

Time-Compressed Analysis of PCBs and Persistent Pesticides in Biological Samples by Isotopic dilution Gas Chromatography/Time-of-Flight Mass Spectrometry

Jean-Francois Focant¹, Edwin De Pauw¹, James Grainger², Donald G. Patterson Jr.²
and Jean-Marie D. Dimandja²

¹Mass Spectrometry Laboratory, University of Liege, B6c Sart-Tilman, B-4000 Liege, Belgium
e-mail : JF.Focant@ulg.ac.be

²Toxicology Branch, Division of Laboratory Sciences, National Centers for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA 30341.

Introduction

Polychlorinated biphenyls (PCBs) are a class of Persistent Organic Pollutants (POPs) that are important environmental toxicants. Due to their industrial production and extensive use until the late 1970's, these compounds are ubiquitous in the environment and various levels of human exposure (acute as well as chronic) have been well documented. Out of the 209 possible congeners, 38 PCBs (all of which have at least one chlorine atom in the *ortho* position) are utilized as biomarkers in human blood [1,2]. In order to evaluate their concentration levels in humans, robust analytical methods are required. These methods must be sensitive enough to allow part-per-trillion (ppt) detection level and fast enough for high sample throughput.

Among the monitored POPs, PCBs and persistent pesticides are some of the most prominent in human samples. In order to respond to the needs of public health studies, CDC has developed several isotope dilution GC/HRMS methods to analyze organic toxicants in human serum. The otherwise powerful GC/HRMS instrumentation that is used for the analysis of these POPs suffers some limitations that are related to the limited accelerating voltage working range for a given group of ions in the selected ion monitoring (SIM) mode. For example, co-eluting compounds with wide differences in their masses cannot be effectively monitored in the same window [3] and the chromatographic run thus needs to be prolonged for adequate component speciation. Another consequence is this limitation is the need for an additional step in the sample cleanup method to yield two separate fractions (one fraction containing the PCBs and another fraction for the persistent pesticides) from the single fraction containing all the PCBs and persistent pesticides of interest, which only protracts the analysis time even further because these two fractions are run separately [4].

An alternative method has been developed to enhance the capabilities of the current GC/IDHRMS method in use. Due to their non-scanning character, time-of-flight mass spectrometers (TOF-MS) are valuable tools for fast GC because they are able to monitor the entire mass range in very short times [5]. In addition, the lack of spectral biasing (since all ions leave the source in the same time, the ion ratios remain the same during the same peak) allow for the use of deconvolution algorithms to resolve co-eluting compounds in the MS domain. These capabilities thus open the window for faster cleanup procedures (through the reduction of the number of analytical fractions) and "time-compressed" analytical runs.

This work was dedicated to the development of a new method for the simultaneous analysis of PCBs and persistent pesticides using fast gas chromatography/isotope dilution/time-of-flight mass spectrometry (GC/IDTOF MS).

Materials and methods

All standards and samples used were obtained from CDC Dioxin laboratory working on the current methods for PCBs and pesticides analysis, and these methods have been described elsewhere [3,4].

All the analyses were run by GC- TOF MS using a Pegasus II time-of-flight mass spectrometer (LECO corporation, St. Joseph, MI, USA) and an Agilent (Palo Alto, CA, USA) 6890+ Series gas chromatograph. Samples (injection volume: 1 μ L) were injected (in splitless mode) both manually and/or through the use of an autosampler. The injection port temperature was set at 260°C. A DB-5 MS capillary column (30m, 0.25mm I.D., 0.25 μ m film thickness) was used for the separation of the analytes, and helium was used as carrier gas. Transfer line temperature was set at 280°C. An acquisition rate of 10 spectra/sec over a range of 100 to 450 amu has been used with a detector voltage of 1500 V.

Results and Discussion

Time compression of the analytical run from 22 minutes (in the GC/IDHRMS method [4]) to 5.6 minutes is illustrated in Fig.1.

