Application Note 268

Enhancing the performance of SPME and sorptive extraction for GC–MS using trap-based preconcentration

This study shows how GC–MS performance for the sampling of aroma compounds and off-odours in beverages can be enhanced by using techniques incorporating trap-based preconcentration. The first part of the study focuses on SPME, and how trapping and enrichment can improve peak symmetry, qualitative analysis and sensitivity. The second part of the study compares these methods against automated probe-based high-capacity sorptive extraction, which as well as being operationally robust, offers improved recovery for higher-boiling compounds and an extended analyte range.

Introduction

As a readily-automated, fast, solvent-free technique, solid-phase microextraction (SPME) has become widely adopted for a broad range of samples and applications, including analysis of foods and beverages, profiling of aromas and off-odours, as well as environmental, clinical and industrial investigations.

This application range is supported by a variety of SPME fiber phases (including PDMS, polyacrylate and multi-phase DVB/CAR/PDMS), which allow analyte selectivity to be optimised. However, workflows for conventional ('direct') SPME sometimes suffer from its limited sensitivity. This stems from the small volume of sorptive phase on the fiber (typically ~0.5 µL of PDMS), as well as from the relatively slow heating rate of commonly-used GC injection ports, resulting in broad peaks.

Sample preconcentration is a powerful approach to dealing with this issue. Following sample extraction, analytes desorbed from the SPME fiber are first focused onto a narrow, cryogen-free, sorbent-packed focusing trap. By using this **SPME–trap** method, analytes are enriched/preconcentrated before being injected into the GC-MS in a narrower band, improving peak shape (especially for the early-eluting compounds), and so improving sensitivity.

Use of a focusing trap also offers further benefits. One of these is taking multiple extracts from a sample and focusing them onto the trap, prior to desorption. This **SPME–trap with multi-step extraction and enrichment** allows sensitivity to be improved further, with the best results being obtained when multiple aliquots of the same sample are used.

Direct SPME, SPME–trap and SPME–trap with multi-step enrichment are all automated on the Centri® sample extraction and enrichment platform from Markes International, along with static headspace (and headspace–trap), high-capacity sorptive extraction, and tube-based thermal desorption.

High-capacity sorptive extraction is worth highlighting alongside SPME, as it works on the same basic principles, and is therefore suitable for the same types of samples. The technique uses a relatively large volume of PDMS sorptive phase (~65 µL) immobilised on a robust HiSorb™ probe, optimising performance for trace-level compounds. HiSorb also allows automated immersive as well as headspace extraction, even of samples with high solid content. After sampling, HiSorb probes are automatically washed, dried and desorbed. Released analytes are selectively preconcentrated on Centri's cryogen-free focusing trap, thus maximising sensitivity.

In this study, we first compare the performance of the three SPME methods (direct SPME, SPME–trap and SPME–trap with multi-step enrichment) for the analysis of headspace above a suspension of tea leaves, including an evaluation of their performance for a set of halophenols known to cause off-odours. We then go on to show how HiSorb high-capacity sorptive extraction compares with these SPME methods, and highlight the practical advantages of this approach.

In each case all the extraction, enrichment and preconcentration stages are fully automated on Centri, allowing the analyst to sequence multiple methods according to whichever is best for the sample in question.

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Background to Centri

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.

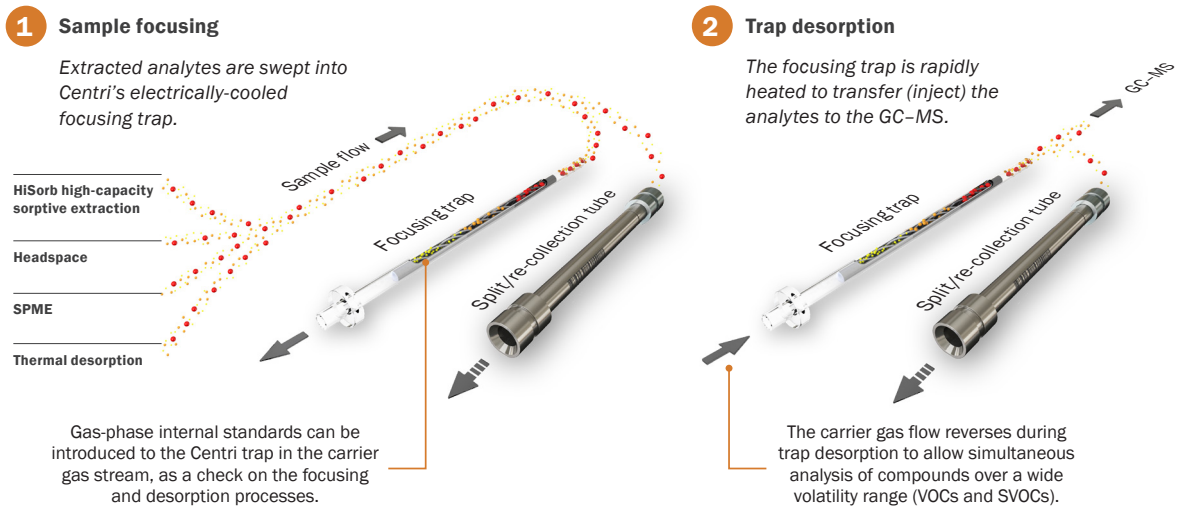
Centri uses leading GC robotics to maximise instrument usage and throughput, with automated extraction options including HiSorb™ high-capacity sorptive extraction (immersive or headspace), SPME, headspace and thermal desorption. All of these options offer sample enrichment on a cryogen-free, sorbent-packed trap, before injection of the analytes into the GC–MS as a narrow band of vapour for optimum sensitivity.

