Characterization of Volatile Components in Dry Chrysanthemum Flowers Using Headspace–Liquid-Phase Microextraction–Gas Chromatography

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Abstract

A headspace–liquid-phase microextraction (HS–LPME)–GC (gas chromatography) method for the characterization of volatile components in dry chrysanthemum flowers has been developed. In the proposed method, two extraction solvents, *n*-hexadecane and benzyl alcohol, are used for preconcentrating volatiles in the sample. A droplet of the extraction solvent is squeezed from the GC syringe and inserted in the headspace of the sample bottle with the dry flower, immersed in deionized water, and warmed in a water bath. The optimum HS–LPME parameters in terms of extraction solvent type, droplet magnitude, equilibrium (water bath) temperature, equilibrium time, extraction time, and ionic strength are achieved using GC–FID (flame ionization detection) by varying several levels of the factors that affect the HS–LPME procedure. After extraction under the optimized conditions, the extraction droplet is retracted into the syringe and injected for GC–MS (mass spectrometry) analysis. Thirty-three volatile components are extracted and identified using this HS–LPME–GC–MS method, with the aid of chemometric methods. It is shown that the volatiles in dry chrysanthemum flowers are mainly unsaturated organic compounds, such as monoterpenes, sesquiterpenes and their oxygenous derivatives, triterpenoids, and aliphatic compounds. Several representative components, in order of precedence of the retention time, are pinene (106.3 µg/g), camphene (112.7 µg/g), eucapyptol (52.1 µg/g), camphor (29.4 µg/g), borneol (4.2 µg g), bornyl acetate (67.3 µg/g), caryophyllene (0.7 µg/g), and caryophyllene oxide (20.0 µg/g). The relative standard error and detection limit of this method is 5~9% and 0.4 µg/g, respectively.

Introduction

Dry chrysanthemum flower is a natural product, used as a drug and a food in Chinese traditional medicines (1,2). The volatile components in the product have significant influence on its taste and quality. Therefore, the determination of the volatiles in this product is meaningful. There are several methods proposed for the determination of volatiles in a plant or liquid sample, mainly simultaneous distillation-extraction (SDE) (3,4), supercritical fluid extraction (SFE) (5,6), solid-phase microextraction gas chromatography mass spectrometry (SPME–GC–MS) (7–10). Among these analytical methods, SPME is a rapid and efficient technique for preconcentrating organic volatiles in solid, liquid, and gas samples. However, the SPME fibers are relatively expensive, the fiber types available are limited, and the fiber has memory effect on some analytes (11–13). The dynamic and static headspace (HS) technique sampling for GC analysis is an effective method for the analysis of volatiles in samples with many advantages. Foremost among its many advantages is the elimination of much of the interference brought by the sample matrix (14,15). However, HS–GC analysis of the trace volatiles in samples is difficult because of the low content levels.

Liquid-phase microextraction (LPME) is a new sample preparation technique introduced by Jeannot et al. (16). Later, the LPME technique was combined with HS–GC for the analysis of volatile components in liquid or solid samples. This was done by selecting extraction solvent according to the sample properties, and optimizing the HS–LPME parameters such as droplet magnitude of the extraction solvent, equilibrium temperature and time, extraction time, and ionic effects (17–20). HS–LPME integrates preconcentration and sampling into one step. It uses only a few microliters of extraction solvent and simple laboratory apparatus, and has the advantages such as flexible design of the preconcentration procedure and inexpensive experimental cost, compared with that of HS–SPME. Therefore, HS–LPME and LPME combined with GC attracted increasing attention and was successfully applied for the analysis of volatiles in environmental and food samples (17–22).

In this study, an HS–LPME–GC–FID–MS method was proposed for the characterization of volatiles in dry chrysanthemum flower by the alternate application of two

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extraction solvents with different polarities. The HS–LPME parameters of extraction solvent type, droplet magnitude, equilibrium (water bath) temperature, equilibrium time, extraction time, and ionic strength were investigated. Thirty-three volatile components were extracted under optimized conditions and identified by GC–MS with the aid of correlation of retention behavior and boiling point (bp) (23–25).

