AUTOMATED SOLVENT EXTRACTION (LLME AND DLLME) OF FLAVOURS FROM A SELECTION OF FRUIT BEVERAGES

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INTRODUCTION

There are a number of techniques that can be employed for extraction of flavours from liquid samples, most of which can be fully automated with the GERSTEL MPS platform. Liquid-liquid micro extraction (LLME) and Dispersive liquid-liquid micro extraction (DLLME) have been used for a number of targeted applications (1,2,3). In this application note, the use of these approaches for flavour profiling was investigated. A selection of fruit beverages were obtained locally and methods investigated in order to evaluate the compounds observed.

This initial work focussed on DLLME for flavoured water/juice drinks and LLME for direct extraction of Orange Juice. As a comparison, HS-SPME was also performed on each of the samples tested.

All techniques were run on a GERSTEL MPS robotic pro mounted on an Agilent GC-MS, with HES ion source, using MS scan acquisition. Peaks were tentatively identified using NIST mass spectral library search.

The System set up is shown in Figure 1.

MATERIALS AND METHODS

Samples are detailed in Table 1

Instrumentation

Autosampler: Dual Head MPS Robotic, USM tool equipped with 10 µL syringe, PSM tool equipped with 1 ml syringe, Quickmix and Centrifuge, SPME tool with mixed fibre installed (Divinylbenzene/ Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS))

GC-MS: Agilent GC 7890 B coupled to Agilent 5977 MS, HES source

Figure 1: GERSTEL Dual Head Robotic MPS on top of Agilent GC7890 B coupled to 5977 B MS (HES)

Table 1: Fruit beverage samples

Sample reference	Sample description
Water (blank)	Bottled mineral water
Sample (A)	Still Flavoured water – Orange and Pineapple
Sample (B)	Fruit Splash – Apple & Blackcurrant juice drink
Sample (C)	Fruit shoot - Orange
Sample (D)	Orange Juice (100% squeezed smooth, not from concentrate)

DLLME on flavoured water/juice drinks

Preliminary work evaluating variations in extraction solvents showed a better response for some compounds using a combination of IPA and DCM/Pentane (compared to DCM only and DCM/Pentane only). A clear bottom layer of extraction solvent was observed (Figure 2).
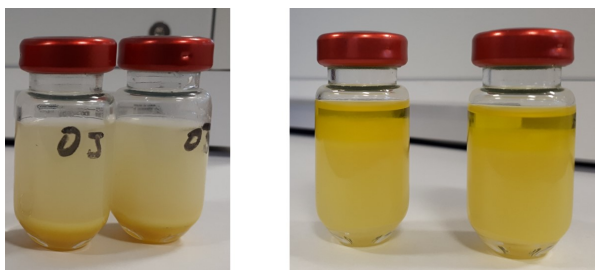
Sample extraction:

Duplicate aliquots (6 ml) of each of the samples were manually loaded into high recovery vials. The GERSTEL MPS was programmed to add 600 µl IPA and 500 µl DCM/Pentane (80/20). Samples were extracted using the GERSTEL QuickMix for 3 minutes at 2000 rpm. Following this to aid phase separation, samples were centrifuged for 1 min (4500 rpm), prior to an aliquot of the bottom layer being taken for GC-MS analysis. Injections were either 1 µl (splitless) or 10 µl (solvent vent) using a CIS inlet (ramped from 10 °C to 250 °C) with glass wool liner.
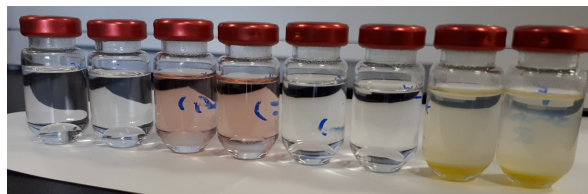
Figure 2: DLLME of flavoured drinks (samples left to right – water, samples (A) to (C))

Extraction of flavours from orange juice

Preliminary work evaluating variations in extraction solvents showed that DCM was not suitable for direct liquid micro extraction of Orange Juice, as the pulp was drawn to the bottom layer. Liquid-liquid extractions with Hexane were demonstrated to give a good response for a range of compounds and enabled automation by removal of the top solvent layer for injection (Figure 3).

