Application Note

Use of Pressurized Liquid Extraction and Automated Column Chromatography in the Analysis of Polychlorinated Dibenzo-p-dioxins, Furans and Biphenyls in Human Serum

Introduction

Analysis of polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs) and biphenyls (PCBs) in human serum is routinely carried out as part of biomonitoring studies. Such studies are of great importance in assessing the effects of these toxic compounds on human health. In the United States these analyses have been carried out, among others, by the Centers for Disease Control (CDC) in the N-HANES studies. Methods followed in these analyses are typically US EPA methods 1613 and 1668.

Use of Pressurized Liquid Extraction (PLE) and Automated Multi-Layer Column Chromatography (PowerPrep) can greatly reduce the time needed for sample prep and results in more accurate and reliable data. The procedure described here can be easily carried out in one 8 h work day, resulting in same day GC/MS 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. Classical Acid-Base-Neutral column
- FMS, Inc. Basic Alumina column
- FMS, Inc. Carbon-Celite column
- Fisher Optima® Formic acid
- Millipore OmniSolv® Benzene
- Fisher Optima® Dichloromethane
- Fisher Optima® Ethylacetate
- Fisher Optima® Hexane
- Fisher Optima ® Toluene
- NIST 1958 SRM Fortified Human Serum
- Cambridge Isotope Labs (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

Sample Prep

- 10.7 g NIST-1958 Human Serum was treated with 0.5 g formic acid per mL

PLE

- 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 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 90 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 serum values, NIST reference values and ^{13}C-labeled recoveries.

	native pg/g	**NIST1958 pg/g**	**recoveries %**
2378-T4CDF	0.097	0.11 ± 0.055	68%
2378-T4CDD	0.084	0.097 ± 0.048	71%
12378-P5CDF	0.083	0.11 ± 0.055	73%
23478-P5CDF	0.180	0.22 ± 0.11	74%
12378-P5CDD	0.091	0.11 ± 0.055	81%
123478-H6CDF	0.086	0.10 ± 0.050	68%
123678-H6CDF	0.089	0.11 ± 0.055	68%
234678-H6CDF	0.670	0.96 ± 0.48	68%
123789-H6CDF	0.088	0.10 ± 0.050	74%
123478-H6CDD	0.100	0.099 ± 0.049	73%
123678-H6CDD	0.270	0.36 ± 0.18	70%
123789-H6CDD	0.090	0.10 ± 0.050	
1234678-H7CDF	0.200	0.31 ± 0.15	68%
1234789-H7CDF	0.120	0.086 ± 0.043	78%
1234678-H7CDD	0.410	0.59 ± 0.29	73%
OCDF	0.130	0.089 ± 0.044	
OCDD	1.540	2.75 ± 1.37	75%

Table with native serum values, NIST reference values and ^{13}C-labeled recoveries.

		native pg/g	NIST1958 pg/g	recoveries %
33'44'-T4CB	77	5.22		86%
344'5-T4CB	81	0.78		85%
233'44'-P5CB	105	527.08	425 ± 127	67%
2344'5-P5CB	114	52.72		69%
23'44'5-P5CB	118	519.74	418 ± 125	63%
2'344'5-P5CB	123	53.62		67%
33'44'5-P5CB	126	nd		85%
233'44'5-H6CB	156	539.18	424 ± 127	63%
233'44'5'-H6CB	157	506.05	426 ± 127	63%
23'44'55'-H6CB	167	516.54	409 ± 122	60%
33'44'55'-H6CB	169	10.38		81%
233'44'55'-H7CB	170	527.73	429 ± 128	60%
22'344'55'-H7CB	180	558.40	470 ± 141	59%
233'44'55'-H7CB	189	454.53	409 ± 122	48%

Conclusions

As can be seen the serum analysis showed excellent agreement between the values found with our extraction and clean up method and the acceptable reference values provided for this fortified human serum. The concentrations found were in the low ppt range demonstrating the sensitivity of this method. The whole process of extraction, clean up and analysis by properly trained personnel can be carried out in one day, resulting in same-day data collection from start to finish.

For more information contact FMS:
FMS, Inc.
580 Pleasant Street
Watertown, MA 02472
Phone: (617) 393-2396
Fax: (617) 393-0194
Email: onlineinfo@fms-inc.com
Web site: www.fms-inc.com