PLE FOR EXTRACTION OF DIOXINS IN ANIMAL FEED AND INGREDIENTS

Focant JF, Scholl G, De Pauw E, Eppe G

CART, Organic and Biological Analytical Chemistry, Mass Spectrometry Laboratory, Chemistry Department, University of Liège, Allée de la Chimie 3, B-6c Sart-Tilman, B-4000 Liège, Belgium. (JF.Focant@ulg.ac.be)

Introduction

Within the entire complex procedure required to measure dioxins and related compounds in biological matrices, the extraction step is often seen as a well controlled step. Although maybe true for many human and food-related matrices, the situation is very different for animal feed and feed ingredients. Specific European guidelines (e.g. Commission Directive 2006/13/EC, Commission Regulation (EC) No 152/2009) exist for animal feed but only list general requirements for the various stages of the procedure. The liberty is left to laboratories to select, for example, the tools used for the extraction steps. This has the advantage to allow 'in-house' methods to be used, as long as they satisfy with all the requirements of the EU Regulation. In that context, it is foreseen that the European Committee for Standardization (CEN) will soon propose a standard for the determination of PCDD/Fs and PCBs in animal feed that would be the reference method to be used to solve potential issues in case of dispute over results reported from different laboratories.

A major point of concern is that it has been reported earlier[1] that most commonly accepted extraction procedure can conduct to significantly different results for the extraction of dioxins and related compounds in feed and feed additives such as mineral clays and various oxides. Several non-instrumental and instrumental automated approaches are available for extraction. Soxhlet extractors have long been the most used tools for non-instrumental extraction of solids. They have proven to be very efficient but some limitations encouraged the development of other approaches based on instrumental techniques. For feed extraction, pressurized liquid extraction (PLE) (also branded as accelerated solvent extraction ASE®) is the technique of choice for high sample throughput.

This study reports on the investigation of the use of various solvent mixtures, extraction temperatures, and instruments (parallel PLE, sequential ASE®) for the extraction of 17 PCDD/Fs and 12 dioxin-like PCBs in mineral clay, bovine feed, fish meal, and in-house quality control animal compound feed.

Materials and Methods

Test Material

The test materials consist in mineral clay, bovine feed and fish meal samples used for the CEN PT 2010 - Animal Feed ring trail on determination of PCDD/Fs and PCBs in animal feed, fat/oil. The samples were shipped at room temperature in polypropylene jars. The WHO-PCDD/F-TEQ and WHO-PCB-TEQ concentrations in the samples were in the range of action to maximum levels defined for animal feed in Commission Directive 2006/13/EC. Next to the three above mentioned samples an in-house quality control animal compound feed matrix was also considered. It consists in dried homogeneous aliquots of a bulk sample of feed saved from the Belgian dioxin crisis of 1999. It is 'naturally' contaminated and also expresses levels close to the EU regulation values.

Target compounds

The seventeen 2,3,7,8 toxic PCDD/Fs and the twelve dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189).

Analytical procedures

Extractions were either carried out by the sequential extractor ASE® 350 from Dionex (Sunnyvale, CA, USA) or the parallel extractor PLE-6 from Fluid Management Systems (Waltham, MA, USA). All samples were spiked with a mixture of labeled PCDD/Fs and DL-PCBs, thoroughly mixed and placed in the extraction cell (30-100ml). After extraction, extracts were filtered on sodium sulphate and the solvent was exchanged to hexane using a rotary evaporator. The extract was further cleaned and fractionated with an automated Power-Prep system (Fluid Management Systems, Waltham, MA, USA). PCDD/Fs fraction was injected into a gas chromatography – isotope dilution high resolution mass spectrometer (GC-IDHRMS, Autospec Ultima, Waters, UK). All details regarding the analytical procedure for Power-Prep and GC-HRMS can be found elsewhere[2]. All solvent and consumable issued from lots used by our laboratory for routine analyses according to a BELAC accredited method.

Results and Discussion

Part of this study was performed during a proficiency testing (CEN PT 2010 - Animal Feed ring trail on determination of PCDD/Fs and PCBs in animal feed, fat/oil) organized to compare several different approaches. Based on this, two sets of extraction parameters were used, as illustrated in Table 1.

