

ANALYTICAL WORKFLOWS FOR DISTILLED SPIRITS ANALYSIS

APPLICATION NOTE AS-303

Authors

Dr Kathy Ridgway

Element Laboratory Solutions
Unit 4, Wellbrook Court, Girton Road,
Cambridge, CB3 0NA

+44 (0)1223 279210

enquiries.cambridge-LS@element.com

Abstract

Measurement of the volatile profiles of alcoholic beverages, such as Whisky, are important both for understanding the impact of processes on flavour characteristics and investigating counterfeit products. Compounds of interest may be present at extremely low levels, resulting in the requirement for highly sensitive and robust methods.

There are a number of techniques that can be employed for the analysis of flavours from liquid samples, many of which can be fully automated. For some spirits, a simple 'dilute and shoot' approach may be possible, but in order to meet the required limits of detection, some degree of enrichment is often required.

Automated liquid- liquid extraction, including Dispersive Liquid Liquid Micro Extraction (DiLLME) and headspace techniques such as Solid Phase Microextraction (SPME) or Dynamic Headspace (DHS) can provide the required degree of enrichment for this type of analysis.

The use of comprehensive 2- Dimensional GC (GCxGC) and fast scanning TOFMS instrumentation can also enhance the number of compounds detected and simplify data processing. Statistical data mining approaches can aid the identification of differences between samples.

This application note discusses the choice of sample preparation technique for distilled spirit analysis and offers examples of sample extraction and data analysis strategies. It clearly shows that the most suitable sample extraction technique will depend on the matrix and analytes of interest. Direct immersion (Stir Bar Sorptive Extraction (SBSE) can give increased response for the less volatile analytes, although may not extract the more polar compounds. DHS Fully Evaporative Technique (FET) and MultiVolatile Method (MVM), in general yield the broadest range of analytes. SPME is a good choice for obtaining a general profile of a sample, although can show some bias towards the most volatile components.

INTRODUCTION

The measurement of the volatile profiles of alcoholic beverages, such as Whisky, are important both for understanding flavour characteristics and detecting fraudulent products. The aroma and flavour active compounds may be present at extremely low levels, and so methods need to be sensitive and robust to ensure appropriate comparisons between products or to aid product development.

There are a number of techniques that can be employed for the analysis of volatiles and semi-volatile compounds from spirits. These fall into two categories: liquid-liquid extraction and headspace (thermal) extraction.

The latter includes techniques such as static and dynamic headspace (DHS), solid phase microextraction (SPME) and dynamic headspace (DHS) including the GERSTEL multi-volatile method (MVM).

The former category includes liquid-liquid (LLE) and stir bar sorptive extraction (SBSE or Twister™).

Several of these have been presented in previous application notes [1,2,3,4,5]

EXPERIMENTAL

Instrumentation

GERSTEL MultiPurpose Sampler (MPS), single head Robotic, or dual-head Robotic/RoboticPro. Agilent 7890 or 8890 with either MSD detector (5977), 7000 GC- QQQ (used in MS1 Scan), Agilent 7250 GC/QTOF or LECO Pegasus BT 4D TOF-MS

DILLME: Universal Syringe Module (USM) equipped with 10 µL syringe and Prep Syringe Module (PSM) with a 1000 µL syringe. GERSTEL QuickMix, Anatune CF200 Robotic centrifuge.

DHS (MVM): Gerstel Thermal Desorption Unit (TDU), Cooled Injection System (CIS) and Dynamic Headspace (DHS) module. TDU desorption tubes- Tenax™ TA and Carboxen™ B/Carboxen™ X/Shincarbon X. GERSTEL Twister™ stir bars – PDMS 10 mm x 0.5 mm (24 µL phase)

SPME: Agilent split/spitless inlet

An example instrument set-up is shown in Figure 1.



Figure 1: GERSTEL MPS Robotic Pro and DHS on Agilent GC-MS

Materials

Samples – commercially available whiskies (shown in Table 1) and matured and new make spirits (provided by Diageo) were used in the evaluation of the different techniques. Some experiments also included other commercially available spirits, such as Brandy and Gin. These are detailed in Table 1.

