DEVELOPMENT OF AUTOMATED ON-SITE ANALYTICAL METHODS FOR WATER ANALYSIS

BY

MOUSA YASIR AMAYREH

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This thesis, written by **MOUSA YASIR MOUSA AMAYREH** under the direction his thesis advisor and approved by his thesis committee, has been presented and accepted by the Dean of Graduate Studies, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY IN CHEMISTRY.**

Dr.Abdul Rahman Al-Arfaj
(Advisor)

Dr. Abdullah Al-Hamdan
Chemistry Department Chairman

Dr. Basheer Chanbasha
(Co-Advisor)

Dr. Salam A. Zummo
Dean of Graduate Studies

Dr. Abdalla Abulkibash
(Member)

21/11/13
Date

Dr. Assad Ahmed Al-Thkair
(Member)

Dr. Zain Hassan Yamani
(Member)

Mousa Yasir Mousa Amayreh

2013

Dedication

My parents: Thank you for your unconditional support with my studies. Thank you for giving me a chance to prove and improve myself through all my walks of life. Please do not ever change. I love you.

My wife and kids: thank you for believing in me; for allowing me to further my studies. Please do not ever doubt my dedication and love for you.

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LIST OF ABBREVIATIONS

GC-MS	:	Gas Chromatography Mass Spectrometry.
DLLME	:	Dispersive Liquid-Liquid Microextraction
SPME	:	Solid Phase Microextraction
PAEs	:	Phthalate Esters
BPA	:	Bisphenol A
NAs	:	N-Nitrosoamines
HEs	:	Haloethers
EE-SPME	:	Electro Enhanced Solid Phase Microextraction
LR	:	Linear Range
EF	:	Enrichment Factor
LOD	:	Limit of Detection
R^2	:	Coefficient of Determination
r	:	Correlation Coefficient
RSD	:	Relative Standard Deviation

USEPA	:	United States Environmental Protection Agency
DMP	:	Dimethyl Phthalate
DEP	:	Diethyl Phthalate
BBP	:	Butyl Benzyl Phthalate
DOP	:	Di-n-Octyl Phthalate
DBP	:	Din-n-Butyl Phthalate
DEHP	:	Bis (2- ethylhexyl Phthalate)
PVC	:	Poly Vinyl chloride
EDC	:	Endocrine Disruptor Chemical
SPE	:	Solid Phase Extraction
LLE	:	Liquid Liquid Extraction
LPME	:	Liquid Phase Microextraction
HPLC	:	High Performance Liquid Chromatography
SWCNTs	:	Single Wall Carbon Nano Tubes
HF	:	Hollow Fiber
UA	:	Ultrasound Assisted
CIA	:	Cold Induced Aggregation
DBPs	:	Disinfection By Products
NDEA	:	N-Nitroso-Di-n-ethyl Amine

NDPA	:	N-Nitroso-Di-n-Propyl Amine
NPIP	:	N-Nitrosopiperidine
NDBA	:	N-Nitroso-Di-n-Butyl Amine
PDMS-DVB	:	Polydimethysiloxane-Divinylbenzene
PA	:	Poly Acrylate
RSM	:	Response Surface Methodology
RSC	:	Response Surface Curve
BBD	:	Box-Behnken Design
ANOVA	:	Analysis of Variance
FID	:	Flame Ionization Detector
BCIE	:	Bis (2-Chloroisopropyl)ether
BCEE	:	Bis (2-Chloroethyl)ether
BCEM	:	Bis (2-Chloroethoxy)methane
FA	:	Flow Assisted
CW/DVB	:	Carbowax/Divinylbenzene
DBPs	:	Disinfection By Products

DISSERTATION ABSTRACT

Full Name : MOUSA YASIR MOUSA AMAYREH

Thesis Title : DEVELOPMENT OF AUTOMATED ON-SITE ANALYTICAL METHODS FOR WATER ANALYSIS

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Determination of organic contaminates in water samples required tedious sample preparation prior to instrumental analyses. Most of the environmental contaminants are present at trace level concentrations; thus, multi-step conventional sample preparation methods lead to poor quantization. In this regards, this thesis is focused on the development of automated analytical methodologies for water samples. To achieve complete automation, different strategies were adopted to automate the entire analytical procedure without any human intervention. For the first time, solvent minimized dispersive liquid-liquid microextraction (DLLME) and solvent less solid-phase microextraction techniques (SPME) were developed and integrated the extraction using CTC CombiPal autosampler coupled with gas chromatography/mass spectrometry. Chapter 2 and 5 are dedicated for DLLME/GC-MS analytical technique while chapter 3, 4 and 6 are focused on SPME/GC-MS methods. Detailed description and analytical performances of each technique are discussed below

Chapter 2: DLLME is one such preconcentartion technique that based on micro-scale volume of organic solvents. A DLLME was developed for the determination of the amount of six compounds of phthalate esters (PAEs) in six brand bottled drinking water samples. DLLME samples were analyzed by GC–MS. Various experimental conditions influencing the extraction were optimized. Under the optimized conditions, very good linearity was achieved for all analytes in the range between 0.05 and 150 µg/L with coefficient of determination (R^2) between 0.9953 and 0.9992. The LODs based on S/N = 3 were 0.005–0.022 µg/L. The reproducibility was evaluated, the RSDs were 1.3–5.2% (n = 3). The concentrations of phthalates were determined in bottled samples available in half shell. To understand the leaching profile of these phthalates from bottled water, bottles were exposed to direct sunlight during the summer (temperature from 34–57 °C) and sampled at different intervals. Results showed that the proposed DLLME is suitable for rapid determination of phthalates in bottled water and di-n-butyl, butyl benzyl, and bis-2-ethylhexyl phthalate compounds leaching from bottles up to 36 hours. Thereafter, degradation of phthalates was observed.

Chapter 3: For the first time electro-enhanced solid-phase microextraction (EE-SPME) method was developed for the determination of endocrine disruptor compounds such as phthalate esters (PAEs) and bisphenol A (BPA) in seawater and human blood samples. After EE-SPME; samples were analyzed by GC-MS. In this approach, SPME fiber was used in direct-immersion mode with an applied potential to extract di-ethyl phthalate, di-butyl phthalate, benzyl butyl phthalate and bisphenol A. The applied potential enhances the extraction efficiency of the target analytes. Various experimental conditions

influencing performance of the EE-SPME were optimized. Very good linearity was observed for all analytes in the range between 1 and 100 µg L^{-1} with correlation of determination (R^2) between 0.9636 and 0.9988. The limits of detection based on signal to noise of 3 were from 0.004 to 0.15 µg L^{-1}. The reproducibility of EE-SPME were evaluated, the relative standard deviations were between 1.0 and 5.0% (n=9). The proposed method was applied to the human blood samples stored in transfusion bags and seawater. The EE-SPME was more efficient than a conventional SPME approach and the results showed that the proposed EE-SPME was simple and suitable for trace level analysis.

Chapter 4: An automated headspace solid-phase microextraction (HS-SPME) coupled with GC-MS for the determination of four *N*-nitrosoamines (NAs) in groundwater samples was developed. Response surface methodology (RSM) technique was employed to investigate the optimized extraction conditions of HS-SPME using CombiPAL autosampler. Under the optimum conditions, good linearity for all analytes in a range between 0.1 and 100 µg/L with correlation of coefficients (r) between 0.9750 and 0.9920 were obtained. The LODs-based S/N ratio of three were 0.78-11.9 ng/L with corresponding RSDs of 1.8-5.7% (n=4). The method was applied to determine the NAs concentrations in groundwater samples from different locations in Saudi Arabia. The average recoveries of spiked NAs in groundwater by 1 µg/L and 20 µg/L were between 96.6±4.4% and 102.3±4.86 %, respectively, were obtained. These results indicate that the proposed automated HS-SPME is suitable for routine groundwater analyses.

Chapter 5: An automated DLLME was developed for the determination of three N-nitrosoamines (N-nitroso-di-n-propylamine (NDPA), N- nitrosopiperidine (NPIP) and N-

nitroso-di-n-butylamine (NDBA) in water samples. After DLLME extracts were analyzed automatically by in-line GC-MS. Response surface methodology was employed to optimize the enrichment factors of the N-nitrosoamines based on the effects extraction time, dispersive solvent volume, pH, ionic strength and agitation speed. The optimal conditions for simultaneous extraction of the mixture of the N-nitrosoamines in water samples were 28 minutes extraction time, 33.5 µL of methanol dispersive volume, 722 rotations per minute agitation speed, 23 % (w/v) NaCl concentration and pH 10.5. Under these conditions good linearity for the analytes in the range between 0.1-100 µg/L with coefficients of determination (r) of between 0.9937 and 0.9993 were of obtained. The limit of detection (LODs) based on a signal to noise of 3(S/N) ratio were between 5.7 and 124 ng/L with corresponding relative standard deviations of between 3.4 and 5.9% (n=4). The method was applied to determine the presence of the N-nitrosamines in water samples of different complexities, such as tap water and fresh ground water in a local water treatment plant (before and after treatment). The relative recoveries of NDPA, NPIP and NDBA present in spiked groundwater and tap water samples at concentrations of 2 µg/L of each (mean ± standard deviation, $n=3$) were (93.9±8.7)%, (90.6±10.7)% and (103.7±8.0)% respectively. Compared with the other methods the proposed automated DLLME/GC-MS procedure has more advantages due its better accuracy, low LODs, high relative recoveries as well as good linear range.

Chapter 6: Finally a fully automated flow assisted-solid-phase microextraction (FA-SPME) was developed for the determination of chloroethers (CEs) in aqueous samples. A CTC CombiPAL autosampler coupled with gas chromatography–mass spectrometry (GC-MS) was used to automate the entire extraction process. In this method, the SPME

fiber was exposed to a 100 mL sample in a direct immersion mode for 10 min. After exposure, the fiber was desorbed at the injection port of GC-MS. Good liner correlation was found over a concentration range of 0.5 to 100 μg L^{-1}. The limits of detection are determined between 0.017 and 0.053 μg L^{-1} with the correlations coefficient (r) from 0.9941 to 0.9981. The relative standard deviations of the FA-SPME are in the ranges between 1.2 and 6.2 %. The applicability of the method was assessed by means of recovery studies and satisfactory values for all compounds were obtained. The optimized method was applied to the analysis of water and human urine. This fully automated FA-SPME/GC-MS is substantially faster and more suitable for routine analysis of large volume of environmental water and urine samples.

ملخص الرسالة

الاسم الكامل: موسى ياسر موسى عمايره

عنوان الرسالة: تطوير طرق تحليل اتوماتيكية للملوثات العضوية بالماء

التخصص: الكيمياء التحليلية

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إن تحديد الملوثات الكيميائة في الماء تحتاج الى عدة خطوات سابقة لعملية التحليل. إن معظم الملوثات العضوية العضوية في المياه تتواجد بنسب قليلة جداً. إن الخطوات اليدوية الإعتيادية المتبعة عادةً في إستخلاص الملوثات تتسبب في التقليل من كفاءة التحديد. و لهذا السبب، لقد علمنا في هذا البحث على تطوير خطوات آلية كبديل للعمل اليدوي لزيادة الكفاءة و الفاعلية في الإستخلاص. للمرة الأولى تم تطوير طرية تعتمد على حجوم من المذيبات العضوية في غاية الصغر تدعى (DLLME) و أيضا طريقة أخرة لا حاجة لإستخدام المذيبات العضوية فيها و هي (SPME) و ذلك بإستخدام الربوت الآلي (CTC CombiPal) موصولا بجهاز الفصل الغازي (GC-MS). إن الوحدة الثانية و الخامسة من هذه الاطروحة تتناول تطوير طريقة (DLLME) في حين أن الوحدات الثالثة و الربعة و السادسة تتناول تطوير طريقة (SPME). إن تفصيلات عملية و آدائها بشكل مختصر في الفقرات التالية.

الوحدة الثانية: مؤخراً كان الإهتمام في البحث عن طرق متطورة للكشف عن تراكيز المركبات الكيميائية في الماء بحيث تكون مصاحبة للبيئة من حيث التقليل من حجم المذيبات العضوية المستخدمة. تعتبر طريقة (DLLME) واحدة من هذه الطرق الحديثة لاعتمادها على حجوم مذيبات تقاس بوحدة الميكروليتر. وفي هذا الخصوص تم تطوير طريقة DLLME و استخدامها في إيجاد تراكيز مركبات الإيستر فثالات (PAEs) في ستة أنواع تجارية من مياه الشرب في المملكة العربية السعودية و كذلك تم استخدام جهاز الفصل الغازي و كشف التأين الكتلي في التحليل. تم إختبار العوامل المختلفة التي قد تؤثر على الاستخلاص لستة من مركبات (PAEs). كانت الخطية جيدة جداً لجميع المركبات قيد الدراسة ضمن التراكيز المستخدمة، وكانت أقواس تحديد خطية بمعادلات الارتباط بين 0.9953 و 0.9992 على تراكيز تتراوح بين(0.05-150) ميكروغرام/لتر. وحدود الكشف لهذه الطريقة (نسبة الإشارة إلى الضوضاء من 3) كانت على مدى 0.005 إلى 0.022 ميكروغرام/لتر. وكان معدل التكرارية (الإنحراف المعياري النسبي) يتراوح بين 1.3 و 5.2 %. لقد تم تحديد تراكيز مركبات الفثالات المتواجدة في مياه الشرب و كذلك تاثير تخزينها تحت أشعة الشمس لفترات متباينة و كانت درجات الحرارة تتراوح بين (34-57 درجة مئوية) على إنحلال بعض هذه المركبات من المادة التي تصنع منها القوارير المحتوية على عينات الماء. ولقد تبين من النتائج التي تم التوصل إليها من هذا التطبيق، أن هذه الطريقة مناسبة و سريعة و فعالة في تحديد مركبات الإيستير فثالات في مياه الشرب التجارية المعبأه وكذلك لوحظ وبشكل واضح إنحلال هذه المركبات من مادة العبوات البلاستيكية نتيجة لتخزينها المباشر تحت أشعة الشمس لفترات الدراسة.

الوحدة الثالثة: للمرة الأولى تم تطوير الطريقة المستخدمة لتحديد المركبات و هي (SPME) و ذلك بإدخال التأثير الفولتي (EE)، و تم تطبيقها عملياً في دراسة تراكيز بعض المركبات العضوية المتواجدة في ماء البحر و عينات من دم الإنسان مثل فثالات الإيستر و البيس فينول أ. و تم التحليل بواسطة جهاز الفصل الغازي و كشف التأين الكتلي. و في هذا النوع من التحليل تم استخدام الألياف التجارية الخاصة بهذه الطر يقة بطريقة الغمر في العينة أثناء الإستخلاص، حيث تم توصيل القابض المعدني المحتوي على الليف بفرق جهد كهربائي في حين أن الطرف الآخر من فرق الجهد تم وصله بقضيب معدني خامل تم غمسه في العينة قيد الدراسة. تم إختبار العوامل المختلفة التي قد تؤثر على الاستخلاص بهذه الطريقة. و تبين من المقارنة بين التحليل باستخدام (SPME) العادي و (EE-SPME) أن التأثير الفولتي يلعب دوراً كبيراً في كفاءة هذه الطريقة مقارنة بـ (SPME) العادي. كانت الخطية جيدة جداً لجميع المركبات قيد الدراسة ضمن التراكيز المستخدمة ، و كانت أقواس تحديد خطية بمعادلات الإرتباط بين 0.9636 و 0.9988 على تراكيز تتراوح بين (1-100) ميكروغرام/لتر. وحدود الكشف لهذه الطريقة (نسبة الإشارة الى الضوضاء من 3) كانت على مدى 0.004 إلى 0.15 ميكروغرام/لتر. وكان معدل التكرارية لتسع قراءات لنفس العينة (الإنحراف المعياري النسبي) يتراوح بين 1.0 و 5.0 %. هذه الطريقة المقترحة تم استخدامها في تحديد تراكيز المركبات المذكورة سابقاً في عينة من ماء البحر و كذلك عينة من دم الإنسان مخزنة في وحدة بلاستيكية لفترة من الزمن في بنك الدم التابع لمشفى في منطقة الخبر في السعودية. و أظهرت النتائج بساطة تطبيق و فعالية هذه الطريقة في تحديد التراكيز الدقيقة للمركبات العضوية في العينات المذكورة.

الوحدة الرابعة: تم استخدام الطريقة المسماه (HS-SPME) و المقترنة بجهاز الفصل الغازي و كشف التأين الكتلي بشكل اتوماتيكي بواسطة الربوت الآلي (CombiPA) في تحديد تراكيز مركبات النيتروزوأمين (NAs) في المياه الجوفية التي تم الحصول عليها من أربع مصادر مختلفة في المملكة العربية السعودية. حيث أنه للمره الأولى تم استخدام منهجية السطوح في دراسة العوامل المؤثرة على هذا النوع من الطرق. و من النتائج لوحظ أن الخطية جيدة لجميع المركبات، و كانت أقواس تحديد الخطية بمعادلات الإرتباط بين 0.9750 و 0.9920 على تراكيز تتراوح بين(1-100) ميكروغرام/لتر. وحدود الكشف لهذه الطريقة (نسبة الإشارة إلى الضوضاء من 3) كانت على مدى 0.78 إلى 11.9 نانوغرام/لتر. وكان معدل التكرارية لعدة تجارب متتالية لنفس العينة (الإنحراف المعياري النسبي) يتراوح بين 1.85 و 5.7 %. لقد عرضت هذه الطريقة قيم استرداد عالية تتراوح بين 96.6 و 102.3 % لمركبات NAs. و كذلك لوحظ أن هذه الطريقة الأوتوماتيكية فعالة و مناسبة للكشف عن هذه المركبات في عينات المياه الجوفية.

الوحدة الخامسة: تم تطوير الطريقة الاعتيادية و هي (DLLME) إلى اتوماتيكية متكاملة الأداء باستخدام الربوت الآلي (CTC CombiPal) لتحديد ثلاث من مركبات النيتروزوأمين NAs المتواجدة في عينات من الماء، لوحظ تكون الطبقة الضبابية خلال عملية الخلط بين عينة الماء و المذيبات العضوية المستخدمة وتم حقن جزء من الطبقة العضوية في جهاز الفصل الغازي و كشف التأين الكتلي للتحليل. تم ايضاً دراسة العوامل المؤثرة على فعالية هذه الطريقة باستخدام منهجية السطوح (RSM). و تبين من النتائج أن الخطية جيدة لمركبات النيروزوأمين قيد الدراسة، و كانت أقواس تحديد خطية بمعادلات الإرتباط بين 0.9937 و 0.9993 على تراكيز تتراوح بين (0.1-100) ميكروغرام/لتر. وحدود الكشف لهذه الطريقة (نسبة الإشارة إلى الضوضاء من 3) كانت على مدى 5.7 إلى 124 نانوغرام/لتر. وكان معدل التكرارية لأربع تجارب متتالية لنفس العينة (الإنحراف المعياري النسبي) يتراوح بين 3.4 و 5.9 %. و كان معدل قيم الإسترداد 103% لجميع مركبات NAs. لوحظ أن هذه الطريقة الأوتوماتيكية فعالة و مناسبة للكشف عن هذه المركبات في عينات المياه الجوفية. إن هذه الطريقة الأوتوماتيكية الحديثة أفضل من غيرها المستخدمة لنفس الغرض من حيث السرعة و الدقة و قلة الأخطاء الناتجة لعدم تدخل العمل اليدوي فيها.

الوحدة السادسة: أخيراً تم تطوير طريقة أتوماتيكية كاملة الأداء جديدة باستخدام الربوت الآلي (CTC CombiPAL) معتمدة على تدفق السائل و هي (FA-SPME) لتحديد ثلاث من مركبات الكلوروايثر(CEs) في عينات من الماء و بول الإنسان. حيث تم استخدام جهاز الفصل الغازي و كشف التأين الكتلي للتحليل. و لوحظ من النتائج الفعالية العالية

لهذه الطريقة في تحديد تركيز المركبات المذكورة. حيث أن الخطية كانت واضحة على تراكيز تتراوح بين 0.5 و 100 ميكروغرام/لتر. وحدود الكشف لهذه الطريقة (نسبة الإشارة إلى الضوضاء من 3) كانت على مدى 0.017 إلى 0.053 ميكروغرام/لتر، وأيضاً كانت قيمة أقواس تحديد خطية بمعادلات الإرتباط بين 0.9941 و 0.9981 و الإنحراف المعياري النسبي (التكرارية) تتراوح بين 1.2 و 6.2%. و كانت قيمة الإسترداد عالية نسبياً لجميع مركبات CEs قيد الدراسة في كلا النوعين من العينات. و كانت هذه الطريقة مناسبة بشكل كبير من حيث السرعة و الدقة لتحليل عينات ذات حجم كبير و هذا ما يميزها عن غيرها من الطرق الإعتيادية.

CHAPTER 1

INTRODUCTION

1.1 OVERVIEW

As the world's population increases; the demand on potable water will also increase. Data from the 20th century revealed that the 3-fold increase in population during that period was accompanied by an increase in the world's demand for water by 6-fold [1]. In addition, current growth models project that in the next 50 years, global population will increase by an additional 40 – 50% [2]. This population growth, coupled with the world's progress towards increased industrialization and urbanization, will further increase our need for water.

Depletion of groundwater and worsening pollution levels are among the more obvious indicators of water stress. The concern over such chemicals in the environment is based on the realization that they have serious impacts on wildlife and humans. Moreover, once such effects become apparent, exposure cannot be prevented in the short term. In particular, there is increasing evidence that their presence do have measurable toxic effects on humans [3].

Miniaturized analytical chemistry has attracted a great deal of scientific efforts during the

past decade due to the increasing awareness of the importance of novel materials for analytical applications. Good retention of both non-polar and polar compounds on a suitable sorbent are required to ensure retention of most organic compounds. Recently, on-site sample preparation or filed sampling procedures got more attention in the field of environmental/analytical chemistry. The demand for easy to handle, fast, and efficient sample preparation methods, which can be directly coupled to gas chromatography (GC) is very high. There has been an increase in the need for rapid and reliable field-portable methods for the quantification of organic pollutants present at sampling location (for example, ground water, seawater and desalination plants). In many of these sites, fast and accurate determination is necessary in order to make appropriate decisions regarding water quality and its remediation.

Rezaee et al. 2006 [4] developed a novel microextraction technique, termed dispersive liquid–liquid microextraction (DLLME), which is based on a ternary solvent system like homogeneous liquid–liquid extraction and cloud point extraction. In this method, the appropriate mixture of extractant and dispersant is injected rapidly into an aqueous sample by syringe, and then a cloudy solution is formed, which markedly increase the contact surface between phases and reduce the extraction times with increasing enrichment factors [5]. After extraction, the phase separation is performed by agitation; the determination of the analytes concentration in the organic phase can be performed by instrumental analysis [6, 7]. However, this method suffers from low repeatability and lack of special selectivity, so it application for complex samples is a challenging task.

Solid-phase microextraction (SPME), was developed by Pawliszyn and co-workers [8]. SPME is another involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent). The quantity of analyte extracted by the fibre is proportional to its concentration in the sample as long as equilibrium is reached or, in case of short time pre-equilibrium, with help of convection or agitation [9]. After extraction, the SPME fiber is transferred to the injection port of the GC instruments, where desorption of the analyte takes place and analysis is carried out.

DLLME and SPME technologies require multi-step manual operational procedures, which often results in large experimental variations. SPME is a well-established technique used for the extraction of numerous compound classes in water, which is superior to DLLME in both cost savings and extraction efficiency.

The second chapter of this thesis is dedicated to method development of an efficient dispersive liquid-liquid microextraction (DLLME) for phthalate esters in drinking water. Chapter three focuses on the novel electro enhanced solid-phase microextraction (EE-SPME) for the determination of phthalate esters and bisphenol A in water and human blood samples. These two methods were then applied to fully automation process. Chapters four, five and six are dedicated to fully automation of the above mentioned technique for determination of emerging contaminants such as N-Nitrosoamines in water samples. Using this fully automated approach, manual sample handling are minimized or completely eliminated when compared to regular DLLME and SPME methods.

1.2 PROBLEM DEFINITION

To address this problem, development of a fully automated method was necessary. Automated sample-handling not only shortens the total analysis time, thus improving productivity, but also usually provides better accuracy and precision relative to manual techniques [10].

Analysis of trace level of organic contaminants from environmental water samples (ground water, seawater and drinking water) and biological samples (Human blood and urine) is challenging; seawater and biological samples are classified as complex matrix samples. In the conventional analytical techniques, samples need to be brought back to the laboratory and extracted with multistep sample preparation methods. Particularly for aqueous matrices, this is a demanding task.

We have the vision to challenge present practices. Our goal is to have an integrated and fully automated system for determination of organic pollutants in water and biological samples; this system will include sample preparation, detection and characterization of organic pollutants such as phthalate esters (PAEs), Bisphenol A (BPA), N-nitrosoamine (NAs) and Haloethers (HEs) that are found in water and biological samples.

Because these analytes are chemically diverse, no single method would be suitable for their determination, and conventional methods are usually multi-step with high risk of analyte loss. Therefore, different simple and efficient off-line, automated and fully

automated methodologies that will suit each group of analytes will be developed in this research venture.

1.3 AIMS AND OBJECTIVES

Roadmap to automatization:

[1] Developing off line DLLME for extraction of PAEs from different brand of drinking water samples in Saudi Arabia.

[2] Developing for the first time off line EE-SPME (electro enhanced- solid phase microextraction) methods for extraction of PAEs and BPA from seaseawater and human blood samples.

[3] Automated SPME using CTC-auto sampler for determination of NAs in groundwater samples that collected from four different locations in Saudi Arabia.

[4] Automated DLLME using Combi-PAL auto sampler, for determination of NAs in tap water, groundwater samples before and after treatment collected from groundwater well and water purification plant in the main campus of King Fahd University of Petroleum and Mineral (KFUPM) in Saudi Arabia.

[5] Fully automated SPME methods using CTC-auto sampler for determination of HEs drinking water, tap water and human urine samples.

The aims and objectives of each part in this research work are defined as follows:

[1] To investigate various factors affecting the performances of such methods.

[2] To evaluate the performance of such methods through the determination of linear range (LR), enrichment factors (EF), recovery and limits of detection (LOD), Coefficient of Determination (R^2) and correlation coefficient (r).

[3] To compare performance of the developed methods with literature reports.

[4] To apply the respective methods for the determination of PAEs, BPA, NAs and HEs in real water samples (seawater, drinking water and ground water) and biological samples (human blood and urine).

Linear range (LR) mean, the linear range of a chromatographic detector represents the range of concentrations that detected, the best way to present detector linear range is the Linearity Plot. Numerically, the linear range can be expressed as the ratio of the upper limit of linearity obtained from the linearity plot and the minimum detectability, both measured for the same substance.

The coefficient of determination (R^2) represents the percent of the data that is the closest to the line of best fit. While, the quantity r ($r=\sqrt{R^2}$), called the correlation coefficient, measures the strength and the direction of a linear relationship between two variables. When r value of exactly +1 indicates a perfect positive fit. Positive values indicate a relationship between the variables (x,y) such that as values for x increases, values for y also increase. For example, if r = 0.922, then $r^2 = 0.850$, which means that 85% of the total variation in y can be explained by the linear relationship between x and y (as described by the regression equation). The other 15% of the total variation in y remains unexplained.

Figure 1 summarizes the procedures followed to achieve these objectives.

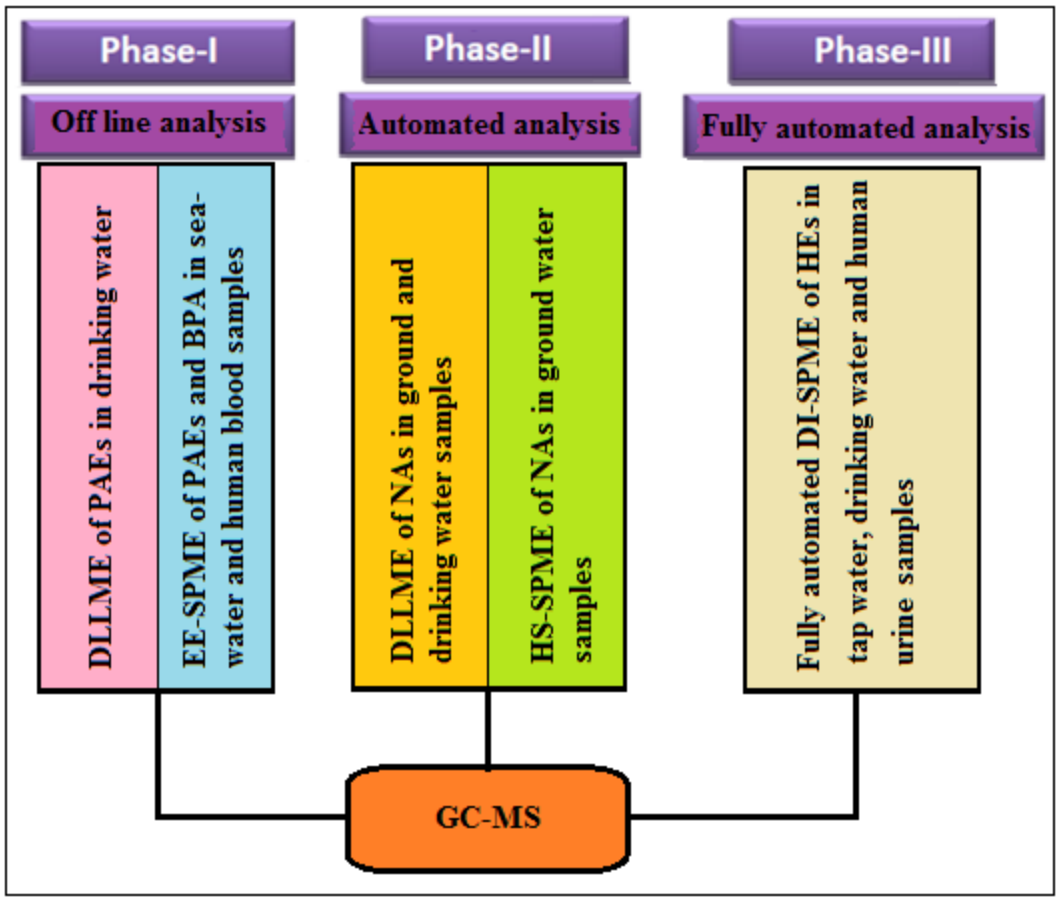

Figure 1: Summary of procedures for roadmap to automatization determination of organic contaminant in real samples.

CHAPTER 2

DETERMINATION OF PHTHALATE ESTERS IN BOTTLED WATER USING DISPERSIVE LIQUID-LIQUID MICROEXTRACTION WITH GAS CHROMATOGRAPHY MASS SPECTROMETRY

2.1 LITERATURE REVIEW

Phthalate esters (PAEs) are known as polymer additives (plasticizers) in industries such as manufacture of plastics, medical devices, building materials, children's toys, and cosmetics to improve their flexibility [11-12]. The interaction between PAEs and polymer chains in the plastics are very weak [13-16]. This poor interaction leads to leaching of PAEs from the plastic materials [13, 17]. PAEs are classified as endocrine disrupting compounds, which are able to mimic or block the action of natural hormones that affect biological functions in animals and humans [18–20]. In recent years, the leaching of PAEs from different industrial products has received considerable attention due to its mode of action [18, 21, 22].

The United States Environmental Protection Agency (USEPA, 1993) classified butyl benzyl phthalate (BBP) as class C category and possible human carcinogen due to formation of mononuclear cell leukemia [16]. There are no international guidelines for PAEs in drinking water. However, USEPA proposed maximum contaminant level for bis

(2-ethylhexyl) phthalate (DEHP) and BBP in drinking water of 6 and 100 μg/L, respectively [16, 23, 24]. DEHP is commonly used plasticizer to improve flexibility in polyvinyl chloride (PVC) manufacture. Furthermore, it is ubiquitous in the environment and it is estimated that approximately 70% of the human population will have a detectable concentration of DEHP and/or its metabolites in their blood or urine at any time [25]. The type of bonding between DEHP as a plasticizer and PVC molecules is non-covalent bonding and this enhances the leaching of DEHP and is characterized as a highly lipophilic molecule from PVC into the surrounding environment [25]. The Kingdom of Saudi Arabia's domestic water supply depends on water distribution using plastic containers. Saudi Arabia ranks number 12 in bottled water consumption (88 L per capita in 2004) among the 71 reported countries [16]. Sometime these containers are reused and exposed to hot sunlight. Thus, it is important to determine the concentration of PAEs and its degradation profile.

Various preconcentration techniques have been attempted to extract PAEs from aqueous samples such as liquid–liquid extraction and SPE [26–30]. However, these traditional pretreatment methods are expensive, require large amounts of solvents and are time consuming. Solid-phase microextraction (SPME) [31–35] and liquid-phase microextraction [36, 37] are simple and efficient preconcentration methods used for wide range of organic contaminants in aqueous samples. However the SPME method needs an expensive fiber for extraction. Furthermore liquid-phase microextraction is not suitable for high throughput analysis.

Dispersive liquid–liquid microextraction (DLLME) is based on a ternary solvent system like homogenous liquid–liquid extraction and cloud-point extraction [4, 12, 15]. In this method, the mixture of low density/high density solvent (extraction solvent) relative to water and dispersive solvent (water-miscible solvent) is rapidly injected by a syringe into an aqueous sample. A cloudy solution consisting of very fine droplet of extraction into aqueous phase is formed [38, 39].

The advantages of this pretreatment technique are rapidity, simplicity, low cost, low solvent use, short extraction time, and high recovery and enrichment factors [13, 15, 40]. This method is widely used for the determination of PAEs in tap and river water [12, 13], polycyclic aromatic hydrocarbons [41], polybrominated diphenyl ethers [42, 43], phenols and chlorophenols [44, 45], organophosphours pesticides [46–49], aromatic amine [49], chlorobenzenes [50], and metal ions in aqueous samples [51–53].

The objective of this part of my study is to use DLLME to investigate and determine the amount of phthalates in different bottled water manufactured locally in Saudi Arabia and investigate the leaching profiles of PEAs when exposed to sunlight.

2.2 EXPERIMENTAL

2.2.1 Materials and Methods

2.2.1.1 Chemicals

A mixture of PAEs standard was purchased from Sigma- Aldrich (St. Louis, MO-USA). This mixture contains dimethyl phthalate, diethyl phthalate, di-*n*-butyl phthalate, BBP, DEHP, and di-*n*-octyl phthalate esters at 2000 μg/mL; the chemical structures are shown in Figure 2 . A working standard solution was prepared daily by appropriate dilution of stock solution of PAEs in methanol. Ultrapure water was prepared from a Milli-Q system (Millipore, Bedford, MA-USA). Analytical grade dispersive and extraction solvents were purchased from Supelco (Bellefonte, PA-USA). Sodium hydroxide, sulfuric acid, and sodium chloride were obtained from Merck (Darmstadt-Germany). To avoid any carryover of PAEs, all laboratory glassware was washed with concentrated hydrochloric acid and rinsed with deionized water and acetone and dried in the laboratory oven at 100 °C for 1 h before use.

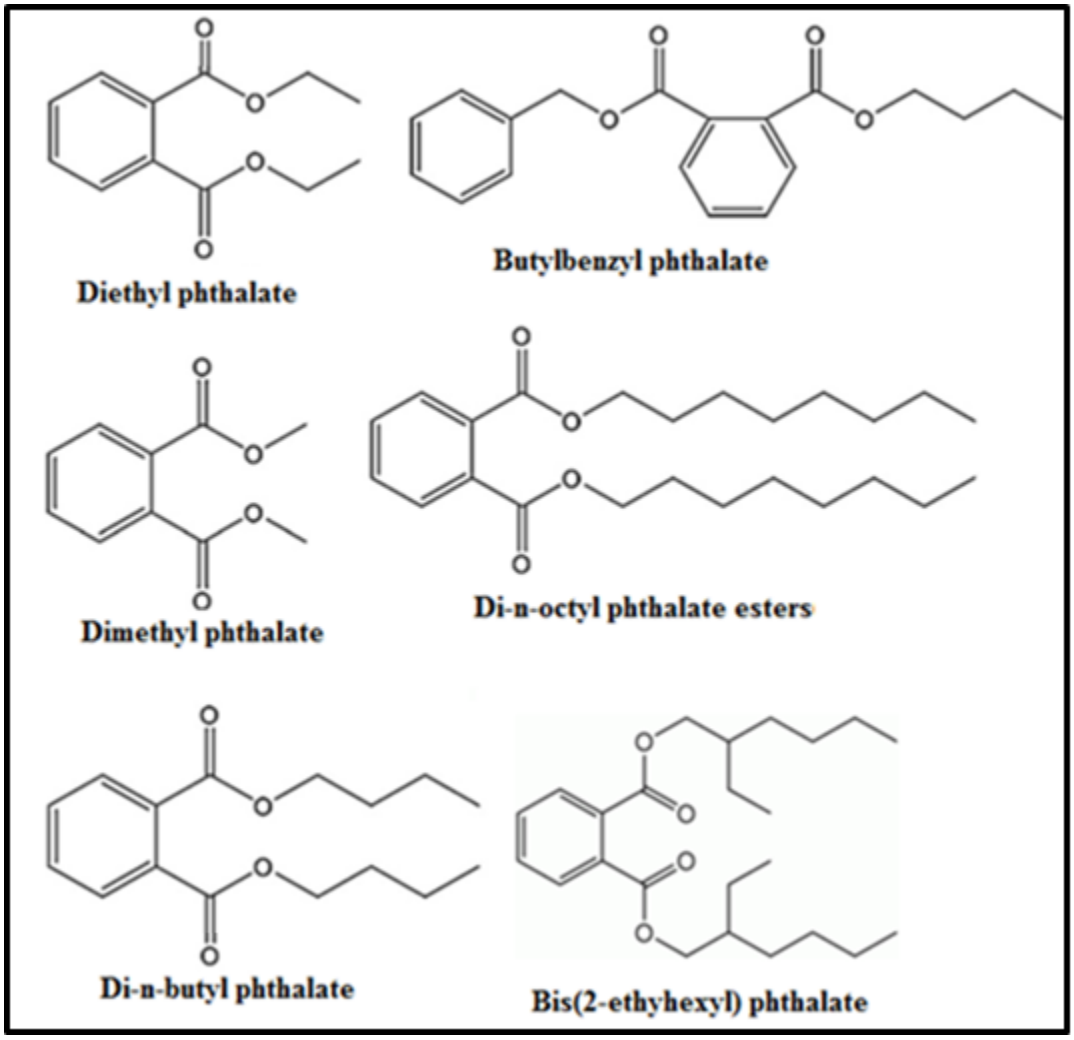

Figure 2: Molecular structures of the six PAEs.

