VERY FAST AUTOMATED EXTRACTION AND CLEAN-UP OF MILK SAMPLES FOR PCDD/FS AND cPCBs ANALYSIS.

Jean-François Focant and Edwin De Pauw

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

Introduction

Among the group of persistent organic pollutants (POPs), polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are of major concern since they represent a potential human risk via the environment and the food consumption. The lipophilic character of these toxicants allows them to bio-accumulate in the food chain up to human for which consumption of fatty food became the most important way of exposure with milk and dairy products a major concern. Their contribution to the daily intake of TCDD-equivalents had been previously estimated to be close to 30% [1].

Extraction and clean-up of organohalogen compounds present in food-stuffs have both been strongly investigated last 30 years by many research groups. Most of them using procedures such as Soxhlet, liquid-liquid and solid phase for extraction and chromatography columns using such sorbents as polymer beads for gel permeation, alumina, silica gel, florisil® and active carbon for clean-up [2]. Most of the methods using these procedures are labour intensive, need repetitive manipulations and generally require skilled personnel which is exposed to important quantity of organic solvents. Automation processes developments have been investigated in order to overcome these problems and to increase the sample throughput of the analytical laboratory [3,4]. With a reduction of the clean-up procedure time by a factor of a half, sequentially operating systems were upgraded to parallel configuration, becoming a productive analytical tool for large number sample studies [5].

Solid phase extraction (SPE) on octadecyl bonded sorbents (C_{18}) is on of the most promising technique for extraction of liquid samples and is used in several laboratories instead of more conventional techniques [6]. Following that trend, the automated Power-Prep™ system (Fluid Management Systems, Waltham, MA, USA) has recently been modified to be able to accommodate the SPE step. This results in a direct load of the prepared liquid sample on the system following by its automated extraction and clean-up.

This paper will describe the automated extraction and clean-up of different types of milk samples using disposable C_{18}, silica, alumina and carbon columns with the Power-Prep™ system for high throughput analysis of PCDD/Fs and cPCBs.

Materials and methods

Samples

Cow's milk samples were full fat grade (3.6 % fat) purchased in local supermarkets, breast milk samples were collected on a volunteer basis in Wallonia (Belgium) [7,8]. Portions of 15 to 100 ml were used for this study. Sample pre-treatment was carried out based on a modified version of the

AOAC method [9]. 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). Between 50 and 300 ml of treated sample is processed through the system. Spray dried reference (RM 534) and certified (CRM 607) materials [10] were treated identically after reconstitution in warm (50 °C) water in order to produce the equivalent of a full fat cow's milk.

Extraction and Clean-up

Automated extraction and clean-up were performed on the new generation of the Power-Prep™ system using classical sets of columns [11]. Disposable C_{18} columns were pre-packed by FMS with 25 g of adsorbent. Comparative manual SPE were carried out on 25 g Flash™ cartridges from International Sorbent Technology (IST, Hengoed, UK) in conjunction with a manifold.

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 (30m x 0.25mm x 0.25µm) capillary column (Restek, Interscience, Louvain-la-neuve, Belgium).

Results and Discussion

The plumbing diagram of the new system is illustrated in Fig.1.

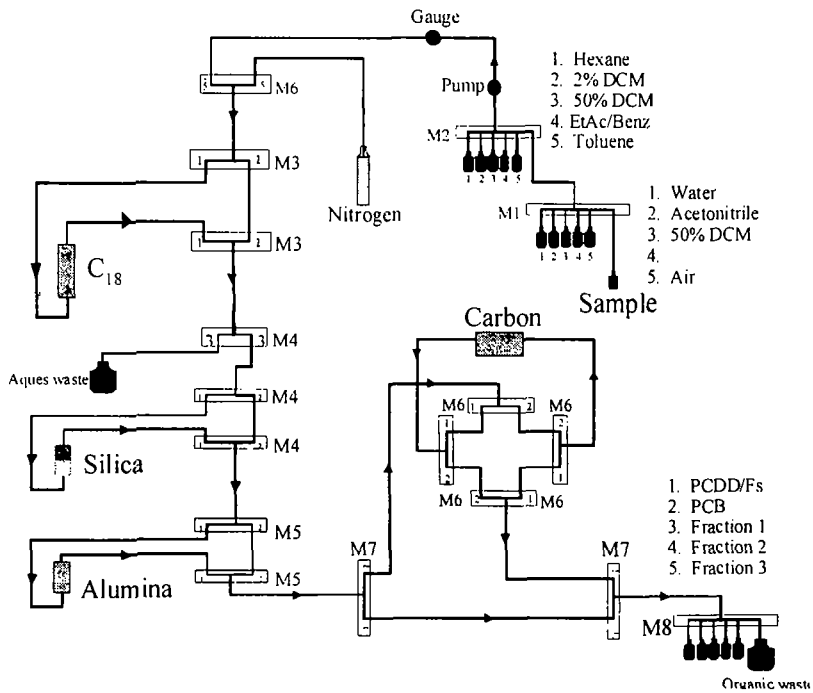

Fig. 1 : Modified plumbing diagram for the integrated extraction on the Power-Prep™ system.

