การตรวจวัดเอ็น-ไนโตรซามีนในเครื่องสำอางโดยเฮดสเปซโซลิดเฟสไมโครเอกซ์แทร็กชันแก๊สโครมาโทกราฟี

นางสาววงเดือน นาคนิยม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2553

DETERMINATION OF *N*-NITROSAMINE IN COSMETICS BY HEADSPACE SOLID PHASE MICROEXTRACTION GAS CHROMATOGRAPHY

Miss Wongduan Nakniyom

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University
Academic Year 2010

Thesis Title	DETERMINATION OF *N*-NITROSAMINE IN COSMETICS BY HEADSPACE SOLID PHASE MICROEXTRACTION GAS CHROMATOGRAPHY
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วงเดือน นาคนิยม : การตรวจวัดเอ็น-ไนโตรซามีนในเครื่องสำอางโดยเฮดสเปซโซลิดเฟสไมโครเอกซ์แทร็กชันแก๊สโครมาโทกราฟี. (DETERMINATION OF *N*-NITROSAMINE IN COSMETICS BY HEADSPACE SOLID PHASE MICROEXTRACTION GAS CHROMATOGRAPHY) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.อรุณศิริ ชิตางกูร, 48 หน้า.

เอ็น-ไนโตรซามีน เป็นสารก่อมะเร็งในสัตว์ทดลองและจัดเป็นวัตถุห้ามใช้เป็นส่วนผสมในการผลิตเครื่องสำอางตามประกาศกระทรวงสาธารณสุข งานวิจัยนี้ได้พัฒนาวิธีตรวจวัดเอ็น-ไนโตรโซไดเมทิลเอมีน (NDMA) ด้วยแก๊สโครมาโทกราฟีแมสสเปกโทรเมตรี (GC-MS) ใช้คอลัมน์ชนิด DB-WAX (ยาว 30 เมตร เส้นผ่านศูนย์กลาง 0.25 มิลลิเมตร ฟิล์มหนา 0.25 ไมโครเมตร) และโปรแกรมอุณหภูมิจาก 60 ถึง 200 องศาเซลเซียส ใช้เทคนิคเฮดสเปซโซลิดเฟสไมโครเอกซ์แทร็กชัน (HS-SPME) ในการสกัดและเพิ่มความเข้มข้นของ NDMA ที่ระเหยได้ในตัวทำละลายน้ำ ศึกษาปัจจัยที่มีผลต่อการสกัดด้วย HS-SPME ได้แก่ ชนิดของไฟเบอร์ ความเข้มข้นของโซเดียมคลอไรด์ในสารละลาย ความเป็นกรด-ด่างของสารละลาย อุณหภูมิและเวลาในการสกัด พบว่าภาวะที่เหมาะสมคือใช้ไฟเบอร์ชนิด Carboxen/polydimethylsiloxane (CAR/PDMS) สำหรับขวดสารขนาด 40 มิลลิลิตร ใช้ปริมาตรสารละลายตัวอย่าง 5–10 มิลลิลิตร เติมโซเดียมคลอไรด์เข้มข้น 357 มิลลิกรัม/มิลลิลิตร ส่วนค่าความเป็นกรด-ด่างของสารละลายไม่ส่งผลต่อการสกัด และเลือกใช้อุณหภูมิการสกัด 65 องศาเซลเซียส เป็นเวลา 10 นาที ผลการทดสอบความถูกต้องของวิธี พบว่าความเข้มข้นที่ให้ความสัมพันธ์เป็นเส้นตรงอยู่ในช่วง 0.1–200 นาโนกรัม/มิลลิลิตร ขีดจำกัดของการตรวจพบ (LOD) 10 นาโนกรัม/กรัม ขีดจำกัดของการวัดเชิงปริมาณ (LOQ) 50 นาโนกรัม/กรัม ร้อยละของการคืนกลับอยู่ในช่วง 84–99 และความเที่ยงของการวิเคราะห์ซ้ำภายในวันเดียวกันมีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ ร้อยละ 3 วิธีที่พัฒนาขึ้นสามารถนำไปใช้ในการตรวจวัด NDMA ตัวอย่างแชมพู ได้

ภาควิชา .....เคมี..... ลายมือชื่อนิสิต .....

สาขาวิชา .....เคมี..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา .... 2553.....

507 26226 23 : MAJOR CHEMISTRY
KEYWORDS: *N*-NITROSAMINES / GAS CHROMATOGRAPHY / HEADSPACE SOLID-PHASE MICROEXTRACTION

WONGDUAN NAKNIYOM : DETERMINATION OF *N*-NITROSAMINE IN COSMETICS BY HEADSPACE SOLID PHASE MICROEXTRACTION GAS CHROMATOGRAPHY. ADVISOR : ASST. PROF. AROONSIRI SHITANGKOON, Ph.D., 48 pp.

N-Nitrosamines are carcinogen in testing animals and are prohibited to use in cosmetics as specified in Notification of Ministry of Public Health. This research has developed the method for determination of *N*-nitrosodimethylamine (NDMA) by gas chromatography-mass spectrometry (GCMS) using DB-WAX column (30 m long, 0.25 mm I.D., 0.25 μm film) with a temperature program from 60 to 200 °C. Headspace solid-phase microextraction (HS-SPME) was used for extraction and preconcentration of volatile NDMA in aqueous solutions. Factors affecting the extraction efficiency with HS-SPME were studied including type of fiber, concentration of NaCl in solution, pH of solution, extraction temperature and time. The carboxen/polydimethylsiloxane (CAR/PDMS) fiber was found to be suitable for the extraction of NDMA. For 40-mL vial, sample volume of 5–10 mL was used. NaCl concentration of 357 mg/mL was added, while pH of solution showed insignificant effect to extraction efficiency. Extraction temperature of 65 °C and extraction time of 10 min were selected. The validation results of the method showed good linearity in 0.1-200 ng/mL range; limit of detection (LOD) of 10 ng/g; limit of quantitation (LOQ) of 50 ng/g; recovery of 84–99 %; and repeatability of 3 % RSD. The developed method could be used to determine NDMA in shampoo.

Department : .....Chemistry.... Student's Signature .....W. Nakniyom.....
Field of Study : .....Chemistry.... Advisor's Signature .....A. Shitangkoon.....
Academic Year : .....2010.....

ACKNOWLEDGEMENTS

I would like to express my sincerest appreciation and deepest gratitude to my advisor, Assistant Professor Dr. Aroonsiri Shitangkoon for her perspective guidance, support, and critical proofreading throughout the research. I also would like to recognize my thesis committee, Assistant Professor Dr. Suchada Chuanuwatanakul, Dr. Amarawan Intasiri and Dr. Khamphee Phomphrai, for their comments and thesis revision.

This research was financially support by Bureau of Cosmetics and Hazardous Substances of Department of Medical Sciences, Ministry of Public Health and the Center for Petroleum, Petrochemicals, and Advanced Materials of Chulalongkorn University.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
°C	degree Celsius
CAR/PDMS	Carboxen/polydimethylsiloxane
CI-MS	chemical ionization mass spectrometry
cm	centimeter
cm/sec	centimeter/second
DAD	diode array detection
EI/CI	electron impact/chemical ionization
g	gram
GC	gas chromatography
GC-TEA	gas chromatography-thermal energy analyzer
GC-MS	gas chromatography-mass spectrometry
HS-SPME	headspace solid-phase microextraction
I.D.	internal diameter
LC	liquid chromatography
LC-MS-MS	liquid chromatography-mass spectrometry-mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
m	meter
mg	milligram
mg/mL	milligram per milliliter
min	minute
mL	milliliter
mm	millimeter
MSD	mass selective detector
MS	mass spectrometry
m/z	mass to charge ratio
n	replicate
ng	nanogram
ng/g	nanogram per gram

ng/L	nanogram per liter
ng/mL	nanogram per milliliter
NaCl	sodium chloride
NCD	nitrogen chemiluminescence detector
NDBA	*N*-nitrosodi-*n*-butylamine
NDEA	*N*-nitrosodiethylamine
NDELA	*N*-nitrosodiethanolamine
NDMA	*N*-nitrosodimethylamine
NDPA	*N*-nitroso-di-*n*-propylamine
NDPHLA	*N*-nitrosodiphenylamine
NMEA	*N*-nitrosomethylethylamine
NMOR	*N*-nitrosomorpholine
NPD	nitrogen phosphorus detector
NPIP	*N*-nitrosopiperidine
NPYR	*N*-nitrosopyrrolidine
PA	polyacrylate
PCI	positive chemical ionization
PDMS/DVB	polydimethylsiloxane/divinylbenzene
R^2	correlation of determination
rpm	round per minute
RSD	relative standard deviation
SIM	selected ion monitoring
S/N	signal to noise ratio
SPE	solid-phase extraction
SPME	solid-phase microextraction
UV	ultraviolet
%	percentage
µg	microgram
µL	microliter
µm	micrometer

CHAPTER I

INTRODUCTION

1.1 Problem definition

N-Nitrosamines or *N*-nitroso compounds are organic compounds with the general formula R_1R_2NNO (Figure 1.1). *N*-nitrosamines were found in a wide variety of products such as food, drugs, drinking water and cosmetics [1–6]. They are also widespread in the environment (water, soil and air) even though at low levels (ppb). *N*-Nitrosamines were proven to be carcinogenic in animals and they induced tumors in every animal species tested [7]. They are regarded as potential human carcinogens.

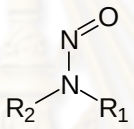


Figure 1.1 General structure of *N*-nitrosamine

N-Nitrosamines are formed by the reaction of amine precursors with nitrosating agents such as nitrite salts or nitrogen oxides. They also can be formed endogeneously in the human body from normal food or tobacco smoking. Exogenous exposure to *N*-nitrosamines can occur from various consumer products including food and drink, use of rubber products and cosmetics. In this study, *N*-nitrosamines in cosmetics are of concerned. *N*-nitrosamines in cosmetic product can either formed by reaction of precursors within the product itself or be a result of contaminated raw materials [7, 8].

Ministry of Public Health of Thailand has issued on the substances, including *N*-nitrosamines, not to be used as an ingredient in the manufacture of cosmetics [9]. In Thailand, people in the community have prepared cosmetics, such as hair shampoo and conditioner, and distributed them to the market as community products (OTOP products). Their manufacturing processes may not be under quality-controlled system or use under-qualified raw materials. A quick and simple analysis of *N*-nitrosamines in these community products is of interest.

1.2 Literature review

Examples from previous reports for the determination of *N*-nitrosamines in cosmetic products are as follows.

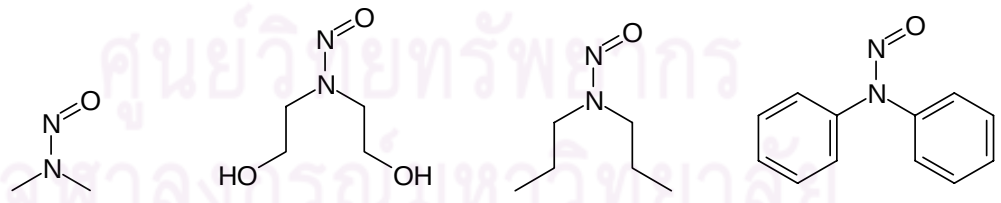
Matyska *et al*. [1] determined the presence of *N*-nitrosodiethanolamine (NDELA) in cosmetic samples including eye make-up remover, scented body lotion, hand cream and lipstick. NDELA was extracted from samples by liquid-liquid extraction with methylene chloride before analyzing with open-tubular capillary electrochromatography (OT-CEC) using a C18 modified etched capillary column of 50 µm I.D. and UV detection at 214 nm. In spiked samples, recoveries were 94 % for hand and body lotion and 55 % for lipstick. NDELA was also found in old cosmetics at 14.0–35.0 ppm level. A liquid-liquid extraction and OT-CEC analysis was easy for routine screening of NDELA in cosmetic samples.

