Sample “Pre-Treatment”: Introduction to Commonly Used Techniques for Gas Chromatography

Joan Stevens, Ph.D.

Sample Preparation Applications Scientist
Chemistries and Supplies Division

Introduction

- Why “pre-treatment” Ξ “sample preparation” is important in GC-MS analysis
- Three “Pre-Treatments” to be discussed
  - Solid Phase Extraction: SPE
  - Supported Liquid Extraction: SLE
  - Quick, Easy, Cheap, Effective, Rugged, Safe: QuEChERS
- Basic Steps
- Examples
- Decision Tree

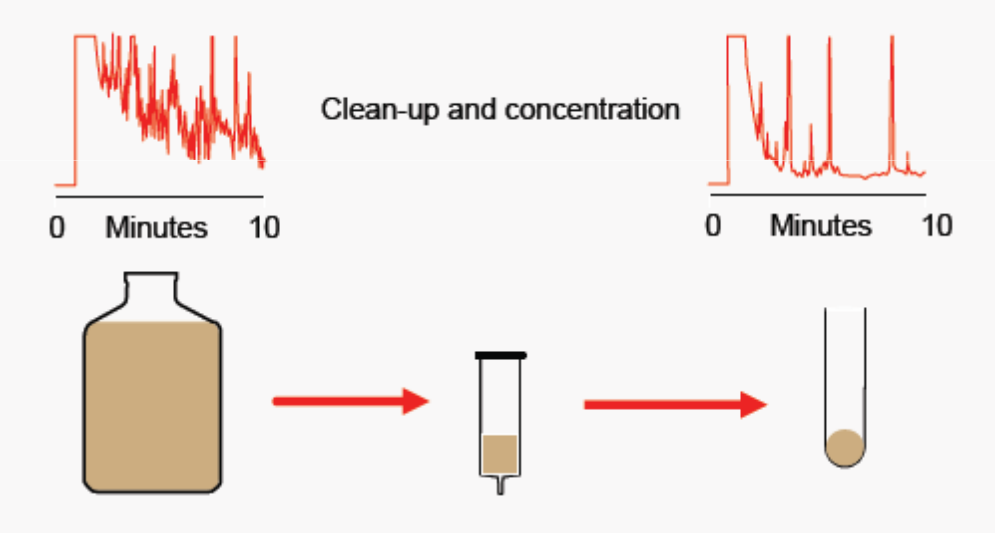
Sample Preparation

- There are many types of sample preparation techniques
- Only address 3 techniques today; related to food/environmental samples
- Following e-Seminars in 2012 will address additional techniques; related to biological, pharma, clinical, and forensic samples/matrixes

Why “Pre-Treatment or “Sample Preparation” is Important in GC-MS Analysis

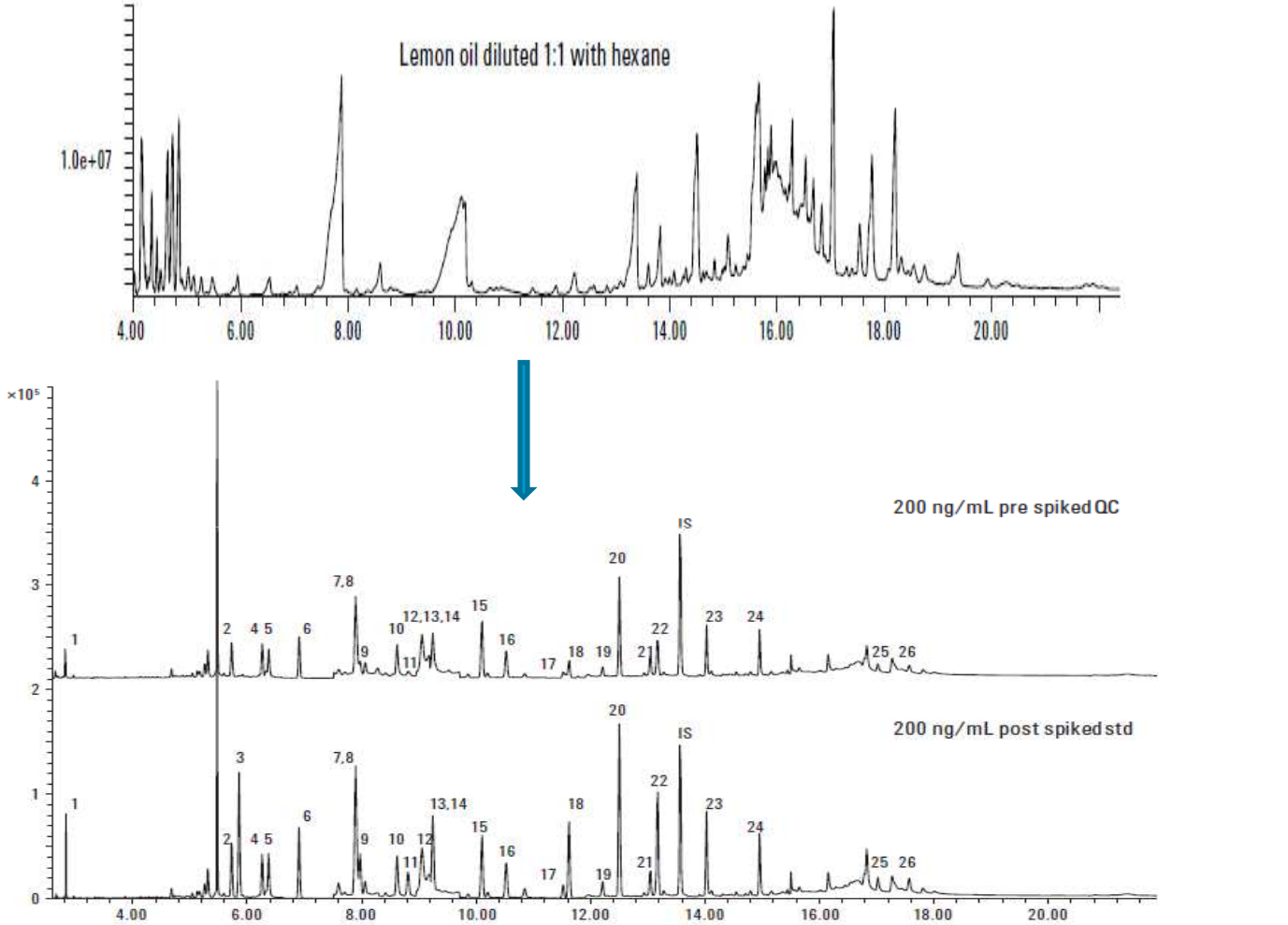
- Sample is in the wrong physical state for analysis (requires a liquid and the sample is a solid)

- Sample has interfering matrix components that effect measurement
- Sample is causing a significant increase in maintenance, loss in chromatographic separation, peak shape
- Sample has too low an analyte concentration to be detected by the instrument



Dirty liner on left versus clean liner on right

Lemon oil extract



GC/MS chromatograms of 200 ng/mL pre and postmatrix fortified samples. Peaks identification: 1. Dichlorvos, 2. α-BHC, 3. Hexachlorobenzene, 4. β-BHC, 5. γ-HCH, 6. Disulfoton, 7. Chlorpyrifos methyl, 8. Parathion methyl, 9. Heptachlor,10. Fenitrothion, 11. Aldrin, 12. Malathion, 13. Chlorpyrifos, 14. Parathion, 15. Procymidone, 16. Endosulfan I, 17. Dieldrin,18. 4,4´-DDE, 19. Endosulfan II, 20. 4,4´-DDD, 21. Endosulfan sulfate, 22. 4,4´-DDT, 23. Bromopropylate, 24. λ-Cyhalothrin, 25. Fenvalerate, 26. Deltmethrin.

How do Sample Preparation Options Compare?

Solid Phase Extraction (Bond Elut)

Supported Liquid Extraction (Chem Elut)

Extraction/Dispersive SPE (dSPE) (QuEChERS)

How do Sample Preparation Options Compare?

Solid Phase Extraction (Bond Elut)

Most selective and therefore cleanest sample, with built in concentration capabilities

Supported Liquid Extraction (Chem Elut)

Extraction/Dispersive SPE (dSPE) (QuEChERS)

How do Sample Preparation Options Compare?

Solid Phase Extraction (Bond Elut)

Supported Liquid Extraction (Chem Elut)

Simple procedure, delivering higher analyte recoveries and cleaner extracts than equivalent LLE methods

Does not form emulsions

Extraction/Dispersive SPE (dSPE) (QuEChERS)

How do Sample Preparation Options Compare?

