Taking the Complexity out of SPE Method Development

Includes guidelines for the following:

- ✓ SPE Format Selection
- ✓ SPE Sorbent Selection
- ✓ SPE Methods
- ✓ SPE Volume Guidelines
- ✓ Common Laboratory Conversions and Solution Calculations
- ✓ Calculating Recovery and Matrix Effects
- ✓ Experimental Set Up
- ✓ Monitoring Phospholipids
- ✓ SPE Troubleshooting
- ✓ Sample Pre-treatment

Selecting the Correct SPE Format

Formats

µElution Plates

- Patented µElution plate design.
- Ideal for SPE cleanup and analyte enrichment of sample volumes ranging from 10 µL to 375 µL.
- No evaporation and reconstitution necessary due to elution volumes as low as 25 µL.
- Up to a 15x increase in concentration.
- Compatible with most liquid-handling robotic systems for automated, reliable high throughput SPE (HT-SPE).

96-well Extraction Plates

- Innovative, award-winning two-stage well design.
- High throughput and high recovery.
- Available with 5 mg, 10 mg, 30 mg, and 60 mg of sorbent per well.
- Compatible with most liquid-handling robotic systems for automated, reliable high throughput SPE (HT-SPE).

Syringe-Barrel Cartridges

- Ultra-clean syringe barrel and frits.
- Available with cartridge sizes ranging from 1cc/10 mg up to 35 cc/6 g.
- Flangeless syringe-barrel cartridges available in 1cc, 3 cc, and 6 cc configurations.
- Plus-style cartridges with Luer inlet hub and outlet tip with 225 mg of sorbent.

Glass Cartridges

- Ultra-clean glass syringe with Teflon® frit.
- For trace level detection and analysis at part-per-trillion levels.
- Available in 5 cc with 200 mg of sorbent configuration.

On-Line Columns and Cartridges

- For rugged, reproducible, and ultra-fast on-line analysis.
- Wide choice of configurations, particle sizes, and sorbent chemistries.
- Available with six patented Oasis® Sorbents — HLB, PRiME HLB, MCX, MAX, WCX, and WAX.
- High recovery and reproducible results for a wide range of compounds.
- Cartridge format for use with Spark Holland Prospekt-2™/Symbiosis™ systems also available.

SPE Sorbent Selection

What is the Ideal SPE Method?

✓ Easy to implement   ✓ Reproducible and robust   ✓ Fast   ✓ Achieves your goals

[START HERE]

⊙

Oasis PRiME HLB

First Choice

Reversed-phase SPE cleanup
of samples in routine analysis

ALL MATRICES

Oasis HLB

Choose If:

Require ultra high capacity
of very polar compounds

ALL MATRICES

Oasis Mixed-Mode

Choose If:

Higher analyte specificity, sensitivity,
and/or cleanliness required

ALL MATRICES
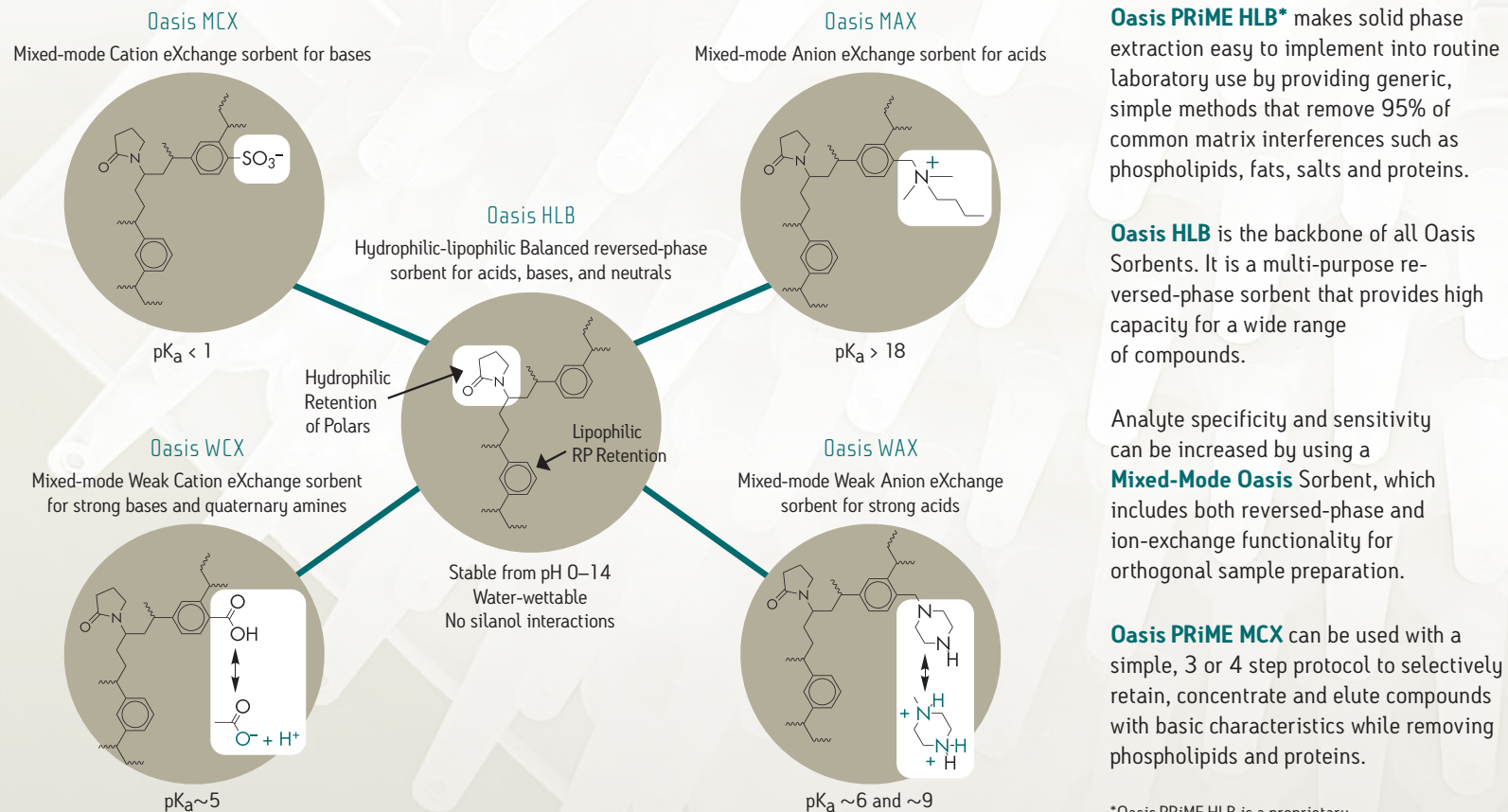
SIMPLEST   Combined Advantage
with Oasis PRiME HLB   CLEANEST

