INCREASING THE DIOXINS LABORATORY THROUGHPUT WITH USING DIFFERENT DRAYING AGENTS AS ALTERNATIVE OR FREEZE DRYING

Pietron W., Maszewski S., Kosmala M.
Department of Radiobiology, National Veterinary Research Institute in Pulawy, Poland

Introduction

Regulatory limits and health guidelines required from analytical methods of dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) detection at the sub-picogram levels. Classical analysis is very laborious multi-stage sample preparation methods prior to instrumental detection with high resolution gas chromatography – high resolution mass spectrometry (HRGC-HRMS). Because the PCDD/Fs and PCBs have highly lipophilic properties the efficient extraction of fat is extremely important according current legislation (EU 1259/2011)[1] enforce expressing the result in pg/g of fat for food of animal origin. Extraction and sample preparation time are important factor for all laboratories but most of food-related matrices need freeze drying. This way of drying is not laborious but 52 h time consuming step which may be critical during dioxin crisis. In an effort to omission this step the additional drying agents is needed such as: sodium sulphate, diatomaceous earth or polymeric gel absorbents. Freeze drying omission could considerable reduce time of dioxins analysis and increase laboratory throughput. Moreover automation of extraction and sample clean-up such as pressure liquid extraction (PLE, *FMS*) also branded as accelerated solvent extraction (ASE, *Thermo Scientific Dionex*) and automatic clean-up system (e.g. POWER-PREP, *FMS*) are undeniably convenience for daily routine analysis in laboratory control. The extraction idea of both system PLE and ASE is similar however extraction process may be performed in serial by ASE and parallel up 6 samples at the same time by PLE. In serial extraction each sample could be extracted by different solvents or their mixtures however parallel PLE not have this possibility but is more time efficient.

The aim of study was possibility of applying absorbents to dry pork meat and reducing of sample preparation time by omission of freeze drying process. Moreover results of extractions made by two automatic extractor systems (PLE and ASE) were compared (Fig. 1).

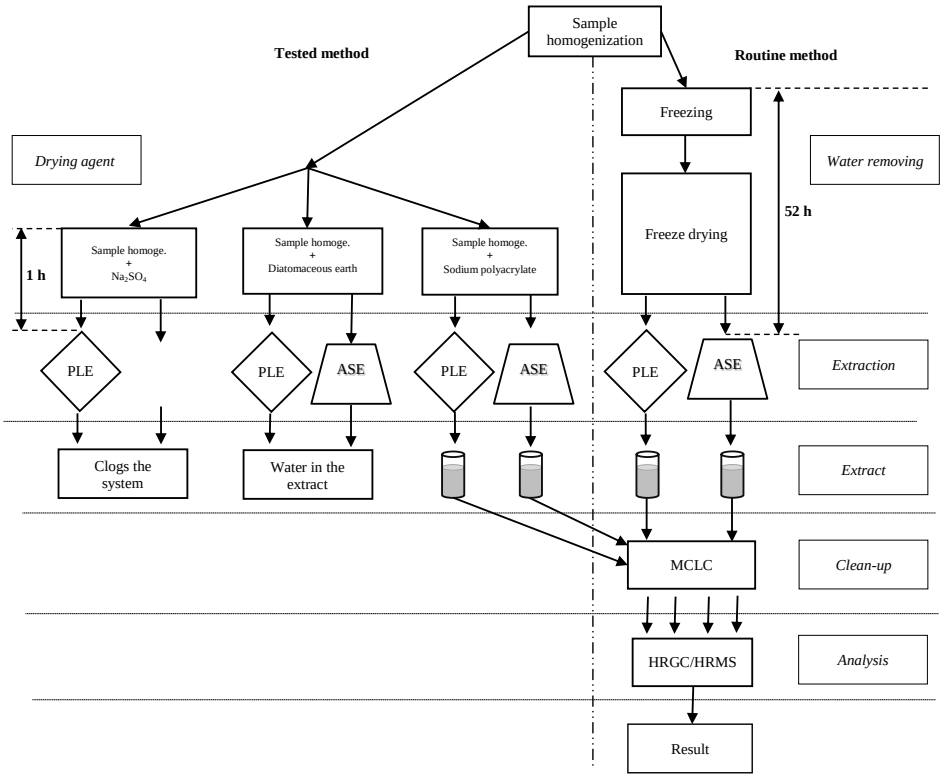

Fig. 1. Flowchart of analytical proceeding

Methods and materials

Analytes: The target compounds were seventeen congeners of 2,3,7,8 toxic PCDD/Fs, twelve dl-PCBs (77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167 and 189) and 6 ndl-PCBs (28, 52, 101, 138, 153 and 180).

