

Sample Preparation Using Solid Phase Extraction



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Based on:
Yung-Fong (Henry) Cheng
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Present at EAS'98 Workshop

Troubleshooting of Sample Preparation Methods Using Solid-Phase Extraction



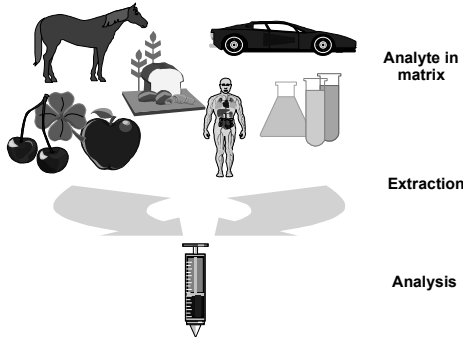
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Outline

- Importance of Sample Preparation
- Principle of Solid-Phase Extraction (SPE)
- Typical Problems in SPE
 - ▶ detail steps of SPE
 - ▶ examples
- Summary

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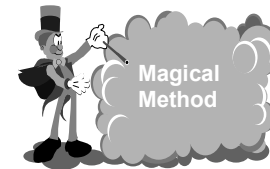
Why Sample Preparation?



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Sample Preparation

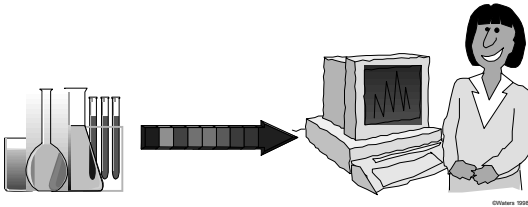
- Typically the most time-consuming step
- Typically the most difficult
- Typically the least amount of effort spent developing a rugged sample preparation method



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■ Wouldn't It Be Nice --

If We didn't have to Prepare Samples
Before Injection into the Instrument



Why Perform Sample Preparation?

- Remove interferences
 - ▶ e.g. Analysis of drug and metabolite in plasma.
Need to remove protein interferences
 - Concentrate sample
 - ▶ e.g. Pesticides in drinking water
- Processing Steps needed to get Sample Ready
Before Injecting into the Instrument
- | | |
|---------|----------|
| ^ HPLC | ^ GC |
| ^ LC/MS | ^ GC/MS |
| ^ AA | ^ Others |

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Sample Prep Techniques

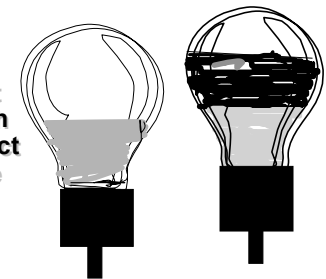
| Method | Basis for Selectivity |
|----------------------------------|--|
| • Precipitation | Solubility |
| • Liquid-Liquid Extraction | Partitioning in one of two liquid phases |
| • Solid-Liquid Extraction (SPE) | Adsorption/partitioning onto solid sorbent |
| • Dialysis / Ultrafiltration | Molecular weight/size |
| • Electrophoresis | Charge |
| • Distillation/Evaporation | Boiling point/vapor pressure |
| • Supercritical Fluid Extraction | Partitioning into supercritical fluid |

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Liquid-Liquid Extraction (LLE)

■ Chemical Technique

Where an Immiscible Solvent is Added to the Sample which then Separates into 2 Distinct Liquid Phases. Some Sample Analytes will go into the Bottom Phase (Aqueous), Some will Separate into the Top Phase, (Organic)



Disadvantages of LLE

- Large solvent consumption
- Time/Labor intensive
- May require an evaporation step prior to analysis to remove excess solvent
- When one needs to assay for several analytes, it may be difficult to find proper solvent/conditions for all analytes, requiring more than one extraction per sample
- Problematic samples - emulsions
- Contamination issues

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Advantages of SPE vs. Other Extraction Techniques

