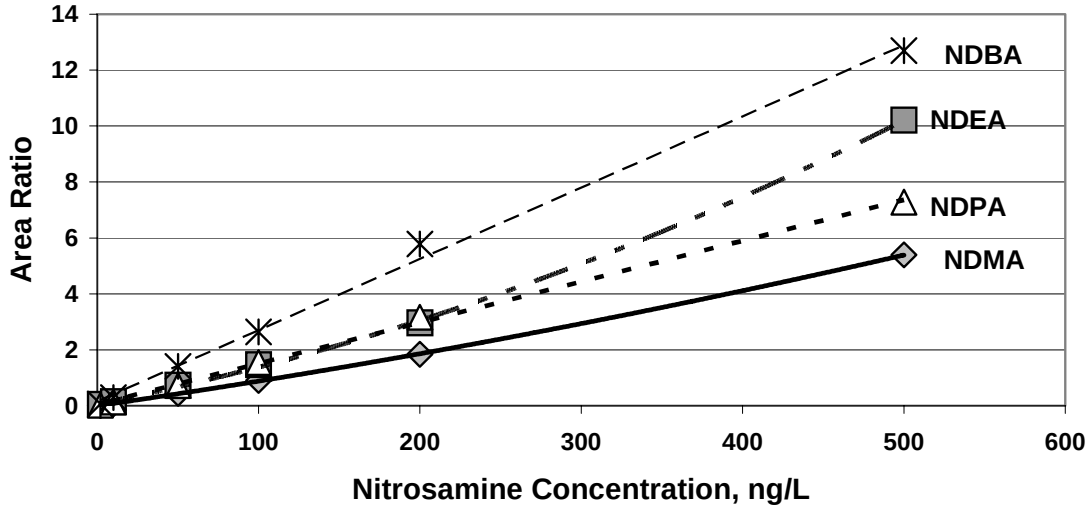


Figure 5.8 Calibration curves for NDMA, NDEA, NDPA, and NDBA (2 to 500 ng/L)

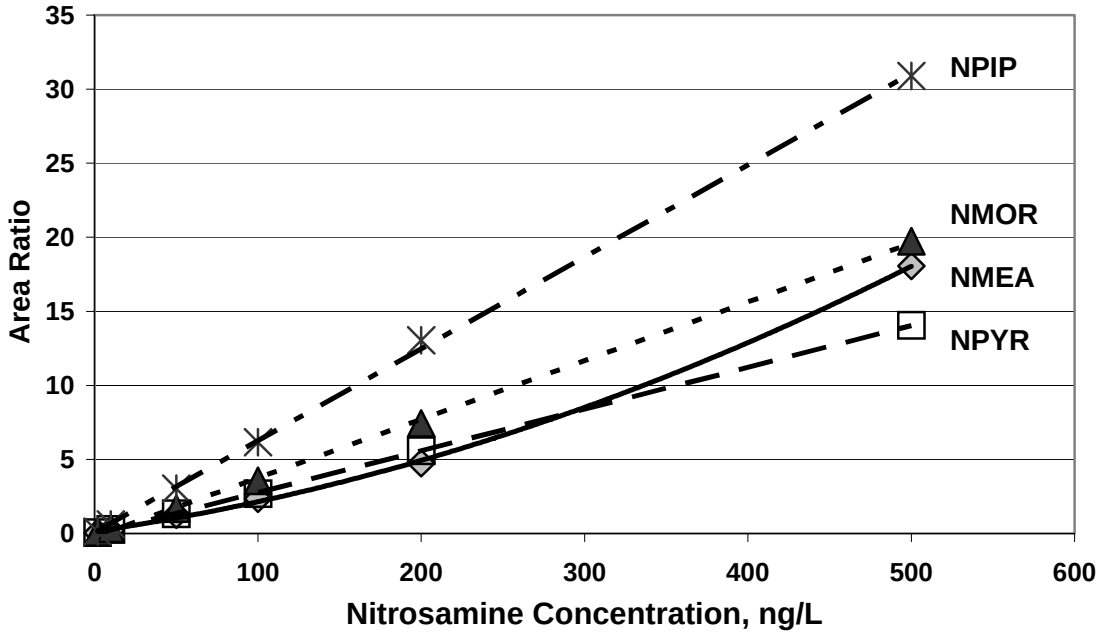


Figure 5.9 Calibration curves for NPYR, NMEA, NMOR, and NPIP (2 to 500 ng/L)

Precision and Accuracy

A limited number of samples (ranging from drinking waters to secondary effluent) were analyzed by the Amb-Envi CSPE method in parallel with other nitrosamine extraction methods and the results are discussed in detail in Chapter 8. The results of the spikes and a duplicate spike analyzed with these samples are given in Table 5.4. The spike recoveries for all eight nitrosamines are within the 70 to 130% guidelines recommended by CDHS and the relative differences for the duplicate spike are within 20%, except for NDPA recovery of 23%.

Table 5.4 Results of Amb-Envi CSPE analysis of two reclamation plant effluent samples

	LB-RR03			Reclam Plt Eff	
Sample	Conc. (ng/L)	Spike recovery[a] (%)	Dup Spk rel diff (%)	Conc. (ng/L)	Spike recovery[b] (%)
NDMA	17.1	77	10.4	11.1	88
NMEA	ND	96	1.0	ND	99
NDEA	ND	91	0.6	ND	98
NMOR	2	101	7.5	ND	92
NPYR	ND	103	10.1	ND	91
NDPA	ND	106	23.0	ND	102
NPIP	ND	95	5.1	ND	90
NDBA	ND	87	9.4	ND	103

[a]*Spiked with 20 ng/L of each nitrosamine*
[b]*Spiked with 200 ng/L of each nitrosamine*

Summary and Conclusions

Summary

- Oasis® HLB, MCX, and MAX sorbents did not extract NDMA from water.
- Envi-carb sorbent retained 80% of the NDMA applied, but it could not be easily removed during the DCM elution.
- Amb–Envi dual-media cartridges produced recoveries of 60% of the NDMA and 70 to 95% of the other seven nitrosamines.
- Optimal flow rate was 5 mL/min.
- Three 2 mL DCM elutions recovered 96% or more of the extractable nitrosamines.
- Cartridge air-drying time of 45 minutes or greater was needed.
- An Ambersorb layer of 350 mg resulted in the greatest recovery of NDMA, although 250 mg resulted in better recoveries for the other nitrosamines and similar NDMA recoveries.
- The MDL for the Amb-Envi CSPE analysis was 0.7 ng/L for NDMA and from 0.3 to 1.4 ng/L for the other nitrosamines.

Conclusions

The following optimized dual-media Amb-Envi CSPE method incorporates the optimized conditions reported in this chapter and is suitable for analysis of the eight target nitrosamines in water and wastewater:

- A 350 mg layer of Ambersorb resin is added to a 3 mL, 250 mg Envi-carb disposable extraction cartridge.
- The cartridge is conditioned with methanol followed by reagent water.
- 500 mL sample spiked with isotopically labeled internal standards is applied to the cartridge under vacuum at a maximum flow rate of 5 mL/min (typically 3 to 4 mL/min).
- The cartridge is dried under vacuum for 45 to 60 minutes and extracted with three 2 mL aliquots of DCM.
- The combined eluates are then concentrated to 0.5 mL under a stream of helium in a water bath at 35 ^{o}C.
- The extract is then injected into a GC/MS/MS operated in chemical ionization mode, and the nitrosamine concentration is calculated from the area ratio of product ion to labeled surrogate.
- The standard operating procedure for the Amb-Envi CSPE method is given in Appendix C.

The Amb-Envi cartridge SPE method reduces solvent usage, can achieve low ng/L detection limits and can be automated. The method has been successfully used to analyze drinking water, wastewater and reclaimed water for the eight targeted nitrosamines: NDMA, NMEA, NDEA, NDPA, NDBA, NPYR, NPIP, and NMOR. The results of a project-team, multi-method comparison and an interlaboratory North American round-robin study are discussed in Chapter 8.

CHAPTER 6

SOLID–PHASE MICROEXTRACTION METHOD

Introduction

A solid–phase microextraction (SPME) method was developed for seven nitrosamines in wastewater (NPYR was not detected). A silica fiber support is coated in a thin layer of polymer material. This fiber is exposed to a water sample, either by immersion in aqueous samples or in the sample headspace. During extraction, target analytes adsorb onto the fiber, equilibrating between the phases of the system. Equilibrium can be shifted by the addition of heat or salt to increase fiber sorption. The fiber is then removed and placed in a heated GC injection port, where the analytes are thermally desorbed. This SPME method is attractive in that:

- No solvents are required;
- Extraction is highly selective;
- Extraction and concentration occur simultaneously;
- Analytical time is reduced as compared to continuous liquid–liquid extraction;
- No specialized extraction or concentration glassware are required; and
- Method can be automated.

The optimum extraction parameters were first determined. Parameters to be optimized included SPME fiber coating, mode of extraction, salt saturation, extraction temperature, extraction time, and pH. Experiments were conducted to determine the likelihood of matrix competition effects. MDLs were then established and an initial assessment of the method performance was conducted. A complete list of procedures for this method is listed in Appendix D.

Experimental

All specifications are suggested. Brand names and/or catalog numbers are included for illustration purposes only.

Chemicals

Reagent water with a resistivity of 18.2 MΩ-cm, was obtained from a water purification system (Milli-Q-UV, Millipore Corp, Bedford, MA). Sodium chloride (J.T. Baker Chemical Co., NJ) used in extractions contained less than 2 mg/L of nitrogen-containing compounds. A nitrosamine stock standard containing NDMA, NDPA, NMOR, NPYR, NPIP, NDBA, NMEA, and NDEA was obtained in methanol (Ultra Scientific, RI). NDPA-d_{14} in methanol (Cambridge Isotope Laboratories, MA) was used as internal standard for the NCD. NDMA-d_6 in methanol was used as a surrogate for GC/CI/MS/MS analysis (Cambridge Isotope Laboratories, MA). Standards stored in amber vials in a refrigerator set to –10 °C stored for greater than six months showed no apparent loss or degradation.

Equipment

Open-top vials with 15 mL capacities with septa were used in extraction (Supelco, St. Louis, MO). Four SPME fibers were examined for NDMA extraction efficiency: polyacrylate (PA), carboxen/polydimethylsiloxane (CAR/PDMS), carbowax/divinylbenzene (CW/DVB), and polydimethylsiloxane/divinylbenzene (PDMS/DVB). Manual SPME holders were used for this study; however, automatic sampler SPME equipment is available. The sample vial was agitated using a heater/magnetic stirrer with a ¼" stir bar. An aluminum heating block held the sample during extraction. All SPME equipment was purchased from Supleco. Various size syringes and glassware were used in the preparation of standards.

Nitrogen Chemiluminescence Detector

A Sievers model 255 Nitrogen Chemiluminescence Detector (Ionics Instruments, Boulder, CO) was used for optimization in addition to GC/CI/MS/MS. The NCD was modified by the manufacturer to eliminate nitrogen oxidation. Hydrogen flow to the pyrolysis chamber was removed. Additionally, the design of the ceramic catalyst tubes within the pyrolysis chamber was modified, both in structure and by the addition of Platinum. The detector was mounted on a Hewlett Packard (HP) 5890 gas chromatograph. Integration and data collection were provided by a HP 3396 Series II Integrator slaved to a computer operating HP Peak 96 software. The standard split–splitless GC inlet was used with a 0.75-mm ID SPME liner (Agilent, Palo Alto, CA). Inlet temperature was set at 250 °C. Helium carrier gas was set to a column head pressure of 13 psi. Inlet purge time was set for 2 minutes with a purge flow of 30 mL/min. Column and oven parameters are described in Chapter 7.

Mass Selective Detector

Some samples were analyzed on Thermo Finnigan Trace GC coupled with TSQ in ammonia CI-SIM mode. The SPME liner with 0.75-mm ID was used with the split–splitless inlet heated at 250 °C. A DB-210 capillary column, 30-m long, 0.25-mm inner diameter, and 0.5-µm film thickness was purchased from Agilent. GC temperature was operated at 45 °C for 2 minutes, ramped at 50 °C/min to 100 °C and held for 1.5 minutes, then 15 °C/min to 180 °C, and 80 °C/min to 250 °C and held for 1.5 minutes. Ions used for quantification were 92 for NDMA, 106 for NMEA, 120 for NDEA, 148 for NDPA, 132 for NPIP, 176 for NDBA, 134 for NMOR, and 98 for NDMA-d_6 (internal standard).

General SPME Procedures

SPME fibers were first conditioned to remove any contaminants. Each fiber was conditioned prior to use in a GC split–splitless inlet, at the temperature and length of time recommended by the manufacturer for each fiber type. Amber open top vials (15 mL) with septa were used for extraction. Sodium chloride and a magnetic stir bar were added to the sample vial, followed by the aqueous sample. The sample was placed on a heater/magnetic stirrer and the septa pierced by the SPME holder. The SPME fiber was immediately exposed to the sample headspace. After the set extraction time, the fiber was retracted and removed. Exposure of the SPME fiber in the heated GC inlet desorbed nitrosamines for analysis.

SPME Optimization Experiments

The parameters studied for the development of this SPME method are summarized in the optimization matrix given in Table 6.1. Optimization of SPME fiber coating, extraction mode, NaCl concentration, and pH was performed using GC/CI/MS/MS. Optimization of the remaining parameters was performed with GC/NCD. Four SPME fibers were evaluated for NDMA extraction efficiency. For all fibers, two modes of extraction were compared, direct aqueous and headspace. Sample of 5 and 30 mL volumes were analyzed at the same concentrations, while maintaining a constant headspace to volume ratio of 0.25. While maintaining a constant sample volume, the headspace volume was varied such that the ratio of headspace to total volume ranged from 0.4 to 0.7. Three temperatures were tested: 45 °C, 65 °C, and 95 °C. Extraction times from 15 to 1030 minutes were tested to determine the length of time necessary for NDMA to reach equilibrium. The concentration of NaCl for optimum recoveries was determined by evaluating 25-, 50-, and 100-% saturated salt solutions. Two pH levels were studied, 7 and 12. Adjustments to the pH were made using 0.01 M orthophosphate buffer solution.

Table 6.1 Optimization parameters for SPME nitrosamines method

Variable	Range
SPME fiber coating	PA, CAR/PDMS, CAR/DVB, PDMS/DVB
Mode of extraction	Direct aqueous, Headspace
Sample volume (mL)	5, 30
Headspace: total volume ratio	0.4, 0.6, 0.7
Extraction temperature (°C)	45, 65, 95
Extraction time (minutes)	15, 30, 45, 65, 90, 135, 230, 1030
NaCl concentration (% Saturation)	25, 50, 100
Extraction pH	7,12

Results and Discussion

SPME Fiber Coating and Mode of Extraction

To optimize sorption of nitrosamines on the SPME fiber coating, four SPME fibers were examined for NDMA extraction efficiency: PA, CAR/PDMS, CW/DVB, and PDMS/DVB. There are two modes of SPME, aqueous extraction and headspace extraction. In aqueous extraction, the SPME fiber is submerged in the sample and analytes partition between the aqueous and fiber phase. In headspace extraction, the fiber is exposed in the headspace above the water sample and is not submerged. In this mode, analytes must partition between three phases: aqueous, air, and fiber. Each of the four fibers was tested with direct aqueous extraction and headspace extraction. Due to the polarity of NDMA and other nitrosamines and their relatively low volatility, it was expected that direct extraction would have higher recoveries. However, the opposite effect was observed. For all four fibers tested, headspace extraction showed greater NDMA recovery. The significantly better NDMA recoveries resulting from the use of CAR/PDMS as compared to the other three fibers can be seen in Figure 6.1, shown here in logarithmic scale.

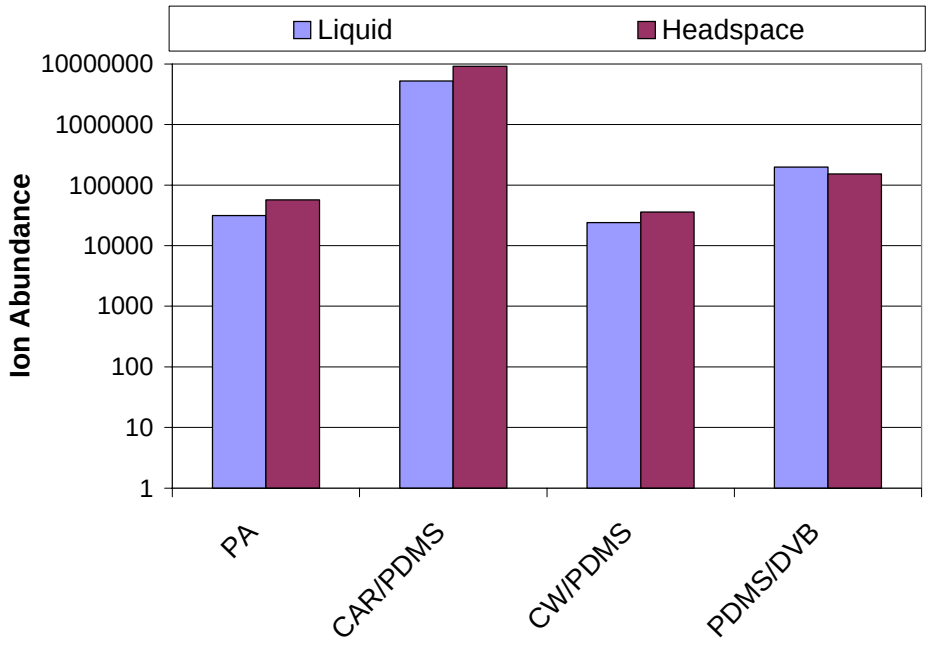


Figure 6.1 SPME fiber and extraction mode comparison for NDMA

Sample Volume and Headspace Optimization

The aqueous volume to headspace ratios were optimized after determining that a headspace extraction method was preferable for the analysis of NDMA by SPME. Sample volumes of 5 and 30 mL were analyzed at the same concentrations while maintaining a constant headspace to volume ratio of 0.25. Varying the sample volume, while maintaining a constant headspace to sample ratio, was not shown to affect the NDMA extraction efficiency. Next, the volume of the headspace was varied while maintaining a constant sample size. The optimum headspace to total volume ratio was found to be 0.6 (Figure 6.2). This corresponded to 7 mL of sample in a 15 mL vial (true volume is 17 mL).

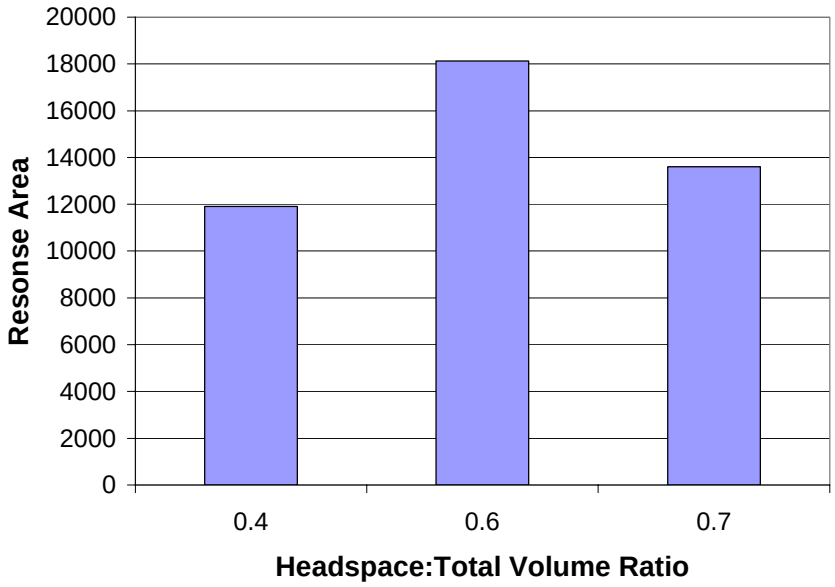


Figure 6.2 SPME headspace optimization for NDMA

Extraction Temperature

The volatility of target analytes is increased by increasing the temperature of the extraction. Three temperatures were tested: 45 °C, 65 °C, and 95 °C, with the results presented in Figure 6.3. Between 45 °C and 65 °C, all compounds showed an increase in extraction efficiency. With the exception of NDMA and NMEA, all nitrosamines showed a substantial increase in extraction at 95 °C. However, NDMA recovery decreased at this temperature, which may have caused by the effect of increased water vapor present. Because of this effect, the optimum extraction temperature was selected as 65 °C.

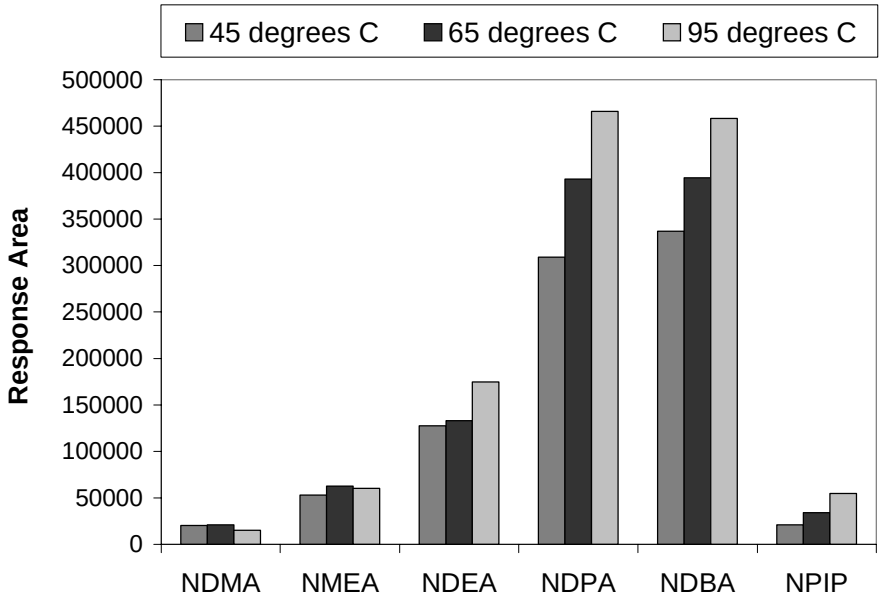


Figure 6.3 SPME temperature optimization

Extraction Time

The partitioning of chemicals from the aqueous phase to the SPME fiber is an equilibrium process. Because an advantage of using SPME is the decreased analysis time, it may be preferable to use nonequilibrium extraction for the compounds that have a long extraction time. Extraction times from 15 to 1030 minutes were tested to determine the length of time necessary for NDMA adsorption to reach equilibrium. Experiments showed equilibrium was reached after 230 minutes of extraction time (Figure 6.4).

Using SPME, samples must be analyzed as soon as possible following extraction, as compared to solvent extraction methods where the extracted sample may be held for an extended period of time. The most efficient use of the laboratory analyst's time when performing the manual SPME method is to properly sequence the time needed for extraction with the time necessary for the GC to complete analysis of a given sample and return to ready for the next analysis. Because the full equilibration time of 230 minutes was deemed too long for analytical requirements, 45 minutes was selected as an extraction time which would optimize the point of equilibration with the time required for the continuous sample extraction and analysis. With the use of automated SPME equipment, the laboratory analyst's time is of less concern and the use of longer extraction times, with a corresponding increase in sensitivity may be preferable. When using nonequilibrium extraction, care must be taken to consistently use the same extraction time as small changes in extraction time will cause changes in the amount of analyte extracted. This problem is also helped by the use of an extraction surrogate to account for slight variations between sample runs.

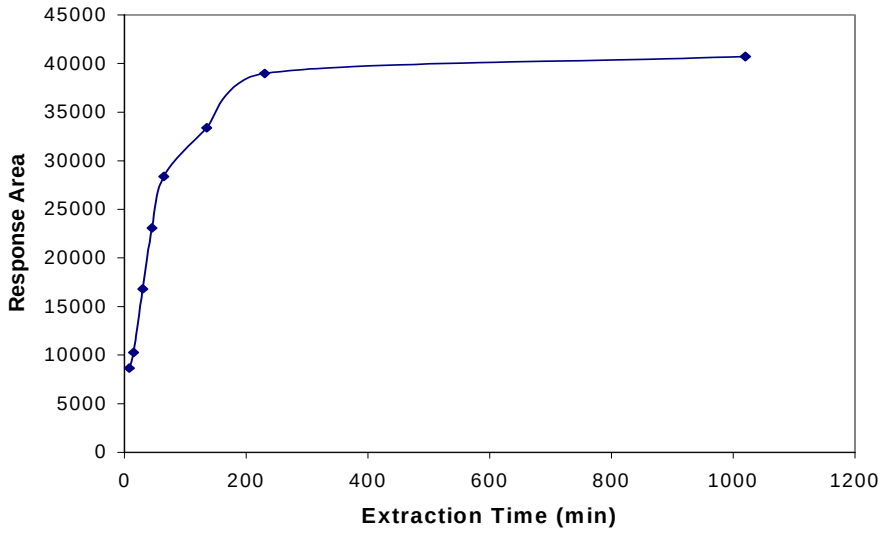


Figure 6.4 SPME extraction time optimization for NDMA

Salt Effects

Salt is added to the SPME sample to decrease the solubility of analytes in the aqueous phase, shifting equilibrium towards the air phase. The optimum amount of NaCl was determined; 25, 50, and 100% saturated salt solutions were tested. A salt saturation of 100% resulted in a significant improvement over other concentrations in the extraction of NDMA (Figure 6.5).

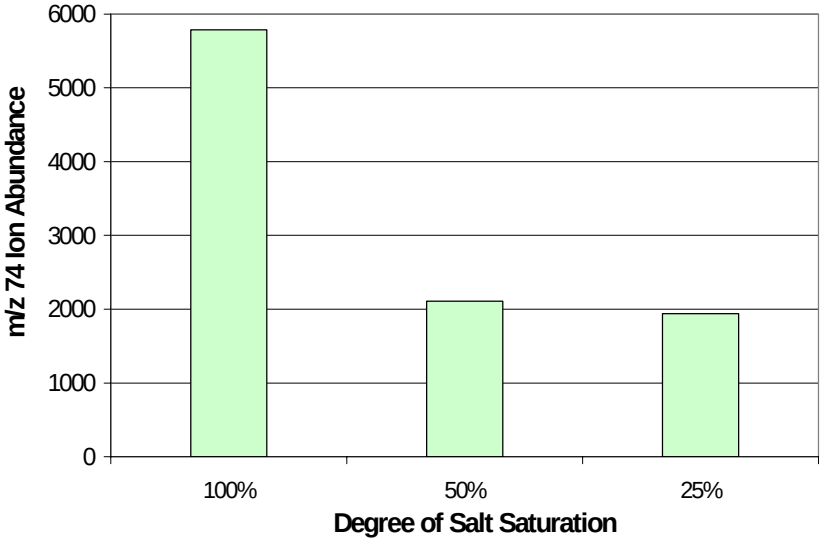


Figure 6.5 SPME salt saturation optimization

pH Effects

The effect of pH on extraction efficiency was also tested. Two pH levels were studied, pH of 7 and 12. For all cases, neutral pH was shown to provide better extraction of NDMA, with approximately 10% greater recoveries at 95 °C.

Competition Study

Several possible competition candidates were tested to determine if this SPME method was sensitive to competition effects that may invalidate results. These chemicals include humic substances and unsymmetrical dimethylhydrazine, UDMH, a known NDMA precursor. To determine if the water matrix could compete with the SPME fiber and affect extraction results, SPME results were compared to CLLE results from the same water samples. Wastewater samples at neutral pH, secondary effluent (unspiked and spiked with 200 ng/L NDMA) and tertiary effluent with TOC levels of 9.6 and 8.3 mg/L respectively, were analyzed for NDMA by SPME and CLLE. Organic-free water and potable water were spiked with 250 ng/L UDMH and 30 ng/L NDMA, and then analyzed by SPME to verify NDMA recovery. GC/CI/MS/MS was used to analyze all competition study samples. Test results are listed in Table 6.2.

The presence of organic carbon did not significantly affect the NDMA results determined by SPME as compared to those by LLE. The presence of UDMH in either organic free or potable water also did not significantly affect recoveries of the spiked NDMA concentrations. From these results, it was expected that no detrimental competition effects would be seen in the analysis of complex wastewater samples. This result was later verified by checking the recoveries of matrix spiked samples.

Table 6.2 Effect of matrix competition on extraction of NDMA by SPME

Method	Water matrix	NDMA (ng/L)
SPME	Secondary effluent[1] (no chlorine)	17.9
LLE	Secondary effluent (no chlorine)	11.2
SPME	Secondary effluent (no chlorine) *- spiked with 200 ng/L NDMA*	220
SPME	Tertiary effluent[2]	210
LLE	Tertiary effluent	183
SPME	Organic-free water with 250 ng/L UDMH *- spiked with 30ng/L NDMA*	37.6
SPME	Potable water with 250 ng/L UDMH *- spiked with 30 ng/L NDMA*	41.2

[1]9.63 mg/L of TOC and pH of 7.27
[2]8.33 mg/L of TOC and pH of 7.11

Method Detection Limits

MDLs were determined according to USEPA protocol 40 CFR 136 (USEPA, 1984). An organic-free water sample was spiked with a low concentration of nitrosamines and analyzed. This was performed seven times over a three-day period. The standard deviation of the seven replicates was multiplied by the student t number for 99% confidence. For NCD, a 200 ng/L nitrosamine standard, with NDPA-d_{14} internal standard, was used to establish MDLs for all nitrosamines simultaneously. For the GC/CI/MS/MS

quantitation, a 250 ng/L standard of NDMA was used to establish the MDL for NDMA alone. A 500 ng/L standard was used to determine the detection limits for remaining nitrosamines.

MDLs for the two detection systems used in this study are shown in Table 6.3. NPYR was not detected by either detector, and thus was not extracted to any significant degree from the water samples. NMOR was detectable by GC/CI/MS/MS, but not by NCD. This could be due to an enhanced GC/CI/MS/MS sensitivity towards this compound based on the different operating mechanisms of the two detectors. The MDL for NDMA was 56.5 ng/L and 30 ng/L for the NCD and GC/CI/MS/MS, respectively. These limits are not sensitive enough to meet the demands of drinking water nitrosamine analysis, requiring detection limits between 1 to 10 ng/L. However, typical wastewater nitrosamine levels would fall within the limits of this SPME method.

Table 6.3 MDLs for SPME and quantitation by GC/NCD and GC/CI/MS/MS

	NCD			GC/CI/MS/MS		
Compound	**Avg (ngL)**	**Stdev (ngL)**	**MDL (ngL)**	**Avg (ngL)**	**Stdev (ngL)**	**MDL (ngL)**
NDMA	168	18	57	253	10	30
NMEA	182	42	132	NA	NA	NA
NDEA	180	28	87	343	20	60
NDPA	240	18	58	505	20	60
NDBA	324	61	193	501	20	60
NPIP	296	40	125	408	19	60
NPYR	ND	ND	ND	ND	ND	ND
NMOR	ND	ND	ND	540	19	60

NA = not available, ND = not determined

Method Evaluation

A secondary effluent sample was analyzed with the finalized SPME method to determine the presence of interference or competition effect in this matrix. Matrix spiked samples were analyzed to check that method recoveries were within an acceptable range of 70 to 130%, as recommended by the CDHS. The unspiked wastewater sample was analyzed by each detector. NDMA was the only nitrosamine detected at a concentration of 133 ng/L and 104 ng/L by the NCD and GC/CI/MS/MS, respectively. The water sample was then spiked with 200 ng/L nitrosamines and analyzed. The results listed in Table 6.4 are the average of two duplicate analyses. NDMA spike recoveries are adjusted to account for the initial concentration present in the sample. Results for both detection systems were excellent. NCD spike recoveries were within ±10% of the true value, with the exception of NPIP (±15%) and NMEA (±12%). GC/CI/MS/MS recoveries were within ±30% of the true value with the exception of NMEA and NMOR. The spike recoveries of NDMA and NMEA showed significant error (66% and –54%, respectively), indicating the possible occurrence of a matrix effect that may hinder the analyses of these nitrosamines with GC/CI/MS/MS.

Table 6.4 Spike recoveries from secondary wastewater effluent

	NCD		GC/CI/MS/MS	
Nitrosamine	Conc. (ng/L)	Spike recovery (%)	Conc. (ng/L)	Spike recovery (%)
NDMA	320	93	435	166
NMEA	223	112	92	46
NDEA	192	96	221	110.5
NDPA	205	103	229	114.5
NDBA	205	102	206	103
NPIP	230	115	146	73
NPYR	ND	ND	ND	ND
NMOR	ND	ND	236	136

Summary and Conclusions

Summary

- CAR/PDMS fiber coating provides highest NDMA recovery, with headspace extraction.
- The best extraction temperature for NDEA, NDPA, NDBA, and NPIP was 95 °C, but NDMA and NMEA extraction is maximized at 65 °C.
- Total analysis time is 1.25 hours per sample.
- Of the eight nitrosamines tested, only NPYR could not be analyzed with SPME.
- MDL for SPME-NCD is 57 ng/L for NDMA, from 58 to 193 ng/L for the five other nitrosamines.
- MDL for SPME-GC/CI/MS/MS is 30 ng/L for NDMA and 60 ng/L for six other nitrosamines.
- SPME-NCD spike recoveries in wastewater were within 10% of true value, excluding NPIP.
- SPME-GC/CI/MS/MS spike recoveries, excluding NMEA, were within 30% of true value.
- SPME-GC/CI/MS/MS could not analyze NMEA in wastewater matrix.

Conclusions

A method was developed able to analyze seven nitrosamine compounds from water by SPME. The detection limits for this method were found to be in the ng/L range. This method was used to analyze wastewater samples and showed excellent selectivity of extraction. The two detectors used in this study, NCD and GC/CI/MS/MS, provided reliable results. The high selectivity of SPME functioned well with the NCD; nitrosamines were the only compounds extracted and detected, which signifies that dual column confirmation (Chapter 7/Experimental/Quantitation) was unnecessary, and a reduction in analysis time is achievable with this detector.

The MDLs are currently too high to reach low ng/L nitrosamine (e.g., < 10 ng/L for CDHS Action Level for NDMA). However, detection limits are within range of typical wastewater nitrosamine concentrations, usually above 100 ng/L for NDMA. SPME is a highly selective extraction method, which is attractive for complex wastewater matrices. Competitive extraction effects were not seen in the samples analyzed in this study, with the exception of NMEA analyzed by GC/CI/MS/MS. The small amount of time and materials that are needed for this technique make it an inexpensive and simple method. Additionally, the small aqueous volumes necessary for analysis are more convenient for collection and transport than the significantly larger volumes used in other extraction techniques. The selectivity in addition to the fast analysis time would make this method ideal for general surveys, wastewater analysis, and laboratory studies (e.g., kinetics of degradation or formation potential studies).

CHAPTER 7

ALTERNATIVE DETECTORS

Two alternative detectors, a Nitrogen Chemiluminescence Detector (NCD) and Nitrogen Phosphorus Detector (NPD) were selected as potential low cost alternatives to expensive mass spectral detectors, which are currently the most common method for nitrosamine analysis. This chapter contains a discussion of the optimization process followed for each detector, concluding with a comparison of the two detectors' performances to GC/CI/MS/MS for the analysis of drinking water and wastewater samples.

Nitrogen Chemiluminescence Detector (NCD)

Introduction

In the NCD pyrolysis chamber, high temperature thermally degrades nitrosamines, forming nitric oxide radicals:

$$R_2\text{–NNO} \rightarrow {}^{\bullet}\text{NO} \qquad (7.1)$$

Nitric oxide radicals are pulled by vacuum into the reaction cell where they react with ozone according to the following equation:

$${}^{\bullet}\text{NO} + O_3 \rightarrow NO_2^* \rightarrow NO_2 + h\nu\ (800\text{–}3200\ \text{nm}) \qquad (7.2)$$

As electronically excited nitrogen dioxide falls back down to the ground state, light in the red and infrared spectrum is released. The intensity of the light, measured by a photo-multiplier, is proportional to the concentration of nitrogen present. Chemiluminescent nitrogen detection has previously been used for the detection of NDMA in drinking water matrices at low ng/L levels although the applicability of this detector has not yet been tested for wastewater matrices (Kimoto, 1981; Fine, 1975a; Tompkins, 1995).

The objective of this study was to determine if the NCD could be successfully used for the analysis of nitrosamines at low ng/L aqueous concentrations in a range of water types, from clean matrices of drinking water to the complex chemical matrices of wastewater. To complete this objective, the following steps were considered: (1) optimization for nitrosamine response, (2) developing a confirmation method using dual GC capillary columns, and (3) evaluating methods to minimize detector interferences, including exploring modifications of extraction method and the detector configuration. Instrument detection limits were established, in addition to MDLs for an Ambersorb SPE-NCD analytical method. Finally, the method performance of NCD with extraction methods was examined.

Experimental

All specifications are suggested. Brand names and/or catalog numbers are included for illustration purposes only.

Chemicals

A nitrosamine standard containing NDMA, NDPA, NMOR, NPYR, NPIP, NDBA, NMEA, and NDEA obtained in DCM (Ultra Scientific, RI) was used to optimize detector response. NDPA-d_{14} (Cambridge Isotope Laboratories, MA) was selected as an extraction surrogate standard. All standards were stored in amber vials in a refrigerator set to –10 °C. Standards stored for longer than six months showed no apparent loss or degradation.

Equipment

The Sievers model 255 Nitrogen Chemiluminescence Detector was obtained from Ionics Instruments (Boulder, CO). The detector was modified by the manufacturer to eliminate nitrogen oxidation in order to operate in a nitrosamine-specific mode. Hydrogen flow to the pyrolysis chamber was removed. Additionally, the design of the ceramic catalyst tubes within the pyrolysis chamber was modified, both in structure and by the addition of Platinum. The detector was mounted on a Hewlett Packard 5890 Gas Chromatograph. Integration and data collection was provided by a HP 3396 Series II Integrator slaved to a computer operating HP Peak 96 software.

Two capillary GC columns were used for primary analysis and confirmation, DB-1701 and Supelcowax 10. Column and oven parameters are listed in Table 7.1.

Table 7.1 Column and oven parameters for DB-1701 and Supelcowax 10

Column:	DB-1701	Supelcowax 10
Column length (m)	30	60
Column ID (mm)	0.32	0.25
Column film (μm)	0.5	1.0
Column Head (psi)	13	13
Detector Type	Sievers 255 Nitrogen Chemiluminescence Detector	
Inlet Type	Split–splitless Manual	Split–splitless Manual
Injection Volume (μL)	3	3
Temp (^{o}C)	220	220
Purge Time (min)	1	1
Purge Flow (mL/min)	30	30

Nitrosamine Retention Time (Min)		
Compound	**DB-1701**	**Supelcowax 10**
NDMA	3.90	9.84
NMEA	5.02	10.56
NDEA	5.91	11.00
NDPA-d_{14} (IS)	8.14	12.89
NDPA	8.23	12.99
NMOR	8.73	16.92
NPYR	9.06	16.29
NPIP	9.33	15.89
NDBA	12.69	15.43

Column Temperature Program							
DB-1701				Supelcowax 10			
Temp. (^{o}C)	**Rate (^{o}C/min)**	**Hold time (min)**	**Total time (min)**	**Temp. (^{o}C)**	**Rate (^{o}C/min)**	**Hold time (min)**	**Total time (min)**
40	0	1		40		1	
150	12	3		250	10	5	27
250	25	2	26				

NCD Optimization Experiments

In addition to the detector hardware modifications optimizing nitrosamine response, three additional parameters were investigated: detector gas flow rate, detector gas type, and pyrolysis temperature. Conditions tested are listed in Table 7.2. Increasing the pyrolysis chamber temperature increases the efficiency of pyrolysis. Temperatures between 475 and 600 °C were tested. Gas flows through the detector were tested between 1 and 5.5 mL/min. In order to minimize the possibility of unwanted oxidation of non-nitrosamine nitrogen compounds present in any sample, inert helium was tested as a replacement of oxygen gas.

Table 7.2 Optimization parameters for NCD

Variables	Range
Gas Flow Rate (mL/min)	1, 2, 3, 4, 4.5, 5, 5.5
Detector Gas	O_2, He
Pyrolysis Temperature (°C)	475, 500, 550, 600

Inline Clean-up Method

An inline nitric oxide selective trap was obtained from Thermedics, Inc. (Woburn, MA) for NCD sample clean-up. The alumino-silicate molecular sieve material in the trap preferentially allows NO and NO_2 to pass through, trapping larger, polar, organic molecules, such as sulfur and double-bonded carbon compounds. The trap prevents the compounds from reaching the ozone reaction chamber where they could potentially react with ozone. This is an inline cleanup method, requiring no additional experimental steps. The trap is placed within the gas lines of the detector, after the pyrolysis chamber but before the ozone reaction chamber.

Extraction Methods

Wastewater and drinking water samples were extracted by CLLE, Amb SPE (discussed in Chapter 4), and MLLE (discussed in Chapter 3).

CLLE was performed according to USEPA Method 3520C, requiring 18 hours of continuous extraction with DCM, followed by a nitrogen evaporation concentration step. The initial aqueous sample size of 1 L is reduced to a final solvent volume of 1-mL DCM, with a method concentration factor of 1000. NDPA-d_{14} in methanol was added as an extraction surrogate.

In an attempt to make this a more selective extraction technique, the pH of CLLE was varied to observe the effect on the amount of extraneous compounds extracted. The water sample pH was adjusted prior to solvent extraction. Basic (pH >12), Acidic (pH<2) and Neutral (pH 6 to 8) conditions were tested.

Results and Discussion: NCD Optimization

Detector Gas Flow Rate

To determine if increased contact time in the pyrolysis chamber would increase NCD response, the flow rate of O_2 through the pyrolysis chamber was decreased from the manufacturer's recommended setting of 5 mL/min. Injections of 1 μL nitrosamine standard (10 μg/L) in DCM was injected a single time at each flow rate. No significant effect on NCD response was observed at flows between the test range of 1 to 5.5 mL/min.

Pyrolysis Temperature and Gas Type

The NCD pyrolysis chamber temperature controls the amount of energy available to break down nitrosamines into nitric oxide, which influences detector response. The temperature of pyrolysis was varied between 475 and 600 °C. Increasing pyrolysis temperature improved pyrolysis efficiency and detector response (Figure 7.1). However, higher temperatures also caused increased noise and significant baseline drift. A pyrolysis temperature of 550 °C provided minimal noise and baseline problems while significantly improving detector response.

The manufacturer recommended oxygen for use in the NCD. However, oxygen was not necessary for the detection of nitrosamines and had potential to react with other compounds, increasing detector interferences. Inert helium was investigated as a replacement. A repeated study of varied pyrolysis temperatures with helium showed that detector response was not affected by the substitution of gases.

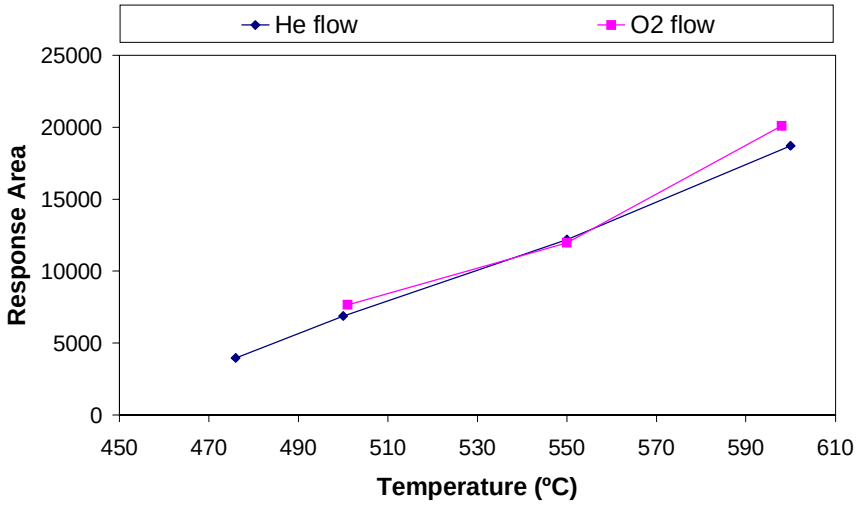


Figure 7.1 Effect of NCD pyrolysis temperature on NDMA response

Dual Column Confirmation

A two-column confirmation method was developed to confirm nitrosamine peaks in the presence of possible interferences and to eliminate false positive responses. This method is similar to that used to test for chlorinated pesticides with ECD (e.g., USEPA Method 608). Two columns of differing polarities are used, resulting in different retention times for the target compounds for each column. The changed polarity will also cause any possible interferences to elute at different times. The presence of target compounds is confirmed by the appearance of a peak at the proper retention time window for each of the two columns. If no interferences occur, the same concentration of a compound can be quantified from standards run on each column.

Four capillary chromatographic columns were tested for suitability with dual column confirmation method: 30-m DB-1701, 30-m DB-XLB, 30-m DB-210, and 60-m Supelcowax 10. The performance of the DB-XLB for the separation of the eight target nitrosamines was acceptable, but coelution of NMOR and NPYR was observed. In addition, the broad peak shape of early eluting compounds, including NDMA, made quantification of these compounds at levels less than 20 ng/L difficult. The DB-210 column performed very well for the separation of seven target nitrosamines, but did not resolve NPYR from the solvent peak and so was not suitable for the current analytical requirements. Separation of the eight nitrosamines was excellent on the DB-1701 and Supelcowax 10, and these two columns were

selected for the final confirmation method. Figure 7.2 presents the chromatograms of the target nitrosamines on the 30-m DB-1701 and 60-m Supelcowax 10. The clear change of retention time order between columns is excellent for dual column confirmation.

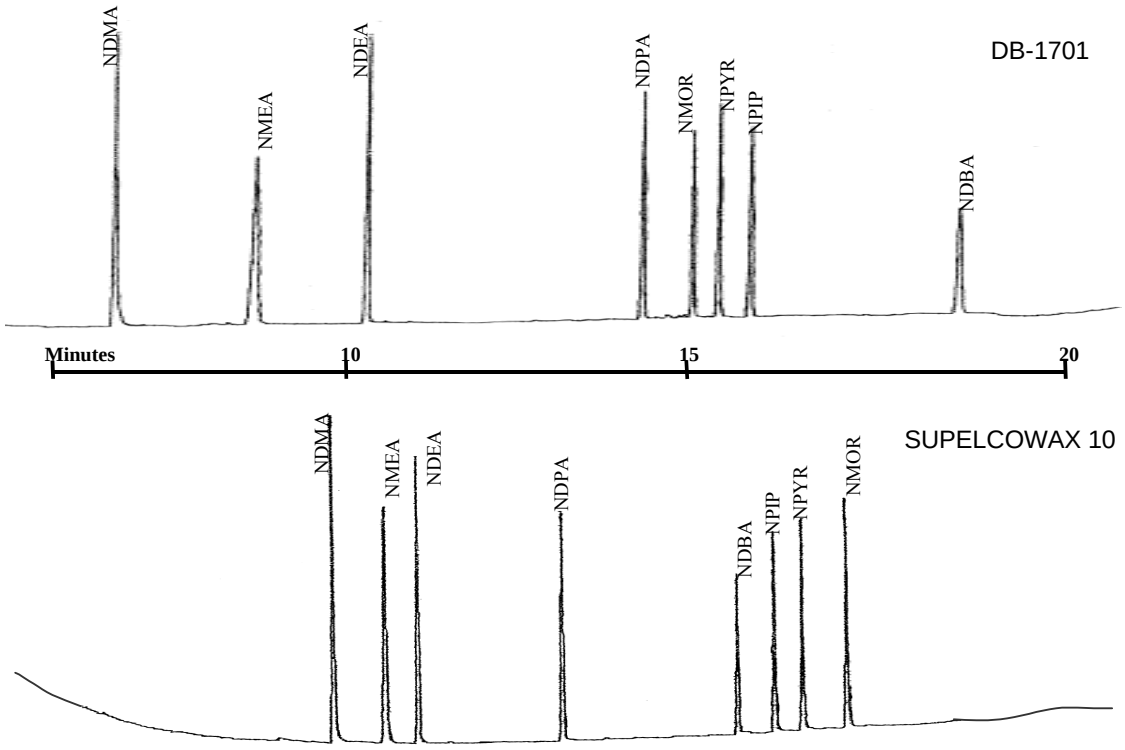


Figure 7.2 Dual column confirmation for DB-1701 and Supelcowax 10

Interferences

The NCD was operated in a low-temperature pyrolysis, nitrosamine-selective mode, meaning that there was no oxidation of nitrogen and only those compounds possessing the nitroso functional group should be detected. However, literature suggests that even low temperature pyrolysis could result in the detection of other compounds with nitrogen chemiluminescence (Fine et al., 1975b; Hansen et al., 1979; Fan et al., 1978). These compounds include nitrogen, sulfur, and double-bonded carbon compounds. A number of interfering peaks were indeed detected in wastewater samples analyzed with the nitrosamine-specific NCD modifications. The likelihood that these peaks were actually nitrosamines was small and different methods were tested to minimize the detection of these unknown compounds.

The effect of NCD pyrolysis temperature on interferences was first examined. Following this, the use of pH to minimize extraction of interferences with CLLE was evaluated. An inline nitric oxide specific molecular sieve was also examined to remove interferences prior to detection.

Effect of Pyrolysis Chamber Temperature on Interferences

The N-NO bond is relatively weak, with a bond energy of 5 to 12 kcal/mole. This is much lower than most other chemical bonds present. It was thought that decreasing the pyrolysis temperature below the optimized value of 550 °C might be more appropriate to break the N-NO bond while not providing enough energy to degrade any compounds that might be present in addition to nitrosamines. The response of two unknown peaks, retention times of 5.13 and 16.53 minutes, were selected and compared to the detector response to NDMA at a variety of temperatures on the DB-1701 column. The results can be seen

in Figure 7.3. At the pyrolysis temperatures tested, increasing temperature did not have a significant effect on the magnitude of the two unknown peaks, while a significant increase in NDMA response was observed. Therefore, decreasing pyrolysis temperature would not be sufficient to minimize interferences, while allowing for good NDMA detector response.

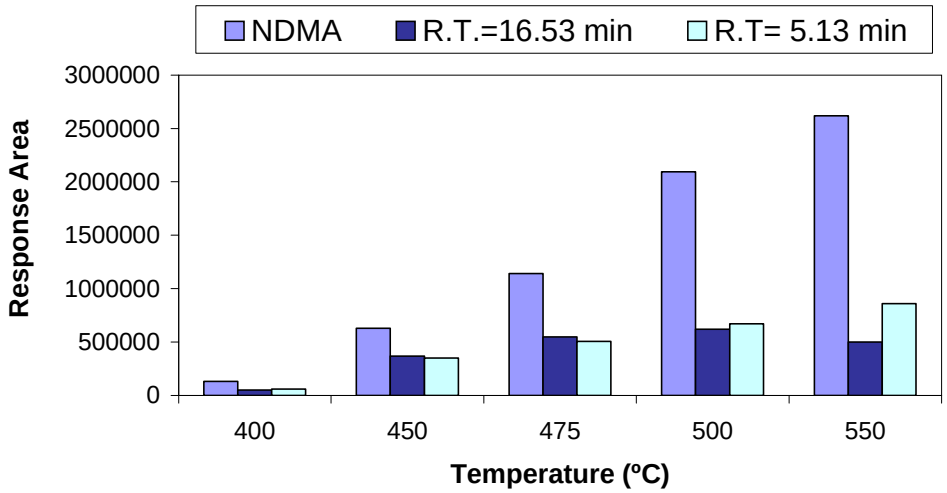


Figure 7.3 Effect of pyrolysis temperature on NCD interferences

Effect of CLLE pH

The effect of pH on minimizing interferences, (e.g., lowering the number of peaks detectable by the NCD) was evaluated. Figure 7.4 presents NCD chromatograms from 1-L CLLE samples of tertiary effluent concentrated 1,000 times, completed under acidic, basic, and neutral conditions, respectively. Region A of the chromatograms contains NDMA and region B includes the elution times of some of the later eluting nitrosamines, such as NDPA, NMOR, NPYR and NPIP. The basic extraction provided the cleanest results for region A. However, the acidic and neutral extractions would also be suitable for quantifiable analysis, though more peaks are present in this region. Region B of the basic extraction, however, is the worst of the three extractions. A number of peaks eluted in this area, including several that coelute, creating broad peaks which could significantly hinder accurate quantification. The acidic and neutral extractions are much better in this region, with the neutral extraction appearing slightly preferable. Neutral conditions therefore, appear most suitable to use with the NCD.

