MEASUREMENT OF PCB-153 AND DDE IN 20 µL DRIED-BLOOD SPOTS

L'Homme B*, Focant JF

Organic and Biological Analytical Chemistry, Chemistry Department, University of Liège, Allée de la Chimie 3, B-6c Sart-Tilman, B-4000 Liège, Belgium.

Introduction

The concept of sampling newborn infants for a few microliters of blood to screen for inherited endocrine, nutritional, or metabolic disorders has been introduced by Guthrie at the University of Buffalo in 1963[1]. Newborn screening for additional diseases has since been extensively performed in North America and Europe (Newborn Screening Programs - NSPs), but also in developing countries because of the ease of collection, transport, and storage, as well as the reduced risk of contamination of the handlers due to infectious pathogens, compared to the use of classical liquid specimens. Human dried-blood spots (DBS) are generally simply obtained by pricking the heel or finger by using single-use lancing devices to sample a few microliters (50-150 µl) of capillary blood. The blood is then collected on a piece of filter paper made of high purity cotton linters[2]. After drying, DBS are stored in plastic bags at ambient temperature. For analysis, part of the spot is punched out (6 mm punches) and the blood is eluted using various aqueous solutions[3]. In recent years, DBS testing further evolved towards more extensive testing due to the availability of more sensitive and specific methodologies. Next to screening for congenital diseases and viruses, DBS from NSPs have thus lately also been considered for exposure to toxicant assessment. To the best of our knowledge, only Dua *et al.* and Burse *et al.* briefly reported preliminary data on the potential use of DBS for hexachlorocyclohexane (HCH), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE) measurement using GC coupled to non-selective micro-electron capture detector (µECD)[4,5].

The aim of the work is to develop a new analytical strategy to measure selected representative POPs (or metabolites or reaction products) in DBS to assess internal dose exposure by means of innovative minimally invasive biomonitoring. The methodology is based on cryogenic modulation of gas chromatographic signals, either applied to comprehensive two-dimensional gas chromatography (GCxGC), or to cryogenic zone compression (CZC)-GC[6]. Both GC approaches to be hyphenated to high-resolution (HR) time-of-flight (TOF) mass spectrometric analyzer.

Materials and methods

Chemicals

All chemicals and instrumentation are similar to those used for dioxin routine analyses under ISO17025 QA/QC requirements. A human serum QC pool was used for optimization and testing. This pool is naturally contaminated and is representative of the general European population background levels. The target analytes consisted in PCB-153 and DDE. They can be considered as representative of PCB and organochlorine pesticide (OCP) families, respectively.

Analytical procedure

For method development, dried-blood spots sizes ranged between 20 and 50 µL of serum on filter paper. Each sample was pre-soaked with 95% formic acid and then liquid-liquid extracted (LLE) using a hexane-based solvent. Briefly, dried samples were cut (16 mm to 10 mm diameter for 50 to 20 µL of serum respectively) and placed in 1.5 mL polypropylene Epperdorf® with formic acid and internal standards (^{13}C-labeled PCB-153 and ^{13}C-labeled DDE). The solution was hand-shaked and the extraction was then carried out with three times 300-600 µL of the hexane mixture and wrist-action shaking. In practice, the amount of formic acid and organic solvent required for extraction depends on the size of the sample and is discussed on the next section. The nature of the hexane-based solvent is also discussed in the next section. The three organic layers were directly collected in a GC vial after a few drops of methanol were added to the solution to break the emulsion in between the two phases. The solvent extract was then evaporated from ±1,400 µL to around 5 µL under a gentle nitrogen stream

at room temperature. A nonane keeper (5 µL) and the recovery standard ^{13}C-labeled PCB-80 were eventually added to the solution. The final extract volume was 5-10 µL.

Measurements were carried out on a JEOL AccuTOF GC system (JEOL Ltd., Tokyo, Japan). The GC oven (Agilent 6890) was equipped with a ZX1 - LN_2 Cooled Loop Modulation GC x GC System (Zoex Corp., Houston, TX, USA). The ^{1}D GC column was an Rxi-XLB (30 m x 0.25 mm ID x 0.25 µm df) (Restek Corp., Belletonte, PA, USA). The ^{2}D GC column was an Rxi-17 (1.5 m x 0.25 mm ID x 0.25 µm df) (Restek Corp.). The P_M was 4 s, 400 ms of hot pulse duration. The temperature program was 130°C for 1 min, 10°C/min to 238°C, 2°C/min to 244°C for 5 min, 2°C/min to 268°C, 8°C/min to 310°C for 0.5 min. 1.5 µL of the final extract in nonane (5 -10 µL) were injected into a split/splitless injector held at 250°C in splitless mode. Helium was used at 1.0 mL/min. The MS parameters were an ion source temperature of 140°C, working in negative chemical ionization (NCI) mode, an ionisation voltage of 200 V, methane at 1 mL/min as reagent gas, an acquisition range from 30.00 to 400.00 m/z, a recording interval of 0.04 s (25 Hz), an accumulation time of 0.037 s, a data sampling interval of 0.5 ns, and a detector voltage of 2300 V. The mass accuracy of the instrument was ensured by frequent single point calibration checks.

