A new and highly innovative automatic purification system evaluated for dioxins and PCBs

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1 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. Crisis situations have very high financial and human costs and require upgrading the capacity of laboratories. To limit these crisis cases, high throughput methods are required for PCDD/Fs and PCBs analysis to increase the number of controls and auto controls. This is the reason why some providers have developed automatic equipments for sample preparation to implement monitoring with high frequency. Analytical results have to be reported and interpreted uniformly throughout Europe, following EC regulations (252/2012 and 278/2012)[1-2] laying down analytical criteria for the official control. A year ago, an automatic purification device (MIURA SPD-600GC) was tested and compared with a manual conventional method used at the French National Reference Laboratory. It showed conclusive results but had some limitations particularly related to the amount of fat loadable[3]. This year, a new system has been developed by MIURA. The aim of this study was to compare this new automatic purification system (MIURA GX-300) with the conventional manual method but also with the previous system tested last year.

2 Materials and methods

2.1 Samples

The present study is based on results from many different food and feed samples (compound feed, pork fat, egg, butter, salmon, fish oil, grass, eel…) coming from French monitoring plans and samples from PTs organized by the EURL and the Norwegian Institute of Health. These samples originate from different geographical areas and present a wide variability in terms of composition, *i.e.* fat content, contamination pattern and co-extracted compounds.

2.2 Method

2.2.1 Reference method

The reference method used for the determination of PCDD/Fs and PCBs is validated and accredited according to the ISO 17025 standard and has been described elsewhere[3]. 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) using 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-orthoPCBs 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.

2.2.2 Automatic purification system

The GX-300 is a fully automated purification system for POPs purification, and has been tested for PCDD/Fs and PCBs in various matrices (Miura Institute of Environmental Science, Miura Co. Ltd., Japan)[4] in order to accreditate this method.

Figure 1 shows the schematic diagram of the column flow channel, which is separated in two parts. The top of the system (purification section) is composed of two columns, 10%(w/w) silver nitrate silica gel (17.6mmI.D.x 100mm) and 44%(w/w) sulfuric acid silica gel (17.6mmI.D.x 80mm). The bottom part (concentration section) is also made up of two columns, carbon (6mmI.D.x 34mm) and alumina (6mmI.D.x 34mm).

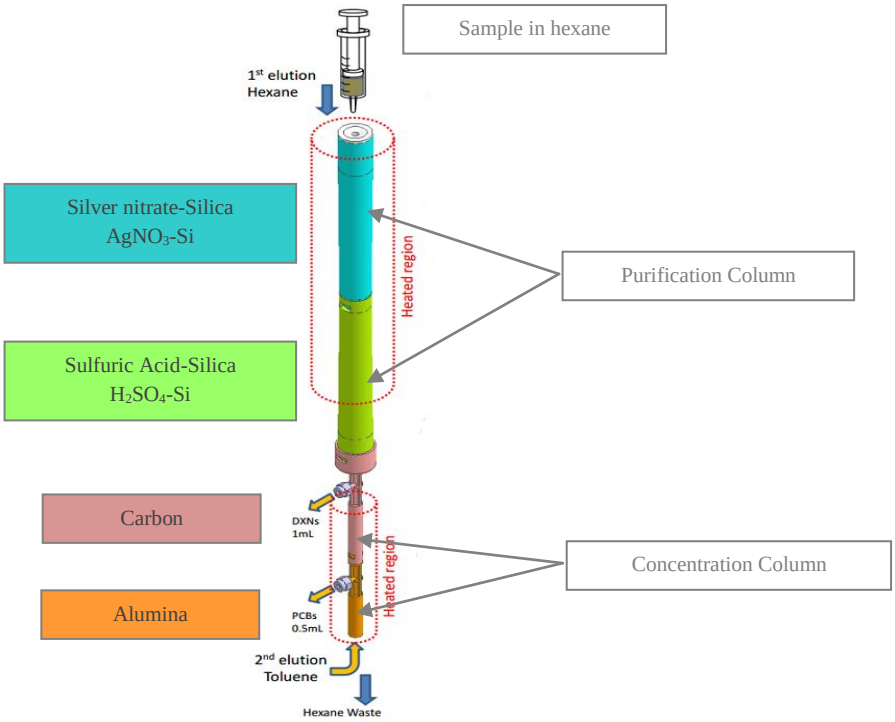

Figure 1: Schematic diagram of the column flow channel.