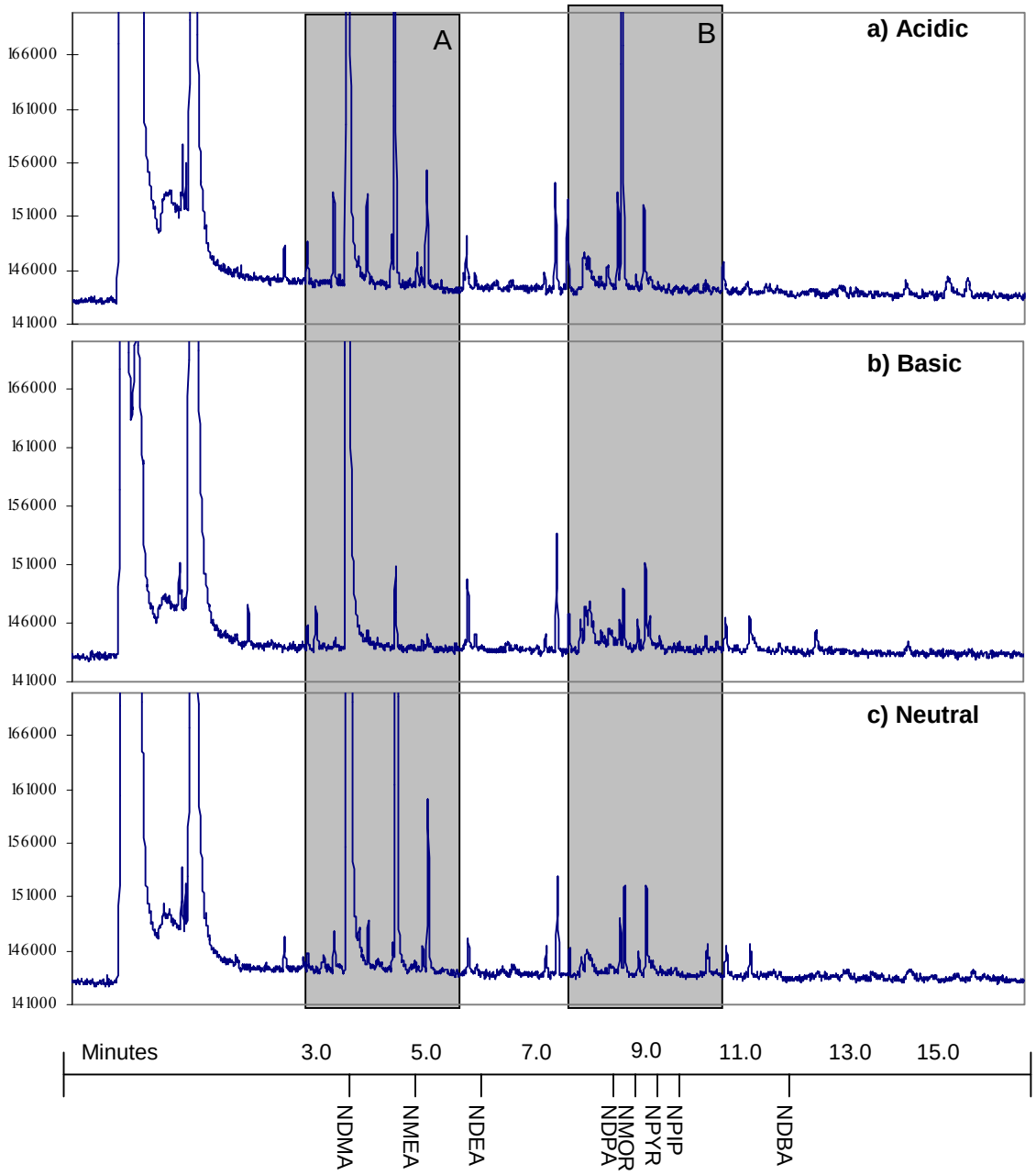


Figure 7.4 Effect of CLLE pH on NCD quantitation for tertiary wastewater effluent

Inline Nitric Oxide Selective Trap to Reduce Interferences

The NCD pyrolyzed all nitrosamine compounds and generated nitric oxide. A nitric oxide selective trap was tested to selectively remove hydrocarbons and sulfur compounds which can also chemiluminesce with ozone, potentially generating interferences. Only those compounds which release NO or NO_2 will pass through the trap to be detected. A tertiary effluent sample was injected with and without the trap. When the nitric oxide selective trap was added, many of the chromatographic peaks were removed, and nitric oxide or nitrogen dioxide was released when pyrolyzed (Figure 7.5). Nitrosamine standards injected with and without the trap showed no effect on the magnitude of detector response. However, the

interaction of the gaseous sample with the sieve material caused broadening of the chromatographic peaks. This effect made integration of very small nitrosamine peak areas difficult and caused a slight rise in the detection limits of the NCD as shown in Table 7.3. Only certain types of water samples were found to require use of the inline trap. The trap was unnecessary for analyzing drinking water samples which were not found to contain significant interferences. Some types of wastewater required interference clean-up, including influent wastewater samples; however, the trap was not necessary for all wastewaters. Inclusion of the trap on the NCD for the analysis of wastewater-type samples should be used as necessary for a particular set of samples. Therefore, site specific evaluations with and without the trap are necessary to evaluate particular interferences.

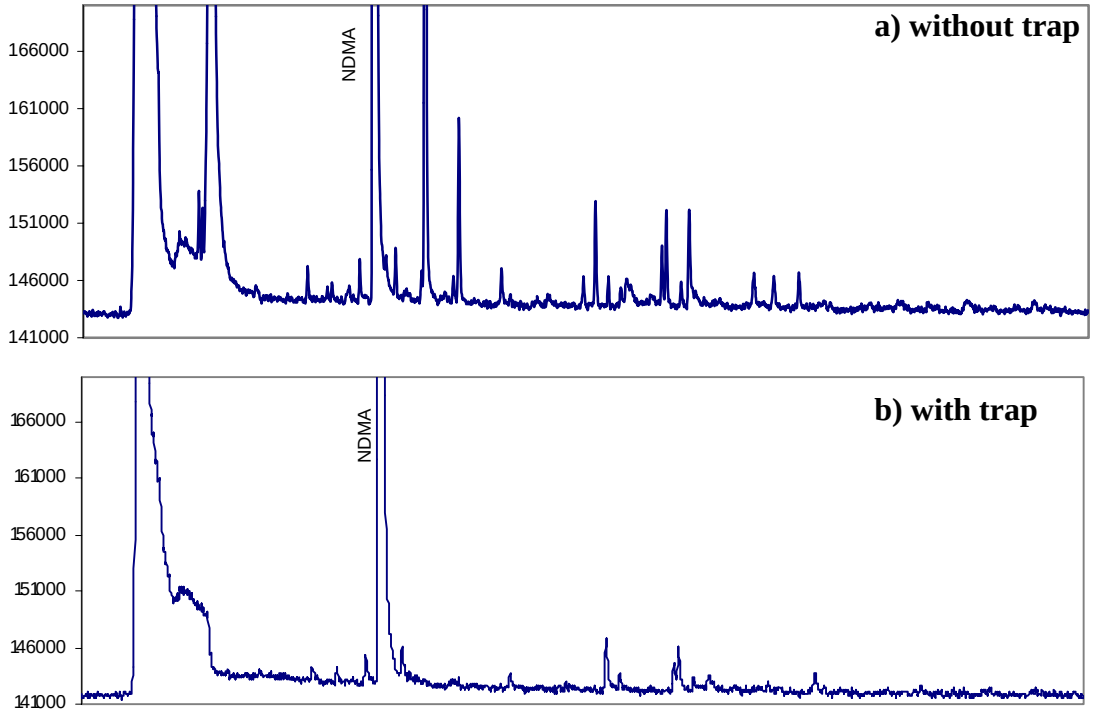


Figure 7.5 Effect of inline NO-selective trap on NCD quantitation for tertiary wastewater effluent

Extraction Comparison

Two extraction methods, CLLE and Amb SPE, were compared in the analysis of spiked surface water and secondary effluent samples to confirm adequate performance with the NCD. To generate the calibration curve for Amb SPE samples, standards were spiked into water and extracted as samples. To calibrate the CLLE results, standards made in the extraction solvent, DCM, was used. Table 7.3 shows the results of the extraction comparison for both techniques. The inline trap was used with the NCD for the analysis of the CLLE samples. NCD data are compared to the results of an interlaboratory study, utilizing several extraction methods and a variety of GC/MS detection methods. All data points from the NCD were subjected to the outlier test at the 5% significance level suggested in the American Society for Testing Materials (ASTM) Standard Practice D2777-86 (ASTM, 1994).

The clean-up method used with the NCD was effective on even complex sample extraction by CLLE. The results indicate either extraction method, CLLE or SPE, could be used in conjunction with this detector.

Table 7.3 Extraction method results comparison

		NCD		Interlaboratory results	
Sample	**Compound**	**CLLE (ngL)**	**SPE (ng/L)**	**Mean (ng/L)**	**S.D. (ng/L)**
Potable Water, Spiked	NDMA	11	20	24	14
	NMEA	<20	9.1	15	7.0
	NDEA	13	5.5	18	12.8
	NDPA	13	18*	12	2.0
	NMOR	15	20	17	4.5
	NPYR	7.9	15	14	4.6
	NPIP	9.5	17	12	3.8
	NDBA	5.6	26	13	4.8
2° Effluent, spiked	NDMA	354	586	581	95
	NMEA	266	357	441	171
	NDEA	284	324	403	216
	NDPA	263	349	354	63
	NMOR	251	377	356	99
	NPYR	278	419	388	110
	NPIP	258	362	367	167
	NDBA	256	395	368	140

** denotes values which failed the ASTM two-sided t test for outliers*

Instrument Detection Limits

IDLs were determined independent of extraction technique and were performed according to EPA protocol 40 CFR 136 Appendix B (USEPA, 1990) by injecting a low concentration standard in DCM seven times over a three-day period. A solvent volume of 3 μL was injected into a standard GC split–splitless injector. The standard deviation of the seven duplicates was multiplied by the student t number for 99% confidence. IDLs were determined for the NCD with and without use of the nitric oxide-selective trap. IDLs without use of the trap for NDMA was 0.8 μg/L, and ranged from 0.9 μg/L to 3.6 μg/L for the other nitrosamines. IDLs with the trap were higher for all nitrosamines, increasing up to 3.3 μg/L for NDMA and between 2.3 μg/L and 6.6 μg/L for the seven other nitrosamines (Table 7.4). IDLs are a function of detector sensitivity and injection volume only and are independent of extraction method recoveries. These detection limits could be lowered further by the use of large volume injectors, such as those mentioned in previous chapters, which would increase the amount of nitrosamine mass able to be injected into the detector system.

Table 7.4 NCD instrument detection levels with and without NO selective trap

(3 μL injection volume)

Compound	IDL in solvent (μg/L)	
	No trap	*With trap*
NDMA	0.8	3.3
NMEA	0.9	4.6
NDEA	2.5	2.7
NDPA	1.7	6.6
NMOR	3.1	5.0
NPYR	2.5	5.2
NPIP	2.1	2.3
NDBA	3.6	4.3

Amb SPE-NCD Method Detection Limits

Amb SPE was used with the NCD as an alternative extraction method to the CLLE. A 500 mL water sample was extracted and concentrated down to 400 μL of DCM (concentration factor = 1,250). Table 7.5 lists the MDLs determined for the Amb SPE-NCD method. Seven standards were made with reagent water spiked at a concentration of 20 ng/L nitrosamines, and no surrogate compounds were added. These samples were extracted and analyzed over a period of three days. Injection of 3 μL extracted solvent was performed manually. Nitrosamine MDLs range between 6.5 ng/L for NDMA and 22.1 ng/L for NDPA. These detection limits are not suitable for drinking water analysis, which may require detection capabilities of less than 10 ng/L. However, similar to IDLs, MDLs could be lowered significantly by using a large volume injector and standard deviation may be improved with the use of an autosampler. The lack of extraction surrogates in these samples, combined with the relatively poor NDMA through Amb SPE (60%, see Chapter 4), and the resulting effect on accuracy and precision may significantly affect MDLs. It may be expected that these MDLs would be lowered by the addition of a surrogate.

GC/CI/MS/MS quantitation with the same extracts resulted in MDLs of 2 ng/L for NDMA and 2 to 4 ng/L for other nitrosamines. However, due to equipment availability, the maximum injection volume used with the NCD and NPD (3 μL) was less than injection volumes used to determine MS detection limits (8 μL). With equipment modifications allowing for comparable injection volumes, NCD detection limits for NDMA might potentially be decreased to 2 ng/L.

Table 7.5 Amb SPE/NCD MDLs

Sample = 500 mL, CF = 1250, Injection vol = 3 μL

Compound	NCD (ng/L)		
	Avg.	**Stdev.**	**MDL**
NDMA	14	2.1	6.5
NMEA	24	6.3	20
NDEA	36	6.1	19
NDPA	37	7.0	22
NMOR	23	4.5	14
NPYR	24	2.6	8.3
NPIP	28	3.2	9.9
NDBA	27	1.8	5.7

Calibration

Calibration curves were generated for the Amb SPE-NCD method to check linearity. Nitrosamine standards from 10 to 500 ng/L were made in reagent water, extracted, and analyzed by NCD. The extraction surrogate, NDPA-d_{14}, was spiked into all standards prior to extraction. Calibration curves showed excellent linearity for all eight nitrosamines within the tested range, with coefficients of determination (r^2) values of 0.998 or better (Figure 7.6).

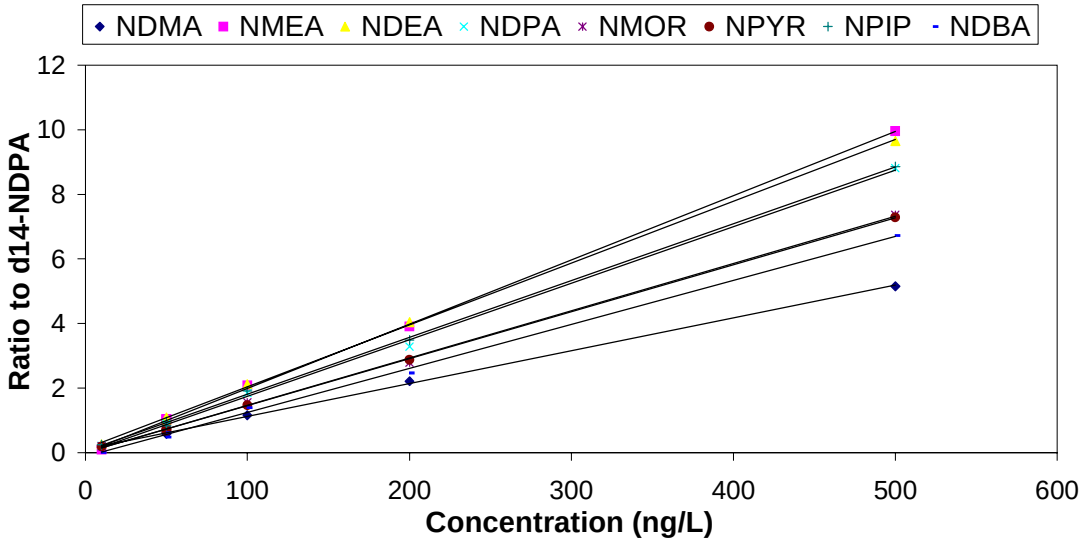


Figure 7.6 SPE extracted calibration curves for NCD (10 to 500 ng/L)

NCD Summary

This study has shown that:

- Dual column confirmation using 30-m DB-1701 and a 60-m Supelcowax 10 capillary columns provides reliable NCD results confirmation without the need for a mass spectrometer;
- Neither decreased NCD pyrolysis temperature, nor varying pH of CLLE is effective to minimize NCD interferences;
- Inline nitric oxide-selective trap effectively removed NCD interferences;
- CLLE with interference removal and Amb SPE can be used with NCD analysis for nitrosamines;
- IDLs without NO-selective trap are 0.8 µg/L for NDMA and 0.9 µg/L to 3.6 µg/L for other nitrosamines. IDLs with trap are 3.3 µg/L for NDMA and 2.3 µg/L to 6.6 µg/L for other nitrosamines. All IDLs were established for 3 µL solvent injection;
- MDLs for Amb SPE method are 6.5 ng/L for NDMA and 5.7 ng/L to 22.1 ng/L for other nitrosamines with 3 µL solvent injection;
- Use of a large volume injector may result in lowered detection limits for the NCD.

Nitrogen Phosphorus Detector (NPD)

Introduction

A Nitrogen Phosphorus Detector was selected as a low cost alternative to mass spectral detection for the analysis of nitrosamines in water. This nitrogen-specific detector is based on the thermionic emission of electrons from a heated surface. A mixture of H_2 and air creates a chemically active boundary layer near the hot surface of the source bead. Within this boundary layer, organic nitrogen compounds are decomposed into small electronegative fragments. These fragments interact with the alkali embedded source bead, becoming anions. A positively-charged ion collector captures these anions and generates a current that is then measured by the detector's electrometer (Kolb et al., 1977; Fujii and Arimoto, 1985; USEPA, 1982).

The objective of this study was to determine if the NPD could be successfully used for the analysis of nitrosamines at low ng/L aqueous concentrations in a range of water types, from clean matrices of drinking water to the complex chemical matrices of wastewater. To achieve this goal, the NPD were first optimized for nitrosamine response. Interferences were minimized by the selection of proper extraction conditions. Finally, IDLs were established in addition to MDLs with Amb SPE.

Experimental

Chemicals

A nitrosamine standard containing NDMA, NDPA, NMOR, NPYR, NPIP, NDBA, NMEA, and NDEA obtained in DCM (Ultra Scientific, RI) was used to optimize detector response. NDPA-d_{14} (Cambridge Isotope Laboratories, MA) was selected as an extraction surrogate standard. All standards were stored in amber vials in a refrigerator set to –10 °C. Standards stored for longer than six months showed no apparent loss or degradation.

Equipment

The Hewlett Packard NPD was operated on a HP 6890 Gas Chromatograph. The ceramic source bead was a TID-4 nitrogen specific thermionic source (DETector Engineering & Technology, Inc., CA). Integration and data collection were provided by a HP 3396 Series II Integrator slaved to a computer operating HP Peak 96 software. The standard split–splitless inlet was used with a gooseneck splitless insert (Agilent, CA). Inlet temperature was set at 220 °C. Helium, at a flowrate of 3 mL/min, was used as the carrier gas through the capillary column. Purge time was set for 1 minute with a purge flow of 60 mL/min.

Two capillary GC columns, DB-1701 and a Supelcowax 10, were used for primary analysis and confirmation (see Chapter 7, Nitrogen Chemiluminescence Detector, Results and Discussion). Column and oven parameters are listed in Table 7.6.

Table 7.6 Column and oven parameters for DB-1701 and Supelcowax 10

Column	DB-1701	Supelcowax 10
Column length (m)	30	60
Column ID (mm)	0.32	0.25
Column film (μm)	1.0	1.0
Column flow (mL/min)	3	3
Detector type	Hewlett-Packard 6890 Nitrogen Phosphorus Detector	
Inlet type	Split–splitless Manual	Split–splitless Manual
Injection volume (μL)	3	3
Temp (^{o}C)	220	220
Purge time (min)	1	1
Purge flow (mL/min)	60	60

Nitrosamine Retention Time (Min)		
Compound	**DB-1701**	**Supelcowax 10**
NDMA	7.77	9.22
NMEA	9.66	9.85
NDEA	11.17	10.24
NDPA-d_{14} (IS)	14.78	11.93
NDPA	14.91	12.02
NMOR	15.61	15.53
NPYR	15.96	14.96
NPIP	16.39	14.58
NDBA	19.21	14.22

Column Temperature Program							
DB-1701				**Supelcowax 10**			
Temp. (^{o}C)	**Rate (^{o}C/min)**	**Hold time (min)**	**Total time (min)**	**Temp. (^{o}C)**	**Rate (^{o}C/min)**	**Hold time (min)**	**Total time (min)**
40	0	1		40		1	
150	7	3		250	10	5	27
250	25	2	26				

It has been previously noted that use of chlorinated solvents with the NPD may be problematic (DETector Engineering and Technology, 2003). Interactions of solvents such as DCM with the NPD source bead typically cause a sharp increase in the baseline, followed by a slow drift back to the original baseline level. During this period, detector sensitivity to analytes may be increased. Because of this effect, some analytical methods using the NPD call for solvent exchange into a nonchlorinated solvent prior to injection on the NPD.

In this work, it was found that this solvent effect was generally decreased by conditioning a new bead with multiple injections of DCM over a period of several days prior to injection of samples. The extent of baseline drift was also found to vary between different source beads of the same type. On a daily basis, an injection of DCM was made prior to any calibration or sample injections. Additionally, the detector was recalibrated daily. All calibration standards were made in dichloromethane. Although there was tailing of the solvent peak, it was minimal and the baseline returned to a stable point before the elution of any analyte compounds. After experimenting with these various steps, it appeared that the use of DCM would not hinder analysis and thus the added time and cost of solvent exchange would not be necessary.

NPD Optimization Experiments

Because the efficiency of the NPD operation is dependent on maintaining the heat of the detector, parameters affecting the temperature were optimized. Parameters optimized and the conditions tested are listed in Table 7.7. All tests were made with 1-μL injection of 100 μg/L nitrosamines. Carrier and make-up gas He flows cool the source bead, decreasing the ionization efficiency of the alkali catalyst. Make-up gas flows were tested over a range of 3 to 10 mL/min. Carrier gas flows were tested from 1 to 4 mL/min. It was necessary to determine the correct combination of hydrogen and air to maximize the degradation of nitrosamines. H_2 flow of 4 mL/min resulted in the most stable detector response at a range of air flows, which were tested over a range of 45 to 70 mL/min.

Table 7.7 Optimization parameters for NPD quantitation

Parameter	Range
Detector temperature (^oC)	200, 250, 300
He carrier gas flow (mL/min)	1, 2, 3, 4
He make-up gas glow (mL/min)	3, 5, 7, 10
Air flow (mL/min)*	45, 50, 60, 70

*H_2 Flow constant = 4 mL/min

SPE Cartridge Pre-cleaning of CLLE Extract

A method utilizing prepacked SPE cartridges was developed to remove non-nitrosamine contaminants from CLLE extracts prior to injection onto the gas chromatograph. All samples were extracted as described in Chapter 7, Nitrogen Chemiluminescence, Experimental. Two wastewater samples were tested, tertiary effluent treated by flocculation, activated sludge, dual-media gravity filtration and chlorination, and a backwash sample taken during the cleaning cycle of dual-media filters. Water samples were first extracted and concentrated using the CLLE method.

SPE cartridges with 1.5 mL total volume and 100 mg of solid phase were obtained from Alltech (Lexington, KY). Two solid phases were tested, including C_8 and silica. The SPE cartridge was connected to a glass air-tight syringe by Teflon tubing. To pre-clean the cartridges, 10 mL of DCM was filtered through the cartridge at a rate of approximately 2 mL/min. The syringe was removed and emptied when the solvent covered the top of the media. A clean 5 mL glass air-tight syringe was used to draw each sample. A CLLE sample of 1.5 mL volume was added to the cartridge. To ensure that the sample had completely passed through the cartridge and tubing into the syringe, an additional 5 mL of DCM was filtered through the cartridge. The final volume of approximately 6.5 mL was concentrated back to the initial sample volume using nitrogen evaporation with small volume concentration glassware (e.g., 10 mL Kuderna-Danish vials and Snyder columns). Blanks were obtained for each solid phase. Method recoveries were determined in duplicate, running a 1 mg/L nitrosamine standard through each solid phase and comparing the response to the original standard.

Results and Discussion: NPD Optimization

Because the efficiency of the NPD operation is dependent upon maintaining the heat of the detector, those parameters affecting the temperature were optimized. Maximum detector temperature minimizes the temperature difference between the source bead and the detector casing and helps to stabilize the source bead temperature. Temperatures from 200 °C to 300 °C were tested. As expected, the maximum detector temperature, 300 °C, resulted in the highest response. The results for NDMA and NDPA are shown in Figure 7.7. All nitrosamines followed similar optimization trends.

Carrier and make-up He flows cool the source bead, decreasing the ionization efficiency of the alkali catalyst. Make-up gas flows were tested over a range from 3 to 10 mL/min. The optimum flow was 5 mL/min (Figure 7.8). Carrier gas flows were tested from 1 to 4 mL/min. It was seen that lower carrier gas flows increased response, but the broadened peak shape associated with low flows made integration difficult at low levels. A flowrate of 3 mL/min was chosen to maintain sharp peak shape with minimal cooling of the detector.

It was necessary to determine the correct combination of hydrogen and air to maximize the degradation of nitrosamines. A H_2 flow of 4 mL/min resulted in the most stable detector response. Air flows were tested over a range of 45 to 70 mL/min. Figure 7.9 presents the results of this trial for NDMA.

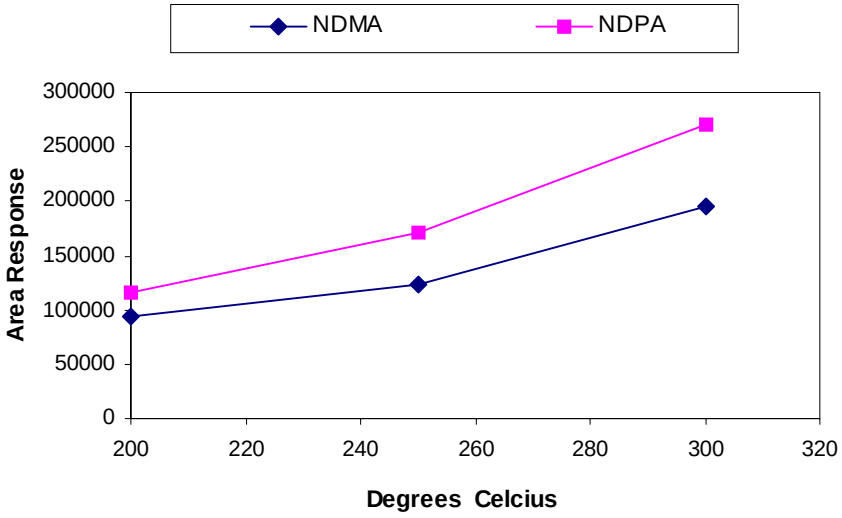


Figure 7.7 Effect of NPD detector temperature on NDMA and NDPA area response

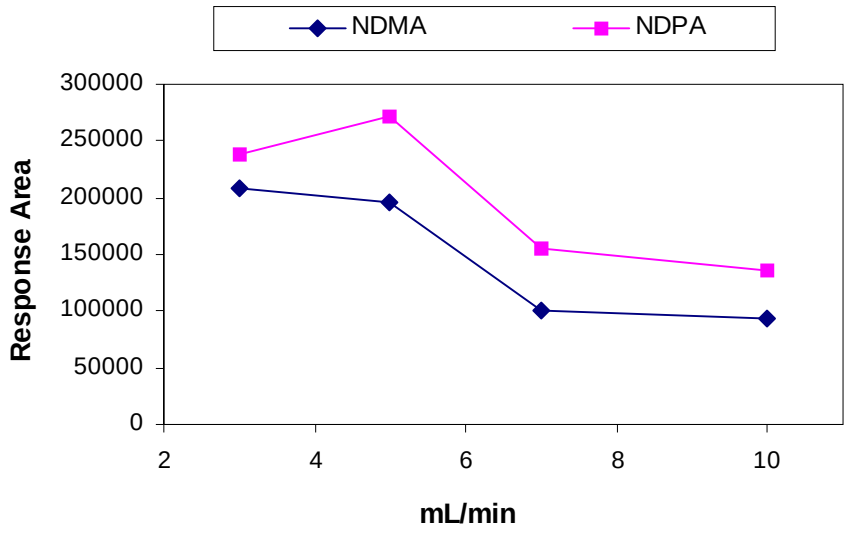


Figure 7.8 **Effect of NPD make-up gas flow on NDMA and NDPA area response**

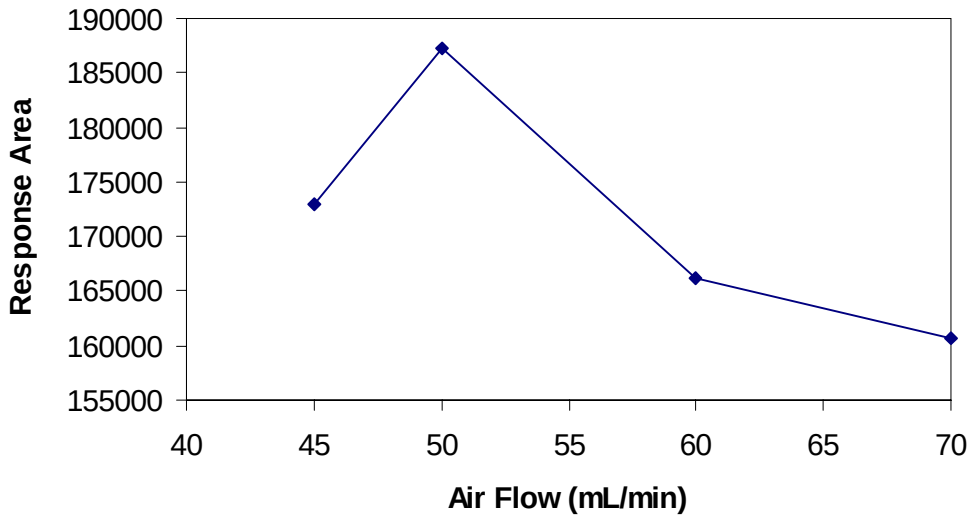


Figure 7.9 **Effect of H_2 to air ratio on NDMA area response**
(H_2 flow = 4 mL/min)

Interferences

The ability of the NPD to detect all nitrogen compounds in a sample makes it a flexible analytical detector. However, wastewaters have been shown to contain high concentrations of a wide variety of nitrogen-containing compounds, and the broad detection can be problematic. The low selectivity of CLLE, currently the most common extraction technique used for nitrosamine analysis, is a drawback when using this technique coupled with the NPD. In order to minimize NPD interferences, modifications to the CLLE method were explored, including varying extraction pH and clean-up of the CLLE extract prior to GC injection. Due to the varying nature of wastewater, the need for sample clean-up with the NPD for these samples would need to be determined for a particular sample set by initial testing of the water matrix.

Effect of CLLE pH

Tertiary wastewater effluent samples were concentrated by CLLE according to USEPA method 3520C. The water sample pH was adjusted prior to solvent extraction. Basic (>12), Acidic (pH<2) and Neutral (pH 6 to 8) conditions were tested.

Figure 7.10 show the NPD chromatograms of extractions performed under acidic, basic and neutral conditions, respectively. Region A, containing NDMA and NMEA, of the basic extraction is the cleanest, and all chromatograms are quantifiable in this region. Region B, containing NDPA, NMOR, NPYR, NPIP, is problematic at all three pHs. The coelution of many peaks, causing broad humps which significantly distort the baseline, can be seen, particularly under basic and neutral conditions. This would significantly impair quantification, and thus acidic extraction conditions are recommended for the analysis of all eight nitrosamines using recycled CLLE for the NPD.

Although results obtained during this study could vary using different source waters, or the same source water sampled at different times, the general conclusion can be made that pH will have only a minimal effect on the extraction of interferences by recycled CLLE.

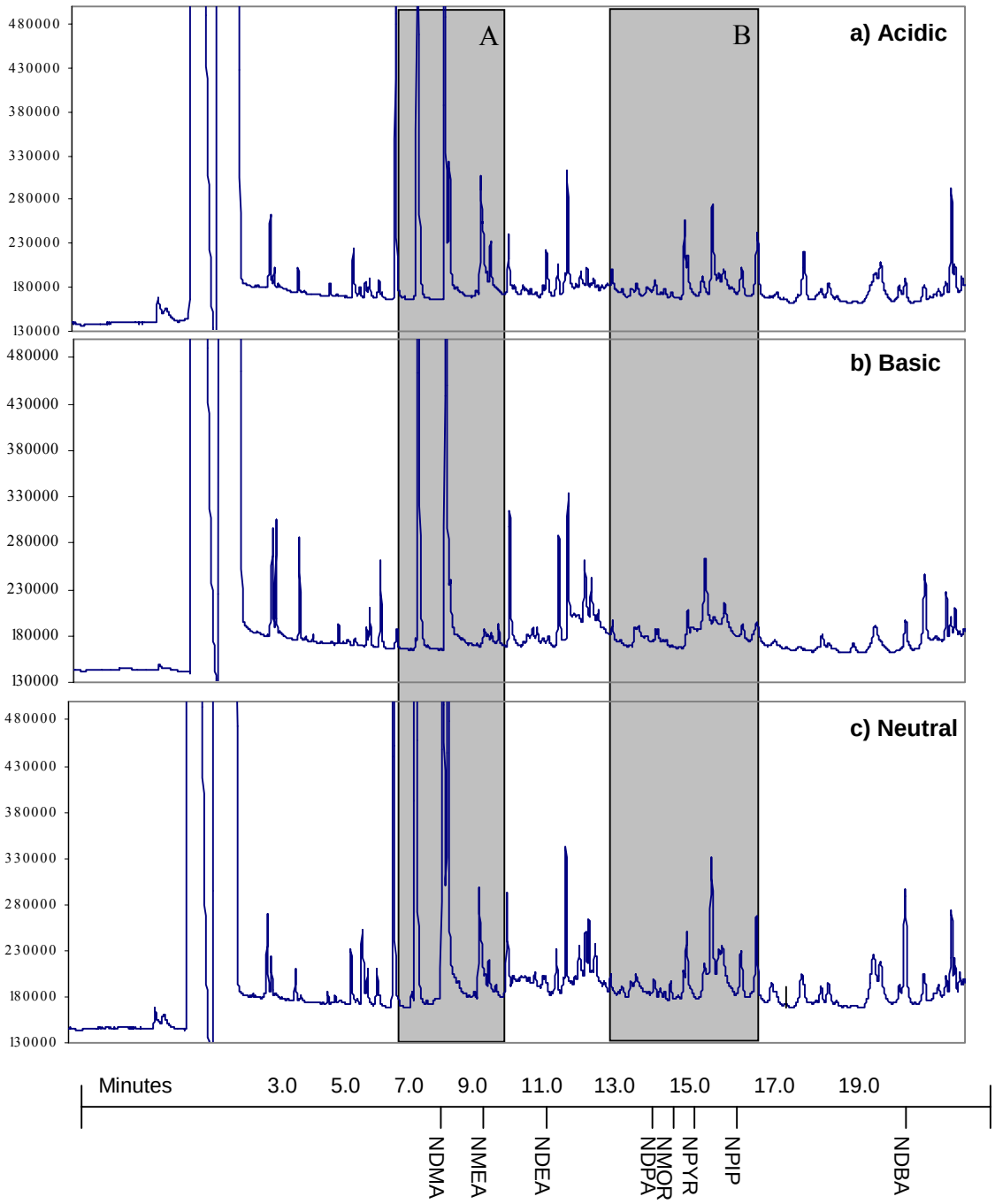


Figure 7.10 Effect of CLLE pH on NPD quantitation of tertiary wastewater effluent

SPE Cartridge Pre-cleaning of CLLE Extract

A method utilizing prepacked SPE cartridges was developed to remove non-nitrosamine contaminants from CLLE extracts prior to injection onto the gas chromatograph. Tertiary wastewater effluent and filter backwash samples were first extracted and concentrated using normal recycled CLLE conditions, followed by passage through an SPE cartridge to remove interferences from the extract solvent.

Initial results from the NPD clean-up study indicate this method has good potential for use with CLLE and other nonselective extraction methods. The SPE clean-up method appeared to be more effective for the clean-up of tertiary effluent than for the filter backwash sample. Figure 7.11 presents the NPD chromatograms of tertiary effluent, before and after clean-up. A great reduction in the number of peaks is evident and the clean-up method seems to function well for the type of incident compounds present. Figure 7.12 presents effects of clean-up on the chromatograms for filter backwash sample, and shows that SPE to be less effective for clean-up of this sample. There were indications that more rinsing should be performed because some blanks for both the polar and nonpolar cartridges showed indications of compounds being stripped off of the cartridge after rinsing with 10 mL of DCM. These stripped compounds caused a broad late eluting peak, which could interfere with the quantification of NDBA. Both cartridges, C_8 and silica, appeared to work equally well at removing interfering compounds. Because both solid phases worked well, this indicates that polarity might not be as important a factor in interference removal as was initially expected.

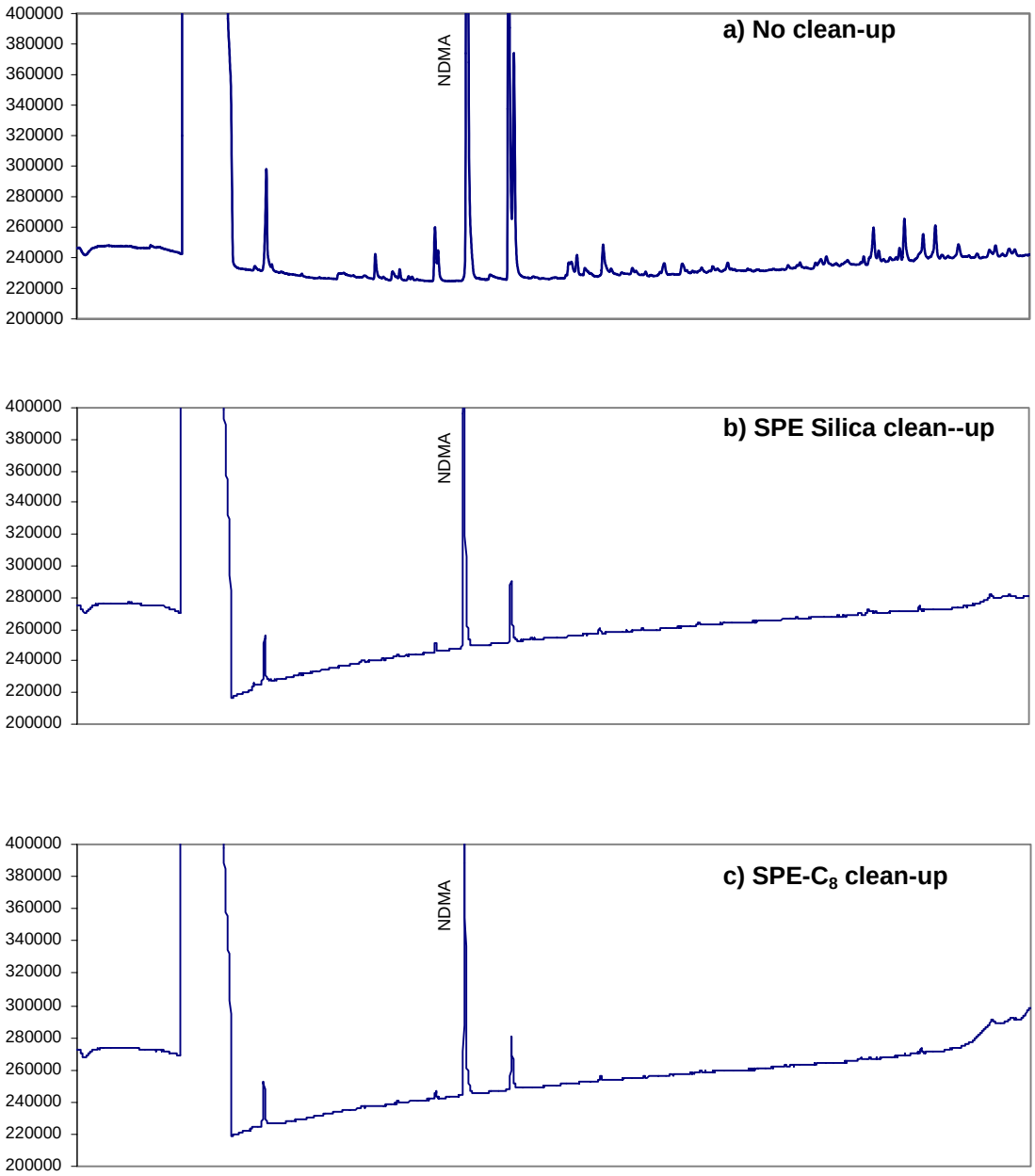


Figure 7.11 Effect of SPE clean-up on NPD quantitation of tertiary wastewater effluent

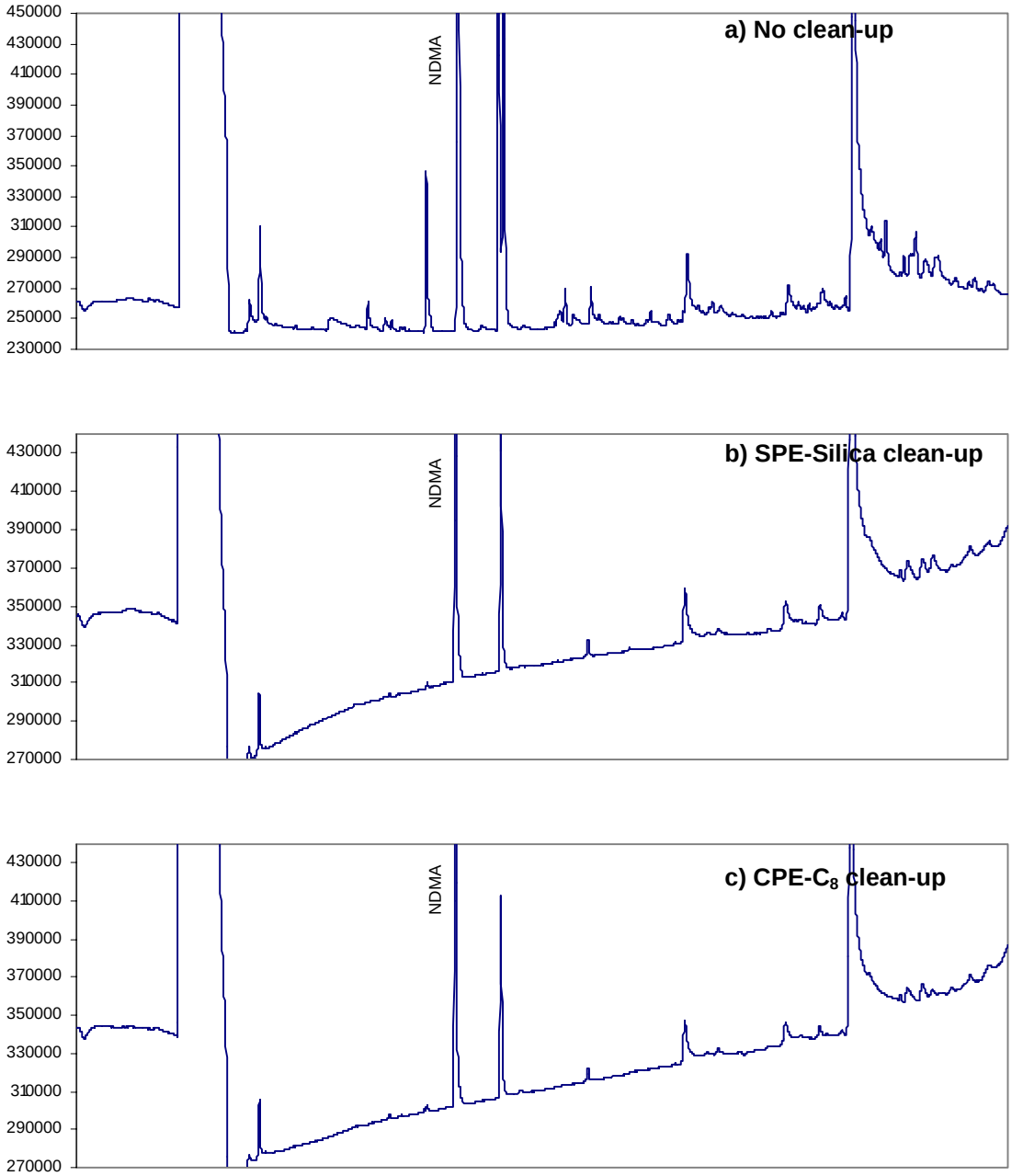


Figure 7.12 Effect of SPE clean-up on NPD quantitation of filter backwash sample

The recoveries listed for each cartridge type in Table 7.8 are the average of duplicate tests. Overall, the silica cartridge provided the best recovery of all nitrosamines with the exception of NPYR and NMOR. Recoveries from the C_8 cartridge are also good, again with the exception of NMOR. Recoveries of all analytes could possibly be improved with larger elution volumes than those tested here. Both solid phases were shown to provide excellent sample clean-up. From the recovery results, silica would appear to be the better solid phase media.

Table 7.8 Nitrosamine recoveries following SPE cartridge clean-up

Compound	C_8 Recovery (%)	Silica Recovery (%)
NDMA	80	84
NMEA	82	87
NDEA	82	88
NDPA	85	92
NDBA	94	100
NPIP	81	84
NPYR	84	60
NMOR	66	55

Extraction Comparison

Due to the additional cost and time associated with recycled CLLE and clean-up for the NPD, the Amb SPE method was also evaluated with the NPD. A potable water sample and a tertiary effluent sample were spiked with the eight nitrosamines and extracted with CLLE and Amb SPE. To generate the Amb SPE calibration curve, standards were spiked into water and extracted as samples. To calibrate the CLLE results, standards made up in the extraction solvent, DCM, were used. No extract-cleaning steps were taken for either extraction technique.

Table 7.9 shows the results of the extraction comparison. The results from the NPD are compared to the results of the interlaboratory round-robin testing, utilizing several extraction methods and a variety of GC/CI/MS/MS setups for detection. All data points from the NPD were subjected to the outlier test at the 5% significance level suggested in the ASTM Standard Practice D2777-86 (ASTM, 1994).

The multiple outliers resulting from analyzing water samples by CLLE, in addition to a significant bias toward higher values, even in drinking water matrices, indicates that this combination of extraction and detector would not be ideal. The CLLE extract clean-up developed for the NPD could be used to improve method accuracy, however, this would cause a further increase in analytical time. For the NPD, solid–phase extraction provided more accurate results without requiring any sample clean-up steps. Of these two extraction methods, Amb SPE is the recommended extraction technique to use in conjunction with the NPD.

Table 7.9 Extraction method results comparison for NPD
(all values in ng/L)

Sample	Compound	NPD		Interlaboratory results	
		CLLE	SPE	Mean	S.D.
Potable, Spiked	NDMA	65*	17	24	14
	NMEA	37*	15	15	7.0
	NDEA	32	12	18	13
	NDPA	10	12	12	2.0
	NMOR	23	17	17	4.5
	NPYR	26	25	14	4.6
	NPIP	ND	23*	12	3.8
	NDBA	21	15	13	4.8
Secondary Effluent, spiked	NDMA	748	687	581	95
	NMEA	476	271	441	171
	NDEA	437	361	403	216
	NDPA	430	297	354	63
	NMOR	497	384	356	99
	NPYR	600	408	388	110
	NPIP	504	398	367	167
	NDBA	509	350	368	140

* denotes values which failed the ASTM two-sided t test for outliers

NPD Detection Limits

IDLs were first determined according to EPA protocol 40 CFR 136 Appendix B (US EPA, 1990), by injecting a low concentration standard in DCM seven times over a three-day period. A solvent volume of 3 μL was injected into a standard split–splitless GC injector. The standard deviation of the seven duplicates was multiplied by the student t number for 99% confidence. The IDLs listed in Table 7.10 are a function of detector sensitivity and injection volume only, and are independent of extraction method recoveries.

Due to the additional cost and time associated with recycled CLLE and clean-up with the NPD, Amb SPE was also tested with the NCD. Table 7.10 also lists the MDLs determined for the Amb SPE-NPD method. Seven standards were made with reagent water spiked at a concentration of 20 ng/L nitrosamines, and extracted and analyzed over a period of three days. No extraction surrogates were added to the samples. An injection volume of 3 μL solvent was used for IDL determination.

These MDLs are not suitable for drinking water analysis, which requires detection capabilities of less than 10 ng/L. However, MDLs could be lowered significantly by using a large volume injector and standard deviation would improve with an autosampler. The IDLs could be lowered further by the use of large volume injectors, such as those mentioned in previous chapters, which would increase the amount of nitrosamine mass able to be injected into the detector system. Additionally, the lack of extraction surrogates in these samples combined with the relatively poor recovery of NDMA by Amb SPE (60%, see Chapter 4), and the resulting effects on accuracy and precision may significantly affect MDLs. It is expected that these MDLs would be lowered by the addition of a surrogate.

GC/CI/MS/MS quantitation with the same extracts resulted in MDLs of 2 ng/L for NDMA and 2 to 4 ng/L for other nitrosamines. However, due to equipment availability, the maximum injection volume used with the NCD and NPD (3 µL) was less than injection volumes used to determine MS detection limits (8 µL). With equipment modifications allowing for comparable injection volumes, NPD detection limits for NDMA might potentially be decreased to less than 5 ng/L.

Table 7.10 NPD instrument detection limits, Amb SPE-NPD MDLs

(Sample vol =500 mL, CF = 1,250, Injection volume =3 µL)

	NPD (ng/L)			
Compound	**IDL**	**Avg.**	**Std. Dev.**	**MDL**
NDMA*	5.0	13	3.5	11
NMEA**	4.0	74	4.2	13
NDEA*	3.6	56	12	38
NDPA	5.0	46	6.8	22
NMOR	4.8	40	9.5	30
NPYR*	3.7	44	13	41
NPIP	8.8	71	22	70
NDBA	8.0	42	12	37

**Due to integrator error, only six replicates were used for MDLs*

***Due to integrator error, only five replicates were used for MDLs*

Calibration

Figure 7.13 presents the calibration curves for the Amb SPE method with NPD detection. These curves were generated with extracted standards, and the extraction surrogate NDPA-d_{14} was spiked into all standards and samples prior to extraction. Curves for all eight nitrosamines were linear from 10 ng/L to 500 ng/L with coefficients of determination (r^2) values of 0.998 or better.

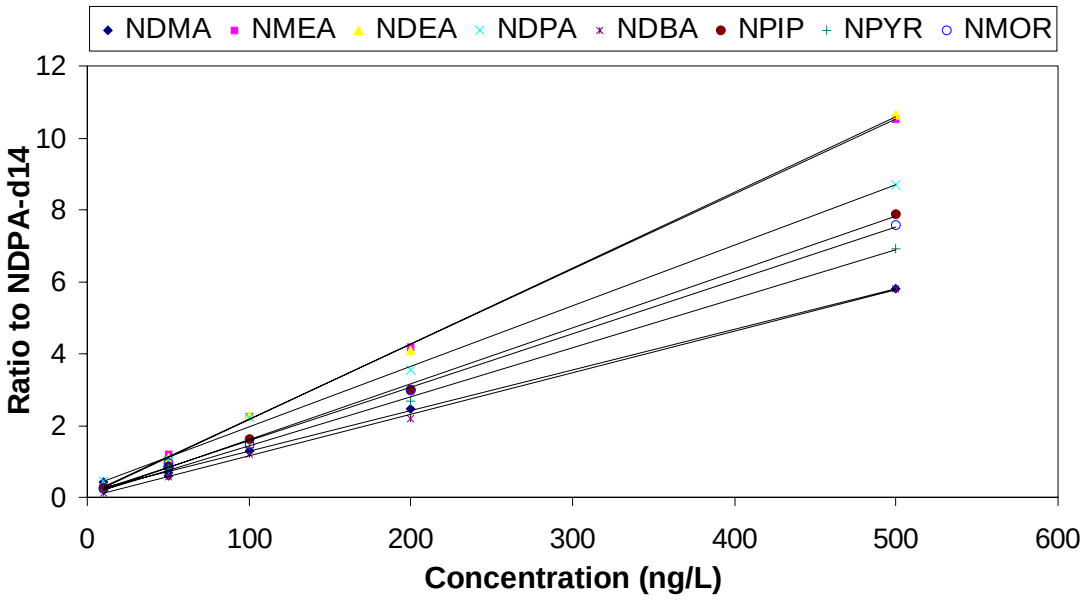


Figure 7.13 SPE calibration curves for NPD (10 to 500 ng/L)

NPD Summary

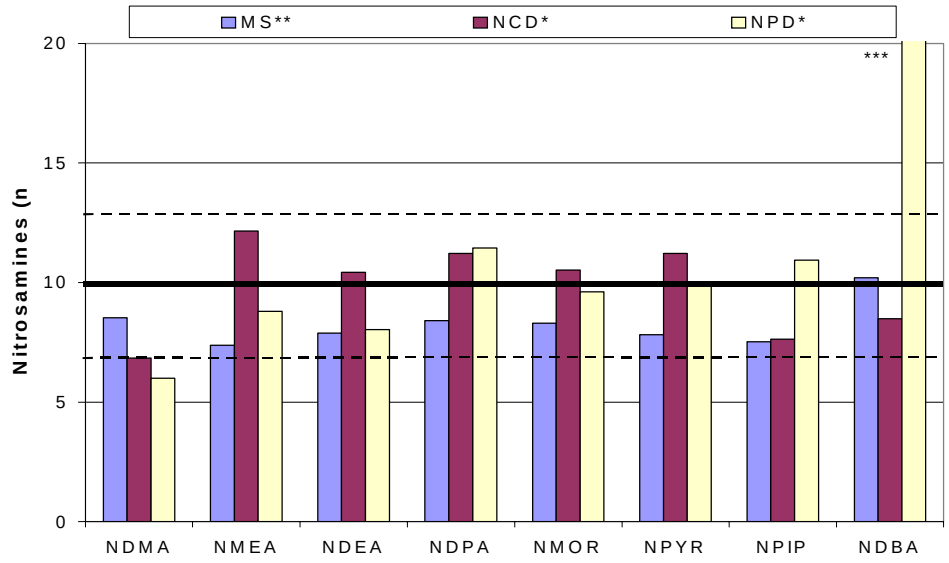
This study has shown that:

- Maximum NPD response to NDMA and other nitrosamines occurred at a detector temperature of 300 °C, carrier gas flow of 3 mL/min, make-up gas flow of 5 mL/min, and H_2 to Air ratio of 0.8 (4 mL/min:50 mL/min).
- CLLE extracted at acidic conditions provides best removal of NPD interferences.
- Both silica and C_8 prepacked SPE cartridges resulted in excellent clean-up of CLLE extracts, with recoveries of 84 and 80%, respectively, for NDMA.
- Amb SPE results in better performance with the NPD than does CLLE without extract clean-up steps.
- IDLs are 5.0 μg/L for NDMA and 3.6 μg/L to 8.8 μg/L for other nitrosamines with 3 μL solvent injection.
- MDLs for Ambersorb-SPE methods are 10.9 ng/L for NDMA and 13.3 ng/L to 70.1 ng/L for other nitrosamines with 3 μL solvent injection.
- Use of a large volume injector would result in lowered detection limits for the NPD.
- However, chlorinated solvents, such as the DCM extracts used in this study, produce not only a strong signal with the NPD or thermionic detector, but also cause a temporary enhancement of the detector sensitivity. The high recoveries in the MDL study (Table 7.10) and the comparison of results with the NCD and MS detectors (e.g., Figures 7.15 and 7.16) may be a reflection of signal enhancement. This sensitivity enhancement may be somewhat reproducible, if continuous injections are performed with the chlorinated solvent samples. However, the sensitivity enhancement may not be reproducible on a day-to-day basis, or even if the NP detector has been idle for a few hours. For this reason, analytical methods that rely on the NP detector generally require that a nonchlorinated solvent, such as hexane or MtBE, be used to prepare the calibration standards and sample extracts. If the initial sample extraction requires a chlorinated solvent, a solvent exchange to a nonchlorinated solvent is usually employed before GC analysis. Including a solvent exchange step would result in an increased cost of analysis for NDMA and the other nitrosamines, but should produce results that are more reliable and reproducible. Also, chlorinated solvents tend to decrease the lifetime of the NP bead, so avoiding the use of methylene chloride will help to extend the lifetime of the bead and save on replacement costs and downtime.

