2015

Detection and removal of emerging environmental contaminants

Ruipu Mu

Department: Chemistry

Recommended Citation

Mu, Ruipu, "Detection and removal of emerging environmental contaminants" (2015). *Doctoral Dissertations*. 2605.
https://scholarsmine.mst.edu/doctoral_dissertations/2605

DETECTION AND REMOVAL OF EMERGING ENVIRONMENTAL CONTAMINANTS

by

RUIPU MU

A DISSERTATION

Presented to the Faculty of the Graduate School of the

MISSOURI UNIVERSITY OF SCIENCE &TECHNOLOGY

In Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

in

CHEMISTRY

2015

Approved by

Dr. Yinfa Ma, Advisor

Dr. Honglan Shi, Co-advisor

Dr. Jeffrey Winiarz

Dr. Paul Nam

Dr. Joel Burken

PUBLICATION DISSERTATION OPTION

This dissertation consists of the following three articles that have been published, submitted for publication, or will be submitted for publication as follows:

Page 9-29 were published in Analytical Chemistry.

Page 30-54 will submit for publication in Journal of Water and Health.

Page 55-82 will submit for publication in Clean-Soil, Air, Water.

ABSTRACT

Water is a very crucial natural resource for human. The increasing number of contaminants detected in water bodies has drawn considerable increasing attention over the last decade. There are numerous emerging environmental contaminants which may cause serious health issues. The recent occurrence studies of these contaminants show both industrial and household activity introduction of chemicals into water resources. These various sources result in a large variety of chemicals such as explosives, pharmaceutical and personal care products (PPCPs) and disinfection byproducts detected in water worldwide. Due to the relatively low contaminant concentrations, development of new and improved detection methods along with occurrence studies have been an active research area in the past decade. The United States Environmental Protection Agency (EPA), or other organizations, does not have valid methods for many of the listed contaminants. In order to meet the detection needs and screening studies, this research focused on LC-MS/MS or GC-MS method development, validation and utilization of these techniques for water analysis of different classes of emerging environmental contaminants. In addition, removal efficiency studies were also evaluated for some contaminants.

ACKNOWLEDGMENTS

The work presented in this doctoral dissertation would never have been possible without the continual support and guidance of my advisor and mentor, Dr. Yinfa Ma. His direction in all aspects of my graduate work was invaluable and will be forever appreciated.

I would also like to thank my doctoral committee members, for the various ways in which they assisted me in my work. I would like to thank Dr. Honglan Shi, who is my co-advisor, for her enthusiastic patience in advising me on experimental designs. I would like to thank Dr. Burken, Dr. Nam and Dr. Winiarz for advice in their respective fields of expertise and their continual support.

This work was supported by funds from Leonard Wood Institute (LWI), Missouri S&T Department of Chemistry, Environmental Research Center and Missouri Department of Natural Resources. I am grateful for their financial support. Sincere thanks are extended towards to Mr. Todd Eichholz and Brenda Mace at Missouri Department of Natural Resources for their great help in arranging water sample collection from water treatment plants in Missouri.

I would like to thank the Department secretaries, Tina Balch, Shannon Roark and Melinda Warner, who were constantly eager to help, and provided timely information. I would also like to thank my group members for their help.

At last, I am deeply indebted to my family: my parents, my grandparents, and my sister for their love, encouragement, and support throughout my life. I am also grateful to all my friends in both China and United States.

TABLE OF CONTENTS

Page

SECTION

PAPER

SECTION

LIST OF ILLUSTRATIONS

Figure Page

PAPER 1

PAPER 2

PAPER 3

SECTION

LIST OF TABLES

Table Page

PAPER 1

PAPER 2

PAPER 3

SECTION

1. INTRODUCTION

1.1 EMERGING ENVIRONMENTAL CONTAMINANTS

Some of the listed emerging environmental contaminants are known carcinogens and most of them were classified as probable human carcinogens based on scientific evidences [1-4]. Due to their high cytotoxicity and genotoxicity compared to regulated DBPs, emerging environmental contaminants, explosives, pharmaceutical and personal care products (PPCPs) and halonitromethanes (HNMs), have received great attention within recent years due to their presence within water bodies [1-3].

The occurrences of these contaminants indicate that there are several sources by which the contaminants have been introduced into water resources: household, agriculture, and industrial activities. A variety of chemicals, such as explosives, disinfection byproducts (trihalomethanes (THMs), haloacetic acids (HAAs), HNMs, etc.), and PPCPs, have been detected in water bodies and treated water all over the world [1, 2]. The fate of these chemicals within the environment is determined by a combination of their physical, chemical, and biological properties. The transport processes are compound dependent, however, not only at the locations near the sources but also places relative away from the origin, certain level of contaminant were detected The transport processes are compound dependent and result in contamination detected, not only at the origin, but also further away [1,2,4].

1.1.1. Explosives. Due to the long time operation, previous disposal, and handling techniques of explosives, a number of them are now problematic pollutants at many sites. Base on previous studies, most explosives that have been used are cytotoxic [1, 6, 7]. Considering the potential health effects and environmental impacts, identification and cleanup of contaminations is a goal in many locations. In addition to the commonly used explosives, such as octahydro-1, 3, 5, 7-tetranitro-1, 3, 5, 7-tetrazocine (HMX), 1, 3, 5-trinitroper-hydro-1, 3, 5-triazine (RDX), trinitrotoluene (TNT), pentaery- thritoltetranitrate (PETN), nitroguanidine (NG), and 2, 4- dinitroanisole (DNAN) have been utilized recently [8-11]. A sensitive analytical method is urgently necessary to monitor these compounds in soil, water, or other samples for environmental preservation purposes. High performance liquid chromatography –ultraviolet (HPLC–UV) detection is commonly used for detecting HMX, RDX and TNT. However, HPLC–UV is not suitable for all of these explosive compounds due to its low sensitivity and limitations for non-UV absorbing compounds such as PETN [12]. Solid-phase micro-extraction (SPME) coupled with gas chromatography/isotope ratio mass spectrometry (GC-IRMS) has been used for TNT detection [13]. Furthermore, Gas Chromatography Mass Spectrometry (GC/MS) has also been used to determine the RDX, HMX, and PETN from plastic explosives [14]. However, GC is not an ideal analytical technique to detect and quantify some organic explosives, such as RDX, due to the thermal instability [15]. HPLC coupled with tandem mass spectrometry (HPLC–MS/MS) can be a powerful analytical technique for quantitative analysis of trace levels of explosives in environmental samples.

1.1.2. Halonitromethanes. HNMs are one group of many identified nitrogenous disinfection by-products (N-DBPs). HNMs have received a high priority for health effects research from the USEPA in the past several years [18]. The present of N-DBPs is likely to increase with increased impact of wastewater and algae [18]. Furthermore, switching from chlorination to chloramination to reduce the formation of trihalomethanes (THMs) can also increase certain kinds of N-DBPs [17].

Compared with other DBPs such as THMs, HNMs have not drawn much concern. According to the recent toxicology studies, even very low levels of HNMs result in more severe adverse effects than the regulated THMs [18]. Chloronitromethane(CNM) [19], dichloronitromethane (DCNM) [19], trichloronitromethane (TCNM, chloropicrin), bromonitromethane (BNM), dibromonitromethane (DBNM), tribromonitromethane (TBNM, bromopicrin), bromochloronitromethane (BCNM), bromodichloronitromethane (BDCNM), and dibromochloronitromethane (DBCNM) received special attention for their great potential of occurring in finished waters at some treatment facilities [5,20,21]. Brominated HNMs were found to be more toxic than the corresponding chlorinated halonitromethanes [18].

There are several different methods available to detect HNMs [18, 21-24]. This study utilized a modified USEPA 551.1 method (USEPA 1990) [25]. To determining all nine HNMs simultaneously, the analytes were extracted by liquid-liquid extraction (LLE) and analyzed by gas chromatography- mass spectrometry (GC-MS) [22, 24]. Sensitivity was increased and thermal decomposition was minimized by lowering the temperature of the injection port, transfer line and mass spectrometer [26-28].

1.1.3. Pharmaceutical and Personal Care Products (PPCPs). PPCPs represent a widespread and pervasive class of environmental toxins with observable adverse effects when present at very low environmental concentrations (ng/L) [3, 4]. There are many types of pharmaceuticals on the market to benefit human and animals [29, 30]. In recent years, PPCPs have been widely detected in the environment, especially in rivers, lakes, reservoirs, and even groundwater [3, 29]. The present of these compounds in water systems has raised concerns regarding the long-term health effects. Contaminated water not only affects organisms present in the contaminated water itself, but also affects municipalities that use the contaminated water for drinking water, due to the limitation of conventional water treatment methods in comprehensive PPCP removal [31]. In addition, several studies have reported PPCPs in surface water [32, 33], but source localization has remained a challenge for many of these studies, which further complicates regulation [34]. Moreover, low concentrations and natural concentration fluctuations (such as diel and seasonal changes) further impede efforts to comprehensively detect and characterize PPCPs in water systems [35].

1.2. REGULATORY STATUS AND ANALYTICAL TRENS

As there are thousands of emerging environmental contaminants, it's not practical or feasible to regulate and routinely monitor all the contaminants. However, the EPA has already listed some of them under the unregulated contaminants and several analytical methods have been developed in the past several years for sample analysis [1-4]. Yet, most are not capable of detection of the trace level concentrations present within the environment.

The most sensitive technique used for the analysis of low level contaminants in water samples includes mass spectrometry, which has been widely used in environmental sample analysis [4, 25]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) [31, 32] and gas chromatography mass spectrometry (GC-MS) [2] have become the most commonly used methods for the analysis of emerging environmental contaminants. Water samples are typically required to undergo solid-phase extraction (SPE) for pre-concentration before injection.

In this study, the LC-MS/MS and GC-MS based techniques for water analysis were investigated. Approaches were developed for high throughput screening for a large number of emerging contaminants: explosives, PPCPs and HNMs. Further studies included removal efficiency by different treatments.

PAPER

I. Fast Separation and Quantification Method for Nitroguanidine and 2, 4-Dinitroanisole by Ultrafast Liquid Chromatography–Tandem Mass Spectrometry

Ruipu Mu[1], Honglan Shi[1], Yuan Yuan[2], Adcharee Karnjanapiboonwong[2], Joel G. Burken[2], and Yinfa Ma[1]*,

[1]Department of Chemistry and Environmental Research Center, Missouri University of Science and Technology, Rolla, MO 65409, USA

[2]Department of Civil, Environmental and Architectural Engineering and Environmental Research Center, Missouri University of Science and Technology, Rolla, Missouri 65409, United States

* Corresponding Author

Address: Department of Chemistry

Missouri University of Science and Technology

400 West 11th Street

Rolla, MO 65409

Phone: 1-573-341-6220

Fax: 573-341-6033

E-mail: yinfa@mst.edu

Abstract

Explosives are now persistent environmental pollutants that are targets of remediation and monitoring in a wide array of environmental media. Nitroguanidine (NG) and 2, 4-dinitroanisole (DNAN) are two insensitive energetic compounds recently used as munitions explosives. To protect our environment and human health, the levels of these compounds in soils and waters need to be monitored. However, no sensitive analytical methods, such as liquid chromatography–tandem mass spectrometry (LC–MS/MS), have been developed for detecting these new compounds at trace levels and to be concurrently applied to monitor the common explosives. In general, the concentrations of explosives in either soil or water samples are very low and widely distributed. Therefore, a fast and sensitive method is required to monitor those compounds and increase our ability to find and address the threats they pose to human health and ecological receptors. In this study, a fast and sensitive analytical method has been developed to quantitatively determine NG and DNAN in soil, tap water, and river water by using ultrafast LC–MS/MS. To make this method a comprehensive analytical technique for other explosives as well, it has included other commonly used explosives in the method development, such as octahydro-1, 3, 5, 7-tetranitro-1,3,5,7-tetrazocine (HMX), 1,3,5-trinitroper-hydro- 1,3,5-triazine (RDX), 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (ADNT), and pentaerythritoltetranitrate (PETN). The method detection limits (MDLs) of these compounds in soil ranged from 0.2 to 5 ppb, and a good linearity was obtained over a concentration range of 0.5–200 ppb.

The recoveries of some compounds are equal to or better than the current EPA methods but with much higher sensitivities.

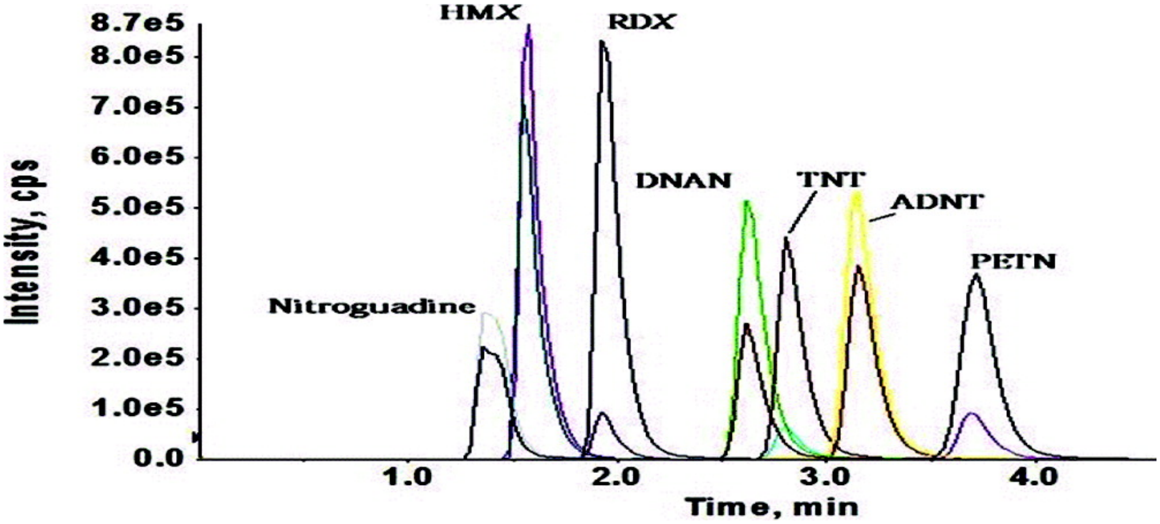


Figure 1. MRM LC/MS/MS chromatogram of explosives in MQ water

Key worlds:

Explosives; LC-MS/MS; Analytical method

1. INTRODUCTION

Because of the long time operation, previous disposal, and handling techniques, a number of munitions are now problematic pollutants at many production sites and at active or retired military sites. On the basis of previous studies, the most explosives that have been used are cytotoxic at different levels.[1–3] Considering the potential health effects and environmental impacts, identification and cleanup of contaminations is a goal in many locations. Because of the dispersed production and processing facilities, undocumented disposal, and the scattered nature of artillery range practices, the contamination is widely distributed and difficult to identify and monitor. In addition to the commonly used explosives, such as octahydro- 1,3,5,7-tetranitro-1, 3,5,7-tetrazocine (HMX), 1,3,5-trinitroper- hydro-1, 3,5-triazine (RDX), trinitrotoluene (TNT), pentaerythritoltetranitrate (PETN), nitroguanidine (NG), and 2,4- dinitroanisole (DNAN) have been utilized recently.[4–8] A fast and sensitive method is urgently necessary to monitor these compounds in soil, groundwater, and other environmental samples for environmental protection purpose. A conventional U.S. EPA method (method 8330),[9,10] which is for determination of HMX, RDX, TNT, 4-amino-2, 6-dinitrotoluene (A-DNT), and some other explosives, is available by using high- performance liquid chromatography–UV detection (HPLC–UV). However, HPLC–UV is not suitable for all of these explosive compounds not only because of its low sensitivity but also its limitations on non-UV absorbing compounds such as PETN.[11] Trinitrotoluene (TNT) has been determined in aqueous samples using solid-phase microextraction (SPME) coupled with gas chromatography/isotope ratio mass spectrometry (GC/IRMS).[12] GC/MS has also been used to determine the RDX, HMX, and PETN from plastic explosives.[13] In addition, GC coupled with thermal energy analyzer, electron capture, and nitrogen phosphorus detection have also been applied to various munitions identification.[14–16] However, GC is not an ideal analytical technique to detect and quantify some organic explosives, such as RDX, due to the thermal instability.[17] Overall, previous GC methods offer a good sensitivity for certain compounds, but none of the existing methods can rapidly analyze a wide range of munitions compounds with low detectable levels that were needed for assessing environmental explosive contaminants.

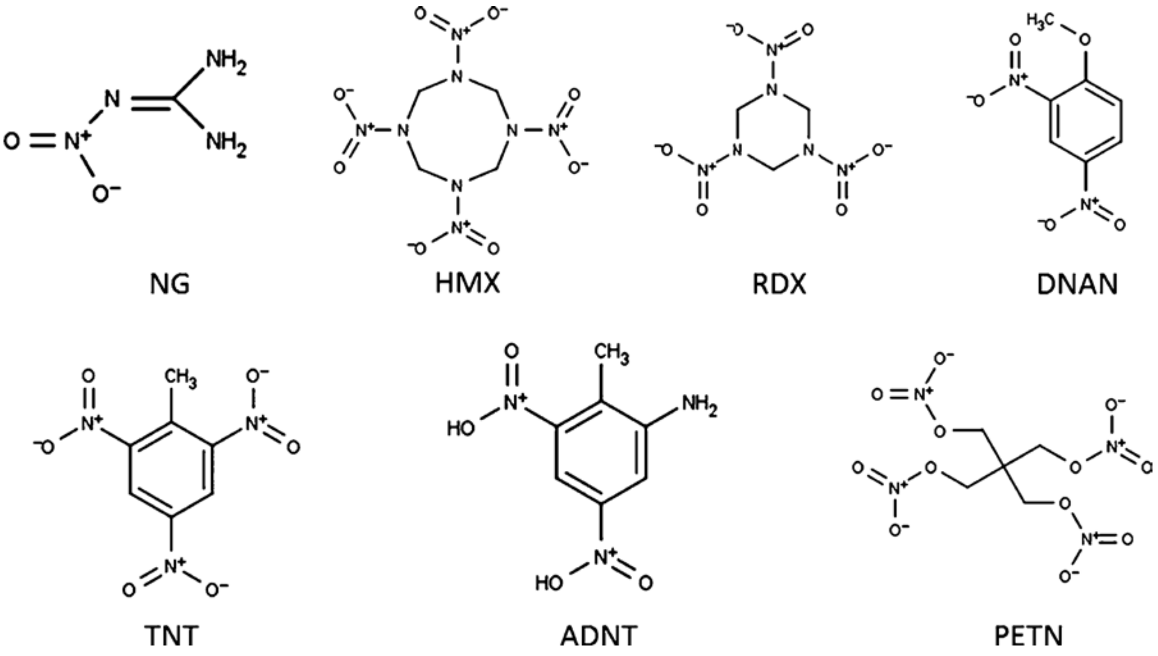
HPLC coupled with tandem MS (HPLC−MS/MS) can be a powerful analytical technique for quantitative analysis of trace levels of explosives in environmental samples. HPLC−MS/MS methods have been developed to analyze some explosive compounds by using an atmospheric pressure chemical ionization (APCI) source[18−20] or electrospray ionization (ESI) source.[21−23] However, no HPLC−MS/MS method was published for quantitative analysis of NG and DNAN in any environmental samples based on a thorough literature search. In this study, an ultrafast liquid chromatography (UFLC) −MS/ MS method has been developed for simultaneous quantitative analysis of NG, DNAN, TNT, A-DNT, RDX, HMX, and PETN explosive compounds. ESI was used as ionization source, and the negative ion mode was applied to produce molecular ions. For TNT, A-DNT, NG, and DNAN, deprotonated ions $[M - H]^-$ were generated. For compounds, which lack of acidic protons like RDX, HMX, and PETN, ammonium acetate was added to form $[M + CH_3CO_2]^-$ ions for MS/MS detection. A new solvent extraction method has been developed for all seven explosive compounds in soil samples. The extracts can be directly injected into the UFLC−MS/MS for analysis. The method has also been applied for determining trace levels of explosives in groundwater and surface water without a solvent extraction process. The water samples were filtered through 0.22 μm filters and injected into UFLC−MS/MS for analysis.

The following explosive compounds were the target of this work and are noted as environmental concerns: NG, HMX, RDX, DNAN, TNT, A-DNT, and PETN. NG is a newly used explosive compound and is often used as an explosive propellant. NG and its derivatives are also used as insecticides. The potential distribution of NG in environmental media and its monitoring methods have not been well investigated. DNAN is also a new generation energetic material that is a potential replacement for TNT. Although DNAN has been available for decades, it has not been widely used as munitions until recently. Compared with TNT, DNAN is less sensitive to shock and has a higher detonation temperature, yet still has many similar properties of TNT. Concerns for the environmental fate of DNAN led researchers, at the U.S. Army Engineer Research and Development Center, to develop an anaerobic treatment to remove DNAN from wastewaters.[24]

TNT is one of the most commonly used munitions for military and industrial applications. The effects of TNT on the immune system and some other organs such as liver, blood, and spleen have led to concern for the toxicity of TNT.[25–28] Because of the widespread usage, the TNT contamination in environmental samples is difficult and expensive to remediate. A-DNT is one of the TNT degradates that is readily found in the environment and is responsible for the red color that can be seen on many TNT contaminated sites.

RDX is a heterocyclic nitramine explosive compound and was found as a contaminant in soils, sediments, surface water, and groundwater near military installations.[29–31] RDX is less stable in storage and is much more powerful than TNT. RDX is a potential human carcinogen (U.S. EPA Class C).[3,32,33] Because of their toxicology concerns, both TNT and RDX were well studied.[34–37]

HMX is known as octogen, which is a relatively insensitive nitroamine explosive, made by nitrolysis of RDX. The cytotoxicity of HMX and its biodegradation mechanism have been studied by several research teams by using bacteria and mammalian cells.[34–37] PETN is the nitrate ester of pentaery-thritol and is more difficult to detonate than primary explosives, but it is more sensitive to shock and friction. PETN is the least stable compound out of the common military explosives and is often mixed with other explosives before use. PETN is also used medically as a vasodilator.[38] Because of its low solubility in water; PETN has low bioavailability and relatively low toxicity.[38]The chemical structures of explosives studied are shown in Figure 2.



NG HMX RDX DNAN

TNT ADNT PETN

Figure 2. Chemical structures of seven studied explosive compounds.

2. MATERIAL AND METHODS

2.1. Chemicals

Standards TNT, NG, DNAN, and ammonium acetate (99.99+ %) were purchased from Sigma-Aldrich (St. Louis, MO); HMX, RDX, 2-ADNT, PETN, and LC–MS grade methanol were purchased from Fisher Scientific (Fairlawn, NJ). Laboratory reagent water was purified by the Millipore Elix-3 water purification system (Millipore, Bierica, MA) and was used for all aqueous solution preparation.

Stock solutions were prepared with acetonitrile at a concentration of 10 μg/mL, and working solutions were made from the stock solutions by dilution with Milli-Q water–methanol (40:60 v/v) solution. All solutions were stored at 4 °C before analysis.

2.2. Instrumentation

A 4000Q TRAP mass spectrometer (AB SCIEX, Foster City, CA) equipped with an electrospray ionization interface was used in this study. A Shimadzu UFLC system (Columbia, MD), which consisted of a degasser (DGU-30A3), two pumps (LC-20 AD XR), an auto-sampler (SIL-20AC XR), and a column oven (CTO-20A), was used for the separation of explosive compounds. Analyst 1.5 software was used for data acquisition and quantification.

2.3. UFLC Separation and MS/MS Detection

The chromatographic separation of explosives was performed on a knietex C-18 reversed phase column (75 mm × 3.0 mm i.d., 2.6 μm particle size, Phenomenex, Torrance, CA) at a flow rate of 0.25 mL/min with an analysis time of 5 min, and the injection volume was 10 μL. The auto-sampler was kept at 15 °C, and the column was kept at 40 °C. The UFLC mobile phase was composed of methanol−water (60:40 v/v) containing 1 mM ammonium acetate. The elution was isocratic with a flow rate of 0.25 mL/min, and the total separation run time was 5 min.

2.4. Mass Spectrometry Operating Conditions

Negative electrospray ionization (ESI−) with the multiple-reaction- monitoring (MRM) mode was utilized for quantification of explosive compounds. Nitrogen gas used for the curtain and collision gases was generated by a N2 Generator (Peak Scientific, Billerica, MA). Compound and source-dependent parameter optimizations were performed by infusion of standard solutions. The most sensitive ion pair was selected as the quantification ion pair of each compound, while the ion transition with the second highest signal was selected as the confirmation ion pair. All other conditions were optimized through flow injection.

