ANALYTICAL WORKFLOWS FOR SUCCESSFUL AROMA ANALYSIS

APPLICATION NOTE AS-305

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Abstract

Measurement of the aroma compounds in food, beverages and home and personal care products are important for understanding consumer preference, product performance, investigating processes or investigating counterfeit products. Aroma compounds may be present at extremely low levels, resulting in the need for sensitive and robust methods.

There are a variety of techniques that can be employed for the analysis of volatiles, many of which can be fully automated, offering advantages such as speed, improved reproducibility, improved sustainability and reduced operator interaction. For some liquid samples, a simple 'dilute and shoot' approach may be possible, but to detect compounds present at low levels, some level of enrichment is often required.

The choice of sample preparation will depend on the matrix, the analytes of interest (if known) and on the limits of detection required. Automated liquid- liquid extraction, including dispersive liquid liquid micro extraction (DiLLME) or headspace techniques such as solid phase microextraction (SPME) or Dynamic headspace (DHS) can provide a high level of enrichment.

The work set out in this application note discusses the choice of automated sample preparation technique and gives some comparisons of the results obtained for different matrices.

INTRODUCTION

The term 'aroma compound' refers to a chemical compound that has a smell or odour – which infers that it is sufficiently volatile to be transported to the olfactory system in the upper part of the nose. Volatile Organic Compounds (VOCs) are numerous, various and ubiquitous and most compounds responsible for odours are VOCS. However, it is worth noting that not all volatile compounds are aroma active, so when discussing aroma profiles and techniques, it is important to ensure that the link to sensory analysis is not overlooked.

There are a number of techniques that can be employed for the analysis of aroma compounds, and these fall into two general categories; liquid-liquid extraction and headspace (or thermal) extraction.

The latter includes techniques such as headspace, solid phase microextraction (SPME) and dynamic headspace (DHS) including the GERSTEL multivolatile method (MVM) which have been covered in previous application notes (1,2, 3,4). These have the advantage of being solventless, but can require the sample to be warmed, which may cause the formation of reaction products or require long extraction times.

The former category includes liquid-liquid and stir bar sorptive extraction (SBSE).

Choosing which technique to use can be a challenge and one approach may not be appropriate for all sample types and matrices. Food matrices can be complex, containing fats, proteins, emulsifiers etc. and similarly fragranced products may contain surfactants or solvents that can interfere with the extraction of aroma compounds.

EXPERIMENTAL

Instrumentation

To enable analysis by a number of different approaches, the Multiflex GCMS MPS Robotic/RoboticPro solution was used. This provides full automation on a single system of automated liquid-liquid extraction with liquid injection, static headspace (SHS), solid phase microextraction (SPME), dynamic headspace (DHS), stir bar sorptive extraction (SBSE) and direct thermal extraction (ATEX) and enables further enrichment by hot injection trapping (HIT) using SHS or SPME. The SPME Arrow module with Heatex stirrer was also used for some samples. Most samples were run on an Agilent GC-MSD (7890/5977) with extractor ion source in full scan acquisition.

METHODS

As they were different for each matrix, method details are given for each of the examples in the results section. Some were fully optimized, others based on previous work or existing customer methods. All compound identifications are based on NIST mass spectral library search results.

RESULTS

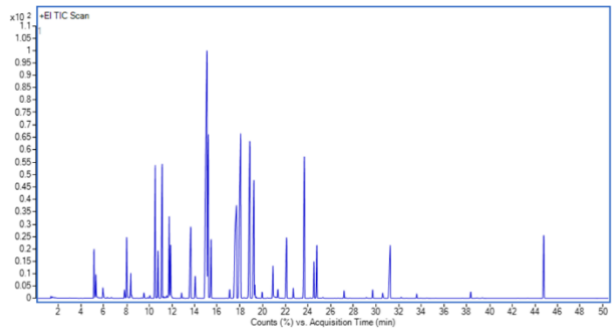

Figure 3. SPME arrow analysis of fragrance in candle

Sample (0.05g candle) in 20ml SPME vials, Fibre: DVB/C-WR/PDMS, 120µm, Length =20mm (Restek Cat no.: 27875)
50°C, 5 min equilibration, 30-minute extraction.
Desorption 5 minutes in inlet at 250°C (10:1 Split).