Schothorst and Stephany [2] developed a gas chromatographic (GC) method with thermal energy analyzer (TEA) for determination of NDELA in cosmetics without derivatization. The commercially available samples included shower gel, hair gel, shampoo and conditioner, body lotion, cream, foam bath, etc. The samples were cleanup with a silica gel column and eluted with appropriate solvents. GC-TEA analyses were done using Carbowax Amine column (30 m, 0.53 mm I.D., 1 µm film) and temperature program from 60 to 200 °C. The limit of quantification was 5.3 µg/kg and the average recovery for NDELA was 99 % (range 86–112 %). Seven out of 25 cosmetic products contained NDELA above the limit of quantification of 5.3 µg/kg.

Schothorst and Somers [3] have developed a liquid chromatography-mass spectrometry (LC-MS-MS) method for determination of NDELA in various kinds of cosmetics: shower gels, hair oil, shampoos and conditioners, cream and foam baths, soap and body washes, etc. Sample preparation was done by solid-phase extraction (SPE) cleanup with Bakerbond C18 column or dichloromethane cleanup depending on sample characteristics. LC-MS-MS analysis was performed using Spherisorb ODS II column (150 mm × 4.6 mm I.D.); methanol-ammonium acetate in water as mobile

phases under gradient condition; and triple quadrupole mass spectrometer with electrospray. The method gave limit of detection of 22.8 µg/kg; limit of quantification of 45.6 µg/kg; repeatability of 7.6 %; and linearity in the range of 0–1600 µg/kg. The average recoveries were 88.3 % (range 48.3–112.7 %) for SPE cleanup and 52.2 % (range 41.3–60.3 %) for dichloromethane cleanup. The method was applied to quantify NDELA content in 140 cosmetic samples. NDELA was found in 35 products at 23 to 992 µg/kg level.

Wang *et al*. [10] developed a screening method based on LC with diode array detection (DAD) for simultaneous detection of five *N*-nitrosamines in cosmetic products such as hair shampoo, foam bath, shower gel, and washing cream. Five *N*-nitrosamines include NDELA, *N*-nitrosodimethyamine (NDMA), *N*-nitrosodi-*n*-propylamine (NDPA), *N*-nitrosodiphenylamine (NDPHLA), and *N*-nitroso-bis-(2-hydroxypropyl)amine. The samples were dissolved in ethyl acetate before cleanup with a silica gel column. The LC analyses were performed using a Phenomenex Luna CN column (250 mm × 4.6 mm I.D.); methanol-phosphate buffer as mobile phases under gradient condition. The method gave limit of detection of 0.02–0.03 mg/L and linearity in the range of 0.2–100 mg/L. Recoveries of greater than 96 % were found in most cosmetic products. Analyses of real samples showed that the mean concentrations of NDELA, NBHPA, NDPLA, and NDPHLA were 1.26, 1.49, 3.43, and 2.49 mg/L respectively.



NDMA NDELA NDPA NDPHLA

Figure 1.2 Structures of some *N*-nitrosamines studied in cosmetics [1–3, 10]

The abovementioned sample preparation techniques for determination of *N*-nitrosamines in cosmetic products were mostly liquid-liquid extraction with organic solvent or cleanup with SPE or silica gel column. Those techniques require the use of

organic solvents and waste management. Solid-phase microextraction (SPME) is another sample preparation technique that requires no organic solvent. SPME extracts and preconcentrates analytes in one single step. In addition, SPME device is compatible with GC analysis. Examples of previous reports on the use of SPME for the determination of *N*-nitrosamines in other samples are as follows.

Andrade *et al*. [4] developed a method for determination of volatile *N*-nitrosamines in sausages using SPME with headspace sampling (HS) and GC analysis with TEA detection. Four *N*-nitrosamines were studied including NDMA, *N*-nitrosodiethyamine (NDEA), *N*-nitrosopiperidine (NPIP), and *N*-nitrosopyrrolidine (NPYR). Several parameters associated with SPME efficiency were optimized: type of fiber coating, ionic strength of solution, extraction temperature and time. GC-TEA analyses were performed using HP-INOWAX megabore column (30 m, 0.53 mm I.D., 1 µm film) and temperature program from 100 to 160 °C. The method gave limit of detection of 3 µg/kg and limit of quantification of 10 µg/kg. The average recoveries ranged from 105–110 % for all four *N*-nitrosamines. Determination of *N*-nitrosamines in sausage samples were carried out by standard addition because the sample matrices are quite complex. Only NDMA was found in the samples at 15.0 and 43.5 µg/kg level, above the enforcement level of 10 µg/kg.

Grebel *et al*. [6] developed a SPME method for extraction of seven *N*-nitrosamines from water and analyzed by GC. Seven *N*-nitrosamines included NDMA, NDEA, *N*-nitrosomethylethylamine (NMEA), NDPA, *N*-nitrosodi-*n*-butylamine (NDBA), *N*-nitrosomorpholine (NMOR), NPIP, and NPYR. Several parameters related to SPME efficiency were optimized: type of fiber coating, NaCl concentration, pH of solution, extraction temperature and time. GC analyses were done on DB-1701 column (30 m, 0.32 mm I.D., 1 µm film) with temperature program from 40 to 250 °C. Different types of detectors were used including nitrogen phosphorus detector (NPD), nitrogen chemiluminescence detector (NCD) and chemical ionization mass spectrometry (CI-MS). It was found that method detection limit for NDMA ranged from 30 to 890 ng/L depending on the detector type, with CI-MS giving the lowest detection limit. However, good detection limit and acceptable recovery were obtained with NCD.

1.3 Objective

The objective of this research is to apply a simple and solvent-free sample preparation technique based on SPME to extract *N*-nitrosodimethyamine (NDMA) from cosmetic product, shampoo, before analyzing with GC-MS. NDMA was selected as it is volatile and highly toxic among other *N*-nitrosamines found in shampoo. GC-MS conditions and SPME parameters were optimized and validated before applying the method to real samples.

CHAPTER II

THEORY

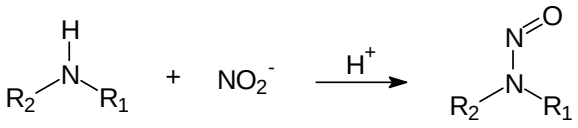
2.1 *N*-Nitrosamines

N-Nitrosamines or *N*-nitroso compounds are organic compounds with the general formula R_1R_2NNO. *N*-Nitrosamines are classified into four groups [10]:

- volatile *N*-nitroso compounds e.g. *N*-nitrosodimethyamine (NDMA), *N*-nitrosodi-*n*-propylamine (NDPA)
- low polarity, non-volatile *N*-nitroso compounds e.g. *N*-nitrosodiphenylamine (NDPHLA)
- high polarity, non-ionic, non-volatile *N*-nitroso compounds e.g. *N*-nitrosodiethanolamine (NDELA)
- high polarity, ionic, non-volatile *N*-nitroso compounds e.g. *N*-nitrosoproline

2.1.1 Formation of *N*-nitrosamines [7, 8, 11]

N-Nitrosamines are formed by the reaction of amines with a nitrosating agent (N_2O_3). The source of a nitrosating agent can be nitrites (NO_2^-), nitrates (NO_3^-), or nitrocompounds. In laboratory, *N*-nitrosamines are synthesized from the reaction of a secondary amine with acidic nitrite at ~ pH 3. Primary and tertiary amines also form *N*-nitrosamines; however, with slower rate and lower yield than secondary amines. The formation of *N*-nitrosamines depends on pH of solution, alkalinity of amine, and temperature. Primary aliphatic and aromatic amines do not form *N*-nitrosamines at low pH and low temperature. The rate of formation of *N*-nitrosamines from secondary amines increases proportionally with a decrease in alkalinity of amines.



N-Nitrosamines contamination in cosmetic products may result from the use of contaminated raw materials or the nitrosation of precursors present within the finished product itself [10, 12]. Sources of nitrite include natural occurrence in

water, high nitrate foods, calcined inorganic materials and pigments, and the decomposition of some preservatives. Some secondary amines usually found as contaminants or decomposition products in raw materials of cosmetics include diethanolamine, diisopropanolamine, dimethylamine and long chain methylamine, and morpholine.

2.1.2 Toxicity [7, 8]

Many *N*-nitrosamines are toxic to animals and cell in culture. NDMA is known to be acutely toxic to the liver in humans. Human acute and subacute exposure to NDMA can result liver damage, diffuse bleeding, edema, inflammation and death. These effects closely resemble those observed in animals dosed with NDMA.

A number of *N*-nitrosamines have been tested for carcinogenicity and most of them are carcinogenic, although the potency varies considerably. For example, the mean dose for the formation of tumors by NDEA is only ~ 0.0006 mol/kg body weight, while *N*-nitrosoproline is considered noncarcinogenic. Table 2.1 demonstrated the carcinogenic potency of some *N*-nitrosamines.

Table 2.1 Carcinogenic potency and main target organ in the rat of some *N*-nitrosamines [8]

N-nitrosamine	carcinogenic activity	target organ
N-nitrosodimethylamine (NDMA)	high	liver
N-nitrosodiethylamine (NDEA)	high	liver
N-nitrosodi-*n*-propylamine (NDPA)	high	liver
N-nitroso-iso-propylamine	low	liver
N-nitrosodibutylamine	medium	liver (bladder)
N-nitrosomethylpentylamine	high	esophagus
N-nitrosomethylbenzylamine	high	esophagus
N-nitrosomorpholine (NMOR)	high	liver
N-nitrosopiperazine	high	esophagus

2.2 Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) is one of the hyphenated analytical techniques. As the name implies, it is actually two techniques that are combined to form a single method of analyzing mixtures of chemicals. GC separates the components of a mixture and MS characterizes each of the components individually. By combining the two techniques, an analytical chemist can both qualitatively and quantitatively evaluate a sample containing a number of chemicals.

2.2.1 Gas chromatography

In general, chromatography is used to separate mixtures of chemicals into individual components. Once isolated, the components can be evaluated individually.

In GC, separation occurs when the sample mixture is introduced (injected) into a mobile phase, which is an inert gas such as helium, hydrogen or nitrogen. The mobile phase carries the sample mixture through a stationary phase, held in a column. The capillary column is held in a thermostatted oven. The oven temperature can be kept constant or programmed to increase the temperature gradually (or ramped) depending on the type of analytes. As the temperature increases, compounds with low boiling points elute from the column sooner than those with higher boiling points. Therefore, there are actually two distinct separating factors: temperature and stationary phase interactions. As the compounds are separated, they elute from the column and enter a detector. The time from when the injection is made (time zero) to when elution occurs is referred to as the retention time.

2.2.2 Mass spectrometry

As the individual compounds elute from the GC column, they enter the electron ionization detector. They are bombarded with a stream of electrons causing them to break apart into fragments. These fragments can be large or small pieces of the original molecules. The fragments are actually charged ions with a certain mass. The mass of the fragment divided by the charge is called the mass to charge ratio (m/z), usually represents the molecular weight of the fragment.

The mass spectrum produced by a given chemical compound is essentially the same every time. Therefore, the mass spectrum is essentially a fingerprint for the molecule. This fingerprint can be used to identify the compound from the GC-MS spectra library. The library compares the mass spectrum from a sample component and compares it to mass spectra in the library. It reports a list of possible compounds along with the statistical probability of the match.

2.3 Solid-phase microextraction [13, 14]

Sample preparation is one of the crucial steps in the analytical procedure. For samples with complicated matrix which cannot be analyzed directly by the instruments, a sample preparation is necessary to isolate the analytes of interest from the sample matrix. Sample preparation usually includes cleanup, isolation, and preconcentration. The principle of sample preparation technique is based on partition of analytes between a sample matrix and an extracting phase. Most sample preparation techniques, such as liquid-liquid extraction, solid phase extraction (SPE), or silica gel column, require the use of organic solvents or reagents, which subsequently create chemical waste.