2.5. Sample Preparations.

All water samples (tap water and river water) were filtered through a 0.22 μm Nylon membrane filter and directly injected into the UFLC−MS/MS for analysis without any further sample preparation. For the recovery study, different levels of explosive compounds were spiked into the water samples and then were filtered and injected for UFLC- MS/MS analysis.

Soil samples were extracted by the following extraction procedures. In total, 2 g of dry soil were accurately weighed and the explosives were extracted with 10 mL of methanol−water (50:50, v/v) with sonication for 2 h. For the recovery study, two grams of dry soil were spiked with 100 μL of standard solution (20 μg/L). The soil sample was then placed in an oven at 70 °C until it was completely dry. The soil was then sonicated for 2 h in 10 mL of methanol−water (50:50, v/v) to extract the explosives. The extracted samples were then filtered through a 0.22 μm nylon membrane filters and injected into an LC−MS/MS for analysis. For statistical purposes, each measurement was conducted in triplicate. Controls with no soil were also included and tested in duplicate.

2.6. Method Performance

During the method development, the following factors were evaluated in a variety of sample matrices: calibration curves, linear ranges for each compounds, method detection limits, blanks, reproducibility, and recovery. River water, tap water, and soil were spiked with standards to evaluate matrix impacts and spike recovery. Recovery was examined at concentrations of 5, 50, 100 μg/L by spiking the appropriate amount of stock solutions to the river or tap water. For the spike recovery of soil samples, explosive standards were spiked into the soil sample and mixed well and then preceded through extraction and UFLC−MS/MS analysis.

Calibration standards (1−200 μg/L) were analyzed to demonstrate the linearity of the method, and calibration curves of 7 compounds were constructed by plotting analyte concentrations versus peak areas. The regression coefficients were calculated to show the quality of linearity. The reproducibility study was performed through replicate analyses of standards. The intraday (n = 3) and interday (n = 5) reproducibility were investigated to examine the stability of these explosive compounds.

A signal-to-noise (S/N) ratio of 3 to 5 was used to determine the method detection limit (MDLs) for each analyte in this study. The method quantification limit (MQL) of each analyte was obtained based on the lowest concentration at a S/N ratio ≥ 10. The MQL varied among different compounds. The MDLs for soil samples were calculated by using the following equation:

$$\text{MDLs} = \frac{Detected\ amount\ of\ spiked\ analyte\ (ng)\ at\,\frac{S}{N} = 5}{Mass\ of\ the\ soil\ (g)}$$

3. RESULTS AND DISCUSSION

3.1. LC−MS/MS Method Optimization

To achieve the highest sensitivity and a faster separation, the UFLC−MS/ MS method was systematically optimized. First of all, different concentrations of ammonium acetate (0.5, 1, 2, 3, and 6 mM) were investigated in aiding the formation of explosive adduct ions. On the basis of the peak areas of adduct ions for all seven compounds, 1 mM ammonium acetate was chosen as an ideal concentration. The ammonium acetate addition in the mobile phase greatly enhanced the ionization, stability, and reproducibility of RDX, HMX, and PETN. The extracted ion chromatogram (XIC) of the standard explosives was shown in Figure 3.

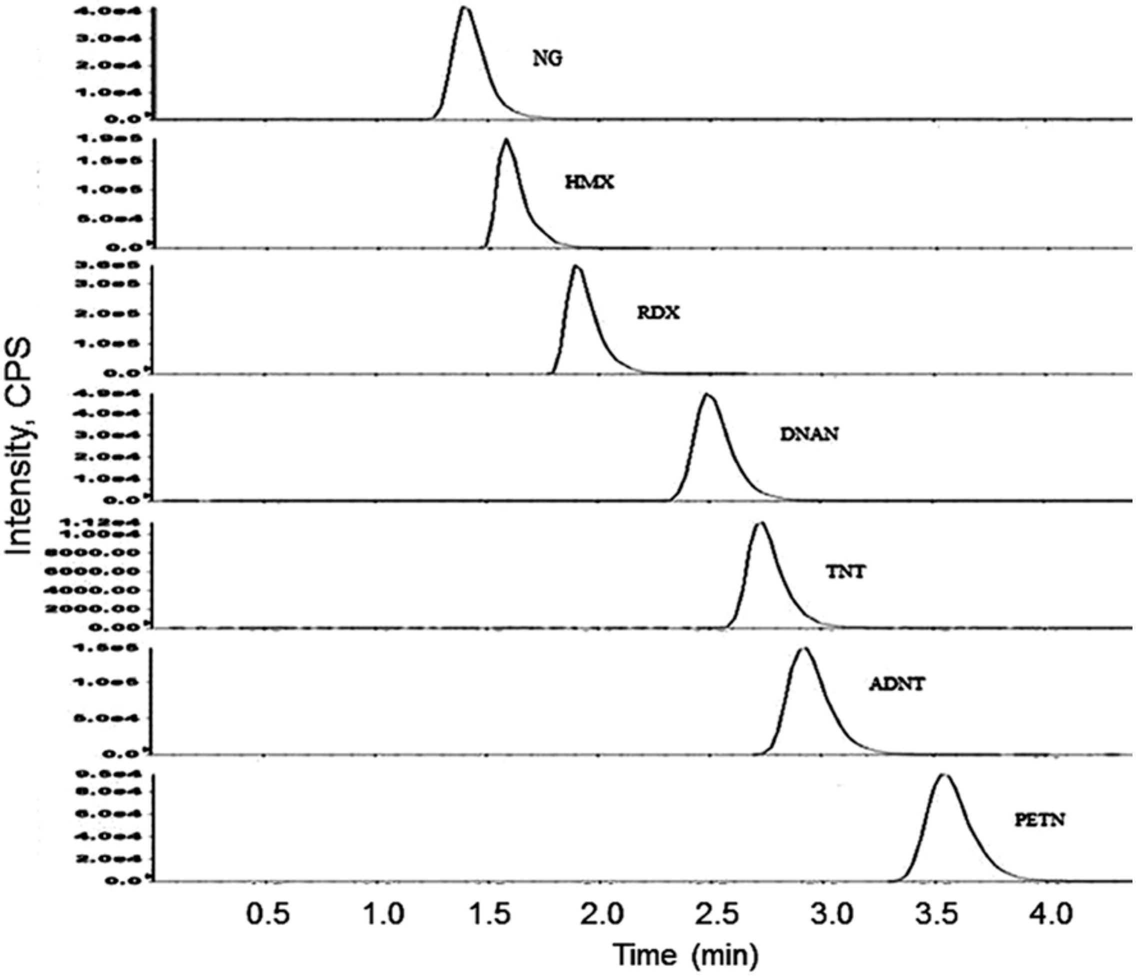


Figure 3. Extracted ion chromatogram (XIC) of 7 standard explosive compounds under optimized conditions by UFLC-MS/MS. Column, P knietex C-18 (75 mm × 3.0 mm i.d., 2.6 μm particle size); flow rate, 0.25 mL/min; injection volume, 10 μL; mobile phase, methanol–water (60:40) both containing 1 mM ammonium acetate. Other experimental conditions were described in the Materials and Methods.

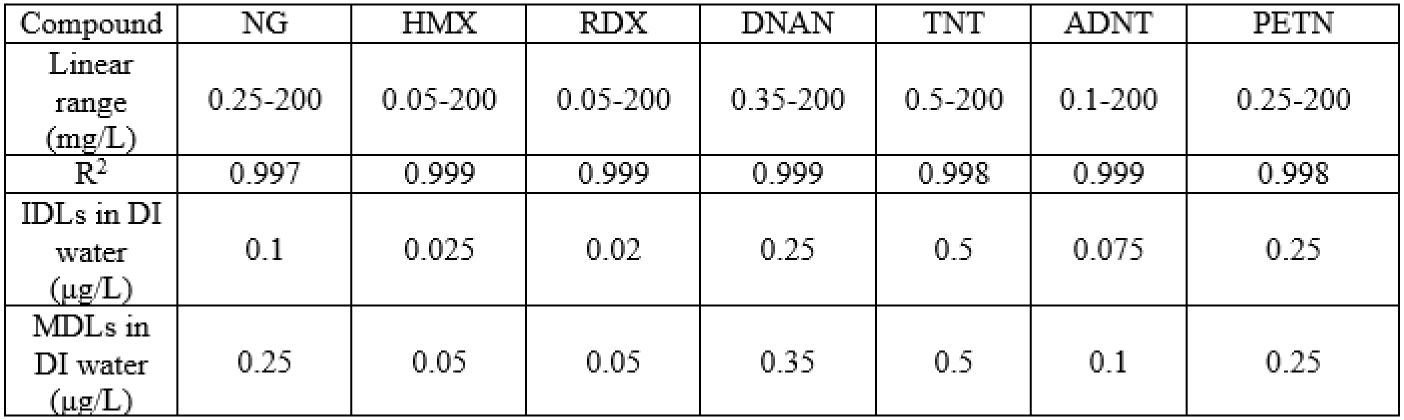
To accomplish the desired separation, different compositions of the mobile phases and flow rates were studied to optimize the separation conditions. After a series of experiments, isocratic elution with methanol–water (60:40, v/v, both water and methanol containing 1.0 mM ammonium acetate) at a flow rate of 0.25 mL/min was found to work well for this study. Nitroguanidine was eluted first at around 1.6 min, and the last one was PETN which had a retention time of 4.0 min. A-DNT has a very similar chemical structure with TNT, and they cannot be completely separated (less than 10% overlap) chromatographically. The same phenomenon was observed for HMX and nitroguanidine (less than 20% overlap). Nevertheless, all of these compounds can be identified and quantified by MS/MS because they can be differentiated by their different MRM transitions.

MS/MS conditions were optimized as follows: dwell time, 130 ms; ion source temperature, 350 °C; ion spray voltage, −4500 V; curtain gas pressure, 30 psi; ion source gas 1 pressure, 40 psi; ion source gas 2 pressure, 50 psi. The other compound dependent parameters were listed in **Table 1**.

Table 1. UFLC-MS/MS experimental conditions of 7 munitions contaminants

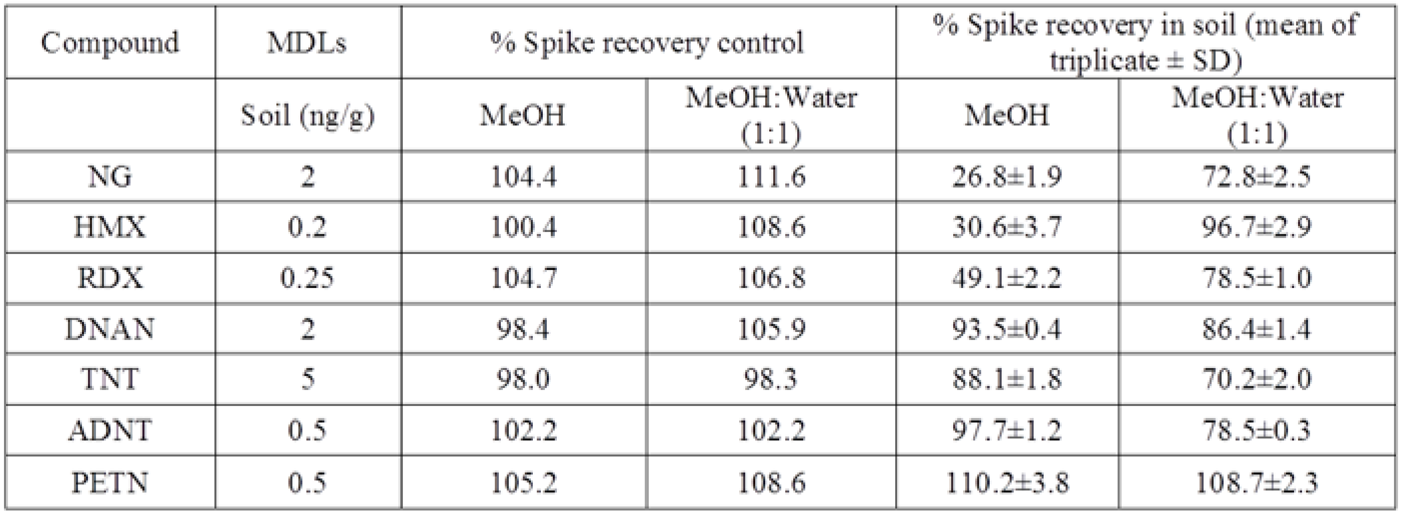
	Compounds						
	NG	HMX	RDX	DNAN	TNT	ADNT	PETN
Retention time (min)	1.559	1.760	2.134	2.808	3.062	3.275	3.965
MRM (m/z)	103.1/46.0	354.9/147.1	280.9/46.0	183.1/109.1	225.8/195.8	196.0/136.2	374.9/61.9
	103.1/61.2	354.9/46.0	280.9/59.2	183.1/123.1	225.8/165.9	196.0/46.0	374.9/46.0
Declustering potential (DP, V)	-40	-35	-20	-40	-55	-60	-25
Collision energy (CE, V) (Quantitation ion/Confirm ion)	-34/-14	-12/-38	-40/-24	-38/-26	-18/-24	-26/-48	-14/-36
Collision cell potential (CXP, V) (Quantitation ion/Confirm ion)	-1/-1	-13/-16	-19/-5	-5/-7	-13/-9	-7/-1	-1/-5
Entrance potential (EP, V)	10	10	10	10	10	10	10

Table 2. Linear Range, IDLs, and MDLs of explosives in DI water



Compound	NG	HMX	RDX	DNAN	TNT	ADNT	PETN
Linear range (mg/L)	0.25-200	0.05-200	0.05-200	0.35-200	0.5-200	0.1-200	0.25-200
R^2	0.997	0.999	0.999	0.999	0.998	0.999	0.998
IDLs in DI water (µg/L)	0.1	0.025	0.02	0.25	0.5	0.075	0.25
MDLs in DI water (µg/L)	0.25	0.05	0.05	0.35	0.5	0.1	0.25

Table 3. MDLs and spike recovery of explosives in soil matrix.



Compound	MDLs	% Spike recovery control		% Spike recovery in soil (mean of triplicate ± SD)	
	Soil (ng/g)	MeOH	MeOH:Water (1:1)	MeOH	MeOH:Water (1:1)
NG	2	104.4	111.6	26.8±1.9	72.8±2.5
HMX	0.2	100.4	108.6	30.6±3.7	96.7±2.9
RDX	0.25	104.7	106.8	49.1±2.2	78.5±1.0
DNAN	2	98.4	105.9	93.5±0.4	86.4±1.4
TNT	5	98.0	98.3	88.1±1.8	70.2±2.0
ADNT	0.5	102.2	102.2	97.7±1.2	78.5±0.3
PETN	0.5	105.2	108.6	110.2±3.8	108.7±2.3

Table 4. Spike recovery of explosive compounds in tap and river water

Compound	MDLs		High level spike(100 ppb)				Medium level spike (40 ppb)				Low level spike (5 ppb)			
	Tap water (ppb)	River water (ppb)	Tap water		River water		Tap water		River water		Tap water		River water	
			Recovery%	RSD %	Recovery %	RSD %	Recovery %	RSD %	Recovery%	RSD %	Recovery%	RSD %	Recovery %	RSD %
NG	0.7	0.7	94.0	1.2	97.4	1.7	97.4	1.3	101	1.1	96.3	6.4	99.5	2.5
HMX	0.15	0.15	99.1	0.7	98.9	1.4	101	1.3	101	0.7	97.7	1.8	97.8	1.3
RDX	0.15	0.15	101	0.8	99.8	1.5	106	1.8	106	1.3	105	0.9	104	1.3
DNAN	0.5	0.6	101	0.8	101	0.8	102	1.5	103	0.7	98.8	1.7	98.5	1.4
TNT	0.7	0.7	101	1.7	101	1.4	104	1.9	104	2.1	102	5.6	103	3.9
ADNT	0.2	0.3	99.6	0.3	98.7	0.7	104	1.4	103	2.1	102	1.6	99.8	2.1
PETN	0.3	0.4	101	1.9	99.2	3.6	102	2.7	103	1.1	101	3.4	99.1	6.5

3.2. Method Performance

Under the optimized separation and MS detection conditions, calibration curves for all explosive compounds were constructed to show the linearity of the method. The regression coefficients were calculated, and they were all ≥0.997 for the 7 compounds. The details were presented in Table 2. The intraday (n = 3) and inter-day (n = 5) reproducibility were also investigated to examine the stability of these explosive compounds. The results demonstrated that the method is reliable and there were no significant differences for intraday and inter-day analyses (relative standard deviation (RSD) ranged from 1 to 5%). The instrumental detection limits (IDLs) and method detection limits (MDLs) for all 7 compounds were shown in **Table 2**. The newly developed method showed higher sensitivities for TNT, HMX, RDX, ADNT, and PETN than the existing EPA method 8330. For NG and DNAN, there was no LC−MS/MS method being reported. This was the first LC−MS/MS method for simultaneous detection of all 7 explosive compounds. The data in Table 2 have demonstrated that the method is highly sensitive for quantitative analysis of all 7 explosive compounds.

3.3. Water Sample Analysis

The newly developed UFLC− MS/MS method was evaluated for real water samples analysis. Both tap water and river water were tested. The detection limits in the real water samples were close to those of DI water. Spike recoveries were evaluated at high, medium, and low level spikes. The data were shown in **Table 3**.

The spike recoveries ranged from 94% to 106% with good reproducibility indicated by the RSDs (0.3–6.5%).

3.4. Soil Sample Analysis

The newly developed UFLC–MS/ MS method was also applied for soil sample analysis. The performance of the method was shown in **Table 4**. The spike recoveries for all the analytes in soil samples were found to be more than 70% by using an extraction solution of methanol– water (50:50, v/v). Because of the greater solubility of nitroguanidine in water (0.42 g/100 mL) than in methanol (0.302 g/100 mL), the recovery of nitroguanidie was only 27% if pure methanol was used for extraction. The recovery percentage increased dramatically after adding 50% water (72.8%). Similar results were obtained for HMX and RDX, which are much polar than TNT. However, after adding 50% water, the recoveries of TNT and A-DNT decreased about 18% and 20%, respectively, due to their nonpolar properties. Even though the recoveries of TNT and A-DNT were dropped some 70.2% for TNT and 78.5% for A-DNT, their recoveries were still acceptable for environmental sample analysis. Compared with the water samples, the soil samples had significantly higher interferences due to the abundance of organic matter and salts. Some of the interferences had the same retention time as some of target compounds, such as NG and RDX. To avoid false- positive results and obtain accurate data, a different confirmation ion pair was selected during quantification.

4. CONCLUSION

A simple, rapid, specific, and sensitive UFLC–MS/MS method was developed for simultaneous analysis of 7 explosive compounds. LC–MS/MS method for NG and DNAN analysis has never been reported previously based on our best knowledge. The UFLC allows the method to use a small packing particle size and short column (75 mm, 2.6 μm particle size), resulting in a shorter analysis time, better resolution, and high sensitivity. The method was validated through evaluation of recoveries (≥70% for soil and ≥94% for river and tap water samples), reproducibility (represented by RSD), and MDLs. This developed method is currently being applied for explosive screening in several different types of real environmental samples including tap water, river water, soil sample, and plant tissue sample.

5. ACKNOWLEDGMENT

This study was funded by the Leonard Wood Institute. The authors would like to express their appreciation to Millipore, Inc., and the Environmental Research Center at Missouri University of Science and Technology.

6. REFERENCES

(1) Robidoux, P. Y.; Svendsen, C. Environ. Toxicol. Chem. 2000, 19, 1764–1773.

(2) Rylott, E. L.; Lorenz, A.; Bruce, N. C. Curr. Opin. Biotechnol. 2011, 22, 434–440.

(3) Yinon, J. Toxicity and Metabolism of Explosives; CRC Press: Boca Raton, FL, 1990.

(4) Duan, W.-d.; Lu, Z.-s. HannengCailiao 2003, 11, 209−212.

(5) Mudryy, R. Int. Annu. Conf. ICT 2011, 42nd, 111/1−111/11.

(6) Powala, D.; Orzechowski, A.; Maranda, A.; Nowaczewski, J. Przem. Chem. 2005, 84, 582−585.

(7) Provatas, A.; Wall, C. Int. Annu. Conf. ICT 2011, 42nd, 6/1−6/12.

(8) Zhang, G.-q.; Dong, H.-s. HannengCailiao 2010, 18, 604−609.

(9) Chow, T. M.; Wilcoxon, M. R.; Piwoni, M. D.; Maloney, S. W. J. Chromatogr. Sci. 2009, 47, 40−43.

(10) EPA. Method 8330, Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC), 1994.

(11) Gaft, M.; Nagli, L. Opt. Mater. 2008, 30, 1739−1746.

(12) Berg, M. J.; Hofstetter, T. B. Opt. Mater. 2008, 30, 1739−1746.

(13) Lai, H.; Leung, A.; Magee, M.; Almirall, J. R. Anal. Bioanal. Chem. 2010, 396, 2997−3007.

(14) Batlle, R.; Carlsson, H.; Tollbaeck, P.; Colmsjoe, A.; Crescenzi, C. Anal. Chem. 2003, 75, 3137−3144.

(15) Calderara, S.; Gardebas, D.; Martinez, F. Forensic Sci. Int. 2003, 137, 6−12.

(16) Gregory, K. E.; Kunz, R. R.; Hardy, D. E.; Fountain, A. W.; Ostazeski, S. A J. Chromatogr. Sci. 2011, 49, 1−7.

(17) Fialkov, A. B.; Gordin, A.; Amirav, A. J. Chromatogr., A 2003, 991, 217−240.

(18) Zhao, X.; Yinon, J. J. Chromatogr., A 2002, 977, 59−68.

(19) Tachon, R.; Pichon, V.; Le, B. M. B.; Minet, J.-J. J. Chromatogr., A 2007, 1154, 174−181.

(20) Sun, Q.; Chen, Z.; Yuan, D.; Yu, C.-P.; Mallavarapu, M.; Naidu,R. Chromatographia 2011, 73, 631−637.

(21) Pan, X.; Tian, K.; Jones, L. E.; Cobb, G. P. Talanta 2006, 70, 455−459.

(22) Schmidt, A.-C.; Herzschuh, R.; Matysik, F.-M.; Engewald, W. Rapid Commun. Mass Spectrom. 2006, 20, 2293−2302.

(23) Tachon, R.; Pichon, V.; Borgne, M. B. L.; Minet, J. J. J. Chromatogr., A 2007, 1154, 174−181.

(24) Boddu, V. M.; Abburi, K.; Fredricksen, A. J.; Maloney, S. W.; Damavarapu, R. Environ. Technol. 2009, 30, 173−181.

(25) Banerjee, H. N.; Verma, M.; Hou, L. H.; Ashraf, M.; Dutta, S. K. Yale J. Biol. Med. 1999, 72, 1−4.

(26) Miliukiene, V.; Cenas, N. Z. Naturforsch., C: J. Biosci. 2008, 63, 519−525.

(27) Miliukiene, V.; Cenas, N. Z. Naturforsch., C 2008, 63, 519−25.

(28) Zeng, K.; Hwang, H.-M.; Zhang, Y.; Cook, S. Environ. Toxicol. 2004, 19, 490−496.

(29) Best, E. P. H; Geter, K. N.; Tatem, H. E.; Lane, B. K. Chemosphere 2006, 62, 616−625.

(30) Clausen, J.; Robb, J.; Curry, D.; Korte, N. Environ. Pollut. (Amsterdam, Neth.) 2004, 129, 13−21.

(31) Rylott, E. L.; Budarina, M. V.; Barker, A.; Lorenz, A.; Strand, S. E.; Bruce, N. C. New Phytol. 2011, 192, 405−413.

(32) Choi, Y.; Jeong, S.; Ryu, H.; Lee, K.; Bae, B. H.; Nam, K. Hum. Ecol. Risk Assess. 2011, 17, 856−872.

(33) Kwon, M. J.; O'Loughlin, E. J.; Antonopoulos, D. A.; Finneran, K. T. Chemosphere 2011, 84, 1223−1230.

(34) Berthe-Corti, L.; Jacobi, H.; Kleihauer, S.; Witte, I. Chemosphere 1998, 37, 209−218.

(35) Cenas, N.; Nemeikaite-Ceniene, A.; Sarlauskas, J.; Anusevicius, Z.; Nivinskas, H.; Miseviciene, L.; Maroziene, A. Mechanisms of the Mammalian Cell Cytotoxicity Of Explosives. In Ecotoxicology of Explosives; CRC Press: Boca Raton, FL, 2009; pp 211−226.

(36) Lachance, B.; Robidoux, P. Y.; Hawari, J.; Ampleman, G.; Thiboutot, S.; Sunahara, G. I. Mutat. Res., Genet.Toxicol. Environ. Mutagen 1999, 444, 25−39.

