A Matter of Taste....
Flavour Profiling by GCxGC-qMS/FID

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The analysis of flavour compounds in many industries is important for a number of reasons. This can include the differentiation between genuine products and those of lower grade materials produced using different ingredients; for quality control purposes to compare different production batches to detect possible changes; and also in identifying any off-tastes or off-odours caused by aging and storage. The samples are often complex, containing many hundreds of organic compounds of varying polarities which may or may not contribute to the flavour. Separation of the compounds can be difficult on a single type of stationary phase and frequently, strong flavours are given by very low concentration analytes which may co-elute with high concentration analytes on several different stationary phases and, therefore, go undetected.

Introduction

Comprehensive two-dimensional gas chromatography, GCxGC, has become a common technique in the research world over the past decade. Rather than analytes in a complex sample being separated on a single stationary phase; or a 'heartcut' of co-eluting peaks from one column being transferred to a second column containing a different type of stationary phase for separation; every peak transferred onto the column is separated on two different columns containing two different stationary phases. This multiplies the resolving power of one gas chromatograph by that of another, resulting in the possible separation of thousands of analytes.

The interface between the two columns, enabling the re-injection of co-eluting analytes from the first column onto the second column, is known as the modulator. The modulator has two main purposes: firstly to collect a cut, usually 1-10 seconds in length, of the eluent from the first column and secondly to re-inject this cut onto the second column in a tight sample band. The first column is usually of standard dimensions, typically 0.18-0.25mm i.d. and 25-60m long, commonly it contains a non-polar stationary phase. Separation is usually slow with a flow rate of 0.8-1mL/min; this enables a peak to be "cut up" into several slices for separation on the second column and help to prevent overloading.

The second column commonly contains a more polar stationary phase. To enable a fast separation within the modulation time from the first column, depending on the modulator type, the second column is shorter, typically between 0.5-2m long with a narrower i.d. of 0.1-0.15mm or has a standard i.d. at 0.18-0.25mm and is 3-10m long but with a very fast flow rate at around 20mL/min.

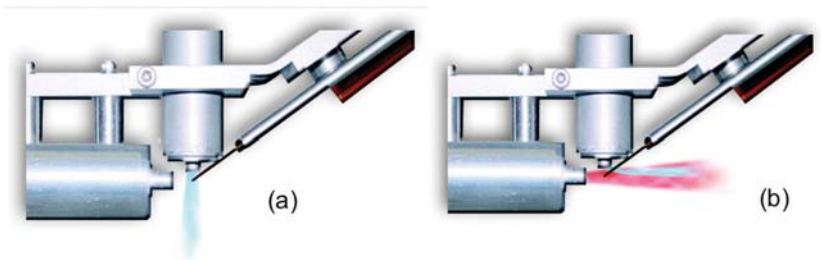

Figure 1: Zoex loop thermal modular. (a) trap with the cold jet; 9b0 reinject with the hot jet

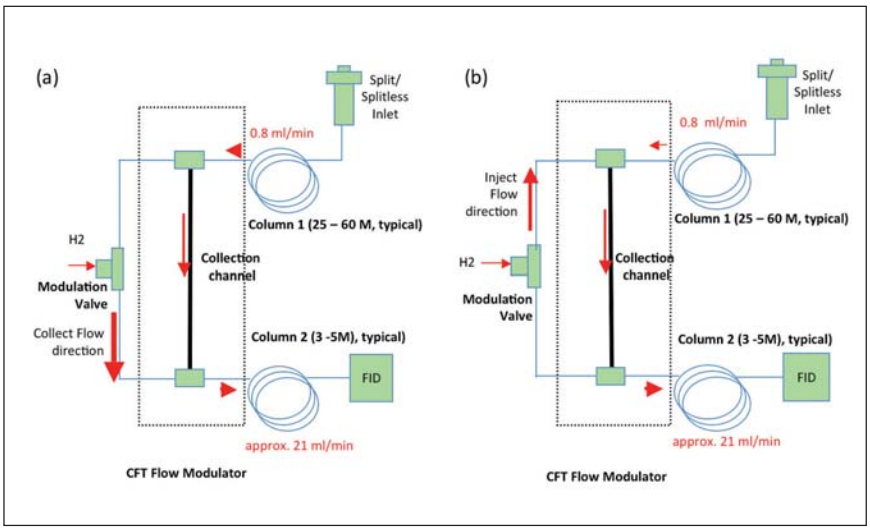

Figure 2: Agilent Capillary Flow Modulator. (a) Load Position; (b) Inject position

By introducing the cut in a tight sample band sharp peaks are obtained that are resolved and eluted from the smaller second column very quickly before the next cut is introduced. These second dimension peaks are around 0.03-0.1 seconds wide and result in an increase in sensitivity.