Table 1: Compounds identified in candle
(spectral library search with NIST17 and FFNSC3)

Compounds identification in Fragranced Candle		
Butanoic acid, 2-methyl-, ethyl ester	Eucalyptol	Verdox
3-Hexen-1-ol, (Z)-	Melon aldehyde	Phenylethyl alcohol <alpha-alpha, dimethyl-acetate
1-Butanol, 3-methyl-, acetate	Diethyl malonate	3-Hexen-1-ol, acetate, (Z)-
methanethiol butyrate	Trivertal	α-damascone
α- Phellandrene	ethyl heptanoate	isobornyl acetate
α-Pinene	Ligustral	Damascone (delta?)
Pentanoic acid, 2-methyl-, ethyl ester	(+)-2-Bornanone	caryophyllene
Ethyl acetoacetate	Benzyl acetate	Oxiranecarboxylic acid, 3-methyl-3-phenyl-, ethyl ester, cis-
Benzaldehyde	Allyl heptanoate	Ethyl Vanillin
2,6-dimethyloctene	Benzenemethanol, .alpha.-methyl-, acetate	2(3H)-Furanone, 5-hexyldihydro- (or γ-decalactone)
β- Phellandrene (or sabinene)	Ethyl maltol	Benzamide, 4-ethyl-N-allyl-
β-pinene	.alpha.-Terpineol	raspberry ketone
5-Hepten-2-one, 6-methyl-	Decanal	dodecanoic acid methylethyl ester
β-Myrcene	Citronellol	Oxiranecarboxylic acid, 3-methyl-3-phenyl-, ethyl ester, trans-
ethyl hexanoate	Neral	hedione
3-Hexen-1-ol, acetate	Phenyl ethyl acetate	Citronellyl valerate
Acetic acid, hexyl ester	Citral	benzyl benzoate
Thiazole, 4-methyl-2-(1-methylethyl)-	Bornyl acetate	Isopropyl myristate
Cymene (O or P)	tert-Butyl cyclohexyl acetate	
D-Limonene		

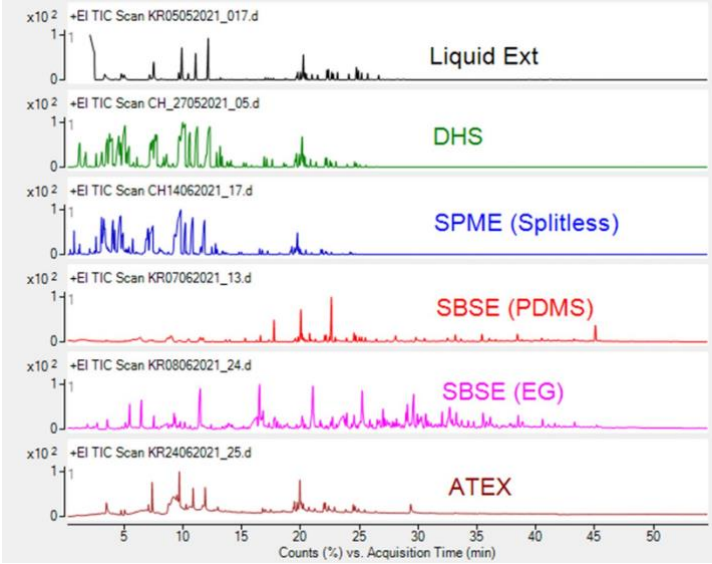

Figure 4. Comparison of techniques for analysis of a laundry product

Liquid extraction: 1 g sample, 3 mL hexane, quickMix (1 min at 2000 rpm) and centrifuge.

DHS: 0.5 g sample in 20 mL vials, incubated at 30 °C , extracted using a Tenax TA trap (1 Litre at 100 mL/min, followed by 600 mL dry step).TDU ramped to 260°C, Splitless desorption, CIS split 10:1.