(37) Sagi-Ben, M. S.; Ronen, Z.; Dahan, O.; Weisbrod, N.; Groisman, L.; Adar, E.; Nativ, R. Environ. Pollut. (Oxford, U.K.) 2009, 157, 2231− 2238.

(38) Ebadi, M. S. Desk Reference of Clinical Pharmacology; CRC Press: Boca Raton, FL, 1998.

II. Study on Determination and Screening of Halo-nitro methane in Missouri and Tulsa Drinking Water System

Ruipu Mu[1], Honglan Shi[1], Craig Adams[2], Todd Eichholz[3], Yinfa Ma[1]*,

[1]Department of Chemistry and Environmental Research Center, Missouri University of Science and Technology, Rolla, MO 65409, USA

[2]Department of Civil and Environmental Engineering, Utah State University, Logan, UT 84322, USA

[3]Missouri Department of Natural Resources, Jefferson City, MO 65102, USA

* Corresponding Author

Address: Department of Chemistry

Missouri University of Science and Technology

400 West 11th Street

Rolla, MO 65409

Phone: 1-573-341-6220

Fax: 573-341-6033

E-mail: yinfa@mst.edu

Abstract

Halonitromethanes (HNMs) are a class of nitrogenous disinfection by-products (N-DBPs) that have been detected in some water distribution systems. As halonitromethanes have begun to play an increasingly important role as disinfection byproducts, the modified LLE- GC-MS method provide a fast and sensitive approach to detect 9 HNMs. Meanwhile the mass spectrometric behavior those candidates show that mono and dihalonitromethanes are more stable than trihalomitromethanses under the same conditions. This comprehensive method for HNMs gives us the whole array of these species with limits of detection (LODs) range from 0.2 to 1g/L and 7.0% adequate precision with minimum consumption of solvent. A screening study was performed to investigate the appearance of halo nitro methane in raw and finished water collected from Missouri and Tulsa water treatment plant. Most of HNMs were not found in those representative raw water samples except one sample (reservoir water) from winter collection. For the finished waters, HNMs were found in waters from all types of water sources except the deep well water those with nondetectable level or very low level of HNMs. The highest concentration of HNMs was found in finished lake water at total HNMs 6.71 μg/L. Most of finished lake waters had relatively high concentration of HNMs.

Key Worlds:

HNM, GC-MS, screening, drinking water, DBPs

1. INTRODUCTION

Nitrosamines (Pozzi *et al.*, 2011), cyanogen halides, haloacetonitriles (Huang *et al.*, 2013), haloacetamides(Yang *et al.*, 2007) and halonitromethanes (Luo *et al.*, 2014), are all nitrogenous disinfection by-products (N-DBPs). Due to their high cytotoxicity and genotoxicity with comparison of regulated DBPs (Yang *et al.*, 2007; Pozzi *et al.*, 2011; Huang *et al.*, 2013; Luo *et al.*, 2014), it was reported that with the increased impact of waste water and algae, the present of N-DBPs is likely to increase (Plewa *et al.*, 2004). Furthermore, switching from chlorination to chloramination to reduce the formation of trihalomethanes (THMs) and haloacetic acids (HAAs) (Sa *et al.*, 2012), can also increase certain kind of N-DBPs. For formation of N-DBPs is complex and variable with the impact of water treatment procedures (Chang *et al.*, 2011). Some studies show coagulation and filtration etc. common techniques have decent efficiency for the removal of N-DBPs precursors, if used before disinfection. In contrast, application of oxidant prior to final disinfection can cause the formation of halonitromethane (Chen *et al.*, 2009; Krasner *et al.*, 2009; Yu *et al.*, 2011).

HNMs are one group of N-DBPs with that have received a high priority for health effects research from the USEPA in the past several years (Plewa *et al.*, 2004). Compared with other DBPs such like THMs, these emerging carcinogenic compounds that have not drawn much concern. According to the toxicology studies in recent years, HNMs are some of the most genotoxic and cytotoxic compounds among those unregulated DBPs (Montesinos *et al.*, 2011; Montesinos and Gallego, 2012a). Therefore, HNMs has more severe adverse effects than other regulated THMs even at very low levels (Plewa *et al.*, 2004). Among these DBPs, HNM species chloronitromethane(CNM) (Mincher *et al.*, 2010), dichloronitromethane (DCNM) (Mincher *et al.*, 2010), trichloronitromethane (TCNM, chloropicrin), bromonitromethane (BNM), dibromonitromethane (DBNM), tribromonitromethane (TBNM, bromopicrin), bromochloronitromethane (BCNM), bromodichloronitromethane (BDCNM), and dibromochloronitromethane (DBCNM) received special attention because of their potential high possibility of occurring in finished waters at some treatment facilities (Krasner *et al.*, 2009; Hong *et al.*, 2014; Luo *et al.*, 2014). Brominated halonitromethanes were found to be more toxic than the corresponding chlorinated halonitromethanes (Plewa *et al.*, 2004). The toxicity of HNMs is greater than haloacetic acids (HAAs) in drinking water (Plewa *et al.*, 2004). For instant, the cytotoxicity of DBNM is 86.2 times and genotoxicity is 67 times higher than dibromoacetic acid (HAAs). It was reported dibromonitromethane is the most toxic compounds, furthermore, brominated HNMs has more adverse effects than chlorinated analogues (Plewa *et al.*, 2004). The maximum concentration of total HNMs found in a national-wide occurrence study of drinking water by Weinberg et al. (Weinberg and Cook, 2002)was 0.1- 3 μg/L, while another study by Richardson (Richardson, 2007)reported concentrations up to 10 μg/L.

There are several different methods available to detect HNMs (Plewa *et al.*, 2004; Chang *et al.*, 2011; Pozzi *et al.*, 2011; Sa *et al.*, 2012; Hong *et al.*, 2014). Each method has its own advantages and disadvantage. This paper utilized modified USEPA 551.1 method (USEPA 1990) (J.W. Hodgeson, 1990). To determining all nine HNMs simultaneously, two procedures were involved which are liquid-liquid extraction (LLE) and instrument analysis. Compare with head space solid phase micro extraction (HS-SPME) (Montesinos and Gallego, 2012b; Montesinos and Gallego, 2012a), LLE is more suitable for large sample sizes with simpler procedures and more wildly used for environmental samples (Barrionuevo and Lancas, 2002; Saar *et al.*, 2009). Since the halogen elements in the structure, which makes HNMs volatile, gas chromatography- mass spectrometry (GC-MS) (Chang *et al.*, 2011; Pozzi *et al.*, 2011) is a good choice for the analysis. The injection port temperature, transfer line temperature and mass spec temperature ware lowered in order to minimize the thermal decomposition. More over the sensitivity was increased due to those modifications (Song *et al.*, 2010; Montesinos *et al.*, 2011; Yang *et al.*, 2012; Shi *et al.*, 2013).

In this study, all 9 HNMs were examined in samples collected from drinking water treatment plants. The main objectives of the study were to investigate the level of HNMs. The occurrence data of this group of DBPs was not available in Missouri drinking water systems and should be evaluated in order to eliminate/minimize this group of DBPs in drinking water.

2. MATERIAL AND METHODS

2.1. Chemicals

Standard BNM (90%) and TCNM (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA); the other HNM standards CNM 90–95%, DCNM >95%, BCNM 85–90%, BDCNM 90–95%, DBNM 90%, DBCNM 90–95%, TBNM, 90–95%) were not commercially available currently and were synthesized by Orchid Cellmark (New Westminster, Canada). Naphthalene-d8 was used as internal standard (IS), which was purchased from Sigma-Aldrich. Sodium sulfate, copper sulfate, methyl-tert-butyl ether (MTBE) were all purchased from Fisher Scientific. The standard stock solutions were prepared in MTBE at 100 mg/L or 1000 mg/L concentrations in amber vials, and stored in the refrigerator.

2.2. Standard Solutions

Stock standard solutions containing 100 mg/L or 1000 mg/L of individual HNM were prepared in MTBE and stored in 2 mL amber vials at 4 °C. The working standard solutions (0.1–200 μg /L) were prepared by diluting the stock standard solutions in MTBE.

2.3. Water Sample Collection and Storage

1. All of the sample bottles were 125 ml amber glass bottles with Teflon liner screw caps and rinsed with DI water, methanol, then baked at 150 ^{o}C for at least 2 hrs.

2. Weigh 12.5 mg ammonium chloride into the bottle and cap the bottle tightly for shipping to the water collection point

3. Water collection:

•For tap water collection, remove the aerator if it present; open the water tap and let the water flow for around 5 min, then fill sample bottles to just overflowing but take care not to flush out solid. No air bubbles should pass through the sample as the bottle is filled, or be trapped in the sample when the bottle is sealed. Seal the bottle and agitate by hand for one minute. Place it in cooler with ice during overnight transfer to the lab.

•For river water, use a large pre-cleaned wide mouth bottle or beaker to take the water at a representative area, and carefully fill the sample bottle from the container to just overflowing but take care not to flush out solid; seal the bottle and agitate by hand for one minute. Keep samples sealed from collection time until analysis. Place it in cooler with ice during overnight transfer to the lab.

4. Store the samples in refrigerator until extraction. All the samples were processed within 14 days.

2.4. Extraction Procedures

Transfer 30 ml water sample into a 40 ml glass vial with 10-11 g sodium sulfate (Na_2SO_4) and 1 g copper sulfate (1 g $CuSO_4 \cdot 5H_2O$), mix to let all of solid dissolve, add several drops of 0.5 M sulfuric acid to adjust pH to 3.5, then add 3 ml of MTBE into the vial for extraction; shake vigorously for 5 min to extract the HNMs into MTBE; let set for 10 minutes until both phases were separated clearly, take 1ml supernatant extract into an auto-sampler vial; add 20 μL naphthalene-d8 IS (concentration 500 ppb) into the 1 ml extractant. Seal the sample and stored at 4 °C before being injected into GC–MS system.

2.5. Instrument Analysis

The MTBE extract was analyzed by Agilent 6890 GC with a 5973N mass selective detector (MSD). The GC column was a HP-5ms (Hewlett Packard) column with a 30 m by 0.25 mm i.d., and a 0.25-μm film thickness. The GC oven temperature was programmed as follows: Initial temperature 35 °C for 4 min; 9 °C /min to 130 °C, then 30 °C /min to 250 °C, and hold for 10 min. The injection port temperature was 117 °C. The MS source temperature was 200 °C. The GC-MS transfer line temperature was 225 °C. The injection volume was 2 μL in splitless mode. The carrier gas was ultra-high purity nitrogen setting at 1.0 mL/min. For qualitative identification, scan mode was used with a scan range of 35-300 amu. For quantification, SIM (selected ion monitoring) mode was used.

3. RESULTS

3.1. Method Validation

HNM spectra and separation: Standard HNMs were injected individually for GC-MS analysis in scan mode. For each peak, the retention time and mass spectrum were obtained. The mass spectra of HNMs are shown in Figure1 and retention times of each HNM are listed in Table 1. A representative chromatogram of all nine HNMs together in a standard mixture is shown in Figure 2. All of the 9 HMNs were well separated.

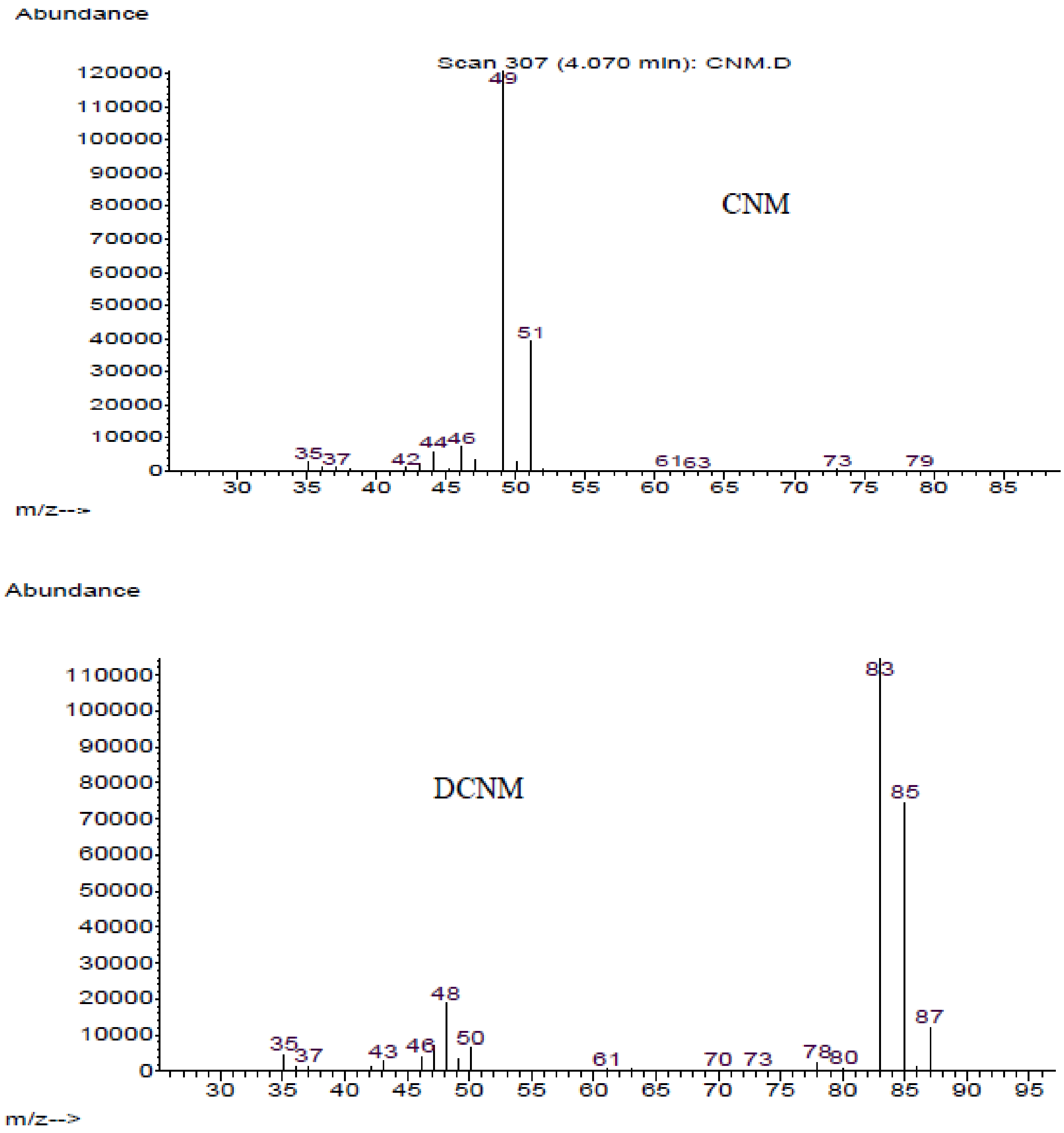


Figure 1. The mass spectra of HNMs

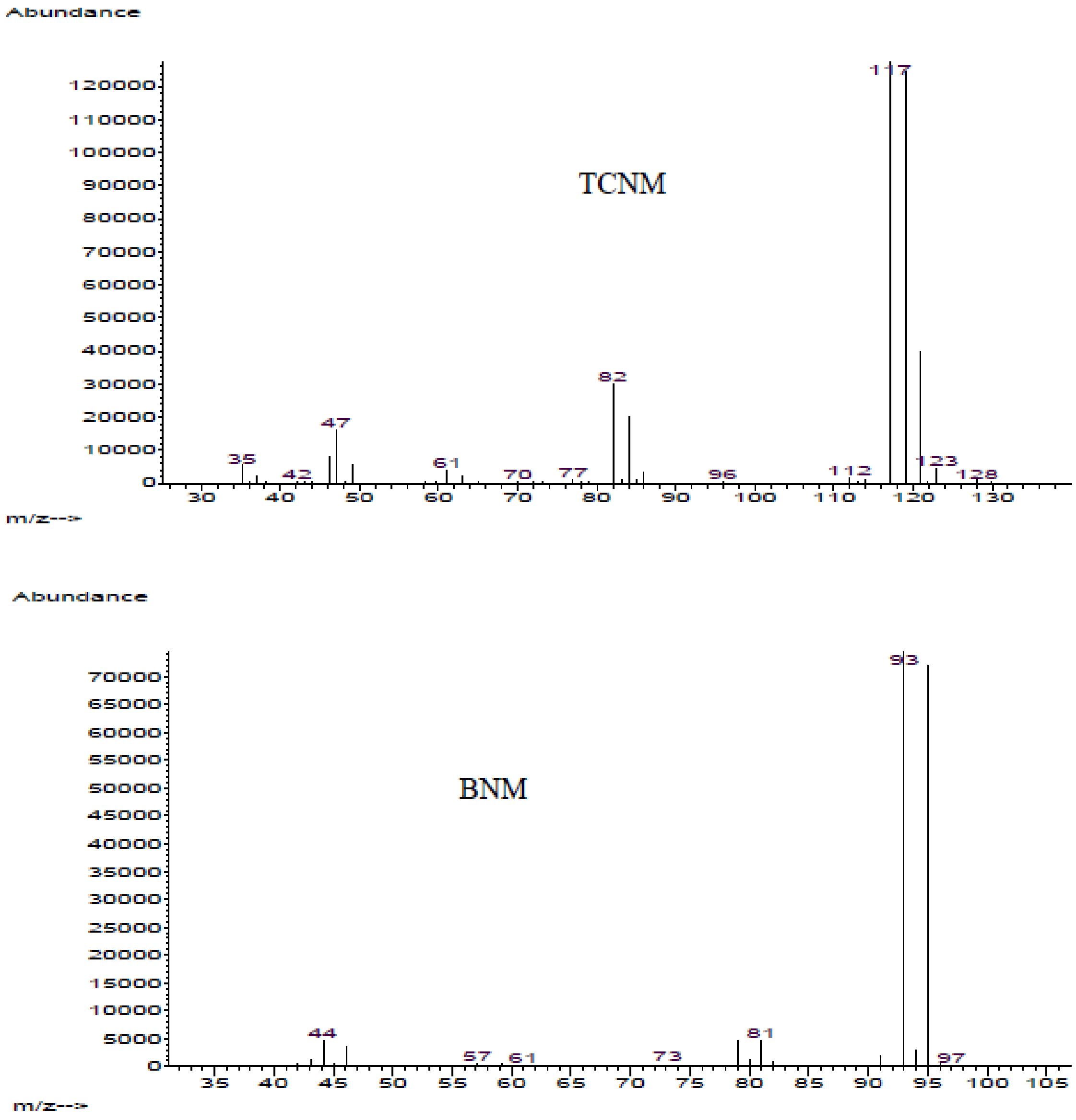


Figure 1. The mass spectra of HNMs (continued)

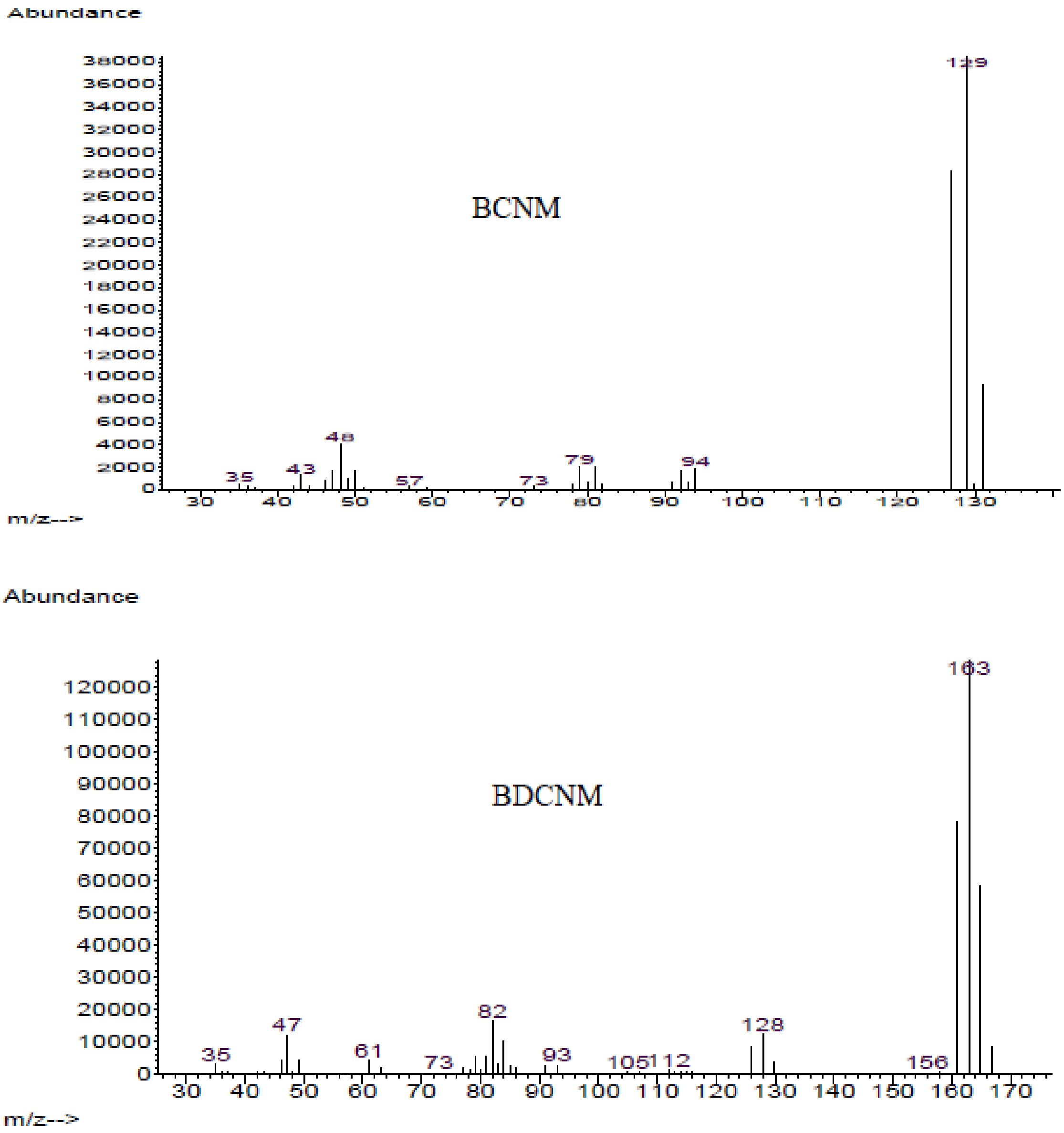


Figure 1. The mass spectra of HNMs (continued)

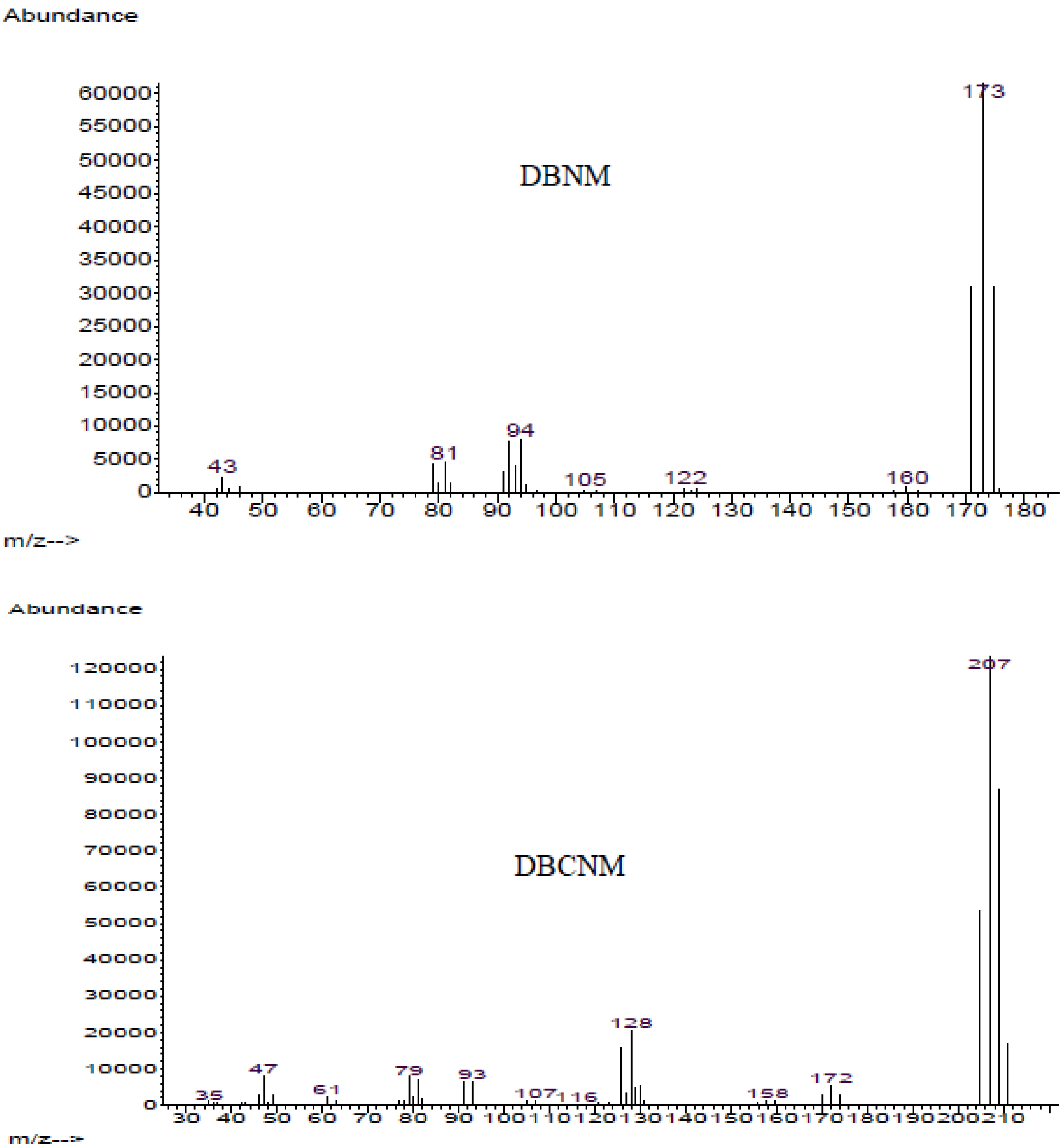


Figure 1. The mass spectra of HNMs (continued)

