Sample Preparation Options for Aroma Analysis

by Kathy Ridgway, Anatune Ltd
Unit 4, Wellbrook Court, Girton Road, Cambridge, CB3 0NA
kathy.ridgway@anatune.co.uk

Following developments in Gas Chromatography (GC) instrumentation and data analysis software, the need for good reproducible sample preparation is often underestimated. The choice of sample preparation procedure can be critical in obtaining key information about a sample, or reaching required limits of detection. A number of sample preparation techniques are available that provide extraction and enrichment in one step, and can be easily automated.

This article will discuss some of the options that are particularly suited to Aroma analysis. Aroma compounds can have a range of chemical and physical properties and it is not always safe to assume that all compounds can be extracted using one analytical approach. The choice of technique will depend on the information required. Methods may involve the use of sophisticated GC systems (such as the Agilent GC-Quadrupole-Time of Flight (QTOF), or for real time aroma release measurement Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS).

Some established and more recently developed techniques will be presented and discussed.

As instrumentation and software tools have improved and the trend for multi-analyte methods has increased, the importance of effective, reproducible, sample preparation can sometimes be forgotten. The requirement to see everything in a sample means that often, very specific targeted sample preparation methods, with one or only a small number of target analytes are becoming less common. Increasingly a 2-step approach is taken, employing both a non-target screen method, followed with a more robust targeted analysis for known analytes. For both these approaches, the choice of sample preparation procedure can be critical in obtaining the key information about a sample. The choice will depend on the requirements, and the reasons for sample preparation, whether for clean-up, enrichment, or both. Objectives for the method in terms of selectivity and sensitivity need to be defined.

The ideal sample preparation will contain the minimum number of steps in order to achieve these objectives. In terms of cost – both monetary and environmental, it is also preferable to avoid the use of large volume of solvents.

Miniaturisation and automation of sample preparation is not new, but an increasing number of steps that form part of standard preparation protocols can now be automated. These include liquid extraction, solid phase extraction, centrifugation and evaporation/reconstitution. Other automated sample preparation techniques are available that provide extraction, clean up and enrichment in one step.

Aroma analysis is one area where the choice of sample preparation can be key to obtaining the desired information about a sample. Analysis of Aroma compounds presents several challenges and the purpose of the analysis needs to be well defined to develop a method that is fit for purpose. Extremely low levels can be relevant, which can present a challenge in some complex matrices, particularly for a non-target approach where selective clean up cannot be used. In profiling work, it is also critical to understand what differences are within the normal variation for your sample and the impact of each of the components from a sensory perspective.

A direct 'dilute and shoot' approach may be sufficient for a simple Quality Control (QC) analysis, but for a complete Aroma profile, or identification of a taint or 'off' odour, it is likely that alternatives will need to be considered. In the case of taints or 'off' odours, the wrong approach may result in the compounds responsible not being detected at all and if you have limited sample size, repeat analysis may not be an option.

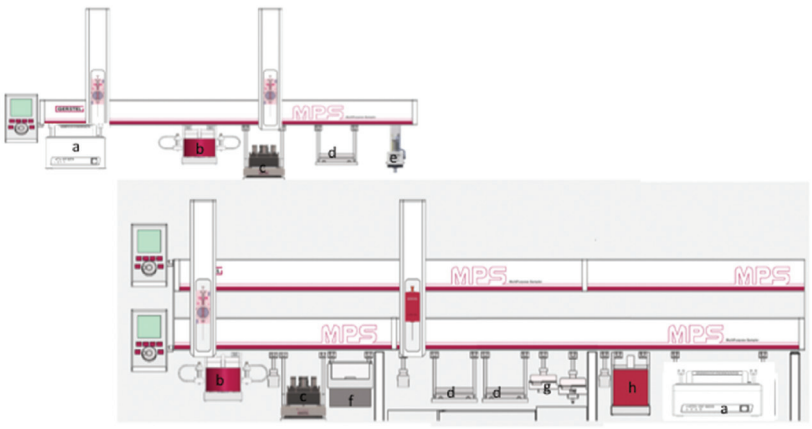

Figure 1: Comparison of Gerstel Multi-Purpose Sampler (MPS) for liquid-liquid extraction and a more complex dual rail system (a:CF200 centrifuge, b:mVap, c:Mvorx, d:sample tray, e:solvent filing station, f:cooled sample tray, g: wash station, h: agitator)

