

# **Systematic Evaluation of Solid Phase Extraction (SPE) Chemistries for the Determination of Acidic, Neutral, and Basic Drugs**

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# Abstract

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**Solid phase extraction is one of the most widely used tools for sample cleanup. Because it is believed that method development for solid phase extraction is overly time consuming, many scientists choose to follow a generic method using universal supports, such as the most popular phase chemistry, C18. The aim of this study is to explore the differences in various phase chemistries, then utilize their specificity in solid phase extraction. SPE methods were systematically developed for acid, neutral and basic drugs to recognize other phase chemistries that are often overlooked. Three different phase chemistries were utilized. Through methodic wash/elute profiles, advantages and disadvantages of the different phase chemistries were established for a particular sample and matrix. Strategies for improving SPE method development are discussed. The compounds studied were alprenolol, diphenhydramine, hydrocortisone, ibuprofen, nalidixic acid, naproxen and nortriptyline. The derivatives were analyzed by HPLC.**



# Introduction

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**SPE methods are frequently developed by copying or modifying an existing application or choosing a generic method. Although these approaches are less time-consuming, they often result in sub-optimal recovery/reproducibility and poor selectivity/sample clean up.**

**As a result, it can often be very difficult to determine the root cause(s) of a specific problem(s) associated with an SPE method. Some examples include:**

- **Poor Recovery.** Is it due to...
  - Poor retention during sample load?
  - Pre-mature elution during wash?
  - Over retention during elution?
- **Poor Reproducibility.** Typically caused by one or more inadequate steps. Which one?
- **Insufficient clean-up.** Stronger wash solvent? Maybe a different SPE phase?



# Introduction (contd.)

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**For sample prep methods intended for extensive use (e.g., preclinical and clinical studies), extra effort in SPE method development can save costly reanalysis, redevelopment, and revalidation time.**

**In this study, 7 representative pharmaceutical compounds (3 basic, 3 acidic, and 1 neutral) were extracted on three different SPE chemistries: C18, C8, and CN followed by HPLC-UV analysis.**

**Using standards and buffered/organically modified solutions, key variables (pH and percent organic modifier) affecting analyte retention and elution were systematically profiled to determine analyte behavior relative to changing extraction conditions.**

**By approaching method development systematically, application specific data was generated for more effective method development, optimization, and troubleshooting.**

# Experimental- Approach

## Systematic SPE Method Development

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### Step 1 - Load Optimization

1. Develop analytical method (Figure A)
2. Screen SPE phases for loadability (compound retention)
3. Analyze load flow-thru for premature compound breakthrough

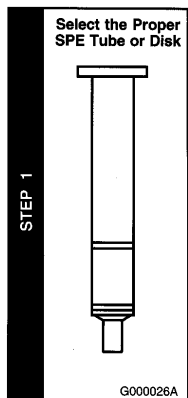
### Step 2 - Establish Optimal Wash and Elution Solvents

1. Load samples according to step 1
2. Systematically profile wash/elute solvents of varying pH and percent organic modifier
3. Analyze for recovery and plot results

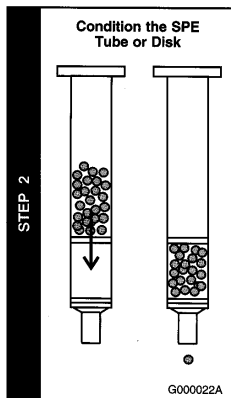
### Step 3 - Evaluation

1. Determine analyte(s) behavior on sorbent(s) in response to changing extraction conditions
2. Define method, incorporate sample matrix, and optimize/troubleshoot as necessary

