

Optimized Workflow of POPs Analysis in Environmental and Food Matrices Using Semi-Automated Cleanup and GC-MS/MS





FMS, Inc.

- Founded in 1986 based in USA
- Manufactures Total Solution Sample Preparation Systems and Consumables for GC, GC/MS, LC and LC/MS
- Manual Dioxin/PCB sample preparation is tedious and comprehensive
- FMS provides total solution Dioxin/PCB Automated and Semi-Automated Sample Preparation Systems
- Fast, Consistent, Reliable Results
- Suitable for all kinds of Environmental, Food and Feed samples
- Complies with EPA methods and the criteria of the European legislation



Overview

- Introduction
- Sample Prep Workflow
- Automated Hyphenated System Configurations for different Matrices and Extractions
- Semi Automated Systems





Laboratory Workflow Breakdown







Background

- POPs (PCDD/Fs, PCBs) continue to attract interest around the world due to strict regulations enforced in many countries
- Rapid, quality sample clean up and analysis is needed for many laboratories processing samples
- Processing times and cost are important considerations
- In the US, EPA methods SW-846, 1613, 1668 and 8082A are used for PCBs and PCDD/Fs work
- Sample extracts in DCM, hexane or toluene
- Cleanup for analysis of all 209 PCBs in common in North America extracts are often in toluene after Soxhlet Extraction



Challenges of POPs Sample Prep

- Labor intensive, prone to error
- Compliance with regulatory procedures and accreditation (lengthy method validation)
- Strict QA/QC requirements
- Sample matrix complexity
- Native background and interferences (can be orders of magnitude higher than analytes)
- Pico/femto-gram analyses require ultra pure extract and excellent instrument sensitivity



Automated Sample Prep

• Advantages of Automated Sample Prep

- Rapid Turn Around Time:
- Cleaner Background Interferences:
- Quality Results:
- Green Technology:
- QA/QC & Accreditation Requirements:
- Computerized Method:

- 30 to 45 Minutes for 6 Samples
- Closed Loop System
- Certified Pre-packaged Columns
- Lower solvent and power use
- Easier to Manage
- Instrumentation based prep



Manual Sample Prep

• Advantages of Manual Sample Prep

- Most labs use a Manual Methods for the following reasons:
 - No electronics or mechanical components to fail
 - No down time due to the system failure
 - No service contract
 - No capital equipment cost





Sample Processing Workflow

- Analysis of various matrices for PCDD/Fs and PCBs using extraction, clean up and concentration.
- Soxhlet extraction (typically up to 24-36 h).
- Concentration step
- Preparative multi column chromatography involving various solvents and steps.
- Can include acid-base-neutral silica, pure acidified silica, alumina, florisil and carbon columns. Use of H2SO4 acid mixed with silica; NaOH mixed with silica Neutral Silica
- Fractionation and Concentration





Sample Prep and Analysis WorkFlow in Hours vs Days

= 2hrs

FMS Automated Sample Prep Time

1 up to 8 samples





GC/MS 45 Min

Manual Sample Prep Time = 2 to 4 days

Extraction	Concentration	Sample Cleanup	Concentration	GC/MS
				45 Min
24 hours	60 Min	24 to 48 hours	60 Min	





Sample Preparation consist of three main instruments:

1- Extraction 2- Sample Clean-Up 3- Concentration

= Total Prep





Extraction Procedure

- 1 g sample mixed with Hydromatrix[™] to dry, transferred to extraction cells
- Spiked with ¹³C PCDD/Fs and PCBs standards.
- Void volume filled with Hydromatrix[™].
- Sample Cells filled with 50% mixture Hexane/Methylene Chloride.
- Cells pressurized to 1500 PSI and heated to 120 °C.
- Temperature held for 20 minutes.
- Extraction cells cooled, flushed with solvent (50% cell volume), and nitrogen
- Sent directly to the SuperVap collected in 250 mL tubes and automatically concentrated



Automation

- Advantages of automated sample prep are:
- Reduced time:
 - Automated Pressurized Liquid Extraction (PLE) takes 60 min start-to-finish
 - Manual Soxhlet up to 36 h.
- Reduced cost: less labor involved, shorter turnover time per sample, less electricity use for PLE than Soxhlet.
- Reduced volume: less solvent used.





SuperVap Evaporation

- System pre-heated to 45-60 °C.
- Extracts evaporated at stable temperature under 5-6 psi nitrogen.
- Solvent exchange with hexane to eliminate dichloromethane.
- Dichloromethane would interfere with subsequent sample clean up.
- Extracts reduced to a few mLs.





