SPE and QuEChERS – Method Development

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Today's Agenda

1. QuEChERS Workflow overview and original methods
2. Method development for alternative matrices
3. SPE for polar compounds
4. SPE for non-polar compounds
5. SPE for ionic compounds
6. Questions

Filtration and Other Sample Preparation Techniques

	More Specific ← **Instrument Separation and Detection Specificity** ← *Less Specific*								
	Less Specific → ***Sample Preparation Specificity*** → ***More Specific***								
Sample Prep Technique / Interference Removed	**Dilute & Shoot**	**Filtration**	**Liquid/Liquid Extractions**	**Supported Liquid Extractions (SLE)**	**Dried Matrix Spotting**	**Precipitation**	**QuEChERS**	**Lipid Removal 'Hybrid' Filtration**	**Solid Phase Extraction**
Lipids	No	No	No	Some	No	No	Yes	Yes	Yes
Oligomeric Surfactants	No	No	No	No	No	No	No	Yes	Yes
Particulates	No	Yes	No	Some	No	Yes	Yes	Yes	Yes
Pigments	No	No	No	Some	No	No	Yes	No	Yes
Polar Organic Acids	No	No	Yes	Yes	No	No	Yes	No	
Proteins	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Salts	No	No	Yes	Yes	No	No	No	No	Yes
Suggested Agilent Product	Agilent Autosampler Vials	Captiva Syringe Filters		Chem Elut	Bond Elut DMS	Captiva ND	Bond Elut QuEChERS	Captiva ND LIPIDS	Bond Elut Silica and Polymeric SPE
Agilent Captiva Filtration Products are recommended for use with any LC or LC-MS method									

What is QuEChERS (pronounced "*Catchers*")

Quick, **E**asy, **Ch**eap, **E**ffective, **R**obust and **S**afe

- Developed jointly by USDA and EU Food Regulatory Agencies as a sample preparation method for multi-residue analyses
- Simplified extraction and cleanup approaches that reduce use of expensive and/or dangerous solvents
- Originally for preparing fruits and vegetables for pesticide analysis
- Rapidly being extended to other matrices and compound classes





Time = Money?

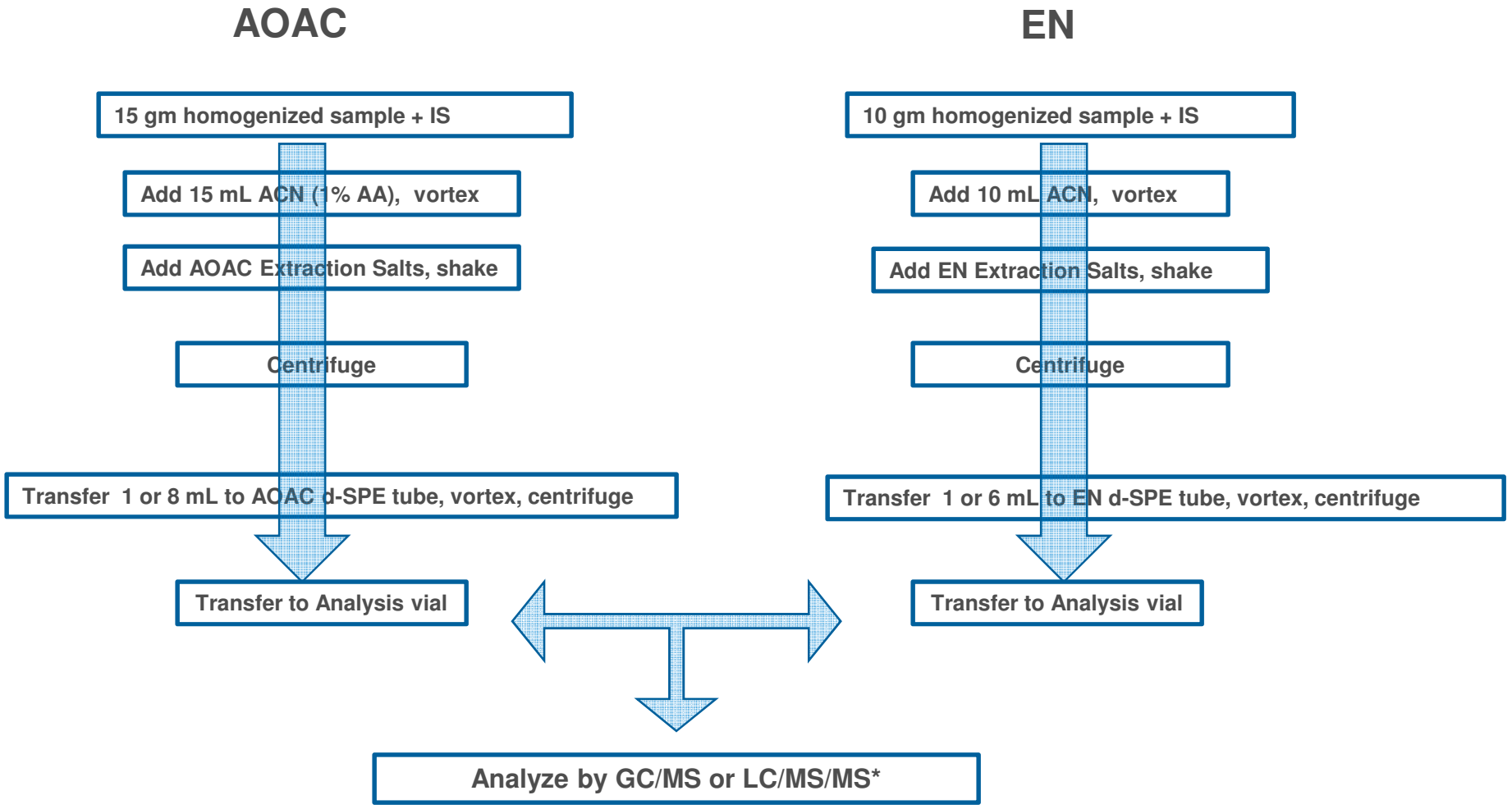
	Luke method, traditional SPE, or GPC	QuEChERS	QuEChERS Benefits!
Estimated Time to process 6 samples (min)	120	20	6 x faster
Solvent Used (mL) per sample	90 mL	10-15mL	9 x less solvent
Chlorinated Waste (mL)	30 mL	none	safer, greener, less costly
Glassware/ specialized equipment	Clean Separatory funnels, water bath, 200mL containers, evaporator, etc.	None	No additional supplies needed

Significant time savings because lengthy liquid extraction procedures are eliminated!

THE ORIGINAL QUECHERS METHOD

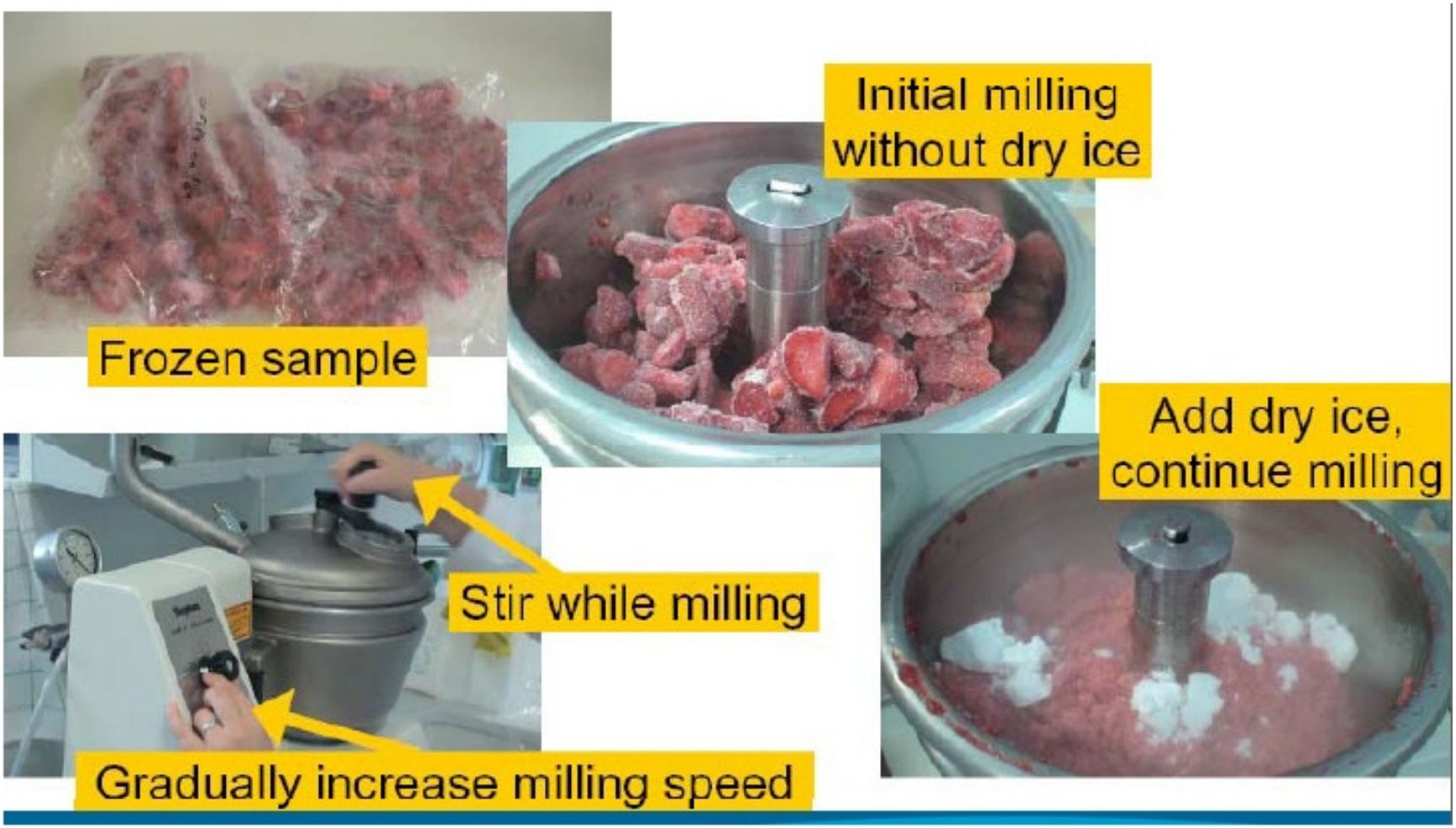
Pesticide Residue in Fruit and Vegetables

