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Hyphenated technique : 5. Sample preparation techniques

Application area : B. Clinical/toxicological
C. Environmental
D. Food analysis/agriculture

AUTOMATED SAMPLE PREPARATION USING A SPE-LC COUPLING FOR DIOXIN ANALYSIS IN BIOLOGICAL FLUIDS

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Introduction

Sample preparation for polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and non-*ortho* (coplanar, 'dioxin-like') polychlorinated biphenyls (cPCBs) is probably the trickiest part of these samples analysis [1]. Extraction and clean-up, depending on matrix type and required amount of sample to be processed, often consist in tedious and time consuming steps. In the particular case of biological fluids such as milk and serum, liquid-liquid extraction (LLE) has extensively been used as well as newer techniques such as pressurised liquid extraction (PLE) and supercritical fluid extraction (SFE), which have been presented as suitable tools in that area [2]. On the side of these methods, solid phase extraction (SPE) using C_{18}-bonded-silica disposable cartridges appeared as an outsider in the category. SPE actually fulfilled valuable criteria such as no significant sample pre-extraction treatment required, low risk of samples cross contamination's, partial removal of interfering lipids (lipid contents are analytically measured on the side), ease of automation,…

For clean-up of extracted samples, liquid chromatography (LC) using such sorbents as polymer beads for gel permeation, alumina, silica gel, Florisil and active carbon have been proven to be well suited to the purification of dioxins and related compounds [3]. Due to the repetitive manipulations and the labor character of these purification steps, several research group have been working on automation processes development in order to increase sample throughput of their laboratory [4]. In order to increase the sample throughput of the global analysis, an automated clean-up system (FMS, Inc., USA) has recently been modified to be able to accommodate the SPE step. This results in a direct parallel load of prepared liquid samples (cows' milk, breast milk, human serum,…) on the system followed by its on-line automated extraction and clean-up.

Results and discussion

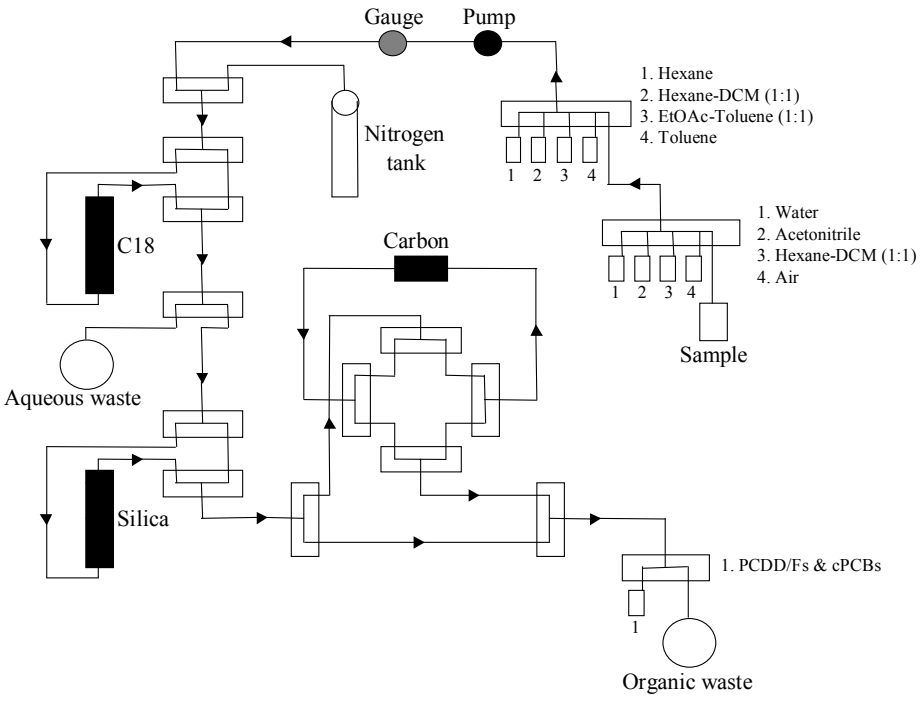

Figure 1. Plumbing diagram of the automated sample extraction and clean-up system used for the preparation of biological fluids dedicated to PCDDs, PCDFs and cPCBs analysis.

