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# The Qualitative and Quantitative Determinations of Volatile Constituents in Some Herbal Medicines by Gas Chromatography

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## 1. Introduction

Many herbal medicines pertain characteristic odour which indicates the volatility of some compositions. Most of the volatiles constituents have low molecular weight, typically, monoterpenes, sesquiterpenes and phenylpropanes and their oxygenated derivatives (Harnone, 1998). Some herbs contain high content of volatile oils from which the oil themselves are used as drugs, for example eucalyptus oil, eugenol etc., but some are very low. Volatile oils are some times called essential oils due to the believed that it is the essence of the herb. At present, qualitative or identification by gas chromatography (GC) is well established due to the availability of mass spectrometer (MS) detector. When constituents are identified, the interested components can be quantitatively determined by the flame ionization detector (FID).

The purpose of this chapter is to simplify technique of GC for new users in qualitative and quantitative determinations of herbal medicines. The advantage for the method compared to other chromatographic methods is that it can be used to determine complex mixtures with very minute amounts and accurate. This chapter will be emphasized on practical points of view and will be elucidated by examples from literatures.

## 2. Gas chromatographic system

As we already know that GC is used to separate mixtures which vapourise at the operating temperatures. The main parts of gas chromatography are injector, column oven and detector. Running gas chromatography requires accessories one of which is a column which is the crucial part of the separation.

### 2.1 Injector

Injector usually set 50°C above the boiling point of the highest boiling point of the component in the mixture. For separation volatile oils it is usually set around 170-290 °C. Usually sample is injected directly into the injector through the injector rubber septum. Sample will be flushed through the column with a carrier gas. The whole amount of injected sample can be loaded or splitted into 1:10 or 1:100 or 1:500 as desired onto the column which depended on the concentration of the sample.

Helium, hydrogen argon, nitrogen and air can be used as a carrier gas. Hydrogen and nitrogen can be generated from the gas generators which are available in the market. Helium and argon cost more. However, air and hydrogen are usually avoided since they are reactive to some analytes.

## 2.2 Column

Glass capillary column coated with methyl silicone, carbowax, 5%-phenyl-methylsiloxane are usually employed for the separation. Column temperature programming can be preliminary set by using medium ramping time of each step of increments, if the components are eluted unresolved or too far apart, the programme can be optimised. The maximum temperature for each type of column has to be carefully checked.

## 2.3 Detector

There are many types of detector available for a GC system. In this chapter, only MS and FID will be referred.

### 2.3.1 Mass spectrometer detector

Mass spectrometer is a very useful detector since the mass spectrum can be acquired which is a further step to confirm the compound being analysed. Besides that the intensity of the fragment which is the base peak of the spectrum can be used to increase the sensitivity when the parent peak is very low. The industry standard for sensitivity of GCMS instruments is based on octafluoronaphthalene (OFN); the sensitivity of an instrument is defined as the signal to noise (S/N) obtained from a 1 $\mu$ L injection of a solution containing 1pg/ $\mu$ L OFN (Shimadzu, 2007). However this sensitivity is not always implied to our interested compounds, the chromatographic conditions, the MS conditions employed as well as the fragmentation of the compound itself involves in the sensitivity of the GC-MS.

High purity of helium is usually employed as a carrier gas for a GC-MS. For the mass spectrometer detector, the interface temperature usually set at not lower than the injector temperature, the ion source is also high closed to the interface. Scan mode for positive or negative ions, range of mass required, rate of the scanning and solvent delay can be set as required. The setting methods can be found in the instrument manual. Example of setting these parameters are shown in the analysis of *Mimusop elengi* and *Curcuma aeruginosa*.

The mass spectra of constituents in volatile oils which are monoterpenoids, sesquiterpenoids, and phenylpropanoids have been extensively studied, well documented and collected as a data base. Data base of mass spectra of various volatile components especially which are main constituents in most fragrance, essential oils and many other odorous components are organized systematically as libraries *i.e.* NIST (National Institute of Standard and Technology) or FFNSC (Flavour and Fragrance Natural. and Synthetic Compounds). Thus qualitative analysis of volatile oil in these days is easy to perform. However, similarity of the same group of compounds or variation of the GC system might cause some difficulty in the identification. In ionization a compound in a mass spectrometer of the GC instrument, an electron impact is used. The energy usually employed is 70 eV, which is quite strong thus parent peaks might not be detected. But if they do exist, the molecular weight of many compounds are equal.

Fragmentation of these compounds are also similar. Therefore assignment of the individual component requires additional information, linear retention index or Kovat's index and confirmation with authentic standards. Manual interpretation of the mass spectrum is some time needed, when there is no fitted matching. Isotope abundance is very useful to postulate the identity of molecule or fragment.

### 2.3.2 Flame ionization detector

The flame ionization detector is the most commonly used for GC. It is considered an economical detector. The gas used for the detector is hydrogen and air. Signal out put of the flame detector is very sensitivity, 1pg/s, however the sensitivity also depends upon the sample preparation and the GC instrument. For example for benzene with the headspace GC-FID of one model is 0.02 ppm and 0.06 ppm for detection and quantitation limits, respectively which is more sensitive than the other model of the same manufacturer (Agilent technology, 2007). In quantitative analysis of a known compound by a GC, a MS detector can be used but usually avoided for routine analysis due to the running cost.

## 3. Isolation of volatile oil from its matrix

Isolation of volatile oil by solvent extraction, steam distillation or enfleurage have been employed for a long time. Steam distillation seems to be the method of choice since non-volatile components are excluded. However, decomposition of some components causing the alteration of its original odour is the main drawback. These decomposed components are considered as an artefact. At present, headspace can be used as an *in situ* separation which required an extra part to transfer the sample into the gas chromatographic column. However, steam distillation and solvent extraction are still in use these days, since they are simple and the methods can be done in any laboratory.