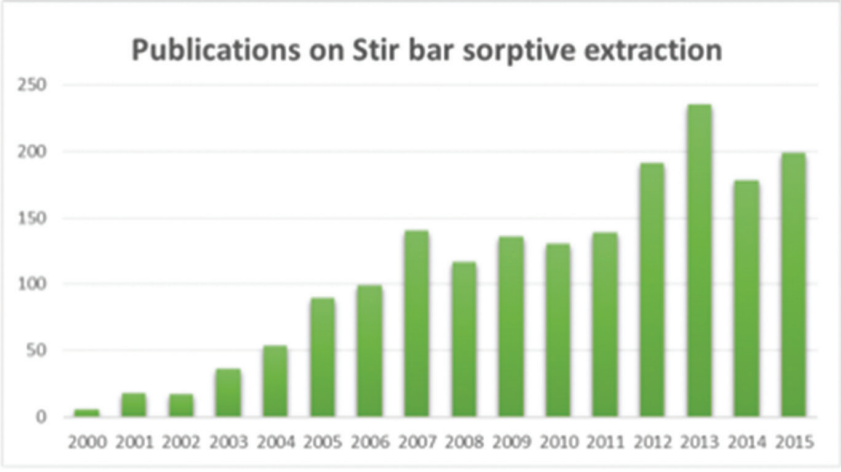

Figure 2: Number of SBSE publications by year (up to Oct 2015).

As most aroma compounds are volatile, gas chromatography is the instrument of choice – either with a flame ionisation detector (FID) or more selective detectors for quantitation of target compound or mass spectrometric detectors for identification of unknowns. Each of the automated sample preparation techniques outlined in this article can be integrated online with a range of GC instrumentation, or can be used as standalone sample preparation solutions. As can be seen from Figure 1, solutions can range from a basic liquid extraction system to a much more complex dual rail approach enabling automation of several complex sample preparation protocols. These include liquid-liquid extraction with an automated vortex mixer (Gerstel mVorx), evaporation of solvent using vacuum with the Gerstel automated solvent evaporator (mVap), as well as different liquid handling and agitator options to enable reconstitution, derivatisation and direct injection into the GC (if required).

Sample Preparation Options Direct (liquid) Extraction Approaches

Liquid extractions are thought to be more exhaustive, but may require several extractions and invariably require some enrichment, either through evaporation or solid phase extraction clean up. Traditionally this approach is labour intensive and often the bottleneck in analysis. However, the use of automated systems not only increases efficiency, and aids in method development, but also leads to an improvement in robustness and in some cases can have an additional benefit of the analyst not having to handle potentially harmful chemicals.

One option that provides some selectivity and enrichment is the use of stir bar sorptive extraction (SBSE). This is effectively a miniaturised direct liquid-liquid extraction and when thermal desorption is used for injection, can provide extremely good enrichment factors. SBSE is now an established technique, as the number of publications (Figure 2) illustrates. It is particularly suited to low level target analytes in aqueous matrices, and has been demonstrated for determination of food taints [1], malodour compounds in water [2] and for analysis of volatiles for characterisation and differentiation of high quality vinegars [3], as well as analysis of wine, ham, and a range of other food aroma applications.

Previously this technique was limited to non-polar analytes, but now there is a second phase available that is designed to extract more polar analytes and is particularly suitable for compounds such as phenols, which can be very aroma active. For a broader screen, both extraction phases can be used and if desired thermally desorbed simultaneously to obtain a single chromatogram. To enable multi-stir bar extraction Gerstel developed the 'Twicester' (Figure 3) which can also be used to sample a liquid and headspace concurrently.

