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ชื่อโครงการ การพัฒนาเทคนิคโครมาโทกราฟีหลายมิติเพื่อเพิ่มประสิทธิภาพในการวิเคราะห์อาหารไทย

Development of multidimensional chromatographic technique for improved analysis of Thai food

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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของโครงงานทางวิชาการที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)

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Development of multidimensional chromatographic technique for improved analysis of Thai food

By

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In Partial Fulfillment for Degree of Bachelor of Science

Department of Chemistry, Faculty of Science

Chulalongkorn University

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Project Title : Development of multidimensional chromatographic technique for improved analysis of Thai food

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บทคัดย่อ

ในงานวิจัยนี้เทคนิคโครมาโทกราฟีสองมิติอย่างง่ายได้ถูกพัฒนาขึ้นเพื่อแยกสารประกอบในสารตัวอย่างที่มีความซับซ้อนสูงเรียกเทคนิคนี้ว่า เทคนิคลิควิดและแก๊สโครมาโทกราฟีชนิดครอบคลุม-แมสสเปกโทรเมตรี (LCxGC-MS) ซึ่งจะใช้ลิควิดโครมาโทกราฟี (LC) เป็นเทคนิคแยกสารในมิติแรกและแก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรี (GC-MS) เป็นเทคนิคแยกสารในมิติที่สอง ในงานนี้แกงเขียวหวานได้ถูกเลือกมาใช้เป็นสารตัวอย่างในการวิเคราะห์ เนื่องจากมีองค์ประกอบทางเคมีที่ซับซ้อน ซึ่งยังไม่มีการตรวจสอบองค์ประกอบทางเคมีมาก่อน โดยผลการทดลองของ LCxGC-MS ถูกนำมาเปรียบเทียบกับผลการทดลองที่ได้จาก GC-MS ในด้านของจำนวนของสารประกอบที่ตรวจพบและคุณภาพของแมสสเปกตรัมของสารประกอบ การวิเคราะห์ด้วยเทคนิค LCxGC-MS และเทคนิค GC-MS ที่ใช้กันทั่วไปสามารถระบุสารประกอบในแกงเขียวหวานได้ 106 และ 63 สารประกอบ ตามลำดับ โดยเปรียบเทียบระหว่างแมสสเปกตรัมที่ได้จากการทดลองกับระบบสืบค้น NIST นอกจากนั้นค่าความเข้มของสัญญาณที่ได้จากเทคนิค LCxGC-MS มีค่าที่เพิ่มมากขึ้นและมีความซับซ้อนน้อยกว่าการวิเคราะห์ด้วยเทคนิค GC-MS ทำให้การระบุสารสารประกอบมีความเชื่อมั่นที่สูงขึ้น ซึ่งแสดงให้เห็นโดยค่าการจับคู่แมสสเปกตรัมที่ได้จากเทคนิค LCxGC-MS มีค่ามากขึ้น นอกจากนี้สารประกอบที่มีประเภทแตกต่างกันและสารประกอบที่แสดงคุณสมบัติทางกลิ่นได้นำมารายงานและวิเคราะห์ผล การตรวจสอบนี้แสดงให้เห็นว่าเทคนิคโครมาโทกราฟีหลายมิติมีประสิทธิภาพ, ความถูกต้อง และความน่าเชื่อถือสูงกว่าเทคนิคโครมาโทกราฟีหนึ่งมิติ

คำสำคัญ: เทคนิคโครมาโทกราฟีสองมิติอย่างง่าย, เทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรี, เทคนิคลิควิดและแก๊สโครมาโทกราฟีชนิดครอบคลุม-แมสสเปกโทรเมตรี, สารตัวอย่างที่มีความซับซ้อนสูง, เทคนิคโครมาโทกราฟีหลายมิติ

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ABSTACT

In this work, simple two-dimensional (2D) chromatographic technique was developed to separate compounds in highly complex sample. The technique is called LCxGC-MS, involving the use of liquid chromatography (LC) as the first dimensional separation technique and the conventional GC-MS as the second dimensional separation. In this work, the green curry paste was selected as the sample for the analyses since it has a complicated chemical profile that has not been investigated in detail before. The LCxGC-MS results were compared with that obtained from the conventional GC-MS in the aspects of the number of identified compounds and quality of the MS spectra of the compounds. The LCxGC-MS and the conventional GC-MS technique analysis could identify 106 and 63 compounds in green curry paste, respectively, by comparison between the experimental MS spectra with that from the NIST database. Moreover, the signal intensities obtained with LCxGC-MS is enhanced and less complex than that with GC-MS analysis. This allows compound identification with higher confidence as indicated by the higher MS match scores obtained with LCxGC-MS. The compounds in different classes and the odor active compounds were also reported and discussed. This investigation emphasizes that multidimensional chromatographic technique is more effective, accurate and reliable than one-dimensional chromatographic technique.

Keywords: simple two-dimensional (2D) chromatographic technique, GC-MS, LCxGC-MS, highly complex sample, multidimensional chromatographic technique

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LIST OF ABBREVIATIONS

°C degree	degree celsius
g	gram
GC	gas chromatography
LC	liquid chromatography
MD	multidimensional chromatography
MS	mass spectrometry
min	minute
mg	milligram
mL	milliliter
μL	microliter
NIST	national institute of standards and technology
RT	retention time
MH	monoterpene hydrocarbon
SH	sesquiterpene hydrocarbon
DH	diterpene hydrocarbon
OM	oxygenated monoterpenes
OS	oxygenated sesquiterpenes
S	sulfur-containing compounds
M	miscellaneous compounds
RI	retention index

CHAPTER I

INTRODUCTION

1.1 Problem definition

Gas chromatography (GC) is a highly effective technique in the separation of mixtures. In general, gas chromatography hyphenated with mass spectrometry technique (GC-MS) is preferred since it has high sensitivity and accuracy for both quantitative and qualitative analyses.[1,2] The high resolution separation of GC-MS enables the more effective detection and identification of chemical composition in the sample.[1] In some cases, the analysis of non-volatile compounds uses derivatization technique, which changes a polar functional group into a less polar group such as silyl ethers, in order to increase volatility of the compounds before analysis by GC-MS.[1-3]

Recently, the demand for analysis of Thai food has grown significantly since these samples contain a wide range of compounds from purely natural products to processed products as well as many other products. The conventional GC techniques can separate approximately 100-200 compounds in each analysis. This cannot be used to analyze compounds in a complex sample (such as wine with more than 400 compounds).[4,7] So, the technique to separate the sample can be improved by developing multidimensional gas chromatography (MDGC),[4,5] which is a highly effective technique that separates compounds, using 2 columns that have different separation selectivity for separation of the compounds. This technique provides enhanced peak capacity to result in separation of up to 1,000-2,000 compounds in each analysis. MDGC is thus appropriate for complex sample analysis[4,6] such as wine, herb, spice or Thai food, which has components of many volatile compounds.[6]

However, application of MDGC requires the complicated and expensive instrumental setup.[4,7] In this work, alternative method using simple approach with the conventional GC-MS instrument has been applied which is called comprehensive multidimensional chromatography. This involved the use of liquid chromatography (LC) as the first dimensional separation technique and the conventional GC as the second dimensional separation (LCxGC). This method could provide improved analysis

of both volatile and semi-volatile compounds in Thai food that has not been studied. A green curry paste was selected as the sample due to its complexity in the chemical constituents since it is made from many ingredients such as chilli, lemongrass, garlic, kaffir lime peel and the others.

The analysis performance and reliability provided by LCxGC-MS was then compared with the conventional GC-MS in the aspects of the number of identified compounds and quality of the MS spectra of the compounds (indicated by the match score with the spectra from MS library).

The compounds in different classes and the odor active compounds were also reported and discussed with the benefit of the developed LCxGC approach focusing on the database with increasing numbers of compounds in Thai food.

1.2 Literature review

The concept of separation of compounds in a mixture using stationary phase was firstly reported in 1903 by Mikhail Tswett who was the first person to use the term chromatography. The German scientists discovered the principle of column LC in 30 years later. In 1952, Archer Martin, Richard Synge and Anthony James developed gas-liquid chromatographic technique. The word GC was later used and widely applied by chemical researchers of oil' big company since it is a highly effective analysis technique and can be further improved to the new technique.[8,9]

At present, the chromatography technique is popular for separation and analysis of compounds in many types of samples. GC is appropriate with sample containing volatile or semi-volatile compounds, while non-volatile compounds are popularly analyzed using LC. Both of these techniques are conventionally performed with one-dimensional approach.[7,10]

Food is one of the four-factor that has necessary for human. Many of compounds can be found in food, including both organic compounds (carbohydrates, proteins, fats, vitamins) and inorganic compounds (water, oxygen, minerals). The evaluation of quality, control and development of quality in food is important to humans. So, the method of food analysis has to involve both the main component

and the sub-component. Since food consists of many compounds, it is highly complex. Therefore, the analysis performance of one-dimensional chromatography cannot be adequate to analyze a food sample.[4,6]

Green curry has reactive volatile compounds, which are unstable in the presence of heat (Ankri and Mirelman, 1999). In previous research, green curry paste was studied. The study developed of green curry paste marinated with white shrimp in 2007 which applied GMP or hurdle technique to prepare the ingredients and the paste. This is performed in order to reduce their microbial load that may improve the antimicrobial activity of curry paste.[11] In 2015, green curry paste was studied about fortification of calcium in Thai green curry paste, which can be successfully fortified with calcium lactate and calcium gluconate. The study used chemical properties and sensory evaluation to evaluate the qualities.[12]

The main problem of one-dimensional chromatographic techniques is the limited separation capacity which is not sufficient to analyse of complex sample containing >200 compounds due to the larger number of compounds than the separation capability of the technique. So, there are co-elutions of compounds which cannot be separated in a complex sample. Therefore, MD chromatographic technique was developed with improved separation performance and can be effectively used to analyze the complex sample.

MD chromatography is a technique that uses two or more stationary phases for separation of samples prior to the detection. This employs two columns with different selectivity in separation, which subsequently increases the capability to analyze more compounds. This technique increases the resolution of measurements and is easy to use which is appropriate for complex sample analysis.[7,10]

1.2 Theory

1.3.1 Green curry paste

Green curry (Figure 1.1) is the most popular Thai dish in Thailand and worldwide, which is the Thai second most ordered dish among consumers and widely consumed in all socioeconomic classes. It was made from fresh spices and flavored

with cumin. The constituents of green curry paste are chili, lemongrass, garlic, salt, shrimp paste (shrimp, salt), kaffir lime peel, coriander root and spices (cumin, turmeric). Others are also used for flavoring, deodorizing, pungency, coloring and enhancing the taste. The ingredients of green curry paste may be different up to home or region. Many ingredients that are used in the green curry paste have been investigated to show antimicrobial and antioxidant properties with positive health benefits. [11,12]

Figure 1.1 Green curry food[13]

1.3.2 Gas chromatography (GC)

GC is the technique for separation of compounds in sample mixtures. GC system contains an injection port and an oven consisting of a mobile phase (or carrier gas) as an inert gas and a column with stationary phase inside. The separation of compounds is based on difference of boiling points (or vapor pressures) and polarities of the compounds as well as the different strengths of interactions of the compounds with the stationary phase. The compounds that have a stronger interaction with the stationary phase take longer time to move through the column. The components of gas chromatography instrument are shown in Figure 1.2. [14-19]

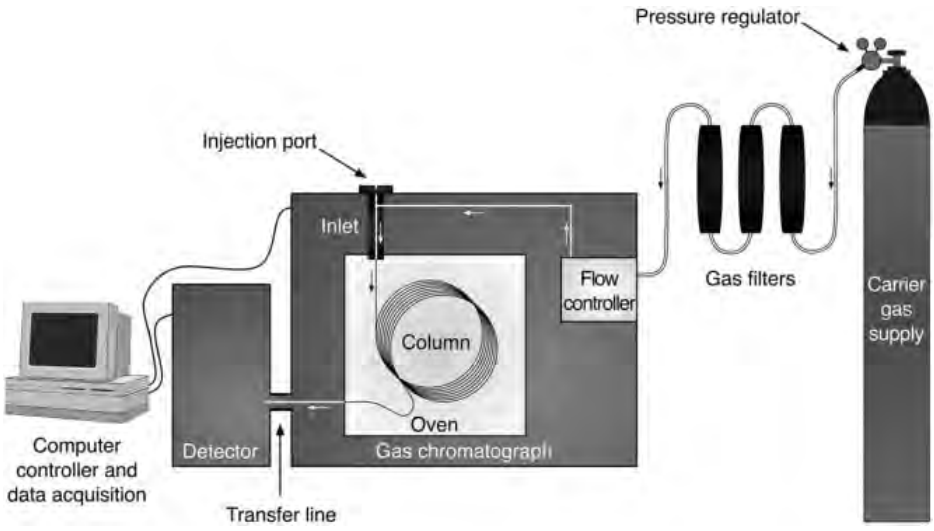

Figure 1.2 The components of gas chromatography instrument[15]

1.3.2.1 Carrier gas

The carrier gas is a mobile phase and must be an inert gas, reacting with neither the stationary phase nor the sample component. The examples include nitrogen, helium and argon, which can be purified to remove traces of oxygen, water and hydrocarbons.[16,17] The choice of carrier gas is often based on type of the applied detector which is selected to result in good detector response for analytes, as well as being easily available and inexpensive. Moreover, it is safe to use.[15,17]

1.3.2.2 Injector

An injector is a chamber that the sample is transferred into the instrument. The sample is heated by the injector and becomes vapor which is taken into the column by the carrier gas. The choice of injection system depends on the column type and the sample component. The most commonly used type with capillary columns is the heated split/splitless injector which can operate in split and splitless modes (with lower and larger injected amounts of samples, respectively) as shown in Figure 1.3. The selection of injection mode depends on compound concentrations in samples.

Both split and splitless injection modes are performed under high injection temperature which makes the solvent and samples to vaporize. The injection temperature is constant throughout the GC run. The split injection is used for neat samples with relatively high concentrations; whilst, the splitless mode is applied when samples contain analytes at trace levels.

In the split mode, the sample is injected and it vaporizes into carrier gas stream. Only the small portion of the sample and solvent is transferred onto the GC column inlet and the rest of the sample is vented to waste. The amount of analyte entering the column can be calculated as

$$\text{Split ratio} = \frac{\text{Column flow+Vent flow}}{\text{Column flow}}$$

In splitless mode, the sample is introduced into the heated linear as in split injection and it is brought into the gas phase without sample discrimination during injection. This introduces all the injected amount of the sample and solvent onto the GC column inlet. This technique is useful for trace analysis of compounds in samples or analysis of compounds with a fairly narrow boiling-point range. However, this is not suitable for injection of thermally labile compounds. [14-19]

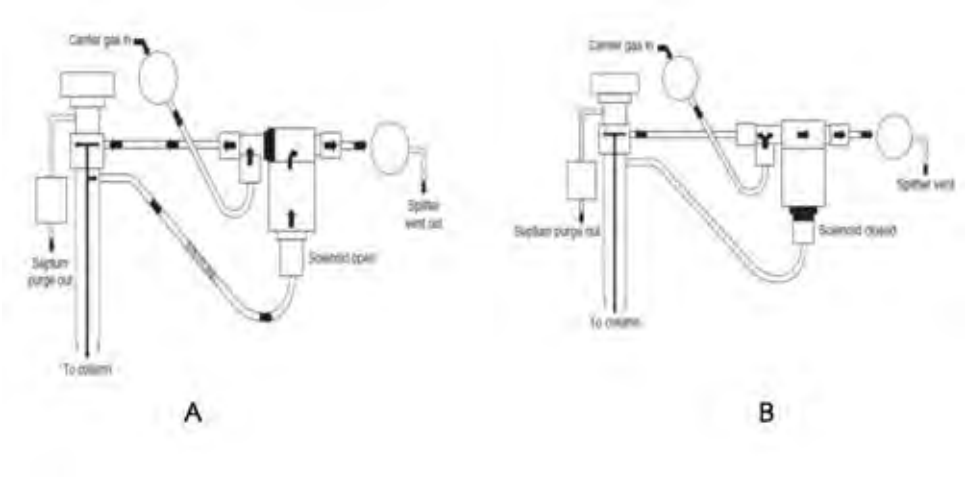

Figure 1.3 The flow path of the carrier gas in split mode, A and splitless mode, B[18]

1.3.2.3 Column

A column is connected with the injector and detector inside a GC oven. The column dimension varies in length and internal diameter depending on the application type. Packed and capillary are the two general column types. Sample components are separated onto the column with the separation result depending on interaction between stationary phase and mobile phase (as well as compound boiling points). This is mainly governed by the chemistry of stationary phase materials.[14-19]

1.3.2.4 Detector

The physicochemical property of the analyte responds to the detector. Most of the detectors are sensitive and generate electronic signals for the data system to further produce a chromatogram. The common detectors used in GC include the flame ionization detector (FID), flame photometric detector (FPD), the electron capture detector (ECD) and mass spectrometer (MS). [14-19]

1.3.3 Mass spectrometry (MS)

MS is an analytical technique that is suitable and powerful tool for compound analysis to positively identify compounds and quantify known compounds within a sample. Hyphenation with GC allows separation of compounds prior to the MS detection. A typical result is shown as a MS spectrum of each separated compound which is a plot of counts (or intensity) vs mass to charge ratio (*m/z*).[20]

1.3.4 Liquid chromatography (LC)

LC is a separation technique that used to separate a sample into its individual parts in liquid phase. The compounds in a sample mixture move along with the mobile phase through a stationary phase with different velocities which depends on interaction between the compounds and mobile/stationary phases.

