

## ITEX Application Note # 02

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Pages: 7

### **Analysis of Suspected Flavor and Fragrance Allergens in Lotion Samples. A comparison between Static Headspace, SPME, HSSE and ITEX Headspace Sampling**

#### Abstract

Suspected flavor and fragrance allergens were determined in an alcohol/water based lotion using ITEX headspace sampling. The dynamic headspace sampling was compared to static headspace, SPME and headspace sorptive extraction.

#### Introduction

According to recent EU regulation [1], 27 suspected allergen compounds should be monitored in cosmetic products. Depending on the sample matrix and solute concentrations, different sample preparation methods are developed and applied [2]. For the determination of suspected allergens in cosmetic products, one of the major problems is related to the presence of detergents that contaminate the analytical system if the samples are introduced without selective sample preparation. Selective extraction or selective sample introduction is however not easy since the target compounds cover a broad volatility range (from limonene to benzyl benzoate) and polarity range (from relatively polar benzyl alcohol,  $K_{ow}=1.1$ , to apolar benzyl benzoate,  $K_{ow}=4.0$ ). Liquid sample introduction with selective retention of non-volatiles in a PTV liner [3] or sorptive extraction using a PDMS coated stir bar [2] have been used for this application. Sampling from the headspace, using static headspace, dynamic headspace, SPME or headspace sorptive extraction (HSSE) can also be considered as these techniques avoid contamination of the analytical system by high molecular weight material such as detergents. The method of choice should however give ppm sensitivity, on one hand, and avoid discrimination of the target solutes based on relative volatility or polarity, on the other hand. In this application note, the use of dynamic headspace extraction using ITEX is demonstrated. The technique is compared to classical static headspace, solid phase micro-extraction (SPME) and to headspace sorptive extraction, using a polydimethyl siloxane (PDMS) coated stir bar in the headspace of the sample. The latter two techniques are similar in concept, only the total amount of sorptive PDMS phase is different [2,4].

#### Sample Preparation

As typical sample an alcohol/water based lotion was analysed. The lotion is used in wet wipes and contains besides different detergents also a fragrance.

For each method, 100 mg sample was placed in a 20 mL headspace vial. Two internal standards (1,4-dibromobenzene and 4,4'-dibromobiphenyl) were added at 10 ppm level, according to the reference method for the determination of allergens in perfumes described by Chaintreau et al [5].

## ITEX Application Note # 02

### Static HS conditions

Sample Conditioning @ 80°C, 15 min  
HS needle: 2.5 mL, 90°C  
Injection: 1 mL; 350 µL/s; 1/10 split ratio

### ITEX conditions

Sample Conditioning @ 80°C, 15 min  
Extraction Strokes: 10 x 1 mL; 50 µL/s  
Desorption @ 250°C with 1 mL headspace; 50 µL/s  
Trap Material Tenax TA 80/100mesh

### SPME conditions

Fiber: 100 µm PDMS  
Sample Conditioning @ 80°C, 15 min  
Desorption @ 250°C, 2 min

### Headspace Sorbative Stirbar Extraction conditions

Sample Conditioning @ 80°C, 15 min  
HSSE sampling in headspace: 10 mm x 0.5 mm df Twister™  
Desorption @ 250°C during 10 min in splitless mode  
Cryo-focussing @  
Injection: -100°C @ 600°C/min to 250°C, 1/10 split ratio

### GC conditions

All analyses were performed on an Agilent 6890 GC – 5975 MSD combination.

Column: 30 m x 0.25 mm i.d. x 0.25 µm df HP-5MS (Agilent)  
Carrier gas: helium, 168 kPa constant pressure at inlet (column outlet pressure: 28 kPa using AUX EPC and QuickSwap connector) (\*)  
Inlet: split, 250°C, 1/10 split ratio  
Oven temperature program: 50°C, 1 min, 8°C/min to 270°C.  
MSD transfer line: 250°C (17 cm x 110 µm i.d. restrictor, 28 kPa)  
Detection: MS in scan mode, scan range: 40-350 amu

(\*)under these conditions, alpha isomethyl ionone elutes at 15.5 min. These settings were used to performe the analyses under retention time locked conditions [2].

