Analysis on fat-soluble components of sinapis seminafrom different habitats by GC–MS

Mrs.Y.R.Anitha,Ms A R Sridevi,Mr. M Kalyan babu ,Mr,Y Naveen Kumar,Mr.S.Buggareddy
Department of Pharmaceutical Analysis
Email ID: anitharobert17@gmail.com , Mobile no:7207165156
GOKULA KRISHNA COLLEGE OF PHARMACY

Abstract:

The fat-soluble components of sinapis semina were identified using a fast and easy gas chromatography/mass spectrometry (GC/MS) analytical technique. In order to test the efficacy of the procedure, four chemicals were selected as marker compounds. Following an analysis of many extraction methods, sonication extraction with diethyl ether proved to be the most effective. After checking the resolutions, tailing factors, and theoretical plate number of the marker chemicals, we were able to determine that the apparatus was suitable for the approach. We also checked that the accuracy and repeatability, measured as relative standard deviation (RSD), were within the allowed limits. Eight sinapis semina samples were tracked using the approach after being acquired from Xi'an markets. Hierarchical cluster analysis (HCA) similarity analysis was used to examine the fingerprints of those samples. A combination of fingerprint and HCA allowed for the analysis of sinapis semina from various habitats, according to the results.

KEYWORDS : Sinapis semina, GC/MS, fingerprinting, and hydrophilic extraction

1. Introduction

Dried Sinapis semina are the seeds of the Sinapis alba lineage. Among the pharmacological effects of this traditional Chinese medicine include anti-cancer, analgesic, and antiviral properties [1]. Sapidus semina relies on its fat-soluble components. Isolating and identifying the fat-soluble chemicals is crucial for sinapis semina study. Gas chromatography/mass spectrometry (GC-MS) and gas chromatography have seen extensive application for the investigation of herbal medicines' fat-soluble components [2,3]. As an especially applicable and trustworthy technique, GC/MS has been used for the determination of plant medicinal components that are fat-soluble, because of their superior capacity for isolation and identification.

To ensure the efficacy of herbal medicines, quality control is essential, and one aspect of this procedure is regularly monitoring the amounts of chemical ingredients [4,5]. Herbal remedies have a complicated chemical makeup, and the quantification of substances depends on factors such as harvest time, storage conditions, processing technique, and environmental factors. A lot of places have started growing Sinapis semina. country. Sinapis semina's impact is associated with its fat-soluble components, which come from several places.

Quantitative extraction of fat-soluble components from herbal medicines has been accomplished using a variety of procedures, such as steam distillation, solvent immersion, and solid-phase extraction [6, 7, 8]. Having said that, these approaches are tedious and time consuming. The fast extraction of herbal medicine's fat-soluble components has been achieved by the use of sonication extraction. Its low organic solvent consumption and ease of operation make it a practical choice [9–12].

It is not sufficient to only quantify one or even many substances in herbal medicine in order to assess the quality of sinapis semina. One form of thorough, quantifiable chromatographic identification approach is the Chinese medicine chromatographic fingerprint technique. A comprehensive analysis of the chemical composition of Chinese herbal medicine forms the basis of the technique. There has been a recent uptick in interest in chromatographic fingerprint analysis of herbal medicines [13–16]. This is because the technology incorporates the holistic and systemic aspects of Chinese traditional medicine. In addition, by comparing how similar two samples are,

hierarchical cluster analysis (HCA) has been used to classify samples into categories [16–20]. For easier comprehension, a dendrogram may be used to depict the degree of similarity and dissimilarity between data.

This study demonstrates that a GC/MS approach, in conjunction with sonication extraction, may be used to efficiently characterize fat-soluble chemicals in sinapis semina for quality control purposes. We used the established analytical approach to examine eight sinapis semina samples that we obtained from the market. Hierarchical cluster analysis (HCA) and similarity analysis were used to assess the chromatographic fingerprints of those sources. Sinapis semina from various environments may be analyzed using a combination of fingerprint and HCA.

2. Materials and methods

2.1. *Reagents*

Acetic ether (chromatographic grade) was purchased from Fisher Scientific (Pittsburgh, PA, USA). Petroleum ether and diethyl ether (analytical pure) were purchased from Hongyan reagent factory (Tianjin, China).

