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Centri
Application

Food and beverage
analysis by HS–trap

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Application Note 264

Enhancing the sensitivity of headspace analysis using large volume preconcentration (LVP) – Trace-level GC–MS analysis of VOCs in foods and beverages

In this application note, we show an increase in sensitivity for headspace analysis using a sample preparation technique called large volume preconcentration (LVP). This is particularly useful for the analysis of trace-level volatile organic aroma and flavour compounds in food and beverage samples. As an example, an orange juice was analysed by headspace extraction using the Centri® sample extraction and enrichment platform operating in headspace–trap mode. Inclusion of a multi-bed focusing trap within the system flow path enables a preconcentration step after sample introduction, allowing for the extraction of headspace sample volumes greater than the conventional 1 mL. Additionally, the ability to introduce multiple headspace volumes to the trap is demonstrated as an enrichment process prior to GC–MS analysis. This provides an incremental response for each compound and comparative data are supplied for single and multiple extractions.

Introduction

The ability to detect and identify aroma and flavour VOCs is important for the food and beverage industry for reasons such as product quality, shelf life, off-odours that may lead to customer complaints, competitive product analysis and consumer organoleptic responses. Some of these odour-active components may have very low odour threshold values (OTV) at the ppb or ppt level; therefore, their identification becomes a very important part of the analysis. Sample extraction and identification of the principal components are routinely achieved in food laboratories using established techniques such as static headspace extraction followed by GC–MS analysis. However, for compounds at trace levels, obtaining quality GC–MS data that generate confident library identifications may require an enhancement in the performance of the traditional headspace technique. This is where the headspace–trap LVP technique can be a valuable asset.

In this work, we show the headspace–trap results obtained using 5 mL sample volumes from both single and multiple injections to the focusing trap from a ready-to-drink supermarket orange juice using the Centri multi-mode sample extraction and enrichment platform. This sample volume is well in excess of the 1 mL volume typically used in static headspace analysis, and the focusing trap is an essential component that facilitates the headspace LVP and enrichment process, providing both selective retention and preconcentration of analytes without peak splitting. Multiple sample extractions can occur from the same vial or, as in this

case, separate vials, the latter providing the highest level of enrichment as there has been no historical extraction of sample. Emphasis in this work is placed on VOCs at low or trace levels in the sample, as obtaining an accurate identification is challenging.

We also show ChromSpace® data processing software for automated rapid screening of the chromatograms against a target library. The package uses sophisticated algorithms to process GC–MS data files. Dynamic baseline compensation of the imported data eliminates background interferences before deconvolution of co-eluting compounds into individual analyte peaks is performed. The deconvolved spectra are compared to NIST library spectra, allowing both target and unknown compounds to be confidently identified, even at trace levels.

The Centri focusing trap

At the core of the Centri system is a multi-sorbent-bed focusing trap containing solid adsorbent materials (*e.g.*, Tenax®, Carbograph™, Carbosieve™) that selectively retain compounds within different boiling point ranges. The trap is electrically cooled, which avoids the use of expensive cryogen cooling units, and can contain up to four sorbents of increasing strength, enabling a broad range of VVOC/VOC/SVOC compounds, ranging from $C_2/_3$ to C_{44}, to be enriched. After an extracted sample is transferred to the trap *via* the integrated injection inlet on Centri, the carrier gas flow through the trap is reversed (*i.e.*, the trap is backflushed) and it is rapidly heated (100°C/sec). The compounds are released

from the trap in a sharp band of vapour to the capillary column. Backflushing the trap is a critical operation, enabling analytes to be released efficiently to the analytical column. (Systems that perform forward-flushing of the focusing trap are limited to a single-sorbent trap as the analytes are required to travel through the entire sorbent bed, thus limiting the analyte volatility range that can be analysed in a single run.) The trap also enables the use of unconventionally large sample volumes; for example, 5 mL or multiples of 1 mL or above. These are injected into the Centri inlet using controlled syringe injection rates and the entire volume is efficiently refocused and enriched on the trap prior to GC injection with no subsequent chromatographic degradation.

