MARKES international

TDTS

Thermal Desorption Technical Support

Note 88: Enhancing olfactory profiling of fruit juices and wine using complementary analytical thermal desorption techniques

Keywords

(S)VOCs, fruit juice, wine, sample adsorption, SPE-tD, sorptive extraction

Introduction

A variety of predominantly volatile organic compounds (VOCs) define the characteristic aroma and flavour of drinks such as fruit juice or wine. Understanding the VOC profile of a beverage and using this information to enhance or eradicate contributory components can increase the appeal of the product. As such, there is great interest in developing the analytical techniques available for characterising aroma profiles.

Historically, analysis of olfactory compounds has required cumbersome sample preparation steps such as liquid/liquid extraction, solid phase extraction and/or distillation techniques, usually with the additional drawback of organic solvent use.

More recently, headspace (HS) analysis has been used to characterise some aroma compounds (those tending towards higher volatility); however, conventional static equilibrium headspace is limited with respect to sensitivity and detectable volatility range. These limitations can be countered by coupling headspace to thermal desorption technology (HS-TD). HS-TD allows a dynamic approach to sampling headspace vapours in one or multiple steps. Repeated evacuation of the headspace vapour ensures that larger volumes are swept directly onto the trap of the thermal desorption (TD) instrument. The sorbent configuration and temperature of the trap can be optimised to selectively retain the volatiles of interest, while volatile interferences such as water and ethanol are purged to vent. Combined, this results in increased sensitivity, an extended volatility range and quantitative analysis. (See Markes International's HS5-TD™ brochure for more information.)

Another solvent-free approach to the analysis of aroma profiles is sorptive extraction coupled with TD. Markes' SPE-tD™ cartridges offer a simple, convenient method for extracting a wide range of (semi-)volatile organic compounds ([S]VOCs) from liquid samples. Note that SVOCs have traditionally required even more labour-intensive sample preparation techniques than VOCs.

Using either or both of these approaches, HS-TD and SPE-tD, a very wide range of olfactory compounds can be analysed without the need for solvent extraction or distillation. Here, we will highlight how the use of these complementary TD techniques, when applied to a range of commercially available beverages, facilitates the analysis of a very broad spectrum of volatility; from the ultra-volatiles to the very low volatility compounds. The flavour/fragrance profiles obtained can then be used to identify major contributory components, to make comparisons between samples and, consequently, make important decisions regarding the drink manufacturing process to maintain or enhance product quality.

HS-TD and SPE-tD accessories

Markes' HS5-TD accessory (figure 1) presents a simple, low-cost way to sample headspace (HS).

Figure 1: Markes' HS5-TD accessory coupled with UNITY 2™

The HS5-TD can accommodate five standard HS vials within a heated environment and is directly coupled with the UNITY 2™ thermal desorber. Once connected to UNITY 2, HS vapours are transferred directly from the vial onto the electrically cooled focusing trap of the thermal desorber. The HS5-TD offers pulsed sampling (pressurisation and evacuation) of the headspace vial. This can be repeated indefinitely so that all volatiles are exhaustively extracted, leading to greatly improved quantification and detection limits. Sensitivity is typically 1–2 orders of magnitude higher than that obtained via static equilibrium HS, and is comparable to that obtained by purge and trap methods, but without the undesirable foaming issues. Trap sorbents and temperature can be optimised so as to retain a wide range of analytes whilst eliminating interferences, *e.g.* water.

As mentioned above, another low-cost solution to the manually intensive solvent extraction/distillation of (semi-)volatiles from liquid is the use of SPE-tD cartridges from Markes (figure 2).

These reusable cartridges optimise sample adsorption as they are long, hollow and coated with polydimethyl siloxane (PDMS) (stationary phase). This results in the largest surface area possible, whilst ensuring the cartridges fit conveniently into industry standard TD tubes.

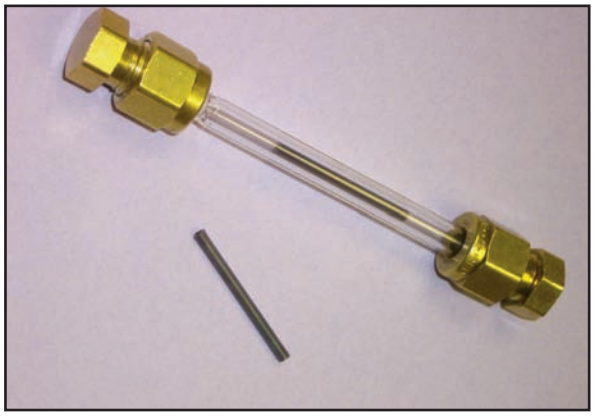

Figure 2: Markes' SPE-tD cartridges are inserted directly into a tube

This large surface area gives Markes' SPE-tD cartridges a uniquely high capacity for target (S)VOCs.

Sample preparation for this technique is simple; the cartridges are introduced to the liquid sample and agitated, encouraging the compounds present to partition between the aqueous matrix and the PDMS, eventually reaching equilibrium. Analysis of the compounds present is performed on the UNITY 2 TD instrument; the SPE-tD cartridges are inserted directly into an empty TD tube (figure 2). This is then backflushed and refocused onto the electrically cooled focusing trap of UNITY 2.

Once a sample has been introduced to the focusing trap, rapid, backflushed desorption occurs. This desorption is so efficient that true splitless injection to the GC is possible, ensuring all compounds of interest are introduced, therefore achieving optimum sensitivity. Alternatively, if necessary, applying a split means that UNITY 2 can re-collect the sample, allowing quantitative repeat analysis and confirmation of results.

This simple method allows semi-quantitative and highly sensitive analysis of (S)VOCs; detection limits in the order of ppb to ppt are possible.

