

DOES AUTOMATIC SAMPLE PREPARATION FULFILL THE EUROPEAN ANALYTICAL CRITERIA FOR PCDD/Fs AND PCBs MONITORING IN FEED AND FOOD?

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Introduction

In spite of huge efforts made for several years to reduce the occurrence of PCDD/Fs and PCBs in most developed countries, some specific situations of acute contamination may still happen. The current European regulation (Commission Regulation 1259/2011 of 2 December 2011 and Regulation 277/2012 of 28 March 2012)^{1,2} sets maximum levels for PCDD/Fs and PCBs in feed and foodstuffs and non compliant products cannot be placed on the market. To improve the control, high throughput methods and cost savings are required for PCDD/Fs and PCBs analysis. At the same time, crisis situations induce high financial and human costs and require upgrading the capacity of laboratories. For the last decade, some providers have developed automatic equipments for sample preparation to implement high throughput monitoring. To ensure that analytical results are reported and interpreted uniformly throughout, European Union regulations (252/2012 and 278/2012) laying down analytical criteria for the official control have to be applied. The aim of this study was to compare an automatic purification system (MIURA SPD-600GC) with a manual conventional method used at the French National Reference Laboratory. Criteria such as repeatability, sensitivity, recoveries and specificity were assessed and compared.

1. Materials and methods

1.1 Samples

The present study is based on results from *ca.* 40 different food and feed samples (milk, feeding stuff, pork fat, poultry muscle, egg, butter, sardines, fish oil, grass...) coming from different geographical areas and presenting a wide variability in terms of composition, *i.e.* fat content, contamination pattern, coextracted compounds...

1.2 Method

1.2.1 Reference method

The reference method used for the determination of PCDD/Fs and PCBs is validated and accredited against the ISO 17025 standard and has been described elsewhere³. Briefly, 10–20 g aliquots of fresh samples (corresponding to an equivalent of 0.5–1.5 g of fat) of food and feeding stuff were previously freeze-dried and grinded. Fat samples were extracted using an ASE 300 extractor (Dionex, Sunnyvale, CA, USA) with three successive extraction cycles (5 min each) by a mixture of toluene/acetone 70:30 (v/v). The extracts were evaporated to dryness. Extracted fat contents were determined gravimetrically and dissolved in n-hexane for further purification. Clean-up steps involved 3 successive open columns: (1) a multilayer sulphuric acid activated silica column for lipids removal, (2) a Florisil[®] deactivated with 3% water column for PCDD/Fs and PCBs fractionation, and (3) a carbon column (PCDD/Fs) or a Florisil[®]/carbon column (mono-ortho PCBs and di-ortho PCBs fractionation). PCDD/Fs and PCBs measurements were performed by gas chromatography (HP-5890, Hewlett Packard, Palo Alto, CA, USA) coupled to a double electromagnetic sector high resolution mass spectrometer (GC-HRMS) set at a resolution of 10 000 (JMS-700D and 800D, Jeol, Tokyo, Japan). Acquisition was performed in the single ion monitoring mode and for quantification as required by the isotopic dilution method principle. Toxic Equivalent Quotient values (TEQ) were calculated according to the 2005 World Health Organization Toxic Equivalency Factors (2005-WHO-TEF) and basically expressed on a lipid-weight basis.

1.2.2 Alternative purification strategy (Automatic)

The SPD-600GC is a fully automated device for the extraction of PCDD/Fs and PCBs in various matrices (Miura Institute of Environmental Science, Miura Co. Ltd., Japan)⁴.

Figure 1 shows the schematic diagram of the column flow channel, which is as follows: (1) the top column is a multilayer silica gel column, filled with 10% (w/w) silver nitrate silica gel and 44% (w/w) sulfuric acid silica gel (200 mm x 12.5 mm). (2) The bottom one is a concentration column filled with a graphite carbon dispersed silica gel (30 mm x 6 mm).

