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Pet Food Perseverance

By Andrew Ward, JSB UK

"How heart-cutting two-dimensional gas chromatography aided in the identification of hidden odorant peaks to save the day – and the dog's dinner."

The Problem

Is it possible to reliably identify and quantify components present at 'undetectable' levels in extremely complex matrices without laborious sample preparation protocols – and without breaking the bank?

Background

No doubt, you've been here: "my old instrument isn't going to do the job; it isn't selective/sensitive enough." At this point, you've started looking for ways to justify the expense of that shiny, new instrument you've had your eye on – the one with the state-of-the-art detector… only to be stopped in your tracks by limited budget. When you hit that wall, it's worth remembering that a little innovation can go a very long way.

The identification and/or quantification of very low-level components in complex matrices is a common challenge. Co-elution is something every experienced chromatographer has faced, and the approaches used to overcome it are numerous. However, de-convolution can only take us so far. In a sample that contains large amounts of 'component X', which co-elutes with trace levels of 'component Y', reliable identification and quantification of both components can be extremely difficult, even with laborious sample preparation protocols. In such circumstances, using higher-end instrumentation can be a logical choice. But it is not the only one.

Lewis Jones of Mars Waltham has been working on odorant identification for some time. One problem is the subjective nature of odorant detection, and so Lewis was keen to explore analytical options that would facilitate a more objective approach. Here, he hit a situation familiar to many of us: "I know something else is there, but I just can't see it". Lewis had confirmed that odorants were present in his samples by using a much more selective and sensitive detector than is commonly available for your chromatographic system of choice – the human nose. However, he could not detect – let alone identify – them as peaks in the chromatogram with the tools that he had available. Lewis needed a way of (i) identifying and separating very low levels of components in very complex matrices, while (ii) improving sensitivity to such a degree that components present at 'undetectable' levels could be reliably identified and even quantified.

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The Solution

Working with pet food samples, which Lewis casually informed me "really don't taste that bad," he likened the 'hardware' of the human nose to dynamic headspace sample preparation with direct detection, and so paired direct MS with olfactory detection. However, it soon became apparent that it was not possible to correlate the identity and importance of hundreds of ion masses to the associated odorants, and that gas chromatography (GC) was required.

Next, he used GC to separate the volatile organic compounds and combined it with three detectors: an olfactory detection port (ODP) to detect odor-active peaks, a pulsed flame photometric detector (PFPD) to detect the presence of sulphur-containing moieties, and a single quadrupole mass selective detector (MSD) to identify components. Yet, even following sample preparation by SAFE extraction and
distillation, the resulting chromatograms were very crowded and showed a high level of background noise due to the complex nature of the matrix involved (see Figure 1A and 1B). Furthermore, the approach came with its own set of problems: odors were being detected where only odor-inactive compounds could be identified in the mass spectrum or where there were no peaks at all (on the MSD or PFPD). Having ruled out misalignment of the detectors, the conclusion became clear: co-eluting peaks in the MSD were masking odorants and some odorants were present at levels below the detection limit of the MSD and PFPD.