Since aqueous solutions and samples are present in the system, additional valves have been added on the previous configuration of the system [12]. Acetonitrile as well as water are used to condition the C_{18} and nitrogen is delivered at a pressure of 30 PSI for 20 min. to ensure water removal prior the hexane elution of the C_{18} to the multi-layer silica column.

During this study, we observed that traces of remaining water could have a negative effect on the efficiency of the alumina column. This problem was overcome either by adding a small disposable sodium sulfate column (2.5 g) before the silica column or by simply suppressing the alumina step which is not essential due to the low amount of interfering analytes present in milk. In these conditions, we can go up to 100 ml of milk and still produce extracts presenting very good level of cleanness. If necessary, bigger size of silica columns are available [13].

The comparison of classical recoveries obtained on triplicates analysis for cow's milk samples using manual and automated systems are illustrated in Fig.2. These are very good for most of congeners and the lower rates observed for hepta and octa congeners are due to some C_{18} stronger secondary interactions and do not increase with the automated system. All recovery rates are in complete agreement with quantification requirements. Slightly lower recoveries (10-20% less) were sometimes observed for breast milk samples depending of the sample amount and the homogeneity of the fluid. Levels study are presented elsewhere [7,8]. Since the lipid fraction is not isolated, "lipid percent" determination can rapidly be carried out on the side on few ml of sample.

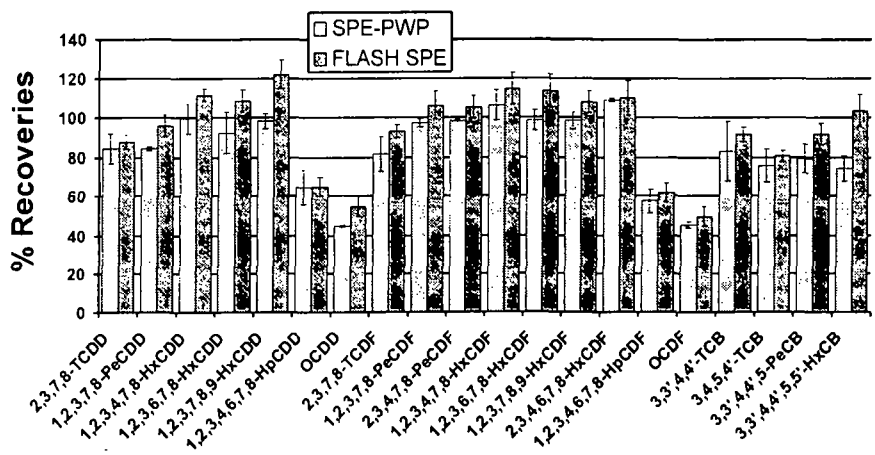

Fig. 2 : Recoveries for real sample on the fully automated system.

