SepSolve Analytical

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Comprehensive analysis of tobacco smoke using TD–GC×GC–TOF MS with Tandem Ionisation

In this study, we demonstrate the enhanced separation and unparalleled sensitivity that can be achieved in the characterisation of cigarette smoke, using thermal desorption (TD) and GC×GC with time-of-flight mass spectrometry, aided by Tandem Ionisation for simultaneous acquisition of hard and soft EI data.

Introduction

The hazardous constituents of cigarette smoke have attracted considerable attention lately, especially with increasing regulation around the world limiting or banning smoking in public places – and even in private cars if children are present.

From an analytical perspective, however, there is much that remains to be learnt about the composition of cigarette smoke, because of its high degree of complexity – tobacco smoke is thought to contain thousands of components across multiple chemical classes and wide concentration ranges.

Thermal desorption (TD) is widely used for the analysis of volatile and semi-volatile organic compounds (VOCs and SVOCs) by GC–MS, in a variety of sample types and over a wide concentration range. It greatly improves sample throughput, by allowing the full automation of sample preparation, desorption/extraction, preconcentration and GC injection.

Comprehensive two-dimensional gas chromatography (GC×GC), when coupled with time-of-flight mass spectrometry (TOF MS), is also a powerful approach to the analysis of complex samples, improving chemical fingerprinting in areas of study as diverse as petrochemical analysis and fragrance profiling.

In this study, we use thermal desorption (TD) for collection and analysis of whole cigarette emissions, and couple it with flow-modulated GC×GC–TOF MS, to enable smoke constituents to be routinely and confidently sampled, separated and identified.

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The use of Tandem Ionisation® [1] is also harnessed to increase the analytical resolution of the system, by providing both reference-quality 70 eV spectra and soft electron ionisation (EI) spectra simultaneously in a single analysis.

Experimental

Sample preparation: Smoke from three commercial cigarette brands (A, B and C) was analysed. Cigarette smoke was drawn (on the fourth 'puff') directly onto an inert-coated Tenax® TA sorbent tube using an ACTI-VOC™ low-flow pump (Markes International). A flow of 300 mL/min was applied for 10 s, sampling ~50 mL of cigarette aerosols. Aluminium foil was wrapped around the interface between the tube and cigarette filter (Figure 1) and made airtight by applying a piece of Scotch® tape. After the sampling period the tube was capped and placed in the automated thermal desorber ready for analysis.

Figure 1

Sampling set-up for the collection of cigarette smoke, showing (left) the ACTI-VOC pump with sorbent tube and (right) the seal between the cigarette and sorbent tube.

TD: Instrument: TD100-xr™ (Markes International); Flow path: 190°C; Pre-purge: 1 min, 20 mL/min; Tube desorb: 10 min, 300°C, 50 mL/min, no split; Trap: General-purpose; Desorb split flow: 200:1; Pre-trap-fire purge: 1 min, 50 mL/min; Trap low: 20°C; Heating rate: Max; Trap high: 300°C, 5 min.

GC×GC: 2D column set: 1st dimension: BPX5™, 20 m × 0.18 mm × 0.18 µm; 2nd dimension: DB-17ms™, 5 m × 0.25 mm × 0.25 µm; Temp. program: Main oven: 40°C (5 min), 5°C/min to 240°C (5 min); Modulator: INSIGHT® flow modulator (SepSolve Analytical); Loop dimensions: 227 mm, 1/32" o.d., 0.53 mm i.d.; Loop volume: 50 µL; Loop fill time: 7.75 s; Loop flush time: 0.25 s; Modulation period (P_M): 8.0 s.

