

**Herzlich  
Willkommen** zum  
Agilent Tipps & Tricks  
Seminar:  
Probenvorbereitung in der  
Lebensmittel- und  
Umweltanalytik  
**München 16.03.2011**



**Agilent Technologies**

# Teilnehmerzertifikat & Feedback Form

## Teilnehmerzertifikat

Erhältlich am Ende des Seminars

## Feedback Form

Ihre Meinung ist uns wichtig! Bitte lassen Sie uns wissen, ob Ihnen das Seminar zugesagt hat, ob wir etwas verbessern können und ob Sie weitere Wünsche haben.

**Bitte füllen Sie den Feedback Bogen aus.** Als Dankeschön erhalten Sie einen USB Stick mit wertvollen Unterlagen

Die **Vorträge** senden wir Ihnen nach der Veranstaltung in einem separaten eMail zum Download zu.



# Agenda Lebensmittel & Umwelt vormittags



Title	Speaker	
<b>Session I – Optimale Ergebnisse mit Probenvorbereitung</b> <i>Wie lässt sich durch Probenaufreinigung der Betriebsablauf im Labor positiv beeinflussen?</i>	Roland Weber	30 min
<b>Session II, Teil A – Applikative Lösungen , Methodenentwicklung &amp; Fehlersuche</b>	Roland Weber	30 min
Kaffeepause		15 min
<b>Session II, Teil B – Applikative Lösungen , Methodenentwicklung &amp; Fehlersuche</b>	Roland Weber	30 min
<b>Session III – Die beste analytische Säule für die aufgereinigte Probe</b>	Dr. Jens Görlich	30 min
<b>Session IV – Zu guter Letzt: Die Analysengeräte</b>	Dr. Manfred Bergmann	30 min
Imbiss		



# Agilent Sample Prep: Accuracy Starts Here

- Options for Your Needs
- Innovative Products Designed for Lab Efficiency
- Technical Support at Every Step
- World Class Manufacturing and Quality

Provide chromatographers with highly accurate, reproducible results and enable transfer of methods from lab-to-lab and around the world



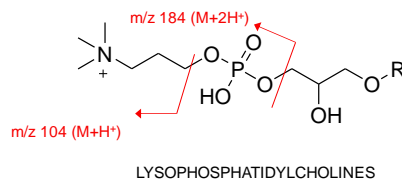
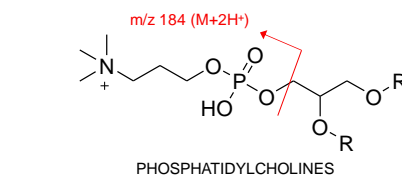
# Tips and Tricks

Throughout this presentation will be a series of tips and tricks to help improve your results and productivity.

Watch out for the  
Orange Boxes!

**BUT** please let us if there is  
a **TIP** or **TRICK** that you need in  
your lab

## TIP: Monitoring Phospholipid Removal



- Monitoring the 184 → 184 m/z trace in MS/MS gives a measure of the total phospholipids and lyso-phospholipids in a sample
- Measures the ionic head group common to all classes of phospholipids

*“We are now monitoring the 184→184 transition in all of our bioanalytical method development studies”*

DMPK Group, AstraZeneca, UK



# Sample Prep Terminology

**Analyte(s):** Molecule(s) of interest

**Matrix:** The sample (soil, urine, blood, saliva etc.)

**Interferences:** Entities inside the sample which will inhibit analysis of desired analyte

**IS:** Internal standard

**LLE:** Liquid-liquid extraction

**SLE:** Supported-liquid extraction

**SPE:** Solid phase extraction

**LC (MS):** Liquid chromatography (mass spectroscopy)

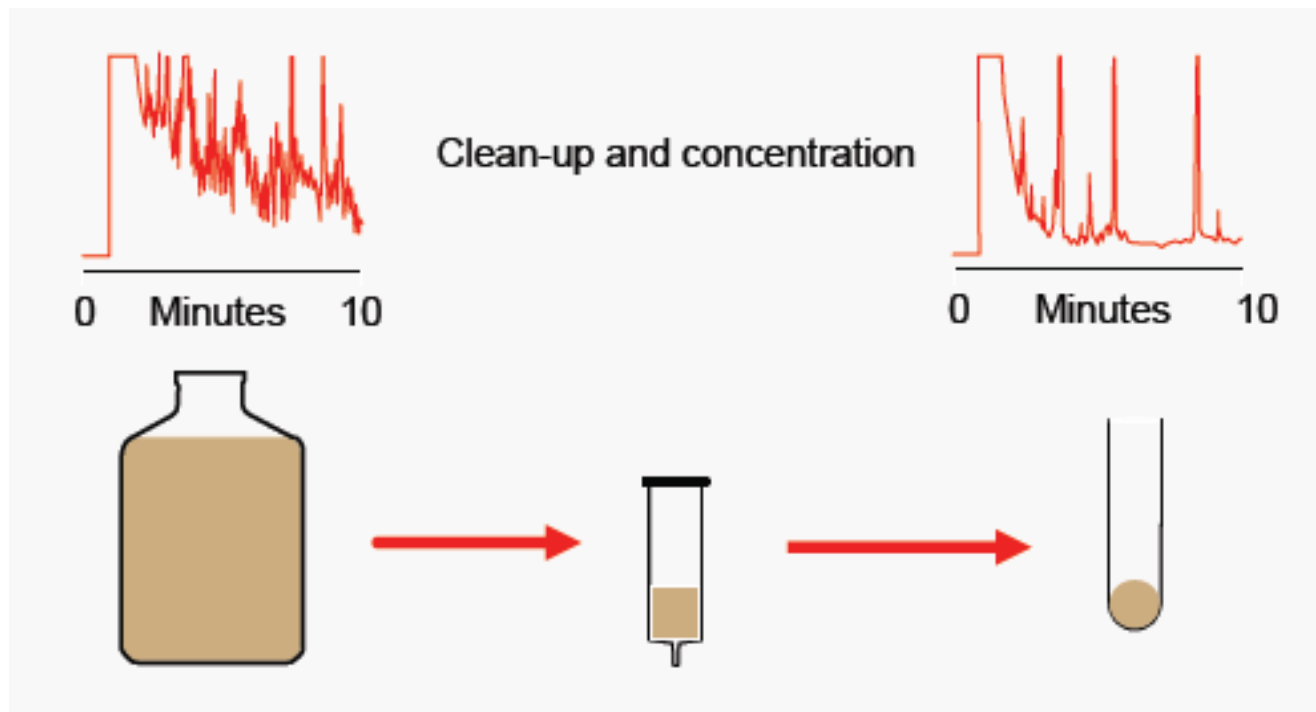
**GC (MS):** Gas chromatography (mass spectroscopy)

**LOQ:** Limit of Quantification    **LLOQ:** Lower limit of quantification

**RSD:** Relative standard deviation



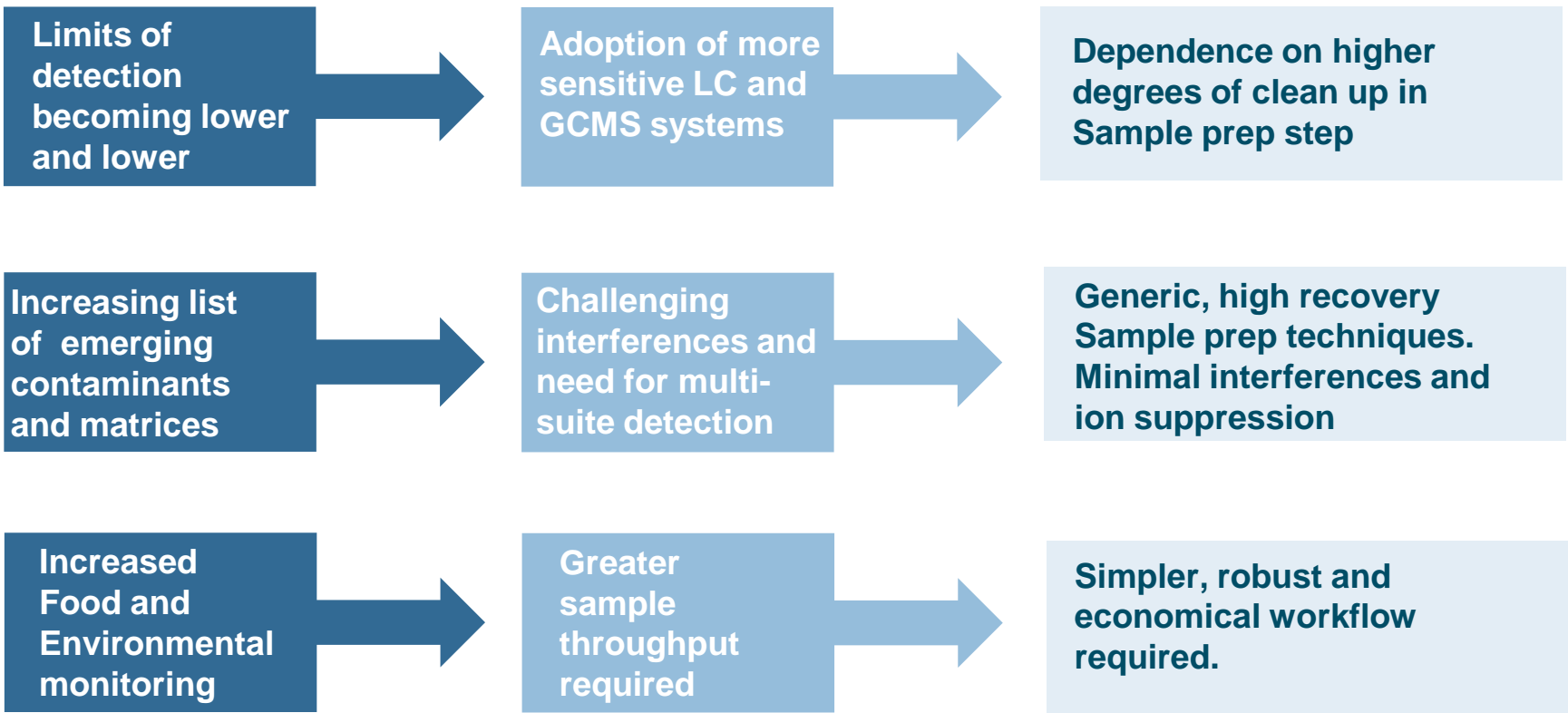
# Why Do Sample Preparation?



- Removal of interferences which would otherwise affect detection of analyte
- Concentration of an analyte to a detectable concentration
- Solvent Switching into an analytically more compatible solvent

# Current Relevancy of Sample Prep

## Industry Trends



# Effects of Endogenous Interferences

- Poor Chromatography
- Mechanical issues (particulates, blockages)
- LC Column Lifetime Issues
- Instrument Downtime
- Carry Over
- Increase in sample run time/ cost
- Ion Suppression
- Overall loss in sensitivity

**Let's take a look at some common matrices**



## Composition

- Water
- Fats, Lipids, Proteins
- Cells
- Collagen
- Myosin + actin
- Myoglobin
- Carbohydrate
- Salts + electrolytes



## Composition/Types

- Drinking
- Surface
- Sea

## Challenges:

- Large sample volumes
- Low LODs
- Large analyte suites



## Composition

- Rocks and minerals
- Silicates
- Humus
- Humic acids (lignin by-products)
- Plant fiber
- Micro-organisms



# Fruit and Vegetables

## Composition

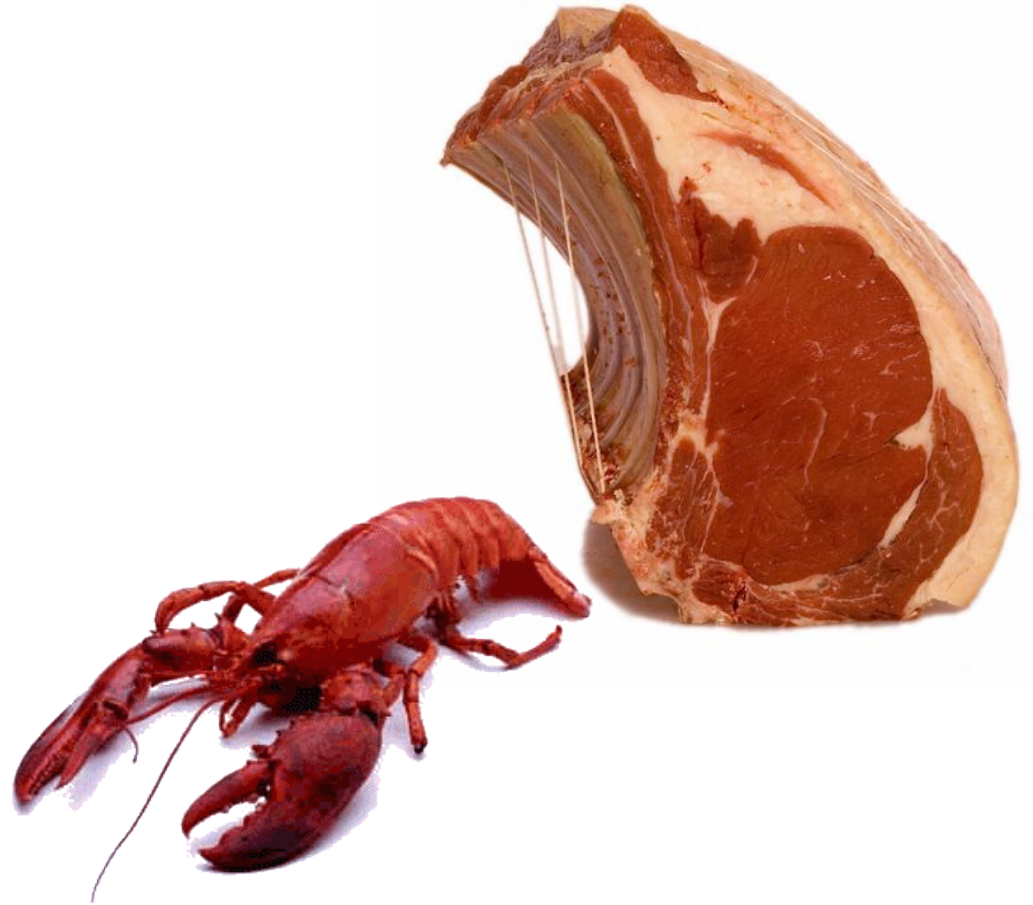
- Carbohydrates (sugars and complex)
- Proteins, amino acids,
- Vitamins
- Enzymes
- Fats
- Organic acids
- Chlorophyll



# Meat Products

## Composition

- Water
- Fats, Lipids, Proteins
- Cells
- Collagen
- Myosin + actin
- Myoglobin
- Carbohydrate
- Salts + electrolytes



## Composition

- Carbohydrates
- Proteins
- Fats
- Fibre
- Minerals
- Vitamins
- Ethanol?



## Composition

- Proteins
- Fats
- Fibre
- Carbohydrates
- Vitamins
- Minerals
- Water



# Sweet Sugar - Honey

## Composition

- Water
- Carbohydrates
- Proteins
- Amino Acids
- Vitamins
- Minerals



**So, what techniques do we have available ?**



# Dilution

Sample dilution (with an internal standard added) aka “dilute and shoot” is a commonly adopted simple approach to sample analysis

## Advantages

- Fast and easy

## Limitations

- Interferences are not removed
- Analyte concentration is reduced
- Instrument and Column contamination
- Ion Suppression



# Liquid-Liquid Extraction (LLE)

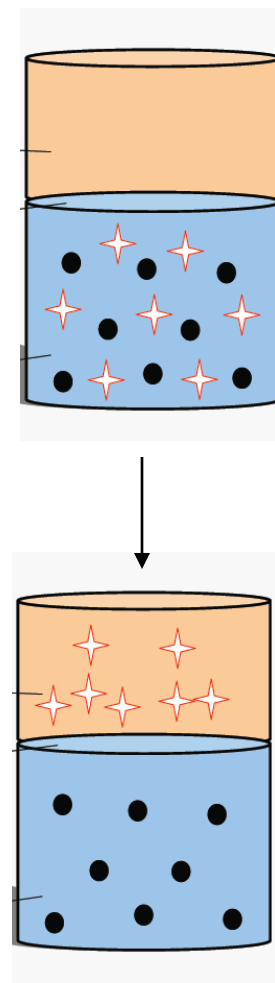
Technique which uses two immiscible solvents to partition analytes of different polarities

## Advantages

- Low Cost
- Effective for Inorganic/Salt removal

## Limitations

- No concentration of analyte achieved, sample will need to be reconstituted prior to analysis
- Uses large volumes of solvents (often chlorinated)
- No selectivity for organic soluble analytes
- Difficult to automate
- Matrix specific (e.g. Urine vs Tissue)



# Supported-Liquid Extraction (SLE)

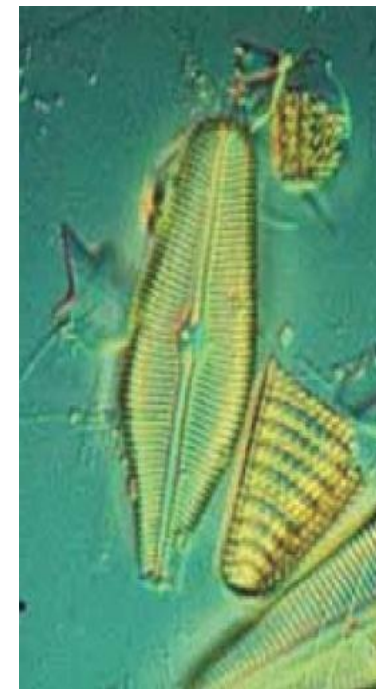
Technique which uses a highly wettable inorganic sorbent (diatomaceous earth).