Centri has four principal extraction and enrichment modes – high-capacity sorptive extraction (HiSorb™), SPME, headspace and thermal desorption. All four modes utilise the focusing trap; however, for headspace and SPME, there is also the choice to bypass the trap and transfer sample directly to the capillary column. Experimental results have shown the benefits of using the trap with both modes – the preconcentration effect improves chromatographic performance in general, particularly for the more volatile compounds, and enables more demanding sampling techniques such as LVP to be implemented.

Experimental

Sample preparation:
8 mL volumes of an orange juice sample were transferred into 20 mL headspace vials and 1.6 g of sodium chloride was added to each vial to increase the ionic strength of the solutions. This is standard procedure to reduce the solubility of the more polar compounds in an aqueous sample to aid partitioning into the headspace. Each vial was then sealed with a crimped cap. Multiple vials were identically prepared and three were used for a 15 mL LVP enrichment analysis, where a single 5 mL headspace volume was sequentially extracted from each of the vials and loaded onto the focusing trap prior to introduction to GC–MS.

Extraction and enrichment conditions:

Instrument:	Centri (Markes International)

Headspace–trap:

Incubation:	60°C for 30 min, with agitation at 300 rpm
Sample volume:	5 mL (single extraction), 3 x 5 mL enrichment (multiple vials)

Preconcentration:

Focusing trap:	'Material Emissions' (part no. U-T12ME-2S)
Trap purge:	50 mL/min (2 minutes)
Trap desorption:	30°C to 300°C (3 min, 100°C/sec)
Outlet split:	18 mL/min
Flow path:	150°C

GC conditions:

Column:	MEGA®-5 HT, 30 m x 0.25 mm x 0.25 µm
Column flow:	Helium constant flow 2.5 mL/min

Background to Centri®

Markes International's Centri system for GC–MS is the first platform to offer high-sensitivity unattended extraction and enrichment of VOCs and SVOCs in solid, liquid and gaseous samples.

Centri allows full automation of sampling using HiSorb™ high-capacity sorptive extraction, SPME, headspace and tube-based thermal desorption. Leading robotics and analyte-trapping technologies are used to improve sample throughput and maximise sensitivity for a range of applications – including profiling of foods, beverages and fragranced products, environmental monitoring, clinical investigations and forensic analysis.

In addition, Centri allows samples from any injection mode to be split and re-collected onto clean sorbent tubes, avoiding the need to repeat lengthy sample extraction procedures and improving security for valuable samples, amongst many other benefits.
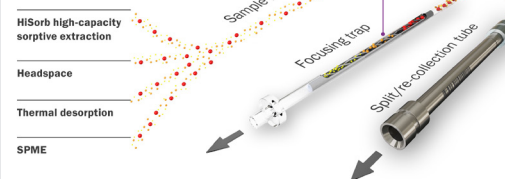
For more on Centri, visit www.markes.com.
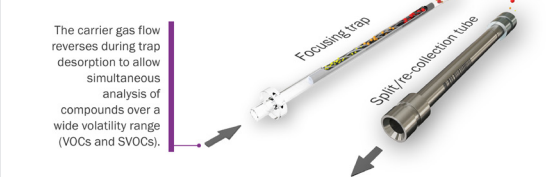

ANALYTE TRAPPING ON CENTRI

1 Sample focusing

Extracted analytes are swept into Centri's electrically-cooled focusing trap.

HiSorb high-capacity sorptive extraction

Headspace

Thermal desorption

SPME

Gas-phase internal standards can be introduced to the Centri trap in the carrier gas stream, as a check on the focusing and desorption processes.

Sample flow

Focusing trap

Split/re-collection tube

2 Trap desorption

The focusing trap is rapidly heated to transfer (inject) the analytes to the GC–MS.

The carrier gas flow reverses during trap desorption to allow simultaneous analysis of compounds over a wide volatility range (VOCs and SVOCs).