Experimental

Procedure

For headspace analysis, orange juice made from concentrate was diluted 1:10, freshly squeezed orange juice was diluted 1:2 and red wine was diluted 1:20. 'From-concentrate' and freshly pressed apple juice were not diluted. 10 mL samples of each were transferred to 20 mL standard HS vials, which were sealed using blue silicone PTFE-coated septa.

For SPE-tD sampling, both orange juice samples were diluted 1:20, whereas the apple juice and red wine samples remained undiluted. 20 mL of each were then transferred to standard 20 mL headspace vials. Conditioned SPE-tD cartridges were placed in each vial along with an inert (glass-coated) stir bar. The samples were sealed using blue silicone PTFE coated septa and stirred for 1 h at ambient temperature. Cartridges were then removed, rinsed and placed in a clean, empty tube prior to analysis by direct TD-GC/MS.

Vials containing 10 mL of HPLC-grade water were also sealed with blue silicone PTFE-coated septa and used as blanks.

Juice and wine samples were taken from the same cartons/bottles throughout.

Analytical conditions

HS-TD

Instrument configuration:	Unity 2 + HS5-TD
Cold trap:	General purpose (U-T11GPC-2S)
Septa:	Blue silicone PTFE
Pre-purge (flow rate):	1 min (20 mL/min to split)
Sample cycles:	2
Pressurise:	1 min
Sampling (flow rate):	1.5 min (50 mL/min to trap)
Equilibration:	1 min
Flush sample:	1 min
Post-sampling line purge (flow rate):	1 min (20 mL/min)
Pre-trap fire purge (flow rate):	3 min (20 mL/min)
Trap low:	25°C
Trap heating rate:	Maximum
Trap high:	300°C
Trap high time:	5 min
Split:	20 mL/min
Flow path:	140°C
Vial temperature:	50°C

GC

Column:	Varian VF-5MS, 30 m x 0.25 mm x 0.25 µm
Pressure:	10 psi @ 40°C
Column flow (calculated):	1.3 mL/min
Mode:	Constant pressure
Oven program:	40°C (2 min) then 5°C/min to 160°C
Total run time:	26 min

MS

Quad temperature:	150°C
Source temperature:	230°C
Full scan range:	35–300 amu

SPE-tD

SPE-tD cartridges (P/N C-SPTD10):	Comprise a hollow tube, coated inside and out with PDMS
Instrument configuration:	UNITY 2 + ULTRA
Cold trap:	General purpose (U-T11GPC-2S)
Pre-purge (flow rate):	1 min (20 mL/min to split)
Desorption temperature:	180°C
Desorption time (flow rate):	5 min (50 mL/min to trap)
Pre-trap fire purge (flow rate):	1 min (20 mL/min)
Trap low:	25°C
Trap heating rate:	Maximum
Trap high:	300°C
Trap high time:	5 min
Split:	20 mL/min
Flow path:	200°C

GC

Column:	Varian VF-5MS, 30 m x 0.25 mm x 0.25 µm
Pressure:	10 psi @ 40°C
Column flow (calculated):	1.3 mL/min
Mode:	Constant pressure
Oven program:	40°C (2 min) then 5°C/min to 160°C, then 10°C/min to 320°C (2 min)
Total run time:	44 min

MS

Quad temperature:	150°C
Source temperature:	230°C
Full scan range:	35–300 amu

Results

All chromatograms displayed in this report have been processed using ClearView™ GC/MS reprocessing software. ClearView uses sophisticated 'dynamic background compensation' (DBC) algorithms to distinguish between chromatographic peaks and background/baseline anomalies. It reprocesses stored GC/MS and LC/MS data files, eliminating background ions from the total ion chromatogram (TIC) and improving both spectral purity and peak integration. The original data files are stored intact, so application of ClearView is risk-free.

Orange juice

Figure 3 shows the analysis of 'from-concentrate' orange juice using HS-TD and SPE-tD.

From this figure, it can be seen that the compounds identified from the HS-TD and SPE-tD analyses overlap considerably. As expected from the nature of the sampling method, HS analysis shows preferential sensitivity towards the more volatile types of compounds, which tend to be responsible for aroma. SPE-tD analysis, whilst also sensitive to aromatic compounds, shows very good sensitivity towards lower volatility compounds, which tend to contribute to flavour.

By interpretation of the results obtained, it was determined that the compounds responsible for flavour and fragrance of the orange juice sample are mainly terpenes and sesquiterpines. Notable compounds identified from the juice sample are described below.

Linalool (3,7-dimethyl-1,6-octadien-3-ol; boiling point [bp] 199°C) is a terpenoid alcohol found naturally in a variety of plants, flowers and spices. Linalool is often added to processed food and beverages, perfumes, cosmetics and soaps as well as to household detergents and waxes due to its flavourful and fragrant properties.

