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Optimization of Disk Sorptive Extraction Based on Monolithic Material for the Determination of Aroma Compounds from *Lantana camara* L. by Gas Chromatography-Mass Spectrometry

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Present study describes the optimization of disk type sorptive extraction using monolithic material (Mono Trap) for the analysis of volatile aroma compounds from *Lantana camara* L. in combination with gas chromatography/mass spectrometry (GC/MS). Monolithic material sorptive extraction (MMSE) is a new sampling technique using a monolithic hybrid adsorptive disk (O.D. 10 mm, 1 mm thickness) made of high purity silica and activated carbon having a large surface area chemically bonded with octadecyl silane (ODS). The experimental parameters that may influence the MMSE efficiency have been optimized. Linearity, accuracy, precision and detection limits were evaluated to assess the performance of the proposed method. The method was validated with real plant samples of *Lantana camara* L. Twenty eight compounds including the main representative compounds of α-curcumene and β-caryophyllene were found in analyzed samples. Results proved that proposed method could be used as a good alternative for the analysis for such volatile aroma compounds in plant samples.

Key Words : *Lantana camara*, Monoliths, Monolithic material sorptive extraction, Aroma, GC-MS

Introduction

Nowadays, the porous materials known as monoliths have attracted much attention, and are widely applied in the fields of separation science. Monoliths are structured materials which contain large transport pores connected to smaller diffusive pores with larger surface area.[1] Valuable information and remarkable features of monoliths were described in a review[2] summarized by Svec and Huber and a comprehensive reference book[1]. There are various advantages of these materials: good permeability, fast mass transfer, high sensitivity, and ease of modification. Therefore, monolithic materials have been widely used in microseparation system, not only as stationary phases but also as matrices for sample preparation.[3]

During the past decade, monolithic materials in the shape of disks, stacked layers, rolled sheets, sponges, irregular chunks, tubes, rods and cylinders prepared from a wide variety of materials such as silica, cellulose and synthetic polymers have all been successfully demonstrated. Recently, a stir bar for sorption extraction based on monolithic material was also reported for preconcentration of polycyclic aromatic hydrocarbons in seawater.[4]

The first porous monolithic polymer used for solid phase extraction (SPE) was poly(styrenedivinyl benzene) (PS-DVB).[1] Current trends of effective sample pretreatment are focused on the study to achieve high throughput, to enrich the analyte for high sensitivity detection, automation, miniaturization, and to reduce sample size, solvent consumption, and matrix interferences. Previously, we reported several articles on the analysis of volatile aroma compounds using ethyl vinyl benzene divinylbenzene copolymer (Porapak-Q) or poly(2,6-diphenyl-*p*-phenylene oxide) adsorbent (Tenax TA).[5-9]

Very recently, a commercial monolithic hybrid adsorptive material (MonoTrap) made of high purity silica and/or silica with activated carbon or graphite having a large surface area chemically modified with octadecyl silane (ODS) was launched as a sample pretreatment device.[10] MonoTrap is available in the shapes of disk (O.D. 10 mm, 1 mm thickness) as well as rods (O.D. 2.9 mm, 5 mm height, with 1 mm I.D. hole). There are two types of MonoTrap : one is silica modified with ODS, and the other one has both silica modified with ODS and activated carbon. When sample passes through pores in a monolithic structure having the large surface area at least 150 m^2/g, the sample is trapped by ODS groups chemically bonded to the surface of the silica structure or by activated carbon present inside and outside the structure. Not only gaseous substances but also liquid substances are permeable through pores and mesopores. Hashi *et al.* applied this monolithic material sorptive extraction (MMSE) technique for the gas chromatography/mass spectrometry (GC/MS) of poly aromatic hydrocarbons.[11] The MMSE is considered as an alternative technique to solid phase microextraction (SPME)[12] and stir bar sorptive extraction (SBSE).[13]

