EASY ACCESS MULTI-CARTRIDGE EXTRACTION AND CLEAN-UP ASSEMBLY FOR SPECIFIC ISOLATION OF DIOXINS IN BIOLOGICAL FLUIDS

Jean-François Focant and Edwin De Pauw

[1]Mass Spectrometry Laboratory, University of Liège, B6c Sart-Tilman, B-4000 Liège, Belgium. e-mail: JF.Focant@ulg.ac.be

Introduction

Over last 30 years, many efforts have been dedicated to the development of robust methods for the analysis of dioxins and related compounds. As scientific knowledge concerning potential toxicity of these molecules was growing up, regulations appeared to protect general population against unacceptable exposures. This goal is probably at least partly achieved since few crisis have been identified and traced back to their sources lately in different countries [1,2,3]. It is nevertheless not too dared to postulate that if, given a number of samples selected for analysis, we can point out a given number of crisis, we also may miss few crisis by limiting the number of samples we analyze; the 'no-monitoring-no-problem' law is unfortunately quite efficient. Everybody then agree to enlarge monitoring program to the largest possible number of samples. This is obviously not attainable without a significant reduction of the cost per sample from prices that currently prevail. Therefore, few option exist, among them: 1) Validate alternative cheaper physico-chemical approach such as quadrupole ion storage mass spectrometry (QISTMS) based methods [4,5,6] to replace high resolution mass spectrometry, 2) Simplify labor intensive 'old fashion' extraction and clean-up steps [7] or 3) Implement biological screening approach [8].

As it is well known that not less than 70 % of the actual analysis cost is due to the sample preparation, we investigated a drastically simplified extraction and clean-up procedure for the specific isolation of PCDDs and PCDFs. This procedure is based on solid phase extraction (SPE) using octadecyl bonded sorbents (C_{18}) for the extraction step, followed by an on-cartridge acidic digestion of the lipid fraction [9] and further purification using a combination of sulfonic acid and carbon cartridges.

Materials and methods

Samples

Cow's milk samples (full fat grade) were used as method development matrix. Portions of 50 ml were used for this study. Milk fat globules membranes are disrupted by potassium oxalate and acetonitrile that is added to the milk (1:1) as well as water (1:1) [10]. Around 150 ml of treated sample is processed through the multi-cartridge set. Spray dried reference materials (RM-534 and RM-533) were treated identically after reconstitution in warm (50 °C). An 'in-house' quality control pool of bovine serum samples was also used to evaluate the robustness of the method [11].

Multi-cartridge setup

All cartridges were disposable. Octadecyl bonded (non-endcapped) cartridges were Isolute Flash 25g/150ml or Isolute C_{18} 10g/70ml (average particule size of 50µm). The drying step was either carried out using a manifold or by flushing nitrogen (30 p.s.i.) through the C_{18} cartridge. Sulfonic acid based Isolute SCX-3 cartridges were 5g/25ml (average particule size of 50 µm). Compounds eluting from C_{18}

to SCX-3 were dried using Isolute sodium sulfate drying cartridges (2.5g Na_2SO_4/reversible tube). All selected cartridges were from International Sorbent Technology (IST,

