

Centri Food analysis by HiSorb Released: September 2020





Application Note 272

Improved aroma profiling of breakfast cereal by automated high-capacity sorptive extraction (HiSorb™)

This study demonstrates the use of high-capacity sorptive extraction, HiSorb[™], with multi-step enrichment (MSE) on the Centri[®] sample extraction and enrichment platform for enhancing the analysis of volatile organic compounds (VOCs) contributing to the overall aroma and flavour of a breakfast cereal product. MSE results showed a more comprehensive sample profile when compared to a single HiSorb extraction and provided improved confidence in the identification of trace-level compounds of interest, such as food additives, contaminants and compounds with potential toxicity.

Introduction

Within the food industry, there is an increasing need to monitor product safety and quality to understand flavour composition, taint and product contamination, and how these relate to both consumer satisfaction and effects on human health. This analysis requires a detailed understanding of individual components, but conventional approaches to sample preparation and GC(–MS) analysis struggle to provide the necessary level of sensitivity.

Traditionally, solid-phase micro-extraction (SPME) has been used to sample VOCs from foods and beverages. SPME offers simple, solventless sampling and is easily automated for high-throughput applications. However, SPME is limited by the amount of sorptive phase (~0.5 μ L) and the fragility of the fiber. More recently, thicker sorptive films and stir bar technology have been used to enhance sensitivity; however, the latter approach is limited in scope due to the manual interactions required by the user.

High-capacity sorptive extraction (HiSorb) overcomes these issues. Firstly, the use of strong, metal-core probes supporting a higher amount of sorptive phase (~65 µL) means they are less prone to damage, providing a more robust extraction technique with improved sensitivity. Combined with Centri, full automation of the workflow is achieved, from sample extraction and post-sampling probe washing/drying through to desorption and GC injection. It is also possible to preconcentrate multiple HiSorb extractions onto a single



Figure 1: HiSorb with multi-step enrichment (MSE) workflow.



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Background to Centri and HiSorb

The **Centri** system from Markes International is the first GC–MS sample extraction and enrichment platform to offer high-sensitivity unattended sampling and

preconcentration of VOCs and SVOCs in solid, liquid and gaseous samples.

Centri allows full automation of sampling using HiSorb highcapacity 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.



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. The **HiSorb** capability deployed in this study involves use of robust metal probes fitted with a section of highcapacity sorbent polymer, to extract and enrich compounds from liquids and solids. Samples contained in standard 20 mL or 10 mL vials are loaded onto Centri, and the HiSorb probe inserted into the vial for either immersive or headspace extraction. The probe is then automatically washed, dried and desorbed, with the analyte vapours concentrated on the Centri focusing trap prior to GC-MS injection.



For more on Centri and HiSorb, visit <u>www.markes.com</u>.

ANALYTE TRAPPING ON CENTRI (optional for headspace and SPME)



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In this study, the volatile organic chemical profile of breakfast cereal was analysed using the Centri sample extraction and enrichment platform. This enabled a broad range of volatile organic compounds (VOCs), contributing to the flavour and aroma of the product, to be identified. Additionally, food contaminants, potentially from the product's packaging and the original cereal crop, were detected, some of which extended into the semi-volatile category of chemicals.

Experimental

Sample preparation: 1 g of a store-bought breakfast cereal was homogenised prior to addition of 2.5g NaCl and 10 mL distilled water.

Sampling: Headspace extraction of VOCs was performed using two modes (a) HiSorb, a single-vial extraction for analysis and (b) HiSorb with MSE, loading four extractions from different sample vials to the trap prior to analysis.

Extraction and enrichment:

Instrument: Centri (Markes International).

