

Waters

Improving Sample Clean-Up for Strong Bases and Strong Acids

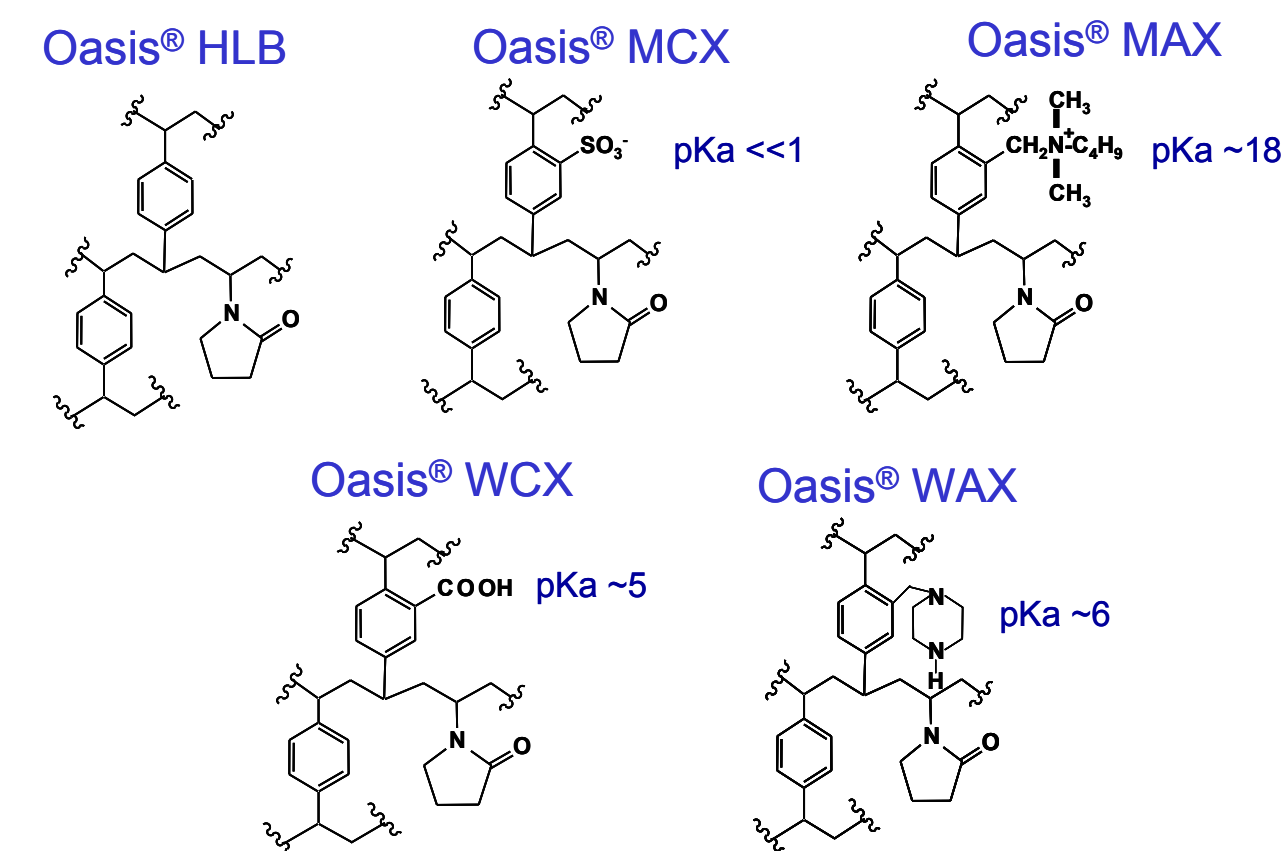
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OVERVIEW

Strong bases and acids are difficult to elute from strong ion-exchange resins. New Oasis® solid-phase extraction (SPE) sorbents were developed to alleviate this issue. Both materials combine reversed-phase and ion-exchange mechanisms of retention: Oasis® WCX (Weak Cation eXchange) and Oasis® WAX (Weak Anion eXchange), for strong bases and strong acids, respectively. We have developed robust SPE protocols that are highly selective and sensitive for the clean-up of strong bases, quaternary amines and strong acids from biological matrices such as urine and rat plasma using these sorbents.