SuperVap Concentration







Automating Sample Prep

- Automated FMS Pressurized Liquid Extraction (PLE) for sample extraction is fast (60 min), efficient (120 °C, 1500 psi), green (less power), reliable (long track record).
- FMS Solid Phase Extraction for serum and water is fully automated, fast (less time than manual), low background (closed system), versatile for many cartridges and sample sizes.
- Users' choice of FMS fully automated or semi automated clean up system: fast (20-60 min), low solvent usage



Fluid Management Systems PowerPrep Clean-up





Expandable and Modular





PowerPrep Clean-up

- Dioxin / PCB / PBDE Cleanup and Fractionation
 - Multicolumn Cleanup
 - Silica (Jumbo / Classic/ ABN)
 - Alumina
 - Carbon
- Florisil cleanup
- Custom Clean-up
 - Ability to combine and manufacture any mix of columns





Columns





System Flexibility

- With the combination of the PLE, SuperVap and PowerPrep NG you are given the flexibility to automate your extraction, concentration and clean-up for a wide variety of compounds.
- This is an ideal system for:
 - Laboratories that receive varying, different sample types and compounds of interest
 - High volume labs with a consistent daily sample amount load
 - Research laboratories looking to improve efficiency, recoveries and reproducibility



SuperVap Concentrator





SuperVap Evaporation

- System pre-heated to 45-60 °C.
- Samples evaporated at stable T under 8 psi nitrogen.
- 1 mL extract vial transferred to GC vial (can have direct-to-vial feature).
- Recovery standards added (nonane/dodecane).
- •Extract taken to 10 uL volume with a gentle stream of nitrogen at ambient temperature.





24 position vial evaporator





Glass Evaporation tube





Using the Agilent 7010 GC-MS/MS





Agilent 7010 MS/MS Dioxin Analysis

Compliant

• Through use of an (ATP) Alternative Test Procedure for the EPA Methods and the new European Union Commission Regulations

Excellent Sensitivity

- 7010 GC/MS/MS detector meets detection requirements of the EPA methods and European regulations and is 10x more sensitive
- Cost Effective alternative to High Res Mass Spec Systems





Agilent 7010 GC-MS/MS Precision







Agilent 7010 GC-MS/MS Limit of Detection





Mean PCDD/F recoveries (6 edible oils)

	Mean	Dev	Blk Conc.
Analyte			
2378TCDF	70	8.5	< .1 pg/g
2378TCDD	78	8.6	< .1 pg/g
12378PeCDF	83	13.5	< .5 pg/g
23478PeCDF	81	10.7	< .5 pg/g
12378PeCDD	81	11.6	< .5 pg/g
123478HxCDF	70	7.1	< .5 pg/g
123678HxCDF	62	3.6	< .5 pg/g
234678HxCDF	71	10.0	< .5 pg/g
123789HxCDF	66	6.9	< .5 pg/g
123478HxCDD	81	11.3	< .5 pg/g
123678HxCDD	77	9.4	< .5 pg/g
123789HxCDD	NA	NA	< .5 pg/g
1234678HpCDF	73	5.0	< .5 pg/g
1234789HpCDF	85	9.0	< .5 pg/g
1234678HpCDD	75	7.1	< .5 pg/g
OCDD	70	3.6	< 1 pg/g
OCDF	NA	NA	< 1 pg/g



Mean PCBs recoveries (6 oils)

	Mean	Dev	Blk Conc.
PCB-77	73	14.9	< .5 pg/g
PCB-81	64	11.0	< .5 pg/g
PCB-105	75	15.2	< .5 pg/g
PCB-114	73	11.4	< .5 pg/g
PCB-118	73	8.5	< .5 pg/g
PCB-123	72	8.0	< .5 pg/g
PCB-126	88	19.7	< .5 pg/g
PCB-156	63	7.4	< .5 pg/g
PCB-157	53	8.7	< .5 pg/g
PCB-167	63	6.1	< .5 pg/g
PCB-169	75	10.4	< .5 pg/g
PCB-170	79	9.4	< .5 pg/g
PCB-180	77	14.2	< .5 pg/g
PCB-189	80	9.8	< .5 pg/g



Conclusions

- Analysis of the 6 Oil matrices processed yielded acceptable recoveries for all analytes with standard deviations below 20%.
- Analysis of an n-Hexane blank sample resulted in no detectable target analytes measured within the calibration range of each respective compound.
- With a total processing time of less than 2.5 hours, the FMS PowerPrep® and SuperVap® Concentrator delivers an efficient, totally automated sample prep process for edible oils.