Table 1: Commercial Whisky Samples

| Reference | Description |
|-----------|-------------------------------|
| Sample A | Blended Scotch Whisky |
| Sample B | Blended Scotch Whisky (12yrs) |
| Sample C | Single Malt (Islay)- (10yrs) |
| Sample D | Irish Blended Whiskey |
| Sample E | Blended scotch Whisky |
| Sample F | Blended Scotch Whisky (Black) |

METHODS

DiLLME:

Each sample was diluted 50:50 with Milli-Q water. Duplicate aliquots (7 mL) of each of the diluted samples were manually loaded into high-recovery vials. The GERSTEL MPS was programmed to add 1000 μL dichloromethane (DCM) /pentane mix and 500 μL isopropyl alcohol (IPA). Samples were extracted using the GERSTEL QuickMix for 1 minute and then centrifuged for 5 minutes (4500 rpm), to produce a clear bottom layer of extraction solvent as illustrated in Figure 2.

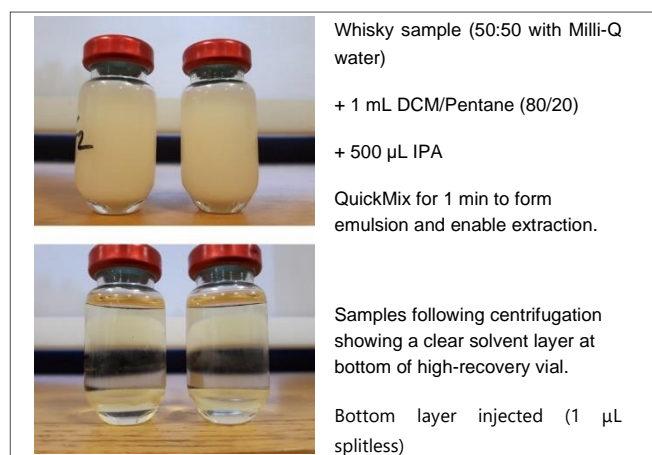


Figure 2: DiLLME of Whisky Samples

DHS: Duplicates of each sample (20 μL or 50 μL) were taken and following incubation at 80 $^{\circ}\text{C}$ for 5 minutes, extracted using a Tenax TA trap (750mL or 2L).

Twister: Duplicates of each sample (1 mL) were diluted with 4 mL water and extracted with PDMS Twister for 3 hours, stirring at 1000 rpm.

MVM: Duplicates of each sample (50 μL) were extracted with 3 traps as per MVM protocol:

The first DHS extraction was made with the Shincarbon X/Carbopack B+X adsorbent (incubation at 55 $^{\circ}\text{C}$, 10mL trapping, short dry) and then a second extraction was performed with the Tenax TA adsorbent (incubation at 80 $^{\circ}\text{C}$, 750 mL trapping, long dry). After these two extractions, the Tenax adsorbent is firstly desorbed in the TDU followed by the Shincarbon X/Carbopack B+X trap (Figure 3). After the two traps are desorbed, the CIS is heated to transfer analytes to the analytical column.

SPME:

Following incubation, the headspace was sampled using the mixed SPME fibre (DVB/CAR/PDMS) for 5 minutes. After this time, the fibre was removed and thermally desorbed in the GC inlet.

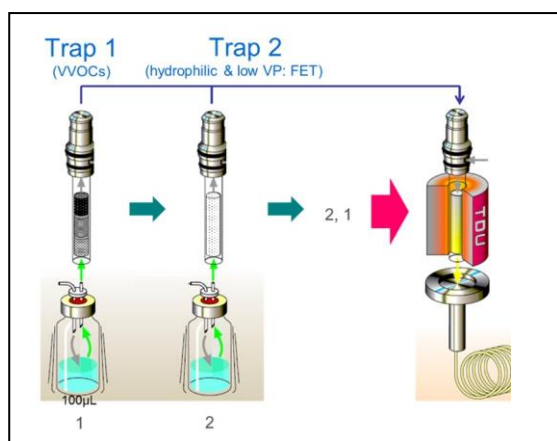


Figure 3: MVM method using 2 traps.

RESULTS AND DISCUSSION

A comparison of chromatograms obtained following Whisky analysis using SPME, single DHS and MVM methods is shown in Figures 4 and 5. The MVM extraction appears to result in better extraction for the majority of analytes and also gives some additional compounds, such as acetaldehyde, oak lactone, 1-hexadecanol and 5-hydroxymethylfurfural. However, it is clear that ethanol is being extracted by the first trap, although in this case, no peaks of interest were observed in this area of the chromatogram. Figure 5 shows a zoom to give the detail of the lower-level compounds with the three techniques.

