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METHOD DEVELOPMENT FOR RAPID ANALYSIS OF DIOXIN IN SOME FOOD AND FEED USING FREEZE DRYING AND ACCELERATED SOLVENT EXTRACTION TECHNIQUES

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ABSTRACT

The global methods approved and reliable for the analysis of dioxins in food and feed, which take a lot of time, money and effort in addition to that, take many harmful solvents. A simple and rapid method for sample preparation and extraction of dioxins from food developed using freeze-drying (FD) and accelerated solvent extraction (ASE) techniques, respectively. Average recoveries of dioxins extracted from the food obtained by a conventional soxhlet extraction and the ASE almost equal, when the data compared by both methods. Moreover, decrease the time of tissue extraction from 24 hours using soxhlet to only 35 minutes using ASE technique. Development of dioxin analysis in food and feed samples by entire method of fat extraction based on ASE for determination of dioxin compounds with high-resolution gas chromatography-high resolution mass spectrometry (HRGC/HRMS) from food and feed samples was developed. Optimization conditions for ASE method are effective and reliable by using Dionex ASE 350 as follows oven temperature: 150 °C, static cycle time: 5 minute, cycle's no.: 4, flush volume: 100%, purge time: 90 sec, cell pressure: 1590 psi (nitrogen gas) and total extraction time: 35 min per sample. Extraction solvents used for fatty-food samples such as fish, meat and liver were Hexane: DCM (1:1 v,v). While toluene used for extraction of Non-fatty food samples. Quality assurance for ASE extraction method such as precision and recovery as well as robustness and natural contaminated samples evaluated through certified reference materials (CRM) analysis. Accuracy of PCDD/Fs estimated with CRM for Trout sample was below 4% for both ASE and soxhlet, (complying with EU requirement ≤ 20%). This study was to compare efficiency of various extraction tools to determine the content of dioxins in food and feed samples by using accelerated solvent extraction technique (ASE) with those prepared by extraction in soxhlet technique.

Keywords: Method Development, Dioxin, Freeze-drying, Accelerated Solvent Extraction, Food, Feed and HRGC/HRMS.

INTRODUCTION

Accelerated Solvent Extraction (ASE) is a new extraction method that significantly streamlines sample preparation. A solvent delivered into an extraction cell containing the sample, which then brought to an elevated temperature and pressure. Minutes later, the extract transferred from the heated cell to a standard collection vial for cleanup. The entire extraction process fully automated and performed in 35 minutes for fast and easy extraction with low solvent consumption. The extract contains dioxin from fish tissue and fish homogenates hindered by the presence of co-extracted fatty materials that interfere with the chromatographic analysis. There are standard procedures for cleanup to remove the co-extracted lipids from such samples

prior to analysis. These clean-up procedures include size-exclusion chromatography (SEC), column chromatography, and acid treatment. These procedures add time to sample preparation and increase the potential for analyte losses. As an alternative, selective extraction procedures have developed using ASE. The data presented in this application note demonstrate that selective extractions performed using ASE with the proper choice of solvent and sorbent in the extraction cell. Results are given for the recovery of PCBs from contaminated fish tissue showing that extracts can be obtained using ASE that do not require further cleanup prior to analysis by gas chromatography. ASE or Pressurized Liquid Extraction (PLE) is one of the most widely used techniques to replace the traditional soxhlet extraction. Different strategies have employed depending on the different kinds of matrices. Usually the extraction temperature was between 100-185°C and the pressure was 1500 psi. Basically, samples prepared in 66 or 100 ml cells and statically extracted 2-3 times by different solvents (e.g. for milk: n-hexane/dichloromethane/methanol; fatty food or feed stuff: n-hexane/dichloromethane; non-fatty food or feed stuff ; environmental samples: toluene), under the condition of a static time of 3-10 min, a flush volume of 80-125 % and a purge time of 90-120 seconds. To compare the efficiency and time consuming between ASE and the soxhlet extraction, several samples extracted with toluene/acetone under a reflux condenser for 16 hours in a soxhlet apparatus(Hölscher et.al., 2004). One of ASE system applications is obtaining extracts intended for determination of PCDDs/Fs contents (Application Note 323, Dionex). This application is standard based on the extraction methods of the investigated samples was developed. Labconco (2010) illustrate the operation of Freeze-drying which involves the removal of water from a frozen product by a process called sublimation. Sublimation occurs when a frozen liquid goes directly to the gaseous state without passing through the liquid phase. In contrast, drying at ambient temperatures from the liquid phase usually some chemical and physical changes in the product, and may be suitable only for some materials. However, in freeze-drying process, the material does not go through the liquid phase, and it allows the stability of product to be easy to use and aesthetic in appearance. The advantages of freeze-drying are obvious. Properly freeze dried products are not need refrigeration, and can be stored at ambient temperatures. The process may appear to be an expensive however, it save the sample by stabilizing it, thus eliminating the need for refrigeration, more than compensate for the investment in freeze drying equipment. As inferred by its name (freeze-drying), moisture in samples is first frozen to ice and then the ice is removed by sublimation at temperature and pressure below the triple point of water (273, 16 K and 611 Pa). The mass determination under vacuum is not easy task, limitations to the operation of some sensors occur and the measure is affected by several disturbances, like buoyancy effects, vibrations, gas flows, temperature gradients. Antal and Kerekes (2007) Carrying out on-line mass measurements of a product is very important during lyophilization, for reaching an optimal performance. The freeze-drying performed at very low temperatures therefore; the final product suffers little damage. For the

reasons already mentioned and because the velocity of freeze-drying depends on the intensity in which the vapor flows through the dried superficial layer, each product requires an optimum cooling rate to provide effective dehydration and rehydration rates, thus ensuring good quality product. The effectiveness of freeze-drying depends on the sample temperature and the thickness during the process.
The objectives of this study:

- To minimize the samples preparation time and enhance the efficiency of dioxin determination by using freeze-drying (FD).
- To reduce extraction time of the samples by using the accelerated solvent extractor, ASE extract the fat from samples, which take 35 min only instead of use traditional technique using the Soxhlet apparatus, which consume more than 18 hours.

