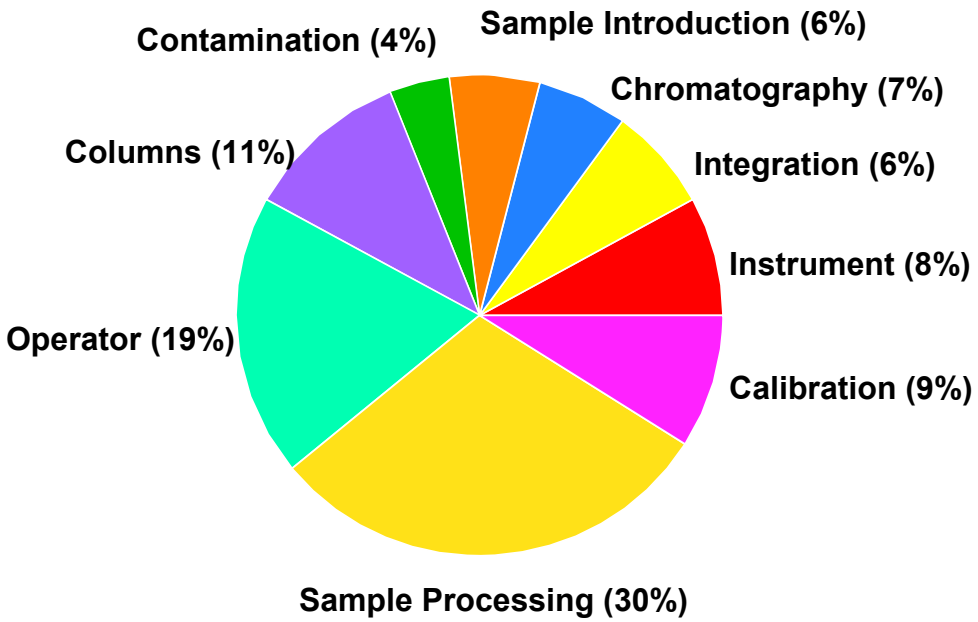
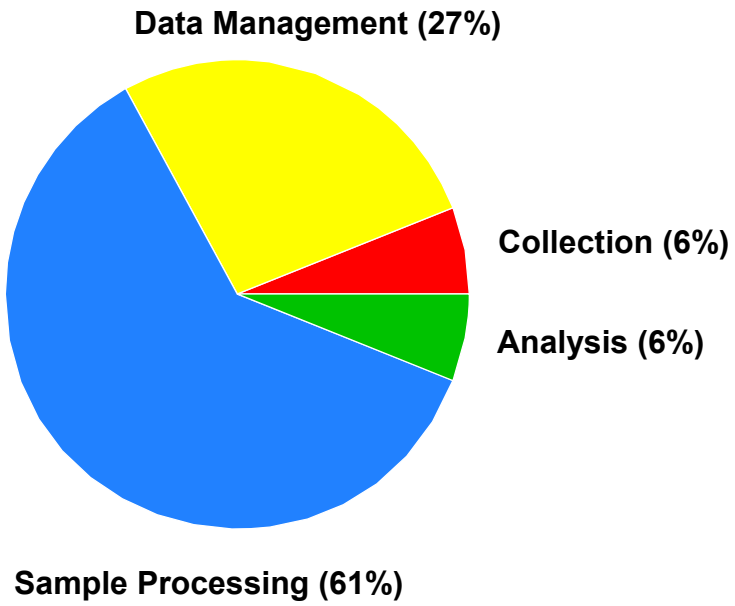
SPE Method Development Tips and Tricks

Sources of Error Generated and Time Spent During a Typical Chromatographic Analysis



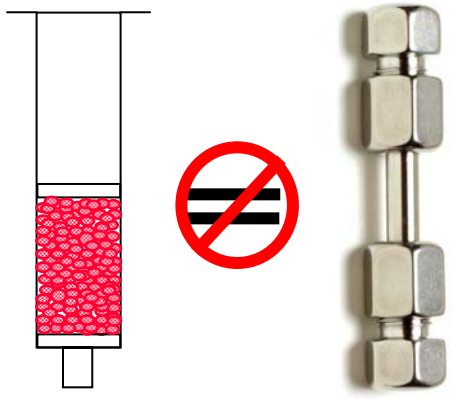
Sources of Error



Time Spent

(R.E. Majors, LC/GC Magazine, 2002)

What is SPE?



• Can be thought of as digital chromatography – compounds either bind or flow through

•Has a wide choice of sorbents with selectivities similar to sorbents used in HPLC, but it is not HPLC

•Many samples can be analyzed in parallel. Manifolds of 10 and 20 ports are readily available.

•Methods are simple to perform, but the process can be time-consuming, however SPE can be readily automated

•SPE methods give high selectivity, recovery and reproducibility

In addition SPE is widely used for:

- •Desalting (reversed-phase principles)
- •Solvent Exchange (for better chromatography)
- •Sample Preservation and Storage (analytes stabilized)

Advantages of SPE vs. Liquid-Liquid Extraction

Improved throughput (parallel vs. serial processing)

Decreased organic solvent usage and waste generation

Higher and more reproducible recoveries

Cleaner extracts (contamination, solvent impurities)

No emulsions

Tunable selectivity (SPE phase choices, solvent mixtures)

Readily automated

Typical Applications of SPE

Sample Cleanup

- Combinatorial reaction cleanup before LC-MS or LC
- Pharmacokinetic studies, dissolution testing
- Isolate analytes from complex matrices – urine, plasma
- Remove “column killers” or major interferences
- Eliminate late-eluters to allow isocratic analysis

Trace Enrichment

- Environmental analysis
- Pharmaceutical and Agrochemical applications

Desalting

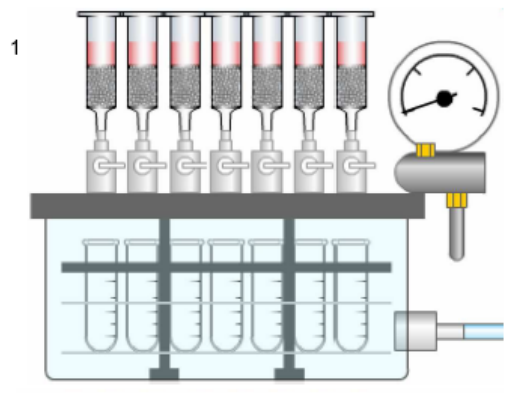
Solvent Exchange

Sample Preservation and Storage

Processing Choices for Cartridges

1. Vacuum Manifold
2. Pressure Manifold
3. Centrifugation

Vacuum Manifold



Pros

1. Easy to add sequential solvents
2. Inexpensive set up
3. Easy to monitor flow rate
4. Low carryover with simple cleaning
5. Most widely used approach

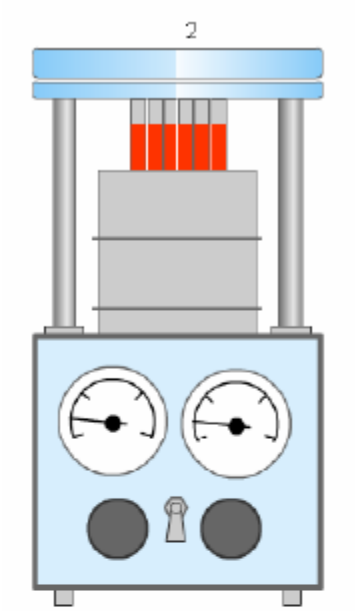
Cons

1. Inconsistent flows when slight variation in packing bed.
2. As each cartridge empties effective vacuum changes resulting in changing flow rate for remaining cartridges

Pressure Manifold

Pros

1. Consistent pressure on each cartridge regardless of whether some cartridges empty more quickly
2. Relatively inexpensive set up
3. Easy to monitor flow rate
4. Most widely used approach



Cons

1. Difficult to add successive solvent steps
2. Sample contamination possible if sealing gasket is accidently wet by prior sample.