Solid-phase microextraction (SPME), on the other hand, is a solventless sample preparation technique. An SPME unit is liked a syringe. It consists of an SPME holder and a fused silica fiber coated with polymer or polymer mixed with a solid adsorbent acting as absorbing material. The fiber is attached to a stainless steel plunger protected inside the syringe needle. SPME can be used to extract and concentrate volatile, semivolatile or nonvolatile compounds from gas, liquid, or solid samples to be subsequently analyzed by chromatographic system. SPME is relatively simple as it could extract and concentrate analytes in one single step.

Extraction with SPME for GC analysis involves two steps: extraction and desorption. For extraction, an SPME needle is passed through a vial septum. The SPME plunger was depressed to expose the fiber to extract analytes onto the fiber. After specified extraction time, the fiber was retracted into the needle and the SPME syringe was removed from the vial. For analysis by GC, the SPME needle was pierced

through the GC injector. The fiber was exposed for a specified time to desorb the analytes into the injector before retracting the fiber and removing the syringe from the injector. SPME extraction can be performed either by direct extraction where the fiber is immersed in the sample matrix or by headspace (HS) extraction where the fiber is placed in the gas phase above the sample.

2.3.1 Principle of SPME

When the polymer coating is in contact with the sample matrix, the transport of analytes from the matrix to the coating begins. Complete analyte extraction from the sample matrix is not possible by SPME. SPME extraction is regarded as completion when the analyte concentration has reached distribution equilibrium; therefore, the extracted amount is not significantly varied by an increase of extraction time. Samples are generally analyzed after the equilibrium is reached or at a specified time prior to equilibrium. The equilibrium condition can be described by

$$C_o\ V_s = C_s^{\infty}\ V_s + C_f^{\infty}\ V_f$$

Where C_o is initial concentration of analyte in the sample

C_s^{∞} is equilibrium concentration in the sample

C_f^{∞} is equilibrium concentration in the fiber

V_s is volume of sample

V_f is volume of fiber coating

The distribution coefficient of the analyte between sample matrix and fiber coating (K_{fs}) is defined by

$$K_{fs} = \frac{C_f^{\infty}\ V_f}{C_s^{\infty}\ V_s}$$

At equilibrium, the number of mole of analyte extracted (n) by the coating can be described by

$$n = \frac{K_{fs}\ V_f\ V_s\ C_o}{K_{fs}\ V_f + V_s}$$

If the sample volume is very large ($K_{fs}\ V_f << V_s$), then $n = K_{fs}\ V_f\ C_o$ and the amount of extracted analyte is independent of sample volume.

For headspace SPME (HS-SPME), the extraction depends on the partition of analyte between sample, headspace, and fiber phases. The number of mole of analyte extracted is modified to

$$n = \frac{K_{fs}\, V_f\, V_s\, C_o}{K_{fs}\, V_f + K_{hs}\, V_h + V_s}$$

Where K_{hs} is the distribution coefficient of the analyte between headspace and sample matrix

V_h is the volume of headspace

2.3.2 Parameters affecting extraction efficiency

There are several parameters affecting extraction efficiency by HS-SPME such as type of fiber coating, salt concentration, pH of solution, and extraction temperature.

- ***fiber coating***

The selection of fiber coating is based on the polarity and volatility of analytes. Several types of fiber coating are now commercially available.

- polydimethylsiloxane (PDMS): a nonpolar liquid phase and is available in 7, 30, and 100 μm film thickness. Analyses of low molecular weight or volatile compounds require 100 μm film thickness, while 7 or 30 μm film thickness is used for large molecular weight or semivolatile compounds.
- polyacrylate (PA): a polar liquid phase and is suitable for extraction of polar semivolatile compounds.
- polydimethylsiloxane/divinylbenzene (PDMS/DVB): is good for volatile polar analytes such as alcohols and amines.
- Carboxen/polydimethylsiloxane (CAR/PDMS): is good for gases and low molecular weight compounds.
- Carbowax/divinylbenzene (CW/DVB): is good for alcohols and polar compounds.

- ***salt concentration***

Addition of salt to aqueous samples generally increases the distribution coefficient of neutral organic compounds between fiber and sample, resulting in an increase in the amount of analyte extracted.

- ***pH of solution***

Solution pH could affect the dissociation equilibrium in aqueous media for acidic and basic analytes. However, high and low pHs could damage the fiber coating in direct extraction approach. HS-SPME is suitable to use with pH-modified samples.

- ***extraction temperature***

At high temperatures, analytes can move into the headspace for effective extraction. However, the fiber/sample distribution coefficient also decreases with an increase of temperature.

CHAPTER III

EXPERIMENTAL

3.1 General apparatus and glassware

- analytical balance (4 digit) model LA 230 S, Sartorius AG, Germany
- analytical balance (2 digit) model BT 4202 S, Sartorius AG, Germany
- hot plate/stirrer model PC-420D, Corning, U.S.A.
- Milli-Q 185 Plus ultrapure water purification system, Millipore, U.S.A.
- vial (amber) with silicone/PTFE cap: size 8, 16, 40, 125, 250, 500 mL, Supelco, U.S.A.
- volumetric flask: size 10, 50, 100, 1000 mL
- volumetric pipet: size 0.5, 1, 2, 5, 10, 15, 20 mL

3.2 Instrumentation and equipments for GC-MS and SPME

3.2.1 GC-MS

- gas chromatograph mass spectrometry (GC-MS) system: Agilent 6890 N series gas chromatograph (GC) with Agilent 5975B series inert XL EI/CI mass selective detector (MSD), Agilent Technologies, U.S.A.
- DB-WAX capillary GC column, 30 m long, 0.25 mm I.D., 0.25 μm film, Agilent Technologies, U.S.A.

3.2.2 SPME

- 40 mL aluminum block vial holder (can hold six 40 mL vial for heating/stirring during SPME sampling), Supelco, U.S.A.
- SPME holder (or SPME syringe) for manual sampling, Supelco, U.S.A.
- SPME fibers (from Supelco, U.S.A.):
  - Carboxen/polydimethylsiloxane (CAR/PDMS), 75 μm
  - polyacrylate (PA), 85 μm
  - polydimethylsiloxane/divinylbenzene (PDMS/DVB), 65 μm

3.3 Chemicals

- buffer solutions of pH 4–12 (Radiometer Analytical, Germany)
    - pH 4.005 (potassium hydrogen phthalate 0.05 molal)
    - pH 7.000 (disodium hydrogen phosphate 0.0275 molal and potassium dihydrogen phosphate 0.0200 molal)
    - pH 10.012 (sodium hydrogen carbonate 0.025 molal and sodium carbonate 0.025 molal)
    - pH 12.45 (saturated calcium hydroxide)
- methanol, AR grade (Merck, Germany)
- *N*-nitrosodimethylamine (NDMA) standard solution in methanol, 100 µg/mL (Chem Service, U.S.A.)
- sodium chloride (NaCl), AR grade (Merck, Germany)

3.4 Preparation of NDMA standard solutions

- 10 µg/mL NDMA standard solution

  Pipet 1.00 mL of 100 µg/mL NDMA standard solution into 10 mL volumetric flask. Dilute to mark with methanol and mix well.
- 1000 ng/mL NDMA standard solution

  Pipet 1.00 mL of 10 µg/mL NDMA standard solution into 10 mL volumetric flask. Dilute to mark with methanol and mix well.
- 10 ng/mL NDMA standard solution

  Pipet 1.00 mL of 1000 ng/mL NDMA standard solution into 100 mL volumetric flask. Dilute to mark with Milli-Q water and mix well.

  To study the effect of pH of solution on HS-SPME, the NDMA solutions were diluted with buffer solutions of pH 4, 7, 10 or 12, instead of Milli-Q water.

3.5 GC-MS analyses

3.5.1 Initial HS-SPME condition of NDMA for GC-MS analysis

Twenty mL of 10 ng/mL NDMA standard solution was added into 40-mL amber vial, followed by the addition of 7.14 g NaCl and a magnetic bar. The vial

was closed and then was placed in an aluminum block which was placed on a hot plate/stirrer. The solution was simultaneously stirred at 650 rpm and heated to 65 °C. The SPME syringe needle, containing PDMS/DVB fiber, was pierced through the vial septum to the vial headspace. After stirring for 5 min, the SPME plunger was depressed to expose the fiber to headspace and to extract analytes onto the fiber. After 10 min extraction time, the fiber was retracted into the needle and the SPME syringe was removed from the vial. Then the SPME syringe needle was pierced through the GC injector. The fiber was exposed for 5 min before retracting the fiber and removing the syringe from the injector.

3.5.2 GCMS conditions

All GCMS analyses were performed using Agilent GC-MS system (6890 N GC with 5975B MSD) and DB-WAX capillary GC column with the condition provided in Table 3.1. The GC oven temperature was optimized for the analysis of NDMA.

Table 3.1 Initial GC-MS conditions for NDMA analysis

parameter	condition
GC:	
carrier gas	helium at 2 mL/min (52 cm/sec)
injector	splitless at 300 °C
splitless time	1 min
split vent	20 mL/min
MS:	
ion source	positive chemical ionization (PCI)
reagent gas	methane
MS source temperature	300 °C
MS interface temperature	250 °C
MS quadrupole temperature	150 °C
detection mode	selected ion monitoring (SIM) at m/z 75
electron multiplier (EM volt)	2200 volts

3.6 Optimization of HS-SPME conditions

General procedure for the optimization of HS-SPME conditions was similar to section 3.5.1. Several HS-SPME parameters were studied including: type of SPME fiber, NaCl concentration, pH of solution, sample volume, extraction temperature and extraction time.

3.6.1 Effect of type of SPME fiber

Twenty mL of 10 ng/mL NDMA standard solution was added into 40-mL amber vial, followed by the addition of 7.14 g NaCl (357 mg NaCl/mL solution). The solution was stirred at 650 rpm and the extraction temperature was set to 65 °C. After stirring for 5 minutes, the SPME plunger was depressed to expose the fiber. After 10 min extraction time, the SPME syringe was removed from the vial and injected into the GC-MS. Three types of SPME fibers were investigated: PDMS/DVB (65 μm), CAR/PDMS (75 μm) and PA (85 μm). The analyses were repeated three times (n = 3) for each type of fiber. The appropriate fiber will be selected and used for further studies.

3.6.2 Effect of NaCl concentration

Twenty mL of 10 ng/mL NDMA standard solution was added into 40-mL amber vial, followed by the addition of NaCl. The solution was stirred at 650 rpm and the extraction temperature was set to 65 °C. After stirring for 5 minutes, the SPME plunger, with appropriate type of fiber selected from 3.6.1, was depressed to expose the fiber. After 10 min extraction time, the SPME syringe was removed from the vial and injected into the GC-MS. Three concentration of NaCl were investigated: 89, 178 and 357 mg NaCl/mL solution. The analyses were repeated three times (n = 3) for each NaCl concentration. The appropriate NaCl concentration will be selected and used for further studies.

3.6.3 Effect of pH of solution

Twenty mL of 10 ng/mL NDMA standard in buffer solution of controlled pH was added into 40-mL amber vial. NaCl of appropriate concentration, determined from 3.6.2, was added to the same vial. The solution was stirred at 650

rpm and the extraction temperature was set to 65 °C. After stirring for 5 minutes, the SPME plunger, with appropriate type of fiber selected from 3.6.1, was depressed to expose the fiber. After 10 min extraction time, the SPME syringe was removed from the vial and injected into the GC-MS. Four pH values were studied: 4, 7, 10, and 12. The analyses were repeated three times (n = 3) for each pH. The appropriate solution pH will be selected and used for further studies.

3.6.4 Effect of sample volume

Various volume of 10 ng/mL NDMA standard in buffer solution, determined from 3.6.3, was added into 40-mL amber vial. NaCl of appropriate concentration, determined from 3.6.2, was added to the same vial. The solution was stirred at 650 rpm and the extraction temperature was set to 65 °C. After stirring for 5 minutes, the SPME plunger, with appropriate type of fiber selected from 3.6.1, was depressed to expose the fiber. After 10 min extraction time, the SPME syringe was removed from the vial and injected into the GC-MS. Five volumes of NDMA standard were studied: 1, 5, 10, 15 and 20 mL. The analyses were repeated three times (n = 3) for each sample volume. The appropriate sample volume will be selected and used for further studies.

