Application Note 261

Enhanced aroma profiling of vodka using automated immersive high-capacity sorptive extraction with GC–MS

This study shows the advantages of immersive sorptive extraction, using high-capacity HiSorb probes, for the gas chromatography–mass spectrometry (GC–MS) analysis of a wide volatility range of aroma compounds in vodka. Key benefits include full automation on the Centri sample extraction and enrichment platform, re-collection for repeat analysis of a single sample under different conditions, and selective purging of ethanol.

Introduction

Historically, a wide variety of sampling methods have been used to extract volatiles from alcoholic spirits, with a key driver being the need to improve upon inefficient solvent-extraction methods.

One such improved method is HiSorb™ high-capacity sorptive extraction, which is a highly efficient sampling approach for a wide range of applications. It involves use of robust, inert metal probes fitted with a relatively large volume of PDMS sorptive phase (see boxed text on next page), allowing high sensitivity to be achieved. Following headspace or immersive extraction, the probes are automatically rinsed, dried and desorbed, with the analyte vapours then concentrated on a focusing trap prior to GC–MS injection.

In this study, we employ immersive extraction using HiSorb in conjunction with GC–MS, to identify a range of VOCs and SVOCs in vodka. The entire process of extraction, enrichment and trap-based analyte preconcentration is automated by the Centri® system from Markes International, and here we show the numerous advantages of this approach for analysts tasked with investigating aroma profiles of spirits. These include detecting analytes across a wide volatility range, extending dynamic range by varying the split ratio, and eliminating problems caused by ethanol overloading.

We explain how these features are enhanced by automated sample splitting and re-collection, and also show how the performance of HiSorb compares very favourably to static headspace extraction, here used in conjunction with trap-based preconcentration ('headspace–trap').

Experimental

Samples:
Vodka was dispensed into a 20 mL headspace vial, which was crimp-capped and placed onto the Centri autosampler tray for analysis. No additional sample modification was performed.

Extraction and enrichment:

Instrument:	Centri (Markes International)
Immersive high-capacity sorptive extraction:	
Sample:	20 mL
Probe:	Standard-length stainless steel HiSorb probe (part no. H1-XXAAC)
Incubation/agitation:	40°C (60 min) at 500 rpm
Desorption:	270°C (10 min)
Flow path:	180°C
Headspace–trap:	
Sample:	8 mL
Extraction volume:	5 mL
Incubation/agitation:	40°C (10 min) at 500 rpm
Injector:	250°C (1 min)
Preconcentration:	
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 flows:	Immersive high-capacity sorptive extraction: High split: 50 mL/min (51:1); Low split: 5 mL/min (6:1) Headspace–trap: 5 mL/min (6:1)
Sample re-collection:	
Sorbent tube:	'Bio-monitoring' (part no. C2-AAXX-5149)
Tube desorption:	280°C (10 min)

GC:

Column type:	DB-WAX™ Ultra Inert, 60 m × 0.25 mm × 0.25 µm
Column flow:	1 mL/min (constant-flow)
Oven program:	40°C (3 min), 30°C/min to 60°C, 3°C/min to 230°C (15 min)

Quadrupole MS:

Transfer line:	230°C
Ion source:	230°C
Mass range:	m/z 35–350

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Background to Centri and HiSorb

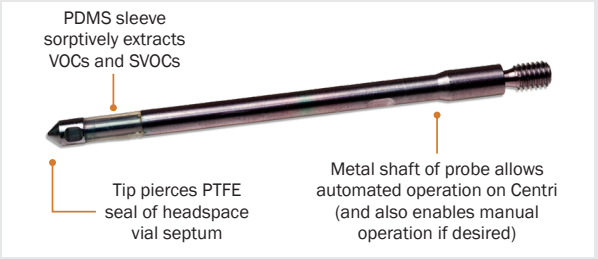
The **Centri®** system from Markes International for GC–MS is the first platform to offer high-sensitivity unattended extraction and enrichment of VOCs and SVOCs in solid, liquid and gaseous samples.