<u>Headspace HiSorb</u> Probe:

Incubation: Probe desorption: Flow path: Focusing trap: Purge flow: Trap low: Trap high: Heating rate: Split flow:

75°C (20 min) 250°C (10 min) 180°C Materials Emissions (U-T12ME-2S) 50 mL/min (1min) 20°C 280°C (5 min) MAX (>100 °C/s) 5 mL/min

Short-length (48 mm) stainless-steel

no. H1-XXABC)

HiSorb probe (Markes International part

GC method:

Column type: Column flow: Oven program: TG-624, 30 m x 0.25 x 1.4 μm Helium, 1.5 mL/min (constant flow) 50°C (5 min), 15°C/min to 240°C (10 min)

MS:

Instrument: Mass range: Acquisition rate: BenchTOF-Select™ mass spectrometer m/z 33–300 8 Hz

Integrated instrument control and data analysis:

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



Retention time (min)

Figure 2: Headspace HiSorb TIC profile comparison of the breakfast cereal – single extraction (lower inverted red trace) and MSE (4x extractions, upper black trace). Inset shows deconvolved elution profiles typically used for identification, in this instance showing the contaminants dibutyl phthalate (DBP) and tris(2-chloroisopropyl)phosphate (Amgard TMCP).

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Results and discussion

VOC/SVOC profile analysis

Cereal samples were extracted using HiSorb probes, initially performing a single vial analysis, followed by multi-step enrichment (MSE), where multiple (x4) headspace extractions were taken from separate vials for one GC analysis. Figure 2 shows the total ion chromatogram (TIC) for both the enriched (upper black trace) and single (lower inverted red trace) extractions. It is clear that an enhancement in sensitivity is achieved across the entire sample profile when using enrichment compared to single extraction, with no compromise in peak symmetry or resolution. MSE provided a more comprehensive sample profile and as such, the results using this technique are discussed in the following sections.

In total, 150 VOC/SVOC compounds were detected by MSE. The identification of compounds that had a peak area >1% of the TIC and with a NIST match factor >750 are shown in Table

1, with odour descriptors (where available) and odour threshold values (OTV).

In a sample this complex with significant chromatographic co-elution, spectral deconvolution is critical for a more confident identification of VOCs present. This was achieved using ChromSpace data analysis software with resulting spectra compared to compounds in the NIST 2017/EPA/NIH mass spectral library. Deconvolution is a data processing technique that can be applied to complex elution profiles, and Figure 2 shows an example (inset) of this. In this instance, the deconvolved peaks were identified as the SVOCs dibutyl phthalate (DBP), a plasticiser, and tris(2-chloroisopropyl) phosphate (Amgard TMCP), a flame retardant.

Also detected were compounds of interest from a food toxicity/contamination perspective, identified (text labelling in Figure 2) and discussed in more detail later in the application note.

Peak no.	RT	Compounds	% TIC	Odour description	Odour threshold value (OTV*) ppb (µg/L water)	
1	6.17	Acetic acid	1.1	Vinegar	9.9 x 10 ⁴	
2	6.28	3-Methyl butanal	1.5	Malty	0.5	
3	9.06	1-Pentanol	1.9			
4	9.46	1-Hexanal	5.6	Green, grassy	5	
5	10.72	1-Hexanol	1.9	Resin, flower	400	
6	10.94	Furan methanol	1.9	Burnt, sweet	1000	
7	10.99	2-Heptanone	1.2	Cheesy, fruity	140	
8	11.11	Heptanal	1.5	Fat, citrus	7	
9	11.12	α-Pinene	1.3	Pine, turpentine	6	
10	11.78	2,2,4,6,6-Pentamethyl heptane	2.8			
11	11.99	2-Pentyl furan	1.2	Fruity, green	6	
12	12.11	(E)-2-Heptenal	1.8	Green, fatty	13	
13	12.19	1-Octen-3-ol	1.7	Soap, plastic	1	
14	12.32	Benzaldehyde	1.3	Almond, burnt sugar	350	
15	12.48	Limonene	2.2	Lemon, orange	10	
16	12.49	Octanal	2.0	Soap, lemon	0.7	
17	12.54	Undecane	2.4			
18	13.12	3-Octen-2-one	1.4	Nut, crushed bug		
19	13.34	1-Octanol	1.7	Nut, mushroom	110	
20	13.39	(E)-2-Octenal	1.7	Fatty, herbal	3	
21	13.55	3,5-Octadiene-2-one (Isomer 1)	3.2	Fruity, grassy		
22	13.72	Nonanal	3.1	Citrus, soapy	1	
23	13.87	3,5-Octadiene-2-one (Isomer 2)	1.9			
24	14.77	Hydroxydihydromaltol	1.1			
25	15.60	(E)-2-Decenal	1.1	Tallow, orange peel	0.3	
26	16.03	3-Cyclohex-1-enyl-prop-2-enal	1.4			
27	16.14	5-Hydroxymethylfurfural	3.0	Fatty, buttery		
28	16.79	Piperonal	5.5	Cherry, sweet	3.9*	
29	17.63	Vanillin	5.3	Sweet vanilla	20-200	
30	18.01	2,4-Di-tert-butylphenol	3.1			
31	18.29	Cyclododecanone	3.5			

