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OPTIMISED EXTRACTION AND FINE-TUNED CHEMICAL AND BIOANALYTICAL DETERMINATION OF ORGANOHALOGENS IN HUMAN SAMPLES

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Introduction

The analyses of halogenated persistent organohalogen compounds in human tissues basically aim at assessing exposure and associated health risks. Epidemiological studies and screening of background exposure of large population groups are typical for these assessments. Today there is a demand for fast and robust analyses of human samples such as blood and adipose tissue. Most of the time we use various chemical analytical techniques to determine an increasing number of organohalogens that have been identified in our environment and food chain. When it comes to assessing exposure, congener specific chemical analyses is appropriate. But, when it comes to health risk assessment bioassays could be more appropriate in explaining the relationship between exposure and health related endpoints.

In search for the 'ultimate trace method' for halogenated persistent organic pollutants in human tissues we have used a broad variety of techniques, both chemical and bioanalytical, in our laboratory. Since many of our studies are epidemiological or health related we have to go beyond the simple equation that the total is the sum of its parts. The toxicity of a complex mixture of POPs in human or other biological tissues does not always relate to the sum of the individual compounds in an explainable way. Quantitative structure activity relationships of complex mixtures, such as POPs in human tissues, need to be studied also with focus on their biological activity. Faced with this we have used biological assessments as complements.

But, whichever technique we use, chemical or bioanalytical, the first step is the extraction and isolation of the compounds from the tissue. The ultimate extraction technique in both chemical and bioanalytical applications is optimised supercritical fluid extraction, SFE. For the detection - qualitative as well as quantitative determinations - the techniques are based on mass spectrometry and appropriate bioassays.

At a workshop during the 16th Symposium on Chlorinated Dioxins and Related Compounds in 1996 in Amsterdam we used SFE and HRGC-HRMS for determination of dioxin-like compounds (DLCs) in adipose tissue samples. The total analysis time was around 2 hours, which demonstrated the superiority of this technique as a fast automated preparative technique for POPs analyses. The principles of our SFE applications for POPs analyses have earlier been described in detail. [1, 2, 3.]

In the First Round of Interlaboratory Comparison of Dioxin-like Compounds in Food using Bioassays it is concluded that most of the 12 laboratories that analysed the biological sample, cod

liver, were able to predict the WHO-TEQ level fairly well [4, 5]. Eight of the twelve laboratories reported levels between 60 and 108% of the WHO-TEQ level of 27 pg/g liver determined by chemical analyses. This provides evidence that bioassays and chemical analyses can be used for the same purpose. One factor that affects the outcome of a bioassay, possibly more than the results of chemical analyses, is the specificity of the tested extract - the extraction step.

Supercritical fluid extraction with carbon dioxide is here demonstrated to be very efficient sample pre-treatment technique for chemical as well as bioanalytical determination of dioxin-like compounds in human adipose tissue. Further, optimised SFE facilitates the fractionation of the total POPs content in the sample into well defined fractions of components ranging from non-polar planar dioxin-like compounds to non-planar and more polar compounds such as the brominated diphenylethers.

Methods and Materials

Extraction and fractionation by SFE-LC. The sample is split in two (each corresponding to approx. 1 g lipids), one for chemical analyses and the other for bioassay analysis, and extracted with CO_2 in dynamic mode at a flow temperature of 2ml/min and a density of 0.9 g/ml. The SFE extracts are collected on a Carbon (PX-21) column trap and fractionated into one non-planar fraction (containing PCBs, HCB, chlordane, PBDEs, etc, etc) and one planar fraction (containing PCDDs, PCDFs, non-o PCBs, PCNs, etc, etc)[1]. The sample for chemical analyses with isotope dilution MS is spiked with ^{13}C surrogates. The sample for bioassay analysis with DR-CALUX is unspiked. The parallel SFE extraction and fractionation is outlined in Figure 1.

