Vol. 8, No. 1, 2017
ISSN 2233-4203/ e-ISSN 2093-8950
LETTER www.msletters.org | Mass Spectrometry Letters

Comparative Study of Extracting Fragrance Allergens by GC-MS/MS

In Ja Lee, Jae-Chan Ahn*, Bogsoon Kim, and Deukmo Chung

Seoul Water Institute, Seoul Metropolitan Government, 04981, South Korea

Received March 06, 2017; Accepted March 20, 2017
First published on the web March 31, 2017; DOI: 10.5478/MSL.2017.8.1.18

Abstract : Products containing any one or more of 26 fragrance allergens likely to cause contact allergies, are required under the 2008 domestic cosmetic law to be labeled when their concentrations exceed a certain range. This study focuses on the comparison and development of analytical methods based on headspace-solid phase micro extraction (HS-SPME) and liquid-liquid extraction (LLE) methods followed by GC-MS/MS for 24 of the fragrance allergens excepting for two natural materials in water samples. Using the developed HS-SPME method, 15 of the 24 fragrance allergens were analyzed and 9 compounds which have relatively low $\log K_{OW}$ values (below about 2.5) were not extracted, and the correlation coefficient (r^2) of the calibration curve for quantification showed linearity of 0.9969 or more, and the method detection limits (MDL) and the limits of quantification (LOQ) were 0.078 ~ 0.582 μg/L and 0.261 ~ 1.940 μg/L, respectively. In the case of using the optimized LLE method, all 24 fragrance allergens were analyzed, and the correlation coefficient (r^2) of the calibration curve for quantification showed linearity of 0.9957 or more, MDL and LOQ were 0.020 ~ 0.138 μg/L and 0.065 ~ 0.440 μg/L, respectively.

Keywords : fragrances, allergens, HS-SPME (headspace-solid phase micro extraction), LLE (liquid-liquid extraction), GC-MS/MS

Introduction

The Scientific Committee on Consumer Safety (SCCS) of the European Union has identified 26 of these compounds as being fragrance allergens likely to cause contact allergies in 2012.[1] Allergic reactions and side effects include skin sensitivity, dermatitis, asthma attacks and migraines.[2,3] Their presence must be indicated in the list of ingredients when their concentrations exceed 0.01% for rinse-off products (e.g. cleansers, shampoos), and 0.001% for leave-on products (e.g. lotions, deodorants).[1] 24 of these suspected allergens were able to be analyzed by GC, whereas the other two are not single compounds but very complex natural extracts (oak moss and tree moss). Fragrance allergens are continuously introduced into the environment via urban waste water effluents because they are important components of daily use products such as soaps, shampoos and lotions.[4] But fragrance allergens are not included in the group of emerging contaminants, so probably for this reason, their occurrence in the environment was little reported.[5,6] Some synthetic musk compounds have been detected in surface water and sewage treatment plant (STP) influents/effluents.[6,7,8,9]

Analytical methods for fragrance allergens have been developed for environmental samples, and cosmetic and personal care products. Stir Bar Sorptive Extraction (SBSE)[10] and Solid Phase Micro Extraction (SPME)[2,3,11,12,13] have been developed to determine synthetic fragrances in tap water, river water and wastewater treatment plant final effluent water. Regarding the analysis of fragrance allergens in cosmetics and personal care products, micro-matrix solid phase dispersion,[4] size-exclusion chromatography followed by GC-MS,[14] high-performance liquid chromatographic method,[15] two-dimensional gas chromatography,[16] HS-GC-MS method,[17] and fully automated ionic liquid head space single drop micro extraction (IL-HS-SDME)[18] have been used.

In this study, we developed HS-SPME and LLE methods and compared them followed by GC-MS/MS for 24 of the fragrance allergens.

Experimental

Chemicals and reagents

A set of 24 fragrance allergens (ALR-EU24-SET) was obtained as 1000 μg/mL in a acetonitrile or ethanol from Accustandard (New Haven, CT, USA). Twenty-four fragrance allergens mixtures at 10 mg/L were prepared in methanol from individual standards, and stored at 4°C. Mixed working solution was prepared daily in methanol HPLC grade from J.T. Baker (Phillipsburg, NJ, USA).