Solid Phase Extraction (Bond Elut)

Supported Liquid Extraction (Chem Elut)

Extraction/Dispersive SPE (dSPE) (QuEChERS)

- Effective for a wide range of analytes from various matrices
- Simple, involves pipetting and shaking
- Greener than SLE
- “Just Enough”

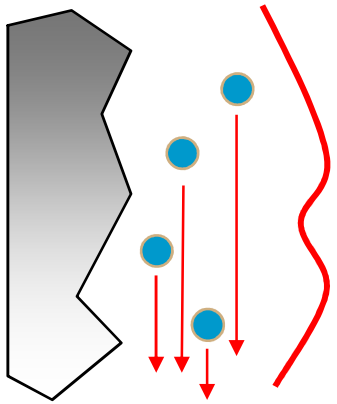
Solid Phase Extraction

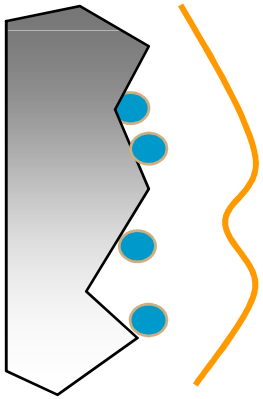
Basic Chromatography Theory

SPE is not much different than HPLC, it is still chromatography and the same rules apply.

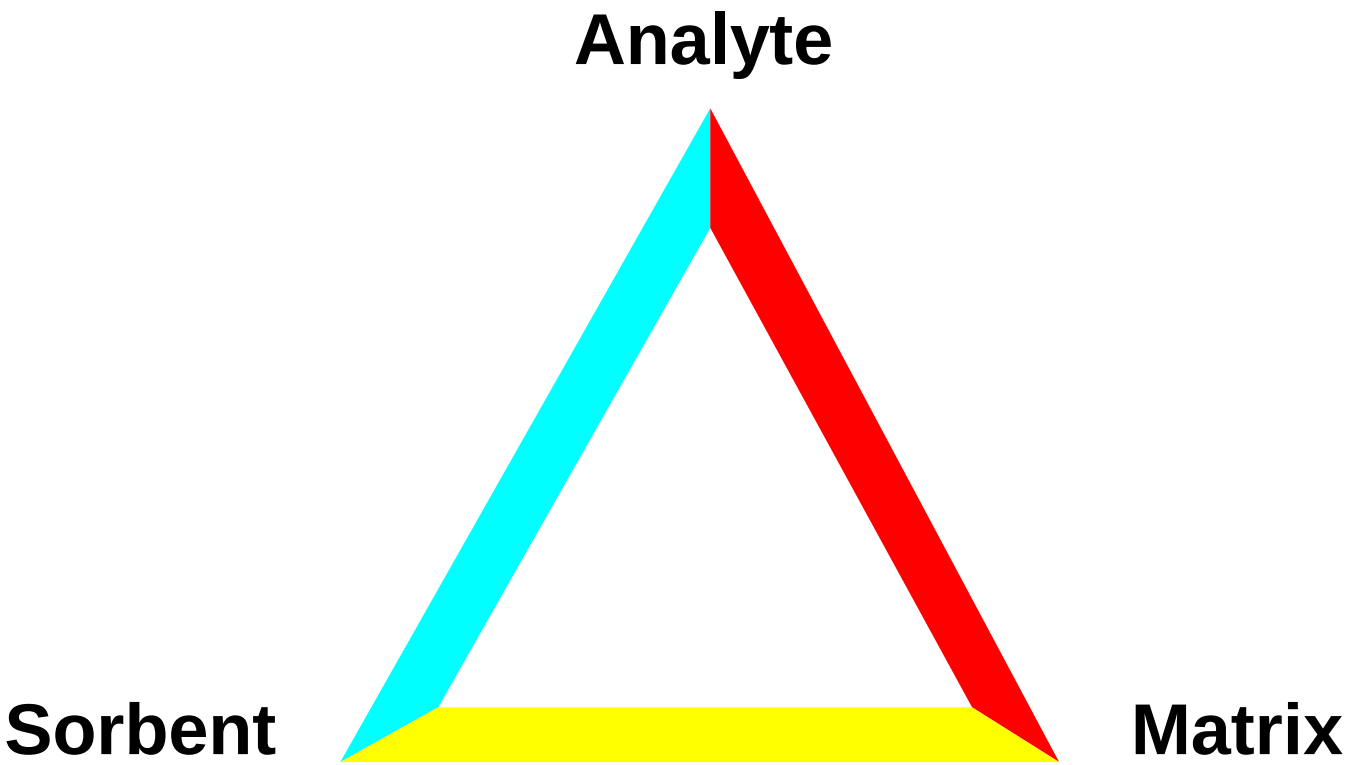
If the analyte has a higher affinity for the mobile phase than the stationary phase, the analyte will migrate through the cartridge with little interaction with the stationary phase (no retention).

If the analyte has increased affinity for the stationary phase, it will adsorb to the cartridge depending on the extent of the interaction (retention).





Interrelationship in SPE



What is BondElut? How Does It Work?

Bond Elut is formatted in a cartridge, 96 well plate, on-line or pipette tip

•High purity bonded silicas (Bond Elut); polymers (Plexa); special phases

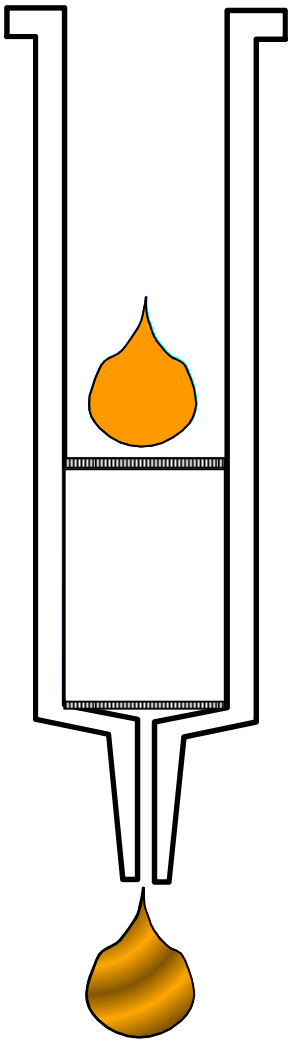
•High surface area

•Modified to attract certain types of molecules out of a liquid sample

•Analyte passes through (collected), interferences retained ("Scavenger SPE") or after washing interferences off collect the analytes that were attracted to the sorbent (Classic SPE).

The SPE Sequence

- Wet the cartridge, then apply sample (eg food extract, water)
- Some compounds “retain”
- Wash the cartridge
- Apply a different liquid to “elute”
- The extract is cleaner, in a different liquid and usually concentrated

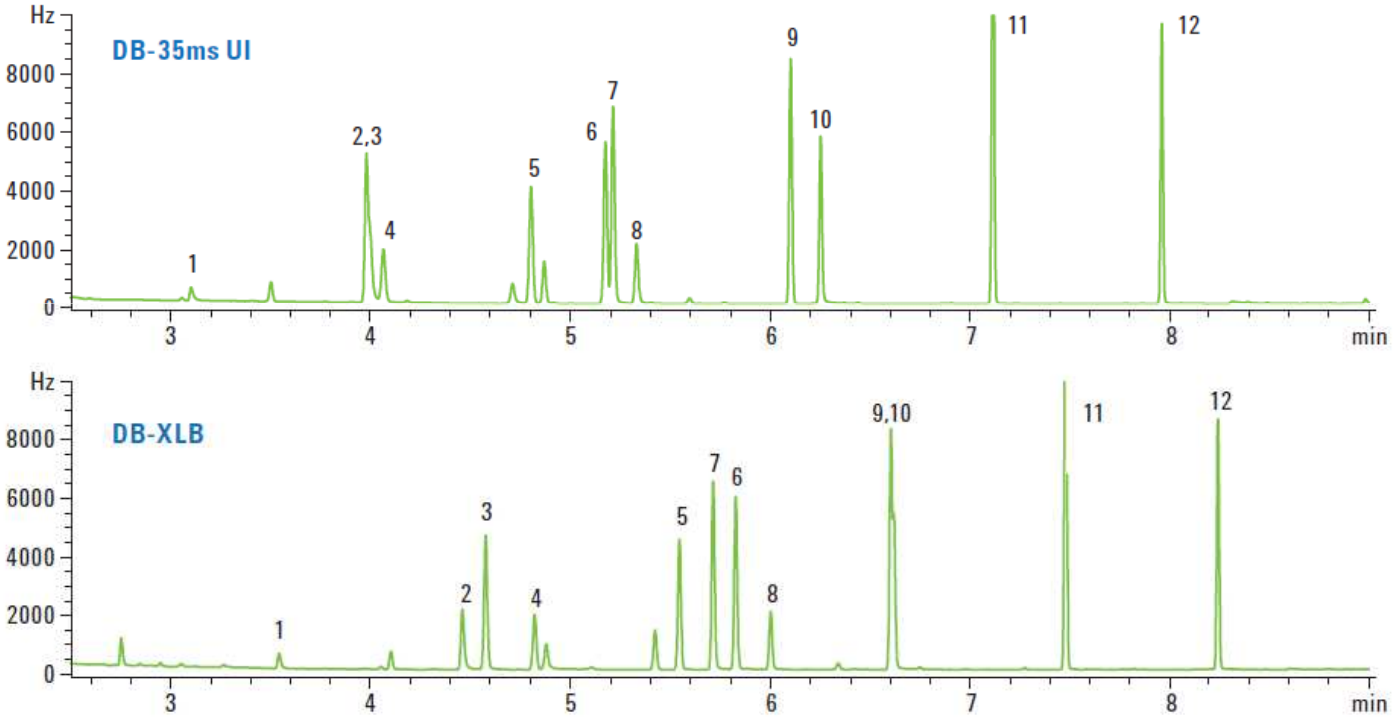


Solid-Phase Extraction (SPE)