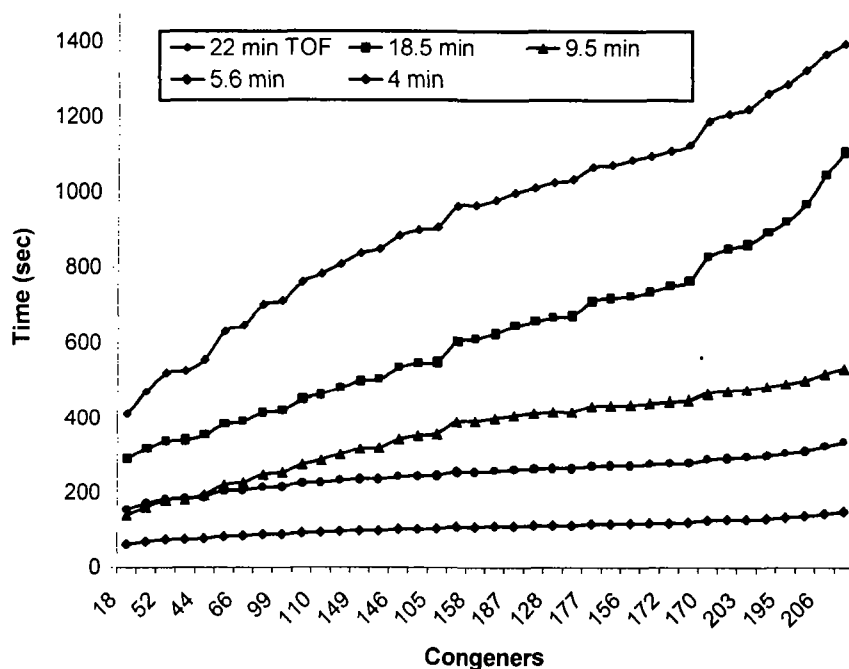


Fig. 1 : Time compression for the 38 PCBs.

The 5.6-minute run was produced in the temperature range between 115°C and 300°C, with a selection of rates not exceeding 40°C/min. Using this temperature program, several co-elutions of PCB congeners at various chlorination levels were observed. The deconvolution software allowed for qualitative and quantitative analysis of all co-eluting compounds except for the PCB 128/ PCB 167 co-eluting pair because a TEF (toxic equivalent factor related to 2,3,7,8-TCDD [5]) value has been attributed to PCB 167 but not to PCB 128. The algorithm for the deconvolution of positional isomers exists, but currently is in need to be simplified for the end-user operator. While awaiting the improvements, a slower ramp rate was used (2°C/min) in the region of this co-elution to separate this pair chromatographically, and the final program that was chosen yielded a run time of 9.5 minutes.

Standard calibration curves were generated and quality control samples were analyzed. Fig.2 shows the broad dynamic range (3 order of magnitude) obtained for this method with good correlation coefficients.

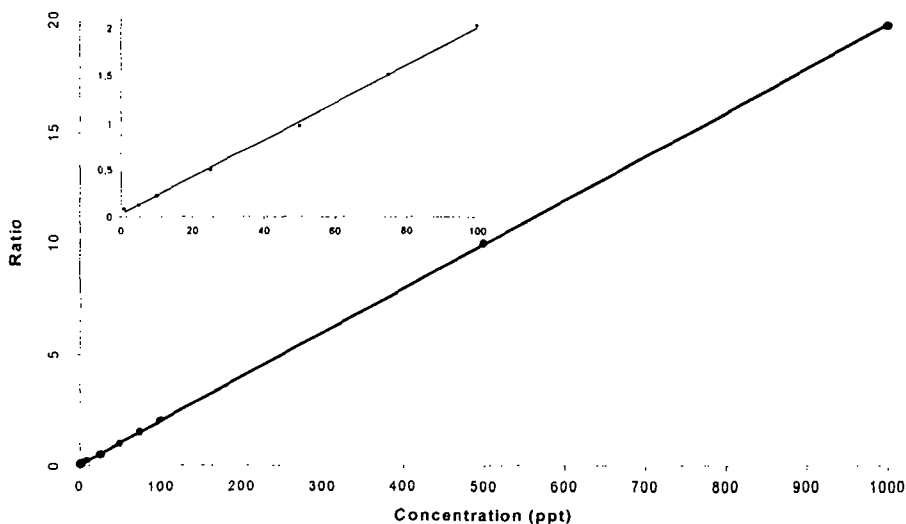


Fig. 2 : Calibration curve for PCB 28 (corr. coef. 0.9988).

Mean instrumental limits of detection (iLOD) were estimated at 5 pg/ μ L based on hexachloro-PCB 149. Using the current method clean-up conditions [3], the method LOD (mLOD) was estimated at 30 ppt. This mLOD is sufficient for the analysis of general background samples in which most of the congeners are in amounts greater than the mLOD with PCB138, 158 and 153 contributing for 50% of the total [6]. In addition to these 38 PCBs, the peak deconvolution software allows the analysis of strongly coeluting peaks, and it is thus also possible to monitor other species in the same single injection. Fig.3 illustrates the capability of the method to simultaneously consider the PCBs and the persistent pesticides present in the same sample. This represents a significant increase of the analytical power.