QuEChERS Extraction Flow Chart



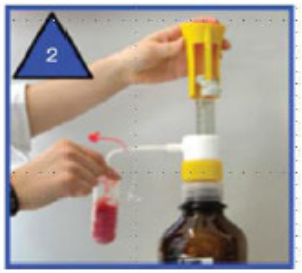
* Requires a dilution prior to analysis

Sample Homogenization – Pre-Preparation

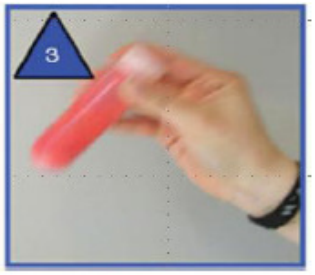


QuEChERS – Easy as 1-2-3

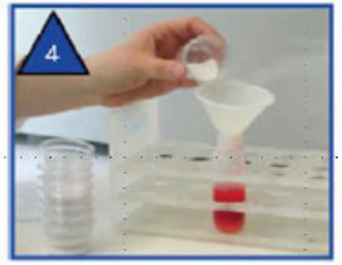
Weigh sample



Add solvent



Shake

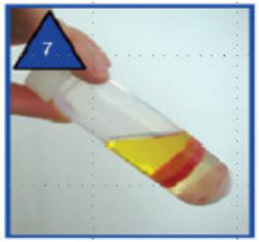


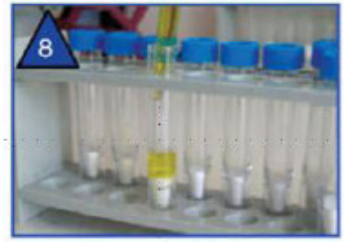
Add salts

Step 1
EXTRACT
Original QuEChERS Method Extraction Kits (available with or without 50 mL centrifuge tubes)

Add internal standard

Shake and centrifuge

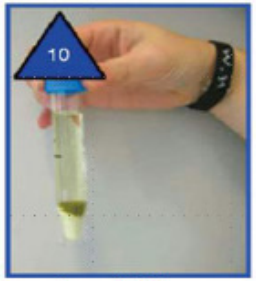




Transfer extract (top) for cleanup

Step 2
CLEAN
Choose a dispersive SPE Kit specific to your matrix

Shake and centrifuge

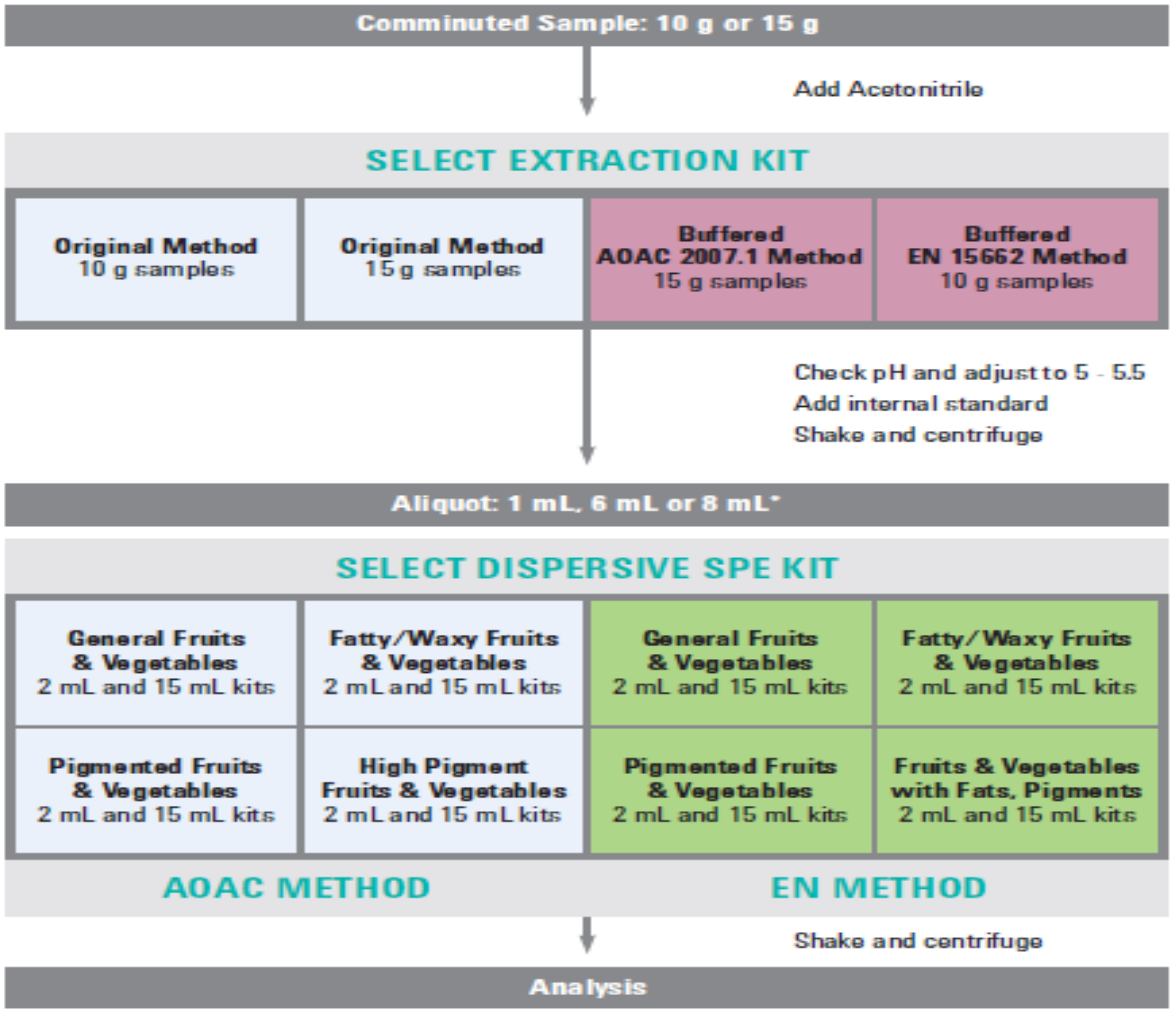


Transfer (dilute or concentrate) to vials

Step 3
ANALYZE
Using 6400 Series Triple Quad LC/MS and 7000 Series Triple Quad GC/MS

LC-GC, **2008**, vol. 11 issue 1

Agilent Tools for Pesticide Residue Analysis



….but I don’t LIKE vegetables!!

ALTERNATIVE MATRICES

Method Development

“Trial and Error” vs. “Educated Guess”

Trial and Error for Extraction Step:

- Only three existing methodologies
- Unpredictability of results
- Eliminates need for bulk salts

Educated Guess for Clean Up Step

- Predictability of results
- Better understanding = less time and $ developing methods!

Optimization Considerations for Juice Concentrates - A Case Study

- Extraction and Dispersive SPE
- Sample amount
- pH variation (Lemon juice is highly acidic)
- AP (analyte protectant)

Juice concentrates are a distinctively challenging matrix due to pH and consistency

Optimization of QuEChERS Procedure: Extraction Salt Selection

- Three variations of the QuEChERS extraction salts were investigated
  - Original, Non-buffered: 4 g $MgSO_4$, 1 g NaCl
  - AOAC: 6 g $MgSO_4$, 1.5 g NaAc
  - EN: 4 g $MgSO_4$, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate

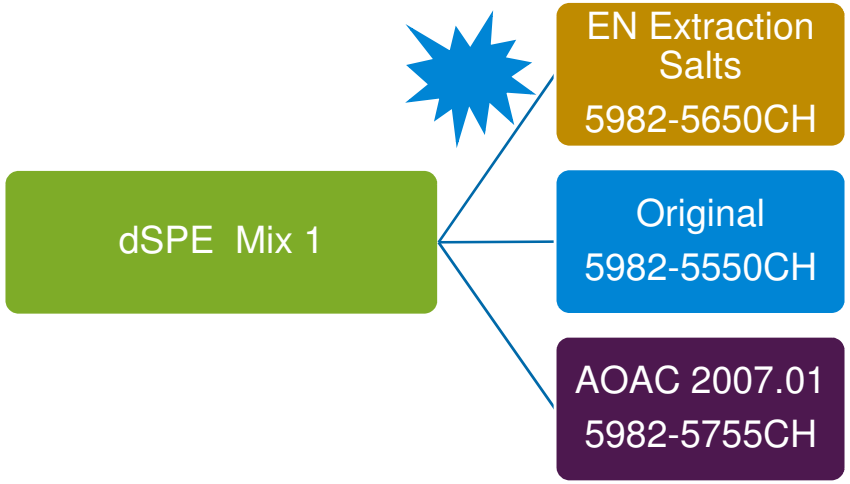
TIP!

