Fast screening for PCBs, pesticides and brominated flame retardants in biological samples by SFE-LC in combination with GC-TOF.

Bert van Bavel, Jonathan Hughes*, Steve Davis*, Håkan Wingfors and Gunilla Lindström.

Environmental Chemistry, Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden (Bert.vanBavel@chem.umu.se)

* HD Technologies, Atlas House, Simonsway, Manchester M22 5PP, United kingdom (info@hdtech.demon.co.uk)

Introduction

The analysis of environmental pollutants such as PCBs, pesticides and brominated flame-retardants in biological samples is both time and resource consuming. During recent year the sample preparation including both extraction and clean up has become more efficient due to the use of new extraction techniques[1]. By combining extraction and clean up using SFE-LC the time to produce an extract ready for GC/MS injection was reduced considerably from several days to only 20 minutes[2]. When achieving such fast clean up and extraction, suddenly GC/MS analysis becomes the bottleneck in the analysis. The introduction of fast GC is a step in the good, 'fast' direction but conventional mass spectrometers are experiencing problems in achieving very fast scanning times. Time of flight mass spectrometers are not experiencing this kind of problems and are able to scan more than 50 times per second, more than enough for fast GC applications.

Material and Methods

SFE-LC extracts were acquired as described elsewhere[2], in short about 1 g of sample was mixed with 5 g Na_2SO_4 , this mixture was added to the extraction vessel and topped with around 5 g of

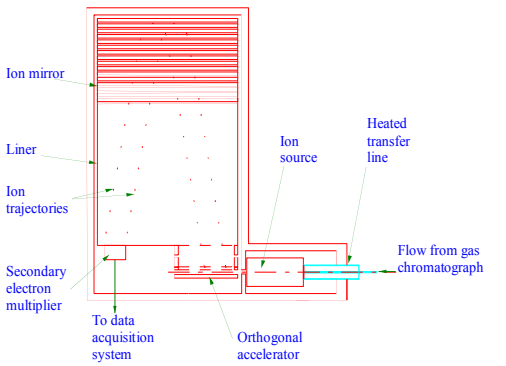

Figure 1. General schematics of orthogonal extraction TOF coupled to GC.

AlOx. Internal ^{13}C-labbeled standards were added to the fat/Na_2SO_4 homogenate before extraction. Using AlOx as a fat retainer the SFE extraction was performed with supercritical CO_2 at 40 °C, 300 atm. and at a flow rate of 2 ml/min. The target compounds were trapped on a PX-21/ODS solid phase trap and eluted from the trap with 10 ml of hexane/MeCl. After adding of 30 µl tetradecane and the ^{13}C-labelled recovery standards the extract was concentrated to the 30 ul tetradecane and ready for GC/MS analysis. Traditional GC/MS was performed on a Fisons MD 800 quadrapole operating in the selected ion monitoring mode (SIM). Monitoring the two most abundant ions in the chlorine cluster of the target compounds (PCBs, pesticides and PBDEs) after electron impact ionisation (EI). Only one ion from the ^{13}C-labelled internal and recovery standard was recorded. The sampling time at each mass was 30 ms. In total 26 ion were measured in two groups, together with an inter-channel delay of 1 ms this adds up to a total scan time of 806 ms (1.24 scans/sec). The following GC programme was used after splitless injection of 2 µl of the sample on a 60m DB-5 column; 180°C initial hold of 2 minutes, increase to 205°C at rate of 15°C min^{-1} followed by an increase to 300°C at a rate of 3.7 °C min^{-1}. Time of flight mass spectrometry was performed on a HD Technology Sprint coupled to a Thermoquest Trace 2000 GC. A schematic of the time of flight mass spectrometer (TOF) is given in Figure 1. The TOF was operated in the EI mode and ions were accelerated into the flight tube at a frequency of 36 kHz. After reflection from the ion mirror the ions were measured at a rate of 4 scans min^{-1}. After on column injection of 2 ul on a 30 m HD-5 column, the GC was programmed as follows initial temperature of 200°C for 1 minute, 25°C min^{-1} to a final temperature of 350 °C.

Results and discussion

In Figure 2 the reconstructed GC/MS runs of both the TOF and the quadrapole of a human adipose tissue sample are given in the same picture. The summation of the mass channels 360, 394 and 318, corresponding to the masses for Hexa-PCB, Hepta-PCB and p,p-DDE respectively is shown here. This Figure clearly illustrates clearly the difference in analysis time between the GC-TOF run and the SIM quadrapole run. It takes less than 7 minutes to elute all compounds of interest and still achieve enough resolution to identify and quantify all PCBs and pesticides of interest in a human sample from the GC-TOF run with the fast GC. It was possible to quantify 30 different PCB congeners in a human sample above the detection limit. Routinely around 40 congeners are detected above the detection level by using SIM on a quadrapole GC/MS in 45 minutes. This data has been published elsewhere[3]. The detection limit using the GC-TOF seemed to be somewhat higher. The minimum detection limit of the GC-TOF system was further evaluated by running calibration curves in a concentration range of 3.5 pg/µl to 1740 pg/µl. The GC-TOF showed good linearity until a concentration of 17.5 pg PCB injected on column, the quadruple GC/MS showed good linearity down to 3.5 pg injected splitless running in the SIM mode.
During GC-TOF analyses full scan spectra are always acquired and in Figure 3 an example of such a full scan spectra is given. This spectra is obtained from an SFE-LC whale extract[4] and the EI spectra for p,p-DDE is shown. This TOF mass spectra was found to be identical to earlier acquired mass spectra using the quadrapole instrument in the full scan mode. The sensitivity during such a qudrapole full scan run is however 10 to 20 times lower than achieved with the GC-TOF.
The acquisition of full scan spectra during analysis opens the possibility of screening for 'unknown' pollutants present in the SFE-LC extracts. A SIM GC/MS run always need input on the masses to be monitored, and thus this kind of quantitative analysis is restricted to compounds

known to be present in the extracts. By using GC-TOF new compounds can be identified by their mass spectra while doing quantitative analysis of known compounds at the same time.

