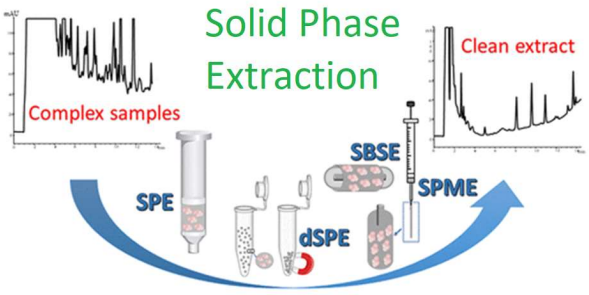
SOLID PHASE EXTRACTION

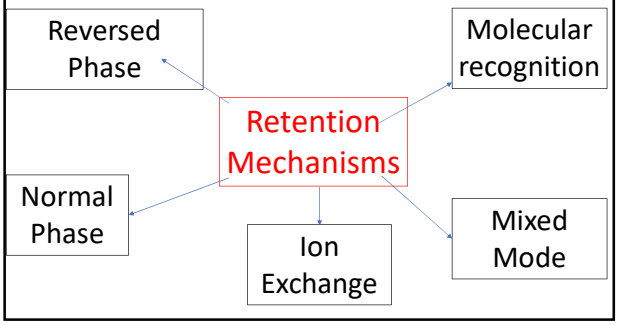
Method Development



Adapted from: Valérie Pichon, Nathalie Delaunay, Audrey Combès, **Sample Preparation Using Molecularly Imprinted Polymers, Analytical Chemistry** 92 (2020) 16 - 33

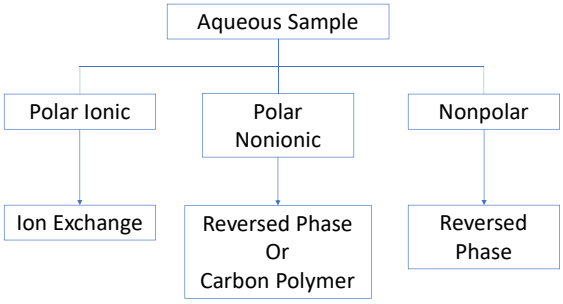
SPE - Method Development

Always begin choosing the correct retention mechanism



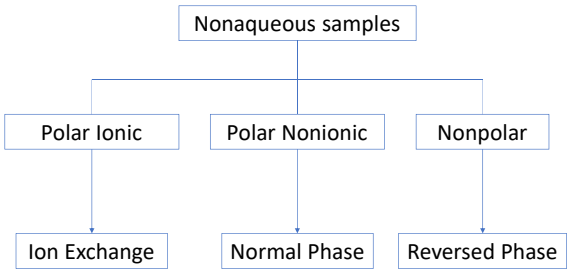
SPE - Method Development

For aqueous samples



SPE - Method Development

For nonaqueous samples



SPE - Method Development

A seven step Approach adapted from Thurman and Mills

1- Analyte structure

2- Identify the goal

3- Physical constants

4- Choose the mode of SPE then condition and load

5 - Washing

6- Elute the SPE cartridge

7- Perform sorption experiments and determine the breakthrough of the column

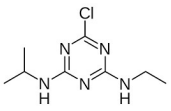
SPE - Method Development

1- Analyte Structure – Answer the following questions:

- What are the functional groups?
- Is it ionic or nonionic?
- What is the water solubility?
- In what organic solvent is the analyte soluble?
- Does the matrix (pH and ionic strength) affects the solubility and structure of the analyte?

SPE - Method Development

2- The goal of the analysis



Example: The herbicide atrazine widely used in corn and sugar cane crops in Brazil and USA. Banned from EU

Matrix: Surface Waters

Maximum concentration level (MCL) defined by US-EPA: 3 μg L^{-1}

In European Union the maximum concentration allowed is 0.1 μg L^{-1}

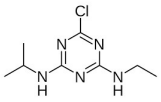
Limit of detection should be at least 0.05 μg L^{-1}

For the aimed detectability – GC-MS should be used

SPE - Method Development

3- Obtain the physicochemical characteristics

Consult Merck Index, PubChem



What is the solubility in water?

Octanol/water partition coefficient (log $P_{O/W}$)

$$\log P_{O/W} = \log \frac{[S]_O}{[S]_W}$$

Log $P_{O/W}$ < 1.5 – polar
1.5 < Log $P_{O/W}$ < 4 – moderate polarity
Log $P_{O/W}$ > 4 – nonpolar

Is the reversed phase mechanism possible?

