Thank you for joining us!
Our Webinar will begin shortly...

Principles of SPE:
Troubleshooting Techniques

Using the Power of Chromatography to Solve Sample Preparation Challenges
This webinar will be presented by Joseph Arsenault. With more than 37 years of chromatography experience at Waters, Joseph Arsenault was a part of the team that introduced Solid Phase Extraction (SPE) technology for analytical laboratories in 1978. He has been involved in all aspects of SPE products from manufacturing, quality control, quality assurance, having spent numerous years as a Product Manager for SPE products.

Over the last 14 years, he has devoted himself to the training and education of chromatographers around the world. In addition to his presentations, he has also authored and co-authored 3 handbooks/primers for chromatographers:

- Beginners Guide to Solid Phase Extractions (SPE)
- Beginners Guide to Liquid Chromatography
- Beginners Guide to Ultra Performance Liquid Chromatography (UPLC)
  - All Available via www.Waters.com
Friendly Reminders…

- Please use **text chat** functionality to submit questions during the Webinar.

Upon conclusion, follow up information will be available:

- [http://www.waters.com/SPEpart2](http://www.waters.com/SPEpart2)
- Recorded version of today’s presentation
- Copies of today’s slides
- Product discount offers
- Product specific information
- Reference materials
1) Introduction to Solid Phase Extraction (SPE) Technology

2) SPE Troubleshooting Techniques

3) SPE Method Development Basics

- Part #: 715003405
- 212 pages, paperback
- Size: 8.5 x 11”
- >150 Full Color Figures and Diagrams
- Chapter Titles
  - Benefits of SPE in Sample Preparation
  - SPE is LC
  - Key Terms and Calculations
  - In the Lab
  - Method Development
  - Troubleshooting
  - Appendix: Glossary of SPE and LC Terms
  - Appendix: Oasis Sorbent Technology for SPE
  - Appendix: Applications
  - Appendix: Additional Reference Materials

- Price: $99
Most Common Problem in SPE

Poor or Inconsistent % Recovery Values
Most Common Problem in SPE: Poor or Inconsistent Recovery

TOP “SPE Trouble SOURCES”

- Flow Rate Control
- De-wetting in Reversed Phase Devices
- Cation Exchange with Silica Sorbents ($\Delta$ pH)
- MS Matrix Effects
- Lack of Breakthrough Study in Method Development
- Lack of Mass Balance Study in Method Development

BUT FIRST:
Common Problem -- POOR % RECOVERY

Root Causes

1) Variations in accurately determining the mass loaded and mass recovered values, for example:

2) Calculation Method

3) Not knowing where your analyte is at each step of the protocol
Solid-Phase Extraction (SPE) Recovery – Accurate Mass

Analyte is the Blue Dye in the “Green” Sample

Proper Load Sample Analyte Fully Retained during Loading

10 ng ± of Blue Dye

Need to check for no analyte

Load

Green Sample (yellow interfer and blue analyte)

Elute

Elution Solvent

Recovery % = \frac{10 \text{ ng Recovered}}{10 \text{ ng Loaded}}

Recovery = 100%

Wash Step to Remove Yellow

10 ng ± at COMPLETION
Common Problem --
POOR % RECOVERY

Root Causes

1) Variations in accurately determining the mass Loaded and mass recovered values, for example:

**50%** ??
Mass Recovered measured TOO Low
Mass Loaded measured TOO High

**140%** ??
Mass Recovered measured TOO High
Mass Loaded measured TOO Low

2) Calculation Method

3) Not knowing where your analyte is at each step of the protocol
Common Problem --
POOR %RECOVERY

Root Causes

1) Variations in accurately determining the mass loaded and mass recovered values, for example

<table>
<thead>
<tr>
<th></th>
<th>50%</th>
<th>140%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass Recovered</td>
<td>TOO Low</td>
<td>TOO High</td>
</tr>
<tr>
<td>Mass Loaded</td>
<td>TOO High</td>
<td>TOO Low</td>
</tr>
</tbody>
</table>

2) Suggested Calculation Method

3) Not knowing where your analyte is at each step of the protocol
Proper Recovery Calculation
Step One

**Step 1**

Blank Sample Matrix
[no analyte[s]]

Run SPE Protocol

Spike into extracted Matrix Standard

10 ng/mL

Analytical Result = 10 ng/mL

Post-Extracted SPIKED Sample

Proper Determination of % Recovery
Proper Recovery Calculation
Step Two

Step 1
Blank Sample Matrix [no analyte[s]]

Step 2
Spike Standard into Sample Matrix

Run SPE Protocol
10 ng/mL →

10 ng/mL
Analytical Result = 10 ng/mL
Post-Extracted SPIKED Sample

Extracted Sample (with standard)

Proper Determination of % Recovery
Proper Recovery Calculation

Perform Calculation

\[
\% \text{ RE}^* = 100 \times \frac{\text{Response \ Extracted \ Sample \ (with \ standard)}}{\text{Response \ Post-Extracted \ SPIKED \ Sample}}
\]

*Recovery of the Extraction Procedure RE
Proper Recovery Calculation
Perform Calculation

Step 1: Blank Sample Matrix [no analyte(s)]

