GC-Orbitrap method development

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Introduction

Gas chromatography coupled with mass spectrometry (GC-MS) enables the detection of volatile/semi-volatile chemicals in samples. GC-MS remains the preeminent technique for many clinical, forensics, environmental monitoring and biomonitoring assays, due to good chromatographic stability and generation of highly reproducible mass spectra.[1]

GC-MS methods operating in full-scan mode (fs-GC-MS) are widely used in routine screening programmes (e.g. doping and drug testing, pharmaceutical impurity analysis, food fraud and authenticity testing, newborn screening for inborn errors of metabolism etc.). However, typical fs-GC-MS methods are relatively slow (> 30 min), limiting sample throughput. Furthermore, most fs-GC-MS analyses are conducted at nominal mass, limiting selectivity and, in practice, detection sensitivity.

Consequently, there is a need to develop faster, more selective fs-GC-MS methods which can be routinely applied at population scale for screening programmes. In 2015, the high-resolution GC Orbitrap MS was released and provides enhanced mass accuracy and greater selectivity in full-scan mode, meaning faster chromatography is possible due to better discrimination in the mass domain.

Aim: To develop a fast fs-GC-MS method suitable for the analysis of hundreds to thousands of biological samples

Method

Development was based upon the previous fs-GC-MS method, outlined in Table 1. Analysis of standard mixtures of 33 alkanes (C_7 – C_{40}) and 44 native polychlorinated biphenyls (mono-deca PCBs, resolvable) were used for assessment.

Development followed three steps:

1. The previous method was translated from 30 m to 15 m column by the Restek EZGC Method Translator online tool.
2. The temperature programme of the GC oven was optimized to i) resolve co-eluting peaks (for PCB mixture) and ii) to assess coverage range (for alkane mixture).
3. The flow rate was optimised to obtain highest intensities on target ions.

To ensure compatibility with previous fragment ion identification databases, the retention times of compounds in an in-house library were interpolated via natural cubic splines from the measured retention indices of the alkane mixture.[2-5]

Table 1. Overview of fs-GC-HRMS method parameters.

	Previous method			Updated method		
Column	30m Rxi-5Sil MS			15m Rxi-5Sil MS		
Temp gradient	Rate (°C/min)	Temp (°C)	Hold (min)	Rate (°C/min)	Temp (°C)	Hold (min)
		70	2		80	0.5
	20	90	0	40	200	0.5
	10	200	1	40	260	0.5
	10	280	2	55	330	4
	20	320	8			
Flow rate	1.3 mL/min			1.2 mL/min		
Total runtime	35 min			11.5 min		
Alkane range	C11-C38			C11 - C40		

Results & Conclusion

- Updated fs-GC-HRMS method enables 3-fold greater sample throughput (Figure 2).
- Expanded chemical space coverage (e.g. inclusion of C_{39} & C_{40} alkanes; Figure 2).
- Base peak intensity of target ions has been increased (Figure 2).
- All mono-deca PCBs are easily resolved (Figure 3).
- Compatibility with spectral libraries has been maintained (Figure 4).
- Scans per peak remain adequate for accurate quantitation.
- The method is being routinely applied in the Biomarker Analytical Laboratories.

