

Application Note 270



Trace-level analysis of VOCs in a tomato product using headspace extraction with large volume preconcentration (LVP) and multi-step enrichment (MSE)

In this application note, we demonstrate the benefit of a novel technique – large volume preconcentration (LVP) – for enhancing traditional headspace analysis of trace-level volatile organic compounds (VOCs) that contribute to aroma and flavour in a food product – tomato paste. Using the Centri® sample extraction and enrichment platform in headspace-trap mode, the extracted analytes are preconcentrated on an electrically-cooled, cryogen-free focusing trap. This allows for large extraction volumes to be used (up to 5 mL) while maintaining optimum chromatographic performance. We also highlight the benefits of multi-step enrichment (MSE), whereby repeat extractions from a single vial or multiple vials are delivered to the trap prior to a single GC injection. This results in a further improvement in the discovery and confident identification of trace-level aroma and flavour compounds.

Aroma and flavour compounds have important roles in the consumer sensory experience, so their analysis is vital in the food and beverage industry for product development, determination of shelf-life and product quality, investigation of off-odours and analysis of competitor products. Whilst headspace sampling, in conjunction with GC-MS, is routinely used for the analysis of principal aroma compounds, the detection of trace-level active odour components (with low odour threshold values (OTVs)) is more challenging. Typically, headspace extraction volumes are limited to 1 mL due to the poor chromatographic performance, such as peak broadening, that occurs when using larger volumes, limiting the response achieved from lower-level analytes. Therefore, an enhancement in the sensitivity of the technique is required, making large volume preconcentration (LVP) and multi-step enrichment ideal for this scenario.

The Centri automated multi-mode platform allows the headspace injection and GC column flow to be separated (Figure 1). The extracted headspace volume is injected to an electrically-cooled, cryogen-free, multi-sorbent-bed focusing trap where the analytes are refocused and preconcentrated. Following this step, the carrier flow is reversed ('backflushed') and the trap is heated rapidly (up to 100°C/s heating rate), transferring the analytes to the capillary column in a concentrated band of vapour (~100 µL).

This has several clear benefits for the low-level analysis of odorous compounds:

- **Large volume preconcentration (LVP):** Volumes up to 5 mL in a single extraction (automated using standard syringes) are preconcentrated on the trap prior to GC

injection, meaning a greater quantity of each analyte is introduced to the analytical system for detection.

- **Multi-step enrichment (MSE):** Repeat extractions can be taken from the same sample vial (single-vial enrichment) or from different sample vials (multi-vial enrichment).
- **Water management:** Most foods (e.g., tomato paste) have some level of water content, leading to the possibility that water residue is extracted and injected to the analytical system. The presence of residual water could lead to poor chromatographic performance and particularly affect the earlier part of the chromatogram. Centri overcomes this issue with the use of the focusing trap, by selection of hydrophobic sorbents and the ability to purge residual water to vent (under ambient conditions) prior to injection to the capillary column.

In this work, we demonstrate these features for the discovery and identification of compounds present at low or trace levels in tomato paste.

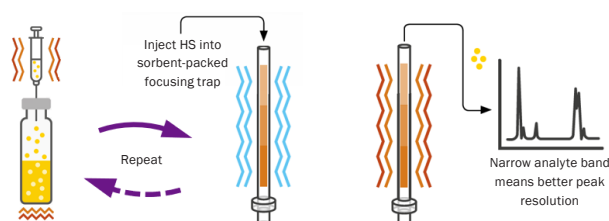


Figure 1: Headspace-trap with multi-step enrichment workflow on the Centri sample extraction and enrichment platform.

Experimental

Sample preparation:

Tomato paste was acquired from a local supermarket, and mixed with sodium chloride and deionised water in a ratio of 15g:6g:17 mL, respectively. The addition of salts such as sodium chloride is a well-established protocol for increasing the ionic strength of an aqueous sample matrix, thereby increasing the concentrations of VOCs with higher K (partition) values in the sample headspace. Volumes of 8 mL of the mixture were dispensed into standard 20 mL headspace vials and sealed with crimped caps. For multi-step enrichment, three vials were identically prepared. A single 5 mL headspace volume was sequentially extracted from each vial and loaded onto the same focusing trap prior to GC injection (Figure 2).

