MARKES international

TDTS

Thermal Desorption Technical Support

Note 94: Using Markes' thermal desorption technology to automate high/low analysis of a complex beer sample

Key Words:

Sorptive extraction, sample re-collection, flavours

Introduction

Beer, made primarily from natural ingredients, unsurprisingly comprises a wealth of chemical components whose concentrations vary by several orders of magnitude. Analytical difficulty is encountered when trying to characterise the chemical profile and quantify high level compounds at the same time as those at trace level. Compounds responsible for flavour, for example, can have extremely low olfactory thresholds and may be present at ppt level, but are nonetheless significant. The challenge is developing a simple and suitable method to fully characterise a sample comprising such a wide dynamic range of compounds.

Volatile extraction

In the first instance, it is essential to extract the widest possible range of compounds from a sample in order to comprehensively characterise it. Solid phase micro-extraction (SPME) and equilibrium (static) headspace are techniques which have previously been used to extract volatile organic compounds (VOCs) from liquid samples; however, neither method provides the sensitivity required for trace-level analysis.

Sorptive extraction using SPE-tD™ cartridges (Figure 1) is a technique which provides simple yet highly efficient extraction of organic compounds from aqueous matrices. The enrichment factor possible from thorough mixing of large volumes of sample matrix with physically large sorbents, such as polydimethylsiloxane (PDMS), is the key to the sensitivity advantage over the aforementioned techniques.

Sorptive extraction is complemented by thermal desorption (TD), which re-focuses and concentrates the sample, and provides efficient injection directly into the GC.

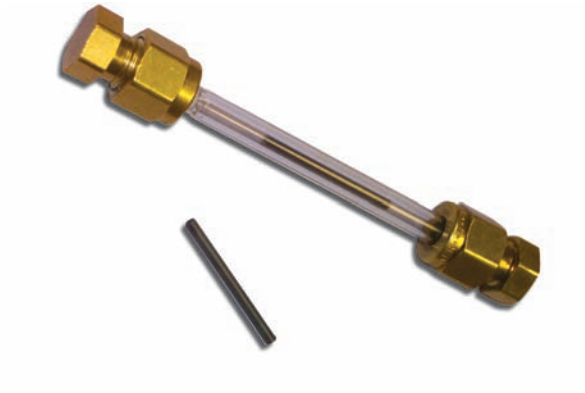


Figure 1: Long, hollow, PDMS-coated SPE-tD cartridges

Compound analysis

To ensure the trace-level components are precisely measured, a highly concentrated sample needs to be introduced to the GC/MS; however this may exceed the capacity of a highly resolving column and result in detector overload. By contrast, injecting a smaller sample to the column will allow correct characterization and measurement of the major components, but then the low-level compounds will be lost.

Historically, the solution to this problem was simply running several analyses using different dilutions to obtain representative profiles. However, this approach significantly lengthens throughput, requiring hands-on operation for each run, and data is spread over several files. In answer to this dilemma, technological developments in thermal desorption technology allow quantitative re-collection (SecureTD-Q™), and automatic repeat analysis of one sample at different dilutions.

SecureTD-Q is a unique feature to all Markes thermal desorbers, which offer manual re-collection of all split flows (*i.e.* the split during both sample tube and cold trap desorption) as standard. This unsurpassed split flow

versatility means that the Markes thermal desorption platform is compatible with analyte levels from sub-ppt to percent. Users may programme splitless, single split (either tube or trap desorption) or double split (both tube and trap desorption) operation into their method. Double-splitting is critical for the successful analysis of high level samples, whereas working in single split or splitless mode optimises sensitivity for trace-level analysis.

Making use of the flow split and sample re-collection facilities of UNITY 2™ (Figure 2), upon desorption of the sample, a double split allows a diluted aliquot to be injected onto the column to measure the high-abundance components. SecureTD-Q employs a second TD tube to retain the analytes that would have otherwise been split to waste; follow-up analysis of this using a single split loads a higher concentration onto the column, allowing accurate measurement of the minor components of the beer. Therefore, quantifying both high and low level analytes in a complex sample using a single, automated method can be achieved.

For the analysis of multiple samples, the TD-100™ (Figure 3), which incorporates the UNITY 2 TD platform, allows automatic sampling and re-collection of up to 100 samples.

The UNITY 2 TD platform was used here, possibly for the first time in beer analysis, to run a single sample consecutively at two widely differing concentration levels (aliquots) without operator intervention. This was followed by GC/TOF-MS analysis. The results were combined and normalised to a common intensity for interpretation of the entire profile.

Figure 2: UNITY 2 single-tube thermal desorber

Figure 3: TD-100 automated TD for up to 100 tubes

Experimental

Sample preparation

A popular bottled beer was obtained from a supermarket for analysis. The bottle was chilled to 5ºC prior to opening in an effort to limit the loss of volatile compounds. As soon as it was de-capped, a zero-headspace aliquot was collected and stored in a 20 mL screw-capped headspace vial.

A pre-conditioned SPE-tD cartridge (30 mm x 2.5 mm x 500 µm PDMS film thickness) was introduced to the sample along with a clean, glass-encapsulated magnetic stir-bar (10 mm) before securing the vial cap. The vial was then transferred to a heated magnetic stirring plate (at 45 °C) where it was stirred vigorously at 1100 rpm and allowed to equilibrate for 30 min.

After extraction, the SPE-tD cartridge was transferred to a clean vial where it was rinsed twice in distilled water. The cartridge was then dried in a lint-free wipe, ensuring any excess water was blotted away, and placed in a conditioned glass TD tube. The cartridge was immobilised between glass wool and a stainless steel spring clip. The loaded TD tube was immediately sealed with long term storage caps until its analysis.

