DEVELOPMENT OF THIN-LAYER CHROMATOGRAPHY FOR SELECTIVE ANALYSIS OF VOLATILE COMPOUNDS USING GAS CHROMATOGRAPHY MASS SPECTROMETRY

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Chemistry
Department of Chemistry
FACULTY OF SCIENCE
Chulalongkorn University
Academic Year 2019

การพัฒนาทินเลเยอร์โครมาโทกราฟีสำหรับการวิเคราะห์สารระเหยง่ายอย่างจำเพาะโดยใช้แก๊สโครมาโทกราฟีแมสสเปกโทรเมตรี

นายณัฐภัทร สุชาตานุกูล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี ภาควิชาเคมี
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2562

Thesis Title	DEVELOPMENT OF THIN-LAYER CHROMATOGRAPHY FOR SELECTIVE ANALYSIS OF VOLATILE COMPOUNDS USING GAS CHROMATOGRAPHY MASS SPECTROMETRY
By	Mr. Nattapat Suchatanugal
Field of Study	Chemistry
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ณัฐภัทร สุชาตานุกูล : การพัฒนาทินเลเยอร์โครมาโทกราฟีสำหรับการวิเคราะห์สารระเหยง่ายอย่างจำเพาะโดยใช้แก๊สโครมาโทกราฟีแมสสเปกโทรเมตรี. (DEVELOPMENT OF THIN-LAYER CHROMATOGRAPHY FOR SELECTIVE ANALYSIS OF VOLATILE COMPOUNDS USING GAS CHROMATOGRAPHY MASS SPECTROMETRY) อ.ที่ปรึกษาหลัก : อ. ดร.ชฎิล กุลสิงห์

งานศึกษาวิจัยชิ้นนี้มีความประสงค์ที่จะหาวิธีการวิเคราะห์สารประกอบระเหยได้โดยวิธีการที่ง่ายต่อการวิเคราะห์และมีประสิทธิภาพในการวิเคราะห์ตัวอย่างด้วยความจำเพาะเจาะจง โดยการใช้เทคนิคทางโครมาโทกราฟีเข้ามาประยุกต์ด้วยกัน โดยเลือกใช้เทคนิคทินเลเยอร์โครมาโตกราฟีผ่านการแยกตัวอย่างต่างๆ บนแผ่นที่ถูกเคลือบไว้ด้วยซิลิกาเจลหรือเฟสคงที่ โดยสารประกอบทางเคมีในตัวอย่างจะถูกแยกโดยการใช้เฟสเคลื่อนที่ในการพาสารประกอบทางเคมีไปอยู่ในตำแหน่งต่างๆ อย่างจำเพาะเจาะจงบนแผ่นทินเลเยอร์โครมาโตกราฟี ตามคุณสมบัติทางเคมีของสารประกอบนั้น ในงานวิจัยนี้จะมีแบ่งตัดแผ่นทินเลเยอร์โครมาโตกราฟีเป็นทั้งหมด 4 หรือ 7 ส่วน และมีการเก็บตัวอย่างสารระเหยได้ผ่านเทคนิคเฮดสเปซ โซลิดเฟสไมโครเอกซ์แทรกชัน เข้าไปวิเคราะห์ในเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโตรมิทรี ซึ่งเป็นเทคนิคที่มีความสามารถวิเคราะห์ตัวอย่างสารประกอบระเหยได้ที่มีประสิทธิภาพสูง จากผลการทดลองพบว่าเมื่อเราทำการเก็บตัวอย่างจากแผ่นทินเลเยอร์โครมาโตกราฟีที่ผ่านการแยกแล้ว และนำแผ่นทินเลเยอร์โครมาโตกราฟีมาเก็บตัวอย่างโดยเทคนิคเฮดสเปซ โซลิดเฟสไมโครเอกซ์แทรกชันที่อุณหภูมิ 80 องศา ในเวลา 15 นาที ผลการทดลองจะพบว่าสามารถเก็บสารประกอบทางเคมีได้มากถึง 65-81 % และเมื่อทำการวิเคราะห์จำแนกสารประกอบเคมีในตัวอย่างโดยผ่านการศึกษาค่าความความสัมพันธ์ ระหว่างสัมประสิทธิ์การกระจายตัว (log P) เปรียบเทียบกับตำแหน่งบนแผ่นทินเลเยอร์โครมาโตกราฟี จากข้อมูลการเปรียบเทียบดังกล่าวจะทำให้ทราบถึงตำแหน่งต่าง ๆ บนแผ่นทินเลเยอร์โครมาโตกราฟีได้ เมื่อเราทราบคุณสมบัติทางเคมีของสารนั้น นำมาซึ่งเราสามารถที่จะสร้างเทคนิคการวิเคราะห์สารระเหยได้ที่มีความซับซ้อนผ่านเทคนิคที่ง่ายและมีความจำเพาะเจาะจง

สาขาวิชา เคมี ลายมือชื่อนิสิต .....

ปีการศึกษา 2562 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

6171952923 : MAJOR CHEMISTRY

KEYWORD: Thin-layer chromatography Gas chromatography–mass spectrometry Headspace-solid phase microextraction Volatile compounds

Nattapat Suchatanugal : DEVELOPMENT OF THIN-LAYER CHROMATOGRAPHY FOR SELECTIVE ANALYSIS OF VOLATILE COMPOUNDS USING GAS CHROMATOGRAPHY MASS SPECTROMETRY. Advisor: Chadin Kulsing

In this study, simple and selective solid phase extraction approach for a range of volatile compounds in perfume was developed prior to analysis with headspace solid phase micro extraction (HS-SPME) and gas chromatography hyphenated with mass spectrometry (GC-MS). The technique relies on use of thin layer chromatography (TLC) for separation of compound along the silica gel plate followed by selective cuts of the regions of interest, desorption, HS-SPME and GC-MS analysis. The TLC separation of standard compounds using hexane and ethyl acetate (6:1 volume ratio) as the mobile phase was initially performed. The compounds was then desorbed from the TLC plate and extracted with SPME at 80 °C for 15 min resulting in insignificant bleeding signals (siloxane derivatives) of the plate with good linearity of the calibration curves (R2>0.98) and acceptable recovery range (65-81 %) of the volatiles after the TLC separation. The selectivity of this approach was demonstrated by cutting the plate after separation into 4 or 7 smaller parts. The SPME/GC-MS analysis of each part revealed different compound profile and the correlation between log P vs the position along the TLC plate of the standard compounds (R2=0.65). The developed 4-piece TLC based approach was further applied to analyze the perfume spiked into the synthetic agarwood sample with the strong matrix interference. Whilst, the SPME/GC-MS analysis of this sample solution revealed only 62 identified compounds (with 35 compounds from the perfume), the 4-piece approach could identify 109 compounds (including 62 perfume compounds). 15 compounds were not recovered from the TLC approach. In addition, the capability to analyze 2-phenylethanol in this complex sample was demonstrated by using the developed TLC approach. The established techniques allow simple and selective sample preparation for improved volatile compound analysis

Field of Study:	Chemistry	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....

ACKNOWLEDGEMENTS

First of all, I am very grateful to my advisor, Dr. Chadin Kulsing, for his professional, helpful guidance, valuable advice, and proofreading throughout the research project. I am also grateful to Associate Professor Dr. Vudhichai Parasuk, Associate Professor Dr. Pakorn Varanusupakul and Assistant Professor Dr. Natthida Sriboonvorakul for their kindness, useful comments and suggestions.

I would like to thank my friends for their friendship and encouragement.

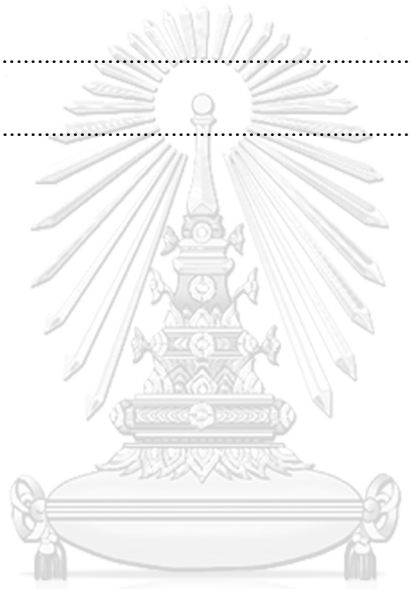
Additionally, I would like to thank Department of Chemistry, Faculty of Science, Chulalongkorn University for supplying the instruments and all facilities in this research.

Finally, I am grateful to my beloved family: my mother for her endless supporting, helpfulness and encouraging throughout my life.

Nattapat Suchatanugal

TABLE OF CONTENTS

Page



LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

1.1 Problem definition

Perfume has been a product for collection used as jewelry for human skin which shows people characteristic and reflects the mood. The global market is large and has been continuously growing at reasonable valuations over a decade, emphasizing an average growth of more than 5 % per year. Despite the economic and financial crisis in 2008, astonishingly, the global business has been multiplied from the past 15 years (US$9.6 billion in 1995) to US$22 [1]. The perfume industries strive to develop fragrances with a variety of responses to market demands. A wide range of natural and synthetic components refers to several chemical classes of these complicated matrices [2]. The sources of fragrances mixtures and chemical structures widely affected scents in perfume products [3]. Currently, the fragrance compounds are used as components of perfume products obtained from more than 3000 chemical substances, while a mixture of 20 to up 200 among these assembles the fragrance mixtures [4]. The perfume industries were improved and analytical techniques were established to identify chemical components in perfume products. Usually, the chemical components of the fragrance were odor active volatile compounds. Gas chromatography-mass spectrometry (GC-MS) is one of the popular techniques for their analysis, which is used to obtain profiles of semi-volatile and volatile organic compounds [5]. Due to insufficient selectivity and limited number of peak capacity of GC-MS, this technique often suffers from analysis of sample with strong matrix interference or multi-component samples containing several hundred compounds. To this end, multidimensional separation system such as comprehensive two-dimensional gas chromatography (GC×GC) or hyphenation with higher dimensional MS

can be applied to provide sufficient analysis capability [6],[7]**.** However, this involves various factor including complicated instrumental setup, high-cost of maintenance, or difficulty in data interpretation. The objective of this research is to establish a novel, simple and fast analysis method based on application of thin-layer chromatography (TLC) as a sample preparation approach for improved selectivity and peak capacity in 1D-GC-MS.

1.2 Literature review

Gas chromatography—mass spectrometry (GC—MS) has been widely used to obtain profiles of volatile and semi-volatile organic compounds in a wide range of samples such as essential oil, perfume, petroleum, food, beverage, biological and environmental [1],[2],[3]. GC—MS allows quantification of target compounds via generation of calibration curves or analysis of untarget compounds by comparison of their mass spectra with available libraries and match with the retention index data from literatures [4].

Thin layer chromatography (TLC) is one of the most simple and multipurpose methods with fast analysis time, low cost, high sensitivity, and good reproducibility [8],[9]. TLC coupled with a UV lamp has been widely applied to visualize products in organic synthesis as well as the application in other disciplines, such as pharmaceutical, industrial, environmental, toxicology or food analysis [10]. As in the case of gel electrophoresis for protein/DNA separation [9], TLC can be used as sample preparation approach towards specific compounds for the subsequent chromatographic and MS analysis. TLC has been used for improved analysis of terpenes, terpenoids, phenolic acids and flavonoids in herb samples which was performed by TLC separation of the sample and selection of regions containing the target analytes before high performance liquid chromatography (HPLC) -MS analysis [11],[12]. It is still a challenge to develop

and apply TLC as a simple platform for selective analysis of volatile compounds and further analysis with GC-MS, which combines complementary selectivities of polarity based separation (TLC) and boiling point based separation (GC). For the sampling approach from the TLC plate prior to injection into GC system, Solid Phase Microextraction (SPME) can be applied offering a simple extraction process where volatile compounds in the sample headspace (HS) can be adsorbed onto the SPME fiber with the solvent removal [13], [14].

1.3 Aim, scope and expected benefits of this work

The objective of this work is to develop a selective and straightforward technique for analyzing volatile compounds in complex perfume products. To this end, TLC based technique was developed and applied for extraction of volatile compounds. The TLC extracted volatiles were desorbed and analyzed by HS-SPME GC—MS. Different experimental conditions were initially investigated with the standard compound mixture in order to select a suitable condition. Recoveries of the compounds from the extraction were then investigated. The technique was further applied for analysis of in a perfume sample mixed with the higher content of synthetic agarwood to represent high matrix interference. The selective TLC extraction was discussed according to different volatile compound profiles in different TLC parts.