One of the most widely used LC is column chromatography. This technique employs adsorbents solid adsorbents such as silica gel or alumina as

stationary phase and organic solvents as the mobile phase. The common column looks similar to a Pasteur pipette. The column's diameter is related to the scale of the sample and the length depends on the difficulty in separation. The column outlet is plugged with glass wool or a porous plate in order to support the column packing material and keep it from escaping the tube.

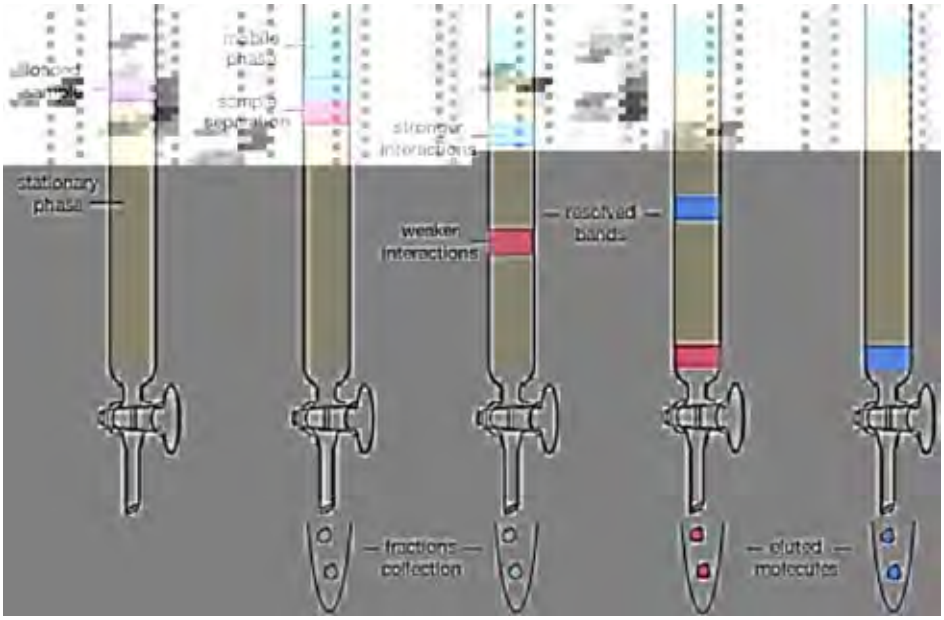

Figure 1.4 The separation of compound by column chromatography[22]

The separation is based on the properties of the compound and their interactions with the stationary phase. The compound that strongly interacts with the stationary phase is retained in the column and move slowly. In contrast, the compound that has weaker interaction with the stationary phase elutes easily and exits first as shown in Figure 1.4. [21-23]

1.3.5 Multidimensional (MD) chromatography

MD chromatography is a separation technique, which is an important analytical tool since it provides high chromatographic resolution and highly effective separation for complex mixtures and difficult to separate substances such as petrochemistry, metabolomics, environmental, and flavor and fragrance science.

MD chromatography is a powerful separation technique that has been developed in the past few years. This method is appropriate with the analysis of highly complex samples. Different separation mechanism is exploited in each dimension for improving the resolution in the separation. The separation performance is based on the sample properties and involves multiple chromatographic steps. Thus, MD chromatography offers superior peak capacity and has the potential to overcome the limitations of one-dimensional chromatography (1D) efficiently.

MD chromatography originated from simple heart-cutting techniques where only target fractions from the first dimensional separation are isolated and transferred to the second dimensional column inlet for the next separation as shown in Figure 1.5.[4-7,24-25]

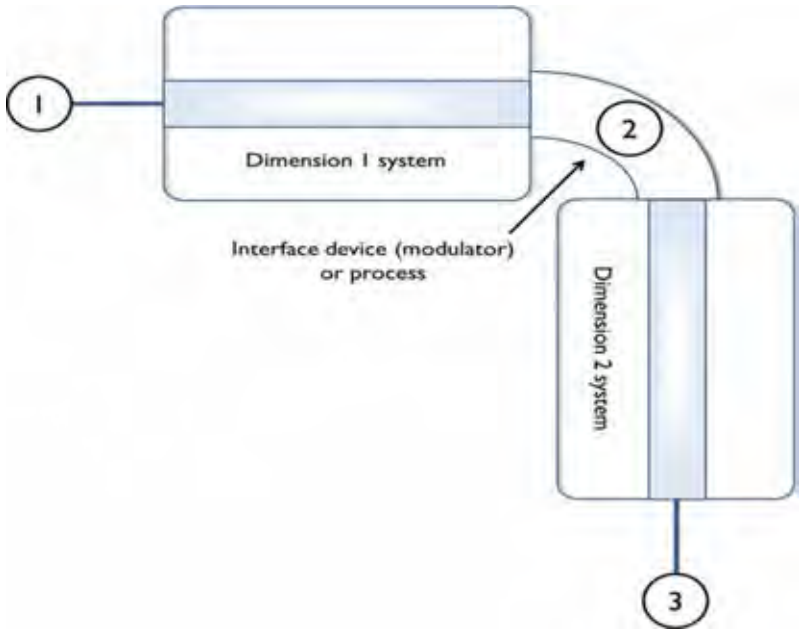

Figure 1.5 Diagram of a multidimensional chromatography system[7]

1.4 Objective and scope of study

1.4.1 Objective

1.4.1.1 To develop a MD technique (LCxGC-MS) to analyze compounds in a complex sample of green curry paste extract.

1.4.1.2 To analyze and compare the number of identified compounds and signal quality in the analysis of compounds in green curry paste extract using GC-MS and LCxGC-MS techniques.

1.4.1.3 To construct a database of compounds found in green curry paste extract using LCxGC-MS.

1.4.2 Scope of study

The scope is to analyze compounds in green curry paste sample using LCxGC-MS and compared with one dimensional GC-MS. The LCxGC-MS analysis uses liquid chromatography as the first dimensional separation. Then, all the fractions were collected and analyzed with GC-MS which can be considered as the second dimensional separation with MS detection. The quality of the results obtained from LCxGC-MS and GC-MS were then compared in term of the number of identified compounds and the MS spectrum quality of each compound.

Chapter II

EXPERIMENTAL SECTION

2.1 Instrument and equipment

2.1.1 Gas chromatograph-Mass spectrometer (GC-MS)

2.2.1.1 Gas chromatograph model 7890A, Agilent Technologies

2.2.1.2 Mass spectrometer model 7000, Agilent Technologies

2.1.2 Capillary column of HP-5MS (30m x 0.25mm x 0.25 μm)

2.1.3 Rotary evaporator

2.1.4 Glass column (diameter 3.2 cm)

2.1.5 Micropipette 100-1000 μL

2.1.6 Beaker

2.1.7 Balance (4 digits)

2.1.8 Glass vial HS 20 mL, Agilent Technologies

2.1.9 Thermometer

2.1.10 Round bottom flask

2.1.11 Filter paper (Thomas baker, 125mm)

2.1.12 Filtering Funnel

2.1.13 Silica gel obtain from Merck

2.2 Chemicals and sample

2.2.1 A mixture of n-alkanes (C8-C20) purchased from Sigma Aldrich

2.2.2 Green curry paste, Lobo (Sample) purchased from local supermarket

2.2.3 Ethanol obtain from Merck

2.2.4 Methanol obtain from Merck

2.2.5 Ethyl acetate obtain from RCI Labscan

2.2.6 Helium carrier gas

2.2.7 Dichloromethane obtain from Merck

2.3 Sample preparation

2.3.1 To the green curry paste (Lobo brand, 0.5537 g) was added dichloromethane (20 mL). The mixture was then stirred and sonicated for 5 minutes.

2.3.2 The mixture was filtered with a filter paper to separate the residue and the solution.

2.3.3 The solution was evaporated to dryness under reduced pressure using a rotary evaporator. The resulting crude extract was used for further analysis in the next steps.

Figure 2.1 Green curry paste sample, Lobo[26]

2.4 Analysis of Thai food by one-dimension chromatography (GC-MS)

The sample solution was diluted with EtOAc (3 mg/mL) and injected into GC-MS system.

2.4.1 GC-MS conditions

Transfer line temperature	: 250 °C
Injection temperature	: 250 °C
Ion source temperature	: 230 °C
MS quadrupole temperature	: 150 °C
Helium quench flow	: 2.25 mL/min
Carrier gas flow	: 1.2 mL/min
m/z range	: 30-350

2.4.2 Temperature program

Initial temperature	: 60 °C
Temperature increase rate	: 4 °C/min
Final temperature	: 300 °C and hold for 5 min
Total analysis time	: 65 min

2.5 Analysis of Thai food by two-dimension chromatography (LCxGC-MS)

2.5.1 Liquid chromatography technique (Column 1)

2.5.1.1 The silica gel was packed in the class column (diameter 3.2 cm) to a height of 11 cm. Then, hexane (100 mL) was added to wash the column.

2.5.1.2 The pump was used for pushing the eluent through silica gel until fully packed and kept washing the column with hexane until the silica gel was colorless without bubbles.

2.5.1.3 The crude extract from part 2.3 was redissolved in a minimum amount of dichloromethane then added into the column.

2.5.1.4 The column was eluted with various mixtures of solvents with increasing polarity as the eluent. The full detail of the ratio of solvents is listed in Table 2.1.

Table 2.1 The ratio eluent composition used in column chromatography (LC)

Eluent	Ratio	Volume (mL)
Hexane	-	50
Hexane : Dichlorometane	9 : 1	150
Hexane : Dichlorometane	4 : 1	150
Hexane : Dichlorometane	2 : 1	125
Hexane : Dichlorometane	1 : 1	125
Dichlorometane	-	100
Dichlorometane : Ethyl acetate	9 : 1	100
Dichlorometane : Ethyl acetate	4 : 1	100
Dichlorometane : Ethyl acetate	1 : 1	50
Ethyl acetate	-	50
Ethyl acetate : MeOH	9 : 1	50

2.5.1.5 For every 30 mL of solution that came out of the column were collected in a 50 mL round bottom flask, then the collected solution was evaporated to dryness under reduced pressure using a rotary evaporator as shown in Figure 2.2.

2.5.1.6 The fraction was dissolved in EtOAc (0.5 mL). The solution was transferred into a 2 mL vial prior to the GC-MS analysis.

2.5.1.7 The processes from part 2.5.1.4-2.5.1.6 were repeated until all the listed eluents were used up, resulting in 30 fractions as shown in Figure 2.3.

Figure 2.2 Photographs illustrating collection of fractions of green curry eluted from column chromatography (left) and evaporation of each fraction for solvent removal (right).

Figure 2.3 Photograph showing all the solutions collected into the vials prior to GC-MS analysis

2.5.2 Gas chromatography technique (Column 2)

All 30 fractions, which were collected from the liquid column chromatography were further diluted by EtOAc (0.5 mL). Then, the sample solution was injected into GC-MS system using the conditions described in Section 2.4. Injection of a standard alkane solution (*n*-alkane, C8-C20) was also performed under the same GC-MS

conditions. The overall separation and data processing process in this study is summarized in Figure 2.4.

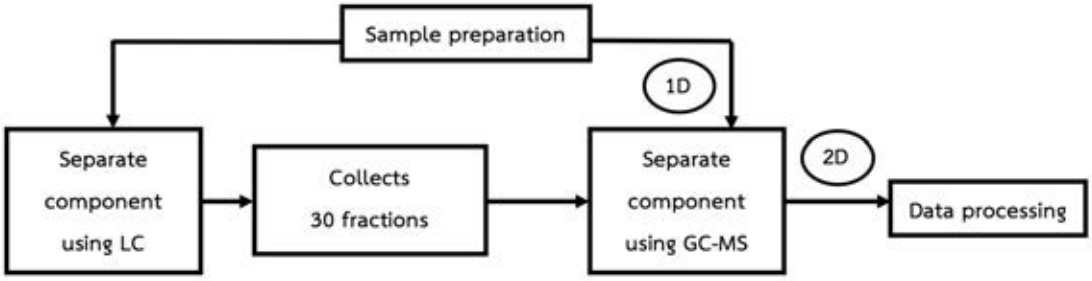

Figure 2.4 The flow diagram showing the overall experimental process

2.6 Data processing

The chromatographic peaks of green curry paste were identified using Agilent MassHunter software. The compounds were identified by the comparison of their MS spectra with those obtained from the NIST library. The identification criteria include 1) the compound that has match score >700 and 2) 20 units difference between the experimental retention index (RI) and the literature RI on the same type of the stationary phase. Experimental RI of peak *i* in a chromatogram can be calculated by comparison with the peak positions of *n*-alkane series according to

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

where t_R is retention time of peak *i*.

n and *n+1* are the carbon numbers of alkane standards bracketing the peak *i*.

Chapter III

RESULTS AND DISCUSSION

3.1 One-dimensional chromatography with GC-MS

In this work, green curry paste was chosen as the test sample. Since green curry paste consists of many ingredients, it contains highly complex chemical profile which has not been studied in detail before. The (crude) sample was initially analyzed using the conventional one-dimensional gas chromatography hyphenated with mass spectrometry (GC-MS) with a HP-5MS column and other conditions described in Section 2.4. The result is shown in Figure 3.1.

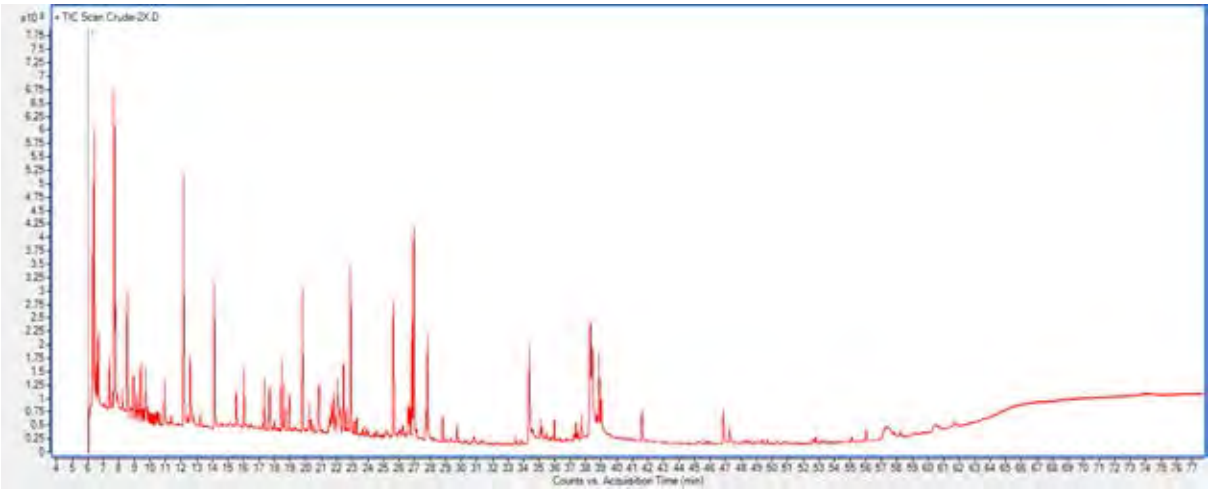

Figure 3.1 Chromatogram of green curry paste obtained by using GC-MS

Figure 3.1 shows the GC/MS result obtained by using splitless mode which is the mode that all the volatile compounds were introduced into the column. The chromatogram reveals many peaks of the sample which is highly complex with coelution of several compounds within each peak caused by incomplete separation of the compounds. The overlapping of the signal area can cause false positive identification of the compound for each peak.