Experimental

Materials and reagents

Dry chrysanthemum flower sample, produced in Henan Province (China), was purchased from Sida Supermarket (Zhengzhou, China). *n*-Hexadecane (analytical reagent grade) was purchased from Chengdu Kelong Chemical Reagents Company (Chengdu, China). Benzyl alcohol (analytical reagent grade) and *n*-octanol (analytical reagent grade) were purchased from Tianjin Kemio Chemical Reagents Company (Tianjin, China).

Apparatus and HS–LPME procedure

Figure 1 shows the sketch of HS–LPME apparatus used in this work. An extraction solvent droplet squeezed from the 10 µL GC syringe (Agilent Technology, Palo Alto, CA) was inserted above the headspace of the sample bottle containing the dry flower sample. It was then immersed in deionized water, stirred, and warmed in a water bath. A hot plate/magnetic stirrer (Henan Gongyi Yuhua Instrumental, China) was used to warm and agitate the sample (stirred at 25 rpm). When the HS–LPME procedure was performed, the extraction droplet in microliter level was retracted into the syringe and injected for GC analysis.

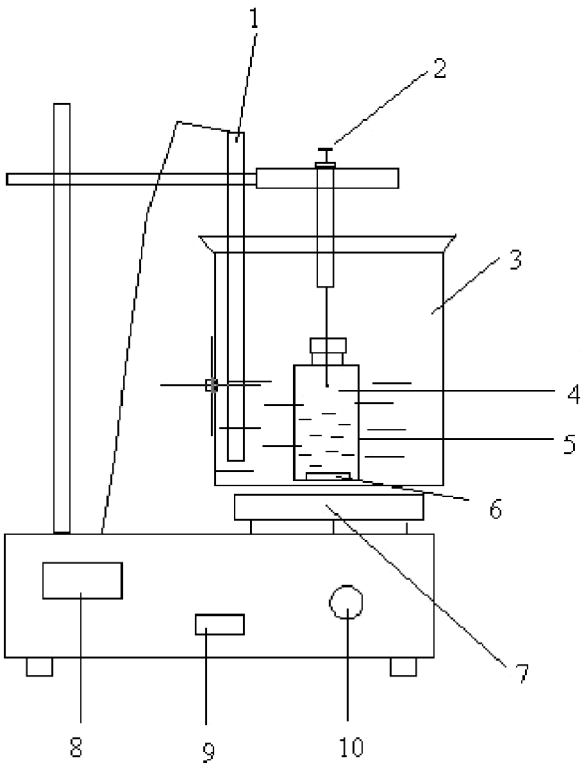

Figure 1. Sketch of the HS–LPME apparatus: temperature sensor, 1; GC syringe, 2; beaker, 3; extraction solvent droplet, 4; sample bottle, 5; micro-stirrer bar, 6; electric hot plate, 7; temperature indicator, 8; power switch, 9; rotate speed adjustor, 10.

GC–FID and GC–MS procedure

In order to achieve the optimum HS–LPME parameters, the extraction droplet was first directly injected for GC–FID analysis. The percentage of the integrated peak area (Area%) of one selected representative component was used as an indicator of the extraction efficiency. Then the extraction solution obtained under the optimum HS–LPME parameters was separated and identified using GC–MS with the aid of chemometric methods (23–25). For the separation of the extraction droplet, the following GC (Agilent 6890, Agilent Technology) conditions were adopted: capillary column, HP19091A (25 m × 0.32 mm × 0.52 µm, crosslinked methyl siloxane, Agilent Technology); inlet temperature, 280°C; split ratio, 1/1; flow rate, 2.0 mL/min (constant flow mode); temperature program, 50°C for the first 2 min, then heated at a rate of 9°C/min up to 250°C and held for 2 min. For both GC–FID and GC–MS analysis, the detector temperature was set at 280°C. The electron impact ionization of the mass spectrometric detector (MSD 5973N, Agilent Technology) in GC–MS was tuned at 70 eV and set at 20–400 amu in full scan mode.

