INSTITUT DES SCIENCES ANALYTIQUES

UNIVERSITÉ DE LYON

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TUNTWIN

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Workshop/Summer school

Sample preparation

Laure WIEST and Barbara GIROUD

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Commission européenne

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TUNTWIN

Sample preparation, a key step in physico-chemical analysis: extraction, pre-concentration, purification

Liquid matrices

Summary

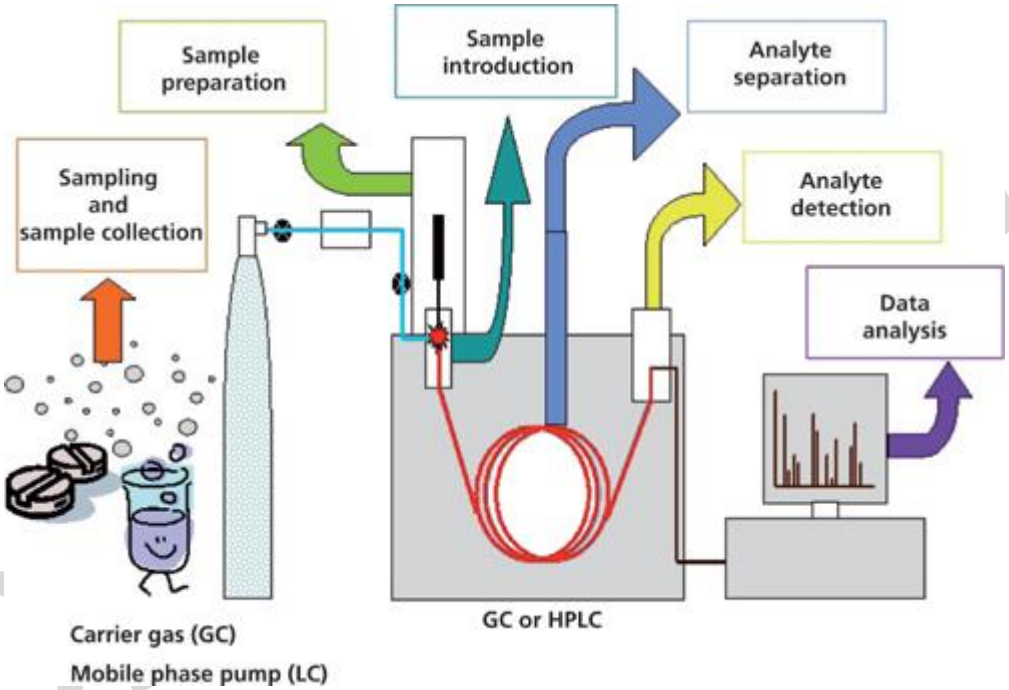
1. **Introduction**
   - Objectives of sample preparation
2. **Strategies for liquid matrices**
   - Liquid-liquid extraction: conventional and miniaturised
   - Solid phase extraction: conventional and miniaturised

Introduction

Objectives of sample preparation

Introduction : sample preparation

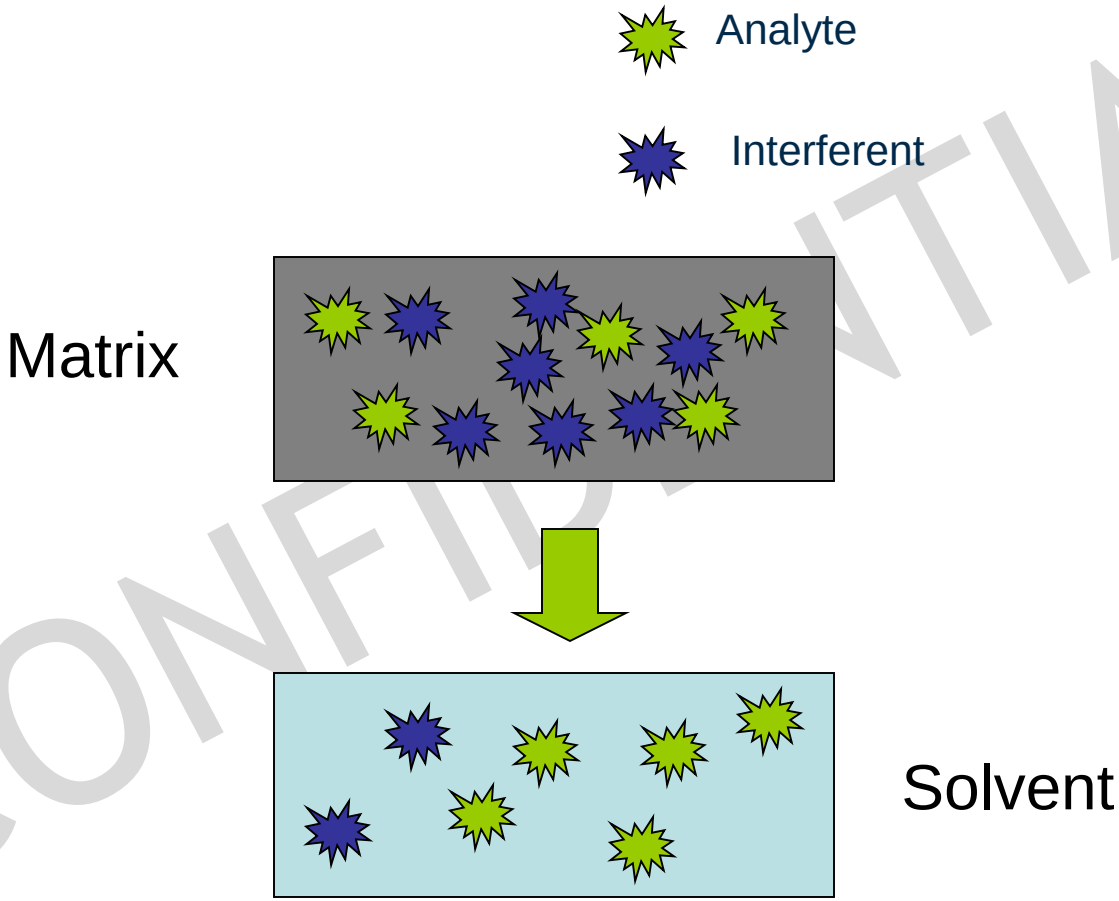
Why?



D. Turner, *LCGC North Amer.* **30**(2), 100–110 (2012)

Introduction : sample preparation

Principle of extraction



Introduction : sample preparation

cause	problem
Sample not liquid	Impossible injection
Too high concentration	Column overload / out of calibration range
Too weak concentration	Not detectable
Presence of particles	Plugged
Presence of compounds that can cause damage to the analytical column	
Presence of interferents for quantification	Quantification error
Solvent not adapted	Column deterioration

Introduction : sample preparation

cause	solution
Sample not liquid	Extraction / dissolution
Too high concentration	dilution
Too weak concentration	Concentration / derivatisation
Presence of particles	Centrifugation / filtration
Presence of compounds that can cause damage to the analytical column	Solvent extraction / derivatisation
Presence of interferents for quantification	Solvent extraction / derivatisation
Solvent not adapted	Solvent exchange / Injection volume

Objectives of sample preparation

How to increase sensitivity?

Signal

- extraction recovery
- concentration

F enrichment = (Extracted volume/ final volume) x extraction recovery

Noise

- purification
- dilution

Extraction of compounds from the matrix

Concentration of traces

Removal of interferents

The most commonly used techniques

Methodology
- Direct Injection
- Filtration
- Centrifugation
- Dilute and shoot
- Sonication
- Lyophilization
- Protein precipitation
- Distillation
- Dialysis and ultrafiltration
- Liquid–solid extraction and pressurized fluid extraction
- Soxhlet extraction
- Solid-phase microextraction
- Supported liquid extraction
- Liquid–liquid extraction
- Solid-phase extraction
- QuEChERS
- Turbulent flow chromatography
- Derivatization
- Column switching and heart cutting
- Immunoaffinity sorbents
- Molecularly imprinted polymers

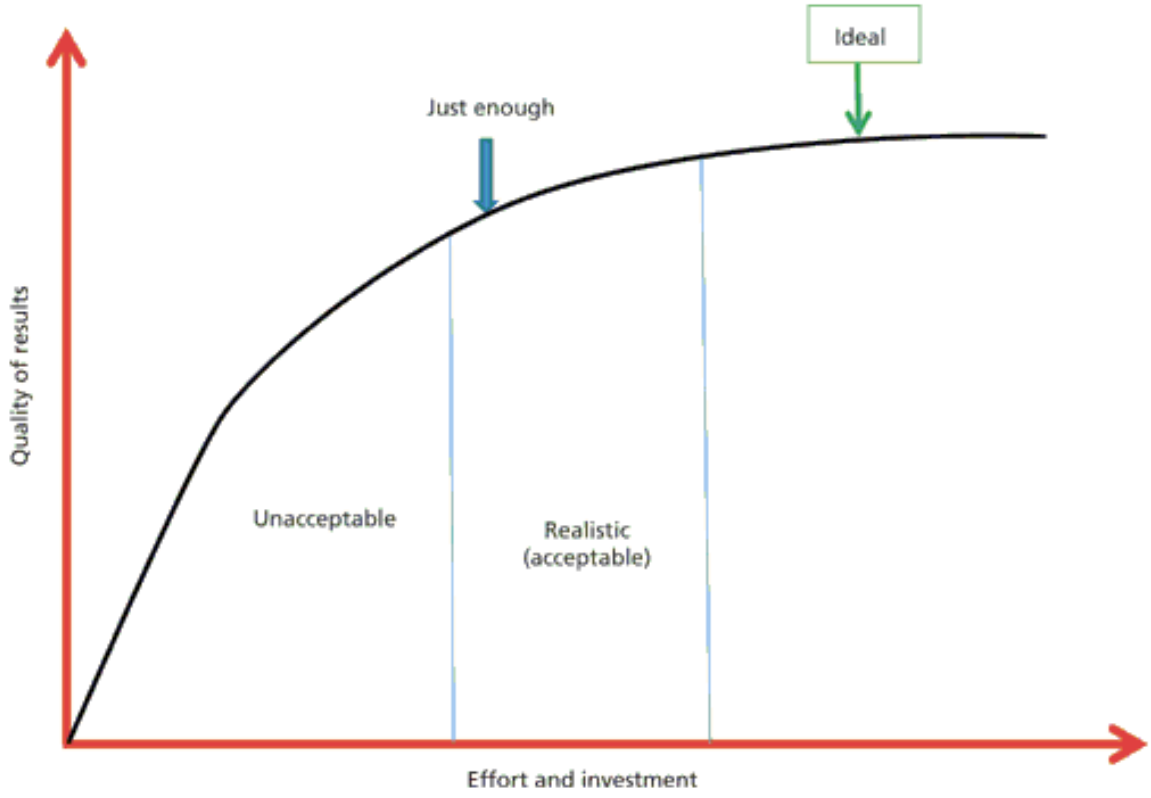
Simpler, generic methodology ↓ More complicated methodology

Less selective, Minimal sample cleanup ↓ Greater selectivity, Optimal sample clean up

