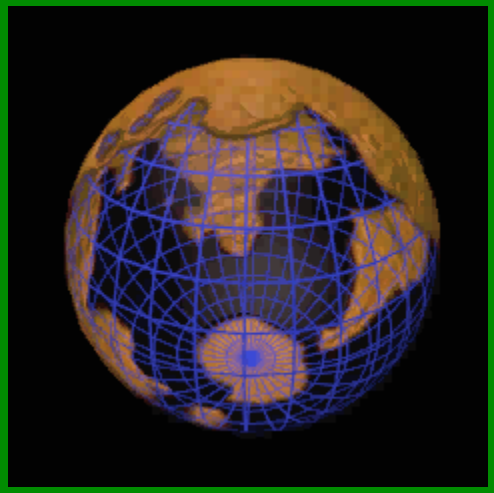
FOOD ANALYSIS
I. Solid Phase Extraction

Principles

Recent Developments

Applications



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Basic Principles of SPE

- Sample Preparation Overview
- Fundamentals of SPE
- Various Modes of SPE
- Packed Bed vs. Disk Format
- The use of Dual Phases
- Step by Step Method Development; Validation

Recent Developments in SPE

- Stacked / Layered Phases
- Argentation Chromatography
- ISOLUTE ENV+
- MSPD: Matrix Solid Phase Dispersion
- Mechanised L / L Extraction
- MIP and Immunoaffinity Columns
- SPE Automation

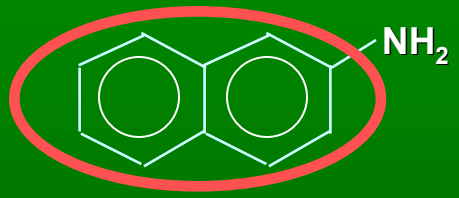
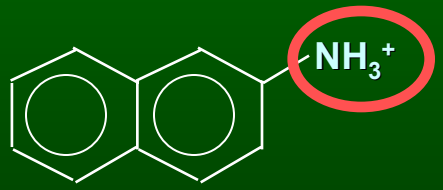
Applications

- Multi-Residue Methods using
  - Cation-Exchange SPE
  - DIOL / NH2 SPE
  - Layered-Column SPE
  - GPC Clean-Up
- SPE of Pesticides & Mycotoxins
- MSPD of Drug Residues
- Isolation of Dimetridazole

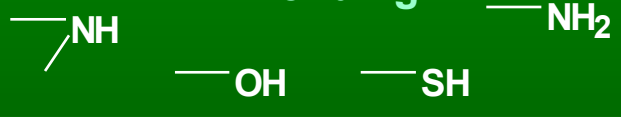
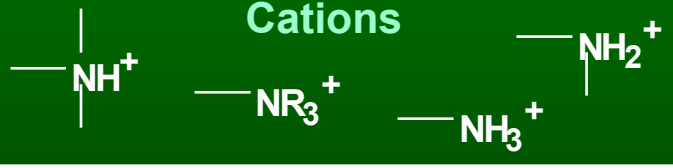
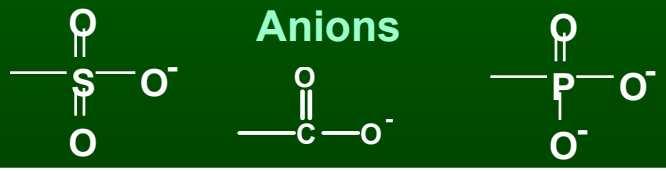
Definition of SPE

Separation or removal of an analyte or analytes from a mixture of compounds by selective partitioning of the compounds between a solid phase (sorbent) and a liquid phase (solvent).

SPE Mechanism Selection (1)

Functionality	Analyte	Mechanism
Hydrophobic		Non-Polar
H-Bonding		Polar
Ionic		Ion-Exchange

SPE Mechanism Selection (2)

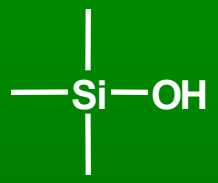
Analyte	Matrix	Sorbent
Hydrophobic 	Aqueous	Non-Polar C18 C8 PH CH C2 CN
H-Bonding 	Non-Polar Solvent	Polar SI NH2 2OH CN
Cations 	Aqueous (Low Ionic Strength)	Cation Exchange PRS CBA SCX
Anions 	Aqueous (Low Ionic Strength)	Anion Exchange SAX NH2

ISOLUTE Non-Polar Sorbents

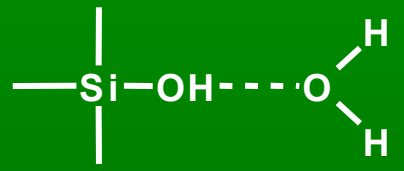
C18 *	Octadecyl
MFC18	Octadecyl
C8 *	Octyl
C2 *	Ethyl
C4	Butyl
C6	Hexyl
PH *	Phenyl
CH (EC)	Cyclohexyl
CN (EC)	Cyanopropyl
101	PS-DVB
ENV+	Polystyrene

* EC

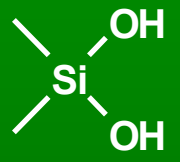
Silica Surface Variations



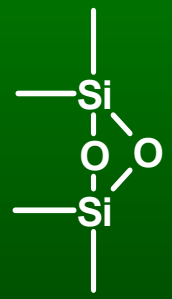
Free silanol



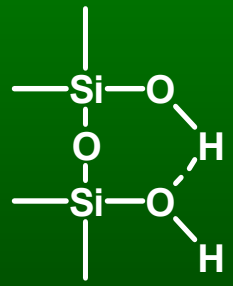
Adsorbed water



Geminal silanol

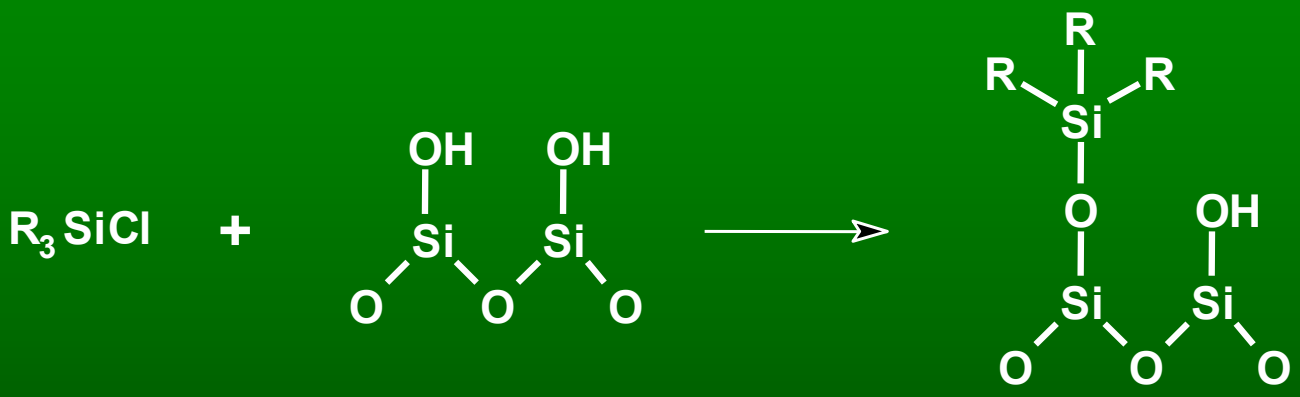


Siloxane

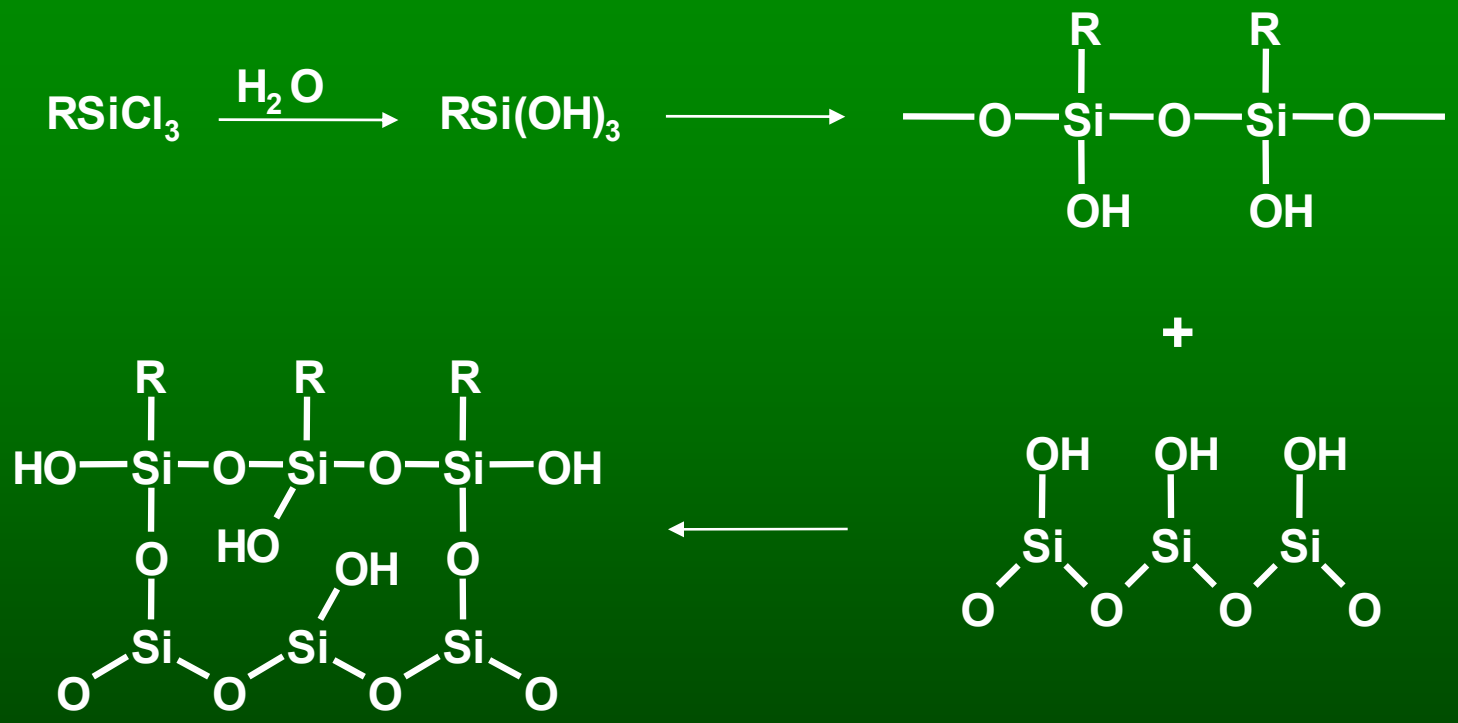


Bound silanols

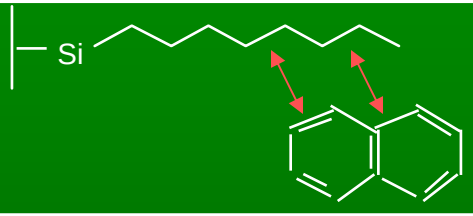
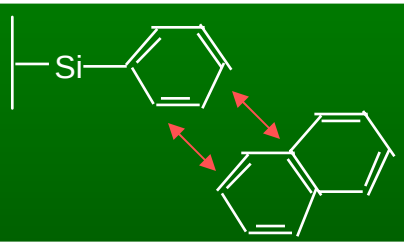
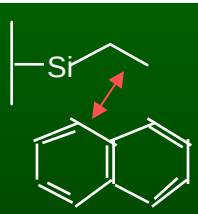
Monochlorosilane Chemistry