Supposing that the response factors of different volatile components are equal, the volatilization of the extraction solvent and the tiny peaks of the components that overlapped in the peak of the extraction solvent can be neglected. Therefore, the relative content of an identified volatile component can be approximately calculated or semiquantitated as follows:

$$C_x = \frac{A_x m_s}{A_s m} \ (\mu g \cdot g^{-1}) \qquad \text{Eq. 1}$$

where A_x and A_S denotes the integrated peak area of a determined component and the extraction solvent, respectively; m_s(µg) and m(g) denotes the weight of the extraction solvent and the dry chrysanthemum flower sample, respectively.

Identification of the extracted volatile components

The volatile components extracted by HS–LPME under the optimum conditions were determined using GC–MS. The separated components were firstly searched against the NIST mass spectra library (NIST02), using a probability-based matching (PBM) algorithm embedded in the Agilent MSD ChemStation. The candidate components in the PBM searching list were selected or eliminated with the aid of chemometric methods that had previously been used successfully (23–25). The boiling points (bps) of the compounds unavailable in the searching list were estimated using the group contributions method (26).

Results and Discussion

First, HS–LPME–GC–FID was used to optimize the HS–LPME parameters. Then the extraction droplet obtained under the optimum conditions was separated and identified using GC–MS

analysis.

Optimization of HS–LPME procedure

Extraction solvent type

The solvent properties highly influence the extraction efficiency. It is primarily known that the chemical constituents in garland chrysanthemum coronarium is complex, with different polarities (1,2), that lead to difficulties in extracting the volatile components in dry chrysanthemum flower, when performed on a single solvent type. Therefore, three extraction solvents with different polarities were used in this study: *n*-hexadecane (bp, 287.2°C), benzyl alcohol (bp, 205.3°C), and *n*-octanol (bp, 201.5°C). Because of the unavailability of the experimentally measured bps of the compounds, the bps of the extraction solvent and the latter candidate components in the PBM list were calculated by the group contributions method (26).

Figure 2 shows the chromatographic profiles of the GC–FID analysis of extraction droplets obtained by the HS–LPME procedure, performed under the same conditions except that of extraction solvent. It can be seen that the GC profile of the HS–LPME extraction droplet using benzyl alcohol as extraction solvent is similar to that of *n*-octanol, and the corresponding peak height of the former is higher with the exception of the extraction solvent. However, the GC profile of the extraction droplet by *n*-hexadecane is obviously different from that of benzyl alcohol and *n*-octanol. Meanwhile, the peak heights and numbers of the extraction droplet by benzyl alcohol with retention times more than 12.5 min is higher than that of *n*-hexadecane. Therefore, *n*-hexadecane and benzyl alcohol were selected as the extraction solvents, and the volatiles in dry chrysanthemum flower were extracted by the HS–LPME procedure by alternately using these two agents with different polarity as extraction solvents. Furthermore, the components that may be obscured by the solvent peak can be easily determined using another agent as the extraction solvent.

Droplet magnitude

It is known that the higher the droplet magnitude, the better the extraction, but it is more difficult to hang a droplet on the syringe tip if it has a higher magnitude. The extraction efficiency of the HS–LPME procedure was indicated by a variation of the percentage of the integrated peak area (Area%) of a selected component (retention time 10.213 min) using *n*-hexadecane as the extraction solvent. The HS–LPME conditions were set as follows: droplet magnitude, 1.0, 1.5, and 2.0 µL, respectively; dry chrysanthemum flower, 0.2 g (immersed in 8 mL deionized water); equilibrium (warming) temperature, 70°C (equilibrium time, 30 min); HS–LPME extraction time, 4 min. It was shown that the maximum extraction efficiency of the selected component was reached when the droplet magnitude was 1.5 µL, therefore, this was set as the HS–LPME optimum droplet magnitude.