H-bonding is possible for the amino - nitrogen?

Could the pKa of atrazine be a problem because of the ionization of the analyte?

SPE - Method Development

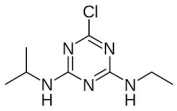
3- Obtain the physicochemical characteristics

Methods Development Worksheet for ***Atrazine*** using GC-MS

Analyte

Aqueous Solubility: ***33 mg/L***
Log $P_{O/W}$ = ***2.60***
p*Ka* = ***1.7***
Organic Solubility (1): ***Methanol***
Organic Solubility (2): ***Ethyl acetate***
Organic Solubility (3): ***Chloroform***

Structure:



Important Functional Groups: ***2nd amine, triazine ring, halogen***

Sample/Method

Matrix: ***water, pH 5 - 8***
Sample Concentration: ***0.05 μg/L***
Mass of solute for detection: ***100 pg***
Amount of Sample Needed: ***100 mL***
Solid Phase Chosen: ***C18***

Retention Mechanism: ***Reversed Phase***
Percent retained on SPE: ***100 %***
Wash solvent: ***none***
Elution solvent: ***Ethyl Acetate***
Percent desorbed from SPE: ***~95%***
Method Recovery: ***95%***

SPE - Method Development

Planning for GC-MS

Consider that the GC-MS has a detection limit of **100 pg** when **2 μL** of solvent are injected, and the volume of the microvials is **100 μL**

Therefore, the minimum mass per vial is 100 μL x 50 pg/μL = 5000 pg (or 5 x 10^{-3} μg)

In a 0.05 μg/L solution, which volume does contain 5 x 10^{-3} μg of atrazine?

0.05 μg _____ 1 L
5 x 10^{-3} μg _____ x x = 0.1L

For a LOD of 0.05 μg/L a **minimum of 100 mL of water** is required, which must be concentrated to a volume of 100 μL for the injection of 2 μL

SPE - Method Development

4- Choose the mechanism

Normal phase?

No, only if atrazine were in an organic solvent

Ion Exchange?

No, Atrazine protonates only at very low pH (pKa = 1,7)

In acidic medium humic acids (the main components of natural organic matter) precipitates

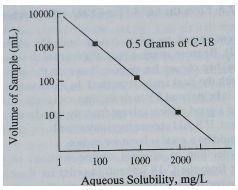
Silica based sorbents are stable in a pH range between 2.5 – 7.5

SPE - Method Development

4- Choose the mechanism

Reversed Phase, Mixed Mode and Molecular recognition?

Yes, they are ok. Now, we will address RP



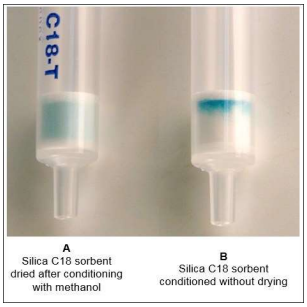
The plot uses the water solubility to estimate the volume that can be applied onto a C_{18} sorbent for 95% retention

As the solubility of atrazine is 33 mg/L and the volume to be processed is 100 mL, 500 mg of C_{18} will work

The plot is empirical (recovery experiments)

SPE - Method Development

Before loading the sample, condition the column!!!!



For reversed phase:

1 – Wash with organic Solvent (Typically Methanol) to remove impurities and to wet the pores

2- Condition with water or buffer – compatible with the sample matrix

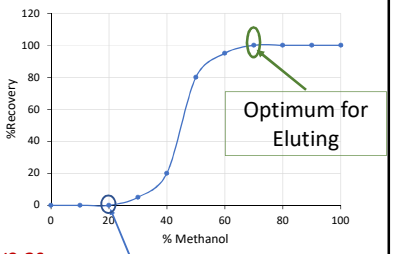
3- Don't let the sorbent dry

SPE - Method Development

5- Washing / Elution (Ten bottle approach by Agilent)

Load 11 cartridges with the same amount of analyte

Elute with increasing amounts of MeOH or ACN (in RP mechanism) and analyze



Washing must remove as much interferences as possible, without eluting the analyte

SPE - Method Development

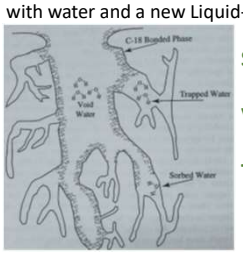
5- Elution

Choose the solvent:

Ethyl acetate and chloroform work well for GC

Acetonitrile and methanol, better for HPLC

Residual water may affect the recoveries, especially if ethyl acetate or chloroform are used as eluting solvent since they do not mix with water and a new Liquid-Liquid equilibrium sets



Sorbed water — Removed by reduced pressure before elution

Void Water

Trapped water — Mixing the solvent with another solvent miscible with water (Methanol)

SPE - Method Development

5- Elution

Test the effectiveness of the eluting solvent:

Prepare a standard solution in ethyl acetate

Pass it through the conditioned sorbent

A second aliquot (only solvent) is passed, and the two aliquots are combined

Analyze by GC-MS and compare the result with that obtained with the same standard that has not passed through the column

Recovery > 85% ?

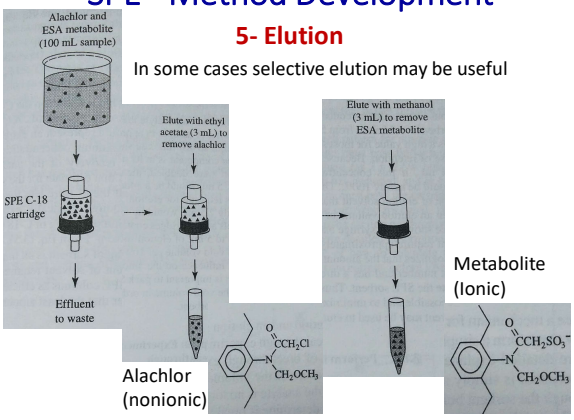
Yes → This solvent is Ok

No → Try another solvent

SPE - Method Development

5- Elution

In some cases selective elution may be useful



SPE - Method Development

5- Elution

In the case of determination of atrazine in soils or waters, selective extraction may be useful

Atrazine elutes in Ethyl acetate but humic substances do not

↓

Clean chromatogram

Methanol elutes both atrazine and humic substances from the C18 sorbent

↓

Noisy chromatogram

SPE - Method Development

5- Elution

The volume of the eluting solvent depends on:

The retention of the analyte

The Void volume of the sorbent

A typical SPE cartridge filled with 60 μm particles has a void volume of 120 μL per 100 mg of sorbent

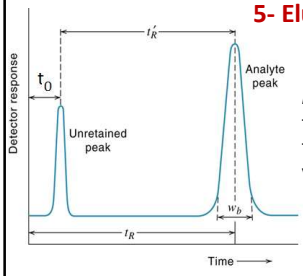
For a 500 mg cartridge – 600 μL of void volume

$$V_r = V_0(1+k')$$

V_r = volume of the maximum concentration of the eluting peak
V_0 = Void Volume
K' = chromatographic retention factor (number of void volumes required for the peak concentration elutes from de the column)

SPE - Method Development

5- Elution



$$k' = \frac{t_R - t_0}{t_0}$$

k' = retention factor
t_R = retention time (min)
t_0 = time corresponding to the void volume (or unretained substance)

$$V_r = t_R \times U$$

U = Flow rate in mL/min

$$k't_0 = t_R - t_0 \qquad t_R = t_0(k'+1) \qquad \frac{V_R}{U} = \frac{V_0}{U}(k'+1)$$

$$V_r = V_0(k'+1)$$

SPE - Method Development

5- Elution

For a 500 mg cartridge and k' = 3, we have

Vr = 0.6(1 + 3) = 2.4 mL

Since 2.4 mL is the volume corresponding to peak maximum, the elution volume should be at least 5 mL

If k' = 1, Vr = 1.2 mL, so at least 2.4 mL should be used for elution

The void volume is minimized by efficient packing of the sorbent inside the column device

SPE - Method Development

6 – Sorption to determine the breakthrough

A measure of the sample volume that may be passed through the sorbent before the analyte is no longer retained

1 – Pump a known volume of sample through the conditioned column

2- Determine the mass of the retained analyte

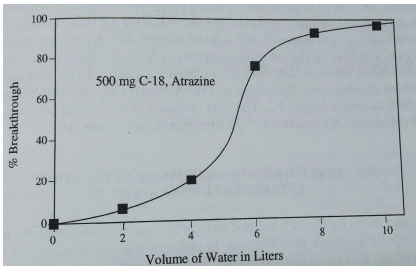
3 – The mass recovered is divided by the mass applied to the column