2.2.1.2 GC-MS Analysis of PAEs

Analyses were performed using gas chromatograph mass spectrometer GC–MS-QP 2010 (Shimadzu, Japan) equipped with CTC Auto sampler. A DB-5 fused silica capillary column (30m, 0.25mm id, and 0.25-µm film thicknesses, J&W Scientific, Folsom, CA, USA) was selected to separate the analytes. Ultrahigh purity helium (99.999%) was obtained from Abdulah Hashim, Al-Khobar, Saudi Arabia and used as the carrier gas at a flow rate of 1.0 mL/min. One microliter of samples was injected in the splitless mode. The temperature program used for the analyses was as follows: the initial temperature was 40 °C held 5 min that was then increased to 300 °C at 20 °C min^{-1} and held for 11 min. The total run time was 29 min. The injection port and detector temperatures were 250 and 280 °C, respectively. Full scan with mass range of 50–500 *m/z* and selective ion monitoring mode was used for quantifying the analytes.

These chromatographic conditions are presented in Table 1 and the peaks for the PAEs were identified using individual standards. Separation and retention time for the different analytes are given in Figure 3.

Table 1: Gas chromatographic conditions for PAEs determination

Instrument	Shimadzu, GC-MS-QP 2010
Column	DB-5 fused silica capillary column (30m, 0.25mmid, and 0.25-µm film thicknesses)
He flow rate	1.0 mL/min
Injection mode	splitless mode
Injection volume	1 µL
Oven temperature program	40 °C (5 min) Ramped at 20° C/min to 300 °C and held at this temperature (10 min) The total run time (29 min).
Injection port temperature	250 °C
MS temperature	280 °C

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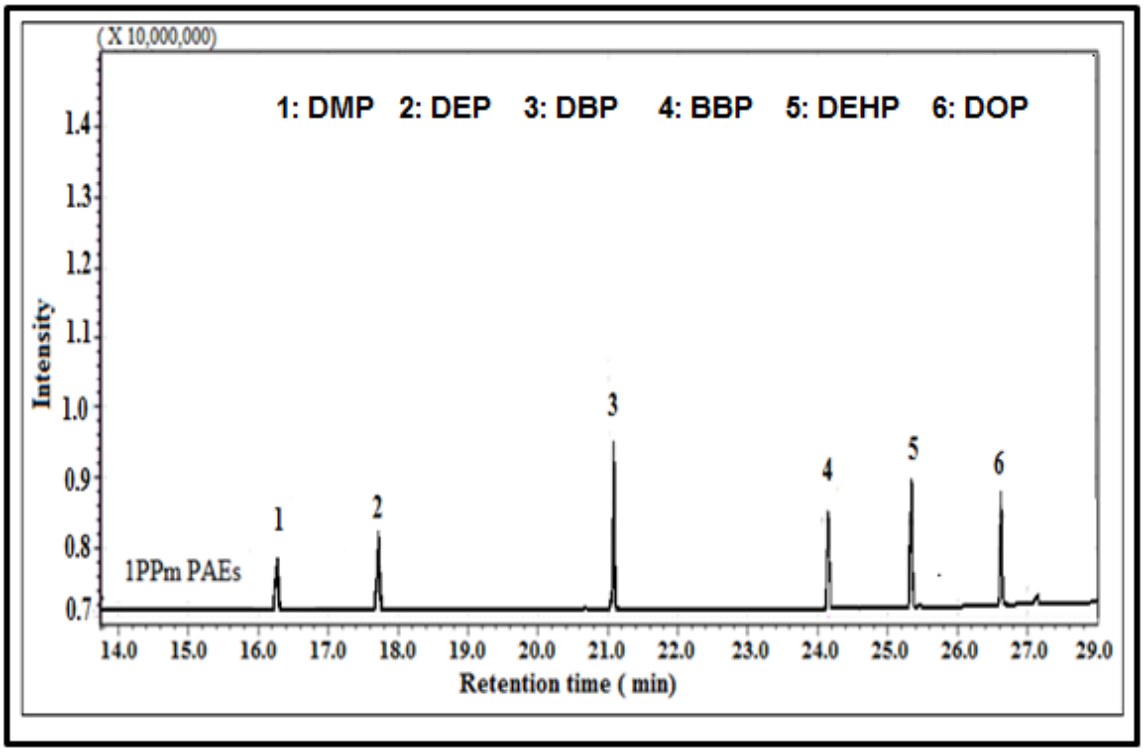


Figure 3: Separation and retention time for the different PAEs with GC-MS

2.2.1.3 Extraction Procedure

Ten milliliters of sample (pH adjusted) was placed in a glass vial and 20 μL of dispersive solvent (methanol) and 500 μL of extraction solvent (xylene) were added. A cloudy solution was formed. The sample was then ultrasonicated for 20 min. The upper organic phase was removed by glass syringe and transferred to a GC auto sampler vial. Finally, 1 μL of extractant was injected into GC–MS for analysis.

2.2.1.4 Calculation of Enrichment Factor

The enrichment factor (EF) was defined as the ratio between the analyte concentration in the extraction phase (Cext) and the initial concentration of analyte in (Co) in the standard sample.

$$EF = Cext/Co$$

*C*ext is obtained from a calibration graph prepared by direct injection of PAEs standard solution in the extraction solvent.

2.3 RESULTS AND DISCUSSION

2.3.1 Optimization of DLLME

Different factors that affect the microextraction conditions such as different combinations of solvents and volume, sample pH, extraction temperature, ultra sonication time, and ionic strength of the sample were investigated. It is important to optimize them in order to obtain the high EF and LODs.

2.3.1.1 Selection of Extraction and Dispersive Solvents

The selection of extraction and dispersive solvents (solvent combination) is the most important experimental parameter of the DLLME. The criteria for a good extraction solvent are: (i) density higher or lower than water, (ii) low solubility in water, (iii) high capability for extraction of the analytes of interest from aqueous phase, (iv) it should be easily dispersed through dispersive step and (v) good chromatography behavior [12, 13, 15, 38, 51, 54–56]. The main criterion for the selection of disperser solvent are: (i) miscibility in both phases (extraction solvent and the aqueous sample) [12, 15] and (ii) less surface tension [38].

Based on the above criteria; different extraction solvents (hexane, isooctane, *n*-pentane, toluene, and xylene) and dispersive solvents (methanol, acetonitrile, and acetone) were

selected for method optimization. Furthermore various combinations of extraction and dispersion solvents were used for optimization of DLLME.

Table 2: Composition of extraction and dispersive Solvents.

Sample number	**Extraction solvent** [a]	**Dispersive solvent** [b]
1	Xylene	Methanol
2		Acetonitrile
3		Acetone
4	Hexane	Methanol
5		Acetonitrile
6		Acetone
7	Isooctane	Methanol
8		Acetonitrile
9		Acetone
10	n-Pentane	Methanol
11		Acetonitrile
12		Acetone
13	Toluene	Methanol
14		Acetonitrile
15		Acetone

(a) 500 µL (b) 20 µL

Figure 4 shows the comparison of the EF obtained with the different extraction and dispersive solvents.

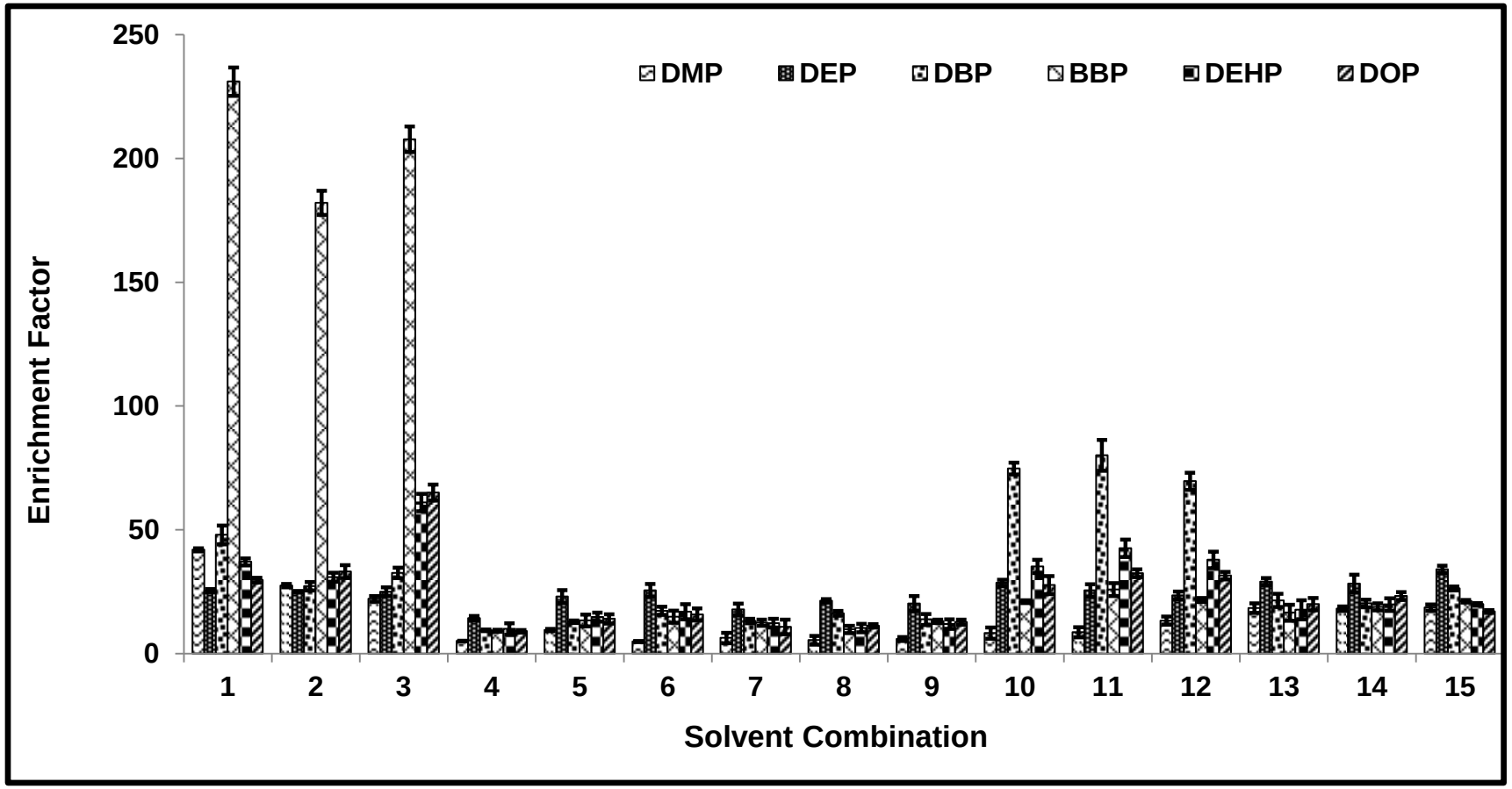


Figure 4: Extraction profiles of various extraction and dispersive solvents as mentioned in Table 2.

Extraction with xylene/methanol gave better performance when compared with rest of the solvents mixtures. Methanol was selected as dispersive solvent due to its high enrichment factor in extraction [57] and also its higher dispersing in xylene/ water mixture [15].

The methanol/xylene combination formed fine droplets quickly and generated considerably large surface area between the extraction solvent and the aqueous sample. The increase of extraction efficiency of the analytes was achieved quickly.

2.3.1.2 Effect of Dispersive Solvent Volume

The formation of cloudy solution (water/disperser solvent/extraction solvent) and the degree of dispersion of the extraction solvent in the aqueous phase were directly affected by the volume of disperser solvent. The volume of dispersive solvent plays an important role on the extraction efficiency and EF. Consequently, after choosing methanol as disperser solvent, a range of volume between 20 and 500 μL was studied for optimization. Figure 5 shows influence of disperser solvent volume on the EF of the PAEs.

When the volume of methanol was too low, the dispersion did not take place completely [38]. A reduction in the EF was observed when the volume of methanol exceeded 20 μL due to the fact that the solubility of analyte in water increases as the volume of methanol increases [38]. Hence, 20 μL of methanol was chosen as the optimum dispersive solvent volume.

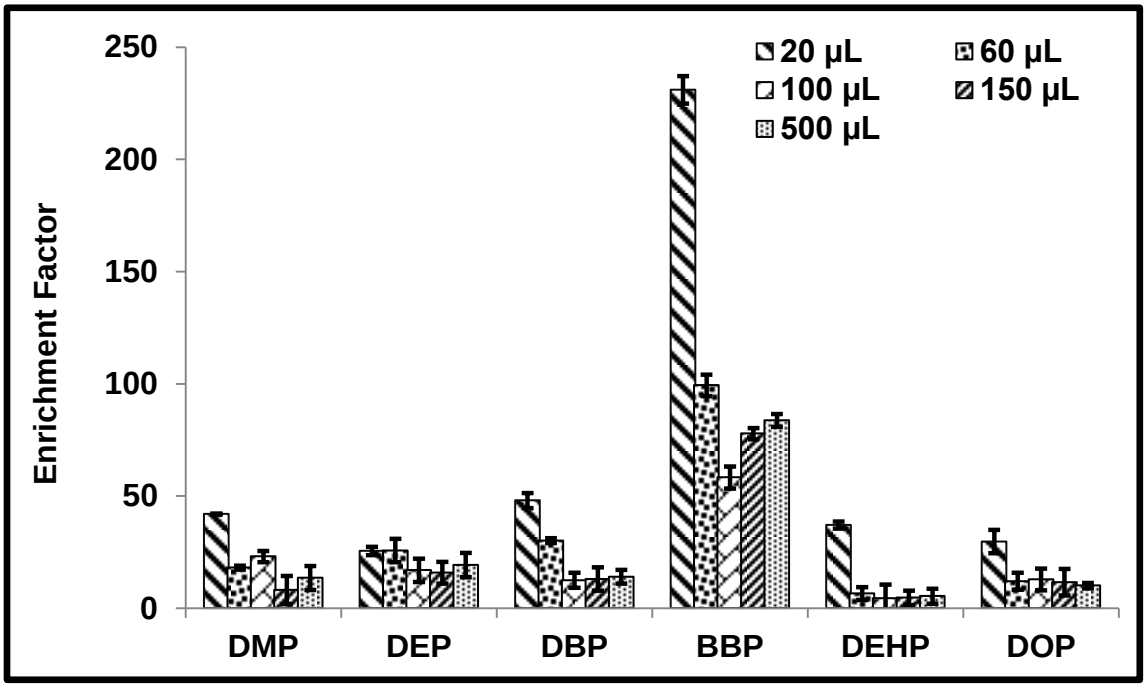


Figure 5: Effect of the volume of methanol on the enrichment factor of PAEs. Extraction condition: 10 mL of sample (20 µL of 10 mg/L PAEs, 500 µL of xylene as extraction solvent, methanol as dispersive solvent, and double distilled water), sonication time 20 min.

2.3.1.3 Effect of Extraction Time

The effect of the extraction time was examined in the range between 0 and 30 min while all other experimental conditions remained constant.

The results (Figure 6) showed that the highest EF was achieved at 20 min extraction. Increase in EF was obtained at up to 20 min, whereas above 20 min EF decreased due to temperature rise in the ultra-sonication bath. Therefore, 20 min was applied to all experiments as optimum extraction time.

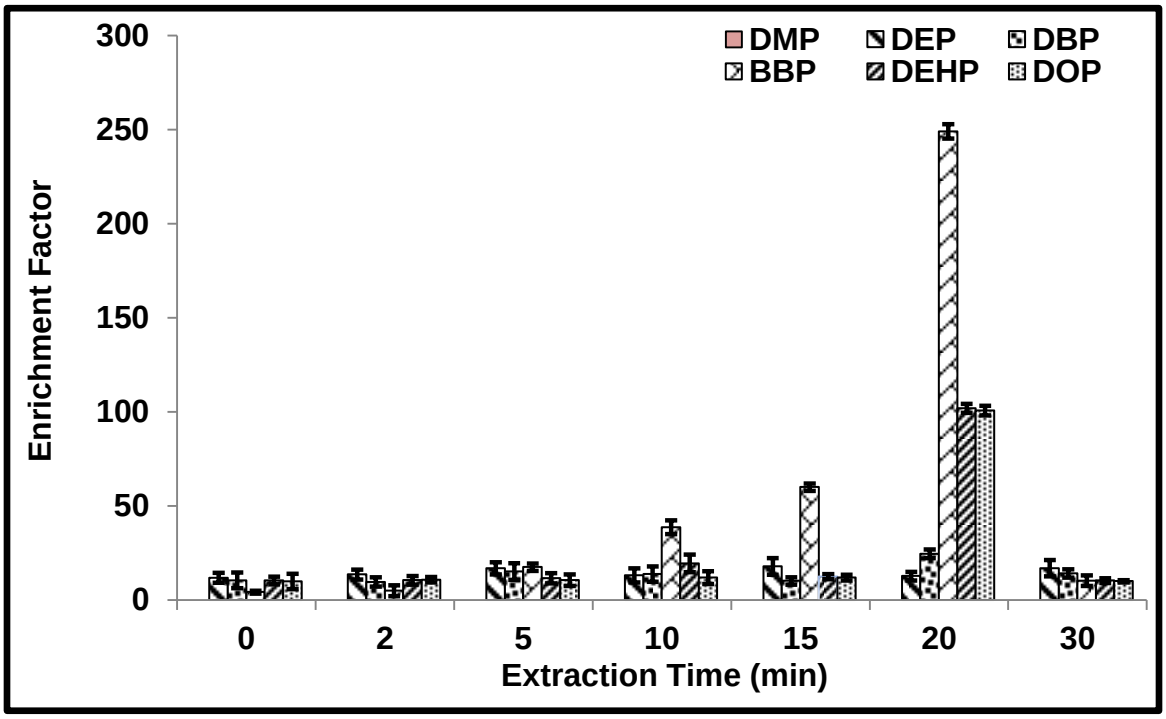


Figure 6: Effect of extraction time of PAEs: Extraction condition: 10 mL of sample (9.46 mL double distilled water, 20 µL of 10mg/L PAEs, 500 µL of xylene as extraction solvent and 20 µL methanol as dispersive solvent), sonication time 20 min.

2.3.1.4 Effect of Sample pH

Sample pH was studied in the range of 2-12, as shown in Figure 7. The EF decreases as pH increases from 2 to 12. At alkaline conditions, low EF was obtained; this could be due to hydrolysis of PAEs [13, 57]. From the results, the maximum EF tack place at pH 2; pH 2 was used for further studies.

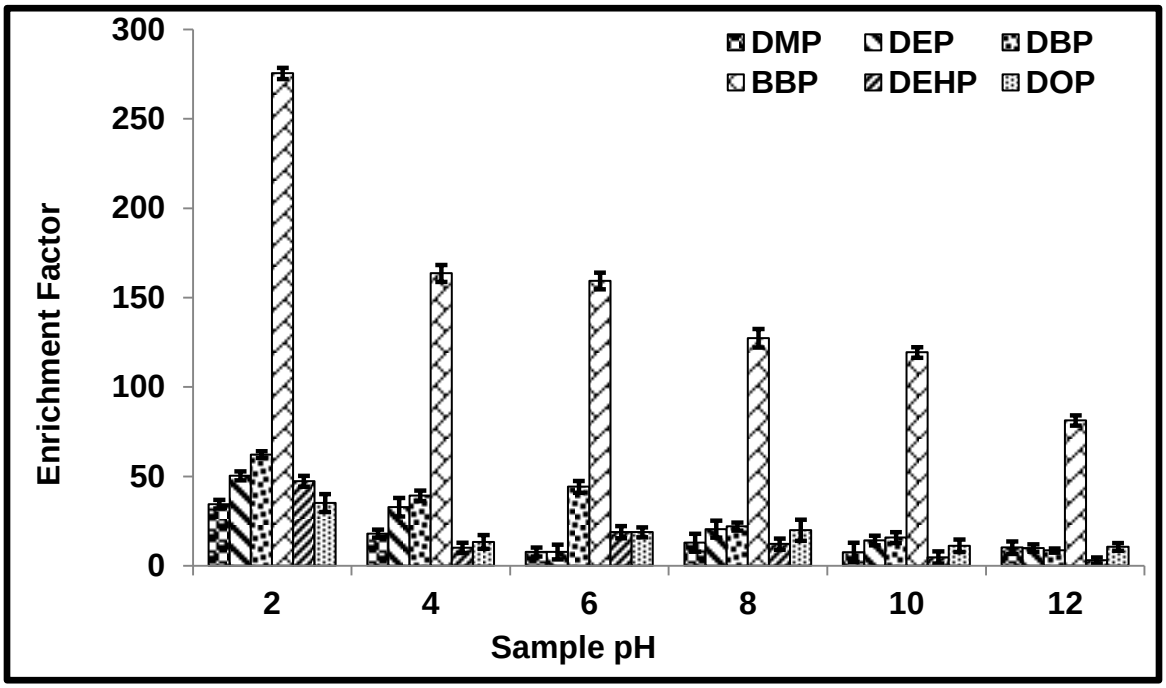


Figure 7: Effect of sample pH on the enrichment factor of PAEs: 10 mL of sample (9.46mL double distilled water, 20 µL of 10mg/L PAEs, 500 µL of xylene as extraction solvent and 20 µL methanol as dispersive solvent), sonication time 20 min.

2.3.1.5 Effect of Salt Addition

Generally, the addition of salt decreases the solubility of analytes in the aqueous sample and enhances their partitioning of the organic phase [13]. Ionic strength was evaluated by adding sodium chloride (NaCl) between 20 and 100 mg/L into the water sample.

The results in Figure 8 show a decrease in EF with the increase in the concentration of NaCl. This could be due to decrease of the diffusion coefficient of analytes by increasing the viscosity of aqueous sample [12, 13, 17, 39]. Addition of salt gave contamination, after comprehensive consideration of the results, salt was not added in the subsequent experiments.

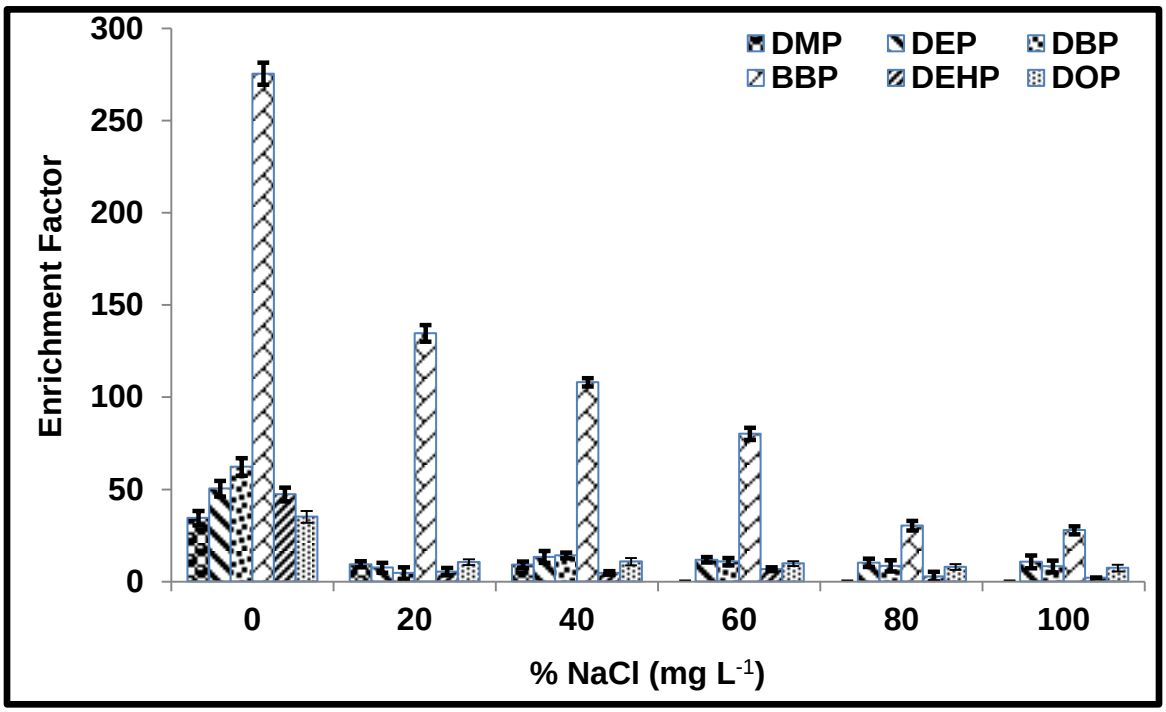


Figure 8: Effect of additional NaCl on the enrichment factor of PAEs: 10 mL of sample (9.46 mL double distilled water; pH = 2, 20 µL of 10mg/L PAEs, 500 µL of xylene as extraction solvent and 20 µL methanol as dispersive solvent), sonication time 20 min.

2.3.2 Analytical Performance of DLLME

To evaluate this method, the linear range, repeatability, and LODs were investigated under the optimized condition. The results are summarized in Table 3. Excellent linearity was observed over the concentration range of 0.05–150 µg/L for all PAEs with favorable coefficient of determination (R^2) ranging from 0.9953 to 0.9992 (Figures 9-14).

The repeatability study was carried out by extracting spiked water samples at a different concentration level of (0.05, 0.1, 1, 5, 10, 20, 50, 100, 150 µg/L), and the RSDs were between 1.3 and 5.2% (n = 3). The LODs, based on S/N = 3, ranged from 0.005 to 0.022 µg/L.

The results confirmed that the proposed method is suitable for trace level analysis of PAEs in water samples.

Table 3: Features of the DLLME. Linear range, coefficient of determination (R^2), linear equations, Relative standard deviation (% RSD), limits of detections (LODs) and of PAEs by DLLME/GC-MS

Compound	Linearity range µg L^{-1}	R^2	Equation	%RSDs (n=3)	LODs (µg L^{-1})
DMP	0.05-150	0.9991	y = 91733x - 95642	1.3	0.022
DEP	0.05-150	0.9992	y = 142681x - 92076	4.9	0.009
DBP	0.05-150	0.9991	y = 207727x + 126086	4.3	0.014
BBP	0.05-150	0.9990	y = 170262 x + 69544	1.6	0.011
DEPH	0.05-150	0.9980	y = 196393x - 48639	3.9	0.005
DOP	0.05-150	0.9953	y = 114720x - 284010	5.2	0.005

DMP, dimethyl phthalate; DEP, diethyl phthalate; DBP, di-*n*-butyl phthalate; DOP, di-*n*-octyl phthalate esters.

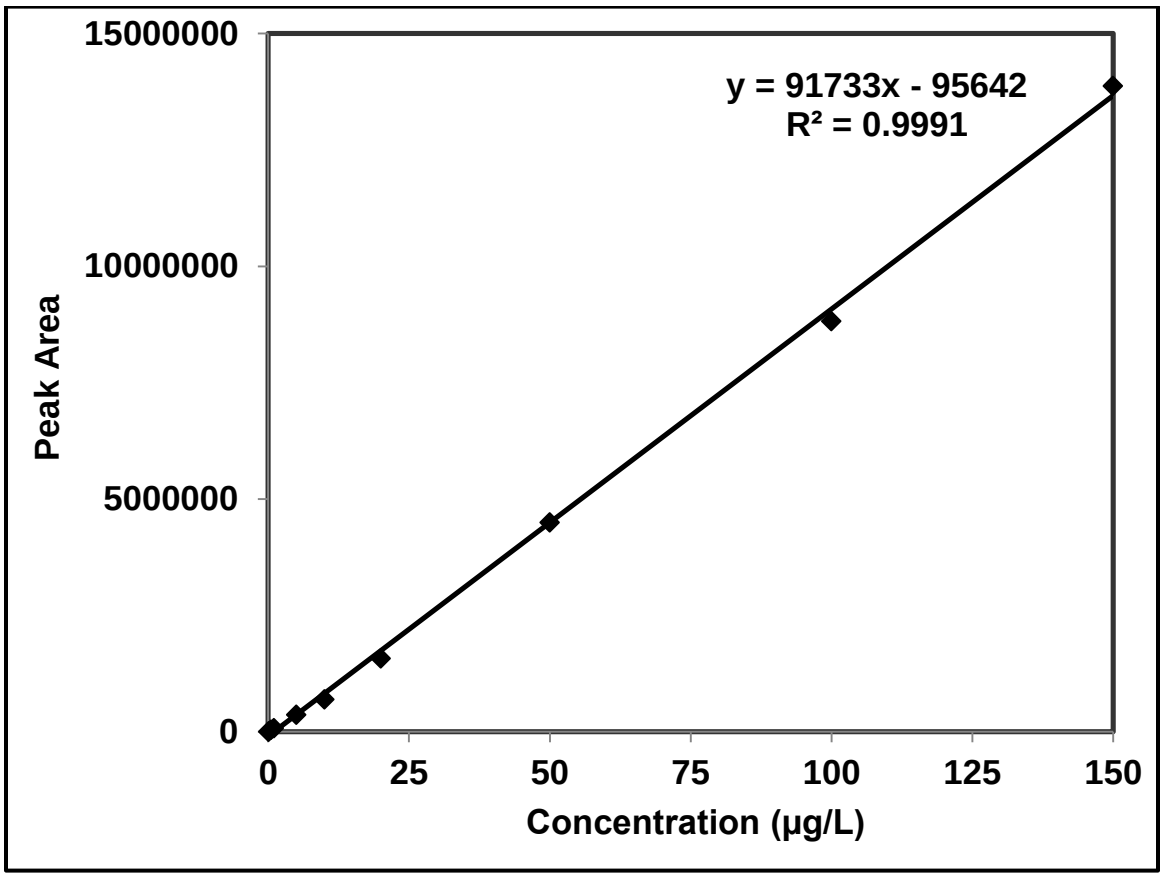


Figure 9: Calibration plot for DMP at concentrations of 0.05-150 µg/L (%RSD: 2.6-8.4)

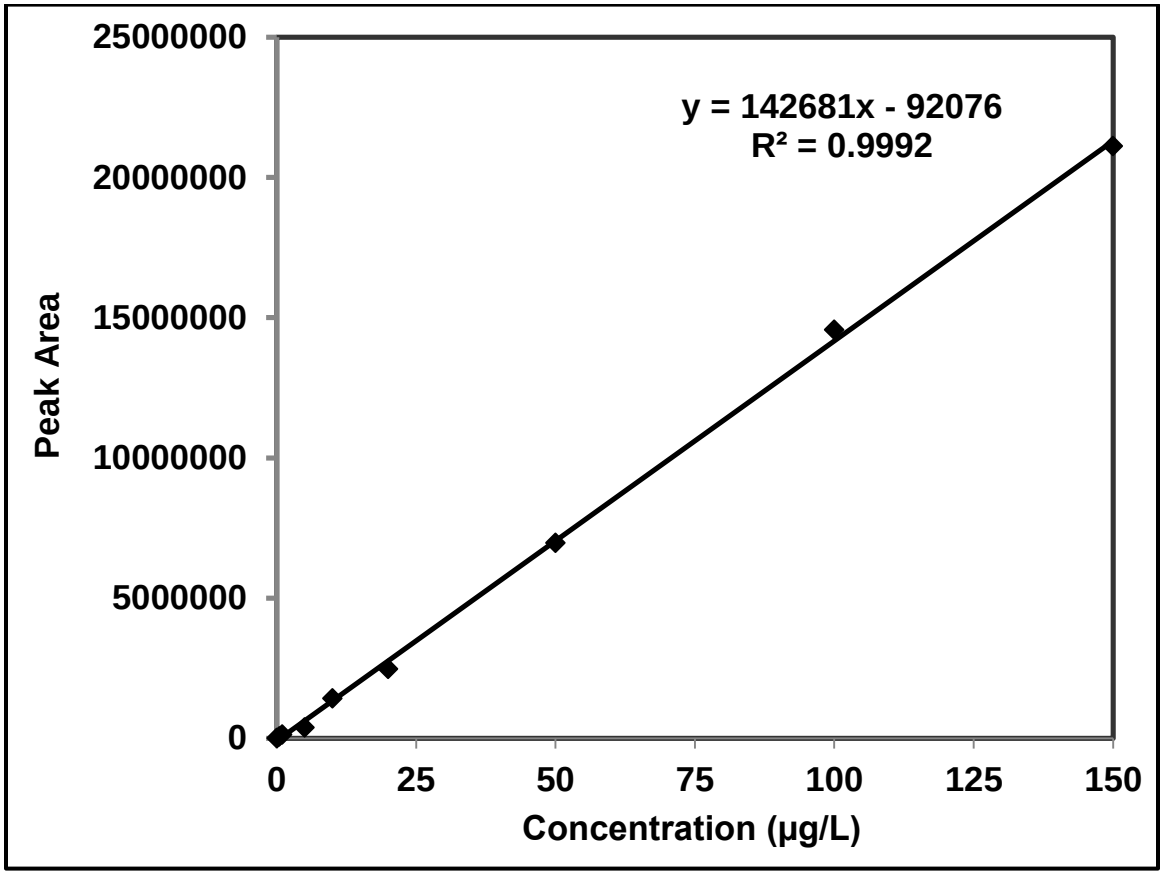


Figure 10: Calibration plot for DEP at concentrations of 0.05-150 µg/L (%RSD: 2.8-8.5)

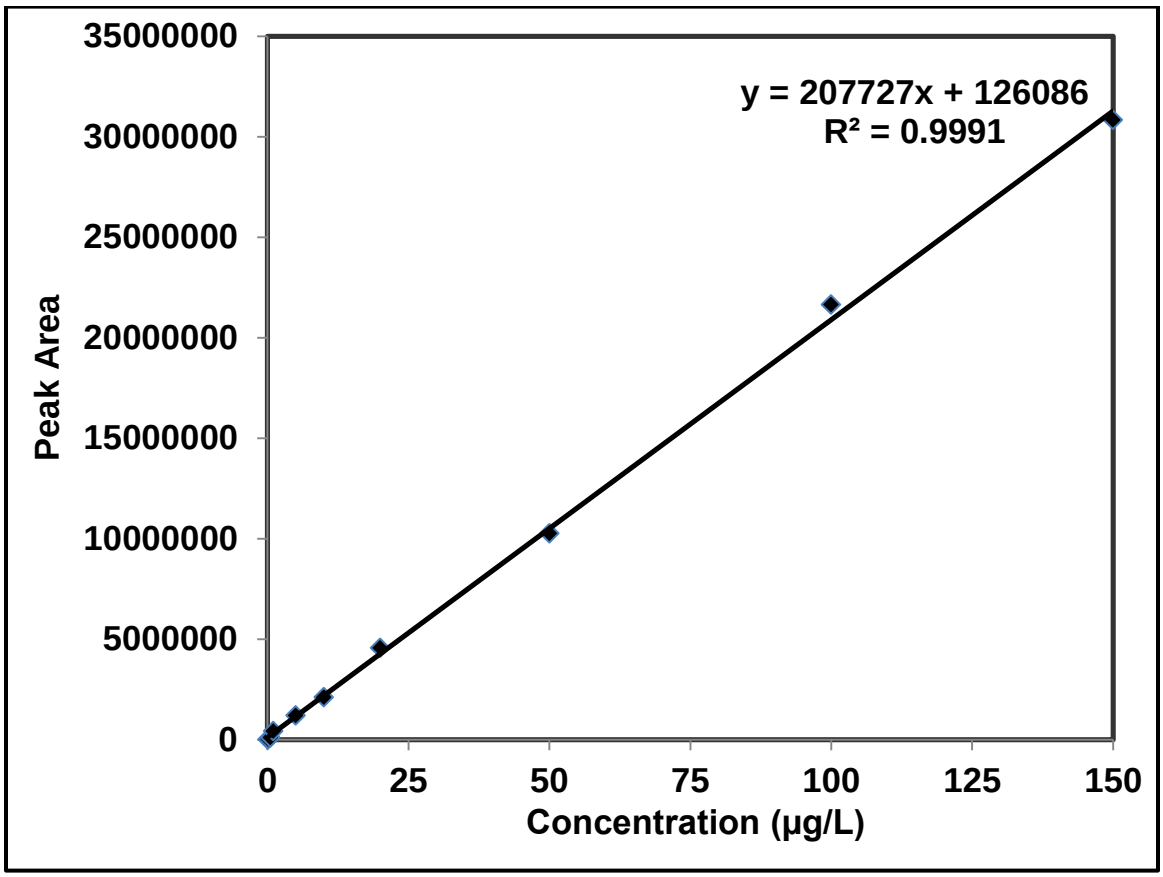


Figure 11: Calibration plot for DBP at concentrations of 0.05-150 µg/L (%RSD: 21.5-7.6)