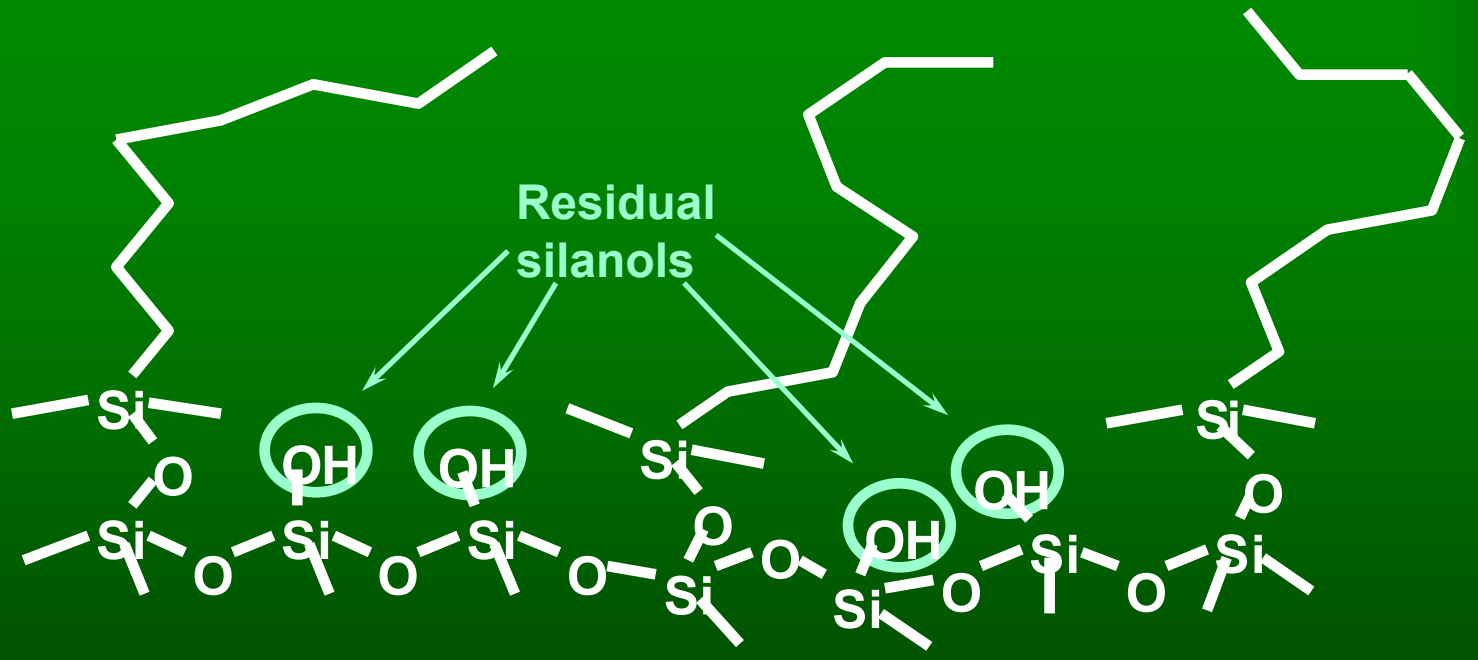
Trichlorosilane Chemistry



Non-Polar Interactions

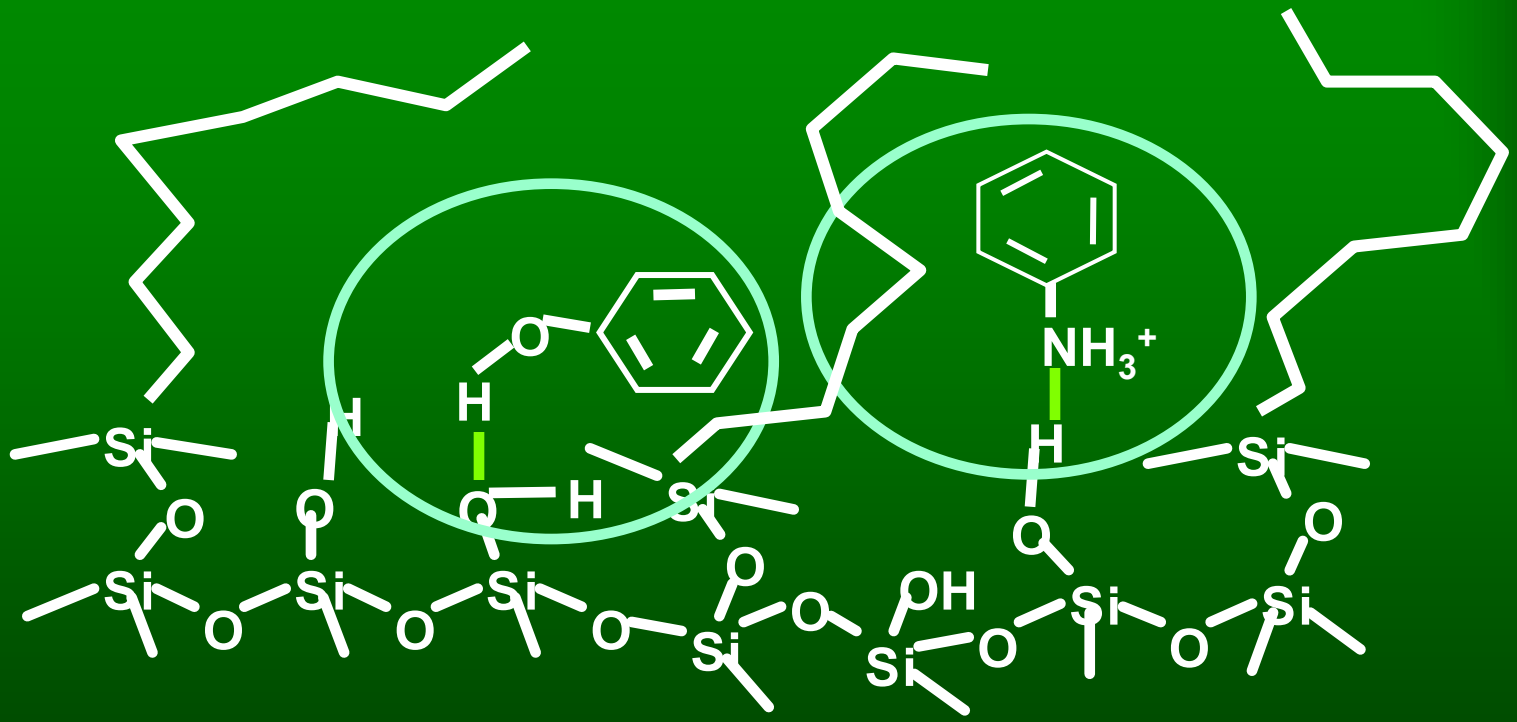
Sorbents		Interactions
C8		van der Waals
PH		van der Waals
C2		van der Waals