CHAPTER II
THEORY

2.1 perfume products

The origin of perfume in the world evidence runs back for thousand years when Egyptians using flowers, waxes, and resins in religious ceremonies [2]. Recently, perfumes are widespread in our daily lives. They are applied in various household products and cosmetics.

Perfume is one of the popular products that consist of many chemical components. Odors play a critical role in lives, affecting psychophysics. Regarding civil scents that can have a calming impact or make one sense more enjoyable, uncivil scents can change our mood negatively and produce tension and discomfort. We can smell perfumes that are responsible for civil redolence. Each perfume is made up of numbers of aromatic chemicals, each of which has specific odor and contributes to the particular aroma of the perfume. Therefore, it is essential to summarize the odor perception of perfume products. In a subsequent order, as time passed, people apply fragrance into cosmetics. Hundreds of ingredients (fragrances and other ingredients) are used as perfume in cosmetic products, which together give a unique character of scent. They are applied in all cosmetics such as skin care products, hair treatment, shaving creams, hairsprays or shampoos. Perfume products are hydroethanolic solutions prepared from essential oils and aromatic chemicals. They are called differently according to the perfume content as Perfume, Eau de parfum, Eau de Toilette, Eau Fraiche, Eau de Cologne, Cologne.

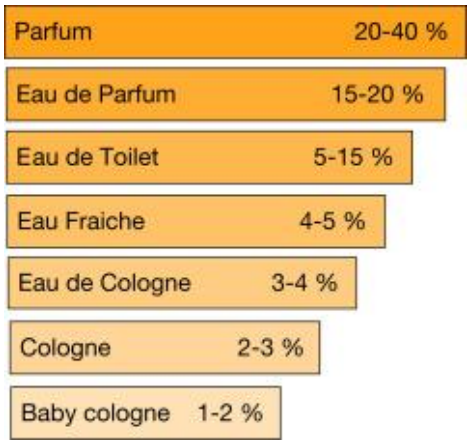


Figure 1 Approximate concentration of perfume in different types of fine fragrances [15].

Most chemical compounds in perfume products are volatile or semi-volatile compounds. They can be classified into three groups according to 1) characteristic of odor; the character of smell can chance a button of perfume product changing with time due to the fact that the rate of evaporations are not the same for all the compounds. This is recognized as triangle (top, middle, base) notes [16],[17]. The most volatile fragrances justified to the top note are perceived immediately after the application of the perfume. The less volatile ones are in the middle note and should be more wholly perceived after the top note, continuing for a few hours. Lastly, the base note group contains less volatile compounds, which are more clearly perceived after the middle note fading away and last for several hours or even days. 2) Chemical structure, such as aldehydes, alcohols, lactones, esters and terpene derivatives [18]**.** 3) Source; fragrances extracted from natural materials can be divided into plant and animal sources. The largest sources are different parts of plants (such as barks, flowers, blossoms, fruits or woods) which contain essential oils as well as aroma compounds; whilst, fragrances can also be extracted from animal components such as musk, ambergris of civet**.** However, synthetic aroma compounds are also applied to reduce

perfume costs and avoid the risk of poor crop quality or other inconsistency in plant production. In some cases, the synthetic compounds are not observed in nature [19].

2.2 Gas-chromatography (GC)

Gas chromatography (GC) is one of the chromatographic techniques used for separating compounds which can be vaporized without decomposition

The purpose of GC is to obtain proper separation of analyte peaks in chromatograms. The process starts with injection of a mixture of liquid or gaseous compounds into a heated injector, and the sample is vaporized. This can be performed manually or automatically using an auto injector. Usually, the injection mode is either split or splitless. Selection the mode depends on analyte concentrations in samples. The splitless mode is suitable for analysis of analyte at trace levels (<0.01 %); whilst, analysis of samples with relatively high concentrations is practically performed under split mode.

The vaporized compounds are then flushed into the GC column located inside an oven by a mobile phase or carrier gas, *e.g.* helium, hydrogen and nitrogen. The GC column contains a solid or liquid stationary phase material. The characteristic of polarities of stationary phases depends on their chemistries such as the number of fluorinated alkyl or phenyl groups where the polarity increases with higher phenyl content. Different chemical and physical properties of compounds affect separation result in GC captured by their different retention times. Compound elution order depends on their boiling point and interaction (*e.g.* polar/non-polar) with the stationary phase, such as highly volatile analysts weakly interacting with the stationary phase eluting earlier. For analytes with similar boiling points, the more polar ones elute later on a column containing more polar stationary phases. To this end, overall separation performance in GC can be optimized through changing experimental conditions such

as oven temperature program, stationary phase-type, column dimensions, and flow rate. This changes either the theoretical plate number or selectivity in the separation. Next, the vaporized compounds elute at the end of the column to be detected by a detector. Lastly, a chromatogram (a plot of the detection signal vs retention time) was obtained.

Various types of detectors have been applied with the GC system. The most popular detection is expected to provide fast response, stability, efficiency of operation, low dead volume, low detection limit and wide linearity range. The common detectors include mass spectrometer (MS), electron-capture detector (ECD), nitrogen phosphorous detector (NPD), thermal conductivity detector (TCD), flame photometric detector (FPD) and atomic emission detector (AED).

2.3 Mass spectrometry

The GC system has been widely coupled with MS. The MS system is a suitable and vital tool for volatile compound analysis because it offers selective detection and qualitative characterization of each separate peak with significant signal. The combination of the GC system with the MS system is also valuable for efficient quantitative analysis [20].

2.3.1 Ion source: Electron ionization

After the introduction by Dempster in 1918 [21], electron ionization (EI) is generally used to ionize gas-phase atoms or molecules by using a beam of energetic electrons (70 eV) at low pressure. This removes an electron from a molecule leading to a molecular radical cation. This process also results in fragmentation of the radical cation into the smaller fragments, as has been recognized by the term "hard" or “electron impact” ionization. The generated molecular and fragment ions are then

directed toward a mass analyzer inside MS. The efficiencies of ionization and production of fragment ions depend strongly on analyte chemistry.

2.3.2 Mass analyzer: Quadrupole

The mass analyzer then differentiates these produced ions according to their mass to charge ratios (*m/z*). Various types of mass analyzers have been applied depending on the analysis aims, for example, focusing on MS resolution, mass range, scan rate, linearity range or detection limits. The most common type applied with GC-MS system is quadrupole (Q) that consists of a set of four electrodes arranged over each other. As shown in **Figure 2**, ions from EI source moving through Q are separated based on their *m/z*. For a particular moment, only ions with a single *m/z* value can pass the Q to a detector. The selection of target ions with specific *m/z* values is achieved by applying the particular Radio frequency (RF) and Direct Current (DC) voltages to the electrodes. This corresponds to an oscillating electric field working as a bandpass filter for the target ions.

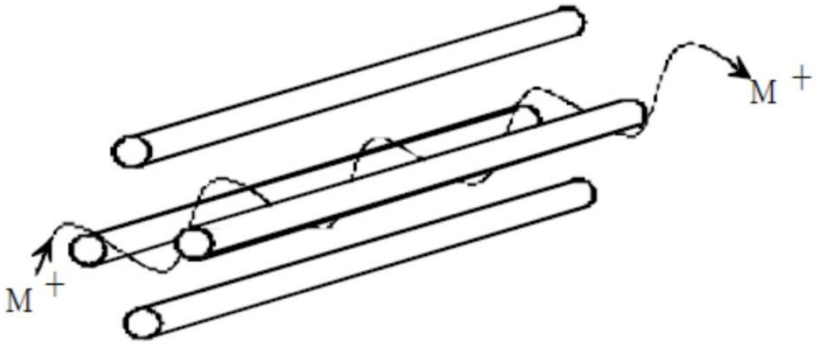


Figure 2 Diagram showing quadrupole mass analyzer [22].

2.3.3 Ion detector

Ions from the mass analyzer are detected by a detector according to their momentum or charge, which can be converted into the current signal. For large-signal generation, Sequence assembles of faraday cup is commonly applied. A collector is also applied to amplify the signals of ions, with the concept similar to the photomultiplier tube where the signal can be continuously modified for several times along the tube. The signal increase can be tuned by modifying the voltage inside the detector. The performance of the detector relies on its speed, gain, geometry, sensitivity, and dynamic range.

2.4 Gas chromatography-mass spectrometry (GC-MS)

In a field of chemical analysis, when was identify and separate chemical that volatile compounds and requires high resolution, excellent reliability, repeatability. The popular technique that using was gas chromatography (GC), which a technique connected with mass spectrometry (MS) applying a range of ionization methods. Volatile and semi-volatile compounds can be to separate and identify via GC. The reduced boiling points of volatile and semi-volatile compounds derivatized to result. The Performance of the GC system adjusted by modify experimental conditions such as temperature, flow rate, or stationary phase-type. The type of stationary phase significantly affects analyte peak positions in chromatograms, which impact to different interactions between each analyst with stationary phases. Any number of stationary phases synthesized for another separation purposes [23].

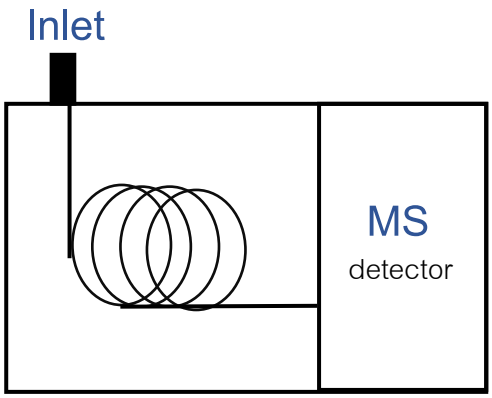


Figure 3 GC diagram

The Application of GC involved the analysis of fatty acid methyl ester (FAME) [24], hydrocarbons in petrochemical [25], environmental, food, beverages, and fragrance [26]. However, chemical components in a perfume product are tough to identify for one-dimensional gas chromatography-mass spectrometry (1D GC-MS); therefore, it must apply the complicated technique is two-dimensional gas chromatography (2D GC-MS) [27].

2.5 Headspace solid-phase microextraction (HS-SPME)

HS-SPME is one of the most widely used techniques for sample preparation before sending to separation and identification with GC-MS. This technique offers a simple extraction process where volatile compounds in the sample headspace (HS) can be adsorbed onto the SPME fiber with the solvent removal which has been applied to analyze volatile organic compounds from a variety of samples [28]. This work applies the HS-SPME technique to extract fragrances after TLC separation step, prior to analysis by GC-MS. With this approach, volatile compounds in sample HS can be adsorbed (extracted) onto the SPME materials. For example, divinylbenzene based fibers followed by direct injection into the introductory part of the GC system. The

equipment of the SPME technique consists of two parts which is fiber and holder. The show SPME in **Figure 4**.

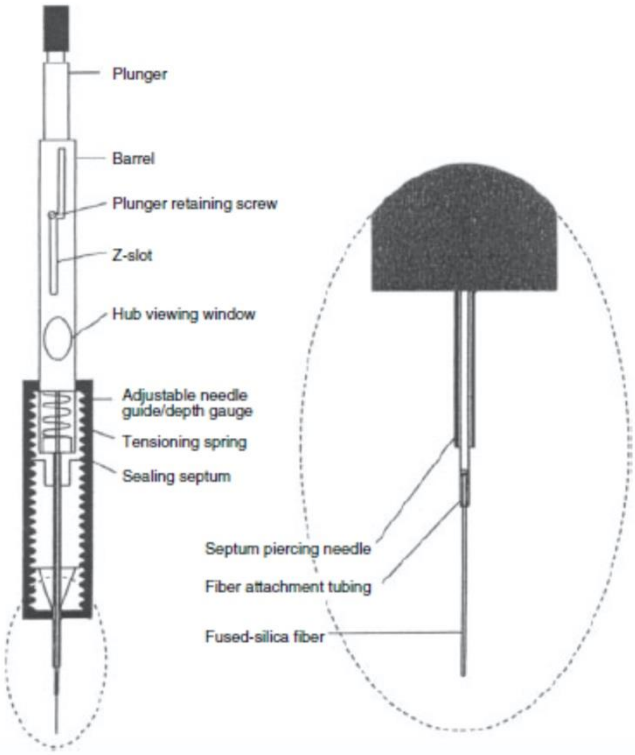


Figure 4 The construction of SPME [29].