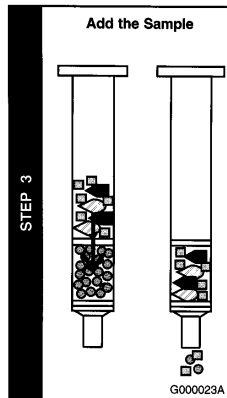
# The SPE Process



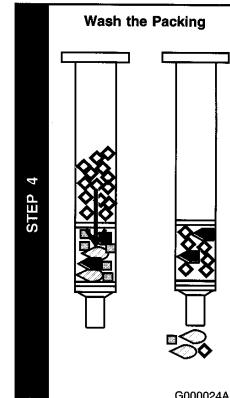
**Phase &  
Hardware  
Selection**



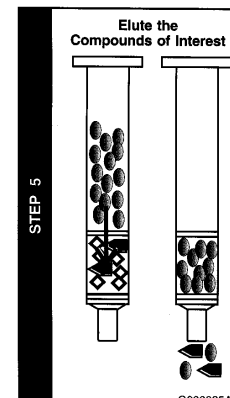
**Condition/  
Equilibrate**



**Load  
Sample**



**Wash Off  
Co-Extracted  
Interferences**

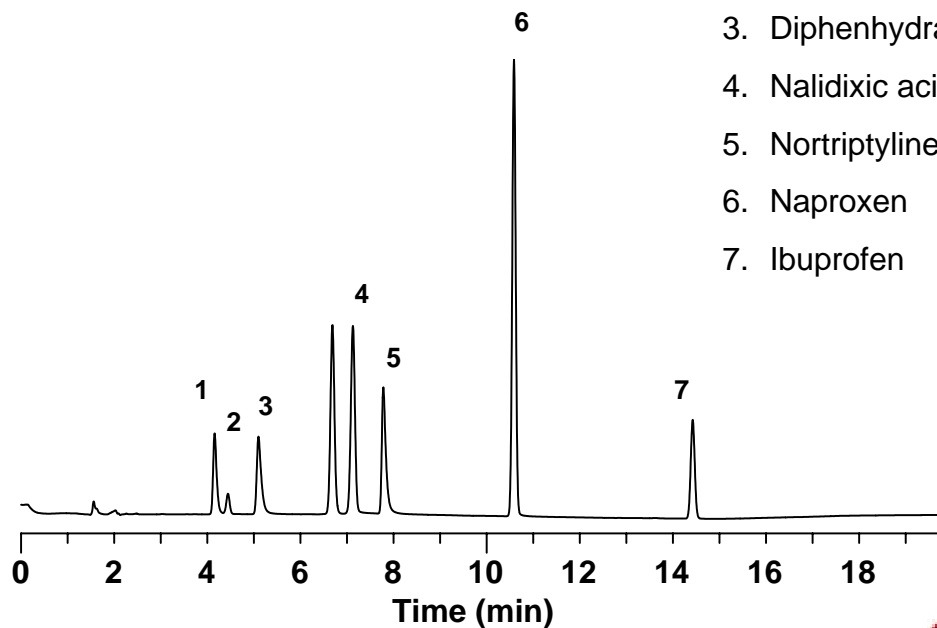


**Elute  
Analytes**

# Figure A. Example Chromatogram of Standard Test Mix

**Column:** Discovery HS C18, 5cm x 4.6mm ID, 3 $\mu$ m particles  
**Mobile Phase:** (A) 20mM potassium phosphate, pH 2.8; (B) acetonitrile  
**Flow Rate:** 1.0mL/min  
**Temperature:** 35°C  
**Detection:** UV, 220nm  
**Injection:** 20 $\mu$ L

<u>Min</u>	<u>%A</u>	<u>%B</u>
0	65	35
3	65	35
12	35	65
15	35	65
20	35	65



1. Alprenolol
2. Hydrocortisone
3. Diphenhydramine
4. Nalidixic acid
5. Nortriptyline
6. Naproxen
7. Ibuprofen



# Load Optimization

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**SPE:    Discovery DSC-18 96-Well SPE (100mg/well)  
          Discovery DSC-8 96-Well SPE (100mg/well)  
          Discovery DSC-CN 96-Well SPE (100mg/well)**

- 1. Prepare standard test mix containing all 7 compounds (20µg/mL spike level) in 20mM potassium phosphate, pH 3 (low pH), pH 7 (neutral pH), and pH 10 (high pH)**
- 2. Condition & equilibrate SPE wells (C18, C8, & CN) with 2mL methanol & DI H<sub>2</sub>O**
- 3. Load 1mL of each standard test mix (low, neutral, and high pH)**
- 4. Collect load flow-through eluate & analyze for compound breakthrough via HPLC-UV (see results)**



# Wash/Elute Profile

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**SPE: Discovery DSC-18 96-well SPE (100mg/well)**

**Discovery DSC-8 96-well SPE (100mg/well)**

**Discovery DSC-CN 96-well SPE (100mg/well)**

- 1. Condition & equilibrate SPE wells (C18, C8, & CN) with 2mL methanol & DI H<sub>2</sub>O**
- 2. Load 1mL of the 20µg/mL standard test mix, pH 7 (neutral pH)**
- 3. Wash/elute respective wells with 1mL test solvents ranging from 0-100% methanol in 2% NH<sub>4</sub>OH, pH 11 (high pH), DI H<sub>2</sub>O (neutral pH), and 2% CH<sub>3</sub>COOH, pH 3 (low pH)**
- 4. Collect wash/elute eluate & analyze for compound breakthrough via HPLC-UV (see results)**



# Results

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## Load Optimization

- Adequate retention was observed for each of the 7 analytes tested on all three SPE chemistries (C18, C8, & CN)
  - Load retention determined by analyzing load flow-through eluate for compound breakthrough via HPLC-UV
  - Shift in load pH (b/w 3-11) did not affect retention during sample load
- A sample load pH of 7 was chosen for all subsequent studies

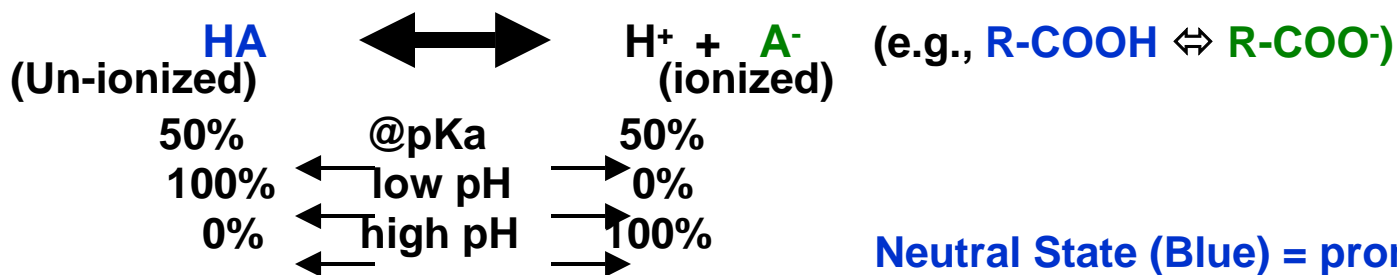
## Wash/Elute Profiles

- Standard test mix samples were loaded onto C18, C8, & CN SPE wells, and eluted with test solvents ranging from 0-100% methanol in 2% acetic acid (**low pH**), DI water (**neutral pH**), & 2% NH<sub>4</sub>OH (**high pH**).
- Figure B explains the important role of pH in reversed-phase SPE
- The data is organized and visually depicted (Figures C-E)