Bonded Silica Surface

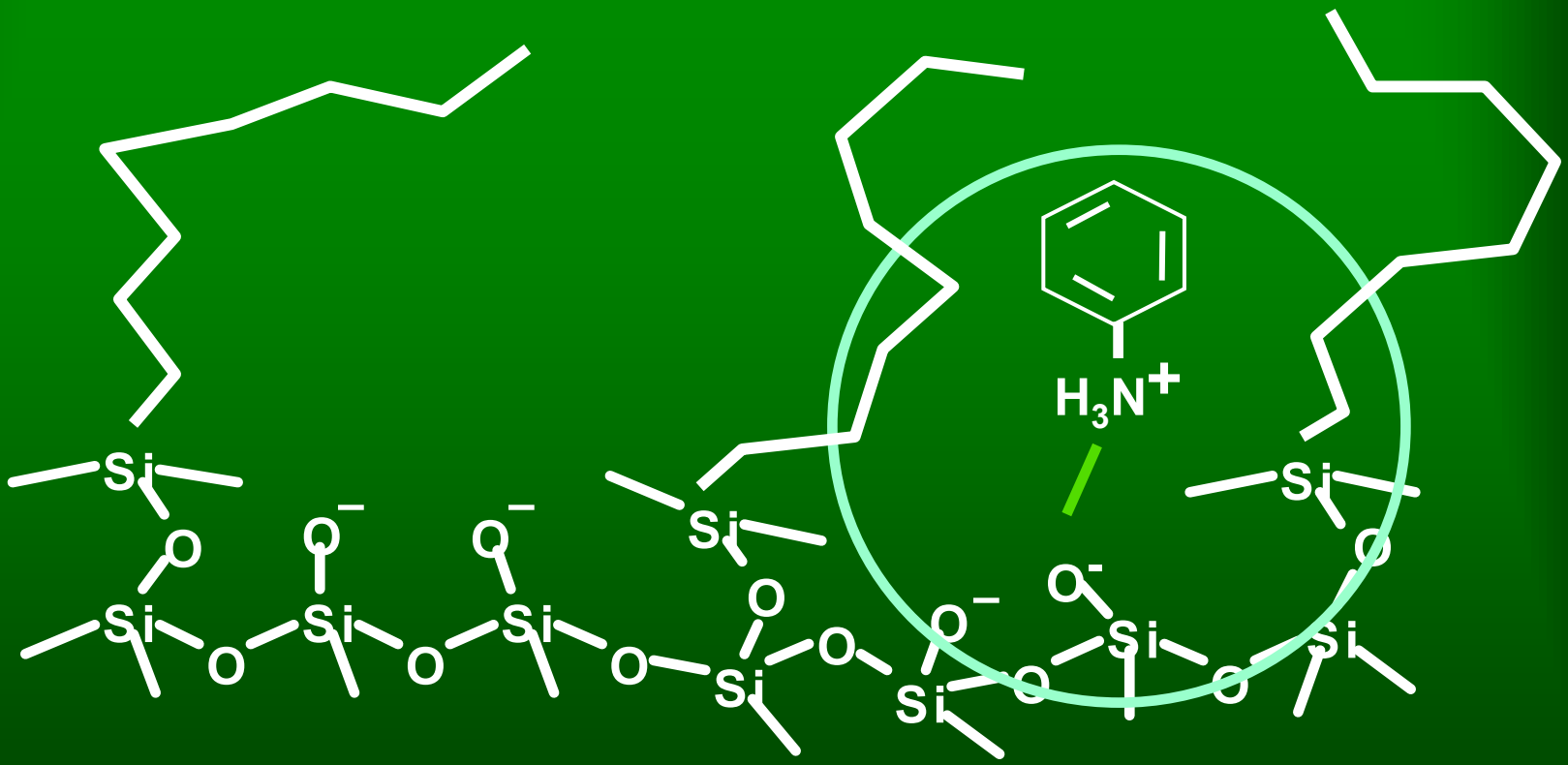


pH<3

Polar Secondary Interactions



Ionic Secondary Interactions

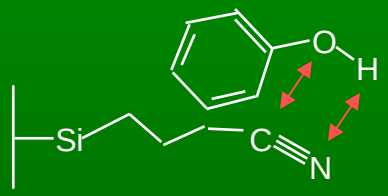
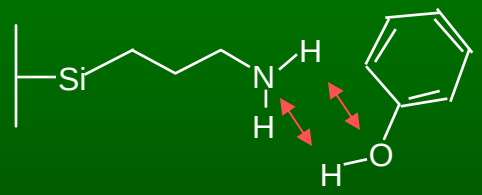
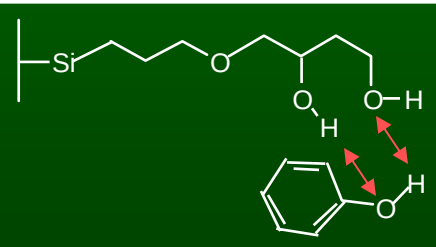


ISOLUTE Polar Sorbents

Si	Silica
NH_2	Aminopropyl
PSA	Primary Secondary Amine
Diol	2,3-Dihydroxypropyl
CN *	Cyanopropyl

* Also available in endcapped chemistry
  Used to extract polar compounds from non-aqueous matrices (e.g. Hexane, ethyl acetate, dichloromethane, etc.)

Polar Interactions

Sorbents		Interactions
CN		Dipole / Dipole
NH_2		Hydrogen-Bonding
2OH		Hydrogen-Bonding

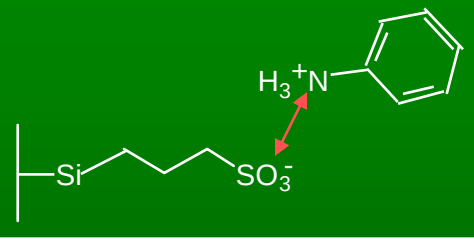
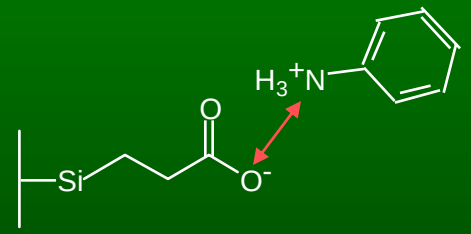
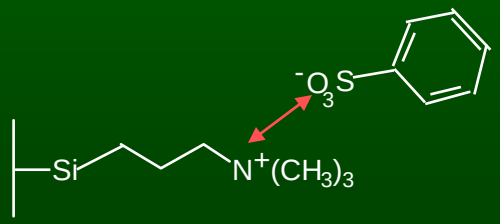
NH_2 vs. Silica Columns

The use of NH_2 columns in place of silica columns is strongly recommended. This is because the activity of silica columns can be affected by moisture content. IST columns are manufactured to a constant standard moisture level, so their activity will always be reliable, but adaption of literature methods using other types of silica can be problematic. Different moisture levels will affect the amount and polarity of solvents necessary for elution of the analytes. NH_2 columns are recommended as they are much less susceptible to this variation.

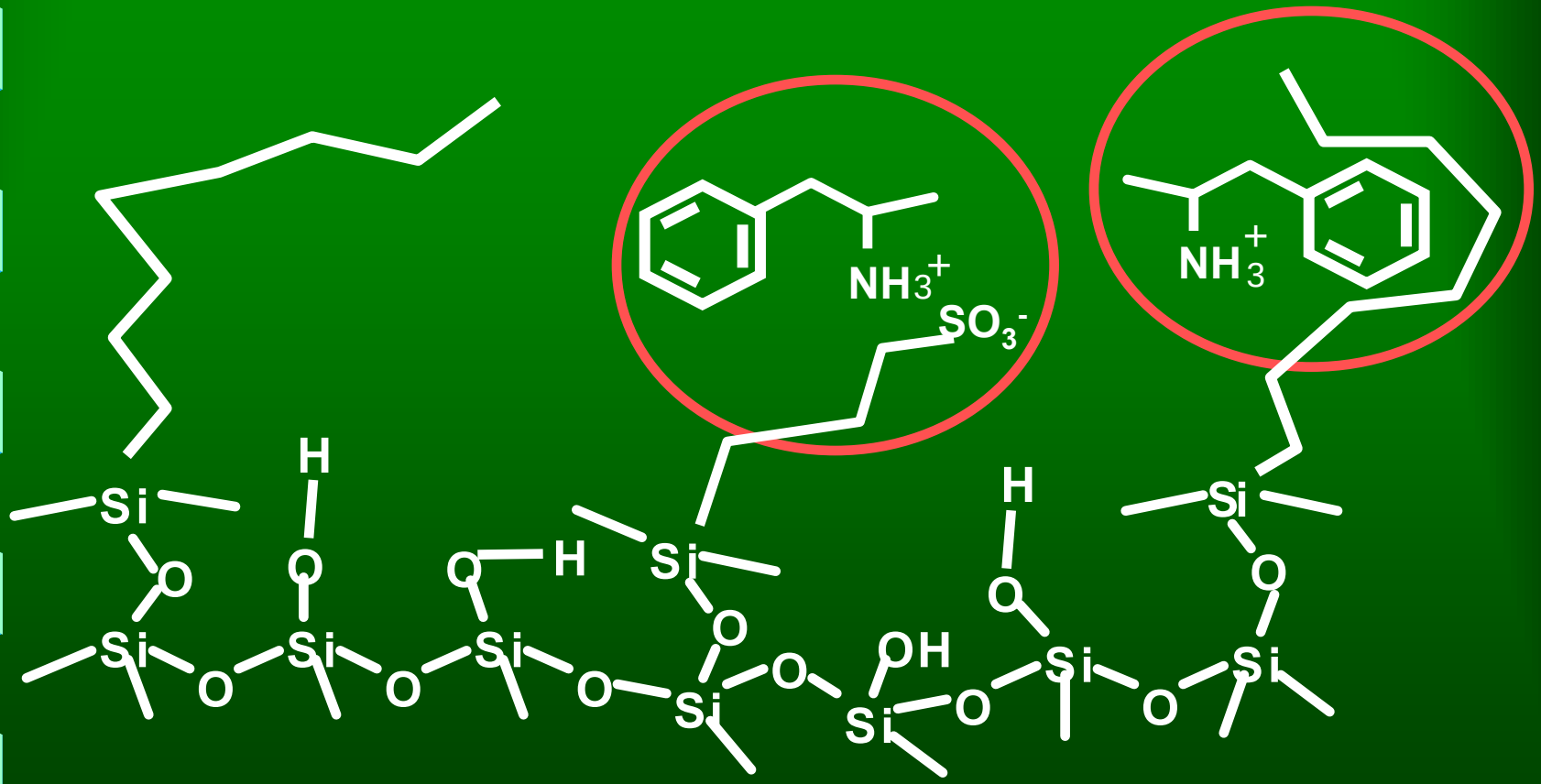
ISOLUTE Ion-Exchange Sorbents

Anion Exchange:		
Weak:	**NH_2**	**Aminopropyl**
	PSA	**Primary Secondary Amine**
Strong:	**SAX**	**Quaternary amine**
Cation exchange:		
Weak:	**CBA**	**Carboxypropyl**
Strong:	**SCX**	**Benzenesulphonic acid**
	PRS	**Propylsulphonic acid**