The majority of modulators, in particular commercial modulators, until recently, involved the use of cryogens to trap and re-inject the cut. These two-stage thermal modulators use cold and hot jets to enable the modulation. An example of the loop thermal modulator from Zoex is shown in Figure 1. The cold jet runs continuously to create two cold spots on the looped column, about 3mm long, Figure 1(a). The hot jet is pulsed periodically to divert the cold jet and heat the cold spot to re-mobilise the trapped analytes and move them on to the next stage. The cold jet is usually cooled with a liquid nitrogen or CO_2 heat exchange, but room temperature compressed gas can be used for the modulation of low volatility analytes. The volume of nitrogen used can be around 12 standard litres per minute, resulting in a large consumable cost as well as high initial set-up costs for handling liquid nitrogen for a thermal modulator.

More recently, commercial flow modulators have enabled the modulation of gases to high boiling components without the use of cryogens; Figure 2 shows the Agilent capillary flow technology GCxGC modulator. In the load position, Figure 2(a), the eluent from the first column is accumulated in the collection channel at a flow rate of 0.8mL/min. The cut is then injected onto the second column by changing the flow direction from the modulation valve to flush the analytes at the second column flow rate of around 21mL/min as shown in Figure 2(b), to produce a tight sample band. The analytes are then quickly separated on the second column by this high flow rate ready for the next cut to be introduced. Hydrogen is typically used as it produces a very fast, efficient separation and is cheap considering the carrier gas flow rates used in the separation on the second dimension column.

When considering detectors for GCxGC, one with a fast response is necessary for the very narrow peaks eluted from the second dimension column. Typically, universal detectors like Flame Ionisation Detectors (FIDs) and specific detectors like Electron Capture Detectors (ECDs) and Sulphur Chemiluminescence Detectors (SCDs) are employed. To hyphenate a Mass Selective Detector (MSD) for additional information for compound identification, confirmation and quantitation, a fast-scanning Time-of-Flight is required to obtain enough data points across the peak.

Until recently, investment in a GCxGC-MS instrument with a thermal modulator and a Time-of-Flight mass spectrometer, was a large cost which is justified for a dedicated