Additional features offered by Centri include:

- Multi-step enrichment: Combining multiple extracts onto the same trap for greater sensitivity.
- Re-collection: Quantitative trapping of the split flow from any sample extraction mode on a sorbent tube, for re-analysis without needing to repeat lengthy sample extraction procedures, or archiving in a more stable form.

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

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



Experimental

Halophenol standard:
Table 1 lists the halophenol off-odour compounds used to spike the tea sample.

Sample:
1 g of dry loose-leaf tea was weighed into a 20 mL headspace vial. 10 mL of HPLC-grade water was added and the mixture was spiked with 10 µL of the halophenol standard, to produce a final concentration of 1 ppb. The vial was capped and crimped to seal it.

Extraction and enrichment:

Instrument: Centri® (Markes International)

Headspace SPME and SPME–trap:

Fiber: Multi-phase (DVB/CAR/PDMS), 20 mm long, 50/30 µm d_f (Supelco part no. 57299-U)

Sampling depth: 30 mm

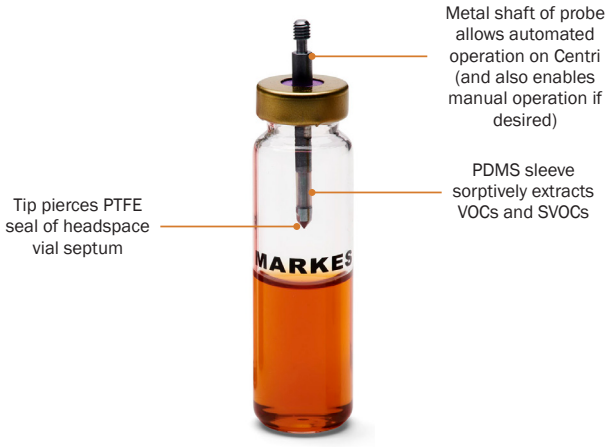


Figure 1: Short-length HiSorb probe, shown ready for headspace extraction above a liquid in a 20 mL vial. Probe desorption, followed by washing, drying and trap-based preconcentration, are all carried out automatically on the Centri platform prior to GC–MS injection.

No.	Compound	t_R (min)	Quant ion (m/z)	log $K_{o/w}$
A	6-Chloro-o-cresol (6-COC)	36.8	107	2.70
B	2-Chlorophenol	39.6	128	2.16
C	2-Bromophenol	43.7	172	2.40
D	2,6-Dichlorophenol	48.5	162	2.80
E	2,4-Dichlorophenol	50.3	162	2.80
F	2,6-Dibromophenol	56.1	252	3.29
G	2,4,6-Trichlorophenol	56.4	196	3.44
H	4-Chlorophenol	58.0	128	2.16
I	2,4-Dibromophenol	58.6	252	3.29

Table 1: Composition of the halophenol standard mixture. The log $K_{o/w}$ value is a useful indicator of the extent to which the compound would be expected to partition between PDMS and water at equilibrium. Values above 3 indicate that a substantial proportion of the compound will partition into the PDMS phase, whereas values below 2 indicate that very little of the compound will be extracted.

Incubation: 60°C (15 min) at 500 rpm
Desorption: 250°C (3 min)
Enrichment: For SPME–trap with multi-step enrichment, two further rounds of the above incubation–desorption procedure were carried out, using the same fiber

Headspace HiSorb high-capacity sorptive extraction:

Probe: Short-length (48 mm) stainless-steel HiSorb™ probe (Markes International part no. H1-XXABC) (Figure 1)
Incubation: 60°C (60 min) at 500 rpm
Desorption: 270°C (10 min)
Probe wash: 10 s
Probe dry: 5 s

Preconcentration:

Flow path: 180°C
Focusing trap: 'Material emissions' (part no. U-T12ME-2S)
Purge flow: 50 mL/min (1 min)
Trap low: 25°C
Trap high: 290°C (3 min)
Split ratio: 6:1

GC–MS:

Column: DB-WAX™ Ultra Inert (Agilent Technologies), 60 m × 0.25 mm × 0.25 µm
Oven program: 40°C (3 min), then 30°C/min to 60°C, then 3°C/min to 230°C (15 min)
Constant flow: 1 mL/min helium
Transfer line: 230°C
Ion source: 230°C
Quad: 150°C
Mass range: m/z 35–300
Tune type: E-tune

Results and discussion

For clarity, this discussion is split into two sections. Section A compares the results obtained with the three SPME methods, and Section B then makes an additional comparison with HiSorb high-capacity sorptive extraction.

