Application Note

Polychlorinated Dibenzo-p-dioxins, Furans and Biphenyls in Fish: Automated Sample Processing

Introduction

The occurrence of polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs) and biphenyls (PCBs) in a variety of foods has been amply documented. This includes fish for human consumption. Some evidence has been found for a relationship between concentrations in sediment and fish tissue. Analyses of fish samples using US EPA methods 1613 (PCDD/Fs) and 1668 (PCBs) have been carried out around the world. Traditional Soxhlet extraction and sample clean up are time consuming and can result in data of low quality and reproducibility. This application note describes the automated Pressurized Liquid Extraction (PLE) and automated open column chromatography clean up (PowerPrep) of fish tissue. Quick and easy processing results in samples being ready for same-day analysis.

Instrumentation

- FMS, Inc. PLE®
- FMS, Inc. PowerPrep®
- FMS, Inc. SuperVap® 6 Concentrator
- FMS, Inc. SuperVap® Vial Concentrator
- FMS, Inc. 250 mL concentrator tubes (1 mL termination)
- Thermo Trace GC Ultra with high res magnetic sector DFS Thermo mass spec

Consumables

- FMS, Inc. Jumbo Acidified Silica column
- FMS, Inc. Classical Acid-Base-Neutral column
- FMS, Inc. Basic Alumina column
- FMS, Inc. Carbon-Celite column
- Millipore OmniSolv® Benzene
- Fisher Optima® Dichloromethane
- Fisher Optima® Ethylacetate
- Fisher Optima® Hexane
- Fisher Optima ® Toluene
- Cambridge Isotope Labs (CIL) EDF-2526 Fortified Fish Reference Material
- CIL EDF-8999 Method 1613 ^{13}C PCDD/F Stock Solution
- CIL EDF-5999 ^{13}C PCDD/F Recovery Standard
- CIL EC-4995 ^{13}C PCB Internal Isotope Dilution Standard who-12 PCB and 170/180
- CIL EO-5275 ^{13}C PCB Recovery Standard

PLE

- 5 g of sample mixed with 10 g inert Hydro-matrix® and spiked with surrogates
- Sample placed in extraction cell
- Capped with disposable Teflon end caps
- Heated with 50% Dichloromethane/50% Hexane for 20 min at 120 ^{o}C and 1500 psi
- 20 min cool down
- Nitrogen flush to transfer analytes and extract to 250 mL collection tubes

SuperVap Concentration

- Pre-heat temperature: 45 ^{o}C
- Pre-heat time: 15 min
- Heat in Sensor mode: 45 ^{o}C
- Nitrogen Pressure: 6-8 psi
- Solvent exchange to hexane

PowerPrep Clean Up

- Standard 25-step program
- Install jumbo silica, classical ABN, alumina and carbon/celite columns
- Mixes used are hexane, 2%/98% dichloromethane/hexane, 50%/50% dichloromethane/hexane, 50%/50% ethylacetate/benzene, and toluene

FMS
Fluid Management Systems

- Run conditioning steps 1-13 with columns in place
- Load sample (in hexane)
- Elute silica with 150 mLs hexane (waste)
- Elute alumina with 60 mLs 2%/98% DCM/ hexane (collect as F1)
- Elute alumina with 120 mLs 50%/50% DCM/hexane (collect as F1)
- Elute carbon with 4 mL 50%/50% ethyl-acetate/benzene (collect as F1)
- Elute carbon with 75 mLs toluene (collect as F2)

SuperVap step (above)

Vial Evaporator
- Reduce sample to 10 uL final volume under 1-1.5 psi nitrogen at 25 ^{o}C

Table with native fish tissue values, reference material values and ^{13}C-labeled recoveries.

	native pg/g	reference value pg/g	recoveries %
2378-T4CDF	19.79	18.7 ± 9.35	87%
2378-T4CDD	20.40	19.8 ± 0.099	87%
12378-P5CDF	36.34	39 ± 19	91%
23478-P5CDF	37.77	37.8 ± 18.9	90%
12378-P5CDD	38.02	40 ± 20	93%
123478-H6CDF	74.62	83.3 ± 41.7	87%
123678-H6CDF	56.72	62.8 ± 31.4	83%
234678-H6CDF	54.72	58.6 ± 29.3	84%
123789-H6CDF	51.78	57.3 ± 28.6	89%
123478-H6CDD	47.42	54.9 ± 27.4	83%
123678-H6CDD	50.13	51.1 ± 25.5	83%
123789-H6CDD	50.48	52.9 ± 26.4	
1234678-H7CDF	76.24	81.6 ± 41.3	83%
1234789-H7CDF	70.84	76.7 ± 38.8	87%
1234678-H7CDD	65.61	70.7 ± 35.3	87%
OCDF	173.68	185 ± 92.5	
OCDD	166.25	181 ± 90.5	79%

Table with native fish tissue values, reference material values and ^{13}C-labeled recoveries.

		native pg/g	reference value pg/g	recoveries %
33'44'-T4CB	77	612.91	451 ± 225	77%
344'5-T4CB	81	1.89	3.0 ± 1.5	68%
233'44'-P5CB	105	105.80	108 ± 54	58%
2344'5-P5CB	114	8.15	7.73 ± 3.86	66%
23'44'5-P5CB	118	298.35	348 ± 174	45%
2'344'5-P5CB	123	39.47		67%
33'44'5-P5CB	126	419.82	431 ± 215	62%
233'44'5-H6CB	156	17.35	23.3 ± 11.6	64%
233'44'5'-H6CB	157	5.58	9.3 ± 4.6	74%
23'44'55'-H6CB	167	12.55	12.0 ± 6.0	78%
33'44'55'-H6CB	169	477.50	512 ± 256	90%
233'44'55'-H7CB	170	29.93		89%
22'344'55'-H7CB	180	99.94	116 ± 58	83%
233'44'55'-H7CB	189	1.84	3.51 ± 1.75	90%

Conclusions

Excellent agreement was found between the PCDD/Fs and PCBs concentrations found in our laboratory and the reference values listed for this fortified fish tissue. ^{13}C recoveries of the labeled compounds were very good. Extraction, clean up and analysis by properly trained personnel can be carried out in one day, resulting in low turnaround times for sample batches of any size.

PowerPrep, PLE, and Concentrator

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