The Use of Supercritical Fluid Extraction (SFE) as a Sample Preparation Method in the Analyses of PCDD, PCDF and PCB in Human Tissue.

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1. Introduction

The demands by environmental risk assessment and regulatory practice, as well as the demands to reduce solvent handling and costs call for efficient analytical methods. Improvements of analytical methods for halogenated aromatic compounds, such as PCBs and dioxins, have so far been made mainly concerning the detection of the analytes by specific (GC-EC) and mass selective (MS) instrumentations. But, the sample handling and clean-up steps have principally remained the same since the beginning of the century.

For some years now a new outstanding analytical method for clean-up, supercritical fluid extraction (SFE), has been at hand. We have beneficially replaced the traditional sample preparation procedures in dioxin and PCB analyses, i.e. Soxhlet extraction, liquid/liquid extraction, column chromatography and enrichment, by SFE. The gas-like mass transfer and liquid-like solvating capability and non-toxicity of supercritical CO_2 makes it superior in analytical extractions of less polar contaminants, such as dioxins and PCBs in biological tissue. Coupling the SFE with liquid chromatography (LC) and mass spectrometry (GC-MS) makes it an excellent tool for the environmental chemist.

The SFE sample extraction and clean-up of dioxins from human tissue takes one hour, and is performed in one single step by coupling of the supercritical extraction with LC on an active carbon trap. We have earlier reported on the determination of PCBs in human adipose tissue using SFE and a solid octadecylsilica, OCD, sorbent trap[1)]. As a development of this, the new active carbon trap enables us to determine both the PCDDs/PCDFs and the PCBs by a simultaneous extraction. After trapping the SFE extract on the active carbon trap the analytes are automatically eluted from this in two fractions, one PCB fraction and one dioxin fraction.

Without any further clean-up the two fractions are analysed by HRGC-MS, for determination of the PCBs on ppb-level, and HRGC-HRMS, for determination of the PCDDs, PCDFs and non-o-PCBs on ppt-level.

Not only is this new SFE-LC technique for dioxin analyses economically and environmentally sound, it is also superior to the traditional techniques in terms of meticulously clean sample extracts and an improved analytical quality.

The use of analytical-scale SFE technique in environmental analysis has been discussed by S.B. Hawthorn[2]. Nam et al. have applied SFE for determination of chlorinated organics in biological samples[3].

2. Material and Methods

A. Supercritical Fluid Extraction (SFE). Extraction of human adipose tissue (~2g) was carried out with a Hewlett-Packard 7680T (Wilmington, DE, USA) extractor. A trap consisting of an active carbon adsorbent[4] was installed in connection to the nozzle. As the supercritical fluid CO_2 (N48 grade) was used. Extraction conditions; chamber temperature 40°C and CO_2 with a density of 0.9 g/ml (281 bar), were chosen based on previous optimisation experiments[1]. The extraction times were optimised to 34 minutes to achieve complete extraction of OCDD and OCDF. Prior to extraction the adipose tissue was homogenises and mixed with anhydrous Na_2SO_4 and fortified with a set of ^{13}C-labelled PCDDs, PCDFs and PCBs as internal standard (IS). The 7 ml-volume extraction chamber was filled with the homogenate and AlOx as a fat retainer. Dynamic extraction was performed for 34 minutes. All analytes were trapped on the carbon adsorbent. To achieve enhanced enrichment of the analytes two or several consecutive extractions were collected on the trap before the LC step.

B. Liquid chromatography (LC). After completion of the extraction the trap was rinsed on-line, first with hexane/methylene chloride (1:1) to recover the PCB fraction, fraction 1, and then with xylene to release the planar compounds, PCDDs, PCDFs and non-o-PCBs, fraction 2. The total amounts of solvents used were, 6 ml hexane/ methylene chloride and 10 ml xylene. The total time required for processing one sample and reconditioning the column, with 10 ml hexane/methylene chloride (1:1) and 10 ml xylene, was 69 minutes.

C. The HRGC-HRMS determination of PCDDs, PCDFs and non-o-PCBs. To the xylene extract was added a recovery standard (RS) and it was concentrated to 30 µL in tetradecane. One-tenth of the sample was analysed by SIR on a VG-250 S2 mass spectrometer, running at a resolution of 10.000. Split-splitless injection on a SP-2330 column enabled quantitative determination of all 2,3,7,8-substituted PCDDs and PCDFs as well as the non-ortho-PCBs two hours after starting the sample preparation. The PCBs were analysed as described earlier[1].

Blank samples, consisting only of Na_2SO_4 and AlOx were processed in the same way.

Analysis of Dioxins by SFE-LC-HRGC-HRMS

CO_2 hex/mc **fraction 1 (HRGC-MS) *PCBs* and *pesticides* (ppb)**

SFE --------> Carbon Trap--------{

xylene **fraction 2 (HRGC-HRMS) *PCDD/F* and *non-o-PCB* (ppt)**

0 >>>>>>>>> 34 min >>>>>>>> 69 min >>>>>>>>>>>>>>>>>>> 129 minutes required totally

0 >>>>>>>>> 35 ml CO_2 >>>>>> 16 ml hex/mc (1:1) + 10 ml xylene >>>>>> required totally

3. Results and Discussion

Recoveries of a ^{13}C-labelled PCDD, PCDF and non-o-PCB internal standard (IS). Four replicate samples, samples 1- 4, were analysed by SFE-LC-HRGC-HRMS. The extraction was carried out by extracting two sub-samples, of 1 g each, and trapping both of them on the carbon adsorbent before the LC step.

Table 1. Recoveries by SFE-LC-HRGC-HRMS analyses of ^{13}C fortified human adipose tissue.