Figure 3: Extraction solvents with Orange Juice

(A) Orange Juice + DCM (bottom layer)

(B) Orange Juice + Hexane (top layer)

HS-SPME analysis

Sample extraction:

Duplicate aliquots (6 ml) of each of the samples were manually loaded into 20 ml headspace vials. Following incubation at 40 °C for 20 minutes, samples were extracted using a mixed fibre (Divinylbenzene / Carboxen / Polydimethylsiloxane (DVB/CAR/PDMS) for 10 minutes prior to splitless desorption at 250 °C.

GC-MS parameters:

GC-MS parameters (for all extraction techniques)

GC Column: DB- FFAP, 30 m x 0.25 μm x 0.25 μm, Helium carrier gas, 1.2 ml/min flow

Oven Program: initial 40 °C (held for 1 minute) then ramped at 5 °C/min to 200 then 10 °C/min to 250 °C (held 2 minutes)

Sample extraction:

Duplicate aliquots (6 ml) were manually loaded into high recovery vials. The GERSTEL MPS was programmed to add 2000 μl Hexane. Samples were extracted using the GERSTEL QuickMix for 3 minutes at 2000 rpm. Following this to aid phase separation, samples were centrifuged for 1 min (4500 rpm). An aliquot of the top solvent layer was then taken for GC-MS analysis, either 1 μl (splitless) or 10 μl (solvent vent) using a CIS inlet (ramped from 10 °C to 250 °C) with glass wool liner.

RESULTS AND DISCUSION

DLLME of juice drinks

As expected the 10 μl injections gave a better response than 1 μl. Several peaks were identified in the chromatograms obtained (Figure 4) using NIST library spectral searches. Each of the drinks gave a different profile (Figure 4), with characteristic flavour compounds.
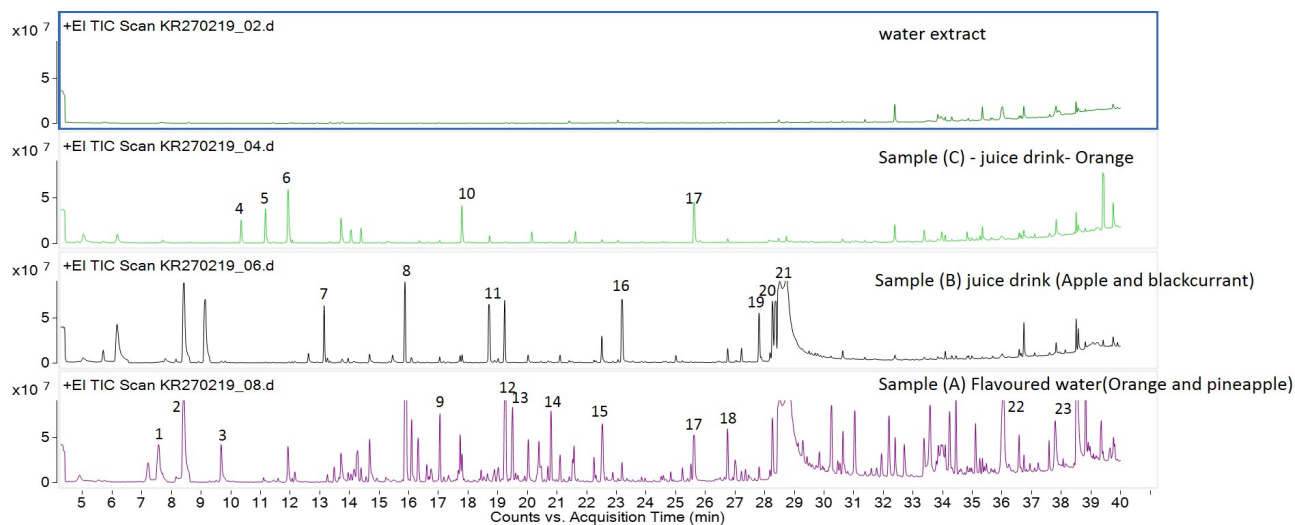
1) Isoamyl acetate, 2) Ethyl hexanoate, 3) Octanal 4) Hexenyl acetate 5) Hexanol, 6) Hexenol 7) ethyl octanoate , 8) Linalool, 9)Terpinen-4-ol, 10) Butanoic acid 11) methylbutanoic acid, 12) alpha-Terpineol 13) Valencene 14) Citronellol 15) Geraniol 16) Benzyl alcohol 17) Glutaconic anhydride, 18) Octanoic acid 19) Isoaleprolic acid , 20) gamma-decalactone, 21) sorbic acid, 22) Linoleic acid 23) hexadecanoic acid