## ITEX: Application Note # 02

### Results

In Figure 1, the total ion chromatogram obtained for the lotion sample using classical static headspace sampling is given. The internal standards, added at the same concentration level, are detected at 10.3 min and 23.1 min respectively. The response for the first internal standard is higher in comparison to the second internal standard, corresponding to their relative volatility.

In this sample, some allergens could be detected. Linalool (peak 1) and hexyl cinnamaldehyde (peak 6) are easily detected. Other allergens are only detected as traces and confirmation of their presence by mass spectral comparison with a library spectrum is difficult.

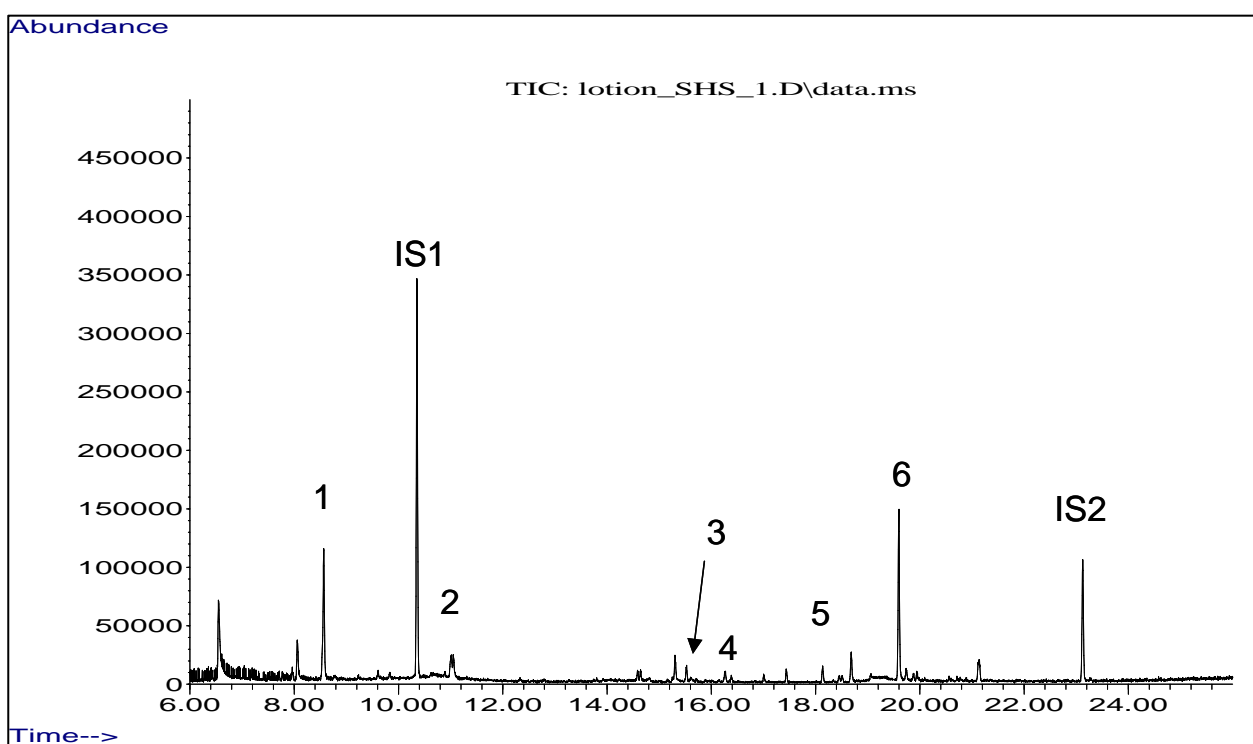


Figure 1: static headspace

## ITEX Application Note # 02

The chromatogram obtained by ITEX headspace sampling is shown in Figure 2. A much higher sensitivity is obtained in comparison to static headspace and several flavor and fragrance solutes could be detected. It is very interesting to observe that the response for the two internal standards is nearly equal, corresponding to their equal concentration in the