MATERIALS AND METHODS

1. Sampling:

All samples extracted by pressurized liquid extraction (PLE) using a Dionex ASE 350 extractor capable of sequentially extracting up to 18 samples. Transfer freeze-dried of homogenized fresh samples to the accelerator solvent extractor (ASE) mixed with sodium sulfate (sorbent). This study tested the effect of freeze-drying which was validate in dioxin using freeze-dried CRM certified by the National Institute of Standards and Technology (NIST).

A total of 18 samples of food, three samples of corn oil and one CRM trout sample as well as three samples of meat, three samples of liver(beef) and other feed samples, were included in the study. Fresh samples equally divided, and each sample extracted using the methods of soxhlet and ASE techniques.

2. Reagents and Standards:

Drying Reagent

Sodium sulfate, reagent grade, granular, anhydrous, baked at 400°C for one hour minimum, cooled in a desecrator, and stored overnight at 130 °C.

Extraction Solvents

Acetone- purity ≥97%, toluene - purity ≥ 99.9 (Merck), cyclohexane - purity ≥ 99, n-hexane- purity ≥ 99.9% (Merck or Rediel Dhein) , methanol - purity ≥ 98, methylene chloride - purity ≥ 98, and nonane - purity ≥ 99. All solvents must be pesticide grade.

Adsorbents for Sample Cleanup

Silica gel: Activated silica gel- Silica Gel 60 (0.063-0.2 mm) or equivalent, baked at 130°C for a minimum of one hour, cooled in a desecrator, and stored overnight at 130°C. Acid silica gel (30% w/w) and Basic silica gel.

Potassium silicate: Dissolve 56 g of high purity potassium hydroxide (Aldrich, or equivalent) in 300 ml of methanol in a 750-1000 ml flat bottom flask. Activate overnight at 130°C.

Basic Alumina: ICN biomedical GMBH or its equivalent activated by heating up to 600°C for a minimum of 24 hours. Do not heat over 700°C, as this can lead to reduced capacity for retaining the analytes. Basic alumina was store at 130°C in a covered flask.

Carbopack C: (Supelco C 80/100) and Celite 545- (BDH or Aldrich) prepared thoroughly mix 9.0 g Carbopack C and 41.0 g Celite 545 to produce an 18% w/w mixture. Activate the mixture at 130°C for a minimum of six hours.

Reference Matrices- Matrices in which the CDDs/CDFs and interfering compounds are not detected by this method. Tissue reference matrix, corn or other vegetable oil.

Standard Solutions - Solutions or mixtures with certification to their purity, concentration, and authenticity, when not being used, standards are stored in the dark at room temperature in screw-capped vials.

Precision and Recovery (PAR) Solution

Wellington Laboratories Inc. (EPA 1613 PAR) for the CDDs/CDFs with certification to its concentrations. Used for determination of initial precision and recovery. Dilute 5 µL of the precision and recovery standard to 1.0 ml with acetone (CDDs/CDFs).One ml is required for the IPR with each batch.

Labeled-Compound Spiking Solution (LCS)

Wellington Laboratories Inc.(EPA 1613) for the CDDs/CDFs with certification to its concentrations. Labeled-Compound Spiking Solution contains the CDDs/CDFs at the concentrations

Dilute 20 µL of the labeled compound standard solution to 1.0 ml with acetone (CDDs/CDFs).One ml is required for the IPR with each batch.

Internal Standard Solution (ISS)

Wellington Laboratories Inc. (EPA 1613) for the CDDs/CDFs with certification to its concentrations. Internal Standard contains 13C-1,2,3,4-TCDD and 13C-1,2,3,7,8,9-HxCDD in nonane at the concentrations for the CDDs/CDFs in nonane at the concentrations.

Calibration Standard Solution (CSS)

Wellington Laboratories Inc. (EPA 1613 CS0.1-CS5) for the CDDs/CDFs with certification to its concentrations. These solutions permit the relative response (labeled to native) and response factor to measure as a function of concentration. The CS3 standard was use for calibration verification (VER).

3. Apparatus and Materials

3.1 Balances: 0.01g, with an accuracy of 0.001 g

3.2 Freeze-drying (lyophilization)**:** Freeze Dryer ilShin Lab co., Ltd.

3.3 Soxhlet Extractor (Bibby Sterlin, Great Britain): Soxhlet- 50 mm ID, 200 ml capacity with 500 ml flask Thimble- 43 x 123 to fit Soxhlet (Whatman or equivalent). Heating mantle- Electromantle.

3.4 Accelerator Solvent Extractor (ASE): Pressurized Liquid Extraction by ASE with Model no. Dionex ASE 350 and the extraction of samples in 100 ml cell capacity with 200 ml bottle flask (extract receiver) containing one cellulose filter up and two cellulose filter down. Condition of ASE: Dionex 350 model; condition of ASE: oven temp.: 150˚C, static cycle time: 5 minute, cycles no.: 4, flush volume: 100%, purge time: 90 sec, cell pressure: 1590 psi (nitrogen gas) and total extraction time: 35 min per sample. Extraction solvents use for fish, meat and liver samples by Hexane: DCM (1:1), for non-fatty food samples by toluene and for milk samples by Hexane: DCM: Methanol (5:2:1).

3.5 Cleanup Apparatus: Anthropogenic isolation cleanup column: 300 mm long x 25 mm ID, with 300 ml reservoir. Silica gel and alumina cleanup columns: 200 mm long x 15 mm ID, with 250 ml reservoir. Carbon cleanup column: 15 cm long x 6 mm ID.