Solid Phase Extraction

- Reduce chromatographic complexity
- Increase signal to noise/improve detection limits
- Minimize risks associated with matrix effects
- Concentrate analytes of interest

- Reduce variability in analytical results/increase robustness of analysis
- Increase column lifetime
- Reduce system downtime

The Oasis SPE Family of Sorbents

As a unique, water-wettable polymeric sorbent, Oasis Products can be used without the conditioning and equilibration steps required by other polymeric and silica based sorbents. Historically, those steps were required to obtain retention of analytes by reversed-phase SPE. The water-wettable nature of Oasis allows direct loading of aqueous samples without sacrificing recovery.

Oasis MCX

Mixed-mode Cation eXchange sorbent for bases

$-SO_3^-$

$pK_a < 1$

Oasis MAX

Mixed-mode Anion eXchange sorbent for acids

$pK_a > 18$

Oasis HLB

Hydrophilic-lipophilic Balanced reversed-phase sorbent for acids, bases, and neutrals

Hydrophilic Retention of Polars

Lipophilic RP Retention

Stable from pH 0–14
Water-wettable
No silanol interactions

Oasis WCX

Mixed-mode Weak Cation eXchange sorbent for strong bases and quaternary amines

O
OH

$O^- + H^+$

$pK_a \sim 5$

Oasis WAX

Mixed-mode Weak Anion eXchange sorbent for strong acids

$pK_a \sim 6$ and ~ 9

Oasis PRiME HLB* makes solid phase extraction easy to implement into routine laboratory use by providing generic, simple methods that remove 95% of common matrix interferences such as phospholipids, fats, salts and proteins.

Oasis HLB is the backbone of all Oasis Sorbents. It is a multi-purpose re-versed-phase sorbent that provides high capacity for a wide range of compounds.

Analyte specificity and sensitivity can be increased by using a **Mixed-Mode Oasis** Sorbent, which includes both reversed-phase and ion-exchange functionality for orthogonal sample preparation.

Oasis PRiME MCX can be used with a simple, 3 or 4 step protocol to selectively retain, concentrate and elute compounds with basic characteristics while removing phospholipids and proteins.

*Oasis PRiME HLB is a proprietary, patent pending sorbent.
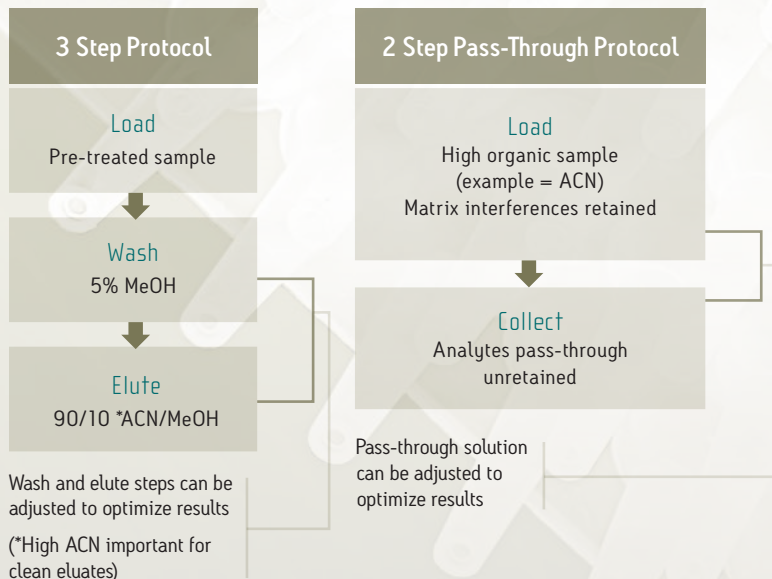
Sample Prep in 3 Steps or Less?
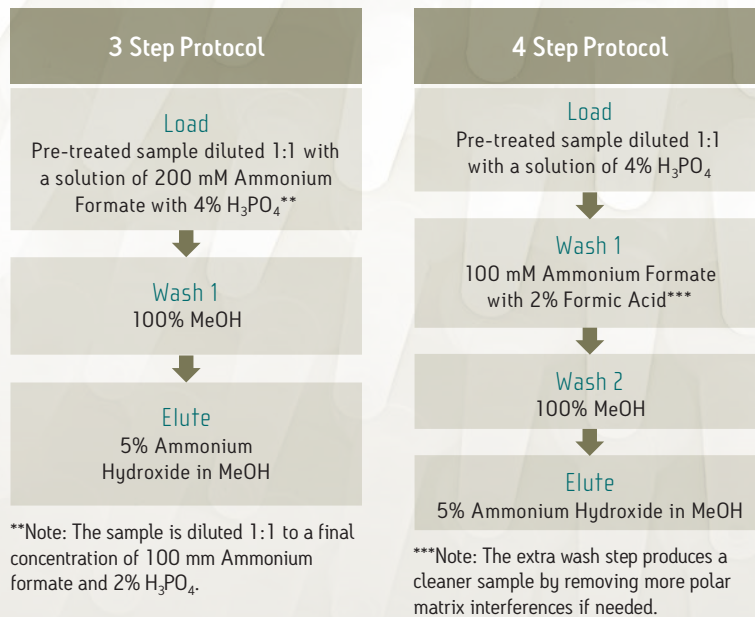
✓ SIMPLER   ✓ CLEANER   ✓ FASTER

