2010

Ecological method development for detecting N-nitrosodimethylamine in water using HPLC-PDAD

JoLisa McDay

ECOLOGICAL METHOD DEVELOPMENT FOR DETECTING *N*-NITROSODIMETHYLAMINE IN WATER USING HPLC-PDAD

by

JoLisa McDay

Thesis

Submitted to the Department of Chemistry

Eastern Michigan University

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Chemistry

Thesis Committee**:**

Heather Holmes, Ph.D, Chair

Harriet Lindsay, Ph.D.

Donald Snyder, Ph.D.

January 2010

Ypsilanti, Michigan

DEDICATION

I am especially grateful to my husband and children who have been exceedingly patient and supportive through this endeavor. Gearry, DeAndré, Devonte, Dominique, and DeMario, thank you for your love, strength, and prayers.

ACKNOWLEDGEMENTS

I am most appreciative for the guidance and insight of Dr. Heather Holmes. Thank you for your commitment. The resources for this project would not have been available without the assistance of Larry Sanford – Assistant Plant Manager, Ann Arbor Water Treatment Plant. I am grateful to have a supervisor who supports the educational endeavors of his staff; thank you for your assistance. I would also like to thank the members of my committee, Dr. Lindsay and Dr. Snyder. I appreciate your patience and support.

ABSTRACT

Using ethanol, a sustainable reversed-phase high-performance liquid chromatographic method with photo-diode array detection (HPLC-PDAD) was investigated to detect sub-parts per billion concentrations of *N*-nitrosodimethylamine (NDMA) in drinking water and environmental water samples. This presents a practical and "green" option to analytical methods that require laborious derivatization, expensive detectors and unecological solvents.

Mobile phase characteristics, instrument operating parameters, and sample preparation were investigated. Selectivity for NDMA was evaluated using three different C_{18} columns. Optimal peak resolution and detection were achieved at 231 nm. The limit of detection and quantitation for NDMA was 0.29 μg/mL and 0.96 μg/mL, respectively, with an ethanol modifier and 5-μm column. A sustainable solid-phase extraction method was also investigated. Using ethanol for cartridge conditioning and extraction, recovery of NDMA was low (10%).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background

N-Nitrosodimethylamine (NDMA) is a known mammalian carcinogen that, upon ingestion, is rapidly and extensively absorbed within the gastrointestinal tract.[1] It has been identified in a variety of processed foods, beverages, and consumer goods. Also acknowledged as a nitrogenous disinfection-by-product, NDMA has been detected in recreational waters, wastewater plant effluents, and drinking water.[2] A notification level of 10 ng/L was implemented by the state of California upon finding the pollutant in numerous groundwater sources.[3]

Cycle 2 of the Unregulated Contaminant Monitoring Regulation is enforced by the Environmental Protection Agency (EPA) and is used to evaluate the presence of twenty-five contaminants in drinking water, including NDMA. Method 521, "Determination of Nitrosamines in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (GC/CI-MS/MS)," is required for compliance with the rule.[4] Per each sample, conditioning of the solid phase extraction (SPE) cartridge requires the use of dichloromethane and methanol. Additional quantities of methylene chloride are required for cartridge elution. More than 90% of the solvent is evaporated under nitrogen to concentrate the sample to 1 mL. The EPA acknowledges dichloromethane as a priority water pollutant.[5] Methanol is primarily derived from natural gas (a non-sustainable fuel) and is toxic to aquatic life in low concentrations.[6]

Reversed-phase high-performance liquid chromatography (RP-HPLC) is applicable to

trace analysis of nitrosamines in water. For this application, the use of acetonitrile is favored because of its high water solubility and low wavelength cutoff at 190 nm. Acetonitrile is a minor byproduct of acrylonitrile production. However, only two to four liters of acetonitrile are retrieved for every 100 L of acrylonitrile produced.[7] The uncertain availability of acetonitrile prompted analysts to employ less "eco-friendly" solvents such as methanol for use with HPLC. Though its UV cutoff is higher (205 nm), methanol is a cost-effective alternative to acetonitrile.

The movement to protect the environment has spurred interest into sustainable practices. Ethanol is acknowledged as a "green" solvent produced from agricultural feedstocks. With chemical properties similar to methanol, ethanol is suitable for reversed-phase HPLC applications. A search of academic and professional databases has not presented evidence of using ethanol for analyzing NDMA with HPLC and photodiode array detection (PDAD). Investigating ecological methods for detecting trace concentrations of nitrosamines in drinking water warrants further exploration.

1.2 Objective

The purpose of this investigation was to develop a sustainable HPLC-UV/vis method for determining trace concentrations of NDMA in environmental waters.

1.3 Research Objective

Using RP-HPLC-PDAD and solid phase extraction with a modest preconcentration step, this study assessed the feasibility of using ethanol (as a modifier) in quantifying parts per trillion concentrations of NDMA in water. This goal required investigating (i) mobile phase characteristics, (ii) instrument operating parameters, (iii) column properties, and (iv) sample preparation. The method was applied to river, well, and finished

drinking water. This research may provide a “greener” option to established chromatographic methods used for quantitation of this carcinogen.

CHAPTER 2

LITERATURE REVIEW

2.1. Applications of Ethanol in HPLC

Analysts were deterred from using ethanol in HPLC due to inconsistencies in lot-to-lot purity that made results difficult to reproduce.[8] However, improved quality and sustainability have contributed to an increase in applications that employ ethanol. Using C_8 and C_{18} columns, ethanol, methanol, and acetonitrile were evaluated as modifiers for resolving a mixture of neutral, aromatic compounds.[9] Greater efficiency (as plates per meter) was achieved with ethanol.

Ethanol has a viscosity that is 50% greater than methanol and 69% greater than acetonitrile, which results in higher instrument operating pressures.[10] This property can impact peak symmetry and resolution. The stationary phase of a monolithic column is porous, which permits faster analysis time with reduced back-pressure and improved resolution. Using a monolithic C_{18} column, acetonitrile, methanol, and ethanol were individually added to water in various proportions and compared in resolving mixtures of alkylbenzenes.[11] The eluent strength of ethanol was deemed comparable to acetonitrile in mobile phases comprised of 50 to 60% organic solvent; however, ethanol's eluent strength was greater as the percentage of organic solvent increased. Gradient and isocratic elution modes were evaluated.

An ethanol modifier (1%) has been investigated for NDMA analysis with RP-HPLC; however, the application required derivatization of the nitrosamine and the extensive use of both acetonitrile and methanol in the mobile phase.[12] In that study, chemiluminescent detection was used for quantitation of NDMA. Ethanol has not yet

been identified as a mobile phase constituent or extraction solvent in NDMA analysis with UV detection.

2.2. *N*-Nitrosodimethylamine: A Water Pollutant

NDMA is a priority water pollutant (PWP), a toxic substance identified and controlled under the Clean Water Act and regulated by the EPA.[13] The concentration of NDMA in finished drinking water may be attributed to the presence of certain chemical and biological precursors in natural waters supplies. Conventional water treatment practices are ineffective for removing NDMA from processed water, but may promote the transformation of precursors into NDMA.[2]

NDMA is a yellow, oily, water-soluble, semi-volatile liquid with no distinctive odor.[14] Some intrinsic properties are shown in Table I.[15]

Table I [15]: Physicochemical Properties of *N*-Nitrosodimethylamine (CAS: 62-75-9)

Property	*Value*
Molecular weight (amu)	74.08
Density (g/mL @ 20 ˚C)	1.006
Water Solubility (mg/L)	1×10^6
Vapor Pressure (mmHg @ 20 ˚C)	2.7
Boiling Point (˚C)	154
Octanol/Water Partition Coefficient (log P_{ow})	-0.57

It is primarily a byproduct of industrial processes that use dimethylamine (DMA), nitrate, and nitrite.[16] Equation I represents a general scheme for NDMA formation in the

presence of various nucleophilic anions or water.[17] The reaction is known to proceed at pH 3.

$$X\text{-}NO + (CH_3)_2NH \rightarrow H^+ + X^- + (CH_3)_2NNO, \textit{ Where } X = Cl^-, Br^-, I^-, SCN^- \textit{ or } H_2O \quad (I)$$

Under alkaline conditions, nitric oxides are direct nitrosating agents that promote nitrosamine formation; acidic conditions are not required.[18]

NDMA is a solvent and additive in textile manufacture. It serves as a plasticizer, softener, lubricant additive, and antioxidant.[6] As a nematocide and inhibitor of soil nitrification, NDMA is incorporated into pesticides and agricultural products. Industrial wastewater discharge, soil runoff, and degradation of NDMA-containing products can elevate quantities of the pollutant and its precursors in receiving streams that supply water treatment facilities.

Organic nitrogen, coagulant aids, and disinfection techniques can also promote NDMA formation within the water treatment process. For example, NDMA concentrations are higher in drinking water that has been treated with chloramines and polymeric compounds like poly(diallyl dimethylammonium chloride).[17,19] At pH greater than eight, monochloramine (a disinfectant) and residual DMA (from polymer application) have been implicated in NDMA formation.[2,20] It has been proposed that a "Raschig" type of reaction occurs in water and wastewater treatment processes.[21] DMA reacts with chloramines (Equation II) to produce unsymmetrical dimethylhydrazine (UDMH).[22]

$$NH_2Cl + (CH_3)_2NH + OH^- \rightarrow (CH_3)_2NNH_2 + Cl^- + H_2O \quad (II)$$

Subsequent oxidation of UDMH yields NDMA (Equation III).[21]

$$(CH_3)_2NNH_{2(aq)} \xrightarrow{NH_2Cl} (CH_3)_2NNO_{(aq)} \quad (III)$$

2.3. Health Implications of NDMA

The EPA recognizes NDMA as a probable human carcinogen. Mammalian studies associate cancers of the lung, liver, and kidney with exposure to NDMA.[17] In less than one month, the size and number of carcinogenic tumors increased for laboratory rodents that drank water containing 0.01 to 5 mg/L NDMA.[1] Considering the various sources of NDMA exposure, drinking water contaminated with NDMA accounts for less than 10% of a person's total exposure to the carcinogen.[16] Table II reveals quantities of NDMA present in typical consumer items.[23]

Table II[23]: Concentration of NDMA in Everyday Products

Product	Concentration (parts per billion)
Cheese	15
Beer/wine	5
Baby pacifier (rubber)	60
Fried Bacon	1 - 44
Tobacco products	0.2 -85
Drinking water	0.003 – 2.7

The World Health Organization recommends that drinking water contain no more than 0.1 µg/L of NDMA.[16]

2.4. HPLC Analysis of NDMA

For monitoring and compliance with agency standards, PWPs are quantified using EPA approved methods. The EPA has not sanctioned HPLC for regulatory analysis of NDMA in drinking water; however, the technique has been explored in various matrixes

with different chromatographic conditions and detectors (Table III).[12]

Table III [12]: Variations on Chromatographic Conditions for NDMA Analysis

Matrix	*Column*	*Mobile phase*	*Detection Method*	*Detection Limit*
Fruit juice, infant formula	LiChrosorb-Si60 (25 cm x 4.6 mm, 5-µm particle size)	1.5% acetone/ *n*-hexane	Thermal Energy Analyzer (TEA)	1 µg/L
Pork products	Spherisorb (25 cm x 4.6 mm, 5-µm particle size)	Hexane/ Isopropanol (96.5:3.5)	Chemilumenescence	0.04–0.09 µg
Distilled water	Shim-pack CLC-ODS (15 cm x 4.7 mm, 5-µm particle size)	0.1M Phosphate buffer	Electrochemical	0.74 µg/L
Beer	µ-Bondapak C_{18} (30 cm x 3.9 mm, 5-µm particle size)	Acetonitrile/ Water	Mass Spectrometry (after photolysis)	0.002-0.006 µg

Quantitation of NDMA with a thermal energy analyzer requires the cleavage of nitric oxide from the nitroso group and the measurement of energy released from the chemilumenescent reaction of the nitrosyl radical and ozone.[24] Though the detector is highly selective in quantifying nitroso compounds, it is primarily used with GC and normal-phase HPLC applications.[12] NDMA has been studied with chemilumenescent and electrochemical detectors, but hazardous chemicals are required for derivatization. [1,17] The lowest detection limits have been achieved with mass spectrometry.[12] Interpreting fragmentation patterns may be difficult when analyzing nitrosamine mixtures.

HPLC is suitable for evaluating mixtures of volatile and nonvolatile nitrosamines. Analysis of NDMA in environmental water has been performed under reversed-phase conditions with methanol or acetonitrile and buffered water. An organic solvent may account for greater than 90% of the mobile phase when gradient elution is employed.

However, methanol and acetonitrile often serve as modifiers in isocratic elution with buffered water mobile phase systems.[25,26] In general, the volume of a modifier represents no more than 10% of the mobile phase composition.[8,27] The use of ethanol, as a modifier, for analyzing concentrations of NDMA in environmental waters with UV detection has not been documented.

2.4.1. Column Selection

In general, columns for HPLC are chosen based upon the characteristics of the analyte. During method development, interactions of the analyte with both the mobile and stationary phases are considered. NDMA is a basic, neutral, polar molecule that has been investigated with a variety of columns. Resolving NDMA with an octadecyl stationary phase (ODS or C_{18}) is commonplace.[12] These columns are frequently selected for reversed-phase applications and many are suitable for analyzing acidic, basic, neutral, and ionized compounds.[28,29] A Hypersil ODS C_{18} column (125 mm x 4 mm, 5-µm particle size) was used for evaluating nitrosamines in wastewater.[22] This bonded phase is suitable for moderately polar analytes and can be used with basic solutes, like NDMA.[29]

The particle size of the stationary phase affects column efficiency. Smaller particles reduce the rate of mass transport and provide improved flow. The consequence is decreased analysis time and reduced solvent consumption. Environmentally friendly practices have employed columns with particle diameters less than 2 µm.[30] A C_{18} column (2.1 mm x 150 mm, 1.7-µm particle size) was used for analyzing NDMA concentrations in raw and finished drinking water.[31] Columns comprised of 5-µm particles also remain a viable option. A Shimadzu C_{18} column (150 mm x 6 mm, 5-µm particle size) was used in method development and for the resolution of eight nitrosamines.[32] NDMA was resolved

in approximately five minutes. Band broadening, which is greater when large particles are used, was significant for two highly retained analytes that eluted after thirty minutes. In general, reducing the particle size requires greater instrument operating pressure but results in a greater number of theoretical plates and improved resolution. On the basis of ecological considerations and efficiency, NDMA should be evaluated with columns having particle diameters less than 5 μm.

2.4.2. Mobile-Phase Selection

Nitrosamines are soluble in an array of eluents and modifiers used in RP-HPLC. Alcohols, ketones, and halogenated hydrocarbons have been investigated during method development.[24] The physicochemical characteristics and quantity of the organic constituent can affect peak shape as well as analyte retention and detection.

Manipulating mobile phase composition can impact analyte separation and analysis time. For a mixture of eight nitrosamines, isocratic elution with a 45:55 (v/v) methanol/water solvent system produced optimal resolution of NDMA and the remaining analytes.[32] The presence of acetonitrile reduced analysis time and resulted in coelution of NDMA and N-nitrosomorpholine (mobile phase composition was 7:37:56 (v/v/v) methanol/acetonitrile/water). Mixtures of organic solvents can increase the eluent strength of the mobile phase and decrease analyte retention. In certain applications, acetonitrile is a stronger eluent than methanol.

Mobile phase pH and buffer concentration can also impact analysis. NDMA analysis often proceeds with a buffered mobile phase (acetate or phosphate) with pH in the range of 2 to 7.[29,33,34] A low pH mobile phase can impart a positive charge on basic compounds and protonate the silanols of some C_{18} columns, thereby decreasing analyte

retention.[29] In general, ionized solutes are retained less strongly than non-ionized analytes.[27,29] Consequently, peak shape may be improved and tailing is reduced.

Though HPLC is used in NDMA analysis, many studies are based on the yield that results from manipulating the pH of the reaction. Mobile phase pH and buffer considerations are not emphasized.

2.4.3. UV-vis

Identification of NDMA and its derivatives have been performed using UV-vis.[32,35] The availability of mobile phase solvents with low UV cutoff has advanced the use of this technique for quantifying nitrosamines. The nitroso group (-N=O) absorbs UV radiation at 230-240 nm and 330-350 nm.[24] As a result, acetonitrile (cutoff at 195 nm), and methanol or ethanol (cutoff at 205 nm) are frequently selected when UV detection is used for quantitation of NDMA.[8] These solvents are not eco-friendly.

Multiple wavelengths can be monitored and higher signal-to-noise ratios are achieved with PDAD.[36] Therefore, analyte coelution and the presence of contaminants can be evaluated. When compared to conventional single channel instruments, faster analysis and lower limits of detection are achieved with PDAD.[28] These factors suggest that ethanol can be used with PDAD in developing a sustainable method for analyzing NDMA.

2.5. Sample Preparation Techniques for NDMA Analysis

Extraction, preconcentration, and derivatization are common aspects of sample preparation for trace analysis of nitrosamines. Preconcentration is often time-consuming and performed with unrecyclable sorbents and solvents classified as hazardous waste. Derivatization can improve sensitivity, but it frequently requires the use of toxic

chemicals to produce a more stable and detectable compound of interest. In HPLC applications, NDMA has been analyzed using various extraction media and pre- and post-column derivatization.

2.5.1. Solid-Phase Extraction

Liquid-liquid extraction (LLE) with dichloromethane (DCM) was a standard practice until solid sorbents were proven effective for quantifying nitrosamines.[37] Solid phase extraction (SPE) provides isolation of the analyte onto a solid surface, thereby reducing solvent waste associated with LLE. Synthetic carbonaceous resins and granular activated carbon (GAC), in the form of coconut shell charcoal (CSC) or bituminous coal, are sorbents frequently employed in extracting nitrosamines from aqueous solutions.[38,39]

NDMA has been recovered from various environmental waters using resins like Ambersorb 572 and 563. Conditioning often entails baking the resin and soaking the beads in an organic solvent.[40] The sample and sorbent are mixed (1-3 hrs.), and the analyte is commonly eluted with DCM.[37,41] Sample preparation and processing may require 4-18 hours. NDMA recovery varies between 40 and 95%.[37,42]

In comparison to bituminous coal and Ambersorb resins, CSC was demonstrated to be more effective for extracting nitrosamines from water.[37,38] EPA Method 521 (GC-CI-MS-MS) employs CSC. Conditioning and extraction of the media requires the use of toxic solvents. For RP-HPLC applications, NDMA recoveries of greater than 80% were achieved when CSC was selected as the extraction medium.[39] Sustainable practices were not considered. Investigating SPE with ethanol and CSC may provide a green alternative to other extraction techniques.

2.6. Summary

Investigations of NDMA frequently emphasize quantitation on the basis of pre- and post-column derivatization, and SPE, without exploring instrument operating parameters or altering chromatographic conditions (e.g. column dimensions, mobile phase considerations). In general, these studies are performed using nonsustainable methods where acetonitrile or methanol and a 5-μm ODS column are commonplace.

A variety of chromatographic techniques and detection methods have also been explored in studying NDMA. Many of these applications explore quantitation on the basis of modifying the pH of the reaction to evaluate NDMA formation.

The use of ethanol for method development in quantifying NDMA has not been demonstrated for SPE or RP-HPLC with UV detection. The objective of this investigation is to (i) use ethanol for developing an environmentally-friendly RP-HPLC method with photo-diode array detection and (ii) evaluate a sustainable SPE process for quantifying NDMA using primarily ethanol for cartridge conditioning and elution.

CHAPTER 3

EXPERIMENTAL

3.1. Instrumentation

NDMA analysis was performed using a Shimadzu Prominence HPLC equipped with a 20-µL sample loop, and LC-20AT solvent delivery module. It was integrated with a Shimadzu SPD-M20A photodiode array, UV-vis detector, and EZ-Start data acquisition software.

3.2. Materials

Zorbax SB-C_{18} column (4.6 mm x 15 cm, 5-µm particle size, Agilent Technologies), Zorbax SB-C_{18} column (4.6 mm x 15 cm, 3.5-µm particle size, Agilent Technologies), Eclipse XDB-C_{18} column (4.6 mm x 15 cm, 5-µm particle size, Agilent Technologies), Visiprep DL™ Solid Phase Extraction Vacuum manifold (12-port model, Supelco), Visiprep™ Large Volume Sampler (cat. no. 57275, Supelco), and Supelclean coconut charcoal SPE tubes (2g/6mL) were obtained from Supelco. NDMA (5 mg/mL in methanol) and NDMA (100 µg/mL in dichloromethane) were obtained from Accustandard. HPLC grade dichloromethane was purchased from Fisher Scientific. Ethanol (200 proof), HPLC grade solvents and reagents (methanol, acetonitrile, and ammonium acetate), ammonium hydroxide, glacial acetic acid, Millipore™ membrane filters (0.45 µm), and syringe filters (0.45 µm) were obtained from laboratory stock. Amber glass bottles (250 mL) with PTFE caps and 1-mL amber GC vials were obtained from laboratory stock. Microfiltered water was processed with a NANOpure® Infinity water purification system.

3.3. Reversed-Phase High-Performance Liquid Chromatography

3.3.1. Mobile Phase Optimization

3.3.1.1. Solvent Selection

All investigations were performed using isocratic elution. A graduated cylinder was used to measure the required volume of each solvent. The solvents were combined and gently mixed in an HPLC solvent reservoir bottle. All mobile phases were sonicated at room temperature for 40 minutes prior to use.

Three methanol:water mobile phase systems were evaluated. Mobile phase 1 (MP1) consisted of 50:50 (v/v) methanol:aqueous buffer. Mobile phase 2 (MP2) consisted of 10:90 (v/v) methanol:aqueous buffer. Mobile phase 3 (MP3) consisted of 5:95 (v/v) methanol/aqueous buffer. One ethanol:aqueous buffer mobile phase system was evaluated; mobile phase 4 (MP4) consisted of 5:95 (v/v) ethanol:aqueous buffer. Mobile phase 5 (MP5) consisted of 1:99 (v/v) acetonitrile:aqueous buffer. Acetonitrile concentration was determined using a nomogram.

3.3.1.2. pH and Buffer Selection

Ammonium acetate was used to prepare a 10mM buffer. Water pH was adjusted with CH_3COOH or NH_4OH (as needed) and filtered using a 0.45-µm membrane filter prior to mixing with the organic solvent. The pH of the aqueous solution was measured with an ion-selective electrode. A three point calibration of the pH meter was performed. Chromatographic performance was evaluated at pH 4, 5, and 6. A 20 mM acetate buffer was also prepared at pH 6 in the same manner. Only the 10 mM acetate buffer was used to prepare the ethanol and acetonitrile mobile phases.

3.3.1.3. Analyte Concentration and Detection

A 10 µg/mL NDMA stock solution was prepared, in methanol, from a 1-mL ampule of NDMA (100 µg/mL in DCM). A 5 µg/mL NDMA sample was prepared in methanol from the stock solution. One injection was used for analysis.

A stock solution (1 mg/mL) was prepared in methanol from a 1-mL ampule (5 mg/mL NDMA in methanol). A working solution (100 µg/mL) was prepared in methanol from the stock solution. Absorbance and peak shape of the working solution were evaluated to monitor stability. 1-mL aliquots of the mobile phase were spiked with NDMA in a 1.5-mL GC vial to obtain 0.1, 1.0, and 10 µg/mL concentrations. Duplicate injections were made for each concentration using a 100-µL fused syringe. For each injection, at any stage of the investigation, the sample loop was flushed with three aliquots of the sample prior to injection.

3.3.2. Flow Rate

Flow rates of 0.75, 1.0, 1.25, and 1.5 mL/min were used with MP3.

3.4. Photodiode Array Detection (PDAD)

3.4.1. Instrument Operating Parameters

Output channels of the detector were set at 231 nm and 360 nm for evaluating NDMA. Acetonitrile was measured at 190 nm. Methanol was measured at 205 nm. The range of wavelength detection was from 190 to 400 nm.

3.4.1.1. Lamps

Deuterium (D_2) and tungsten (W) lamps were on for the initial mobile phase investigation. Per the instrument manual, the D_2 lamp was selected for method optimization.[43]

3.4.1.2. Bandwidth

At 231 nm, bandwidth settings of ±3 to ±5 nm were evaluated. The remaining output channels were set at ±4 nm.

3.4.1.3. Time Constant and Sampling Time

Prior to analyzing instrument operating parameters, the time constant was set at 640 ms and the sampling time was set at 240 ms. Sampling times were set equal to the time constant per recommendation of the manufacturer.[43] Settings of 240, 320, and 640 ms were investigated.

3.5. Column Comparison

The Zorbax SB-C_{18} column (4.6 mm x 15 cm, 5-µm particle size) was used in each investigation for method development. The Zorbax SB-C_{18} column (4.6 mm x 15 cm, 3.5-µm particle size) and Eclipse XDB-C_{18} column (4.6 mm x 15 cm, 3.5-µm particle size) were evaluated using MP3 and MP4, respectively for select spiked and extracted samples.

3.6. Sample Preparation and Solid-Phase Extraction of NDMA

3.6.1. Sample Preparation

Purified water (250 mL) was spiked to achieve a concentration of 10 µg/L. Aliquots were extracted within one hour and filtered using a 0.45-µm syringe filter prior to analysis.

3.6.2. Cartridge Conditioning

Coconut charcoal SPE cartridges were conditioned as outlined in Table IV.

TABLE IV: SPE Cartridge Conditioning Instructions

Step	Procedure for Vials 1, 2, and 6
1	Apply 2-mL acetonitrile to the cartridge and aspirate completely.
2	Apply 2-mL methanol to the cartridge and retain it for one minute. Aspirate completely.
3	Apply 2-mL of methanol to the cartridge and aspirate completely. Repeat.
4	Apply 2-mL of water to the cartridge and aspirate completely.
5	Apply 3-mL of water and aspirate to frit.
6	Apply the 250-mL sample to the cartridge.
Step	Procedure for Vials 3 and 4
1	Apply 2-mL acetonitrile to the cartridge and aspirate completely.
2	Apply 2-mL ethanol to the cartridge and retain it for one minute. Aspirate completely.
3	Apply 2-mL of ethanol to the cartridge and aspirate completely. Repeat.
4	Apply 2-mL of water to the cartridge and aspirate completely.
5	Apply 3-mL of water and aspirate to frit.
6	Apply the 250-mL sample to the cartridge.
Step	Procedure for Vial 5
1	Apply 2-mL methanol to the cartridge and aspirate completely.
2	Apply 2-mL methanol to the cartridge and retain it for one minute. Aspirate completely.
3	Apply 2-mL of water to the cartridge and aspirate completely.
4	Apply 3-mL of water and aspirate to the frit.
5	Apply the 250-mL sample to the cartridge.

3.6.3. Sample Extraction

The rate of flow for each sample was measured with a stop watch. Extraction was performed at room temperature and under vacuum using the Visiprep DL™ Solid Phase Extraction Vacuum Manifold (with disposable liners) and Visiprep™ Large Volume Sampler. Vacuum was maintained between 5 and 10 mmHg. Flow was modified using the valve at each port.

3.6.4. Cartridge Elution

Cartridges were dried for thirty seconds under vacuum (5 mmHg) prior to elution. Aliquots of solvent were added continuously; the column was not permitted to go dry. SPE cartridges were eluted drip-wise under gentle vacuum as outlined in Table V. The eluant was collected and stored in a 40-mL amber glass vial. An average of seven minutes was required for elution. Extracts were stored away from light for two days at 4 °C before preconcentration.

3.6.5. Preconcentration

Extracted samples were preconcentrated to 1 mL under nitrogen gas and with a hot water bath at 48 °C.

Table V: SPE Cartridge Elution Instructions

Step	Procedure for Vials 1, 3, and 5
1	Add 1, 4-mL aliquot of methanol to the cartridge.
2	Apply a gentle vacuum to begin slow, drip-wise elution.
3	Add 1, 4-mL aliquot of acetone to the cartridge as the remaining methanol approaches the frit.
4	Store the vial away from light at 4 ºC.
Step	Procedure for Vials 2 and 4
1	Add 1, 4-mL aliquot of ethanol to the cartridge.
2	Apply a gentle vacuum to begin slow, drip-wise elution.
3	Add 1, 4-mL aliquot of acetone to the cartridge as the remaining ethanol approaches the frit.
4	Store the vial away from light at 4 ºC.
Step	Procedure for Vial 6
1	Add 1, 2-mL aliquot of acetonitrile to the cartridge.
2	Apply a gentle vacuum to begin slow, drip-wise elution.
3	Add 1, 2-mL aliquot of methanol to the cartridge as the remaining acetonitrile approaches the frit.
4	Add 1, 4-mL aliquot of acetone to the cartridge as the remaining methanol approaches the frit.
5	Store the vial away from light at 4 ºC.

3.7. Robustness

3.7.1. Sampling Procedure and Preparation

Environmental and purified water samples were collected in 250-mL amber bottles that were washed, rinsed with deionized water, and dried at 220 °C for 3 hours prior to use. Duplicate injections were made of spiked (1.0 and 10 μg/ml NDMA) and unspiked samples. Samples were filtered with a 0.45-μm syringe filter and spiked with NDMA prior to injection.

3.7.2. pH Variation of Purified Water

Purified water was used to prepare samples of pH 5, 7.2, 9, and 11. CH_3COOH or NH_4OH were used for pH adjustment.

3.7.3. Environmental and Drinking Water Samples

Water samples (river, well, and reservoir) were obtained from sample taps at the Ann Arbor Water Treatment Plant. Two drops of 10% sodium thiosulfate solution were added to each bottle prior to sample collection. Purified water was used as a blank.

3.8. Method Validation

Determination of the limit of detection (LOD) and limit of quantitation (LOQ), and linear regression were performed using the Zorbax SB-C_{18} column (5-μm particle size) for MP3 and MP4, respectively.

3.8.1. Linearity

A calibration curve was established using 0.5, 1, 2, 5, 10, and 20 μg/ml NDMA. All standards were made using the mobile phase.

3.8.2. Statistical Determination of LOD and LOQ

Ten injections of 1.0 μg/ml NDMA were made using MP3 and MP4. Peak areas were used to assess the statistical LOD and LOQ. The LOD was calculated as $3\sigma/m$; the LOQ was calculated as $10\sigma/m$, where m is the slope (sensitivity) of the calibration curve.

CHAPTER 4

RESULTS AND DISCUSSION

The purpose of this study was to evaluate the feasibility of ethanol as a mobile phase modifier for quantifying trace concentrations of NDMA in water using HPLC-PDAD and isocratic elution. Mobile phase characteristics, instrument operating parameters, column properties, and water sample characteristics were investigated during method development.

In this study, method optimization entailed modifying experimental conditions in order to maximize peak height, maintain peak symmetry, minimize background noise, and (where applicable) achieve an acceptable retention factor for NDMA. Reducing the use of organic solvent in the mobile phase was also evaluated during method optimization.

A secondary goal was to investigate the use of ethanol for extracting NDMA from water samples, thereby developing a sustainable SPE process. Analyte recovery was evaluated and comparisons were made using combinations of ethanol, methanol, and acetonitrile for SPE cartridge conditioning and elution.

4.1. Mobile Phase Optimization

Mobile Phase Composition

Preliminary mobile phase investigations were based on the method of Lim et al. with isocratic elution and a flow rate of 1 mL/min.[32,39] MP1 (50:50 (v/v) methanol:aqueous buffer) was evaluated for resolving 5 µg/mL NDMA, while minimizing interference from DCM and methanol contained within the primary standard, and working solution, respectively. Methanol and NDMA coeluted (Figure 4.1).

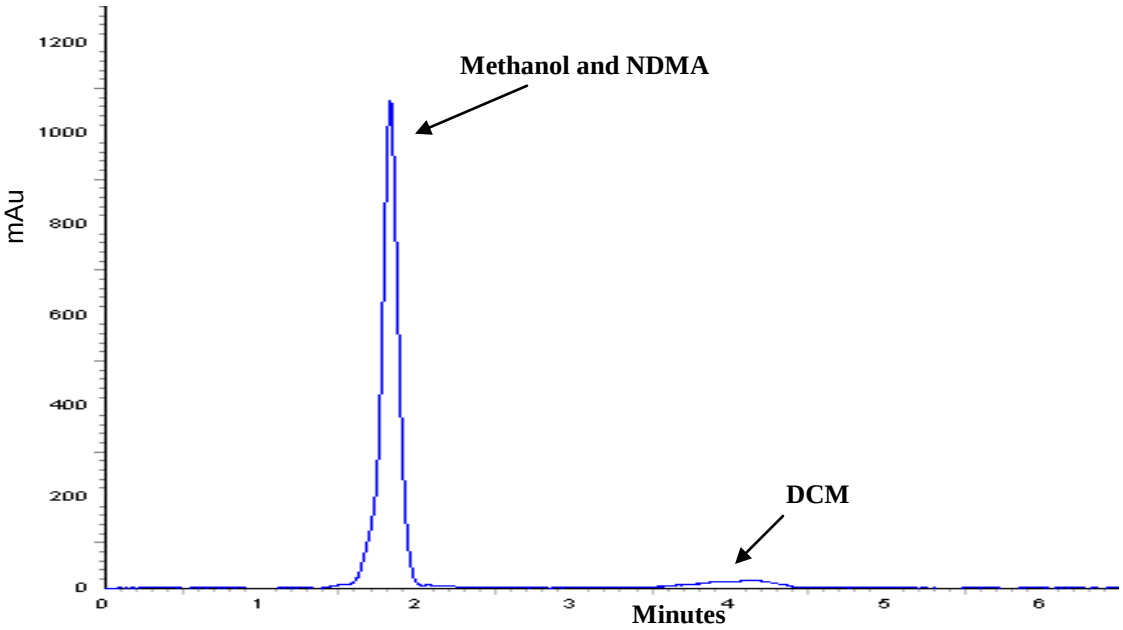

Figure 4.1. Coelution of NDMA and Methanol at 231 nm.