Terpinen-4-ol (4-methyl-1-[1-methylethyl]-[R]-3-cyclohexen-1-ol; bp 212°C) is used as a marker of juice quality. It is an off-odour compound of mandarin juice but is sometimes added to orange juices at a maximum ratio of 1:10 to improve colour and aroma. Thermal and catalytic decomposition of food

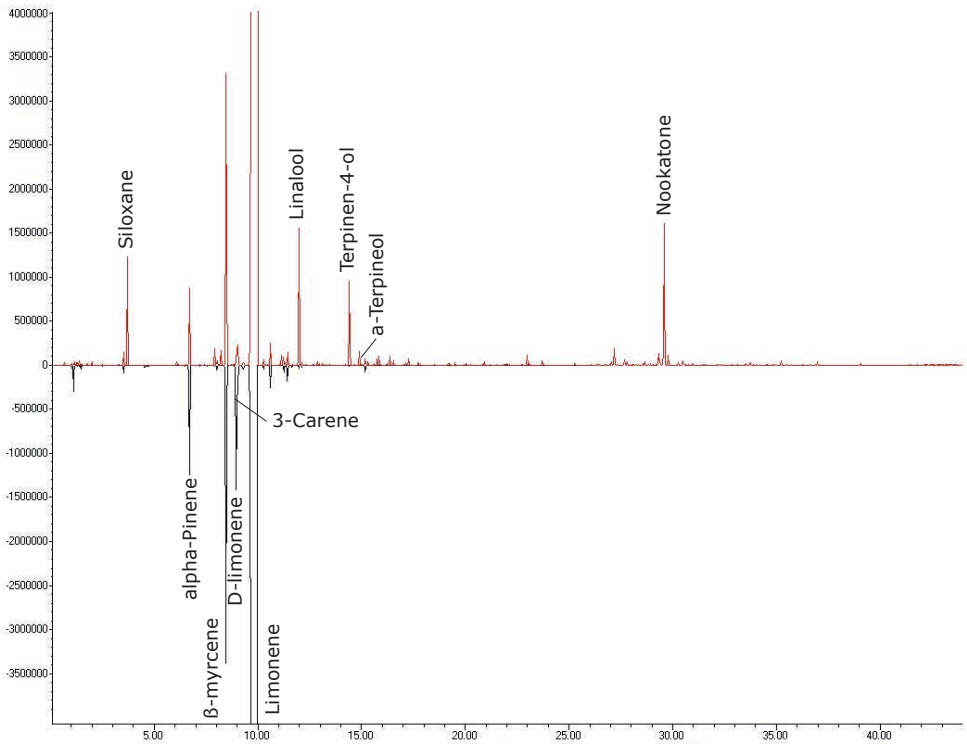

Figure 3: Two chromatograms comparing the SPE-tD-GC/MS analysis (red) and HS-TD-GC/MS analysis (black) of 'from-concentrate' orange juice

components occurs during heat treatment and storage of juices. An example of a thermally induced alteration is the transformation of d-limonene and linalool into alpha-terpineol and terpinen-4-ol[1]. Observing quantities of d-limonene and linalool, as well as alpha-terpineol and terpinen-4-ol by SPE-tD, as seen here, can help juice producers monitor the quality of their products.

As above, the HS-TD and SPE-tD methods led to the analysis of a very wide range of volatiles in freshly squeezed orange juice. Although those found were again mainly terpenes, the exact compounds identified in the freshly squeezed sample differed significantly from that of the 'from-concentrate'.

Of note, the HS-TD technique, by packing the focusing trap of the UNITY 2 TD instrument with a combination of strong sorbents and setting the temperature at 25°C, was able to extract ultra-volatiles, such as ethyl acetate and ethyl propanoate (figure 4), not seen in the previous 'from-concentrate' sample. These highly volatile compounds are important for 'sweet' and 'fruity' aromas[3].

Towards the other end of the scale, SPE-tD of freshly squeezed orange juice highlighted the lower volatility compounds. Relatively high proportions of valencene compared to nootkatone were observed. Valencene (1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-[1-methylethenyl]-,[1R-{1.alpha.,7.beta.,8a.alpha.}]-naphthalene, bp 274°C) is the major sesquiterpene in orange peel oil and has been suggested to be an easily measurable marker for increased fruit maturity, known to correlate positively with better orange juice flavour[4].

Figure 5 is a comparison of the complete range of volatiles identified by HS-TD in the two different orange juice samples. The figure shows a magnified view of a section of the chromatogram, which highlights important compounds present at low concentrations, and the TIC (inset) normalised to show the whole range; this highlights the large limonene peak in 'from-concentrate' juice.

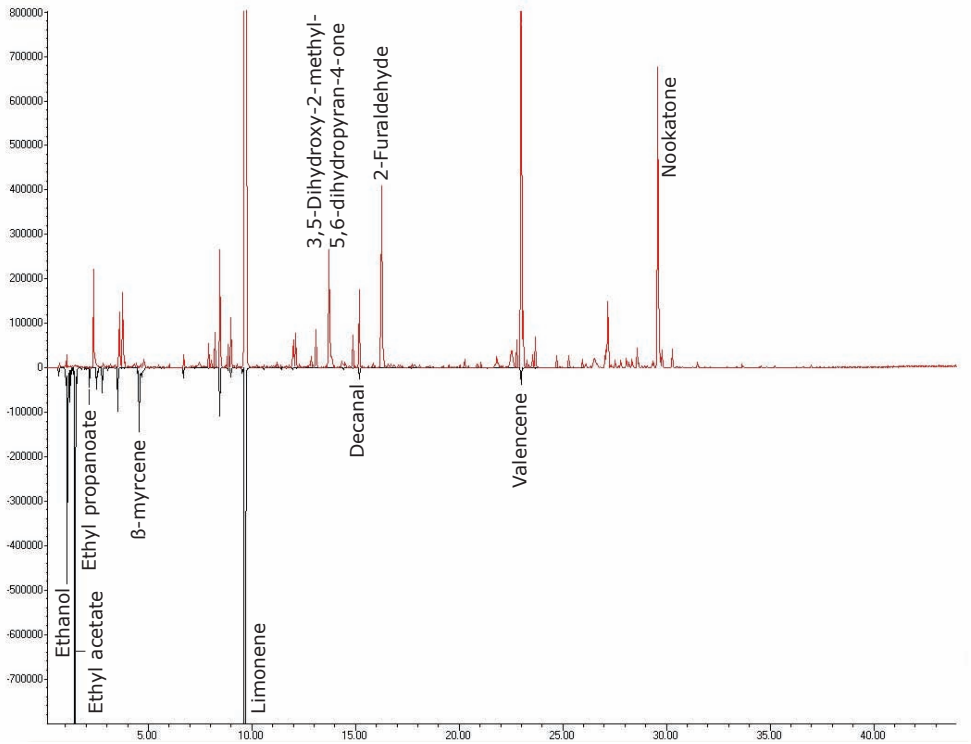

Figure 4: Two chromatograms displaying the SPE-tD-GC/MS analysis (red) and HS-TD-GC/MS analysis (black) of freshly squeezed orange juice