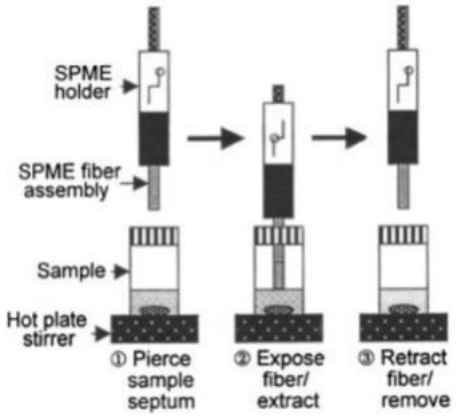
Other SPME materials can also be applied for improved performance in extraction of several compounds as shown in **Table 1.**

Table 1 Commercial SPME fiber taken from [29].

Fibre coating	Film thickness (μm)	Polarity	Coating method	Maximum operating temperature (°C)	Technique	Compounds to be analysed
Polydimethylsiloxane (PDMS)	100	Non-polar	Non-bonded	280	GC/HPLC	Volatiles
PDMS	30	Non-polar	Non-bonded	280	GC/HPLC	Non-polar semivolatiles
PDMS	7	Non-polar	Bonded	340	GC/HPLC	Medium- to non-polar semivolatiles
PDMS–divinylbenzene (DVB)	65	Bipolar	Cross-linked	270	GC	Polar volatiles
PDMS–DVB	60	Bipolar	Cross-linked	270	HPLC	General purpose
PDMS–DVB[a]	65	Bipolar	Cross-linked	270	GC	Polar volatiles
Polyacrylate (PA)	85	Polar	Cross-linked	320	GC/HPLC	Polar semivolatiles (phenols)
Carboxen–PDMS	75	Bipolar	Cross-linked	320	GC	Gases and volatiles
Carboxen–PDMS[a]	85	Bipolar	Cross-linked	320	GC	Gases and volatiles
Carbowax–DVB	65	Polar	Cross-linked	265	GC	Polar analytes (alcohols)
Carbowax–DVB[a]	70	Polar	Cross-linked	265	GC	Polar analytes (alcohols)
Carbowax-templated resin (TPR)	50	Polar	Cross-linked	240	HPLC	Surfactants
DVB–PDMS–Carboxen[a]	50/30	Bipolar	Cross-linked	270	GC	Odours and flavours

[a] Stableflex type is on a 2 cm length fibre.

a. Extraction step for HS-SPME



b.Thermal desorption on the GC system injection port.

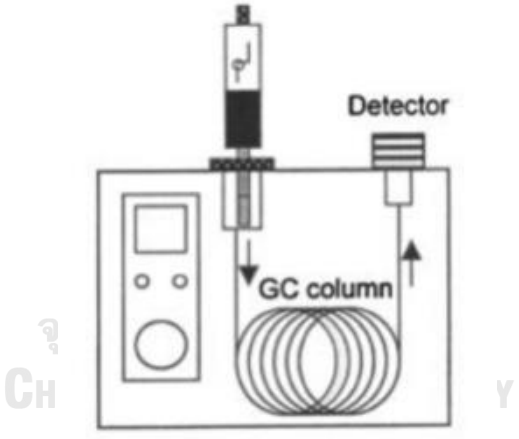


Figure 5 The extraction proceed of HS-SPME [29]. a) Extraction step for HS-SPME, b) Thermal desorption on the GC system injection port.

2.6 Thin-layer chromatography (TLC)

Since thin-layer chromatography (TLC) was established in the late 1930s [30], [31], this technique is among the most simple and multipurpose methods with fast analysis time, low cost, portable, high selectivity, and good reproducibility**.** TLC coupled with a UV lamp has been widely applied to visualize products in organic

synthesis as well as the application in other disciplines, such as pharmaceutical, industrial, environmental, toxicology or food analysis. This technique is similar to other types of chromatography, which applies mobile phase and stationary phase to separate a sample. The TLC plate contains thin layer of adsorbent substance, ordinarily silica gel or aluminum oxide, which is known as stationary phase. The sample was applied to the plate and solvent mixture was applied as mobile phase the movement of which is based on capillary action.

Movement of the solvent through the stationary phase forces solute to move upward the plate top. The analytes moves along the plate with the adsorption and desorption mechanisms. The zones of separation will be spread out because of the analyte diffusion, irregular movements of individual analyte molecules and variations in the arrangement of the sorbent. The analytes strongly interact with the sorbent move more slowly and use more time in the sorbet. On the other hand, the analytes moving faster more weakly interact with the stationary phase (or more soluble in the mobile phase). Thus, the compounds with various properties can be classified from one another by using the diverse interactions between the solutes and the stationary and mobile phases.

TLC can be classified according to the mechanisms of separation. The first parameter is the adsorption or the physical sorption of the solutes onto the stationary phase particles. The second parameter is the partition or the dissolution of the solutes into a stationary liquid on the sorbent. The third parameter is the ion exchange or the attraction among the ions and of the oppositely charged functional groups of the sorbent. The final parameter is the size exclusion or the retention base on size. The dipole-dipole and hydrogen bonding also affect to adsorption and partition mechanisms. Normal phase TLC (NP-TLC) sorbent is polar resulting in that the more polar solutes move more slowly and stay closer to the sample loading position while the nonpolar solutes move faster and get closer to the solvent front. Separation results

can be further varied by changing the polarity and functional group of the mobile phase. On the other hand, the reverse-phase TLC (RP-TLC) employs nonpolar sorbent interacts more strongly with nonpolar solutes, and the polar compounds thus move faster. The greater separation of the nonpolar compound zones can be achieved by increasing solvent polarity.

2.6.1 Selection a TLC sorbent and solvent

General stationary phase materials are based on silica or silica gel (SiO_2). The silica gel consists of oxygen bonded silicon with residual hydroxyl groups which is considerably polar and can be used in NP-TLC. Modification of the material leads to other types of stationary phase employed in different applications as shown in **Table 2**.

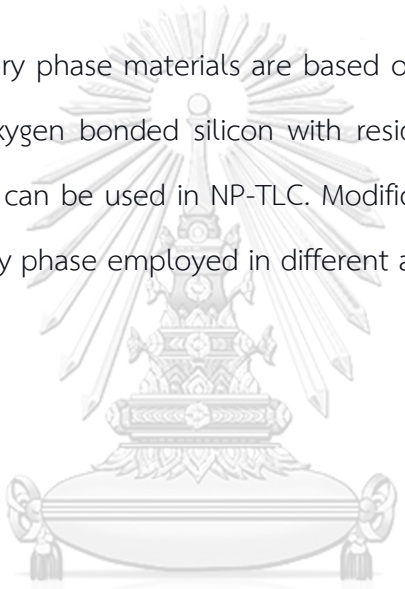


Table 2 The sorbents can be attached to plastic, aluminum, or glass plates [32].

Sorbent	Modification	Classes of compounds able to separate
Silica gel		All classes of compounds
	Amino-bonded	Carbohydrates, aflatoxins, herbicides, and tetracyclines
	Cyano-bonded	Many classes. Particularly, pesticides, steroids, and preservatives
	Diol-bonded	Many classes. Particularly, steroids and hormones
	Reversed-phase	Steroids, tetracyclines, phthalates, antioxidants, lipids, barbiturates, capsaicins, aminophenols, and fatty acids
	Chiral modified	Enantiomers of amino acids, halogenated, *N*-alkyl, and α-methyl amino acids, simple peptides, and catecholamines
	Impregnated with silver nitrate	Lipids
	Impregnated with caffeine	Polyaromatic hydrocarbons
	Impregnated with boric acid/phosphate	Carbohydrates
Kieselguhr		Carbohydrates, aflatoxins, herbicides, and tetracyclines
Cellulose		Amino acids and derivatives, food dyes, and carbohydrates
Polyamide		Phenols, flavonoids, and nitro-compounds
Aluminum oxide		Basic compounds, steroids, terpenes, and aromatic and aliphatic hydrocarbons

Apart from the stationary phase, mobile phase also affects the separation result. Single solvent with a range of polarity can be used in the separation. More polar solvent elutes polar analytes faster in NP-TLC; whilst, more hydrophobic solvent elutes analytes faster in RP-TLC. Mixed solvent can also be applied in order to finely tune the polarity of the mobile phase improving separation performance.

CHAPTER III

EXPERIMENTAL

3.1 Instruments and apparatus

3.1.1 The system that identifies target compounds using Gas chromatography-Mass spectrometer (GC-MS)

Receive from Agilent Technologies with GC Model 7890A and MS Model 7000 (CA, USA), which this system consists of autosampler and column oven. The MS system consists of electron ionization (EI), triple quadrupoles, and MassHunter software processing.

3.1.2 The column system

Using the HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, USA)

3.1.3 The equipment sample preparation

Using SPME 50/30 μm DVB/CAR/PDMS fiber, Supelco (Sigma-Aldrich, Bellefonte, PA)

3.1.4 The holder of SPME

The holder take form Supelco (Sigma-Aldrich, Bellefonte, PA)

3.1.5 TLC plates

The plates were purchased from Merck, USA. The plates were cut into a dimension of 2.5 cm × 10 cm before use.

3.1.6 Carrier gas

Using ultra-high purity helium (99.999 %), Linde

3.1.7 Micropipette

3.1.8 Water bath

3.1.9. Thermometer

3.1.10 Glass vial HS 20-mL

The vial take form Agilent technologies (USA)

3.1.11 Aluminum cap

The cap was sealed PTFE/silicone septum, Agilent technologies (USA)

3.1.12 Crimper

3.2 Chemicals

The sample of this work using the perfume solution takes from a local supermarket, Thailand. A mixture of n-alkanes (C8-C20, purchased from Sigma Aldrich) was used as a reference to calculate the retention index (I) of the analyte peaks. The mobile phase select hexane and ethyl acetate (analytical grade) were obtained from Merck (NJ) and J.T. Baker (NJ), sequentially.

3.3 TLC separation

The process of TLC separation was used for separation, which the same separation method of the standard mixture and the sample. In the first step, The TLC plate was cut into a dimension of 2.5 cm × 10 cm before applying. In the second step, The loading sample was three-times (5 uL each) onto the TLC plate (2.5 cm × 10 cm) by micropipette. In the final step, The separation process used hexane and ethyl acetate as the mobile phase with the separation time of 15 min.

3.4 Selective TLC extraction

According the **Figure 7** as show in this work used two-sample, which standard mix and the agarwood spiked with the perfume with the ratio of 1:3. The TLC plate (10 cm × 2.5 cm) was loaded by the perfume sample amount 15 μL. This work divide two identical TLC plates (called Plate total of the TLC plate and B), which contains each sample behind separation with the process described above handled differently. The total of the TLC plate was applied as the full TLC plate analysis experiment, which it after separation was cut into the smaller parts (2.5 cm × 2.5 cm each) total of which were then merged and concurrently analyzed by SPME GCMS. Plate B was cut divide into four parts behind the separation (Part A1, Part B2, Part B3, and Part B4) each piece the same length of 2.5 cm that covering the regions from the top of the TLC plate to the loading position below. Every part was separated and analyzed by SPME GCMS, respectively. The desorption process of SPME used temperatures of room temperature 28, 40, 60, 80 ^{o}c, which apply with total of the TLC plate approach before the SPME GCMS analysis. The temperature of 80 ^{o}c was additionally applied for Plate B approach.

