Application Note 265

Enhanced fragrance profiling of shampoo using automated immersive high-capacity sorptive extraction with GC–MS

This study shows the advantages of immersive sorptive extraction using HiSorb™ probes for GC–MS analysis of a wide range of compounds in a fragranced shampoo sample. Key benefits include: direct extraction from the liquid phase by robust immersion of the probes, fully automated extraction and enrichment (including probe washing and drying on the Centri® multi-mode platform), 'prep-ahead' sample extraction functionality and high sensitivity to target analytes, particularly those at lower levels compared to the principal components.

Introduction

For bathing products such as shampoo, fragrance is a major aspect of the user experience and influences a consumer's buying decision. A wide range of volatile organic compounds (VOCs) contribute to the fragrance profile of shampoo. Characterisation of these compounds is a vital part of product development, formulation and routine quality monitoring, where the relative levels of key olfactory constituents are screened to ensure consistency is achieved, particularly for batch-to-batch production.

In this study, we demonstrate the use of HiSorb, a high-capacity sorptive extraction technique using HiSorb probes (Figure 1) for sampling of key fragrance compounds in a shampoo sample. HiSorb is compatible with both headspace and immersive extraction of VOCs in liquid samples, using standard 10- or 20-mL vials. The strength of the metal probes compared to delicate SPME fibers enables a more robust immersive extraction technique. The probes support a large volume of sorptive phase (~65 µL) to enhance the extraction efficiency relative to traditional solid-phase microextraction (SPME) methods where the SPME fibers contain approximately 0.5 µL of phase. The availability of more sorptive phase enables better extraction of trace-level compounds, facilitating much lower levels of detection in subsequent GC–MS analyses – typically >100x enhancement in sensitivity. Thus, high-capacity sorptive extraction is ideally suited to the measurement and identification of key olfactory components that may have a significant impact on consumer perception of fragrance even when present at low levels. Semi-volatile higher-molecular-weight compounds, which do not readily partition into the headspace due to their low volatility, are more efficiently extracted by immersion of the sorptive phase into the liquid sample, improving the discovery and identification of these sample constituents.

The Centri platform uniquely offers a fully automated sample extraction and enrichment workflow for shampoo VOCs by immersive high-capacity sorptive extraction. Often, manual washing and drying of sorptive material are required following immersive extraction to prevent contamination of the analytical system with residual sample matrix. With Centri, the probes are washed and dried as part of the automated sequence, prior to desorption, removing the need for manual intervention.

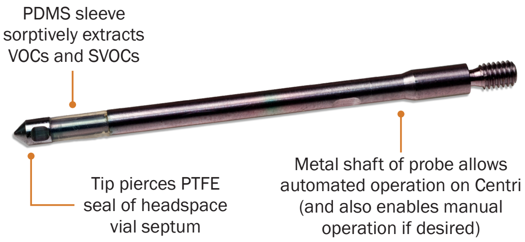


Figure 1: A HiSorb probe.

Background to Centri and HiSorb

Markes' versatile Centri automation platform combines extraction, enrichment and injection for a wide range of complex GC–MS applications, including solid, liquid and gaseous samples.

Automated extraction options include HiSorb high-capacity sorptive extraction (immersive or headspace), SPME, headspace and thermal desorption; all operating in combination with advanced cryogen-free focusing for optimum sensitivity. Extracted analytes may be focused in one or multiple enrichment steps before injection to the GC–MS as a narrow band of vapour. Centri is integrated with leading GC robotics to maximise uptime and sample throughput.

Another unique advantage of Centri is the ability to quantitatively re-collect the split flow from any operating mode and stabilise/archive this portion of the sample on a sorbent tube. This facilitates repeat analysis of samples for data confirmation or further investigation without needing to repeat lengthy sample extraction procedures.

For more on Centri and HiSorb, visit www.markes.com.