Extraction and enrichment:

Instrument: Centri (Markes International)

Headspace-trap conditions:

Incubation: 60°C for 30 min, with agitation at 300 rpm
 Extraction volume: 5 mL
 Enrichment: 3 times using different vials, introducing a total volume of 15 mL for analysis

Preconcentration:

Flow path: 150°C
 Focusing trap: Material emissions (part no. U-T12ME-2S)
 Trap purge: 50mL/min (2 minutes)
 Trap low: 30°C
 Trap high: 300°C (3 min)
 Outlet split: 18 mL/min

GC-MS:

Column: MEGA® 5 HT, 30 m x 0.25 mm x 0.25 µm
 Constant flow: Helium, 2.5 mL/min
 Oven program: 35°C (3 min), 10°C/min to 100°C, 30°C/min to 220°C (15 min)
 Transfer line: 280°C
 MS source: 250°C
 Mass range: m/z 35–300

Data analysis:

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

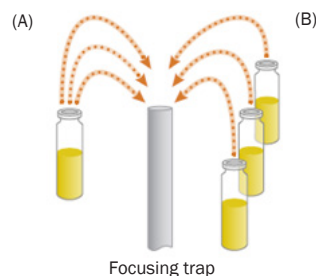


Figure 2: An illustration of the multi-step enrichment (MSE) process, which can be performed from a single vial (A) or from replicate samples in multiple vials (B). Sample extractions are loaded onto the same focusing trap for a single analysis.

Background to Centri®

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

Centri allows a range of fully automated sample preparation modes, all with selective enrichment. Options include HiSorb™ high-capacity sorptive extraction, headspace (-trap), SPME (-trap) 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 the split portion from any injection mode to be re-collected onto clean sorbent tubes, allowing quick repeat analysis and stable archiving of valuable samples, amongst many other benefits.

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



Results and discussion

The total ion chromatogram (TIC) results of the tomato paste analysed by headspace-trap (HS-trap), taking a 5 mL extraction from one vial, and HS-trap with multi-step enrichment (MSE), taking three 5 mL extractions each from different vials (15 mL total), are shown in Figure 3. The scaling is set to highlight the lower-level compound peaks in the profiles. Principal components producing strong signals will not be discussed here as they can be confidently identified in the sample by classical, static headspace analysis.

All components show an increase in response in the HS-trap with MSE analysis (blue trace) compared with the single HS-trap sample (red trace). This leads to an increase in the signal-to-background-noise ratio, improving the spectral quality as more characteristic ions can now be distinguished; thus allowing for a more confident identification of the unknown sample compounds when performing library searches. The effectiveness of the water management step is also demonstrated here by the excellent chromatography.