GC-MS

Focusing trap

Split/re-collection tube

Inlet: 190°C, septum purge 5 mL/min
Oven program: 35°C (3 min), 10°C/min to 100°C,
30°C/min to 220°C (15 min)

MS conditions:
MS transfer line: 280°C
MS source: 250°C
Full scan acquisition: 35–300 m/z

Data analysis:
Software: ChromSpace (SepSolve Analytical)
MS library: NIST 2018

Results and discussion

Figure 1 compares GC–MS total ion chromatogram (TIC) profiles for the orange juice sample using the LVP approach based on a single 5 mL headspace volume (lower inverted trace) compared to the enrichment (upper trace) volume of 15 mL. The TIC scaling chosen was set to highlight the trace-level compounds in the profile. Arbitrary lines have been drawn to focus attention within this concentration boundary. The principal compounds will not be discussed here as they produce strong signals that already enable confident identification. Comparing the two plots, an incremental response is achieved for all compounds when increasing the extraction volume from 5 mL to 15 mL; one might consider how much lower this response would be for the lower-concentration compounds if a conventional 1 mL sample volume was used. Many of these would be more difficult to confidently identify. The residual water vapour expected from the large extraction volumes of this type of sample (typically seen in the early part of the TIC) causes no adverse chromatographic effects here, indicating good water management by careful selection of focusing trap sorbents and trap purge parameters.

To underline the value of sample enrichment, Figure 2 shows a time window of ~2–8 minutes. The enhanced response for the compounds shown in the upper trace now enables a more confident identification. Table 1 lists the compounds with a NIST match factor > 750, along with their retention times (RT) and sensory odour descriptions. The OTV values (ug/L in water) for some analytes are also shown.
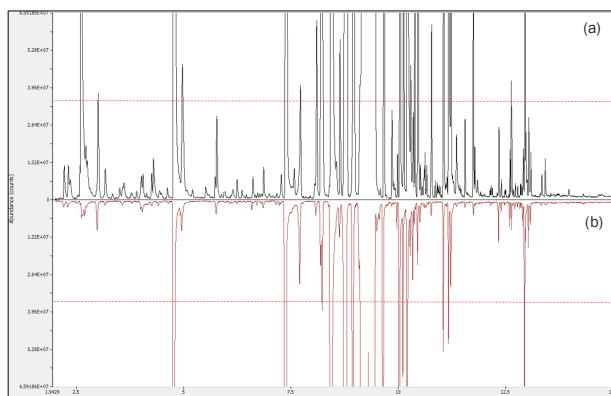

Figure 1: GC–MS TIC profiles of an orange juice sample using headspace–trap extraction and enrichment. The lower inverted trace (b) shows a single 5 mL extraction volume and the upper trace (a) shows a 15 mL total volume from three separate vial extractions.
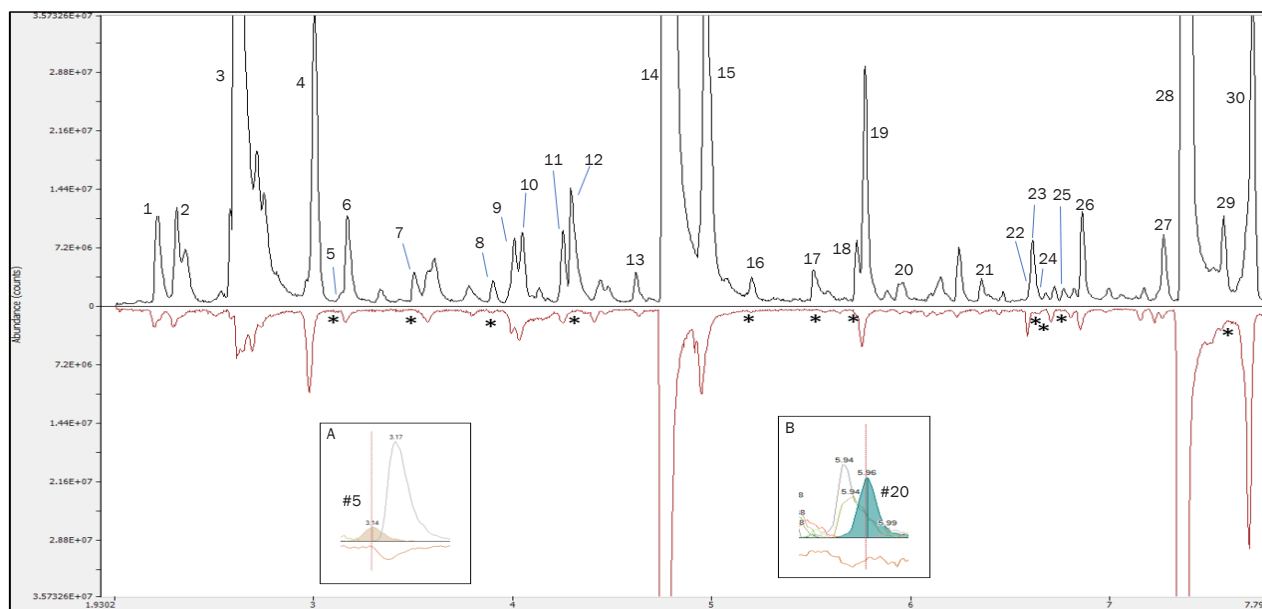