3.6 Oven: baking and storage of adsorbents, in the range of 105-150 °C.

3.7 Concentration Apparatus: Macro-Concentration(a rotary evaporator) - Heidolph or equivalent, Equipped with a variable temperature water bath. Sample micro vials and conical vials – 0.3 and 0.9 ml, respectively.

3.8 HRGC/HRMS Instrument

Analyses were conducted using HP 6890 plus gas chromatograph coupled with Micromass /Autospec Ultima mass spectrometer operating in EI mode at 35 ev and with a resolution of 10.000 (5% valley). Sample injections performed in the splitless mode on DB5 MS column (60m, 0.25 mm id, 0.1µm film thickness). The oven program started from 90ºC then takes 15min. to reach 220ºC then held for 15 min, then from 220-290 in 8min then held for 17min. Helium (Ultra high purity) at a flow rate 0.8 ml/min. used as a carrier gas. Injector temperature was 225 C; 1µl of the sample injected using splitless mode.

4. Procedure:

4.1 Soxhlet Extractor: Extract in triplicates Freeze-dried of meat, liver and trout (25 g) was extracted for 24 h in 200 ml n-hexane/dichloromethane (1/1, v/v) soxhlet extractors at the speed of six siphons per hour.

Accelerated Solvent Extraction: The sample extracted using either Soxhlet or Accelerator Solvent Extractor (ASE). A 25 ± 0.1g from tissue sample for homogenization (except the oily matrix sample a weight of 10 ± 0.1g for analysis) after removed the water content from the samples by Freeze-drying for about 4-6 hours then mixed with sodium sulfate (3-5 equivalents in weight). Spiked the samples with the labeled compounds, and then extract the lipid by either Soxhlet for 18-24 hours or by Accelerator Solvent Extractor for 35 minutes in hexane: methylene chloride (1:1).

ASE extract four times (4 cycles) with 120 ml of hexane: DCM (1:1), 5 min cycle time at pressure of 1590 psi. The fat extracts were dried by filtration through 30-40 g of powdered anhydrous sodium sulfate, evaporated at 40°C using a rotary evaporator to lipid content determination using gravimetric analysis. Aliquots of about 1-7 g fat used for further step of clean up. Calculate the lipid content as following equation:

$$\textbf{Percent lipid} = \frac{\textbf{Weight of residue (g)}}{\textbf{Weight of tissue (g)}} \times 100$$

Concentrate the extract to near dryness by using Macro-Concentration devise. Complete the removal of the solvent using the nitrogen blow down procedure and a water bath temperature of 60°C.

4.2 After extraction, Sample cleanup include silica gel, alumina, and activated carbon clean-up column chromatography. Prior to the cleanup procedures cited above, tissue extracts cleaned up using acidified silica gel followed by an anthropogenic isolation column.

4.3 After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, injection standards added to each extract, and an aliquot of the extract injected into the gas chromatograph.

5. Determination of dioxin samples:

Quantitative analysis performed using selected Ion Recording (SIR) mode and the concentration of each compound is determined using the internal standard technique. The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems. At the beginning of analyses, GC/MS system performance and calibration verified for all CDDs/CDFs and these labeled compounds. For these tests, analysis of the CS3 calibration verification (VER) standard perform until all performance criteria met such as blanks, analyze precision and recovery. Blank sample extracted with each tested sample in same batch immediately following tested samples aliquot to demonstrate freedom from contamination and freedom from carryover from the IPR analysis.

The QCAP lab operates and follows the quality assurance system and method of analysis of PCDD/F in tissue and accredited since 2003 by Finnish Accreditation Service body (FINAS) according to the requirements of the International Standard ISO/IEC 17025.

RESULTS AND DISCUSSION

All fresh (freeze dried and dry food samples were tested with homogenized tissue extracted in triplicate and analyzed by soxhlet and ASE. Moreover, results of precision and accuracy verification check for sample preparation development of dioxin analysis in food and feed samples tabulated in tables 6 and 8 show acceptable data from comparisons between soxhlet and ASE extractions techniques. ASE extraction technique operates at optimal conditions as a good alternative extraction technique for

quantitative analysis of PCDDs/PCDFs in food and feed samples. The non-selective extraction using ASE gives acceptable results, and additional cleanup steps needed such as sulfuric acid treatment or size exclusion chromatography eliminated.

1.0 Development of dioxin analysis method using of freeze-drying (FD):

In table1 focused on the evaluation of freeze-drying equipment by measure the efficiency of water content removal after 6 hours only from different types of tissue samples such as meat and liver samples with 8.0 and 6.7% respectively. The freeze-dried products grounded in order to obtain a fine powder. Many of advantages resulted from using the freeze-drying technique such as increase the precision and accuracy of dioxin analysis. It has proved very flexible and reliable for different kinds of food samples as well as lead to lower dioxin background levels and LOQ levels. In all the reported procedures PLE applied to dry samples since, as in the case of soxhlet extraction, the absence of water in the samples makes the sample matrix more accessible to organic solvents. Therefore, samples were dried by grinding with sodium sulphate or with hydrometrics, air-dried, freeze-dried or lyophilized before PLE as shows in Fidalgo et.al (2007).

Table 1. Efficiency of freeze-drying measured in different types of tissue samples

Matrices	Wt. Before F. Dryer, g	Wt. After F. Dryer, g	Vol. of Water loss, ml	Av. Water Loss, %	Efficiency CV%
Trout (CRM)	25	11.90	13.10	**52.40**	**---**
Meat 1	25	13.61	11.39	**53.85**	**8.0**
2	25	14.68	10.32		
3	25	12.51	12.49		
4	25	13.05	11.95		
Liver 1	25	12.00	13.00	**45.4**	**6.7**
2	25	11.75	13.25		
3	25	10.30	14.70		

2.0 Development of dioxin analysis method using ASE instead of soxhlet:

To avoid the use of large amounts of organic toxic solvents the Accelerated Solvent Extractor (ASE) as a new technique used for extraction instead of the traditional soxhlet extraction. The method outlined in this application note demonstrates that non-selective extractions can perform using ASE with the proper choice of solvent in the extraction cell. Table 2 show two different conditions of extraction, clean up of PCDDs/Fs for fatty and non-fatty food, and feed samples using Soxhlet technique.