Step 2: Spike Standard into Sample Matrix

10 ng/mL →

% RE* = 100 \times \frac{\text{Response Extracted Sample (with standard)}}{\text{Response Post-Extracted SPIKED Sample}}

*Recovery of the Extraction Procedure RE

Analytical Result = 10 ng/mL

Extracted Sample (with standard)

Analytical Result = 9.5 ng/mL

Post-Extracted SPIKED Sample

Spike into extracted Matrix Standard

10 ng/mL

100 \times \left( \frac{9.5}{10.0} \right) = 95\% \text{ Recovery}
Common Problem -- POOR %RECOVERY

Root Causes

1) Variations in accurately determining the mass Loaded and mass recovered values, for example

- **50% ??** Mass Recovered measured TOO Low
- **140% ??** Mass Recovered measured TOO High
- Mass Loaded measured TOO High
- Mass Loaded measured TOO Low

2) Calculation Method

3) Not knowing *where your analyte is* at each step of the protocol - several things to consider
How Much Liquid is Contained in this Glass of Ice Water?

Liquid is only able to "Fit" in the Spaces between the "Particles of Ice" – this is called the "Interstitial Volume"

For example, a full 12 oz. glass of Ice Water may contain only 6 oz. of Liquid Water!

Familiar Example of Interstitial Volume
Liquid is only able to “Fit” in the Spaces between the “Particles of Ice” – this is called the “Interstitial Volume”.

For example, a full 12 oz. glass of Ice Water may contain only 6 oz. of Liquid Water!

For SPE Devices, we need to know what this volume value is to predict where an analyte will be located – this is called the SPE Device “Hold-UP Volume”.

Familiar Example of Interstitial Volume
Knowing Where Your Analyte “SHOULD BE” (Hold-Up Volume??)

Liquid HOLD-UP VOLUME of an SPE Device

Hold-Up Volume—SPE Cartridge
Knowing Where Your Analyte “SHOULD BE” (Hold-Up Volume ??)

Liquid HOLD-UP VOLUME of an SPE Device

When flow stops – how much liquid is still in the device?

\[ \text{-- For example } 1.2 \text{ mL} \]

Value must be determined for each device design

Carefully load a liquid onto the device and STOP FLOW – just as the FIRST DROP Elutes – Measure how much was added

Upon subsequent liquid additions, this value can be used to predict where your analyte will be
Load Analyte with a $k = 0$
Not Captured/No Retention

Sample volume greater than hold-up volume

Pure purple analyte has a $k = 0$.
Therefore, it is not retained.

Note: Some purple analyte is already coming through, while sample is still being loaded.

Pass-Through Strategy when the Sample Volume is Greater than the Hold-up Volume
Loaded Analyte with a $k = 0$ Elution Step

Sample volume greater than hold-up volume

Add Pure Sample Solvent ($k = 0$) GREATER THAN ($>)$ Hold-up Volume to flow ALL of the REMAINING ANALYTE out of the Cartridge

Note: Some of the Analyte is contained in BOTH Vessels (Original Load, and Elution Steps)

Pass-Through Strategy when the Sample Volume is Greater than the Hold-up Volume
Load Analyte with a $k = 0$
Not Captured/No Retention

**Less sample volume than hold-up volume**

Pure purple analyte has a $k = 0$. Therefore, it is not retained.

Note: purple analyte does not make it through the sorbent bed before loading is stopped.

Pass-Through Strategy when the Sample Volume is Less Than the Hold-up Volume
Loaded Analyte with a $k = 0$ Elution Step

Less sample volume than hold-up volume

Add Pure Sample Solvent ($k = 0$) with Volume GREATER THAN (>) Hold-up Volume to flow ALL of the ANALYTE out of the Cartridge

Colorless interferences are captured $k =$ very high

Pure purple analyte passes completely through the sorbent bed with the addition of pure sample solvent $k = 0$

Note: Purple analyte is contained in only ONE vessel

Pass-Through Strategy when the Sample Volume is Less Than the Hold-up Volume
Loaded Analyte with a $k = 0$ Elution Step

**Less sample volume than hold-up volume**

Add Pure Sample Solvent ($k = 0$) with Volume LESS THAN (<) Hold-up Volume will elute SOME of the ANALYTE out of the Cartridge.

Some analyte is still in the cartridge!!

**Pass-Through Strategy when the Sample Volume is Less Than the Hold-up Volume**

Colorless interferences are captured $k = \text{very high}$
Load Analyte with a $k = \text{HIGH}$
Well Captured/High Retention

Sample Volume
Greater or Less Than
Hold-up Volume

Note: purple analyte
DOES NOT make it
through the sorbent bed

Analyte $k = \text{High}$
for Good Capture -
analyte does not move
too far down the bed
Loaded Analyte with $k = \text{HIGH}$

Elution Step

Initial Sample Volume Greater or Less Than Hold-up Volume --
Now, Elution Solvent is Added GREATER Than Hold-up Volume

Elution Solvent

Sample Solvent

STRONG ELUTION SOLVENT ($k = 0$) with Volume GREATER THAN ($>)$ Hold-up Volume to flow ALL of the ANALYTE out of the Cartridge