Here, we present the development, optimization and application of the headspace(HS) MMSE using MonoTrap disk followed by GC/MS to the analysis of aroma components of *Lantana camara* L. *Lantana camara* L. belonging to the *verbenaceae* family, is mostly native to subtropical and tropical America, but a few texa are indigenous to

tropical Asia and Africa. The plant is regarded both as a notorious weed and a popular ornamental garden plant and has found various uses in folk medicine in many parts of the world.[14] A tea prepared from the leaves and flowers was taken against fever, influenza, and stomach ache. In addition, lantana is a favorite nectar plant for several insects, its scent together with visual floral cues are important signal to adult butterflies searching for food-rewarding plants.[15] Their flower color ranges from white to yellow, orange to red, pink to violet in unlimited combinations and the flowers usually change in color as they age. Chemical composition of their essential oils obtained through distillation was mainly analyzed by GC-FID or GC/MS.[16-18] In this study, the composition of the volatile aroma compounds from the leaves and flowers of lantana from Korea was investigated in detail by using HS-MMSE technique combined with GC/MS. To the best of our knowledge, this is the first application of MMSE to the analysis of aroma compounds from *Lantana camara* L.

Experimental

Plant Material. The aerial parts of *Lantana camara* L. were collected from April to July in 2010 at the herb garden of southern region of Seoul. Air dried flowers and leaves at ambient temperature in the shade room were crushed by a powdering machine and homogenized, then powder sample was stored in a tight bottle until to use.

Reagents and Device. All working reference standards were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Tokyo Kasei (Nihonbashi, Tokyo, Japan). Organic solvents of chromatographic grade were obtained from Mallinckrodt Baker (Phillisburg, NJ, USA).

MonoTrap™ (MT) sampling kit for HS-MMSE was obtained from GL Science (Shinjuku, Tokyo, Japan). This kit includes MT stand, MT holder, MT extract cup, clean pin hole septum with vial, 200 μL glass insert, and four types of MonoTrap disk and rods. MonoTrap DCC18 disk (O.D. 10 mm, 1 mm thickness) containing ODS group and activated carbon was used for HS-MMSE. For the comparative study, MonoTrap DSC 18 disk (O.D. 10 mm, 1 mm thickness) without activated carbon was also used.

HS-MMSE Procedure. The schematic illustration of HS-MMSE procedure is shown in Figure 1. Prior to use, MonoTrap disk was cleaned with methylene chloride and conditioned in the oven at 100 °C for 30 min in order to remove any impurities or contaminants. A stainless steel MT holder was inserted into a hole on the MT stand. A MT disk was inserted to the stainless steel MT holder through the center hole of the MT disk using a clean tweezers to avoid contamination. A clean septum was passed the end of the MT holder using a clean tweezers. A cap was put on the top of the holder. Then, a MT disk was placed at a fixed position in the headspace of a vial (30 mL) containing lantana powder sample (about 1.5 g), and the septum was hermetically sealed on the vial. This vial was kept in a sand bath for 90 min at 60 °C, and volatile aroma compounds from the sample were exposed and adsorbed to both side surfaces of MT disk. Extraction temperature, time and the volume of desorption solvent were investigated and optimized.

After adsorption, a MT disk was removed and immediately placed very carefully in a MT extract cup (Teflon ring-shaped bonnet O.D. 21.02 mm, cylindrical Teflon cup O.D. 16.05 mm, I.D. 10.3 mm, depth 2 cm) filled with 100 μL methylene chloride and the cup bonnet was previously fitted on the neck of an extracting vial (20 mL volume, depth 55.67 mm, neck I.D. 17.12 mm) contained distilled water (about 17 mL). This step should be carried out very carefully, because rigid MT disk is easy to broken. Then, the

