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Accelerated Solvent Extraction – Basics and Applications

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Agenda

1. ASE Basics and Principle
2. Applications
3. Instrument
4. Q & A

The Challenge for Analysis

1.5 mL GC / LC Vial

How do we get analytes out of these samples?

The Answer is Sample Preparation

SP = Three Techniques

Initial Sample

Extraction

- Analytes are removed from sample
- Uses a solvent to remove analytes
- Requires energy source (e.g. heat)

Clean Up

- Removes coextracting analytes from sample
- Ensures quality chromatography
- Uses resins or a size exclusion technique

Evaporation

- Reduces a large sample volume for improved sensitivity
- Concentrates sample down to 1 mL or evaporates to dryness

Analysis

Severe Processing Bottleneck

- 2/3 of processing time spent preparing samples
- >80% of all laboratory error occurs within these steps

Data Recording and Reporting

Analysis Technique

Sample Preparation…….

Sample Preparation…….

Importance of Sample Preparation

“Eighty Percent of the Variance in an Assay Usually Arises from the Sample Prep.”

R. Stevenson, “Pittcon ‘98: Part 3, Sample Prep:
The Place to Make a Difference” American Laboratory, Vol. 30, No. 14, p. 21, 1998.

The Important Parameters for Sample Prep

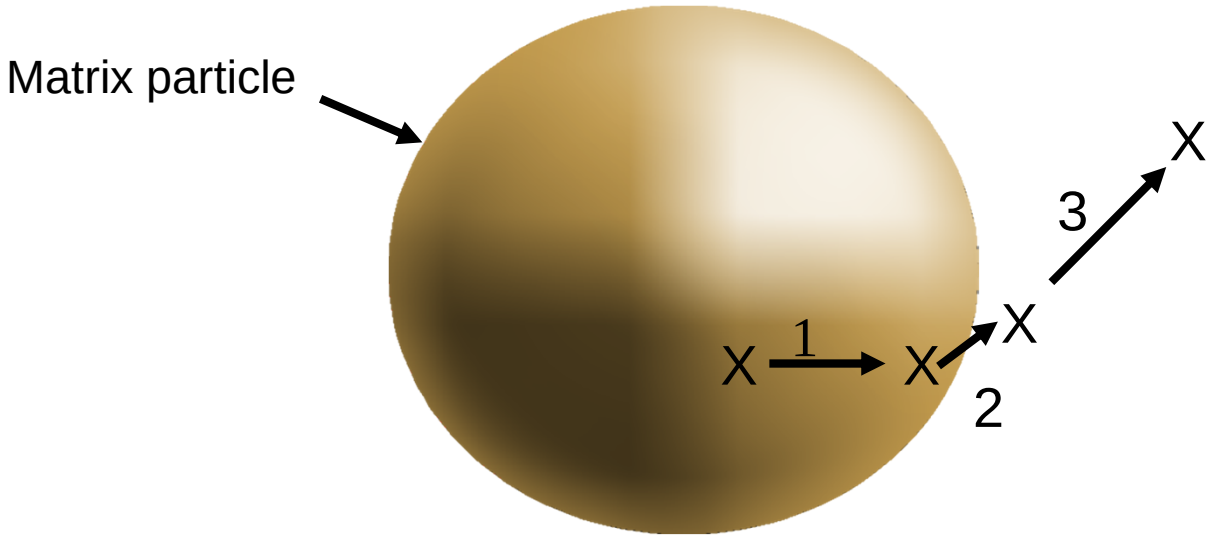
- Solvent Use
  - Amount of solvent consumed for the extraction
  - Solvents are expensive; reducing use reduces costs
- Extraction Time
  - Amount of time required for each extraction to occur
  - Reducing extraction time increases lab throughput
- % Recovery
  - Amount of analyte recovered following the extraction
  - Low % recovery yields poor analytical results
- % Relative Standard Deviation (RSD)
  - Measure of reproducibility between extractions
  - Extraction results have greater reproducibility with lower %RSDs

These Parameters Evaluate SP Techniques

Important Parameters for Liquid-Solid Extraction

Parameter	Effect on the Extraction Process
Temperature	Elevated temperature increases analyte diffusion from the matrix and improves analyte solubility in the extraction solvent.
Pressure	Increased pressure enables liquid solvents to be used at high temperature.
Analyte Solubility	Increases as temperature increase to improve extraction efficiency (e.g. solubility of anthracene increases 13-fold in DCM (50^{o}C to 150^{o}C)).
Solvent Viscosity	Decreases as temperature increases. Improves solvent migration through the matrix to increase extraction efficiency.
Solvent Surface Tension	Decreases as temperature increases. Allows solvent to better coat the matrix and helps improve analyte diffusion.

Three Mechanisms Controlling Extractions

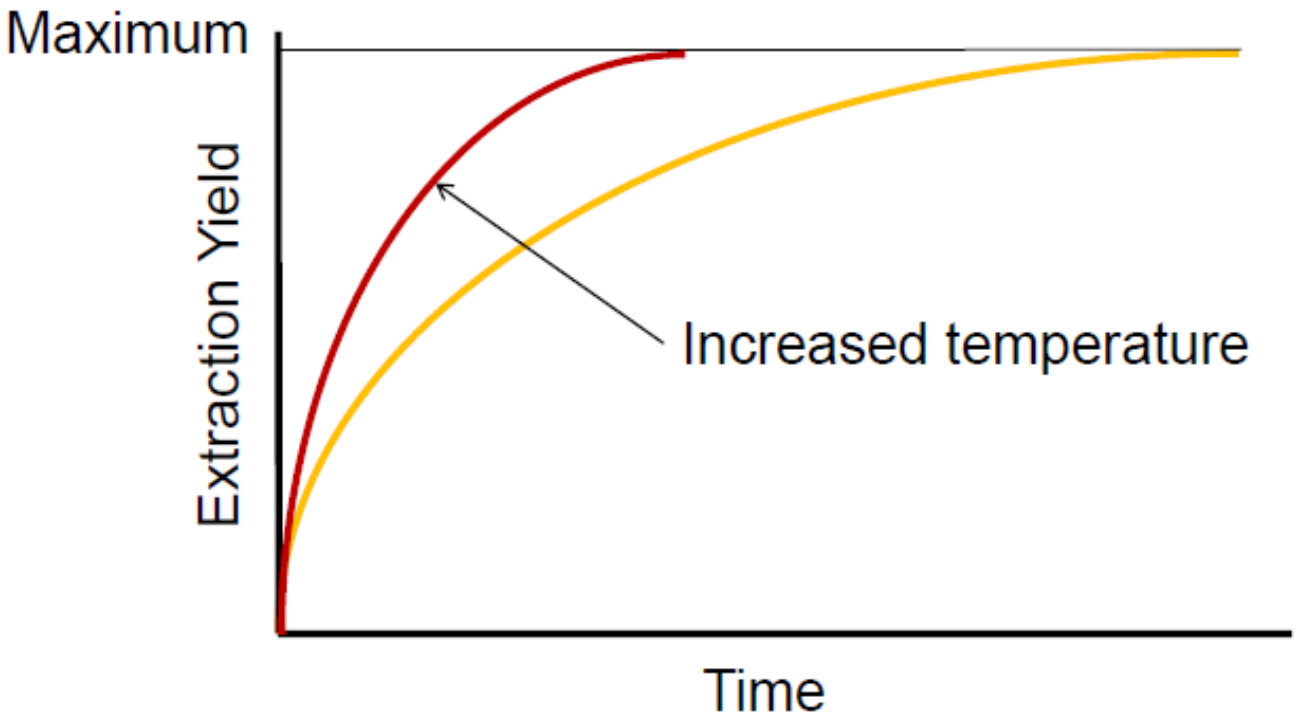


1. Transport of analyte X through sample particle, including overcoming analyte-matrix interactions and diffusion though sample particle.