3.6.5 Effect of extraction temperature

Specified volume of 10 ng/mL NDMA standard (obtained from 3.6.4) in buffer solution, determined from 3.6.3, was added into 40-mL amber vial. NaCl of appropriate concentration, determined from 3.6.2, was added to the same vial. The solution was stirred at 650 rpm and the extraction temperature was varied. After stirring for 5 minutes, the SPME plunger, with appropriate type of fiber selected from 3.6.1, was depressed to expose the fiber. After 10 min extraction time, the SPME syringe was removed from the vial and injected into the GC-MS. Five temperatures were studied: 45, 55, 65, 75 and 85 °C. The analyses were repeated three times (n = 3) for each extraction temperature. The appropriate extraction temperature will be selected and used for further studies.

3.6.6 Effect of extraction time

Specified volume of 10 ng/mL NDMA standard (obtained from 3.6.4) in buffer solution, determined from 3.6.3, was added into 40-mL amber vial. NaCl of appropriate concentration, determined from 3.6.2, was added to the same vial. The solution was stirred at 650 rpm and the extraction temperature was set according to 3.6.5. After stirring for 5 minutes, the SPME plunger, with appropriate type of fiber selected from 3.6.1, was depressed to expose the fiber. After specified extraction time, the SPME syringe was removed from the vial and injected into the GC-MS. Nine values of extraction time were studied: 2, 5, 10, 20, 30, 60, 120, 240 and 360 min. The appropriate extraction time will be selected.

3.7 Validation of the method

The method was validated in terms of limit of detection (LOD), limit of quantitation (LOQ), linearity, accuracy and precision. Johnson baby shampoo was used as a blank sample for the determination of LOD, LOQ, accuracy and precision.

3.7.1 Limit of detection

Limit of detection or LOD is the lowest analyte concentration that can be detected and gives the S/N of 3. A 0.5 g blank sample and 1.00 mL of 5 ng/mL NDMA standard solution were added into 50 mL volumetric flask. The solution was diluted with buffer solution of pH 7 (determined from 3.6.3). The spiked blank sample solution was analyzed with optimized HS-SPME-GC-MS conditions. The analyses were repeated ten times (n = 10).

3.7.2 Limit of quantitation

Limit of quantitation or LOQ is the lowest analyte concentration that can be measured or quantified and gives the S/N of 20. A 0.5 g blank sample and 0.50 mL of 50 ng/mL NDMA standard solution were added into 50 mL volumetric flask. The solution was diluted with buffer solution of pH 7 (determined from 3.6.3). The spiked blank sample solution was analyzed with optimized HS-SPME-GC-MS conditions. The analyses were repeated ten times (n = 10).

3.7.3 Linearity

Nine solutions of NDMA standard were prepared by direct or serial dilution from 500 ng/mL NDMA standard solution with buffer solution of pH 7 (determined from 3.6.3) to obtain the concentrations of 0.1, 0.5, 1, 5, 10, 20, 50, 100 and 200 ng/mL. Each standard solution was analyzed three times (n = 3) under the optimized HS-SPME-GC-MS conditions.

3.7.4 Accuracy and precision

A 0.5 g blank sample and 0.50 mL of 50 ng/mL NDMA standard solution were added into 50 mL volumetric flask. The solution was diluted with buffer solution of pH 7 (determined from 3.6.3). The spiked blank sample solution was analyzed with optimized HS-SPME-GC-MS conditions. Five spiked blank sample solutions were independently prepared and analyzed each day. A calibration curve was newly prepared each day with 0.1, 0.5, 1, 2, and 5 ng/mL NDMA standard solutions in buffer solution of pH 7. Recovery for each analysis and % RSD for the analyses in the same day were obtained. The same procedure was repeated for three consecutive days. The accuracy and precision of the method were evaluated from % recovery and % RSD, respectively.

3.8 Application to real samples

The validated method was applied to analyze NDMA presented in real samples. Five hair shampoos (which are OTOP products and contain plant extracts) of different brands were purchased from local market. They were analyzed using the optimized HS-SPME-GC-MS conditions according to 3.5.2 and 3.6.

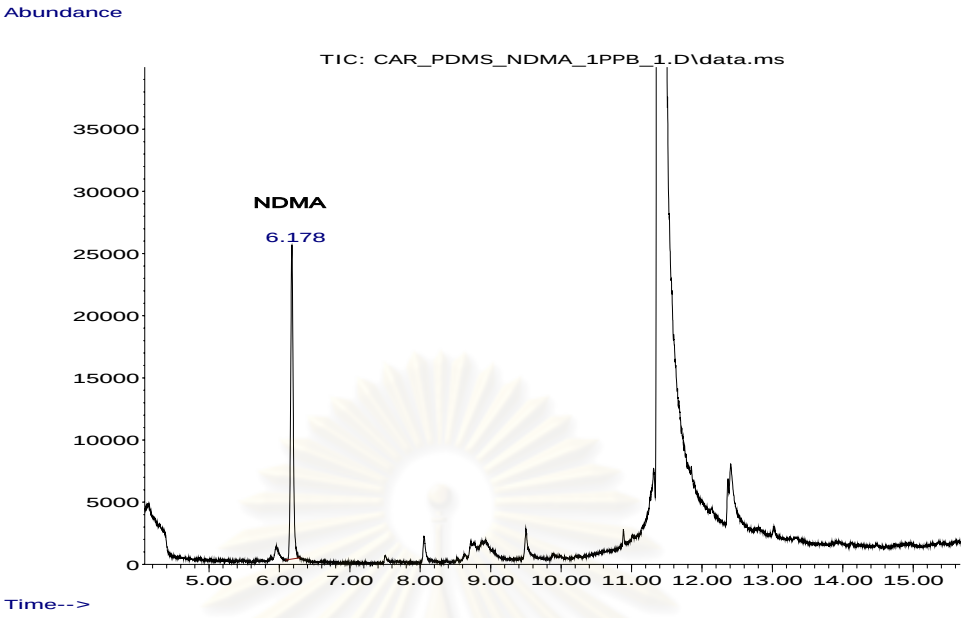
CHAPTER IV

RESULTS AND DISCUSSION

4.1 GC-MS conditions for the analysis of NDMA

Standard NDMA was extracted with SPME using headspace sampling mode according to the procedure in 3.5.1. The analysis of the extracted NDMA was carried out using DB-WAX capillary GC column of 30 m long, 0.25 mm I.D., 0.25 μm film. The optimum oven temperature was selected to include the separation of other *N*-nitrosamines of interest in the same run. The oven temperature started from 60 °C held for 5 min; programmed to 100 °C at 10 °C /min; then to 200 °C at 15 °C /min. Retention time of NDMA was observed at 6.2 min as shown in Figure 4.1 (a). However, a large peak was also observed around 11–13 min. After SPME cleanup, another injection was performed using SPME without the NDMA extraction step. A peak at 6.2 min disappeared, but a large peak around 11–13 min still remained (Figure 4.1 (b)) indicating that this peak must be from the SPME fiber.

(a)



(b)

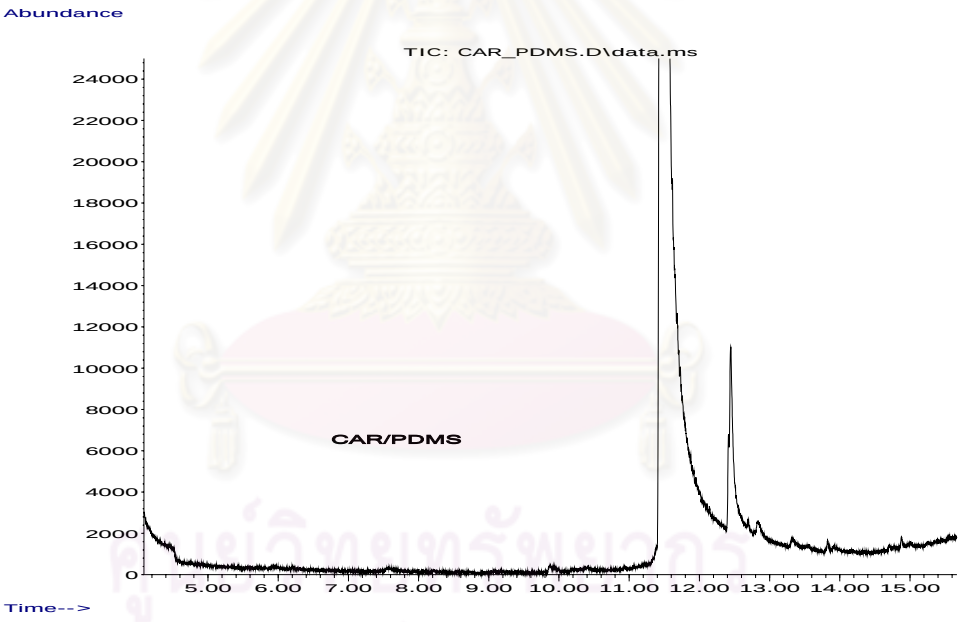


Figure 4.1 GC-MS chromatograms on DB-WAX column obtained (a) after HS-SPME of NDMA standard; (b) from SPME fiber without NDMA extraction.

4.2 Optimization of HS-SPME conditions

In this study, NDMA analyte partitioned between aqueous, headspace and fiber phases. Several parameters related to analyte partition were investigated including type of SPME fiber, salt concentration in the solution, pH of solution, sample volume, extraction temperature and extraction time.

4.2.1 Effect of type of SPME fiber

Three types of SPME fibers of different polarity were examined for headspace NDMA extraction efficiency: CAR/PDMS, PDMS/DVB and PA. As shown in Figure 4.2, CAR/PDMS has higher extraction efficiency than PDMS/DVB and PA for about 6 and 42 times, respectively. The result about the efficiency of CAR/PDMS for the extraction of NDMA was similar to that reported by Grebel *et al.* [6]. Therefore, CAR/PDMS was chosen for the remaining of the study.

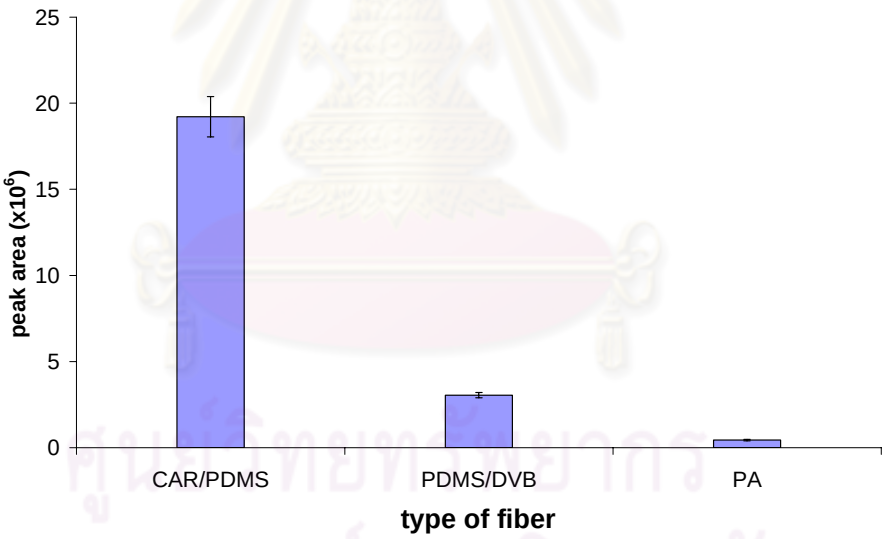


Figure 4.2 Effect of type of SPME fiber on NDMA response (conditions: NDMA 10 ng/mL; 357 mg NaCl/mL; sample volume 20 mL; extraction temperature 65 °C; extraction time 10 min; n = 3).