- Elution solvent may need to be evaporated prior to required derivatization (TMS)
  - Melamine contamination if animal feed
- Evaporation to a small volume versus complete dryness, volatile compounds
- Elution solvent can be directly taken to derivatization (MTBE)
  - Haloacetic acids in water

Methylated HAAs Fortified QC sample

1. Methyl chloroacetate
2. Methyl bromoacetate
3. Methyl dichloroacetate
4. Dalapon methyl ester
5. Methyl trichloroacetate
6. 1,2,3-Trichloropropane (IS)
7. Methyl bromochloroacetate
8. Methyl 2-bromobutanoate (SS)
9. Methyl bromodichloroacetate
10. Methyl dibromoacetate
11. Methyl dibromochloroacetate
12. Methyl tribromoacetate



GC/μECD chromatograms of a 1–10 ng/mL fortified reagent water sample prepared according to method procedure and analyzed on an Agilent J&W DB-35ms UI (p/n 122-3832UI) and DB-XLB (p/n 122-1236) capillary GC columns. Chromatographic conditions are listed in Table 1.

Recovery and Repeatability of Haloacetic Acids in Fortified Reagent Water Using Agilent's Bond Elut SAX SPE for Extraction and J&W DB-35ms UI (p/n 122-3832UI) and DB-XLB (p/n 122-1236) GC Columns for Quantitative Analysis

Recovery and Repeatability of Haloacetic Acids on DB-35ms UI column

	Spike	1 x Spike fortified QC		5 x Spike fortified QC		20 x Spike fortified QC	
Analytes	ng/mL	% Recovery	RSD (n=6)	% Recovery	RSD (n=6)	% Recovery	RSD (n=6)
Methyl chloroacetate	0.60	108.8	1.8	102.4	0.6	95.8	0.7
Methyl bromoacetate/Methyl dichloroacetate*	1.00	98.2	1.6	98.2	0.7	95.9	0.6
Dalapon methyl ester	0.40	95.5	2.1	99.4	0.9	96.1	0.7
Methyl trichloroacetate	0.20	96.7	1.7	88.4	2.0	92.2	1.4
Methyl bromochloroacetate	0.40	91.9	2.6	95.0	1.8	93.4	1.3
Methyl bromodichloroacetate	0.40	113.0	1.1	88.9	1.7	93.9	1.8
Methyl dibromoacetate	0.20	82.5	3.4	92.1	2.3	93.6	1.6
Methyl dibromochloroacetate	1.00	116.5	1.0	89.5	1.7	94.3	1.9
Methyl tribromoacetate	2.00	101.4	2.1	84.6	2.1	90.4	2.2
Methyl 2-bromobutanoate (SS)	0.50	104.4	2.5	105.5	1.2	106.9	0.7

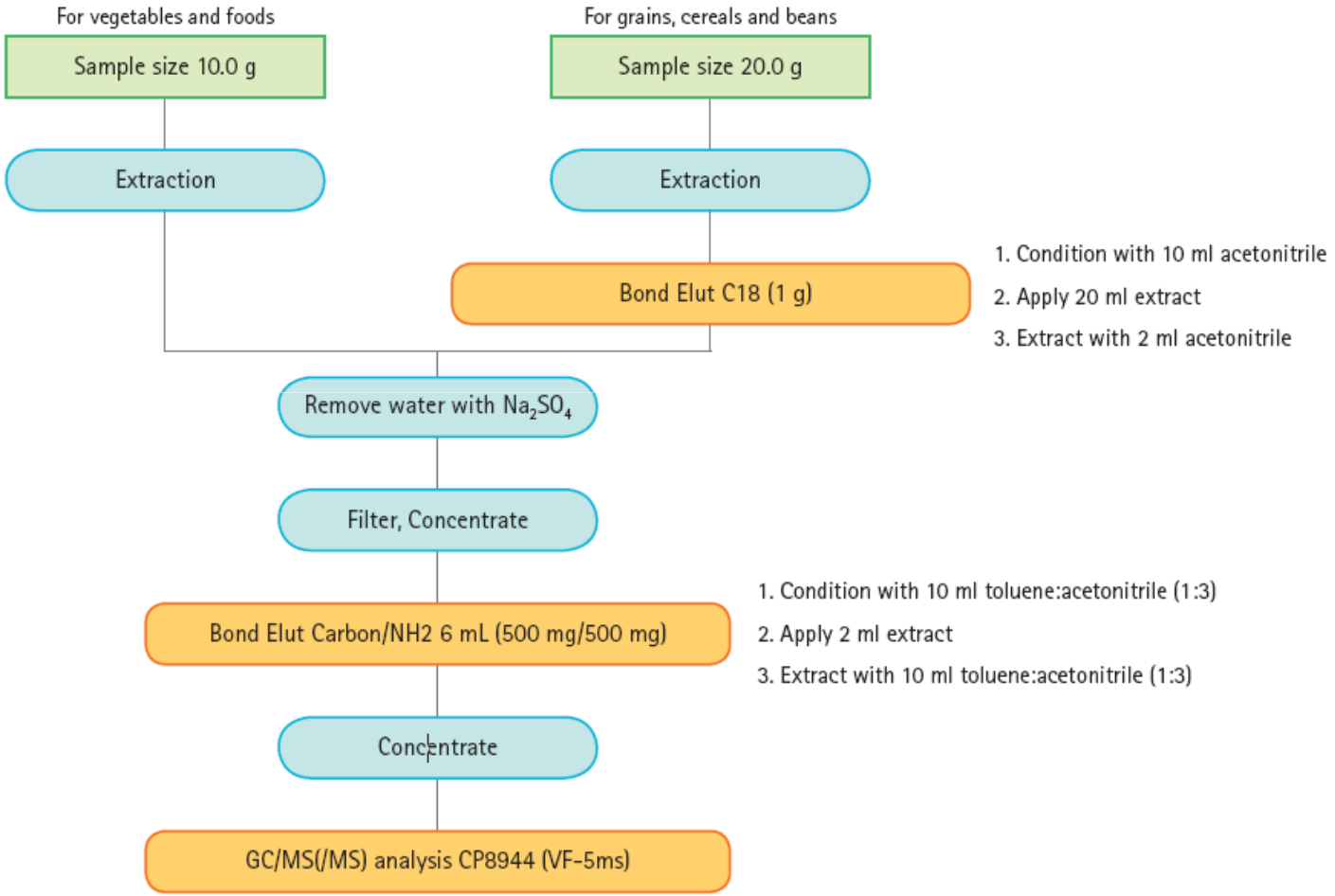
Recovery and Repeatability of Haloacetic Acids on DB-XLB column