## Advantages

- Low Cost
- Effective for Inorganic/Salt removal

## Limitations

- No concentration of analyte achieved, sample will need to be reconstituted prior to analysis
- Uses large volumes of solvents (often chlorinated)
- No selectivity for organic soluble analytes
- Difficult to automate
- Matrix specific (e.g. Urine vs Tissue)

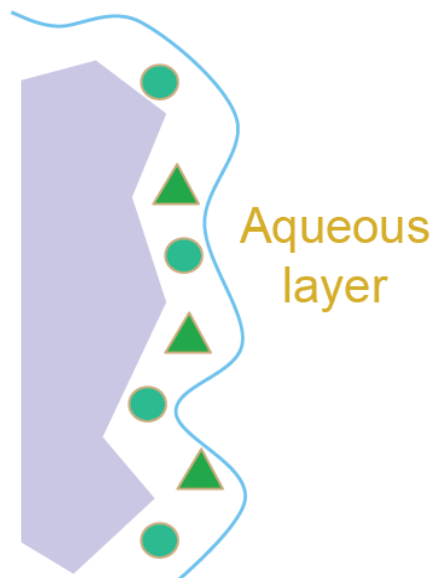


# SLE Mechanism

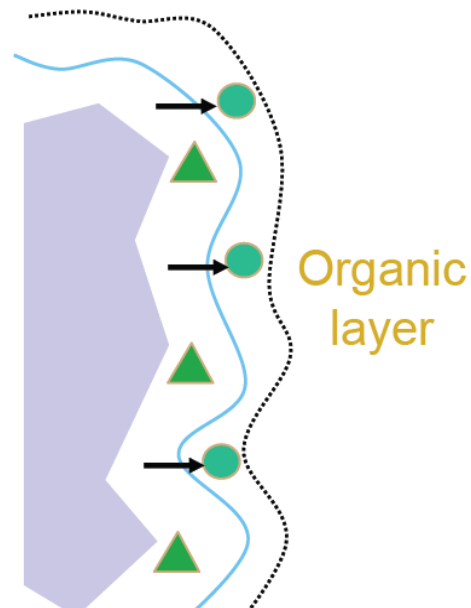
Before Extraction



Apply Sample



Extract with Organic Solvent



# Matrix Solid Phase Dispersion (MSPD)

Mechanical grinding/homogenisation of a functional sorbent with a solid sample followed by elution from the sorbent

## Advantages

- Excellent for tissue/solid sample
- Different functional sorbents can be used

## Limitations

- Interferences often co-extracted
- Can use large volumes of solvents
- Labour intensive
- Difficult to automate
- Matrix specific (e.g. Solids, Tissue, Post mortem bloods)



Simple two-step procedure ideal for complex food matrices

**STEP 1:** Salt Extraction

**STEP 2:** Dispersive d-SPE

## Advantages

- Low Cost
- Excellent for food pigment/chlorophyll removal
- Good level of clean up
- Robust and generic

## Limitations

- Not suitable for high fat matrices
- Not easily automatable



# Solid Phase Extraction

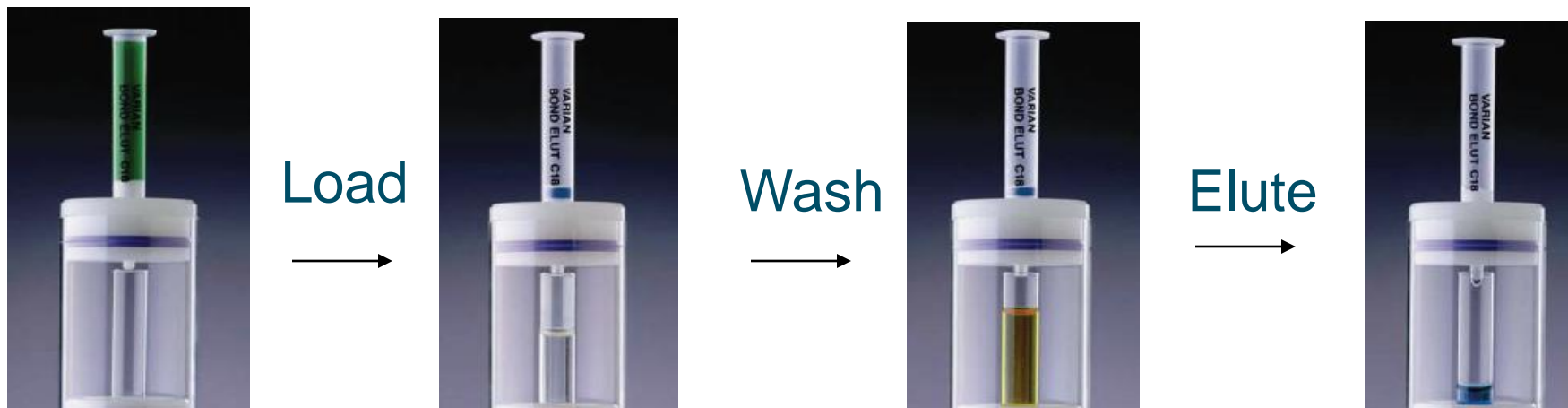
- **Solid Phase Extraction is the most commonly used sample preparation technique.**
- **The use of an immobilized sorbent, pre-packed into a device.**

## Advantages

- Best interference removal
- Gain concentration effect
- Flexibility and Automation
- Extract whole suites of drugs

## Limitations

- Requires method development
- Difficult to separate analytes with similar properties/structure!



# Sample Preparation Workflow: SPE

- **Fractionate** Plasma from whole blood (centrifuge)
- **Precondition** tube
- **Dilute** sample with appropriate buffer (add IS)
- **Apply** the sample
- **Wash** steps
- **Elution** (pH modified)
- **Filter** under vacuum
- **Inject** sample into LCMS

Direct Injection or  
Dry-down /reconstitute

	<b>Plexa (30 mg, 1mL)</b>
<b>Sample</b>	100 µL human plasma
<b>Pretreatment</b>	Dilute 1:3 w/ 2% NH <sub>4</sub> OH (bases) 2% Phosphoric (acids)
<b>Conditioning</b>	1. 500 µL MeOH 2. 500 µL H <sub>2</sub> O
<b>Washes</b>	5 % MeOH in water
<b>Elution</b>	2 x 250 µL 50:50 ACN/MeOH (or 100% MeOH)

**5 minutes per sample (SPE Tubes)**

**30 minutes per 96 samples (Well plate)**

# Silica Based SPE: A Plethora of Options

Bond Elut AccuCAT

Bond Elut Alumina (AL-A)

Bond Elut Alumina (AL-B)

Bond Elut Alumina (AL-N)

Bond Elut NH<sub>2</sub>

Empore Anion-SR

Bond Elut Atrazine

BioBond Elut

Bond Elut C1

Bond Elut C2

Bond Elut C8

Elut C18

Elut C18 EWP

Bond Elut C18 INT

Bond Elut C18 OH

Bond Elut CBA

Bond Elut Certify

Bond Elut Certify II

Bond Elut CH

Bond Elut DEA

Bond Elut Diol (2OH)

Bond Elut ENV

Bond Elut FL

Bond Elut NH<sub>2</sub>

Bond Elut PBA

Bond Elut PCB

Bond Elut PH

Bond Elut PPL

Bond Elut PRS

Bond Elut PSA

Bond Elut SAX

Bond Elut SCX

Bond Elut SI



**40 + Phases and over 2000 Applications**



# Bond Elut SPE

## Non-polar

**C18, C8, C2, C1**

**C18 variations** in carbon load  
and endcapping

**EnvirElut**

**CH** – cyclohexyl

**CN-E** – endcapped cyano

**PH** – phenyl

**ENV, LMS, PPL, Focus, Nexus**

**Plexa**

## Anion Exchange

**SAX** – quaternary amine

**PSA** – primary and secondary  
amine

**NH2** – aminopropyl

**DEA** – diethylaminopropyl

## Polar

**PSA** - primary and secondary  
amine

**NH2** - aminopropyl

**DEA** - diethylaminopropyl

**Diol**

**Si** - silica

## Mixed mode IEX/NP

**Certify** – SCX/C8

**Certify II** – SAX/C8

**Plexa PCX**

**Plexa PAX**

## Cation Exchange

**SCX** – benzenesulfonic acid

**PRS** – propylsulfonic acid

**CBA** – carboxylic acid

## Reversible Covalent

**PBA** – phenylboronic acid

## Specialty Phases

AccuCAT

Atrazine

Etc..

**Alumina** – aluminum oxide

**Florisil** – magnesium-silica

**Carbon**

**Carbon/NH2**



# Formats

The most comprehensive range of formats in the industry

## Tubes

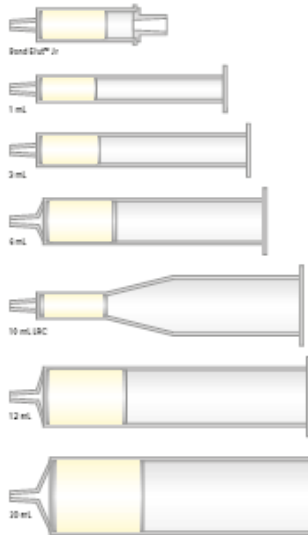
1mL to 60mL Straight Barrel (50mg –10g)

Bond Elut Jr (500mg –1g)

LRC (Large Reservoir Capacity)  
(100-500mg)

Mega Bond Elut

12-150mL (2g-70g)



## Multi-Array

1mL, 2mL 96 Well

1.8mL Versaplate

OMIX 96

SPEC 1mL, 2mL

## Automation

Hamilton

TomTEC

Gilson ASPEC

Gerstel

Spark Holland Prospekt



# SPE Manifolds



CaptiVac Vacuum Collar

## 96 Well

Captivac Collar: For use with Bond Elut 96 1mL and Captiva filtration plates



## VacElut 12, 20 and SPS 24

vacuum manifold for SPE syringe barrels.



# Basic Accessories

## Bond Elut Adaptors

- Connect cartridges in series
- Fit syringe for positive pressure

## Luer stopcocks

- Stop flow on all Luer tip style cartridges

## Frits, Reservoirs, Fritted reservoirs



# SPE Methods? Where Do I Start?

## Consider your analyte?

- Does the analyte appear to be polar or non polar
- Do the analytes dissolve into the matrix
- Do the analytes contain any ionic groups
- Are the compounds unstable in acid or base
- What is the method of analysis ?
- What is the concentration of the analyte in the sample

## Consider your matrix?

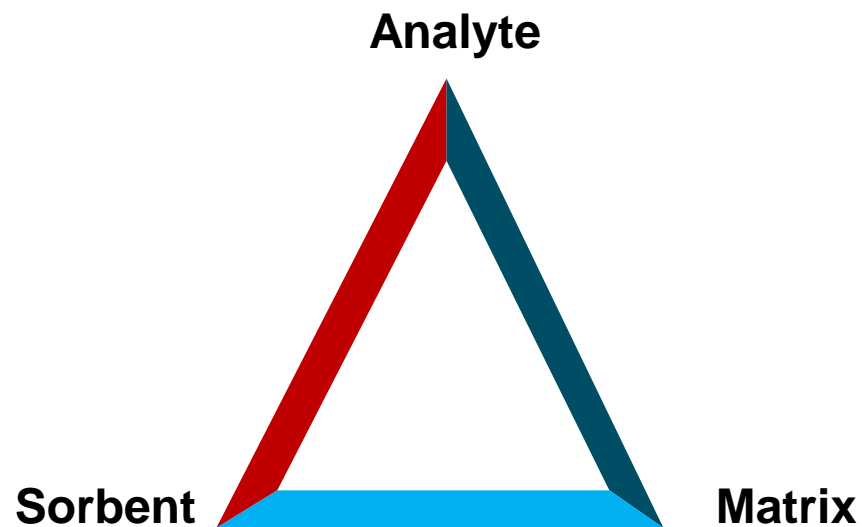
- Is the matrix polar or non polar: (olive oil or urine?)
- is the matrix high in organic or ionic content? (will dilution of the sample be necessary)
- What is the sample size?
- Does the sample require pH adjustment?
- Is an internal standard required?
- Is filtration or centrifugation necessary?



# The SPE Triangle

## Consider LC Theory

- The same physical processes in liquid chromatography also apply in SPE
- Think of SPE as a digital LC process
  - Analyte is ON (retention)
  - Analyte is OFF (elution)
- We are not looking at separation
- Normal or Reverse phase?
- Ionic modifier?



# TRICK: Use Google!

- **Bond Elut SPE has more published methods than any other SPE platform**
- 1000s of articles
- Official methods
- 31 years of heritage

**BOND ELUT + (Analyte)**

**Don't re-invent the wheel!**

Google **Bond Elut Acrylamide**  
About 14,500 results (0.34 seconds)

Everything  
More

Bengaluru, Karnataka  
Change location

The web  
Pages from India  
More search tools

[Retention studies of acrylamide for the design of a robust liquid ...](#)  
by J Rosén - 2007 - Cited by 8 - Related articles  
Based on these investigations, a method for analysis of **acrylamide** in food using .... **Bond Elut PPL**, Polymer, Styrene divinyl benzene polymer, modified ...  
linkinghub.elsevier.com/retrieve/pii/S0021967307016275

[Determination of acrylamide in Chinese traditional carbohydrate ...](#)  
by Y Zhang - 2007 - Cited by 9 - Related articles  
Results showed that **acrylamide** was not completely adsorbed by cartridges ...  
linkinghub.elsevier.com/retrieve/pii/S0003267006021684  
[Show more results from elsevier.com](#)

[Analysis of Coffee for the Presence of Acrylamide by LC-MS/MS ...](#)  
by D Andrzejewski - 2004 - Cited by 71 - Related articles  
10 Mar 2004 ... After elution of sample extract through the **Bond Elut Accucat SPE cartridge**, some **acrylamide** will remain on the cartridge. ...  
pubs.acs.org/doi/abs/10.1021/jf0349634 - Similar

[New Research Developments on Acrylamide: Analytical Chemistry ...](#)  
by Y Zhang - 2009 - Cited by 1 - Related articles  
coffee, 13C3-**acrylamide**, LC-MS, SIM, defatting with hexane, extraction with ...  
pubs.acs.org/doi/abs/10.1021/cr800318s - Similar  
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[A Modified Sample Preparation for Acrylamide Determination in ...](#)  
by AP Ariseto - 2008 - Cited by 2 - Related articles  
method used for the determination of **acrylamide** in potato and cereal products to achieve good ... and **Bond Elut-Accucat®** cartridges are used in combina- ...  
www.springerlink.com/index/doi/10.1007/s12014-008-9075-5.pdf - Similar

[Eriksson - Analysis of acrylamide in food, air and in ...](#)  
File Format: PDF/Adobe Acrobat - View as HTML  
Different limits for **acrylamide** contaminations. ... AccuBOND II SCX. **Bond Elut AccuCAT. Bond Elut C18. Bond Elut Jr-PSA. ENVI-Carb. Isolute® MF C18** ...  
www.anapceurope.com/2006/eriksson.pdf



# Productivity Enhancements

- Fewer particulates: reduce back pressure issues
- Less endogenous material build up
- Lower LOQs possible
- Methods easily automatable
- Fewer repeats/failed runs
- Less Instrument downtime /maintenance
- Lower solvent costs (including disposal!)
- **Overall improvement in data quality**



Thank You for your Attention!

**Fragen?**



## Part II : Applications, Troubleshooting, Tips and Tricks



# Application Section

## Food Safety Testing

**QuEChERS: Quick, Easy, Cheap, Effective, Rugged, Safe**

- AOAC Method 2007.01
- EN Method 15662
- PAHs and Surfactants in Fish
- Pesticides in Green Tea

## **Solid Supported Liquid/Liquid Extraction (Chem Elut)**

- Lutz-Alder Method
- Acidic pesticides
- Multi-residue method for pesticides in honey



# Application Section

## Food Safety Testing

### **SPE: Solid Phase Extraction**

- Japanese positives list
- Pesticides in agricultural products
- Mycotoxin extraction

### **MSPD: Matrix Solid Phase Dispersion**

- Pesticide residues in olive oil
- Pesticides in citrus fruits



# What is QuEChERS (pronounced “Catchers”)

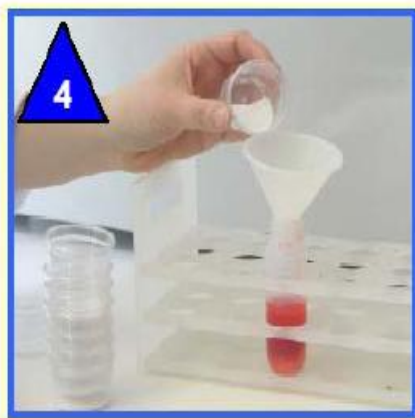
## Quick, Easy, Cheap, Effective, Robust and Safe

- Introduced in **2003**: M. Anastassiades, S.J. Lehotay, D. Stajnbaher, and F.J. Schenck, J. AOAC Int 86 (2003) 412
- QuEChERS is a fast sample preparation method for screening for analytes of interest - Without the concentration found in SPE
- QuEChERS is still a new technique; being adopted worldwide
- QuEChERS process can substantially decrease cost per sample

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

# First Step – Extraction/Partitioning

## Pictorial Representation of the QuEChERS Steps

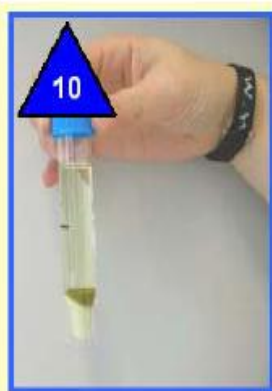


- 1) Weigh sample
- 2) Add Ceramic Homogenizers
- 2) Add standards
- 3) Shake !
- 4) Add ACN (1% AA)
- 5) Shake
- 6) Add salts
- 7) Shake 1 min
- 8) Centrifuge

# Second Step – Dispersive SPE



- 1) Choose d-SPE
- 2) Transfer volume
- 3) Vortex 1 min
- 4) Centrifuge
- 5) Analyze



# The Value of Agilent QuEChERS Kits

## Easy Product Selection: QuEChERS Kits Identified by method and matrix

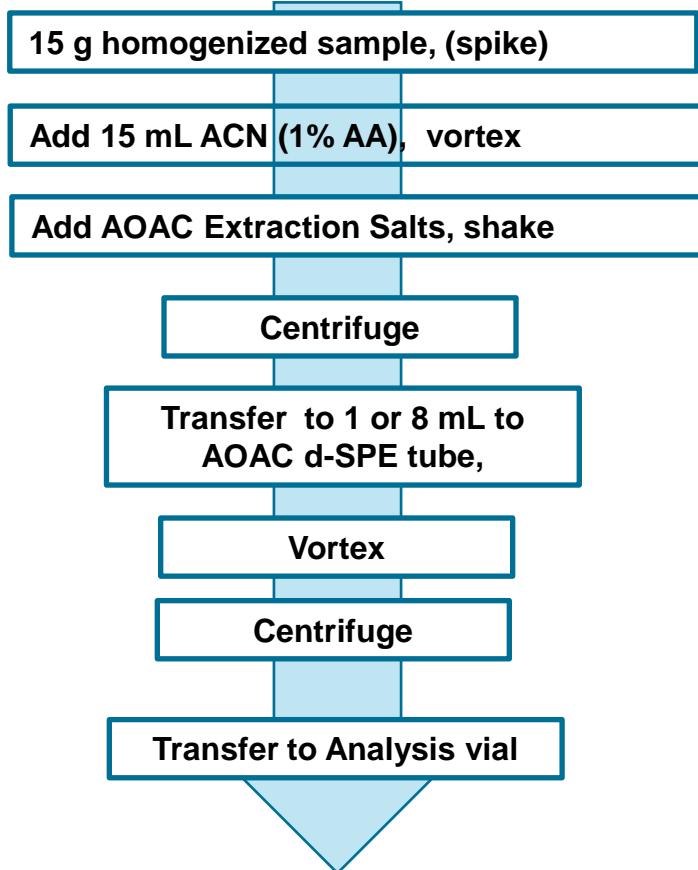
**Technical support:** Over 23 application notes on QuEChERS:

- All available on our website: [www.agilent.com/sampliQ](http://www.agilent.com/sampliQ)
- QuEChERS Food Application “Notebooks”: Lit number: 5990-4977EN
- Seminars and hands-on training available worldwide