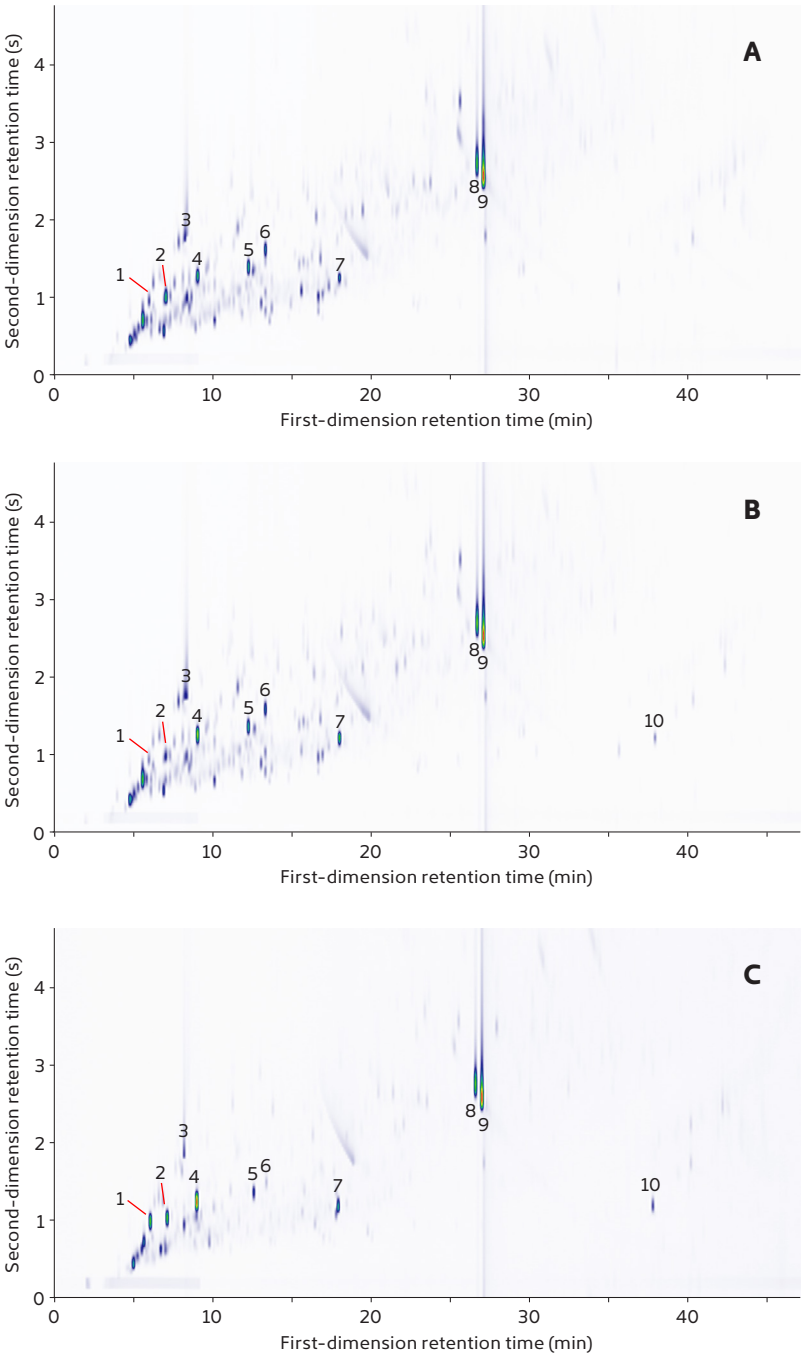
TOF MS: Instrument: BenchTOF-Select™ (Markes International); Filament voltage: 1.7 V; Ion source: 250°C; Transfer line: 250°C; Mass range: m/z 35–400; Data rate: 100 Hz; Tandem Ionisation®: Simultaneous acquisition of 70 eV and 14 eV data.

Software: Instrument control and data processing by ChromSpace®.

Results and discussion

Characterisation of tobacco smoke

The GC×GC–TOF MS plots of the three smoke samples (Figure 2) show the same major components, with nicotine and triacetin unsurprisingly the dominant peaks. Sample C had a similar suite of components as Samples A and B, but generally in lower abundance (as evident in the less intense gradient of the colour plot).



1 Benzene
2 2,5-Dimethylfuran
3 Propylene glycol
4 Toluene
5 *m*- and *p*-Xylene
6 *o*-Xylene
7 Limonene
8 Triacetin
9 Nicotine
10 Neophytadiene

Figure 2

TD–GC×GC–TOF MS colour plots for tobacco smoke from three commercial cigarette brands. Note the minimal breakthrough and tailing even for the most abundant compounds (e.g. nicotine and triacetin).

Table 1 compares the composition of the three samples for compounds with NIST 14 match factors greater than 750. It is worth noting that, unlike all other TOF instruments, BenchTOF overcomes mass discrimination, resulting in reference-quality (70 eV) spectra that are a close match for those produced by conventional quadrupole systems and in commercial libraries such as NIST or Wiley.