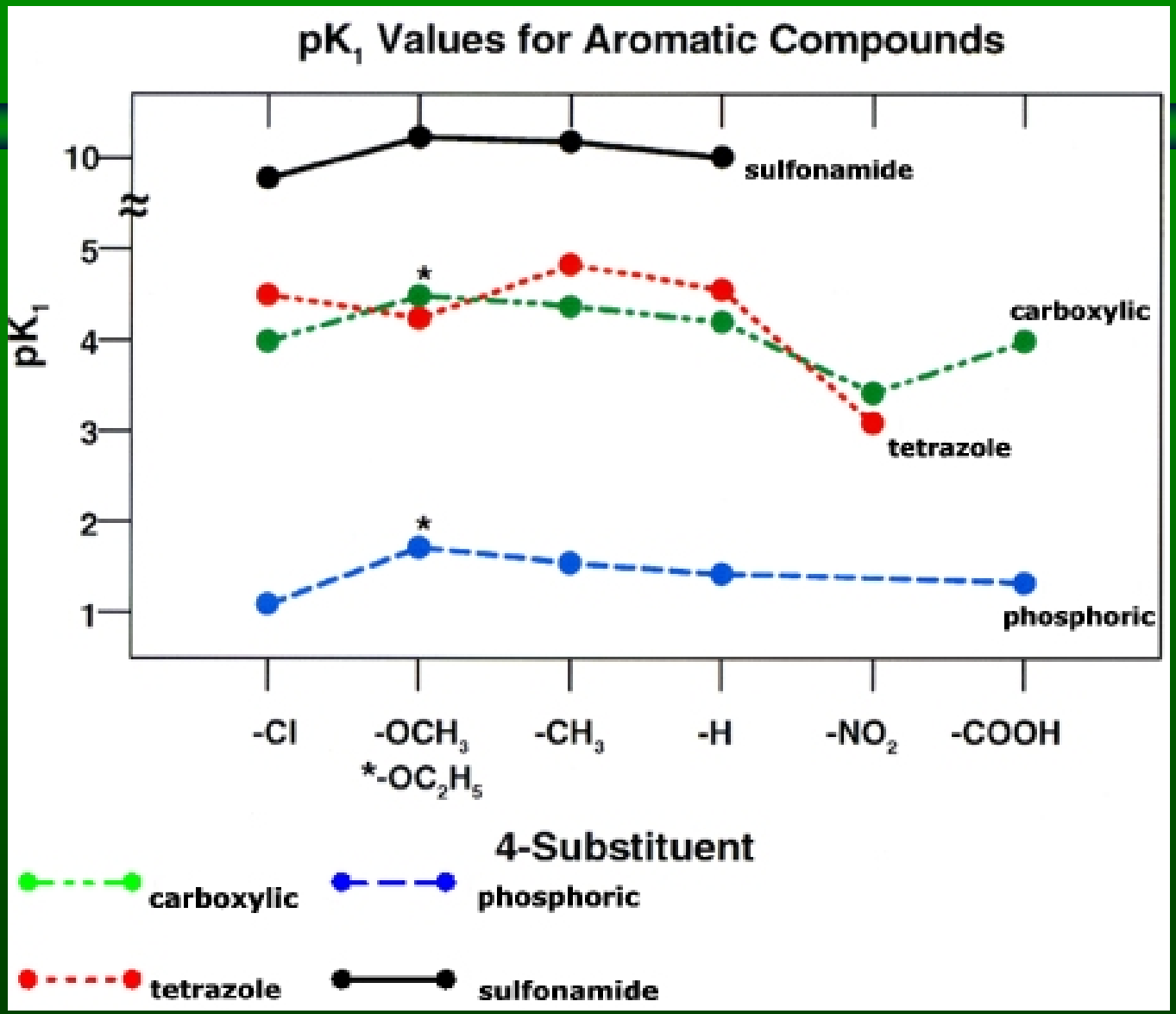
Ionic Interactions

Sorbents		Interactions
PRS		Electrostatic
CBA		Electrostatic
SAX		Electrostatic

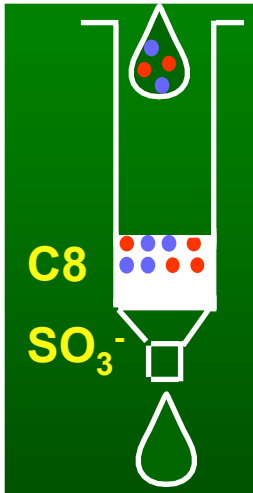
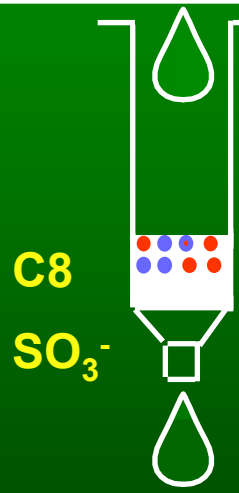
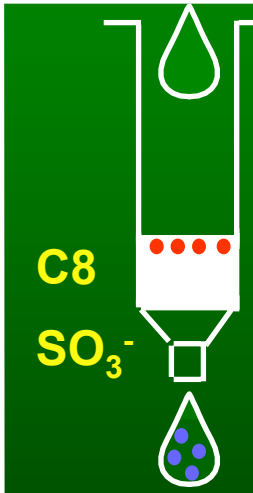
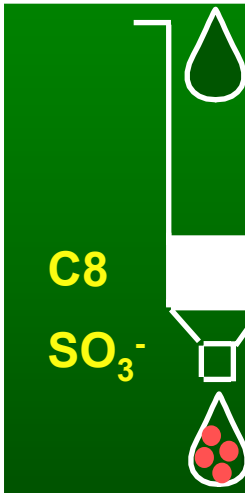
Multiple Interactions



pK of Sulfonamides



Mixed-Mode SPE

Apply Sample + phosphate buffer (pH 6)	pH 6 phosphate buffer,1M AcOH Dry column	MeOH Dry column	Elution: EtOAC : NH_4OH (98:2 v/v)
			
Interferences	Interferences	Interferences	Basic drugs

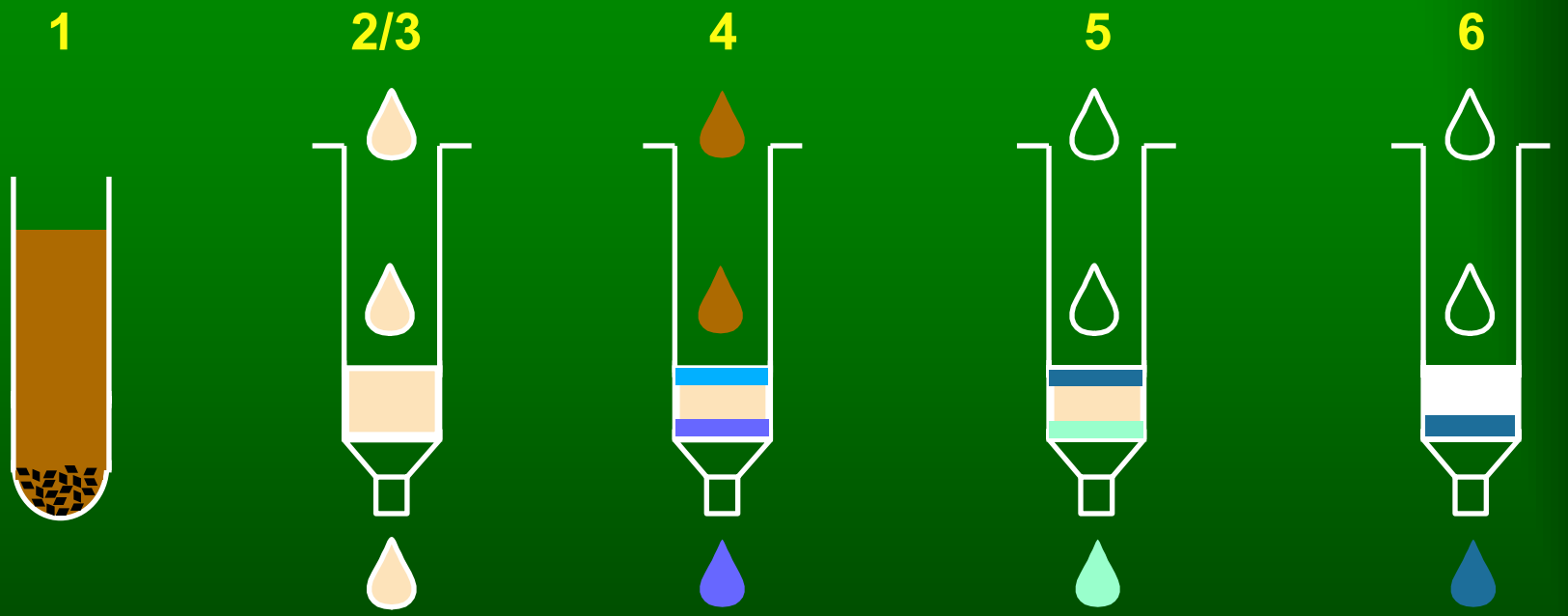
SPE Modes of Operation

- Six-step procedure (typical)
- Four-step procedure
- Stacked columns
- Layered phases
- Mixed phases

A Typical SPE Procedure Involves Six Steps

1. Sample pre-treatment
2. Column solvation
3. Column equilibrium
4. Sample application
5. Interference elution
6. Analyte elution

Six Step SPE Procedure



Sample Pre-Treatment

Optimize sample for analyte retention

- Proper dilution / ionic strength
- Correct pH
- Analytes free in solution
- Remove particulates

