MINIATURIZED SAMPLE PREPARATION FOR THE MEASUREMENT OF SELECTED OCPs AND PCBs IN LOW VOLUME OF BLOOD BY GC-MS/MS

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Introduction

Humans all over the world are exposed to chemicals during their life time. Among the thousands of existing anthropogenic compounds are the persistent organic pollutants (POPs), including compounds like polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), but also a large number of new molecules like halogenated flame retardants (HFRs). Although the peak exposure to PCDD/Fs, PCBs, and OCPs happened in the 1970's, their persistence and ubiquity result, still today, in exposure levels at above the estimated tolerable monthly intake (TMI).

The aim of the work is to develop a miniaturized method for the analysis of POPs in small amount of sample (20-50µL of serum or blood). The proof of concept on a few selected analytes has already been carried out using GC×GC-TOFMS and reported at previous Dioxin meetings[1,2]. However, the lack of sensitivity, even when using GC×GC as a signal enhancer (Cryogenic zone compression[3]), drove us to a more selective and sensitive instrumentation as GC triple quadrupole. We present the work done on GC-MS/MS for analysis of a range of representative organochlorine pesticides (from the Stockholm convention) and selected PCBs in low sample volume.

Besides the instrumental analysis, we mainly focus on the SPE-based miniaturized sample preparation procedure suitable for target trace analysis and that uses as low amount of solvent as possible: micro-extraction by packed sorbent (MEPS)[4-6]. Several strategies are investigated including pre liquid-liquid extraction (LLE) or different solvent combinations. We also optimized parameters for MEPS extraction to maximize recovery rates.

Materials and methods

Chemicals

All solvents (formic acid, methanol, hexane, dichloromethane, acetone, and acetonitrile) were GC grade (LGC Promochem, Wesel, Germany). 'Expanded POPs pesticides' were used as set of organochlorine pesticides (OCPs) (ES-5466, ES-5465, and ES-5464, Cambridge Isotope Laboratories, CIL) and EC-4687, EC-5179, EC-4058 (CIL), and MBP-MKX (Wellington Laboratories) standard solutions were used for PCBs analysis. For OCPs, a low level calibration curve was prepared and ranged from 0.1 to 3pg/µL. Serum and blood samples originated from left over stocks from previous epidemiological studies and a pool was prepared as a working solution for sample preparation optimization.

Extraction

Micro-extraction by packed sorbent (MEPS) was developed from the regular commercially available MEPS syringe and from a prototype 'CDF-MEPS' (Controlled directional flow-MEPS) (SGE, Australia). C18 cartridges were used for all extractions. A pre liquid-liquid extraction (LLE) of the biological sample was performed before the MEPS step using an acetonitrile-based solvent with NaCl. The sample was vortexed (1000 rpm), sonicated, and centrifuged (5min, 5000rpm) to collect the organic layer that was subsequently passed through the MEPS C18 column.

Analysis

For analysis, we used the GC-QQQ 7000C Agilent system equipped with PTV (we injected 5µL) and 7693A automated liquid sampler. PTV was operated on solvent vent mode. The temperature program was: start at 45°C (1.3min) and ramped at 720°C/min to 320°C; vent flow of 100mL/min at a pressure of 10psi for 1min. Purge flow was set to 1200mL/min after 4min. GC column was an Rxi-XLB 30m x 250µm x 0.25µm (Restek). The

GC oven temperature program for pesticides was: start at 50°C (4.3min), ramped at 50°C/min to 140°C (0min), then 10°C/min to 238°C (0min), then 2°C/min to 244°C (5min), then 2°C/min to 268°C (0min), and then 20°C/min to 310°C (0.5min). For PCBs, the GC oven temperature program was: start at 130°C (1min), ramped at 10°C/min to 238°C (0min), then 2°C/min to 244°C (5min), then 2°C/min to 268°C (0min), then 8°C/min to 310°C (0.5min). On MS side, we used the 7000C EI ion source, nitrogen collision flow of 1.5mL/min, and helium quench flow of 2.25mL/min. Quads resolution was set to wide.

Results and discussion

Targets and analysis

Preliminary studies have been done on a few markers (PCB-153, DDE)[1,2], which are representative targets of the PCB and the OCP families. The proof of concept was done on 20µL serum deposited on dried-blood spots papers. We have observed that the bottleneck of the method was not the state of the sample (dried rather than liquid) but the extraction method, requiring a miniaturized approach. We therefore moved back to liquid sample to set up the method, assuming that it was to be more easily extrapolated to a different support.

