

## Technical Report

# The Importance of Ion Exchange Capacity in Mixed-Mode SPE

An Trinh - [atrinh@sial.com](mailto:atrinh@sial.com)

Mixed-mode chemistries rely on at least two retention mechanisms (e.g. reversed-phase and ion exchange) to simultaneously extract a broad range of compounds from a single aqueous sample. Charged compounds can form strong ionic bonds with the ion exchange component, while other compounds are bound by the hydrophobic interaction of the reversed phase component. This multiple interaction requires a high ion exchange capacity to improve sample clean up of ionizable compounds. The Discovery DSC-MCAX provides the efficiency critical to isolating the more polar basic and zwitterionic compounds from the basic nonpolar fraction.

## Ion-Exchange Capacity & Recovery

Discovery DSC-MCAX SPE is primarily used to improve selectivity and sample clean up when extracting basic compounds from dirty sample matrices such as biological fluids (urine, plasma, etc.). When drugs containing amine groups are undergoing pharmaceutical bioanalysis, this improved selectivity is critical for reducing ion suppression and achieving low detection limits necessary during pre-clinical and clinical evaluation.

When extracting basic nonpolar compounds (e.g.,  $\text{Log } P_{\text{ow}} \geq 1.0$ ), hydrophobic interactions with the C8 bonded group play a significant role towards compound retention; minimal cation exchange capacity is required to lock the compound onto the sorbent. In contrast, polar basic and zwitterionic compounds lack the hydrophobic functionality required for reversed-phase retention, and must be retained predominately by the cation exchange functionality (benzene sulfonic acid). Consequently, the low ion-exchange capacity of some mixed-mode SPE leads to premature breakthrough of the ionic compounds during load and wash steps, resulting in poor recovery and variable reproducibility. Table 1 lists the ion exchange capacity levels of Discovery DSC-MCAX SPE compared to some of the leading competitors. The Discovery DSC-MCAX SPE provides more than 50% more ion exchange capacity, allowing for greater sample load and improved retention of polar basic and zwitterionic compounds.

**Table 1. Ion Exchange Capacity Values for Discovery DSC-MCAX and Competitor Phases**

Phase	Capacity (mg/g; meq/g) <sup>1</sup>
Discovery DSC-MCAX SPE	4.1mg/g ; 0.06meq/g
Leading Competitor A	2.4mg/g ; 0.033meq/g
Leading Competitor B	1.2mg/g ; 0.018meq/g

<sup>1</sup> Load breakthrough capacity using atrazine as a test probe.

## Ion Exchange Capacity & Polar Basic Compound: Cytosine

Cytosine, a pyrimidine that is one of the five bases found in DNA, is a polar basic compound that is very difficult to retain on reversed-phase SPE. In this example, cytosine at 20µg/mL in 10mM potassium phosphate, pH 3, was extracted using mixed-mode SPE. A 1mL sample was applied to 100mg/3mL tubes, following the protocol outlined in Table 2. The Discovery DSC-MCAX SPE was compared to two competitor mixed-mode SPE phases, analyzing the recoveries by HPLC-UV (Figure A).

Because of the low ion exchange capacity attributed to Competitor A, poor compound retention was observed resulting in an average recovery of ~1.8%. Although improved recovery was observed for Competitor B, inadequate ion exchange capacity led to poor reproducibility with a recovery range of 4.2 – 44%. In contrast, Discovery DSC-MCAX SPE exhibited an average recovery and RSD of 99.2 ± 4.8%.

**Table 2. Recommended Generic Protocol for Discovery DSC-MCAX**

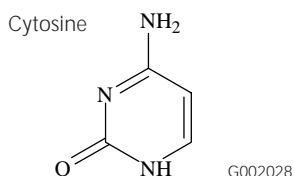
1. Condition and equilibrate with methanol and 10mM potassium phosphate, pH 3-6.
2. Load sample.
3. Wash off hydrophilic compounds/interferences with 10mM potassium phosphate, pH 3-6; and/or 1M acetic acid.
4. Wash off hydrophobic compounds/interferences with methanol.
5. Elute basic/zwitterionic compounds with 5% ammonium hydroxide in methanol.
6. Evaporate eluate via nitrogen purge and reconstitute appropriately.