Alternative Detector Performance Comparison

Before the finalized alternative detection methods were completed, a preliminary detector comparison was performed for chlorinated surface water, spiked at 10 ng/L nitrosamines. CLLE extraction of the water sample was performed under two different conditions. GC/CI/MS/MS extractions were performed with NDMA-d_6, NDEA-$^{15}N_2$, and NDPA-d_{14} extraction surrogates. NCD and NPD extractions were performed without the addition of extraction surrogates. The lack of any surrogates for the NCD and NPD was expected to cause some decreased accuracy as compared to GC/CI/MS/MS. Figure 7.14 presents the results of this comparison. Due to variability associated with low concentration detection, the CDHS considers accuracy within plus or minus 30% (± 30%) to be reasonable. Thus, observed values that fell within ± 30% of actual value are considered acceptable.

The underestimation of NDMA, with spiked recoveries of 68 and 60% for the NCD and NPD, respectively, can be accounted for by the typically low extraction recoveries of NDMA as compared to other nitrosamines and the lack of extraction surrogates to account for these recovery losses. The NPD spike recovery of NDBA was an outlier due to column contamination during analysis which caused misquantification. All other nitrosamine results show excellent accuracy. This initial comparison indicated that both alternative detectors would be suitable for analysis of low level drinking water samples.



* No extraction surrogates used for analysis
** NDMA-d_6, $^{15}N_2$-NDEA, NDPA-d_{14} extraction surrogates
*** Outlier- column contamination caused mis-quantification

Figure 7.14 CLLE extraction of chlorinated surface water with 10 ng/L spike
Solid line = actual spike conc. (10 ng/L), dashed lines = ± 30% (13 and 7 ng/L, respectively)

To determine the suitability of the alternative detectors to analyze wastewater, secondary and tertiary effluent from two wastewater treatment facilities were analyzed. All samples were extracted with CLLE. NDMA-d_6, NDEA-$^{15}N_2$, and NDPA-d_{14} extraction surrogates were used for all samples. Although NDMA was present in all samples, it could not be quantified by NCD or NPD due to interferences caused by NDMA-d_6. NMOR was also detected and the results are shown in Table 7.11. NCD results showed excellent agreement with GC/CI/MS/MS values, however NPD results were significantly higher. The results indicate that the NCD could be used to reliably quantify NMOR in wastewaters with CLLE. However, the presence of some compound which interferes with NPD detection of this compound, suggests that using CLLE coupled with NPD may not produce reliable results, even with dual column confirmation. This agrees with the previous extraction comparison performed for the NPD, which indicated that Amb SPE was a more suitable and convenient method to use with this detector.

Table 7.11 Detector comparison for NMOR in wastewater

Sample	MS (ng/L)	NCD (ng/L)	NPD (ng/L)
Plant 1 – secondary effluent	73	75	148
Plant 1 – tertiary effluent	ND	ND	ND
Plant 2 – secondary effluent	26	23	54
Plant 2 – tertiary effluent	ND	ND	ND

A final detector comparison was performed after finalizing the detection methods. To evaluate their performance and to assess the differences between the accuracy of the three detection systems — GC/CI/MS/MS, NCD, and NPD — low and high concentration water samples were analyzed. Six water samples were tested: chloraminated potable surface water unspiked and spiked with 12.7 ng/L nitrosamines; RO effluent; secondary effluent unspiked and spiked with 376 ng/L nitrosamines; and tertiary effluent were collected and prepared as described in Chapter 8, Round-Robin Testing. Using the finalized alternative detector methods, NDPA-d_{14} was added as an extraction surrogate for NCD and NPD samples. GC/MS samples were extracted, as previously described, with three deuterated surrogates. In order to assess the impact of extraction method on detector accuracy, extracts from the CLLE and SPE method were analyzed through GC/CI/MS/MS, NCD, and NPD. Extracts from MLLE were analyzed by GC/CI/MS/MS and NCD only.

A general comparison of the three detectors' performance for NDMA analysis can be seen in Figure 7.15 and Figure 7.16, SPE and CLLE results. NDMA concentration for unspiked chlorinated surface water was below detection limits for the NCD and NPD and is not shown. Using SPE, the NCD showed excellent agreement with GC/CI/MS/MS values for NDMA in all sample types, while NPD results show a bias towards higher values. For CLLE extracted samples, the NCD values are consistently lower than GC/CI/MS/MS values, while NPD values are consistently high. Overall, NCD with Amb SPE showed the best agreement with GC/CI/MS/MS results for NDMA throughout the range of water types.

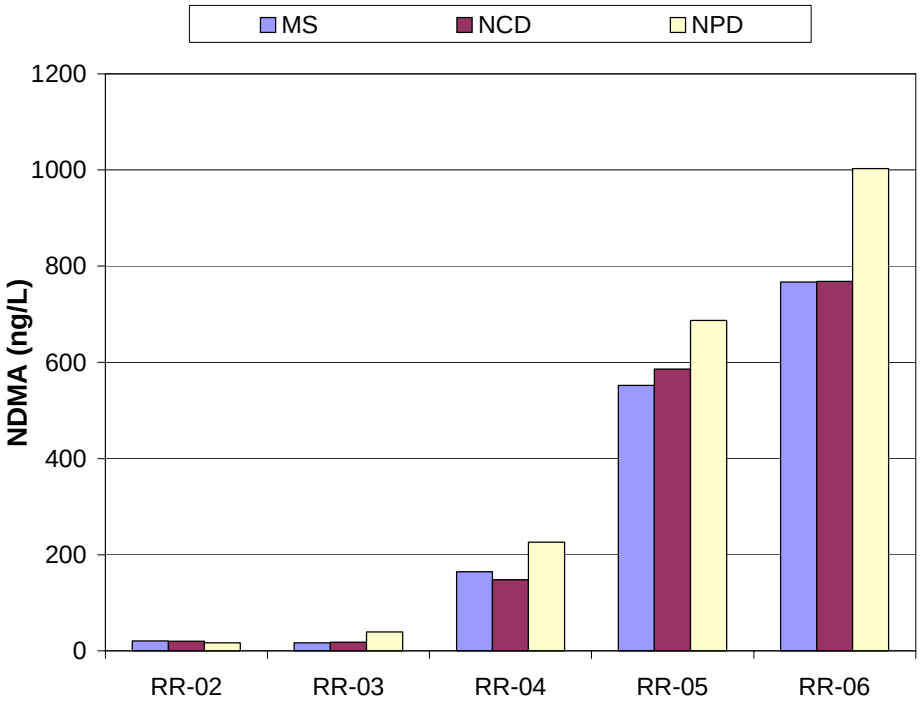


Figure 7.15 Detector comparison for NDMA in various waters, Amb SPE

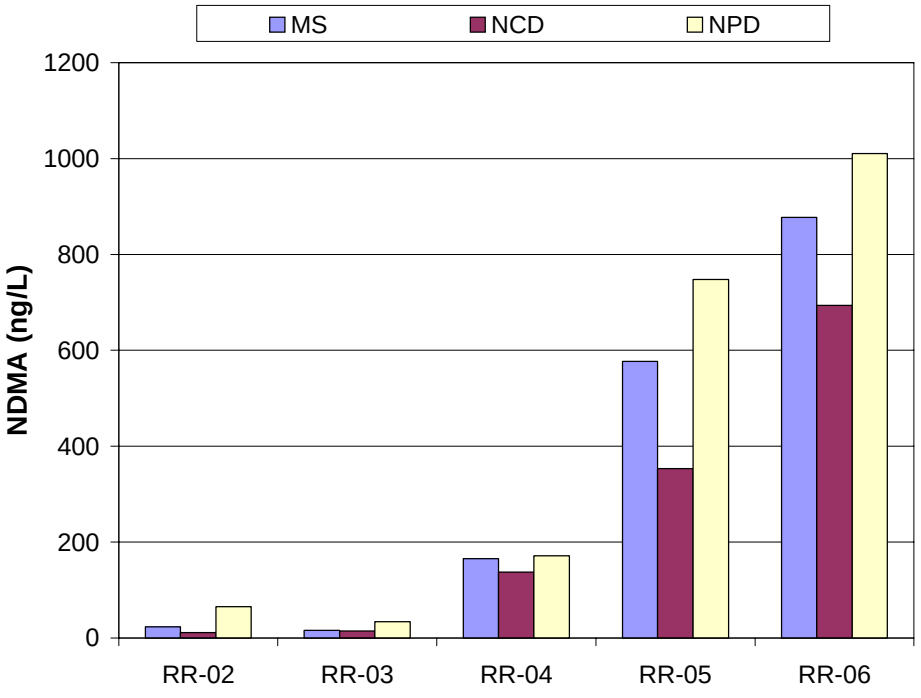


Figure 7.16 Detector comparison for NDMA in various waters, CLLE

To compare the accuracy of the three detectors for all eight nitrosamines, spike recoveries for the chloraminated surface water and secondary effluent samples were calculated. Due to the presence of NDMA in all water samples, spike recoveries were adjusted for initial NDMA concentration.

For the low concentration spike in chlorinated surface water, the GC/CI/MS/MS method was the most accurate detector overall (Figures 7.17a through 7.17i) and more importantly, the GC/CI/MS/MS was consistently within acceptable accuracy range with the exception of NMOR and NMEA. While the NCD method yielded an acceptable result for NDMA using the CLLE extract, the detected NDMA in the Amb SPE and MLLE extract was above the acceptable accuracy range. Detection of all other nitrosamines, except NMEA, NPYR, and NDBA, were within the acceptable ± 30% accuracy range when using the NCD method coupled with the CLLE extraction method. Utilizing the NPD method, the observed NDMA concentration was above the acceptable boundary for both the CLLE and Amb SPE extract (Figure 7.17a). The NPD appears to produce results that were higher than the NCD results, but overall the best performance with the NPD was provided by Amb SPE, all nitrosamines were within acceptable range with the exception of NDMA, NPYR, and NPIP. Depending on the exact nitrosamine, the results from the NCD and NPD could differ by greater than 50% from the GC/CI/MS/MS results.

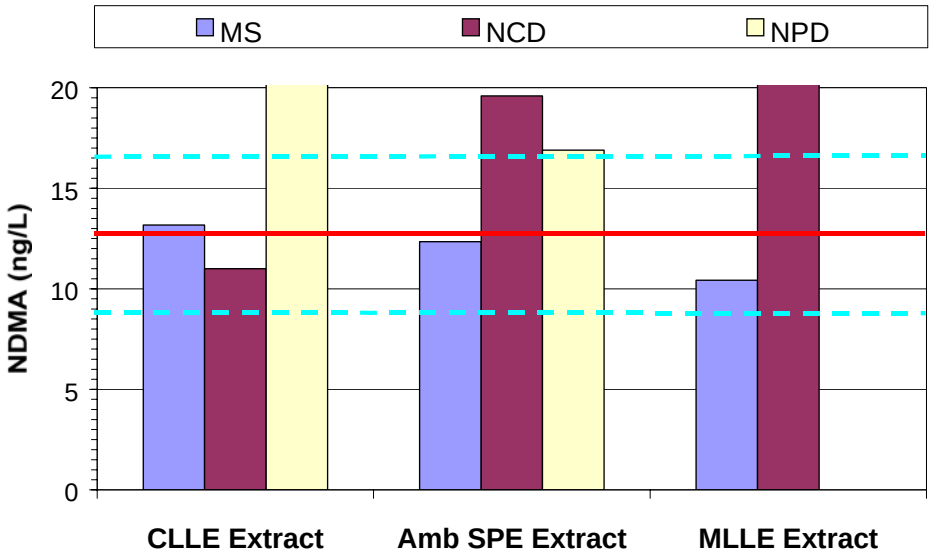


Figure 7.17a **NDMA detection accuracy for low conc. spike recovery using three detectors**
Solid line = actual spike conc. (12.7 ng/L), dashed lines = ± 30% (16.5 and 8.9 ng/L, respectively)

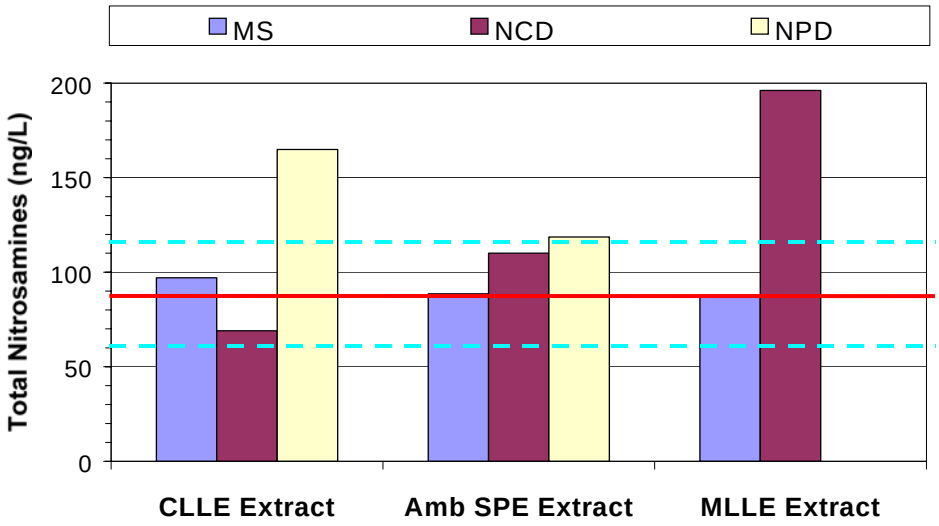


Figure 7.17b **Calculated total nitrosamine (without NDMA) detection accuracy for low concentration spike recovery using three detectors**
Solid line = actual spike conc. (88.9 ng/L), dashed lines = ± 30% (115.6 and 62.2 ng/L, respectively)

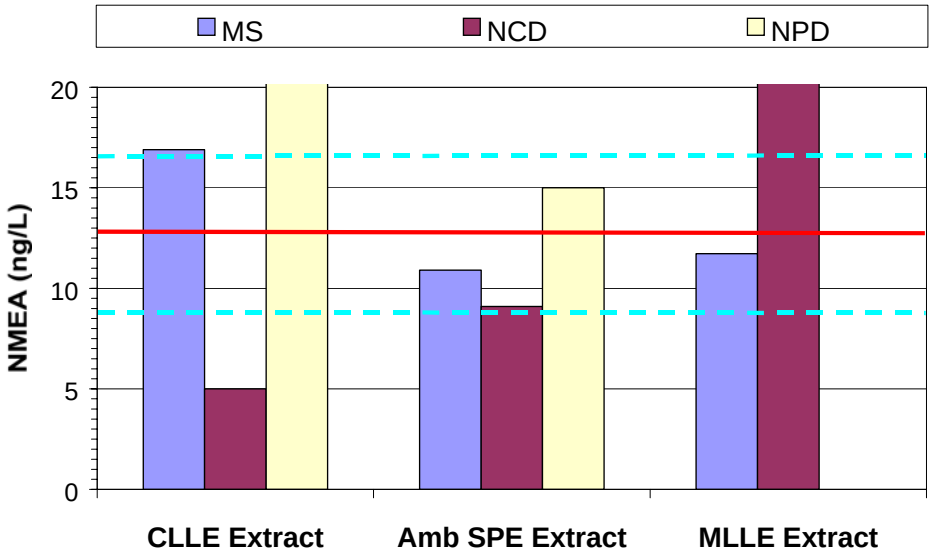


Figure 7.17c **NMEA detection accuracy for low conc. spike recovery using three detectors**
Solid line = actual spike conc. (12.7 ng/L), dashed lines = ± 30% (16.5 and 8.9 ng/L, respectively)

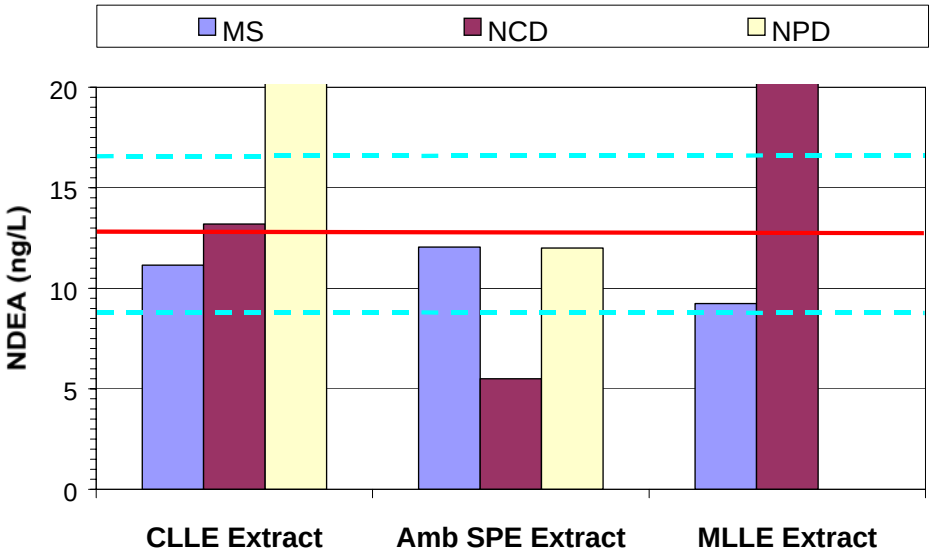


Figure 7.17d **NDEA detection accuracy for low conc. spike recovery using three detectors**
Solid line = actual spike conc. (12.7 ng/L), dashed lines = ± 30% (16.5 and 8.9 ng/L, respectively)

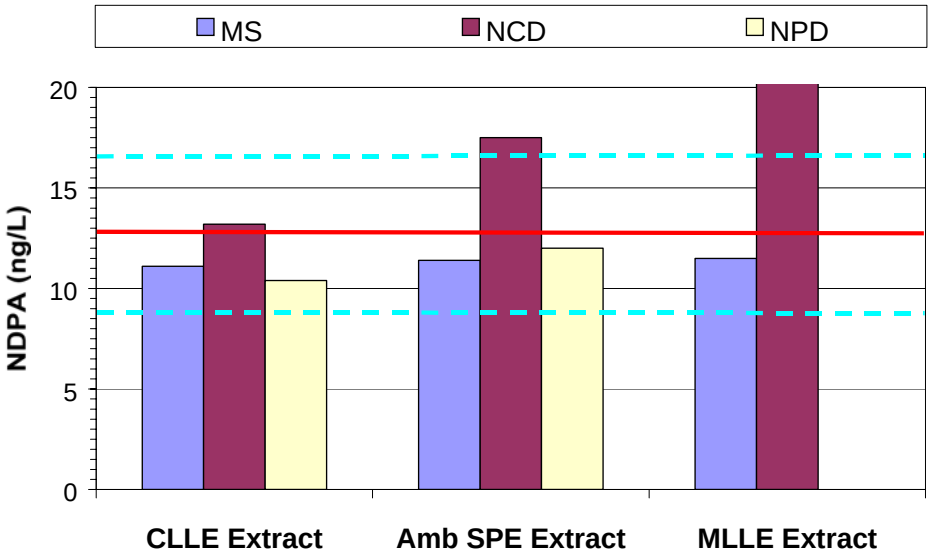


Figure 7.17e **NDPA detection accuracy for low conc. spike recovery using three detectors**
Solid line = actual spike conc. (12.7 ng/L), dashed lines = ± 30% (16.5 and 8.9 ng/L, respectively)

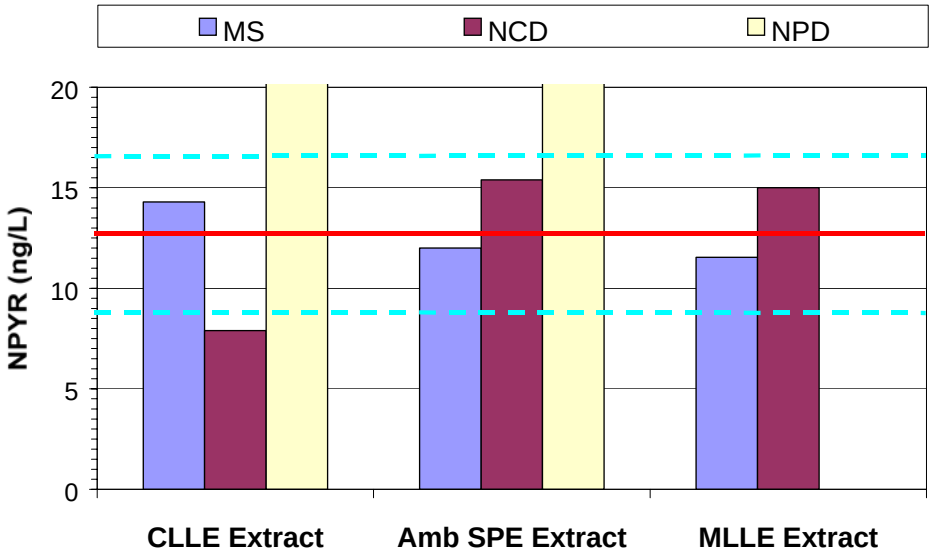


Figure 7.17f **NPYR detection accuracy for low conc. spike recovery using three detectors**
Solid line = actual spike conc. (12.7 ng/L), dashed lines = ± 30% (16.5 and 8.9 ng/L, respectively)

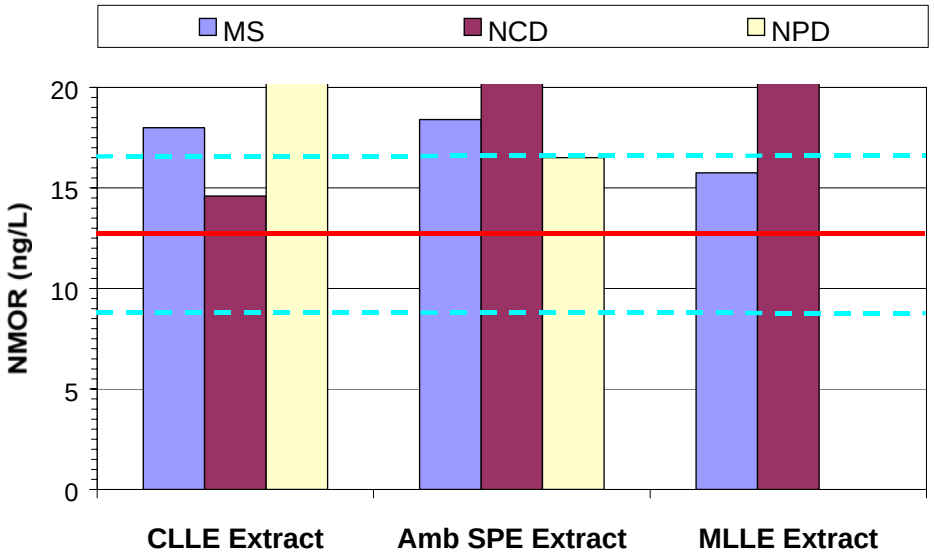


Figure 7.17g **NMOR detection accuracy for low conc. spike recovery using three detectors**
Solid line = actual spike conc. (12.7 ng/L), dashed lines = ± 30% (16.5 and 8.9 ng/L, respectively)

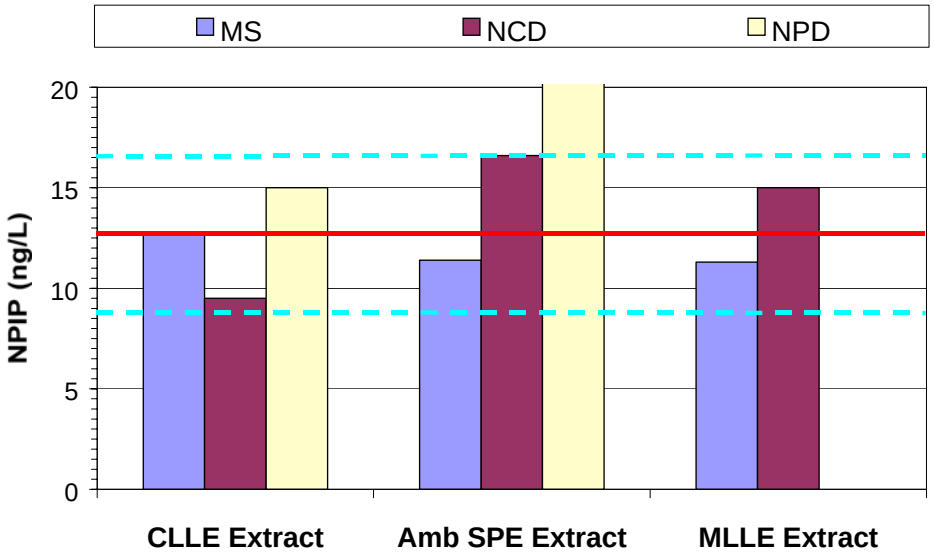


Figure 7.17h **NPIP detection accuracy for low conc. spike recovery using three detectors**
Solid line = actual spike conc. (12.7 ng/L), dashed lines = ± 30% (16.5 and 8.9 ng/L, respectively)

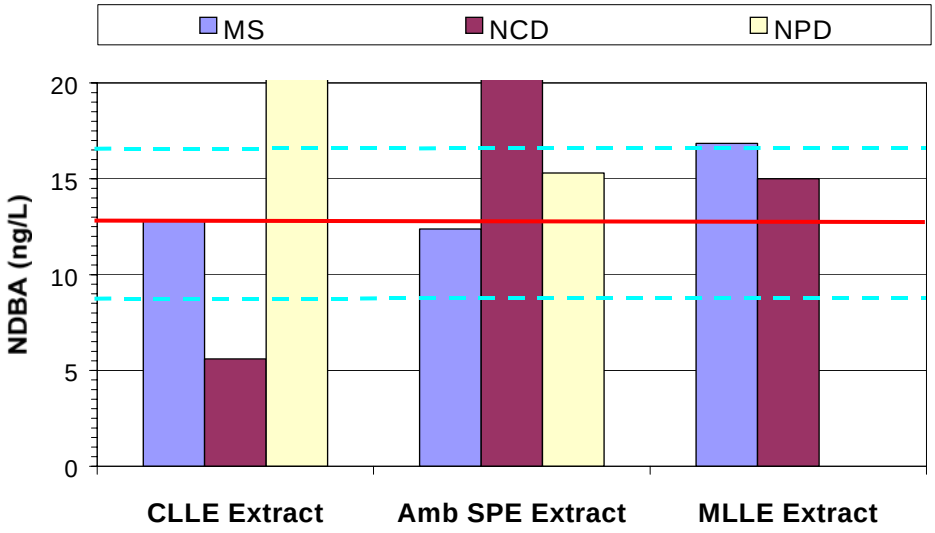


Figure 7.17i **NDBA detection accuracy for low conc. spike recovery using three detectors**
Solid line = actual spike conc. (12.7 ng/L), dashed lines = ± 30% (16.5 and 8.9 ng/L, respectively)

For the high concentration spike in secondary effluent, the alternative detectors appear to produce more comparable NDMA results to the GC/CI/MS/MS, with the exception of CLLE extract (Figure 7.18a). A similar observation may be made for the other nitrosamines data, where the NCD and NPD results were comparable to the GC/CI/MS/MS results (Figures 7.18b through 7.18i). The detector results appear to differ with the different extraction techniques. For example, more variability between the different detectors was observed with CLLE extraction than with the other two extraction methods. The results obtained with the NCD with the CLLE extract was consistently lower than the GC/CI/MS/MS and below the acceptable accuracy range for many of the nitrosamine species, while the NPD values were consistently high and beyond acceptable accuracy limits. With additional validation testing, both NCD and NPD appear to be a suitable substitute for GC/CI/MS/MS with Amb SPE extract for high nitrosamine concentration samples, although the NCD shows better accuracy. For the MLLE extract, the NCD method measured all nitrosamines except NDBA within acceptable accuracy. Thus, for high concentration nitrosamine detection, depending on the extraction method, one of the alternative detectors may be suitable.

Some of this variability between the extraction methods may be due to differences in calibration. When extracted calibration standards are not used, as was the case for the CLLE samples in this study, it is impossible to determine the recovery ratios of the NDPA-d_{14} extraction surrogate to the other nitrosamines and the accuracy of calibration is decreased. In comparing the NPD results obtained from CLLE and Amb SPE for all nitrosamines at high concentrations (Figures 7.18a-i), it was observed that the accuracy of the Amb SPE data is very good. Although samples were run at the same approximate time period and under the same conditions, the accuracy of the CLLE data is very close to, and in some cases, exceeds the upper limit of acceptability. The fact that NPD accuracy is worse with CLLE as compared to Amb SPE may indicate that the use of external calibration (used with CLLE) as opposed to extracted standards (used with Amb SPE) might have a significant effect on detector reliability.

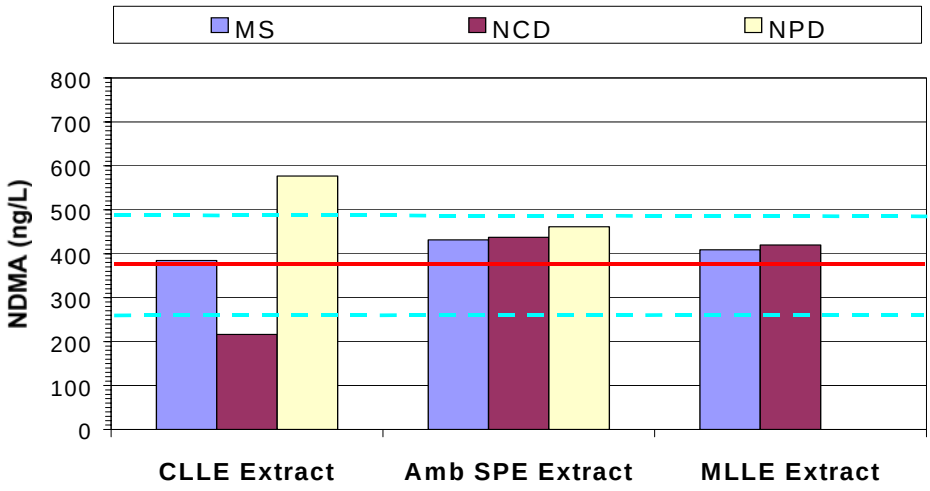


Figure 7.18a **NDMA detection accuracy for high conc. spike recovery using three detectors**
Solid line = actual spike conc. (376 ng/L), dashed lines = ± 30% (489 and 263 ng/L, respectively)

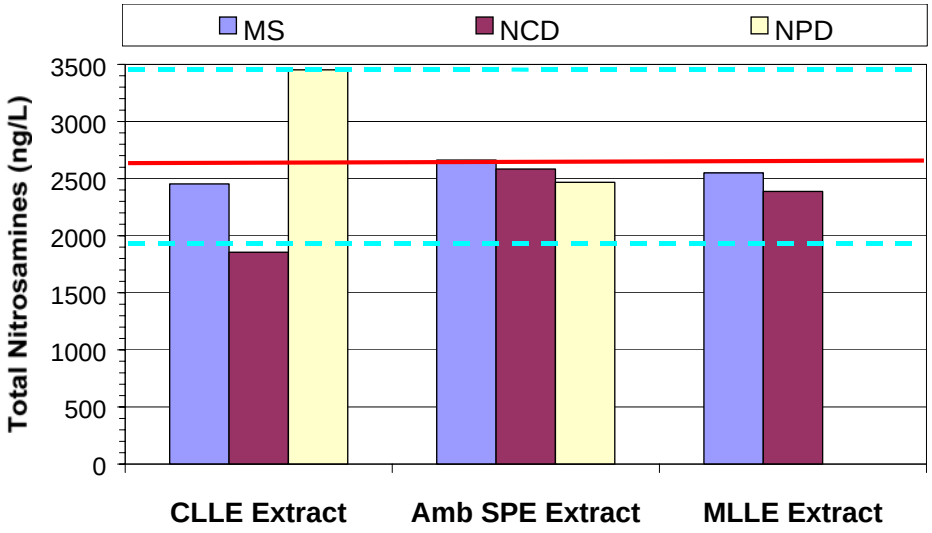


Figure 7.18b **Total nitrosamine detection accuracy for low conc. spike recovery using three detectors**
Solid line = actual spike conc. (2632 ng/L), dashed lines = ± 30% (1842 and 3422 ng/L, respectively)

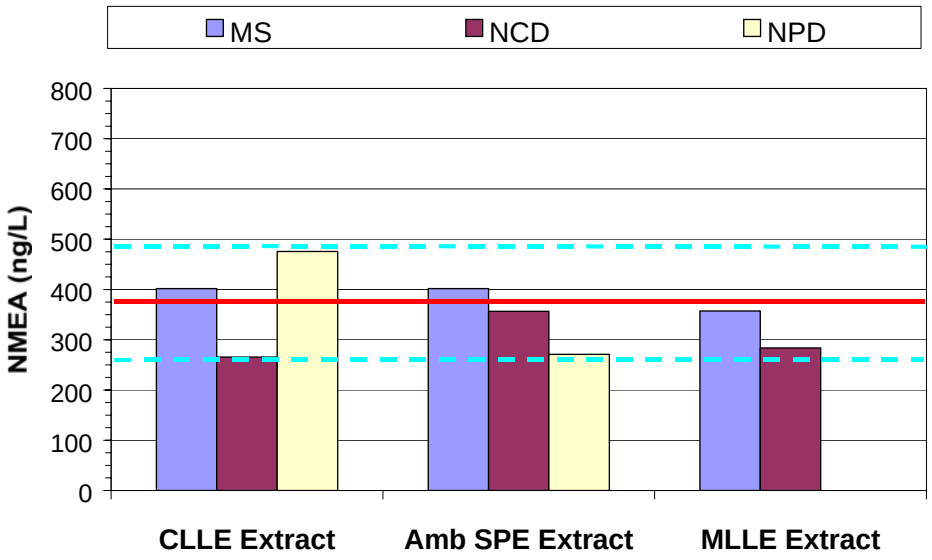


Figure 7.18c **NMEA detection accuracy for high conc. spike recovery using three detectors**
Solid line = actual spike conc. (376 ng/L), dashed lines = ± 30% (489 and 263 ng/L, respectively)

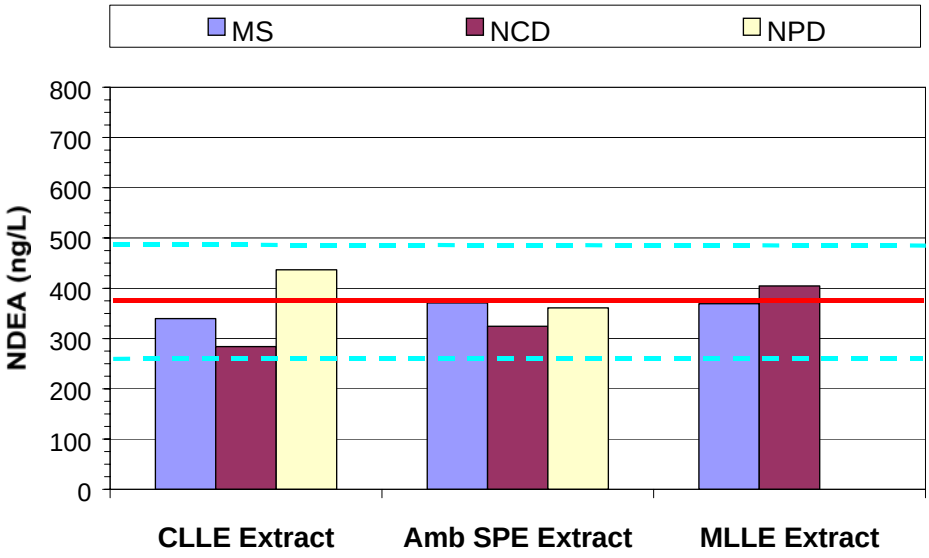


Figure 7.18d **NDEA detection accuracy for high conc. spike recovery using three detectors**
Solid line = actual spike conc. (376 ng/L), dashed lines = ± 30% (489 and 263 ng/L, respectively)

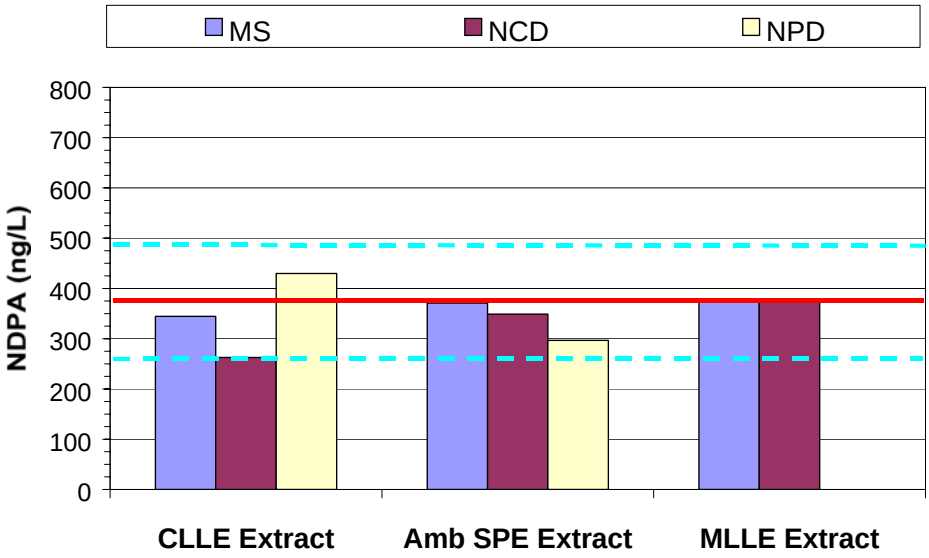


Figure 7.18e **NDPA detection accuracy for high conc. spike recovery using three detectors**
Solid line = actual spike conc. (376 ng/L), dashed lines = ± 30% (489 and 263 ng/L, respectively)

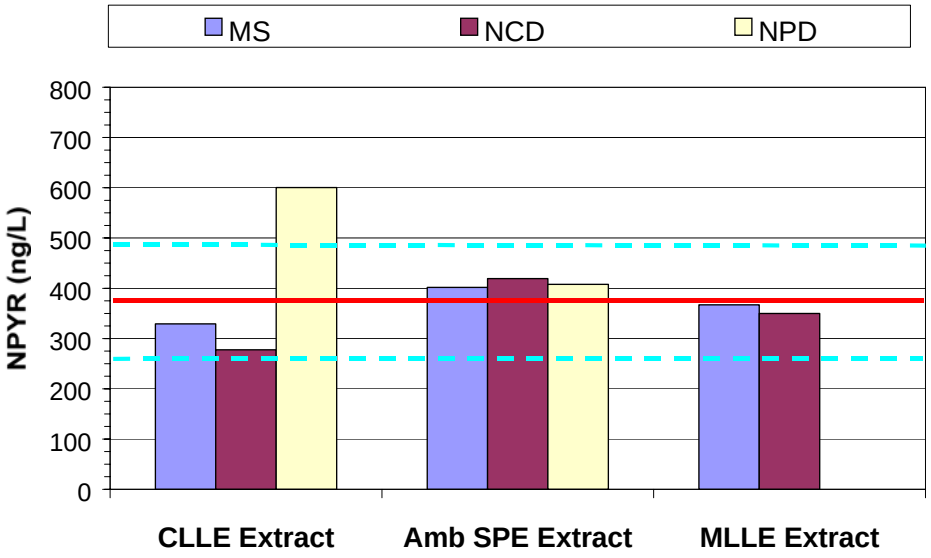


Figure 7.18f **NPYR detection accuracy for high conc. spike recovery using three detectors**
Solid line = actual spike conc. (376 ng/L), dashed lines = ± 30% (489 and 263 ng/L, respectively)

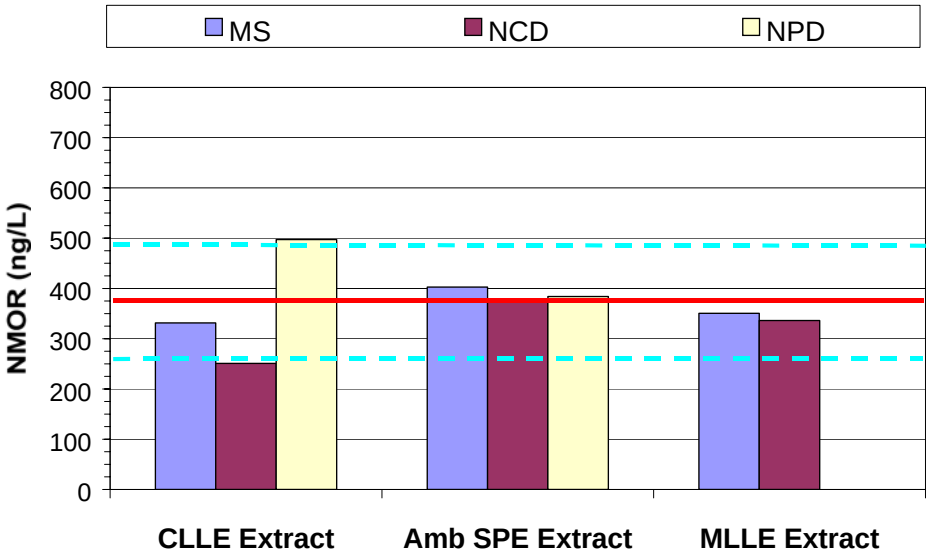


Figure 7.18g **NMOR detection accuracy for high conc. spike recovery using three detectors**
Solid line = actual spike conc. (376 ng/L), dashed lines = ± 30% (489 and 263 ng/L, respectively)

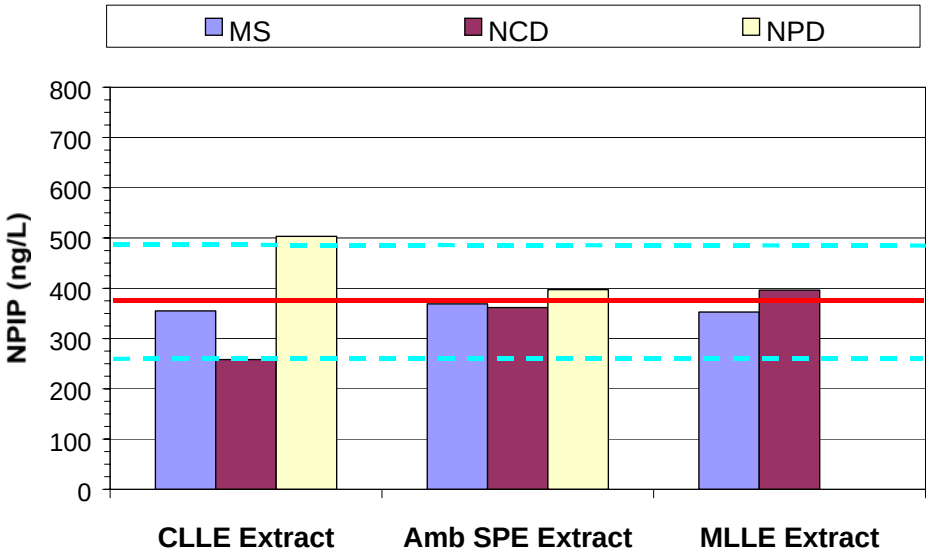


Figure 7.18h **NPIP detection accuracy for high conc. spike recovery using three detectors**
Solid line = actual spike conc. (376 ng/L), dashed lines = ± 30% (489 and 263 ng/L, respectively)

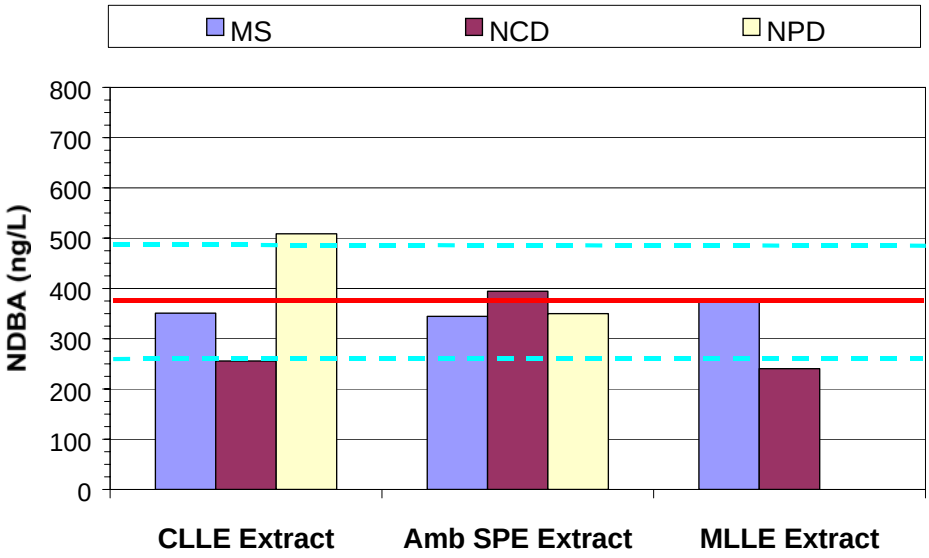


Figure 7.18i **NDBA detection accuracy for high conc. spike recovery using three detectors**
Solid line = actual spike conc. (376 ng/L), dashed lines = ± 30% (489 and 263 ng/L, respectively)

Summary of Alternative Detector Performance Comparison

The following observations are based on spike recoveries of low-level spike sample (12.7 ng/L) and high-level spike sample (376 ng/L).

- For the low-level sample, the NCD and NPD did not yield sufficient accuracy for all nitrosamines. The results could differ by more than 50%, depending on the nitrosamine.
- NPD appears to produce results higher than that obtained for NCD for all nitrosamines.
- For the high concentration CLLE extract, it appears that the NPD produced higher results compared to the GC/CI/MS/MS results, where the NCD produced consistently lower results.
- For the high concentration Amb SPE extract, it appears that all three detection methods produced comparable results.
- For the high concentration MLLE extract, the NCD generally produced comparable results to the GC/CI/MS/MS.

Detector Performance Conclusions

- Initial tests of NCD and NPD performance with CLLE on low concentration drinking water samples indicated that detectors' performance would be acceptable with proper extraction surrogate.
- Initial tests of NCD and NPD performance with CLLE on wastewater samples indicated that the NCD could reliably quantify NMOR, but the NPD could not.
- NCD with Amb SPE showed best agreement with GC/CI/MS/MS values for detection of NDMA in all water types tested.
- Overall, Amb SPE provided better performance with alternative detectors than CLLE.

The extraction method used was shown to affect the performance of the NCD and NPD, at both low and high concentrations. The results above indicate that these alternative detectors could be used reliably for the analysis of higher concentration water samples. The variations in accuracy between the two sets of low concentration results (10 ng/L and 12.7 ng/L spike) indicates that further work should be performed to obtain an accurate picture of detector accuracy at low levels. Multiple water samples of this type and spiking concentration should be analyzed to determine if the inaccurate results of the 12.7 ng/L spiked sample are reliably reproduced. Samples with and without surrogate, should be analyzed to determine if NDPA-d_{14} is an appropriate surrogate for low level analysis with the NCD and the NPD.

Although the NCD or NPD showed good accuracy in the analysis of high concentration wastewater samples, based on the inconclusive results presented here, it cannot yet be definitively concluded if the NCD and NPD alternative detectors are suitable for low concentration nitrosamine detection. With further refinement, the analysis of lower concentrations with improved accuracy might also be consistently achieved. For this study, only one laboratory had the equipment available to test the two alternative detectors and thus, these results are only an initial evaluation. A more rigorous, multi-laboratory testing of multiple water samples is ultimately needed to validate NCD and NPD performance.

CHAPTER 8

COMPARISON OF METHODS

Extraction Methods Optimization

The purpose of this project was to refine, develop, and evaluate multiple methods for NDMA analysis, and where applicable, determine how well these methods are able to detect other nitrosamines. Although the extraction/concentration methods evaluated by the various investigators were fundamentally different, a similar methodology was used. First, the parameters influencing the extraction results were examined in reagent waters in order to isolate the effect from the sample matrix. The reagent waters were all spiked with a detectable concentration of NDMA or other nitrosamines (> 50 ng/L). The goal of these experiments were to examine the primary variables that would influence extraction efficiency for NDMA and other nitrosamines, and then to determine if simplifications in the methods may be made in order to make the analysis more cost-effective and automation friendly.

The two primary extraction methods examined in this study included liquid–liquid extraction (LLE) and solid–phase extraction (SPE) methods. Variables examined that were common to both extraction methods included salt addition, solvent type, and sample volume. For the liquid extraction technique, two methods were derived and tested. The simplified LLE (SLLE) method reduces the sample volume requirement from 1 L to 500 mL, reduces the number of extractions from three to one, and, although it yielded a sufficiently low method detection limit (MDL) of 0.52 ng/L (providing a MRL of 1.5 ng/L), the method was cumbersome, time consuming, and ultimately not used in the round-robin testing phase.

A micro LLE (MLLE) method further reduced the sample volume required to 100 mL sample and 20 mL dichloromethane (DCM) for the solvent volume, and it could be performed in a 125 mL bottle rather than a separatory funnel (see Appendix for procedures). Because of the lower volumes, extraction of larger sample batches is possible using automated shakers. A primary limitation of this method is the concentration factor (CF), which is defined as the original sample volume divided by the final volume of the extract. The CF for MLLE is 200, while the CF for other methods are 1,000 or more. To be able to measure 1 ng/L in the sample with the utilized GC/CI/MS/MS sensitivity, the extraction step has to be able to concentrate the sample 1,000 times or more. The physical limitation of the final volume of the extract is somewhat dependent on the analyst skill, but generally 0.5 mL (500 μL) is the practical limit without additional evaporation steps. Starting with a 100 mL sample, the concentration factor is only 200. Because of this, the corresponding theoretical MDL is 5 ng/L. The experimental MDL for NDMA was determined to be 2.3 ng/L for this method, which translates to a MRL of approximately 7 ng/L. This may not be suitable for drinking water monitoring, but may be suitable for other matrices or formation potential studies where higher reporting levels are acceptable. Refer to Chapter 3 for further details of this study, and see Appendix A for the recommended procedures.

Two different solid–phase extraction methodologies were examined: the free Ambersorb® 572 solid–phase extraction method and the cartridge SPE method. CSPE is essentially a more automated version of the traditional SPE method. In both extraction methods, the sample volumes used were 500 mL. For the SPE method, the primary variables that were examined included salt addition, solvent volume and type, contact time, and pH. The baseline conditions were selected from Taguchi (1994). From the baseline conditions, the primary recommended change was in the contact time (Fields et al., 2004). Although it was recommended that the contact time be increased from one hour to two hours, the rotator apparatus procured for this portion of the study is able to process 12 samples at one time and, therefore, provides for a degree of automation during this process. Refer to Chapter 4 for further details of this study and see Appendix B for the recommended procedures.