The VOC profiles of the two types of orange juice are very different. For example, the higher volatility compounds that are seen in the freshly squeezed juice (those which have importance in the 'sweet', 'fruity' smell of the drink, *e.g.* ethyl propanoate) have been lost completely or are present at much lower concentrations in the 'from-concentrate' sample, perhaps due to the age of the juice (volatiles lost to the air) or as a result of a pasteurisation process. Conversely, additives such as alpha-pinene are seen at a high concentration in the 'from-concentrate' sample, possibly introduced during manufacture as replacements for the olfactory volatiles lost.

Figure 6 is a comparison of the VOC profiles obtained by SPE-tD analysis of the different orange juice samples, showing a magnified view displaying compounds present at low concentrations, and an overall view (inset) of the high concentration VOCs.

SPE-tD analysis provides a broad range of analyte detection, with notable sensitivity towards the lower volatility compounds.

Comparison between the SPE-tD analyses of the two orange juice samples highlights a large difference between the types and relative quantities of (S)VOCs present. Of note, SPE-tD analysis has identified terpinen-4-ol (described previously as a marker of juice quality) to be present at a substantial level in 'from-concentrate' juice, relative to freshly squeezed orange juice which contains none. Similarly, linalool, a chemical often added to processed food was found in far greater abundance in 'from-concentrate' than freshly squeezed juice. Losses of compounds such as valencene (linked to flavour quality) from the 'from-concentrate' sample were also visible from the comparison.

Limonene, a compound which should not be present in orange juice in high concentrations, was detected in both 'from-concentrate' and freshly squeezed orange juice by HS-TD and SPE-tD, as seen previously (figures 3-6). Figure 7 gives a direct comparison of the levels of limonene detected by HS-TD; this clearly shows the amount in the 'from-concentrate' sample to be significantly higher than in freshly squeezed juice. This high level of limonene indicates that a significant amount of peel might have been included in the extraction process.

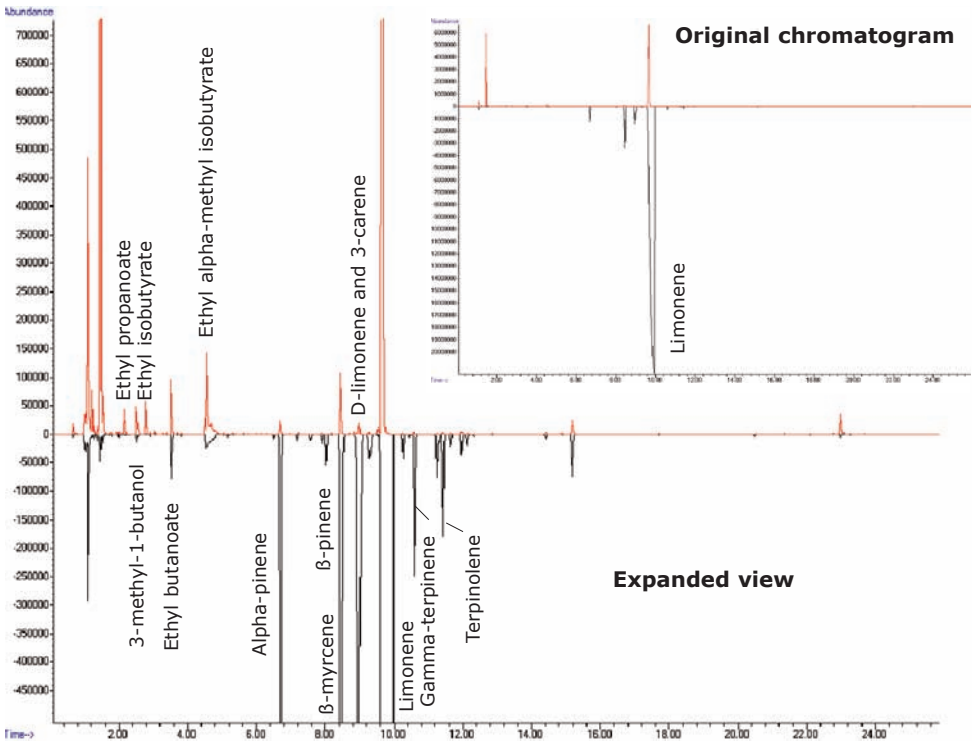

Figure 5: Chromatograms comparing the HS-TD-GC/MS analyses of freshly squeezed orange juice (red) and 'from-concentrate' orange juice (black). Original chromatograms are shown in inset