LC-GC North America, Vol. 30, No. 12, 2012

Adapting sample preparation to final needs

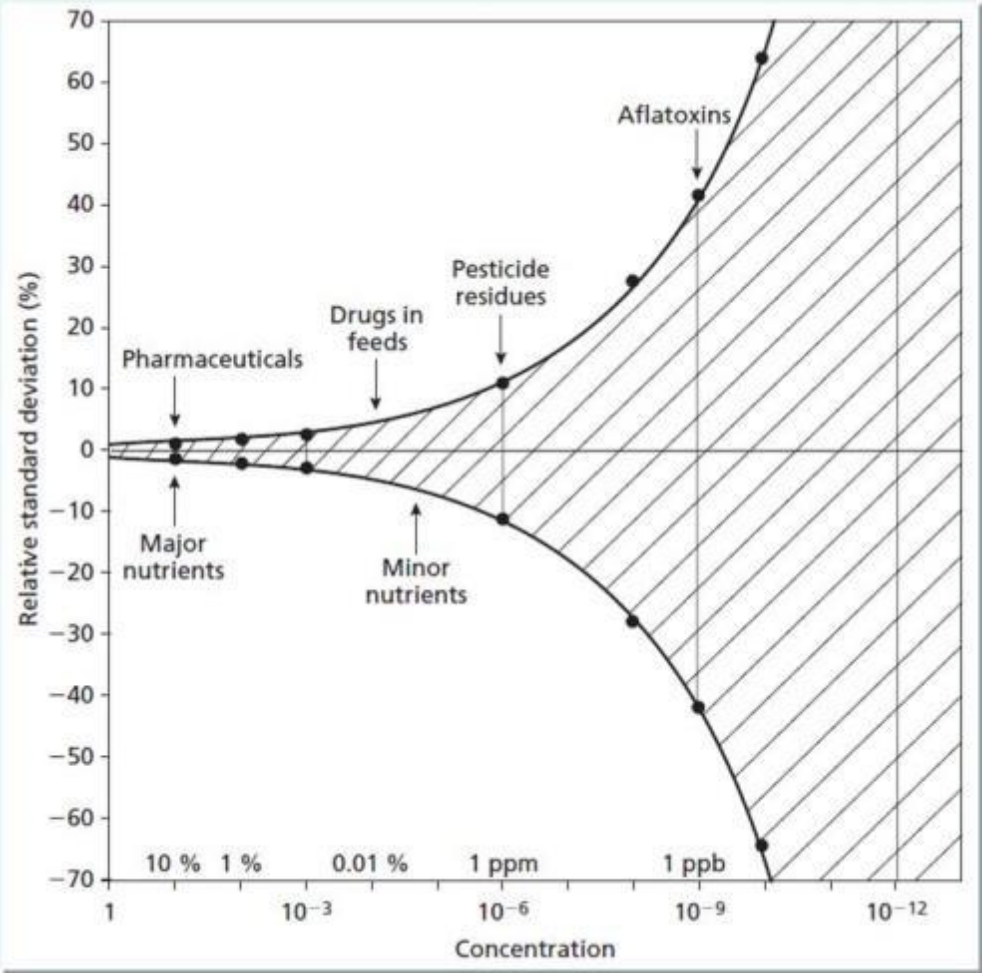
« Just enough »



LC-GC North America, Vol. 30, No. 12, 2012

Adapting sample preparation to final needs

Taking into account the concentration of analytes



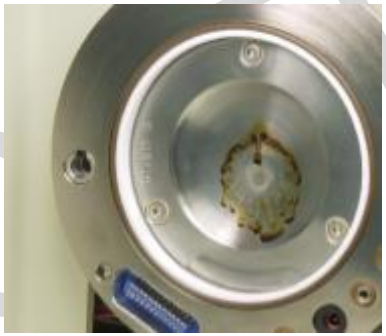
W. Horwitz, L.R. Kamps and K.W. Boyer, J. Assoc. Off. Anal. Chem., 63, 1344–1354 (1980).

Development of strategies before analysis

Exhaustive extraction

Extraction of compounds and interferents

- **Short development time**
- **BUT purification step necessary and sometimes complicated**



Selective extraction

Preferential extraction of compounds of interest over interferents

- **Long development time**
- **BUT purification step easier to implement and sometimes not necessary**

Strategies for liquid matrices

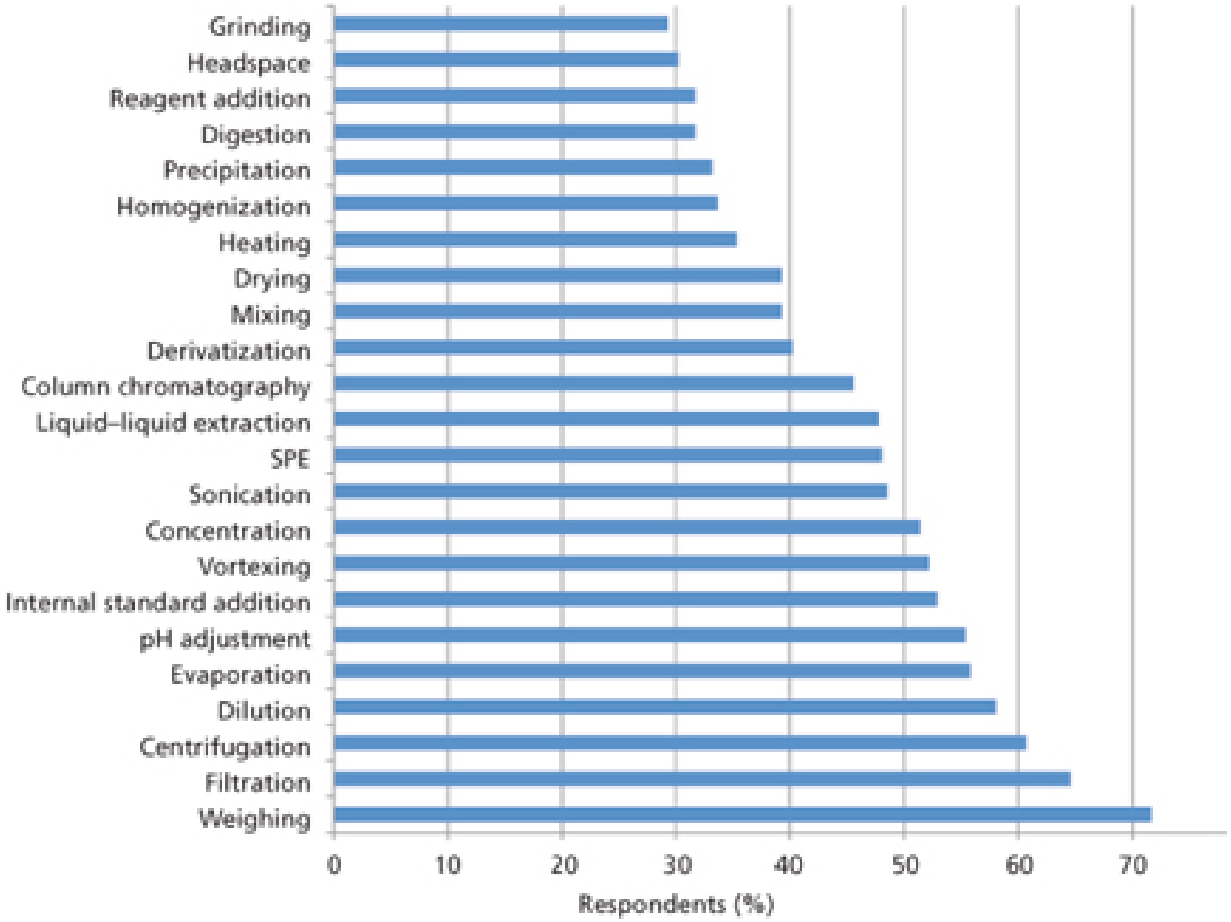
Strategies for liquid matrices

- Pre-treatment

- Liquid-liquid extraction
- LLE
- DLLME

- Solid phase extraction
- SPE
- MEPS
- SBSE

Liquid matrices: pre-treatment



Most used

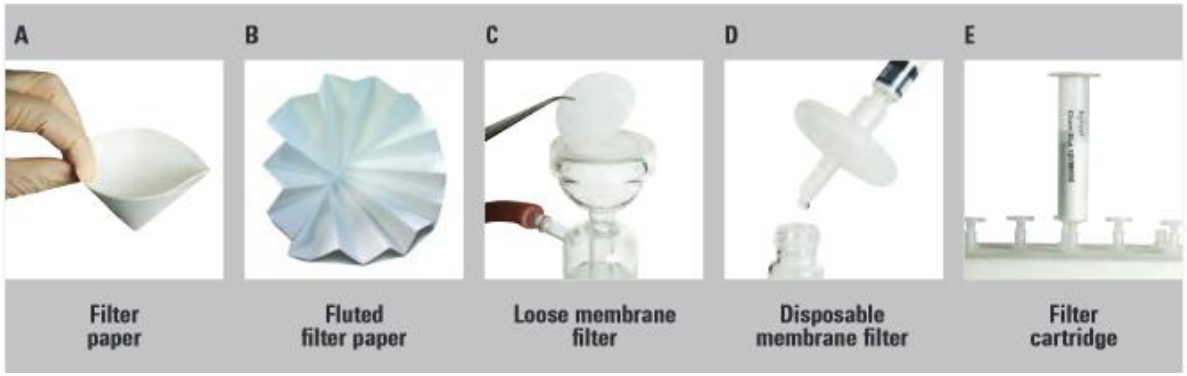
R.E. Majors, LCGC North Am. 31(3), 190–202 (2013).

Liquid matrices: pre-treatment

- Pre-treatment : filtration : filter paper, membranes, functionalized membranes, SPE discs or cartridges

  centrifugation

  dialysis…



Diameter [mm]	Sample Volume [mL]	Hold Up Volume [µL]	Effective Filter Area [cm²]
30	1-50	<50	5.1
25	1-30	<30	3.5
13	1-10	<10	0.75
3	<1	<7	0.0

Sample preparation fundamentals for chromatography, Agilent Technologies®

Liquid matrices: pre-treatment

Tableau 3 – Caractéristiques des membranes filtrantes

Matériau	Dimensions des pores (µm)	Utilisation
Esters mixtes de cellulose	0,1 à 8,0	Applications courantes mettant en jeu des solutions aqueuses Haut débit Faible perte de charge
Nitrate et acétate de cellulose	0,2 à 5,0	Filtration stérile Faible adsorption protéique
Polyamide	0,2 à 20	Membrane hydrophile Filtration de solutions aqueuses, de solvants organiques et de préparations biologiques
PTFE	0,2 à 10	Membrane hydrophobe Très stable à la température Filtration des acides, des bases fortes et des solvants
Polysulfone	0,2 à 0,8	Filtration stérilisante Concentration de macromolécules
PVDF	0,2 à 5	Filtration stérilisante Clarification de solutions biologiques

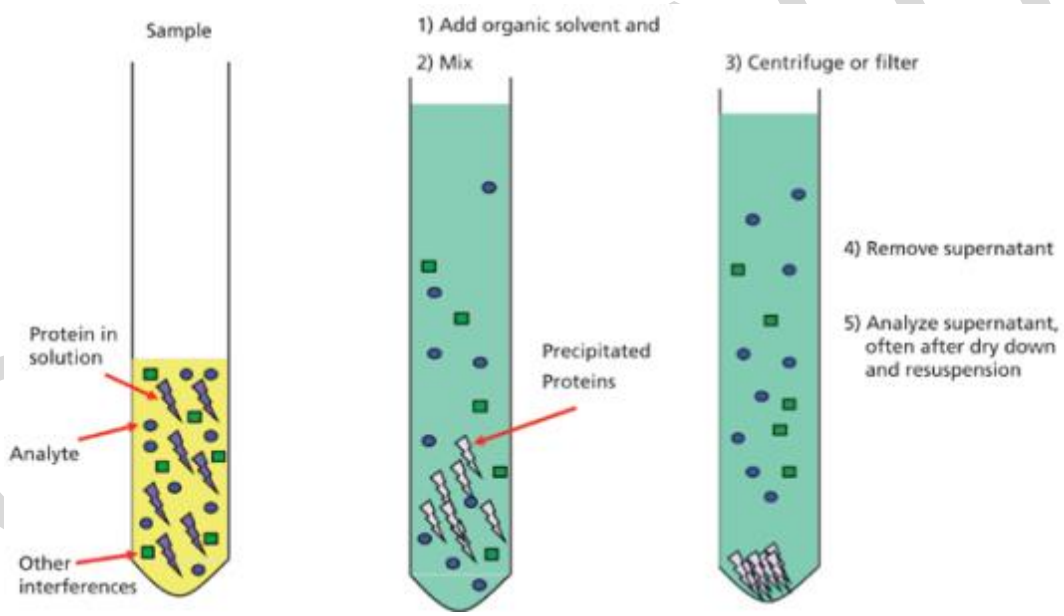
Tableau 4 – Compatibilité chimique des matériaux

Nature de la fibre	Résistance aux		
	acides/bases	oxydants	solvants
Nitrate et acétate de cellulose	Utilisation déconseillée aux solutions concentrées	Sous réserve	Utilisation déconseillée
PVC	Excellente sauf pour les solutions acides ayant des fonctions oxydantes	Excellente	Gonflement de la fibre
PTFE	Excellente	Excellente	Excellente
PVDF	Excellente	Excellente	Sous conditions
Polyéthersulfone	Excellente	Sous conditions	Utilisation déconseillée
Polycarbonate	Acceptable	Sous conditions	Utilisation déconseillée
Polyamide	Sous conditions	Acceptable	Acceptable

Techniques de l'ingénieur, 2013

Liquid matrices: pre-treatment

- Pre-treatment : case of biological matrices
  - ➢ Precipitation of proteins: blood, plasma, serum, milk
    - NaCl, saturated ammonium sulphate, phosphate buffer
    - Acetonitrile, methanol, Zn sulphate/methanol