2. Transport of analyte from particle surface to extracting fluid, overcoming adsorption energy at particle surface.

3. Transport of extracting fluid and analyte away from sample particle.

Increasing Temperature Accelerates Extraction



Higher temperature results in a curve that reaches maximum extraction yield faster

Thermo Fisher Scientific Dionex Sample Prep Product Line

Thermo Scientific Dionex ASE 150 and ASE 350 Accelerated Solvent Extractor

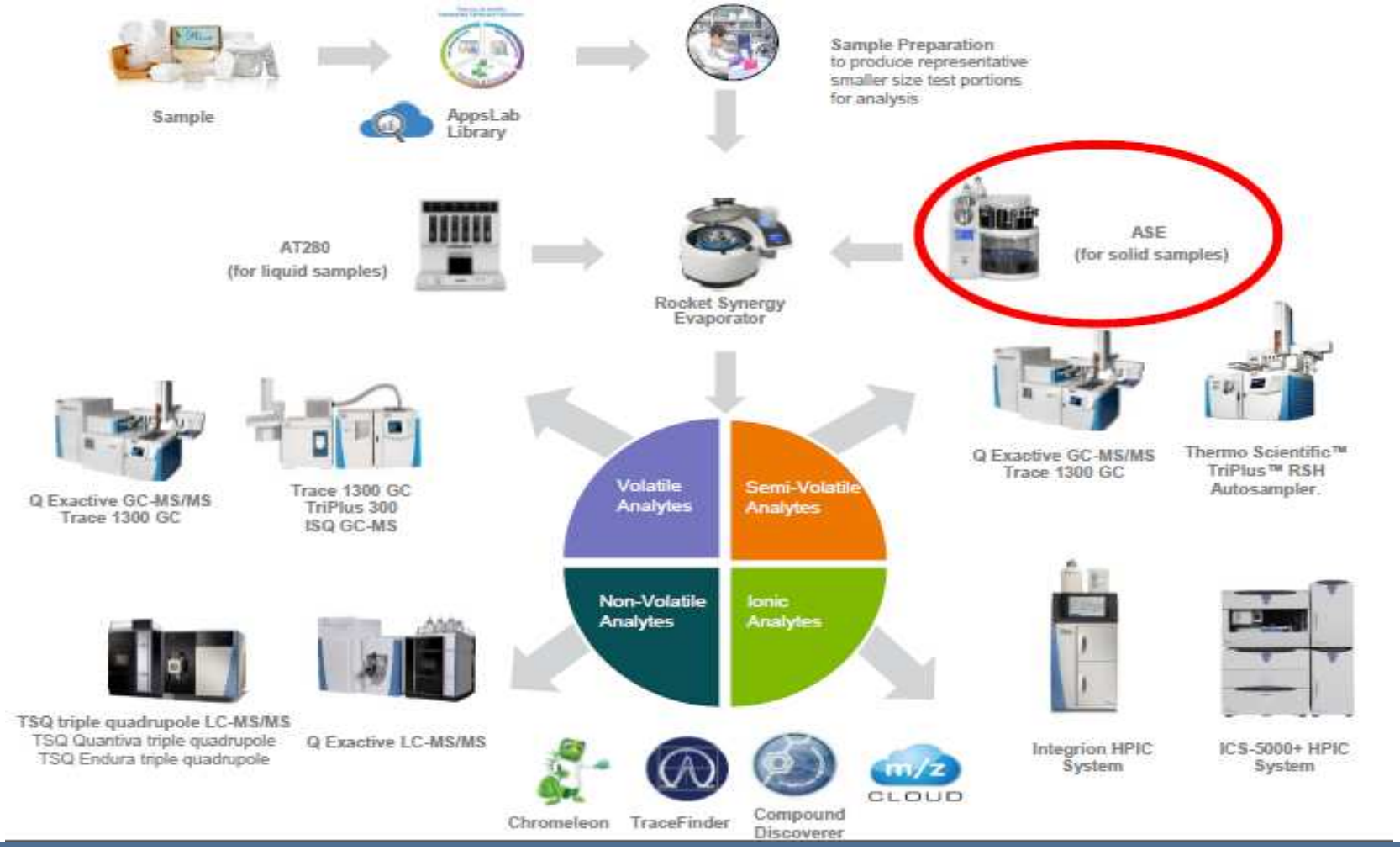
Thermo Scientific Dionex AutoTrace 280 Solid-Phase Extraction (SPE) Instrument

Thermo Scientific Dionex SolEx SPE Cartridges

Genevac Rocket Evaporator

Novel & Innovative Solutions

Sample Preparation is Critical for Sample To Knowledge



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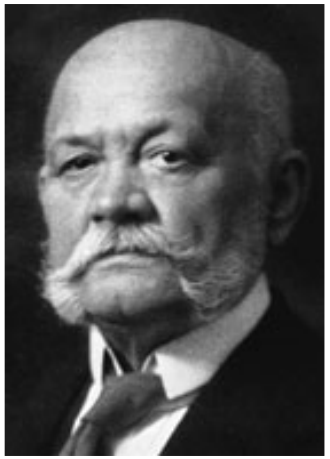
In the Beginning There Was Soxhlet...



Franz von Soxhlet
(1848 – 1926)

de facto standard for solvent extraction

Slow, high solvent usage

Now . . . Accelerated Solvent Extraction

- Automates sample preparation for solid and semisolid samples using solvents at elevated temperatures and pressure.
- Operates above the boiling point of extraction solvents by using sealed extraction cells.
- pH Hardened pathways allows use of strong acids and bases for sample pretreatment
- Well established and proven technique that is superior to Soxhlet and approved for U.S. EPA Method 3545A.

Thermo Scientific™ Dionex™ ASE™ 350 Accelerated Solvent Extractor system

U.S. EPA Method 3545A…. Highlights

METHOD 3545A

PRESSURIZED FLUID EXTRACTION (PFE)

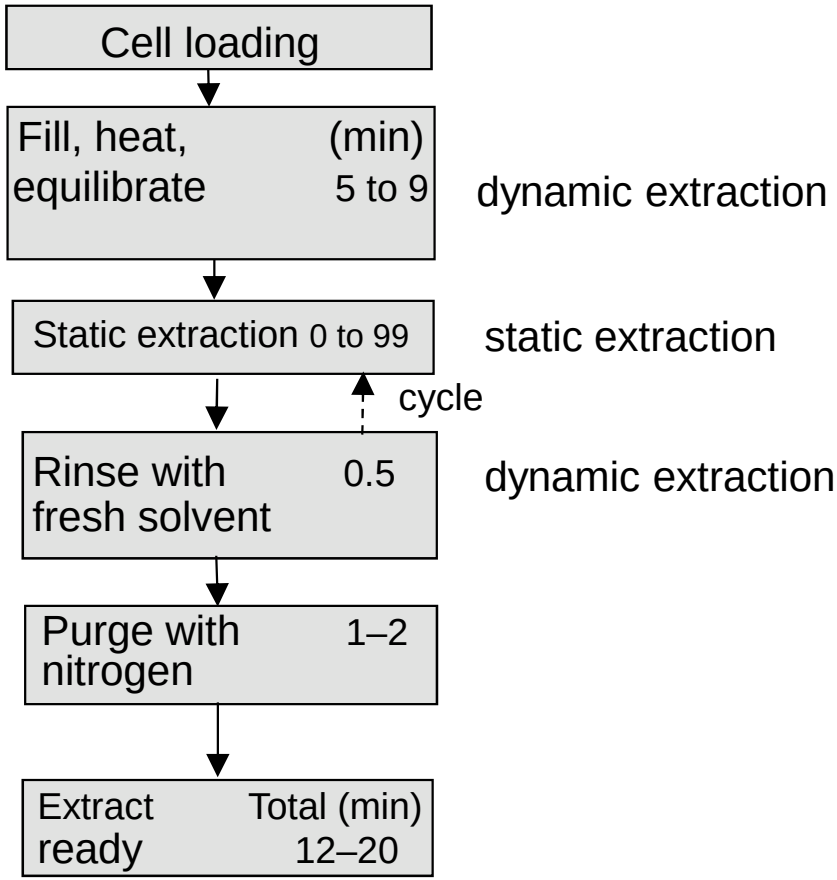
1.0 SCOPE AND APPLICATION

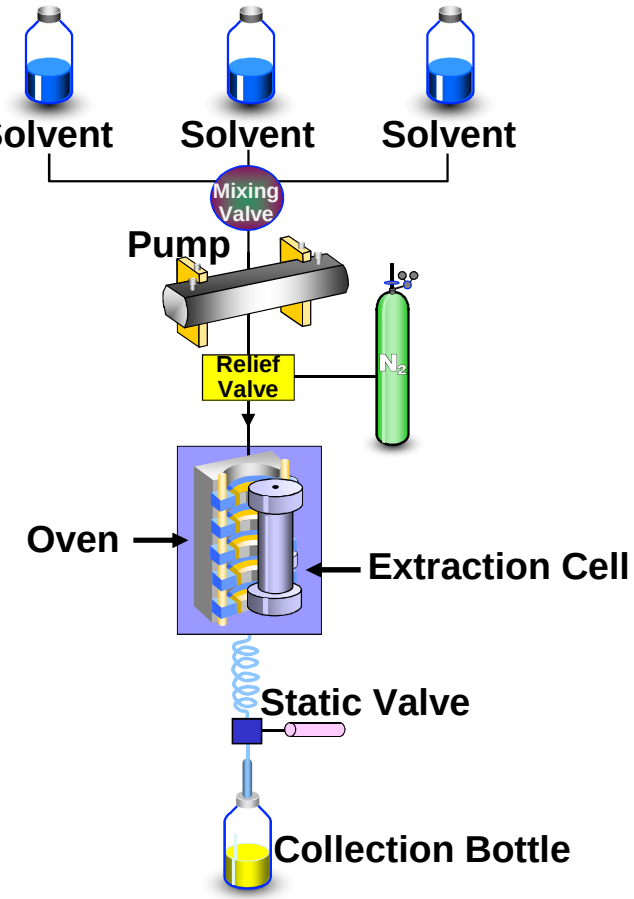
1.1 Method 3545 is a procedure for extracting water insoluble or slightly water soluble organic compounds from soils, clays, sediments, sludges, and waste solids. The method uses elevated temperature (100 - 180°C) and pressure (1500 - 2000 psi) to achieve analyte recoveries equivalent to those from Soxhlet extraction, using less solvent and taking significantly less time than the Soxhlet procedure. This procedure was developed and validated on a commercially-available, automated extraction system.