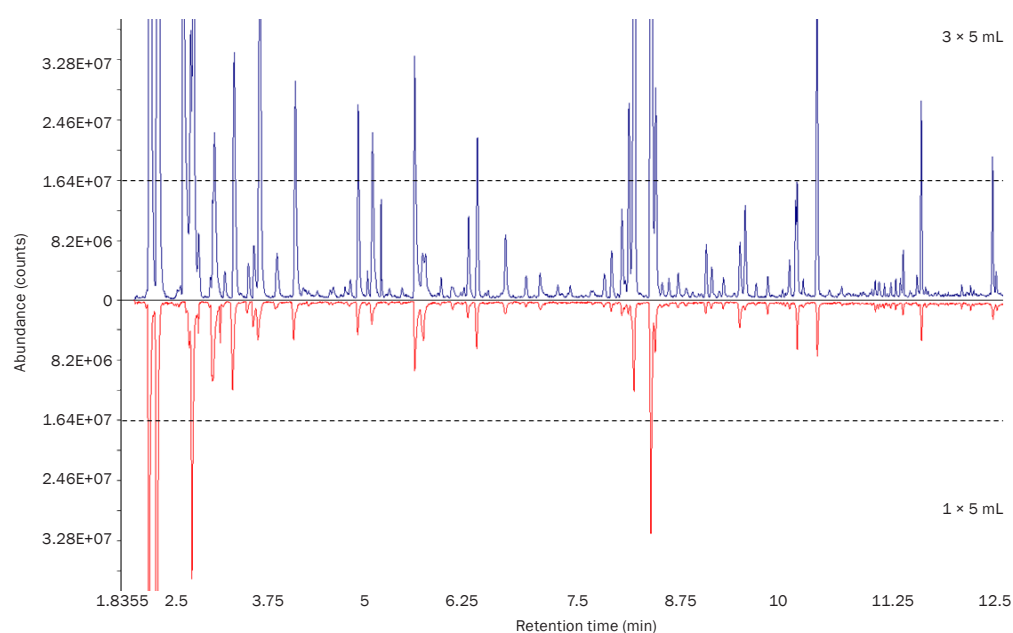


Figure 3: The TIC profiles of tomato paste extracted and analysed by HS-trap (1 x 5 mL) and HS-trap with MSE (3 x 5 mL). Dotted lines mark an arbitrary abundance, highlighting the improved peak height when using the HS-trap with multi-step enrichment technique.

Figure 4 shows a time window of the same chromatograms (from 6 to 11.6 min) wherein many trace-level components elute. The spectra produced by these peaks were compared to NIST library data. Taking a NIST library match factor (MF) value >750 to indicate a confident identification, in the 3 x 5 mL (total 15 mL) enrichment analysis, 23 compounds were identified that passed this criterion, and these are listed in Table 1. Three of these compounds (denoted by an asterisk in Figure 4 and Table 1) were only identifiable with enrichment.

The low-level compounds identified have a profound effect on the flavour and aroma profile of the tomato paste so routine monitoring is essential to maintain product quality and uphold the customer experience. Compounds with low odour threshold values (OTVs), such as linalool (#19) and nonanol (#20), can contribute significantly to the aroma and flavour of a food product when present at low levels (Table 1). Therefore, the detection and identification of these compounds is extremely important for food producers.

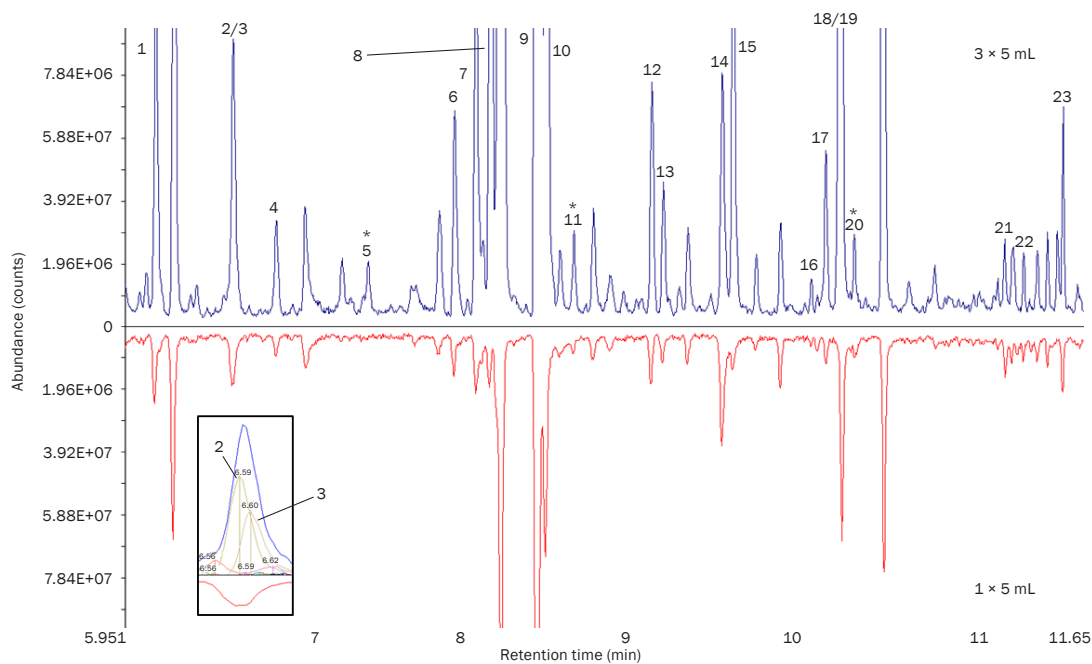


Figure 4: Zoomed portion of the chromatogram shown in Figure 3, focusing on a region with many low-concentration compounds. Identifications for numbered peaks are given in Table 1. The asterisks indicate compounds that are detectable only when extracted and analysed by HS-trap with MSE (3 x 5 mL). The inset shows the deconvoluted profiles of styrene (retention time 6.59) and 2-heptanone (6.60).