Centri allows full automation of immersive and headspace extraction using HiSorb, high-capacity sorptive extraction probes. It also offers full automation of headspace, SPME and tube-based thermal desorption with enrichment.

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 high-capacity sorbent polymer, to extract and concentrate compounds from liquids and solids.



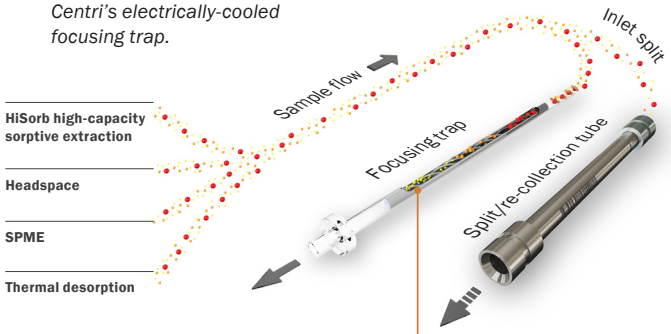
Samples contained in standard 20 mL or 10 mL vials are loaded onto Centri, and the HiSorb probe inserted into the vial for either immersive or headspace extraction. The probe is then automatically washed, dried, and desorbed, with the analyte vapours concentrated on the Centri focusing trap prior to GC–MS injection.

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

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

1 Sample focusing

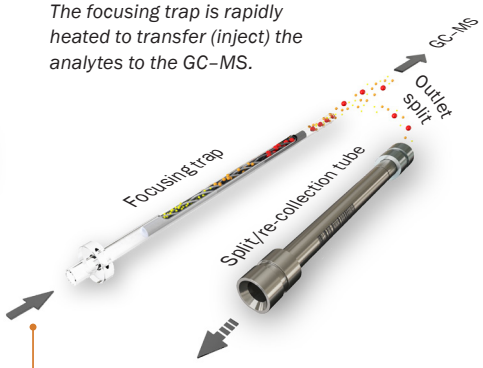
Extracted analytes are swept into Centri's electrically-cooled focusing trap.



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.

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).

Results and discussion

1. Sample re-collection

To allow optimised column loading and ethanol purging for this application, automated sample re-collection was configured as part of the workflow on Centri. This process takes place during trap desorption, and allows a precisely-defined proportion of the desorbed sample to be transferred to a sorbent-packed tube in the 50-tube TD module of Centri (see boxed text). This re-collected sample can then be desorbed from the tube onto the same trap, offering the capability to analyse the same sample under the same or different conditions, while avoiding the need to repeat any sample preparation steps.

Re-analysing a re-collected sample under the same conditions is valuable for validating the analytical method, by checking for complete analyte transfer through the system. On the other hand, using different conditions – for example, a

different detector or a different split ratio – offers the ability to discover more information about the analytes present. This is discussed in more detail below.

2. 'High/Low' analysis

As alluded to above, one of the advantages of sample re-collection on Centri is the ability to use different split ratios to investigate the effect of different column loadings of the same sample, within a single automated sequence ('High/Low' analysis).

In this case, analyte concentrations in the vodka sample were unknown, so a high split ratio (~51:1) was initially adopted as a precautionary measure (Figure 1A), in order to send most of the sample to the re-collection tube and avoid potential overload of the analytical system with water or ethanol.

Having determined the principal components of the sample in this way, a lower split ratio of 6:1 was used for the re-collected sample (Figure 1B), to increase the column loading and so identify the larger number of compounds at lower levels.

Table 1 lists the top 50 compounds identified, along with their aroma descriptions (where known). A broad range of chemical groups are present, including alkanes, aldehydes, esters, alcohols, antioxidants (such as butylated hydroxytoluene and 2,4-di-*tert*-butylphenol) and several fatty acids (ranging from acetic acid to C_{16} and C_{18} congeners). Despite being present at low levels, some of these compounds are likely to have a significant effect on the aroma, because of their lower odour thresholds.

