

Accelerated Solvent Extraction (ASE[®]) as an Extraction Technique for the Determination of Contaminants, Pollutants, and Poisons in Animal Tissues

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ABSTRACT

Accelerated Solvent Extraction (ASE) utilizes elevated temperatures and pressures with liquid solvents to obtain extractions in very short periods of time and with minimal amounts of solvent. For example, a 10-g sample can be extracted in less than 15 min using less than 15 mL of solvent. ASE is widely used with organic solvents for the extraction of many types of organic compounds in several industries. ASE is approved under EPA Method 3545A for the extraction of organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), semi-volatile compounds (BNAs), polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins and furans (PCDDs and PCDFS), and petroleum hydrocarbon contamination in solid and semisolid samples such as soils and sediments.

ASE has also been utilized for the extraction of animal tissues for various contaminants and pollutants. For example: PCDDs, PCDFs, OCPs, and PCBs have been determined in extracts generated using ASE with various animal tissues. There have also been data generated on the extraction of brominated flame retardants (BFRs) in animal and human tissues. Other contaminants determined in extracts generated by ASE include antibiotics and steroids in animal tissue processed for human consumption. Recently, ASE has also been applied to the determination of poisons such as pesticides and other environmentally important compounds in human tissues as part of forensic studies. Data in these application areas will be discussed in this presentation. We will present the results of method optimization studies along with recovery and precision results for ASE, and compare these results to other extraction techniques.



ASE-100



ASE-200



ASE-300

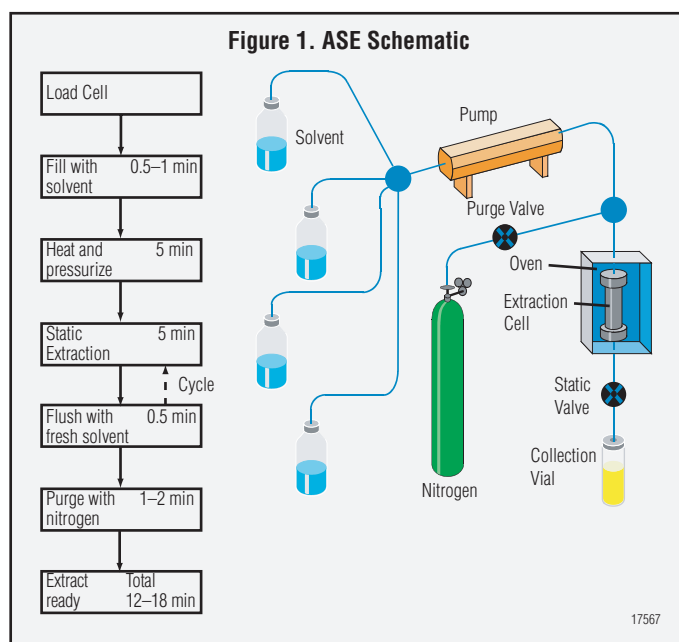
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Sample Extraction Issues

- Single biggest source of errors is sample handling
- Biggest bottleneck of time for most analysis methods
- Costs of solvent purchase and disposal are increasing
- The data are only as good as the sample preparation
 - High-price chromatography and data systems will not improve the quality of poorly prepared samples
- Dionex has developed ASE to address these issues



ASE APPLICATION AREAS

- Environmental
- Pharmaceutical
 - Natural products
 - Formulations
- Foods
 - Contaminants and major components
- Polymers
 - Additives and physical properties
- Consumer products

PCDDs/PCDFs in Environmental Samples Using ASE

- Fish tissue, harbor sediment, industrial sludge, boiler ash, and soil samples investigated by ASE and Soxhlet
- Toluene at 175 °C, 1500 psi (10.34 MPa)
- Clean-up using acidified silica (40% H₂SO₄), alumina, and carbon columns
- HRGC-HRMS analytical method

S. Raccanalli, et al., *Organohalogen Compd.*, **1999**, 40, 239–242.

Table 1. PCDDs/PCDFs in Fish Tissue Samples (ng/kg or ppt) Using ASE

| Compound | Soxhlet | ASE | Certified |
|------------------|---------|------|-----------|
| 2,3,7,8-TCDD | 7.6 | 7.6 | 6.6 |
| 1,2,3,7,8-PCDD | 4.3 | 4.3 | 4.4 |
| 1,2,3,4,7,8-HCDD | 1.4 | 1.4 | 1.9 |
| 2,3,7,8-TCDF | 13.4 | 12.6 | 11.9 |
| 1,2,3,7,8-PCDF | 5.4 | 5.1 | 5.0 |
| 1,2,3,4,7,8-HCDF | 12.5 | 12.2 | 12.2 |
| OCDD | 6.4 | 6.4 | 6.3 |
| Total TEQ | 21.4 | 21.1 | 21.0 |