SPE Modes—"Digital Chromatography"

Analyte Adsorption (Bind-Elute)

Analyte(s) retained ($K_D >> 1$)

Matrix unretained ($K_D \sim 0$)
and/or strongly retained ($K_D >> 1$)

Preconcentration factor

Cleaner extracts

Load at 1-3 drops/sec (recovery $\propto$ 1/flow)

Capacity issues may be more important

Matrix Adsorption (Interference Removal)

Analyte(s) unretained ($K_D \sim 0$)

Matrix retained ($K_D >> 1$)

No preconcentration advantage

Eluates may not be as clean

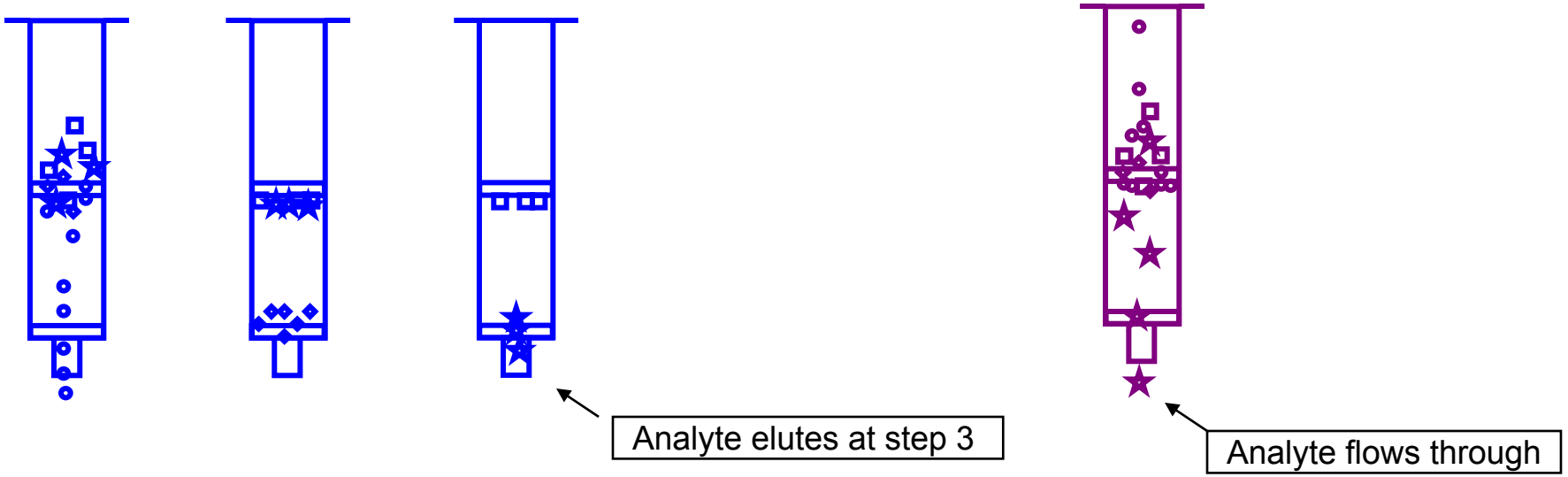
Sample loading often gravity fed

Used less often than analyte adsorption

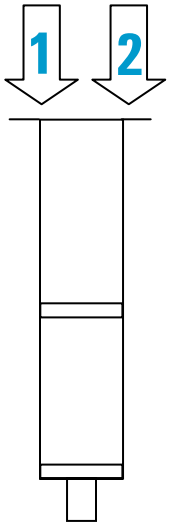
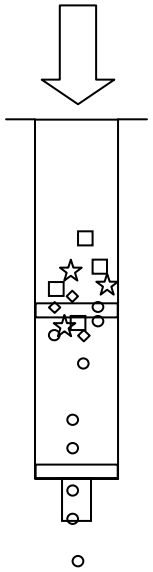
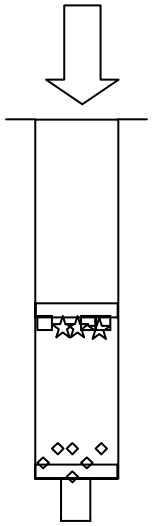
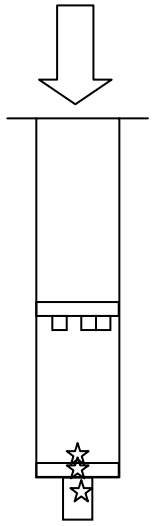
SPE Modes—"Digital Chromatography"

Analyte Adsorption (Bind-Elute)

Matrix Adsorption (Interference Removal)



Fundamental Steps for the 'Bind-Elute' SPE Experiment

Condition	Load	Wash	Elute
Prepare cartridge to accept sample	Load sample and rinse reservoir(s)	Wash with solvent that won't elute analyte	Elute analyte in smallest volume possible
			
1. MeOH or ACN 2. Weak solvent (water, buffer)	Weakly retained matrix compds elute	Analyte and other matrix compds retained	Elute analyte leaving highly retained compds

Optimizing Steps for the 'Bind-Elute' SPE Experiment (non-polar example)

1. **Conditioning:** Solvent is passed through the SPE material to *wet* the bonded functional groups => ensures consistent interaction. (Use methanol)

2. **Equilibration:** Sorbent/ phase is treated with a solution that is similar (in polarity, pH, etc.) to the sample matrix => maximizes retention. (Use water or the same aqueous solution that the sample is prepared in).

3. **Sample Load:** Introduction of the sample = analytes of interest are bound/ extracted onto the phase/ sorbent. Must be an aqueous solvent (no organic)

4. **Washing:** Use the 'strongest' aqueous solution that will NOT elute the target compounds. Increasing the % organic, increasing or decreasing the pH, changing the ionic strength are all tips for increasing clean-up. Dry the cartridge to remove all water.

5. **Elution:** Use the smallest volume of organic solvent that will elute ALL of the target analyte. Use the 'weakest' organic solvent that will remove ALL of the target analyte. As a general rule the 'strength' of the solvent is directly related to the target compound. Polar target compounds elute best in polar solvents so in order of polarity try: methanol>acetonitrile>ethylacetate>acetone>THF. Modify the pH, increase the ionic strength.

6. **Solvent exchange:** If the subsequent analyses are HPLC, the organic elution solvent should be evaporated and the sample reconstituted in starting mobile phase. If the next analysis is GC, then methanol is the reconstitution solvent. In all cases the reconstitution must be to the same volume.

Retention Mechanisms

Polar

matrix is organic (ie organic phase from a liquid/liquid extraction)

analyte is water soluble

wash solvents are non-polar (hexane, methyl t-butyl ether etc)

elution solvents are polar (water, methanol, acetonitrile etc)

Non-polar

matrix is aqueous (foods, biological fluids)

analyte is organic soluble

wash solvents are aqueous

elution solvents are organic

Mixed mode

matrix is aqueous (foods, biological fluids)

analyte can be polar, hydrophillic, or hydrophobic

wash solvents are aqueous and organic

elution solvents are organic

sorbents are either mixed silica (such as C8/SCX) or polymer

Retention Mechanisms

Cation exchange

matrix is aqueous (foods, biological fluids)

analyte is basic (cationic)

wash solvents are aqueous

elution solvents are :

- high ionic strength,
- pH is increased above the pKa of the target compound,
- competition with a cation (such as Na^+) with greater affinity for the sulfonic acid

Anion exchange

matrix is aqueous (foods, biological fluids)

analyte is acidic (anionic)

wash solvents are aqueous

elution solvents are :

- high ionic strength,
- pH is increased below the pKa of the target compound ,
- competition with an anion (such as SO_3^-) with greater affinity for the positively charge amine

Sorbent Choices

Non-Silica

Normal Phase

Reverse Phase

Polymers

Silica vs polymer

Silica Pros

Wide variety of modified silica sorbents

Inexpensive

Many validated methods

Silica Cons

Fines are more prevalent with irregularly shaped particles as fines are frequently originated by particle particle interaction after the cartridge is packed

Sensitive to some solvents and pH conditions. Below pH 2 and above pH 8 the sorbent will rapidly deteriorate