### 3.1 Steam distillation

This method, specimens and water are heated in a steam distillation apparatus, once the water is boiled, volatile constituents will be carried along with the water vapour and then condensed in to a trap containing water and an immiscible solvent. Water and the solvent will separate into two phases. The volatile oils is dissolved in the solvent. A standard method described in British Pharmacopoeia (BP) 2010, Essential oil in Herbal Drugs, is very useful in determining the total content of volatile oils in herbs. The volatile distillate thus obtained can be used for determination of the constituents either qualitatively or quantitatively.

### 3.2 Solvent extraction

Diethyl ether, acetone and low molecular weight hydrocarbons, pentane or hexane, are commonly employed. Ether and acetone is more advantage due to its better ability to dissolve more oxygenated compounds. The advantage of these solvents are their high volatility, 34.6°C, 56°C for ether and acetone and 36°C and 54°C for pentane and hexane, respectively. The tendency of these solvents interfering the elution of the peaks of the volatile components is low. Further more if the extract has to be concentrated, these solvents are easily expelled.

### 3.3 Enflourage

The method is to allow the volatile substances to be absorbed by fat and then dissolved into alcohol. This method is usually applicable for the old process in fragrance manufacturing.

### 3.4 Headspace

This method, sample containing liquid or solid is sealed in a closed container with an empty space for vapour to be volatilised and collected. The sample is warmed up for a period of time to reach equilibrium in the container. The vapour above the liquid is sampled and injected into the column using pressure as the injection mode. This technique started about 30 years ago. It is already one technique used in the USP 34 to determine the residual solvents in pharmaceutical ingredients as well as in BP 2010. In herbal medicine, this technique becomes very popular due to the ease, convenience, requiring low amount of specimen and less time consuming. The drawback is that an extra cost for the installation of the headspace part. At present rapid headspace solid phase extraction were employed for determination of volatile components in herbs, flowers and wine flavour (Cha, 2009; Won, 2009; Boutou, 2007).

## 4. Qualitative determination

The availability of the GC-MS makes qualitative analysis of volatile oil simple. If the volatile oil of a herb has been investigated and our herb is in same species and we would like to check only the varieties of the herbs that deviated from the previous investigation, the GC-FID can be employed. However, some misinterpretation or discrepancy results might occur. The whole constituents have to be redone and GC-MS is most recommended. In identifying a compound, a mass spectrum itself is very useful, theoretically the parent mass and fragmentation of compound is due to its structure. However, volatile components are similar, for example all monoterpenes consist of 10 carbons. Fragment masses are also similar. Thus additional confirmations is required, such as Linear retention index (LRI) or Kovat's index. Confirmation with authentic compound is also recommended, since it is not only confirm the identity of the compound. It can be used to relate to others constituents in the prior or subsequent eluted components. This is because the sequence in eluting of these compounds on the same type of column are the same. In the qualitative assay, libraries of the volatile components are available and installed in the GC-MS instrument, NIST (National Institute of Standard and Technology) or FFNSC (Flavor and Fragrance Natural. and Synthetic Compounds). At present, even the linear retention time can be confirmed from the library. Identification of the component from the mass spectrum can be done by comparing the obtained mass spectrum with those stored in the library. Similarity index or percentage of fitting indicates the probability of the identification. If the LRI fitting is not available in your instrument, LRI of such compound can be checked with those in literatures, *i.e.* Davies, 1990 and Adams, 2007.

### 4.1 Linear retention index calculation

The volatility of the series of *n*-alkanes (HC) depends on their molecular weight which in turns the chain length. They are nonpolar, their interaction with the GC column are the same, their retentions on the GC solely depend upon the molecular weight. Therefore, it is useful to use as references for gas chromatographic system.

A mixture of *n*-alkanes (HC), C<sub>10</sub>-C<sub>25</sub> are usually used, 10 µL or 1 mg each of the *n*-alkanes in 100 mL of pentane or hexane or the solvent used for sample. Commercial products of the combination of these *n*-alkanes are available. Temperature programme for the column oven is available in nearly all GC instrument. If the initial temperature of the programme is high, the low molecular weight HCs can be omitted since they will be eluted simultaneously with the solvent front. Most of the linear retention of constituents for volatile oils starts from C<sub>10</sub>. On the contrary, lower molecular weight HC can also be used if constituents pertains low vapour pressure, initial temperature should be set accordingly low. The time of each constituent eluted from the column related the series of HCs are used to calculate the LRI. For example, if the retention time of C<sub>14</sub> and C<sub>15</sub> is 11.4 and 12.9 minutes, and the retention time of a component is 11.7, the LRI of the component is 1420, by using the following equation.

$$\begin{aligned} \text{LRI} &= 100[(t-t_n/t_{n+1}-t_n) + n] \\ t &= \text{retention time of the constituent} \\ n &= \text{carbon number of the preceding } n\text{-alkane} \\ n+1 &= \text{carbon number of the subsequent } n\text{-alkane} \\ \text{LRI} &= 100 [(11.7-11.4)/(12.9-11.4) + 14] \\ \text{LRI} &= 1420 \end{aligned}$$

Example 1. Qualitative determination of volatile oil extracted from *Mimusop elengi* flowers:

Dried *M. elengi* flowers are used in many Thai traditional medicine recipe'. The degree of dryness of the flowers was not specified. In establishing the standards for this flower for Thai Herbal Pharmacopoeia, analysis of the left over constituents is one of the aspect to be carried out. Fresh and 4-week air dried flower were GC-MS examined. Fig.1 is the GC-MS