Table 1. Principal parameters for the extraction methods

	Temp (°C)	Pressure (psi)	Static time (min)	Cell flush with solvent	Cell purge with nitrogen	Number of cycles	Solvent cycle 1	Solvent cycle 2	Solvent cycle 3
CEN Method	100	1500	15	Yes	Yes	3	Tol	Tol/Et	Tol/Et
CART method	150	1500	15	Yes	Yes	3	Tol/Et	Tol/Et	Tol/Et

Tol is pure toluene, Tol/Et is a mixture of toluene and ethanol at 90:10, respectively.

Figure 1 and Figure 2 show the levels of PCDD/Fs and DL-PCBs measured in mineral clay using ASE® and PLE, using the CEN method, and plotted against a CEN PT consensus value (CEN PT also included Soxhlet extractions). For most congeners, a tendency is observed for the ASE® values to be higher than the consensus value, although the PLE values tend to be below the consensus value.

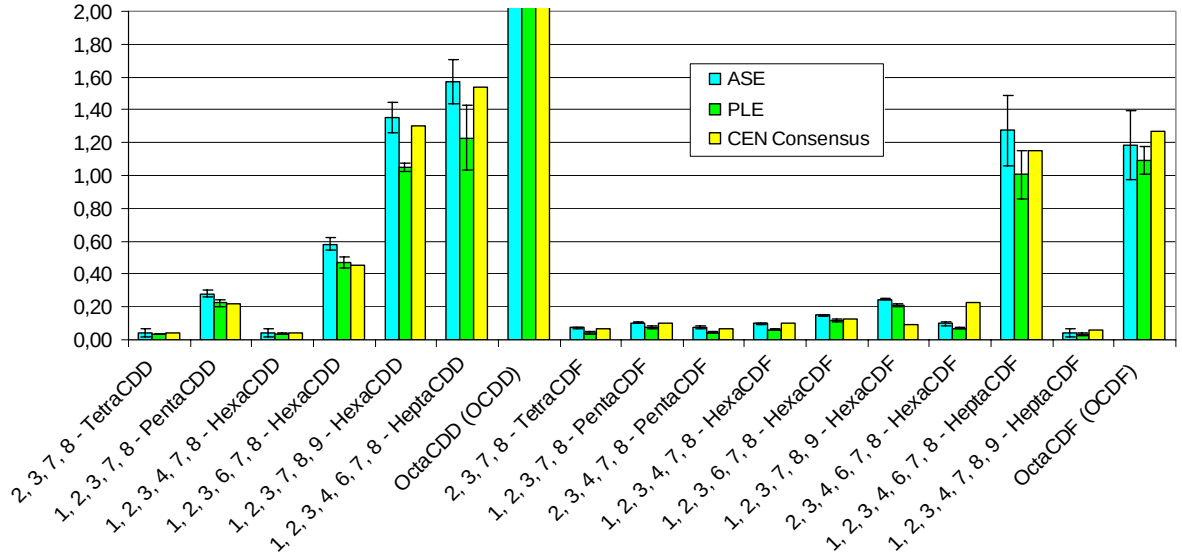

Figure 1. PCDD/Fs measured in mineral clay (levels in ng/kg product)