Peak homogeneity was assessed using 3-D analysis. This investigation revealed that the quantity of methanol within the mobile phase was too high. If MP1 is used for evaluating nitrosamine mixtures, DCM can be a source of interference. Longer retention times are associated with nitrosamines larger than NDMA.[32,38,39] The presence of DCM was confirmed at 4.14 minutes. Due to the coelution of NDMA and methanol (Figure 4.2), a retention factor (*k'*) was not obtained for NDMA. A methanol-based NDMA

standard was employed for the remaining analysis, and the mobile phase flow rate was maintained at 1mL/min.

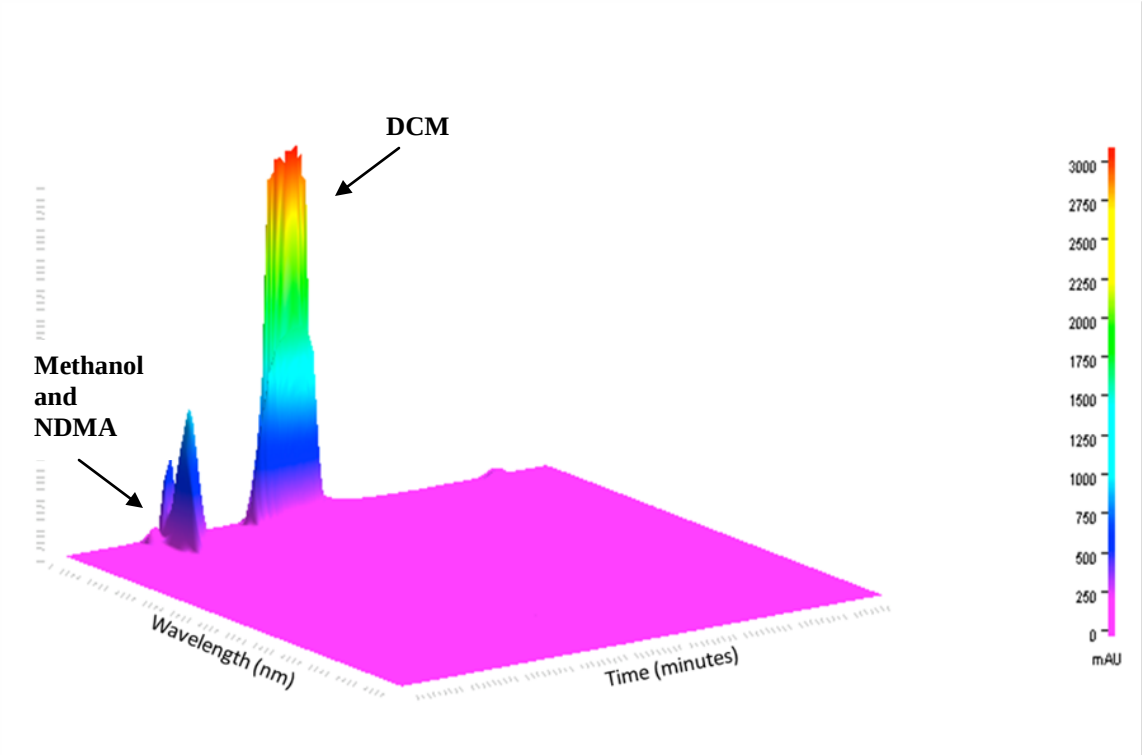

Figure 4.2. 3-D Representation of the Coelution of NDMA and Methanol at 205 nm.

As a preliminary analysis for detecting NDMA in extracted and preconcentrated samples, an aliquot of methanol was spiked to obtain 10 μg/ml NDMA and analyzed with MP2 (10:90 (v/v) methanol:aqueous buffer) and MP3 (5:95 (v/v) methanol:aqueous buffer), respectively. Decreased absorbance of NDMA was observed when a highly aqueous mobile phase was used. Since MP2 and MP3 contained substantially less methanol than MP1, a higher concentration of NDMA was selected to ensure detection of the analyte. In RP-HPLC, decreasing the amount of organic solvent in the mobile phase

increases both the retention time and retention factor. Accordingly, elution times for NDMA were 3.35 and 4.90 minutes for MP2 and MP3, respectively (Figure 4.3). One goal of this investigation is to reduce the use of organic solvent. Therefore, the ratio of organic to aqueous solvent in MP3 was selected for further method optimization.

The nature of the sample matrix can impact resolution. It is a common practice to dissolve the analyte of interest in the mobile phase to minimize interference. Figure 4.3 also conveys that significant peak distortion will occur with both mobile phase systems if a 100% organic sample matrix is injected.

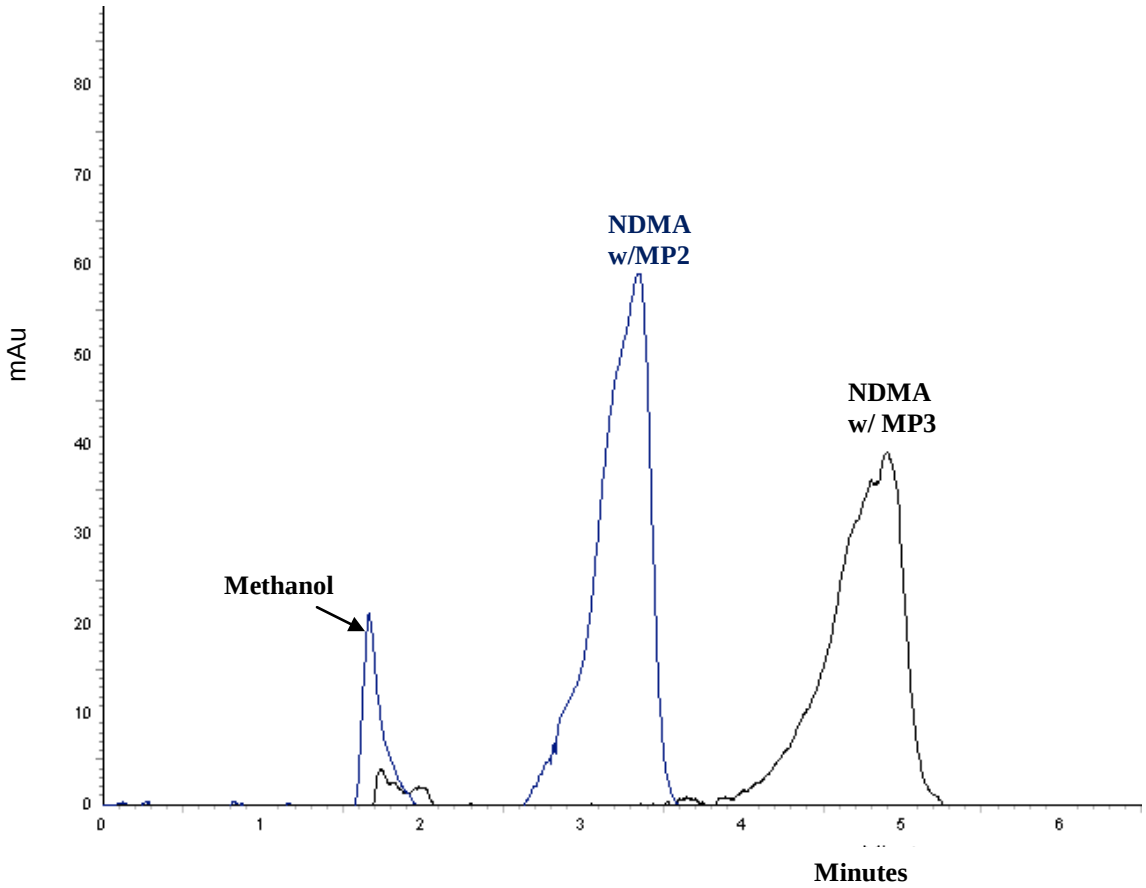

Figure 4.3. Influence of Mobile Phase Composition and Methanol Sample Matrix on Retention and Peak Shape of NDMA.

Mobile Phase Modifications: Buffer Concentration and pH

Effect of pH Modification on NDMA Peak Characteristics using Methanol (MP3)

Mobile phase pH was modified to assess detection and peak characteristics at 0.1, 1.0 and 10 µg/ml NDMA (mobile phase spiked with analyte). Qualitative detection was not achieved for 0.1 µg/ml. (Figure 4.4).

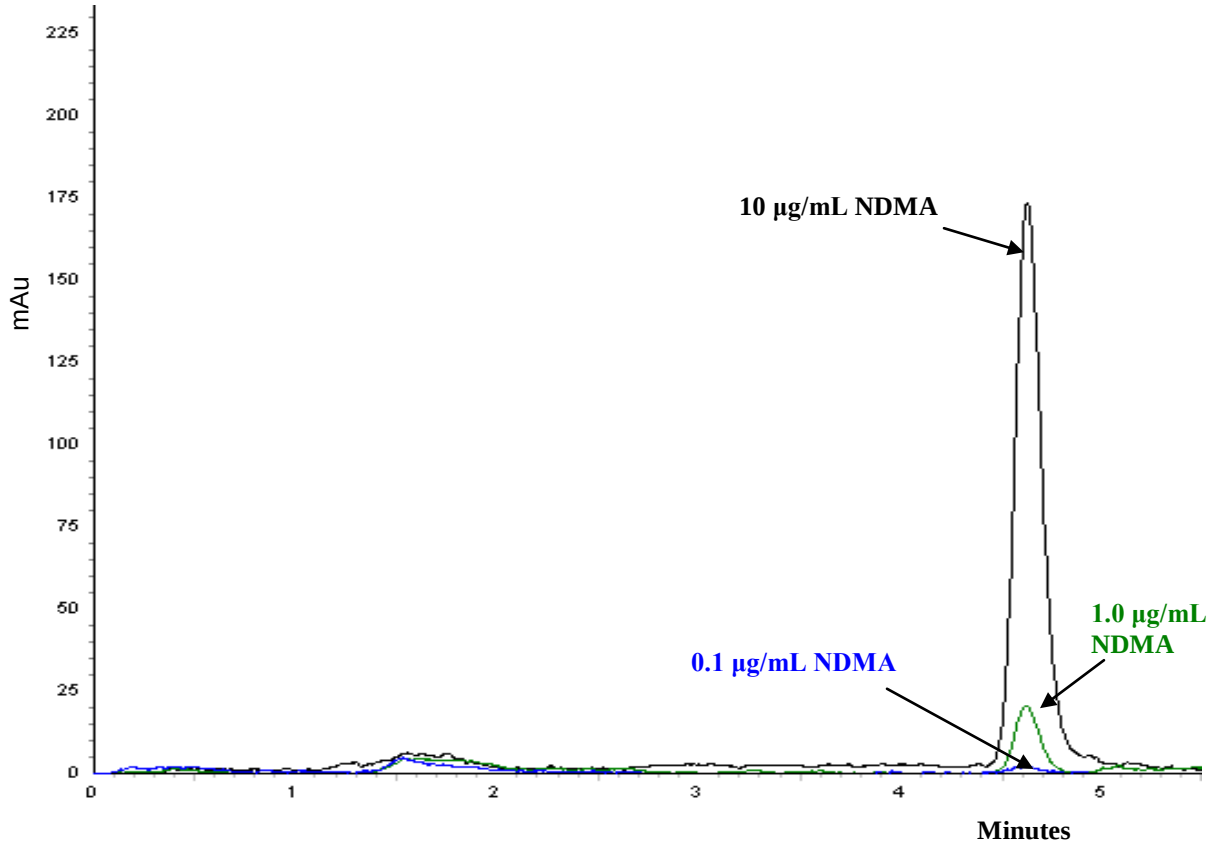

Figure 4.4. Chromatogram of 0.1, 1.0 and 10 µg/ml NDMA (1-mL aliquot of mobile phase spiked with analyte).

Method development was performed using MP3 at pH 4, 5, and 6 (*Tables VI* and *VII*). Though slightly inconsistent with the data for 1μg/ml NDMA, the peak area for 10 μg/ml NDMA at pH 4 was 20.6% and 12.7% greater than pH 5 and 6, respectively.

TABLE VI: Impact of Mobile Phase pH on Retention and Resolution using 1.0 μg/mL NDMA in the Mobile Phase

pH	Mobile Phase	t_r	t_m	k'	Peak Area	Peak Height
4	MP3	4.812	1.844	1.61	161030	14937
5	MP3	4.576	1.816	1.52	177307	17495
6	MP3	4.620	1.82	1.54	195977	20973

TABLE VII: Impact of Mobile Phase pH on Retention and Resolution using 10 μg/mL NDMA in the Mobile Phase

pH	Mobile Phase	t_r	t_m	k'	Peak Area	Peak Height
4	MP3	4.84	1.82	1.66	1752419	145554
5	MP3	4.56	1.79	1.55	1529954	155121
6	MP3	4.46	1.77	1.52	1391204	145368

A minimal decrease in the retention time for 10 µg/ml NDMA was observed as pH increased (Figure 4.5).

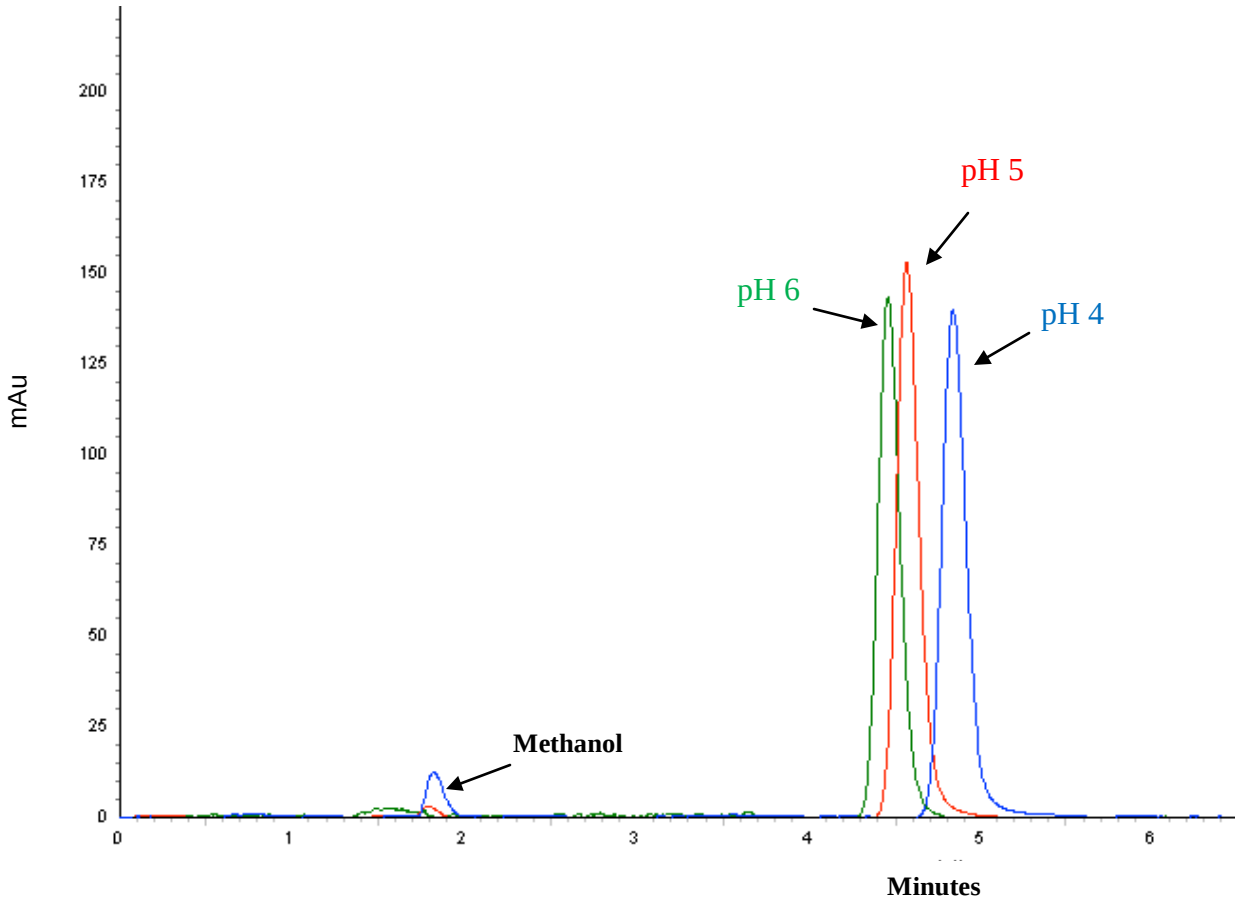

Figure 4.5. Chromatogram of NDMA Analysis (10 µg/mL) using MP3 at pH 4, 5, and 6.

The absorbance scale (y-axis) was adjusted so that the methanol peak could be seen. Methanol absorbance and peak symmetry improved at pH 4 (See Figures 4.6-4.8). The LOD and LOQ are based upon statistical analysis of 1.0 µg/mL NDMA. Therefore, peak characteristics at this concentration were examined for further method development. Investigations were performed at pH 6 using MP3 upon considering the retention factor, peak area, and peak height.

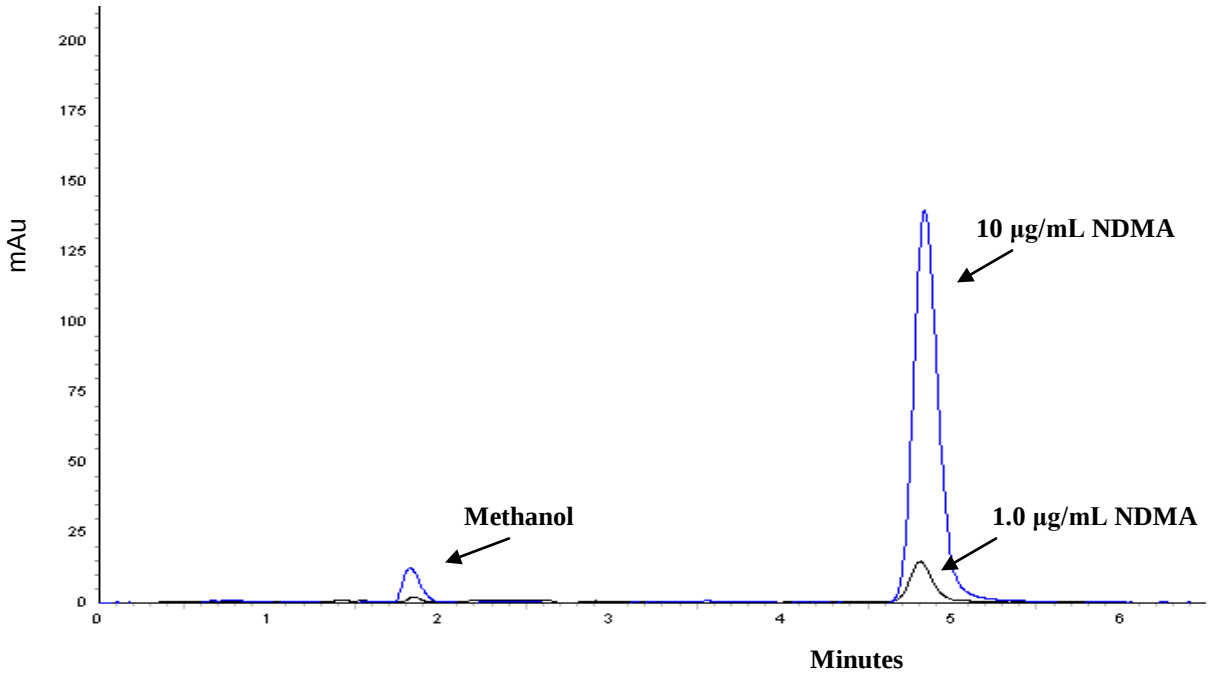

Figure 4.6. Chromatogram of NDMA Analysis (1.0 and 10 µg/mL) using MP3 at pH 4.

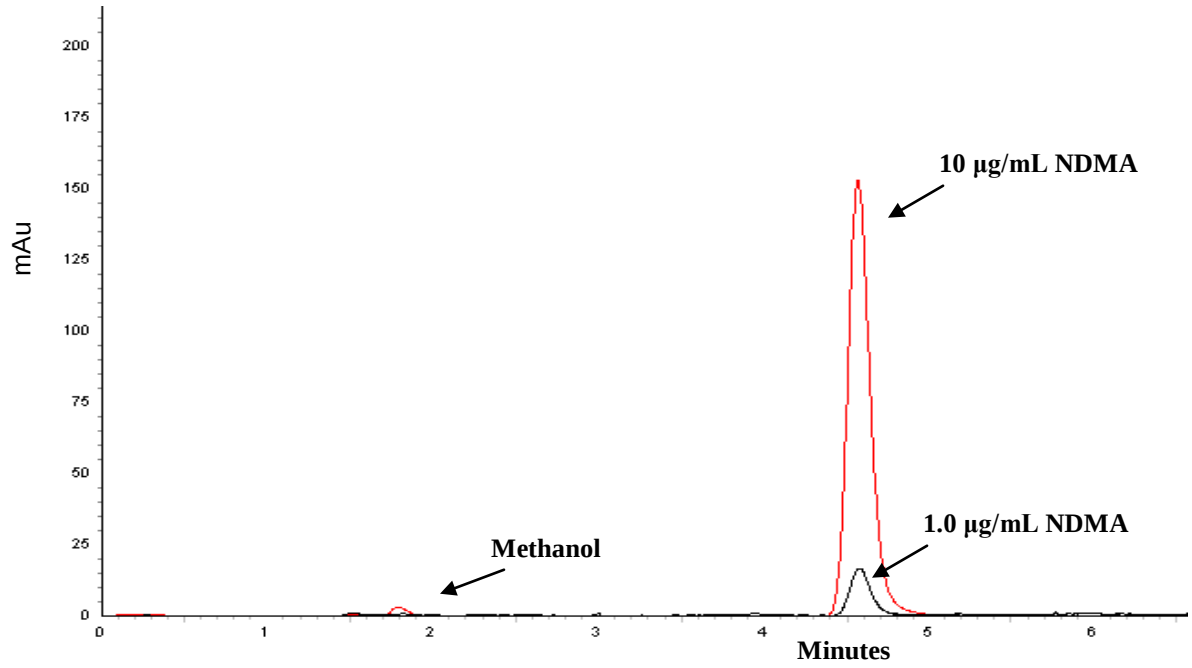

Figure 4.7. Chromatogram of NDMA Analysis (1.0 and 10 µg/mL) using MP3 at pH 5.

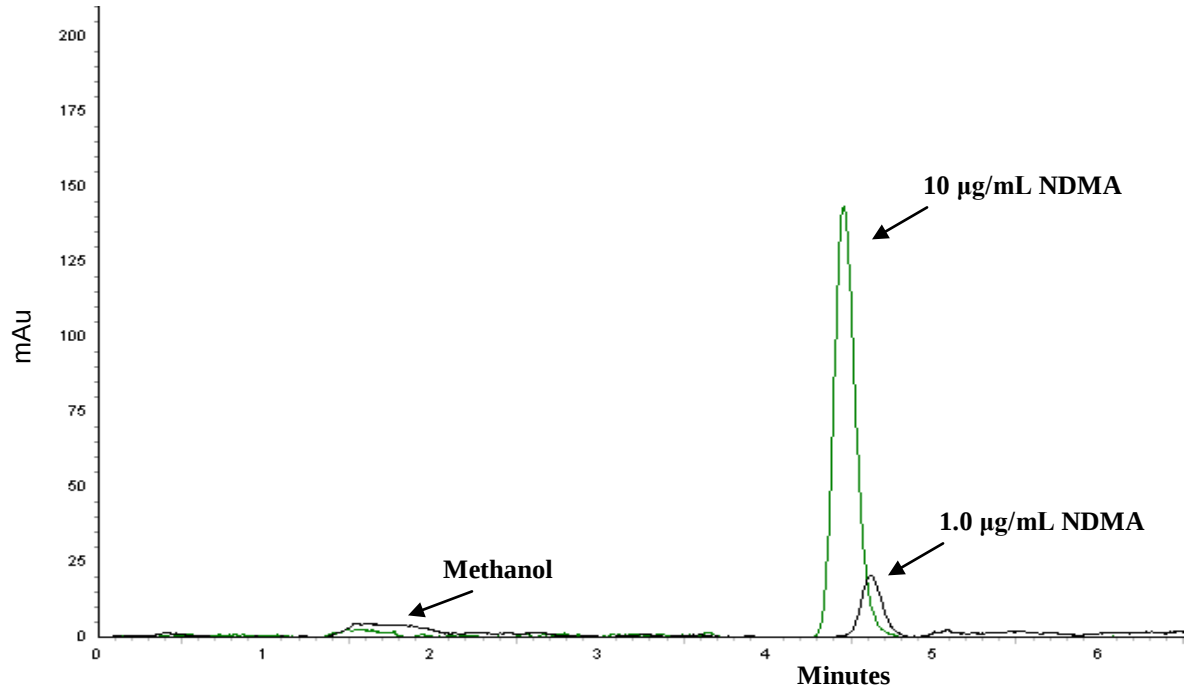

Figure 4.8. Chromatogram of NDMA Analysis (1.0 and 10 µg/mL) using MP3 at pH 6.

Influence of pH Modification on NDMA Peak Characteristics using Ethanol (MP4)

The chromatographic behavior of 1.0 and 10 µg/mL NDMA (mobile phase spiked with analyte) was assessed using MP4 at pH 4, 5, and 6 (*Tables VIII* and *IX).*

TABLE VIII: Impact of Mobile Phase pH on Retention and Resolution using MP4 and 1.0 µg/mL NDMA in the Mobile Phase

pH	Mobile Phase	t_r	t_m	k’	Peak Area	Peak Height
4	MP4	3.216	1.819	0.77	374476	32780
5	MP4	3.173	1.477	1.15	162098	22115
6	MP4	3.147	1.445	1.18	163172	21641

TABLE IX: Impact of Mobile Phase pH on Retention and Resolution using MP4 and 10 µg/mL NDMA in the Mobile Phase.

pH	Mobile Phase	t_r	t_m	k'	Peak Area	Peak Height
4	MP4	3.216	1.776	0.81	2434681	245901
5	MP4	3.173	1.744	0.82	1453241	210949
6	MP4	3.141	1.445	1.17	1576116	224807

Retention time was relatively unaffected; yet peak symmetry and background noise improved as pH decreased (Figures 4.9-4.11). Resolution of NDMA and methanol greatly improved at pH 4. However, the retention factor (*k'* = 1.17) was most favorable at pH 6 when MP4 was used.

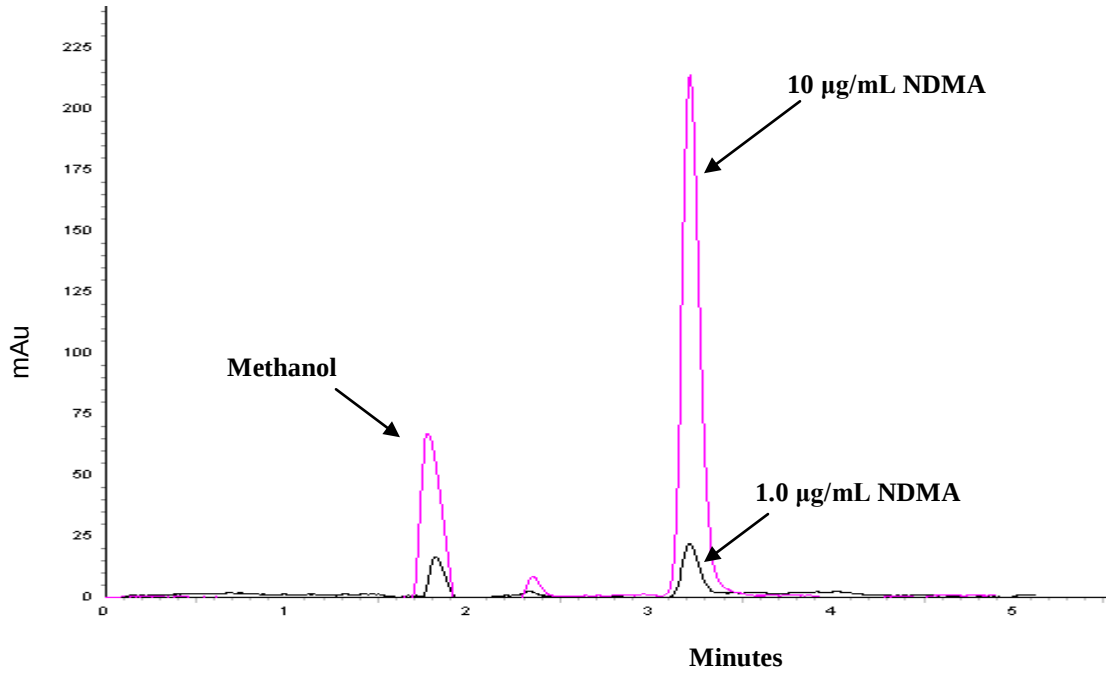

Figure 4.9. Chromatogram of NDMA Analysis (1.0 and 10 µg/mL) using MP4 at pH 4.

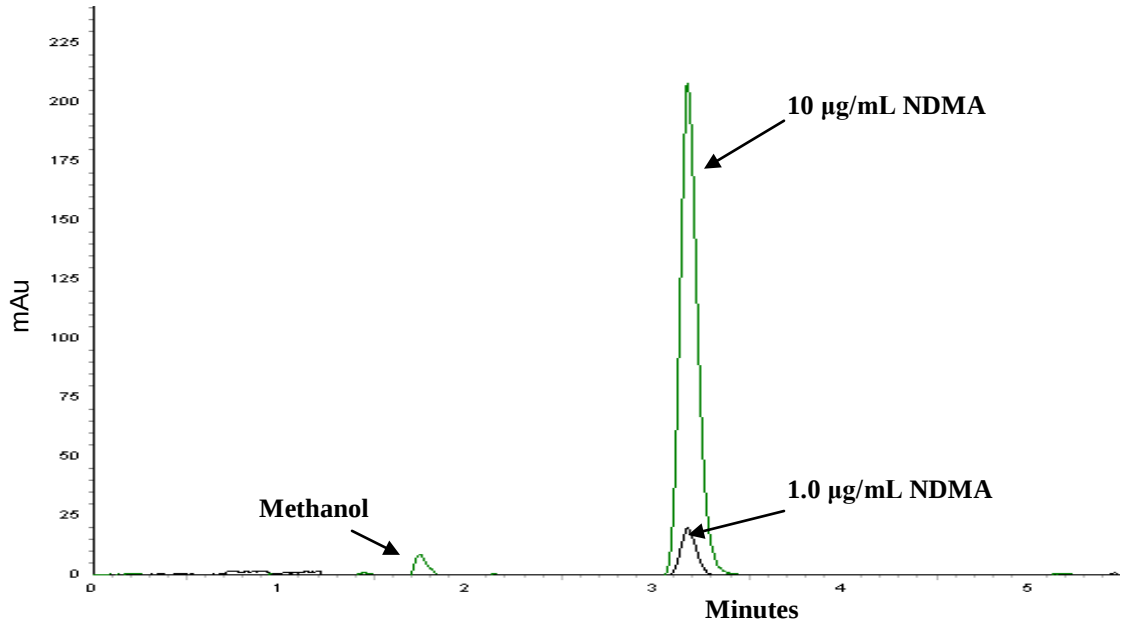

Figure 4.10. Chromatogram of NDMA Analysis (1.0 and 10 µg/mL) using MP4 at pH 5.

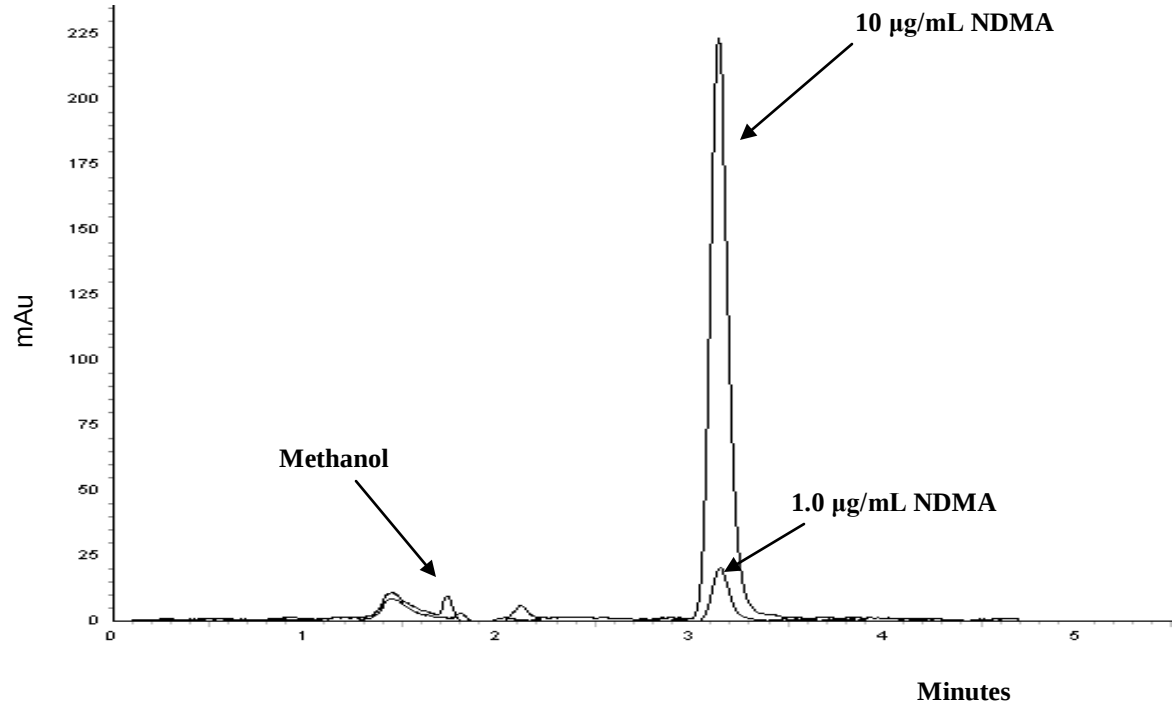

Figure 4.11. Chromatogram of NDMA Analysis (1.0 and 10 µg/mL) using MP4 at pH 6.

