ONE STEP CLOSER TO A 'COOK BOOK' METHOD FOR DIOXIN ANALYSIS PART 1: THE PROCEDURE

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Abstract

An integrated sample preparation and analysis method for the measurement of dioxin in various matrices is presented. This first paper describes the global procedure and a second paper will illustrate performances on real biological samples. The development of such a fast 'cook book' procedure has large interest in the food processing industry where testing has to be performed as quickly as possible to avoid down time in production lines. The level of automation and coupling is such that it is now possible to imagine performing the dioxin screening on site in a regular industrial laboratory that does not have specific 'dioxin skills'.

Introduction

Preparing either human or food samples for dioxin and PCB measurement is a task that is challenging many research centres and routine laboratories. Accurate measurement of dioxins and related compounds requires high standard analytical strategies, time, extensive know-how, and money. One of the major reason is that PCDDs, PCDFs and PCBs are found at levels as low as pico- or femtogram per gram of matrix, depending on the investigated biological samples. Additionally, matrix-related interferences are present in concentrations at orders of magnitude higher than the analytes of interest. For those reasons, a complex multi-step approach is required to 1) extract the analytes from the matrix core, 2) separate undesirable interferences and, 3) finally isolate, separate and quantify analytes of interest. Those complex multistep strategies include sample extraction, sample cleanup, sample fractionation, several steps of solvent reduction, and, finally, analyte measurements by GC-MS under strict quality assurance/quality control (QA/QC) criteria. Accredited laboratories often require a week or more for reporting, due to the tedious manual multi-step procedures that are mandatory for this ultra-trace analysis (ppt, ppq). In terms of cost per sample and sample throughput, it is not only the final measurement of the analyte concentration, but – maybe even more importantly - the complex sample preparation procedure, which makes this measurement possible. In the past few years, efforts focused on the development of alternative procedures to speed up and simplify the process while maintaining a high level of QA/QC.

Several non-instrumental and instrumental automated approaches are available for both extraction and cleanup[1]. Soxhlet extraction and liquid-liquid extraction have long been the most used tools for non-instrumental extraction of solids and liquids, respectively. They have proven to be very efficient but some limitations encouraged the development of other approaches based on instrumental techniques. Depending on the physico-chemical properties of sample matrices, instrumental techniques are based on solid phase extraction (SPE), matrix solid phase dispersion (MSPD), pressurized liquid extraction (PLE), microwave assisted extraction (MAE), and supercritical fluid extraction (SFE). For the following clean-up step, preparative liquid chromatography (LC) using silica-based sorbents and size exclusion chromatography (SEC) are the most common techniques to remove most of matrix-related interferences. Additionally, because of peak capacity issues in GC and MS fragmentation similarities of target analytes, a fractionation step is required prior measurement. This is performed using additional LC sorbents like Florisil, basic alumina, porous graphitic carbon (PGC) and 2-(1pyrenyl)ethyl (PYE). Those sorbents allow the

separation of the cleaned extract in subgroups of compounds (PCDDs, PCDFs, PCBs) depending on their polarity and geometry. The fractions can then be analyzed separately by GC-MS.

Integrated approach
In order to move towards simplification of the entire sample preparation procedure, coupling and hyphenation of the various analytical steps is required. In that context, an integrated strategy has recently been proposed. It rests on the use of PLE coupled to an automated solvent reduction-exchange device that produce sample extracts that can automatically be further cleaned-up via a multi-step LC setup. The LC setup includes a multi-layer silica column (acid, neutral, basic), a basic alumina column, and a column containing carbon dispersed on celite. The fractionated extracts are further evaporated using the hyphenated solvent reduction-exchange device to satisfy to the required concentration factor and, then, transferred to the GC injection device for GC-MS measurements.

Highlight of the global procedure
1. Sampling
The first analytical step is the representative sampling of the material to be analyzed. This step is followed by homogenisation and sub-sampling, according to the matrix type. The internal standard is added at that level to ensure proper traceability of recovery rates by GC-MS quantification. For high water content samples, a pre-drying step might be performed by oven drying or freeze drying.

