Application Note

Dioxins, PCBs and PBDEs in Human Serum Using Automated Pressurized Liquid Extraction, Multi-Column Cleanup, and Concentration via EPA Methods 1613 and 1668

Introduction

Because of its complexity, human serum is one of the most challenging sample matrices encountered. Organic contaminants often exist at low lipid concentrations (~600 mg/dl) that require extremely low detection levels and ultra clean blanks. The manual sample preparation process for human serum consists of multiple, time-consuming steps that are messy, difficult to reproduce and yield inaccurate results.

By combining the analysis of multiple analytes into a single extraction and concentration method using the Pressurized Liquid Extraction (PLE) system, the PowerPrep® system to fractionate analyte classes, and the SuperVap® Concentrator, the sample preparation process can be streamlined into a rapid, reproducible method.

Instrumentation

- FMS, Inc. PLE® System
- FMS, Inc. PowerPrep® System
- FMS, Inc. SuperVap® Concentrator
- FMS, Inc. 200 mL direct-to-vial concentrator tubes
- FMS, Inc. 200 mL concentrator tubes (1 mL termination)
- Thermo Scientific Trace Ultra GC with Quantum TSQ
- Thermo Scientific Trace Ultra GC with DFS HRMS

Consumables

- FMS, Inc. PBDE free high capacity acidic silica columns
- FMS, Inc. PBDE free Alumina columns
- FMS, Inc. PBDE free Carbon columns
- Fisher Optima* Toluene
- Fisher Optima* n-Hexane
- Fisher Optima* Methylene Chloride
- Agilent Hydromatrix
- Fisher Formic Acid
- NIST 1958 RM; Fortified Human Serum
- Method 1613 and 1668 $^{13}C_{12}$ spiking and recovery standards

Procedure

1. PLE cells are filled with Hydromatrix (baked at 500 ºC).
2. Sample amounts are measured (up to 20 mLs)
3. Serum samples are spiked with appropriate labeled surrogates for analytes of interest
4. Samples are transferred to extraction cells via a large volume pipettor.
5. Formic acid is added to samples on a 1:5 ratio
6. Cells are capped and allowed to equilibrate for 30 minutes before loading onto the PLE system

Pressurized Liquid Extraction system

1. Cells filled with hexane: DCM (50:50)
2. Cells pressurized to 1500 PSI
3. Cells heated to 120 ºC (2 cycles, 20 and 10 min)
4. Cells cooled to ambient temperature
5. Cells flushed with 20 mL solvent
6. Cells purged with N_2 and extract discharged to SuperVap Concentrator.

SuperVap concentration system

1. Preheat temp: 20 minutes at 60 °C
2. Evap mode w/Sensor temp: 60 °C
3. Nitrogen Pressure: 7-10 PSI

FMS
Fluid Management Systems

PowerPrep system

1. Columns conditioned with hexane
2. Load sample extract(s)
3. Columns eluted with Hexane and collected (some PCBs and PBDEs)
4. Alumina and Carbon columns eluted with DCM and collected (mono- and di-ortho PCBs, remaining PBDEs)
5. Carbon eluted in reverse direction with toluene (PCDD/Fs and coplanar PCBs)

SuperVap concentration system

4. Preheat temp: 20 minutes at 60 °C
5. Evap mode w/Sensor temp: 60 °C
6. Nitrogen Pressure: 7-10 PSI

SuperVap Vial Evaporator

1. Samples 1 mL in GC vials
2. Add recovery standards
3. Reduce volume to ~ 10 uL at 25 ^{o}C and 1-2 psi Nitrogen for final analysis

Results

Table 1. Mean recoveries and deviations for labeled compounds over 20 samples.

Compound	Mean Rec.	STD DEV
2,3,7,8-TCDD	67.4%	8.5%
1,2,3,7,8-PeCDD	78.1%	9.8%
1,2,3,4,7,8-HxCDD	81.8%	10.0%
1,2,3,6,7,8-HxCDD	67.8%	8.0%
1,2,3,7,8,9-HxCDD	81.1%	9.9%
1,2,3,4,6,7,8-HpCDD	60.9%	7.0%
OCDD	60.6%	6.3%
2,3,7,8-TCDF	71.7%	8.8%
1,2,3,7,8-PeCDF	76.6%	9.2%
2,3,4,7,8-PeCDF	78.2%	10.0%
1,2,3,4,7,8-HxCDF	75.5%	9.0%
1,2,3,6,7,8-HxCDF	75.8%	8.9%
2,3,4,6,7,8-HxCDF	75.1%	9.1%
1,2,3,7,8,9-HxCDF	96.9%	13.0%
1,2,3,4,6,7,8-HpCDF	69.6%	7.1%
1,2,3,4,7,8,9-HpCDF	NA	NA
OCDF	68.7%	10.0%
PBDE-28	65.1%	19.1%
PBDE-47	73.1%	22.3%
PBDE-100	74.6%	25.1%
PBDE-99	79.5%	25.5%
PBDE-154	78.5%	25.6%
PBDE-153	85.8%	26.9%
PBDE-183	98.3%	29.9%
PBDE-209	95.5%	25.7%
PCB-28	81.6%	27.8%
PCB-52	80.0%	25.9%
PCB-101	81.0%	28.7%
PCB-105	75.0%	21.3%
PCB-114	78.0%	20.8%
PCB-118	69.5%	19.8%
PCB-123	74.9%	21.4%
PCB-128	75.2%	21.2%
PCB-138	75.1%	21.4%
PCB-153	77.5%	22.2%
PCB-156	71.0%	20.5%
PCB-157	67.7%	19.9%
PCB-167	65.0%	21.4%
PCB-170	86.4%	19.3%