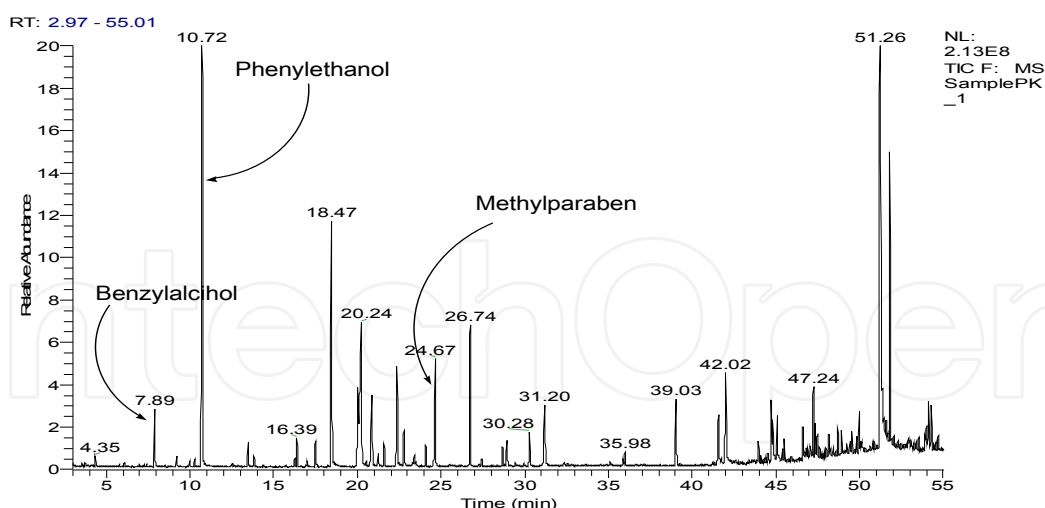


Fig. 1. Chromatogram of fresh *Mimusop elengi* flower. GC system: Column: TR-5 (5%phenyl-95%dimethylpolysiloxane), 30m, 0.25µm, 0.25 mm ID; Oven temperature program: 60 °C to 180 °C at 3 °C.min<sup>-1</sup>, to 280 °C.min<sup>-1</sup> at 10 °C.min<sup>-1</sup> (hold 5 min); Run time: 55.00 min; Carrier gas flow rate (Helium): 1 ml/min; Transfer line temperature (Interface temp.): 275 °C; Ion source: 220 °C. Scan mode: full scan 35-650 m/z; Scan rate: 2000amu/s; Solvent delay time: 3min. Instrument: TraceGC ultra, Model K05200B20000070, Italy/MS Model TraceDSQ,USA; Software: Xcalibur 1.4; Library: NIST MS Search



chromatogram of fresh *M. elengi* ethereal extract and Fig. 2 is the dried flowers' ethereal extract. Fig. 3 is the chromatogram of *n*-alkane series, C<sub>10</sub>-C<sub>23</sub>. From Fig. 3 the retention times of C<sub>11</sub> and C<sub>12</sub> are 10.08 and 14.00 min, respectively. The LRI of the component at 10.72 min was calculated to be 1116. Its mass spectrum is shown in Fig. 4. From the matching to the NIST-MS library, the compound is 2-phenylethanol, see also Fig. 4. Authentic standard of 2-phenylethanol was injected to confirm the identity (Aromdee, 2009). Langlois (1996) found that the LRI of 2-phenylethanol was 1280. However, the column used was DB1701 which is coated with 14%-cyanopropyl-phenyl-methylpolysiloxane. As we can see that LRI of compounds also depended on the analysis system used, thus mass spectrum and checking with authentic compound is some time necessary. Anyway, there are many components in a volatile oil, checking all components with authentic standard is not possible. However one compound confirmation can be useful in the confiding of the GC system being used.

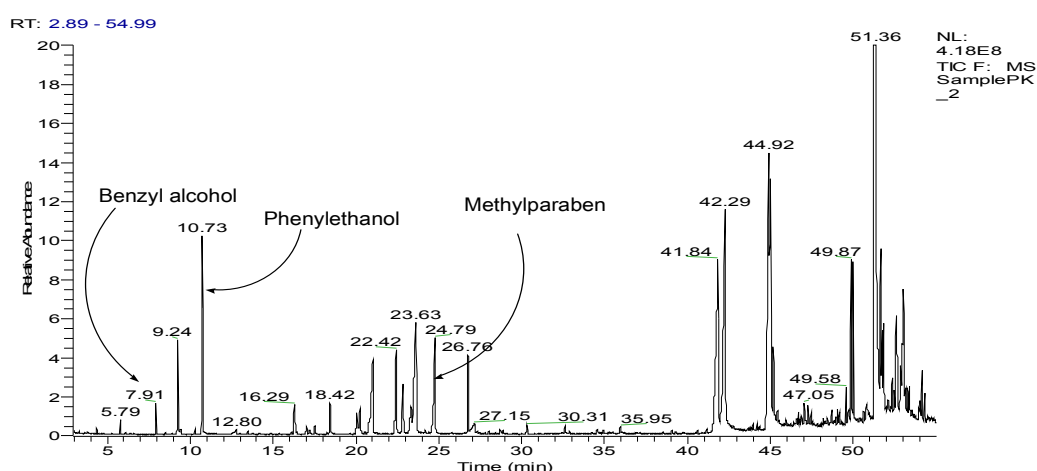


Fig. 2. Chromatogram of dried *Mimosa elengi* flowers. System was the same as described in Fig 1.

