Chromatography Technical Note No AS106

Dynamic headspace (DHS) analysis using Full Evaporation Technique (FET) to quantify trace level analytes present in a herbal based liquor

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Introduction

Maintaining the quality of herbal based liquors is an important factor in their production. Slight differences in the ingredients of these beverages can drastically change the taste. Therefore, it is important for the distilleries to maintain the quality of their product for customer satisfaction. Many of the volatile analytes are at trace level and cannot be detected with conventional GC headspace analysis. Dynamic headspace (DHS) offers an approach to continuously enrich the analytes making it possible to detect and quantify them by Mass Spectrometry (MS).

In 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 1 shows a photograph of the instrumentation in use for DHS.

For a sensitivity comparison, static headspace was also performed on a herbal based liquor. Linearity and precision were performed in analytes present in the liquor using DHS.

Instrumentation

Gerstel Multipurpose Sampler MPS 2 XL
Gerstel Dynamic Headspace
Gerstel Static Headspace
Gerstel Thermal Desorption Unit
Gerstel Cooled Injection System 4
Maestro Version 1.4.8.14/3.5
Agilent 5975 C inert XL MSD
Agilent GC 7890A

Methodology

Full evaporation technique (FET) is used to enhance the detection of volatile analytes by DHS. A low volume aliquot (100µl) of the herbal based liquor is placed into an empty headspace vial. This vial is heated to 80°C allowing the analytes in the sample to vaporize while leaving most of the low volatile matrix behind. The FET technique is performed by using a small volume of sample and vaporizing the analytes in the headspace vial completely, without having to rely on establishing equilibrium between two phases [1, 2].

The analytes in the purged headspace are trapped onto a 2cm adsorbent bed (Tenax) in a compact glass tube. The tube is then placed into the Thermal Desorption Unit (TDU) and the analytes are thermally desorbed onto the GC. The analytes are focused using a Cooled Injection System (CIS 4) inlet to improve peak shape and increase sensitivity. Figure 2 shows a schematic of the trapping and desorption process.

A set of calibration standards containing Estragole and Anethole were prepared ranging from 0.02 µg/ml to 5 µg/ml. These were prepared in 35% ethanol 65% HPLC grade water. 100µl aliquots of each standard were pipetted into individual headspace vials and capped.

Figure 1. MPS 2 with Dynamic Headspace (DHS) using a 7890A GC and 5975C MSD

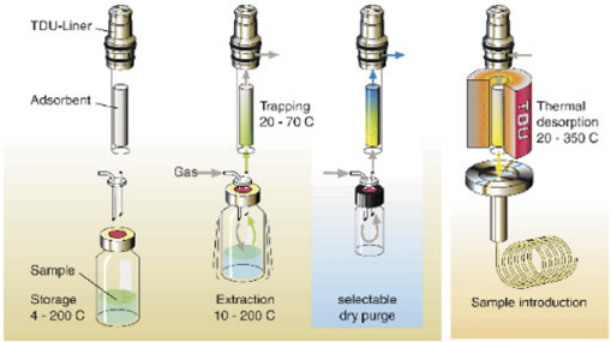

Figure 2. Schematic view of the DHS Process

For sample preparation, six 100 µl aliquots of the herbal based liquor were a pipetted into headspace vials and capped for analysis.

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DHS Analysis 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)

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)

DHS and Static Headspace 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

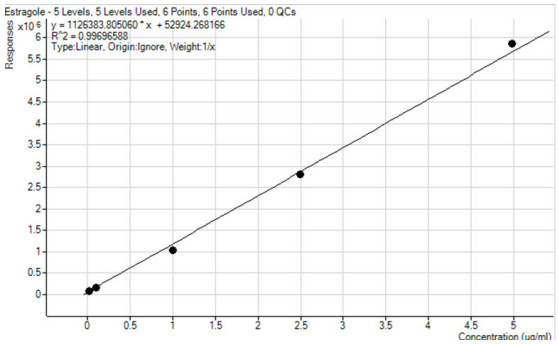
Estragole and Anethole were quantified in the herbal based liquor. Precision data was also collected for Estragole, Anethole, and Linalool. Table 1 shows the different concentrations chosen for the calibration.

