Validation of 96-well plate SPE method for analysis of persistent organic pollutants in low volume blood serum samples

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Introduction

Polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs) and polybrominated diphenylethers (PBDEs) are considered as persistent organic pollutants (POPs). They are ubiquitous in the environment. They are lipophilic, tend to bioaccumulate and may be toxic for living organisms and humans. This combination of properties highlights the importance of their monitoring and regulation not only in the environment but also in living organisms and humans [1].

For population exposure assessment, matrix of choice should be accessible in adequate volumes for analysis, follow the ethical steps and cause minimal discomfort for volunteers. Blood serum provides good assessment of population and due to circulation in the human body the information on exposure as well [2,3].

Low volumes of blood serum samples, low concentrations of target compounds and high amount of samples make analysis challenging. Therefore it is important to set up high-throughput sample preparation method.

Objective of the study:

- Development and validation of a high-throughput SPE extraction and clean-up method using 96-well plate solid phase extraction, validate the method using certified reference materials
- Comparison of POPs in fortified standard reference material (NIST 1958) and non-fortified standard reference material (NIST 1957) of human serum analysed by GC-APCI-MS/MS and CG-Orbitrap-HRMS

Sample processing

- An aliquot of 250 µL SRM was used
- Addition of ^{13}C-labeled standards (^{13}C NFR/BDE 28-183, ^{13}C BDBPE, ^{13}C BDE 209, ^{13}C PCBs and OCPs, ^{13}C HBCDs)
- Addition of acetonitrile for protein precipitation
- Precondition Oasis HLB well plates with 1 mL 5%DCM in *n*-hexane, 1 mL methanol and 1 mL HPLC-grade water
- Quantitative transfer of samples to the wells
- Centrifugation and purge of Oasis HLB wells
- Oasis HLB well coupled to prewashed Phree phospholipid removal well (Fig. 1 a 2)
- Elution with 1.2 mL 5% DCM in hexane
- Concentration under stream of N_2, solvent exchange to nonane
- Addition of syringe standards (BDE 77 and 138, PCB 162)

The target compounds in this study were 7 PCBs, 12 OCPs and 10 PBDEs.

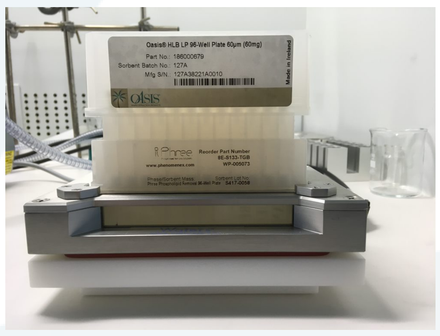

Fig.1. Oasis HLB well coupled to Phree phospholipid removal well

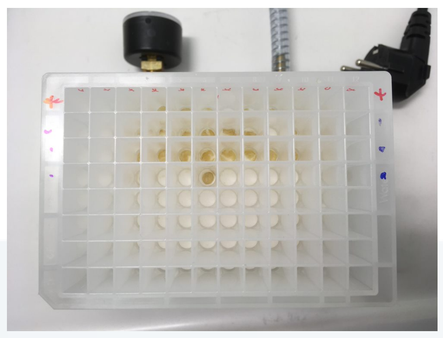

Fig.2. 96-well plate Oasis HLB

Instrument parameters

GC-MS/MS (APCI)

Injector

Split/splitless

Volume 1 µL

Oven

Initial 80°C hold for 1 min

1. ramp at 12 °C/min. to 250 °C
2. ramp at 5 °C/min. to 280 °C
3. ramp at 45 °C/min. to 320 °C hold for 5 min.

MS/MS APCI

Polarity: positive

MS mode: MRM

Fig.3. GC atmospheric pressure chemical ionization tandem MS (GC-APCI-MS/MS), Waters Xevo TQ-S MS coupled to Agilent 7890 GC

GC-Orbitrap-HRMS (EI)

Injector

Split/splitless

Volume 2 µL

Oven

Initial 90°C hold for 1 min

1. ramp at 40 °C/min. to 210 °C
2. ramp at 2.5 °C/min. to 230 °C
3. ramp at 5.0 °C/min. to 300 °C
4. ramp at 40 °C/min. to 320 °C hold for 9 min.