Table 2. Different conditions of extraction, clean up of PCDDs/Fs for fatty and non-fatty food and feed samples using soxhlet technique

Extraction conditions	Fatty food samples (e.g Meat and Liver)	Non-fatty food and feed samples
Solvent	Hexane (HEX): Dichloromethane(DICM) (1:1)	Toluene
Temperature, °C	ca.110	ca.110
Extraction time, hours	18-24	18-24
Clean-up columns	Anthropogenic, Silica, Alumina and Carbopack	Florisil, Silica, Alumina and Carbopack

Verification the ASE compare with Soxhlet

Van Loco et.al, (2004) shown that accelerated solvent extraction (ASE) is a valid alternative extraction and clean-up procedure for fish oil and vegetable oil. The results obtained with CALUX and GC-HRMS after ASE are equivalent to the results obtained with the classical extraction and purification procedures.

Although the evaluation of 3rd test sample was the best trial of ASE condition as mention in table (3a).

Table 3a. Extraction of PCDDs/Fs compounds in reagent blank with different conditions of ASE technique

Extraction conditions	1st Test sample	2nd Test sample	3rd Test sample	4th Test sample
Extraction Solvents	HEX: DICM (1:1)	HEX: DICM (1:1)	HEX: DICM (1:1)	HEX:DICM (1:1)
Temperature, °C	125	150	125	150
Pressure, psi	1590	1590	1590	1590
Static time, min	5	5	5	5
Flushing solvent cell volume, %	100	100	60	60
Purging time, sec	90	90	90	90
No. of cycles	4	4	4	4
Extraction time, min	35	35	35	35

The extract of PCDD/Fs in reagent blank at low concentration level as agreed with Dionex company recommendation for ASE conditions (Dionex Technical Note 208) shown that good recoveries and within the acceptable concentration ranges of EPA 1613 method for native and labeled PCDD/F as in table3b. However when applying the 3rd test sample with different matrices of foodstuffs and feedstuff instead off reagent blank found that low recoveries for native and labeled PCDD/Fs compounds because matrix effect. Therefore, change the ASE conditions to high temperature at 150°C and increase flushing solvent cell volume to 100% according 2nd test sample as in table 4, gives a good recoveries as shown in table5 and 6. Identical results obtained by Grochowalski A. and Maślanka A.(2003) about the recovery values for the samples analyzed using the ASE technique (under the most extreme conditions) compared with, using the Soxhlet apparatus which are very close to each other. At lower ASE temperature, the recovery values of dioxins reduced.

3b

Dionex has published several application notes describing extraction of various foods and animal feed samples. These application notes provide recommended methods and ASE parameters for these particular applications. However, because every sample matrix may be different and some scenarios may not fit exactly with each application note, a new method may need to develop for some samples (Dionex Technical Note 209).

In table 4 the ASE extraction applies temperature and pressure to accelerate extraction processes, the effect was particularly improved with PCDD/Fs using four cycles, showing a good efficiency of Hexane: Dichloromethane (1:1 v,v) and Toluene extraction solvents.

Table 4: Different conditions of extraction and clean-up of PCDD/Fs for fatty and non-fatty food and Feed samples using ASE technique

Extraction conditions	Fatty food (e.g Meat and Liver) and feed (DFM) samples	Non-fatty food samples
Solvent	Hexane: Dichloromethane (1:1)	Toluene
Temperature, °C	150	150
Pressure, psi	1590	1590
Static time, min	5	5
Flushing solvent cell volume, %	100	100
Purging time, sec	90	90
No. of cycles	4	4
Extraction time, min	35	35
Clean-up columns	Anthropogenic, Silica, Alumina and Carbopack	Florisil, Silica, Alumina and Carbopack

2.1 Initial Precision and Recovery (IPR) test in Corn oil:

Table 5 summarize the spiked of IPR in corn oil of native and $^{13}C12$ labeled of PCDDs/PCDFs compounds ratios of each isomer's concentration to the accepted certified values of native compounds and the accepted certified recoveries of labeled compounds, respectively. Replicates of the spiked corn oil samples were analyzed and determined to be within the accepted RSD of ≤ 20% complying with EU requirement on European Commission (EC) regulation (2012). Replicate average of RSD% for native compounds were 11.7% using Soxhlet and 6.2% using ASE. Whereas cleanup standard recoveries were 74% (Soxhlet) and 58% (ASE).The mean recovery of PCDD/F when using ASE with hexane/methylene chloride (1/1, v/v) for congeners ranging from 27.6% (1,2,3,4,6,7,8,9-OCDD) to 85.6% (1,2,3,6,7,8-HxCDF).

2.2 Accuracy test of ASE compare to soxhlet techniques using certified reference material (CRM):

Accuracy test of dioxin analysis from ASE method with trout sample as certified reference material compare to soxhlet technique. Table 6 show the Z-score values for PCDD/Fs using ASE extraction were consistently in CRM samples within the acceptable range ±2, revealing that recovery values are almost the same those obtained with Soxhlet extraction.

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To ensure the maximum selectivity of the PCDD/Fs determination by elimination of effects of potential interfering matters is necessary. Accuracy of PCDD/Fs estimated with CRM (Norway -Trout 25) was below ±4% for both ASE and soxhlet, complying with EU requirement (≤ 20%).