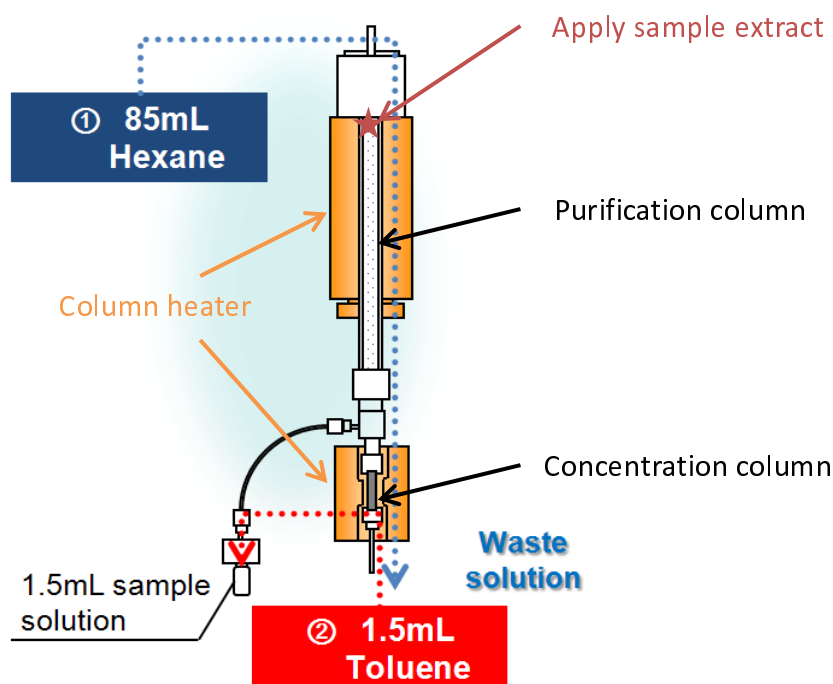


Figure 1: Schematic diagram of the column flow channel.

The fat extract dissolved in n-hexane (see 2.2.1) was directly applied on the top of the purification column which was then placed on the system. Then the program sequence was launched for a 2-hour run. After heating, and keeping the purification column at 60 °C, PCDD/Fs and PCBs were eluted using 85 mL of n-hexane. PCBs (mono and di-ortho PCBs) were passed through the concentration column and were collected in a flask. Then, PCDD/Fs and non-ortho PCBs trapped on the concentration column were reversibly eluted in the same fraction using 1.5 mL of toluene maintained at approximately 80 °C.

2 Results and discussion

2.1 Results

2.1.1 Comparison between automated and manual procedures based on TEQ values

Six different matrices (feeding stuff, milk, poultry muscle, eggs, sardine and grass) were analyzed by both operating procedures, *i.e.* automatic and manual ones. Contaminants were quantified with the same GC-HRMS method and the results were expressed according to the 277/2012 European Regulation² requirements. Table 1 summarizes the values obtained for food and feed samples using both approaches.

Table 1: Results for Dioxins and PCBs in different samples (Automatic: SPD-600GC procedure, Manual: LABERCA procedure, Deviation=(Automatic result-Manual result)/Manual result). Left hand side Table: details for PCDD/F and DL-PCB TEQ results (pg-TEQ/g); Right hand side Table: results expressed in total TEQ (PCDD/F-DL-PCB expressed in pg-TEQ/g) and as the sum of NDL-PCBs (ng/g).

Matrix	Automatic	Manual	Deviation	Automatic	Manual	Deviation
	TEQ PCDD/F			TEQ DL-PCBs		
Feeding Stuff	0,019	0,013	46%	0,002	0,002	0%
Milk	0,81	0,56	44%	3,42	3,96	-14%
Poultry	0,408	0,276	48%	0,265	0,368	-28%
Egg 1	1,67	1,96	-15%	2,19	1,95	12%
Egg 2	1,98	2,08	-5%	5,48	5,05	9%
Sardine 1	0,032	0,023	35%	0,080	0,069	16%
Sardine 2	0,208	0,160	30%	0,922	0,901	2%
Grass 1	9,26	8,76	6%	377,63	369,10	2%
Grass 2	0,488	0,459	6%	0,149	0,121	23%
Grass 3	0,263	0,244	8%	0,913	0,913	0%