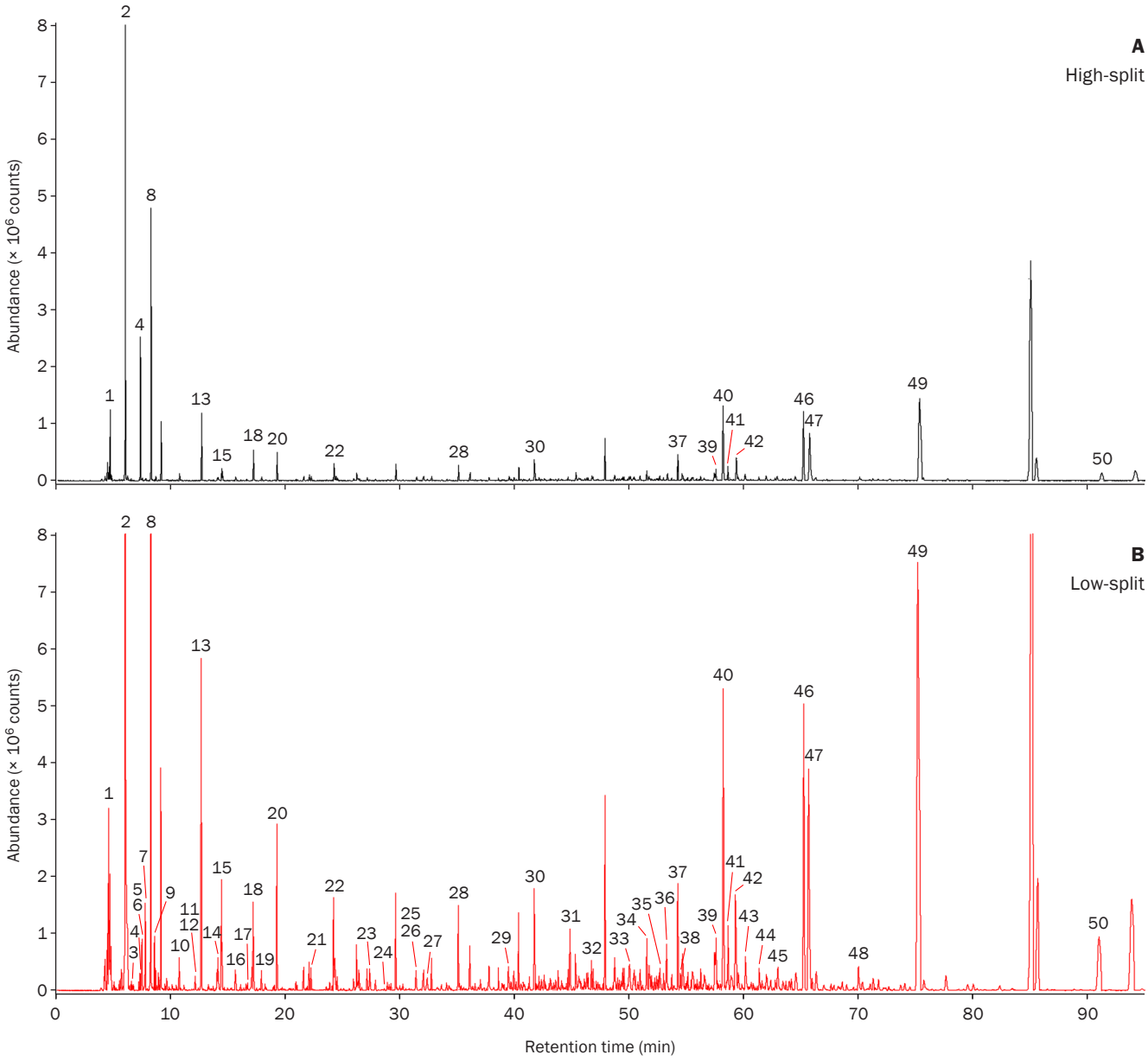


Figure 1: TIC profiles of vodka extracted immersively using HiSorb probes, using (**A**) a high split, and (**B**) a low split.