2. Extraction
The solid or semi-solid sample is placed in a stainless steel extraction cartridge that is capped on both ends with disposable 'push-in' Teflon caps equipped with metal frits to preserve the system from particulate clogging. Additional drying agent can be placed inside the cartridge to ensure proper final drying prior extract collection. The extraction cartridge holder can accommodate various cell sizes (5-100 ml). Packed cells are manually placed on the cell holder and secured using rapid closure device that ensure a leak free connection. PLE cartridges are pressurized by the high pressure pump to pressures as high as 3500 psi. The electrical heating of the cartridges is ensured by a surrounding heater block. Cooling of the cartridges after extraction is performed using 2 cooling fans. The cartridges are then flushed with fresh solvent and purged with nitrogen to ensure complete transfer of the analytes to the collection tube. Static and continuous extraction can be carried out. Depending on matrix types, various solvents and solvent mixtures can be used. A schematic of the PLE setup is illustrated in Figure 1. All extraction data (temperature, pressure, …) are computer controlled via a specific real time software and all events are recorded for traceability. Plots of the parameters are further available for automatic documentation and data reporting.

3. First solvent volume reduction and exchange
Depending of the PLE cartridge volume (related to the sample size), and the number of extraction cycles (1 to 3), extraction solvent volumes can be up to 300 ml. This has to be reduced to around 50 ml to ensure compatibility with the following LC clean-up step that is performed in hexane. If the extraction solvent is hexane, it is only a matter of reducing the solvent volume from 300 ml to 50 ml. This is automatically performed by collecting the extracted mixture in evaporation tubes that are placed in a temperature-controlled metal heat block and flushed with a gentle nitrogen stream to speed-up the evaporation (Figure 1). The solvent reduction-exchange device automatically stops the concentration cycle when the required volume is reached. If the extraction solvent is not hexane, it can easily be exchanged by evaporating the extraction solvent to dryness (using keeper or not, depending on the extraction residue) and reconstitute it by the desired volume of hexane prior the transfer of the extract to the clean-up columns for purification.
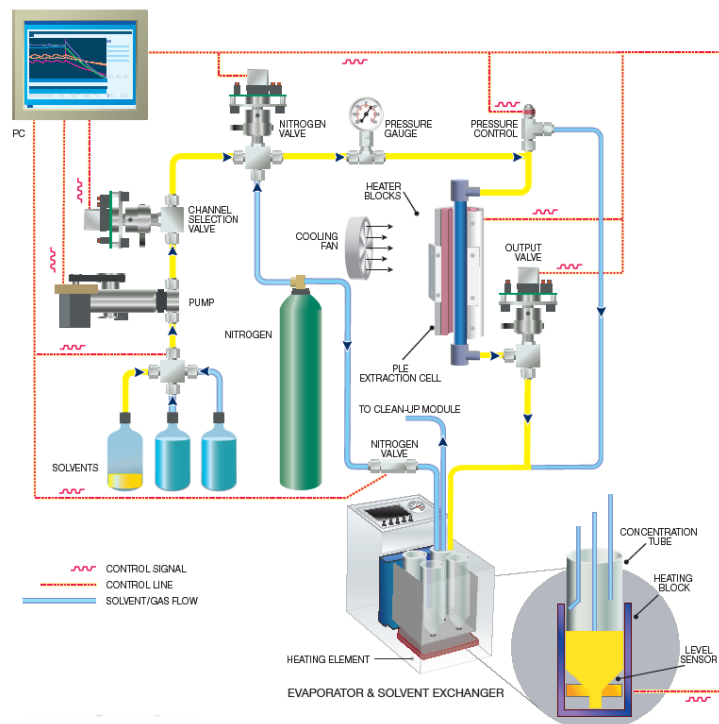
Figure 1. PLE schematic illustrating the purge of the extraction cell and collection-evaporation of the extract.

4. Clean-up and fractionation

The schematic of the automated multi-column LC clean-up system is illustrated in Figure 2.