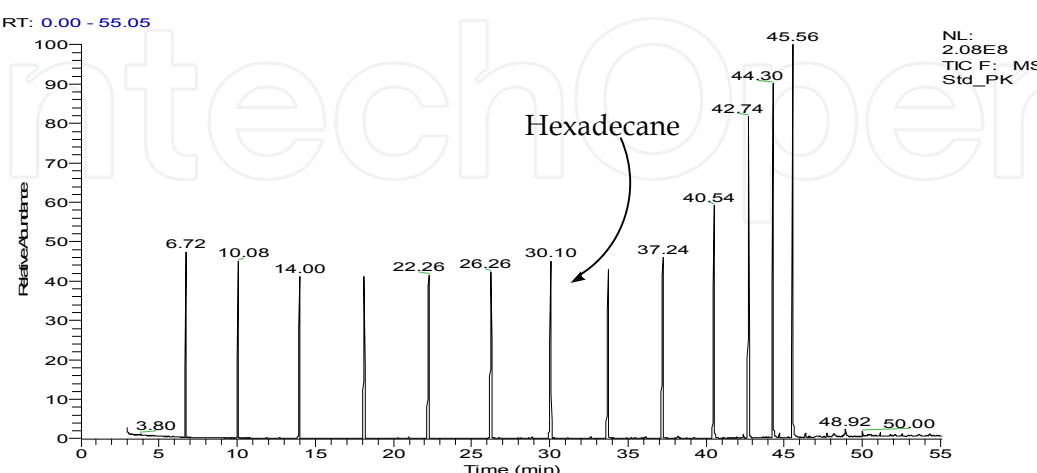


Fig. 3. Series of *n*-alkane C<sub>10</sub>-C<sub>23</sub>. Retention time of C<sub>10</sub> is 6.72 and C<sub>23</sub> is 45.56 min. GC system was the same as fresh flowers, Fig. 1.

SamplePK\_1 #1411 RT: 10.72 AV: 1 AV: 5 SB: 12 1404-1409 1413-1418 NL: 1.78E7  
F: + c Full ms [ 35.00-650.00]

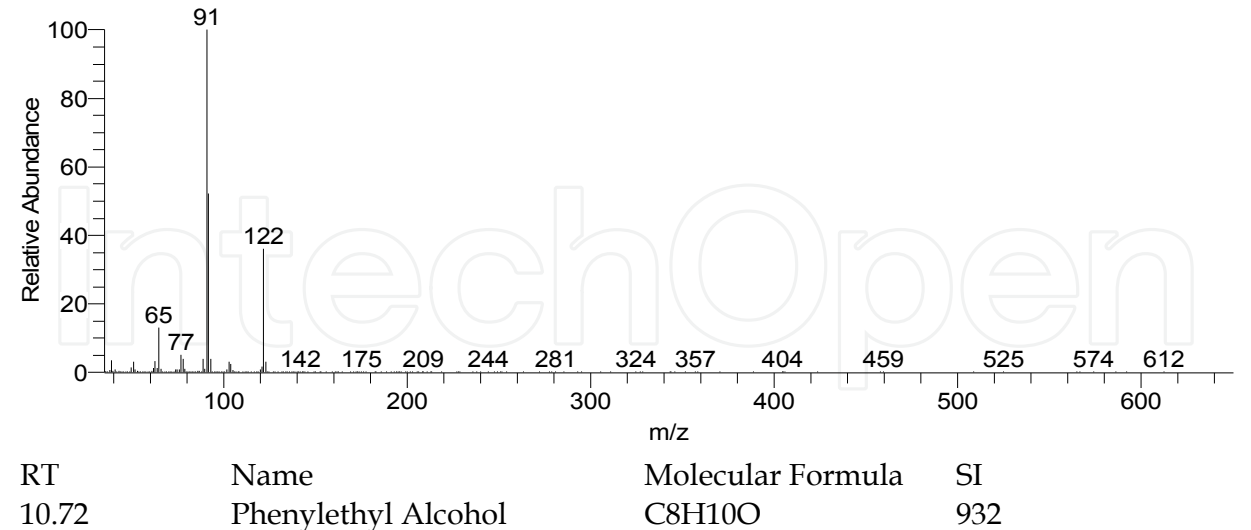


Fig. 4. Mass spectrum of the peak with the retention time of 10.72 min. From the NIST library search, the compound was matched with 2-phenylethanol (phenylethyl alcohol) with 932 similar index (SI).

Components	Retention time		LRI	%		Method of identification
	Fresh flower	Dry flower		Fresh flower	Dry flower	
Benzyl alcohol	7.89	7.91	1040	0.79	0.38	MS, LRI, Std
2-Phenylethanol	10.72	10.73	1116	10.49	3.29	MS, LRI, Std
3-Phenyl-2-propene-1-ol	18.46	18.43	1308	6.17	0.53	MS, LRI
4-Hydroxybenzene methanol	20.24	20.25	1354	8.69	0.41	MS, LRI
Methyl 4-hydroxybenzoate						
2-Butyl-phenol	24.67	24.78	1462	2.69	1.74	MS, LRI, std
Hexadecanoic acid	31.20	-	1632	1.74	-	MS, LRI
Long chain carboxylic acid	42.02	42.02	1968	2.43	0.07	MS, LRI
Unidentified	-	42.29	1980	-	5.37	MS, LRI
(Z)-9-Octadecenoic acid	-	44.92	2150	-	6.65	
Octadecanoic acid	-	45.02	2158	-	4.71	MS, LRI
	-	45.18	2170	-	0.66	MS, LRI

Table 1. Volatile constituents in fresh and dried *Mimusop elengi*

If we look back at Fig. 3 the elution of *n*-hexadecane was at 30.10 min which does not interfere with any component in dried flowers extraction (Fig. 2), it was later selected as the internal standard for quantitative analysis. The tenderly odour of the flowers still exists in most of the dried flowers purchased from traditional medicine stores in Thailand. Some active constituents of which the pure chemicals are commercial available were determined to represent existing components in the dried flowers, which will be described in section 5 “Quantitive Determination”. There are more than 50 components identified in *M. elengi*. Some components in the fresh flowers and 4-week dried flowers were compared as can be seen in Table 1.