# Figure B. The Critical Role of pH in SPE

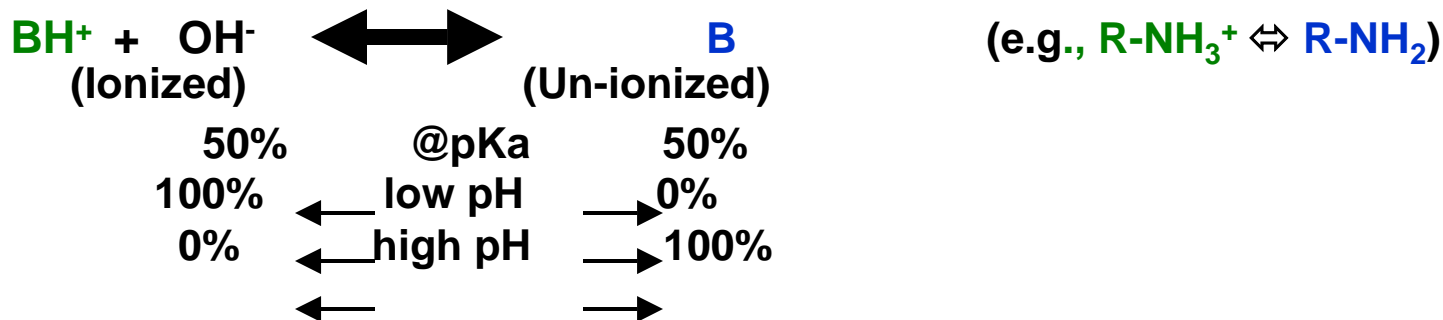
## Ionization of Acidic & Basic Molecules-

### Acids (e.g., carboxylic acids):



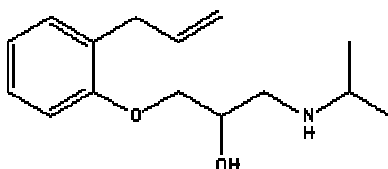
Neutral State (Blue) = promotes hydrophobic (reversed-phase) interaction b/w compound and sorbent

### Bases (e.g., amines):

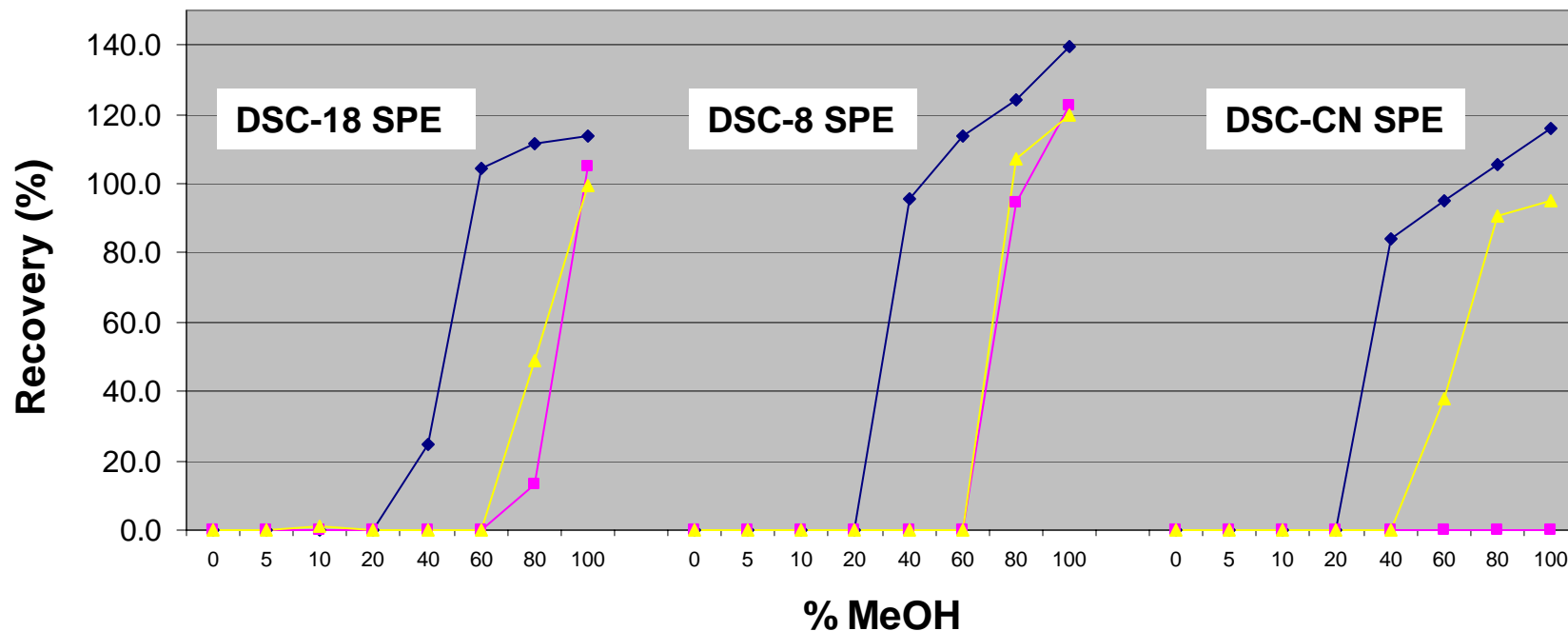


# Figure C. Wash/Elute Profile – Basic Compounds

## Alprenolol

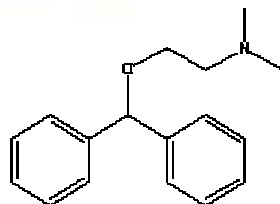


- ◆ % MeOH in 2% CH<sub>3</sub>COOH
- % MeOH in DI H<sub>2</sub>O
- ▲ % MeOH in 2% NH<sub>4</sub>OH