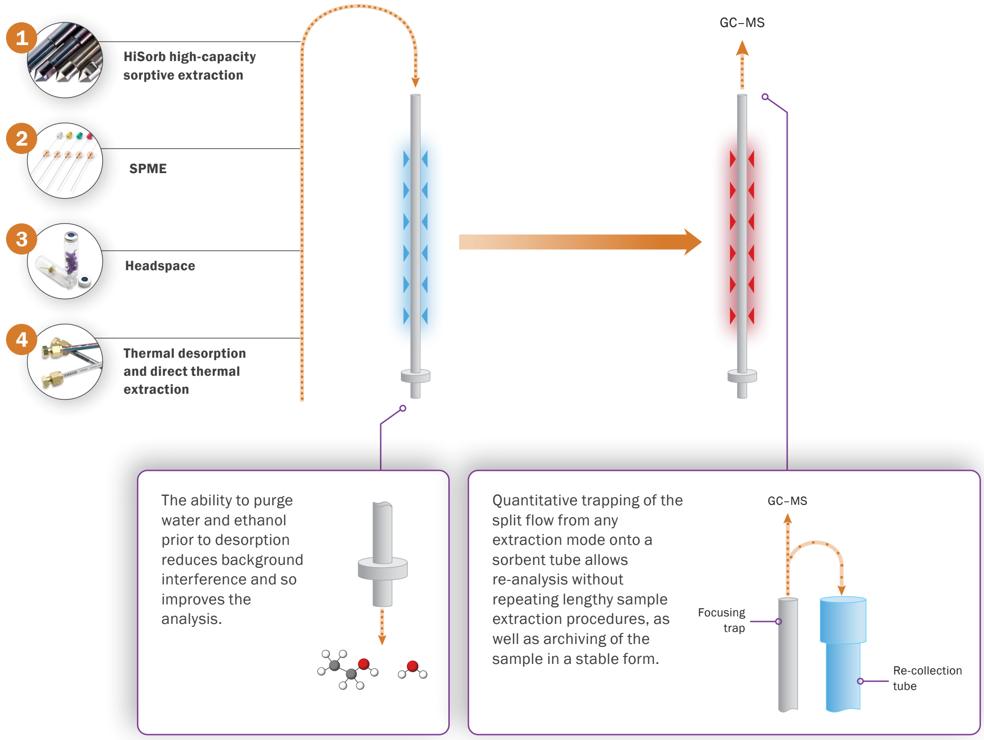
ANALYTE TRAPPING ON CENTRI *(optional for headspace and SPME)*

FIRST STAGE: Sample focusing

Samples collected using one of the four extraction modes are preconcentrated on Centri's cryogen-free sorbent-packed focusing trap.

SECOND STAGE: Trap desorption

The trap is heated rapidly to desorb the analytes and inject them into the GC–MS in a narrow band.



The ability to purge water and ethanol prior to desorption reduces background interference and so improves the analysis.

Quantitative trapping of the split flow from any extraction mode onto a sorbent tube allows re-analysis without repeating lengthy sample extraction procedures, as well as archiving of the sample in a stable form.

Further, Centri's 'prep-ahead' functionality allows for simultaneous extraction of multiple sample vials while a previous sample is being analysed by GC–MS, significantly increasing sample throughput (Figure 2).

During probe desorption, the extracted compounds are introduced to Centri's electrically-cooled, cryogen-free focusing trap in a stream of carrier gas. Selecting hydrophobic sorbents in the trap enables retention of a wide range of target analytes while allowing any water extracted from the sample to pass through and be purged to vent without reaching the capillary column or the GC detector.

This minimises chromatographic interference, enhancing the signal-to-noise ratio and thus sensitivity, as well as increasing column and detector lifetime, improving system stability. Centri therefore offers the high-sensitivity performance required for compound discovery in product development, and the robust automation and high-throughput operation required for routine quality control.

Here, the performance of HiSorb is demonstrated, generating excellent chromatographic results that clearly identify both abundant and trace compounds in a fragranced shampoo sample.