Table 6: Evaluation of dioxin analysis of trout CRM samples (Norway - Trout 25) by using ASE extraction technique

PCDD/Fs	Assigned value pg/g (f.W.)	ASE (n=3)		Soxhlet (n=3)	
		Result pg/g (f.W.)	Z-Scores	Result pg/g (f.W.)	Z-Scores
2,3,7,8-TCDD	0.130	0.123	-0.17	0.138	0.19
1,2,3,7,8-PeCDD	0.180	0.194	0.24	0.168	-0.22
1,2,3,4,7,8-HxCDD	0.008	0.016	1.76	0.006	-0.56
1,2,3,6,7,8-HxCDD	0.051	0.055	0.30	0.047	-0.31
1,2,3,7,8,9-HxCDD	0.009	0.014	1.30	0.016	1.70
1,2,3,4,6,7,8-HpCDD	0.048	0.079	1.64	0.064	0.83
1,2,3,4,6,7,8,9-OCDD	0.210	0.225	0.12	0.191	-0.16
2,3,7,8-TCDF	2.000	1.301	-1.49	2.014	0.03
1,2,3,7,8-PeCDF	0.180	0.234	1.09	0.183	0.05
2,3,4,7,8-PeCDF	0.490	0.510	0.16	0.496	0.05
1,2,3,4,7,8-HxCDF	0.023	0.029	0.74	0.021	-0.21
1,2,3,6,7,8-HxCDF	0.029	0.038	0.94	0.029	0.03
2,3,4,6,7,8-HxCDF	0.029	0.046	1.58	0.043	1.24
1,2,3,7,8,9-HxCDF	0.005	0.012	1.76	0.005	0.05
1,2,3,4,6,7,8-HpCDF	0.022	0.042	1.86	0.043	1.91
1,2,3,4,7,8,9-HpCDF	0.006	0.015	1.63	0.017	1.87
1,2,3,4,6,7,8,9-OCDF	0.031	0.055	1.51	0.048	1.06
Sum PCDD/F- WHO-TEQ, pg/g (f.W.)	**0.78**	**0.74**	**-0.02**	**0.78**	**0.0**

Z- Scores = (Found reported - Assigned value)/ Target St.dev.

2.3 Precision test of ASE compare to the soxhlet with incurred samples:

Evaluate the results from the ASE and soxhlet extractions of meat, liver and Danish fishmeal (DFM) is listed in Table 8. The liver and Danish fishmeal samples were containing highly contaminated extracted using ASE almost gives close results compare to those from soxhlet technique due to high fat content in samples (more than 2%) such as 4.06% in average fat for liver samples and 12.1% in average fat for DFM. Moreover, fat content 2.08% in average fat for meat samples were not nearly as homogeneous represent by division percent of -15% between soxhlet and ASE techniques which calculated the dioxin based on fresh weight according European Commission (EC) regulation (2012) recommendation. Table 7 study the homogenize of meat sample which grind in a meat grinder with two different sizes in inner plate at 3 and 3.4 mm particularly at low fat content (less than 4%) and grind three times to ensure homogeneity and get reproducible results.

Table 7: Evaluation of homogeneity of low fat content of meat sample using ASE extraction technique

Meat samples homogeneity test	Holes in inner plate	
	3.4 mm	3.0 mm
Av., gm	0.98	0.67
SD	0.26160	0.02646
CV%	26.8	3.9
Fat%	3.9	2.7

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Conclusions

The non-selective extraction using ASE gives acceptable results, and need additional cleanup, such as sulfuric acid treatment, florisil, carbon chromatography and alumina. By using this method, time decreased for sample preparation, best cost-efficiency ratio and the potential analyte losses. Freezing drying sample preparation in combination with ASE extraction developed and tested showed good recoveries percentages, low samples back ground levels and no chromatographic interferences for the dioxin congeners.

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REFERENCES

Antal T. and Kerekes B. (2007) establishment of a data processing system for freeze drier; 7th international multidisciplinary conference , Baia Mare, Romania, May 17-18, 2007, ISSN- 1224-3264

Dionex Technical Note (2009). Accelerated solvent extraction (ASE) sample preparation techniques for food and animal feed samples. Application note: 209, Dionex Corporation, Sunnyvale, CA.

Dionex Technical Note (1999). Extraction of Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans from Environmental Samples Using Accelerated Solvent Extraction (ASE®). Application note: 323, Dionex Corporation, Sunnyvale,CA.

Dionex Technical Note (2004). Methods Optimization in Accelerated Solvent Extraction (ASE®). Technical note 208, Dionex Corporation, Sunnyvale, CA.

EPA1613 method revision B (1994)- Tetra- through Octa-Chlorinated Dioxins and Furans By Isotope Dilution HRGC/HRMS-October 1994. U.S. Environmental Protection Agency - Office of Water Engineering and Analysis Division.

European Commission (EC) regulation no 252/2012 of 21 March 2012, laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin- like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC)No 1883/2006

Fidalgo U.N., Blanco G.E. and Sanz M. A.(2007) Sample handling strategies for the determination of persistent trace organic contaminants from biota samples, Analytica Chimica Acta 590,1–16

Grochowalski A. and Maślanka A. (2003) comparison of ase and soxhlet-apparatus extraction indetermination of polychlorinated dibenzodioxins and benzofurans, Organohalogen Compounds, Volumes 60-65, Dioxin 2003 Boston, MA

Hölscher K., Maulshagen A., Shirkhan H., Lieck G.and Peter A. B. (2004) Automated rapid analysis for dioxins and PCBs in food, feedingstuff and environmental matrices, organohalogen compounds, Vol. 66: p117-125

Labconco Corporation (2010) A Guide To Freeze Drying for the Laboratory 2010 by Labconco Corporation Printed in the U.S.A. 3-53-8/10-James-3M-R8. www.labconco.com

Van Loco J., Van Leeuwen S.P.J, Roos P., Carbonnelle S., de Boer J., Goeyens L. and Beernaert H. (2004) The international validation of bio- and chemical-analytical screening methods for dioxins and dioxin-like PCBs: the difference project rounds 1 and 2, Elsevier, Talanta 63,1169–1182.