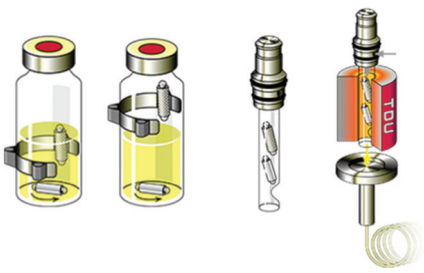

Figure 3: Gerstel Twicester enables 2 or more Twister stir bars to be extracted simultaneously

Headspace Techniques

As most aroma compounds are volatile, headspace sampling methods are often employed. Static headspace approaches rely on the equilibrium between the sample and the headspace, following heating and agitation for a defined period of time. By measuring the concentration in the headspace, the levels in the samples can be compared. Direct static headspace can be employed for most aroma compounds, although it has limited sensitivity as it provides no enrichment and typically only 1ml of sample headspace is taken. Multiple headspace injections can be taken from one sample, to provide a more exhaustive extraction.

Alternatives that provide enrichment include headspace solid phase microextraction (HS-SPME), where the analytes in the headspace are extracted onto a fibre coated with an extraction phase. This provides some selectivity (depending on the choice of fibre) and as the fibre is then thermally desorbed also provides enrichment, resulting in improved limits of detection. This approach can also be used for sampling liquids directly, although this is much less common, due to matrix interferences and issues with cleaning the fibres between samples.

Another way of enriching sample headspace is to sample dynamically, by purging the headspace with a flush gas and trapping analytes onto a sorbent. Several automated purge and trap systems are available, including the Gerstel dynamic headspace system (DHS), which uses the thermal desorption unit as the injector, enabling efficient transfer of analytes into the GC instrument. This technique is a powerful tool to look at the more minor components in a sample, due to the level of enrichment that can be achieved. Using an approach known as full evaporative technique (FET) also extends the range of compounds that can be observed. In this technique, a very small volume of sample is taken and heated to ensure all the analytes are in the gas phase, prior to dynamic headspace sampling. This enables compounds that would not partition readily into the headspace, such as more polar analytes, to be sampled as well as the more volatile/less hydrophilic and has an additional advantage that, due to the small volume taken, a drying step is generally not necessary. The advantage of this approach compared to a standard DHS method is illustrated in Figure 4 for analysis of fragrance in a cream.