Effect of Buffer Concentration on NDMA Analysis

MP3 was used to determine the influence of buffer concentration on the chromatographic behavior of 10 μg/ml NDMA (mobile phase spiked with analyte). The study evaluated 10 mM and 20 mM acetate buffers (pH 6). Ionic strength and pH of the mobile phase can impact analyte retention; however, differences in retention time were minimal (3%) and peak shape was maintained (*Table X*) (Figure 4.12). Though peak area and height were greater for the 20 mM buffer, background noise improved with use of the 10 mM buffer. As a result, further method optimization was performed using the 10 mM buffer.

TABLE X: Analysis of Buffer Concentration on NDMA Analysis using 10 μg/mL NDMA in the Mobile Phase

Buffer Concentration	Mobile Phase	t_r	t_m	k'	Peak Area	Peak Height
10 mM	MP3	4.94	1.74	1.84	1132119	106815
20 mM	MP3	4.78	1.78	1.68	1200586	114380

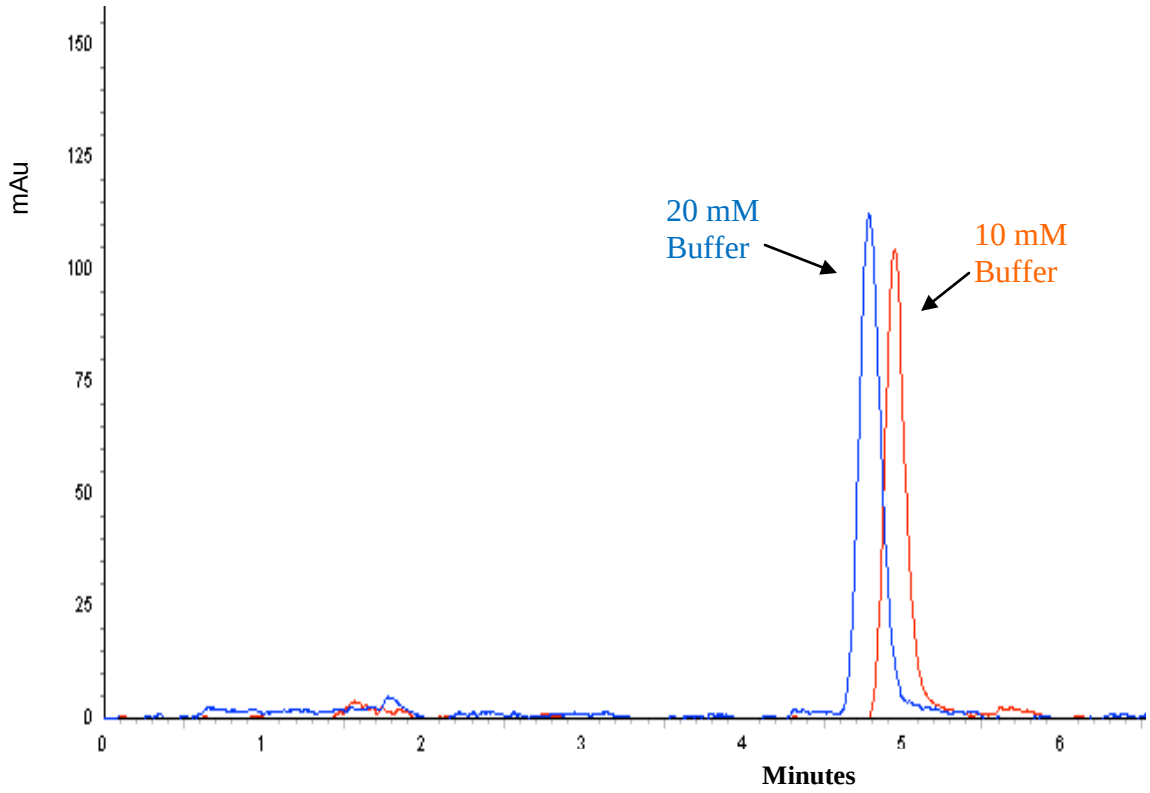

Figure 4.12. Impact of Buffer Concentration on NDMA Analysis.
(10 µg/mL NDMA in Mobile Phase)

4.2. Evaluation of Methanol, Acetonitrile and Ethanol Modifiers in NDMA Analysis

Due to cost, methanol mobile phases were studied prior to using ethanol and acetonitrile. Ethanol and acetonitrile each were combined with the 10 mM acetate buffer for evaluating NDMA (1.0 and 10 µg/mL in mobile phase, pH 6). Ethanol was applied in the same proportion as methanol. The volume of acetonitrile required was obtained using a nomogram. When preparing a binary mobile phase system that consists of a different organic solvent, a nomogram is useful for determining the volume of organic solvent (as a percent) required for developing a mobile phase of comparable eluent stength.[28] This tool is often used when an organic solvent substitute is necessary.

In comparison to MP3 and MP5, the greatest absorbance and least retention was observed with MP4. Elution times were 4.62, 3.14, and 8.42 min. for MP3, MP4, and MP5, respectively, for the 10 µg/mL NDMA standard (Figure 4.13).

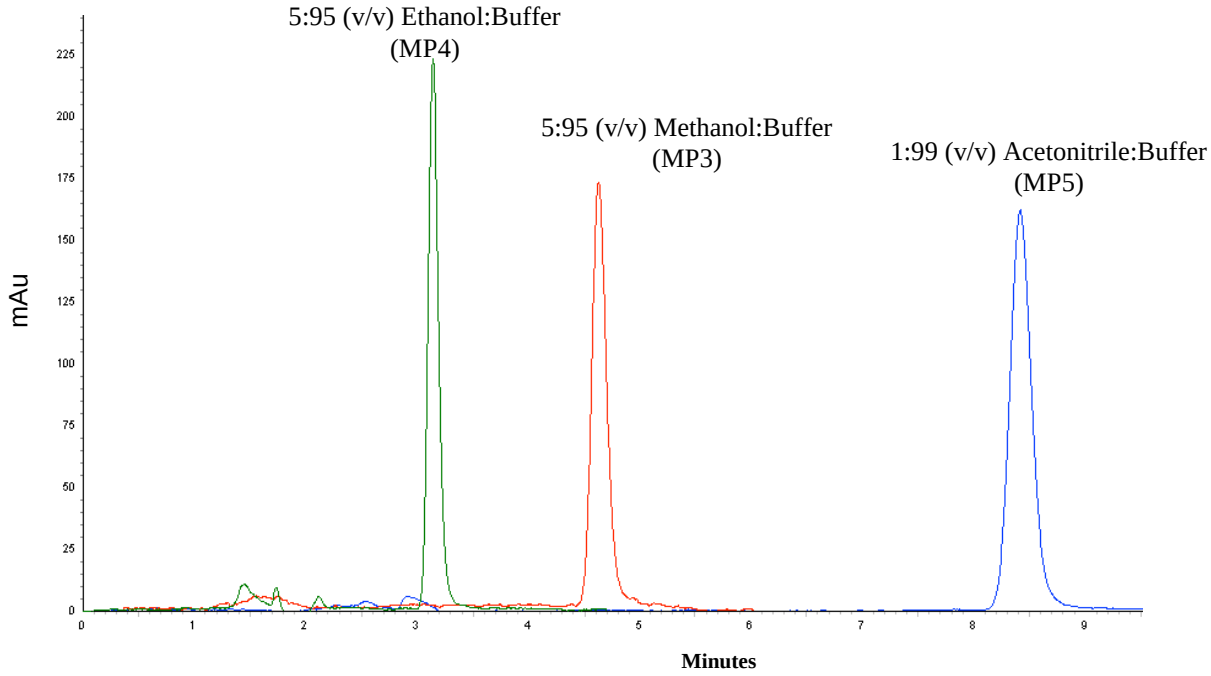

Figure 4.13. Impact of Modifier on NDMA Analysis (10 µg/mL).

Acetonitrile is frequently used for NDMA analysis[32,34,40]; therefore, MP5 was expected to decrease the retention time for NDMA while improving resolution for NDMA and methanol. Acetonitrile has a lower viscosity than methanol and ethanol, which often reduces band broadening. Generally, acetonitrile is recognized as a stronger eluent than methanol and ethanol in RP-HPLC applications. This investigation demonstrated that an ethanol modifier improved the detection of NDMA (increased peak height for 10 µg/mL), while maintaining peak symmetry and reducing analysis time.

Data for the modifier evaluation using MP3, MP4, and MP5 are within *Tables XI and XII.*

TABLE XI: Evaluation of Modifier on NDMA Analysis using 1.0 μg/mL in the Mobile Phase

Modifier	Mobile Phase	t_r	t_m	k'	Peak Area	Peak Height
Methanol	MP3	4.620	1.824	1.53	195977	20973
Ethanol	MP4	3.147	1.445	1.18	163172	21641
Acetonitrile	MP5	8.427	3.029	1.78	289375	21884

TABLE XII: Evaluation of Modifier on NDMA Analysis using 10 μg/mL in the Mobile Phase

Modifier	Mobile Phase	t_r	t_m	k'	Peak Area	Peak Height
Methanol	MP3	4.62	1.72	1.60	1753099	174366
Ethanol	MP4	3.14	1.445	1.17	1576116	224807
Acetonitrile	MP5	8.42	2.928	1.88	2272303	162127

Flow rate

Peak symmetries of 1.0- and 10 μg/mL NDMA were evaluated at flow rates of 0.75, 1.0, 1.25 and 1.5 mL/min. using MP3 (Figure 4.14). Though the analysis time decreased with the increased flow rate, peak height was not significantly affected. (See *Tables XIII and XIV*.) As expected, peak width was greatest at 0.75 mL/min.

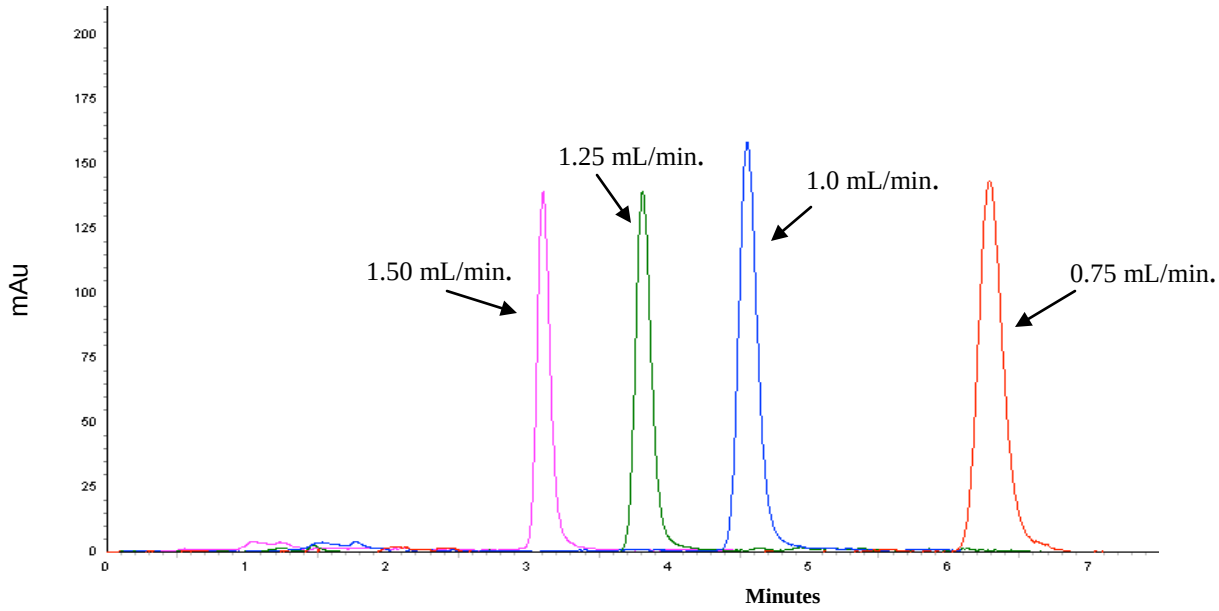

Figure 4.14. Flow Rate Analysis using 10 µg/mL NDMA and MP3.

TABLE XIII: Impact of Flow Rate on Retention and Resolution using 1.0 µg/mL NDMA in the Mobile Phase

Flow Rate mL/min.	Mobile Phase	t_r	Peak Area	Peak Height
0.75	MP3	6.299	221424	16911
1.0	MP3	4.596	167287	17927
1.25	MP3	3.808	168059	15728
1.50	MP3	3.044	102791	15100

TABLE XIV: Impact of Flow Rate on Retention and Resolution using 10 µg/mL NDMA in the Mobile Phase

Flow Rate mL/min.	Mobile Phase	t_r	Peak Area	Peak Height
0.75	MP3	6.288	1866533	144611
1.0	MP3	4.556	1521757	158435
1.25	MP3	3.812	1185213	140750
1.50	MP3	3.104	960481	144611

Noise was reduced and the resolution of methanol and NDMA improved when a flow rate of 1.5 mL/min. was used with MP3. However, an undesirable retention factor and probable coelution (of methanol and NDMA) may result from using a flow rate greater than 1.0 mL/min. with MP4. Prior analysis (Section 4.2) indicates that NDMA had a retention time of approximately 3.1 minutes when MP4 was used. Based upon these findings, a 1-mL/min flow rate was selected for the remaining investigations. Chromatograms representing the 1.0-µg/mL NDMA analysis are presented in Figures 4.15-4.18.

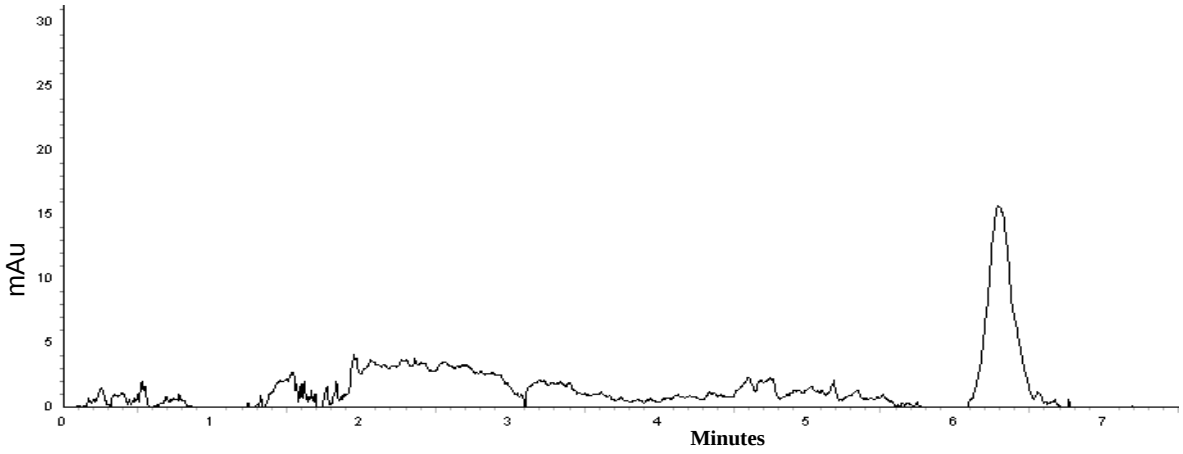

Figure 4.15. Impact of Flow Rate (0.75 mL/min.) on Retention and Resolution using 1.0 µg/mL NDMA in MP3.

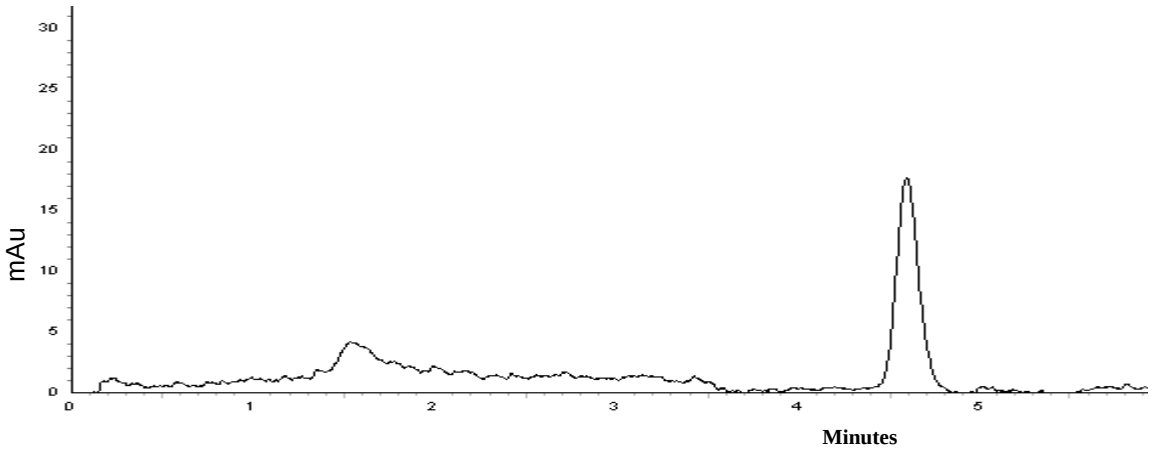

Figure 4.16. Impact of Flow Rate (1.0 mL/min.) on Retention and Resolution using 1.0 µg/mL NDMA in MP3.

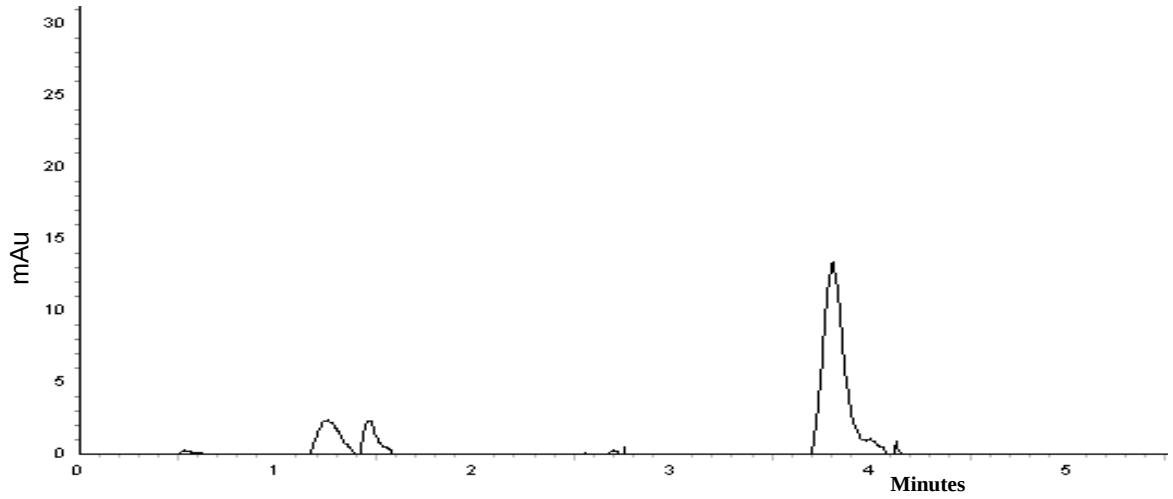

Figure 4.17. Impact of Flow Rate (1.25 mL/min.) on Retention and Resolution using 1.0 μg/mL NDMA in MP3.

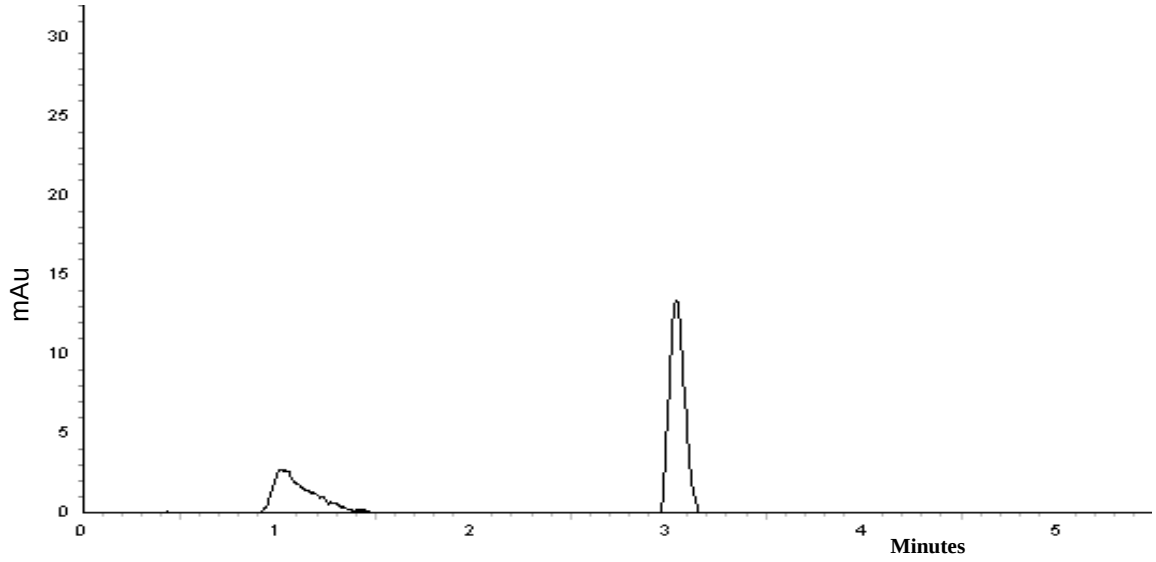

Figure 4.18. Impact of Flow Rate (1.50 mL/min.) on Retention and Resolution using 1.0 μg/mL NDMA in MP3.

4.3. Instrument Operating Parameters

Instrument operating parameters were evaluated using the 5-µm Zorbax SB-C_{18} column, MP3 and MP4 (for select investigations), and a 1-mL/min. flow rate. Optimal detection of NDMA was achieved at 231 nm.

Select studies employed the tungsten (W) and deuterium (D_2) lamps simultaneously. The manufacturer recommends the use of only the D_2 lamp when analytes do not absorb in the visible range.[43] For both concentrations, an increase in peak area and height were observed when the D_2 lamp was used (*Tables XV and XVI).* The selection of lamps did not significantly impact background noise or peak symmetry when the absorbance scale (y-axis) was set at 250 mAu (Figures 4.19 and 4.20). However, a baseline analysis (absorbance scale set at 50 mAu) reflected a slight improvement of these characteristics when the D_2 lamp was used. As a result, the D_2 lamp was used for the remaining investigations.

TABLE XV: Impact of Lamp Selection on NDMA Analysis using 1.0 µg/mL NDMA in the Mobile Phase.

Lamp	Mobile Phase	t_r	Peak Area	Peak Height
Deuterium (D_2)	MP3	4.596	89586	13337
Tungsten (W) and D_2	MP3	4.620	65707	11427

TABLE XVI: Impact of Lamp Selection on NDMA Analysis using 10 µg/mL NDMA in the Mobile Phase

Lamp	Mobile Phase	t_r	Peak Area	Peak Height
Deuterium (D_2)	MP3	4.57	1018295	135212
Tungsten (W) and D_2	MP3	4.60	810271	116199

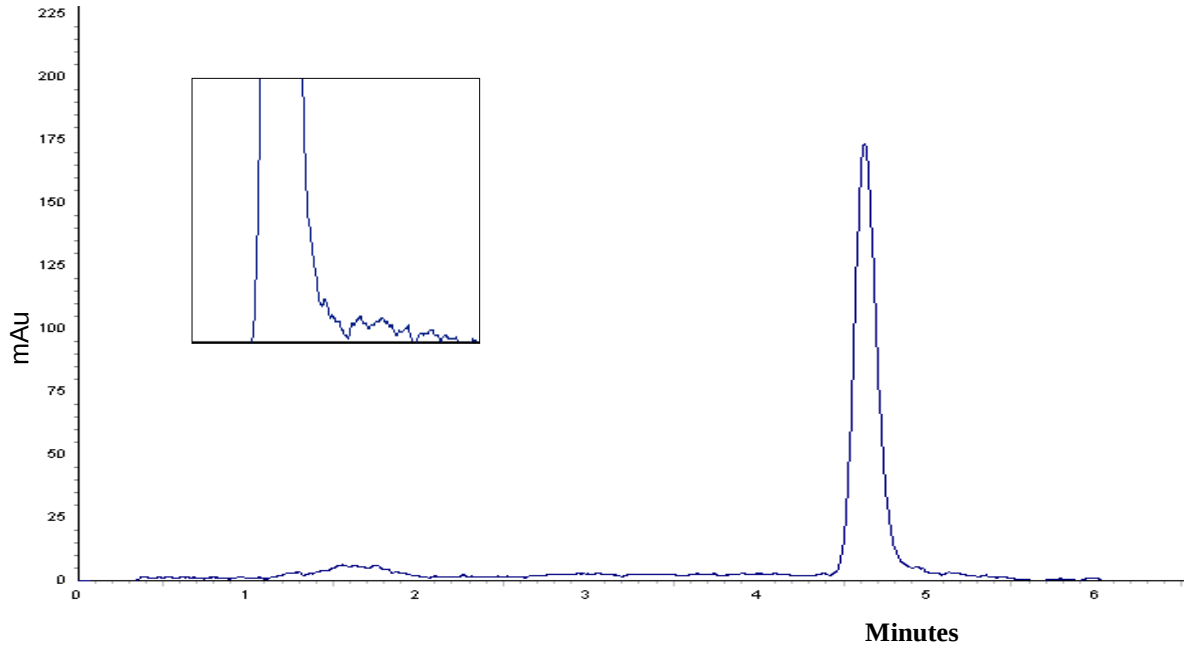

Figure 4.19. Chromatogram Obtained using the W and D_2 lamps for the Analysis of 10 µg/mL NDMA.

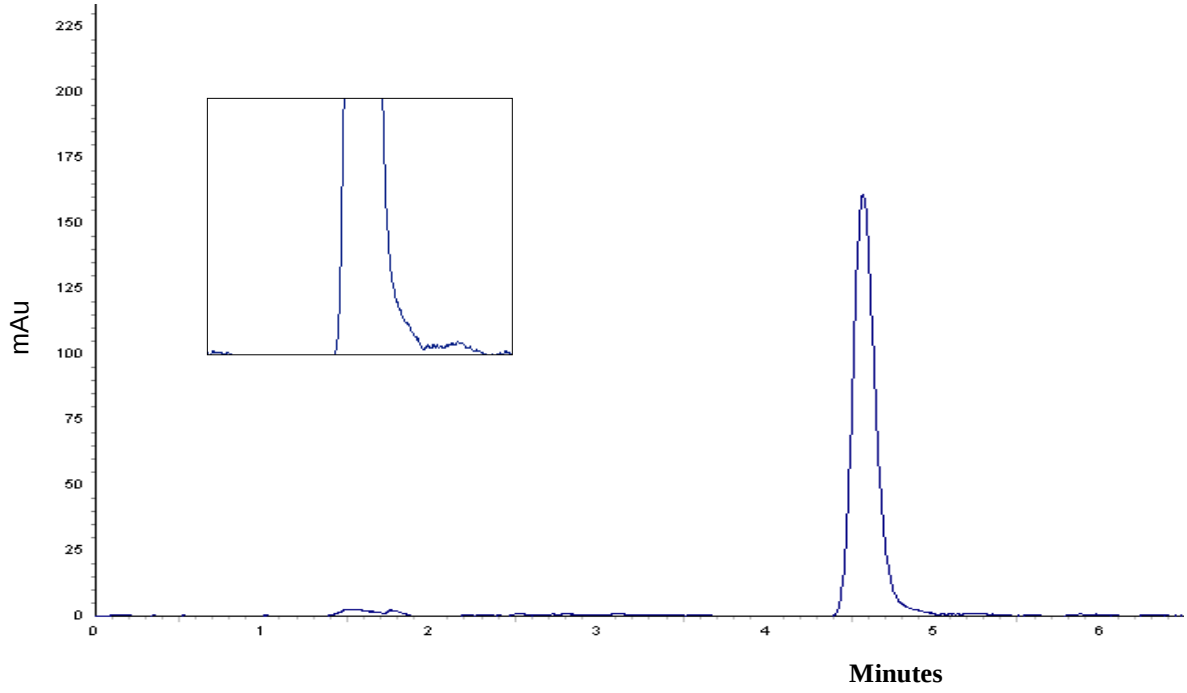

Figure 4.20. Chromatogram Obtained using the D_2 Lamp for the Analysis of 10 µg/mL NDMA.

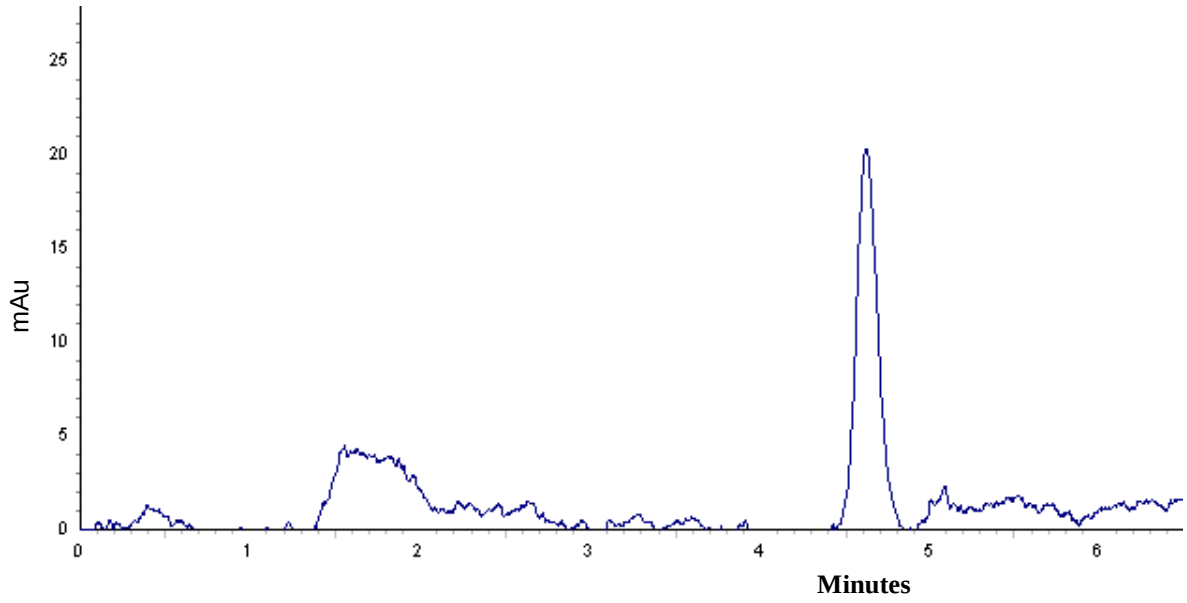

Figure 4.21. Chromatogram Obtained using the W and D_2 lamps for the Analysis of 1.0 µg/mL NDMA.

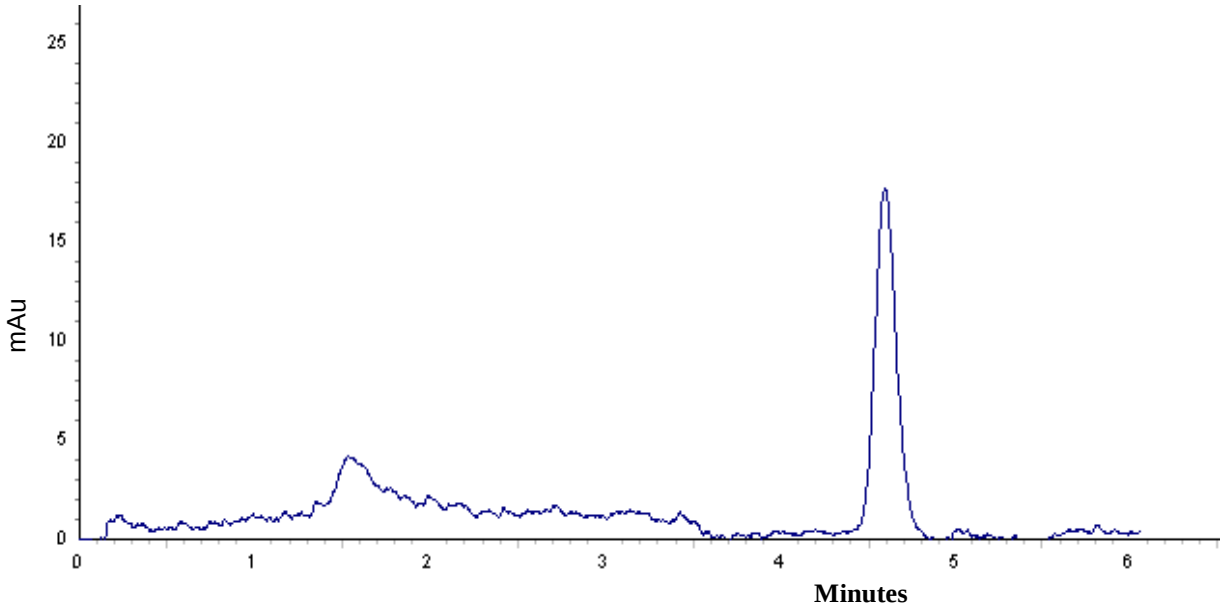

Figure 4.22. Chromatogram Obtained using the D_2 Lamp for the Analysis of for 1.0 µg/mL NDMA.

