

Evaluation of a new Dynamic Background Compensation Algorithm for Improving Aroma Characterisation by Thermal Desorption – GC-MS

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Key Words: Aroma profiling, odour, olfactory compounds, thermal desorption, background compensation

Flavour and fragrance profiles typically comprise contributions from a wide range of volatile organic compounds (VOCs) and those at the lowest concentration can have the most profound effect on perceived aroma. Historically, this has made it difficult to carry out meaningful odour profiling using standard sample preparation methods and GC-MS instrumentation. Conventional GC-MS technology is therefore often coupled with analytical thermal desorption to provide both automated sampling versatility and selective concentration. However, quantitative and qualitative analysis of olfactory components at the lowest levels can still be compromised by chromatographic anomalies such as; column bleed, extended solvent tails, air/water interference and unresolved sample matrix components.

To address this issue, a new software algorithm has been developed for reprocessing stored GC-MS data and removing background interference. It uses an innovative dynamic approach to distinguish and eliminate mass ions due to the chromatographic background as it changes throughout a run. Such dynamic background compensation (DBC) should be of tangible benefit for a wide range of GC-MS studies, allowing trace target compounds to be detected more reliably against complex and variable analytical interference.

Application of the new algorithm to odour profiling by automated TD-GC-MS is described here with examples. The benefits to qualitative and quantitative analysis of trace olfactory compounds are evaluated.

Introduction

Flavour and fragrance profiles typically comprise contributions from hundreds of volatile organic compounds (VOCs) and those at lowest concentration can be the most significant olfactory components i.e. have the most profound effect on perceived aroma. Historically, this has made it difficult to carry out meaningful aroma tests using standard GC-MS instrumentation.

Conventional sample preparation methods (solvent extraction, steam distillation, etc.) simply don't offer the sensitivity required and may also distort the vapour profile so that it is no longer representative of the flavour/fragrance perceived by consumers.

Odour experts and aroma assessment panels have therefore continued to play a significant role in product testing with respect to flavour and fragrance. These olfactometry procedures work well; however, they are to some extent subjective, are rarely able to identify the precise cause of a problem and are also, by definition, manual and consequently expensive/time-consuming to carry out.

In recent years, analytical thermal desorption (TD) has provided a useful complement to GC-MS enabling more aroma profiling applications to be carried out, using quantitative, automatic instrumentation^[1]. TD combines automated sample preparation with selective analyte enrichment allowing target organic compounds to be injected into

the GC-MS as a narrow concentrated band of vapour, free of most/all sample matrix effects. The technology is available in on- and off-line configurations and is now widely used for vapour profiling in the food, flavour, fragrance and consumer product industries.

The process of thermal desorption and its advantages for GC-MS analysis of aroma compounds

The process of thermal desorption is illustrated in Figure 1. One of the strengths of coupling TD to GC-MS for food, flavour and fragrance profiling is that it offers a versatile range of automatic or semi-automatic sampling methodologies. These include:

- **Sorbent tubes (traps)** – used for off-line concentration of organic vapours. The tubes may be packed with multiple sorbents for collecting the complete vapour profile or with a single sorbent that retains key olfactory compounds but allows volatile interferences (e.g. water, ethanol and acetic acid) to be purged to vent. Example applications include; profiling the fragrance of consumer products, tracking taint in warehouse air or shipping containers, breath profiling and monitoring crop volatiles.
- **On-line sampling** i.e. discontinuous sampling/concentration and analysis - used for monitoring changes in odour profile over time. Example applications include food shelf life studies, diurnal variation in natural (biogenic) fragrances, process gas purity and monitoring the profile of perfume products, such as air fresheners, as they decay over time.
- **Direct, low-temperature desorption of materials** weighed into empty TD tubes – used for screening the odour profile of dry, homogeneous materials such as spices, instant coffee, soap powder, etc. Example applications include quality control of spice blends, identifying taint/off-odour in consumer products, validating the quality of natural products (e.g. checking for cheap synthetics), QC of packaging and screening the vapour profile of medicinal pastes/creams.
- **Off-line thermal extraction or dynamic sampling of headspace vapours with sorbent trap.** Used for monitoring the vapour profile of a wide range of inhomogeneous products and raw materials. Aroma profiling applications carried out this way include; tobacco and other vegetable products, shampoo and cleaning products, prepared foods (pizza, pet foods, meat products, etc.), dairy products, biogenic emissions (insect pheromones, plant volatiles, etc.), drinking water odour, taint from packaging, potable spirits and genetically modified (GM) foods.