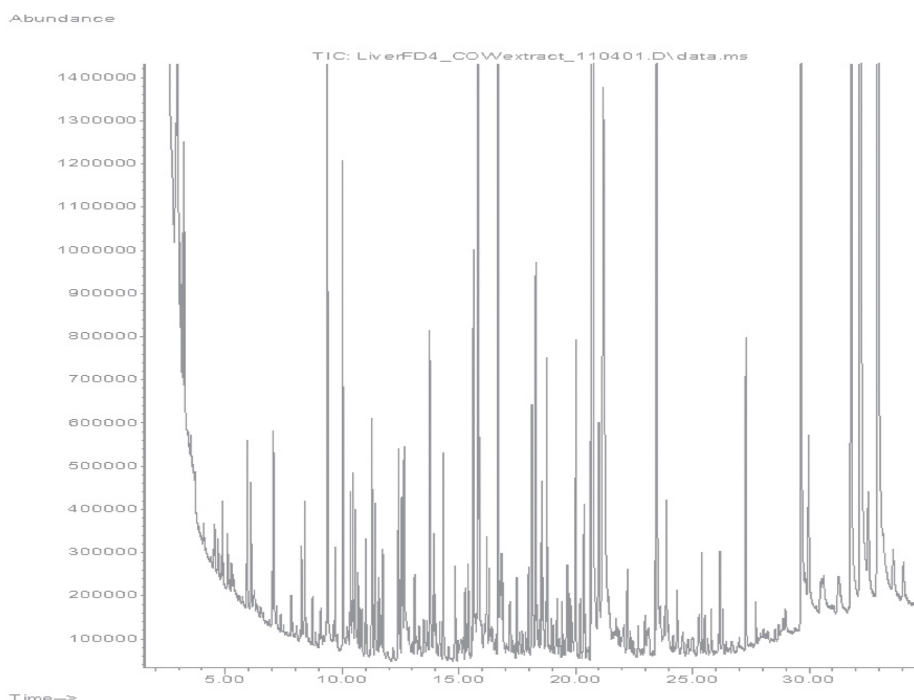

Figure 1. A. TIC chromato gram of pet food sample fol lowing SAFE extraction and distillation.

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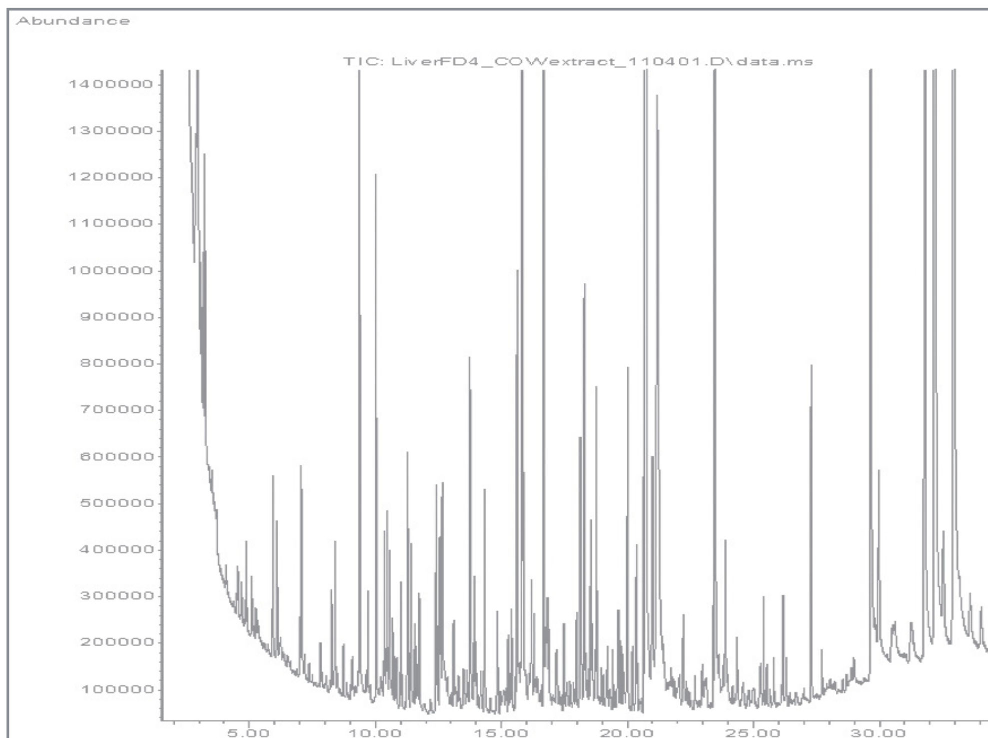

Figure 1A
TIC chromatogram of pet food sample following SAFE extraction and distillation.