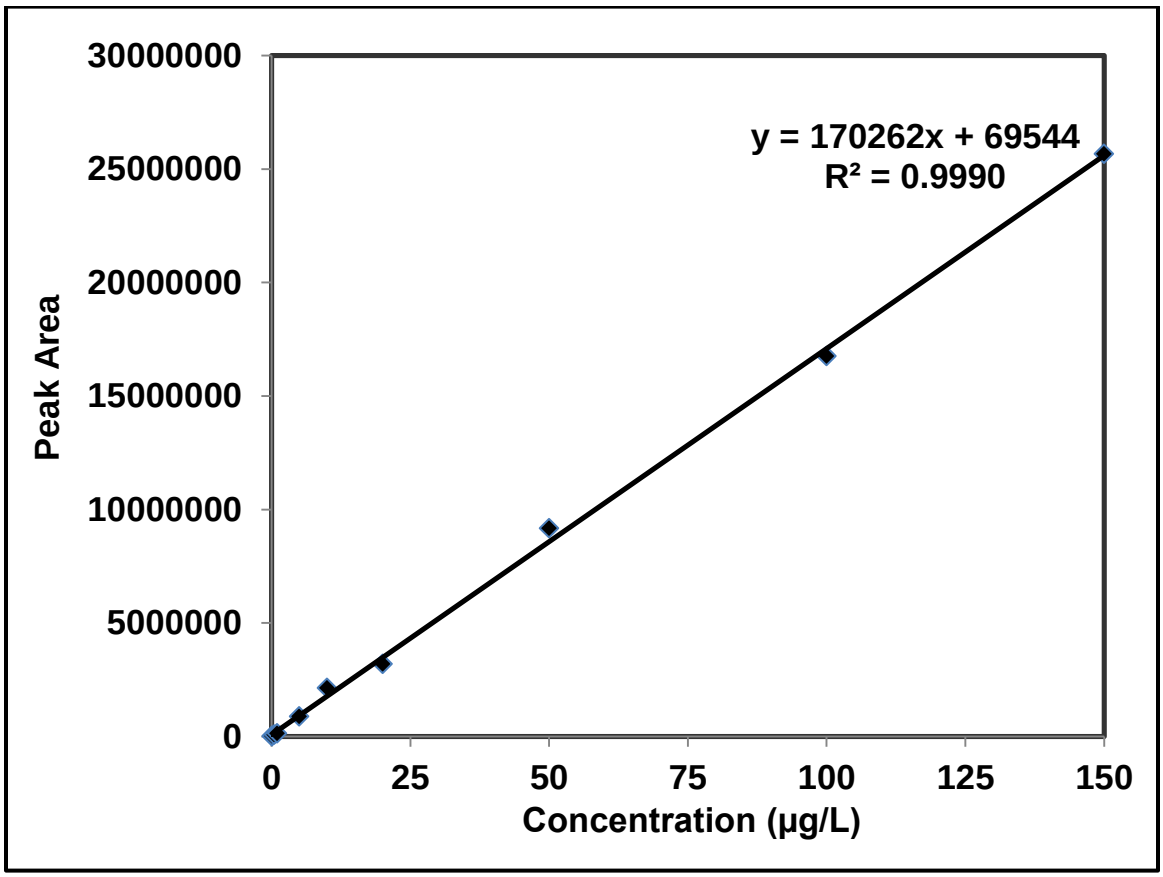


Figure 12: Calibration plot for BBP at concentrations of 0.05-150 µg/L (%RSD: 1.4-7.6)

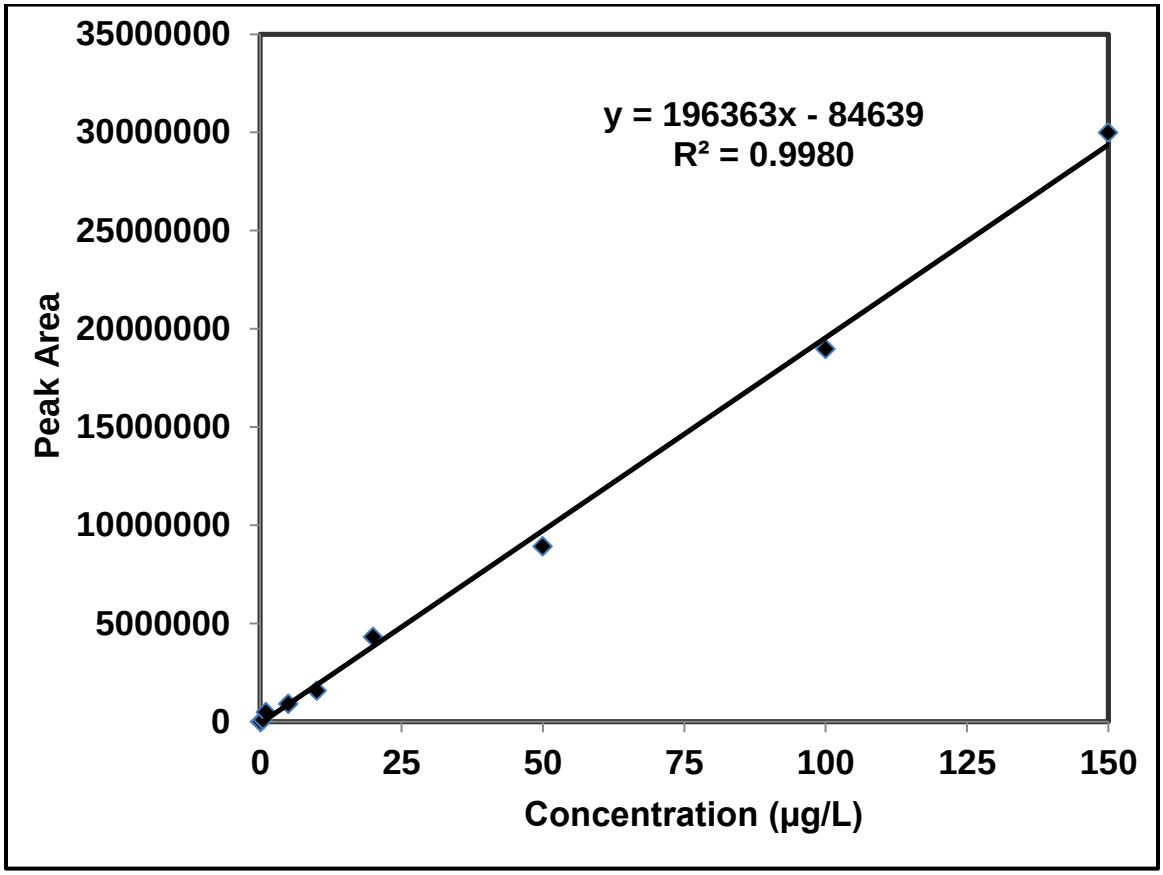


Figure 13: Calibration plot for DEPH at concentrations of 0.05-150 µg/L (%RSD: 1.9-6.1)

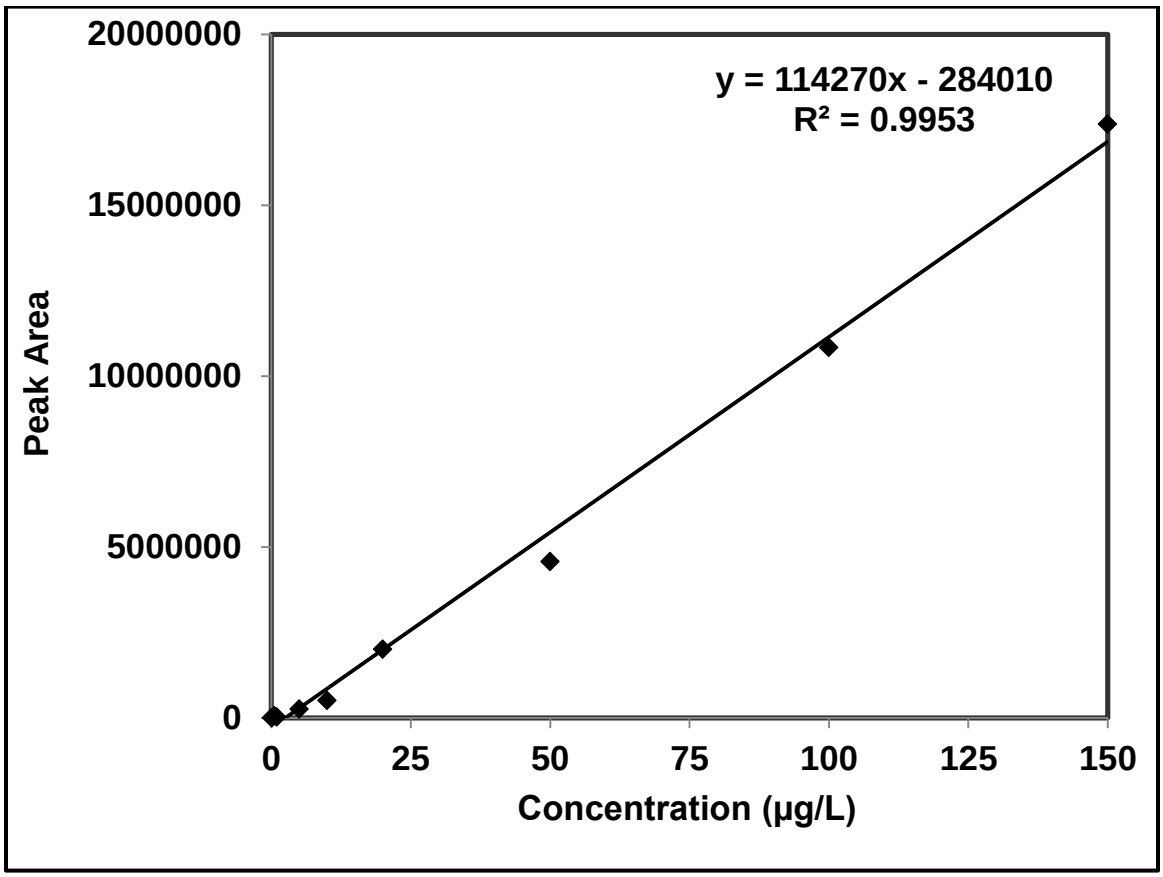


Figure 14: Calibration plot for DOP at concentrations of 0.05-150 µg/L (%RSD: 3.2-9.1)

2.3.3 Comparison of DLLME with Other Methods

Extraction and determination of PAEs in water samples by the proposed method were compared with those of other methods and the results are shown in Table 4. The developed method showed promising results compared with previously reported DLLME and SPME methods.

Table 4: Comparison of DLLME/GC-MS with other reported methods for the determination of PAEs in liquid samples.

Method	Extraction time (min)	Extraction Solvent	L.R. µg L^{-1}	LODs µg L^{-1}	% RSDs	Ref-
SPE-GCMS[a]	-	-	0.0-10^5	0.025-0.050	1.20-2.20	[11]
IL-CIA-DLLME-HPLC[b]	3-4	[C8MIM][PF6]	0.1-100	0.680-1.360	2.20-3.70	[12]
UA-DLLME/GC-MS[c]	17	CCL_4	0.80-51	0.640-0.790	2.80-4.00	[15]
DLLME-HPLC-VWD[d]	5	CCL_4	5-5000	0.880-1.800	4.30-5.90	[21]
HF-LPME/GC-MS[e]	20	Toluene	0.02-10	0.005-0.010	5.00-190	[38]
SPME/GC-MS	90	-	0.02-10	0.020-0.170	4.20-5.90	[48]
DLLME-GC-MS	20	Xylene	0.05-150	0.005-0.022	1.31-5.22	Present

LPME, liquid-phase microextraction; L.R., linearity range.
a) Solid phase extraction coupling with GC–MS.
b) Ionic liquid cold induced aggregation DLLME coupling with LC.
c) Ultrasound-assisted DLLME coupling with GC–MS.
d) DLLME coupled with LC-variable wavelength detector.
e) Hollow-fiber-based liquid-phase microextraction coupled with GC–MS.

2.3.4 Application to Real Water Samples

To assess the performance of the method; six brands (Nova, Pure aqua, Hada water, Pure life, Alqassim, and Zamzam water) of widely consumed bottled drinking water packed in polyethylene terephthalate or polyvinyl chloride were purchased from various local supermarkets in Khobar City, Saudi Arabia. The PAEs were frequently detected in all samples and the total concentration between 4.7 and 72.4 μg/L were detected after 12 h of direct exposure to sunlight. Thus we used these samples to investigate the leaching profile by exposing to direct sunlight.

For the leaching profile study, samples were analyzed after direct exposure to sunlight (outdoor) at the following times: 12, 24, 36, 48, 70, 90, 124, 222 h, where the temperature of the samples were measured as 47, 34, 43, 44, 52, 53, 51, 57 °C, respectively. Each group contains three batches from each brand and the total number of samples was 162.

Figure 15 shows the total ion chromatograms of PAEs in bottled water samples at different exposure time to direct sunlight. Degradation profile of the total average PAEs concentrations is shown in Figure 16. Table 5 shows the concentration profile of individual PAEs from water samples.

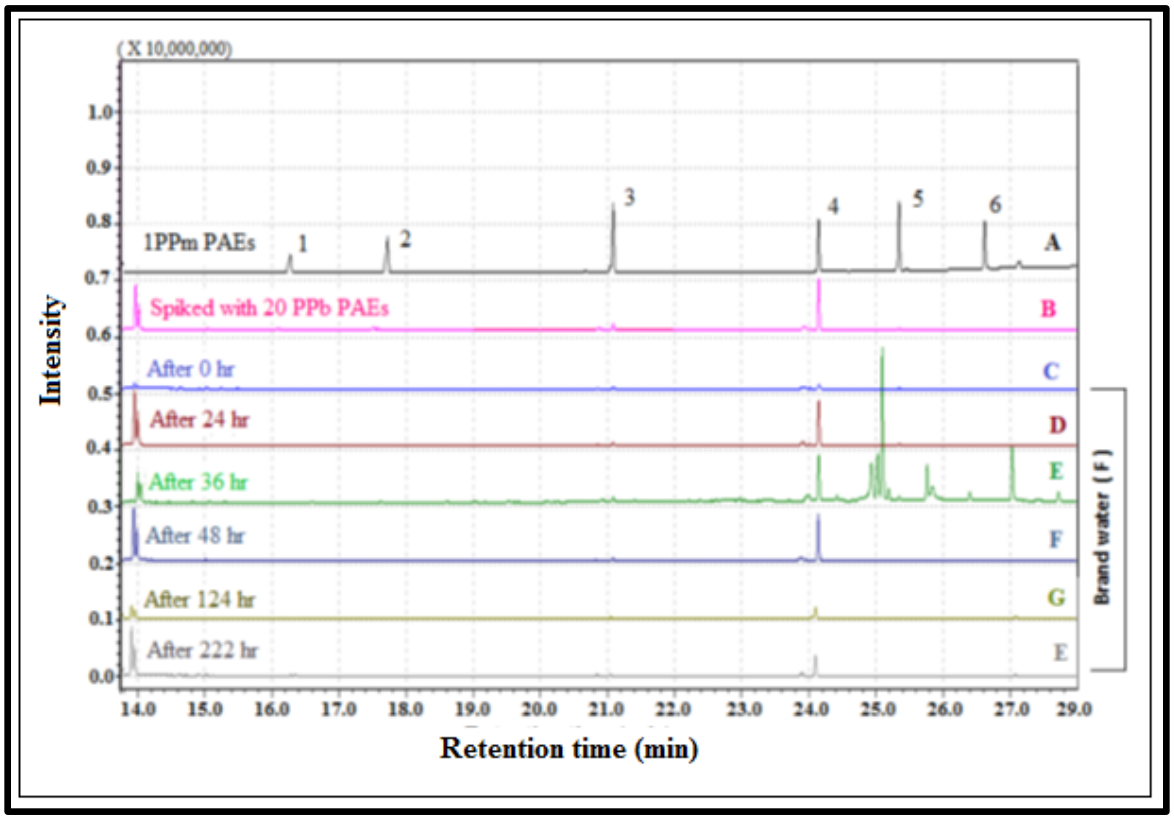


Figure 15: Typical chromatograms (A–E) belong to standard PAEs, spiked by 20 µg/L PAEs and mineral water samples (Brand F; Zamzam water) at different time out door. Peak identification: 1: DMP, 2: DEP, 3: DBP, 4: BBP, 5: DEHP, 6: DOP.

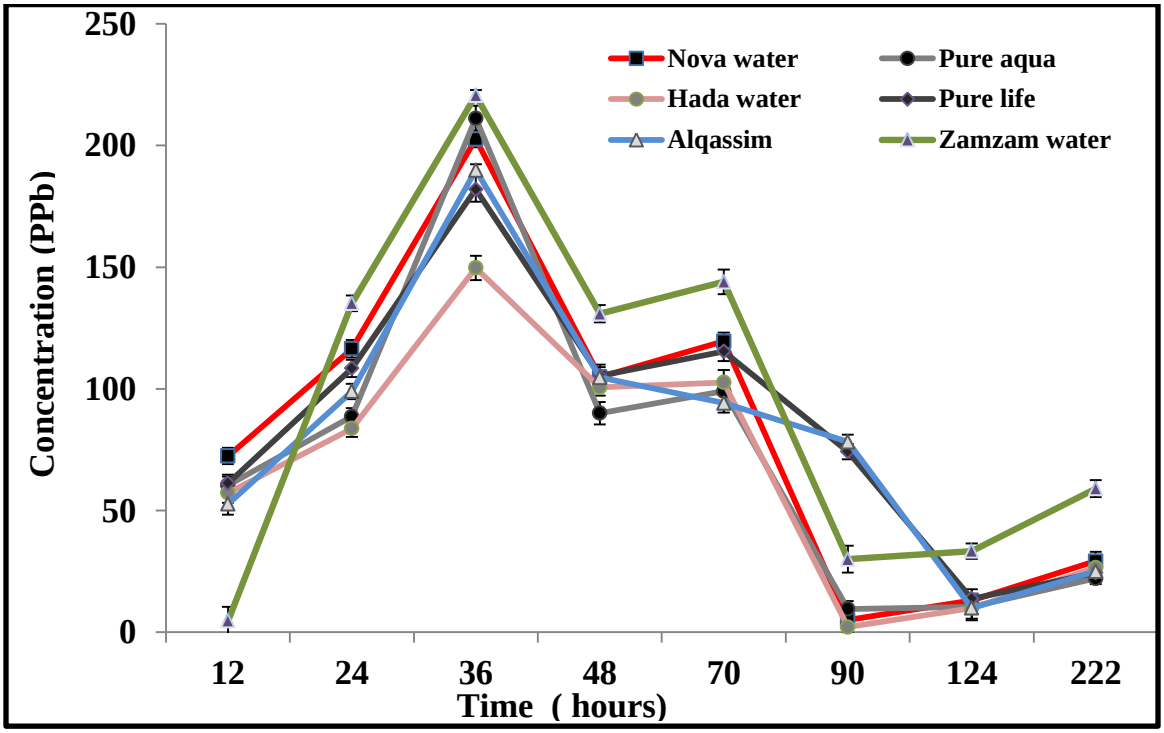


Figure 16: Average concentration of phthalate compound that extracted by DLLME in six brands of widely consumed bottled drinking water in Saudi Arabia. (Average RSD for all phthalate were between 0.6 and 9.9 %)

Table 5: Concentration leaching profile in of total PAEs compounds obtained from analysis of brands of bottled water samples in Saudi Arabia by DLLME/GC-MS.

	Concentration (µg L^{-1})							
Time in Hours	12	24	36	48	70	90	124	222
Temperature (ºC)	47	34	43	44	52	53	51	57
Nova water (% RSD: 1.4 - 9.9, n=3)								
DMP	ND	ND	ND	ND	ND	ND	ND	ND
DEP	ND	ND	ND	ND	ND	ND	ND	ND
DBP	4.64	9.86	14.69	13.74	12.23	2.93	5.15	6.74
BBP	52.35	76.95	104.93	80.67	79.87	0.69	0.16	24.74
DEPH	15.2	29.22	57.57	10.58	24.76	0.17	4.64	4.32
DOP	ND	ND	20.14	ND	ND	ND	ND	ND
Pure aqua (% RSD: 1.1 -7.1, n=3)								
DMP	ND	ND	ND	ND	ND	ND	ND	ND
DEP	ND	ND	ND	ND	ND	ND	ND	ND
DBP	2.73	7.69	22.34	6.44	6.61	0.33	4.09	2.08
BBP	51.27	71.83	114.1	76.64	81.58	0.12	4.8	18.23
DEPH	3.86	8.24	71.1	5.81	8.57	7.12	0.25	0.01
DOP	ND	ND	ND	ND	ND	ND	ND	ND
Hada water (% RSD: 1.1 - 4.6, n=3)								
DMP	ND	ND	ND	ND	ND	ND	ND	ND
DEP	ND	ND	ND	ND	ND	ND	ND	ND
DBP	2.2	6.33	19.94	8.32	6.87	0.32	1.36	2.6
BBP	42.98	66.35	82.73	79.51	78.7	0.09	4.12	20.72
DEPH	9.15	8.93	42.77	8.94	14.57	0.21	2.75	2.24
DOP	ND	ND	ND	ND	ND	ND	ND	ND
Pure life (% RSD: 0.6 – 3.8, n=3)								
DMP	ND	ND	ND	ND	ND	ND	ND	ND
DEP	ND	ND	ND	ND	ND	ND	ND	ND
DBP	3.35	7.92	17.47	7.46	4.98	0.29	1.01	1.8
BBP	52.1	86.16	99.21	88.12	83.98	2.57	0.07	21.49
DEPH	4.69	11.52	62.97	8.3	24.44	67.94	10.88	0.02
DOP	ND	ND	ND	ND	ND	ND	ND	ND

Al-qassim (% RSD: 1.0 - 4.4, n=3)								
DMP	ND	ND	ND	ND	ND	ND	ND	ND
DEP	ND	ND	ND	ND	ND	ND	ND	ND
DBP	1.63	5.67	18.29	7.83	6.04	0.44	0.27	2.15
BBP	39.03	73.08	103.75	85.33	74.43	1.27	0.1	21.77
DEPH	8.16	19.31	64.02	13.03	12.73	74.44	7.74	0.01
DOP	ND	ND	ND	ND	ND	ND	ND	ND
Zamzam water F (% RSD: 0.7 – 9.3, n=3)								
DMP	ND	ND	ND	ND	ND	ND	ND	ND
DEP	ND	ND	ND	ND	ND	ND	ND	ND
DBP	0.33	8.08	15.38	7.86	6.12	1.27	1.28	3.41
BBP	0.08	109	124.52	108.83	110.06	28.83	27.75	49.61
DEPH	4.26	15.87	75.71	8.19	24.4	0.18	3.48	3.65
DOP	ND	ND	ND	ND	ND	ND	ND	ND

ND: not detected.
a) Concentration > 50 μg/L were diluted for quantization.

The highest concentrations of PAEs were at 36-h exposure to outdoor condition (day and night) in for all brands. After 36 h, lower concentrations of PAEs were detected. This is probably due to more evaporation of PAEs. Interestingly at 36-h exposure, new peaks appeared in the chromatogram due to degradation of PAEs. Further detailed studies are required to investigate these degradation mechanisms of PAEs.

2.4 CONCLUSION

In this study, dispersive liquid-liquid microextraction was developed to determine the concentration of phthalate esters in bottled drinking samples in Saudi Arabia. Parameter that affects the DLLME were optimized to achieve better extraction efficiency. The combination of DLLME with GC–MS enables to determine at ultra-trace concentrations and degradation profiles of phthalate esters. Our findings on the degradation study show that phthalate esters leach from the bottles to water samples. However, more detailed investigations are required to understand the mechanism of degradation.

CHAPTER 3

APPLICATION OF ELECTRO ENHANCED-SOLID PHASE MICROEXTRACTION FOR DETERMINATION OF PHTHALATE ESTERS AND BISPHENOL A IN BLOOD AND SEAWATER SAMPLES

3.1 LITERATURE REVIEW

Phthalate esters (PAEs) are used as plasticizers in the manufacturing process of plastics, polyvinyl chloride, polyethylene materials to improve their flexibility and transparency. These plasticizers do not have strong interaction with polymer chains and easily leach under harsh conditions [12-16, 59]. Bisphenol A (BPA) is a chemical produced in large quantities for use primarily as flame retardant and stabilizer in the production of poly vinyl chloride, polycarbonate plastics, rubber, and epoxy resins [60-62].

PAEs and BPA are classified as endocrine disruptor chemical (EDC) which are able to cause abnormalities in invertebrate, fish, avian, reptilian, and mammalian species [63]. Carcinogenic toxicity of EDCs are known even at very low concentrations, and their mode of action mimics the estrogenic activity and may affect the health and reproduction systems of humans as well as wildlife [17-19, 61, 64-66]. Various mechanisms have been proposed in the literatures on the disruption activities of EDCs; for example, (i) by binding to receptors and mimicking or antagonizing the effects of the endocrine

hormones [67-69], (ii) by affecting the concentration of hormones through the altering of their synthesis or metabolism of natural hormones [70], (iii) by interfering with the signal between the different components of the hypothalamus-pituitary-endocrine gland axes [71] and (iv) modifying the number of hormone receptors in a cell [72-73]. Studies have shown that BPA concentration at a level of 0.23 ng L^{-1} will exhibit the estrogenic affect [74]. The United States Environmental Protection Agency has proposed maximum concentration level for benzyl butyl phthalate (BBP) in drinking water of 100 µg L^{-1} [16].

The leaching of BPA and PAEs from different industrial products such as plastic packaging and stored canned food were hardly determined due to the complicated sample matrix and low concentrations [15, 75-78]. In recent years, considerable attention has been given to the leaching effects of PAEs and BPA due to its high toxicity to humans [18, 21-22, 79-82].

In this regard different preconcentration techniques have been developed to extract EDCs from aqueous samples which include liquid-liquid extraction (LLE) and solid-phase extraction (SPE) [26-30, 83-85]. However, LLE and SPE require larger volumes of organic solvents and multi-step extractions, thus these techniques are not suitable for trace level determination of EDCs in water and food samples [67]. Liquid phase microextraction (LPME) [37-38] and dispersive liquid-liquid microextraction (DLLME) [12, 86] were used for extraction of PAEs from aqueous samples. Recently, low density solvent-based vortex-assisted surfactant-enhanced-emulsification liquid-liquid

microextraction (LDS-VSLLME) technique was developed by Zhang and Lee (2013) for the determination of phthalate esters in bottled water samples in which multistep complex extraction procedures were reported [87]. For the phthalates analysis, a single step analytical method is preferred because of the risk of contamination from glassware. Additionally, selection of suitable solvents for the extraction of polar analytes such as PAEs and BPA is a challenging task in LPME, DLLME and LDS-VSLLME [86, 87]. Stir bar sorptive extraction is another solvent minimized method (SBSE) used for the extraction of BPA from waste water [61], sea water [88], milk samples [60] and also for determination of PAEs in water samples [59].

Solid-phase microextraction (SPME) is a solvent less polymer sorption technique [89]. SPME is relatively simple; samples were extracted based on the partitioning between the polymeric sorbent and target analytes [89-90]. There are three modes of extraction by SPME; (i) direct immersion-SPME in which SPME fibers were exposed directly to the sample solution [111], (ii) headspace-SPME, where the SPME fibers were suspended on the headspace of the heated sample to extract volatile target compounds [92] and (iii) membrane protected-SPME, in which a porous polymeric membrane was used as a protective sleeve to extract polar analytes from complex samples [93].

Zhou et al. [94] and Rastkari et al. [95] develop functionalized-SPME fibers assisted microextraction for the determination of phthalate ester and bisphenol A. An additional fiber modification and longer extraction time were required to achieve better extraction

efficiency. Recently, to enhance the performance of SPME; electrical potential was applied to pencil lead fibers for the extraction of the methaamphetamine drug in an aqueous sample [96]. In this method, pencil lead was conditioned for a long time (60 min) at high temperature (600 °C) before each run. To overcome these challenges and improve the conductivity of the SPME, fibers were functionalized with multi-walled carbon nanotubes/nafion to determine the basic drug extraction in urine samples [97].

In electro enhanced solid phase microextraction (EE-SPME), faster transport of charged analyte from samples toward the surface of the fiber via electrophoresis was observed which increased the enrichment of analytes on SPME fiber [97]. In our study for the first time, a single-step EE-SPME method was developed using commercial SPME fiber (without any modification) for the extraction of phthalates and bisphenol A. The extraction performance of the EE-SPME was compared with conventional SPME (without potential) methods.

3.2 EXPERIMENTAL

3.2.1 Materials and Methods

3.2.1.1 Chemicals and Materials

A mixture of PAEs and BPA standards were purchased from sigma-Aldrich (St. Louis, MO, USA). This mixture, contain diethyl phthalate (DMP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), bisphenol A (BPA) (Figure 17) at 1000 µg ml^{-1}, was prepared in dichloromethane. A working standard solution was prepared daily by appropriate dilution of stock solution of EDCs in the same solvent. Physical and chemical properties of target analytes are shown in (Table 6). Analytical grade solvents were purchased from Supelco (Bellefonte, PA, USA). Double deionized water were obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Sodium hydroxide, sulfuric acid and sodium chloride were obtained (Merck, Darmstadt, Germany). To avoid any carryover of EDCs; all laboratory glasswares were washed with concentrated *hydrochloric acid* and rinsed with deionized water and acetone and dried out in the laboratory oven at 100 ºC for 1 h. A manual SPME fiber holder and 30 µm polydimethylsiloxane (PDMS) fibers were also obtained from Supelco (Bellefonte, PA, USA). Prior to use, the fibers were conditioned in the GC injection port in accordance with the manufacturer's recommendation. A variable voltage DC power supply was used. Two silver cable wires clipped at each end and a 10 cm length inert metallic wire with a diameter of 0.5 mm were used to complete the electrical circuit (Figure 18).

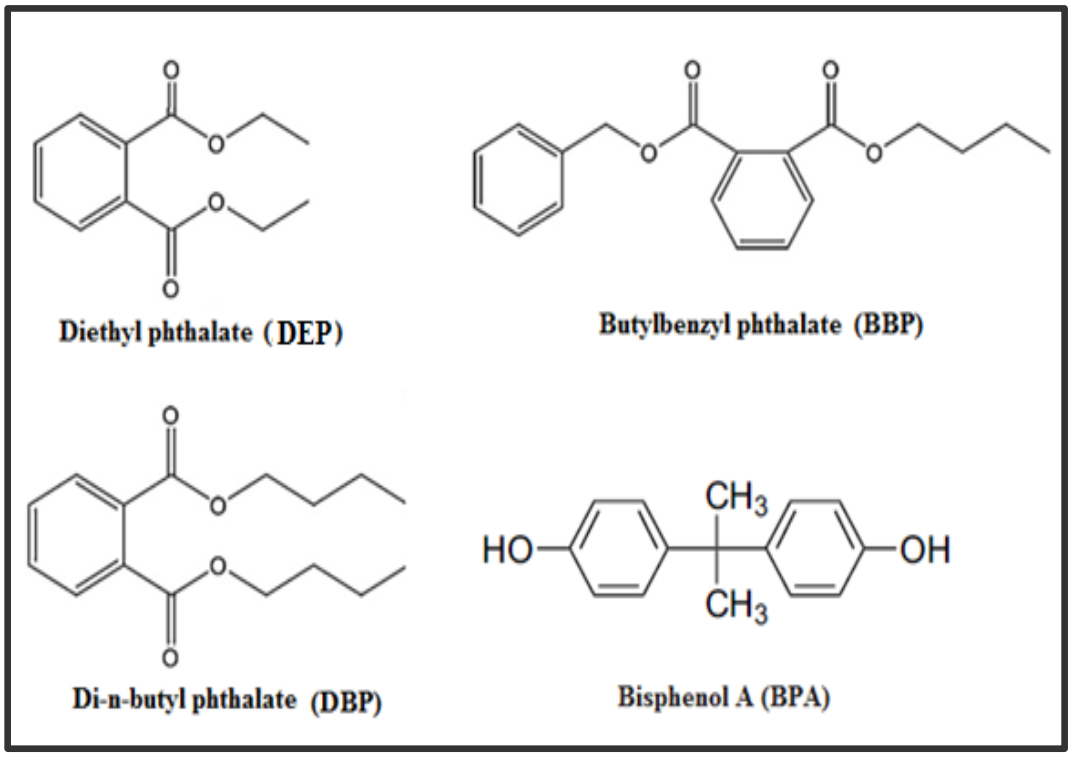


Figure 17: Molecular structures of three PAEs and Bisphenol A.

Table 6: Physical properties of selected EDCs (three PAEs and BPA) [10]

Compounds	**DEP**	**DBP**	**BBP**	**BPA**
Molecular weight (g mol^{-1})	222.24	278.34	312.36	228.29
Density (g ml^{-1})	1.12	1.043	1.0	1.2
Melting point (ºC)	-40.5	-35	61.3	159
Boiling point (ºC)	295	340	92.5	220
Water solubility (g L^{-1})	1.1	13	3	0.0027

3.2.1.2 Blood and Seawater Samples

Stored blood samples were collected from a blood bank at a local hospital at Al-Khobar, Saudi Arabia. Seawater samples were collected from the coastal area of Al-Khobar in pre-cleaned glass bottles. Blood samples were treated with anticoagulant and stored at 4 ^{o}C. Samples were directly extracted using EE-SPME without any further pre-treatment.

3.2.1.3 EE-SPME

A 10 mL sample solution spiked with EDCs was placed in a volumetric flask with a magnetic stir bar. SPME fiber and inert metallic wire were inserted in the sample solution. Both the metallic wire and SPME holder were connected via cable wires to the DC power supply. A positive voltage (+32 V) was applied to the SPME fiber and a negative (-32 V) potential was applied to the inert metallic wire as shown in Figure 18. The SPME fiber was immersed in the sample solution. Then the sample was agitated at 800 rpm for 20 min. After the extraction, the fiber was thermally desorbed in the GC-MS injection port for 3 min at 290 ^{o}C.

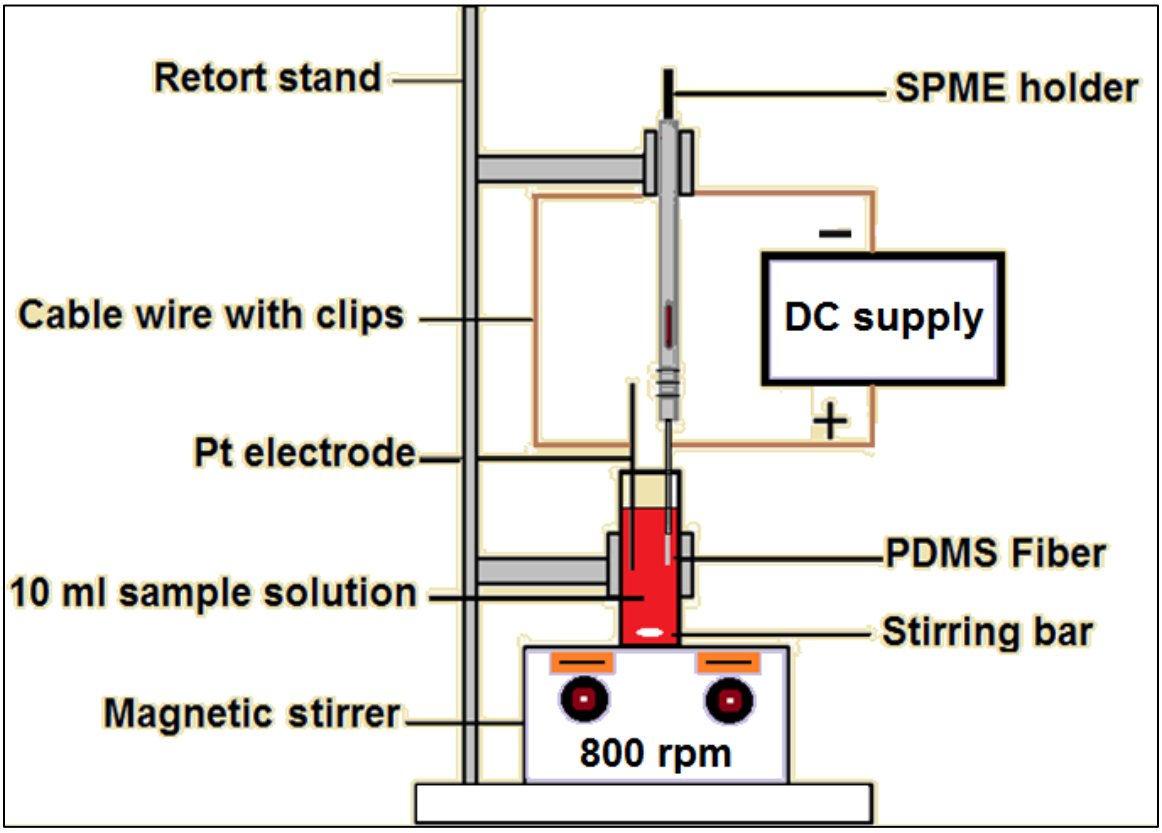


Figure 18: Schematic of EE-SPME

3.2.1.4 GC-MS Analysis

Analyses were carried out using a gas chromatograph (Agilent technologies, 6890N GC) coupled with a mass spectrometer (Agilent technologies, 5975B MSD). An HP-1 methyl siloxane column (Agilent 19091Z-213; 30 m × 320 µm I.D. × 1 µm thickness) was used. High purity helium (>99.999%) was used as a carrier gas and the samples were analyzed in a constant flow at 1.2 mL min^{-1}. The oven temperature program used for the analyses was as follows: the initial temperature was 55 ºC held at 15 min which was then increased to 250 ºC at 6 ºC min^{-1} and held for 2 min. Samples were analyzed in splitless mode. The injection port and detector temperatures were 250 and 280 °C, respectively. For qualitative determinations, the MSD was operated in full-scan mode from m/z 50 to 550. For quantitative determinations, the MSD was operated in selected ion monitoring (SIM) mode.