INTRODUCTION

To address the need for new SPE sorbents in the pharmaceutical, environmental and life science fields, new weak ion-exchange SPE materials were developed. These sorbents are new additions to the Oasis® family of polymeric SPE products. Oasis® MCX material is a mixed-mode, *strong* cation exchange SPE sorbent that is too retentive for strong bases and quaternary amines. In these applications, both the sorbent and analyte remain charged and cannot be easily released from the sorbent. The new Oasis® WCX material is also a mixed-mode sorbent, but contains a *weak* cation exchange functionality whose charge can easily be controlled by the pH of the solution. Oasis® MAX material is a mixed-mode, *strong* anion exchange SPE sorbent that is too retentive for strong acids that contain highly ionic species, such as phosphate groups. The new Oasis® WAX material is also a mixed-mode sorbent, but contains a *weak* anion exchange functionality.



HPLC Conditions

Columns: XTerra® MS C₁₈ 2.1 x 20 mm *IS*TM, 3.5 µm (bases) or SunFire™ C₁₈ 2.1 x 20 mm *IS*TM, 3.5 µm (acid)
Mobile Phase A: 10 mM NH₄HCO₃, pH 10 (bases) or 10 mM CH₃COONH₄⁺, pH 7 (acid)
Mobile Phase B: MeOH with 10 mM NH₄HCO₃, pH 10 (bases) or MeOH with 10 mM CH₃COONH₄⁺, pH 7 (acid)

Flow Rate: 0.4 mL/min

Gradient: Time (min)	%A	%B
0.0	95	5
3.0	5	95
4.0	5	95
4.1	95	5
5.0	95	5

Injection Volume: 10 µL

Instrument: Waters 2777 Sample Manager and Waters 1525µ Binary HPLC Pump

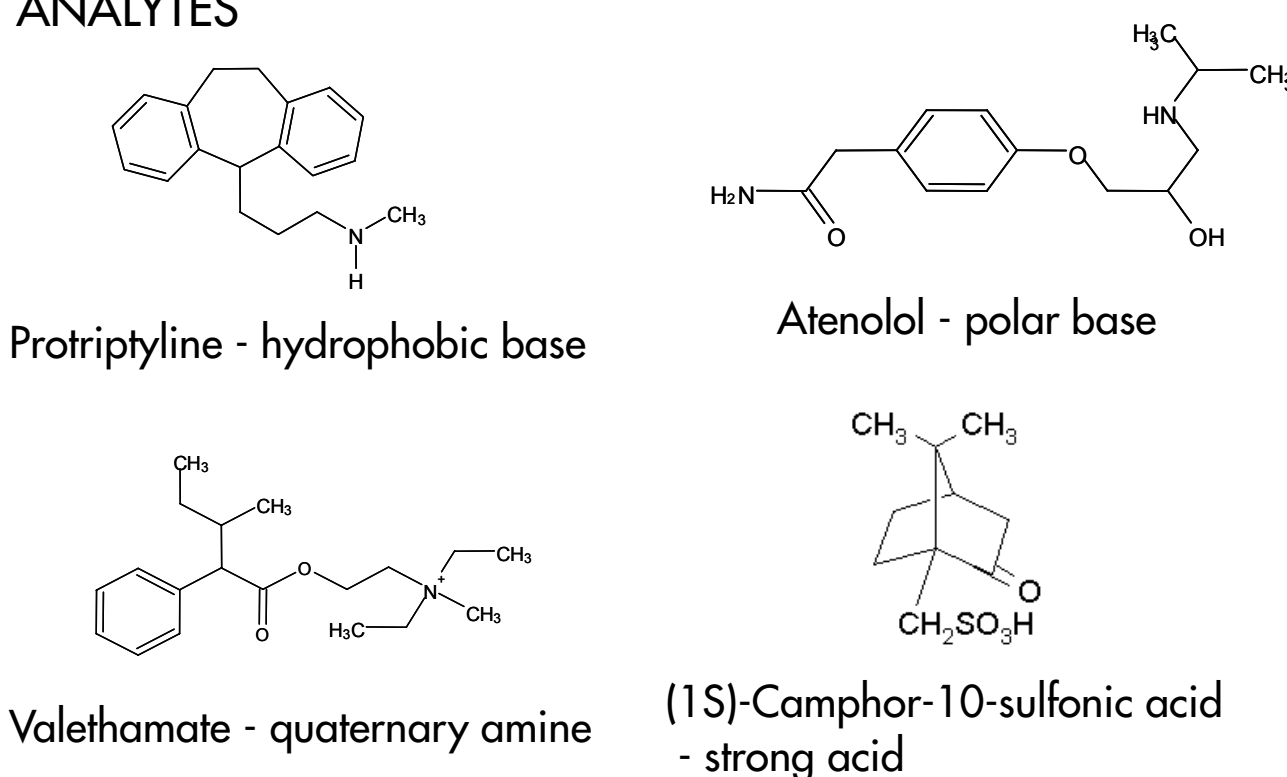
MS/MS Conditions

Waters Micromass® Quattro Ultima™ (ESI+) and Waters Micromass® Quattro Premier™ (ESI-)

