Pressurized Solvent Extraction
High Throughput Extraction for Dioxin and Furan Analysis

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.

![Chemical structure of dioxins (PCDDs) left and furans (PCDFs) right.](image)

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.

![SpeedExtractor E-914 / E-916 provides maximum speed and high throughput.](image)

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.

![Investigation of PAH carry over and cross-contamination, selection, n=4, [µg/kg].](image)

<table>
<thead>
<tr>
<th>Selection of PAH congeners</th>
<th>Sample</th>
<th>Cross-contamination blank</th>
<th>Carry over blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>249</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>1058</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>495</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>447</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>
Pressurized Solvent Extraction (PSE) of soil, air filters, fish and different foodstuffs for the determination of POPs

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 traditional 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).](image)

![Figure 4: Putting the PUF filter into a SpeedExtractor E-914 extraction cell using a dedicated funnel.](image)

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 then placed into the extraction cells as shown in Figure 4.

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

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

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.

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.

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.

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.

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8. References

[1] EPA 3545A: Pressurized Fluid Extraction

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