1.2 This method is applicable to the extraction of semivolatile organic compounds, organophosphorus pesticides, organochlorine pesticides, chlorinated herbicides, PCBs, and PCDDs/PCDFs, which may then be analyzed by a variety of chromatographic procedures.

1.3 This method has been validated for solid matrices containing 250 to 12,500 µg/kg of semivolatile organic compounds, 250 to 2500 µg/kg of organophosphorus pesticides, 5 to 250 µg/kg of organochlorine pesticides, 50 to 5000 µg/kg of chlorinated herbicides, 1 to 1400 µg/kg of PCBs, and 1 to 2500 ng/kg of PCDDs/PCDFs. The method may be applicable to samples containing these analytes at higher concentrations and may be employed after adequate performance has been demonstrated for the concentrations of interest (see Method 3500, Sec. 8.0).

How Does Accelerated Solvent Extraction Work?





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What About Thermally Labile Compounds and Carryover?

Technical Note 206

Investigations of Thermal Degradation During Accelerated Solvent Extraction (ASE)

John Ezzell, Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words
Accelerated Solvent Extraction, ASE, DDT, Endrin, Dicumyl Peroxide, Method Optimization, Thermally Labile Compounds

Goal
To demonstrate that the accelerated solvent extraction technique can be used to extract thermally labile compounds with proper method optimization.

Executive Summary
Accelerated solvent extraction is a sample preparation technique that uses elevated temperature and pressure to increase extraction efficiency in solid and semi-solid samples. This technique significantly reduces the amount of time and solvent required for extraction when compared to traditional techniques such as Soxhlet. Since elevated temperature is used to accomplish the extraction, the effect of thermal degradation was investigated to ascertain the viability of this technique for thermally labile compounds. Thermal degradation was not observed for DDT, endrin, and dicumyl peroxide in spiked sand samples at temperatures as high as 150 °C. These results demonstrate the versatility of the accelerated solvent extraction method and show that thermally labile compounds can be extracted in an optimized extraction method.

Introduction
Accelerated lower case these two is a new extraction method that significantly streamlines sample preparation. A commonly used solvent is pumped into an extraction cell containing the sample, which is then brought to an elevated temperature and pressure. Minutes later, the extract is transferred from the heated cell to a standard collection vial for cleanup or analysis. The entire extraction process is fully automated and performed in minutes for fast and easy extraction with low solvent consumption.

Because extractions are performed at elevated temperatures using the accelerated solvent extraction method, thermal degradation could be a concern. This has been investigated, and no evidence of degradation has been seen. The experiments reported here include monitoring the stability of thermally labile compounds during standard accelerated solvent extraction conditions (100 °C) as well as extractions done at higher temperatures (150 °C).

The degradation of DDT and endrin during GC analysis is used as an indication of active sites or excessive thermal conditions.[1] DDT breaks down to DDD and DDE, and endrin forms endrin aldehyde and endrin ketone. These same compounds were used to determine if thermal decomposition can occur during the accelerated solvent extraction method. Another temperature sensitive compound was also used as a probe to measure thermal and oxidative decomposition. Dicumyl peroxide (DCP) is used as a free radical generator in polymerization, and it is very sensitive to thermal degradation.

Instrumentation
- Thermo Scientific™ Dionex™ ASE™ 200 Accelerated Solvent Extractor system
- Gas chromatograph (GC) with electron capture detector (ECD)
- Thermo Scientific™ Dionex™ DX-500 HPLC system with AD20 (UV detector)

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TN 206: Investigation of Thermal Degradation

Technical Note 207

Investigation of Carryover or Cross-Contamination in the Thermo Scientific Dionex ASE 200 Accelerator Solvent Extractor System

John Ezzell and Bruce Richter
Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words
Accelerated Solvent Extraction, ASE, Polyaromatic Hydrocarbons, Polychlorinated Biphenyls, U.S. EPA Method 3545A

Goal
To demonstrate that the accelerated solvent extraction technique is exhaustive and does not result in carry over or cross contamination in-between samples.

Executive Summary
Accelerated solvent extraction is a sample preparation technique that uses elevated temperature and pressure to increase extraction efficiency in solid and semi-solid samples. This technique significantly reduces the amount of time and solvent required for extraction when compared to traditional techniques such as Soxhlet. The accelerated solvent extraction technique ensures a high degree of reproducibility by running each sample individually under the preset method conditions. This sequential mode of operation uses a common pathway to collect the extracts and carry-over or cross contamination was investigated to ascertain the viability of this technique for multiple samples processed in a single batch. Two sets of extractions were run on soil and sediment contaminated with PAHs (up to 1,500 mg/kg) and PCBs (up to 3,700 µg/kg) and carryover was not observed. These results demonstrate that the accelerated solvent extraction technique is exhaustive and all compounds will be removed from the common extract pathway when using an optimized extraction method.

Introduction
Accelerated solvent extraction (ASE) is an innovative sample preparation technique that combines elevated temperatures and pressures with liquid solvents to achieve fast and efficient removal of analytes of interest from various matrices. With accelerated solvent extraction technique, extractions can be done in very short periods of time and with minimal amounts of solvent as compared to conventional sample extraction techniques like Soxhlet or sonication. For example, 10 g samples can be completely extracted in less than 15 min with less than 15 mL of solvent. Accelerated solvent extraction technique has been demonstrated to be equivalent to existing extraction methodologies such as Soxhlet and automated Soxhlet for most RCRA (Resource Conservation and Recovery Act) analytes from solid and semisolid samples. It meets the requirements of U.S. EPA Method 3545, Pressurized Fluid Extraction.

With the small amount of solvent used relative to the sample size, carryover or cross-contamination could be potential concerns with the accelerated solvent extraction technique and the Dionex ASE 200. Two sets of experiments were conducted to investigate these concerns. The experiments performed included the extraction of heavily loaded soil and sediment samples followed by extracting blank samples and the determination of target analytes in both extracts.

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TN 207: Investigation of Carryover

ASE Does Not Degrade Labile Compounds and is Exhaustive!

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TN 206: Evaluating Thermal Degradation

Compound	Ext. Temp.	%Recovery	%RSD
DDT	150 °C	103	3.9
Endrin	150 °C	110	2.4
O-Toluidine	150 °C	104	10.3
Dichlorobenzidine	150 °C	106	13.2

- Spike DDT and Endrin on sand at 5 ppb level
- Measure recovery and monitor for the presence of DDD, DDE, Endrin Aldehyde and Endrin Ketone
- Monitor the recovery of other temperature sensitive compounds such as o-toluidine and dichlorobenzidine

TN 207: Investigation of Carryover with PAHs and PCBs

- 5 g of SRS sample (soil contaminated at 11%, N= 3)
  - First extract = 106.1% average recovery of PAH by HPLC
  - MDQ was 0.2 mg/kg, Certified concentration of PAHs ranged from 31 – 1500 mg/kg
  - Second extract (solvent blank) = PAHs not present

- 8 g of sediment from NIST(SRM 1939, 35% extractable, N=3)
  - First Extract = 101.8% average recovery of PCB by GC-ECD
  - MDQ – 0.1 µg/kg, Certified concentration of PCBs ranged from 180 to 3700 µg/kg
  - Second extract (solvent blank) = PCBs not present

Using the ASE 350

1. Power on, automatic initialization

2. Turn on gas supply, set pressure to 1.03 MPa (150 psi)

3. Select and prepare appropriate solvents

4. Grind and mix samples with dispersants

5. Load samples into cells

6. Prime the system

7. Create/Load Method and Sequence

8: Run the Method or Sequence!

Selecting Solvents

Autoignition Point

Do not use solvent with autoignition point below 200 ºC (carbon disulfide, diethyl ether, 1,4-dioxane)

Acids

Strong mineral acids should not be used (e.g. hydrochloric acid)

Sulfuric acid and nitric acid can be used at concentrations less than 0.1% by volume.

Weak Acids such as phosphoric or acetic acid can be used as extraction solvents in small percentages (< 5% by volume.

n-Hexane

Methanol

Quality

Use HPLC or pesticide grade organic or aqueous solvents.

Solvents do not need to be degassed.

Bases

Strong bases such as sodium hydroxide or potassium hydroxide can be used at concentrations less than 0.1% by volume.

