Chromatography Technical Note No AS107

Benefits of using sample enrichment techniques (Headspace SPME, Twister SBSE, and Dynamic headspace) to determine trace level analytes present in a herbal based liquor.

Dan Carrier, Anatune Ltd. Hardwick, Cambridgeshire, UK.

Introduction

This application note discusses different sample enrichment techniques available using the Multipurpose sampler (MPS2 XL). A herbal based liquor was chosen for this comparison as this contains trace level analytes which cannot be detected using conventional sample introduction techniques such as Static Headspace.

Static Headspace analysis works by analysing the vapour above the sample at equilibrium. This usually gives good quantitative data and it is the most common method for volatile analysis. However, for detection of trace level analytes, this may not be the most suitable method and some form of enrichment may be necessary.

Headspace SPME

A SPME fibre is introduced into the headspace and analytes partition into the coating on the fibre. After a fixed time, the fibre is removed and desorbed in the GC inlet. A wide range of fibre coatings are available and this can enable selective extraction of target analytes.

Twister Stir Bar Sorptive Extraction (SBSE)

Twister SBSE is a technique used to enrich analytes onto a magnetic stir bar. The most common twister is coated with a polydimethylsiloxane (PDMS) phase. The twister bar can be either submerged in an aqueous sample, or can be suspended in the headspace. Within this application note, the twister is submerged in the aqueous sample. The aqueous samples are then placed onto a stirrer plate and stirred for a period of time. Each twister bar should then be dried. The enriched analytes are subsequently desorbed in the GC inlet. Figure 1 shows a photo of multiple twister extractions.

Figure 1 Photo of Multiple twister extractions

Dynamic Headspace (DHS)

In Dynamic Headspace (DHS), the sample is continuously purged with an inert gas, usually carrier gas, and the volatile compounds are continuously retained onto an adsorptive trap. The trap can then be dried to remove any residual water which may have been collected. Reducing the amount of water is necessary to obtain good chromatography of the analytes. DHS can be fully automated using the Multipurpose Sampler MPS2 from Gerstel. Figure 2 shows a schematic view of the DHS Process

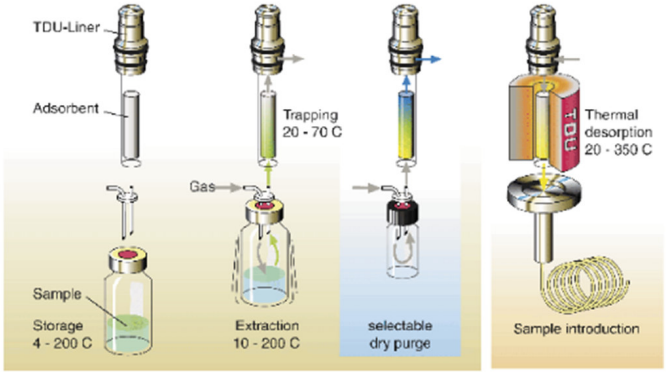

Figure 2 Schematic view of the DHS Process

Instrumentation

Gerstel Multipurpose Sampler MPS 2 XL
Gerstel Dynamic Headspace
Gerstel Static Headspace
Gerstel Thermal Desorption Unit
Gerstel Cooled Injection System (CIS) 4
Gerstel SPME Option
Maestro Version 1.4.8.14/3.5
Agilent 5975 C inert XL MSD
Agilent GC 7890A
Twister Stirrer plate

Anatune Ltd, Broadway House, 149-151 St Neots Road, Hardwick, Cambridge, CB23 7QJ, UK
Tel: +44 (0) 1954 212909 Fax: +44 (0) 1954 212908 Email: info@anatune.co.uk Internet: www.anatune.co.uk

Methodology

A herbal based liquor was enriched using Static Headspace, Headspace SPME, Twister SBSE, and Dynamic Headspace (DHS) using the same GC temperature program and MS conditions. 100 µl of the herbal based liquor was used for Dynamic Headspace, Headspace SPME, and Twister SBSE. 2 ml of the herbal based liquor was used for Static Headspace. 5-6 replicates were performed to gain understanding of precision using each technique.