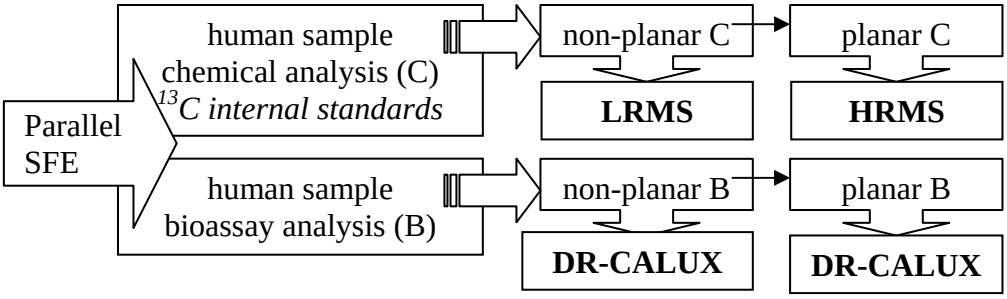

Figure 1. Extraction and fractionation of dioxin-like compounds and POPs in human tissue samples by parallel SFE-LC for determination by LRMS, HRMS and CALUX

Detection by GC LRMS. The first SFE fraction from the carbon column trap was eluted with hexane/$MeCl_2$ and contains the non-planar POPs from the tissue. Congener specific PCBs, HCB, DDE, chlordane and PBDEs were determined in this fraction by GC-LRMS, SIM (EI) at a MDL of 1 ppb.

Detection by GC HRMS. The second SFE fraction was eluted with xylene or toluene and contains all planar compounds in the tissue such as PCDD, PCDF, non-ortho PCB and PCN. This planar fraction was analysed for DLCs by GC- HRMS, SIM (EI) at a MDL of 0.2-2 ppt.

DR-CALUX. The bioassay is a reporter gene-based assay using the luciferase gene under control of DRE sequences. It is based on a genetically modified H4IIE rat hepatoma cell line, steadily transfected with the firefly luciferase gene coupled to the dioxin responsive elements (DRE) in the DNA[6]. The luciferase induction is measured after sample exposure and is correlated to the TEQ exposure.

Results and Discussion

Recovery of SFE extraction. Recovery and reproducibility of the SFE extraction was determined. Since it is not appropriate to use ^{13}C labelled surrogates in the extracts to be used in the DR-CALUX assessment the real concentrations in the extracts to be used in the DR-CALUX analyses where calculated using the recovery determined by GC-MS. Table 1 shows the recoveries for the analyte extraction by GC-MS and the mean and SD (n=5) of the WHO-TEQ level in pg/g.

Table 1. Recovery and WHO-TEQ level and SD (n=5) for PCDD and PCDF in adipose tissue by SFE and determination by GC-HRMS.

Analyte	Recovery %	WHO-TEQ in pg/g lipid
2,3,7,8-TeCDF	75	<0,3
2,3,7,8-TeCDD	77	1,2
1,2,3,7,8-PeCDF	73	0,18
2,3,4,7,8-PeCDF	74	3,9
1,2,3,7,8-PeCDD	79	2,8
1,2,3,4,7,8-HxCDF	90	0,22
1,2,3,6,7,8-HxCDF	63	0,06
1,2,3,7,8,9-HxCDF	57	<0,03
2,3,4,6,7,8-HxCDF	56	0,042
1,2,3,4,7,8-HxCDD	61	0,085
1,2,3,6,7,8-HxCDD	67	0,74
1,2,3,7,8,9-HxCDD	63	0,044
1,2,3,4,6,7,8-HpCDF	44	0,013
1,2,3,4,7,8,9-HpCDF	44	<0,012
1,2,3,4,6,7,8-HpCDD	48	0,083
OCDF	24	<0,0002
OCDD	28	0,0047
TEQ	-	9,7 ± 0,74

Comparison of chemical analytical and bioanalytical results. A comparison of the chemical analytical and bioanalytical results is shown in Table 2.

Table 2. Comparison of GC-MS data and DR-CALUX data in ppt (pg/g fat)

Method	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
WHO-TEQ (PCDD/F)	8,3	10,4	9,9	10,2	9,7
*REP-TEQ (PCDD/F)	8,5	10,3	10,1	10	9,5
DR-CALUX (DLC)	10,8	7,1	10,2	9,3	6,6
WHO-TEQ (non-planar)	19	24	22	24	26
REP-TEQ (non-planar)	6,3	8,1	7,0	7,5	8,6
DR-CALUX (non-planar)	7,8	9,6	8,2	13	9,2

*REP= Relative Potency Factor for DR-CALUX (BDS)

Conclusion

Parallel optimised SFE is a good sample pre-treatment and fractionation technique for chemical as well as bioanalytical determination of halogenated persistent compounds in human tissues.

The chemical data (GC-MS) and bioanalytical data (DR-CALUX) for human adipose tissue in this study show results in the same order of magnitude for the extracts containing the DLCs.

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