For the CSPE method, five commercially-available cartridges and a dual-media manually augmented commercial cartridge were tested. These cartridges included HLB, MCX, MAX LC18, and Envi-carb cartridges. With exception of the Envi-carb cartridge, none of the cartridges tested showed the ability to extract NDMA from water with significant retention of NDMA (> 80%). However, there was a much lower recovery of NDMA from the Envi-carb cartridge than there was for the other nitrosamines. Because Ambersorb has been demonstrated to be a good media for NDMA extraction, it was decided to pack 350 mg of Ambersorb on top of the Envi-carb media. The results showed absolute recoveries of NDMA that were comparable to the SPE method (> 60%). The dual-media Ambersorb-Envi-carb cartridge (Amb-Envi) was selected for optimization and round-robin testing. Refer to Chapter 5 for further details of this study, and see Appendix C for the recommended procedures.

Solid–phase microextraction (SPME) was also examined as a possible low-cost method for NDMA extraction. SPME has the advantage over the traditional liquid– and solid–phase extraction methods because no solvents are required, the extraction and concentration steps occur simultaneously, and this method may be automated. Investigation of the SPME parameters included fiber coating, extraction mode (headspace or liquid contact), salt concentration, contact time, and pH. It was determined that the optimal extraction recovery occurred with headspace contact using the carboxen/polydimethylsiloxane fiber, with the sample at 100% salt saturation and a neutral pH. NPYR was not recovered. Although SPME is a rapid method (each sample requires approximately 75 minutes for analysis), the MDL for NDMA is 30 ng/L (using GC/CI/MS/MS), which may not be acceptable for drinking water monitoring.

After each method was optimized for NDMA, samples containing additional nitrosamines were tested under optimized conditions to determine the absolute recoveries for each method, and the results are provided in Table 8.1. It appears that the Amb SPE and Amb-Envi CSPE methods resulted in the highest absolute recoveries, as well as providing a 1,000x concentration factor, which is necessary in order to meet a detection limit of 1 ng/L or less.

Table 8.1 Absolute recoveries and concentration factors for target nitrosamines

Compound		LLE	MLLE	Amb SPE	Amb-Envi CSPE
N-Nitrosodimethylamine	NDMA	51%	30%	62%	59%
N-Nitrosomethylethylamine	NMEA	58%	40%	84%	76%
N-Nitrosodiethylamine	NDEA	52%	54%	81%	82%
N-Nitrosomorpholine	NMOR	27%	51%	74%	86%
1-Nitrosopyrrolidine	NPYR	32%	53%	80%	81%
N-Nitrosodi-n-propylamine	NDPA	60%	57%	88%	89%
N-Nitrosopiperidine	NPIP	32%	57%	89%	79%
N-Nitrosodi-n-butylamine	NDBA	Not given	71%	82%	95%
Theoretical Concentration Factor	CF	1,000	200	1,250	1,000

MDL studies were performed by extracting seven replicate aliquots of 2.0 ng/L to 200 ng/L spiked reagent water. Although not yet proposed, it is believed that an analytical MRL desired for drinking water monitoring would be in the range of 2 ng/L NDMA or less. Therefore, it appears that only the SPE, CSPE, and SLLE methods can currently meet this requirement, with the other methods suitable for applications not requiring this degree of sensitivity (Table 8.2).

Table 8.2 MDLs for target nitrosamines

Compound	SLLE	MLLE	Amb SPE	Amb-Envi CSPE	SPME (GC/MS)
Spike ng/L	**2**	**10**	**5**	**5**	**200**
NDMA	0.52	2.3	0.78	0.70	30
NMEA	0.60	3.9	1.35	1.36	**60**
NDEA	0.73	2.5	1.78	0.84	60
NMOR	0.33	2.7	1.38	0.63	60
NPYR	0.55	1.8	0.84	0.54	ND
NDPA	0.58	3.4	1.64	0.81	60
NPIP	0.42	2.2	1.35	0.33	60
NDBA	0.27	3.8	1.61	0.80	60

Round-Robin Testing

Background

The round-robin testing evaluated the following four extraction methods studied in this project. The extracts from each method were analyzed by gas chromatography/mass spectrometry (GC/MS).

- Liquid–liquid extraction (LLE), manual or continuous (baseline method)
- Micro liquid–liquid extraction (MLLE)
- Free Ambersorb solid–phase extraction (Amb SPE)
- Cartridge solid–phase extraction (Amb-Envi CSPE)

Extracts were analyzed using a wide range of MS systems including: high resolution magnetic sector instruments and low resolution electron impact (EI-MS) and CI-MS/MS (reagent: methanol, acetonitrile or ammonia) with ion trap and quadruple systems. All systems were able to produce acceptable nitrosamine analyses. Injection volumes varied from 2 to 8 μL, depending on instrument sensitivity.

A total of eight U.S. and four Canadian laboratories were represented, with each method tested by a minimum of three laboratories to ensure that statistical comparisons could be made. Two additional academic laboratories performed evaluations using a modified CSPE (Mod CSPE) method (Charrois et al., 2003) and the SPME method. These methods were not formally evaluated as part of the round-robin testing. The results for SPME were discussed in Chapter 6, and the limited results obtained from the Mod CSPE testing are discussed in this chapter. The types of participating laboratories and methods evaluated are shown in Table 8.3.

Table 8.3 Summary of laboratories participating in round-robin testing

Type Lab	MLLE	Amb-Envi CSPE	Amb SPE	LLE
Regulatory	2	---	1	---
Commercial	2	1	1	4
Utility	2	2	2	2
Total	6	3	4	6

Three weeks prior to the round-robin sampling, analytical methods were sent to the 14 participating laboratories. Primary and secondary nitrosamine standards and isotopic standard solutions (for internal standard calculation) to be used as calibration and QC standards were prepared by the research team and delivered by courier or mail service to the participants.

Source Water Selection

Four different source waters were selected for the round-robin testing. The source waters were selected to expose the different analytical methods to different water matrices with varying levels of possible interference, as well as a wide range of expected NDMA concentrations. The sources were: (1) potable chloraminated surface water, (2) chlorinated reclaimed effluent treated by reverse osmosis (RO effluent), (3) secondary effluent, and (4) tertiary effluent. The potable water sample was derived from a surface water source, and treated through conventional treatment (flocculation, sedimentation, and filtration) and disinfected with chlorine followed by chloramine. The potable water source was expected to be a relatively clean matrix, with least interference during the extraction process and the lowest nitrosamine concentrations. The RO effluent was a tertiary effluent that had undergone reverse osmosis membrane treatment. Although the RO process is poor at removing NDMA, it will substantially reduce the concentration of compounds that may interfere with the extraction process. The secondary effluent provided the next level up from RO effluent in terms of potential analytical difficulties. The secondary effluent had undergone biological treatment (activated sludge) but it had not been chlorinated. Thus, high levels of nitrosamine precursors and compounds that may interfere with the extraction process were expected in the secondary effluent, but because this water was collected prior to the chlorination process, the nitrosamine levels were expected to be less than the tertiary effluent. The tertiary effluent was a secondary-treated wastewater further treated for ammonia removal and was chlorinated. Thus, the tertiary effluent was expected to have the highest levels of nitrosamines and interfering compounds.

The NDMA levels in the four source waters were expected to range from less than 10 ng/L to greater than 1,000 ng/L. The concentrations of the other nitrosamines have not been previously reported so no references were available. Because the absolute concentration of nitrosamines in these samples was unknown, it was decided to spike two of the source waters with known levels of nitrosamines (matrix spike), which would permit the determination of accuracy and provide some values for all the nitrosamines. The potable water and the secondary effluent were selected for nitrosamine spiking, representing one clean matrix and one containing higher background levels of interference. The matrix spike allowed the determination and comparison of the accuracy of the various methods implemented by the different laboratories. Details of the matrix spiking are described in the sample preparation section below.

Sample Collection and Preparation

For collection of the potable water and RO effluent samples, dedicated sample taps were used. However, a battery-powered field-sampling pump was used to collect the secondary and tertiary effluent samples. The sample line was flushed a minimum of two minutes prior to collecting the samples. The samples were collected in preweighed clean stainless-steel 55-L containers. The stainless-steel containers were placed on scales during the sample collection to accurately record the volume of source water collected in each stainless-steel container. The chlorine residual of the samples was measured, and an excess amount of sodium thiosulfate was added for dechlorination. The stainless-steel containers were stored in a refrigerated storage space (4 ^{o}C) prior to preparation of samples for the 14 analytical laboratories. The general water quality characteristics of the source waters are listed in Table 8.4.

Table 8.4 Description of waters used during round-robin testing

Sample ID	Source	pH	Turbidity (NTU)	Cond. (µmho/cm)	Cl_2[1] (mg/L)	NA spike[2] (ng/L)
RR-01	Chloraminated potable water	8.32	0.30	423	2.80	0
RR-02	RR-01 (spiked)	7.97	0.13	403	2.80	12.7
RR-03	RO effluent	4.73	0.11	65	0.05	0
RR-04	secondary effluent	7.41	1.07	824	0.04	0
RR-05	RR-04 (spiked)	7.28	0.23	838	0.04	376
RR-06	tertiary effluent	7.42	0.39	912	3.64	0

[1] *Total chlorine residual (as Cl_2): analysis performed at the time of sample collection.*
[2] *All eight nitrosamines spiked to indicated concentrations.*

The six waters (four source waters plus two spiked waters) were aliquoted into specially-cleaned 1-L amber Wheaton bottles and divided for shipment to the participating laboratories. A minimum of one set of waters (six 1-L bottles, one for each of the six waters) was sent to the respective laboratory for testing. The number of sets was increased for laboratories evaluating more than one analytical method (e.g., SPE and CLLE = 2 sets). The samples were placed into ice chests filled with blue ice for delivery. For the laboratories located within Southern California, the samples were hand-delivered within 24 hours of the sample spiking. For the laboratories located outside of this region, the samples were shipped on ice via overnight delivery service. A total time of approximately 96 hours elapsed between the time of sample collection and the time of delivery to the participating laboratories. All laboratories reported that the samples were received intact, and they were requested to provide the results back to the principal investigators within 14 working days.

Matrix Spike

The potable water and secondary effluent were spiked with a known concentration of nitrosamines to allow the evaluation of analytical accuracy for the various methods. Spiking stock solutions at the 10 mg/L and 40 mg/L level were prepared and added directly to the 55-L containers. Hand-mixing was performed using a clean agitator with Teflon blade and stainless-steel shaft to ensure homogeneity of matrix spike prior to dividing the samples into the 1-L containers. The volume of spiking solution added to the potable water and the secondary effluent resulted in a nitrosamine concentration increase of 12.7 ng/L and 376 ng/L, respectively. The concentrations of the spike solutions were selected based on the order of the magnitude of NDMA concentration expected in the background sample (< 10 ng/L in the potable water sample and > 100 ng/L in the secondary effluent). In addition to allowing the study group to confirm the accuracy of the various analytical methods, the matrix spike also ensured that nitrosamines would be measurable in the potable water sample (RR01) matrix.

Data Analysis Procedures

In order to compare the observed data with a standard variability pattern, box and whisker plots were employed. The box and whisker plot illustrates the maximums and minimums (denoted by the extents of the whisker), the 75 and 25%iles (denoted by the limits of the box), and identifies the median value. For the purposes of this work, the median value is used to analyze the accuracy of the results and the box is utilized to evaluate the precision, where indicated. Statistically significant outlier values were identified and removed from the data set.

Due to the low nitrosamine concentrations expected, variability in the data is inevitable. There is no way to prove mathematically whether an extreme value detected represents an actual error or is simply a large random deviation. However, the farther away a measurement is from the distribution's median, the more suspicious-looking it becomes. These observed data values may cause statistical calculations such as

mean and standard deviation performed on the data to be potentially skewed and inaccurate of the true values within a reasonable amount of variance. Therefore, outlier values should be identified and removed from the data set.

All outliers show an obvious deviation from the other values in the set on a plot and should be identified by some established quantifiable means. For the purpose of this work, the upper and lower fence guideline was employed to identify which observations are pushing the limits of "reasonable" and these outliers were excluded from the data analysis per standard statistical method (Larsen, 1997). An outlier is identified as a value higher than the upper fence or lower than the lower fence. The upper and lower fence were calculated using the equations shown below:

Upper fence	= 75^{th} percentile + 1.5(H-spread).	(8.1)
Lower fence	= 25^{th} percentile - 1.5(H-spread).	(8.2)
H-spread	= 75^{th} percentile value - 25^{th} percentile value.	(8.3)

Table 8.5 illustrates an example on how the outlier was identified. In this example, five laboratories measured NDMA. An upper and lower fence was calculated using the five measured values. Subsequently, if the measured value was within the calculated fences, the measured value was kept; otherwise, the value was deemed an outlier and removed.

Table 8.5 Sample table showing removal of data outliers
(Sample LB-RR-02, MLLE method)

Organization	All values	Outlier criteria	w/o Outlier
Lab 2	14.8	Value < lower	14.8
Lab 3	14.9	fence, > upper	14.9
Lab 4	17.1	fence?	17.1
Lab 5	14.5		14.5
Lab 6	25.1		Excluded

75^{th} percentile 19.0 ng/L
25^{th} percentile 15.0 ng/L
H-spread - 4.0 ng/L
Upper fence 25.0 ng/L
Lower fence 9.1 ng/L

Results and Discussion

General Results

A complete listing of the raw data is provided in Appendix E. The 50^{th} percentile NDMA values for the six waters ranged from 4.9 ng/L to 834 ng/L (Figure 8.1). As expected, NDMA levels in source RR01 and RR03 were low, while RR04 and RR06 contained relatively high concentrations based on the types of waters examined. A minimum of 15 data points (n) was used for each water for the calculations, although only 14 laboratories participated in the round-robin evaluation. This is due to the fact that some laboratories evaluated multiple methods, which provided more data points for each water.

The results for many of the individual nitrosamines in the sample were not detected; thus, the average of the seven nitrosamines (NA_7), excluding NDMA, are presented for the purpose of the general results discussion. The NA_7 value ranged for all sources, except RR05, from 2.1 to 12.8 ng/L (Figure 8.2). The average nitrosamine detected in RR05 was 365 ng/L, which is expected due to the 376 ng/L nitrosamine spike added. The general trend of nitrosamines followed that of NDMA, where the potable and RO treated water show lower concentration of nitrosamines than the secondary and tertiary effluent. Although

the trend of nitrosamine concentration with respect to source was similar to NDMA, the levels detected were much lower. While NDMA was the only nitrosamine analyzed in detectable concentrations in potable water and RO effluent, the two wastewater treatment plant samples (secondary and tertiary effluent) contained detectable concentrations of NDEA, NPYR, NMOR, NPIP, and NDBA. Even in the extreme case of RR06, where over 800 ng/L of NDMA was detected, the average of other nitrosamines was approximately two orders of magnitude less (<9 ng/L).

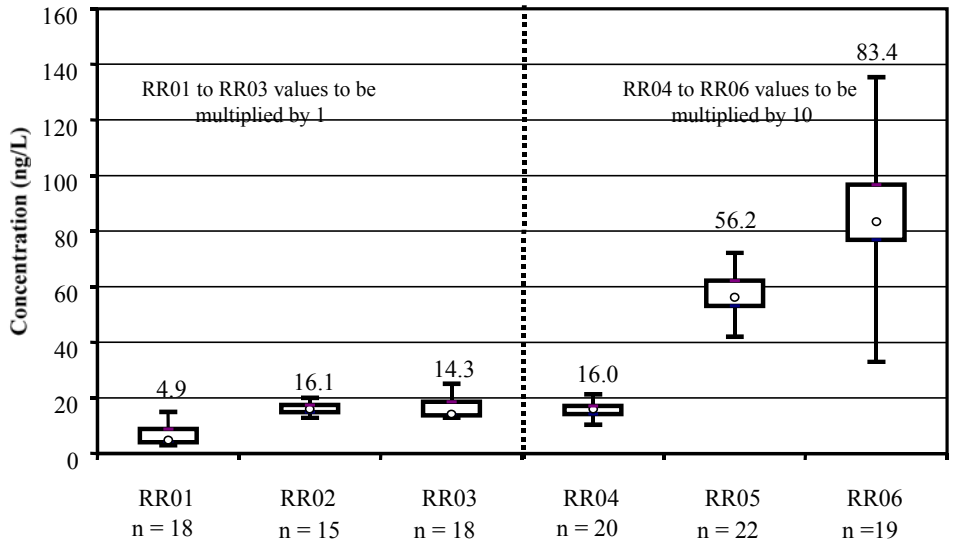


Figure 8.1 **Summary of NDMA levels for the six source waters using all methods**
Top and bottom of whiskers = maximum and minimum, top and bottom of box = 25th and 75th percentiles, circle = 50th percentile. "n"= the number of observations used in analysis

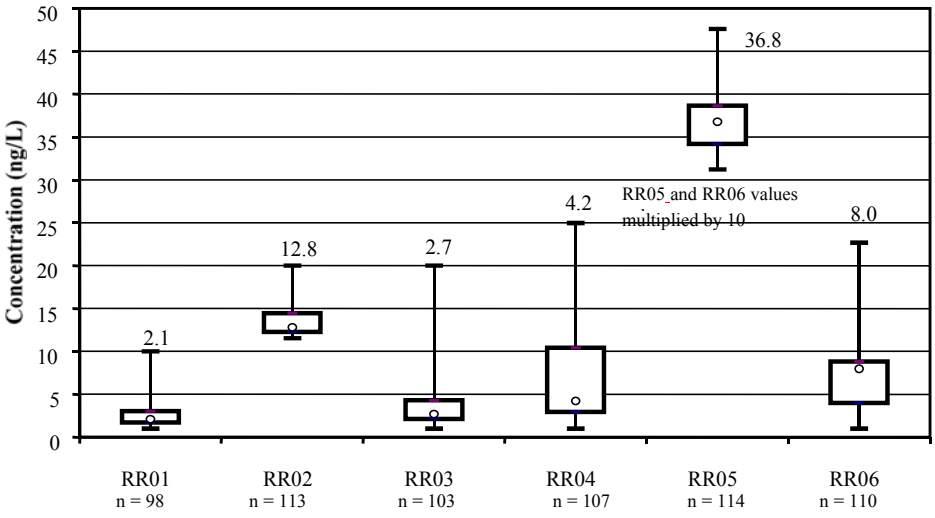


Figure 8.2 **Summary of composite other nitrosamine levels for the six source waters using all methods**
Top and bottom of whiskers = maximum and minimum, top and bottom of box = 25th and 75th percentiles, circle = 50th percentile. "n"= the number of observations used in analysis

Method Accuracy

The accuracy of each method was assessed by determining the differences in results between spiked and unspiked sample for the paired laboratory and method, and comparing the results to the known spike concentrations (low-concentration spike = 12.7 ng/L, high-concentration spike = 376 ng/L). For the low concentration evaluation, measured results for each laboratory RR01 was subtracted from RR02 and for the high concentration spike, RR04 was subtracted from RR05. For the purposes of discussion in this section, box and whisker plots were used to present the data collected and the 50th percentile value was used to compare the accuracy of the different methods. Due to variability associated with low concentration detection, the CDHS considers accuracy within plus or minus 30% (± 30%) to be reasonable. Thus, observed values that fell within ± 30% of actual value are considered acceptable.

The results show that for low concentration NDMA detection, all methods tested were able to generate results that fell within the ± 30% guidelines recommended (Figure 8.3a) and thus, all methods are deemed accurate. The Amb-Envi CSPE was the most accurate method while the MLLE method yielded the least accurate result. It was difficult to assess the accuracy for the individual nitrosamine species because of the low levels in the background. For the low-level nitrosamine spike, the calculated average nitrosamine detected for all methods appears to be able to detect nitrosamines within acceptable accuracy (Figure 8.3b). However, for the detection of NMOR, the Amb SPE and CLLE method did not produce results within acceptable accuracy (Figure 8.3g), but the Amb-Envi CSPE method yielded a result within acceptable limits (Figure 8.3g). Overall, the two solid–phase methods tested appear to yield the best accuracy (Figure 8.3b through 8.3i) for all nitrosamines including NDMA. The liquid-phase methods yielded acceptable accuracy, although for NDMA the MLLE method was the least accurate. This is not unexpected given that the NDMA MRL for this method is greater than the concentration in RR01 and half that of the RR02 spike and is the highest of the four methods tested.

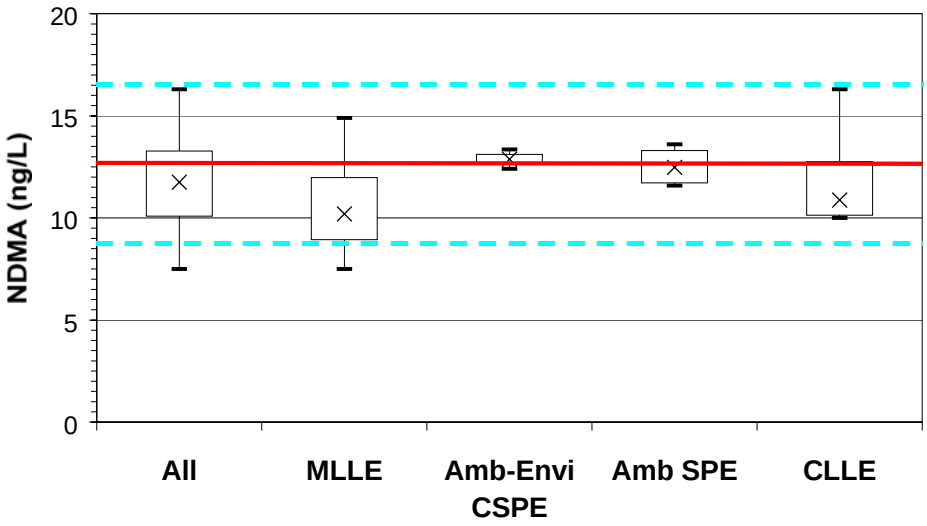


Figure 8.3a **NDMA detected concentration (Low concentration)**
RR02 minus RR01, solid lines = actual spike conc. (12.7 ng/L), dashed lines = plus 30% (16.5 ng/L) and minus 30% (8.9 ng/L)

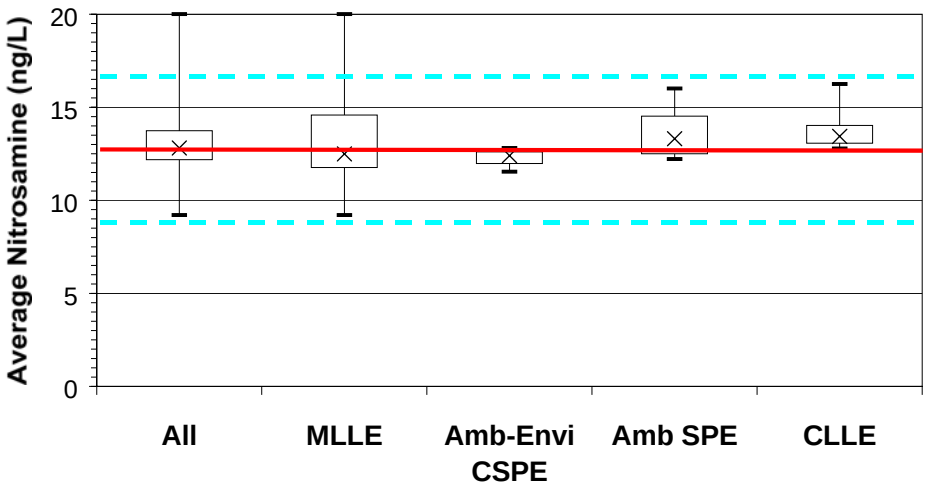


Figure 8.3b **Calculated average nitrosamine detected concentration other than NDMA (Low concentration)**
RR02 minus RR01, solid line = actual spike conc. (12.7 ng/L), dashed lines = plus 30% (16.5 ng/L) and minus 30% (8.9 ng/L)

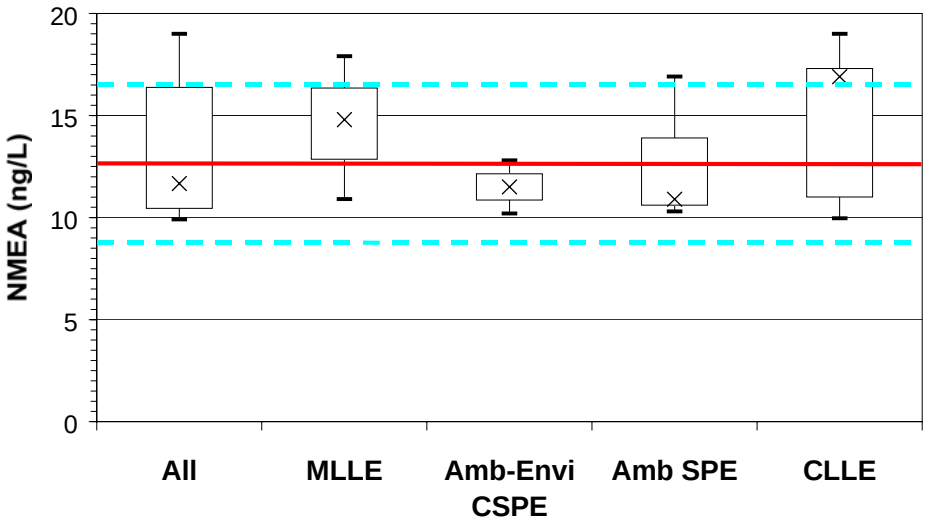


Figure 8.3c **NMEA detected concentration (Low concentration)**
RR02 minus RR01, solid line = actual spike conc. (12.7 ng/L), dashed lines = plus 30% (16.5 ng/L) and minus 30% (8.9 ng/L)

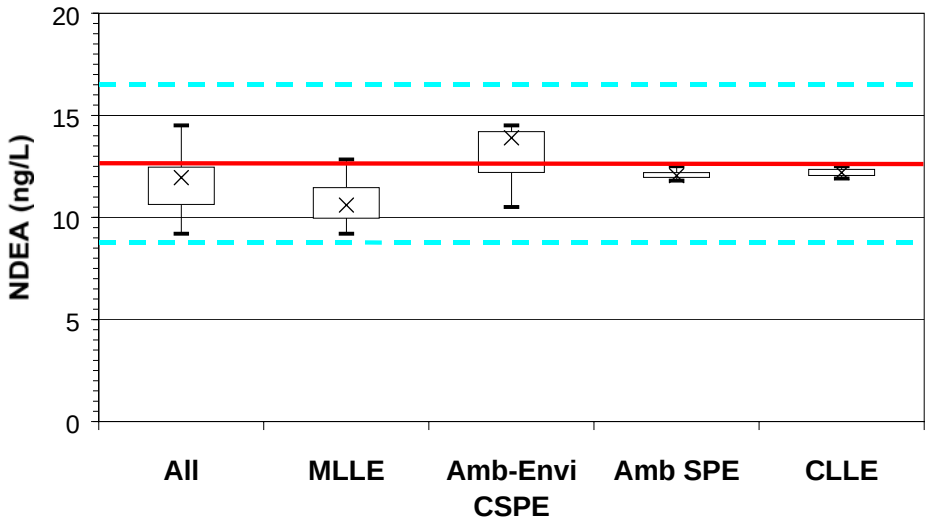


Figure 8.3d **NDEA detected concentration (Low concentration)**
RR02 minus RR01, solid line = actual spike conc. (12.7 ng/L), dashed lines = plus 30% (16.5 ng/L) and minus 30% (8.9 ng/L)

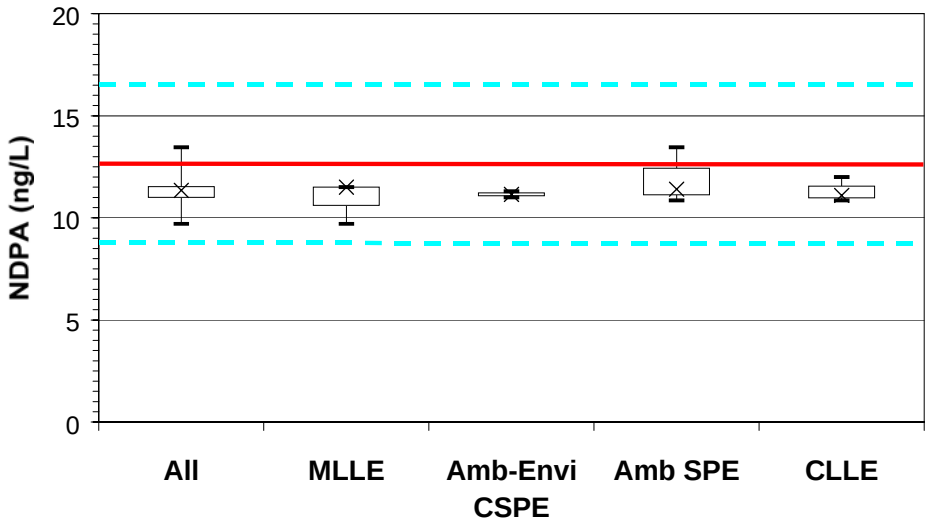


Figure 8.3e **NDPA detected concentration (Low concentration)**
RR02 minus RR01, solid line = actual spike conc. (12.7 ng/L), dashed lines = plus 30% (16.5 ng/L) and minus 30% (8.9 ng/L)

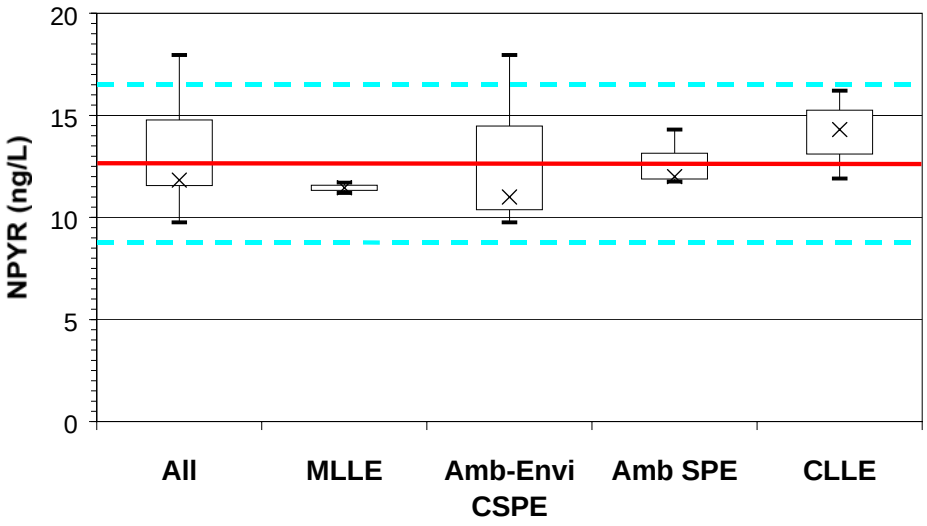


Figure 8.3f **NPYR detected concentration (Low concentration)**
RR02 minus RR01, solid line = actual spike conc. (12.7 ng/L), dashed lines = plus 30% (16.5 ng/L) and minus 30% (8.9 ng/L)

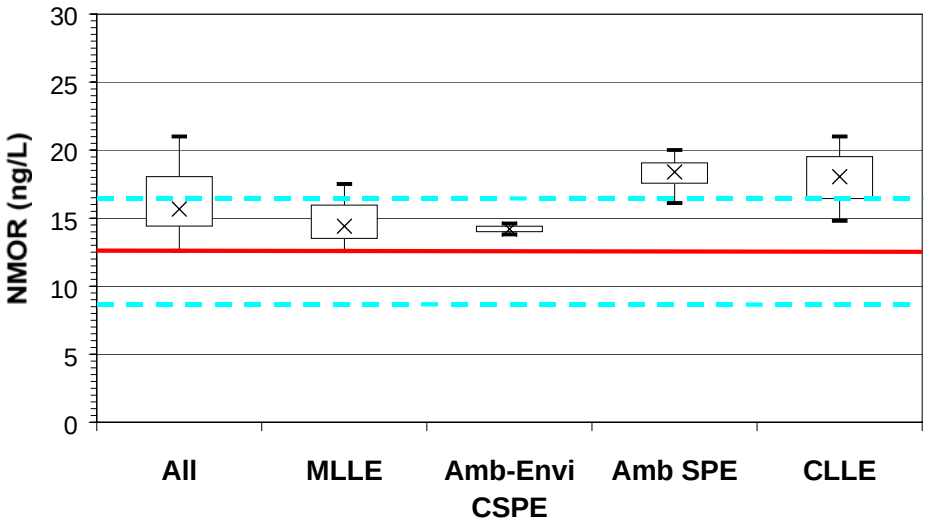


Figure 8.3g **NMOR detected concentration (Low concentration)**
RR02 minus RR01, solid line = actual spike conc. (12.7 ng/L), dashed lines = plus 30% (16.5 ng/L) and minus 30% (8.9 ng/L)

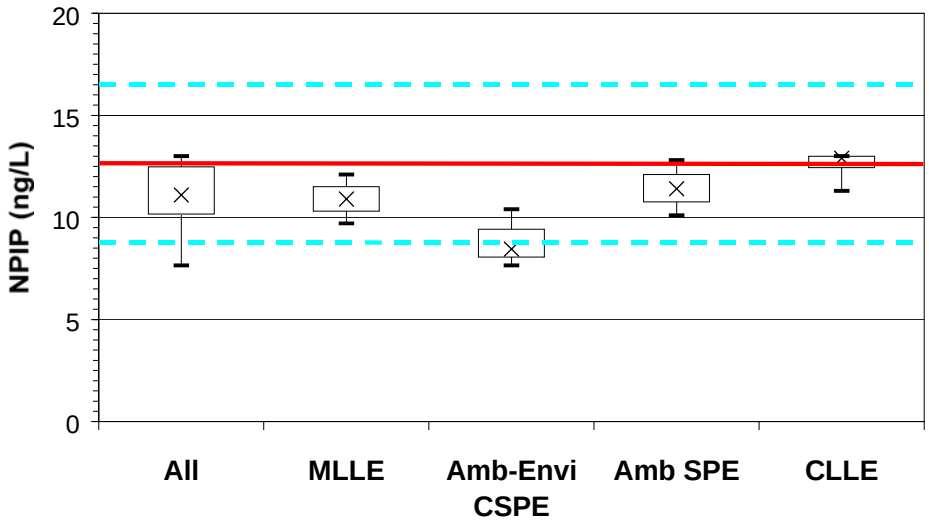


Figure 8.3h **NPIP detected concentration (Low concentration)**
RR02 minus RR01, solid line = actual spike conc. (12.7 ng/L), dashed lines = plus 30% (16.5 ng/L) and minus 30% (8.9 ng/L)

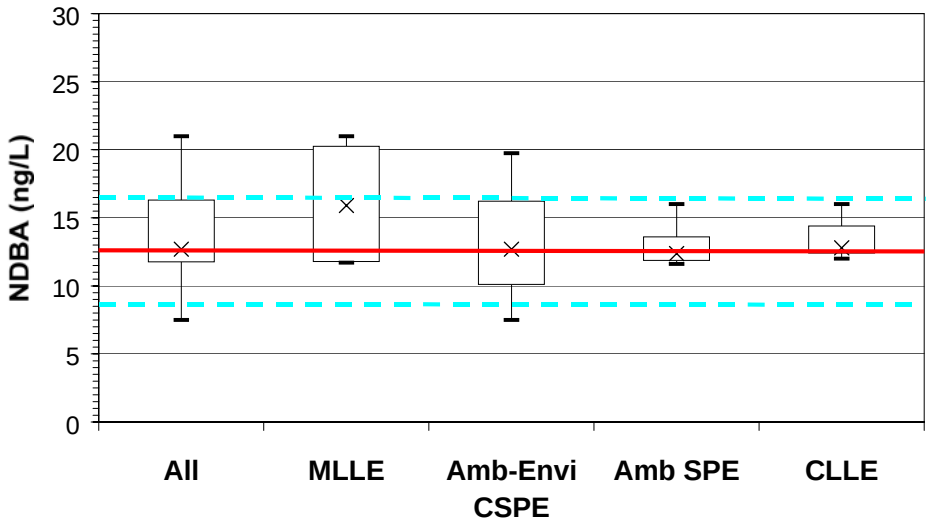


Figure 8.3i **NDBA detected concentration (Low concentration)**
RR02 minus RR01, solid line = actual spike conc. (12.7 ng/L), dashed lines = plus 30% (16.5 ng/L) and minus 30% (8.9 ng/L)

For the high-concentration spike, all methods were able to quantify NDMA concentration within CDHS' accuracy range (Figure 8.4a). The CLLE method produced the median result that was the closest to the actual spike value, while Amb-Envi CSPE method results were the least accurate. For the other nitrosamines, the different methods were able to detect all nitrosamine species within the acceptable accuracy range (Figures 8.4b through 8.4i). Overall, the MLLE method was the most accurate for all

nitrosamines, while the other methods resulted in comparable degrees of accuracy. The high variation in the Amb-Envi CSPE results was most likely due to the inexperience of two of the three laboratories with the method (this was their first use of the method). At MWD's laboratory (i.e., Metropolitan Water District of Southern California), the high concentration samples LL05 and LL06 were diluted 1:2 before analyses and the standards bracketed the sample concentration. The labeled internal standards should correct for incomplete extraction.

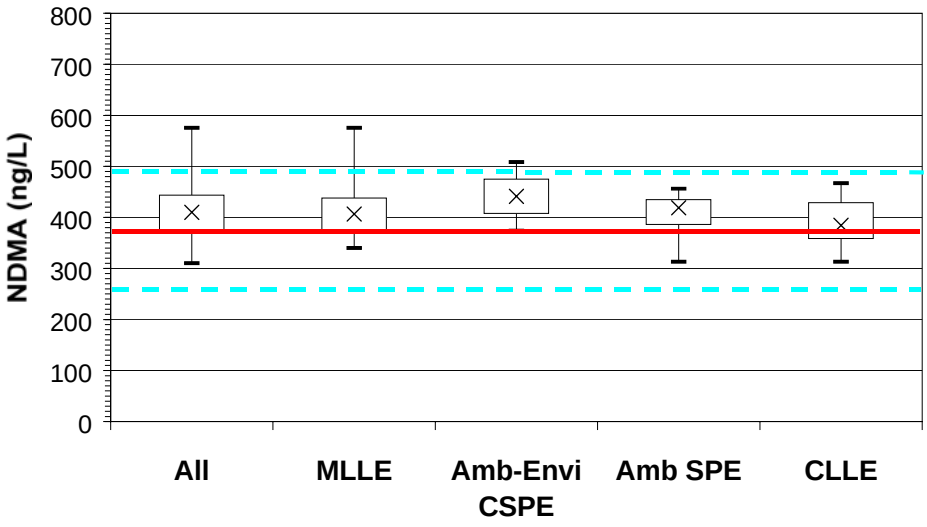


Figure 8.4a **NDMA detected concentration (High concentration)**
RR05 minus RR04, solid line = actual spike conc. (367 ng/L), dashed lines = plus 30% (489 ng/L) and minus 30% (263 ng/L)

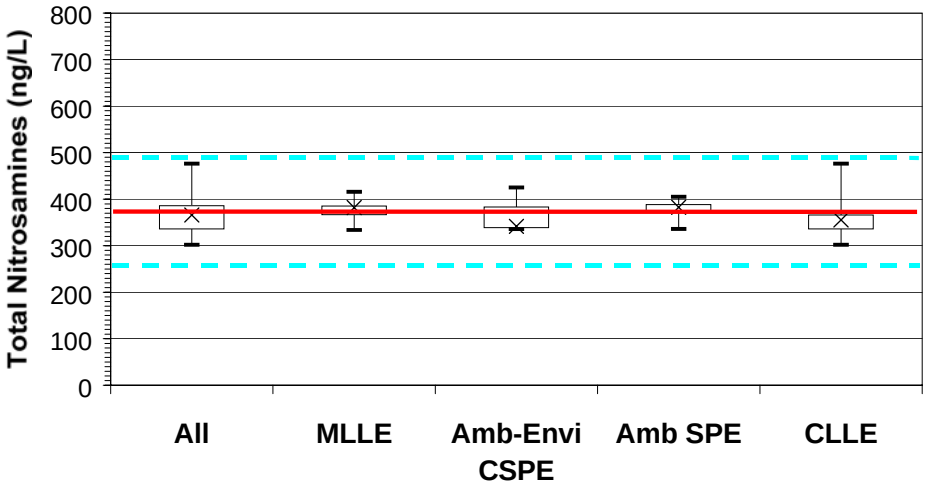


Figure 8.4b **Calculated average nitrosamine detected concentration other than NDMA (High concentration)**
RR05 minus RR04, solid line = actual spike conc. (367 ng/L), dashed lines = plus 30% (489 ng/L) and minus 30% (263 ng/L)

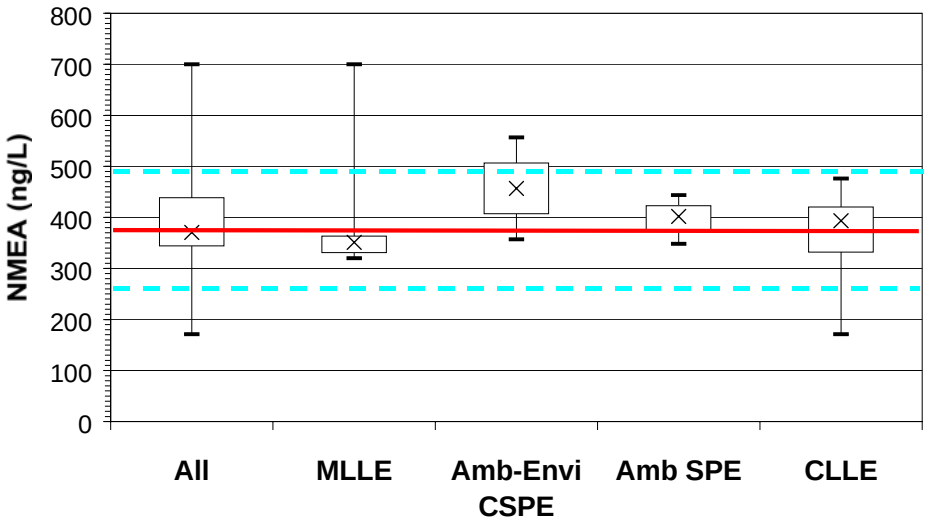


Figure 8.4c **NMEA detected concentration (High concentration)**
RR05 minus RR04, solid line = actual spike conc. (367 ng/L), dashed lines = plus 30% (489 ng/L) and minus 30% (263 ng/L)

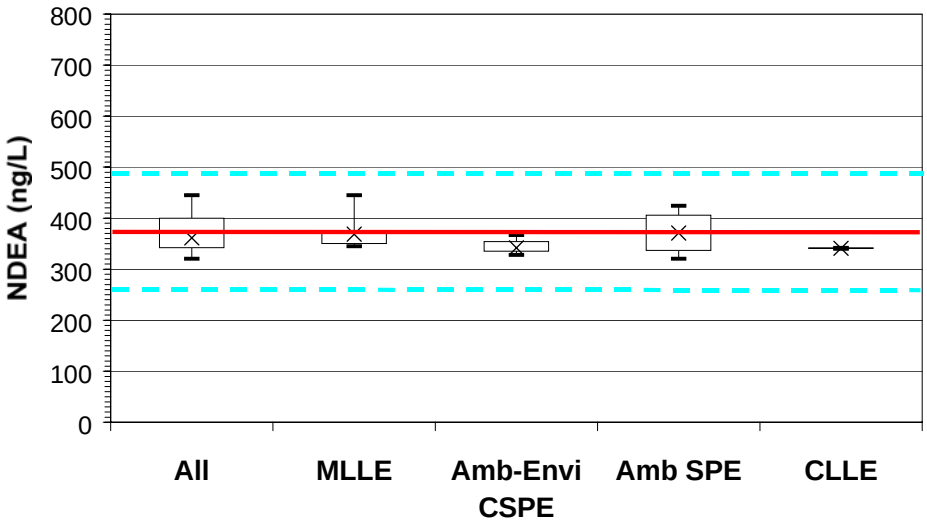


Figure 8.4d **NDEA detected concentration (High concentration)**
RR05 minus RR04, solid line = actual spike conc. (367 ng/L), dashed lines = plus 30% (489 ng/L) and minus 30% (263 ng/L)

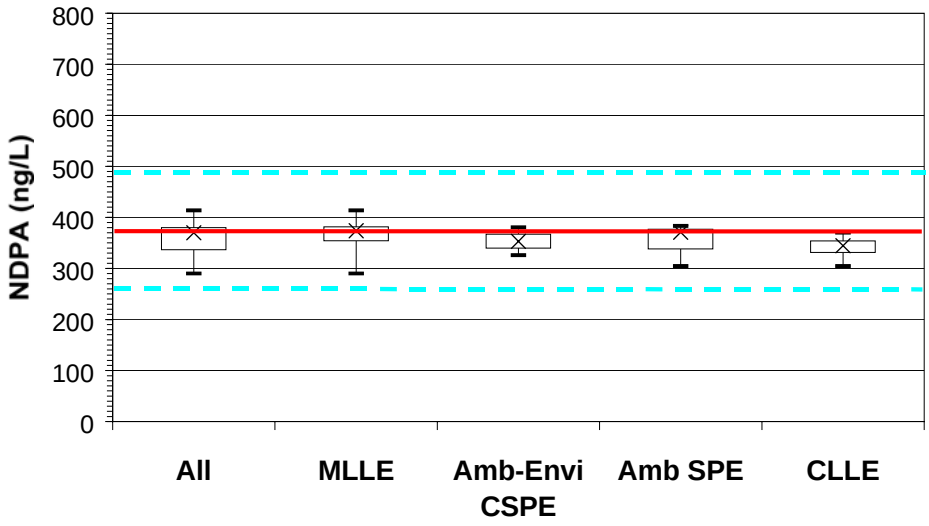


Figure 8.4e **NDPA detected concentration (High concentration)**
RR05 minus RR04, solid line = actual spike conc. (367 ng/L), dashed lines = plus 30% (489 ng/L) and minus 30% (263 ng/L)

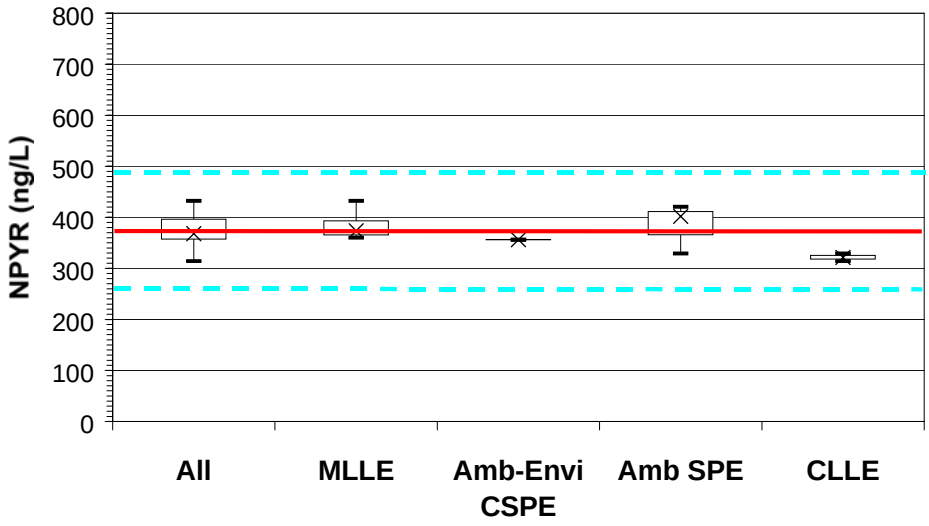


Figure 8.4f **NPYR detected concentration (High concentration)**
RR05 minus RR04, solid line = actual spike conc. (367 ng/L), dashed lines = plus 30% (489 ng/L) and minus 30% (263 ng/L)

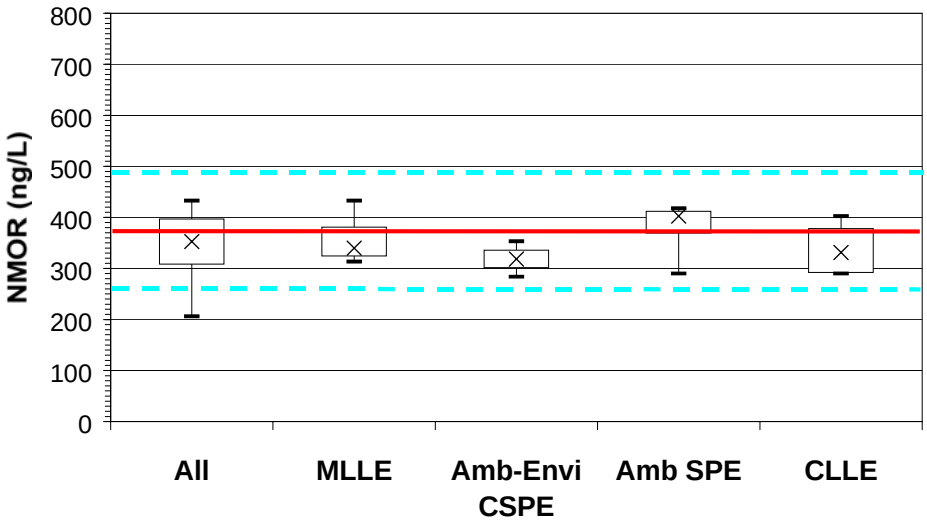


Figure 8.4g **NMOR detected concentration (High concentration)**
RR05 minus RR04, solid line = actual spike conc. (367 ng/L), dashed lines = plus 30% (489 ng/L) and minus 30% (263 ng/L)

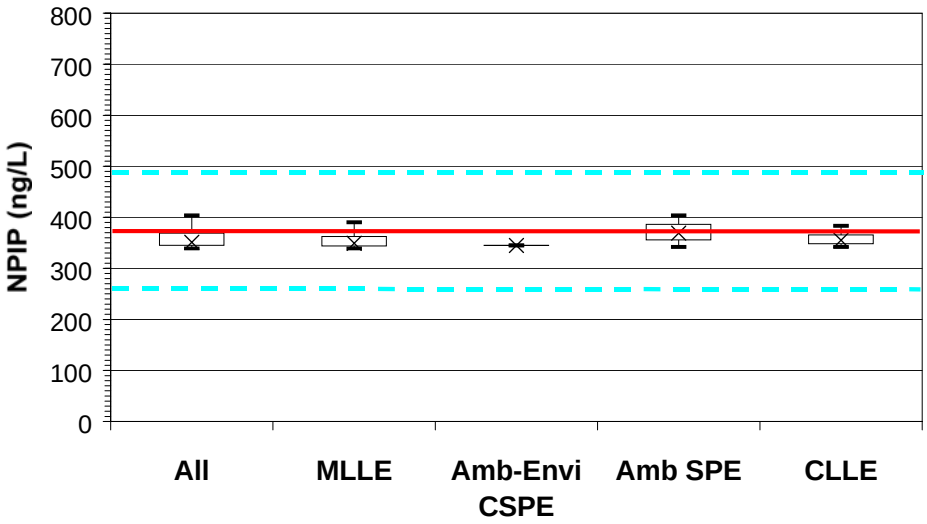


Figure 8.4h **NPIP detected concentration (High concentration)**
RR05 minus RR04, solid line = actual spike conc. (367 ng/L), dashed lines = plus 30% (489 ng/L) and minus 30% (263 ng/L)

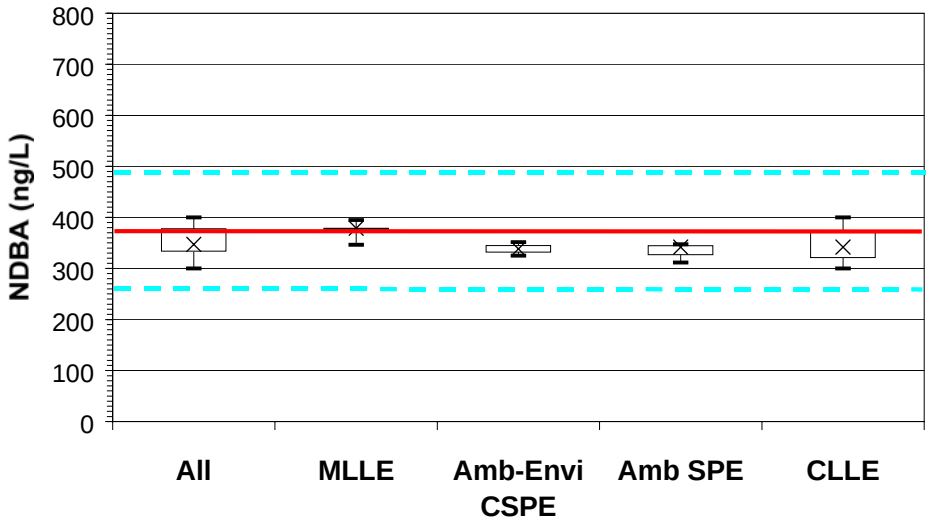


Figure 8.4i **NDBA detected concentration (High concentration)**
RR05 minus RR04, solid line = actual spike conc. (367 ng/L), dashed lines = plus 30% (489 ng/L) and minus 30% (263 ng/L)

For simplicity, a relative accuracy ranking was assigned to each method for each particular nitrosamine. A value of 1 was given to the method that produced the most accurate result and a value of 4 was given to the least accurate result. Table 8.6 shows the rankings for the low concentration detection accuracy and table 8.7 illustrates the results for high concentration detection accuracy. Although this ranking method is qualitative in nature, it gives an overall method accuracy that can be summarized in table form. The results show that for low concentration samples, the Amb SPE method was the most accurate averaged over all nitrosamines while MLLE was the most accurate for high concentrations averaged over all nitrosamines. However, the differences between the analyses were less pronounced for the low-concentration samples than for the high-concentration samples.