 Table 1: Identification of compounds detected at >1% area of the TIC with odour descriptors^{1,2} and odour threshold values (OTV²) for HiSorb with MSE analysis. (*No odour data available. This is a flavour value (Leffingwell & Associates²).)

A wide range of VOC/SVOC compounds were identified, demonstrated in Figure 3. The bar chart shows the distribution of the number of components for each compound class. The profile was dominated by aldehydes, alkanes, alcohols, heterocyclics and aromatics. Within these classes, the most abundant compounds were hexanal (green, grassy) at 5.6% (of the TIC), piperonal (cherry, sweet) at 5.5% (of the TIC) and vanillin (sweet vanilla) at 5.3% (of the TIC). During the production (heating) process of the cereal, Maillard reactions (a chemical reaction between amino acids and reducing sugars) occur and therefore a large amount of these compounds would be expected. Several compounds that are known to give an unpleasant or off-odour were also detected, e.g. four thiols/organo-sulfurs and three phenols (Figure 3), albeit at relatively low levels.



Figure 3: Number of components per compound class making up the VOC/SVOC profile of the cereal sample extracted by HiSorb with MSE.

Trace-level compound identification

The VOC/SVOC principal component analysis is important to characterise the aroma and flavour profile of a food product and the odour threshold value (OTV) is an associated parameter. Detection and identification of trace VOCs can therefore be very significant, particularly for compounds with medium and low OTVs or for problematic compounds (e.g. malodours) with low values, so that subsequent remedial action can be taken if necessary.

MSE provides a mechanism to increase the amount of each compound introduced to the GC, thus increasing the number of compounds detected, extending down to trace-level components. As representative examples, compounds such as γ -terpinene, anethole and γ -nonalactone were detected in the single extraction but at very low levels where their spectral quality was not sufficient for a good library match. Figure 4 demonstrates how MSE enabled a more confident identification for these compounds, and this is indicated in the NIST values shown in parentheses in Table 2.



Figure 4: Example peak profiles for compounds with minimal response and poor spectra from a single extraction (lower traces) compared to MSE (upper traces).

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RT	Compound	% TIC	Odour description	Odour threshold value (OTV*) ppb (µg/L water)	MF	NIST (%)	Hit nos
12.67	Eucalyptol	0.25	Herbal, camphor	12	795	80	1
17.36	α -Farnesene	0.23	Woody, green		829	62	1
14.32	Phenylethyl alcohol	0.03	Sweet floral	750	860	85	1
12.83	γ-Terpinene	0.02	Sweet citrus		735 (581)	35 (16)	1(1)
15.79	Anethole	0.07	Anise, licorice	15	738 (563)	46 (18)	1 (3)
17.19	γ-Nonalactone	0.18	Creamy, coconut		812 (552)	62 (12)	1 (3)

 Table 2: Examples of compounds only detected/identified in enriched samples and those detected with poor response/spectral quality for single-vial extraction (NIST statistics in parentheses).