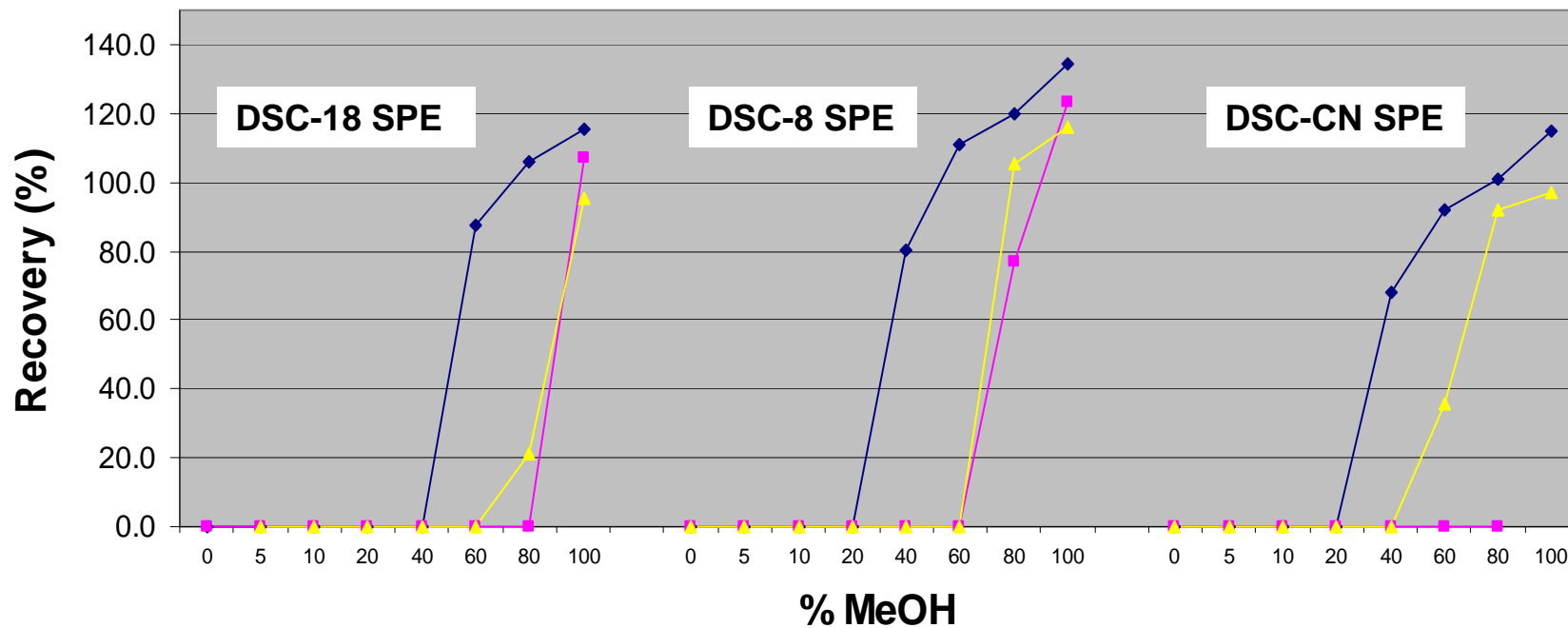


# Figure C (contd.)

## Diphenhydramine

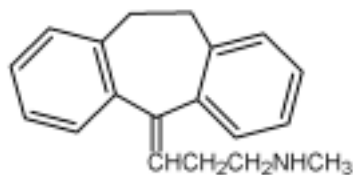


- ◆ % MeOH in 2% CH<sub>3</sub>COOH
- % MeOH in DI H<sub>2</sub>O
- ▲ % MeOH in 2% NH<sub>4</sub>OH