% Breakthrough = (mass recovered/mass applied) x 100

This is a laborious procedure – when possible, use the concept of frontal chromatography to determine the breakthrough of the column

SPE - Method Development

6 – Sorption to determine the breakthrough



SPE - Method Development

Alternative approach for the determination of the breakthrough

Measure the mass of the analyte in the second cartridge relative to mass of compound that was present in the original sample

SPE - Method Development

SPE in 5-steps: Executing and troubleshooting

Step 1: Check the efficiency of the eluting solvent

Spike the compound in the eluting solvent and pass the solution through the conditioned sorbent

Compare the recovery to the same standard (spiked to the solvent) and analyzed directly (not undergone sample preparation losses due to SPE)

90 – 100 % recovery → **Good eluting solvent**

Problem 1 – Incomplete recovery

The eluting solvent is not capable of breaking the bonding mechanism of retention

SPE - Method Development

Problem 1 - Remedies

- Increase the volume of solvent or try back extraction (difficult in off-line cartridges – but quite easy in automated online systems)
- Change the solvent or mix it with others to increase or decrease the polarity
- pH adjustments to change the hydrophobicity or ionic state of the analyte. Hydrogen bonding may be retaining the compound in active sites of silica-based sorbent, requiring basic solvent for elution
- Decrease the interaction strength between the analyte and the sorbent. Try C8, C4, cyanopropyl instead C18
- Change the mechanism of retention, select a new sorbent and begin the method development again

SPE - Method Development

Step 2: Carry out the planned procedure

- Prepare a standard in distilled water (or in organic solvent in the case of NP) and pass a known volume through the sorbent
- Elute the compound and analyze
- Compare the recovery to the same mass of standard spiked directly into the eluting solvent

Yes — Recovery > 90% ? — **No**

Procedure is Ok

Go to Troubleshooting

SPE - Method Development

Problem 2 – Early breakthrough

Problem 2 remedies

- Change the solid sorbent to one of greater affinity for the analyte
  For RP, try SDVB or activated carbon instead of C18
- Reduce the flow rate (5 mL/min is usual for RP but for IEX reduce to about 0.5 mL/min)
- Change the form of the analyte (pH adjustment of sample solution). Turn an ionic compound in nonionic for the RP mechanism
- Try salting out for RP mechanism (5 – 10 % (m/v) NaCl)
  Hydration of the salts increases the polarity of the solvent and drives the compound onto the RP sorbent
- Change the sorption mechanism

SPE - Method Development

Step 3: Analyze an enriched sample

- Spike a standard in a real matrix (river water, urine, soil extract, etc) and run the planned procedure
- Compare the result with that obtained from a standard spiked in distilled water

Recovery > 90% ? Clean spectrum or chromatogram?

Yes → Procedure is Ok

No → Go to Troubleshooting Sample matrix problems

SPE - Method Development

Problem 3 – Matrix interfering substances

Problem 3 remedies

- Design the washing step to selectively retain the analyte and elute the interferences
- Change the eluting solvent to retain the interferences and elute the analyte
- Cleanup the eluent of the SPE which contains both analyte and interferences with another sorbent that uses a diferent mechanism for the interference but does not retain the analyte

SPE - Method Development

Step 4: Calibration curve

- Make up a calibration curve over the instrument's linear range in the matrix that will be used and process the solutions through the SPE sorbent with the planned method.
- Check the linearity

Step 5: Analyze the samples

- Analyze real samples of unknown concentration and compare with an independent method for accuracy and precision
- Spike the sample and compare the recovery to check for matrix interferences

Expected Precision: ± 10%
Expected recovery (accuracy): 95 ± 10%

SPE - Method Development

Problem 4 – Solvents or Hardware

Problem 4 remedies

- SPE hardware bleed – wash the sorbent with the final eluting solvent during sorbent conditioning to remove interferences (in the sorbent – remains of the synthesis/modification processes)
- Check if the pH of the sample is within the range compatible with the sorbent

Most silica-based materials have to work in pH between 2.5 and 7.5 to avoid acid or base hydrolysis

- Change the solvent to eliminate bleed from the SPE hardware, or to eliminate impurities

SPE - Method Development

Quantification

$$R\% = \frac{q_p}{q_i}100$$

R% = recovery percentage
q_p = amount of analyte in the processed sample
q_i = amount of analyte in the initial sample