SBSE: 0.5 g of sample diluted into 5 mL with water and extracted with either a EG-Silicone or PDMS Twister™ at 750 rpm for 2 hours. Following extraction, the stir bars were directly thermally desorbed into the GC-MS using the GERSTEL TDU 2 and CIS inlet. TDU ramped to 280 °C (PDMS) and 220 °C (EG-SIL), Splitless desorption, CIS split 10:1.

SPME:: 0.5g sample in 20 mL vials and extracted with a mixed SPME fibre (StableFlex Divinylbenzene/Carboxen/PDMS (DVB/CAR/PDMS)) for 30 minutes at 30°C, followed by automated desorption at 260 °C into the Agilent split/splitless inlet.

ATEX: 0.02g (approx.) sample, TDU ramped to 50°C (held for 5 mins), Splitless desorption, CIS split 50:1

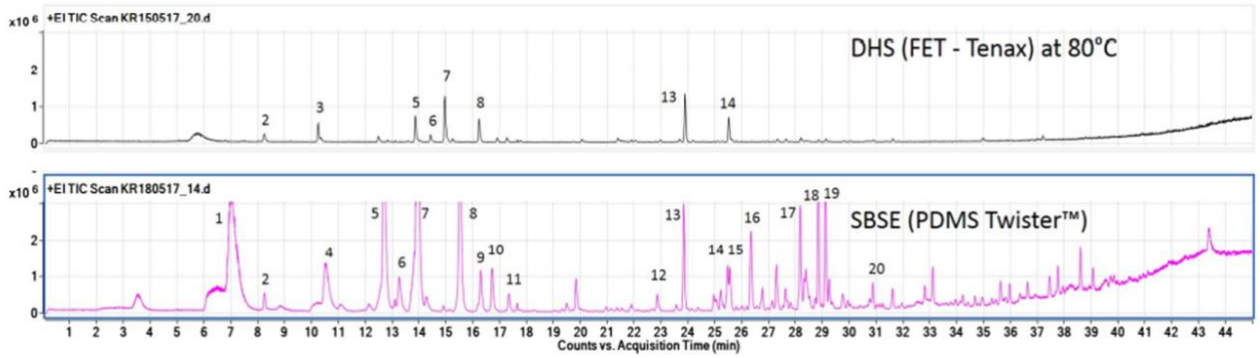

Figure 6. Comparison of techniques for analysis of Gin

DHS: 20 µL sample in 20 mL vials, incubated at 80 °C , extracted using a Tenax TA trap (2 L at 100 mL/min, TDU Splitless desorption, CIS split 20:1.

SBSE: 1 mL sample diluted with 4 mL water and extracted with a PDMS Twister for 3 hours, stirring at 1000 rpm, TDU Splitless desorption, CIS split 20:1.

Table 2: Compounds identified in Gin
(spectral library search with NIST17)

Compound identification in Gin	
1) α-Pinene	11) Sabinene hydrate
2) Ethanol	12) Copaene
3) Thujene	13) Linalool
4) β-Pinene	14) Terpinen-4-ol
5) Myrcene	15) Caryophyllene
6) Carene	16) β-Elemene
7) Limonene	17) Germacrene
8) γ-Terpinene	18) Geranyl acetate
9) Cymene	19) Cadinene
10) Terpinolene	20) γ-Elemene

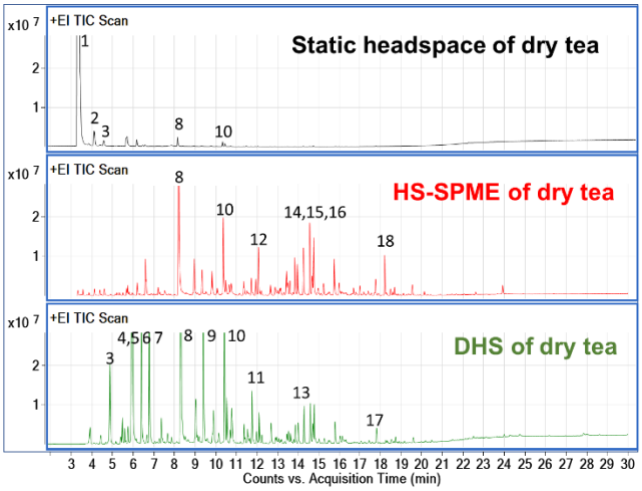

Figure 5. Comparison of techniques for analysis of tea
Static Headspace: 1 g sample in 20mL vials, incubation at 80°C for 40 mins.