---

*Reprint requests to Jae-Chan Ahn
E-mail: anjchan@seoul.go.kr

2,4,6-Trichloroanisole-d_5 and phenanthrene-d_{10} were obtained from CDN isotopes(Pointe-Claire, Quebec, Canada) and SUPELCO(595 North Harrison Road, PA, USA) respectively. Sodium chloride and sodium sulfate were supplied by Sigma-Aldrich (St. Louis, MO, USA). Methyl tert-butyl ether (MTBE), hexane and dichloromethane (DCM) used as extraction solvents were purchased from Merck (Billerica, MA, USA), Wako (Osaka, Japan), J.T. Baker (Phillipsburg, NJ, USA) respectively. All reagents were of analytical grade. Nitrogen evaporator used to evaporate the solvents was EYELA MG-2200 from Tokyo rikakikai (Tokyo, Japan). The water was purified using PureLAB from ELGA (Lowell, MA, USA). Water samples were collected in 1 L amber glass containers and stored in the dark at 4°C until analysis.

Instrumentation and analytical conditions

The GC-MS/MS analysis was performed with a Trace GC 1310 coupled to a TSQ 8000 Evo triple quadrupole mass spectrometer (Thermo Scientific, USA). A DB-5MS capillary column (30 m × 0.25 mm, id:0.25 μm film thickness (Agilent Technologies, USA) was used and the carrier gas was 99.99% pure helium (Seoul specialty gases, Korea) at a constant inlet flow rate of 1 mL/min. The injection port was equipped with a SPME liner or a tapered liner and operated in splitless mode (maintained for 4 min) at 270°C. A SPME liner(L × O.D × I.D., 78.5 mm × 6.5 mm × 0.75 mm) was used for the SPME method and a tapered liner(L × I.D., 78.5 mm × 4.0 mm) was used for the LLE method.

The GC oven temperature was programmed from 45°C (held 2 min) to 100°C at 8°C/min; to 150°C at 20°C/min; to 200°C at 25°C/min (held 5 min) and a final ramp to 240°C at 10°C/min (total analysis time = 24.38 min).

MS analyses were conducted in SRM (selective reacting monitoring) mode. The transfer line and ion source temperature were 250°C and 230°C respectively. Ionization was performed in electron impact mode at 70 eV. The acquisition of chromatographic data was performed using Xcalibur software (Thermo Scientific, USA). Table 1 summarizes the retention time and optimal MS parameters for each compound.

Headspace-solid phase micro extraction (HS-SPME)

HS-SPME experiments were performed with a CombiPAL (CTC Analytics, Switzerland). Three commercial extraction fibers including, 100 μm polydimethylsiloxane (PDMS), 65 μm

Table 1. Retention times, precursor and product ions, and collision energy of the target compounds

Key	R.T (min)	Compound	Precursor Ions (m/z)	Product ions (m/z)	Collision Energy (eV)
1	9.24	Limonene	67.1	41.1	15
2	9.31	Benzyl alcohol	107.1	79	5
3	10.36	Linalool	71.1	43.1	10
4	11.56	Methyl-2-octynoate	95.2	67.1	5
5	11.83	Citronellol	81.2	79.1	10
6	12.06	Geraniol	69.2	41.1	5
7	12.23	Citral	69.1	41.1	5
8	12.34	Cinnamaldehyde	131.1	77	25
9	12.39	Hydroxycitronellal	59.2	30.9	15
10	12.39	Anis alcohol	138.1	109	5
11	12.58	Cinnamyl alcohol	134.1	92	5
12	12.93	Eugenol	164.1	131.1	10
13	13.59	Coumarin	118.2	89	20
14	13.59	Isoeugenol	164.1	149	10
15	13.77	Ionone	135.1	91	15
16	14.15	Lilial	189.2	131.2	10
17	15.07	Amylcinnamaldehyde	115.2	88.9	15
18	15.27	Lyral	136.1	92.9	10
19	15.39	Amyl cinnamic alcohol	133.1	115	10
20	15.65	Farnesol	69.1	41.1	10
21	16.05	Hexyl cinnamaldehyde	115.1	89	15
22	16.38	Benzyl benzoate	105.1	77.1	10
23	17.84	Benzyl salicylate	91.1	65.2	15
24	21.41	Benzy lcinnamte	131.1	103.2	10

polydimethylsiloxane/Divinylbenzene (PDMS/DVB), and 50/30 μm polydimethylsiloxane/-divinylbenzene/Carboxen (PDMS/DVB/CAR) were purchased from Supelco (Bellefonte, PA, USA). Before use each fiber was conditioned according to the manufacturer's instructions to remove contaminants and stabilize the solid phase.