Name	^1t_R (min)	^2t_R (s)	Peak area		
			A	B	C
Alkanes					
2-Methylpentane	4.535	0.48	5.96×10^5	7.32×10^5	7.28×10^6
Alkenes					
Hex-1-ene	4.802	0.64	8.73×10^6	1.05×10^7	–
2-Ethylbutadiene	5.069	0.76	5.67×10^6	4.05×10^6	–
5-Methylcyclopenta-1,3-diene	5.602	1.06	1.81×10^7	1.89×10^7	3.77×10^6
Azides, nitriles and cyanides					
Hydrogen azide	4.001	0.68	5.92×10^5	6.22×10^5	–
2-Methylbutanenitrile	7.603	2.64	5.51×10^5	5.04×10^5	–
Isobutyl cyanide	7.870	2.66	3.26×10^6	3.62×10^6	–
Pentanenitrile	9.337	3.08	1.83×10^5	5.54×10^5	–
3-Methylbenzonitrile	21.209	5.16	6.31×10^5	–	–
Benzenepropanenitrile	24.010	5.48	5.83×10^5	–	–
Monoaromatics					
Benzene	6.003	1.52	3.46×10^6	2.22×10^6	2.87×10^5
Toluene	8.937	1.92	1.27×10^7	1.83×10^7	1.72×10^7
Ethylbenzene	12.272	2.18	9.63×10^6	9.02×10^6	2.25×10^5
m- & *p*-Xylene	12.539	2.14	4.15×10^6	–	2.55×10^6
Phenylethyne	12.805	2.72	3.43×10^5	2.94×10^5	–
o-Xylene	13.339	2.32	–	–	8.60×10^5
Styrene	13.339	2.50	8.00×10^6	6.57×10^6	–
α-Methylstyrene	16.407	2.62	4.69×10^5	4.67×10^5	–
Phenol	16.274	3.32	2.54×10^6	1.75×10^6	3.78×10^5
2-Methylphenol	19.341	3.44	1.28×10^6	6.35×10^5	1.92×10^5
4-Ethylphenol	22.009	3.56	–	1.28×10^6	3.38×10^5
Catechol	23.343	3.84	1.47×10^6	9.08×10^5	4.92×10^5
Hydroquinone	25.211	5.20	4.30×10^6	2.14×10^6	8.50×10^5
Acids and esters					
Acetic acid	4.802	1.18	6.24×10^5	5.82×10^5	–
Butyrolactone	14.006	6.44	1.52×10^5	9.14×10^4	–
Triacetin	26.544	4.38	2.72×10^7	2.94×10^7	1.47×10^7

Table 1

A selection of compounds identified in the tobacco smoke samples, organised by chemical class and retention time *(continued on next page)*.

Name	^1t_R (min)	^2t_R (s)	Peak area		
			A	B	C
Ketones					
3-Methylbut-3-en-2-one	6.269	1.80	2.84×10^6	1.59×10^6	3.88×10^5
Pentan-2-one	6.670	1.96	1.64×10^6	2.19×10^6	–
Methyl isobutyl ketone	8.137	1.92	1.94×10^6	1.71×10^6	–
Methyl butyl ketone	9.737	2.18	1.08×10^6	2.24×10^6	–
Cyclopent-2-en-1-one	11.338	4.50	4.21×10^5	3.98×10^5	–
Cyclopent-4-ene-1,3-dione	12.939	4.62	–	–	2.23×10^5
2-Methylcyclopentanone	13.472	3.48	3.67×10^5	3.92×10^5	–
2-Methylcyclopent-2-en-1-one	13.739	3.88	–	3.19×10^5	3.53×10^5
Acetophenone	19.075	4.08	–	4.39×10^5	–
Aldehydes					
Benzaldehyde	15.740	3.80	6.08×10^5	–	–
Alcohols					
Propylene glycol	8.270	2.78	8.44×10^6	1.03×10^7	3.48×10^6
Glycerin	18.808	2.80	1.64×10^6	8.90×10^5	2.80×10^6
PAHs					
Naphthalene	22.676	3.92	1.46×10^6	1.02×10^6	–
2,7-Dimethylnaphthalene	28.545	3.94	6.92×10^5	2.62×10^5	–
1,6-Dimethylnaphthalene	28.945	4.14	–	3.97×10^5	–
Acenaphthylene	29.612	5.18	3.73×10^5	–	–
Oxygen heterocycles					
2,5-Dimethylfuran	7.070	1.58	1.30×10^7	5.47×10^6	7.00×10^6
2,4-Dimethylfuran	7.337	1.54	2.84×10^6	1.82×10^6	–
2-Vinylfuran	7.603	1.80	2.37×10^6	1.45×10^6	1.46×10^5
Furfural	11.205	3.94	8.71×10^5	–	2.98×10^5
2-Furanmethanol	12.005	3.32	7.23×10^5	6.42×10^5	–
1,4:3,6-Dianhydro-α-D-glucopyranose	23.343	5.92	1.07×10^6	8.25×10^5	4.23×10^5
2,3-Dihydrobenzofuran	23.476	4.04	2.53×10^6	5.41×10^5	4.80×10^5
5-Hydroxymethylfurfural	23.743	6.00	–	8.88×10^5	–
Terpenes					
Limonene	18.007	1.92	6.51×10^6	1.16×10^7	5.44×10^6
Neophytadiene	37.749	1.82	–	1.57×10^6	1.94×10^6