- Cleaner extracts
- Easier to automate
- Higher recoveries for polar compounds

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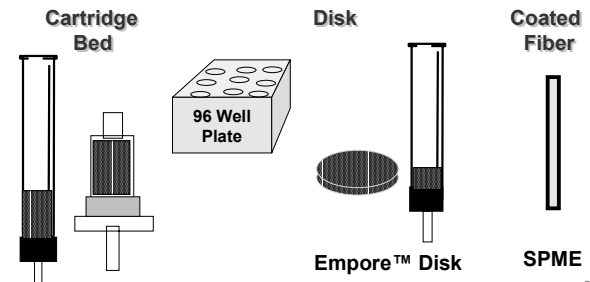
■ Solid Phase Extraction (SPE)

- Chromatographic Particles
- Packed-Bed Column Cartridges
- 1st Commercialized In 1978
- Well Established Technology
- Many Thousands of Literature References

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■ Solid Phase Extraction (SPE)

- Formats and Configurations



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Differences Between HPLC and SPE

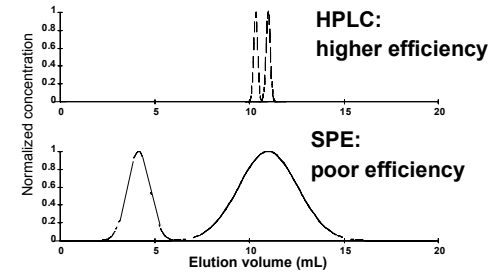
| | <u>HPLC</u> | <u>SPE</u> |
|-----------------------|------------------|---------------------|
| Particle size | ~5 μm | 40-80 μm |
| Packed bed efficiency | high | low |
| Extra-column volume | low | high |
| Column length | 5-30 cm | ~1 cm |
| Number of plates (N) | ~10,000 | < 50 |

Bottom line: HPLC can separate similar compounds. SPE requires a significant selectivity difference between compounds for separation. *Compounds not well resolved by HPLC cannot be separated by SPE with a similar retention mechanism.*

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Solid Phase Extraction (SPE) Technology

Comparison of Efficiency - HPLC vs. SPE



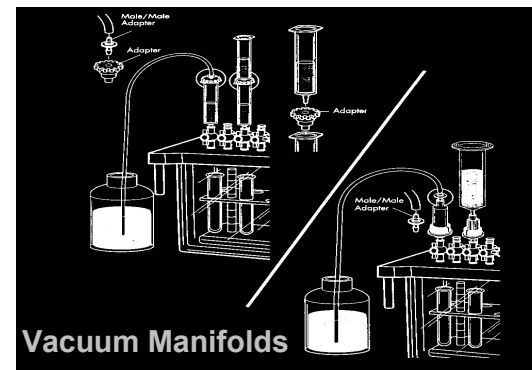
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Solid Phase Extraction (SPE) Technology

- **Sample Must be in Liquid State**
- **Driving Forces**
 - ^ Gravity
 - ^ Pressure
 - ^ Vacuum

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Solid Phase Extraction (SPE) Technology



Solid Phase Extraction (SPE) Technology

| Manufacturer | Brand Name |
|----------------------------------|------------------|
| Waters | SEP-Pak OASIS |
| Varian | BondElute |
| Baker | BakerBond |
| International Sorbent Technology | Isolute |
| 3 M | Empore |
| Supelco | Supelclean |

+ Many Others

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Solid Phase Extraction (SPE) Technology

SPE Strategies

Elute the product of interest, retain interferences

want k' 0 for analyte

want k' large for interferences

Elute interferences, retain product

want k' 0 for interferences

want k' large for analyte

*** Concentrate product of interest**

^ want k' large for analyte / load large sample volume

^ elute concentrated analyte

^ enhanced sensitivity

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Solid Phase Extraction (SPE) Technology