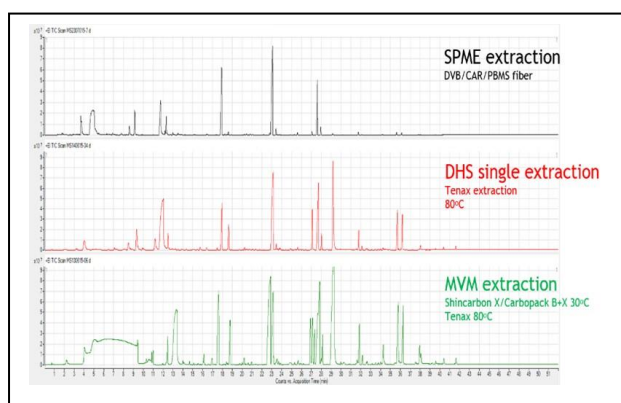


Figure 4: Comparison between 3 techniques

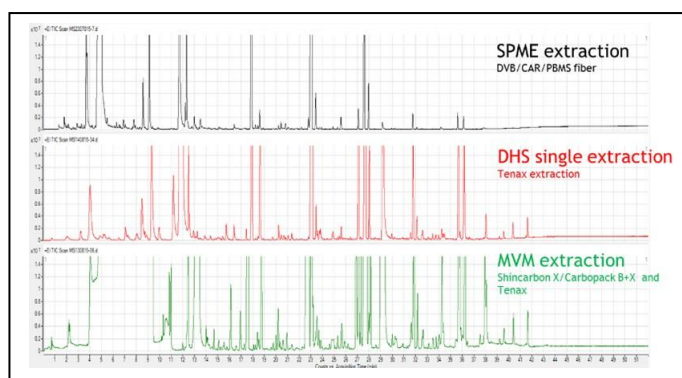


Figure 5: Magnification of the chromatograms shown in Figure 4.

In other to check the reproducibility of the method, 10 µL of a mix of two internal standards were added to the 100 µL of sample. The first internal standard was butyl acrylate and was used to check the extraction with the first trap. The second internal standard was benzyl 2,3,4,5,6 d-5 alcohol and was used to check the extraction with the second trap.

Table 2 shows the reproducibility of the internal standards and selected compounds determined in the whisky (both high and low concentrations).

Table 2: Reproducibility for selected compounds in whisky (n=5)

| Compounds | Retention time (min) | RSD (%) |
|-------------------------------------|----------------------|---------|
| 1-propanol, 2-methyl* | 5.35 | 8.1 |
| Butyl acrylate | 6.14 | 2.3 |
| Ethyl octanoate | 9.95 | 3.8 |
| Ethyl decanoate | 12.59 | 2.6 |
| Benzyl 2,3,4,5,6 d-5 alcohol | 16.31 | 4.7 |
| Ethyl oleate* | 24.23 | 5.4 |

*compounds in very low concentrations in the whisky

A comparison of extraction with DHS (single Tenax trap), SBSE with PDMS Twister and MVM (Shincarbon and Tenax traps) was made for a range of spirits and liqueur samples. MassHunter Unknowns analysis was used to determine the number of compounds observed using each of the techniques for all the samples. The results show that the most suitable technique depends on the analytes of interest. DHS (FET) and MVM, result in the broadest range of analytes, although MVM did give a large response for

ethanol. Only a small response for ethanol was observed using DHS (Tenax trap only) and SBSE (PDMS). The SBSE direct immersion approach appears to be more suitable for the less volatile analytes (as illustrated for the Gin in Figure 6), although did not extract the more polar analytes (such as phenyl ethyl alcohol) in the Brandy (Figure 7). The Shincarbon trap used in the MVM method was able to extract highly volatile analytes, such as acetaldehyde.

To look at data analysis workflows for differentiating samples, those samples detailed in Table 1 were analysed by dispersive liquid-liquid microextraction (DiLLME) and Agilent GC/Q-TOFMS and processed using Agilent MassHunter analysis with deconvolution and identification using NIST mass spectral matching.

This data was imported to Agilent Mass Profiler Professional (MPP) software for Statistical Significance and Principle Component Analysis (PCA).

Initial qualitative comparison of the chromatograms showed some differences which were identified as cresols and phenols.

After deconvolution of all samples in MassHunter Unknowns Analysis and subsequent MPP analysis, the PCA plots (Figure 8) showed clear grouping of the Whisky samples and the tight grouping of the individual replicates within the groups demonstrates good reproducibility of the DiLLME technique.