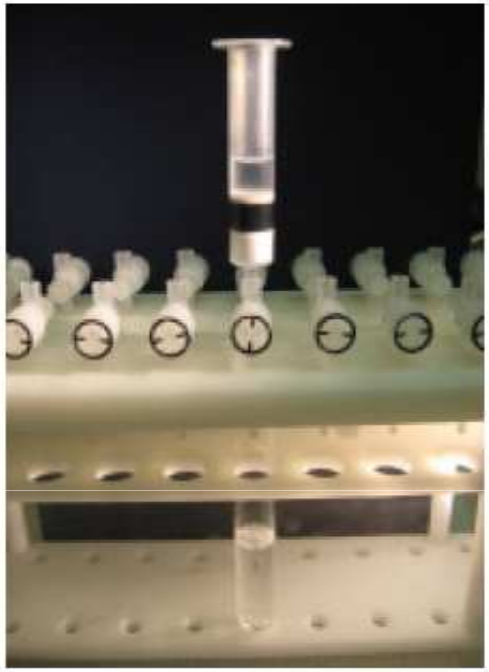
	Spike	1 x Spike fortified QC		5 x Spike fortified QC		20 x Spike fortified QC	
Analytes	ng/mL	% Recovery	RSD (n=6)	% Recovery	RSD (n=6)	% Recovery	RSD (n=6)
Methyl chloroacetate	0.60	83.5	2.8	98.9	0.7	91.9	0.8
Methyl bromoacetate	0.40	85.2	0.8	92.7	0.4	88.5	0.7
Methyl dichloroacetate	0.60	94.2	1.0	98.9	0.3	94.9	0.5
Dalapon methyl ester	0.40	95.3	0.4	99.6	0.2	96.2	0.4
Methyl trichloroacetate	0.20	106.1	0.8	100.8	0.5	99.7	0.6
Methyl bromochloroacetate	0.40	103.8	1.1	107.8	0.9	102.7	1.2
Methyl bromodichloroacetate/Methyl dibromoacetate*	0.60	109.2	1.0	104.0	0.8	102.8	1.2
Methyl dibromochloroacetate	1.00	116.2	1.0	101.0	0.9	102.8	1.2
Methyl tribromoacetate	2.00	107.2	2.0	95.9	1.2	98.7	1.6
Methyl 2-bromobutanoate (SS)	0.50	109.5	0.7	108.6	0.5	108.3	0.9

*Coelute; % Recovery is based on sum of both analytes

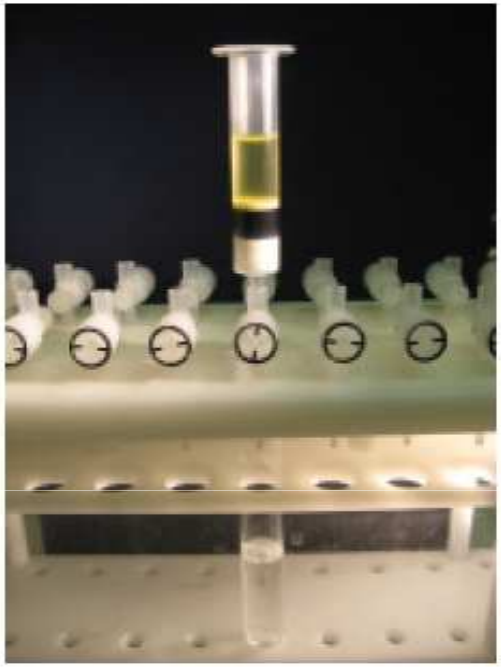
Japanese Positives

Method for the simultaneous monitoring of pesticide residues in agricultural products.
Extraction, refining (clean-up) and quantitative analysis

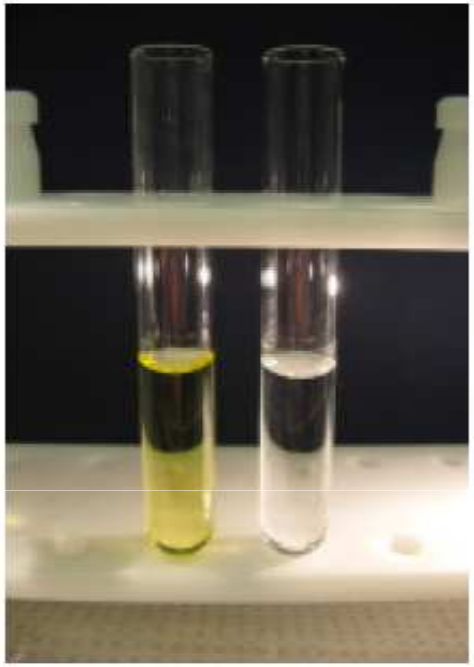




Conditioning



Clean-up



Before and after clean-up

SPE Applications

- Melamine and Cyanuric acid in food material, milk, formula
- Pesticides in water, food
- Pharmaceutical Personal Care Products in water
- EPA 500 series: herbicides, organic compounds in water
- Beta agonists drug residues in animal tissue
- PAHs in water

Advantages behind SPE: extremely clean sample, selectivity, less instrument maintenance, concentrates sample

Supported Liquid Extraction

Terminology

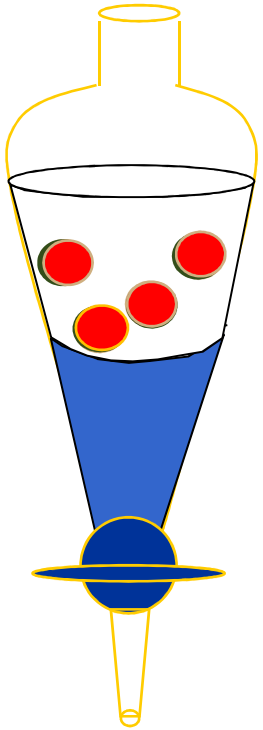
Hydromatrix™ - diatomaceous earth sorbent

- composed of fossilized diatoms
- purified at high temperatures
- high surface area for water adsorption

Chem Elut™ - pre-assembled cartridges with Hydromatrix

'Solid Supported Liquid/Liquid Extraction' or 'Solid Liquid Extraction' = **SLE**

A Traditional Way to Extract Samples



Shake sample with an immiscible “extraction” solvent

Let the liquids separate

Remove the “extraction” solvent and collect

Why Does Liquid/Liquid Extraction Work?

If we develop the method right

- The molecules we want to collect dissolve better in an extraction solvent than in the sample
- Unwanted molecules stay in the sample

But the method

- Uses lots of solvent and results in lots of waste
- Gives results that are affected by the skill of the chemist
- Creates emulsions
- Needs expensive, clean glassware
- Is hard to speed up - and it IS slow!

What is ChemElut? How Does It Work?

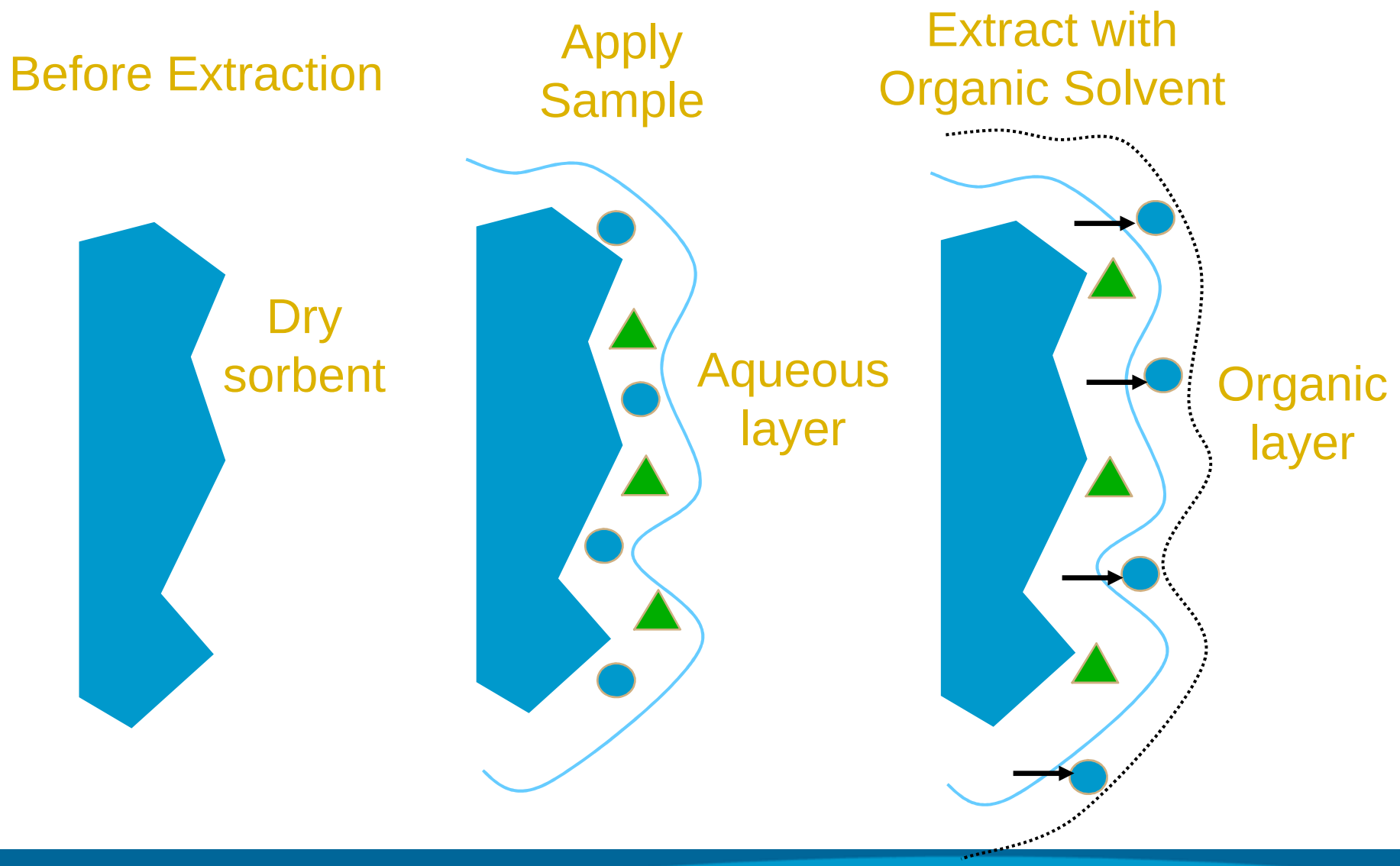
Chem Elut is a tube packed with a solid “sponge”