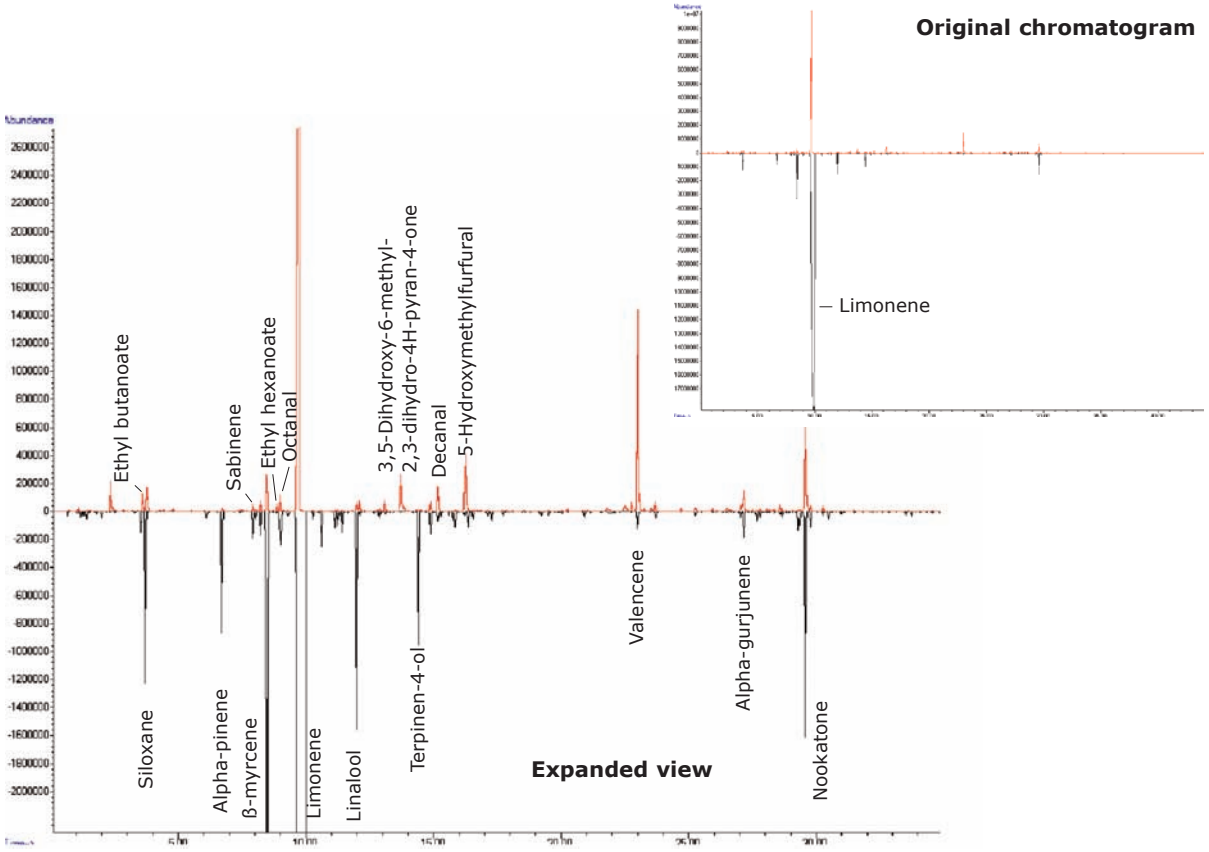

Figure 6: Two chromatograms comparing SPE-td-GC/MS analyses of freshly squeezed orange juice (red) and 'from-concentrate' orange juice (black). Original chromatograms are shown in inset

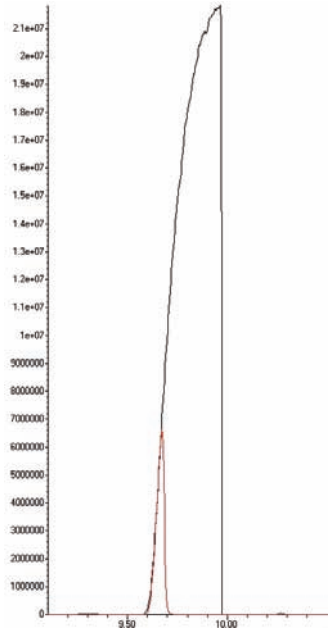

Figure 7: Close-up comparison of the limonene peaks of HS-TD-GC/MS analysis of freshly squeezed orange juice (red) and 'from-concentrate' orange juice (black)

Apple juice

Figure 8 compares the HS-TD-GC/MS and SPE-tD-GC/MS analyses of 'from-concentrate' apple juice.

From the TICs, it is immediately apparent that the compounds in apple juice are distinctly different from those in orange juice. The flavour/aroma profile of apple juice predominantly comprises oxygenated compounds such as aldehydes, alcohols and ketones, rather than terpenes and sesquiterpenes. The application of both HS-TD and SPE-tD clearly identifies a sufficiently broad range of compounds important to the flavour/fragrance profile of apple juice with excellent sensitivity:

Hexanal is an alkyl aldehyde used in the flavour industry to produce 'fruity' flavours. Its fragrance is described as a 'fresh green grass, leafy, fruity' smell. *Trans*-2-hexenal and hexyl acetate are chief among those molecules responsible for the freshness of the apple juice flavour, and thus hexenal can be used as an additive to give flavours a 'greener' profile.