The compounds in the green curry paste sample were identified by comparison of their MS spectra with those obtained from the NIST library with the match score of >700, as well as comparison between experimental and literature values of the retention indices with the difference of ±20 unit. The MS match score is an examination of a spectrum of interest compared with the library spectrum with the higher score corresponding to the larger number of experimental *m/z* values that are matched with in the database *m/z* values within each spectrum.

From the GC/MS analyses, a total of 63 compounds were identified in the green curry paste sample with the data shown in Table A1 (Appendix). Three major compounds were found in green curry sample, with the %area shown in parentheses, are limonene (8.69%), isolimonene (7.85%) and 4-terpineneol (7.13%), respectively. In fact, green curry may have more minor compounds co-eluting underneath the peaks of these three compounds. Therefore, they cannot be detected and analyzed.

3.2 Multidimensional chromatography with LCxGC-MS

The multidimensional chromatographic technique is a method of separating substances using 2 or more columns. This technique generally requires use of complicated and expensive instrumental system. In this work, the conventional instruments were applied with column LC and GC as the first and second dimensional separation, respectively.

The crude sample was initially loaded onto a column packed with silica gel as the stationary phase. The sample was then separated using hexane, dichlorometane, ethyl acetate and methanol with different compositions as the eluents, see Table 2.1 for the detail. With this liquid chromatography (LC) approach, compounds were separated according to their polarity difference. This leads to compounds eluting through the column at different rates. Since the silica gel stationary phase has high polarity, it has strong interaction with highly polar compound. The non-polar or less polar eluted from the column outlet first. Hexane was used as the first eluent since it is nonpolar. Then, the eluent polarity gradually increased by progressively increasing the contents of dichlorometane, ethyl acetate and methanol, respectively, in order to

result in good separation of compounds. Lastly, the solvent is removed using a rotary evaporator to yield the isolated compounds and using ethyl acetate to dissolve each fraction for analyzing in GC.

From the column chromatography, 30 fractions were collected and analyzed with GC-MS where GC with the low polar (semi-nonpolar) column was employed providing second dimensional separation mainly based on difference of compound boiling points (or vapor pressures) and hydrophobic interaction. The fractions 1-10 were chosen for analysis and discussion because these fractions contain most of the compounds found in the green curry paste sample.

GC-MS analysis part of the LCxGC used the same conditions as those in one-dimensional GC-MS. The chromatograms from the GC-MS analyses of the 10 LC fractions of the green curry sample were shown in Figure 3.2.

A total of 106 different compounds in the green curry sample were identified as shown in Table A2-A10 (Appendix). These include monoterpenes, sesquiterpenes, diterpenes, oxygenated monoterpenes, oxygenated sesquiterpenes, sulfur-containing compounds and the other miscellaneous compounds.

Three compounds were identified in fraction 1. The corresponding chromatogram (the top chromatogram in Figure 3.2) showed a few small peaks due to the first nonpolar hexane fraction mostly containing the void volume of the LC column.

39 compounds were identified in fraction 2 which were mostly monoterpenes and sesquiterpenes hydrocarbon such as isolimonene (MH, 18.51%), copaene (SH, 17.05%) and limonene (MH, 11.47%).

The largest amount of volatiles (38 compounds) were identified in fraction 3 corresponding to the third chromatogram from the top in Figure 3.2, which showed the major compounds of δ-cadinene (SH, 12.39%), α-gurjunene (SH, 10.81%) and eremophilene (SH, 8.79%).

Most of sulfur-containing compounds were mostly found in fractions 4-6, such as diallyl trisulfide (7.13% in fraction 4, 19.26% in fraction 5, 11.69% in fraction 6), diallyl disulphide (0.43% in fraction 4, 18.69% in fraction 5, 19.04% in fraction 6) and mintsulfide (52.13% in fraction 6). This observation provides a selective analytical

method which can be applied to effectively analyze sulfur compounds. To this end, sulfur compound analysis in a food sample can be performed by fractionation of the sample with the developed LC method followed by collection and analysis of only fractions 4-6.

Fraction 7 contains only a small part of the compound peak from fraction 6. Thus, no additional peaks of compounds were observed in the chromatogram of this fraction (Figure 3.2).

Fractions 8-10 contain the similar of the compound peak profile as shown in Figure 3.2 (the last three chromatograms in Figure 3.2). Therefore, the analysis of the signal of each fraction showed the similar compounds such as tumerone (33.44% in fraction 8, 19.48% in fraction 9, 38.07% in fraction 10), ethyl linoleate (4.39% in fraction 8, 7.71% in fraction 9, 6.53% in fraction 10) and citronellol acetate (1.3% in fraction 8, 1.47% in fraction 9, 2.27% in fraction 10).

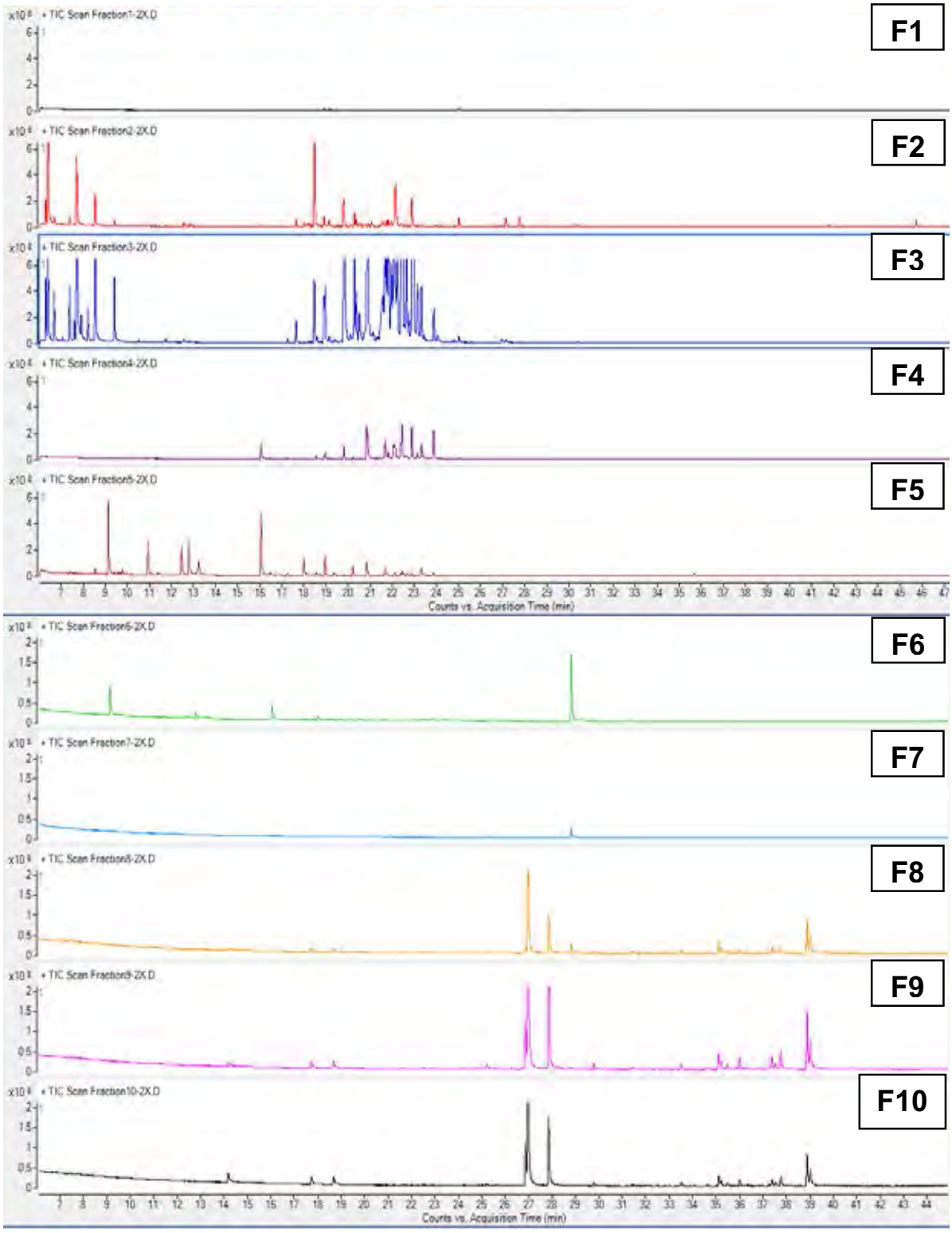

Figure 3.2 Chromatogram of all Fraction of green curry paste using LCxGC analysis (2D)

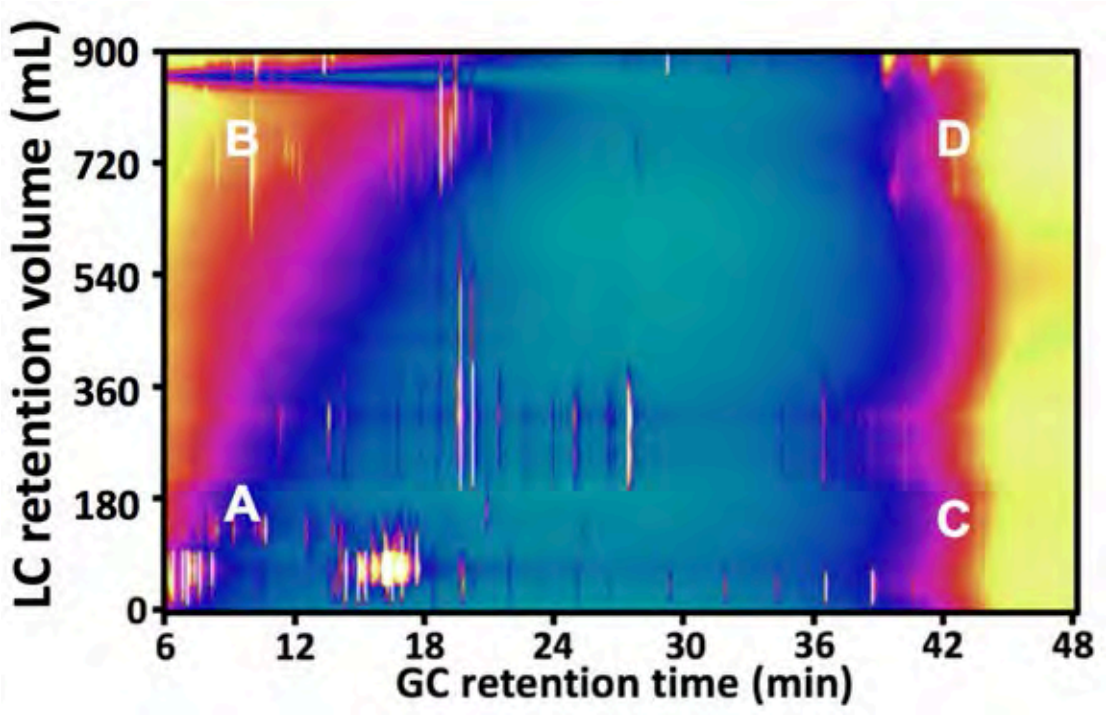

Figure 3.3 The LCxGC result plot

Figure 3.3 showed the overall LCxGC analysis result as a contour plot with GC retention time (min) of each fraction as an X-axis, LC retention volume (mL; approximately 30 mL per fraction) as a Y-axis, and the intensity in each GC-MS analysis as a Z-axis.

The region A in Figure 3.3 belongs to the compounds with relatively low GC retention time and LC retention volume. This corresponds to the compounds with low boiling point and low polarity such as β-pinene and pinene isomers which are monoterpene hydrocarbons. They are found in essential oils of many plants including lime peel oil, ginger, nutmeg, mace, bitter fennel, rosemary and sage.[27] They can also be used as flavoring ingredients.[28] The boiling point of β-pinene is considerably low (166.0 °C) and its polarity is also low (log P = 4.16) [27,29] as shown by the structure in Figure 3.4. β-pinene were found in fraction 2 and fraction 3 with the same retention time of 6.3 min.

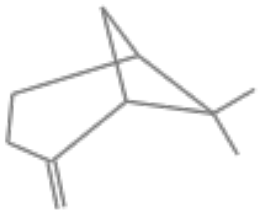

Figure 3.4 Structure of β-pinene[30]

The region C in Figure 3.3 was occupied by the peaks with high GC retention time and low LC retention volume. So, this corresponds to the compounds with relatively high boiling point and low polarity such as methyl linoleate is a methyl ester of linoleic acid and normally found in cloves and used as a flavoring ingredient.[31,32] The boiling point of methyl linoleate is high (373.0 °C), and it has low polarity (log P = 6.82)[32,33] as shown by the structure in Figure 3.5. This compound was observed in fractions 8-10 with the same retention time of 37.4 min.

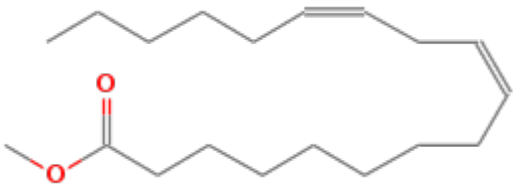

Figure 3.5 Structure of methyl linoleate[34]

Another example of the compound in region C is which is a long-chain fatty acid ethyl ester. Its boiling point is high (303.0 °C) and its polarity is low (log P = 6.76) [35,36], see also the structure in Figure 3.6. This compound was found in fractions 8-10 with the retention time of 35.1 min.

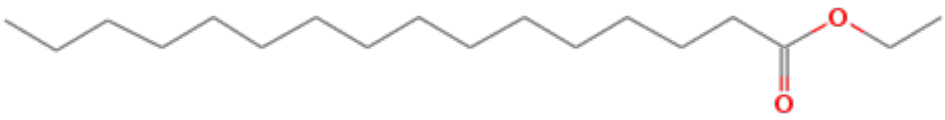

Figure 3.6 Structure of ethyl palmitate[37]

The region B in Figure 3.3 covers the peaks low GC retention time and high LC retention volume. These peaks belong to the compounds with low boiling point and high polarity. Likewise, the peaks in region D have high the GC retention time and high LC retention volume. So, the compounds in this area have relatively high boiling point and polarity.

3.3 Comparison of the quality of the experimental results obtained from one-dimensional GC-MS and LCxGC-MS

The results of one-dimensional and multidimensional chromatography were separately discussed in Section 3.2 and 3.3 with the overlaid chromatograms of crude (representing the 1DGC-MS result, the top chromatogram) and all the fractions (representing the LCxGC-MS result, the other chromatograms) shown in Figure 3.7. The LCxGC-MS results revealed greater separation of each peak with smaller number of the co-eluting peaks than that in the 1DGC-MS result. As a result, identification of compound for each peak was more accurate and reliable. To this end, LCxGC-MS analysis resulted in 43 compounds more than that identified in 1DGC-MS with the data shown in Table 3.1.