Most Common TYPES OF CHROMATOGRAPHY

- Normal Phase

^ The "Original" Type - Used By Tswett

^ Non-Polar Mobile Phase

^ Polar Stationary Phase

- Reversed-Phase



Most Common

^ Polar Mobile Phase

^ Non-Polar Stationary Phase

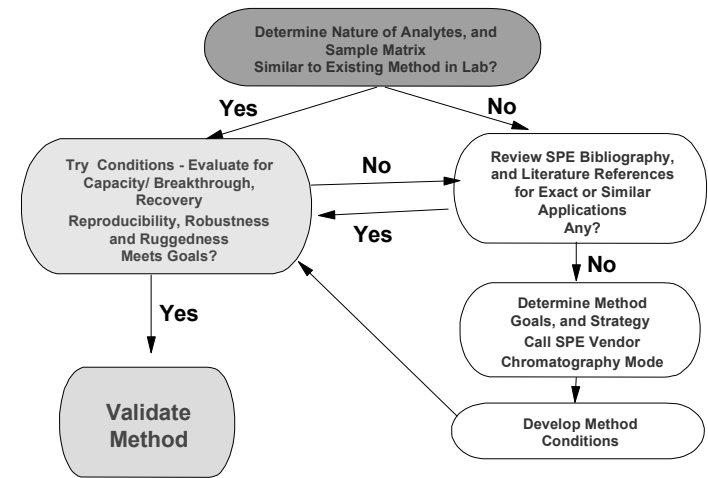
- Ion Exchange

^ Buffer/Ionic Mobile Phase

^ Cationic/Anionic Exchanger Stationary Phase

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Methods Development Approach



Outline

- Importance of Sample Preparation
- Principle of Solid-Phase Extraction (SPE)
- Typical Problems in SPE
 - ▶ detail steps of SPE
 - ▶ examples
- Summary

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Solid Phase Extraction (SPE) Technology

Common Problems in SPE

- Incomplete Removal of Interferences
- Low Recovery of Analyte(s)
- High Variability (RSDs)

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Solid Phase Extraction (SPE) Technology


SPE Procedure

- Sample**
- ① ↓ Prepare: Homogenize, suspend, centrifuge, etc.
 - ② ↓ Load onto conditioned cartridge
 - ③ ↓ Wash off weakly retained interferences with weak solvent
 - ④ ↓ Elute product with strong solvent
- Analyze: HPLC, GC, etc.**

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Solid Phase Extraction (SPE) Technology

Step 1 - Sample Preparation

- Sample**
- 
- ① ↓ Prepare: Homogenize, suspend, centrifuge, etc.
 - ② ↓ Load onto conditioned cartridge
 - ③ ↓ Wash off weakly retained interferences with weak solvent
 - ④ ↓ Elute product with strong solvent
- Analyze: HPLC, GC, etc.**

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Solid Phase Extraction (SPE) Technology

Step 1 - Sample Preparation

Typical problems

Analytes

- adsorbed to test tube walls
- adsorbed to or inclusion in matrix solids
- bound to proteins in matrix

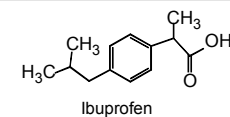
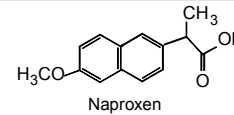
Possible solutions

- ▶ use silanized or plastic test tubes
- ▶ homogenize more completely
- ▶ add acid to sample solution

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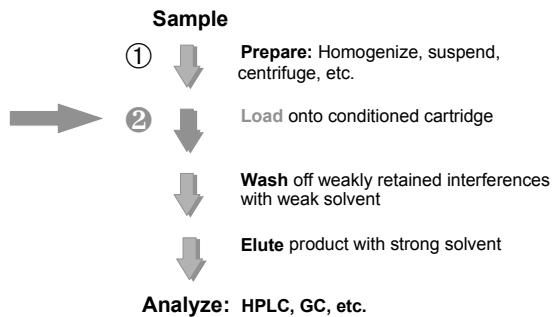
Sample Pretreatment: Effect of Acid on Recovery

| Compounds | Concentration [µg/mL] | % Recovery | | |
|-----------|-----------------------|--------------------------|-------------------------|---|
| | | No Acid Saline Sample | No Acid Serum Sample | Phosphoric Acid, 2% Serum Sample |
| Naproxen | 1.0 | 96 | 4 | 89 |
| Ibuprofen | 10.0 | 94 | 19 | 87 |