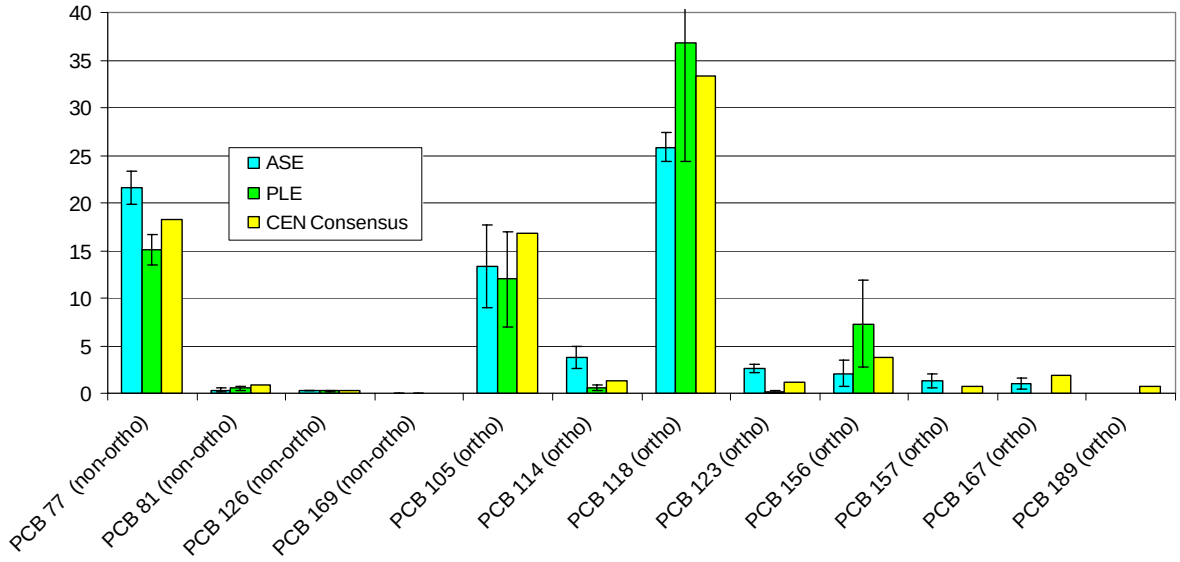

Figure 2. DL-PCBs measured in mineral clay (levels in ng/kg product)

The same trend is reflected on a TEQ basis, as illustrated in Figure 3.

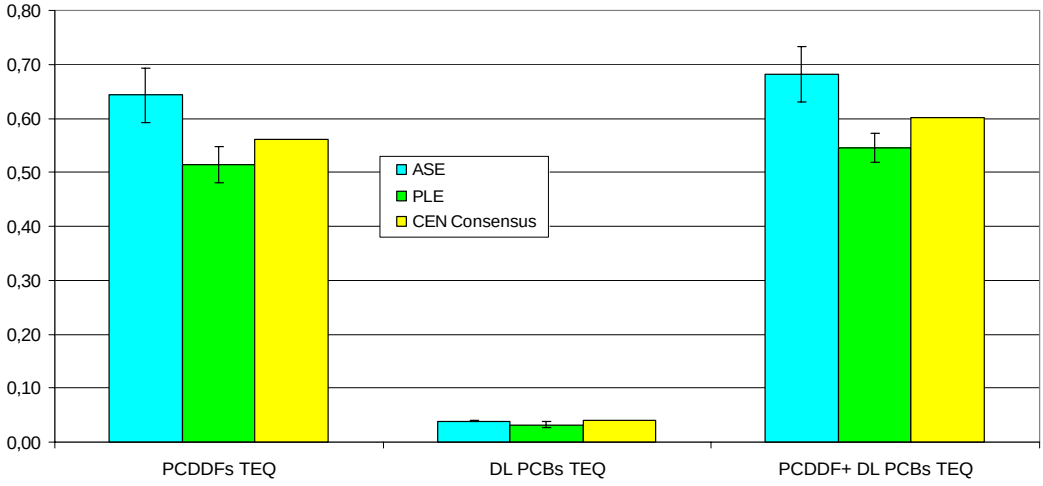

Figure 3. TEQ levels reported for mineral clay (levels in ngTEQ/kg product)

Although not as pronounced for bovine feed, this trend is also observed for the other matrices. Based on z-score, a positive and a negative bias can be highlighted for ASE® and PLE, respectively (Table 2). For z-score calculations, the target deviation was set at 10% in TEQ (for individual congeners, target SD is 20%).. The variation in z-scores between data is to be attributed to the extraction because z-scores very close to zero were obtained for fish oil samples that did not undergo extractions but were directly processed through clean-up and fractionation.

Table 2. Z-score data for the various matrices

		Z-scores		Consensus
		ASE	PLE	Level (ng/kg)
Mineral clay	PCDD/Fs	1,4	-0,9	0,56
	PCBs	0,0	-2,5	0,04
	PCDD/Fs+PCBs	1,3	-1,0	0,60
Bovine feed	PCDD/Fs	1,8	0,3	1,49
	PCBs	0,1	-0,3	1,02
	PCDD/Fs+PCBs	1,1	0,0	2,51
Fish meal	PCDD/Fs	1,4	-2,6	0,58
	PCBs	0,7	-0,7	0,84
	PCDD/Fs+PCBs	1,0	-1,5	1,42

Those results encouraged us to perform an additional extraction temperature study. We compared the CEN method to our CART method (see Table 1). The little difference in solvent used for the first extraction cycle is not to be important here as such influence is expected for extraction of oxides of trace elements[3] although we carried this out on our animal feed QC sample.