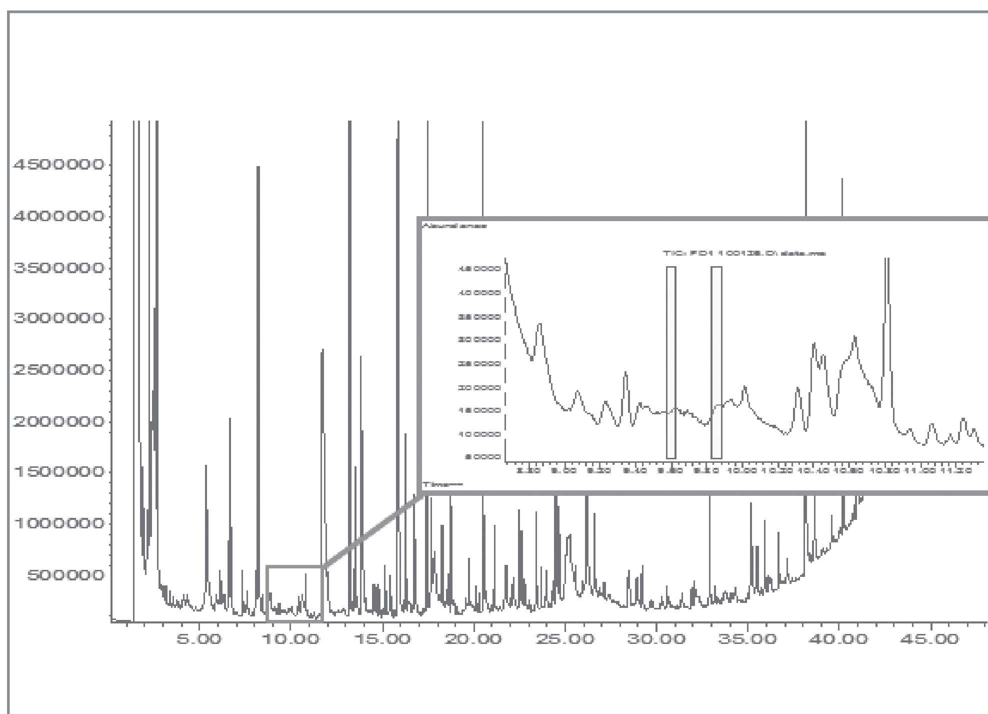

Figure 1B
Regions of a chromatogram where odors were detected via the olfactory detection port showing that no corresponding peaks were seen in the mass selective detector results.

To reduce the complexity of the chromatograms, Lewis fractionated his sample extracts into basic, neutral and acidic fractions, thus reducing the number of components present in each injection and reducing the chance of co-elution. This approach confirmed the presence of co-eluting peaks, and furthermore the presence of co-eluting odorants (see Figure 2). It is important to note that the human nose is capable of detecting more than one odor at once.

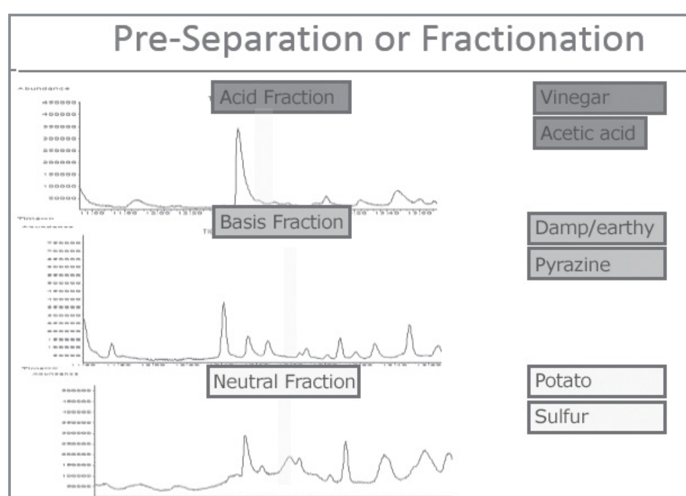

Figure 2
Fractionation of SAFE extracts with the odors detected at the highlighted points.

Now the situation becomes even more challenging: according to the odours emitted from the olfactory detection port, multiple, co-eluting odour-active components are present at levels not detectable on the current system.

It was at this stage that Lewis approached us, armed with the information he had gained from his work to date and his knowledge of the workings of the human nose and odorants. He told us that he needed to find an objective analytical system equivalent to the human nose. In simple terms, the problem can be broken down as follows: the sensitivity of a single quadrupole MSD is in the region of approximately 1pg (scan); the sensitivity of PFPD is approximately 1pg (spec); and the sensitivity of a high-speed ToF-MS is in the region of approximately 0.1pg. The human nose can detect some compounds down at levels of approximately 40ag, way below that of commercially available GC detectors. We needed to develop a system that could identify unknown compounds present at very low levels in extremely complex matrices, which may co-elute both with each other and with other components present at levels many orders of magnitude higher.