Analytical samples of sinapis semina were purchased from Xi'an medicinal materials market (China) and identified by an expert discriminator of herbal medicine. Prior to use, the sinapis semina samples were dried and pulverized, then filtered using a standard sieve with a mesh size of 18.

2.2. *Sample preparation*

Dried sinapis semina were pulverized and 10.0 g was added into 40 mL diethyl ether in a volumetric flask. For solvent immersion extraction, the sample mixture was allowed to stand for 24 h at room temperature in a volumetric flask. The mixture was extracted for 30 min in an utrasonic bath at room temperature. After extraction, the extract was filtered under reduced pressure. The filtrate was collected and solvents were recovered to obtain oil substance. The oil substance was diluted 100 times by using acetic

ether and vortexed for 1 min. Finally, 1 μL of the diluted sample was injected into the GC/MS system.

2.3. *GC/MS assay*

A capillary gas chromatography−mass spectrometry (GCMS- QP2010PLUS Shimadzu, Kyoto, Japan) with a Rtx-5MS capillary column (30 m 0.25 mm i.d.; 0.25 μm film thickness, Restek, CA, USA) was used. Helium (purity 99.999%) was the carrier gas

with a constant flow of 1.00 mL/min. The inlet temperature was maintained at 300 °C. The initial temperature was 200 °C, ramped at 10 °C/min up to 280 °C and held for 15 min, and finally ramped at 20 °C/min up to 300 °C, held for 5 min. The mass spectrometer was operated in electron ionization mode at 70 eV. The mass range was scanned from 50*m/z* to 600*m/z* for full-scan mode. Data were collected using the GC/MS analysis software, and analyzed using a NIST library (Shimadzu, Kyoto, Japan).

Under these conditions, the volatile compounds of eight sinapis semina samples were analyzed by GC/MS.

2.4. *Data analysis*

HCA is a multivariate analysis technique that is used to sort samples into groups. Here, different samples of sinapis semina were analyzed by using SPSS 16.0 software, and the results were subjected to HCA. Similarity tests were performed on the basis of the relative retention time (RRT) and relative peak area (RPA) (angle cosine method to calculate the relative peak area), using the professional software named Similarity Evaluation System for Chromatographic Fingerprint (2004).

3. Results and discussion

3.1. *Extraction procedure*

The herbal sample is homogenized and extracted with a suitable solvent to reduce the bulk of the sample matrix and to extract fat- soluble compounds into the solvent. Both the selection of solvent and the extraction method can be critical in obtaining a satisfactory extraction of fat-soluble compounds from herbal drugs. In this study, the traditional steam distillation and the ultrasonic extrac- tion were compared. The result shows that the extraction rate is 8.96% by using the ultrasonic extraction method, while the traditional steam distillation is only 6.48%. The traditional steam distillation consumed too much time and it was difficult to control the temperature. So the ultrasonic extraction was selected to extract fat-soluble parts from sinapis semina. Petroleum ether, ethyl acetate, and diethyl ether were used as extraction solvents. Total ion chromatograms

of the extracts obtained from sinapis semina are shown in Fig. 1. As seen from Fig. 1, ultrasonic extraction with diethyl ether can help obtain more compounds. The extraction efficiency of ultrasonic extraction with diethyl ether, ethyl acetate, and petroleum ether was 8.96%, 7.63% and 7.54% respectively. Finally, diethyl ether was chosen due to its high extraction efficiency.

The extraction yield, the rapidity, and the convenience of sample preparation were taken into consideration; the sonication extraction method using diethyl ether was deemed most suitable.

3.2. *Optimization of GC/MC condition*

In order to let the analytes be separated within a short time, the temperature program was adopted. A total of 13 compounds were identified in this work. The compounds whose similarity and content (%) are above 80% and 0.40%, respectively, are listed in Table 1 and the chromatogram is shown in Fig. 2. Peaks that existed in all batches of samples were considered as "marker compounds" for methodology study. Four common peaks were chosen, whose structures are shown in Fig. 3.

3.3. *Method validation*

To precisely evaluate the quality control of sinapis semina, the method validation was performed to determine the marker compounds in herbal plant. In this study, apparatus suitability, precision, repeatability, and sample's stability were tested for analytical method validation.