Use one dSPE mixture and keep this part the same for the extraction salt optimization

QuEChERS Extraction Optimization Summary

Use one dSPE type with three salt types to identify the best combination for the application

Method Development Products – Fruits & Vegetables



Method Development Process

dSPE Selection: Educated Guess

MgSO4	-	Present in all QuEChERS kits, removes residual water
PSA	-	“Primary/Secondary Amine” scavenges organic acids and sugars, typical matrix component in fruits and vegetables
C18	-	scavenges residual proteins and lipids, amount in kits appropriate for f&v, may need adjustment
GCB	-	“graphitized carbon black”, removes pigments (notably chlorophyll and carotenoids)

dSPE Selection for Juice Concentrate

- EN extraction salt = EN dSPE kit because ratios matter
- No lipids and proteins = no need for C18
- No considerable pigmentation = no need for GCB
- Significant organic acids and sugars

EN Fruits and Vegetables
5982-5021CH

QuEChERS Optimization
- Sample Amount Variation

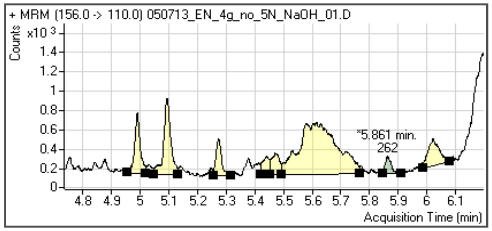
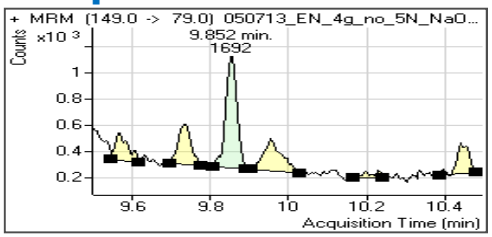
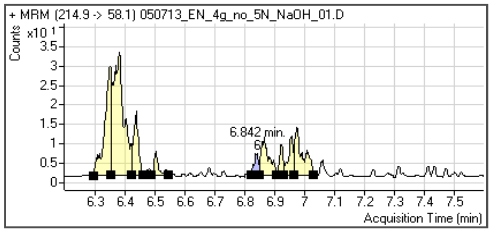
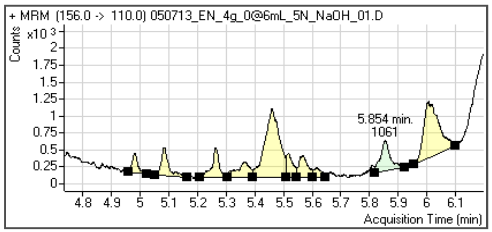
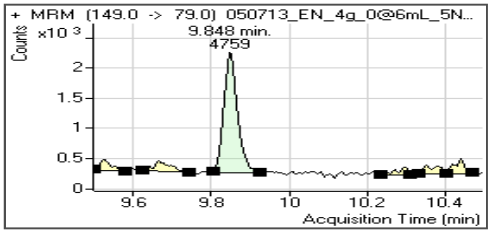
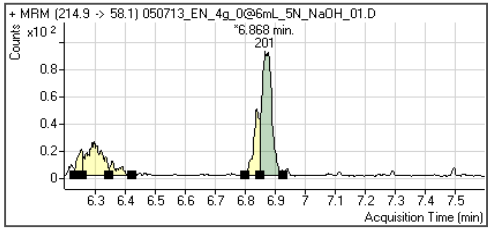
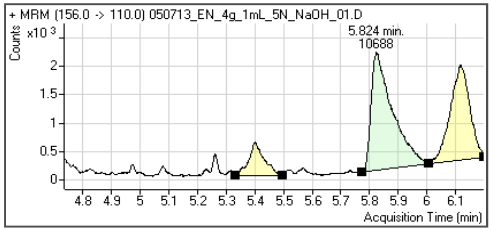
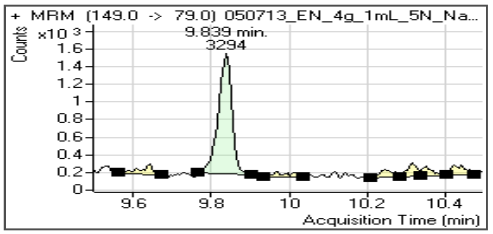
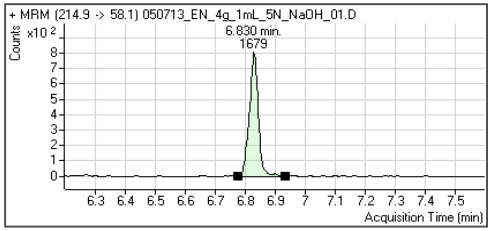
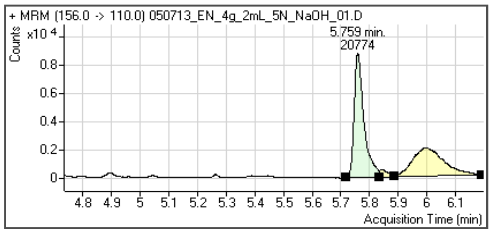
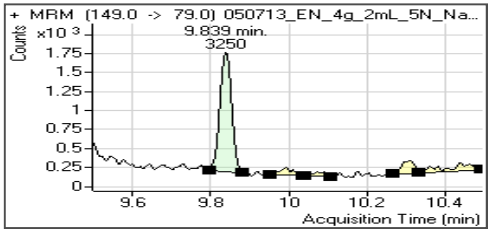
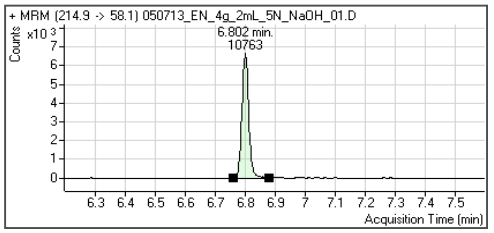
- Overall sample volume (sample plus water) MUST be 10ml or 15ml (EN vs. AOAC)
- Sample amount ↑
  - Extracted compound amount ↑ → helps reaching low detection limits
  - GC-MS/MS contamination ↑ → not desired
- Lemon juice concentrate was spiked at 100 ppb and 3, 5, 7 g of sample loading amounts were tested
- For some compounds (e.g. Dichlofluanid, Tolylfluanid, Captan, Folpet) drastically better response from 2 – 6 times higher when 5 g of sample were used compared to 3 g of sample

→ Optimized method with 4g of sample

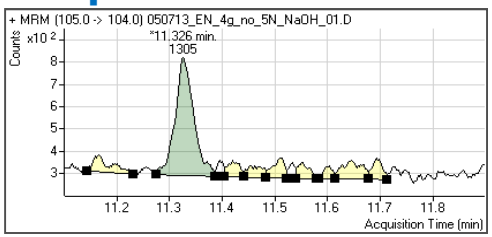
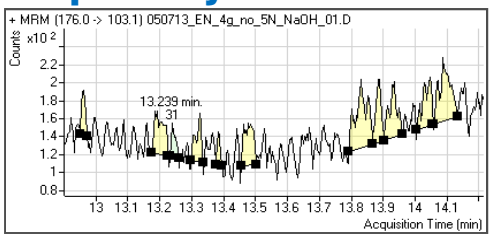
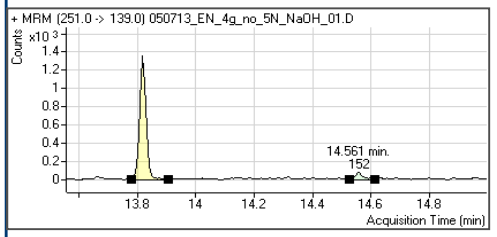
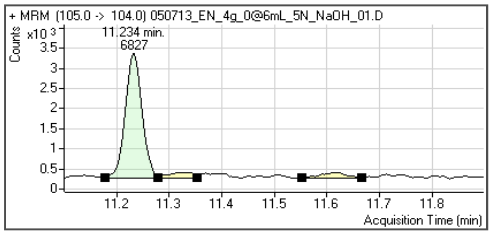
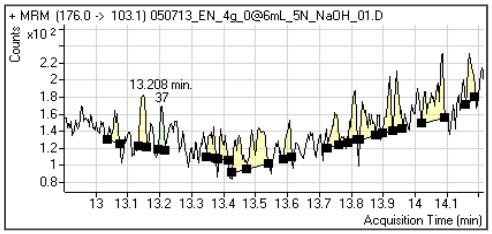
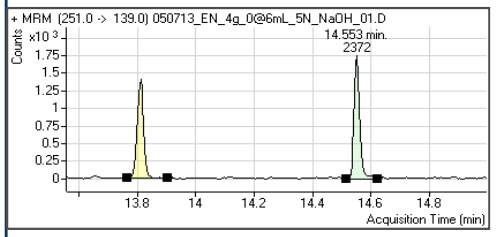
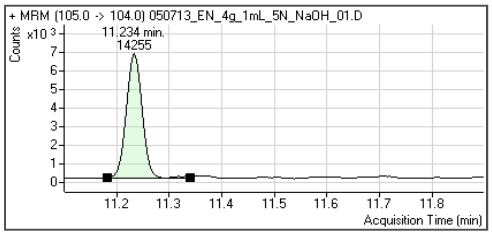
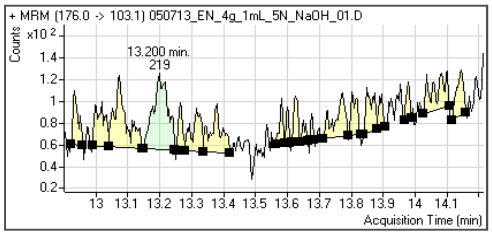
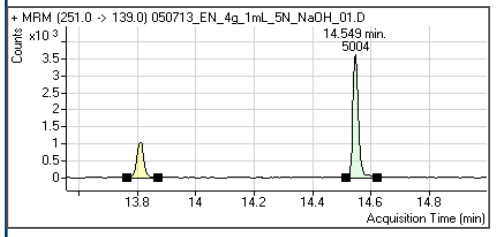
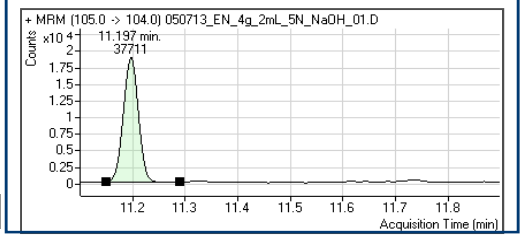
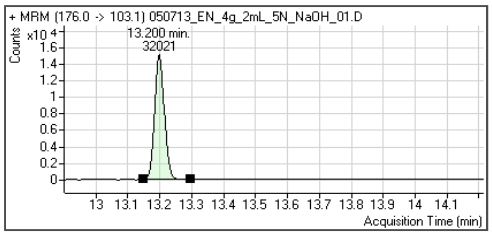
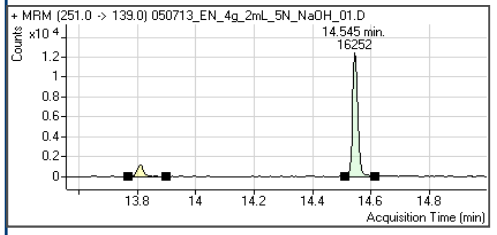
QuEChERS Optimization - pH Variation