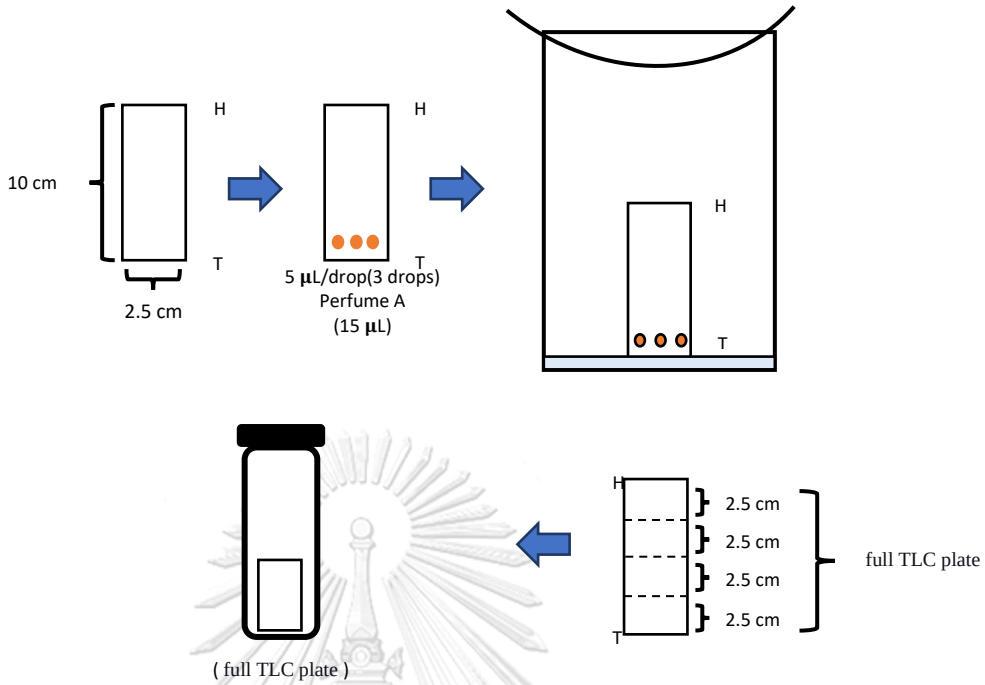


Figure 6 The TLC separation process and the Plate A approach.

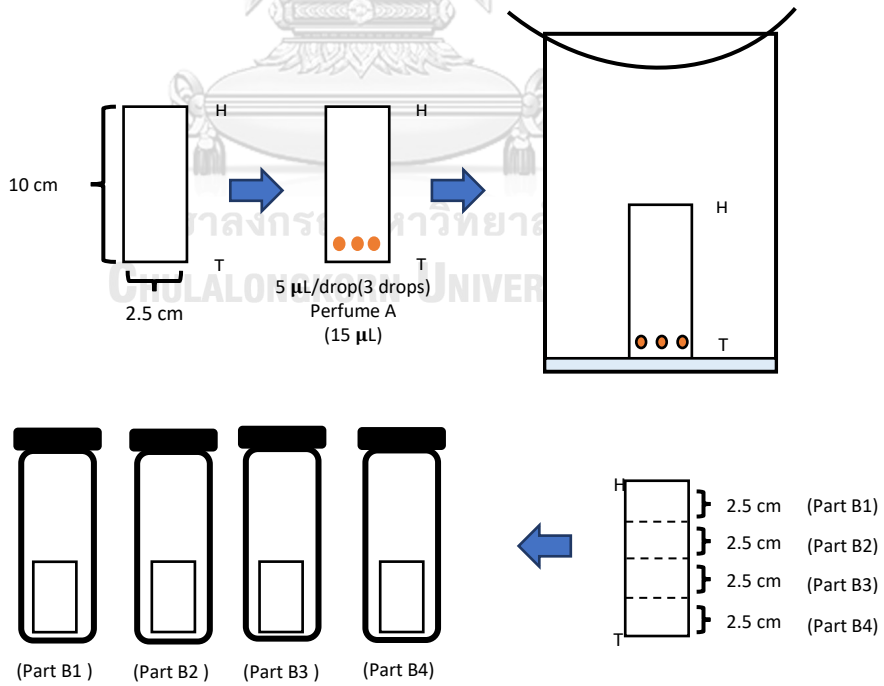


Figure 7 The TLC separation process and the plate B approach.

3.5 Analysis of volatile compounds

3.5.1 GC analysis

The process of analysis of volatile compounds was starting to set blank fiber by injection to check the background signal from the fiber of SPME. The GC system separation was optimized for analysis of perfume samples, which it used an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, USA). This insertion into the GC injection port in conditioned of 270 ^{o}c for 1 hr. The injection port of the GC-QqQMS system was set the temperature at 250 ^{o}c and desorption time in 5 min, which analyzation was applied under single Q scan mode with other quadrupoles operated in the total transfer of ion mode. The carrier gas flowed it with a flow rate of 2.25 mL/min. The injection mode used condition of split mode. The oven of the GC system established a program to increase from 40 to 260 ^{o}c at a rate of 7 ^{o}c /min. The MS system in the ion source was set the temperature at 230 ^{o}c and in the electron ionization voltage was -70 eV. The mass spectra obtained over the mass range of 30-300 Da with a scan time of 10 ms.

3.5.2 HS-SPME

To receive a high peak area of volatile compounds in perfume samples, which has many parameters of HS-SPME sample preparation, such as time, extraction temperature, and type of SPME (thickness and coating material). This work choose SPME 50/30 μm DVB/CAR/PDMS fiber and the holder were purchased from Superlco (Sigma-Aldrich, Bellefonte, PA). Starting, Prepare glass vials (20 mL) would contain the perfume solution (15 μL), Plate total of the TLC plate ,B, C, and D samples (the TLC perfume behind the extraction in the TLC chamber). The glass vials were closed by

aluminum caps that sealed PTFE/silicone septa, which was heated in a water bath. The extraction process used an equilibrium time of 10 min at different temperatures (28, 40, 60, and 80 ^{o}C) and extraction time of 15 min at a similar temperature used in the equilibrium step that revealed inside each vial to extract volatile compounds in the headspace of the sample by the SPME fiber.

Agilent MassHunter software was used to identify the chromatographic peaks of perfume and standard solutions based on MS data. The data presentation and processing were additionally performed using Microsoft Excel. The MS spectra of compounds were tentatively compared with those obtained from the NIST library. The criteria to identify the peaks were match scores of more than 650 and the differences of 20 units from the calculated retention indices (*I*) and the *I* data from the literatures on the similar stationary phase.

The process of calculating *I* value for a peak in the chromatogram was performed by injection of an alkane mixture under the same experimental conditions used for the sample separation according to the van den Dool and Kratz relationship. The *I* values can be calculated according to [33].

$$I = 100n + 100\left(\frac{t_{R(i)} - t_{R(n)}}{t_{R(n+1)} - t_{R(n)}}\right)$$

From this equation, the $t_{R(i)}$ is the retention time of peak *i*, *n* and *n*+1 are the carbon numbers of alkane standards elution before and after the peak *i*.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 TLC extraction

Commonly, the TLC technique is processed to separate a sample and the separated components can be seen on the TLC plate irradiated with a lamp at a specific wavelength. This work used the TLC technique combined with SPME GC-MS to analyze complex perfume samples and a mixture of standard volatile compounds. The potential of the TLC technique that can extract volatile compounds from the TLC plate was initially investigated to indicate significant recoveries of the volatile compounds from the TLC plate. Then, TLC was applied to separate a complex sample onto different TLC plate positions followed by selective cuts of the target regions prior to the SPME GC-MS result analysis.

4.2 Analysis of standard sample

Standard volatile compounds were separated from each part on the TLC plate prior to GC-MS analysis in order to reduce complications of complex samples.

TLC technique capability to separate volatile compounds was developed with standard samples to understand that the concept involves the effect of desorption temperature on the recovery from the TLC plate, selectivity of TLC extraction, and application of selective TLC extraction approach.

The extraction of volatile compounds from a perfume mixture was developed TLC method as solid-phase extraction technique for selective sample preparation before SPME GC-MS analysis. The procedure was originally applied to analyze the standard mixture of volatile compounds with different log *P* values. Plate A approach

was applied to investigate the effect of temperature for desorption of the analytes from the TLC plate. The recovery of the analyte from the TLC plate was compared with the SPME analysis of the standard solution without TLC separation. The demonstrated analysis of selectivity extraction was obtained from different TLC cuts. The TLC was cut into 4 parts (Plate B approach) after sample separation. The synthetic agarwood consisting of complex matrix was then applied to extract on Plate B.

4.2.1 Effect of desorption temperature on the recovery of standard compounds from the TLC plate

This experiment was developed to optimize the condition in process of SPME sampling. The effect of temperature on desorption of various standard volatile compounds from the TLC plate after loading without separation (see Plate A approach in the experimental section) was investigated. The SPME was used to sample the desorbed volatile compounds in headspace of the standard mixture followed by the GC-MS analysis with the results shown in **Figure 8.** with the compound profiles in **Table 3.** The process using the desorption temperature and the SPME extraction temperature was similar. The higher temperature increased the desorption rate and led to a larger total peak area of the extracted sample.

However, the impact of high temperature further resulted in high inference peaks caused by bleeding of the coating materials (e.g. siloxane derivatives) on the TLC plate. The result showed that the desorption and extraction temperature of 80 ^{o}C was selected due to providing high peak area with an insignificant level of the interference (**Figure 8D**). This experiment was applied to Plate A (see **Plate A** approach in the Experimental section) approach at 80 ^{o}C. The standard compound calibration curves were constructed with the concentration range from 1-150 ppm. Good linearity rages were obtained with the R^2 of $\geq$ 0.900. The compounds were well recovered from

the TLC plate, with the data presented in **Table 3**. The desorption and the SPME extraction temperature at 80 °C was recoveries varied from 65.4 to 81.3 % depending on either their volatility or interaction with the TLC plate after sample loading. For example, compounds ***S1*** and ***S3*** have roughly the same boiling (~205 ^{o}C).

The recovery of this topic compares peak area between of standards solution with standards solution on TLC plate, which it was showed ability of compounds extraction of this work. The recovery of compounds ***S3*** was higher due to the weaker interaction with the silica gel on the TLC plate captured by the higher log *P* value of 1.6 (lower polarity) compared with log *P* of 1.1 of compounds ***S1***. When considering topic of molecular weight, the comparison between the other phenyl ketones, 1-phenyl-1-pentanone (***S8***) with the highest molecular weight is least volatile. This compound showed the lowest recovery. The different compounds have different recoveries towards some compound extraction at low-temperature could be perceived as shown with ***S2, S5,*** and ***S6***. The dominant specific compounds desorbed from the TLC plate were shown in (**Figure 8A**). However, their recoveries were <3 % (**Table 3**).

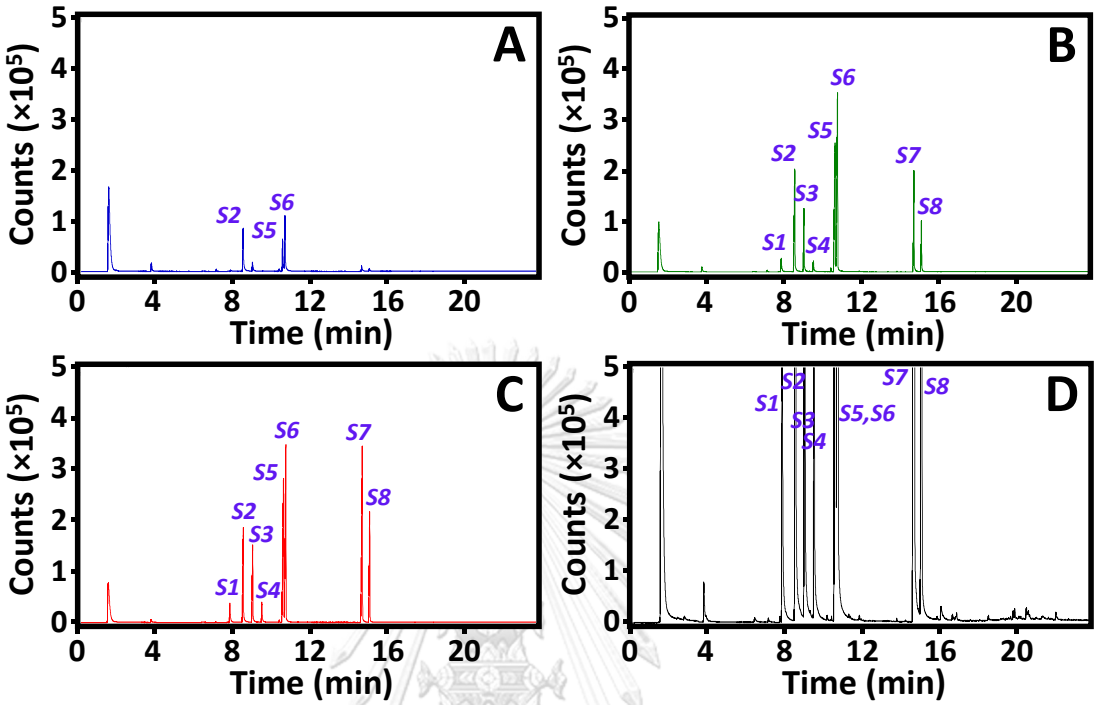


Figure 8 SPME-GC—MS results of TLC plate loaded with the standard mixture (50 ppm) without separation by using TLC desorption temperature of 28, 40, 60 and 80 °C, A-D, respectively. The compound labels were shown in **Table 3**