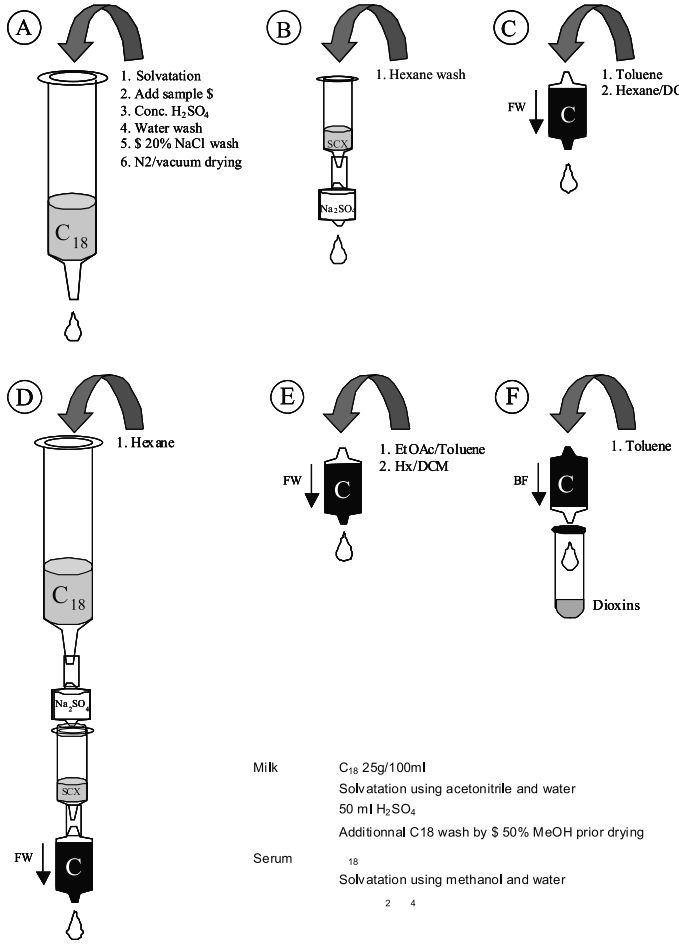

Figure 1. Sequence of events of the multi-cartridges clean-up method Hengoed, UK). Disposable silica dispersed carbon columns were obtained from Fluid Management Systems (Waltham, MA, USA).

Analysis

GC/HRMS analysis (isotopic dilution method) were performed using Autospec Ultima high-resolution mass spectrometer (Micromass, Manchester, UK) operating at a resolution of 10.000 in the selected ion monitoring mode (SIM) and an Agilent (Palo Alto, CA, USA) 6890 Series gas chromatograph equipped with a RTX-5 (40 m x 0.18 mm x 0.18 µm) capillary column (Restek, Interscience, Louvain-la-Neuve, Belgium).

Results and Discussion

This simple set of cartridges was tested against milk and serum. After optimization of sorbent

Table 1. Recovery rates for the multi-cartridges method.

	Cow's milk		Serum	
	% recoveries	% RSD	% recoveries	% RSD
2,3,7,8-TCDD	68,0	12,3	71,3	15,6
1,2,3,7,8-PeCDD	49,5	9,8	78,0	13,4
1,2,3,4,7,8-HxCDD	30,5	10,1	62,5	8,3
1,2,3,6,7,8-HxCDD	29,7	11,5	67,0	9,4
1,2,3,7,8,9-HxCDD	27,3	12,4	68,8	10,9
1,2,3,4,6,7,8 HpCDD	60,0	20,9	78,8	7,5
OCDD	28,5	15,3	76,5	9,2
2,3,7,8-TCDF	67,6	14,0	62,3	12,7
1,2,3,7,8-PeCDF	45,7	13,7	63,0	13,9
2,3,4,7,8-PeCDF	43,8	14,6	66,0	13,9
1,2,3,4,7,8-HxCDF	31,2	11,4	53,0	14,4
1,2,3,6,7,8-HxCDF	29,3	10,6	55,8	14,2
1,2,3,7,8,9 HxCDF	25,7	14,2	57,5	14,8
2,3,4,6,7,8-HxCDF	25,7	10,5	58,3	14,3
1,2,3,4,6,7,8 HpCDF	65,6	14,0	59,5	10,8
OCDF	29	15,0	70,0	10,5
PCB-77	7,5	20,2	13,5	22,2
PCB-81	6,3	24	10,0	16,3
PCB-126	9,8	16,7	28,5	29,6
PCB-169	4,7	15,3	56,3	8,9

Table 2. Accuracy of the multi-cartridge clean-up for the reference material RM-533 and RM-534.

	RM-533			RM-534		
	Assigned pg/g	Measured pg/g	Trueness %	Assigned pg/g	Measured pg/g	Trueness %
2,3,7,8-TCDD	0,42	0,41	98	0,65	0,63	97
1,2,3,7,8-PeCDD	0,86	1,02	118	1,44	1,56	109
1,2,3,4,7,8-HxCDD	0,56	0,47	85	0,8	0,77	96
1,2,3,6,7,8-HxCDD	1,27	1,33	105	1,92	2,32	121
1,2,3,7,8,9-HxCDD	0,47	0,54	115	0,8	0,79	98
2,3,7,8-TCDF	0,39	-	-	0,65	0,35	53
1,2,3,7,8-PeCDF	0,1	-	-	0,15	-	-
2,3,4,7,8-PeCDF	1,96	1,97	100	3,16	3,16	100
1,2,3,4,7,8-HxCDF	1,06	1,04	98	1,75	1,76	101
1,2,3,6,7,8-HxCDF	1,03	1,15	111	1,55	1,69	109
2,3,4,6,7,8-HxCDF	1,22	1,16	95	1,9	1,83	96
Total	9,34	8,85	95	14,77	14,36	97