sample. In this analysis, 6 allergens are detected and their presence could easily be confirmed by the mass spectra. Following allergens are present: 1. linalool, 2. citronellol, 3. alpha isomethyl ionone, 4. linal, 5. amyl cinnamaldehyde and 6. hexyl cinnamaldehyde.

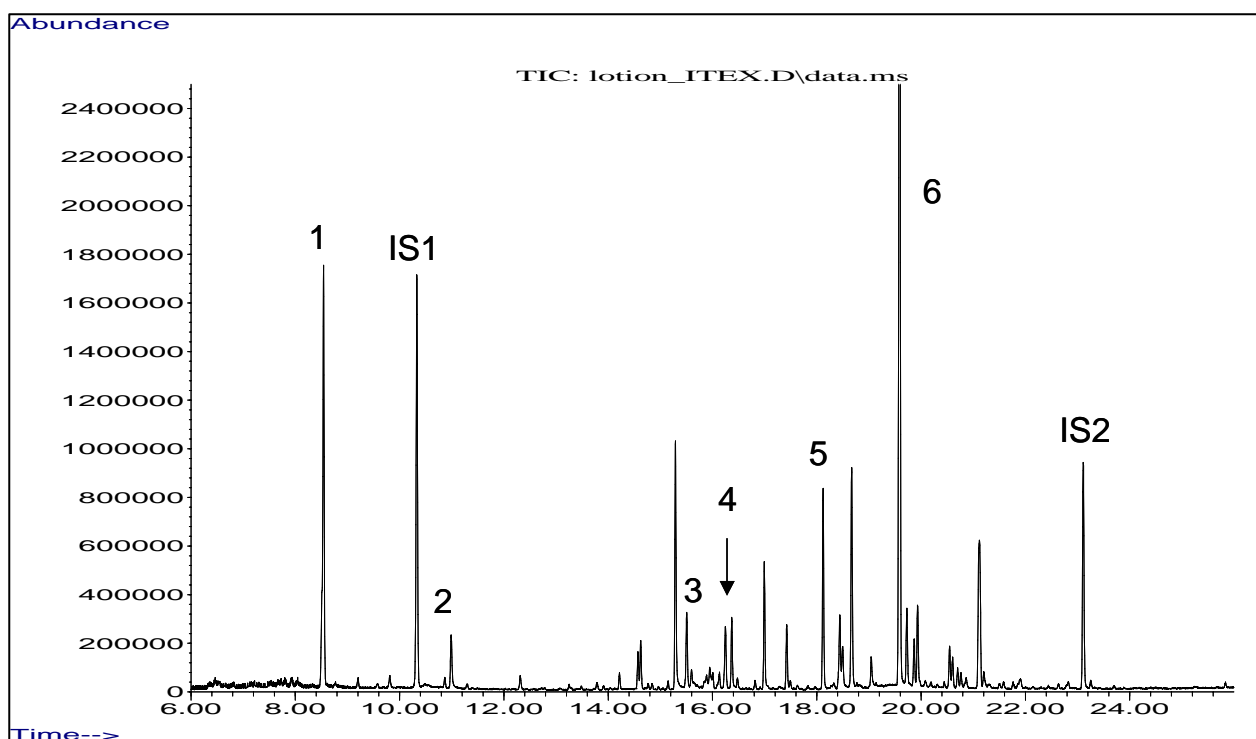


Figure 2: ITEX

## ITEX Application Note # 02

The chromatogram obtained by SPME headspace sampling is shown in Figure 3. Excellent enrichment is obtained and all 6 allergens could easily be detected. However, it is interesting to observe that the response of the second internal standard is much higher than for the first eluting internal standard. This difference can be explained due to the higher partitioning coefficient between PDMS and air for the higher molecular weight, later eluting compound. From the whole chromatogram it is clear that the less volatile compounds, having higher  $K_{\text{PDMS/air}}$  coefficients, are more enriched in comparison to more volatile solutes. The responses of the target solutes largely vary in function of the  $K_{\text{PDMS/air}}$  coefficients. This corresponds well with theoretical predictions [6].