Table 8.6 Ranking low concentration extraction method accuracy

Compound	MLLE	Amb-Envi CSPE	Amb SPE	CLLE
NDMA	4	1	2	3
NMEA	3	1	2	4
NDEA	4	3	2	1
NDPA	1	3	2	4
NPYR	2	4	1	3
NMOR	2	1	4	3
NPIP	3	4	2	1
NDBA	4	1	3	2
Total w/NDMA	23	18	18	21
Total w/o NDMA	19	17	16	18

Table 8.7 Ranking for high concentration extraction method accuracy

Compound	MLLE	Amb-Envi CSPE	Amb SPE	CLLE
NDMA	2	4	3	1
NMEA	2	4	3	1
NDEA	2	3	1	4
NDPA	1	3	2	4
NPYR	1	2	3	4
NMOR	3	4	1	2
NPIP	3	4	1	2
NDBA	1	4	2	3
Total w/NDMA	15	28	16	20
Total w/o NDMA	13	24	13	19

Method Precision and Comparison

In the earlier sections, various nitrosamine extraction and detection accuracy was evaluated, but it is equally important to evaluate the precision of the extraction methods. In this section, round-robin testing results with respect to precision and reproducibility for the various waters are presented. This provides insight into the relative precision of each method with respect to each other and whether certain extraction methods are more suitable for a specific water type because different source waters will contain different possible interfering compounds. Unfortunately, the evaluation can only present which method appears to be precise but it is out of the scope of this project to determine what are the probable causes for a specific method to be more precise than another.

Precision, as defined by Standard Methods (19^{th} Ed., 1995), is the degree of agreement among replicate analyses as expressed by the standard deviation. To evaluate the method precision, an analysis was made comparing the standard deviation as a percent of the mean NDMA value. CDHS has set the relative standard deviation for NDMA replicate analyses to be within 20% (CDHS, 2001). This was done as opposed to comparing the absolute standard deviation values because the standard deviation would be biased high for sources containing high levels of nitrosamines. The results indicate that the Amb SPE method was the only method to meet the 20% RSD for all waters and concentrations (Figure 8.5a). However, the methods that yielded the least RSD varied for different waters. As the NDMA concentrations increased, it appears that the difference in the RSD from method to method is minimized and that the percent standard deviation did not follow a consistent trend to the relative cleanliness of the source water. In other words, the standard deviation as a percent of the mean NDMA value did not increase with increasing potentially competing and interfering compounds (e.g., dirtier matrix). In fact, the cleanest water showed the highest percent variability. This result suggests that the highest variability in precision will occur as the sample is closer to the method detection limit and not by other compounds potentially present in the source water.

The NDMA precision guideline set by CDHS of ±20% for replicate analyses was meant to address intralaboratory precision. The discussion in this section addresses interlaboratory precision, which is generally expected to result in higher deviations than intralaboratory precisions. For all other nitrosamine compounds (NA_7), there was not one method that was able to meet the CDHS 20% reproducibility criteria at low nitrosamine concentrations (Figure 8.5b). However, with the exception for NMEA, Amb SPE was able to meet the 20% criteria. For high nitrosamine concentrations, all methods were able to meet the 20% criteria except for NMEA (Figure 8.5c). For NMEA, only Amb SPE was able to meet the CDHS precision criteria.

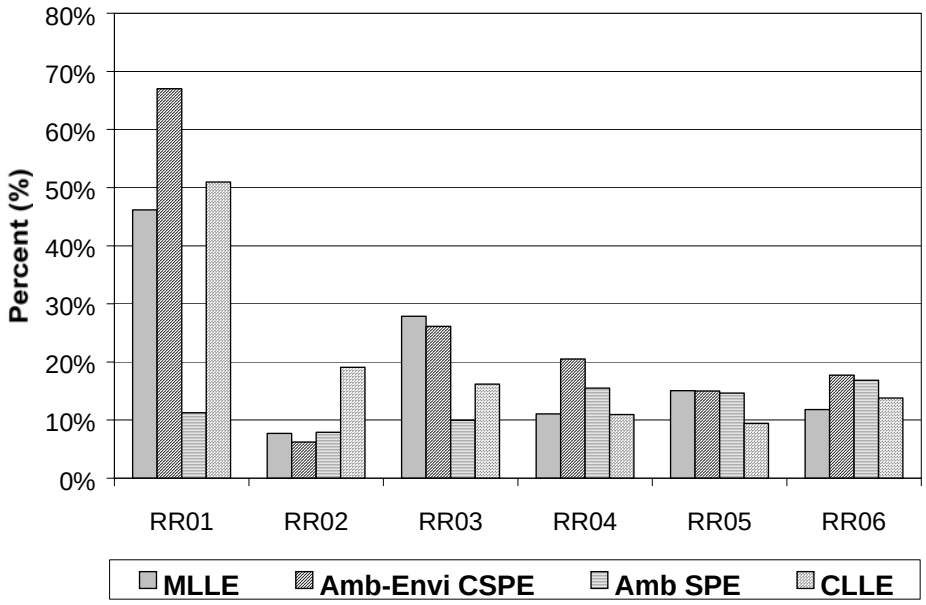


Figure 8.5a Relative standard deviation of NDMA for various waters

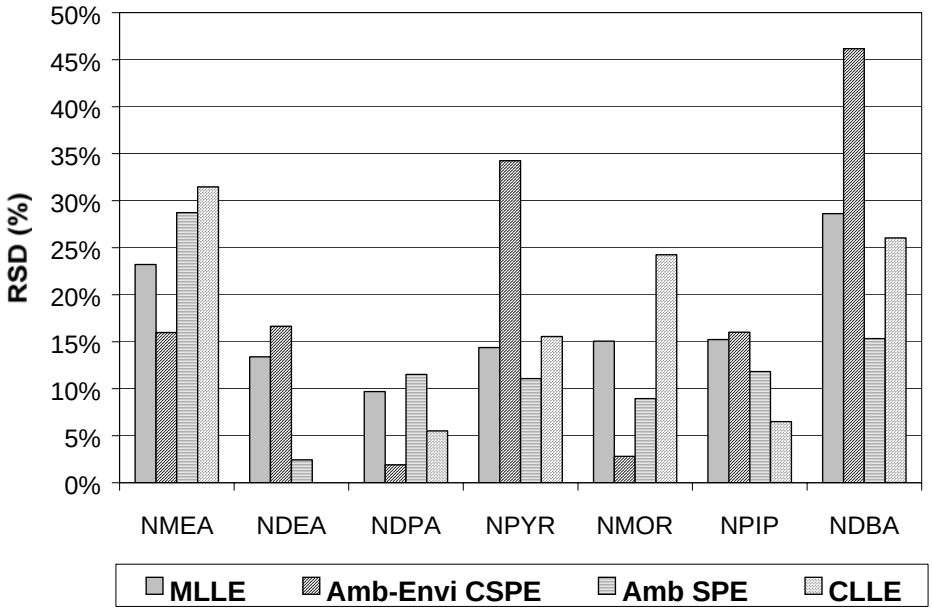


Figure 8.5b Relative standard deviation of low concentration NA_7 analysis

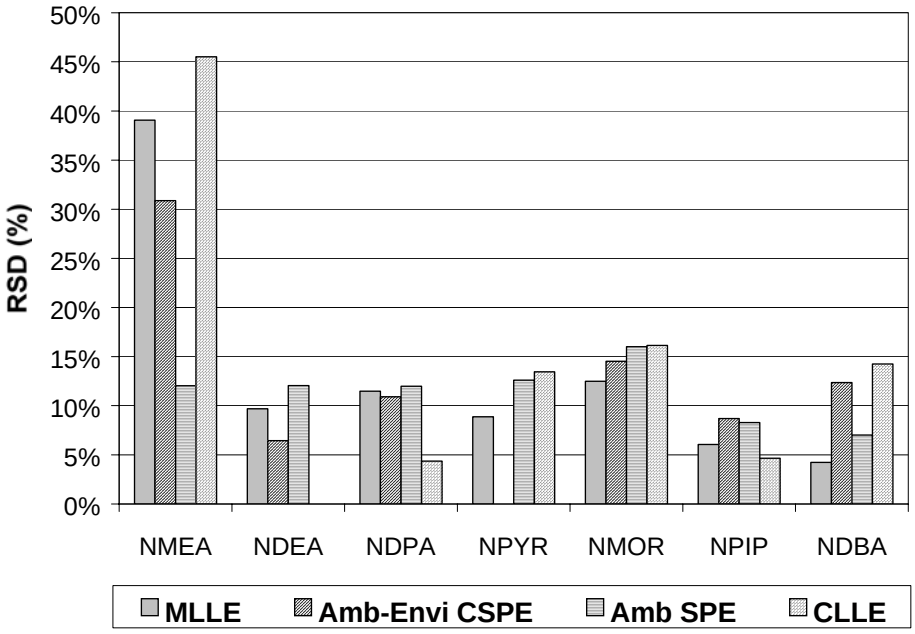


Figure 8.5c Relative standard deviation of high concentration NA_7 analysis

Reproducibility of each method against one another for each water was performed. Source RR01 is a relatively clean water that is low in all nitrosamines. The data shows that for NDMA, while the median values for CLLE and SPE are comparable, Amb SPE extraction method results in the highest degree of reproducibility as illustrated by the tight range for the 25th and 75th percentile (Figure 8.6a). The Amb-Envi CSPE method, while generating a median value that is close to the SPE and CLLE methods, has the highest degree of scatter for the 25th and 75th percentiles, and thus, is the least reproducible method for NDMA. For the other nitrosamine compounds in RR01, both Amb SPE and Amb-Envi CSPE are approximately equal for reproducibility, while MLLE method appears to be the least reproducible, illustrated by the highest degree of scatter (Figure 8.6b).

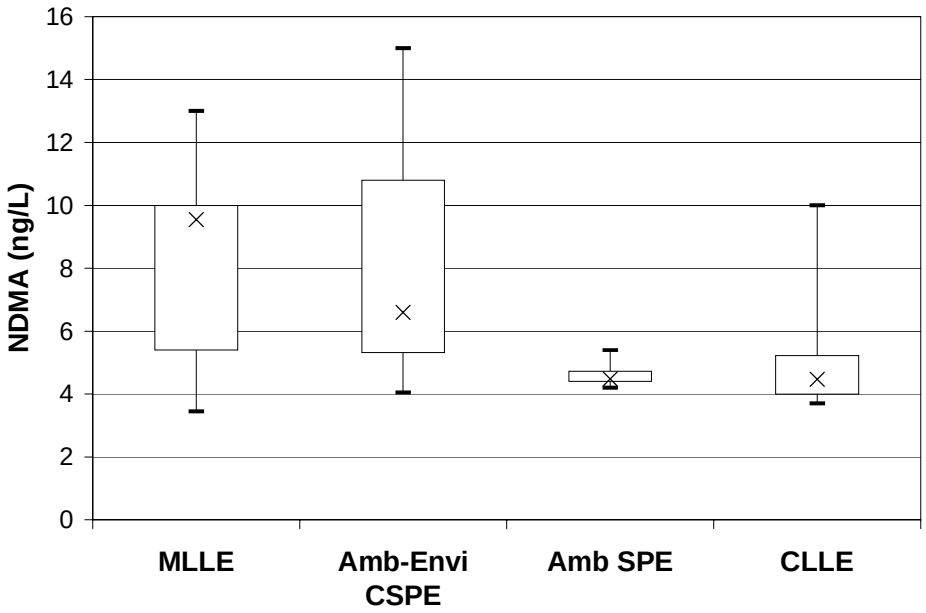


Figure 8.6a Comparison of four extraction methods for detection of NDMA in RR01

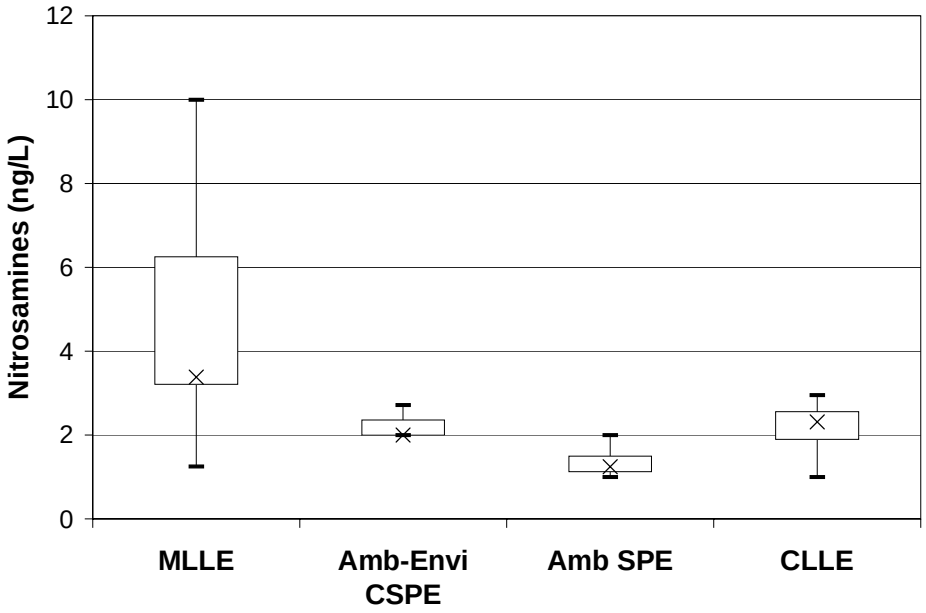


Figure 8.6b Comparison of four extraction methods for detection of average NA_7 in RR01

For source RR02, which is RR01 spiked with 12.7 ng/L of nitrosamine mix, showed slight variation in the median values but all methods show relatively good reproducibility for measuring NDMA (Figure 8.7a). For this water, MLLE and Amb-Envi CSPE have the highest level of reproducibility, while CLLE appears to have the lowest level of reproducibility. However, CLLE shows the highest reproducibility for all other nitrosamines, and MLLE was the least reproducible (Figure 8.7b).

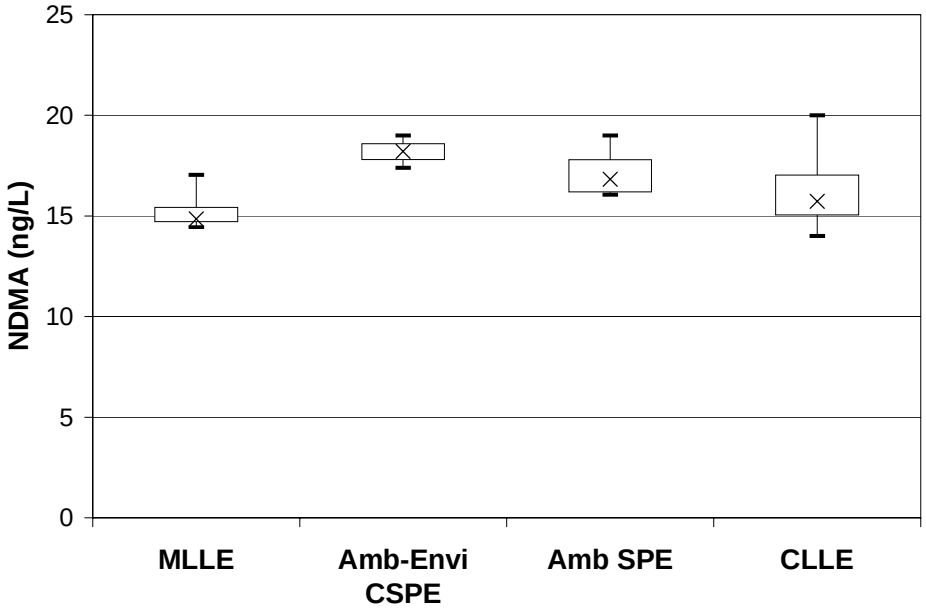


Figure 8.7a Comparison of four extraction methods for detection of NDMA in RR02

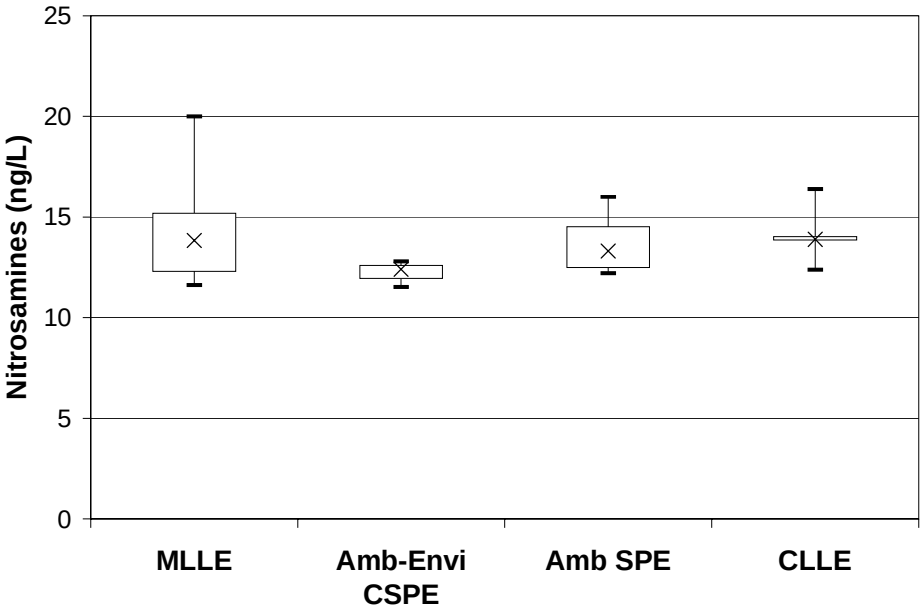


Figure 8.7b Comparison of four extraction methods for detection of average NA_7 in RR02

RR03 is tertiary effluent treated through the RO process and, therefore, should be relatively clean with respect to possible interfering compounds. Although it is not known what the exact concentration of NDMA and the other nitrosamines are, the pattern of results are similar as for RR01, which is a potable water source. The Amb SPE method resulted in the highest reproducibility for NDMA while MLLE was the lowest (Figure 8.8a). For all other nitrosamines, Amb-Envi CSPE showed the highest reproducibility while MLLE again was the least reproducible (Figure 8.8b).

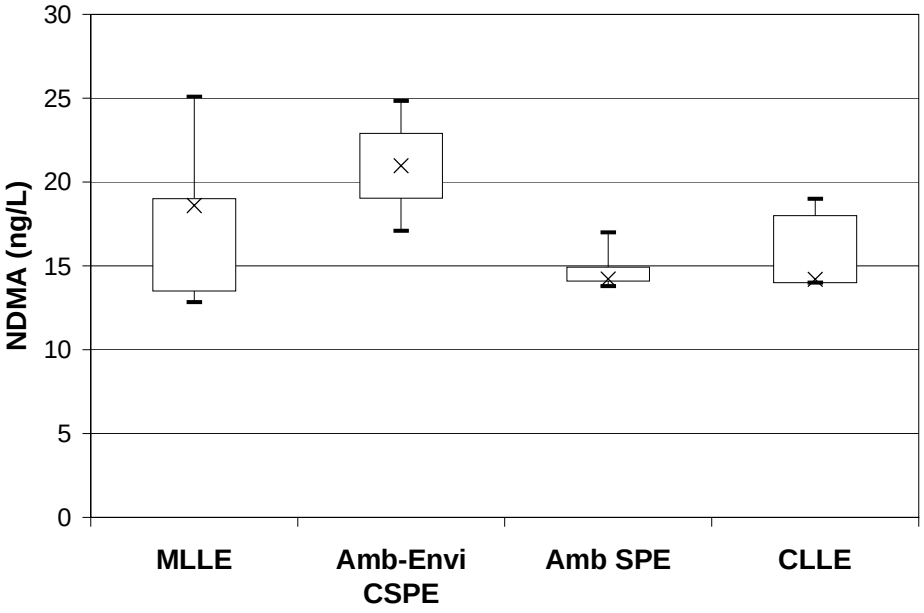


Figure 8.8a Comparison of four extraction methods for detection of NDMA in RR03

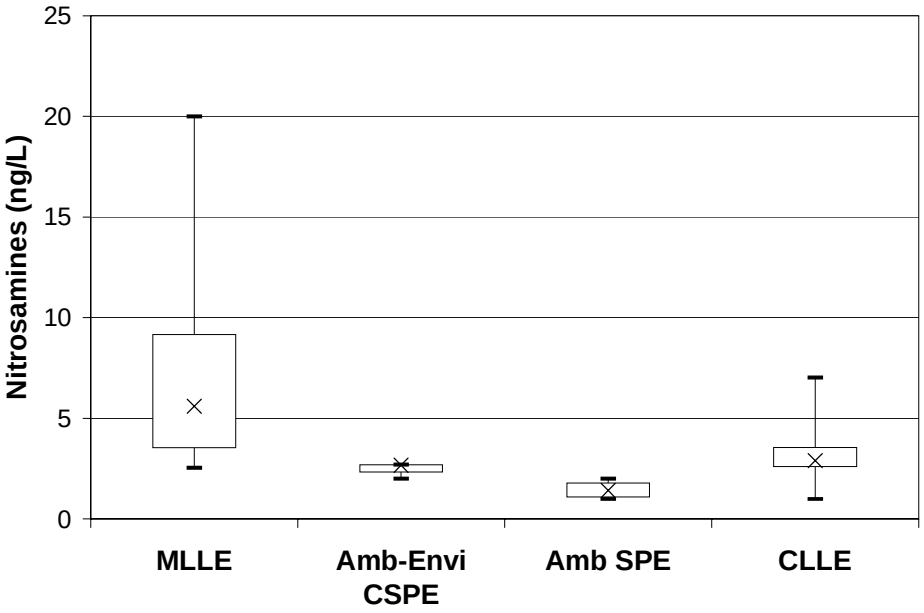


Figure 8.8b Comparison of four extraction methods for detection of average NA_7 in RR03

RR04 is secondary effluent, which potentially will contain many unidentified interfering compounds. For NDMA, the degree of scatter between the different methods was comparable, with MLLE having the highest reproducibility and Amb-Envi CSPE with the lowest (Figure 8.9a). For NA_7 values in RR04, Amb-Envi CSPE shows the highest reproducibility while MLLE shows the lowest (Figure 8.9b).

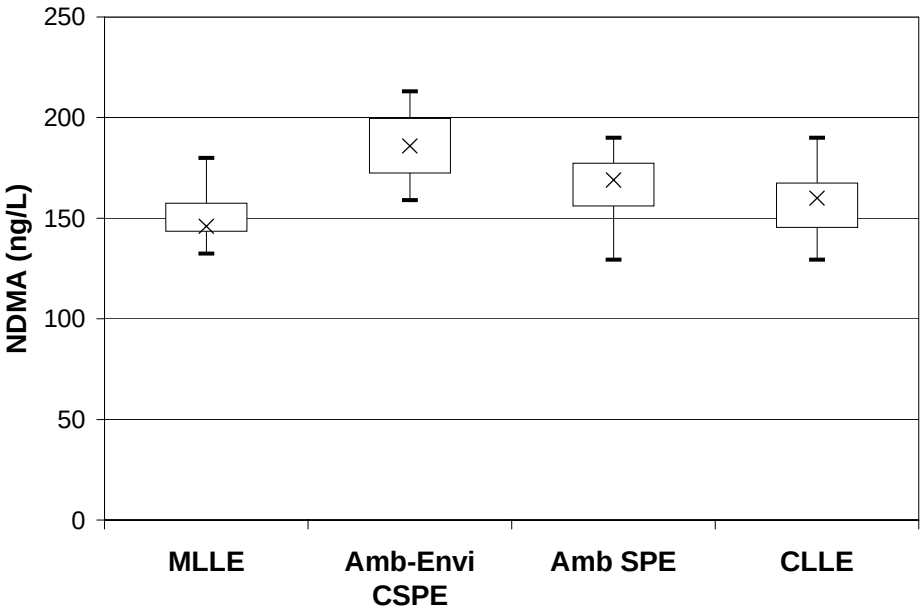


Figure 8.9a Comparison of four extraction methods for detection of NDMA in RR04

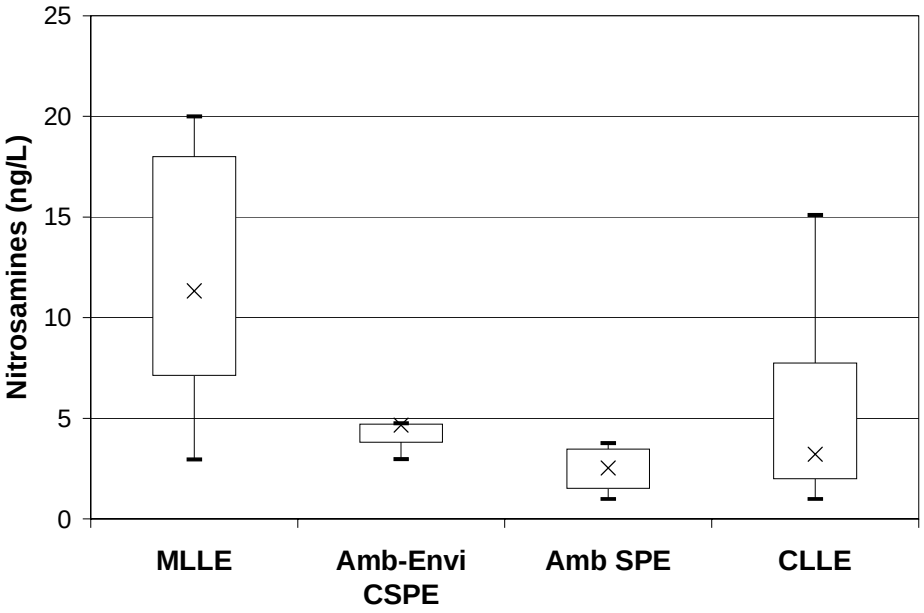


Figure 8.9b Comparison of four extraction methods for detection of average NA_7 in RR04

For NDMA in source RR05, both Amb SPE and CLLE methods were about equal for best reproducibility while Amb-Envi CSPE had the highest variability (Figure 8.10a). Similarly, Amb SPE showed the highest reproducibility and Amb-Envi CSPE had the greatest variability (Figure 8.10b).

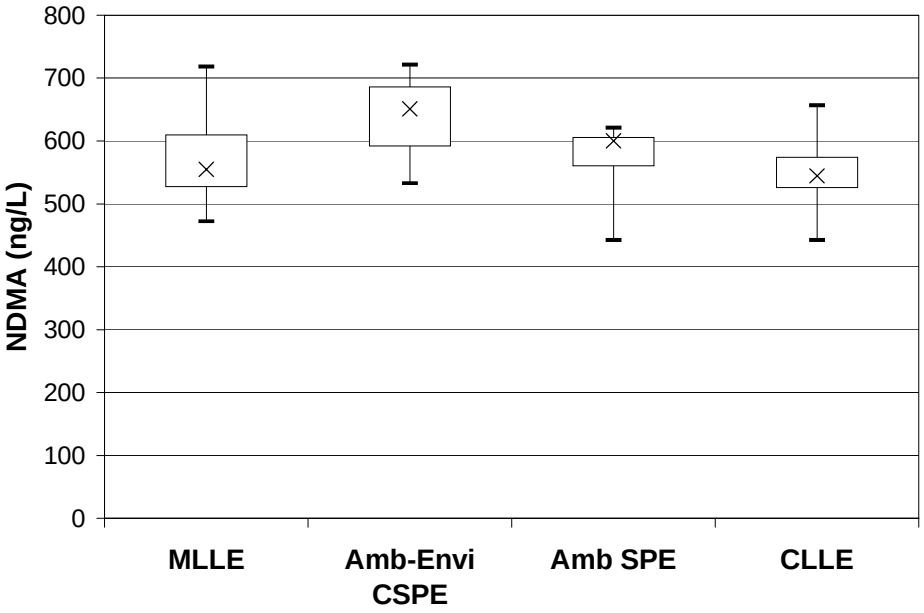


Figure 8.10a Comparison of four extraction methods for detection of NDMA in RR05

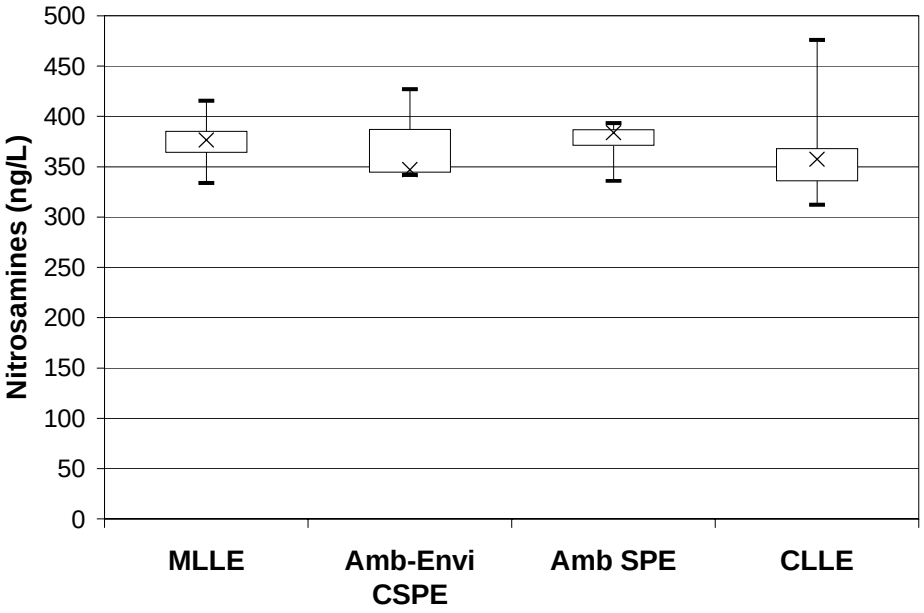


Figure 8.10b Comparison of four extraction methods for detection of average NA_7 in RR05

Sample RR06 is the chlorinated tertiary effluent sample. The results show comparable variability of NDMA detected by all methods, with Amb SPE having the highest reproducibility and CLLE the lowest (Figure 8.11a). However, for the other nitrosamines, Amb-Envi CSPE had the highest reproducibility while MLLE showed the lowest reproducibility (Figure 8.11b).

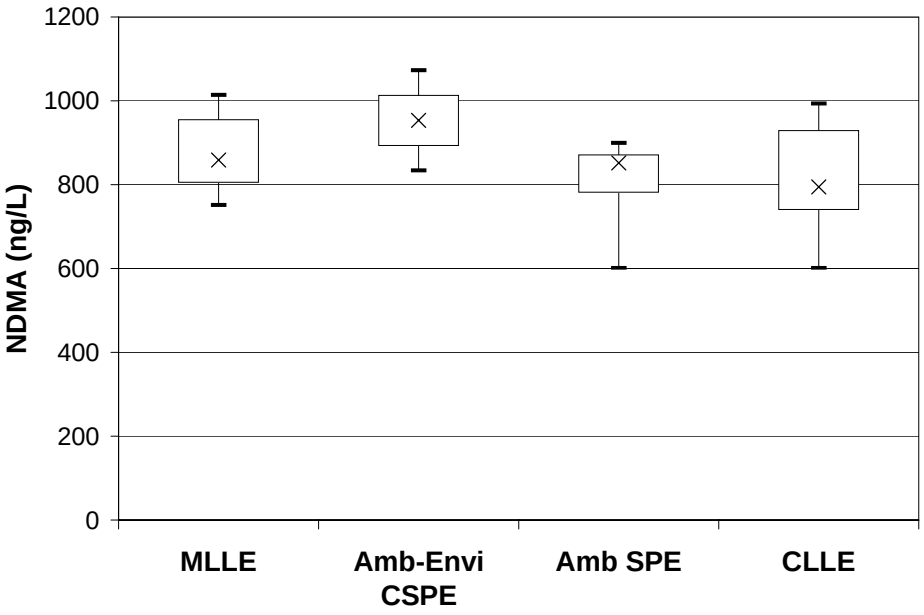


Figure 8.11a Comparison of four extraction methods for detection of NDMA in RR06

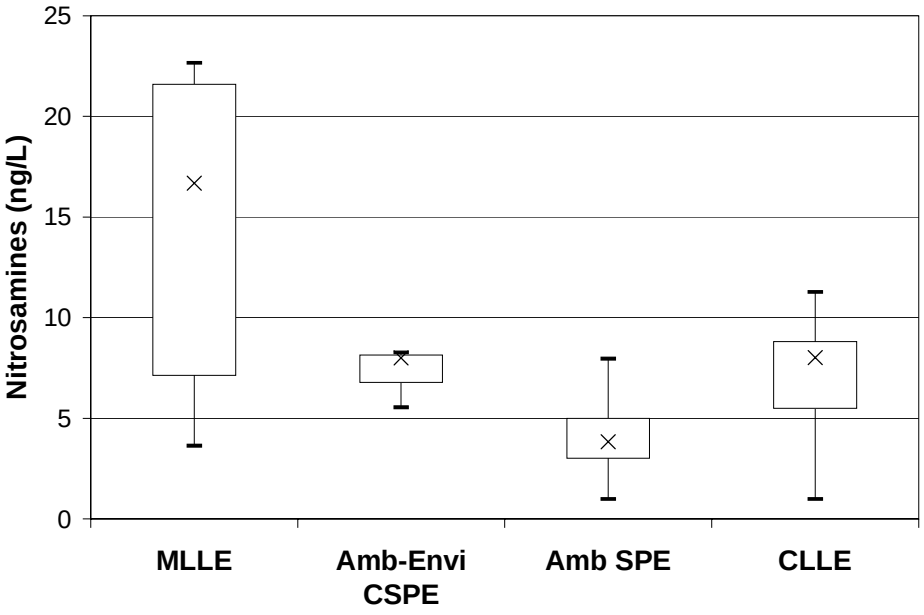


Figure 8.11b Comparison of four extraction methods for detection of average NA_7 in RR06

The results previously presented indicate that although all methods show acceptable degree of precision for NDMA, there was no correlation observed between methods that yielded the highest precision for NDMA and the average NA_7 for a specific water. In other words, the most precise extraction method for NDMA for a particular water was not always the most precise for all other nitrosamines. This is also true for the least precise method. Moreover, in the samples where nitrosamines were spiked, with exceptions of the nitrosamine concentration, all other factors should be considered equal. Thus, the potentially interfering compound for source RR01 should be the same for RR02 and RR04 should equal RR05. Thus, one would suspect that the method that resulted in the highest precision would be the same for both waters if interfering compounds affect precision. However, the most precise method differs for the two waters, indicating that potentially interfering compounds may not play a significant role.

As performed in the extraction accuracy section, a simplified ranking of precision for the various methods was performed in an attempt to simplistically summarize which method yielded the highest overall precision. Again, the method that yielded the smallest variability between the 25^{th} and 75^{th} percentile was given a ranking of 1 and the method with the highest variability was given a value of 4. With respect to NDMA analytical precision, the Amb SPE method was more precise on average compared to the other extraction methods (Table 8.8). With respect to all other nitrosamines except NDMA, the Amb-Envi CSPE and Amb SPE were equal in overall precision compared to the other two extraction methods (Table 8.9).

Table 8.8 Simplified ranking of precision for NDMA for all sources and extraction methods

Source	Method			
	MLLE	Amb-Envi CSPE	Amb SPE	CLLE
RR01	3	4	1	2
RR02	1	2	3	4
RR03	4	2	1	3
RR04	1	4	2	3
RR05	3	4	2	1
RR06	3	2	1	4
Total	15	18	10	17

Table 8.9 Simplified ranking of precision for all nitrosamines except NDMA for all sources and extraction methods.

Source	Method			
	MLLE	Amb-Envi CSPE	Amb SPE	CLLE
RR01	4	2	1	3
RR02	4	2	3	1
RR03	4	1	1	3
RR04	4	1	2	3
RR05	2	4	1	3
RR06	4	1	2	3
Total	22	11	11	16

Alternative Cartridge SPE Methods

Other CSPE methods have recently been proposed in addition to the Amb-Envi CSPE method, including one that was proposed by the USEPA (Munch and Bassett, 2003) that uses a coconut charcoal as the adsorbent, and another method proposed by Charrois et al. (2003) that uses a combination of Ambersorb and LiChrolut® EN media (Mod CSPE). All three CSPE methods provide an opportunity in the future to minimize sample preparation time by allowing manufacturers to prepackage cartridges that would be ready for use by laboratory staff for nitrosamine extraction work rather than spending time measuring out adsorbents. The USEPA method was not tested during this round-robin, but the Mod CSPE method was evaluated.

The results obtained from the Mod CSPE method fall within the range of Amb-Envi CSPE data. (see Table 8.10). In the spiked samples, the recoveries for all nitrosamines obtained by Mod CSPE were within the actual spike concentration ± 30%. The results suggest that the Mod CSPE method is comparable to the Amb-Envi CSPE method. Although the data is promising, additional testing is recommended to verify the accuracy as well as provide information on the reproducibility of this method

Table 8.10 Comparison of NDMA concentrations by CSPE to average of all other methods for different waters

(all values reported in ng/L)

Method	RR-01	RR-02	RR-03	RR-04	RR-05	RR-06
All Methods	6.4 ± 3.6	16.3 ± 2.0	16.6 ± 3.9	157 ± 24	574 ± 81	844 ± 125
All Amb-Envi CSPE	8.6 ± 5.7	18.2 ± 1.1	21.0 ± 5.5	186 ± 38	635 ± 95	954 ± 169
Mod CSPE	3.7	12.8	13.3	165	538	770
	Δ RR02 - RR01 = 12.7 ng/L			Δ RR05 - RR04 = 376 ng/L		
All Methods	10 ng/L			417 ng/L		
All Amb-Envi CSPE	9.6 ng/L			449 ng/L		
Mod CSPE	9.1 ng/L			373 ng/L		

Costs Comparison

Due to various factors, providing a detailed cost comparison for these different methods is a difficult task. The cost differences are not only dependent on the method selected, but also on the institution performing the analysis. The contributing factors to the cost differences in methodology include, but are not limited to equipment needed, reagents required, and the skill level and time required. Because different organizations handle cost accounting differently, including issues as overhead and fringe benefits, it is not unexpected to see different cost pricing from different organizations for the same method.

It is beyond the scope of this project to derive the price for a nitrosamine analysis; charges are set by the organizations choosing to perform the analyses. Instead, the factors that influence the cost, and an example of how these factors may impact the final cost and to some extent the price, are provided. For sample analysis, it was determined that the costs include the following components: equipment (e.g., instrumentation), consumables (e.g., reagents), and labor. A sensitivity analysis performed showed that although the initial equipment cost may be substantial, the contribution from this cost component is overshadowed by the labor cost. Because the major analytical cost component is labor, it was felt that data showing the range of times required for each analysis would be of value.

It is worth noting that when using the analytical time data, there is a tradeoff between laboratory automation, cost, and space. The traditional LLE method may be performed by either hand-shaking the samples, or by the use of continuous extraction through refluxing the sample. Although the continuous process may require more overall time, the time that is required of the analyst is a fraction of the total processing time. To a degree, the CLLE process may be considered to be more automated than the hand-shaking method, but will take up more room. If a laboratory has sufficient space, CLLE may be a preferred method over the manual LLE.

Table 8.11 shows a range of times required for the various methods tested during this project, as well as the detection sensitivities.

Table 8.11 Summary of laboratory analytical capabilities

Extraction method	No. labs tested	Time (hrs) (6 samples)	NDMA[1] detection (ng/L)
MLLE	6	7 – 26	2 - 20
Amb SPE	4	15 – 26	1 - 2
Amb-Envi CSPE	3	11 – 32 (44)[2]	2 - 4 (2)
LLE (manual or continuous)	5	30 - 32	2
SPME	1	19	30

[1]*NDMA Detection – the lowest level or range of levels as reported by participating laboratories*

[2]*Number in parenthesis is the result for Mod CSPE*

Cost Example

It was determined that for the six samples tested during the round-robin phase, the Amb SPE method would require between 15 to 27 hours for analysis (Table 8.12). A summary of time breakdown by methods is provided in the following table. The differences in the total times required are dependent on a number of factors, including the number of QA/QC samples analyzed and the familiarity with the method. It appears that major component of the differences observed between the different times was in the extraction/separation/desorption step, and fewer differences were observed in the quantitation/detection/reporting steps. On average, it appears that the Amb SPE method requires the least amount of time per sample to analyze.

Table 8.12 Summary of representative analytical times required for various methods

Step	LLE	Amb SPE	MLLE	SPME	CSPE
No. samples	6	6	6	6	6
No. samples analyzed, includes standards	20 - 34	16 - 43	6 - 19	16	16 - 30
Sample preparation (hr)	1 - 2	1 - 2	1 - 2	4	1 - 8.5
Extraction/Separation/Desorption (hr)	16 - 22	5 - 7	4 - 6	4	3 - 20
Quantitation/Detection (hr)	7 - 12	6 - 18	7 - 8	8	6 - 8
QA/QC/Reporting (hr)	1 - 2	2 - 5	1 - 5	3	1 - 8
Total (hr)	30 - 32	15 - 27	16 - 23	19	11 - 44
Time/sample (inc. standards) (min)	60 - 90	40 - 80	70 - 80	70	40 - 120

An example of a cost analysis for NDMA is described below using the Amb CSPE extraction method. The costs considered included the amortized cost for the equipment, standards, consumables and labor. For equipment, the cost includes a GC/MS for quantitation, autorotator for mixing, and water treatment systems. The annual service agreement for the GC/MS is estimated at $10,000. The authors recognize that the GC/MS will allow the laboratory to perform other analyses in addition to nitrosamines, and therefore the amortized cost per sample for nitrosamine analysis will be lower as a result. Using a five-year life cycle for the equipment and amortized at 7%, it was determined that the annual cost is approximately $29,366, representing a conservative estimate (Table 8.13).

Table 8.13 Summary of equipment cost for example laboratory (GC/MS)

Equipment	Cost	Depreciation (yrs)	Interest rate	Annual cost
GC/MS	$71,000	5	7%	$17,317
Auto-rotator	$5,000	5	7%	$1,219
DI-water	$3,000	5	7%	$732
UV system for water	$400	5	7%	$98
Instrument svc. contract				$10,000
Total				$29,366

For standards required, it is expected that the calibrations would be required 12 times annually, for a total cost of $6,620. For materials, the cost is made up of consumables, including reagents and gases, and the estimated cost per sample is $7.80. For labor, it is expected that approximately 2.0 hours of an analyst's time is required, with approximately 0.5 hours of a supervisor's time to check and report the data. The labor rates will vary by market as well as the industry segment; however, it may be reasonable to expect that the labor rate for a laboratory supervisor may be double of that for an analyst. For this example of a laboratory located in a large metropolitan area, a labor cost of $62.50 per sample may be expected, not including any overhead or benefits.

Table 8.14 Summary of consumables and labor cost for example laboratory

Standards	Cost	Calibrations	Unit	Subtotal
Nitrosamine standard 1	$64.64	12	yr	$775.68
Nitrosamine standard 2	$37.00	12	yr	$444.00
NDMA-d_6 standard	$150.00	12	yr	$1,800.00
NDEA-N2 standard	$150.00	12	yr	$1,800.00
NDPA-d14 standard	$150.00	12	yr	$1,800.00
Standards (total)				$6,619.68

Materials	Unit Cost	Qty	Unit	Ea analysis	Cost/sample
Methanol	$150.00	6	L	0.02	$0.50
DCM	$120.00	6	L	0.03	$0.60
Vials (2 mL amber)	$20.00	100	Vial	1.0	$0.20
Vial Caps (2 mL)	$25.00	100	Cap	1.0	$0.25
Conical inserts	$20.00	100	Insert	1.0	$0.20
Ambersorb 572	$87.00	100	Gm	0.3	$0.26
Filter paper	$40.00	100	Piece	1.0	$0.40
Syringe			Piece		$3.50
Gases	$500.00	80	Hr	0.3	$1.88
Materials (total)					$7.79

Labor	Cost	Qty	Unit	Subtotal	Cost/sample
Analyst time	$23.00	1	hr	2.0	$46.00
Administrative time	$35.00	1	hr	0.5	$17.50
Labor (total, no multiplier)					$62.50

From the data presented in Table 8.14, it is clear that the labor cost overshadows the cost of the materials. The cost per sample is also dependent on the number of samples that are analyzed and the multiplier used for labor. Using the data presented above provides an example for hypothetical costs based on one

utility's estimate (Table 8.14). In Figure 8.12, it is assumed that a minimum number of QA/QC samples will be needed. Even for one sample analyzed per week, a minimum of 14 QA/QC additional samples, including blank, matrix spike, and duplicate will be required. For more than 10 samples analyzed per week, the additional QA/QC samples required would be the 14 plus 33% of the total samples. This graph should be only used for illustrative example and not to reflect the actual charges imposed by vendors.

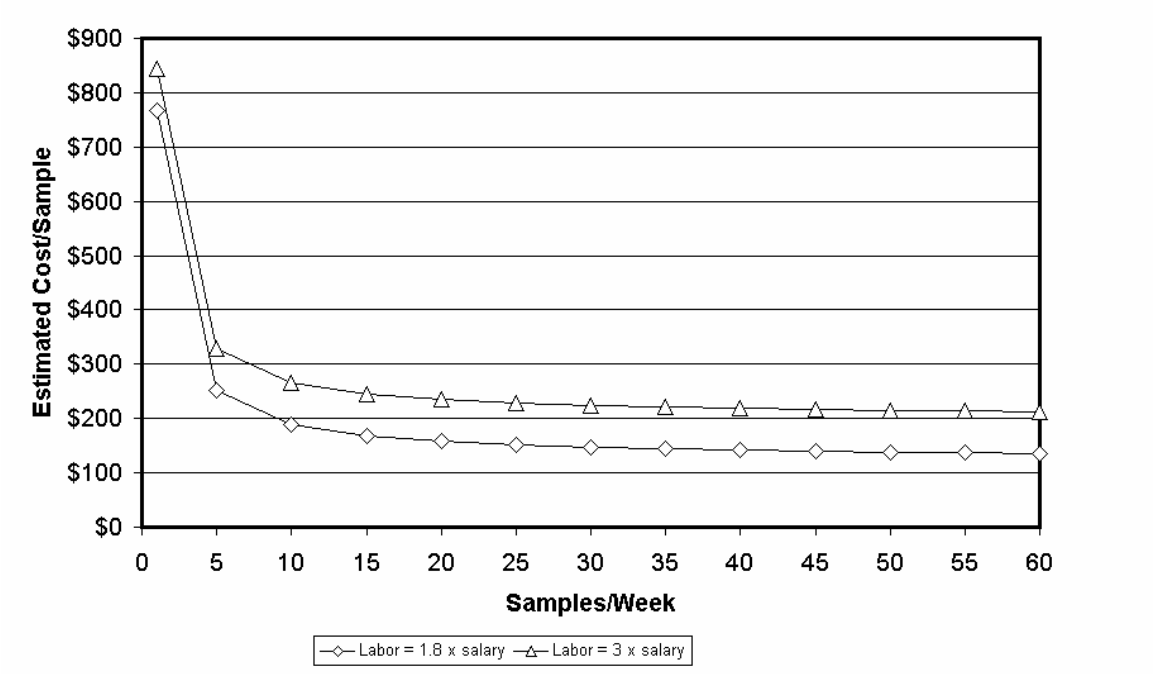


Figure 8.12 Sample graph of estimated costs for nitrosamines analysis

It can be seen that the per-sample cost decreases significantly with the increased number of samples processed. The upper bound of this graph, at 60 samples per week, represents a total of 94 samples processed per week, which includes the required QA/QC samples. Depending on the method selected and the equipment available, the maximum number of samples processed may be more or less. In this example, it is possible to reduce the cost through the SPE method to approximately $100 (based on the lower multiplier factor). As the number of samples increase, the effects from the capital amortization portion is diminished, and the contribution from labor becomes the largest component of the cost

Many factors may be included in the labor multiplier, including rent, support services (e.g., clerical), and fringe benefits for the employees. In the authors' dealings with various agencies, the multiplier can be less than 1 to as much as 2, resulting in a final labor charge that is less than double the labor rate to tripling the labor rate. It is likely that the higher multiplier takes into account all the factors mentioned above, whereas the lower multiplier only takes into account the fringe benefits for the employees. The impact on the sample cost from the multiplier can be the most significant factor. The graph above demonstrates that depending on the multiplier used, the cost based on processing a large batch of samples may be as little as $100, based on a 1.36 x multiplier (e.g., utility), to $200 per sample based on a 3 x multiplier (e.g., commercial).

The costs from this graph should not be used for pricing purposes. Rather, the purpose is to reinforce the fact that the consumables and amortized equipment cost are not significant factors in the final costs. The two more important factors that should be considered are the amount of time required to perform the analysis and the organization's overhead factors. On that basis, it appears that the methods examined in this study require the same or less time than the baseline method, CLLE, and therefore have the potential to have lower associated costs. From the initial survey data showing that the range of costs for NDMA

analysis ranged from $350 to $530 per sample, it is evident that there is potential for lowering the cost. Although there is evidence that the current per sample cost may be as low as $250, the results from this study should aid in lowering the analytical cost.

Summary and Conclusions

For the round-robin testing, the goals were to use multiple independent laboratories to: (1) evaluate the feasibility of using the various refined methods; (2) determine the accuracy and precision of the methods in determining nitrosamine levels for various source waters; and (3) compare the various methods against each other. Twelve laboratories participated in the round-robin testing process, with four methods evaluated: MLLE, Amb SPE, Amb-Envi CSPE, and CLLE. Each method had three to five participants.

General Conditions and Findings

- Four water matrices were tested. From low to high in NDMA concentrations, the order is potable (RR-01) < RO effluent (RR-03) < secondary wastewater effluent (RR-04) < tertiary wastewater effluent (RR-06).
- Two additional samples were included: RR-02 is the same sample as RR-01 (potable), with a 12.7 ng/L spike for all nitrosamines, and RR-05, which is the same sample as RR-04 with a 376 ng/L spike of all nitrosamines.
- The trend observed for NDMA concentrations was also observed for the other seven measured nitrosamines.
- NDMA was the highest single nitrosamine in all the samples and can be as high as 90% of total nitrosamine.

Accuracy, Low Concentration Detection (12.7 ng/L for individual NAs)

The following observations are based on the 50^{th} percentile values for each method.

- All methods were able to produce results that met CDHS guidelines of 70 to 130% recovery for NDMA and NA_7.
- Amb SPE and Amb-Envi CSPE appeared to be the methods able to produce results closest to the actual spike for NDMA (RR-02 minus RR-01 = 12.7 ng/L)
- CLLE was more accurate than MLLE, which has the highest MDL of the methods tested in the round-robin and an MRL greater than the NDMA concentration in RR-01.
- For the other nitrosamines (NA_7), the accuracy for detection is as follows: Amb SPE ≈ CLLE > Amb-Envi CSPE > MLLE.
- Of the NA_7, all methods were consistently biased high for NMOR, and biased low for NDPA (based on comparison with spiked 12.7 ng/L).

Accuracy, High Concentration Detection (376 ng/L for individual NAs)

The following observations are based on the 50^{th} percentile values for each method.

- All methods were able to produce results that met CDHS guidelines of 70 to 130% recovery for NDMA and NA_7.
- All methods appear to produce similar results to the actual spike for NDMA (RR-05 minus RR-04 = 376 ng/L), although Amb-Envi CSPE was the least accurate.
- For NA_7, the accuracy for detection is as follows: Amb SPE ≈ CLLE ≈ MLLE > Amb-Envi CSPE.
- Of the NA_7, with the exception of NMEA, Amb-Envi CSPE appeared to underestimate the spiked concentration.

Precision, Low Concentration Detection (12.7 ng/L for individual NAs)

The following observations are based on RSD and 25th to 75th quartile values for each method.

- All methods were able to produce results that met CDHS guidelines of within 20% RSD for NDMA. However, no single method was able to meet the 20% criteria for all nitrosamines at low levels. Therefore, these criteria may be too rigid for the low-level detection of the other nitrosamines.
- Amb SPE and Amb-Envi CSPE appeared to be the most accurate as well as precise methods.
- CLLE was more accurate and precise than MLLE, which has the highest MDL of the methods tested in the round-robin.
- For NA_7, reproducibility for Amb SPE and CLLE appeared to be consistently better than for Amb-Envi SPE and MLLE.

Precision, High Concentration Detection (376 ng/L for individual NAs)

The following observations are based on RDS and the 25th to 75th quartile values for each method.

- All methods were able to produce results that met CDHS guidelines of 20% for all nitrosamines except NMEA. For NMEA, only Amb SPE met the 20% criteria.
- With the exception of NMEA, all methods appear to have similar precision. CLLE was the least precise method for this nitrosamine.

