Pressurized Solvent Extraction
Improved Workflow in Dioxin and PCB analysis

Pressurized solvent extraction for the determination of dioxins and PCBs in dairy, egg, pork and fish

Abstract

The determination of dioxins and PCBs in foodstuffs is performed to monitor the potential exposure to humans. This whitepaper describes an innovative combination of pressurized solvent extraction and automated clean-up, showing clear benefits compared to the conventional manual approach. The extraction of proficiency test samples with the SpeedExtractor E-914 and subsequent automatic clean-up using a MIURA system and analysis by GC-HRMS revealed excellent correspondence of determined levels of dioxins and PCBs with the assigned values and proved the combination as a reliable, fast and resource-efficient method.

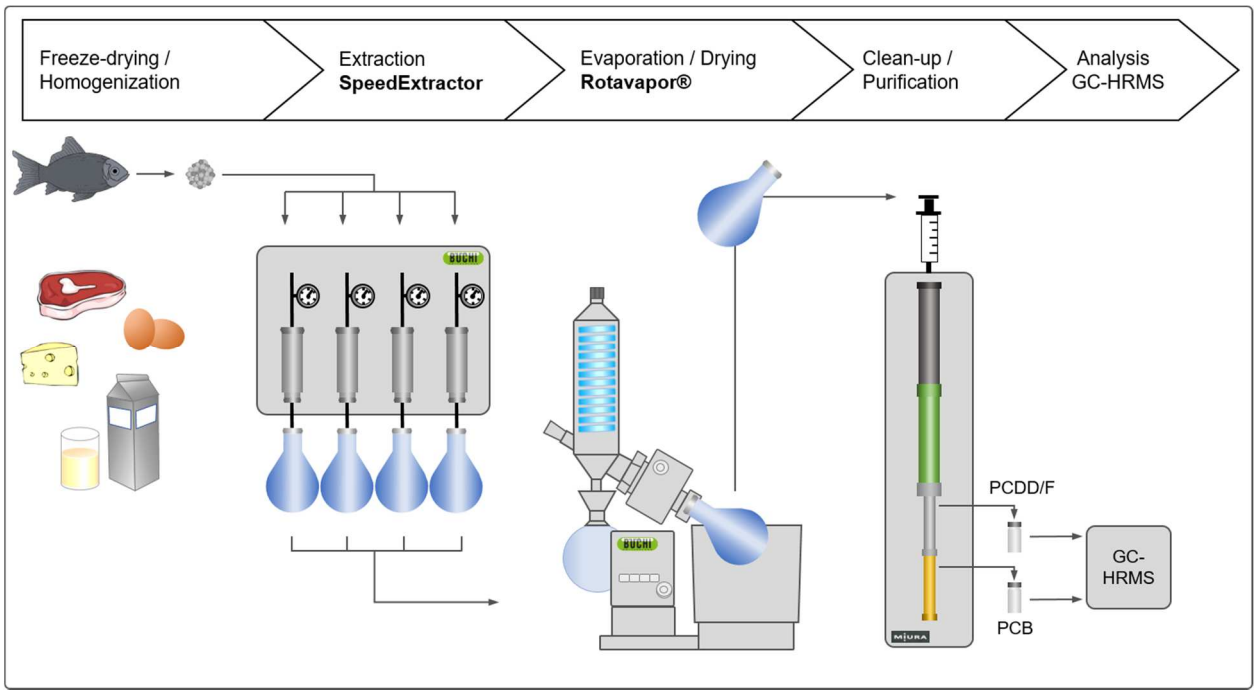

Figure 1: *Principle of the analysis method and improved workflow for dioxins and PCB in foodstuffs.*

1. Introduction

Dioxins and PCBs are toxic substances of different origin. Whereas dioxins are released into the atmosphere by incomplete combustion, PCBs have been produced in large volumes in industrial applications. Both persistent organic pollutants are regulated under the Stockholm convention aiming at reduction and, where feasible, ultimate elimination.

"Dioxin" is the collective term for polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Fig. 2). The overall assessment of dioxin toxicity includes 12 congeners of polychlorinated biphenyls (PCBs) due to the "dioxin-like" character (Fig. 3).

Dioxins and PCBs are lipophilic and accumulate in human and animal tissues. Therefore, high fat foods from animals such as meat, eggs, milk and derived products are at a greater risk of contamination.

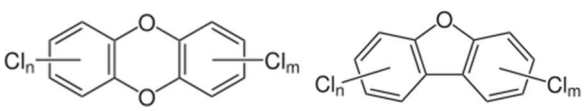

Figure 2: *Structure of dioxins (PCDDs, left) and furans (PCDFs, right)*

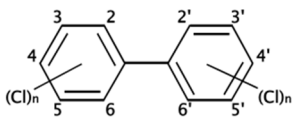

Figure 3: *Structure of PCBs*

2. Workflow

The conventional analysis of dioxins and PCBs is very demanding and time consuming. For most food samples, the dioxin and PCBs content is expressed related to the fat content and therefore requires an exhaustive fat extraction of the sample. Traditionally, this step is performed using a Soxhlet extraction of several hours followed by gravimetric fat determination. Subsequently, the extract is redissolved and a time and solvent demanding clean-up procedure is performed to purify the