All of the Analyte Should be in JUST the Elution Vessel

Colorless interferences are captured $k = \text{very high}$

$k = 0$

Note: Purple analyte is contained in only ONE vessel
Loaded Analyte with \( k = \text{HIGH} \) Elution Step

Initial Sample Volume Greater or Less Than Hold-up Volume --
Now, Elution Solvent is Added LESS Than Hold-up Volume

Elution Solvent

STRONG ELUTION SOLVENT \((k = 0)\) with Volume LESS THAN \(<\) Hold-up Volume
only SOME of the ANALYTE will flow out of the Cartridge

Colorless interferences are captured \( k = \text{very high} \)

Only SOME of the Analyte will be in the ELUTION vessel

contained in only ONE vessel
Most Common Problem in SPE: Poor or Inconsistent Recovery

TOP “SPE Trouble SOURCES”

- Flow Rate Control
- De-wetting in Reversed Phase Devices
- Cation Exchange with Silica Sorbents ($\Delta$ pH)
- MS Matrix Effects
- Lack of Breakthrough Study in Method Development
- Lack of Mass Balance Study in Method Development
SPE is Liquid Chromatography
Same Rules Apply

Stationary phase is non-polar (C$_{18}$)
Reversed Phase

Same chromatography principles, different format.

Mobile phase is polar (aqueous)

Reversed-Phase Chromatography, SPE and LC
van Deemter Curve and Plate vs. Flow Rate Curve

Optimal Linear Velocity corresponds to **Optimal Flow Rate**
(for maximum Plate Count – Resolution)

HETP vs. **Linear Velocity**

\[ u = \frac{L}{t_0} \]

**HETP** vs. Linear Velocity

**HETP** ↓ PLATES ↑

Height Equivalent to Theoretical Plate HETP

**Flow Rate** {mL/min}

**Separation Efficiency**

1.0
2.0
3.0

**Do Not Flow Too Fast**
van Deemter Curve and Plates vs. Flow Rate Curve

Optimal Linear Velocity corresponds to **Optimal Flow Rate**
(for maximum Plate Count – Resolution)

HETP vs. Linear Velocity

\[ u = \frac{L}{t_0} \]

Plates vs. Flow Rate

Separation Efficiency

- **Optimal**: Do Not Flow Too Fast
- Loss of Plates (Resolution)

**Height Equivalent to Theoretical Plate (HETP)**
SPE Steps Requiring FLOW RATE Control

Conditioning and Equilibration steps are NOT Flow Rate sensitive

Load, Wash and Elute steps WILL be Flow Rate sensitive

1) Start with “Drops” flowing from device (Typical cartridge – 1 mL/min.) (Smaller ID cartridge will require even slower flow rate)
2) Check recovery
3) Controlled testing to higher flow rates to find Max (application dependent)

Note: Ion-exchange systems may require even slower flow rates
Load at Proper Flow Rate

Pure green analyte fully retained during loading

10 ng

No analyte comes through

Drops usually indicate acceptable flow-rate

1 mL/min

Importance of Flow Rate Control—Load Steps
Steps Requiring FLOW RATE Control

Load

Load at Proper Flow Rate

Pure green analyte fully retained during loading

10 ng →

No analyte comes through

Drops usually indicate acceptable flow-rate

Load Sample with Flow Rate TOO High

Analyte breaks through in the load step

6 ng →

Some analyte captured in cartridge, some passes through

4 ng (60% recovery at end)

2 mL in 10 seconds = 12 mL/min

Importance of Flow Rate Control—Load Steps
Steps Requiring Proper FLOW RATE Control

10 ng of pure green analyte fully retained during loading

Load

10 ng →

Need to check for absence of analyte

Wash

10 ng →

Need to check that all the analyte elutes

Elute

10 ng

0 ng

0 ng

10 ng

Recovery = 100%

Determining % Recovery
Most Common Problem in SPE: Poor or Inconsistent Recovery

TOP “SPE Trouble SOURCES”

- Flow Rate Control
- **De-wetting in Reversed-Phase Devices**
- Cation Exchange with Silica Sorbents ($\Delta$ pH)
- MS Matrix Effects
- *Lack of Breakthrough Study in Method Development*
- *Lack of Mass Balance Study in Method Development*
TOP “SPE Trouble SOURCES”

- Flow Rate Control
- De-wetting in Reversed-Phase Devices
  - C18 and C8 Silica Based
- Cation Exchange with Silica Sorbents (Δ pH)
- MS Matrix Effects
- Lack of Breakthrough Study in Method Development
- Lack of Mass Balance Study in Method Development
Polarity Retention Mechanism

Likes Attract Likes

Polar…Polar

Non-Polar…Non-Polar

Opposites Repel

Polar
(WATER)
(Vinegar)

Non- Polar
(C18 RP Sorbent)
(Oil)
Familiar Example
Rain on Old Auto

Rain water is very polar

Thin shiny film of POLAR water – LIKES POLAR car surface

Unwaxed Car – A Very Polar Surface
Familiar Example
Rain on Waxed Auto

Waxing your car changes the POLAR surface to a NON-POLAR surface.