These chromatographic conditions are presented in Table 7 and the peaks for the PAEs were identified using individual standards.

Table 7: Gas chromatographic conditions for PAEs and BPA determination

Instrument	Agilent technologies, 6890N GC coupled with Agilent technologies, 5975B MSD
Column	HP-1 methyl siloxan column (Agilent 19091Z-213; 30 m × 320 µm I.D. × 1 µm thickness)
He flow rate	1.2 mL/min
Injection mode	splitless mode
Oven temperature program	55 °C (15 min) Ramped at 6° C/min to 250 °C and held at this temperature (2 min)
Injection port temperature	250 °C
MS temperature	280 °C

3.3 RESULTS AND DISCUSSION

3.3.1 Optimization of EE-SPME

3.3.1.1 Extraction Time of SPME (Without Potential)

The optimum absorption time can be obtained when no additional increases in peak areas with further time of extraction are found [90]. The influence of extraction time on the SPME enrichment factor was investigated with the time varying from 5 to 40 min at room temperature and samples were stirred at 800 rpm. Figure 19 shows the enrichment of PAEs and BPA using direct immersion-SPME (without potential). The enrichment factor for the PAEs and BPA slowly increased as the extraction time varied from 5 to 20 min and tended to reach equilibrium at 20 min. Based on the results, 20 min was selected for the further investigation of applied potential and salt addition studies.

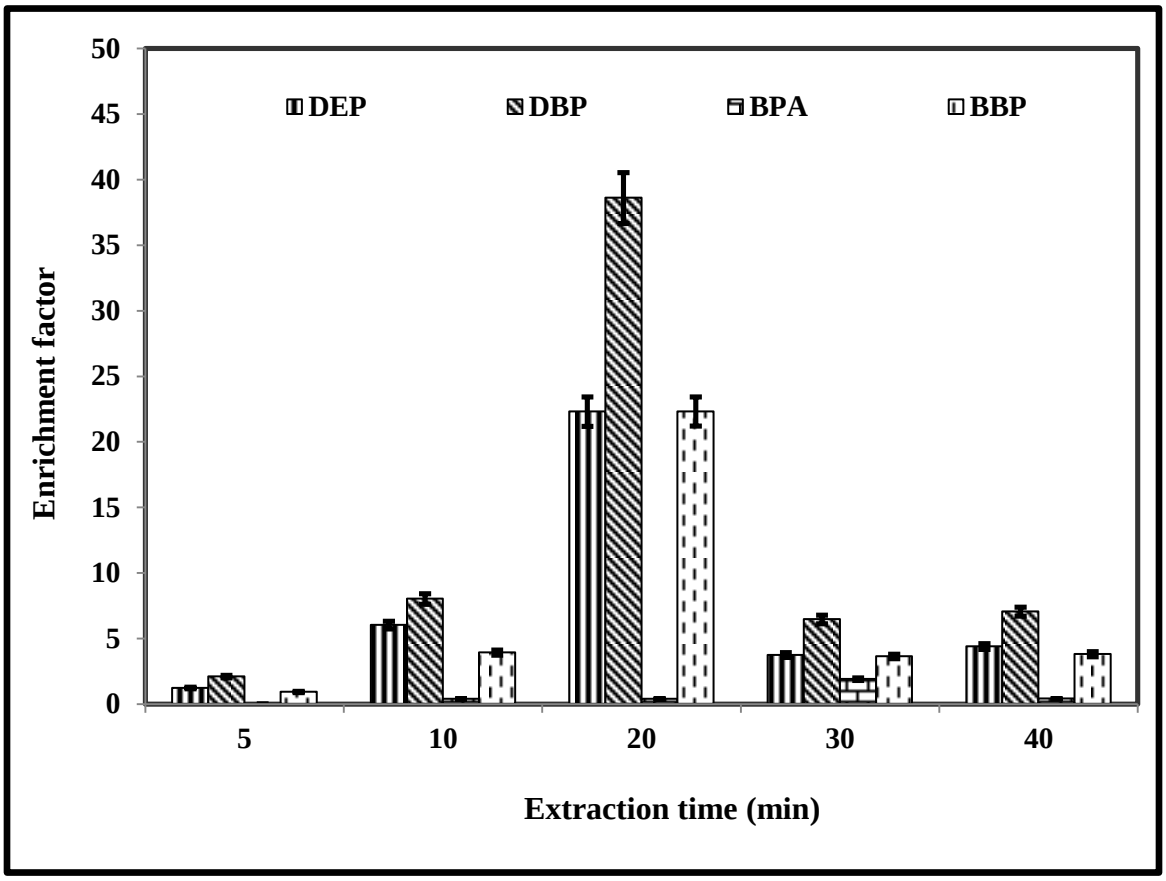


Figure 19: Effect of absorption time of SPME mode on the enrichment factor of target compounds

3.3.1.2 Effect of Applied Voltage on SPME

The effect of applied potential on EE-SPME was investigated by plotting analyte enrichment factor as a function of applied potential. Potentials between 7.5 and 50 V were applied for the SPME method (extraction time was optimized as 20 min). Figure 20 shows that the enrichment factor for the PAEs and BPA obviously increased as the potential varied from 7.5 to 32 V and then decreased.

The ester groups in the phthalate esters have a partial double bond character due to the delocalization of electrons, as shown in the resonance structures (Figure 21). The applied potential may enhance the charge formation on the phthalate ester and expedite the extraction process via electro kinetic migration. Without applied potential, the extraction process was slow and only small amount of analytes were extracted by the same SPME fiber as can be seen in Figure 20.

Furthermore during extraction, the tip of the SPME holder needle was actually immersed in the sample solution, together with the PDMS SPME-fiber. Thus, a complete electrical circuit was established between the needle and the platinum wire electrode, as shown in Figure 18. The BPA is relatively more polar than PAEs. Application of positive potentials made the fiber coating positively charged and therefore enhanced the extraction of deprotonated BPA and PAEs via electrophoresis and complementary charge interaction [97].

At higher potential > 32 V, bubble formation on the SPME fiber reduced the active surface area of the polymer coating. Thus, an optimum applied potential of 32 V was selected for further analysis.

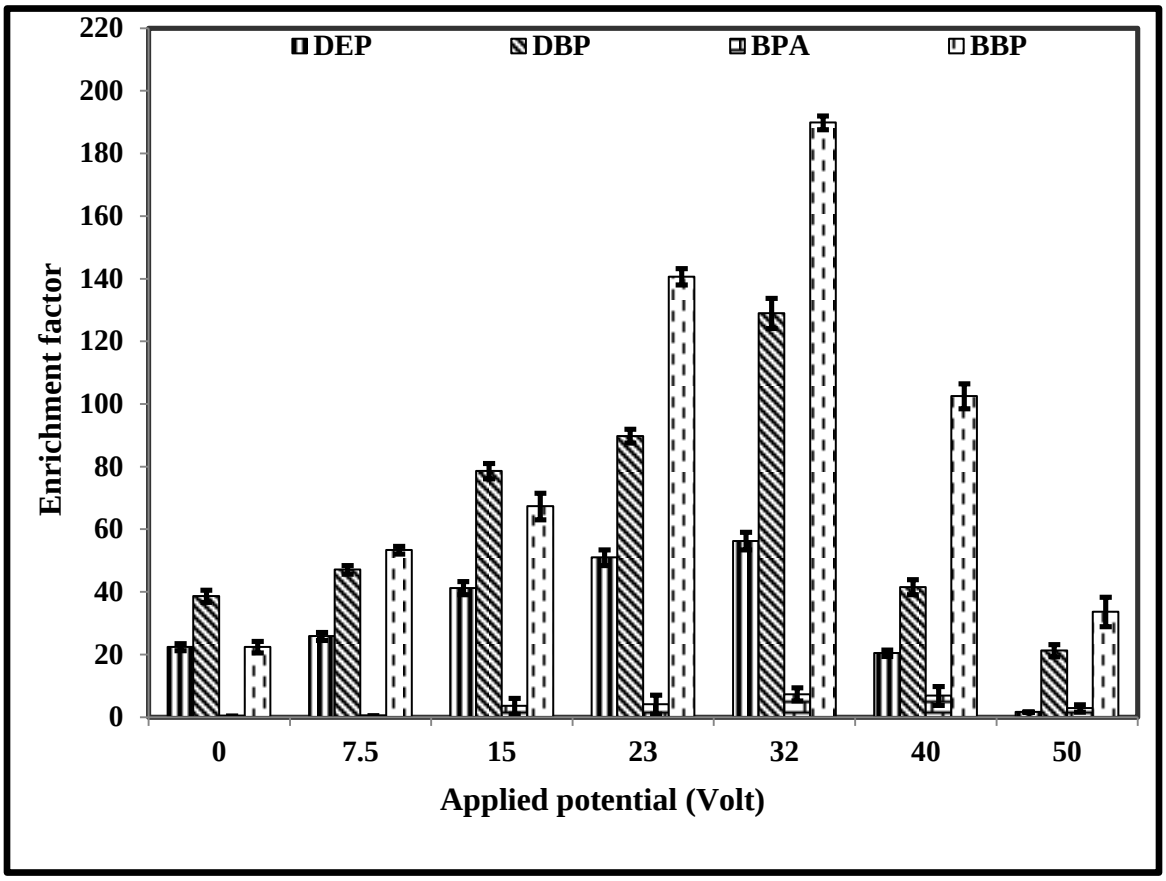


Figure 20: Effect of voltage on EE-SPME (extraction time 20 min)

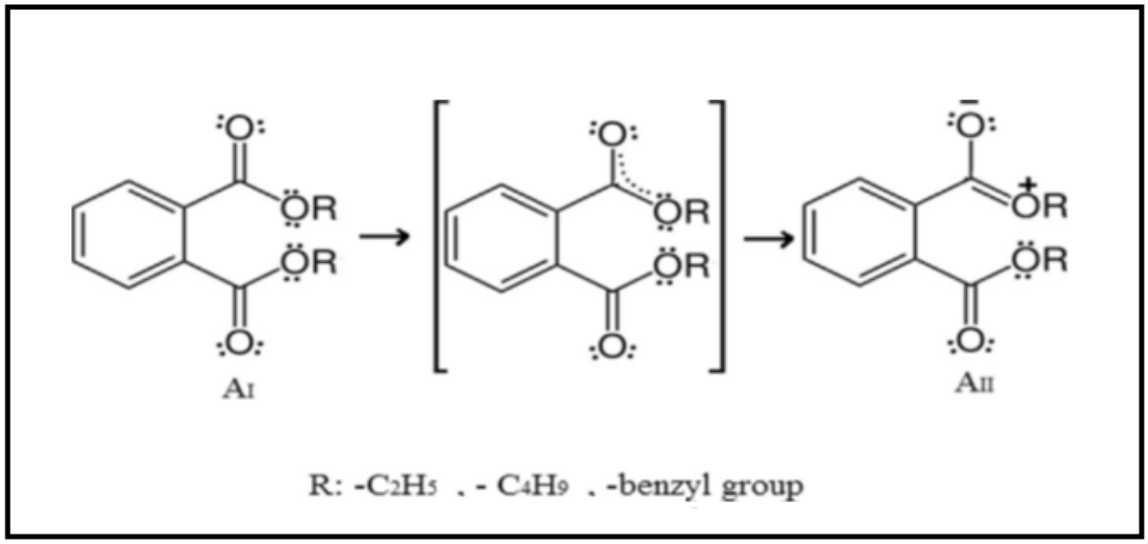


Figure 21: Ionization of phthalate esters

3.3.1.3 Effect of Salt Addition

To increase the ionic strength and decrease the analyte solubility in the aqueous samples NaCl is often added [59, 92, 98-101]. The effect of NaCl on the extraction was evaluated from 0 to 30% (w/v). For this, extraction time was 20 min and applied potential of 32 V were used. Figure 22 show the enrichment factors were highest at 5% of NaCl for all analytes. Increases in the overall ionic strength > 5% of NaCl led to the decrease of the enrichment factors. This could be due to decrease in the diffusion coefficient of analyte by increasing the viscosity of aqueous sample [12-13, 17, 102]. On the basis of the results, 5% NaCl was added to the aqueous sample for subsequent experiments.

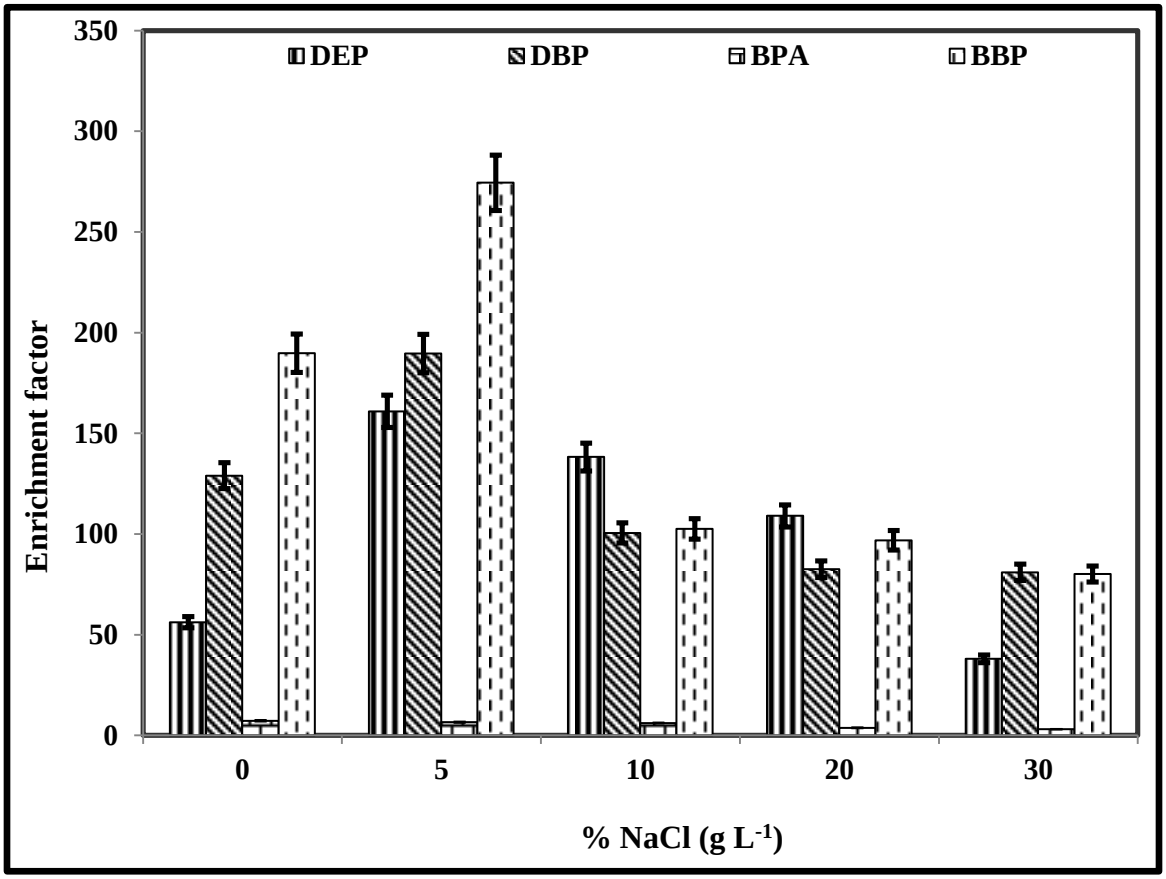


Figure 22: Effect of % NaCl added to the sample solution (extraction time 20 min; applied Voltage 32V)

3.3.1.4 Extraction Profile of EE-SPME

In the electro mediated extraction techniques, application of a potential to SPME was expected to offer faster extraction rates [96]. To determine the optimum extraction time of EE-SPME, different durations from 5, 10, 20, 30 min were studied. Figure 23 show that EE-SPME provides higher enrichment factor for all the analytes when compared to conventional SPME (Figure 19). From the result, 20 min extraction was selected as an optimum time. The lower enrichment factor at 30 min is most likely due to bubbles observed on the fiber at longer extraction times which inhibit and reduces target analayte absorption; this has been reported previously [96].

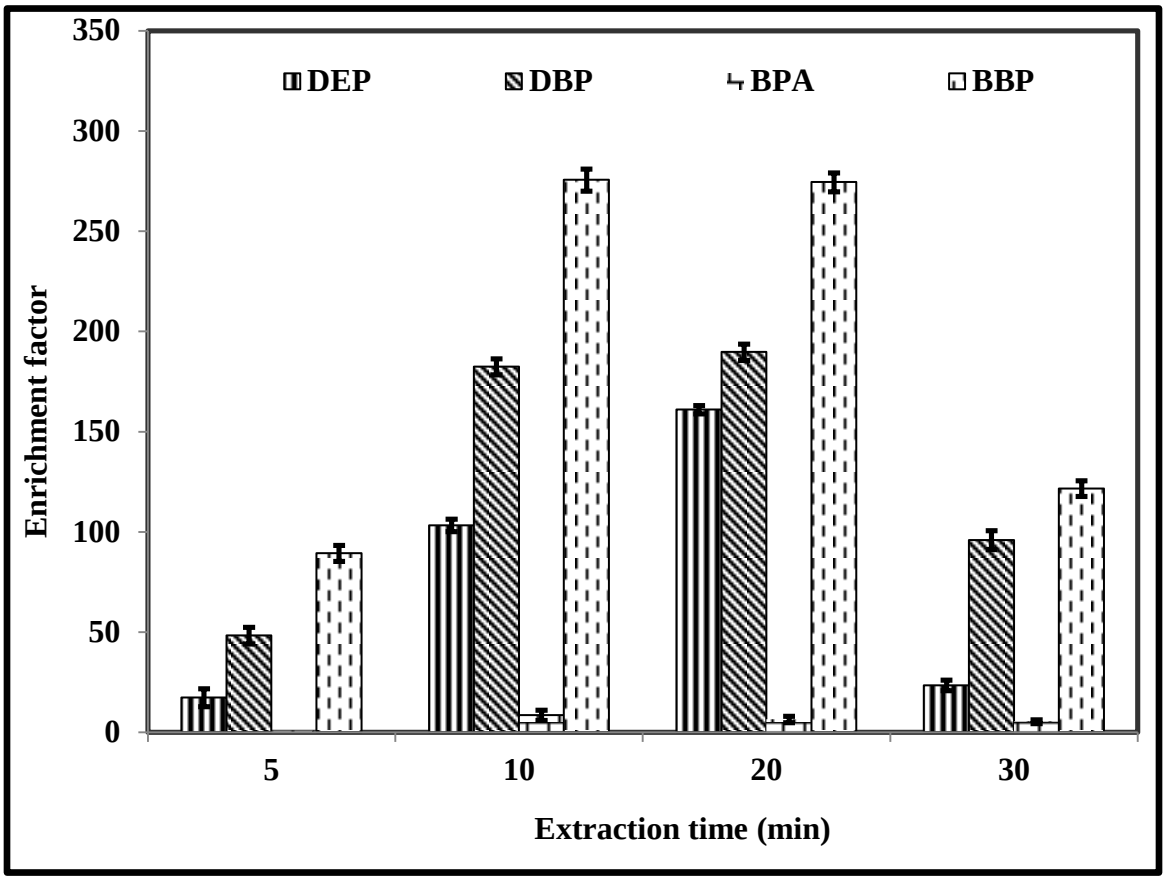


Figure 23: Effect of absorption time of EE-SPME mode on the enrichment factor of target compounds (Applied Voltage 32V, 5% NaCl)

3.3.2 Analytical Performance of EE-SPME

To evaluate this method, the linear range, repeatability and limits of detection (LODs) were investigated under the optimized condition. The results are summarized in Table 8. Very good linearity was observed over the concentration range of 1to 100 μg L^{-1} for PAEs and BPA with favorable correlation of determination (R^2) ranging from 0.9636 to 0.9988. The enrichment factor for the BBP was highest; its average was approximately 274. The repeatability study was carried out by extracting spiked water samples at different concentration levels of (1, 5, 10, 20, 40, 60, 100 μg L^{-1}), and the percentage relative standard deviations (RSDs) were between 1.0 and 5.0% (n = 9). The LODs, based on a signal-to-noise ratio (S/N) of 3, ranged from 0.004 to 0.15 μg L^{-1}. Performance of EE-SPME was compared with those of other methods reported in the literature and the results are shown in Table 9. Results of PAEs clearly indicate that EE-SPME performance is superior to the conventional SPME, and comparable with LDS-VSLLME [106]. Results obtained for BPA was comparable with previously reported literature (Table 9). The advantages of the EE-SPME/GC-MS over the other methods include high enrichment factor as well as being relatively fast and simple [96-97].

Table 8: Features of the EE- SPME

Compound	Linearity range (µg L-1)	R^2	Equation	RSDs (n=9)a	LOD (µg L-1)
DEP	1.0 -100	0.9992	y = 3.412E-05x + 12.02	4.5	0.15
DBP	1.0 -100	0.9988	y = 1.135E-05x – 12.15	1	0.004
BBP	1.0 -100	0.9968	y = 3.355E-05x – 27.5	3.3	0.1
BPA	2.0 -100	0.9636	y = 8.172E-05x – 31.6	5	0.096

[a] Under repeatability condition (n: number of trials)

Linear range, correlation of determination (R^2), linear equations, Relative standard deviations (%RSDs), limits of detections (LODs) and of PAEs and BPA by EE-SPME/GC-MS

Table 9: Comparison of EE-SPME/GC-MS with other reported methods for the determination of PAEs and BPA in liquid samples.

Method	Fiber	Sample	Extraction time (min)	L.R ($\mu g\ L^{-1}$)	LODs ($\mu g\ L^{-1}$)	%RSD	Ref
PAEs							
SPME[a]/HPLC-DAD	PDMS	water	20	-	1.0-2.5	5.0-20	[103]
SPME/GC-MS	PA	water	90	0.02-10	0.02-0.17	4.2-5.9	[59]
EE-SPME/GC-MS	PDMS	water	20	1-100	0.004-0.15	1.0-4.5	Present
BPA							
SWCNTs[b]-SPME/ GC-MS	Modified	Canned food	40	0.3-60 ($\mu g\ Kg^{-1}$)	0.1 ($\mu g\ Kg^{-1}$)		[95]
SPME/GC-MS	PDMS	Milk	30	1-10	0.01 - 0.1	4.1-5.8	[60]
SPME/GC-MS	PDMS/DVB	water	60	0.03-195	0.04-1.0	6-9	[61]
EE-SPME/GC-MS	PDMS	water	20	2-100	0.096	4.2-5	Present

L.R: Linearity Range. LOD: Limits of Detection. %RSD: Relative standard deviation.

[(a)] Solid phase extraction coupling with GC-MS, [(b)] Single wall carbon nanotubes.

3.3.3 Real Samples Analysis

To demonstrate the feasibility of the EE-SPME/GC-MS method; the optimized conditions were applied to human blood samples (stored in transfusion bags in a local hospital blood bank) and seawater. Ten millimeters of each seawater and blood samples were used for the EE-SPME extraction. PAEs were detected in all samples, the highest concentration was found to be 54.5 µg L^{-1} of DEP in blood samples whereas, 36.5 µg L^{-1} of BBP was detected in seawater samples. BPA was not detected in both types of samples. To assess the matrix effect of the EE-SPME, real samples were spiked with 20 µg L^{-1} of target analytes and extraction recoveries were calculated (Table 10). Recoveries for PAEs in seawater and blood samples ranged from 89.6 to 95%, while for BPA the range was 73.9 to 87.1%. Lower recovery of BPA in blood samples indicates that it might strongly bind to blood proteins and the influence of matrix effect (interference due to complex composition) on the extraction.

Figure 24 and Figure 25 show the GC-MS total ion chromatograms of spiked and unspiked seawater and blood samples respectively.

Table 10: Relative recovery of EDCs from seawater and human blood samples by EE-SPME-GCMS

	Human Blood				Sea water			
	Concentration (µg L^{-1})		% Recovery (n=3)	%RSD (n=3)	Concentration (µg L^{-1})		% Recovery (n=3)	%RSD (n=3)
EDCs	Real sample	After spiked with 20 (µg L^{-1})			Real sample	After spiked with 20 (µg L^{-1})		
DEP	54.5	73.6	95	2.4	6.98	25.66	93.4	5.4
DBP	28.6	47.5	94.4	6.7	7.9	26.03	90.4	3.7
BPA	ND	17.4	87.1	15.4	ND	14.78	73.9	6.2
BBP	24	42.7	93.4	8.0	36.5	54.42	89.6	2.7

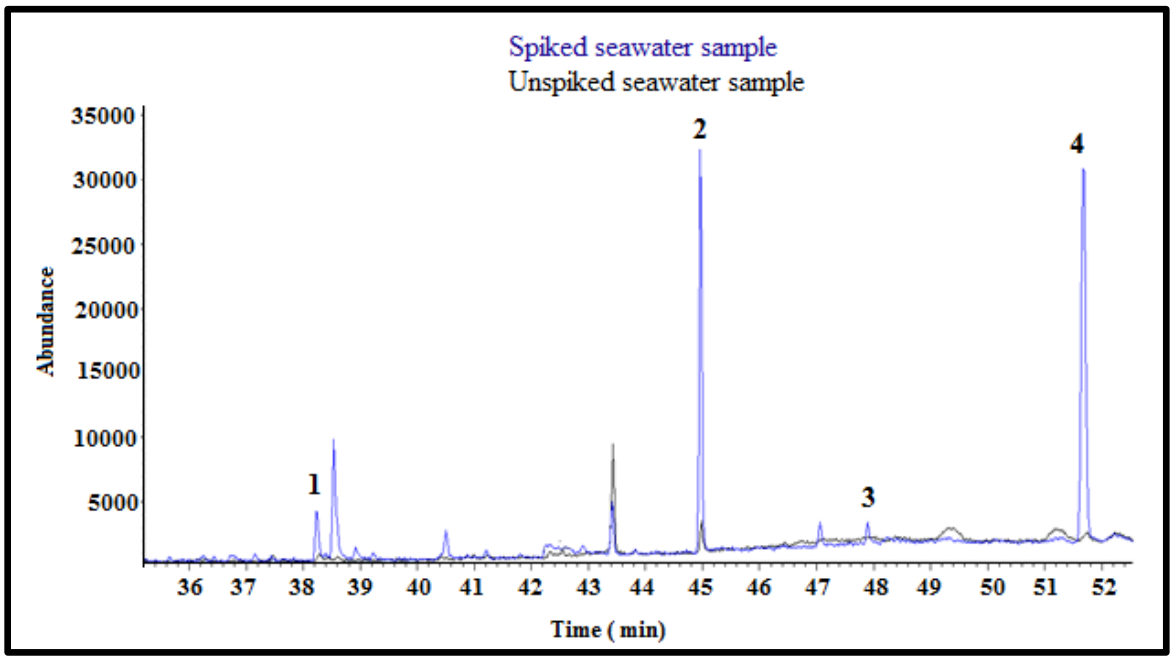


Figure 24: Chromatogram of extracted EDCs of seawater sample (50 µg L^{-1} spiked seawater samples; peak identification: 1: DEP, 2: DBP, 3: BPA, 4: BBP)

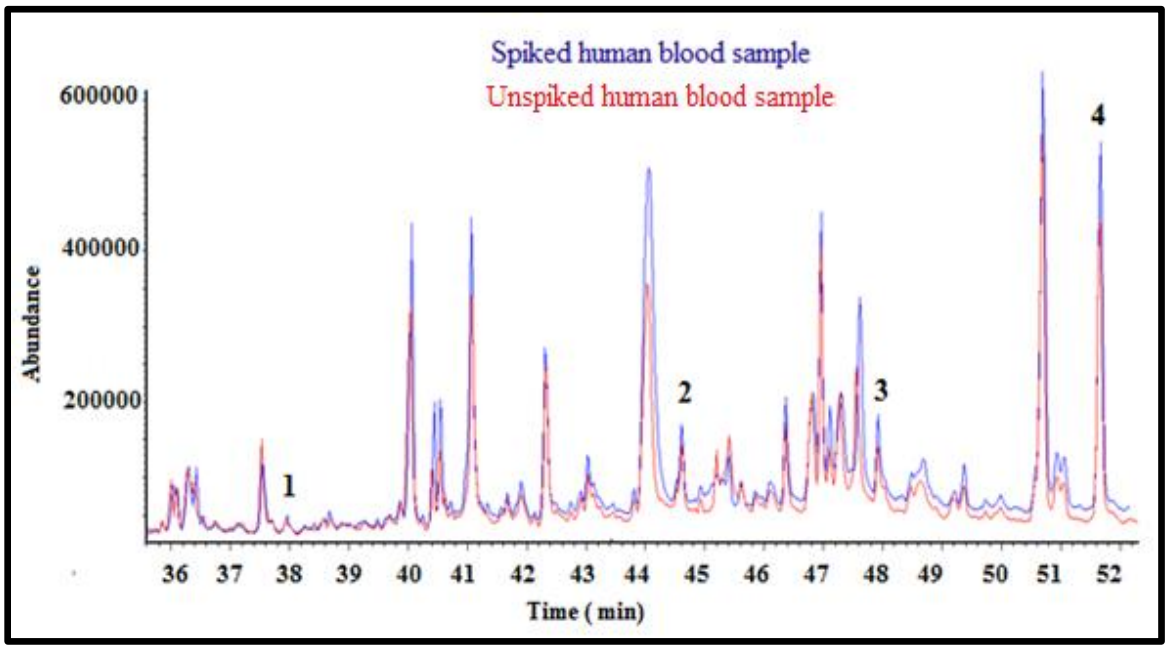


Figure 25: Chromatogram of extracted EDCs of human blood samples (30 µg L^{-1} spiked human blood sample, peak identification: 1: DEP, 2: DBP, 3: BPA, 4: BBP)

3.4 CONCLUSION

In this study, for the first time, an electro-enhanced solid-phase microextraction was developed to determine the concentration of three phthalate esters and bisphenol A in seawater and human blood samples. Various experimental conditions influencing EE-SPME were optimized. The combination of EE-SPME with GC-MS enables PAEs and BPA compounds to be determined at ultra-trace level concentrations. Application of the proposed method reveals trace level contamination of phthalates and BPA in transfusion blood bags and seawater samples. However, further studies with larger samples are required to better understand the leaching profile of these compounds in blood samples.

CHAPTER 4

APPLICATION OF AN AUTOMATED HEADSPACE SOLID PHASE MICROEXTRACTION FOR DETERMINATION OF N-NITROSOAMINES IN GROUND WATER SAMPLES

4.1 LITERATURE REVIEW

N-nitrosamines (NAs) are a class of organic compounds that come from the reaction of amines (secondary amines) with nitrosating agents, as shown in Figure 26 [104-105]. NAs are classified as potentially hazardous disinfection by-products (DBPs) produced through chlorine based disinfection processes of drinking water [104, 106]. NAs also present in other anthropogenic sources such as polymer waste, plasticizers, rocket fuel (incomplete oxidation of hydrazines), batteries and other industrial products [104].

As a result, NAs are detected in a wide range of sample matrices which includes drinking, ground, waste and treated wastewater samples [107-108], soils [109], cosmetics [110-112], biological sample (urine, saliva, blood) [110], and tobacco smoke [113]. Trace amounts of NAs were detected in many food products such as bacon [114], fish and beer [115-116] meat [117], frankfurters and sausages [118].

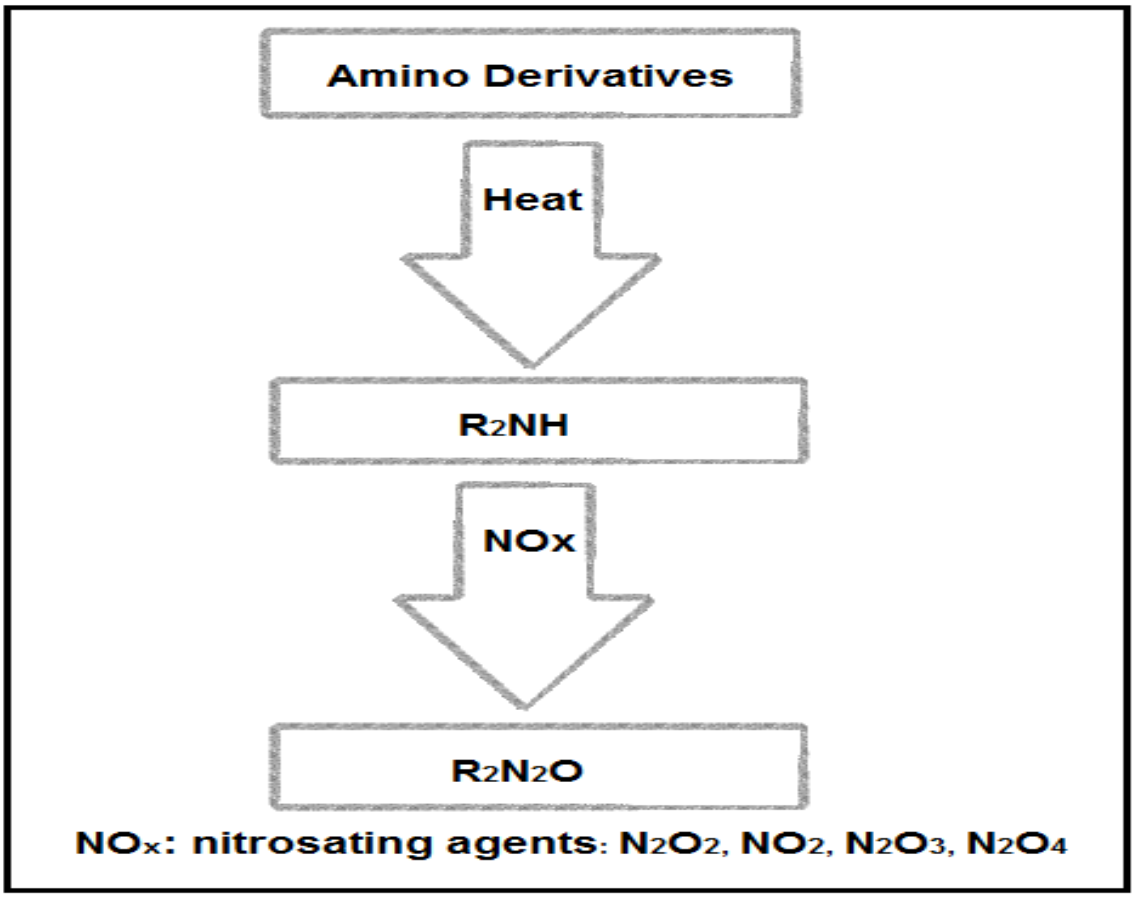


Figure 26: *N*-nitrosoamines formation

NAs are receiving special attention due to high toxicity effects, NAs have high ability to enhance tumors in various animal and human species [119-120]. International Agency for Research on Cancer (IARC) and the US Environmental Protection Agency (USEPA) listed NAs as potential carcinogenics to human [119, 121-122] The USEPA has established the control level (ng/L) of NAs in drinking water [104, 123-124].

The most common analytical methods for determination of NAs are (i) colorimetry [125], (ii) capillary electro-chromatography (CE) [126], (iii) micellar electrokinetic capillary chromatography (MECC) [127], (iv) gas chromatography (GC) with different detector such as flame ionization detectors (FID) [128], nitrogen phosphorous detector (NFD) [120], thermal energy detector (TED) [129], nitrogen chemiluminescence detector (NCD) [130] and with mass spectrometry detector (MSD) [107]. Recently, high-performance liquid chromatography (HPLC) methods with different detectors MSD [131], ultra violet detector (UVD) [132], and fluorescence detectors (FD) [133] were used for analyses of NAs. Analysis of NAs by using GC is more sensitive than HPLC methods due to [134, 135] because the NAs analyte is highly volatile.

The most common preconcentrating techniques used for NAs in water samples are solid-phase extraction (SPE) with sorbent materials such as carbonaceous ambersorb- 572 and coconut charcoal [136, 137]. Alternatively, liquid–liquid extraction (LLE) [138] was also reported; however, LLE consumes large amounts of organic solvents and it is not easy to automate [138] the extraction procedure. Solid-phase microextraction (SPME) [114,

139], which is solvent-free. It is more environmentally friendly and easy to automate using CombiPAL autosampler [104].

Automated-SPME has other advantages such as high degree of accuracy and reproducibility compared to the conventional approaches. The present study describes the development of a simple automated HS-SPME method using CombiPAL autosampler for the first time to the determination of NAs. Various extraction parameters influencing the performance of HS-SPME such as different type of commercial fibers, extraction time, sample pH, incubation temperature and ionic strength of the aqueous solution were optimized using Response Surface Methodology (RSM).

4.2 EXPERIMENTAL

4.2.1 Materials and Methods

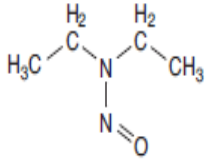
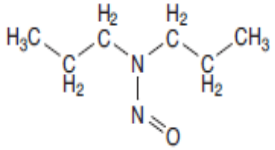
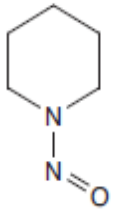
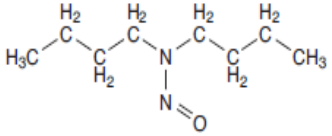
4.2.1.1 Safety Considerations

NAs are suspected carcinogens to human, extra precaution were taken to handle the samples. Experiments were conducted inside fume hood with appropriate personal protective equipments.