A UNITY 2 TD system was interfaced to the host system, harnessing the electronic pneumatic control of the GC, and the sample analysed under the following conditions:

TD

Instrument:	UNITY 2
Cold trap:	U-T15ATA-2S
Pre-purge time:	1 min
Desorption temp:	180°C
Desorption time:	5 min (50 mL/min to trap)
Pre-trap fire purge:	1 min
Trap high temp:	300°C
Trap high time:	5 min
Split:	20 mL/min (double-split followed by single-split)
Split collection:	TD tube packed with Tenax®
Flow path temp:	200°C

GC

Column:	BPX5; 30 m x 0.25 mm x 0.25 µm
Carrier:	He, constant flow, 1.5 mL/min
Oven:	40°C (2 min) 5°C/min to 160°C, 10°C/min to 320°C (2 min)

MS

Instrument:	BenchTOF-dx™
Transfer line:	300°C
Ion source:	200°C
Mass range:	35–800 amu
Data rate:	2 Hz (5000 spectra per second)

Data processing

The TOF-MS software, dx-Connect™, propagates both raw and 'dynamic background compensated' (DBC) traces simultaneously. DBC removes any ion interference from the trace, *e.g.* column bleed, producing clearer spectra for more reliable compound identification. Small data files are generated, which speeds up batch (re)processing. This is especially useful for complex chromatography where there is wide dynamic range within a given sample.

The BenchTOF-dx MS produces classical EI spectra; as such, the standard NIST05 mass spectral database was applied for reliable compound identification.

Results

In order to simultaneously quantify high abundance and trace-level compounds in the beer, the UNITY 2 TD system automatically triggered two consecutive runs of the same sample under dilute and very concentrated conditions.

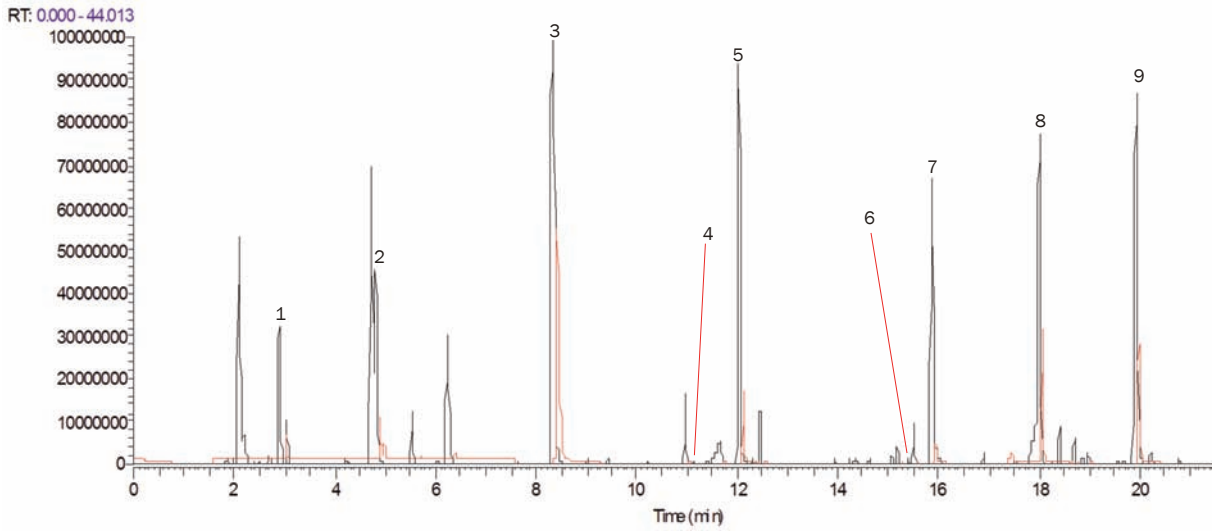
An internal standard spiked into the initial matrix enables easy normalization of the two data files. The difference in loading (primary analysis to recollected/repeat analysis) was calculated to be a factor of 30.

Figure 4 shows the chromatograms from high and low analyses, normalized to a common intensity. The black trace shows the peak overload that is typical in the beverage industry if small components are to be accurately measured. The recollection process can be repeated or altered to yield a larger number of levels from any given sample.

Some of the peaks, both those of high and low abundance, have been identified and are listed in Table 1. Unsurprisingly, a large number of compounds found are known for their flavour. Large peaks, such as those for hexanoic acid ethyl ester and octanoic acid ethyl ester, are well-known olfactory compounds, as are some of the much smaller peaks, including benzaldehyde, which has a characteristic almond flavour, and 1-nonanol, which is citrus-like.

Conclusion

SPE-tD offers a simple but very sensitive means of extracting volatile components from highly complex aqueous products such as beer. The extraction process is easy, safe and scalable to handle large batches of sample. SPE and TD are both enriching techniques; thus the performance of the mass spectrometer will be challenged both in its dynamic range and in its ability to allow identification whether the compound concentration is at high or trace level. Whilst the usual approach to alleviating this issue within industry has been to run a given sample numerous times at different levels of dilution, a far more rigorous and fully automated technique has been demonstrated here with excellent results. Such results can be vital for a company for quality control purposes, for competitive information or for addressing product safety concerns.



Peak number	Compound
1	Ethyl acetate
2	3-Methyl butanol
3	2-Methyl acetate butanol
4	Benzaldehyde
5	Hexanoic acid ethyl ester
6	Nonanol
7	Phenyl ethyl alcohol
8	Octanoic acid ethyl ester
9	2-Phenylethyl ester acetic acid

Figure 4 (above): High/low analysis of beer in 2 sections (0-22 min and 22-44 min). Red trace: Initial, double split analysis; black trace: Low split analysis of re-collected sample.

Table 1 (left) compound identification from the chromatogram in figure 4

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