- pH value is below 2 in the lemon juice concentrate and some compounds are not recovered from the extraction step.
- pH variation experiment was done to find the right pH range for extraction step
- 0, 0.6, 1, 2 mL of 5 N NaOH was used for pH variation in the extraction step
- With pre-spiked lemon juice concentrate (100 ppb), different pH values were tested for recovery and peak shape

QuEChERS Optimization - pH Variation

	Omethoate	Captan	Atrazine
0 mL 5 N NaOH			
0.6 mL 5 N NaOH			
1 mL 5 N NaOH			
2 mL 5 N NaOH			

QuEChERS Optimization - pH Variation

	Buprofezin	Piperonyl Butoxide	Fenarimol
0 mL 5 N NaOH			
0.6 mL 5 N NaOH			
1 mL 5 N NaOH			
2 mL 5 N NaOH			

QuEChERS Optimization - pH Variation

- Problematic compounds showed improved recovery with 5 N NaOH.
- Amount of 5 N NaOH affects recovery. When tested with 0, 0.6, 1, and 2 mL of 5 N NaOH, overall 2 mL 5 N NaOH addition showed the best performance when 4 g of sample was used. Only Captan showed better recovery when 0.6 mL of 5 N NaOH was used.
- Some compounds almost completely disappeared when no 5 N NaOH was added such as Omethoate, Atrazine, Buprofezin, Bupirimate, Piperonyl Butoxide, Fenarimol.

→ Use 2 mL of 5 N NaOH in the extraction step to raise the pH to ~5.

QuEChERS Optimization – AP (Analyte Protectant)

- "Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides" (Anastassiades, Mastovska, Lehotay *Journal of Chromatography A,* 1015 (2003) 163-184)
- Many compounds are available and suitable for AP and from practical point of view a mixture of D-sorbitol and L-gulonolactone is the best
- ***Add 50 mg of D-sorbitol and 100 mg of L-gulonolactone to 5 mL of ACN to make 10 mg/mL and 20 mg/mL concentration in the mix, respectively***

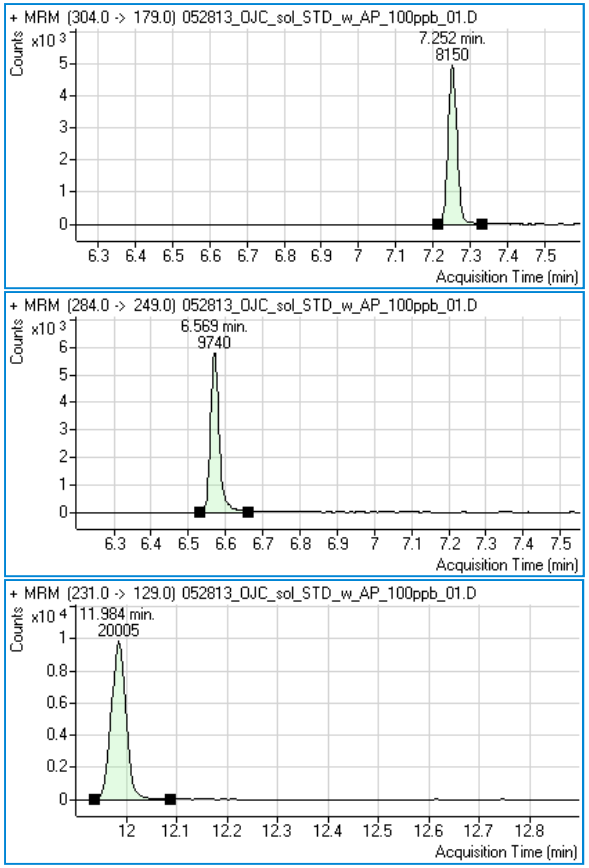
QuEChERS Optimization – AP (Analyte Protectant)

Diazinon

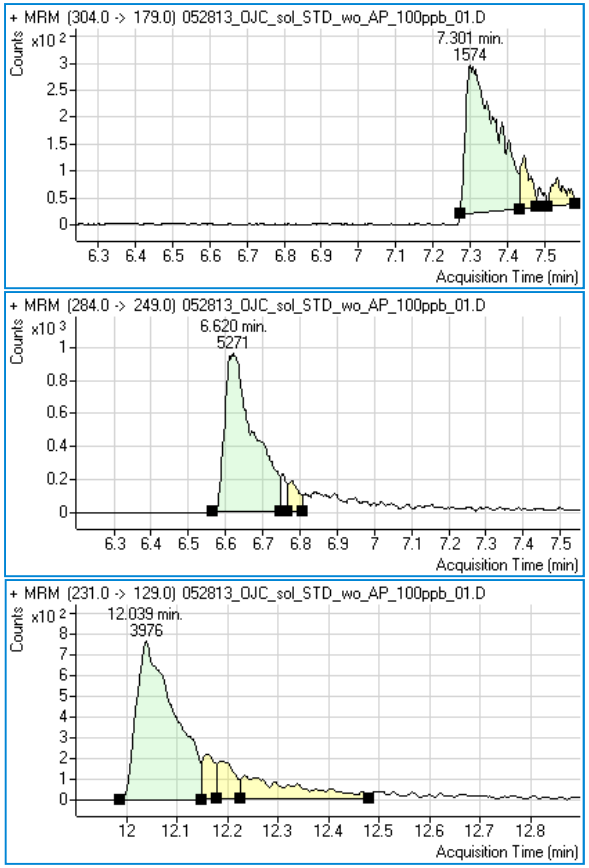
Hexachlorobenzene

Ethion

with AP



without AP



APs are a must in multi-residue pesticide analysis

QuEChERS EN Method – Extraction Protocol Optimized for Juice Concentrates

- Add ***4 g*** of lemon juice concentrate to EN 50 mL extraction tubes
- Spike 80 µL of standard mix in ***ACN + 1% acetic acid,*** shake for 10 min
- Add 6 mL of water to EN extraction tubes (to make the total sample loading 10 g)
- Add ***2 mL of 5 N NaOH*** solution for pH adjustment
- Add 10 mL of ACN to EN extraction tubes and vortex briefly, add Bond Elut EN salt packet and ceramic homogenizers
- Shake for 1 minute, then centrifuge at 4,000 RPM for 2 min

General considerations for alternative matrices or target compounds

- Dried material (e.g. teas, herbs): use less sample, adjust with water, pre-soaking can help recoveries
- If target compounds are acidic, consider PSA-free kit
- Matrices from animal sources tend to be protein and lipid rich, dSPE should contain C18
- Acidifying ACN can help reduce secondary interactions (e.g. protein binding)
- dSPE amount in tubes may need to be adjusted/supplemented (or substitute SPE)

Agilent SPE for Ultimate Cleanliness

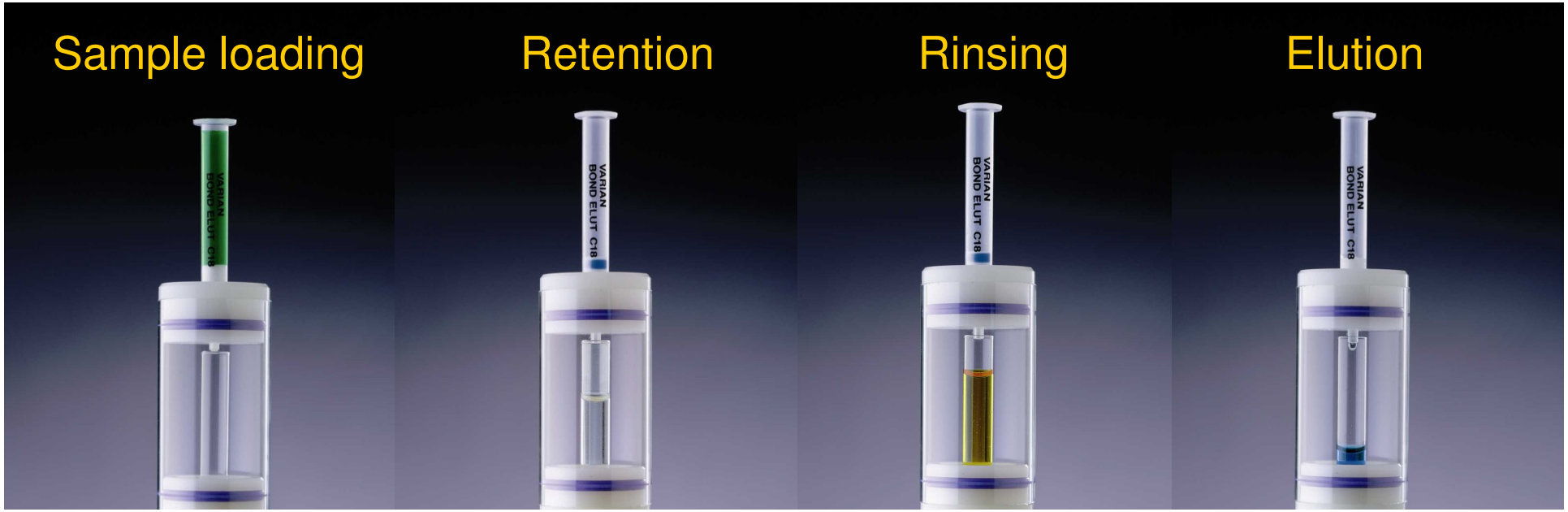
Method Development

The Four Steps of SPE – Selective Elution

Green = Blue and Yellow

Blue is more non polar than yellow

Blue is retained



Is Your Target Compound….