Weak bases such as ammonia can be used at small percentages (< 5% by volume)

The Importance of Grinding Samples

Blender

Homogenizer

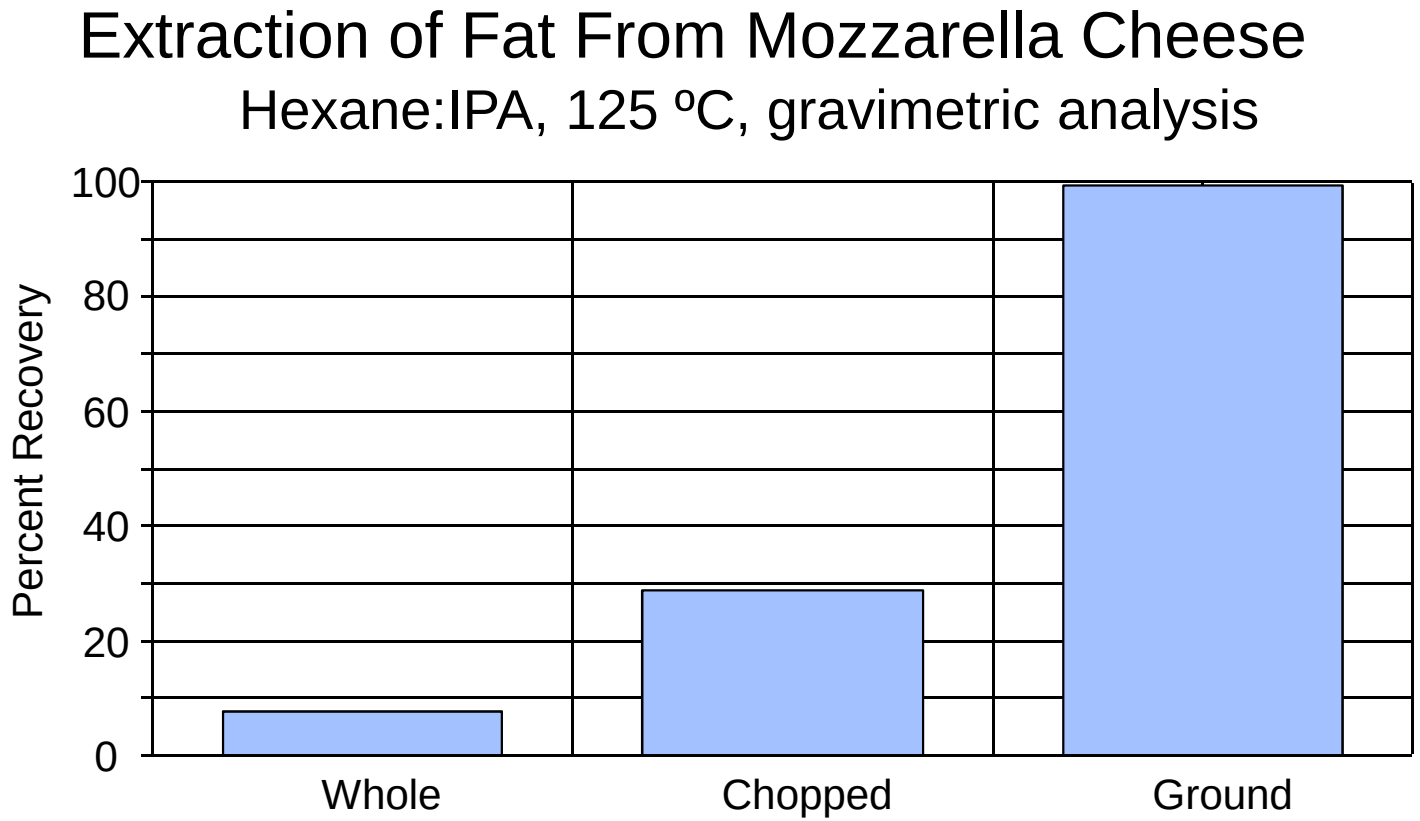
Grinder

Mortar and Pestle

Samples with large particle sizes should be ground prior to extraction.

This exposures more surface area that can be exposed to the solvent and improves extraction efficiency.

The Importance of Grinding Samples



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Mixing the Sample – Dispersants and Resins

ASE Prep DE

Pelletized DE is used as a drying and dispersing agent for solid and semi-solid samples. Prevents sample adhesion and compaction.

ASE Prep Cr H^+ Form

Cation exchange resin in the hydrogen form that neutralizes strong bases in samples that have been pretreated using base hydrolysis.

ASE Prep MAP

Uses a proprietary polymer to absorb moisture in wet samples.

ASE Prep Cr Na^+ Form

Cation exchange resin in the sodium form that neutralizes strong mineral acids in samples that have been pretreated using acid hydrolysis.

ASE Prep Sorbents

Sorbent Type	Typical uses	Recommended method of use
ASE Prep DE	Acts as a dispersant and drying agent (for samples containing up to 10% moisture).	Mix ASE Prep DE with the sample to form a homogeneous mixture prior to extraction
ASE Prep MAP	Used to remove moisture from samples containing 10-85% moisture.	Mix ASE Prep MAP with DE (1:1 ratio) with the sample prior to extraction
ASE Prep CR Na+	Used to neutralize acid hydrolyzed samples	Mix ASE Prep CR Na+ with ASE Prep DE (1:1 ratio) with the acid hydrolyzed sample prior to extraction
ASE Prep CR H+	Used to neutralize base hydrolyzed samples.	Mix ASE Prep CR H+ with ASE Prep DE (1:1 ratio) with the base hydrolyzed sample prior to extraction

Selecting the Sample Cell

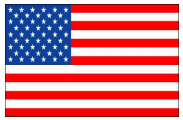
Stainless Steel Extraction Cells

1 mL, 5, mL, 10 mL, 22 mL, 34 mL, 66 mL, 100 mL

Dionium Extraction Cells

66 mL, 100 mL

International Agency Acceptance of ASE



United States

U.S. EPA Method 3545A (OCP, OPP, BNA, TPH, PCDD, herbicides and semi-volatiles)

U.S. EPA Method 8267 (Toxaphene)

U.S. EPA Method 6860 (Perchlorate)

NOAA Method NWFS-NWFSC-59 (Hydrocarbons)

ASTM D-7210 (Polymer Additives)

China

Method GB/T 19649-2006 for 475 pesticides in grains and grain products

Method GB/T 23376-2009, pesticides in tea leaves

Method GB/T22996-2008, ginsenosides in ginseng

Mexico

National Standard NMX-AA-146-SCFI-2008 for PAHs in soils and sediments

Germany

Method L00.00-34 for pesticides in foodstuffs

Key ASE Applications Summary

Industry	Analyte	Determinative Step	Matrix	Application Note
Environmental	Polyaromatic Hydrocarbons (PAHs)	GC-MS	Soil, Tissue	AN 1025
	Polychlorinated Biphenyls (PCBs)	GC-ECD	Soil, Tissue, PUFs	AN 1025
	Dioxins and Furans	GC-MS/MS	Sediment, brick, dust, ash	AN 10336
	Total Petroleum Hydrocarbons (TPH)	GC-FID	Soil	AN 324
	Base, Neutral, Acids (BNAs)	GC-MS	Soil	AN 317
Food	Fat Content	Gravimetric	Chocolate Meat Snack Foods Infant Formula	AU 344 AN 334 AN 321 AN 329 AU 195
	Oil Content	Gravimetric	Oil seeds (e.g. canola)	AU 325
	Pesticide Residues	GC-MS	Fruits, Vegetables, Animal Feeds	AN 332 AN 349
	Acrylamide	LC-MS	Coffee, Chocolate	AN 358
Natural Products	Herbal Marker Compounds	LC-UV	Plants	AN 362
	Active Ingredients in Herbal Supplements	LC-UV	Pills	AN 335
Chemical	Polymer Additives	LC-UV	Polymer Materials	AN 331
	Bioalcohol	Gravimetric	Biomass	AN 363
Pharma	Leachables & Extractables	LC-MS/MS	Drug Packaging	TBD
	Active Ingredients	LC-UV	Transdermal Patches	AN 327

Environmental Market

1. PAHs & PCBs in Soils, Sediments, and Tissue
2. Dioxins in Dust, Brick, Sediment, and Ash
3. Flame Retardants in Electronic Waste and Dust
4. BNAs in Soils and Sediments
5. Total Petroleum Hydrocarbons in Soils
6. Pesticides in Soil, Sediments, and Tissue
7. Toxaphene in Fish Tissue
8. Petroleum Hydrocarbons in Tissue
9. Persistent organic pollutants in sludges

Government Agencies

Water Treatment Plants

ALS Environmental

Contract Laboratories

PAHs and PCBs

Simultaneous extraction and clean-up of PAH and PCB from mussels* and soil*

Parameter	Method 1	Method 2
Cell Size	66 ml	66 ml
Oven Temperature	125 ℃	100 ℃
Sample Size	5 g	5 g
Static Time	6 min	4 min
Static Cycles	4	5
Adsorbent	Acidic alumina	Acidic alumina
Flush Volume	40 ml (60%)	40 ml (60%)
Solvent	DCM	DCM
Nitrogen Purge	150 psi, 5 minutes	150 psi, 5 minutes

* Spiked samples with EPA 8270 PAH Base-Neutral Surrogate Mix, PAH Spike Mix, PCB surrogate (2,4,5,6-Tetrachloro-*m*-xylene) and Aroclor 1254.

Extraction of PAHs and PCBs From Mussel & Soil

Table 2. Data for mussel and soil samples extracted by Method 1.