تطوير طريقة تحليل سريعة للدايوكسين في بعض المواد الغذائية والأعلاف باستخدام تقنيات التجفيف بالتجميد والاستخلاص السريع بالمذيبات

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ان الطرق العالمية المعتمدة والموثوق بها لتحليل الدايوكسين فى الأغذية والأعلاف حيث تأخذ الكثير من الوقت والمال والجهد علاوة على ذلك تأخذ الكثير من المذيبات الضارة. لذا تم وضع طريقة بسيطة وسريعة لإعداد واستخلاص الديوكسينات من عينات المواد الغذائية باستخدام تقنية التجفيف (FD) وبتقنية المذيبات المعجلة (ASE)، على التوالي. متوسط استرجاع الدايوكسينات المستخلصة من المواد الغذائية التي تم الحصول عليها بالاستخلاص بتقنية جهاز السوكسليت (soxhlet) التقليدية وتقنية المذيبات المعجلة (ASE) على قدم المساواة تقريبا، عند مقارنة نتائج كل من تلك التقنيات. علاوة على ذلك تقلل من وقت استخلاص الأنسجة الدهنية من 24 ساعة باستخدام السوكسليت إلى 35 دقيقة فقط باستخدام تقنية المذيبات المعجلة. ان تطوير تحليل الديوكسينات في المواد الغذائية والأعلاف عن طريق استخلاص الدهون منها بتقنية المذيبات المعجلة لتحديد مركبات الديوكسينات باستخدام جهاز كروماتوجرافى الغاز عالي الدقة- مطياف الكتلة عالي الدقة (HRMS/HRGC) في المواد الغذائية والأعلاف. الظروف المثلى لجهاز المذيبات المعجلة Dionex 350 بالأفة الى كونها فعالة وموثوق بها هى كالآتي: درجة الحرارة فرن: 150 درجة مئوية، ودورة ثبات الزمن: 5 دقائق، وعدد الدورات: 4، حجم تدفق: 100٪، وتطهير الوقت: 90 ثانية، ضغط الخلية: 1590 PSI (غاز النيتروجين)، ومجموع أوقات الأستخلاص: 35 دقيقة لكل عينة. المذيبات المستخدمة في استخلاص عينات الأغذية الدهنية مثل اللحوم والأسماك وعينات الكبد هي ن-هكسان: مثيليين كلوريد (1:1 حجم/حجم). استخدام التولوين لاستخلاص عينات الأغذية غير الدهنية.

ولتأكيد ضمان جودة طريقة استخلاص تقنية المذيبات المعجلة بقياس دقتها واسترجاعها لمركبات الدايوكسين، فضلا عن متانة الطريقة تم استخدام عينات ملوثة طبيعيا وأيضا تحليل عينات مرجعية معتمدة (CRM).

وبتقييم نتائج دقة الدايوكسين للعينات المرجعية لعينة سمك السلمون وجد أنها أقل من 4٪ لكل من المذيبات المعجلة والسوكسليت، (مطابقة مع متطلبات الاتحاد الأوروبي بان تكون أقل من 20٪).

هذه الدراسة تمت لمقارنة فعالية استخدام أدوات استخلاص مختلفة لتحديد محتوى الديوكسينات في عينات الأغذية والأعلاف باستخدام الاستخلاص بتقنية المذيبات المعجلة (ASE) مع تلك التي استخلصت بتقنية السوكسليت (soxhlet).

قام بتحكيم البحث

أ.د / عادل عبد المنعم صالح	**كلية الزراعة – جامعة المنصورة**
أ.د / اشرف المرصفى	**مركز البحوث الزراعية**

Table 8. Comparison of PCDD/Fs values extract from some incurred contaminated samples (e.g meat, liver (Beef) and danish fish meal (DFM) using soxhlet and ASE.

Matrices	Meat (Fat basis)				Liver-Beef (Fat basis)				DFM (Fresh basis)				Soxhlet	ASE
PCDDs/PCDFs Native Compounds	Soxhlet		ASE		Soxhlet		ASE		Soxhlet		ASE			
	Average (ng/ml)	CV%	Average (ng/ml)	CV%	Average (ng/ml)	CV%	Average (ng/ml)	CV%	Average (ng/ml)	CV%	Average (ng/ml)	CV%	Division (%)*	Division. (%)*
2,3,7,8-TCDD	<0.02	-	<0.02	-	<0.02	-	<0.02	-	0.13	10.8	0.19	4.7	10.8	4.7
1,2,3,7,8-PeCDD	<0.02	-	<0.02	-	0.06	14.0	0.04	28.4	0.46	15.9	0.43	20.3	15.0	24.4
1,2,3,4,7,8-HxCDD	<0.02	-	<0.02	-	1.13	14.6	0.68	7.8	0.10	0.7	0.10	11.8	7.7	9.8
1,2,3,6,7,8-HxCDD	0.05	10.0	0.02	5.2	0.96	0.4	0.51	15.0	0.39	3.1	0.34	30.0	4.5	16.7
1,2,3,7,8,9-HxCDD	0.03	31.5	0.02	16.7	0.33	0.9	0.16	18.8	0.08	9.2	0.09	1.6	13.9	12.4
1,2,3,4,6,7,8-HpCDD	0.39	21.2	0.27	3.4	184.0	0.8	111.53	5.2	0.90	9.8	1.10	34.1	10.6	14.2
1,2,3,4,6,7,8,9-OCDD	2.68	49.4	0.80	3.6	1262.1	4.9	764.82	9.9	5.20	0.8	3.31	1.0	18.4	4.8
2,3,7,8-TCDF	0.09	36.4	0.03	17.9	0.09	13.7	0.04	10.1	2.12	5.8	1.16	7.5	18.6	11.8
1,2,3,7,8-PeCDF	0.08	4.9	0.09	11.3	0.11	20.0	0.08	22.9	0.89	8.5	1.16	27.5	11.1	20.6
2,3,4,7,8-PeCDF	0.12	26.2	0.12	17.9	0.17	14.9	0.07	25.8	2.80	13.4	2.78	37.5	18.2	27.1
1,2,3,4,7,8-HxCDF	0.25	37.7	0.39	13.4	0.57	32.6	0.41	13.3	1.18	1.1	1.04	6.2	23.8	11.0
1,2,3,6,7,8-HxCDF	0.11	26.1	0.11	7.6	0.18	23.0	0.12	9.6	0.65	3.8	0.67	5.6	17.6	7.6
2,3,4,6,7,8-HxCDF	0.10	18.3	0.09	8.2	0.19	31.7	0.08	29.4	0.41	6.3	0.30	40.7	18.8	26.1
1,2,3,7,8,9-HxCDF	0.03	14.1	0.03	18.0	0.06	10.9	0.06	10.5	0.09	10.8	0.12	13.1	11.9	13.9
1,2,3,4,6,7,8-HpCDF	0.70	32.7	0.65	13.2	3.87	31.0	1.76	5.6	3.88	4.1	2.19	3.0	22.6	7.3
1,2,3,4,7,8,9-HpCDF	0.05	28.8	0.04	14.4	0.29	0.5	0.12	17.4	0.20	9.8	0.13	9.7	13.0	13.8
1,2,3,4,6,7,8,9-OCDF	0.24	25.8	0.40	12.8	9.53	8.2	5.20	1.4	1.62	21.5	1.08	6.8	18.5	7.0
Average fresh or fat basis (ng /kg)	**0.21**	10.2	**0.22**	5.6	**71.1**	8.8	**55.4**	9.2	**1.24**	5.6	**1.09**	5.3	15	6.7
Efficiency of Fat Content Extract — **Average, %**	2.24		1.92		4.02		4.08		12.65		11.55			
Efficiency of Fat Content Extract — **Difference, %**	2.08				4.06				12.1					
Efficiency of Fat Content Extract — **Division, %**	-15.4				1.48				-9.1					