Very Polar	Log P < 1.5	Polar (lp), Ion Exchange (?) (aq, lp)
Moderate Polarity	Log P > 1.5 and < 4	Non-Polar (aq), Ion Exchange (?) (aq, lp), Polar (lp)
Non-Polar	Log P > 4	Non-polar (aq), might need lipid clean up, polar unless hydrocarbon
Strongly acidic or basic	pKa <2 or >11	Weak anion or cation exchange or mixed-mode
Weakly acidic or basic	pKa >2 and <11	Strong anion or cation exchange or mixed-mode

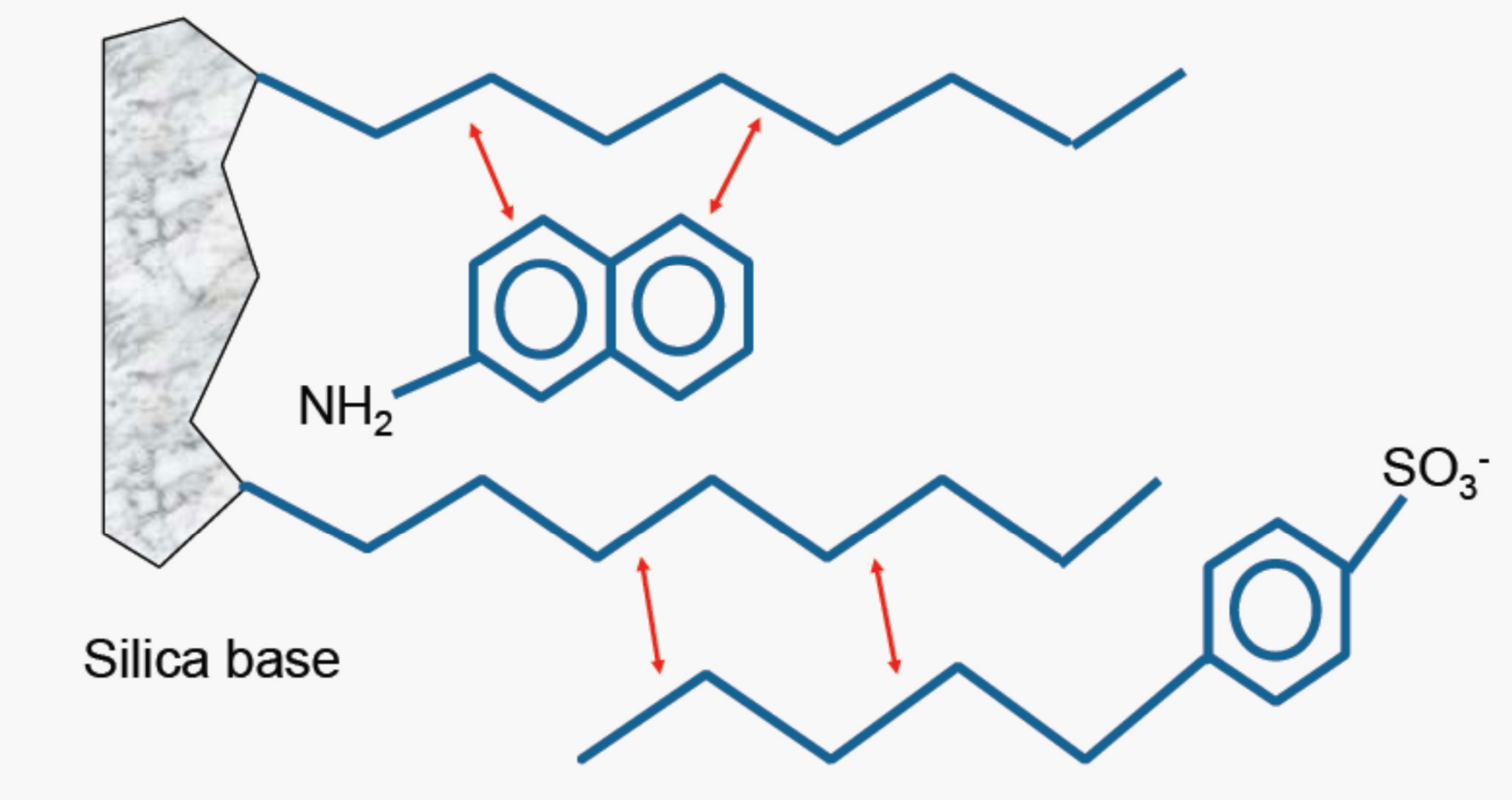
Is Your Matrix

- Mostly aqueous (e.g. fruit juice, energy drinks, brewed teas)
- Mostly lipids or organics (e.g. olive oil, lotions, non-polar extracts)
- Polar extracts (MeOH or ACN): dry down or dilute

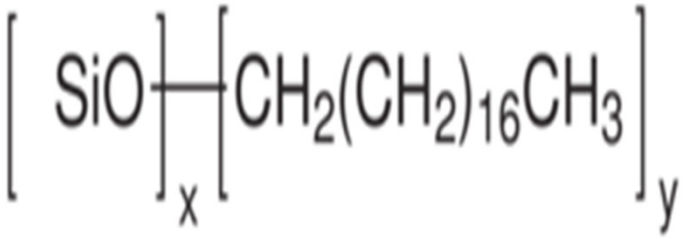
NON-POLAR EXTRACTIONS

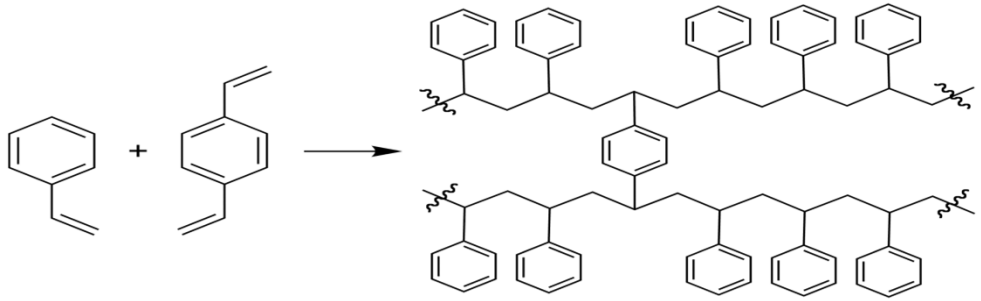
Method Development

Interactions with Non-Polar Sorbents



Silica VS. Polymer



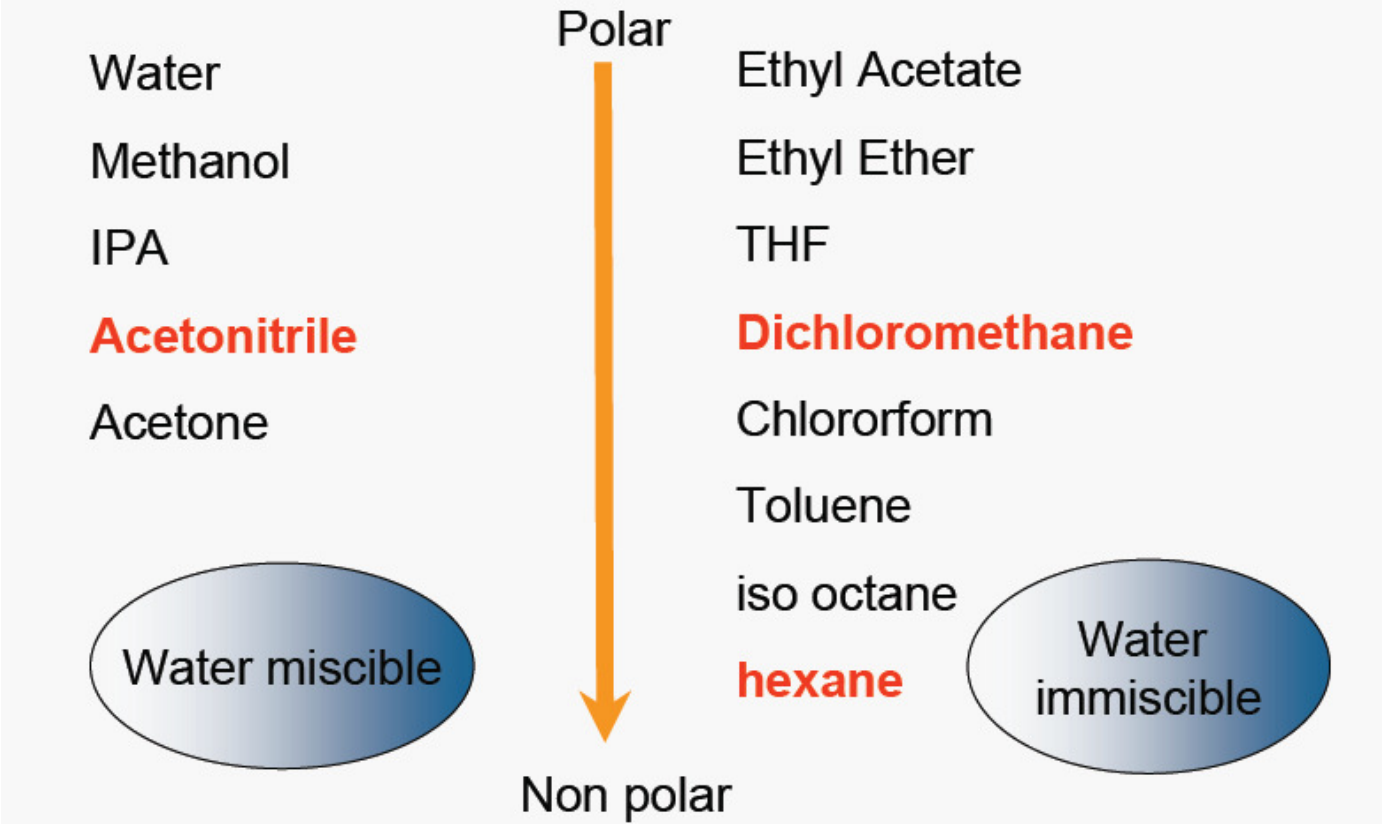


- "True" polar/ion exchange possible
- Wide range of chemistries
- Wide range of established methods
- Can be more selective