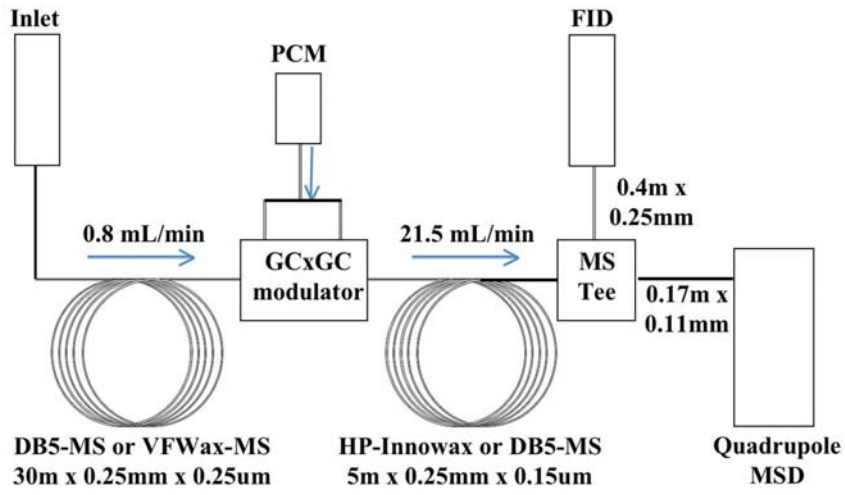

Figure 3: GCxGC-FIDqMSD configuration showing both column sets

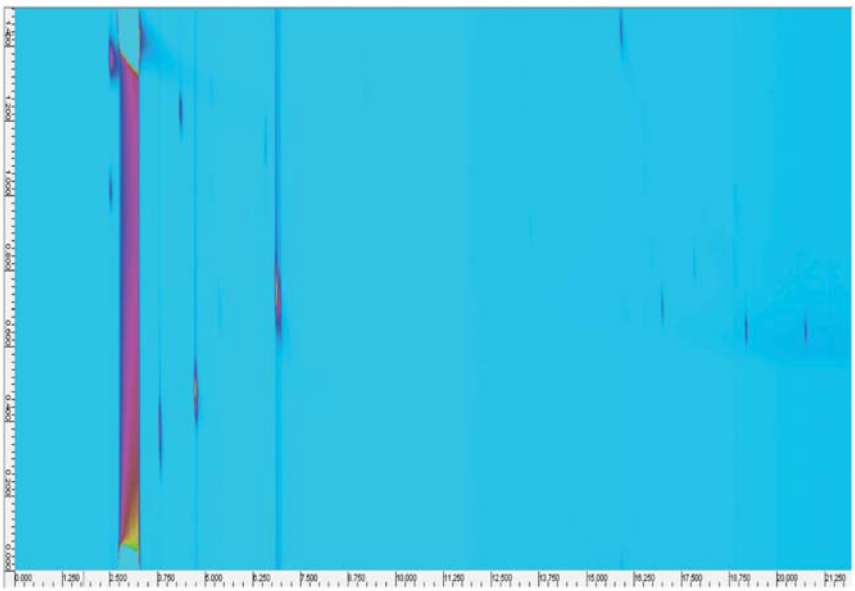

Figure 4: GCxGC-FID chromatogram of Talisker whisky on column set 1

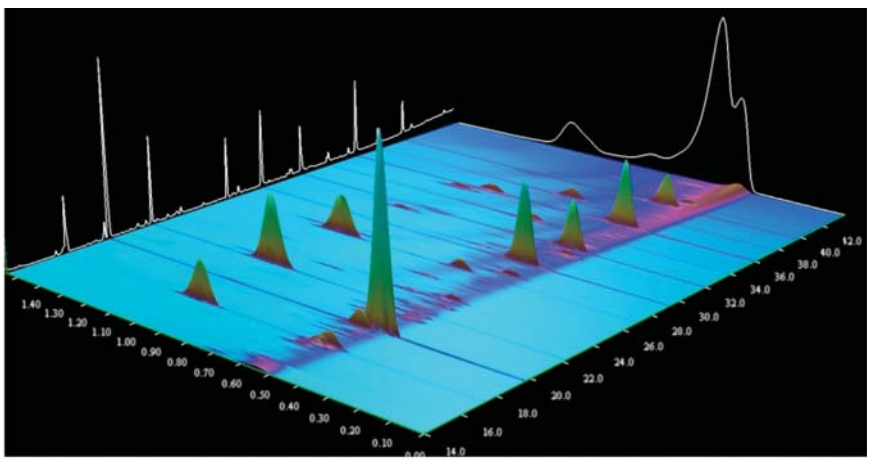

Figure 5: GCxGC-FID 3D chromatogram of Talisker whisky on column set 2

instrument that is regularly used for the analysis of complex samples but not justifiable for the occasional sample analysis by GCxGC-MS. Introduction of a flow modulator, that can be upgraded on a standard gas chromatograph and uses cheap hydrogen as the only consumable greatly reduces the investment in GCxGC technology and the instrument can justifiably continue to be used for non-GCxGC applications. The flow rate eluting from the second column is far too high to be