Table 1

A selection of compounds identified in the tobacco smoke samples, organised by chemical class and retention time *(continued on next page)*.

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Name	^1t_R (min)	^2t_R (s)	Peak area		
			A	B	C
Nitrogen heterocycles					
1-Methyl-1*H*-pyrrole	8.003	2.56	–	–	9.19×10^5
Pyridine	8.404	3.32	5.28×10^6	–	–
1-Ethyl-1*H*-pyrrole	10.538	2.70	5.88×10^5	8.48×10^5	–
2-Methylpyridine	10.938	3.52	1.97×10^5	1.67×10^5	–
4-Methylpyridine	12.405	4.12	2.31×10^5	–	–
2,3-Dimethyl-1*H*-pyrrole	12.405	3.06	–	1.98×10^5	–
2-Ethylpyridine	13.873	3.04	3.02×10^5	2.64×10^5	–
2,5-Dimethylpyrazine	14.139	3.42	6.14×10^5	1.08×10^6	–
3-Ethyl-1*H*-pyrrole	14.406	3.22	1.66×10^5	–	–
3-Ethenylpyridine	16.007	3.64	9.73×10^5	9.41×10^5	–
Pyridin-3-ol	20.809	3.90	2.42×10^6	1.87×10^6	5.91×10^5
2-Methylpyridin-3-ol	21.476	3.64	3.57×10^5	–	–
Indolizine	25.611	5.70	4.65×10^6	3.57×10^6	6.57×10^5
Nicotine	26.945	6.40	9.06×10^7	7.65×10^7	3.84×10^7
1-Methylindolizine	28.012	5.50	–	8.72×10^5	–
Myosmine	28.945	5.76	9.63×10^5	1.07×10^6	–
2-Phenyl-1*H*-pyrrole	31.747	6.68	–	3.77×10^5	–
Cotinine	35.348	8.12	2.83×10^5	–	–

Table 1

A selection of compounds identified in the tobacco smoke samples, organised by chemical class and retention time *(continued from previous pages)*.

The enhanced region shown in Figure 3 shows the efficient modulation and excellent separation provided by this GC×GC method, with average peak widths of ~200–300 ms in the second dimension. At one point, at least five components would be expected to co-elute in a one-dimensional separation (dotted red line) – which would likely have resulted in identification difficulties.

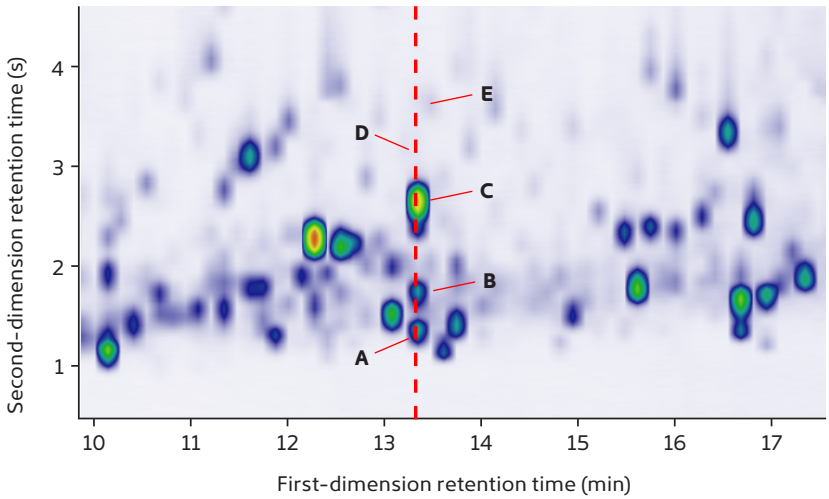

Figure 3

Enhanced region from Sample A, showing the separation capacity of flow-modulated GC×GC–TOF MS. The dotted line represents shows a point where five components (**A–E**, with spectra shown in Figure 4) are separated in the second dimension.

