Chromatography Technical Note No AS 153

Using Dynamic Headspace and Principal Component Analysis to find key differences between chocolate samples

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Introduction

For static headspace, an equilibrium of analyte concentration is formed between the gas phase and the liquid or solid sample phase. Using dynamic headspace (DHS), the equilibrium is altered by continuously removing the gas phase onto an absorption trap. Of course, after removal of analyte concentration from the gas phase, the equilibrium is then reformed which drives the concentration of analytes from the sample to the gas phase. The sensitivity for trace level analytes can be dramatically improved using dynamic headspace. Figure 1 shows a schematic view of the DHS Process.

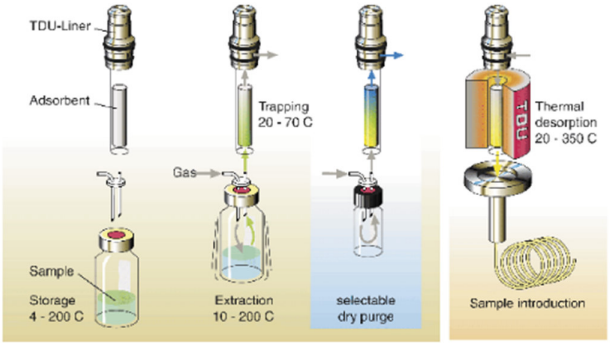

Figure 1 Schematic view of the DHS Process

Figure 2 shows the system used for this experiment. A MultiFlex GC/Q-TOF with DHS was used for this work. Maintaining the quality of chocolate is an important factor in its production and storage. Slight differences in the ingredients can drastically change the taste and smell. Therefore, it is important to maintain the quality of products for customer satisfaction. Figure 2 shows the system set up in our lab for the DHS and Static Headspace analysis.

Figure 2 MultiFlex GC/Q-TOF with DHS and Static Headspace with Odour port at Anatune.

Principal components analysis (PCA) can be used to visualise trends in complex data sets that would not be possible by manual investigation. Mass Profiler Professional is software provided by Agilent to interrogate and view complex data. This includes Principal Component Analysis plots (both scores and loading plots). From this data, we are able to find key differences between different chocolate samples which we would not able to find by eye.

Instrumentation

Dual Head GERSTEL MPS 2 with Headspace option/Agitator
GERSTEL Dynamic Headspace
GERSTEL Odour Port (ODP3)
Maestro software integrated
Agilent 7890 GC with a 7200 GC/Q-TOF
Agilent Mass Profiler Professional

Method

Six different chocolate bars were purchased from a local shop in Girton. Some bars were different products from the same manufacturer. Each chocolate bar was grated and weighed out into 20 ml vials for Headspace and DHS. Figure 3 shows a photograph of the chocolates analysed. Each chocolate sample was weighed out in triplicate.

Figure 3 Grated chocolate in 20 ml vials for Headspace and DHS experiments.

Headspace conditions

Incubation temperature:	50 ºC
Sample volume:	1ml
Inlet temperature:	250 ºC

DHS conditions

Incubation temperature:	50 ºC
Trap:	Tenax TA
DHS Trap temperature	40°C
Incubation temperature	50°C
Purge volume	1000 ml

Both methods were split at 20:1

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Results

Figure 4 shows a comparison of chocolate (Sample 3) chromatograms analysed by Static Headspace and by DHS.

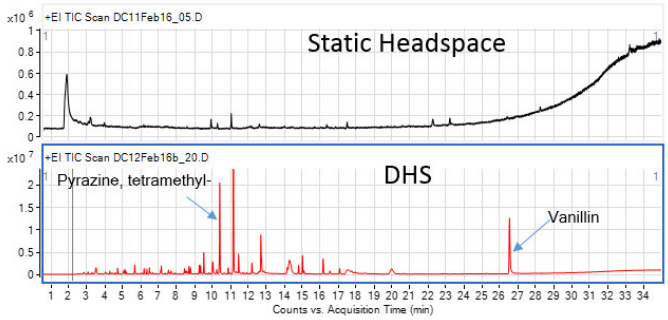

Figure 4 chromatograms of Sample 3 analysed by Static Headspace and by DHS.

It is clear to see that much more information on the chocolate profile for sample 3 is obtained by DHS. In combination with the extra sensitivity obtained from using a QTOF compared to a Single Quadrupole instrument, analytes at trace level can now be detected in the samples analysed. An odour port (ODP3) is also set up on the system, so that characteristic smells can be associated with selected analytes. Figure 5 shows an extracted ion chromatogram of the base peak for an analyte found in triplicate in chocolate sample 6.