4.2.2 Effect of NaCl concentration

Another way to increase the extraction efficiency is to add salt into the solution to decrease the solubility of NDMA in aqueous phase and, at the same time, increase the amount of NDMA in air phase (headspace). NDMA extractions were performed at three NaCl concentrations: 89, 178 and 357 mg NaCl/mL solution. As 1 g of NaCl can be dissolved in smallest volume of water of 2.8 mL; therefore, the concentration of 357 mg NaCl/mL solution was considered a saturated salt concentration. From Figure 4.3, NDMA extraction was highest at saturated NaCl concentration, about 14-20 times better than other concentrations. Therefore, NaCl concentration of 357 mg/mL was chosen for the remaining of the study.

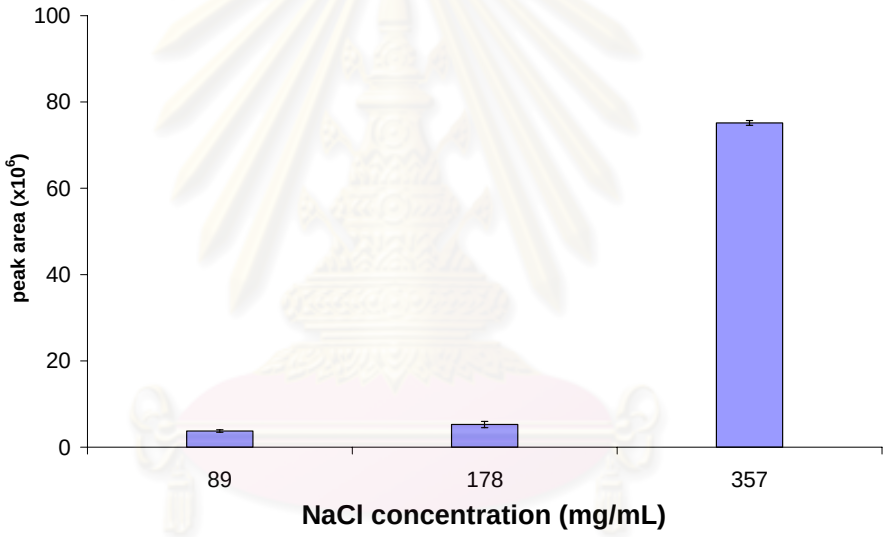


Figure 4.3 Effect of NaCl concentration on NDMA response (conditions: NDMA 10 ng/mL; CAR/PDMS fiber; sample volume 20 mL; extraction temperature 65 °C; extraction time 10 min; n = 3).

4.2.3 Effect of pH of solution

The effect of pH of solution on the NDMA extraction efficiency was also examined. For this study, 10 ng/mL NDMA standard solutions were prepared in buffer solutions of pH 4, 7, 10 or 12, instead of Milli-Q water. As seen from Figure 4.4, pH of solution showed no significant effect on extraction efficiency of NDMA; therefore a neutral pH of 7 was selected for the remaining of the study.

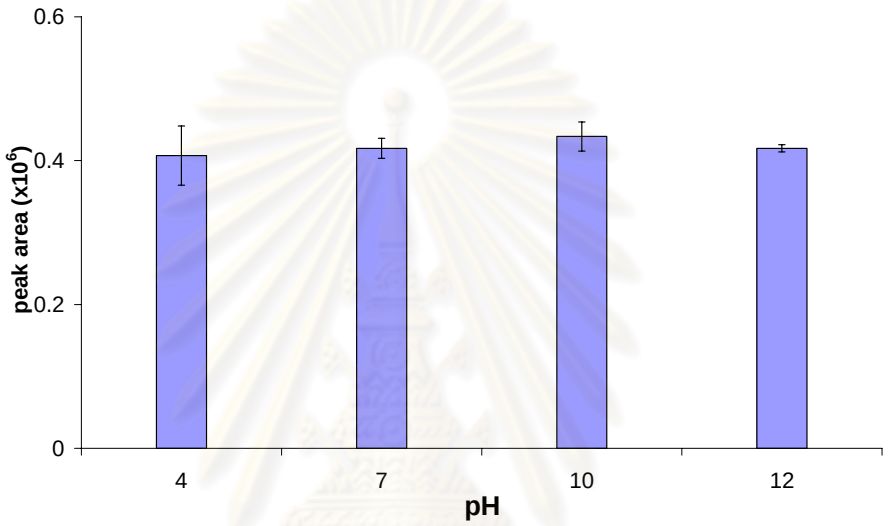


Figure 4.4 Effect of pH of solution on NDMA response (conditions: NDMA 10 ng/mL; CAR/PDMS fiber; 357 mg NaCl/mL; sample volume 20 mL; extraction temperature 65 °C; extraction time 10 min; n = 3).

4.2.4 Effect of sample volume

For headspace extraction, the ratio of headspace volume to sample volume must be optimized. In this study, the vial volume of 40 mL was selected. The sample volumes of 1, 5, 10, 15 and 20 mL, corresponding to headspace volume relative to vial volume values of 0.98, 0.88, 0.75, 0.63 and 0.50 respectively, were tested. From Figure 4.5, as the sample volume increased from 1–10 mL, extraction efficiency increased. However, as the sample volume was greater than 10 mL, extraction efficiency decreased. The sample volume of 10 mL (with the headspace ratio of 0.75) provided the best extraction efficiency. Since the method will be applied to analyze hair shampoo, sample with large volume gave a lot of bubbles and could contaminate SPME fiber. Therefore, sample volume of 5 mL, with lower sample volume and slightly lower extraction efficiency, was selected instead of sample volume of 10 mL.

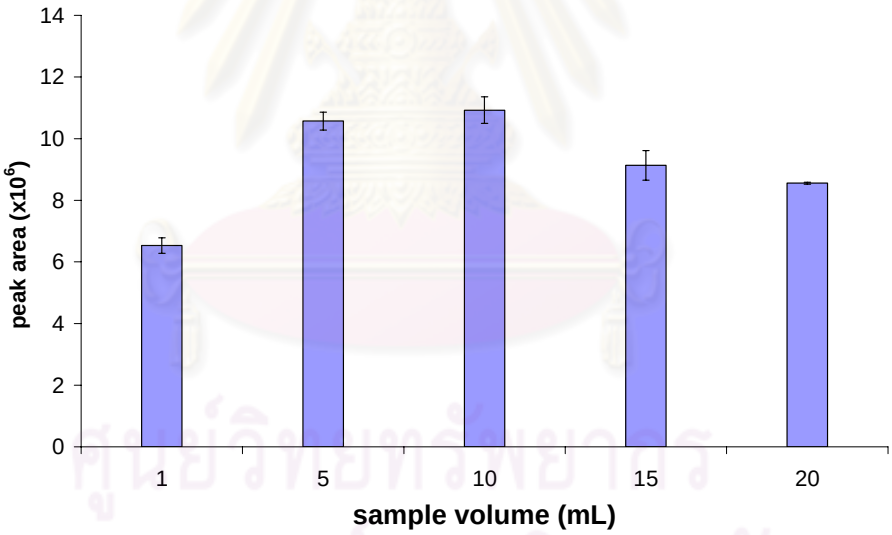


Figure 4.5 Effect of sample volume on NDMA response (conditions: NDMA 10 ng/mL in buffer pH 7; CAR/PDMS fiber; 357 mg NaCl/mL; extraction temperature 65 °C; extraction time 10 min; n = 3).

4.2.5 Effect of extraction temperature

Extraction of volatile compounds such as NDMA could be improved by increasing extraction temperature to increase the volatility of NDMA to headspace phase. Five extraction temperatures were studied: 45, 55, 65, 75 and 85 °C. NDMA showed an increase in extraction efficiency as the extraction temperature increased from 45–65 °C. However, as the temperature further increased to 85 °C, extraction efficiency showed a slight decrease. It was possible that at high temperature, water vapor increased and adsorbed on SPME fiber and; therefore, reduced the adsorption of NDMA on fiber. Another possible explanation was that NDMA started to desorb from fiber at higher temperature and the number of adsorbed NDMA on fiber decreased. Therefore, extraction temperature of 65 °C was chosen for the remaining of the study.

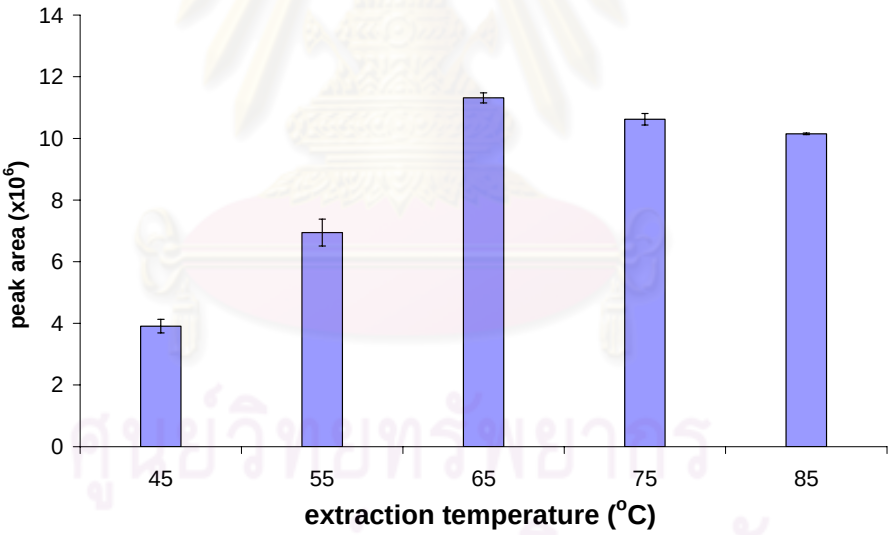


Figure 4.6 Effect of extraction temperature on NDMA response (conditions: NDMA 10 ng/mL in buffer pH 7; CAR/PDMS fiber; 357 mg NaCl/mL; sample volume 5 mL; extraction time 10 min; n = 3).

4.2.6 Effect of extraction time

The partition of an analyte between aqueous phase and SPME fiber during extraction is an equilibrium process. If an analyte has a long equilibrium time, it may be preferable to use non-equilibrium extraction. The extraction times from 2 to 360 min were examined to determine the equilibrium time for NDMA extraction. An equilibrium time for NDMA extraction was reached at 240 min (from Figure 4.7). However, this equilibrium time of 240 min is too long and is not practical for routine analyses. At 10 min extraction time, with a decrease in extraction efficiency of 4–5 times compared to 240 min equilibrium time, was selected instead.

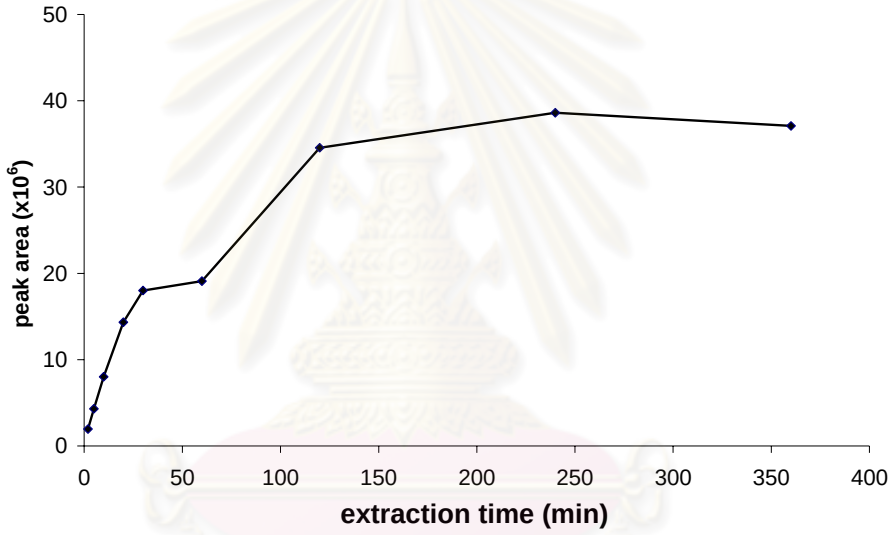


Figure 4.7 Effect of extraction time on NDMA response (conditions: NDMA 10 ng/mL in buffer pH 7; CAR/PDMS fiber; 357 mg NaCl/mL; sample volume 5 mL; extraction temperature 65 °C; n = 1).