Whichever of these approaches is used to introduce the sample to the TD-GC-MS, the compounds of interest end up separated from

the sample matrix and focused on a small, electrically-cooled sorbent trap (see Figure 1). The focusing trap is subsequently desorbed by heating it rapidly in a reverse flow of carrier gas causing the organic compounds to be injected / transferred into the GC-MS analytical system as a narrow band of vapour.

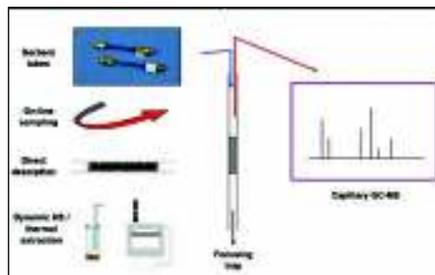


Figure 1. Overview of sample introduction methods compatible with thermal desorption analysis

Trap desorption efficiency and system inertness are key to ensure optimum sensitivity and quantitative recovery of the widest range of compounds. Quantitative re-collection of both primary (tube desorption) split flow and secondary (trap desorption) split flow is offered as standard on the latest TD equipment and facilitates repeat analysis of samples and standards. Where available, this provides a convenient method for demonstrating quantitative recovery of reactive odour components such as amines, oxygenates and mercaptans (thiols) through the analytical system^[1,2] – see figure 2. Any loss of labile compounds would be shown up by a change in relative response in the repeat run.

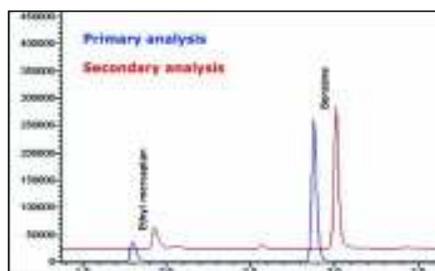


Figure 2. Primary and repeat analysis of ethyl mercaptan shows quantitative recovery of this highly reactive compound through the analytical system. Benzene used as internal standard.

A fundamental advantage of thermal desorption and its associated sampling methods is that compounds of interest are extracted straight into the GC carrier gas stream. No manual sample preparation steps

are required and problems associated with the use of solvents are eliminated e.g. masking of peaks of interest, loss of volatiles and variable extraction efficiency^[1,3]. The vapour profile produced is thus more likely to be representative of the aroma perceived by consumers. The latest TD technology is also capable of transferring the vapour profile constituents into the GC capillary column in much smaller volumes of carrier gas than was possible before. Transfer volumes can be as low as 100 µL of carrier gas allowing significant concentration enhancement factors to be achieved - typically from 10³ to 10⁶ depending on the number of concentration/desorption steps.

Chromatographic interference

However, while the latest innovations in analytical thermal desorption technology have provided a major breakthrough in allowing more aroma profiling to be carried out using automated laboratory instrumentation, TD-GC-MS does not always provide the complete answer. Aroma profiling often relates to natural products or complex composite manufactured products which produce a high background signal in the total ion chromatogram (TIC). The complex background signal can make it difficult to reliably identify key olfactory components in such cases.

Similarly, thick-film columns may be required to separate volatile polar components in the aroma profile and this too can compromise subsequent analysis of trace components.

For example, the top (black) chromatogram in Figure 3 shows the analysis of a trace landfill odour standard loaded onto a sorbent tube and analysed using TD-GC-MS with a 1.4 µm film x 60 m x 0.25 mm I.D. DB-VRX column exhibiting a very high bleed profile.