When processing polar basic/zwitterionic compounds on mixed-mode cation exchange SPE phases of insufficient ion exchange capacity, premature compound elution/breakthrough can occur during steps 2, 3, and 4.

This protocol is consistent with generic protocols recommended for other commercially available silica-based mixed mode-mode cation exchange SPE products.

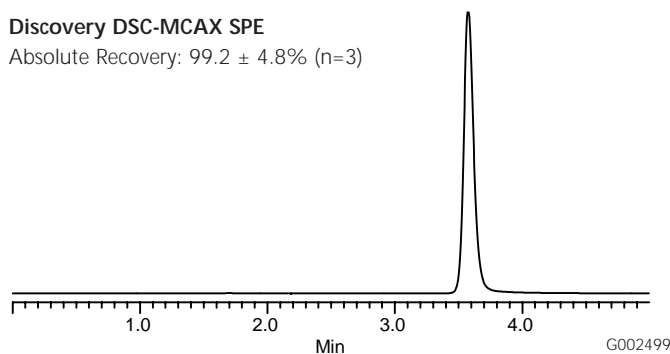
## Figure A. Competitor Comparison - Recovery of Cytosine Using Discovery DSC-MCAX SPE

**SPE Tube:** Discovery DSC-MCAX, 100mg/3mL  
**HPLC Column:** Discovery HS F5, 15cm x 4.6mm ID, 5µm particles  
**Mobile Phase:** 10mM potassium phosphate, pH 3  
**Flow Rate:** 1mL/min  
**Temp.:** Ambient  
**Det.:** 280nm, UV  
**Inj. Vol.:** 10µL



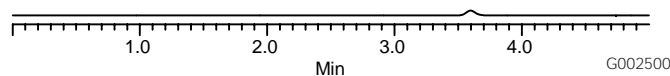
### Discovery DSC-MCAX SPE

Absolute Recovery: 99.2 ± 4.8% (n=3)



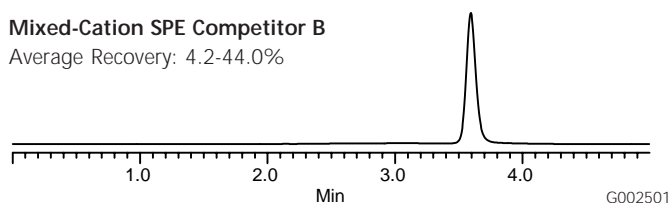
### Mixed-Cation SPE Competitor A

Average Recovery: 1.8%



### Mixed-Cation SPE Competitor B

Average Recovery: 4.2-44.0%



## Ion Exchange Capacity & Bioanalysis: Piroxicam and 2-Aminopyridine

Drug metabolism and pharmacokinetic studies are the most common bioanalytical applications performed. In some cases, the parent drug is metabolized into polar compounds that are difficult to retain by reversed-phase SPE. Mixed-mode SPE provides the two different retention mechanisms required to retain both the parent drug and its metabolite(s).