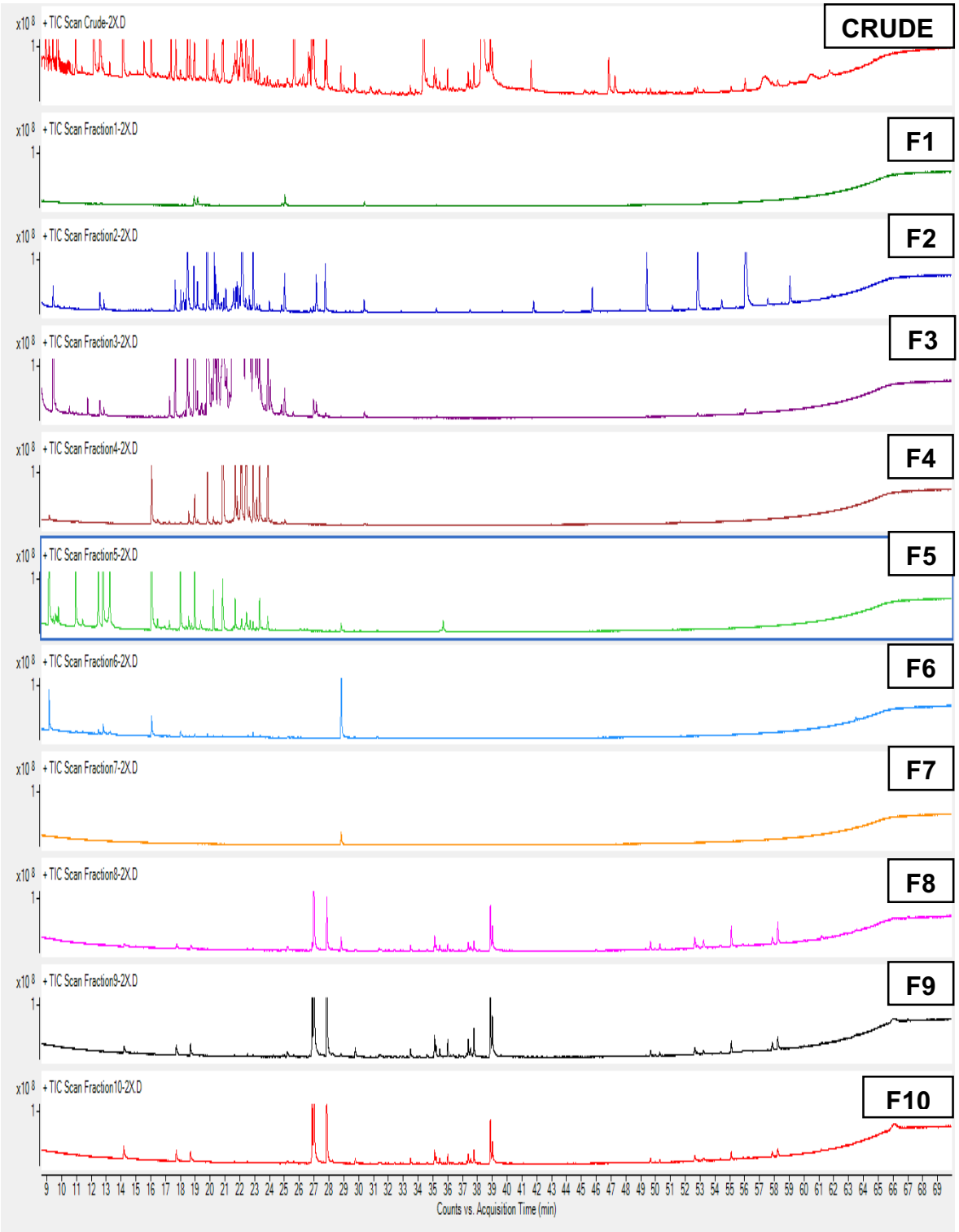

Figure 3.7 Comparison of chromatograms between crude sample representing GC-MS analysis (top) and all the fractions representing LCxGC-MS analysis (the other chromatograms below)

Table 3.1 Compound profiles of green curry in crude (1DGC-MS analysis) and all the fractions (LCxGC-MS)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
1	β-Pinene	MH	3.91	-	2.84	1.75	-	-	-	-	-	-
2	Sulcatone	OM	0.66	-	-	-	-	-	-	-	-	-
3	Isolimonene	MH	7.85	-	18.51	2.68	-	-	-	-	-	-
4	β-Myrcene	MH	1.43	-	0.87	1.63	-	-	-	-	-	-
5	2-Carene	MH	1.22	-	-	-	-	0.42	-	-	-	-
6	α-Phellandrene	MH	0.08	-	-	-	-	-	-	-	-	-
7	α-Terpinene	MH	-	-	1.18	1.8	-	-	-	-	-	-
8	*o*-Cymene	MH	0.3	-	-	0.55	-	-	-	-	-	-
9	Eucalyptol	MH	1.88	-	-	-	-	-	-	-	-	-
10	Limonene	MH	8.69	-	11.47	-	-	-	-	-	-	-
11	β-(*Z*)-Ocimene	MH	0.13	-	-	1.06	-	-	-	-	-	-
12	β-Ocimene	MH	0.41	-	-	-	-	-	-	-	-	-
13	β-(*E*)-Ocimene	MH	-	-	-	0.54	-	-	-	-	-	-
14	γ-Terpinene	MH	3.08	-	4.41	4.73	-	1.12	-	-	-	-
15	*trans*-Linalool oxide	OM	0.84	-	-	-	-	-	-	-	-	-

Table 3.1 (continued)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
16	Diallyl disulphide	S	0.62	-	-	-	0.43[A]	18.69	19.04	-	-	-
17	Terpinolene	MH	1.12	-	0.6	2.3	-	-	-	-	-	-
18	Linalool	OM	1.39	-	-	-	-	-	-	-	-	-
19	(*E*)-1-Allyl-2-(prop-1-en-1-yl)disulfane	S	-	-	-	-	-	0.75	-	-	-	-
20	(*Z*)-*p*-Menth-2-en-1-ol	OM	0.11	-	-	-	-	-	-	-	-	-
21	Allyl methyl trisulfide	S	-	-	-	-	-	9.38[A]	-	-	-	-
22	Citronellal	OM	0.14[A]	-	-	-	-	-	-	-	-	-
23	4-Terpineneol	OM	7.13[B]	-	-	-	-	-	-	-	-	-
24	***α***-Terpineol	OM	2.15	-	-	-	-	-	-	-	-	-
25	1-Dodecene	M	-	-	0.64	0.15	-	-	-	-	-	-
26	4H-1,2,3-Trithiine	S	-	-	-	-	-	10.65	4.87	-	-	-
27	3-Vinyl-1,2-dithiacyclohex-4-ene	S	0.14[A]	-	-	-	-	7.78	-	-	-	-
28	2-Vinyl-4H-1,3-dithiine	S	0.25	-	-	-	-	4.38	-	-	-	-
29	Benzaldehyde	M	3.81	-	-	-	-	-	-	-	-	-
30	Cumaldehyde	OM	-	-	-	-	-	-	-	0.94	1.14	3.56

Table 3.1 (continued)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
31	Chavicol	M	0.14[A]	-	-	-	-	-	-	-	-	-
32	Diallyl trisulfide	S	1.46	-	-	-	7.13	19.26	11.69	-	-	-
33	(*E*)-10-Heptadecen-8-ynoic acid, methyl ester	M	0.04[A]	-	-	-	-	-	-	-	-	-
34	Allyl propyl trisulfide	S		-	-	-	0.4[A]	-	-	-	-	
35	2-[[2-[[2-[(2-Pentylcyclopropyl)methyl]cyclopropyl]methyl cyclopropanebutanoic acid	M	0.1[A]	-	-	-	-	-	-	-	-	-
36	δ-Elemene	SH	0.12	-	-	0.16	0.19	-	-	-	-	-
37	Citronellol acetate	OM	1.02	-	-	-	-	-		1.3	1.47	2.27
38	α-Cubebene	SH	0.24	-	1.19	0.88	-	-	-	-	-	
39	Nerol acetate	OM	-	-	-	-	-	-	-		1.64	
40	5-Methyl-1,2,3,4-tetrathiane	S	-	-	-	-	0.22[A]	-	-	-	-	-
41	2-Methyltridecane	M	-	-	0.7	-	-	-	-	-	-	-
42	Cyclosativene	SH	-	-	0.18	-	-	-	-	-	-	-
43	Ylangene	SH	-	-	2.33	-	-	-	-	-	-	-

Table 3.1 (continued)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
44	Longicyclene	SH	0.06[A]	-	0.5	-	-	-	-	-	-	-
45	Copaene	SH	1.58	-	17.05	-	-	-	-	-	-	-
46	Daucene	SH	-	-	0.33	-	-	-	-	-	-	-
47	Farnesane	SH	-	26.82	-	0.14	-	-	-	-	-	-
48	Geranyl acetate	OM	0.97	-	-	-	-	-	-	1.09	-	2.42
49	β-Cubebene	SH	0.36	-	-	1.32	-	-	-	-	-	-
50	Methyleugenol	M	0.1[A]	-	-	-	-	-	-	-	-	-
51	β-Elemene	SH	0.8	-	-	1.82	2.97	4.74	0.97	-	-	-
52	1-Tetradecene	M	-	29.53	1.66[A]	-	-	-	-	-	-	-
53	Sativen	SH	-	-	0.19	-	-	-	-	-	-	-
54	Tetradecane	M	-	-	-	-	0.27	-	-	-	-	-
55	Isocaryophyllene	SH	-	-	-	-	-	-	1.09	-	-	-
56	α-Gurjunene	SH	-	-	-	10.81	-	-	-	-	-	-
57	Caryophyllene	SH	3.92	-	6.47	2.56	4.14	-	-	-	-	-
58	α-Bergamotene	SH	0.55	-	1.98	-	-	-	-	-	-	-

Table 3.1 (continued)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
59	*β*-Gurjunene	SH	-	-	0.32	0.24	-	-	-	-	-	-
60	*γ*-Elemene	SH	0.21	-	-	-	0.64	2.4	-	-	-	-
61	Aromandendrene	SH	-	-	-	-	0.15[A]	-	-	-	-	-
62	*α*-Guaiene	SH	0.23	-	1.2	1.37	-	-	-	-	-	-
63	*β*-*(Z)*-Farnesene	SH	1.1	-	-	-	-	-	-	-	-	-
64	*β*-Humulene	SH	-	-	-	0.23	-	-	-	-	-	-
65	*α*-Himachalene	SH	-	-	0.18	1.31	-	-	-	-	-	-
66	*α*-Caryophyllene	SH	0.73	-	-	2.08	-	4.23	-	-	-	-
67	Humulene	SH	-	-	-	-	10.44	-	-	-	-	-
68	*β*-(*E*)-Famesene	SH	-	-	-	-	6.1	-	-	-	-	-
69	Alloaromadendrene	SH	-	-	-	4.68	-	-	-	-	-	-
70	2-Methyltetradecane	SH	-	-	0.77	-	-	-	-	-	-	-
71	*γ*-Gurjunene	SH	-	-	0.38	-	-	-	-	-	-	-
72	4,11-selinadiene	SH	0.2	-	0.69	0.61	-	-	-	-	-	-
73	*γ*-Muurolene	SH	0.43	-	-	-	-	-	-	-	-	-

Table 3.1 (continued)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
74	β-Chamigrene	SH	-	-	0.9	5.89	-	-	-	-	-	-
75	Bicyclosesquiphellandrene	SH	-	-	-	0.13	-	-	-	-	-	-
76	α-Curcumene	SH	-	-	-	1.35	6.73	1.7	-	-	-	-
77	β-Selinene	SH	0.66	-	0.86	3.83	1.5	-	-	-	-	-
78	β-Guaiene	SH	-	-	-	-	0.06[A]	-	-	-	-	-
79	Valencen	SH	-	-	0.18	-	-	-	-	-	-	-
80	Elixene	SH	-	-	-	-	-	0.74	-	-	-	-
81	Eremophilene	SH	-	-	-	8.79	-	-	-	-	-	-
82	Zingiberene	SH	1.53	-	-	-	4.05	-	-	-	-	-
83	β-Cadinene	SH	0.31[A]	-	0.56	1.48	-	-	-	-	-	-
84	Bicyclogermacrene	SH	-	-	-	-	4.21	-	-	-	-	-
85	α-Muurolene	SH	0.58	-	-	1.76	0.06[A]	-	-	-	-	-
86	δ-Guaijene	SH	-	-	0.34	-	4.13	0.24	-	-	-	-
87	β-Bisabolene	SH	2.13	-	-	-	12.23	1.23	-	-	-	-
88	γ-Cadinene	SH	0.56	-	0.44	3.48	1.08	-	-	-	-	-

Table 3.1 (continued)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
89	β-Curcumene	SH	-	-	-	-	0.02[A]	-	-	-	-	-
90	7-*epi*-α-Selinene	SH	-	-	-	0.61	-	-	-	-	-	-
91	Cadine-1,4-diene	SH	-	-	0.16	-	-	-	-	-	-	-
92	2,4-Bis(1,1-dimethylethyl)-phenol	M	-	-	-	-	-	-	-	0.21	-	-
93	δ-Cadinene	SH	4.24	-	4.64	12.39[A]	11.62	0.45	2.22	0.35	-	-
94	α-Panasinsene	SH	-	-	0.12[A]	-	-	-	-	-	-	-
95	Diallyl tetrasulfide	S	-	-	-	-	6.56[A]	-	1.38[A]	-	-	-
96	γ-Bisabolene	SH	0.35	-	-	-	2.08[A]	-	-	-	-	-
97	Selina-3,7(11)-diene	SH	0.39	-	0.14	1.82	-	-	-	-	-	-
98	Elemol	OS	0.2	-	-	-	-	-	-	-	-	-
99	β-Germacrene	SH	0.21	-	-	1.19	10.24	0.76	-	-	-	-
100	2-Methyl-pentadecane	M	-	-	0.35	-	-	-	-	-	-	-
101	Caryophyllene oxide	OS	0.22[A]	-	-	-	-	-	-	-	-	-
102	1-Hexadecene	M	-	9.33	0.2	-	-	-	-	-	-	-
103	Hexadecane	M	-	-	-	-	0.48	-	-	-	-	-

Table 3.1 (continued)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
104	γ-Eudesmol	OS	4.59	-	-	-	-	-	-	-	-	-
105	τ-Cadinol	OS	0.44	-	-	-	-	-	-	-	-	-
106	Cubenol	OS	0.11	-	-	-	-	-	-	-	-	-
107	α-Cadinol	OS	0.99	-	-	-	-	-	-	-	-	-
108	Tumerone	OS	-	-	-	-	-	-	-	33.44[D]	19.48[A,D]	38.07[D]
109	*ar*-Tumerone	OS	2.83	-	-	-	-	-	-	0.82	5.01	6.84
110	*cis*-9-Tetradecen-1-ol	SH	-	-	0.18	-	-	-	-	-	-	-
111	2-Methyl-9-(prop-1-en-3-ol-2-yl)-bicyclo[4.4.0]dec-2-ene-4-ol	OS	-	-		-	-	-	-	-	37.52[A,C]	-
112	Curlone	OS	-	-		-	-	-	-	12.7[D]	-	19.28[D]
113	1-Heptadecene	M	-	-	1.61	-	-	-	-	-	-	-
114	Eudesm-7(11)-en-4-ol	OS	0.74	-		-	-	-	-	-	-	-
115	Pristane[A]	M	-	-	0.1	-	-	-	-	-	-	-
116	1,6,6-Trimethyl-7-(3-oxobut-1-enyl)-3,8-dioxatricyclo[5.1.0.0(2,4)]octan-5-one	OS	-	-		-	-	0.55[A]	-	-	-	-

Table 3.1 (continued)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
117	Mintsulfide	S	-	-		-	-	-	52.13	-	0.41[A]	-
118	3,5,6,7,8,8a-Hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-2(1H)naphthalenone	OS	0.44[A]	-		-	-	-	-	-	-	-
119	2,2,6-Trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-7-oxabicyclo[4.1.0]heptane	OS	-	-		-	-	-	-	0.35[A,C]	1.12[A,C]	-
120	Methyl palmitate	M	-	-		-	-	-	-	1.42	1.11	1.01
121	Ethyl 9-hexadecenoate	M	-	-		-	-	-	-	0.31	-	-
122	Ethyl palmitate	M	-	-	-	-	-	-	-	2.42	1.83	1.67
123	*p*-Camphorene	DH	-	-	-	-	-	-	-	-	0.68[A]	-
124	Geranyl linallol	DH	-	-	-	-	-	-	-	1.07	0.97	1.57
125	Methyl oleate	M	-	-	-	-	-	-	-	0.74	0.83	-
126	Methyl linoleate	M	-	-	-	-	-	-	-	1.51	1.62	1.3
127	Ethyl linoleate	M	-	-	-	-	-	-	-	4.39	7.71	6.53
128	Ethyl Oleate	M	-	-	-	-	-	-	-	7.86	3.92	3.3
129	Geranyl palmitoleate	M	-	-	-	-	-	-	-	-	0.32	-

Table 3.1 (continued)

No.	Compound	Class	Percentage of total peak area (%)									
			Crude	F1	F2	F3	F4	F5	F6	F8	F9	F10
130	Geranyl linoleate	M	-	-	-	-	-	-	-	-	0.58[A]	-

Note

A = All compounds match below 700

B = Too many similar spectra

C = No RI confirm

D = The difference between retention index of experimental and literature have more than 20

MH = Monoterpenes hydrocarbon

SH = Sesquiterpenes hydrocarbon

DH = Diterpenes hydrocarbon

OM = Oxygenated monoterpenes hydrocarbon

OS = Oxygenated sesquiterpenes hydrocarbon

S = Sulfur-containing compounds

M = Miscellaneous

Apart from the larger number of identified compounds, the 2D analysis provides higher MS match score of the experimental spectra with that from NIST library, since the 2D approach reduced the number of coeluted peaks and decreased background noise which resulted in cleaner spectrum signals and thus more accurate and reliable compound identification. The related examples are given below.