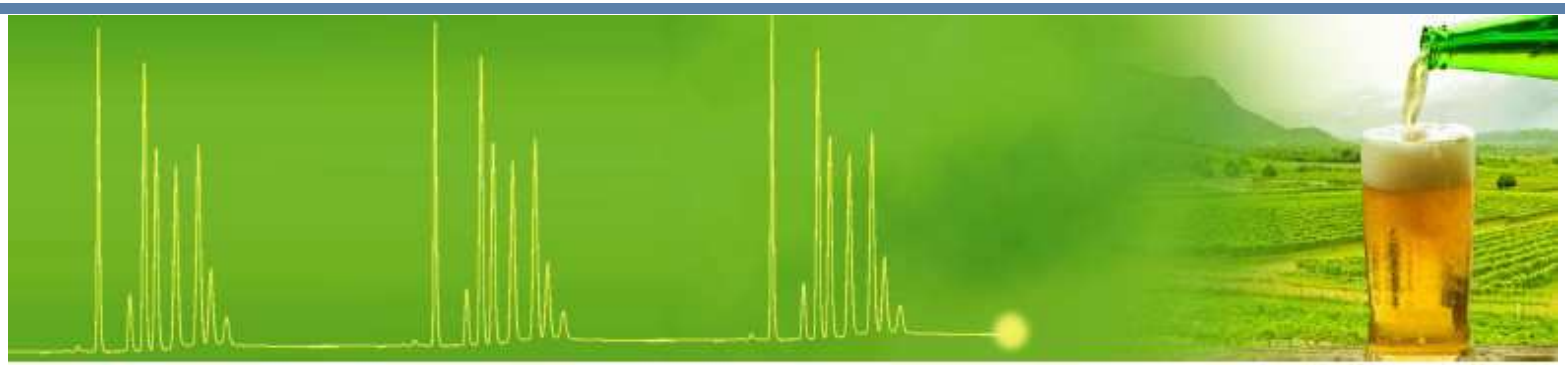
PAH Recoveries – Mussel (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
Nitrobenzene-d5**	83.3	0.54	13.05
2-Fluorobiphenyl**	95.1	0.43	9.13
p-Terphenyl-d4**	91.4	0.27	5.92
Naphthalene	89.1	0.28	6.33
Acenaphthylene	101.2	0.30	5.91
Acenaphthene	98.3	0.28	5.65
Fluorene	107.5	0.46	8.65
Phenanthrene	104.6	0.30	5.70
Anthracene	100.1	0.29	5.77
Fluoranthene	97.1	0.30	6.24
Pyrene	88.9	0.24	5.31
Benzo(a)anthracene	85.4	0.21	4.85
Chrysene	95.5	0.27	5.66
Benzo(b)fluoranthene	91.7	0.31	6.72
Benzo(k)fluoranthene	88.3	0.20	4.43
Benzo(a)pyrene	89.9	0.28	6.29
Benzo(ghi)perylene	94.1	0.31	6.60
Dibenzo(a,h)anthracene	92.3	0.28	6.06
Indeno(1,2,3-cd) pyrene	91.1	0.31	6.72
PCB Recoveries – Mussel (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
2,4,5,6-tetrachloro-m-xylene**	93.1	0.48	5.21
Aroclor 1254	95.9	0.06	3.26

PAH Recoveries – Soil (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
Nitrobenzene-d5**	94.6	0.81	17.20
2-Fluorobiphenyl**	101.2	0.25	4.87
p-Terphenyl-d4**	102.1	0.10	1.94
Naphthalene	79.0	0.47	6.29
Acenaphthylene	76.3	0.21	5.44
Acenaphthene	102.9	0.33	6.40
Fluorene	80.3	0.21	5.31
Phenanthrene	114.8	0.37	6.39
Anthracene	91.4	0.51	11.19
Fluoranthene	103.6	0.12	2.23
Pyrene	97.4	0.14	2.90
Benzo(a)anthracene	99.0	0.17	3.35
Chrysene	91.2	0.09	1.90
Benzo(b)fluoranthene	96.3	0.14	2.82
Benzo(k)fluoranthene	92.8	0.13	2.70
Benzo(a)pyrene	83.0	0.23	5.52
Benzo(ghi)perylene	82.4	0.13	3.22
Dibenzo(a,h)anthracene	78.9	0.15	3.68
Indeno(1,2,3-cd) pyrene	84.6	0.11	2.65
PCB Recoveries – Soil (N = 6)			
Compound	**% Recovery**	**SD**	**% RSD**
2,4,5,6-tetrachloro-m-xylene**	86.7	1.2	4.72
Aroclor 1254	101.6	0.19	3.15

**Surrogate Spike

Recovery Ranges from 83 – 107 %

Food and Beverage Market



1. Acrylamide in Bread and Chips
2. Total fat from dairy, powdered milk, meat, and infant formula
3. Mycotoxins from wheat and corn
4. Fat from chocolate and oil seeds
5. Total unbound fat in snack foods
6. Pesticides from oyster tissue
7. Pesticides in multiple types of food samples
8. Perchlorate in vegetation
9. Active ingredients in dietary supplements
10. Pollutants in dietary supplements

USDA

Government Agencies

the nature network®

PhytoLab

Dietary Supplements

Nestlé

Food Manufacturers

Dioxins and Furans – Fish Tissue (CRM)

Compound	Soxhlet (ng/kg)	ASE (ng/kg)	Certified
2,3,7,8-TCDD	7.6	7.6	6.6
1,2,3,4,8-PCDD	4.3	4.3	4.4
1,2,3,4,7,8-HCDD	1.4	1.4	1.9
2,3,4,7,8-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	12.4	6.4	6.3

ASE yields equivalent results to Soxhlet while using less time and solvent

Pesticides from Oyster Tissue

Compound	Recovery (%) ASE Prep MAP (n = 3)	Recovery (%) Sodium Sulfate (n = 3)
Lindane	91	81
Heptachlor	93	64
Aldrin	94	66
Dieldrin	105	75
Endrin	106	70
DDT	114	69
Total	101	71

ASE Prep MAP yields higher recoveries than sodium sulfate

Pesticides in Food

Recovery of polychlorinated pesticides in raw banana

Compound	Recovery (%) (n = 3)	SD (µg/kg)	RSD (%)
α-BHC	100.3	2.3	2.3
β-BHC	102.2	2.3	2.3
γ-BHC	98.9	3.2	3.2
Heptachlor	89.2	7.6	8.5
Aldrin	89.4	2.2	2.5
Dieldrin	93.7	1.6	1.7
4-4‘ –DDE	92.1	1.8	1.9
2,4‘ – DDD	95.4	2.5	2.6
Endrin	94.4	2.7	3.0
4,4‘ – DDT	89.6	5.8	6.4

Pesticides in Food

Recovery of polychlorinated pesticides in raw potatoes

Compound	Recovery (%) (n = 3)	SD (µg/kg)	RSD (%)
α-BHC	96.3	6.3	6.6
β-BHC	108.6	2.3	2.1
γ-BHC	97.4	6.6	6.8
Heptachlor	93.9	3.5	3.7
Aldrin	95.9	3.3	3.4
Dieldrin	97.1	0.55	0.6
4-4‘ –DDE	95.4	0.7	0.7
2,4‘ – DDD	95.7	0.85	0.9
Endrin	97.8	1.8	1.9
4,4‘ – DDT	93.0	4.5	4.8

Fat Extraction from Food using Acid Hydrolysis, GC-MS Data

Food		ASE® Method-Resin Lipid (%)	Mojonnier Method Lipid (%)
Corn Chips	AVG	**29.85**	**30.41**
	RSD	1.1%	1.2%
Mayonnaise	AVG	**74.25**	**75.11**
	RSD	0.58%	1.2%
Parmesan Cheese	AVG	**26.27**	**26.41**
	RSD	0.84%	1.1%
Bologna	AVG	**28.60**	**28.58**
	RSD	1.3%	0.97%
Shortcake	AVG	**14.07**	**13.95**
	RSD	0.32%	0.24%

pH Hardened Pathways Allow the Use of Acids

Extraction of Perchlorate from Vegetation

- Challenging for sample preparation
- Many interferences can be co-extracted
- EPA method labour intensive and time consuming*
  - 20 h for extraction
  - 20+ h for cleanup
- Can ASE® be used to address labour and time?