Water beads up! –POLAR water DISLIKES NON-POLAR surface

Waxing Your Car is Like Bonding C18 to Silica – it Makes it Non-Polar
Conditioning / Equilibration for “Wetting”

BEFORE the Sample is Loaded, use an Organic Solvent to “WET” the Sorbent Pores --

then Flush with Sample Solvent or Water to EQUILIBRATE the Sorbent

Required for Reversed-Phase Applications
Proper Wetting
Good Capture/Retention

Chromatographic Surface of Packing Materials

- Sorbent particles are very porous, like the pores of a sponge – 99% of chromatographic surface is inside the pores
- Mobile phase must be allowed into the pore in order for chromatographic retention of the analyte to take place – (competition)

If the pores are wet, then the red analyte molecule will be retained – “captured”
Solid-Phase Extraction (SPE)

Note: A reversed-phase sorbent needs to be properly wetted.

Organic solvent initially “WETS” (“CONDITIONS”) the pores, but is TOO strong for sample loading.
Proper Wetting Conditioning and Equilibration Steps

Replace strong organic in the pores with water or the sample solvent. The pores remain “WET” but are now ready for the sample since they are "EQUILIBRATED".

Remember, the Organic Solvent from the Conditioning Step brings the Equilibration Water or Sample Solvent into the Pores and is then Replaced by the Water or Sample Solvent.

Equilibrating the Pores with Water or Sample Solvent
Fully Wetted Sorbent Pore
Proper Conditioning and Equilibration

$C_{18}$ Bonded and “Fully Endcapped”
High Purity Silica Gel Pore

Packing materials/particles are very porous, like the pores of a sponge—99% of the chromatographic surface is inside the pores.

Mobile phase must be allowed into the pore in order for chromatographic retention of the analyte to take place—(competition).

If the pores are dry, then the analyte cannot get into the pore and will not be retained (captured) by the chromatographic surface.

Remember: ~30-50% of surface silanols remain due to “Steric Hindrance”

Note: Difficulty bonding silanols in micropores—ligands too large to enter
Loading Step  --  When the Sample is Actually Applied to the SPE Device

Sorbent Pores must be properly wetted for this step in Reversed-phase methods
Successful Loading
Proper Wetting

Green sample, (yellow interference and blue analyte in sample solvent) can be “LOADED” onto the cartridge.

Proper Wetting Results in Good Capture

Load the Sample

Condition (Organic)
Equilibrate (Water, Sample Solvent)
Load
Wash
Elute
Dry Down
Reconstitute
De-Wetted Pores in Reversed-Phase No Analyte Retention (No Capture)

If the pores are dry, then the analytes cannot get into the pore and will not be retained (captured) by the chromatographic surface.

If the pores are dry, (“de-wetted”), then the red analyte will NOT be retained (“not captured”)

Chromatographic Surface of Packing Materials

Mobile Phase

Analyte

©2013 Waters Corporation
Solid-Phase Extraction (SPE)
Drying Out Effect on C18 Silica

Condition with Organic

Wets the Pores

Vacuum can pull air into one or some of the cartridges, and Dries Out Organic in the Pores

Equilibrate with Water/Sample Solvent

No Organic in Pores means No Water gets into Pores

LOAD

Analytes DO NOT Enter Pores and are NOT Retained – POOR RECOVERY!

Wash

Elute

100% Recovery  No Recovery

Completely Dried Out
De-Wetted Pores in Reversed-Phase Poor Analyte Retention (No Capture)

INCONSISTENT % Recovery Values (Calculated at the completion of the SPE Protocol)
Impact on %Recovery Due to Drying-Out Effect on Silica

(Minutes of Air Drawn into the Cartridge - Vacuum)
Impact on %Recovery Due to Drying-Out Effect on C18 Silica

C18 Cartridge (100 mg)

% Recovery

Drying Time

100% Recovery
Completely Dried Out
Partially Dried Out

100% Recovery
No Recovery
Partial Recovery

Macro 96-well Plate
Top Plate
Bottom Plate

96-Well Plate on Manifold
Greatly Improved % Recovery Values with Unique Polymeric Sorbent

No Impact of Sorbent Drying on HLB-High, Consistent Recovery

Oasis HLB Cartridge (30 mg)

C18 Cartridge (100 mg)

Oasis HLB 1 cc/30 mg and C18 1 cc/100 mg Cartridges were Conditioned on a Waters Vacuum Manifold
Oasis® Sorbents Prevent De-Wetting

More Consistent % Recovery Values
Oasis® Sorbents

100% Recovery

% Recovery values calculated at the completion of the SPE Protocol
Most Common Problem in SPE: Poor or Inconsistent Recovery

TOP “SPE Trouble SOURCES”

- Flow Rate Control
- De-wetting in Reversed-Phase Devices
- Cation Exchange with Silica Sorbents ($\Delta$ pH)
- MS Matrix Effects
- Lack of Breakthrough Study in Method Development
- Lack of Mass Balance Study in Method Development
Cation Exchange: Strong Capture for Charged Bases