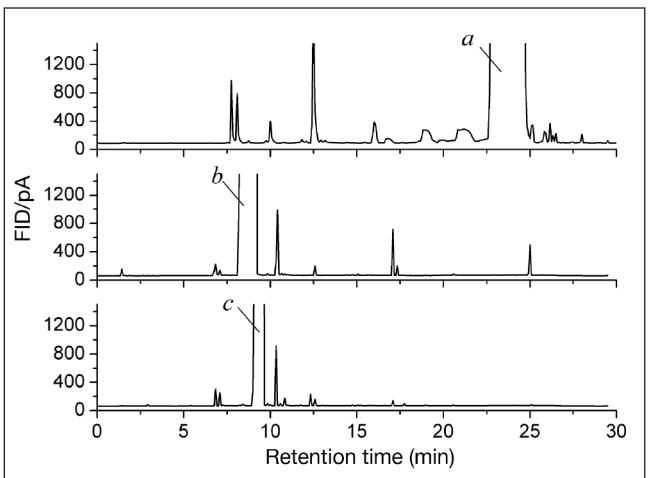

Figure 2. Chromatographic profiles of HS–LPME–GC–FID analysis of dry chrysanthemum flower using different extraction solvents: *n*-hexadecane (a); benzyl alcohol (b); *n*-octanol (c).

Equilibrium temperature

In the HS–LPME procedure, when the equilibrium temperature increases, the diffusivity of organic components to organic phase increases, the convection process intensifies, and these factors will shorten the extraction equilibrium time. However, this will lead to the decreasing of the distribution coefficient of the organic volatile components in the hanged extraction solvent, which will lead to the decreasing of the extraction efficiency. To optimize the equilibrium temperature, the HS–LPME conditions were as follows: equilibrium temperature, 50ºC, 70ºC, and 80ºC (equilibrium time, 30 min), respectively; droplet magnitude, 1.5 µL; dry chrysanthemum flower, 0.2 g (immersed in 8 mL deionized water); extraction time, 4 min. The extraction efficiency was best for the selected component when the equilibrium temperature was set at 70°C.

Extraction time

The HS–LPME process is a partition equilibrium based on the components in the extraction solvent (receptor) being dissolved in the aqueous phase, but remaining in the vapor phase. Generally, in a complex mixture, the extraction time of a component with a lower distribution coefficient is selected before the equilibrium (during a non-equilibrium state) because of the length of time required for the equilibrium. On the other

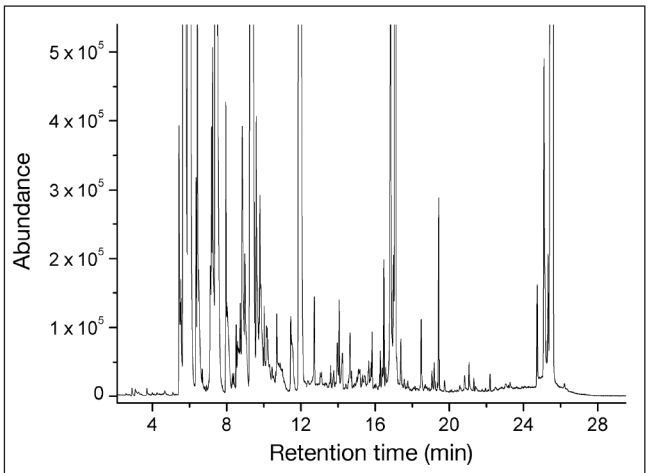

Figure 3. Total ion current chromatogram (TIC) of the extracted mixturecombining TIC profiles of *n*-hexadecane (retention time less than 12.5 min) and benzyl alcohol (retention time more than 12.5 min) as extraction solvent, respectively.

hand, the extraction time has an influence on the vaporization of the extraction solvent. Therefore, the extraction time should be strictly controlled in order to obtain acceptable reproducibility and extraction efficiency.

Further experiments were run setting the extraction time at 2, 5, 10, and 20 min, respectively, and the other HS–LPME conditions as follows: equilibrium temperature, 70°C (equilibrium time, 30 min); droplet magnitude, 1.5 µL; dry chrysanthemum flower, 0.2 g (immersed in 8 mL deionized water). The extraction efficiency increased with the extraction time, but when the extraction time was more than 10 min, the tendency of the extraction efficiency increase was indistinctive. Therefore, the extraction time for the HS–LPME procedure was set at 10 min.