Orbitrap-HRMS (EI)

Polarity: positive

Resolution: 60 000 (FWHM)

MS mode: Full scan

Scan range: 150-750 AMU

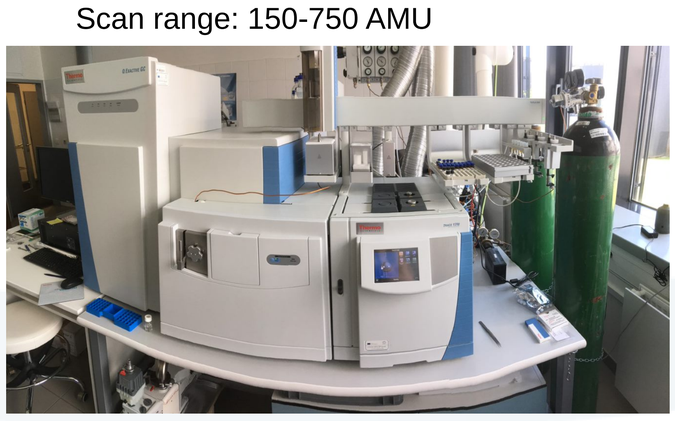

Fig.4. Thermo Scientific Q Exactive GC (GC-Orbitrap-HRMS)

Results

- The mean recovery of ^{13}C standards for PCBs, OCPs and PBDEs ranged from 40-116%, 20-85% and 75-155%, respectively.
- For testing accuracy, compounds with certified values were selected
- Trueness of the method was tested by comparison of the certified values in NIST SRM materials and measured values (Fig. 5 and 6).
- For target POPs, measured concentrations in NIST 1957 deviated from the certified values by 16% or less, with exception of PCB 118 and HCB, which had deviation -27% and 36%, respectively.
- Precision of the method was tested by calculating standard deviation in measured values of repeated analysis
- For the targeted POPs, RSD ranged from 3% to 18% for NIST 1957 (11 compounds) and from 8% to 21% for NIST 1958 (24 compounds), with exception of o,p'-DDD (55%) and o,p'-DDT (33%)
- Targeted GC-APCI-MS/MS provided better quantitative results (trueness, precision) than non targeted HRMS analysis
- Low concentrations analytes (Fig. 6 and 8) showed much worse trueness in HRMS analysis; for screening purposes, though, the HRMS method provided acceptable results

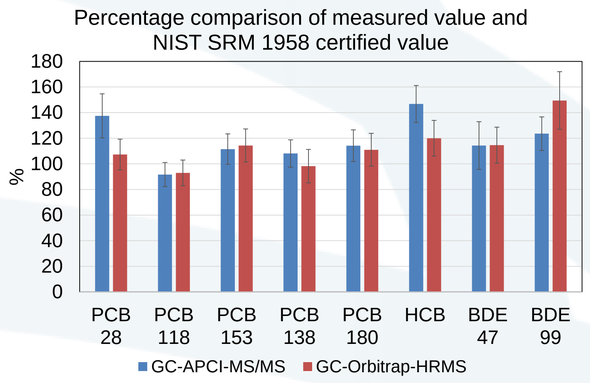

Fig. 5. Analysed percentage concentration obtained by using GC-APCI-MS/MS (n=10) and GC-Orbitrap-HRMS (n=9) compared to certified reference value in fortified blood serum reference material. Percentage concentration was calculated by: $[C_{(POP(analysed)}/C_{(POP(certified)}]*100$