Solid Phase Extraction (SPE) Technology

Step 2 - Sample load



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Solid Phase Extraction (SPE) Technology

Step 2 - Sample Load

Possible problems

- Improper conditioning of cartridge
- Poor analyte retention
- Matrix variability
- Volume overload
- Mass overload

Solutions

- Condition cartridge as appropriate. Do not let dry, if silica based C18
- Dilute with weaker solvent, use stronger sorbent, use larger cartridge
- Buffer sample to constant pH, ionic strength
- Decrease load volume, use larger cartridge
- Decrease load volume, use larger cartridge

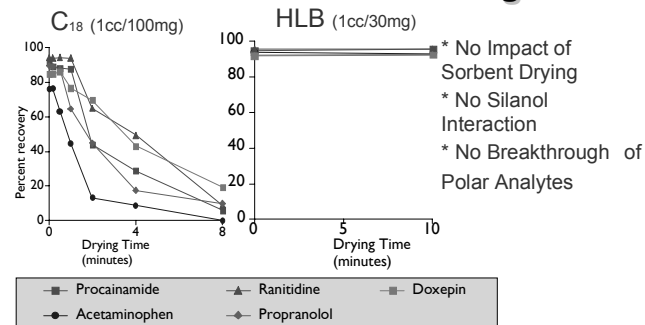
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Incomplete Conditioning of Cartridges

"Note: Do not dry SPE cartridge between initial methanol conditioning wash and completion of addition of sample and sample wash. Monitor elutions closely to ensure that cartridges do not dry."

J. D. MacNeil, V. K. Martz, G. O. Korsrud, C. D. C. Salisbury, H. Oka, R. L. Epstein, C. J. Barnes, J. AOAC Intl., 79(2) (1996), 405-417

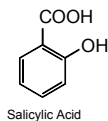
Incomplete Conditioning of Cartridges Effect on Recovery: C₁₈ vs. Oasis® HLB Cartridges



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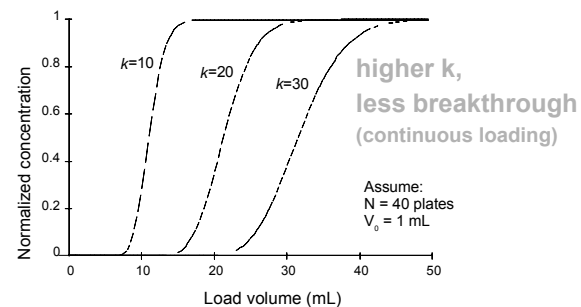
Effect of the Sample pH on Recovery

| Compounds | | Load at pH 7 | Load at pH <2 |
|--------------------------|-----------------------|--------------|---------------|
| | Concentration [µg/mL] | Recovery (%) | Recovery (%) |
| Salicylic Acid in Saline | 10 | 62.5 | 101 |



Solid Phase Extraction (SPE) Technology

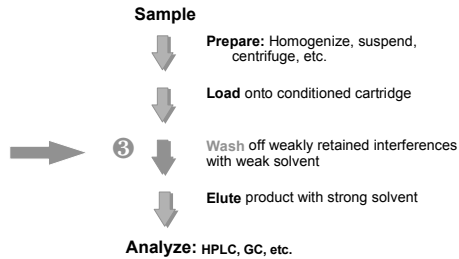
Sample Loading



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Solid Phase Extraction (SPE) Technology

Step 3 - Wash



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Solid Phase Extraction (SPE) Technology

