ON-LINE PLE-LC SAMPLE PREPARATION FOR THE MEASUREMENT OF DIOXINS AND WHO-PCBS IN FOOD AND FEED

Jean-François Focant, Catherine Pirard, Anne-Cécile Massart, Georges Scholl, Gauthier Eppe, and Edwin De Pauw
CART Mass Spectrometry Laboratory, Chemistry Department, University of Liège, Allée de la Chimie 3, B-6c Sart-Tilman, B-4000 Liège, Belgium.
E-mail : JF.Focant@ulg.ac.be

Sample preparation-fractionation for the measurement of selected dioxins (PCDDs and PCDFs) and PCBs in biological matrices is a complex field of investigation [1]. A potential improvement of the current situation is the direct coupling of pressurized liquid extraction (PLE) with automated multi-sorbent clean-up and fractionation. A prototype system has evolved through several generations of changes dictated by a long term testing exercise. This paper reports on the latest data that were obtained using this system for food and feed samples dedicated to dioxin and PCB analyses.

Two types of quality control (QC) samples were tested: fortified yolk QC samples (5g d.w. sample size) and unfortified animal feed QC samples (30g d.w. sample size), both at levels close to EU regulation levels (low pg/g) [2]. Blank (BC) levels were measured with sodium sulfate inside the PLE cells. The isotope dilution (ID) ^{13}C-labelled standards containing PCDD/Fs and selected PCBs were added to the extraction cell prior to extraction and clean-up. Extraction cells had a volume of 100 ml. The stainless steel parts of the PLE cells were washed after each run. Viton and Peek washers were disposable. The HPLC system (pump and valves) can operate at pressures up to 2500 psi. The PLE cell is attached outside the module via quick connectors and the oven is surrounding it. The utilization and maintenance of the low pressure part of the system has been described elsewhere [3]. After concentration, the extracts were analyzed by GC-IDHRMS. Two separate injections were used: one for PCDD/Fs and non-*ortho*-PCBs (VF-5MS 50 m x 0.20 mm ID x 0.33 µm df), the other for mono-*ortho*-PCBs and indicator PCBs (HT-8 25 m x 0.22 mm ID x 0.25 µm df). The analyses followed EU recommendations [4].

The current plumbing of the system is depicted in Figure 1. The HPLC pump uses hexane during the extraction and dichloromethane for the wash. The pressure transducer allows computer recording of the pressure during the run for QA/QC purposes. Nitrogen is available for the purge of the PLE cell after extraction. A 60 micron stainless steel filter is present after the PLE cell to ensure particle free solvent after extraction. The pressure relief valve is set at the extraction value (1500 psi) and is responsible for maintaining the pressure to the set point during the extraction. Eluents issued from this valve are directed to the clean-up column to ensure proper transfer of extracts exiting the PLE cell during the static extraction step. Extracts are purified on the multilayer silica column [3]. Dioxins and PCBs are fractionated using the basic alumina and the carbon columns. The entire clean-up run has been simplified and validated. In practice, the entire run time is 1.5h (3 samples in parallel) and can be divided in 3 main parts: 1) the column conditioning (30min), 2) the extraction step (30min for 1 extraction cycle), and 3) the clean-up step. As the PLE cell is filled up with solvent, it is already lined up with both the multilayer silica and the alumina columns to ensure the transfer of early extracted analytes once the PLE cell is full of solvent and starts to leak out on the multilayer silica column. In the mean time, it is important to ensure that the air content of the PLE cell is not transferred to the clean-up columns for proper retention and fractionation of the analytes. PCB retention on alumina, especially for MO-PCBs, is sensitive to the presence of air inside the column.

Recovery rates for PCDDs, PCDFs, and NO-PCBs ranged from 62% to 87% (SD <20%) with good reproducibility. Deviations from the assigned concentration values for a yolk QC on a congener basis in terms of concentrations were below 20% for most congeners, excepted for NO-PCBs where larger differences are probably correlated to the transfer of air to the clean-up column.

For yolks, 1 and 3 extraction cycles were tested and 1 cycle showed to be sufficient. Purging of the PLE cell after extraction was either carried out using nitrogen or fresh hexane. Both ways were efficient

but the use of hexane is preferred because it does not yield to undesirable air transfer to the clean-up column(deactivation). Figure 2 shows the PCDD/F TEQ QC chart data for yolk QC samples.