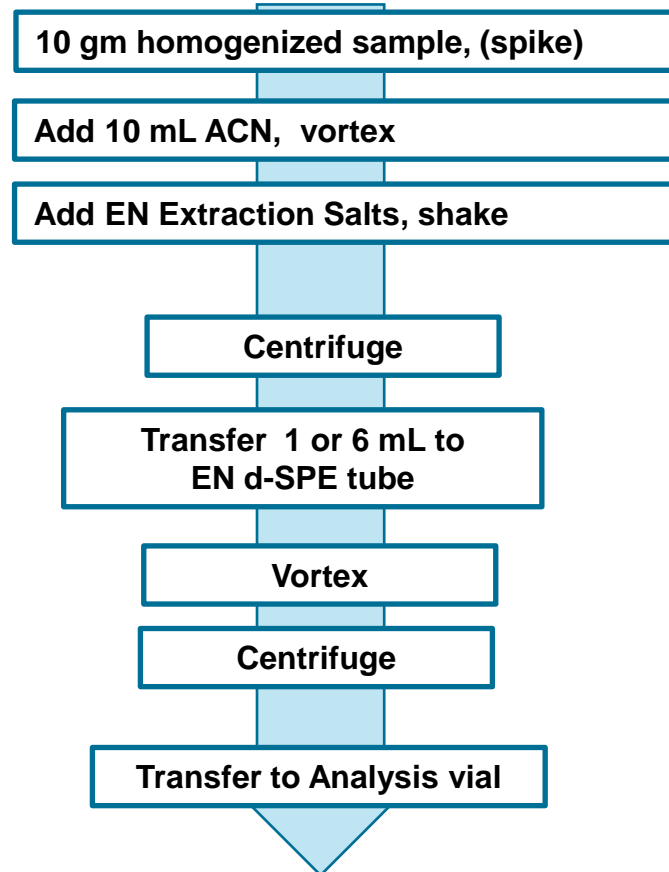


# Method Selection: AOAC and EN

## AOAC 2007.01



## EN 15662



Analyze by GC/MS or LC/MS/MS\*

\* Requires a dilution prior to analysis



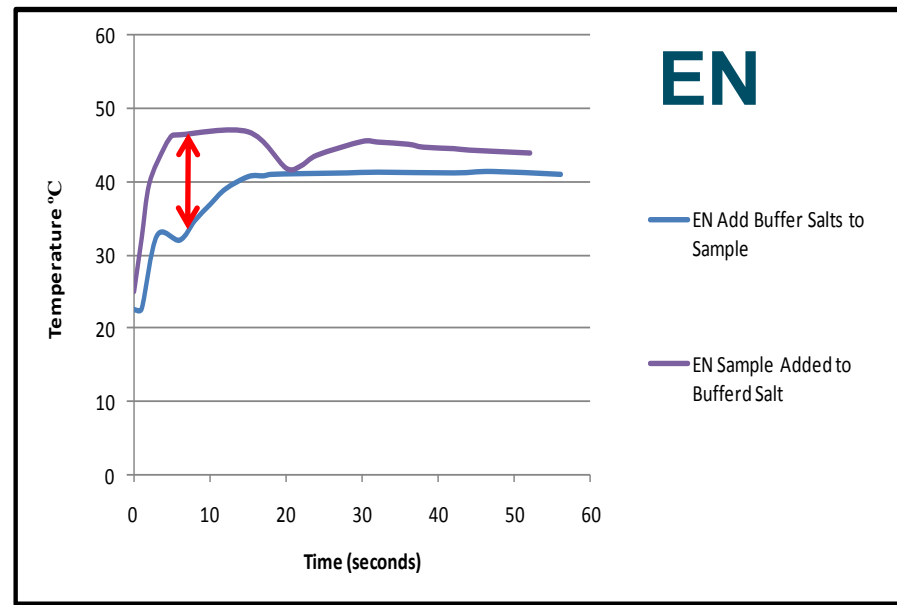
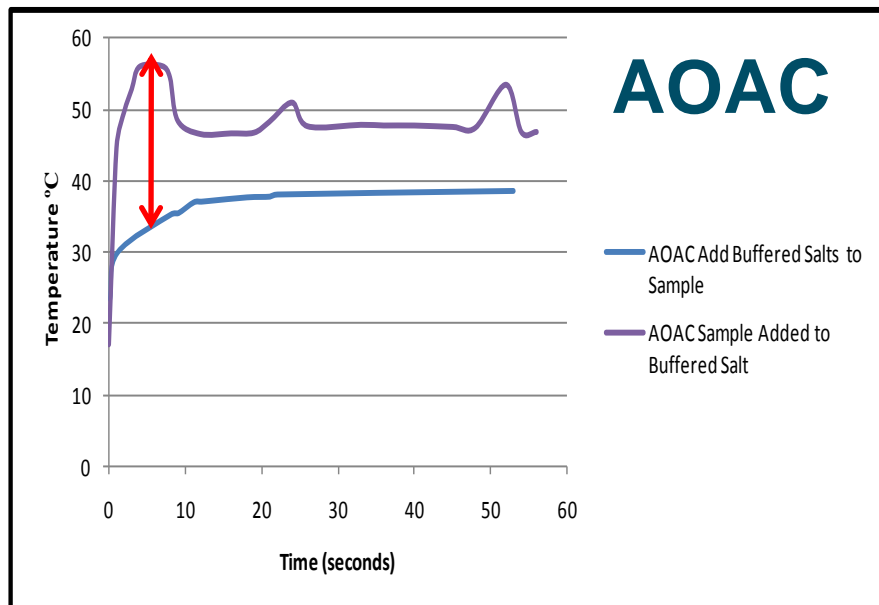
# Packaging Quality = Reproducibility

Packaging salts separately allows customers to add sample before adding salts

- Prevents exothermic reaction
  - Prevents degradation of sample
  - Ensures maximum recoveries
- 
- Water and UV resistant packaging 3 layer material
  - Pack the salts under nitrogen not vacuum
  - Increases pouring capabilities
  - Substantially reduces clumping of salts
  - All ingredients listed on packet



# TRICK: Minimize Exotherms During Addition



- A substantial increase in temperature is observed
- (increase of approx 25 °C for the AOAC method and approx 10 °C for the EN method)

# Agilent Improvements to the Extraction of Samples

## Extraction salts with comminuted fruit/vegetable

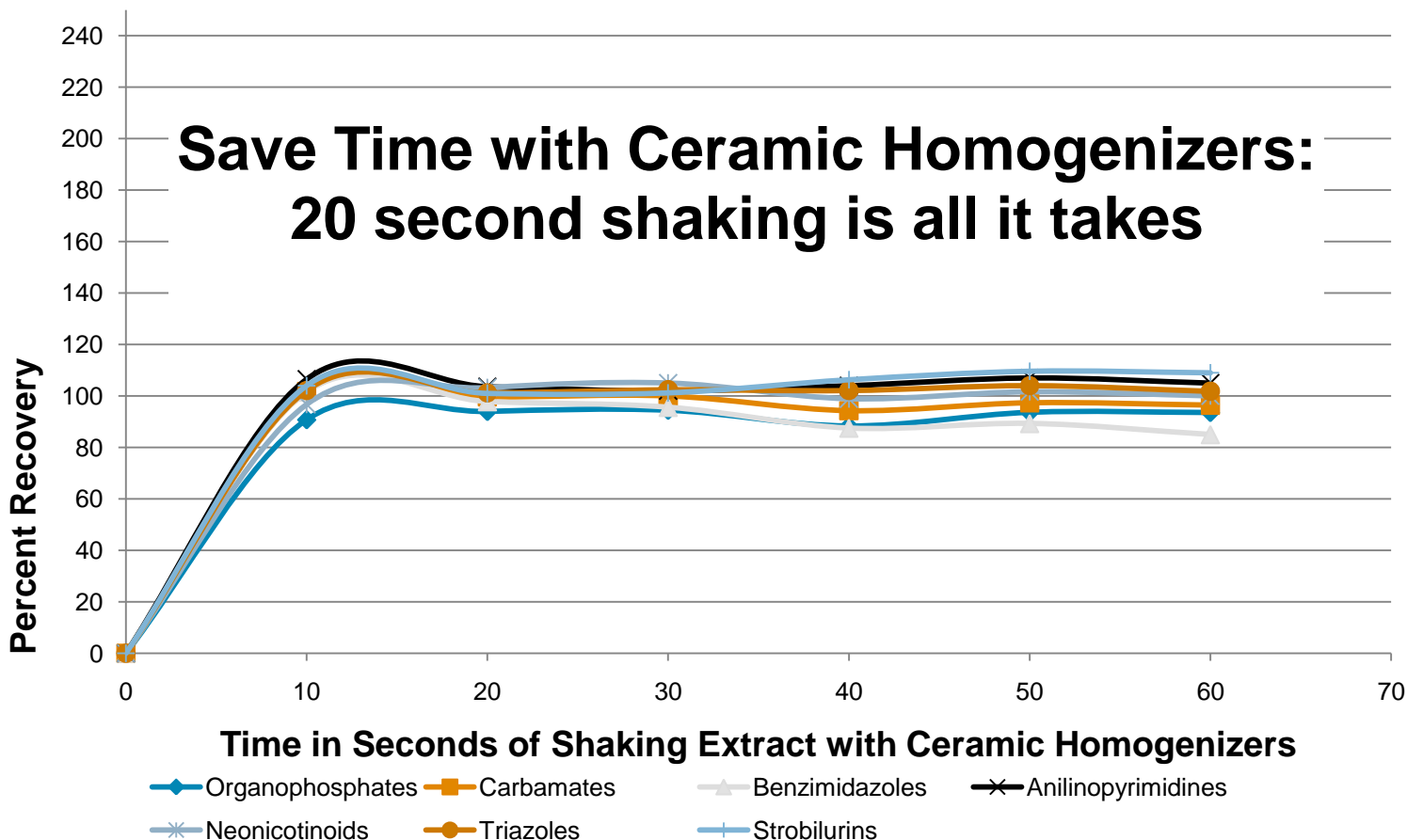
- Consistency in shaking, everyone shakes differently
- Variability in QuEChERS applications, recovery and RSDs



## SampliQ Ceramic Homogenizers

- **Reduces shaking time from 1 minute to <20 seconds!**
- Consistent extraction of the sample with the salts
- Breaks up salt agglomerates
- Facilitates homogenization with angle cut
- Increase recovery of pesticides from sample
- 3 different sizes: 50 mL extraction tube and 2 dispersive tubes 15 and 2 mL

# TRICK: Improve shaking efficiency



# TIP: Ionic Strength- Salt Buffering

- AOAC buffered method  
Relatively strong acetate buffering conditions (pH 4.8)
- EN buffered method  
Weaker citrate buffering conditions (pH 5-5.5)\*\*
- Buffering at pH 5 during extraction offers optimum recoveries (> 70%) for certain pH-dependent pesticides ( e.g. pymetrozine, imazalil, thiabendazole)



\*S.J. Lehotay, K. Mastovska, A.R. Lightfield, J. AOAC Int. 88 (2005), 615-629 & 60A

\*\* M. Anastassiades, E. Scherbaum, B. Tasdelen, D. Stajnbaher, in: H. Ohkawa, H. Miyagawa, P. W. Lee (Eds.), Crop Protection, Public Health, Environmental Safety, Wiley-VCH, 2007, p.439.

# TIP: Chose The Correct Dispersive (d-SPE)

## The Basics

- $\text{MgSO}_4$  is found in all dispersive kits, removal of remaining water
- PSA for removal of organic acids
- C18 for removal of fat, and lipids
- GCB for removal of pigment, chlorophyll

## The generic: Universal d-SPE

- 50 mg PSA, 50 mg C18, 7.5 mg GCB, 150 mg  $\text{MgSO}_4$  (2 mL)
- 400 mg PSA, 400 mg C18, 60 mg GCB, 1200 mg  $\text{MgSO}_4$  (15 mL)
- Cleanest extract, for all matrices without unacceptably affecting recoveries even for structurally planar pesticides  
(S. Lehotay: *J. Chromatography A* 1217 (2010), 2548-2560)



# PAHs in Fortified Red Snapper Fish

Analytes	25 ng/mL			250 ng/mL			500 ng/mL	
	%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

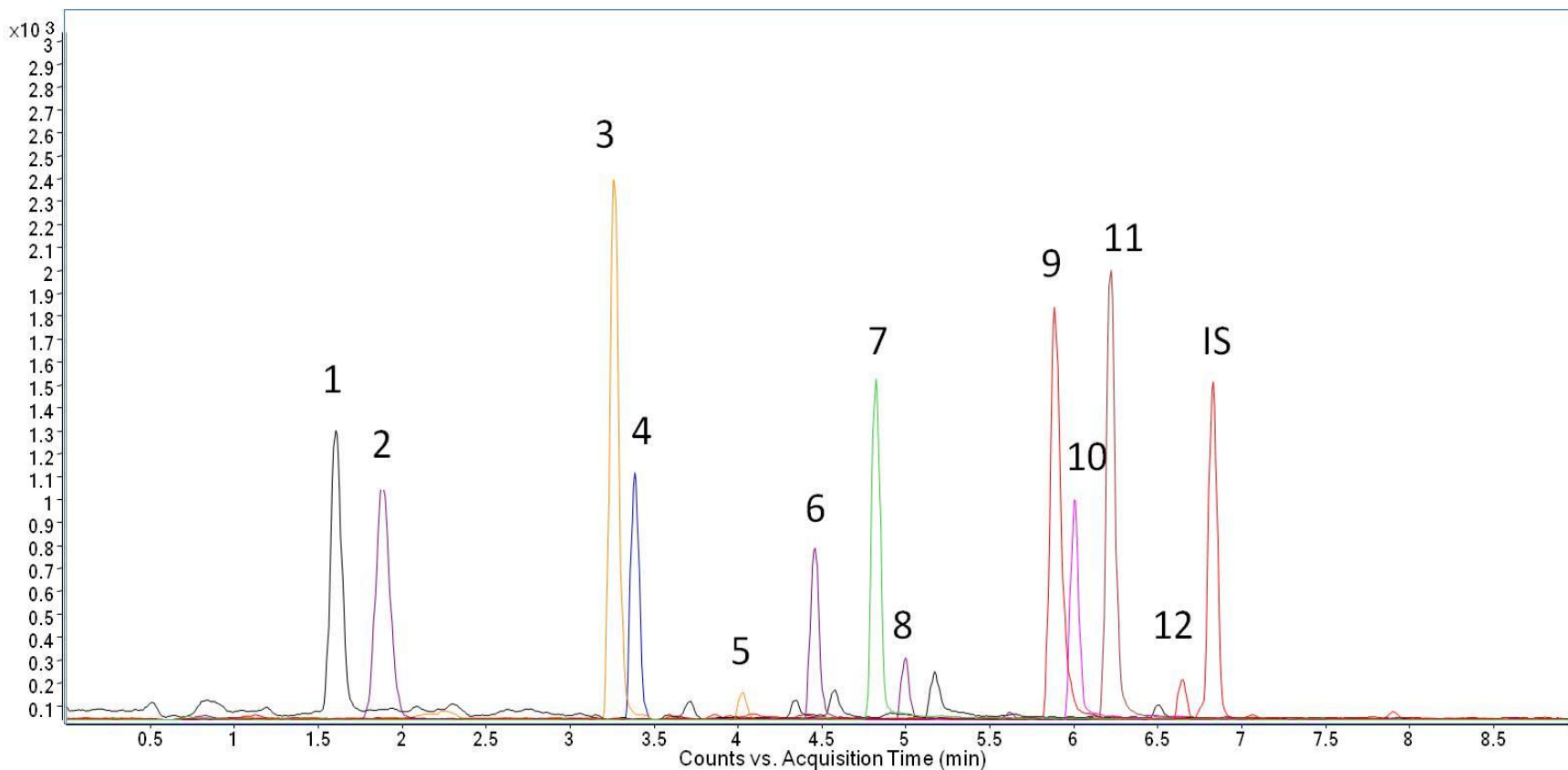


# Pesticides in Fortified Green Tea with QuEChER

Analytes	10 ng/g		50 ng/g		250 ng/g	
	%Recovery	RSD (n=6)	%Recovery	% RSD (n=6)	%Recovery	% RSD (n=6)
Acephate	80.5	5.4	91.7	2.9	88.9	8.2
Pymetrozine	43.1	3.0	42.2	3.4	43.4	9.8
Carbendazim	114.6	11.6	97.6	2.0	105.0	6.2
Thiabendazole	98.1	6.9	90.4	2.4	81.7	5.8
Imidacloprid	104.3	11.7	108.6	2.5	93.9	7.9
Imazalil	97.5	4.4	87.8	5.6	92.4	4.6
Propoxur	98.1	2.4	110.2	1.7	107.8	3.9
Carbaryl	89.7	11.2.14	104.9	3.3	108.1	5.2
Cyprodinil	84.9	2.1	92.5	3.7	93.9	5.5
Ethoprophos	103.4	3.1	111.2	3.2	104.9	5.7
Penconazole	108.7	2.9	94.3	4.5	89.8	3.3
Kresim-methyl	105.7	12.4	96.4	2.5	99.2	5.5



# Pesticides in Green Tea: LC/MS/MS



*MRM chromatograms of 50 ng/g fortified sample processed by EN method. Peak identification: 1. Acephate, 2. Pymetrozine, 3. Carbendazim, 4. Thiabendazole, 5. Imidacloprid, 6. Imazalil, 7. Propoxur, 8. Carbaryl, 9. Cyprodinil, 10. Ethoprophos, 11. Penconazole, 12. Kresoxim-methyl, IS: TPP*



# QuEChERS Applications: Beyond Produce

Application	Literature
Veterinary Drugs (antibiotics) in animal tissues	5990-5085EN, 5990-5086EN, 5990-5395EN
PAHs in Fish	5990-5441EN, 5990-6668EN
Pesticides in Olive Oil	5990-5553EN
PAH's in Soil	5990-5452EN, 5990-6324EN
Acrylamides in Fried Food and Oil	5900-5940EN, 5990-5988EN
Pesticides in Baby Food	5990-5028EN
Pesticides in Green Tea	5990-6400EN
PCBs in Fish and Fish Oil Supplements	5990-6236EN
Universal d-SPE	5990-6558EN
Hormones in Shrimp	5990-6589EN
Pesticides in Essential Oils	5990-6432EN



# Solid Support Liquid/Liquid Extraction

## Product Offering

**ChemElut:** Preassembled cartridges containing diatomaceous earth 'hydromatrix' sorbent

**Hydromatrix:** Loose bulk sorbent to be used in dispersive or custom applications

## Workflow advantages

- General, simple method
- No emulsions
- Less glassware (especially with larger samples)
- Wide range of analytes extracted
- Can be automated or "walk away" automation
- Can be less 'technique' dependent

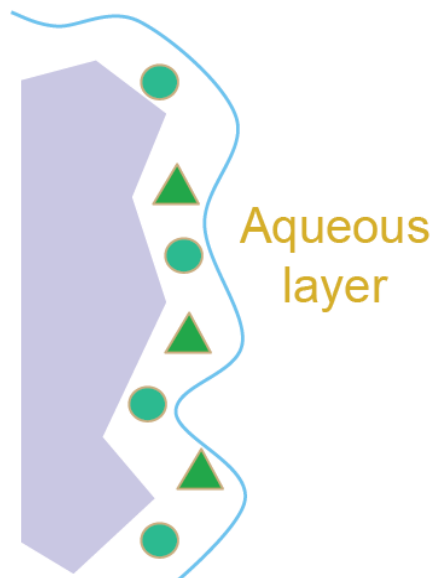


# SLE Mechanism

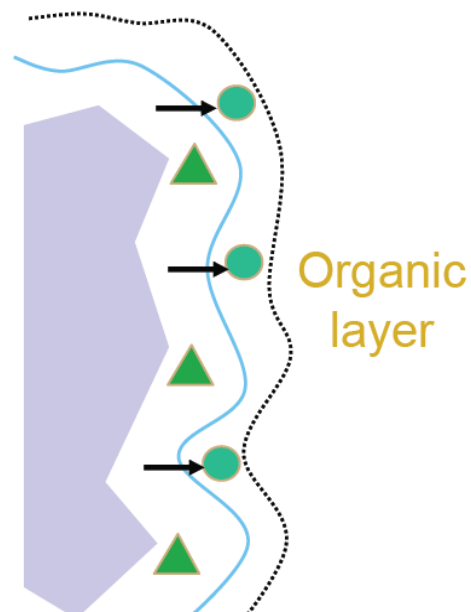
Before Extraction



Apply Sample



Extract with Organic Solvent



# Food Applications (Lutz Alder)

## Workflow



Add water to 10 g sample.  
(sum of naturally and added: 10 ml)



Homogenize with 20 ml methanol.  
(+ centrifugation)



Add NaCl solution to an aliquot and soak diatomaceous earth.  
Elute with  $\text{CH}_2\text{Cl}_2$ .