Figure 4: Chromatograms from DLLME extraction of fruit drink (10 μl injections)

Comparing the DLLME extract chromatograms to those obtained from HS-SPME extraction (Figure 5) clearly shows a different flavour profile. As the SPME is sampling the headspace, this technique favours the more volatile components and the direct solvent extraction the less volatile. Also with HS-SPME, as 'injection' is via direct thermal desorption of the fibre, no solvent delay is required therefore earlier peaks can be observed.
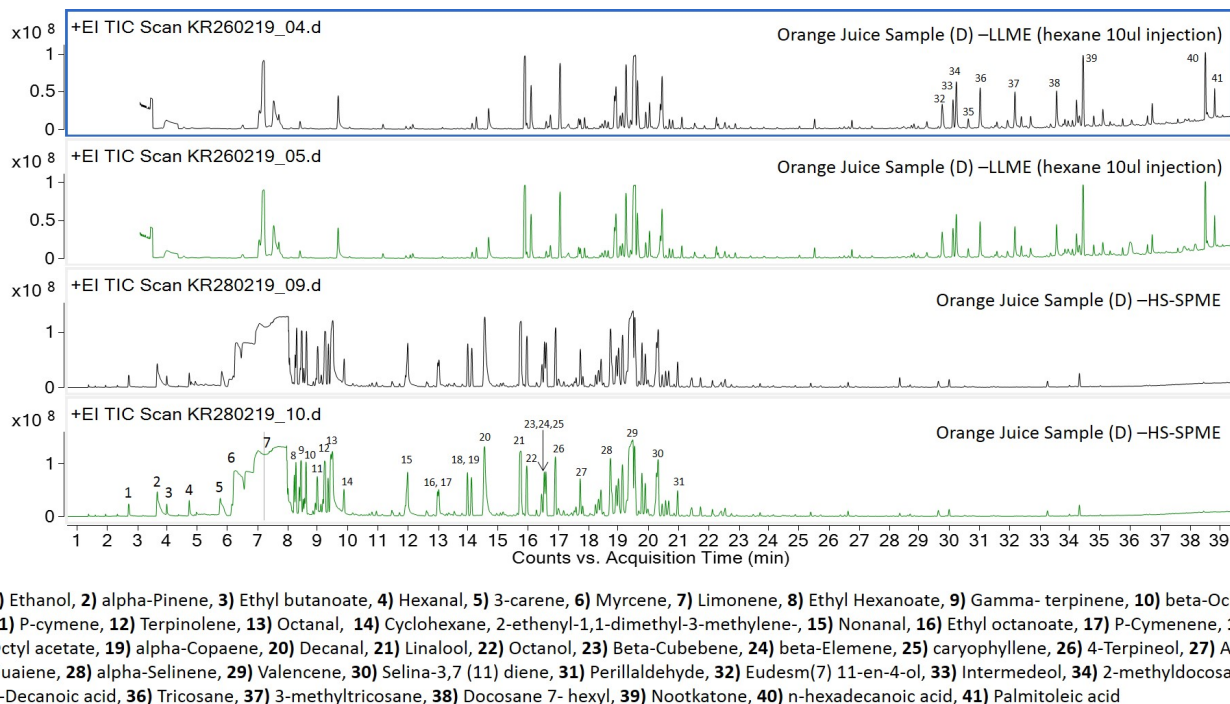
Extraction of orange Juice