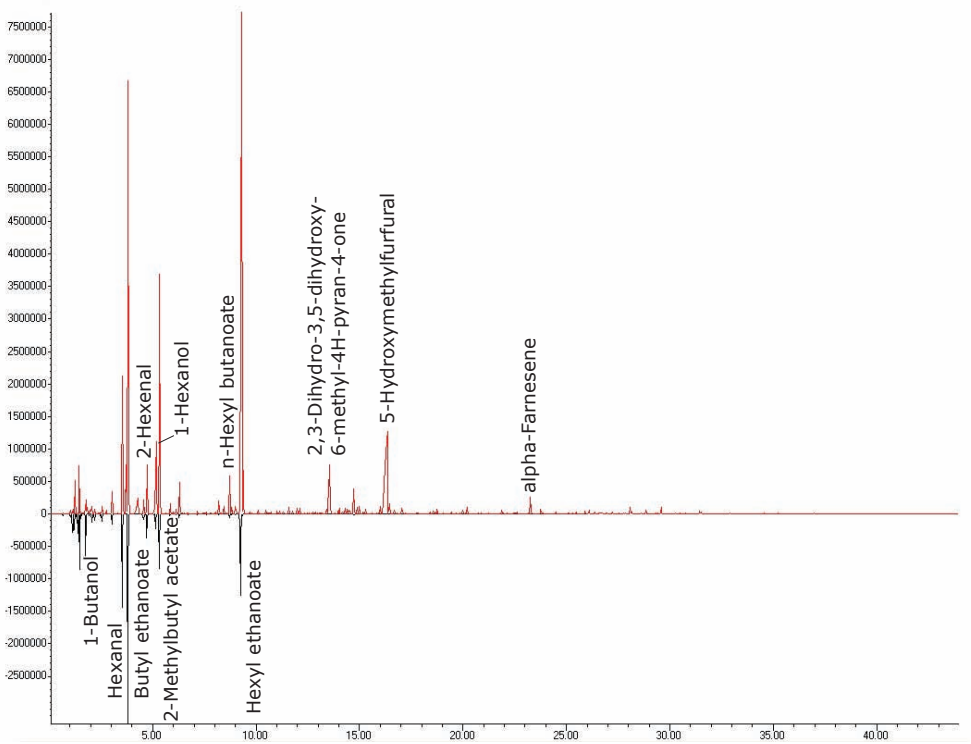

Figure 8: Comparison of the SPE-tD-GC/MS analysis (red) and HS-TD-GC/MS analysis (black) of from-concentrate apple juice

Butyl ethanoate (butyl ester acetic acid) is used as a synthetic fruit flavouring in foods such as candy, ice cream, cheese and baked goods. It is a colourless, flammable liquid with the sweet smell of banana. Butyl acetate is found in many types of fruit, where, along with other chemicals, it imparts characteristic flavours. Apples, especially of the Red Delicious variety, are flavoured in part by this chemical.

The very low volatility sesquiterpene alphafarnesene, detectable here by SPE-tD, and its metabolites have been implicated in the development of 'superficial scald' in apples.

Figure 9 shows the analysis of freshly pressed apple juice by HS-TD and SPE-tD. The compounds are extremely similar to those in the 'from-concentrate' sample.

Figure 10 compares the HS-TD-GC/MS analyses of the two types of apple juice, and figure 11 compares the two SPE-tD-GC/MS analyses.

The HS-TD comparison of 'from-concentrate' and freshly pressed apple juice are remarkably similar, with the compounds present being almost identical but their relative amounts differing slightly.

The most obvious difference observed from SPE-tD analysis between the freshly pressed and the 'from-concentrate' apple juice samples is the presence of the estragole peak in freshly pressed juice. Estragole (*p*-allylanisole, methyl chavicol) is a natural organic compound. It is a colourless-to-pale yellow liquid and an important terpeniod in the flavour of apples. Estragole is used in perfumes and as a flavouring additive, and is described as having a 'strong, sweet, tarragon' smell. It is a natural pesticide and known carcinogen but is not threatening until levels are amplified in the crops; a possible side effect of genetic engineering. Therefore, its presence is important for flavour, although levels must be monitored.

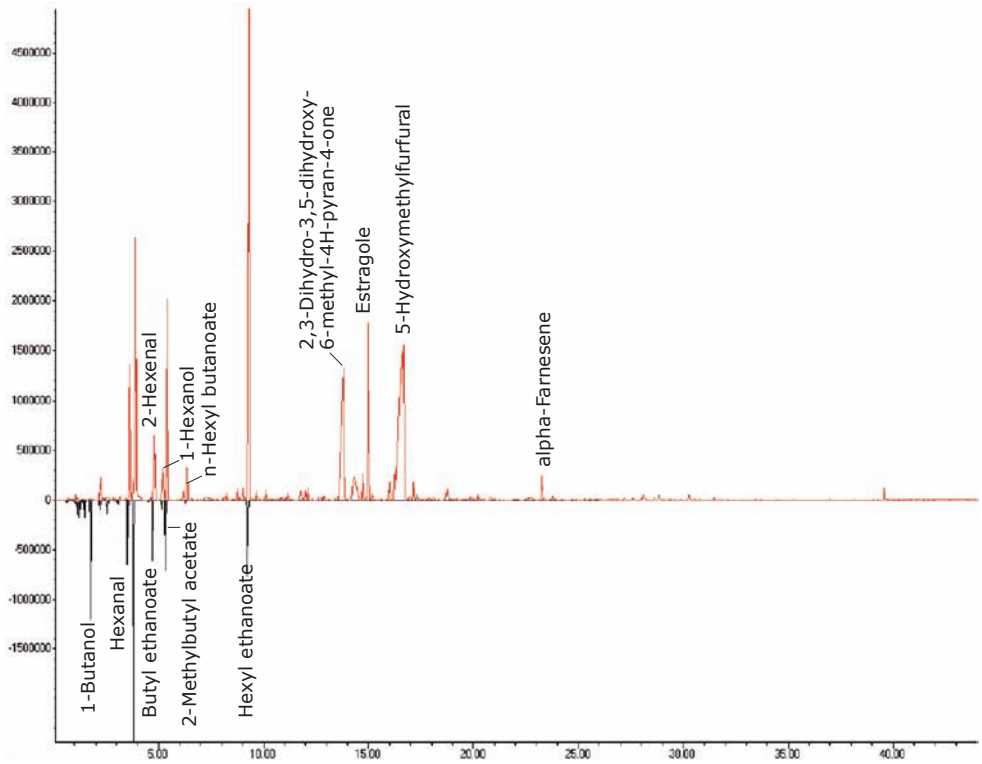

Figure 9: Two chromatograms comparing the SPE-tD-GC/MS analysis (red) and HS-TD-GC/MS analysis (black) of freshly pressed apple juice