Evaporate to dryness and reconstitute in methanol.  
LC-ESI-MS/MS

neutral pesticides

acidic pesticides



„Old“ method: Unbuffered diatomaceous earth in 5 mL cartridge



Modified method: Buffered (pH 4,5) diatomaceous earth in 3 mL cartridge

**Typical Matrices:** cucumber, lemon, wheat flour, hazelnuts and raisins.



# >100 analytes: 23 substance classes

## **Carbamates (incl. metabolites)**

3,4,5-Trimethacarb (Landrin)  
3-Hydroxycarbofuran  
Bendiocarb  
Carbaryl  
Carbofuran  
Desmethylformamido-pirimicarb  
Desmethyl-pirimicarb  
Ethiofencarb  
Ethiofencarbsulfon  
Ethiofencarbsulfoxid  
Fenoxycarb  
Furathiocarb  
Methiocarb (Mercaptodimethur)  
Pirimicarb  
Promecarb  
Propamocarb  
Propoxur

## **Oxim-carbamates (incl. metabolites)**

Aldicarb  
Aldicarb-sulfoxid  
Aldoxycarb (Aldicarb-sulfon)  
Butocarboxim  
Butocarboxim-sulfoxid  
Butoxycarboxim  
Methomyl  
Oxamyl  
Thiodicarb  
Thiofanox  
Thiofanox-sulfon  
Thiofanox-sulfoxid

## **Sulfonyl ureas**

Amidosulfuron  
Bensulfuron-methyl  
Chlorsulfuron  
Cinosulfuron  
Flazasulfuron  
Iodosulfuron  
Metsulfuron-methyl  
Nicosulfuron  
Primisulfuron-methyl  
Prosulfuron  
Rimsulfuron  
Thifensulfuron-methyl  
Triasulfuron  
Tribenuron-methyl  
Triflursulfuron-methyl

## **Benzoyl ureas**

Flufenoxuron  
Diflubenuron  
Teflubenzuron  
Hexaflumuron  
Triflumuron

## **Oxyalkanoic acids**

2,4-D  
MCPA  
Mecoprop-P  
Fluazifop-P-butyl  
Haloxifop-ethoxyethyl  
Haloxifop-methyl  
Quizalofop-ethyl

## **Organophosphates**

Acephate  
Demeton-S-methyl  
Demeton-S-methyl-sulfon  
Dimethoat  
Fosthiazate  
Methamidophos  
Monocrotophos  
Omethoat  
Oxydemeton-methyl  
Vamidothion

## **Benzimidazoles**

5-Hydroxythiabendazol  
Carbendazim  
Thiabendazol  
Thiophanat-methyl

## **Urea herbicides**

Diuron  
Isoproturon  
Linuron

## **Neonicotinoids**

Imidacloprid  
Thiacloprid

## **Other (14 substance classes)**

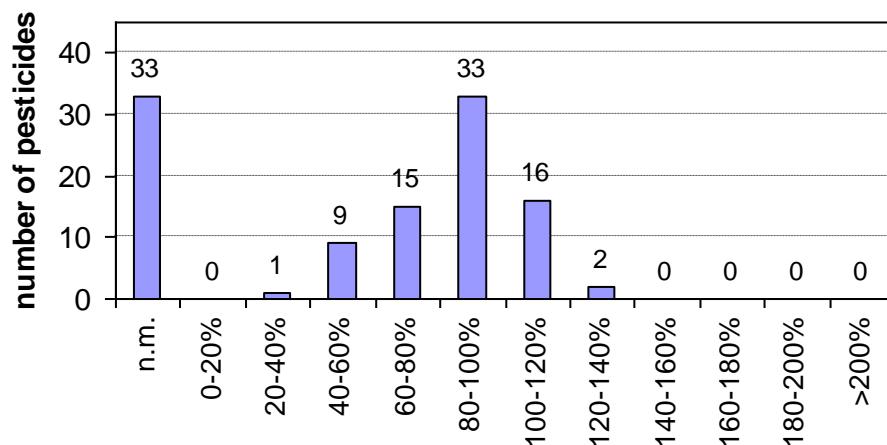
Atrazin  
Azoxystrobin  
Bromoxynil  
Clethodim  
Clethodim-imin-sulfon  
Clethodim-imin-sulfoxid  
Clethodim-sulfon  
5-Hydroxy-clethodim-sulfon  
Clethodim-sulfoxid  
Cyprodinil  
Daminozid  
Desmedipham  
Fenhexamid  
Fenpropiomorph  
Florasulam  
Fludioxonil  
Imazalil  
Ioxynil  
Iprovalicarb  
Isoxaflutole  
Metalaxyl  
Metamitron  
Metolachlor  
Phenmedipham  
Pymetrozin  
Pyridat  
Pyridat Metabolit  
Pirimethanil  
Quinmerac  
Spiroxamine  
Tebuconazol  
Tebufenozid

Work performed by Lutz Alder and Jeannette Klein, BgVV Berlin

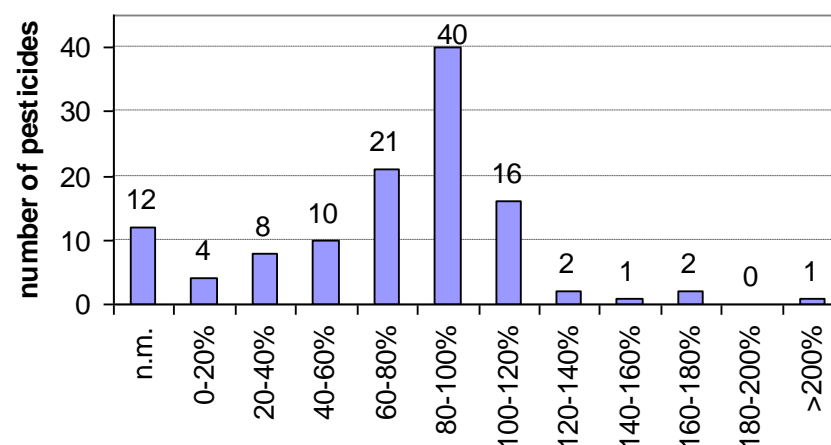


# Recovery Data

Recoveries of 109 pesticides  
Lemon without cleanup at 0.01mg/kg



Recoveries of 109 pesticides Lemon with  
ChemElut at 0.01 mg/kg



- Excellent recoveries and low RSDs
- Only 2 highly acidic analytes not recovered

Work performed by Lutz Alder and Jeannette Klein, BgVV Berlin

# Multi-residue Analysis of Pesticides in Honey

The application shows the development and validation of 17 pesticides and metabolites of different chemical classes.

## Insecticides:

Carbofuran (Ca), Methiocarb (Mh), Pirimicarb (Pi), Dimethoate (Dm), Fipronil (Fi), Imidacloprid (Im)

## Herbicides:

Amidosulfuron (Am), Rimsulfuron (Ri), Atrazine (At), Simazine (Si), Chloroturon (Ch), Linuron (Li), Isoxaflutole (Is), Metosulam (Mo)

**Fungicides:** Diethofencarb (De)

**Metabolites:** Methiocarb sulfoxide (Mhs), 2-Hydroxytertbutylazine (TOH)



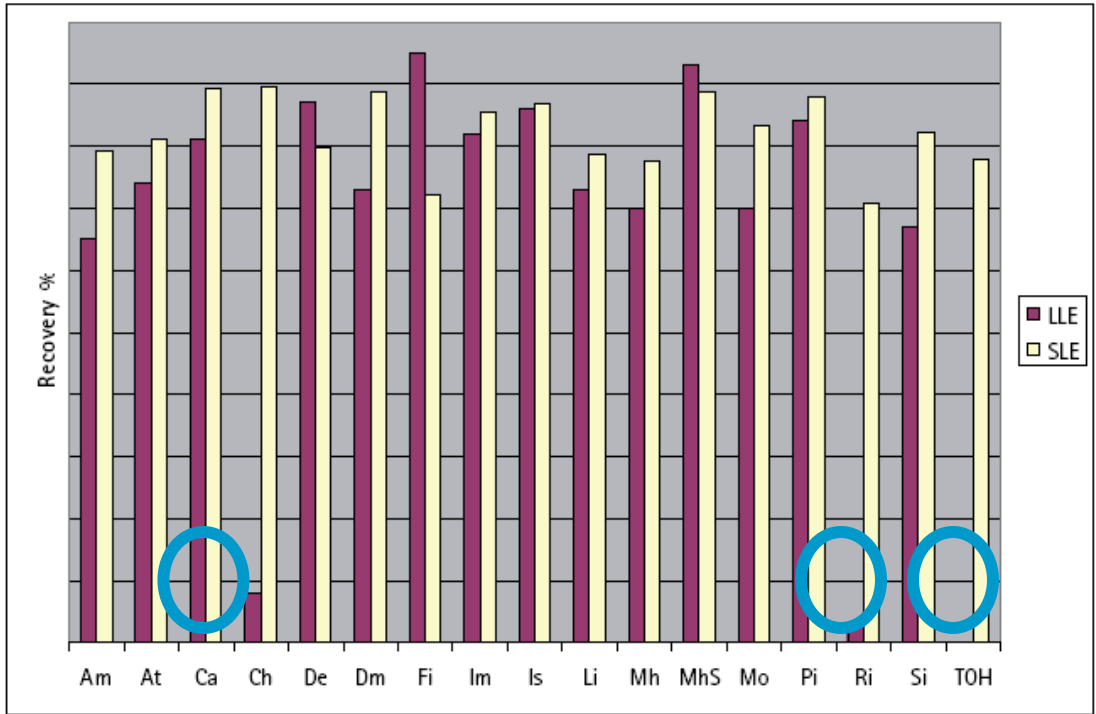
# SLE shows improvement of LLE

## Application Note SI-01002

Recovery comparison of pesticides between solid supported liquid-liquid extraction (SLE) on Chem Elut and classical liquid-liquid extraction (LLE).

14 of 17 compounds exhibited higher recoveries with SLE.

3 undetectable compounds were detectable with SLE



Pesticide (ng/mL):

Am	0.4	Fi	10.0	Mo	2.0
At	0.4	Im	2.0	Pi	0.4
Ca	0.4	Is	2.0	Ri	0.4
Ch	20.0	Li	2.0	Si	2.0
De	2.0	Mh	10.0	TOH	1.0
Dm	2.0	MhS	20.0		



# Bond Elut Carbon: Pesticide Analysis

## Bond Elut Carbon: Optimized for Pesticide Analysis

- Excellent retention for small organics, including ones that are too polar to retain on C18 or polymeric SPE
- Removal of chlorophyll and other pigments leads to fewer interferences
- Broader retention and easier elution for analytes right across the polarity scale, for improved multi-residue analysis



# Bond Elut Carbon/NH<sub>2</sub> & Bond Elut Si

Japanese Positives List: Liquid/Liquid extraction from food, followed by:

## Method I

- ◆ Condition a graphite carbon/ aminopropylsilylated silica gel layered mini column (500 mg/500mg) with 10 ml of acetonitrile/ toluene (3:1) (Bond Elut Carbon/NH<sub>2</sub>, 500 mg/500mg, 6 ml, Catalog #: 12252202). Apply the solution obtained from the Extraction step to the column and elute the column with 20 ml of acetonitrile/ toluene (3:1).



- ◆ Collect the entire volume of effluent. Concentrate the effluent to 1 ml or less at 40°C or lower. Add 10 ml of acetone and concentrate to 1 ml or less at 40°C or lower. Add 5 ml of acetone to the concentrated solution and concentrate to dryness.



- ◆ Dissolve the residue in methanol to make a 4 ml solution. Use this as the test solution.

## Method II

- ◆ Condition a silica gel mini column (500 mg) with 5 ml of methanol, 5 ml of acetone, and 10 ml of *n*-hexane in this order (Bond Elut Si, 500 mg, 10 ml, Catalog #: 14113036). Apply the solution obtained from the Extraction step above to the column.



- ◆ Elute the column with 10 ml of acetone/triethylamine/*n*-hexane (20:0.5:80), and discard the effluent.



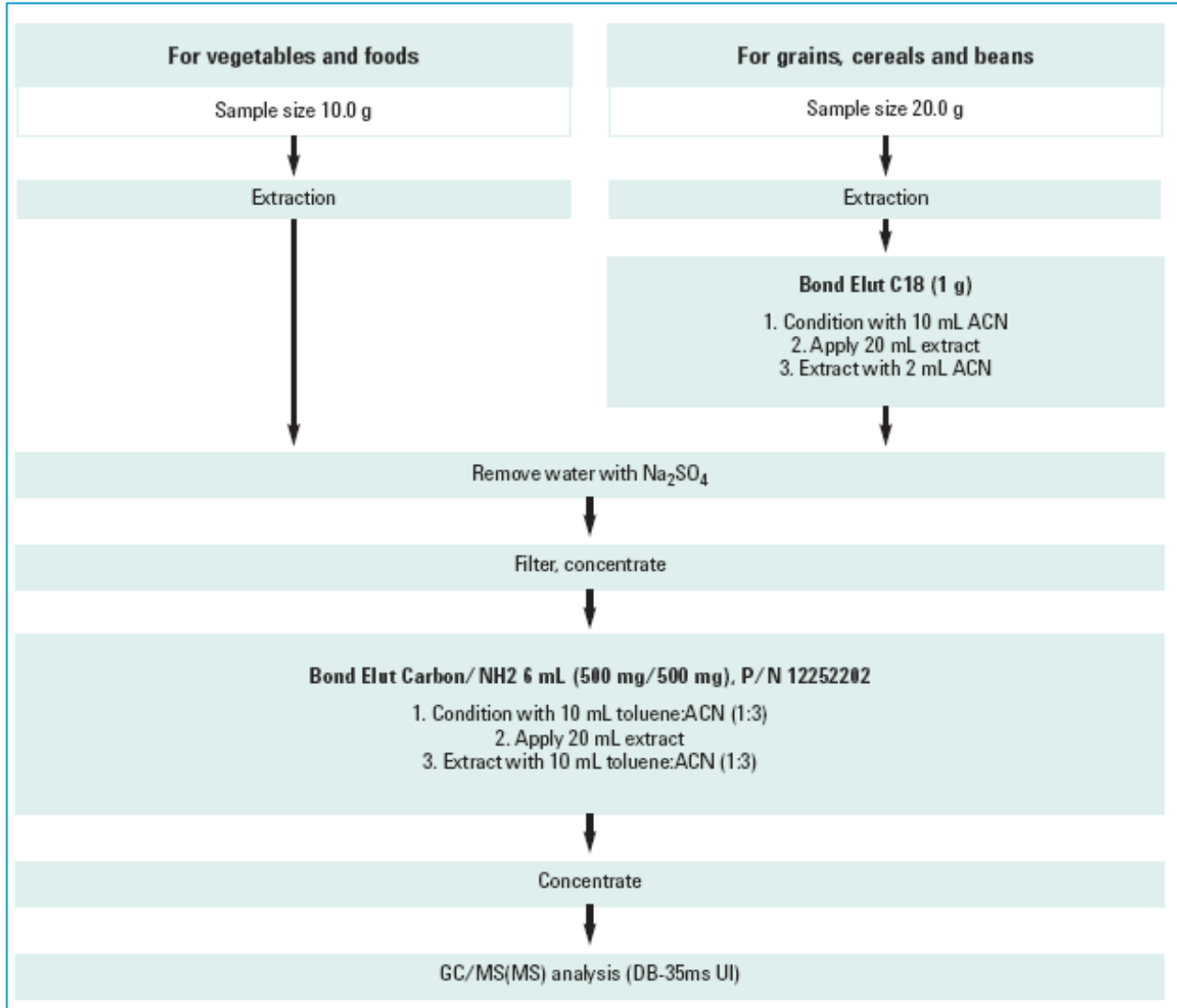
- ◆ Elute the column with 20 ml of acetone/methanol (1:1). Collect the entire volume of effluent.



- ◆ Concentrate the effluent to dryness at 40°C or lower. Dissolve the residue in methanol to make a 4 ml solution. Use this as the test solution.



# Japanese Positives: Solid Foods



# Mycotoxins

## Mycotoxins

Analytes which are secondary metabolites of molds, contaminating a wide range of crop plants and fruits before or after harvest.

Major classes of mycotoxins are:

- Aflatoxins B1,B2, G1 and G2 (*Aspergillus*)
- Ochratoxin A (*Penicillium verrucosum*)
- Patulin (other *Penicillium*)
- Fumonisin
- **Trichothecenes**: (e.g. Deoxynivalenol, HT-2)
- **Zearalenone** and its metabolites

} Fusarium



# Bond Elut Mycotoxin

A variety of *Fusarium* fungi produce different toxins of the class of trichothecenes. The trichothecenes differ a lot in polarity and solubility.

## Traditional Techniques

### Immunoaffinity Columns (IAC)

Highly selective extractions with high recoveries, but separate IAC columns are needed for each toxin.

### Charcoal-Alumina cartridges

Recoveries of the more polar analytes are often compromised with this approach.