***The results within ±50% of acceptable criteria according to EPA method-1613**

Table 3b. Divisions of native and recoveries percentage of label PCDD/F compounds at low concentration level of PCDD/Fs extract in reagent blank using different ASE conditions.

PCDDs/PCDFs Native Compounds	Expected Extract Conc., ng/ml	Accepted* Range (ng/ml)	1st Test Sample		2nd Test Sample		3rd Test Sample		4th Test Sample	
			(ng/ml)	Dev.%	(ng/ml)	Dev.%	(ng/ml)	Dev.%	(ng/ml)	Dev.%
2,3,7,8-TCDD	0.4	0.268-0.632	0.34	-14.13	0.35	-12.38	0.46	14.75	0.33	-17.63
1,2,3,7,8-PeCDD	2	1.4-2.84	1.76	-12.03	1.83	-8.60	2.36	17.78	1.60	-19.90
1,2,3,4,7,8-HxCDD	2	1.4-3.28	1.68	-15.93	1.78	-10.90	2.30	14.93	1.62	-19.20
1,2,3,6,7,8-HxCDD	2	1.52-2.68	1.74	-13.15	1.79	-10.30	2.36	18.00	1.62	-19.00
1,2,3,7,8,9-HxCDD	2	1.28-3.24	1.53	-23.48	1.54	-23.05	2.11	5.48	1.48	-25.80
1,2,3,4,6,7,8-HpCDD	2	1.64-2.44	1.65	-17.65	1.67	-16.38	2.06	3.17	1.70	-14.80
1,2,3,4,6,7,8,9-OCDD	4	3.12-5.76	3.53	-11.74	3.91	-2.30	4.47	11.66	3.49	-12.83
2,3,7,8-TCDF	0.4	0.3-0.632	0.34	-16.13	0.35	-13.25	0.48	20.13	0.33	-18.38
1,2,3,7,8-PeCDF	2	1.6-2.68	1.86	-6.88	2.00	0.20	2.49	24.50	1.79	-10.55
2,3,4,7,8-PeCDF	2	1.36-3.2	1.59	-20.68	1.71	-14.38	2.12	6.18	1.60	-19.88
1,2,3,4,7,8-HxCDF	2	1.44-2.68	1.87	-6.70	2.11	5.53	2.42	21.18	1.80	-9.88
1,2,3,6,7,8-HxCDF	2	1.68-2.6	1.74	-13.03	2.00	-0.08	2.35	17.40	1.79	-10.38
2,3,4,6,7,8-HxCDF	2	1.4-3.12	1.49	-25.70	1.65	-17.60	2.12	5.85	1.54	-23.18
1,2,3,7,8,9-HxCDF	2	1.56-2.6	1.58	-21.00	1.90	-5.08	2.17	8.62	1.64	-17.83
1,2,3,4,6,7,8-HpCDF	2	1.64-2.44	1.85	-7.35	2.18	8.80	2.40	19.88	1.72	-13.78
1,2,3,4,7,8,9-HpCDF	2	1.56-2.76	1.57	-21.55	1.94	-2.95	1.90	-4.90	1.64	-18.13
1,2,3,4,6,7,8,9-OCDF	4	2.52-6.8	3.25	-18.65	3.55	-11.30	3.99	-0.32	3.15	-21.25
PCDDs/PCDFs Labeled Compounds										
2,3,7,8-TCDD	100	20-175	74.71	-25.29	59.5	-40.46	62.0	-37.96	60.4	-39.61
1,2,3,7,8-PeCDD	100	21-227	72.81	-27.19	60.0	-39.99	56.1	-43.90	55.8	-44.20
1,2,3,4,7,8-HxCDD	100	21-193	93.39	-6.61	74.6	-25.44	70.5	-29.47	79.7	-20.33
1,2,3,6,7,8-HxCDD	100	25-163	101.96	1.96	78.8	-21.17	74.4	-25.60	88.0	-12.01
1,2,3,4,6,7,8-HpCDD	100	26-166	67.61	-32.39	58.8	-41.20	55.3	-44.70	52.9	-47.09
1,2,3,4,6,7,8,9-OCDD	200	26-397	46.00	-77.00	38.4	-80.80	38.9	-80.55	32.8	-83.60
2,3,7,8-TCDF	100	22-152	80.23	-19.77	69.8	-30.20	65.3	-34.72	67.6	-32.41
1,2,3,7,8-PeCDF	100	21-192	70.40	-29.60	58.8	-41.22	47.9	-52.07	56.1	-43.94
2,3,4,7,8-PeCDF	100	13-328	69.56	-30.44	59.5	-40.53	40.7	-59.31	57.5	-42.46
1,2,3,4,7,8-HxCDF	100	19-202	94.08	-5.92	77.2	-22.77	77.8	-22.19	83.6	-16.40
1,2,3,6,7,8-HxCDF	100	21-159	102.19	2.19	85.4	-14.62	83.4	-16.56	90.9	-9.12
2,3,4,6,7,8-HxCDF	100	22-176	92.31	-7.69	73.6	-26.43	70.2	-29.84	78.7	-21.27
1,2,3,7,8,9-HxCDF	100	17-205	85.94	-14.06	67.3	-32.72	55.1	-44.90	70.8	-29.22
1,2,3,4,6,7,8-HpCDF	100	21-158	74.77	-25.23	62.9	-37.09	57.0	-43.01	64.8	-35.23
1,2,3,4,7,8,9-HpCDF	100	20-186	60.88	-39.12	49.4	-50.61	44.8	-55.17	48.1	-51.95