Step 3 - Wash

Possible Problems

- Poor analyte retention
- Matrix variability
- Volume overload
- Mass overload

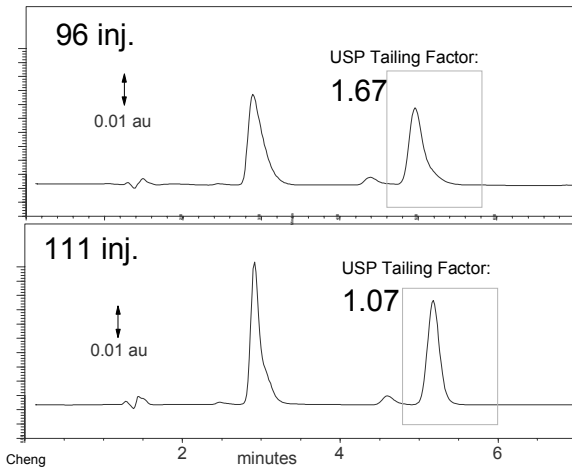
Solutions

- Use stronger sorbent, use larger cartridge
- Buffer sample to constant pH, ionic strength
- Decrease load volume, use larger cartridge
- Decrease load volume, use larger cartridge

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Effect of Incomplete Wash

■ Interferences



1st wash:
40% MeOH,
2% NH₄OH

1st wash:
40% MeOH,
2% NH₄OH

2nd wash:
5% MeOH,
2% HAc.

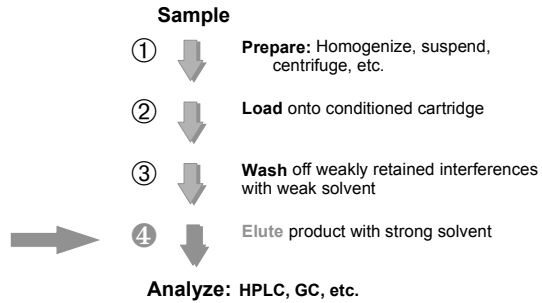
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Washing Procedure: Effect of Wash Solvent on Recovery

| Compounds | Concentration [µg/mL] | 5% Methanol in Water | Water |
|--------------|-----------------------|----------------------|--------------|
| | | Recovery (%) | Recovery (%) |
| Theobromine | 0.5 | 87 | 99 |
| Paraxanthine | 0.5 | 67 | 92 |
| Theophylline | 0.5 | 75 | 106 |
| Caffeine | 0.5 | 92 | 105 |

Solid Phase Extraction (SPE) Technology

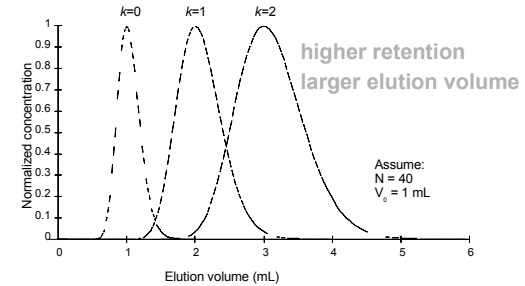
Step 4 - Elute



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Solid Phase Extraction (SPE) Technology

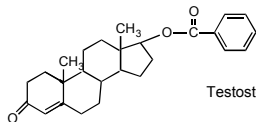
Step 4 - Elution



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Effect of Elution Solvent on Recovery and Reproducibility

| Compound | | Methanol | | Methylene Methanol | Chloride: 50:50 |
|--------------------------------------|------------------------------------|--------------|---------|--------------------|-----------------|
| Testosterone benzoate | Concentration [$\mu\text{g/mL}$] | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| First milliliter of elution solvent | 2.0 | 92 | 5.1 | 102 | 0.49 |
| Second milliliter of elution solvent | | 6.6 | 13.3 | <0.50 | |