•High purity, diatomaceous earth

•Adsorbs water which spreads as a thin film over the surface

•Resulting in a sample that exposes a high surface area to an extraction solvent (which must be immiscible)

The Supported Liquid Extraction Process



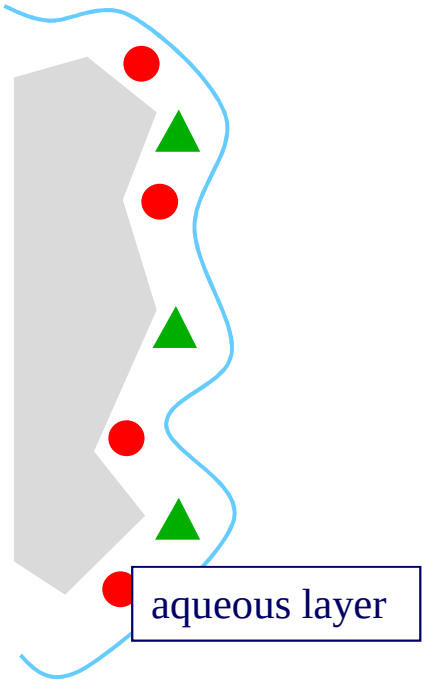
The SLE Process

STEP 1: Apply aqueous sample to dry Chem Elut cartridge via gravity flow

- no vacuum needed
- no conditioning
- analytes do not bind to the Hydromatrix material
- choose cartridge size based on total sample volume

The SLE Process

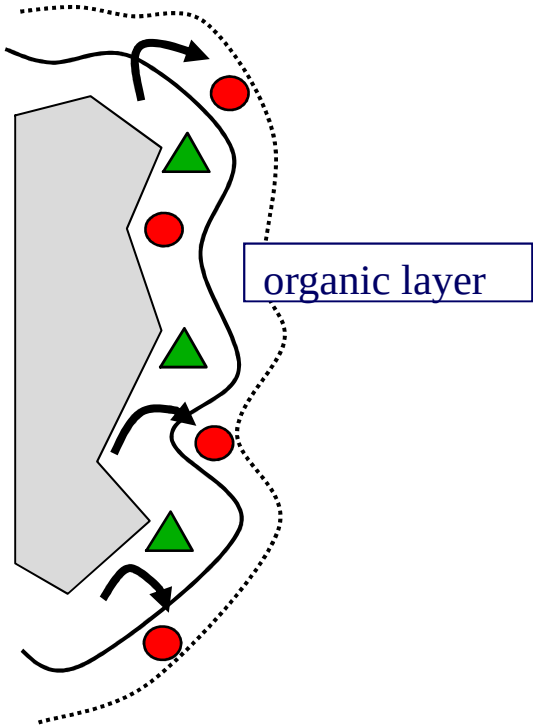
STEP 2:
Wait 5-10 minutes



- analytes do not bind to the hydromatrix material
- samples are stable and do not need to be eluted immediately
- closed Chem Elut cartridges (protected from sample evaporation) can be used for sample storage and transportation.

The SLE Process

STEP 3:
Add organic extraction solvent



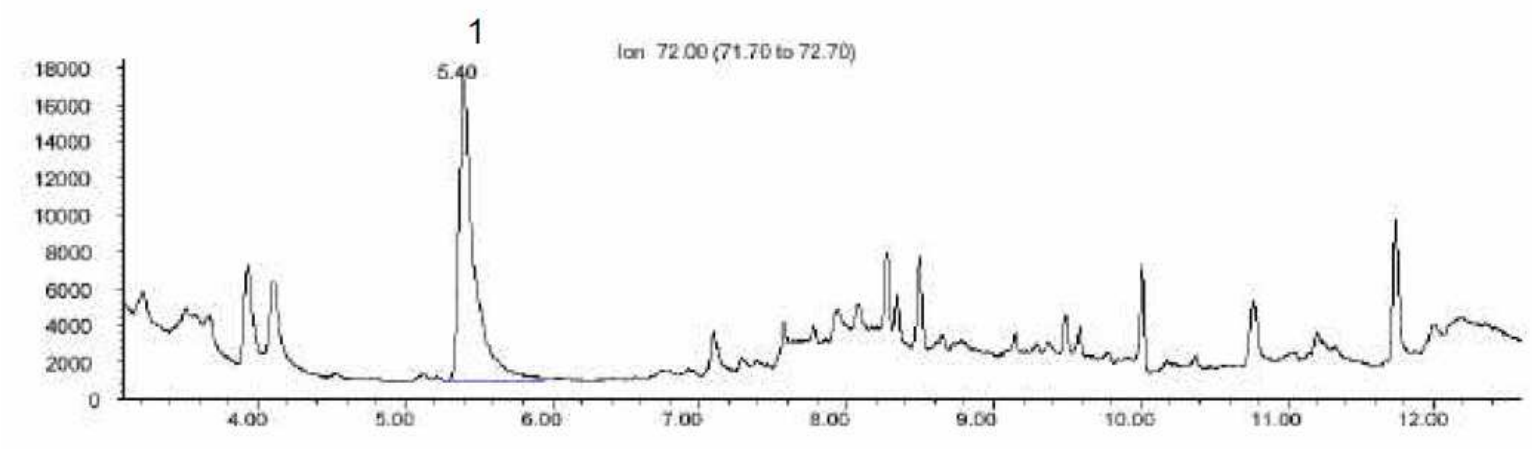
- Elution with immiscible organic solvent
- In general, extraction with 2-3 times the sample volume is sufficient
- 2 aliquots better than one

Solvent Mixtures Compatible with SLE

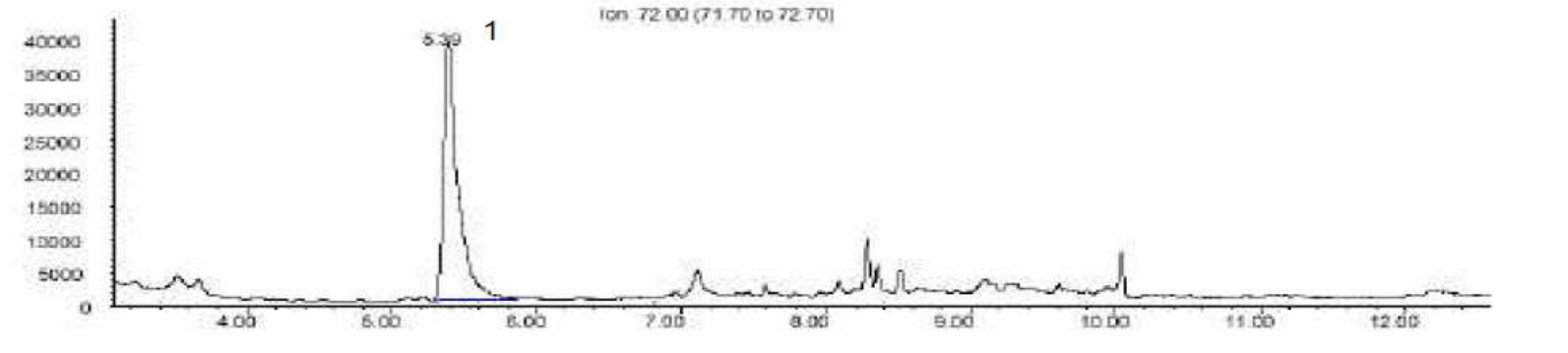
Mixture	Max % ***water-miscible solvent***	Mixture	Max % ***water-miscible solvent***	Mixture	Max % ***water-miscible solvent***
CH_2Cl_2/MeOH	20% MeOH	CH_2Cl_2/THF	70% THF	EtOAc/THF	70% THF
CH_2Cl_2/ acetone	20% acetone	CH_2Cl_2/ CH_3CN	10% CH_3CN	EtOAc/IPA	60% IPA
CH_2Cl_2/DMF	10% DMF	Toluene/THF	70% THF	EtOAc/MeOH	10% MeOH
CH_2Cl_2/DMA	10% DMA	Toluene/DMF	30% DMF	Et_2O/THF	50% THF
CH_2Cl_2/NMP	20% NMP	EtOAc/DMF	10% DMF		

adapted from Breitenbucher, J. G., et al., *J. Comb. Chem.* **2001**, *3*, 528-533