Column, Oven Program, and MS conditions

Column: 30 m HP-Innowax (Agilent)
di = 0.25 mm df = 0.25 µm
Pneumatics: He, constant flow = 1 mL/min
Oven: 40°C (5 min); 10°C/min; 235°C
MSD: Scan, 35 - 350 amu

Dynamic Headspace conditions

Trap: Tenax TA
DHS Trap temperature 40°C
Incubation temperature 80°C
Purge volume 50 ml
Purge flow 100 ml/min
Dry purge volume 950 ml
TDU temperature program 50°C; 120°C/min;
350°C (3 min)
CIS 4: Tenax TA liner,
CIS 4: Temperature Program 10°C; 12°C/s; 250°C (5 min)

Headspace SPME conditions

60 °C incubation temperature
Fibre: Carboxen/PDMS (incubation time 10 minutes)
Inlet temperature 250°C (Splitless)

Twister SBSE

100 µl of the herbal based liquor in 4.9 ml deionized water
PDMS Twister (2 cm x 1 mm id)
Stirred for 2 hours
TDU temperature program 50°C (2 minutes); 720°C/min;
280°C (2 min)
CIS 4: Baffled glass liner,
CIS 4: Temperature Program -150°C; 16°C/s to 150°C; 12 °C/s to 220°C (5 min)

Static Headspace conditions

60 °C incubation temperature
Injection volume: 2500 µl
CIS 4: Tenax TA liner
CIS 4: Temperature Program 40°C; 10°C/s; 300°C (5 min)

Results

Figure 3 shows example chromatograms of the herbal based liquor using each sample introduction technique.

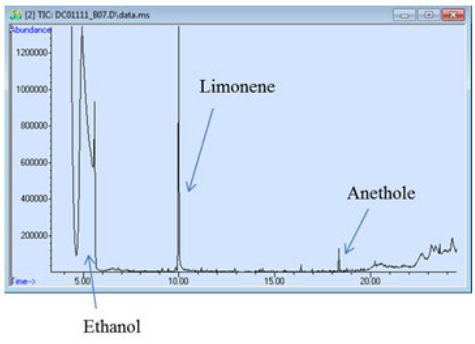

Figure 3(a) Herbal based liquor using Static Headspace

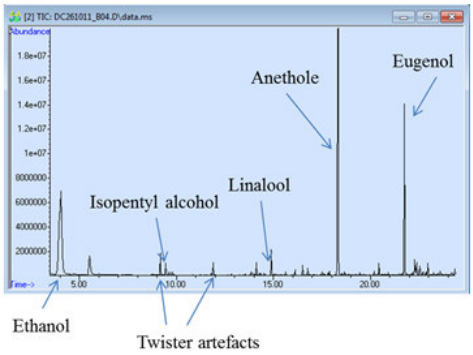

Figure 3(c) Herbal based liquor using Twister SBSE

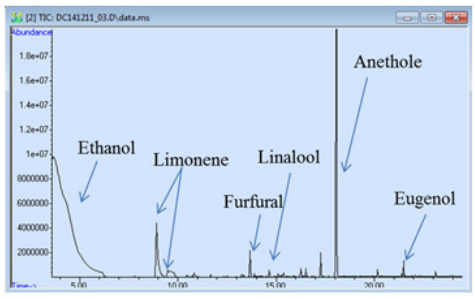

Figure 3 (b) Herbal based liquor using Headspace SPME
Static Headspace

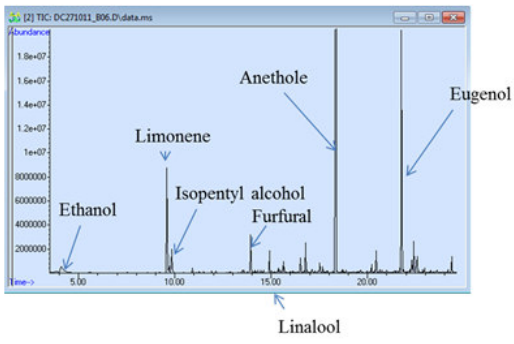

Figure 3 (d) Herbal based liquor using Dynamic Headspace

Anatune Ltd, Broadway House, 149-151 St Neots Road, Hardwick, Cambridge, CB23 7QJ, UK
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Static Headspace

Five replicate samples injections of the herbal based liquor were performed using Static Headspace. The percentage relative standard deviation (% RSD) for Limonene and Anethole was poor (both >18%). No further method development was carried out using Static Headspace as it was clear that the detection limits would not be as good as the enrichment techniques DHS, Headspace SPME, and Twister SBSE.