Table 5. Comparison of low concentration level of PCDD/Fs in corn oil (IPR) using soxhlet and ASE techniques

PCDDs/PCDFs Native Compounds	Expected Extract Conc., ng/ml	Accepted* Range (ng/ml)	Soxhlet		ASE		Evaluation
			Average (ng/ml)	CV%**	Average (ng/ml)	CV%**	Deviation%
2,3,7,8-TCDD	0.4	0.268-0.632	0.39	8.4	0.47	3.3	20
1,2,3,7,8-PeCDD	2	1.4-2.84	2.13	9.9	2.61	1.2	24
1,2,3,4,7,8-HxCDD	2	1.4-3.28	2.12	16.5	2.66	4.9	27
1,2,3,6,7,8-HxCDD	2	1.52-2.68	2.09	11.0	2.47	5.0	19
1,2,3,7,8,9-HxCDD	2	1.28-3.24	2.03	7.1	2.29	7.1	13
1,2,3,4,6,7,8-HpCDD	2	1.64-2.44	1.99	15.3	2.14	9.0	7.5
1,2,3,4,6,7,8,9-OCDD	4	3.12-5.76	3.95	16.5	4.59	5.7	16
2,3,7,8-TCDF	0.4	0.3-0.632	0.40	2.8	0.56	6.3	40
1,2,3,7,8-PeCDF	2	1.6-2.68	2.18	13.7	2.56	3.0	19
2,3,4,7,8-PeCDF	2	1.36-3.2	2.17	13.7	2.70	2.3	26.5
1,2,3,4,7,8-HxCDF	2	1.44-2.68	2.22	20.0	2.88	13.7	33
1,2,3,6,7,8-HxCDF	2	1.68-2.6	2.10	10.8	2.38	3.8	14
2,3,4,6,7,8-HxCDF	2	1.4-3.12	2.12	17.8	2.58	4.6	23
1,2,3,7,8,9-HxCDF	2	1.56-2.6	1.97	3.4	2.33	10.7	18
1,2,3,4,6,7,8-HpCDF	2	1.64-2.44	2.18	14.4	3.33	18.8	57.5
1,2,3,4,7,8,9-HpCDF	2	1.56-2.76	1.96	7.0	2.12	5.5	8
1,2,3,4,6,7,8,9-OCDF	4	2.52-6.8	3.79	11.0	4.27	0.8	12
PCDDs/PCDFs Labeled Compounds	**Expected Extract Conc., ng/ml**	**Accepted* Range Recovery**	**Average Recovery%**	**CV%**	**Average Recovery%**	**CV%**	**Evaluation Deviation%**
2,3,7,8-TCDD	100	20-175	74.9	26.0	54.3	27.2	-20.6
1,2,3,7,8-PeCDD	100	21-227	48.5	28.3	47.7	28.8	-0.8
1,2,3,4,7,8-HxCDD	100	21-193	83.3	27.1	67.3	27.6	-16
1,2,3,6,7,8-HxCDD	100	25-163	88.2	23.5	79.6	27.6	-8.6
1,2,3,4,6,7,8-HpCDD	100	26-166	67.0	23.9	45.0	18.0	-22
1,2,3,4,6,7,8,9-OCDD	100	26-397	53.8	22.6	27.6	8.2	-26.2
2,3,7,8-TCDF	100	22-152	84.0	24.6	60.9	29.7	-23.1
1,2,3,7,8-PeCDF	100	21-192	74.7	24.8	51.3	21.2	-23.4
2,3,4,7,8-PeCDF	100	13-328	70.0	25.0	47.9	28.0	-22.1
1,2,3,4,7,8-HxCDF	100	19-202	86.1	24.9	76.9	24.5	-9.2
1,2,3,6,7,8-HxCDF	100	21-159	90.2	23.3	85.6	21.8	-4.6
2,3,4,6,7,8-HxCDF	100	22-176	82.4	27.5	71.1	28.8	-11.3
1,2,3,7,8,9-HxCDF	100	17-205	79.8	29.1	61.5	28.3	-18.3
1,2,3,4,6,7,8-HpCDF	100	21-158	71.4	27.0	50.8	23.3	-20.6
1,2,3,4,7,8,9-HpCDF	100	20-186	62.1	22.9	37.8	13.1	-24.3

Performance characteristics:

*** The results within IPR Acceptable Criteria according to EPA method-1613**

**** Intermediate precision (RSD %) ≤ 20 %**