Table 1 continued

Compound	Mean Rec.	STD DEV
PCB-178	63.7%	22.9%
PCB-180	65.2%	18.6%
PCB-189	71.7%	18.1%
PCB-194	86.4%	21.4%
PCB-206	82.9%	23.0%
PCB-209	100.8%	23.4%
PCB-77	73.4%	9.2%
PCB-81	67.9%	7.5%
PCB-126	78.9%	10.7%
PCB-169	101.3%	14.0%

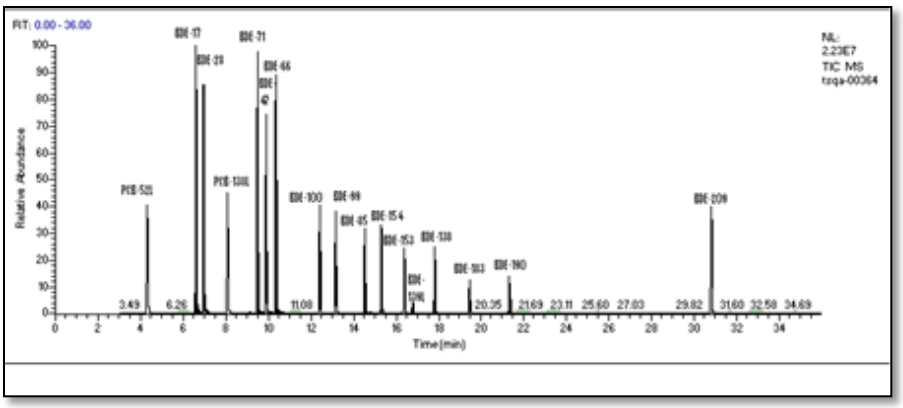
Table 2. Results of NIST 1958 analysis.

Compound	Calc. Conc.	Cert Value
2,3,7,8-TCDD	80	97.3
1,2,3,7,8-PeCDD	90	114
1,2,3,4,7,8-HxCDD	10	98.5
1,2,3,6,7,8-HxCDD	270	363
1,2,3,7,8,9-HxCDD	90	103
1,2,3,4,6,7,8-HpCDD	410	595
OCDD	2540	2750
2,3,7,8-TCDF	100	107
1,2,3,7,8-PeCDF	80	107
2,3,4,7,8-PeCDF	180	221
1,2,3,4,7,8-HxCDF	90	102
1,2,3,6,7,8-HxCDF	90	110
2,3,4,6,7,8-HxCDF	670	958
1,2,3,7,8,9-HxCDF	90	99.6
1,2,3,4,6,7,8-HpCDF	ND	NA
1,2,3,4,7,8,9-HpCDF	120	86.2
OCDF	130	88.6
PBDE-28	425.74	470
PBDE-47	750.31	661
PBDE-100	417.14	482
PBDE-99	449.02	499
PBDE-154	359.62	450
PBDE-153	376.31	460
PBDE-183	365.71	461
PBDE-209	366.41	415

Table 2. continued

Compound	Calc. Conc.	Cert Value
PCB-105	437.04	415
PCB-114	41.13	47.4
PCB-118	412.13	418
PCB-123	41.02	54.4
PCB-156	381.57	424
PCB-157	343.81	426
PCB-167	341.44	409
PCB-170	339.70	429
PCB-180	358.75	470
PCB-189	380.17	409
PCB-77	NA	NA
PCB-81	NA	NA
PCB-126	7890	8050
PCB-169	8013	8400

Figure 1: TIC of PBDE run on the Quantum TSQ



Conclusions

The results of the 20 sample study indicate that Pressurized Liquid Extraction (PLE) combined with the PowerPrep multi-column fractionation and SuperVap® Concentration systems generated efficient extractions. The relatively low deviation between recoveries demonstrates the robustness of the extraction process as well as the ability to deliver a high level of reproducibility across multiple samples. The calculated concentrations of the NIST 1958 extraction further demonstrate the efficiencies of the extraction and the ability to recover native compounds from the matrix. Also, the need to perform an additional cholesterol removal step, typically required with traditional SPE extractions of human serum, was eliminated.

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