Equilibrium time

The volatile components' original existence in the dry chrysanthemum flower are distributed in four sections during the HS-LPME procedure: extracted to the extraction solvent, remained in the vapor phase, dissolved in the aqueous phase, and retained in the body of the chrysanthemum flower. To investigate the influence of the equilibrium time on the extraction efficiency, the HS–LPME conditions were as follows: equilibrium temperature, 70°C (equilibrium time, 20, 30, 60, and 90 min, respectively); droplet magnitude, 1.5 µL; dry chrysanthemum flower, 0.2 g (immersed in 8 mL deionized water); extraction time, 10 min. The extraction efficiency varied obviously with the variation of the equilibrium time and had a maximum value when equilibrium was 30 min. Therefore, for the selected component, and without loss of generality,

Table I. Identification and Semiquantitation of the Volatiles Extracted by HS–LPME

No.	t_r/min*	Compound name	CAS No.	b.p./K	Qual†	C/g/g‡
1	5.446	3-carene	13466-78-9	445.9	91	6.2
2	5.535	2-methyl-5-(1-methylethyl)-bicyclo [3.1.0]hex-2-ene	2867-05-02	445.8	91	1.7
3	5.692	pinene	7785-70-8	440.9	99	106.3
4	5.752	6,6-dimethyl-2-methylenecyclo[3.1.1]heptane	2437-95-8	440.9	95	2.7
5	5.939	camphene	79-92-5	440.9	95	112.7
6	5.985	4-camphene	29050-33-7	440.9	87	1.4
7	6.358	(1S)-6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane	18172-67-3	440.9	91	3.4
8	6.431	4-methylene-1-(1-methylethyl)-cyclohexene	99-84-3	436.2	91	13.5
9	7.193	3,7,7-trimethyl- bicyclo[4.1.0]hept-2-ene	554-61-0	445.9	64	1.4
10	7.399	eucalyptol	470-82-6	447.3	97	52.1
11	7.963	1-methyl-4-[1-methylethyl]-1,4-cyclohexadiene	99-85-4	442.5	99	9.2
12	8.025	1,7,7-trimethyl-bicyclo[2.2.1]hept-2-ene	464-17-5	441.1	74	41.3
13	8.322	3-cyclopentene-1-acetaldehyde,2,2,3-trimethyl			95	1.3
14	8.522	1-methyl-4-[1-methylethylidene]-cyclohexene	586-62-9	451.4	97	1.6
15	8.587	1-methyl-3-(1-methylethenyl)-cyclohexene	499-03-6	448.7	76	1.3
16	8.739	2-methyl-butanoic acid 2-methylbutylester	2445-77-4	459.8	72	1.6
17	8.838	oct-1-enyl acetate	32717-83-0	489.8	83	1.7
18	8.983	2,2,3-trimethyl-3-cyclopentene-1-acetaldehyde	4501-58-0	492.1	90	10.9
19	9.335	camphor	76-22-2	509.8	97	29.4
20	9.598	6,6-dimethyl-2-methylene-bicyclo[2.2.1]heptan-3-one	16812-40-1	508.7	99	4.2
21	9.793	borneol	507-70-0	529.5	91	4.2
22	10.017	4-methyl-1-[1-methylethyl]-3-cyclohexen-1-ol	562-74-3	544.1	93	1.8
23	10.156	6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde	564-94-3	494.5	90	1.4
24	11.461	bicyc[3.1.1]hept-2-en-4-ol,2.6.6-trimethyl-,acetate	10019-56-6	545.2	86	3.1
25	11.882	bornyl acetate	76-49-3	541.3	98	67.3
26	11.968	isobornyl acetate	125-12-2	541.3	91	1.4
27	14.241	caryophyllene	87-44-5	576.5	98	0.7
28	14.646	1,6,10-dodecatriene,7,11-dimethyl-3-methylene-,[Z]	28973-97-9	544.1	97	1.1
29	15.114	1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene	39029-41-9	571.5	90	0.7
30	15.666	1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-1H-cyclopropa[a]naphthalene	17334-55-3	566.8	81	0.8
31	16.369	decahydro-1,1,7-trimethyl-4-methylene-1H-cycloprop[e]azulen-7-ol	6750-60-3	557.8	90	0.6
32	16.469	caryophyllene oxide	1139-30-6	593.1	95	20.0
33	17.395	2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl-1H-3a,7-methanoazulene	560-32-7	566.8	93	0.6