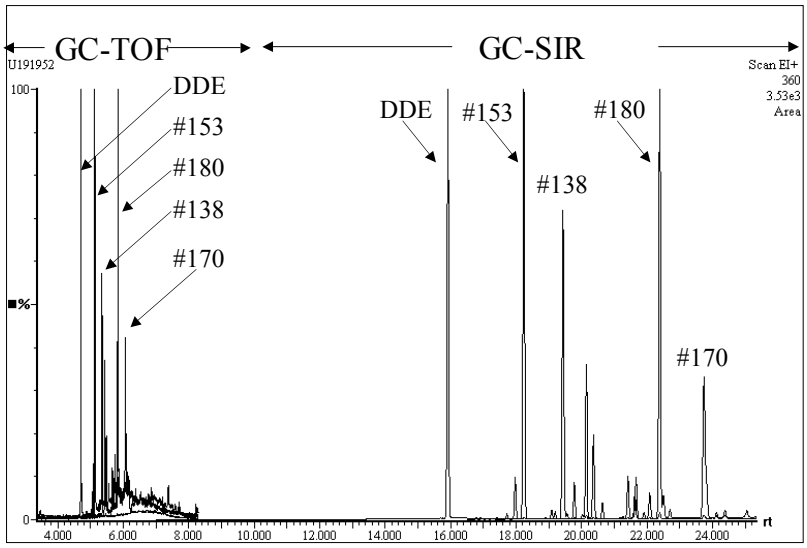

Figure 2. A reconstrucetd chromatogramme of a human adipose tissue extract including both the GC-TOF run and the GC-SIR run on the same time scale. Massees 360,394 and 318 are shown

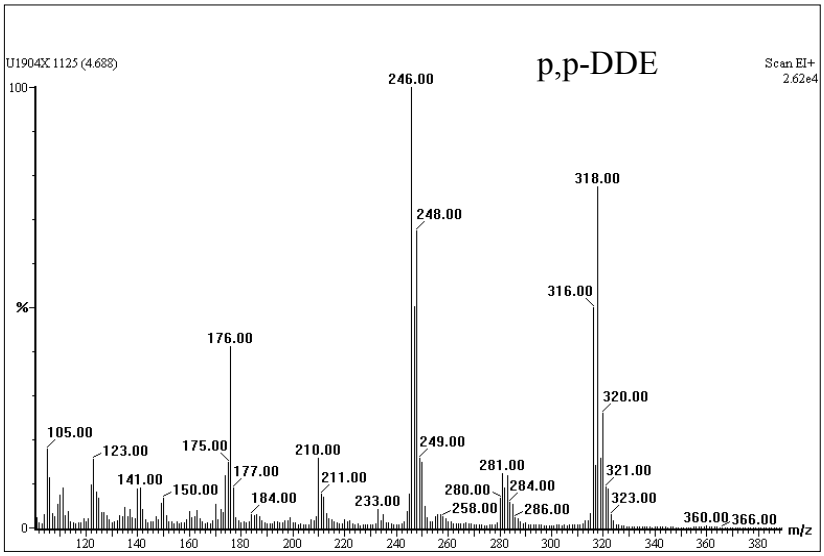

Figure 3. A full scan spectra of a whale blubber SFE-LC etxract acquired by GC-TOF. Ion extraction frequency 36 kHz, sampling time 4 scans/ minute.

Combining SFE-LC as the sample extraction and pre-treatment technique with GC-TOF analysis has reduced the analysis time for screening of environmental pollutants from several days to within 1 hour. This combination provides both qualitative data through full scan spectra and quantitative data through extracted mass chromatograms. The EI mass spectra are compatible with 'normal' EI quadrapole spectra and also the quantitative data acquired using isotope dilution quantification and the usage of ^{13}C labelled is directly comparable with data acquired with a quadrapole instrument operating in the selective ion mode. The fast analysis with a GC-TOF utilising a 'fast' GC still obtains enough resolution of the here studied PCBs and pesticides because of the fast scanning times of a TOF instrument. The only price to be paid for the fast analysis is a slightly lower detection limit than quadrapole SIM analysis. The sensitivity of the whole method (SFE-LC, GC-TOF) is however more than enough to screen human and other biological samples (whale, seal, fish) for environmental pollutants.

Acknowledgement

The Wallenberg foundation is acknowledged for a generous grant for SFE equipment used in the pilot whale extractions. The IngeBritt and Arne Lundberg Foundation is acknowledged for a generous grant for SFE-LC equipment used in the human monitoring projects.

References

1 Hawthorne, S.B. *Anal. Chem*. **1990**, 62, 633A
2 van Bavel B, Dahl P, Karlsson L, Hardell L, Rappe C, and Lindström G. *Chemspehere* **1995**, 30, 1229
3 Hardell L, van Bavel B, Lindström G, Fredrikson M, Hagberg H, Nordström M, Liljegren G and Johansson B. *Int. J. Oncol.* **1996,** 9, 603.
4 Lindström G, Wingfors H, Dam M and van Bavel B. Arch. Environ. Cotamin. Toxiol. **1999**, 36, 355.