The zoomed in regions of the chromatograms focusing on the peaks of 3-vinyl-1,2-dithiacyclohex-4-ene ($C_6H_8S_2$) in crude and in Fraction 5 were shown in Figure 3.8. This revealed the cleaner and higher signal of the peak in Fraction 5. In the crude, the match score of this compound was very low (605). The score is much higher (905) for the peak in Fraction 5.

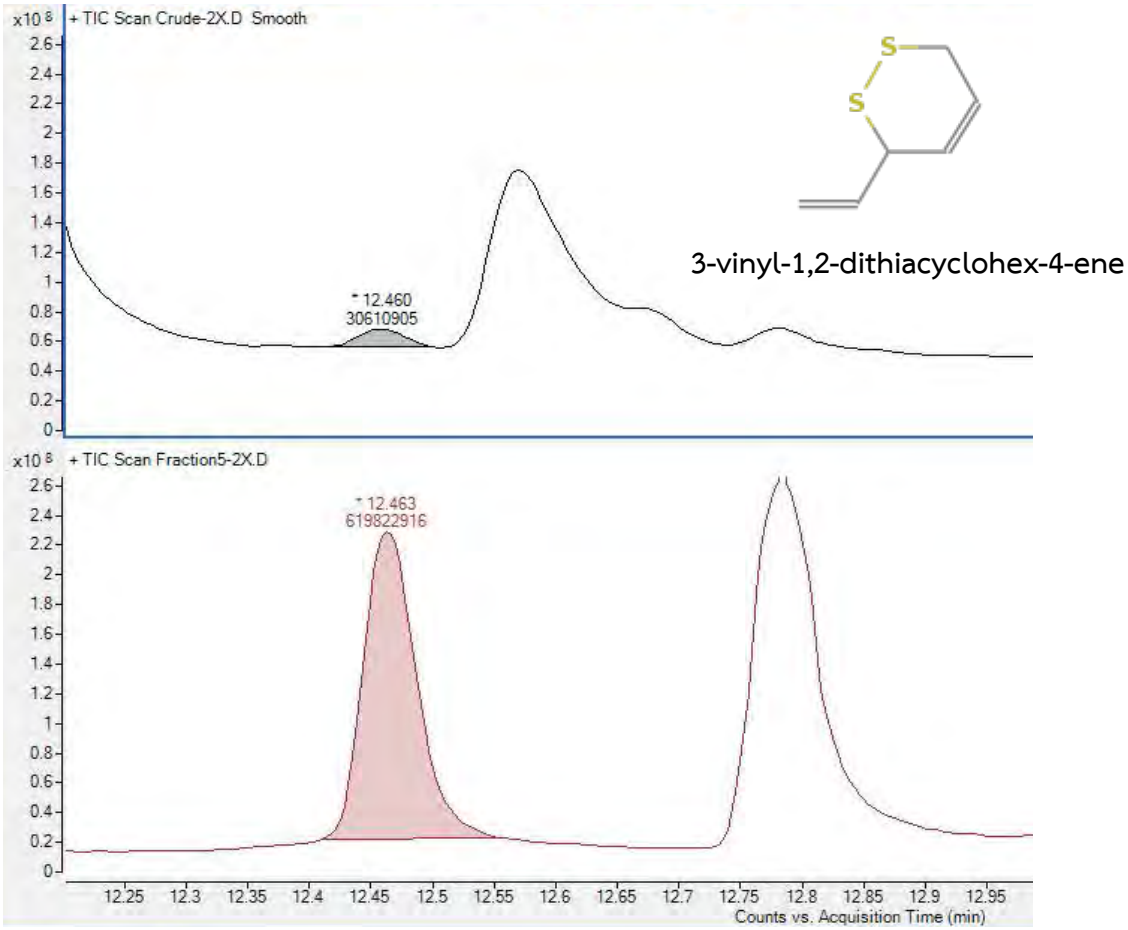

Figure 3.8 Comparison of the peaks of 3-vinyl-1,2-dithiacyclohex-4-ene[38] in crude (1DGC-MS, top) and in Fraction 5 of the LCxGC-MS (bottom) analyses

The peak at 12.8 min in the chromatogram of crude (Figure 3.9 and 3.10) could not be identified because the signal is too small. However, 4H-1,2,3-trithiine ($C_3H_4S_3$) was well identified in both Fraction 5 and Fraction 6 with retention time of 12.8. This clearly illustrates that LCxGC-MS provided larger number of compounds than 1DGC-MS with higher quality of the peak spectra allowing compound identification with higher confidence. Whilst identification of 4H-1,2,3-trithiine was not possible with the 1DGC-MS, this compound was reliably identified with the high MS match scores of 818 and 794 in Fraction 5 and Fraction 6, respectively.

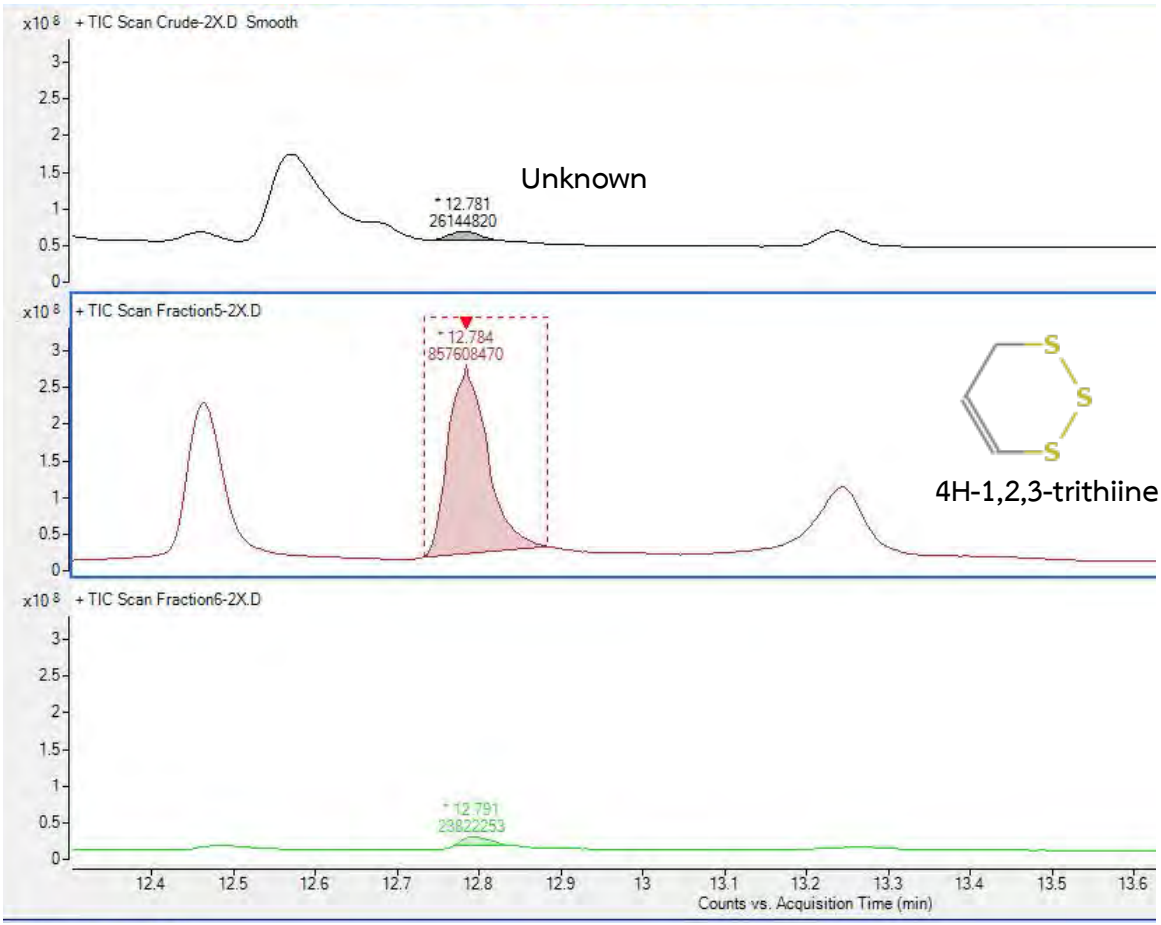

Figure 3.9 Comparison of 4H-1,2,3-trithiine[39] peaks in crude (1DGC-MS, black), in Fraction 5 (brown) and in Fraction 6 (green) of the LCxGC-MS analyses

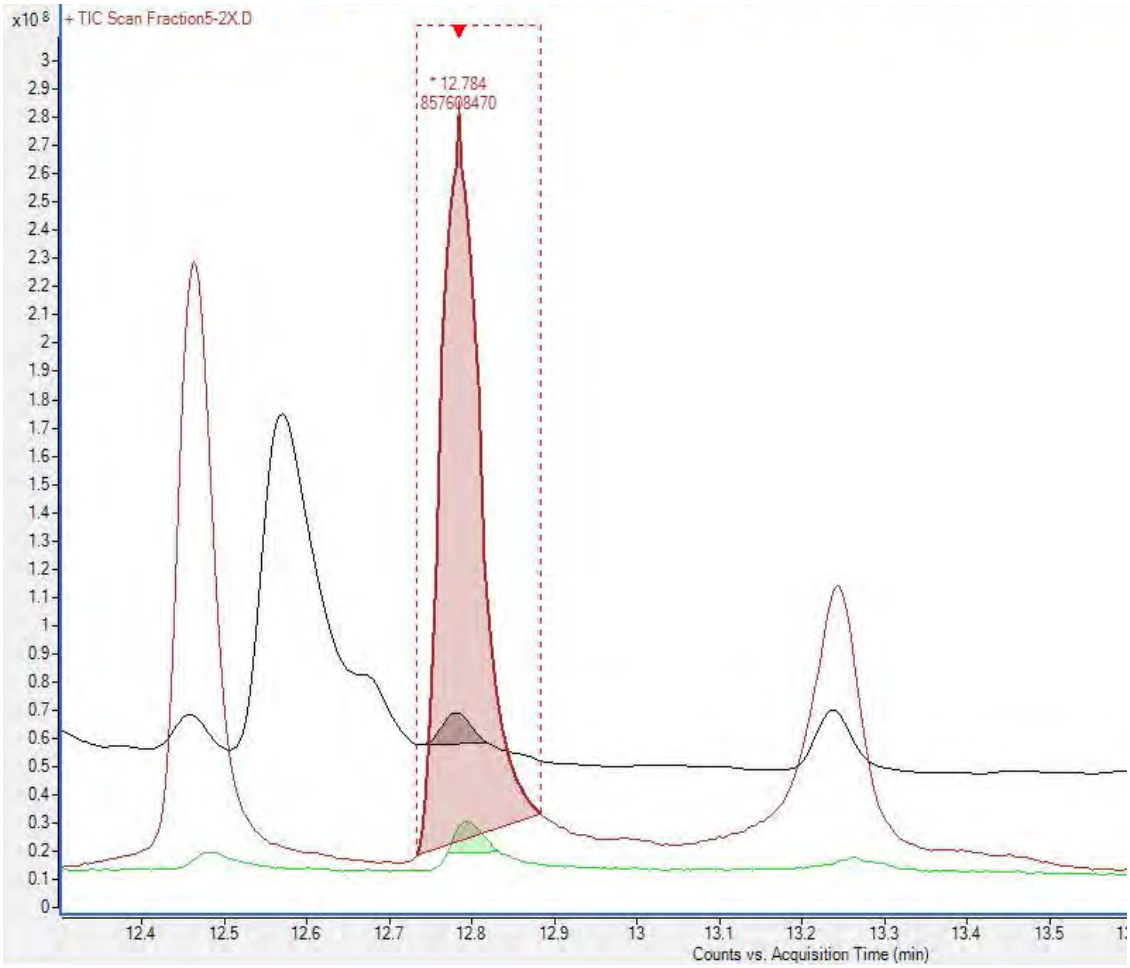

Figure 3.10 The overlaid chromatograms showing 4H-1,2,3-trithiine peaks in crude (1DGC-MS, black), in Fraction 5 (brown) and in Fraction 6 (green) of the LCxGC-MS analyses

α-cubebene ($C_{15}H_{24}$) was identified in the crude, Fraction 2 and Fraction 3 for the peak at 17.6 min as shown in Figure 3.11 and Figure 3.12. In 1DGC-MS analysis of the crude, the peak of **α**-cubebene was small and co-eluted with the other peak. In contrast, the peaks of this compound were clearly separated and concentrated in Fraction 2 and Fraction 3 with LCxGC-MS analysis, especially in Fraction 3. Similarly, the corresponding MS match score increased from 728 in crude to the scores of 888 and 902 in Fraction 2 and Fraction 3, respectively.

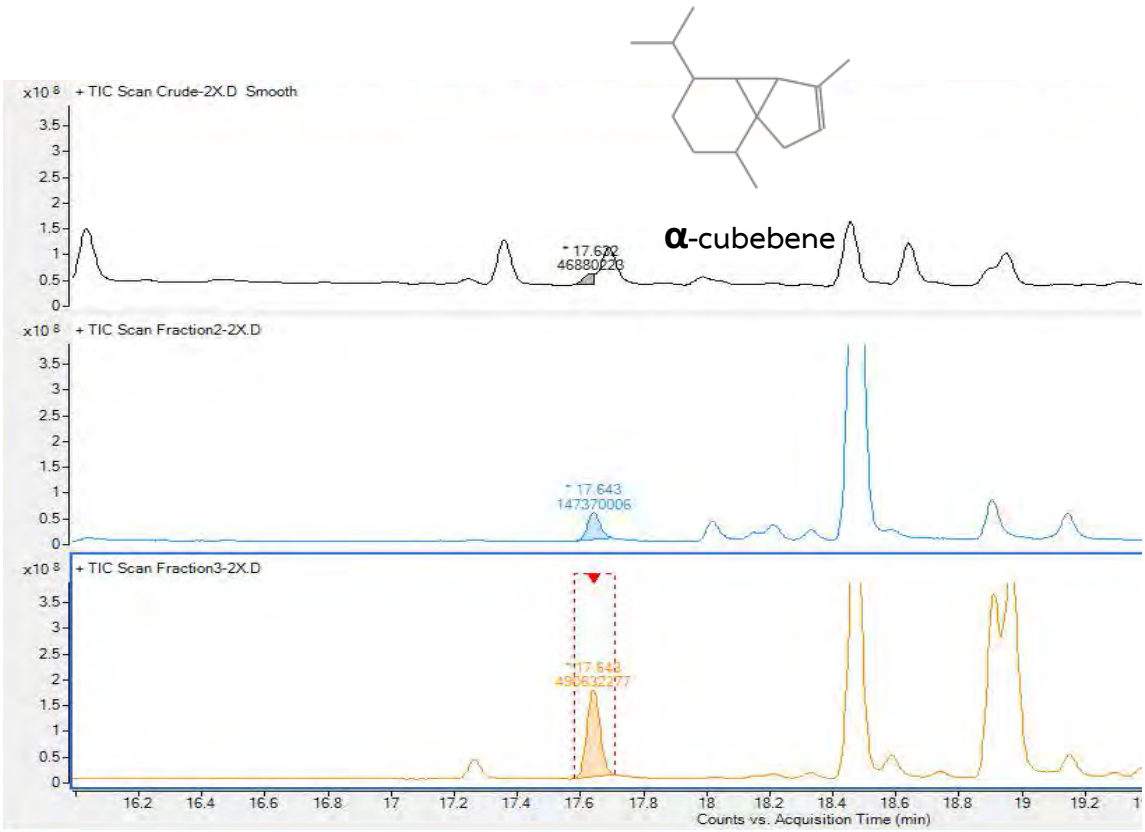

Figure 3.11 Comparison of **α**-cubebene[40] peaks in crude (1DGC-MS, black), in Fraction 2 (blue) and in Fraction 3 (brown) of the LCxGC-MS analyses