*Ellington and Evans, *J. Chromatography. A,* **2000,** *898* 193–199

Perchlorate Sample Prep Options

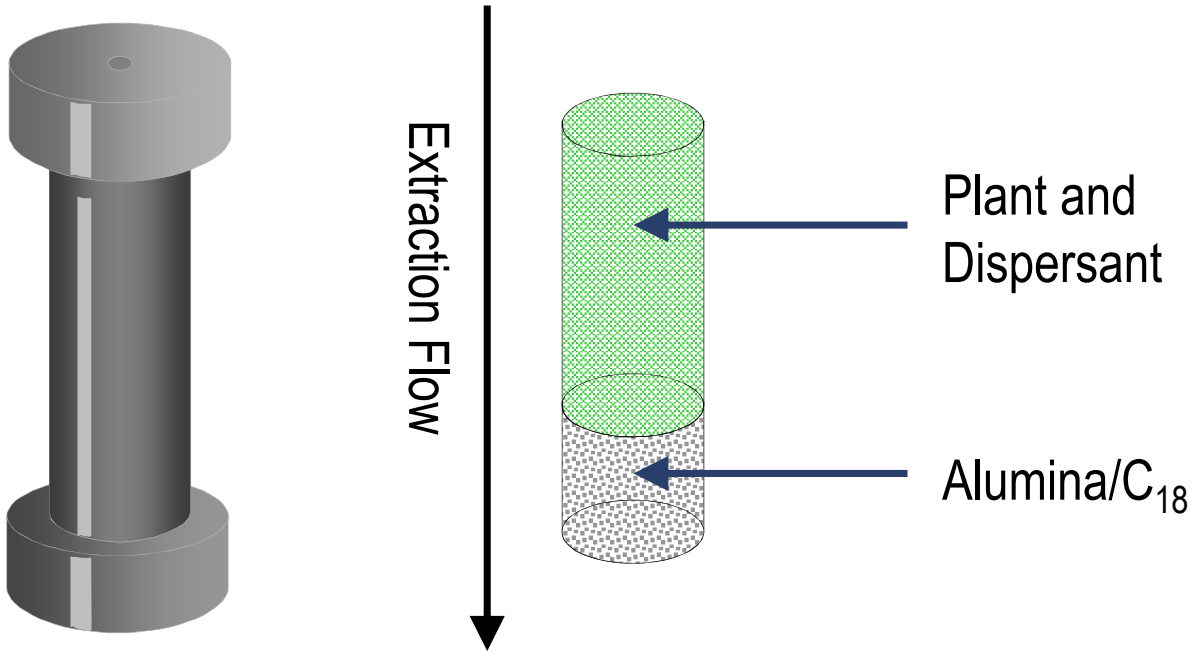
Off-line Cleanup

- Oven-dried samples (5 g) mixed with diatomaceous earth
- Extraction with standard ASE® conditions
  - D. I. water, 125 ° C, 1500 psi, 17-min total time
- SPE cleanup of extracts with alumina and C18

In-line Cleanup

- Oven-dried samples (5 g) mixed with diatomaceous earth
- Samples loaded into cell containing alumina/C18 in outlet
- Extracted with standard ASE conditions
  - D. I. water, 125 ° C, 1500 psi, 17-min total time
- Extracts analyzed without cleanup

Schematic of In-Line Cleanup in ASE® Cell



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Perchlorate Conclusion

- ASE® can be used as a sample extraction/cleanup procedure prior to the determination of perchlorate in soils and plant materials
  - Alumina/C_{18} in ASE cell (in-line) can provide clean extracts ready for analysis
  - Total time is less than 20 min for extraction and cleanup
- Automation of ASE improves the precision of the analytical scheme and increases sample capacity
- Same ASE instrument can be used for both ionic and non polar contaminants

ASE® Extraction of Mycotoxins

- Naturally occurring toxins produced by moulds
- Over 400 have been identified
  - Based on toxicity and occurrence the following are of highest concern
  - Aflatoxins, Vomitoxin, Fumonisin, Zearalenone, T-2 Toxin
- In the livestock industry mycotoxins are found predominantly in animal feeds

Extraction of Fumonisin from ASE® Conditions

Sample Size	10 g of Corn Meal Flakes + 3 g DE 0.5 mL Acetic Acid Spiked onto the Sample in the Cell
Solvent	MeOH/Water (75/25)
Temperature	75 °C
Pressure	1500 psi
Static Time	3 Min
Flush Volume	40%
Purge Time	120 Sec

Recovery of Fumonisin from Corn Meal

Amount (ug/kg)	
Sample	CEN Method ASE®
Corn Meal 1	550 600
Corn Meal 2	690 738
Corn Meal 3	700 806
Corn Meal 4	Non-Detect 91

Extraction of Aflatoxins from ASE® Conditions

Sample Size	20 g Corn, Oats, or Peanuts
Solvent	Acetonitrile (MeCN)
Temperature	75 °C
Pressure	1500 psi
Static Time	5 Min
Flush Volume	75%
Purge Time	100 Sec
Total Time	17 Min per Sample
Analysis	HPLC/Fluorescence after Filtering

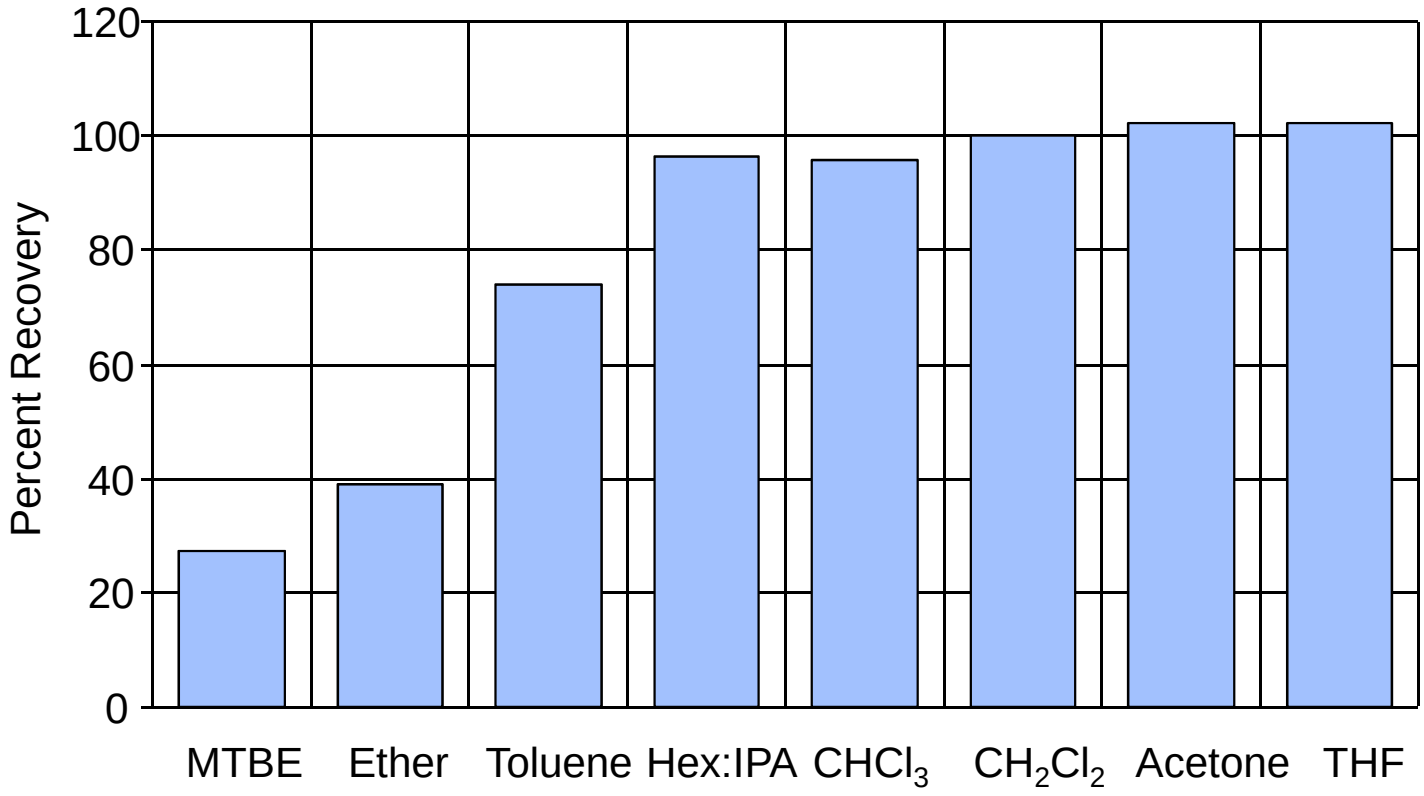
Recovery* of Spiked Aflatoxins

Matrix	Aflatoxins			
	G_2	G_1	B_2	B_1
Sand	90.7 (4.8)	93.8 (6.3)	73.1 (3.8)	100.1 (11.6)
Oats	79.4 (3.1)	90.7 (7.6)	86.7 (4.9)	87.9 (6.0)
Corn	104.4 (7.3)	92.9 (1.3)	78.3 (14.9)	91.9 (3.2)
* %Recovery (%RSD), n=3; Spiking Level at 1 ppm				

The Effects of Solvent Selection

Fat Determination in Powdered Infant Formula (SRM 1846)