Table 3 Standard volatile compound profiles with logarithmic partitioning coefficients (log p), calibration curves, peak areas (counts·s) in the solution mixture and that prepared on a TLC plate without separation prior to analysis with SPME GC—MS at different TLC desorption temperature

#	Compound	log *p*	Calibration curve at 80°C (R^2)	Peak area in solution at 80°C ($\times 10^4$)	Recovery from TLC (%)			
					28°C	40°C	60°C	80°C
S1	Phenylmethanol	1.1	y =6.54x+-5.95 (0.99)	331.1	2.2	4.6	5.2	66.7
S2	Acetophenone	1.6	y =22.86x+9.16 (0.989)	1200.5	1.6	4.9	4.3	70.1
S3	2-Methoxy phenol	1.3	y =13.66x-10.53 (0.992)	724.9	2.5	6.4	8	80.5
S4	2-Phenylethanol	1.4	y =7.25x+-7.3 (0.988)	370.3	2.2	3.9	5.2	65.4
	2-Ethyl-hexanoic acid*	2.6	y =0.01x+0 (0.997)	0.6	0.5	5.3	5.7	76.2
S5	1-Phenylpropanone*	2.1	y =4x+8.09 (0.999)	222.4	3.8	7.5	8.1	81.3
S6	2-Phenylpropionic acid*	1.8	y =4.11x+6.02 (0.999)	213.5	1.8	5.9	5.8	80.4
S7	Propyl benzoate*	3.0	y =12.89x16.57 (0.992)	777.8	9	10.3	12.3	75.8
S8	1-Phenyl-1-pentanone*	3.2	y =40.28x+-48.67 (0.986)	2012.1	0	0	0	74.4
	Cyclohexaneacetic acid	2.9	y =0.32x1.91 (0.979)	25.3	0	0	0.5	79

*Calibration curves and peak areas obtained from EIC at 134, 150 and 123 for S5, S6 and S7, respectively, due to their coelution with the other peaks.

4.2.2 Selective volatile compound extraction from the TLC plate

The TLC plate was divided into four equal regions containing compounds, called as Part B1-B4 (see Plate B approach in the Experimental section). The method from each part of this work was separately analyzed with SPME GC-MS. **Figure 9** showed the results of selectivity in extraction of different compounds on the TLC plate, which depends on the selected part.

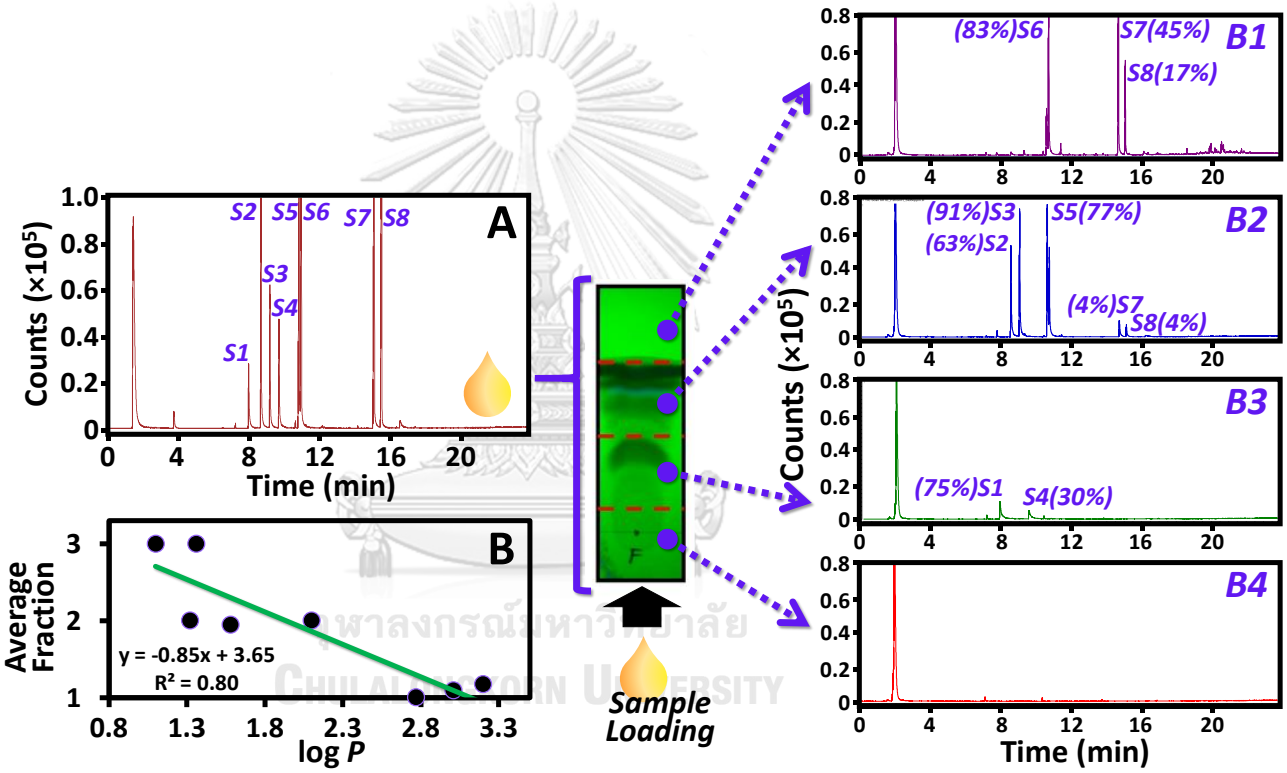


Figure 9 SPME GC—MS results of (A) the standard mixture solution (50 ppm) and (B) correlation between log *P* and the weight average fraction containing each compound after extraction using Plate B approach with the corresponding GCMS chromatograms shown on the right (B1-B4, from the top to the bottom of the TLC plate, respectively), by using TLC desorption temperature of 80 °C. The compound labels were shown in **Table 3** and the recoveries (%) compared with the standard solution were shown in parenthesis.

The compounds in the standard solution were separated onto the TLC plate, subsequently desorbing at 80 ^{o}C. Several, the result showed that the compounds were greatly recovered from the different fraction of the TLC plate. The recovery is shown in **Figure 9**. From results can be inferred, the polar property of compounds affected the position of separated compounds on the TLC plate. The more polar compounds were captured by lower log *P*-value, which more strongly interacted with the silica gel. The polar compounds were located at the bottom region the closest to the sample loading position of the TLC plate. The correlation between log *P* and the weighted average fraction containing each compound was shown in **Figure 9B**. From the result, Part B1, B2 and B3 mostly contained compounds "**S6**, **S7**, **S8**", "**S2**, **S3**, **S5**" and "**S1, S4**", respectively. The extraction selectivity can be increased by cutting the TLC plate (after separation) into the smaller pieces as shown by the perfume analysis with less number of compounds obtained by cutting TLC plate into smaller pieces (7 pieces). For example, 2-phenylethanol and 1,4-diethoxybutane were almost isolated from the complex matrix of the perfume sample. See the experimental approach and further discussion in **4.3 Selective TLC extraction (4- vs 7-piece approaches)**.

4.3 Selective TLC extraction (4- vs 7-piece approaches)

This work expected to minimize the interference in the perfume solution. When seeing the experiment of **4.2.2 Selective volatile compound extraction from the TLC plate**, which was selected by cutting the TLC plate (after separation) into 4 parts. From the concept of cutting the TLC plate to become smaller pieces, the hypothesis of increasing 4 parts of smaller pieces can improve efficiency. Commonly, the TLC plate was cutting into 4 parts will be divided into 2.5 x 2.5 cm dimension for each of the TLC plate. If increase the TLC cutting of 4 **(C1, C2, C3, C4)**

to 7 parts **(D1-D7)**, which divide into 1.25 x 2.5 cm for each of the TLC plates. However, the two types (**C type, D type**) of cutting approach were compared.

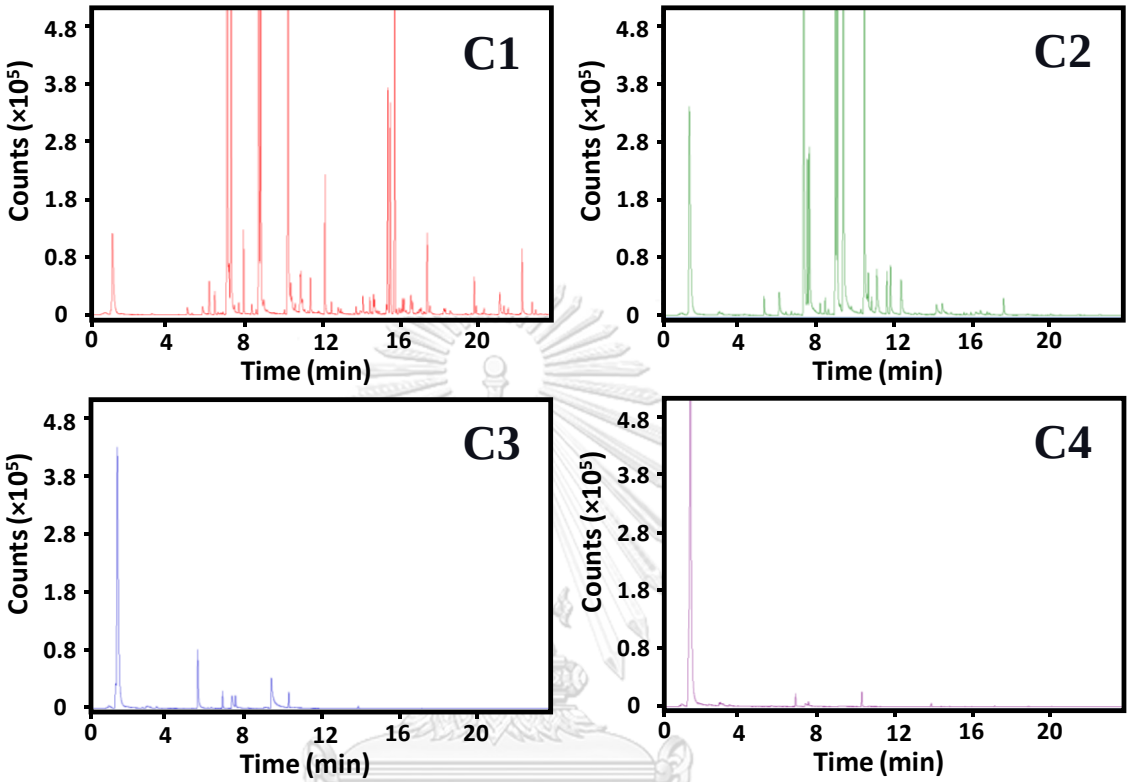


Figure 10 SPME/GC-MS results of the perfume solution (unknown sample) extracted onto the TLC cut after separation into 4 pieces (Plate C1-C4, from the top to the loading position at the bottom of the TLC plate respectively) by using TLC desorption temperature at 40 ^{o}C.