The solution proposed by Bryan White (Director at JSB) was to heart-cut peaks or regions of interest – where odors were detected – in the chromatogram onto a second column with a different phase for a second dimension of chromatographic separation in isolation. This helped with separation of co-eluting peaks, but the key to improving sensitivity was the addition of a cryo-trap after the heart-cut. Using an Agilent 7890B GC with an Agilent 5795C MSD, we initially used an Agilent Capillary Flow Technology modulator plate to heart-cut regions from column one into a SIM Ice Cube cryo-trap packed with glass beads at -20 ºC to trap

the components. This has the advantage of allowing multiple injections into column one, heart-cutting the same region each time and concentrating the components in the trap. When a second analytical method heats the trap the components are released and separated on column two before being detected by the MSD (and ODP).

Results from initial tests using a mixture of alkanes and a sample extract looked promising, then we hit a couple of snags. Bryan is anosmic – having no sense of smell makes this kind of work particularly difficult, which is why I took over. I developed the GC method to separate the alkanes to allow calculation of Kovat's retention indices on column one and set about doing the same on column two by cutting all of the alkanes into the trap. However, it became apparent that glass beads at -20 ºC would not trap the full required range of alkanes (and therefore all of the components of interest based on their retention indices). Reducing the temperature was not an option with a Peltier cooling system. Instead, I replaced the glass beads with Tenax and solved the issue. The system was ready.

Lewis gave me a sample of what he described as 'a very difficult co-elution' of known components. This was a mixture of four odorants that all co-eluted with levels of acetic acid often found in his samples. It was in fact five odors across one large, ugly peak (see Figure 3A). Other than the acetic acid, the odorants could not be identified by standard GC-MS. Initially we tried three heart-cuts of the peak onto the trap and eluted the components. This allowed us to identify three odorants, but two peaks were still missing. So we did 24 heart-cuts and eluted the trap contents. This allowed the other two components to be easily identified. But was the system linear at such low levels?

Figure 3
Resolving co-eluting odorants

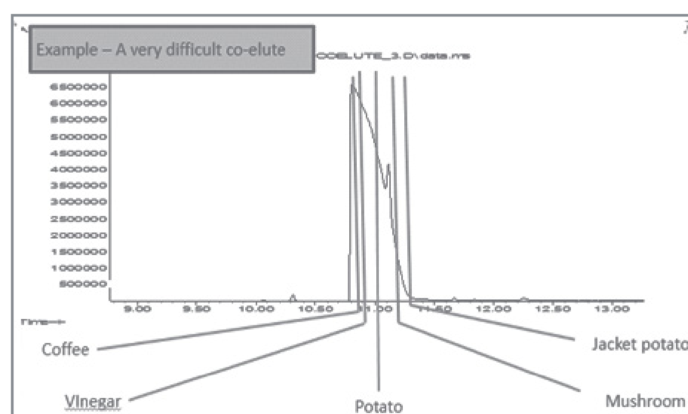

Figure 3A
A very difficult co-elution: five odors across one large peak.

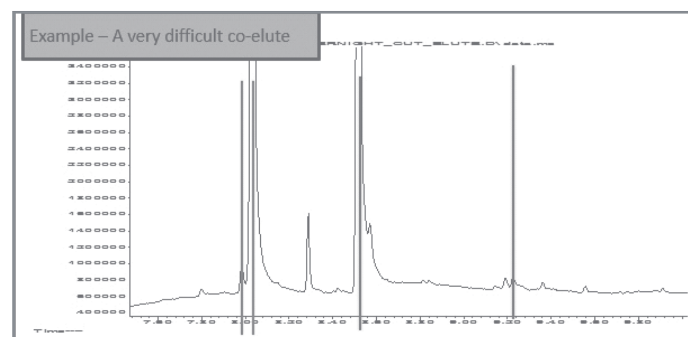

Figure 3B
The 'missing' four odorants under the acetic acid peak following concentration by 24 heart-cuts.