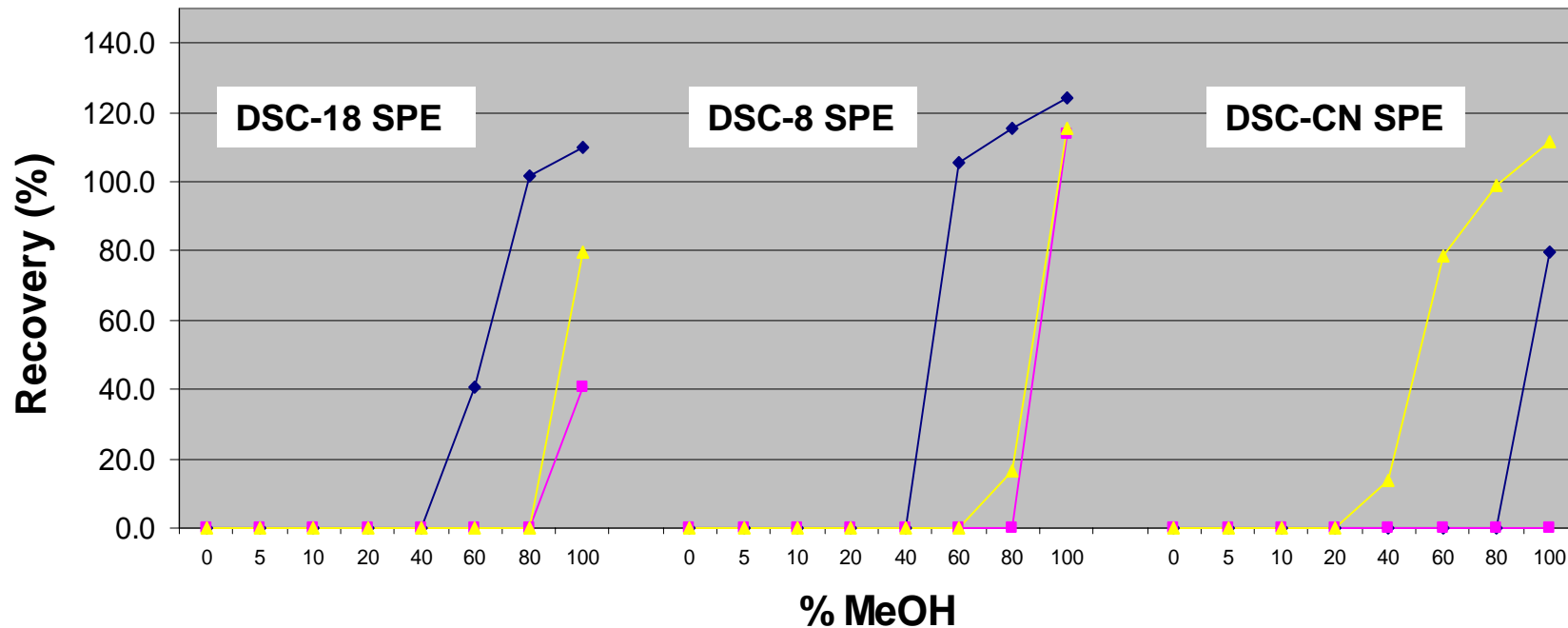


# Figure C (contd.)

## Nortriptyline



- ◆ % MeOH in 2% CH<sub>3</sub>COOH
- % MeOH in DI H<sub>2</sub>O
- ▲ % MeOH in 2% NH<sub>4</sub>OH





# Wash/Elute Profile - Basic Compounds (Fig. C)

- **High pH conditions (%MeOH in 2% NH<sub>4</sub>OH)**
  - Basic compounds in their neutral form
  - More strongly retained under reversed-phase conditions
  - Stronger solvents can be used during the wash step to improve selectivity
    - Up to 60% MeOH can be used as a potential wash solvent on C18 and C8 SPE before premature elution occurs, and 20% MeOH for CN SPE
- **Low pH conditions (%MeOH in CH<sub>3</sub>COOH)**
  - Basic compounds in their ionized form
  - Ionized form weakens reversed-phase retention
  - Weaker solvents must be used during wash step
    - Maximum wash strength of 20% MeOH is allowable before premature elution occurs
  - Weaker elution solvents may be possible (e.g. 60% MeOH) under low pH conditions improving selectivity and/or allowing for direct-inject protocols (no eluate evaporation/reconstitution)



# Wash/Elute Profile - Basic Compounds (contd.)

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- **Neutral pH conditions (%MeOH in DI H<sub>2</sub>O)**
  - None of the basic compounds eluted on CN SPE
  - CN (cyanopropyl) SPE's short alkyl chain exposed more silanol groups on the silica sorbent's surface. The silanol groups acted like weak cation-exchangers.
  - Basic compounds retained via mixed-mode behavior when processed at neutral pH on CN SPE
  - At high and low pH, basic compounds and silanol groups are neutralized, respectively, disrupting secondary ion-exchange behavior
  - Using secondary silanol interactions, researchers can potentially maximize sample clean-up on DSC-CN by first washing with 80% methanol and then eluting with acidified methanol.

# Potential Reversed-Phase Protocols for Basic Compounds

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## DSC-18 SPE

1. Condition/equilibrate with MeOH & DI water
2. Load sample at pH 7
3. Wash with 60% MeOH in 2% NH<sub>4</sub>OH
4. Elute with 100% CH<sub>3</sub>COOH in MeOH

## DSC-8 SPE

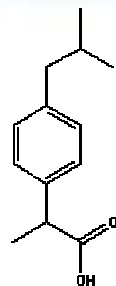
1. Condition/equilibrate with MeOH & DI water
2. Load sample at pH 7
3. Wash with 60% MeOH in 2% NH<sub>4</sub>OH
4. Elute with 80-100% MeOH in CH<sub>3</sub>COOH