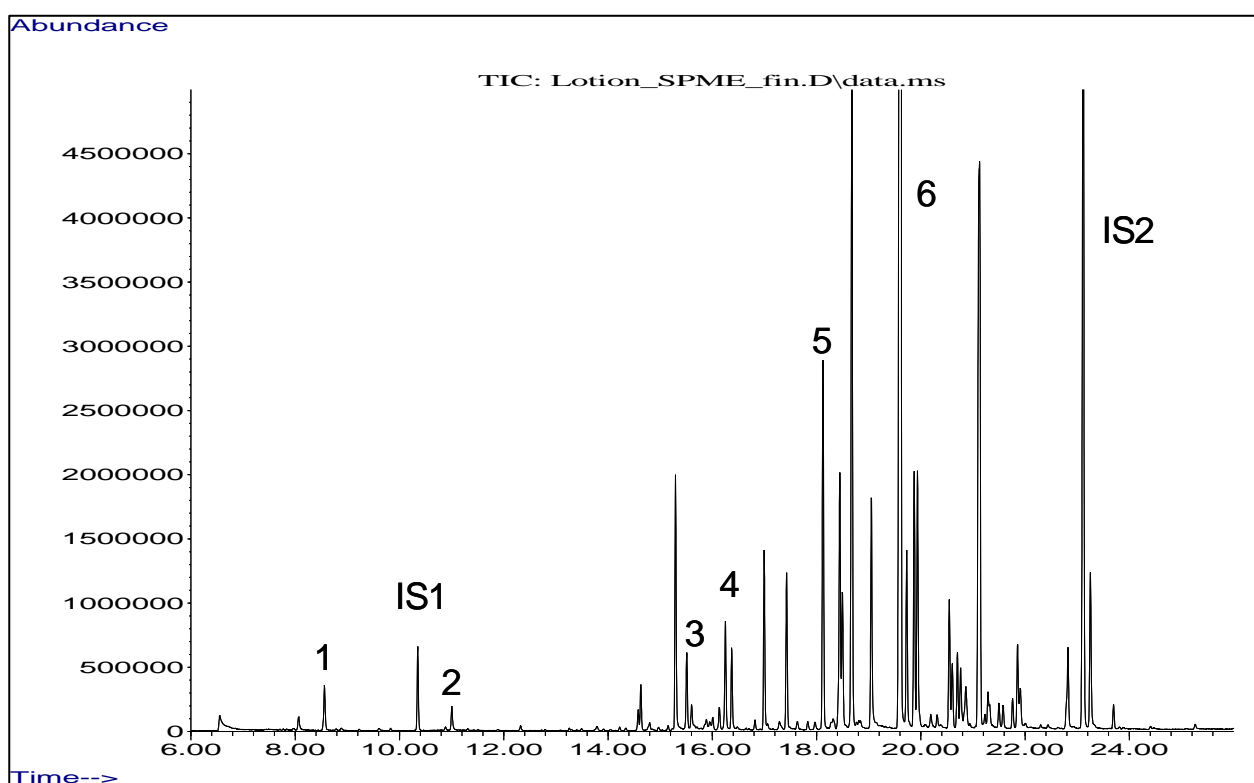


Figure 3: SPME

## ITEX Application Note # 02

The chromatogram obtained by headspace sorptive extraction sampling on a 1 cm stir bar coated with 0.5 mm PDMS is shown in Figure 4. As in SPME, excellent enrichment is obtained, but now the response of two internal standards is nearly equal, corresponding to their equal concentration in the sample. Since more PDMS material is available, quantitative recovery is obtained at lower  $K_{PDMS/air}$  coefficients and the profile is very similar to the profile obtained by ITEX sampling. In this analysis, 6 allergens are also detected and their presence could easily be confirmed by the mass spectra.