The analyte recovery is determined by adding a known amount of the analyte in a blank sample and measuring it in the processed sample

or

$$R\% = \frac{(q_p - q_o)}{(q_i - q_o)}100$$

q_0 is the amount of analyte preexisting in the sample

SPE - Method Development

Quantification

Enrichment Factor (F)

$$F = \frac{c_p}{c_i}$$

c_p = concentration in the processed solution
c_i = concentration in the initial solution

$$F = \frac{V_{sample}}{V_{eluent}}R \qquad R = R_{retention}R_{elution}$$

F can be obtained from the ratio of the slopes of the calibration curves obtained with SPE processed and non-processed solutions

If the calibration solutions are processed in the SPE, in a standard addition protocol, the knowledge of F is not necessary (although may be beneficial)

SPE - Method Development

A systematic approach to estimate the minimum volume of sample

$q_p^{min} = V_p^{min} c_p^{min}$

q_p^{min} = the minimum amount of analyte in the processed sample

V_p^{min} = minimum volume of the processed sample

c_p^{min} - minimum concentration of the processed sample

$q_i^{min} = V_i^{min} c_i^{min}$

q_i^{min} = the minimum amount of analyte in the initial sample

V_i^{min} = minimum volume of the initial sample

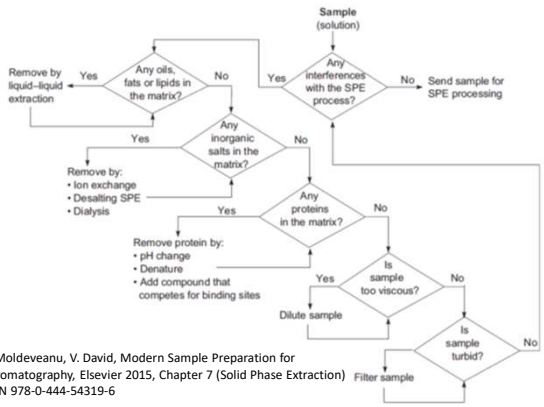
c_i^{min} - minimum concentration of the initial sample

$$F = \frac{c_p^{min}}{c_i^{min}} \qquad F = \frac{q_p^{min}/V_p^{min}}{q_i^{min}/V_i^{min}} = \frac{q_p^{min}}{V_p^{min}} \frac{V_i^{min}}{q_i^{min}} \qquad V_i^{min} = \frac{F V_p^{min} q_i^{min}}{q_p^{min}}$$

q_i^{min} = 1/R(%)

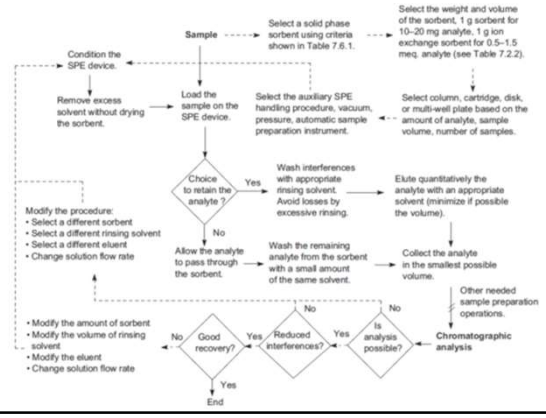
$$V_i^{min} = \frac{F V_p^{min} 100}{R(\%)}$$

Preliminary Steps to SPE



S. Moldeveanu, V. David, Modern Sample Preparation for Chromatography, Elsevier 2015, Chapter 7 (Solid Phase Extraction) ISBN 978-0-444-54319-6

Development and validation of a SPE procedure

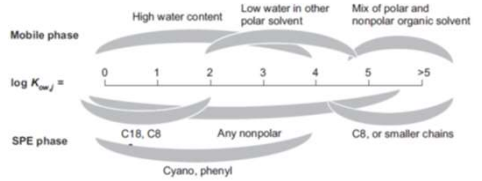


Selection of Solid Phase Sorbent

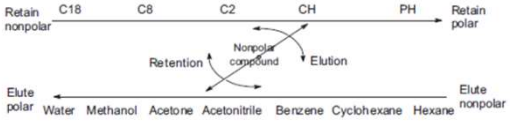
TABLE 7.6.1 Selection of Solid-Phase Sorbent, Rinsing Solvent, and Eluent