Oasis PRiME Methods

- Removes more than 95% of common matrix interferences such as salts, proteins, and phospholipids
- Ability to concentrate analytes
- Faster, more predictable analysis times
- Directly load pre-treated samples without conditioning and equilibration

Oasis PRiME HLB Methods

3 Step Protocol

Load
Pre-treated sample

↓

Wash
5% MeOH

↓

Elute
90/10 *ACN/MeOH

Wash and elute steps can be adjusted to optimize results

(*High ACN important for clean eluates)

2 Step Pass-Through Protocol

Load
High organic sample
(example = ACN)
Matrix interferences retained

↓

Collect
Analytes pass-through
unretained

Pass-through solution can be adjusted to optimize results

Oasis PRiME MCX Methods

3 Step Protocol

Load
Pre-treated sample diluted 1:1 with a solution of 200 mM Ammonium Formate with 4% H_3PO_4.**

↓

Wash 1
100% MeOH

↓

Elute
5% Ammonium Hydroxide in MeOH

**Note: The sample is diluted 1:1 to a final concentration of 100 mm Ammonium formate and 2% H_3PO_4.

4 Step Protocol

Load
Pre-treated sample diluted 1:1 with a solution of 4% H_3PO_4

↓

Wash 1
100 mM Ammonium Formate with 2% Formic Acid***

↓

Wash 2
100% MeOH

↓

Elute
5% Ammonium Hydroxide in MeOH

***Note: The extra wash step produces a cleaner sample by removing more polar matrix interferences if needed.

Oasis Mixed-Mode Sorbents

Oasis Mixed-mode Products were designed to help scientists achieve the highest level of cleanliness and analyte specificity. By combining the power of reversed-phase and ion-exchange retention mechanisms, it is possible to design a targeted SPE method by choosing the appropriate Oasis Sorbent for a known acidic, basic, neutral or zwitterionic compound.

These sorbents can also be used in a method development scenario for mixtures of unknown analytes to quickly determine the best sorbent and protocol for your compound of interest.

Oasis 2 x 4 Method Development Protocol

BASES	STRONG ACIDS
pK_a 2–10	pK_a <1.0
OASIS MCX	OASIS WAX

4 Step Protocol

Load
Pre-treated sample

Wash 1
2% Formic acid

Wash 2 or Elute 1
100% MeOH → NEUTRALS

Elute 2
5% NH$_4$OH in MeOH

→ BASES   STRONG ACIDS

STRONG BASES	ACIDS
pK_a >10	pK_a 2–8
OASIS WCX	OASIS MAX

4 Step Protocol

Load
Pre-treated sample

Wash
5% NH$_4$OH

Elute
100% MeOH → NEUTRALS

Elute 2
2% Formic Acid in MeOH

→ STRONG BASES   ACIDS

The **load**, **wash** and **elute** solutions can be modified depending on the properties of the target analyte or the needs of the assay. For example, a weak base may be loaded onto the Oasis MCX Sorbent in an acidic aqueous solution to ensure that the basic analyte is charged (ionized), ensuring both ion-exchange and reversed-phase retention. Additionally, the organic content of the steps can be adjusted to further optimize the method.

More information is available on our website at **www.waters.com/oasis**

SPE Volume Guidelines

Recommended volumes for generic methods
(assuming sample is diluted 1:1 with appropriate diluent prior to loading)

	Cartridges (cc)						96-well plate (mg)				µElution plate
Cartridges size/ Sorbent mass	1 cc	3 cc	6 cc	12 cc	20 cc	35 cc	5 mg	10 mg	30 mg	60 mg	2 mg
Maximum load of matrix & diluted sample	1 mL	2 mL	5 mL	15 mL	30 mL	100 mL	0.5 mL	1 mL	1–2 mL	1–2 mL	0.025– 0.750 mL
Wash (mL)	1 mL	2 mL	4 mL	5 mL	10 mL	40 mL	0.2 mL	0.5 mL	0.5– 1.0 mL	1–2 mL	0.2 mL
*Elute (mL)	1 mL	2 mL	4 mL	5 mL	10 mL	60 mL	0.05– 0.20 mL	0.15– 0.30 mL	0.4– 1.0 mL	0.8– 2.0 mL	0.025– 0.100 mL

*Recovery may be increased by splitting the elution volume into two aliquots. For example, instead of eluting with one aliquot of 1 mL, elute with 2 aliquots of 500 µL each.