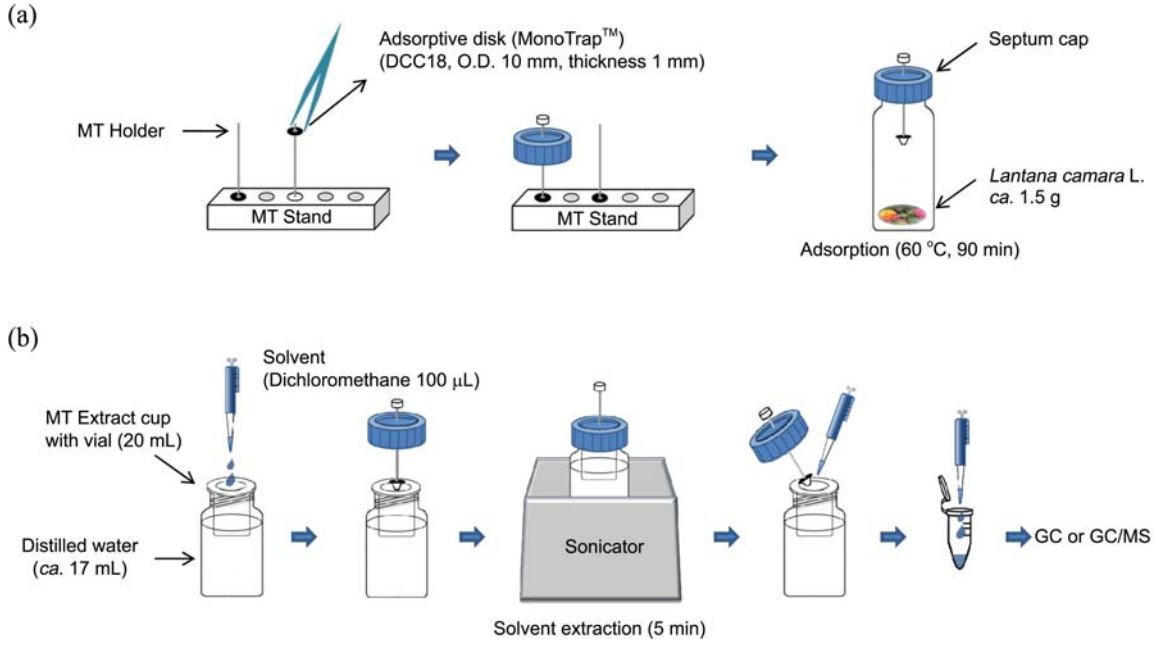

Figure 1. Schematic illustration showing procedure of the headspace MMSE using MonoTrap disk and GC-MS.

septum cap with MT extract cup fitted previously in an extracting vial was tightened, and the extracting vial was placed in an ultrasonic cleaner (Saehan Korea, SH-1025, frequency 28 kHz) for 5 min to accelerate the extraction. After extraction, 1.0 μL of extract was directly injected to the GC or GC/MS for the analysis. The blank run under the same conditions as those for the analysis of actual sample was performed to confirm the absence of contamination peaks.

Instrumentation and Analysis. GC/MS analyses were carried out by using a Trace GC 2000 and a GC-Q plus ion trap MS^n (Thermoquest-Finnigan, Austin, TX, USA) with electron impact (EI) ionization mode. In GC/MS, chromatographic separations were performed on a 6% cyanopropylphenyl-94%-dimethylsiloxane copolymer (DB-624, 30 m length × 0.25 mm I.D. × 1.4 mm film thickness, J & W Scientific, Folsom, CA, USA) capillary column. Flow rate of carrier gas (He, 99.9995%) was 1.0 mL/min. The injector temperature was 240 °C. The oven temperature program was 50 °C (3 min) – 5 °C/min – 220 °C (10 min). A split injection with a ratio of 1:30 was used. The injected sample volume was 1.0 μL. The ion trap mass spectrometer was operated as follows; ionization voltage, 70 eV; ion source temperature, 200 °C. Transfer line temperature was 230 °C. The measuring mode was scanned from 30 to 500 mass ranges. The analytes were identified tentatively by comparison of their retention times and mass spectra with those of authentic standards. The tentative identification was also performed by comparing the obtained mass spectra of relevant chromatographic peaks with those of corresponding spectra based on the NIST and Wiley libraries.

Gas chromatographic analysis was performed using a Hewlett-Packard HP 5890 gas chromatograph (GC) equipped with flame ionization detector (FID). A DB-624 (30 m length × 0.25 mm I.D. × 1.4 μm film thickness, J & W Scientific, Folsom, CA, USA) capillary column was used under the following conditions. Nitrogen (99.9%) was used as carrier gas at a constant flow rate of 1.0 mL/min. Flow rates of hydrogen and air were kept at 30 mL/min and 300 mL/min, respectively. Oven temperature was kept at 50 °C (3 min), and programmed to 220 °C (10 min) at a rate of 5 °C /min. Injector and FID temperatures were kept at 240 °C and 250 °C, respectively. Samples (1.0 μL) were injected in the split mode and the split ratio was adjusted at 1:30. Data were recorded using a HP 3396A integrator.