No.	Compound	t_R (min)	Aroma[1–3]
1	n-Hexane	4.57	–
2	Oct-1-ene	6.05	–
3	Ethyl acetate	6.64	Fruity, sweet
4	Ethanol	7.40	–
5	Nonene	7.52	–
6	Benzene	7.57	–
7	2,2,4,6,6-Pentamethyl-heptane	7.86	–
8	Pentanal	8.27	Bready, fermented
9	n-Decane	8.66	–
10	Hexanal	10.81	Leafy, grassy
11	1-Methoxy-2-propan-2-ol	12.19	–
12	Ethylbenzene	12.23	–
13	Butanol	12.66	Vanilla, fruit
14	Heptan-2-one	14.03	Cheese, fruity, coconut
15	n-Dodecane	14.56	–
16	Dodecene	16.17	–
17	Styrene	16.78	Sweet, balsamic, plastic
18	Cymene	17.33	Citrus, terpene, woody, spice
19	Octanal	17.99	Citrus, orange peel
20	Methylstyrene	19.69	–
21	Nonanal	22.17	Fat, citrus, green
22	Acetic acid	24.39	Pungent, sour
23	Benzaldehyde	27.43	Nutty, woody
24	Octan-1-ol	28.74	Green, citrus, orange

No.	Compound	t_R (min)	Aroma[1–3]
25	Methyl benzoate	31.35	Phenolic
26	Butanoic acid	31.47	Acidic, sour, cheesy
27	Acetophenone	32.43	Bitter almond
28	Benzyl acetate	35.33	Fruity, sweet
29	Hexanoic acid	39.53	Fruity, sweet,
30	Butylated hydroxytoluene	41.78	–
31	Phenol	44.89	Phenolic
32	Octanoic acid	46.77	Rancid, soapy, cheesy
33	Nonanoic acid	50.14	Fatty, waxy, cheesy
34	Methyl hexadecanoate	51.62	–
35	Ethyl hexadecanoate	52.73	Waxy, fruity, creamy
36	Decanoic acid	53.35	Soapy, waxy, fruity
37	2,4-Di-*tert*-butylphenol	54.31	–
38	Dibenzo-*p*-dioxin	54.54	–
39	Methyl stearate	57.63	Oily, waxy
40	Methyl octadec-9-enoate	58.25	–
41	Isopropyl octadec-11-enoate	58.69	–
42	Dodecanoic acid	59.33	–
43	11-Methylpentacosane	60.20	–
44	Nonadecylcyclohexane	61.42	–
45	11-Methylpentacosane	63.03	–
46	Dibutyl decandioate	65.30	–
47	Tetradecanoic acid	65.72	–
48	Pentadecanoic acid	70.07	Waxy
49	Hexadecanoic acid	75.22	–
50	Octadecanoic acid	91.02	–

Table 1: Listing of the top 50 compounds with a NIST match factor >800, and associated aroma characteristics.

3. Comparison of HiSorb and headspace–trap

To compare the results obtained using HiSorb probes with a conventional sampling technique, Figure 2 shows the results of a syringe-based headspace–trap analysis, which was also automated on the Centri system. For a fair comparison, the same low split ratio of 6:1 was used, and the same y-axis scaling is applied. It is clear that even when using analyte trapping to improve sensitivity, the overall recovery and analyte range is lower, with ethanol (#4), and to a lesser extent 2,2,4,6,6-pentamethylheptane (#7), having by far the greatest abundance.