Note: The above listed sample volumes are recommendations for biological samples. For certain types of samples (i.e drinking water) up to 20X above the recommended volumes may be used.

Note: SPE dilution, wash and elution solutions should be made fresh daily.

Load volumes for large volume water analysis

Cartridge size/ Sorbent mass	1 cc	3 cc	6cc (200 mg, 30 µm)	6cc (200 mg, 60 µm)	12 cc	20 cc	35 cc
Load (mL water) (total of matrix and dilution)	50 mL	200 mL	500 mL	1000 mL	1000 mL	2000 mL	5000 mL

Important SPE Considerations

- **Flow Rate:** The flow rate during the load and elute steps is critical to SPE success. Flow through the device should be about 1 mL/min, such that you can observe discreet droplets eluting from the device. Flowing too quickly will result in break-though (no retention) of your analytes during the load step, or failure to elute during the elution step. Either can result in loss of recovery.

- **Sample Pre-treatment:** This step is essential to make sure that your analytes of interest are contained within a solution appropriate for your SPE protocol. For example, analytes in tissue or blood samples may need to be extracted into a separate solution prior to SPE. In addition, any drug-protein binding must be disrupted before SPE in order for the anaytes of interest to be retained. This is often achieved by diluting the sample (i.e. plasma) 1:1 with a 4% H_3PO_4 (phosphoric acid) solution, to a final concentration of 2% H_3PO_4. In some cases, stronger disruptive action may be needed. Please see the Sample Pre-treatment section for additional suggestions.

- **Ionization States:** When using the mixed-mode sorbents, it is important to think not only about the charge of your analyte of interest, but about the charge of the SPE sorbent as well. Strong ion-exchange sorbent will always be in a charged state. Weak ion-exchange sorbent can be charged or uncharged, depending on the pH of the solution flowing through the sorbent. It is important to understand the impact of these charge states on your sample. As a general rule, operate at least 2 full pH units away from the pK_a of the analytes and/or the sorbent. More information can be found in the Beginners Guide to SPE at **www.waters.com/primers**.

Common Laboratory Conversions and Solution Calculations

1 Conversion Tables

ppm Conversion (parts per million)	ppb Conversion (parts per billion)	ppt Conversion (parts per trillion)	ppq Conversion (parts per quadrillion)
1 mg/L = 1.0 ppm	1 mg/L = 1000 ppb	1 mg/L = 1,000,000 ppt	1 mg/L = 1,000,000,000 ppq
1 µg/L = 0.001 ppm	1 µg/L = 1.0 ppb	1 µg/L = 1000 ppt	1 µg/L = 1,000,000 ppq
1 ng/L = 0.000001 ppm	1 ng/L = 0.001 ppb	1 ng/L = 1.0 ppt	1 ng/L = 1000 ppq
1 pg/L = 0.000000001 ppm	1 pg/L = 0.000001 ppb	1 pg/L = 0.001 ppt	1 pg/L = 1 ppq
1 mg/mL = 1000 ppm	1 mg/mL = 1,000,000 ppb	1 mg/mL = 1,000,000,000 ppt	1 mg/ml = 1,000,000,000,000 ppq
1 µg/mL = 1.0 ppm	1 µg/mL = 1000 ppb	1 µg/mL = 1,000,000 ppt	1 µg/mL = 1,000,000,000 ppq
1 ng/mL = 0.001 ppm	1 ng/mL = 1.0 ppb	1 ng/mL = 1000 ppt	1 ng/mL = 1,000,000 ppq
1 pg/mL = 0.000001 ppm	1 pg/mL = 0.001 ppb	1 pg/mL = 1 ppt	1 pg/mL = 1000 ppq

2 Preparing a 1 Molar (1 M) Solution

- Molar (M) solutions are based on the number of moles of Chemical in 1 litre of solution
- Determine the molecular weight of each atom in the chemical formula

$NaOH$ = 1xNa (22.99), 1xO (15.999), 1xH (1.008)

$NaOH$ = **39.997**

- 1 M $NaOH$ consists of 39.997 g in 1 L of distilled water
- What if 100 ml of 0.1 M of $NaOH$ is required?

Grams of chemical = (molarity of solution in mole/litre) x (MW of chemical in g/mole) x (ml of solution) ÷ (1000 ml/L)

Grams of $NaOH$ = 0.1 x 39.997 x 100 ÷ 1000

100 ml of a 0.1 M NaOH consists of 0.39997 g of NaOH

3 Preparing a Weight/Volume Percentage (W/V%) Solution

- The following calculation is used to calculate w/v% solutions

w/v(%) = weight of the solute ÷ volume of the solution x 100

- What is the w/v(%) of an 250 ml aqueous sodium chloride ($NaCl$) solution containing 8 g of sodium chloride

w/v(%) = 8 g ÷ 250 ml x 100

- 250 ml aqueous sodium chloride solution containing 8 g of sodium chloride is **3.2%** (weight/volume%)
- To determine how much chemical to add to make a w/v% solution

grams of chemical = volume of solution ÷ 100 x w/v%

- What weight of $NaCl$ is required to make 250 ml of a 3.2% w/v% solution

grams of $NaCl$ = 250 ÷ 100 x 3.2

A 3.2% in 250 ml w/v% solution of NaCl consists of 8 g of NaCl

4 Reagent/Sample Dilution Calculation

$$C_1 * V_1 = C_2 * V_2$$

Where: C_1 = Initial concentration, C_2 = Final concentration, V_1 = Initial volume, V_2 = Final volume

For example, to prepare 5 mL of a plasma solution containing 50 ng/mL of a target analyte from a 50 ng/mL stock solution.