As depicted in figure 1, sets of PC-controlled electromagnetic valves allows solvents to reach the right place at the right time. Details on this modified system are available elsewhere [5]. In the present study, we incorporated additional valves necessary for sample load and aqueous solvents mobility through the C_{18} column. After complete load of the liquid sample, C_{18} is washed with water and dried under nitrogen pressure (30 PSI, 20 min). Once all remaining traces of aqueous solvents have been eliminated, organic media LC can take place and hexane is used to elute C_{18} on the multi-layer silica column responsible for remaining lipids decomposition. Analytes present on the silica columns are released by applying a hexane solution and planar species are trapped on the carbon column. Final cleaning step occurs by applying hexane-DCM and then ethyl acetate-toluene mixture on the carbon column. PCDD/Fs and cPCBs are then extracted from carbon layers by toluene provided in the back-flush direction. This fraction is further evaporated and analysed by GC/MS [6].

Repeatability, trueness regarding available certified reference materials, reproducibility over time and personal, robustness against matrix types have been investigated for this integrated SPE method versus Soxhlet and manual SPE in order to evaluate this strategy. Figure 2 illustrates typical results that can be obtained for either cows' milk, breast milk or human serum samples.

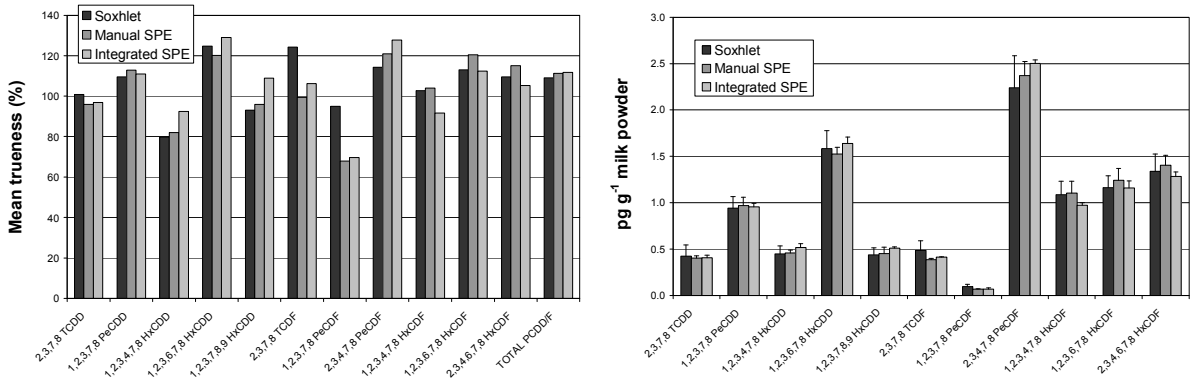

Figure 2. Comparison of results obtained for milk reference material (RM-533) using Soxhlet, manual SPE and integrated SPE.

Recovery rates for the integrated SPE were included in a 80-120 % interval for all congeners excepted for hepta and octa-chlorinated congeners (for both PCDD and PCDF families) which were around 55-60 % (classically 70 % for Soxhlet). This is related to C_{18} properties since it was also observed for manual SPE. In addition, due the low toxicity factors (TEFs) of hepta and octa chlorinated congeners (0.01 and 0.0001, respectively), it does not affect the 2,3,7,8-TCDD toxic equivalent (TEQs) calculation. Blank levels for SPE (manual and integrated) were always lower than the ones for Soxhlet due to the smaller amount of solvents used and disposable character of SPE cartridges and columns.

Finally, the new method has proven to be analytically stable and suitable to deal with real (cows' milk, breast milk and human serum) 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.

Such integrated technique allows reduction of time length and cost of this type of routine analyses, making possible the extension of large-scale field and/or epidemiological studies to additional samples and permitting faster response in case of incident.

References

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