Material: Pork meat was selected as the test material and obtained from local market. The whole material was homogenized, dived to two portions. In order to remove water the first part was freeze dried and the another portion was dried with three different kinds of drying agents.

Extraction parameters: Drying efficacy was compared by two automatic extraction systems PLE (FMS) and ASE (Dionex). Samples were extracted using dichloromethane and n-hexane (1:1 v/v) in 2 cycles (5 min) at 120^{o}C and then the cells were purged with N2 by 300 s. For PLE and ASE different pressures were maintain at 1700 psi and 1500 psi respectively. After extraction solvent was reduced in vacuum or under N_2 gentle stream for ASE and PLE respectively and lipid content was gravimetrically determined.

Experiment: Ten grams of freeze dried pork were mixed with diatomaceous earth, paced to extraction cell and all $^{13}C_{12}$ isotopically labeled congeners of interest were added. After 24 h equilibration extractions were made on both automatic systems. Sample weight of raw meat was quantitatively limited to 20 grams by the size of the extraction cell which was 100 mL for both extraction systems.

To compare extraction three 20 grams (equivalent to 5.5 g of dry weight) of raw meat samples were taken and mixed with drying agent: sodium sulphate, diatomaceous earth and sodium polyacrylate. After the addition of isotopic labeled standards the extraction process were performed with conditions described above. The fat content and congener's recovery were controlled to compare the extractions.

Clean up: The fat extract purification step consisted of multi-column liquid chromatography (MCLC) based on multilayer acidic silica, Florisil, Carbopack C/Florisil and Carbopack C column according the method described earlier[2]. The purified extracts were analyzed using high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC/HRMS)[2].

Results and discussion

The aim of the study was successfully achieved. The freeze drying step could be replaced by using drying agent. Taking into account the increasing number of samples, shorter analytical method during possible dioxin crisis require reduce the time of each sample preparation step in dioxin determination. Raw meat samples need water removing realized before extraction by drying in a different way. The most popular technique is convenient but time consuming freeze dried however could be omitted by appropriate sample preparation realized by adding drying agent to the sample. Popular sodium sulphate in high pressure and temperature could passes to extract or clogs the extractor ducts. Diatomaceous earth was applied as potential drying agent for raw pork, nevertheless in this case the absorption of water is low efficient and not resistant to temperature and pressure. Realized water was presented in the extract and interfered during extraction process lowering fat recoveries. The average content of fat (Table 1) determined in this test was estimated as 6.30% (CV = 4.8%) and was significantly smaller in relation to values obtained by extraction from freeze drying material and equals 7.27% of fat with CV=5.5%. Extraction of raw material requires draying agent which will be able to absorb large water amount and keep it under high temperature and pressure therefore the sodium polyacrylate was selected. Percent content of fat received from raw pork using this absorbent was similar to value determined by use of freeze dried samples and equal 7.29% (CV=1.3%) and 7.32% (CV=2.2%) for PLE and ASE respectively. Extracts were clean up and analyzed by HRGC-HRMS. Obtained congeners recoveries were presented on Fig 2 and 3.

Table. 1. Percent of fat determination by different extraction technique

	Fat %	CV
Freeze dried ASE	6.96	2.2
Freeze dried PLE	7.27	5.5
Diatomaceous earth PLE	6.30	4.8
Sodium polyacrylate ASE	7.32	2.2
Sodium polyacrylate PLE	7.29	1.3

Congeners recoveries obtained for extraction from freeze dried and raw pork dried by sodium polyacrylate by both extraction systems are similar but in the case of ASE the extraction process required further optimization, because slightly lower recovery values of $^{13}C_{12}$ labeled congeners.