Initial investiations were performed with the time constant and sampling period set at 640 and 240 ms, respectively. The manufacturer suggests that the sampling time and time constant be set at the same value. Therefore, the original setting (Figure 4.23) was compared to equivalent settings for the time constant and sampling period at 240, 320, and 640 ms (Figures 4.24-4.26). The purpose was to investigate noise reduction, peak symmetry, and the resolution of methanol and 1 µg/mL NDMA. The mobile phase flow rate was 1.5 mL/min.

The time constant is used to reduce noise. Increasing the time constant to 320 ms produced a minimal increase in peak height for NDMA. Increasing the sampling time reduces the available data set that can be used for spectral analysis. The consequence is decreased spectral resolution and increased noise. In comparison to the initial conditions, noise increased significantly and peak resolution was poor when equivalent settings of 640 ms were employed.

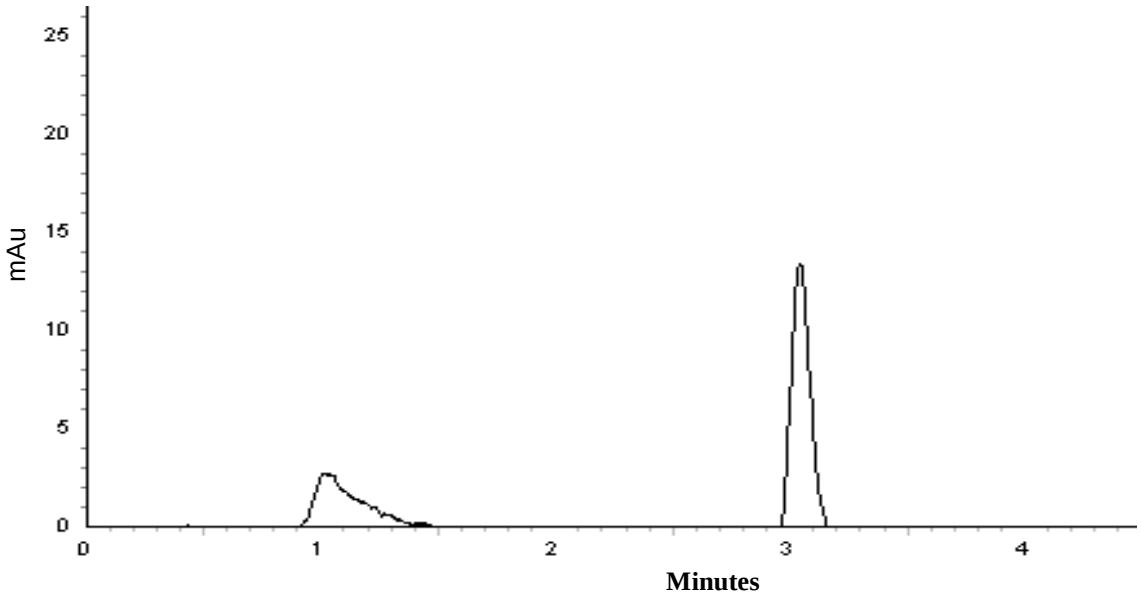

Figure 4.23. Chromatogram for 1.0 µg/mL NDMA Obtained using 240 ms Sampling Time and 640 ms Time Constant.

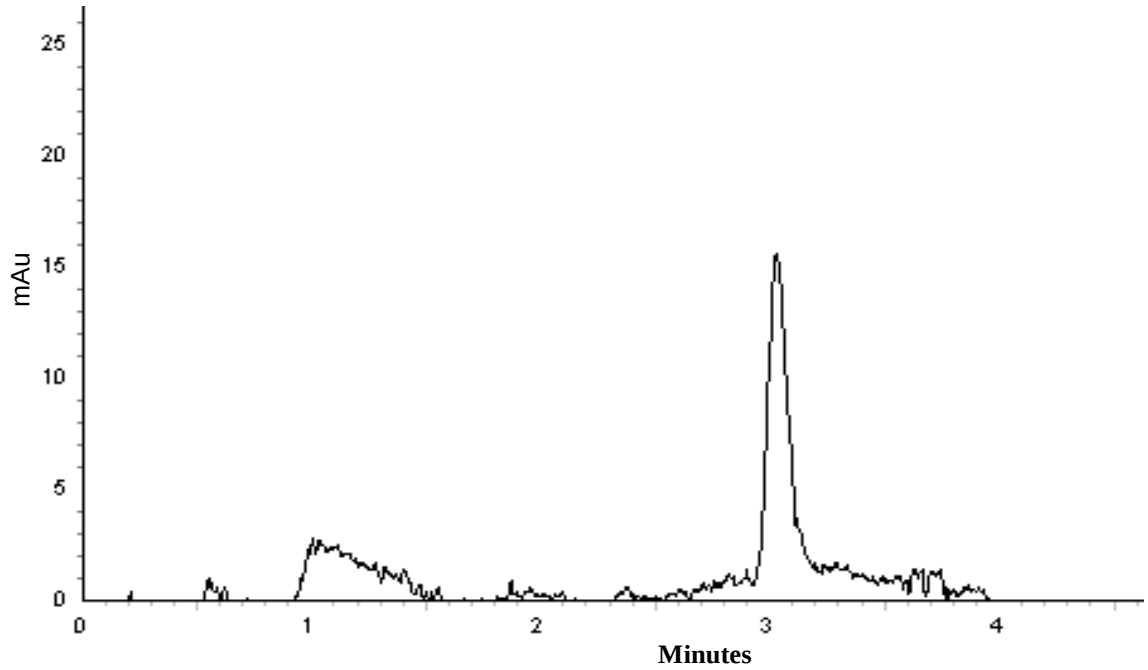

Figure 4.24. Chromatogram for 1.0 µg/mL NDMA Obtained using 240 ms Sampling Time and 240 ms Time Constant.

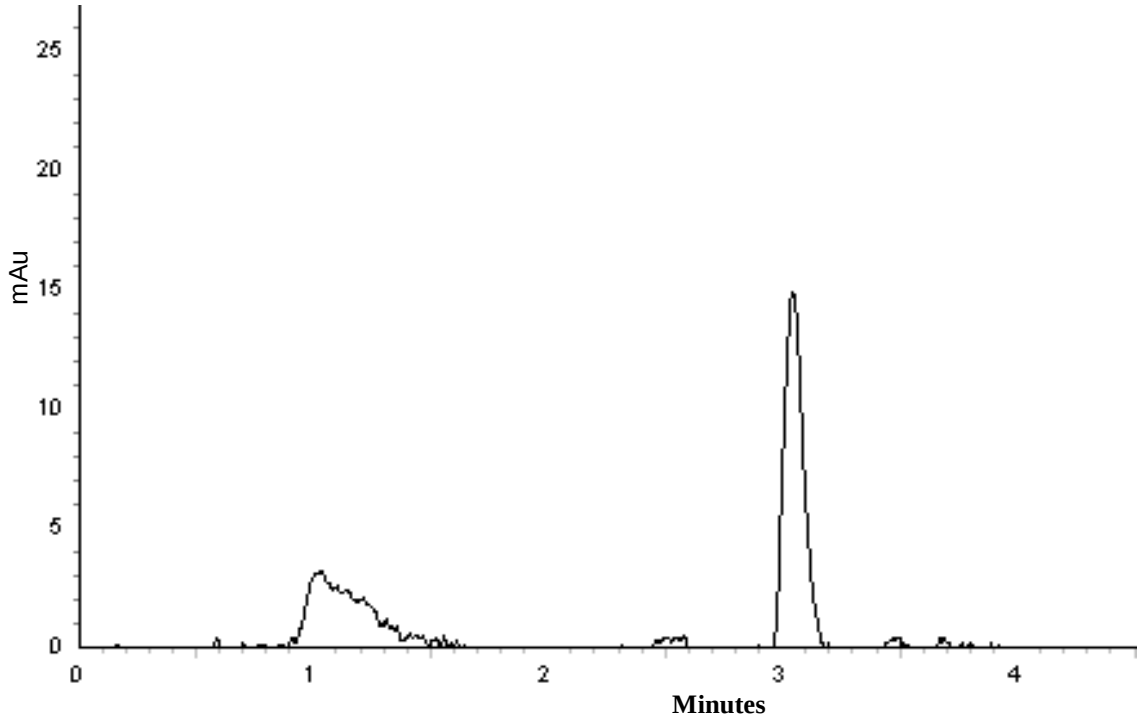

Figure 4.25. Chromatogram for 1.0 µg/mL NDMA Obtained using 320 ms Sampling Time and 320 ms Time Constant.

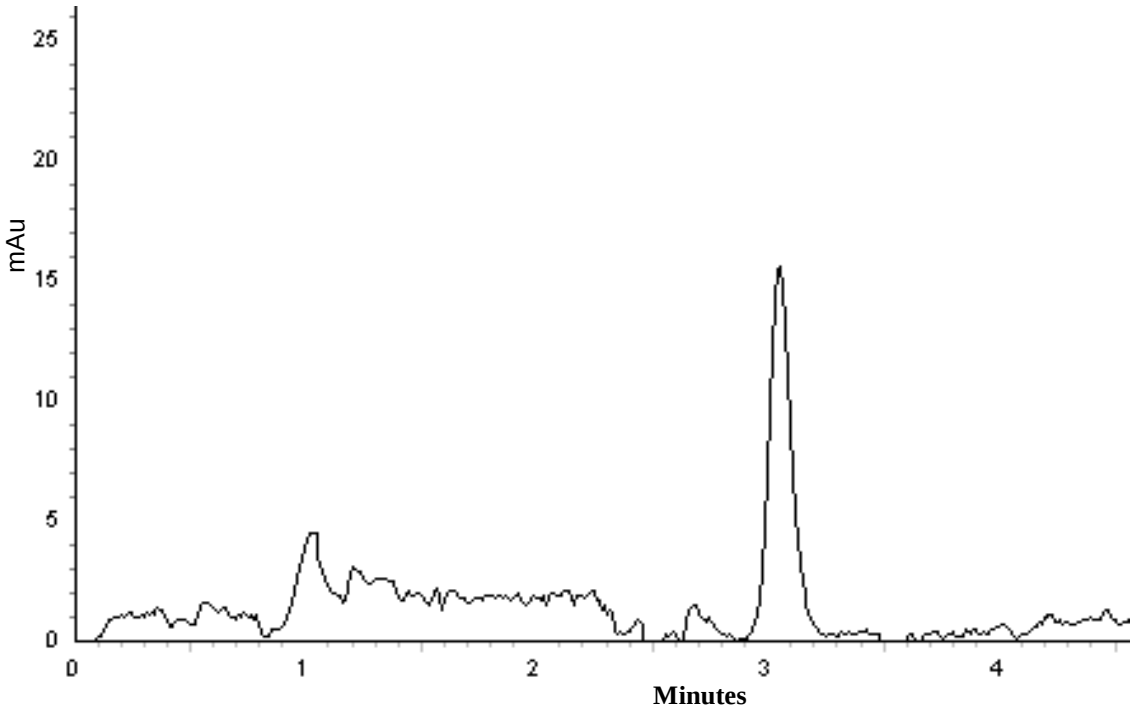

Figure 4.26. Chromatogram for 1.0 μg/mL NDMA Obtained using 640 ms Sampling Time and 640 ms Time Constant.

Absorbance values are averaged based on a range indicated by the bandwidth. Decreased resolution and increased noise are associated with larger bandwidth values. The bandwidth setting at 231 nm was modified to assess noise reduction and absorbance. Settings of ±3 and ±5 nm were compared to the normal setting at ±4 nm, for MP3 and MP4, respectively.

Impact of Bandwidth Modification on NDMA Analysis using Methanol (MP3)

Though it did not improve resolution for NDMA and methanol, peak symmetry for NDMA improved as bandwidth decreased. Variations in bandwidth did not significantly impact background noise (Figures 4.27-4.29). Using MP3, analysis should be performed at 231 nm with a bandwidth of ±3.

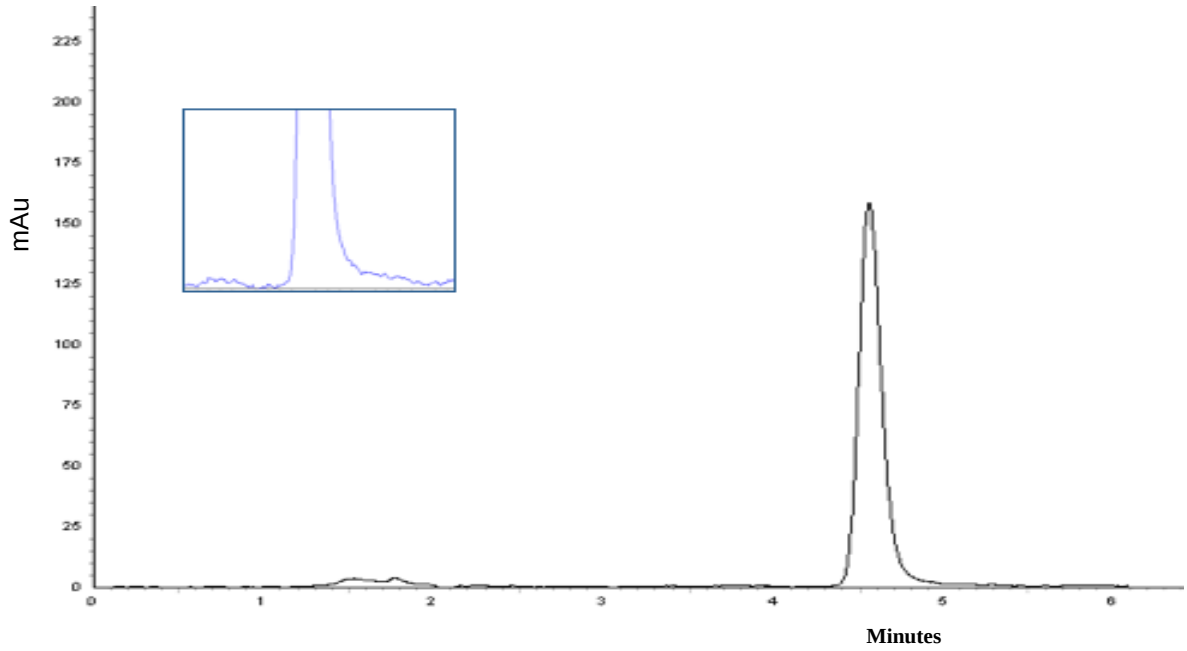

Figure 4.27. Bandwidth Analysis for 10 µg/mL NDMA at 231 nm (±3 nm) using MP3.

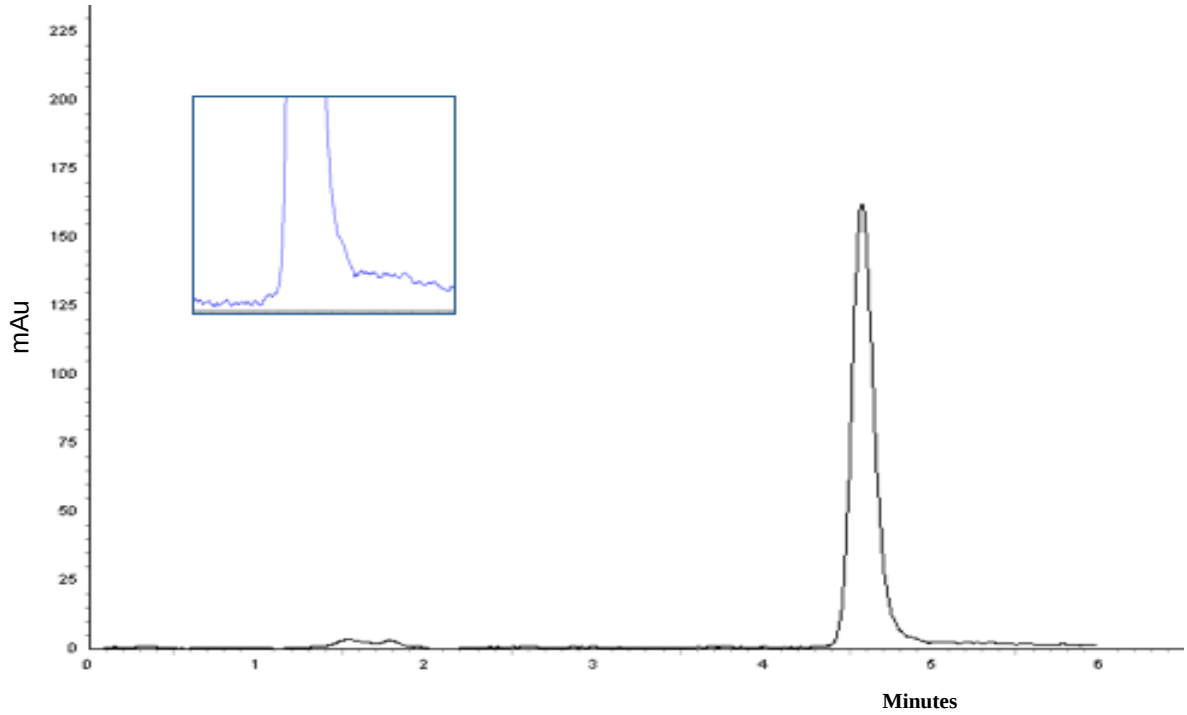

Figure 4.28. Bandwidth Analysis for 10 µg/mL NDMA at 231 nm (±4 nm) using MP3.

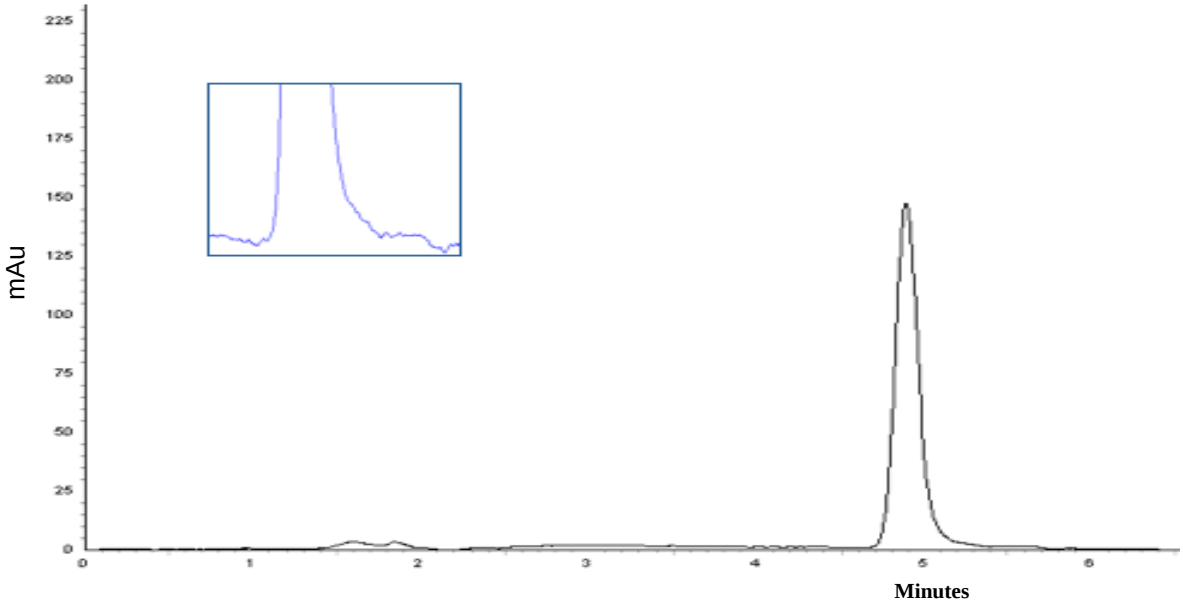

Figure 4.29. Bandwidth Analysis for 10 µg/mL NDMA at 231 nm (±5 nm) using MP3.

Impact of Bandwidth Modification on NDMA Analysis using Ethanol (MP4)

Resolution of NDMA and methanol were not impacted by changes in bandwidth. A minimal increase in noise was observed at the setting of ±5 nm. (Figures 4.30-4.32). Improved peak symmetry was observed using bandwidth of ±4 nm and MP4.

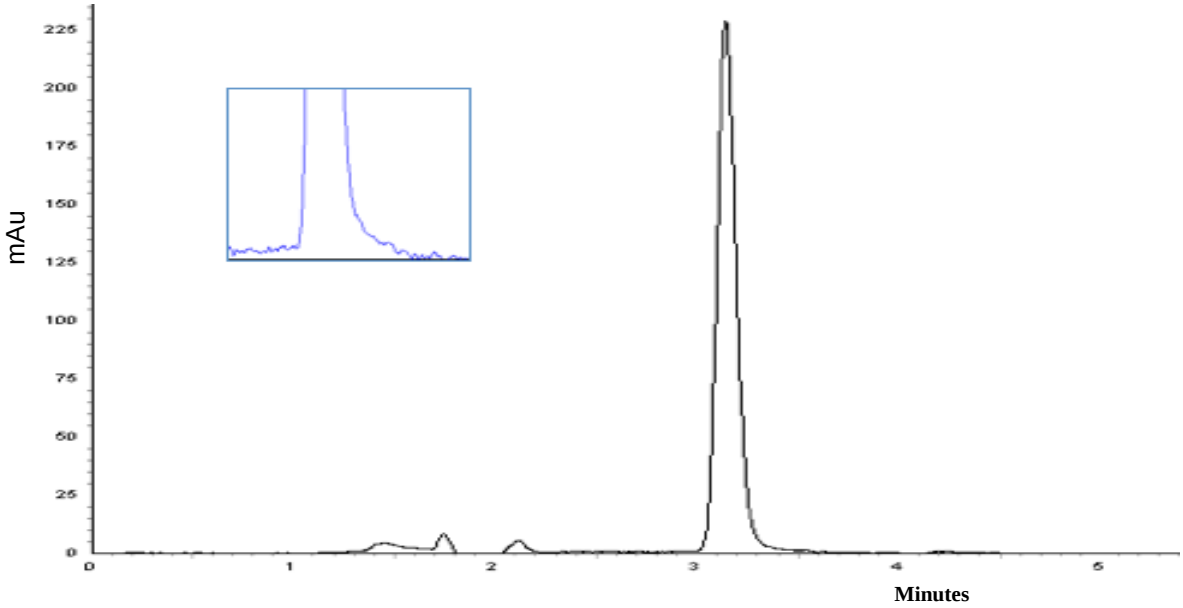

Figure 4.30. Bandwidth Analysis for 10 µg/mL NDMA at 231 nm (±3 nm) using MP4.

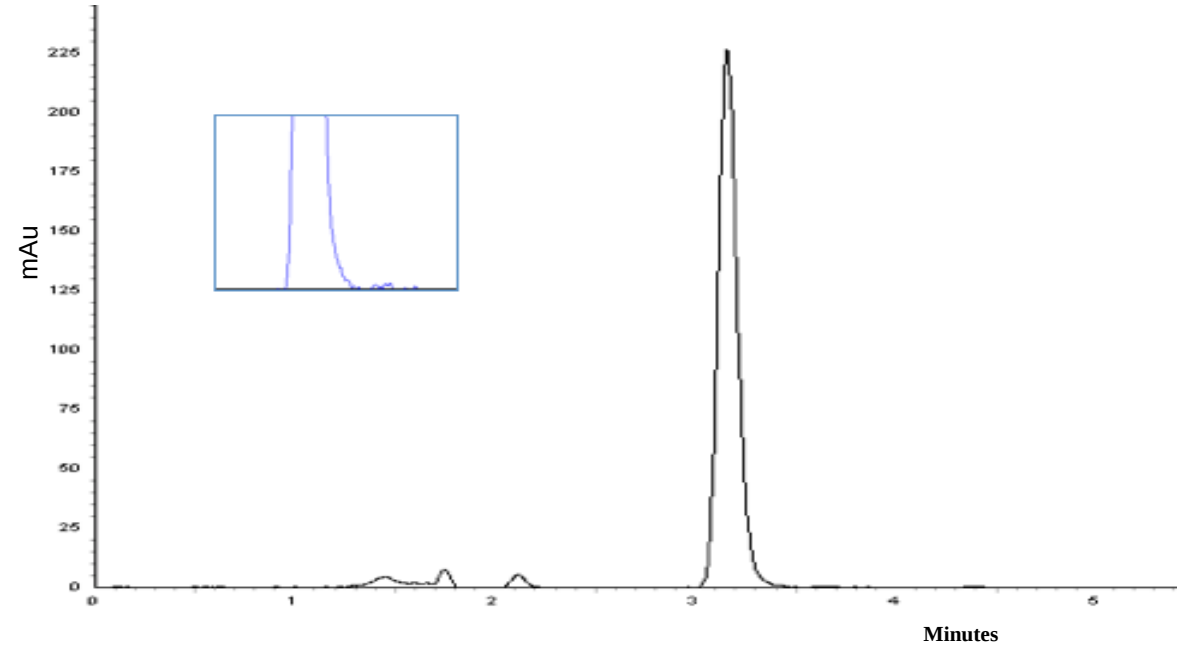

Figure 4.31. Bandwidth Analysis for 10 µg/mL NDMA at 231 nm (±4 nm) using MP4.

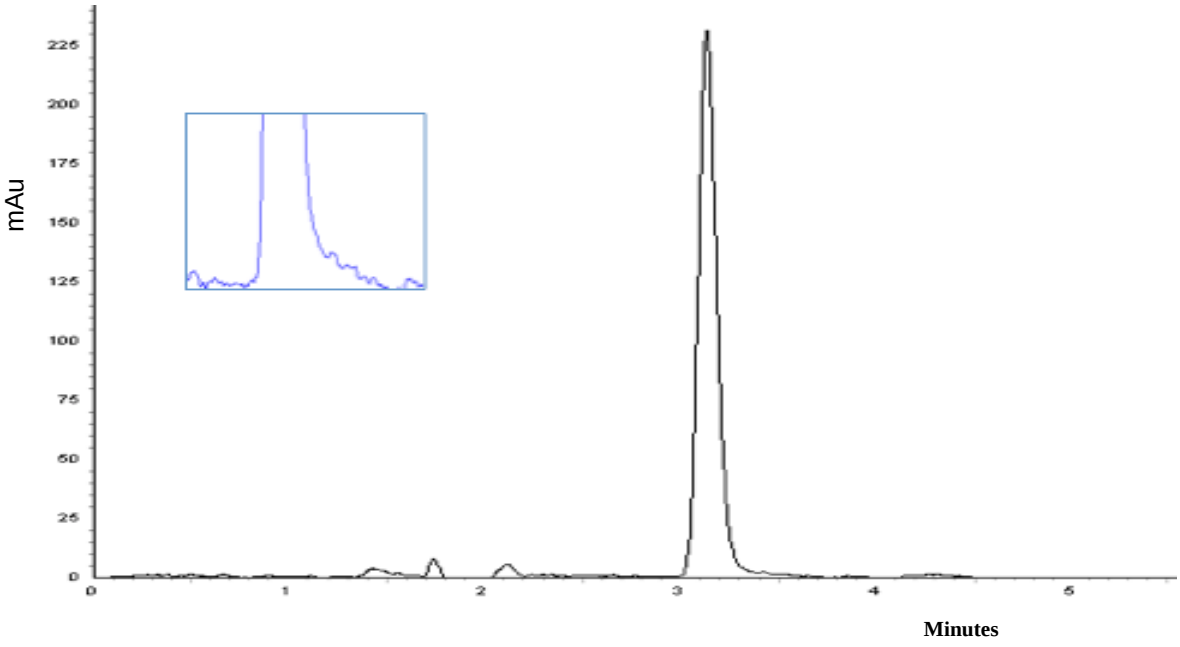

Figure 4.32. Bandwidth Analysis for 10 µg/mL NDMA at 231 nm (±5 nm) using MP4.

4.4. Robustness

Using MP3, water samples with different pH values were used to evaluate retention and resolution.

pH Modified Water

Purified water was evaluated at pH 5, 7.2, 9, and 11 to simulate conditions present in water treatment processes (*Tables XVII and XVIII*). Analyte retention and peak symmetry were impacted minimally by modifications in pH (Figures 4.33-4.40). However, background noise increased for 1.0 µg/mL NDMA at pH 5 and 11. This study confirms that method is robust, and applicable for evaluating water samples in the pH range from 5 to 11 and NDMA concentrations as low as 1.0 µg/mL using MP3.

TABLE XVII: Effect of Sample pH on NDMA Retention and Resolution using 1.0 µg/mL NDMA in the Mobile Phase

pH	Mobile Phase	t_r	Peak Area	Peak Height
5	MP3	4.571	220270	23976
7.2	MP3	4.560	161789	16882
9	MP3	4.565	138277	16272
11	MP3	4.549	194136	19843

TABLE XVIII: Effect of Sample pH on NDMA Retention and Resolution using 10 µg/mL NDMA in the Mobile Phase

pH	Mobile Phase	t_r	Peak Area	Peak Height
5	MP3	4.560	1617846	179286
7.2	MP3	4.566	1371063	152696
9	MP3	4.544	1494373	156743
11	MP3	4.549	1596429	173203

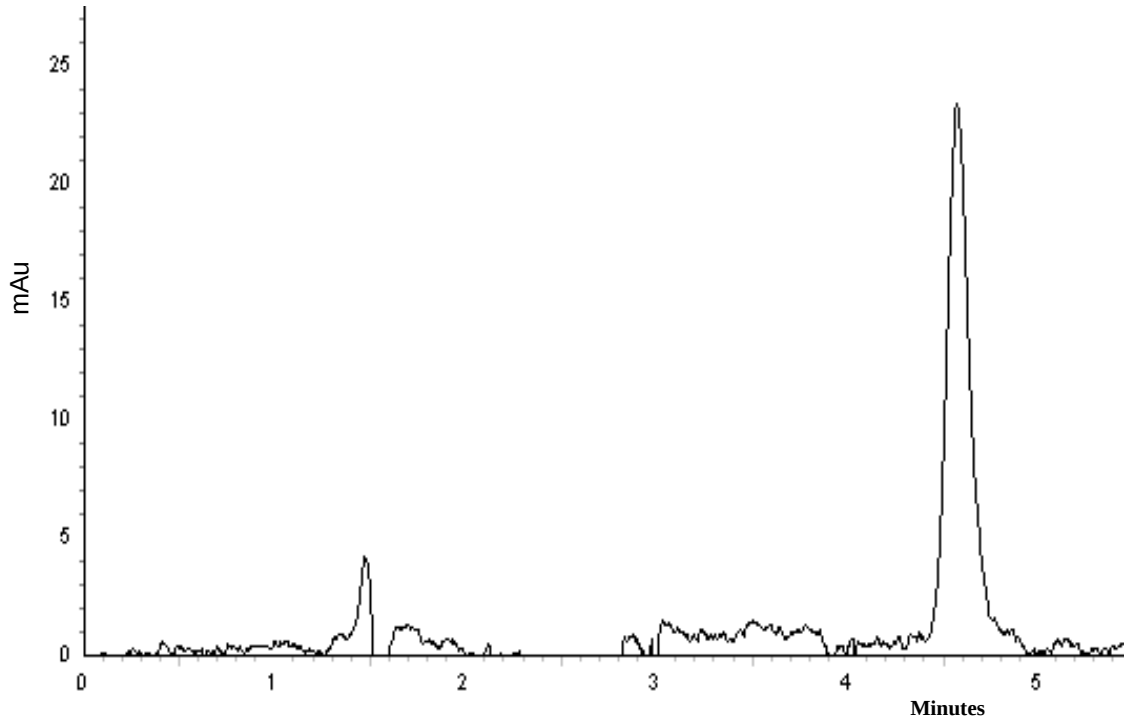

Figure 4.33. Chromatogram of 1.0 µg/mL NDMA at pH 5.

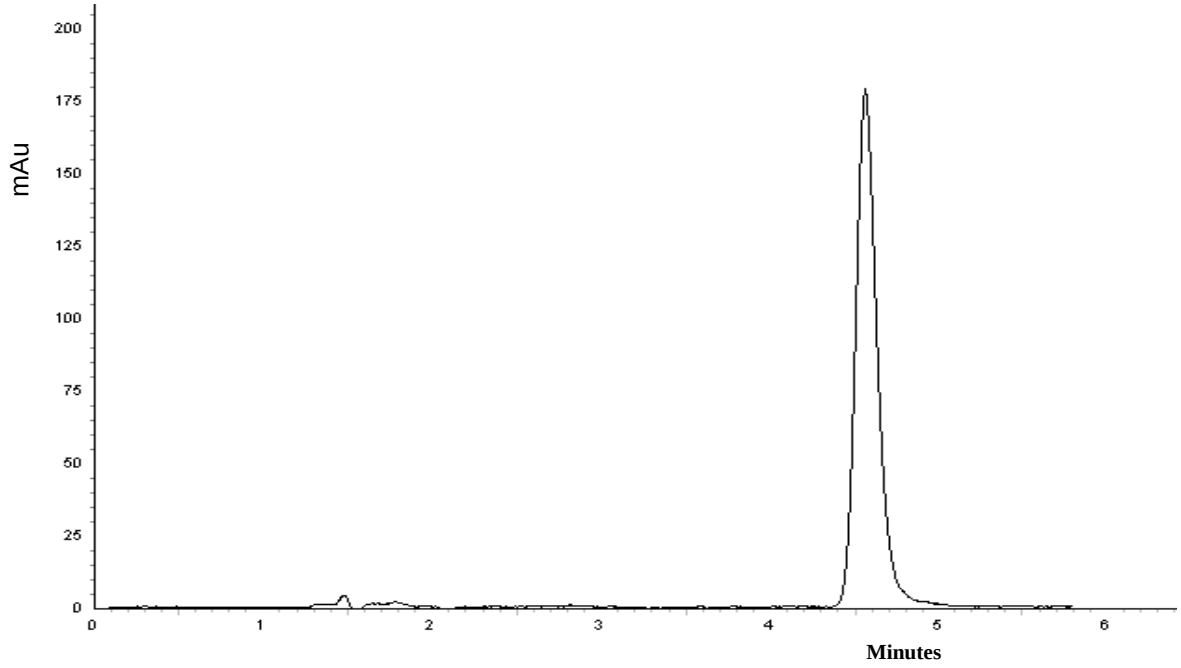

Figure 4.34. Chromatogram of 10 µg/mL NDMA at pH 5.