The fat was dissolved in about 5 mL of n-hexane (see 2.2.1) and was directly applied on the top of the purification column using a disposable syringe. After applying the extract, the column was loaded on the instrument. No conditioning step was needed, except for the samples with a high viscosity (egg, grass…). This preconditioning consisted in packing the purification column with 10 mL of n-hexane before fat loading. Then, the sequence program was launched for less than 2 hours. After heating and keeping the purification column at 60°C, PCDD/Fs and PCBs were eluted using 85 mL of n-hexane. PCBs (except non-ortho PCBs) were trapped on the alumina column heated at 90°C, while PCDD/Fs and non-ortho PCBs were fixed on the carbon column also heated at 90°C. These 2 groups of contaminants were eluted in 2 fractions using respectively 0.5 and 1.0 mL of toluene.

3 Results and discussion

3.1 Results

3.1.1 Comparison between automatized and manual procedures based on TEQ values

More than 15 different matrices (feeding stuff, milk, eggs, salmon, grass…) were analysed using 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 Regulation[2] requirements. Table 1 summarizes the values obtained for food and feed samples using both approaches. Many samples were samples already analyzed in International proficiency tests.

Table 1: Results for Dioxins and PCBs in different samples (Automatic: GX-300 procedure, Manual: LABERCA procedure, Deviation=(Automatic result-Manual result)/Manual result. Table: details for PCDD/F, DL-PCB, PCDD/F-DL-PCB expressed in pg-TEQ/g and the sum of NDL-PCBs (ng/g).

	Matrix	Automatic	Manual	Deviation	Automatic	Manual	Deviation	Automatic	Manual	Deviation	Automatic	Manual	Deviation
		TEQ PCDD/F			TEQ DL-PCBs			TEQ (PCDD/F+DL-PCBs)			NDL-PCBs		
	Pork Compound Feed	0,008	0,014	-41%	0,006	0,011	-48%	0,014	0,025	-44%	0,05	0,13	-59%
	Choline Chloride	0,010	0,009	9%	0,002	0,001	50%	0,011	0,011	-1%	0,01	0,01	14%
	Equine Compound Feed	0,013	0,021	-36%	0,012	0,011	7%	0,025	0,032	-21%	0,08	0,09	-12%
	Grass	0,251	0,273	-8%	0,960	0,884	9%	1,135	1,233	-8%	6,71	6,29	7%
Proficiency Test	Fish Meal	0,481	0,397	21%	0,648	0,600	8%	1,129	0,997	13%	4,25	5,25	-19%
	Mineral Clay	0,582	0,479	21%	0,041	0,023	82%	0,623	0,502	24%	3,27	3,27	0%
	Gum Guar	0,877	0,978	-10%	0,005	0,029	-81%	0,883	1,007	-12%	0,10	0,26	-61%
	Bovine Compound Feed	1,203	1,375	-13%	0,921	0,991	-7%	2,124	2,366	-10%	1,83	2,44	-25%
	Vegetal Oil	0,216	0,224	-3%	1,982	1,895	5%	2,206	2,111	4%	3,00	2,68	12%
	Oil	1,747	1,632	7%	3,841	3,377	14%	5,473	5,124	7%	30,95	33,16	-7%
	ButterOil	2,618	2,785	-6%	2,825	2,380	19%	5,611	4,999	12%	12,41	11,42	9%
	Milk Fat	2,735	2,877	-5%	2,574	2,238	15%	5,450	4,972	10%	60,20	54,91	10%
	Egg	0,449	0,467	-4%	0,334	0,386	-14%	0,782	0,853	-8%	2,95	3,12	-5%
	Eel	0,574	0,683	-16%	8,107	8,362	-3%	8,682	9,045	-4%	256,20	272,87	-6%
	Salmon	0,803	0,701	15%	1,588	1,720	-8%	2,289	2,524	-9%	10,22	11,07	-8%
	Salmon	0,674	0,632	7%	1,372	1,369	0%	2,005	2,043	-2%	9,37	9,41	0%
	Salmon	0,752	0,824	-9%	1,505	1,409	7%	2,257	2,233	1%	10,41	11,33	-8%
	Grass	0,337	0,315	7%	0,739	0,863	-14%	1,076	1,178	-9%	5,03	5,36	-6%

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. Whatever the method used, the uncertainty was drastically higher at lower contamination levels.