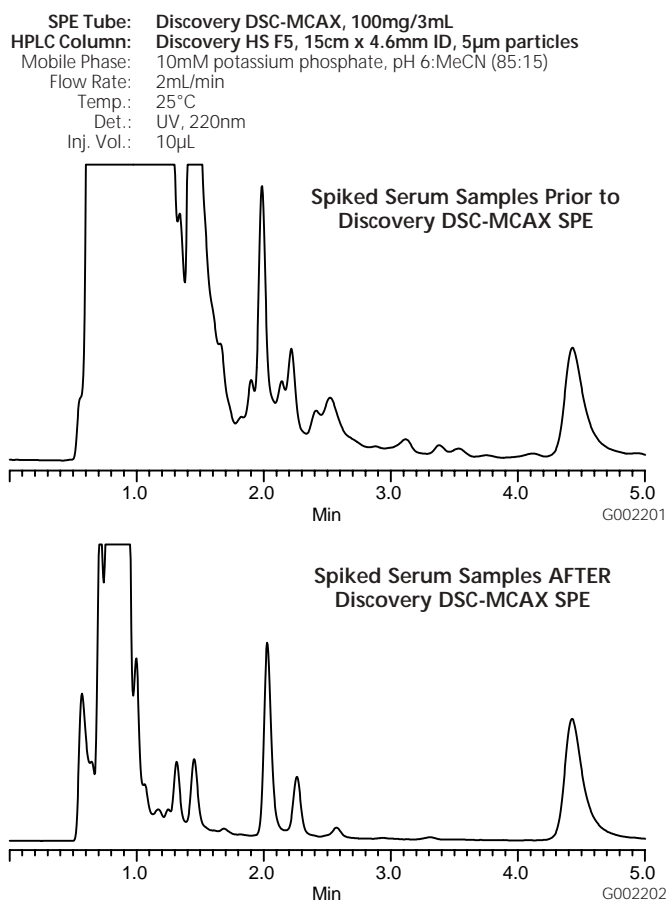
In this example, piroxicam and piroxicam's highly polar metabolite, 2-aminopyridine, were spiked into human urine. Spiked samples were then extracted via Discovery DSC-MCAX using the recommended generic protocol, and analyzed via HPLC-UV. The results were then compared against two competitor mixed-cation phases run in parallel.

Using the DSC-MCAX provided excellent clean up of biological samples such as urine (Figure B). Although greater than 80% recovery was observed for piroxicam on all three mixed-cation phases, recovery suffered for 2-aminopyridine on the two competitor phases. Unlike piroxicam, 2-aminopyridine's polar nature required cation exchange to be the dominant mode of retention. Discovery DSC-MCAX's high ion exchange capacity allowed for excellent retention of both piroxicam and its polar metabolite, 2-aminopyridine.

## Ion Exchange Capacity & Zwitterionic Compounds: p-Aminobenzoic Acid

p-aminobenzoic acid is a zwitterionic compound because it has both positive and negative ionic charge. Its polar nature makes it difficult to isolate via reversed-phase SPE. In this application, 1mL 5.0µg/mL p-aminobenzoic in urine:10mM potassium phosphate, pH 3 (1:1) was extracted using DSC-MCAX, 100mg/3mL, and competitors A/B using the protocol described in Table 1. Analysis was conducted via HPLC-UV (Figure C). On competitor A, 3% of the compound was lost during sample load, and greater than 20% of the compound was prematurely eluted during the first aqueous wash step (10mM potassium phosphate, pH 3). Although no breakthrough was observed during sample load on competitor B, 12% of the compound was lost after the first aqueous wash step. On the contrary, full retention was observed during sample load and all wash steps when employing Discovery DSC-MCAX, and the compound was fully recovered during elution.

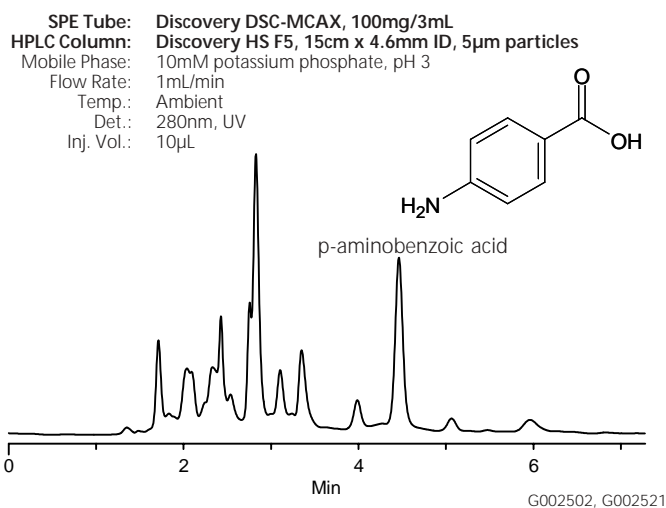
**Figure B. Competitor Comparison of 2-Aminopyridine and Piroxicam from Human Urine**



