Pressurized Solvent Extraction
High Throughput Extraction for Dioxin and Furan Analysis

Pressurized Solvent Extraction (PSE) of soil, air filters, fish and different foodstuffs for the determination of POPs

Abstract

Dioxins, furans and PCBs are persistent organic pollutants (POPs) and as such have been banned by the Stockholm Convention. They are monitored worldwide due to their strong toxicological impact on humans and livestock.

In this whitepaper, the extraction of dioxins, furans, PCBs and PAHs in different environmental and food matrices using the SpeedExtractor E-914 / E-916 is described. BUCHI's SpeedExtractor is a pressurized solvent extraction instrument used for the extraction of up to 6 samples in parallel. The use of pressurized solvent extraction is widely recognized as a fast and reliable extraction method for environmental contaminants such as POPs.

1. Introduction

Dioxins are toxic substances that are released into the environment by incomplete combustion processes. "Dioxin" is the umbrella term for polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), see Figure 1.

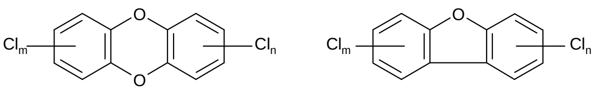

Figure 1: *Chemical structure of dioxins (PCDDs) left and furans (PCDFs) right.*

There are many dioxin congeners, but only some of them are carcinogenic because they can attach to a human receptor (AhR) which regulates cell division. The toxicity of each congener is expressed by the TEF (toxic equivalent factor), the most toxic dioxin (TCDD, the so-called "Seveso"-dioxin) has a factor of 1. The overall toxicity is expressed as TEQ (toxic equivalent quantity), which is a sum parameter based on the TEF and the corresponding concentration of all the congeners present.

From a toxicological point of view, 12 PCBs are classified as "dioxin-like" because they can attach to the same receptor as dioxins and they are included in the assessment of the overall dioxin toxicity.

The SpeedExtractor E-914 / E-916, see Figure 2, is an automated instrument used for the parallel extraction of primarily organic compounds from a variety of solid or semi-solid samples. Conventional methodologies are accelerated using solvent at elevated temperatures. In order to maintain the solvent in a liquid state during the extraction process, the solvent inside the extraction cell is put under pressure. To achieve high recoveries multiple extraction cycles are usually applied. Once the extraction step is finished, the extracts are cooled down in a cooling unit and flushed into collection vials, which can then be easily evaporated in parallel using the Multivapor™ P-6 or the Syncore® Analyst R-12. Alternatively the extract can be collected in round bottom flasks for evaporation using the Rotavapor®. The whole process workflow can be performed in parallel with up to six samples. Extraction cells can accommodate samples sizes from 10 – 120 mL ensuring reliable analysis of high and low polluted samples.

Figure 2: *SpeedExtractor E-914 / E-916 provides maximum speed and high throughput.*

Dioxins, furans and other POPs are monitored worldwide in a large variety of matrices. The extraction of these analytes from different sample types as well as a study to exclude any carry over or cross-contamination within the extraction instrument are summarized in the following paragraphs. The application protocol and the detailed results are presented in the corresponding application notes, see references.

2. No carry over, no cross-contamination

Concentration of analyte levels in samples processed in laboratories usually varies widely. The processing of a high level sample before a low level sample or the simultaneous parallel processing of a high and a low level sample must not result in a significant transfer of material. The former is referred to as carry over of consecutive runs, the latter as cross-contamination between adjacent samples in the same run.

Sediment and soil samples containing PAHs, PCBs, dioxins and furans were extracted using the SpeedExtractor E-916. The same procedure as described in section 3 was used. While processing, the samples, parallel blanks (cross-contamination blanks) and consecutive blanks (carry over blanks) were extracted to investigate possible carry over or cross-contamination.

No significant carry over or cross-contamination was observed for the three different analytes, see tables 1 and 2.

Table 1: *Investigation of PAH carry over and cross-contamination, selection, n=4, [µg/kg].*

Selection of PAH congeners	Sample	Cross-contamination blank	Carry over blank
Naphtalene	249	<2	<2
Fluoranthene	1058	<2	<2
Benzo[a]pyrene	495	<2	<2
Benzo[g,h,i]perylene	447	<2	<2

Table 2: *Investigation of dioxin carry over n=3, [pg/kg], method blank was < 1 for all congeners, except for OCDD, where it was 1.2.*

Selection of dioxin congeners	Sample (%rsd)	Carryover blank 1	Carryover blank 2	Carryover blank 3
2378-TCDF	154 (17%)	<1	<1	<1
2378-TCDD	6.69 (29%)	<1	<1	<1
23478-PeCDF	370 (15%)	< 1	< 1	< 1
12378-PeCDD	46.9 (4%)	< 1	1.2	< 1
123478-HxCDF	657 (9%)	<1	<1	<1
123678-HxCDF	311 (14%)	<1	<1	<1
1234678-HpCDF	2807 (11%)	< 1	< 1	< 1
1234678-HpCDD	672 (5%)	< 1	< 1	< 1
OCDF	2006 (2%)	< 1	< 1	< 1
OCDD	2003 (10%)	1.2	1.6	1.2

3. Dioxins in soil

PCDDs and PCDFs were extracted from a soil sample using the SpeedExtractor E-916 according to the U.S. EPA Method 3545A [1]. Approx. 1 g of soil sample was mixed with sand and placed into a 10 mL extraction cell. The sample was extracted with toluene at 130 °C and 100 bar, four cycles of 5-10 min each were carried out. Total extraction time was 1 h 13 min, which is a significant time saving compared to 24 h using tradtional Soxhlet extraction of soil samples. The extracts were cleaned-up by flash chromatography using silica gel and alumina, and the PCDDs and PCDFs were analyzed by GC-HRMS using an isotopic dilution method.