Testosterone Benzoate

Evaporation and Reconstitution

Advantages

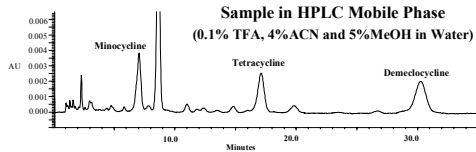
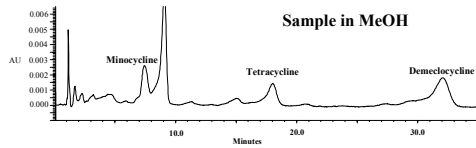
- ▶ Increase Assay Sensitivity
 - Increase sample concentration
 - Inject larger sample volume
- ▶ Improve HPLC Peak Shape
 - Dissolve in mobile phase or weaker solvent

Disadvantages

- Loss of more volatile analytes
- Poor solubility

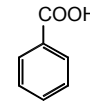
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HPLC Analysis: Effect of Sample Solvent

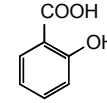


Effect of Evaporation on Sample Recovery

| Compounds | Concentration [µg/mL] | Evaporation to Dryness | | Evaporation to 100 µL | |
|----------------|-----------------------|------------------------|---------|-----------------------|---------|
| | | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| Benzoic Acid | 5.0 | 62.8 | 9.1 | 87.6 | 3.0 |
| Salicylic Acid | 5.0 | 93.6 | 5.1 | 91.3 | 5.0 |

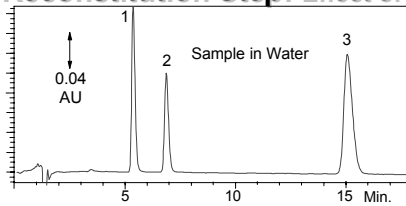


Benzoic Acid



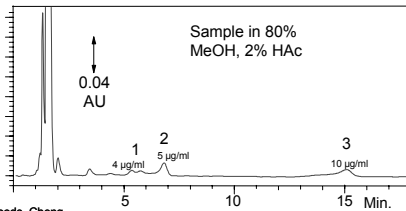
Salicylic Acid

Eliminating the Evaporation and Reconstitution Step: Effect of Sample Solvent



Sample Identification
1. EDDP
2. Diphenhydramine(S)
3. Methadone

Column: SymmetryShield™ RP18,
3.5 µm, 5.9 x 150 mm
Guard Column: Sentry™ Guard Column
SymmetryShield RP18,
5µm
Temperature: 30°C
Mobile Phase: 0.1% TFA:Methanol
(60:40)
Detection: UV at 210 nm
Flow Rate: 1 mL/min
Inj. Volume: 30 µL

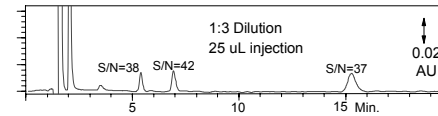


Extraction on
Oasis® HLB,
96-well, 10 mg/well
2-D SPE Method

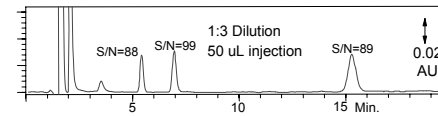
Woods, Cheng

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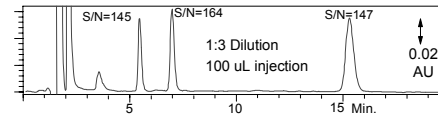
Strategy of Signal-to-Noise (S/N) Enrichment Comparison of S/N for Dilution (1:3 with water) of Urine Sample Solution after SPE Extraction