Results and discussion

Cryogenic zone compression (CZC)

CZC was performed to enhance instrumental LODs. The iLOD was 20 fg/µL for PCB-153 and 1 pg/µL for DDE based on the M+2 ion. However, if the chlorine signal at the retention time of DDE is considered, iLOD can be reduced to 10 fg/µL. Monitoring the chlorine signal instead of the M+2 parent signal obviously preclude the use of ^{13}C-labeled standards for quantification and possibly reduce the specificity of the qualification. It is however important to remember that possible DDE coeluters have good chances to be separated in the second dimension (^{2}D) and that a second dimension retention time value (^2t_R) is available and brings an extra clue for identification of DDE[7]. The gain of sensitivity thanks to the modulation (sequentially trapping and releasing the compounds in the second dimension an thus compressing the gaussian peak) is about 10 times when keeping 7 data points per chromatographic peak[8].

We used a 30 m XLB GC column to ensure proper separation of both PCBs and DDE and avoid co-elution of other isobaric species. The width at half height was 150 ms. Several slices usually corresponded to a single broad non-modulated peak, excepted for the lowest points of the calibration curve where only one slice per compound was often observed. Analyses were done in 20 minutes while acquiring between 14 and 20 minutes at 25 Hz.

The calibration curve working zone ranged from 50 fg to 10 pg for PCB-153 and DDE. Corresponding ^{13}C-labeled standards were used to quantify the targets by isotopic dilution (ID). For some samples, quantification of DDE was impossible by isotopic dilution and was therefore performed using Cl ion and ^{13}C-labeled PCB-153 as internal standard.

Sample preparation

A simple miniaturized sample preparation was set up. Formic acid was used at 95% to ensure all lipidic and proteic structures to be broken down before liquid-liquid extraction. The organic extracts were directly collected in a GC vial and, after evaporation, directly injected for analysis. Clean-up and drying steps were skipped without altering the quality of the chromatograms. In order to miniaturize the sample prep in a 1.5 mL Epperdorf®, the minimum quantity of solvent was determined, as illustrated in Figure 1. The amount of organic phase to be used for the liquid-liquid extraction is determined by the formic acid. For a given sample we can always use more organic solvent than predicted (Fig. 1, grey zone). Dried-blood spots of more than 60 µL cannot be analyzed in a 1.5 mL Eppendorf®. The required volume of solvent would be around 1.5 mL.

The nature of both the aqueous and organic phases were investigated regarding accuracy and recovery of extraction. All dried samples were prepared from liquid serum samples whose contaminants levels were known. Table 1 shows some combinations of solvents and their effect. Accuracy is the relative error of the measurement with regards to the reference level of PCB-153 in the sample. Recovery rates were calculated using ^{13}C-labeled PCB-80 as recovery standard.

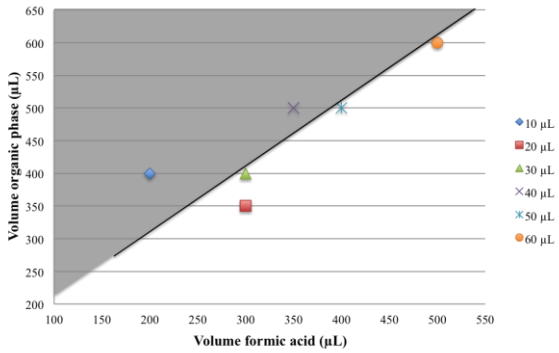

Figure 1. Volume of formic acid and organic phase required for the extraction of dried-blood spots.

Table 1. Effect of various solvents on accuracy and recovery

Solvent	Relative error	Recovery
PBS* + hexane	> 30 %	30%
PBS* + hexane(70%):acetone(30%)	25%	40%
Ac. formique 95% + hexane(70%):acetone(30%)	< 10%	30%

*Phosphate Buffer Saline solution

Formic acid and the mixture hexane/acetone have shown great capabilities to reach organic molecules encapsulated in complex lipophilic structures (high accuracy, triplicates ± 20% RSD). However, recoveries are still poor and mainly due to the loss of compounds in emulsion between the aqueous and organic phase during LLE. Some other solvents like hexane/methanol are currently under investigation to improve the sample preparation.