- Inherent hydrophobicity (conditioning)
- Higher capacity (sorbent mass/flow)
- Polarity gradient in Plexa

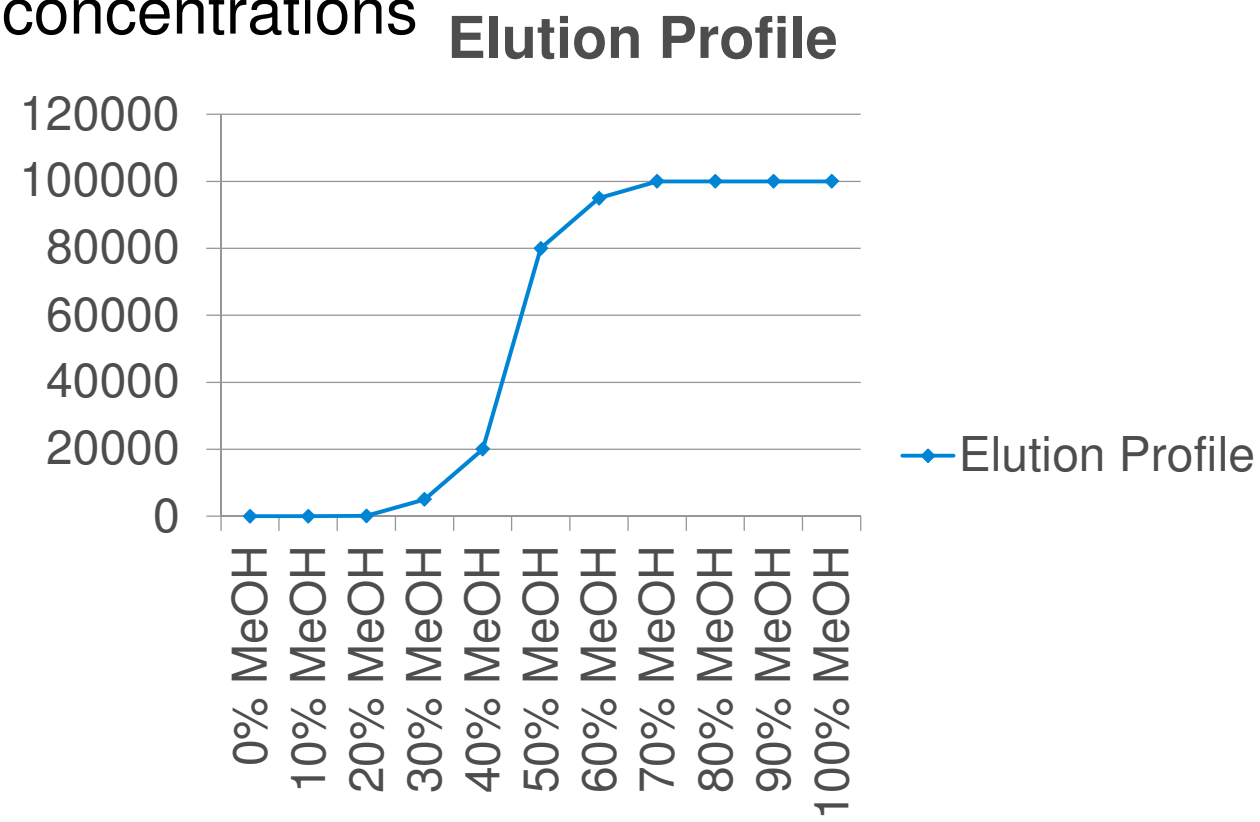
Method Development Considerations

Solubility characteristics of target compound?



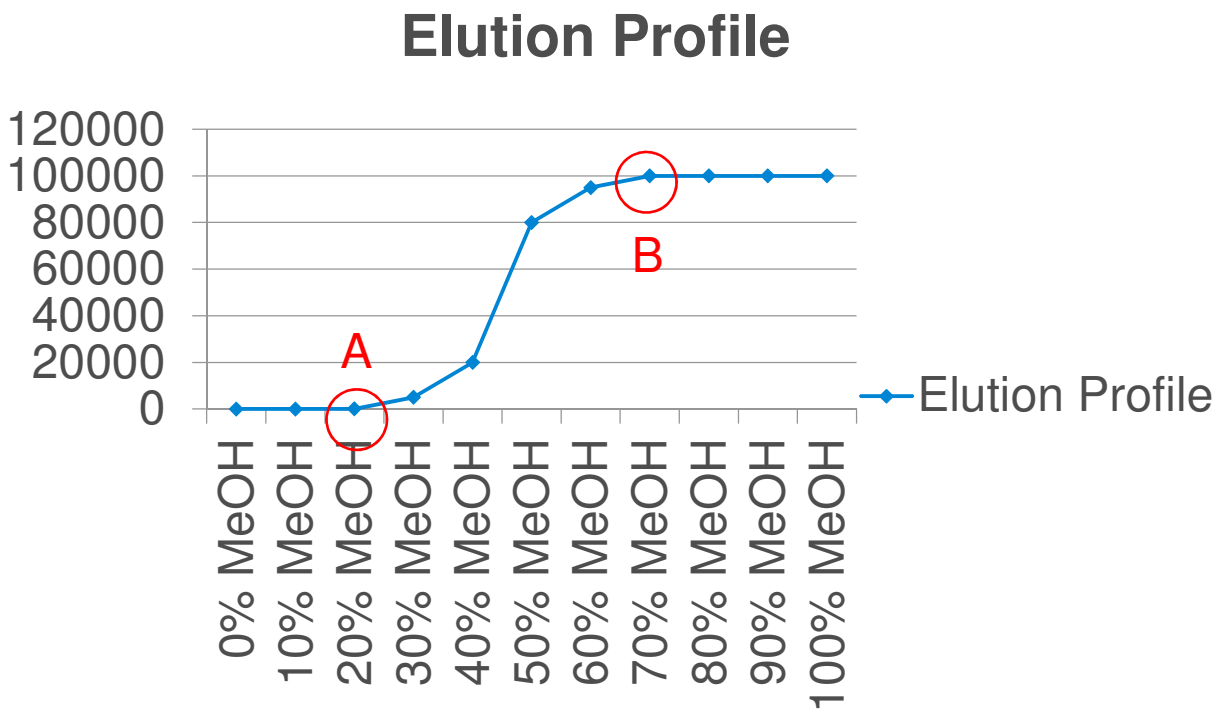
Method Development Considerations

- Select suitable solvents (water miscible only)
- Prepare 0%-100% concentrations
- Plot recoveries



Method Development Consideration

- Highest % organic with low recoveries for wash **A**
- Lowest % organic with high recoveries for elution **B**
- Try acid/base modifiers and MeOH/ACN mix



Low recovery even at 100% organic?

-Use stronger organic solvent, **dry cartridge** before elutions step

-But stronger solvents often = more non-polar contaminants

-Make sure the isolate is soluble and does not degrade under the extraction conditions.

-Reduce secondary interactions on silica-based SPE with buffers addition at different pHs in elution solvent. Addition of 0.5% HCl can help with elution of amine groups.