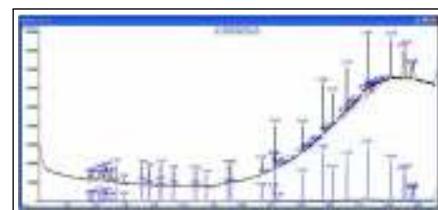


Figure 3. TD-GC-MS analysis of a landfill-gas standard using a thick film capillary column with high bleed. Original TIC data shown (black) and DBC reprocessed data (blue). Software used: ClearView, Markes International Ltd, UK

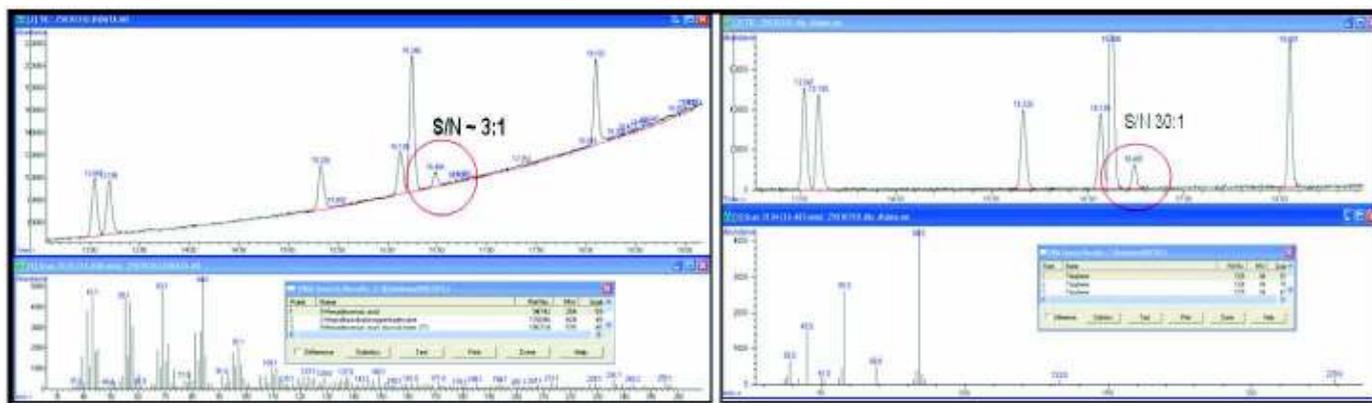


Figure 4. Comparison of original data file (left) and reprocessed file (right) demonstrates how the DBC processing improves not only the spectral quality (and hence the effectiveness of automatic library searching), but also the signal-to-noise ratio.

Dynamic background compensation

The new dynamic background compensation (DBC) algorithm was developed to address exactly such chromatographic background issues. It distinguishes and eliminates mass ions originating from chromatographic 'background' from those in real peaks, however small. This has great potential for many real-world GC-MS and TD-GC-MS applications, including odour and aroma profiling. To summarise how it works; the software reprocesses stored GC-MS data files (singly or in batches) distinguishing mass ions from the background (column bleed, sample matrix, solvent tail, air/water interference, etc.) and eliminating their contribution from the mass ion fragmentation pattern of chromatographic peaks without impacting on any peak related data. A separate

'DBC-reprocessed' data file is then produced leaving the original data file intact for separate analysis if required [4].

The effects of reprocessing can be seen in the bottom (blue) chromatogram of Figure 3. This data was evaluated in detail to assess the benefits and potential limitations of DBC for odour profiling (removal of additional full stops)

Impact of DBC on the qualitative assessment of trace odour components in the presence of high column bleed

Original and reprocessed data for one of the smallest compounds present (retention time: 16.486 mins) is shown in close up in Figure 4. The enhanced spectral purity of the

reprocessed data is immediately apparent. Whereas in the case of the original data, manual background subtraction would be required before the component could be identified, library search of the same peak in the reprocessed data file automatically picked out thiophene and with high confidence.