3.3.1. *Apparatus suitability*

In this work, the resolutions, tailing factors and theoretical plate number of the marker compounds were calculated, which are shown in Table 2. As shown in Table 2, the theoretical plate numbers of the maker compounds are within the range of 141,911−199,388 and the RSDs of the theoretical plate numbers decreased to 1.555−9.770%. The tailing factors were in the range 0.956−1.372. In addition, resolutions of four marker compounds were within the range of 8.409−17.139. The result indicated that the method was system applicable.

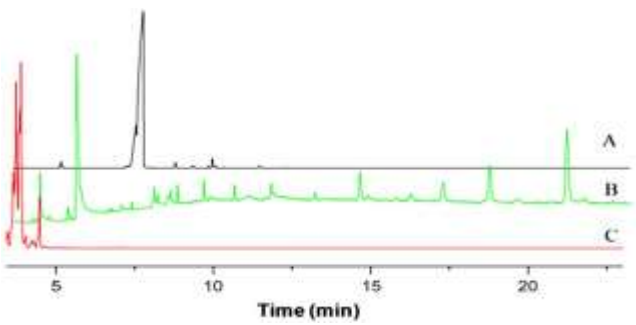

Fig. 1 Total ion chromatograms of sinapis semina: (A) sonication extraction with ethyl acetate; (B) sonication extraction with diethyl ether; and (C) sonication extraction with petroleum ethyl.

3.3.2. *Repeatability, precision and stability*

The repeatability was assessed by analyzing six independently prepared samples of sinapis semina. Intra-day precision was determined by analyzing five replicates during a single day. However, the inter-day precision was determined by analyzing five replicates on five consecutive days. The relative standard deviation (RSD) was taken as a measure of repeatability and precision. The stability of sample solutions was tested at room temperature. The sample solution was analyzed at 0, 12, 24, 48, and 72 h. The results are shown in Table 3. From the table, we can see that this method was stable and reliable, as the RSDs were less than 5%.

3.4. *Establishment of fingerprint*

3.4.1. *Selection of standard samples*

To gain the standardized fingerprint, the standard samples with good quality were selected to establish the mean chromatogram. Eight sinapis semina samples (see Table 4) which met the requirement of the Pharmacopoeia of People's Republic of China (2010 Edition) were selected as the standard samples. All standard samples were analyzed with the developed method.

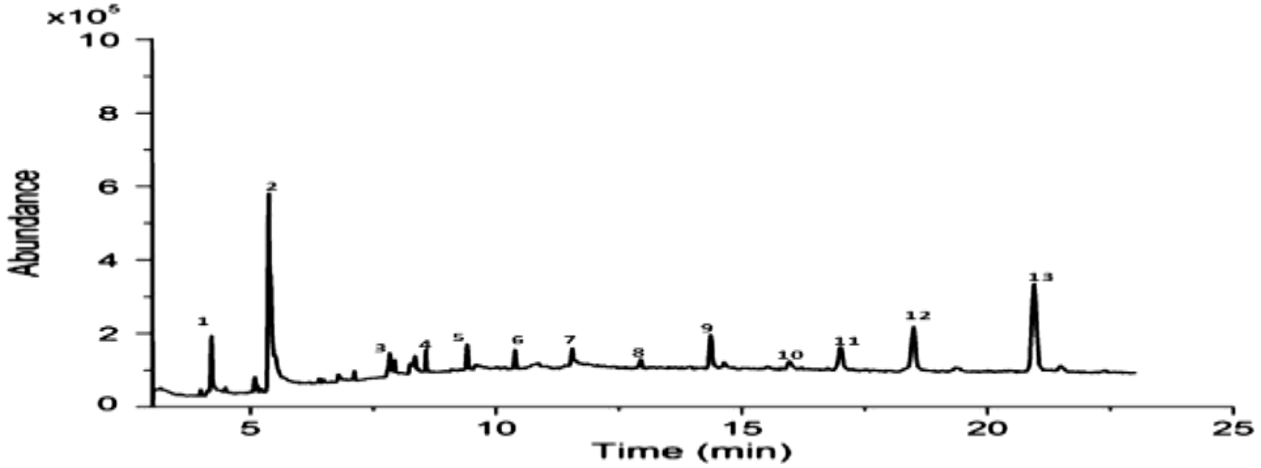

Fig. 2 The total ion chromatograms of sinapis semina extracted by sonication extraction with ethyl acetate.