# Bond Elut Mycotoxin

## An SPE Approach?

- SPE sorbent for clean-up of Fusarium toxin contaminated cereals and cereal-based foods
- Solid phase extraction based on ion exchange mechanism
- **Single column and method** for 12 type A and B Trichothecenes plus Zearalenone
- Excellent purification : The mycotoxins pass the cartridge while the impurities remain on the sorbent
- Internal Standard can be used for recovery calculations



# Simple Methodologies

## Application notes 000295 and SI-01075

**Cereals:** Extract 25 g of finely ground sample with a solution of 100 mL acetonitrile/water (80/20; v/v) by blending at high speed for 3 minutes. Then Filter.

**Wine and Beer:** Degass and Filter

- Pass 4 mL of the filtrate through a Bond Elut Mycotoxin column.
- Evaporate 2 mL of the eluate at 50 °C under a gentle stream of nitrogen.
- Reconstitute in 0.5 mL acetonitrile/water (20/80; v/v). Inject 10 µL into LC/MS/MS for analysis.



# Recoveries: Wheat Beer and Sake Wine

## Wheat beer

	% Recovery	% RSD	% Recovery	% RSD
Mycotoxin	35 ng/g		350 ng/g	
DON	92.0	2.6	95.5	1.5
ZEA	116.0	6.1	101.9	1.3
T-2	61.3	12.6	60.1	1.1
HT-2	81.8	5.6	76.1	1.4

## Sake wine

	% Recovery	% RSD	% Recovery	% RSD
Mycotoxin	35 ng/g		350 ng/g	
DON	94.3	7.4	96.8	0.5
ZEA	99.3	1.3	99.8	0.8
T-2	101.3	1.3	66.0	0.9
HT-2	113.9	8.3	111.0	1.0

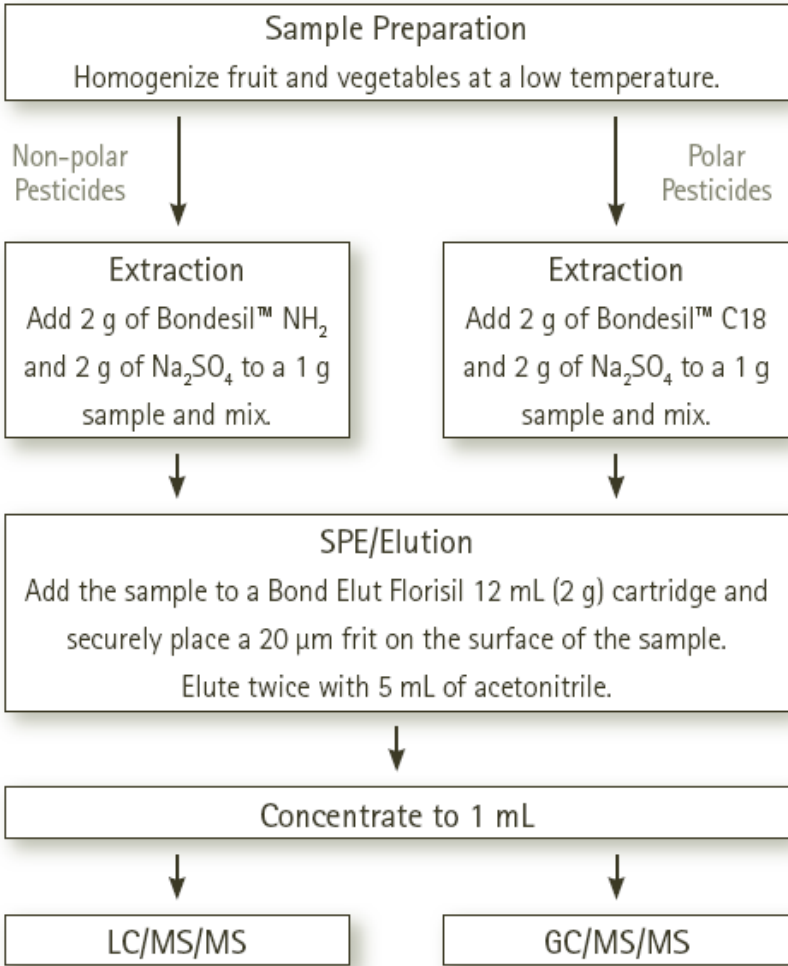


# Matrix Solid Phase Dispersion - MSPD

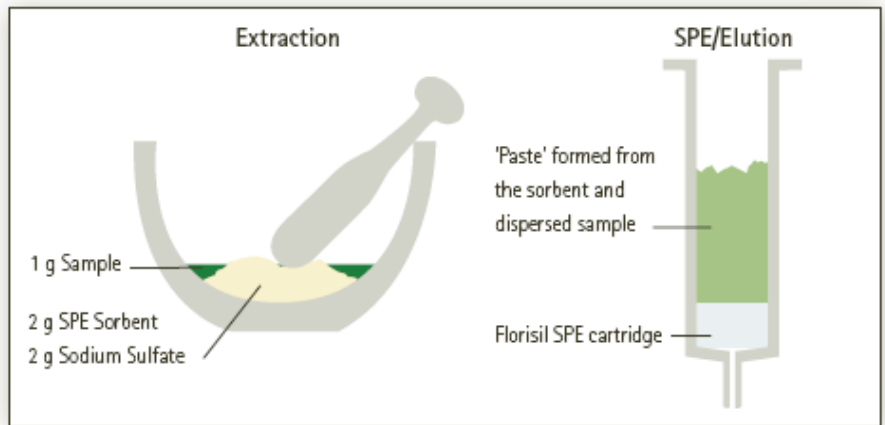
- Applied to disrupt solid matrices (e.g. food, tissues, other biological matrices) and disperse sample components over a solubilising surface (e.g. C18)
- Recoveries in range of 80-100% for pesticides in food (e.g. beef, baby food, vegetables)
- Applied to over 50 drugs in milk, infant formula, fish tissue, pork muscle tissue, animal feed
- Simple to carry out manually; difficult to automate
- Chemically mild
- Choice of sorbent should provide selective extractions



# Matrix Solid Phase Dispersion



A Mortar and Pestle Combines the Food Sample With Bonded Silica and Polar Impurities are Removed From the Sample Using Florisil



## Typical Phases:

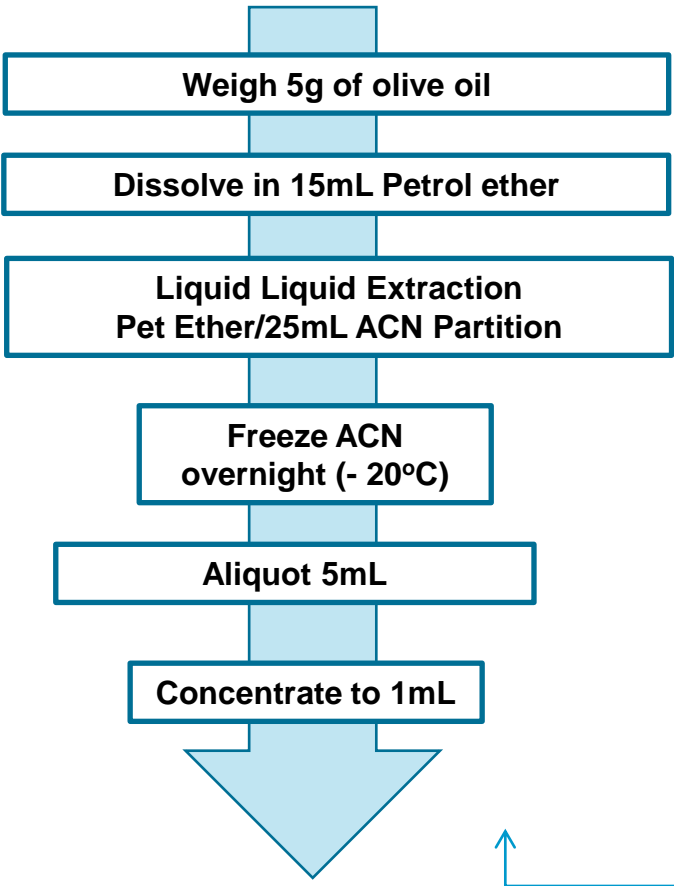
- Bondesil NH<sub>2</sub> & C18
- Bond Elut Florisil

Original technique developed by Dr. Steve Barker, LSU, Baton Rouge

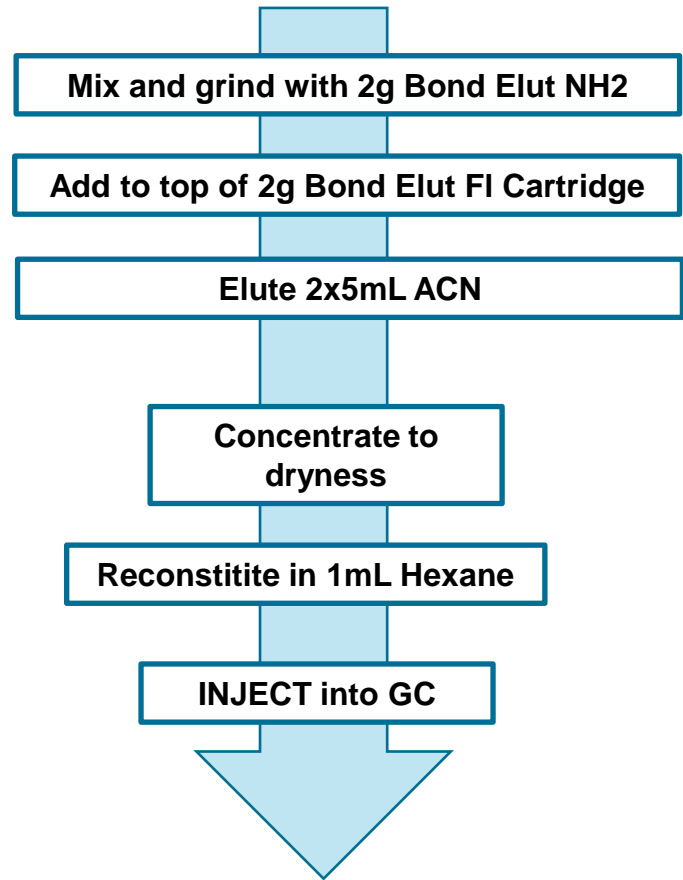


# Determination of Pesticide Residues in Olive Oil by MSPD

## LLE Step



## MSPD Step



DGCCRF Laboratory, 205 rue de la croix verte , Montpellier CEDEX 5 , 34196 , FRANCE



# Determination of Pesticide Residues in Olive Oil by MSPD

## Level of fat during process

STEPS	Sampling	Aliquot	Post Extraction	Post Freezing	Post MSPD
FAT (mg)	5000	1000	9	4	<0.2
Fat level during the process					

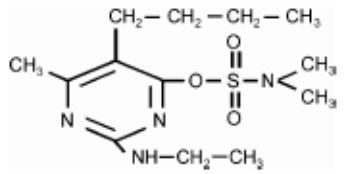
- Highly effective fat extraction
- Avoids deterioration of chromatographic system
- Simple method
- High recoveries
- reproducible



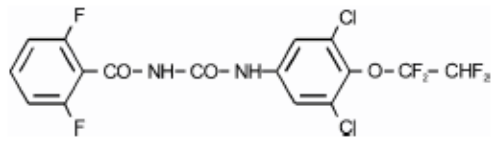
# Pesticide Analysis in Citrus Fruits with MSPD



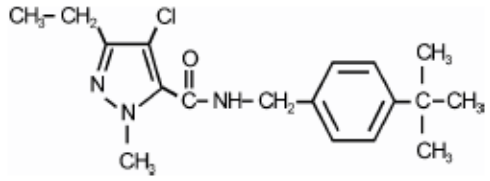
Bupirimate



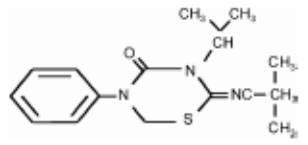
Hexaflumuron



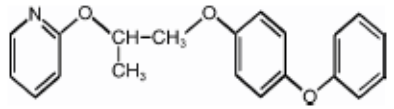
Tebufenpyrad



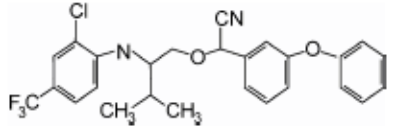
Buprofezin



Pyriproxyfen



Fluvalinate



Carla Soler, et.al, Journal of Chromatography A, 1088 (2005)



# Pesticide Analysis in Citrus Fruits with MSPD

## MSPD

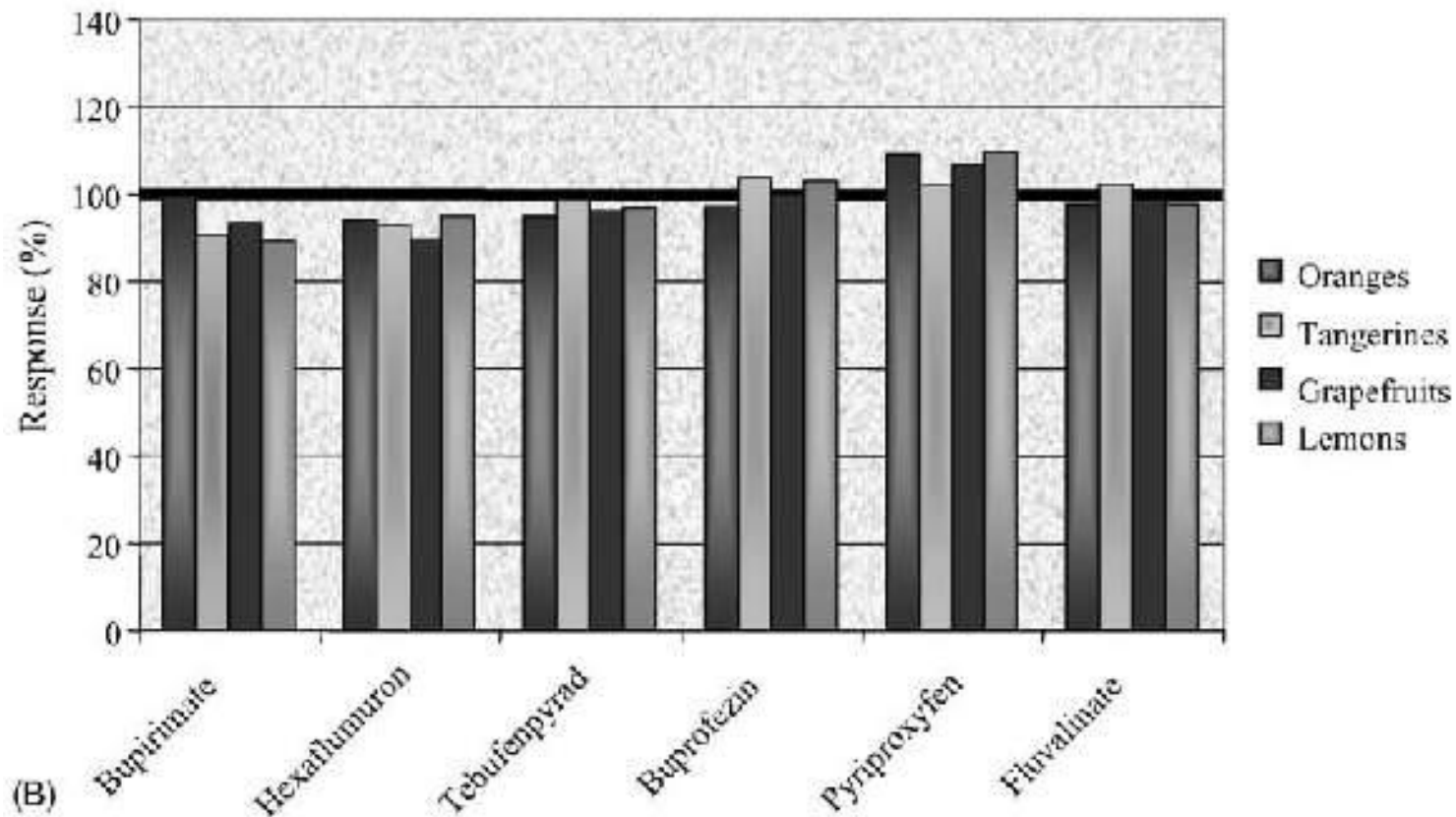
1. Blend 0.5 g of chopped sample gently with 0.5 g of C18 bonded silica for 5 min using a pestle, to obtain homogeneous mixture.
2. Introduce the homogeneous mixture into a cartridge
3. Eluted drop wise with 10 ml of a dichloromethane-methanol (80:20, v/v) mixture by applying a slight vacuum.
4. Collect the eluate and concentrate under stream of nitrogen, to 0.5 ml.
5. Inject 5  $\mu$ l of the final extract into the LC.

Carla Soler, et.al, Journal of Chromatography A, 1088 (2005)



# Pesticide Analysis in Citrus Fruits with MSPD

Response observed at LOQ level by MSPD



Carla Soler, et.al, *Journal of Chromatography A*, 1088 (2005)

# Time for Some Coffee!

Vielen Dank für Ihre Aufmerksamkeit !

## Kaffepause

### 15 MINS



# Application Section

## Environmental Testing

### **SPE: Solid Phase Extraction**

- Polymeric SPE , advantages and benefits in water analysis
- Multi-residue analysis from drinking water
- Extraction of PAHs from drinking water
- EPA 500 series – VOCs and SOCs in drinking water
- Extraction of polar organic residues
- Explosive residues in ground water
- Organophosphates

### **Emerging Contaminants**

- Melamine in milk products and meats
- Drug residues in municipal and surface water



# Polymeric SPE : Advantages and Benefits

**Some advantages that are common to all polymeric SPE compared to silica SPE are:**

- Stability in a wide pH range – useful for all method options
- Resistance to drying after conditioning – creates greater error-free ease of use and throughput for end users
- High capacity/loadability – smaller SPE bed masses minimize solvent usage and create greater throughput
- Loading of 10-12% of bed Capacity (Interferences count!)



# Polymeric SPE : Advantage to Environmental/Food

- Multi-suite analysis large number of internal standards need extra capacity.
- Regular particles afford reproducible flow
- Forgiveness to accidental drying - loss of sample
- Ability to dry the cartridge prior to elution
- Simple methodology.
- Very cost effective.



# The Bond Elut Plexa Family

## Bond Elut Plexa

Non-polar polymer with a hydroxylated surface functionality is the best choice for a wide range of acidic, neutral and basic analytes.