4.2.1.2 Chemicals and Materials

USEPA 8270-standard solution containing 2000 mg/L of the four NAs was purchased from Sigma-Aldrich (St. Louis, MO, USA). The mixture contains *N*-nitroso-di-n-ethylamine (NDEA), *N*-nitroso-di-n-propylamine (NDPA), *N*-nitrosopiperidine (NPIP) and *N*-nitroso-di-n-butylamine (NDBA) with purity > 99%. The physical properties of NAs are shown in Table 11.

Table 11: Physical property of NAs used in this study

Property	NDEA	NDPA	NPIP	NDBA
Chemical structure				
Molecular eight	102.1g/mol	130.2 g/mol	114.2 g/mol	158.2 g/mol
Specific gravity	0.9422 (at 20 °C/4 °C)	0.916 (at 20 °C /4 °C)	1.0631 (at 18.5 °C/ 4	0.9009 (at 20 °C/ 4 °C)
Boiling point	177 °C	06 °C	219 °C	116 °C (at 14 mmHg)
Log K_{ow}	0.48	1.36	0.36	2.63
water solubility	106 g/L (at 24 °C)	13 g/L (at 24 °C)	76.5 g/L (at 24 °C)	1.27 g/L (at 24 °C)
Vapor pressure	0.86 mm Hg (at 20 °C)	0.086 mmHg (at 20 °C)	0.092 mmHg (at 20 °C)	0.05 mmHg (at 25 °C)
Standard US EPA cancer classification group [145]	B2	B2	B2	B2
MCL for $R=10^{-5}$ (ng/L)	2	50	-	60

MCL: maximum contaminant level for risk 10^{-5} (USEPA)

A working standard solution of 1 mg/L mixture was prepared by appropriate dilution of stock solution in the same solvent (dichloromethane) and stored in darkness at 4 ºC. HPLC-grade organic solvents were purchased from Merck (Darmstadt, Germany). Sodium hydroxide and hydrochloric acid were obtained from Scharlau Chemie (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA) and used throughout this study. All laboratory glassware were washed with concentrated hydrochloric acid and rinsed with ultrapure water, acetone and dried out in the laboratory oven for 2 h to avoid any contamination.

SPME fibers coated with polydimethylsiloxane-divinylbenzene (PDMS-DVB, 65-µm), polydimethylsiloxane (PDMS, 100-µm) and polyacrylate (PA, 85-µm) coated fibers were purchased from Supelco (Supelco, Bellefonte, PA, USA) and used to extract the volatile NAs from water sample. The fibers were conditioned prior to use according to the instructions provided by the suppliers.

4.2.1.3 Real Samples

Groundwater samples were collected in a pre-cleaned glass bottles from four different sources (Hafr Al-Batin, Ras Tanura, Riyadh and Al-Khafji) in Saudi Arabia. Water samples were stored in an ice box and transported to the laboratory. Samples were directly extracted using HS-SPME without any further pretreatment.

4.2.1.4 GC-MS Analysis

Analyses were performed using a gas chromatograph (Agilent technologies, 7890A GC) coupled with a quadrupole mass selective spectrometer (Agilent technologies, 5975C) equipped with an inert ion source and provided with a split-splitless injection port. An A HP-5 GC fused silica capillary column (Agilent 19091J-413; 30 m × 320µm ID × 0.25 µm thickness) was selected to separate the analytes. CombiPAL autosampler (GC sampler 80, Zwingen, Switzerland) was used for the automated HS-SPME. Ultrahigh purity helium (99.999%, Abdulah Hashim, Al-Khobar, Saudi Arabia) was used as the carrier gas at a flow rate of 1.0 mL/min. The samples were injected in the splitless mode. The temperature program used for the analyses was as follows: the initial temperature was 40 °C held 3 min which was then increased to 180 °C at 15 °C/min and held for 2 min. The total run time was 14.5 min. The injection port, ion source and interface temperatures were heat at 200 °C, 200 °C, and 280 °C, respectively. For qualitative determinations, the MSD was operated in full-scan mode from m/z 50 to 550 and selective ion monitoring mode was used for the quantitative quantification of the analytes. These chromatographic conditions are presented in Table 12 and the peaks for the PAEs were identified using individual standards.

Table 12: Gas chromatographic conditions for NAs determination

Instrument	Agilent technologies, 7890A GC coupled with Agilent technologies, 5975C MSD
Column	HP-5 used silica capillary column (Agilent 19091J-413; 30 m × 320µm ID × 0.25 µm thickness)
He flow rate	1.0 mL/min
Injection mode	Splitless mode
Oven temperature program	40 °C (3 min) Ramped at 15° C/min to 180 °C and held at this temperature (2 min) The total run time was 14.5 min.
Injection port temperature	200 °C
interface temperatures	280 °C
MS temperature	200 °C

4.2.1.5 Sample Preparation

5 mL of water samples (adjusted to pH 7), spiked with NAs, was poured into a 10 mL HS-SPME vial containing 0.15 g of sodium chloride, and placed in the CombiPAL autosampler tray. Optimum extraction conditions were programmed for automated extraction. Samples were extracted at headspace of the vial at 65 °C for 20 min with 500 rpm agitation speed. After extraction, the SPME fiber was withdrawn into the SPME syringe needle and inserted into the GC injection port for desorption. The desorption was conducted at 250 °C for 3 min and then the SPME fiber was cleaned by heating at 250°C

for 5 min prior to the next extraction. The entire HS-SPME extractions procedure was automated by CombiPAL autosampler.

4.2.1.6 Experimental Design

A multi-variate statistical modeling technique RSM was used to evaluate the response of various HS-SPME parameters. Extraction peak areas (PA_i) of NAs influenced by several independent variables such as extraction time (**A)**, sample pH **(B)**, incubation temperature (**C)** and salt addition (**D**) (input variables) were used to plot RSM. The proposed RSM optimization required less number of samples analyses when compared to other optimization procedures such one-variable-at-a-time technique. In addition, the design also caters for curvature (i.e. non-linear behaviors of response surface) in the response function which cannot be achieved in one-variable-at-a-time approaches. Using RSM, the effect of four different parameters (**A-D**) was investigated to achieve higher extraction efficiency of automated-HS-SPME.

A Box–Behnken design (BBD) [144], with response surface method was employed for non linear models with the aid of statistical package Design Expert 8.0 (Stat-Ease, Inc. Minneapolis, MN). As the BBD is an orthogonal design, factor levels are evenly spaced and coded for low, medium (central point) and high level, as −1, 0 and +1, respectively, code values were calculated as per equation 1. Table 13 shows the coded values of four variables used for method optimization. A total of twenty five (25) experimental runs were used for implementing the BBD. Each of the twenty five runs was repeated three times (n=3) and their average were used for the optimization.

$$x_i = \frac{X_i - \left(X_{high} + X_{low}\right)/2}{\left(X_{high} - X_{low}\right)/2} \quad \textbf{(1)}$$

where x_i is the coded value and X_i is the actual value of variable.

Table 13: Actual and Coded Values of four variables in Design Expert

Variable	Component	Unit	Coded and actual level		
			-1	**0**	**+1**
A	Extraction time	min	20	35	50
B	PH	-	3.5	7	10.5
C	Incubation temperature	ºC	50	65	80
D	Salt addition	% (g/mL)	0	15	30

Behaviors of the mathematical response models were generally represented by the following quartic function.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j + \cdots + \varepsilon \quad (\mathbf{2})$$

Where y is the predicted response, β_0 the constant coefficient, βi the linear coefficients, βii the squared coefficients, βij the interaction coefficients, and xi, xj are the values of the independent variables, ε is the error.

4.3 RESULTS AND DISCUSSION

4.3.1 SPME Fiber Selection

The initial studied were conducted to select the suitable SPME fiber for the extraction of NAs. Three commercially available SPME-fibers with different properties (65-µm polydimethylsiloxane/divinylbenzene (PDMS-DVB), 85-µm polyacrylate (PA) and 100-µm Polydimethylsiloxane (PDMS) fibers) were evaluated. The PA fiber showed higher peak areas for the extraction of NDPA and NDBA in comparison with the PDMS-DVB and PDMS. Polarity of PA fiber with polar-polar interactions facilitates the higher extraction performance of NAs (Figure 27).

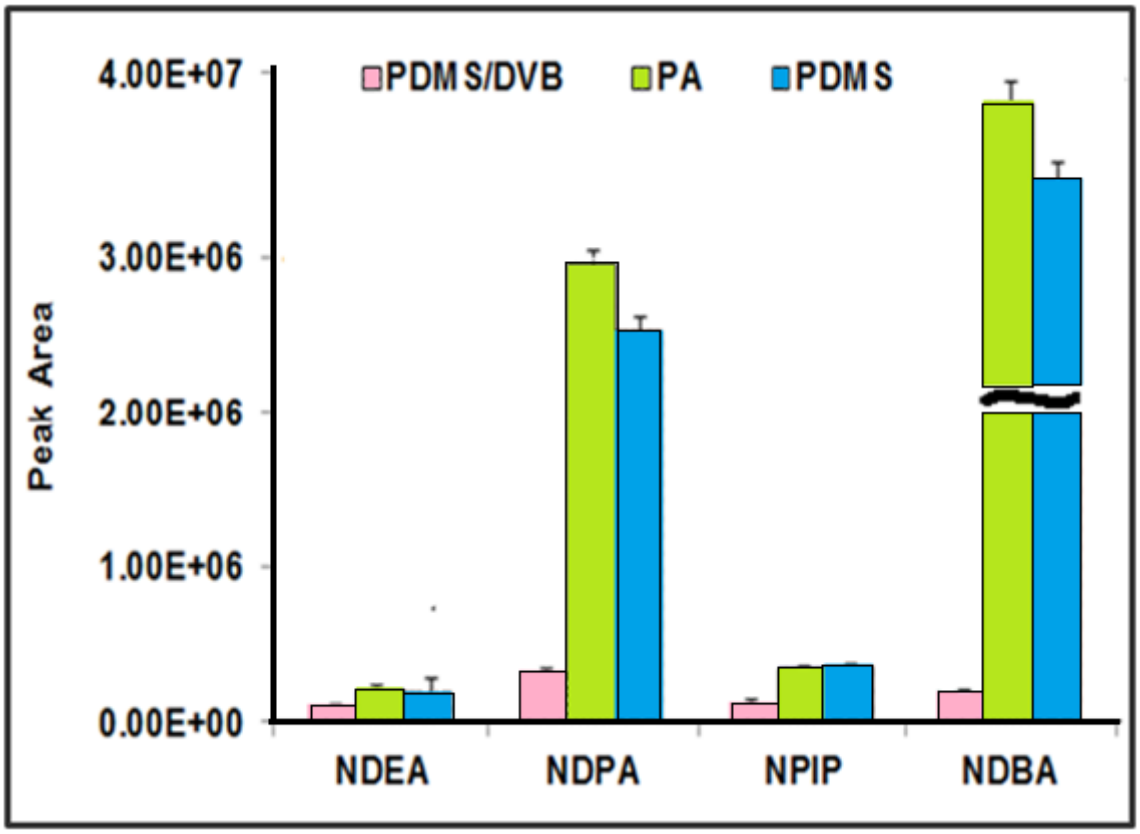


Figure 27: Comparison of different SPME fibers. (Conditions: 5 mL of groundwater spiked with 1 mg/L of NAs, agitation speed of 500 rpm; incubation temperature at 50 ºC and extraction time for 20 min).

4.3.2 RSM Extraction Models

The experimental data of the PA_i for the NAs were fitted to eq 2 to develop multiple nonlinear models which are capable of explaining the main and different degrees of interactive effects on the extraction conditions. The Design Expert 8.0 also gives the analysis of variance (ANOVA) and estimates the coefficient parameters of the regression for the model. The quality of the developed nonlinear model was further improved by dropping insignificant interaction effects that dwindle the respective response prediction accuracy.

The repeatability of the experimental runs was measured by relative standard deviations (% RSDs) which are ranging between 1.8 and 14.7%. These data were subjected to multiple nonlinear regressions using Design Expert 8.0. The reduced models in terms of coded factors are written in eqs 3 to 6 for NDEA, NDPA, NPIP and NDBA, respectively.

$$PA_{NDEA} = 2364 - 202A - 323B + 68C + 719D + 1147AB - 951AC - 1402AD - 903BC - 2230BD - 1080A^2 - 4B^2 + 2064C^2 + 2675D^2 + 568A^2B - 638A^2C - 2028A^2D - 638AC^2 - 878B^2C + 6042A^2B^2 + 4284A^2D^2 \quad \textbf{(3)}$$

$$PA_{NDPA} = 2.86E+5 - 10515A - 1.1E+5B + 25516C - 29499D - 1.1E+5\,AB + 1.8E+5\,AD + 1.23E+5BC + 26855BD + 1.7E+5\,CD + 1385B^2 - 7651D^2 + 1.1E+5\,AB^2 + 66567AD^2 - 1.6E+5\,B^2C + 1.8E+5\,B^2D + 8433\,8BD^2 \quad \textbf{(4)}$$

$$PA_{NPIP} = 56017 - 9552A - 227B + 24380C + 2155D + 4162\,AB + 24465AC + 2900AD - 3E+5BC - 3821BD - 4087CD - 13427\,A^2 - 31815B^2 - 9157C^2 \quad \textbf{(5)}$$

$$PA_{NDBA} = -1.4E+5A + 1.4E+6B + 6.7E+4C + 2E+5D - 1.3E+7AB + 1.4E+5AC - 1.1E+5AD - 1.6E+6BC - 1.1E+7BD + 1.1E+6CD + 9.5E + 6A^2 + 5.8E+6B^2 - 5.4E+6C^2 + 8.17E+6D^2 - 1.5E+7A^2B - 9.8E+5A^2C + 1.5E+7A^2D + 1.3E+7AB^2 + 5E + 5B^2C \quad \textbf{(6)}$$

Where PA_i is peak area; NDEA, NDPA, NPIP and NDBA are the NAs compounds.

The coefficients of all variables of the nonlinear equations provided a measure of the effect on the independent variable of the response (PA_i). In addition, positive and negative coefficients values indicate synergistic and antagonistic effects between the corresponding linear or interactive effect of the response [140].

Quality of the developed HS-SPME nonlinear model was evaluated based on statistical test of hypothesis. As displayed in Table 14, the models' regression coefficients (R^2) are 0.991, 0.788, 0.868 and 0.985 for PA_{NDEA}, PA_{NDPA}, PA_{NPIP} and PA_{NDBA}, respectively.

The P-value was used as a tool to check the significance of each of the coefficients, which in turn indicated the pattern of the interactions between the variables. The smaller value of P was more significant to the regression. According to the ANOVA table, the regression model is significant at the considered confidence level (95%) since the regression has P-value < 0.05 [144].

Table 14: ANOVA for the quartic order regression model obtained from experimental data.

	PA_{NDEA}		**PA_{NDPA}**		**PA_{NPIP}**		**PA_{NDBA}**	
	(R^2 = 0.991)		**(R^2 = 0.788)**		**(R^2 = 0.868)**		**(R^2 = 0.985)**	
Precision	18.88		5.801		5.325		7.808	
	F-value	*p*-value[a]	*F*-value	*p*-value[a]	*F*-value	*p*-value[a]	*F*-value	*p*-value[a]
Model	22.74	0.0040*	2.88	0.0388*	2.02	0.026*	3.52	0.039*
A	1.03	0.0368*	2.39	0.0146*	2.27	0.020*	0.0041	0.009*
B	2.63	0.0180*	5.62	0.0339*	0.077	0.097**	0.0094	0.008*
C	16.55	0.0820**	0.63	0.0442*	11.03	0.029*	0.093	0.081**
D	13.01	0.0226*	1.19	0.0295*	0.1	0.076**	0.009	0.009*

[a](*) Significance was established at $p < 0.05$, (**) Significance was established at $p < 0$.

According to ANOVA, when R^2 value closer to unity, indicates the higher model's accuracy and the good prediction capabilities of the developed models [113, 141, 144]. Moreover, all the sources of variations of the four models' *F*-values determined from ANOVA indicated that the models are statistically significant at 5% significant level (i.e at probability values $p < 0.05$). This also further supports the fact that the four equations could be adequately predicting the experimental results with a high degree of accuracy [140, 142].

Considering the main factors, the coefficients of the independent variables of **A** and **B** were negative for all NAs except NDBA (eq 6). The other two independent variables **C** and **D** were positive for all NAs except the coefficient of variable **D** for NDPA (eq 4). This implies that lower level of **A** and **B**, and higher level of **C** and **D** are expected to give higher PA_i. In this regard, the relative contributions of the main effects on the extraction of NAs from groundwater could be ranked according to the order of salt addition > pH > incubation temperature > extraction time, respectively.

4.3.3 Response Surface Curves

Three-dimensional response surface curves (for the PA_i nonlinear models were constructed. This enables more clear visualization and understanding the influence of the independent variables. Each of the response curves was developed by fixing two of the independent variables while varying the remaining two within the investigated range. For example, in Figure 28 the variables are extraction time and sample pH (incubation temperature and salt additions were kept constant). These curves corroborate the ANOVA analysis and reveal the independent variables which have significant contributions on the HS-SPME response.

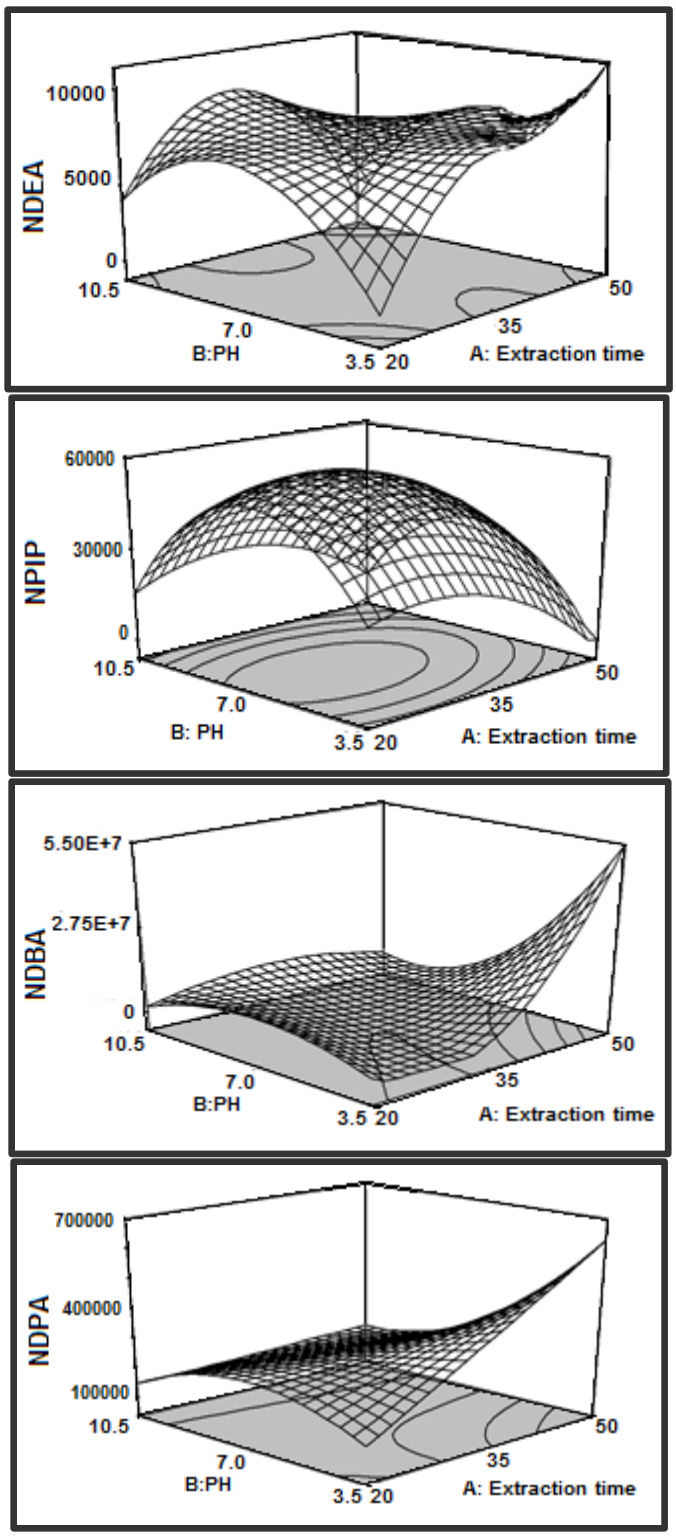


Figure 28: Influence of extraction time and sample pH on HS-SPME. (Conditions: 5 mL of groundwater spiked with 50 µg/L of NAs, agitation speed of 500 rpm; incubation temperature at 65 ºC and salt addition 15%).

Figure 28 shows an increase in response of NDEA, NDPA and NDBA by increasing extraction time, whereas for NPIP, the maximum response was observed after 35 min extraction time. Furthermore, stronger degree of curvature is due to influence of sample pH (**B**) which is portrayed in the upward plateau shape (Figure 28) and reaches maximum around pH 7 and then decreases. This could be due to hydrolysis of the NAs at high alkaline conditions (pH 10.5) (Figure 28).

In Figure 29 the variables are sample pH and the influence of salt addition (extraction time and incubation temperature were kept constant). At fixed central value of A and C the extraction performance of HS-SPME is shown in Figure 4. The PAi of all NAs increased with the increase in salt addition. Interestingly, at alkaline conditions, the presence of salt decreases the hydrolysis rate of NDEA and NDBA. Reverse effect for NDPA was observed by increasing sample pH higher than 7.5. From Figure 29, we can conclude that NDPA and NPIP degraded at alkaline conditions in the presence of salt [57].

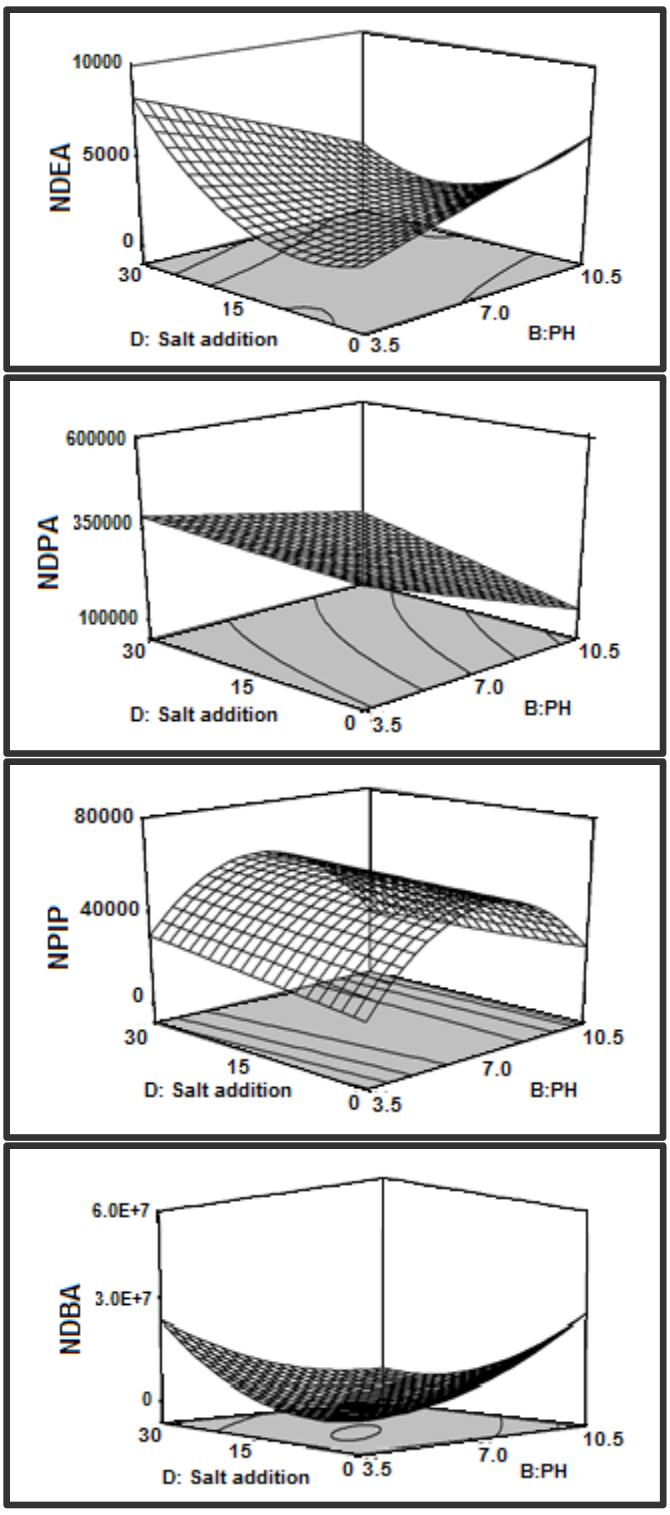


Figure 29: Effect of sample pH and salt addition on HS-SPME. (Conditions: 5 mL of groundwater spiked with 50 µg/L of NAs, agitation speed of 500 rpm; extraction time 35 min and incubation temperature at 65 ºC).

Figure 30 (a-d) displays the influence of HS-SPME conditions with respect to total nitrosamines (TNAs) response. Figure 30a, extraction time and sample pH are the variables and incubation temperature and salt concentrations were maintained as constant. Results clearly indicate that TNAs increases with increasing extraction time and no significant improvement of TNAs were observed with change in sample pH. Figure 5b show the relationship between extraction time and incubation temperature we can conclude that by increasing the extraction time the efficiency of HS-SPME was increased. However, after 65 ºC a slight decrease in TNAs was observed.

Figure 30 shows the influence of salt addition on extraction time (30c) and incubation temperature (30d) with other experimental conditions kept constant. Results clearly show that extraction time and incubation temperature not significantly influence the HS-SPME by the addition of salt. Salt addition (**D**) had positive effect on the extraction of TNAs; these findings are in agreement with a previously reported HS-SPME method [120].

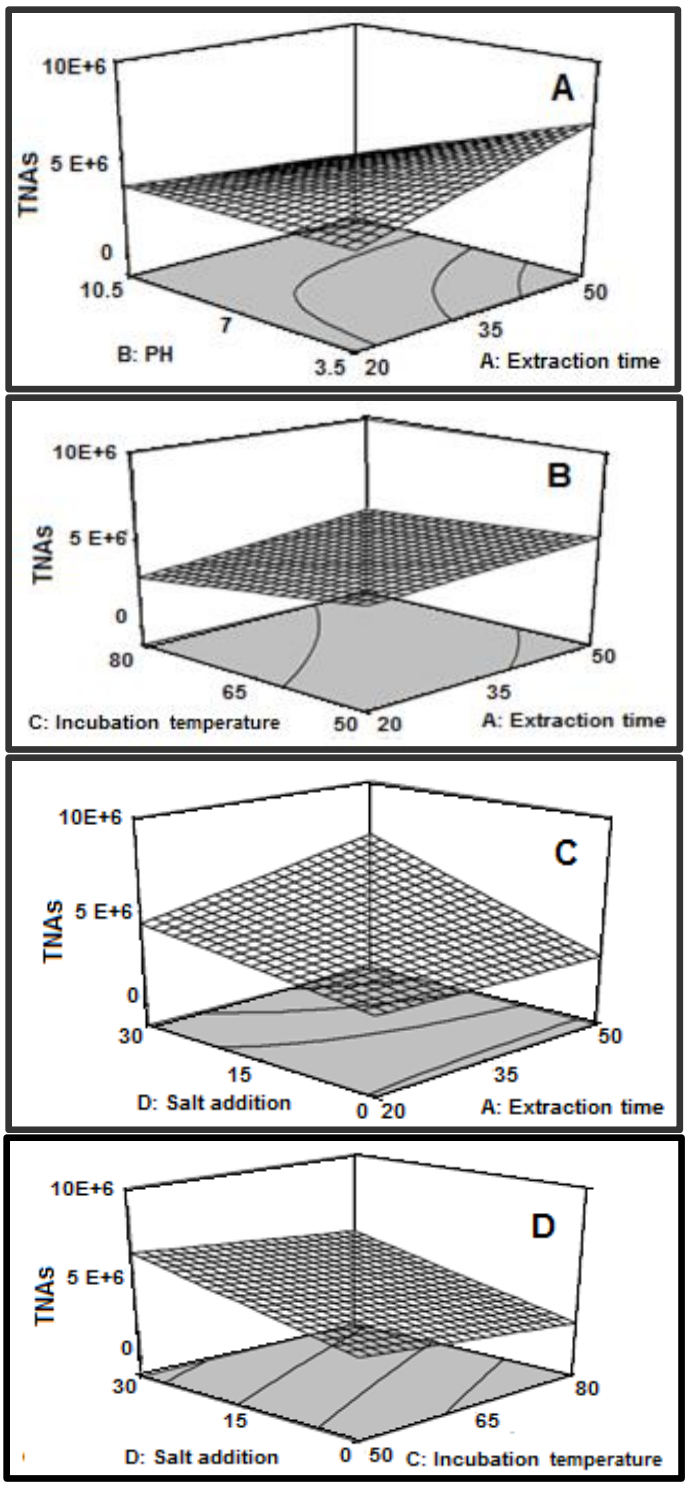


Figure 30: Effect of variable on HS-SPME. (TNAs: sum of NDEA, NDPA, NPIP and NDBA) (Conditions: 5 mL of groundwater spiked with 50 µg/L of NAs, agitation speed of 500 rpm; extraction time 35 min and sample pH 7).

The results of ANOVA and RSM showed that among tested variables, salt addition (**D**) were the most important variable in the extraction of NAs with HS-SPME [104]. Thus, the influences of other three independent variables (**A, B** and **C**) on HS-SPME were studied with known amount of salt addition (i.e. 15%) and illustrated in a cube plot (Figure 31). Each cube corner represents the eight different experimental conditions with the coded levels from -1 to +1. The highest peak areas of TNAs (9.7 E+6) was obtained for the combination of a high extraction time (+A) and moderate pH (-B) with moderate incubation temperature (-C) at fixed salt addition 15%.

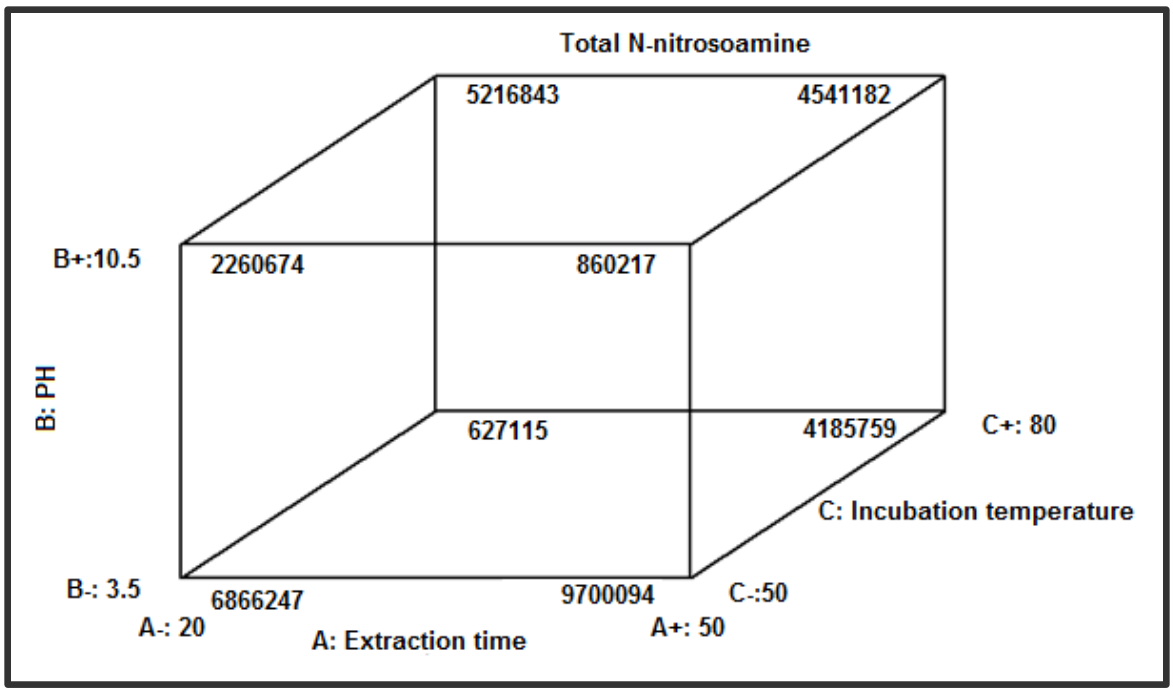


Figure 31: Influence of extraction time, sample pH and incubation temperature on HS-SPME. (Conditions: 5 mL of groundwater spiked with 50 µg/L of NAs, agitation speed of 500 rpm and 15% Salt addition).

The forgone analyses implies that among the four NAs investigated, NDEA is the least favorable for extraction in the water sample using HS-SPME (Figures 28-29). Based on the model, the order of influencing HS-SPME parameters are ranked in the order of salt addition **(D) >** pH **(B) >** incubation temperature **(C) >** extraction time **(A),** respectively.

4.3.4 Optimization of HS-SPME

The optimum conditions for the extraction of individual and TNAs were predicted using coded values of the independent variables. With least parameters (i.e., 3 parameters) under investigation, finding the optimum region through visual inspection of the response surfaces is possible in absence of constraints. However, for higher number of parameters (as in the case of present study), obtaining the global (rather than local) maximum point is a challenging task. As such, simultaneous numerical optimization for the extraction was performed with the aid of the Design-Expert$^{@}$ 8.0. The coordinates of the optimal points were calculated through equating the first derivatives of the reduced models (eqs 3 to 6) to zero according to eq 7 in conjunction with set of convergent criteria [143, 144].

The convergent criteria are composed of goals based on desired constraints for the parameters of interest (responses and the independent variables). The criteria weighted the individual parameters to their relative importance in contributing towards the desired targeted goals.

$$\frac{\partial y}{\partial x_i} = \beta_i + 2\beta_{ii}x_i + \sum_{j=2}^{k}\beta_{ij}x_j + \ldots\ldots = 0 \quad \textbf{(7)}$$

Using ANOVA program, optimum conditions were identified to enhancing the performance of HS-SPME. The solution suggested the optimal condition for extraction of NAs in water samples were as follows; 20 min extraction time, sample pH of 7, 65 ºC incubation temperature and 30% salt addition.

4.3.5 Analytical Performance of Automated HS-SPME

Based on the optimized conditions, quantitative parameters of HS-SPME such as linearity, repeatability and limits of detection (LODs) were investigated. The results are summarized in Table 15.

Table 15: Quantitative parameters of automated HS-SPME.

Analytes	linearity equation	(correlation of coefficient r)	Linear range (µg /L)	% RSDs (n=3)	LODs (ng/ L)
NDEA	y = 6.257E-04x + 7.664	0.975	0.1 - 100	3.8	11.9
NDPA	y = 5.008E-05x + 6.602	0.988	0.1 - 100	5.7	9.6
NPIP	y = 5.450E-04x + 7.797	0.977	0.1 - 100	3.5	5.4
NDBA	y = 8.347E-06x + 6.137	0.992	0.1 - 100	1.8	0.78

Linearity was tested over the concentration range of 0.1 to 100 µg/ L (0.1, 1, 5, 10, 20, 30, 50, 100 µg/L) for all NAs and very good correlation of coefficient (r) ranging from 0.975 to 0.992. The repeatability study was carried out by extracting spiked water samples at different concentration levels and the percentage relative standard deviations (% RSDs) were between 1.8 and 5.7% (n = 3). The LODs, based on a signal-to-noise ratio (S/N=3), ranged from 0.78 to 11.9 ng/L. Performance of automated HS-SPME was compared with other methods reported in the literature and the results are shown in Table 16.

Table 16: Comparison of automated HH-SPME/GC-MS with other reported methods from literatures.

Method	Sample	Linear range (ng/L)	LODs (ng/L)	% RSDs	Ref-
HS-SPME/GC-MS-MS[b]	Water	10 - 1500	1 – 5	3 – 13.0	[104]
SPE/GC-FID[d]	Water	10000 - 600000	2000-3500	3 – 6.5	[128]
SPE/GC-NPD[e]	Water	300 - 20000	20 – 80	3.5 – 6.3	[128]
SPE/GC-MS	Water	40 - 20000	3 – 13.0	4.1 – 6.1	[128]
HPLC-CL[c]	Water	5 - 1000	1.5 - 3	0.7 – 4.5	[132]
SPE/GC-EI-MS-MS[a]	Water	500 - 50000	0.4 - 4	*max 10*	[146]
HS-SPME/GC-MS	Water	100-100000	0.8 – 11.9	1.8 – 5.7	Present

L.R: Linearity Range. LODs: Limits of Detection. % RSDs: Relative standard deviation. (a) Solid phase extraction gas chromatography–electron ionization tandem mass spectrometry. (b) Head space solid-phase microextraction followed by gas chromatography tandem mass spectrometry. (c) high-performance liquid chromatography with chemiluminescence detection. (d) solid-phase microextraction by gas chromatography with flam ionization detector. (e) solid-phase microextraction by gas chromatography with nitrogen phosphorus detector.

Results of NAs (Table 15) clearly indicate that the performance of HS-SPME is comparable with those reported SPME/GC-MS-MS methods and superior to SPE-GC-MS methods. One advantage of our method that it is simple and the entire extraction process is automated.