quantities and volumes of solvent, good recovery rates were obtained reproductively (Table 1). It appeared that both PCDDs and PCDFs were quite selectively isolated from other related compounds such as PCBs and PBDEs but the elution also further shown to exclude most of the coplanar PCBs. Such behavior is not without interest in the area of biological screening that uses immuno or bio-assays presenting cross-reactivities to these compounds. As authorities plan to use these assays as screening tools in the context of regulations based on PCDD/Fs only, some attention has to be dedicated to development of specific sample preparation. Accuracy over milk reference material and serum QC samples also appeared to be quite well controlled, as illustrated in Table 2 and Fig.2. Extract cleanness was subject to some variation due to the physiology of the cartridges. Classical cartridges are in fact characterized by a quite large diameter and a thin bed of sorbent. Longer tubes and thus thicker beds

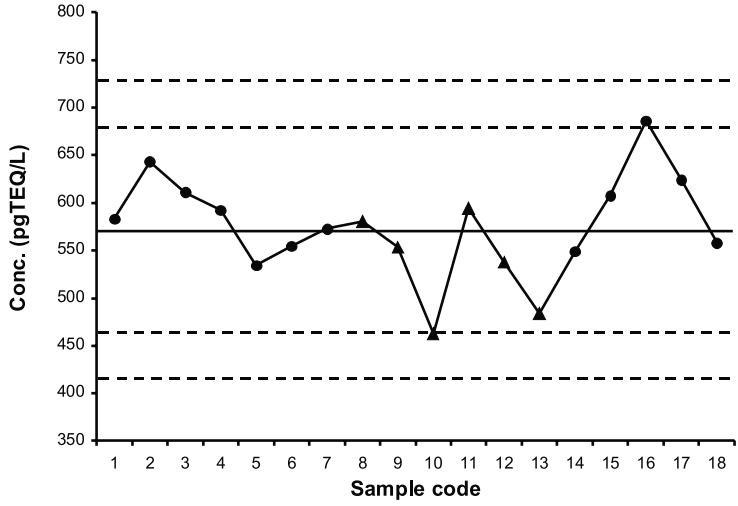

Figure 2. QC chart for PCDD/F levels in serum samples (? validated routine method, ? multi-cartridges method).

should help to increase time of residence inside the bed and overcome such problem. In addition, the present optimization was carried out on high volumes of samples (50 ml milk, 25 ml serum). In case scenario of sensitive bio-assay analysis, reduction of sample volume will deeply improve the extract quality. Furthermore, moving from syringe-barrel format to 48-well SPE plates would open the door to robotic applications.

Acknowledgement

This study has been financially supported by the "Fonds pour la Formation à la Recherche dans l'Industrie et l'Agriculture" (F.R.I.A.).

References

1. Malisch, R., *Organohalogen Compd.*, 1998, 38, 65-70.

2. van Larebeke, N., Hens, L., Schepens, P., Covaci, A., Bayens, J., Everaert, K., Bernheim, J.L., Vlietinck, R., De Poorter, G., *Environ. Health. Pespect.*, 2001, 109, 265-272.

3. Llerena, J.J., Abad, E., Caixach, J., Rivera, J., *Organohalogen Compd.*, 2002, 51, 283-286.

4. Hayward, D.G., Hooper, K., Andrzejewski, D., *Anal. Chem.*, 1999, 71, 212-220.

5. Eppe, G., Focant, J.-F., Pirard, C., De Pauw, E., *Organohalogen Compd.*, 2001, 50, 186-189.

6. Focant, J.-F., De Pauw, E., *Organohalogen Compd.*, 2001, 50, 276-279.

7. Lapeza, C.R., Patterson Jr., D.G. and Liddle J.A., *Anal. Chem.*, 1986, 58, 713.

8. Hoogenboom, R., Portier, L., Onstenk, C., Polman, T., Hamers, A., Traag, W., *Organohalogen Compd.*, 2000, 45, 180-183.

9. Janàk, K., Jensen, E., Becher, G., ., *J. Chromatogr. B.*, 1999, 734, 219-227.

10. Williams, S. ed. 1984 method 16.064 and 20.012, 14th Ed., AOAC.

11. Focant, J.-F., De Pauw, E., *J. Chromatogr. B.*, 2002, submitted for publication.