Results and Discussion

Optimization of the Extraction Conditions. Several factors were studied to optimize the extraction procedure, including adsorption temperature, time and desorption solvent volume. The *β*- forms of pinene and caryophyllene are considered as major characteristic components of the lantana sample. Stock solutions of *α*- and *β*- forms of pinene and caryophyllene at a concentration of each 1.0 mg/mL in methylene chloride were prepared, respectively. Then each 50 μL of four stock solutions was mixed in a vial (30 mL)

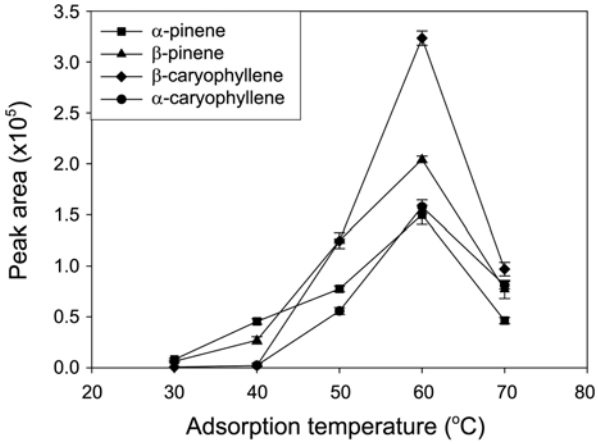

Figure 2. Influence of adsorption temperature on the efficiency of HS-MMSE and GC-FID. Bars represent the standard deviation (n = 3). MMSE conditions: concentration of each target compound = 0.25 μg/μL, 90 min adsorption time, 100 μL of methylene chloride for desorption.

for HS-MMSE. The concentrations of the each compound were approximately 0.25 μg/mL, respectively. The concentrations of each compound in each extract were approximately 0.5 μg/mL if 100 μL of methylene chloride were used for solvent desorption. The chromatographic peak areas of these compounds by 1.0 μL injection of extracts were considered as relative analytical signals for optimization. When a variable is optimized, its optimum value is fixed for further studies.

The influence of the extraction temperature on the efficiency of HS-MMSE was investigated in the range from 30 °C to 70 °C at 90 min of adsorption time using 100 μL of solvent volume. As the extraction temperature profile shown in Figure 2, the highest peak areas for model compounds were obtained at 60 °C. The rapid increase of peak area values at temperature range from 30 °C to 60 °C suggests that fast mass transfer and diffusion of compounds from the headspace of sample to the MT disk phase yields higher efficiency as the adsorption temperature increases. Interestingly, the influences of temperature were significant in the *β*-forms of caryophyllene and pinene than those *α*-forms. However, peak area values at 70 °C are decreased considerably, which means that extraction temperature higher than 70 °C reduces efficiency due to desorption back to headspace from the MT disk.

Four extraction times including 30, 60, 90 and 120 min were evaluated to determine optimum extraction time on the equilibrium of the target volatile compounds between the MT disk and headspace of sample. Figure 3 shows the extraction time profile varied from 30 min to 120 min at 60 °C of extraction temperature using 100 μL of desorption solvent volume. Considering analyte detection sensitivity, the extraction time for subsequent analyses was then fixed at 90 min.

The solvent volume of methylene chloride was also investigated at 60 °C of extraction temperature and 90 min of adsorption time. As shown in Figure 4, the solvent

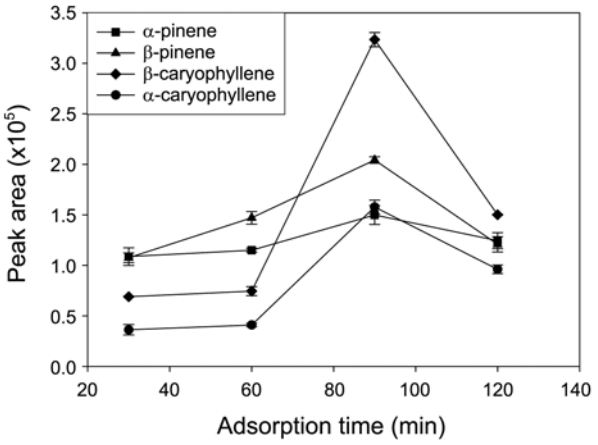

Figure 3. Influence of adsorption time on the efficiency of HS-MMSE and GC-FID. Bars represent the standard deviation (n = 3). MMSE conditions: concentration of each target compound = 0.25 μg/μL, 60 °C min adsorption temperature, 100 μL of methylene chloride for desorption.