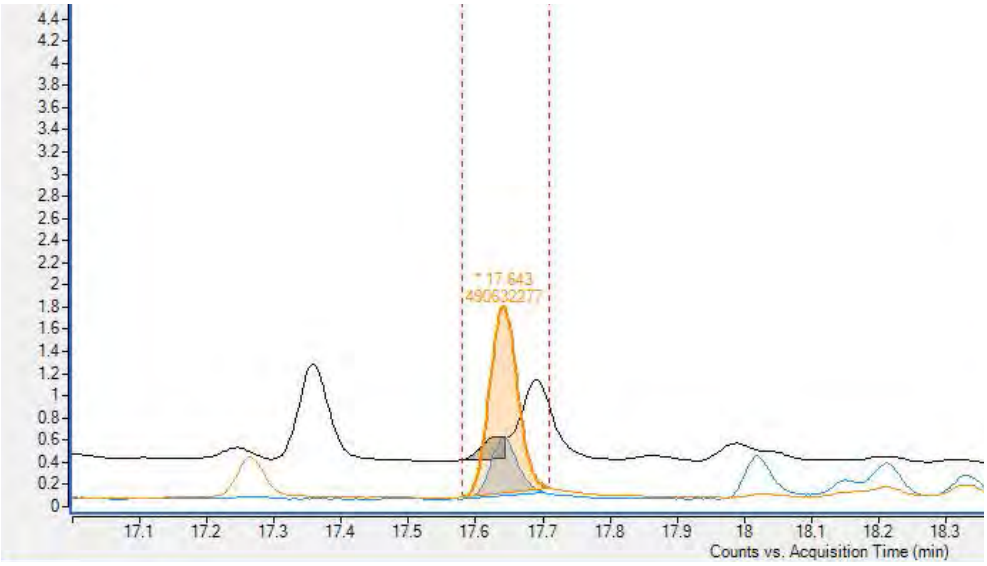

Figure 3.12 The overlaid chromatograms showing the peaks of **α**-cubebene in crude (1DGC-MS, black), in Fraction 2 (blue) and in Fraction 3 (brown) of the LCxGC-MS analyses

Figure 3.13 showed the chromatograms focusing on the peaks of β-elemene ($C_{15}H_{24}$) in the crude, Fraction 3, Fraction 4, Fraction 5 and Fraction 6. This revealed that the signal of this compound in the 2D analysis could appear in several fractions due to the broad zone of this compound during the liquid chromatography (LC) which was fractionated into several fractions. Also, this can be noticed by considering the peaks of this compound which had similar MS spectra and were observed with the same retention time of 19.0 min in all the related fractions. Moreover, β-elemene peak in crude and Fraction 2 co-eluted with the interference peaks, but it was clearly separated in Fractions 4-6, especially in Fraction 5. The match score of β-elemene peak in crude was 877 and increased up to 914 in Fraction 5.

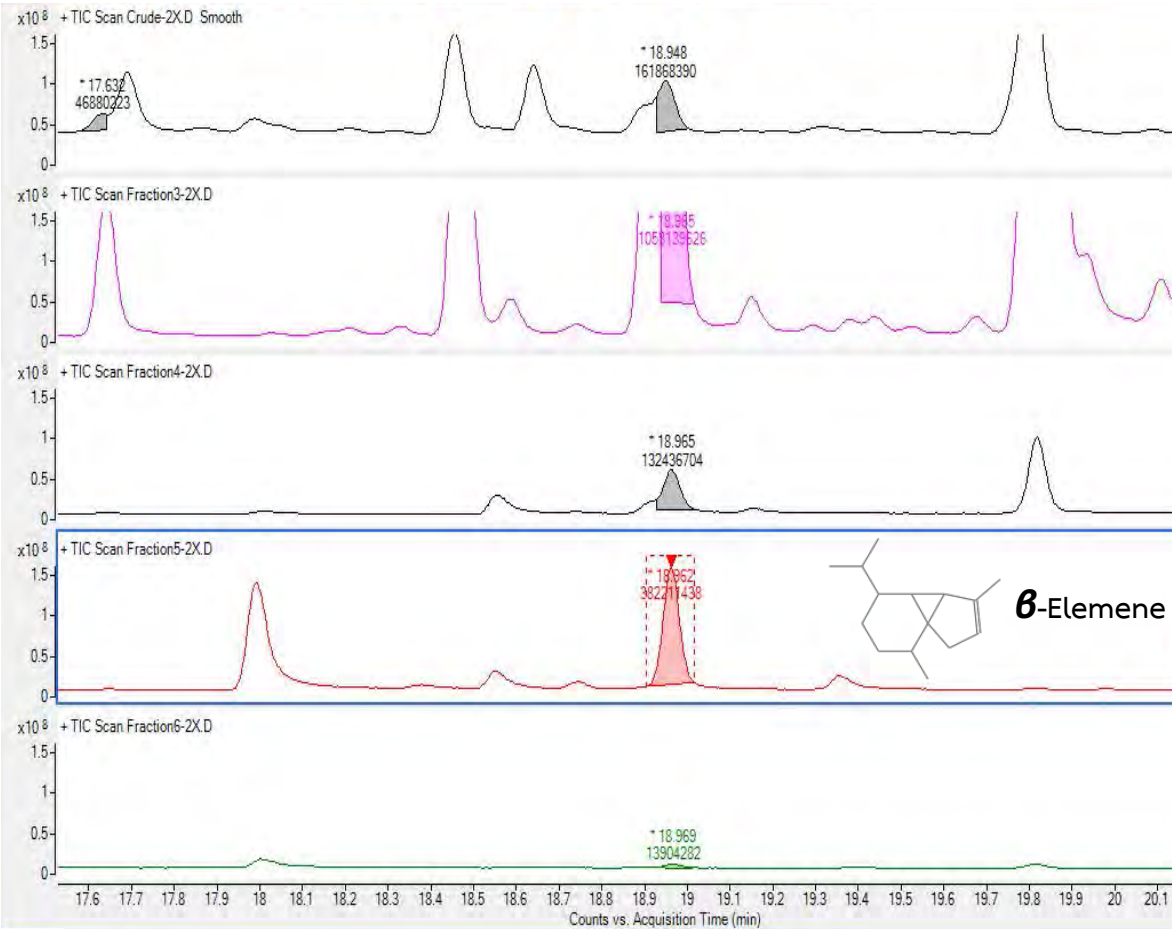

Figure 3.13 Comparison of β-elemene[41] peaks in crude (1DGC-MS, the top chromatogram, black), in Fraction 3 (pink), in Fraction 4 (black), in Fraction 5 (red) and in Fraction 6 (green) of the LCxGC-MS analyses

The peaks of β-germacrene in the related chromatograms at 23.9 min were shown in Figure 3.14. The peak in crude is too small with low match score (757). However, the peak signals were much clearer in the chromatograms of Fraction 3 and Fraction 4 with the match scores of 851 and 919 in Fraction 3 and Fraction 4, respectively.

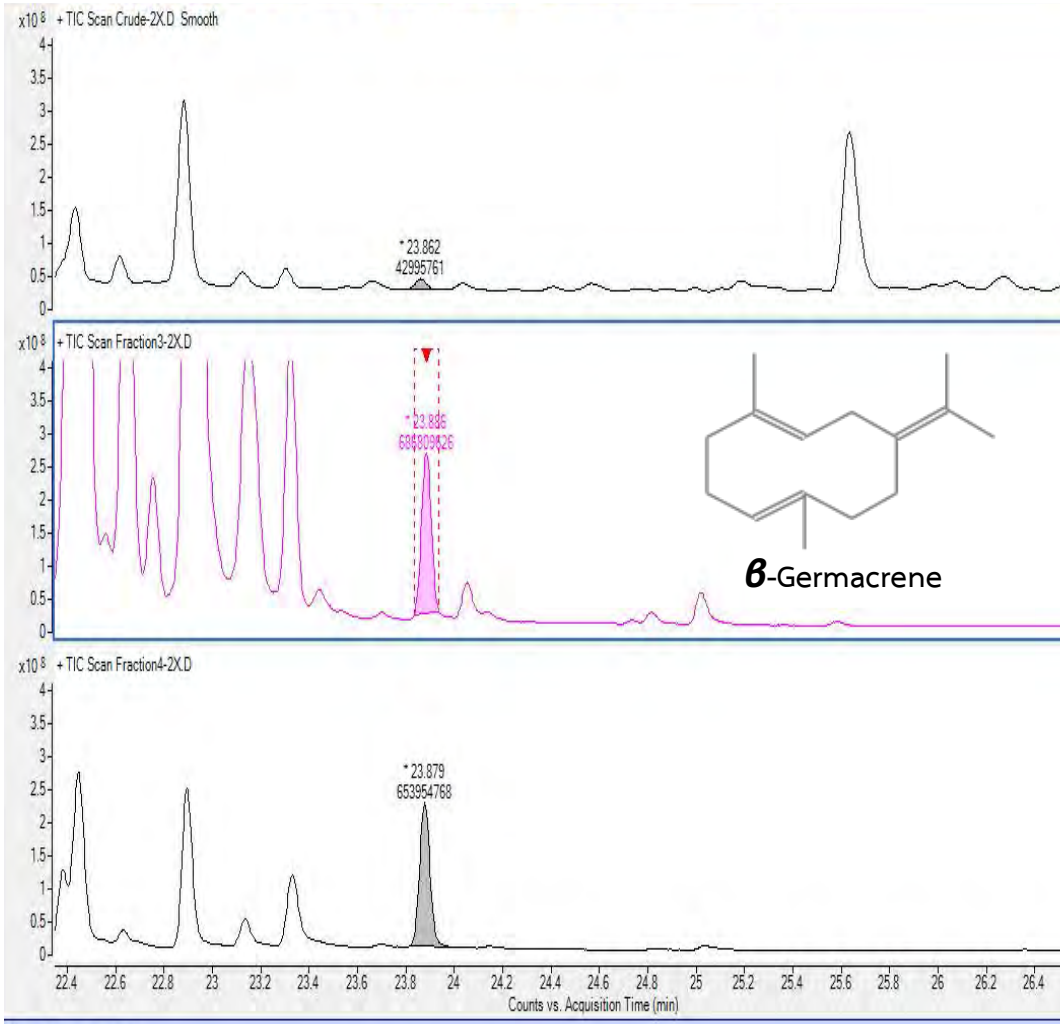

Figure 3.14 Comparison of β-germacrene[42] peaks in crude (1DGC-MS, the top chromatogram, black), in Fraction 3 (pink) and in Fraction 4 (black) of the LCxGC-MS analyses

3.4 Smell and taste of component in green curry paste

The ingredients of the green curry paste are chilli, lemongrass, garlic, kaffir lime peel and the others. The compounds found in this green curry paste sample using GC-MS and LCxGC-MS are expected to be similar to those in the individual ingredient of the green curry paste, which are mostly related to the characteristic tastes and odors of the green curry.

Mintsulfide is a sulfur-containing compound which was found in Fraction 6. This compound is commonly found in herbs and spices, *e.g.* as a constituent of *Mentha piperita* oil[43] which has a characteristic, sweetish and strong odors and aromatic, warm and pungent tastes with a cooling aftertaste.[44]

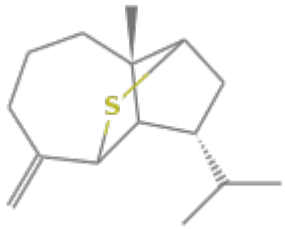

Figure 3.15 Structure of mintsulfide[45]

δ-cadinene is a sesquiterpene hydrocarbon which was found in the crude and Fractions 2-8. This compound has been reported as a constituent of the essential oils of ylang-ylang, citronella, cubebs, and sweet flag.[46]

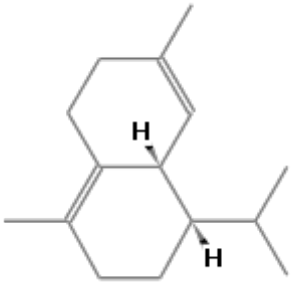

Figure 3.16 Structure of δ-cadinene[47]

Humulene is a sesquiterpene hydrocarbon that was found in Fraction 4. This compound is a component of the essential oil from the flowering cone of the hops plant.[48] It is also found in cannabis, sage, and ginseng. Moreover, humulene possesses powerful anti-inflammatory and is an effective analgesic.[49]

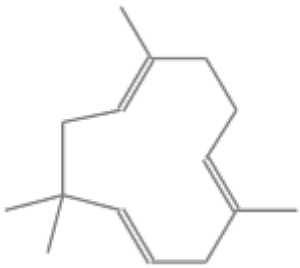

Figure 3.17 Structure of humulene[50]

Limonene is a monoterpene hydrocarbon that was found in crude and Fraction 2. This compound has been reported as a major component in the oil of oranges which has many uses, including as flavor and fragrance for its pleasant lemon-like odor. Limonene is used as a traditional medicine for treating diabetes, cancer, anti-hyperlipidemic, hypertension and inflammation. Moreover, it is used in food manufacturing such as a flavoring to mask the bitter taste of alkaloids, and as a fragrance in perfumery. Also, it is used as a botanical insecticide.[51]

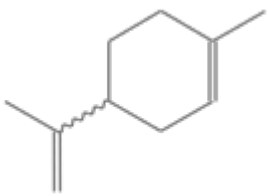

Figure 3.18 Structure of limonene[52]

Diallyl disulphide is a sulfur-containing compound which was found in the crude and Fractions 4-6. It has been isolated from garlic and other species of the genus Allium and has a strong garlic odor. It has a role as an antineoplastic agent, an antifungal agent and a plant metabolite.[53-54] This compound has been reported to provide many health benefits, as they are very efficient in detoxifying natural agents. Therefore, these compounds may be useful for prevention/treatment of cancers.[54-56]

Figure 3.19 Structure of diallyl disulphide[57]

Terpinolene is a monoterpene hydrocarbon compound that was found in the crude and Fraction 2-3. This compound was observed in allspice and a constituent of many essential oils, e.g. citrus, mentha, juniperus, and is a lemon flavouring ingredient.[58-60]

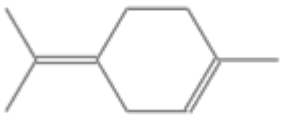

Figure 3.20 Structure of terpinolen[61]

Chapter IV
Conclusion

In this work, simple two-dimensional (2D) chromatographic technique which only requires the use of liquid chromatography and conventional GC-MS system (LCxGC-MS) was developed. The technique was applied to obtain the profile of volatile and semi-volatile compounds in green curry paste, LOBO. The result was compared with that obtained by using one-dimensional gas chromatography (1DGC-MS) in the aspect of quality of the signal in the chromatogram, the number of identified compounds and their MS spectrum quality.

GC-MS and LCxGC-MS analyses resulted in 63 and 106 compounds, respectively, in the green curry paste. This revealed that the 2D approach can identify more compounds than the 1D. The 2D result also provided clearer signal of several compounds with reduced number of compound co-elutions. Moreover, the 2D method showed higher match score of the identified compound with that from the NIST library. Thus, the result obtained from LCxGC-MS was more accurate and reliable than that from the conventional GC-MS.

The major compounds that were found in green curry were **δ**-Cadine and **α**-Gurjunene**.** The other compound classes include monoterpenes, sesquiterpenes, diterpenes, oxygenated monoterpenes, oxygenated sesquiterpenes, sulfur-containing compounds and the other minor compounds. In addition, several compounds (such as limonene, linalool, terpinolene) correspond to that applied for flavoring, deodorizing, pungency, coloring and taste enhancing. The compounds with antioxidant and medicinal properties were also observed such as diallyl disulphide which is useful for prevention/treatment of cancers, humulene which can be anti-inflammatory as well as an effective analgesic.

In LCxGC-MS analysis, the crude was separated into 30 fractions, which provide increasing resolution of the separation and classification of compounds in the contour plot. These fractions are also useful for specific compound analysis. For example, many sulfur compounds found in fraction 5, researchers can thus apply this method and collect only fraction 5 for sulfur compounds analysis.