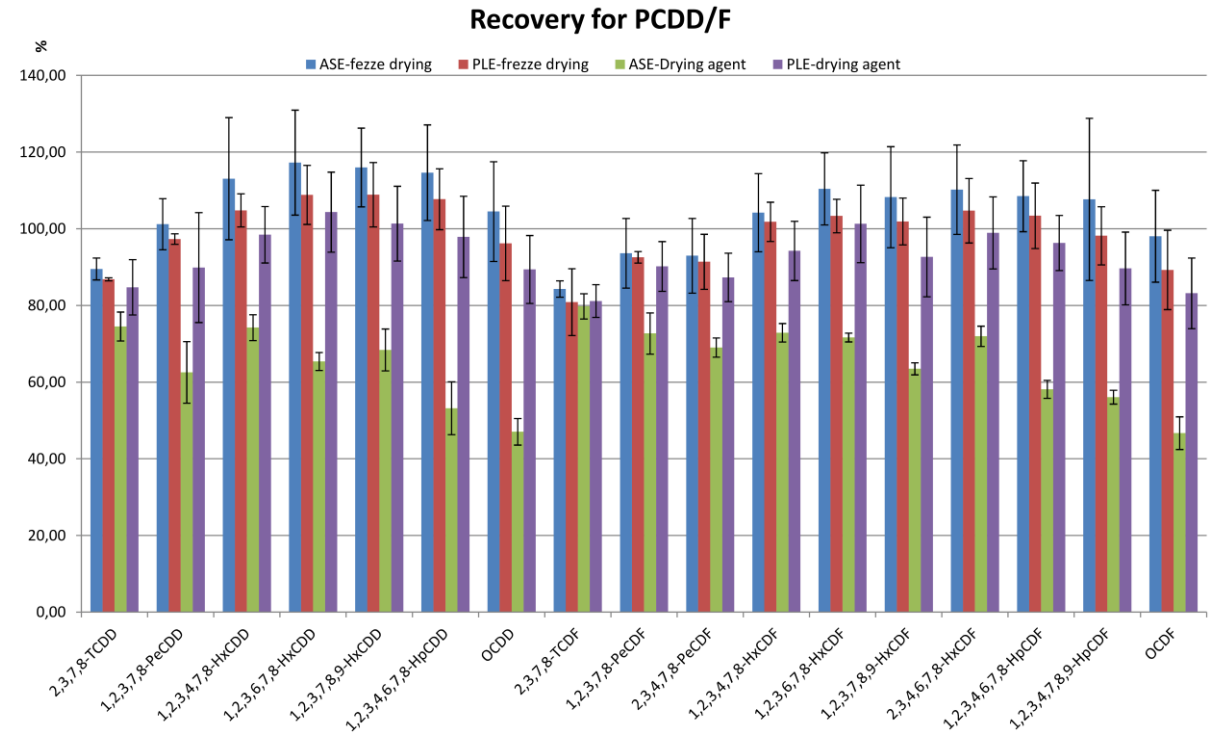

Fig. 2. Comparison recovery of dioxins and furans for extraction from freeze dried and raw pork meat by ASE and PLE

