USE OF MICRO SCALE SOLID PHASE EXTRACTION AND AUTOMATED CLEAN UP IN POPS ANALYSIS OF MILK AND SERUM

Juma R, Addink R[1*]

[1]Toxic Report, 580 Pleasant Street, 2nd Floor, Watertown MA, USA

Introduction

Analysis of breast milk for Persistent Organic Compounds (POPs) has been ongoing since the 1970s when polychlorinated dibenzo-p-dioxins and furans (PCDD/F) were first detected in it. Later polychlorinated biphenyls (PCBs) and more recently brominated flame retardants (PBDEs) were also found in milk.[1] Laboratory analysis of human serum for Persistent Organic Compounds (POPs) has also become increasingly important.

The concept of biomonitoring in which levels of toxic chemicals are assessed in humans has received much attention in the last decade. One example is the NHANES studies carried out by the Centers for Disease Control (CDC). Automation of the sample prep process can result in faster turnaround time of samples, lower costs, and improved quality of the data generated. As the amount of sample used in bio-monitoring with Solid Phase Extraction is typically lower than in, e.g., water analysis, a micro system was used.

Materials and methods

PCBs/PBDEs in serum: 2 g bovine serum was added to 2g water and treated with 4mL formic acid. Then 100 uL methanol, 100 uL HCl (pH~2) and ^{13}C labeled standards were added and mixed. 4 g water was added extra to prepare about 12 g of sample. The sample was loaded on an HLB-500 cartridge (conditioned with dichloromethane, methanol, and water) with positive pressure and dried (N_2). The cartridge was then eluted with 12 mLs dichloromethane, followed by volume reduction to 3 mLs, and solvent-exchange with hexane. The sample was then cleaned up over a 0.2 g acidified silica column using the same system. Silica was eluted with 20 mLs of hexane, followed by volume reduction to 1 mL in a 6 position evaporator, then to a final volume of 10 uL in a 24 position vial evaporator.

PCDD/Fs in serum: 20g bovine serum was mixed with 20g water and treated with 40 mL formic acid. ^{13}C labeled standards were added and mixed. 40 g water was added extra to make about 120 g sample. The sample was loaded on a C-18 cartridge (conditioned with dichloromethane, methanol, and water) with positive pressure and dried (N_2). The cartridge was then eluted with 20 mLs dichloromethane, followed by volume reduction to 5 mLs, and solvent-exchange with hexane. The sample was then cleaned up in an automated column chromatography system using acid-base-neutral, alumina and carbon columns. Volume reduction was achieved as describe above.

PCBs/PBDEs in milk: 1g of cow milk (as received, not freeze dried) was spiked with ^{13}C labeled standards and absorbed into a 1g Hydromatrix™ cartridge. It was dried with N_2 (positive pressure, no conditioning). The cartridge was then eluted with 12mLs dichloromethane. Subsequent steps were identical to treatment of the serum samples.

PCDD/Fs in milk: 5g of cow milk and ^{13}C labeled standards were absorbed into 5 g of Hydromatrix™ and dried with N_2. It was eluted with 20 mLs dichloromethane followed by volume reduction to 5 mLs and exchange with hexane. Subsequent steps were identical to treatment of the PCDD/Fs in serum samples.

Analysis was carried out with a Thermo Trace Ultra GC coupled with a DFS HRMS; 15 m (PBDEs) and 30 m (PCBs and PCDD/Fs) columns were used.

Results and discussion:
Average recoveries for ^{13}C PCDD/Fs, PCBs and PBDEs are given below:

	Milk	**Serum**
PCBs		
tri-	70%	81%
tetra-	83%	77%
penta-	96%	84%
hexa-	94%	86%
hepta-	97%	68%
octa-	89%	50%
nona-	88%	na
deca-	88%	na
PBDEs		
tri-	81%	85%
tetra-	84%	90%
penta-	85%	81%
hexa-	92%	75%
hepta-	74%	88%
octa-	53%	109%
nona-	na	95%
PCDD/F		
tetra-	na	93%
penta-	na	80%
hexa-	na	91%
hepta-	na	88%
octa-	na	74%

Table 1: Average recoveries of various POPs for milk and serum samples.

Solid Phase Extraction of milk and serum using a micro system has numerous advantages. The amounts of solvent involved are low and the small scale limits background contamination. For PCBs and PBDEs the extraction is followed by one step column clean up using the same system. Thus the extraction and subsequent chromatography step do not require additional equipment. For PCDD/Fs the automated column chromatography system used provides reliable and fast clean up of the samples preparing them for high resolution GC/MS analysis.