Polymer Pros

Mixed mode retention mechanisms

- wide variety of compounds will be retained

Mixed mode wash steps

- wash with high aqueous to remove hydrophilic impurities

- wash with high organic to remove non-polar impurities

Polymer Cons

Fewer modified resins

Non-Silica

- Carbon
- Florisil PR
- Alumina (acid, base, neutral)
- Silica

Normal Phase

- **Popular Types**: Diol, amino, cyano, bare silica
- **pH range** – 2 – 7.5 however, for the single use SPE cartridges higher or lower pH's may be used but are not optimum
- **Particle shape** – irregular
- **Matrix is** – non-polar
- **Target compound is** – polar (amine, hydroxyl, carbonyl, aromatics, sulfhydryls, rings containing heteroatoms)
- **Frequently used** – when a pre-treatment step results with the target analyte in a non-polar solvent, such as with liquid-liquid extraction

Normal Phase - Tips

- No equilibration of cartridges required. Condition the cartridge with the same organic solvent as the sample
- Acceptable non-polar loading and washing solvents are: hexane, chloroform, methyl-t-butyl ether
- Acceptable polar elution solvents are:
- Tetrahydrofuran, ethyl acetate, isopropanol, acetonitrile and methanol as long as they are miscible with the loading/washing solvents

Reverse Phase Bonded Silica

- **Popular Types**: C18, C18 endcapped, C8, C2, phenyl, mixed mode (C8/SCX)
- **pH range** – 2 – 7.5 however, for the single use SPE cartridges higher or lower pH's may be used but are not optimum
- **Particle shape** – irregular
- **Matrix is** – aqueous
- **Target compound is** – non-polar
- **Key to success** – Adequate solvation of the functional groups prior to sample loading

NEW! Mixed Mode SPE Cartridges

C8/SCX (Octyl/Sulfonic acid)

- Mixed mode phase retains neutral and amine-containing compounds while allowing carboxylate groups to pass through

Multi-functional polymers —

- OPT non-polar resin extracts acidic, neutral and basic compounds in a single SPE bed
- pSCX cation exchange resin extracts basic and neutral compounds
- pSAX anion exchange resin extracts acidic and neutral compounds

Polymer Resins

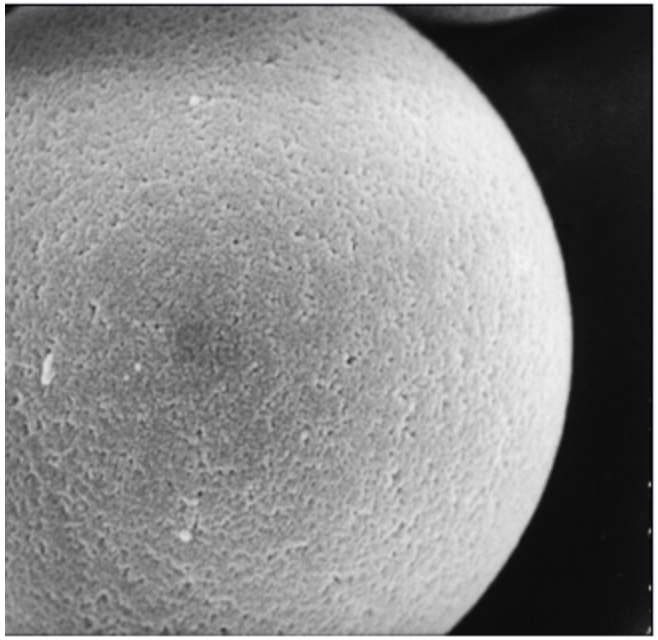
PS-DVB

SampliQ OPT

SampliQ pSCX

SampliQ pSAX

What is the SampliQ Polymer?



Features:

- **High retention, outstanding recovery, and excellent reproducibility**
- **High sorbent robustness: if cartridges accidentally go dry during the SPE process, you will not risk losing analytes and/or reproducibility**
- **No leaking bonded phases or other leachables that can contaminate valuable extracts**
- **Compatibility with most organic solvents and aqueous solutions over a pH range of 0 to 14**
- **Spherical particles and narrow size distribution, which ensure reproducible flow characteristics**

Specifications:

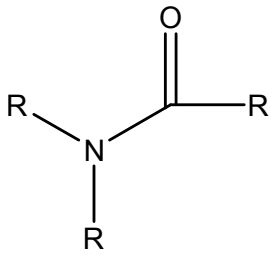
- **spherical particles 25-35µm**
- **pore size 1000Å**
- **defined ion exchange capacity**
- **particle quality controls**
  - •Electrozone sensing (size)
  - •Light microscopy (shape)
  - •Nitrogen adsorption (porosity)
  - •Mass Spectrometry (contaminants)

Resin Quality Controls:

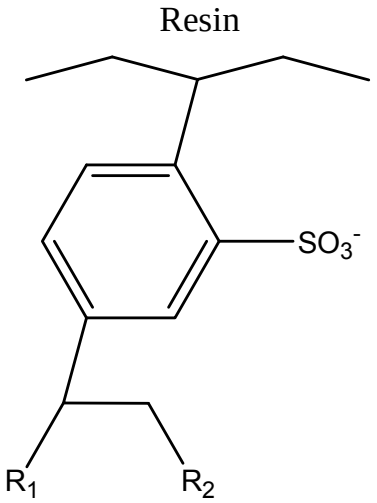
Packed Cartridge Test

- •Cartridge Purity Test (GC)
- •Frit Purity Test (GC)
- •Material Weight Check
- •Cartridge Flow Resistance
- •Extraction Residue (%)
- •Turbidity (NTU)

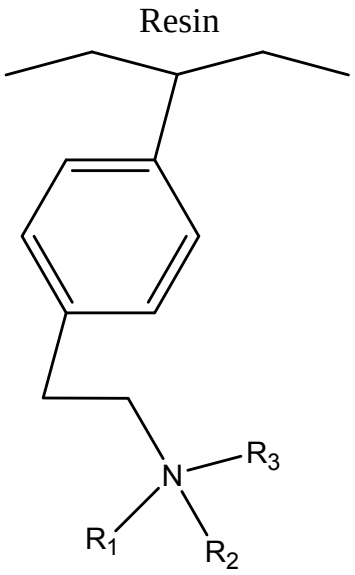
SampliQ Polymer Line



OPT



SCX Resin



SAX resin

OPT
- a novel polyamide polymer
- has affinity for both hydrophobic and hydrophillic compounds

SCX
- a sulfonic acid modified divinylbenzene polymer
- has mixed-mode affinity for both basic and neutral compounds

SAX
- a tertiary amine modified divinylbenzene polymer
- has mixed-mode affinity for both acidic and neutral compounds