Inspection of all the peaks in the odour standard showed that spectral purity was universally enhanced in the reprocessed data file, but, as expected, the positive effect was most pronounced for the lowest level components and for peaks eluting where the column bleed was highest. In all cases, automatic library searching of the reprocessed data correctly identified the analyte whereas manual background subtraction was required before most components (21 out of 36 compounds) could be identified in the original data.

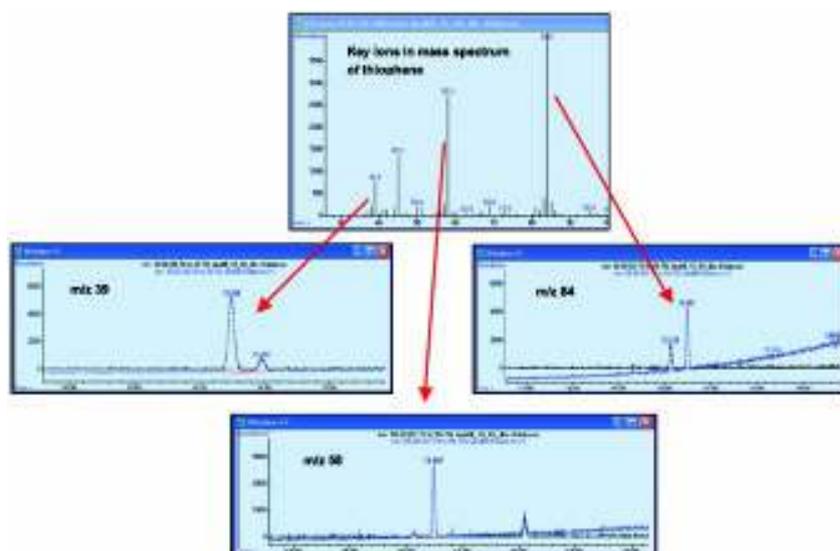


Figure 5. Comparison of original and DBC processed data for extracted ions shows that no peak information is compromised by the background subtraction process. This was true for the major ion (m/z 84) and less significant ions (m/z 58, & 39)

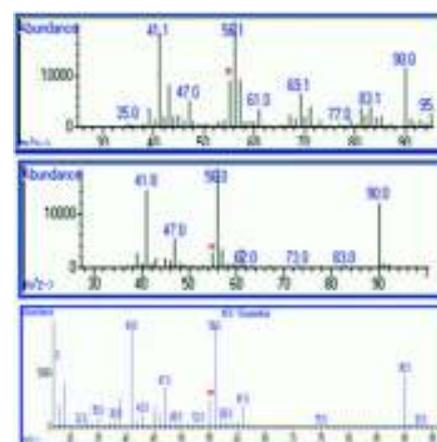


Figure 6. Butanethiol apex spectrum (top) from the original data file; after DBC reprocessing (middle), and library spectrum (NIST05) (bottom)

Impact of DBC on the quantitative assessment of trace odour components in the presence of high column bleed

Another positive impact of DBC was found to be a significant improvement in signal to noise ratios – i.e. in sensitivity. In Figure 4 above, the signal to noise ratio for thiophene improved from 3:1 in the original data to 30:1 in the reprocessed data – representing a ten fold sensitivity enhancement in this extreme case. Moreover, close comparison of the extracted ion chromatographic data showed that none of the peak related information had been compromised by the DBC process – even in the case of minor ions contributing to the thiophene spectrum/peak (Figure 5).

To challenge the quantitative impact of DBC even further another component from the mix was selected (butanethiol, RT 18.1 mins) which contained a common mass ion (mass 55) with the chromatographic background. Figure 6 shows the apex spectra for this peak in the original and reprocessed data together with the library spectrum for butanethiol.

To confirm the presence of mass ion 55 in the background, baseline spectra were taken from the original data file immediately before and after the peak – see Figure 7. Mass ion 55 is clearly visible in each case.

To assess the impact of DBC on mass ion 55 abundance, the extracted ion chromatographic (EIC) data for mass 55, original and DBC reprocessed, were overlaid. (See Figure 8).