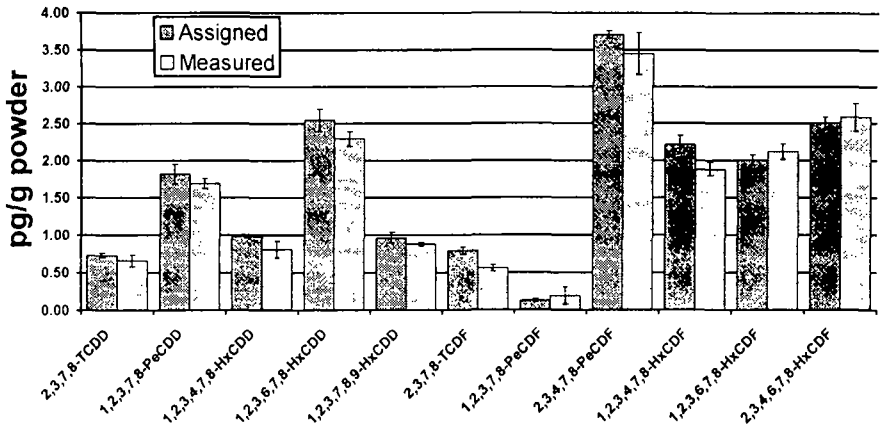

Fig. 3 : Accuracy for the RM 534 (4 g) congeners using the automated system

Produced extracts are free of matrix interferences. The reproducibility and repeatability have been evaluated on reference materials with good results as illustrated in Fig.3. The RSD for measured values are comprised between 2 and 13 % (average 6 %). The justness to the total theoretical value (18.4 ±0.28 pg/g powder) is 92 %. Blank levels on the system are negligible. Results obtained on certified reference materials also give good results as shown in Table 1.

Table 1: Manual *vs* automated results for the certified reference material CRM 607.

CRM 607	Flash-SPE					SPE-PwP				
	Conc. pg/g	SD	RSD %	Trueness %	Mod WHO pg I-TEQ/g	Conc. pg/g	SD	RSD %	Trueness %	Mod WHO pg I-TEQ/g
2,3,7,8-TCDD	0,27	0,03	13	108	0,27	0,34	0,02	6	134	0,34
1,2,3,7,8-PeCDD	0,94	0,02	2	119	0,94	1,03	0,04	4	130	1,03
1,2,3,4,7,8-HxCDD	0.47	0,07	15	113	0,05	0,40	0,04	10	95	0,04
1,2,3,6,7,8-HxCDD	0,96	0,19	19	98	0,10	0,98	0,15	15	100	0,10
1,2,3,7,8,9-HxCDD	0.33	0.01	3	98	0,03	0.37	0,22	58	110	0,04
2,3,4,7,8-PeCDF	1,98	0,02	1	109	0,99	2,23	0,12	6	123	1.11
1,2,3,4,7,8-HxCDF	0,86	0.13	15	91	0,09	0,81	0,15	19	86	0,08
1,2,3,6,7,8-HxCDF	1,14	0,09	8	113	0,11	1,10	0,07	6	109	0,11
2,3,4,6,7,8-HxCDF	1,18	0,02	2	110	0,12	1,18	0,09	8	110	0,12
Total	**8,14**	**0,42**	**5**	**105**	**2,70**	**8,18**	**0,07**	**1**	**106**	**2,94**

Conclusions

This new extraction and clean-up system represents a powerful and robust tool for large number samples studies. It has proven to be analytically stable and suitable to deal with real (cow and breast) milk samples. The amount of sample can easily be increased in case of lower background levels using bigger size of C_{18} columns. The global time of samples preparation (up to 20 samples in parallel) between sampling and injection to GC/MS has been drastically reduced to less than 4 hours. The method can be extended to PCBs and persistent pesticides without significant changes.

Acknowledgement

The authors thank Hamid Shirkhan and the technical staff of FMS for collaboration. This 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 Fürst, P., Fürst, C. and Groebel, W., *Chemosphere*, **1990**, 20 (7-9), 787.

2 Erickson, M.D. **1992** In: Analytical Chemistry of PCBs, Butterworth Publishers, Boston, pp. 87-168.

3 Tindle, R.C. and Stalling, D.L., *Anal. Chem.*, **1972**, 44 (11), 1768.

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

5 Burse, V.W., Patterson Jr., D.G., Brock, J.W. and Needham, L.L. *Toxicol. Ind. Health*, **1996**, 12 (3-4), 481-498.

6 DiPietro, E.S., Lapeza Jr., C.R., Cash, T.P., Turner, W., Green, V.E., Gill, J.B. and Patterson Jr., D.G., *Organohalogen Compounds*, **1997**, 31, 26.

7 Focant, J.-F., Pirard, C., André, J.-E., Massart, A.-C. and De Pauw, E., *Organohalogen Compounds*, **2001**.

8 Focant, J.-F., Pirard, C., André, J.-E., Thielen, C., Eppe, G. and De Pauw, E., *Organohalogen Compounds*, **2001**.

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

10 BCR European Commission reference materials.

11 Focant, J.-F., Eppe, G., Pirard, C. and De Pauw, E., submitted to J. Chromatogr.. A., **2001**.

12 Turner, W.E., Cash, T.P.C., DiPietro, E.S. and Patterson Jr., D.G., *Organohalogen Compounds*, **1998**, 35, 21.

13 Focant, J.-F., Eppe, G. and De Pauw, E., *Organohalogen Compounds*, **2000**, 45, 49.