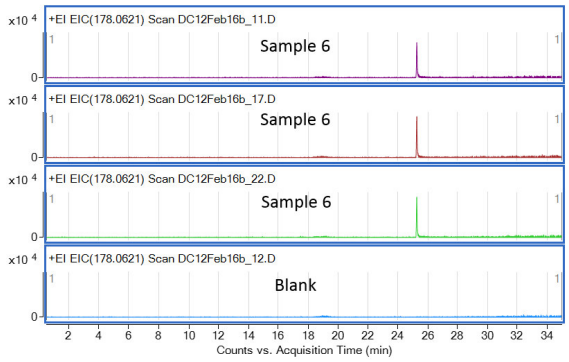

Figure 5 Extracted ion chromatogram 178 m/z for Chocolate sample 6

From the DHS data obtained for all the samples, some of the chocolate samples can show clear differences in their profiles obtained. However, different chocolate bars from the same manufacturer can be difficult to distinguish by eye. Figure 6 shows two different chocolate products from the same manufacturer which are difficult to distinguish by eye.

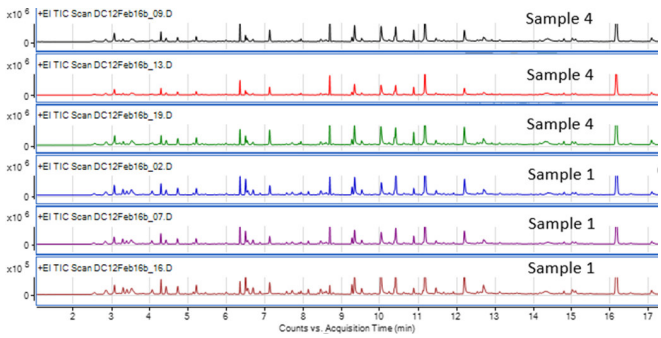

Figure 6 shows chromatograms from Chocolate sample 1 and sample 4.

Figure 7 shows a PCA scores plot for the different chocolate bars (in triplicate). Principal Component 1 is plotted against Principal Component 2. As you can see, some groups can be easily observed. Sample 3 can be seen to be significantly different from the other samples. Also sample 5 and sample 6 are separated from the other groups. Unfortunately, samples 1 and 4 were overlapping and the two groups could not be distinguished from the initial PCA.

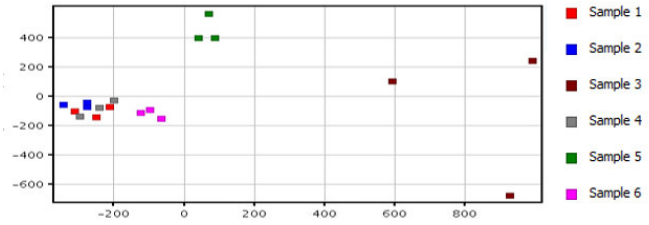

Figure 7 PCA Scores plot for Chocolate samples

However, by further PCA separation could be achieved by focusing on a selected portion of analytes from the left hand side of the loadings plot.

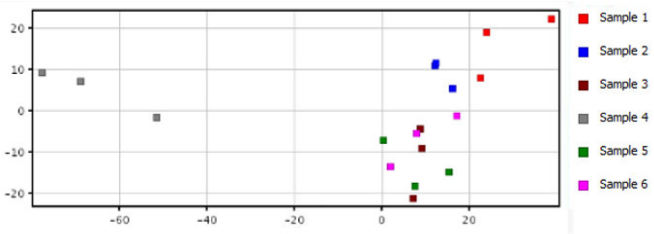

Figure 8 PCA scores plot for all chocolate samples (in triplicate)

From this data, we were able to find a ketone which was significantly higher in Chocolate sample 1. This is shown in figure 9.

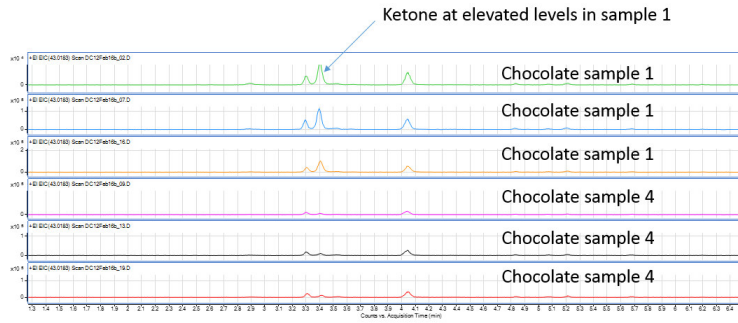

Figure 9 Extracted ion chromatogram at 43 m/z of the two different chocolate bars (in triplicate) from the same manufacturer.

Discussion

DHS offers significant signal to noise advantages over Static headspace. The increase in sensitivity means that those compounds that may be critical to taste and odour and therefore perceived quality of the product, but that may be present at low levels, can be more readily detected

The use of Mass Profiler Professional helps group the chocolate and find groups that may not be seen by eye.

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