Analyte type	Matrix type	Sorbent	Extraction mechanism	Rinsing solvent	Eluent
Compounds with long alkyl chains, aromatic rings, low polarity	Aqueous, biological fluids	C18, C8, C2, phenyl, cyclohexyl, cyanopropyl, polymeric styrene–divinylbenzene	Distribution (reversed phase)	Water, methanol, other polar solvents	Hexane, chloroform, ethyl acetate
Hydrophilic groups, hydroxyls, amines, heteroatoms	Nonpolar, lipids	CN, 2OH-diol, silica, aminopropyl, Florisil, alumina	Distribution (direct phase)	Hexane, CH_2Cl_2, other nonpolar solvents	Polar solvents, methanol/water, methanol, etc.
Positively charged groups, such as amine cations	Aqueous, low ionic strength, biological fluids	Strong (benzene or propyl sulfonic groups) or weak (carboxylic acids)	Cation exchange	Water, methanol, other polar solvents, low ionic strength	Alkaline buffers, ammonia, ammonia in methanol, high ionic strength
Negatively charged groups, ionized organic acids	Aqueous, low ionic strength, biological fluids	Strong (tetraalkylammonium), weak (diethylaminopropyl, amino)	Anion exchange	Water, methanol, other polar solvents, low ionic strength	Acidic buffers, high ionic strength
Vicinal diols	Aqueous, biological fluids	PBA (phenyl boronic)	Covalent bonds	Water, methanol	Acidic methanol
Specific analytes	Water, biological fluids	Specific, tailored for the analyte	Usually distribution		

Selection of the solid phase sorbent and of the solvent for the retention of an analyte depending on its octanol : water distribution coefficient

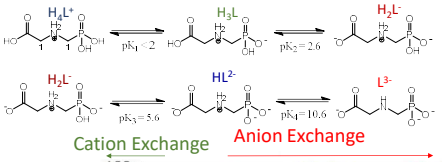


Retention/elution choices for SPE



SPE - Method Development

The case of Glyphosate

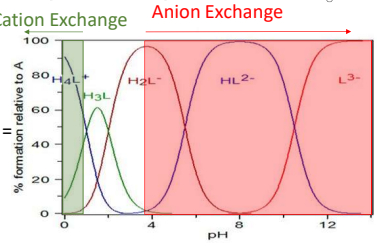


Cation Exchange

Anion Exchange

Log K_{OW} = -3.4

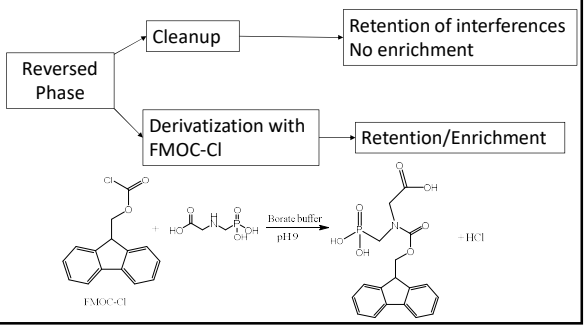
Solubility in water = 10.5 g/L at pH 1.9



SPE - Method Development

The case of Glyphosate

Ion Exchange – retention mechanism for enrichment/cleanup

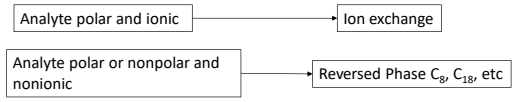


SPE - Method Development

Summary

From the chemical structure and physicochemical properties – choose the retention mechanism

For aqueous samples the decision for the sorbent is based on the polarity and ionic character of the analyte



Remember that pH control may be useful to change ionic compounds in nonionic and vice versa

Decide if the SPE is for retention of the analyte or of interferences and chose the retention mechanism

SPE - Method Development

Summary

Choose the proper solvent to wash the sorbent from the interferences

or

Wash the sorbent for quantitative transfer of the analyte, keeping the interferences adsorbed

Choose the eluting sorbent for breaking the bonding interactions with a minimum volume

Elute and analyze (eventually after evaporating the solvent and suspending the analyte in the solvent for HPLC or GC)

Validation and troubleshooting

SPE Method development

https://www.youtube.com/watch?v=Gkdow0i4F68

https://www.youtube.com/watch?v=A2B5ugQLQBw

https://www.youtube.com/watch?v=byzY4KOHFIg&t=708s