As can be seen in the Table very good recoveries were obtained for each of the three compound classes studied. Samples analyzed gave detection limits at the low picogram per gram level. The solid phase extraction of PCBs and PBDEs which is followed by sample clean up using the same system is very straightforward and can easily be done in one day. For the PCDD/Fs the SPE step is followed by automated column chromatography using an acid-base-neutral column, an alumina column and a carbon column. New reduced solvent use by the automated clean up system which has reduced total consumption to less than 300 mLs can further decrease cost and labor involved in manual column chromatography.[2] Although more time consuming than the PCB/PBDE sample prep, this entire process can also be done in one day. Further method development and validation using human breast milk and human serum are forthcoming.

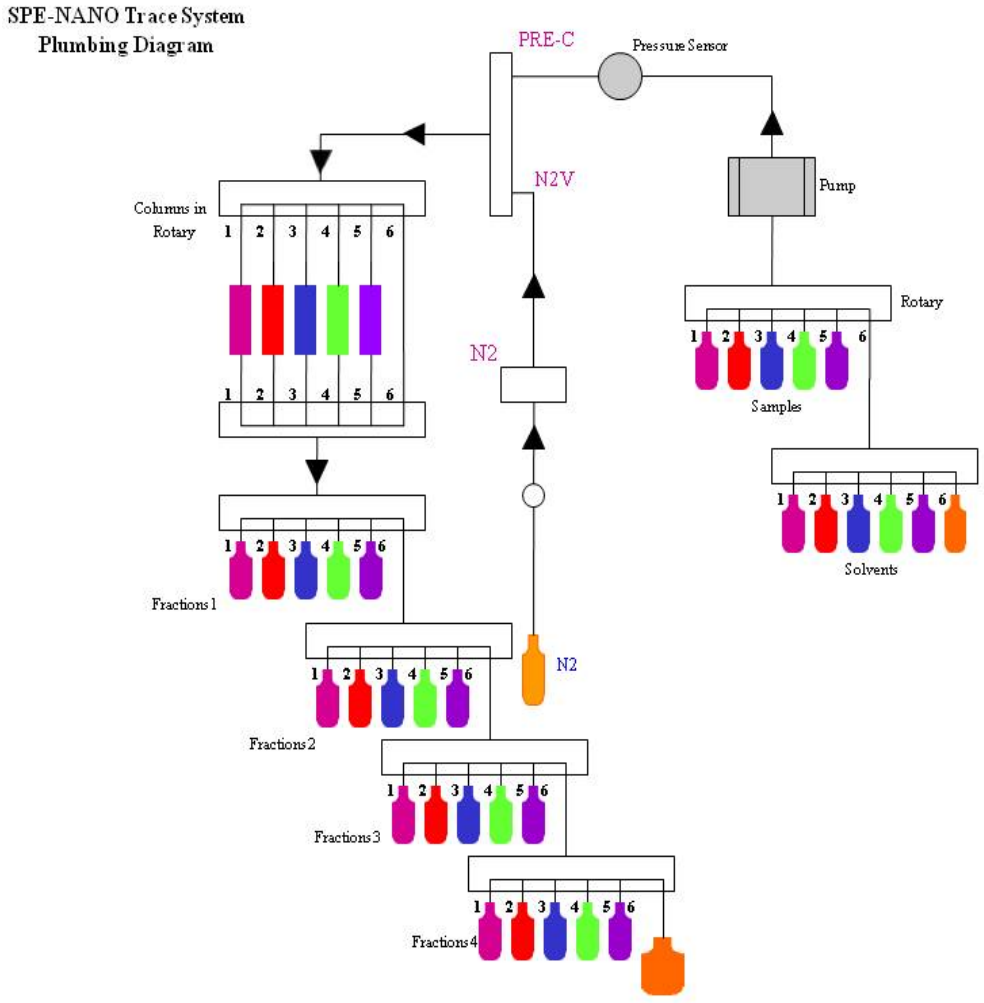

Figure 1 Schematic of Solid Phase Extraction System for milk and serum.

References:

1. Konishi, Y, Kuwabara, K, Hori, S, (2001) *Arch. Environ. Contam. Toxicol.*, 40 (4), 571-578.
2. Juma, R, Addink, R, Dioxin 2015 Contribution.