The determined concentrations corresponded well to the established method for the accredited laboratory, see Figure 3.

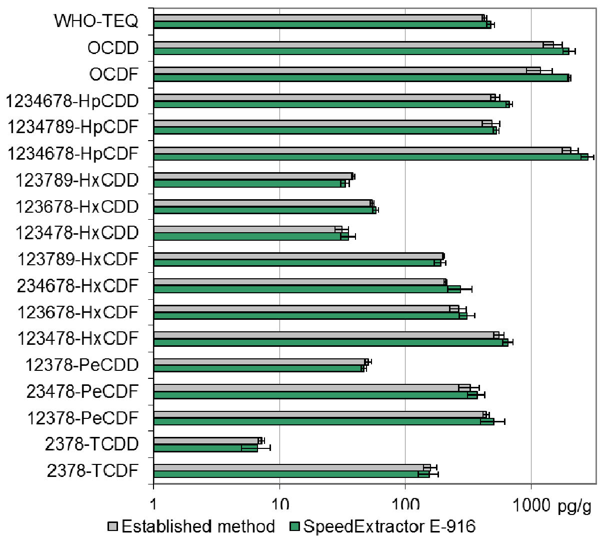

Figure 3: *Concentrations (pg/g) and standard deviations of the determined dioxin and furan congeners achieved by the established laboratory method (grey) and the SpeedExtractor E-916 (green).*

4. Dioxins on air filters

Since dioxins and furans are by-products from incomplete combustion, significant amounts can be released into the air and may be transported over long distances. It is important to monitor the air levels of dioxins and dioxin-like compounds in order to better understand dioxin exposure. The polyurethane foam (PUF) filters were placed in a high volume air sampler for 3 days. The filters were placed into the extraction cells as shown in Figure 4.

Figure 4: *Putting the PUF filter into a SpeedExtractor E-914 extraction cell using a dedicated funnel*

The filters were extracted with toluene using the following extraction method: 80 °C, 100 bar with 3 extraction cycles of 3 min each. The total extraction time was 53 min. In parallel, the air filter samples were extracted with conventional Soxhlet apparatus for 16 h. The extracts were then cleaned-up using gel chromatography and analyzed by HRGC-HRMS. The results are compared in Figure 5. The determined concentrations in the extracts from the SpeedExtractor and from Soxhlet are in good accordance.

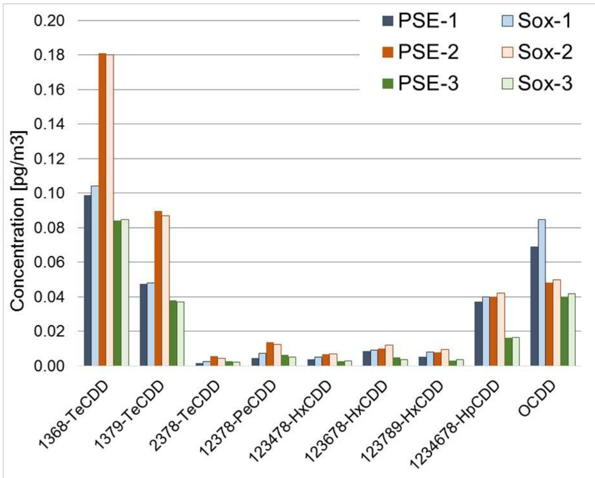

Figure 5: *Comparison of three air filters extracted in parallel using the SpeedExtractor E-914 (PSE) and Soxhlet apparatus (Sox).*

5. Dioxins in fish tissue

Dioxins accumulate in the fatty tissue of fish. The fat content of fish depends on the species and changes during the year. To determine dioxins, the fat is extracted and the dioxins are determined from the fat fraction. Traditional Soxhlet extraction methods need extraction times of 18 to 24 h [2, 3].

Two fish samples (trout and eel) and a control material (trout) were extracted with the SpeedExtractor E-914 using Hexane:Dichloromethane (50%:50%) as solvent with the following extraction parameters: 100 °C, 100 bar, 3 cycles of 10 min each. 10 g (eel) or 20 g (trout) of sample were mixed with diatomaceous earth and placed into 80 mL extraction cells. The total extraction time was approx. 1 h 25 min. The results were compared to the results from Soxhlet extraction (24 h extraction time) and assigned value of the ring-test material, respectively, see Figure 6.