Table 1 The constituents and relative contents in the ester-solubility fractions of sinapis semina.

Number	Compound	Similarity (%)	Molecular formula	Relative content (%)
1	Ethylbenzene	89	C8H10	0.42
2	o-Xylene	95	C8H10	1.70
3	2,6-Dihexadecanoate,l-(+)-Ascorbic acid	91	C38H68O8	3.71
4	(Z,Z)-9,12-Octadecadienoic acid	93	C18H32O2	45.56
5	Olealdehyde	87	C18H34O	2.20
6	Z,Z-8,10-Hexadecadien-1-ol	85	C16H30O	4.72
7	Oleic acid	88	C18H34O2	2.39
8	(Z)- 7-Hexadecenal	82	C16H30O	1.97
9	(8Z)-14-Methyl-8-hexadecenal	81	C17H32O	4.51
10	beta-Tocopherol	88	C28H48O2	3.56
11	Brassicasterin	80	C28H46O	4.02
12	Campesterol	86	C28H48O	7.35
13	gamma-Sitosterol	89	C29H50O	17.89

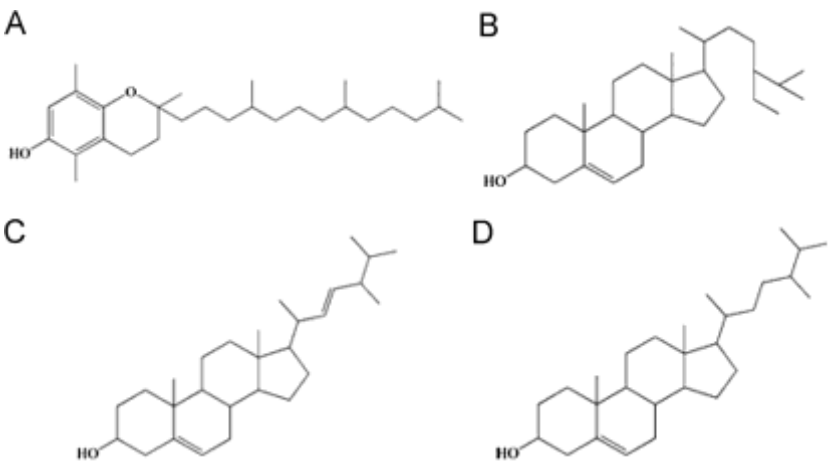

Fig. 3 The structures of marker compounds in sinapis semina: (A) beta-tocopherol; (B) brassicasterin; (C) campesterol and (D) gamma-sitosterol.

Table 2 The system applicability of four marker compounds in sinapis semina.

Marker compounds	Theoretical plate numbers				Tailing factors				Resolution			
	1	2	3	CV (%)	1	2	3	CV (%)	1	2	3	CV (%)
A	198,341	199,612	193,779	1.56	1.37	1.21	1.19	7.92	14.13	16.90	15.40	8.96
B	141,911	169,565	168,349	9.77	1.02	1.01	0.96	3.25	17.14	18.06	17.91	2.79
C	184,364	189,917	199,388	3.97	1.09	1.01	1.05	3.88	8.41	8.87	8.98	3.47
D	180,040	178,770	194,639	4.78	1.04	1.03	1.13	5.37	13.38	13.47	13.87	1.93

Table 3 Precision, repeatability and stability of four marker compounds in sinapis semina.

Marker	compounds	Precision		Repeatability	Stability
		Inter-day precision RSD%	Intra-day precision RSD%		
A	RRT	0.123	0.047	0.144	0.212
	RPA	3.806	0.657	3.560	1.392
B	RRT	0.107	0.039	0.117	0.229
	RPA	3.309	0.207	3.420	1.752
C	RRT	0.121	0.045	0.145	0.243
	RPA	2.618	0.849	2.826	4.405
D	RRT	0.106	0.034	0.601	0.236
	RPA	2.405	0.352	3.584	1.752

RRT: relative retention time.
RPA: relative peak area.

3.4.2. *Selection of reference substance*

There are two kinds of reference substances: one is an internal reference substance to which the common peaks belong to and the other is an external reference substance which is added to the sample. In this study, peak no. 3 was chosen as the internal reference substance because this peak, which was present in the middle of the chromatogram with maximum content, existed in all chromatograms.