## Bond Elut Plexa PCX

based on Plexa, with a strong cation exchange functionality, specifically designed for effective results for a wide range of pKa and logP basic compounds from plasma

## Available SOON

## Bond Elut Plexa PAX

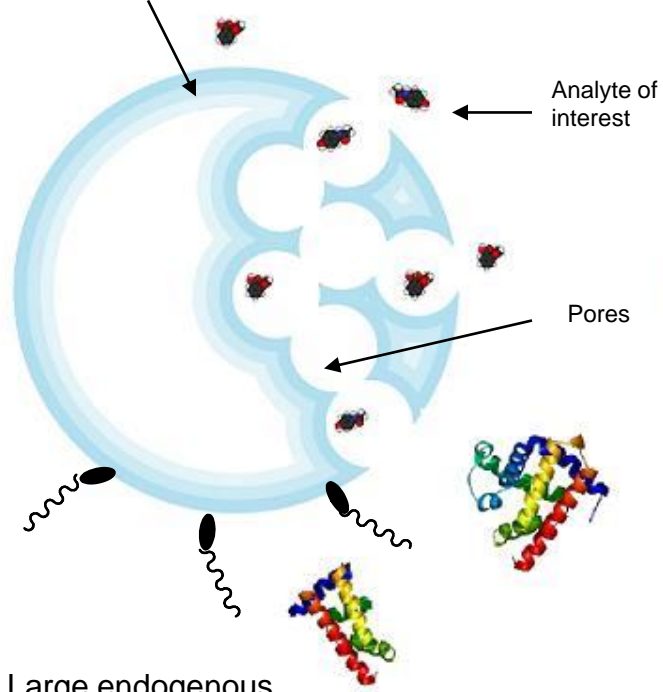
based on Plexa, with a strong anion exchange functionality, specifically designed for effective results for a wide range of pKa and logP acidic compounds from plasma



# How Bond Elut Plexa works

## Apply Sample

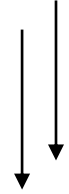
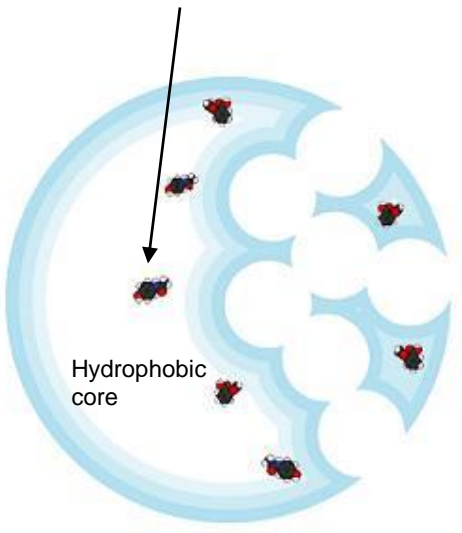
Water Rich Hydrophilic component allows excellent phase transfer of analytes into the polymer core



Large endogenous proteins do not bind to the surface of the polymer and cannot access pore structure.

## Washing

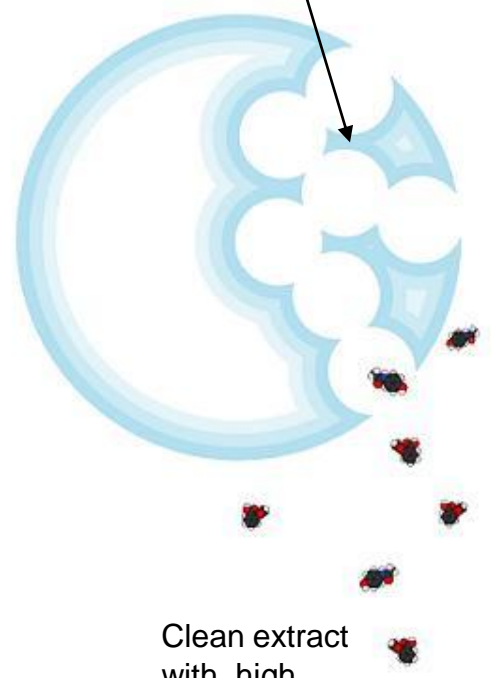
Analytes remain tightly bound in the hydrophobic core.



Interferences ( lipids and proteins) washed away without leaching into the analytes of interest

## Elution

Specially engineered pore structure allows excellent mass transfer out of polymer



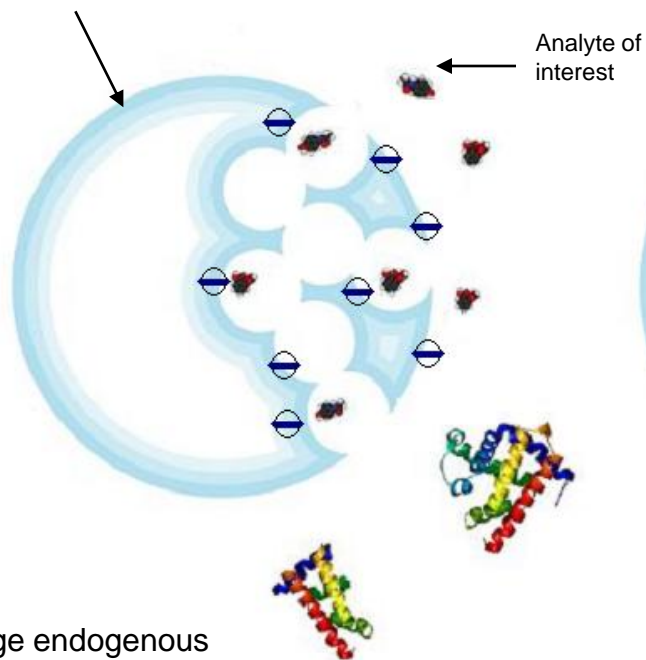
Clean extract with high recovery



# How Bond Elut Plexa PCX works

## Apply Sample

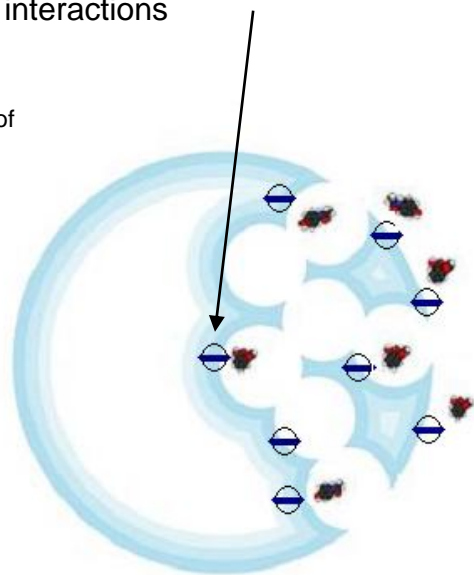
Water-rich Hydrophilic component allows excellent phase transfer of analytes to the cation exchange sites within the polymer.



Large endogenous proteins cannot access pore structure.

## Washing

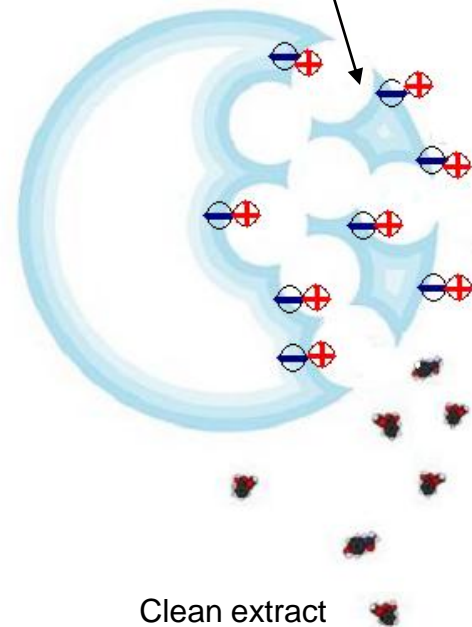
Analytes remain tightly bound to the polymer through strong ionic interactions



Interferences can be washed away without the leaching of basic analytes

## Elution

Change in pH allows the elution of analytes from the strong cation exchange sites



Clean extract with high recovery



# Plexa Recoveries Versus Other Polymer

Analyte	Plexa	Other Polymer	pKa	LogP
Albuterol	97.9	115.4	5.9	1.3
Atenolol	97.0	94.0	4.2	4.2
Loratadine	71.0	49.0	5.7	1.5
Metoprolol	92.0	74.0	5.7	1.5
Naltrexone	85.7	13.0	4.9	5.2
Pravastatin	85.0	59.0	4.9	5.2
Propranolol	55.0	35.0	4.9	5.2
Zolpidem	93.0	96.8	9.9	3.4

Plexa gives equivalent or better absolute recoveries than other polymer for a wide range of pKa and logP compounds

Conditions: Absolute Recovery from human plasma  
Basic load conditions, n=6, 200 ng/ml



# Absolute vs. Relative Recovery

## Absolute Recoveries (AR)

Sample + ISTD + drug

Extract

Inject

Compared to

Mobile Phase + ISTD + drug. **No extraction**, no loss of recoveries, **no matrix effects**

## Relative Recoveries (RR)

Sample + ISTD + drug

Extract

Inject

Data is then compared to a graph of extracted analyte controls.

Analytes are the same and should extract at the same efficiencies.

If the standard curves were extracted at 80% and the unknowns at 80% the relative recoveries will show to be at about 100%!!

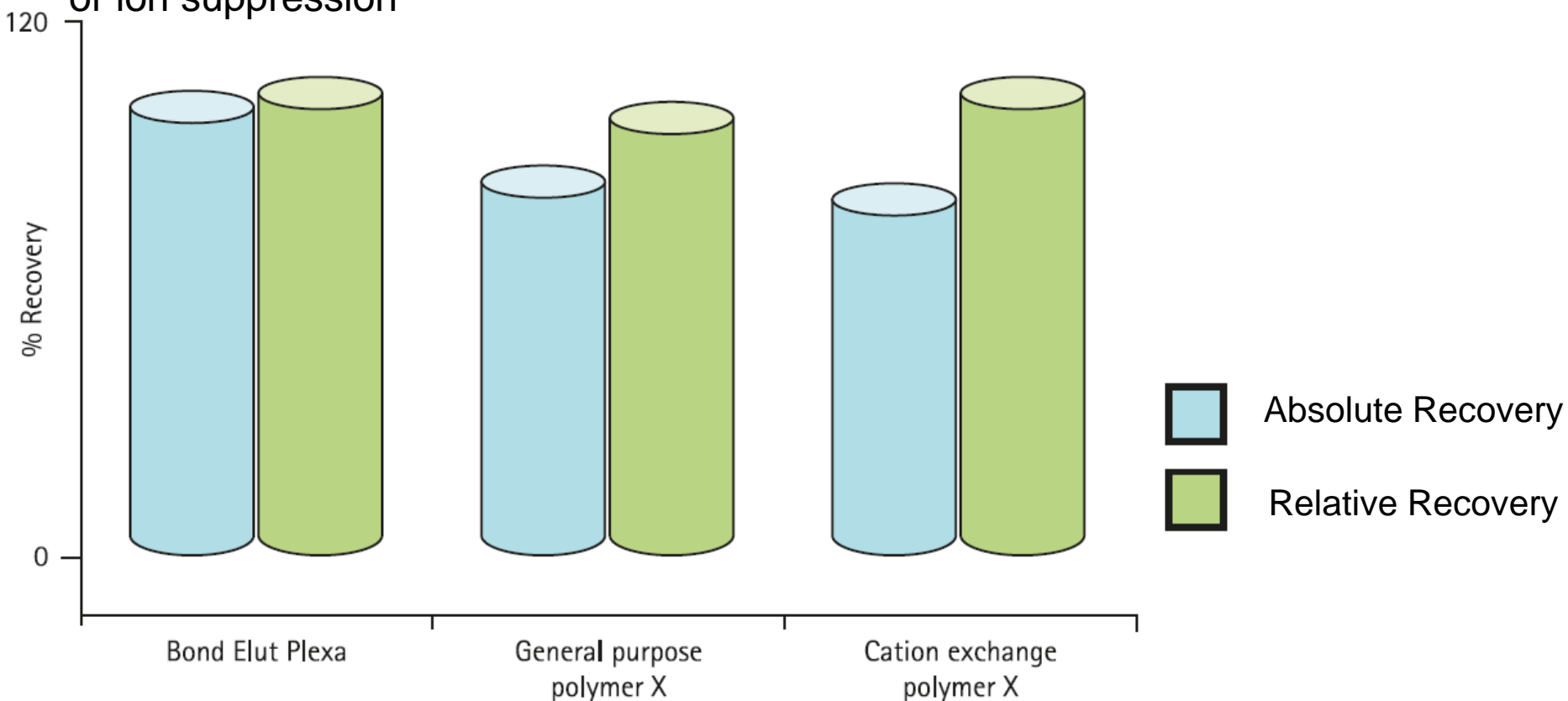
*Relative recoveries (via calibration curves) can mask the affects of ion suppression, whereas absolute recoveries do not “cancel” these affects.*

*Minor differences in absolute vs. relative recoveries is indicative of extract cleanliness or superior extraction characteristics.*

# Absolute vs. Relative Recovery

Small differences between AR and RR indicate high extraction efficiency and low signal loss due to ion suppression.

Larger differences indicate sensitivity problems arising from low extraction efficiency or ion suppression



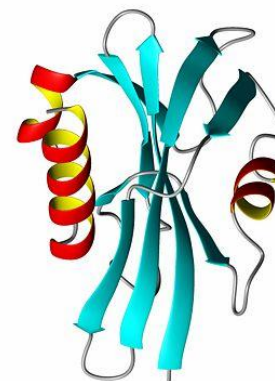
# Ion Suppression

Ion Suppression is a deleterious phenomenon that can be experienced during LCMS analysis.

## Causes

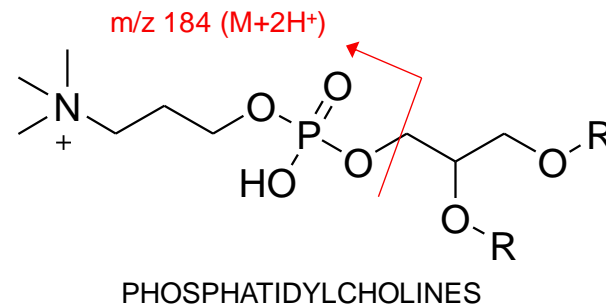
Endogenous matrix interferences

- Proteins and Peptides
- Lipids
- Salts
- Humic acids

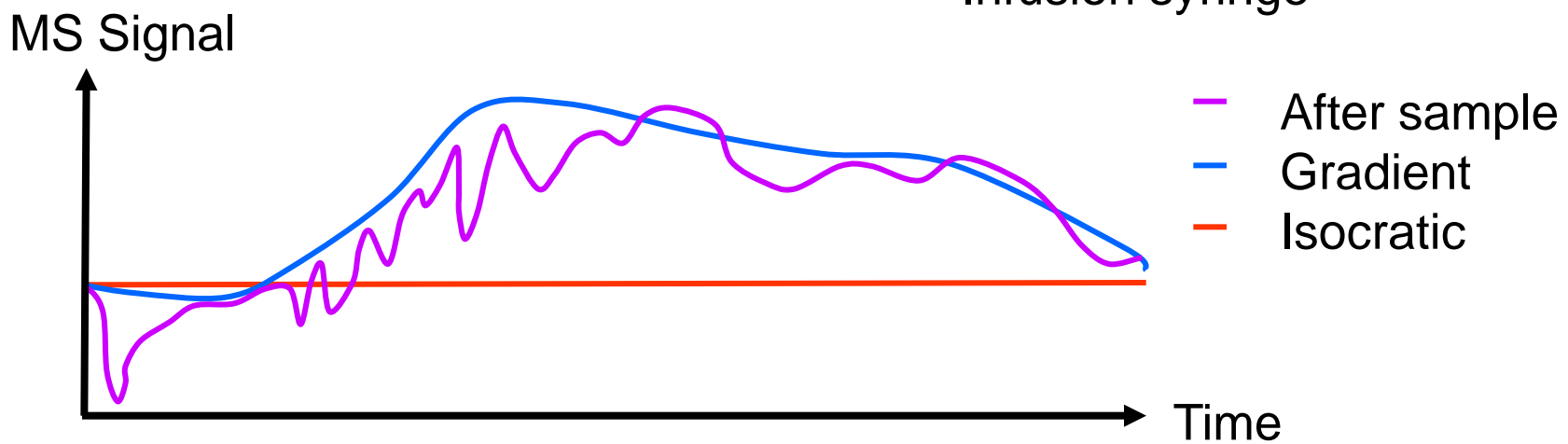
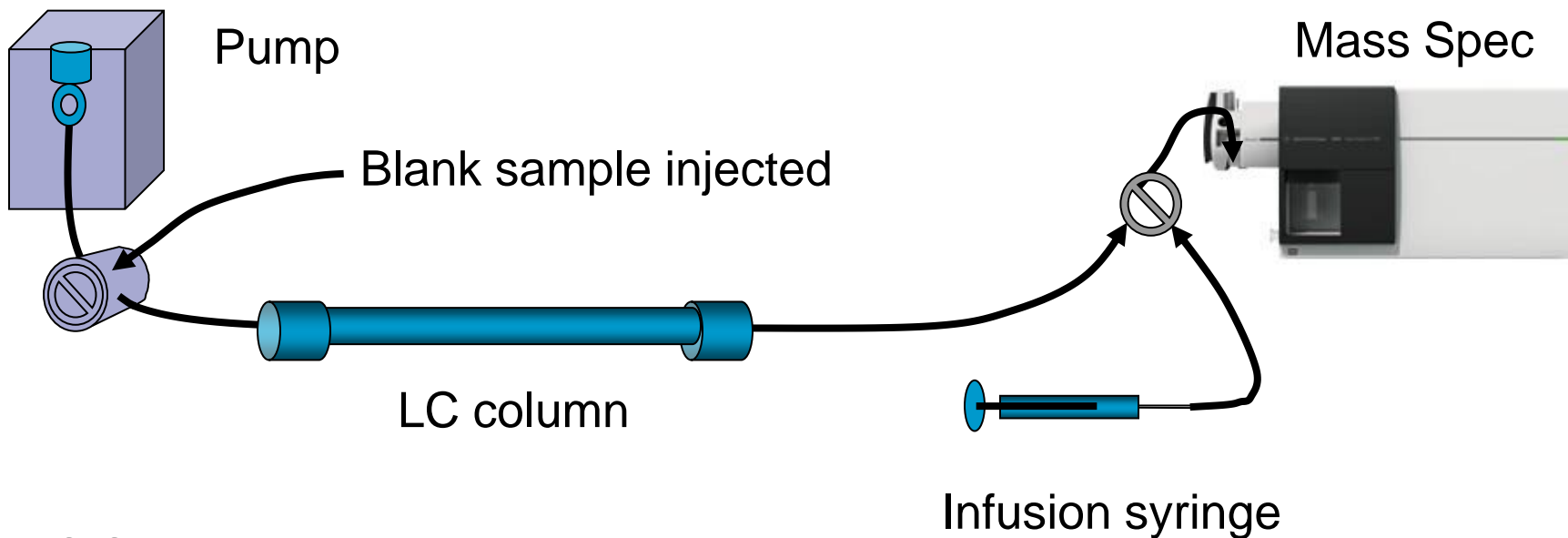


