

# AUTOMATED PROCEDURES IN ISO17025 ROUTINE DIOXIN LABORATORY: IMPACT ON THE THROUGHPUT

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## Introduction

Looking back some 10 years ago, most of dioxin analyses required a least a few days to be completed. The major reason for this was the tremendous efforts to be provided to ensure proper quality of extracts prior to GC-MS measurement. Those efforts often translated in multi-steps procedures, including Soxhlet, liquid-liquid extraction (LLE), solid-phase extraction (SPE), sequential accelerated solvent extraction, (ASE), multiple column preparative liquid and size exclusion chromatography, ... most of them being carried out manually and repeatedly. Recent improvements in terms of viability of parallel automated systems contributed to speed up analytical processes. The ease of use, the robustness, and the modularity of most systems make their use successful in laboratories of various sizes and types, no matter what matrices are. The possibility of performing rapid analyses is especially important in food-feed control under European guidelines (e.g. Commission Directive 2006/13/EC, Commission Regulation (EC) No 152/2009, Commission Regulation (EC) No 199/2006).

This paper reports on the implementation of a highly automated procedure for the measurement of PCDD/Fs and DL-PCBs in food, feed, and feed ingredients under strict QA/QC requirements under ISO17025 regulation.

## Materials and Methods

### Test Materials

All food, feed, ingredients, ... under EU regulation.

### Target compounds

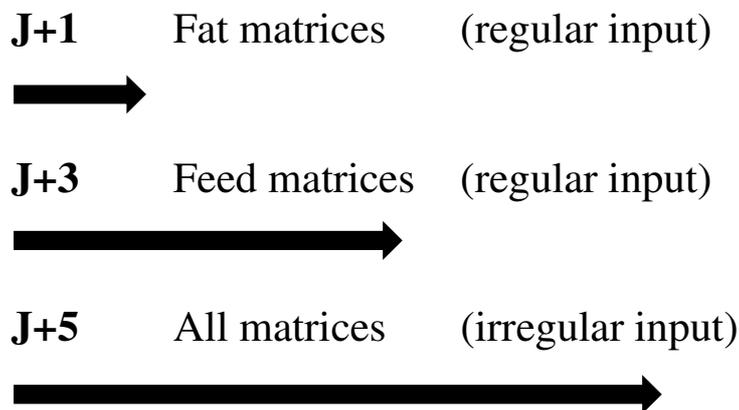
The seventeen 2,3,7,8 toxic PCDD/Fs and the twelve dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189).

### Analytical procedures

Extractions are either carried out by the sequential extractor ASE<sup>®</sup> 350 from Dionex (Sunnyvale, CA, USA) or the parallel extractor PLE-6 from Fluid Management Systems (Waltham, MA, USA). All samples are spiked with a mixture of labeled PCDD/Fs, DL-PCBs, and NDL-PCBs thoroughly mixed and placed in the extraction cell (30-100 mL). After extraction, extracts are filtered on sodium sulphate and the solvent are exchanged to hexane using a rotary evaporator. Manual parallel disposable glass columns are used to pre-clean the extracts. The extract is further cleaned and fractionated with an automated Power-Prep system (Fluid Management Systems). The NO-PCB and PCDD/F fraction is injected into a gas chromatography – isotope dilution high resolution mass spectrometer (GC-IDHRMS, Autospec Ultima, Waters, UK). The MO-PCB fraction is on a MAT95 XL GC-IDHRMS (ThermoFinniganMAT, Bremen, Germany). All details regarding the analytical procedure for clean-up, fractionation, and GC-HRMS can be found elsewhere<sup>1-4</sup>. All solvent and consumable issued from lots used by our laboratory for routine analyses according to a BELAC accredited method.

## Results and Discussion

The flow of samples in the laboratory is attached to one of the three procedures listed in Figure 1.



