

Achieving Optimal Selectivity/Sample Clean Up Using Discovery DSC-MCAX (Mixed-Mode Cation Exchange) SPE

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Introduction

Discovery DSC-MCAX (mixed-mode cation exchange) SPE was developed for improving selectivity/sample clean up when extracting basic/zwitterionic compounds from aqueous samples (e.g. biological plasma, urine, etc.). Unlike single mode phase chemistries (e.g., C18 SPE), DSC-MCAX contains two bonded functional groups: octyl (C8) and benzene sulfonic acid (SCX). As a result, two retention mechanisms are available to retain a diverse range of compounds. Through the careful manipulation of pH and organic modifier conditions, the user can elute (wash off) a range of acidic and neutral polar and non-polar interferences prior to eluting basic and zwitterionic compounds of interest. Extraction methods that offer inadequate selectivity/sample clean up often lead to higher background, misleading peak responses, increased backpressure (leading to system failure), and decreased sensitivity during subsequent LC analyses. For LC-MS analysis, lower sensitivity and increased ion-suppression can occur.

How is Selectivity/Sample Clean Up Improved When Using Mixed-Mode Technology?

When a basic or zwitterionic compound is applied to Discovery DSC-MCAX SPE, the combination of strong ionic bonds and hydrophobic retention allows for the use of strong wash

solvents (e.g. 100% methanol or acetonitrile) that would prematurely elute the compounds on standard single mode chemistries (e.g. C18). Table 1 details the generic protocol differences between standard C18 and DSC-MCAX chemistries and describes how selectivity and sample clean up are improved using DSC-MCAX.

DSC-MCAX vs. C18 SPE for Extracting Amphetamines from Serum

In this application, 1mL human urine spiked with amphetamine and methylamphetamine (2µg/mL) was extracted using both Discovery DSC-MCAX and standard C18 SPE independently. Spiked urine samples, diluted 1:1 with 50mM ammonium acetate, pH 6.0, were loaded onto DSC-MCAX SPE cartridges pre-conditioned and equilibrated with 1mL methanol and 50mM ammonium acetate, pH 6.0. Upon sample load, the tubes were washed with 1mL 50mM ammonium acetate, pH 6.0, 1mL 1M acetic acid, and 1mL methanol. Elution of the two basic compounds was facilitated via 1mL 5% NH₄OH in methanol. The eluate was further evaporated to dryness and reconstituted with LC mobile phase prior to HPLC-UV analysis.

On standard C18, SPE cartridges were first conditioned and equilibrated with 1mL methanol and DI water. 1mL diluted urine samples (from the same pool used for DSC-MCAX) were loaded onto the C18 tubes, and washed with 1mL DI water

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Table 1. Comparison of Standard C18 and Discovery DSC-MCAX Generic Protocols when Extracting Basic Compounds

Generic Method	Standard C18 SPE	Discovery DSC-MCAX SPE	Comments
1. Condition	methanol	methanol	Wets/ activates bonded functional groups to ensure consistent interaction between sorbent and analyte.
2. Equilibrate	aqueous buffer/ solution	pH 3-6 buffer	Sorbent is treated with solution that is similar to sample matrix (polarity, pH, etc.) in order to maximize retention.
3. Sample Load	Load at neutral-high pH	Load at pH 3-6	On standard C18, sample pH should be neutral-high in order to neutralize all basic compounds. This maximizes reversed-phase retention. On DSC-MCAX, the opposite is true. The low pH sample environment ionizes basic compounds to facilitate the electrostatic interactions between compound and SCX bonded functional groups.
4. Wash	5-15% methanol	Wash 1 - low pH 3-6 buffer Wash 2 - 100% methanol	On standard 18, most wash solvents require a dilute form of the elution solvent to remove co-retained interferences. Stronger wash solvents may potentially elute basic compounds of interest prematurely. On DSC-MCAX, Wash 1 plays two roles. The low pH environment further reinforces ion-exchange interactions for basic compounds, and removes all non-basic hydrophilic interferences. Wash 2 is a powerful wash step used to remove all non-basic hydrophobic interferences. Because basic compounds are retained by strong ionic interactions along with reversed-phase interactions, they should still be retained.
5. Elute	methanol	Basified methanol (e.g. 5% NH ₄ OH in methanol)	On DSC-MCAX, the high pH environment neutralizes basic compounds disrupting the electrostatic interaction between compound and sorbent functional groups. The organic solvent environment disrupts any hydrophobic interactions.

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and 20% methanol. Basic compounds were then eluted with 1mL methanol followed by eluate evaporation and LC mobile phase reconstitution prior to HPLC-UV analysis. Figure A describes the results obtained from the two SPE procedures.

Conclusion

In conclusion, the dual retention properties of Discovery DSC-MCAX provide a broad affinity for a wide range of compounds. By controlling pH and organic strength, the technology offers superior clean up and selectivity when extracting basic and

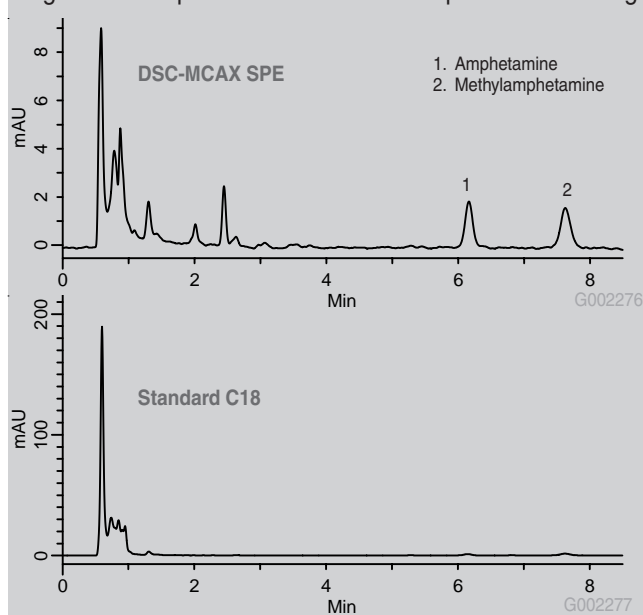
zwitterionic compounds. When compared directly against standard C18 SPE, Discovery DSC-MCAX SPE offered significantly less background potentially reducing misleading peak responses, column backpressure, and ion suppression during subsequent LC-UV and LC-MS analysis.



Related Information

For more information request *A Case Study In Improved Extraction Performance and Selectivity using Dual Mode SPE Technology*, T404048 (GUW).

Figure A. Comparative Extraction of Amphetamines Using Discovery DSC-MCAX and Standard C18 SPE



SPE Tube: Discovery DSC-MCAX, 100mg/3mL
Standard C18, 100mg/3mL
Cat. No.: 52783-U
HPLC Column: Discovery HS F5, 15cm x 4.6mm ID, 5µm particle size
Cat. No.: 567516-U
Mobile Phase: 10mM ammonium acetate, pH 4.5:MeCN (35:65)
Flow Rate: 2mL/min
Temp.: 40°C
Det.: 210nm, UV
Inj. Vol.: 10µL

Discussion:

- Note the Y-axis scale difference between DSC-MCAX and C18 SPE. DSC-MCAX SPE offered a maximum background height of ~9 mAU.
- In contrast, standard C18 background levels were 20 times greater than DSC-MCAX.
- Also, on DSC-MCAX absolute recovery averaged at 100.3 and 101.7%, for amphetamine and methylamphetamine, respectively.
- On standard C18, absolute recovery averaged at 48 and 79% for the two compounds.

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