Methods Comparison for Potable Water (RR-01, RR-02)

- The exact nitrosamine concentrations for RR-01 and RR-02 are unknown.
- A method of comparison for accuracy is to calculate the spike recovery by taking the difference from RR-02 and RR-01, and determining how close the value is to the 12.7 ng/L spiked concentration.
- By comparison of the spiked value and the 50th percentile values, it appears that Amb SPE and Amb-Envi CSPE are the most accurate methods overall, while MLLE is the least accurate.
- With respect to precision (range of values covered by 25th to 75th quartile), it appears that the Amb SPE and MLLE are more reproducible compared to CLLE and Amb-Envi CSPE for NDMA. However, for NA_7, Amb-Envi CSPE and Amb SPE were more reproducible compared to MLLE and CLLE.
- The ratio of standard deviation to the mean NDMA value support the conclusion above, that this ratio is the smallest for Amb SPE.

Methods Comparison for RO Effluent (RR-03)

- The behavior of the methods to this water is similar to that for the potable water (both water matrices should be relatively clean).
- Amb SPE and CLLE methods appear to produce similar mean NDMA results. However, Amb SPE was more reproducible than all other methods for NDMA.

Methods Comparison for Secondary Wastewater Effluent (RR-04, RR-05)

- By using the difference method and based on the 50th percentile values, it appears that all methods produced similar results for NDMA and other nitrosamines.
- It appears that all methods are similarly reproducible (comparing range of values covered by 25th to 75th quartile).
- The ratio of standard deviation to the mean NDMA value support the conclusion above, that the ratios for all methods are similar.

Methods Comparison for Tertiary Wastewater Effluent (RR-06)

- Based on the 50th percentile values, it appears that all methods produced similar results for NDMA.
- For the NA_7, it appears MLLE is the least reproducible (but based on an overall average NA concentration of < 20 ng/L).

Cartridge SPE Comparison

- Only one laboratory evaluated Mod CSPE.
- The Mod CSPE method appears to be comparable to the Amb-Envi CSPE for both the low and high nitrosamine waters.
- Because only one laboratory evaluated the Mod CSPE method, additional testing is strongly recommended to verify the accuracy as well as to provide information on the reproducibility of this method.

Cost Comparison

- Fourteen laboratories participated in the round-robin testing.
- It appears that the methods tested during the round-robin require less time than that reported for the CLLE method (30 hours).
- A wide variation of analytical time was reported for the different methods, reflecting differences in batches and laboratory experiences.
- The analytical time reported ranged from 11 to 30 hours, with most participants requiring 24 hours or less to process six samples.
- The higher times are likely due to the laboratories' application of a new method. As a laboratory becomes more familiar with a method, it is expected that the analytical time required will decrease toward the lower range. The differences between the various laboratories may be minimized after each laboratory has sufficient time in testing the methods.
- For the cost analysis, it appears that the labor charge and the institution's multiplier will influence the price of the analysis more than the instrumentation or the consumables costs.
- From the cost example, it appears that the methods examined in the round-robin testing should be able to be lower-cost methods than the lowest cost obtained during the initial laboratory survey of $325 per sample for NDMA.

CHAPTER 9

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

A summary of the findings from the individual chapters is presented in the following section.

Sample Collection and Storage Recommendations (Chapter 2)

- Collect samples as representative grab or composite volumes of 1-L.
- Collect samples in pre-cleaned, amber glass containers with Teflon lined caps.
- Avoid sample contact with common rubber, because additives are known to contain compounds that have been identified as NDMA precursors.
- Chlorine and chloramines residuals must be quenched to minimize additional formation. Add 0.5 g of sodium sulfite (or sodium thiosulfate) to wastewater in 1 L sample containers or 0.04 to 0.1 g to drinking water sample bottles for typical chlorine residuals. Alternatively, ascorbic acid may be used for drinking water sample preservation.
- Include laboratory and field blanks as part of a sampling SOP.
- Analyze a method blank for quality control with each batch.
- Check extraction solvents for contamination prior to use.
- When possible, use UV-irradiation for the laboratory DI waters used as blanks.

LLE Findings (Chapter 3)

- The order of extraction efficiency for the target nitrosamines is:
  NDMA < NMEA ≈ NPYR ≈ NMOR < NDEA ≈ NPIP < NDPA ≈ NDBA.
- DCM is a much better extraction solvent as compared to MtBE.
- Salting-out greatly increased extraction efficiency especially for NDMA extractions, with NaCl being more effective and easier to handle than sodium sulfate.
- pH had minimal effect on nitrosamine extraction efficiencies.

SLLE Method Conditions

- Sample volume is 500 mL.
- A single 200 mL DCM extraction is performed in a separatory funnel.
- Final volume concentrated to 0.5 mL and preferably performed on an automated system.
- The MDL for NDMA is 0.5 ng/L, and for other nitrosamines ranged from 0.3 to 0.7 ng/L.

MLLE Method Conditions

- Sample volume is 100 mL.
- A single 20 mL DCM extraction is shaken in a bottle on a mechanical shaker.
- The extract is separated by syringe.
- Final volume is concentrated to 0.5 mL, preferably performed on an automated system.
- The MDL for NDMA is 2.3 ng/L, and for the other nitrosamines ranged from 1.8 to 3.8 ng/L.
- Accuracy for NDMA is 97.8% ± 12.4%, and precision is 10.0% ± 8.9%.
- For other nitrosamines, accuracy ranges from 82.8% to 97.2%, and precision from 6.2% to 14.6%.

Amb SPE Findings (Chapter 4)

- Recoveries for all nitrosamines at 120 minutes shake time exceeded 80%, except for NDMA, which was 60%.
- Recovery of NDMA increases when Ambersorb is increased from 50 to 400 mg, but marginal benefits were observed after 200 mg.
- Adding 1 M NaCl during extraction resulted in an increase in recovery between 12 and 20% for NDMA and the other nitrosamines.
- Acceptable absolute recoveries were obtained when no salt was added, resulting in 60% for NDMA and over 80% for the other nitrosamines.
- Adjustment of pH alone during extraction had little effect on the recovery of NDMA and other nitrosamines.
- This study shows a 1 ng/L MDL value can be reached for all nitrosamines extracted for 120 minutes under baseline conditions (Taguchi et al., 1994). On average, approximately 60% of the variability in values can be attributed to the extraction process and 40% to the detection process for all nitrosamines.

Amb SPE Method Conditions

- The sample volume is 500 mL.
- The Ambersorb mass is 200 mg.
- Contact time is 2 hours.
- No pH adjustment and no salt addition is needed.
- Extraction volume is set at 400 µL DCM.
- The MDL for NDMA is 0.8 ng/L, and for other nitrosamines ranges from 0.5 to 1.1 ng/L.

Amb-Envi CSPE Findings (Chapter 5)

- Oasis® HLB, MCX, and MAX sorbents did not extract NDMA from water.
- Ambersorb® 572+Envi-carb dual-media cartridges produced recoveries of 60% of the NDMA and 70 to 95% of the other seven nitrosamines.
- The optimal flow rate was 5 mL/min.
- Three 2 mL DCM elutions recovered 96% or more of the extractable nitrosamines.
- A cartridge air-drying time of 45 minutes or greater was needed to remove moisture and to promote better extraction of the nitrosamines from the media.
- An Ambersorb® layer of 350 mg resulted in a higher recovery of NDMA, but 250 mg resulted in better recoveries for the other nitrosamines.
- The MDLs for the Amb-Envi CSPE analysis were 0.7 ng/L for NDMA and from 0.3 to 1.4 ng/L for the other nitrosamines.

SPME Findings (Chapter 6)

- A CAR/PDMS fiber coating with headspace extraction provided the highest NDMA recovery of the fibers tested.
- NDMA and NMEA extractions were maximized at 65 °C, but 95 °C provided the best extraction of NDEA, NDPA, NDBA, and NPIP.
- Of the eight nitrosamines tested, only NPYR could not be analyzed with SPME.
- The MDL for SPME-NCD was 57 ng/L for NDMA, and it ranged from 58 to 193 ng/L for five other nitrosamines. NMOR and NPYA were not detected
- The MDL for SPME-GC/CI/MS/MS was 30 ng/L for NDMA and 60 ng/L for six other nitrosamines.

- SPME-NCD spike recoveries in wastewater were within 10% of true value, excluding NPIP.
- SPME-GC/CI/MS/MS spike recoveries, excluding NMEA, were within 30% of true values.
- SPME-GC/CI/MS/MS could not analyze NMEA in a wastewater matrix.

NCD Findings (Chapter 7)

- Dual column confirmation using 30-m DB-1701 and 60-m Supelcowax 10 capillary columns provided reliable NCD results confirmation without the need for a mass spectrometer.
- Neither decreased NCD pyrolysis temperature, nor varying pH of CLLE were effective for minimizing detector interferences.
- An inline nitric oxide-selective trap resulted in excellent removal of NCD interferences.
- IDLs without a NO-selective trap were 0.8 μg/L for NDMA and 0.9 μg/L to 3.6 μg/L for other nitrosamines. IDLs with a trap were 3.3 μg/L for NDMA and 2.3 μg/L to 6.6 μg/L for other nitrosamines.
- MDLs for a NCD detector combined with the Amb SPE method were 6.5 ng/L for NDMA and 5.7 ng/L to 22.1 ng/L for other nitrosamines.
- CLLE with interference removal and Amb SPE could be used with NCD analysis for nitrosamines.

NPD Findings (Chapter 7)

- Maximum NPD response to NDMA and other nitrosamines occurred at a detector temperature of 300 °C, carrier gas flow of 3 mL/min, make-up gas flow of 5 mL/min, and H_2:Air ratio of 0.8 (4 mL/min:50 mL/min).
- Recycled CLLE extracted under acidic conditions provided best removal of NPD interferences.
- Both silica and C_8 prepacked SPE cartridges resulted in excellent clean-up of CLLE extracts, with recoveries of 84.1 and 79.9%, respectively, for NDMA.
- Amb SPE showed better performance with the NPD than did CLLE without extract clean-up steps.
- IDLs were 5.0 μg/L for NDMA and 3.6 μg/L to 8.8 μg/L for other nitrosamines.
- MDLs for Amb SPE methods were 10.9 ng/L for NDMA and 13.3 ng/L to 70.1 ng/L for other nitrosamines.

Round-Robin Comparison of Methods (Chapter 8)

- Fourteen laboratories were selected for round-robin testing.
- Four methods were evaluated during the round-robin: MLLE, Amb SPE, Amb-Envi CSPE, and CLLE. Each method was evaluated by three to five laboratories.
- SPME was not tested during the round-robin because not enough laboratories had the necessary equipment.
- Four water matrices were tested. From low to high in NDMA concentrations, the order is potable (RR-01) < RO effluent (RR-03) < secondary wastewater effluent (RR-04) < tertiary wastewater effluent (RR-06).
- The trend observed for NDMA concentrations was also observed for NA_7.
- Two additional samples were included: RR-02 is same sample as RR-01 (potable), with a spike 12.7 ng/L for all nitrosamines, and RR-05, which is the same sample as RR-04 with a 376 ng/L spike of all nitrosamines.
- NDMA was the highest single nitrosamine present in all the samples.
- NDEA was detected in some samples, but the levels detected were less than 9 ng/L, which is lower than the new Notification Level of 10 ng/L.

Accuracy, Low Concentration Detection
(12.7 ng/L spike for individual NAs)

The following observations are based on the 50th percentile values for each method.

- All methods were able to produce results that met CDHS guidelines of 70 to 130% recovery for NDMA and NA_7.
- Amb SPE and Amb-Envi CSPE appeared to be the methods able to produce results closest to the actual spike for NDMA (RR-02 minus RR-01 = 12.7 ng/L).
- CLLE was more accurate than MLLE, which has the highest MDL of the methods tested in the round-robin.
- For the other nitrosamines (NA_7), the accuracy for detection was as follows: Amb SPE ≈ CLLE > Amb-Envi SPE > MLLE.
- Of the NA_7, all methods were consistently biased high for NMOR, and biased low for NDPA (based on comparison with spiked 12.7 ng/L).

Accuracy, High Concentration Detection
(376 ng/L spike for individual NAs)

The following observations are based on the 50th percentile values for each method.

- All methods were able to produce results that met CDHS guidelines of 70 to 130% recovery for NDMA and NA_7.
- All methods appeared to produce similar results to the actual spike for NDMA (RR-05 minus RR-04 = 376 ng/L), although Amb-Envi CSPE was the least accurate.
- For NA_7, the accuracy for detection was as follows: Amb SPE ≈ CLLE ≈ MLLE > Amb-Envi SPE.
- Of the NA_7, with the exception of NMEA, Amb-Envi CSPE appeared to underestimate the spiked concentration.

Precision, Low Concentration Detection
(12.7 ng/L spike for individual NAs)

The following observations are based on RSD and 25th to 75th quartile values for each method.

- All methods were able to produce results that met CDHS guidelines of within 20% RSD for NDMA. However, no single method was able to meet the 20% criteria for all nitrosamines at low levels.
- Amb SPE and Amb-Envi CSPE appeared to be the most accurate and most precise methods.
- CLLE was more accurate and precise than MLLE, which has the highest MDL of the methods tested in the round-robin.
- For NA_7, reproducibility for Amb SPE and CLLE appeared to be consistently better than for Amb-Envi SPE and MLLE.

Precision, High Concentration Detection
(376 ng/L spike for individual NAs)

The following observations are based on RDS and the 25th to 75th quartile values for each method.

- All methods were able to produce results that met CDHS guidelines of 20% for all nitrosamines except NMEA. For NMEA, only Amb SPE met the 20% criteria.
- With the exception of NMEA, all methods appear to have similar precision. CLLE was the least precise method for this nitrosamine.

Methods Comparison for Potable Water (RR-01, RR-02)

- The exact nitrosamine concentrations for RR-01 and RR-02 are unknown.
- A method of comparison for accuracy is to calculate the spike recovery by taking the difference from RR-02 and RR-01, and determining how close the value is to the 12.7 ng/L spiked concentration.
- By comparison of the spiked value and the 50th percentile values, it appears that Amb SPE and Amb-Envi CSPE are the most accurate methods overall, while MLLE is the least accurate.
- With respect to precision (range of values covered by 25th to 75th quartile), it appears that the Amb SPE and MLLE are more reproducible compared to CLLE and Amb-Envi CSPE for NDMA. However, for NA_7, Amb-Envi CSPE and Amb SPE were more reproducible compared to MLLE and CLLE.
- The ratio of standard deviation to the mean NDMA value support the conclusion above, that this ratio is the smallest for Amb SPE.

Methods Comparison for RO Effluent (RR-03)

- The behavior of the methods to this water is similar to that for the potable water (both water matrices should be relatively clean).
- Amb SPE and CLLE methods appear to produce similar mean NDMA results. However, Amb SPE was more reproducible than all other methods for NDMA.

Methods Comparison for Secondary Wastewater Effluent (RR-04, RR-05)

- By using the difference method and based on the 50th percentile values, it appears that all methods produced similar results for NDMA and other nitrosamines.
- It appears that all methods are similarly reproducible (comparing range of values covered by 25th to 75th quartile).
- The ratio of standard deviation to the mean NDMA value support the conclusion above, that the ratios for all methods are similar.

Methods Comparison for Tertiary Wastewater Effluent (RR-06)

- Based on the 50th percentile values, it appears that all methods produced similar results for NDMA.
- For the NA_7, it appears that MLLE is the least reproducible (but based on an overall average NA concentration of < 20 ng/L).

Alternative Detectors

The following observations are based on low-level spike sample (12.7 ng/L) and high-level spike sample (376 ng/L).

- For the low-level CLLE and Amb SPE extracts, it appeared that the NCD and NPD yielded higher results for all nitrosamines than GC/CI/MS/MS detection. The results could differ by more than 50%, depending on the nitrosamine examined.
- NPD appears to produce results higher than that obtained for NCD for all nitrosamines.
- For the high concentration CLLE extract, it appeared that the NPD produced comparable results to the GC/CI/MS/MS results, where the NCD produced consistently lower results.
- For the high concentration Amb SPE extract, it appeared that all three detection methods produced comparable results.

- For the high concentration MLLE extract, the NCD generally produced comparable results to the GC/CI/MS/MS.

Cost Comparison

- It appears that the methods tested during the round-robin required less time than the reported time for the CLLE method of 30 hours.
- A wide variation of analytical time was reported for the different methods.
- The span of time ranged from 11 to 30 hours, with most methods requiring times of 24 hours or less to process six samples.
- The higher analysis times are likely due to the laboratories' exposure to a new method. As a laboratory becomes more familiar with a method, it is expected that the analytical time required will decrease toward the lower range.
- For the cost analysis, it appears that the labor charge and the institution's multiplier (overhead) will influence the price of the analysis more so than the instrumentation or the consumables.
- From the cost example, it appears that the methods examined in the round-robin testing should be able to be lower-cost methods than the lowest cost obtained during the initial laboratory survey of $325 per sample for NDMA.

Conclusions

The following section presents the conclusions of this research as they relate to the original project goals.

a. To optimize and refine *existing extraction and concentration methods* (liquid–liquid extraction (LLE), and solid–phase extraction (SPE)) and investigate a *new method* (solid–phase microextraction (SPME)).

   Existing methods were examined and the relevant factors refined in order to optimize, to the extent practical, the absolute recoveries for NDMA and other nitrosamines. From this research, the modified viable methods that were developed included MLLE, Amb-Envi CSPE, and Amb SPE (see Appendix for SOPs). These methods were all validated through an extensive round-robin process.

   The Amb-Envi CSPE and Amb SPE methods are able to attain a MDL of less than 0.8 ng/L for NDMA and less than 1.8 ng/L for the other nitrosamines. The MLLE method has a higher MDL of 2.3 ng/L for NDMA and less than 4.0 ng/L for the other nitrosamines, but it is a method that is more rapid than the traditional LLE techniques and shows promise for samples containing higher concentrations of NDMA. SPME was studied and shows promise because of its simplicity and high level of automation available through commercial autosamplers. However, it has a relatively high MDL of 30 ng/L for NDMA and 60 ng/L for other nitrosamines, which will limit its utility as a screening tool, or for higher concentration samples.

b. To determine whether a method may be developed to analyze for NDMA in addition to seven other nitrosamines.

   The methods examined and refined from this research, including MLLE, Amb-Envi CSPE, and Amb SPE, all measure lower levels nitrosamines. It is interesting to note that during the optimization process for NDMA, it was found that the extraction efficiency for NDMA was actually the worst of any nitrosamine. The ability of these methods to detect the eight nitrosamines was also confirmed during the round-robin testing, where Amb-Envi CSPE, Amb SPE, and CLLE appear to accurately quantify low-level spiked samples (e.g., 12.7 ng/L). For high-level spike samples (e.g., 376 ng/L), all methods tested, including MLLE, appeared to be able to accurately quantify this spiked concentration.

c. To *evaluate* the capabilities of nitrogen (N)-selective GC detectors, NPD or NCD, for quantitation of nitrosamines at or below the desired method detection limit (MDL) of 0.5 ng/L, and *compare* these detectors to the currently-used GC/MS detection systems

   The NCD and NPD were examined and interferences minimized during this testing. Due to the limited tests that were conducted with the various extracts from the different methods during the round-robin, it is difficult to make any conclusive recommendations on these detectors. It appears that the detectors are influenced by the extraction method and the concentration of the sample. Generally, it appears that the NPD results did not compare favorably with the GC/CI/MS/MS results at the lower-level spiked sample, but they compared more closely at the higher-spiked sample. More work needs to be performed on these detectors before they may be recommended for compliance purposes.

Future Work

While this work has met the goals of the project, more work may be warranted to determine the applicability of these methods to automation (e.g., Amb-Envi CSPE). Further work could also be conducted to better evaluate the applicability of NPD and NCD as a lesser-cost compliance alternative to GC/MS detectors. As more occurrence data and future regulations unfold, these methods may warrant revisiting for more focused optimization of specific nitrosamine species and/or minimum reporting limits. Also, additional cycles of interlab testing after the laboratories gain more experience with each method.

APPENDIX A

MICRO LIQUID–LIQUID EXTRACTION GAS CHROMATOGRAPHIC/ MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF NDMA AND SEVEN OTHER NITROSAMINES

1. General Discussion

a. *Sources and Significance:* The chemical N-nitrosodimethylamine (NDMA) has been identified as a probable human carcinogen by the U. S. Environmental Protection Agency. It is commonly found in liquid rocket fuel, lubricants, and pesticides. The general population may be exposed to NDMA from outdoor air; tobacco smoke; diet such as cured meats, fish, and cheese; beverages such as beer and whisky; cosmetics; and rubber products. The average concentration of NDMA measured in food ranges from 90 to 100 ng/L (ppt) for pasteurized milk, 600 to 1,000 ng/Kg for fried pork bacon, and 50 to 5,900 ng/Kg for various beers. NDMA has also been detected in recycled water, wastewater, and potable water. It can either occur as a chemical contaminant from industrial processes or be formed by chlorine and chloramine disinfection processes. The formation mechanisms that produce NDMA may also form other nitrosamines. Seven other nitrosamines (listed in Table A1) that are structurally related to NDMA and/or known to cause cancer in animals have been included in this method.

b. *Principle:* NDMA and other nitrosamines (Table A1) are analyzed by the micro liquid–liquid extraction (MLLE) method followed by gas chromatography/tandem mass spectrometry (GC/MS/MS). Isotope dilution was used for quantitation with three isotopically labeled surrogates (e.g., d6-NDMA, d14-NDPA, and 15N2-NDEA) added prior to extractions to correct for extraction efficiencies. A 100-mL volume of sample with 30 grams of sodium chloride added is extracted with 20 mL of dichloromethane (DCM) by shaking on a reciprocal shaker for 10 minutes. The DCM layer was extracted out with a 20 mL glass syringe, dried with sodium sulfate, and subsequently concentrated to 0.5 mL on a Zymark TurboVap II concentrator. An 8 µL aliquot of sample extract is injected into a Varian Saturn 2200 GC/MS operated in the chemical ionization (CI) MS/MS mode with acetonitrile as the CI reagent. NDMA and the other nitrosamines are identified based on their retention times, parent ion isolation and fragmentation patterns.

c. *Interferences:* Method interferences may be caused by contaminants, especially from NDMA, in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. The samples or analytical system also may be contaminated from rubber objects in the work area. All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by reagent water. A final rinse with solvents may be needed. In place of a solvent rinse, nonvolumetric glassware can be heated in a muffle furnace at 400^{o} C for 2 hours. Volumetric glassware should not be heated above 120^{o} C. Samples that are not properly preserved may experience inaccurate target analyte recovery due to formation caused by a chloramine residual. Coeluting GC peaks with nominal masses equivalent to the target analytes and internal standards have been observed in drinking water and wastewater necessitating MS/MS or high resolution MS quantitation. Surfactants and other organic contaminants in wastewater may cause emulsions during extraction. To demonstrate freedom from interferences, a reagent-water blank must be analyzed under the same conditions as the samples. The blank concentration must be equal to or less than one half the minimum reporting level.

d. *Safety:* Because NDMA, most of the other nitrosamines, and dichloromethane have been identified as animal carcinogens and some as probable human carcinogens, exposure to these

compounds and their isotopically labeled analogs must be minimized. A reference file of material safety data sheets (MSDS) should also be made available to all personnel involved in analyses.

1) Effluents of GC sample splitters and GC/MS vacuum pumps should pass through either a column of activated carbon or be bubbled through a trap.
2) The following precautions for safe handling of NDMA and other nitrosamines in the laboratory are presented as guidelines only.
   a) Protective equipment: Laboratory hood, safety glasses, disposable plastic gloves, and apron or lab coat.
   b) Personal hygiene: Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).
   c) Decontamination: Personnel — any mild soap with scrubbing action. Glassware, tools, and surfaces — wash with detergent and water. Solvent waste should be minimized.
   d) Handling the dilute solutions normally used in analytical work presents no significant inhalation hazards except in case of an accident.

e. Detection limits: Minimum detection limits (MDL) are compound, instrument, and matrix dependent. The detection limit is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero. Experimentally determined detection limits for the target analytes are provided in Table A2. The detection limit differs from, and is lower than the Minimum Reporting Level (MRL). The concentration range for target analytes in this method was evaluated between 5 ng/L and 500 ng/L.

2. Sampling and Storage

a. Sample Collection:

1) The sample site should be free of auto exhaust, cigarette smoke, fresh paint, and any other possible sources of contamination. Sample location should provide a representative grab sample or composites (maintained cold and with proper quenching).
2) When sampling from a water tap, the tap is allowed to flush until the water temperature has stabilized (usually about 3 to 5 minutes). When sampling from an open body of water, the sample is collected using a clean stainless steel bucket with a clean rope. Avoid plastic and rubber tubing, gaskets, etc. that may leach interfering analytes into the water sample.
3) Samples should be collected in pre-cleaned, amber glass bottles with Teflon-lined polyethylene caps. The minimum volume collected should be sufficient for the analysis of the sample, a matrix spike, and matrix spike duplicate for quality assurance purposes, preferably in two or more bottles. Sample bottles are filled to the top but care is taken not to flush out the preservative.
4) When sampling chlorinated or chloraminated water, the residual should be quenched at time of sampling. The addition of solid sodium sulfite or sodium thiosulfate (approximately 0.5 g for wastewater, or 0.04 to 0.1 g for potable water) to a 1 L bottle should minimize additional nitrosamine formation. Alternately 0.02 g of ascorbic acid may be used for drinking water. If chloramines residual is greater than 4 mg/L additional quenching agent should be added. If preservatives are used, the bottle should not be rinsed with sample before collection.

b. Storage:

1) Samples should be iced or refrigerated at 4 °C or lower (but not freezing) and maintained at these conditions away from light until extraction. To prevent photodecomposition, samples must be protected from light from the time of collection until extraction. Amber colored bottles work well for this. Avoid storage of samples under low pH conditions because this has been observed to produce elevated levels of NDMA in some wastewater effluents and potable waters. Extract drinking water samples within 28 days. As a guideline, wastewater

samples should be extracted within 14 days. However, degradation and formation of nitrosamines in wastewater matrices can be complicated processes. It is suggested that appropriate holding times be developed on a case by case basis.

2) Extracts should stored at –11 °C, away from light in amber glass vials with Teflon-lined caps. Extracts should be analyzed in a timely manner, however, under the conditions mentioned, archived extracts have generally shown minimal NDMA losses over a period of six months.

3. Apparatus

All specifications are suggested. Brand names and/or catalog numbers are included for illustration only.

a. *Shaker:* A mechanical linear shaker capable of vigorously shaking (approximately 150 strokes per minute) of six to twelve 125 mL glass bottles to automate the DCM extraction.

b. *Concentrator with glassware:* Heated water bath with inert gas sample evaporation stations and accessories needed to concentrate extract from 20 to 0.5 mL (e.g., TurboVap II concentration workstation, Zymark Corp., Hopkinton, MA).

c. *Sample containers:* Amber glass bottles fitted with PTFE-lined screw caps.

d. *Extraction bottles:* Clear 125 mL glass bottles with PTFE-lined screw caps.

e. *Volumetric flasks*: Class A, various sizes used for preparation of standards.

f. *Inert Gas*: Ultra high purity helium or nitrogen with purifying cartridge and low-pressure regulator.

g. *Syringes*: Glass microsyringes with stainless steel needle and plunger in various sizes for spiking solutions and preparing intermediate solutions. A 20 mL glass hypodermic (glass plunger) with 17 gauge, 3.5 inch long stainless steel pipetting needle is used to withdraw the DCM layer.

h. *Vials:* Screw cap amber glass vials with TFE-lined silicone septa in sizes appropriate for the autosampler and for storage of spiking solutions.

i. *Transfer pipets:* Disposable glass Pasteur pipets.

j. *Gas chromatograph/mass spectrometer/mass spectrometer system (e.g., Varian 3800 GC coupled with Saturn 2200 ion trap mass spectrometer) equipped with*:

1) *Temperature programmable large volume injector*: Capable of going from 35 to 230 °C at 200 °C/min and large volume (up to 100 µL) injection.
2) *Capillary column*: Either a 30 m x 0.25 mm i.d., 1.0 µ film thickness DB 1701 or a 60 m x 0.32 mm i.d., 1.8 µ film thickness DB-VRX fused silica or other capillary column capable of providing adequate and reproducible resolution.
3) *MS/MS analyzer:* A system with chemical ionization capable of producing and isolating a $[M+H]^+$ molecular ion and then fragmenting it to produce unique product ion spectra in a consistent and quantitative manner.

4. Reagents

Reagent grade or better chemicals and high resolution gas chromatography-grade solvents should be used. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

a. *Reagent water:* Freshly prepared purified water which does not contain any measurable quantities of any target analytes or interfering compounds greater than one third of the minimum reporting level (MRL) for each compound of interest such as demineralized ultraviolet-treated water (e.g., Mill-Q-UV treated).

b. *Methanol:* CH_3OH, high purity, HR-GC grade, demonstrated to be free of analytes and interferences (e.g., OmniSolv grade, EM Science).

c. *Dichloromethane:* CH_2Cl_2, alkene stabilized, (e.g., OmniSolv, HR-GC grade).

d. Standard solutions:

1) Stock nitrosamine mix containing eight target nitrosamines (plus *N*-nitrosodiphenylamine) at 2000 µg/mL each in methanol (Supelco, Bellefonte, PA #502138*)* and in dichloromethane (Protocol, # 8270-AF-C). One stock source is used to prepare extracted and direct standards and the other for spiking samples and laboratory fortified blanks.
2) Target analytes Primary Dilution Standard (PDS): Prepare a nitrosamine mix PDS of a suitable concentration by accurately transferring the appropriate volume of stock standard solution into a volumetric flask partially filled with methanol. Dilute to volume, mix thoroughly, transfer to an amber glass vial and store at 4 °C. As an example, 500 ng/mL PDS were prepared in 25 mL volumetric flasks. A serial dilution of this PDS, to make a 100 ng/mL solution is useful for low level spiking.
3) Internal standard stock solutions: *N*-nitrosodimethylamine (D_6), *N*-nitrosodipropylamine (D_{14}) and *N*-nitrosodiethylamine ($^{15}N_2$) are purchased as individual stock solutions at 1 mg/mL in methylene chloride (Cambridge Isotope Laboratories, Andover, MA, #DLM-2130-S, DLM-2131-S and NLM-3432-S, respectively).
4) Internal standard PDS: Prepare an ISPDS containing all three internal standards at suitable concentration by accurately transferring the appropriate volumes of each internal standard stock solution to a volumetric flask, containing methanol as described in the previous section. As an example, 200 ng/mL PDS of internal standards was prepared in 25 mL volumetric flasks. Store in an amber glass vial at 4 °C.
5) Calibration Standards: This method uses procedural standards for preparation of calibration curves. Standard concentrations must range from the MRL to greater than the highest sample concentration, which typically is from 10 ng/L to 500 ng/L. Prepare and extract a five-point calibration curve as outlined in Section 5.c., by fortifying a series of 100 mL reagent water aliquots with appropriate levels of target analytes and 50 ng/L each of internal standards.

e. Sodium chloride: NaCl ACS grade. Heated at 400 °C overnight and stored at 110 °C.

f. Sodium sulfate: Na_2SO_4 granular anhydrous ACS reagent grade. Heated at 400 °C for 3 hours and stored at 110 °C.

5. Procedure

a. Extraction:

1) Remove samples from storage and allow to equilibrate to room temperature. Using a clean graduated cylinder transfer 100 mL of sample or reagent water (for standards, etc.) to a clean 250 mL bottle.
2) Add the three isotopically labeled standards, i.e., d6-NDMA, d14-NDPA, and 15N2-NDEA, to the sample so that the final concentration of each one is 50 ng/L (e.g., 25 µL of a 200 ng/mL ISPDS).
3) Add 30 g of sodium chloride.
4) Add 20 mL of DCM. Cap and shake to dissolve as much as possible.
5) Shake on the reciprocal shaker for 10 minutes.
6) Let the sample bottles stand for approximately 15 minutes so that the layers are separated well.
7) Withdraw as much of the DCM layer as possible from the bottle with a 20 mL glass syringe and transfer to a 40 mL vial or other suitable glassware.
8) Dry the DCM layer with approximately 0.4 g of sodium sulfate for 3 minutes.
9) Transfer the DCM fraction to a TurboVap tube. Rinse the sodium sulfate twice with DCM and combine with original fraction. Concentrate the solution at 35 ^{o}C under approximately 10 psi nitrogen until the final volume reaches 0.5 mL. Rinse the tube walls with appproximately 5 mL of DCM and concentrate again to 0.5 mL.
10) Transfer the concentrate to a 2 mL autosampler vial, store at –11 ^{o}C or load onto GC/MS/MS autosampler for injection of an 8 µL aliquot.

b. *Gas Chromatography:* Establish operating conditions such as those described in Table A3.

This method uses chemical ionization (CI), tandem mass spectrometry (MS/MS). In the CI mode of operation a CI reagent gas (acetonitrile or methanol) is introduced into the ion trap, ionized, and allowed to react with sample molecules. Ionization of the sample molecules generates the protonated molecular ion $[M+1]^+$. This ion is isolated from matrix ions in the trap and is selected as the precursor (parent) ion for subsequent fragmentation. One of the benefits of CI is that it results in a higher concentration of the $[M+1]^+$ than electron impact ionization. A waveform is applied to the trap increasing the energy of the isolated precursor ion. The amplitude of this waveform is called the collision induced dissociation (CID) excitation amplitude. As the energy of the precursor ion increases, chemical bonds are broken and product ions (daughter ions) of lower m/z than the precursor ion are formed. It is possible to use the precursor or product ions to quantify the target analytes. Quantitation based on $[M+H]^+$ provides greater sensitivity, especially in clean water matrices, while quantitation on product ions provides greater specificity and may be needed for wastewater or matrices with interfering compounds. Examples of MS/MS parameters with acetonitrile as CI reagent are listed in Table A4. CID values were chosen to maximize the transition from parent ion to product ions while retaining 10 to 25% of the parent ion for confirmation. The degree of fragmentation observed for a certain CID value depends on the instrument and operating conditions and therefore will vary with time and laboratory. In some instances, the degree of fragmentation has also been observed to depend on analyte concentration.

Other GC/MS/MS conditions may be used as long as QC requirements are met. Establish an appropriate retention time window and precursor to product ion mass ratio for each target and surrogate analyte to facilitate detection and identification in all QC and field samples.

c. *Calibration:* Prepare standards as described in Section 4.d. Extract and analyze each standard under the same conditions used for sample extracts. Use internal standards as designated in Table A1 for quantitation of each nitrosamine. Using the GC/MS software, generate a linear regression or quadratic calibration curve plotting area ratios ($Area_x/Area_{IS}$) verses concentration for each nitrosamine. Curves are typically linear to 500 ng/L. The coefficient of determination (r^2) should round to 0.99 or higher. Alternately mean response factors may be used for linear calibration.

d. *Continuing Calibration*: For continuing calibration, verify the calibration by extracting and analyzing a mid-point calibration standard. The calculated concentrations should be 70 to 130% of its true value.

6. Data Analysis and Calculations

a. *Review*: Check the chromatogram for any incorrect peak identification or poor integration.
b. *Quantitation:* Calculate sample concentration with the best-fit calibration equation or the average relative response factor as explained in Section 5, Procedure c, Calibration. Quantitate only those samples that fall between the MRL and the highest calibration standard. If the determined analyte concentration exceeds that of the highest standard, dilute the original sample into a final volume of 100 mL, re-extract and re-analyze.
c. *Identification:* From the product ion chromatograms, identify the analytes in the sample by comparing the retention time and spectrum of the suspect peak to retention time and spectrum of the reference analyte peak in a calibration standard. All ions that are present in the reference mass spectrum should be present in the sample mass spectrum with intensities between 10 to 50% of the reference mass spectrum.
d. *Correction*: Adjust the calculated concentrations of detected analytes to reflect any dilutions performed.
e. *Reporting*: Report analyte concentrations in ng/L.

7. Quality Control

At a minimum, an initial demonstration of an acceptable calibration curve (RSD <20%), continuing calibration checks with acceptance criteria of ± 30% of initial calibration, reagent method blanks, field blanks, field sample duplicates, matrix spikes, and low level calibration checks (at or below the MRL) with an acceptance criteria of ± 50%, should be employed. Additional quality control parameters are recommended for ongoing quality assurance.

a. *Method detection limit (MDL):* An MDL study using the EPA protocol (40CFR136, Appendix B) must be available. Example results are shown in Table A1.
b. *Minimum reporting level (MRL):* The minimum reporting level should be no less than three times the MDL and a standard must be run at this level.
c. *Laboratory reagent blank:* A blank using the laboratory reagent water is analyzed by the method (including reagents, glassware, etc.) with each batch of samples. The blank must be free of nitrosamine contamination (e.g., equal to or less than one half the MRL).
d. *Calibration Check:* A mid-level continuing calibration check standard (CCC) is analyzed with each batch. If it agrees within ± 20% of the expected value, a new calibration curve is not needed (see Procedure: calibration section).
e. *Laboratory fortified blank (LFB):* An LFB is prepared by spiking reagent water at the MRL level with a different stock solution than used for the calibration curve standards. The LFB is taken through the full method with each batch of samples. Recovery should be within ± 50% of the theoretical value.
f. *Laboratory fortified sample matrix (LFSM):* Analysis of an LFSM is required in each analysis batch or 10 samples and is used to determine that the sample matrix does not adversely affect method accuracy. Within each analysis batch, a minimum of one field sample is fortified as an LFSM for every 10 samples processed. The LFSM is prepared by spiking a sample with an appropriate amount of the analyte from a different stock source than used for the standards. A spiking concentration is selected approximately twice the matrix background concentration, if known. The percent recovery (R) is calculated for each analyte using the equation:

$$R = \frac{(A - B)}{C} \times 100$$

where:

A = measured concentration in the fortified sample,
B = measured concentration in the unfortified sample, and
C = fortification concentration.

For samples fortified at or above their native concentration, recoveries should range between 70 to 130%.

g. *Sample duplicate (LD1, LD2) or laboratory fortified matrix spike duplicate (LFSMD):* If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, a second LFSM (i.e., LFMSD), must be prepared, and analyzed from a duplicate of the field sample used to prepare the LFSM to assess method precision. The relative percent difference (RPD) for duplicates (LD1 = LFSM and LD2 = LFSMD) is calculated using the equation:

$$RPD = \frac{(LFSM - LFSMD)}{(LFSM + LFSMD)/2} \times 100$$

RPDs for LDs and duplicate LFSMs should fall in the range of ± 20% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are spiked near the MRL. At the MRL, RPDs should fall in the range of ± 50% for samples fortified at or above their native concentration.

8. Method Performance

a. A typical nitrosamine chromatogram and NDMA calibration curve are presented in Figures A1 and A2, respectively.

b. Laboratory precision and accuracy data for a single laboratory are shown in Table A5. The samples ranged from potable water to treated wastewater, with N ranging from 16–19 and spike levels from 20 and 100 ng/L of each nitrosamine. The background levels of NDMA ranged from not detected to 402 ng/L with an average value of 137 ng/L. The average background level for the other nitrosamines were below the reporting limit of 10 ng/L, except for NDBA which was 13 ng/L.

c. Interlaboratory bias and precision data for a potable water and a secondary wastewater are given in Table A6 and A7, respectively.

9. References

1. "NDMA Laboratory Analysis," California Department of Health Services, http://www.dhs.cahwnet.gov/ps/ddwem/chemicals/NDMA/NDMAlabs.htm, 5/16/2003.
2. "Carcinogens—Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, NIOSH, Pub. #77-206, August 1977.
3. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Ed., 1979.

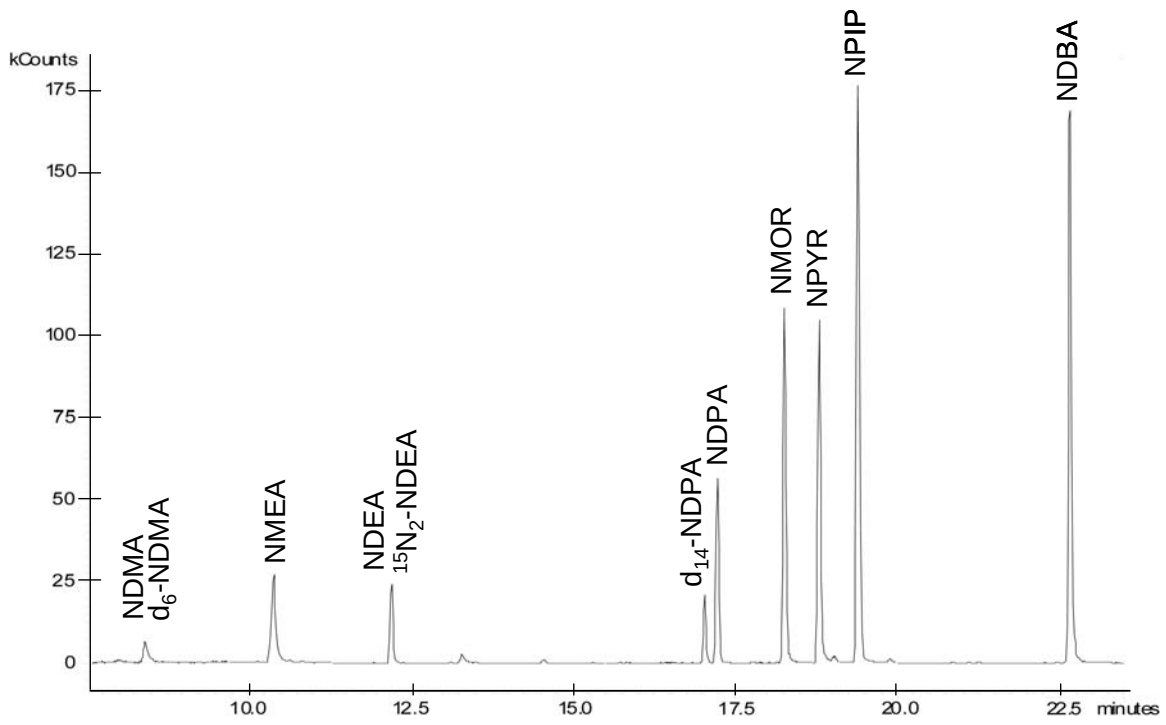


Figure A1 Typical chromatogram of 200 ng/L MLLE extracted nitrosamine standard

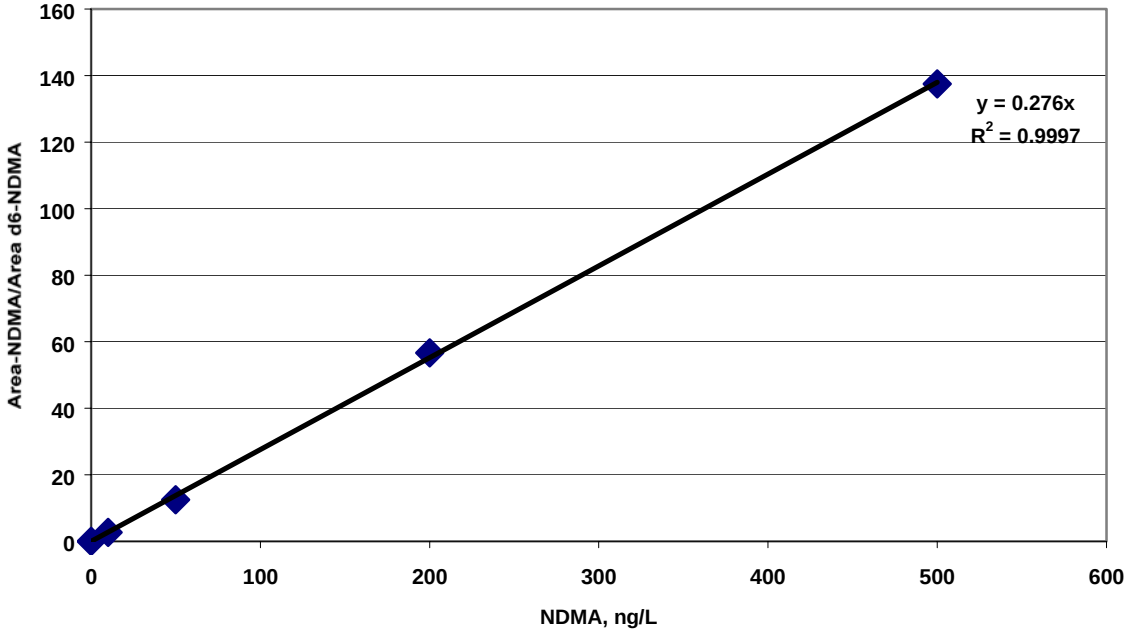


Figure A2 Calibration curve for MLLE of NDMA (10-500 ng/L)

Table A1 Target nitrosamine analytes: formula, molecular weight, CAS No., and internal standard

Nitrosamine	Abbrev.	Formula	MW	CAS #	Internalstd.
N-Nitrosodimethylamine	NDMA	$C_2H_6N_20$	74	62-75-9	d_6-NDMA
N-Nitrosomethylethylamine	NMEA	$C_3H_8N_20$	88	10595-95-6	$^{15}N_2$-NDEA
N-Nitrosodiethylamine	NDEA	$C_4H_{10}N_2O$	102	55-18-5	$^{15}N_2$-NDEA
N-Nitrosodi-n-propylamine	NDPA	$C_6H_{14}N_2O$	130	621-64-7	d_{14}-NDPA
N-Nitrosomorpholine	NMOR	$C_4H_8N_2O_2$	116	59-89-2	d_{14}-NDPA
N-Nitrosopyrrolidine	NPYR	$C_4H_8N_2O$	100	930-55-2	d_{14}-NDPA
N-Nitrosopiperidine	NPIP	$C_5H_{10}N_2O$	114	100-75-4	d_{14}-NDPA
N-Nitrosodi-n-butylamine	NDBA	$C_8H_{18}N_2O$	158	924-16-3	d_{14}-NDPA

Table A2 Minimum Detection Limits in reagent water

Nitrosamine	Fortification level (*ng/L*)	MDL (*ng/L*)
NDMA	10.0	2.3
NMEA	10.0	3.9
NDEA	10.0	2.5
NDPA	10.0	3.4
NPYR	10.0	2.7
NMOR	10.0	1.8
NPIP	10.0	2.2
NDBA	10.0	3.8

Minimum Detection Limits were determined by analysis of seven replicates.

Table A3 GC conditions

Injector program		DB-VRX		DB-1701	
Temp (^{o}C)	Rate (^{o}C/min)	Hold |(min)	Total (min)	Hold (min)	Total (min)
35	0	0.8	0.8	0.8	0.8
260	200	2	3.92	2.08	4.00
150	200	31.5	35.97	21.00	25.56

Time (min)	Split state	Split ratio
Initial	On	5
0.8	Off	Off
2.2	On	100
20	On	30

Injection volume	8 μL
Plunger Inject speed	0.2 μL/sec
Post injection delay	99 sec

Column Temperature Program: DB-VRX				DB-1701			
Temp.(^{o}C)	Rate (^{o}C/min)	Hold time (min)	Total time (min)	Temp. (^{o}C)	Rate (^{o}C/min)	Hold time (min)	Total time (min)
35	0	4	4.0	35	0	4	4
100	20	2	9.3	200	15	0	15
210	5	0	31.3	240	40	10	26
250	50	5	37.1				

Table A4 Acetonitrile-CI/MS/MS parameters (DB-1701 GC column)

CI gas: acetonitrile	Eject. amp: 15.0 m/z
CI storage level: 19.0 m/z	Background mass: 40 m/z
Max. ion time: 2000 microsec	Max. reaction time: 120 millisec
Target TIC 5000 counts	Prescan time: 200 microsec

Segment	Description	Start time	End time	Low mass	High Mass	Ioniz. Mode	Ion Prep
1	Fil/Mul delay	0	7.6	40		CI auto	
2	NDMA	7.6	9.7	40	83	CI auto	MRM
3	NMEA	9.7	11.3	40	91	CI auto	MS/MS
4	NDEA	11.3	15.5	40	107	CI auto	MRM
5	NDPA	15.5	17.7	40	150	CI auto	MRM
6	NMOR	17.7	18.7	40	125	CI auto	MRM
7	NPYR	18.7	19.1	40	106	CI auto	MS/MS
8	NPIP	19.1	20.0	40	120	CI auto	MS/MS
9	NDBA	20.0	23.5	40	165	CI auto	MS/MS

Segment	Channel		Precursor mass	Isolation window	Quan ion (Product ion)	Waveform type	Excit stor level	Excit ampl
2	NDMA	1	81	1.5	49	Resonant	35	0.36
	d_6-NDMA	2	75	1.5	44	Resonant	35	0.34
3	NMEA	1	89	2	61	Resonant	40	0.31
4	$^{15}N_2$-NDEA	2	105	2	77	Resonant	40	0.33
	NDEA	1	103	2	75	Resonant	40	0.34
5	NDPA	1	131	2	89	Resonant	40	0.34
	d_{14}-NDPA	2	145	2	97	Resonant	40	0.33
6	NMOR	1	117	2	87	Resonant	40	0.3
7	NPYR	1	101	2	55	Resonant	40	0.33
8	NPIP	1	115	2	69	Resonant	40	0.34
9	NDBA	1	159	2	103	Resonant	48	0.37

Table A5 **MLLE method precision and accuracy for nitrosamines in drinking and wastewater samples**

	Accuracy		Precision	
Nitrosamine	**Mean rec. (%)**	**Stdev (%)**	**Mean rel.diff. (%)**	**Stdev (%)**
NDMA	98	12	10	8.9
NMEA	97	15	12	13
NDEA	92	16	15	15
NDPA	83	19	6.2	3.2
NPYR	93	16	8.3	6.0
NMOR	97	17	11	11
NPIP	87	15	6.2	4.8
NDBA	90	17	13	8.2

Table A6 **Interlaboratory bias and precision data for nitrosamines spiked into chloraminated potable surface water**

		Recovery, %				
Nitrosamine	**Sample conc. (ng/L)**	**Lab 1**	**Lab 2**	**Lab 3**	**Mean**	**%RSD**
NDMA	<10	74	117	87	93	24.0
NMEA	<10	118	86	82	95	20.9
NDEA	<10	87	80	92	86	6.8
NDPA	<10	91	91	96	92	3.2
NPYR	<10	88	92	100	94	6.7
NMOR	<10	99	113	135	116	15.4
NPIP	<10	95	86	92	91	5.3
NDBA	<10	93	92	108	98	9.1

Potable surface water samples observed (n=3), Spiked concentration = 13 ng/L

Table A7 **Interlaboratory bias and precision data for nitrosamines spiked into secondary wastewater effluent**

Nitrosamine	**Sample conc. (ng/L)**	**1**	**2**	**3**	**Mean rec. %**	**%RSD**
NDMA	142	101	115	90	102	12.0
NMEA	<20	93	85	88	89	4.7
NDEA	<20	92	93	118	101	14.8
NDPA	<20	93	77	100	90	13.2
NPYR	<14	101	98	132	110	17.4
NMOR	<20	101	86	115	101	14.3
NPIP	<20	92	90	94	92	2.0
NDBA	<20	101	92	100	98	5.0

Secondary effluent water samples observed (n=3), Spiked concentration = 376 ng/L

APPENDIX B

AMBERSORB 572® EXTRACTION GAS CHROMATOGRAPHIC/ MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF NDMA AND SEVEN OTHER NITROSAMINES

1. General Discussion

a. Sources and Significance: The chemical N-nitrosodimethylamine (NDMA) has been identified as a probable human carcinogen by the U. S. Environmental Protection Agency. It is commonly found in liquid rocket fuel, lubricants, and pesticides. The general population may be exposed to NDMA from outdoor air; tobacco smoke; diet such as cured meats, fish, and cheese; beverages such as beer and whisky; cosmetics; and rubber products. The average concentration of NDMA measured in food ranges from 90 to 100 ng/L (ppt) for pasteurized milk, 600 to 1,000 ng/Kg for fried pork bacon, and 50 to 5,900 ng/Kg for various beers. NDMA has also been detected in recycled water, wastewater, and potable water. It can either occur as a chemical contaminant from industrial processes or be formed by chlorine and chloramine disinfection processes. The formation mechanisms that produce NDMA may also form other nitrosamines. Seven other nitrosamines (listed in Table B1) that are structurally related to NDMA and/or known to cause cancer in animals have been included in this method.

b. Principle: The principle is based on a procedure originally published by Taguchi et al. (1994). A 500 mL volume of sample (or sample diluted with reagent water to 500 mL) spiked with isotopically labeled surrogates, is extracted via adsorption to 200 mg of a carbonaceous resin (Ambersorb 572®) for a period of two hours. The resin containing the nitrosamines extracted from the sample is collected and dried on filter paper and the filtrate is discarded. Nitrosamines are desorbed from the resin in 400 µL of dichloromethane, DCM, which is then injected onto a GC column where target nitrosamines are separated. The target nitrosamines are then detected with a tandem mass spectrometer (MS/MS) equipped with chemical ionization (CI) using methanol. Target nitrosamines are quantified by first generating a relative response factor (RRF) from the signal of reference nitrosamine standards and surrogates. The RRF can be used to determine unknown nitrosamine concentrations by interpolation from a linear calibration curve containing RRF values for nitrosamines with known concentrations.

c. Interferences: Method interference may be caused by contaminants in reagents, sample bottles and caps, and other materials used during the analysis. Laboratory and reagent blanks must be demonstrated to be free of interference, with concentrations less than one-third the minimum reporting limit (MRL).