Figure 4: *SpeedExtractor E-914*

extract for quantification by GC-MS. The full analysis can be accelerated by automated extraction and clean-up. BUCHI's SpeedExtractor E-914 (Fig. 4) reduces extraction time from hours to minutes by pressurized solvent extraction at elevated temperatures and pressure. The SpeedExtractor E-914 is designed for extraction of four samples simultaneously enabling a four-fold increase in sample throughput and enormous time savings compared to the conventional or serial approach. The resulting extracts are collected into round-bottom flasks that are compatible with BUCHI parallel or rotary evaporators, avoiding cumbersome and error prone sample transfer. The SpeedExtractor E-914 offers high flexibility with widely adjustable process parameters (30-200° C, 50-150 bar) and various extraction cell sizes (40-120 mL).
After gravimetric fat determination, the extracts are redissolved with solvent for purification and concentration using the automatic clean-up system by MIURA. The parallel system consists of four columns that requires no conditioning and the entire clean-up procedure takes less than two hours [2]. This approach enables significant time as well as solvent saving compared to manual clean-up procedures. MIURA's clean-up system yields PCDD/F and PCBs separated into two different vials for further analysis with GC-HRMS.

3. Analysis of test samples

The SpeedExtractor E-914, in combination with the Rotavapor® and MIURA GO-HT system, was used for the determination of samples of milk powder, milk fat, egg powder, pork and fish (herring). All samples were from EU-RL and Norwegian Institute of Public Health proficiency tests.

The samples were freeze-dried and added directly to 40 mL extraction cells of the SpeedExtractor E-914. The 3 extraction cycles were carried out at 120°C, 100 bar with hold time of 5 min each. As solvent a mixture of 70 % toluene:30 % acetone was used. The extracts were collected in 250 mL round bottom flasks. Total extraction time was 53 min. The extraction of milk powder which was performed according to the parameters given in the EN method [3].

After extraction, the extracts were evaporated to dryness using a Rotavapor® and the fat content was calculated. The extract was then redissolved with hexane and injected into the MIURA GO-HT system for automated clean-up. The resulting extracts were then analyzed for dioxins, dioxin-like PCBs and non-dioxin-like PCBs using GC-HRMS (Agilent 7980A coupled to JEOL 800D).

4. Results

The determined WHO-TEQ 2005 values for the sum of the dioxins and the dioxin-like PCBs are shown in Figure 5. In all samples, 17 dioxin and furan congeners, 12 dioxin-like PCBs and 6 non-dioxin like PCBs were quantified. Z-scores associated with the determined values were < 2 (detailed data shown in the application note).

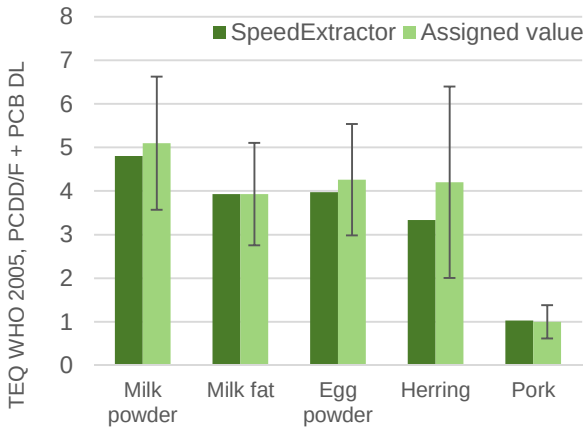

***Figure 5**: Determined TEQ for the sum of the dioxins and dioxin-like PCBs in proficiency test samples compared to the assigned values in [pg/g fat] for all samples except herring, where it is [pg/g fresh weight]. The error bars correspond to the concentration range with a z-score between +2 and -2. Mean values, n=4.*

5. Conclusions

The determined WHO-TEQ values for PCDD/Fs and PCBs show excellent agreement with the assigned values, with absolute z-score values of < 1. The presented procedure using Pressurized Solvent Extraction with the SpeedExtractor E-914, combined with the automated clean-up system by MIURA-SHIMADZU, proves as a reliable, fast and resource-efficient method for the determination of dioxins and PCBs for various food samples.

5. Acknowledgement

We gratefully thank Mr. Philippe Marchand and Mr. Vincent Vaccher from Laboratoire d'Etudes des Résidus et Contaminants dans les Aliments (LABERCA) at Oniris Université Nantes, France, for sharing their expertise in dioxin determination, the fruitful collaboration and valuable discussions.

6. References

[1] Stockholm Convention on Persistent Organic Pollutants, 2001
[2] Marchand, P. *et al.* A new and highly innovative automatic purification system evaluated for dioxins and PCBs, Organohalogen Compounds 76 (2014) 546-549.
[3] EN 16215:2012 Animal feeding stuffs - Determination of dioxins and dioxin-like PCBs by GC-HRMS and of indicator PCBs by GC/HRMS.

Detailed instructions and all the results are presented in the Application Note No. 205/2015.