TotalPrep®







EP-110[®] Clean Up Zero DCM







System Characteristics

- Uses Zero DCM
- Control module that pilots valve drive modules connected to a pump and pressure modules responsible for solvent flow in the valve module.
- Built in computer that does not need a stand-alone pc.
- Easy programming and software editing provides custom made sequences of events that drive the required solvent at the right place at the right moment.
- Low pressure (5-30 psi). Flow rates of 5-10mL/min are used. Nitrogen valve enables push through sample lines.
- Modular and Expandable 1 to 4 modules (2 samples per module up to 8 samples total)





Column Kits




Columns

- Silica PCB/PBDE-free Acid, Base and Neutral silica gel column (mini, classical, classical plus, high capacity, XL).
- Alumina PCB/PBDE-free basic alumina column.
- Carbon PCB/PBDE-free carbon/celite column.
- Packed in disposable Teflon tubes; individually sealed in Mylar packaging; production in clean room environment.



EP-110 Features

- EP-110 fully automated sample load and elution.
- Load Sample Extracts in hexane directly onto the system with no Manual Pretreatment
- Easy to perform QC sample simultaneously with a Real sample.
 - 2 samples per module
- Different column configuration: silica-carbon-alumina.
- Uses no DCM, only Hexane and Toluene.
- Total Clean Up time 20-45 min.
- Low volumes 100-250 mLs.





Recoveries various matrices



Sediment 1g Feed 2g Egg 8 g Fish Oil 40 mg Fatty Hexane



120

13C PCBs Recoveries Matrices







EconoPrep[®] for traditional Pops Sample Cleanup

Touch Screen Control _ SD Card for Storing and Transferring Methods _____

> 2 Sample Positions per module

Automatic and Manual Sample Loading



Real Time Graphical Display of Extraction Steps

Clean Up and Fractionate up to Eight Extracts in Parallel

> Direct to Vial Concentration

Positive Pressure Pumps for precise, consistent delivery of Sample and Solvent



Expandable and Modular

- Low Cost POPs analysis
- Runs 2 samples per Module
 In parallel
- Expandable up to 4 Modules
- Run up to 8 samples in Parallel
- Run up to 8 samples in 30 to 40 minutes





Expandable

EconoPrep 4 Capable of running 8 Samples in Parallel





Columns

- Silica PCB/PBDE-free Acid, Base and Neutral silica gel column (mini, classical, classical plus, high capacity, XL).
- Alumina PCB/PBDE-free basic alumina column.
- Carbon PCB/PBDE-free carbon/celite column.
- Packed in disposable Teflon tubes; individually sealed in Mylar packaging; production in clean room environment.





Classical PCB data



Classical Up to 0.4 gm lipid

PCB / Dioxin Fractions





Classical PCDD/F data



Position 1 Position 2



Simple, Quick & Low Cost Semi-Automated Clean-Up for Dioxins/PCBs





Automated Sample Prep

• Advantages of Automated Sample Prep

- Rapid Turn Around Time:
- Cleaner Background Interferences:
- Quality Results:
- Green Technology:
- QA/QC & Accreditation Requirements:
- Computerized Method:

30 to 60 Minutes for 6 Samples Closed Loop System Certified Pre-packaged Columns Lower solvent and power use Easier to Manage Instrumentation based prep





Manual Sample Prep

- Advantages of Manual Sample Prep
 - Most labs use a Manual Methods for the following reasons:
 - No electronics or mechanical components to fail
 - No down time due to the system failure
 - No service contract
 - No capital equipment cost





Semi-Automated System

Specification:

- Simple to run, no computerized instrumentation
- Fast: 60 min
- Closed loop system to give a clean background, low level detection
- Use certified pre-packaged columns
- Green technology, only vacuum pump uses power
- Low solvents, as low as 100 mL for serum
- Economical column kits, choice of low fat and high fat column kits
- No capital equipment cost
- No electronics or mechanical equipment to fail
- No downtime



Characteristics of Semi-Automated System (EZPrep)











Column Kits





Stage 1 Manifold















Stage 1: to waste





Stage 2: collect





Attributes

- Closed loop system:
 - Eliminates background contaminants
 - No washing needed.
 - Capped solvent reservoirs
- Optimized for solvent reduction while obtaining highest possible recoveries
- Uses Hexane and Toluene, no Dichloromethane
- Easy sample loading on top of silica column via syringe vial
- Columns connect easy with SNAP connections





Attributes

- Order of columns is Silica-Carbon-Alumina
- Columns are assembled and vacuum turned on
- Conditioning on top of silica column via syringe vial (Stage 1 to waste, use 20-60 mL hexane through all columns)
- Load sample into syringe vials and pull through column assembly (Stage 1, waste)
- Elute columns with hexane (80-180 mL) and transfer all target compounds to carbon and alumina (Stage 1, waste)
- Discard silica columns and remove carbon and alumina columns