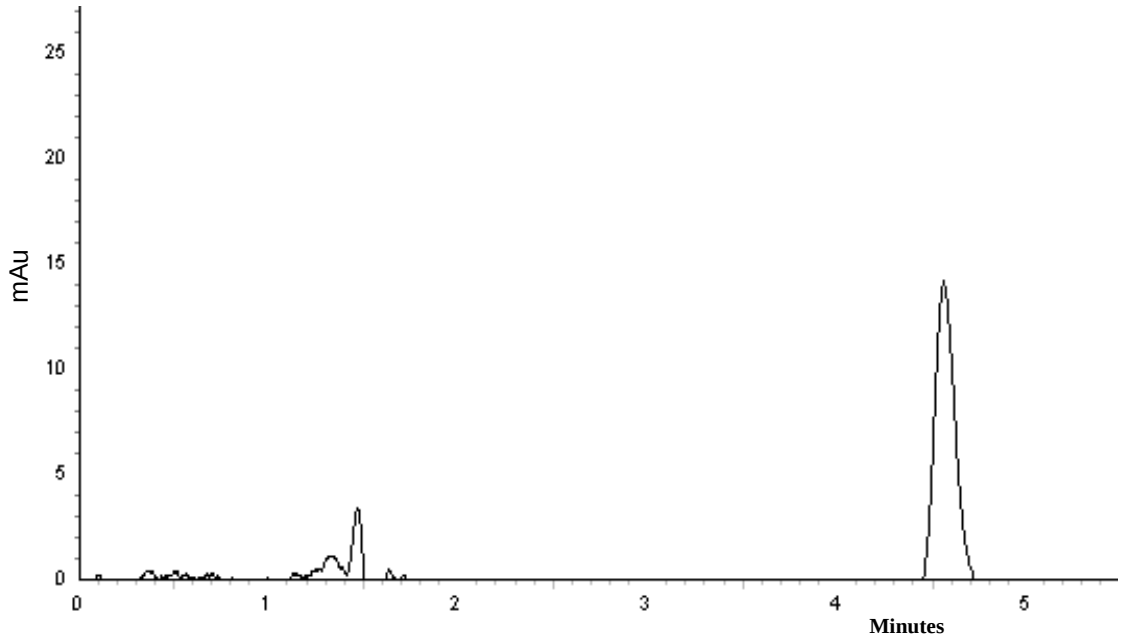

Figure 4.35. Chromatogram of 1.0 µg/mL NDMA at pH 7.2.

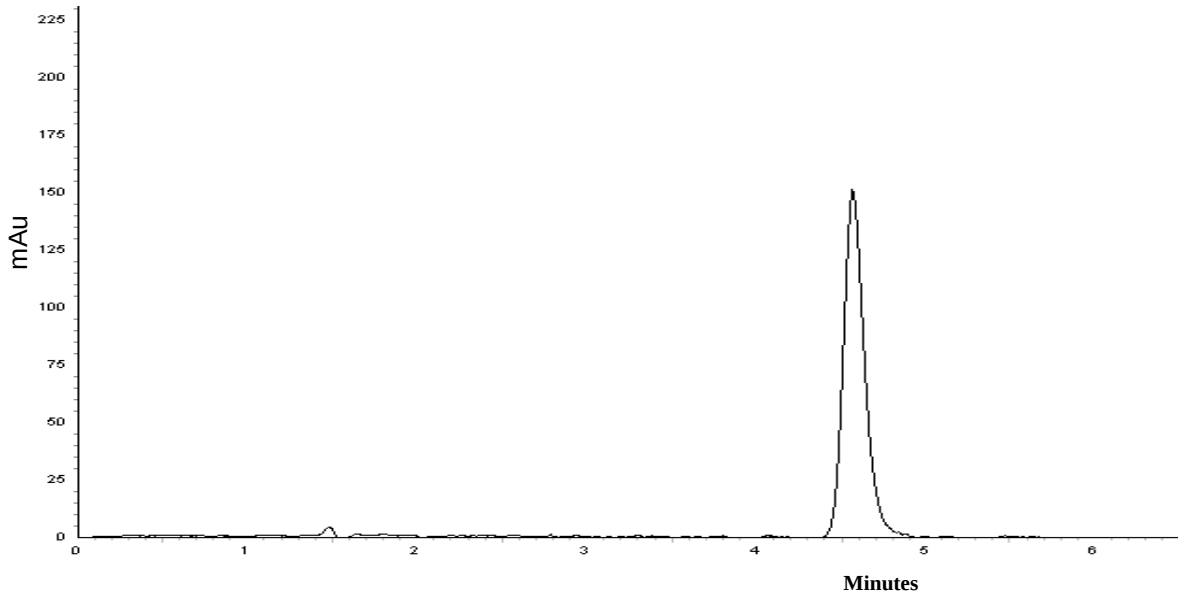

Figure 4.36. Chromatogram of 10 µg/mL NDMA at pH 7.2.

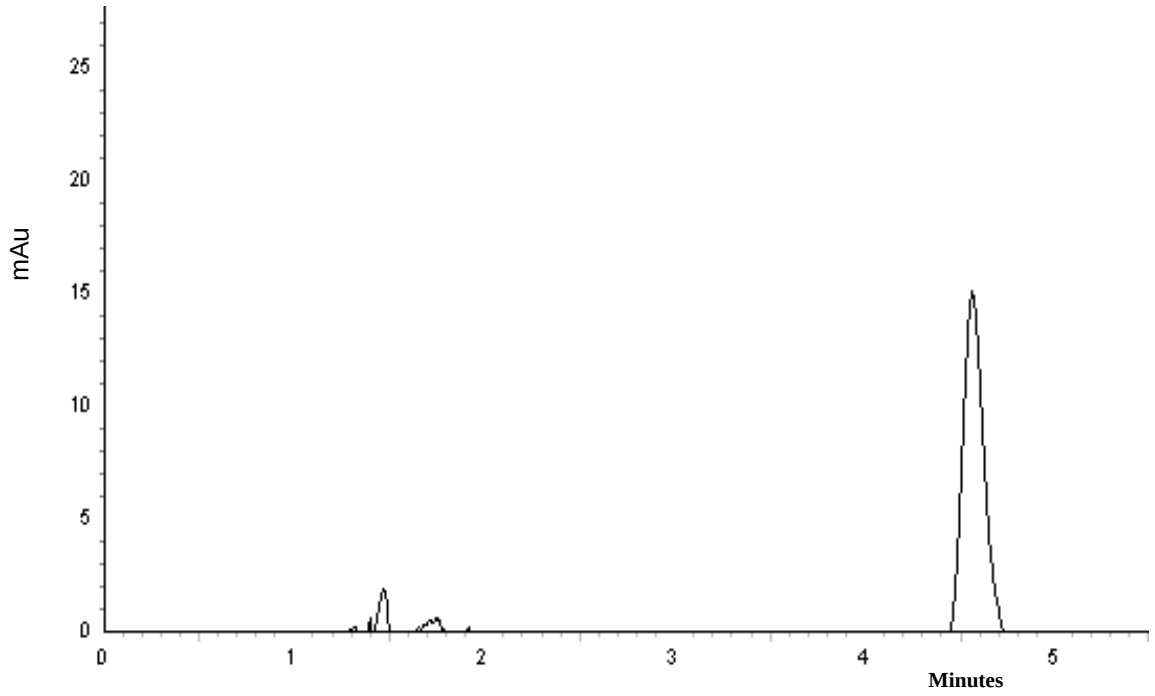

Figure 4.37. Chromatogram of 1.0 µg/mL NDMA at pH 9.

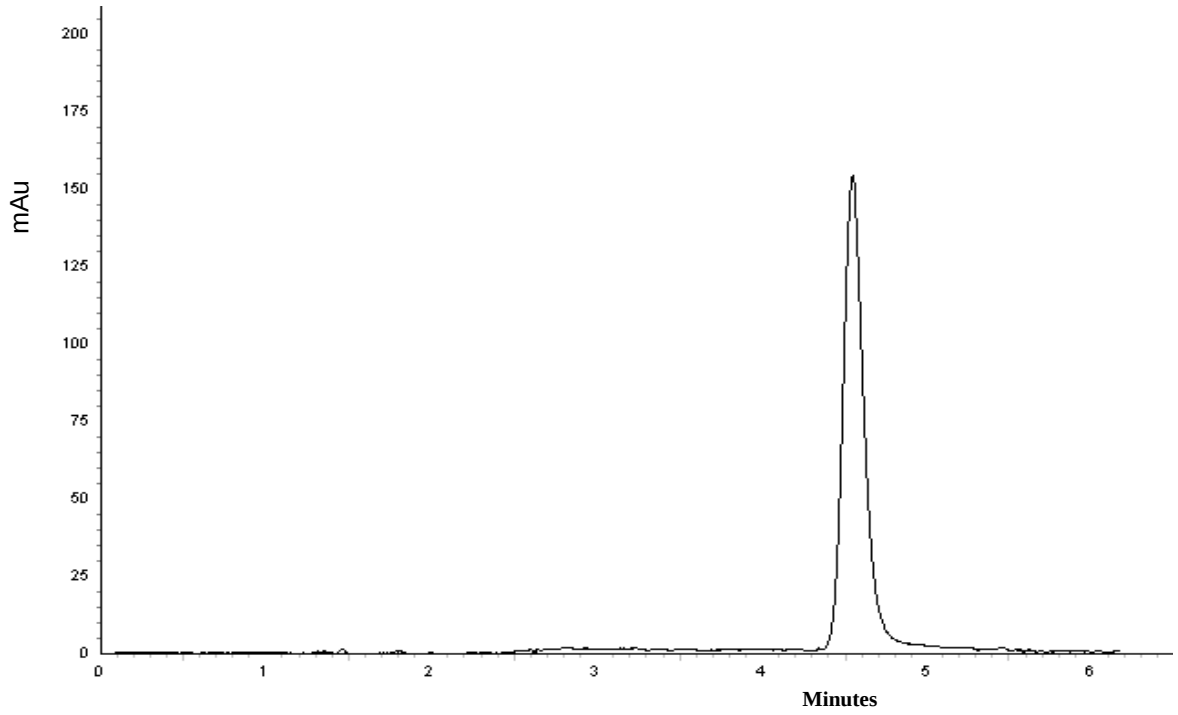

Figure 4.38. Chromatogram of 10 µg/mL NDMA at pH 9.

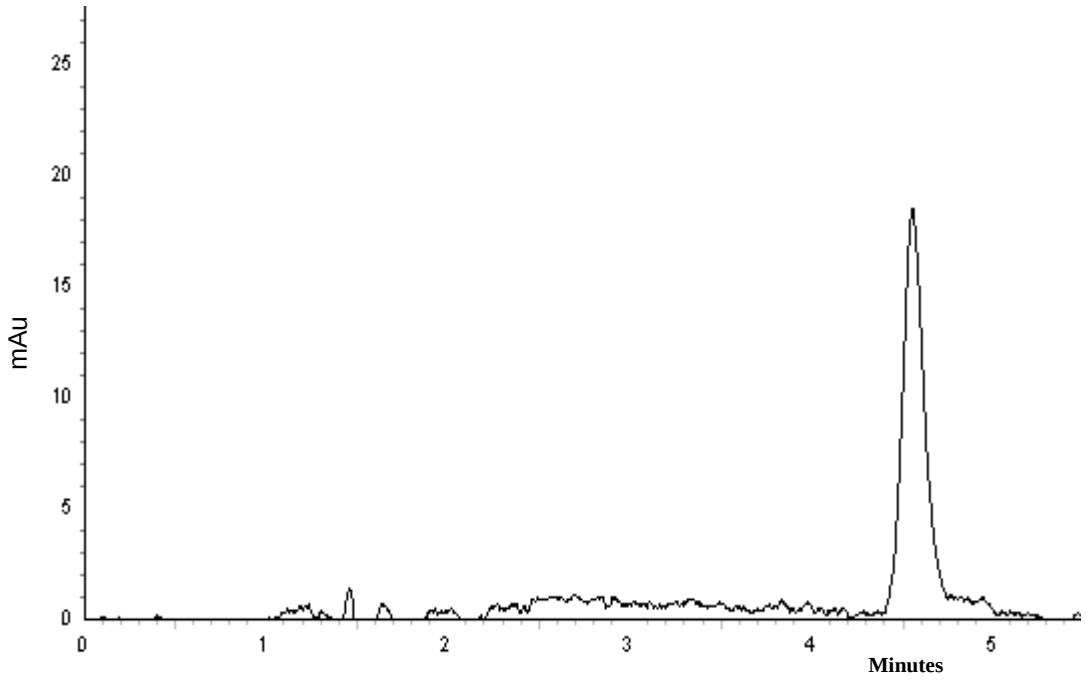

Figure 4.39. Chromatogram of 1.0 µg/mL NDMA at pH 11.

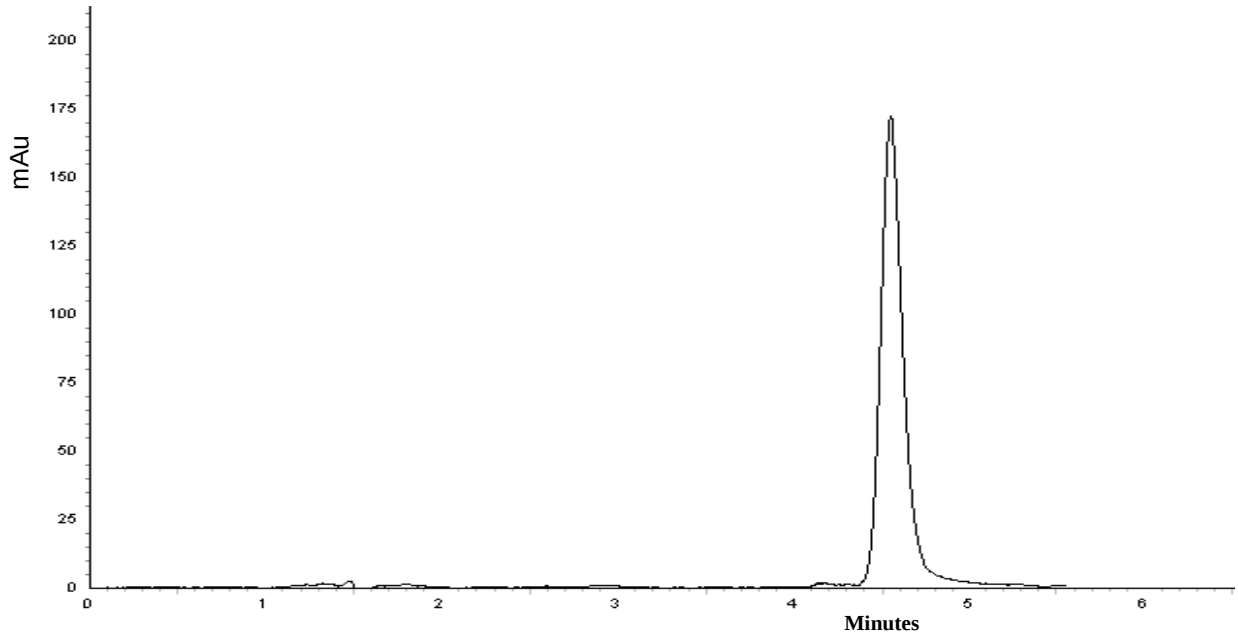

Figure 4.40. Chromatogram of 10 µg/mL NDMA at pH 11.

Environmental and Drinking Water

Well (pH 7.3, Municipal Airport Well Field), river (pH 8.1, Huron River), and drinking water (pH 9.3, from the plant's reservoir) were obtained from sample taps at the Ann Arbor Water Treatment facility. The water samples were spiked with NDMA (1- and 10 μg/mL NDMA) and evaluated using MP3 and MP4 (Tables XIX and XX). Unspiked samples were also evaluated; NDMA was not detected in these samples. Water sample characteristics did not affect peak symmetry and retention (Figures 4.41-4.52). As prescribed in the EPA method, sodium thiosulfate was added to each sample to inhibit further NDMA formations. When compared to NDMA, this compound was highly absorbed with both mobile phase systems. The addition of $Na_2S_2O_3$ to water samples did not impair the detection of NDMA. The method is suitable for quantifying NDMA in environmental and drinking water.

TABLE XIX: Effect of Environmental Water Characteristics on NDMA Analysis using 1.0 μg/mL NDMA in the Mobile Phase

pH	Mobile Phase	t_r	Peak Area	Peak Height
Reservoir	MP3	4.907	222562	22678
	MP4	3.141	168099	22749
Well	MP3	4.923	174251	16762
	MP4	3.136	181664	25342
River	MP3	4.949	202992	18497
	MP4	3.152	175365	21766

TABLE XX: Effect of Environmental Water Characteristics on NDMA Analysis using 10 μg/mL NDMA in the Mobile Phase

pH	Mobile Phase	t_r	Peak Area	Peak Height
Reservoir	MP3	4.880	1782635	185672
	MP4	3.147	1548354	234726
Well	MP3	4.891	1521212	160498
	MP4	3.141	1594612	250362
River	MP3	4.955	1718006	176005
	MP4	3.141	1431660	224466

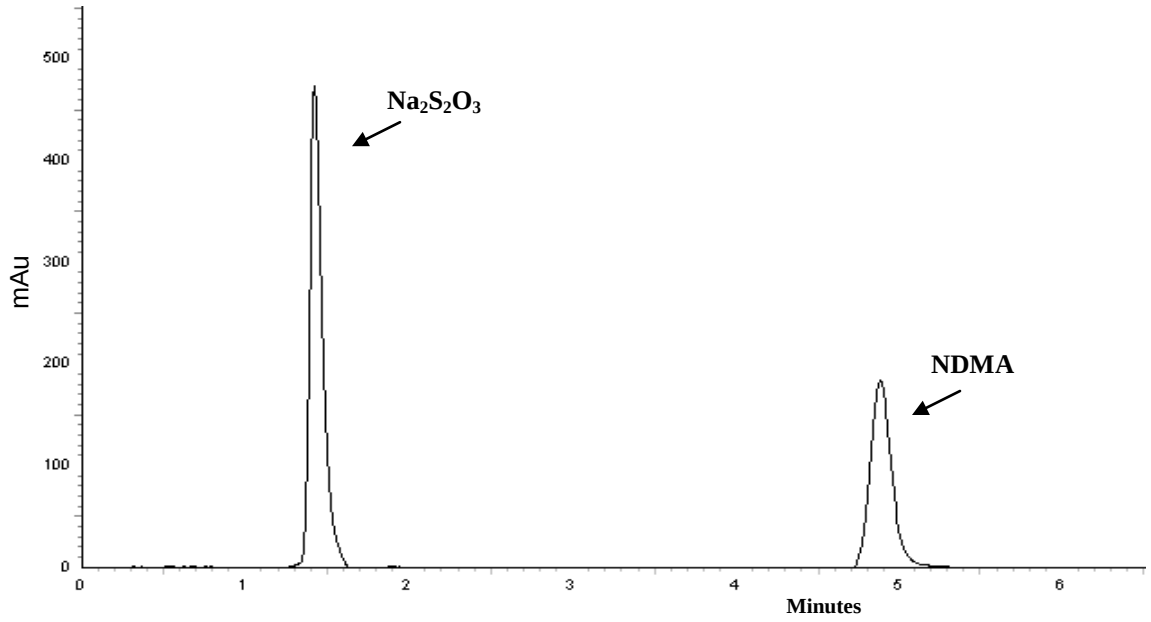

Figure 4.41. Reservoir Sample Spiked with 10 µg/mL NDMA using MP3.

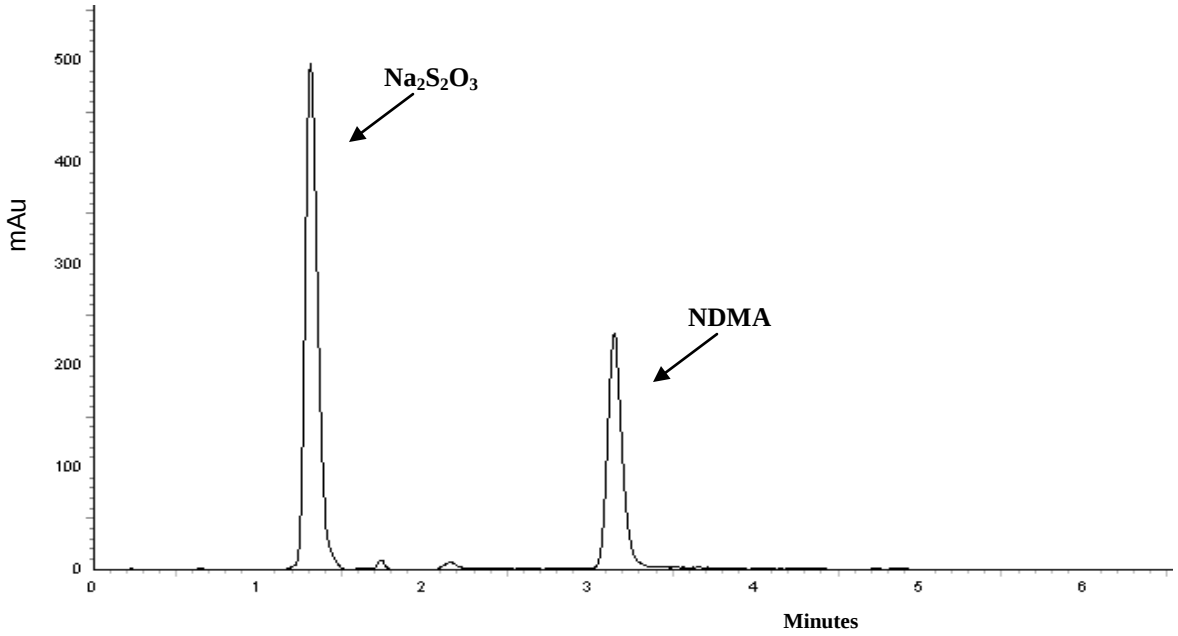

Figure 4.42. Reservoir Sample Spiked with 10 µg/mL NDMA using MP4.

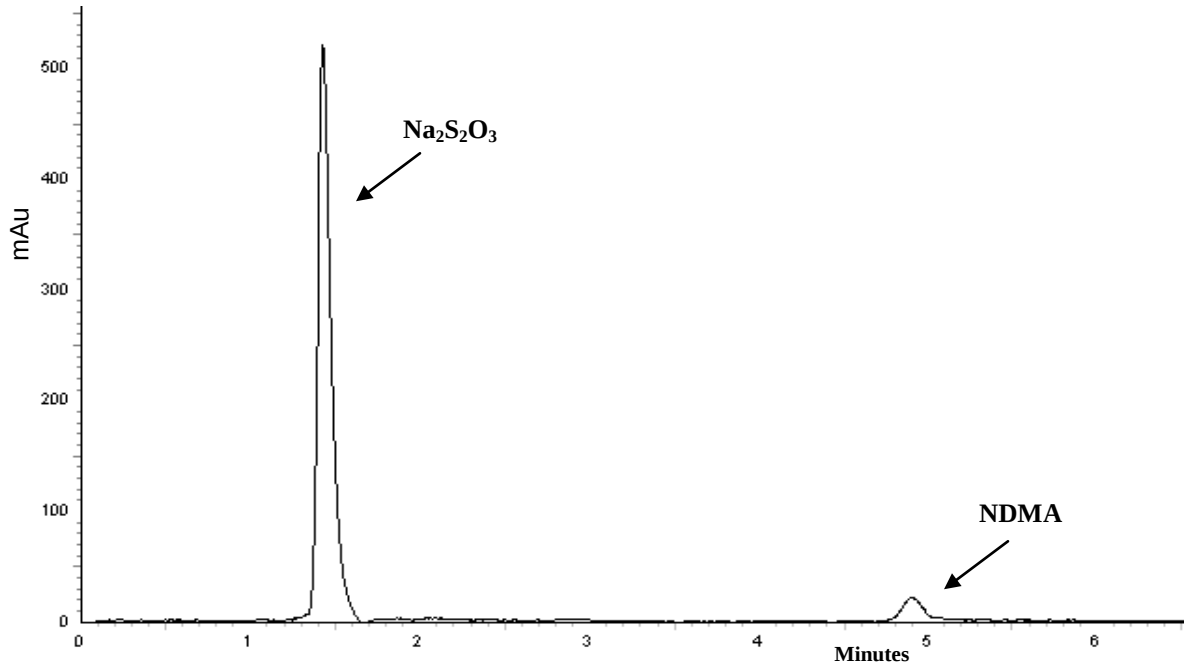

Figure 4.43. Reservoir Sample Spiked with 1.0 µg/mL NDMA using MP3.

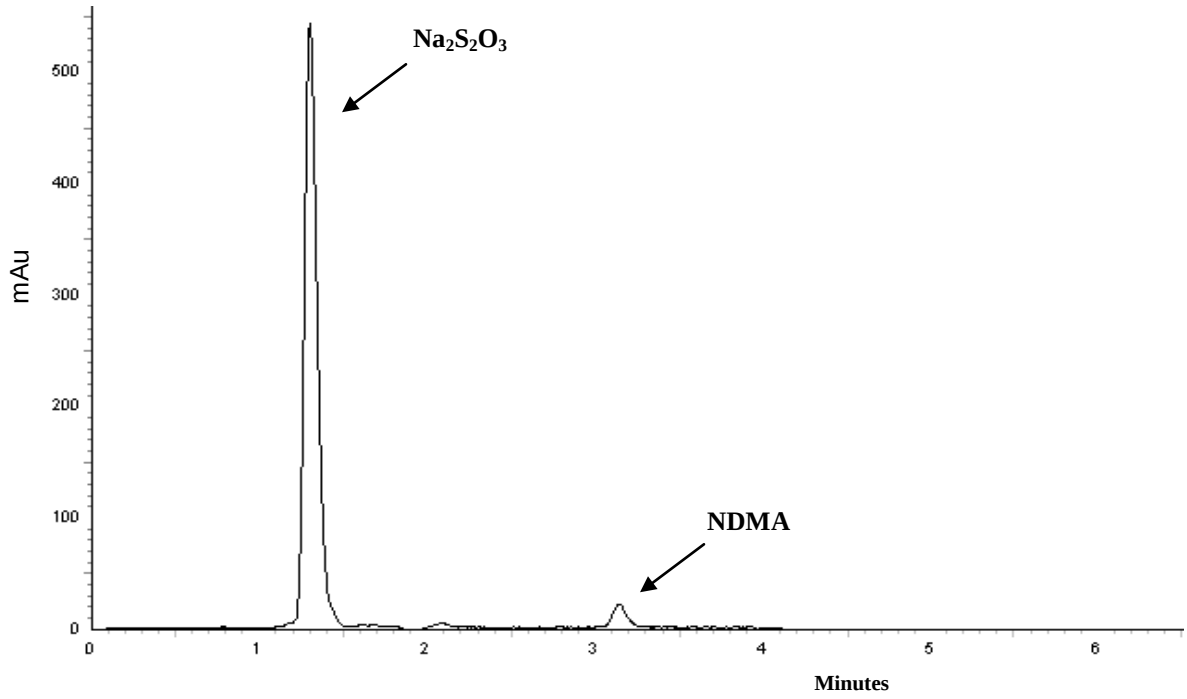

Figure 4.44. Reservoir Sample Spiked with 1.0 µg/mL NDMA using MP4.

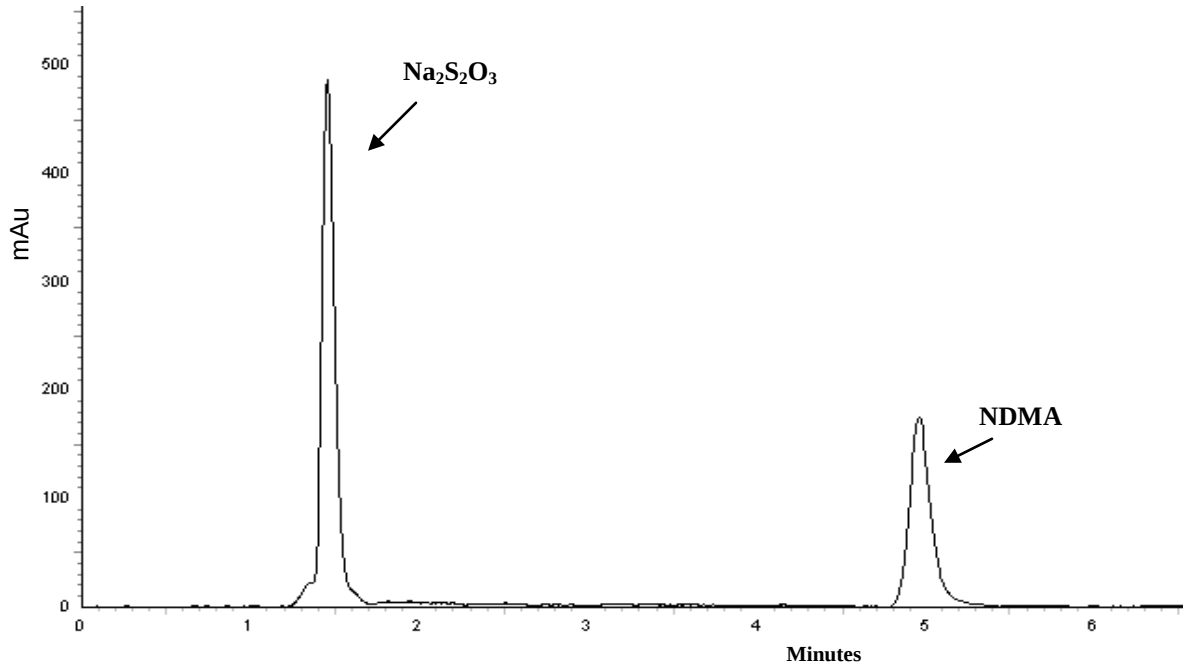

Figure 4.45. River Sample Spiked with 10 µg/mL NDMA using MP3.

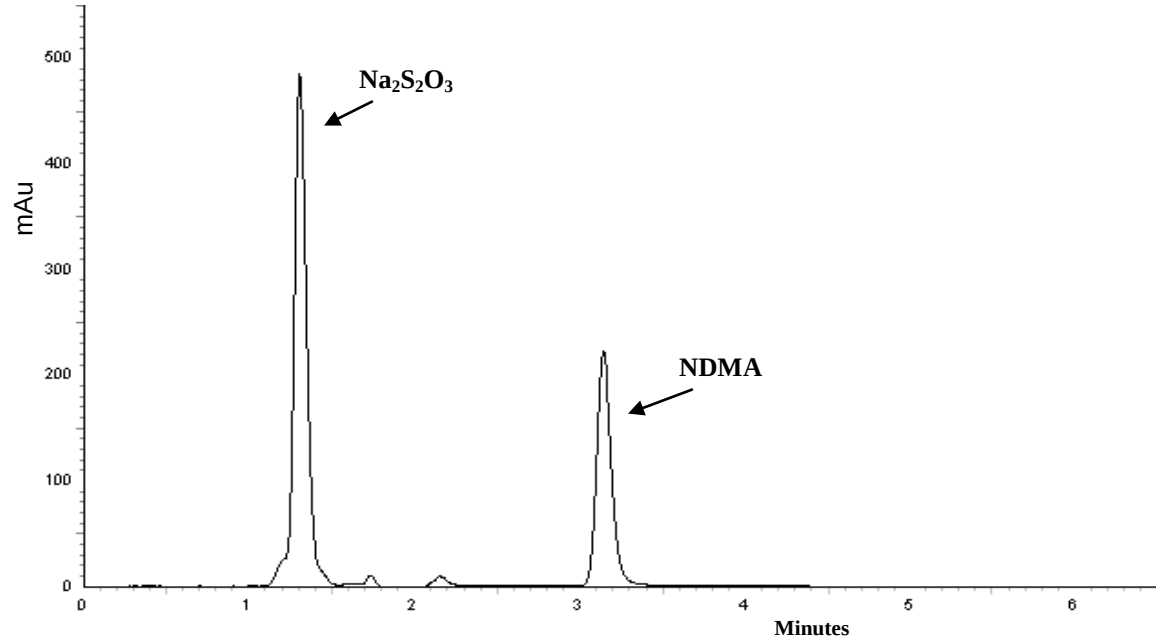

Figure 4.46. River Sample Spiked with 10 µg/mL NDMA using MP4.