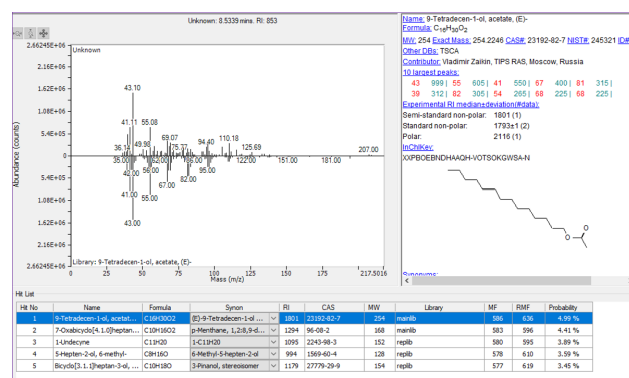
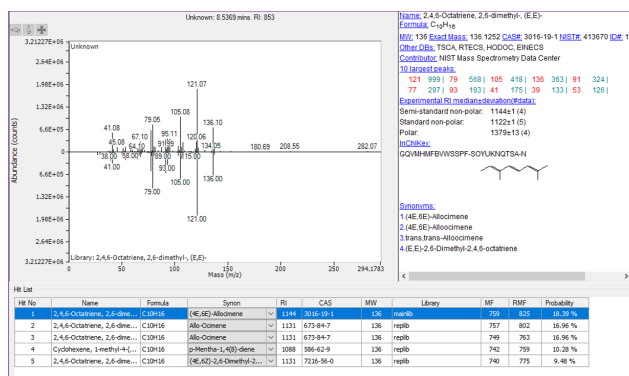
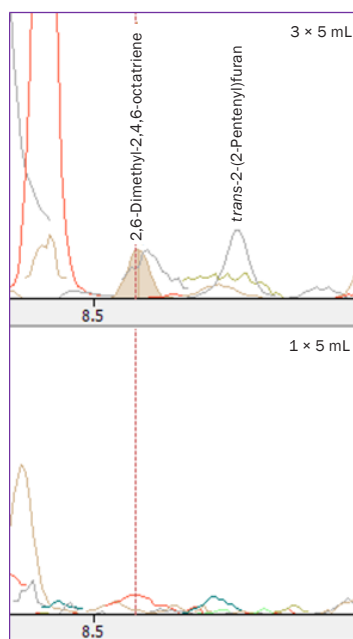
Peak no.	Compound	RT	Odour sensory description ¹	OTV (µg/L in water) ²
1	<i>p</i> -Xylene	6.13	Plastic	
2	Styrene	6.59	Sweet, balsam, floral, plastic	730
3	2-Heptanone	6.60	Fruity, spicy, sweet, herbal, coconut	140–3000
4	Heptanal	6.84	Fresh, aldehydic, fatty, green, herbal	3
5*	3,5-Xylenol	7.39	Flat, dry	333 ppb (Hoek 1957)
6	2,7-Dimethyloxepine	7.90		
7	Benzaldehyde	8.03	Strong, sharp, sweet, bitter, almond	350–3500
8	Dimethyl trisulfide	8.11	Sulfurous, cooked onion, savoury	0.005–0.01
9	6-Methyl-5-heptene-2-one	8.39	Citrus, green, musty, lemongrass, apple	50
10	2-n-Pentylfuran	8.45	Fruity, green, earthy, beany, vegetable	6
11*	<i>trans</i> -2-(2-Pentenyl)furan	8.57		
12	<i>o</i> -Cymene	9.07		
13	D-Sylvestrene	9.14		
14	Phenylacetaldehyde	9.49	Green, sweet, floral, hyacinth, clover	4
15	<i>cis</i> -2,6-Dimethyl-2,6-octadiene	9.55		
16	Terpinolene	10.01	Fresh, woody, sweet, pine, citrus	200
17	<i>p</i> -(1-Propenyl)-toluene	10.10	Phenolic, spicy, styrene, clove, guaiacol	
18	Perillene	10.18	Woody	
19	Linalool	10.19	Citrus, floral, sweet, bois de rose	6
20*	Nonanol	10.25	Waxy, aldehydic, rose, fresh, orris	1
21	α-Terpineol	11.16	Lilac, floral	330–350
22	α-Ionene	11.27		
23	<i>m</i> -Di- <i>tert</i> -butylbenzene	11.51		