**Figure 1:** Matrix-dependent sample workflow

### J+1 strategy

It is developed for animal fats and vegetable oils that do not require extraction prior clean-up. Depending on the origin and the regulatory level, samples sizes range between 2g (fish oils) and 7g (pork fat). The time between sample reception and reporting to the customer cannot exceed 31 hours. In practice, a sample must be received at the lab office before 9 AM on day 1. It is inspected, encoded, and internally labeled. By 9.30 AM, series of samples are aliquoted to the right amount, diluted in hexane (50 mL), and spiked with the  $^{13}\text{C}$  internal standard mixture. A quick fat removal step is carried out using manual parallel disposable glass columns. The 200 mL of hexane are reduced to 20 mL using rotary evaporators as some samples might have turbidity and opacity levels that could perturb the use of optical sensors such as the ones used in automated evaporation devices. Those devices can be used if the evaporation is timed rather than optically sensed, especially with final volumes such as 20 mL.

Automated parallel clean-up and fractionation start at 12 PM and take 75 minutes for 6 samples to be processed. An additional 15 minutes is required to decontaminate the system and re-start the next series of samples. The related programs are shown in Figure 2. Both Fraction 1 (PCDD/Fs and NO-PCBs, 80 mL of toluene) and fraction 2 (MO-PCBs and NDL-PCBs, 100 mL of hexane/dichloromethane 50:50) are collected in evaporation tubes to which GC injection vials are screwed. Those tubes are paced inside the evaporation device during collection so that the evaporation process is started before the end of the elution (Figure 3). The final volume contained in the GC injection vial is approximately 300  $\mu\text{L}$ . GC-MS injections can start by 4 PM the first day and continue overnight. The time before 4 PM that day was used by the GC-MS operator to perform all required QA/QC testing of the system. On day 2, GC-MS data are revised and electronic reports are created once all QA/QC checks are verified and approved. Reports are sent out before 4 PM on the second day. The technical lab staff works in 2 schedules shifted by 3 hours (6 AM – 2 PM and 9 AM – 5 PM), that ensures 11 hours of work on a single day.

### J+3 strategy

It is developed for animal feed and ingredients that do require extraction prior clean-up. Depending on the origin and the regulatory level, samples sizes range between 10g and 30g (dry weight). The time between sample reception and reporting to the customer cannot exceed 79 hours. In practice, a sample must be received at the lab office before

**PCDD/F, PCB clean-up and fractionation**

001	10	15	01222116	00000000	
002	10	12	01212116	00000000	fill tubing
003	10	20	01221226	00000000	wet alumina column
004	10	100	01122006	00000000	wet carbon column w
005	10	12	05222226	00000000	condition silica
006	10	40	05221226	00000000	change to toluene
007	10	12	03222226	00000000	pre elute with tolu
008	10	20	03221226	00000000	change to 50%
009	10	12	01222226	00000000	pre-elute with 50%
010	10	30	01221226	00000000	change to hexane
011	5	50	06112126	00000000	pre-elute with hexa
012	10	90	01112116	00000000	Add sample
013	10	12	03222126	00000000	Elute silica with h
014	10	100	03211222	00000000	change to 50%
015	10	12	01222226	00000000	Elute with 50%; load
016	10	10	01221226	00000000	change to hexane
017	5	80	05221111	00000000	flush with hexane
018	1	.5	00000000	00000000	elute PCDD/PCDF

**PCDD/F, PCB decontamination**

001	10	20	06222006	00000000	wash inlet 50/50
002	10	20	06222006	00000000	wash inlet hexane
003	10	30	03111221	00000000	wash column 50/50
004	10	30	03111222	00000000	wash column 50/50
005	10	20	03222006	00000000	wash bypass 50/50
006	10	20	01222006	00000000	wash bypass hexane
007	10	30	01111116	00000000	wash column hexane
008	1	.5	00000000	00000000	turn off valves

Figure 2: Automated clean-up simplified programs



Figure 3: Automated clean-up simplified programs

3 PM on day 1. It is inspected, encoded, and internally labeled. By 3.30 PM, series of samples are aliquoted to the right amount, grinded, and dried overnight at 60°C. The next morning, 10-30g of samples are spiked with the <sup>13</sup>C internal standard mixture, eventually mixed with some sorbent, and packed in extraction cells extracted either