A control module pilots valve drive modules connected to the pump and pressure modules responsible for the solvent flow in the valve module. Easy programming and software editing allows the creation of custom made sequences of events that drive the required solvent at the right place at the right moment. The clean-up part takes place at low pressure (5-30 psi) and operates with an independent pump from the PLE system. The classical clean-up for PCDD/F and PCB run uses disposable multi-layer silica columns (4 g acid, 2 g base and 1.5 g neutral), basic alumina (8 g) and PX-21 (2 g) carbon columns. These columns are packed in disposable Teflon tubes individually sealed in Mylar packaging and manufactured by FMS. All columns are conditioned by the required volumes of solvents during the extraction step. As illustrated in Figure 3, the hexane fraction is loaded on the silica column (previously conditioned with 100 ml of hexane at 10 ml/min) at 5 ml/min. After a flush of 100 ml of hexane at 10 ml/min through alumina to the waste (F1), PCDD/Fs and PCBs are eluted from alumina to carbon using 100 ml of hexane-dichloromethane (1:1) at a flow rate of 10 ml/min. The planar species (PCDD/Fs and NO-PCBs) are fixed on the carbon column, although the non planar species (other PCBs) are collected in F2. Some hexane is added to the carbon column for additional clean-up of the planar fraction (F3) and the F4 fraction is collected by back flushing the carbon column with 80 ml of toluene at 5 ml/min to elute the PCDD/Fs and NO-PCBs that are collected in evaporation tubes. At the end of the process, the system is automatically decontaminated via a special solvent program.
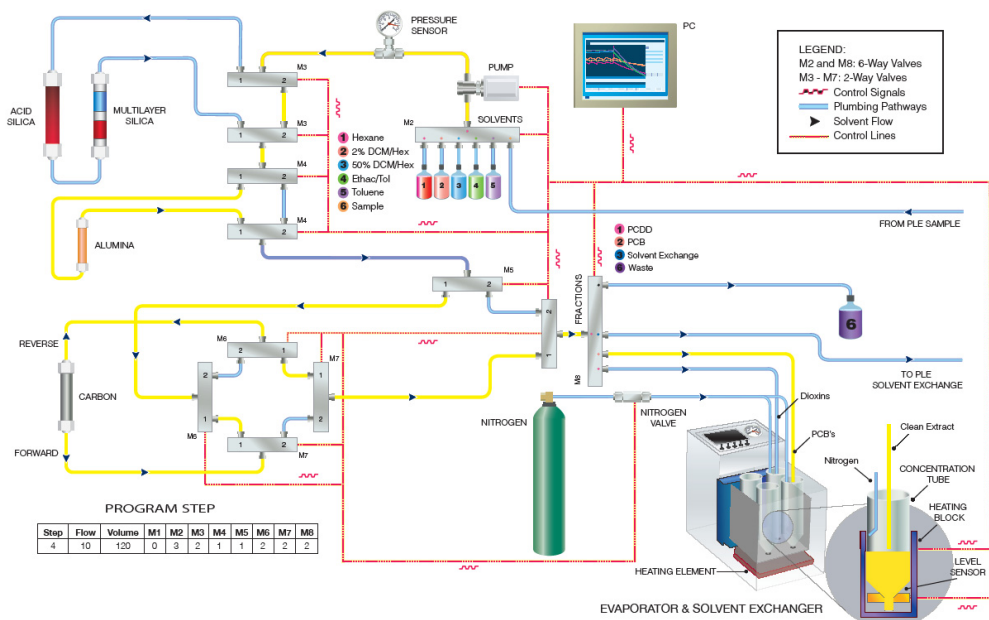
Figure 2. Clean-up schematic illustrating the elution of the alumina with hexane/DCM 1:1 and collection-evaporation of the MO-PCB fraction
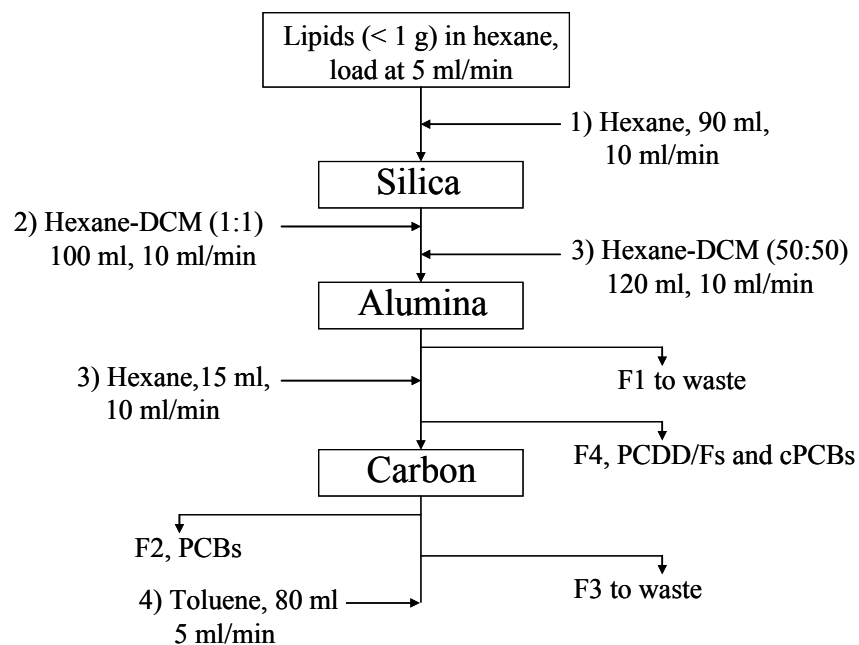