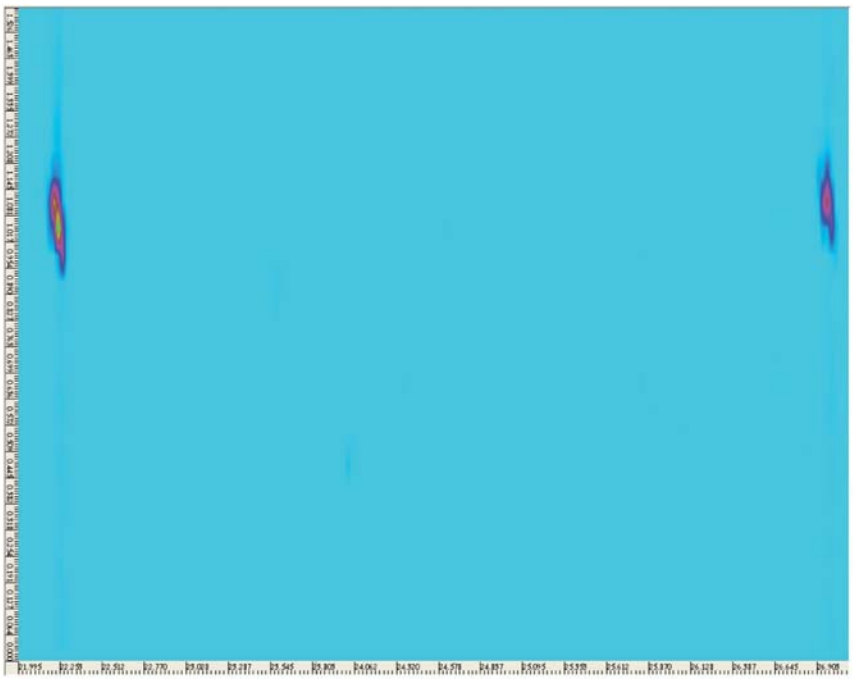

Figure 6: Selected ion chromatogram 87-89 ions

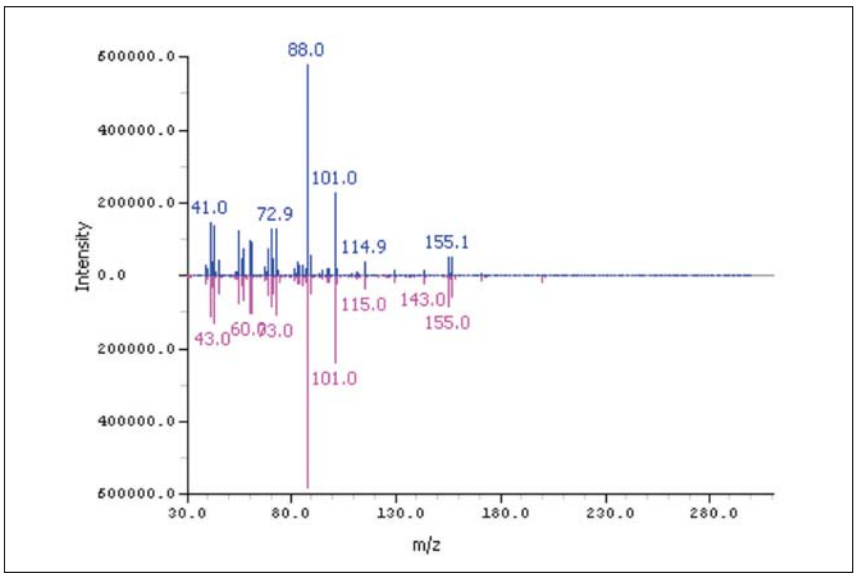

Figure 7: Decanoic acid, ethyl ester at 22.228, 1.017 89% match

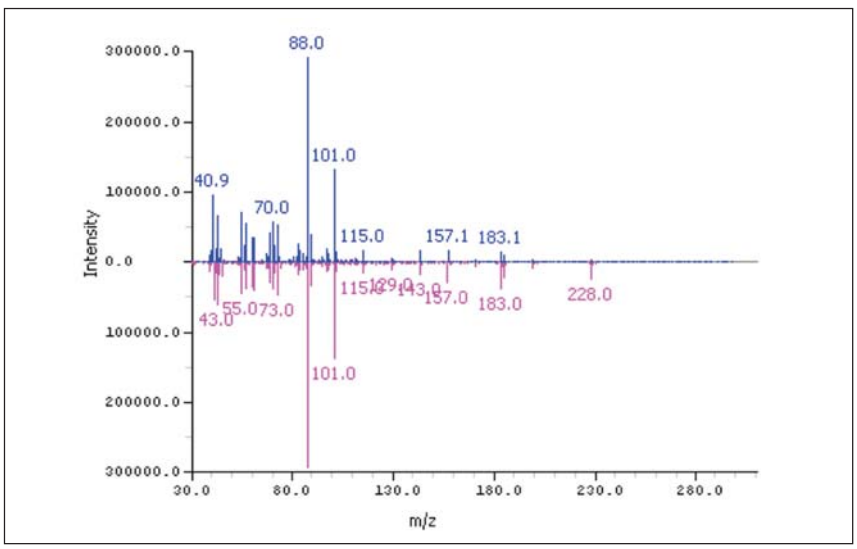

Figure 8: Dodecanoic acid, ethyl ester at 26.955, 1.081 85% match

introduced directly into any mass spectrometer, fast scanning or not. FIDs have long been the detector of choice for quantitation for many applications, with their molar response to carbon, linearity to seven orders of magnitude and reasonable sensitivity. GCxGC adds the high resolution required to separate hundreds if not thousands of peaks, reducing the necessity for analytical resolution. The retention times on both the non-polar and polar columns, along with the class position give added confirmation of the identification of the analyte. Therefore, the main advantage of using an MSD with GCxGC is in the identification of an unknown sample component. Splitting the eluent from the second dimension column when using a flow modulator between a detector like an FID and an MSD means that an MSD with a very high acquisition rate isn't needed, the number of data points across the peak can be quite low to obtain a mass spectrum for identification rather than the higher number needed for quantitation or deconvolution. Hence a bench-top mass spectrometer, like a quadrupole, can be used which is standard in most analytical laboratories these days and again doesn't require a huge investment in GCxGC-MS technology for an occasional sample. An instrument capable of working at higher flow rates is recommended with a high performance turbo to enable a higher proportion of the split to be directed to the MSD giving a higher sensitivity, but this is the same as a GC-MS capable of chemical ionisation. Therefore, by using a GCxGC flow modulator with a quadrupole mass spectrometer the investment required both to purchase the instrumentation and the running costs is far reduced, making GCxGC-MS more accessible for commercial laboratories whether it is to analyse samples routinely or to analyse the occasional sample using this technique.