C_1 = Initial concentration = 10 µg/mL = 10,000 ng/mL

C_2 = Final concentration = 50 ng/mL

V_1 = Initial volume = unknown

V_2 = Final volume = 5 mL = 5,000 µL
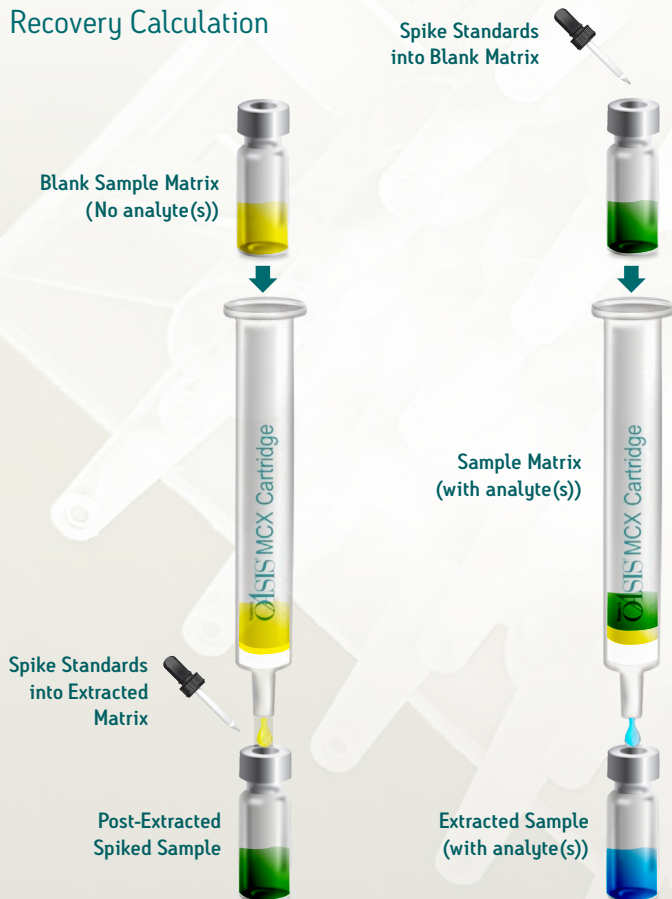
$$C_1 * V_1 = C_2 * V_2$$
$$V_1 = (C_2 * V_2)/C_1$$
$$V_1 = (50 \text{ ng/mL} * 5,000 \text{ µL})/(10,000 \text{ ng/mL})$$
$$V_1 = 25 \text{ µL}$$

Calculating Recovery

To determine the success of the SPE method, there are two key parameters that must be evaluated. These are **recovery** and **matrix effects**. Recovery will determine how successfully the SPE method has isolated your compound(s) of interest. **Matrix effects** will determine if you have removed matrix components that may interfere with your ability to accurately and consistently quantify your compound(s).

Recovery Calculation

Spike Standards into Blank Matrix

Blank Sample Matrix (No analyte(s))

Sample Matrix (with analyte(s))

OASIS MCX Cartridge

Spike Standards into Extracted Matrix

Post-Extracted Spiked Sample

Extracted Sample (with analyte(s))

Recovery of the Extraction Procedure (RE)* (or, SPE Recovery)

$$\% \, RE = 100 \times \frac{\text{Response Extracted Sample (with analyte(s))}}{\text{Response Post-Extracted SPIKED Sample}}$$

- Both extracted samples should be in the same solution

  *Matuszewski, B.K., Constanzer, M.L., Chavez-Eng, C.M. *Anal. Chem.* 2003, 75, 3019-3030.

Calculating Matrix Effects

To determine the success of the SPE method, there are two key parameters that must be evaluated. These are **recovery** and **matrix effects**. Recovery will determine how successfully the SPE method has isolated your compound(s) of interest. **Matrix effects** will determine if you have removed matrix components that may interfere with your ability to accurately and consistently quantify your compound(s).

Matrix Effects Calculation

Blank Sample Matrix
(No analyte(s))

Standard Solution
(Analyte(s))

OASIS MCX Cartridge

Spike Standards
into Extracted
Matrix

Post-Extracted
Spiked Sample
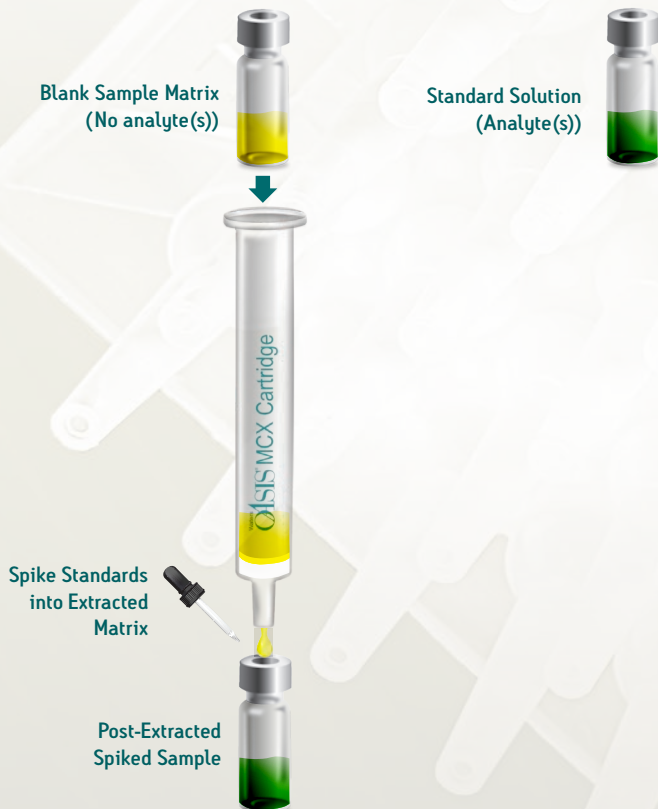
Matrix Effects and Matrix Factor