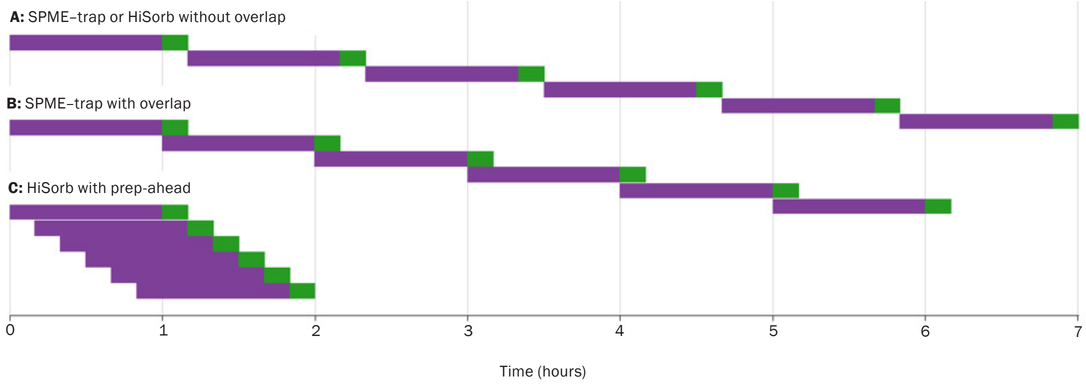


Figure 2: Comparison of overall sequence times for three operational modes of Centri, for a set of six samples with a typical 60-minute incubation time (purple) and a 10-minute GC run time (green).

Experimental

Samples: A commercially available shampoo with no specific scent detailed on the packaging was diluted to a proportion of 8.64 mg shampoo per 1 mL tap water. 18 mL diluted shampoo was dispensed into 20 mL vials, crimp-capped and placed on Centri for automated extraction, enrichment and subsequent GC injection. Resulting total ion chromatograms (TICs) were examined and a profile of shampoo constituents was generated.

Extraction and enrichment:

Instrument:	Centri (Markes International Ltd.)

Immersive HiSorb high-capacity sorptive extraction

Sample volume:	18 mL
Probe:	Standard-length stainless steel HiSorb probe (part no. H1-XXAAC)
Incubation:	30°C for 30, 45 or 60 min at 600 rpm, followed by automated washing and drying
Desorption:	280°C (12 min)
Flow path:	200°C

Preconcentration:

Focusing trap:	'Material emissions' (part no. U-T12ME-2S)
Purge flow:	50 mL/min (1 min)
Trap low:	20°C
Trap high:	310°C (5 min)
Split flow	5 ml/min (5:1)

GC:

Column type:	HP-5ms – 30 m x 0.25 mm x 0.25 µm
Column flow:	2 mL/min
Oven programme:	40°C (2 min), 10°C/min to 300°C (2 min)

Quadrupole MS:

Transfer line:	310°C
Ion source:	250°C
Mass range:	m/z 35–350

Data analysis:

Software:	ChromSpace® (SepSolve Analytical)
MS library:	NIST 2017

Results and discussion

Firstly, an assessment of extraction time was performed to understand how this affects the abundance of compounds extracted from a sample. The responses of six compounds covering the volatility range were measured and are shown in Figure 3. Greater responses were achieved when longer extraction times were used, aiding the discovery and more confident identification of lower-level components, such as γ-terpinene. For compounds present at high concentrations, the extraction time could be reduced further to improve sample throughput, for rapid fragrance profiling and product quality monitoring. Based on this information, the 30-minute extraction time was chosen for subsequent analyses.

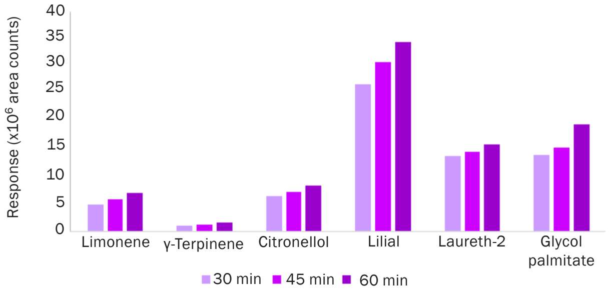


Figure 3: GC–MS peak areas obtained for compounds of interest in shampoo following immersive extraction with HiSorb probes for the times indicated.

