Trace Level Analysis of Aqueous Samples by Dynamic Headspace

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Static Headspace analysis [1] is the most common sample introduction technique for volatile analysis by Gas Chromatography (GC). It works by analysing the vapour above the sample at equilibrium. However, for detection of trace level analytes, this may not be the most suitable method and some form of enrichment may be necessary. For certain analytes, over a 100 fold increase in sensitivity can be achieved with Dynamic Headspace (DHS) analysis when compared to Static Headspace analysis. This article compares DHS with other sample enrichment techniques such as Solid Phase Microextraction (SPME), and Stir Bar Sorptive Extraction (SBSE) using Twister stir bars.

Introduction

In this article, a herbal based liquor and three different orange juices were chosen for enrichment purposes. Both sets of drinks are aqueous based and require some form of enrichment and removal of water for trace level analysis by GC.

Slight differences in the ingredients can drastically change the taste of a drink. The unique smell and taste of different brands of drinks often are due to minor differences in their volatile components. Therefore, it is important to maintain a consistent composition of the drinks to ensure customer satisfaction. Many of the analytes in these drinks are volatile and most are present at trace level. Some of the trace level analytes cannot be detected with Static Headspace analysis. DHS offers an approach to continuously enrich the analytes making it possible to detect and quantify them by Mass Spectrometry (MS).

This article compares DHS with other sample enrichment techniques such as Solid Phase Microextraction (SPME), and Stir Bar Sorptive Extraction (SBSE) using Twister stir bars.

In DHS, a sample is continuously purged with an inert gas, usually the GC 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 which have been enriched. Figure 1 shows a schematic view of the automated DHS Process.

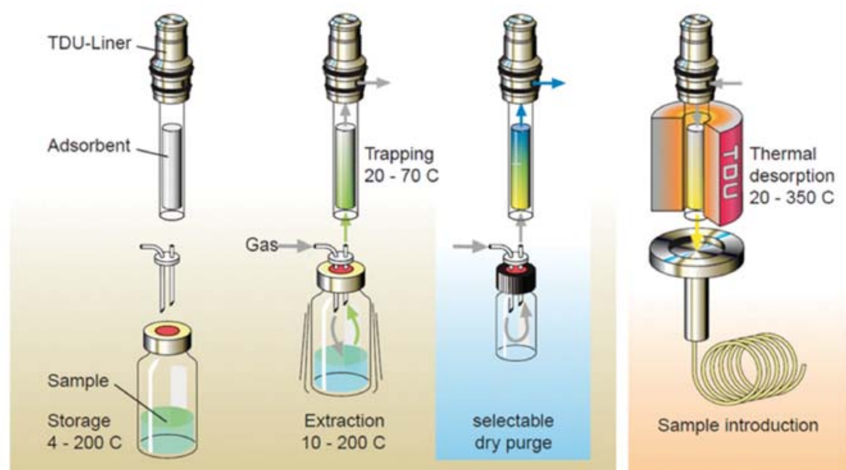

Figure 1: Schematic view of the automated DHS process

Firstly, the sample is heated to a required temperature. This allows the sample to form an equilibrium between the liquid and the gas phase in the same manner as Static Headspace analysis. A dual needle then pierces the septa and an inert gas, usually helium, is passed through enabling analytes to be trapped onto the adsorptive trap. Some residual water may have been retained on the trap. Therefore, there is an option to dry purge the trapped sample. If this is required, the dual needle pierces a clean vial and the adsorptive trap is then dried with a set volume of helium at a specified temperature. The trap, containing sample analytes, is transferred into the thermal desorption unit (TDU). A fast temperature ramp is used to desorb the extracted analytes from the TDU onto the Cold Inlet System (CIS) which is set at a cold temperature to focus the analytes of interest. Once the analytes have been focussed into a tight band on the CIS, another fast temperature ramp is used to desorb the extracts onto the GC column.