At this dilution (1:3 with water);
achieve
- better peak shapes
- higher S/N



Extraction on Oasis® HLB,
96-well, 10 mg/well
2-D SPE Method



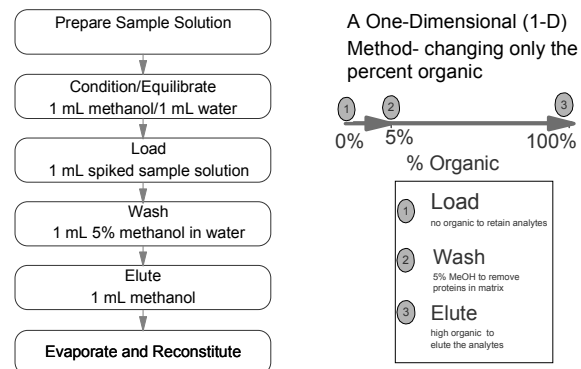
Solid Phase Extraction (SPE) Technology

Impact On Today's Analytical Chemist

- **Faster Method Development**
- **More Sensitive Methods**
- **Shorter Processing Times**
- **Reduced Cost Per Analysis**

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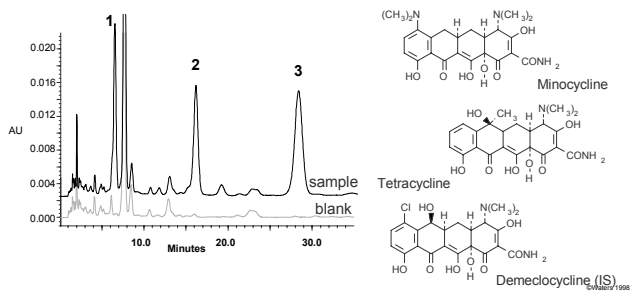
Generic Reversed-Phase, 1-D, SPE Method (Oasis® HLB Sorbent)



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Results: Tetracyclines

| Compound | Concentration | % Recovery | % RSD |
|--------------|---------------|------------|-------|
| Minocycline | 2.5 µg/mL | 94.8 | 1.4 |
| Tetracycline | 2.5 µg/mL | 104 | 0.55 |



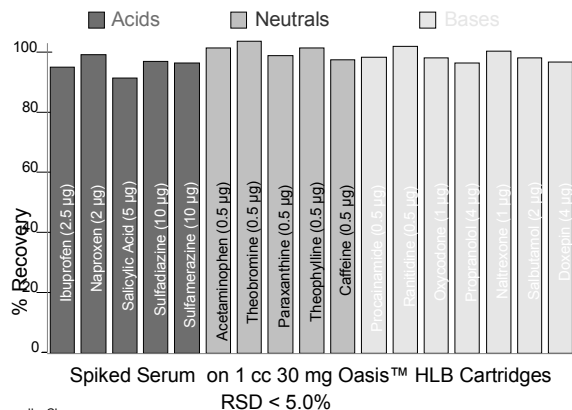
Comparison: Tetracyclines

| Compound | Conc. [µg/mL] | Oasis® HLB Cartridge | | C18 Cartridge | |
|--------------|---------------|----------------------|-------------|---------------|-------------|
| | | Recovery (%) | RSD (%) n=6 | Recovery (%) | RSD (%) n=6 |
| Minocycline | 2.5 | 94.8 | 1.40 | 40.7 | 0.82 |
| Tetracycline | 2.5 | 104 | 0.55 | 67.4 | 0.44 |

Cheng et al. *Chromatographia* 1997, 44 (3/4), p 187

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Results of 1-D SPE Method



Capparella, Cheng,
Phillips

©Waters 1998

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Solid Phase Extraction (SPE) Technology

Successful Tips

- Collect all Fractions (= Mass Balance)
 - ▶ Load
 - ▶ Wash(es)
 - ▶ Elute
 - ▶ 2nd Elute

Solid Phase Extraction (SPE) Technology

Summary

- Sample preparation is a necessary step prior to the analysis
 - ▶ perception was/is time consuming and tedious
- Solid-Phase Extraction (SPE) provides
 - ▶ cleaner extracts
 - ▶ simpler protocol
- Successful Tips
 - ▶ perform mass balance
- Ideal SPE Method
 - ▶ one method, one good result for a wide range of compounds

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Solid-Phase Extraction (SPE) Technology

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