1) All glassware must be meticulously cleaned. Rinse sample bottles with reagent water immediately after extraction. Wash volumetric glassware with detergent and rinse with tap water followed by reagent water. Bake bottles overnight at temperature above 250 °C. Store glassware inverted or capped with aluminum foil.
2) Condition Ambersorb in a shallow tray at a temperature above 250 °C (300 °C recommended) for 3 hours before use and store in a capped amber glass bottle in a desiccator. There is evidence that nitrosamines can be transmitted to ambersorb through the gaseous phase thus proper storage is critical. To ensure a uniform size distribution of beads sieve with a No. 50 ASTM mesh or allow the finer particles to settle in the storage container taking beads from the upper portion only.
3) Rubber materials can be a source of NDMA contamination so avoid use during extraction and analysis.

4) NDMA has been found in deionized (DI) water at levels up to 10 ng/L, therefore use of an ultraviolet water purification system is recommended to avoid contamination. Reagent water freshly drawn from a Milli-Q UV Plus system has been used.
5) Carry over may be observed during GC analysis. To avoid contamination a solvent injection should be made in between high concentration and low concentration samples.

d. Safety: Because the toxicity or carcinogenicity of each reagent used in this method has not been precisely defined, each chemical should be treated as a potential health hazard, and exposure should be minimized. Wear suitable protection to skin and eyes and work under hood when handling unknown samples and stock or other high concentration standard solutions. *Each laboratory should maintain a MSDS file for all chemicals used in this procedure.*

e. Detection Limits: Method detection limits (MDL) for the above listed analytes in deionized water are provided in Table B2. Prepare at least seven replicates of laboratory fortified blanks with nitrosamine standards at a concentration of 1 to 5 ng/L and extract over a three-day period. Calculate the mean recovery and the standard deviation for each analyte. Multiply the student's t value at 98% confidence and n-1 degrees of freedom (3.143 for seven replicates) by this standard deviation to yield a statistical estimate of the detection limit. This calculated value is the MDL. A set of ten replicate standards of 1 ng/L in DI reagent water was extracted over a three-day period. The MDL was calculated using the formula: MDL = 2.821 x STDEV for 10 replicates. The results are shown in Table B2.

2. Sampling and Storage

a. Sample Collection:

1) The sample site should be free of auto exhaust, cigarette smoke, fresh paint, and any other possible sources of contamination. Sample location should provide a representative grab sample or composites (maintained cold and with proper quenching).
2) When sampling from a water tap, the tap is allowed to flush until the water temperature has stabilized (usually about 3–5 minutes). When sampling from an open body of water, the sample is collected using a clean stainless steel bucket with a clean rope. Avoid plastic and rubber tubing, gaskets, etc. that may leach interfering analytes into the water sample.
3) Samples should be collected in pre-cleaned, amber glass bottles with Teflon-lined polyethylene caps. The minimum volume collected should be sufficient for the analysis of the sample, a matrix spike, and matrix spike duplicate for quality assurance purposes, preferably in two or more bottles. Sample bottles are filled to the top but care is taken not to flush out the preservative.
4) When sampling chlorinated or chloraminated water, the residual should be quenched at time of sampling. The addition of solid sodium sulfite or sodium thiosulfate (approximately 0.5 g for wastewater, or 0.04 to 0.1 g for potable water to a 1 L bottle should minimize additional nitrosamine formation. Alternately 0.02 g of ascorbic acid may be used for drinking water. If chloramines residual is greater than 4 mg/L additional quenching agent should be added. If preservatives are used, the bottle should not be rinsed with sample before collection.

b. Storage:

1) Samples should be iced or refrigerated at 4 °C or lower (but not freezing) and maintained at these conditions away from light until extraction. To prevent photodecomposition, samples must be protected from light from the time of collection until extraction. Amber colored bottles work well for this. Avoid storage of samples under low pH conditions because this has been observed to produce elevated levels of NDMA in some wastewater effluents and potable waters. Extract drinking water samples within 28 days. As a guideline, wastewater samples

2) should be extracted within 14 days. However, degradation and formation of nitrosamines in wastewater matrices can be complicated processes. It is suggested that appropriate holding times be developed on a case by case basis.
3) Extracts should stored at –11 °C, away from light in amber glass vials with Teflon-lined caps. Extracts should be analyzed in a timely manner, however, under the conditions mentioned, archived extracts have generally shown minimal NDMA losses over a period of six months.

3. Apparatus

a. *Sample containers:* 1-L Amber glass bottles fitted with PTFE-lined screw caps.
b. *Standard solution storage containers:* 10 to 20 mL amber glass vials with PTFE-lined screw caps.
c. *Vials:* Screw cap 2.0 mL glass autosampler vials with PTFE-faced septa, amber.
d. *Volumetric flasks:* Class A, various sizes used for preparation of standards and samples.
e. *Microsyringes:* Various sizes.
f. *Filters:* Disposable filters are used to isolate resin from the water samples (Whatman 55 mm, #1 (Cat. No.: 1001 055), #4 (Cat.No.: 1004 055), glass fiber filters (Whatman GF/F #1825 047) or equivalent.
g. *Balance:* Analytical, capable of accurately weighing to 0.0001g.
h. *Disposable aluminum dishes*
i. *Rotator:* modified to maintain 50 rpm – LE2002 Heavy-Duty, 12 positions or orbital shaker able to accept 1 L bottles and shake at 50 rpm (Lab-Line Instruments, Melrose Park, IL) or equivalent.
j. *Vacuum filtration apparatus*
k. *Gas chromatograph* Capillary GC with split–splitless temperature programmable injector capable of large volume injections. GC oven and injector should be able to maintain 35 °C. During development of this method, the Varian 3800cx GC equipped with the 1079 Varian universal capillary injector was used.
l. *Chromatographic column:* A 60 m long x 0.32 mm ID, 1.8 μm film thickness, low polarity capillary column, HP-VOC, was used during method development. Other capillary columns, such as DB1701 (30 m x 0.25 mm ID, 1.0 μm film) or DB-VRX (60 m x 0.32 mm ID, 1.8 μm film) have also been used and others may also be appropriate.
m. *Autosampler:* The Varian 8200cx autosampler was used during method development. This model contains a side-port needle capable of slow injection (approximately 0.2 μL/sec) of 8 μL volumes.
n. *Detector:* ultra trace mass spectrometer, capable of chemical ionization (CI), and tandem mass spectrometry with the sensitivity to detect low part per billion levels of NDMA. The Varian Saturn 2000 system-utilizing methanol CI was used for development of this method.

4. Reagents

a. *Reagent Water,* purified water that does not contain target analytes or interfering compounds at levels greater than 1/3 the MRL for each compound of interest.
b. *Methanol* (CH_3OH, CAS# 67-56-1), high purity, demonstrated to be free from analytes or interferences.
c. *Acetonitrile* (CH_3CN, CAS# 75-05-8), high purity, demonstrated to be free from analytes or interferences.
d. *Dichloromethane* (DCM, CH_2Cl_2, CAS# 75-09-2), also known as methylene chloride, high purity, demonstrated to be free from analytes or interferences. Alkene stabilized reagent preferred because cyclohexene under some conditions contributes a large peak in the total chromatogram.
e. *Ambersorb® 572 Adsorbent (Supelco P/N: 10432-U)*, conditioned as described in Section 1.c.2.
f. *Helium*, Ultra High Purity (UHP), GC carrier gas.
g. *Carbon Dioxide*, bone dry with siphon tube, for injector cryogenics.

h. *Nitrogen*, UHP grade, for autosampler pneumatics.

i. *Sodium thiosulfate* ($Na_2S_2O_3$, CAS#: 7772-98-7), or *sodium sulfite* (Na_2SO_3. CAS#. 7757-83-7) dechlorinating agent.

j. *Standard materials:*

1) Stock standard solutions: Prepare from pure standard materials or purchase as certified solutions, available at concentrations of 100 to 5000 µg/mL. To prepare a stock standard from a pure material, partially fill a volumetric flask with methanol. Allow the flask to equilibrate, weigh to the nearest 0.1 mg then add the desired volume of the pure standard material with a microsyringe and re-weigh. Dilute to volume, stopper, and mix by inverting 3 times. Calculate the concentration of the stock standard from the net gain in weight. When compound purity is assayed to be 96% or greater, use the weight without correction to calculate concentration of the stock standard. Store stock standard solution in an amber glass vial, at a temperature below 0 °C, for 3 months.
2) Target analytes Primary Dilution Standard (PDS): Prepare a nitrosamine mix PDS of a suitable concentration by accurately transferring the appropriate volume of stock standard solution into a volumetric flask partially filled with methanol. Dilute to volume, mix thoroughly, transfer to an amber glass vial, and store at 4 °C. As an example, 1000 ng/mL PDS were prepared in 25 mL volumetric flasks. A serial dilution of this PDS, to make a 100 ng/mL solution is useful for low level spiking.
3) Internal Standard and Surrogate Primary Dilution Standard (ISPDS): NDMA-d_6, N-nitrosodiethylamine-$^{15}N_2$ and N-nitroso-di-n-propylamine-d_{14} are used as internal standards. Other compounds may be used as surrogates as long as they satisfy the necessary QC requirements. Prepare an internal standard and surrogate mix PDS of suitable concentration by accurately transferring the appropriate volumes of internal standard and surrogate stock solutions into a volumetric flask, containing methanol as described in the previous section. As an example, 1000 ng/mL PDS of internal standard and surrogate were prepared in 25 mL volumetric flasks. Store in an amber glass vial at 4 °C.
4) Calibration Standards: This method uses the procedural standard calibration curve. Prepare and extract at least a five-point calibration curve as is outlined in Section 5.c, by fortifying a series of 500 mL reagent water contained in 1-L amber bottles with the PDS and the ISPDS to produce a calibration curve ranging from 1 ng/L to 300 ng/L with internal/surrogate standards at 20 ng/L, as is shown in Table B3.

5. Procedure

a. *Sample Extraction*: by means of the Solid–phase Extraction (SPE) technique.

1) Remove samples from storage and allow to equilibrate at room temperature. Using a clean graduated cylinder, transfer 500 mL of sample (including method blanks, calibration standards, continuing check standards, field blanks, etc.) into 1-L amber glass bottles.
2) Add an aliquot of internal standard and surrogate ISPDS that results in a 20-ng/L final concentration (for example 10 µL of a 1000 ng/mL ISPDS). When spiking standards into an aqueous sample, be sure to place the needle of the syringe below the surface of the water. After injection, cap the bottle and invert to allow for mixing.
3) Add 200 mg of Ambersorb. Place bottles in rotator apparatus and rotate for 2 hours @ 50 rpm.
4) Isolate the Ambersorb from the water by filtration with filter paper and the aid of a vacuum system under a hood.
5) To ensure complete transfer of Ambersorb, thoroughly rinse the walls of the bottle with reagent water (a squeeze bottle can be used for this purpose) and add to the collected resin on the filter. Leave Ambersorb in the filtration apparatus under vacuum for approximately 5 minutes to remove as much water as possible.
6) Transfer the Ambersorb to a disposable aluminum dish with the help of forceps.
7) Air dry Ambersorb for at least 45 minutes under the hood or under a gentle stream of dry helium. Transfer the dry Ambersorb to a 2-mL autosampler vial and cap the vial.

8) Store vials containing Ambersorb in the refrigerator or freezer. When prepared to analyze proceed to the next step.
9) Remove from the refrigerator and immediately uncap the vial and add 400 µL of DCM using a microsyringe. Heat is released when the solvent comes into contact with Ambersorb. In order to minimize this effect, slowly deliver DCM along the walls of the cold vial.
10) Cap the vial. Shake gently to allow contact between Ambersorb and solvent, and tap the vial to expel air bubbles from the Ambersorb.
11) Allow a half-hour contact time between Ambersorb and DCM to ensure desorption equilibrium has been reached.
12) Load vials onto autosampler tray for analysis, inject 8 µL.

b. *Gas Chromatography/Mass Spectrometry:* Establish operating conditions such as those described in Tables B4 to B9.

This method uses chemical ionization (CI), tandem mass spectrometry (MS/MS). In the CI mode of operation, a CI reagent gas (methanol or acetonitrile) is introduced into the ion trap, ionized by EI and then allowed to react with sample molecules. Ionization of the sample molecules generates the protonated molecular ion $[(M+1)]^+$. This ion is isolated from matrix ions in the trap and acts as the parent ion for subsequent fragmentation. One of the benefits of CI is that it results in less parent ion fragmentation than EI alone. A waveform is applied to the trap increasing the energy of the isolated parent ion. The amplitude of this waveform is called the collision induced dissociation (CID) excitation amplitude. As the energy of the parent ion increases, chemical bonds are broken and product ions of lower m/z than the parent ion are formed (daughter ions). It is possible to use the parent or daughter ions to quantify the target analytes.

Quantitation based on $[M+H]^+$ provides greater sensitivity, especially in clean water matrices, while quantitation on product ions provides greater specificity and may be needed for wastewater or matrices with interfering compounds. CID values used are listed along with other instrument parameters in Table B11. CID values were chosen to maximize the transition from parent ion to product ions while retaining 10 to 25% of the parent ion for confirmation. The degree of fragmentation observed for a certain CID value depends on instrument conditions and therefore it is very likely that different laboratories will obtain different degrees of parent ion fragmentation. In some instances, the degree of fragmentation has also been observed to depend on analyte concentration. Where variances in fragmentation over a range of concentrations are significant and/or interferences in the sample result in inconsistencies, it may be necessary to adjust the CID value and use the parent ion or an alternative daughter ion for quantitation.

Other GC/MS/MS conditions may be used as long as QC requirements are met. Establish an appropriate retention time window and parent to daughter ion mass ratio for each target and surrogate analyte to facilitate detection and identification in all QC and field samples.

c. *Method Performance*: A typical nitrosamine chromatogram and NDMA calibration curve along with analyte absolute recovery and matrix spike recovery data are presented in Figures B1 and B2. Additional information is presented in Tables B10 to B14.

1) *Maximum ionization time*: The time electrons are allowed to react with reagent gas molecules, set from 10 to 2500 µs. Generally, 1/10 of the EI ionization time. Increasing the maximum reaction time may result in a larger peak signal area and consequently better sensitivity.
2) *Maximum reaction time*: The time reagent gas ions are allowed to react with sample molecules set from 1 to 120 ms. Increasing the maximum reaction time may result in a larger peak signal area and consequently better sensitivity.

3) *CI storage level*: The smallest mass stored in the trap during ionization, set from 0 to 50 m/z, usually much smaller than the mass of the reagent ion.
4) *CI background mass*: Greater than or equal to the largest reagent ion. Masses less than this are ejected from the trap after reaction. If too low, unwanted reagent ions can cause ionization time to be reduced. Significant peaks at the low end of a spectrum should be eliminated.
5) *Reagent ion eject*: Low mass eject cutoff, slightly higher than mass of largest reagent ion produced, gets rid of EI fragments w/o affecting reagent ions. This can be checked by lowering the CI background mass and then lowering the ion eject amp to the point where reagent ions are diminished. Restore CI background mass and verify that reagent ions are gone.

d. Calibration: Prepare standards as described in Section 4.j.4. Extract and analyze each standard under the same conditions used for sample extracts. Use internal standard for quantitation. Generate a RRF for each analyte as follows:

$$RRF = \frac{(A_x)(C_{is})}{(A_{is})(C_x)}$$

where A_x = integrated abundance of the selected ion for each analyte
A_{is} = integrated abundance of the internal standard
C_x = concentration of analyte injected
C_{is} = concentration of internal standard injected

Calculate the mean factor (RF_{mean}) and standard deviation of the calibration levels. Check for linearity and recalibrate if the RSD for the initial calibration exceeds 20%.

Alternately the GC/MS software may be used to generate a linear regression or quadratic calibration curve plotting area ratios ($Area_x/Area_{IS}$) verses concentration for each nitrosamine. Curves are typically linear to 300 ng/L. The coefficient of determination (r^2) should round to 0.99 or higher.

e. Continuing Calibration: For continuing calibration, verify the calibration by extracting and analyzing a mid-point calibration standard. The calculated concentrations should be 70 to 130% of its true value.

6. Data Analysis and Calculations

a. Review: Check the chromatogram for any incorrect peak identification or poor integration.
b. Quantitation: Calculate sample concentration with the best-fit calibration equation or the average relative response factor as explained in Section 5, Procedure, c. Calibration. Quantitate only those samples that fall between the MRL and the highest calibration standard. If the determined analyte concentration exceeds that of the highest standard, dilute the original sample into a final volume of 500 mL, reextract, and reanalyze.
c. Identification: From the daughter ion chromatograms, identify the analytes in the sample by comparing the retention time and spectrum of the suspect peak to retention time and spectrum of the reference analyte peak in a calibration standard. All ions that are present in the reference mass spectrum should be present in the sample mass spectrum with intensities between 10 to 50% of the reference mass spectrum.
d. Correction: Adjust the calculated concentrations of detected analytes to reflect any dilutions performed.
e. Reporting: Report analyte concentrations in ng/L.

7. Quality Control

At a minimum, an initial demonstration of an acceptable calibration curve (RSD <20%), continuing calibration checks with acceptance criteria of ± 30% of initial calibration, reagent method blanks, field blanks, field sample duplicates, matrix spikes, and low level calibration checks (at or below the MRL) with an acceptance criteria of ± 50%, should be employed. Additional quality control parameters are recommended for ongoing quality assurance.

a. *Method detection limit (MDL):* An MDL study using the EPA protocol (40CFR136 Appendix B) must be available. Example results are shown in Table B2.

b. *Minimum reporting level (MRL):* The minimum reporting level should be no less than 3 times the MDL and a standard must be run at this level.

c. *Laboratory reagent blank:* A blank using the laboratory reagent water is analyzed by the method (including reagents, glassware, etc.) with each batch of samples. The blank must be free of nitrosamine contamination (e.g., equal to or less than one half the MRL).

d. *Calibration Check: A* mid-level continuing calibration check standard (CCC) is analyzed with each batch. If it agrees within ± 20% of the expected value, a new calibration curve is not needed (see Procedure, calibration section).

e. *Laboratory fortified blank (LFB):* An LFB is prepared by spiking reagent water at the MRL level with a different stock solution than used for the calibration curve standards. The LFB is taken through the full method with each batch of samples. Recovery should be within ± 50% of the theoretical value.

f. *Laboratory fortified sample matrix (LFSM):* Analysis of an LFSM is required in each analysis batch or 10 samples and is used to determine that the sample matrix does not adversely affect method accuracy. Within each analysis batch, a minimum of one field sample is fortified as an LFSM for every 10 samples processed. The LFSM is prepared by spiking a sample with an appropriate amount of the analyte from a different stock source than used for the standards. A spiking concentration is selected approximately twice the matrix background concentration, if known. The percent recovery (R) is calculated for each analyte using the equation:

$$R = \frac{(A - B)}{C} \times 100$$

where:

A = measured concentration in the fortified sample,
B = measured concentration in the unfortified sample, and
C = fortification concentration.

For samples fortified at or above their native concentration, recoveries should range between 70 to 130%.

g. *Sample duplicate (LD1, LD2) or laboratory fortified matrix spike duplicate (LFSMD):* If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, a second LFSM (i.e., LFMSD), must be prepared and analyzed from a duplicate of the field sample used to prepare the LFSM to assess method precision. The relative percent difference (RPD) for duplicates (LD1 = LFSM and LD2 = LFSMD) is calculated using the equation:

$$RPD = \frac{(LFSM - LFSMD)}{(LFSM + LFSMD)/2} \times 100$$

RPDs for LDs and duplicate LFSMs should fall in the range of ± 20% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are spiked near the MRL. At the MRL, RPDs should fall in the range of ± 50% for samples fortified at or above their native concentration.

8. References

1. Taguchi,V.Y., S.W.D Jenkins, D.T. Wang, J-P.F.P. Palmentier & E.J. Reiner. 1994. Determination of N-nitrosodimethylamine by isotope dilution, high-resolution mass spectrometry. *Canadian Journal of Applied Spectroscopy*.39:3.
2. APHA, AWWA, WEF (1992). *Standard Methods for the Examination of Water and Wastewater*. Method Detection Limit, 18th ed. Washington DC:APHA, 1030 E.2.
3. A. Hubaux and G. Vos, *Anal. Chem.*, **42**, No.8, July 1970
4. R.B. Dean and W.J. Dixon, *Anal. Chem*., **23**, No. 4, April 1951

Chromatogram Plot

File: c:\... \nt-dec03\nt120803\calibration\200ppb, 12-9-2003.sms
Sample: 200ppb
Scan Range: 1 - 2979 Time Range: 0.00 - 29.99 min.

Operator: JDL
Date: 12/9/2003 2:59 AM

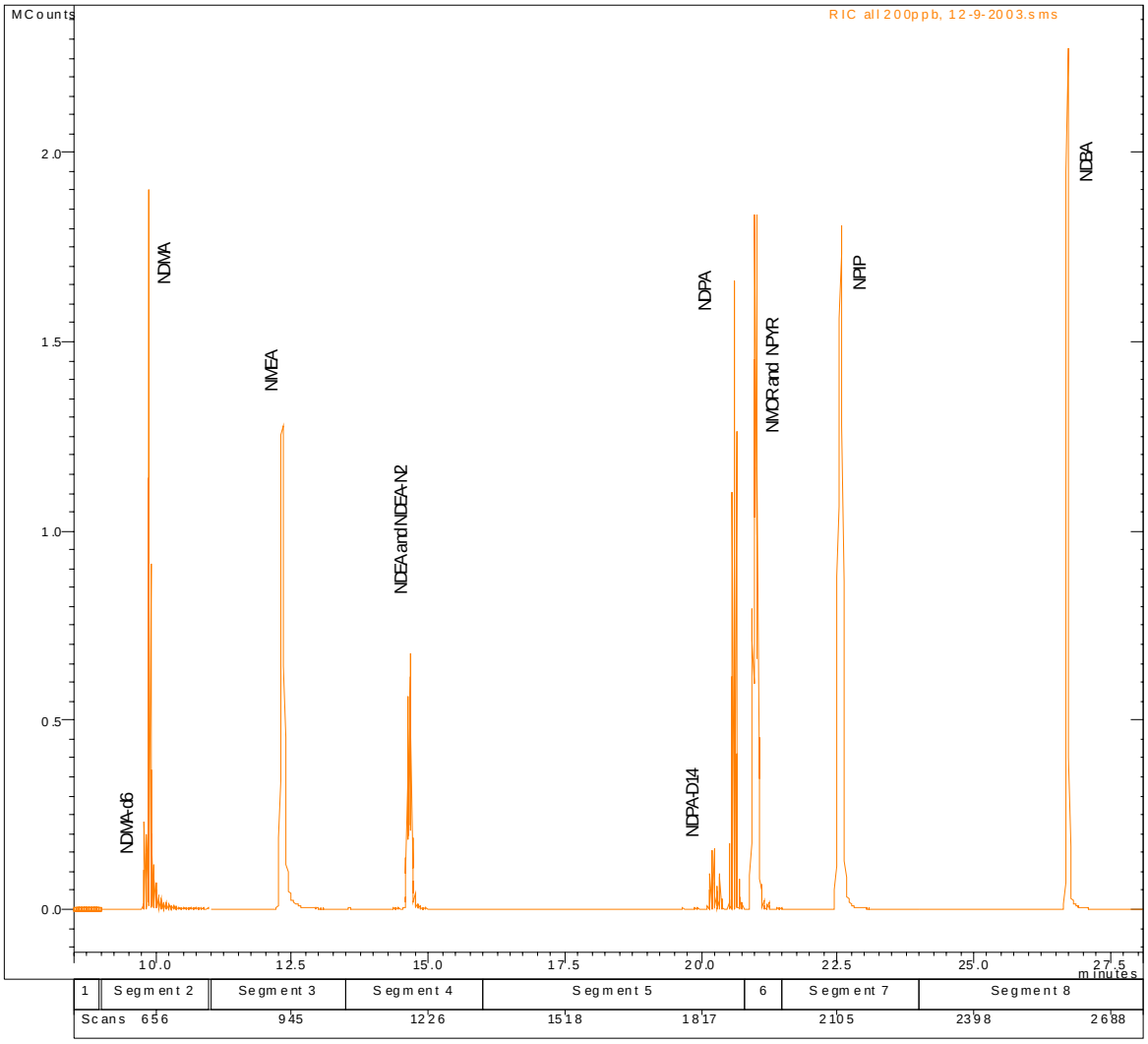


Figure B1 Typical chromatogram of a nitrosamine mix, 200 µg/L

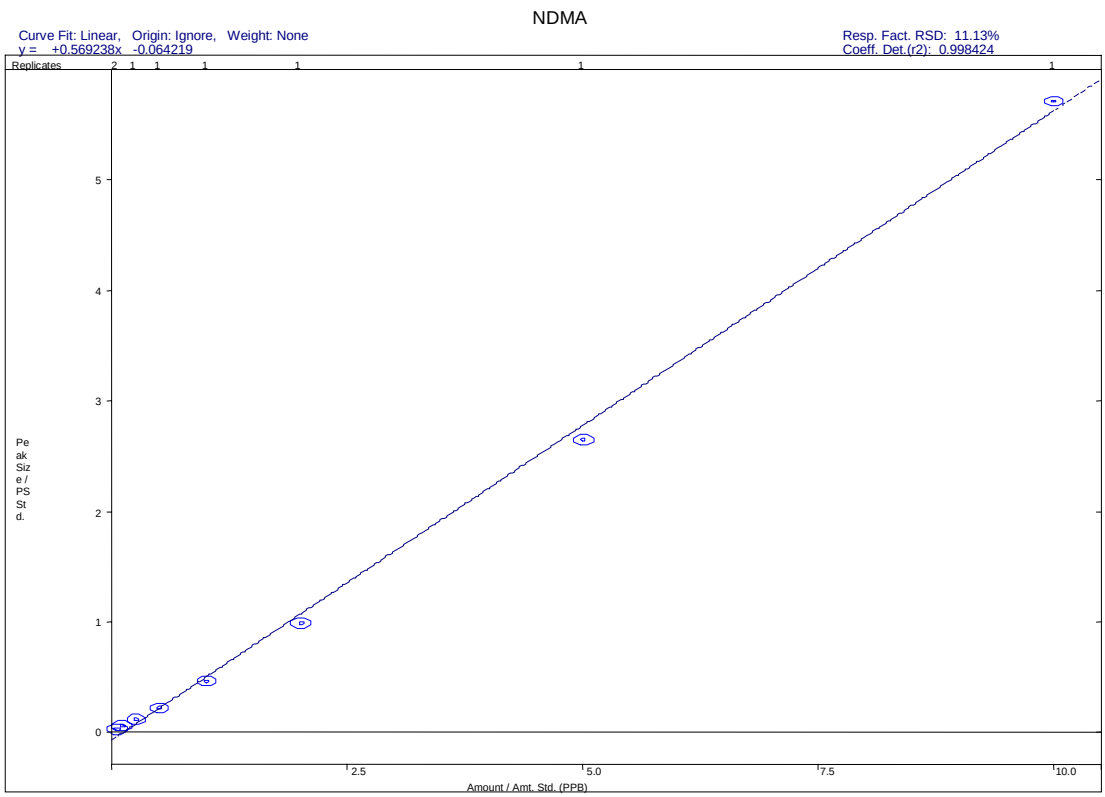


Figure B2 NDMA calibration curve (2 – 200 ng/L)

Table B1 Target nitrosamine analytes: formula, molecular weight, CAS No., and internal standard

Nitrosamine	Abbrev.	Formula	MW	CAS #	Internal std.
N-Nitrosodimethylamine	NDMA	$C_2H_6N_20$	74	62-75-9	d_6-NDMA
N-Nitrosomethylethylamine	NMEA	$C_3H_8N_20$	88	10595-95-6	$^{15}N_2$-NDEA
N-Nitrosodiethylamine	NDEA	$C_4H_{10}N_2O$	102	55-18-5	$^{15}N_2$-NDEA
N-Nitrosodi-n-propylamine	NDPA	$C_6H_{14}N_2O$	130	621-64-7	d_{14}-NDPA
N-Nitrosomorpholine	NMOR	$C_4H_8N_2O_2$	116	59-89-2	d_{14}-NDPA
N-Nitrosopyrrolidine	NPYR	$C_4H_8N_2O$	100	930-55-2	d_{14}-NDPA
N-Nitrosopiperidine	NPIP	$C_5H_{10}N_2O$	114	100-75-4	d_{14}-NDPA
N-Nitrosodi-n-butylamine	NDBA	$C_8H_{18}N_2O$	158	924-16-3	d_{14}-NDPA

Table B2 MDLs of nitrosamines

Nitrosamine	Fortification level (*ng/L*)	MDL (*ng/L*)
NDMA	1.0	0.84
NMEA	1.0	0.45
NDEA	1.0	0.81
NDPA	1.0	1.08
NPYR	1.0	0.83
NMOR	1.0	0.62
NPIP	1.0	0.74
NDBA	1.0	0.71

For n=10, t=2.764 at the 99% confidence level.

Table B3 Procedural calibration standards*

Calibration std. concentration (ng/L)	100 ng/mL nitrosamine mix	1000 ng/mL nitrosamine mix	1000 ng/mL internal std. and surrogate mix
1	5	-	10
2	10	-	10
5	25	-	10
10	-	5	10
20	-	10	10
50	-	25	10
100	-	50	10
300	-	150	10

**Spike the tabulated volumes of analyte and internal standard/surrogate PDS into 500 mL reagent water to obtain the different concentrations of calibration standards.*

Table B4 GC/CI/MS/MS conditions

GC Injector Program (CP-3800cx)

Temp. (^{o}C)	Rate (^{o}C/min)	Hold time (min)	Total time (min)
37	0	0.67	0.67
250	200	27	28.74

Table B5 HP-VOC column temperature program

Temp. (^{o}C)	Rate (^{o}C/min)	Hold time (min)	Total time (min)
32	0	1.7	1.7
100	15	2	8.2
190	5	0	26.2
270	50	5	30.0*

**May be increased depending on the content of the sample, HP-VOC column (60 m x 0.32 mm x 1.8 μm)*

Table B6 CI/MS/MS conditions

Segment	Analyte	Retention time (min)	Parent mass	Quant (Daughter) ion	Internal standard	CID	RRF	Start time	End time	Low high mass
1	Fil/Mul delay							0	10.5	
2	NDMA- d_6	11.0	81	50		0.60		10.5	12.0	40-85
	NDMA	11.1	75	44	**NDMA-d_6**	0.68	1.4			
3	**NMEA**	13.6	89	61	**NDEA-$^{15}N_2$**	0.70	4.7	12.0	14.5	50-95
	NDEA-$^{15}N_2$	16.0	105	77	-	0.65	-	14.5	17.0	65-110
4	**NDEA**	16.0	103	75	**NDEA-$^{15}N_2$**	0.77	1.2			
5	**None**							17.0	21.0	
6	**NDPA-d_{14}**	21.6	145	97	-	0.77	-	21.0	23.2	50-160
	NDPA	21.9	131	89	**NDPA-d_{14}**	0.74	0.4			
	NMOR	22.3	117	86	**NDPA-d_{14}**	0.70	1.0			
	NPYR	22.3	101	55	**NDPA-d_{14}**	0.70	0.8			
7	**NPIP**	23.9	115	69	**NDPA-d_{14}**	0.49	1.6	23.2	25.0	50-120
8	**NDBA**	28.2	159	57	**NDPA-d_{14}**	0.60	0.2	25.0	28.8	50-165

**Ionization mode: CI auto, Ion prep: MRM, Isolation window: 2, Waveform type: Resonant*

Nitrosamine	Internal standard	Quant ion	CID	RRF
NDMA	NDMA-d_6	75	0.35	2.5
NDPA	NDPA-d_{14}	131	0.30	1.9
NPIP	NDPA-d_{14}	115	0.30	3.0
NDBA	NDPA-d_{14}	103	0.45	0.5

Table B7 Methanol chemical ionization parameters

Gas/Liquid	CI (MeOH)
Molecular mass	32
Maximum ionization time	2500
Maximum reaction time	120
CI storage level	15
CI background mass	45
Reagent ion eject	10

Table B8 Chemical ionization default settings

Gas/Liquid	Methane (G)	Isobutane (G)	Ammonia (G)	Acetonitrile (L)	Methanol (L)
Molecular mass	16	58	17	41	32
Maximum ionization time	2000	2000	2000	2000	2000
Maximum reaction time	40	40	40	40	40
CI storage level	5	10	5	25	15
CI background mass	45	65	40	65	45
Reagent ion eject	9.0	7.4	12.5	9.0	10.0

***** *Pressure must be adjusted for different reagent gases and changes in CI reagent may require the GC system to be modified by the vendor.*

Table B9 Recommended amplitude

Mass	Eject Amp
<20	12.5
21-50	9
>50	7.5

Table B10 Absolute recovery of nitrosamines in deionized water fortified at 100 ng/L

Nitrosamine	Fortification Level (*ng/L*)	% Abs rec.
NDMA	100	56
NMEA	100	100
NDEA	100	118
NDPA	100	124
NPYR	100	99
NMOR	100	99
NPIP	100	108
NDBA	100	103

Table B11 Single laboratory bias and precision data for nitrosamines spiked into drinking water

Nitrosamine	Sample conc. (ng/L)	Run 1	Run 2	Run 3	Run 4	Mean rec. %	%RSD
NDMA	<1	104	101	100	99	101	2.2
NMEA	<1	108	102	102	101	103	2.5
NDEA	<1	106	101	101	98	101	1.9
NDPA	<1	103	95	104	101	101	4.6
NPYR	<1	104	88	93	92	94	3.8
NMOR	<1	99	101	100	98	100	1.1
NPIP	<1	97	103	98	92	97	2.7
NDBA	1.1	107	101	99	91	99	3.6

Potable sample observed (n=4), Spiked concentration = 20 ng/L

Table B12 Single laboratory bias and precision data for nitrosamines spiked into secondary effluent water

Nitrosamine	Sample conc. (ng/L)	Run 1	Run 2	Run 3	Run 4	Mean rec. %	%RSD
NDMA	93	104	106	102	107	105	0.9
NMEA	<4	75	66	71	70	71	4.2
NDEA	9.4	101	100	106	95	101	3.3
NDPA	<4	102	102	109	110	106	3.0
NPYR	<4	95	90	101	83	92	6.9
NMOR	5.9	100	95	99	85	95	6.6
NPIP	<4	98	99	105	104	102	2.9
NDBA	<4	101	102	107	104	104	2.1

Secondary effluent water sample observed (n=4), Spiked concentration = 20 ng/L

Table B13 Interlaboratory bias and precision data for nitrosamines spiked into chlorinated potable surface water

Nitrosamine	Sample conc. (ng/L)	Lab 1	Lab 2	Lab 3	Mean rec. %	%RSD
NDMA	4.4	101	91	91	94	5.8
NMEA	<2	86	81	133	100	28.7
NDEA	<2	95	93	98	96	2.8
NDPA	<2	90	106	85	94	10.8
NPYR	<2	94	93	113	100	11.1
NMOR	<2	127	148	142	139	10.8
NPIP	<2	90	80	101	90	10.6
NDBA	<3	91	94	101	95	4.9

Potable sample observed (n=3), Spiked concentration = 12.7 ng/L

Table B14 Interlaboratory bias and precision data for nitrosamines spiked into secondary wastewater effluent

Nitrosamine	Sample conc. (ng/L)	Lab 1	Lab 2	Lab 3	Mean rec. %	%RSD
NDMA	156	121	114	83	106	20.1
NMEA	<2	93	118	107	106	12.7
NDEA	<11	113	88	91	97	13.6
NDPA	<2	99	102	81	94	11.2
NPYR	<2	107	112	88	102	12.9
NMOR	<5	112	106	77	98	18.8
NPIP	<2	98	107	91	99	8.2
NDBA	<3	92	83	91	89	5.1

Secondary effluent water sample observed (n=3), Spiked concentration = 376 ng/L

APPENDIX C

AMBERSORB 572/ENVI-CARB CARTRIDGE SOLID–PHASE EXTRACTION GAS CHROMATOGRAPHIC/MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF NDMA AND SEVEN OTHER NITROSAMINES

1. General Discussion

a. Sources and Significance: The chemical N-nitrosodimethylamine (NDMA) has been identified as a probable human carcinogen by the U. S. Environmental Protection Agency. It is commonly found in liquid rocket fuel, lubricants, and pesticides. The general population may be exposed to NDMA from outdoor air; tobacco smoke; diet such as cured meats, fish, and cheese; beverages such as beer and whisky; cosmetics; and rubber products. The average concentration of NDMA measured in food ranges from 90 to 100 ng/L (ppt) for pasteurized milk, 600 to 1,000 ng/Kg for fried pork bacon, and 50 to 5,900 ng/Kg for various beers. NDMA has also been detected in recycled water, wastewater, and potable water. It can either occur as a chemical contaminant from industrial processes or be formed by chlorine and chloramine disinfection processes. The formation mechanisms that produce NDMA may also form other nitrosamines. Seven other nitrosamines (listed in Table C1) that are structurally related to NDMA and/or known to cause cancer in animals have been included in this method.

b. Principle: NDMA and other nitrosamines (Table C1) are extracted from the water matrix by adsorption predominantly onto Ambersorb 572 resin supported by auxiliary adsorbent Envi-carb, all in a disposable cartridge. After drying, the analytes are eluted from the adsorbents with dichloromethane. The eluent is then concentrated by evaporation. Analysis is by gas chromatography(GC) /tandem mass spectrometry (MS/MS) with chemical ionization to promote formation of the precursor protonated molecular ion $[M+H]^+$. Isotope dilution/internal standard quantitation was implemented with the addition of three isotopically labeled internal standards (i.e., d6-NDMA, d14-NDPA, and 15N2-NDEA) prior to extraction to correct for extraction as well as instrument variations. A large volume (8 µL aliquot) of sample extract is injected to increase sensitivity, thereby lowering detection limits to low part per trillion levels. Identification of NDMA and the other nitrosamines is based on their retention times, parent ion isolation, and fragmentation patterns.

c. Interferences: Method interferences may be caused by contaminants, especially from NDMA, in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. The samples or analytical system also may be contaminated from rubber objects in the work area. All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by reagent water. A final rinse with solvents may be needed. In place of a solvent rinse, nonvolumetric glassware can be heated in a muffle furnace at 400 ^{o}C for 2 hours. Volumetric glassware should not be heated above 120 ^{o}C. Samples that are not properly preserved may experience inaccurate target analyte recovery due to formation caused by a chloramine residual. Coeluting GC peaks with nominal masses equivalent to the target analytes and internal standards have been observed in drinking water and wastewater necessitating MS/MS or high resolution MS quantitation. To demonstrate freedom from interferences, a reagent-water blank must be analyzed under the same conditions as the samples. The blank concentration must be equal to or less than one half the minimum reporting level (MRL).

d. Safety: Because NDMA, most of the other nitrosamines, and dichloromethane have been identified as animal carcinogens and some as probable human carcinogens, exposure to these compounds

and their isotopically labeled analogs must be minimized. A reference file of material safety data sheets (MSDS) should also be made available to all personnel involved in analyses.

1) Effluents of GC sample splitters and GC/MS vacuum pumps should pass through either a column of activated carbon or be bubbled through a trap.
2) The following precautions for safe handling of NDMA and other nitrosamines in the laboratory are presented as guidelines only.
   a.) Protective equipment: Laboratory hood, safety glasses, disposable plastic gloves, and apron or lab coat.
   b.) Personal hygiene: Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).
   c.) Decontamination: Personnel — any mild soap with scrubbing action. Glassware, tools and surfaces — wash with detergent and water. Solvent waste should be minimized.
   d.) Handling the dilute solutions normally used in analytical work presents no significant inhalation hazards except in case of an accident.

e. Detection limits: Minimum detection limits (MDL) are compound, instrument, and matrix dependent. The detection limit is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[1] Experimentally determined detection limits for the target analytes are provided in Table C2. The detection limit differs from, and is lower than the Minimum Reporting Level (MRL). The concentration range for target analytes in this method was evaluated between 2 ng/L and 500 ng/L.

2. Sampling and Storage

a. Sample Collection:

1) The sample site should be free of auto exhaust, cigarette smoke, fresh paint and any other possible sources of contamination. Sample location should provide a representative grab sample or composites (maintained cold and with proper quenching).
2) When sampling from a water tap, the tap is allowed to flush until the water temperature has stabilized (usually about 3 to 5 minutes). When sampling from an open body of water, the sample is collected using a clean stainless steel bucket with a clean rope. Avoid plastic and rubber tubing, gaskets, etc. that may leach interfering analytes into the water sample.
3) Samples should be collected in pre-cleaned, amber glass bottles with Teflon- lined polyethylene caps. The minimum volume collected should be sufficient for the analysis of the sample, a matrix spike, and matrix spike duplicate for quality assurance purposes, preferable in two or more bottles. Sample bottles are filled to the top but care taken not to flush out the preservative.
4) When sampling chlorinated or chloraminated water, the residual should be quenched at time of sampling. The addition of solid sodium sulfite or sodium thiosulfate (approximately 0.5 g for wastewater, or 0.04 to 0.1 g for potable water) to a 1 L bottle should minimize additional nitrosamine formation. Alternately 0.02 g of ascorbic acid may be used for drinking water. If chloramines residual is greater than 4 mg/L additional quenching agent should be added. If preservatives are used, the bottle should not be rinsed with sample before collection.

b. Storage:

1. Samples should be iced or refrigerated at 4 °C or lower (but not freezing) and maintained at these conditions away from light until extraction. To prevent photodecomposition, samples must be protected from light from the time of collection until extraction. Amber colored bottles work well for this. Avoid storage of samples under low pH conditions because this has been observed to produce elevated levels of NDMA in some wastewater effluents and potable waters. Extract drinking water samples within 28 days. As a guideline, wastewater samples should be extracted within 14 days. However, degradation

and formation of nitrosamines in wastewater matrices can be complicated processes. It is suggested that appropriate holding times be developed on a case by case basis.

2. Extracts should stored at –11 °C, away from light in amber glass vials with Teflon-lined caps. Extracts should be analyzed in a timely manner, however, under the conditions mentioned, archived extracts have generally shown minimal NDMA losses over a period of six months.

3. Apparatus

All specifications are suggested. Brand names and/or catalog numbers are included for illustration only.

a. *SPE vacuum manifold*: Chemical resistant cartridge holders with individual flow control valves, glass basin, collection rack, large volume samplers and nonrubber vacuum hose connections (Supelco #57160-U + accessories). Desirable: Inert gas drying accessory.

b. *Extraction cartridges*: Dual-media cartridges comprised of a commercially available Supelclean ENVI-carb sorbent (Supelco, Bellefonte, PA # 57088, 3 mL, 250 mg) with additional 350 mg Ambersorb® 572 (Supelco #10432-U,) added to the top and sealed with an additional polyethylene frit (Supelco, # 57180-U).

c. *Concentrator with glassware:* Kuderna-Danish (KD), 10-mL graduated tube. (Verify calibration at volume used.) Heated water bath with inert gas sample evaporation stations and accessories needed to concentrate extract from 10 to 0.5 mL.

d. *Sample containers:* Amber glass bottles fitted with PTFE-lined screw caps.

e. *Volumetric flasks*: Class A, various sizes used for preparation of standards.

f. *Inert Gas*: Ultra high purity helium or nitrogen with purifying cartridge and low-pressure regulator.

g. *Syringes*: Glass microsyringes with stainless steel needle and plunger in various sizes for spiking solutions and preparing intermediate solutions. A 20 mL glass hypodermic (glass plunger) with 17 gauge, 3.5 inch long stainless steel pipetting needle is used to withdraw the DCM layer.

h. *Vials:* Screw cap amber glass vials with TFE-lined silicone septa in sizes appropriate for the autosampler and for storage of spiking solutions.

i. *Transfer pipets:* Disposable glass Pasteur pipets.

j. *Gas chromatograph/mass spectrometer/mass spectrometer system (e.g., Varian 3800 GC coupled with Saturn 2200 ion trap mass spectrometer) equipped with*:

1) *Temperature programmable large volume injector*: Capable of going from 35 to 230 °C at 200 °C/minute and large volume (up to 100 µL) injection.
2) *Capillary column*: Either a 30 m x 0.25 mm i.d, 1.0 µ film thickness DB 1701 or a 60 m x 0.32 mm i.d., 1.8 µ film thickness DB-VRX fused silica or other capillary column capable of providing adequate and reproducible resolution.
3) *MS/MS analyzer:* A system with chemical ionization capable of producing and isolating a $[M+H]^+$ molecular ion and then fragmenting it to produce unique product ion spectra in a consistent and quantitative manner.

4. Reagents

Reagent grade or better chemicals and high resolution gas chromatography-grade solvents should be used. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

a. *Reagent water:* Freshly prepared purified water which does not contain any measurable quantities of any target analytes or interfering compounds greater than one third of the minimum reporting level (MRL) for each compound of interest such as demineralized ultraviolet-treated water (e.g., Mill-Q-UV treated).

b. *Methanol:* CH_3OH, high purity, HR-GC grade, demonstrated to be free of analytes and interferences (e.g., OmniSolv grade, EM Science).
c. *Dichloromethane:* CH_2Cl_2, alkene stabilized, (e.g., OmniSolv, HR-GC grade).
d. *Standard solutions:*
    1) Stock nitrosamine mix containing eight target nitrosamines (plus *N*-nitrosodiphenylamine) at 2000 µg/mL each in methanol (Supelco, Bellefonte, PA #502138*)* and in dichloromethane (Protocol, # 8270-AF-C). One stock source is used to prepare extracted and direct standards and the other for spiking samples and laboratory fortified blanks.
    2) Target analytes Primary Dilution Standard (PDS): Prepare a nitrosamine mix PDS of a suitable concentration by accurately transferring the appropriate volume of stock standard solution into a volumetric flask partially filled with methanol. Dilute to volume, mix thoroughly, transfer to an amber glass vial, and store at 4 °C. As an example, 500 ng/mL PDS were prepared in 25 mL volumetric flasks. A serial dilution of this PDS, to make a 100 ng/mL solution is useful for low level spiking.
    3) Internal standard stock solutions: *N*-nitrosodimethylamine (D_6), *N*-nitrosodipropylamine (D_{14}) and *N*-nitrosodiethylamine ($^{15}N_2$) are purchased as individual stock solutions at 1 mg/mL in methylene chloride (Cambridge Isotope Laboratories, Andover, MA, #DLM-2130-S, DLM-2131-S and NLM-3432-S, respectively).
    4) Internal standard PDS: Prepare an ISPDS containing all three internal standards at suitable concentration by accurately transferring the appropriate volumes of each internal standard stock solution to a volumetric flask, containing methanol as described in the previous section. As an example, 200 ng/mL PDS of internal standards was prepared in 25 mL volumetric flasks. Store in an amber glass vial at 4 °C.
    5) Calibration Standards: This method uses procedural standards for preparation of calibration curves. Standard concentrations must range from the MRL to greater than the highest sample concentration, which typically is from 10 ng/L to 500 ng/L. Prepare and extract a five-point calibration curve as outlined in the procedure below, by fortifying a series of 100 mL reagent water aliquots with appropriate levels of target analytes and 50 ng/L each of internal standards.
e. *Sodium chloride:* NaCl ACS grade. Heated at 400 °C overnight and stored at 110 °C.
f. *Sodium sulfate:* Na_2SO_4 granular anhydrous ACS reagent grade. Heated at 400 °C for 3 hours and stored at 110 °C.

5. Procedure

a. *Extraction:*
    1) Measure 500 mL of sample into an amber bottle. Add 25 ng of internal standards, d_6-NDMA, $^{15}N_2$-NDEA, and d_{14}-NDPA (50 µL of 0.5 mg/L mix prepared in methanol) to give a concentration of 50 ng/L for each internal standard. If the sample is a method standard, laboratory fortified blank (LFB), matrix spike, or matrix spike duplicate the appropriate volume of intermediate nitrosamine mix (in methanol) should be added.
    2) *Set up SPE manifold* with appropriate number of dual-media SPE cartridges and adjust vacuum to 15 psi.
    3) *Conditioning*. Fill each dual-media cartridge with 2 mL aliquot of methanol, with cartridge closed, adjust for dropwise flow. Repeat methanol rinse. Do not let media go dry from 2nd methanol rinse until end of sample extraction. Rinse cartridge with two 2 mL aliquots of reagent water.
    4) *Sample transfer:* With cartridge almost full of water, attach transfer tubes. Prefilling the transfer tubes with reagent water may minimize introduction of air. Transfer 500 mL sample from amber container at flow rate of not more than 5 mL/min.
    5) *Drying:* Remove transfer line and dry cartridge for 60 minutes under full vacuum and dry eluent with anhydrous sodium sulfate, or dry cartridges overnight under gentle stream of clean, dry nitrogen. Dry manifold needles.
    6) *Elution:* Insert graduated tube in manifold under sample cartridge. Fill cartridge with dichloromethane and draw through slowly to wet media allowing approximately 10 drops to

elute then stop the flow. Allow dichloromethane to equilibrate for several minutes for sufficient interaction between dichloromethane and dual media. Draw solvent down dropwise to the top of media. Repeat elution again until 6 mL of eluent are collected. Apply additional vacuum to drain remaining solvent from the cartridge.

7) *Concentration:* Remove concentrator tube from manifold. If cartridge was dried for only 1 hour or water is observed, add anhydrous sodium sulfate to dry the extract, swirl, let sit then decant, rinse with dichloromethane twice and combine extracts. If cartridge dried overnight, proceed to next step. Concentrate the eluent to 0.5 mL under a gentle stream of inert gas, while keeping eluent sufficiently warm to prevent moisture condensation.
8) *Storage:* Transfer concentrate to an autosampler vial and inject or store in a freezer.

b. Gas Chromatography: Establish operating conditions such as those described in Table C3.

This method uses chemical ionization (CI), tandem mass spectrometry (MS/MS). In the CI mode of operation a CI reagent gas (acetonitrile or methanol) is introduced into the ion trap, ionized, and allowed to react with sample molecules. Ionization of the sample molecules generates the protonated molecular ion $[M+1]^+$. This ion is isolated from matrix ions in the trap and is selected as the precursor (parent) ion for subsequent fragmentation. One of the benefits of CI is that it results in a higher concentration of the $[M+1]^+$ than electron impact ionization. A waveform is applied to the trap increasing the energy of the isolated precursor ion. The amplitude of this waveform is called the collision induced dissociation (CID) excitation amplitude. As the energy of the precursor ion increases, chemical bonds are broken and product ions (daughter ions) of lower m/z than the precursor ion are formed. It is possible to use the precursor or product ions to quantify the target analytes. Quantitation based on $[M+H]^+$ provides greater sensitivity, especially in clean water matrices, while quantitation on product ions provides greater specificity and may be needed for wastewater or matrices with interfering compounds. Examples of MS/MS parameters with acetonitrile as CI reagent are listed in Table C4. CID values were chosen to maximize the transition from parent ion to product ions while retaining 10 to 25% of the parent ion for confirmation. The degree of fragmentation observed for a certain CID value depends on the instrument and operating conditions and therefore will vary with time and laboratory. In some instances, the degree of fragmentation has also been observed to depend on analyte concentration.

Other GC/MS/MS conditions may be used as long as QC requirements are met. Establish an appropriate retention time window and precursor to product ion mass ratio for each target and surrogate analyte to facilitate detection and identification in all QC and field samples.

c. Calibration: Prepare standards as described in Section 4.j.5. Extract and analyze each standard under the same conditions used for sample extracts. Use internal standards as designated in Table C1 for quantitation of each nitrosamine. Using the GC/MS software, generate a linear regression or quadratic calibration curve plotting area ratios ($Area_x/Area_{IS}$) verses concentration for each nitrosamine. Curves are typically linear to 500 ng/L. The coefficient of determination (r^2) should round to 0.99 or higher. Alternately mean response factors may be used for linear calibration.

d. Continuing Calibration: For continuing calibration, verify the calibration by extracting and analyzing a mid-point calibration standard. The calculated concentrations should be 70 to 130% of its true value.

6. Data Analysis and Calculations

a. Review: Check the chromatogram for any incorrect peak identification or poor integration.
b. Quantitation: Calculate sample concentration with the best-fit calibration equation or the average relative response factor as explained in Section 5, Procedure c, Calibration. Quantitate only those samples that fall between the MRL and the highest calibration standard. If the determined analyte

concentration exceeds that of the highest standard, dilute the original sample into a final volume of 500 mL, re-extract, and re-analyze.

c. *Identification:* From the product ion chromatograms, identify the analytes in the sample by comparing the retention time and spectrum of the suspect peak to retention time and spectrum of the reference analyte peak in a calibration standard. All ions that are present in the reference mass spectrum should be present in the sample mass spectrum with intensities between 10 to 50% of the reference mass spectrum.
d. *Correction*: Adjust the calculated concentrations of detected analytes to reflect any dilutions performed.
e. *Reporting*: Report analyte concentrations in ng/L.