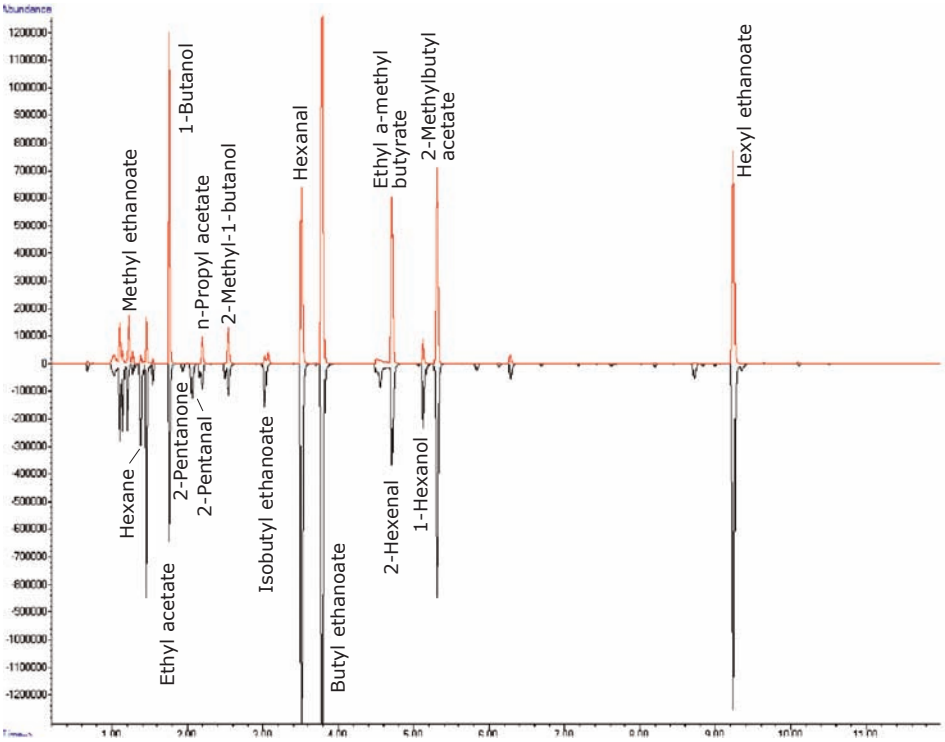

Figure 10: Two chromatograms comparing the HS-TD-GC/MS analyses of freshly pressed apple juice (red) and from-concentrate apple juice (black)

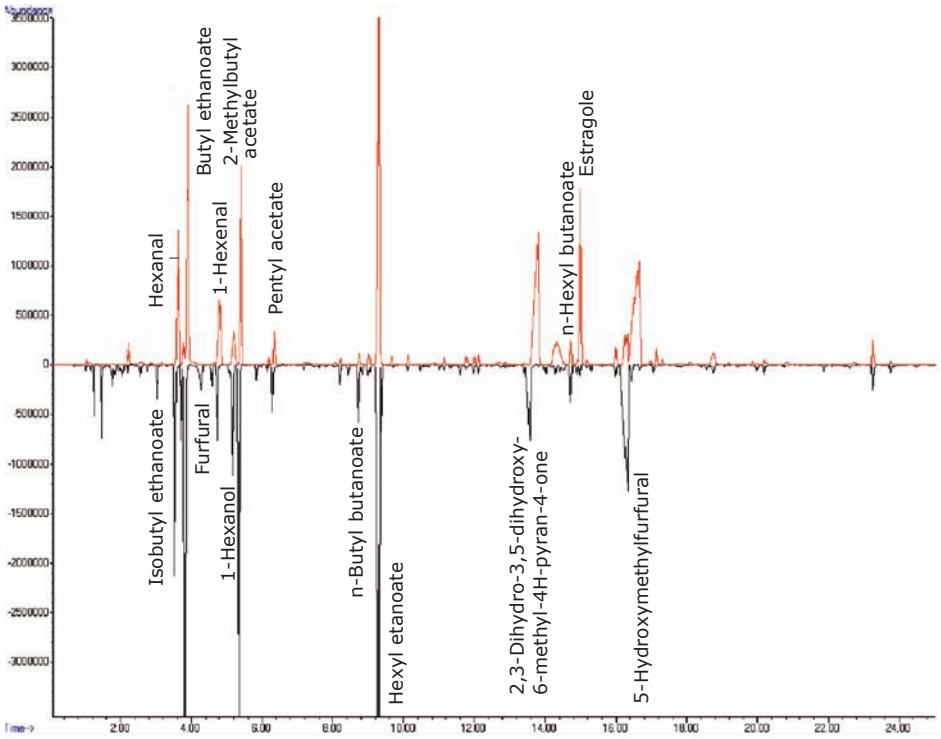

Figure 11: Two chromatograms comparing the SPE-tD-GC/MS analyses of freshly pressed apple juice (red) and from-concentrate apple juice (black)

Red wine

Wine aroma comprises a complex mixture and range of organic volatiles. The presence and concentration of primary or 'varietal' compounds has been used for wine differentiation and characterisation over many centuries. Determining the compounds responsible for the varietal aroma of wines is used to differentiate wines on the basis of grape variety and production area. The volatile compounds found are mainly terpenes and C_{13}-norisoprenoids, which supply 'fruity' or 'floral' notes to the aroma.