**Efficiency of Absolute Recovery (n=4)**

	% Recovery ± RSD	
	2-Aminopyridine	Piroxicam
Discovery DSC-MCAX	<b>102 ± 3.5%</b>	101 ± 1.2%
Leading Competitor A	30 ± 52.5%	98 ± 3.2%
Leading Competitor B	36 ± 24.2%	83 ± 4.3%

**Figure C. Extraction of p-aminobenzoic acid From Urine Using DSC-MCAX SPE**



**Ion Exchange Capacity & Fractionation: 2-Aminobenzoic Acid, Benzoic Acid, and p-Nitrobenzoic Acid**

Mixed-mode cation exchange SPE can also be used to fractionate basic from acidic and neutral compounds. In such applications, a mix of basic, acidic, and neutral compounds are applied to the packed bed. Acidic and neutral compounds are retained solely by reversed-phase mechanisms, and can thus be eluted by introducing methanol (2<sup>nd</sup> wash step) to the phase. Ideally, because of cation exchange interactions, basic compounds should still be adsorbed, and can only be subsequently eluted using basified methanol. When processing more polar basic/zwitterionic compounds on mixed-cation phases of low ion exchange capacity, poor or partial ionic retention is observed resulting in poor fractionation. In other words, polar basic/zwitterionic compounds are partially eluted in both the acidic/neutral fraction and basic fraction.

In this application, 1mL 10mM potassium phosphate, pH 3 solution standard containing 10µg/mL 2-aminobenzoic acid, benzoic acid, and p-nitrobenzoic acid was processed using Discovery DSC-MCAX SPE, 300mg/3mL, and two equivalent competitor phases (Table 1), and analyzed via HPLC-UV (Figure D, on page 4). Ideally, only benzoic acid and p-nitrobenzoic acid should be present in fraction 1, and 2-aminobenzoic acid should be fully recovered in fraction 2.

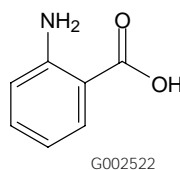
Competitor A has very low ion exchange capacity. As a result, almost no fractionation occurred resulting in the premature elution (92%) of 2-aminobenzoic acid within the acidic/neutral fraction. On competitor B, only partial fractionation occurred resulting in partial distribution of 2-aminobenzoic acid between both fractions. Discovery DSC-MCAX SPE, on the other hand, completely fractionated and recovered 2-aminobenzoic acid from the other two compounds.

## Figure D. Effects of Mixed-Mode Ion Exchange Capacity on Fractionation

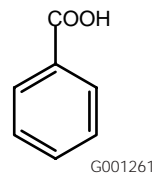
SPE Tube: Discovery DSC-MCAX, 100mg/3mL  
 HPLC Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles  
 Mobile Phase: 0.1% TFA:methanol (1:1)  
 Flow Rate: 2mL/min  
 Temp.: Ambient  
 Det.: 254nm, UV  
 Inj. Vol.: 10µL