A. Comparison of SPME methods

A1. Chromatographic performance

Figure 2 compares the profiles for the tea suspensions using direct SPME, SPME–trap and SPME–trap with multi-step enrichment (which involves two further rounds of sampling

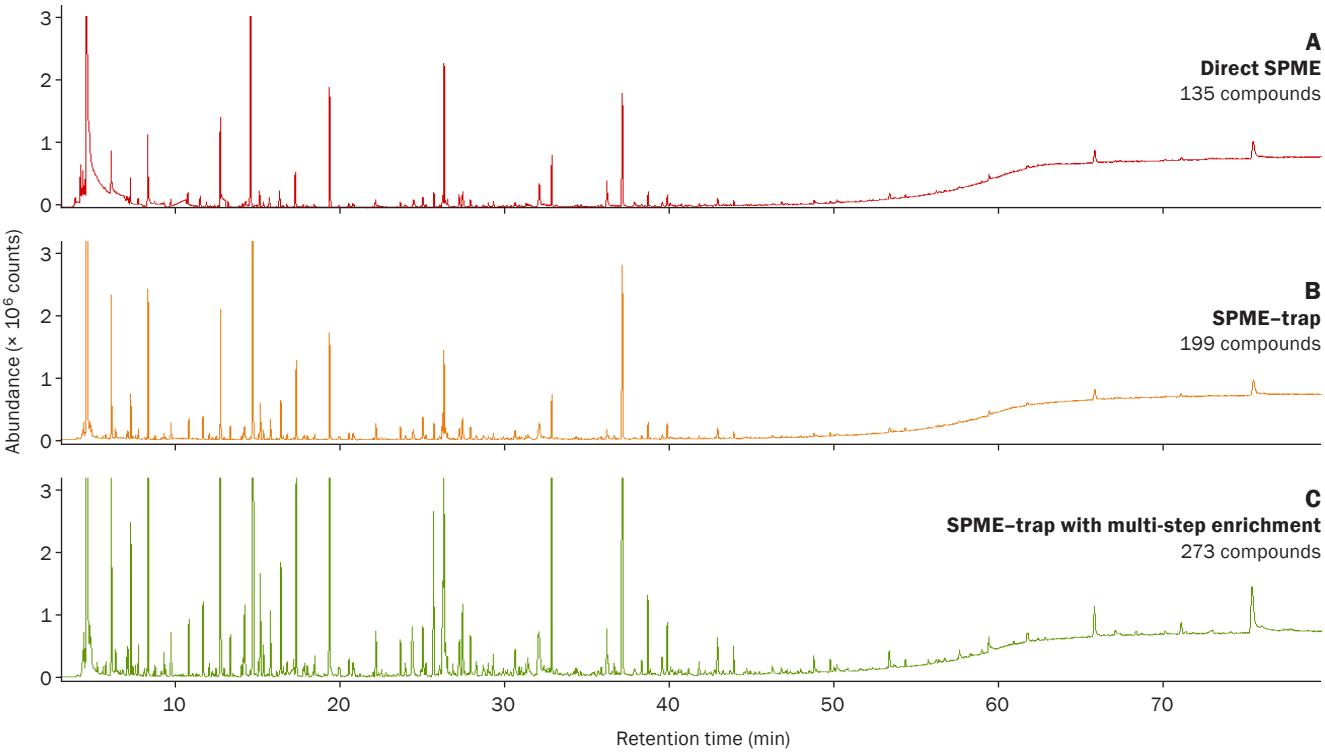


Figure 2: Analysis (TIC) of the headspace of the tea suspension, using (**A**) direct SPME, (**B**) SPME–trap, and (**C**) SPME–trap with multi-step enrichment.