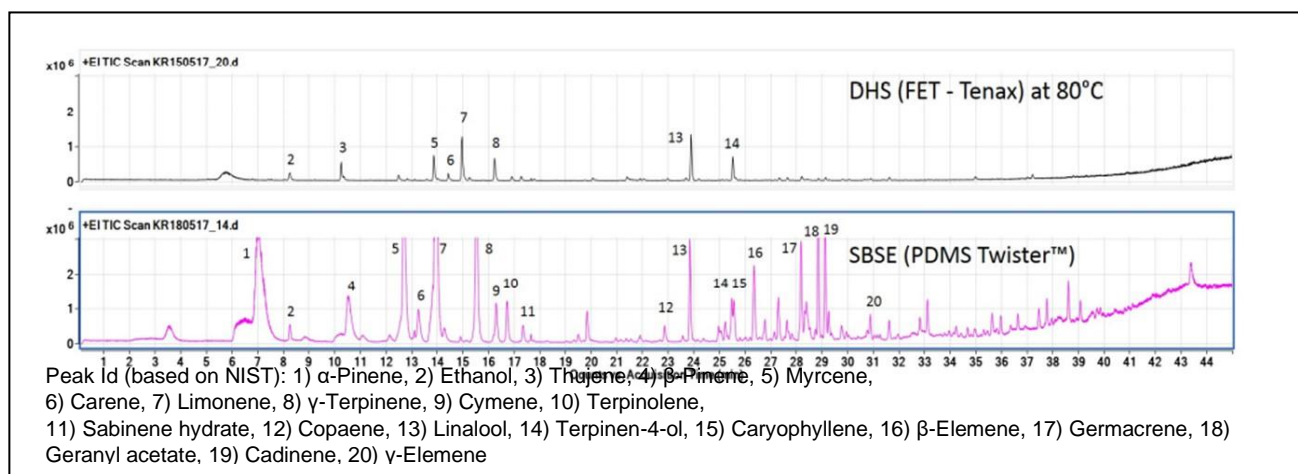


Figure 6: Comparison of Gin Profiles

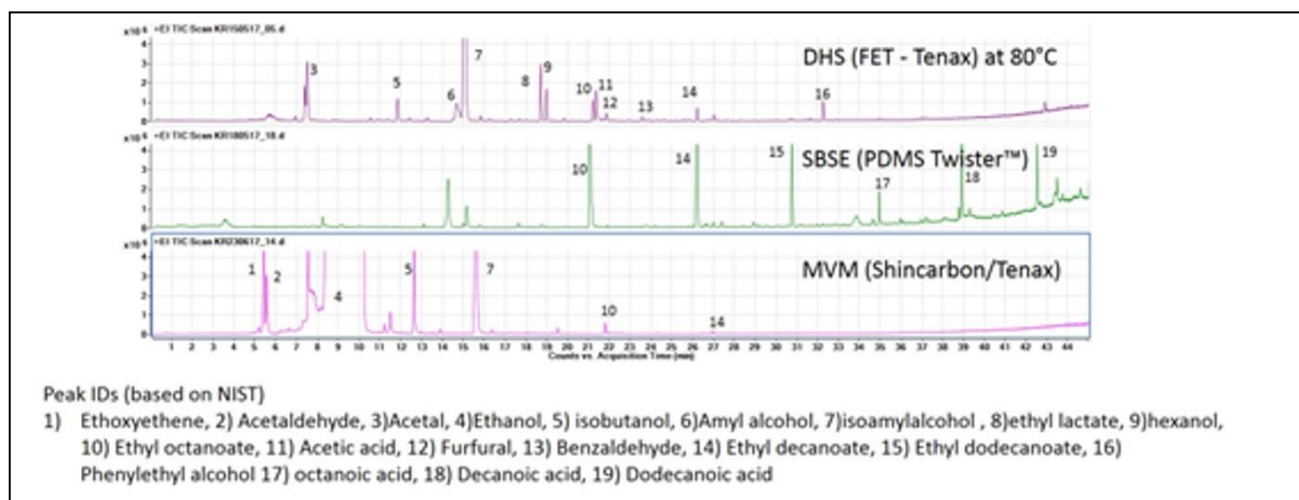


Figure 7: Comparison of Brandy Profiles

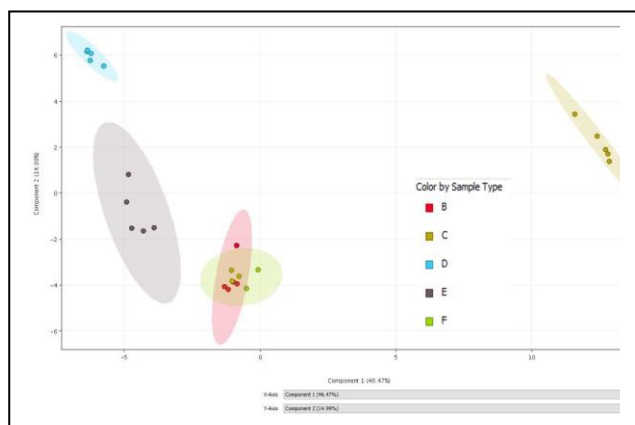


Figure 8: PCA from DiLLME data from MPP

Irish Whiskey (D) and Single Malt Islay whisky (C) are clearly separated from the other samples, with some similarities being noted in the blended Scotch Whiskies, in particular (B) and (F). MPP enabled further data interrogation to determine the compounds responsible for similarities/differences between groups. The Islay Whiskies are renowned for their smoky notes and compounds associated with this including cresols and phenols. An example of this is the elevated level of guaiacol (2-methoxyphenol) that was observed in Sample C compared to the other samples. Sample F had the second highest level and was a so-called ‘black’ blend containing some Islay malts.

In order to further investigate whisky profiles, the LECO Pegasus BT 4D GCXGC-TOF MS was assessed – performing analysis in both 1D and 2D acquisition modes. The samples analysed for this experiment were provided by Diageo and consisted of several new make spirit samples and matured whiskies. The data were treated as two distinct experiments and processed using the ChromaTOF software -using Sync for 1D and Tile for 2D. ChromaTOF Sync is a data mining software intended to perform peak finding and alignment on sets of similarly prepared samples by providing an Aligned Results Table (ART), with compiles peak information across the sample set. Once the list of aligned features is generated, the software can perform unsupervised PCA without any sample classes defined and univariate class comparison (one-way ANOVA or F-test) for all features reported to assess statistical significance.