Full evaporation technique (FET) is used to enhance the detection of volatile analytes by DHS. A low volume aliquot of each drink is placed into an empty headspace vial. The vial is heated to 80°C allowing the analytes in the sample to vaporise whilst leaving the low volatile matrix behind. The FET technique is performed by using a small volume of sample and vaporising the analytes in the headspace vial completely, without having to rely on establishing equilibrium between two

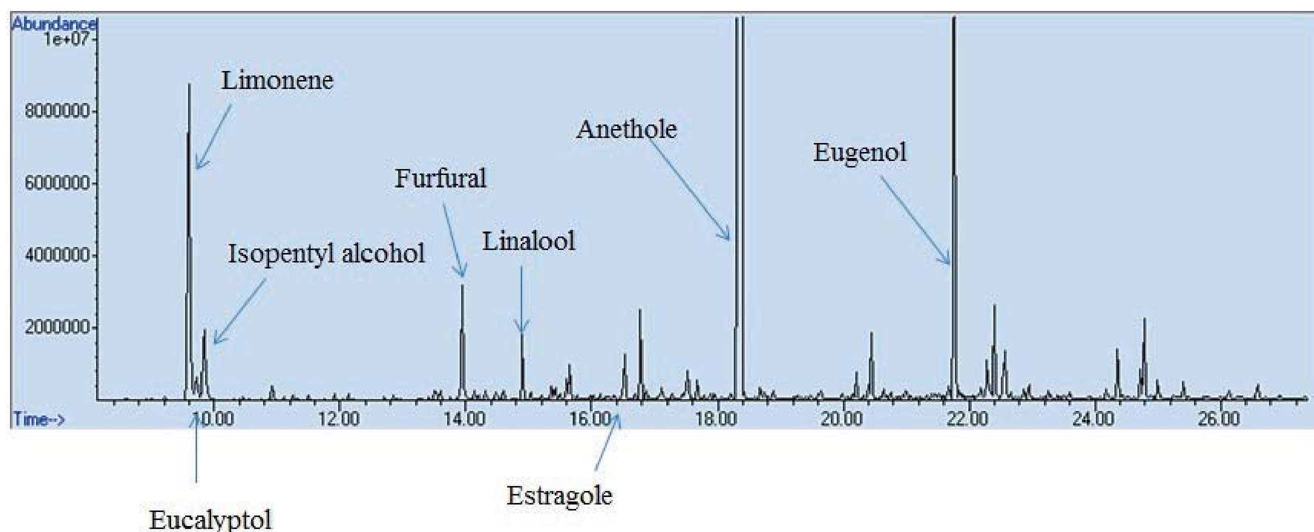

Figure 2: Example chromatogram of the herbal based liquor by DHS

phases [2, 3]. The low sample volume used in DHS increases the sensitivity of analytes. This phenomenon may seem counterintuitive, however, this is possible by DHS as the technique effectively enriches most of the analytes.

Twister Stir Bar Sorptive Extraction (SBSE) and Headspace Solid Phase Microextraction (Headspace SPME) are techniques also available to enrich analytes of interest. Twister SBSE is a technique used to enrich analytes onto a magnetic stirrer 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. For the purposes of this article, the Twister is submerged in aqueous samples. The aqueous samples are then placed onto a stirrer plate and stirred for a period of time. Each Twister bar should then be dried with tissue. The enriched analytes are subsequently desorbed in the GC inlet. With Headspace SPME, an 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.

Method

The first series of experiments was designed to determine the differences between the different sampling techniques. A herbal based liquor was chromatographed using Static Headspace analysis, Headspace SPME, Twister SBSE, and DHS using the same GC temperature program and MS conditions. 100 µl of the herbal based liquor was used for DHS, 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. Estragole and Anethole were also quantified in the herbal based liquor by DHS.

Finally, the three different orange juices were chromatographed using the same DHS method as for the herbal based liquor to identify if differences could be determined between the different sources of juice. 50 µl of each orange juice was used for DHS; six replicates were performed on orange juice A; individual injections of oranges juices B and C were performed. Table 1 shows the method conditions used for the analysis.