	Analyte µg/ml	
Calibration Level	Estragole	Anethole
Std_01	0.02	0.02
Std_02	0.10	0.10
Std_03	1.00	0.98
Std_04	2.49	2.46
Std_05	4.98	4.92

Table 1. Levels of Estragole and Anethole in the calibration standards

Results

Calibration curves were constructed for both Estragole and Anethole. Linear calibrations were achieved from the five point calibration standards. Correlation coefficients of 0.997 and 0.998 were achieved for Estragole and Anethole respectively (see Figure 3).



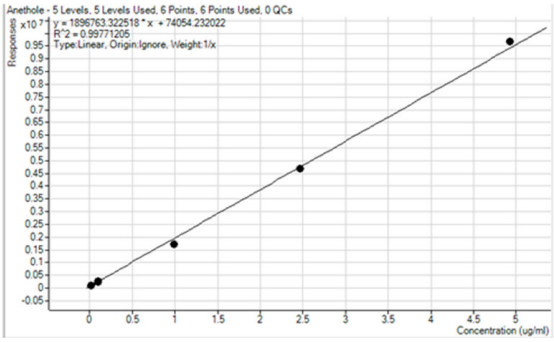


Figure 3. Calibration curves for Estragole and Anethole.

Table 2 shows the precision data obtained from the herbal based liquor for Estragole, Anethole and Linalool.

Description	Linalool	Estragole	Anethole
	Area	Area	Area
Sample 1	364459	332393	31162952
Sample 2	407646	353438	32121254
Sample 3	379104	332959	31672976
Sample 4	369767	298300	29948924
Sample 5	383566	313123	30258390
Sample 6	446215	357317	31830603
Mean	391793	331255	31165850
sd	30575	22761	885016
CV	7.80	6.87	2.84

Table 2. Precision data for Estragole, Anethole, and Linalool

Calculated concentrations of the herbal based liquor were calculated to be 0.16 µg/ml for Estragole and 13 µg/ml* for Anethole.
* above quantitation limit.

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Comparison of DHS verses Static Headspace

Limonene and Anethole were the only analytes detected by Static Headspace. Figure 4 shows extracted ion chromatograms of the herbal based liquor using the two different sample introduction techniques. Over a 100 fold increase in signal to noise for Anethole was observed for DHS.

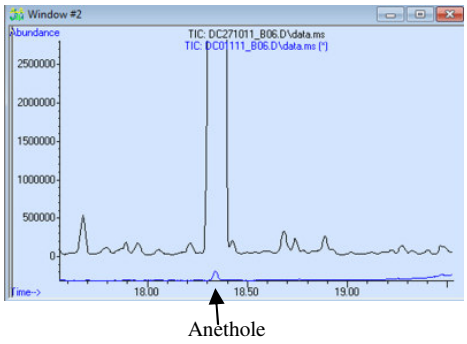

Figure 4. Comparison of Extracted ion Chromatograms using Static Headspace and Dynamic Headspace.

Many analytes were detected by Dynamic Headspace creating a good finger-print of the herbal based liquor. Figure 5 shows a TIC chromatogram of the herbal based liquor with some of the analytes being identified.

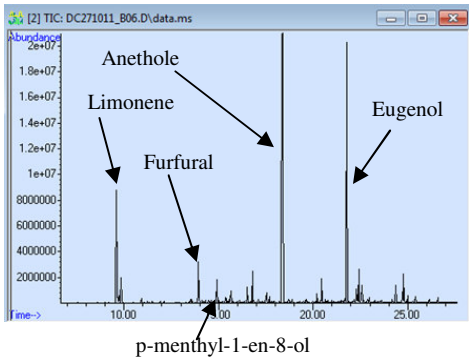

Figure 5. TIC Chromatogram of the Herbal based Liquor.

Conclusions

Presented is a fully automated method for the quantitation of Estragole and Anethole in a herbal based liquor using Dynamic headspace. This technique offers superior sensitivity when compared to static headspace.

References

[1] M. Markelov, J.P. Guzowski, Jr., Analytica Chimica Acta, 276 (1993) 235
[2] M. Markelov, O.A. Bershevits, Analytica Chimica Acta, 432 (2001) 213

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