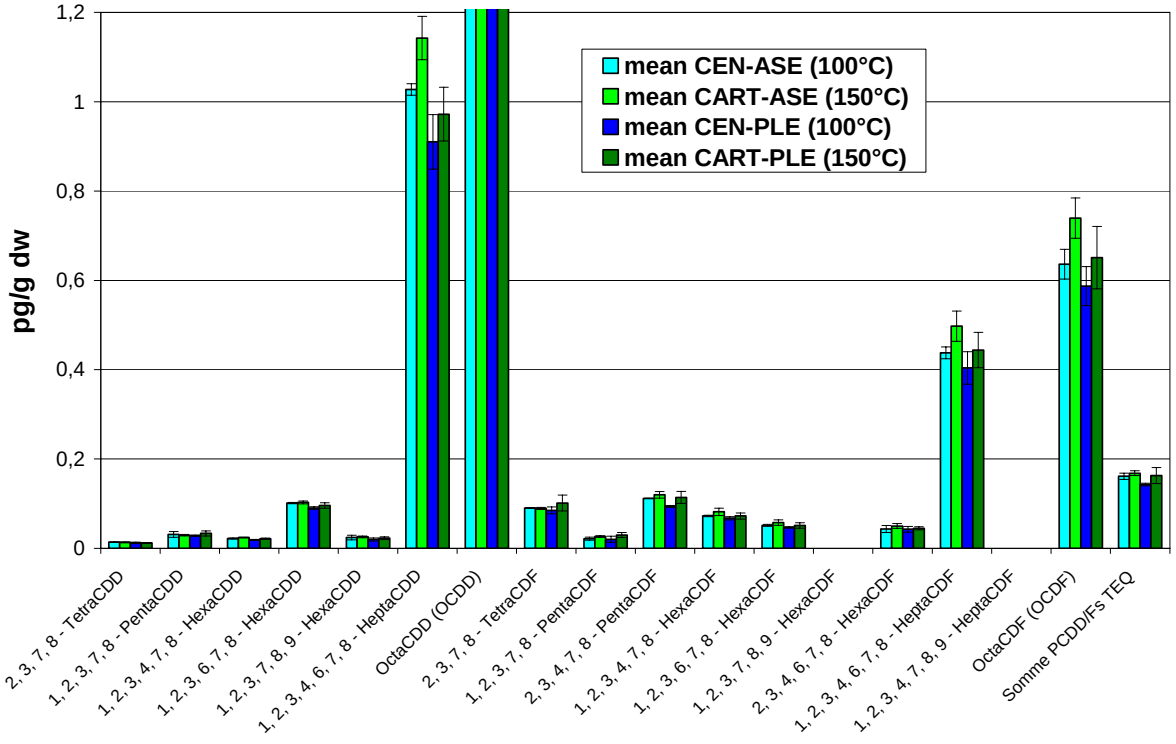

Figure 4. Comparison of the CEN method to the CART method for both ASE® and PLE

From Figure 4, one can mainly extract two pieces of information. First, if we select one method (either ASE® or PLE), and then we compare the temperatures, it appears that a temperature of 100°C is not as efficient as a temperature of 150°C to quantitatively extract target analytes from the feed matrix. Second, if we select one temperature (either 100°C or 150°C), and then we compare the methods, it appears that PLE results seem to reproducibly be lower than ASE® results. This second observation corroborates what was observed earlier with the z-score values.

Because ASE® and PLE rely on identical physico-chemical principles, and because performing in parallel instead of sequentially should not influence the extraction efficiency, we suspected a temperature transfer issue for the PLE. Therefore, we developed an experimental set up that allowed us to measure the temperature inside the extraction cell of the PLE during extraction cycles. A thermocouple thermometer was immersed in the open cell that was filled up with a mixture of ethylene glycol/water. This was only possible with the PLE because the ASE® design does not allow to perform heat cycles with the cell opened.

What resulted from this temperature study is illustrated in Figure 5. It is quite clear that temperature transfer issue can arise if the position of the cell in the heater is not optimized (if an air gap remains between metal pieces, Figure 6). Good positioning is easily obtained by using a rubber band around the Teflon insulator of the heater. Also, it is clear that a pre-heating step should be included in the program to ensure that proper temperature is reached before the extraction time counter starts. In Figure 5, a pre-heating step of 5 minutes is necessary to reach the extraction temperature of 150°C. This later fact is most probably also true for the ASE® system.