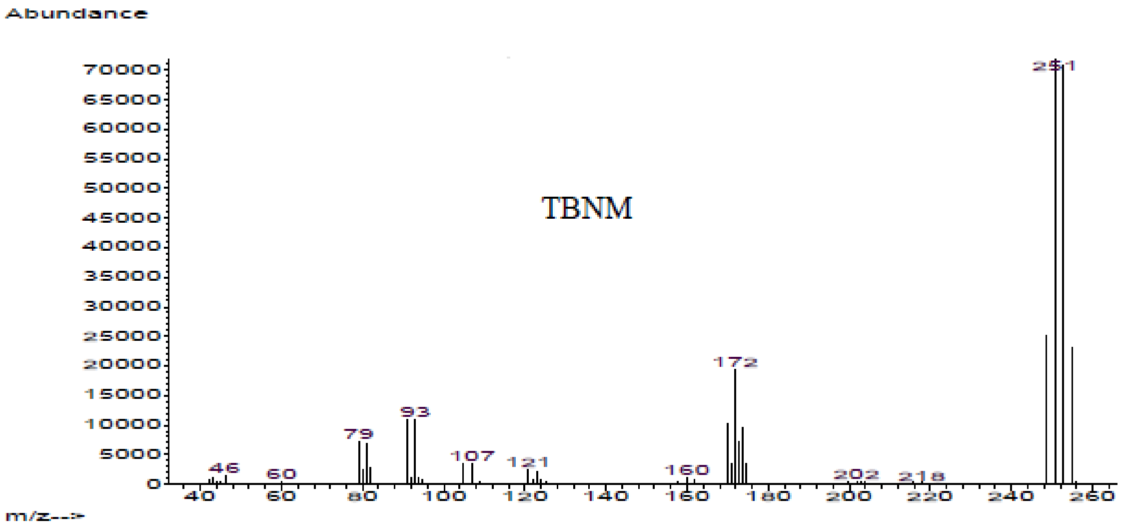


Figure 1. The mass spectra of HNMs (continued)

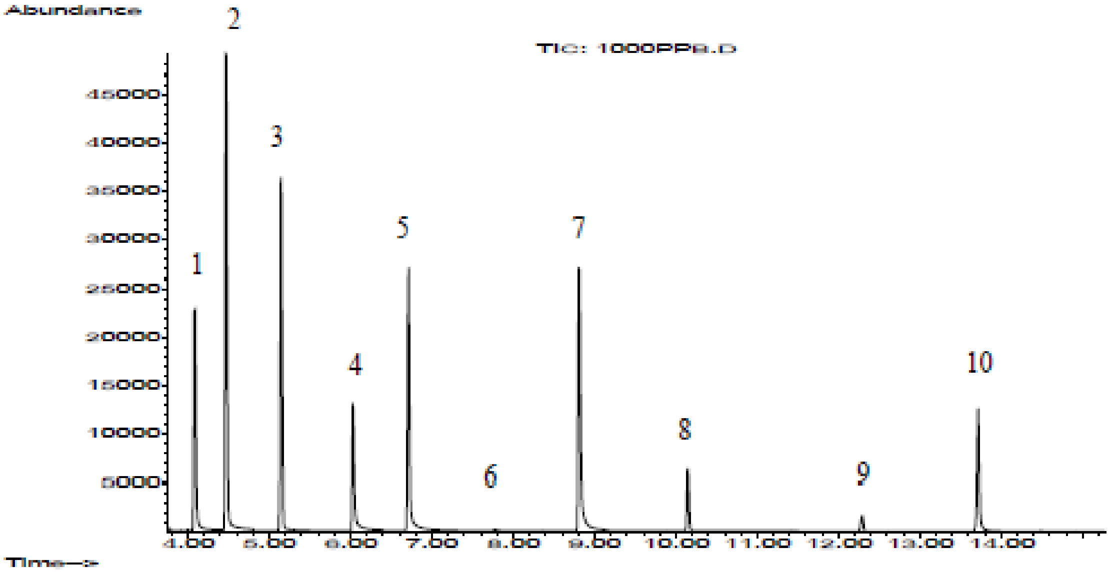


Figure 2. Chromatogram of nine halonitromethanes standards. Peak 6 (7.76 min) was very small due to high instability of the standard which degraded quickly after preparation.

Although TBNM, BDCNM, and DBCNM standards were tested to determine their spectra and retention times, these HNMs were found very unstable and decomposed quickly. For example, BDCNM degraded in several hours and when diluted in a mixture with other standards. Therefore, these compounds were not quantitatively analyzed in this study. For all the water samples analyzed in this study, there were no peaks observed at the retention times of these three unstable standards. These compounds might not be present in the water samples, or possibly were degraded during sample transportation and processing.

3.2. Factors influencing the extraction efficiency of HNMs

During the water collection excess ammonia chloride was added to the sample to quench the chlorine. To reduce the uncertainty associated with other factors despite of LLE, 20 µL 500 ppb internal standard (I.S.) was spiked to 1 ml extractant after the extraction. All the results was corrected by using relative peak area (RPA), which was performed by using the ratio of the HNMs peak area and the I.S. peak area.

Modified USEPA 551.1 method (J.W. Hodgeson, 1990) was utilized in this study in order to obtain maximum extraction efficiency. Sodium sulfate (Na_2SO_4) was added in the water sample to increase the ionic strength in the aqueous phase, as a result, further push HNMs into organic phase, in this case MTBE. The presence of sodium sulfate also decreases the solubility of MTBE in the water phase and increases the recovery. According to F.Q. Huang's study (Feng *et al.*, 2013), the amount of sodium sulfate plays a great role on the extraction of HNM, even though the ratio of Na_2SO_4 to water remains unknown. For both CNM and BNM, an increased trend was seen as sodium sulfate was increased, but for the other HNMs, the extraction efficiencies were decreased. Instead of using 20 g sodium sulfate for 50 ml water samples in USEPA 551.1 method (J.W. Hodgeson, 1990), 11g Na_2SO_4 was applied for 30 ml sample amount with decent recovery.

In the EPA standard method, the copper sulfate was not used during the extraction. Since copper sulfate is a good color indicator in the aqueous phase, it's much easier to distinguish the layers between aqueous solution and organic solution. Furthermore, there is no obvious effect on the extraction efficiency for the samples with or without copper sulfate according to Weinberg's study (Weinberg and Cook, 2002). And it is recommended to add copper sulfate during the extraction procedures in recent studies (Montesinos *et al.*, 2011; Song *et al.*, 2011).

In the aid of inhibiting the degradation of haloacetonitriles by base, the EPA 551.1 method advises to adjusting the pH to 4.8-5.5. To date, some research groups performed extraction under the pH ranging from 3.5-5.5 (Montesinos *et al.*, 2011). While others did not even conduct the pH adjustment (Song *et al.*, 2011)Chen reported TBNM requires a pH of 3.5-4.0 to minimize base-catalyzed hydrolysis in water (Chen *et al.*, 2009). Since how the pH will affect HNM extraction efficiency in LLE method remains unclear, pH range 3.5–5.0 was used in this study.

In conclusion, for extracting 30 ml water sample, 11 g of Na_2SO_4 and 1 g of $CuSO_4$ were added. Meanwhile pH was adjusted to 3.5–5.0. And these conditions were applied in all the following experiments

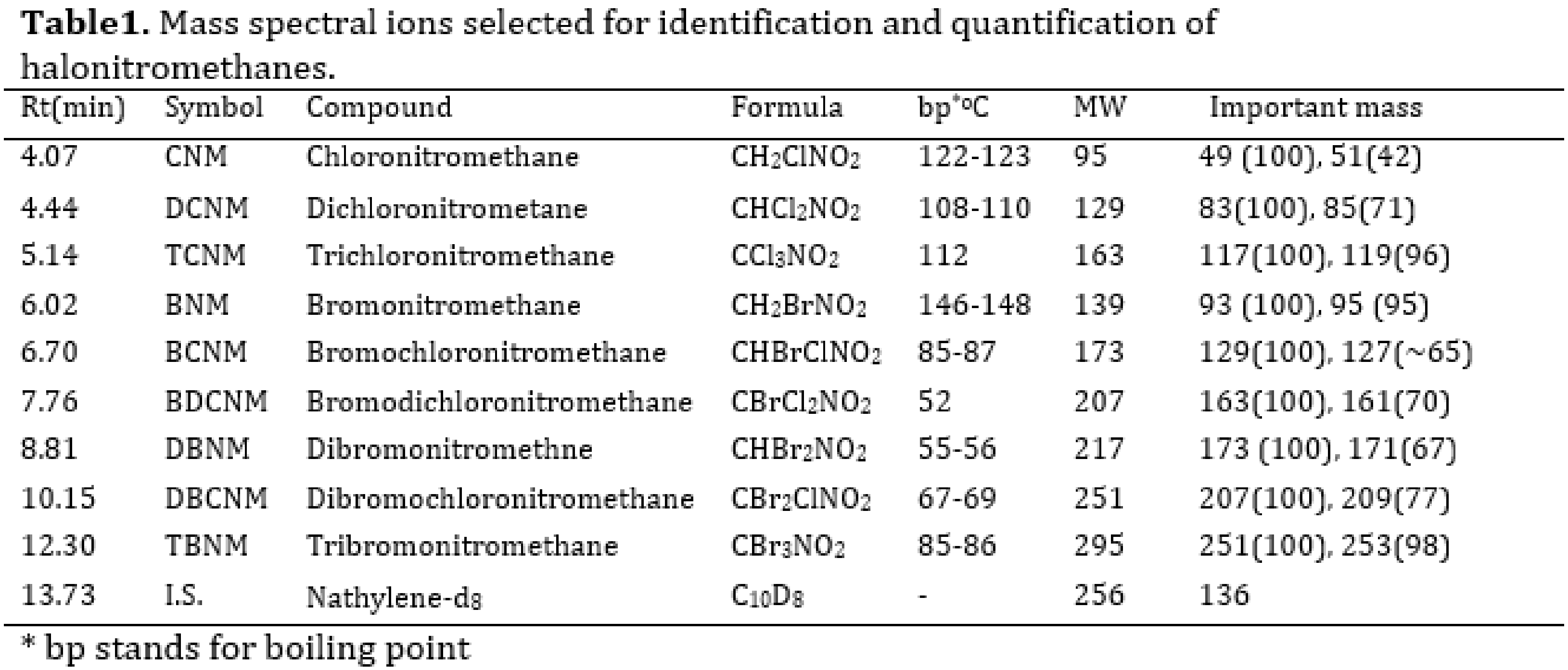


Table1. Mass spectral ions selected for identification and quantification of halonitromethanes.

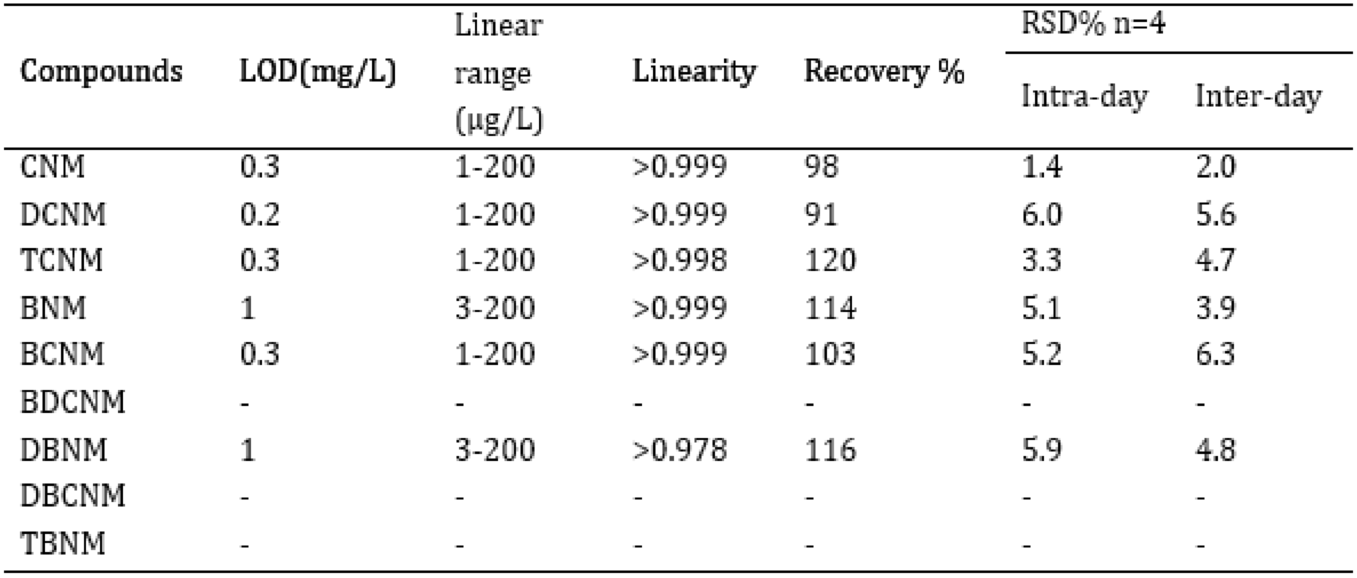
Rt(min)	Symbol	Compound	Formula	bp*°C	MW	Important mass
4.07	CNM	Chloronitromethane	CH_2ClNO_2	122-123	95	49 (100), 51(42)
4.44	DCNM	Dichloronitrometane	$CHCl_2NO_2$	108-110	129	83(100), 85(71)
5.14	TCNM	Trichloronitromethane	CCl_3NO_2	112	163	117(100), 119(96)
6.02	BNM	Bromonitromethane	CH_2BrNO_2	146-148	139	93 (100), 95 (95)
6.70	BCNM	Bromochloronitromethane	$CHBrClNO_2$	85-87	173	129(100), 127(~65)
7.76	BDCNM	Bromodichloronitromethane	$CBrCl_2NO_2$	52	207	163(100), 161(70)
8.81	DBNM	Dibromonitromethne	$CHBr_2NO_2$	55-56	217	173 (100), 171(67)
10.15	DBCNM	Dibromochloronitromethane	CBr_2ClNO_2	67-69	251	207(100), 209(77)
12.30	TBNM	Tribromonitromethane	CBr_3NO_2	85-86	295	251(100), 253(98)
13.73	I.S.	Nathylene-d_8	$C_{10}D_8$	-	256	136

* bp stands for boiling point

3.3. Quantitative calibration and reproducibility

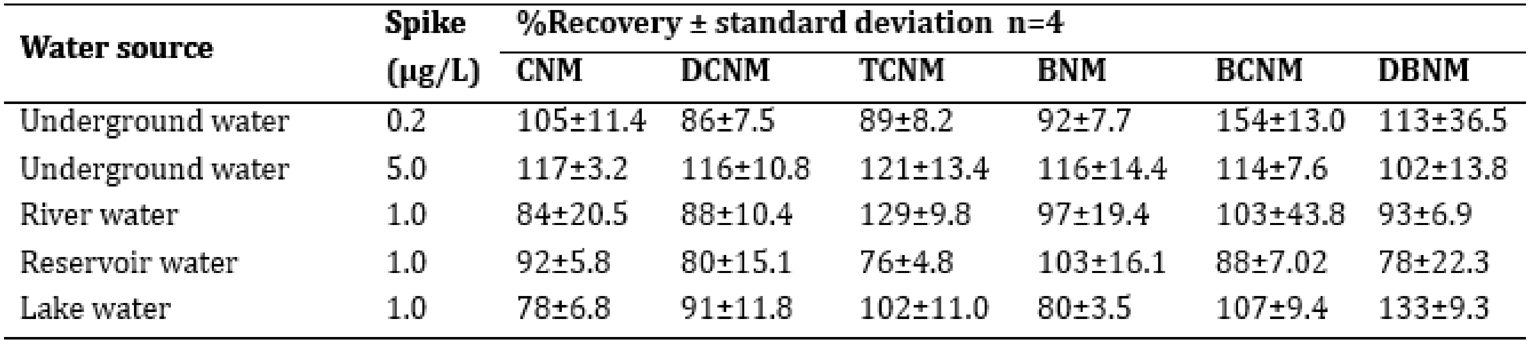
Several analytical curves for standards in water over the concentration up to 200 µg/L of HNMs were obtained by plotting the analyte to the internal standard peak area against the analyte concentration. The calibration curve for each halonitromethane throughout the experimental concentration range showed good linearity with the correlation coefficients (r^2) of ≥0.978. The limits of detection were defined as the concentration of the analyte that provided a chromatographic peak signal equal to three times the baseline (Signal/noise ratio), ranging from 0.2 µg/L for DCNM to 1µg/L for DBNM. As can be seen in Table 2, the LLE method was very sensitive and allowed the determination of DCNM, TCNM, BCNM and BDCNM sub ppb levels; the brominated compounds were those that presented the least sensitivity. The high degree of sensitivity achieved for TCNM, CNM and TCNM was noteworthy since it is the compound most frequently detected in drinking water. The reproducibility of the method proposed (analyzing water samples spiked with 5 µg/L of each HNM) was good, with RSD values from 1.4 to 6.0% (intra-day) and from 2.0 to 6.3%(inter-day).

Table 2. Analytical figures of merit for the determination of HNMs by LLE-GC-MS.



Compounds	LOD(mg/L)	Linear range (μg/L)	Linearity	Recovery %	RSD% n=4	
					Intra-day	Inter-day
CNM	0.3	1-200	>0.999	98	1.4	2.0
DCNM	0.2	1-200	>0.999	91	6.0	5.6
TCNM	0.3	1-200	>0.998	120	3.3	4.7
BNM	1	3-200	>0.999	114	5.1	3.9
BCNM	0.3	1-200	>0.999	103	5.2	6.3
BDCNM	-	-	-	-	-	-
DBNM	1	3-200	>0.978	116	5.9	4.8
DBCNM	-	-	-	-	-	-
TBNM	-	-	-	-	-	-

Table 3. spike recovery in real water samples



Water source	**Spike (μg/L)**	**%Recovery ± standard deviation n=4**					
		CNM	**DCNM**	**TCNM**	**BNM**	**BCNM**	**DBNM**
Underground water	0.2	105±11.4	86±7.5	89±8.2	92±7.7	154±13.0	113±36.5
Underground water	5.0	117±3.2	116±10.8	121±13.4	116±14.4	114±7.6	102±13.8
River water	1.0	84±20.5	88±10.4	129±9.8	97±19.4	103±43.8	93±6.9
Reservoir water	1.0	92±5.8	80±15.1	76±4.8	103±16.1	88±7.02	78±22.3
Lake water	1.0	78±6.8	91±11.8	102±11.0	80±3.5	107±9.4	133±9.3

Table 3 shows the spike recovery in real water samples. The recoveries of low level spikes were in the acceptable range of 76–133 % for all the HNMs. During water sample analysis, different concentrations of standards were also spiked in different water types and also for each batch of 10 samples or less, and the recoveries were tested.

3.4. Analysis of water samples

The water samples from 34 water treatment plants were analyzed in both winter and summer seasons in this study. The winter samples were collected during January and February, and the summer samples were collected in June and July. In addition to all of finished water samples, 10 representatives of each type of raw water samples were also analyzed (in winter and summer). The HNM concentrations detected in finished water samples are listed in Table4. The data for the raw water samples are not included in the table because only one detectable TCNM (0.58 μg/L) was found in one water sample (a reservoir water) from winter collection. TCNM was not detectable in the summer sampling water. All the other HNMs were not found in these representative raw water samples. For the finished waters, HNMs were found in waters from all types of water sources except the deep well water with non-detectable level or very low levels of HNMs. The highest concentration of HNMs was found in finished lake water at total HNMs 6.71 μg/L. Most of finished lake waters had relatively high concentration of HNMs. TCNM and BCNM were the major HNMs in most of water samples. Total HNMs were detected at higher overall concentrations in winter samples compared to summer samples.

More BCNM was found in the winter samples than summer samples. This correlated with the higher total bromine concentration in the winter water samples. For all of the samples detected, no TBNM, BDCNM and DBCNM peak were found. These three HNMs were either not presented in the water, or were degradated during transportation or analysis process. The sum of the HNMs in a U.S. water occurrence study reported in the range from not detectable to 10 μg/L, with the median concentration 1 μg/L in high-precursors loading(Richardson, 2007), including some trihalonitromethanes.