- Charged Sorbent

+ Charged Base
Analyte Charge as a Function of pH

- **Weak Base**: Positively charged
- **Strong Base**: Positively charged
- **Weak Acid**: Negatively charged
- **Strong Acid**: Negatively charged
- **Zwitterion**: Neutral
- **Neutral**: Neutral

**Low pH** to **High pH**
Analyte Charge as a Function of pH

- **Weak Base**
- **Strong Base**
- **Weak Acid**
- **Strong Acid**
- **Zwitterion**

**Neutral** positions at Low and High pH.
Sorbent Surface Charge as a Function of pH -- C18 Silica

SORBENTS

Ionized (+)
- Weak Anion Exchanger/RP
  - Oasis WAX

Ionized (+)
- Strong Anion Exchanger/RP
  - Oasis MAX

Un-Ionized
- Weak Cation Exchanger/RP
  - Oasis WCX

Un-Ionized
- Strong Cation Exchanger/RP
  - Oasis MCX

Ionized (—)

C18 Silica

Low pH

High pH
Un-bonded Silica Gel Particle: Pore Surface - Silanol Group

Silanol Groups    Si - OH

Note:
Surface silanols "Acidic sites" (Weak Acid)
Un-bonded Silica Gel Particle: Pore Surface - Silanol Group

Silanol Groups  Si - OH

Note:
Surface silanols “Acidic sites” (Weak Acid)

Uncharged at Low pH
but
- Charged @ pH >5
Sorbent Silanol Interactions
Creating a Negative Charge

Silica Gel Surface Silanol Charge changes with mobile phase

OH       OH       0⁻       0⁻

l       l       l + 2H⁺

Si   –   Si   Si   –   Si

(pH 2)  →  (pH 7)

Silica Based Bonded Phases can Behave as Weak Cation Exchanger

Result: Strong interaction between ionized surface silanols (⁻) and basic analytes (+)
Creates a Cation Exchange Retention Mechanism
C18 Bonded and “Fully End-Capped” High Purity Silica Gel Pore

Bonded and End-Capped Silica Based Sorbents can also Become Negatively Charged at pH > ~5

Remember:
~50% of surface silanols remain due to “Steric Hindrance”

Note: Difficulty bonding silanols in micro-pores
Sorbent Surface Charge as a Function of pH  C18 Silica

**SORBENTS**

- Ionized (+)
  - Weak Anion Exchanger/RP
  - Oasis WAX
  - Un-Ionized

- Ionized (+)
  - Strong Anion Exchanger/RP
  - Oasis MAX

- Un-Ionized
  - Weak Cation Exchanger/RP
  - Oasis WCX
  - Ionized (—)

- Un-Ionized
  - Weak Cation Exchanger/RP
  - Oasis MCX

**C18 Silica**

- Ionized (—)
  - Strong Cation Exchanger/RP
  - Oasis MCX
  - Ionized (—)

**Un-Anticipated Cation Exchange**

pH > 5
Hydrophobic Interaction with Bonded Phase

- pH < 3
- Mobile Phase
- Si - OH
- Substrate Protonated
- No Charge

Mobile Phase

pH < 3

Substrate Protonated

No Charge

Base +

SAME C18 Silica SPE Different pH

Mixed-Mode Retention SPE Cartridges C18 - Silica
Hydrophobic Interaction with Bonded Phase

pH < 3
Mobile Phase
Si - OH
Substrate Protonated no charge

pH > 5
Mobile Phase
Si - O
Substrate De-protonated Negative Charge

Mixed-Mode Retention
SPE Cartridges C18 - Silica

Ion exchange Interaction with Charged Sites High Silanol Activity, and Reversed-phase

N
Base +

Easy to Elute Base—Cation EX
Low Recovery in SPE

Hard to Elute Base—RP

SAME C18 Silica SPE Different pH
During the SPE Protocol Load or Wash Steps, if the pH approaches 5-7

(pH Water = 7)

Particle becomes Negatively Charged

Elute 1 MeOH

Elution Step for Base Using 100% MeOH

Even High Organic Will NOT Elute the Positively Charged Basic Analyte

Resulting in Very Poor Recovery

It is Not where you think it should be!!!
TOP “SPE Trouble SOURCES”

- Flow Rate Control
- De-wetting in Reversed Phase Devices
- Cation Exchange with Silica Sorbents ($\Delta$ pH)

**MS Matrix Effects**

- Lack of Breakthrough Study in Method Development
- Lack of Mass Balance Study in Method Development
Standards in aqueous solution give acceptable response
Signal Suppression -- What can happen to Analytes in a Complex Sample Matrix

Analytes in human plasma with only Protein Precipitation (PPT)

Analyte Standards in aqueous solution

Example of Ion Suppression Due to Sample Matrix

Scan ES+ 609.6

50/50 Water/ACN

Analyte Standards in aqueous solution

% Loss

Scan ES+ 591.6

50/50 Water/ACN + human plasma supernatant

Analytes in human plasma extracted using protein precipitation

Example of Ion Suppression Due to Sample Matrix

Scan ES+ 609.5

% Loss

260.2 -97 %
291.2 -96 %
354.4 -86 %
411.4 -93 %
472.6 -93 %
485.6 -95 %
518.5 -89 %
591.6 -93 %
MRM for Terfenadine

Significant ion suppression observed for analytes that co-elute with residual matrix components using just PPT.