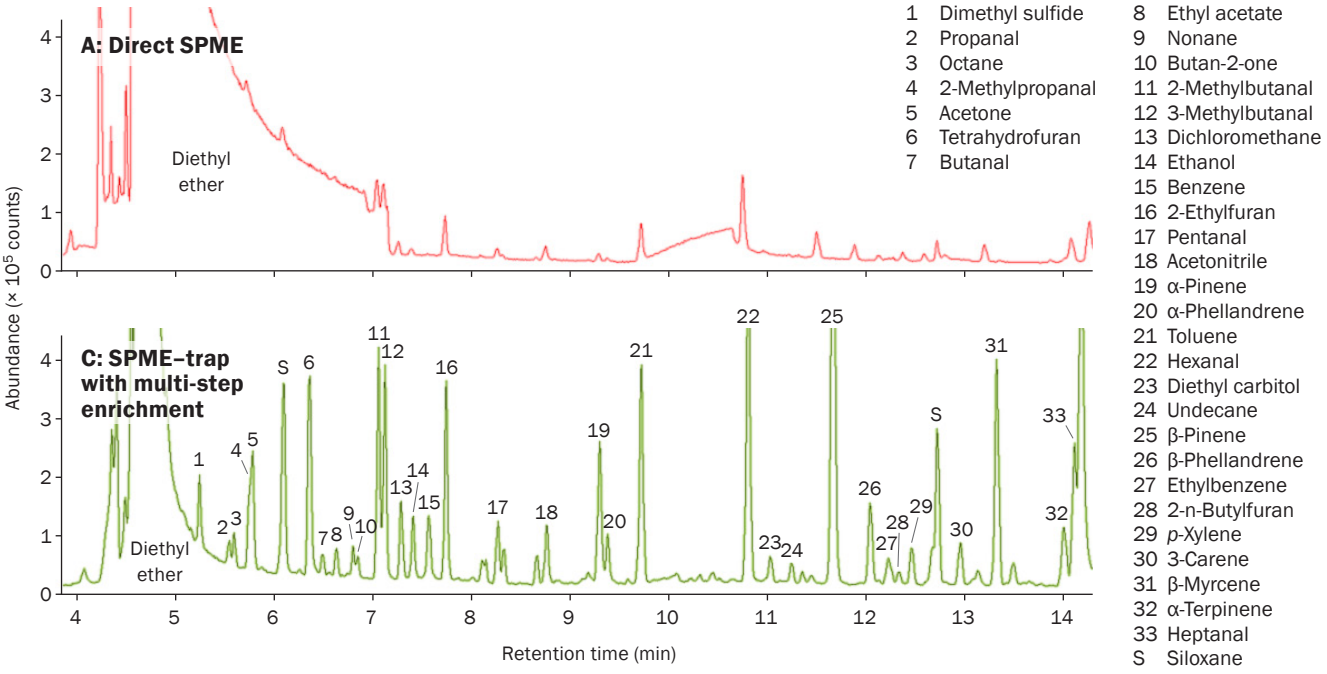


Figure 3: Expansions of Figures 2A (direct SPME) and 2C (SPME–trap with multi-step enrichment), showing the reduction in tailing for the large diethyl ether peak, and the additional compounds tentatively identified.

from the same vial). As expected, the use of trapping results in a clear improvement in peak symmetry, and the enrichment step results in an increase in intensity.

The result of using these enhancements is to increase the number of compounds identified, from 135 (direct SPME) to 199 (SPME–trap) and then to 273 (SPME–trap with multi-step enrichment), using the NIST database with a match coefficient ≥800.

A2. Peak shape for early-eluters

Poor peak shape for early-eluting compounds is often a concern with SPME methods, and Figure 3 shows how the use of SPME–trap resolves this issue. The early part of the direct SPME analysis (Figure 3A) is dominated by diethyl ether, which is abundant because of its use as the solvent for the halophenol standard. Although this compound would not otherwise have been present, the severe tailing is typical of SPME analysis of samples containing high-abundance volatiles.

By using SPME–trap (in this case, run with three rounds of enrichment (Figure 3C)), the peak shape for diethyl ether is much improved, allowing previously obscured compounds to be seen and identified.

A3. Identifying trace-level compounds

A common problem in food and beverage analyses is that some compounds can have very low odour threshold values (OTVs). This issue is exacerbated in complex matrices, because automated identification of mass spectra can be unreliable when peaks are not baseline-resolved, or the baseline itself is obscured. Often the only option in such cases is to manually interrogate every compound, but this is of course highly time-consuming.

SPME–trap with multi-step enrichment can help to address these issues, by increasing the signal for a compound above

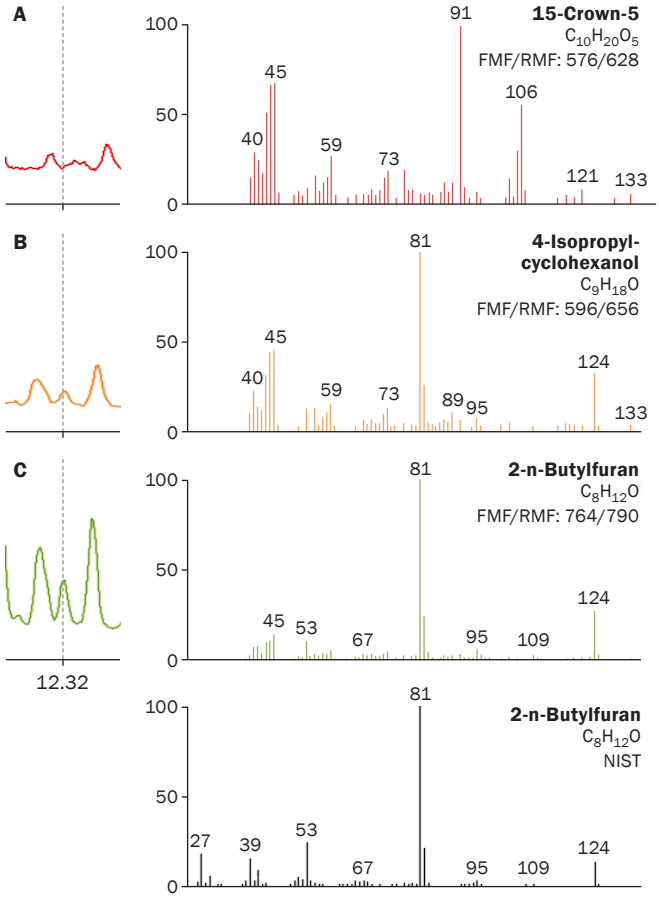


Figure 4: Left: Expansions of Figures 2A (direct SPME), 2B (SPME–trap), and 2C (SPME–trap with multi-step enrichment) centred on 2-n-butylfuran (dotted line), showing the increase in intensity. Right: Corresponding mass spectra, showing the improved forward and reverse matches to the NIST spectrum (bottom) when using multi-step enrichment.