LC-GC North America, Vol. 30, No. 12, 2012

Liquid matrices: pre-treatment

- Pre-treatment : case of biological matrices

  - Precipitation of proteins: blood, plasma, serum, milk

  - Hydrolysis of glucuronide and sulphate metabolites:
    - hydrochloric, acetic, formic acids
    - β-glucuronidase /sulfatase enzymes (incubation, T°, pH, [enzyme])

Strategies for liquid matrices

Liquid-liquid extraction

Liquid-liquid extraction

Analyte

↓

Organic phase not miscible with the matrix

↓

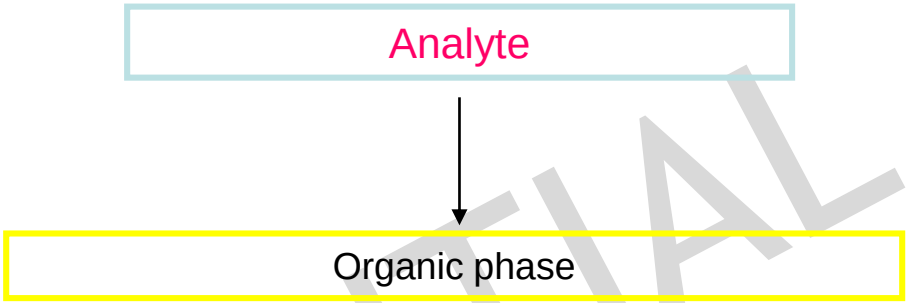
Concentration

↓

Injection

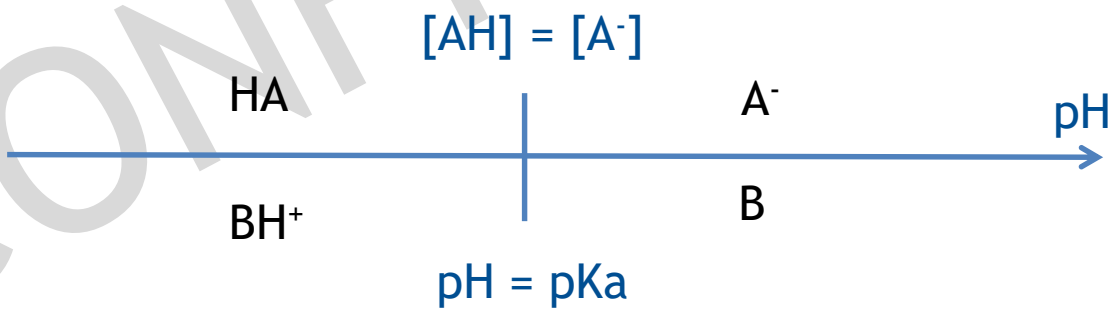
Abe et al. (2010)

Liquid-liquid extraction

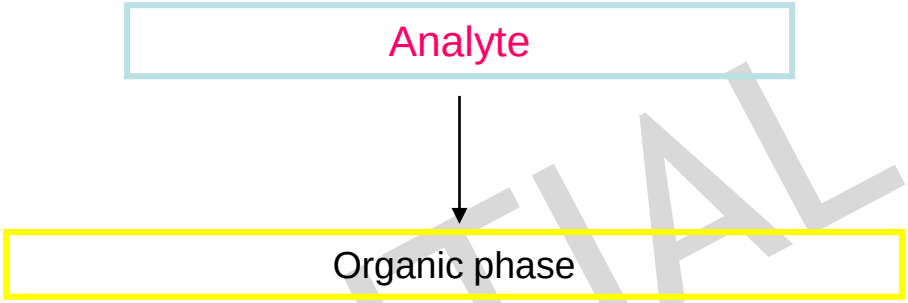


- Acidity constant pKa of the analyte

The analyte must be in neutral form to be extracted by an organic solvent



Liquid-liquid extraction



- Acidity constant pKa
- Octanol/water partition coefficient LogP ($LogK_{ow}$)

The higher the LogP, the more lipophilic the substance is

Liquid-liquid extraction

Analyte

↓

Organic phase

- Acidity constant pKa
- Octanol/water partition coefficient LogP (LogKow)

- Sources:
  - Publications
  - PubChem
  - Chemspider
  - Chemicalize

Octanol/water partition coefficient Log P

Eau
Methanol
Isopropanol
Acetonitrile
Acetone
Ethyl acetate
Ether
Tétrahydrofurane
Dichloromethane
Chloroforme
Toluene
Iso-octane
Hexane

POLAIRE LogP <3

3<LogP <5

LogP >5

APOLAIRE

Liquid-liquid extraction

- One solvent
- Solvent mixture

Solvent Polarity Chart

Relative Polarity	Compound Formula	Group	Representative Solvent Compounds
Nonpolar	R - H	Alkanes	Petroleum ethers, ligroin, hexanes
	Ar - H	Aromatics	Toluene, benzene
	R - O - R	Ethers	Diethyl ether
	R - X	Alkyl halides	Tetrachloromethane, chloroform
	R - COOR	Esters	Ethyl acetate
	R - CO - R	Aldehydes and ketones	Acetone, methyl ethyl ketone
	R - NH_2	Amines	Pyridine, triethylamine
	R - OH	Alcohols	Methanol, ethanol, isopropanol, butanol
	R - $COHN_2$	Amides	Dimethylformamide
	R - COOH	Carboxylic acids	Ethanoic acid
Polar	H - OH	Water	Water

Increasing Polarity

Liquid-liquid extraction

Analyte

↓

Organic phase

- One solvent
- Solvent mixture:
  - Advantage:

Extraction of a larger number of compounds of interest

  - Disadvantage :

Extraction of a larger number of compounds:

Interferences of the matrix

Liquid-liquid extraction

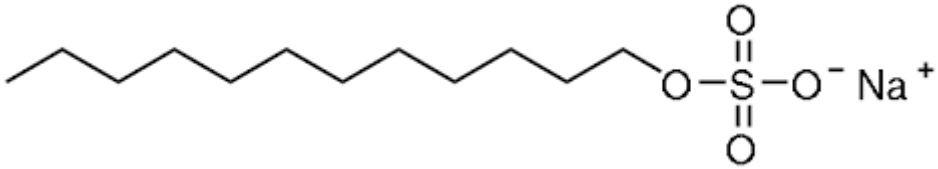
Analyte

↓

Organic phase

- One solvent
- Solvent mixture
- Ionic analyte: counter-ion

Ex: quaternary ammonium for anionic surfactants



sodium dodecylsulfate

Liquid-liquid extraction

Analyte

↓

Organic phase

- Optimisation
  - Solvent :
    - Choice of solvent(s)
    - Volume, number of extractions
  - Vortex, ultrasound
  - Centrifugation

Salt assisted liquid-liquid extraction

1 mL of blood in a 15 mL tube

↓

Add 2 mL of acetonitrile + $MgSO_4$+NaCl + IS solution

Centrifugation

↓

Purification

Centrifugation

↓

Analysis

Time: 30 min for 8 samples

Analytes: pharmaceuticals

Matrix: blood

Analysis: GC-MS

Plössl et al. (2006)

Salt assisted liquid-liquid extraction

Drug data		Recovery data Spiking level: 100 ng mL^{-1} ($n = 3$)	
Name	M_R	Recovery (%)	RSD (%)
Acetaminophen	151	90.7	10.1
Amitriptyline	277	95.6	5.8
Articaine	284	98.1	11.2
Atropine	289	101.7	8.9
Benzocaine	265	99.2	6.3
Biperidene	311	102.9	7.9
Caffeine	194	87.1	12.3
Cholesterol	386	[a]	[a]
Codeine	299	95.7	9.8
Diazepam	284	103.7	6.2
Dienestrol	266	92.5	11.3
Diethylstilbestrol	268	89.9	15.7
Diphenhydramine	255	98.9	7.0
Doxepine	279	97.8	8.2
Escitalopram	324	93.5	12.4
Estradiol	272	85.0	14.1
Imipramine	280	98.1	5.2
Lidocaine	234	103.1	11.2
Memantine	179	103.5	7.2
Methylphenobarbital	246	94.3	13.1
Nicotine	162	99.2	11.1
Papaverine	339	90.2	18.9
Paroxetine	329	94.1	7.3
Phenobarbital	232	101.2	13.3
Phenytoine	252	95.9	8.2
Pridinol	295	101.3	9.8
Prilocaine	220	98.6	7.4
Procaine	236	94.3	7.9
Scopolamine	303	94.8	13.0
Sparteine	234	98.4	12.2
Strychnine	334	86.0	15.1
Testosterone propionate	344	91.0	14.3

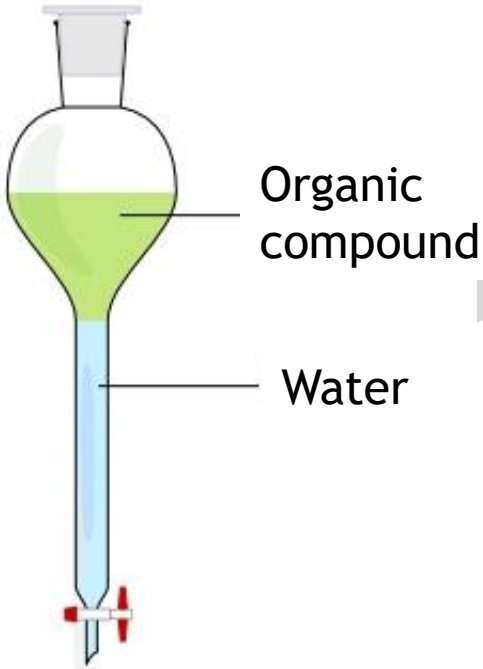
Analytes: pharmaceuticals

Matrix: blood

Analysis: GC-MS

Plössl et al. (2006)

Liquid-liquid extraction : LLE



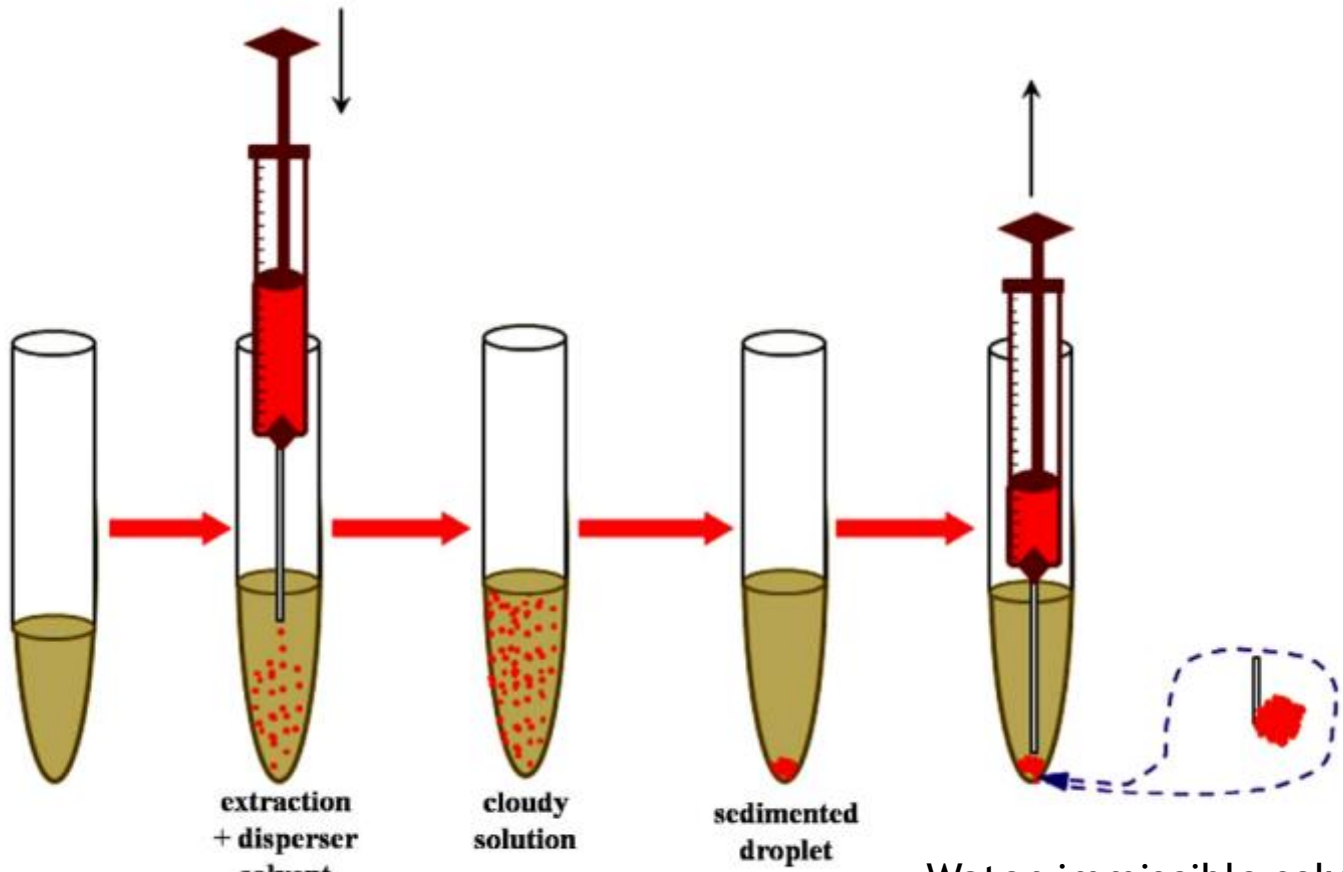
- Large volume of solvent
- Difficult to automate