Acid Dissociation Constants

$$HA \leftrightarrow H^+ + A^-$$

$$K_a = \frac{[H^+]\,[A^-]}{[HA]}$$

$K_{HOAC} = 1.75 \times 10^{-5}$

$K_{HCN} = 6.20 \times 10^{-10}$

$$pK_a = -\log K_a$$

$pK_{HOAC} = 4.76$

$pK_{HCN} = 9.21$

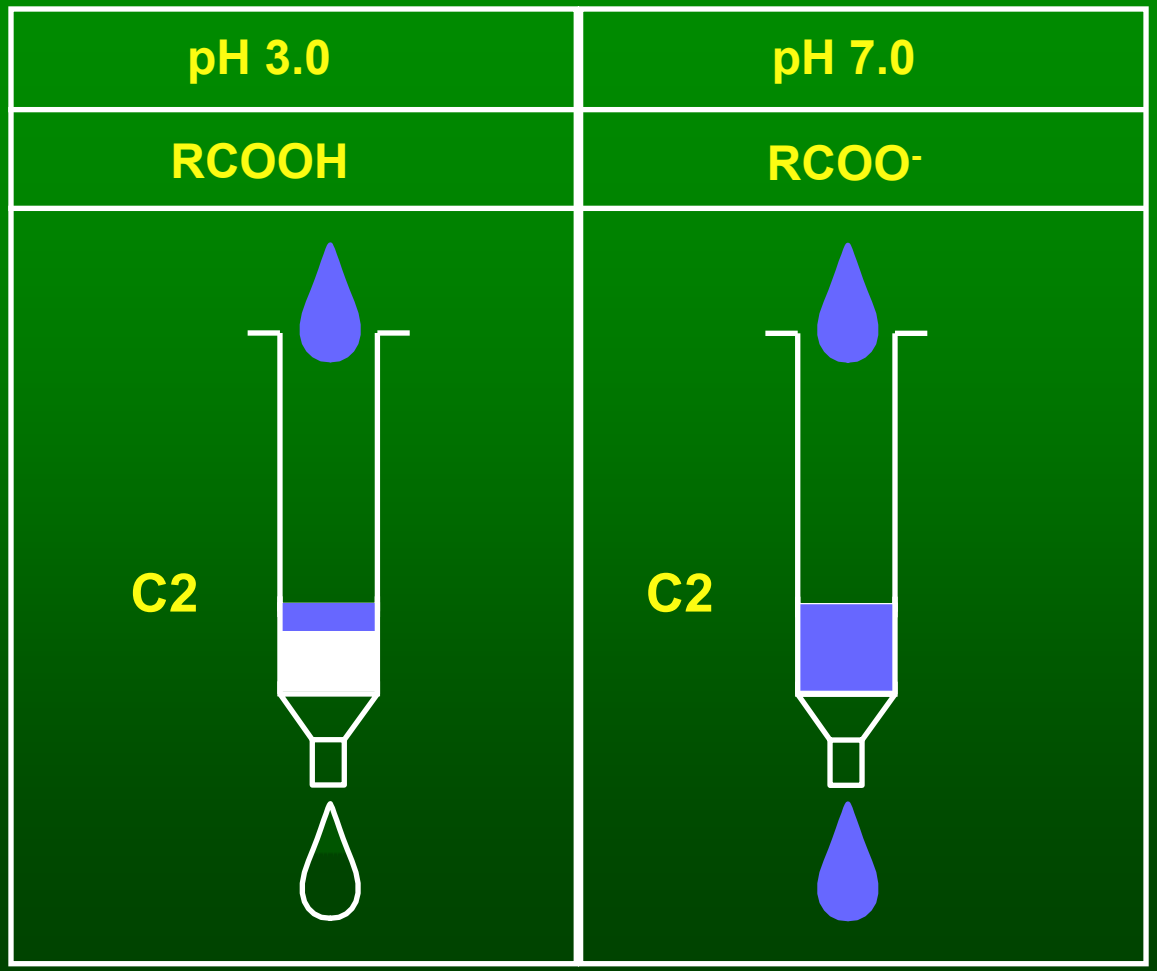
Henderson-Hasselbach Equation

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

Choose a pH at least 2 units away from pK_a

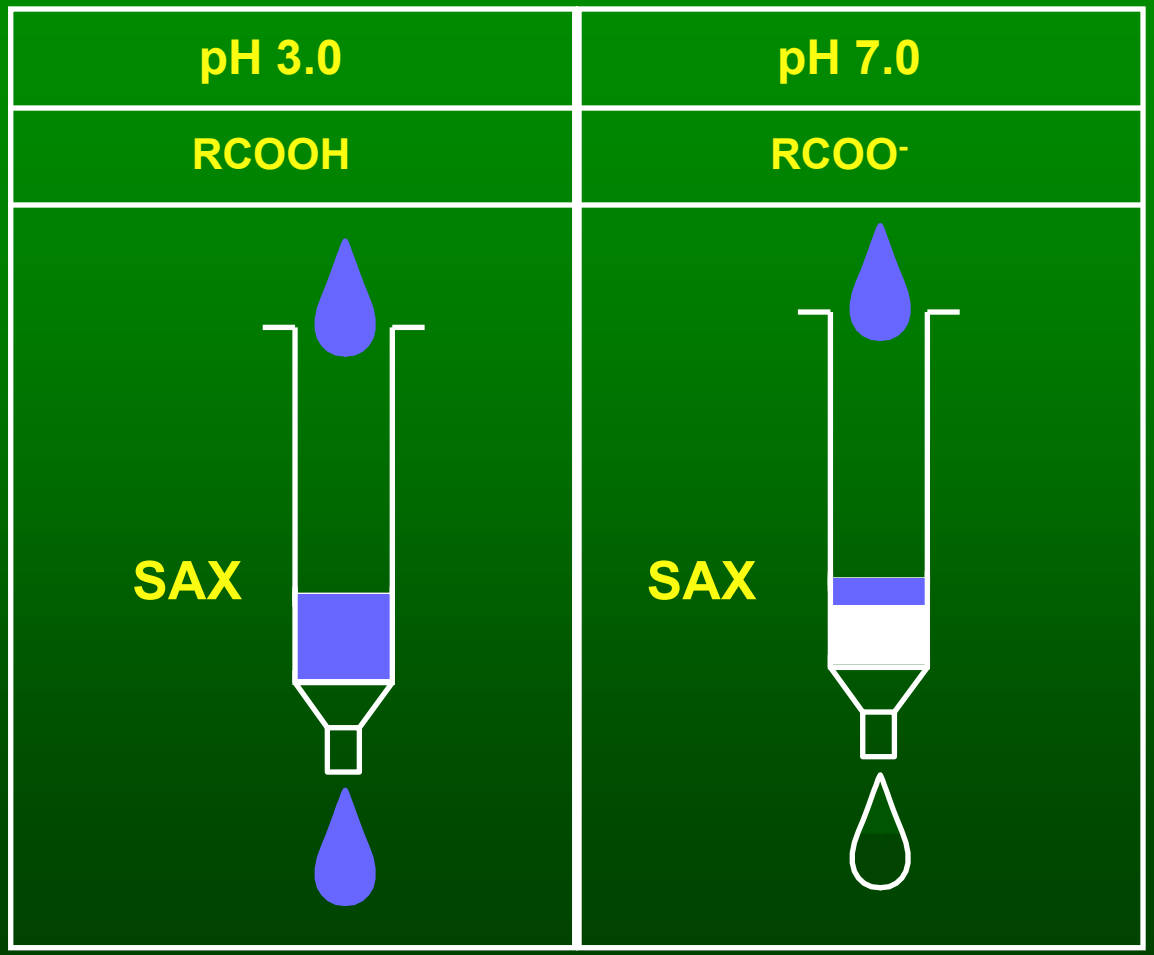
Influence of pH on Retention

Non-Polar Phases ($pK_a = 5$)

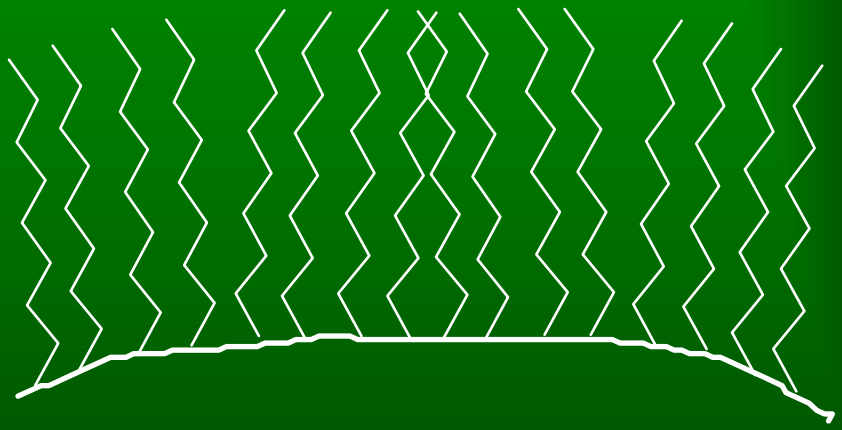


Influence of pH on Retention

Ion-Exchange Phases ($pK_a = 5$)



Column Conditioning



Column Conditioning

- Non-polar sorbents
  - MeOH, MeCN, THF
- Polar sorbents
  - nC6, EtAc; same solvent as the sample matrix
- Ion-exchange sorbents
  - MeOH, MeCN, THF

Column Equilibration

- Remove excess solvation solvent
- Normalize sorbent to sample condition (optimum environment for retention)
  - Ionic strength, pH, solvent composition
- Ion-exchange
  - Counter-ion, pH

Sample Application

Type of Analyte	Type of Sorbent	Cartridge Size (mL)	Loading Rate (mL / min)
Neutral	Hydrophobic	1	1-5
		3	3-15
		6	10-120
Cation or Anion	Ion Exchange	1	0.5-2
		3	1-5
		6	3-35

Interference Elution

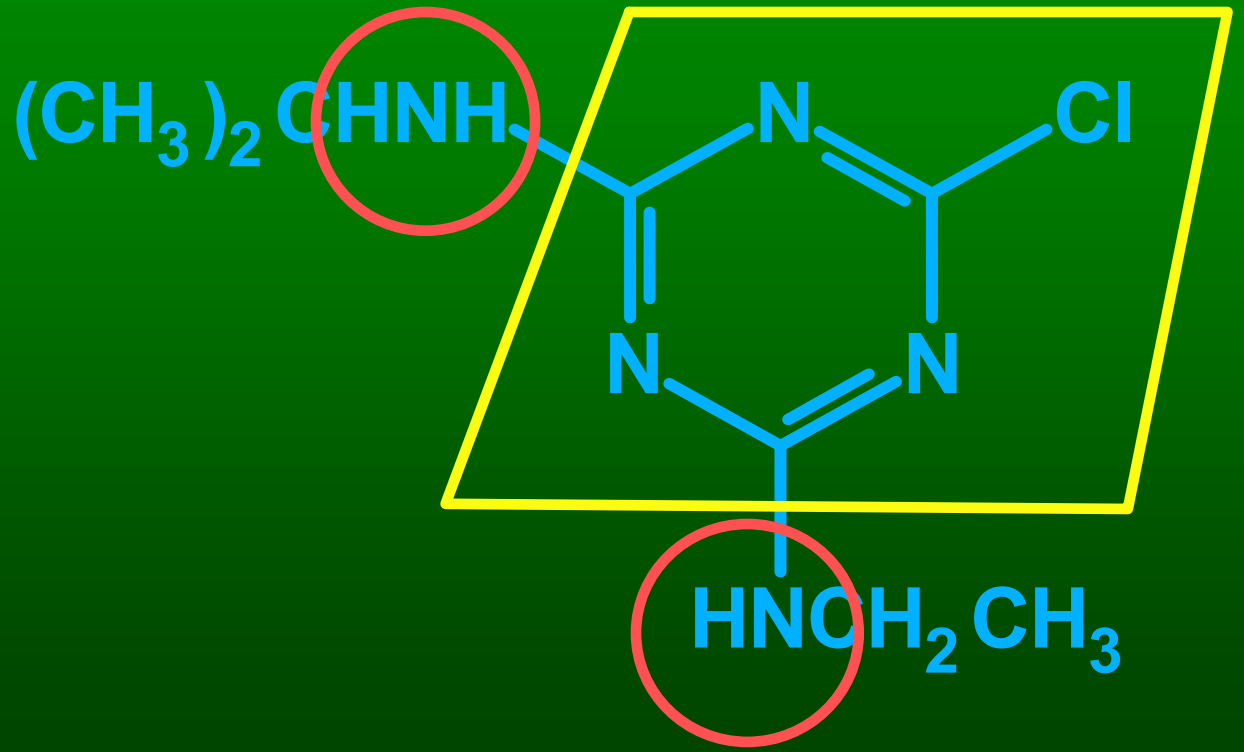
- Analyte-insoluble solvent
- Selective mixtures
- Maintain analyte retention
  - (pH control can be important)
- Optimize flow rate