Results and Discussion

Figure 2 shows an example chromatogram of the herbal based liquor by DHS. Several of the volatile ingredients can be observed by DHS. Only Limonene and Anethole were detected by Static Headspace analysis. Over a 100 fold increase in sensitivity was observed for Anethole using DHS when compared to the Static Headspace analysis for the herbal based liquor. Table 2 shows precision obtained for a number of analytes by DHS, Twister SBSE and Headspace SPME. For DHS, the percentage relative standard deviation (% RSD) for Limonene and Eugenol

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

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

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)

Headspace SPME conditions

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

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)

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)

Table 1: Instrumentation and method conditions

Technique		Eucalyptol	Isopentyl alcohol	Fenchone	Furfural	Linalool	Estragole	Anethole	Eugenol	Limonene
DHS	Mean	123710	1139197	28324	1673222	391793	331255	31165850	6181178	3448051
	sd	6846	67352	1717	166900	30575	22761	885016	1180565	582466
	%rsd	5.5	5.9	6.1	10.0	7.8	6.9	2.8	19.1	16.9
Twister SBSE	Mean	43033	28265	10382	Not detected	540300	249878	20706991	3812173	Not detected
	sd	3328	1391	1377		37636	15655.2	355162	128999	
	%rsd	7.7	4.9	13.3		7.0	6.3	1.7	3.4	
Headspace SPME	Mean	Not detected	Not detected	Not detected	1911923	220088	437374	25300659	390920	Poor peak shape
	sd				108597	11214	51742	1934005	90742	
	%rsd				5.7	5.1	11.8	7.6	23.2	

%rsd above 10% have been highlighted in red

Table 2: Percentage relative standard deviation (%RSD) for a range of analytes in the herbal based liquor using DHS, Twister SBSE, and Headspace SPME

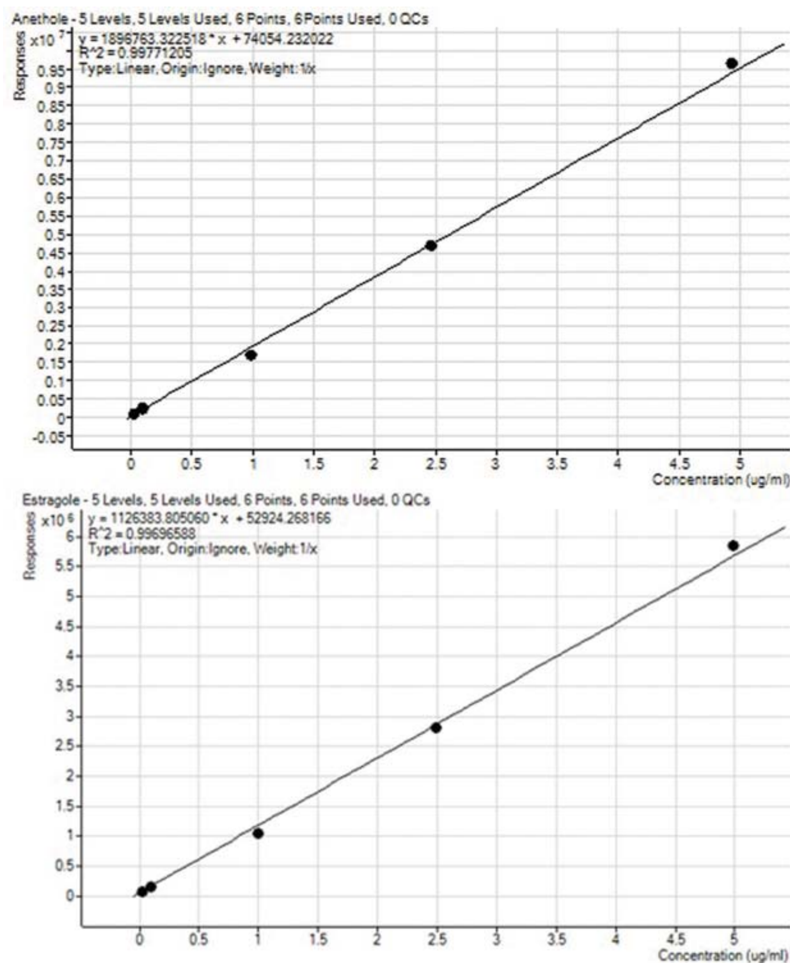

Figure 3: Linearity for Anethole and Estragole by DHS

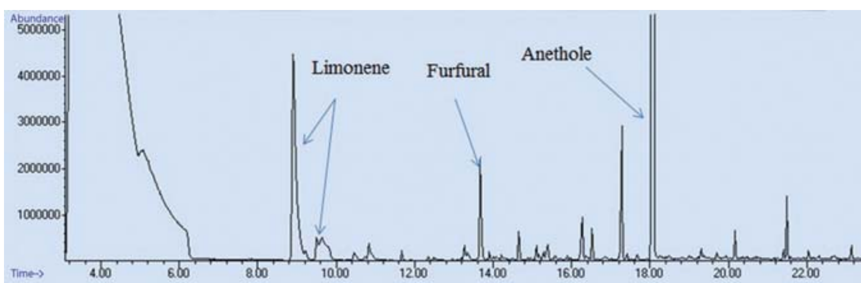

Figure 4: Example chromatogram of the herbal based liquor by Headspace SPME showing poor peak shape observed for Limonene