Table 4. HNM concentrations (µg/L) in water samples (W=winter, S=summer)

Plant	Water source	Treatment	CNM		DCNM		TCNM		BNM		BCNM		DBNM		Total HNM	
			W	S	W	S	W	S	W	S	W	S	W	S	W	S
01	Mississippi River	FC	<0.07	<0.07	0.31	<0.09	0.26	0.88	0.32	0.19	1.63	0.44	0.91	<0.20	3.43	1.51
12		FC	<0.07	<0.07	0.14	<0.09	0.44	0.99	0.17	0.12	1.24	0.26	<0.20	<0.20	1.98	1.50
02		Chloramines	<0.07	<0.07	<0.09	0.16	0.49	1.89	0.15	0.16	0.64	0.62	<0.20	<0.20	1.27	2.83
03	Missouri River	Chloramines	<0.07	<0.07	<0.09	0.19	0.33	0.38	0.13	<0.06	0.39	<0.10	<0.20	<0.20	0.85	0.57
04		Chloramines	<0.07	<0.07	<0.09	0.15	0.21	0.80	0.27	0.23	0.56	0.57	0.46	<0.20	1.50	1.75
11		Chloramines	<0.07	<0.07	0.32	0.24	1.16	0.50	0.25	<0.06	0.99	0.24	<0.20	<0.20	2.72	0.99
32	Big River	Chloramines	<0.07	<0.07	0.21	<0.09	0.31	0.29	<0.06	<0.06	0.16	0.28	<0.20	<0.20	0.68	0.87
30	Reservoirs	FC	<0.07	<0.07	<0.09	0.19	<0.10	0.48	<0.06	0.14	<0.10	0.51	<0.20	<0.20	ND	1.33
09		FC	<0.07	<0.07	<0.09	<0.09	0.25	0.39	0.14	0.15	1.33	0.64	<0.20	<0.20	1.72	1.27
38		Chloramines	<0.07	<0.07	<0.09	<0.09	<0.10	<0.10	<0.06	<0.06	<0.10	<0.10	<0.20	<0.20	ND	ND
39		Chloramines	<0.07	<0.07	<0.09	<0.09	<0.10	<0.10	<0.06	<0.06	<0.10	<0.10	<0.20	<0.20	ND	ND
10		Chloramines	<0.07	<0.07	0.23	<0.09	1.72	1.30	0.12	0.17	1.55	0.38	<0.20	<0.20	3.61	2.31
14	Lakes	Chloramines	<0.07	<0.07	0.24	<0.09	1.28	0.53	0.11	0.16	3.89	0.20	<0.20	<0.20	5.52	0.98
16		Chloramines	<0.07	<0.07	0.44	0.23	1.74	0.34	0.08	<0.06	4.45	<0.10	<0.20	<0.20	6.71	0.76
17		Chloramines	<0.07	<0.07	0.14	0.17	0.40	0.54	0.13	<0.06	0.27	0.29	<0.20	<0.20	0.93	1.00
28		Chloramines	<0.07	<0.07	<0.09	0.14	<0.10	0.64	<0.06	0.17	<0.10	0.63	<0.20	<0.20	ND	1.73
13		FC	<0.07	<0.07	0.23	<0.09	1.03	0.51	0.16	0.12	1.94	0.20	0.65	<0.20	4.01	0.97
15		FC	<0.07	<0.07	0.17	0.21	0.52	1.57	0.17	0.16	1.93	0.33	<0.20	<0.20	2.78	2.43
18		FC	<0.07	<0.07	0.15	<0.09	0.73	1.01	0.14	0.09	2.06	<0.10	<0.20	<0.20	3.08	1.24
27		FC	<0.07	<0.07	0.16	<0.09	0.38	0.48	0.13	0.08	2.21	<0.10	<0.20	<0.20	2.87	0.84
33		FC	<0.07	<0.07	0.13	<0.09	0.30	0.44	0.14	<0.06	3.94	0.33	<0.20	<0.20	4.50	0.93
29		FC	<0.07	0.18	0.16	0.18	0.34	0.76	0.21	0.16	1.58	0.33	<0.20	<0.20	2.28	1.61
31		FC	<0.07	<0.07	<0.09	0.20	<0.10	0.73	<0.06	0.13	<0.10	0.38	<0.20	<0.20	ND	1.58
34		FC	<0.07	<0.07	0.11	<0.09	0.26	0.95	0.14	0.10	1.36	<0.10	<0.20	<0.20	1.86	1.32

Table 4. HNM concentrations (µg/L) in water samples (W=winter, S=summer) (Continue)

Plant	Water source	Treatment	CNM		DCNM		TCNM		BNM		BCNM		DBNM		Total HNM	
			W	S	W	S	W	S	W	W	S	W	S	W	S	W
19	Deep wells	FC	<0.07	<0.07	<0.09	<0.09	0.14	<0.10	<0.06	<0.06	<0.10	<0.10	<0.20	<0.20	0.14	0.08
20		FC	<0.07	<0.07	<0.09	<0.09	<0.10	<0.10	<0.06	<0.06	<0.10	0.36	<0.20	0.27	0.00	0.90
21		FC	<0.07	<0.07	<0.09	<0.09	<0.10	0.16	<0.06	<0.06	<0.10	<0.10	<0.20	<0.20	0.00	0.26
22		FC	<0.07	<0.07	<0.09	<0.09	<0.10	0.15	<0.06	<0.06	<0.10	<0.10	<0.20	<0.20	0.00	0.25
07		FC	<0.07	<0.07	<0.09	<0.09	<0.10	0.16	<0.06	<0.06	0.27	<0.10	<0.20	<0.20	0.27	0.24
35		FC	0.60	<0.07	0.29	<0.09	<0.10	<0.10	<0.06	<0.06	<0.10	<0.10	<0.20	<0.20	0.89	ND
36		FC	<0.07	<0.07	<0.09	<0.09	<0.10	<0.10	<0.06	<0.06	<0.10	<0.10	<0.20	<0.20	ND	ND
37		FC	<0.07	<0.07	0.35	<0.09	<0.10	<0.10	<0.06	<0.06	0.16	<0.10	<0.20	<0.20	0.51	ND
08		Chloramines	<0.07	<0.07	<0.09	<0.09	<0.10	<0.10	<0.06	<0.06	0.17	<0.10	<0.20	<0.20	0.17	ND
05	Uncon and collector Wells	Chloramines	<0.07	<0.07	<0.09	<0.09	0.36	0.24	0.17	0.15	0.50	0.30	<0.20	<0.20	1.04	0.80
06		FC	<0.07	<0.07	<0.09	<0.09	0.22	0.20	0.20	0.20	0.56	0.27	<0.20	<0.20	0.99	0.79
23		FC	<0.07	<0.07	0.12	<0.09	<0.10	<0.10	0.19	0.15	0.56	0.34	0.91	0.21	1.78	0.81
24		FC	<0.07	<0.07	<0.09	<0.09	ND	0.18	<0.06	<0.06	<0.10	<0.10	<0.20	<0.20	ND	0.40
25		FC	<0.07	<0.07	0.31	0.15	0.30	0.52	0.18	0.20	0.80	0.32	<0.20	<0.20	1.59	1.18
26		FC	<0.07	<0.07	0.11	0.14	<0.10	0.25	0.24	0.32	0.59	0.44	0.24	<0.20	1.18	1.14

In general, the HNM concentrations were found lower in summer than winter samples. This may result from more rain in the summer time than the winter. For different type of water sources, the average total HNM concentrations were lowest in well waters. This was expected because underground water should have less total dissolved carbon (TOC), thus less precursors for HNM formation.

The disinfectants used were mainly free chlorine and chloramines. The HNM analysis results did not indicate significant difference for these two different disinfectants. However, the water sources were different for different treatments.

4. CONCLUSION

The modified LLE-GC-MS method provided good limits of detection for determining the selected HNMs. In comparison to EPA method 551, this method requires smaller volumes of extractant, providing LODs ranging from 0.2 to 1 μg/L. This methods involved manual extraction and internal standard spike, which provided good recoveries and precision. The spike recoveries of HNMs in the water influent showed a low variability throughout the day, with the RSDs less than 25% for most samples. However, for TBNM, BDCNM, and DBCNM, due to the instability, LLE was not suitable for the detection of those compounds.

The occurrence of selected six HNM was studied among the water treatment facilities in Missouri and Tulsa. The concentrations ranged from 0.12 to 4.45 μg/L in the finished water, which presented a lower level, compared to other countries. According to the results, all types of water sources contain HNMs after treatment except deep well water. Higher level of HNMs was found during the winter seasons than those in summer. The results from this drinking water study are consistent with the findings of this national wide study.

5. ACKNOWLEDGEMENT

This work has been supported by the Missouri Department of Natural Resources (MDNR), City of Tulsa and Environmental Research Center (ERC) at Missouri University of Science of Technology

6. REFERENCES

Barrionuevo, W.R. and F.M. Lancas, Comparison of liquid-liquid extraction (LLE), solid-phase extraction (SPE), and solid-phase microextraction (SPME) for pyrethroid pesticides analysis from enriched river water. Bull Environ Contam Toxicol, 2002. 69(1): p. 123-8.

Chang, H., C. Chen, and G. Wang, Identification of potential nitrogenous organic precursors for C-, N-DBPs and characterization of their DBPs formation. Water Res, 2011. 45(12): p. 3753-64.

Chen, B., et al., Fate of effluent organic matter and DBP precursors in an effluent-dominated river: a case study of wastewater impact on downstream water quality. Water Res, 2009. 43(6): p. 1755-65.

Feng, K., et al., [Species identification of Echinococcus isolates collected from canines and Tibetan foxes in Chengduo County, Qinghai Province]. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi, 2013. 31(3): p. 185-7.

Hong, H., et al., Use of multiple regression models to evaluate the formation of halonitromethane via chlorination/chloramination of water from Tai Lake and the Qiantang River, China. Chemosphere, 2014. 119C: p. 540-546.

Huang, H., et al., Formation of haloacetonitriles and haloacetamides during chlorination of pure culture bacteria. Chemosphere, 2013. 92(4): p. 375-81.

J.W. Hodgeson, A.L.C., Determination of chlorination disinfection by-products, chlorination solvents, and halogenated pesticides/herbicides in drinking water by liquid-liquid extration and gas chromatography with electron-capture detection . 1990: USEPA.

Krasner, S.W., et al., Impact of wastewater treatment processes on organic carbon, organic nitrogen, and DBP precursors in effuent organic matter. Environ Sci Technol, 2009. 43(8): p. 2911-8.

Luo, Q., et al., Simultaneous and high-throughput analysis of iodo-trihalomethanes, haloacetonitriles, and halonitromethanes in drinking water using solid-phase microextraction/gas chromatography-mass spectrometry: an optimization of sample preparation. J Chromatogr A, 2014. 1365: p. 45-53.

Mincher, B.J., et al., Free-radical chemistry of disinfection byproducts. 3. Degradation mechanisms of chloronitromethane, bromonitromethane, and dichloronitromethane. J Phys Chem A, 2010. 114(1): p. 117-25.

Montesinos, I., M.J. Cardador, and M. Gallego, Determination of halonitromethanes in treated water. J Chromatogr A, 2011. 1218(18): p. 2497-504.

Montesinos, I. and M. Gallego, Headspace gas chromatography-mass spectrometry for rapid determination of halonitromethanes in tap and swimming pool water. Anal Bioanal Chem, 2012. 402(7): p. 2315-23.

Montesinos, I. and M. Gallego, Solvent-minimized extraction for determining halonitromethanes and trihalomethanes in water. J Chromatogr A, 2012. 1248: p. 1-8.

Plewa, M.J., et al., Halonitromethane drinking water disinfection byproducts: chemical characterization and mammalian cell cytotoxicity and genotoxicity. Environ Sci Technol, 2004. 38(1): p. 62-8.

Pozzi, R., et al., Determination of nitrosamines in water by gas chromatography/chemical ionization/selective ion trapping mass spectrometry. J Chromatogr A, 2011. 1218(14): p. 1808-14.

Richardson, S.D., Water analysis: emerging contaminants and current issues. Anal Chem, 2007. 79(12): p. 4295-323.

Sa, C.S., R.A. Boaventura, and I.B. Pereira, Analysis of haloacetic acids in water and air (aerosols) from indoor swimming pools using HS-SPME/GC/ECD. J Environ Sci Health A Tox Hazard Subst Environ Eng, 2012. 47(2): p. 176-83.

Saar, E., et al., Comparison of extraction efficiencies and LC-MS-MS matrix effects using LLE and SPE methods for 19 antipsychotics in human blood. Anal Bioanal Chem, 2009. 393(2): p. 727-34.

Shi, H., Z. Qiang, and C. Adams, Formation of haloacetic acids, halonitromethanes, bromate and iodate during chlorination and ozonation of seawater and saltwater of marine aquaria systems. Chemosphere, 2013. 90(10): p. 2485-92.

Song, H., et al., Halonitromethanes formation in wastewater treatment plant effluents. Chemosphere, 2010. 79(2): p. 174-9.

Song, X.Z., et al., [Analysis of the clinical effects of the modified halo pelvic frame for the treatment of severe scoliosis with rigidity]. Zhongguo Gu Shang, 2011. 24(9): p. 737-41.

Weinberg, H.S. and S.J. Cook, Segmented flow injection, UV digestion, and amperometric detection for the determination of total cyanide in wastewater treatment plant effluents. Anal Chem, 2002. 74(23): p. 6055-63.

Yang, X., et al., Nitrogen origins and the role of ozonation in the formation of haloacetonitriles and halonitromethanes in chlorine water treatment. Environ Sci Technol, 2012. 46(23): p. 12832-8.

Yang, X., C. Shang, and P. Westerhoff, Factors affecting formation of haloacetonitriles, haloketones, chloropicrin and cyanogen halides during chloramination. Water Res, 2007. 41(6): p. 1193-200.

Yu, W., et al., Mechanism and direct kinetics study on the homogeneous gas-phase formation of PBDD/Fs from 2-BP, 2,4-DBP, and 2,4,6-TBP as precursors. Environ Sci Technol, 2011. 45(5): p. 1917-25.

III. Detection, occurrence, and comprehensive removal of selected pharmaceuticals in Missouri drinking water

Ruipu Mu[1]*, Honglan Shi[1], Craig Adams[2], Todd Eichholz[3], Yinfa Ma[1],

[1]Department of Chemistry and Environmental Research Center, Missouri University of Science and Technology, Rolla, MO 65409, USA

[2]Department of Civil and Environmental Engineering, Utah State University, Logan, UT 84322, USA

[3]Missouri Department of Natural Resources, Jefferson City, MO 65102, USA

* Corresponding Author

Address: Department of Chemistry

Missouri University of Science and Technology

400 West 11th Street

Rolla, MO 65409

Phone: 1-573-341-6220

Fax: 573-341-6033

E-mail: yinfa@mst.edu

Key words:

Missouri water, PPCP, Removal, Screening study, SPE-HPLC-MS/MS

Abstract

A simple, sensitive, and selective solid phase extraction – ultra-fast liquid chromatography – tandem mass spectrometry (SPE-UFLC-MS/MS) method was developed and validated for the quantification of selected pharmaceutical and personal care products (PPCPs) in water samples. The method detected the following six PPCPs: cotinine, cephapirin, ciprofloxacin, enrofloxacein, azithromycin and diphenhydramine at sub-ppb in multiple water matrices after clean-up and pre-concentration by SPE. Cotinine-d_3 and $^{13}C_3{}^{15}N$-Ciprofloxacin were used as internal standards for accurate quantitation. This method was validated through evaluation of spike recoveries (67-129%), reproducibility (RSD: 2.3-15.7%), and method detection limits (MDLs: 2-5ng/L). The resulting method significantly improved detection capabilities over existing EPA methods. Moreover, the method was validated in source and treated water from 13 Missouri water treatment facilities in a seasonal study. Only trace PPCPs were detected during winter months, reflecting seasonal precipitation and biodegradation phenomena.

1. INTRODUCTION

Pharmaceutical and personal care products (PPCPs) represent a widespread and pervasive class of environmental toxins with observable adverse effects present at very low environmental concentrations (ng/L) [1-5]. There are many types of pharmaceuticals on the market for various applications [6, 7]. In recent years, PPCPs have been widely reported in the environment, particularly in rivers, lakes, reservoirs, and even groundwater [6, 2, 4]. The widespread prevalence of these compounds in water systems has raised concerns regarding the long-term health effects of these bio-active compounds. Importantly, contaminated water not only affects organisms present in the contaminated water itself, but also affects municipalities that use the contaminated water for drinking water, because conventional water treatment methods often have limited success in comprehensive PPCP removal [8]. In addition, several studies have reported PPCPs in surface water [9, 10, 3], but source localization has remained a challenge for many of these studies, which further complicates regulation [11]. Moreover, low concentrations and natural concentration fluctuations such as diel and seasonal changes, further impede efforts to comprehensively detection and characterize PPCPs in water systems [12].

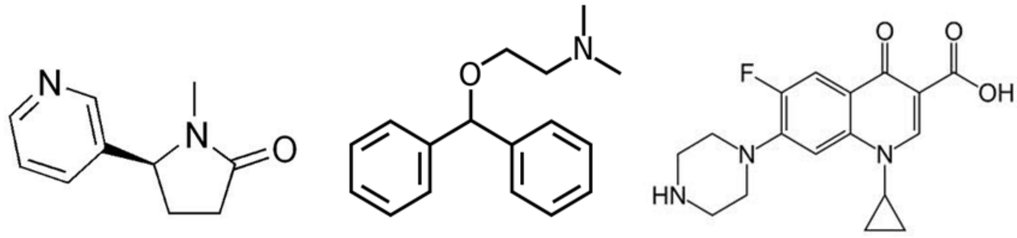
To address these growing concerns, recent attention has been placed on the development of analytical methods for the accurate detection of PPCPs in untreated and treated water matrices. Numerous methods have been developed on an array of analytical platforms, including liquid chromatography - mass spectrometry (LC-MS) [2. 13-16] and liquid chromatography - tandem mass spectrometry (LC-MS/MS) [17-21]. Nevertheless, the sub-ppb detection limits offered by many of these methods remains inadequate for PPCP detection in real water specimens. Since PPCPs concentration appear in nature water is lower than instrumental detection limited stated above, we have extended the work of these important methods through the addition of solid phase extraction (SPE) to reach the detection limits needed to detect PPCPs in real water samples [4, 13,22].

In this study, a solid phase extraction - ultra-fast liquid chromatography – tandem mass spectrometry method was developed to simultaneously detect six PPCPs and further validated in 13 source and treated Missouri drinking water facilities in a seasonal study.

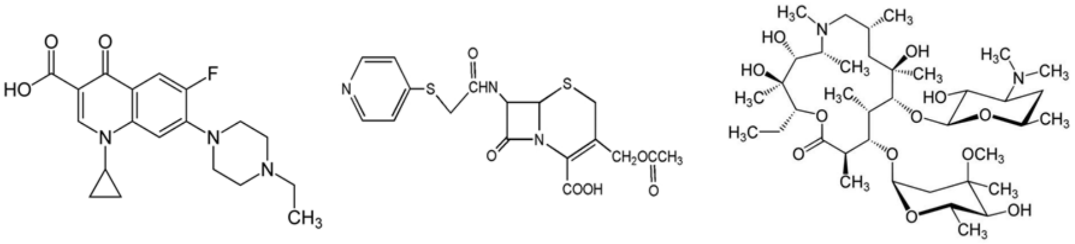
The basic information of selected 6 PPCPs compounds is shown in **Table 1**. The structures are shown in **Figure 1**.

Table 1. Pharmaceuticals selected for occurrence and treatability study in this research.

Compound	Abbreviation	CAS#	Formula	MW	Class
Cotinine	COT	486-56-6	$C_{10}H_{12}N_2O$	176.22	Nicotine metabolite
Cephapirin	CEP	21593-23-7	$C_{17}H_{17}N_3O_6S_2$	423.47	Antibiotic
Ciprofloxacin	CPF	85721-33-1	$C_{17}H_{18}FN_3O_3$	331.35	Antibiotic
Enrofloxacein	EFX	93106-60-6	$C_{19}H_{22}FN_3O_3$	359.40	Antibiotic
Azithromycin	AZI	83905-01-5	$C_{38}H_{72}N_2O_{12}$	749.05	Antibiotic
Diphenhydramine	DPH	58-73-1	$C_{17}H_{21}NO$	255.35	Antihistamine



Cotinine Diphenhydramine Ciprofloxacin



Enrofloxacein Cephapirin Azithromycin

Figure 1. The chemical structures of 6 selected PPCPs.

2. EXPERIMENTAL

2.1. Standards and Reagent

All chemicals and reagents used in this study were analytical grade or better unless otherwise stated. The six PPCPs including cotinine, diphenhydramine, ciprofloxacin, enrofloxacein, cephapirin and Azithromycin were obtained from Sigma-Aldrich (St. Louis, MO, USA); Stock solutions of cotinine, enrofloxacein and azithromycin were prepared by dissolving standards in acetonitrile (ACN), while ciprofloxacin, diphenhydramine and cephapirin were dissolved in a 1:1 ACN: ultra-pure water solution. Notably, ciprofloxacin required 0.1% formic acid for complete dissolution. The stock solutions were further diluted to desired concentrations with ultra-pure water generated from a Millipore Elix 3 water purification system (Millipore, Bedford, MA). Cotinine-d3 and 13C315N-Ciprofloxacin were used as internal standard (IS) and were purchased from Cambridge Isotope laboratories, Inc. (Tewksbury, MA, USA). EDTA and acetonitrile (ACN) were purchased from Fisher Scientific. The PPCP removal component of the study further utilized five different types of activated carbon, including WPH (Calgon Carbon Corporation), Hydrodarco 3000, Granular activated carbon 830 (H3000, GAC 830, Cabot Norit), charcoal, and bamboo (US Research Nanomaterials Inc.).

2.2. Instrumentation

An API 4000Q trap MS/MS system (Applied Biosystems, Foster City, CA) was used for the quantification of six selected PPCPs**.** All ions were monitored in scheduled multiple-reaction monitoring (MRM) mode with ESI-positive ionization. Optimized flow injection parameters include: ion spray voltage: 4900V; curtain gas: 16 psi; collision gas: 6 psi; GS1: 37 psi; GS2: 46 psi; and source temperature: 410°C. A Shimadzu UFLC system consisting of a degasser (DGU-30A3), two pumps (LC-20 AD XR), an auto sampler (SIL-20AC XR) and a column oven (CTO-20A) was used for the separation of the six selected PPCPs. The software program, Analyst 1.5, was used to interpret spectral acquisition and facilitate peak quantification.

2.3. Chromatographic Separation

The chromatographic separation was performed on a Phenomenex Synergi 2.5µ Max-RP column (100×2.00mm i.d. 2.5 µm particle size, Phenomenex, Torrance, CA, USA) at a flow rate of 0.25 mL/min with a run-to-run time of 15 min, and a 10 μL injection volume. The auto-sampler was kept at 15 °C and column was maintained at 40 °C. The mobile phase included both ACN and water (both containing 0.2% formic acid) and a gradient elution was used for the separation. The elution program is shown in **Table 2.**The extracted ion chromatogram (XIC) is shown in **Figure 2**.

Table 2. Gradient Program for UFLC separation of selected PPCPs.

Time(min)	Flow (μl min^{-1})	Eluent A H_2O, 0.2% Formic acid	Eluent B ACN, 0.2% Formic acid
0	250	97	3
7	250	40	60
9	250	40	60
10	250	97	3
15	250	97	3

Table 3. LC-MS/MS experimental conditions of the sixteen pharmaceutical compounds

Compound	RT (min)	Precursorion	Production	DP (V)	CE (V)	CXP (V)	I.S.
COT	2.53	177	80	66	31	8	Cotinine-d_3
CEP	4.52	424	152	61	39	14	$^{13}C_3{}^{15}N$-Ciprofloxacin
CPF	4.95	332	231	76	59	14	$^{13}C_3{}^{15}N$-Ciprofloxacin
EFX	5.10	360	245	66	37	16	$^{13}C_3{}^{15}N$-Ciprofloxacin
AZI	5.19	749	591	131	43	18	$^{13}C_3{}^{15}N$-Ciprofloxacin
DPH	6.31	256	167	26	27	8	$^{13}C_3{}^{15}N$-Ciprofloxacin

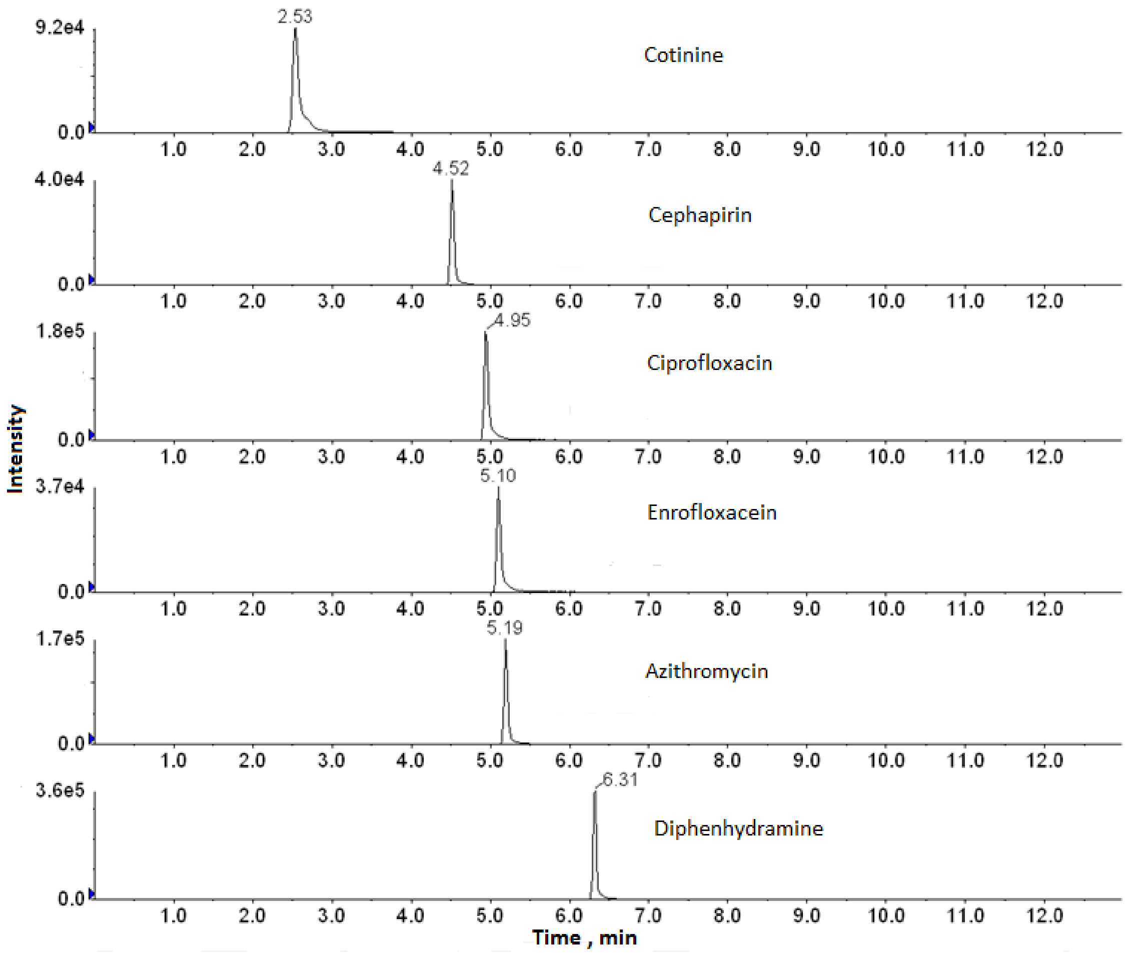


Figure 2. XIC Chromatograph of PPCPs in MQ water

2.4. Sample Collection

Water sample collection followed the EPA standard method (EPA 1649, 2007) using 500 ml brown amber bottles with Teflon caps. Two types of waters, including source and finished water, were collected at 13 Missouri drinking water facilities at February, May, August and November during the year. It should also be noted that the bottles contained chemical preservatives so that rinses were unnecessary prior to collection.