## Effect on Data

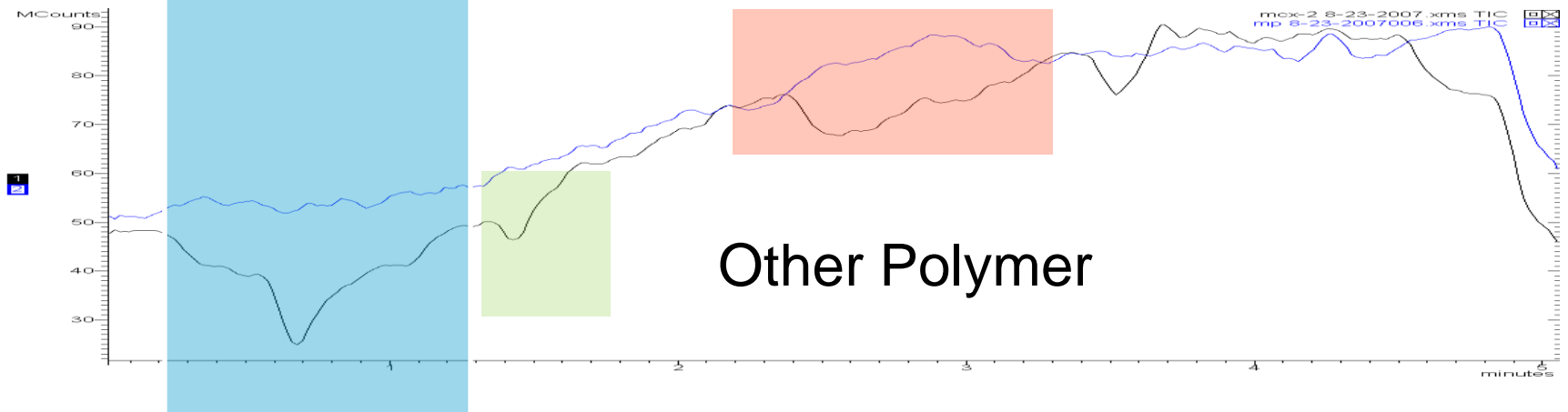
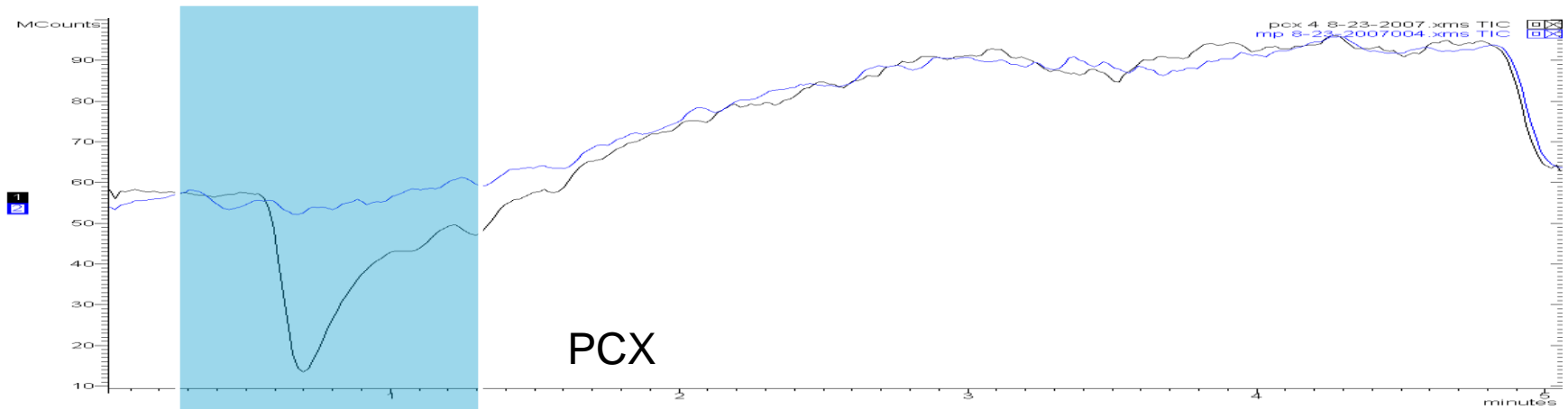
- Loss of Ion signal will inhibit quantification of analyte
- Loss of sensitivity, precision and accuracy





# Post Column Infusion Experiments

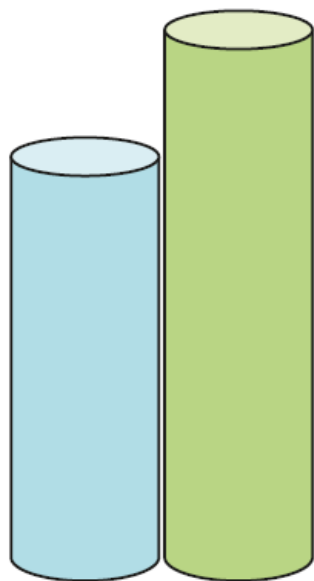


# Improved Precision - Reduced Ion Suppression



# TIP: Evaluating Ion Suppression

-  Absolute Recovery
-  Relative Recovery



Cation exchange  
polymer X

- **Simple Route:**  
Compare absolute and relative recoveries (if the difference is >10% after  $n = 3$  then you have a significant matrix effect).
- **Comprehensive:**  
Run and isocratic or gradient (preferred) Post Column Infusion experiment
- **Extra View:**  
Monitor the 184→184 transition for lipids

# Simple Plexa Methodology

## Bases/Neutrals

Condition: 500  $\mu$ L MeOH  
500  $\mu$ L water

Apply sample: Aqueous sample  
w/ 2%  $\text{NH}_4\text{OH}$

Wash: 500  $\mu$ L 5% MeOH

Elute: 500  $\mu$ L MeOH

## Acids

Condition: 500  $\mu$ L MeOH  
500  $\mu$ L water

Apply sample: Aqueous sample  
w/ 1%  $\text{HCO}_2\text{H}$

Wash: 500  $\mu$ L 5% MeOH

Elute: 500  $\mu$ L MeOH

Methods for 30 mg Plexa in 96 well plate

# PCX Method

## Bases

Condition:	500 $\mu$ L MeOH 500 $\mu$ L water
Apply sample:	Aqueous sample with 2% phosphoric acid
Acidic wash:	500 $\mu$ L 2% formic acid
Neutral wash:	500 $\mu$ L MeOH:ACN (1:1 v/v)
Elute:	500 $\mu$ L 5% $\text{NH}_3$ in MeOH:ACN (1:1)

Methods for 30 mg Plexa in 96 well plate

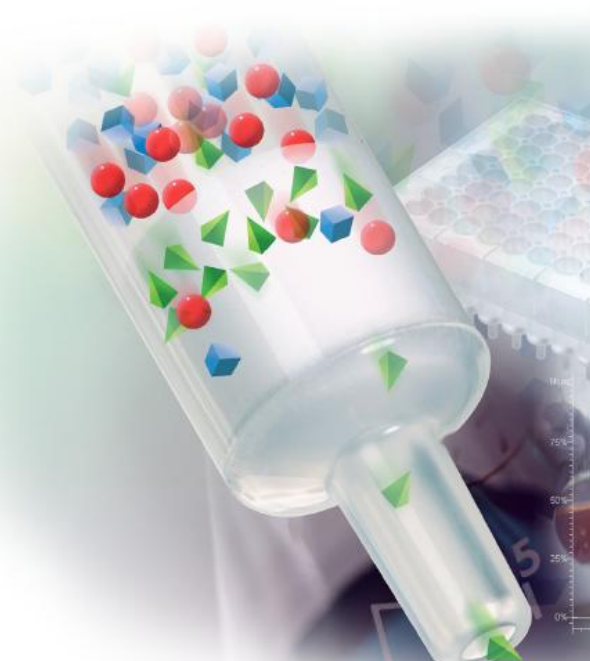
# TRICK: Maximizing Elution Recovery

## Elution Solvent Composition

- Most methods recommend 100% MeOH or 5% NH<sub>3</sub> in MeOH elution (CX)
- **We recommend 50:50 MeOH/ACN**
- Combination of polar protic and dipolar solvent breaks more analyte interactions with SPE phase

## Elution Volume

- Most methods recommend 1000uL of eluting solvent
- 2 x 500uL often gives higher recovery
- **For Plexa methods 2 x 250uL (30mg bed)**



# Multi-Suite Extraction

An SPE method to extract Triazines, Chloro-Pesticides and PAHs from drinking water with one single cartridge.



All data shown in this application have been developed at Laboratori de l'Agència de Salut Pública de Barcelona.

The Bond Elut Plexa SPE procedure is the validated method in this laboratory and used in daily routine.



# Compound List

Triazines	OCPs	PAHs
Desisopropylatrazine	Alachlor	Benzo(a)anthracene
Desethylatrazine	Aldrin	Benzo(b)anthracene
Cianazine	DDD o-p'	Benzo(k)anthracene
Simazine	DDD p-p'	Benzo(a)pyrene
Atrazine	DDE o-p'	Dibenzo(a,h) anthracene
Terbutrine	DDE p-p'	Benzo (ghi) perylene
Propazine	DDT o-p'	Indeno (1,2,3-cd) pyrene
Tertbutylazine	DDT p-p'	Chrysene
	Dieldrin	
	Endosulfan (I + II)	
	Endrin	
	HCH ( $\alpha+\beta+\delta+\gamma$ )	
	Heptachloro Epoxy	
	Hexachloroceno	



# Automated multi-suite method

SPE Method on 200mg/6mL Bond Elut Plexa  
with AutoTrace Workstation

Step	Method	Flow Rates
1	Wash syringe with 2mL MeOH	40 ml/min
2	Rinse with 5mL EtOAc	40
3	Rinse with 5mL DCM	40
4	Condition with 10mL MeOH	40
5	Condition with 10mL H2O	40
6	Load 800mL water sample	20
7	dry with air for 10 minutes	15
8	Soak and collect 2.5mL fraction using EtOAc	5
9	Collect 1mL fraction using EtOAc	5
10	Soak and collect 2.5mL fraction using DCM	5
11	Collect 1mL fraction using DCM	5

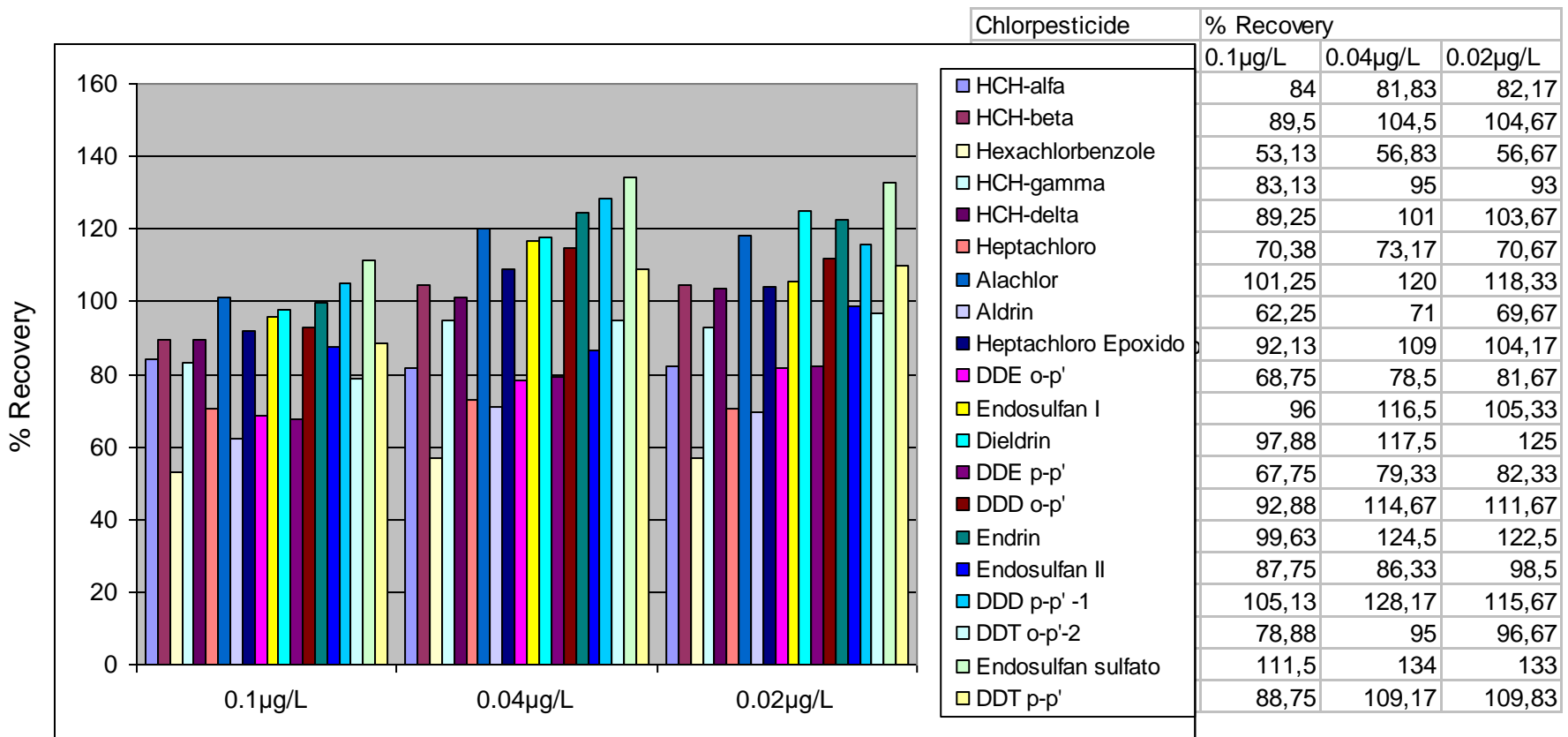
HPLC-FL/UV for determination of PAHs

GC/MS for Chloro-Pesticides

LC-MS/MS for Triazines



# Recovery Data



IS Hexachlorbenzene C<sup>13</sup>



# TIP: Improve Productivity with a single method

Save time and reduce costs in the extraction of Triazines, Chloro-Pesticides and PAHs from water samples.

Previous methods at Laboratori de l'Agència de Salut Pública de Barcelona used liquid/liquid extraction for the PAHs and Triazines, and C18 extraction for the Chloro-Pesticides.

**Using Bond Elut Plexa, all three sample types can be extracted on one single cartridge.**

The RSDs of the PAHs are very good (<6%); this makes the SPE with Plexa a reliable method in daily routine work.



## PAHs in Drinking Water

Application Note SI-02187

Determination of 24 PAHs in Drinking Water using Automated SPE and Fast HPLC-FLD/UV Detection

M.C. Díaz Ramos, A. Suárez, A. Rúbies, F. Centrich, Laboratori Agència de Salut Pública de Barcelona

Elisabeth Korte, Varian Deutschland GmbH

R. Companyó, Department de Química Analítica, Universitat de Barcelona

## EPA Method 528: Phenols in Drinking Water

Bond Elut PPL and Bond Elut Plexa

## EPA Method 500 Series: VOCs in Drinking Water

Bond Elut C18 is a Generic Phase for 525.2



# Determination of 24 PAHs in Drinking Water using Automated SPE and Fast HPLC-FLD/UV Detection

Figure 1 shows the FLD chromatogram of the 22 fluorescent analytes in water sample, spiked at the quantification limit of 0.02 µg/L.

KEY

- 1. Naphthalene
- 2. Acenaphthene
- 3. Fluorene
- 4. Phenanthrene
- 5. Anthracene
- 6. Fluoranthene
- 7. Pyrene
- 8. Benzo(c)fluorene
- 9. Benzo(a)anthracene
- 10. Chrysene
- 11. Methylchrysene
- 12. Benzo(j)fluoranthene
- 13. Benzo(b)fluoranthene
- 14. Benzo(k)fluoranthene
- 15. Benzo[a]pyrene d<sup>12</sup>
- 16. Benzo(a)pyrene
- 17. Dibenzo(a,h)anthracene
- 18. Dibenzo(a,l)pyrene
- 19. Benzo(g,h,i)perylene
- 20. Indeno(1,2,3 cd)pyrene
- 21. Dibenzo(a,e)pyrene
- 22. Dibenzo(a,i)pyrene
- 23. Dibenzo(a,h)pyrene

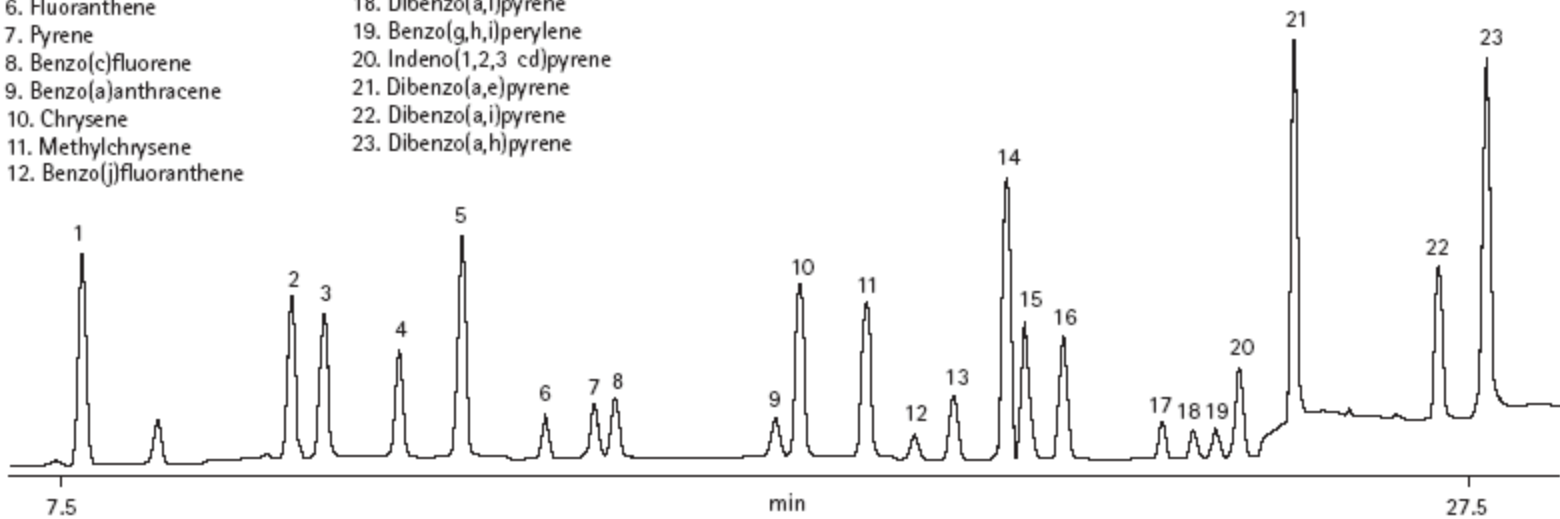


Figure 1. HPLC/FLD chromatogram of a 5 µL injection at a concentration of 20 ppt in water sample using the Varian Pursuit PAH 3 µm 100 x 4.6 mm LC column (pn: A7001100X046).



# High Flow SDVB SPE: Bond Elut ENV

## Bond Elut ENV

Bond Elut ENV, a PS/DVB polymer, is designed for the extraction of polar organic residues.