The increased separation capacity allows these components to be confidently identified, as demonstrated by the spectral comparisons with the NIST 14 library (Figure 4).

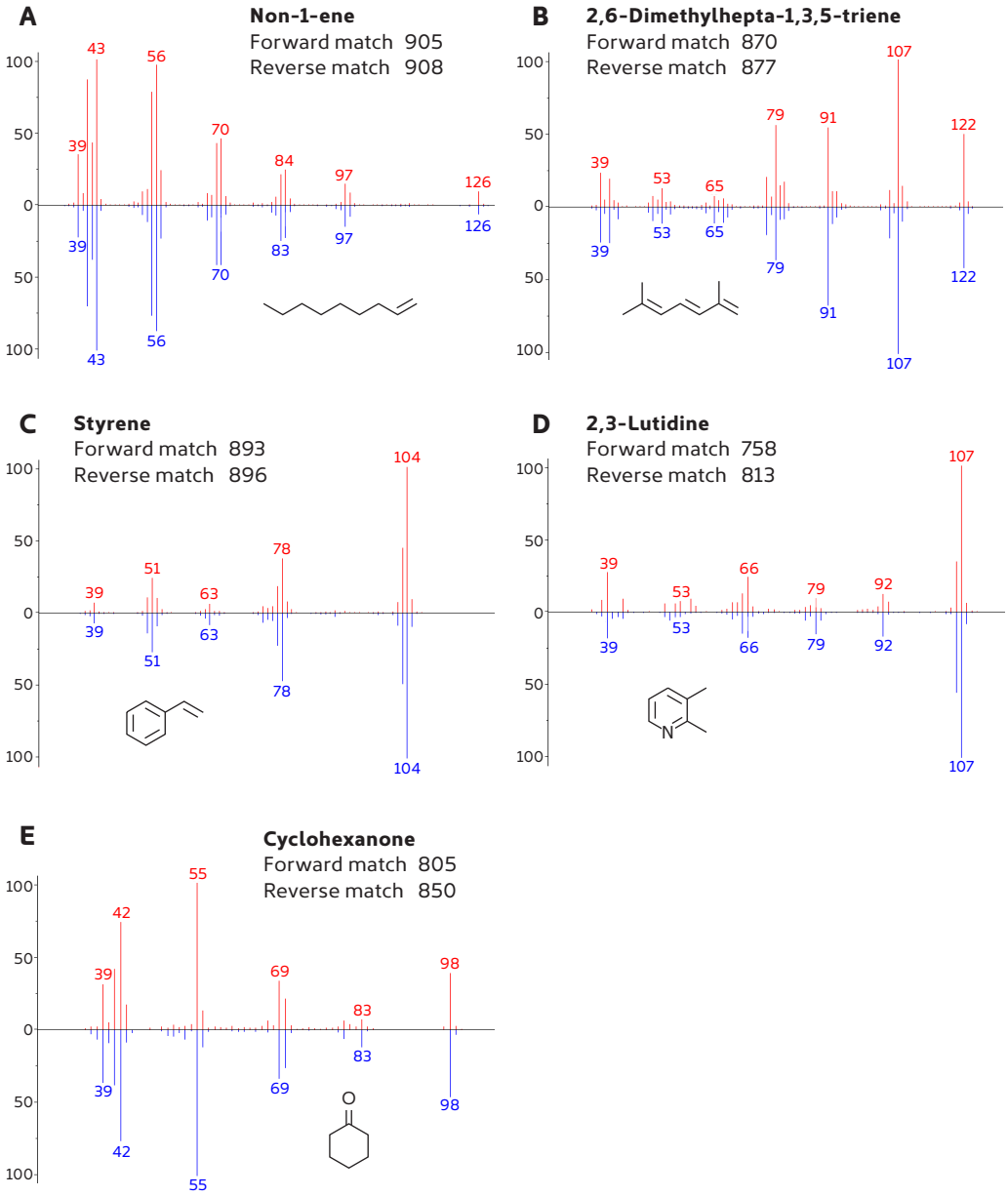

Figure 4

BenchTOF spectra (top, red) and NIST 14 spectra (bottom, blue) for the five components **A–E** identified in Figure 3.