## DSC-CN SPE

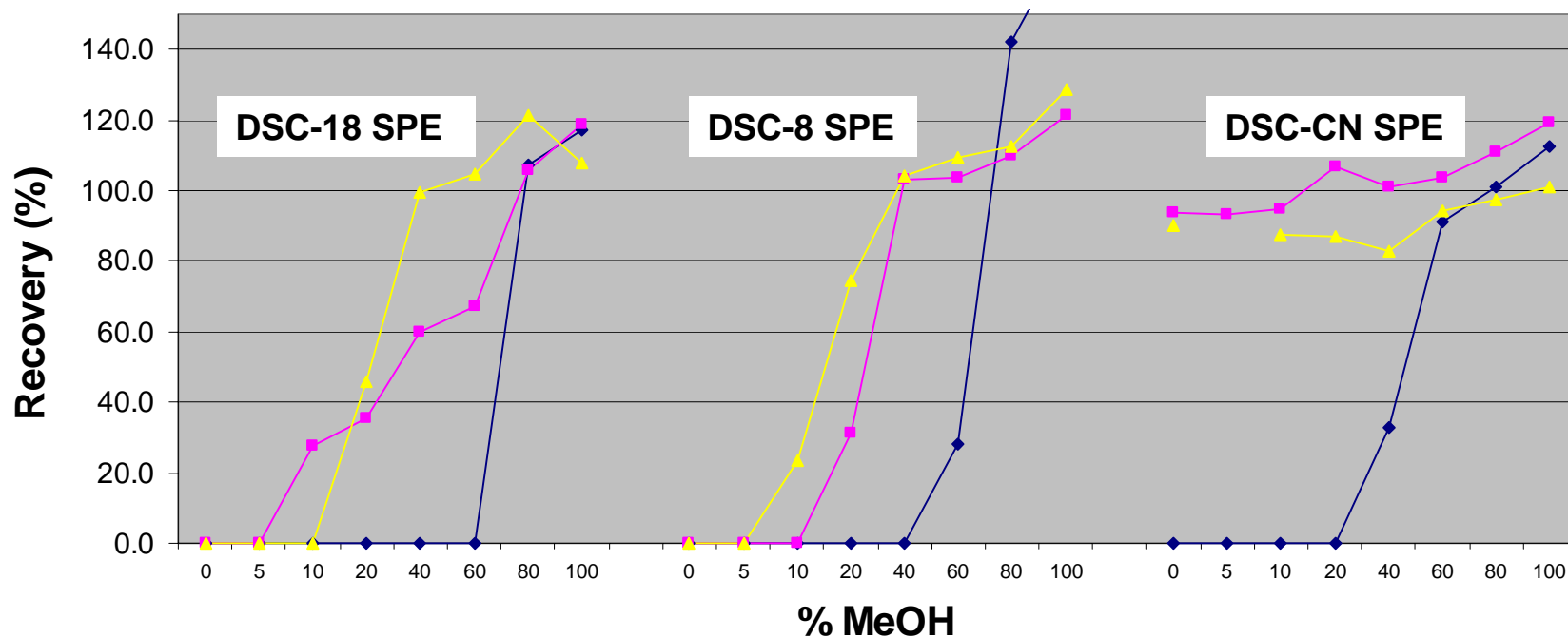
1. Condition/equilibrate with MeOH & DI water
2. Load sample at pH 7
3. Wash with 80% MeOH
4. Elute with 80-100% MeOH in CH<sub>3</sub>COOH

# Figure D. Wash/Elute Profile – Acidic Compounds

## Ibuprofen

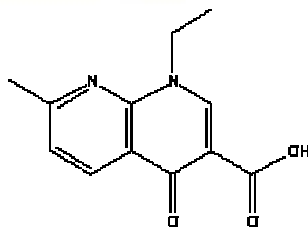


- ◆ % MeOH in 2% CH<sub>3</sub>COOH
- % MeOH in DI H<sub>2</sub>O
- ▲ % MeOH in 2% NH<sub>4</sub>OH