Briefly, the water samples were collected and prepared by the following outline. Prior to collection, aerators were removed and the line flushed for three minutes.

Upon adjusting the flow to desired levels, the bottle was filled completely. Special care was taken to avoid any headspace or trapped air in order to prevent analyte loss from evaporation into the headspace. The bottle was subsequently sealed and agitated for one minute, whereupon it was immediately refrigerated. Samples were shipped from the water facility to the analytical lab in ice for approximately 12 hours. All water samples were processed with 48 hrs after arrival to the analytical lab.

2.5. Sample Extraction

Before solid phase extraction, water pH was adjusted to 5.0 with hydrochloric acid. The samples were subsequently filtered with 0.45 µm Nylon filters and then further acidified to pH 2.0±0.5 using hydrochloric acid. Exactly 250 mg of $Na_4EDTA:2H_2O$ was added to 0.500 liters of each sample. Solid phase extraction was conducted using Waters Oasis HLB 3cc cartridges. Cartridges were conditioned with 3 ml methanol, 2 ml ultra-pure water, and 2 ml pH 2.0 ultra-pure water. The 500 ml aliquots were extracted at a flow rate of 1-2 drops per second. Next each cartridge was washed with 3 ml ultra-pure water to remove any residual EDTA. Cartridges were subsequently dried under vacuum for five minutes. Later cartridges were placed on the elution rack eluted using 5ml of Methanol for each cartridge. After that they were further eluted using 3ml of acetone: methanol (1:1). Then both eluents were combined and those centrifuge tubes were placed in Turbovap LV at 50±5°C. And those eluents were evaporated to 100 µL using a nitrogen stream of 10-15 psi. 900 µL of mobile phase (ACN: H_2O = 3:97) was added to each sample tube and vortex mixed. Finally they were transferred into 2-mL amber glass sample vials with Teflon liner screw caps and placed in refrigerator before LC/MS analysis.

3. RESULTS AND DISCUSSION

3.1. Mass Spectrometry Operation Parameters

Mass spectrometry utilized positive electrospray ionization (ESI) with multiple reaction monitoring (MRM) mode. The mass spectrometers parameters that were optimized for each compound included mass calibration, polarity of each compound, compound-dependent parameters, and source-dependent parameters. Compound and source depend parameters optimization were performed by using standard solutions of 100 ppb and 200 ppb (containing 0.2% formic acid) infused at 20μL/min by a syringe pump (Kd Scientific, Holliston, MA). After optimization, the ion transition with the most intense signal was selected as the quantification ion pair of the corresponding compound, while the ion transition with the second highest signal was selected as the confirmation ion pair of the corresponding compound. Due to the possible interferences from the real samples, the third highest signal may be chosen as confirmation ion pair. A dwell time of 100ms was used per ion pair monitored. Nitrogen for the curtain and collision gases was generated by a Peak Scientific N_2 Generator. The ion source temperature was set at 410°C with an ion spray voltage of 4900 V. The curtain gas, ion source gas 1, and ion source gas 2 were 16, 37, and 46 psi, respectively.

3.2. Method Validation (Linearity, Method Detection Limit, Quantification Limit and Reproducibility)

The PPCP calibration standards were analyzed to construct calibration curves and determine response linearity of the six selected PPCPs. The regression coefficients (R^2>0.995) were used to characterize the linearity of each calibration curve. The linearity, limit of detection (LOD) and the limit of quantitation (LOQ) have been summarized in **Table 4**.

Spiked recoveries were performed by spiking a desired concentration of PPCPs into 500 mL ultra-pure water and Mississippi river water, followed by SPE-UFLC-MS/MS analysis. In order to fully encompass the range of PPCP concentrations that would be detected in real water samples, high and low concentrations were selected (200 ppb and 20 ppb, respectively). In addition, spiked recoveries were normalized to the two selected isotope-labeled internal standards. Spiked recoveries were run in quadruplicate to provide sufficient statistical significance. The spiked recovery results were shown in **Table 5**.

According to Table 5, recoveries ranged from approximately 80% to 120% for high concentration spikes (200 ng/L) while low concentration spikes (20 ng/L) ranged from 65 to 130%. Moreover, decent reproducibility (<16% RSD) was observed for both ultra-pure and complex Mississippi river matrices. Notably, matrix effects can be observed through the slightly lower recoveries reported in the more complex Mississippi river samples. It should finally be noted that the selected isotope-labeled internal standards were non-ideal for several PPCPs, which may have further adversely affected spiked recoveries.

Table 4. LOD, LOQ, and linear range for the selected PPCPs

Compound	Ion Pair (m/z)	Limit of Detection (ng/L)[a]	Limit of Quantitation (ng/L)[b]	Linear Range[c] (µg/L)	Linearity (R^2)
Cotinine	117/80	4	8	0.1-100	0.9998
diphenhydramine	256/167	2	4	0.075-100	0.9999
Ciprofloxacin	332/314	5	12	0.1-100	0.9997
Enrofloxacein	360/342	5	11	0.1-100	0.9999
Cephapirin	424/292	4	9	0.1-100	0.9952
Azithromycin	750/591	2	5	0.1-100	0.9999
Cotinine-d_3	180/80		Internal standard		
$^{13}C_3{}^{15}N$-Ciprofloxacin	336/318		Internal standard		

[a] Limit of detection is the method limit of detection of each analyte with S/N>3

[b] limit of quantitation is the method limit of quantification of each anaylte with S/N>10

[c] Unless stated, all the data were with SPE, but calibration range and linearity were calculated based on pure standard in mobile phase without going throughSPE.

Table 5. Spike recovery[a] and RSD (n=4) of studied pharmaceuticals in MQ water and tap water matrix

Compound	Low level (20ng/L) spike						High level (200 ng/L) spike					
	Ultra-pure water			Mississippi river water			Ultra-pure water			Mississippi river water		
	Spike ng/L	Recovery %	RSD %	Spike ng/L	Recovery %	RSD %	Spike ng/L	Recovery %	RSD %	Spike ng/L	Recovery %	RSD %
COT	20	92.3	13.2	20	82.5	8.4	200	87.3	2.3	200	106.7	3.7
CEP	20	80.2	10.4	20	67.8	6.5	200	110.2	9.3	200	87.6	8.2
CPF	20	105.8	5.8	20	85.2	5.8	200	90.5	3.1	200	95.6	4.7
EFX	20	92.8	8.3	20	110.3	15.7	200	102.8	8.6	200	113.5	6.8
AZI	20	110.3	7.7	20	128.7	10.1	200	112.2	6.4	200	120.6	5.1
DPH	20	85.2	11.5	20	76.1	7.5	200	80.2	15.3	200	98.2	2.7

[a]All the spike recoveries were corrected by internal standards

3.3. PPCP Occurrence in Missouri Drinking Water

Different source waters were used in these water treatment plants including rivers, lakes, unconsolidated wells, and deep wells which represent the most common water types in Missouri. Conventional water treatment methods were utilized in these utilities such as primary disinfection, pre-sedimentation, rapid mix, flocculation, sedimentation, powdered activated carbon (PAC) adsorption, two-stage lime softening and chlorine and/or chloramine disinfection and distribution. The sequence and number of procedures varied among the 13 treatment facilities.

Moreover, the water samples from the 13 treatment facilities were analyzed seasonally in this study. The cold season samples were collected in November and February, while the hot season samples were collected in May and August. Both source water and treated water were collected at the same time to investigate the effects of water treatment process on PPCP occurrence. During the occurrence water analysis, at least one blank, one duplicate, and one spike were preceded with sample preparation and LC-MS detection for each batch. The choices of these sample matrices represented river water, lake water, well water, and reservoir water. The results were similar with the recoveries showed in Table 5. The PPCPs concentrations detected in these water samples have been tabulated in **Table 6** and **Table 7**.

In cold seasons, some of the pharmaceutical compounds were detected in the untreated water samples. All the pharmaceutical compounds monitored in this study were detected in at least one of the source waters. Some of them were even detected in the finished water samples. No single PPCP was detected in all facilities.

In most cases, either low concentrations or concentrations below the method detection limit were reported in finished water compared with raw water samples.

COT was detected in three of 13(23%) selected water treatment facilities in both raw and finished waters at concentrations up to 13.1 ng/L. CEP was detect in 5 of 13 (38%) water facilities with a maximum concentration of 7.5 ng/L. CPF was also found in both treated and untreated waters. And all of samples with detectable PPCPs were from surface water (river and lake) with a highest concentration of 15.4 ng/L. EFX was also detected in three facilities with a concentration ranging from 5.2 ng/L to 11.4 ng/L. AZI and DPH were negligibly detected. Moreover, all PPCPs were detected in river water samples. These results indicate that the concentrations of detected pharmaceutical compounds were water source dependent and were usually higher in surface waters which include river water and lake water in comparison with groundwater.

In warm seasons, fewer PPCPs were detected across all 13 water facilities. The relative concentrations of the detected compounds in different types of water sources in water samples during the summer shared a trend similar to that of water samples collected during the winter season. As water temperatures rise during the summer months, biodegradation of these PPCPs may occur at an increased rate, causing the apparent decrease in PPCP concentrations. Detection of these biodegradates is therefore urgently needed to determine the fates of these environmentally harmful PPCPs and their derivatives. In addition, Missouri water tables generally drop and rivers slow during the dry winter months, further concentrating any PPCPs in the watershed

Finally, this study corroborates a nationwide PPCP occurrence study conducted by the U.S. Geological Survey [23](Focazio et al., 2008) which concluded that PPCP concentrations generally lie in the sub-mg/L range.

Table 6.PPCPs Occurrence in May and August

#	Water source	treatment	Water type	Concentration (ng/L)											
				COT[a]		CEP		CPF		EFX		AZI		DPH	
1	MS river	FC	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	3.8	<MDL	3.0	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
2	River	CA	Untreated	<MDL	7.5	<MDL	<MDL	<MDL	<MDL	6.4	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
3	Well	FC	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
4	Lake	FC	Untreated	<MDL	<MDL	<MDL	<MDL	7.3	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
5	MO river	CA	Untreated	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL
			Treated	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL
6	River	FC	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
7	Well	None	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA
8	MS river	CA	Untreated	5.5	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
9	MS river	FC	Untreated	<MDL	<MDL	<MDL	<MDL	5.9	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
10	Well	FC	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
11	River	FC	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	5.3	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
12	Well	CA	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	5.2	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
13	Lake	FC	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL

[a] First column presents the water samples collected in May; second column shows the data for August

*All the results were corrected by the internal standards.

Table 7. PPCPs Occurrence in November and February

#	Water source	treatment	Water type	Concentration (ng/L)											
				COT[a]		CEP		CPF		EFX		AZI		DPH	
1	MS river	FC	Untreated	<MDL	<MDL	<MDL	5.4	<MDL	5.5	<MDL	<MDL	<MDL	<MDL	<MDL	3.5
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
2	River	CA	Untreated	<MDL	9.5	<MDL	<MDL	9.2	<MDL	<MDL	11.4	<MDL	<MDL	<MDL	3.8
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
3	Well	FC	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
4	Lake	FC	Untreated	<MDL	<MDL	<MDL	<MDL	15.4	6.1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
5	MO river	CA	Untreated	<MDL	<MDL	2.2	2.7	<MDL	7.6	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
6	River	FC	Untreated	<MDL	<MDL	4.3	7.5	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
7	Well	None	Untreated	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA
			Treated	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA
8	MS river	CA	Untreated	13.1	<MDL	2.5	2.8	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
9	MS river	FC	Untreated	<MDL	<MDL	<MDL	3.1	<MDL	<MDL	6.0	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
10	Well	FC	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
11	River	FC	Untreated	7.1	<MDL	3.5	<MDL	<MDL	<MDL	5.2	7.3	<MDL	<MDL	<MDL	32.1
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
12	Well	CA	Untreated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	21.0	<MDL	<MDL	<MDL
			Treated	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
13	Lake	FC	Untreated	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL
			Treated	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL	NA	<MDL

[a] First column presents the water samples collected in November, second column shows the data for February.

*All the results were corrected by the internal standards.

3.4. Comprehensive Removal Study

In general, the concentrations of selected PPCPs in finished drinking water were lower than in untreated source water. These results may be readily attributed to water treatment processes, such as clarification, disinfection, and activated carbon sorption, used by the water treatment facilities. In this study, five activated carbons were evaluate for their PPCP removal capabilities, including WPH, H3000, GAC830, charcoal and bamboo. Treatments were carried out in 5 mM phosphate buffer systems at pH 6.6 and 8.6. The starting concentration of PPCPs was 5 µg/L with a typical dosage of activated carbon 2 mg/L and a contact time of 4 hrs. After centrifugation (3000 *g)* the supernatant was filtered with a 0.22 µm Nylon filter. Then samples were transferred to auto-sampler vials and analyzed by the SPE-UFLC-MS/MS method. All samples were run in duplicate. **Figure 3** and **Figure** 4 showed the removal efficiency of different activated carbon at different pHs.

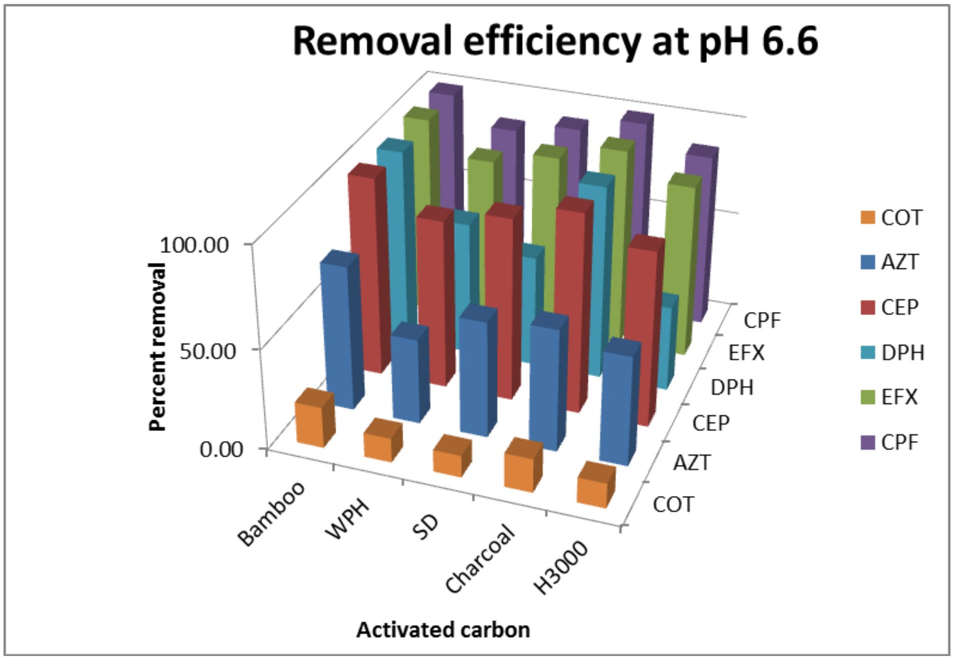


Figure 3. PPCPs removal at pH 6.6

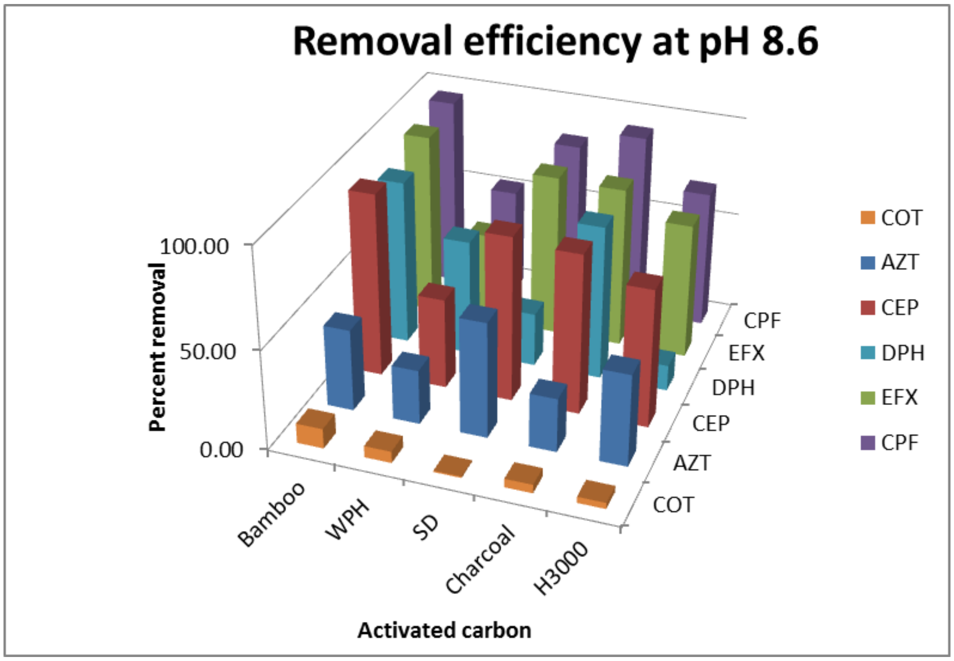


Figure 4. PPCPs removal at pH 8.6

Base on the results, the removal of selected PPCPs varied significantly with activated carbon type. Notably, bamboo and charcoal were highly effective in the elimination of the selected PPCPs and represent low-cost alternatives to other commercial solutions. In addition, no single activated carbon type effectively removed all six selected PPCPs.

4. CONCLUSION

A simple, rapid, specific, and sensitive SPE-UFLC-MS/MS method was developed and validated for quantification of six selected PPCPs in source and treated water matrices. This method was validated through evaluation of spike recoveries (67-129%), reproducibility (RSD: 2.3-15.7%), and method detection limits (MDLs: 2-5ng/L). The resulting method significantly improved detection capabilities over existing EPA methods. Several ng/L of PPCPs can be detected in different water samples with going through SPE which make the method feasible for PPCP screening in water samples.

PPCP occurrence was characterized in 13 water treatment facilities across Missouri in a seasonal study. Total PPCP content was reported to be less than 35 ng/L for all facilities. Moreover, most PPCPs were undetectable by the developed method, indicating safe levels in Missouri drinking water, although PPCP levels were highly dependent on water source type. Higher level was observed in surface water than those in ground water. Finally, five activated carbons were evaluated for their efficiency in removing PPCPs from water matrices. Bamboo was reported to provide the most comprehensive PPCP removal.

5. ACKNOWLEDGEMENT

This study was funded by the Missouri Department of Natural Resources (MDNR). We also thank the Environmental Research Center at Missouri University of Science and Technology for the valuable support.

6. REFERENCES

[1] Xu, J., Wu, L., Chang, A.C., 2009. Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. Chemosphere 77, 1299-1305.

[2] Chen, F., Ying, G.G., Yang, J.F., Zhao, J.L., Wang, L., 2010. Rapid resolution liquid chromatography-tandem mass spectrometry method for the determination of endocrine disrupting chemicals (EDCs), pharmaceuticals and personal care products(PPCPs) in wastewater irrigated soils. Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes 45, 682-693.

[3] Ferguson, P.J., Bernot, M.J., Doll, J.C., Lauer, T.E., 2013. Detection of pharmaceuticals and personal care products (PPCPs) in near-shore habitats of southern Lake Michigan. The Science of the total environment 458-460, 187-196.

[4] Liu, J.L., Wong, M.H., 2013. Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environment international 59, 208-224.

[5] Dai, G., Huang, J., Chen, W., Wang, B., Yu, G., Deng, S., 2014. Major pharmaceuticals and personal care products (PPCPs) in wastewater treatment plant and receiving water in Beijing, China, and associated ecological risks. Bulletin of environmental contamination and toxicology 92, 655-661.

[6] Boyd, G.R., Palmeri, J.M., Zhang, S., Grimm, D.A., 2004. Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and Bayou St. John in New Orleans, Louisiana, USA. The Science of the total environment 333, 137-148.

[7] Kosma, C.I., Lambropoulou, D.A., Albanis, T.A., 2010. Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece. Journal of hazardous materials 179, 804-817.

[8] Westerhoff, P., Yoon, Y., Snyder, S., Wert, E., 2005. Fate of Endocrine-Disruptor, Pharmaceutical, and Personal Care Product Chemicals during Simulated Drinking Water Treatment Processes. Environ. Sci. Technol. 39, 6649-6663.

[9] Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000: A National Reconnaissance. Environ. Sci. Technol. 36, 1202-1211.

[10] Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stanford, B.D., Snyder, S.A., 2008. Pharmaceuticals and Endocrine Disrupting Compounds in U.S. Drinking Water. Environ. Sci. Technol. 43, 597-603.

[11] Wu, M., Janssen, S., 2010. Dosed Without Prescription: A Framework for Preventing Pharmaceutical Contamination of Our Nation's Drinking Water. Environ. Sci. Technol. 45, 366-367.

[12] Nelson, E.D., Do, H., Lewis, R.S., Carr, S.A., 2010. Diurnal Variability of Pharmaceutical, Personal Care Product, Estrogen and Alkylphenol Concentrations in Effluent from a Tertiary Wastewater Treatment Facility. Environ. Sci. Technol. 45, 1228-1234.

[13] Kerns, E.H., Kleintop, T., Little, D., Tobien, T., Mallis, L., Di, L., Hu, M., Hong, Y., McConnell, O.J., 2004. Integrated high capacity solid phase extraction-MS/MS system for pharmaceutical profiling in drug discovery. Journal of pharmaceutical and biomedical analysis 34, 1-9.

[14] Naxing Xu, R., Fan, L., Kim, G.E., El-Shourbagy, T.A., 2006. A monolithic-phase based on-line extraction approach for determination of pharmaceutical components in human plasma by HPLC-MS/MS and a comparison with liquid-liquid extraction. Journal of pharmaceutical and biomedical analysis 40, 728-736.

[15] Pan, C., Liu, F., Ji, Q., Wang, W., Drinkwater, D., Vivilecchia, R., 2006. The use of LC/MS, GC/MS, and LC/NMR hyphenated techniques to identify a drug degradation product in pharmaceutical development. Journal of pharmaceutical and biomedical analysis 40, 581-590.

[16] Tran, N.H., Hu, J., Ong, S.L., 2013. Simultaneous determination of PPCPs, EDCs, and artificial sweeteners in environmental water samples using a single-step SPE coupled with HPLC-MS/MS and isotope dilution. Talanta 113, 82-92.

[17] Hirsch, R., Ternes, T.A., Haberer, K., Mehlich, A., Ballwanz, F., Kratz, K.L., 1998. Determination of antibiotics in different water compartments via liquid chromatography-electrospray tandem mass spectrometry. Journal of chromatography. A 815, 213-223.

[18] Croley, T.R., Hughes, R.J., Koenig, B.G., Metcalfe, C.D., March, R.E., 2000. Mass spectrometry applied to the analysis of estrogens in the environment. Rapid communications in mass spectrometry : RCM 14, 1087-1093.

[19] Jeannot, R., Sabik, H., Sauvard, E., Genin, E., 2000. Application of liquid chromatography with mass spectrometry combined with photodiode array detection and tandem mass spectrometry for monitoring pesticides in surface waters. Journal of chromatography. A 879, 51-71.

[20] Lagana, A., Bacaloni, A., Fago, G., Marino, A., 2000. Trace analysis of estrogenic chemicals in sewage effluent using liquid chromatography combined with tandem mass spectrometry. Rapid communications in mass spectrometry : RCM 14, 401-407.

[21] Asperger, H., Wagner, M., Brandl, E., 2001. An approach towards public health and foodborne human listeriosis--the Austrian Listeria monitoring. Berliner und Munchener tierarztliche Wochenschrift 114, 446-452.

[22] Vanderford, B.J., Snyder, S.A., 2006. Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry. Environmental science & technology 40, 7312-7320.