Protein Precipitation (PPT)

80% ion suppression

Gradient time = 1.5 min

Note: These samples are dried and reconstituted.
MRM for Terfenadine

PPT

80% Ion Suppression

SPE (Oasis® MCX)

Minimal Ion Suppression

No loss in signal observed for analytes when the interferences, which cause the suppression, are removed by mixed-mode SPE.

Note: These samples are dried and reconstituted.
Matrix Effect Calculation Step One

Step 1

Blank Sample Matrix (no analyte(s))

Run SPE Protocol

10 ng/mL Post-Extracted SPIKED Sample

Proper Determination for Matrix Effects
Matrix Effect Calculation
Step Two

**Step 1**
Blank Sample Matrix (no analyte(s))

**Step 2**
Spiked Standard into Neat Solvent

10 ng/mL
Non-Extracted Neat Solvent (with standard)

Post-Extracted SPIKED Sample

*Proper Determination for Matrix Effects*
Matrix Effect Calculation

\[
\% \text{ ME}^* = 100 \times \left( \frac{\text{Response Post Extracted Spiked Sample (with standard)}}{\text{Response NON-Extracted Neat Solvent with analyte(s))}} \right) - 1
\]

* Matrix Effects (ME)
  - Both samples should be in the same solution
  - Negative value = Suppression
  - Positive value = Enhancement
Matrix Effect Calculation -- No Matrix Effect

**Step 1**
Blank Sample Matrix (no analyte(s))

**Step 2**
Spiked Standard into Neat Solvent

- **Non-Extracted Neat Solvent** (with standard)
- **Post-Extracted SPIKED Sample**

**MS Result = 10 ng/mL**

*Matrix Effects (ME)*
- Both samples should be in the same solution
- Negative value = Suppression
- Positive value = Enhancement

\[
\% \text{ ME} = 100 \times \left( \frac{10.0}{10.0} - 1 \right) = 0\%
\]

If both results are the SAME,
\% \text{ ME} = 0%
No matrix effect

<table>
<thead>
<tr>
<th>Post-Extracted Spiked</th>
<th>Non-Extracted Neat</th>
<th>Matrix Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ng/mL</td>
<td>10 ng/mL</td>
<td>0%</td>
</tr>
</tbody>
</table>

First Scenario: No Matrix Effects
Matrix Effect Calculation – Suppression

**Matrix Effects (ME)**
- Both samples should be in the same solution
- Negative value = Suppression
- Positive value = Enhancement

\[
\% \text{ ME} = 100 \times \left( \frac{2.0}{10.0} - 1 \right) = -80\%
\]

% ME = -80%
which means 80% Suppression

<table>
<thead>
<tr>
<th>Post-Extracted Spiked</th>
<th>Non-Extracted Neat</th>
<th>Matrix Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ng/mL</td>
<td>10 ng/mL</td>
<td>80% Ion Suppression</td>
</tr>
</tbody>
</table>

Second Scenario: Significant Ion Suppression
Matrix Effect Calculation --
Enhancement

**Step 1**
Blank Sample Matrix
(no analyte(s))

**Step 2**
Spiked Standard
into Neat Solvent

- Matrix Effects (ME)
  - Both samples should be in the same solution
  - Negative value = Suppression
  - Positive value = Enhancement

MS Result = 10 ng/mL

% ME = \( \frac{12.0}{10.0} - 1 \) = 20%

% ME = 20%
which means 20% Enhancement

<table>
<thead>
<tr>
<th>Post-Extracted Spiked</th>
<th>Non-Extracted Neat</th>
<th>Matrix Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 ng/mL</td>
<td>10 ng/mL</td>
<td>20% Ion Enhancement</td>
</tr>
</tbody>
</table>

**Third Scenario: Ion Enhancement**
Most Common Problem in SPE: Poor or Inconsistent Recovery

TOP “SPE Trouble SOURCES”

- Flow Rate Control
- De-wetting in Reversed Phase Devices
- Cation Exchange with Silica Sorbents (Δ pH)
- MS Matrix Effects

**Lack of Breakthrough Study During Method Development Work**

**Lack of Mass Balance Study During Method Development Work**
Solid-Phase Extraction (SPE) Breakthrough/Loading Study

How Much Sample can I Load onto this Cartridge?
Solid-Phase Extraction (SPE)

**Capacity**

Amount of Sample the SPE Device can Effectively Handle, based on the Chromatographic Conditions [\( \sim f(k) \)]

**Breakthrough**

When the Capacity of the SPE Device is Exceeded for the Analyte(s) of Interest (can’t capture all of the analyte)
Capacity  Amount of Sample the SPE Device can Effectively Handle, based on the Chromatographic Conditions \( \sim f(k) \)

Breakthrough  When the Capacity of the SPE Device is Exceeded for the Analyte(s) of Interest (can’t capture all of the analyte)

Remember – Other Sample Matrix components are also being captured by the cartridge
Loading Determination – Answer Depends on Chromatography