The components of this sample were:

Component	Concentration
Acetic acid	1000 mg/l
Furanmethanthiol	10 mg/l
1-octen-3-ol	10 mg/l
3-(methylthio)propanal	2mg/l
2-ethyl-3,5(6)-dimethylpyrazine	0.2 mg/l

Looking at the furanmethanthiol (TIC) peak areas, the ratio of the peak area after 24 heart cuts to that after three heart cuts was 8.3:1. The corresponding value for the 1-octen-3-ol peak was 7.9:1. Promising results.

So how could the system be improved? The oven was looking more than a little intimidating with all of the connections inside. I changed both columns to low thermal mass (LTM) units so they sat outside the oven, which aids in column changing and has the advantage of independent temperature programming for the two columns. I also connected a flame ionization detector (FID) to facilitate quantification.

A schematic of the final system is shown in Figure 4.

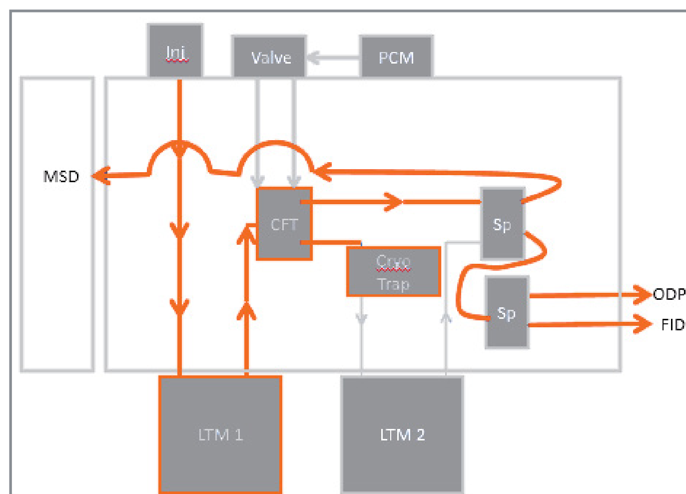

Figure 4A
Schematic showing heart-cut flow path

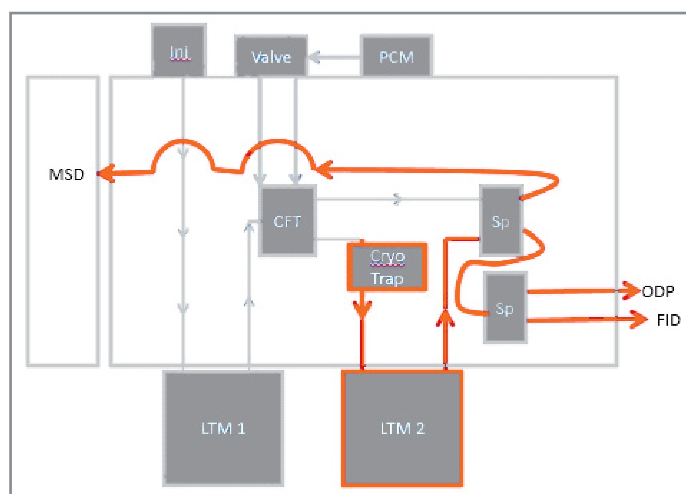

Figure 4B
Schematic showing trap elute method flow path. LTM: low thermal mass column, CFT: capillary flow technology modulator, PCM: pneumatics control module, Sp: flow splitters, Inj: injection, MSD: mass selective detector, ODP: olfactory detection port, FID: flame ionization detector.

Beyond the Solution

I think we can claim success with this solution. It is easy to implement, adaptable (by the simple exchange of low thermal mass columns), doesn’t require vast quantities of cryogen, and, most importantly, the potential applications of the system are absolutely vast – essentially any application looking at low level components in complex matrices.

The system is also useful for particularly difficult co-elutions (chiral columns can be used to separate enantiomers, for example). Another potential application would be automated sample screening for very low-level components, using sequential runs to take different cuts and concentrating components eluting in regions of interest. But that is for another day!

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