$$\text{Matrix Factor (MF)} = \frac{\text{Response}_{\text{Presence of Matrix}}}{\text{Response}_{\text{Absence of Matrix}}}$$

$$\% \text{ Matrix Effects (ME)} = \left(\left(\frac{\text{Response}_{\text{Post-Extracted Spiked Sample}}}{\text{Response}_{\text{Solvent Standard}}}\right) - 1\right) * 100$$

- Both samples should be in the same composition solution
- MF Value <1, negative % ME = suppression
- MF Value >1, positive % ME = enhancement

Calculating Recovery and Matrix Effects

Example of Recovery Sample Preparation

Post-Extracted SPIKED Sample

500 µL blank matrix

SPE – elute with 50 µL

Post-spike solvent – 50 µL of a 100 ng/mL = 5 ng (analyte)

50 µL extract

Extracted Sample

500 µL matrix with 10 ng/mL analyte

500 µL of a 10 ng/mL = 5 ng

SPE – elute with 50 µL

Post-spike solvent – 50 µL (no analyte)

50 µL extract 5 ng analyte

- Both vials contain matrix components from 500 µL matrix, (eluted with the 50 µL elution solvent), 50 µL post-spike solvent, and theoretically an equivalent to 5 ng analyte in each solution.

Example of Matrix Effects Sample Preparation

Post-Extracted SPIKED Sample

500 µL blank matrix

SPE – elute with 50 µL

Post-spike solvent – 50 µL of a 100 ng/mL = 5 ng (analyte)

50 µL extract

Standard Solution (analytes)

Post-spike solvent 50 µL of a 100 ng/mL

50 µL elution solvent

- Both vials contain 5 ng analyte (50 µL elution solvent and 50 µL post-spike solvent)
- The post-spike sample also contains the components extracted from the sample matrix.
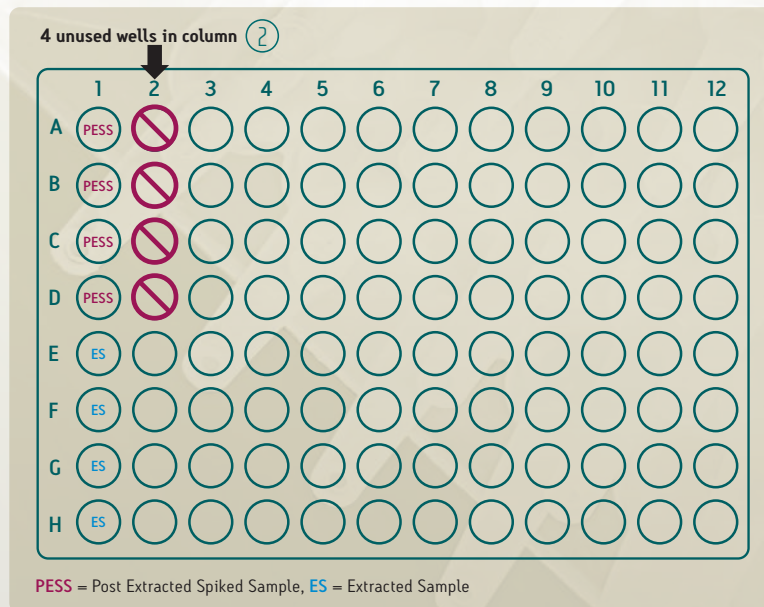
Experimental Set-Up

Definitions for SPE Plate and Collection Plate Samples

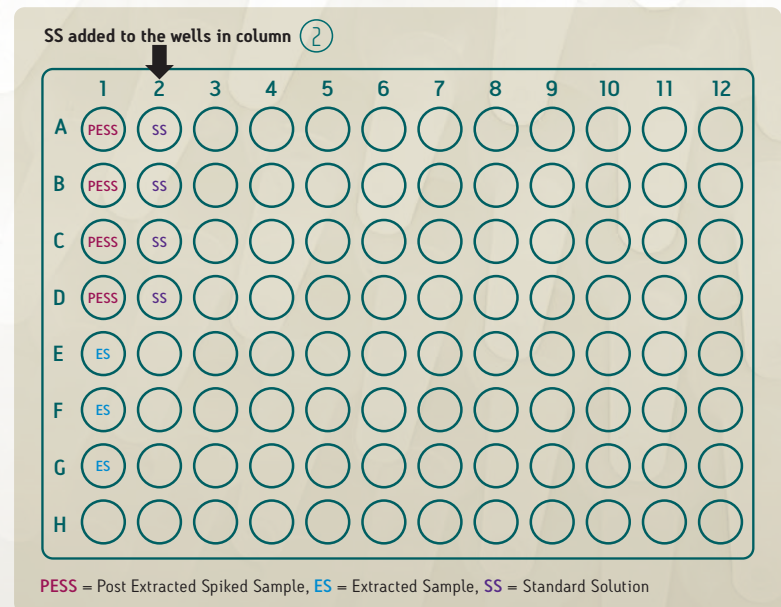### 96-Well Plate Template for Recovery and Matrix Effects Experiment