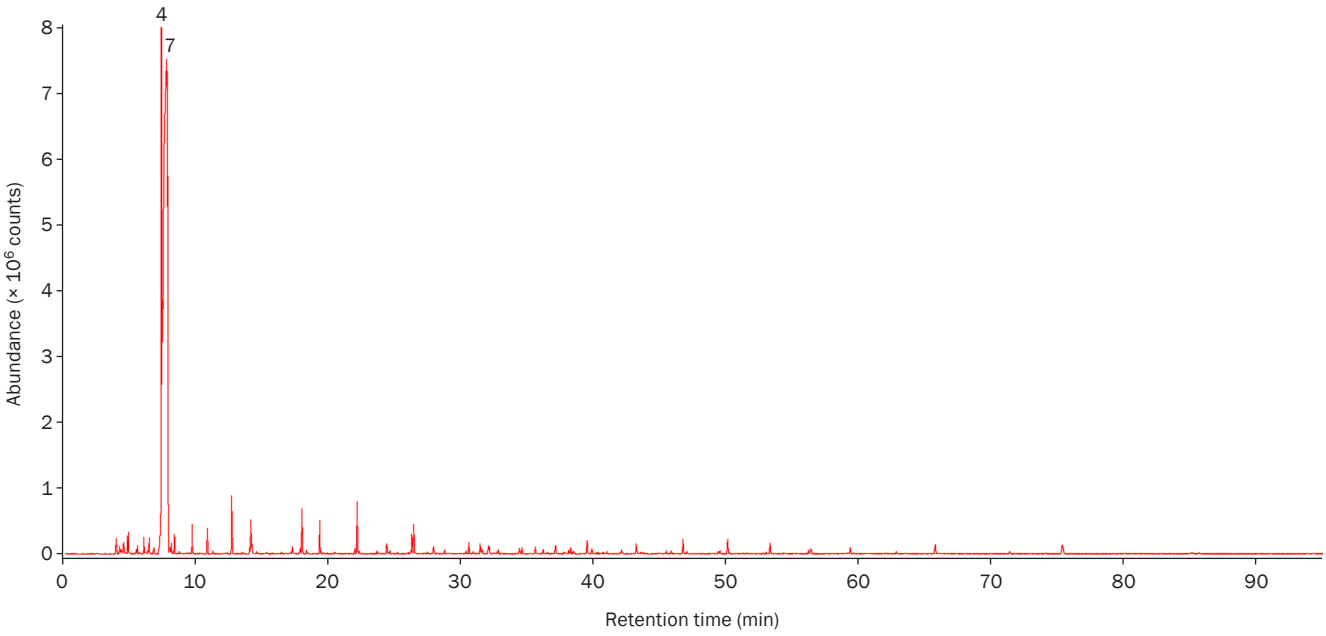


Figure 2: TIC profile of vodka sampled using headspace–trap, using a 6:1 split ratio, shown on the same scale as Figure 1.

The greater recovery using HiSorb is likely to be a consequence of the direct contact of the probe's PDMS phase with the liquid sample, particularly for higher-boiling and less polar species. This extraction efficiency can be broadly predicted by the analyte partition coefficient between octanol and water ($\log K_{o/w}$) – typically, efficient partitioning into PDMS requires $\log K_{o/w}$ values >3. Compounds such as decanoic acid (#36), tetradecanoic acid (#47) and hexadecanoic acid (#49) (which have $\log K_{o/w}$ values of 4.02, 5.98 and 6.96 respectively[4]) would therefore be expected to have improved responses using HiSorb, as indeed is the case.

A further factor in the performance of immersive extraction with HiSorb probes compared to syringe-based headspace extraction is that high ethanol content in the headspace could suppress the partitioning of some analytes between the liquid and gas phases. Finally, compared to other sorptive techniques such as SPME, the relatively large volume of PDMS phase on the HiSorb probes (65 µL) is beneficial, because it results in a lower sample/PDMS phase ratio, and consequently greater recovery for compounds with lower $\log K_{o/w}$ values.

4. Ethanol purging

The ethanol concentration in the vodka was >50%, and Figure 3 shows the relative response around the corresponding elution window when using headspace–trap, and with HiSorb high-capacity sorptive extraction at the two split ratios.