The developed LCxGC-MS technique is expected to be applied for analysis of other complex samples in the future.

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APPENDIXS

Table A.1 The database of the compounds of green curry in crude (1D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
β-Pinene	MH	976	976	0	834	C10H16	6.306	917000653	3.91
Isolimonene	MH	980	983	-3	813	C10H16	6.403	1842840873	7.85
Sulcatone	OM	987	977	10	718	C8H14O	6.568	156088829	0.66
β-Myrcene	MH	991	987	4	882	C10H16	6.679	335770181	1.43
Limonene	MH	1029	1028	1	815	C10H16	7.704	2040752666	8.69
α-Phellandrene	MH	1006	1002	4	618	C10H16	7.066	18655327	0.08
2-Carene	MH	1018	1002	16	880	C10H16	7.383	286966270	1.22
o-Cymene	MH	1025	1025	0	871	C10H14	7.594	69846742	0.30
Eucalyptol	MH	1032	1028	4	897	C10H18O	7.78	441516089	1.88
β-(*Z*)-Ocimene	MH	1036	1034	2	800	C10H16	7.912	29637590	0.13
β-Ocimene	MH	1047	1037	10	843	C10H16	8.212	96213698	0.41
γ-Terpinene	MH	1059	1057	2	913	C10H16	8.54	722846097	3.08
trans-Linalool oxide	OM	1073	1078	-5	835	C10H18O2	8.934	196352384	0.84

Table A.1 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Diallyl disulphide	S	1080	1081	-1	745	C6H10S2	9.144	144491297	0.62
Terpinolene	MH	1089	1084	5	802	C10H16	9.403	263526115	1.12
Linalool	OM	1100	1094	6	830	C10H18O	9.707	326740974	1.39
(*Z*)-*p*-Menth-2-en-1-ol	OM	1123	1119	4	759	C10H18O	10.412	25540988	0.11
Unknown	-	1139	-	-	-	-	10.923	279164268	1.19
Citronellal[A]	OM	1153	1152	1	662	C10H18O	11.361	32999908	0.14
4-Terpineneol[B]	OM	1178	1175	3	858	C10H18O	12.152	1674317427	7.13
3-Vinyl-1,2-dithiacyclohex-4-ene[A]	S	1188	1198	-10	605	C6H8S2	12.46	32374837	0.14
α-Terpineol	OM	1192	1184	8	874	C10H18O	12.57	505102713	2.15
Unknown	-	1194	-	-	-	-	12.649	77487097	0.33
Unknown	-	1198	-	-	-	-	12.781	32125811	0.14
2-Vinyl-4H-1,3-dithiine	S	1213	1206	7	772	C6H8S2	13.236	59847376	0.25
Benzaldehyde	M	1241	1229	12	886	C10H12O	14.131	894111866	3.81
Chavicol[A]	M	1255	1242	13	610	C9H10O	14.59	33112333	0.14
Unknown	-	1271	-	-	-	-	15.098	22727053	0.10

Table A.1 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Unknown	-	1285	-	-	-	-	15.543	192311801	0.82
Diallyl trisulfide	S	1300	1297	3	865	C6H10S3	16.034	341959639	1.46
(*E*)-10-Heptadecen-8-ynoic acid, methyl ester [A]	M	1306	-	-	-	C18H30O2	16.223	8756280	0.04
2-[[2-[[2-[(2-Pentylcyclopropyl)methyl]cyclopropyl]methyl cyclopropanebutanoic acid [A]	M	1314	-	-	-	C25H42O2	16.458	22986202	0.10
Unknown	-	1331	-	-	-	-	16.994	12072451	0.05
δ-Elemene	SH	1339	1338	1	696	C15H24	17.246	27857062	0.12
Unknown	-	1343	-	-	-	-	17.356	265628472	1.13
α-Cubebene	SH	1352	1351	1	728	C15H24	17.632	56356613	0.24
Citronellol acetate	OM	1353	1342	11	790	C12H22O2	17.691	239384598	1.02
Unknown	-	1363	-	-	-	-	17.988	55280615	0.24
Unknown	-	1364	-	-	-	C16H26O2	18.026	23128006	0.10
Longicyclene [A]	SH	1370	1374	-4	599	C15H24	18.209	13250287	0.06

Table A.1 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Copaene	SH	1378	1375	3	898	C15H24	18.454	371478925	1.58
Geranyl acetate	OM	1384	1382	2	833	C12H20O2	18.641	226904783	0.97
β-Cubebene	SH	1393	1389	4	878	C15H24	18.914	85110115	0.36
β-Elemene	SH	1394	1391	3	877	C15H24	18.948	188775950	0.80
Methyleugenol[A]	M	1406	1391	15	613	C11H14O2	19.318	24263122	0.10
Caryophyllene	SH	1422	1420	2	922	C15H24	19.804	919618781	3.92
Unknown	-	1431	-	-	-	-	20.091	12004168	0.05
γ-Elemene	SH	1435	1434	1	727	C15H24	20.208	49955212	0.21
α-Bergamotene	SH	1437	1427	10	798	C15H24	20.264	129253227	0.55
α-Guaiene	SH	1441	1440	1	830	C15H24	20.364	53512325	0.23
Unknown	-	1445	-	-	-	-	20.505	52368798	0.22
α-Caryophyllene	SH	1456	1451	5	906	C15H24	20.834	172324103	0.73
β-*(Z)*-Farnesene	SH	1458	1446	12	875	C15H24	20.875	258123140	1.10
Unknown	-	1481	-	-	-	-	21.569	32507348	0.14

Table A.1 The database of the compounds of green curry in crude (1D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
γ-Muurolene	SH	1484	1476	8	704	C15H24	21.659	102032648	0.43
4,11-selinadiene	SH	1486	1473	13	748	C15H24	21.721	46927072	0.20
β-Selinene	SH	1489	1486	3	850	C15H25	21.807	155116591	0.66
β-Cadinene[A]	SH	1493	1498	-5	664	C15H24	21.945	72140362	0.31
Zingiberene	SH	1497	1495	2	744	C15H24	22.059	358861286	1.53
Unknown	-	1499	-	-	-	-	22.122	169728852	0.72
α-Muurolene	SH	1502	1499	3	816	C15H24	22.201	136025819	0.58
β-Bisabolene	SH	1510	1509	1	869	C15H24	22.432	499173501	2.13
γ-Cadinene	SH	1516	1513	3	845	C15H24	22.619	131687436	0.56
δ-Cadinene	SH	1525	1524	1	865	C15H24	22.881	996499494	4.24
γ-Bisabolene	SH	1534	1533	1	759	C15H24	23.123	81519199	0.35
Selina-3,7(11)-diene	SH	1540	1539	1	728	C15H25	23.303	92041805	0.39
Elemol	OS	1553	1540	13	729	C15H26O	23.662	46199764	0.20
β-Germacrene	SH	1560	1557	3	757	C15H24	23.862	49342635	0.21

Table A.1 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Unknown	-	1566	-	-	-	-	24.035	34613385	0.15
Unknown	-	1579	-	-	-	-	24.408	21424048	0.09
Caryophyllene oxide[A]	OS	1584	1577	7	640	C15H24O	24.566	52177311	0.22
Unknown	-	1606	-	-	-	-	25.188	48397464	0.21
γ-Eudesmol	OS	1622	1623	-1	836	C15H26O	25.633	1077707217	4.59
Cubenol	OS	1635	1634	1	658	C15H26O	25.982	24753795	0.11
Unknown	-	1638	-	-	-	-	26.069	55432681	0.24
τ-Cadinol	OS	1645	1633	12	798	C15H26O	26.269	102492178	0.44
α-Cadinol	OS	1658	1648	10	836	C15H26O	26.618	232919455	0.99
Unknown	-	1662	-	1	-	-	26.714	201780490	0.86
ar-Tumerone	OS	1667	1658	9	812	C15H20O	26.849	663580718	2.83
Unknown	-	1671	-	-	-	-	26.963	1472122005	6.27
Eudesm-7(11)-en-4-ol	OS	1699	1687	12	828	C15H26O	27.736	172649210	0.74
Unknown	-	1703	-	-	-	-	27.837	714166285	3.04

Table A.1 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Unknown	-	1740	-	-	-	-	28.807	139388179	0.59
Unknown	-	1748	-	-	-	-	29.014	33166448	0.14
3,5,6,7,8,8a-Hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-2(1H)naphthalenone [A]	OS	1776	1773	3	677	C15H22O	29.75	103067190	0.44

Table A.2 The database of the compounds of green curry in Fraction 1 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
1-Tetradecene	M	1393	1393	0	812	C14H28	18.92	76143571	29.53
Farnesane	SH	1400	1381	19	760	C15H32	19.159	69149879	26.82
1-Hexadecene	M	1594	1590	4	733	C16H32	24.836	24055307	9.33
Unknown	-	1601	-	-	-	-	25.036	88501596	34.32

Table A.3 The database of the compounds of green curry in Fraction 2 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
β-Pinene	MH	976	976	0	821	C10H16	6.306	415126464	2.84
Isolimonene	MH	980	983	-3	746	C10H16	6.406	2702310080	18.51
β-Myrcene	MH	991	987	4	824	C10H16	6.682	126713766	0.87
α-Terpinene	MH	1018	1014	4	858	C10H16	7.387	171571036	1.18
Limonene	MH	1029	1028	1	836	C10H16	7.708	1673777462	11.47
γ-Terpinene	MH	1059	1057	2	903	C10H16	8.544	643437322	4.41
Terpinolene	MH	1090	1084	6	872	C10H16	9.421	87124080	0.60
1-Dodecene	M	1192	1190	2	829	C12H24	12.573	93007406	0.64
Unknown	-	1200	-	-	-	-	12.839	47164148	0.32
α-Cubebene	SH	1352	1351	1	888	C15H24	17.643	173024546	1.19
2-Methyltridecane	M	1364	1365	-1	876	C14H30	18.019	102783675	0.70
Cyclosativene	SH	1368	1368	0	824	C15H24	18.154	26238104	0.18
Longicyclene	SH	1370	1374	-4	838	C15H25	18.213	72866554	0.50
Unknown	-	1374	-	-	-	-	18.33	50458635	0.35

Table A.3 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Copaene	SH	1379	1376	3	832	C15H24	18.475	2489581370	17.05
Daucene	SH	1382	1381	1	799	C15H24	18.582	48289121	0.33
1-Tetradecene[A]	M	1392	1393	-1	698	C14H28	18.907	242451674	1.66
Unknown	-	1400	-	-	-	-	19.145	155189758	1.06
Sativen	SH	1413	1396	17	760	C15H24	19.525	27770279	0.19
Caryophyllene	SH	1422	1420	2	864	C15H24	19.804	944587458	6.47
β-Gurjunene	SH	1432	1429	3	783	C15H24	20.108	46651755	0.32
Unknown	-	1433	-	-	-	C15H24	20.139	23482580	0.16
α-Bergamotene	SH	1438	1427	11	894	C15H24	20.281	288553351	1.98
α-Guaiene	SH	1441	1440	1	875	C15H24	20.381	175123324	1.20
Unknown	-	1447	-	-	-	-	20.543	112344366	0.77
α-Himachalene	SH	1452	1448	4	719	C15H24	20.72	26228834	0.18
Unknown	-	1454	-	-	-	-	20.782	35574030	0.24
γ-Gurjunene	SH	1459	1471	-12	818	C15H24	20.923	55275507	0.38

Table A.3 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
2-Methyltetradecane	SH	1463	1465	-2	756	C15H32	21.061	112205888	0.77
β-Chamigrene	SH	1481	1478	3	784	C15H24	21.583	131555604	0.90
4,11-selinadiene	SH	1486	1473	13	869	C15H24	21.738	100864401	0.69
β-Selinene	SH	1489	1486	3	883	C15H24	21.825	125252654	0.86
β-Cadinene	SH	1494	1498	-4	724	C15H24	21.959	81488479	0.56
Unknown	-	1500	-	-	-	-	22.149	1243350084	8.52
δ-Guaijene	SH	1509	1505	4	766	C15H24	22.398	50182438	0.34
Valencen	SH	1510	1491	19	773	C15H24	22.432	26461212	0.18
γ-Cadinene	SH	1517	1513	4	874	C15H24	22.633	64632008	0.44
(-)-α-Panasinsen[A]	SH	1521	1527	-6	510	C15H24	22.747	17869460	0.12
δ-Cadinene	SH	1526	1524	2	878	C15H24	22.899	676830861	4.64
Cadine-1,4-diene	SH	1535	1520	15	758	C15H24	23.158	23316140	0.16
Selina-3,7(11)-diene	SH	1541	1539	2	777	C15H24	23.316	19850003	0.14
2-Methyl-pentadecane	M	1564	1564	0	850	C16H34	23.983	51428161	0.35
1-Hexadecene	M	1593	1590	3	823	C16H32	24.815	28817701	0.20

Table A.3 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Unknown	-	1600	-	-	-	-	25.019	209103267	1.43
cis-9-Tetradecen-1-ol	SH	1671	1667	4	731	C14H28O	26.956	26468781	0.18
1-Heptadecene	M	1678	1682	-4	831	C17H34	27.146	235732270	1.61
Unknown	-	1700	-	-	-	-	27.754	300787303	2.06
Pristane[A]	M	1706	1708	-2	631	C19H40	27.923	14480746	0.10

Table A.4 The database of the compounds of green curry in Fraction 3 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
β-Pinene	MH	976	976	0	810	C10H16	6.306	1095613340	1.75
Isolimonene	MH	980	983	-3	803	C10H16	6.403	1683375490	2.68
β-Myrcene	MH	991	987	4	817	C10H16	6.679	1019851340	1.63
α-Terpinene	MH	1018	1014	4	808	C10H16	7.383	1126122400	1.80
o-Cymene	MH	1025	1025	0	832	C10H14	7.597	345941140	0.55
Unknown	-	1029	-	-	-	-	7.718	3929467590	6.27
β-(*E*)-Ocimene	MH	1037	1044	-7	864	C10H16	7.919	339591920	0.54
β-(*Z*)-Ocimene	MH	1047	1034	13	899	C10H16	8.212	665161310	1.06
γ-Terpinene	MH	1059	1057	2	723	C10H16	8.547	2966674470	4.73
Terpinolene	MH	1090	1084	6	815	C10H16	9.414	1443242120	2.30
1-Dodecene	M	1192	1190	2	791	C12H24	12.577	94597480	0.15
δ-Elemene	SH	1340	1338	2	866	C15H24	17.263	101781300	0.16
α-Cubebene	SH	1352	1351	1	902	C15H24	17.643	550312610	0.88
Ylangene	SH	1378	1368	10	876	C15H24	18.472	1462742670	2.33
β-Cubebene	SH	1393	1389	4	818	C15H24	18.914	828115754	1.32

Table A.4 The database of the compounds of green curry in Fraction 3 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
β-Elemene	SH	1394	1391	3	908	C15H24	18.965	1139733115	1.82
Farnesane	SH	1400	1381	19	811	C15H32	19.152	90365720	0.14
α-Gurjunene	SH	1423	1408	15	741	C15H24	19.846	6777081770	10.81
β-Humulene	SH	1426	1446	-20	815	C15H24	19.918	141210966	0.23
β-Gurjunene	SH	1432	1429	3	765	C15H24	20.108	150702390	0.24
Caryophyllene	SH	1438	1420	18	844	C15H24	20.288	1602303180	2.56
α-Guaiaene	SH	1441	1440	1	930	C15H24	20.385	860921470	1.37
α-Himachalene	SH	1446	1448	-2	836	C15H24	20.523	819745710	1.31
α-Caryophyllene	SH	1457	1451	6	851	C15H24	20.865	1302327650	2.08
Alloaromadendren	SH	1459	1461	-2	842	C15H24	20.906	2931724470	4.68
Bicyclosesquiphellandrene	SH	1466	1482	-16	829	C15H24	21.131	83159010	0.13
α-Curcumene	SH	1484	1483	1	727	C15H22	21.683	847432780	1.35
4,11-selinadiene	SH	1487	1473	14	849	C15H24	21.752	379825900	0.61