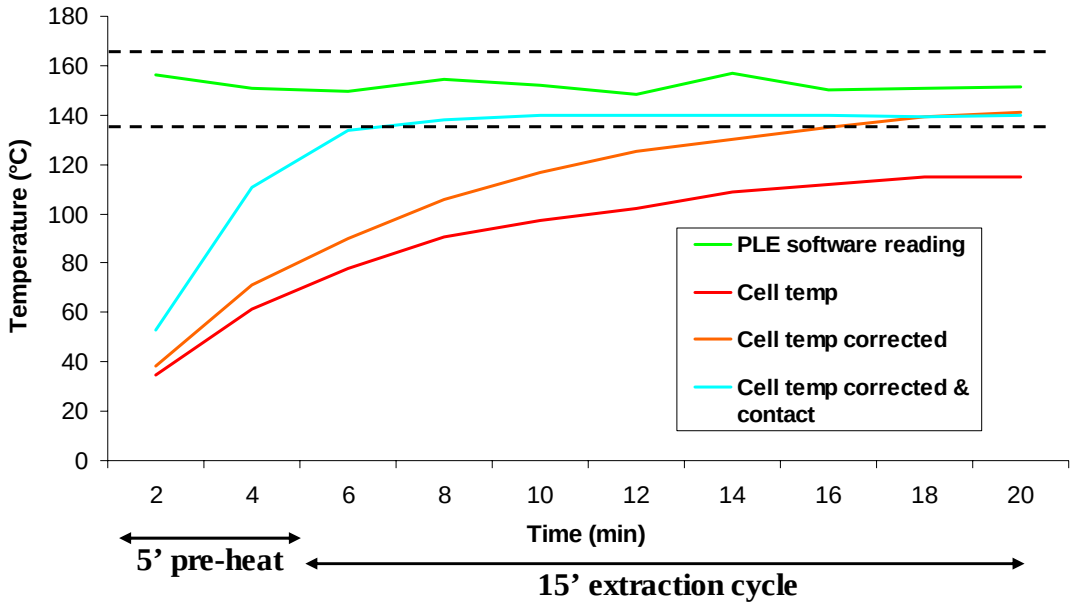

Figure 5. Evolution of the temperature for a PLE cycle (CART method). The 'PLE software reading' is the temperature recorded by the temperature sensor of the instrument. The probe is located inside the heating metal piece surrounding the extraction cell. The 'Cell temp' is the temperature measured inside the cell in the classical design. The 'Cell temp corrected' is the temperature inside the cell once a correction factor is incorporated in the software. The 'Cell temp corrected & contact' is the temperature inside the cell once the contact between the cell walls and the metal heater is verified and maintained. The dashed line represents a ±10% variation range from the set temperature

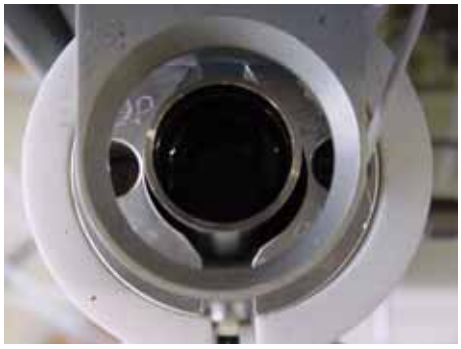

Figure 6. Bad positioning of the cell in the PLE heating element

We should precise that those temperature variations are important because of the difficult nature of the matrices considered here. They'd probably not be pointed out for regular food matrices as the extraction of the lipid fraction I less challenging.

Conclusions

Both automated parallel PLE and sequential ASE have successfully used for the extraction of complex animal feed and feed ingredients. A careful monitoring of the real extraction temperature is required to ensure reproducible results. A temperature of 100°C could be source of additional variations and seem to be too low. A pre-heating step is required to carry the extraction at the desired temperature inside the cell.

References

1. Kotz A, Malisch R, Wahl K, Hädrich J, Adamovic K, Gerteisen I, Leswal S, Schächtele J, Tritschler R, Winterhalter H, Organohalogen Compounds 69 (2007) 28.
2. Focant JF, Eppe G, Pirard P, DePauw E, Journal of Chromatography A 925 (2001) 207.
3. Kotz A, Malisch R, Hädrich J, Adamovic K, Gerteisen I, Tritschler R, Winterhalter H, Organohalogen Compounds 70 (2008) 902.