It is immediately evident that the peak areas/heights are identical – even in close-up. Moreover, it should be noted that the abundance of mass ion 55 in butanethiol is relatively small and any over- or under-

Prepurge:	1min, trap and split in-line
Desorption:	8mins @120°C, split on.
Desorb flow:	~30ml/min,
Split flow:	~50ml/min
Cold trap:	-10°C to 300°C for 3mins split on
Flow path:	150°C
GC programme:	40°C for 5 min, then 20°C/min up to 300°C.
Column:	60m x 0.25mm x 0.25µm

Table 1. Analytical conditions for direct desorption of tobacco

compensation for the background contribution, by the DBC software, would have a significant and obvious impact on the extracted ion peak area/height. Furthermore, from the flatness of the EIC baseline, it can be seen that DBC is accurately compensating for mass 55 in the background but without impacting the contribution of mass 55 to the butanethiol peak.

Impact of DBC on the detection of key olfactory components in complex product profiles

To evaluate the potential of DBC for enhancing detection of trace olfactory components in complex vapour profiles, the TD-GC-MS aroma data from direct desorption of tobacco were reprocessed using the new

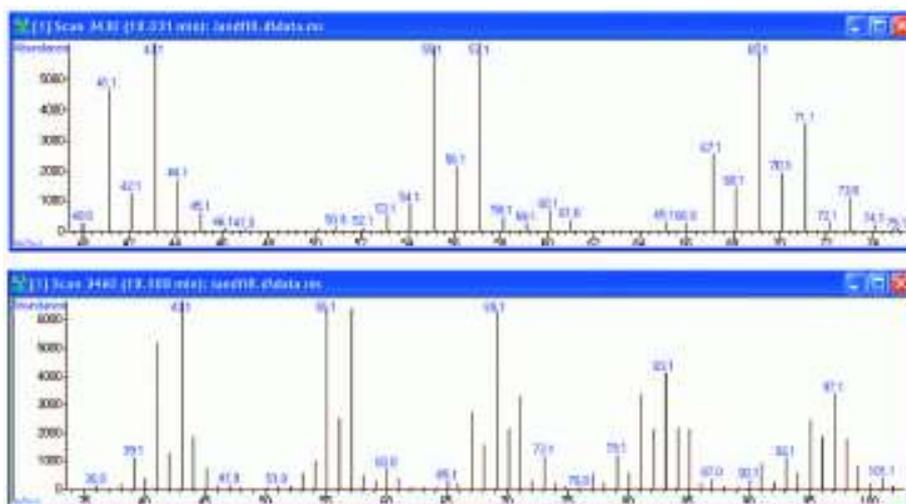


Figure 7. Baseline spectra before/after butanethiol peak identifying presence of m/z 55 in background

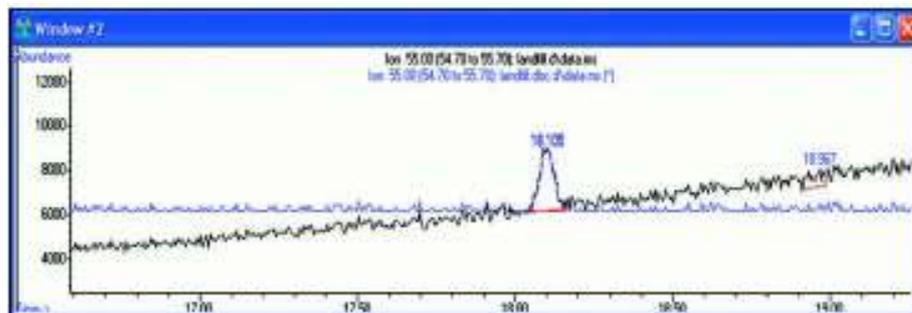


Figure 8. Comparison of original (black) and DBC-reprocessed (blue) EIC data for m/z 55 shown and overlaid and offset