Miniaturisation: Dispersive Liquid-Liquid Micro Extraction

DLLME

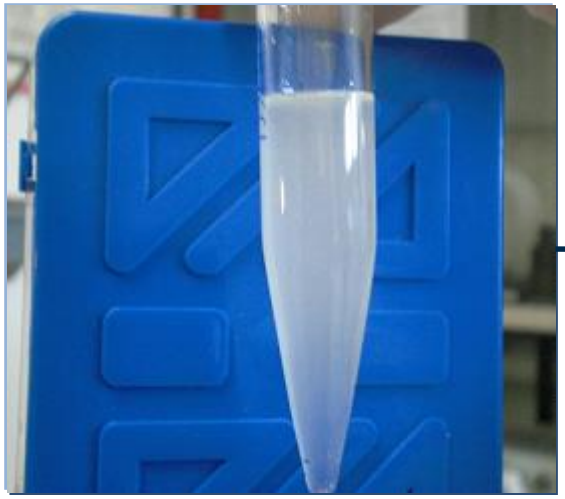
Liquid-liquid micro extraction : DLLME

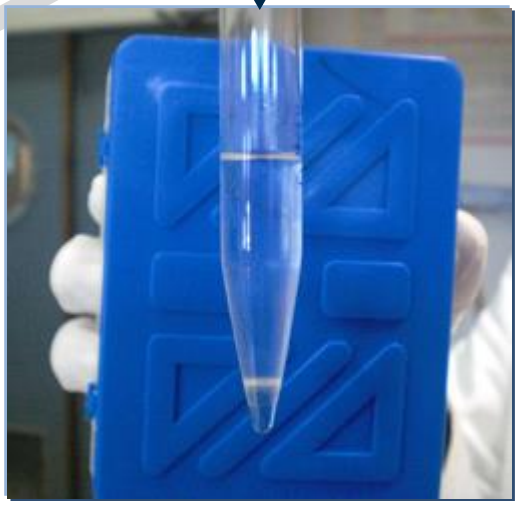
R. Jain, R. Singh/Trends in Analytical Chemistry 75 (2016) 227–237



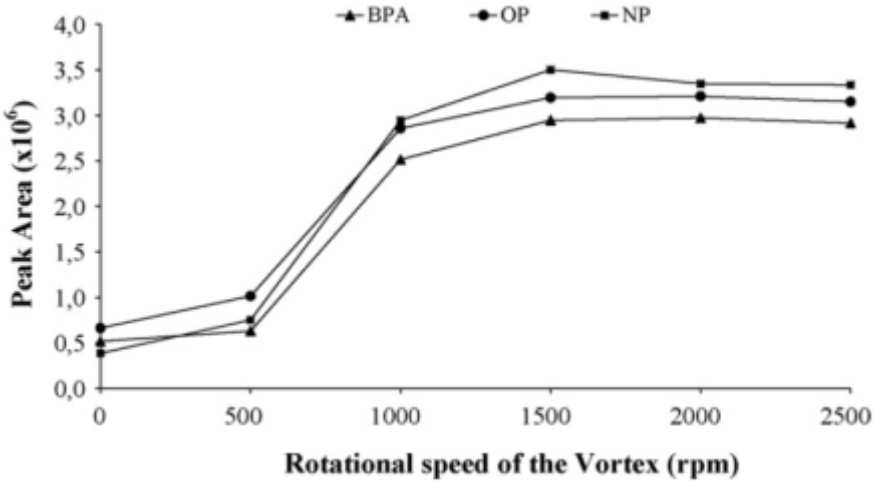
Water-immiscible solvent: 10-50µL
Water miscible dispersant solvent: 0.5-2mL
Sample: 10mL

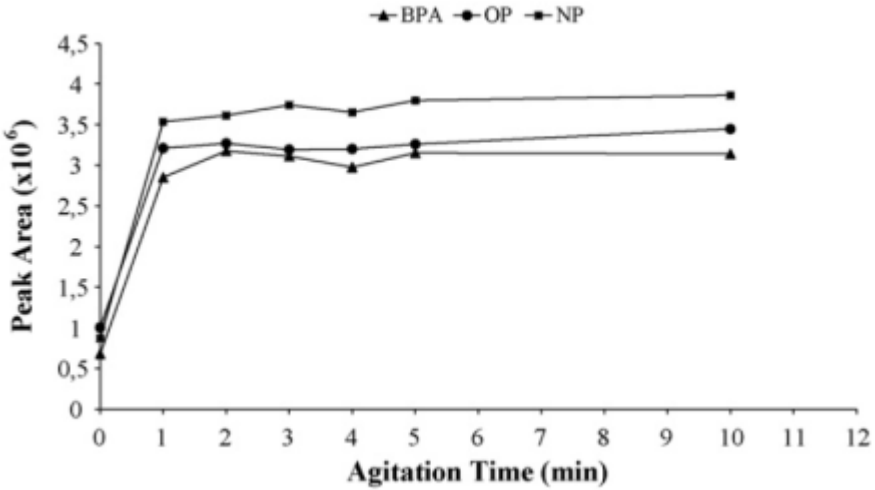
Liquid-liquid micro extraction : DLLME





Liquid-liquid micro extraction : DLLME





Analytes: alkylphenols

Matrix: river water, wastewater

Volume: 20 mL

Extraction solvent: octanol, Vol: 50 µL

Analysis: HPLC-FLUO

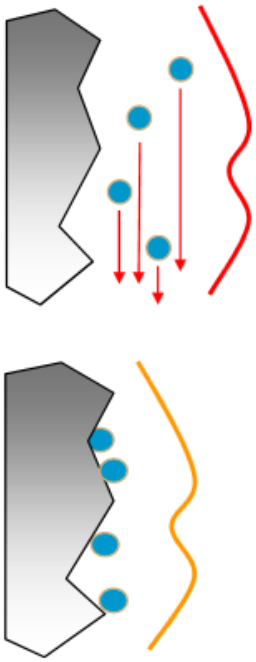
Yiantzi et al. (2010)

Liquid matrices

Solid Phase Extraction

Solid Phase Extraction : SPE

SPE ≡ liquid chromatography



Agilent, 2011, sample “pre-treatment”

Solid Phase Extraction : SPE

Different adsorbent packages available for solid phase extraction

Source : Waters® Corporation

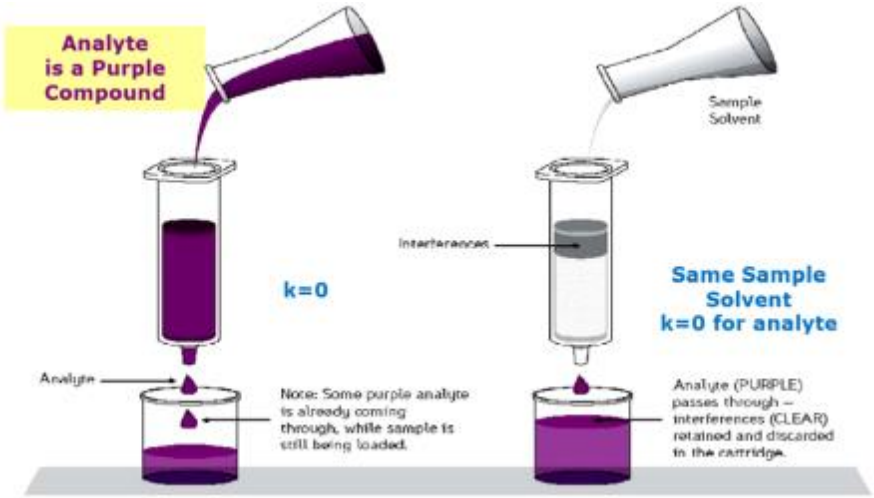
Source : Thermo ® Autotrace

Solid Phase Extraction : 2 strategies

❖ **Pass through**

✓ retention of interferents on the cartridge
no interaction of compounds of interest
easy clean-up, little development

✗ No concentration of analytes



Source Waters®

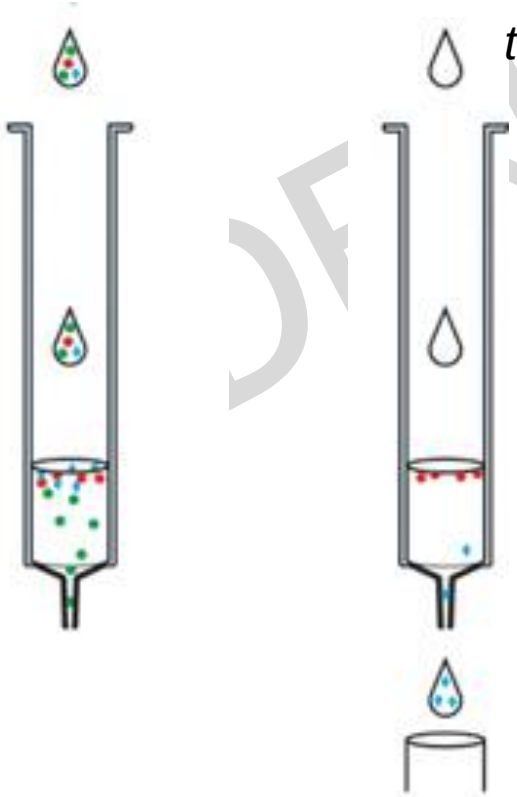
❖ **Capture (retention of compounds of interest)**