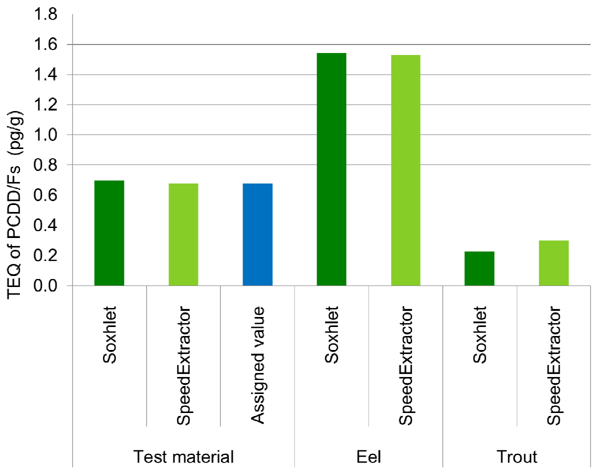

Figure 6: *Median (proficiency test material) and mean concentration (eel and trout) of. TEQ of PCDD/Fs in pg/g fresh weight of fish. Ring-test material: Soxhlet n=1, SpeedExtractor n=1, Ring-test n=79. Eel: Soxhlet n=1, SpeedExtractor n=3. Trout: Soxhlet n=1, SpeedExtractor n=3.*

The SpeedExtractor results show excellent agreement with the results found with Soxhlet for all three samples. Furthermore, excellent comparability to the consensus results was found.

6. Dioxins in foodstuffs

Dioxins and PCBs are lipophilic and accumulate in human and animal tissue. Therefore high fat foods from animals such as meat, eggs, milk and derived products are at a higher risk of contamination. Freeze-dried proficiency test samples of milk powder, milk fat, egg powder, pork and herring were extracted using Toluene:Acetone (70%:30%) with the following parameters: 120 °C, 100 bar, 3 cycles of 5 min each. Total extraction time was 53 min.

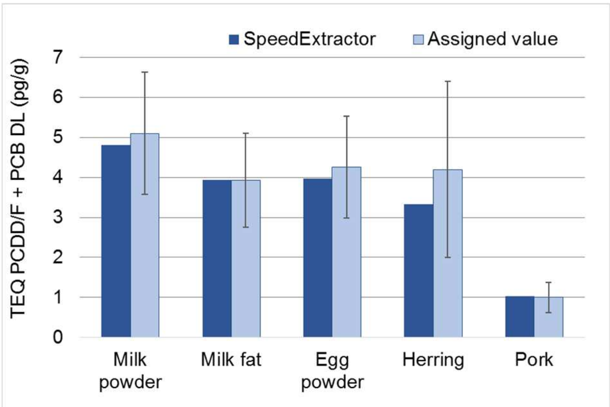

Figure 7: *Determined TEQ for the sum of the dioxins and dioxin-like PCBs in proficiency test samples compared to the assigned values in [pg/g fat] for all samples except herring, where it is [pg/g fresh weight]. The error bars correspond to the concentration range with a z-score between +2 and -2. Mean values, n=4.*

After extraction, the extracts were evaporated to dryness using a Rotavapor® and the fat content was calculated. The extract was then redissolved and cleaned-up using an automated clean-up instrument (GO-HT4, MIURA). After clean-up, the extracts were analyzed for dioxins, dioxin-like PCBs and non dioxin-like PCBs using GC-HRMS.

The determined WHO-TEQ values for the PCDD/Fs and DL PCBs, see Figure 7, were all within an absolute z-score value of < 1. The presented procedure for dioxin determination using the SpeedExtractor E-914 is a fast, reliable method for the determination of dioxins and PCBs.

7. Acknowledgements

We sincerely thank Dr. M. Schlummer, L. Gruber, G. Wolz and N. Weise from the Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany, for their study on the carry over and cross-contamination and for their work on the extraction of soil samples.

We gratefully acknowledge the cooperation for the extraction of air filters that was carried out by Mr. T. Tojo and Dr. A. Yamamoto from the Osaka City Institute of Public Health and Environmental Science, Osaka, Japan.

We sincerely thank Dr. Stephan Hamm and Dr. Armin Maulshagen and their analytical team from the - münster analytical solutions gmbh, Münster Germany for the analytical work on the determination of dioxins in fish.

We gratefully thank Mr. Philippe Marchand and Mr. Vincent Vaccher from LABERCA, Nantes for sharing their expertise in dioxin determination in foodstuffs, the fruitful collaboration and valuable discussions.

8. References

[1] EPA 3545A: Pressurized Fluid Extraction
[2] EPA 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS
[3] EPA 1668B: Revision B: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS

Application notes (www.buchi.com/applications)

- Technical note No. 055/2009 Investigation of carry over and cross-contamination effects in the SpeedExtractor E-916.
- Application Note 012/2009, Determination of dioxins and furans in soil using the SpeedExtractor E-916
- Short Note 153/2014, Dioxin determination in air filter samples using Pressurized Solvent Extraction (PSE)
- Application note 069/2012, Extraction of PCDD/Fs and PCBs in fish using the SpeedExtractor E-914
- Application note 205/2015, Dioxin determination in foodstuffs