Analysis of real human samples

Dried-blood spots human samples were analyzed by GCxGC-HRTOFMS. Figure 2 illustrates the usefulness of the GCxGC when necessary to quantify DDE based on chlorine ions.

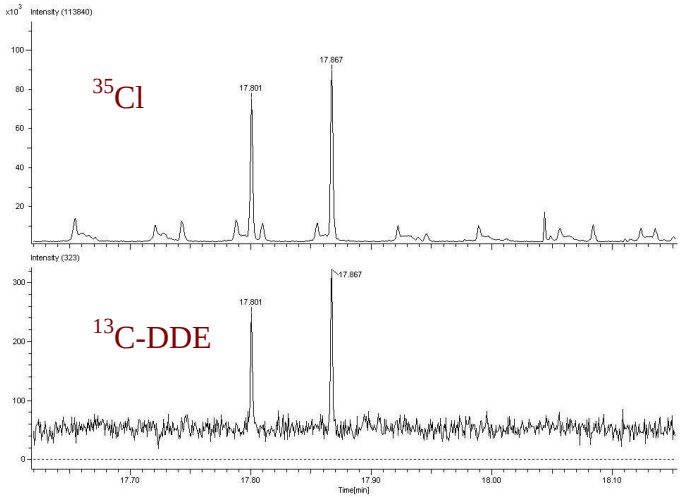

Figure 2. Mass chromatogram of ^{35}Cl (top) and of ^{13}C-DDE (bottom) in 50 µL real DBS sample. Two slices were produced and allow univocal identification.

The two slices of modulation on the bottom chromatogram correspond to a ^{13}C-labeled DDE standard. On the top chromatogram the signal of DDE is identified thanks to retention times of DDE. All peaks within the modulation period of 4 seconds (0.066 min) were separated on the second dimension. No co-elution occurred when this column set was used. Quantification of DDE was possible due to the high specificity of the column with the ^{13}C-labeled PCB-153 (human level of DDE was 5 ng/g fat).

Conclusion

Those results demonstrate the feasibility of analyzing selected POPs in human dried-blood spots. The sample preparation appears to be the crucial point in the procedure and this could be miniaturized. The analysis will now be extended to other pollutants like brominated flame retardant or emergent compounds.

Acknowledgements

JEOL (Europe) B.V Belgium Office (Zaventem, Belgium) and Zoex Europe (Eindhoven, The Netherlands) are supporting this research by providing instrumental support with the AccuTOF GC and the loop cryo-modulator, respectively. The GC columns were kindly provided by Restek (Belletonte, PA, USA). The Research Training Fund for Industry and Agriculture (Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture) F.R.I.A is providing financial support for the PhD project.

References:

1. Guthrie, R. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* **32**, 338 (1963).
2. Turner, R.C. & Holman, R.R. Automatic lancet for capillary blood sampling. *Lancet* **312**, 712 (1978).
3. Mei, J., Alexander, J., Adam, B. & Hannon, W. Use of filter paper for the collection and analysis of human whole blood specimens. *The Journal of Nutrition* **131**, 1631S (2001).
4. Dua, V., Pant, C., Sharma, V. & Pathak, G. Determination of HCH and DDT in finger-prick whole blood dried on filter paper and its field application for monitoring concentrations in blood. *Bulletin of environmental contamination and toxicology* **56**, 50–57 (1996).
5. Burse, V. et al. Preliminary Investigation of the Use of Dried-Blood Spots for the Assessment ofin UteroExposure to Environmental Pollutants. *Biochemical and molecular medicine* **61**, 236–239 (1997).
6. Patterson, D.G., Welch, S.M., Turner, W.E., Sjödin, A. & Focant, J.-F. Cryogenic zone compression for the measurement of dioxins in human serum by isotope dilution at the attogram level using modulated gas chromatography coupled to high resolution magnetic sector mass spectrometry. *J Chromatogr A* **1218**, 3274–3281 (2011).
7. L'Homme, B., Brasseur, C. & Focant, J.-F. Innovative minimally-invasive analytical strategies for human biomonitoring: measurement of toxicants in dried-blood spots. *Organohalogen Compounds* **73**, 1142–1145 (2011).
8. Adahchour, M. et al. Comprehensive two-dimensional gas chromatography coupled to a rapid-scanning quadrupole mass spectrometer: principles and applications. *J Chromatogr A* **1067**, 245–254 (2005).