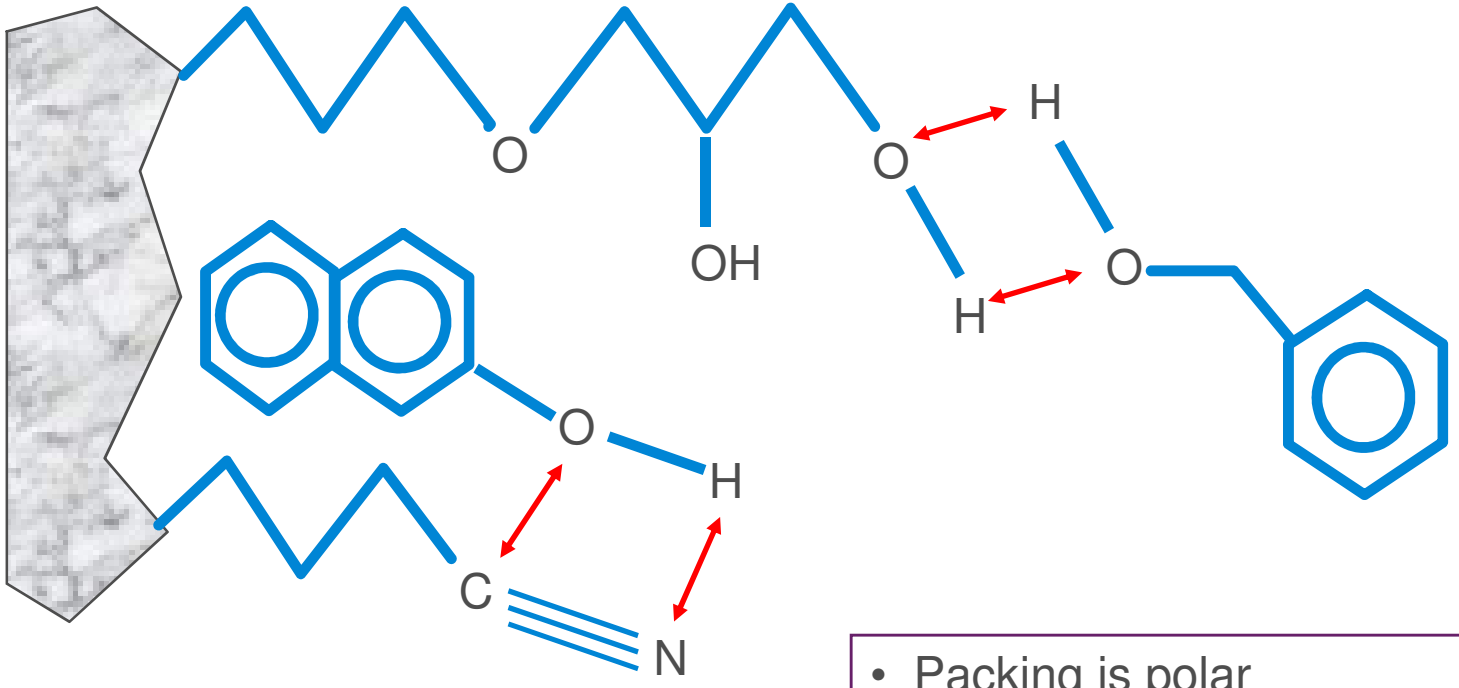
-Consider lower hydrophobicity sorbent (e.g. CH, C2)

POLAR EXTRACTIONS

Method Development

Polar (dipole or H-bonding) Interactions

Silica base



Dipolar attraction or hydrogen bonding

- Packing is polar
- Mobile phase is non-polar (e.g. hexane, methylene chloride, ethyl acetate)
- lower polarity/higher organic for retention
- higher polarity/lower organic for elution

Method Development Consideration

- The goal is to clean up lipids and oils
- Select most non-polar solvent compatible with analyte and matrix, hexane is ideal
- Load extract or hexane/matrix mixture under low vacuum (sample must be water free and SPE cartridges must be well stored to avoid moisture)
- Rinse with 100% loading solvent for 2x column volumes
- Elute with loading solvent + polar modifier such as IPA (about 5-10%) at 2-4 ml/min. Make sure that your analyte is soluble in elution solvent.

ION EXCHANGE EXTRACTIONS

Method Development

Ion Exchange Nomenclature

STRONG: Ionic group is always charged (+ or -)

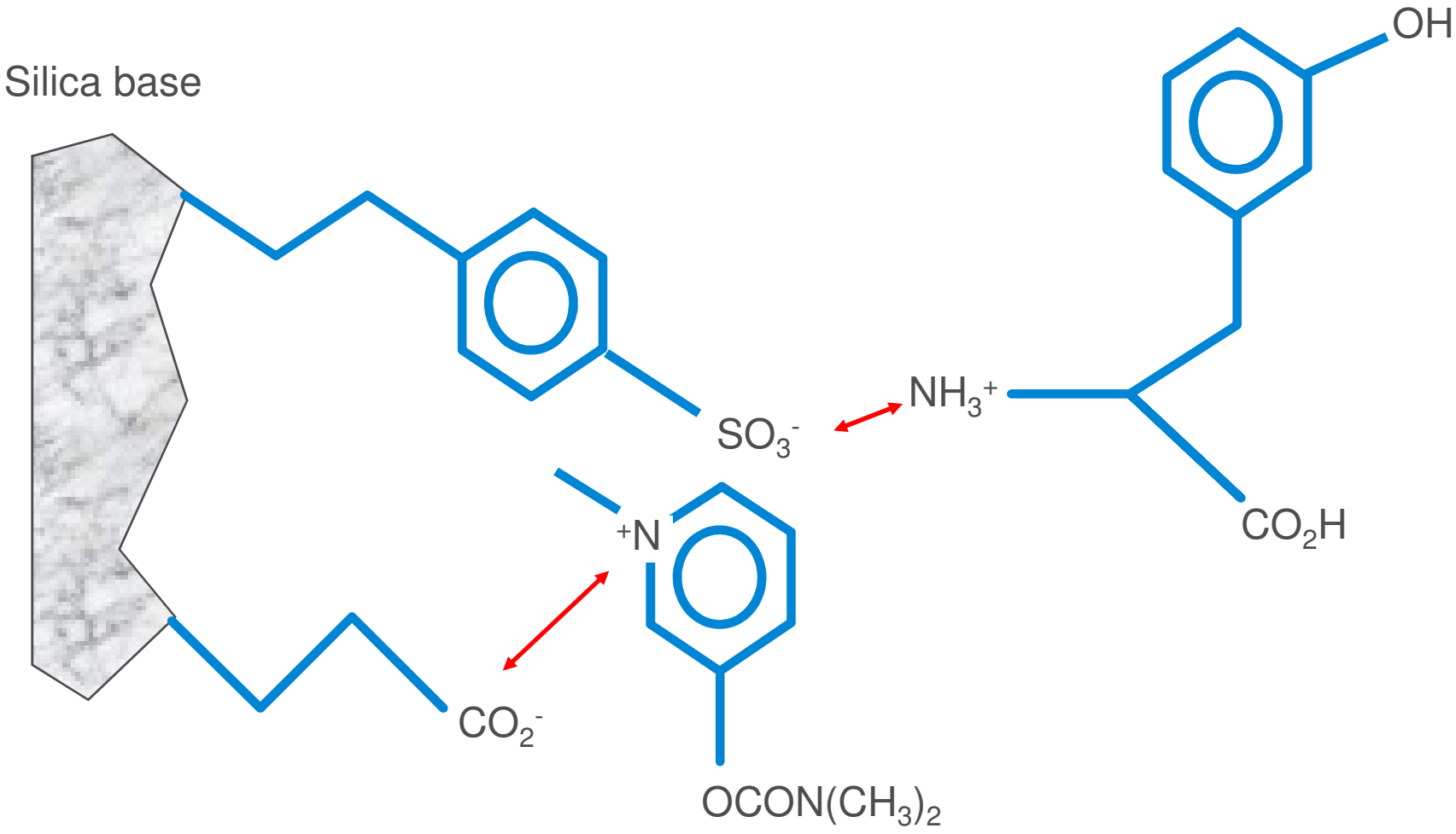
WEAK: Ionic group is variably charged (+ or -)

CATIONS: (+) Found in basic compounds

ANIONS: (-) Found in acidic compounds

Extract weak ions with strong exchangers and strong ions with weak exchangers!

Interactions on Ion Exchange Sorbents



Method Development Considerations

What is the pKa of your compound?

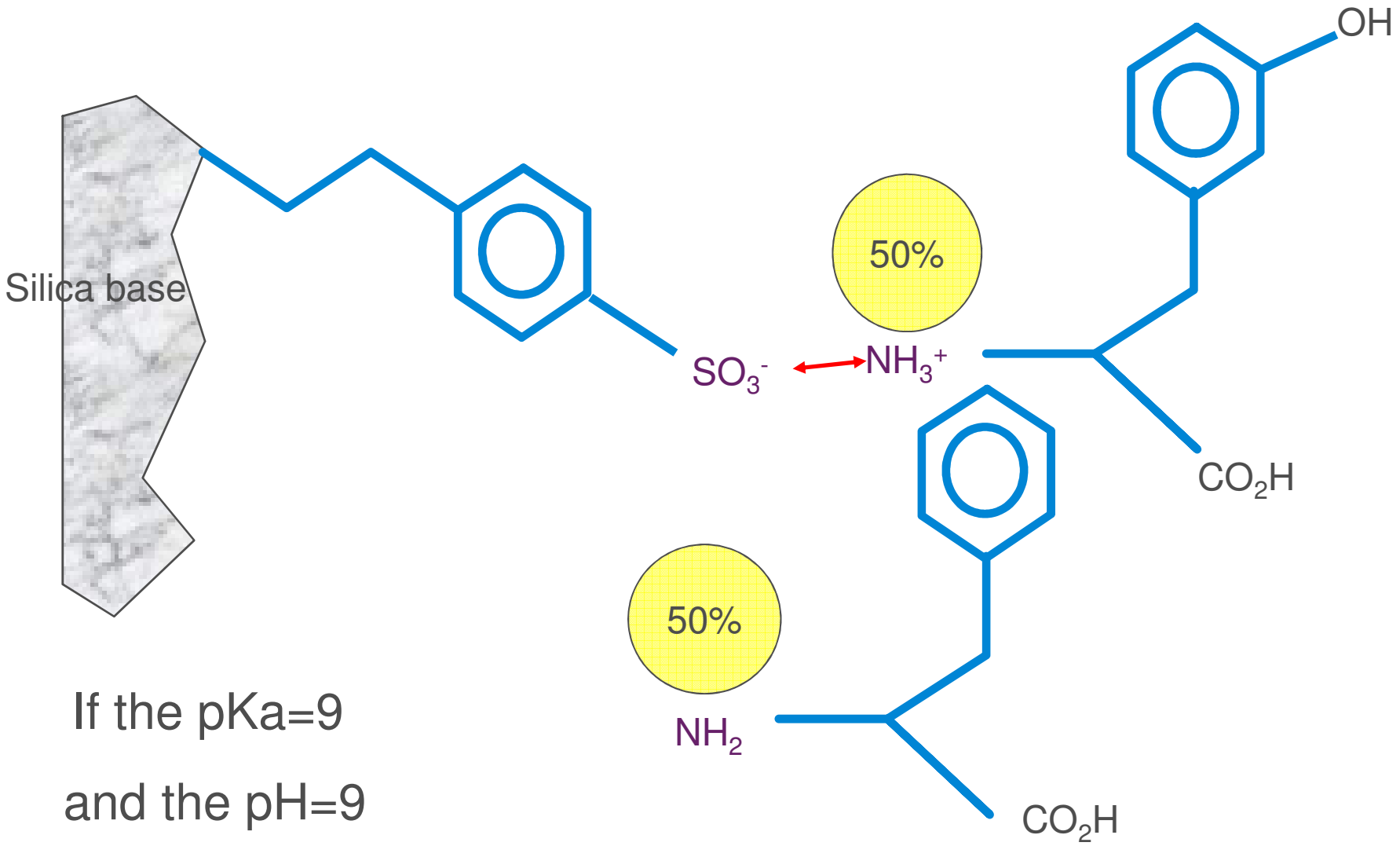
$$pK_a = -\log K_a$$

and

$$K_a = [A^-][H^+]/[HA]$$

- If pH=pKa, 50% of the compound is ionized and 50% is neutral
- To ensure full charge or full neutralization, employ the rule of 2