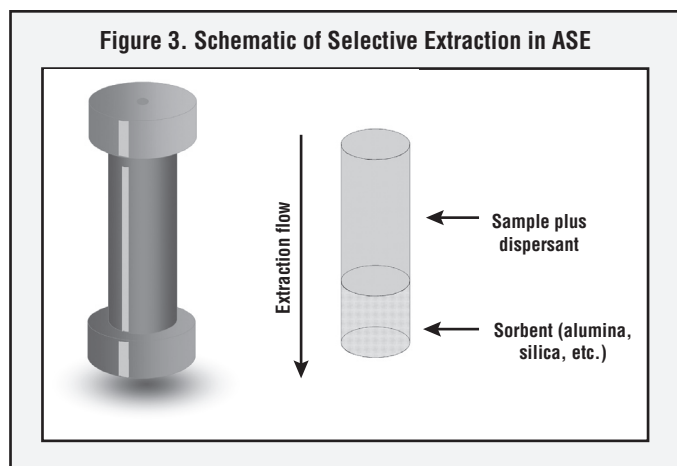
Polybrominated Diphenylethers (PBDEs) in Marine Animals and Sediments Using ASE

- Sediment, clam, oyster, and mussel samples were homogenized and mixed with dispersant
- DCM at 100 °C, 1500 psi (10.34 MPa), GPC clean-up for the animal extracts, alumina/copper for the sediment samples
- GC-MS
- 0.5-212 ppb in sediments, 14-106 ppb in animals and values are rising each year

D. R. Oros, et al., *Environ. Sci. Technol.*, **2005**, 39, 33–41.



Figure 3. Schematic of Selective Extraction in ASE



PCBs in Animal Tissues: Selective Extraction Using ASE

- Egg, oyster, clam, fish, and mussel samples were collected, freeze dried, and ground
- 2-g samples mixed with Florisil, placed on top of 6 g of Florisil in cell
- DCM/pentane (15:85, v/v) at 40 °C
- 1500 psi (10.34 MPa)
- 25 min extraction, 40 mL of solvent
- GC with ECD analysis
- Lipid content of samples ranged from 2.9 to 10.2% by weight
- 85 to 120% recovery as compared to Soxhlet
- Less than 0.1% of the lipids in sample were collected in vials because of selective extraction
- MDLs of 0.001 to 0.004 ng/g
- Less than 11% RSD

J. L. Gomez, et al., *J. Chromatogr., A* **2002**, 946, 209–219.

Sulfonamide Residues in Animal Tissues Using ASE

- 2-g samples of veal, pork, beef, chicken, or turkey
- Mix with 4 g of C18 resin
- Water, 160 °C, 1500 psi (10.34 MPa), cool sample in freezer to separate lipids
- Analyze by HPLC-MS/MS
- 15 min extraction and preparation

A. Gentili, et al., *J. Agric. Food Chem.*, **2004**, 52, 4614–4624.

Sulfonamide Residues in Animal Tissues Using ASE

- Recoveries
 - Beef: 80 to 95% at 100 ppb
 - Pork: 79 to 94% at 100 ppb
 - Poultry: 82 to 98% at 100 ppb
- LOD (limits of detection) and LOQ (limits of quantitation)
 - LOD: 0.5 to 2.3 ppb
 - LOQ: 1.5 to 6.9 ppb

Anabolic Steroids in Beef Kidney Using ASE

- 2-g sample, alumina in cell to retain steroids
- Defatting step first
 - Hexane at 60 °C, 10-min extraction
- Analyte extraction second
 - Acetonitrile at 50 °C, 15-min extraction
- LC-MS/MS

R. Draisci, et al., *J. Chromatogr., B* **2001**, 753, 217–223.

Performance of Method Using ASE: Anabolic Steroids in Beef Kidney

- Seven compounds investigated
- Kidney tissue and kidney fat as matrices
- 100 to 135% recovery at 2 ng/g (ppb)
- LOD 0.3 ng/g
- No false positives or false negatives
- Robust performance with over 100 samples

Forensic Applications Using ASE

- Barbiturates in human serum
- Environmental toxins from organ tissue
- Dioxins in blood and organ tissue
- Drugs of abuse in hair
- Poisons in human viscera



CONCLUSION

- ASE has wide applicability to the extraction of analytes from animal tissues
 - Food industry, environmental monitoring, forensics, environmental fate studies, etc.
- ASE can rapidly provide animal tissue extracts with good recovery and precision while using less time and solvents
- ASE with integrated cleanup can greatly shorten total sample preparation by providing sample extracts that require no further cleanup prior to analysis

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