The flow modulation setup used in this analysis, because it is based on a simple valve system, eliminates the need for expensive liquid cryogen, lowering running costs and making it practical to operate. The valves can also be configured for heart-cutting and backflushing, making the whole system versatile enough to handle other sample types encountered in the tobacco industry, such as e-cigarette aerosols.

Simultaneous acquisition of spectra at two ionisation energies (Tandem Ionisation)

Although the 'reference-quality' spectra generated by BenchTOF enable the confident characterisation of cigarette smoke described above, challenges may still remain in cases where compounds exhibit similar spectral characteristics at conventional 70 eV ionisation energy.

Markes' Tandem Ionisation technology addresses this problem by multiplexing two ionisation energies within a single analysis, using Select-eV soft electron ionisation (EI).[1] In this study, Tandem Ionisation was applied using 70 eV and 14 eV EI, creating two independent data files with the obvious advantage of eliminating the need to collect a duplicate sample. This results in two perfectly aligned chromatograms from a single analytical run – simplifying both the acquisition and processing of soft-ionisation data.

In tobacco smoke analysis, the availability of complementary soft EI spectra can be of considerable value, as illustrated by the example of neophytadiene. This diterpene is a common target in tobacco analysis because it is present at low levels in green tobacco leaf, but it is known to increase in concentration during yellowing and curing.[2] Neophytadiene undergoes a high degree of fragmentation at 70 eV, making confident identification problematic, but acquiring data at 14 eV using Select-eV enhances the structurally-significant peaks, enabling its identity to be verified (Figure 5).

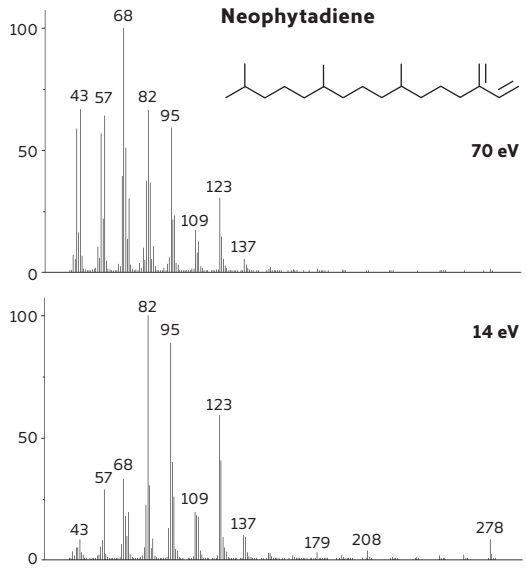

Figure 5

Comparison of 70 and 14 eV spectra of neophytadiene, obtained by Tandem Ionisation of tobacco smoke.

The soft EI data was also found to provide enhanced confidence in the identification of other important tobacco smoke constituents (Figure 6). For example, triacetin (Figure 6A) is the most extensively used plasticiser in cigarette filters, but the significant ions in the 70 eV spectrum for this compound have very low intensity. Select-eV Tandem Ionisation provides enhancement of the higher-m/z ions (such as m/z 145 and 158) for simpler identification of this compound in complex matrices, and making it possible to achieve reliable quantitation using these diagnostic ions, rather than the more common m/z 43.