1. 2-aminobenzoic acid
2. benzoic acid
3. p-nitrobenzoic acid

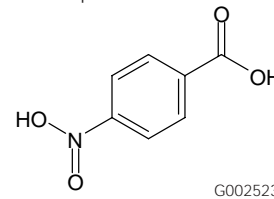
2-aminobenzoic acid



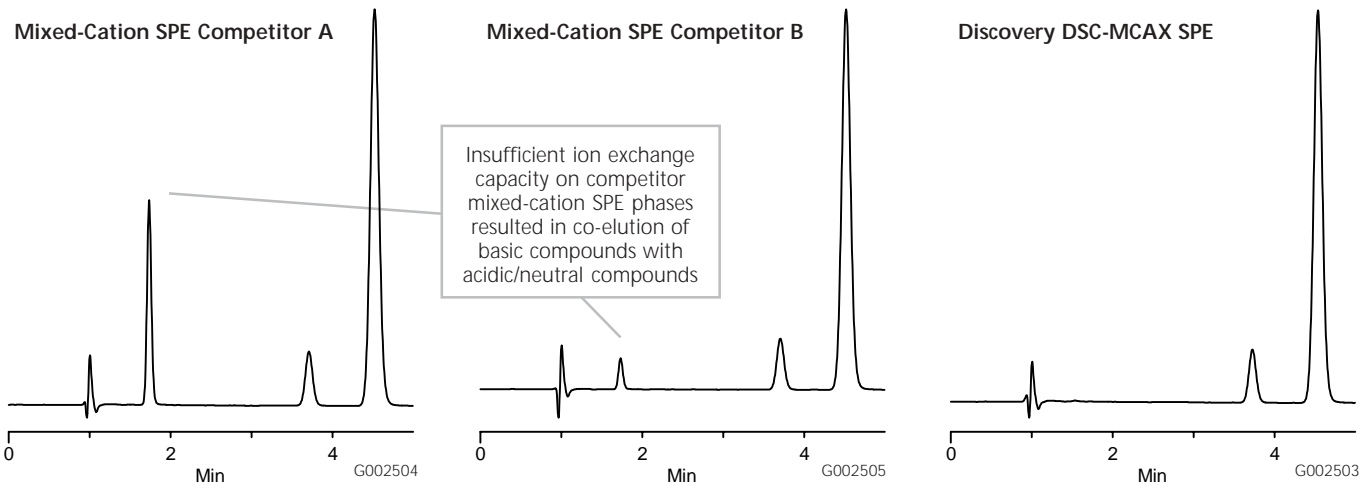
benzoic acid



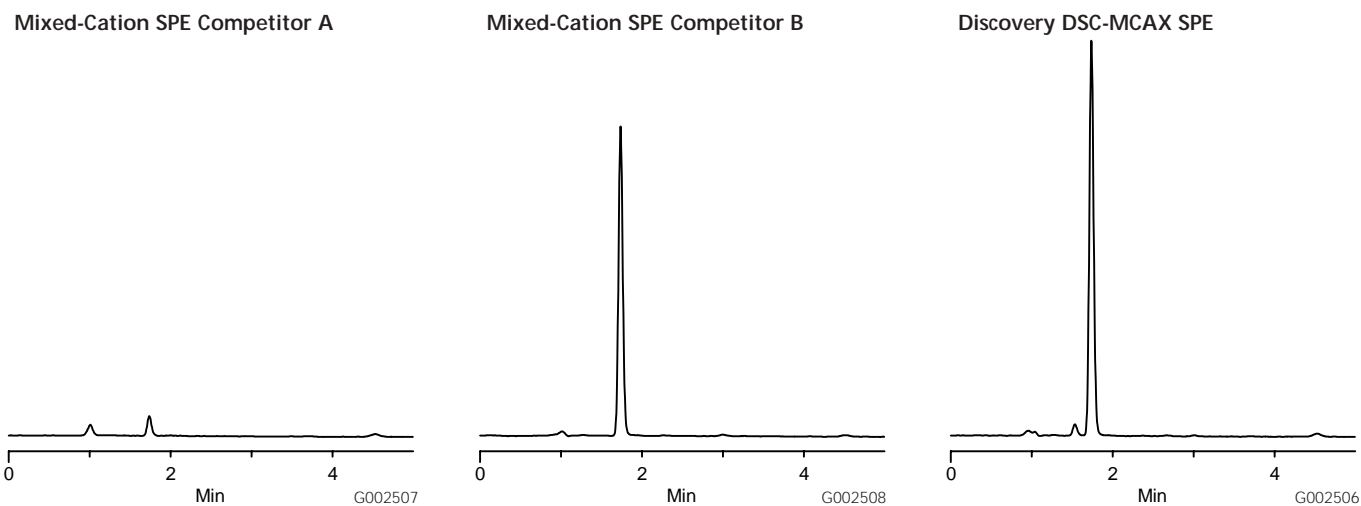
p-nitrobenzoic acid



### Fraction 1 - Acidic/Neutral Compounds



### Fraction 2 - Basic Compounds



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