3.1.2 Repeatability and reproducibility of the automatized procedure

Five samples were investigated, including a quality control analyzed daily to validate each batch of samples. Table 2 presents the results obtained with the automatic device.

Table 2: Repeatability and reproducibility of TEQ values for PCDD/F, DL-PCB and the sum of NDL-PCBs in five samples (Butter, Bovine Compound Feed, Pork Fat, Salmon and Grass)

Matrix	Quantity of fat (g)	TEQ PCDD/F	TEQ PCB DL	TEQ (PCDD/F+PCB DL)	Sum PCB ind
Butter (n=4)	1,00	2,908	0,357	3,265	1,25
	2,00	2,830	0,392	3,221	1,32
	3,00	2,787	0,374	3,161	1,18
	4,00	2,744	0,369	3,113	1,17
	RSD	2%	4%	2%	6%
Bovine Compound Feed (n=8)	0,21	1,200	0,877	2,077	1,85
	0,25	1,176	0,916	2,092	1,84
	0,26	1,209	0,979	2,188	1,84
	0,22	1,221	0,913	2,135	1,80
	0,21	1,226	0,902	2,128	1,80
	0,22	1,186	0,889	2,075	1,85
	0,24	1,194	0,949	2,142	1,79
	0,22	1,210	0,945	2,155	1,85
	RSD	1%	4%	2%	1%

Matrix	Quantity of fat (g)	TEQ PCDD/F	TEQ PCB DL	TEQ (PCDD/F+PCB DL)	Sum PCB ind
Pork Fat (n=3)	2,00	0,037	0,008	0,045	0,25
	3,00	0,032	0,012	0,044	0,26
	4,00	0,030	0,011	0,042	0,23
	RSD	10%	18%	4%	4%
Salmon (n=5)	0,25	0,776	1,605	2,381	10,09
	0,15	0,747	1,470	2,217	9,97
	0,30	0,766	1,458	2,224	10,73
	0,46	0,744	1,499	2,243	10,35
	0,58	0,725	1,493	2,217	10,89
	RSD	3%	4%	3%	4%
Grass (n=4)	0,04	0,339	0,698	1,037	5,04
	0,10	0,320	0,747	1,067	4,95
	0,17	0,334	0,752	1,087	5,01
	0,25	0,353	0,761	1,114	5,11
	RSD	4%	4%	3%	1%

Relative standard deviations (RSD) were below 10 % for most samples and analytes except for the pork fat with a RSD value of 18 % observed for DL-PCBs TEQ, which can be explained by the very low TEQ value (more than 10 times below MRL). It was noticed that no differences were observed from 1 to 4g of fat of the same sample. The capacity to load up to 4g of fat without any loss was highly appreciated compared to the previous system.

Figure 2 shows the control charts in use for PCDD/Fs and DL-PCBs in butter sample. Results obtained after automatic preparation of the samples have been added and highlighted in green circles. All the results obtained with the automatized device are included in the acceptable range of values for the four control charts.