Example 2. Qualitative determination of steam distilled volatile oil from *Curcuma aeruginosa* rhizome:

Steam distilled volatile oil of *C. aeruginosa* was similarly determined for the composition. The GC-MS system employed was similar to that of *M. elengi* except the ramping rate and final temperature. The content of volatile oil in *C. aeruginosa* is very rich, the injection was splitted 1:100. The chromatogram is shown in Fig. 5 and the *n*-alkane series is in Fig 6.

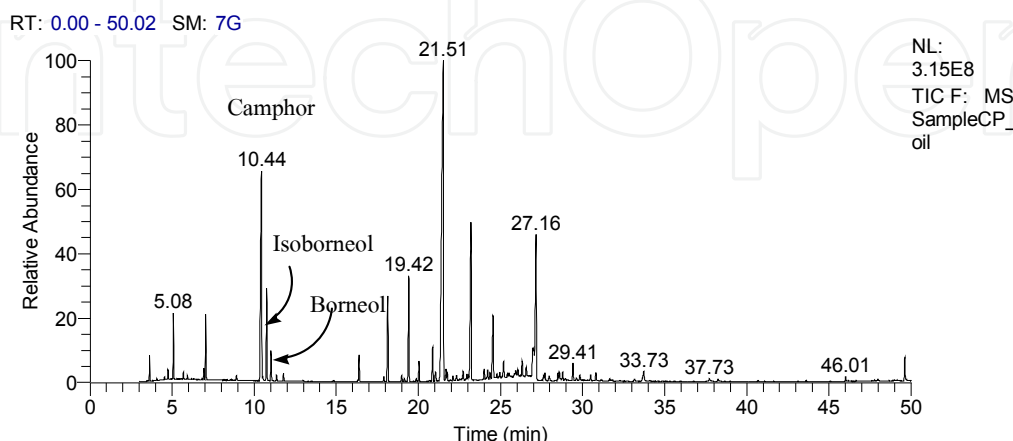


Fig. 5. GC-MS chromatogram of the steam distilled volatile oil of the rhizome of *C. aeruginosa*. Camphor, isoborneol and borneol were eluted at 10.44, 10.65 and 10.99min, their LRI were 1150, 1160, 1177, respectively. GC-MS system: 1  $\mu$ L (split mode 1:100) at 270  $^{\circ}$ C onto a TR-5 column using helium as a carrier gas at a flow rate of 1 mL.min<sup>-1</sup>. The oven temperature was programmed for 60 – 240  $^{\circ}$ C (4  $^{\circ}$ C.min<sup>-1</sup>) and 240 – 270  $^{\circ}$ C (10  $^{\circ}$ C.min<sup>-1</sup>) then held for 2 min. The detector and interface were maintained at 275  $^{\circ}$ C and the ion source at 220 $^{\circ}$ C and the MS scanned in positive ion mode over 35 – 650 m/z. Obtained from the same instrument as Fig. 1.

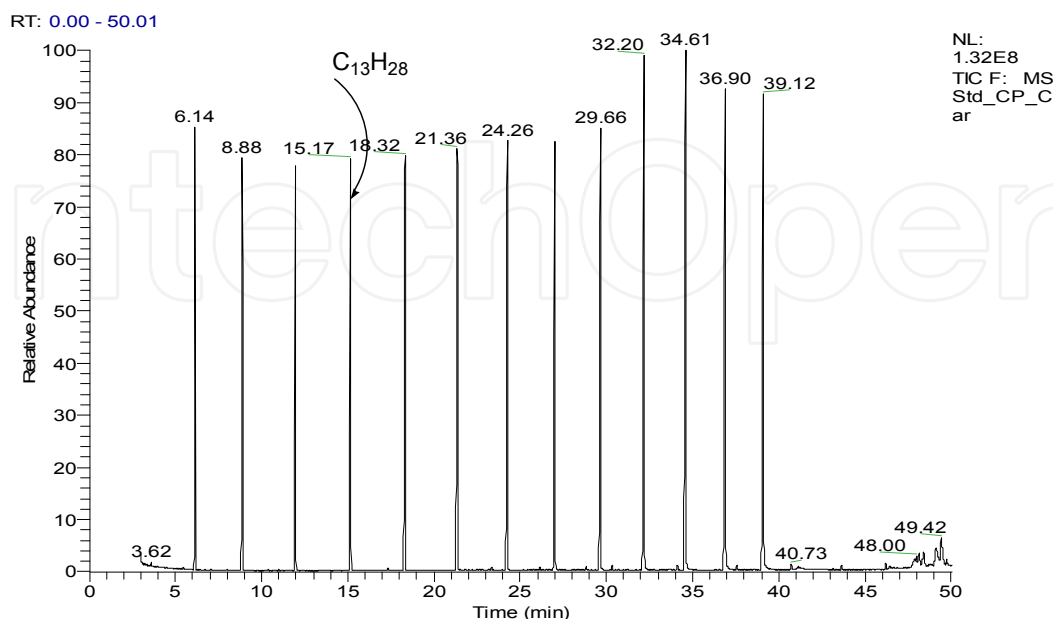


Fig. 6. GC chromatogram of C<sub>10</sub>-C<sub>22</sub> running in the same conditions as Fig. 5