SECTION

2. REMOVAL STUDY OF SELECTED PPCPS

2.1 OBJECTIVES

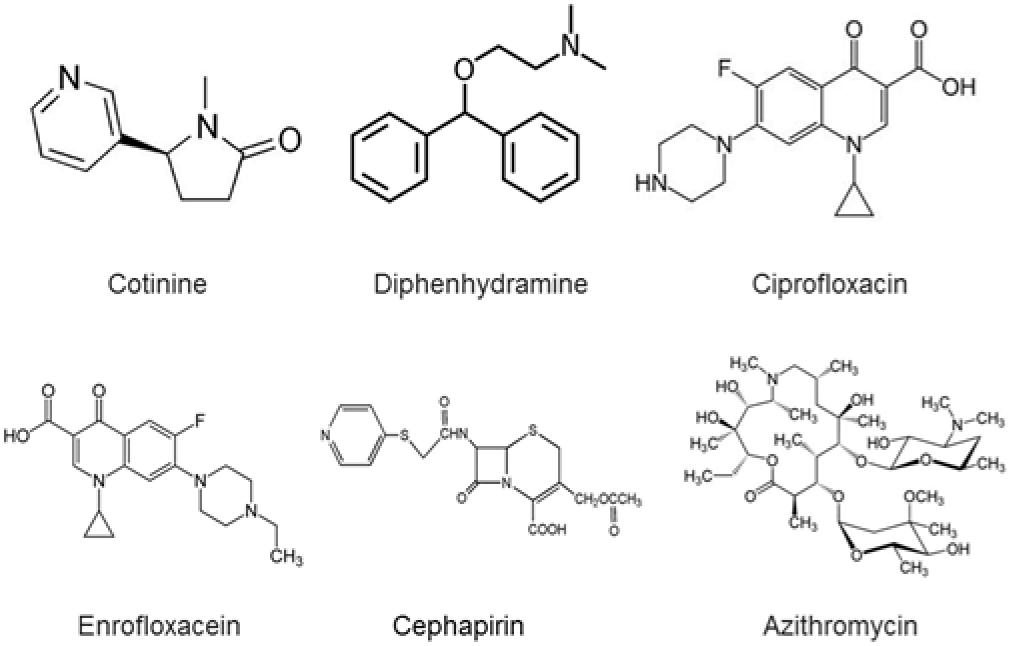
The overall research goal is removal of newly selected PPCPs in Missouri natural and drinking water. We have developed a sensitive UFLC-MS\MS method for a group of newly selected PPCPs including 6 compounds as listed in **Table 1**. Occurrence screening/removal study of this group PPCPs in 13 Missouri drinking water systems have been conducted before, including hot and cold seasons for both source and finished waters. It provides important data for MDNR and is important to protect Missouri citizens. In this study, we conducted a continuous study to explore the PPCPs in Missouri drinking water systems and the effective ways to remove these selected PPCPs from water by various activated carbons.

2.2. EXPERIMENT, RESULTS, AND DISCUSSION

2.2.1. Information of Selected PPCPs. All chemicals and reagents used in this study were analytical grade or better unless otherwise stated. Cotinine, diphenhydramine, ciprofloxacin, enrofloxacein, cephapirin and azithromycin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of cotinine, enrofloxacein and azithromycin were prepared by dissolving standards in acetonitrile (ACN) while ciprofloxacin, diphenhydramine and cephapirin were dissolved in a 1:1 ACN: water ratio. However, the ciprofloxacin stock solution needed the addition of 0.1% formic acid to get fully dissolved. Other concentrations were prepared by diluting standards in Milli-Q water which was produced with a Millipore Elix 3 water purification system (Millipore; Bedford, MA). Super activated carbon nano powders (raw material bamboo, coconut and charcoal) were purchased from US research nanomaterials Inc. (Houston, TX, USA). Hydrodarco 3000(H3000), Norit MSDS (NS) and Hydrodarco B (HB) were obtained from Norit Americas Inc. (Marshall, TX, USA). Aqua Nuchar (AQ) and WPH are purchased from MWV Specialty chemicals (North Charleston, SC, USA) and Calgon Carbon Corporation (Pittsburgh, PA, USA) respectively. Chemical information of 6 selected PPCPs, are listed in Table 2.1and Figure 2.1.

Table 2.1.Pharmaceuticals selected for occurrence and treatability study in this research

Compound	**Formula**	**MW**	**Class**	**Abbreviations**
Cephapirin	$C_{17}H_{17}N_3O_6S_2$	423.466	antibacterial	CEPR
Azithromycin	$C_{38}H_{72}N_2O_{12}$	749	antibiotic	AZT
Enrofloxacein	$C_{19}H_{22}FN_3O_3$	359.4	antibiotic	EFX
diphenhydramine	$C_{17}H_{21}NO$	255.355	antihistamine	DPH
Cotinine	$C_{10}H_{12}N_2O$	176.22	nicotine metabolite	COT
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	331.346	antibiotic	CIPX



Cotinine Diphenhydramine Ciprofloxacin

Enrofloxacein Cephapirin Azithromycin

Figure 2.1**.** The chemical structures of 6 selected PPCPs

2.2.2. Instrumentation. A 4000Q TRAP mass spectrometer (AB SCIEX, Foster City, CA) equipped with an electrospray ionization (ESI) interface. The Shimadzu UFLC system consisted of a degasser (DGU-30A3), two pumps (LC-20 AD XR), an auto sampler (SIL-20AC XR) and a column oven (CTO-20A). The software program that provided the data platform for spectral acquisition and peak quantification was Analyst 1.5.

2.2.3. Chromatographic Separations. The chromatographic separation was performed on a Phenomenex Synergi 4μ Max-RP column (150×2.00 mm i.d., 4-μm particle size, Phenomenex, Torrance, CA, USA) at a flow rate of 0.25 mL/min with an analysis time of 15 min, and the injection volume was 10 μL. The auto-sampler was kept at 15 °C. The mobile phase was composed of ACN and water (both containing 0.2% formic acid) and gradient elution was used for the separation.

2.2.4. PPCP Removal by Activated Carbon.

2.2.4.1. PACs dosage effects and pH effects. The majority of the experiments were conducted using AN and HB PAC. The experiments to compare different PAC types on their adsorption efficiency were conducted using all two PACs: HB and AN. For each of these experiments, the initial concentration was 5 mg/L for all six PPCPs; PAC dosages were 0, 1, 2, 5, 10, 20, and 40 mg/L; with a sampling time of 2 h. Each PAC was dried in an oven at 110 °C overnight prior to use. A 400 mg/L PAC stock suspension solution was prepared by stirring the PAC in DI water for at least 20 min. The water samples were buffered with 5 mM phosphate to pH of 5.7, 7.5, 8.2, and 10.7. The adsorption experiments were initiated by adding 25 μL of 1000 mg/L PPCPs stock to 8-mL glass vials. Next, different amount of the PAC suspension and buffer solution were added into each treatment vial to make a total final volume of 5 mL, with 5 mg/L PPCPs and varied PAC dosages. Duplicates tests were performed. The samples were placed in the labquake rotisserie for 2 hours, and then centrifuged to separate liquid from the solid adsorbents. The liquid samples were filtered before analyzed by the UFLC-MS/MS method. A blank of 5 μg/L PPCPs was measured initially and after 2 hours.

According to the results from Figure 2.2-2.7, pH and dosing concentration played an important role in removing different PPCPs. For most of the compound, 5 mg/L PAC dosage is enough for removing 80% for most selected PPCPs. There is only 60% removal for COT for 10 mg/L PAC dosing and HB seems work better than AN. The removal efficiency of PPCPs may vary by different activated carbons, pHs and dosing concentration of PACs.