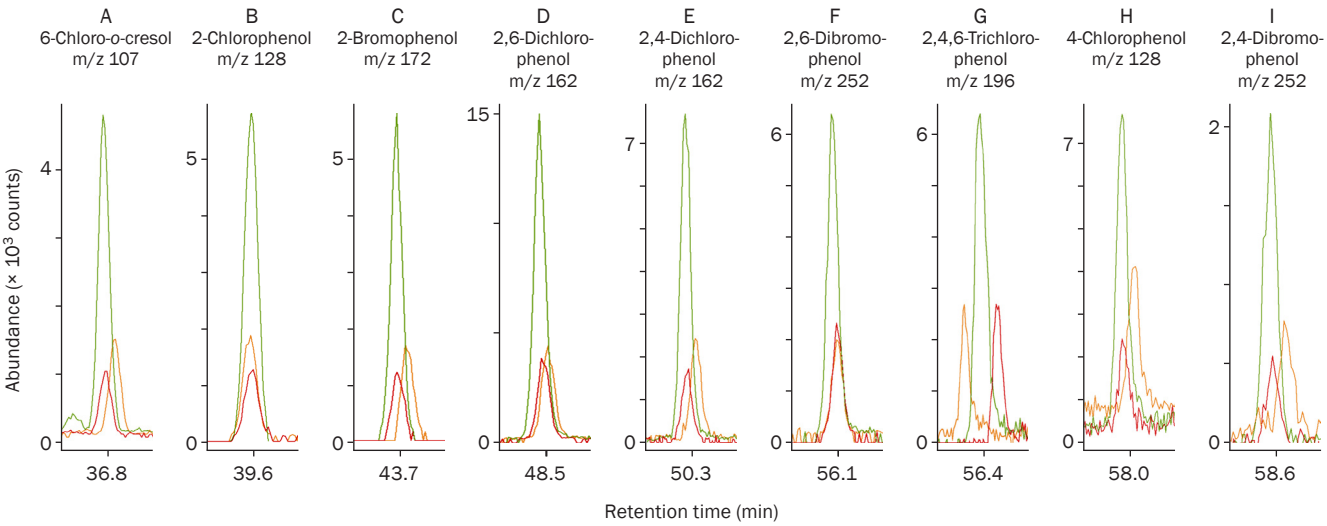


Figure 5: EIC reponses for the nine spiked halophenols in the headspace of the tea suspension (direct SPME —, SPME–trap —, SPME–trap with multi-step enrichment —).

the background, and so improving the automated library search results by enabling more characteristic ions to be distinguished.

Figure 4 shows an example of this, using the trace-level compound 2-n-butylfuran, which has an OTV of 50 mg/m^3 (50 ppb), and imparts a 'fruity-sweet' and 'spicy' aroma' [1] to food products. Whereas the compound is undetectable using direct SPME, and has low abundance using SPME–trap, SPME–trap with multi-step enrichment shows a distinct peak. The result of this is low responses from interfering ions in the corresponding mass spectrum, and a more confident match for the correct compound.

A4. Identifying halophenol off-odours

As a further example of the improved performance obtained using SPME–trap with multi-step enrichment, Figure 5 shows EIC profiles for the nine spiked halophenols in the tea sample. These compounds can be present as off-odours in brewed tea, and originate either from product deterioration or from contamination from packaging.

All nine halophenols are well-focused on the GC column stationary phase for both direct SPME and SPME–trap. As expected, similar abundances (and peak profiles) are seen because only a single extraction is being taken. However, the three-fold enrichment step significantly enhances the responses (Figure 6), reducing detection limits as well as improving confidence in identification.

B. Comparison of HiSorb with SPME

B1. Chromatographic performance

In the second part of the study, an automated HiSorb method was developed to analyse the headspace of the same halophenol-spiked tea sample as before. Figure 7 shows the

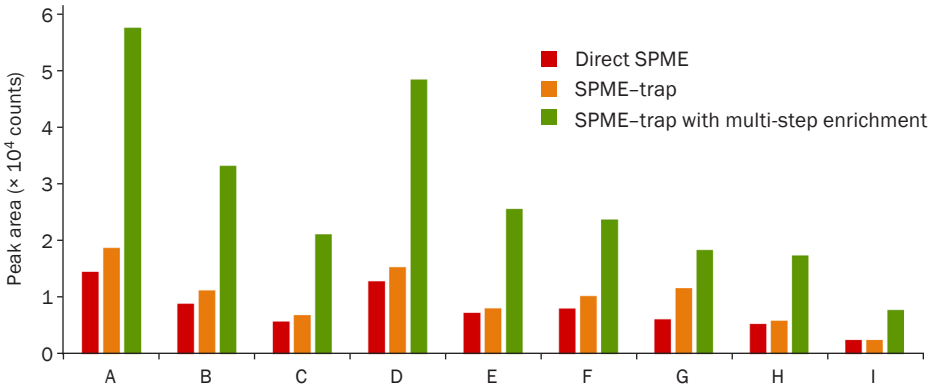


Figure 6: EIC peak abundances for the nine spiked halophenols in the headspace of the tea suspension for the three SPME methods.