Matrix	Automatic	Manual	Deviation	Automatic	Manual	Deviation
	TEQ (PCDD/F-DL-PCBs)			NDL-PCBs		
Feeding Stuff	0,021	0,016	31%	0,03	0,03	0%
Milk	4,22	4,52	-7%	9,58	10,41	-8%
Poultry	0,673	0,645	4%	5,633	6,753	-17%
Egg 1	3,87	3,92	-1%	45,53	41,91	9%
Egg 2	7,46	7,13	5%	11,73	11,17	5%
Sardine 1	0,112	0,093	21%	0,800	0,539	48%
Sardine 2	1,130	1,061	6%	7,403	7,665	-3%
Grass 1	386,89	377,86	2%	677,71	522,00	30%
Grass 2	0,637	0,580	10%	0,975	0,891	9%
Grass 3	1,175	1,157	2%	5,501	5,800	-5%

No significant differences could be observed for food or feed samples at the level of interest. At background levels, the deviation calculated was in the same range as the one calculated in reproducibility tests for the official manual method. As a result of the automatic procedure, the quantification of dioxins and non ortho-PCBs in the same fraction seems to be acceptable whatever the matrix and the level of contamination.

2.1.2 Repeatability of the automated procedure and compliance with Quality Control (QC) Charts expectations

Three quality control samples are usually analyzed to validate each batch of samples when running the official method. Indeed, butter, grass and oil fish samples are available in the lab with corresponding target TEQ values characterized for each of them. Table 2 presents the results obtained with the automatic preparation. Relative standard deviations (RSD) are always below 10% for butter and oil fish samples, with respectively 6 and 9 repetitions. A RSD value of 20% is observed on grass for TEQ PCDD/F (n=7), which can be explained by the very low congeners concentration in this particular sample.

Table 2: Repeatability of TEQ values for PCDD/Fs, DL-PCBs and the sum of NDL-PCBs in three quality control samples (butter, grass and oil fish (n: number of replicates)).

	TEQ PCDD/F	TEQ DL-PCBs	Sum NDL-PCBs		TEQ PCDD/F	TEQ DL-PCBs	Sum NDL-PCBs
Butter (n=6)				Grass (n=7)			
μ	2,728	0,418	1,32	μ	0,236	0,775	5,74
s	0,170	0,012	0,03	s	0,047	0,040	0,32
RSD	6%	3%	2%	RSD	20%	5%	6%
	TEQ PCDD/F	TEQ DL-PCBs	Sum NDL-PCBs				
Oil Fish (n=9)							
μ	3,116	12,733	149,72				
s	0,127	0,652	14,48				
RSD	4%	5%	10%				

Figure 2 shows the control charts in use in our laboratory for PCDD/Fs and DL-PCBs in two control samples (fish oil and butter). Results obtained after automatic preparation of the samples have been added and highlighted in green circles. All the results obtained with the automated preparation are included in the acceptable range of values for the four control charts.