- Loading is a Function of;
- How much Sorbent
- Type of Sorbent
- Chromatographic Conditions
  - $^k$ of Analytes (Sorbents and Solvents)
  - $^k$ of Interferences
  - $^e$ Conditioning / Equilibration
- Flow Rate
- Variations in Sample Matrix (male vs. female, dog vs. human

Breakthrough Study must be Performed to Develop a Robust Method
Loading Determination

Breakthrough Study

• Series of Experiments – Passing Increasing Volumes of Sample Matrix through separate SPE Devices [All the same cartridge size / type] [Volumes bracketing Desired Volume]

• Follow your planned SPE Protocol

• Determine Analytical Results (% Recovery) for Analytes

• Plot Recovery Results for Analytes vs. Sample Volume

• Determine Maximum Volume for Each of the Analytes – find Maximum Volume for Method
Breakthrough Study — Loading Step

1.0
2.0
3.0
4.0
5.0 mL

Breakthrough Study — Loading
Breakthrough Study
Loading Step

Breakthrough Study—Loading
Breakthrough Study
Loading Step

Desired Method Goal: Load 2mL of Sample on Smallest Cartridge Possible
Breakthrough Study
Loading Step

Save these for future reference
Analyze for Analytes, and Calculate %Recovery
Loading Determination

Breakthrough Study

• Series of Experiments – Passing Increasing Volumes of Sample Matrix through separate SPE Devices [All the same cartridge size / type] [Volumes bracketing Desired Volume]

• Follow your planned SPE Protocol

• Determine Analytical Results (% Recovery) for Analytes

• Plot Recovery Results for Analytes vs. Sample Volume

• Determine Maximum Volume for Each of the Analytes – find Maximum Volume for Method
Loading Determination
Plot %Recovery vs Load Volume

% Recovery vs. Sample Volume (as a function of k)

(Results for 6 Cartridges)

<table>
<thead>
<tr>
<th>Cartridge</th>
<th>1</th>
<th>2</th>
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% Recovery

Volume through SPE Device (mL)

Breakthrough Study: Recovery vs. Load Volume
Loading Determination
Plot %Recovery vs Load Volume

% Recovery vs. Sample Volume (as a function of k)

(Results for 6 Cartridges)

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% Recovery vs. Load Volume

Two Analytes
- Yellow: $k = 0$
- Blue: $k = 2$
Breakthrough Study LOAD Step

0.5

1.0

2.0

3.0

4.0

5.0 mL

1

2

3

4

5

6

Breakthrough Study—Loading
Loading Determination
Plot %Recovery vs Load Volume

% Recovery vs. Sample Volume (as a function of k)

(Results for 6 Cartridges)

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<tr>
<td>% Recovery</td>
<td>120</td>
<td>100</td>
<td>80</td>
<td>60</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Load volume through SPE Device (mL)</td>
<td>0.5</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
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<td>5.0</td>
</tr>
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Two Analytes
- Yellow: k = 0
- Blue: k = 2
Breakthrough Study LOAD Step

Loading Step
Loading Determination
Plot %Recovery vs Load Volume

% Recovery vs. Sample Volume (as a function of k)

(Results for 6 Cartridges)

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% Recovery

Load volume through SPE Device (mL)

Two Analytes

- Yellow: k = 0
- Blue: k = 2
What is the Answer? How Much Can I Load?

- If just Yellow?
- If Just Blue?
- If Both?
Breakthrough Study

% Recovery vs. Sample Volume (as a function of k)

(Results for 6 Cartridges)

Cartridge 1 2 3 4 5 6

Two Analytes

Yellow k = 0

Just Yellow
How Much Can I Load?

- If just Yellow?
- If Just Blue?
- If Both?
Breakthrough Study

% Recovery vs. Sample Volume (as a function of k)

(Results for 6 Cartridges)

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<th>Load volume through Spin Device (mL)</th>
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<td>0.5</td>
</tr>
<tr>
<td>% Recovery</td>
</tr>
<tr>
<td>120</td>
</tr>
</tbody>
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Two Analytes

- k = 0
- k = 2

Just Blue
How Much Can I Load?

- If just Yellow?
- If Just Blue?
- If Both?
Breakthrough Study

% Recovery vs. Sample Volume (as a function of k)

(Results for 6 Cartridges)

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Lead volume through SPE Device (mL)

Two Analytes

- $k = 0$
- $k = 2$

BOTH
What would you do if you still needed to process a 2mL sample for both yellow and blue??

% Recovery vs. Sample Volume (as a function of k)

(Results for 6 Cartridges)

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% Recovery

Volume through SPE Device (mL)

% Recovery vs. Load Volume

Two Analytes
- k = 0
- k = 2
What would you do if you needed to process a 2mL sample for both yellow and blue??

You would need a Larger Cartridge with MORE of this Sorbent

or,

A DIFFERENT Sorbent/Solvent System with more capacity (k) for Yellow

---

**% Recovery vs. Sample Volume (as a function of k)**

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(Results for 6 Cartridges)

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![Graph showing % Recovery vs. Load Volume](image)

% Recovery vs. Load Volume
Very important in SPE Methods Development to insure the most efficient performance of the method and its’ robustness.