Figure 2: Zoomed time window from ~2–8 minutes. Asterisks indicate compounds only identified from enrichment with no response from single analysis. Inset A shows the deconvolved elution profile of trace-level phenol (brown profile). Inset B shows the deconvolved elution profile of ethyl benzene (green profile) from two other compounds.

Several compounds from the 5 mL sample show a response, albeit weak, *e.g.* 2- and 3-methyl butanal (#1 and #2, respectively); however, several show no discernable peak (labelled with an asterisk) and identification is only possible from the enrichment profile. These compounds are also referenced in Table 1 with an asterisk.

For some of these compounds, there is added complexity as there are co-elutions with other VOCs taking place. The improvement in signal response in the enriched LVP TIC allowed for spectral deconvolution and a provisional identification. The two inset windows in Figure 2 show examples of this – the deconvolved elution profiles for trace-level phenol (#5) co-eluting with methyl butanoate (#6) and for two compounds closely eluting with ethyl benzene (#20). Only a small response for methyl butanoate is seen in the single extraction profile and there is no response for phenol. However, the deconvolved enriched spectrum for phenol shows good match quality statistics when compared to the NIST spectral library (Figure 3). Similar results were obtained for ethyl benzene.

The OTV values shown in Table 1 give an indication of the diverse range of odour activities for different compounds. If the OTV is very low, for example with the esters ethyl 2-methylpropanoate (#8) and ethyl 2-methylbutanoate (#19) (where initial values lie in the low ppt range), trace-level amounts can contribute significantly to the aroma of the food or beverage product. Detection and identification of these compounds therefore becomes extremely important, which is where the LVP technique can provide a powerful enhancement to conventional static headspace analysis.
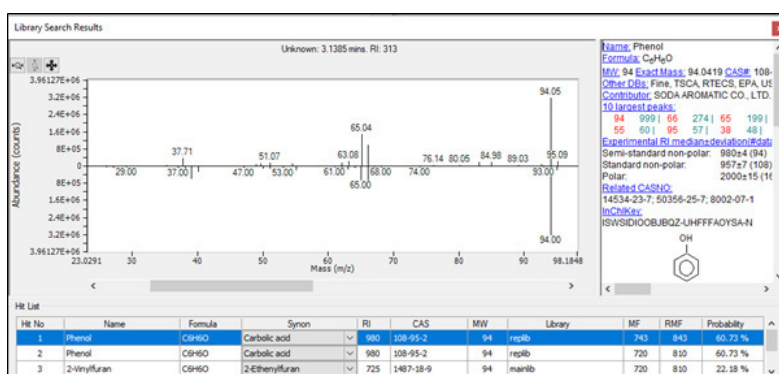

Figure 3: Deconvolved spectrum (upper trace) and library search statistics for trace-level phenol in the enriched sample.