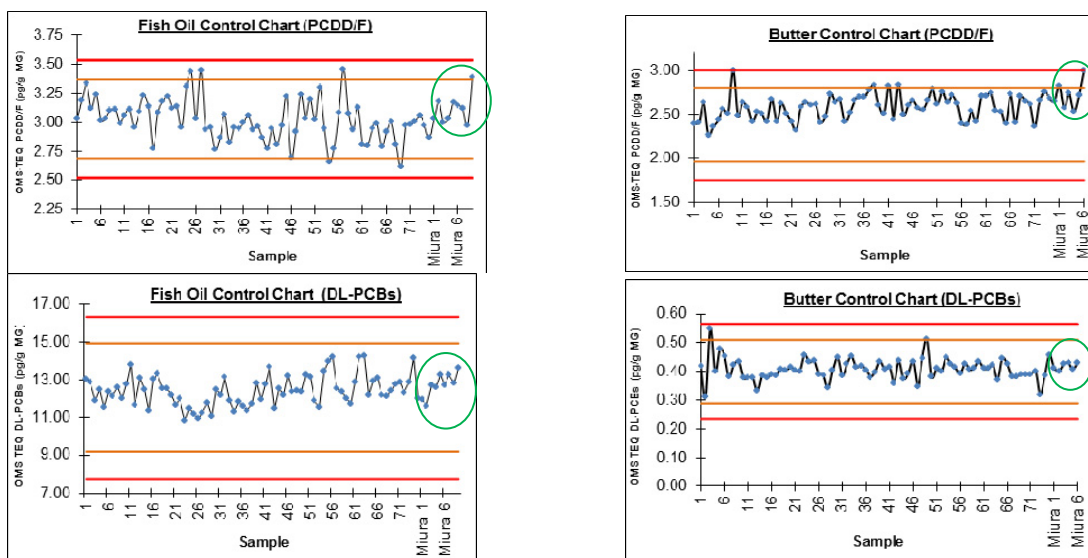


Figure 2: Fish Oil and Butter Control Charts, respectively on the left and on the righthandsides. PCDD/Fs and DL-PCBs concentrations values expressed in TEQ(pg/g fat), respectively on the top and on the bottom.

2.1.3 Comparison between the two sample preparation procedures based on the recovery values

The EU regulation³ specifies that in case of confirmatory methods, all 17 ¹³C-labelled 2,3,7,8-substituted internal PCDD/F standards and all 12 ¹³C-labelled internal DL-PCBs standards shall be added at the beginning of the analytical method in order to validate the analytical procedure. Minimum and maximum recoveries corresponding to the 35 ¹³C-labelled internal standards are presented in Table 3, in the ten samples tested. In some cases, recoveries were over or under estimated. The extreme recovery values were obtained for the highest chlorinated congeners which contribution does not exceed 10% of the total TEQ value. In conclusion no significant difference was observed with the automatic system compared to the manual approach.

[min-max] recovery				[min-max] recovery			
Matrix	Compound	Miura	Laberca	Matrix	Compound	Miura	Laberca
Feeding Stuff	PCDD/F	[84.5-126.4]	[92.6-147.3]	Grass 1	PCDD/F	[33.0-120.1]	[51.2-104.5]
	PCB-dl	[83.7-99.9]	[68.2-105.5]		PCB-dl	[18.3-100.8]	[46.4-125.6]
	PCB ind	[75.0-96.8]	[59.5-95.9]		PCB ind	[44.6-77]	[82.7-98.7]
Milk	PCDD/F	[61.2-127.3]	[61.7-90.2]	Grass 2	PCDD/F	[93.4-162.9]	[96.1-200.0]
	PCB-dl	[66.5-77.8]	[42.8-85.9]		PCB-dl	[92.2-113.4]	[38.6-92.7]
	PCB ind	[65.6-73.6]	[38.2-69.7]		PCB ind	[26.7-108.3]	[30.6-118.7]
Poultry	PCDD/F	[79.2-126.4]	[75.9-161.0]	Grass 3	PCDD/F	[76.4-139.2]	[88.0-153.7]
	PCB-dl	[92.1-105.7]	[85.9-112.5]		PCB-dl	[88.6-101.1]	[40.1-86.0]
	PCB ind	[73.5-102.4]	[90.0-106.1]		PCB ind	[82.9-99.7]	[47.3-96.1]
Egg 1	PCDD/F	[79.1-113.2]	[76.4-118.1]	Sardine 1	PCDD/F	[83.9-157.5]	[33.0-131.9]
	PCB-dl	[86.9-105.8]	[76.8-107.2]		PCB-dl	[91.3-102.2]	[70.8-96.5]
	PCB ind	[86.5-98.0]	[69.2-106.0]		PCB ind	[89.7-95.2]	[82.4-101.3]
Egg 2	PCDD/F	[65.3-112.9]	[88.6-123.0]	Sardine 2	PCDD/F	[71.4-125.7]	[78.6-120.1]
	PCB-dl	[86.6-124.0]	[73.2-106.9]		PCB-dl	[76.3-99.1]	[72.5-101.2]
	PCB ind	[77.1-94.7]	[68.9-99.0]		PCB ind	[71.6-82.5]	[88.0-99.3]

For screening methods

[30-140]

For confirmatory methods

[60-120]

Table 3: Recoveries of the 35 ¹³C-labelled internal standards in ten different samples. Cells in white: recoveries in the range [60-120%]; cells in orange: recoveries not exceeding the range [30-140%]; cells in red: recoveries outside the range [30-140%].