Experimental

Whisky was analysed by GCxGC-FID/qMSD on an Agilent Technologies (Cheadle, UK) 7890 gas chromatograph with a 5975C mass spectrometer and a capillary flow technology GCxGC modulator using two column sets as shown in Figure 3. Column set 1 comprised of a non-polar 30m x 0.25mm i.d. x 0.25um DB5-MS first dimension column and a 5m x 0.25mm x 0.15um HP-Innowax second dimension column. 1 µL of neat Talisker whisky was injected with a 10:1 split ratio.

GC Image software (Zoex Corp., Houston, USA) was used to analyse the data from both the FID and MSD. GC Image can visualise

the data in either a 2D contour plot where the intensity of the peaks is shown by the varying colours within the peak (Figure 4). Additionally the data can be visualised using a 3D plot where the peaks are more recognisable (Figure 5).

Results and discussion

The contour plot of the GCxGC-FID chromatogram of Talisker whisky on column set 1 is shown in Figure 4. As the first dimension column is non-polar, the x-axis in Figure 4 shows separation based on volatility, with decreasing volatility to the right. The y-axis is the separation by polarity on the second dimension column, with increasing polarity towards the top. On this zoomed-out contour plot, not many peaks can be seen, with the major component being ethanol which overloads both dimension columns as expected. Due to the nature of the components within whisky being more polar than non-polar it was decided to reverse the column phases.

Column set 2 was a polar 30m x 0.25mm i.d. x 0.25um VFWax-MS first dimension column and a non-polar 5m x 0.25mm x 0.15um DB5-MS second column. Therefore the separation on the first column is based on volatility and polarity and the second column only on volatility.

Figure 5 shows a zoomed in portion of the chromatogram of an injection of Talisker whisky on column set 2, with a modulation period of 1.55 seconds, shown in a 3D plot. Not only is the improvement in sensitivity apparent by using GCxGC, but the range of volatilities and polarities of the analytes and the relationships between the component classes can be easily seen.