Will be used for Recovery AND Matrix Effects Calculations	Will be used for Recovery Calculations	Will be used for Matrix Effects Calculations
Post Extracted Spiked Sample (PESS)	Extracted Sample (ES)	Standard Solution (SS)
Run your blank sample matrix through the SPE process then spike the standards directly into these wells at the end	Spike your standards into the sample matrix before the SPE process and collect the final eluate in these wells	Pipette the final elution solution used in the SPE protocol into these wells, then spike in the standards. No SPE performed into these wells
4 Replicates	4 Replicates	4 Replicates

Template for 96-well Plate Experiment to Determine Recovery and Matrix Effects

SPE Plate

4 unused wells in column ②

PESS = Post Extracted Spiked Sample, ES = Extracted Sample

Collection Plate

SS added to the wells in column ②

PESS = Post Extracted Spiked Sample, ES = Extracted Sample, SS = Standard Solution
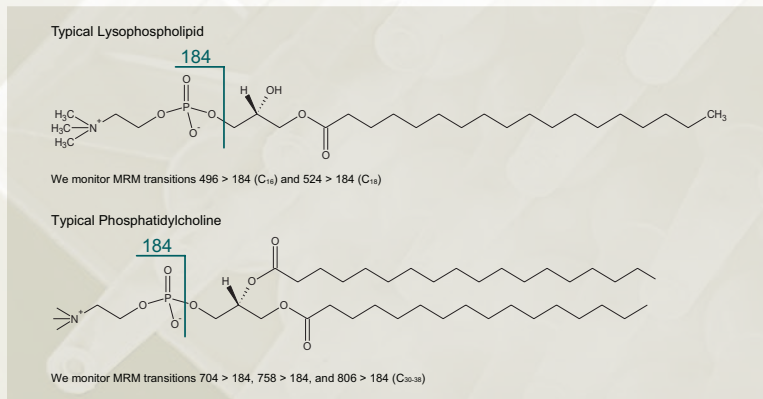
Phospholipid Monitoring

You may wish to monitor the presence of phospholipids in your final sample to evaluate the degree of their removal during the SPE process. Phospholipid removal not only increases method robustness by reducing a common cause of matrix effects, it also increases instrument uptime and column lifetime. There are two common techniques used to monitor the presence of phospholipids. The first approach is to monitor 5 or more MRM transitions from individual phospholipids. The second approach is to monitor 1 MRM transition, the 184.4 fragment common to the polar head group of phosphatidylcholine containing phospholipids, the most abundant type. Either of these methods provides a good representation of the overall cleanliness of your sample.
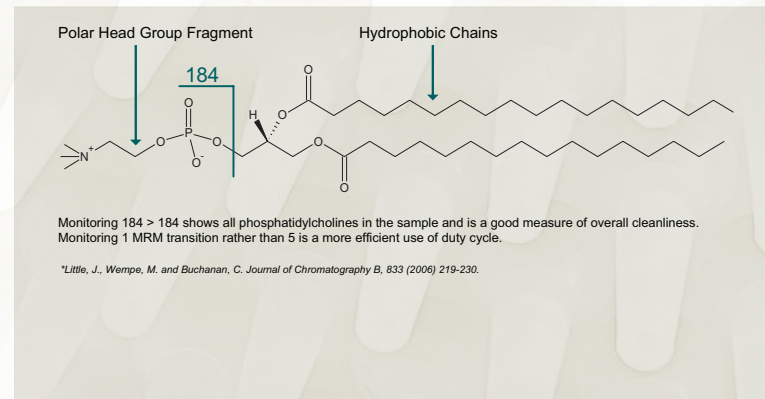
Phospholipid Monitoring MS Method 1:

Monitoring Individual MRM Transition

Typical Lysophospholipid

184

We monitor MRM transitions 496 > 184 (C_{16}) and 524 > 184 (C_{18})

Typical Phosphatidylcholine

184

We monitor MRM transitions 704 > 184, 758 > 184, and 806 > 184 (C_{30-38})

Phospholipid Monitoring MS Method 2:

Monitoring All Phosphatidylcholines*

Polar Head Group Fragment   Hydrophobic Chains

184

Monitoring 184 > 184 shows all phosphatidylcholines in the sample and is a good measure of overall cleanliness. Monitoring 1 MRM transition rather than 5 is a more efficient use of duty cycle.

*Little, J., Wempe, M. and Buchanan, C. Journal of Chromatography B, 833 (2006) 219-230.

Mass Spectrometry Conditions for Phospholipid Monitoring

Precursor Ion (m/z)	Product Ion (m/z)	Cone Voltage	Collision Energy
184.40**	184.40	90	3
496.40*	184.40	35	30
520.40	184.40	35	30
522.40	184.40	35	30
524.40*	184.40	35	30
704.40*	184.40	35	30

Precursor Ion (m/z)	Product Ion (m/z)	Cone Voltage	Collision Energy
758.40*	184.40	35	30
760.40	184.40	35	30
784.40	184.40	35	30
786.40	184.40	35	30
806.40*	184.40	35	30
808.40	184.40	35	30

** For individual MRM method
* Most frequently monitored

SPE Troubleshooting

If you discover that your analyte of interest is not in the elution step, don't panic! You can perform a mass balance experiment to determine where your process went wrong and then take action to correct the problem.