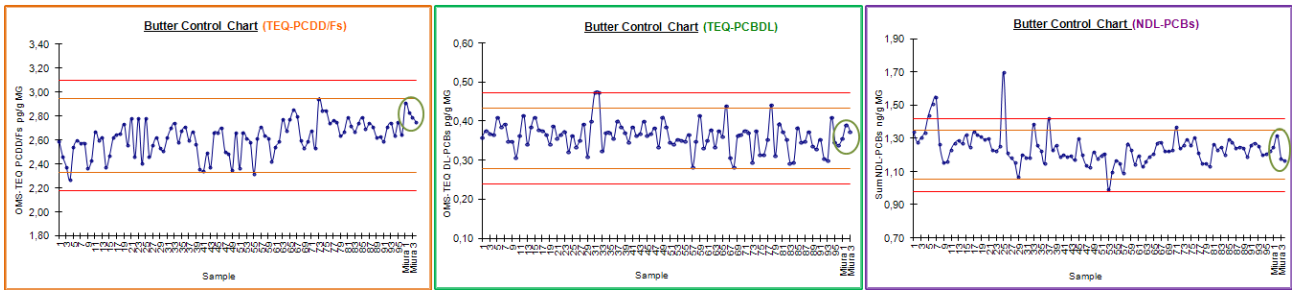

Figure 2: PCDD/F, DL-PCBs concentrations values expressed in TEQ (pg/g fat) and NDL-PCBs concentrations values expressed in ng/g fat of butter, respectively on the left, in the middle and on the right hand side.

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

The EU regulation[3] 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. For screening methods, the recoveries corresponding to the 35 13C-labelled internal standards must be between 30 and 140 %. For confirmatory methods, the range is reduced to 60-120 %.

A majority of recoveries were between 60 and 120 %. However, problems of recoveries were encountered at the beginning of this study for PCB153 with values between 30 and 110 %. The problem seemed to be related to packaging on some of the prototype alumina column. With a new packaging of the latter, the recoveries were between 60 and 120 %.

3.2 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.

With the manual method, the separation of mono and di-ortho PCBs from non-ortho PCBs for salmon was not completely achieved. Some mono and di-ortho PCBs could be found in non-ortho PCBs fraction and especially at the retention time of PCB126. With the automatic method, the separation of congeners according to their planarity was better and avoided such co-elution.

In many grass meals, chromatograms obtained with the automatic method were significantly better than those obtained with the conventional method whether for internal or native congeners, as shown in Figure 4.

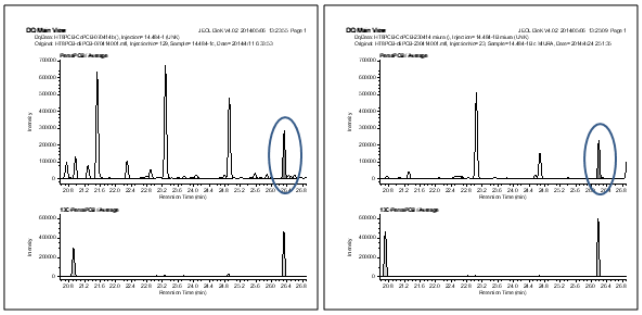

Figure 3: Chromatograms of salmon sample

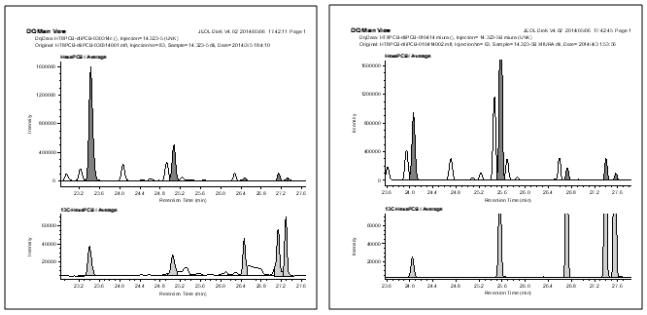

Figure 4: Chromatograms of grass sample

3.3 Other considerations to compare automatic and conventional procedures

Using the GX-300 device, the application of a crude extract on a column provides a purified solution vial after 2 hours of purification instead of 5 hours with the conventional method.

In addition, PCDD/Fs and non-ortho PCBs are recovered in the same vial, saving time for the injection step. Due to very low volume of elution, laboratories equipped with a PTV system could inject directly, avoiding the final evaporation step. In conventional extraction methods, cross-contamination due to the reuse of the glass, human errors caused by manual operations, and other factors reduces the rate of recovery of molecules and repeatability. By contrast, the use of AutoSyM GX-300 eliminates the possibility of cross-contamination as much as possible, because it uses disposable columns and tubes.

One important parameter is the volume of solvent which is almost divided by a factor of 10 for a single sample. Carbon footprint is becoming increasingly important in the technological choice of laboratories. According these elements this system could be considered as a **"green purification system".**

4 References

1. Commission Regulation (EU) No 278/2012 of 28 March 2012 amending regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls

2. Commission 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

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