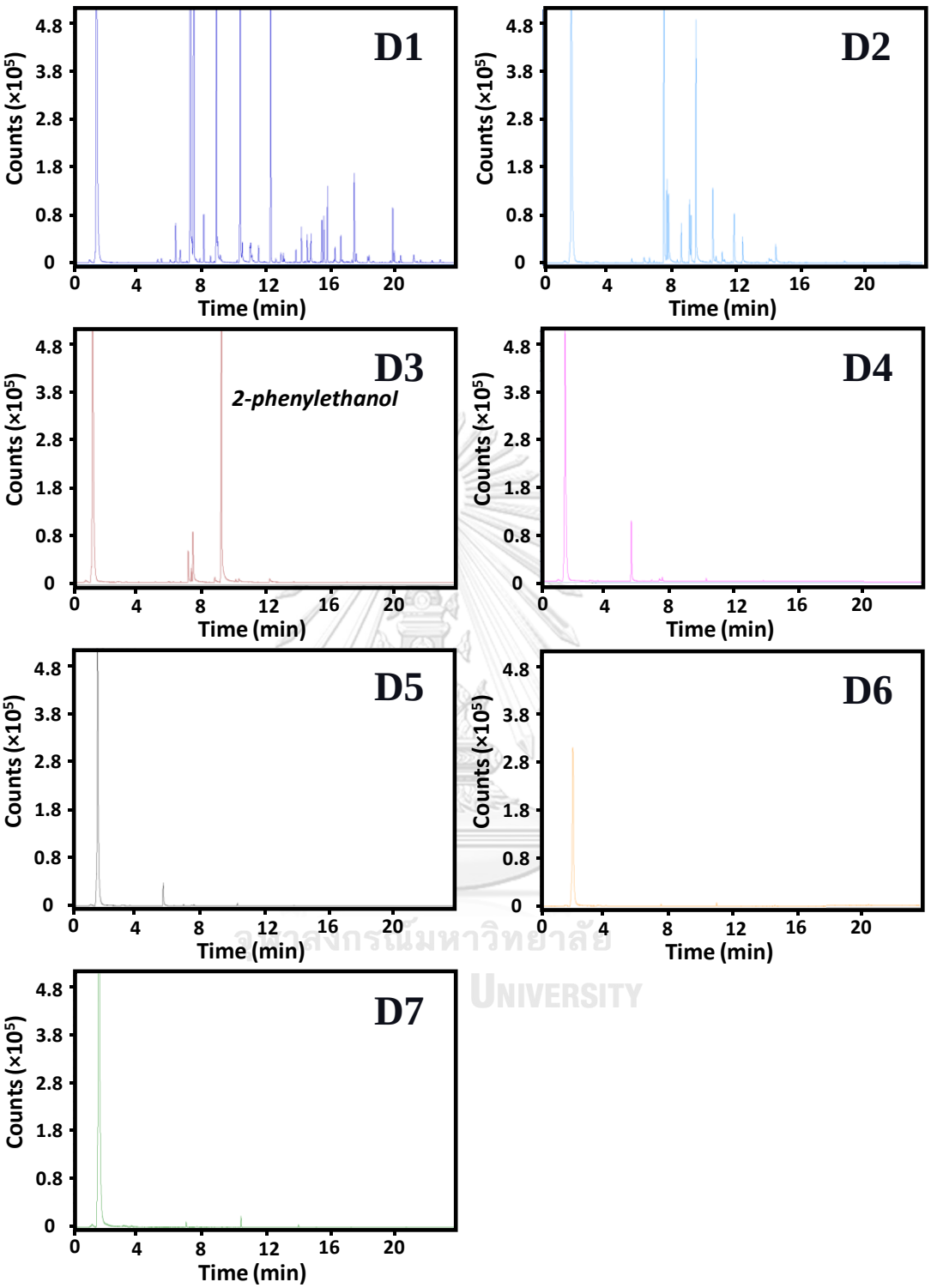


Figure 11 SPME/GC-MS results of the perfume solution (unknown sample) extracted onto TLC cut after separation into 7 pieces (Plate D1-D7, from the top to the loading

position at the bottom of the TLC plate respectively) by using TLC desorption temperature at 40 °C.

The major volatile compounds of the perfume solution (unknown sample) were benzene, 1-methoxy-4-methyl-, methyl benzoate, phenyl ethyl alcohol and phenyl methyl acetate. These contributed to the overall sweet, floral and fresh odors, respectively. After loading the perfume onto the TLC plate and the subsequent desorption temperature at 40 °C, compounds were well recovered from the plate. (based on their peak areas in TLC before separation compared with the perfume solution, see **Table 4**).

Table 4 Volatile compound profiles with literature retention index (I_{lit}), logarithmic partitioning coefficient (log p), peak area and odor description in the perfume sample prepared by using TLC at different fractions cut within every 2.50 and 1.25 cm from the top to bottom of the TLC plates (C1-C4 and D1-D7 respectively) using TLC desorption temperature of 40 ^{O}C prior to analysis with SPME GC-MS. The experiments were performed in triplicate.

Compound	I_{lit}	log *P*	Peak area in perfume solution ($\times 10^4$)	Revcovery (%)					Revcovery (%)							
				C1	C2	C3	C4	Sum	D1	D2	D3	D4	D5	D6	D7	Sum
Benzaldehyde	962	1.5	36.6	20 (±11)	49 (±23)	27 (±23)	0 (±0)	96	11 (±4)	26 (±7)	2 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	39
β-Myrcene	991	4.2	28.2	20 (±8)	8 (±2)	44 (±38)	1 (±1)	73	17 (±8)	1 (±1)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	19
Benzene, 1-methoxy-4-methyl-	1021	2.7	3603.4	16 (±13)	8 (±3)	56 (±48)	0 (±0)	80	9 (±8)	1 (±1)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	11
γ -Terpinene	1060	4.5	62.7	29 (±12)	3 (±1)	75 (±65)	1 (±1)	109	27 (±13)	7 (±2)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	34
Acetophenone	1065	1.5	0.5	101 (±87)	52 (±45)	0 (±0)	0 (±0)	153	0 (±0)	84 (±37)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	84
2,4-Heptadienal, 2,4-dimethyl-	1090	1.9	30.2	12 (±5)	11 (±7)	27 (±23)	0 (±0)	50	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0
Methyl benzoate	1094	2.1	3436.7	11 (±6)	12 (±7)	22 (±19)	0 (±0)	45	3 (±1)	23 (±9)	2 (±2)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	28
2-Phenylethanol	1116	1.4	940.2	38 (±33)	31 (±14)	3 (±1)	0 (±0)	72	0 (±0)	4 (±3)	24 (±10)	6 (±2)	0 (±0)	0 (±0)	0 (±0)	33
Phenyl methyl acetate	1164	2.0	5016.9	13 (±7)	56 (±45)	14 (±13)	0 (±0)	84	17 (±13)	31 (±14)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	49
Ethyl benzoate	1171	2.6	29.6	16 (±14)	7 (±4)	38 (±33)	0 (±0)	60	27 (±12)	17 (±15)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	44
Methyl anthranilate	1343	1.9	16.2	19 (±7)	238 (±182)	38 (±33)	0 (±0)	294	18 (±16)	32 (±14)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	50
Eugenol	1357	2.3	27.3	11 (±9)	153 (±121)	0 (±0)	0 (±0)	164	30 (±26)	51 (±7)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	82
Di-epi-α-cedrene	1382	4.1	1.1	27 (±24)	0 (±0)	58 (±50)	0 (±0)	85	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0
Caryophyllene	1419	6.8	141.5	24 (±15)	1 (±1)	46 (±39)	0 (±0)	71	18 (±3)	1 (±1)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	19
cis-Thujopsene	1429	6.1	155.5	33 (±21)	2 (±1)	65 (±56)	1 (±0)	101	22 (±3)	1 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	23
Cinnamyl acetate	1446	2.9	25.7	8 (±7)	166 (±140)	0 (±0)	0 (±0)	174	8 (±7)	25 (±12)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	32
Caryophyllene	1454	6.8	7.6	21 (±14)	0 (±0)	40 (±35)	0 (±0)	61	18 (±4)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	19

3-(4-Isopropylphenyl)-2-methylpropionaldehyde	1466	3.4	25.2	32 (±18)	87 (±72)	12 (±11)	0 (±0)	131	21 (±6)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	21
Lilial	1533	4.2	154.1	27 (±12)	64 (±54)	15 (±13)	0 (±0)	106	17 (±5)	10 (±8)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	27
3-Epi-cedrenol, methyl ether	1654	4.4	6.1	9 (±5)	4 (±4)	0 (±0)	0 (±0)	14	88 (±70)	117 (±94)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	205
(-)-Isolongifolol, methyl ether	1672	4.1	22.9	38 (±31)	0 (±0)	22 (±19)	0 (±0)	61	11 (±5)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	11
Caryophyllene acetate	1704	4.0	0.1	75 (±37)	0 (±0)	0 (±0)	0 (±0)	75	93 (±43)	87 (±75)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	0 (±0)	180

The summation of the recovery based on peak area of some compounds extracted with C1-C4 or D1-D7 could be >100 % which indicated selective sample cleanup resulting in purer peak signals of the compounds with higher peak area compared with the same peak observed in the perfume solution (coeluting with the other peaks).

The plate was cut into 8 pieces (Part D1-D7) prior to the analysis of each piece with SPME/GC-MS with the result shown in **Figure 11**. Compared with the individual peak obtained from the 4-piece approach (**Figure 10**), each piece contained smaller numbers of compounds suggesting higher selectivity of the 7-piece approach. However, the overall recoveries of the several compounds were reduced as shown in the 'sum' column for the Plate D approach in **Table 4**. As the result, 2-phenylethanol was almost isolated from the perfume mixture by selectively analyzing Part D3.

4.4 Application of selective TLC extraction approach

This work aims to increase the selectivity of separation. This was demonstrated by spiking interference in sample solution. The analysis with interference can be performed to test of selectivity for the TLC approach which should selectively extract volatile compounds for in the synthetic agarwood. Plate B is used to develop TLC condition and it was applied to analyze perfume compounds (**Figure 12A**) in a mixture of the perfume solution and the synthetic agarwood (interference). Application of Plate B that contains sample solution and the synthetic agarwood allowed identification of the perfume compounds. The analysis of the sample solution by direct SPME/GC-MS method showed only 62 identified compounds. **Figure 12B** indicated the compounds in column "Solution" with the peak areas of >0 in **Table 5** with only 35 perfume compounds and 27 interferences. The overall analysis with Plate B results in 109 identified compounds, see the chromatograms B1-B4 in **Figure 12** with 62 perfume

compounds and 47 interferences as in **Table 5**. Analysis of a mixture of the sample solution and the synthetic agarwood represented the strong matrix interference from the agarwood leading to either signal suppression or false negative identification of several perfume compounds in the direct analysis of the sample solution. These compounds were highlighted in blue italic in **Table 5**. The perfume compounds (blue italic) in **Table 5** were not identified due to either the strong matrix interference from the agarwood during the SPME sampling or coelution with the interference peaks in GC-MS.

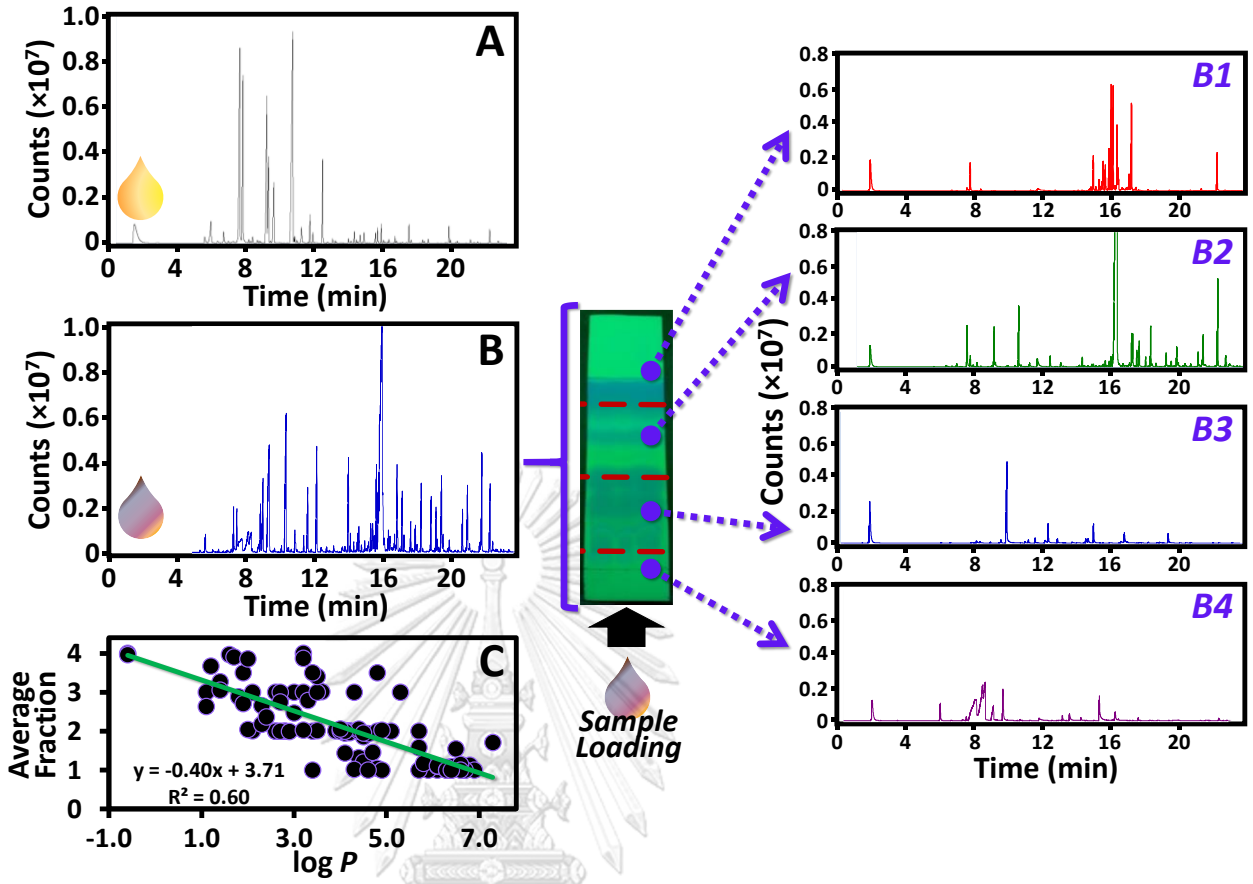


Figure 12 SPME/GC—MS results of (A) perfume solution and (B) mixture the perfume solution and synthetic agarwood, and (C) correlation between log P and the weight average fraction containing each compound after extraction using Plate B approach with the corresponding GCMS chromatograms shown on the right (B1-B4, from the top to the bottom of the TLC plate, respectively), by using TLC desorption temperature of 80 ^{o}C.