Acrylamide in waffles, conc. 1814 µg/kg, recovery 90%



Acrylamide in chips, conc. 3000 µg/kg, recovery 90%



Peak Identification:

1	acrylamide	m/z = 72, 1.512 µg/mL
2	D3-acrylamide	m/z = 75, 0.524 µg/mL

Supported Liquid Extraction

- Azo dyes in textiles
- Caffeine in black tea
- Pesticides in honey
- Polyphenols in wine

Advantages behind Supported liquid extraction: less solvent than LLE, no emulsions, can be used to transport or store the sample

QuEChERS

QuEChERS
(Pronounced *"catchers"*)

- **Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe

- Introduced in 2003: M. Anastassiades, S.J. Lehotay, D. Stajnbaher, and F.J. Schenck, J. AOAC Int 86 (2003) 412
- Validated in 2005, with subsequent modification in 2007
  - AOAC 2007.01 and European Method EN 15662
- Streamlined approach that makes it easier and less expensive to examine pesticide residues in food

QuEChERS

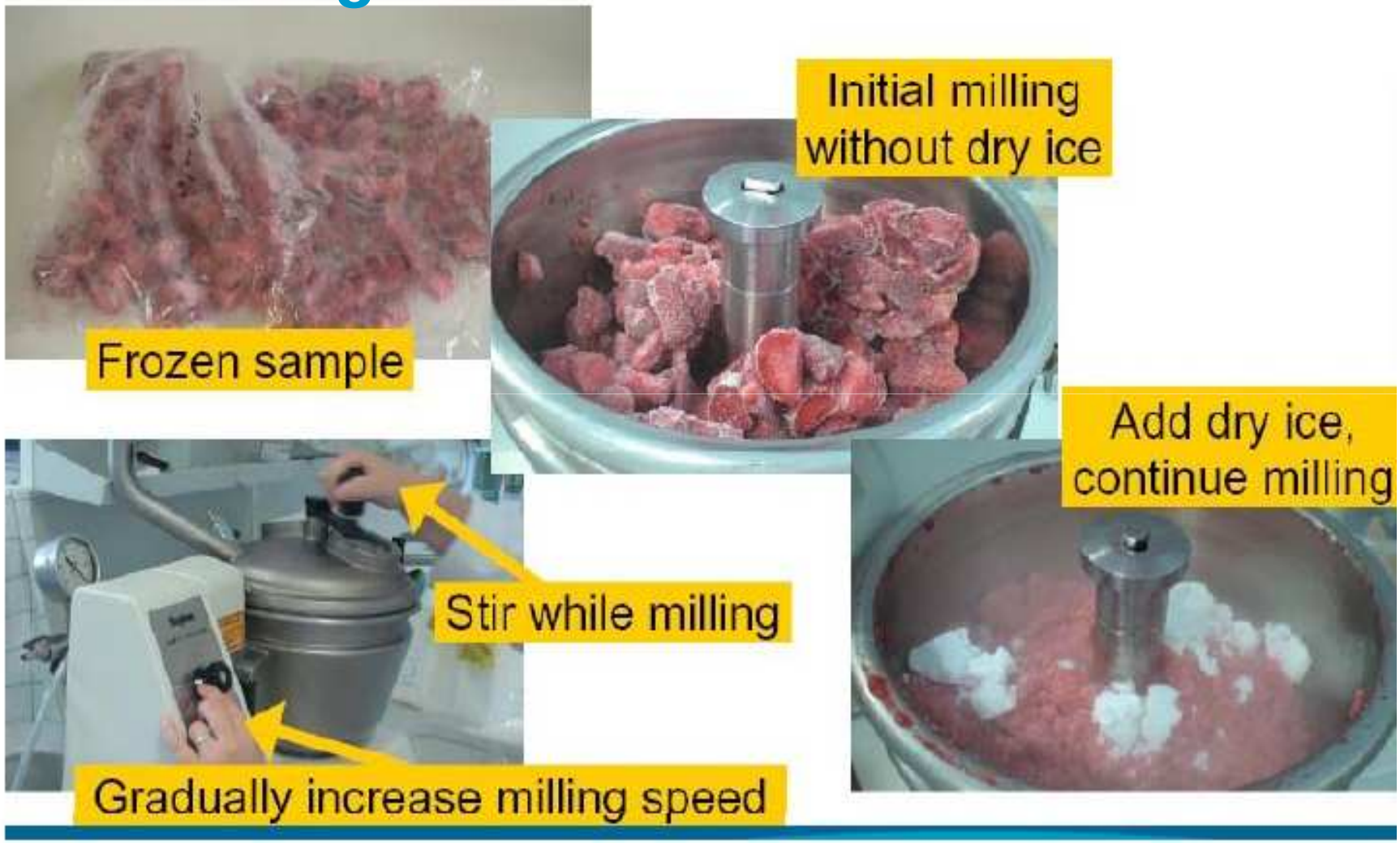
- Alternative to existing methods: LLE, SPE, GPC
- QuEChERS is still a very young; being adopted worldwide
- Detector availability: MS and MS/MS (selective and sensitive)
- QuEChERS process substantially decreases cost per sample
- “Just Enough”

	Luke method, traditional SPE, or GPC	QuEChERS	QuEChERS Benefits!
Estimated Time to process 6 samples (min)	120	30	4x faster
Solvent Used (mL)	90 mL	10-15 mL	9 x less solvent
Chlorinated Waste (mL)	30 mL	none	safer, greener, less costly
Glassware/ specialized equipment	Funnel, water bath, 200 mL containers, evaporator, etc.	None	No additional supplies needed

QuEChERS: Not Just for Pesticides Anymore

- Pesticides in other commodities
- PAHs
- PCBs
- Illegal Dyes
- Mycotoxins
- Veterinary Drugs
- Antibiotics
- Acrylamides
- And More to Come…..

QuEChERS: Prepping the Sample for Processing

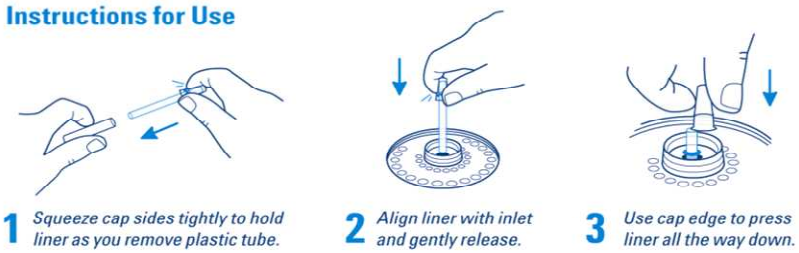


QuEChERS Procedure: Step 1

QuEChERS Procedure: Step 2 & 3

GC/MS Analysis

- GC/MS
  - Selectivity and sensitivity
  - Back flush strongly suggested
  - Large volume injections
  - Ultra Inert: liners and columns
  - Solvent exchange: Not the first choice, but an option