Source Temp: 150 °C
Desolvation Temp: 350 °C
Cone Gas Flow: 50 L/Hr
Desolvation Gas Flow: 550 L/Hr (600 L/Hr ESI-)
Collision Cell: 2.2e⁻³ bar (Ar gas)

MRM Transitions:		Cone (V)	CID (eV)
Valethamate	m/z 306.1 → 218.9	35	20
Protriptyline	m/z 264.0 → 191.1	60	25
Atenolol	m/z 266.9 → 144.9	45	25
Camphorsulfonic Acid	m/z 231.1 → 79.8	60	30

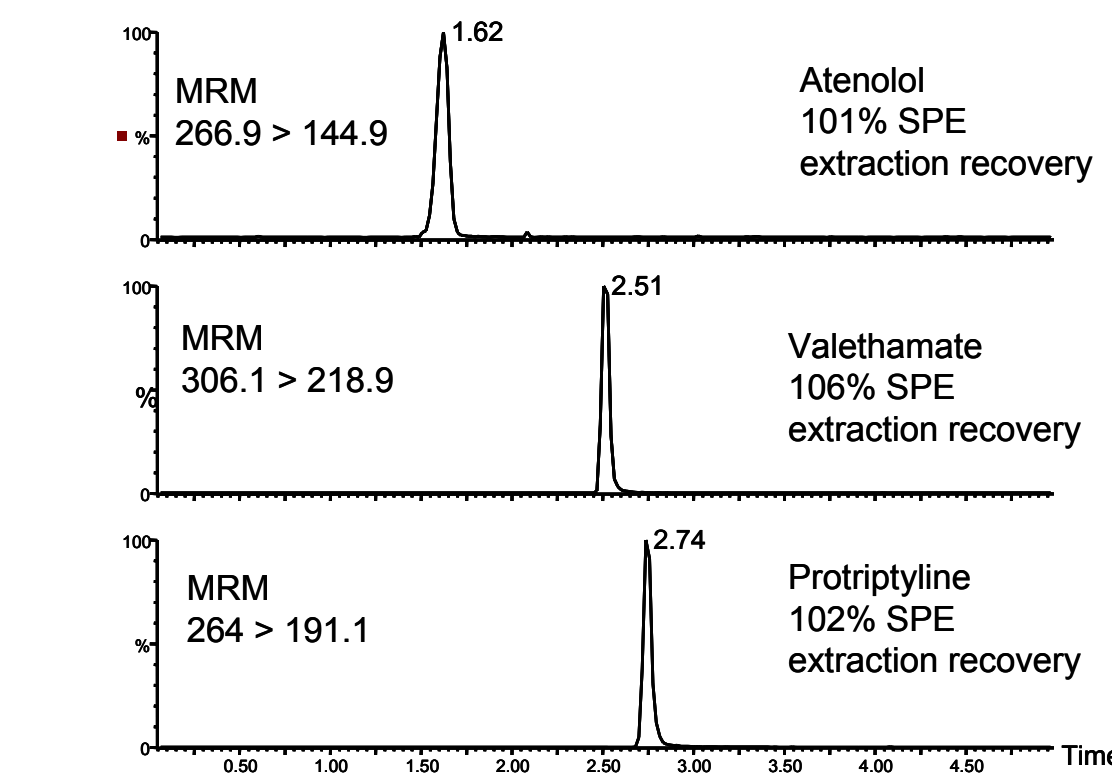
ANALYTES



SPE METHODS

Oasis® WCX µElution Plate

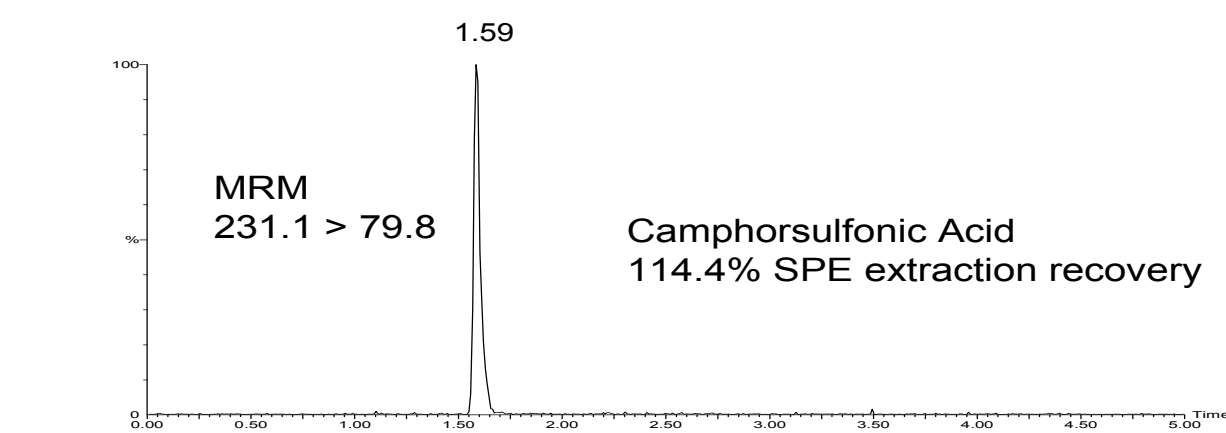
Condition: 200 µL MeOH
Equilibrate: 200 µL H₂O
Load: 150 µL urine or 1:1 diluted rat plasma, spiked with 10 pg/µL each analyte. To disrupt protein binding, add 2% H₃PO₄ (total sample volume) to protriptyline samples only
Wash 1: 200 µL 25 mM phosphate buffer, pH 7