Table 5 Perfume compound of sample solution (**1**-**70**) and interference (**Int1**-**Int54**) profiles with literature retention index (I_{lit}), logarithmic partitioning coefficient (log p), average peak area (n = 4) and odor description in mixture of the perfume and synthetic agarwood prepared by using TLC at different fractions cut within every 2.50 cm from the top to bottom of the TLC plate (B1-B4, respectively) using TLC desorption temperature of 80 °C prior to analysis with SPME/GC—MS. The experiments were performed in triplicate with the blue italic perfume compounds solely observed using the developed TLC approach (not observed in the sample solution).

#	Compound	I_{Lit}	log *p*	Odor description	Peak area (×10⁴ counts·s)				
					Solution	B1	B2	B3	B4
1	3-Methyl-2-butenyl acetate	918	1.7	sweet fresh banana fruity	91	0 ±0	0 ±0	0 ±0	0 ±0
2	***1,4-diethoxy-butane***	***940***	***1.8***		***0***	***0 ±0***	***0 ±0***	***22 ±16***	***43 ±43***
3	Benzaldehyde	962	1.5	strong sharp sweet bitter almond cherry	61	0 ±0	35 ±14	8 ±1	0 ±0
Int 1	3-Ethoxy-ethyl propanoate	971	1.1		5	0 ±0	0 ±0	0 ±0	4 ±4
4	4-methylene-1-(1-methylethyl)-bicyclo[3.1.0]hexane	974	3.9	woody terpene citrus pine spice	44	10 ±3	7 ±4	0 ±0	0 ±0
5	***β-Pinene***	***979***	***4.2***		***0***	***0 ±0***	***2 ±2***	***0 ±0***	***0 ±0***
6	β-Myrcene	991	4.2	peppery terpene spicy	21	6 ±1	35 ±4	2 ±0	0 ±0
Int 2	2-(2-ethoxyethoxy)-ethanol	1007	-0.5	slightly ethereal	30	0 ±0	0 ±0	0 ±0	5 ±2
7	1-(2-methoxypropoxy)- 2-propanol	1010	-0.4		51	0 ±0	0 ±0	2 ±2	11 ±7
8	1-methoxy-4-methyl- benzene	1021	2.7	naphthyl, phenolic, camphoraceous, minty	674	49 ±7	452 ±36	21 ±15	0 ±0
9	***2-methyl-5-(1-methylethenyl)-, (1α,2α,5β)- cyclohexanol***	***1026***	***3.2***	***minty menthol spearmint herbal***	***0***	***0 ±0***	***0 ±0***	***0 ±0***	***5 ±5***
10	1-methyl-5-(1-methylethenyl)-, (R)- cyclohexene	1027	4.4		1551	389 ±81	171 ±43	5 ±2	0 ±0
11	***Benzyl alcohol***	***1036***	***1.1***	***floral rose phenolic balsamic***	***0***	***0 ±0***	***0 ±0***	***26 ±4***	***0 ±0***
Int 3	β-Ocimene	1037	4.2		0	0 ±0	17 ±2	0 ±0	0 ±0
12	***(Z)- 1,3,6-Octatriene3,7-dimethyl-***	***1038***	***4.2***	***tropical, green, terpy and woody***	***0***	***0 ±0***	***0 ±0***	***0 ±0***	***0 ±0***

#	Compound	I_{Lit}	log *p*	Odor description	Peak area (×10⁴ counts·s)				
					Solution	B1	B2	B3	B4
Int 4	1,1'-oxybis-2-propanol	1038	-0.6		0	0 ±0	0 ±0	0 ±0	865 ±93
13	***Benzeneacetaldehyde***	***1045***	***1.8***	***honey, floral rose, sweet***	***0***	***0 ±0***	***2 ±2***	***15 ±5***	***0 ±0***
14	***2-(2-hydroxypropoxy)- 1-propanol***	***1046***	***-0.6***	***slight alcoholic***	***0***	***0 ±0***	***0 ±0***	***36 ±31***	***979 ±372***
15	***3,7-dimethyl-1,3,7-octatriene***	***1047***	***4.1***	***fruity floral wet cloth***	***0***	***2 ±1***	***39 ±5***	***0 ±0***	***0 ±0***
16	***γ-Terpinene***	***1060***	***4.5***	***terpy, sweet, citrus, with tropical and lime***	***0***	***19 ±4***	***5 ±3***	***0 ±0***	***0 ±0***
17	2,6-dimethyl-7-octen-2-ol	1064	3.0	fresh citrus lime floral clean cologne weedy	33	0 ±0	0 ±0	8 ±3	0 ±0
Int 5	1,1,3-triethoxy- propane	1076	1.6		617	0 ±0	0 ±0	6 ±4	185 ±46
18	***1-methyl-4-(1-methylethylidene)- cyclohexene***	***1088***	***4.5***	***sweet, fresh, piney citrus, woody***	***0***	***1 ±0***	***6 ±3***	***0 ±0***	***0 ±0***
Int 6	Methyl benzoate	1094	2.1	phenolic wintergreen almond floral	703	5 ±2	413 ±25	3 ±2	3 ±2
19	Linalool	1099	3.0	citrus, orange, floral, terpy, waxy and rose	1396	0 ±0	42 ±21	34 ±21	1 ±1
20	2-phenylethanol	1116	1.4	sweet, floral, fresh, rosey, honey	4162	0 ±0	0 ±0	1441 ±389	534 ±187
Int 7	1,3,3-Trimethylcyclohex-1-ene-4-carboxaldehyde	1138	2.8		69	0 ±0	9 ±3	0 ±0	0 ±0
Int 8	1,3,4-trimethyl-3-cyclohexene-1-carboxaldehyde	1154	2.8		99	0 ±0	24 ±4	0 ±0	0 ±0
21	Phenyl methyl acetate	1164	2.0	sweet, fruity and floral	3698	2 ±2	917 ±161	15 ±3	14 ±6
Int 9	5-methyl-2-(1-methylethyl)- cyclohexanol	1169	3.2	cooling mentholic minty	0	0 ±0	0 ±0	12 ±7	0 ±0
22	Ethyl benzoate	1171	2.6	sweet, wintergreen, fruity	33	0 ±0	11 ±4	0 ±0	0 ±0
23	Levomenthol	1175	3.4	peppermint cooling mentholic minty	45	0 ±0	0 ±0	0 ±0	0 ±0
Int 10	4-methyl-1-(1-methylethyl)-, [1S-(1 α,3 α,4 α,5 α)]-bicyclo[3.1.0]hexan-3-ol	1176	2.0	cedar leaf, thujonic	30	0 ±0	0 ±0	0 ±0	0 ±0
24	α-Terpineol	1189	2.7	pine terpene lilac citrus woody floral	492	0 ±0	19 ±19	72 ±36	0 ±0
25	***Methyl salicylate***	***1192***	***2.6***	***wintergreen mint***	***0***	***0 ±0***	***49 ±13***	***0 ±0***	***0 ±0***
Int 11	1-methyl-4-(1-methylethylidene)- cyclohexanol	1197	2.6	terpineol lilac	0	0 ±0	0 ±0	5 ±3	0 ±0
Int 12	Dodecane	1200	6.1		0	7 ±1	0 ±0	0 ±0	0 ±0

#	Compound	I_{Lit}	log *p*	Odor description	Peak area (×10⁴ counts·s)				
					Solution	B1	B2	B3	B4
Int 13	Decanal	1206	4.0	sweet, aldehydic, orange, waxy and citrus	0	0 ±0	0 ±0	0 ±0	0 ±0
Int 14	1,2,4-triethyl- benzene	1207	3.4		0	2 ±1	0 ±0	0 ±0	0 ±0
Int 15	4-bromo- benzaldehyde	1217	2.3		0	36 ±32	81 ±61	16 ±12	24 ±14
26	(2,2-dimethoxyethyl)- benzene	1222	1.7	green foliage floral rosy earthy mushroom	216	0 ±0	0 ±0	0 ±0	0 ±0
27	Citronellol	1228	3.3	floral, rosy, sweet, citrus	1246	0 ±0	106 ±106	394 ±236	0 ±0
28	Neral	1240	3.2	sweet citral lemon peel	33	0 ±0	0 ±0	0 ±0	0 ±1
29	***Geraniol***	***1255***	***3.6***	***floral, sweet, rosey, fruity***	***0***	***0 ±0***	***0 ±0***	***67 ±26***	***1 ±1***
30	Linalyl acetate	1257	3.9	sweet, green, floral and spicy with a clean, woody	2088	0 ±0	233 ±82	0 ±0	1 ±1
Int 16	3,7-dimethyl-, (E)- 2,6-octadienal	1270	3.5	citrus lemon	0	0 ±0	0 ±0	1 ±1	0 ±0
Int 17	(E)- Cinnamaldehyde	1270	1.9	sweet spicy	0	0 ±0	0 ±0	2 ±1	2 ±2
Int 18	4-hydroxy-α,α,4-trimethyl- cyclohexanemethanol	1279	1.7		0	0 ±0	0 ±0	2 ±1	19 ±9
31	Anethole	1286	3.4	sweet anise	118	0 ±0	50 ±7	0 ±0	0 ±0
32	***5-methyl-2-(1-methylethyl)-, acetate cyclohexanol***	***1294***	***4.2***	***tea-like, slightly cooling, minty and fruity***	***0***	***0 ±0***	***3 ±3***	***0 ±0***	***0 ±0***
33	***Indole***	***1295***	***2.1***	***floral, slightly naphtha***	***0***	***0 ±0***	***0 ±0***	***9 ±4***	***0 ±0***
Int 19	2,3-dihydro-2-methyl- benzofuran	1306	3.2	burnt phenolic	0	0 ±0	0 ±0	4 ±4	27 ±27
Int 20	(E)- 2-propen-1-ol, 3-phenyl	1310	2.0	cinnamon spice, floral, green	122	0 ±0	0 ±0	3 ±2	18 ±10
34	***4-(1,1-dimethylethyl)-, acetate, trans- cyclohexanol***	***1322***	***4.2***	***woody cedar floral oily herbal balsam green fruity***	***0***	***0 ±0***	***4 ±4***	***0 ±0***	***0 ±0***
35	Piperonal	1330	1.1	cherry, vanilla, sweet cherry	251	0 ±0	15 ±9	26 ±11	0 ±0
36	***4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)- cyclohexene***	***1338***	***6.4***	***sweet herbal woody***	***0***	***11 ±2***	***0 ±0***	***0 ±0***	***0 ±0***
37	***Methyl anthranilate***	***1343***	***1.9***	***fruity, concord grape***	***0***	***0 ±0***	***22 ±9***	***18 ±9***	***8 ±6***
Int 21	α-Cubebene	1351	6.3		0	5 ±1	0 ±0	0 ±0	0 ±0