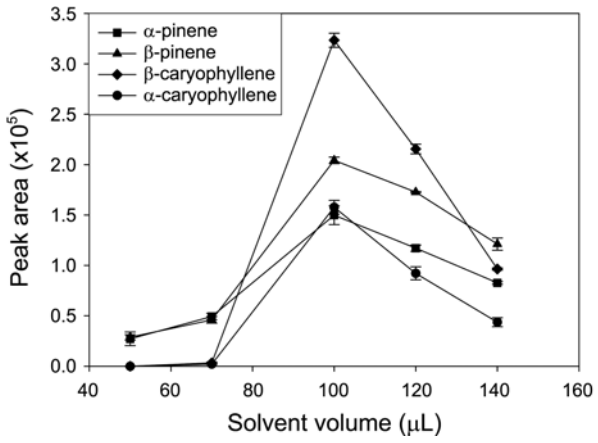

Figure 4. Influence of solvent volume on the efficiency of HS-MMSE and GC-FID. Bars represent the standard deviation (n = 3). MMSE conditions: concentration of each target compound = 0.25 μg/μL, 60 °C min adsorption temperature, 90 min adsorption time.

volume of methylene chloride has a significant influence on the extraction efficiency of the MT disk. It can be seen from Figure 4 that with increasing solvent volume in the range from 50 μL to 140 μL, the highest peak areas of *α*- and *β*-forms of pinene and caryophyllene as target compounds extracted by MT DCC18 disk were obtained at 100 μL. The influences of desorption solvent volume on the *β*-forms of caryophyllene and pinene were significantly greater than those *α*-forms. However, peak area values at the solvent volume more than 100 μL are decreased relatively. This result suggests that the volume of methylene chloride more than 100 μL reduces extraction efficiency due to the dilution of target compounds by methylene chloride. In addition, in this solvent extraction step, sonication longer than 5 min caused methylene chloride to start evaporation.

For subsequent experiments, 60 °C extraction temperature, 90 min extraction time and 100 μL methylene chloride as a desorption solvent were used.

Method Validation. The validation results of method performance were summarized in Table 1. The linearity of four target compounds was investigated. The tested analytes exhibited good linearity for the six-point calibration curves having negative intercepts with satisfactory squared regression coefficients (r^2) better than 0.98. The LOD, calculated at S/N = 3, were as low as 3.46-28.45 μg/mL, and the LOQ calculated at S/N = 10 were 11.54-94.83 μg/mL.

The reproducibility of the chromatographic peak areas was investigated by triplicate experiments using the optimum conditions. The relative standard deviations for the four target compounds were 0.32-4.98%. Recovery test was performed by the proposed method using lantana powder sample spiked with standard solutions of *β*- forms of pinene and caryophyllene. Recoveries obtained were satisfactory, showing 87.99% and 85.35%, respectively.

Extraction Efficiency. The overall extraction efficiency of HS-MMSE was evaluated by the relative concentration factor (CF) of the several characteristic components of lantana sample. The CF values were calculated by the following equation.

$$CF = A_1/A_0$$

where, A_1 is the peak area of the analytes by HS-MMSE-GC/FID, and A_0 is the corresponding peak area obtained by static HS-GC-FID using a 10 mL Hamilton 1010RN gas tight syringe (Supelco).