DHS: 1 g sample in 20 mL vials, incubated at 80 °C , extracted using a Tenax TA trap (650 mLat 100 mL/min, followed by 600 mL dry step).TDU Splitless desorption, CIS split 10:1.

HS-SPME: 1g sample in 20 mL vials and extracted with a mixed SPME fibre (StableFlex Divinylbenzene/Carboxen/PDMS (DVB/CAR/PDMS)) at 60°C, followed by automated desorption at 250 °C into the Agilent split/splitless inlet (10:1 split).

Table 3: Compounds identified in Tea (spectral library search with NIST17)

Compound identification in dry tea	
1) Oxalic acid	10) 2-Hexenal
2) Dimethyl sulphide	11) 2-pentanal
3) Isobutanal	12) 5-Hepten-2-one, 6-
4) 2-Methylbutanol	13) 2,4-heptadienal
5) 3-methyl butanal	14) 3,5-octadiene-2-one
6) Furan, 2-ethyl	15) Benzaldehyde
7) Pentanal	16) Linalool
8) Hexanal	17) Methyl salicylate
9) 1-Penten-3-ol	18) Hexanoic acid

CONCLUSIONS

These examples illustrate the importance of choosing the most appropriate sample preparation procedure for your sample considering the matrix and analytes of interest.

The radically different chromatograms obtained for the various techniques highlights the 'selective' nature of sample extraction, which can be both a pro (focussed analysis of target analyte groups) and a con (the 'whole' picture of volatiles production is not always seen with a single technique). To ensure full characterisation in some matrices, more than one technique may need to be applied. The flexibility of being able to choose a number of different approaches from a single automated platform, helps analytical chemists answer critical questions and optimize information retrieval from their samples.

Table 4 provides a summary of some of the options available and some pros and cons of each technique.

Table 4: Comparison of techniques.

Technique	Pros	Cons	Applications
Dilute and shoot	Simple (dilution can be automated)	Only suitable for limited matrices. May require more instrument maintenance	Spirits analysis/flavour extracts in suitable solvent (ppm)
Liquid – liquid or DiLLME	Selectivity based on choice of solvent	May not be suitable for some matrices	Extraction of aqueous samples (e.g. beverages) (ppb)
Static Headspace (SHS)	Can be performed for most matrices	Limited sensitivity, suitable for volatiles	Targeted VOC screening or high-level profiling (ppm)
Solid phase microextraction (SPME)	Good enrichment, selectivity through choice of fibre. Can be optimised for specific analytes. Clean solvent-less provides extraction and enrichment in one step	Matrix components can interfere/limit capacity. Can favour more volatile analytes	Volatile profiling Low level target analysis (ppt-ppb)
Dynamic headspace (DHS)	Extracts both volatiles and semi volatiles. Excellent enrichment – some selectivity Clean solvent-less provides extraction and enrichment in one step	Will enrich most analytes – can overload high level components	Volatile profiling (ppt-ppb)
Multi-volatile method (MVM)	Can provide exhaustive extraction, good recoveries for wide range of compounds	Time taken (typically 2-3 hours per sample)	Complete volatile profiling (ppt-ppb)
Twister (PDMS)	Good enrichment, larger capacity than SPME Multiple extractions simultaneously (offline)	Extraction off-line PDMS favours non-polar analytes	Low level target analysis Profiling (ppt-ppm)

REFERENCES

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3. Comparison between the MVM (Multi-volatile Method), single DHS extraction and SPME (solid phase Microextraction) for extraction of volatiles in Whisky. Anatune Ltd. Chromatography Technical Note No AS149, 2015

4. Comparison of extraction techniques for volatiles in a selection of tea samples; Anatune Ltd. Chromatography Technical Note No AS188, 2017