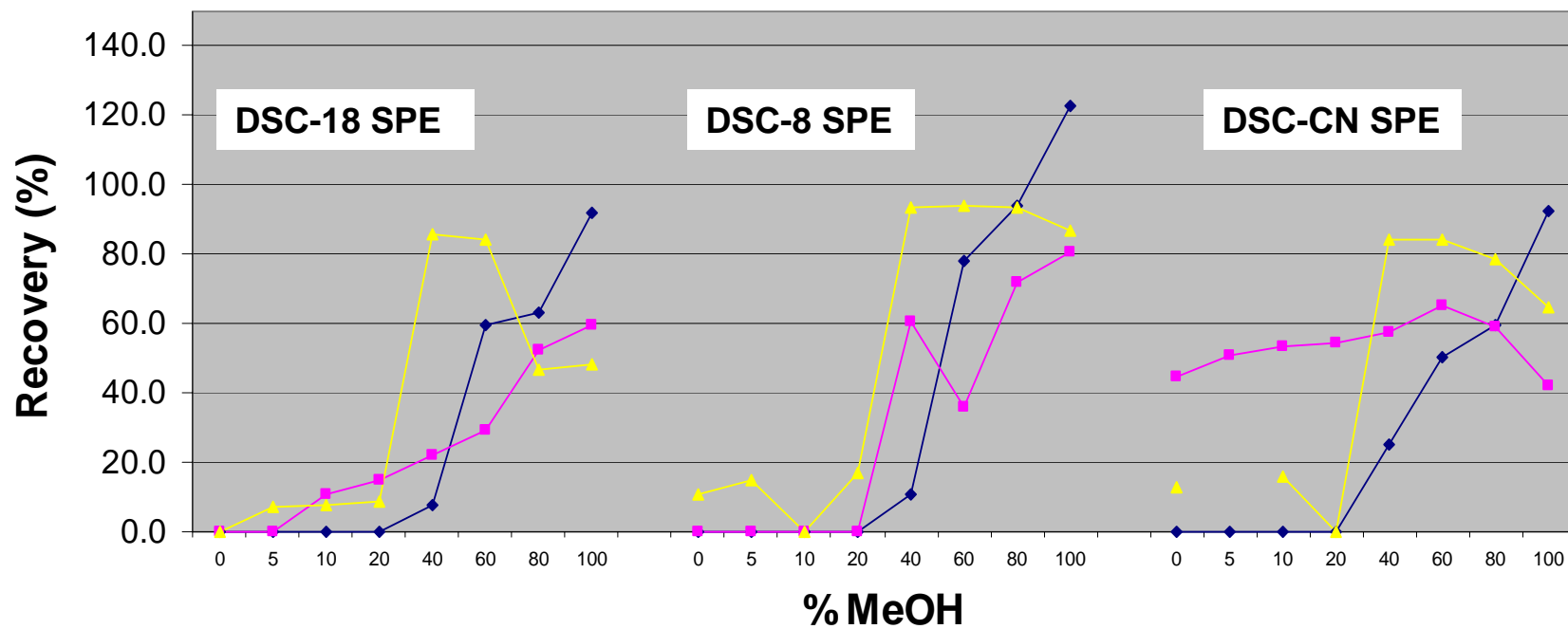


# Figure D (contd.)

## Nalidixic Acid

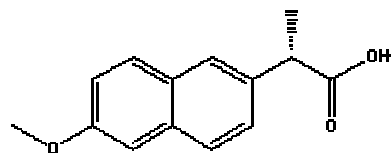


- ◆ % MeOH in 2% CH<sub>3</sub>COOH
- ◆ % MeOH in DI H<sub>2</sub>O
- ▲ % MeOH in 2% NH<sub>4</sub>OH

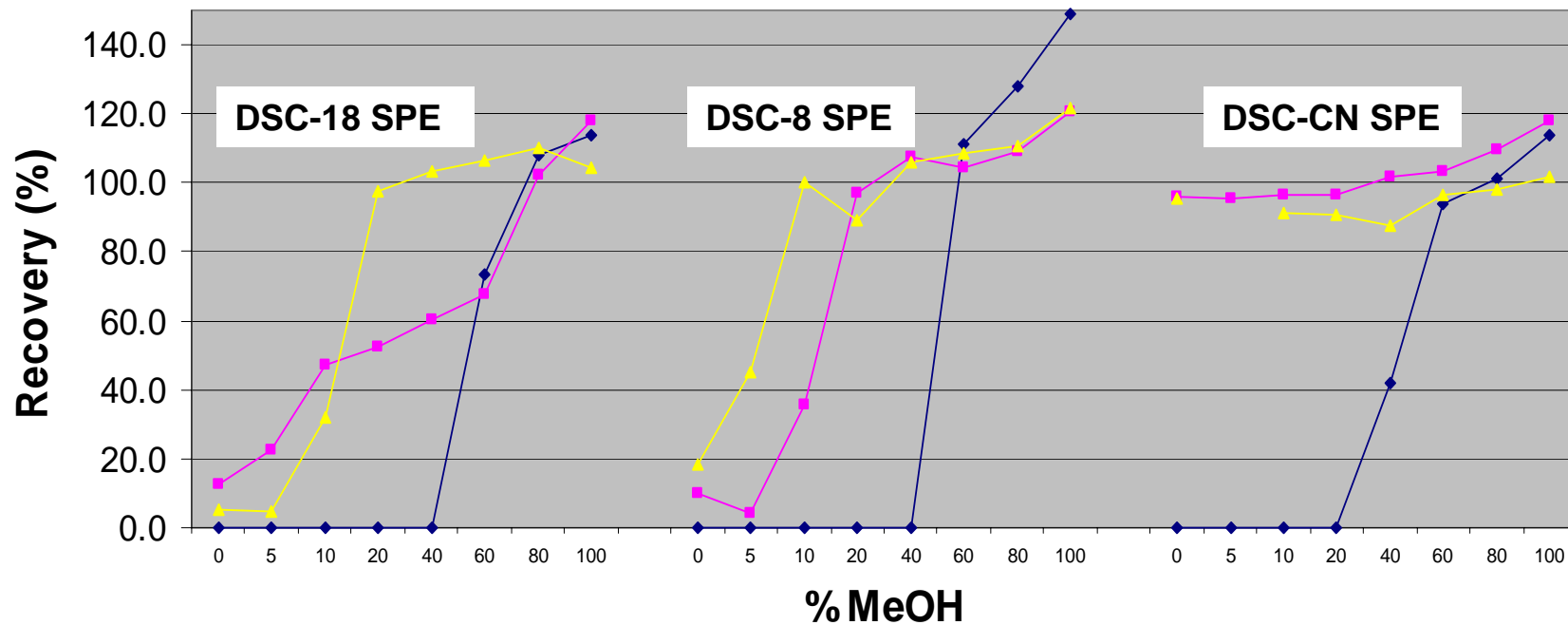


# Figure D (contd.)

## Naproxen



- ◆ % MeOH in 2% CH<sub>3</sub>COOH
- % MeOH in DI H<sub>2</sub>O
- ▲ % MeOH in 2% NH<sub>4</sub>OH





# Wash/Elute Profile - Acidic Compounds (Fig. D)

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- **Low pH conditions (%MeOH in 2% CH<sub>3</sub>COOH)**
  - Acidic compounds in their neutral form
  - More strongly retained under reversed-phase conditions
  - Stronger solvents can be used during the wash step to improve selectivity
    - Up to 40% MeOH can be used as a potential wash solvent on C18 & C8 SPE for naproxen & ibuprofen before premature elution occurs.
- **High pH and neutral conditions (%MeOH in NH<sub>4</sub>OH and DI H<sub>2</sub>O)**
  - Acidic compounds in their ionized form
  - Ionized form weakens reversed-phase retention resulting in very poor retention on all three SPE chemistries
  - Most of the acidic compounds tested began eluting at very low solvent strengths (0-5% MeOH). This effect was most pronounced on less hydrophobic phases such as CN SPE.
  - Weaker elution solvents may be possible (e.g. 40-60% MeOH) under high/neutral pH conditions improving selectivity and/or allowing for direct-inject protocols (no eluate evaporation/reconstitution).