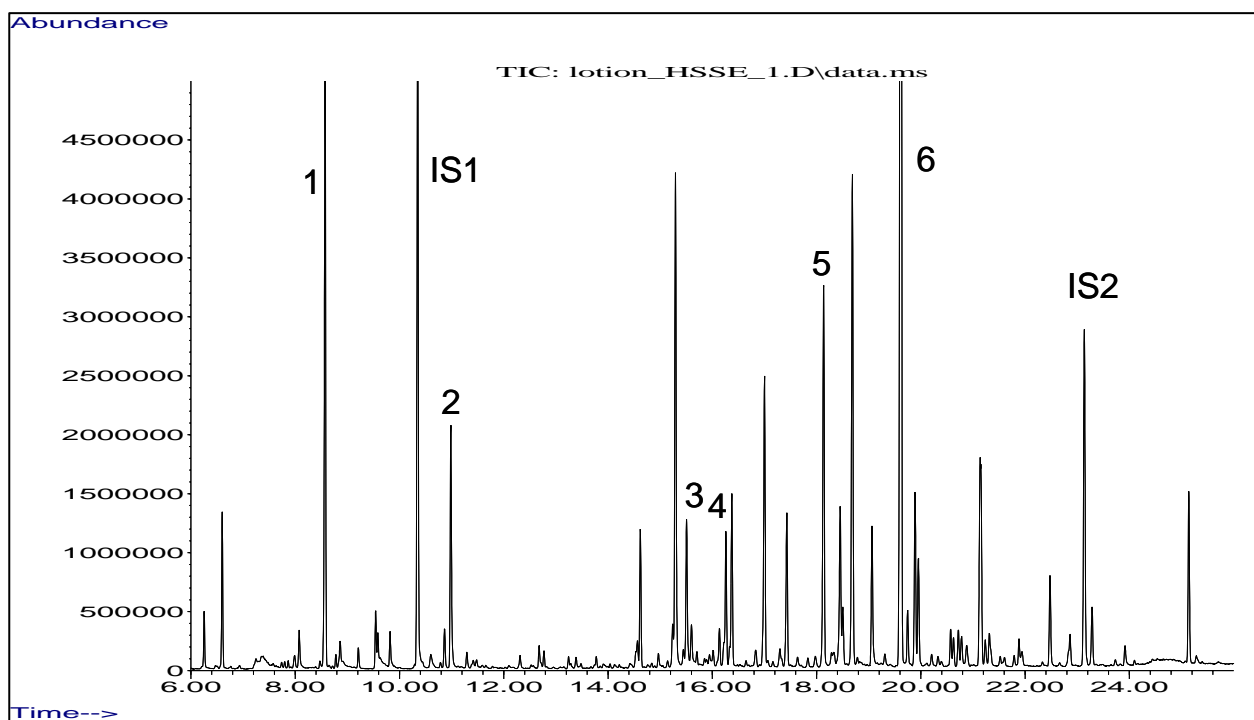


Figure 4: HSSE (Twister™)

## ITEX Application Note # 02

### Conclusion

For the determination of flavour and fragrance allergens in cosmetics, ITEX headspace sampling results in much higher sensitivity than static headspace. The obtained profile is similar to the profile obtained by headspace sorptive sampling (using a Twister<sup>TM</sup> stir bar in headspace). In comparison to SPME, the relative response of the solutes is less dependent on the individual  $K_{PDMS/air}$  coefficients of the target solutes. The sensitivity of the ITEX determination can be increased if the number of extraction strokes would be increased.

### References

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