ChromaTOF Tile works by dividing the contour plots into tiles – these contour plots are like heat maps; for each tile it will look at the response for each ion and this enables a comparison between samples using the Fisher ratios. Samples 1 to 6 were new-make spirits from different distilleries and samples 7 to 11 consisted of a new-make, two 3-year-old and two 12-year-old whisky samples.

The PCA plots obtained for the different experiments (2D acquisition) are shown in Figures 9 and 10.

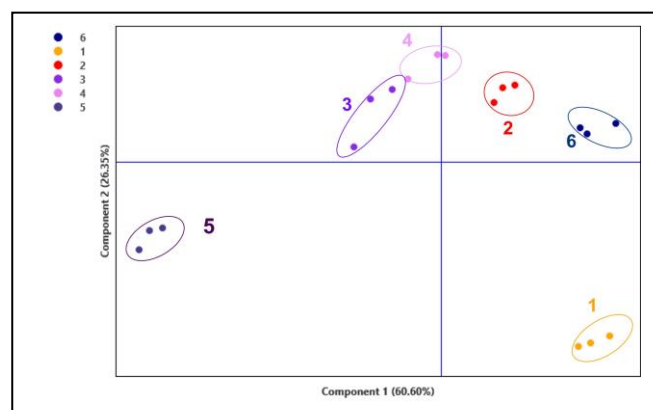


Figure 9: Dat set 1-6 2D.

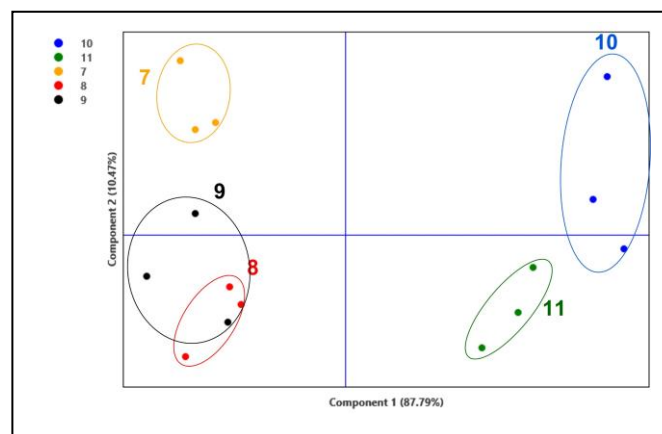


Figure 10: Data set 7-11 2D

Further interrogation of the data shows compounds responsible for the differences observed in the samples. Each of the new make samples 1 to 6 has a slightly different profile, with sample 3 showing the most unique features. This sample is known to be a peated-style spirit and showed elevated levels of compounds known to be related to this, but also other compounds that may be relevant and

provide a deeper level of information about each of the samples. Figure 11 shows a selection of compounds with area response (for selected ions) in each sample replicate and where available, sensory data from Goodscents (<http://www.thegoodscentscompany.com>)

which translated into better similarity scores for library searches. A number of compounds were identified in 2D that were not in 1D.