# Potential Reversed-Phase Protocols for Acidic Compounds

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## DSC-18 SPE

1. Condition/equilibrate with MeOH & DI water
2. Load sample at pH 7
3. Wash with 20% MeOH in 2% CH<sub>3</sub>COOH
4. Elute with 100% MeOH

## DSC-8 SPE

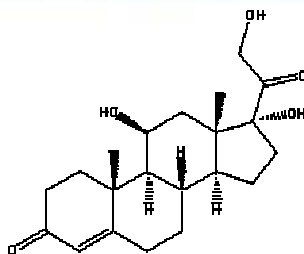
1. Condition/equilibrate with MeOH & DI water
2. Load sample at pH 7
3. Wash with 20% MeOH in 2% CH<sub>3</sub>COOH
4. Elute with 100% MeOH

## DSC-CN SPE

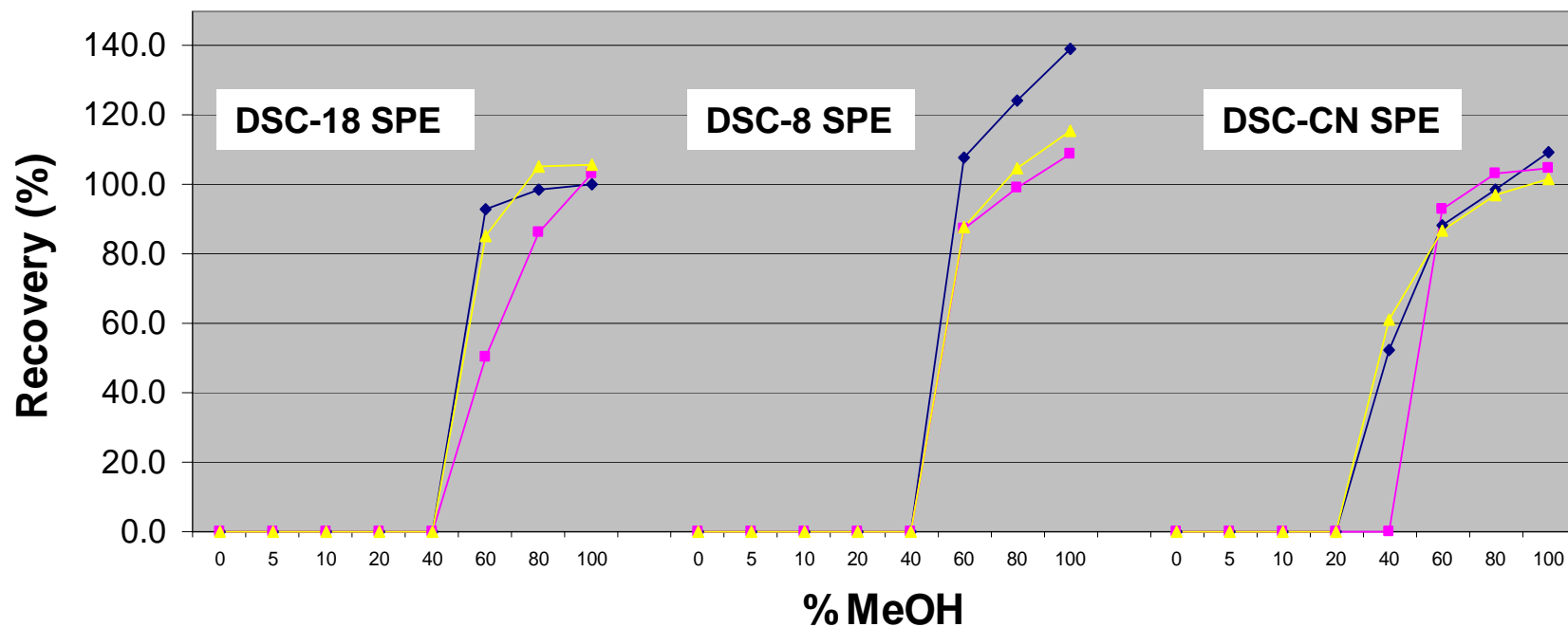
1. Condition/equilibrate with MeOH & DI water
2. Load sample at pH 7
3. Wash with 20% MeOH in 2% CH<sub>3</sub>COOH
4. Elute with 100% MeOH

# Figure E. Wash/Elute Profile – Neutral Compounds

## Hydrocortisone



- ◆ % MeOH in 2% CH<sub>3</sub>COOH
- ◆ % MeOH in DI H<sub>2</sub>O
- ◆ % MeOH in 2% NH<sub>4</sub>OH





## Wash/Elute Profile – Neutral Compounds (Fig. E)

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- Because neutral compounds such as hydrocortisone are not ionizable, pH plays a minor role in controlling retention and elution.
- On C18 and C8 SPE, up to 40% methanol can be used as a potential wash solvent at all pH levels. On CN SPE, 20% methanol is the maximum wash solvent strength before premature elution occurs.
- 100% methanol is required to fully elute hydrocortisone on C18 and C8 SPE. 80% methanol can be used to elute hydrocortisone on DSC-CN.

# Conclusion

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- **pH and % organic modifier play a critical role in determining retention and elution of ionizable compounds in reversed-phase SPE.**
- **More hydrophobic phases such as C18 have a stronger and broader affinity to a wider range of compounds thus allowing for stronger wash solvents. However, stronger elution solvents may be required to elute compounds of interest.**
- **In contrast, less hydrophobic phases such as CN SPE may require weaker wash solvents, but compounds may be eluted using weaker elution solvents.**
- **By approaching SPE method development systematically, a greater understanding of analyte behavior relative to changes in extraction conditions is obtained.**
- **This allows for more effective method development, optimization, and troubleshooting.**