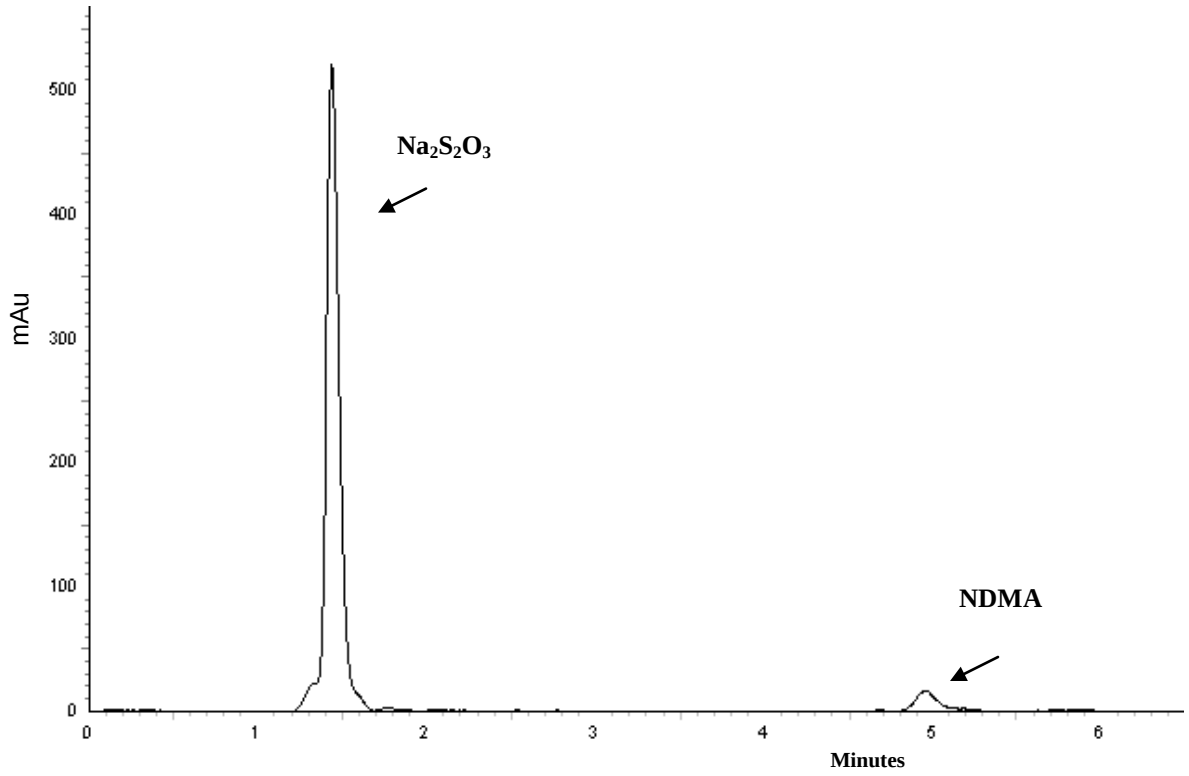

Figure 4.47. River Sample Spiked with 1.0 µg/mL NDMA using MP3.

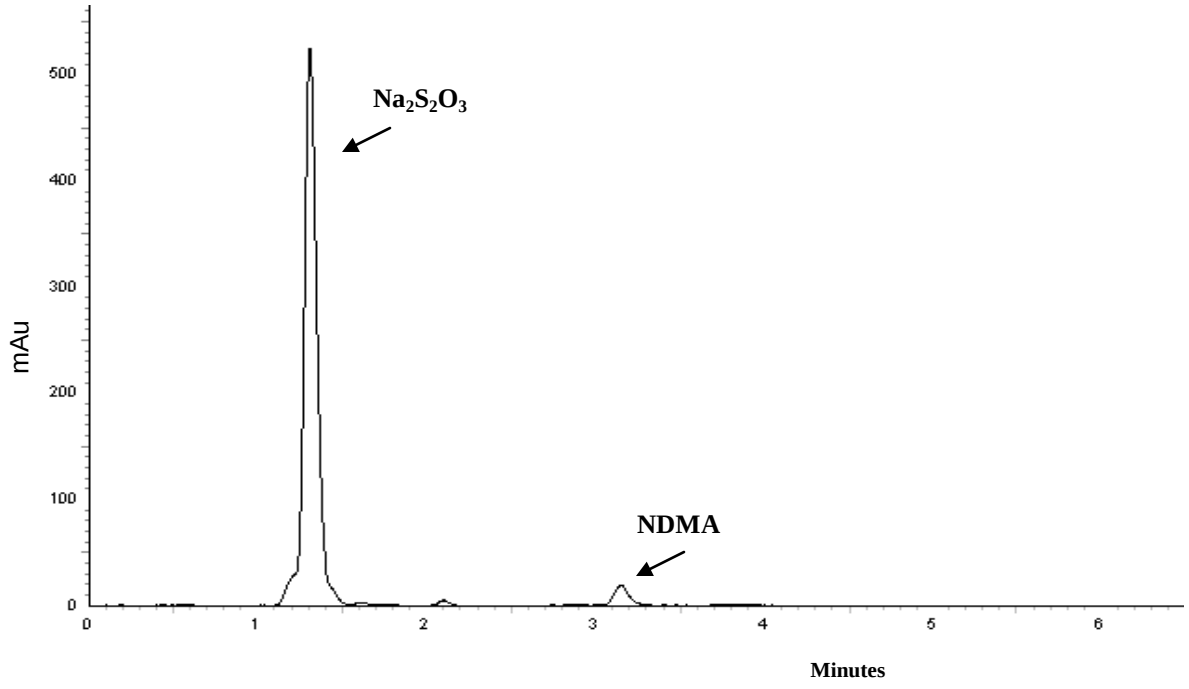

Figure 4.48. River Sample Spiked with 1.0 µg/mL NDMA using MP4.

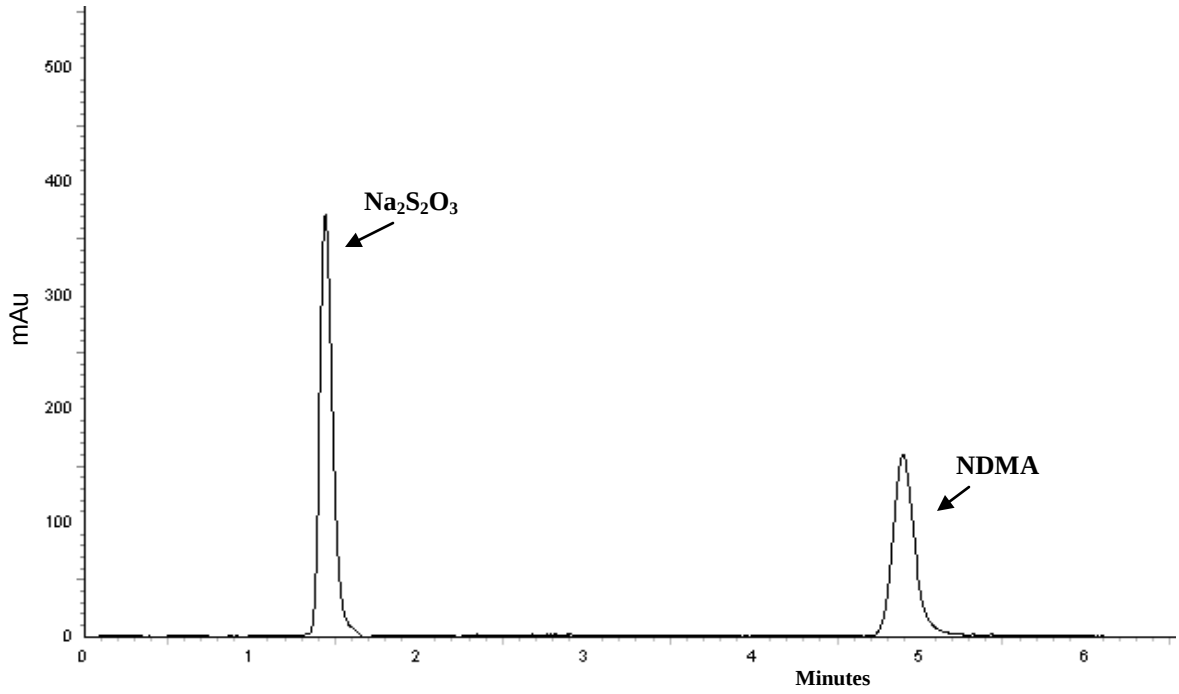

Figure 4.49. Well Sample Spiked with 10 µg/mL NDMA using MP3.

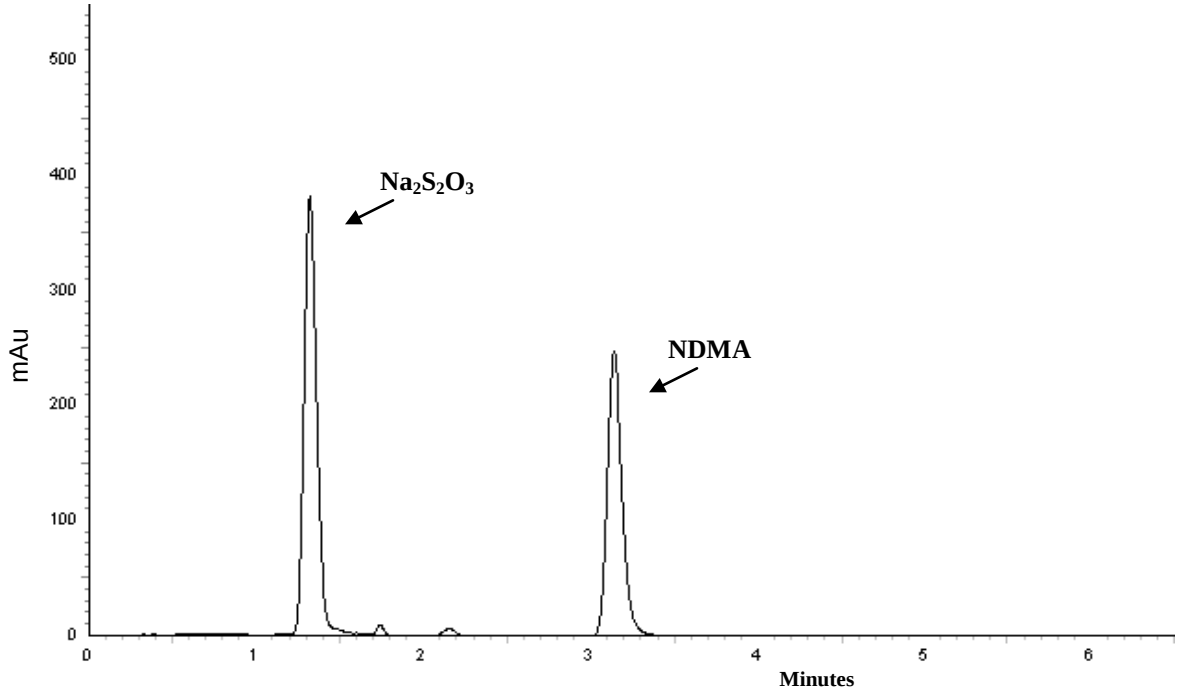

Figure 4.50. Well Sample Spiked with 10 µg/mL NDMA using MP4.

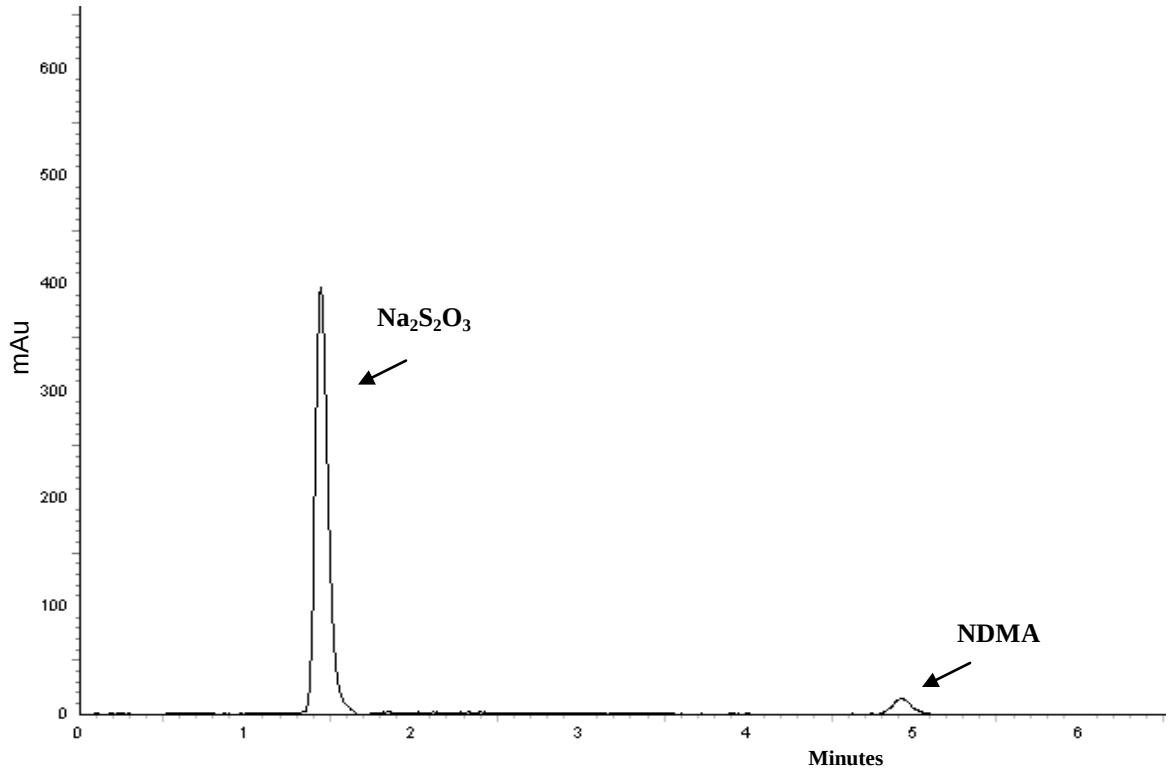

Figure 4.51. Well Sample Spiked with 1.0 µg/mL NDMA using MP3.

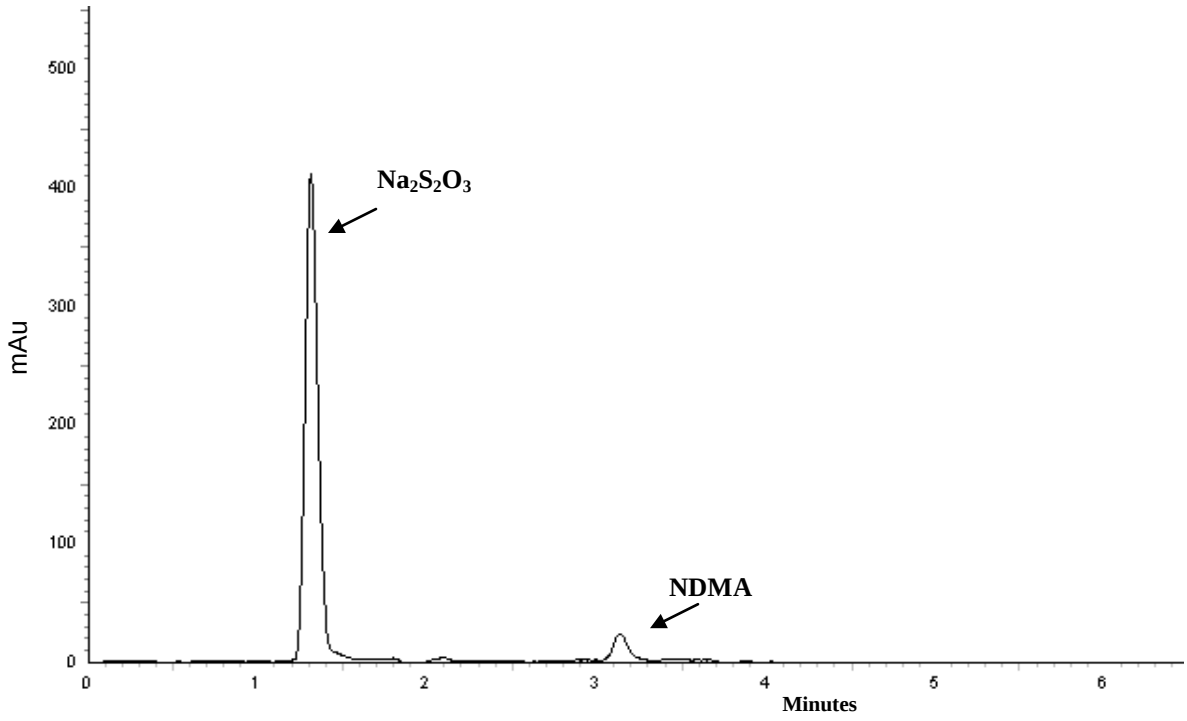

Figure 4.52. Well Sample Spiked with 1.0 µg/mL NDMA using MP4.

4.5. Statistical Analysis

Linearity

Linearity was measured as an aspect of method validation. Using the Zorbax-SB (5 μm) column with MP3 and MP4, calibration curves were created using 0.5, 1, 2, 5, 10, and 20 μg/ml NDMA. Peak area was plotted against concentration (*Table XXI*). A linear fit of the data was established; regression analysis assigned r^2 values of 0.9994 and 0.9999 for MP3 and MP4, respectively (Figures 4.53 and 4.54). The accuracy of the calibration curve was confirmed by evaluating peak areas for duplicate injections of 1- and 10 μg/ml NDMA. The data convey that the method is accurate and acceptable for analyzing highly aqueous samples containing between 0.5 and 20 ug/mL NDMA.

TABLE XXI: NDMA Calibration Curve

Concentration (μg/mL)	Peak Area MP3	Peak Area MP4
0.5	78768	71404
1.0	148395	135662
2.0	276236	282819
5.0	697184	717328
10	1426926	1480862
20	2980683	2945307

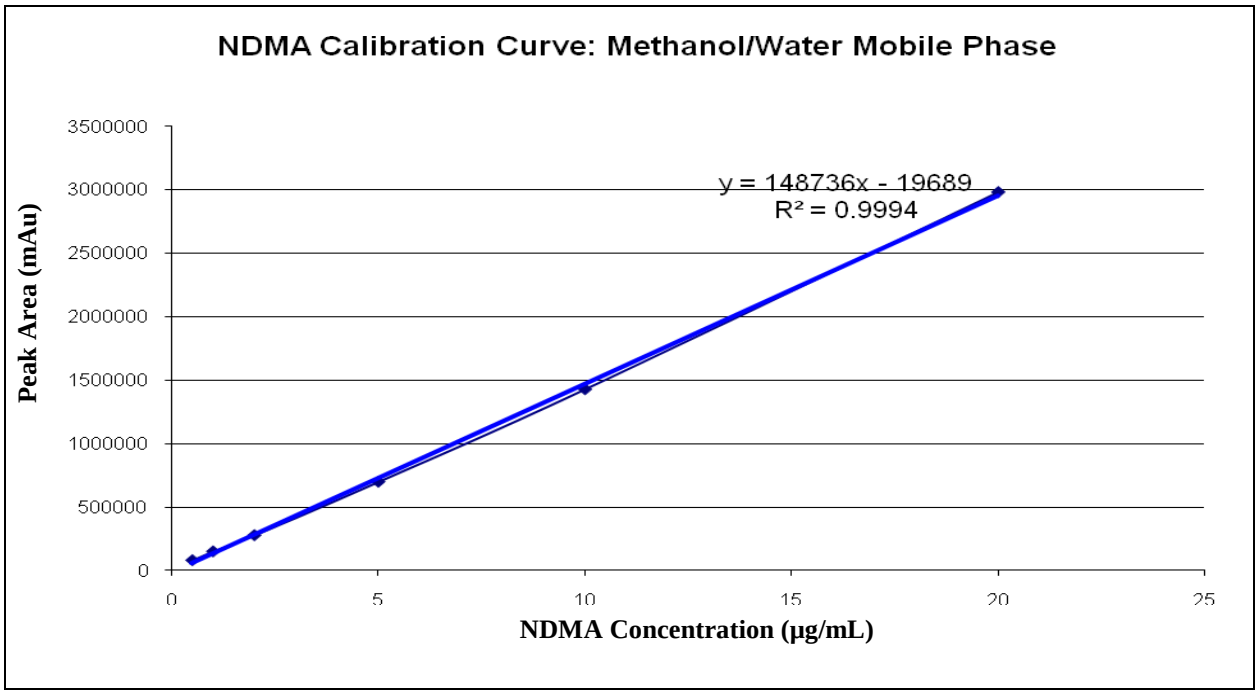

Figure 4.53. NDMA calibration curve using MP3.

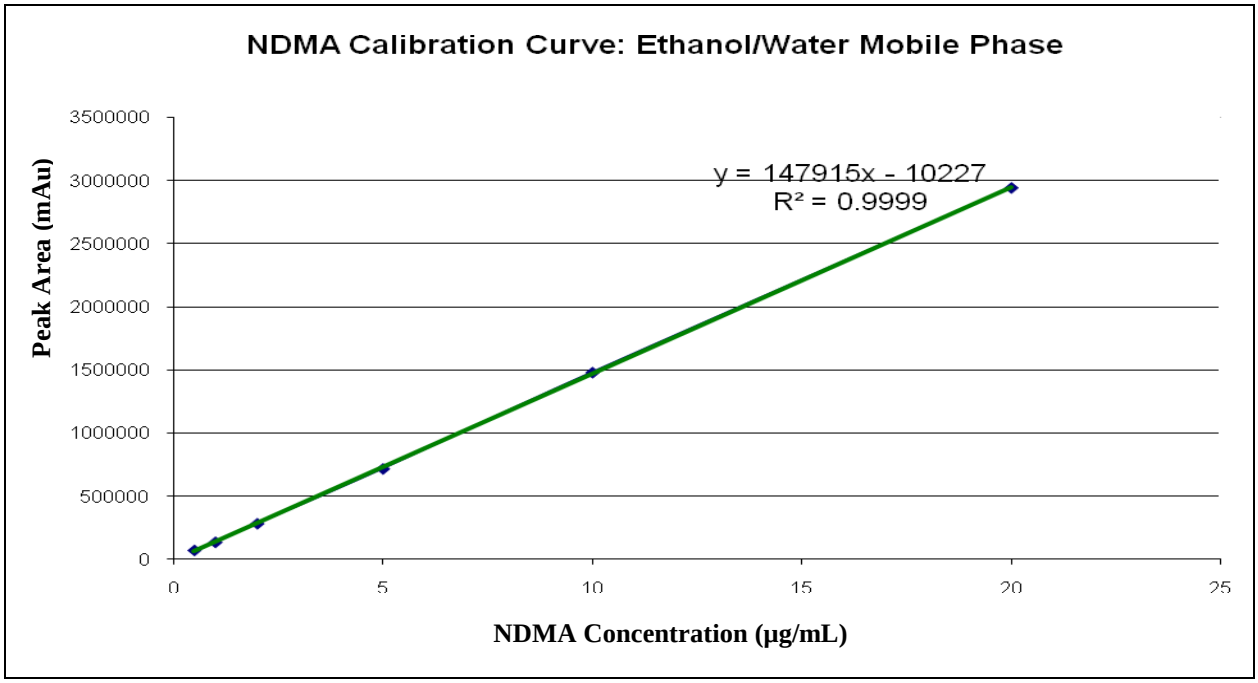

Figure 4.54. NDMA calibration curve using MP4.

LOD and LOQ

Method validation required determining the LOD and LOQ. The Zorbax-SB 5-μm column was used with MP3 and MP4, respectively. Ten replicate injections were made using 1.0 μg/ml NDMA. The standard deviation (σ) was calculated based on the peak area of the ten injections (*Tables XXII and XXIII*). The LOD was calculated as $3\sigma/m$; the LOQ was calculated as $10\sigma/m$, where m is the slope (sensitivity) of the calibration curve.

The LOD for NDMA with MP3 and MP4 was 1.03 and 0.29 μg/mL, respectively. The LOQ for NDMA with MP3 and MP4 was 3.43 and 0.96 μg/mL, respectively. These values may be improved with the use of a column with smaller particle diameter.

TABLE XXII: Replicate Injection Analysis of 1.0 μg/ml NDMA in the Mobile Phase using the Zorbax-SB 5-μm Column and MP3.

Injection	t_r	Peak Area (mAu)
1	4.89	257685
2	4.87	305052
3	4.89	203383
4	4.90	216722
5	4.91	142596
6	4.91	188276
7	4.91	162789
8	4.91	154502
9	4.89	189166
10	4.90	158020

Using MP3, the standard deviation for the Zorbax-SB 5-μm column was 50961.95. This value was based on the peak area of all ten injections.

TABLE XXIII: Replicate Injection Analysis of 1.0 μg/ml NDMA in the Mobile Phase using the Zorbax-SB 5-μm column and MP4.

Injection	t_r	Peak Area (mAu)
1	3.14	162298
2	3.14	165850
3	3.15	161133
4	3.14	164260
5	3.14	157476
6	3.14	145158
7	3.14	195077
8	3.14	143951
9	3.13	160510
10	3.14	152721

Using MP4, the standard deviation for the Zorbax-SB 5-μm column was 14219.26 . This value was based on the peak areas of all ten injections.

4.6. Solid Phase Extraction and Preconcentration of NDMA

Purified water samples (250 mL) were fortified with NDMA at 10 μg/L. Coconut shell charcoal (CSC) extraction cartridges were attached to the vacuum manifold and conditioned with combinations of acetonitrile, methanol or ethanol, and purified water. Aliquots of methanol or ethanol and acetone were used for column elution. Acetonitrile was applied to a select cartridge (Vial 6). A positive control sample was prepared (1-mL aliquot of methanol spiked with 10 μg/mL NDMA). The peak area of the control sample was compared to that of the extracted samples for evaluating the recovery of NDMA. A quantitative sample analysis was performed using MP3; a qualitative comparison was made using MP4 (See *Tables XXIV and XXV*).

TABLE XXIV: Solid Phase Extraction Analysis using MP3.

Vial	Mobile Phase	Retention Time	Peak Area	% Recovery (based on peak area)
1	MP3	4.592	211671	14.12
2	MP3	4.443	141645	9.45
3	MP3	4.544	195704	13.05
4	MP3	4.357	140351	9.36
5	MP3	4.571	156223	10.42
6	MP3	4.523	198114	13.22

TABLE XXV: Solid Phase Extraction Analysis using MP4.

Vial	Mobile Phase	Retention Time	Peak Area
1	MP4	3.179	172364
2	MP4	3.088	169713
3	MP4	3.163	157100
4	MP4	3.056	10309
5	MP4	3.152	181454
6	MP4	3.152	241794

Recovery of NDMA was maximized using acetonitrile and methanol for cartridge conditioning and elution with methanol and acetone (Vial **1**, 14.12% recovery using MP3) (Figures 4.55 and 4.56). Peak symmetry improved when MP4 was used. Reduced tailing and a decrease in background noise were also observed. For Vial 2, ethanol was expected to improve recovery because of its greater eluent strength (ethanol is a less polar solvent than methanol). However, distortion of the NDMA peak was evident. Based upon peak characteristics, recovery of NDMA was not successful (Vial **2**, 9.45% recovery using MP3) (Figure 4.57). Though peak height and symmetry improved with MP4, an increase in background noise was observed (Figure 4.58). For Vial 3, the use of ethanol for cartridge conditioning and methanol for cartridge elution improved peak height and the resolution of methanol and NDMA (Vial **3**, 13.05% recovery using MP3) (Figures 4.59 and 4.60). Peak symmetry also improved when ethanol was not used as an eluent. Poor resolution of methanol and NDMA was the consequence of eluting the cartridge with ethanol (Vial **4**, 9.36% recovery using MP3) (Figures 4.61 and 4.62). A

notable decrease in background noise was observed when only methanol and water were used for cartridge conditioning (Vial **5**, 10.42% recovery using MP3). When compared to Vial 3 (which was partially conditioned with ethanol), the peak area was smaller but peak symmetry improved (Figures 4.63). Using MP4, the peak height for NDMA was greater and the peak width was reduced (Figure 4.64). The presence of acetonitrile for elution produced greater peak heights with both mobile phase systems (Vial **6**, 13.22% recovery using MP3) (Figures 4.55 and 4.56). The chromatograms reveal that peak symmetry was not significantly different from the analysis of Vial 1. Overall, the use of MP4 improves the resolution of methanol and NDMA. Greater absorbance of NDMA was also achieved with MP4.

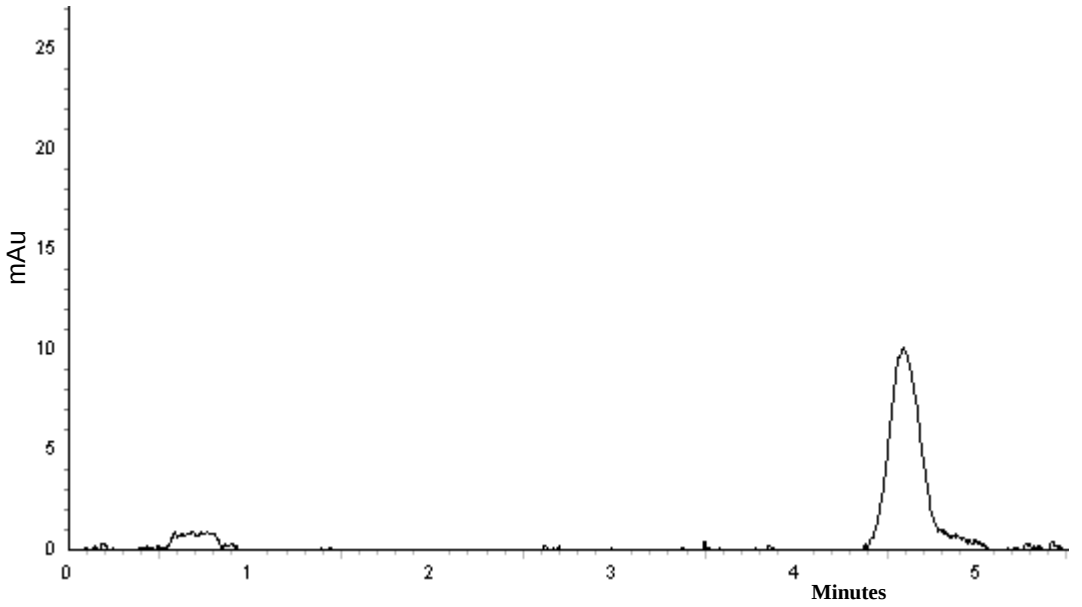

Figure 4.55. Chromatogram of Vial 1 using MP3. (Cartridge conditioned with acetonitrile, methanol and water. Cartridge eluted with methanol and acetone.)

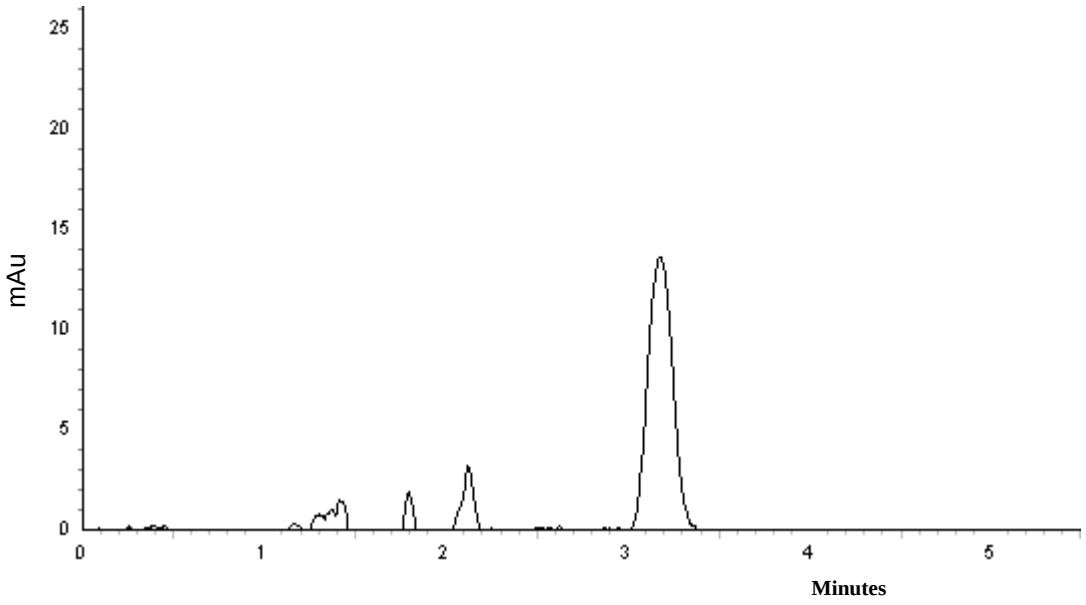

Figure 4.56. Chromatogram of Vial 1 using MP4. (Cartridge conditioned with acetonitrile, methanol, and water. Cartridge eluted with methanol and acetone.)

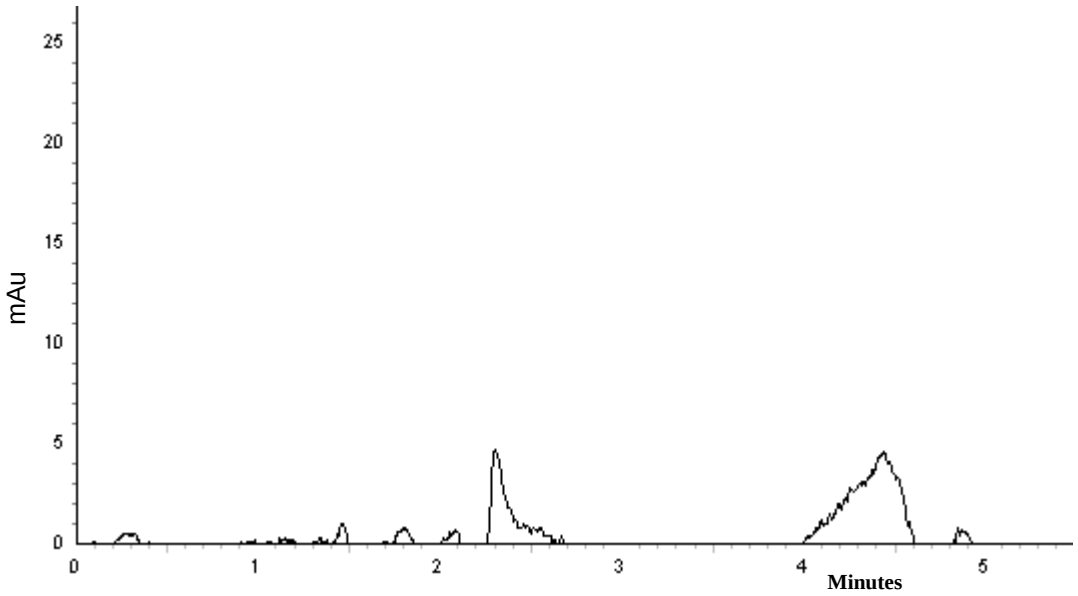

Figure 4.57. Chromatogram of Vial 2 using MP3. (Cartridge conditioned with acetonitrile, methanol, and water. Cartridge eluted with ethanol and acetone.)