Table A.4 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
β-Selinene	SH	1490	1486	4	840	C15H24	21.838	2398443520	3.83
β-Cadinene	SH	1494	1498	-4	871	C15H24	21.973	928822160	1.48
β-Chamigrene	SH	1498	1478	20	770	C15H24	22.09	3695842270	5.89
α-Muurolene	SH	1503	1499	4	840	C15H24	22.229	1104586930	1.76
Eremophilene	SH	1511	1495	16	836	C15H24	22.467	5511256050	8.79
γ-Cadinene	SH	1517	1513	4	844	C15H24	22.646	2182103870	3.48
7-epi-α-Selinene	SH	1521	1517	4	632	C15H24	22.757	380111550	0.61
δ-Cadinene[A]	SH	1527	1524	3	667	C15H24	22.933	7769784130	12.39
Unknown	-	1535	-	-	-	-	23.151	1647700410	2.63
Selina-3,7(11)-diene	SH	1541	1539	2	849	C15H24	23.323	1143380350	1.82
β-Germacrene	SH	1560	1557	3	851	C15H24	23.886	744766210	1.19
Unknown	-	1566	-	1566	-	-	24.055	132769520	0.21
Unknown	-	1600	-	1600	-	-	25.022	169868890	0.27

Table A.5 The database of the compounds of green curry in Fraction 4 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Diallyl disulphide[A]	S	1081	1081	0	683	C6H10S2	9.165	27712073	0.43
Diallyl trisulfide	S	1301	1297	4	867	C6H10S3	16.054	461841122	7.13
Allyl propyl trisulfide[A]	S	1314	1314	0	677	C6H12S3	16.469	25696578	0.40
δ-Elemene	SH	1340	1338	2	743	C15H24	17.259	12200064	0.19
5-Methyl-1,2,3,4-tetrathiane[A]	S	1364	1364	0	659	C3H6S4	18.009	14144379	0.22
Unknown	-	1381	-	-	-	-	18.558	81477408	1.26
Unknown	-	1387	-	-	-	-	18.741	3723326	0.06
β-Elemene	SH	1394	1391	3	854	C15H24	18.962	192330607	2.97
Tetradecane	M	1400	1400	0	706	C14H30	19.155	17199170	0.27
Caryophyllene	SH	1422	1420	2	921	C15H24	19.818	267938613	4.14
γ-Elemene	SH	1436	1434	2	832	C15H24	20.219	41224123	0.64
Aromandendrene[A]	SH	1445	1440	5	675	C15H24	20.505	9679350	0.15
Humulene	SH	1447	1454	-7	891	C15H24	20.847	675943862	10.44
β-(*E*)-Famesene	SH	1458	1457	1	903	C15H24	20.889	394930357	6.10
α-Curcumene	SH	1484	1483	1	843	C15H22	21.683	435884032	6.73

Table A.5 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
β-Selinene	SH	1489	1486	3	883	C15H24	21.821	97300688	1.50
β-Guaiene[A]	SH	1494	1490	4	640	C15H24	21.959	3847923	0.06
Zingiberene	SH	1497	1495	2	784	C15H24	22.07	262455302	4.05
Bicyclogermacrene	SH	1499	1499	0	744	C15H24	22.122	272512621	4.21
α-Muurolene[A]	SH	1502	1499	3	562	C15H24	22.215	3747998	0.06
β-Curcumene[A]	SH	1504	1514	-10	623	C15H24	22.253	1226337	0.02
δ-Guaijene	SH	1508	1505	3	845	C15H24	22.384	267103187	4.13
β-Bisabolene	SH	1510	1509	1	921	C15H24	22.446	792139201	12.23
γ-Cadinene	SH	1517	1513	4	764	C15H24	22.633	70034941	1.08
δ-Cadinene	SH	1526	1524	2	854	C15H24	22.895	752160113	11.62
γ-Bisabolene	SH	1534	1533	1	846	C15H24	23.137	134987932	2.08
Diallyl tetrasulfide[A]	S	1541	1532	9	668	C6H10S4	23.33	424874548	6.56
Unknown	-	1554	-	-	-	-	23.7	14737578	0.23
β-Germacrene	SH	1560	1557	3	919	C15H24	23.879	662906594	10.24
Unknown	-	1569	-	-	-	-	24.142	13260997	0.20

Table A.5 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Unknown	-	1593	-	-	-	-	24.832	8369534	0.13
Hexadecane	M	1601	1600	1	822	C16H34	25.036	30998356	0.48

Table A.6 The database of the compounds of green curry in Fraction 5 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
2-Carene	MH	1018	1002	16	818	C10H16	7.397	36370816	0.42
γ-Terpinen	MH	1059	1057	2	846	C10H16	8.551	97086905	1.12
Diallyl disulphide	S	1080	1081	-1	780	C6H10S2	9.144	1621825254	18.69
(*E*)-1-Allyl-2-(prop-1-en-1-yl)disulfane	S	1102	1103	-1	770	C6H10S2	9.78	65198205	0.75
Allyl methyl trisulfide[A]	S	1139	1131	8	602	C4H8S3	10.933	813421589	9.38
3-Vinyl-1,2-dithiacyclohex-4-ene	S	1188	1198	-10	905	C6H8S2	12.463	675248509	7.78
4H-1,2,3-Trithiine	S	1198	1202	-4	818	C3H4S3	12.784	923894342	10.65
2-Vinyl-4H-1,3-dithiine	S	1213	1206	7	860	C6H8S2	13.243	380425594	4.38
Diallyl trisulfide	S	1301	1297	4	746	C6H10S3	16.058	1671193152	19.26
Unknown	-	1314	-	-	-	-	16.462	45833421	0.53
Unknown	-	1363	-	-	-	-	17.992	484527282	5.58
Unknown	-	1381	-	-	-	-	18.554	66777657	0.77
β-Elemene	SH	1394	1391	3	914	C15H24	18.962	411436820	4.74
γ-Elemene	SH	1436	1434	2	905	C15H24	20.219	199643057	2.30
α-Caryophyllene	SH	1457	1451	6	887	C15H24	20.844	366915056	4.23

Table A.6 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
α-Curcumene	SH	1485	1483	2	899	C15H22	21.69	147831536	1.70
Elixene	SH	1499	1492	7	813	C15H24	22.122	63952207	0.74
δ-Guaijene	SH	1508	1505	3	721	C15H24	22.384	20829770	0.24
β-Bisabolene	SH	1510	1509	1	814	C15H24	22.446	106296158	1.23
Unknown	-	1513	-	-	-	-	22.536	20225000	0.23
Unknown	-	1519	-	-	-	-	22.695	37336855	0.43
δ-Cadinene	SH	1526	1524	2	828	C15H24	22.895	39071842	0.45
Unknown	-	1541	-	-	-	-	23.33	197890745	2.28
β-Germacrene	SH	1560	1557	3	814	C15H24	23.876	66086045	0.76
1,6,6-Trimethyl-7-(3-oxobut-1-enyl)-3,8-dioxatricyclo[5.1.0.0(2,4)]octan-5-one[A]	OS	1741	-	-	645	C13H16O4	28.821	48134765	0.55
Unknown	-	2020	-	-	-	-	35.696	68734556	0.79

Table A.7 The database of the compounds of green curry in Fraction 6 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Diallyl disulphide	S	1080	1081	-1	826	C6H10S2	9.151	228940157	19.04
Unknown	-	1189	-	-	-	-	12.484	21024237	1.75
4H-1,2,3-Trithiine	S	1199	1202	-3	794	C3H4S3	12.794	58614524	4.87
Unknown	-	1213	-	-	-	-	13.261	15886901	1.32
Diallyl trisulfide	S	1301	1297	4	846	C6H10S3	16.058	140580359	11.69
Unknown	-	1363	-	-	-	-	18.005	42625913	3.54
β-Elemene	SH	1394	1391	3	707	C15H24	18.965	11683939	0.97
Isocaryophyllene	SH	1422	1408	14	745	C15H24	19.815	13050412	1.09
δ-Cadinene	SH	1526	1524	2	793	C15H24	22.895	26721747	2.22
Diallyl tetrasulfide[A]	S	1542	1532	10	511	C6H10S4	23.358	16609799	1.38
Mintsulfide	S	1741	1744	-3	741	C15H24S	28.828	626917597	52.13

Table A.8 The database of the compounds of green curry in Fraction 8 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Cumaldehyde	OM	1244	1229	15	790	C10H12O	14.221	29408510	0.94
Citronellol acetate	OM	1355	1342	13	819	C12H22O2	17.736	40412149	1.30
Geranyl acetate	OM	1386	1382	4	733	C12H20O2	18.693	34059133	1.09
2,4-Bis(1,1-dimethylethyl)-phenol	M	1513	1521	-8	769	C14H22O	22.515	6675274	0.21
δ-Cadinene	SH	1526	1524	2	737	C15H24	22.895	10988853	0.35
Unknown	-	1607	-	-	-	-	25.219	41307032	1.33
ar-Tumerone	OS	1667	1658	9	825	C15H20O	26.87	25690976	0.82
Tumerone[D]	OS	1671	1649	22	811	C15H22O	26.98	1042124574	33.44
Curlone[D]	OS	1704	1681	23	854	C15H22O	27.857	395789523	12.70
Unknown	-	1741	-	-	-	-	28.824	91872331	2.95
2,2,6-Trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-7-oxabicyclo[4.1.0]heptane[A,C]	OS	1777	-	-	666	C15H22O	29.777	11029457	0.35
Methyl palmitate	M	1927	1918	9	821	C17H34O2	33.496	44201616	1.42
Ethyl 9-hexadecenoate	M	1974	1977	-3	728	C18H34O2	34.612	9799813	0.31

Table A.8 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Ethyl palmitate	M	1995	1986	9	865	C18H36O2	35.116	75542491	2.42
Unknown	-	1999	-	-	-	-	35.213	12484459	0.40
Geranyl linallol	DH	2010	2020	-10	740	C20H34O	35.465	33239042	1.07
Unknown	-	2034	-	-	-	-	36.003	37757293	1.21
Unknown	-	2090	-	-	-	-	37.285	5323843	0.17
Methyl linoleate	M	2095	2085	10	815	C19H34O2	37.388	46991668	1.51
Methyl oleate	M	2101	2081	20	740	C19H36O2	37.537	22943571	0.74
Unknown	-	2112	-	-	-	-	37.765	68612623	2.20
Ethyl Oleate	M	2163	2162	1	901	C20H36O2	38.876	244774589	7.86
Ethyl linoleate	M	2169	2152	17	755	C20H36O2	39.011	136874293	4.39
Unknown	-	2692	-	-	-	-	49.657	57490222	1.85
Unknown	-	2736	-	-	-	-	50.286	35497012	1.14
Unknown	-	2880	-	-	-	-	52.637	90204738	2.89
Unknown	-	2889	-	-	-	-	52.803	9477648	0.30
Unknown	-	2906	-	-	-	-	53.086	6441259	0.21

Table A.8 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Unknown	-	2916	-	-	-	-	53.235	56586224	1.82
Unknown	-	2994	-	-	-	-	54.374	19226261	0.62
Unknown	-	-	-	-	-	-	55.103	154558671	4.96
Unknown	-	-	-	-	-	-	57.855	48180206	1.55
Unknown	-	-	-	-	-	-	58.235	170395499	5.47

Table A.9 The database of the compounds of green curry in Fraction 9 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Cumaldehyde	OM	1243	1229	14	815	C10H12O	14.19	61659983	1.14
Citronellol acetate	OM	1354	1342	12	811	C12H22O2	17.722	79726246	1.47
Nerol acetate	OM	1385	1354	31	785	C12H20O2	18.675	88817597	1.64
Unknown	-	1607	-	-	-	-	25.209	63500847	1.17
ar-Tumerone	OS	1667	1658	9	867	C15H20O	26.866	271973264	5.01
2-Methyl-9-(prop-1-en-3-ol-2-yl)-bicyclo[4.4.0]dec-2-ene-4-ol[A,C]	OS	1672	-	-	735	C15H24O2	26.984	2037597800	37.52
Tumerone[A,D]	OS	1704	1649	55	771	C15H22O	27.854	1057657153	19.48
Mintsulfide[A]	S	1741	1744	-3	669	C15H24S	28.821	22325768	0.41
2,2,6-Trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-7-oxabicyclo[4.1.0]heptane[A,C]	OS	1777	-	-	710	C15H22O	29.767	61012512	1.12
Methyl palmitate	M	1927	1918	9	833	C17H34O2	33.496	60528310	1.11
Ethyl palmitate	M	1995	1986	9	867	C18H36O2	35.112	99568616	1.83
p-Camphorene[A]	DH	1999	1995	4	677	C20H32	35.213	36726424	0.68
Geranyl linallol	DH	2010	2020	-10	725	C20H34O	35.461	52851265	0.97

Table A.9 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Unknown	-	2034	-	-	-	-	36.003	104754084	1.93
Unknown	-	2090	-	-	-	-	37.285	13457862	0.25
Methyl linoleate	M	2095	2085	10	828	C19H34O2	37.388	88106321	1.62
Methyl oleate	M	2101	2081	20	737	C19H36O2	37.537	44905506	0.83
Unknown	-	2112	-	-	-	-	37.768	184364657	3.40
Ethyl linoleate	M	2163	2152	11	871	C20H36O2	38.876	418460169	7.71
Ethyl Oleate	M	2169	2162	7	811	C20H38O2	39.011	212991682	3.92
Unknown	-	2692	-	-	-	-	49.657	34203843	0.63
Geranyl palmitoleate	M	2736	2730	6	720	C26H46O2	50.282	17528170	0.32
Unknown	-	2880	-	-	-	-	52.637	57734852	1.06
Geranyl linoleate[A]	M	2916	2924	-8	666	C28H48O2	53.238	31587997	0.58
Unknown	-	-	-	-	-	-	55.103	80437590	1.48
Unknown	-	-	-	-	-	-	57.855	55016001	1.01
Unknown	-	-	-	-	-	C15H30O	58.232	92612457	1.71

Table A.10 The database of the compounds of green curry in Fraction 10 (2D)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Cumaldehyde	OM	1242	1229	13	838	C10H12O	14.179	126523768	3.56
Citronellol acetate	OM	1355	1342	13	823	C12H22O2	17.726	80784125	2.27
Geranyl acetate	OM	1385	1382	3	745	C12H20O2	18.679	86071771	2.42
ar-Tumerone	OS	1667	1658	9	879	C15H20O	26.866	243245008	6.84
Tumerone[D]	OS	1671	1649	22	766	C15H22O	26.977	1353409313	38.07
Curlone[D]	OS	1704	1681	23	796	C15H22O	27.854	685606803	19.28
Unknown	-	-	-	-	-	-	29.774	34316083	0.97
Methyl palmitate	M	1927	1918	9	785	C17H34O2	33.496	36071181	1.01
Ethyl palmitate	M	1995	1986	9	855	C18H36O2	35.116	59420501	1.67
Unknown	-	1999	-	-	-	-	35.213	19648681	0.55
Unknown	-	2010	-	-	-	-	35.465	30525113	0.86
Geranyl linallol	DH	2034	2020	14	704	C20H34O	36.003	55891935	1.57
Methyl linoleate	M	2095	2085	10	820	C19H34O2	37.388	46173508	1.30
Unknown	-	2101	-	-	-	-	37.537	23917178	0.67

Table A.10 (continued)

Library		Retention index			Library				
Compound name	Class	Experiment	Literature	Diff.	Match	Formula	RT	Area	%Area
Unknown	-	2112	-	-	-	-	37.765	95276155	2.68
Ethyl linoleate	M	2163	2152	11	858	C20H36O2	38.876	232166743	6.53
Ethyl Oleate	M	2169	2162	7	801	C20H38O2	39.011	117312712	3.30
Unknown	-	2692	-	-	-	-	49.654	28114942	0.79
Unknown	-	2881	-	-	-	-	52.644	47517643	1.34
Unknown	-	2916	-	-	-	-	53.238	19710399	0.55
Unknown	-	-	-	-	-	-	55.1	51958970	1.46
Unknown	-	-	-	-	-	-	57.855	29451114	0.83
Unknown	-	-	-	-	-	-	58.228	52406840	1.47

Note

A = All compounds match below 700

B = Too many similar spectra

C = No RI confirm

D = The difference between retention index of experimental and literature have more than 20

MH = Monoterpenes

SH = Sesquiterpenes

DH = Diterpenes

OM = Oxygenated monoterpenes

OS = Oxygenated sesquiterpenes

S = Sulfur-containing compounds

M = Miscellaneous

VITA

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