Other components, such as eucalyptol, α -farnesene and phenyl ethyl alcohol were only detected in the enriched sample, meaning they could have been missed had only a single extraction been used for analysis (Figure 5). Eucalyptol is an important component in the sample with a relatively low OTV (12 ppb), imparting a herbal or camphor aroma to the product. These compounds are listed in Table 2. The NIST values represent the enriched sample with comparison to the single point extraction in parenthesis when a partial response was observed.

Additives and compounds with potential toxicity

Along with VOCs related to odour and taste, several additional compounds were identified in the breakfast cereal (Figure 2, Table 3). These include the antioxidants butylated hydroxytoluene and 2,6-di-*tert*-butylphenol, known to be added to food to prevent spoilage, and several compounds that potentially originate from the food packaging as contamination, styrene, two phthalates and two cyclic lactams. Interestingly, compounds that are usually associated with combustion were also detected in this sample (BTEX), with the possibility that these are present due to contamination of the original cereal crop.



Figure 5: Example peak profiles for compounds only detected using MSE (upper traces) compared to a single extraction (lower traces).

Peak label	RT	Compounds	% TIC			
		BTEX+styrene				
В	5.98	Benzene	0.02			
Т	8.50	Toluene	0.11			
E	10.24	Ethylbenzene	0.02			
Х	10.37	Xylene isomer	0.08			
S	10.84	Styrene	0.06			
		Flame retardant				
TMCP	22.68	Tris(2-chloroisopropyl)phosphate	0.79			
Antioxidants						
BHT	17.73	Butylated hydroxytoluene	0.24			
#30	18.01	2,4-Di-tert-butylphenol	3.09			
		Pesticide/defoliant				
BuP	21.17	Butyphos	0.45			
		PAH				
PAH	14.95	Naphthalene	0.05			
Phthalate						
DBP	22.90	Dibutylphthalate	0.13			

 Table 3: Identification of trace level potentially toxic/contaminant compounds.

Furthermore, compounds present on the US EPA Toxic Substances Control Act (TSCA) list were tentatively identified (Figure 2, Table 3), such as butyphos (tributylphospine oxide), a herbicide, Amgard TMCP (tris(2-chloroisopropyl)phosphate), a flame retardant and naphthalene, a PAH. Although this analysis was only qualitative, and as such no indication of the concentration can be inferred from the data, the detection, and tentative identification, of these compounds demonstrates the power of HiSorb and the Centri platform in providing the ability to extract and analyse a wide range of compounds in addition to the main flavour and odour-active compounds of interest.

Conclusion

In this study, we have demonstrated the use of a fully automated high-capacity sorptive extraction technique (HiSorb) for profiling the VOCs present in the headspace of a breakfast cereal using preconcentration with (a) a single-vial extraction and (b) multiple extractions (MSE). A wide range of compounds pertaining to taste and odour were found. Several components were found only in the enriched sample, demonstrating how multi-step enrichment provides a more comprehensive extraction, which leads to a more accurate VOC profile of a food sample. The fast MS acquisition typical of BenchTOF MS and the powerful data processing software further enhanced the identification of compounds, enabling co-eluting peaks in the complex profiles to be distinguished into individual analyte peaks.

Moreover, the ability to discover compounds present in the sample that either contribute to off-odours, have migrated from the packaging, or are contaminants from the original food commodity is important, as they have potential toxicity and can affect human health. This breadth of compounds discovered demonstrates the enhanced capability of the extraction methodology. Lastly, the automated extraction and enrichment process using HiSorb on the Centri platform means that no human interaction is required from sample extraction through to GC injection, allowing faster sample throughput. This ensures high productivity is achieved in industrial laboratories such as contract service or food research.

References

- 1. The Good Scents Company Information System (search facility), www.thegoodscentscompany.com.
- Odour & flavour detection thresholds in water (ppb) Leffingwell & Associates.

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