10 mL of sample were placed in 20 mL SPME glass vials containing 3g of sodium chloride and spiked with 50 μL of 2,4,6-trichloroanisole-d_5 internal standard (conc. 200 μg/L). The vials were then closed with aluminum caps and the samples were introduced in an incubator and the fiber was exposed at 80°C for 45 min in the headspace mode with constant stirring. After the completion of extraction, SPME device was removed from the vial and inserted into the injection port of the GC for analysis.

Liquid-liquid extraction (LLE)

20 g of sodium chloride and 200 mL of the sample were placed into a 250 mL separatory funnel, capped and shook to dissolve salt. 2,4,6-trichloroanisole-d_5, which was used as an internal standard of the HS-SPME method, was not extracted by this LLE method, so phenanthrene-d_{10} was used as an internal standard. 20 μL of phenanthrene-d_{10} internal standard (conc. 20 mg/L) was spiked into the samples. Then, 20 mL of MTBE and 1 mL of hexane were added to the separatory funnel. After capping the funnel, the mixture was vented and shook vigorously for 2 minutes, and after that, 10 minutes was required for layers to separate. The organic layer was drained into a 150 mL Erlenmeyer flask containing 2 g of sodium sulfate that had been baked at 450°C for 2 hrs., and then, the organic solvent was transferred to a 20 mL conical tube and the extract was concentrated to 500 μL under a nitrogen atmosphere. The final extract was transferred into 2 amber GC vials with inserts.

Results and Discussion

Comparison and optimization of the extraction methods

Figure 1 shows the extracted chromatograms of a standard analyzed using HS-SPME and LLE extraction methods. Using the developed HS-SPME method, 15 of the 24 fragrance allergens were analyzed and 9 compounds were not extracted. A study for the selection of the best coatings among the 100 μm PDMS, 65 μm PDMS/DVB and 50/30 μm PDMS/DVB/CAR was performed. The PDMS/DVB/CAR fiber was efficient on the extraction of limonene, linalool,