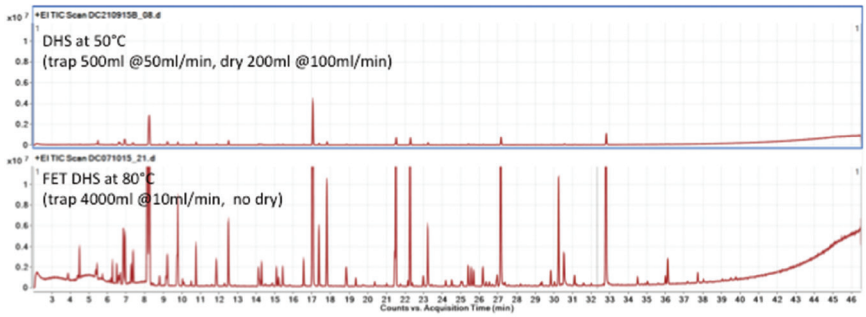

Figure 4: A comparison of standard DHS and FET DHS

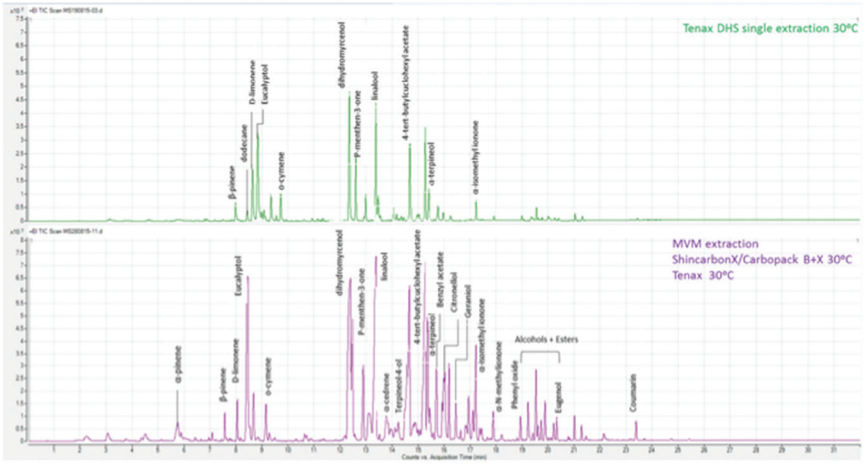

Figure 5: A comparison of a single DHS extraction and the multi-volatile method (MVM)

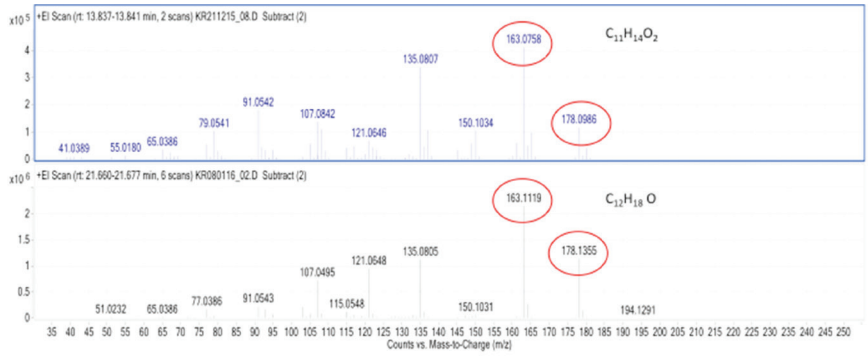

Figure 6: An example of structural elucidation using accurate mass for aroma compounds

One of the challenges with developing a DHS method, is knowing the temperature and volumes to use, as requirements will be matrix and analyte dependant. The Multi volatile method, (MVM) published by Gerstel originally for coffee [4], but more recently extended for green tea [5] aims to increase the range of compounds that can be trapped. This fully automated method extracts the same sample using a series of traps/methods and then employs a multi-desorption technique in order to obtain a single chromatogram. The benefits over a single DHS extraction are illustrated in Figure 5 for analysis of fragrance in a solid matrix.

GC-O the Ultimate Detector

For aroma analysis it is always worth linking analytical data with sensory. One direct way to do this is to use an olfactory port (GC-O), such as the Gerstel Olfactory Detection Port (ODP), to directly assess the odour of components as they elute from the analytical column. This can be used for characterisation studies or to help identify compounds relating to a specific odour. Due to the low thresholds for some compounds, it is possible that an odour will be perceived, but no peak is observed using a traditional detector. In this instance it may be necessary to collect the peak of interest over several injections and re-analyse once enriched. This can be done with some systems by connecting an adsorbent tube in place of the ODP sniffer port. The choice of detector can also aid identification of aroma active compounds. For example, using an ODP alongside a GC-QTOF detector provides not only an increase in sensitivity compared to a single quadrupole mass spectrometric detector (MSD), but also accurate mass information that can be used for full structural elucidation. An example is a recent investigation into a 'pencil' odour in water, where it was concluded that using an MSD with NIST search could lead to the misidentification of the compound responsible. The use of accurate mass was able to confirm the identity where the unit mass spectra were almost indistinguishable (Figure 6). When combined with the corresponding odour descriptors, the system becomes a powerful tool for investigative analysis of aromas.