Analyte Elution

- Elution solvent must overcome both PRIMARY and SECONDARY interactions
- 100% elution in < 20 bed volumes
- Use selective solvents / mixtures
- Optimize flow rate

Example: Atrazine

Atrazine Structure



Atrazine from Water
Apolar Retention

Column: C18, 1g / 6mL

1. Sample pre-treatment: none
2. Solvation: 10mL MeOH
3. Equilibration: 10mL water

4: Sample: 500mL aqueous

5: Wash: 10mL water; dry: 30min

6: Elution: 2 x 4 mL acetone

Atrazine from Corn Oil
Polar Retention

Column: DIOL, 500mg / 6mL

1. 2mL oil ⇨ dilute w. 18mL of nC6
2. Solvation: 6mL nC6
3. Equilibration: none
4. Sample: 20mL (diluted)
5. Wash: 2mL nC6
6. Elution: 1mL methanol

Atrazine from Soybeans Cation-Exchange Mechanism

Column: SCX, 500mg / 6mL

1. 5g sample + 10mL ACN ⇨ homogenize, filter; dilute 5mL filtrate w. 20mL 1% AcOH
2. Solvation: 3mL methanol
3. Equilibration: 6mL 1% AcOH
4. Sample: 25mL (diluted)
5. Wash: 1mL of 1% AcOH and 1ml of ACN
6. Elution: 2mL of 1:1 ACN - 0.1M K_2HPO_4

Multi-Residue Method (PRS)

Wide range of veterinary drugs with cationic functionality

- Anthelmintics
  - Benzimidazoles, levamisole
- Tranquillizers
- Antibacterials
  - Sulfonamides, quinolones
- β-Agonists

Multi-Residue Method (PRS)

Column: PRS, 500mg / 6mL

1. 2g liver ⇨ 2 x 20mL ACN, Ultra-Turrax; filter, then acidify w. 200µL of AcOH
2. Solvation: 5mL methanol
3. Equilibration: 5mL of ACN : AcOH (200:1)
4. Sample: 40mL (diluted)
5. Wash: 5-5mL of EtAc, acetone, methanol
6. Elution: 5ml of acetone:NH_4OH (sg 0.88) 1:1

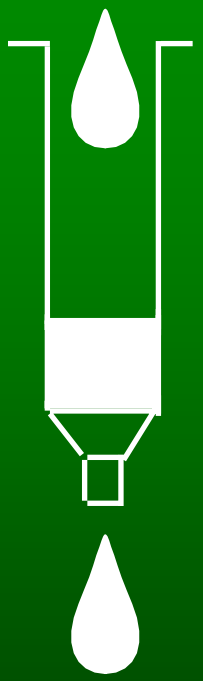
Sample Clean-Up: Four-Step Method

NO trace enrichment

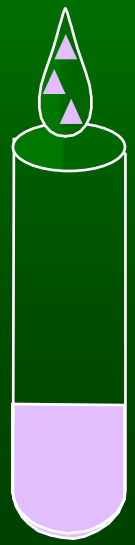
1. Sample pre-treatment
2. Column solvation
3. Column equilibration
4. Interference removal

Four-Step SPE Procedure

Column solvation, equilibration



Sample Application



Purified extract

OP Pesticides in Cherries

Extraction Column:
ISOLUTE SAX / PSA, 1g/6 mL

1. Sample pre-treatment
2. Column solvation
3. Column equilibration
4. Sample application

20g sample + 100 mL ACN. Filter.
Conc. to 25 mL. Add sat'd NaCl. Extract with DCM. Dry with sodium sulfate cartridge. Evap to dryness. Reconstitue with hexane / acetone 95:5

10mL hexane/acetone 95:5

Not required

10mL hexane/acetone 95:5

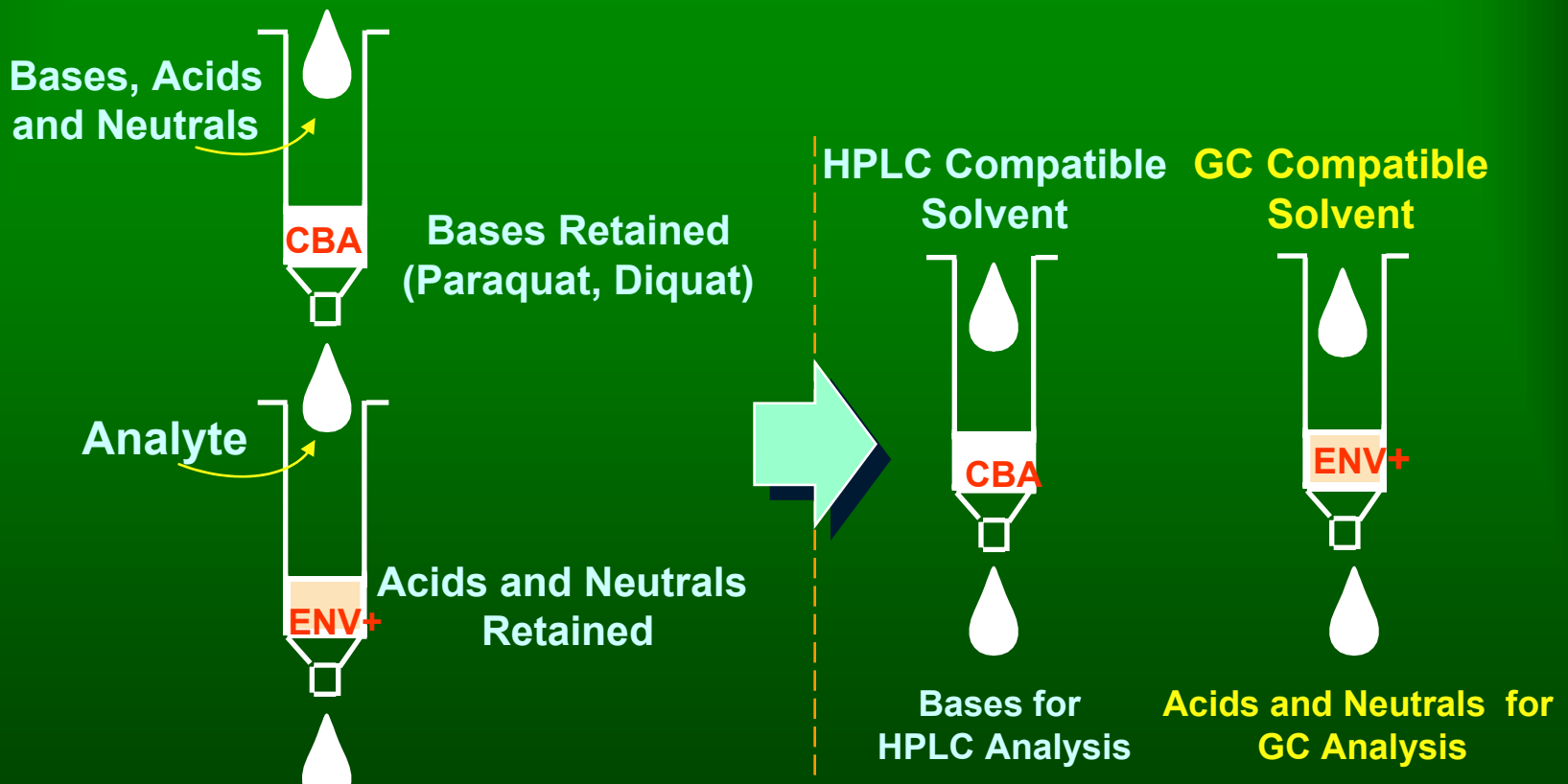
OP Pesticides in Cherries Percent Recoveries

	5% Acetone	10% Acetone
Ethoprophos	97	91
Diazinon	84	82
Etrimphos	92	86
Parathion - methyl	90	87
Fenitrothion	98	97
Pirimiphos - methyl	101	98
Malathion	61	88
Fenthion	93	90
Chlorpyriphos	92	92
Quinalphos	85	86
EPN	81	92