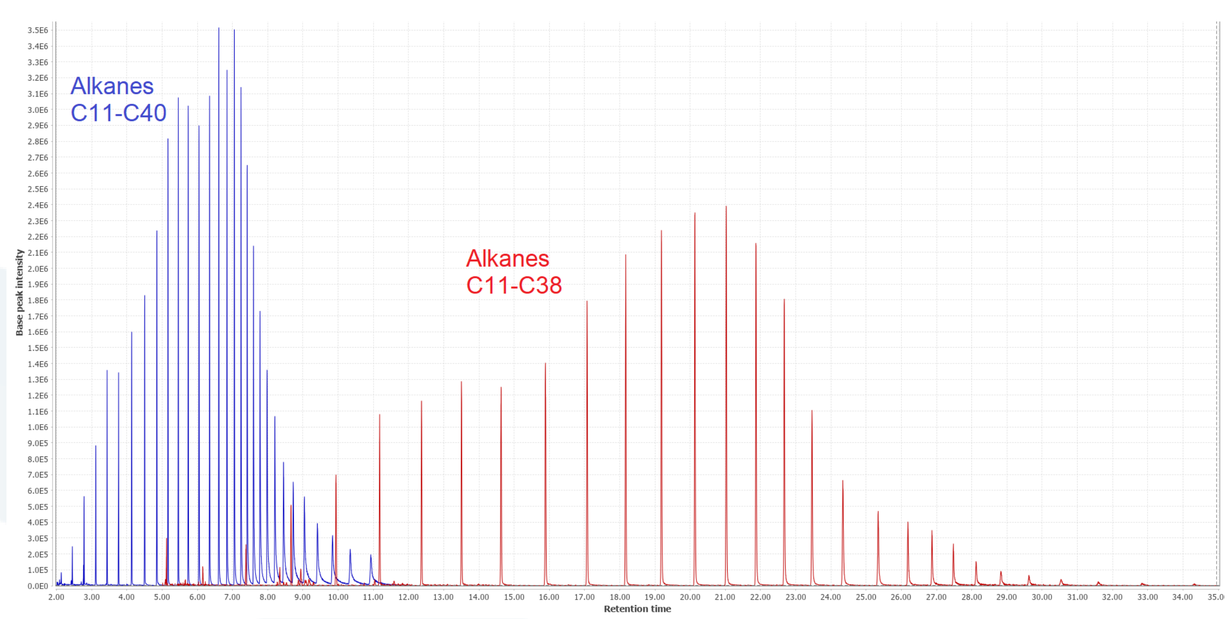

Figure 2. Chromatogram overlay of alkanes (1 µg/mL) analysed via previous method (red) and updated method (blue). Overlays made within MZmine 2 software.

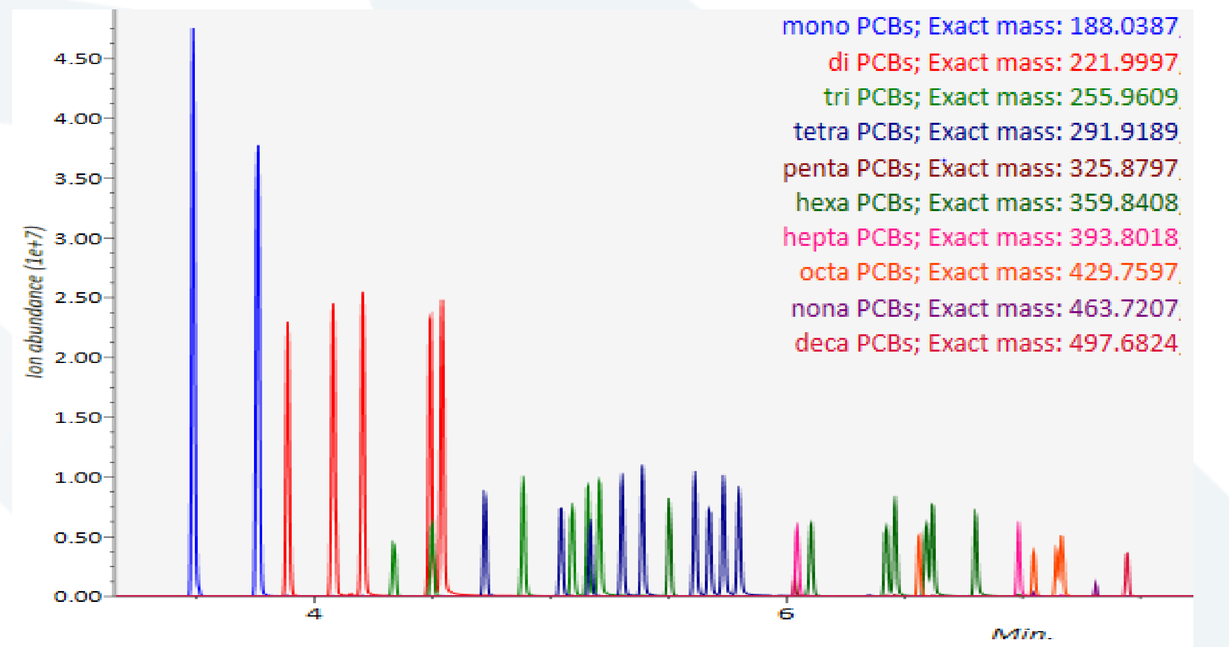

Figure 3. Chromatogram overlay of native PCBs mixture (100 ng/mL) analysed via updated method. Overlays made within MS-DIAL software.