	Sample 1	Sample 2	Sample 3	Sample 4
^{13}C 2,3,7,8-TCDF	83%	85%	85%	88%
^{13}C 2,3,4,7,8-PeCDF	81%	78%	79%	86%
^{13}C 1,2,3,6,7,8-HxCDF	84%	82%	84%	89%
^{13}C 2,3,4,6,7,8-HxCDF	103%	96%	95%	116%
^{13}C 1,2,3,4,7,8,9-HpCDF	90%	84%	84%	104%
^{13}C OCDF	73%	68%	66%	75%
^{13}C 2,3,7,8-TCDD	83%	85%	85%	88%
^{13}C 1,2,3,7,8-PeCDD	87%	88%	93%	94%
^{13}C 1,2,3,6,7,8-HxCDD	88%	84%	87%	96%
^{13}C 1,2,3,4,6,7,8-HpCDD	87%	77%	81%	95%
^{13}C OCDD	79%	71%	69%	88%
^{13}C PCB#77	72%	61%	69%	70%
^{13}C PCB#126	84%	79%	85%	86%
^{13}C PCB#169	78%	72%	78%	82%

Reproducibility of determination of native PCDDs, PCDFs and non-o-PCBs in human adipose tissue. Levels of 2,3,7,8-substituted native PCDDs and PCDFs as well as PCBs #77, #126 and #169 for S1-S4 are reported in table 2. The levels are expressed in pg/g tissue (ppt). As can be seen from the same table, for the blank B5, all analytes were below the detection limits with the exception of PCB #77.

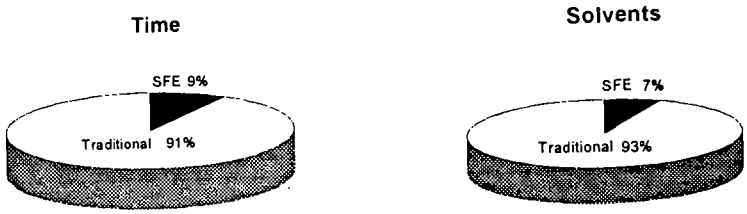
Table 2. Reproducibility of the determination by SFE-LC-HRGC-HRMS of levels of PCDDs, PCDF, and non-o-PCBs in human adipose tissue. Levels in ppt on whole weight basis.

	S 1	S 2	S 3	S 4	Mean	RSD	%RSD	B5
2,3,7,8-TCDF	0.19	0.20	0.30	0.27	0.23	0.05	20%	nd<0.22
1,2,3,7,8-PeCDF	nd<0.33	nd<0.35	nd<0.34	nd<0.38	nd<0.34	0.01	3%	nd<0.35
2,3,4,7,8-PeCDF	9.03	8.58	8.58	7.99	8.73	0.20	2%	nd<0.29
1,2,3,4,7,8-HxCDF	1.25	0.98	1.31	1.32	1.18	0.13	11%	nd<0.72
1,2,3,6,7,8-HxCDF	1.00	0.97	0.84	0.74	0.94	0.07	7%	nd<0.60
2,3,4,6,7,8-HxCDF	nd<0.36	nd<0.40	nd<0.39	nd<0.39	nd<0.38	0.02	5%	nd<0.52
1,2,3,7,8,9-HxCDF	nd<0.49	nd<0.55	nd<0.54	nd<0.54	nd<0.53	0.03	5%	nd<0.71
1,2,3,4,6,7,8-HpCDF	8.25	9.31	8.78	9.83	8.78	0.35	4%	nd<0.70
1,2,3,4,7,8,9-HpCDF	1.19	1.34	1.30	1.28	1.28	0.06	5%	nd<1.81
OCDF	nd<3.60	nd<4.09	nd<4.07	nd<4.35	nd<3.92	0.21	5%	nd<6.71
2,3,7,8-TCDD	1.18	1.12	1.24	1.37	1.18	0.04	3%	nd<0.29
1,2,3,7,8-PeCDD	4.52	3.41	3.49	2.83	3.81	0.48	13%	nd<0.74
1,2,3,4,7,8-HxCDD	0.51	0.56	0.53	0.58	0.53	0.02	3%	nd<0.57
1,2,3,6,7,8-HxCDD	13.70	13.61	13.86	12.85	13.72	0.09	1%	nd<0.80
1,2,3,7,8,9-HxCDD	0.90	0.98	0.82	1.01	0.90	0.05	6%	nd<0.95
1,2,3,4,6,7,8-HpCDD	11.56	14.34	11.87	12.65	12.59	1.17	9%	nd<4.57
OCDD	112.20	122.38	110.35	115.43	114.97	4.94	4%	nd<6.38
PCB#77	<1.72	<1.82	<1.83	<1.84	<1.79	0.05	3%	4.05
PCB#126	60.03	57.10	59.87	56.83	59.00	1.27	2%	nd<0.41
PCB#169	55.77	53.12	55.06	51.77	54.65	1.02	2%	nd<0.42

Concerning the determinations of the PCBs, fraction 2, we refer to our previous work[1].

4. Conclusions

The use of SFE-LC-HRGC-HRMS in dioxin and PCB analyses human tissue is superior to traditional techniques in its several aspects. Time and solvent consumption is reduced by 90%



The reproducibilies of the analytes, RSD 1-20%, are well within the limits for reported reproducibilities in dioxin analyses of human tissue[5].

The often reported blank and sample contamination problems seen in the traditional techniques are absent due to the limited amount of solvents, gels and glassware used for the analyses. The metiquousley clean sample extract makes the HRGC-HRMS determination less affected by column deterioration and co-eluting artefacts.

Not only is the SFE-LC-HRGC-HRMS technique environmentally and economically sound, it is also of high analytical quality.

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5. References

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