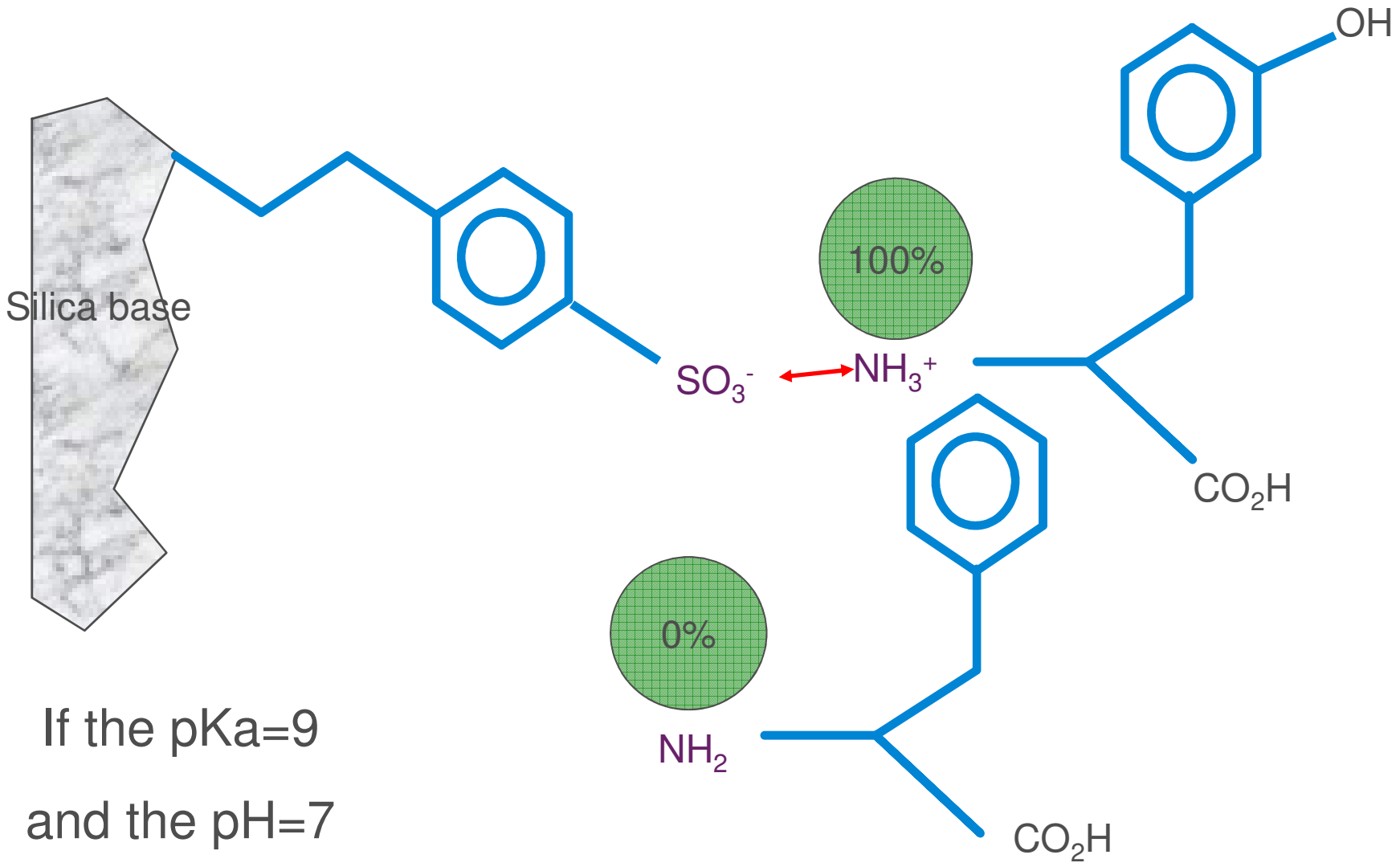
Interactions on Ion Exchange Sorbents



If the pKa=9

and the pH=9

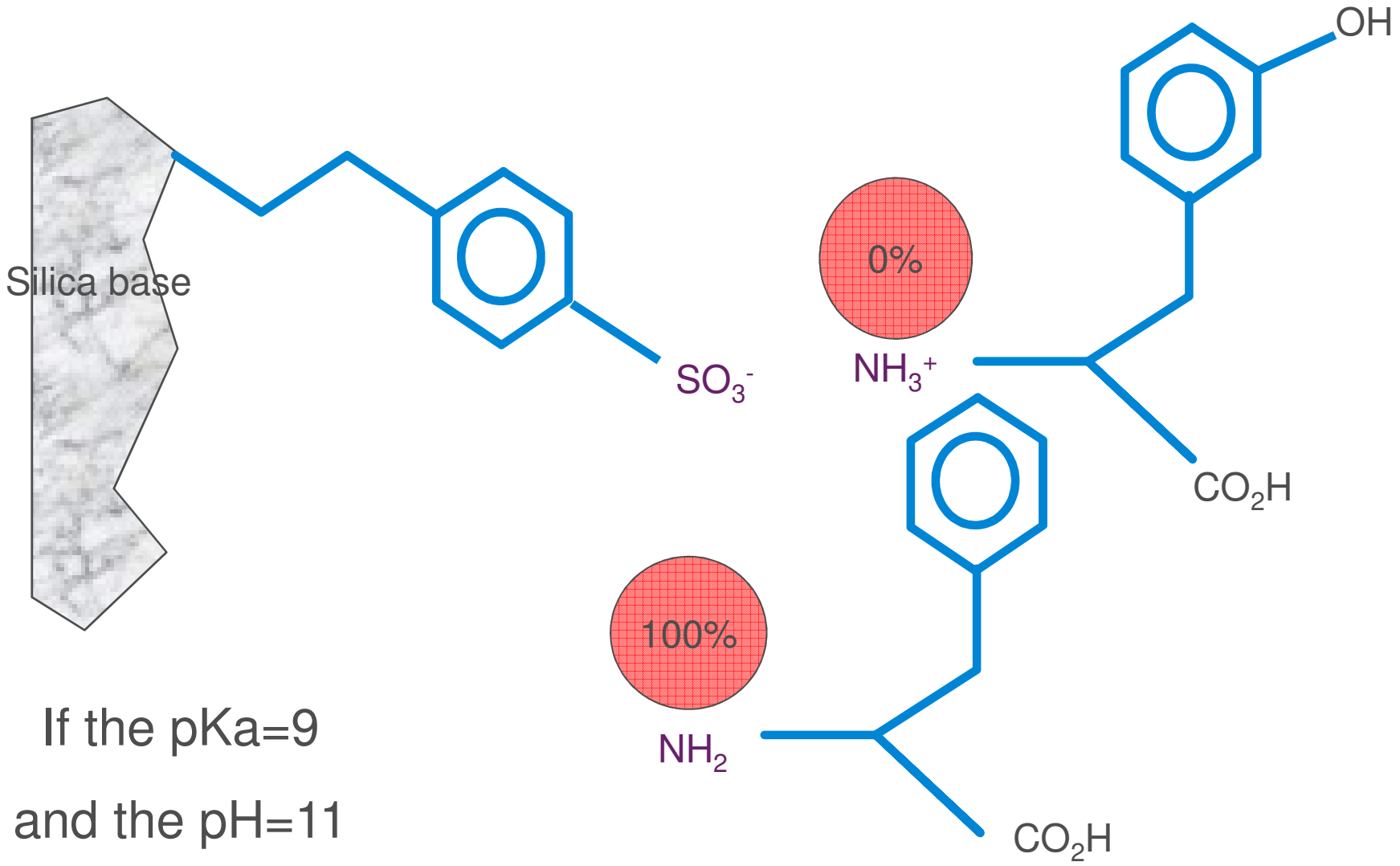
Interactions on Ion Exchange Sorbents



If the pKa=9

and the pH=7

Interactions on Ion Exchange Sorbents



If the pKa=9

and the pH=11

Important Consideration for Ion Exchange

- Reduce ionic strength of “salty” matrices by dilution
- Consider competitive binding when choosing bed mass
- Remember that **ALL** polymeric exchangers are mixed-mode, elute in organic solvent
- Some organic should be present even with silica based ion exchangers because of carbon linkers
- Reduce flow rate at sample application because ion exchange is a relatively slow interaction

In conclusion

1. QuEChERS Workflow overview and original methods
2. Method development for alternative matrices
3. SPE for polar compounds
4. SPE for non-polar compounds
5. SPE for ionic compounds
6. Questions

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Questions