These experiments were carried out using lantana sample instead of standards to ensure that matrix effects were

Table 1. Validation data for the target compounds: linearity, dynamic range, the limit of detection (LOD), the limit of quantitation (LOQ), reproducibility and recovery for target compounds

	α-Pinene	*β*-Pinene	*β*-Caryophyllene	*α*-Caryophyllene
Linearity of calibration curve				
Equation	$y = 58.93x - 3073.10$	$y = 55.16x - 2411.28$	$y = 161.42x - 7114.58$	$y = 182.46x - 13353.94$
Correlation coefficient	$r^2 = 0.9878$	$r^2 = 0.9836$	$r^2 = 0.9925$	$r^2 = 0.9891$
Dynamic rage	51.94 ng ~ 2500 ng	63.49 ng ~ 2500 ng	11.54 ng ~ 3500 ng	94.83 ng ~ 2500 ng
LOD (ng/μL)	15.58	19.05	3.46	28.45
LOQ (ng/μL)	51.94	63.49	11.54	94.83
RSD (%, n = 3)	0.32	0.32	2.64	4.98
Recovery (%)	-	87.99	85.35	-

Table 2. Comparison of concentration factor (CF) of characteristic aroma components of *Lantana camara* L. obtained by HS-MMSE

Compounds	Group	t_R (min)	Concentration Factor Mean (± RSD%), n = 3	
			HS-MMSE (DCC18)	HS-MMSE (DSC18)
β-Pinene	I	14.37	11.08 (± 0.63)	1.28 (± 8.14)
α-Cubebene	II	29.33	3.29 (± 5.09)	2.27 (± 7.75)
β-Caryophyllene	II	30.40	5.22 (± 4.75)	3.39 (± 7.76)
α-Caryophyllene	II	31.32	1.29 (± 0.65)	0.73 (± 5.04)
α-Longipinene	II	31.49	2.81 (± 3.42)	2.40 (± 8.14)
α-Curcumene	II	31.73	2.29 (± 5.13)	1.86 (± 8.09)
Caryophyllene oxide	II	35.60	3.08 (± 1.38)	2.64 (± 6.70)

I: Monoterpenes, II: Sesquiterpenes

identical to those encountered during actual sampling. Experimental CF values for several characteristic compounds of lantana sample are given in Table 2. Experimental CF values for most compounds by HS-MMSE using DCC18 disk exhibited better results than those of DSC18 disk. Thus, DCC18 disk with activated carbon which has many micropores and mesopores is more useful than DSC18 disk without activated carbon.

Application of HS-MMSE for Analysis of Lantana Aromas. The proposed method was applied to the analysis

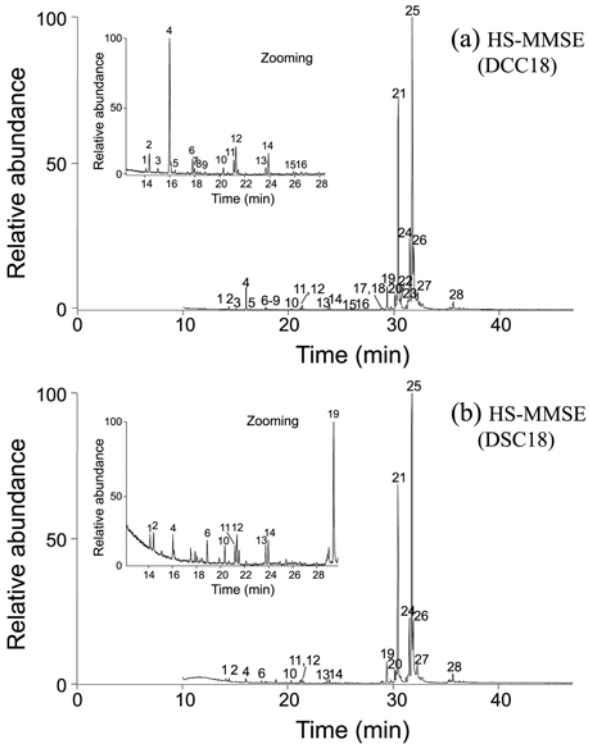

Figure 5. Typical TICs of aroma components from *Lantana camara* L. obtained by (a) HS-MMSE (DCC18), and (b) HS-MMSE (DSC18) combined with GC/MS.