The optimized HS-SPME conditions for NDMA extraction were: vial size 40 mL; NDMA 10 ng/mL in buffer pH 7; CAR/PDMS fiber; 357 mg NaCl/mL solution; sample volume 5 mL; extraction temperature 65 °C; and extraction time 10 min.

4.3 Validation of the method

To validate the optimized HS-SPME-GC-MS method, several validation parameters were performed: limit of detection (LOD); limit of quantitation (LOQ); linearity; and accuracy and precision. In case where a blank sample was needed, Johnson baby shampoo (which contains no NDMA) was used.

4.3.1 Limit of detection

Limit of detection or LOD is the lowest analyte concentration that can be detected and gives the S/N of 2–3 [15]. In this study, LOD was determined at S/N of 3. A 0.5 g blank sample was spiked with 1.00 mL of 5 ng/mL NDMA standard solution and diluted with buffer solution of pH 7 in a 50-mL volumetric flask. The LOD was determined to be 0.1 ng/mL solution or 10 ng/g sample for 10 replicate analyses.

4.3.2 Limit of quantitation

Limit of quantitation or LOQ is the lowest analyte concentration that can be measured or quantified and gives the S/N of 10–20 [15]. In this study, LOQ was determined at S/N of 20. A 0.5 g blank sample was spiked with 0.50 mL of 50 ng/mL NDMA standard solution and diluted with buffer solution of pH 7 in a 50-mL volumetric flask. The LOQ was determined to be 0.5 ng/mL solution or 50 ng/g sample with 0.52 % RSD for 10 replicate analyses.

4.3.3 Linearity

Nine NDMA standard solutions were prepared in buffer solution of pH 7 to obtain the concentration range of 0.1–200 ng/mL. Each standard solution was analyzed in three replicates (n = 3) under the optimized HS-SPME-GC-MS conditions. As shown in Figure 4.8, a coefficient of determination (R^2) of 0.9982 indicated a good linear fit.

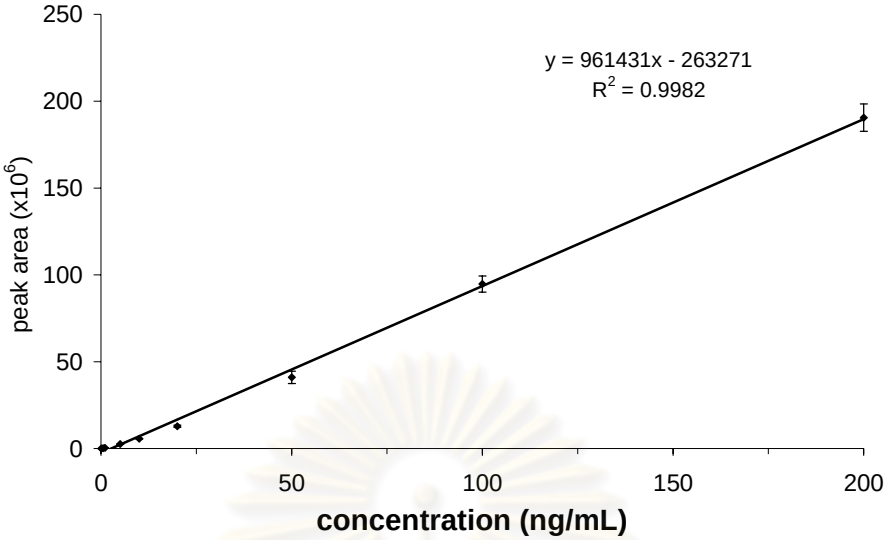


Figure 4.8 Calibration curve for NDMA analysis (NDMA 0.1–200 ng/mL in buffer pH 7; CAR/PDMS fiber; 357 mg NaCl/mL; sample volume 5 mL; extraction temperature 65 °C; extraction time 10 min; n = 3).

4.3.4 Accuracy and precision [15, 16]

The accuracy of an analytical method is the extent to which the values obtained by the method and the true values agree [15]. If certified reference materials or control samples are not available, the true value for the accuracy evaluation could be obtained by spiking a known concentration of analyte to a blank sample matrix of interest. After appropriate sample preparation and analysis, ifs recovery could be determined. In this case, a 0.5 g blank sample was spiked with 0.50 mL of 50 ng/mL NDMA standard solution and diluted with buffer solution of pH 7 in a 50-mL volumetric flask. Five spiked sample solutions were independently prepared and analyzed each day for three consecutive days. Recovery for each analysis was determined from a calibration curve which was prepared each day.

Acceptable recovery depends on the sample matrix, sample preparation and analyte concentration. From Table 4.1, 84–99 % of NDMA could be recovered from spiked samples of 50.0 ng/g concentration. These values were within the range of acceptable % recovery of 80–110 for 50.0 ng/g (50 ppb) concentration specified by AOAC [16].

Table 4.1 Accuracy and precision of the method

day	batch	recovery (ng/g)	% recovery	% RSD	HORRAT
1	1	49.4	99	3.1	0.19
	2	46.8	94		
	3	46.0	92		
	4	46.0	92		
	5	46.2	92		
2	1	46.4	93	2.9	0.17
	2	45.6	91		
	3	45.7	91		
	4	46.6	93		
	5	48.9	98		
3	1	43.6	87	3.0	0.18
	2	45.5	91		
	3	43.0	86		
	4	43.9	88		
	5	42.0	84		
acceptable value			80–110		< 2

The precision of an analytical method is the degree of similarity among individual test results when the procedure is applied repeatedly to multiple samplings [15]. Repeatability is a subgroup of precision if the analysis is carried out in one laboratory by one operator using the same equipments and instruments over a relatively short time span. % RSD for the analyses in the same day was calculated to determine the precision or repeatability of the method.

Acceptable % RSD also varies with analyte concentration. The acceptable % RSD value for repeatability could be obtained from the following formula (Horwitz equation):

$$\text{calculated \% RSD} = 0.67 \times 2\,C^{-0.1505}$$

Where C is the analyte concentration ratio (without unit). For this study, the analyte concentration is 50 ppb and % RSD calculated from the above equation would be 16.82.

Acceptable values for repeatability could be determined from a ratio of % RSD obtained from the experiment to % RSD obtained from calculation which is known as HORRAT.

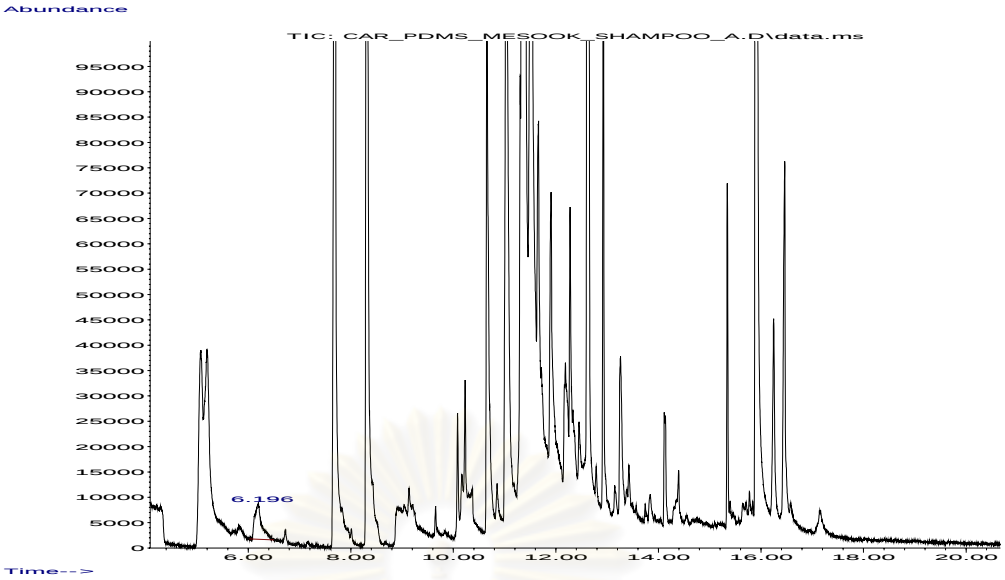
$$\text{HORRAT} = \frac{\text{experimental \% RSD}}{\text{calculated \% RSD}}$$

From Table 4.1, % RSD values obtained from the experiment were quite low (2.9–3.1 %) with HORRAT values of 0.17–0.19 for three days. These HORRAT values were below 2 which is the acceptable value for 50.0 ng/g (50 ppb) concentration specified by AOAC [16].

4.4 Application to real samples

Five hair shampoos of different brands were purchased from local market. All of them are OTOP products and contain plant extracts such as coconut, aloe vera, blue pea, or agarwood oil. Hair shampoos were analyzed using the optimized HS-SPME-GC-MS conditions. GC-MS chromatograms obtained from hair shampoo were shown in Figure 4.9. For real samples, the analyses were complicated by the matrix of samples. In this study, in addition to matrix peak from SPME fiber, many peaks were also observed and some peaks were closed to NDMA peak at 6.2 min. Another experiment was performed by adding NDMA standard to shampoo sample to confirm the present of NDMA in real sample. However, the results could not be concluded with high confidence. Additional sample cleanup to remove interference matrix may be needed before HS-SPME analysis. Alternatively, a more specific detector such as nitrogen chemiluminescence detector may be considered.

(a)



(b)

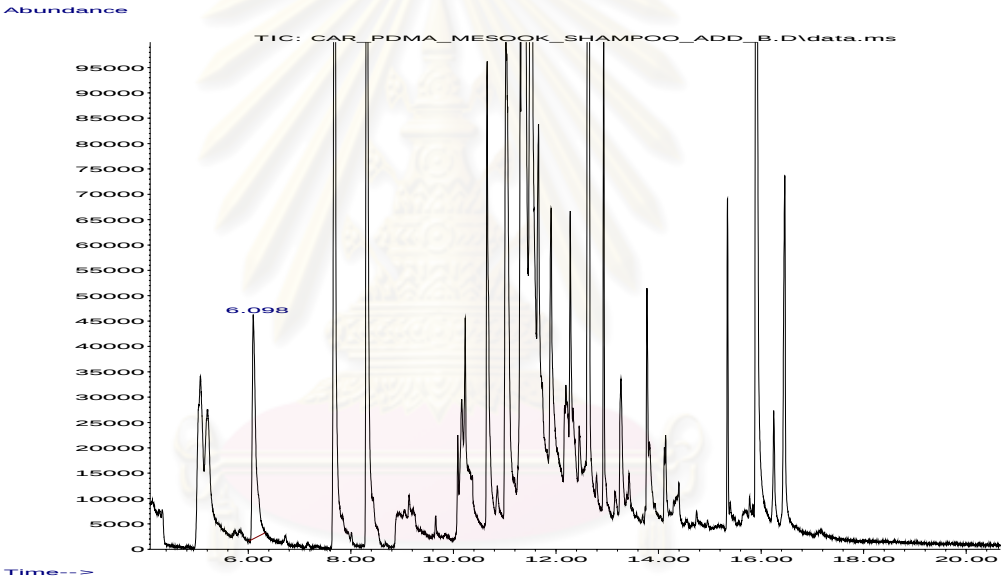


Figure 4.9 GC-MS chromatograms of hair shampoo with agarwood oil (a) after HS-SPME of hair shampoo; (b) after HS-SPME of hair shampoo spiked with 200 ng/g NDMA standard.