The volatile compounds responsible for the secondary or 'fermentative' aroma of wine are generated during alcoholic fermentation and are linked to the type of yeast involved. They include those with a slightly lower volatility, e.g. higher alcohols, esters, fatty acids, carbonyl compounds and volatile phenols. Of these, the esters are known to be major contributors to the wine's flavour.

Figure 12 shows the analysis of a sample of red wine from the Bordeaux region of France. The grape varieties included Cabernet Franc, Cabernet Sauvignon and Merlot, and the bottle was sealed with a natural cork stopper.

Of all the volatile compounds in wine, only a limited number (the alcohols and acetates) are important for aroma. Of these, the following are the most powerful: ethyl hexanoate, ethyl octanoate (by-products of yeast metabolism), 3-methyl-1-butanol, ethyl acetate, isoamyl acetate and ethyl lactate. The highest volatility compounds are monitored best by HS-TD, and the combination of this technique with SPE-tD (targeted at lower volatility components) ensures a broad range of compounds are seen.

Table 1 lists the aroma compounds found in this red wine sample with a description of their odour. These aroma compounds are important in young wine and are sensitised during fermentation. They are among the key compounds in the fruity flavours of wine.

As described above, HS-TD and SPE-tD techniques can also be used to enhance detection of contaminant flavours or odours in a sample. *Brettanomyces* (*Dekkera*) *bruxellensis* ('*Brett*'), a spoilage yeast found in beer and wine, creates off-odours which are described as 'animal' (*i.e.* horsey, sweaty) or 'phenolic' (*i.e.* plastic, metallic). From the chromatogram (figure 12), trace levels of several *Brett* by-products, including the primary

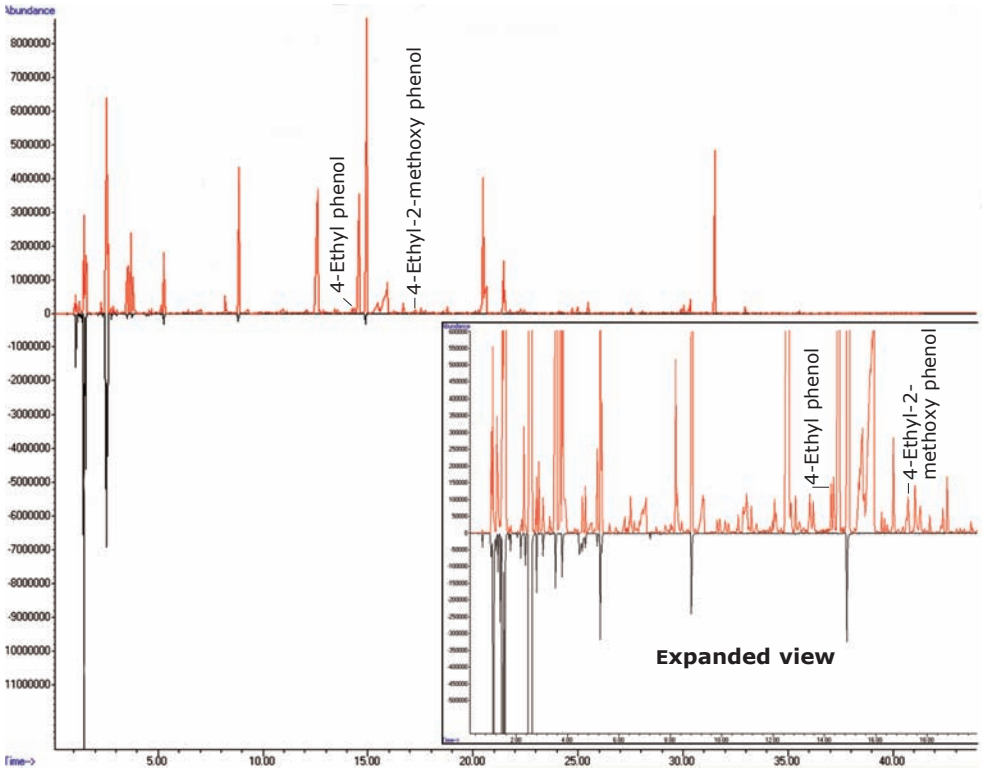

Figure 12: Two chromatograms comparing the SPE-tD-GC/MS analysis (red) and HS-TD-GC/MS analysis (black) of red wine

Compound	Odour descriptor
3-Methyl-1-butanol	Fusel
Ethyl acetate	Fruity
Isoamyl acetate (3-Methyl-1-butanol acetate)	Fruity, banana, pear
Ethyl lactate (2-Hydroxy ethyl ester propanoic acid)	Lactic, raspberry
Ethyl hexanoate (Ethyl ester hexanoic acid)	Apple, banana, violets
Ethyl octanoate (Ethyl ester octanoic acid)	Pineapple, pear, floral

Table 1: Compounds found in red wine and their odour descriptor

ones of 4-ethyl phenol and 4-ethyl-2-methoxy phenol, have been detected, suggesting *Brett* contamination (Contact ALMSCO for more information.)

Conclusions

The historical use of solvent-based or distillation techniques for VOC extraction is no longer desirable since the methods are arduous and can be affected by interference. More modern techniques such as sorptive extraction and headspace sampling offer a more readily automated and solvent-free option. However, conventional static HS sampling retains limitations with regards to the small sample volume obtained and the narrow volatile detection range. Dynamic HS-TD counters these drawbacks and provides the ideal complement to SPE-tD sorptive extraction.

The ability of HS-TD in combination with SPE-tD to determine the full range of compounds contributing to the aroma and flavour of a particular drink has been demonstrated in this study. Application of both methods will allow drink manufacturers to control the quality of their product and identify areas for improvement.

Trademarks

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