Overall, this method has the potential to improve sample throughput to 100 samples a day (10 min cycle time and maintenance) for 38 PCBs and 13 persistent pesticides using a single GC/TOF instrument, with detection limits in the low ppt range.

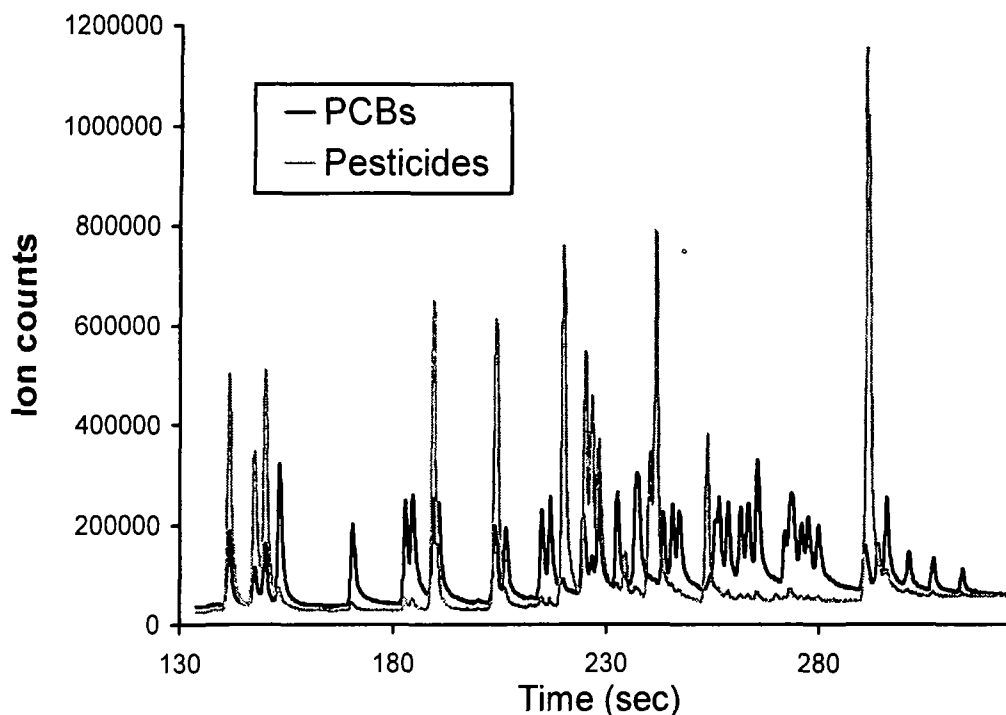


Fig. 3 : Chromatogram (5.6 min) including the 38 PCBs and 13 persistent pesticides.

Acknowledgements

This research was partly supported by the "Fonds pour la Formation à la Recherche dans l'Industrie et l'Agriculture" (F.R.I.A.), "Fonds National de la Recherche Scientifique" (F.N.R.S.) and the French Community of Belgium.

References

- 1 Brouwer, A., Longnecker, M.P., Birnbaum, L.S., Cogliano, J., Kostyniak, P., Moore, J., Schantz, S. and Winneke, G., *Environ. Health Perspect.*, **1999**, 107 (4), 639.
- 2 DeVoto, E., Fiore, B.J., Millikan, R., Anderson, H.A., Sheldon, L., Sonzogni, W.C. and Longnecker, M.P., *Am. J. Ind. Med.*, **1997**, 32, 606.
- 3 Leclercq, P.A. and Cramers, C.A., *Mass Spectrom. Rev.*, **1998**, 17, 37.
- 4 Barr, J.R., Green, V.E., Lapeza Jr., C.R., Maggio, V.L., Turner, W.E., Woolfit, A.R., Grainger, J., Needham, L.L., Patterson Jr., D.G., *Organohalogen Compounds*, **1997**, 31, 276.
- 5 Van den Berg, M., Birnbaum, L., Bosveld, A. T. C., Brunström, B., Cook, P., Feeley, M., Giesy, J. P., Hanberg, A., Hasegawa, R., Kennedy, S. W., Kubiak, T., Larsen, J. C., van Leeuwen, F. X. R., Liem, A. K. D., Nolt, C., Peterson, R. E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F. and Zacharewski, T., *Environ. Health Perspect.*, **1998**, 106 (12), 775.
- 6 Patterson Jr., D.G., Todd, G.D., Turner, W.E., Maggio, V., Alexander, L.R. and Needham, L.L., *Environ. Health Perspect.*, **1994**, 102 (1), 195.