No.	Compound	RT	Odour sensory description[1]	OTV (ug/L in water)[2]
1	3-Methyl butanal	2.23	Ethereal, aldehydic, chocolate, peach, fatty	
2	2-Methyl butanal	2.31	Musty, cocoa, coffee, nutty	
3	Siloxane	2.61		
4	4- Bromodichloromethane	3.00		
5*	Phenol	3.14	Medicinal	5900
6	Methyl butanoate	3.17	Fruity, apple, sweet, banana, pineapple	5–60
7*	3-Methyl butanol	3.50	Fusel, oil, alcoholic, whiskey, fruity, banana	
8*	Ethyl 2-methylpropanoate	3.90	Sweet, ethereal, fruity, alcoholic, fusel, rummy	0.02–0.1
9	Toluene	4.00		
10	1-Methyl cyclohexene	4.05	Lime, citrus	
11	Methyl 2-methylbutanoate	4.25	Ethereal, estery, fruity, tutti frutti, green	
12*	3-Methyl-2-buten-1-ol	4.29	Fruity, green, lavender	
13	6-Caprolactone	4.62		
14	Ethyl butanoate	4.77	Fruity, juicy, fruit, pineapple, cognac	0.13–13.6
15	Siloxane	4.97		
16*	3-Methyl-2-butenyl hexanoate	5.20	Fruity, green, cheesy	
17*	Furfural	5.51	Sweet, woody, almond, fragrant, baked bread	3000–23000
18*	Ethyl methacrylate	5.73	Acrylate	
19	Ethyl 2-methyl butanoate	5.77	Sharp, sweet, green, apple, fruity	0.006–0.3
20	Ethyl benzene	5.96		
21	Isoamyl acetate	6.35	Sweet, fruity, banana, solvent	
22	*p*-Xylene	6.61		
23*	2-Heptanone	6.61	Fruity, spicy, sweet, herbal, coconut, woody	140–3000

Table 1: Identification of VOCs using LVP enrichment from 3 x 5 mL headspace extractions (15 mL). *Indicates compounds with no discernible TIC response from a single sample volume of 5 mL. (*Continued on next page*)

No.	Compound	RT	Odour sensory description[1]	OTV (ug/L in water)[2]
24*	Bromoform	6.68		
25*	Propyl butanoate	6.77	Fruity, sweet, apricot, pineapple, rancid, sweaty	18–124
26	Heptanal	6.86	Fresh, aldehydic, fatty, green, herbal	0.8–10
27	Methyl hexanoate	7.27	Fruity, pineapple, ether	70–84
28	α-Pinene	7.38	Fresh, camphor, sweet, pine, earthy, woody	6–62
29*	Ethyl isoamyl ketone	7.57		
30	Camphene	7.71	Woody, herbal, fir needle	

Table 1: (*Continued from previous page*)

Conclusions

A novel headspace–trap technique has been demonstrated using large volume extraction, preconcentration and enrichment of a beverage sample, facilitated by the focusing trap of the Centri platform prior to GC–MS analysis. The trap enables headspace sample volumes of 5 mL or multiples thereof to be routinely analysed without any detrimental chromatographic effects. Selection of hydrophobic trap sorbents and trap purge parameters enables good water management, with no residual water effects observed in the chromatographic profile. Incremental sensitivity is shown using the higher volume of 15 mL from three extractions and several peaks not seen in the single 5 mL analysis can now be tentatively identified. The signal strength is therefore enhanced and is sufficiently high for spectral deconvolution of the data and trace co-eluting peaks to be identified. The enrichment technique is applicable to a broad range of foods and beverages, enabling an enhancement in VOC characterisation of consumer products, allowing the user to discover more from their samples.

References

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

2. Odour & flavour detection thresholds in water (ppb) – Leffingwell & Associates.