7. Quality Control

At a minimum, an initial demonstration of an acceptable calibration curve (RSD <20%), continuing calibration checks with acceptance criteria of ± 30% of initial calibration, reagent method blanks, field blanks, field sample duplicates, matrix spikes, and low level calibration checks (at or below the MRL) with an acceptance criteria of ± 50%, should be employed. Additional quality control parameters are recommended for ongoing quality assurance.

a. *Method detection limit (MDL):* An MDL study using the EPA protocol (40CFR136 Appendix B) must be available. Example results are shown in Table C2.
b. *Minimum reporting level (MRL):* The minimum reporting level should be no less than 3 times the MDL and a standard must be run at this level.
c. *Laboratory reagent blank:* A blank using the laboratory reagent water is analyzed by the method (including reagents, glassware, etc.) with each batch of samples. The blank must be free of nitrosamine contamination (e.g., equal to or less than one half the MRL).
d. *Calibration Check:* A mid-level continuing calibration check standard (CCC) is analyzed with each batch. If it agrees within ± 20% of the expected value, a new calibration curve is not needed (see Procedure: calibration section).
e. *Laboratory fortified blank (LFB):* An LFB is prepared by spiking reagent water at the MRL level with a different stock solution than used for the calibration curve standards. The LFB is taken through the full method with each batch of samples. Recovery should be within ± 50% of the theoretical value.
f. *Laboratory fortified sample matrix (LFSM):* Analysis of an LFSM is required in each analysis batch or 10 samples and is used to determine that the sample matrix does not adversely affect method accuracy. Within each analysis batch, a minimum of one field sample is fortified as an LFSM for every 10 samples processed. The LFSM is prepared by spiking a sample with an appropriate amount of the analyte from a different stock source than used for the standards. A spiking concentration is selected approximately twice the matrix background concentration, if known. The percent recovery (R) is calculated for each analyte using the equation:

$$R = \frac{(A - B)}{C} \times 100$$

where:

A = measured concentration in the fortified sample,
B = measured concentration in the unfortified sample, and
C = fortification concentration.

For samples fortified at or above their native concentration, recoveries should range between 70 to 130%.

g. *Sample duplicate (LD1, LD2) or laboratory fortified matrix spike duplicate (LFSMD):* If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, a second LFSM (i.e., LFMSD), must be prepared and analyzed from a duplicate of the field sample

used to prepare the LFSM to assess method precision. The relative percent difference (RPD) for duplicates (LD1 = LFSM and LD2 = LFSMD) is calculated using the equation:

$$RPD = \frac{(LFSM - LFSMD)}{(LFSM + LFSMD)/2} \times 100$$

RPDs for LDs and duplicate LFSMs should fall in the range of ± 20% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are spiked near the MRL. At the MRL, RPDs should fall in the range of ± 50% for samples fortified at or above their native concentration.

8. Method Performance

a. A typical nitrosamine chromatogram and NDMA calibration curve are presented Figures C1 and C2, respectively.
b. Single Laboratory precision and accuracy data are shown in Table C5.
c. Interlaboratory comparisons are shown in Figure C6. The round-robin testing was conducted between laboratories with widely varying experience with this cartridge SPE method and demonstrate that accurate results can be produced by an experienced laboratory, but that some experience is needed to achieve consistently acceptable recoveries. Lab 2 was out of control for NMEA and just barely for NDMA, and Lab 3 had recoveries greater than 130% for five of the seven compounds.

9. References

1. "NDMA Laboratory Analysis," California Department of Health Services, http://www.dhs.cahwnet.gov/ps/ddwem/chemicals/NDMA/NDMAlabs.htm, 5/16/2003.
2. "Carcinogens—Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, NIOSH, Pub. #77-206, August 1977.
3. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Ed., 1979.
4. Choi, J., & R. L. Valentine. (2002). Formation of N Nitrosodimethylamine (NDMA) from Reaction of Monochloramine: A New Disinfection By-Product. *Water Res.*, 36: 817.
5. Mitch, W. A., & D. L. Sedlak. (2002). Formation of N-Nitrosodimethylamine (NDMA) from Dimethylamine During Chlorination. *Environ. Sci. Technol.*, 36: 588.
6. Taguchi, V.Y., S.W. Jenkin, D. T. Wang, J-P. F. Palmentier & E. J. Reiner. (1994). *Canadian J. of Applied Spectroscopy,* 39: 87
7. Guo, Y. C., C. J. Hwang, S. J. Pastor, & S. E. Barrett. (2004). Optimization and Comparison of Different Extraction Methods for Nitrosamine Analysis in Drinking Water and Wastewater. Presented at 227TH American Chemical Society National Meeting, Anaheim, CA.

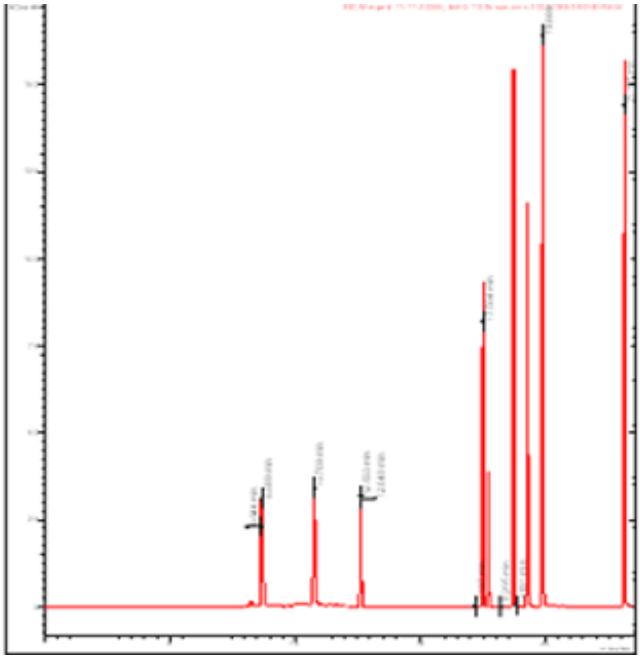


Figure C1 Typical chromatogram of 50 ng/L CSPE extracted nitrosamine standard

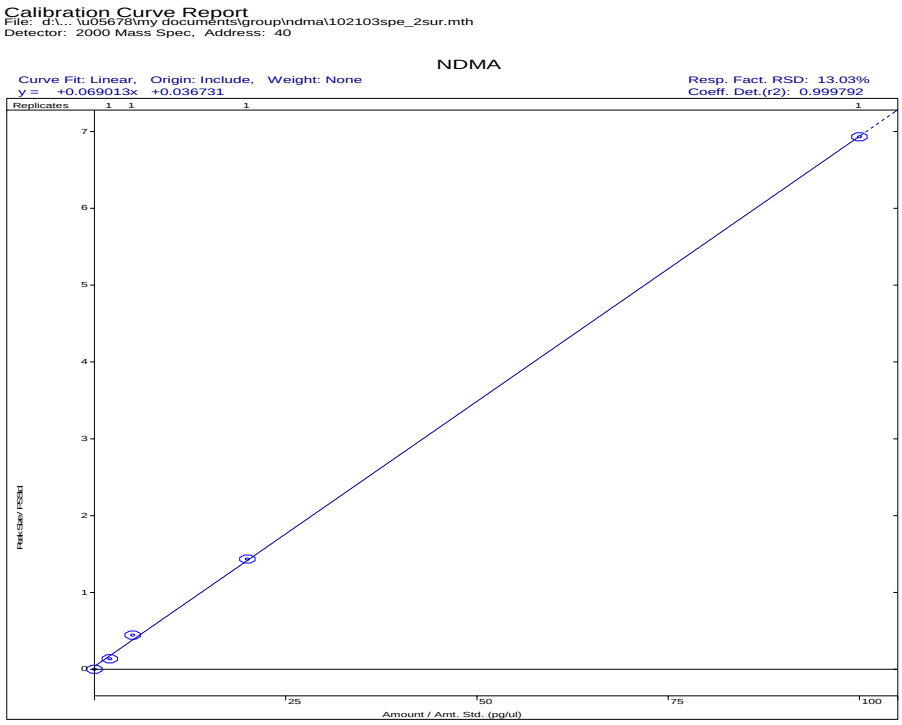


Figure C2 Calibration curve for CSPE of NDMA (2-100 ng/L)

Table C1 Target nitrosamine analytes: formula, molecular weight, CAS No., and internal standard

Nitrosamine	Abbrev.	Formula	MW	CAS #	Internal std.
N-Nitrosodimethylamine	NDMA	$C_2H_6N_20$	74	62-75-9	d_6-NDMA
N-Nitrosomethylethylamine	NMEA	$C_3H_8N_20$	88	10595-95-6	$^{15}N_2$-NDEA
N-Nitrosodiethylamine	NDEA	$C_4H_{10}N_2O$	102	55-18-5	$^{15}N_2$-NDEA
N-Nitrosodi-n-propylamine	NDPA	$C_6H_{14}N_2O$	130	621-64-7	d_{14}-NDPA
N-Nitrosomorpholine	NMOR	$C_4H_8N_2O_2$	116	59-89-2	d_{14}-NDPA
N-Nitrosopyrrolidine	NPYR	$C_4H_8N_2O$	100	930-55-2	d_{14}-NDPA
N-Nitrosopiperidine	NPIP	$C_5H_{10}N_2O$	114	100-75-4	d_{14}-NDPA
N-Nitrosodi-n-butylamine	NDBA	$C_8H_{18}N_2O$	158	924-16-3	d_{14}-NDPA

Table C2 Minimum detection limits in reagent water

Nitrosamine	Fortification level (ng/L)	MDL (ng/L)
NDMA	5.0	0.7
NMEA	5.0	1.36
NDEA	5.0	0.84
NDPA	5.0	0.63
NPYR	5.0	0.54
NMOR	5.0	0.81
NPIP	5.0	0.33
NDBA	5.0	0.8[a]

Minimum Detection Limits were determined by analysis of seven replicates.
[a] Determined by analysis of five replicates.

Table C3 GC conditions

Injector Program		DB-VRX		DB-1701	
Temp (^{o}C)	Rate (^{o}C/min)	Hold (min)	Total (min)	Hold (min)	Total (min)
35	0	0.8	0.8	0.8	0.8
260	200	2	3.92	2.08	4.00
150	200	31.5	35.97	21.00	25.56

Time (min)	Split state	Split ratio
Initial	On	5
0.8	Off	Off
2.2	On	100
20	On	30

Injection volume	8 μL
Plunger Inject speed	0.2 μL/sec
Post injection delay	99 sec

Column temperature program: DB-VRX				DB-1701			
Temp. (^{o}C)	Rate (^{o}C/min)	Hold time (min)	Total time (min)	Temp. (^{o}C)	Rate (^{o}C/min)	Hold time (min)	Total time (min)
35	0	4	4.0	35	0	4	4
100	20	2	9.3	200	15	0	15
210	5	0	31.3	240	40	10	26
250	50	5	37.1				

Table C4 Acetonitrile-CI/MS/MS parameters (DB-1701 GC column)

CI gas: acetonitrile	Eject.amp: 15.0 m/z
CI storage level: 19.0 m/z	Backgroundd mass: 40 m/z
Max. ion time: 2000 microsec	Max. reaction.time: 120 millisec
Target TIC 5000 counts	Prescan time: 200 microsec

Segment	Description	Start time	End time	Low mass	High mass	Ioniz mode	Ion prep
1	Fil/Mul delay	0	7.6	40		CI auto	
2	NDMA	7.6	9.7	40	83	CI auto	MRM
3	NMEA	9.7	11.3	40	91	CI auto	MS/MS
4	NDEA	11.3	15.5	40	107	CI auto	MRM
5	NDPA	15.5	17.7	40	150	CI auto	MRM
6	NMOR	17.7	18.7	40	125	CI auto	MRM
7	NPYR	18.7	19.1	40	106	CI auto	MS/MS
8	NPIP	19.1	20.0	40	120	CI auto	MS/MS
9	NDBA	20.0	23.5	40	165	CI auto	MS/MS

Segment	Channel		Precursor mass	Isolation window	Quan ion (Product ion)	Waveform type	Excit stor level	Excit ampl
2	NDMA	1	81	1.5	49	Resonant	35	0.36
	d_6-NDMA	2	75	1.5	44	Resonant	35	0.34
3	NMEA	1	89	2	61	Resonant	40	0.31
4	$^{15}N_2$-NDEA	2	105	2	77	Resonant	40	0.33
	NDEA	1	103	2	75	Resonant	40	0.34
5	NDPA	1	131	2	89	Resonant	40	0.34
	d_{14}-NDPA	2	145	2	97	Resonant	40	0.33
6	NMOR	1	117	2	87	Resonant	40	0.3
7	NPYR	1	101	2	55	Resonant	40	0.33
8	NPIP	1	115	2	69	Resonant	40	0.34
9	NDBA	1	159	2	103	Resonant	48	0.37

Table C5 CSPE method single laboratory precision & accuracy in drinking and wastewater

Nitrosamine	Precision	DW-1	DW-2	WW-1	WW-2	Accuracy	
Spike level, ng/L	DW-1	20	13	200	376	Mean	RSD
	Rel. diff, %	Spike recovery, %				Recovery, %	%
NDMA	10	77	98	88	99	91	11.4%
NMEA	1.0	96	80	98	95	92	9.0%
NDEA	0.6	91	83	98	87	90	7.1%
NMOR	7.5	101	89	92	87	92	6.7%
NPYR	10	103	87	90	95	94	7.5%
NDPA	23	106	115	102	94	104	8.4%
NPIP	5.1	95	82	90	92	90	6.2%
NDBA	9.4	87	100	102	86	94	9.0%

Table C6 Interlaboratory bias and precision data for nitrosamines spiked into secondary wastewater effluent

Nitrosamine	Sample conc. (ng/L)	Lab 1	Lab 2*	Lab 3*	Mean rec. %	%RSD
NDMA	142	99	135	113	116	15.5
NMEA	<20	95	194	148	145	34.0
NDEA	<20	87	91	97	92	5.7
NDPA	<20	87	101	146	111	27.7
NPYR	<14	95	68	146	103	38.5
NMOR	<20	94	75	157	109	39.3
NPIP	<20	92	80	150	107	34.9
NDBA	<20	86	107	93	96	10.9

Secondary effluent water samples observed (n=3), Spiked concentration = 376 ng/L
** Laboratory new to the method.*

APPENDIX D

NITROSAMINE SOLID–PHASE MICROEXTRACTION GAS CHROMATOGRAPHIC/MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF NDMA AND FIVE OTHER NITROSAMINES

1. General Discussion

a. Sources and Significance: The chemical N-nitrosodimethylamine (NDMA) has been identified as a probable human carcinogen by the U.S. Environmental Protection Agency. It is commonly found in liquid rocket fuel, lubricants, and pesticides. The general population may be exposed to NDMA from outdoor air; tobacco smoke; diet such as cured meats, fish, and cheese; beverages such as beer and whisky; cosmetics; and rubber products. The average concentration of NDMA measured in food ranges from 90 to 100 ng/L (ppt) for pasteurized milk, 600 to 1,000 ng/Kg for fried pork bacon, and 50 to 5,900 ng/Kg for various beers. NDMA has also been detected in recycled water, wastewater, and potable water. It can either occur as a chemical contaminant from industrial processes or be formed by chlorine and chloramine disinfection processes. The formation mechanisms that produce NDMA may also form other nitrosamines. Seven other nitrosamines (listed in Table D1) that are structurally related to NDMA and/or known to cause cancer in animals have been included in this method.

b. Principle: Target nitrosamines are analyzed by a solid–phase microextraction (SPME), isotope dilution gas chromatography (GC)/chemical ionization (CI) mass spectrometry (MS) method. Before extraction, each 7 mL sample is spiked with the isotopically labeled internal standard (IS), (d_6-NDMA). Sodium chloride (2.4 g) are added and mixed until dissolved. The carboxen/polydimethylsiloxane coated SPME fiber is placed into the headspace above the sample in a closed vial. The sample is heated to 65 ºC and extracted for 45 minutes. The SPME fiber is then retracted and placed in the split–splitless GC port, where the fiber is exposed and analytes are thermally desorbed for mass spectral analysis. The nitrosamine concentrations are calculated from the area ratio of mass ion to labeled IS, which compensates for variations during sample preparation and analysis. This method is useful for the extraction of high concentration nitrosamines (greater than 30 ng/L N-nitrosodimethylamine) from complex wastewater samples, as well as general surveys and laboratory studies (e.g., kinetics of degradation or formation potential studies).

c. Interferences: Any organic compounds present in the water sample may potentially be adsorbed onto the SPME fiber, creating interference in the extraction and detection of the target analytes. For matrices containing possible interferences, complete recovery of analytes should first be tested by extracting a known, spiked amount of analyte from the matrices to be used. Studies show that high levels of dissolved organic carbon and the NDMA precursor, 1,1-Dimethylhyrdrazine (UDMH) do not adversely affect analyte adsorption.

Method interference may also be caused by contaminants in reagents, sample bottles and caps, and other materials used during the analysis. Laboratory and reagent blanks must be demonstrated to be free of interference, with concentrations less than one-third the minimum reporting limit (MRL).

1) All glassware must be meticulously cleaned. Rinse sample bottles with reagent water immediately after extraction. Wash volumetric glassware with detergent and rinse with tap water followed by reagent water. Bake bottles overnight at temperature above 250 °C. Store glassware inverted or capped with aluminum foil.
2) Rubber materials can be a source of NDMA contamination so avoid use during extraction and analysis.

3) NDMA has been found in deionized (DI) water at levels up to 10 ng/L, therefore use of an ultraviolet water purification system is recommended to avoid contamination. Reagent water freshly drawn from a Milli-Q UV Plus system has been used.

d. *Safety:* Because the toxicity or carcinogenicity of each reagent used in this method has not been precisely defined, each chemical should be treated as a potential health hazard, and exposure should be minimized. Wear suitable protection to skin and eyes and work under hood when handling unknown samples and stock or other high concentration standard solutions. *Each laboratory should maintain a MSDS file for all chemicals used in this procedure.*

e. *Detection limits:* This method provides for identification and measurement of N-nitrosodimethylamine (NDMA) at concentrations ranging from 30 to 1000 ng/L in drinking water, wastewater, and recycled water. Five other nitrosamines may by measured from 60 to 1000 ng/L. See Table D2.

2. Sampling and Storage

a. Sample Collection:

1) The sample site should be free of auto exhaust, cigarette smoke, fresh paint, and any other possible sources of contamination. Sample location should provide a representative grab sample or composites (maintained cold and with proper quenching).
2) When sampling from a water tap, the tap is allowed to flush until the water temperature has stabilized (usually about 3-5 minutes). When sampling from an open body of water, the sample is collected using a clean stainless steel bucket with a clean rope. Avoid plastic and rubber tubing, gaskets, etc. that may leach interfering analytes into the water sample.
3) Samples must be collected in 250 mL amber glass bottles with Teflon-lined polyethylene caps. When sampling chlorinated or chloraminated water, a dechlorinating agent must be added to the sample bottle before sampling. The container should not be rinsed with sample before collection.
4) When sampling chlorinated or chloraminated water, the residual should be quenched at time of sampling. The addition of solid sodium sulfite or sodium thiosulfate (approximately 0.5 g for wastewater, or 0.04 to 0.1 g for potable water to a 1 L bottle should minimize additional nitrosamine formation. Alternately 0.02 g of ascorbic acid may be used for drinking water. If chloramines residual is greater than 4 mg/L, additional quenching agent should be added. If preservatives are used, the bottle should not be rinsed with sample before collection.

b. Storage:

1) Samples should be iced or refrigerated at 4 °C or lower (but not freezing) and maintained at these conditions away from light until extraction. To prevent photodecomposition, samples must be protected from light from the time of collection until extraction. Amber colored bottles work well for this. Avoid storage of samples under low pH conditions because this has been observed to produce elevated levels of NDMA in some wastewater effluents and potable waters. Extract drinking water samples within 28 days. As a guideline, wastewater samples should be extracted within 14 days. However, degradation and formation of nitrosamines in wastewater matrices can be complicated processes. It is suggested that appropriate holding times be developed on a case by case basis.
2) Extracts should stored at –11 °C, away from light in amber glass vials with Teflon-lined caps. Extracts should be analyzed in a timely manner, however, under the conditions mentioned, archived extracts have generally shown minimal NDMA losses over a period of six months.

3. Apparatus

All specifications are suggested. Brand names and/or catalog numbers are included for illustration only.

a. *SPME fiber*: 75 µm Carboxen/Polydimethylsiloxane fiber.
b. *SPME fiber holder*: Manual or autosampler varieties available.
c. *Extraction vials*: 15 mL screw cap vials with PTFE-faced septa, amber or clear.
d. *Heating*: Hotplate equiped with magnetic stirring, with heating block and thermometer.
e. *Stirrers*: 1.5 cm magnetic stirrer bars for sample mixing during extraction.
f. *Microsyringes*: Glass syringes with stainless steel needle and plunger in various sizes for spiking solutions and preparing intermediate solutions.
g. *Volumetric flasks*: Class A, various sizes used for preparation of standards.
h. *Graduated cylinder*: 10 mL volume, used for preparation of sample and standards
i. *Balance:* Analytical, capable of accurately weighing to 0.001 g.
j. *Gas chromatographic/mass spectrometer equpped with*:
    1) *Split–splitless injector:* injector operated in splitless mode.
    2) *Injector liner:* 0.75 mm i.d. specialty SPME liner
    3) *Capillary column:* Column should be either a 30 m x 0.32 mm i.d., 1.0 µm film thickness DB-1701 or a 30 m x 0.25 mm i.d., 0.5 µm film thickness DB-210 fused silica or similar capillary chromatographic column capable of providing adequate and reproducible resolution.
k. *MS analyzer:* A system capable of chemical ionization using ammonia gas with selective ion monitoring.

4. Reagents

Reagent grade or better chemicals and high-resolution gas chromatography-grade solvents should be used. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

a. *Reagent water:* Purified water that does not contain any measurable quantities of target analytes greater than one third the minimum reporting level (MRL) for each compound (e.g., MilliQ-UV treated).
b. *Methanol:* CH_3OH, high purity, demonstrated to be free of analytes and interferences.
c. *Sodium Chloride*: NaCl, high purity.
d. *Stock standards solution:* Nitrosamine mix containing six target nitrosamines at 2000 µg/L each in methanol. Solution is used to prepare spiked samples and laboratory fortified blanks.
e. *Surrogate analyte (SUR) standard solution: N*-Nitrosodimethylamine (D_6) was purchased as an individual stock solution at 1 mg/mL in dichloromethane.

5. Procedure

a. *SPME fiber conditioning*: The new SPME fiber is conditioned in the GC splitless injector at 270 ºC for a period of 4 hours. The GC oven temperature is then set to 250 ºC for 30 minutes to remove any fiber contaminants that may have entered the capillary column. One SPME fiber may typically be used up to 50 to 100 times before replacement. The fiber should be periodically inspected for signs of physical or chemical wear.
b. *Sample preparation*:
    1) Prepare a 15 mL extraction vial with a magnetic stir bar and 2.4 g of sodium chloride.
    2) Measure out a 7 mL aliquot of sample into the extraction vial.
    3) Using an appropriately sized microsyringe, d_6-NDMA is added to the 7 mL water sample at a level of 100 ng/L from a 100 µg/L spiking solution prepared in methanol.
    4) Cap vial and stir on magnetic stir plate until salt is dissolved.

c. *Extraction of samples:*
   1) Place the vial in a heating block on the hot plate that has been preheated to 65 °C ± 1 °C.
   2) Using the SPME fiber extension controls, dial down the SPME fiber holder to the lowest setting. This is to ensure that the fiber is not submerged into the sample during extraction.
   3) Pierce the extraction vial septa with the SPME fiber needle and depress the plunger, exposing the fiber. Confirm that the fiber is in the headspace only and not submerged in the water sample. If necessary, support the fiber holder with clamps.
   4) Extract the sample for 45 minutes.
   5) Withdraw the SPME fiber back into the needle and remove the fiber holder from the extraction vial. If there is any water vapor on the fiber needle, this should be wiped clean with a Kimwipe.
   6) Dial down the SPME fiber extension to the third marking. This is to ensure the proper placement of the fiber within the GC inlet for optimum thermal desorption.

d. *GC/CI/MS analysis:*
   1) Set the GC inlet to purge after 2 minutes of desorption time to limit peak tailing. In order to prevent ghost peaks from analytes remaining on the fiber, leave the fiber exposed within the inlet an additional 5–8 minutes.
   2) *GC conditions:* See Table D3 for GC conditions and Table D4 for analyte retention times.
   3) *MS conditions:* See Table D5 for mass ions used for selective ion monitoring.
   4) *Calculations:* NDMA concentration is determined by the isotope dilution technique.
   5) The other five nitrosamines concentrations are calculated by IS ratio method. Calibration curves for area ratio versus nitrosamine concentration are prepared from extracted standards.

6. Data Analysis and Calculation

a. *Identification:* Identify the method analytes in the sample chromatogram by comparing the retention time and spectra of the suspect peak to that of an analyte peak in a calibration standard. Internal standard retention times and spectra should be confirmed to be within acceptance limits even if no target compounds are detected to validate the sample's proper treatment throughout this procedure.
b. *Quantitation:* Calculate the analyte concentrations using the initial calibration curve generated as described in Section 7.d. Quantitate only those values that fall between the MRL and the highest calibration standard. Samples with target analyte responses that exceed the highest standard require dilution and reanalysis.
c. *Corrections:* Adjust the calculated concentrations of the detected analytes to reflect the initial sample volume and any dilutions performed.
d. *Review:* Prior to reporting the data, the chromatogram and spectra should be reviewed for any incorrect peak assignments or poor peak area integration.
e. *Reporting:* Analyte concentrations are reported in ng/L.

7. Quality Control

a. *Method detection limit (MDL):* An MDL study using the EPA protocol (40CFR136 Appendix B) must be available. Example results are shown in Table D2.
b. *Minimum reporting level (MRL):* The MRL should be no less than 3 times the MDL.
c. *Laboratory reagent blank:* A blank sample using the laboratory reagent water is analyzed by the method (including reagents, glassware, etc.) with each batch of samples. The blank must be free of nitrosamine contamination (NDMA <0.5 ng/L).
d. *Calibration check:* Calibration curves consist of four to seven standards bracketing the range of expected sample concentrations using the same extraction and analysis method as used for the samples. If standards are not run in the same batch as the samples, a mid-level continuing calibration check standard (CCC) can be used with each batch. If it agrees within ± 20% of the expected value, a new calibration curve is not needed. Typical standards for reclaimed water or wastewater are 50, 100, 300, 500, 700 and 1000 ng/L. A linear or quadratic calibration curve is

obtained for each analyte by plotting area ratio ($Area_x/Area_{IS}$) versus concentration for each nitrosamine.

e. *Laboratory fortified blank (LFB):* A LFB sample is prepared by spiking reagent water at the MRL level with a different stock solution than is used for the calibration curve standards. The LFB is taken through the full method with each batch of samples. Recovery should be within ± 50% of theoretical value.

f. *Laboratory fortified sample matrix (LFSM):* Analysis of a LFSM sample is required in each analysis batch if less than 10 samples or one for every 10 samples for larger batches of samples for analysis. It is used to determine if the sample matrix adversely effects method accuracy. Within each analysis batch, a minimum of one field sample is fortified as a LFSM for every 10 samples processed. The LFSM is prepared by spiking a sample with an appropriate amount of the analyte from a different stock source than used for the standards. Select a spiking concentration approximately twice the matrix background concentration, if known. The percent recovery (R) for each analyte is calculated using the equation:

$$R = \frac{(A - B)}{C} \times 100$$

where:
A = measured concentration in the fortified sample,
B = measured concentration in the unfortified sample, and
C = fortification concentration.

For samples fortified at or above their native concentration, recoveries should range between 70 to 130%.

g. *Sample duplicate (LD1, LD2) or laboratory fortified matrix spike duplicate (LFSMD):* If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, a second LFSM (i.e., LFMSD) must be prepared and analyzed from a duplicate of the field sample used to prepare the LFSM to assess method precision. The relative percent difference (RPD) for duplicates (LD1 = LFSM and LD2 = LFSMD) is calculated using the equation:

$$RPD = \frac{(LFSM - LFSMD)}{(LFSM + LFSMD)/2} \times 100$$

RPDs for LDs and duplicate LFSMs should fall in the range of ± 20% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are spiked near the MRL. At the MRL, RPDs should fall in the range of ± 50% for samples fortified at or above their native concentration.

8. References

1. "NDMA Laboratory Analysis", California Department of Health Services, http://www.dhs.cahwnet.gov/ps/ddwem/chemicals/NDMA/NDMAlabs.htm, 5/16/2003.
2. "Carcinogens -- Working with Carcinogens", Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, NIOSH, Pub. #77-206, August 1977.
3. "Safety in Academic Chemistry Laboratories", American Chemical SocietyPublication, Committee on Chemical Safety, 3rd Ed., 1979.

Table D1 Target nitrosamine analytes: formula, molecular weight, CAS No., and internal standard

Nitrosamine	Abbrev.	Formula	MW	CAS #	Internal Std.
N-Nitrosodimethylamine	NDMA	$C_2H_6N_20$	74	62-75-9	d_6-NDMA
N-Nitrosodiethylamine	NDEA	$C_4H_{10}N_2O$	102	55-18-5	$^{15}N_2$-NDEA
N-Nitrosodi-n-propylamine	NDPA	$C_6H_{14}N_2O$	130	621-64-7	d_{14}-NDPA
N-Nitrosomorpholine	NMOR	$C_4H_8N_2O_2$	116	59-89-2	d_{14}-NDPA
N-Nitrosopiperidine	NPIP	$C_5H_{10}N_2O$	114	100-75-4	d_{14}-NDPA
N-Nitrosodi-n-butylamine	NDBA	$C_8H_{18}N_2O$	158	924-16-3	d_{14}-NDPA

Table D2 Method detection limits in reagent water

Nitrosamine	Fortification level (ng/L)	Found concentration (ng/L)	Standard deviation (ng/L)	Relative standard deviation (%)	Detection limit (ng/L)
NDMA	250	253	10	3.9	30
NDEA	500	343	20	5.8	60
NDPA	500	505	20	4.0	60
NMOR	500	540	19	3.5	60
NPIP	500	408	19	4.7	60
NDBA	500	501	20	4.0	60

Detection Limits were determined by analyzing seven replicates

Table D3 GC conditions

Column:	DB-1701	Supelcowax 10
Column length (m)	30	30
Column ID (mm)	0.32	0.25
Column film (µm)	0.5	0.5
Column Flow (mL/min)	3	1.2
Inlet Temp (^{o}C)	250	250
Inlet Purge Time (min)	2	2
Inlet Purge Flow (mL/min)	60	60

Column temperature program:

DB-1701				DB-210			
Temp. (^{o}C)	Rate (^{o}C/min)	Hold time (min)	Total time (min)	Temp. (^{o}C)	Rate (^{o}C/min)	Hold time (min)	Total time (min)
40	0	1		40		1	
150	12	3		250	10	5	27
250	25	2	26				

Table D4 Analyte retention times

Nitrosamine or internal standard	DB-1701 ret. time (min)	DB-210 ret. time (min)
NDMA-d_6	3.90	5.64
NDMA	3.90	5.84
NMEA	5.02	6.64
NDEA	5.91	7.26
NDPA	8.23	8.77
NMOR	8.73	10.70
NPYR	9.06	--
NPIP	9.33	9.27
NDBA	12.69	10.29

Table D5 Identification and quantitation parameters

Nitrosamine	Quantitation ion
NDMA-d_6	98
NDMA	92
NMEA	106
NDEA	120
NDPA	148
NMOR	134
NPYR	--
NPIP	132
NDBA	176

APPENDIX E

RAW DATA FOR ROUND-ROBIN SAMPLES

Table E1 Raw data for sample RR-01 (potable water)

		Nitrosamine (ng/L)							
LB-RR-01	Organization	NDMA	NMEA	NDEA	NDPA	NPYR	NMOR	NPIP	NDBA
MLLE	Lab 1	<20	<20	24	<20	<20	<20	<20	<20
	Lab 2	5.4	<5	<5	<5	<10	<10	<10	<5
	Lab 3	<10	<10	<10	<10	<10	<10	<10	<10
	Lab 4	10	<1.4	1	<0.5	<1.37	1	<0.9	<1.6
	Lab 5	3.5	<5	<2.5	<2.5	<2.5	<5	<2.5	<2.5
	Lab 6	13	<2	20	<2	10.3	<2	<2	<2
Envi-Carb SPE	Lab 7	6.6	<4	<2	<2	<2	<2	<2	<5
	Lab 8	15	<2	<2	<2	<2	<2	<2	<2
	Lab 9	4.1	<2	<2	<2	<2	<2	<2	<2
Mod Cart SPE	Lab 10	3.7	<2	<2	<2	<2	1.0	<2	<2
Amb SPE	Lab 11	4.2	<1	<1	<1	<1	<1	<1	<3
	Lab 12	4.5	<2	<2	<2	<2	<2	<2	<2
	Lab 13	5	NA	<1	NA	NA	<1	NA	2
	Lab 14	4.5	<1	<1	<1	<1	<1	<1	<1
CLLE	Lab 15	3.7	<2	2.6	<2	4.4	<2	<2	<2
	Lab 16	No data	No data	No data	No data	No data	No data	No data	No data
	Lab 17	4.0	<2	1.1	<2	3	4	<2	<2
	Lab 18	5.2	<2	4.0	<2	<2	4	4	2
	Lab 19	10	NA	NA	NA	NA	NA	NA	NA
	Lab 20	33	ND	26.5	12	ND	ND	ND	9
SPME	Lab 21	<30	<60	<60	<60	NA	<60	<60	<60
	Lab 22	NA	NA	NA	NA	NA	NA	NA	NA

Table E2 Raw data for sample RR-02 (potable water + 12.7 ng/L spike)

		Nitrosamine (ng/L)							
LB-RR-02	Organization	NDMA	NMEA	NDEA	NDPA	NPYR	NMOR	NPIP	NDBA
MLLE	Lab 1	<20	<20	55	<20	<20	31	<20	20
	Lab 2	15	15	11	12	11	13	12	12
	Lab 3	15	11	10	12	12	14	11	12
	Lab 4	17	13	14	13	14	63	15	22
	Lab 5	14	10	12	12	13	17	12	14
	Lab 6	25	18	28	9.7	16	18	10	21
Envi-Carb SPE	Lab 7	19	10	11	11	11	15	10	13
	Lab 8	44	27	15	11	9.8	14	8	20
	Lab 9	17	13	14	15	18	14	8.5	8
Mod Cart SPE	Lab 10	16	12	12	11	12	15.7	10	12
Amb SPE	Lab 11	17	11	12	11	12	16	11	12
	Lab 12	16	10	12	13	12	19	10	12
	Lab 13	19	NA	12	NA	NA	20	NA	16
	Lab 14	16	17	13	11	14	18	13	13
CLLE	Lab 15	20	19	41	9	17	21	13	12
	Lab 16	No data	No data	No data	No data	No data	No data	No data	No data
	Lab 17	14	11	13	12	14	18	13	16
	Lab 18	15	17	17.2	11	12	12	13	10
	Lab 19	44	NA	NA	NA	NA	NA	NA	NA
	Lab 20	47	10	38	14	16	15	11	17
SPME	Lab 21	<30	<60	<60	<60	NA	<60	<60	<60
	Lab 22	NA	NA	NA	NA	NA	NA	NA	NA

Table E3 Raw data for sample RR-03 (RO effluent)

		Nitrosamine (ng/L)							
LB-RR-03	Organization	NDMA	NMEA	NDEA	NDPA	NPYR	NMOR	NPIP	NDBA
MLLE	Lab 1	34	<20	33	<20	<20	<20	<20	<20
	Lab 2	14	<5	<5	<5	<10	<10	<10	<5
	Lab 3	19	<10	<10	<10	<10	<10	<10	<10
	Lab 4	19	<1.4	1	<0.5	<1.37	1	<0.87	10.1
	Lab 5	13	<5	<2.5	<2.5	<2.5	<5	<2.5	<2.5
	Lab 6	25	<2	21	<2	17	<2	<2	15
Envi-Carb SPE	Lab 7	17	<4	<2	<2	<2	2.0	<2	<5
	Lab 8	39	<2	4.4	<2	<2	2.4	<2	4.0
	Lab 9	25	<2	<2	<2	<2	<2	<2	<2
Mod Cart SPE	Lab 10	13	<2	<2	<2	<2	2.7	2.7	<2
Amb SPE	Lab 11	14	<1	<1	<1	<2	2.3	<1	<3
	Lab 12	14	<2	<2	<2	<2	<2	<2	<2
	Lab 13	17	NA	<1	NA	NA	<1	NA	3.3
	Lab 14	14	<1	<1	<1	<1	<1	<1	<1
CLLE	Lab 15	18	<2	7.8	<2	2.5	6.6	<2	<2
	Lab 16	No data	No data	No data	No data	No data	No data	No data	No data
	Lab 17	14	<2	<2	<2	4.3	3.9	<2	<2
	Lab 18	14	<2	5	<2	<2	5	<2	2.5
	Lab 19	19	NA	NA	NA	NA	NA	NA	NA
	Lab 20	36	ND	23	7	ND	2	ND	12
SPME	Lab 21	<30	<60	<60	<60	NA	<60	<60	<60
	Lab 22	NA	NA	NA	NA	NA	NA	NA	NA

Table E4 Raw data for sample RR-04 (secondary effluent)

		Nitrosamine (ng/L)							
LB-RR-04	Organization	NDMA	NMEA	NDEA	NDPA	NPYR	NMOR	NPIP	NDBA
MLLE	Lab 1	180	<20	<20	<20	<20	<20	<20	<20
	Lab 2	145	<5	<5	<5	<10	<10	<10	<5
	Lab 3	147	<20	<20	<20	<10	<20	<20	<20
	Lab 4	161	2	2	1	<1.37	4	2	<41.3
	Lab 5	133	<5	<2.5	<2.5	<2.5	3.3	<2.5	<2.5
	Lab 6	143	<2	26	<2	14	12	8	15
Envi-Carb SPE	Lab 7	159	<4	<2	<2	<2	3.8	<2	<5
	Lab 8	213	<2	5.7	<2	3.4	6.8	2.8	11
	Lab 9	225	<4	6.4	<4	<4	6.3	<4	<4
Mod Cart SPE	Lab 10	165	<2	<2	<2	<2	<2	<2	<2
Amb SPE	Lab 11	165	<1	<1	<1	<1	4	<1	<3
	Lab 12	173	<2	10	<2	<2	4	<2	<2
	Lab 13	156	NA	<1	NA	NA	<1	NA	8.0
	Lab 14	130	<1	<1	<1	<1	<1	<1	<1
CLLE	Lab 15	170	<2	2	<2	49	7.7	<2	3.4
	Lab 16	No data	No data	No data	No data	No data	No data	No data	No data
	Lab 17	160	<2	<2	<2	38	<2	<2	<2
	Lab 18	141	<2	10	<2	<2	18	18	<2
	Lab 19	190	NA	NA	NA	NA	NA	NA	NA
	Lab 20	160	ND	18	ND	ND	3	ND	24
SPME	Lab 21	104	<60	<60	<60	NA	<60	<60	<60
	Lab 22	133	<50	<25	<25	NA	NA	<100	<25

Table E5 Raw data for sample RR-05 (secondary effluent + 376 ng/L spike)

		Nitrosamine (ng/L)							
LB-RR-05	Organization	NDMA	NMEA	NDEA	NDPA	NPYR	NMOR	NPIP	NDBA
MLLE	Lab 1	620	700	370	370	360	340	390	379
	Lab 2	526	351	345	349	380	381	345	379
	Lab 3	579	320	350	290	367	324	339	346
	Lab 4	531	364	369	413	432	313	442	394.65
	Lab 5	473	331	445	378	498	436	353	377
	Lab 6	719	738	392	383	345	361	378	378
Envi-Carb SPE	Lab 7	533	357	328	326	356	357	345	325
	Lab 8	722	729	348	381	259	291	305	413
	Lab 9	651	557	373	548	548	597	565	352
Mod Cart SPE	Lab 10	538	378	361	336	369	352	341	344
Amb SPE	Lab 11	621	348	424	371	402	422	369	347
	Lab 12	601	444	331	383	421	399	404	312
	Lab 13	600	NA	400	NA	NA	370	NA	410
	Lab 14	443	402	342	305	329	290	342	342
CLLE	Lab 15	520	760	1300	370	590	410	350	300
	Lab 16	No data	No data	No data	No data	No data	No data	No data	No data
	Lab 17	545	385	340	340	380	370	360	400
	Lab 18	584	476	528	264	139	116	150	151
	Lab 19	657	NA	NA	NA	NA	NA	NA	NA
	Lab 20	544	171	205	349	314	296	383	360
SPME	Lab 21	540	92	442	458	NA	206	146	614
	Lab 22	674	437	437	380	NA	NA	452	314

Table E6 Raw data for sample RR-06 (tertiary effluent)

		Nitrosamine (ng/L)							
LB-RR-06	Organization	NDMA	NMEA	NDEA	NDPA	NPYR	NMOR	NPIP	NDBA
MLLE	Lab 1	900	<20	<20	<20	<20	<20	<20	36
	Lab 2	802	<5	<5	<5	<10	<10	<10	<5
	Lab 3	973	<20	<20	<20	<20	40	<20	<10
	Lab 4	752	3	4	2	3	6	9	<41.9
	Lab 5	817	<5	<2.5	<2.5	5	5	<2.5	<2.5
	Lab 6	1015	<2	22	13	30	17	17	167
Envi-Carb SPE	Lab 7	834	<8	<4	<4	4.2	4.7	<4	<10
	Lab 8	1073	<2	8	<2	21	8	5	13
	Lab 9	1404	<8	<8	<8	<8	<8	<8	<8
Mod Cart SPE	Lab 10	770	<2	<2	<2	<2	<2	<2	<2
Amb SPE	Lab 11	842	<2	<2	<2	6	5.8	<2	<6
	Lab 12	862	<8	<8	<8	7.6	8.2	<8	<8
	Lab 13	900	NA	<1	NA	NA	<1	NA	12
	Lab 14	602	<1	<1	<1	<1	<1	<1	<1
CLLE	Lab 15	730	<2	13	<2	85	8	3	5
	Lab 16	No data	No data	No data	No data	No data	No data	No data	No data
	Lab 17	815	<2	5	<2	10	21	8	9
	Lab 18	994	<2	7.0	<2	21	22	17	8
	Lab 19	967	NA	NA	NA	NA	NA	NA	NA
	Lab 20	774	ND	15	ND	6	6	ND	47
SPME	Lab 21	330	<60	<60	<60	NA	<60	<60	<60
	Lab 22	1354	<50	<25	<25	NA	NA	<100	<25

REFERENCES

ASTM (American Society for Testing and Materials). (1983). *Standard Practice for Intralaboratory Quality Control Procedures and a Discussion on Reporting Low-Level Data*. D410-83, Philadelphia, PA.

ASTM (American Society for Testing Materials). (1994). *Standard Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water*. D 2777-86 (Reapproved 1994).

APHA, AWWA, WEF. (1995). *Standard Methods for the Examination of Water and Wastewater*. 19th ed. Washington DC: APHA.

Bradley, P.M., Carr S.A., Baird, R.B., and C.H. Chapelle. (Forthcoming). *Mineralization of N-nitrosodimethylamine in soil from a ground water facility*.

CDHS. (2001). California Department of Health Services, http://www.dhs.ca.gov/ps/ddwem/chemicals/NDMA/NDMAlabs.htm (accessed Dec. 12, 2001).

Charrois, J.W.A., Arend, M.W., Froesce, K.L., and S.E. Hrudey. (2003). Detection of N-Nitrosamines in Water Using Solid Phase Extraction Combined with GC/MSD Ammonia Positive Chemical Ionization. AWWA Conference Proceedings, Water Quality Technology Conference, Nov. 2–6, 2003.

Choi, J. and R.L. Valentine. (2001). Studies on the Formation of N-Nitrosodimethylamine (NDMA) in Drinking Water: A New Chloramination Disinfection By-Product, AWWA Conference Proceedings, Annual Conference of the American Water Works Association, June 19, 2001.

DETector Engineering and Technology, Inc. (2003). DET Report No. 45, July 2003. 486 North Widget Lane, Walnut Creek, CA 94598-2408, USA.

Fan T.Y, Vita R., and D.H. Fine. (1978). C-Nitro Compounds: A New Class of Nitrosating Agents. *Toxicology Letters*; 2: 5–10.

Fiddler, W., Pensabene, J.W., Doerr, R.C., and A.E. Wasserman. (1972). Formation of N-nitrosodimethylamine from Naturally Occurring Quaternary Ammonium Compounds and Tertiary Amines. *Nature;* 236: 307.

Fine D.H. and D.P. Rounbehler. (1975a). Trace Analysis of Volatile N-Nitroso Compounds by Combined Gas Chromatography and Thermal Energy Analysis. *Jour of Chromatograph;* 109, 271–279.

Fine D.H., Rufeh F., Lieb D., and D.P. Rounbehler (1975b). Description of the Thermal Energy Analyzer (TEA) for Trace Determination of Volatile and Non-volatile N-nitroso Compounds. *Analytical Chemistry*; 47(7): 1188–1191.

Fitzsimmons, S., Shen, Y., and M. Wehner. (2001). Laboratory Performance Evaluation Study for the Analysis of N-Nitrosodimethylamine (NDMA). AWWA Conference Proceedings, Water Quality Technology Conference, Nov. 11–15, 2001.

Fujii T. and H. Arimoto. (1985). New Sensitive and Selective Detector for Gas Chromatography: Surface Ionization Detector with a Hot Platinum Emitter. *J. Anal Chem*; 57: 2625–2629.

Hansen T.J., Archer M.C., and S.R. Tannenbaum. (1979). Characterization of Pyrolysis Conditions and Interference by Other Compounds in the Chemiluminescence Detection of Nitrosamines. *J. Anal Chem*; 51(9): 1526–1528.

Hubaux, A. and G. Vos. (1970). Decision and Detection Limits for Linear Calibration Curves. *J. Anal Chem.*; 42(8): 849–855.

Kimoto, W.I., Dooley, J., and W. Fiddler. (1980). Role of strong ion exchange resins in nitrosamine formation in water, *Water Research;* 14: 869–876.

Kimoto W.L., Dooley C.J., Carre J., and W. Fiddler. (1981). Nitrosamines in Tap Water After Concentration by a Carbonaceous Adsorbant. *Water Research;* 15: 1099–1106.

Kolb B., Auer M., and P. Pospisil. (1977). Reaction Mechanism in an Ionization Detector with Tunable Selectivity for Carbon, Nitrogen and Phosphorus. *Jour. of Chrom. Science*; 15: 53–67.

Larsen, R., Marx, M., and B. Cooil. (1997). *Statistics for Applied Problem Solving and Decision Making.* Pacific Grove, CA.: Brooks/Cole Publishing.

Lijinsky, W. (1994). "Chemical Structure of Nitrosamines Related to Carcinogenesis," in *Nitrosamines and Related N-Nitroso Compounds,* ed. Loeppky, R. N. and Michejda, C. J., American Chemical Society: Washington , DC.

Mitch, W.A. and D.L. Sedlak. (2002). Formation of N-nitrosodimethylamine from Dimethylamine during Chlorination. *Environmental Science & Technology;* 36: 588–595.

Munch, J.W. and M.V. Bassett. (2003). Methods for Emerging Contaminant Group: Explosives and Nitrosamines. AWWA Conference Proceedings, Water Quality Technology Conference, Nov. 2–6, 2003.

Neisess L.B., Baird R.B., Carr S.A. and J. Gute. (2003). Can N-nitrosodimethylamine Formation be affected by Polymer Use During Advanced Wastewater Treatment. Paper presented at the WateReuse Symposium, San Antonio, Texas.

Ontario. (2002). Ontario Regulation 169/03, Ontario Drinking-Water Quality Standards, Schedule 2, Chemical Standards, Ontario Safe Drinking Water Act, 2002.

Sedlak, D.L., Deeb R.A., Hawley, E.L., Mitch, W.A., Durbin, T., and S. Mowbray (Forthcoming). *Sources and Fate of N-Nitrosodimethyl amine and NDMA Precursors in Municipal Wastewater Treatment Plants.* In press.

Snyder, L. R. (1974). Classification of the Solvent Properties of Common Liquids. *J. Chrom*. 92(2): 223–230.

Snyder, L. R. (1979). Private communication to Burdick & Jackson.

Standard Methods. (1995). *See* APHA, AWWA, and WPCS (1995).

Suffet, I. H. and S. D. Faust. (1972). The p-Value Approach to Quantitative Liquid–Liquid Extraction of Pesticides. from Water, Part 1: Organophosphates: Choice of pH Value and Solvent. *J. Agr. and Food Chem*.; 20: 52–60.

Taguchi, V.Y., Jenkins, S.W.D., Wang, D.T., Palmentier, J-P.F.P., and E.J. Reiner. (1994). Determination of N-Nitrosodimethylamine by Isotope Diliution, High-resolution Mass Spectrometry. *Canadian Journal of Applied Spectroscopy*; 39(3): 87–93.

Tomkins, B.A. and W.H. Griest. (1995). Determination of N-nitrosodimethylamine at the Part-Per-Trillion Levels in Drinking Waters and Contaminated Groundwater. *Jour. Anal. Chem.*; 67: 4387–4395.

USEPA (1982). 40 CFR Part 136: Protection of the Environment, Method 607, Nitrosamines; *Fed Reg.*

USEPA (1984). 40 CFR Part 136: Rules and Regulations, Method 625. *Fed Reg*, 49(209): 43321-43336.

USEPA (1989). Method 1625C, Semivolatile Organic Compounds by Isotope Dilution GC/MS, June 1989.

USEPA (1990). 40 CFR 136, Appendix B: Definition and Procedure for the Determination of the Method Detection Limit, *Fed Reg*, Revision 1.11:100-149

ABBREVIATIONS

ARec	Absolute recovery
ACS	American Chemical Society
Amb-Envi CSPE	Ambersorb-Envicarb solid–phase extraction
Amb SPE	free Ambersorb solid–phase extraction
APPS	automated positive pressure system
ASTM	American Society for Testing Materials
CDHS	California Department of Health Services
CF	concentration factor
CI	chemical ionization
CI/MS/MS	chemical ionization/tandem mass spectrometer
CLLE	continuous liquid–liquid extraction
CSPE	cartridge solid–phase extraction
DBP	disinfection by-product
DCM	dichloromethane
DEC	disposable elution cartridge
DI (water)	de-ionized
DI (standard)	direct injection
GC	gas chromatograph
GC/CI/MS/MS	gas chromatography/tandem mass spectrometer operated in chemical ionization mode
GC/MS	gas chromatography/mass spectrometer
HP	Hewlett Packard
HR-GC	high-resolution gas chromatography
H/V	Hubaux and Vos Method
IDL	instrument detection limit
kcal/mol	kilocalories/mole
L	liter
LLD	lower level detection
LLE	liquid–liquid extraction
LOD	limit of detection
M	molarity
MAC	maximum acceptable concentration
MCL	maximum contaminant level
MDL	method detection limit
MCLG	maximum contaminant level goal
MeOH	methanol
MtBE	methyl tertiary-butyl ether
MIB	methyisoborneol
µg/L	micrograms per liter
min	minutes
mL	milliliter
mL/min	milliliter per minute

MLLE	micro liquid–liquid extraction
MRL	minimum reporting level
MS	mass spectrometer
MS/MS	tandem mass spectrometer
NA	nitrosamine
NA_7	nitrosamine, sum of all except NDMA
NCD	nitrogen chemiluminescence detector
ND	not determined
NDEA	N-Nitrosodiethylamine
NDEA-$^{15}N_2$	N-nitrosodiethylamine ($^{15}N_2$)
NDMA	N-nitrosodimethylamine
NDMA-d_6	N-Nitrosodimethylamine (d_6)
NDBA	N-Nitrosodi-n-butylamine
NDPA	N-Nitrosodi-n-propylamine
NDPA-d_{14}	N-nitrosodipropylamine (d_{14})
ng	nanogram
ng/L	nanogram per liter
NMEA	N-Nitrosomethylethylamine
NMOR	N-Nitrosomorpholine
NPD	nitrogen phosphorus detector
NPIP	N-Nitrosopiperidine
NPYR	N-Nitrosopyrrolidine
OEHHA	Office of Environmental Health Hazard Assessment
QA/QC	quality assurance/quality control
RR	round robin
RRF	relative response factor
RSD	relative standard deviation
SLLE	simplified liquid–liquid extraction
SOP	standard operating procedures
SPE	solid–phase extraction
SPME	solid–phase microextraction
STDEV	standard deviation
UCLA	University of California Los Angeles
USEPA	U. S. Environmental Protection Agency

Advancing the Science of Water Reuse and Desalination

WATEREUSE FOUNDATION

1199 North Fairfax Street, Suite 410
Alexandria, VA 22314 USA
(703) 548-0880
Fax (703) 548-5085
E-mail: Foundation@WateReuse.org
www.WateReuse.org/Foundation