

## AUTOMATED EXTRACTION AND CLEAN-UP OF BROMINATED DIOXINS AND FURANS (PBDD/Fs) USING ASE AND THE POWER-PREP™ SYSTEM

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### Introduction

Large-scale analysis of dioxins and related compounds require rapid and reliable automated systems for extraction and clean-up. The accelerated solvent extraction (ASE) and the Power-prep™ workstation (Fluid Management Systems) have been used successfully together for extraction, clean-up and fractionation of chlorinated dioxins, dioxin-like compounds and pesticides, in food, feedstuff and environmental samples<sup>1-3</sup>. Their combined time efficiency is remarkable in comparison to traditional Soxhlet extraction in combination with clean up over open columns. Moreover, these automated systems show equally good reproducibility and recovery compared to the more traditional techniques.

The more recent findings of brominated dioxins in environmental samples and biota<sup>1-3</sup> have resulted in an increased interest for high throughput analysis of these less studied brominated compounds. This study aims to evaluate a standard procedure for extraction and clean-up of PCDD/Fs, applied on PBDD/Fs, with ASE extraction and automated clean-up on the Power-prep™ system<sup>4-6</sup>. No alterations of the existing PCDD/F extraction/clean-up procedure was made in order to evaluate if samples from PCDD/F extraction and clean up are suitable for PBDD/F analysis.

### Methods and materials

All solvents used were of pesticide grade (Merck, Darmstadt, Germany). All standard solutions were obtained from Wellington Lab. Inc (USA) and Wellington Laboratories Inc. (Canada).

ASE extractions were carried out on an ASE 200 (Dionex, Sunnyvale, CA), according to a standard method for dioxin recovery of environmental samples. Cells filled with Hydromatrix™ (Varian Associates Inc., Harbor City, CA) were spiked with 25 µL of standard solutions (IS and quantification mix) and extracted in one cycle (200°C, 2000 psi, static time 5 min) with toluene.

For clean up on the Powerprep™, 25 µL aliquots of <sup>13</sup>C-labelled IS containing 2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,7,8-PeBDD and 1,2,3,4,7,8-HxBDF (10-20 pg/µL) and <sup>12</sup>C quantification mix including 4-MoBDF, 2,7-DiBDF, 2,8-DiBDF, 2,3,8-TriBDF, 1,2,7,8-TeBDF, 1,2,3,7,8-PeBDF, 1,3,4,7,8-PeBDF, 1-MoBDD, 2,7-&2,8-DiBDD, 2,3,7-TriBDD, 1,3,6,8-TeBDD, 1,3,7,9-TeBDD, 1,3,7,8-TeBDD, 1,2,3,4-TeBDD, 2,3,7,8-TeBDD and 1,2,3,7,8-PeBDD, (10-50 pg/µL) were diluted in 10 mL hexane. The station consisted of three different modules equipped with disposable, prepacked columns: a multilayer silica column (5 g acidic, 2 g basic and 0.5 g neutral silica), an alumina column (12 g), and a carbon/celite column (0.34 g). Hexane/dichloromethane and toluene were used as eluting solvents.

Extraction on ASE and clean-up in Powerprep™ was performed with triplicate samples and triplicate blanks. All equipment was protected from light with aluminum foil throughout the experiment. Finally, samples were volume reduced in a rotary evaporator (Laborota 4000, Heidolph). <sup>13</sup>C-labelled RS (2,3,7,8-TBDD; 20 pg/µL) was added and the sample was transferred to 25 µL tetradecane.

### Analysis

HRGC/HRMS analysis was performed on a Micromass Ultima operating at >10 000 – 12 000 resolution using EI ionization at 35 eV. All measurements were achieved in selective ion recording (SIR) mode, monitoring the two

most abundant ions in the bromine cluster. Splitless injection of 1 µl of the final extract was used on a 30 m DB5-MS (0.25 mm i.d., 25µm) column for PBDD/F analysis. To prevent condensation of the brominated compounds in possible cold spots in the transfer line between the GC and the MS, a deactivated column (1.5 m) was coupled to the end of the chromatographic column. This resulted in stable conditions for PBDD/F analysis.

Injection was made at 250°C and the GC was programmed in the following way: an initial hold for 2 min at 180°C, increasing temperature of 7°C/min to 250°C followed by a slower temperature elevation of 5°C/min to 300°C. The temperature was then held for 12 min at 300°C.