We are now targeting a wider range of molecules representative of both PCB and OCP families: 'Expanded POPs pesticides', mainly banned pesticides by the Stockholm convention, and non dioxin-like (NDL)-PCBs, as well as dioxin-like (DL)-PCBs. The calibration curve for OCPs, coming from purchased solutions (ES-5464, CIL) was injected first and we saw a strong non-linear behavior for some compound (aldrin, dieldrin, BHCs among others) and a perfect linear behavior over the whole range for others (DDTs, DDDs, DDEs) (R^2>0.999). Since the level of interest was at the lower end of this calibration curve (low pg range), we prepared by dilution and ^{13}C-labeled compounds addition a 'low level' calibration curve ranging from 100fg-3pg/µL. Figure 1 shows an example of a calibration curve obtained for Lindane (Gamma-BHC) that was previously not linear over the whole range. Calibration curves consist in 7 levels recorded as triplicates. We fixed a minimum of 0.99 as correlation coefficient (R^2) for all pesticides and PCBs.

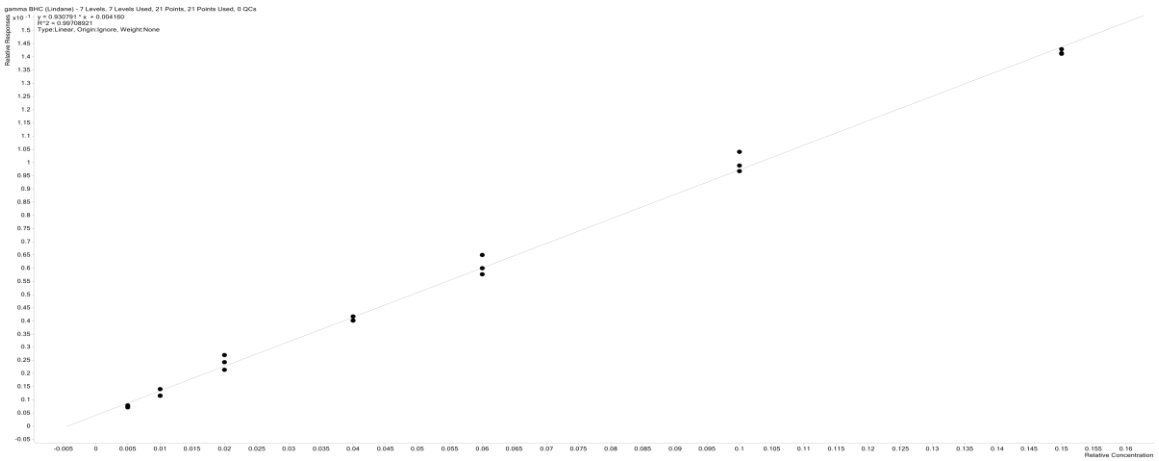

Figure 1: Calibration curve of Lindane from 0.1 to 3 pg/µL. Triplicates injections.

Analyses were carried out using Agilent GC-QQQ 7000B equipment operated with the 7000C EI ion source. We used the G9250AA pesticide Agilent MRM database for pesticides multiple reaction monitoring (MRM) transitions as well as our expertise in the lab for PCB's MRM transitions optimization. Quadrupole resolution was set to 'wide' to maximize sensitivity even though it could be used as unit mass resolution in case of interferences to better filtrate ions.

Instrumental limit of quantitation (iLOQ)

Instrumental limits of quantitation for all targets must be calculated on a statistical basis and not on signal to noise ration (S/N) basis. We used the definition proposed by EU experts (Equation 1) at a previous Dioxin meeting (2012) that is applicable to dioxin and DL- PCB analysis[7].

(Equation 1)

$$iLOQ = 10*stdev\ (8\ replicates)$$

(Equation 2)

$$Accuracy = \frac{C_{average} - C_{theory}}{C_{theory}}.100$$

8 replicate injections of the lowest calibration point were done. For each compounds, the standard deviation was taken from these replicates if accuracy of this lowest point was within ±20%. Equation 2 shows how to calculate the accuracy where $C_{average}$ is the average concentration obtained from 8 replicate injections of the lowest calibration point (treated as unknown samples), and C_{theory} is 100fg/µL. This criterion must be checked to statistically determine iLOQ's from a valid and accurate level. This is also the reason why we have prepared low-level calibration curves since the non-linear behavior over a greater range, as discussed in the previous section, had lead to strong deviation from theoretical values (accuracy ranging from 0 to 500%).