100 ºC, 3 static cycles, gravimetric analysis



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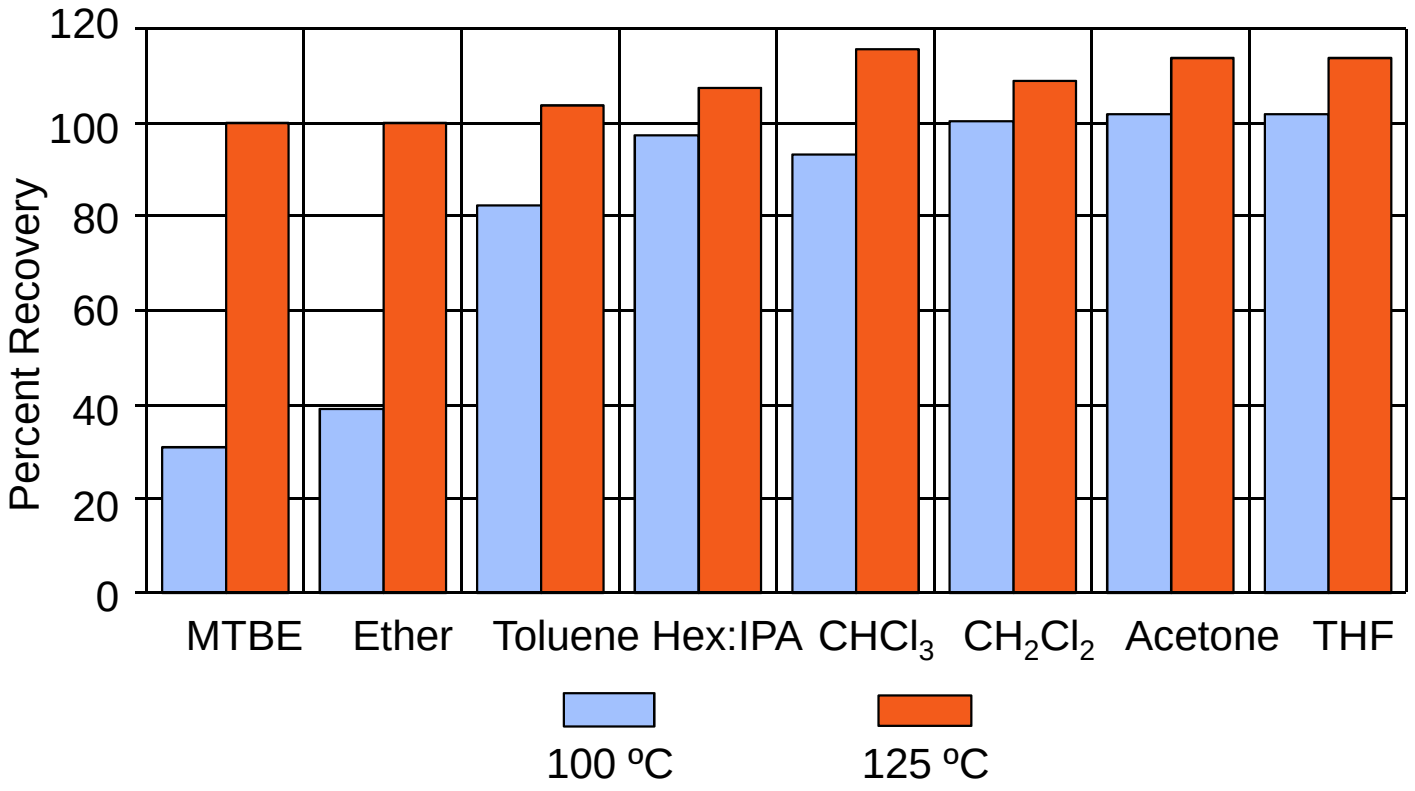
The Effects of Temperature

Fat Extraction from Powdered Infant Formula (SRM 1846)

100 vs.125 ºC, 3 static cycles, gravimetric analysis



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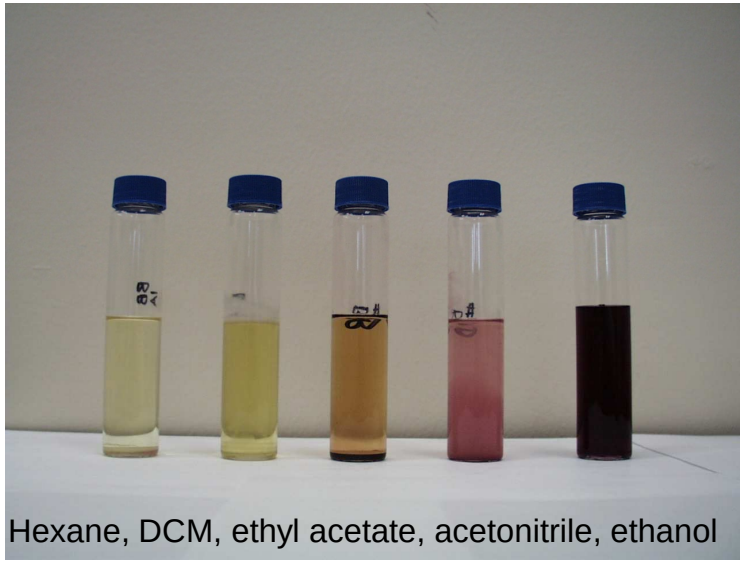
Solvent Selectivity in ASE

Tobacco Extracts



Blueberry Extracts

What does selectivity achieve?

- Isolates only the analytes of interest
- Retains or removes interfering compounds

How to make extractions selective

- Solvent choice – adjust polarity
- Adding sorbents – alumina for fat retention
- Lowering temperature – less coextractables

Use of Adsorbents Improves Selectivity

Accelerated Solvent Extraction Techniques for In-Line Selective Removal of Interferences

Technical Note 210

Introduction

Interferences may be extracted along with desired analytes during an extraction process. These unwanted co-extractables may interfere with analyte detection or decrease instrument performance. Traditionally, chromatographic techniques such as gel-permeation chromatography (GPC) or a glass column packed with specific adsorbents are used to purify sample extracts prior to separation and analysis. Recent advances using accelerated solvent extraction systems, as described in several publications,[1,3–10,12–17] include procedures for selective removal of interferences during sample extraction, thus combining extraction and purification into a single step.

This application note summarizes seven accelerated solvent extraction procedures developed to remove co-extractable material from various matrices: procedures to selectively extract polar compounds from lipid-rich samples and to fractionate lipids from biological samples.

This note is intended to serve as a guide to develop accelerated solvent extraction methods. For more information, please refer to the original publication cited with each method described below, or contact us.

Selective Extraction of Nonpolar Compounds

In an effort to eliminate post-extraction cleanup steps, we and others have researched the addition of various adsorbents to the extraction cell. For many sample types, this approach has proven successful in producing clean extracts that are ready for direct analysis. For example, nonpolar lipids are often co-extracted from fish tissue. Adding alumina (aluminum oxide, Al_2O_3, acidic, activated by placing in a drying oven at 350 °C for 15 h) to the extraction cell before adding the sample or sample mixture has been shown to prevent the extraction of unwanted lipids. Mixing the sample with C18 resin (1:2) has been shown to retain organic contaminants. (C18 bonded silica, 35–70 µm diameter, and porosity of 60 Å, from Alltech has been used, but similar materials from other vendors can be used.)

When in-cell cleanup is performed during accelerated solvent extraction solvent choice impacts the retention of unwanted components. For example, a mixture of hexane/acetone (1:1) is a common solvent for extracting organochlorine pesticides from animal tissues. After extraction, a cleanup step is usually required to remove co-extracted lipids. Adding alumina to an extraction cell and extracting with hexane can prevent the extraction of interferences. However, if hexane/acetone (1:1) is used as the extraction solvent, almost no lipid material will be retained on the alumina. Table 1 lists two types of fat retainers and the ratio of each required to retain fat when using nonpolar solvents. Table 2 lists common adsorbents that are used for selective extraction of compounds using accelerated solvent extraction systems.

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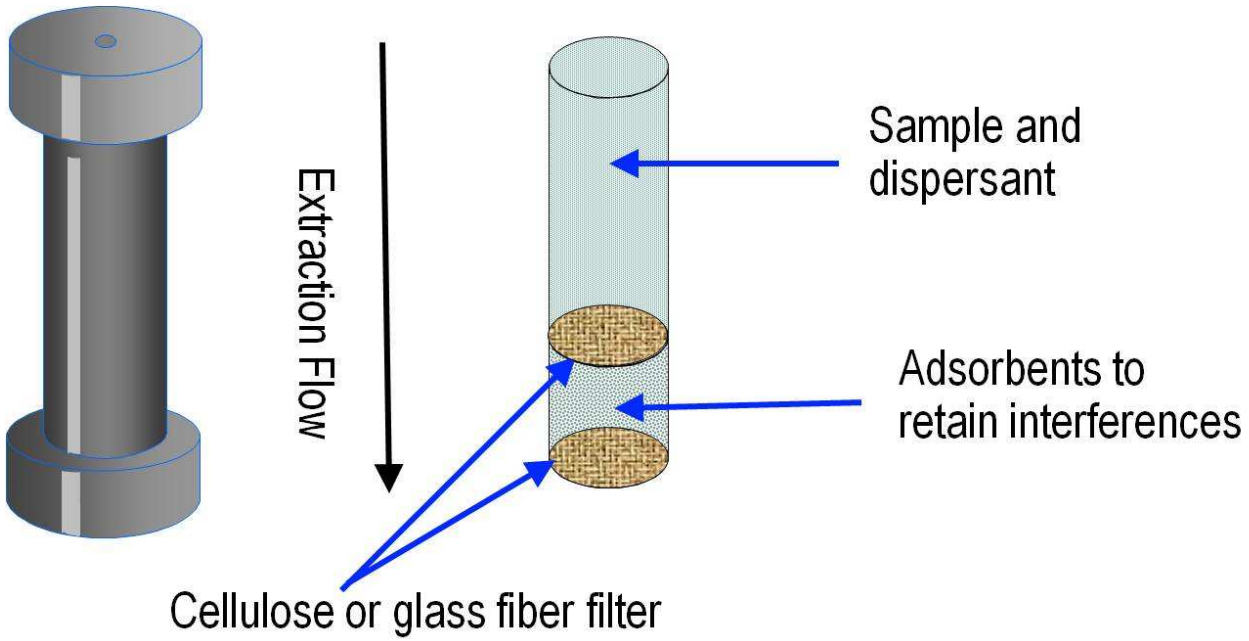
TN 210: In-Line Removal of Interferences