Following extraction with hexane, 10 µl injections were compared with HS-SPME analysis (Figure 6). Although no solvent peak is observed with HS-SPME, in this example a large response for a matrix component was observed in the chromatogram (Limonene). Depending on the choice of Fibre SPME methods can be susceptible to matrix effects and so called 'fibre poisoning'. As observed with the drink samples, the SPME favours the more volatile components and the direct solvent extraction the less volatile. Several peaks were identified in the chromatograms obtained using NIST library spectral searches.

1) Ethyl acetate, 2) DCM, 3) Ethyl butanoate, 4) Ethyl-2-methylbutanoate, 5) isoamylacetate, 6) beta-pinene, 7) Limonene, 8) Ethyl hexanoate, 9) pentyl butanoate, 10) Octanal , 11) Hexenol, 12) Ethyl Octanoate, 13) Linaool , 14) alpha -Terpineol, 15) delta-Cadinene 16) Beta Atlantol, 17) gamma decalactone, 18) sorbic acid, 19) 2-methyldocosane, 20) Tricosane, 21) 3-methyltricosane, 22) Hentriacontane, 23) Pentacosane, 24) Nootkatone, 25) Linoleic acid, 26) n-Hexadecanoic acid

Figure 5: GC-MS chromatograms from DLLME and SPME of fruit drinks

1) Ethanol, 2) alpha-Pinene, 3) Ethyl butanoate, 4) Hexanal, 5) 3-carene, 6) Myrcene, 7) Limonene, 8) Ethyl Hexanoate, 9) Gamma- terpinene, 10) beta-Ocimene, 11) P-cymene, 12) Terpinolene, 13) Octanal, 14) Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-, 15) Nonanal, 16) Ethyl octanoate, 17) P-Cymenene, 18) Octyl acetate, 19) alpha-Copaene, 20) Decanal, 21) Linalool, 22) Octanol, 23) Beta-Cubebene, 24) beta-Elemene, 25) caryophyllene, 26) 4-Terpineol, 27) Alpha-Guaiene, 28) alpha-Selinene, 29) Valencene, 30) Selina-3,7 (11) diene, 31) Perillaldehyde, 32) Eudesm(7) 11-en-4-ol, 33) Intermedeol, 34) 2-methyldocosane, 35) n-Decanoic acid, 36) Tricosane, 37) 3-methyltricosane, 38) Docosane 7- hexyl, 39) Nootkatone, 40) n-hexadecanoic acid, 41) Palmitoleic acid

Figure 6: GC-MS chromatograms from LLME and SPME of Orange Juice

CONCLUSIONS

This work illustrates how important the choice of sample preparation is on obtaining the required information about samples. Automated liquid extraction could be used as an alternative to more established flavour profiling techniques. Selectivity can be achieved through choice of solvent and sensitivity enhanced by use of low volumes of extraction solvent and large volume injection into the GERSTEL CIS. With SPME, selectivity is through choice of fibre and extraction parameters, such as temperature. Where samples may change or analytes form on heating, direct liquid extraction may be preferred. Methods would need to be optimized for specific matrix types and further work performed to establish recoveries if quantitative analysis is required. If you are interested in solvent extraction and want to try Automated DLLME on your samples, please contact enquiries@anatune.co.uk

A video of the technique can be found here.

REFERENCES

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