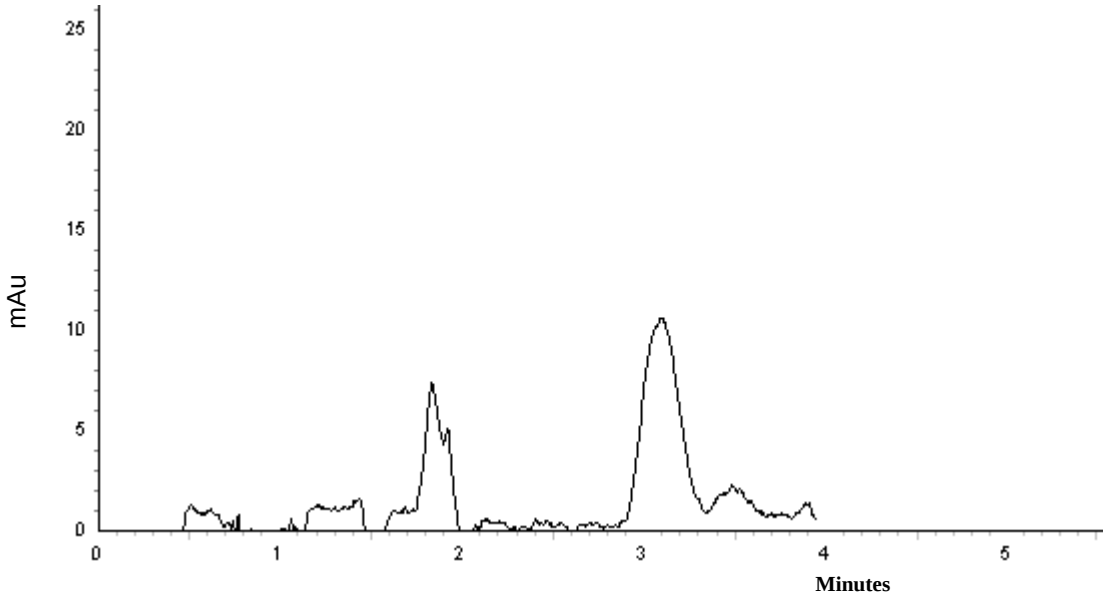

Figure 4.58. Chromatogram of Vial 2 using MP4. (Cartridge conditioned with acetonitrile methanol, and water. Cartridge eluted with ethanol and acetone.)

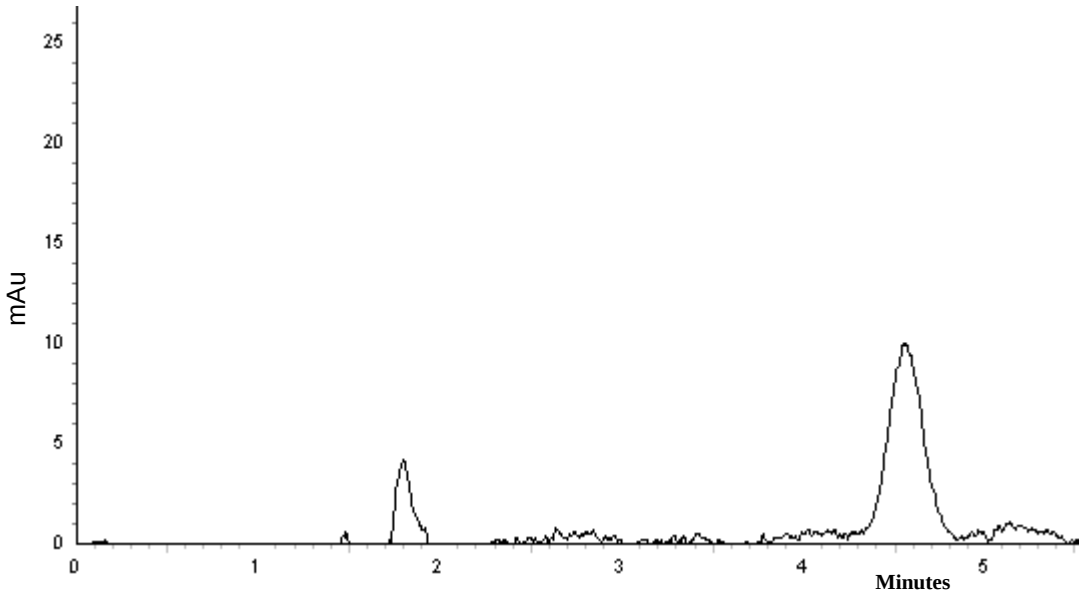

Figure 4.59. Chromatogram of Vial 3 using MP3. (Cartridge conditioned with acetonitrile ethanol, and water. Cartridge eluted with methanol and acetone.)

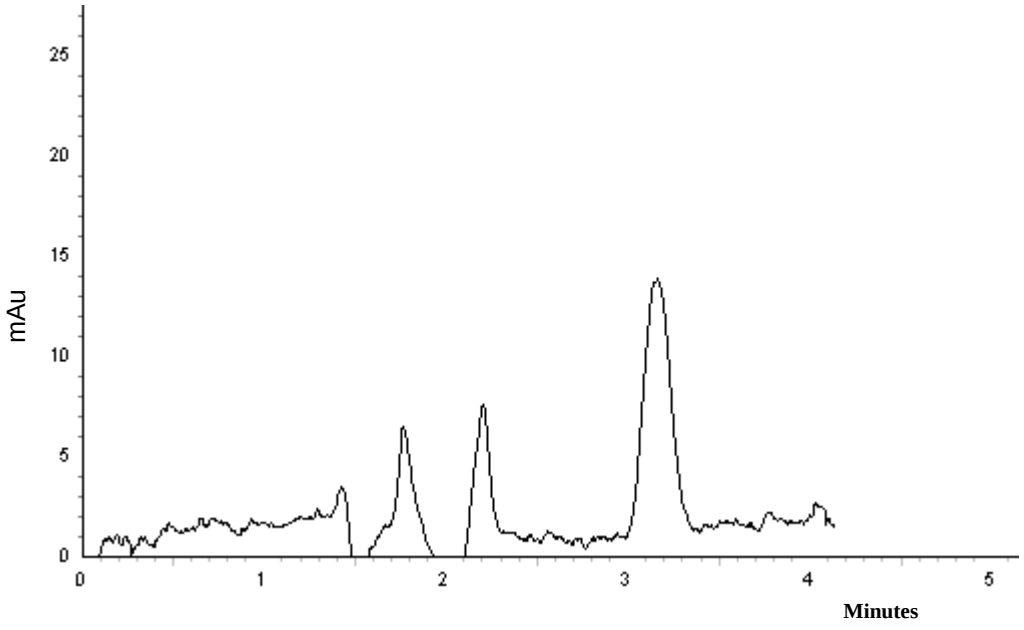

Figure 4.60. Chromatogram of Vial 3 using MP4. (Cartridge conditioned with acetonitrile ethanol, and water. Cartridge eluted with methanol and acetone.)

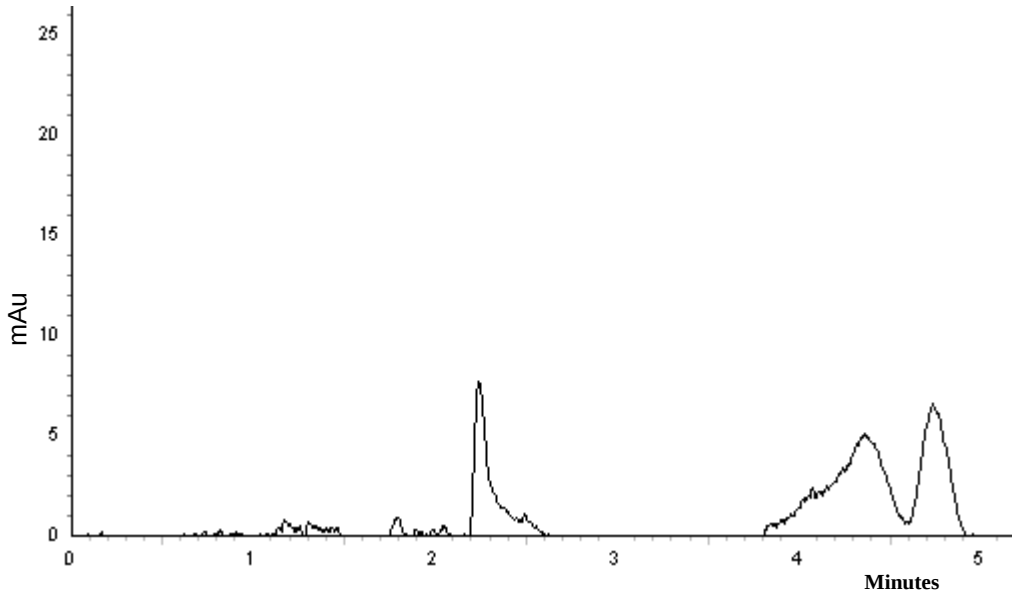

Figure 4.61. Chromatogram of Vial 4 using MP3. (Cartridge conditioned with acetonitrile ethanol, and water. Cartridge eluted with ethanol and acetone.)

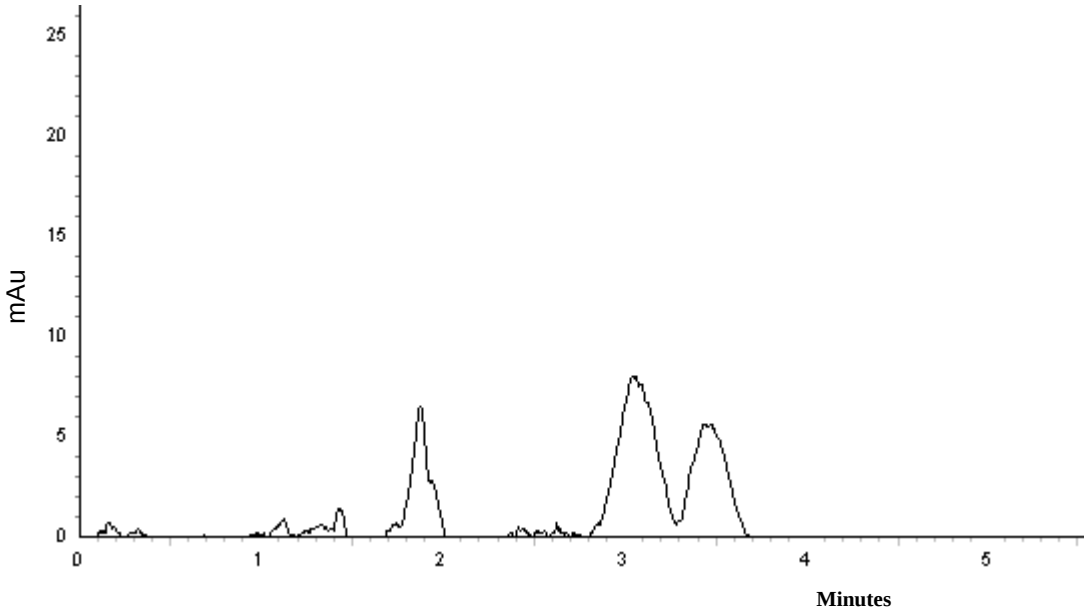

Figure 4.62. Chromatogram of Vial 4 using MP4. (Cartridge conditioned with acetonitrile ethanol, and water. Cartridge eluted with ethanol and acetone.)

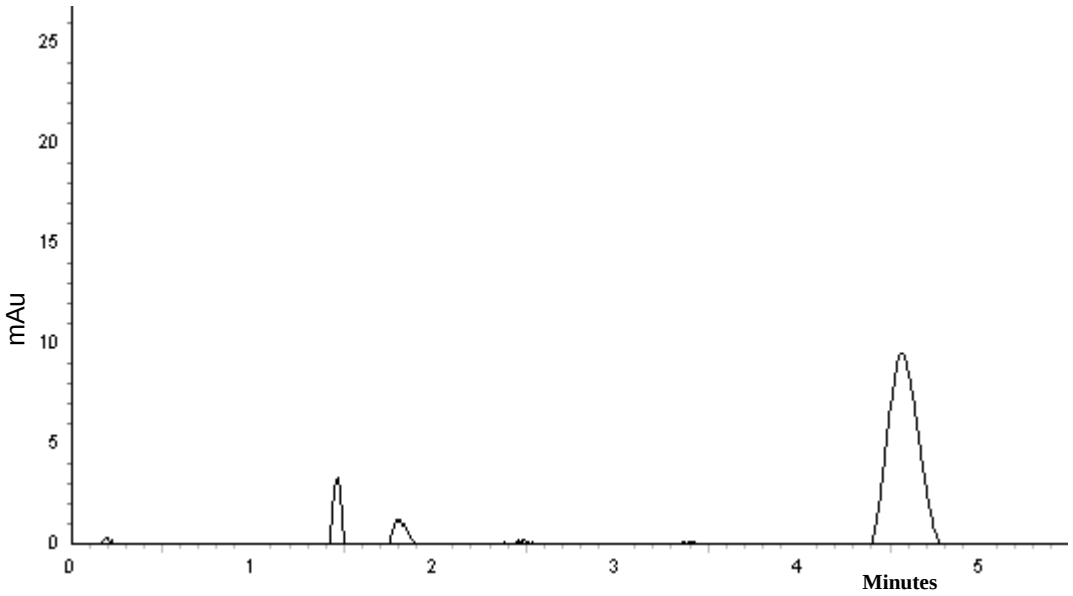

Figure 4.63. Chromatogram of Vial 5 using MP3. (Cartridge conditioned with methanol and water. Cartridge eluted with methanol and acetone.)

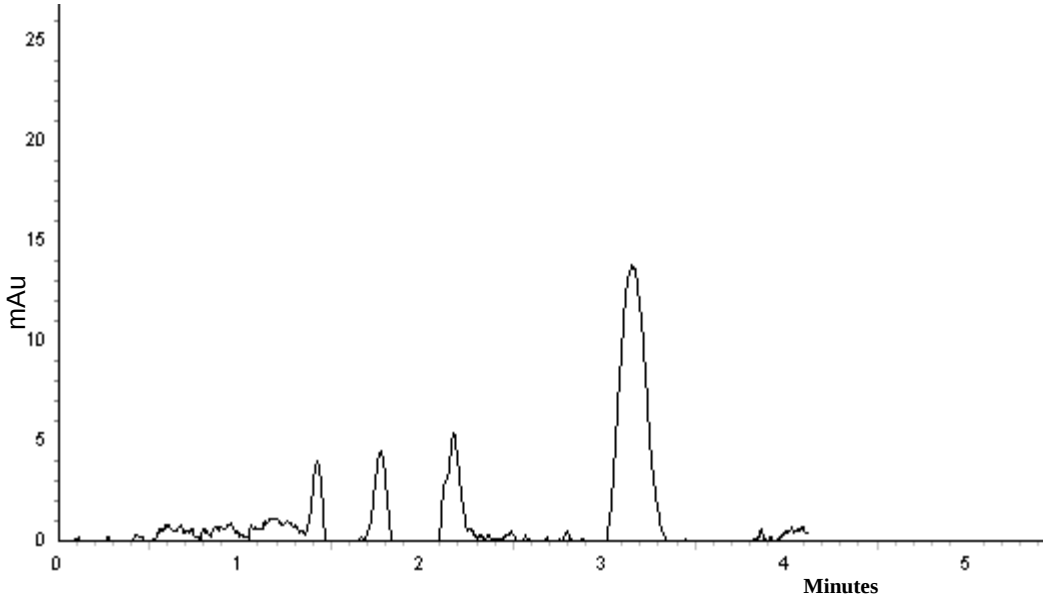

Figure 4.64. Chromatogram of Vial 5 using MP4. (Cartridge conditioned with methanol and water. Cartridge eluted with methanol and acetone.)

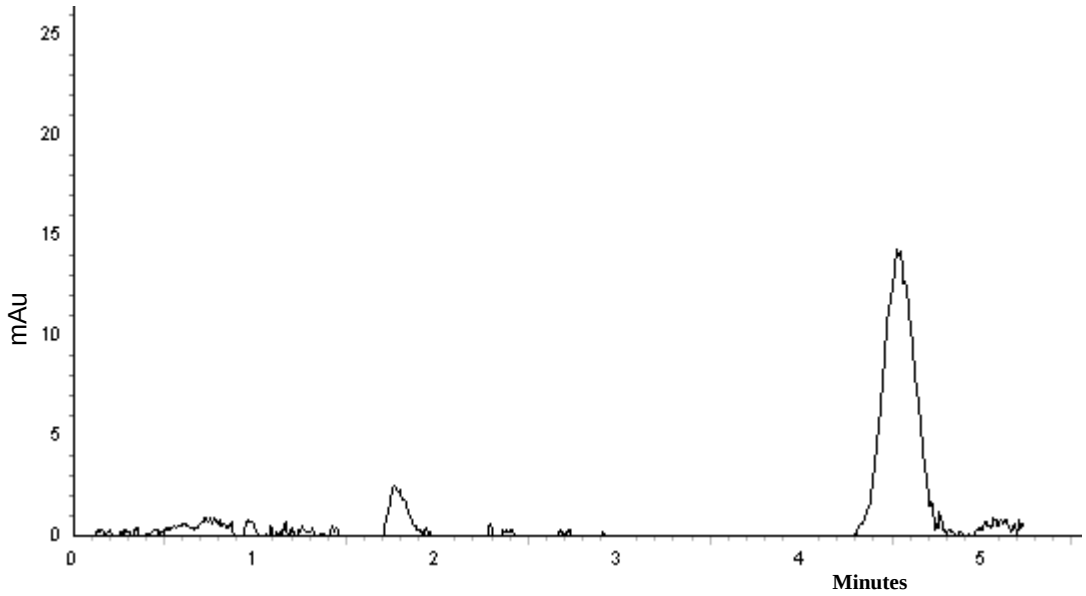

Figure 4.65. Chromatogram of Vial 6 using MP3. (Cartridge conditioned with acetonitrile methanol, and water. Cartridge eluted with acetonitrile, methanol and acetone.)

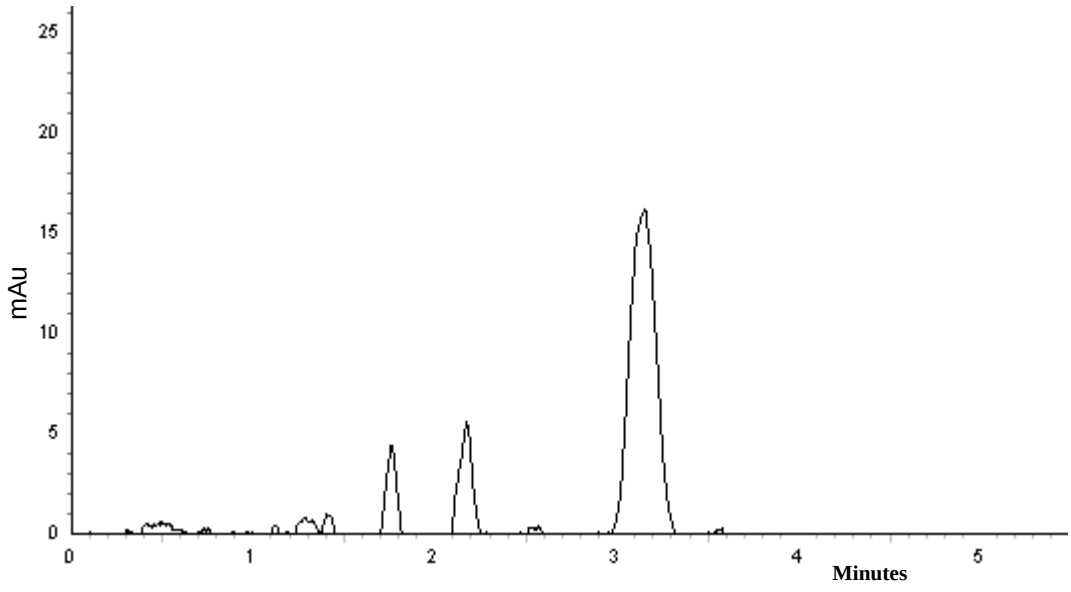

Figure 4.66. Chromatogram of Vial 6 using MP4. (Cartridge conditioned with acetonitrile methanol, and water. Cartridge eluted with acetonitrile, methanol, and acetone.)

Recoveries greater than 75% have been achieved with acetonitrile, methanol, and acetone, using HPLC with tandem mass spectrometry.[39] The poor recovery of NDMA in this study (less than 15%) could be attributed to the following:

(i) NDMA did not adsorb onto the column. The analyte may have passed through to the cartridge and into the waste stream during the extraction process. Cartridge conditioning and sample pretreatment should be considered in future investigations.

(ii) NDMA strongly adsorbed onto the column. A stronger eluent may be required for cartridge elution. Longer contact time between the eluent and SPE cartridge may be required to break attractive forces between NDMA and the conditioned coconut shell charcoal. Again, cartridge conditioning and sample pretreatment should be considered in future investigations.

(iii) A 250 mL sample was used for this study. Though it would lengthen the extraction process, a 500 mL sample may improve recovery.[38,39]

4.7. Column Evaluation

In RP-HPLC applications, it is common to perform method development using a 5-μm ODS column.[27] In this investigation, three different ODS columns were used to assess selectivity. The C_{18} columns were evaluated using 10 μg/mL NDMA with MP3 (Table XXVI) and MP4 (Table XXVII); a qualitative analysis (peak symmetry and background noise) was performed using 1.0 μg/mL NDMA. The Zorbax-SB (5 μm) column had been used in the academic analytical laboratory. The degree of wear for the Zorbax-SB (3.5 μm) and Eclipse XDB (3.5 μm) are unknown. The ability to reproduce results with a used column is one demonstration of method ruggedness.

TABLE XXVI: Influence of Column Selection on Peak Characteristics using 10 ug/mL NDMA and MP3.

Column	Mobile Phase	t_r	Peak Area	Peak Height
Zorbax-SB (5 μm)	MP3	4.624	1753099	174366
Zorbax-SB (3.5μm)	MP3	4.203	1502167	172791
Eclipse XDB (3.5μm)	MP3	3.499	1627547	182997

TABLE XXVII: Influence of Column Selection on Peak Characteristics using 10 ug/mL NDMA and MP4.

Column	Mobile Phase	t_r	Peak Area	Peak Height
Zorbax-SB (5 μm)	MP4	3.141	1576116	224807
Zorbax-SB (3.5μm)	MP4	2.939	1495592	229787
Eclipse XDB (3.5μm)	MP4	3.653	2420825	155200

In comparison to the Zorbax columns (Figures 4.67 and 4.68), peak symmetry for NDMA was improved and elution times were shortened with the Eclipse XDB column (Figure 4.69) and MP3. This is a double-endcapped column that is designed to reduce the retention of basic analytes.[29] The greatest peak area (using 10 µg/mL NDMA) was observed with the Zorbax-SB (5 µm) column. Larger particle diameters can contribute to increased peak width. Chromatograms for 1.0 µg/mL NDMA reveal that background noise is greatly diminished when smaller particle diameters are used. Though the methanol peak was not detected, peak symmetry for NDMA was better with the Zorbax-SB (3.5 µm) (Figure 4.70). In comparison to the Zorbax-SB 5-µm column (Figure 4.71), the Eclipse XDB (3.5 µm) column produced a sharper peak for NDMA (Figure 4.72).

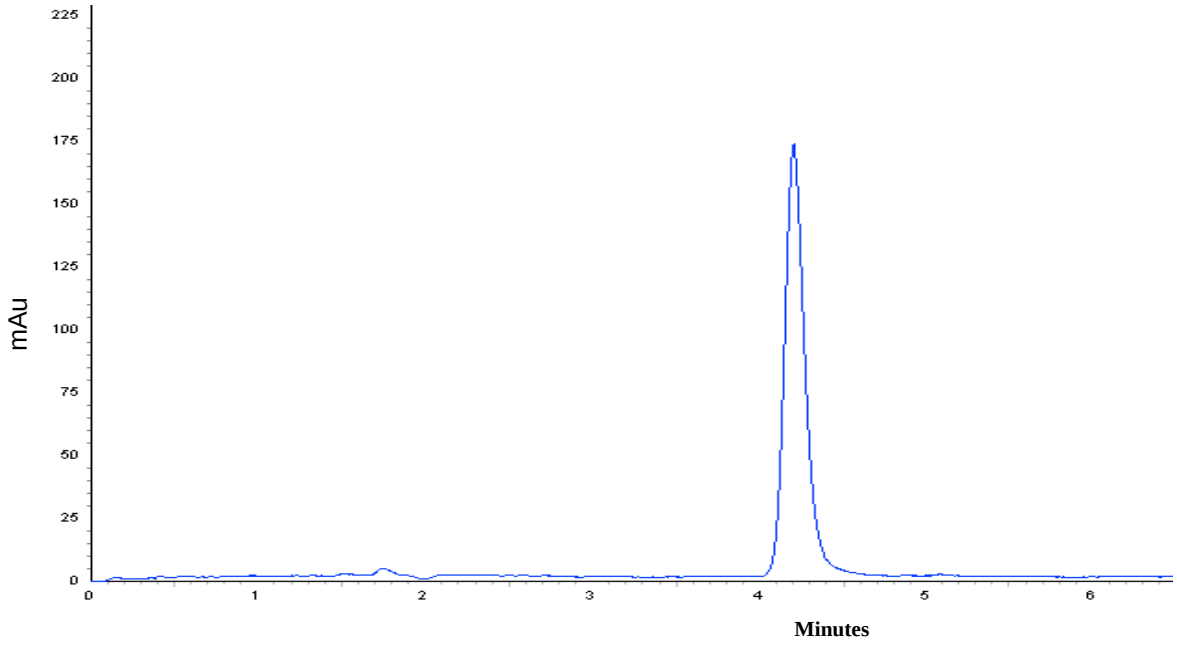

Figure 4.67. Analysis of 10 µg/mL NDMA using the Zorbax-SB (3.5 µm) and MP3.

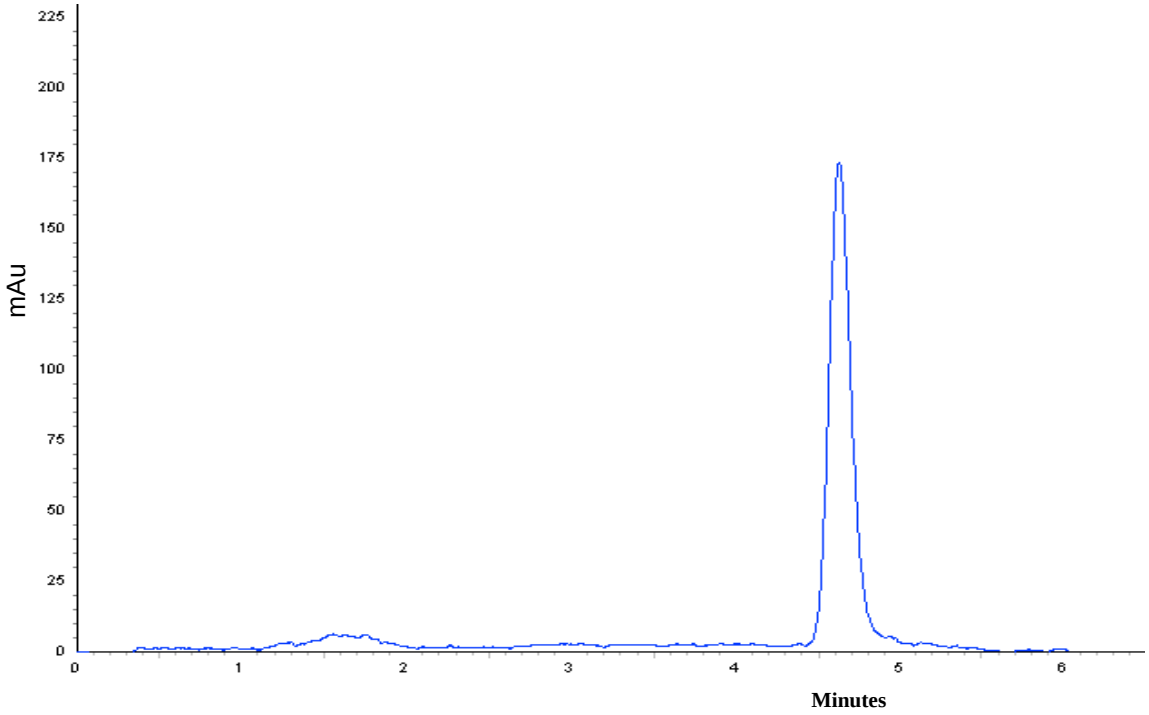

Figure 4.68. Analysis of 10 µg/mL NDMA using the Zorbax-SB (5 µm) and MP3.

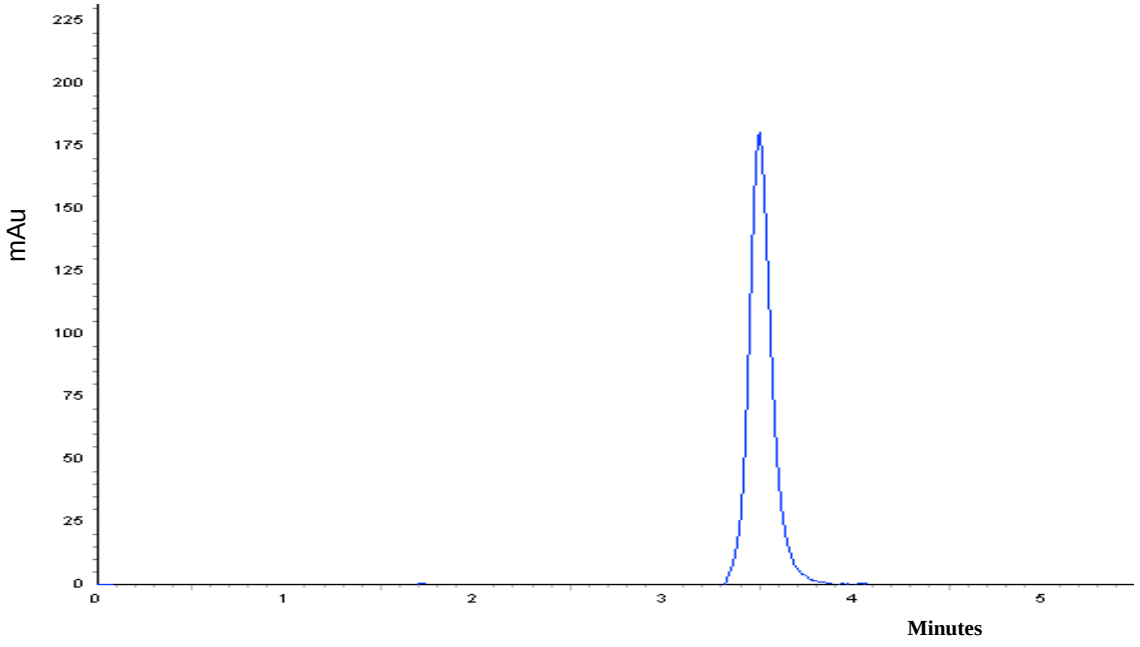

Figure 4.69. Analysis of 10 µg/mL NDMA using the Eclipse XDB (3.5 µm) and MP3.

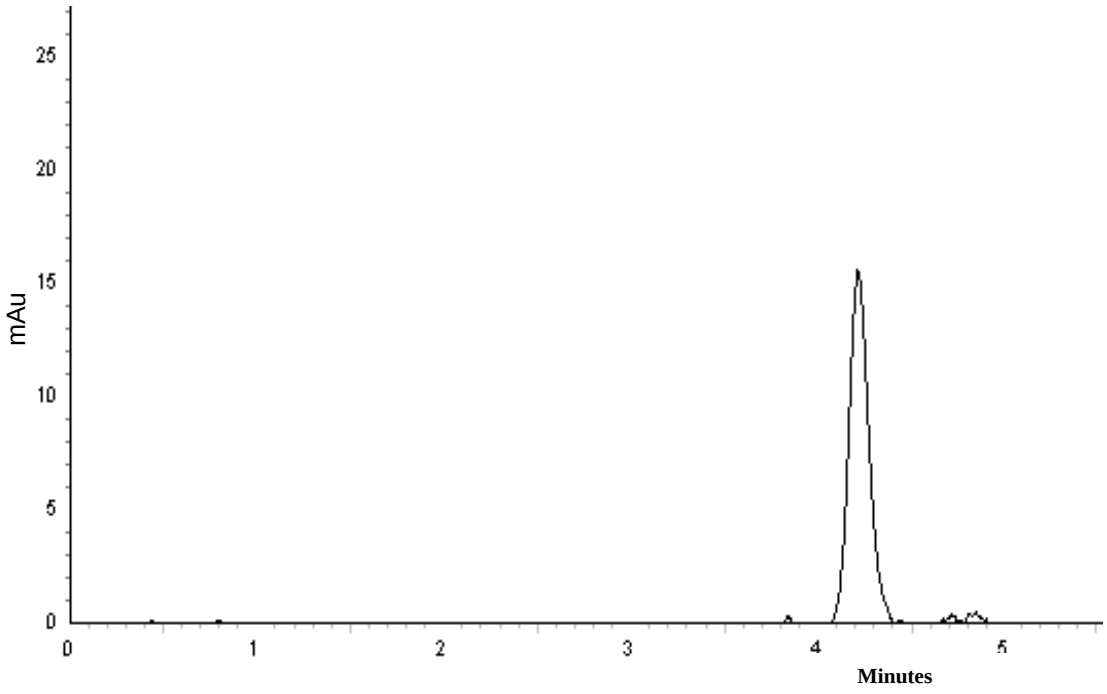

Figure 4.70. Analysis of 1.0 µg/mL NDMA using the Zorbax-SB (3.5 µm) and MP3.