Headspace SPME

Six replicate samples of the herbal based liquor were enriched using Headspace SPME. The % RSD for Limonene was not calculated due to the peak splitting. % RSDs for Furfural, Linalool, Anethole and Eugenol have been calculated in Table 1.

Description	Furfural Results	Linalool Results	Anethole Results	Eugenol Results
	Area	Area	Area	Area
Liquor -1	2062901	217132	23310685	460298
Liquor -2	1765574	201636	22934881	272798
Liquor -3	1907989	227969	26379227	348752
Liquor -4	1981520	233263	27460122	468598
Liquor -5	1815848	215583	24698973	312376
Liquor -6	1937707	224947	27020068	482699
Mean	1911923	220088	25300659	390920
sd	108597	11214	1934005	90742
% RSD	**5.7**	**5.1**	**7.6**	**23.2**

Table 1 % RSDs for Furfural, Linalool, Anethole and Eugenol using Headspace SPME

Twister SBSE

Five replicate samples of the herbal based liquor were enriched using Twister SBSE. % RSDs for Furfural, Linalool, Anethole and Eugenol have been calculated in Table 2.

Description	Isopentyl alcohol Results	Linalool Results	Anethole Results	Eugenol Results
Name	Area	Area	Area	Area
Liquor 1	27740	488087	20450511	3598719
Liquor 2	29780	544143	20591745	3835529
Liquor 3	28945	520918	20329401	3806690
Liquor 4	28718	562903	21114701	3884477
Liquor 5	26143	585448	21048598	3935448
Mean	28265	540300	20706991	3812173
sd	1391	37636	355162	128999
% RSD	**4.9**	**7.0**	**1.7**	**3.4**

Table 2 % RSD for Isopentyl alcohol, Linalool, Anethole and Eugenol using Twister SBSE.

Dynamic Headspace (DHS)

6 replicate samples of the herbal based liquor were enriched using Dynamic Headspace (DHS). The % RSDs for Limonene and Eugenol were poor (both >16%). Further method development would be required on these two analytes using DHS to improve the precision. Table 3 shows good precision (without internal standard) on a range of analytes detected by DHS.

	Isopentyl alcohol Results	Fenchone Results	Furfural Results	Linalool Results	Estragole Results	Anethole Results
Description	Area	Area	Area	Area	Area	Area
Liquor 1	1061449	27442	1498314	364459	332393	31162952
Liquor 2	1123984	30483	1908031	407646	353438	32121254
Liquor 3	1194505	29230	1721238	379104	332959	31672976
Liquor 4	1101294	25908	1557958	369767	298300	29948924
Liquor 5	1109686	27297	1539181	383566	313123	30258390
Liquor 6	1244262	29585	1814611	446215	357317	31830603
Mean	1154746	28501	1708204	397259	331027	31166430
sd	62104	1858	160130	30731	25440	989477
% RSD	**5.4**	**6.5**	**9.4**	**7.7**	**7.7**	**3.2**

Table 3 % RSD for range of analytes using Dynamic Headspace (DHS).

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Conclusion and Discussion

Headspace SPME, Twister SBSE and Dynamic headspace (DHS) all give superior results for the herbal based liquor when compared to results obtained by Static Headspace. Poor precision was observed for Static Headspace. It was thought that this was due to overloading the inlet with high amounts of water and ethanol.

Dynamic Headspace (DHS) can be used to gain the most structural information of the herbal based liquor. Far more analytes are observed compared to Twister SBSE and Headspace SPME. Good precision can be achieved on a number of analytes.

Twister SBSE gives good precision on analytes which are detected by this technique. Limonene is not detected using twister SBSE. Limonene is very hydrophobic and it is thought that Limonene is not soluble in a highly aqueous solvent which is used in the twister process. This could mean that Limonene will not interact with PDMS coating on the twister bar.

Headspace SPME offers good precision for Furfural, Linalool and Anethole. Poor peak shape was observed for Limonene. It is thought that this is due to high amounts of water being injected onto the GC. Further method development should be focused on reducing the amount of water using this technique.

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