Polymer Cartridge Selection Guide

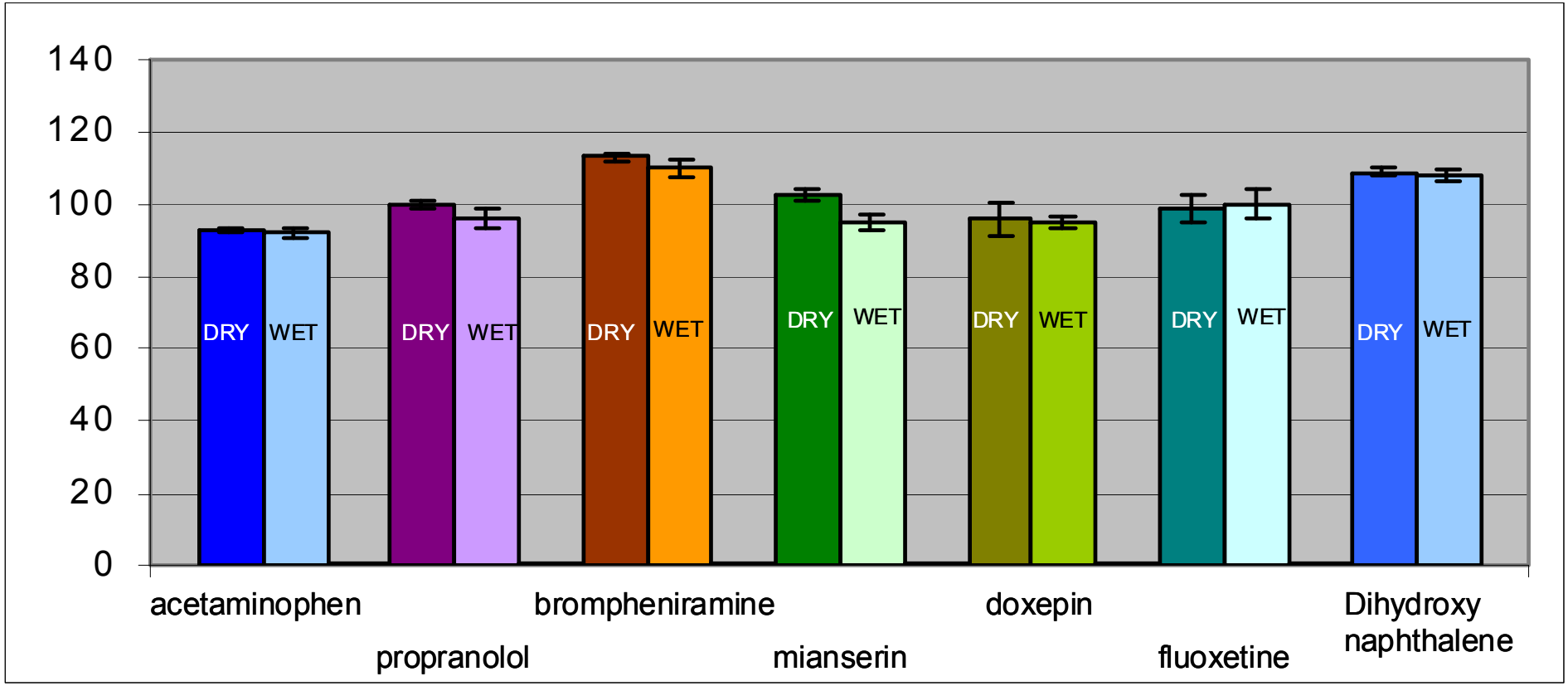
pKa 1-7	pKa 4-10	pKa 6-14
Acidic Compounds	Acid/Base/Neutral	Basic Compounds
SampliQ-SAX	SampliQ-OPT	SampliQ-SCX

All of Agilent's polymer phases exhibit mixed-mode behavior. This characteristic results in the ability to retain target molecules over a wide range of pKa's in any cartridge type. As a starting place for method development, the chart above can be used to identify the cartridge type for any application.

What does it mean for a sorbent to be ‘Water-Wettable’?

- Water-wettable refers to a polymeric surface which incorporates polar groups.
- The advantage of a water-wettable polymer is that if the cartridge accidentally goes dry during sample prep., the surface remains active toward analytes even if an aqueous solution is applied.

Polymer Performance is Robust



- Highly reproducible recoveries wet or dry
  - Cartridges dried under vacuum for 10 minutes before the equilibration step
- RSD's of the recoveries for each of the compounds (n=5) very low
- Compounds range from very polar, basic compounds to hydrophobic, neutral

Flow-rate - speed kills

Applies to ALL sorbents

Flow rate is critical. Too fast a flow will not give the compounds of interest time to interact with the sorbent. Irreproducible flow results in poor RSD's

General SPE Method Development Strategy
SPE Adsorption Mode: Background

Research the Problem

- Previous SPE and analysis conditions for the analyte and matrix?

Characterize the Analyte

- Structure, pK_a, polarity, functional groups
- Solvent solubility and stability
- Any restrictions on final solvent and concentration (technique or instrument)?

Characterize the Sample Matrix

- Possible interferences — similar functional groups, pK_a, etc.
- pH, ionic strength
- Solvent solubility and stability
- Qualitative and quantitative variability

General SPE Method Development Strategy

SPE Adsorption Mode: Experimental

Develop or apply effective HPLC or GC conditions to monitor progress

- Assess recovery and eluate cleanliness

Select and test sorbents

Determine which sorbents provide maximum analyte retention

- Determine which eluent solvents yield highest recoveries

Identify optimum wash solvent

- Assess eluate cleanliness under conditions of maximum analyte retention
- Determine strongest wash solvent that will not elute analyte

Test blank and fortified matrix

- Assess eluate cleanliness and recovery using optimum wash and eluent solvents

Test real samples and fortified samples

Sorbent Selection – Screen several cartridge types – saves time in the long run.

For a compound which contains cationic and non-polar character, screen an OPT, silica C18, silica c8, polymer SCX, mixed mode silica (C8/SCX) or non-polar mixed mode polymer.

Condition and equilibrate as appropriate for selected sorbent

Put matrix spiked sample on all cartridges, use generic protocol for each sorbent. Evaluate all fractions: flow-through, wash, eluent for presence of compound

Using sorbent showing best retention, use solvent standards to find best elution solvent. (keep a record of solvents which do not elute the compound as they may be excellent wash solvents)

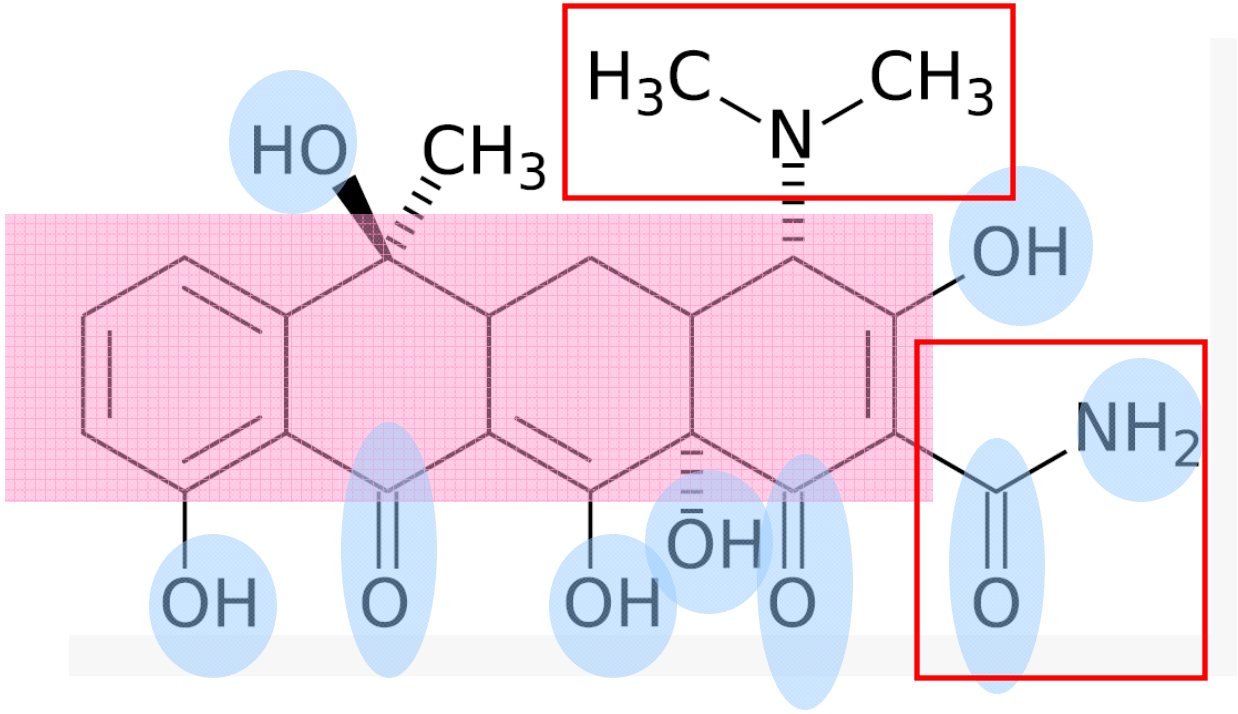
Using matrix spiked samples use several wash solutions with the selected elution solvent and see which one gives the cleanest eluent without losing analyte.