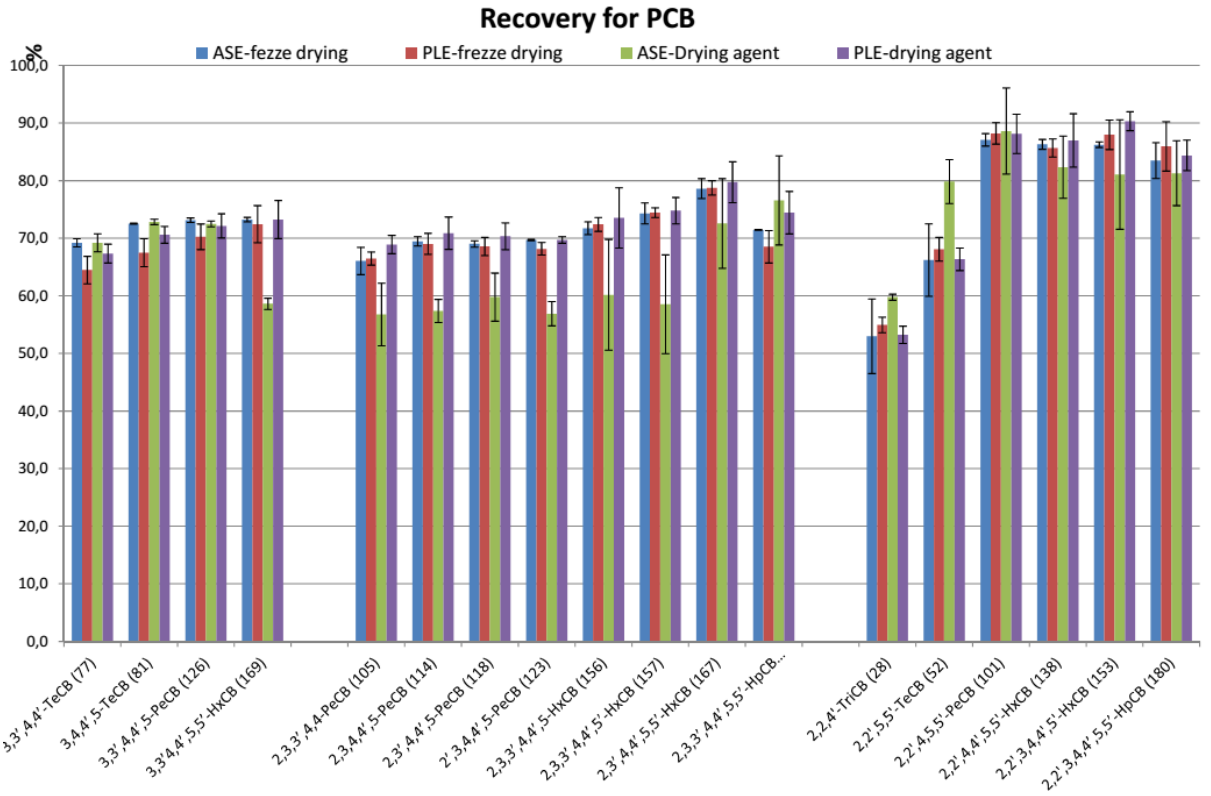

Fig. 3. Comparison recovery of PCB for extraction from freeze dried and raw pork meat by ASE and PLE

For sample preparation with freeze drying the steps of homogenization, freezing, freeze drying, second homogenization and filling an extraction cell are necessary. Advantages of freeze dried samples extraction are reduced sample volume (40 -70%) and no water interference. In presented method it possible to reduce time by leave out of freezing, freeze drying and second homogenization. There is disadvantage of extraction from raw

meat that mass of extracted sample should be bigger to maintain the required levels of detection limits. On the other hand, omission of freeze drying reduces sample preparation time about 52 h and incising laboratory sample throughput. Furthermore, combine this extraction with automated sample clean up method e.g. POWER-PREP (FMS) it possible to reduce time of analysis from 79 h to about 13 h according J.T. Focant[3].The comparison of sample preparation steps and its demand for time consuming were presented in table 2.

Table 2. Sample preparation step and it demand for time.

Step	**Freeze drying**	**Drying agent**
Homogenization	30 min	30 min
Freezing	4 h	-
Freeze drying	48 h	-
Homogenization	15 min	-
Extraction cell preparation	8 min	~ 33 min
Sum	~ 52 h 53 min	~ 1 h 3 min
Difference	**~ 52 h**	

Conclusion

The aim of the study was successfully achieved and freeze drying step could be replaced by using dying agent. There is no significant different in the extractor systems in the case of extraction of PCDD/Fs and PCBs from freeze dried pork. Extraction from raw meat sample is possible by using sodium polyacrylate as drying agent without misestimate of fat determination and it is possibility to make this extraction by both extraction systems. There is no difference in congener's recoveries when the extraction is made from pork freeze dried or dried by sodium polyacrylate. Recoveries obtained by ASE are slightly lowered and extraction process required further optimization.

References

1. Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non-dioxin-like PCBs in foodstuffs (OJ L 320, 3.12.2011, p. 18–23)
2. Jadwiga Piskorska-Pliszczynska, Szczepan Mikolajczyk, Malgorzata Warenik-Bany, Sebastian Maszewski, Pawel Strucinski: Soil as a source of dioxin contamination in eggs from free-range hens on a Polish farm. Sci. Total Environ 466–467 (2014) 447–454.
3. Focant JF, Scholl G, Eppe G, De Pauw E: AUTOMATED PROCEDURES IN ISO17025 ROUTINE DIOXIN LABORATORY: IMPACT ON THE THROUGHPUT, Organohalogen Compounds Vol. 73, 676-679 (2011)