In matching the mass spectrum obtained from experiment and data base of monoterpenes sesquiterpenes were usually found. It is quite often that mass spectra of components were not matched with any one in the library, thus interpretation from parent peaks and fragmentations is necessary. For example in determination of the volatile oil of *Azadirachta indica* obtained by steam distillation (Aromdee, 2006), terpenoids were indentified as well as some dimers of sulphides were found, dipropyl disulphide, propyl propenyl disulphide, they were elucidated from the LRI and the MS. Chemical dimerisation of sulphide is common. However, some other sulphur containing compounds with no availability of LRI were found. Thus, interpretation from mass spectra, fragmentation and isotopic abundance was carried out. The natural isotopic abundance of sulphur, its atomic mass +1 (A+1) is 0.79% whereas the A+2 is 4.4% (McLafferty, 1993). In the study, the isotopic mass of one of the components with the retention time of 19.52 minutes (m/z 166) was found to contain 3 atoms of sulphur. The peak was assigned as HSSSC<sub>5</sub>H<sub>9</sub>. The mass spectrum details are as follows: m/z (ion mass, relative intensity): 41 (C<sub>3</sub>H<sub>5</sub><sup>+</sup>, 83), 45 (CH≡S<sup>+</sup>, 32), 47 (S<sup>+</sup>+CH<sub>3</sub>, 11), 59 (SC<sub>2</sub>H<sub>3</sub><sup>+</sup>, 71), 60 (S<sup>+</sup>+C<sub>2</sub>H<sub>4</sub>, 86), 64 (2S<sup>+</sup>, 59), 69 (C<sub>5</sub>H<sub>9</sub><sup>+</sup>, 94), 73 (C<sub>3</sub>H<sub>5</sub>S<sup>+</sup>, 50), 74 (C<sub>3</sub>H<sub>6</sub>S<sup>+</sup>, 40), 92 (C<sub>2</sub>H<sub>4</sub>S<sub>2</sub><sup>+</sup>, 48), 101 (M<sup>+</sup> - SSH, 53), 102 (M<sup>+</sup> - 2S, 69), 106 (M-C<sub>2</sub>H<sub>4</sub>S, 34), 166 (M<sup>+</sup>, 100). The isotope peak (M+2) at m/z 168 (13.2% of m/z 166) indicated the existence of 3 sulphur atoms and 5 carbons and the compound/fragment could be an artifact due to the vigorous isolation, steam distilled, and the high temperature GC conditions. Anyway, the definite identities have to be confirmed. The odour of *A. indica* is pungent and strong, thus it is not surprising that *A. indica* contains many sulfur containing compounds.

Some other elements with the high % isotopic mass is also easy to elucidate, for examples, A+2 of chlorine is 32.0 % and of bromine is 97.3%. Unfortunately, not many volatile components in herbs were detected to contain these two elements.

## 5. Quantitative determination

### 5.1 Total content of volatile oil

Herbs which are rich in volatile oil for example anise seed, cinnamon, caraway, curcuma species, *etc.* The total content of volatile oil in the herbs indicate the quality of the herbs. Thus the lower limit of volatile oil content is one of the specification for these herbs. Many official monographs of drug containing essential oil or volatile oil in British Pharmacopoeia and European Pharmacopoeia limit the content of volatile oil as one of the specification of the drug, for example "Ginger BP 2010" contains essential oil not less than 1.5 %v/wt (anhydrous drug). Besides that the chromatographic profile of essential oils were also imposed in these pharmacopoeiae. The limits of relative contents of some characteristic essential components of the oil are specified.

Steam distillation is the standard method used for the determination of volatile oil. In British Pharmacopoeia, the dimension of the essential oil apparatus was specified, since all parts of the apparatus affect the yield of the volatile oil and avoid any controversy in the quality of the herbs. This method is usually employed to determine high volatile oil content herbs. Aromdee *et al* steam distilled various species of curcumas, *Curcuma aromatica*, *C. aeruginosa* by the method described in BP using xylene as an organic entraptor. They found the volatile oils in *C. aromatica* was 0.88-0.96% v/wet wt (Aromdee, 2010), where as *C. aeruginosa* was 0.55-0.42%v/wet wt (Aromdee, 2007). The content of volatile oil in other species of curcuma,

*C. longa*, which is official in Thai Herbal Pharmacopoeia 1 (THP1,1998) is not less than 7%v/w. In establishing the standard limit of content of volatile oil of herbs in national or international pharmacopoeiae, 12-20 authentic samples from various parts of the country or reliable sources have to be collected. Replicate determinations of the volatile oil content in each of these samples have to be carried out according to the pharmacopoeial standard method. Mean and standard error of the results are used to set the limit of content of volatile oil.

By the way, some low content of herb was also studied by steam distillation, *i.e.* *Azadirachta indica* gave very minute amount of the volatile components (0.001% v/wet wt).

## 5.2 Quantitative determination of constituents in volatile oil

For quantitative determination of some components in volatile oil, internal standard usually required for gas chromatography since the injector is set at high temperature, uniformity of the injection through the septum, time delay in the injection involving in the irreproducibility of the method. At present, although auto-injector improves the reproducibility of the injection, but internal standard is still in used for the accuracy and precision of the analysis. Analytical method developed to determine the constituents in volatile oil has to be validated. International Conference of Harmonisation (ICH), the quality guidelines are widely used for the validation since it is accepted universally. The guidelines are revised periodically, thus updating the guidelines should be caught up occasionally. However, not much alteration in each revision since it have to be relied on basic concepts which are accuracy, precision, limit of quantitation and limit of detection. These parameters are important for the reliability of the method and result.

### 5.2.1 Finding for an internal standard for quantitative determination of volatile oils

If the LRI of the component to be acquired is known, it is easy the find the internal standard. The *n*-alkane closed to, but does not overlap or interfere the adjacent components or peak of the volatile oil, can be used. For example Polrat used tridecane as internal standard to determine camphor, isoborneol and borneol in some curcuma spp. (Aromdee, 2007). Aromdee (2010) used *n*-hexadecane as an internal standards for determination of benzyl alcohol (1040), 2-phenylethanol (1119) and methylparaben (1525) in *M. elengi*. *n*-Alkanes are universal used for internal standard for gas chromatographic determination of volatile constituents, but not limit to the herbal volatile oil.