Table 1: Identification of VOCs in tomato paste using HS-trap with multi-step enrichment. Compounds marked with * were not detectable with a single 5 mL headspace injection.

Food and beverage samples can be complex and co-elutions are common; in some cases, co-eluting analytes can be difficult to separate and identify. In these situations, the higher responses achieved with the multi-step enrichment technique are particularly beneficial. When using spectral deconvolution, as shown for styrene and 2-heptanone (Figure 4), for example, good signal strength is required to distinguish the co-eluting peaks. Within the data analysis software, a spectral deconvolution algorithm enabled resolution of these co-eluting compounds, leading to accurate identification and compound confirmation.

Two compounds – 2,6-dimethyl-2,4,6-octatriene (Figure 5A) and *trans*-2(2-pentenyl)furan (Figure 5B) – were not initially identified using the single HS-trap extraction technique. For these compounds, an enrichment step – three extractions from multiple vials – was necessary to increase the response so that the spectral deconvolution was able to confidently identify the compounds (MF >750, NIST). Compound A gives a sweet, peppery odour and spicy, nutty flavour¹ while compound B contributes to a 'painty' off-flavour (taint) in the product.³ For an assessment of food quality, the discovery of these components in the tomato paste using enrichment is vital as they would have been missed had smaller headspace volumes been used alone.

(A)



(B)

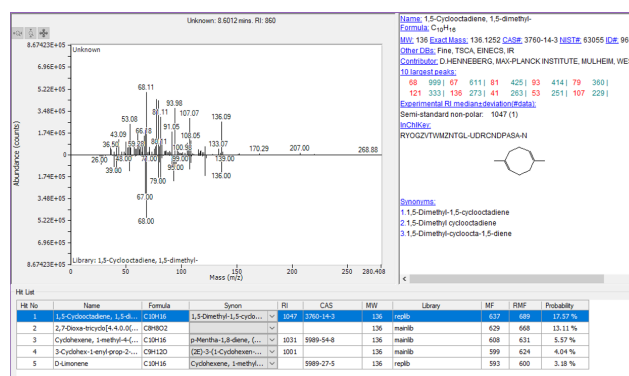
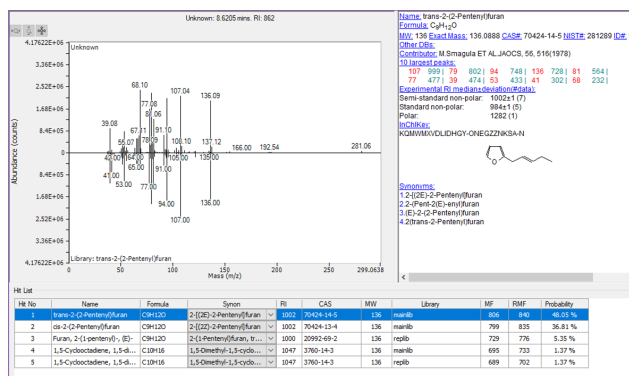
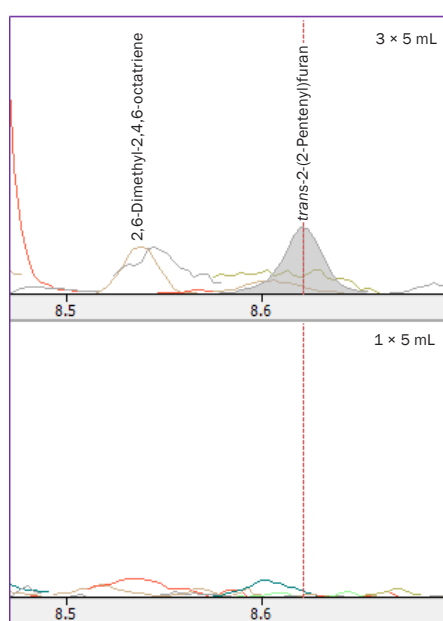