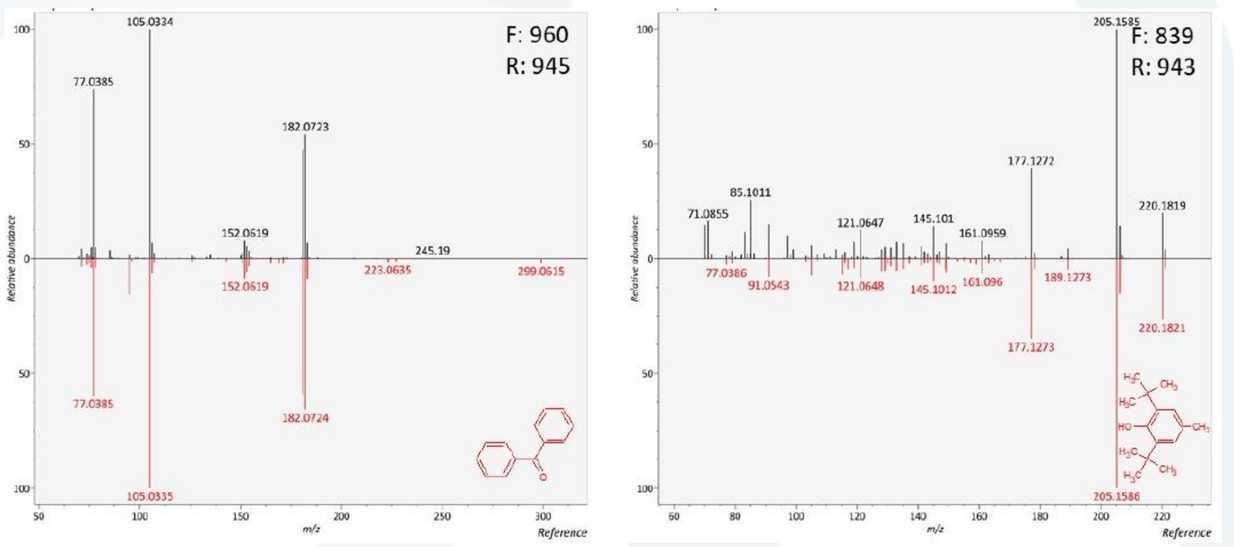

Figure 4. Example matches of sample spectra (black) to library spectrum (red) for identification of benzophenone (left) & butylated hydroxytoluene (right) in human serum (NIST SRM 1957) i.e. forward (F) and reverse (R) dot product scores >800. Spectral deconvolution and matches via MS-DIAL software.[1]

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References

(1) Price, E. J.; Palát, J.; Coufaliková, K.; Kukučka, P.; Codling, G.; Vitale, C. M.; Koudelka, Š.; Klánová, J. Open, High-Resolution EI+ Spectral Library of Anthropogenic Compounds. *Front. Public Heal.* **2021**, *9* (March), 1–5. **https://doi.org/10.3389/fpubh.2021.622558.**

(2) Kováts, E. Gas-chromatographische Charakterisierung Organischer Verbindungen. Teil 1: Retentionsindices Aliphatischer Halogenide, Alkohole, Aldehyde Und Ketone. *Helv. Chim. Acta* **1958**, *41* (7), 1915–1932. **https://doi.org/10.1002/hlca.19580410703.**

(3) van Den Dool, H.; Dec. Kratz, P. A Generalization of the Retention Index System Including Linear Temperature Programmed Gas—Liquid Partition Chromatography. *J. Chromatogr. A* **1963**, *11* (3), 463–471. **https://doi.org/10.1016/S0021-9673(01)80947-X.**

(4) Halang, W. A.; Langlais, R.; Kugler, E. Cubic Spline Interpolation for the Calculation of Retention Indices in Temperature-Programmed Gas-Liquid Chromatography. *Anal. Chem.* **1978**, *50* (13), 1829–1832. **https://doi.org/10.1021/ac50035a026.**

(5) García Domínguez, J. A.; Santiuste, J. M. Cubic Splines Compared with Other Methods for the Calculation of Programmed Temperature Retention Indices. *Chromatographia* **1991**, *32* (3–4), 116–124. **https://doi.org/10.1007/BF02325013.**