* Retention time.
† Quality of the PBM search.
‡ Semiquantitative result of the volatile component.

the equilibrium time for the HS–LPME procedure was set at 30 min.

Salt effect

In the HS–LPME procedure, the solubility of some volatile components in the aqueous phase and retained in the plant body may be affected by the salt effect. In this study, NaCl was used to investigate the salt effect on the extraction efficiency. The test was run using *n*-hexadecane as the extraction solvent, setting the ionic strength at 0%, 5%, and 10% (wt%), respectively, and setting the other HS–LPME conditions as follows: equilibrium temperature, 70°C (equilibrium time, 30 min); droplet magnitude, 1.5 µL; extraction time, 10 min; dry chrysanthemum flower, 0.2 g (immersed in 8 mL deionized water). It was shown that the extraction efficiency is only slightly affected by the ionic strength (i.e., there is no need to adjust the ionic strength in this work.)

HS–LPME–GC–MS analysis

The HS–LPME was performed with the optimum parameters: extraction solvent, *n*-hexadecane (benzyl alcohol as subservient extraction solvent); droplet magnitude, 1.5 µL; equilibrium temperature, 70°C (equilibrium time, 30 min); extraction time, 10 min; dry chrysanthemum flower, 0.2 g (immersed in 8 mL water). The extraction droplet was injected for GC–MS analysis. Figure 3 shows the total ion current chromatogram (TIC) of the extraction droplet obtained using the optimum HS–LPME conditions. The separated volatile components were identified based on PBM search with the aid of chemometric methods (23–26) and listed in Table I. The semiquantitation of the volatile components is also listed in Table I. It can be seen that the volatile components in the dry chrysanthemum flowers are mainly unsaturated organic compounds, such as monoterpenes, sesquiterpenes and their oxygenous derivatives, triterpenoids, and other aliphatic compounds. The content levels of the volatile components in the dry chrysanthemum flowers varies in a wide range. Most of the identified volatile components shown in Table I such as pinene, camphene, eucapyptol, camphor, borneol, caryophyllene, etc. are similar to that of the previous studies (1,2) and references cited therein. However, the relative content levels of the identified components are not identical to those in the previous studies [the content of bornyl acetate (67.3 µg/g) in this work is much higher than that of borneol (4.2 µg/g), while they are reported in the same order of magnitude].

In HS–LPME–GC–FID analysis, the relative standard deviation (RSD, n = 3) of the Area% of the selected component (retention time, 10.276 min) and other components are 7% and 5~9%, respectively. The detection limit of this method is 0.4 µg/g. These indicate that the HS–LPME–GC method is acceptable for characterizing volatile components in natural plant products.

It also can be seen from this work that compared with the conventional HS–SPME procedure, which requires relatively expensive fibers of limited types that also have memory effects for some analytes (11–13), the proposed HS–LPME–GC method only requires a simple apparatus, an inexpensive GC microsyringe, and microliters of organic solvents of which many varieties can be selected.

Conclusion

The HS–LPME–GC method can be used to characterize and semiquantify volatile components in natural plant products. Compared with the SPME technique, the HS–LPME apparatus is inexpensive and simple to install. Moreover, flexible extraction systems can be designed with optimization of the HS–LPME parameters according to the property of the sample. By alternately using two extraction solvents with different polarities, the problem that the tiny peaks might be obscured by the solvent peak can be avoided. HS–LPME combined with GC–FID and GC–MS is a promising method for the characterization of volatile components at trace levels in natural plant products.

Acknowledgments

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