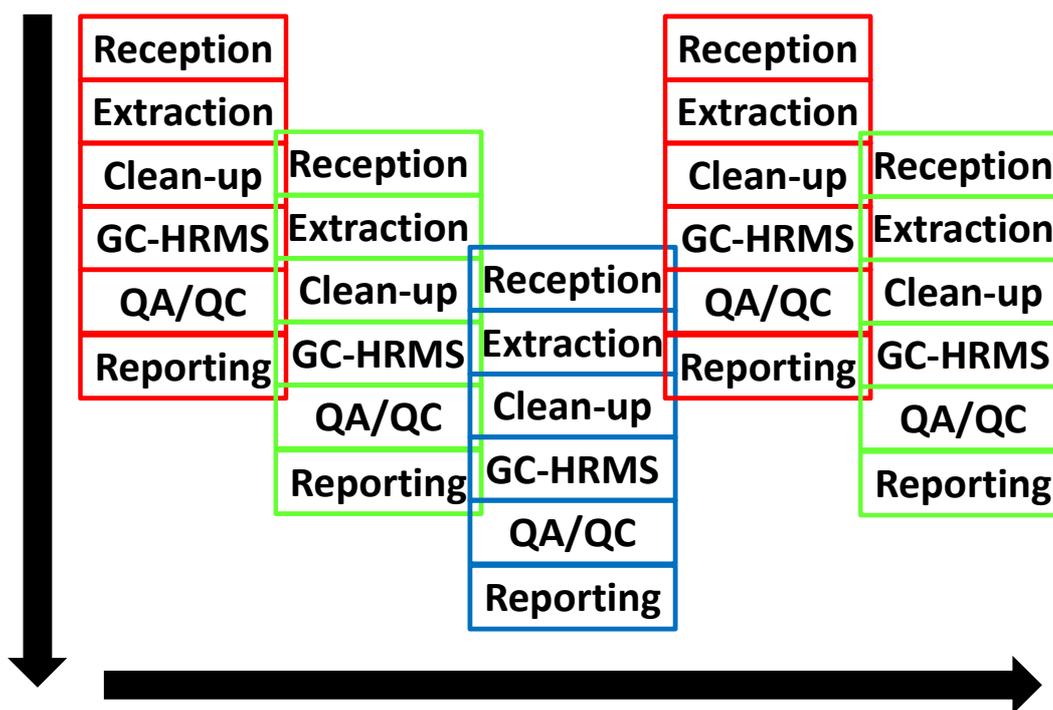
sequentially (ASE) or in parallel (PLE). Those extractions start by 11 AM on day 2. By 1 PM, extracts are filtered on sodium sulphate. Because of the use of many different mixtures of extraction solvents, extracts are solvent exchanged prior to be cleaned-up. This occurs using rotary evaporators. By 5PM, batches of 6 extracts can be ready in hexane (20 mL) for the next step. From day 3, the procedure reach the J+1, with or without the manual parallel disposable glass column step, depending on matrices.

#### J+5 strategy

All samples received in the lab without prior notice are considered in J+5. They fall in one of the two previous categories, but need to be incorporated in planned series.

#### Operational staff and instruments

The lab runs 2000 samples per year. The staff is made of technicians (2.5 FTE), secretary (0.5 FTE), engineer (1.5 FTE) (450 samples/pers/year). One PLE-6, one ASE-300 and two Power-Prep (P-5) are used for sample preparation. Three rotary evaporators, three Turbovap II, one Power-vap 6, and one Rapid-vap are used for solvent reduction and exchange. Two GC-HRMS instruments are used, one for PCDD/Fs and NO-PCBs, one for MO-PCBs and NDL-PCBs. A key aspect is the maximization of the turnover by working on several stages in parallel, as illustrated in Figure 4. This obviously requires dedicated and efficient lab staff.



**Figure 4:** imbrication of sample series to maximize turnover

#### **References**

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