### Results and discussion

Clean up of PBDD/F standard mixtures using the Power-prep™ system resulted in overall good reproducibility with relative standard deviations (RSD) between 1-21% (tab. 1). The highest RSDs were observed for lower brominated PBDD/Fs (mono to di). However, these congeners are calculated against tetra brominated IS and RS and are the most volatile which can explain some of the variation. The RSDs, for the most toxic congeners, 2,3,7,8-TeBDD and 1,2,3,7,8-PeBDD, which contributes to a relatively large part of TEQ-levels, were 5% and 9%, respectively. Also, the highest recoveries were seen for these congeners, for the Power-prep system, for which the recoveries decreased with higher bromination degree (for tetra to hexa). This is also often seen for the chlorinated homologues and is related to that higher chlorinated and brominated dioxins and furans bind more efficiently to the carbon column and more solvents need to be used to elute these compounds. Often, a compromise to accept a lower recovery is made in order to minimize the volume of the eluting solvent and to optimize the clean up procedure.

Table 1. Congener-specific reproducibility in relative standard deviation (RSD; %, n=3) for <sup>12</sup>C-PBDD/Fs and recoveries (%; n=6) for <sup>13</sup>C-labelled PBDD/Fs after ASE extraction.

<b>ASE</b>	
<i>Reproducibility</i>	
<i>(RSD, %)</i>	
4-MoBDF	8
2,7-DiBDF	8
2,8-DiBDF	3
2,3,8-TriBDF	1
1,2,7,8-TeBDF	19
1,2,3,7,8-PeBDF	5
2,3,4,7,8-PeBDF	3
1-MoBDD	6
2,7- & 2,8-DiBDD	5
2,3,7-TriBDD	1
1,3,6,8-TeBDD	3
1,3,7,9-TeBDD	1
1,2,3,4-TeBDD	6
2,3,7,8-TeBDD	2
1,2,3,7,8-PeBDD	10
<i>Recoveries (%)</i>	
2,3,7,8-TeBDF	93-127
1,2,3,7,8-PeBDF	75-98
2,3,4,7,8-PeBDF	69-91
1,2,3,4,7,8-HxBDF	71-99
1,2,3,7,8-PeBDD	69-99

Table 2. Congener-specific reproducibility in relative standard deviation (RSD; %, n=3) for <sup>12</sup>C-PBDD/Fs and recoveries (%; n=6) for <sup>13</sup>C-labelled PBDD/Fs after clean-up using the power-prep™ system.

<b>POWER-PREP™</b>	
<i>Reproducibility</i>	
<i>(RSD, %)</i>	
4-MoBDF	21
2,7-DiBDF	18
2,8-DiBDF	8
2,3,8-TriBDF	7
1,2,7,8-TeBDF	7
1,2,3,7,8-PeBDF	3
2,3,4,7,8-PeBDF	7
1-MoBDD	20
2,7- & 2,8-DiBDD	1
2,3,7-TriBDD	6
1,3,6,8-TeBDD	12
1,3,7,9-TeBDD	5
1,2,3,4-TeBDD	11
2,3,7,8-TeBDD	4
1,2,3,7,8-PeBDD	8
<i>Recoveries (%)</i>	
2,3,7,8-TeBDF	74-128
1,2,3,7,8-PeBDF	52-111
2,3,4,7,8-PeBDF	56-105
1,2,3,4,7,8-HxBDF	42-85
1,2,3,7,8-PeBDD	52-103

The ASE enabled fast extraction of the PBDD/Fs, although with somewhat low reproducibility for the 1,2,7,8-TeBDF congener (tab. 1). The method run was a standard method for PCDD/F extraction and will be further adjusted for better reproducibility and recoveries of PBDD/Fs in different matrices. The recoveries were equally good among the studied congeners, from 71-127%. No detectable levels of the studied congeners were found in the procedure blanks.

Using automated extraction and clean-up by ASE and the Power-prep<sup>TM</sup> system, respectively, was reliable and suitable for clean-up of PBDD/Fs, with good recoveries for the tested congeners and low variation among samples. However, volumes of eluting solvents can be adjusted to improve the recoveries of higher brominated PBDD/Fs. Using this approach, brominated and chlorinated PB/CDD/Fs can be simultaneously recovered from samples, with high throughputs.

### References

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