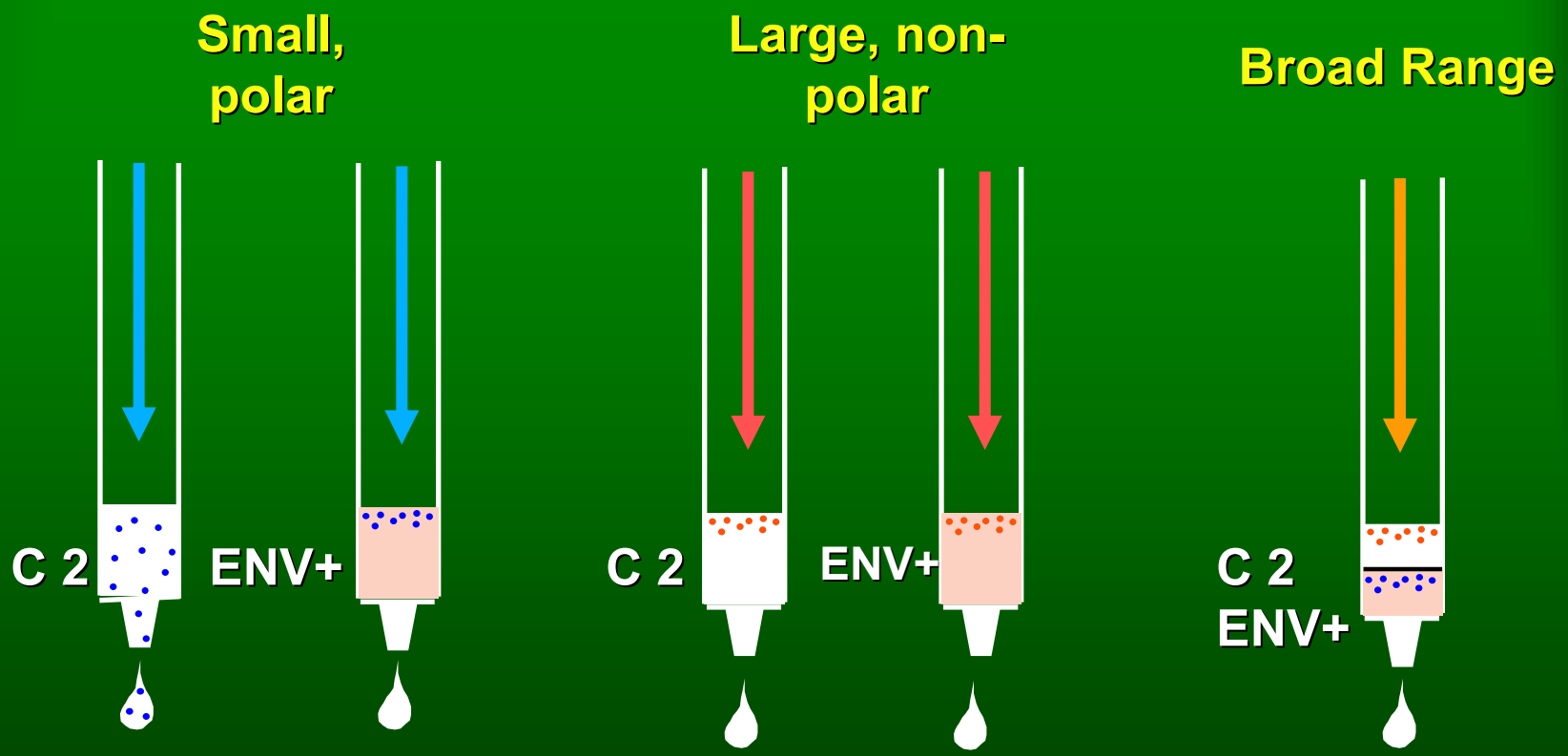
Stacked Columns

- Extending the range
- Enhancing the selectivity
- Method development
- Multiple detection protocol

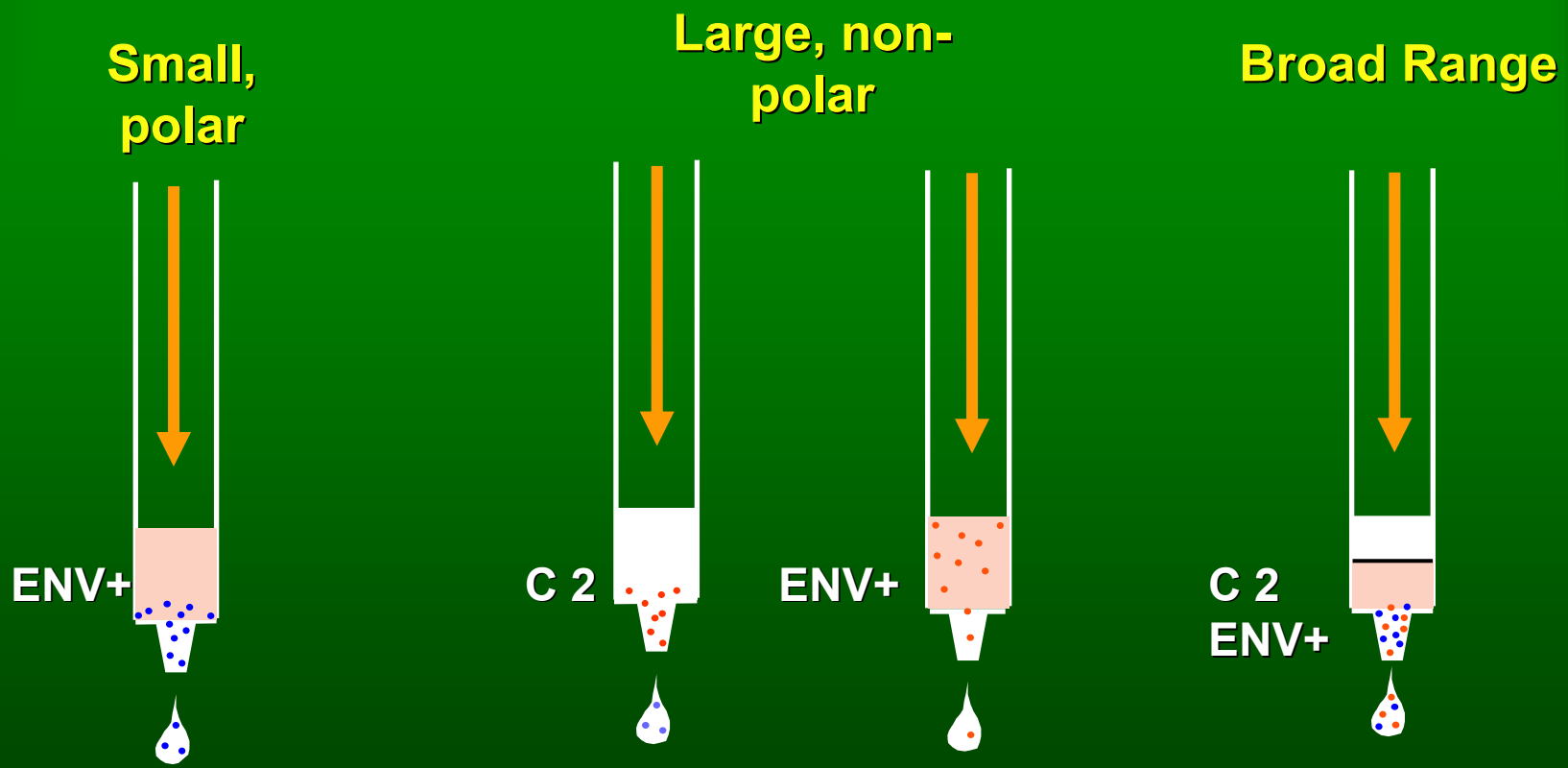
Fractionation (Stacked Columns)



Layered Phases (Loading)



Layered Phases (Eluting)



OC Pesticides from Water Extraction on Layered Phases

ISOLUTE® C2 / C18

Sample pre-treatment	HCl to pH=2, Methanol, 0.5%
Column solvation	5 mL Methanol
Column equilibration	10 mL reagent water
Sample application	One liter in 1/2 hour
Interference elution	10 mL water, 10 min N_2
Analyte elution	Two x 2 mL THF

OC Pesticides from Water Percent Recovery

Pesticides	C18(EC)	C2	C18(EC)	SUM
Aldrin	73	85	0	85
4,4'-DDE	62	86	5	91
Endosulfan II	78	65	37	102
AVG	88	54	43	97
17 pesticides				

What About Solid Samples?

- Tissue
  - growth promoters
  - Anthelmintics
  - Sulfonamides, quinolones
  - β-Agonists
- Vegetables
  - pesticides

Extraction of Solid Samples: Traditional Approach

- Homogenisation
- Liquid / liquid extraction
- Sample clean-up
- Trace enrichment

Extraction of Solid Samples: MSPD Approach

- Homogenise the sample with the sorbent
- Transfer to empty reservoir or clean-up column (Si, NH2, FI, SAX/PSA)
- Elute interferences
- Elute analytes

MSPD Procedure

Homogenise

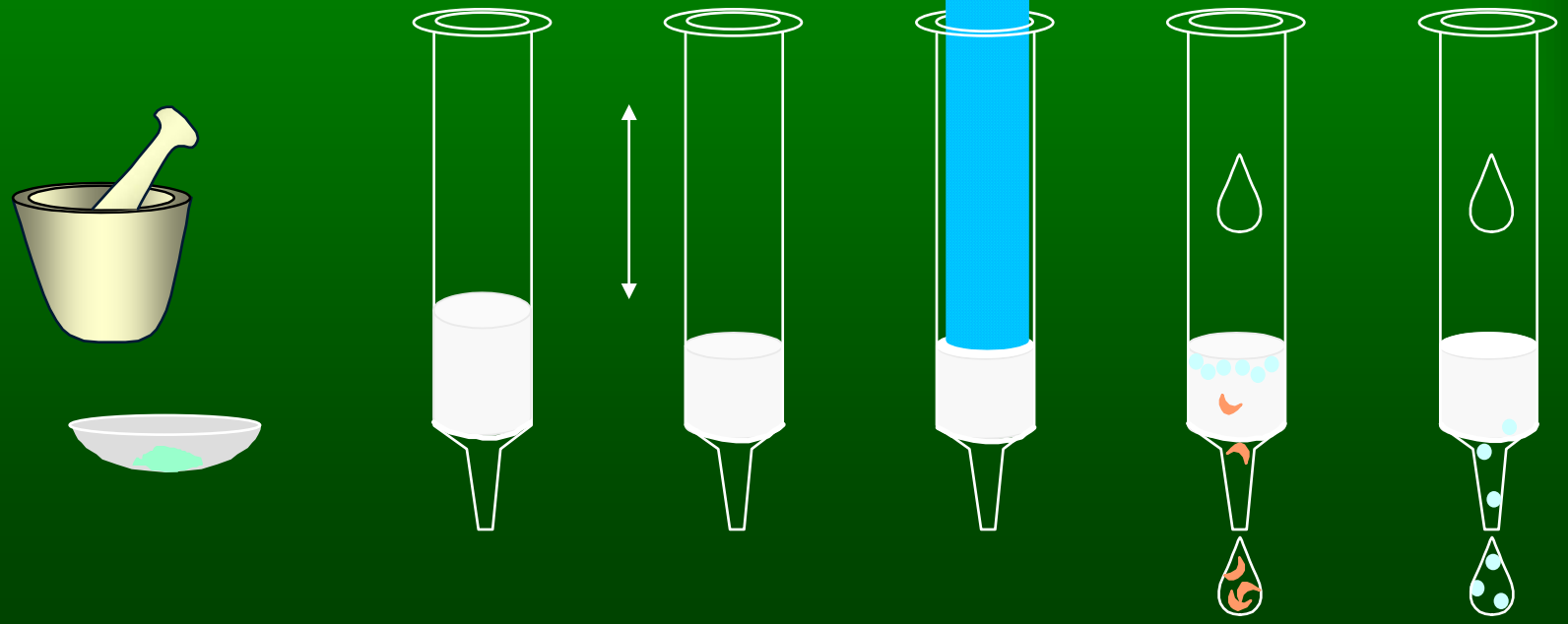
Transfer blend to pre-fitted reservoir

Tap to settle bed

Gently insert top frit with inserter

Elute inter-ferences

Elute analytes



Advantages of MSPD

- Homogenisation, analyte extraction and clean-up are simultaneous
- Less labour intensive
- Less operator dependant
- Time saving
- Low solvent consumption