Real-time Aroma Analysis

A growing area of research in aroma analysis is the study of flavour or fragrance release over time. Traditionally this would have been performed by collecting and analysing a number of samples over a given time period. However, for shorter time or real-time analysis, there are now a few alternative instrumental options. One example is selected ion flow tube mass spectrometry (SIFT-MS), which is based on chemical ionisation using selected reagent ions (H_3O+, NO+ and O_2+ (positive ions) and (O-, O_2-, OH-, NO_2- NO_3- (negative ions). As these result in soft ionisation, simple spectra are obtained, enabling rapid monitoring of specific ions. The system set up results in quantitative results over a large dynamic range without the need to run standards for many analytes. Volatiles can be introduced to the system in a number of ways: directly (air or breath sampling), from headspace vials or tedlar bags using the Gerstel MPS, from thermal desorption of swabs, or even from 'nose space' during consumption of a foodstuff. Figure 7 shows an example of the measurement of release of fragrance from a detergent in real time at different temperatures [6].

Conclusions

There are many available options for sample preparation, the majority of which can now be successfully automated. There is not one sample preparation technique that is suitable for every analyte and matrix combination and the choice must always be dictated by the requirements of the analytical method. The analytical strategy will depend on the reasons for the sample preparation, whether to improve selectivity by removal of matrix interferences, or increase sensitivity by enrichment or derivatisation. The choice should also be

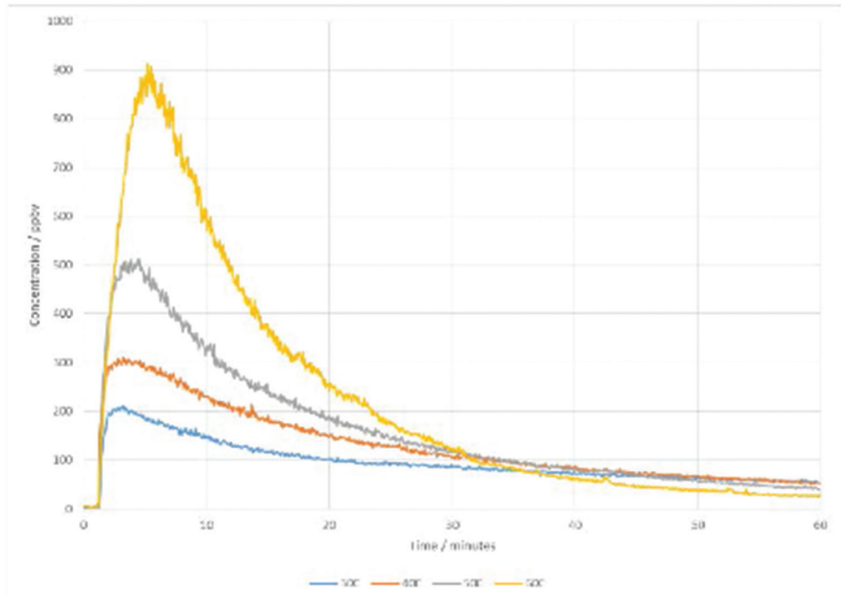

Figure 7: Real-time release of Limonene in fabric detergent using SIFT-MS

guided by the final instrumentation and whether the method is for screening/ profiling or a fully quantitative targeted analysis. The ability to have a choice of sample preparation options on one modular system enables the analyst to make an informed decision about the correct approach for each particular sample.

References

[1]. K. Ridgway, S.P.D. Lalljie, R. M. Smith, Analytica Chimica Acta, 677, (2010) 29-36

[2]. D.Carrier, Y.Nei, Anatune Technical Note No AS115, Trace level enrichment of malodour analytes in water using Twister Technology

[3]. A. Marrufo-Curtido, M.J. Cejudo-Bastante, E. Durán-Guerrero, R. Castro-Mejías, R. Natera-Marín, F. Chinnici, C. García-Barroso, LWT - Food Science and Technology, 47, Issue 2, (2012) 332–341

[4]. N. Ochiai, J. Tsunokawa, K. Sasamoto, A. Hoffmann, Chrom A, 1371 (2014) 65-73

[5]. N. Ochiai, K. Sasamoto, J.Tsunokawa, A.Hoffmann, K. Okanoya, K. MacNamara, J. Chrom. A, 1421, (2015) 103-113

[6]. M.Perkins, Anatune Technical Note No AS146, Preliminary investigation of a range of Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) applications.