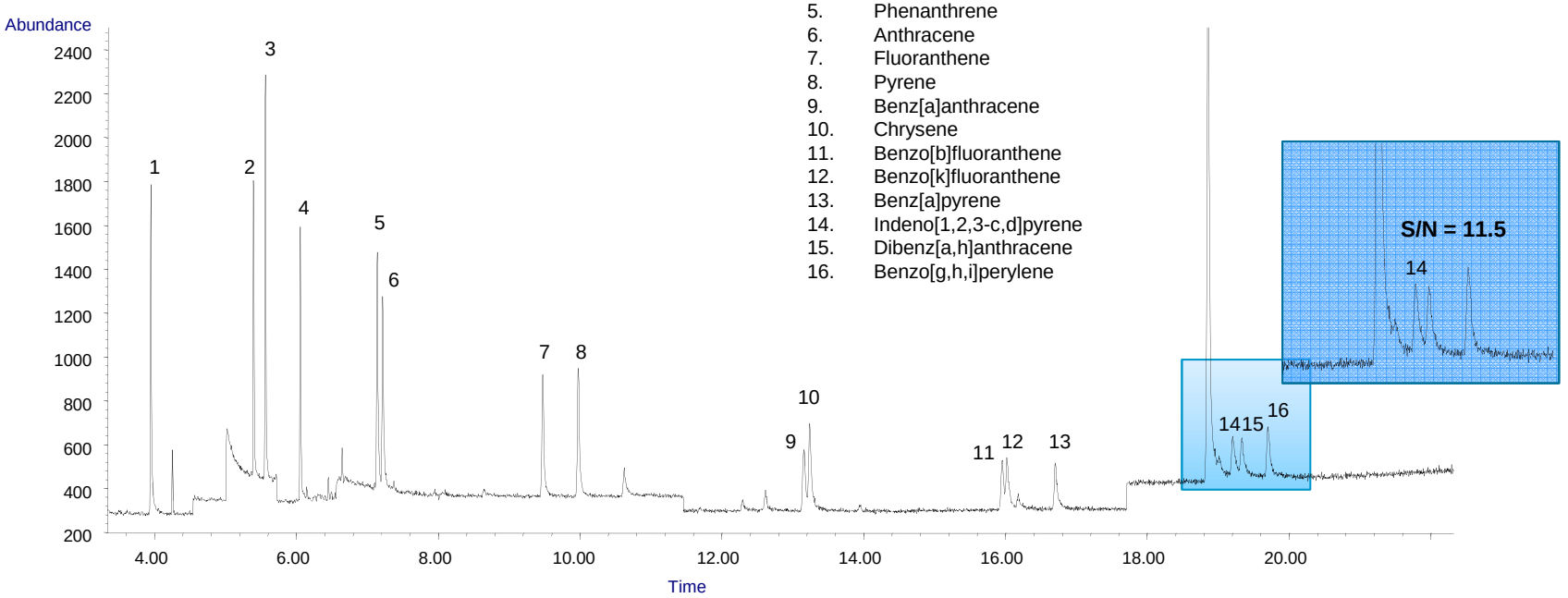
Agilent Ultra Inert liner with deactivated wool
Repeatability (%RSD)

100 injections of matrix standard with added analyte protectant

	%RSD		
Pesticide	10 injections	50 injections	100 injections
Methamidophos	1.0	1.2	2.7
Acephate	1.9	2.4	5.1
Omethoate	2.1	4.8	9.4
Dimethoate	1.5	2.4	3.8

Excellent Signal to Noise Ratios at Trace Levels

1. Napthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benz[a]pyrene
14. Indeno[1,2,3-c,d]pyrene
15. Dibenz[a,h]anthracene
16. Benzo[g,h,i]perylene



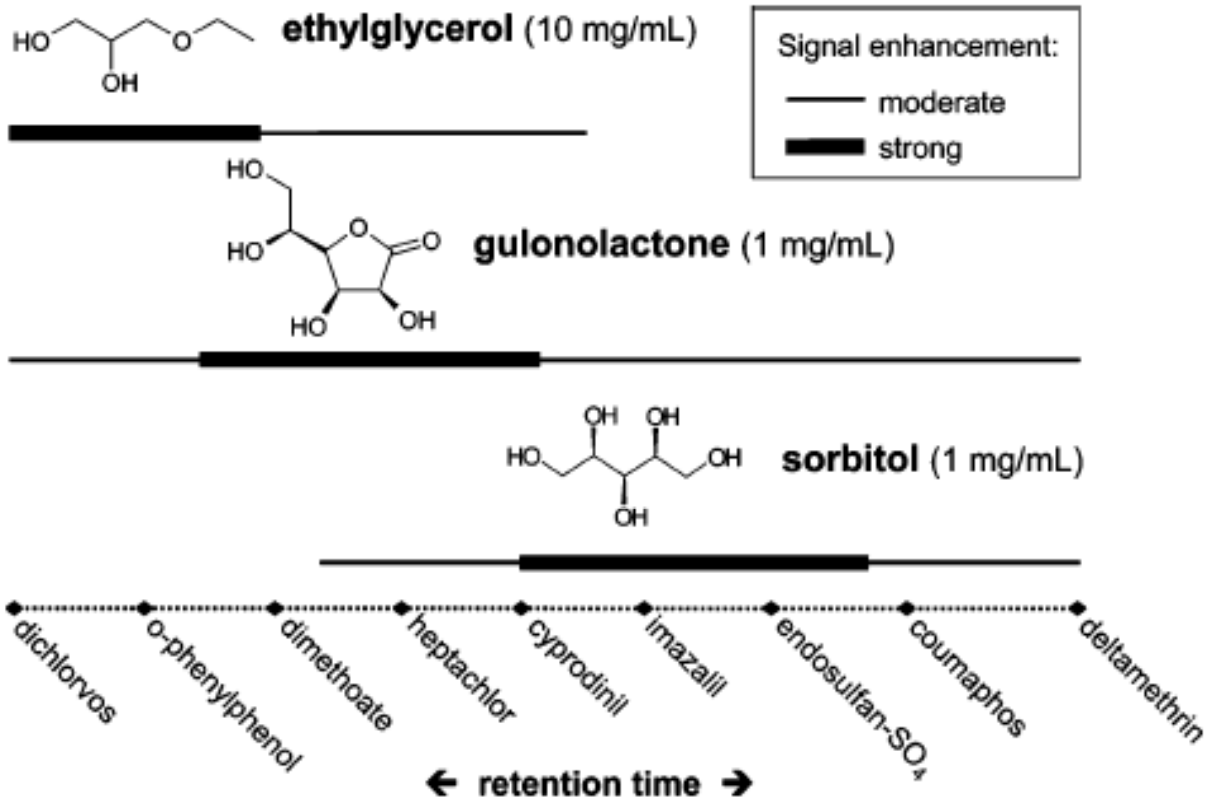
GC/MS SIM Chromatogram of 10 ppb PAHs spiked in fish matrix blank extract

GC/MSD: 7890/5975B with purged ultimate union
Column: **DB-5ms UI 20 m 0.18 mm 0.18 µm**
Restrictor : Siltek 0.7m x 0.15mm ID (Col 2)
MMInlet: 0.5µL, 320℃, splitless , purge flow 50mL/ min at 0.8min
gas saver 30mL/min at 2min
Carrier: Helium, 1.7mL/min cnst flow col 1
PCM 1=3.8 psi cnst pressure, col 2=3.8ml/min flow @ 50℃
Oven: 50℃ (0.4 min) to 195℃ (25℃/min) hold 1.5 mi n, 8℃/min to 265℃ 20℃/min to 315℃ (1.25 min)
Postrun backflush 7 min @320℃
MSD: Transfer line 340℃ Source 340℃ Quad 150℃

Recovery and Repeatability of PAHs in Fortified Red Snapper Fish with Agilent J&W DB-5ms UI column

Analytes	25 ng/mL fortified QC		250 ng/mL fortified QC		500 ng/mL fortified QC	
	%Recovery	RSD (n=6)	%Recovery	RSD (n=6)	%Recovery	RSD (n=6)
Naphthalene	80.35	3.29	96.77	4.23	98.64	1.88
Acenaphthylene	95.28	2.30	103.36	2.80	101.02	2.27
Acenaphthalene	92.28	2.51	101.18	2.87	100.69	2.34
Fluorene	95.98	2.99	105.94	2.82	105.00	1.28
Phenanthrene	100.51	3.46	104.93	2.71	103.25	1.70
Anthracene	107.38	3.51	105.95	3.45	105.38	1.74
Fluoranthene	113.27	3.87	105.76	3.33	103.64	1.81
Pyrene	113.55	3.51	103.99	3.24	102.29	1.94
Benz[a]anthracene	129.79	3.41	101.45	3.91	100.61	3.24
Chrysene	116.75	4.01	98.55	4.17	95.95	5.61
Benzo[b]fluoranthene	131.20	3.70	98.77	4.08	98.08	3.24
Benzo[k]fluoranthene	139.45	2.52	99.13	3.98	95.31	4.54
Benzo[a]pyrene	125.30	3.68	95.33	3.89	96.82	1.80
Indeno[1,2,3-cd]pyrene	119.51	3.47	94.57	3.23	93.71	2.55
Dibenz[a,h]anthracene	126.35	3.54	98.55	3.50	98.85	2.24
Benzo[g,h,i]perylene	114.91	4.93	97.30	3.37	95.63	1.83