Remember the goal here is **Adequate Recovery** and **Sufficient Cleanliness**, not necessarily perfection! Ruggedness and reproducibility are more important

Take the time to develop the method right the first time

There is not going to be a single answer, the experiments need to be performed, the procedure needs to be optimized prior to adoption

Analyte Assessment - Tetracycline



= non-polar

= polar

= ionizable

pKa = 3.3 / 7.7 / 9.7
Log P = -1.3

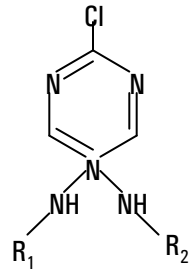
SampliQ Example Applications
Triazines in Different Matrices

Characterize the Analyte

- Structure, pK_a, polarity, functional groups
- Solvent solubility and stability
- Any restrictions on final solvent and concentration due to technique or instrument?

- **Characterize the Sample Matrix**
  - Solvent solubility and stability
  - pH, ionic strength
  - Possible interferences—similar functional groups, pK_a, etc.
  - Qualitative and quantitative variability

Triazines



- Three major species – simazine, atrazine and propazine – all structurally similar
- Mode of Action: Herbicides
- Practically insoluble in water

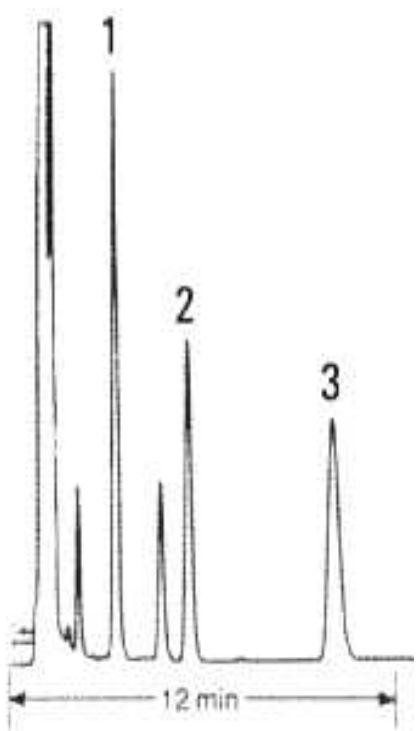
- Soil – large number of charged species-adjust pH to retain triazines and do ion-exchange
- Muscle tissue – large amounts of non-polar lipids – retain these and elute triazines using C18
- Corn oil – non-polar glycerides and fatty acids –weakly retained on diol while triazines are strongly retained

SPE Methods for Triazines in Complex Matrices

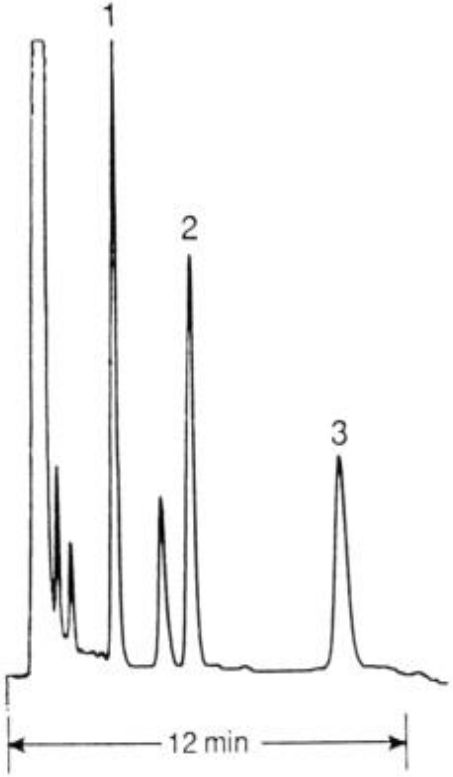
	Soil	Muscle Tissue	Corn Oil
CARTRIDGE	SCX	C18/OPT	Diol
EXTRACTION	Shaken in acetonitrile	Homogenized in methanol	None
PRE-TREAT	Acetic acid	Methanol	Methanol, hexane
LOAD	Diluted with acetic acid	Diluted with water	Diluted with hexane
WASH	Acetic acid, acetonitrile, water, 0.1 M K_2HPO_4	Water	Hexane
ELUTE	Acetonitrile/K_2HPO_4	Methanol	Methanol

HPLC Analysis of Triazines Extracted from Different Matrices

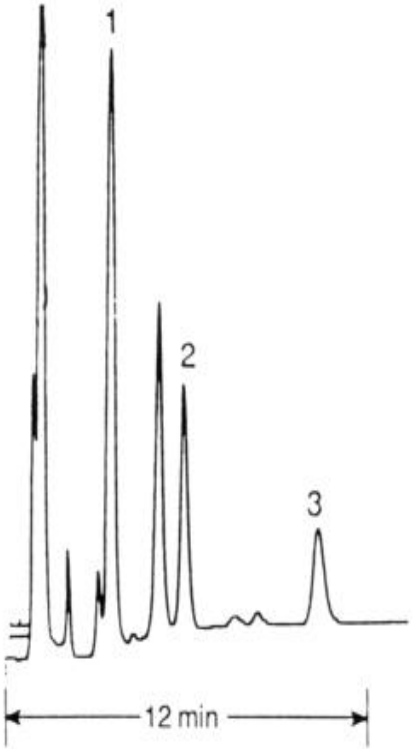
Triazines from Soil with SCX



Triazines from Muscle Tissue with C18



Triazines from Corn Oil with Diol



nm, 5 μm Mobile Phase: 50% methanol:50% 0.01M K_2HPO_4 Flow Rate: 2 mL/min

Detection: UV 254 nm Sample: Triazines 1. Simazine 2. Atrazine 3. Propazine

Troubleshooting Sample Prep.

Problems areas:

1. Poor recovery
2. Poor reproducibility
3. Insufficiently clean

1. Poor Recovery

Determine where the analyte is lost

A. Sample pretreatment

B. Load step and/or Wash

C. Not eluting

D. Matrix effects

A. Pre-treatment Losses

Protein binding

Compounds may be lost in a protein removal pretreatment step (ultrafiltration, protein precipitation etc) or when the bound compound flows through with the protein during the load. In either case it is not possible to detect the compound in any fraction.

Chemically disrupt the protein-analyte bond, acidify (0.1M HCl or concentrated phosphoric acid), use an organic solvent or chaotrope. The chemicals used must be compatible with the sorbent.

Then precipitate proteins or dilute prior to load

Sample instability

change the handling conditions at all stages to maximize compound lifetime.

B. Load and Wash Losses

Check cartridge conditioning and equilibration effects

For ion exchange, the counter ion should be in a consistent form (for SCX equilibrate with 2% formic acid in water for SAX equilibrate with 50mM ammonium acetate)

For OPT, if the target compounds are bases and neutrals, raise the pH of the aqueous solution, lower the pH of the equilibration solution.

Check the load solvent,

For ion exchange appropriate pH, low ionic strength

Check the wash solvent,

Decrease the volume of wash

Decrease the % organic

Adjust the pH or ionic strength

Is the matrix affecting the capacity of the cartridge by binding all the active sites on the sorbent

C. Irreversible Binding to Sorbent

Elution volume may not be optimized

Solvent strength may not be sufficient

Try a ‘soak’ step (2 minutes)

Change the pH, ionic strength

Try a less retentive sorbent (i.e. change from C18 to C8)

Easy steps to optimize recovery on OPT

When a method is good but you want to optimize here are a few easy modifications to try:

Change the elution solvent (isopropanol or acetonitrile instead of methanol)

Change the composition of the elution solvent (decrease and/or increase the % water in the elution solvent)

Change the pH of the elution solvent (0.1% formic acid for enhancing neutral and base compound recovery on OPT)

Add salt to the elution solvent (20mM ammonium acetate for OPT)

Change the volume of solvent used (elution and wash solvents)

Test fewer or more wash steps with different strength solvents

Change the pH of the wash solvent (increase and decrease pH compared to current pH)

D. Matrix effects

- Insufficient capacity
  - Using a larger bed volume does not improve the retention of compound (indicates that a capacity factor is not the problem)
- Matrix effects:
  - In spiked matrix the compound is not found in the eluent or flow-through (suggests compound is protein bound or compound is not separated from the matrix i.e. chelated compounds such as tetracyclines with Ca in milk)
- Insufficient homogenization

2. Poor Reproducibility

Analytical stability – make sure the instrumentation is not contributing to the problem