4.3.6 Application to Real Groundwater Samples

The automated HS-SPME/GC-MS method was applied to determine the NAs in groundwater samples at different locations of Saudi Arabia (Hafr Al-Batin, Ras Tanura, Riyadh and Al-Khafji). The concentrations of NAs that detected in groundwater samples are shown in Table 17.

To assess the matrix effect of the HS-SPME, groundwater samples were spiked with 1 and 20 µg/ L of NAs and extraction recoveries were calculated. Recoveries of NAs in different groundwater samples are shown in Table 18. Moreover, analyte recoveries in the range of 85 and 114% and suitable for routine analyses of NAs in groundwater samples.

Table 17: Concentration of *N*-nitrosoamine detected in groundwater samples.

Concentration of *N*-nitrosoamine in groundwater samples (µg/L) (n=4)								
Analytes	Hafr Al-Batin	% SDs	Ras Tanura	% SDs	Riyadh	% RSDs	Al-Khafji	% SDs
NDEA	13.27	1.06	14	0.70	15.24	1.33	14.67	1.66
NDPA	7.67	0.80	7.9	0.70	7.97	0.80	7.5	1.23
NPIP	9.07	1.15	8.49	1.07	8.9	0.70	8.27	0.39
NDBA	0.15	0.01	0.31	0.03	0.32	0.03	0.32	0.04

Table 18: Recovery of HS-SPME spiked with 1 and 20 µg /L of NAs in real groundwater samples

Recoveries of NAs at 20 µg /L spiked real water samples (n=4)								
Analytes	**Hafr Al-Batin PH: 8.5**	**% RSDs**	**Ras Tanura PH: 7.98**	**% RSDs**	**Riyadh PH: 7.95**	**% RSDs**	**Al-Khafji PH: 9.25**	**% RSDs**
NDEA	89	4.6	91	2	103	5.2	89	8.4
NDPA	112	8	112	2	107	4.6	109	0.8
NPIP	96	1.3	101	6	114	4.1	88	5.2
NDBA	103	15	106	5	110	3.7	106	2
Recoveries of NAs at 1 µg /L spiked real water samples (n=4)								
	Hafr Al-Batin	**% RSDs**	**Ras Tanura**	**% RSDs**	**Riyadh**	**% RSDs**	**Al-Khafji**	**% RSDs**
NDEA	85	4.1	92	4.8	99	8.3	92	3
NDPA	102	3.7	104	9.6	94	1.2	98	1.7
NPIP	105	3.8	97	5.7	102	6.6	92	1.5
NDBA	96	5.4	101	4.1	94	4.9	91	1.97

4.4 CONCLUSION

In this study, automated HS-SPME/GC-MS method was developed for the determination of *N*-nitrosoamine in groundwater samples. The extraction conditions of HS-SPME were optimized via response surface methodology. With the use of CombiPAL autosampler we obtained very good detection limits (between 0.79 and 11.9 ng/L) and satisfactory precision (between 1.8-5.7%). The fully automated method proved to be simple and viable for determining trace level of NAs in groundwater samples.

CHAPTER 5

DETERMINATION OF N-NITROSOAMINES BY AUTOMATED DISPERSIVE LIQUID–LIQUID MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY MASS SPECTROMETRY

5.1 LITERATURE REVIEW

N-nitrosoamines (NAs) are one of chemicals group that have been studying for more than 100 years ago [147-148]. In 1954, Barnes and Magee [149], reported the toxicity effect of NAs, and classified them as potent human carcinogens [107, 147-151] NAs are produced by reaction of amines or their derivatives with nitrosating agents such as nitrous acid, nitrites, or nitrogen oxides [127, 152-153]. They are compounds which are relatively stable and difficult to destroy once formed [127, 152], furthermore; these compounds can enter drinking water supplies mainly during the use of ozone in the disinfection processes [150].

Due to polarity of NAs; they are usually soluble in water. Trace level analysis of NAs in water has become more important, because they have been included in the carcinogenic compounds category B2 in the Unregulated Contaminant Monitoring Rule-2 by the United States Environmental Protection Agency [154-155].

Due to the low concentrations of NAs found in environmental water samples, several convenient and environmentally benign sample preparation methods have been reported for the determination of NAs. These microextraction approaches offer significant advantages over conventional methods such as conventional liquid-liquid extraction (LLE) and solid-phase extraction (SPE) techniques [136,146, 156-157]. Solid-phase microextraction (SPME), hollow fiber liquid–phase microextraction (HF-LPME); and dynamic in-syringe LPME [4, 139] have attracted more attention than classic preconcentration techniques. Complete automation of HF-LPME is not simple when compared to dynamic in-syringe LPME. In dynamic in-syringe LPME, only the autosampler microsyringe is used as an extraction device [158]. In this procedure, the solvent containing syringe plunger is pulled back and forth for the withdrawal and dispensation of the aqueous sample; analytes were extracted from the aqueous sample solution to the organic film formed along the inner wall of the syringe barrel. The dynamic in-syringe LPME is suitable for relatively clean samples and only few applications on automatization have been reported in the literature [159, 178].

Dispersive liquid-liquid microextraction (DLLME) was first developed by Rezaee et al. in 2006 [4]. Since DLLME does not require any special instruments or membrane, number of applications have been reported in the literature [160-161]. The DLLME extraction procedure is very simple; introduction of a solvent mixture (extraction and dispersive solvent) into the aqueous sample, the combination of these solvent mixture produce fine cloudy droplets instantaneously and then the extraction solvent separated in to immiscible layer. To date, the DLLME has undergone a number of modifications such

as use of vortex or ultrasound for the extraction solvent less dense than water [162], for the application of sample cleanup [163] and simultaneous derivatization and extraction of analytes [161]. Recently, reviews summarizing applications [161, 164] and advances regarding DLLME have been reported [165]. The advantages of the DLLME it takes less time, low cost and high enrichment factors than classic extraction methods [166-173].

Recent trends in analytical science toward high-throughput analysis are focused on the development of automatization of analytical techniques. Very few automated analytical methods were reported in the literature. This includes solid-phase extraction (SPE) [174-175] automated liquid−liquid extraction (LLE) [176], liquid-phase microextraction [177-178], solid-phase microextraction [179-180] and DLLME with flow injection analysis [181]. The automation of SPE methods require complex multistep elution and preconcentration when compared with SPME approach. Whereas SPME is also expensive; due to its fiber is fragile and has limited lifetime and sample carry-over can be a problem. The automation of DLLME requires only few milliliters of organic solvents and it is easy to automate the entire procedure.

Therefore, the objective of this study set for the first time an automate DLLME/GC-MS procedure using N-nitrosamines as model compounds (which, incidentally, have not previously been subjected to conventional DLLME) were considered. The DLLME experimental parameters affecting the performance of the method were optimized using response surface methodology (RSM) [140-142**]**. RSM is a multi-variate optimization

technique and provides the ideal opportunity to demonstrate the suitability of DLLME as a complete automation, without human intervention.

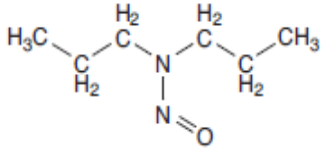
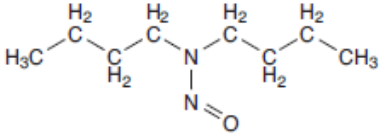
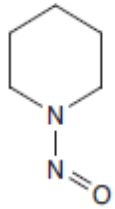
5.2 EXPERIMENTAL

5.2.1 Material and Methods

5.2.1.1 Chemicals

A mixture of NA standards was purchased from Sigma-Aldrich (St. Louis, MO-USA). This mixture contains 2000 µg/L each of NDPA, NPIP and NDBA (Table 19). A working standard solution was prepared daily by appropriate solution dilution. A stock solution of three analytes was prepared in dichloromethane (Darmstadt, Germany). Doubly deionized water obtained from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the study. All other solvents of analytical-grade were purchased from Supelco (Bellefonte, PA, USA). Sodium hydroxide, sulfuric acid and sodium chloride were obtained from Merck. To avoid any carryover of NAs; all laboratory glassware was washed with concentrated hydrochloric acid and rinsed with deionized water and acetone and dried out in the laboratory oven for 2 h at 100 °C before use.

Table 19: Physical property of three NAs in this study.

Property	NDPA	NDBA	NPIP
Chemical structure			
Molecular eight [183]	130.2 g/mol	158.2 g/mol	114.2 g/mol
Specific gravity [183]	0.916 (at 20 °C /4 °C)	0.9009 (at 20 °C/ 4 °C)	1.0631 (at 18.5 °C/ 4 °C)
Boiling point [148]	06 °C	116 °C (at 14 mmHg)	219 °C
Log K_{ow} [183]	1.36	2.63	0.36
water solubility [184]	13 g/L (at 24 °C)	1.27 g/L (at 24 °C)	76.5 g/L (at 24 °C)
Vapor pressure [184]	0.086 mmHg (at 20 °C)	0.05 mmHg (at 25 °C)	0.092 mmHg (at 20 °C)
Standard US EPA cancer classification group [148, 145]	B2	B2	B2
MCL for R=10^{-5} (ng/L)	50	60	-

MCL: maximum contaminant level for risk 10^{-5} (USEPA) [148, 145]

5.2.2 GC-MS Analysis

Analyses were performed on a GC-MS system Shimadzu (Kyoto, Japan) equipped with Combi-PAL auto-sampler GC-MS/QP 2010 (Shimadzu, Japan). A DB-5 fused silica capillary column (30 m X 0.25 mm i.d., 0.25 µm film thickness) (J&W Scientific, Folsom, CA, USA) was used for chromatographic analysis. Ultrahigh purity helium (99.999%, Abdulah Hashim, Al-Khobar, Saudi Arabia) was used as the carrier gas at a flow rate of 1.0 ml/min. The samples were injected in splitless mode. The sample volume in the direct injection mode was 1 µL. The temperature program used for the analyses was as follows: Initial temperature was 40 °C held for 3 min which was then increased to 180 °C at 15 °C/min and held for 2 min. The total run time was 14.5 min. The injection port, ion source and interface temperatures were heat at 200 °C, 200 °C, and 280 °C, respectively. Full scan mode with mass range of 50 to 500 m/z and selective ion monitoring mode were used for the MS analysis. These chromatographic conditions are presented in Table 20.

Table 20: Gas chromatographic conditions for NAs determination

Instrument	Shimadzu, GC-MS-QP 2010
Column	DB-5 fused silica capillary column (30m, 0.25 mmid, and 0.25-µm film thicknesses)
He flow rate	1.0 mL/min
Injection mode	splitless mode
Injection volume	1 µL
Oven temperature program	40 °C (3 min) Ramped at 15° C/min to 180 °C and held at this temperature (2 min) The total run time 14.5 min
Injection port temperature	200 °C
Interface temperature	200

5.2.3 Sample Preparation

For the optimization study, 10 mL of a water sample (adjusted to a pH of 10.5) and to which 2.3 mg of NaCl and 1 mL of extraction solvent (xylene) were added manually to a sample vial, which was then placed on the autosampler tray. Dispersive solvent (methanol, 33.5 µL) and 5 µL of a 200 mg/L of NAs were added automatically, in separate operations, using the autosampler syringe. The total concentration of each NA in the vial was 90.6 µg/L. The vial was then automatically transported to agitator, and agitated at 722 rpm for an extraction time of 28 minutes. After a cloudy solution was formed, the vial was transported back to the autosampler tray, and held for 1 min. The cloudiness disappeared over this period. Then 1 µL of the upper layer of a solution was retrained automatically useing a 10 µL syringe and injecting into the GC-MS for analysis.

5.2.4 Calculation of Enrichment Factor

The enrichment factor is defined as the ratio between the analyte concentration in the extraction phase (C_{ext}) and the initial concentration of analyte in (C_o) in the standard sample.

$$\mathbf{EF = C_{ext} / C_o}$$

$\mathbf{C_{ext}}$ is obtained from a calibration graph prepared by direct injection of NAs standard solution in the extraction solvent.

5.2.5 Experimental Design

Response surface methodology (RSM), a multi-vibrate statistical modeling technique was used to evaluate the effects of the independent variables and their interactions on the EFs and also to optimize the new procedure developed in this study. RSM, which involves designing experiments according to factorial design, enables development of quartic order polynomial models and response surfaces. RSM optimization process is very economical as it requires a small number of experimental runs compared to one-variable-at-a-time approaches. In addition, the design also caters for curvature (i.e. non-linear behaviors of response surface) in the response function which cannot be achieved in first-order design methods.

Using RSM, the effect of different parameters (A; extraction time, B; volume of dispersive solvent, C; pH, D; salt addition, E; agitation speed) were investigated to understand the influence of the parameters, their interactions on the DLLME enrichment factor (EF) and limits of detection (LODs) of the three NAs and also to obtain the highest EFs. To achieve that, a Box-Behnken design (BBD) of statistical software, Design Expert 8.0 (Stat-Ease, Inc. Minneapolis, MN, USA) was used. As the BBD experimental design is an orthogonal design, factor levels are evenly spaced and indicated in coded for low, medium (central point) and high level, as −1, 0 and +1 respectively as per eq 1 and Table 21. A total of 41 experimental runs were needed for implementing the BBD for the present study. Each of the 41 runs was repeated three times and their average was used in order to assess the repeatability, and determine the relative standard deviation.

Table 21: Actual and Coded Values of five variables in Design Expert.

			Coded and actual level		
Variable	Component	Unit	-1	0	+1
A	Extraction time	min	10	20	30
B	Dispersive volume	µL	15	30	45
C	PH	-	4	8	12
D	Salt addition	% (g/ml)	0	15	30
E	Agitation speed	rpm	250	500	750

The experimental run sequences were randomized in order to eliminate the effects of the uncontrolled factors to ensure data quality. The results as shown in Table 22.

$$x_i = \frac{X_i - \left(X_{high} + X_{low}\right)/2}{\left(X_{high} - X_{low}\right)/2} \quad \textbf{(1)}$$

where x_i is the coded value and X_i is the original value.

Behaviors of the mathematical response models were generally represented by the following quartic function

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j + \cdots + \varepsilon \quad (\mathbf{2})$$

where y is the predicted response, β_0 the constant coefficient, β_i the linear coefficients, β_{ij} the interaction coefficients, β_{ii} the quartic coefficients and x_i, x_j are the coded values of the independent variables, ε is the error.

Table 22: Experimental combination conditions for determination of the optimization values for all variables.

Independent Variable						Averege of enrichment factor (n=4)			
Exp.	**A (min)**	**B (µL)**	**C**	**D (%)**	**E (rpm)**	**NDPA**	**NPIP**	**NDBA**	**Total nitrosoamine**
1	30	45	8	15	500	50.80	25.07	40.42	116.29
2	30	30	12	15	500	42.78	22.22	43.91	108.9
3	20	30	8	0	750	41.20	16.46	41.74	99.4
4	20	45	8	30	500	32.52	16.09	19.92	68.53
5	10	30	4	15	500	28.12	17.57	23.70	69.4
6	10	30	8	0	500	34.98	15.56	35.98	86.51
7	20	30	8	0	250	53.16	13.62	31.30	98.09
8	30	30	8	30	500	36.29	21.38	37.44	95.11
9	20	15	8	0	500	36.48	15.48	36.12	88.08
10	30	15	8	15	500	28.90	10.78	19.11	58.8
11	10	30	8	15	250	23.14	10.72	14.13	47.99
12	30	30	8	15	750	44.50	23.46	52.47	120.43
13	20	30	4	15	750	37.10	21.65	34.42	93.17
14	20	45	8	15	250	22.79	19.09	9.20	51.09
15	20	30	4	15	250	25.04	18.32	14.03	57.39
16	10	45	8	15	500	35.67	17.60	23.36	76.62
17	20	15	8	15	250	27.75	17.32	17.07	62.14
18	20	30	8	15	500	33.96	13.94	29.02	76.91
19	10	30	8	30	500	27.52	17.84	18.23	63.59
20	20	30	12	15	250	25.47	15.79	14.44	55.7
21	20	45	8	15	750	36.66	18.66	31.39	86.71
22	20	30	8	30	750	46.34	29.35	44.73	120.42
23	20	30	8	30	250	12.34	20.89	10.35	43.58
24	10	30	12	15	500	22.90	14.10	14.80	51.81
25	20	45	4	15	500	34.31	17.86	19.17	71.34
26	20	15	12	15	500	45.32	24.36	43.19	112.87
27	20	15	8	30	500	15.96	10.71	13.85	40.52
28	20	30	12	0	500	22.51	10.88	16.31	49.71
29	20	30	12	15	750	45.86	23.10	40.65	109.61
30	20	30	4	30	500	39.82	29.93	36.67	106.43
31	30	30	8	15	250	27.95	21.37	24.61	73.93
32	20	15	8	15	750	23.55	6.62	13.50	43.67
33	10	15	8	15	500	28.41	19.14	20.98	68.53
34	10	30	8	15	750	39.28	21.39	42.38	103.05
35	30	30	8	0	500	34.46	13.25	31.96	79.67
36	20	45	8	0	500	32.42	14.92	33.42	80.75
37	30	30	4	15	500	34.85	17.80	30.03	82.68
38	20	30	4	0	500	27.58	13.15	27.96	68.69
39	20	30	12	30	500	35.46	28.52	45.06	109.04
40	20	45	12	15	500	39.37	20.41	40.13	99.9
41	20	15	4	15	500	40.18	20.47	37.00	97.65

(A; extraction time, B; volume of dispersive solvent, C; pH, D; salt addition, E; agitation speed).

5.3 RESULTS AND DISCUSSION

5.3.1 Solvents Combinations

The most important experimental parameters of DLLME are the solvents (extraction and dispersive). There are specific criteria for choosing the best extraction solvent such as: (i) choice higher/lower density than water, (ii) low solubility in water, (iii) the capability of extraction of analytes from aqueous sample, (iv) efficient dispersibility of the solvent and (v) good chromatographic behavior [4, 166, 173, 145]. The main properity of a suitable dispersive solvent should be its miscibility with both the extraction solvent and the aqueous sample [4, 166]. The selection of the solvents for consideration in this work was based on previous work (hexane, isooctane, n-pentane, toluene, xylene) as extraction solvents and (methanol, acetonitrile and acetone) as dispersive solvents as in previous work [182], the combination of xylene-methanol gave better performance when compared with other solvent mixtures.

5.3.2 RSM Extraction Models

The values of 41 different combinations of DLLME conditions based on the independent variables (A, B, C, D and E) studied and the corresponding calculated EFs of the extracted NAs are shown in Table 22. The repeatability of the experimental runs measured by relative standard deviation values (% RSDs) ranging between 0.44 and 11.4 %. These data were subjected to multiple nonlinear regressions using Design Expert 8.0. The experimental data for EFs of the NAs in the extraction solvent were fitted to Eq. 2 to

develop polynomial models capable of explaining the main and different degrees of interactive effects of the investigated parameters on the EFs as well as predicting optimum extraction conditions. Analysis of variance (ANOVA) was conducted to estimate the coefficient parameters of the regression for the models. All three responses for the investigated DLLME fitted reduced quartic models. Qualities of the developed quartic models were further improved by dropping insignificant interaction. The best reduced quartic models in terms of coded factors are shown in eqs 3 to 5 for NDPA, NPIP and NDBA, respectively.

$$\mathbf{EF_{NDPA}} = 33.96 + 2.29\,A + 2.04\,B + 2.30\,C - 1.41D + 8.14\,E + 3.66\,AB + 3.29\,AC + 2.32\,AD - 0.020\,BC + 5.16\,BD + 4.52\,BE + 0.18\,CD + 2.08\,CE + 11.49\,DE - 2.60\,A^2 + 4.58\,B^2 - 2.94\,C^2 + 1.95\,D^2 + 2.35\,E^2 + 5.25\,A^2B - 1.62\,A^2C + 1.62\,AB^2 + 4.36\,AC^2 + 0.25\,B^2C - 3.70\,B^2D - 5.73B^2E - 4.99\,BC^2 + 1.08\,BD^2 + 7.70\,C^2D - 4.65\,CD^2 - 2.64\,D^2E - 7.51DE^2 + 3.74\,A^2C^2 + 4.20\,B^2C^2 - 11.15\,B^2D^2 - 13.21\,B^2E^2 - 1.62\,C^2D^2 \quad \mathbf{(3)}$$

$$\mathbf{EF_{NPIP}} = +13.94 + 1.34A + 1.55B + 0.16\,C + 3.82D + 2.89E + 3.96AB + 1.97AC + 1.46AD - 2.14AE - 0.34BC + 1.49BD + 2.57BE + 0.21CD + 1.00CE + 1.41DE + 0.92A^2 + 3.48B^2 + 3.16C^2 + 2.79D^2 + 3.45E^2 - 4.72B^2D - 5.68B^2E + 4.78C^2D - 5.91B^2D^2 - 5.45B^2E^2 \quad \mathbf{(4)}$$

$$\mathbf{EF_{NDBA}} = +29.02 + 5.40A + 1.01B + 2.22C - 5.50D + 12.29E + 4.73AB + 5.69AC + 5.81AD - 0.097AE + 3.69BC + 2.19BD + 6.44BE + 5.01CD + 1.46CE + 5.99DE + 0.15A^2 - 0.71B^2 + 0.81C^2 + 0.77D^2 + 0.84E^2 - 7.64B^2E + 14.87C^2D^2 - 11.36\,B^2E^2 \quad \mathbf{(5)}$$

Where EF is the enrichment factor.

The coefficients of all variables of the quartic-order equations provided a measure of the effect of the level of the independent variable on the response (EF_i). In addition, the positive and negative coefficients in the response functions indicate a synergistic and antagonistic effect between the corresponding linear or interactive effect and the response, respectively [132]. The qualities of the developed DLLME reduced quartic

models were evaluated based on a statistical test of hypothesis. As displayed in Table 22, the models' quartic-order regression coefficients (R^2) are 0.999, 0.877 and 0.833 for EF_{NDBA}, EF_{NPIP} and EF_{NDPA}, respectively. With the R^2 value closer to unity, the higher the model's accuracy in predicting the experimental values, which indicates the good prediction capabilities of the developed models [140-141]. Moreover, all the sources of variations of the three models' *F*-values determined from ANOVA indicated that the models are statistically significant at 5% significant level (i.e at probability values $p < 0.05$). This further supports the fact that the three equations could be adequately predict the experimental results with a high degree of accuracy [140-141, 145]. Similarly, the respective *p*-values established at either 5% or 10% significant level (i.e $p < 0.05$ or $p < 0.1$) suggest that all the investigated parameters are significant models terms by considering the different sources of the model's variations either as a single (i.e., main effects as provided in Table 23). In addition, the adequate precision (measure of signal to noise ratio) for all the models in Table 23 imply adequate signals (> 4 is desirable) [144] indicating the suitability of the models for navigating the design space for drawing credible conclusions [140].

Table 23: ANOVA for the Quartic Order Regression Model Obtained from Experimental Data

	EF_{NDBA}		EF_{NPIP}		EF_{NDPA}	
	(R^2 = 0.999)		(R^2 = 0.877)		(R^2 = 0.833)	
Precision	7.23		8.59		7.26	
	F-value	*p*-value[a]	*F*-value	*p*-value[a]	*F*-value	*p*-value[a]
Model	432.02	0.0023*	4.3	0.0025*	3.69	0.0039*
A	209.59	0.0047*	3.24	0.0922**	8.67	0.0091*
B	83.42	0.0118*	4.35	0.0546**	0.3	0.5886
C	105.91	0.0093*	0.05	0.8288	1.46	0.2429
D	39.68	0.0243*	13.19	0.0025*	6.75	0.0188*
E	2660.3	0.0004*	11.33	0.0042*	33.73	0.0001*

P-value: statistiac valuesl for quality of models.

[a](*) Significance was established at $p < 0.05$, (**) Significance was established at $p < 0.1$

Considering the main effects, the coefficients of the independent variables were positive for NAs except the variable D in NDPA and NDBA (are negative). This imply that higher levels of extraction time, volume of disperser solvent, sample pH and agitation speed are expected to result in higher EFs by using DLLME. In this regard, the relative contributions of the main effects on the extraction of NAs from water could be ranked according to the order; agitation speed (E) > extraction time (A) > sample pH (C) > volume of disperser solvent (B) > % of salt addition.

5.3.3 Response Surface Curves

Three-dimensional (3D) response surface curves and their corresponding contour maps for the EF models were constructed. This is to enable easy visualization and understanding of the influence of the independent variables and their relative interactions on the EFs. Each of the response curves was developed by fixing three of the independent variables while varying the remaining two within the investigated ranges (Figure 32 to Figure 35). These curves corroborate the ANOVA analysis, revealing that all the independent variables have significant contributions on the responses. They depict the effect of all variables in the extraction of NAs, showing that the EFs were affected by all the investigated variables (A-E).

The dependencies of the EFs on extraction time (A) and dispersive volume (B) at fixed values of initial pH 12, 30% salt addition and 750 rpm agitation speed are depicted in Figure 32. The trends show a linear increase of the influence of extraction time on the extraction efficiencies. Meanwhile, as shown in Figure 32, the stronger degree of curvature due to variation in dispersive volume (B) is portrayed in the upward plateau shape that depicts the decrease with increase in dispersive volume, reaching the highest value in the region around the dispersive volume central point (i.e., 35 μL). Thereafter, the extraction efficiency decrease continuously until the highest level of B is attained. The EFs were low at low volumes of dispersive solvent (methanol) due to the difficulty attaining a cloudy state. Larger volumes of the dispersive solvent increased the solubilities of the NAs in the water phase, leading to a decreased in EFs [27, 166].

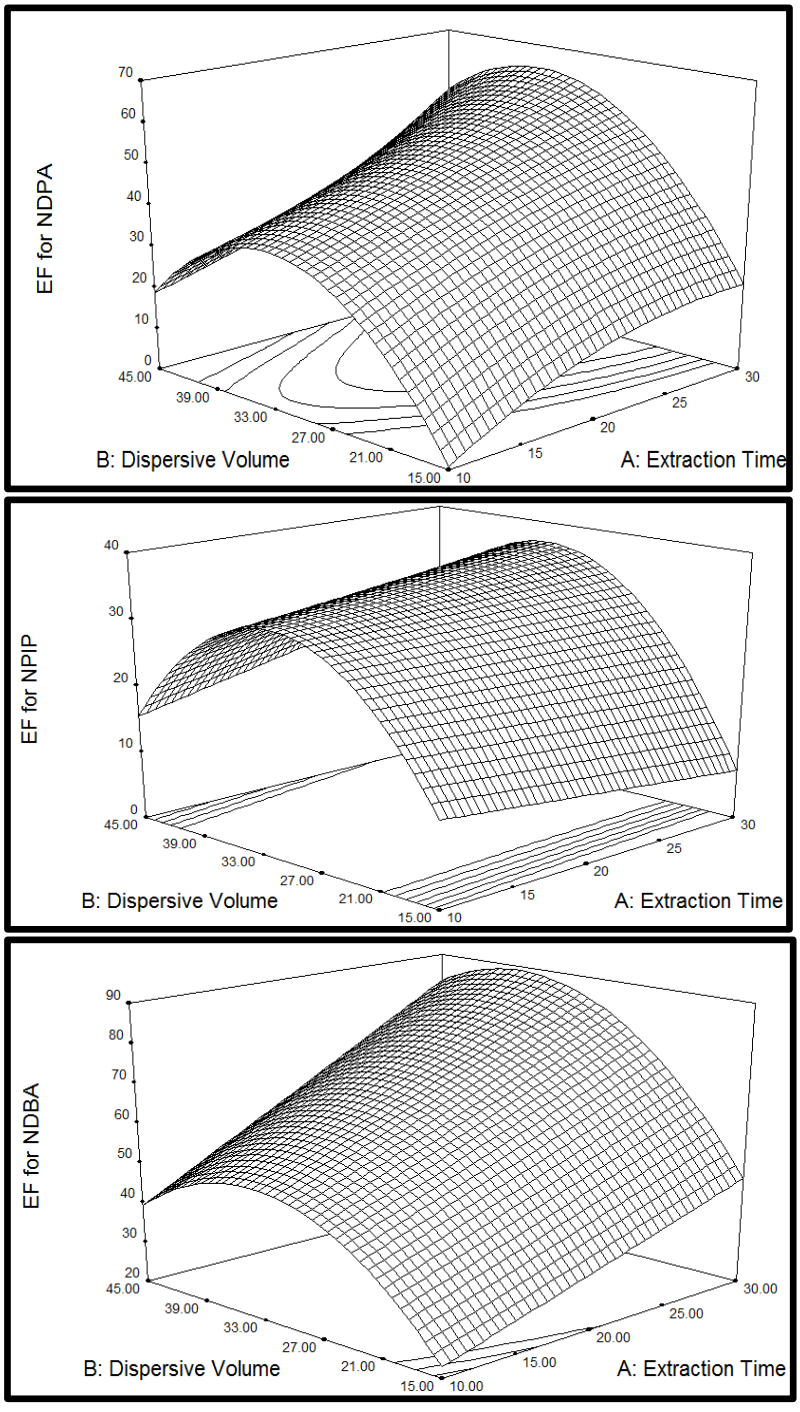


Figure 32: Effect of extraction time and dispersive volume on DLLME of NDPA, NPIP and NDBA from water sample. (pH 12, Salt addition =30%, Agitation speed =750rpm).

At fixed central values of initial sample pH 12, 30% salt addition and dispersive volume of 45 µL, the combined influence of agitation volume (E) and extraction time (A) shown in Figure 33 further corroborates the linear effect of influence of A on the extraction efficiencies for all the NAs. The marked increase in the EFs with increase in the agitation speed tend to remain steady at higher agitation speeds reaching a maximum at the highest agitation speed for NDPA and NDBA. However, the maximum EF for NPIP was achieved at the mid-point of the agitation speed (500 rpm) from where increases in the agitation speed resulted in decrease in the EF.

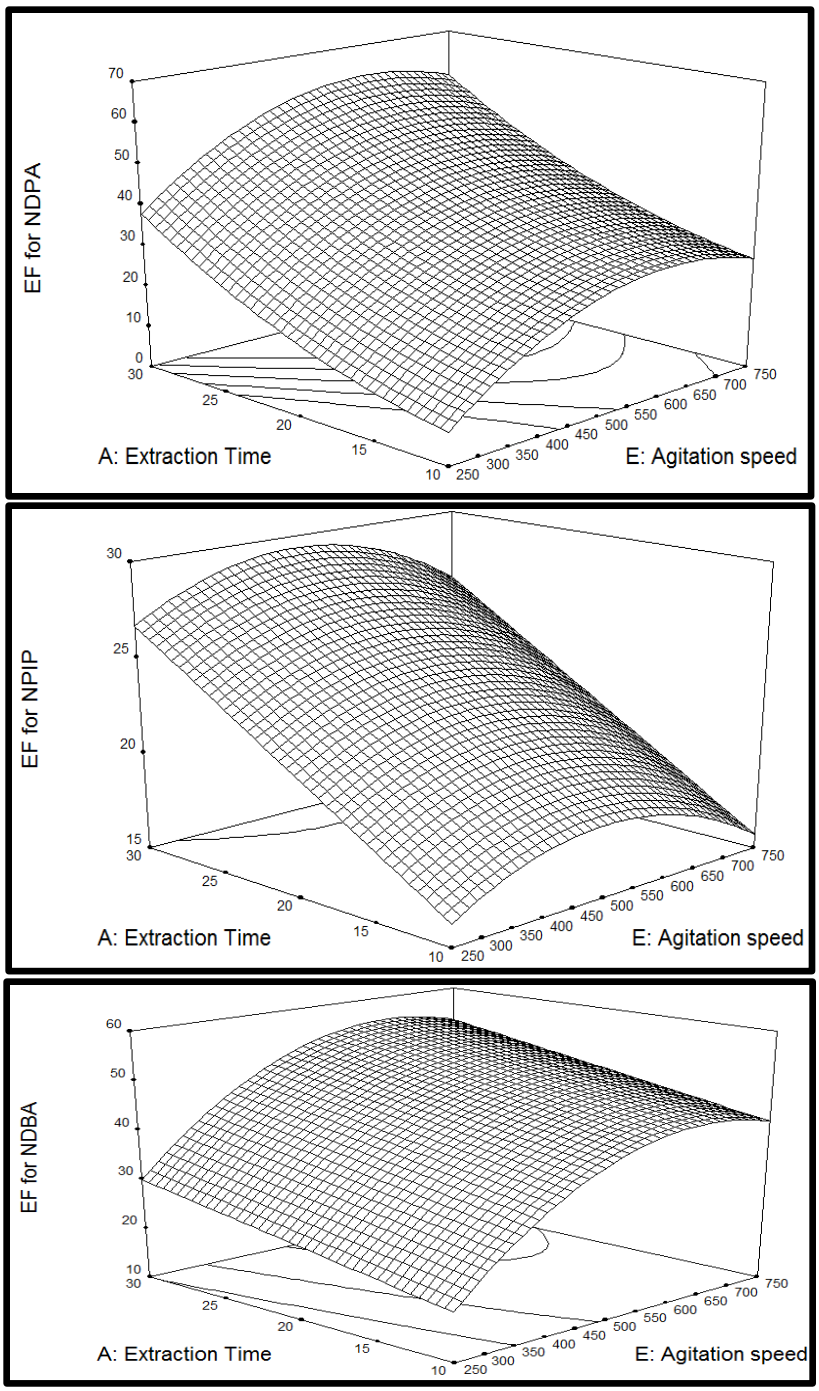


Figure 33: Effect of extraction time and agitation speed on DLLME of NDPA, NPIP and NDBA from water sample. (pH 12, Salt addition =30%, Dispersive volume= 45 µL).

The decrease in extraction efficiency with increased agitation rate could be attributed to back extraction that is more susceptible to take place at higher agitation speed. While the high EF values of 60 and 98 (Figure 32) were achievable for NDPA and NBDA respectively, conversely, that of NPIP is much lower (i.e., 35). Generally, the different plots in Figure 32 and 33 further establish the fact that increasing agitation speed and extraction time has positive effect on the extraction efficiencies of the NAs using DLLME.

The relative effect of agitation speed (E) and dispersive volume (B) can be visualized in Figure 34 predicted at a constant sample pH of 12, salt addition 30% and 30 min extraction time. The depicted patterns of the EFs for all the NAs therein are to some extent like that of NPIP, being little influenced due to changes in the independent variables i.e agitation speed and dispersive volume.

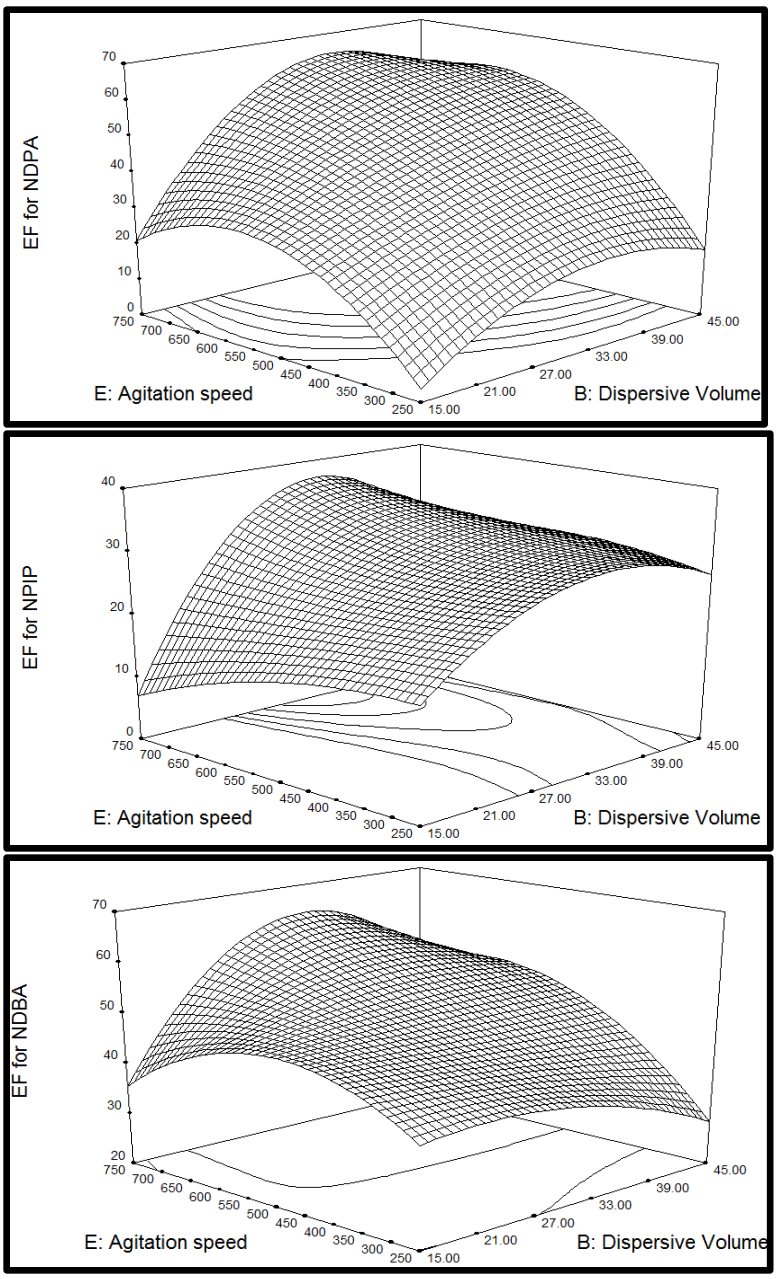


Figure 34: Effect of agitation speed and dispersive volume on DLLME of NDPA, NPIP and NDBA from water sample. (pH 12, Salt addition = 30%, extraction time =30 min).