✓ Clean-up, desalting, concentration, solvent change, fractionation

✗ Development necessary

Supported Liquid Extraction SLE

Ex: Benzodiazepines in blood

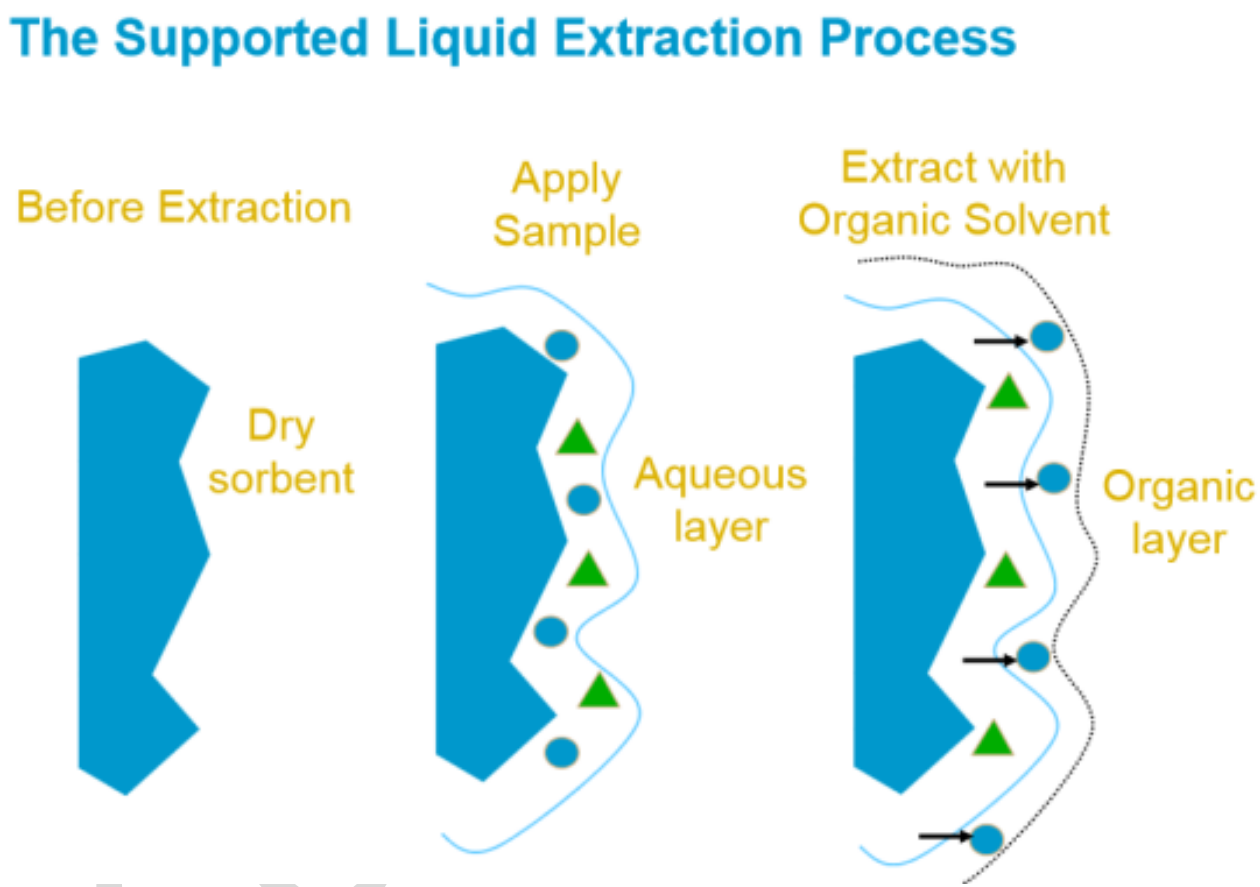


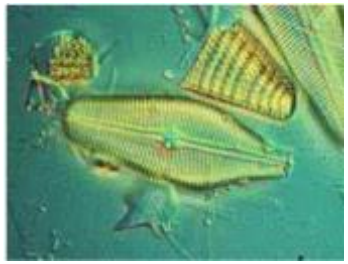
t-butylmethylether

diatomaceous earth

Smink et al. (2004)

Supported Liquid Extraction SLE





Agilent, 2011, sample “pre-treatment”

Supported Liquid Extraction SLE

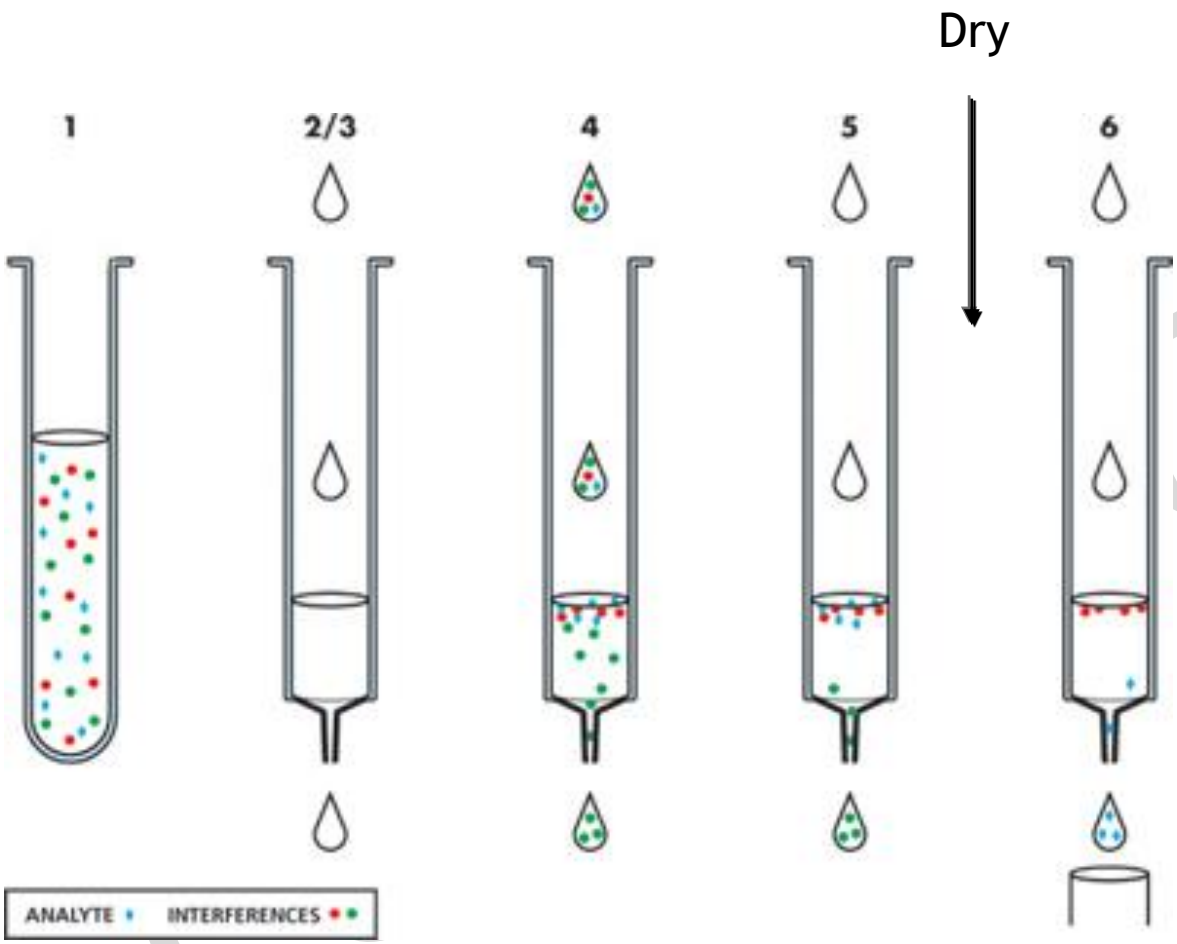
Solvent Mixtures Compatible with SLE

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

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

Agilent, 2011, sample “pre-treatment”

Solid Phase Extraction : SPE



2/3 : rince/wash + conditionning

4 : load

5 : wash

6 : elute

Solid Phase Extraction : SPE

2/3 : Rince/wash + conditionning:

wetting the stationary phase with an organic solvent and then with a solvent close to the sample matrix, to activate the retention sites, the seat of molecular interactions

4 : Load : quantitative retention of analytes of interest on the stationary phase

moderate sample flow rate (<10 mL/min)

5 : Wash : non-systematic step to eliminate weakly retained interferents

Dry : dry the substrate to evaporate traces of washing solvent

6 : Elute : use the solvent with the lowest possible eluting force

capable of carrying away all the molecules of interest,

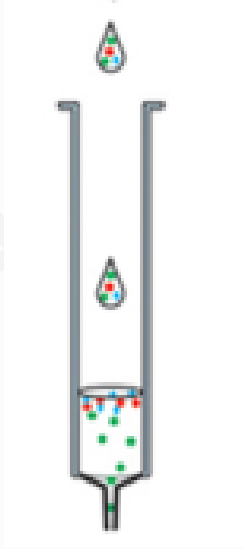
thus avoiding eluting strongly retained interferents

Solid Phase Extraction : SPE

Optimisation

- Choice of the cartridge
  - Solid phase nature

The solid phase should retain the analytes



Solid Phase Extraction : choice of solid phase

- **SILICA**
  - **REVERSED PHASE**
  - **NORMAL PHASE**
  - **ION EXCHANGE**
  - **MIXED MODE**
- **POLYMERIC**

Solid Phase Extraction : SPE

REVERSED PHASE

stationary phase

Apolar

Analytes apolar

Interferents polar

NORMAL PHASE

stationary phase

Polar

Analytes polar

Interferents apolar

Solid Phase Extraction : silica based

REVERSED PHASE

stationary phase

Apolar

NORMAL PHASE

stationary phase

Polar

Bonded silica:

- Octadecyl (C18)
- Octyl (C8)
- Phenyl

Bonded silica:

- Cyano
- Diol
- Amine

Florisil

Solid Phase Extraction : silica based

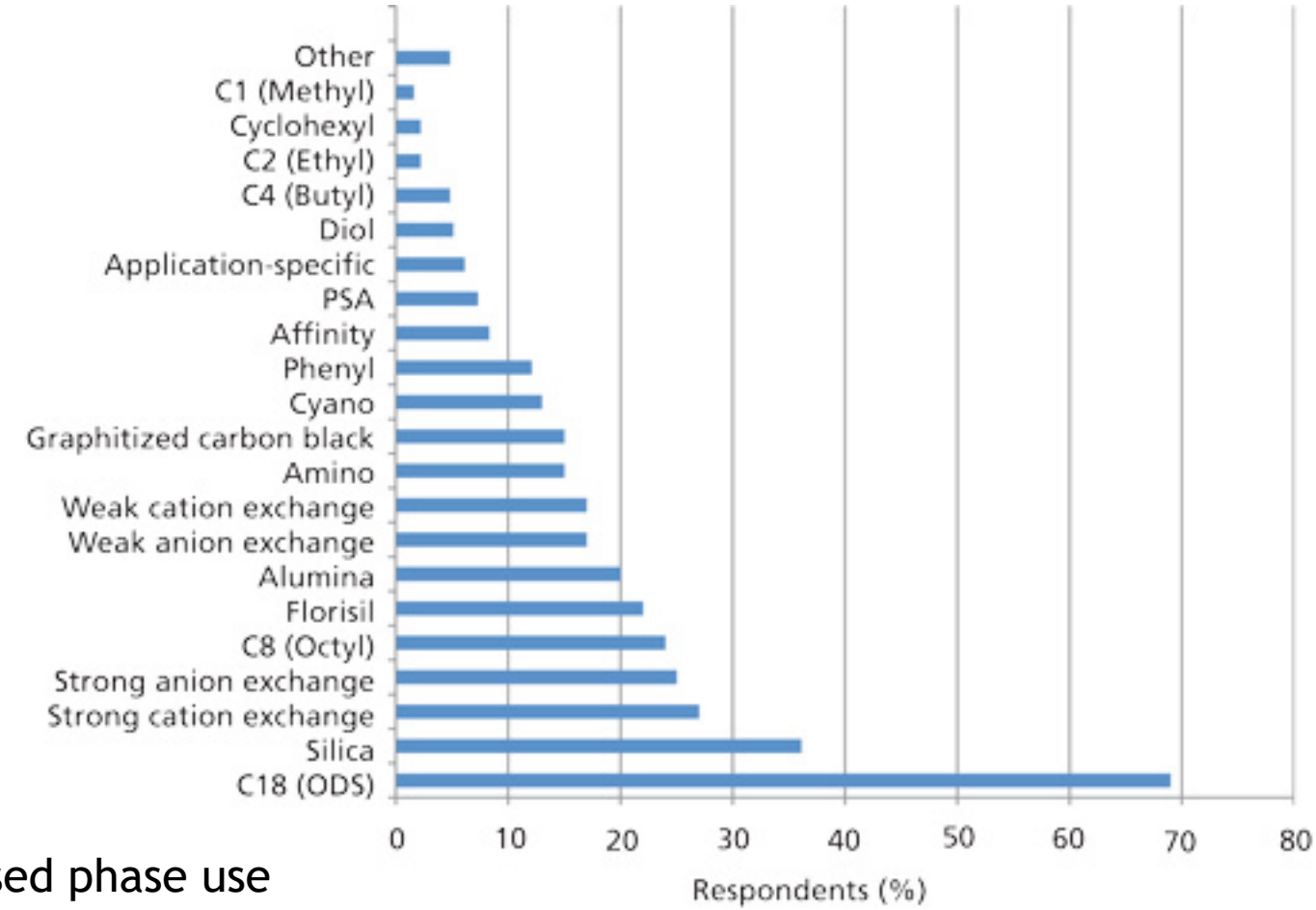
- Stable at pH between 2 and 7.5
- More selective than polymeric
- Lower load capacity due to lower specific surface area

Tableau I. Exemple de quelques phases stationnaires classées en fonction de leurs polarités.

C18	Octadecyl	PHASES APOLAIRES
C2	Éthyl	
CH	Cyclohexyl	
PH	Phényl	Polarité croissante
CN	Cyanopropyl	
2OH	Diol	
NH_2	Aminopropyl	PHASES POLAIRES
Si	Silica	

Annales de Toxicologie Analytique 2010; 22(2): 61-68

Solid Phase Extraction : silica based



Silica-based phase use

R.E. Majors, LCGC North Am. 31(3), 190–202 (2013).