Matrix interferences

Ion suppression

Protein binding

Lot to lot variability – the key here is the supplier

Inconsistent flows

3. Insufficiently Clean

Symptoms of a sample which needs further clean-up are:

- poor reproducibility
- column failure (high backpressure, changing retention times)
- incorrect quantitation on QC samples

3. Insufficiently Clean

Fixes:

✓try a different sorbent which uses the same retention mechanism (i.e. try silica C18 end capped instead of silica C18)

✓change to a different retention mechanism sorbent

✓change the wash solvent

✓change the ionic strength (when using ion exchange sorbents)

✓change the pH of the load and wash

✓change the % organic of the load and wash

✓Try multiple wash steps

3. Insufficiently Clean

Fixes

- ✓multiple wash steps
- ✓add an acidified wash if protein binding is a problem
- ✓use a low % organic followed by higher % organic if protein precipitation is a problem
- ✓add a wash with a solvent that the sample is insoluble in but that may clean out interferences
- ✓change the elution solvent
- ✓using a weaker elution solvent

Solvent strength

Stronger elution solvents for normal phase

Water

Methanol

Acetonitrile

Isopropanol

Ethyl acetate

Tetrahydrofuran

Stronger elution solvents for reversed phase

The hazards of generic protocols

Requirements:

The sorbent must be capable of retaining a wide variety of analytes

The wash steps remove enough of the interferences to achieve the detection limits without sacrificing the columns etc

The elution solvent cleanly removes the target molecules

Limitations:

A sorbent that retains many different analytes will tend to retain many different interferences as well

Gentle wash steps tend to leave interferences as well as the target compounds

Strong elution solvents bring off more interferences along with the target compounds

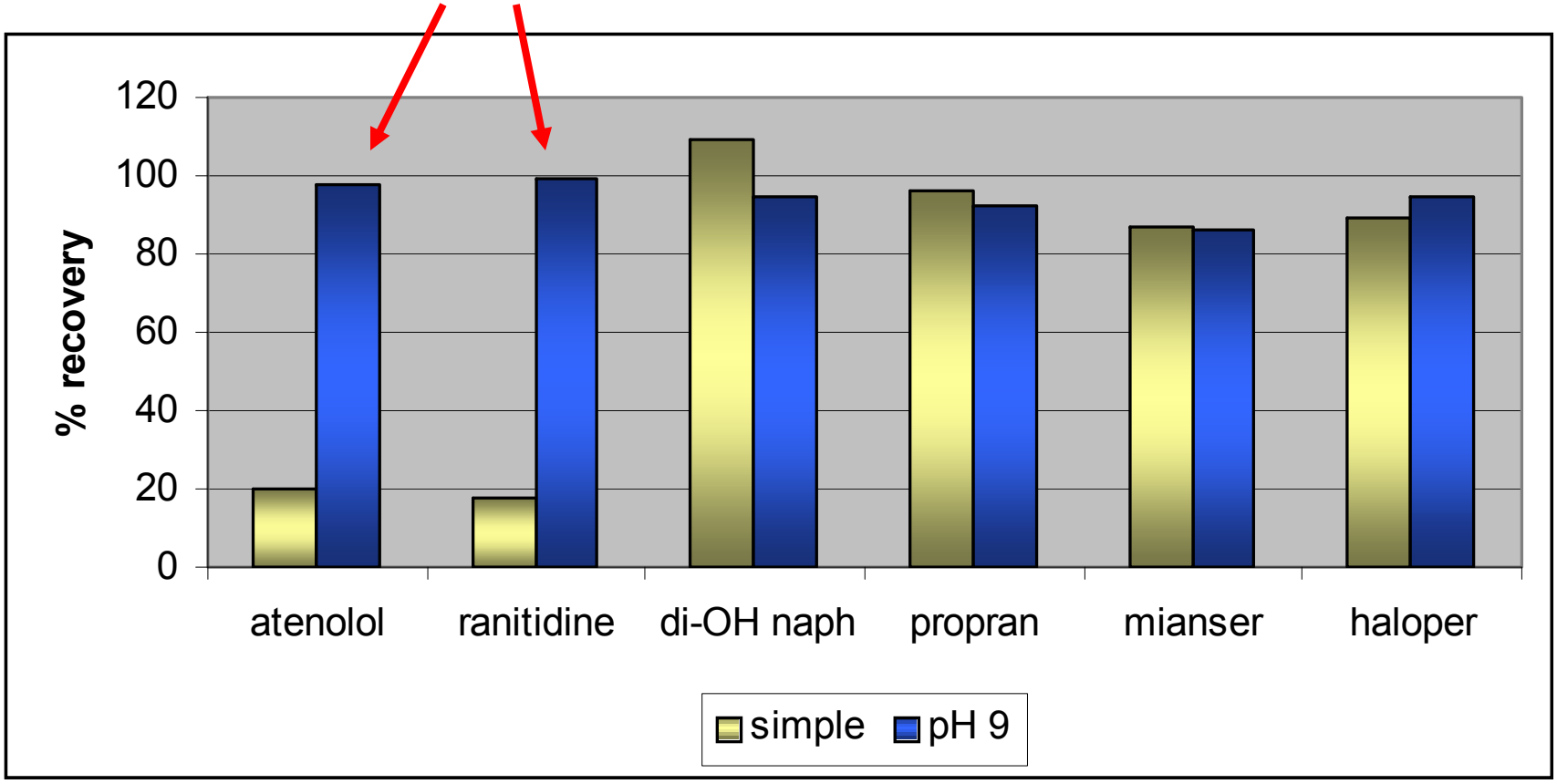
Retention Factors and Physical Character

Very hydrophilic

	K'	pKa	Log P	Base/Neutral	Retention on X-treme
Procainamide	**0.3**	2.6--9.2	1.3	Base	
Atenolol	**0.9**	9.6	0.2	Base	
Ranitidine	**1.0**	2.4--8.2	1.9	Base	
Acetaminophen	1.2	4.4--9.4	0.9	Neutral	
Caffeine	2.4	10.4		Neutral	
Quinidine	2.9	8.6	2.5	Base	
Toluamide	3.2		1.4	Neutral	
Toluidine	3.3			Neutral	
2,7-dihydroxy naphthalene	3.6			Neutral	
Propranolol	4.0	9.5		Base	
Brompheniramine	4.1		3.7	Base	
Mianserin	4.1	7.0	3.8	Neutral	
Doxepin	4.3	8.0	4.2	Base	
Haloperidol	4.5	8.7	4.4	Base	
Fluoxetine	5.1	8.7	5.4	Base	
B-methasone-13-valerate	5.2			Neutral	
Di-propyl-phthalate	6.4			Neutral	

Effect of changing conditioning and wash solvent

Raising the pH above the pKa makes the compound neutral and results in dramatically improved recovery



Questions??

Appendix

Quality controls

The Problem: Fines

• that is particles which have diameters smaller than the pores in the frits. Presence of fines can be detrimental to the performance of the cartridge, show batch to batch irreproducibility and affect the final sample. Fines are associated with an increased occurrence of channels.