Attributes

Rotate turntable (Stage 2)

- Install carbon and alumina columns
- Elute carbon and alumina columns in reverse each individually with 40 mL toluene and collect
- Collect Carbon Fraction 1 with PCDD/F and co-PCBs
- Collect Alumina Fraction 2 with mono- and di-ortho PCBs (and PBDEs)
- Collection step ~ 3-5 min





12 position evaporator 50 mL







SuperVap Evaporation

- System pre-heated to 55-60 °C.
- Samples evaporated at stable T under 6-8 psi nitrogen.
- 1 mL extract vial transferred to GC vial (can have direct-to-vial feature).
- Recovery standards added (nonane/dodecane).
- Extract taken to 10 uL volume with a gentle stream of nitrogen at ambient temperature.





24 position Vial evaporator 1-2 mL







Direct-to-Vial







¹³C PCBs Recoveries EZPrep (%)







¹³C PCDD/F Recoveries EZPrep (%)

13C Dioxins various matrices





Comparison of Native Data with Automated System

PCBs comparison 2 g fish oil





EZPrep Conclusions

- EZprep123 is a low solvent and the fastest clean up system for Dioxins and PCBs (30 - 75 min)
- High sample throughput \rightarrow 18 samples/hour
 - 6 samples in parallel per station
 - 3 stations fit in one hood
- System gives excellent recoveries for PCDD/F, PCB and PBDEs comparable to FMS automated systems
- Use of certified pre-packaged columns guarantees low native background





EZPrep Conclusions

- No DCM used in clean up
- No worries about breakdown or downtime
- No washing needed
- No cross-contamination
- Low cost





FMS Flexible Dioxin, PCB and PBDE Sample Cleanup Solutions

- There are many different requirements in POPs Sample Prep for Analysis
- FMS has Total System Solutions to meet all or any of those requirements
- PLE (Pressurized Liquid Extraction)
 - Reduced time:
 - Automated Pressurized Liquid Extraction (PLE) takes 60 min start-to-finish
 - Manual Soxhlet up to 36 h.
 - Up to 8 samples in Parallel
 - Reduced cost: less labor involved, shorter turnover time per sample, less electricity use for PLE than Soxhlet.
 - Reduced volume: less solvent used.





FMS Flexible Dioxin, PCB and PBDE Sample Cleanup Solutions

PowerPrep NG

- Millions of POPs samples have been processed on the PowerPrep platform
- Superior Flexibility for Chemistry types, Analyte Fractionation and Programability
- With the combination of the PLE, SuperVap and PowerPrep NG you are given the flexibility to automate your extraction, concentration and clean-up for a wide variety of compounds.
- This is an ideal system for:
 - Laboratories that receive varying, different sample types and compounds of interest
 - High volume labs with a consistent daily sample amount load
 - Research laboratories looking to improve efficiency, recoveries and reproducibility



FMS Flexible Dioxin, PCB and PBDE Sample Cleanup Solutions

• EP110

- Uses No DCM
- Low solvent use 100-250 mLs
- Total time from sample till data between 3-4.5 hrs
- Clean up step time between 30 and 45 min
- Modular 2 to 8 sample configurations in parallel
- PLE/SPE and EP-110 can be configured in one system
- Economically Priced automated solutions for any Sample Matrix type




FMS Flexible Dioxin, PCB and PBDE Sample Cleanup Solutions

• EconoPrep

- Extraction, Cleanup and Concentration can be done in 3-4 hours
- Different plumbing available for food (PCBs in two fractions) and environmental (all PCBs together)
- Clean up part is flexible depending on sample requirements: mini, classical or HC
- Excellent recoveries for both PCDD/Fs and PCBs for various complex matrices
- Low solvent use 100-250 mLs
- Clean up step time between 30 and 45 min
- Modular 2 to 8 sample configurations in parallel
- PLE/SPE and EconoPrep can be configured in one system
- Rental Programs
- Economically Priced automated solutions for any Sample Matrix type



FMS Flexible Dioxin, PCB and PBDE Sample Cleanup Solutions

• EZPrep 123

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- No washing needed
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- Low cost
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- System gives excellent recoveries for PCDD/F, PCB and PBDEs comparable to FMS automated systems
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- Economically Priced Solutions for any Sample Matrix type



Agilent 7010 MS/MS Dioxin Analysis

- Provides a complete Dioxin/PCB workflow with FMS Sample Prep Systems
- Easy to Use and Implement
- Cost Effective Alternative to High Res Mass Spec
 - Lower instrumental purchase cost
 - Lower cost of real estate
 - Lower operational cost from lower training requirements and lower maintenance costs





Questions?

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