Solvent elution strength

REVERSED PHASE

Eau
Methanol
Isopropanol
Acetonitrile
Acetone
Ethyl acetate
Ether
Tétrahydrofurane
Dichlorométhane
Chloroforme
Toluene
Iso-octane
Hexane

WEAK

HIGH

NORMALP HASE

Hexane
Iso-octane
Toluene
Chloroforme
Dichlorométhane
Tetrahydrofurane
Ether
Ethyl acetate
Acetone
Acetonitrile
Isopropanol
Methanol

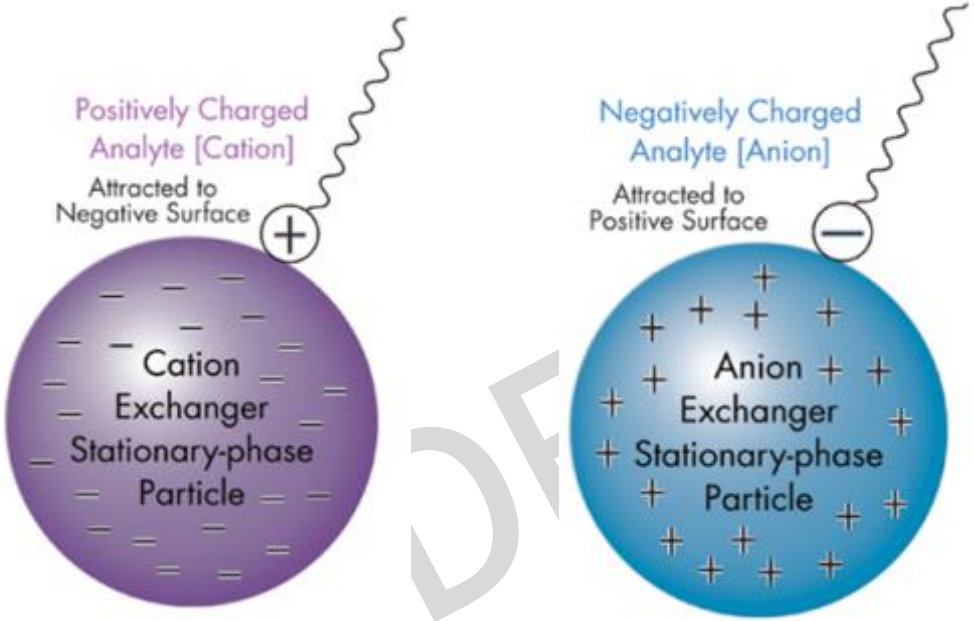
Solid Phase Extraction : silica based

- Ion exchange SPE

SCX

Sulfonate

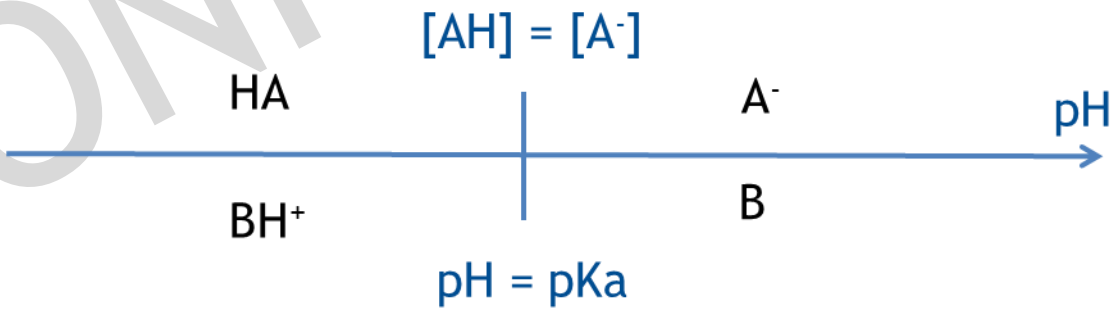
Carboxylic acid



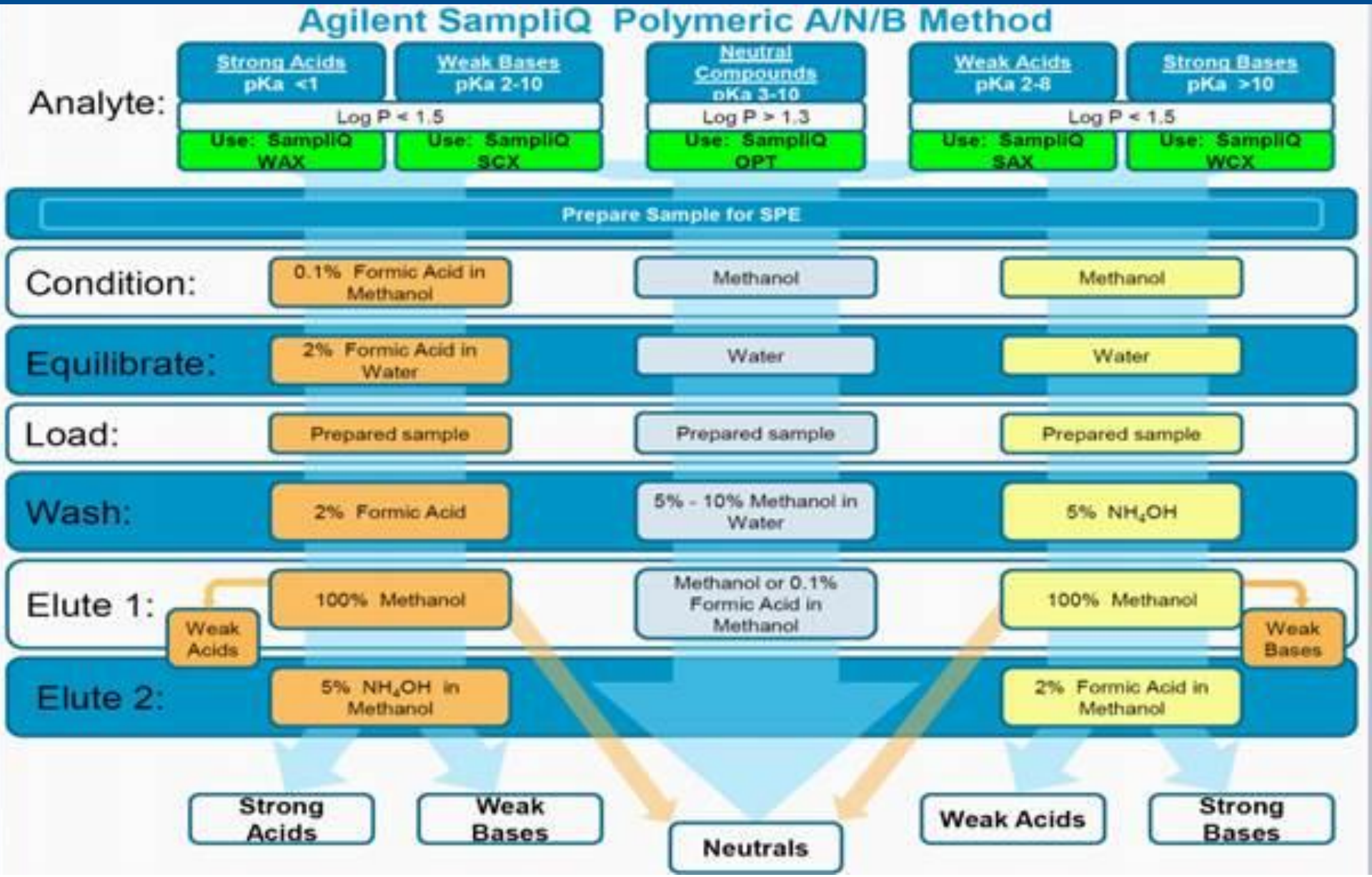
SAX

Quaternary amine

Source: Waters



•Ion exchange SPE



Solid Phase Extraction : silica based

•Ion exchange SPE: Mixed mode

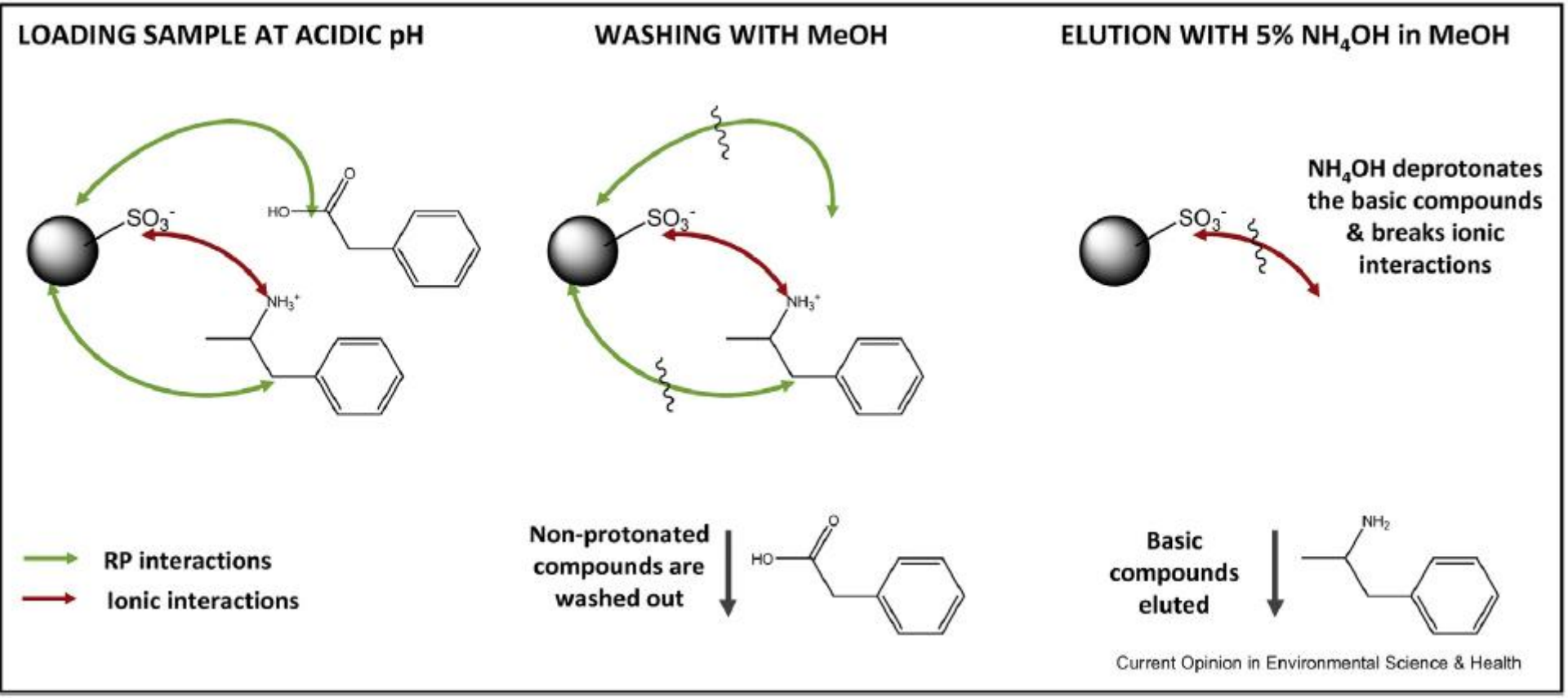
Double bonding: ion exchange + hydrophobic carbon chains

- Retention of compounds of interest (with acid or basic function) on the ion exchange media
- Removal of ionisable impurities by a powerful wash based on pH
- Removal of other impurities retained on the hydrophobic chain by an organic solvent

RP/SCX ; RP/SAX ; RP/WCX ; RP/NH_2

Solid Phase Extraction : silica based

- Ion exchange SPE: Mixed mode

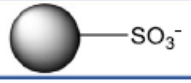


Fontanals et al. 2019

•Ion exchange SPE: Mixed mode

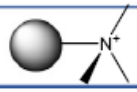
N. Fontanals, F. Borrull and R.M. Marcé / Journal of Chromatography A xxx (xxxx) xxx

SCX



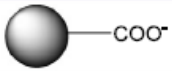
- Oasis MCX (Waters)
- Strata X-C (Phenomenex)
- BondElut Plexa PCX (Agilent Technologies)
- Evolute CX (Biotage)
- Cleanert PCX (Bonna-Agela Technologies)
- AttractSPE SCX (Affinisep)
- Extrabond ECX (Scharlau)
- CHROMABOND HR-XC (Macherey-Nagel)
- HyperSep SCX (Thermo Scientific)
- HXLPP-SCX (In-house)
- AMPSA/HEMA/PETRA (In-house)
- Sulfonated HEMA/DVB (In-house)