Figure 5: Deconvolved spectra for two compounds in tomato paste – 2,6-dimethyl-2,4,6-octatriene (A) and *trans*-2-(2-penteny)furan (B) – along with the NIST library search results. In both A and B, the top chromatograms (3 x 5 mL) and corresponding library search results show a much improved compound match factor compared to the respective lower chromatograms (1 x 5 mL) and library search results. For each chromatogram, a report of a NIST library search on the highlighted peak (solid colour) is shown to the right. Within these reports, the mass spectrum associated with the peak (top spectrum) is compared with the expected spectrum of the closest match (lower spectrum). A list of close matches is shown beneath the spectra, sorted by NIST match factor (MF) values.

Conclusions

This work demonstrates the analyses of tomato paste, highlighting large volume preconcentration (LVP), a novel technique in headspace-trap analysis available on the Centri platform. Where conventional headspace is typically limited to using an extraction volume of up to 1 mL, Centri enables larger volumes of up to 5 mL, significantly improving the chromatography and sensitivity achieved, leading to the discovery of more aroma and flavour compounds in a sample.

Increasing the extraction volume further, from a single 5 mL injection to a total volume of 15 mL by HS-trap with multi-step enrichment, resulted in an increase in response for all compounds. This allowed confident identification of trace-level aroma- and flavour-producing components, such as 2-6-dimethyl-2,4,6-octatriene (A) and *trans*-2-(2-pentenyl) furan (B), which were not previously detected. Finding these compounds at low levels, particularly the taint-causing compound B, could provide an early indication of food spoilage.

Despite the aqueous nature of the sample, no detrimental effects associated with water interference were observed due to the excellent water management functionality the system has to offer; hydrophobic sorbents were used in the focusing trap, and the trap was purged prior to GC injection, eliminating water while sufficiently retaining the sampled compounds for analysis.

Using the Centri platform, the entire analytical process, from sample incubation and enrichment to trap purging and desorption, is fully automated and requires no user intervention, increasing productivity and sample throughput when used for routine analyses.

To conclude, the large volume preconcentration and multi-step enrichment offered by Centri are powerful techniques for improving the sensitivity of traditional headspace analysis and are broadly applicable to the discovery of aroma and flavour compounds in a wide range of food and beverage products.

References

1. The Good Scents Company Information System (search facility), www.thegoodscentscompany.com.
2. Odor & flavour detection thresholds in water (ppb), Leffingwell & Associates.
3. M.J. Saxby, *Food taints and off-flavors*, Springer Science+Business Media, Dordrecht, 2nd edn, 2006, p. 14, accessed using Google Books 2 April 2020 [[https://books.google.co.uk/books?id=e7P4BwAAQBAJ&pg=PA14&lpg=PA14&dq=trans-2-\(2-Pentenyl\)furan+flavor&source=bl&ots=rb-kqyDvmj&sig=ACfU3U2nMNMVT3_nWiMYbcavT05WBmyMVQ&hl=en&sa=X&ved=2ahUKewjOodTevcnoAhXyQxUIHfJqDVOQ6AEwBnoECAsQLg#v=onepage&q=trans-2-\(2-Pentenyl\)furan%20flavor&f=false](https://books.google.co.uk/books?id=e7P4BwAAQBAJ&pg=PA14&lpg=PA14&dq=trans-2-(2-Pentenyl)furan+flavor&source=bl&ots=rb-kqyDvmj&sig=ACfU3U2nMNMVT3_nWiMYbcavT05WBmyMVQ&hl=en&sa=X&ved=2ahUKewjOodTevcnoAhXyQxUIHfJqDVOQ6AEwBnoECAsQLg#v=onepage&q=trans-2-(2-Pentenyl)furan%20flavor&f=false)]

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