

## Technical Report

# Fractionate Basic Analytes from Neutral and Acidic Compounds with Discovery® DSC-MCAX SPE

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The Discovery DSC-MCAX SPE phase utilizes the dual retention mechanisms of both hydrophobic and electrostatic interaction to retain basic, acidic, neutral and zwitterionic compounds from aqueous sample matrices. The packed bed comprises of both octyl (C8) and benzene sulfonic acid (SCX) bondings to offer one of the most selective SPE systems available.

Although the packed bed was designed to drastically improve the selectivity/sample clean-up of basic and zwitterionic compounds, Discovery DSC-MCAX offers other powerful applications as well. Through the careful manipulation of pH and organic strength, the user can also fractionate basic and zwitterionic from acidic and neutral compounds.

### Method

A mix of five pharmaceutical compounds (3 basic, 2 acidic, and 1 neutral) was extracted from human urine using Discovery DSC-MCAX SPE, 1g/6mL (52788-U) using the protocol outlined in Table 1.

### Results

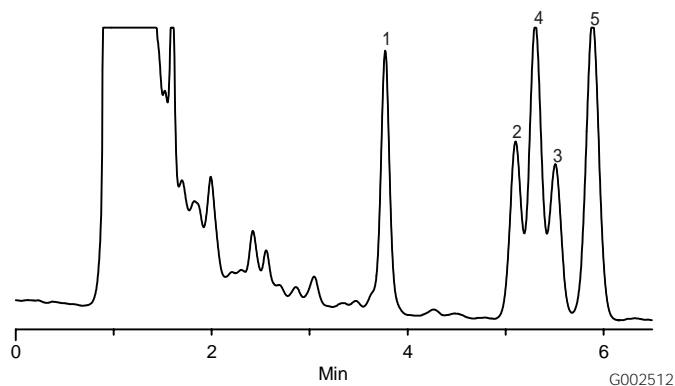
Discovery DSC-MCAX SPE, utilizing mixed mode cation exchange technology, completely fractionated basic from acidic and neutral compounds prior to LC analysis (Figure A). In fraction 1, neutral and acidic compounds retained solely via hydrophobic interactions were eluted with 20% acetone in chloroform. Basic compounds were retained by both ionic and hydrophobic interactions. A combination of increase in pH and organic strength (5% NH<sub>4</sub>OH in methanol) was required for elution (fraction 2). Absolute recovery averaged at 102.5% for all the compounds tested.

**Table 1. Fractionation Protocol Used for Discovery DSC-MCAX SPE, 1g/6mL**

Condition with 6mL methanol	
Equilibrate with 6mL 10mM acetic acid, pH 3	
Load 1mL spiked urine sample	<ul style="list-style-type: none"> <li>● Urine samples diluted 1:1 with 10mM potassium phosphate, pH 3 prior to loading</li> <li>● Low sample pH neutralizes all acidic compounds inducing reversed-phase retention</li> <li>● Low sample pH ionizes basic compounds inducing ionic-exchange retention</li> </ul>
Wash with 6mL 10mM acetic acid, pH 3	<ul style="list-style-type: none"> <li>● The low pH aqueous wash solvent removes all non-basic hydrophilic interferences, and also locks basic compounds onto the sorbent reinforcing both ionic and reversed-phase retention.</li> </ul>
Wash with 6mL chloroform	<ul style="list-style-type: none"> <li>● Low solubility in chloroform prevents compounds from premature elution</li> </ul>
Elute neutral and acidic compounds with 6mL 20% acetone in chloroform	<ul style="list-style-type: none"> <li>● Neutral and acidic compounds are retained exclusively via hydrophobic interaction, and can therefore be eluted via 20% acetone in chloroform</li> </ul>
Elute basic compounds with 6mL 1%TEA in methanol or 5% ammonium hydroxide in methanol	<ul style="list-style-type: none"> <li>● The increase in elution pH neutralizes basic compounds disrupting ionic interactions between compound and sorbent.</li> <li>● Favorable hydrophobic interactions disrupted via the presence of methanol</li> </ul>
Eluate Evaporation	<ul style="list-style-type: none"> <li>● Eluate fractions were evaporated to dryness at 37°C under N<sub>2</sub>-purge, and reconstituted with 1mL mobile phase.</li> </ul>

## Figure A. Fractionation of Basic Compounds from Acidic and Neutral Compounds Using Discovery DSC-MCAX SPE, 1g/mL

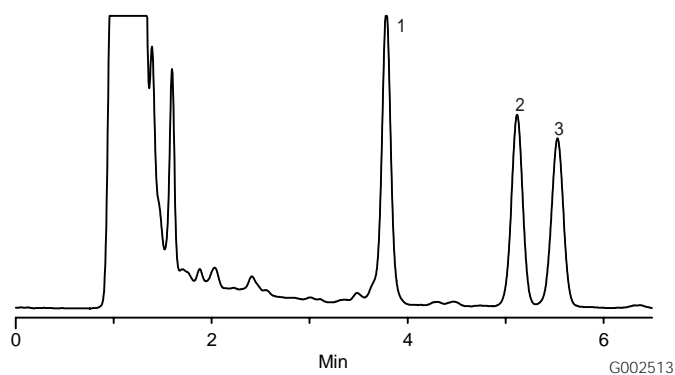
### Spiked Urine Samples before Discovery DSC-MCAX SPE



SPE Tube: Discovery DSC-MCAX, 1g/6mL  
HPLC Column: Discovery C8, 15cm x 4.6mm ID, 5µm particles  
Mobile Phase 1 (Pre-SPE & Fraction 1): 0.1% TFA:MeOH (50:50)  
Mobile Phase 2 (Fraction 2): 10mM ammonium acetate:MeOH (45:55)  
Flow Rate: 2mL/min  
Temp.: Ambient  
Det.: 214nm, UV  
Inj. Vol.: 10µL

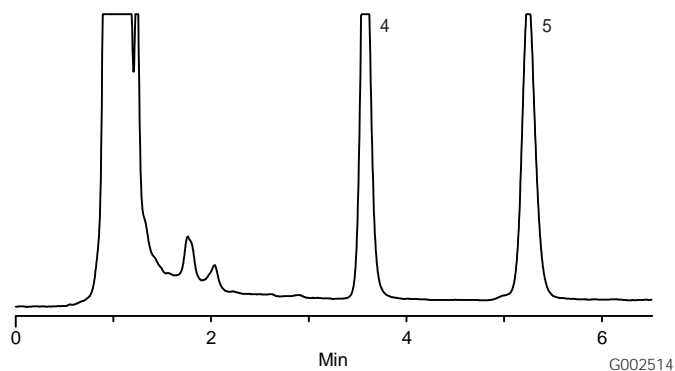
Compound Description		% Recovery ± RSD (n=3)
Neutral	1. Secobarbital (10µg/mL spike)	105.8 ± 2.1%
Acidic	2. Ketoprofen (5.0µg/mL spike)	101.7 ± 1.3%
Acidic	3. Naproxen (2.5µg/mL spike)	101.5 ± 0.8%
Basic	4. Nortriptyline (5.0µg/mL spike)	100.3 ± 0.5%
Basic	5. Amitriptyline (5.0µg/mL spike)	103.3 ± 1.7%

### Fraction 1: Neutral & Acidic Compounds



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### Fraction 2: Basic Compounds



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