SAX



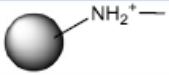
- Oasis MAX (Waters)
- Strata X-A (Phenomenex)
- BondElut Plexa PAX (Agilent Technologies)
- Evolute AX (Biotage)
- Cleanert PAX (Bonna-Agela Technologies)
- AttractSPE SAX (Affinisep)
- Extrabond EAX (Scharlau)
- CHROMABOND HR-XA (Macherey-Nagel)
- HyperSep SAX (Thermo Scientific)
- HXLPP-SAX (In-house)
- DEAEMA-DVB-TEA (In-house)
- VBC-EDMA-TEA/Imidazole (In-house)

WCX



- Oasis WCX (Waters)
- Strata X-WC (Phenomenex)
- Abselut NEXUS WCX (Agilent Technologies)
- Not available (J.T. Baker)
- Evolute WCX (Biotage)
- Cleanert PWCX (Bonna-Agela Technologies)
- AttractSPE WCX (Affinisep)
- CHROMABOND HR-XCW (Macherey-Nagel)
- Styrene screen CCX (UTC)
- HXLPP-WCX (In-house)

WAX

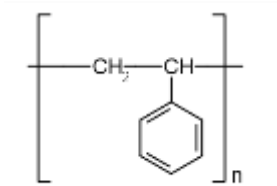


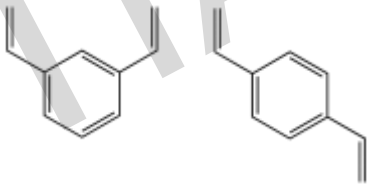
- Oasis WAX (Waters)
- Strata X-AW (Phenomenex)
- Speedisk H2O Phobic WA-DVB (J.T. Baker)
- Evolute WAX (Biotage)
- Cleanert PWAX (Bonna-Agela Technologies)
- AttractSPE SAX (Affinisep)
- CHROMABOND HR-WAX (Macherey-Nagel)
- Styrene screen THC (UTC)
- HXLPP-WAX (In-house)
- VBC-EDMA-pyrrolidone/piperidine (In-house)

Solid Phase Extraction : polymeric

stationary phase

Apolar



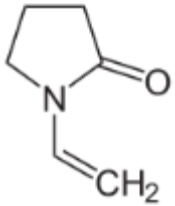


Polymeric:

•Polystyrene-divinylbenzene (PS-DVB)

Ex: Strata-X, Lichrolut-EN

•Polystyrene divinyl pyrrolidone (PS-PVP)



•Poly(vinylpyrrolidone-co-divinylbenzene)

Ex: Oasis HLB

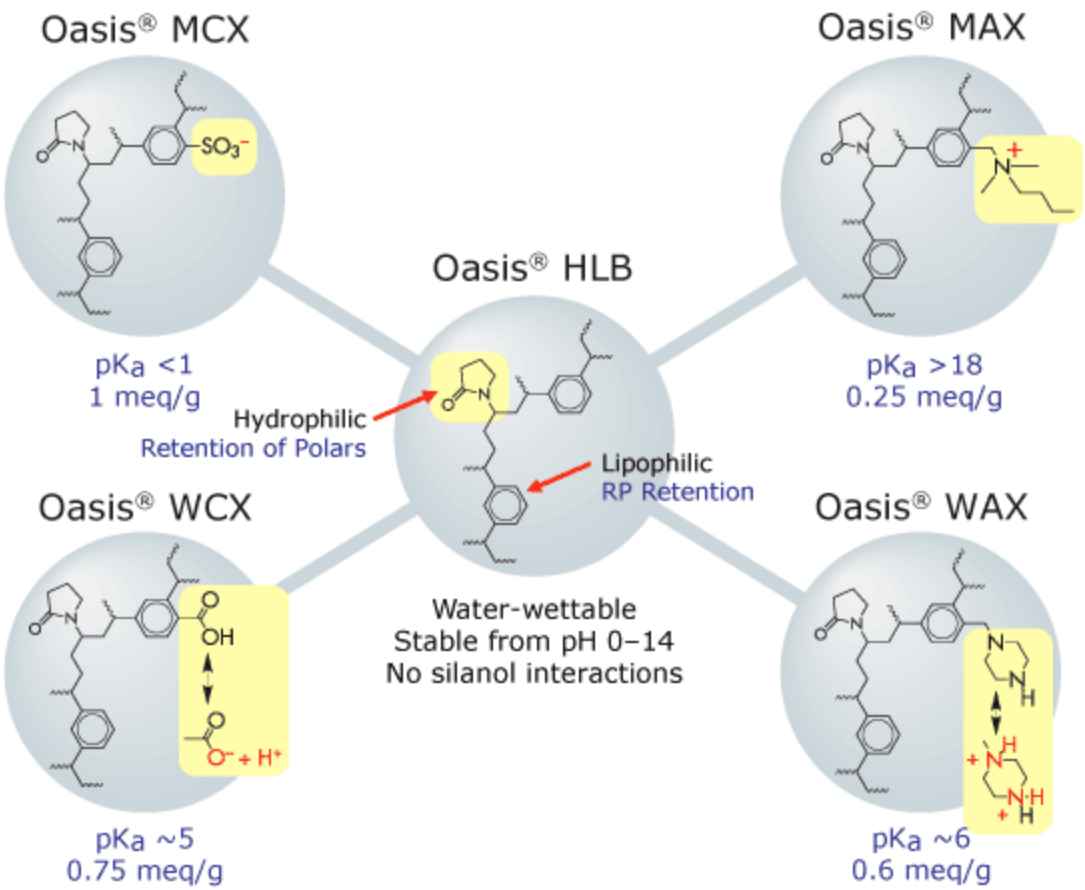
•Dimethylacryloxymethyl naphtalène divinylbenzene (DMN-DVB)

Solid Phase Extraction : polymeric

- Very chemically stable cartridges
- Resistance to pH between 1 and 14
- Low selectivity compared to silica based solid phase
- Much higher loading capacity than traditional silica and allows the purification of a very large number of molecules or families of molecules

Solid Phase Extraction : polymeric

- Ion exchange SPE: Mixed mode



Solid Phase Extraction : polymeric

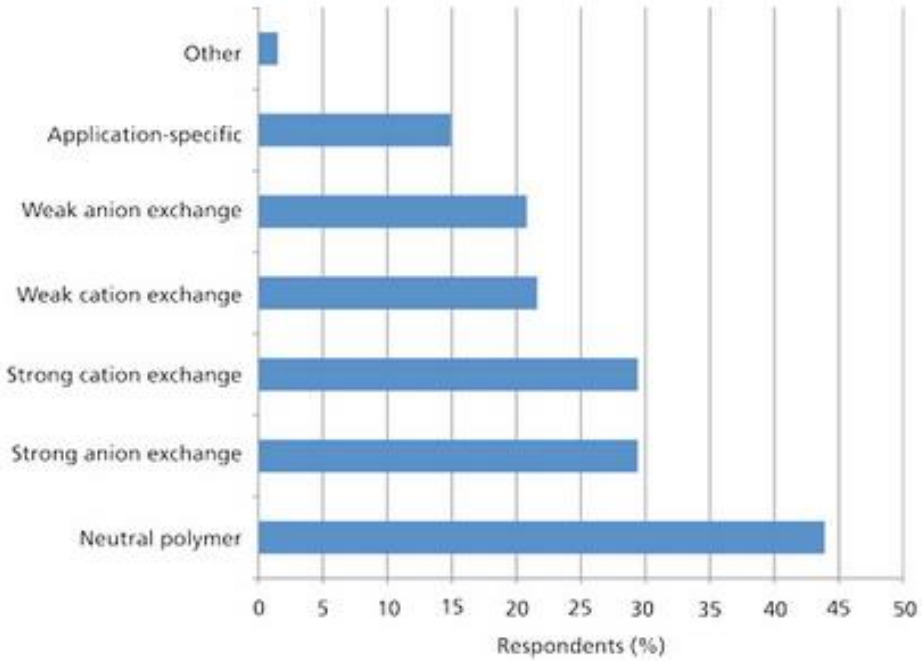


Figure 5: Polymer-based SPE phases used.

R.E. Majors, LCGC North Am. 31(3), 190–202 (2013).

Solid Phase Extraction : solid phase choice

Reversed phase

Stationary phase

Apolar

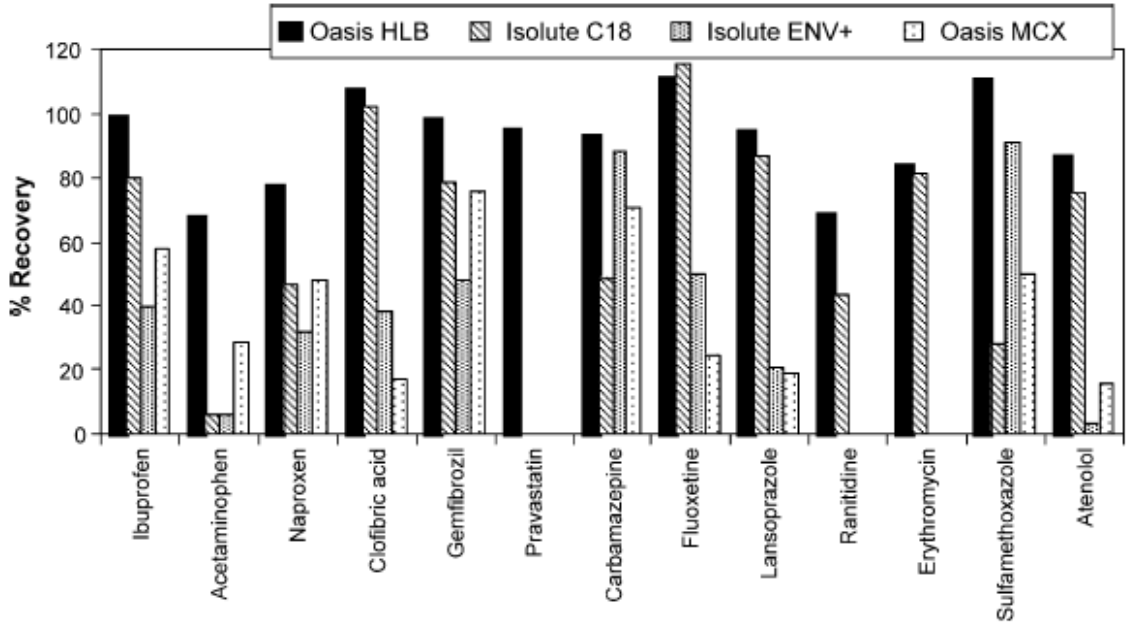


Fig. 1. Recoveries obtained for the extraction of selected Pharmaceuticals in 500 mL of river water, spiked at 1 µg/L without sample pH adjustment using different SPE materials.