was poor (both >16%). Further method development would be required on these two analytes using DHS to improve the precision. Figure 3 shows the linearity results for Anethole and Estragole. Anethole was present in the herbal based liquor at 13 µg/ml and Estragole was present at 0.16 µg/ml. Sensitivity increases were also observed for Twister SBSE and Headspace SPME when compared to Static Headspace analysis. For Headspace SPME, poor peak shape was observed for Limonene. It is thought that this is due to high amounts of water being injected onto the GC. Figure 4 shows the poor peak shape observed for Limonene by Headspace SPME. For Twister SBSE, certain analytes were not detected in comparison to DHS. Furfural was not detected using Twister SBSE. It was thought that Furfural, being hydrophilic, would stay in the highly aqueous solvent and would not interact with the PDMS coating on the Twister bar.

Several of the ingredients in orange juice could be detected by DHS. Figure 5 shows varying levels of the analytes in the three different juices. Six replicas of orange juice A were analysed. Percentage RSDs for Limonene, P-menth-1-en-4-ol, Linalool were all below 5%. Further method development would be required on Eremophilene which was calculated to be 14 % RSD. Precision for β-Pinene was calculated to be 8.5 % RSD.

Conclusion

DHS offers a good automated enrichment process for the volatile components in the herbal based liquor and orange juice. Acceptable quantitation of trace level analytes can be carried out using DHS in aqueous based matrices. Other sample enrichment techniques such as Twister SBSE and Headspace SPME can also be used to give more information on analytes present in

the herbal based liquor and orange juice when compared to Static Headspace analysis.

References

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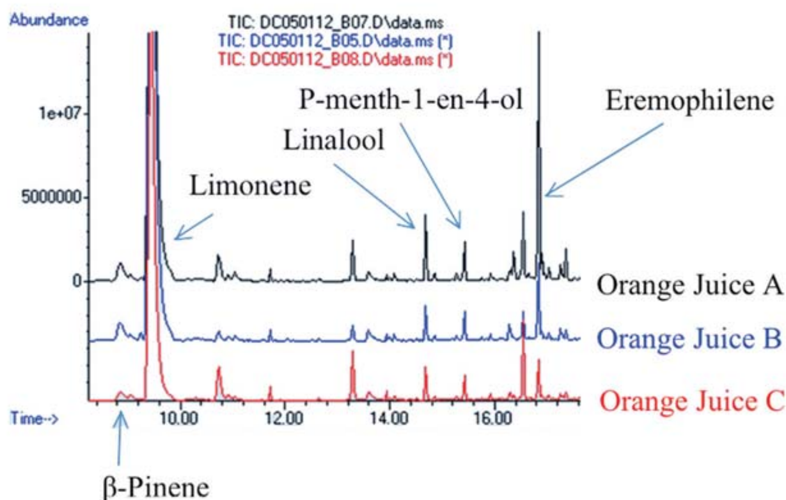

Figure 5: Comparison different orange juices by DHS

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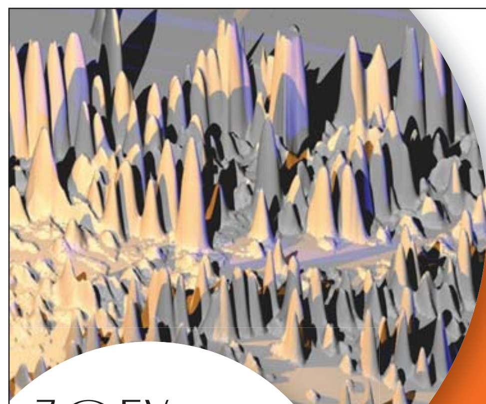

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