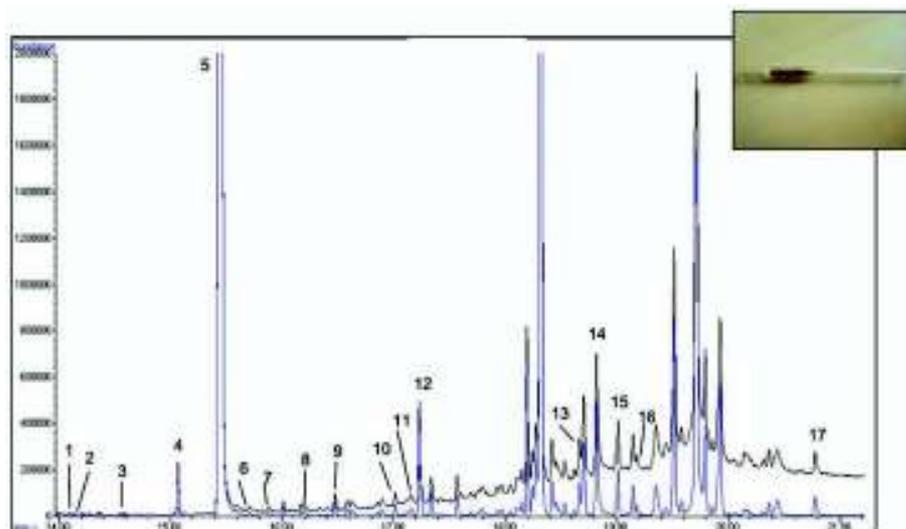


Figure 9. Aroma profile of dried tobacco obtained by direct desorption. Original data (black) overlaid with DBC reprocessed data (blue). Associated Table 1 shows how the reprocessing helps with analysis of trace-level components.

1	1-(2-butoxyethoxy)-ethanol
2	Menthol
3	Benzeneacetic acid
4	Triacetin
5	Nicotine
6	4-hydroxy-benzeneethanol
7	Myosmine
8	3-methyl-4-phenylpyrazole
9	Butylated hydroxytoluene
10	3-Hydroxy-beta-damascone
11	Megastigmatrienone
12	-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one
13	13. Methyl-14-methylpentadecanoate
14	n-Hexadecanoic acid
15	Ethyl palmitate
16	Isopropyl palmitate
17	2-Ethylhexyl-trans-4-methoxy cinnamate

Table 2. List of compounds identified in a sample of dried tobacco. Compounds 1, 2, 11 and 16 were not identified at all in the original, unprocessed data. Compounds 3, 6 and 7 also gave a much improved library match quality after the data was DBC processed.

software. The original and reprocessed data are shown in Figure 9. Table 2 contains a list of the compounds identified in the sample. Components, 1, 2, 11 and 16 were not initially detected in the original data file but were readily identified and measured after reprocessing using dynamic background compensation. Similarly, components 3, 6 and 7 gave a library match significantly better after reprocessing the data with DBC.

Conclusion

The new DBC software for reprocessing (TD-) GC-MS data has been found to enhance detection of trace olfactory components in complex chromatographic profiles even under the most challenging analytical conditions. It has also been demonstrated to do this without compromising quantitative information even when the background and peak of interest contain common mass ions.

In summary, the new software should provide a useful complement to thermal desorption-GC-MS analysis of flavour and fragrance profiles. It should allow even more applications to be successfully carried out using automated laboratory apparatus thus boosting throughput and reducing cost per analysis, whilst minimising the need for manual data manipulation and/or skilled interpretation of data.

References

1. L.J. Kelly and E.A. Woolfenden, *Separation Science*, (2008), in press.
2. D 6196-03, *Standard practice for selection of sorbents, sampling and thermal desorption analysis procedures for VOCs in air*, ASTM, May 2003.
3. A. Jayatilaka, S.K. Poole, C.F. Poole and T.M.P. Chichila, *Anal Chim. Acta*, 302, (1995), 147-162.
4. D. Rosser, G.M. Roberts and E.A. Woolfenden, *The Column*, July 2008.



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