CHAPTER V

CONCLUSION

A sample preparation based on headspace solid phase microextraction (HS-SPME) was developed for *N*-nitrosodimethylamine (NDMA) before analyzing with gas chromatography-mass spectrometry (GC-MS) using DB-WAX capillary GC column of 30 m long, 0.25 mm I.D., 0.25 μm film. The separation temperature started from 60 °C held for 5 min; programmed to 100 °C at 10 °C /min; then to 200 °C at 15 °C /min. Retention time of NDMA was observed at 6.2 min. Several parameters affecting HS-SPME of NDMA were optimized. Among three types of fibers tested, CAR/PDMS was found to give the best extraction efficiency. NaCl should be added to obtain a saturated concentration to increase extraction efficiency, while pH of solution showed insignificant effect to extraction efficiency. Extraction temperature of 65 °C and extraction time of 10 min were selected. Small sample volume of 5–10 mL should be used to avoid contamination from the bubbles of shampoo. With the optimized conditions, the method provided good linearity in the range from 0.1 to 200 ng/mL with a high coefficient of determination (R^2) of 0.9982. LOD was determined to be 0.1 ng/mL solution or 10 ng/g sample and LOQ was 0.5 ng/mL solution or 50 ng/g sample. The method showed good recovery of 84–99 % at 50.0 ng/g concentration and low % RSD around 3 %. Sample preparation based on HS-SPME is simple, environmental friendly, and compatible with GC analysis. This developed method was applied to analyze for NDMA in hair shampoos (OTOP products). However, the real samples gave matrix peaks that interfered with NDMA peak. Additional sample cleanup to remove interference matrix may be needed before HS-SPME analysis. Alternatively, a more specific detector such as nitrogen chemiluminescence detector may be considered.

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[9] ประกาศกระทรวงสาธารณสุข เรื่องกำหนดวัตถุที่ห้ามใช้เป็นส่วนผสมในการผลิตเครื่องสำอาง ตามที่ปรากฏในราชกิจจานุเบกษา เล่ม 125 ตอนพิเศษ 80 ง ลงวันที่ 12 พฤษภาคม 2551 กำหนดให้ Nitrosamines เป็นวัตถุที่ห้ามใช้เป็นส่วนผสมในการผลิตเครื่องสำอาง ลำดับที่ 408

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APPENDIX

Table A.1 Effect of type of SPME fiber on extraction efficiency. HS-SPME conditions: vial size 40 mL; 20 mL of 10 ng/mL NDMA standard; 357 mg NaCl/mL; extraction temperature 65 °C; extraction time 10 min; n = 3.

type of fiber	film thickness	entry #	peak area	average	SD	% RSD
PDMS/DVB	65 µm	1	2999299	3048179	156301	5.13
		2	2922159			
		3	3223079			
CAR/PDMS	75 µm	1	18384436	19219886	1172349	6.10
		2	18715157			
		3	20560064			
PA	85 µm	1	424158	440179	42937	9.75
		2	488823			
		3	407556			

Table A.2 Effect of NaCl concentration on extraction efficiency. HS-SPME conditions: vial size 40 mL; 20 mL of 10 ng/mL NDMA standard; CAR/PDMS fiber; extraction temperature 65 °C; extraction time 10 min; n = 3.

NaCl (mg/mL)	entry #	peak area	average	SD	%RSD
89	1	3409117	3744253	312643	8.35
	2	3795594			
	3	4028048			
178	1	4511007	5232793	721459	13.79
	2	5233447			
	3	5953925			
357	1	6986274	7515074	579283	7.71
	2	8134222			
	3	7424725			

Table A.3 Effect of pH of solution on extraction efficiency. HS-SPME conditions: vial size 40 mL; 20 mL of 10 ng/mL NDMA standard in buffer solutions; CAR/PDMS fiber; 357 mg NaCl/mL; extraction temperature 65 °C; extraction time 10 min; n = 3.

pH	entry #	peak area	average	SD	%RSD
4	1	414929	407064	41150	10.11
	2	362550			
	3	443714			
7	1	417238	417076	13809	3.31
	2	430803			
	3	403186			
10	1	422219	433604	20258	4.67
	2	456993			
	3	421601			
12	1	416949	417162	4863	1.17
	2	422128			
	3	412410			

Table A.4 Effect of sample volume on extraction efficiency. HS-SPME conditions: vial size 40 mL; 10 ng/mL NDMA standard in buffer pH 7; CAR/PDMS fiber; 357 mg NaCl/mL; extraction temperature 65 °C; extraction time 10 min; n = 3.

sample volume (mL)	entry #	peak area	average	SD	%RSD
1	1	6312731	6531525	249824	3.82
	2	6803737			
	3	6478108			
5	1	10775125	10568855	289380	2.74
	2	10238058			
	3	10693383			
10	1	10480845	10922714	430074	3.94
	2	11339932			
	3	10947364			
15	1	8594632	9134378	479980	5.25
	2	9295225			
	3	9513277			
20	1	8587718	8553659	33312	0.39
	2	8552112			
	3	8521147			

Table A.5 Effect of extraction temperature on extraction efficiency. HS-SPME conditions: vial size 40 mL; 5 mL of 10 ng/mL NDMA standard in buffer pH 7; CAR/PDMS fiber; 357 mg NaCl/mL; extraction time 10 min; n = 3.

extraction temperature (°C)	entry #	peak area	average	SD	%RSD
45	1	3718906	3911026	221121	5.65
	2	4152729			
	3	3861442			
55	1	6717869	6944109	439388	6.33
	2	7450514			
	3	6663943			
65	1	11507253	11316348	165336	1.46
	2	11219369			
	3	11222421			
75	1	10810730	10620957	187942	1.77
	2	10434902			
	3	10617239			
85	1	10348919	10149158	237303	2.34
	2	9886845			
	3	10211710			

Table A.6 Effect of extraction time on extraction efficiency. HS-SPME conditions: vial size 40 mL; 5 mL of 10 ng/mL NDMA standard in buffer pH 7; CAR/PDMS fiber; 357 mg NaCl/mL; extraction temperature 65 °C; n = 1.

extraction time (min)	peak area
2	1993734
5	4465906
10	8624708
20	15100239
30	18078189
60	19091811
120	34607556
240	38625913
360	37107363

Table A.7 Determination of LOD at S/N = 3 (n = 10).

entry #	S/N
1	3.5
2	3.0
3	3.5
4	3.2
5	3.6
6	3.2
7	3.2
8	3.4
9	2.9
10	3.7

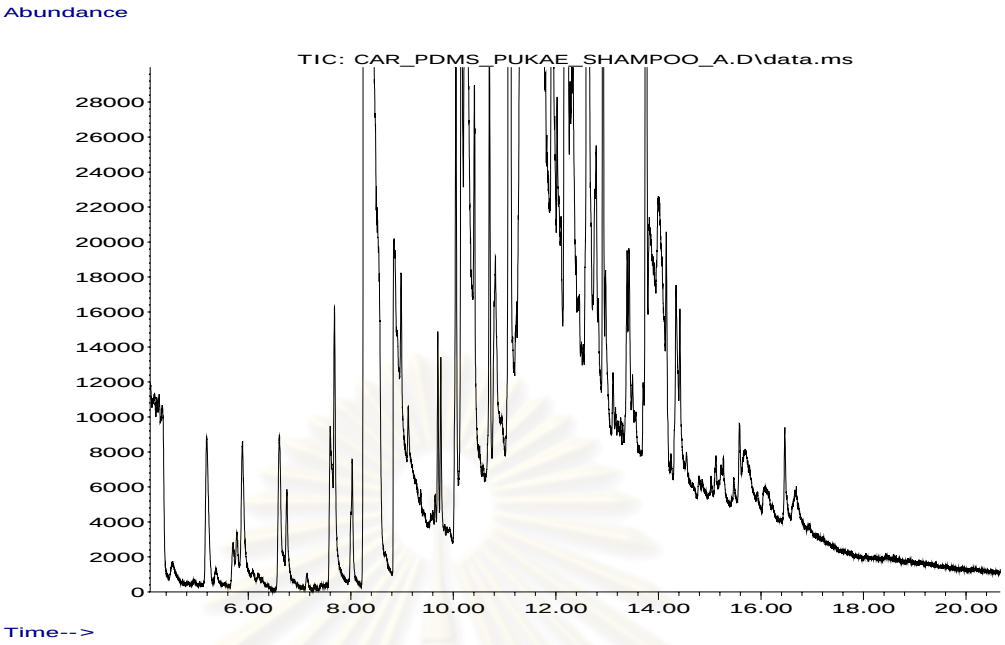
Table A.8 Determination of LOQ at S/N = 20 (n = 10).

entry #	NDMA (ng/g)	S/N	LOQ (ng/g)	% recovery
1	50	21.5	44.9	90
2	50	21.9	45.1	90
3	50	20.1	44.9	90
4	50	16.0	44.9	90
5	50	18.5	45.1	90
6	50	19.1	45.0	90
7	50	16.4	45.0	90
8	50	18.4	45.1	90
9	50	20.1	45.6	91
10	50	17.2	44.7	89

Table A.9 A calibration curve of NDMA standard solutions in the range of 0.1–200 ng/mL (n = 3).

concentration (ng/mL)	peak area	average	SD	%RSD
0.1	38256 38654 38159	38356	262	0.68
0.5	229360 200983 209026	213123	14625	6.86
1	415646 388555 391059	398420	14971	3.76
5	2548262 2602421 2501749	2550811	50384	1.98
10	5659656 5464170 5831749	5651858	183914	3.25
20	12651084 13560450 12347011	12852848	631380	4.91
50	45095789 39043628 38833014	40990810	3556575	8.68
100	89728612 95464555 98991237	94728135	4675018	4.94
200	189967006 182999892 198744330	190570409	7889544	4.14

(a)



(b)

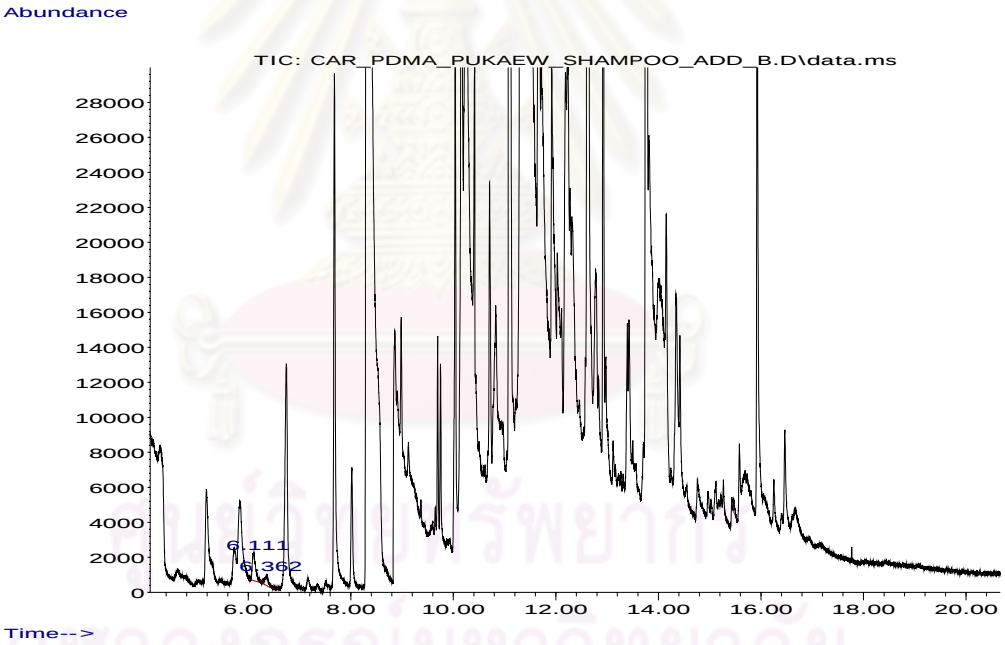
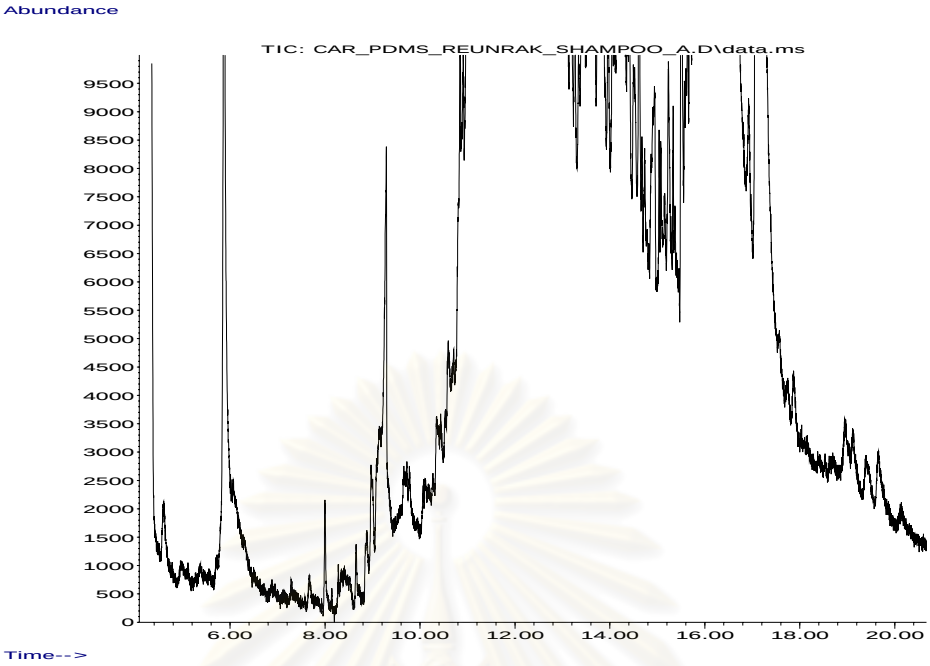