The GCxGC-MS chromatogram can be used to locate peaks as well as identify unknowns. Figure 6 is a selected ion chromatogram of the masses 87-89 used to find the ethyl esters of decanoic acid (figure 7) and dodecanoic acid (figure 8), the match similarities are high despite the mass spectra not being background subtracted in this case. Figure 9 shows the zoomed-in chromatogram of the GCxGC-FID contour plot between these two ethyl esters with nearly 200 individual peaks found in this small area of the chromatogram. The

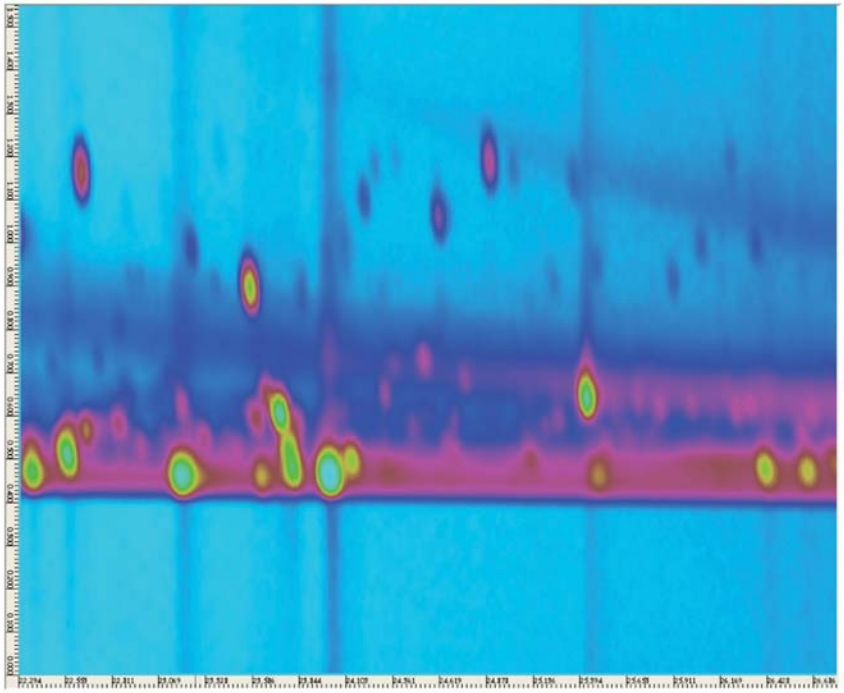

Figure 9: GCxGC-FID chromatogram of Talisker whisky on column set 2

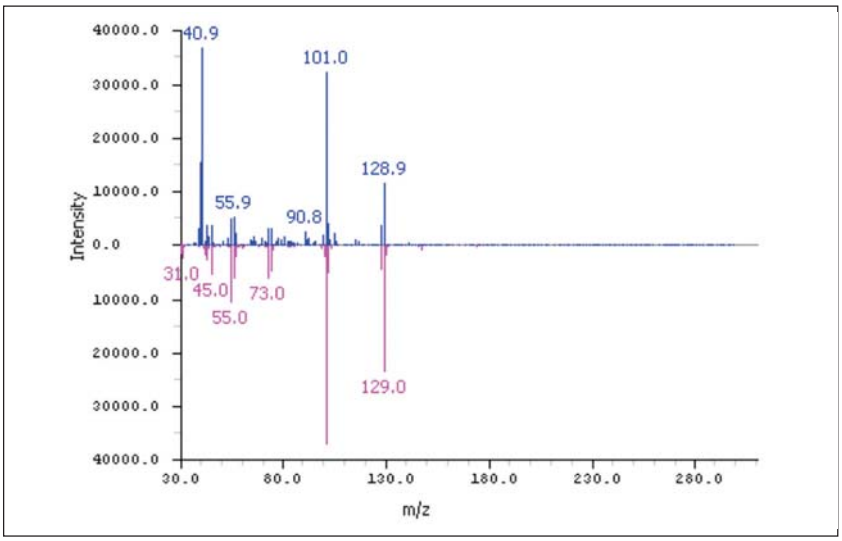

Figure 10: Butanedioic acid, diethyl ester at 23.726, 0.509 84% match

GCxGC-MS chromatogram can again be used to either identify individual peaks of interest through the GC Image software as shown in Figure 10 or from the original data acquisition software, AMDIS and NIST.

Conclusions

In conclusion, GCxGC-MS doesn't have to be a technique available only to research laboratories or those with enough complex samples to justify the purchase of a dedicated, high-cost instrument with high consumable costs. The use of capillary flow technology to upgrade an existing system or the purchase of a standard GC(-MS) with GCxGC capabilities and the possibility of splitting to a quadrupole MSD for the identification of unknowns makes GCxGC an option available to all.