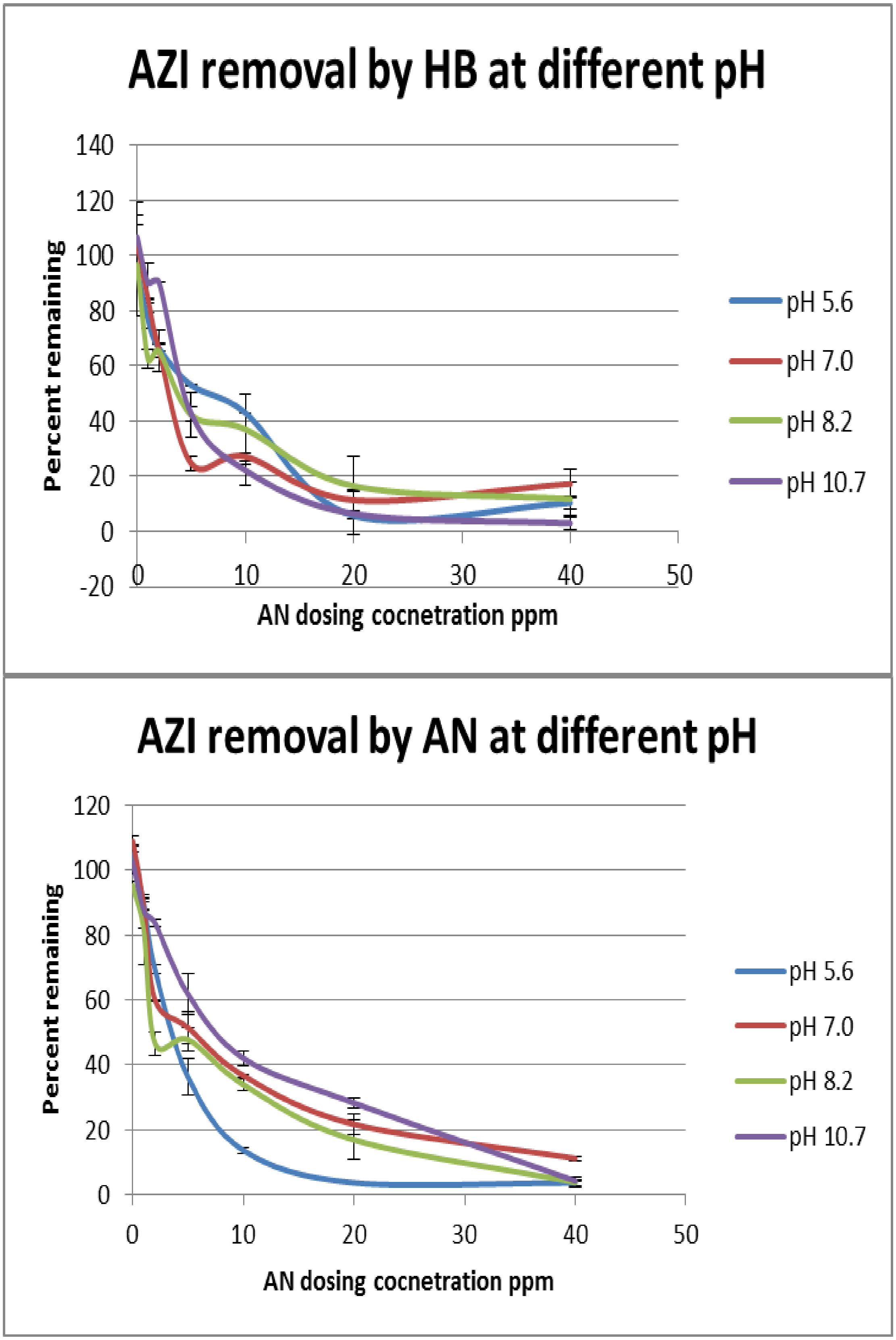


Figure 2.2. AZI removal by AN and HB at different pHs

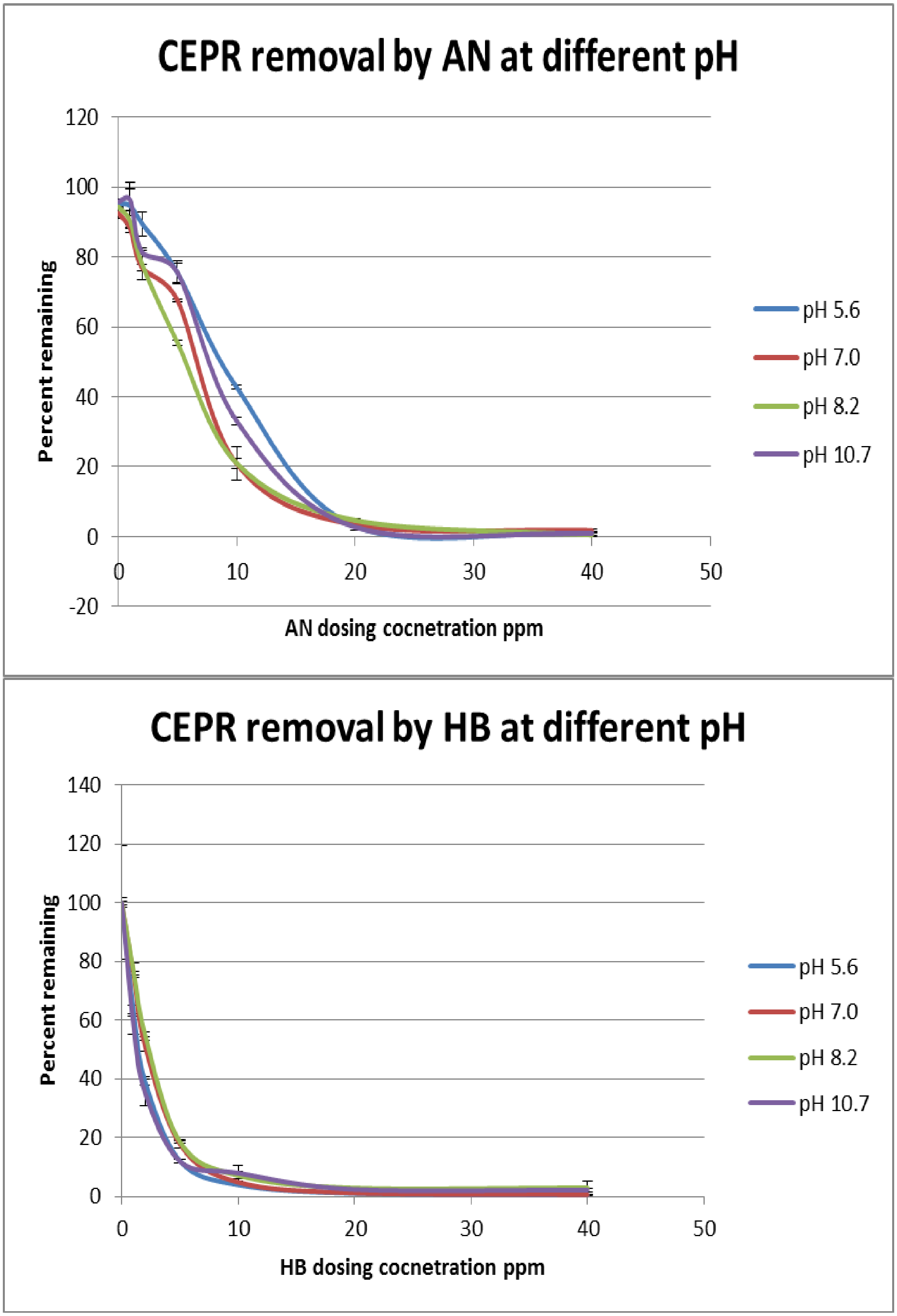


Figure2.3. CEPR removal by AN and HB at different pHs

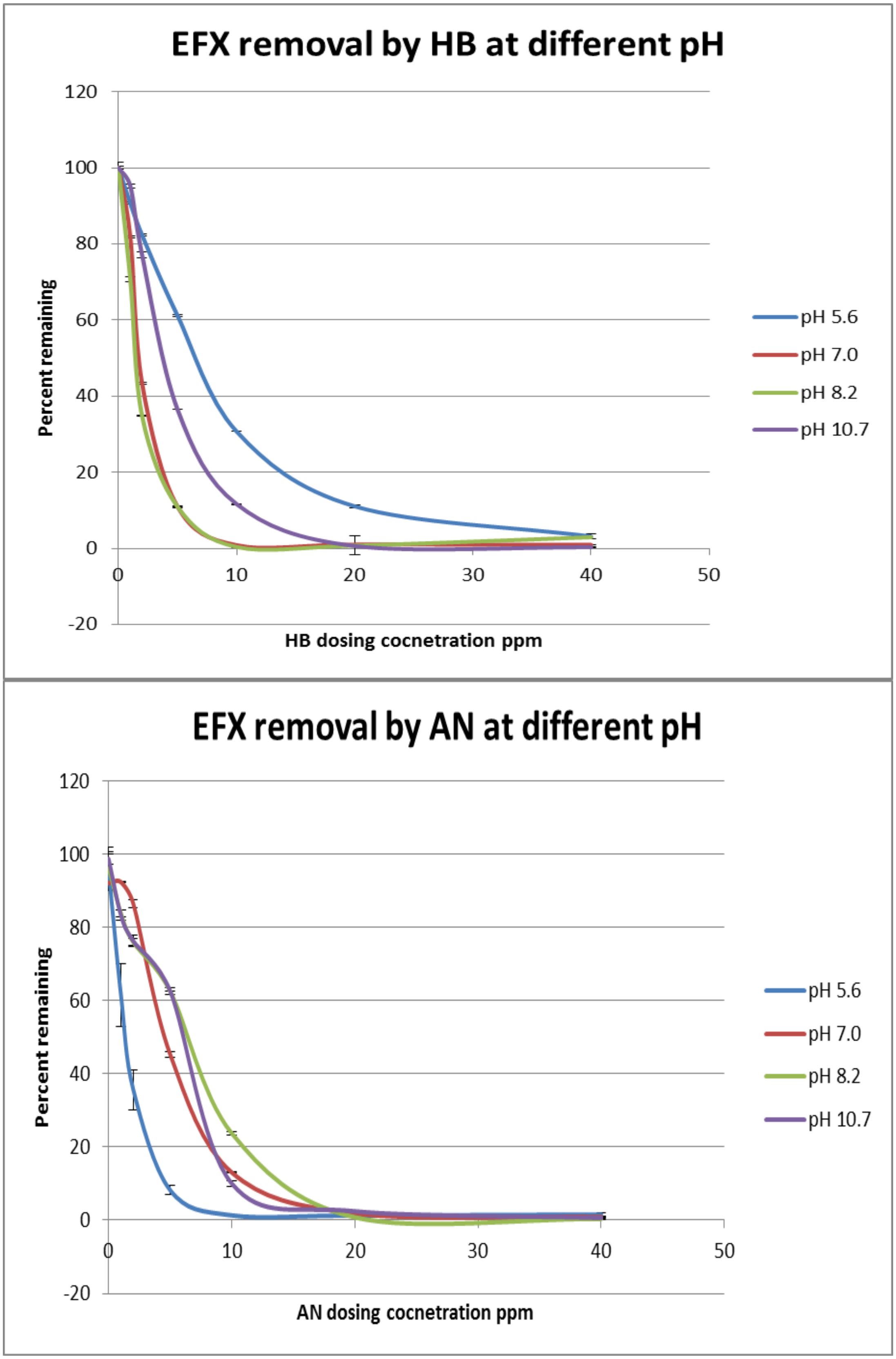


Figure 2.4. EFX removal by AN and HB at different pHs

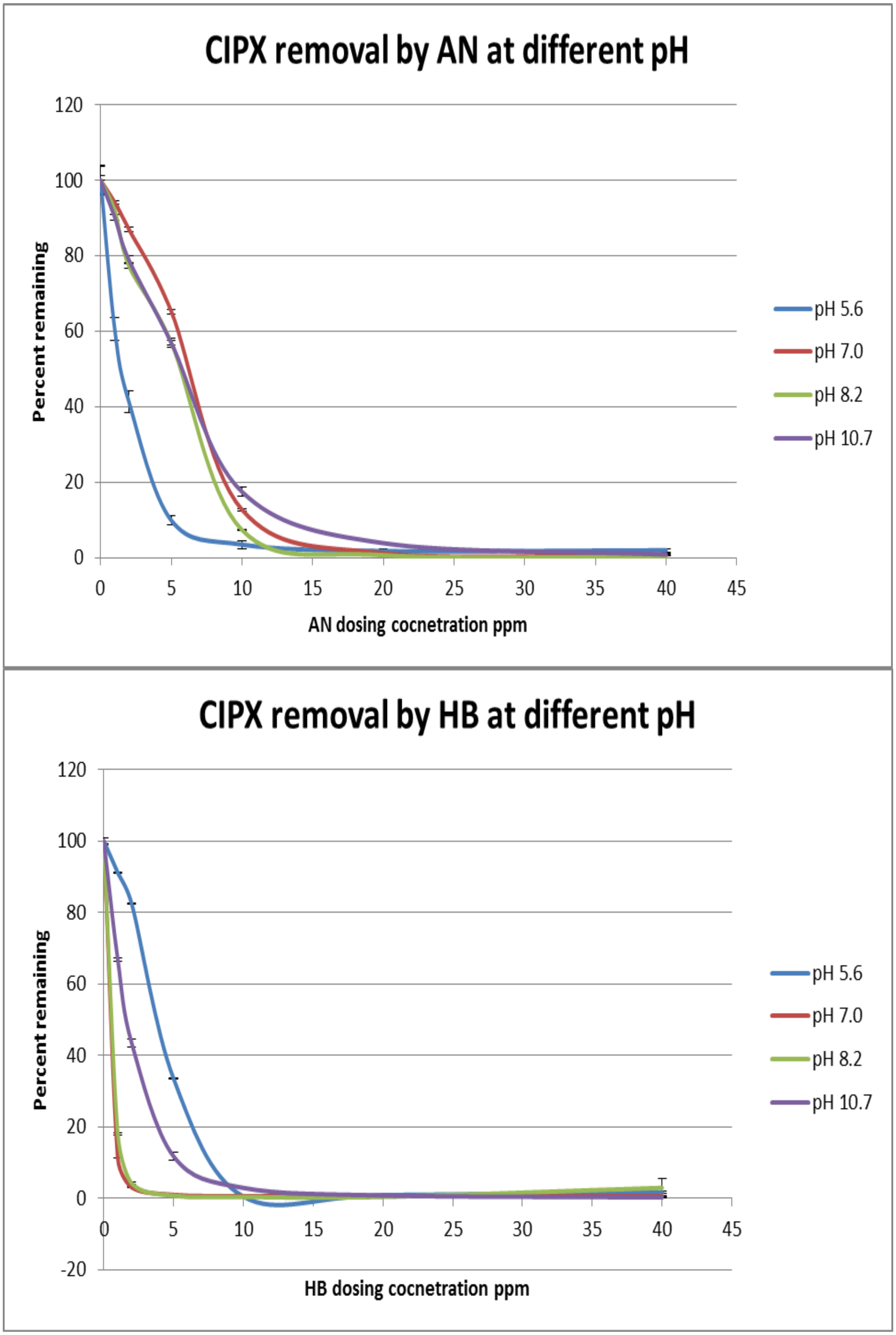


Figure 2.5. CIPX removal by AN and HB at different pHs

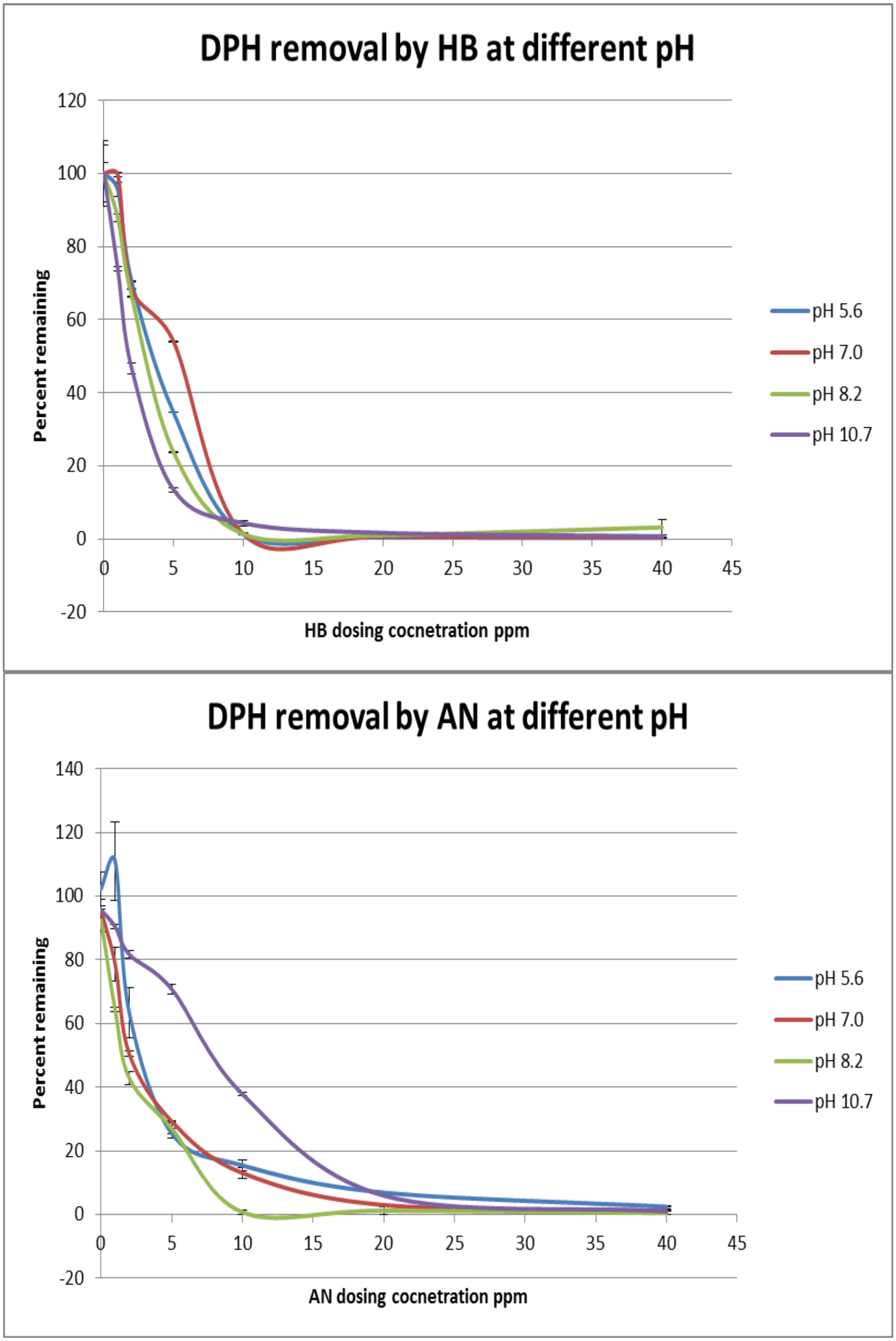
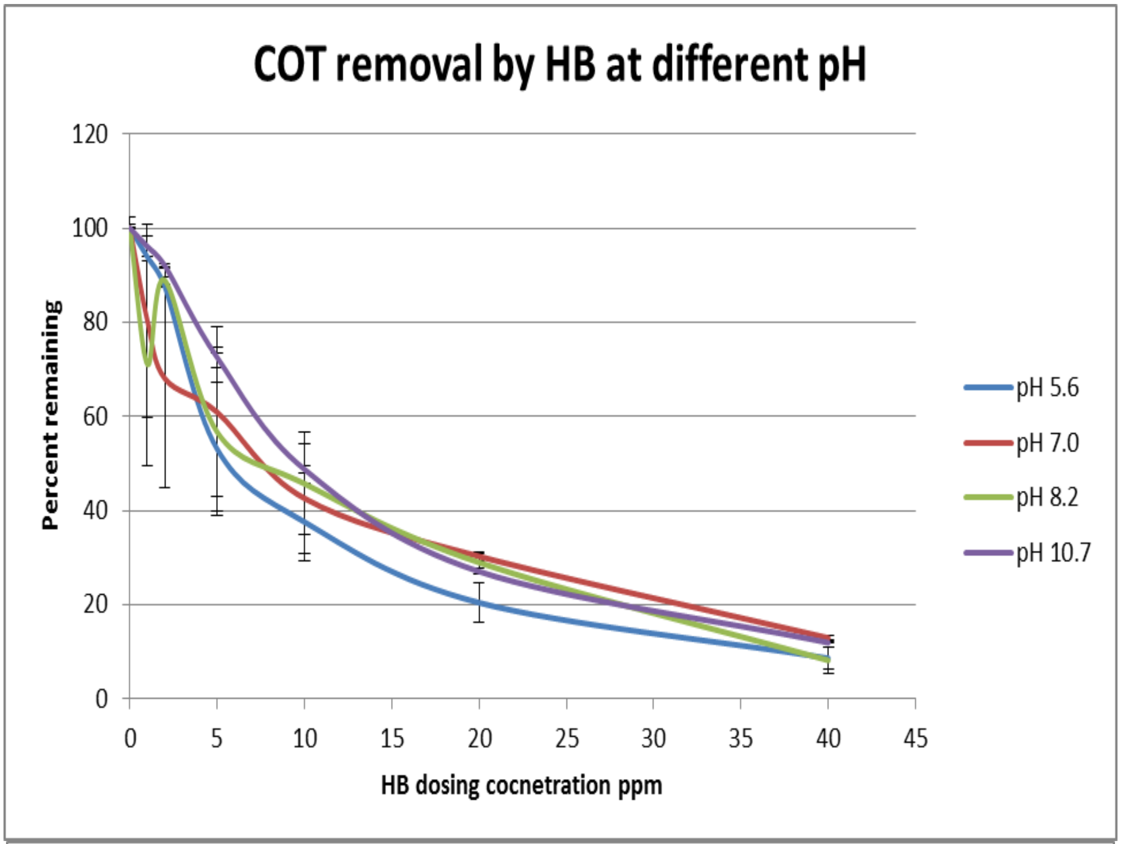


Figure 2.6. DPH removal by AN and HB at different pHs



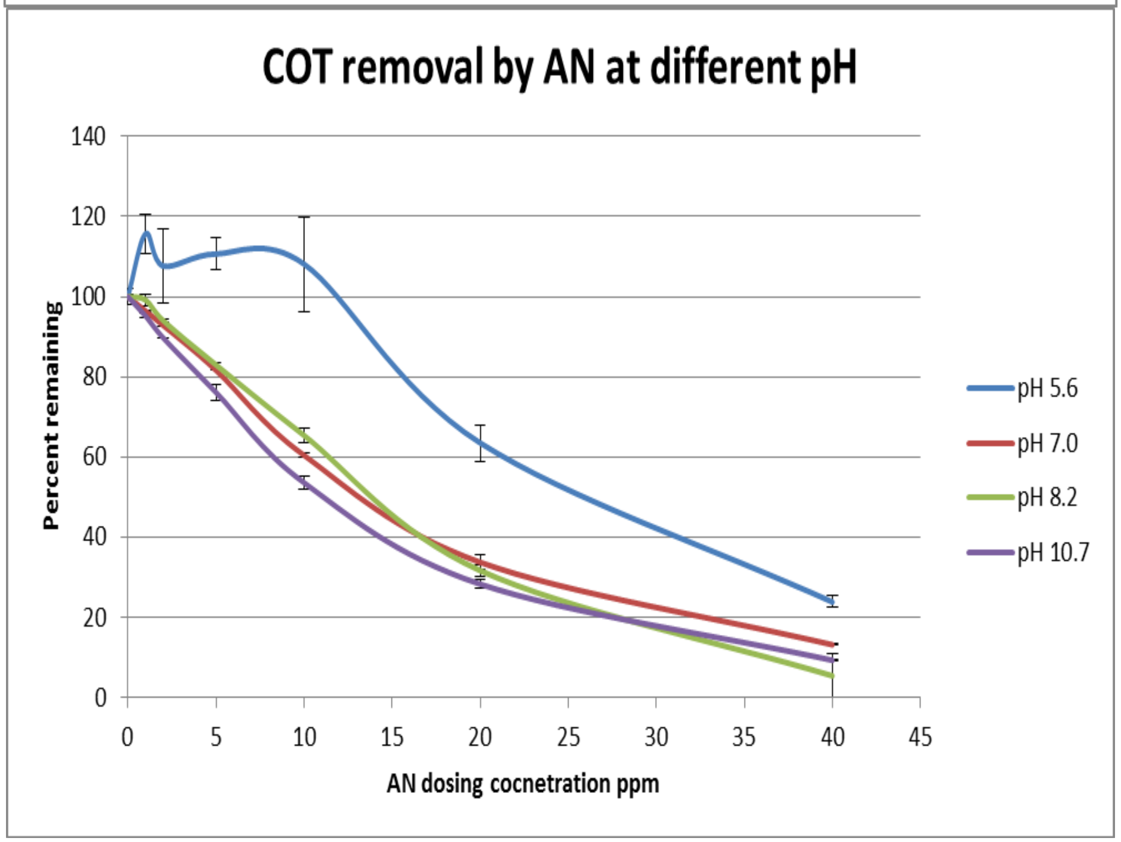


Figure 2.7. COT removal by AN and HB at different pHs

2.2.4.2. Contact time effects. Based on the result obtained from above, contact time experiment was conducted. For this set of experiments, the initial concentration was 5 mg/L for all six PPCPs; PAC dosages were 2 mg/L; with a sampling time of 0 min, 30 min, 1hr, 2hr, 4hr, 8hr and 24hr. The water samples were buffered with 5 mM phosphate to pH of 8.2. And pH 8.2 works best for the most of the compounds, 2mg/L PAC dosage is common dosing concentration for water treatment plants. And the other procedures remained the same.

Figure 2.8 showed the contact time effect, even with 2 mg/L HB dosage; AZI, CEPR, EFX, CIPX and DPH have over 60% removal after 4 hours contact time. For AN, most of the PPCPs follow the same trend but with better removal efficiency except CEPR. For COT, there is no removal by neither of the PACs.

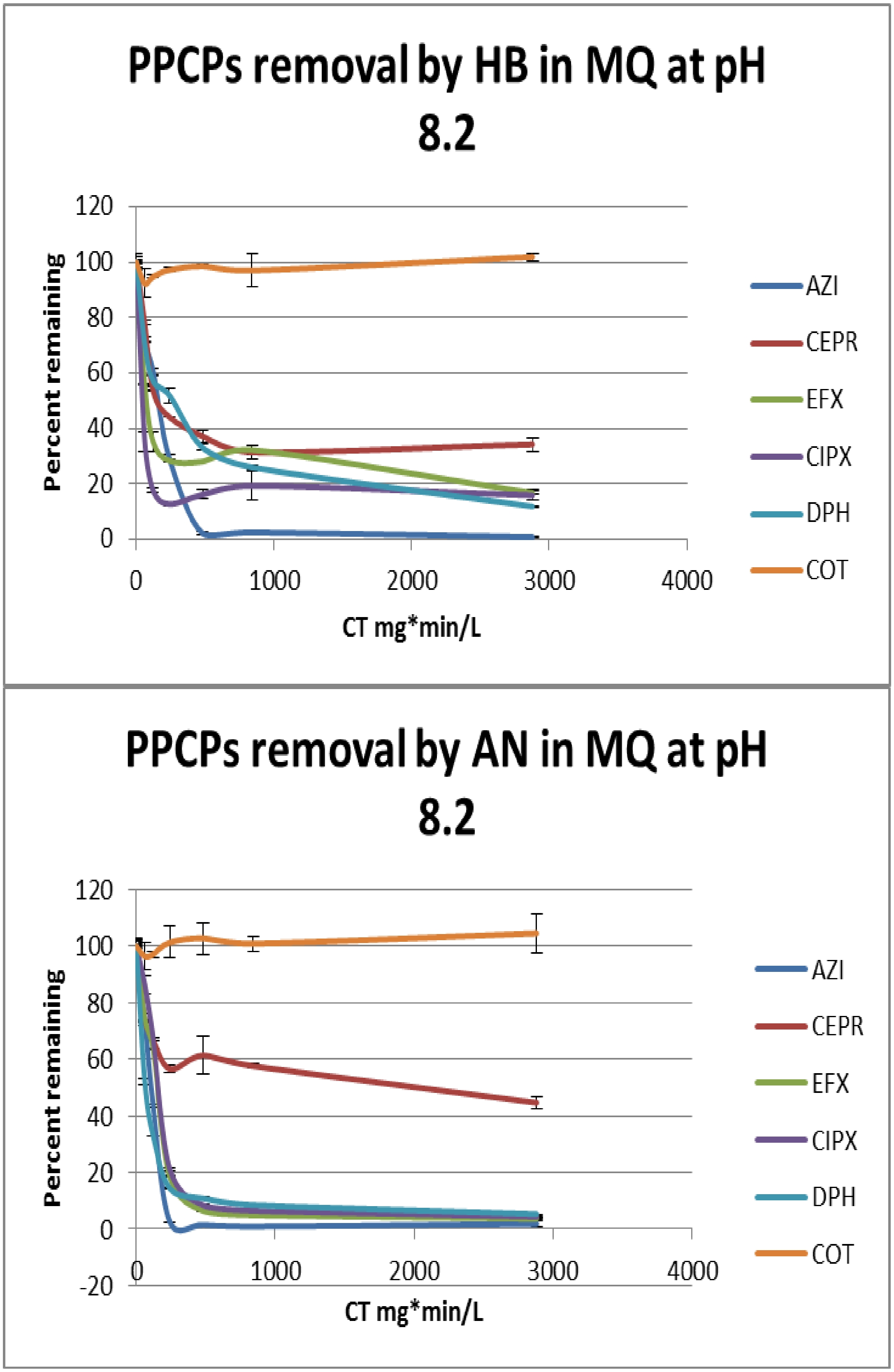


Figure 2.8. Contact time effects

2.2.4.3. Natural water matrix effects. The effects of matrix were investigated using natural water collected from Missouri river water. The pH of the water was adjusted, as needed, by buffering with 5 mM of phosphate. The other parameters remained the same with the contact time test.

Figure 2.9 showed natural water matrix effects, with 2 mg/L AN dosage; AZI, EFX, CIPX and DPH have over 60% removal after 8 hours contact time. For HB, most of the PPCPs follow the same trend but with better removal efficiency except EFX and CIPX. Also HB worked better at removing CEPR than AN. For COT, there is no removal by neither of the PACs.

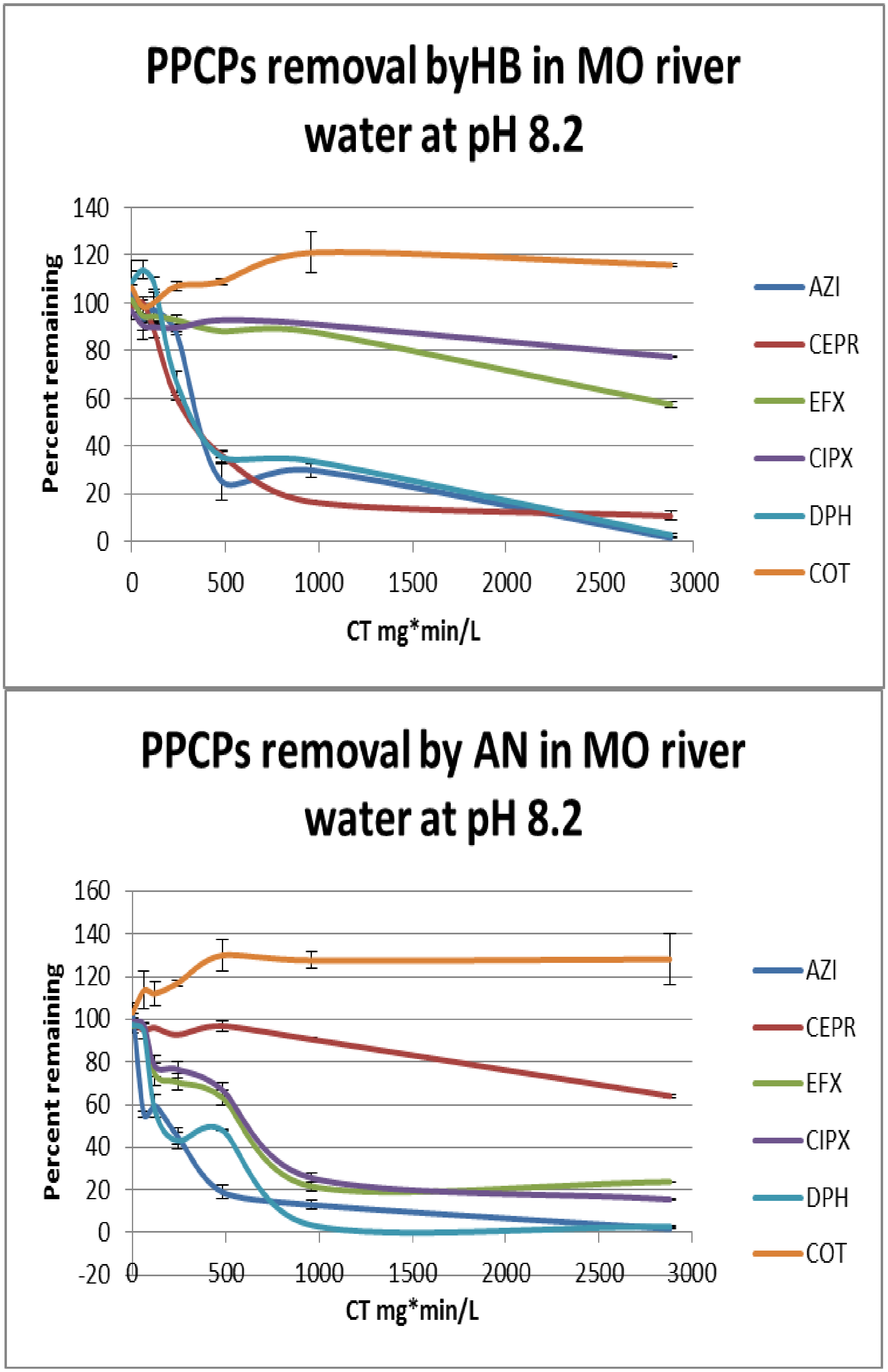


Figure 2.9. Natural water matrix effects

Comparison of adsorption results for buffered DI water (Fig. 24) and for natural water (Fig. 26) showed that the greatest effects of matrix on PPCPs adsorption occurred at pH 8.2, with smaller amounts of PPCPs removed with matrix present, than for buffered-DI water.

2.3. CONCLUSION

The method can detect sub-µg/L levels of PPCPs in different water samples with going through SPE which make the method feasible for PPCP screening in water samples.

The removal of selected PPCPs was investigated using different activated carbons (AN and HB). The removal efficiency of selected PPCPs varied under different pHs, contact time, dosing concentration and different water matrixes. pHs showed a notable and varied effect on removal efficiency. This study conclusively showed that pH (as well as PAC type, dosage, and contact time; and NOM concentration) has a large impact on the adsorptive efficiency of PAC for PPCPs treatment. These effects can be understood based on well-known non-electrostatic and electrostatic interactions. Furthermore, at an intermediate common pH for water treatment of 8.2, the relative performances of three PAC were (from lowest to highest capacity): HB <AN for most of the compounds. The results of this study suggest that the choice of PAC should be closely matched to the objectives of the PAC treatment. Furthermore, these results reinforce that water quality conditions also play a critical role in PAC sorption performance and must be well understood

and/or studied in laboratory or field experiments to optimize water treatment system performance.

REFERENCES

[1] Robidoux, P. Y.; Svendsen, C. Environ.Toxicol. Chem. 2000, 19, 1764−1773.

[2] Yang, X., C. Shang, and P. Westerhoff, Factors affecting formation of haloacetonitriles, haloketones, chloropicrin and cyanogen halides during chloramination.Water Res, 2007. 41(6): p. 1193-200.

[3] Xu, J., L. Wu, and A.C. Chang, Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils. Chemosphere, 2009. 77(10): p. 1299-305.

[4] Liu, J.L. and M.H. Wong, Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environ Int, 2013. 59: p. 208-24.

[5] Luo, Q., et al., Simultaneous and high-throughput analysis of iodo-trihalomethanes, haloacetonitriles, and halonitromethanes in drinking water using solid-phase microextraction/gas chromatography-mass spectrometry: an optimization of sample preparation. J Chromatogr A, 2014. 1365: p. 45-53.

[6] Rylott, E. L.; Lorenz, A.; Bruce, N. C. Curr.Opin. Biotechnol. 2011, 22, 434−440.

[7] Yinon, J. Toxicity and Metabolism of Explosives; CRC Press: Boca Raton, FL, 1990.

[8] Duan, W.-d.; Lu, Z.-s. HannengCailiao 2003, 11, 209−212.

[9] Mudryy, R. Int. Annu. Conf. ICT 2011, 42nd, 111/1−111/11.

[10] Powala, D.; Orzechowski, A.; Maranda, A.; Nowaczewski, J. Przem.Chem. 2005, 84, 582−585.

[11] Provatas, A.; Wall, C. Int. Annu.Conf. ICT 2011, 42nd, 6/1−6/12.

[12]Gaft, M.; Nagli, L. Opt. Mater. 2008, 30, 1739−1746.

[13] Berg, M. J.; Hofstetter, T. B. Opt. Mater. 2008, 30, 1739−1746.

[14] Lai, H.; Leung, A.; Magee, M.; Almirall, J. R. Anal.Bioanal. Chem. 2010, 396, 2997−3007.

[15]Fialkov, A. B.; Gordin, A.; Amirav, A. J. Chromatogr., A 2003, 991, 217−240.

[16] Huang, H., et al., Formation of haloacetonitriles and haloacetamides during chlorination of pure culture bacteria. Chemosphere, 2013. 92(4): p. 375-81.

[17] Yang, X., C. Shang, and P. Westerhoff, Factors affecting formation of haloacetonitriles, haloketones, chloropicrin and cyanogen halides during chloramination.Water Res, 2007. 41(6): p. 1193-200.

[18] Plewa, M.J., et al., Halonitromethane drinking water disinfection byproducts: chemical characterization and mammalian cell cytotoxicity and genotoxicity. Environ SciTechnol, 2004. 38(1): p. 62-8.

[19] Mincher, B.J., et al., Free-radical chemistry of disinfection byproducts. 3. Degradation mechanisms of chloronitromethane, bromonitromethane, and dichloronitromethane. J PhysChem A, 2010. 114(1): p. 117-25.

[20] Krasner, S.W., et al., Impact of wastewater treatment processes on organic carbon, organic nitrogen, and DBP precursors in effuent organic matter. Environ SciTechnol, 2009. 43(8): p. 2911-8.

[21] Hong, H., et al., Use of multiple regression models to evaluate the formation of halonitromethane via chlorination/chloramination of water from Tai Lake and the Qiantang River, China. Chemosphere, 2014. 119C: p. 540-546.

[22] Pozzi, R., et al., Determination of nitrosamines in water by gas chromatography/chemical ionization/selective ion trapping mass spectrometry. J Chromatogr A, 2011. 1218(14): p. 1808-14.

[23] Sa, C.S., R.A. Boaventura, and I.B. Pereira, Analysis of haloacetic acids in water and air (aerosols) from indoor swimming pools using HS-SPME/GC/ECD. J Environ Sci Health A Tox Hazard Subst Environ Eng, 2012. 47(2): p. 176-83.

[24] Chang, H., C. Chen, and G. Wang, Identification of potential nitrogenous organic precursors for C-, N-DBPs and characterization of their DBPs formation. Water Research, 2011. 45(12): p. 3753-64.

[25] J.W. Hodgeson, A.L.C., Determination of chlorination disinfection by-products, chlorination solvents, and halogenated pesticides/herbicides in drinking water by liquid-liquid extration and gas chromatography with electron-capture detection. 1990: USEPA.

[26] Montesinos, I., M.J. Cardador, and M. Gallego, Determination of halonitromethanes in treated water. J Chromatogr A, 2011. 1218(18): p. 2497-504.

[27] Song, H., et al., Halonitromethanes formation in wastewater treatment plant effluents. Chemosphere, 2010. 79(2): p. 174-9.

[28] Yang, X., et al., Nitrogen origins and the role of ozonation in the formation of haloacetonitriles and halonitromethanes in chlorine water treatment. Environ Sci. Technol, 2012. 46(23): p. 12832-8.

[29] Boyd, G.R., et al., Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and Bayou St. John in New Orleans, Louisiana, USA. Sci Total Environ, 2004. 333(1-3): p. 137-48.

[30] Kosma, C.I., D.A. Lambropoulou, and T.A. Albanis, Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece.J Hazard Mater, 2010. 179(1-3): p. 804-17.

[31] Westerhoff, P., et al., Fate of Endocrine-Disruptor, Pharmaceutical, and Personal Care Product Chemicals during Simulated Drinking Water Treatment Processes. Environmental Science & Technology, 2005. 39(17): p. 6649-6663.

[32] Kolpin, D.W., et al., Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000: A National Reconnaissance. Environmental Science & Technology, 2002. 36(6): p. 1202-1211.

[33] Benotti, M.J., et al., Pharmaceuticals and Endocrine Disrupting Compounds in U.S. Drinking Water. Environmental Science & Technology, 2008. 43(3): p. 597-603.

[34] Wu, M. and S. Janssen, Dosed Without Prescription: A Framework for Preventing Pharmaceutical Contamination of Our Nation’s Drinking Water. Environmental Science & Technology, 2010. 45(2): p. 366-367.

[35] Nelson, E.D., et al., Diurnal Variability of Pharmaceutical, Personal Care Product, Estrogen and Alkylphenol Concentrations in Effluent from a Tertiary Wastewater Treatment Facility. Environmental Science & Technology, 2010. 45(4): p. 1228-1234.

VITA

Ruipu Mu was born in the city of Dezhou, Shandong Province, China. In 2005, he went to one of the top Universities in China, Tianjin University. He graduated with a Bachelor of Science in Pharmacy in 2009. He then joined Dr. Yinfa Ma's group at Missouri University of Science and Technology (Formerly University of Missouri-Rolla). There he received his PhD in Analytical Chemistry in May 2015.