

DISPERSIVE LIQUID-LIQUID MICROEXTRACTION (DiLLME) FOR WHISKY FLAVOUR ANALYSIS

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INTRODUCTION

The enjoyment of a good whisky is based on savouring the taste and aroma as we slowly sip and inhale the volatiles coming from the glass. Whisky manufacturers want this experience to be as good as it can be, and so being able to understand the flavour and aroma characteristics of their products can help them deliver the best product and detect counterfeits.

The measurement of the volatile profiles of alcoholic beverages, such as Whisky, are important both for understanding these 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 comparisons to be made between different blends.

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. These basically fall into two groups; liquid-liquid extraction and headspace (or thermal extraction).

The latter includes techniques such as headspace, solid phase microextraction (SPME) and dynamic headspace (DHS) including the GERSTEL multivolatile method (MVM) which have been covered in previous application notes (1,2). These have the advantage of no solvents being used, but can require the sample to be warmed which may have an effect on the sample causing reaction products to be formed or requiring a long extraction time.

The former category includes liquid-liquid of which this application note will detail and stir bar sorptive extraction (SBSE or Twister, 3,4).

Automated liquid extraction can be used as an alternative to more established flavour profiling techniques and provide selectivity through choice of solvent and sensitivity by use of large volume injection (LVI).

In this application note, the use of Dispersive liquid-liquid MicroExtraction (DiLLME) for flavour analysis in Whisky will be presented as an alternative automated approach.

DiLLME uses a much smaller volume of solvent (μL 's) and sample (<10 mL) compared to standard liquid-liquid extraction which not only has the benefits of smaller volumes but makes the extraction time much shorter. The extraction time is further accelerated as the emulsion formed in the mixing phase ensures maximum surface area contact between the sample and the solvent.

INSTRUMENTATION

GERSTEL Dual head MultiPurpose Sampler (MPS) Robotic/Robotic^{PRO} with 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. Agilent 7250 GC/Q-TOF and Agilent 5977B HES GC/ MS. The system set up for DiLLME is shown in Figure 1.

Data were processed using MassHunter 10 (Qual and unknowns) and subsequently analysed using Mass profiler professional (MPP) (version 15).



Figure 1: DiLLME set up (Centrifuge, QuickMix and sample tray with High recovery Vials)

MATERIALS

Samples are detailed in Table 1 - a number of commercially available Whisky samples were used for this work and were selected due to different flavour profiles (based on taste and odour).

Table 1: 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)

METHOD

Each sample was diluted 50:50 in milliQ 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 isopropylalcohol (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.

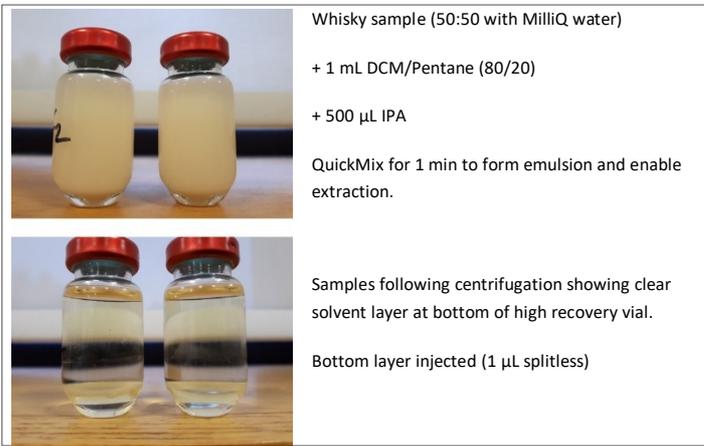


Figure 2: Whisky samples

GC-MS parameters:

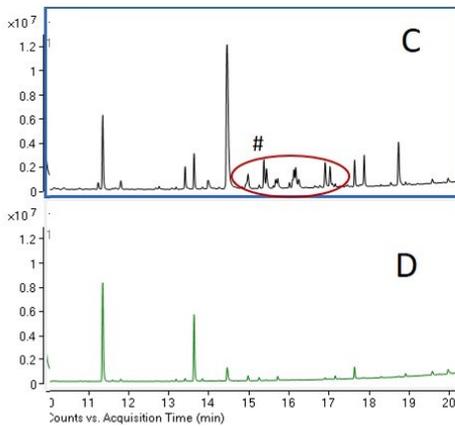
GC Column: DB- FFAP, 30 m x 0.25 µm x 0.25 µm,
 Helium carrier gas, 1 mL/min flow
 Oven Program: initial 40 °C (held for 1 minute) then ramped at 10 °C/min
 to 250 °C (held 3 minutes)
 1 µL injection splitless

DATA ANALYSIS

Each of the chromatograms were qualitatively compared using Agilent Masshunter Qualitative software. Each chromatogram was deconvoluted and peaks were identified using NIST Mass Spectral matching within the software. This data was then exported to Agilent Mass Profiler Profession software for Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

Initial qualitative comparison of the chromatograms showed differences between Whisky types which were most prominent in the region between 15 – 17 minutes and these were identified as cresols and phenols.



Peaks observed in sample C – identified as Cresols and Phenols

Figure 3: Comparison of DiLLME chromatograms (Whisky C and D)

Replicates of samples showed good reproducibility using peak areas from the TIC (RSD 6%, n=5), as shown in Figure 4.