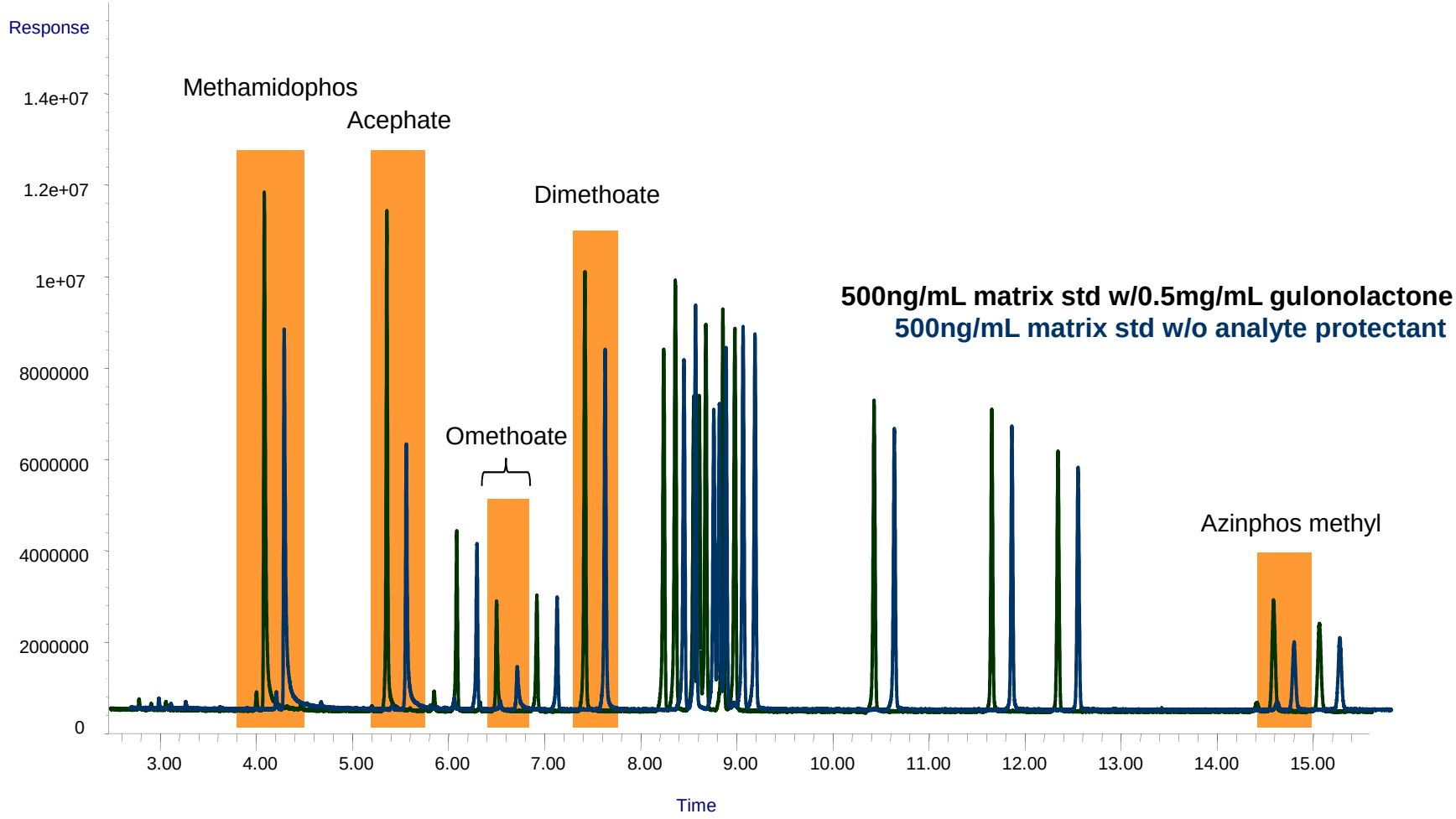
Analyte Protectants



Schematic illustration of the effect of the optimal combination of analyte protectants (ethylglycerol, gulonolactone, and sorbitol at 10, 1, and 1 mg/mL, respectively, in the injected pesticide solutions in acetonitrile) on the signal enhancement of susceptible analytes throughout the elution range of GC-amenable pesticides.

Analytical Chemistry, Vol 77, No. 24, December 15, 2005.

FPD Chromatogram use of Analyte Protectants Ultra Inert Liner/DB-35ms UI

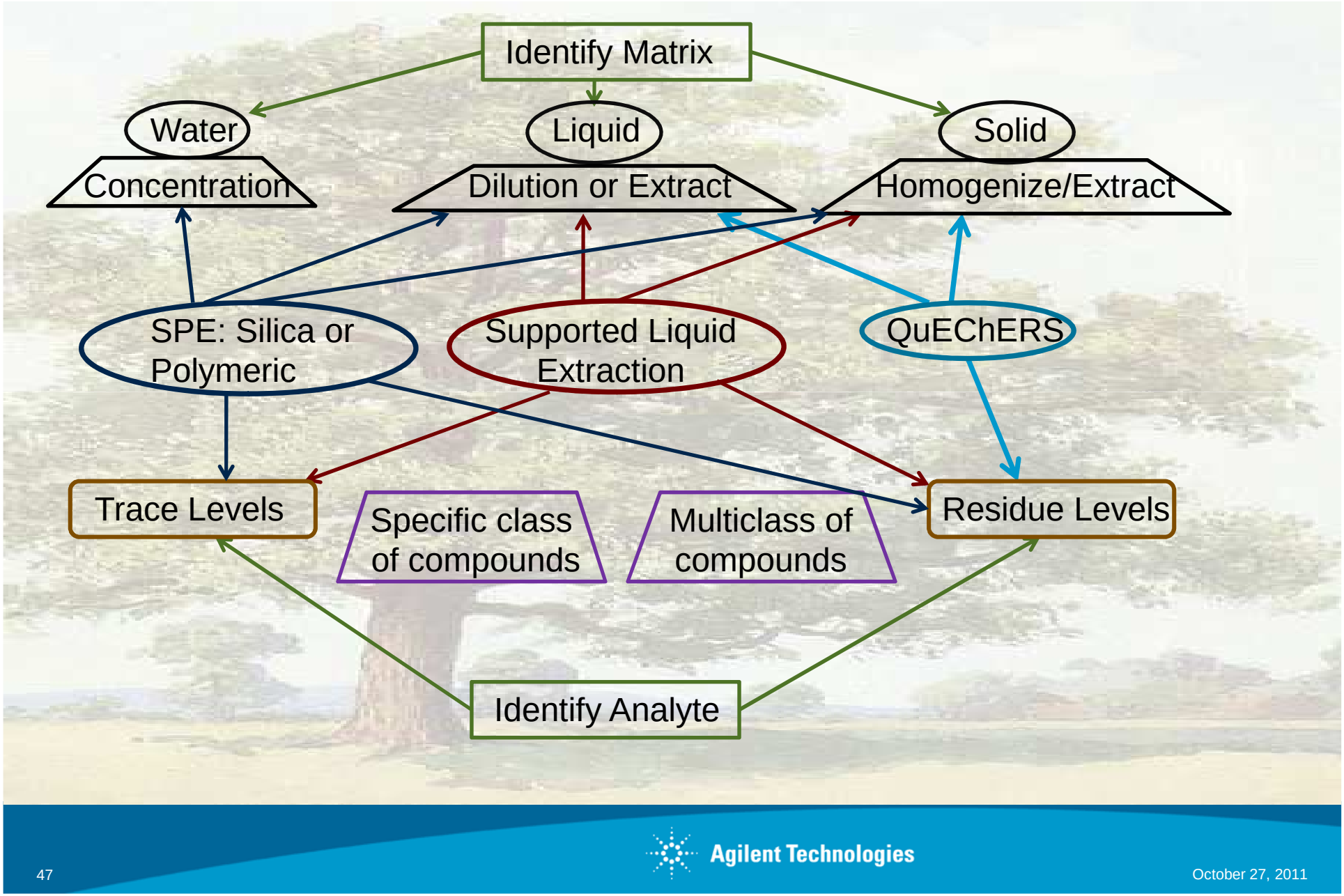


QuEChERS

- Veterinary drugs in Edible meats
- Mycotoxins in wheat
- Illegal Dyes in Food Sauces
- PCBs in Fish
- Hormones in shrimp
- Acrylamide in Fried food and oil

Advantages behind QuEChERS: Less solvent, greener technology, multiclass extraction, “just enough”

Decision Tree



Conclusion

- Many sample preparation options available: SPE, SLE, and QuEChERS
- Matrix and analyte(s) need to be evaluated
- Levels required to be reached
- Determine appropriate sample preparation approach

Thank You!