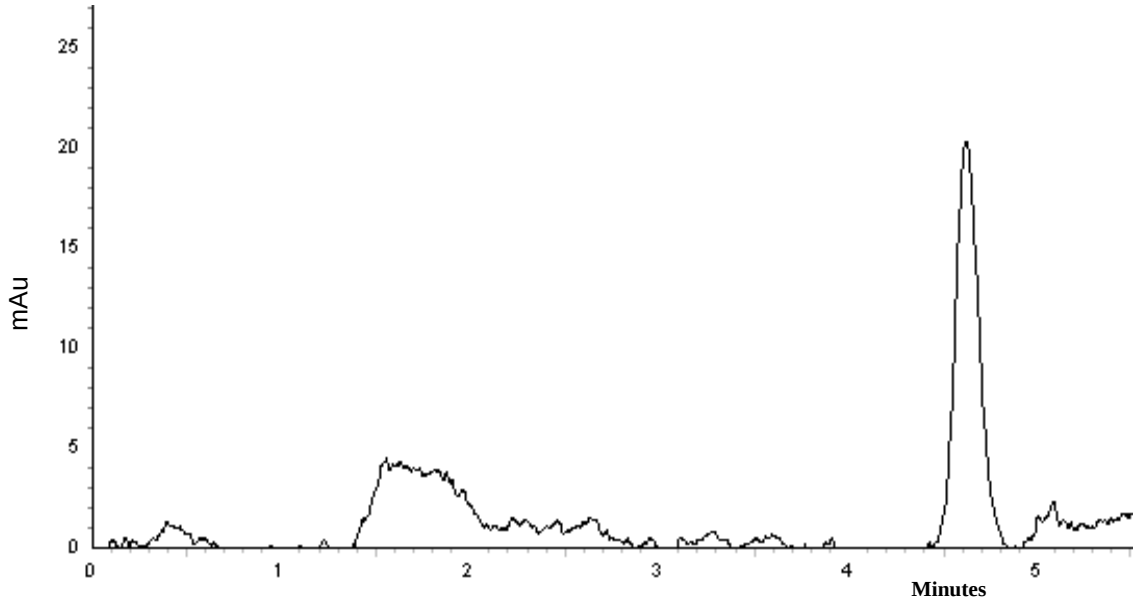

Figure 4.71. Analysis of 1.0 µg/mL NDMA using the Zorbax-SB (5 µm) and MP3.

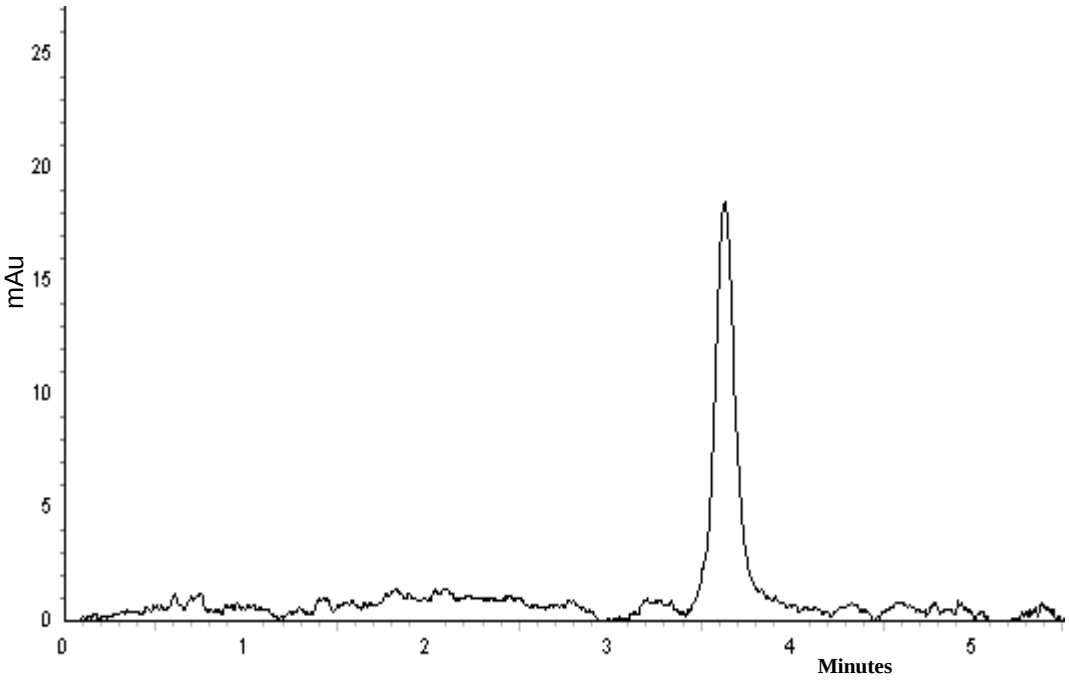

Figure 4.72. Analysis of 1.0-µg/mL NDMA using the Eclipse XDB (3.5 µm) and MP3.

Using MP4, analysis of 10 µg/mL NDMA with the Zorbax columns resulted in smaller peak areas and greater peak heights (Figures 4.73 and 4.74) than the Eclipse XDB (Figure 4.75). Analyte retention varied minimally between the Zorbax-SB columns with elution times of 2.94 and 3.14 for the 3.5-µm and 5-µm columns, respectively. In evaluating 1.0 µg/mL NDMA, the Zorbax-SB (3.5 µm) best resolved methanol and NDMA when MP4 was used (Figure 4.76). Furthermore, background noise was significantly reduced with this column when compared to the Zorbax-SB (5 µm) and Eclipse XDB (3.5µm) (Figures 4.77 and 4.78).

Overall, background noise diminished with smaller particle diameters for both mobile phases. However, the Zorbax-SB (3.5 µm) column is preferred for use with MP4.

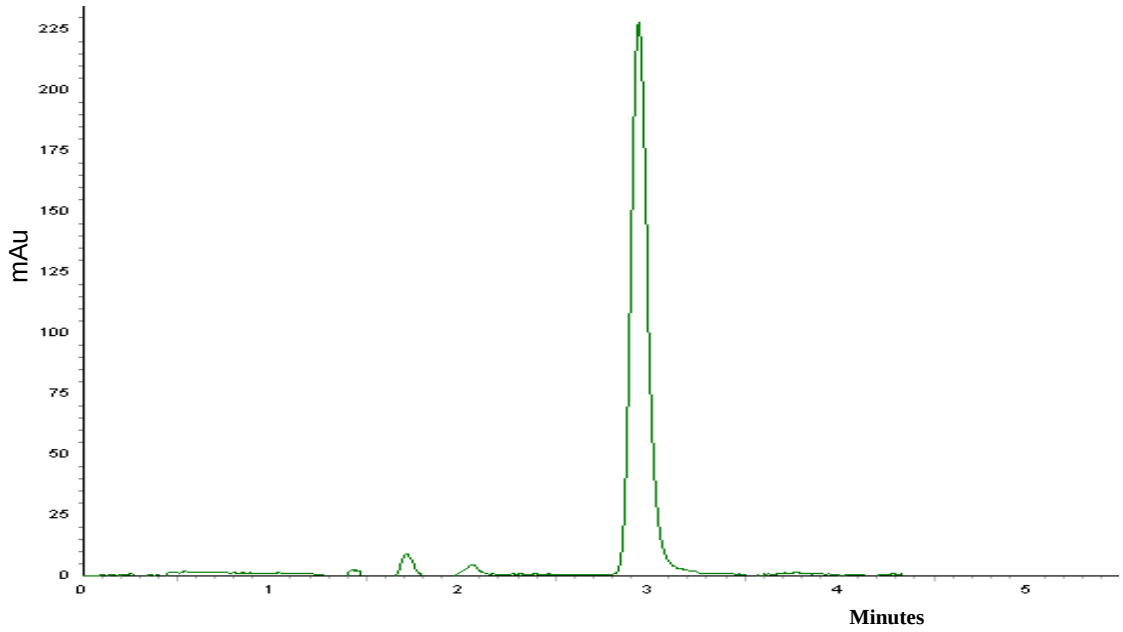

Figure 4.73. Analysis of 10 µg/mL NDMA using the Zorbax-SB (3.5 µm) and MP4.

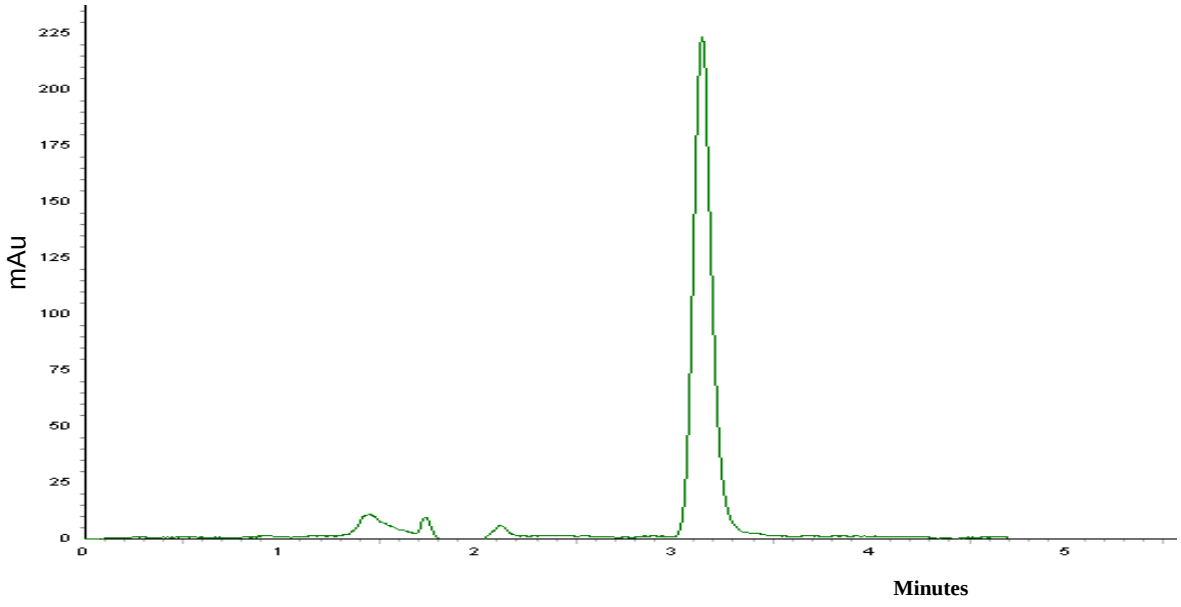

Figure 4.74. Analysis of 10 µg/mL NDMA using the Zorbax-SB (5 µm) and MP4.

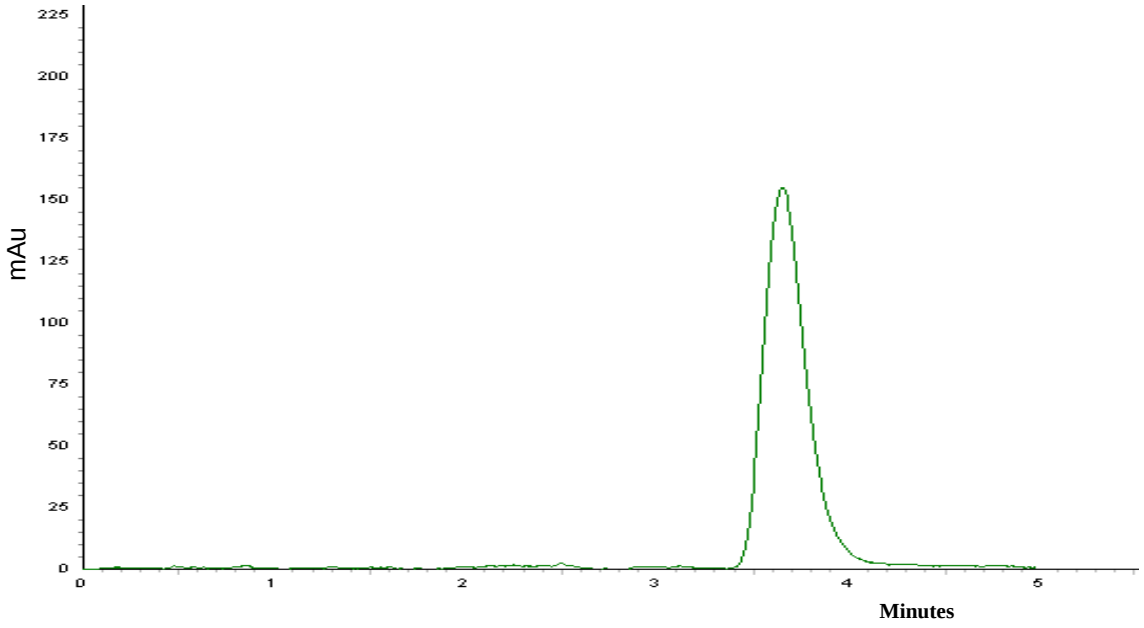

Figure 4.75. Analysis of 10 µg/mL NDMA using the Eclipse XDB (3.5 µm) and MP4.

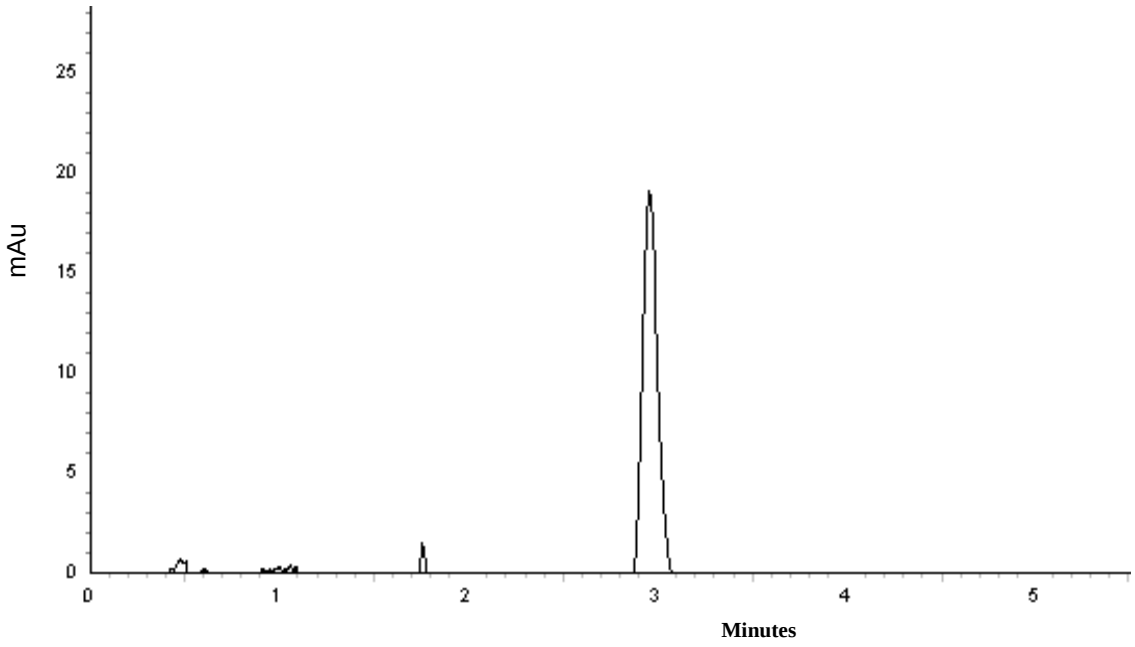

Figure 4.76. Analysis of 1.0 µg/mL NDMA using the Zorbax-SB (3.5 µm) and MP4.

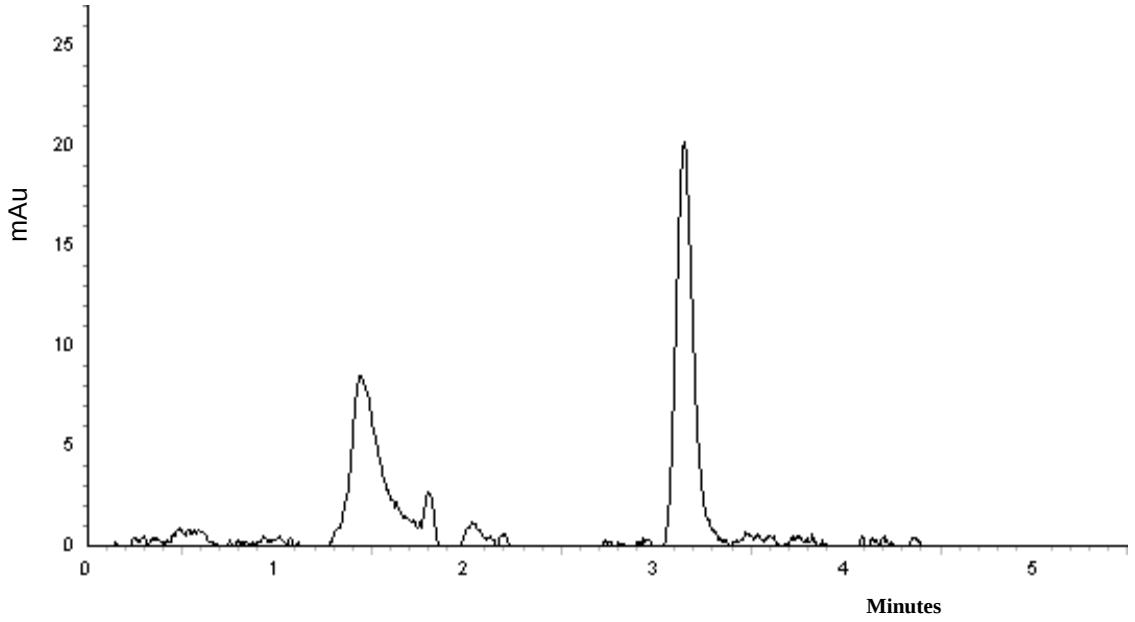

Figure 4.77. Analysis of 1.0 µg/mL NDMA using the Zorbax-SB (5 µm) and MP4.

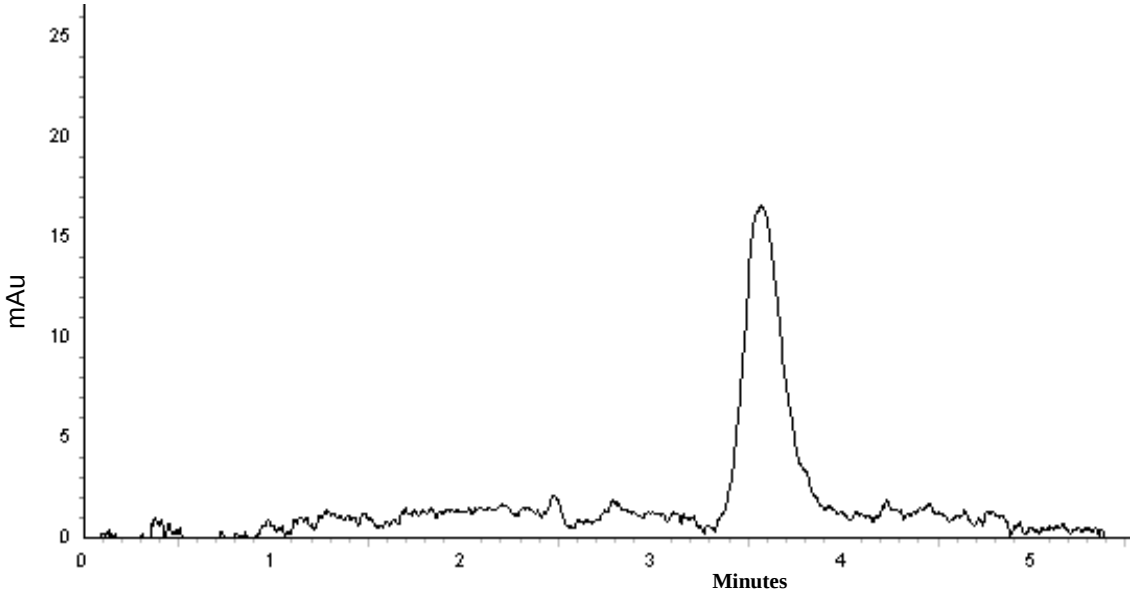

Figure 4.78. Analysis of 1.0 µg/mL NDMA using the Eclipse XDB (3.5 µm) and MP4.

4.8. Summary

The results of this study reveal that the optimal method for evaluating NDMA at 231 nm would incorporate the use of a 5:95 (v/v) ethanol:acetate buffer mobile phase (pH 4, 10 mM) at 1 mL/min., and a Zorbax SB-C_{18} column (4.6 mm x 15 cm, 3.5-µm particle size). Noise and resolution were improved with a sampling period of 320 ms, a timing constant of 640 ms, and a bandwidth of ±4 nm.

Recovery of NDMA was less than 15%. Future investigations to improve the SPE process should consider the following:

1. Lower temperatures for solvent removal and preconcentration of NDMA
2. Adjusting the pH or buffering the water sample prior to extraction
3. Using greater volumes of ethanol or using a combination of ethanol and a solvent with greater eluotropic strength for cartridge elution
4. Investigate solvent combinations for SPE cartridge conditioning

CHAPTER 5

CONCLUSION

One in every 100,000 persons may develop cancer when exposed to NDMA in drinking water at 7 ng/L.[44] The EPA is currently monitoring drinking water for NDMA using UCMR2. Gas chromatography with chemical ionization and tandem mass spectrometry (GC-CI-MS-MS) is the only analytical method approved by the EPA. It requires the use of toxic solvents for analyte preparation and quantitation.

The purpose of this investigation was to evaluate the feasibility of using ethanol (as a modifier) for RP-HPLC with PDAD in detecting trace concentrations of NDMA in environmental and drinking water. Experimental conditions were modified to reduce background noise, while optimizing detection, peak symmetry, and the resolution of methanol and NDMA. Ethanol has been investigated for quantitation of nitrosamines using normal-phase HPLC. However, these applications often require the use of hexane or dichloromethane and incorporate known water toxins into the mobile phase for use as modifiers. Using ethanol (1%) in the analysis of NDMA with RP-HPLC has necessitated the use of hazardous compounds for derivatization and large quantities of unsustainable organic solvents for gradient elution. Quantitation is frequently achieved using chemiluminescent or fluorescent detection.

Using isocratic elution with an ethanol modifier (5%) and a 5-µm column, the LOD and LOQ for NDMA was 0.29 µg/mL and 0.96 µg/mL, respectively. It should be noted that HPLC analysis of NDMA was performed with times comparable to some GC methods.[38,41] However, sensitivity of the method requires significant improvement.

Using solid phase extraction, a detection limit of 0.28 ng/L has been achieved for NDMA.[4]

A green approach to SPE was also explored. Analyte recovery was low (10% with the ethanol modifier). This study suggests that further investigation is required before ethanol can replace current extraction practices for quantitation of NDMA. Future investigations for improving extraction efficiency should evaluate flow rates through the extraction cartridge, modifiying sample characteristics (pH, buffer concentration, modifier addition), and modifiying eluent strength.

RP-HPLC can be an environmentally friendly option to gas chromatographic (GC) and normal-phase HPLC methods. This method can decrease the quantity of organic solvent used, thereby reducing the costs associated with the use and disposal of hazardous laboratory chemicals. As PDAD is compatible with isocratic and gradient modes of elution, the method could be modified to evaluate nitrosamine mixtures. The ability to employ and recycle sustainable solvents further advances method development for detecting NDMA with this technique. This is an ecological approach that avoids the use of hazardous derivatization agents, halogenated solvent mixtures for extraction, and acetonitrile-based mobile phase systems.

REFERENCES

1. *Nitrosamines and N-nitroso Compounds: Chemistry and Biochemistry;* Loeppky, R. N., Michejda, C. J., Eds.; American Chemical Society: Washington, DC, 1994.

2. Valentine, R. L.; Choi, J.; Chen, Z.; Barrett, S. E.; Hwang, C.; Guo, Y.; Wehner, W.; Fitsimmons, S.; Andrews, S. A.; Werker, A. G.; et al. *Factors Affecting the Formation of NDMA in Water and Occurrence.* American Water Works Research Foundation, American Water Works Association; Denver, CO, 2006.

3. Sedman, R.; Zeise, L.; Sandy, M. *Public Health Goals for Chemicals in Drinking Water: N-Nitrosodimethylamine;* Pesticide and Environmental Toxicology Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency: California, 2006.

4. Munch, J. W.; Bassett, M. V. *Method 521: Determination of Nitrosamines in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS);* EPA Document No. EPA/600/R-05/054; U.S. Environmental Protection Agency: Cincinnati, OH, September 2004**.**

5. http://www.epa.gov/ogwdw/dwh/c-voc/dichloro.html. Consumer Factsheet on Dichloromethane. (accessed 6-13-09).

6. http://www.methanol.org/pdf/MethanolMSDS.pdf. Methanol Material Safety Data Sheet. (accessed 6-13-09).

7. Tullo, A. A Solvent Dries Up. *Chem. Eng. News* **2008,** *86,* 27.

8. Sadek, P. *The HPLC Solvent Guide,* 2nd ed., Wiley:New York, 2002.

9. Ribiero, R. L. ; Bottoli, C. B. G. ; Collins, C. H. Reevaluation of Ethanol as Organic Modifier for Use in HPLC-RP Mobile Phases. *J. Braz. Chem. Soc.* **2004,** *15,* 300-306.

10. Howard, P. H., Meylan, W. M., Eds. Handbook of Physical Properties of Organic Chemicals; CRC Press: New York, 1997.

11. Destandau, E.; Lessellier, E. Chromatographic Properties of Ethanol/Water Mobile Phases on Silica Based Monolithic C-18. *Chromatographia* **2008,** 985-990.

12. *Chromatographic Analysis of the Environment*, 3rd ed.; Nollet, L. M. L, Ed.; CRC Press:Boca Raton, FL. 2006.

13. *Regulatory Chemicals Handbook*; Spero, J. M.; DeVito, B.; Theodore, L. Eds.; Marcel Dekker: New York, 2000.

14. Agency for Toxic Substances and Disease Registry, U.S. Public Health Service in collaboration with U.S. EPA (December 1989) Toxicological Profile for N-Nitrosodimethylamine.

15. Howard, P. H., Meylan, W. M., Eds. *Handbook of Physical Properties of Organic Chemicals;* CRC Press:New York, 1997.

16. World Health Organization. *N-Nitrosodimethylamine in Drinking Water: Background Document for Development of WHO Guidelines for Drinking-water Quality.* Geneva, Switzerland, 2006.

17. *Nitrosamines: Toxicology and Microbiology.* Hill, M. J., Ed.; Ellis Harwood: Chichester, U.K. 1988.

18. Williams, D. L. H. *Nitrosation Reactions and The Chemistry of Nitric Oxide;* Elsevier: Amsterdam, The Netherlands, 2004.

19. Kim, J.; Clevenger, T. E. Prediction of N-nitrosodimethylamine (NDMA) Formation as a Disinfection By-product. J. Hazard. Mater. **2007,** *145,* 270-276.

20. Choi, J.; Valentine, R. L. N-Nitrosodimethylamine Formation by Free-Chlorine-Enhanced Nitrosation of Dimethylamine. *Environ Sci. Technol.* **2003,** *37,* 4871-4876.

21. Choi, J; Duirk, S. E.; Valentine, R. L. Mechanistic Studies of N-Nitrosodimethylamine (NDMA) Formation in Chlorinated Drinking Water. *J. Environ. Monit.* **2002,** *4,* 249-252.

22. Delalu, H.; El Khatib, M., Marchand, A.; Counioux, J. J. Mathematical Treatment of Kinetics Governing the Synthesis of N,N-dialkylhydrazines by the Raschig Process. *Chem. Eng. J.* **1995,** *57,* 61-66.

23. Hotchkiss, J. H. *Advances in Food Research, Vol. 31,* **1988**. ASTDR, 1989.

24. *Chemistry and Biology of N-Nitroso Compounds*; Lijinsky, W.; Cambridge University Press: New York, 1992.

25. Kim, S.; Carlson, K. Temporal and Spatial Trends in the Occurrence of Human and Veterinary Antibiotics in Aqueous and River Sediment Matrices. Environ. Sci. Technol. **2007,** *41,* 50-57.

26. Gui, L.; Gillham, R.; Odziemkowski, M. Reduction of N-Nitrosodimethylamine with Granular Iron and Nickel-Enhanced Iron. 1. Pathways and Kinetics. *Environ. Sci. Technol.* **2000,** *34,* 3489-3494.

27. *HPLC Made to Measure: A Practial Handbook for Optimization;* Kromidas, S., Ed.; Wiley-VCH: Germany, 2006.

28. *Quantitative Chemical Analysis,* 6th ed.; Harris, D. C.; W. H. Freeman: New York, 2003.

29. Agilent ZORBAX Column Selection Guide for HPLC, Agilent Technologies, Inc., 2008. Document 5989-5992EN.

30. Wu, N.; Clausen, A. M. Fundamental and Practical Aspects of Ultrahigh Pressure Liquid Chromatography for Fast Separations. *J. Sep. Sci.* **2007,** *30,* 1167-1182.

31. Asami, M.; Oya, M.; Kosaka, K. A Nationwide Survey of NDMA in Raw and Drinking Water in Japan. *Sci. Total Environ.* **2009,** *407,* 3540-3545.

32. Lim, L. K.; Li, S. F. Y.; Lee, H. K. Reversed-Phase High Performance Liquid Chromatographic Separation of Nitrosamines: The Use of the Overlapping Resolution Mapping Technique for the Optimization of Solvent Compositions. *Chromatographia* **1993,** *35,* 671-674.

33. Lee, C.; Choi, W.; Yoon, J. UV Photolytic Mechanism of N-Nitrosodimethylamine in Water: Roles of Dissolved Oxygen and Solution pH. *Environ. Sci. Technol.* **2005,** *39,* 9702-9709.

34. Lee, C.; Yoon, J.; Von G unten, U. Oxidative Degradation of N-nitrosodimethylamine by Conventional Ozonation and Advanced Oxidation Process Ozone/Hydrogen Peroxide. *Water Res.* **2007,** *41,* 581-590.

35. Lee, C.; Choi, W.; Kim, Y.; Yoon, J. UV Photolytic Mechanism of N-Nitrosodimethylamine in Water: Dual Pathways to Methylamine versus Dimethylamine. *Environ. Sci. Technol.* **2005,** *39,* 2101-2106.

36. *Principles of Instrumental Analysis,* 5th ed.; Skoog, D. A., Holler, F. J., Neiman, T. A.; Harcourt Brace: Chicago, 1998.

37. Fleming, E. C.; Pennington, J. C.; Wachob, B. G.; Howe, R. A., Hill, D. O. Removal of N-nitrosodimethylamine from Water using Physical-chemical Techniques. J. Hazard. Mater. **1996,** *151,* 151-164.

38. Munch, J. W.; Bassett, M. V. Method Development for the Analysis of *N*-Nitrosodimethylamine and other N-Nitrosamines in Drinking Water at Low Nanogram/Liter concentrations Using Solid-Phase Extraction and Gas Chromatography with Chemical Ionization Tandem Mass Spectrometry. *J. AOAC Intl.* **2006,** *89,* 486-497.

39. Plumlee, M.H.; Lopez-Mesas, M.; Heidlberger, A, Ishida, K.P.; Reinhard, M. N-nitrosodimethylamine (NDMA) Removal by Reverse Osmosis and UV Treatment and Analysis via LC-MS/MS. *Water Res.* **2008,** *42,* 347-355.

40. Cha, W.; Fox, P.; Nalinakumari, B. High-Performance Liquid Chromatography with Fluorescence Detection for Aqueous Analysis of Nanogram Level N-nitrosodimethylamine. *Anal. Chim. Acta* **2006,** *566,* 109-116.

41. Charrois, J. W. A.; Boyd, J. M.; Forese, K. L.; Hrudley, S. E. Occurrence of N-nitrosamines in Alberta Public Drinking-Water Distribution Systems. *J. Environ. Eng. Sci.* **2007,** *6,* 103-114.

42. Planas, C., Palacios, Ó.; Ventura, F.; Rivera, J.; Caixach, J. Analysis of Nitrosamines in Water by Automated SPE and Isotope Dilution GC/HRMS: Occurrence in the Different Steps of a Drinking Water Treatment Plant, and in Chlorinated Samples from a Reservoir and a Sewage Treatment Plant Effluent. *Talanta* **2008,** *76*, 906-913.

43. SPD-M20A Prominence UV-vis Photo Diode Array Detector User's Manual. Shimadzu: Japan, 2004.

44. U.S. Environmental Protection Agency, Integrated Risk Information System, http://cfpub.epa.gov/ncea/iris/index.cfm (accessed April 24, 2008).