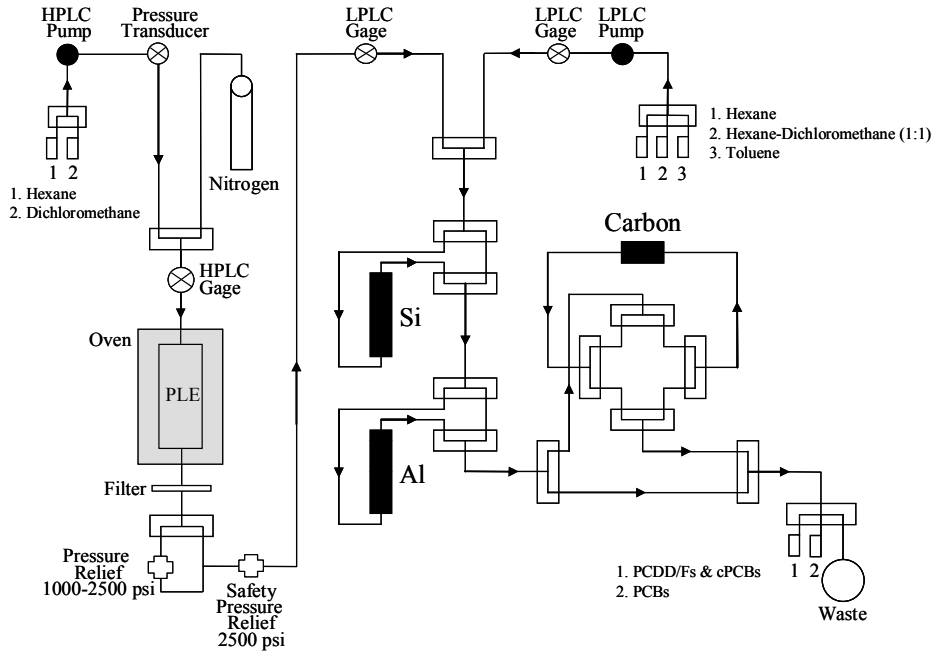

Figure 1: Plumbing diagram of the system.

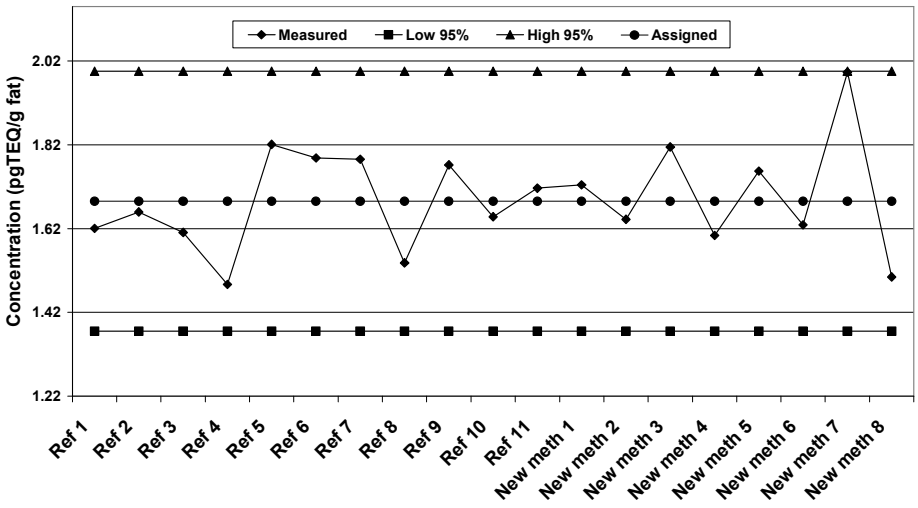

Figure 2: QC chart for the yolk QC samples prepared using the reported method.

For animal feed QC, the extraction solvent was also hexane. Three extraction cycles were performed to ensure efficient extraction. The deviation for the sum of the PCDD/F and NO-PCB TEQs was 16% (underestimation). No differences were observed regarding the extract quality compared to other routinely used extraction and clean-up methods in the laboratory. On a practical point of view, this approach permits the entire analysis of batches of six samples in 24h.

References

1. Focant J.-F., Pirard C. and De Pauw E. (2004) *Talanta* 63: 1101-1113.
2. Council Regulation (EC) 2375/2001, Off. J. European Communities, (6.12.2001) L321/1.
3. Focant J.-F., Eppe G., Pirard C. and De Pauw E. (2001) *J. Chromatogr. A* 925: 207.
4. Commission Directive 2002/69/EC, Off. J. European Communities, (6.8.2002) L209/5.