Figure A.1 GC-MS chromatograms of coconut hair shampoo (a) after HS-SPME of hair shampoo; (b) after HS-SPME of hair shampoo spiked with 10 ng/g NDMA standard.

(a)



(b)

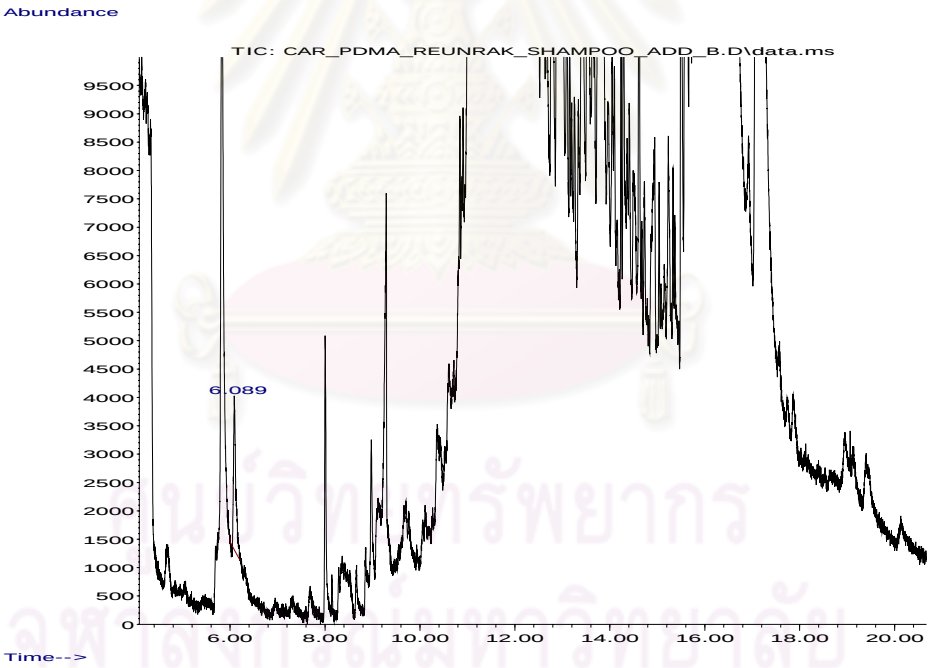
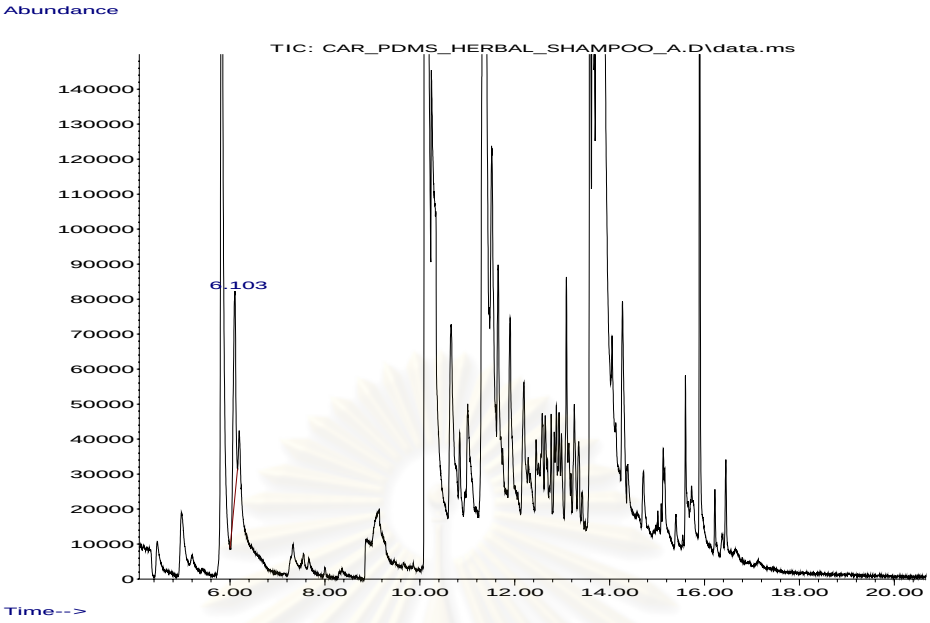


Figure A.2 GC-MS chromatograms of herbal hair shampoo with aloe vera and honey (a) after HS-SPME of hair shampoo; (b) after HS-SPME of hair shampoo spiked with 10 ng/g NDMA standard.

(a)



(b)

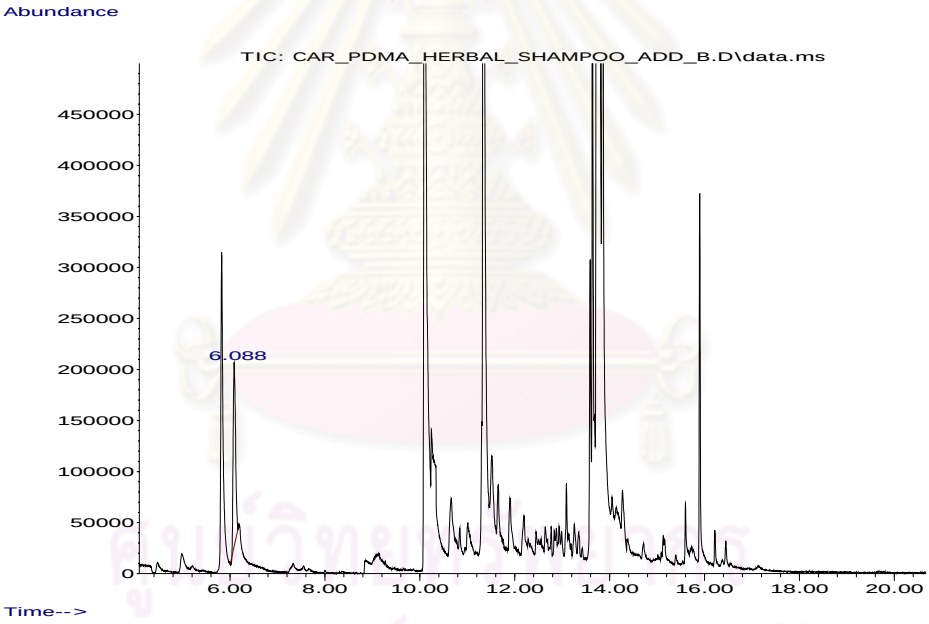
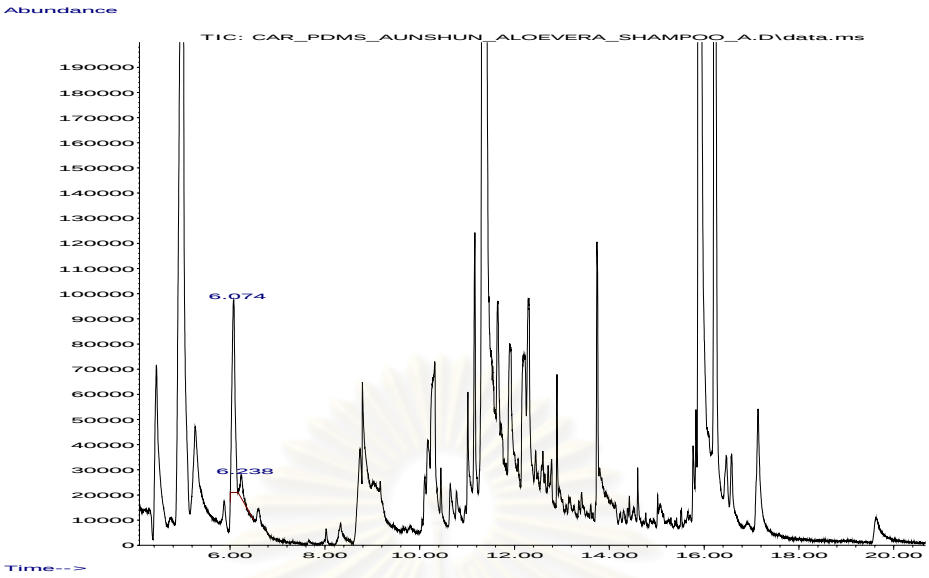


Figure A.3 GC-MS chromatograms of herbal hair shampoo (a) after HS-SPME of hair shampoo; (b) after HS-SPME of hair shampoo spiked with 500 ng/g NDMA standard.

(a)



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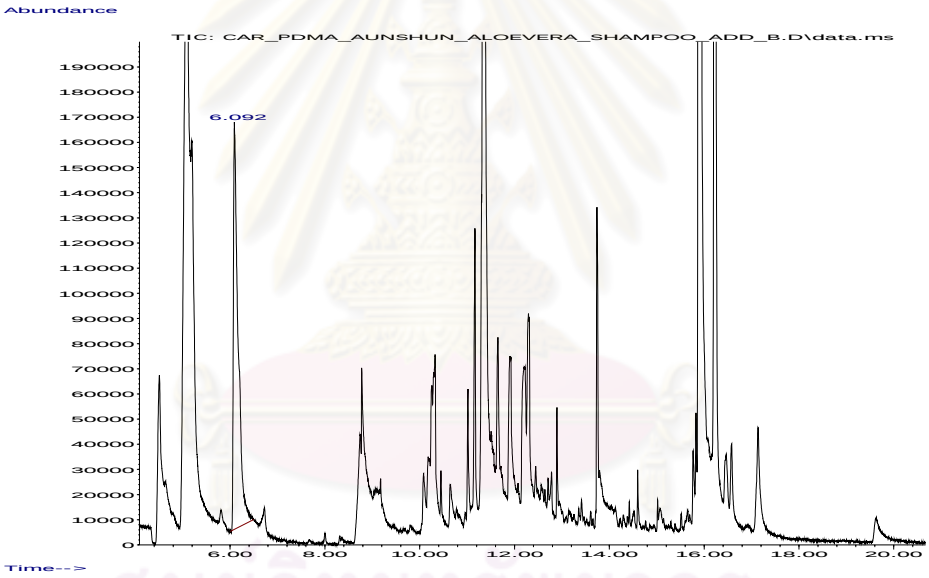

Figure A.4 GC-MS chromatograms of herbal hair shampoo with blue pea and aloe vera (a) after HS-SPME of hair shampoo; (b) after HS-SPME of hair shampoo spiked with 500 ng/g NDMA standard.

VITA

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