The Solution: Quality

• Rigorous testing of base silica and bonded silica

• Bonding chemistry

SampliQ Bonded Silica Products – other quality measures

- Tri-functional bonded surfaces – results in a higher carbon load and fewer active silanol surface groups.
- Vacuum-packed cartridge bags
- Manufactured in Delaware right beside Zorbax manufacturing
- Certificate of Performance Shipped with each box of cartridges
- Material Safety Data Sheets (MSDS) available on-line

SampliQ C18 Certificate of Performance Front Sheet

Certificate of Performance for Packing Lot

Agilent Technologies

DESCRIPTION: SampliQ C18
PACKING LOT #: SPE0840203

BASE SILICA PROPERTIES

Surface Area	555 m²/g
Average Pore Size	60 Å
Particle Shape	Irregular
Average Particle Size	45 µm

BONDED SILICA PROPERTIES

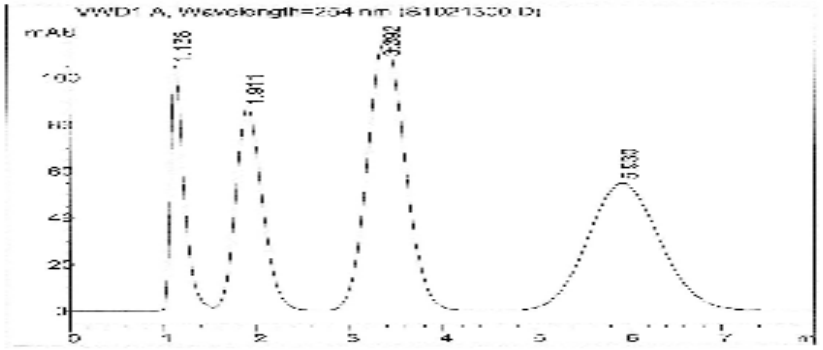
Carbon Loading	23.9%
Surface Coverage	2.9 µmoles/m²

TEST CONDITIONS

Column	=	4.6 x 250mm
Mobile Phase	=	80% Methanol / 20% Water
Column Flow	=	2 ml / min
Temperature	=	Ambient (Nominally 25 °C)
Injection Volume	=	5 µl

QUALITY ASSURANCE PERFORMANCE RESULTS

	TEST VALUES	SPECIFICATIONS
Retention Time Acetophenone =	1.81	Range = 1.6 - 2.1
Retention Time Toluene =	3.39	Range = 3.0 - 3.6
Retention Time Biphenyl =	5.93	Range = 5.5 - 6.3
k' Toluene =	2.0	Range = 1.7 - 2.3
k' Biphenyl =	4.2	Range = 3.7 - 4.7



Sample components with concentrations diluted in mobile phase in the following elution order.

Peak #	Conc (ug/ml)	Sample Component
1	50	Uracil
2	100	Acetophenone
3	500	Toluene
4	100	Biphenyl

MATERIAL SAFETY DATA SHEETS ARE AVAILABLE UPON REQUEST.

APPROVED

Quality Control Manager

SampliQ C18 Certificate of Performance Back Sheet

Description:	Agilent SampliQ C18 6ml, 500 mg cartridge
Catalog No.:	5982-1165
Run No.:	0006033013
Packing Lot No.:	SPE0840203
Contents List:	6 sealed multilayer bags containing 5 cartridges in each bag; total order supplied within – 30 cartridges

This Agilent SampliQ product and sorbent have been manufactured, tested and assembled under the control of an ISO 9001 registered quality system.

This Agilent SampliQ product has been subjected to the following Q.C. tests:

Packed Cartridge Test:

Cartridge Purity Test (GC)	Pass
Frit Purity Test (GC)	Pass
Material Weight Check	Pass
Cartridge Flow Resistance	Pass
Extraction Residue (%)	Pass
Turbidity (NTU)	Pass

SampliQ Bonded Silica Products – support features

- Generic methods to get customer's started
- Technical support and marketing communications program-worldwide
- Cross-references of competitive phases

Checklist for Selecting a Sample Preparation Method

✓ Know the Identity and chemical properties of the analytes and potential chemical forms

✓ Know the concentration range(s) of the analytes and the detection limit requirements

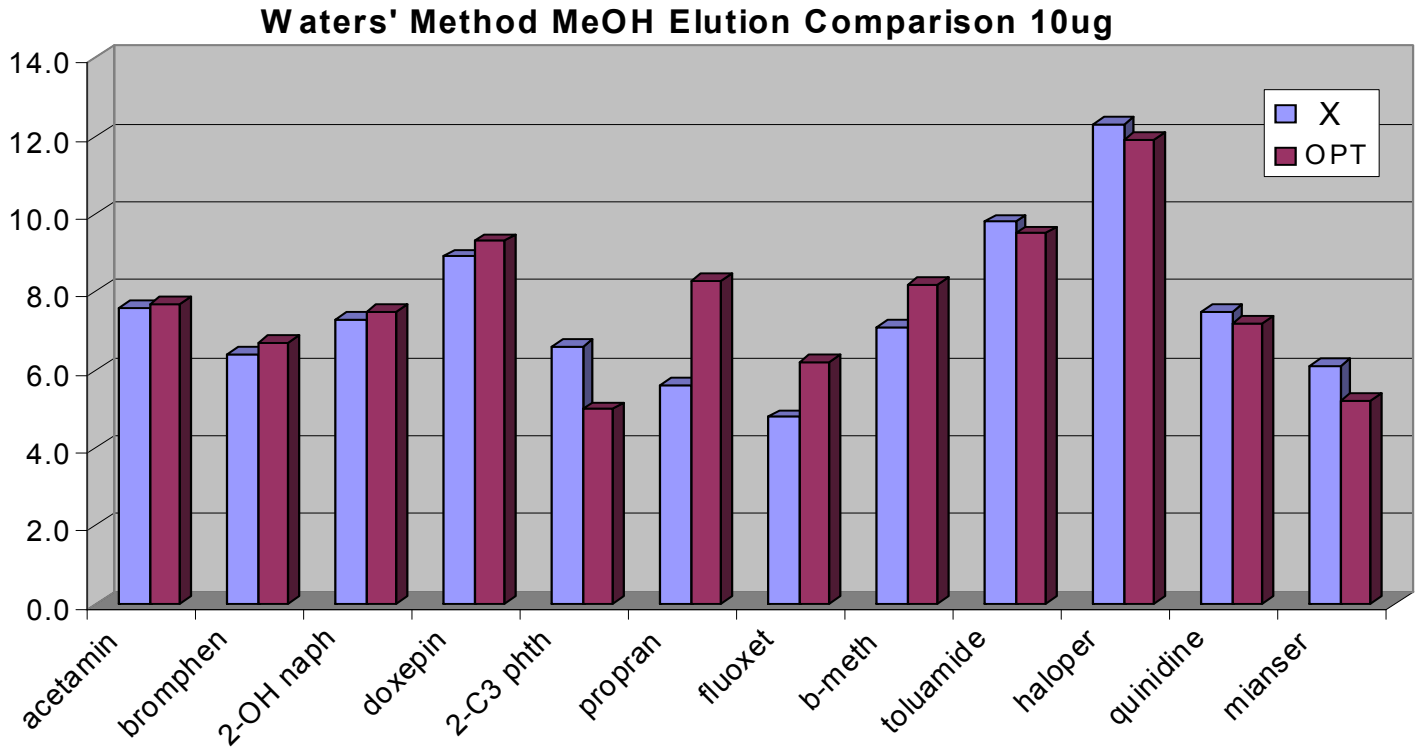
✓ The chemical and physical composition of the sample matrix

✓ The availability of apparatus and equipment

✓ The sample size this is available or required

✓ The potential for contamination during some part of the sample preparation process

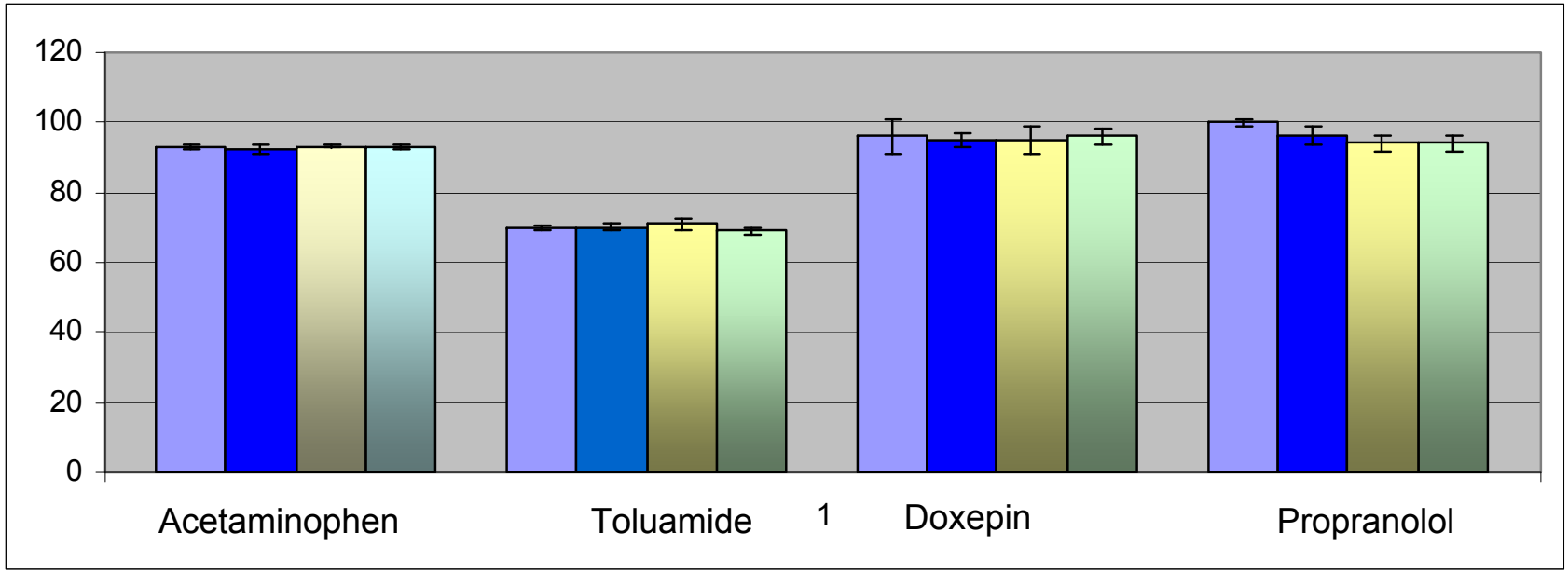
SampliQ OPT and Brand X with Drug Compounds