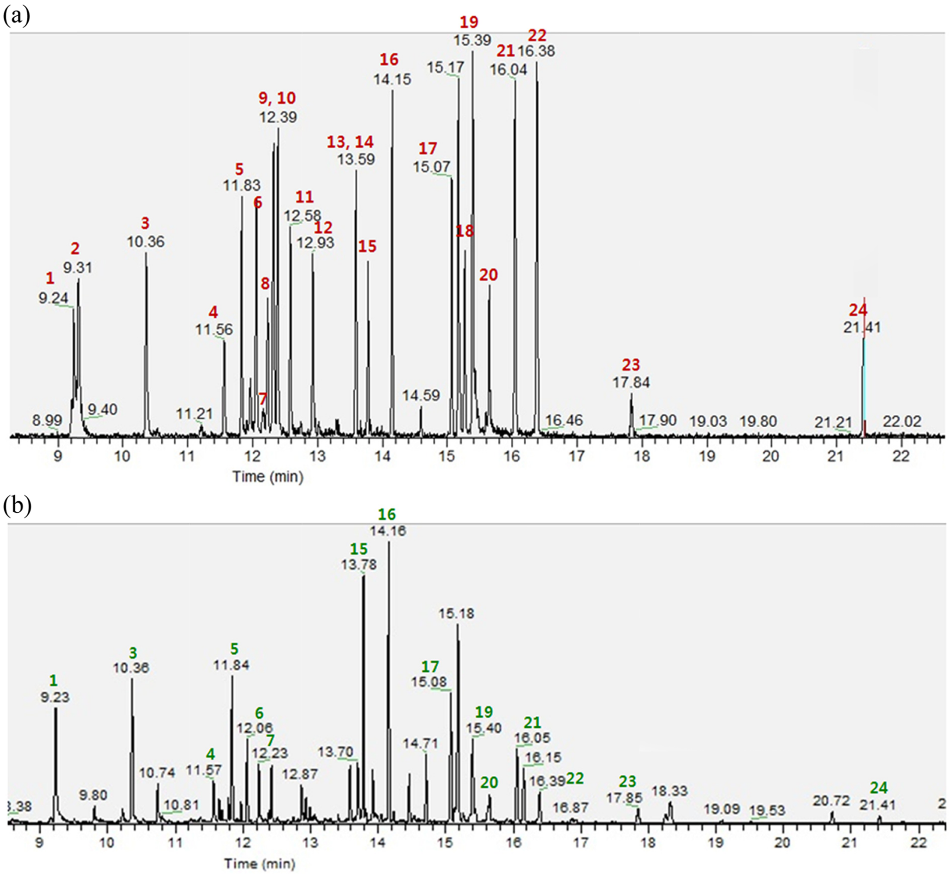

Figure 1. GC-MS chromatograms obtained by LLE of the 24 fragrance allergens at 5 μg/L in 200 mL of water (a) and HS-SPME of the 24 fragrance allergens at 5 μg/L in 10 mL of water using a 50/30 μm DVB/CAR/PDMS fiber (b) (see number code equivalence in Table 1).

methyl-2-octynoate, citronellol, geraniol, citral, ionone, lilial and amylcinnamaldehyde. The PDMS/DVB fiber was efficient on the extraction of amyl cinnamic alcohol, farnesol, hexyl cinnamaldehyde and benzyl benzoate. The PDMS fiber was efficient on the extraction of benzyl salicylate and benzyl cinnamate. The PDMS/DVB/CAR fiber with better extraction efficiency in more compounds was used for the HS-SPME method. In the study of the sodium chloride content it was observed that the addition of salt improved the extraction efficiency of all 15 compounds but if it exceeds 3 g, the extraction efficiency of some compounds (eg. limonene, linalool, geraniol, ionone) decreased. The effect of salt on the extraction efficiency was similar to that of the previous studies.[2,6]

In the case of using the LLE method, all 24 fragrance allergens were analyzed. The selection of an appropriate solvent is essential for LLE. MTBE, dichloromethane and hexane were tested as extraction solvents. The extraction efficiency of MTBE for 24 fragrance allergens was similar to that of DCM, but the extraction efficiency of some compounds which have relatively low $\log K_{OW}$ values (below about 2.5) was very low using hexane as the extraction solvent, because it was difficult to extract into hexane because they relatively mixed better with water. For the same reason, 9 compounds of the 24 fragrance allergens which have relatively low $\log K_{OW}$ values (below about 2.5) were not extracted by the developed HS-SPME method. These $\log K_{OW}$ values also affected the effect of the sodium chloride as a salting agent. With regard to the sodium chloride content of the LLE using MTBE as the extraction solvent, benzyl alcohol, hydroxycitronellal, anis alcohol, cinnamyl alcohol and coumarin with $\log K_{OW}$ values less than 2 were largely influenced. As the amount of salt increased, the extraction efficiency of these compounds increased significantly. In general, it was known that the addition of a salting agent increases the ionic strength in water and therefore the extraction efficiency for dissolved