3.4.3. *Fingerprint*

The fingerprint analysis was carried out by using the software "Similarity Evaluation System for Chromatographic Fingerprint of TCM". The mean chromatograms and correlation coefficients of the samples are shown in Fig. 4 and Table 4. In comparison to the consensus fingerprint, all sinapis semina samples showed a similarity of at least 0.904% (shown in Table 4). Sinapis semina from Shanxi, Henan, Hebei, and Guangxi provinces, China have higher similarity of 98.8–99.5%, followed by those from Anhui, Shandong, Jilin and Sichuan provinces, which were between 90.4% and 97.3%.

3.5. *Quality assessment by HCA*

To differentiate the sinapis semina samples

collected from the market, HCA was applied to the chromatographic data that were

Table 4 The information and correlation coefficients of samples.

Sample	Production	Similarity
S_1	Anhui	0.973
S_2	Guangxi	0.988
S_3	Hebei	0.992
S_4	Hennan	0.993
S_5	Jilin	0.911
S_6	Shandong	0.904
S_7	Shanxi	0.991
S_8	Szechwan	0.945

The similarity was calculated by similarity analysis; each fingerprint (from Fig. 4) was compared to the consensus chromatogram "R". All samples showed a similarity of at least 40.904%.

Conclusion

In this study, we described a simple and rapid GC/MS method for quantifying volatile compounds in sinapis semina. The sonication method was shown to be rapid and effective for the extraction of volatiles from

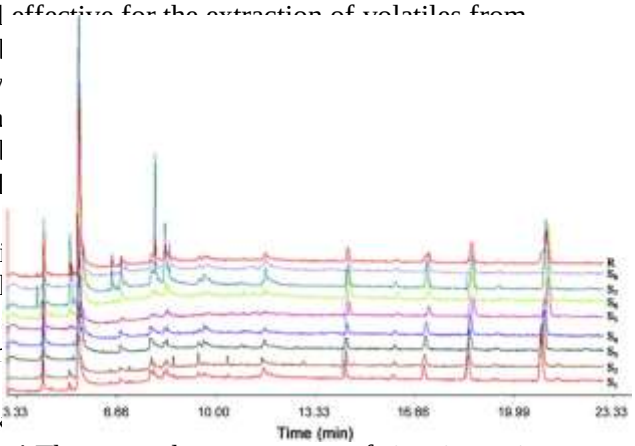

Fig. 4 The mean chromatograms of sinapis semina samples: (S_1) Anhui; (S_2) Guangxi; (S_3) Hebei; (S_4) Henan; (S_5) Jilin; (S_6) Shandong; (S_7) Shanxi and (S_8) Sichuan.

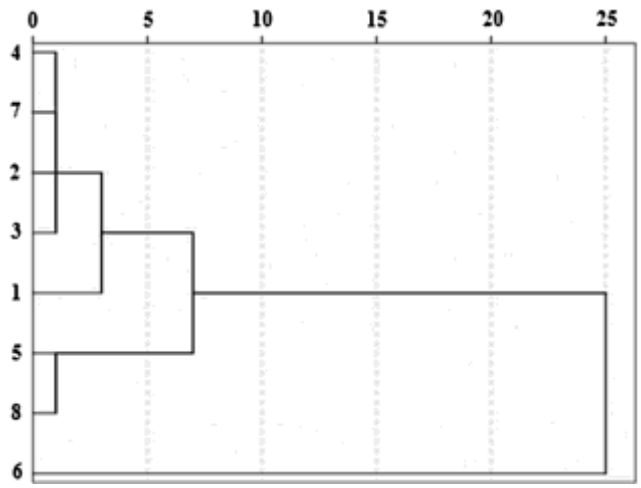

Fig. 5 Dendrogram of sinapis semina generated by the weighted pairgroup method based on hierarchical cluster analysis.

obtained from all the samples. Four marker compounds wereselected for this analysis and peak area ratios of the marker compounds were obtained by calculating the peak area ratio of analytes to internal standard.

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References

V.K. Agnihotri, S.G. Agarwal, P.L. Dhar, et al., Essential oil composition of *Mentha pulegium* L. growing wild in the north- western Himalayas India, Flavour Fragrance J. 20 (2005) 607–610.