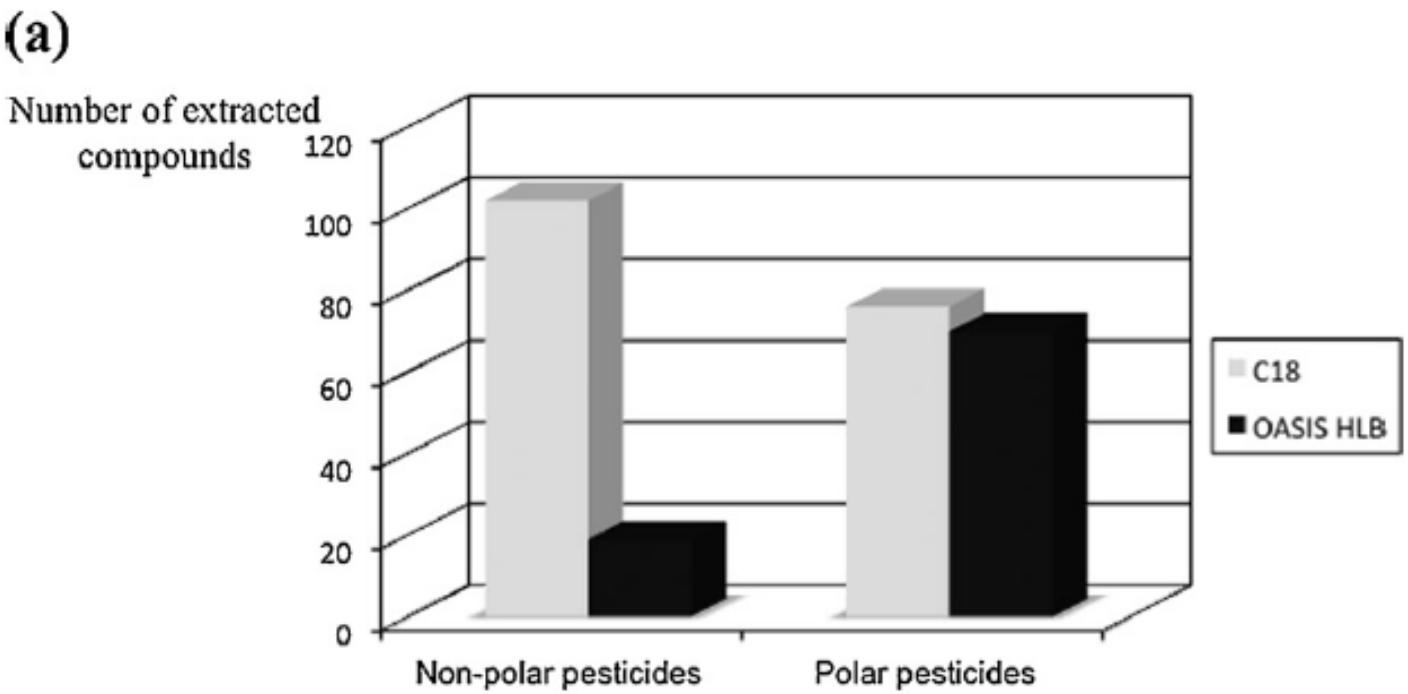
Gros et al. (2006)

Solid Phase Extraction : solid phase choice

Reversed phase

stationary phase

Apolar



Cazorla-Reyes et al. (2011)

Solid Phase Extraction : optimisation

- Optimisation
  - Choice of cartridge
    - Nature of the phase
    - Mass of solid phase

  It must be adapted to the volume of matrix extracted

Retention capacity on silica = approx. 1-5% of the adsorbent mass

Retention capacity on polymer = up to 30 % of the adsorbent mass

Solid Phase Extraction : optimisation

- Optimisation
  - Choice of cartridge
    - Nature of the phase
    - Mass of solid phase
  - Volume of sample percolated

Determination of the end of fixation volume:

Conc. at cartridge outlet = 1% conc. percolated

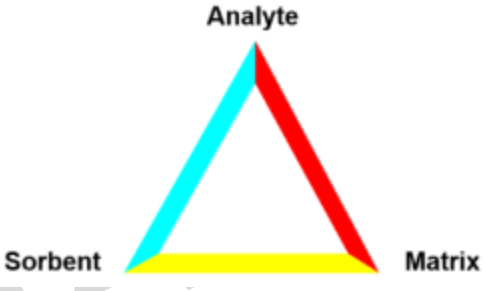
Solid Phase Extraction : optimisation

- Optimisation
  - Choice of cartridge
    - Nature of the phase
    - Mass of solid phase
  - Volume of sample percolated
  - Choice of solvents
  - pH of the sample

Solid Phase Extraction : SPE : summary

ANALYTE

- Polar functions (O, N, S or P)
- Number and position of polar groups
- Ionisable functional groups (cation and/or anion)
- pka of functional groups



MATRIX

- Relative polarity of the matrix compared to the analytes, solvent or solid support
- Potential interferents
- Analytes bound with matrix compounds (proteins)

SOLID PHASE

- Interaction with the compounds of interest or the matrix
- Normal phase, reversed phase, ion exchange, mixed mode

SOLVENT

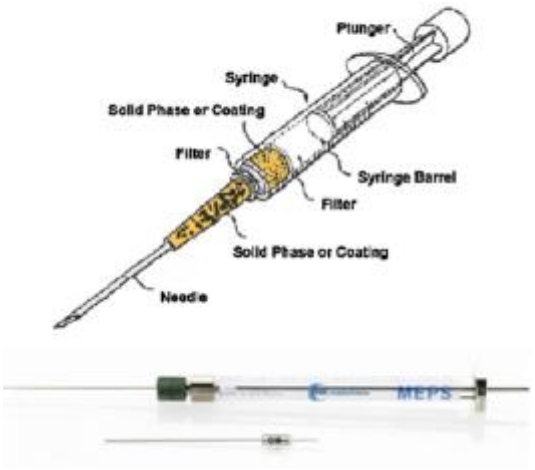
- Extraction of analytes
- Removal of matrix interferents

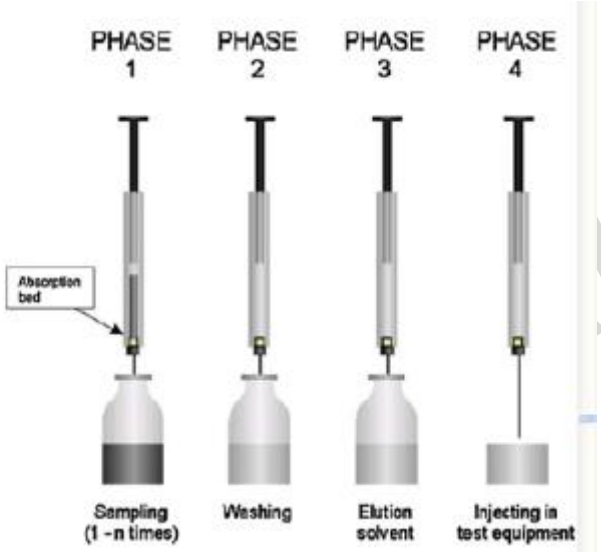
Solid Phase Extraction : strategies

	Capture	Pass-through	Dispersive SPE (d-SPE)
Analyte	Retained, then eluted	Not retained	Not retained
Interferent	Not retained or removed with washing solvent	Mostly retained	Mostly retained
Purification	Efficient and very selective	More efficient than d-SPE	Fast, simple and easy
Solid Phase Selectivity	- Maximise retention of the analyte - Minimise retention of interferents	- Maximise retention of interferents - Minimise retention of the analyte	- Maximise retention of interferents - Minimise retention of the analyte
Enrichment	Yes	< capture, evaporation	< capture, evaporation
Analysis	Similar analytes	Multi-residue	Multi-residue

Solid Phase Extraction : SPE miniaturised

- SPE miniaturised: MEPS





Novakova et al. (2009)

	SPE	MEPS
Volume of solvent	2-5 mL	20-50 µL
Mass of	100 mg	1-5 mg
Available phase	unlimited	C8, C18, SCX

Solid Phase Extraction : SPE miniaturised

- Stir Bar Sorptive Extraction (SBSE)

Solvent-free extraction

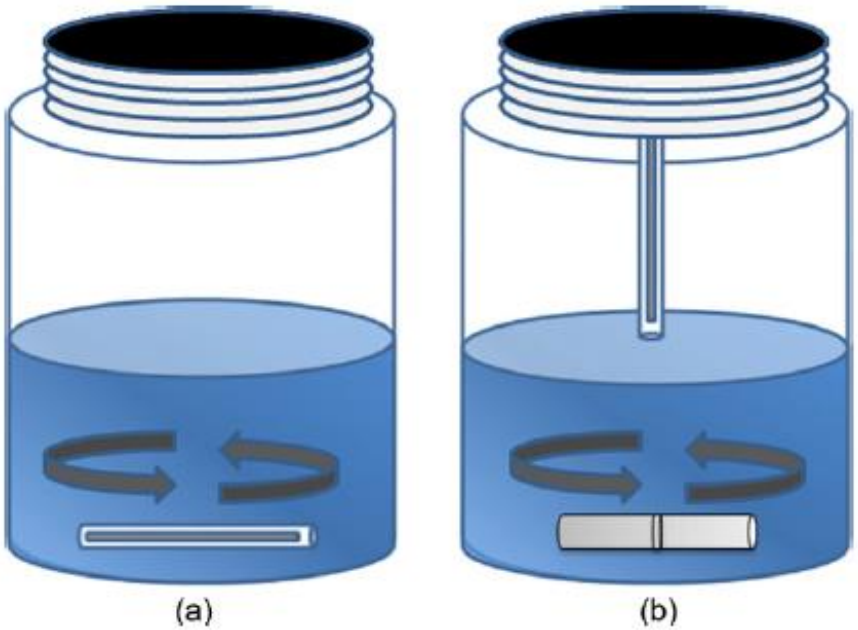


Fig. 2. Extraction modes in SBSE: immersion (a) and headspace (b).

Prieto et al. (2010)

Desorption : thermal or liquid

Stir bar Sorptive Extraction (SBSE)

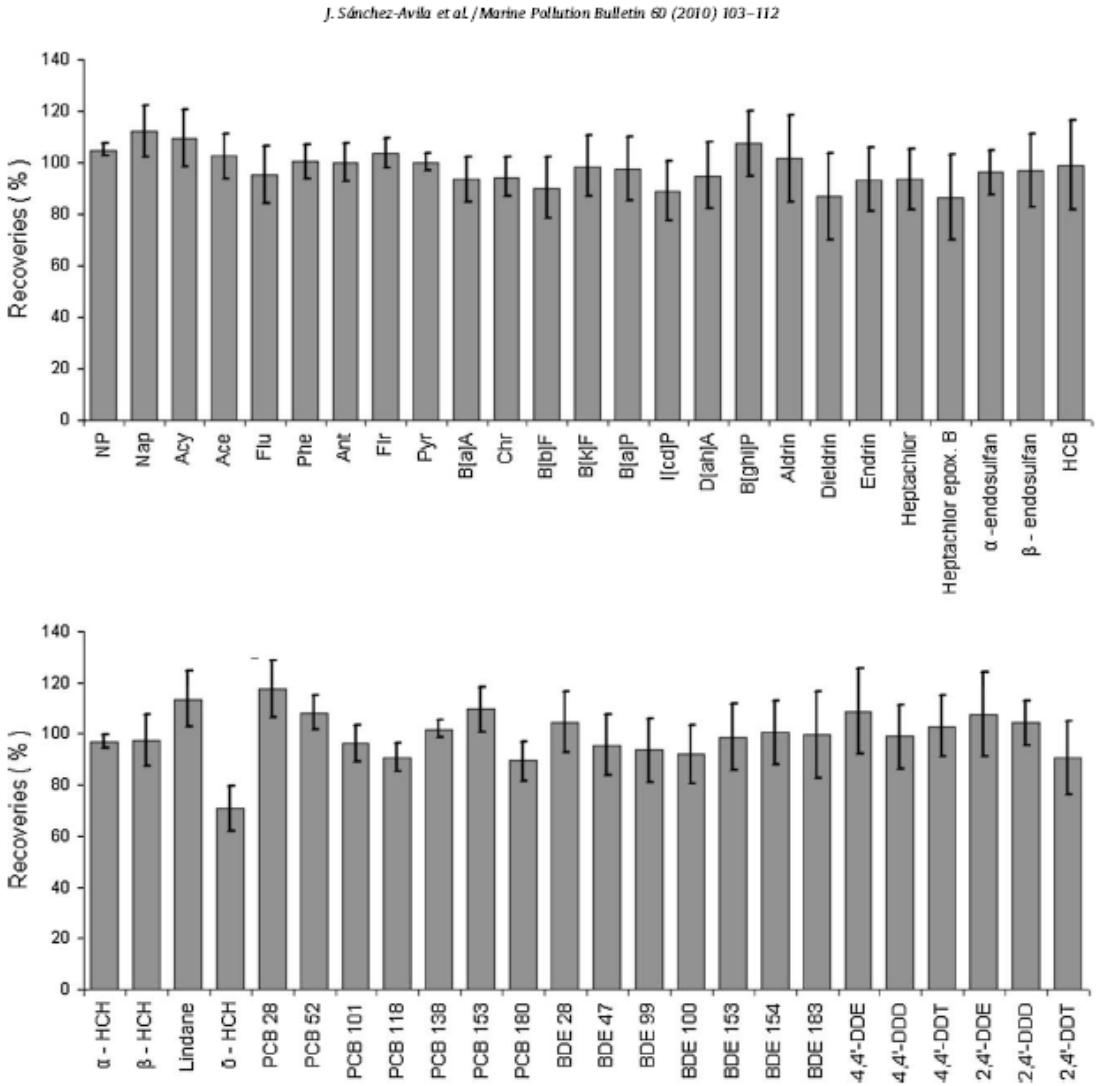


Fig. 3. Mean recoveries (%) and standard deviation ($n = 5$) of 2.5 ng l^{-1} of the compounds studied in synthetic seawater.

Analytes: HAP, PBDE…

Matrix: sea water

Volume: 100 mL

Analysis: TD-GC-MS

Thanks for your attention
Questions ?

barbara.giroud@isa-lyon.fr
laure.wiest@isa-lyon.fr