A wide range of shampoo constituents were detected (Figure 4, Table 1), including both VOCs and SVOCs. Among the VOCs detected were many that have previously been described as prominent fragrance compounds in personal care products,[1,2] including limonene, linalool, γ-terpinene, citronellol, geraniol, lilial, piperonal and benzyl salicylate. The identification of SVOC compounds such as laureth-2, a surfactant/emulsifier ($C_{16}H_{34}O_3$; Bpt: 371°C), and emulsifying agents glycol palmitate ($C_{18}H_{36}O_3$; Bpt: 411°C) and laureth-4 ($C_{20}H_{42}O_5$; Bpt: 450°C) shows the excellent extraction efficiency of HiSorb in immersive mode for these high-molecular-weight compounds. Immersive extraction using HiSorb allows for a more comprehensive characterisation of a shampoo sample in a single run, recovering analytes across a wide volatility range. Chromatographical interference from water in this aqueous sample was minimised through the use of hydrophobic sorbents in the focusing trap and purging the trap with ambient-temperature carrier gas to vent prior to desorption to the GC, allowing water to be selectively removed while retaining analytes of interest.

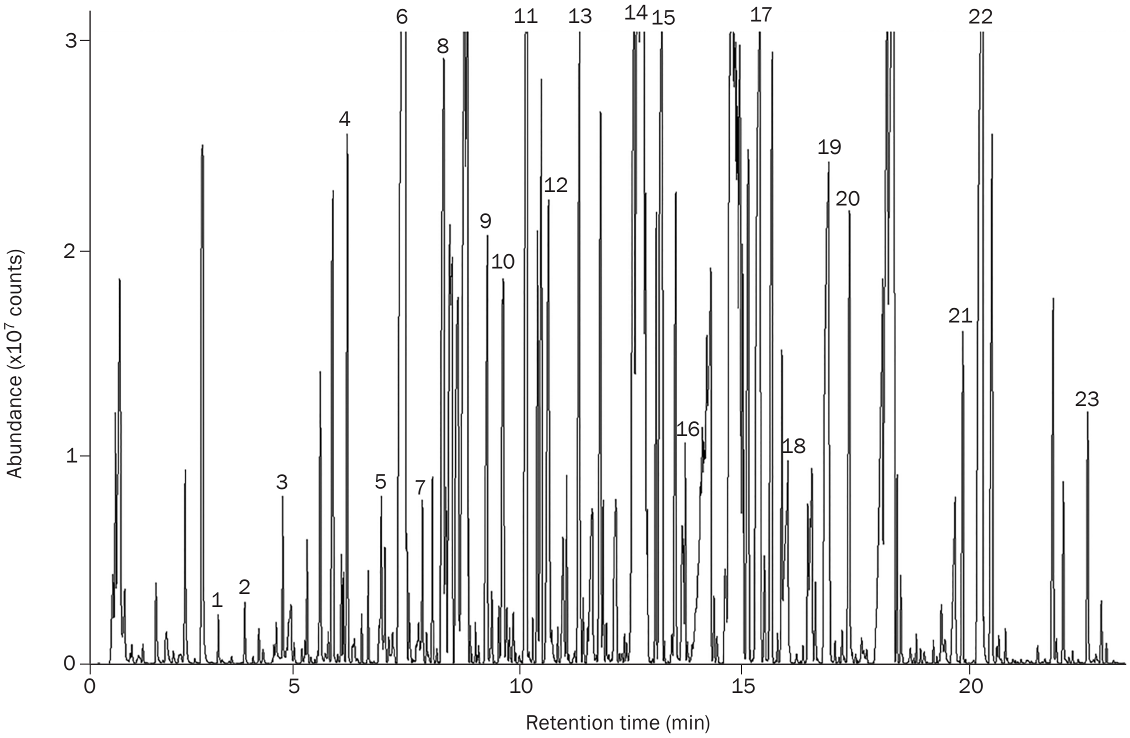


Figure 4: Total ion chromatogram produced following a 30-minute incubation. The numbered peaks are identified in Table 1.

Peak	Compound	RT
1	Ethyl α-methylbutyrate	3.30
2	Isopropyl 2-methylbutanoate	3.89
3	Ethyl α-methylvalerate	4.73
4	D-Limonene	6.17
5	γ-Terpinene	6.63
6	Linalool	7.46
7	1,2-Dihydrolinalool	7.87
8	α-Acetoxytoluene	8.30
9	Citronellol	9.27
10	Geraniol	9.64
11	Ortholate	10.15
12	Piperanol	10.64
13	Geranyl acetate	11.34
14	*trans*-β-Ionone	12.53
15	Lilial	13.14
16	β-Methylionone	13.70
17	Laureth-1	15.35
18	Tetradecanoic acid	15.98
19	Benzyl salicylate	16.87
20	Isobutyl tetradecyl ether	17.35
21	Laureth-2	19.87
22	Glycol palmitate	20.28
23	Laureth-4	22.64

Table 1: Peaks identified in Figure 4.