To quantify an interested component in the volatile oil, standard solutions of the component have to be prepared in a series in the range that cover the expected concentration of the sample. Once volatile oil is isolated, an exact portion of volatile oil will be taken for the determination. An exact amount of internal standard can be added directly to aliquots of the sample and the standard solutions, then the mixtures are diluted to the appropriate concentration. By the way, internal standard can be added to the diluting solvent and an equal volume of this diluting solvent must be used in the standard series and sample preparations.

Aromdee *et al* quantitatively determined the benzyl alcohol, 2-phenylethanol and methylparaben which are believed to be preservatives for preparations containing dried mimusop's flowers. *M. elengi* flowers was extracted with ether and concentrated under the

nitrogen atmosphere. The residue was reconstituted with ethyl acetate. *n*-Hexadecane was used as an internal standard as it does not interfere with any peak of the extract, see Figs.2 and 3. Validation of the method was carried out for accuracy, precision, limit of quantitation, limit of detection and linearity range as directed in ICH guidelines.

Example 3: Quantitative determination of benzyl alcohol, 2-phenylethanol and methylparaben in dried *M. elengi* flowers:

**Stock standard solutions (SS):** Pipette 10 and 100 µL of benzyl alcohol and 2-phenylethanol respectively into a 10-ml volumetric flask, add 10 mg of methylparaben. Dilute to the volume with ethyl acetate. Standard solutions are prepared as shown in Table 2.

Solution	Volume used (µL)			
	Sample	SS	IS	Ethyl Acetate
1		0	100	200
2		20	100	180
3		100	100	100
4		150	100	50
5*	Sample residue	20	100	180
6*	Sample residue	100	100	100
7*	Sample residue	150	100	50

Table 2. Preparation of standard solutions for *M. elengi* (\* Used for accuracy validation)

**Internal standard solution (IS):** Pipette 100 µL of *n*-hexadecane and diluted with ethyl acetate to 100 mL.

**Sample preparation:** Extract the finely ground powder of dried *M. elengi*, 3 g, with ether, 12, 5, 5 mL of ether successively. Evaporate the combined extraction solutions under the steam of nitrogen. Add 200 µL of ethyl acetate and 100 µL of internal standard solution to the residue. Vortex for 5 minutes, centrifuge, and inject the supernatant.

Three sample solutions of the specimen were prepared and each was injected trice as well as the standard solutions and the standard addition samples. The standard curve was constructed for the interpolation of the three components. Reduction of the contents found in the sample solution from the results of standard addition sample, the percent recoveries were obtained which are 91.66, 104.59 and 105.28% benzyl alcohol, 2-phenylethanol and methylparaben, respectively. This dried sample of *M. elengi* was found to contain 13, 196 and 232 ppm of benzyl alcohol, 2-phenylethanol and methylparaben, respectively. Other parameters were also carried out which are the reproducibility, the limits of detection and quantitation. Fig.7 is the chromatogram of dreid *M. elengi* flower extract acquired by the GC-FID.

Example 4: Quantitative determination of camphor, norborneol and borneol in *Curcuma aromatica* and *C. aeruginosa* rhizomes. From Fig. 6 we found that C<sub>13</sub>H<sub>28</sub> is suitable to be used as an internal standard, thus it was selected. Fig. 8 showed the chromatograms of the quantitative analysis. Preparation of standard solutions in shown in Table 3. Validation of the method was also carried out according to the ICH guidelines.