#	Compound	I_{Lit}	log *p*	Odor description	Peak area (×10^4 counts·s)				
					Solution	B1	B2	B3	B4
38	3,7-dimethyl-, acetate6-octen-1-ol	1354	4.2	floral, rosy, green, fatty, citrus	1594	0 ±0	243 ±93	0 ±0	1 ±1
39	Eugenol	1357	2.3	sweet, spicy, clove like, woody	230	0 ±0	53 ±36	101 ±46	0 ±0
Int 22	octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-(1α,2α,3aβ,4α,5α,7aβ,8S*)]-1,2,4-metheno-1H-indene	1368	5.9		0	2 ±1	0 ±0	0 ±0	0 ±0
40	4-tert-Butylcyclohexyl acetate	1368	4.2	woody cedar floral oily herbal balsam green fruity	126	0 ±0	15 ±8	0 ±0	0 ±0
41	Decahydro-1,5,5,8a-tetramethyl-, [1S-(1α,2α,3aβ,4 α,8aβ,9R*)]-1,2,4-methenoazulene	1374	4.7	strong moss woody	21	25 ±3	21 ±17	0 ±0	0 ±0
Int 23	Lavandulyl propionate	1375	2.7	delicate farnesol-like	0	0 ±0	22 ±7	0 ±0	0 ±0
42	***Copaene***	***1376***	***5.7***	***woody spicy honey***	***0***	***35 ±3***	***0 ±0***	***0 ±0***	***0 ±0***
43	1,2,3,4,5,6,7,8-octahydro-1,4,9,9-tetramethyl-, [1S-(1 α,4 α,7 α)]- 4,7-methanoazulene	1381	6.1		340	464 ±60	48 ±20	0 ±0	1 ±1
44	Geranyl acetate	1382	4.0	floral, rosy, waxy, herbal and green	232	0 ±0	0 ±0	0 ±0	0 ±0
45	1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1 α,2β,4β)]-cyclohexane	1391	5.8	herbal waxy fresh	93	81 ±25	17 ±7	0 ±0	0 ±0
Int 24	Vanillin	1404	1.2	sweet vanilla creamy chocolate	215	0 ±0	4 ±4	68 ±52	159 ±70
Int 25	Longifolene	1405	6.4	sweet woody rose medical fir needle	27	40 ±6	4 ±2	0 ±0	0 ±0
46	2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, [3R-(3α,3a β,7 β,8a α)]- 1H-3a,7-methanoazulene	1411	6.3	woody cedar sweet fresh	184	289 ±72	22 ±13	0 ±0	0 ±0
Int 26	2,7-dimethyl-5-(1-methylethenyl)- 1,8-nonadiene	1414	4.8		0	164 ±21	8 ±8	0 ±0	0 ±0
Int 27	(1S,1aS,1bR,4S,5S,5aS,6aR)-1a,1b,4,5a-Tetramethyldecahydro-1,5-methanocyclopropa[a]indene	1418	4.1		0	18 ±18	14 ±14	0 ±0	0 ±0
47	***Caryophyllene***	***1419***	***6.8***	***spicy, woody and terpenic***	***0***	***365 ±57***	***50 ±20***	***0 ±0***	***1 ±0***
Int 28	α-Ionone	1426	4.0	sweet woody floral	691	0 ±0	34 ±20	0 ±0	0 ±0
48	cis-Thujopsene	1429	6.1		169	312 ±45	39 ±14	0 ±0	0 ±0
Int 29	1-methoxy- naphthalene	1434	3.5		0	0 ±0	0 ±0	163 ±60	111 ±28
49	α-Guaiene	1439	6.6	sweet woody balsam peppery	1280	1577 ±376	205 ±55	0 ±0	3 ±2
Int 30	Coumarin	1441	1.4	sweet	0	0 ±0	0 ±0	71 ±28	4 ±4

#	Compound	I_{Lit}	log *p*	Odor description	Peak area (×10^4 counts·s)				
					Solution	B1	B2	B3	B4
Int 31	1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahydro-1H-cyclopropa[e]azulene	1447	4.3		0	1171 ±185	23 ±23	0 ±0	0 ±0
Int 32	2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl-, (1α,3aα,7α,8aβ)-1H-3a,7-methanoazulene	1457	6.3		0	650 ±272	0 ±0	2 ±2	0 ±0
Int 33	Himachala-2,4-diene	1462	6.7		0	204 ±137	0 ±0	0 ±0	0 ±0
50	***3-(4-Isopropylphenyl)-2-methylpropionaldehyde***	***1466***	***3.4***	***floral cyclamen fresh***	***0***	***0 ±0***	***0 ±0***	***2 ±1***	***2 ±2***
51	***1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1α,3aβ,4α,7β)]- azulene***	***1473***	***6.9***		***0***	***20 ±12***	***0 ±0***	***0 ±0***	***0 ±0***
Int 34	Decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)- naphthalene	1479	6.7		0	36 ±23	0 ±0	0 ±0	0 ±0
52	α Isomethyl ionone	1480	4.1	woody violet floral	259	0 ±0	33 ±8	0 ±0	0 ±0
53	***β-Guaiene***	***1490***	***4.9***		***0***	***16 ±9***	***0 ±0***	***0 ±0***	***0 ±0***
Int 35	Caparratriene	1493	5.3		0	0 ±0	0 ±0	4 ±2	0 ±0
Int 36	1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1α,7α,8aα)]- naphthalene	1499	6.5		0	45 ±35	54 ±11	0 ±0	0 ±0
Int 37	1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2 α,4a α,8a β)]- naphthalene	1494	6.4		289	0 ±0	0 ±0	0 ±0	0 ±0
Int 38	2-methylene-5-(1-methylvinyl)-8-methyl- bicyclo[5.3.0]decane	1495	4.6		0	34 ±17	0 ±0	0 ±0	0 ±0
54	***1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,7α,8aβ)]- azulene***	***1505***	***6.6***		***0***	***1750 ±728***	***264 ±47***	***3 ±2***	***9 ±5***
Int 39	Butylated Hydroxytoluene	1513	5.1	mild phenolic camphor	0	0 ±0	193 ±49	0 ±0	0 ±0
Int 40	(2S,4aR,8aR)-4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	1517	-		0	16 ±4	0 ±0	0 ±0	0 ±0
Int 41	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- naphthalene	1524	6.3		0	30 ±12	0 ±0	0 ±0	0 ±0
55	1-(2,6,6-trimethyl-2-cyclohexen-1-yl)- 1-penten-3-one	1524	4.5	sweet fruity floral	57	0 ±0	0 ±0	0 ±0	0 ±0
56	Lilial	1533	4.2	floral green	882	20 ±15	183 ±56	6 ±1	3 ±1
Int 41	α-(trichloromethyl)-, acetate benzenemethanol	1538	3.5		0	0 ±0	147 ±42	0 ±0	1 ±1
57	Isoamyl salicylate	1538	4.4	floral herbal woody orchid metallic	201	0 ±0	227 ±85	0 ±0	0 ±0
Int 43	1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1α,4aβ,8aα)]- naphthalene	1538	6.6		0	12 ±6	0 ±0	0 ±0	0 ±0

#	Compound	I_{Lit}	log *p*	Odor description	Peak area (×10⁴ counts·s)				
					Solution	B1	B2	B3	B4
Int 44	1,5-dimethyl-8-(1-methylethylidene)-, (E,E)- 1,5-cyclodecadiene	1557	6.4	woody earthy spicy	0	23 ±13	0 ±0	0 ±0	0 ±0
Int 45	2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)- 2-buten-1-ol	1559	4.3	woody sandalwood greasy oily waxy	0	0 ±0	0 ±0	4 ±1	0 ±0
Int 46	Tetradecahydro- anthracene	1561	4.4		83	0 ±0	0 ±0	0 ±0	0 ±0
58	Anisyl butyrate	1569	2.9	buttery anisic floral sweet tropical fruity	458	2 ±2	261 ±196	0 ±0	0 ±0
Int 47	1-methyl-8-(1-methylethyl)- tricyclo[4.4.0.0(2,7)]dec-3-ene-3-methanol	1574	5.7	woody spicy honey	0	0 ±0	22 ±16	0 ±0	0 ±0
59	***2-hydroxy-, pentyl ester benzoic acid***	***1583***	***4.6***		***0***	***9 ±9***	***135 ±112***	***0 ±0***	***0 ±0***
60	Diethyl Phthalate	1594	2.4		1107	2 ±2	91 ±57	49 ±26	3 ±1
Int 48	Guaiol	1596	-		35	0 ±0	0 ±0	0 ±0	0 ±0
Int 49	Benzophenone	1635	3.2	balsam rose metallic	908	0 ±0	326 ±98	3 ±2	3 ±1
Int 50	Methyl (3-oxo-2-pentylcyclopentyl)acetate	1649	2.7	sweet, fruity, floral, citrus	127	0 ±0	0 ±0	5 ±5	0 ±0
Int 51	Longiverbenone	1649	-		52	0 ±0	0 ±0	0 ±0	0 ±0
61	***3-oxo-2-pentyl-, methyl ester cyclopentaneacetic acid***	***1649***	***2.7***		***0***	***0 ±0***	***3 ±3***	***9 ±7***	***0 ±0***
62	***α-pentyl- cinnamaldehyde***	***1662***	***4.4***		***0***	***0 ±0***	***91 ±27***	***1 ±1***	***2 ±1***
Int 52	1,2,3,3a,4,5,6,7-octahydro-α,α,3,8-tetramethyl-, [3S-(3α,3aβ,5α)]- 5-azulenemethanol	1667	4.8		0	0 ±0	0 ±0	3 ±2	3 ±3
63	methyl ether (-)-isolongifolol	1672	4.1		1579	0 ±0	26 ±17	0 ±0	0 ±0
64	3,7-dimethyl-10-(1-methylethylidene)-, (E,E)- 3,7-cyclodecadien-1-one	1693	4.8		190	0 ±0	36 ±20	0 ±0	0 ±0
Int 53	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	1695	-		37	0 ±0	36 ±9	0 ±0	0 ±0
65	2,3-dihydro-1,1,3-trimethyl-3-phenyl-1H-indene	1714	5.7		34	20 ±17	28 ±7	0 ±0	0 ±0
66	2-(phenylmethylene)- octanal	1755	4.9	fresh floral green jasmin herbal waxy	620	0 ±0	120 ±44	1 ±1	1 ±1
67	Benzyl benzoate	1762	4.0	faint sweet balsam oily herbal	1299	0 ±0	206 ±76	4 ±3	4 ±2
68	1-(2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulen-5-yl)- ethanone	1780	5.7	woody vetiver amber leather musk cedar	20	0 ±0	0 ±0	0 ±0	0 ±0
69	***Isopropyl myristate***	***1827***	***7.3***		***0***	***412 ±183***	***769 ±333***	***21 ±14***	***18 ±8***

#	Compound	I_{Lit}	log *p*	Odor description	Peak area (×10^4 counts·s)				
					Solution	B1	B2	B3	B4
Int 54	*trans*- β -Ionone	1846	4	floral woody sweet fruity berry tropical beeswax	252	0 ±0	0 ±0	0 ±0	0 ±0
70	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl- cyclopenta[g]-2-benzopyran	1851	4.5		1509	7 ±4	32 ±14	1 ±1	2 ±1

Plate B was developed as a method for separating the interferences from the hindered perfume compounds into different TLC parts, which were sent to the SPME/GC-MS analysis. Commonly, the position of compounds along the TLC plate depends on the correlation log *P* of each compound observed in **Figure 12**. To this end, the compounds of interference were separated into B1-B4, with amount of interference are 26, 19, 11, and 13 compounds respectively follow B1-B4. Note that there were eight perfume compounds and seven interferences that were not recovered from the TLC approach.

CHAPTER V
CONCLUSION

This thesis represents a new simple and cost effective approach for improved performance in volatile compound extraction, making contribution to selective sample preparation research which has not been previously reported. To this end, TLC based sample preparation approach was developed with the application demonstrated for selective extraction of perfume compounds in the strong matrix interference of synthetic agarwood in SPME/GC-MS analysis. The selectivity can be approximated according to log *P* value of the target volatile compounds. Improved number of identified compounds was obtained with good linearity range in the calibration curves for the selected standard compounds after the TLC based preparation. Good recoveries of the compounds were also obtained, especially for less polar and volatile compounds with not too low/high molecular weights. The limitation of low recoveries of several compounds could also be overcome by application of a larger TLC plate with increasing sample loading amount together with the improved selectivity which can be performed by cutting the smaller part of the TLC plate. Since the developed system relies on application of TLC plates, this is thus simple, fast, cost effective and applicable for selective extraction of volatile compounds with improved peak capacity.

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