To understand more about this highly complex profile generated (Figure 4), these data were subjected to spectral deconvolution in ChromSpace data processing software. This feature allows for co-eluting peaks to be separated into individual analyte peaks for improved compound discovery and identification.

Following deconvolution, we were able to identify more compounds that had previously not been apparent, including fragrance molecules with high odour threshold values such as eugenol and isopropyl-2-methylbutanoate (Table 2). Although low in concentration, these compounds may nevertheless contribute to the fragrance experienced by the consumer and their discovery is important to the manufacturers of fragranced products. Examples of such compounds along with odour characteristics and threshold values are given in Table 2, with the deconvolved spectra shown in Figure 5.

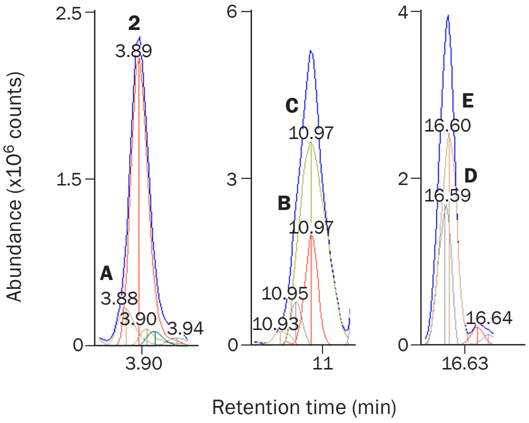


Figure 5: An example of three peaks in the total ion chromatogram shown in Figure 4 after processing with spectral deconvolution. The original trace is shown in blue, and demonstrates that in each case, only one peak is visible. The other traces within these blue peaks represent the co-eluting compounds that have been distinguished through deconvolution. Identifications for labelled peaks are given in Table 2.

Peak	Compound	NIST match factor	Odour characteristics	OTV (ppb)
A	Styrene	905	Sweet, balsamic, floral, plastic	730
2	Isopropyl-2-methylbutanoate	921	Fruity, sweet apple, pineapple	
B	α-Ionene	861		
C	Eugenol	919	Sweet, spicy, clove, woody	6-30
D	Pentadecylic acid	739	Waxy	
E	Lauryl *N*,*N*-dimethylamide	804		

Table 2: Compounds identified *via* spectral deconvolution in a shampoo sample incubated for 30 minutes prior to sample extraction. Confidence identifications are indicated with NIST match factors. Odour characteristics and odour threshold values (OTVs) are given where these were available in the indicated sources.

Conclusions

This study demonstrates the excellent performance of fully-automated HiSorb high-capacity sorptive extraction using robust probes for the discovery and identification of fragrance compounds in a commercially available shampoo. A wide range of VOCs and SVOCs were immersively extracted for detection, allowing for a more comprehensive sample characterisation in a single run. Spectral deconvolution of the generated data enabled more, low-level fragrance compounds present to be identified. These may have previously been overlooked as they co-elute with more prominent compounds in the sample chromatogram. Any residual water was successfully managed *via* the incorporation of a hydrophobic focusing trap, allowing it to be selectively purged prior to GC–MS analysis. Centri is the only platform that offers full 'set up and walk away' automation of high-capacity sorptive extraction, from sampling through to GC introduction, including automatic washing and drying of the probes after the extraction step. This is particularly important when, as in this study, the sample matrix is viscous or sticky and any residue could contaminate the analytical flow path. Coupled with sample overlap, these automation features allow Centri to deliver high-throughput immersive sorptive extraction, whilst retaining the analytical performance required for product development and routine quality testing.

References

1. S.C. Rastogi, S. Heydorn, J.D. Johansen and D.A. Basketter, *Contact Dermatitis*, 2001, 45: 221–225.
2. A.C. Steinemann, I.C. MacGregor, S.M. Gordon, L.G. Gallagher, A.L. Davis, D.S. Ribeiro and L.A. Wallace, *Environmental Impact Assessment Review*, 2011, 31: 328–333.