For all the NAs, the response surfaces show that the EF values for the three NAs are located inside the experimental region around the central values of the E and B. Similarly, the interaction between sample pH (D) and salt addition (C) under the highest level of other experimental conditions shown in Figure 35 suggest comparable trends for NDPA and NPIP extraction. These response curve predictions indicate fairly linear increase in the EFs for all the NAs compounds with increasing salt concentration. This is because under these conditions, the solubility of the NAs in the aqueous phase decreased. Figure 35 suggests that changing the pH slightly affects variability in the NDPA and NPIP EFs. On the other hands, the pH has more variability effect on the EF for NDBA with its curvature effect more pronounced on this analytes. This could be due to stronger hydrolysis of the NDBA that takes place at high alkaline pH compared to that of NDPA and NPIP (Figure 35) [57]. Similarly, trends in variability in the EF values as shown in Figure 34 and 35 clearly demonstrate that higher EF values are more likely to be achieved for NDPA and NDBA compared to NPIP. Thus, the forgoing analyses imply that among the three NAs investigated, NPIP is the least favorable for extraction in the water sample using DLLME. Also factors B, C, D and E are the main sources of the models' curvature which are ranked in the order E > B > C > D. This means that the influence of these factors on EFs tend to deviate from linearity, while that is not the case for factor A (Figure 32 and 33).

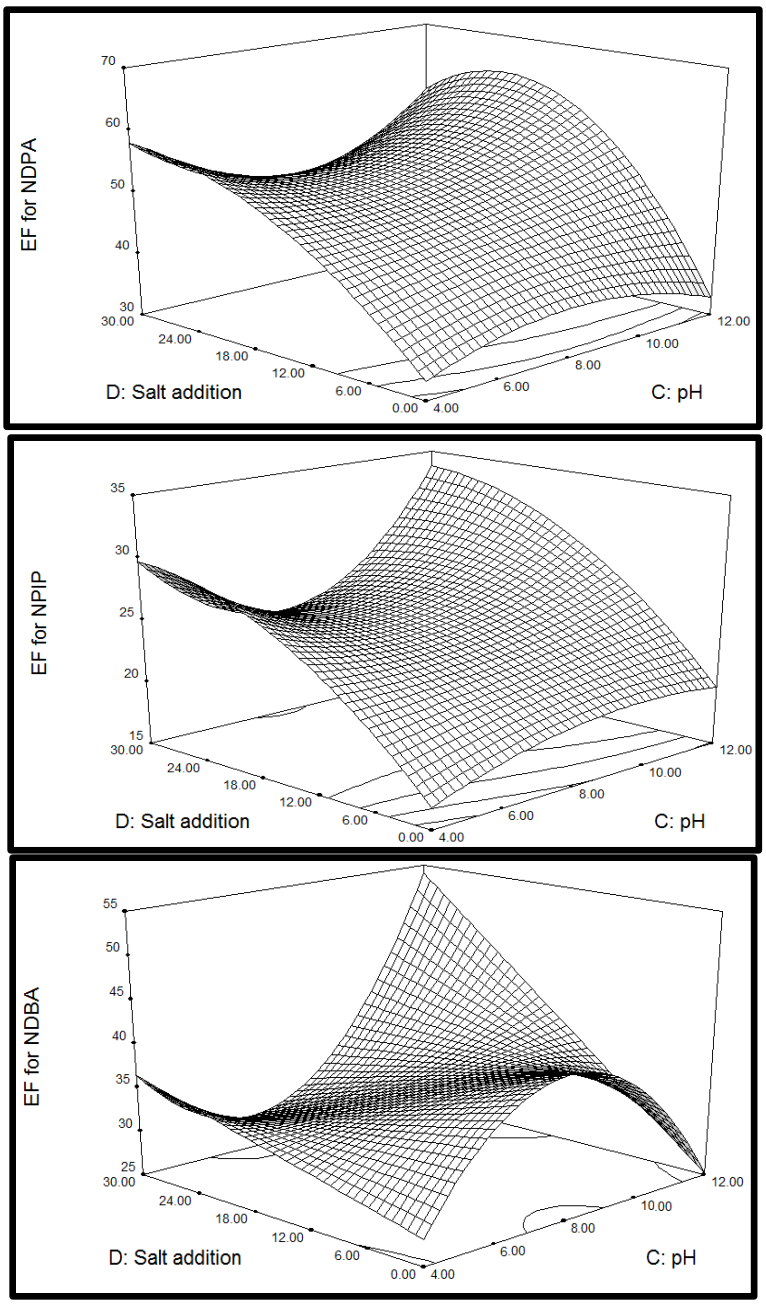


Figure 35: Effect of salt addition and pH on DLLME of NDPA, NPIP and NDBA from water sample. (Agitation speed =750 rpm, extraction time =30 min and dispersive volume = 45 µL).

5.3.4 Optimization of DLMME for NAs Extraction

The optimum conditions for the extraction of NDBA, NDPA and NPIP, and the sum of these three NAs compounds were predicted using coded values of the independent variables. With the least number of parameters (i.e., 3 parameters) under investigation, finding the optimum region through visual inspection of the response surfaces is possible in the absence of constraints. However, for higher number of parameters (as in the case of present study), obtaining the global (rather than local) maximum point within the experimental variable ranges becomes more tasking. As such, numerical optimization for simultaneous extraction of the NAs using DLLME was performed with the aid of the Design-Expert[@] 8.0. The coordinates of the optimal points were calculated through equating the first derivatives of the reduced models (eqs 3 to 5) to zero according to eq 6 in conjunction with a set of convergent criteria [143-144]. The convergent criteria are composed of set of goals based on desired constraints for the parameters of interest (responses and the independent variables). The criteria also weighted the individual parameters according to their relative importance in contributing towards attaining the desired overall targeted goals.

$$\frac{\partial y}{\partial x_i} = \beta_i + 2\beta_{ii}x_i + \sum_{j=2}^{k}\beta_{ij}x_j + \ldots\ldots = 0 \qquad \textbf{(6)}$$

The ANOVA provide the optimal numerical solutions that met the convergent criteria for maximizing the EFs of all the three NAs with highest desirability are shown in Table 24. The average of these solutions suggested the optimal conditions for simultaneous extract of the NAs in a water sample containing all the three NAs were 28 min extraction time, 33.5 µL of methanol dispersive volume, 722 rpm agitation speed, 23 % (w/v) NaCl concentration and pH 10.5 [166].

Table 24: Numerical optimization results for extraction conditions of the NAs by automated DLLME/GC-MS

	Variables					EF				
Solutions	**A**	**B**	**C**	**D**	**E**	**NDPA**	**NPIP**	**NDBA**	**% RSDs**	**Desirability**
1	28.01	33.36	10.50	22.85	722.29	41.75	27.1	56.73	1.47	0.89
2	28.01	33.29	10.53	22.83	721.85	41.74	27.11	56.73	1.47	0.89
3	28.13	33.37	10.47	22.87	722.05	41.77	27.08	56.79	1.47	0.89
Average	≈28	≈33.5	10.5	≈23	722	41.75	27.09	56.75	1.47	0.89

5.3.5 Analytical Performance of Automated DLLME

To evaluate this method, the correlation coefficient (r), linear range (L.R.), repeatability and limits of detection (LODs) were investigated under the optimized condition. The results are summarized in Table 25.

Table 25: Automated DLLME validation

	Linearity equation	r	L.R (µg/L)	%RSDs (n=4)	LODs (3N/S) (ng/L)
NDPA	y = 8.495E-04x – 2.041	0.9937	0.5-100	3.8	52
NPIP	y = 1.652E-03x – 6.114	0.9985	0.1-100	5.9	32
NDBA	y = 6.111E-04x – 4.379	0.9993	0.1-100	3.4	5.7

Linearity was observed over the concentration range of 0.1 to 100 µg/L for NAs and with excellent correlation coefficient (r) ranging from 0.9937 to 0.9993. The repeatability study was carried out by extracting spiked water samples at different concentration levels of 0.1, 0.5, 1, 10, 20, 37, 74, 100 µg/L and the percentage relative standard deviations (%RSDs) were between 3.4 and 5.9% (n = 4). The LODs, based on a signal-to-noise ratio (S/N) of 3 [197] ranged from 5.7 to 52 ng/L. The performance of automated DLLME was compared with those of other methods reported in the literature and the results are shown in Table26.

Table 26: Comparison of automated DLLME/GC-MS with other reported methods for the determination of NAs in water samples.

Method	Sample	L.R (ng/L)	LODs (ng/L)	%RSDs	%Recovery	Ref-
HS-SPME/GC-MS-MS[a]	Water	10 - 1500	1 – 5	3 – 13.0	-	104
SPE/GC-NPD[b]	Water	300 - 20000	20 – 80	3.5 – 6.3	95 - 103	128
SPE/GC-MS	Water	40 - 20000	3 – 13.0	4.1 – 6.1	95 - 103	128
SPE/GC-FID[c]	Water	10000 - 600000	2000-3500	3 – 6.5	-	128
HPLC-CL[d]	Water	5 - 1000	1.5 - 3	0.7 – 4.5	94.8 – 102.8	132
SPE/GC-EI-MS-MS[e]	Water	500 - 50000	0.4 - 4	max 10	82-102	146
Automated DLLME/GC-MS	Water	100-100000	5.7 – 52	3.4 – 5.9	90.3 -112	Present

L.R: Linearity Range. LODs: Limits of Detection. R^2: Coefficient of determination. %RSDs: Relative standard deviation.
(a) Head space solid-phase microextraction followed by gas chromatography tandem mass spectrometry.
(b) solid-phase microextraction by gas chromatography with nitrogen phosphorus detecto.
(c) solid-phase microextraction by gas chromatography with flam ionization detector.
(d) high-performance liquid chromatography with chemiluminescence detection.
(e) Solid phase extraction gas chromatography–electron ionization tandem mass spectrometry.

The results of NAs clearly indicate that, the performance of DLLME (Table 25) is combarable and in some cases more accurate than those mentioned in Table 26. The advantages of the present DLLME/GC-MS approach includes relatively lower LODs, relatively high recoveries and a good linear range [146, 132].

5.3.6 Application to Real Water Samples

The automated DLLME/GC-MS method was applied to determine the NAs in different types of water samples: tap water, groundwater samples before and after treatment, and a water water purification plant in the main campus of King Fahd University of Petroleum and Mineral (KFUPM) in Saudi Arabia. Ten millimeters of each sample were used for the DLLME. Only very low concentration of NDBA was detected in raw groundwater samples (Table 27). NDPA and NPIP were not detected in any of the samples.

To assess the matrix effect of the DLLME/GC-MS, real samples were spiked with 2 µg/L of the target analytes and extraction recoveries were calculated (Table 28). Recoveries for NAs in groundwater and tap water samples were ranged between 90.3 and 112.1%. Figure 36 shows the GC-MS total ion chromatograms of 10 mg/L of standard NAs (without extraction), groundwater and groundwater spiked at 2 µg/L.

Table 27: Concentration (µg/L) of NAs in real water samples using automated DLLME/GC-MS at the university campus.

NAs	groundwater at 25 m deep (n=3)				Tap (n=3)	
	Raw	%RSDs	After treatment	% RSDs	water	% RSDs
NDPA	ND	-	ND	-	ND	-
NPIP	ND	-	ND	-	ND	-
NDBA	0.45	5.3	ND	-	ND	-

ND: Not determined,

Table 28: Extraction recovery of NAs from water samples spiked by (2 µg/L) using automated DLLME/GC-MS.

NAs	Raw underground water	%RSDs (n=3)	Treated underground water	%RSDs (n=3)	Tap water	%RSDs (n=3)
NDPA	96.9	14.4	89.4	7.8	105.6	9.2
NPIP	91.9	6.9	92	13.6	94.2	7.3
NDBA	92.9	4.8	90.3	10.7	112.1	7.7

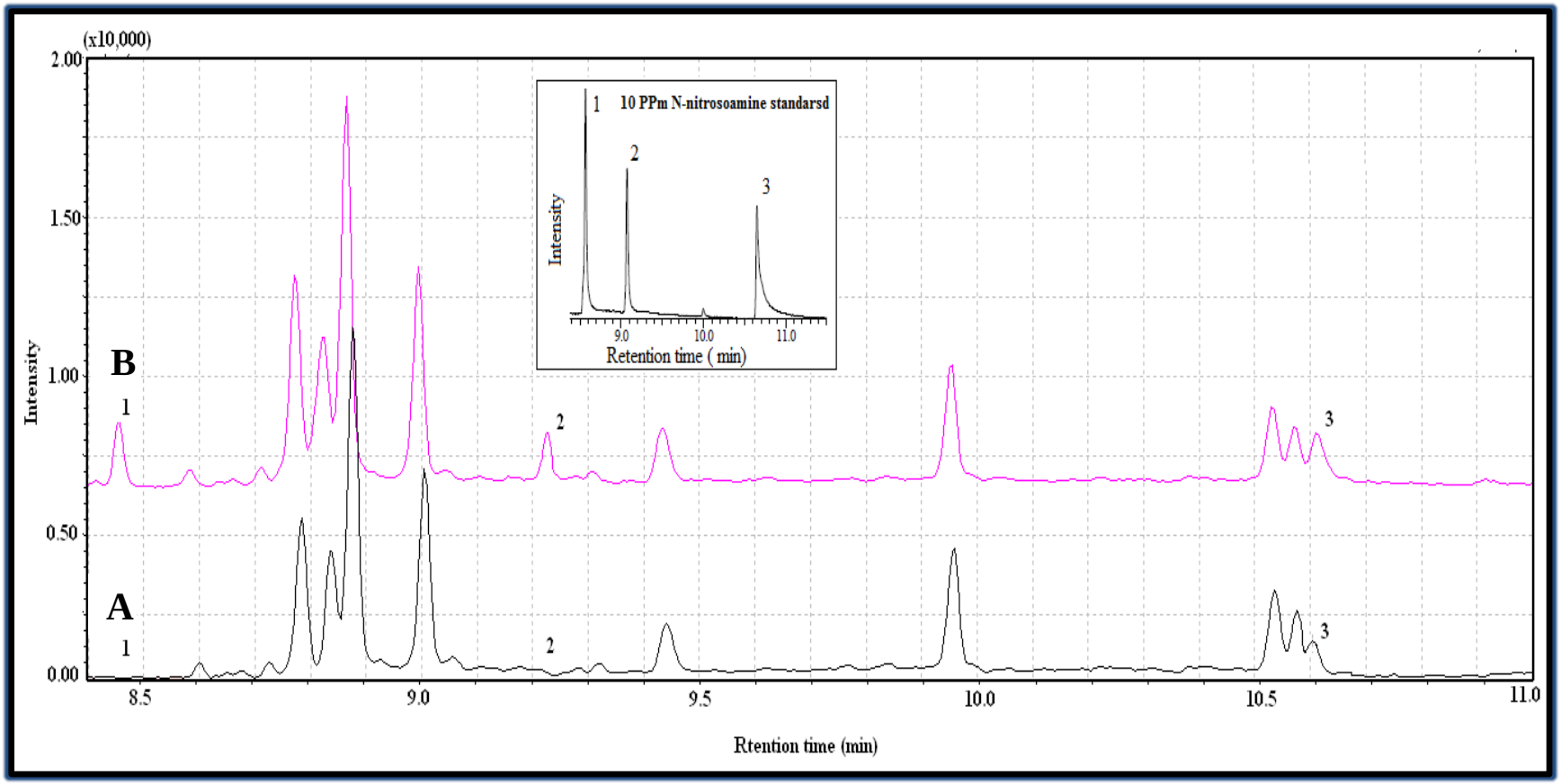


Figure 36: Total ion chromatograms of ;(A: fresh groundwater, B: fresh groundwater spiked by 2 µg/L of N-nitrosoamine standard). 1: NDPA, 2: NPIP and 3: NDBP.

5.4 CONCLUSION

In this study, automated DLLME using GC-MS was developed to determine the concentration of nitrosamines (NAs) in different sample matrices. The extraction conditions of the NAs was optimized via response surface methodology (RSM). The combination of DLLME with GC-MS proved to be suitable for determining nitrosoamines in water samples at ng/L levels. The method provided very good LODs (between 5.7 and 52 ng/L) and satisfactory precision (% RSDs, between 3.4 and 5.9%). The proposed automated DLLME was shown to be efficient, simple and environmentally friendly.

CHAPTER 6

FULLY AUTOMATED FLOW ASSISTED SOLID-PHASE MICROEXTRACTION FOR DETERMINATION OF CHLOROETHERS IN WATER AND URINE SAMPLES

6.1 LITERATURE REVIEW

Chloroethers (CEs) are compounds which contain an ether moiety (R-O-R) and halogen atoms attached to the aryl or alkyl groups. More than 50 million pounds of CEs are produced per year, and commonly used as solvents in various different industrial applications [185-187]. Generally, CEs are stable and non-biodegradable in aqueous samples [185].

Bis(2-chloroethyl)ether (BCEE), bis(2-chloroisopropyl) ether (BCIE) and Bis(2-chloroethoxy)methane (BCEM) are the class of CEs and frequently found in drinking waters and urine [188-191]. Thus, the release of CEs into the environment is of great concern because of their toxicity and carcinogenicity [186, 188, 192-193]. The United Sates Environmental Protection Agency (USEPA) and the International Agency for Research on Cancer are classified CEs as carcinogenic compound category D [194].

No data were found to address the toxicity of BCEM to humans. However, its volatility and water solubility could result in human exposure by inhalation, ingestion or dermal contact in the course of occupational exposures. The minimum half-life of BCEM in

water has been reported to be 2 years [195-196] presenting the potential for persistent environmental exposure.

In this regard, different preconcentration methods were reported for the analysis of CEs in water samples which include USEPA methods 611 and 625 based on liquid–liquid extraction (LLE) [186, 188]. However, LLE procedures require larger volumes of hazardous organic solvents and multi-step extractions. They are time-consuming and involve the risk of analyte loss in the extraction and concentration processes. They are not suitable for trace level determination [193, 197]. The solid-phase extraction (SPE) is a solvent minimized alternative to LLE approach [193]; SPE-C_8 was used for CEs. The main problem associated with SPE-C_8 is the low selectivity of the retention mechanism of CEs which yield low recoveries [186, 188, 198]

In recent years, continuous progress in microextraction techniques for CEs has produced important developments in trace level analyses from various environmental samples. Liquid-phase microextraction (LPME) and solid-phase microextraction (SPME) are alternative microextraction methods reported for CEs in the literature [188, 199-200]. LPME is solvent minimized extraction technique. CEs are extracted using immiscible organic solvents; selection of suitable organic solvents for polar analytes and fully automation of LPME are challenging tasks [186, 199].

Solid-phase microextraction (SPME) is widely used solvent-free extraction microextraction technique which combines sampling, sample clean-up and pre-

concentration into a single step [201]. On the other hand, SPME requires careful calibration and optimization for the quantization of trace level analytes. This requires more time and once the procedures are optimized, SPME can be conveniently used for routine analyses [202]. Manual SPME optimization methods sometime pose to human error and the possibility of contamination associated with manual processing [203]. Automated sample preparations eliminates human intervention in order to improve overall sample analysis efficiency and reliable robustness of the method [204].

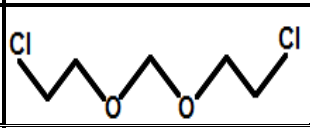
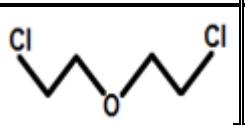
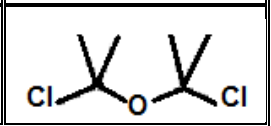
The objective of this study was to optimize an automated flow assisted solid-phase microextraction (FA-SPME) combined with GC-MS in order to quantify CEs in large volume samples. Although SPME automation have been widely used in various modes such as headspace-SPME, direct immersion-SPME, and different formats which includes thin film-SPME, in-tip-SPME and 96 vial plate-SPME [204-207]. In general, SPME automation has been reported only for small volume samples. To our knowledge, this is the first time that a method for large volume samples were used for fully automated SPME.

6.2 EXPERIMENTAL

6.2.1 Chemicals and Materials

A mixture of CEs standards were purchased from Supelco (Bellefonte, PA, USA). This mixture, containing Bis(2-chloroethyl)ether, bis(2-chloroisopropyl)ether and Bis(2-chloroethoxy)methane at 2000 µg mL^{-1}. A working standard solution was prepared daily by appropriate dilution of stock solution of CEs in the same solvent (hexane). Physical and chemical properties of target analytes are shown in (Table 29). Analytical grade solvents were purchased from Supelco (Bellefonte, PA, USA). Double deionized water obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Sodium hydroxide, hydrochloric acid and sodium chloride were obtained (Merck, Darmstadt, Germany). To avoid any carryover of CEs; all laboratory glassware were washed with concentrated hydrochloric acid and rinsed with deionized water followed by acetone and dried out in the laboratory oven at 100 ºC for 1 h. Precise peristaltic pump was purchased from J.P. Selecta (Abrera-Barcelona, Spain) provides flow rates from 20 to 200 mL min^{-1}. The speed can be automatically controlled through an external controller.

Table 29: Physical properties of three CEs [210]

Physical properties	**BCEM**	**BCEE**	**BCIE**
Molecular structure			
Molecular weight (g mol $^{-1}$)	173	143	171
Solubility at 20-25 °C (mg L^{-1})	1353.4	10200	1700
Vapor pressure at 20-25 °C (mmHg)	0.179	1.34	0.85
Boiling Point (°C)	220	178	187
Henry's law constant at 20 °C	0.001	0.00089	0.004
Diffusion coefficient in air (cm^2 s^{-1})	0.058	0.069	0.06
Diffusion coefficient in water (cm^2 s^{-1})	7.11E-06	7.53E-06	6.40E-06

6.2.2 Instrumentation

Analyses were performed using a gas chromatograph (Agilent technologies, 7890A GC) coupled with a quadrupole mass selective spectrometer (Agilent technologies, 5975C) equipped with an inert ion source and provided with a split-splitless injection port. An A HP-5 GC fused silica capillary column (Agilent 19091J-413; 30 m × 320µm ID × 0.25 µm thickness) was selected to separate the analytes. CTC CombiPAL autosampler (GC sampler 80, Zwingen, Switzerland) was used for the full automated FA-SPME. Ultra high purity helium (99.999%, Abdulah Hashim, Al-Khobar, Saudi Arabia) was used as the carrier gas at a flow rate of 1.3 mL min^{-1}. The samples were injected in the splitless mode. The temperature program used for the analyses was as follows: the initial temperature was 40 °C held 1 min which was then increased to 118 °C at 10 °C min^{-1} and held for 3 min, then to 190 C at 15 °C min^{-1} and held for 4 min. The total run time was 18.6 min. The injection port, ion source and interface temperatures were heated at 280 °C, 230 °C, and 250 °C, respectively. For qualitative determinations, the MSD was operated in full-scan mode from m/z 50 to 550 and selective ion monitoring mode was used for the quantification of the analytes. These chromatographic conditions are presented in Table 30.

Table 30: Gas chromatographic conditions for CEs determination

Instrument	Agilent technologies, 7890A GC coupled with Agilent technologies, 5975C MSD
Column	HP-5 used silica capillary column (Agilent 19091J-413; 30 m × 320 µm ID × 0.25 µm thickness)
He flow rate	1.3 mL/min
Injection mode	Splitless mode
Oven temperature program	40 °C (1 min) Ramped at 10° C/min to 118 °C and held at this temperature (3 min) Ramped at 15° C/min to 190 °C and held at this temperature (4 min) The total run time was 18.6 min.
Injection port temperature	280 °C
interface temperatures	250 °C
MS temperature	230 °C

6.2.3 Samples

Drinking and tap water samples were collected from the main campus of King Fahd University, Saudi Arabia. Urine sample were collected in cleaned glass bottles from a volunteer working at water desalination facility. All samples were stored at 4 °C prior analysis.

6.2.4 Analytical Procedure

Experimental setup of FA-SPME is shown in Figure 37. A 100 mL sample solution spiked with CEs, sample pH 10 and salt concentration of 10 % (w/v) was placed in a 125 mL flask and connected to 20 mL modified auto sampler vial with flexible PEEK tubing. Samples were circulated with different flow rates using the peristaltic pump. Extractions were performed by SPME fiber in direct immersion mode at modified auto sampler vial for 10 min in a continuous flow mode. After the extraction, the fiber was thermally desorbed in the GC-MS injection port for 3 min at 290 ^{o}C.

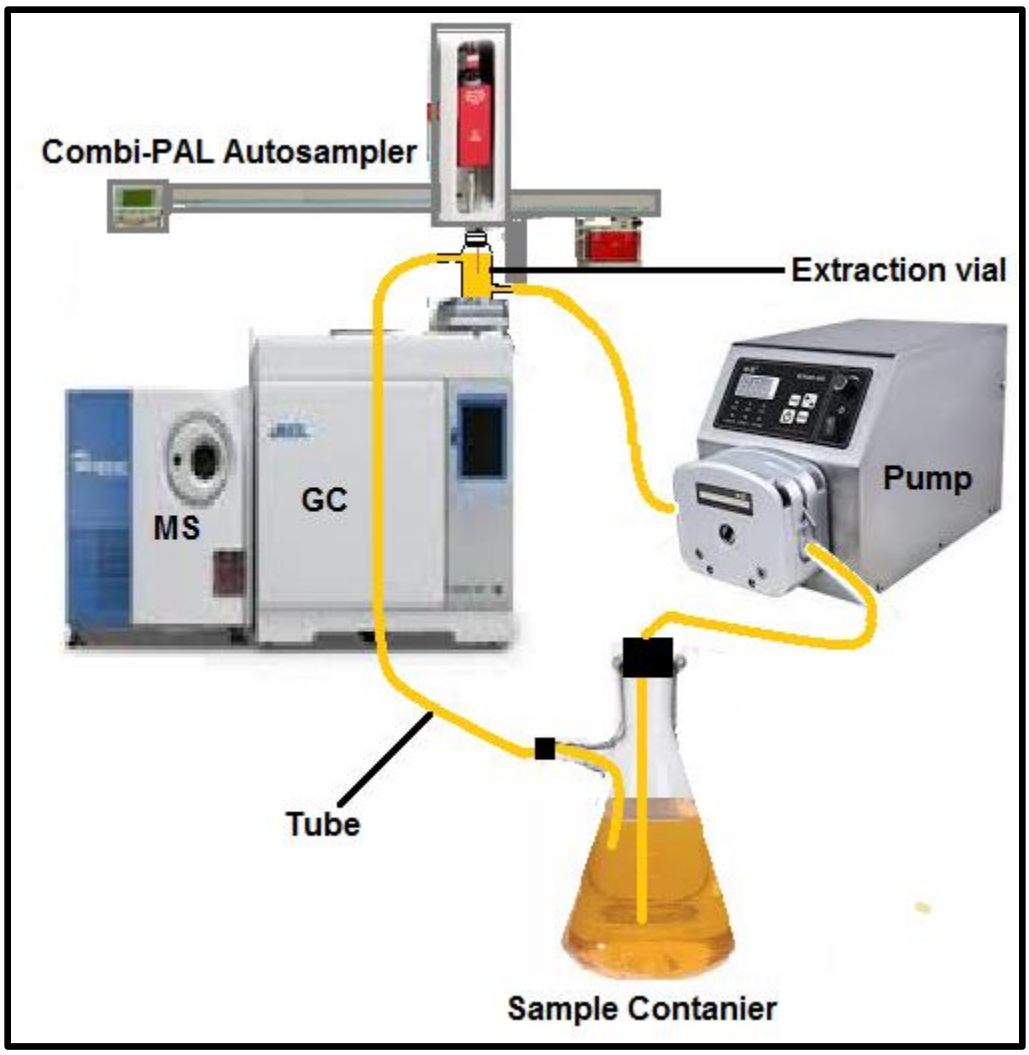


Figure 37: Schematic of automated FA-SPME/GC-MS.

6.3 RESULT AND DISCUSSION

6.3.1 Selection of SPME Fiber

To optimize the SPME conditions, three commercially available fibers were tested to extract CEs. Polydimethylsiloxane (PDMS, 30-µm), Carbowax/Divinylbenzene (CW/DVB, 65-µm) and polyacrylate (PA, 85-µm) coated fibers were purchased from Supelco (Supelco, Bellefonte, PA, USA) and used without any modifications. The fibers were conditioned prior use according to the instructions provided by the suppliers. Figure 38 shows the extraction performance and CW/DVB give high peak areas for all CEs. There is high agreement with the fact that says more polar compounds are best extracted by polar fibres like CW/DVB [209]. From the result CW/DVB fiber was finally selected for use in further optimization studies.

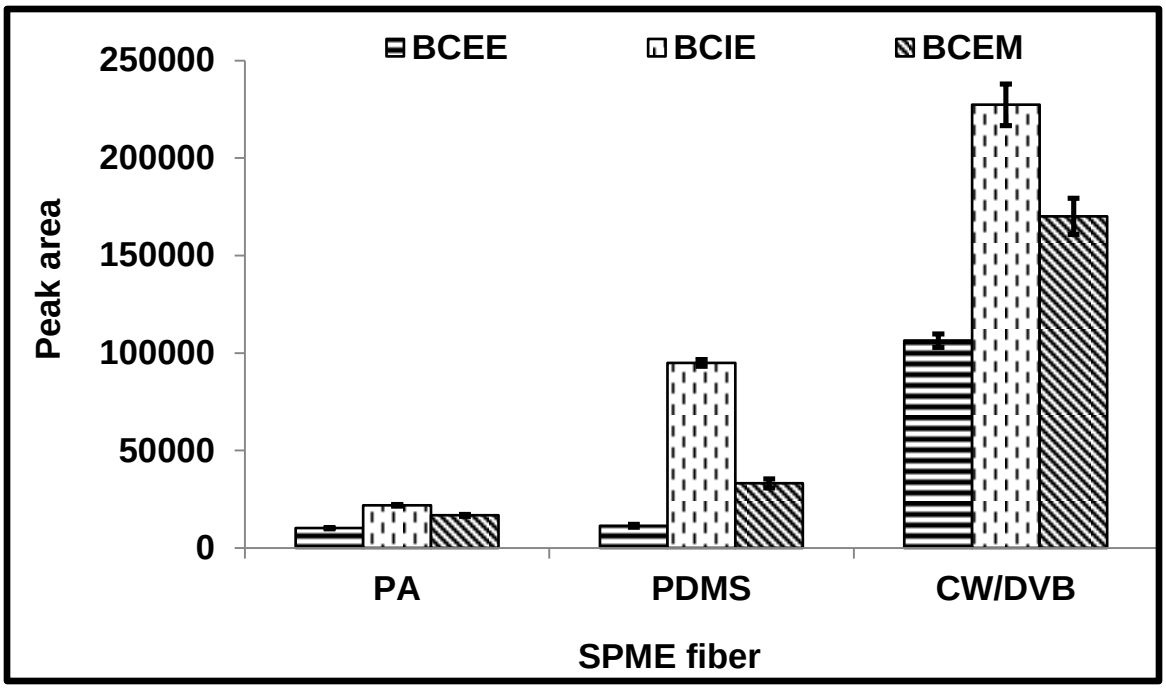


Figure 38: Selection of SPME fiber (extraction conditions; 100 mL of sample spiked with 100 µg/L of CEs, absorption time 10 min, desorption time 3 min, sample flow rate of 40 mL/min and sample pH of 5.7).

6.3.2 Effect of Absorption Time of SPME

The effect of the absorption time profile using CW/DVB fiber was examined in the range between 5 and 30 min. Peak areas are plotted against absorption time and shown in Figure 39. The equilibrium period was 10 min for all CEs; thus an absorption time of 10 min was selected for further optimization.

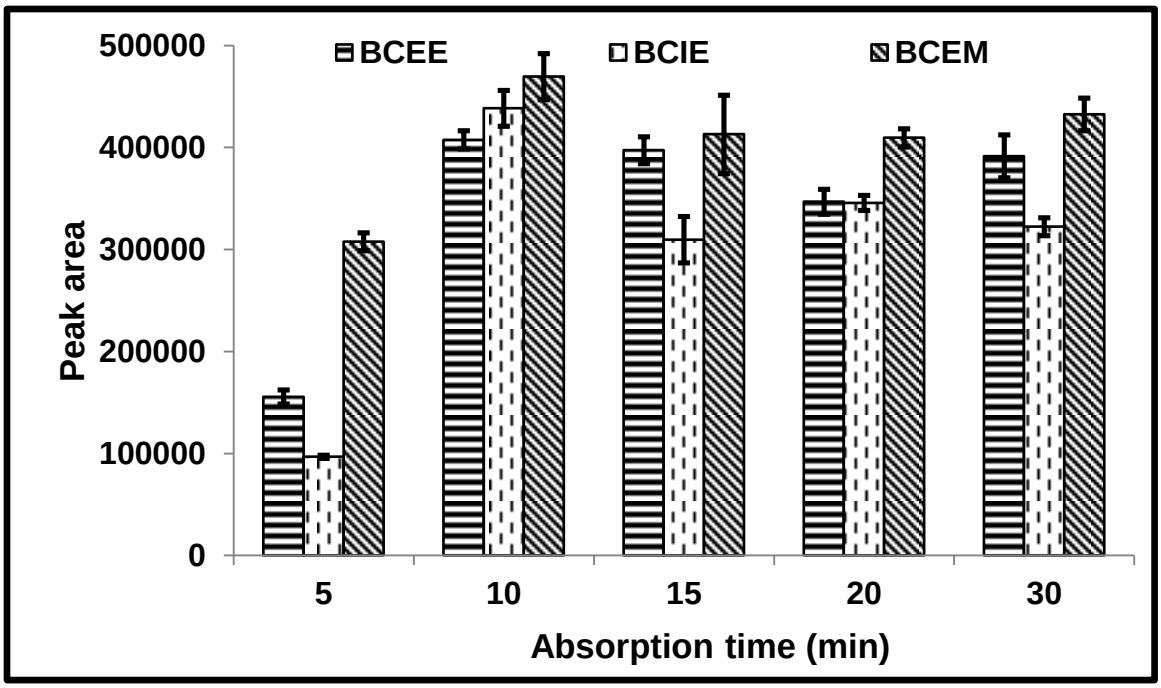


Figure 39: Effect of absorption time on fully automated FA-SPME/GC-MS (extraction conditions; 100 mL of sample spiked with 100 µg/L CEs, desorption time 3 min, sample flow rate of 50 mL/min and sample pH 5.7).

6.3.3 Effect of Desorption Time

In order to ensure complete desorption of analytes from the SPME fiber and avoid carryover, suitable desorption temperature and desorption time are critical. For this reason, desorption temperature of extracted analytes was carried out inside the GC injection port at temperatures 280 $^{\circ}$C [188]. Also optimized by placing the fiber inside the GC injection port for a period of 3 to 5 min desorption time. Figure 40 shows the best efficiency at 3 min desorption time. After analytes desorption, between the runs the SPME fibers were further cleaned at 280 $^{\circ}$C for 3 min in CombiPAL SPME conditioning station. This was to ensure a complete fiber cleanup and avoid any sample carryover.

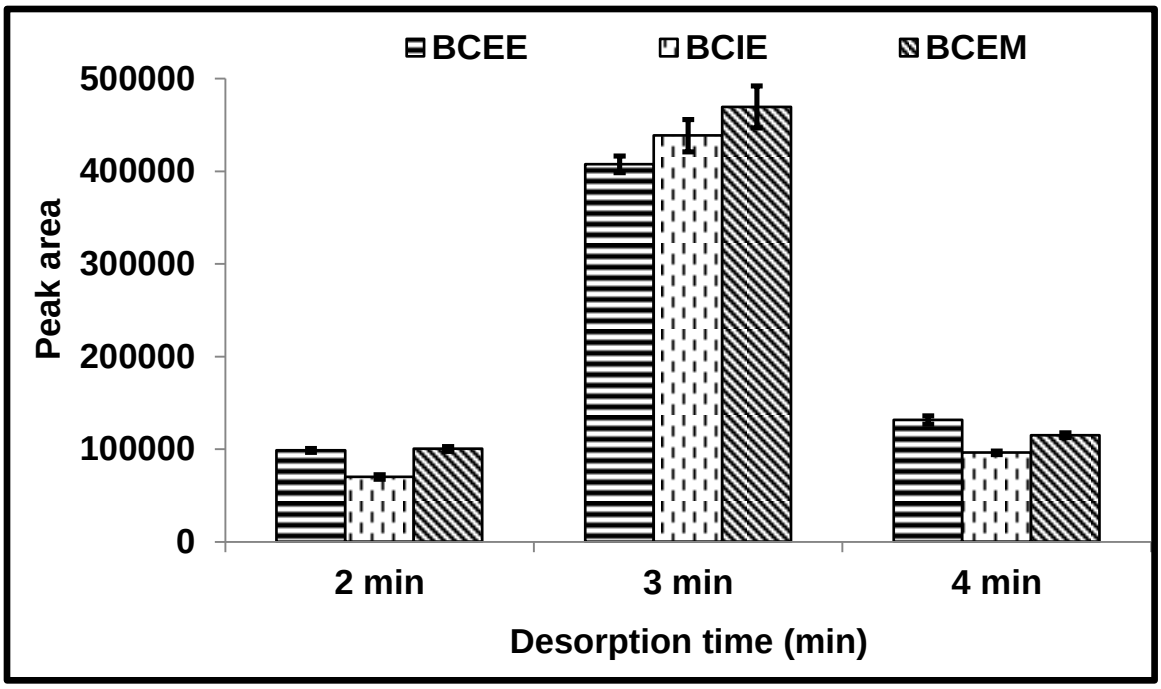


Figure 40: Effect of desorption time on fully automated FA-SPME/GC-MS (extraction conditions; 100 mL of sample spiked with 100 µg/L CEs, absorption time 10 min, sample flow rate 50 mL/min and sample pH 5.7).