% Recovery vs. Sample Volume (as a function of k)

(Results for 6 Cartridges)

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% Recovery

Volume through SPE Device (mL)

Two Analytes
- k = 0
- k = 2

% Recovery vs. Load Volume
TOP “SPE Trouble SOURCES”

- Flow Rate Control
- De-wetting in Reversed Phase Devices
- Cation Exchange with Silica Sorbents ($\Delta$ pH)
- MS Matrix Effects
- *Lack of Breakthrough Study in Method Development*
- *Lack of Mass Balance Study in Method Development*
Root Causes

1) Variations in accurately determining the mass Loaded and mass recovered values, for example

- Mass Recovered measured TOO Low
- Mass Loaded measured TOO High

2) Calculation Method

3) Not knowing where your analyte is at each step of the protocol
Mass Balance Study
Methods Development

- Tracks where your analyte is at each step of the SPE Protocol

- You analyze for the presence/amount of the analyte in what comes out of the device during each step
Need to Know the Hold-Up Volume

SPE Device Hold-Up Volume

- Chromatographic Bed/Sorbent
- Silica-based, Alumina, Polymer
- Filters/Frits
- Polyethylene
- Hold-up volume is represented by orange color.
- Outlet
Collect and Test for How Much Analyte

Analyte is a Purple Compound

Collect and test for the presence, and mass of your analyte, at each step
Less sample volume than hold-up volume

Strategy 1
Situation 2

Note: purple analyte DOES NOT make it through the sorbent bed before loading is stopped.

Pure purple analyte has a $k = 0$. Therefore, it is not retained.

Test that No Analyte has passed through

Pass-Through Strategy when the Sample Volume is Less Than the Hold-up Volume
Strategy 1
Pass Through

Due Mass Balance
How Much Loaded
How Much Eluted
Should Match

Colorless interferences are captured
$k = \text{very high}$

Pure purple analyte passes completely through the sorbent bed with the addition of pure sample solvent
$k = 0$

Note: Purple analyte is contained in only ONE vessel

Pass-Through Strategy when the Sample Volume is Less Than the Hold-up Volume

Less sample volume than hold-up volume
Strategy 2
Capture, Wash, Elute

Wash solvent is strong enough to remove the interferences, but NOT the analyte of interest.

A properly selected WASH solvent causes the yellow interference to move out of the cartridge, (“WASHING”) the interference away from the blue analyte, which is still retained on the cartridge.

Yellow interference is discarded

Condition (Organic)
Equilibrate (Water, Sample Solvent)
Load
Wash
Elute
Dry Down
Reconstitute
Wash Step

Due Mass Balance for Yellow
How Much Loaded
How Much Eluted
Should Match

The Yellow Interference
is Completely Washed Away
Incomplete Wash Step

The Yellow Interference is **NOT** completely Washed Away

Due Mass Balance for Yellow
How Much Loaded
How Much Eluted
DO NOT Match
Incomplete Wash Step

The Yellow Interference is NOT Completely Washed Away

How to Fix

1) Wash with MORE Volume of THIS solvent

2) Wash with SAME Volume of a STRONGER Solvent
Strategy 2
Capture, Wash, Elute

Elution solvent is strong enough to release JUST the blue analyte of interest.

A properly selected ELUTION solvent allows just the pure blue analyte to be released from the cartridge.

Elution Solvent

- Condition (Organic)
- Equilibrate (Water, Sample Solvent)
- Load
- Wash
- **Elute**
- Dry Down
- Reconstitute

Blue analyte is collected for further testing
Incomplete Elute Step

Blue Analyte is **NOT** Completely Eluted

**How to Fix**

1) Elute with **MORE Volume** of **THIS** solvent

2) Elute with **SAME Volume** of a **STRONGER Solvent**
Stronger Solvent Requires LESS Volume than Weaker Solvent

Weak Elution Solvent (Sample Solvent)

Sample Volume = 1 mL
Hold-Up Volume = 2 mL

Very Dilute
62 mL of the weak sample solvent to elute blue k = 30

Strong Elution Solvent

Sample Volume = 1 mL
Hold-Up Volume = 2 mL

Very Concentrated
2 mL of the strong solvent to elute blue k = 0

Elution Volume – Mass Balance
Benefits of Strong Elution Solvents

Check for Complete Elution of Analyte
Solid Phase Extraction
SPE Summary

- Powerful technique
- Chromatographic principles
- Highly selective
- Very Robust when done properly

- Waters provides excellent Applications Support
  - Technical Support
  - Applications Library
  - Methods Development Information
  - Educational Seminars/Workshops
  - Literature
Technology Capability Literature
Primers

[ Beginners Guide to ]
LIQUID CHROMATOGRAPHY

[ Beginners Guide to ]
UPLC
ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY

The Quest for Ultra Performance in Liquid Chromatography
ORIGINS OF UPLC TECHNOLOGY

[ Comprehensive Guide to ]
HILIC
Hydrophilic Interaction Chromatography

THE MASS SPECTROMETRY PRIMER

[ Michael P. Balogh ]
Thank You!

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  - Full Webinar Recording of Today’s Session
  - Compilation of Literature, White Papers, Brochures
- General Questions – mychemrep@waters.com