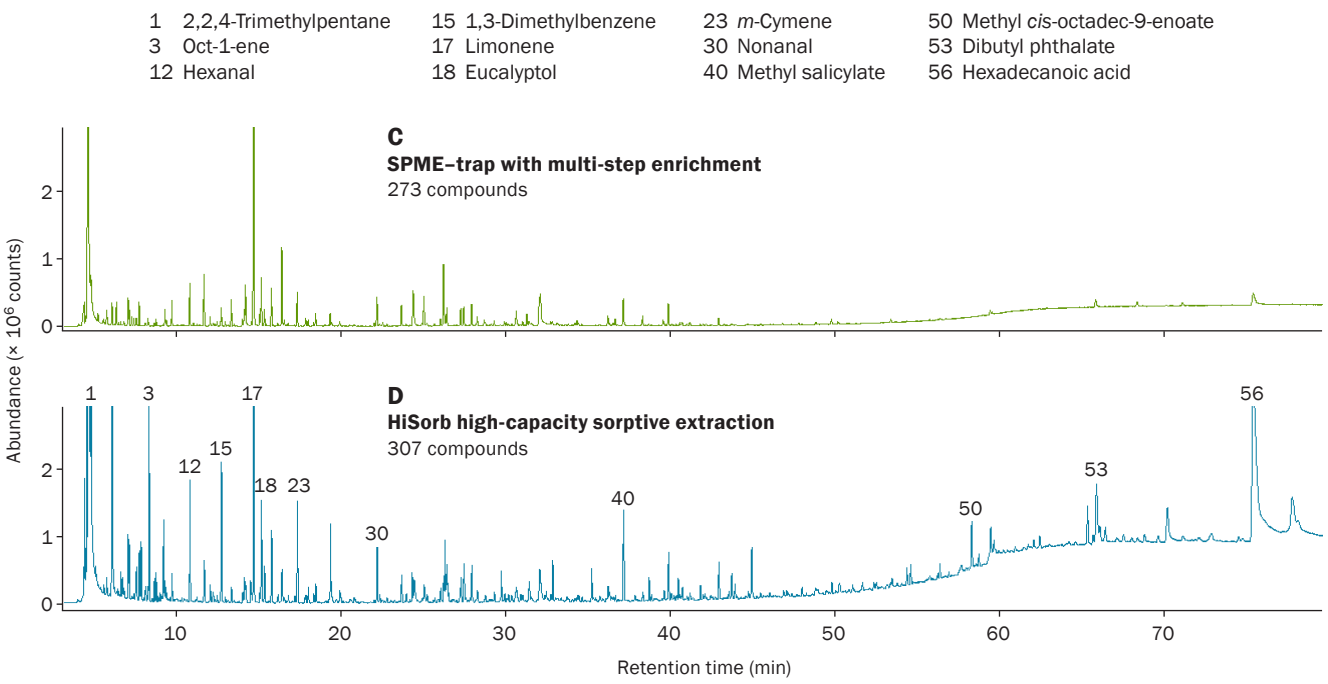


Figure 7: Analysis (TIC) of the headspace of the tea suspension, using (**C**) SPME–trap with multi-step enrichment and (**D**) HiSorb sorptive extraction (major compounds are labelled; a full list is provided in Table A1 (see Appendix)).

resulting profile. Using the same NIST search criteria, 307 compounds were tentatively identified – an increase from 273 using SPME–trap with multi-step enrichment. Table A1 (see Appendix) provides a representative listing of compounds with the highest match factor (≥900).

Figure 7 also shows that HiSorb results in a large increase in the relative abundances of compounds eluting after 40 minutes, compared to the SPME methods. These compounds extend to the C_{16} carboxylic acids hexadecanoic acid (palmitic acid, b.p. 351°C) and hexadec-9-enoic acid (palmitoleic acid, b.p. 363°C), indicating the high sorptive capacity of the PDMS phase for these types of high-boiling compounds.

B2. Identifying halophenol off-odours

Figure 8 shows how the halophenol data for the HiSorb method compares against the three SPME methods. Despite the relatively low log $K_{o/w}$ values of most of these compounds, the performance is nevertheless comparable to that obtained with direct SPME or SPME–trap, which use multiple phases with different polarities. Moreover, the availability of enrichment in all four Centri sampling modes would enable the trap loading to be increased by repeated rounds of HiSorb sampling onto the same trap.

B3. Prep-ahead capability

When sample throughput is important, the ability to overlap the sampling and analysis of a sequence of samples (often referred to as 'prep-ahead') is very useful for improving sample productivity, and the Centri autosampler offers this capability in all sample modes.