Table 2. Quality parameters of each developed method

	Compounds	$\log K_{OW}$[3]	HS-SPME[a]			LLE[b]			Recovery (RSD)[c] (%)
			Linearity (r^2)	MDL (μg/L)	LOQ (μg/L)	Linearity (r^2)	MDL (μg/L)	LOQ (μg/L)	
1	Limonene	4.57	0.9971	0.349	1.165	0.9988	0.132	0.420	110.5(5.8)
2	Benzyl alcohol	1.05				0.9979	0.028	0.090	91.0(9.0)
3	Linalool	3.28	0.9999	0.225	0.751	0.9993	0.047	0.150	86.6(3.0)
4	Methyl-2-octynoate	2.60	0.9998	0.478	1.594	0.9997	0.039	0.123	110.2(2.9)
5	Citronellol	3.38	0.9993	0.218	0.728	0.9993	0.020	0.065	94.2(6.5)
6	Geraniol	3.28	0.9988	0.294	0.981	0.9994	0.086	0.275	97.8(17.0)
7	Citral	3.17	0.9999	0.299	0.997	0.9975	0.042	0.132	105.5(8.9)
8	Cinnamaldehyde	2.22				0.9997	0.043	0.137	107.9(5.1)
9	Hydroxycitronellal	1.54				0.9989	0.081	0.257	91.7(5.4)
10	Anis alcohol	1.10				0.9962	0.036	0.113	95.3(14.0)
11	Cinnamyl alcohol	1.93				0.9975	0.032	0.101	97.7(18.0)
12	Eugenol	2.20				0.9996	0.064	0.205	104.1(12.4)
13	Coumarin	1.39				0.9998	0.036	0.114	105.3(8.0)
14	Isoeugenol	2.45				0.9985	0.089	0.284	111.4(2.3)
15	Ionone	4.41	0.9995	0.078	0.261	0.9996	0.047	0.150	110.2(1.8)
16	Lilial	4.07	0.9997	0.137	0.460	0.9997	0.077	0.244	107.6(1.9)
17	Amylcinnamaldehyde	4.80	0.9999	0.181	0.604	0.9998	0.080	0.256	104.8(1.0)
18	Lyral	2.53				0.9991	0.137	0.435	109.5(7.8)
19	Amyl cinnamic alcohol	4.37	0.9976	0.294	0.979	0.9958	0.084	0.268	96.8(19.1)
20	Farnesol	5.31	0.9989	0.310	1.035	0.9957	0.138	0.440	107.1(17.0)
21	Hexylcinnamaldehyde	4.82	0.9988	0.255	0.851	1.0000	0.064	0.203	109.4(2.2)
22	Benzyl benzoate	3.97	0.9969	0.275	0.916	0.9999	0.044	0.141	109.9(3.8)
23	Benzyl salicylate	4.31	0.9994	0.582	1.940	0.9984	0.114	0.362	109.2(4.5)
24	Benzyl cinnamte	3.65	0.9983	0.387	1.290	0.9990	0.042	0.134	110.5(3.4)

[a] Conc.= 2.0 μg/L, N=8, MDL=SD×2.998, LOQ=SD×10

[b] Conc.= 0.5 μg/L, N=7, MDL=SD×3.143, LOQ=SD×10

[c] Conc.= 2.0 μg/L, N=3, raw water, LLE method

substances in water under the same extraction conditions was increased.[10] Therefore, it was considered that the compounds having a high affinity with water (logK_{OW} value less than 2) were more affected by the salt.

Method performance study

Method linearity has been evaluated performing a calibration study in the experimental conditions. On the developed HS-SPME method, the calibration range was established from 1 or 2 µg/L (depending on the individual limits of quantification) to 20 µg/L with 4-5 calibration levels. The method exhibited a direct proportional relationship between the extracted amounts of each fragrance allergen and their concentration in the samples, with correlation coefficients (r^2) of the calibration curve for quantification showing linearity of 0.9969 to 0.9999 (Table 1). The method detection limits (MDL) and the limits of quantification (LOQ) were 0.078 ~ 0.582 µg/L and 0.261 ~ 1.940 µg/L, respectively.

In the case of using the optimized LLE, the calibration range was established from 0.1 or 0.2 µg/L (depending on the individual limits of quantification) to 5 µg/L with 6-7 calibration levels and the correlation coefficients (r^2) of the calibration curve for quantification showing linearity of 0.9957 to 1.0000, MDL and LOQ were 0.020 ~ 0.138 µg/L and 0.065 ~ 0.440 µg/L, respectively. It was concluded that the LLE extraction method with low detection limit and more simultaneous analysis items was more effective to analyze fragrance allergens in water. Recovery values were evaluated using raw water spiked at 2 µg/L (n=3). They were calculated by consideration the difference between the measured concentrations for spiked and non-spiked samples. The thus obtained values can be considered satisfactory, between 86.5 and 111.4%.