6.3.4 Effect of Pump Flow Rate

Precise peristaltic pump was employed to provide contentious fully-automated FA-SPME technique. The aim of this part was to investigate the effect of sample flow rate on the extraction efficiency of CEs. The flow rate of pump is an important parameter that permits continuous exposure of the SPME fiber to fresh aqueous sample. The flow rate of samples was examined in the range between 0 (static mode) and 80 mL min^{-1}. Figure 41 shows the extraction efficiency increased with increasing flow rate from 30 to 50 mL min^{-1}. A decrease in extraction efficiency at higher flow rates (> 50 mL min^{-1}) was observed. It is likely either the SPME fiber might have reached maximum extraction or high flow rates of sample causes loss of analytes from the SPME fiber resulting in back migration of the analytes and thus leading to lower pre concentration.

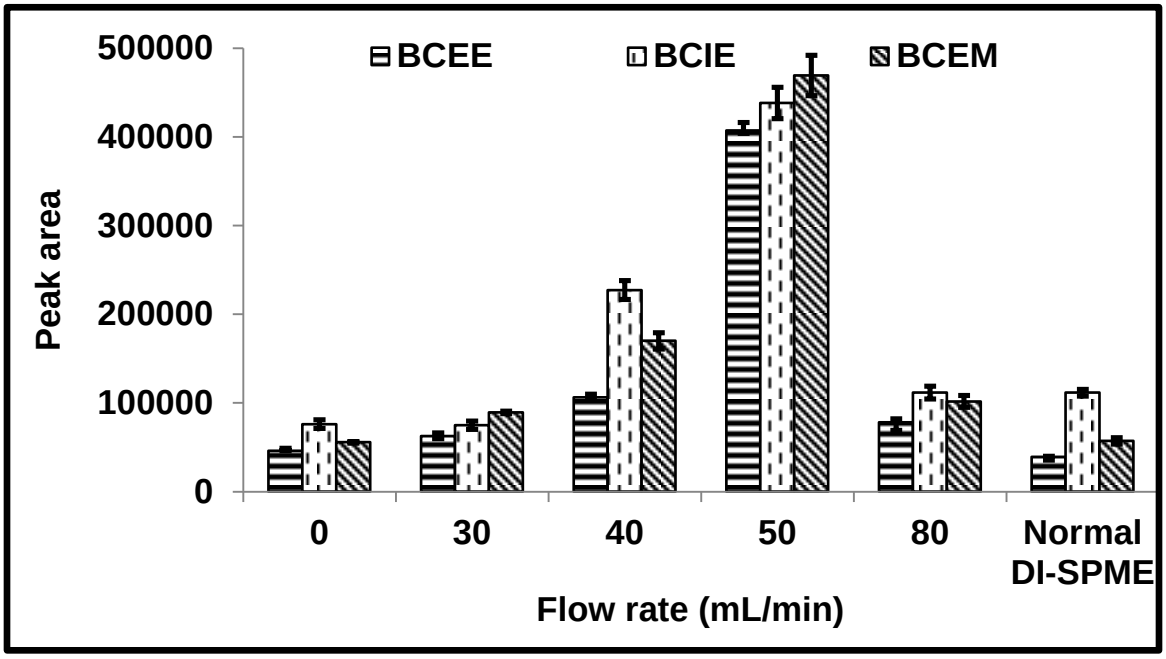


Figure 41: Influence of sample flow rate and conventional agitation on fully automated FA-SPME/GC-MS (extraction conditions for FA-SPME is as Figure 40 with desorption time of 3 min. For conventional agitation DI-SPME, 20 mL sample was spiked with 100 µg/L CEs, absorption time 10 min, desorption time 3 min, sample pH 5.7 and agitation speed of 250 rpm).

To compare the performance of FA-SPME, conventional automated DI-SPME and manual DI-SPME experiments were conducted using 20 mL regular SPME autosample vial with sample agitation speed of 250 rpm for 10 min. Results clearly indicated that the use of flow instead of conventional agitation provided high sensitivity as seen in Figure 41. Thus FA-SPME has advantageous for on-site applications, real sample can be directly analyzed without sub sampling in sample vials; and the FA-SPME/GC-MS approach is more robust and user-friendly.

6.3.5 Effect of Ionic Strength

The effect of ionic strength on the extraction efficiency of fully-automated FA-SPME was investigated by adding NaCl concentrations ranging from 0 (no salt addition) to 30% (w/v) (Figure 42). The highest extraction efficiency CEs was at 10% (w/v) concentration of NaCl. The extraction efficiency decreased for solution contain higher than 10% (w/v). The anomalous effect of NaCl on the extraction of CEs is probably due to two factors. The first is the salting-out effect, which decreases the solubility of the analytes, and thus increase the absorption [186, 188, 209]. Secondly, salt dissolved in the solution may change the physical properties of the static aqueous layer on the fiber, and thereby reduce the rate of diffusion of the analyte through the static aqueous layer to the fiber [188]. Therefore 10% (w/v) NaCl was added in the subsequent studies.

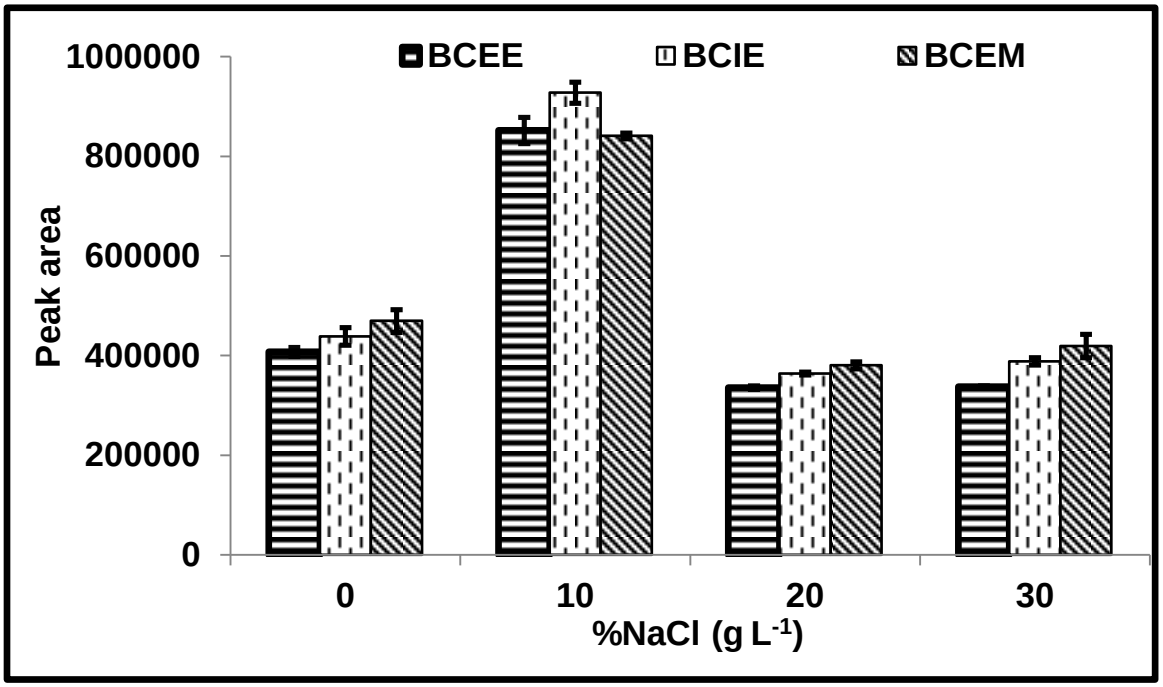


Figure 42: Effect of ionic strength on fully automated FA-SPME/GC-MS (extraction conditions; 100 mL of sample spiked with 100 µg/L CEs, absorption time 10 min, desorption time 3 min, sample flow rate 50 mL/min and sample pH 5.7).

6.3.6 Sample pH

To determine the effects of pH on the performance of FA-SPME, samples at different pH between 2 and 12 were investigated. The extraction performance increases with increasing sample pH (Figure 43).

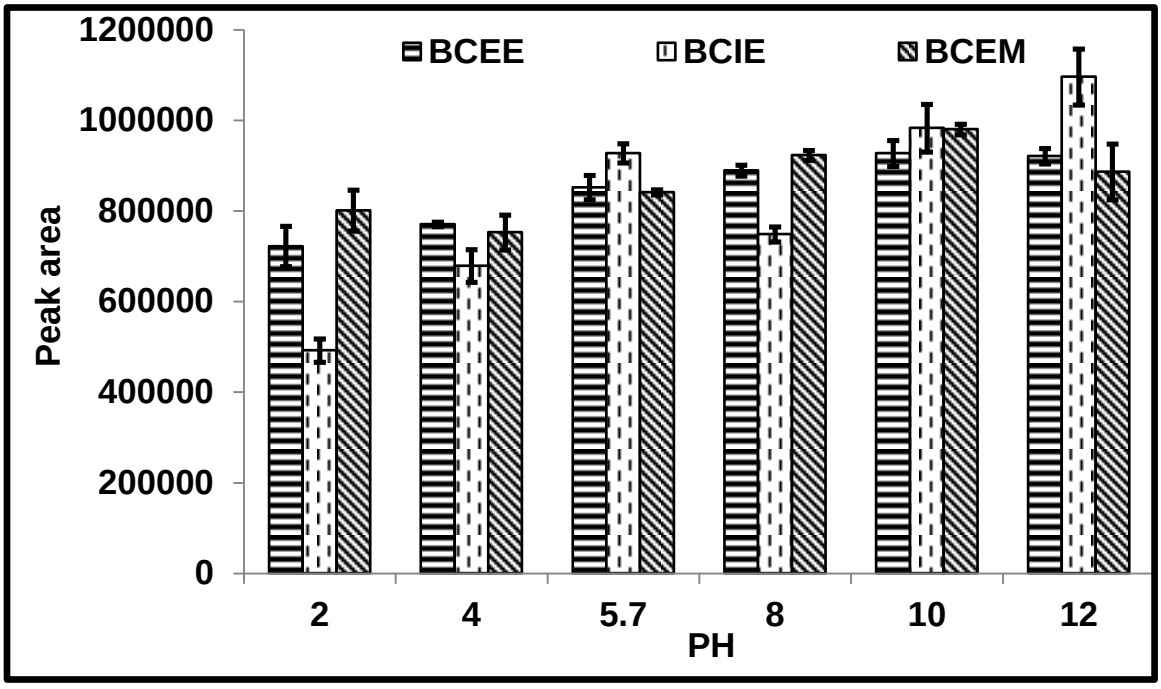


Figure 43: Effect of sample pH on fully automated FA-SPME/GCMS (extraction conditions; 100 mL of sample spiked with 100 µg/L CEs, absorption time 10 min, desorption time 3min, sample flow rate 50 mL/min and 10% of NaCl).

6.3.7 Analytical Performance of Fully-Automated FA-SPME

To evaluate the quantitative performance of the FA-SPME, the linear range, repeatability, and the limits of detection were investigated under the optimized conditions. The results are summarized in Table 31. Excellent linearity was observed over the concentration range of 0.5-100 µg/L with favorable correlation coefficient (r) ranging from 0.9941 to 0.9981 (Figure 44-46). The repeatability study was carried out by extracting spiked water samples at different concentration levels of (0.5, 1, 5, 10, 20, 40, 70, 100 µg L^{-1}), and the average percentage relative standard deviations (% RSDs) were between 1.2 and 6.2% (n = 3). The LODs, based on a (S/N) ratio of 3, ranged from 0.017 to 0.053 µg L^{-1}. These results confirmed that the proposed method is suitable for trace level analysis of CEs in aqueous samples. A comparison of the main characteristics of the proposed method with previously reported works is summarized in Table 32. The developed method shows promising results compared with previously reported other microextraction methods. An important advantage of the present work over other microextraction techniques [188, 193] that it is simple, solvent-free preconcentration system, with high precision, and low detection limits.

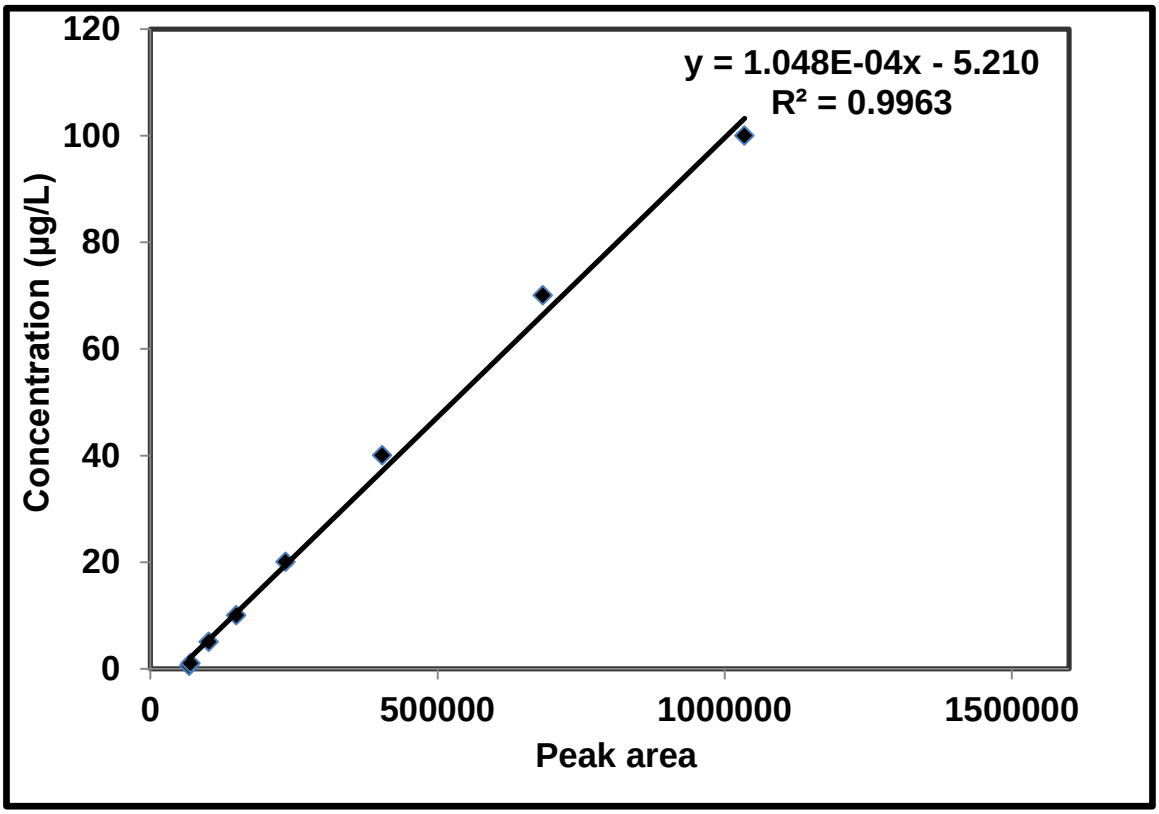


Figure 44: Calibration plot for BCEE at concentrations of 0.05-100 µg/L

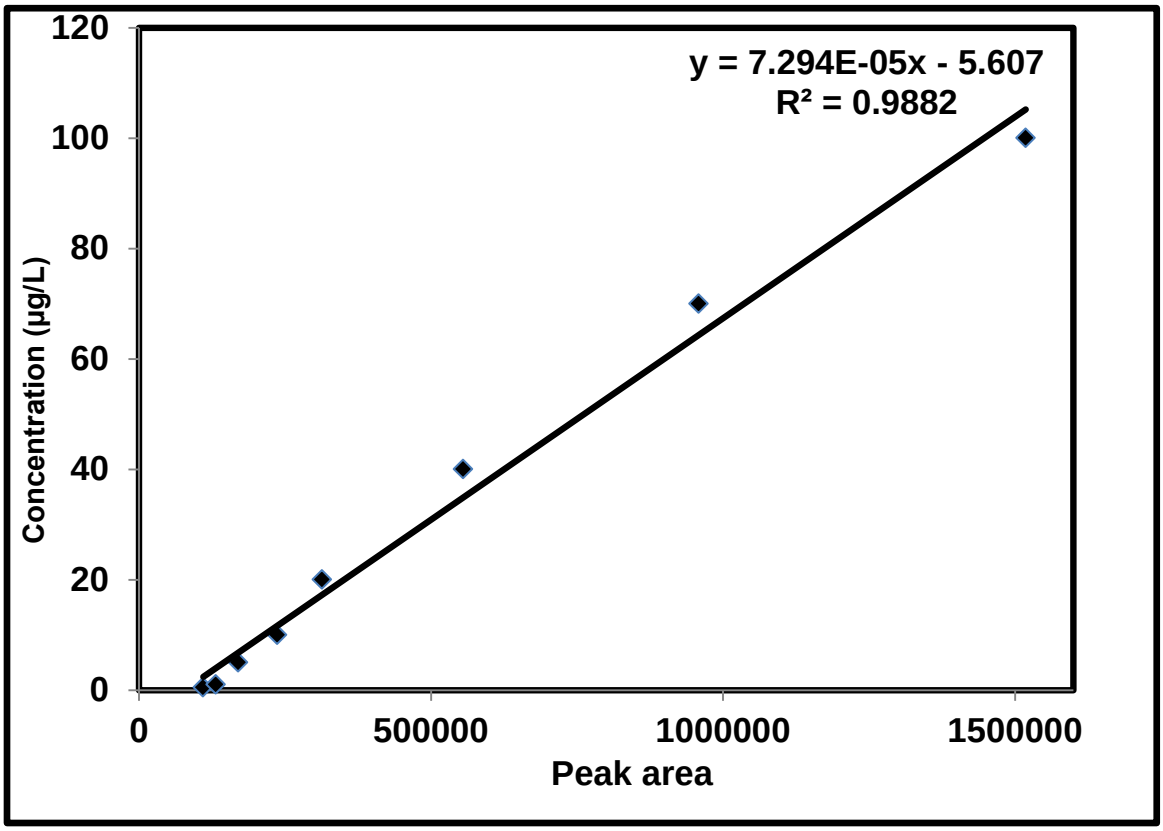


Figure 45: Calibration plot for BCIE at concentrations of 0.05-100 µg/L

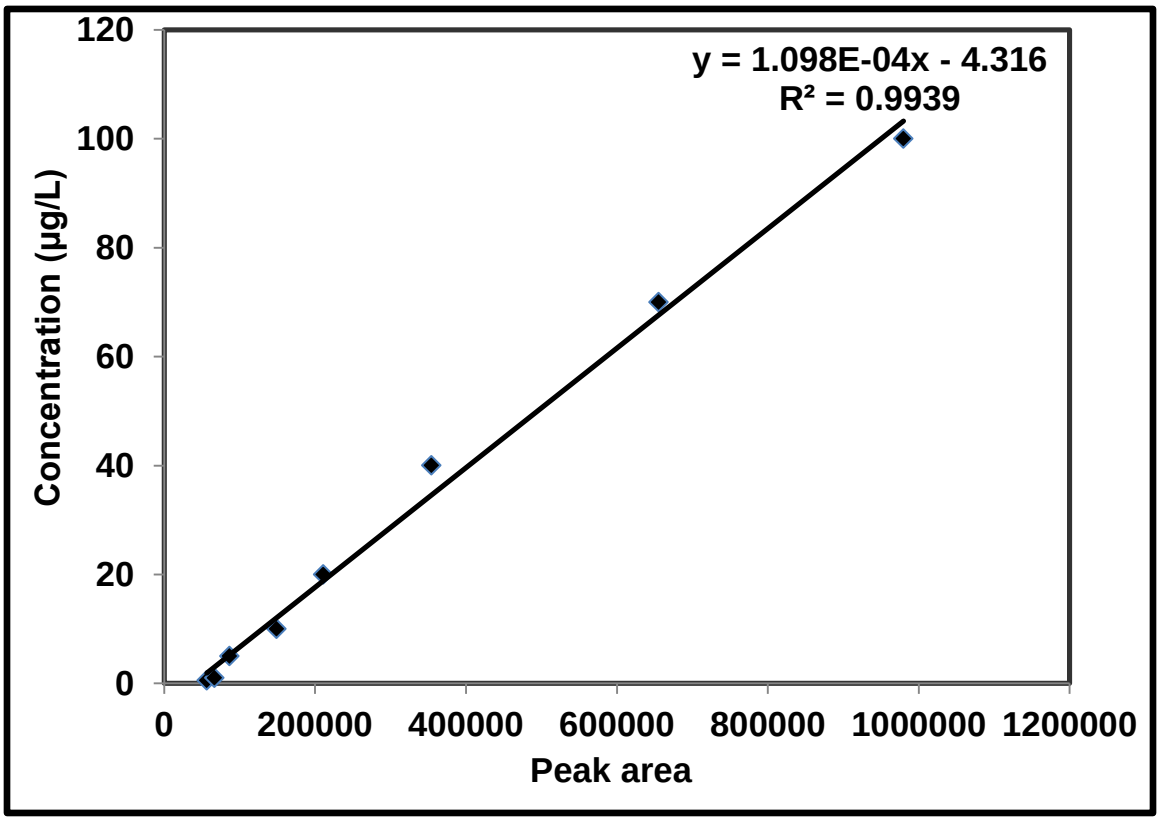


Figure 46: Calibration plot for BCEM at concentrations of 0.05-100 µg/L

Table 31: Feature of the full-automated FA-SPME. Linear range, correlation coefficient (r), linear equations, % RSD, LODs.

Compound	Linearity range µg L^{-1}	r	Equation	RSDs, % (n = 3)	LODs µg L^{-1}
BCEE	0.5 - 100	0.9981	y = 1.048E-04x - 5.210	6.2	0.041
BCIE	0.5 - 100	0.9941	y = 7.294E-05x - 5.607	1.2	0.053
BCEM	0.5 - 100	0.9969	y = 1.098E-04x - 4.316	2.3	0.017

Table 32: Comparison of the proposed method with other previously reported.

Analytical technique	**Sample**	**% Salt** g L^{-1}	**Extraction time (min)**	**% RSDs**	**LODs** µg L^{-1}	**% Recovery**	**Ref-**
HF[b]-LPME/GC-FID	Water	0	30	10.8-11.5	4.28-4.3	93-95	186
HF-LPME/GC-ECD[c]	Water	0	30	8.4-9.7	0.25-0.33	93-95	188
SPME/GC-FID	Water	-[e]	10	10 13	0.82-480	-[e]	188
SPE/GC-FID[a]	Water	0	80	0.9-6.5	0.001-0.003	73.4-80.9	193
LPE/GC-FID	Water	0	30	0.3-4.9	0.1-0.3	34.4-48.5	193
SPME/GC-MS	Water	0	10	-[e]	0.18-0.22	-[e]	193
SPME/GC-FID	Water	35	30	2-2.2	0.7-1.2	-[e]	9
FA-SPME/ GC-MS	water	10	10	1.2-6.2	0.017-0.053	88.2-107.7	Present
FA-SPME/ GC-MS	Urine	10	10	1.2-6.3	0.017-0.053	92.6-106.2	Present

a) Flame ionization detector, b) Hallow fiber assisted liquid phase microextraction c) Electro capture detector, e) not determined in this work

6.3.8 Application to Real Water Samples

The applicability of the proposed FA-SPME technique for real water and urine sample matrices were evaluated. Dilution of the urine sample (1:1 ratio of dilution with ultrapure water) was carried out prior to the fully automated extraction. Concentrations of CEs in water and urine samples are shown in Table 33.

Table 33: The concentration of CEs in real samples determined by proposed method.

	Drinking water		Tap water		Human urine	
Cpds.	**µg L^{-1}**	**RSDs, %**	**µg L^{-1}**	**RSDs, %**	**µg L^{-1}**	**RSDs, %**
BCEE	5.5	0.54	3.1	0.35	30.8	4.6
BCIE	7.4	0.39	4.3	0.85	48.26	2.1
BCEM	6	0.12	7.6	0.83	11.5	3.88

To evaluate the matrix effects, one of the water and urine samples were spiked and recoveries were calculated based on standard addition method and shown in Table 34. The data clearly shows high recovery, with % RSDs less than 10%. The excellent results demonstrated that the matrix effect had a negligible effect on FA-SPME.

Table 34: Extraction recovery of CEs from water and human urine samples spiked by (5 and 20 µg L^{-1}) using full-automated flow system FA-SPME/ GC-MS.

	% Recovery ± RSDs, %					
	Drinking water		Tap water		Human urine	
Cpds.	5 µg L^{-1}	20 µg L^{-1}	5 µg L^{-1}	20 µg L^{-1}	5 µg L^{-1}	20 µg L^{-1}
BCEE	98.4 ± 1.3	95.1 ± 4.1	101.6 ± 6.9	92.7 ± 6.2	105.7 ± 8.1	98.8 ± 5.7
BCIE	107.7 ± 3.6	91.6 ± 2.0	88.2 ± 4.2	94 ± 4.5	97.8 ± 1.0	92.6 ± 2.7
BCEM	104.9 ± 10	90.7 ± 3.0	93.4 ± 2.5	98.5 ± 5.7	95 ± 4.0	106.2 ± 7.6

Figure 47 shows the GC-MS chromatograms of extract from real water and urine samples and their respective spiked samples (at 5 and 20 µg L^{-1}). Direct extraction of urine sample could conceivably pose problems due to its complex sample matrix. The reason for dilution the urine samples in this work were increase the sample volume and to prevent the contamination of SPME fiber and increase the life of the fibers. Main objective of this work was on the fully automation of FA-SPME procedure and its applicability to large volume sample. This has clearly been demonstrated.

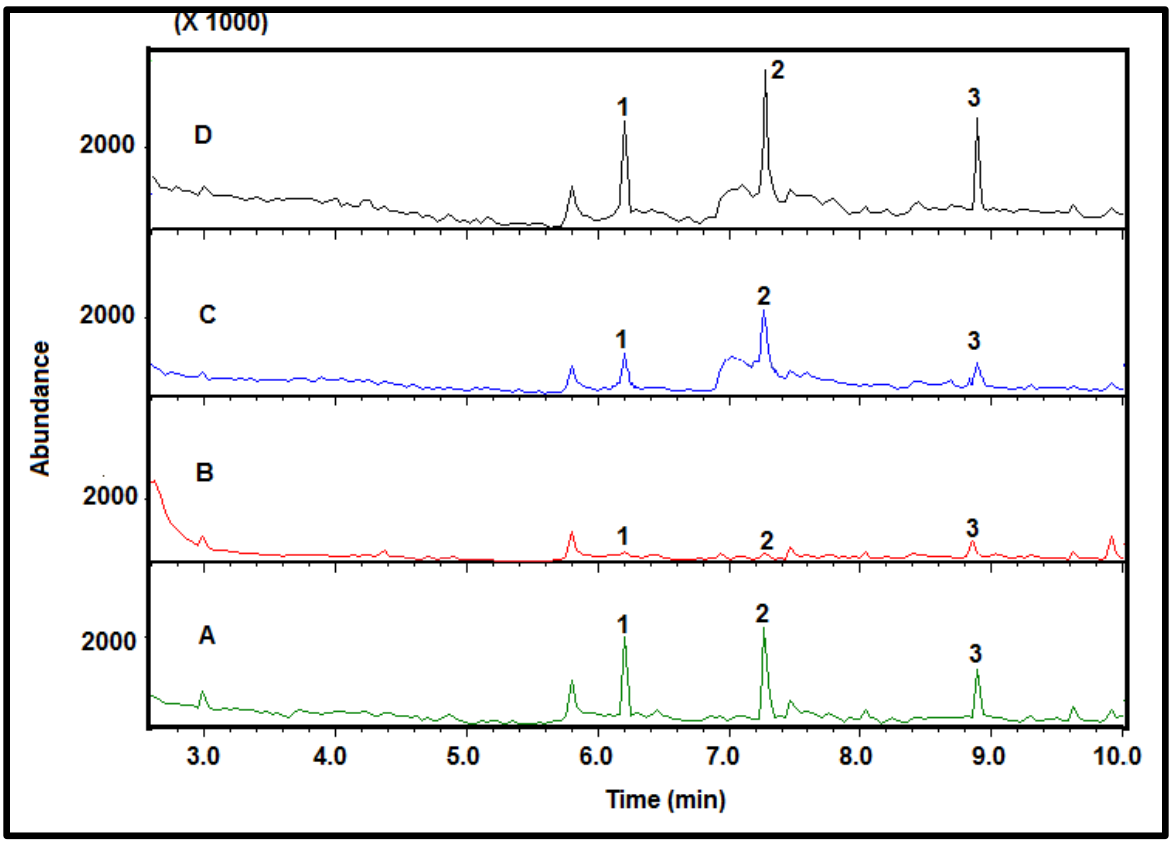


Figure 47: Total ion chromatograms of Three CEs in real samples extracted by fully automated FA-SPME/GC-MS (A: drinking water spiked with 20 µg/L of CEs, B: unspiked drinking water, C: unspiked urine, D: urine sample spiked with 20 µg/L of CEs, peak identifications; 1: BCEE, 2; BCIE, 3: BCEM).

6.4 CONCLUSION

A novel fully automated flow assisted-SPME (FA-SPME) method was developed for the convenient analysis of chloroethers in large volume water and urine samples. With the use of a CTC CombiPal autosampler the fully automated SPME was enabled that allowed sample extraction, injection, and SPME fiber conditioning to be carried out completely automatically. This method provides satisfactory analyte enrichment, sensitivity, and reproducibility and suitable for real water and urine samples and offers the potential of implementing a fully automatic onsite sample preparation GC-MS platform. Moreover, the use of FA-SPME instead of conventional agitation-SPME provided high sensitivity for the determination of CEs in large volume real samples. This automated flow assisted-SPME approach demonstrated the feasibility of a complete analytical system comprising sample preparation and GC-MS that might be operated onsite, fully automatically without human intervention.

7. GENERAL CONCLUSION

In this work, independent conventional, automated and fully automated methods for determination of organic contaminants such as phthalate esters (PAEs), Bis-phenol A (BPA), N-Nitrosoamines (NAs) and Chloroethers (CEs) water samples, using Dispersive liquid-liquid microextraction (DLLME) and solid phase micro extraction (SPME) as the extraction techniques coupled with gas chromatography mass spectrometry (GC-MS) were developed and validated.

7.1 Phthalate Esters (PAEs)

i. Xylene-methanol combination organic solvents were used in DLLME for extraction and determination of PAEs in six brand of drinking water sample in Saudi Arabia.

ii. DLLME used to study the leaching profile of PAEs from plastic bottles to water samples.

iii. Different factors controlling the extraction procedures were investigated. Results have shown that volume of dispersive solvent, extraction time, pH and salt addition could also have significant effect on extraction enrichment factor.

iv. Comparison results have shown that this method could serve as a less costly and more viable alternative to other techniques that use large amount of organic solvents for the extraction.

v. The method was successfully applied to the determination of PAEs and to study the leaching profile of PAEs in drinking water samples with promising results.

7.2 Endocrine Disruption Compounds (EDCs)

i. PAEs and BPA used in plastic manufactues to improve physical properites and classified as EDCs.

ii. In this investigation, we have developed a new simple and efficient method called electro enhanced solid phase phase microextraction (EE-SPME) coupled with GC-MS for the analysis of EDCs in seawater and human blood samples.

iii. Applied of positive potentials made the fiber coating positively charged and therefor enhanced the extraction of EDCs via electrophoresis and complementary charged interaction.

iv. Various factors governing extraction have been studied. Results obtained indicate the optimized conditions as 20 min extraction time and 5% (m/V) salt additions enhanced extraction recoveries obtained at 32 V applied potential.

v. Detector response was found linear within the range of 1-100 $\mu g l^{-1}$ of the analytes, with R^2 values that signify a very good correlation.

vi. These performances and all other appraisal indices such as LOD, relative recovery and %RSD indicate the suitable applicability of the present method in the analysis of real seawater and human blood samples.

7.3 N-Nitrosoamine (NAs)

i. A two automated extraction methods (HS-SPME/GC-MS and DLLME/GC-MS) were developed using CombiPal autosampler for the first time to simultaneous determination of the NAs in groundwater samples.

ii. A multi-variate statistical modeling technique was used to evaluate the response of each HS-SPME and DLLME parameters.

iii. A Box-Behnken design (BBD) with response surface methodology (RSM) was used for developing nonlinear models with the aid of statistical Design Expert 8.0.

iv. The extraction condition of NAs in each method was optimized via RSM.

v. The automated HS-SPME and DLLME, displayed good linearity between a wide range of concentrations and is characterized by low LODs in (ng/L) which would allow sensitive determinations of these analytes at their low concentrations in the water sample.

vi. The HS-SPME method was shown to be efficient, simple and environmentally friendly more than DLLME.

7.4 Chloroethers (CEs)

i. A novel fully automated method developed called flow assisted-solid phase microextraction (FA-SPME) coupled with GC-MS by using CTC CombiPal autosampler for the large sample volume and suitable for on-site extraction of three chloroethers (CEs) contaminants in water and human urine samples.

ii. Various factors that provide high extraction efficiency were optimized type of SPME fiber, absorption time, desorption time, pump flow rate, ionic strength and sample pH.

iii. This method displayed good linearity between (0.5-100 µg L^{-1}) concentrations, correlation coefficient (0.9941-0.9981) and is characterized by low LODs range (0.017-0.053 µg L^{-1}) which would allow sensitive determinations of these analytes at their low concentrations in the natural samples.

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VITAE

Name MOUSA YASIR MOUSAAMAYREH

Nationality JORDANIAN

Date of Birth 03/02/1979

Email Amayreh.Mousa@yahoo.com

Address Chemistry Department, KFUPM, Dhahran, Saudi Arabia

Academic Background:

- **(Jun, 1997)** Arab Institute high school: Certificate of Secondary Education-Tawjihi) in Science) Al-Quds University, Palestine.
- **(Jun, 2001)** BSc. in chemistry and chemical technology ,Department of Chemistry, Faculty of Science, Al-Quds University, Palestine.
- **(Sep , 2001-Sep, 2003)** Chemistry teacher in secondary school, Dora, Hebron, Palestine.
- **(Sep, 2003- Sep, 2006)** Research Assistant and Masters student at the Department of Chemistry and Chemical Technology, Faculty of Science, Al-Quds University, Palestine.
- **(Feb, 2007)** M.Sc. in Applied and Industrial Technology, Faculty of Science and Technology Deanship of Graduate Studies, Al-Quds University, Palestine. Master thesis title: "Synthesis of New Swellable Dithiocarbamate Functionalized

Polymer Microspheres Containing a Variety of Amines and Study of their Selective Optical Sensing Properties*"*.

- **(March, 2007-Sept. 2010)** Instructor in the Department of Agriculture, Al-Quds Open University-part time and chemistry teacher in secondary school, Dora, Hebron, Palestine.
- **(Sept. 2010)** joined the Chemistry Department, KFUPM, Dhahran, Saudi Arabia, in pursuit of PhD Degree in Analytical Chemistry. Thesis Title: "Development of automated on-site analytical methods for water analysis".
- **Published papers:**
  1. **Amayreh, M.**, Chanbasha, B., Al-Arfaj, A. R, "Determination of phthalate esters in bottled water using dispersive liquid–liquid microextraction coupled with GC-MS" J. Sep. Sci. 2013, 36, 2003–2009.
  2. **Amayreh, M.**, Chanbasha, B., Al-Arfaj, A. R," Application of electro-enhanced solid-phase microextraction for determination of phthalate esters and bisphenol A in blood and seawater samples" Talanta, 2013, 115, 308–313.
- **Submitted papers**:
  1. **Amayreh, M**., Chanbasha, B., "Application of an automated headspace solid-phase microextraction for the determination of N-nitrosamines in groundwater samples. *J. Sep. Sci.2013*.
  2. **Amayreh, M.**, Chanbasha, B., Nuhu, D. M., "Automated Determination of N-nitrosoamine in Water in Saudi Arabia by Dispersive Liquid - Liquid Microextraction Coupled with Gas Chromatography Mass Spectrometry" Journal of Analytical Chemistry, 2013.

3. **Amayreh, M.,** Chanbasha, B., Fully automated flow assisted solid-phase microextraction for the determination of chloroethers in water and urine samples, Journal of Analytical Chemi. Acta., 2013)

- **Patent (Filed Submitted):**
  1. **Amayreh, M.**, Chanbasha, B., "Application of Electro enhanced solid phase microextraction method" US Patent Pending, No: 14/024,472.
  2. **Amayreh, M**., Chanbasha, B., "Automated Determination of N-nitrosoamine in Water in Saudi Arabia by DLLME Coupled with GCMS".
  3. **Amayreh, M**., Chanbasha, B., "Fully automated analytical method for determination of chloroethers in water and urine samples".
  4. **Amayreh, M**., Chanbasha, B., "Automated microextraction technique for the analysis of N-nitrosamines in water".
- **Conference:**
  1. **Poster Presentations,** April, 2013: "Application of electro-enhanced solid-phase microextraction for determination of phthalate esters and bisphenol A in blood and seawater samples" The Fourth Saudi Student Conference 2013 (at Umm Al-Qura University, Saudi Arabia). I got a 3ed prize for nice scientific presentation from ministry of education and from KFUPM.
  2. **Oral Presentation,** February, 2013: "Determination of phthalate esters in bottled water using DLLME coupled with GC-MS" 4th International Conference for Young Chemists (2013, University Sains Malaysia, Malaysia).