Adsorbent and Uses	
Carbon	Removes organics and nonpolar compounds
Copper	Removes sulfur
Ion-exchange Resins	Removes organics, ionic interferences for IC and IC/MS analysis
C_8 - C_{18} Resin	Removes organics, polar compounds, lipids, colors
Acid-impregnated Silica Gel	Removes lipids
Alumina	Removes nonpolar lipids, colors
Florisil	Removes nonpolar lipids
Silica Gel	Removes nonpolar lipids

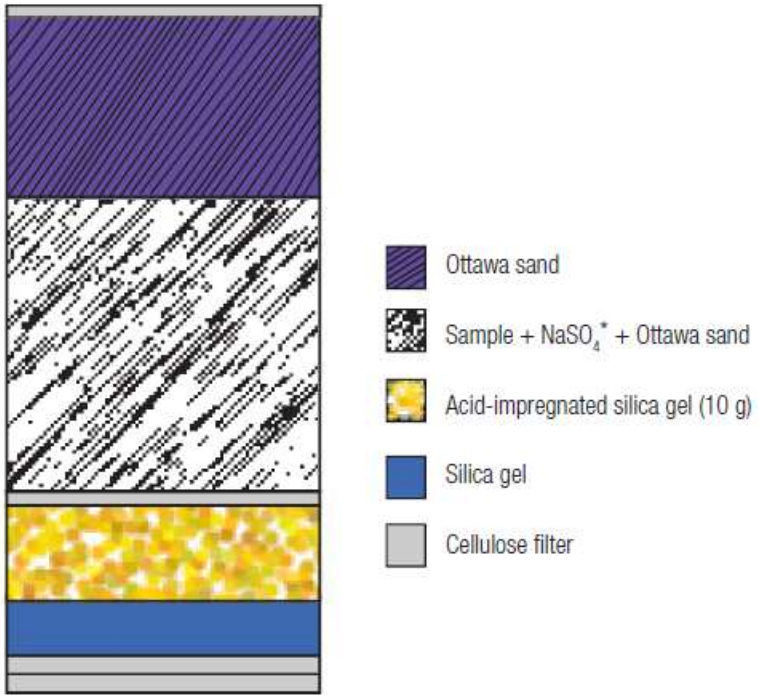
In-Cell Adsorbents May Eliminate the Need for Offline Clean Up Procedures

ASE In-Line Clean-up

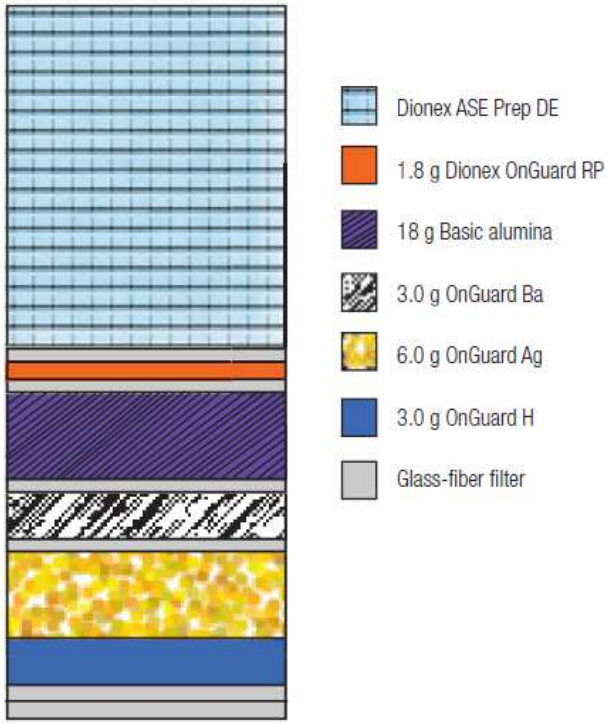
Schematic of the Cell



Use of Adsorbents Improves Selectivity



Preparation of the Extraction Cell for the Selective Extraction of PCBs from Fish Meal



Preparation of the Extraction Cell for the Selective Extraction of Perchlorate from Vegetation

Integrated Clean-Up: Salmon Extracts

Extracts With and Without In-Cell Clean-Up of Fish Tissue
Using Alumina, Silica Gel, and Acidic Silica Gel (40% H_2SO_4)

ASE reduces Extraction Time

Technique	Average Extraction Time
Soxhlet	4 - 48 Hours
Automated Soxhlet	1 – 4 Hours
Sonication	0.5 – 1 Hour
Microwave*	0.5 – 1 Hour
ASE	0.2 – 0.3 Hour

Extraction times are based on a per sample basis. This estimate of time does not include sample weighing, sample loading, or sample concentration.

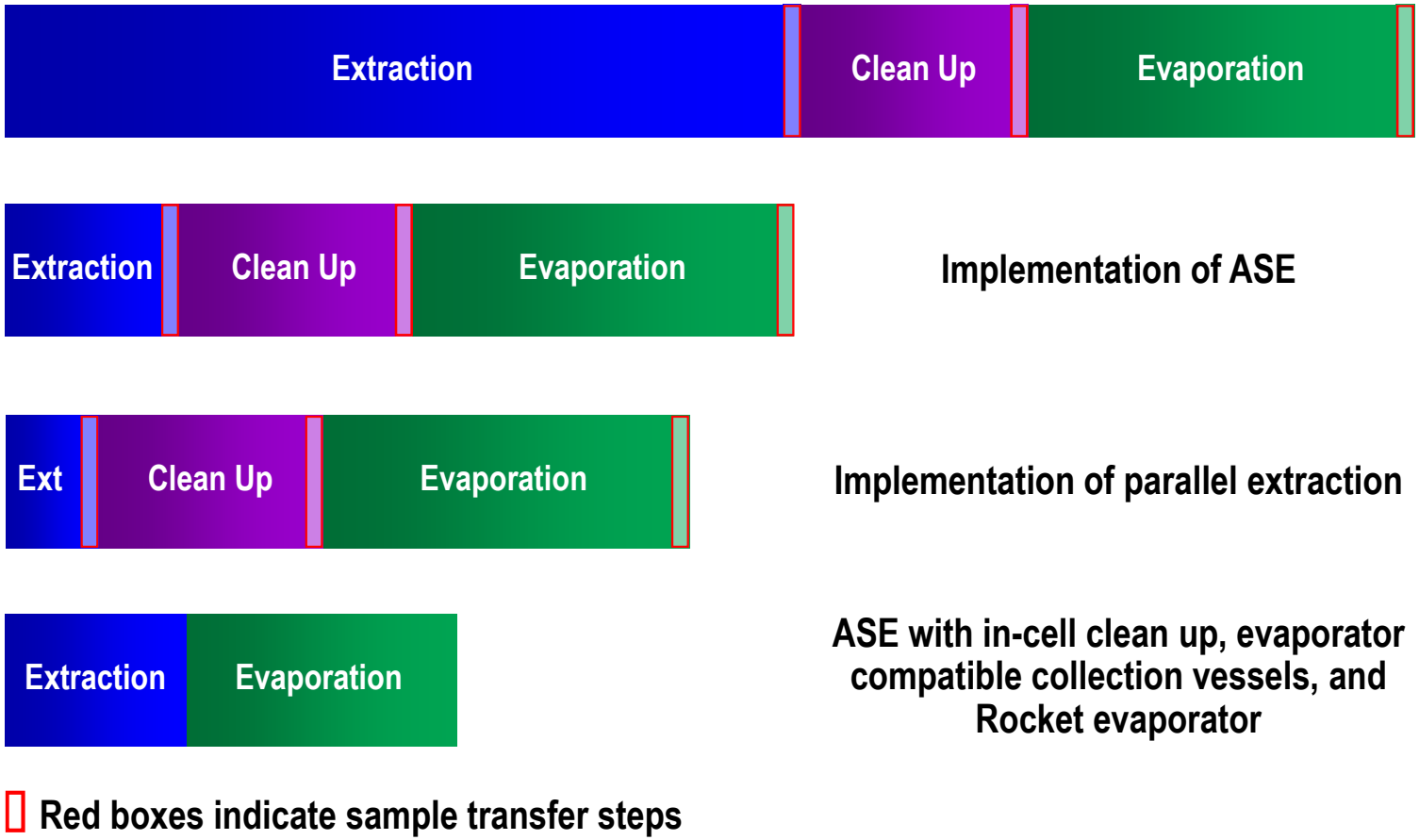
*Requires cooling and offline filtration which adds ~40 minutes of processing time per sample

ASE reduces Solvent Use

Technique	Average Amount of Solvent Used
Soxhlet	200 – 500 mL
Automated Soxhlet	50 – 150 mL
Sonication	150 – 200 mL
Microwave	25 – 50 mL
ASE	15 – 45 mL

Solvent usage is determined on a per sample basis.

Sample Preparation Productivity



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