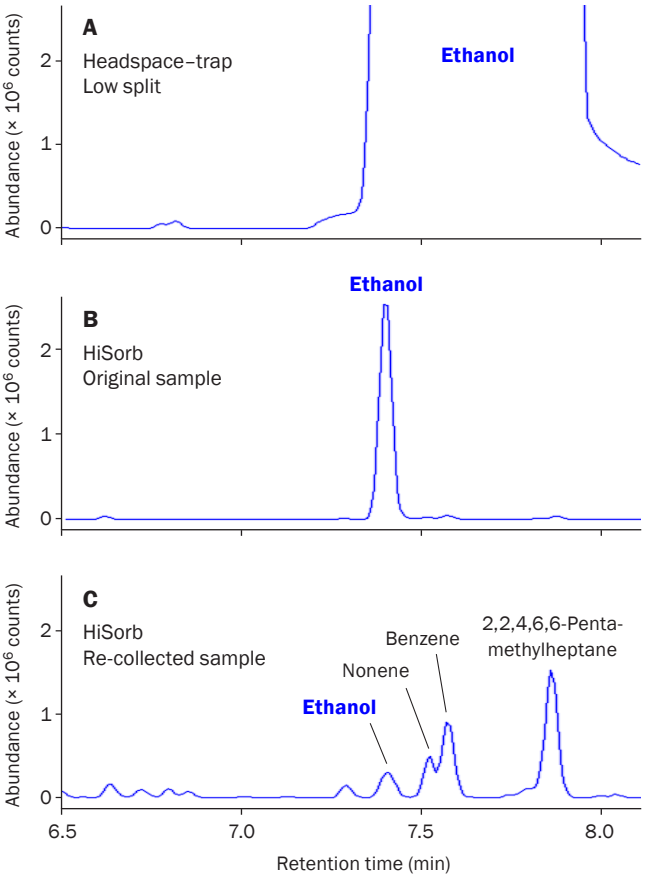


Figure 3: Relative TIC response of ethanol using (**A**) headspace–trap with a low split, (**B**) original HiSorb sample (with a high split) and (**C**) re-collected HiSorb sample (with a low split), and showing the additional low-level compounds identified.

The response of ethanol is highest in the headspace–trap analysis (Figure 3A), and it is clear how much of the baseline is obscured by this very large peak – a response that would certainly mask any nearby compounds. In contrast, the response of ethanol for the two HiSorb runs (Figure 3B and C) is much lower, which is a consequence of its very low partition coefficient ($\log K_{o/w}$ –0.14). It is possible that further optimisation of the focusing trap purge in the headspace–trap run could reduce the ethanol response, but it is unlikely that this would be to the extent shown for HiSorb.

Figure 3C demonstrates how the use of a subsequent analysis of the re-collected HiSorb sample, using a lower split ratio, increases the response from three low-level compounds. In addition, it also shows that re-collection has further reduced the response from ethanol, as a consequence of two additional adsorption/desorption stages – firstly on the re-collection tube and then on the second pass through the focusing trap. This is achieved because of the choice of sorbents, which allow ethanol to pass through while retaining key aroma compounds.

Conclusions

In conclusion, immersive high-capacity sorptive extraction has been shown to be a very efficient sampling technique for a wide range of aroma compounds in an alcoholic spirit. A key feature is that HiSorb probes, as well as being robust and re-usable, have a large volume of PDMS phase that results in much higher responses for important higher-boiling compounds than is possible using headspace (even when coupled with trap-based focusing).

The method also has productivity advantages. Centri, by automating entire extraction and enrichment workflows, allows throughput to be maximised by simultaneous extraction of multiple sample vials as part of an automated sequence (*e.g.* overnight). In addition, time-consuming manual sample preparation is eliminated, with its associated risk of error.

In addition, we have shown how sample re-collection allows optimisation of the method for both major and trace-level components, and how it helps to minimise the response from ethanol, which would otherwise interfere with the analysis.

References

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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.