ISOLUTE® MSPD Sorbents

- Optimized to blend quickly (less than 1min) and easily
- Sample / sorbent blend is homogeneous, dry and free-flowing
- C18(UC) and C18(EC) chemistries are available

Standard vs. MSPD Sorbents

- Sample: bovine liver, fortified with 5 ppb (ng/g) clenbuterol
- MSPD conditions: blend 0.5g sample with 2.0 g sorbent (standard or MSPD grade)
- Analysis: RIA

Standard vs. MSPD Sorbents: Recovery, Reproducibility

Recovery

MSPD C18(EC) n=5	91.0%
Standard C18(EC) n=6	91.0%

RSD

MSPD C18(EC) n=5	5.5%
Standard C18(EC) n=6	15.5%

Pesticides in Fruits and Vegetables: MSPD Procedure

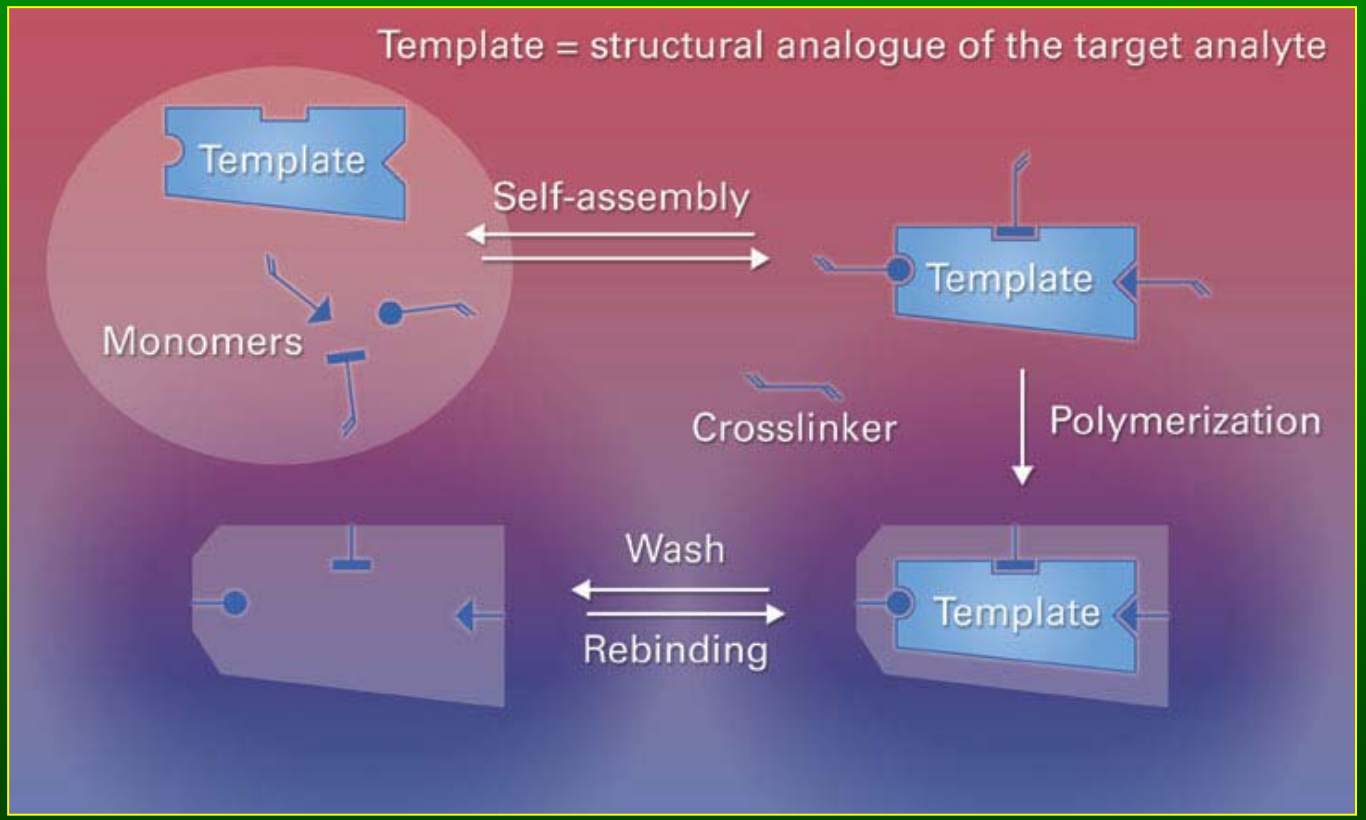
- Mix 100g of sample thoroughly
- Blend 0.5g of sample with a glass pestle into 0.5g of MSPD C18(EC)
- Transfer the mixture into a Silica column (0.5g/6mL); insert top frit
- Elute the analytes with 10mL of ethyl-acetate

Pesticides in Fruits and Vegetables: Recoveries

Aldrin	101	Carbophenothion	86
Captafol	87	Chlorfenvinphos	94
Chlorpyriphos	108	Diazinon	94
Dicofol	105	α-Endosulfan	96
β-Endosulfan	95	Ethion	93
Fenitrothion	98	Folpet	91
Phosmet	66	Malathion	87
Methidathion	85	Methyl-azinphos	57
Methyl-parathion	97	Tetradifon	98

n = 5 (10 — 500 ng / g)

MIP – Molecularly Imprinted Polymers



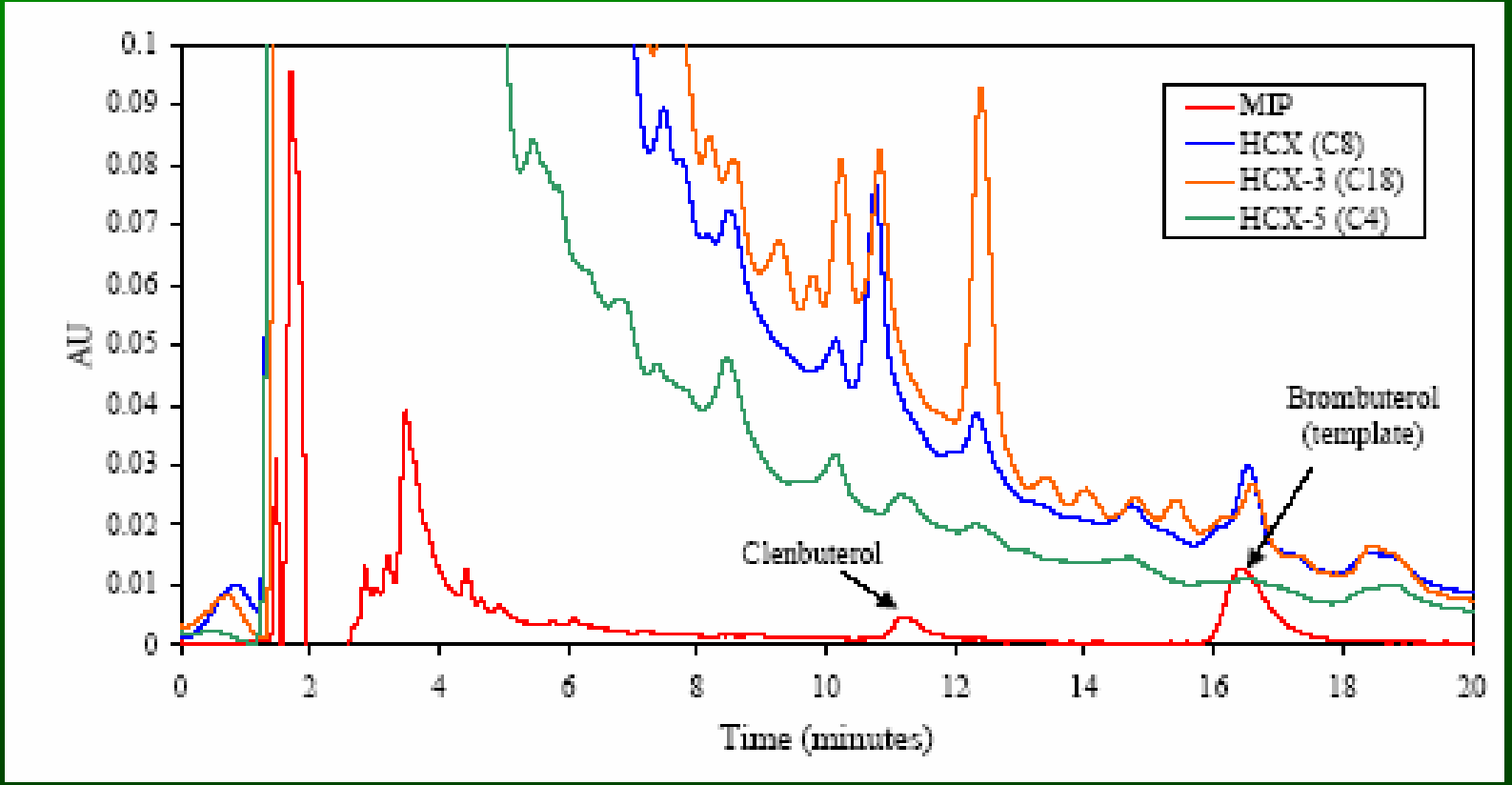
Class-Selective MIPs

- Beta-Agonists
- Triazines
- Nitroimidazoles
- Steroids
- Peptides, Proteins

Unique MIPs

- Clenbuterol
- NNAL
- Riboflavin
- Chloramphenicol
- Nicotine

MIP vs. Mixed-Mode SPE



SUMMARY

- SPE: effective sample clean-up and concentration technique
- New forms of SPE: wide range of analytes can be monitored
- Solid samples can also be processed (MSPD)
- Specialty tubes: selective isolation is possible