A mass balance in SPE means that you are monitoring each step of your protocol to understand the location of your analyte. You can do this by collecting each step of the method and testing it for presence of your target analyte. This unlikely to be quantitative, just qualitative.

Consider these steps individually:

LOAD

If you find your analyte is breaking through the sorbent and coming out in the load step, there are a few possible reasons:

1. Protein binding was not disrupted and your analyte is stuck to the protein. See suggestions in Sample Pre-treatment section.

2. More sorbent capacity is required. Choose a larger sorbent mass.

3. Wrong sorbent was selected for the analysis. Make sure you are loading in an aqueous solution for reversed-phase retention and/or have chosen the correct ion-exchange sorbent for your target analyte. If using reversed-phase retention and your analyte is very polar, it may help to load it in the un-ionized (neutral) state.

4. Check the pKa values of ionizable functional groups on your analyte and/or the ion-exchange sorbent. To achieve retention on ion-exchange sorbents, both the sorbent and the analyte should be fully ionized. If possible, always work at least 2 pH units away from the pKa of the analyte/sorbent.

5. Flow rate was too fast through the device during the loading step to allow sufficient interaction with the binding/retention sites in the sorbent. Reduce flow rate.

WASH

If you find your analyte is eluting in the wash step, it may be caused by the following:

1. If using reversed-phase SPE, the wash solvent was too strong and disrupted hydrophobic retention. Choose a weaker or alternate solvent.

2. If using ion-exchange SPE, the target analyte was not bound by ion-exchange retention. Consider the charge state of your sorbent and target analyte prior to and during this step.

3. Capacity of the sorbent was slightly exceeded. Move to a larger sorbent mass.

ELUTE

If your analyte is not present in the final elution step or the other two steps, it may be a result of the following:

1. If using a strong ion-exchange sorbent, your target analyte may be a strong acid or base and is irreversibly bound to the sorbent. Choose a weak ion-exchanger.

2. Your target analyte may not be stable under the conditions used and it has degraded. Check the stability of your analyte in the solvents used.

3. The analyte is very hydrophobic and the elution solvent is not strong enough. Choose a stronger elution solvent.

4. If using an ion-exchange sorbent, make sure that you have fully un-ionized the analyte or sorbent during the elution step. Incomplete adjustment can result in analytes remaining bound to the sorbent.

5. Insufficient volume of elution solvent to complete elution. Increase volume.

6. Very polar analytes may not be soluble if there is too much organic in the final elution solvent. Reduce organic content.

Sample Pre-Treatment

PLASMA

The standard pre-treatment for plasma is a 1:1 dilution with 4% phosphoric acid. This dilutes the sample, decreasing viscosity and increasing the contact time with the sorbent. It also helps to disrupt protein binding.

If the sample needs to be at a different pH for loading, try diluting 1:1 with 5% strong ammonia or with another appropriate buffer.

Protein binding: If phosphoric acid is not strong enough to disrupt protein binding, precipitation with an organic solvent may be necessary. Typical protein precipitation consists of a 3:1 dilution of the sample with acetonitrile (ACN) (3 volumes ACN: 1 volume sample) Depending upon your analyte, less solvent may be adequate. Try 2:1 or 1:1 dilution to avoid having to dilute the supernatant excessively with water prior to loading onto the SPE sorbent. Another option is to use methanol as a precipitation solvent.

WHOLE BLOOD

When preparing whole blood samples, the blood cells need to be lysed and the entire sample must be precipitated prior to SPE. For cell lysis, a 1:1 or 1:2 dilution with 0.1 M $ZnSO_4$ is usually sufficient. For example, 100 µL of whole blood can be treated with 50 or 100 µL of $ZnSO_4$. A solution of 0.1 M $ZnSO_4$ and 0.1 M ammonium acetate (NH_4OAc) can also be used. Following cell lysis, precipitate the sample with 2:1 or 3:1, organic solvent: sample. 90:10 ACN:MeOH is a good first choice organic solvent.

URINE

Urine is the most straightforward matrix to pre-treat. It should be diluted 1:1 with an appropriate aqueous solution. Water is usually sufficient for reversed-phase SPE, or if using mixed-mode ion-exchange sorbent, good choices include 4% phosphoric acid or 5% strong ammonia. It is important to make sure your analyte and/or sorbent are in the correct ionization state for loading onto the sorbent. If buffering to a specific pH is required, make sure to use a high enough molarity solution to overcome the natural buffering capacity of urine. Also, be aware of sorbent capacity when using ion-exchange sorbents for urine extractions.

Many drugs and biomarkers exist mainly as glucuronide metabolites in urine. If you are not analyzing the glucuronide metabolites directly, it is important to convert to the unconjugated forms. If hydrolysis is performed, the pH and temperature must be optimized for the enzyme and target analytes.

TISSUE/FOOD

Homogenize and/or dilute the sample with organic or aqueous solvent for liquid extraction, depending on the solubility of the target analytes. Mix and/or centrifuge then collect the supernatant. Prepare this solution for the loading step in the SPE protocol. A high organic concentration is acceptable solvent for 2 step pass-through SPE, while reversed-phase SPE requires a high aqueous solution for the loading step. In this case, dry down and reconstitute the analytes in an aqueous solution, or dilute the organic sample with water. Proceed with the appropriate SPE procedure.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™