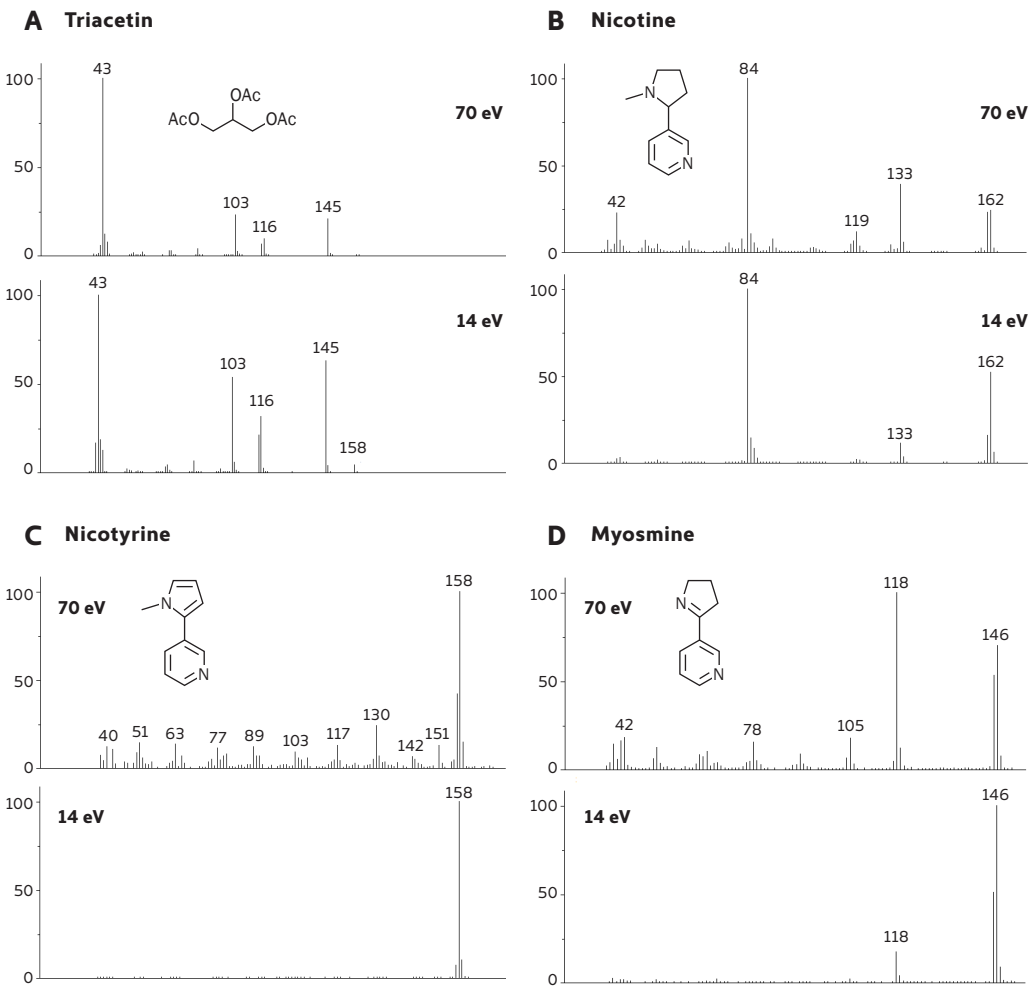

Figure 6

Comparison of 70 and 14 eV spectra for four compounds in tobacco smoke, obtained by Tandem Ionisation.

Conclusions

In this study, we have shown that flow modulation for GC×GC can provide excellent separation of trace-level analytes while minimising breakthrough and tailing of highly concentrated compounds, such as nicotine and triacetin. Furthermore, Tandem Ionisation using BenchTOF-Select provides another level of confidence in compound identification, through the simultaneous acquisition of NIST-quality 70 eV spectra and complementary soft EI spectra with enhanced molecular (and other structurally-significant) ions.

When these GC×GC–TOF MS technologies are combined with the sensitivity enhancement possible with the TD100-xr automated thermal desorber, the result is a multi-functional, highly-automated platform with a high degree of analytical resolution. As demonstrated here, such capabilities make it very well-suited for tackling the analytical challenges encountered in the tobacco industry, whether in matters of regulatory compliance or research & development.

For more information on this application, or any of the techniques or products used, please contact SepSolve.

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References and notes

[1] Select-eV capability, available on the BenchTOF-Select mass spectrometer from Markes International, allows soft EI spectra to be collected down to 10 eV, and is fully automated by the instrument's software with no inherent loss in sensitivity or need for manual intervention. These spectra provide enhanced molecular and structurally-significant ions to aid the identification of compounds that exhibit similar spectra (or extreme fragmentation) when using conventional 70 eV settings. Tandem Ionisation now allows soft and hard ionisation spectra for a single peak to be simultaneously obtained, in both GC and GC×GC analyses, enabling challenging compounds (such as structurally similar isomers) to be discriminated without impacting laboratory workflows. Contact SepSolve for more details.

[2] J.C. Leffingwell, Leaf chemistry: Basic chemical constituents of tobacco leaf and differences among tobacco types (Chapter 8A), in *Tobacco: Production, Chemistry, and Technology*, D.L. Davis and M.T. Nielson (eds.), Blackwell Science, 1999, pp. 265–283.

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