Figure 3. Flow chart for the automated clean-up system (low lipid scheme).

5. Second solvent volume reduction and exchange

As soon as the first fractions start to come off the clean up columns, the second concentration cycle starts. Both the hexane-dichloromethane (1:1) and the toluene fractions are concentrated to approximately 150 µl, using the solvent reduction-exchange device. The bottom of those evaporation tubes is made of a screwed GC vial that can easily be detached at the end of the evaporation to be placed in the GC auto-sampler after addition of the injection standard and keeper. Depending on the fraction and GC injection device used, direct GC-MS injection can be carried out or a few more time is dedicated to further evaporate the fractions to ensure compliance with established LOQs. For example, large volume injection devices can easily handle volumes of 50 µl with good robustness. Figure 4 illustrates the complete integrated system.

Figure 4. Integrated extraction, evaporation, and clean-up system (2 sample unit).

Sample preparation for PLE:	0.5 h
Parallel PLE (2 cycles) :	1.5 h
First solvent reduction:	0.5 h
Parallel Clean-up and fractionation:	2.0 h
Second solvent reduction:	1.5 h
Std addition and transfer to GC:	0.1 h
GC-PTVLVI-GC-IDHRMS:	1.0 h
QA/QC check and reporting:	0.5 h
TOTAL:	7.1 h

Figure 5. Time frame for the entire procedure.

Conclusion

The integrated sample preparation system is modular and expandable from one to six sample configurations. Batches of samples (n=6) can then be processed in parallel so that the sample throughput is significantly improved. The goal is to reach a situation where a 'same-day testing' can be reached for large series of samples. The system is capable of utilizing a wide range of extraction cell sizes, clean up columns, and multiple solvent selection valves. It is well suited for new method development and for experimenting with different sample sizes, solvents, clean up packing materials, extraction pressures and temperatures. Each channel operates independently of other channels, if one channel malfunctions the rest will still work. The large bore plumbing of the extraction module makes it virtually clog free. The exposed construction makes parts replacement extremely easy. On a practical point of view, a set of 5 unknown samples plus 1 QC sample can be processed in a working day (Figure 5). Such a 'cook book' procedure will most probably contribute to simplify the complex dioxin testing and help non-expert laboratories to perform their own testing.

References
1. Focant J.-F., Pirard C., and De Pauw E. *Talanta* 2004; 63:1101.