The use of untargeted data analysis and statistical data processing can provide an efficient way to compare samples and identify the compounds responsible for the differences. To provide relevance, this data should be linked to observed sensory properties.

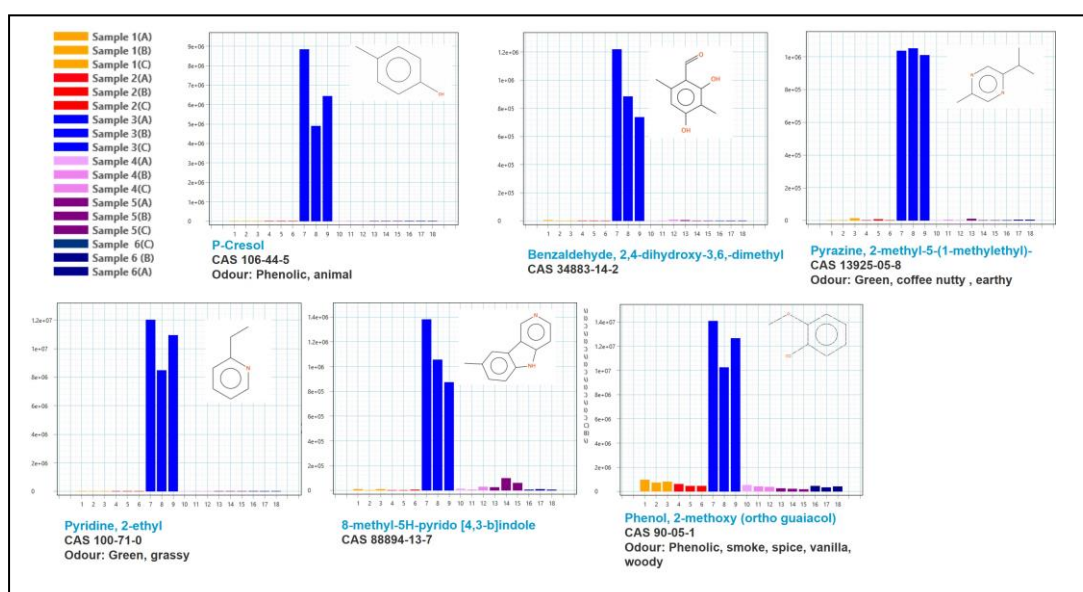


Figure 11: Data from ChromaTOF TILE for selected compounds with a higher response in sample 3

CONCLUSION

The results show that the most suitable sample extraction technique will depend on the analytes of interest. DHS (FET) and MVM, in general yield the broadest range of analytes, although MVM did give a large response for ethanol. A small response for ethanol was observed using DHS (Tenax trap only) and SBSE (PDMS). The SBSE direct immersion approach appears to be more suitable for the less volatile analytes (as illustrated for Gin), although did not extract the more polar analytes (such as phenyl ethyl alcohol) in the Brandy. The Shincarbon trap used on the MVM method was able to extract highly volatile analytes, such as acetaldehyde.

The 2D data showed superior separation of the dataset and higher confidence compound identification, thanks to improved peak purity

REFERENCES

1. Comparison of Extraction Techniques for Volatiles in a Selection of Spirits and Liqueurs; Dr Kathy Ridgway, Anatune Ltd. AS180, 2019
2. The Development of the MVM (Multi-Volatile Method) in the Whisky Matrix; Maïlie SAINT-HILAIRE, Kathy RIDGWAY, Anatune Ltd. Chromatography Technical Note No AS148, 2015
3. Comparison between the MVM (Multi-volatile Method), single DHS extraction and SPME (solid phase Microextraction) for extraction of volatiles in Whisky. Maïlie SAINT-HILAIRE, Kathy RIDGWAY, Anatune Ltd. Chromatography Technical Note No AS149, 2015

4. Dispersive liquid-liquid microextraction (DiLLME) for whisky flavour analysis; Dr Kathy Ridgway Anatune Ltd. AS232, 2020
5. Determining Phenolic Compounds in Whisky using Direct Large Volume Injection and Stir Bar Sorptive Extraction Yunyun Nie, Eike Kleine-Benne Gerstel GmbH & Co. KG, GERSTEL Application note 2012-02