- **For compounds with a wide range of chemical properties (solubility, pKa), SampliQ OPT performs similarly to brand X using the *Waters* "simple" SPE method**

- **For strongly hydrophilic compounds that are not well retained, minor pH modification of the conditioning, wash and elution solvents Can significantly improve performance**

Wet and Dry Recovery Data, SampliQ-OPT and HLB Mix 2 (n=5)



SampliQ-OPT Dry

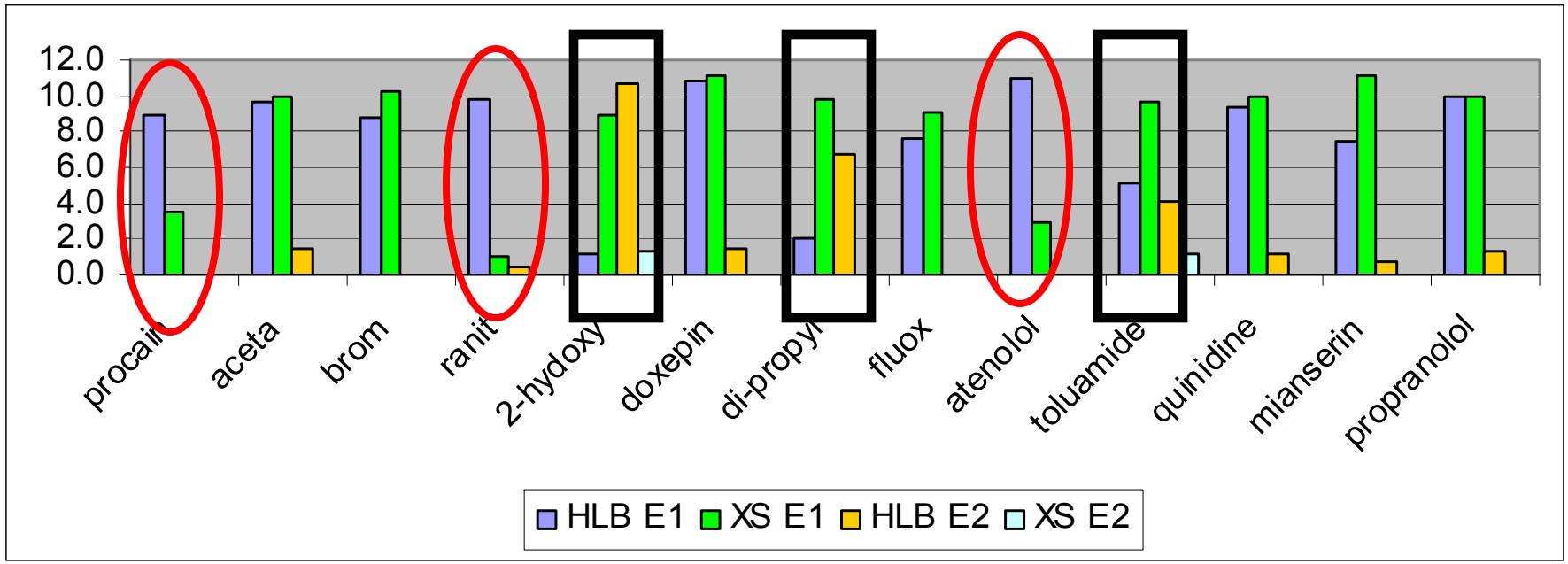
SampliQ-OPT Wet

HLB Wet

HLB Dry

- SampliQ-OPT and HLB perform identically on these compounds.
- Wet and Dry Recoveries are equivalent within the RSD
- In the patent work, Doxepin showed recoveries of 83%, in my hands recoveries on HLB and OPT were 96%
- In the patent work, Toluamide showed recoveries of 99%, in my hands recoveries on HLB and OPT were 70%

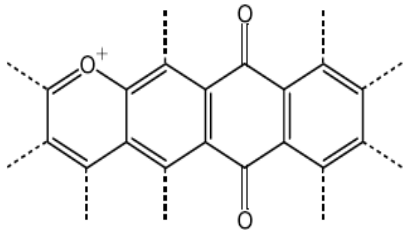
Results, E1 and E2 Analyses – All compounds
0.1% HOAc/MeOH eluent



- Procainamide shows 30% recovery on OPT, 100% recovery on HLB
- Ranitidine shows 10% recovery on OPT, 100% recovery on HLB
- Atenolol shows 20% recovery on OPT, 100% recovery on HLB
- 2-hydroxy naphthalene and di-propyl phthalate show 100% recovery on OPT while HLB requires twice as much elution solvent to reach 100% recovery
- 10 of the 13 compounds tested on OPT perform better than or equivalent to HLB

NEW! Graphitized Carbon SPE Cartridges and Bulk

What is graphitized carbon?



- Pure carbon sorbent with some aromatic character (π-π interactions) and small positive charge on surface due to oxonium group (weak anion exchange properties)

- Characteristics:
  - Homogeneous, non-porous particles, 38-125-um
  - Surface area: 100 m^2/g
  - Mostly functions as reversed-phase medium & retains non-polar compounds
  - Also retains polar organics in aqueous matrices, esp. plant extracts
  - Suited for extraction of acidic, basic and neutral pesticides and herbicides

- Potential applications
  - VOCs in air
  - N-Glycans from aqueous protein-containing samples & plant extracts
  - O-Linked oligosaccharides from protein-containing samples
  - Estrogen steroids from waste water
  - Mycotoxins in fish tissue
  - Herbicides in soybeans

General Extraction Protocol for SampliQ Carbon (200 mg Bed Size)

(Use flow rate of about 5-mL/min)

Conditioning: 5-mL CH_2Cl_2-MeOH (80:20) followed by 2-mL MeOH, & 5-mL Deionized water, pH 2 with HCl

Loading: Pass sample through bed

Wash: 5-mL of deionized water; dry tube by passing air for 2-5 min

Elution: Elute basic and neutral compounds with 1-mL of methanol followed by 5-mL of CH_2Cl_2-MeOH (80:20); elute acidic compounds with 5-mL of CH_2Cl_2-MeOH (80:20) containing 20-mM tetramethylammonium hydroxide.

Volume reduction: reduce volume of extract and re-constitute with appropriate solvent for LC or GC analysis

Recovery and Reproducibility for SampliQ Carbon (using Internal Standard)

Using generic protocol for the pesticide Bromacil

At levels of 1.48 and 1.76 ug/mL (n = 3)

- Average % Recovery: 103.6%
- Relative standard deviation: 2.6%

Typical results for competitive carbon SPE phases:

- Average % Recovery: 100.2-101.6
- Relative standard deviation: 2.1-14.3%