Conclusions

While analytical methods for these fragrance allergens are conducted more commonly, it was the general method to develop and verify the analysis method with one extraction method. In this present study, we investigated the effect of octanol-water partition coefficient (K_{OW}) values on the extraction efficiency by comparing the commonly used HS-SPME and LLE methods. 15 of the 24 fragrance allergens were analyzed and 9 compounds which have relatively low logK_{OW} values (below about 2.5) were not extracted by using the developed HS-SPME method because the lower the logK_{OW} value, the greater the affinity with water, and the more difficult it is to volatilize, resulting in the lower extraction efficiency. The LLE method with a low detection limit and more simultaneous analysis items was more effective to analyze fragrance allergens in water and this method was validated and demonstrated to be reliable and linear in the concentration range of interest. MDLs were satisfactory (0.020 ~ 0.138 µg/L) and recoveries were obtained for targets in raw water (86.5 ~ 111.4%).

Acknowledgment

This work was supported by the research project of 'Standardization of analysis method of fragrances in water using GC-MS/MS and investigation in raw and tap water using the same method' from the Seoul Water Institute.

References

1. Scientific Committee on Consumer Safety (SCCS). *Opinion on Fragrance Allergens in Cosmetic Products; 15th Plenary Meeting*, Brussels (Belgium), June 26 - 27, 2012, p 10.
2. Godayol, A.; Besalu, E.; Antico, E.; Sanchez, J. M. *Chemosphere* **2015**, 119, 363.
3. Becerril, E.; Lamas, P.; Sanchez-Prado, L.; Llompart, M.; Lores, M.; Garcia-Jares, G. *Talanta* **2010**, 83, 464.
4. Celeiro, M.; Guerra, E.; Lamas, J. P.; Lores, M.; Garcia-Jares, G.; Llompart, M. *J. Chromatogr. A* **2014**, 1344, 1.
5. Godayol, A.; Gonzalez-Olmos, R.; Sanchez, J. M.; Antico, E. *Chemosphere* **2015**, 125, 25.
6. Becerril-Bravo, E.; Lamas, J. P.; Sanchez-Prado, L.; Lores, M; Garcia-Jares, G.; Jimenez, B.; Llompart, M. *Chemosphere* **2010**, 81, 1378.
7. Seo, C. D.; Son, H. J.; Lee, I. S.; Oh, J. E. *J. Korean Soc. Environ. Eng.* **2010**, 32, 615.
8. Lee, I.; Lee, C.; Heo, S.; Lee, J. *J. Korean Soc. Environ. Eng.* **2011**, 33, 821.
9. Seo, C. D.; Son, H. J.; Yoom, H. S.; Choi, D. H.; Ryu, D. C. *J. Korean Soc. Environ. Eng.* **2012**, 34, 195.
10. Seo, C. D.; Son, H. J.; Yoom, H. S.; Choi, J. T.; Ryu, D. C.; Kwon, K. W.; Jang, S. H. *J. Korean Soc. Environ. Eng.* **2014**, 36, 387.
11. Martinez, C.; Ramirez, N.; Gomez, V.; Pocurull, E.; Borrull, F. *Talanta* **2013**, 116, 937.
12. Divisova, R.;Vitova, E.; Divis, P.; Zemanova, J.; Omelkova J. *Acta Chromatogr.* **2015**, 27, 509.
13. Lamas, J. P., Sanchez-Prado, L.; Garcia-Jares, C.; Llompart, M. *Anal. Bioanal. Chem.* **2009**, 394, 1399.
14. Niederer, M.; Bollhalder, R.; Hohl, C. *J. Chromatogr. A* **2006**, 1132, 109.
15. Villa, C.; Gambaro, R.; Mariani, E.; Dorato, S. *J. Pharma. Biomedi. Anal.* **2007**, 44, 755.
16. Rey, A.; Corbi, E.; Peres, C.; David, N. *J. Chromatogr. A* **2015**, 1404, 95.
17. Desmedt, B.; Canfyn, M.; Pype, M.; Baudewyns, S.; Hanot, V.; Courselle, P.; DeBeer, J. O.; Rogiers, V.; DePaepe, K.; Deconinck, E. *Talanta* **2015**, 131, 444.
18. Vallecillos, L.; Pocurull, E.; Borrull, F. *Talanta* **2012**, 99, 824.