Table 3. Volatile aroma composition of *Lantana camara* L. obtained by HS-MMSE-GC-FID

Peak No.	Compounds	Group	t_R (min)	Normalized peak area %, mean (± RSD%), n = 3	
				HS-MMSE (DCC18)	HS-MMSE (DSC18)
1	*α*-Thujene	I	14.09	tr	tr
2	*α*-Pinene	I	14.37	0.56 (± 4.70)	tr
3	Camphene	I	15.04	tr	nd
4	*β*-Pinene	I	15.99	3.54 (± 3.27)	0.48 (±0.13)
5	*β*-Myrcene	I	16.43	tr	nd
6	Limonene	I	17.83	0.29 (± 3.37)	tr
7	*p*-Cymene	I	18.00	tr	nd
8	*β*-Ocimene	I	18.43	tr	nd
9	*γ*-Terpinene	I	18.82	tr	nd
10	Sabinene hydrate	III	20.28	tr	tr
11	Linalool	III	21.13	1.07 (± 0.59)	tr
12	Unknown	-	21.46	tr	tr
13	Terpinene-4-ol	III	23.68	tr	tr
14	Borneol	I	23.89	0.41 (± 4.22)	tr
15	Verbenone	I	26.07	tr	nd
16	Citral	I	26.91	tr	nd
17	Unknown	-	28.80	0.34 (± 2.13)	nd
18	Unknown	-	28.92	0.41 (± 1.21)	nd
19	*α*-Cubebene	II	29.33	3.37 (± 0.55)	3.48(± 0.47)
20	*α*-Copaene	II	30.09	3.70 (± 1.97)	4.22(± 5.64)
21	***β*-Caryophyllene**	II	30.40	**26.78 (± 0.03)**	**29.48(± 0.86)**
22	Aromadendrene	II	30.67	tr	nd
23	*α*-Caryophyllene	II	31.32	0.83 (± 1.69)	0.74(± 2.98)
24	*α*-Longipinene	II	31.49	8.68 (± 0.11)	9.44(± 0.43)
25	***α*-Crucumene**	II	31.73	**37.27 (± 0.06)**	**37.23(± 0.18)**
26	Unknown	-	31.85	7.54 (± 1.62)	8.85 (± 1.24)
27	Unknown	-	32.22	2.81 (± 5.00)	4.04 (± 2.80)
28	Caryophyllene oxide	II	35.60	2.58 (± 5.66)	2.63(± 1.03)

I: Monoterpenes, II: Sesquiterpenes, III: Alcohol. *tr (trace) < 0.01. *nd (not detection)

of aroma components of lantana powder samples. Typical total ion chromatograms (TIC) obtained by HS-MMSE followed GC/MS are shown in Figure 5. The peak numbers shown in Figure 5 correspond to those indicated in Table 3. Aroma compositions by HS-MMSE are summarized in Table 3. Twenty eight compounds including the main representative compounds of α-curcumene (37.27%) and β-caryophyllene (26.78%) were found in analyzed samples. Results proved that proposed method could be used as a good alternative for the analysis for such volatile aroma compounds in plant samples.

The aroma compositions of lantana are varied according to the species, cultivation environment, weather condition, region and harvesting season and extraction method. According to the results previously obtained by Sundufu *et al.*[17] germacrene D (37.27), β-caryophyllene (12.35%), α-humulene (9.31%) and germacrene B (6.19%) were identified as main components from the steam distilled essential oil of lantana occurring in south China. Oyedeji *et al.* [18] reported that β-caryophyllene (24.6%), α-humulene (19.5%), sabinene (8.8%), germacrene D (5.7%) and cubebol (5.7%) were the main components from Nigerian essential oils of lantana. These differences are possibly caused by not only the origin of species but also hydrolysis, thermal degradation, molecular rearrangements and loss of components due to harsh conditions during the steam distillation process.

Conclusion

HS-MMSE was newly developed, optimized, validated and applied for the determination of volatile aroma components of lantana by GC/MS. The technique uses a disk based on silica chemically modified with ODS and contained activated carbon and very small volume of methylene chloride. Linearities of calibration curves were generally good, LOD and LOQ showed very low values. This method exhibited good precision and accuracy. The overall extraction efficiency of this new method was evaluated by computing concentration factors (CF). Compared with conventional solvent extraction or solid phase extraction, proposed HS-MMSE can reduce significantly solvent consumption in the sample preparation. Since 100 μL of solvent is used there is minimal waste or exposure to toxic solvent. Proposed method allowed combining of extraction, clean up and enrichment in a single step. The HS-MMSE method is an alternative sampling technique for HS-SPME.

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