- Excellent extraction of polar molecules
- High flow through
- Efficient elution characteristics
- High Flow 125 µm spherical particles
- Herbicides, explosive residues

<b>Sorbent Conditioning:</b>	200 mg/3 mL Bond Elut ENV cartridge
<b>Apply Sample:</b>	Adjust 500 mL sample to pH 2 using concentrated HCl
<b>Interference Wash:</b>	500 mL of water sample at a flow rate between 10 and 15 mL/min.
<b>Analyte Elution:</b>	5 mL DI H <sub>2</sub> O, then dry the cartridge for 3 min 1. 2.5 mL ACN (2 mL of which re-eluted x 4 after 1st elution) 2. 1.5 mL fresh ACN

<b>Compounds</b>	<b>Recoveries (%)</b>
1,3,5-Trinitrobenzene	99.8
Nitrobenzene	92.1
2,4-Dinitrotoluene	97.7
2,6-Dinitrotoluene	86.8
2-Amino-4,6-dinitrotoluene	93.2
4-Amino-2,6-dinitrotoluene	93.3
4-Nitrotoluene	85.3



# Unique Selectivity SPE: Bond Elut PPL

**Bond Elut PPL, a PS/DVB based polymer, has a unique selectivity to highly polar analytes.**

- Excellent extraction of polar molecules
- Slightly more hydrophilic than an SDVB product
- Suitable for EPA 528
- SI-02094 organophosphates in lake water (below)

**Vacuum Manifold:** Vac Elut 20

**Vacuum:** 800 mbar

**Cartridge:** Bond Elut PPL, 100 mg sorbent in 1 mL cartridge

Condition cartridge with 1 mL methanol, 1 mL ethanol/ acetonitrile (1/1)

- Method:**
1. Apply 1.5-2.5 L water sample
  2. Dry the cartridge using nitrogen
  3. Elution with 3 x 333 µL methanol/acetonitrile (1/1)

Analyte	Recovery (%)	LOD (ng/L)
Tris (1-chloro-2-propyl)-phosphate (TCPP)	91	1
Tris (2-chloroethyl)-phosphate (TCEP)	95	2
Tris (1,3-dichloro-2-propyl)-phosphate (TDCCP)	99	1
Tri-n-butylphosphate (TnBP)	89	1
Tri-isobutylphosphate (TiBP)	85	2
Tris(2-butoxyethyl)-phosphate (TBEP)	93	3

Recoveries and LODs of organophosphates; extracted from the water sample with SPE



# Emerging Contaminants and Pollutants

## Drug residues in municipal waters

Application Note SI-02188

Determination of Drug Residues in Surface and Municipal Wastewaters by SPE and LVI-LC/MS/MS

Sabine Oswald and Oliver Schöttler, Niersverband, 41066 Mönchengladbach, Germany  
Elisabeth Korte and Gunnar Weibchen, Varian Deutschland GmbH

## Melamine in milk products



Application Note 01916

Simultaneous, Fast Analysis of Melamine, Cyanuric Acid, and Related Compounds in Milk and Infant Formula by LC/MS/MS

Eugene Chang and Ritu Arora  
Varian, Inc.



# SPE Method

## Bond Elut PPL

**Pretreatment:** Add 2.0 mL sample to 48.0 mL water, add 0.35 g (7 g/L) NaCl

**Condition:** 3.0 mL methanol, 6.0 mL water

**Apply sample** 1-1.5 mL/min

**Wash:** 6.0 mL LC/MS grade water

**Dry:** 5 min @ 10"Hg

**Elute:** 1.5 mL methanol, soak for 5 min, repeat

Evaporate and reconstitute in 250  $\mu$ L methanol, and fill up to 4.0 mL with water.



# Quantitative Determination of Drug Residues in Surface and Municipal Waste Water

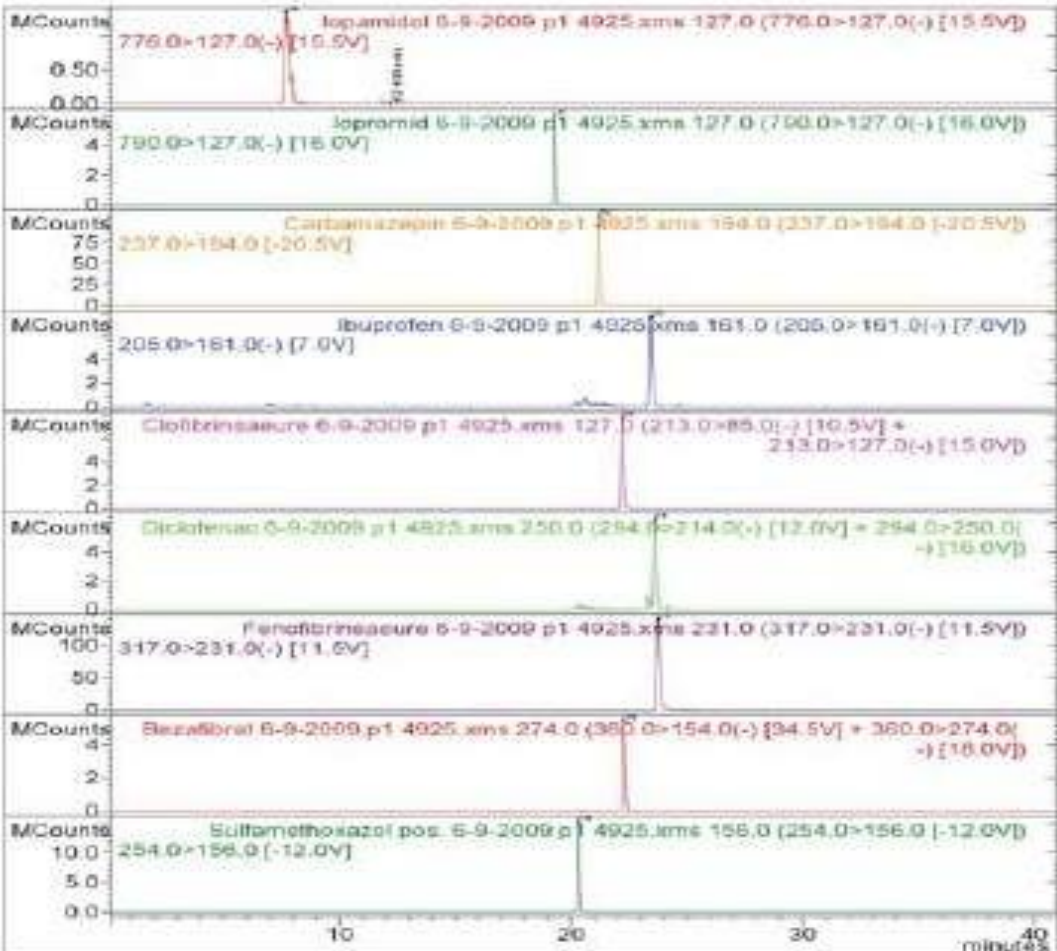


Figure 2. MRM chromatograms of drug residues in surface water after SPE and LVI.

The chromatogram obtained from an actual sample under LVI-LC/MS/MS conditions indicated **good separation and detection of all analytes.**

The injection of a large volume (200 µL) onto a guard column filled with C18 material and the separation on pentafluorophenylpropyl HPLC column gave **narrow and symmetrical peaks and did not show peak broadening.**

The limit of detection for surface water samples is **0.05 µg/L.**



# Quantitative Determination of Drug Residues in Surface and Municipal Waste Water

## Conclusion

The method incorporated pharmaceuticals from a wide variety of classes.

The optimization of a single SPE extraction procedure for the neutral, basic and acidic compounds in combination with a large volume injection streamlined the method and is a significant improvement in sample throughput.



# Melamine Detection

- Melamine can be added to foodstuffs to mimic increased protein content (protein content analysis is usually done by measuring nitrogen content)
- Recent scandals include melamine addition to pet food and infant formula
- The US FDA has released testing requirements for melamine and related compounds, like cyanuric acid
- Several food manufacturers require a method for related compounds like ammeline and ammelide
- Bond Elut Plexa and Bond Elut Plexa PCX have been successfully used to extract melamine and related compounds from infant formula and milk

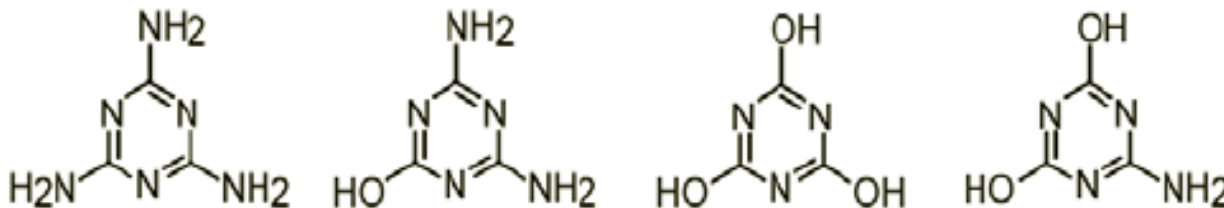
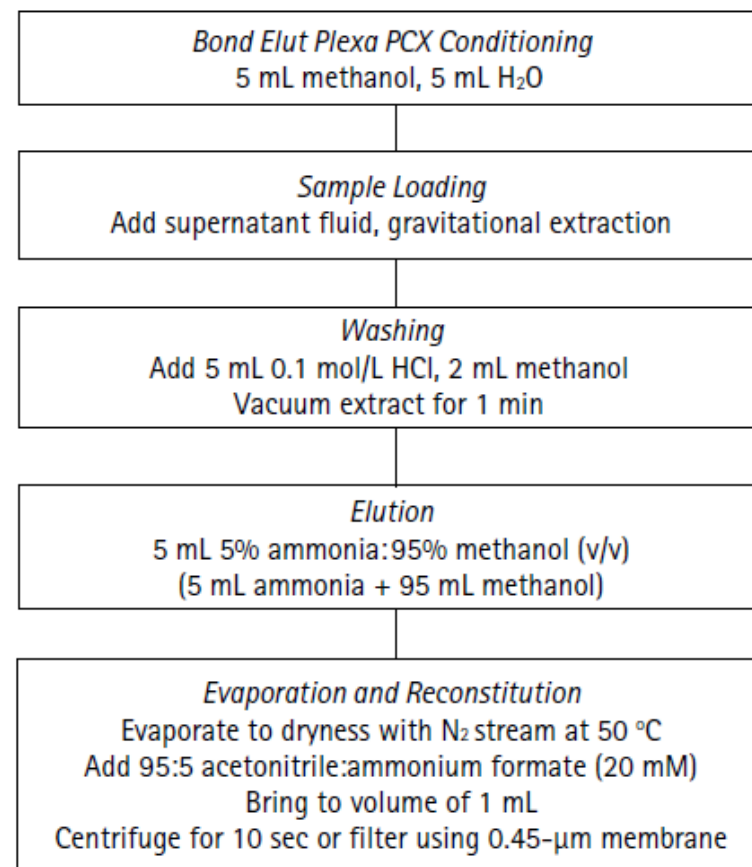


Figure 1. Structures of melamine, ammeline, cyanuric acid, and ammelide, respectively.



# Sample Clean-up with Bond Elut Plexa PCX

- Bond Elut Plexa used as clean-up: matrix interferences remain bound while polar analytes pass through the cartridge
- Analysis of melamine, ammeline, cyanuric acid, and ammeline according to FDA method
- Plexa PCX clean-up step added to successfully analyze all four compounds
- Infant formula and milk



**Bond Elut Plexa PCX, 200mg, 3mL cartridge, P/N 12109603**



# Results of App note 01916

## Results

- Excellent linearity shown from 0.25 – 5.0 µg/g
- FDA requirements for S/N ratio at 0.25 µg/g far exceeded (achieved 16:1, required > 5:1)
- Good recoveries and RSDs:
- Method suitable for dried milk powder and fresh milk

Sample	1.0 µg/g Fortified Infant Formula	1.0 µg/g Fortified Milk
Melamine Average % Recovery ± % RSD (n)	88.3 (n = 3) ±14.7%	83.8 (n = 3) ±19.6%
Cyanuric Acid Average % Recovery ± % RSD (n)	92.5 (n = 3) ±6.2%	94.9 (n = 3) ±14.2%
Ammeline Average % Recovery ± % RSD (n)	77.5 (n = 3) ±16.4%	67.8 (n = 3) ±6.5%
Ammelide Average % Recovery ± % RSD (n)	89.4 (n = 3) ±14.7%	87.3 (n = 3) ±18.0%



# Applications Summary: Melamine

## App Note 01916:

Melamine, cyanuric acid, ammeline, ammeline Milk, LC/MS/MS (320), Plexa, Polaris NH<sub>4</sub> (HILIC style).

## App Note 01841:

Melamine, milk, Plexa PCX, LC/MS/MS (320), C8 Pursuit, 1 ppb

## App Note 01842

Melamine, milk, GC/MS/MS (220 Ion Trap), VF-5ms, Plexa PCX

## App Note 01846:

Melamine, cyanuric acid, ammeline, ammeline, milk and pet food, GC/MS/MS (220 Ion Trap), Plexa PCX, VF-Xms with EZ-Guard.

## App Note 01915:

Melamine, cyanuric acid, ammeline, ammeline, milk, Plexa, Polaris NH<sub>4</sub> (HILIC style)



# TIPS & TRICKS: Trouble Shooting

From our global helpdesk logs, we have identified the most common SPE issues encountered by our end users.

**All of these issues can be linked to simple practical errors**



1. Low Recovery
2. Poor Flow
3. Loss of Analyte
4. Dirty Extracts

# TIP: Sorbent Conditioning

## Sorbent conditioning is vital for good SPE performance

- An unconditioned sorbent bed can result in **Poor Flow** and **Poor Recovery**.
- Particularly for biological samples, where precipitation can occur on dry media
- Clogging and Channelling

## Recommendations

Silica SPE	Polymeric SPE
>2 bed volumes of low viscosity organic solvent (e.g. MeOH)	>2 bed volumes of low viscosity organic solvent (e.g. MeOH)
Condition slowly under minimal vacuum <2" Hg	Wash with aqueous solution prior to sample load
Allow time for equilibration (30-60s)	More tolerant to accidental drying



# TIP: Poor Flow - Vacuum Considerations

## Vacuum control and experience comes with practice

- Flow rates should be kept on the lower side overall (2-5 mm Hg)
- 2-3 mL/min is optimal for load, wash and elution
- Ion Exchange as a primary or secondary interaction is especially affected by high flow rates (break through)
- Even C18 can show band broadening with high flow rates
- Employ variable vacuum or variable time (pulsing) depending on sample type and drying needs



# TIP: Poor Recovery - Capacity

## Understanding the Capacity of your SPE Phase is Critical

Sorbent Type	Capacity
Silica (Polar or Non-Polar)	1-4% of bed mass
Silica (Ion Exchange)	< 1.0 mmol/g
Polymeric	10 -12% of bed mass

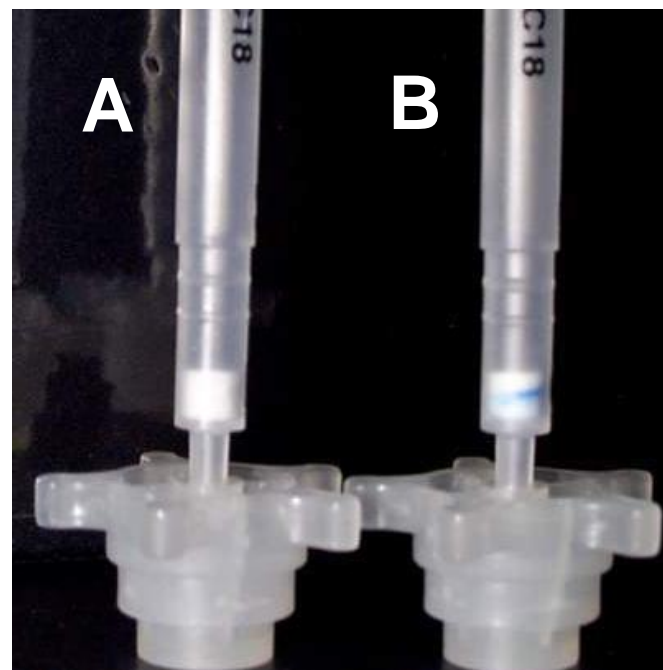
- Assumes good analyte/sorbent interaction
- **Capacity does not distinguish between analyte and interference!**
- Do you know amounts of interferences present?
- Stacking two cartridges on top of each other will help determine if capacity is an issue



# TIP: Poor Recovery - Elution

## Elution volume optimization can be the simple to achieve

- Elution volumes can be minimized and recovery maximized if elution is done in aliquots.
- Four aliquots of 50uL give better results than 200uL in a single shot. 2 x 100uL is a more practical solution.
- Using mixtures of solvents can have a dramatic effect on recovery (50:50 MeOH/ACN)



**A: 4 x 50 uL Aliquots**  
**B: 1 x 200 uL Aliquot**



# TIP: Dirty Extracts

## Dirty Extracts are common in un-refined SPE methods

- Phase may be too universal (e.g. C18)
- Extraction scheme is not specific enough
- **Reduce** concentration of **Elution** solvent
- **Wash** step is ineffective, **increase** organic concentration
- Ion Suppression - try alternative phase or change wash %
- Try polymeric or mixed mode SPE
- **Pre-rinse cartridge with elution solvent**



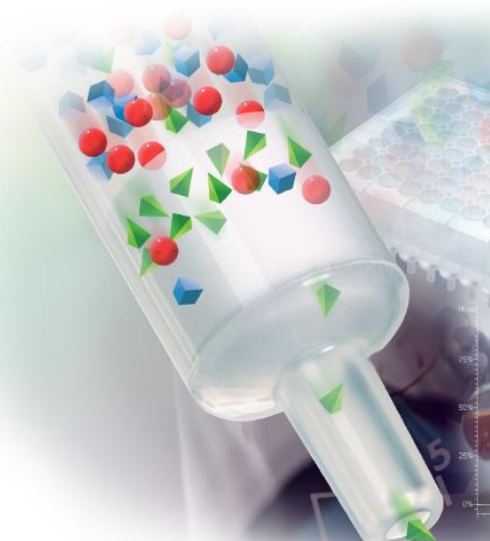
# TRICK: If In Doubt ... Mass Balance!

If you load **???**ng of a sample onto a cartridge, tracking where it goes helps determine important method alterations

1. Collect “effluent” that has passed through upon sample loading
2. Collection all wash steps
3. Collect all elution steps

**Easy to Quantify if you have analyte still on the phase**

If the matrix contains high levels of interference, mass balance can be difficult if analyte does not retain on the load step.



# Sample Prep Summary

- In the ever demanding analytical chemistry environment, sample prep is becoming an essential component of good laboratory workflow
- The advanced sample prep techniques (SPE, SLE and QuEChERS) offer clear ease of use and productivity advantages
- The quantification of endogenous materials and ion suppression is vital to getting the most from your sample
- Polymeric SPE and multi-suite methods is a key platform for environmental samples
- Agilent offers the broadest platform of chemistries in the widest range of formats in the industry
- **This was just a short seminar. Tell us if you have a challenge or application need in your laboratory... we probably have a solution already!**



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Thank You for your Attention!

**Fragen?**