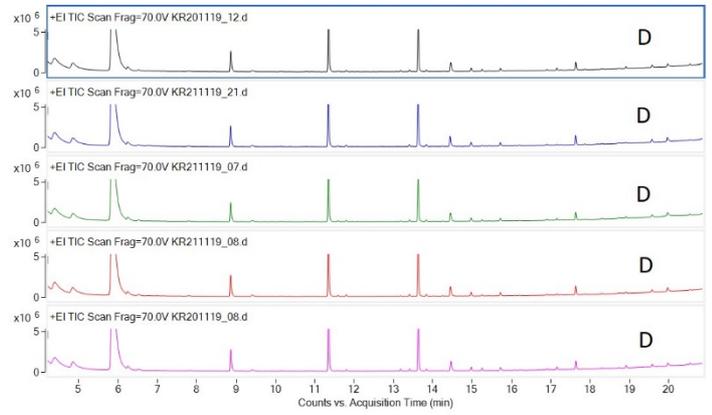


Figure 4: Replicates of Whisky (TIC) chromatograms

Deconvolution in MassHunter unknowns (using Suremass) determined a number of components present in each of the samples and was able to identify differences between some of the Whisky samples analysed (Figure 5). The subsequent Mass Profiler Professional analysis showed clear grouping of the Whisky samples and the tight grouping of the individual analysis within the groups also shows that DiLLME has good reproducibility. (Figure 6).

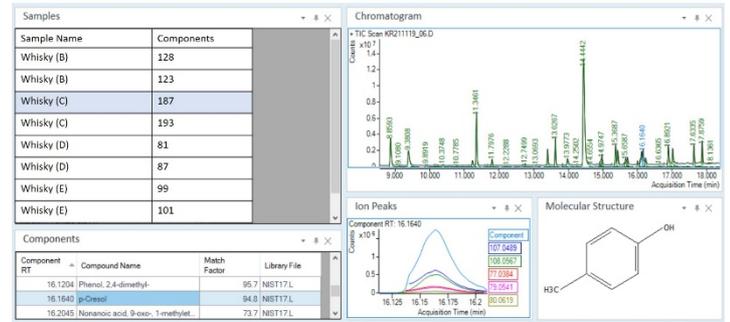


Figure 5: Components identified in MassHunter unknowns.

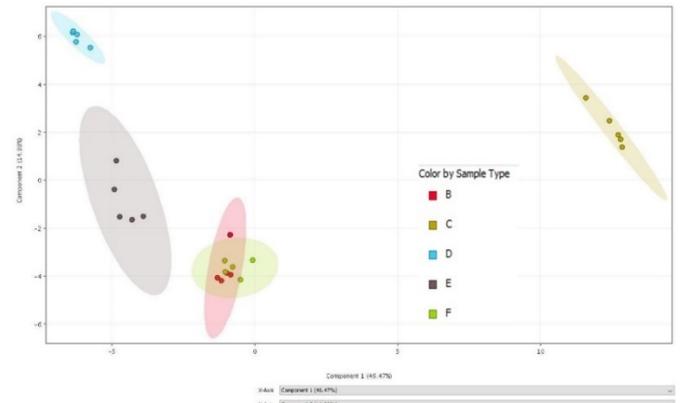


Figure 6: PCA from DiLLME data from MPP.

The 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 enables 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 include cresols and phenols. An example of this, is the level of Guaiacol (2-Methoxyphenol) that was observed in Sample C compared to the other samples as illustrated in Figure 7. Sample F had the second highest level and was a so-called 'black' blend containing some Islay malts.

Example flavour compounds – Guaiacol (EIC)

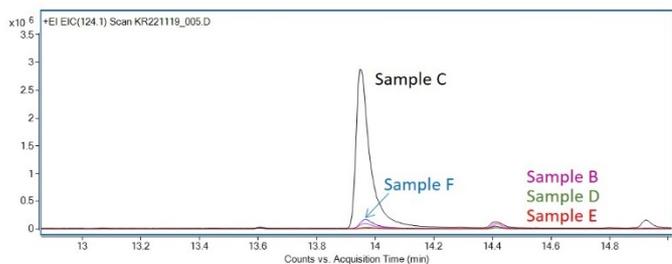


Figure 7: Comparison of Guaiacol levels in Whisky samples (EIC).

CONCLUSIONS

This work was performed using the Agilent 7250 Q-TOF, but subsequent analysis of extracts using the single quadrupole instrument (Agilent 5977B with HES) were comparable. In this situation as the additional selectivity and accurate mass which the Q-TOF has was not required. However, for a more in-depth analysis of the samples these parameters would be of benefit when identifying components which are lower in concentration.

This work demonstrates that DiLLME is a suitable technique to extract a range of compounds relevant to flavour in Whiskies. Compounds observed included esters, whisky lactones, phenolic compounds and aldehydes, as well as less volatile compounds associated with the aging process, such as Homovanillic acid and Syringylacetone.

The main advantages of DiLLME over other extraction techniques for extracting flavour/aroma active compounds is that it requires a very small amount of solvent, is fully automatable and is quick enough to be performed whilst the previous sample is being analysed. The reduction in use of solvents compared to a manual liquid extraction also gives safety and environmental benefits as well as cost savings.

Further optimization could include larger volume injection of the extract or using less extraction solvent to increase sensitivity for determining compounds at lower concentrations. Although not tested in this work the reproducibility of the data indicates that this extraction technique may also be developed for use in a quantitative or semi-quantitative method with the possibility of using alternative solvents to change selectivity too.

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. 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
4. Using Three Types of Twister Phases for Stir Bar Sorptive Extraction of Whisky, Wine and Fruit Juice; Yunyun Nie, Eike Kleine-Benne Gerstel GmbH & Co. KG, GERSTEL Application note 2011-03