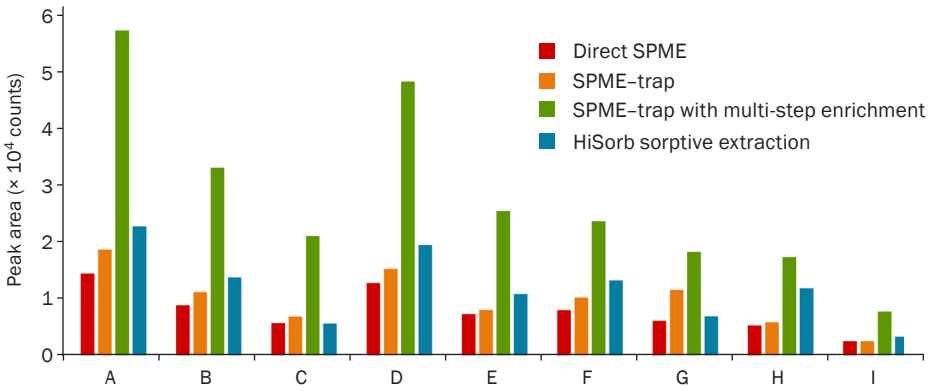


Figure 8: EIC peak abundances for the nine spiked halophenols in the headspace of the tea suspension for the HiSorb method, compared with the three SPME methods.

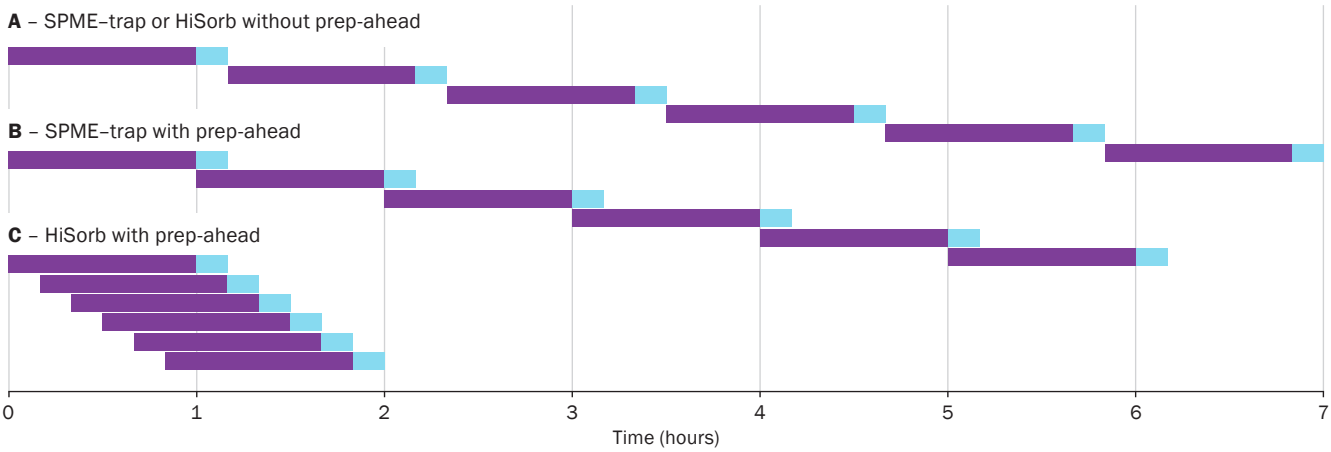


Figure 9: Comparison of overall sequence times for three operational modes on Centri, for a set of six samples with a typical 60-minute incubation time (■) and a 10-minute GC run-time (■).

Figure 9 compares the overall time required to sample and analyse a sequence of six typical samples in three modes of operation. On Centri, the HiSorb probes can be detached from the robotic tool using patented 'grab and release' technology, allowing the robot to perform other functions and so enabling simultaneous extraction from up to six vials. This results in an overall sequence of just 2 hours, which is substantially less than the 6 hours needed for SPME–trap, for which the fiber remains fixed to the tool during sample extraction. The result is therefore increased productivity of the GC–MS system.

Conclusions

In this study we have highlighted a range of options for improving on direct SPME methods for the extraction and enrichment of VOCs and SVOCs from complex matrices:

- **SPME–trap**, by adding a focusing trap to the sample flow path, enables compounds to be preconcentrated and subsequently released in a narrow band. This provides greater peak symmetry and improved qualitative analysis than is possible with direct SPME.
- **SPME–trap with multi-step enrichment** further extends this capability by allowing multiple extracts to be taken from the same vial (or, if desired, separate vials) and focused onto the same trap. The result is a significant increase in peak response and correspondingly lower detection limits, which is especially valuable for trace-level compounds such as halophenols and low-OTV aroma compounds.
- **HiSorb high-capacity sorptive extraction** offers comparable sensitivity to SPME–trap, but improved recovery for the higher-boiling compounds, leading to an extended analyte range. HiSorb probes are also more robust than SPME fibers, so they can be used for direct immersion in liquids, while the unique capability for 'overlapping' the extraction and analysis of multiple samples increases sample throughput.

All of the above methods can be automated on Centri, allowing the analyst to make the best choice of technique for the sample in question.

In addition, Centri offers sample re-collection, which allows split flows to be sent to a sorbent-packed tube. This makes it possible to re-run a single sample multiple times, eliminating the need for repeat sampling. The repeat analyses can either use the same parameters (for method validation) or different parameters (during method development). Different detectors can also be configured to gain further information about target compounds, without having to take multiple aliquots of irreplaceable or expensive samples.

Reference

1. The Good Scents Company Information System (search facility), www.thegoodscentscompany.com.

Appendix

See next page.

Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.

No.	Name	t_R (min)	CAS no.	Area (counts)	Forward match factor	Reverse match factor
1	2,2,4-Trimethylpentane	4.817	540-84-1	7.06×10^6	922	958
2	Acetone	5.795	67-64-1	1.72×10^5	909	909
3	Oct-1-ene	6.054	111-66-0	1.14×10^6	901	901
4	3-Methylbutanal	7.135	590-86-3	3.77×10^6	909	909
5	Dichloromethane	7.294	75-09-2	8.58×10^4	905	906
6	Non-1-ene	7.530	124-11-8	1.52×10^6	908	908
7	Benzene	7.579	71-43-2	2.02×10^6	907	949
8	2-Ethylfuran	7.753	3208-16-0	2.78×10^6	928	935
9	2,2,4,6,6-Pentamethylheptane	7.867	13475-82-6	3.51×10^6	907	910
10	Trichloromethane	9.154	67-66-3	1.81×10^5	922	922
11	Toluene	9.735	108-88-3	1.69×10^6	935	944
12	Hexanal	10.822	66-25-1	8.27×10^6	917	923
13	β-Pinene	11.688	18172-67-3	3.28×10^6	910	923
14	Ethylbenzene	12.236	100-41-4	4.63×10^5	938	972
15	1,3-Dimethylbenzene	12.693	108-38-3	8.96×10^5	942	943
16	β-Myrcene	13.339	123-35-3	1.03×10^6	945	954
17	Limonene	14.692	5989-27-5	3.99×10^7	919	919
18	Eucalyptol	15.157	470-82-6	8.56×10^6	915	915
19	*trans*-Hex-2-enal	15.354	6728-26-3	2.75×10^6	911	911
20	2-Pentylfuran	15.775	3777-69-3	5.51×10^6	928	928
21	γ-Terpinene	16.406	99-85-4	2.56×10^6	932	932
22	Styrene	16.795	100-42-5	5.62×10^5	939	939
23	*m*-Cymene	17.341	535-77-3	8.38×10^6	917	917
24	Trimethylbenzene isomer	17.785	95-63-6	3.89×10^5	925	925
25	Octanal	18.006	124-13-0	1.26×10^6	935	941
26	n-Tridecane	18.343	629-50-5	6.17×10^5	916	916
27	6-Methylhept-5-en-2-one	19.915	110-93-0	9.01×10^5	918	922
28	Trimethylbenzene isomer	19.978	95-63-6	4.82×10^5	914	918
29	Phenylacetylene	20.862	536-74-3	2.21×10^5	907	929
30	Nonanal	22.181	124-19-6	4.56×10^6	909	910
31	n-Tetradecane	22.345	629-59-4	5.24×10^5	915	915
32	*trans*,*trans*-2,4-Hepta-2,4-dienal	25.032	4313-03-5	1.40×10^6	912	914
33	2-Ethylhexan-1-ol	26.033	104-76-7	1.15×10^6	901	907
34	Decanal	26.420	112-31-2	3.25×10^6	912	918
35	Benzaldehyde	27.448	100-52-7	3.62×10^6	925	944
36	Benzeneacetaldehyde	32.030	122-78-1	4.22×10^6	937	937
37	Acetophenone	32.445	98-86-2	7.48×10^5	935	935
38	1-Methylpyrrolidin-2-one	33.700	872-50-4	4.53×10^5	903	903
39	Naphthalene	35.862	91-20-3	2.71×10^5	933	933
40	Methyl salicylate	37.132	119-36-8	8.86×10^6	944	944
41	2,2,4-Trimethylpentane-1,3-diol diisobutanoate	40.703	6846-50-0	1.02×10^6	911	921
42	Biphenyl	44.536	92-52-4	2.33×10^5	923	923
43	Phenol	44.906	108-95-2	5.30×10^6	940	945
44	Octanoic acid	46.837	124-07-2	6.20×10^5	909	912
45	2-Phenoxyethanol	49.410	122-99-6	3.63×10^5	923	953
46	Tetradecan-1-ol	50.259	112-72-1	5.15×10^5	901	905
47	2,4-Di-*tert*-butylphenol	54.318	96-76-4	1.51×10^6	932	933
48	Dibenzo-*p*-dioxin	54.549	262-12-4	2.16×10^6	954	954
49	Hexadecan-1-ol	56.319	36653-82-4	1.36×10^6	946	946
50	Methyl *cis*-octadec-9-enoate	58.250	112-62-9	4.96×10^6	936	938
51	Phenyl benzoate	59.690	93-99-2	2.38×10^5	900	914
52	Di-n-butyl decanedioate	65.276	109-43-3	4.29×10^6	910	913
53	Dibutyl phthalate	65.744	84-74-2	7.72×10^5	957	972
54	Fluoren-9-one	67.922	486-25-9	3.34×10^5	913	913
55	Diphenyl ethanedione	71.169	134-81-6	3.11×10^5	903	903
56	Hexadecanoic acid	75.339	57-10-3	6.07×10^7	926	931

Table A1: Compounds identified (with a NIST match factor ≥900) in the headspace of the tea suspension using HiSorb high-capacity sorptive extraction.