Y. Zhang, Z.Z. Wang, Comparative analysis of essential oil components of three Phlomis species in Qinling Mountains of China, J. Pharm. Biomed. Anal. 47 (2008) 213–217.

N.K. Dubey, R. Kumar, P. Tripathi, Global promotion of herbal medicine: India's opportunity, Curr. Sci. 86 (2004) 37–41.

M.M.W.B. Hendriks, L.C. Juarez, D.D. Bont, et al., Preprocessing and exploratory analysis of chromatographic profiles of plant extracts, Anal. Chim. Acta 545 (2005) 53–64.

M. Benzo, G. Gilardoni, C. Gandini, et al., Determination of the threshold odor concentration of main odorants in essential oils using gas chromatography–olfactometry incremental dilution technique, J. Chromatogr. A 1150 (2007) 131–135.

C. Gherman, M. Culea, O. Cozar, Comparative analysis of some active principles of herb plants by GC/MS, Talanta 53 (2000) 253– 262.

A. Bertoli, L. Pistelli, I. Morelli, et al., Volatile constituents of different parts (roots, stems and leaves) of *Smyrnium olusatrum* L, Flavour Fragrance J. 19 (2004) 522–525.

M.K. Lee, Y.M. Ahn, K.R. Lee, et al., Development of a validated liquid chromatographic method for the quality control of *Prunellae Spica*: determination of triterpenic acids, Anal. Chim. Acta 633 (2009) 271–277.

M.K. Lee, J.H. Ling, M.H. Chun, et al., Simultaneous determination of biological marker compounds in *Ostericum koreanum* by HPLC

method and discrimination by principal component analysis, Bull. Korean Chem. Soc. 29 (2008) 2465–2470.
J. Seo, H.Y. Kim, B.C. Chung, et al., Simultaneous determination of anabolic steroids and synthetic hormones in meat by freezing-lipid filtration, solid-phase extraction and gas chromatography–mass spec-trometry, J. Chromatogr. A 1067 (1–2) (2005) 303–309.
J. Hong, H.Y. Kim, D.G. Kim, et al., Rapid determination of chlorinated pesticides in fish by freezing-lipid filtration, solid-phase extraction and gas chromatography–mass spectrometry, J. Chroma-togr. A 1038 (2004) 27–35.
M.S. Kim, T.W. Kang, H. Pyo, et al., Determination of organo-chlorine pesticides in sediment using graphitized carbon black solidphase extraction and gas chromatography/mass spectrometry, J. Chromatogr. A 1208 (2008) 25–33.
L.S. de Oliveira, F. de M. Rodrigues, F.S. de Oliveira, et al., Headspace solid phase microextraction/gas chromatography–mass spectrometry combined to chemometric analysis for volatile organic compounds determination in canine hair: a new tool to detect dog contamination by visceral leishmaniasis, J. Chromatogr. B 875 (2008)392–398.
New York, 1991.
S.Y. Oh, J.W. Ko, S.Y. Jeong, et al., Application and exploration of fast gas chromatography-surface acoustic wave sensor to the analysis of thymus species, J. Chromatogr. A 1205 (2008) 117–127.
Z.D. Zeng, Y.Z. Liang, F.T. Chau, et al., Mass spectralprofiling: an effective tool for quality control of herbal medicines, Anal. Chim. Acta 604 (2007) 89–98.
B.Y. Li, Y. Hu, Y.Z. Liang, et al., Quality evaluation of fingerprints of herbal medicine with chromatographic data, Anal. Chim. Acta 514(2004) 69–77.
S. Yan, G. Luo, Y. Wang, et al., Simultaneous determination of nine components in Qungkailing injection by HPLC/ELSD/DAD and its application to the quality control, J. Pharm. Biomed. Anal. 40 (2006)889–895.
I.K. Lee, M.A. Kim, S.Y. Lee, et al., Phytochemical constituents of Schizonepeta tenuifolia Briquet, Nat. Prod. Sci. 14 (2008) 100–106.
Z.J. Lin, W. Ji, D. Desai-Krieger, et al., Simultaneous determination of pioglitaz-one and its two active metabolites in plasma by GC–MS,J. Pharm. Biomed. Anal. 33 (2003) 101–108.