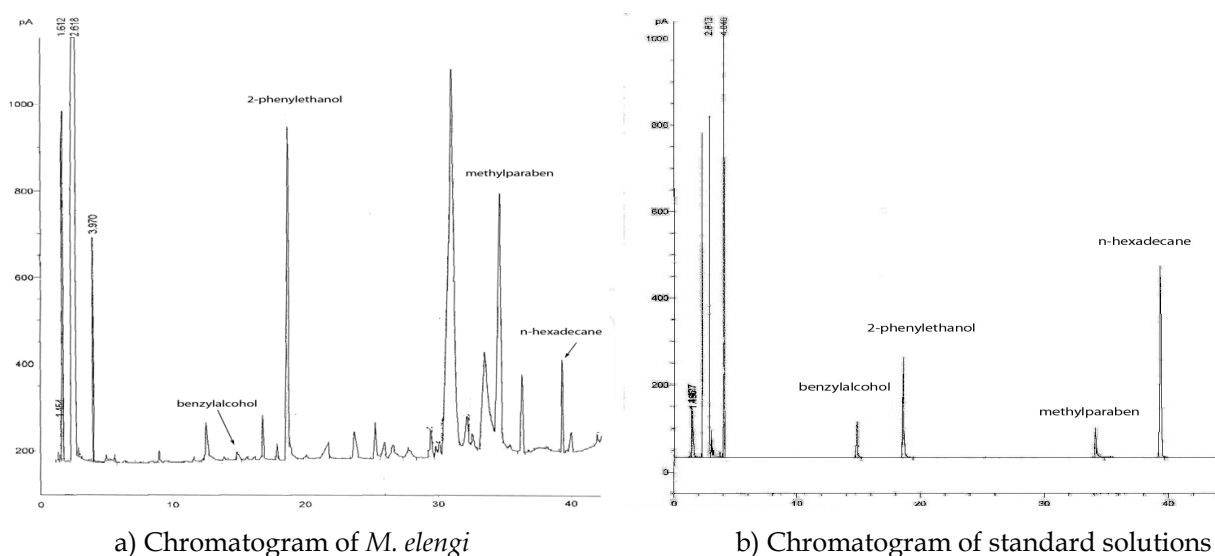


Fig. 7. Gas Chromatograms of a dried *M. elengi* flowers and standard. The specimen was purchased from a Thai traditional medicine store (a). sample extract with *n*-hexadecane as an internal standard; and (b) standard solution containing benzyl alcohol, 2-phenylethanol, methylparaben and the internal standard. Gas chromatographic system: GC (Hewlett Packard HP 6890, USA)/ FID detector; Column: (5%-Phenyl)-methylpolysiloxane, 30m, 0.25 $\mu$ m, 1 mm ID; Oven temperature program: 60 °C to 180°C at 3 °C.min<sup>-1</sup>, to 280 °C.min<sup>-1</sup> at 10 °C.min<sup>-1</sup> (hold 5 min); Run time: 55.00 min; Carrier gas flow rate (Nitrogen): 2 m-l/min.

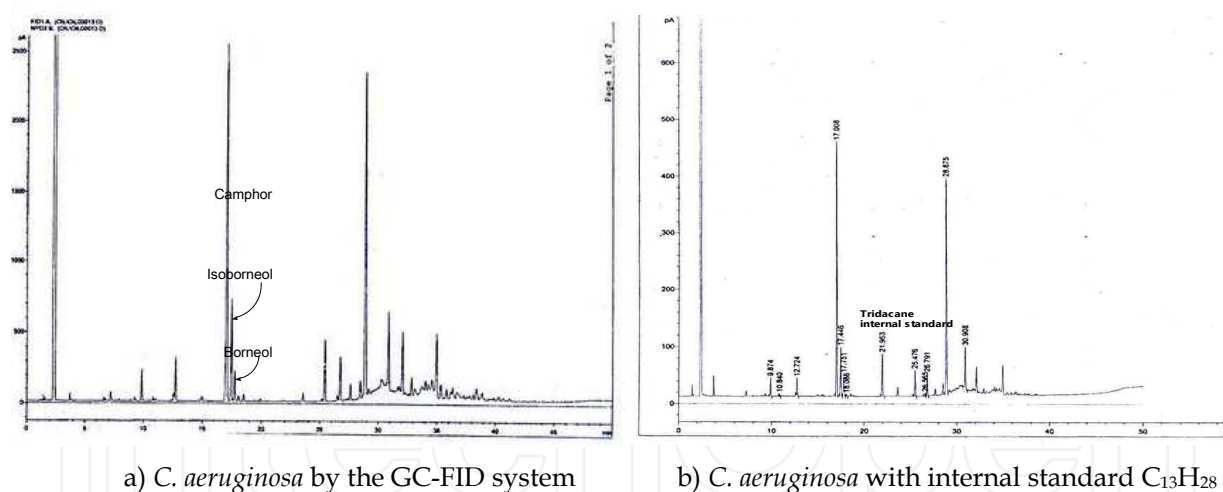


Fig. 8. Gas Chromatogram of volatile oil from rhizomes of *C. aeruginosa*, 1  $\mu$ L of volatile oil solutions was injected (split mode 1:50) at 270°C onto a HP-5 column using nitrogen as carrier gas at a flow rate of 2 mL.min<sup>-1</sup>. The oven temperature was programmed for 60–240° C (4°C.min<sup>-1</sup>) and 240–270°C (10°C.min<sup>-1</sup>) then held for 2 min. The FID detector was maintained at 275°C.

**Stock Standard Solutions (SS):** Accurately weigh about 0.5g camphore, 0.075 g of isoborneol and borneol into a 5 mL volumetric flask, diluted to volume with ethyl acetate.

**Internal Standard Soltion (IS):** Pipette 100  $\mu$ L of tridecane and diluted with ethyl acetate to 100 mL.



**Sample preparation:** Dilute 100 µL of volatile oil to 10 mL with ethyl acetate.

Solution	Volume used (µL)			
	Sample	SS	IS	Ethyl Acetate
1		0	500	500
2		20	500	480
3		100	500	400
4		150	500	350
5*	30	20	500	450
6*	30	100	500	370
7*	30	150	500	320

Table 3. Preparation of standard solutions for camphore, norborneol and borneol in *Curcuma spp.* (\*For accuracy validation)

6. Conclusion

Gas chromatography is very useful for the determination of complex mixtures of analytes. The detectors employed for the qualitative analysis is a mass spectrometer. Whereas the quantitative analysis, both detectors are useful. Factors affect the separation are the type of the column, temperature programming of the column oven, meanwhile sensitivity is mostly depended on detector as well as the isolation and injection method of the volatile constituents. In qualitative analysis of volatile oil in herbal drugs, a GC-MS is required and LRI of the constituents have to be determined. For quantitative determination both GC-MS and GC-FID are applicable but FID is more economical. Separation of the volatile oils from herbs, steam distilled is still the method of choice for conventional method. Headspace is now widely available and convenient. In this chapter, examples of both qualitative and quantitative analysis of *Mimusop elengi* flowers and rhizomes of *Curcuma spp.* were illustrated in details.

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## **Gas Chromatography in Plant Science, Wine Technology, Toxicology and Some Specific Applications**

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The aim of this book is to describe the fundamental aspects and details of certain gas chromatography applications in Plant Science, Wine technology, Toxicology and the other specific disciplines that are currently being researched. The very best gas chromatography experts have been chosen as authors in each area. The individual chapter has been written to be self-contained so that readers may peruse particular topics but can pursue the other chapters in the each section to gain more insight about different gas chromatography applications in the same research field. This book will surely be useful to gas chromatography users who are desirous of perfecting themselves in one of the important branch of analytical chemistry.

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