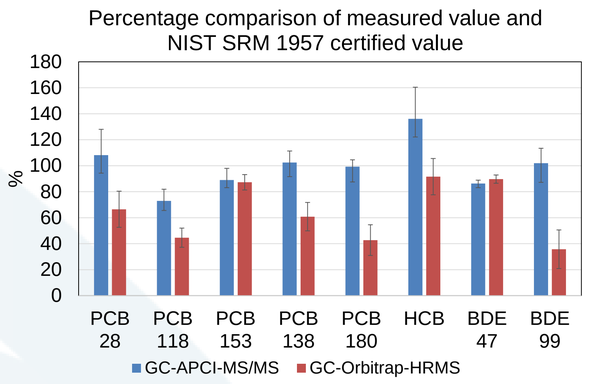

Fig. 6. Analysed percentage concentration obtained by using GC-APCI-MS/MS (n=10) and GC-Orbitrap-HRMS (n=10) compared to certified reference value in fortified blood serum reference material. Percentage concentration was calculated by: $[C_{(POP(analysed)}/C_{(POP(certified)}]*100$

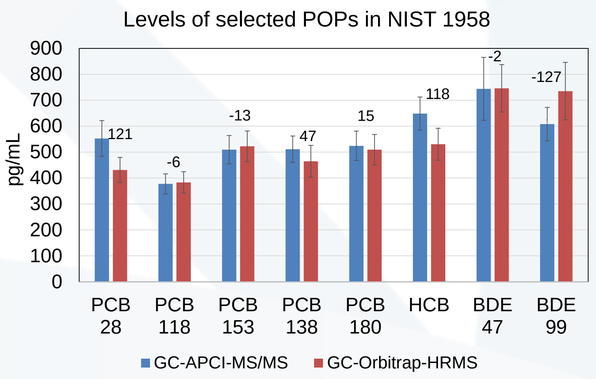

Fig.7. Comparison of POP concentrations in fortified blood serum obtained by GC-APCI-MS/MS (N=10) and GC-Orbitrap-HRMS (n=9). Difference in concentrations obtained by using GC-APCI-MS/MS and GC-Orbitrap-HRMS. Difference was calculated by: $C_{(POP(GC\text{-}APCI\text{-}MS/MS))}$-$C_{(POP(GC\text{-}Orbitrap\text{-}HRMS))}$

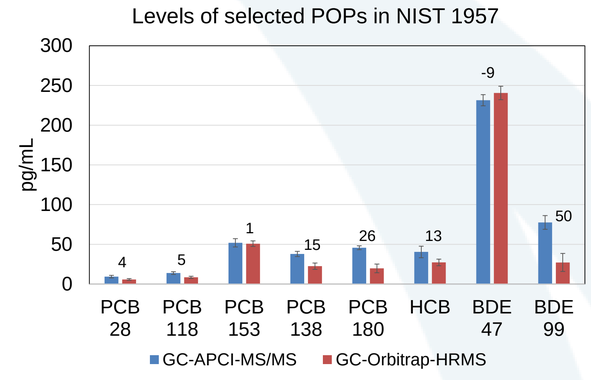

Fig.8. Comparison of POP concentrations in non-fortified blood serum obtained by GC-APCI-MS/MS (N=10) and GC-Orbitrap-HRMS (n=10).Difference in concentrations obtained by using GC-APCI-MS/MS and GC-Orbitrap-HRMS. Difference was calculated by: $C_{(POP(GC\text{-}APCI\text{-}MS/MS))}$-$C_{(POP(GC\text{-}Orbitrap\text{-}HRMS))}$

Conclusion

- 96-well plate SPE method provided accurate results and it was sensitive to detect low concentrations of POPs in very low sample volumes.
- This method has higher throughput than common SPE cartridges.
- These advantages are crucial for biomonitoring and epidemiological studies with large amount of low volume samples.

- Next step of this study will be testing method on 200 µL blood serum samples of pregnant women.

References

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Acknowledgements

This work was carried out with the support of core facilities of the RECETOX Research Infrastructure, project LM2015051 funded by the Ministry of Education, Youth and Sports of the Czech Republic under the activity "Projects of major infrastructures for research, development and innovations".