2.2 Selectivity between the two sample preparation procedures

Specificity of the analysis requires differentiation between various congeners of PCDD/Fs and DL-PCBs such as between toxic (e.g. the 17 2,3,7,8-substituted PCDD/Fs, and 12 DL-PCBs) and other congeners, but also

differentiation from a range of other, co-extracted and potentially interfering compounds present at concentrations up to several orders of magnitude higher than those of the analytes of interest. Except in one grass sample (Figure 3), the selectivity was the same for manual and automatic sample preparation. In the grass sample, the chromatogram obtained with the automatic method is significantly better than that obtained with the conventional method whether for internal or native congeners, as shown in Figure 3.

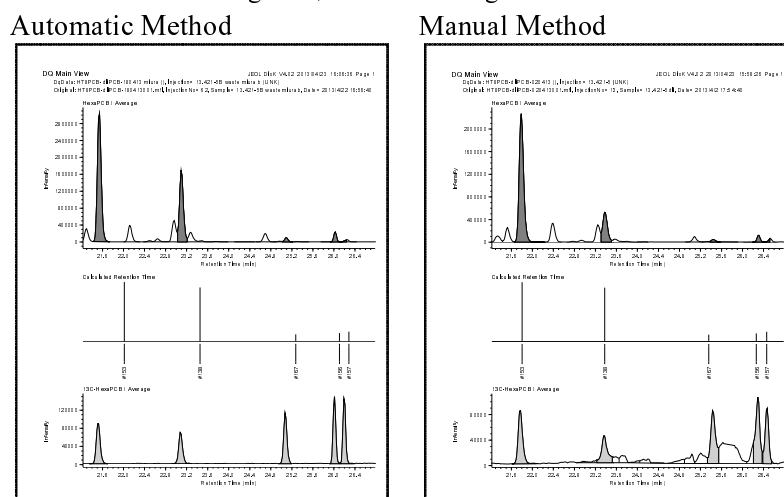


Figure 3: GC-HRMS chromatograms of grass sample

2.3 Other parameters to be considered while comparing the automatic and conventional procedures

Using the SPD-600GC (Miura) device, the application of a crude extract on a column provides a purified solution vial after 3 hours of preparation instead of 5 hours with the conventional method. The amount of solvent used is almost divided by a factor of 10 for a single sample. In addition, the PCDD/Fs and PCBs are non-Ortho recovered in the same bottle, saving thus one injection. In conventional extraction methods, cross-contamination due to the reuse of the glass, human errors caused by manual operations, and other factors have reduced the rate of recovery of molecules and repeatability. By contrast, the use of SPD-600GC eliminates the possibility of cross-contamination as much as possible, because it uses columns and disposable tubes (parts which are in direct contact with the sample).

However, with this device, the amount of fat loadable on the purification column is less than for the conventional method. Moreover, this amount can vary hugely depending on the sample aspect, which could be more or less viscous. Finally, some issues related to automated equipment leaks have been observed.

Volume of solvents	+++
Number of columns	++
Time for purification step	++
Number of injections	+
Glassware	+
Cross contamination	+
Maximum amount of fat	--

Table 4: Comparison results overview (scale from – to +++ in benefit for the automatic system)

3. References

¹Antignac J-P, Marchand P, Gade C, Matayron G, Qannari El M., Le Bizec B, André F. (2006); *Anal Bioanal Chem.* 384: 271–279

²Commission Regulation (EU) No 277/2012 of 28 March 2012 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels and action thresholds for dioxins and polychlorinated biphenyls

³Commission Regulation (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006

⁴Fujita H, Honda K, Hamada N, Yasunaga G, Fujise Y. (2009); Chemosphere 74 : 1069-1078.