Extraction by (CDF)-MEPS

Micro-extraction by packed sorbent (MEPS) was following a pre liquid-liquid extraction (LLE) step and serves as clean up since quality of chromatograms was significantly improved. This technology has been investigated for several reasons including the small size of the device, automation possibilities, low amount of solvent required, and availability of different solid phases. Figure 2 shows the sequence of sample preparation for 20-50µL liquid serum (the procedure takes about 20 min). Note that the organic phase that is passed through the MEPS cartridge is acetonitrile, and that the elution solvent in the MEPS cycle is a mixture hexane/dichloromethane (DCM) 70:30.

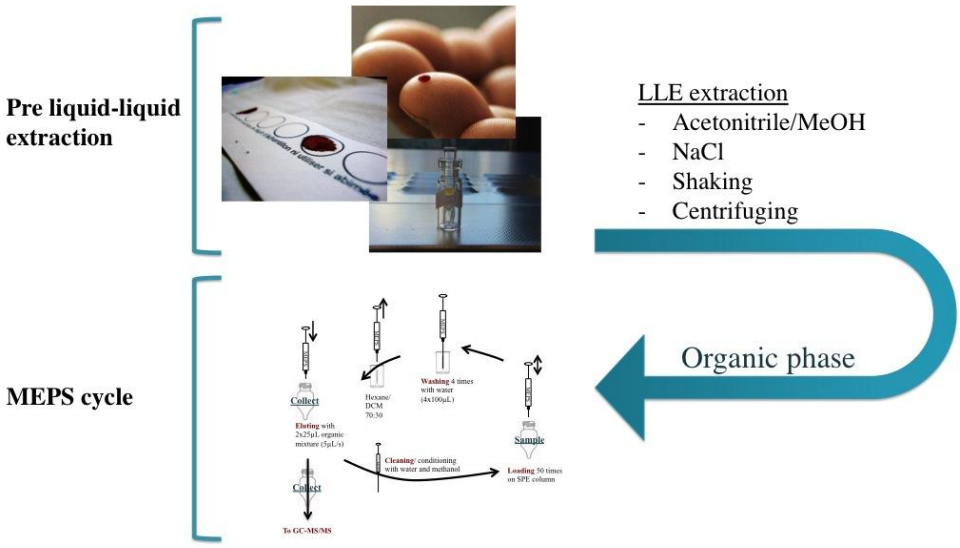

Figure 2: Sample preparation for liquid sample including a liquid-liquid extraction step, followed by a solid phase extraction step.

We observed some limitations in terms of recoveries (percentage of the initial extract that reaches the end of the extraction procedure) using this simple approach due to the MEPS step. This limitation doesn't affect the accuracy since all targets are quantitated using ^{13}C-labelled isotopic dilution, and therefore ratio target/internal standard remains identical. However, we get low recoveries in the range 5-15%, which give smaller signals and reduce our ability to measure low levels of POPs in small-size samples.

The MEPS syringe itself has been modified (SGE) and uses a side port to pump the elution solvent in order to elute compounds in the downward direction only. This prototype, 'Controlled Directional Flow-MEPS' (CDF-MEPS) is represented in Figure 3.

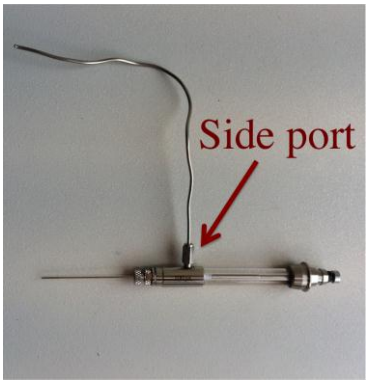

Figure 3: CDF-MEPS syringe with its side port.

We observed better recoveries in the range 10-35% with the CDF syringe even though still very poor. Nevertheless, this modification has also effect of dramatically reducing carry over. We measured <1% compound left after two regular successive elutions of 25µL whereas we had >10% remaining in the body of the syringe after 5 fractions eluted with the classic MEPS. Improvements of the SPE step have a direct impact on the analytical performances: we can now achieve very good precision (<25%RSD for replicates) and accuracy (±20% error using fortified and QC samples).

Conclusion

Sample preparation and analysis of selected OCPs and PCBs in small samples consisting of 20-50µL of blood require specific and adapted procedure for accurate and precise measurement. We have been optimizing and proposing a method based on GC triple quadrupole analysis for high sensitivity, and based on MEPS for miniaturized and fast extraction.

Acknowledgements

Agilent Technologies for instrumental support;
SGE for providing us with MEPS;
University of Liege, Belgium;
Research Training Fund for Industry and Agriculture (F.R.I.A) for financial support of Ph.D;

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