Wash 2: 200 µL MeOH
Elute: 50 µL (25 µL x 2) 2% FA in MeOH
Dilute: 100 µL 2% NH₄OH in H₂O



Representative LC/MS/MS data. Recoveries are the SPE extraction recoveries for rat plasma samples on the Oasis® WCX µElution plate. Excellent recoveries are obtained for all three classes of bases on this material.

Oasis® WAX µElution Plate

Condition: 200 µL MeOH
Equilibrate: 200 µL H₂O
Load: 100 µL 1:1 diluted rat plasma, spiked with 50 pg/µL of camphorsulphonic acid. To disrupt protein binding, add 2% H₃PO₄ (total sample volume)
Wash 1: 200 µL 2% Formic Acid, pH 2.7
Wash 2: 200 µL MeOH
Elute: 50 µL (25 µL x 2) 2% NH₄OH in MeOH
Dilute: 50 µL H₂O with 2% Formic Acid

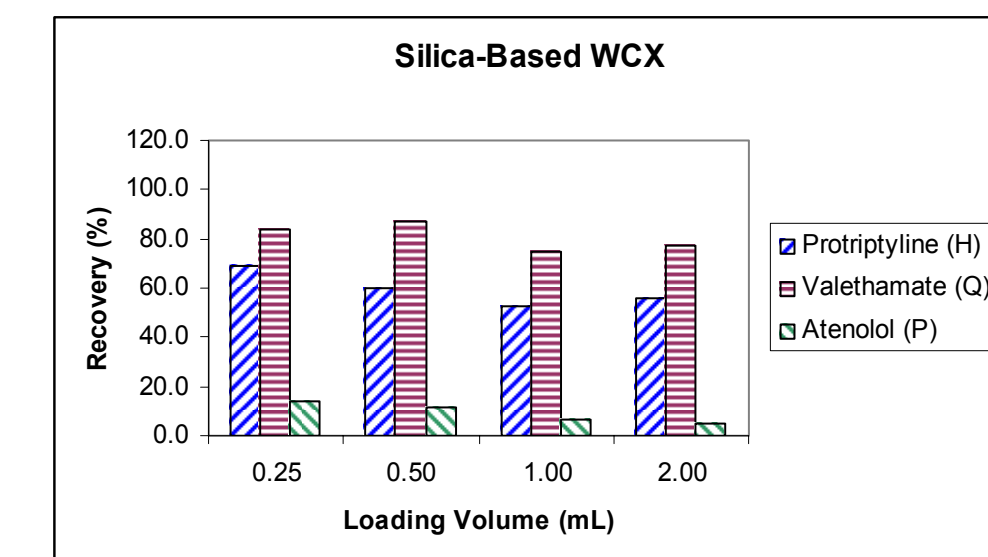
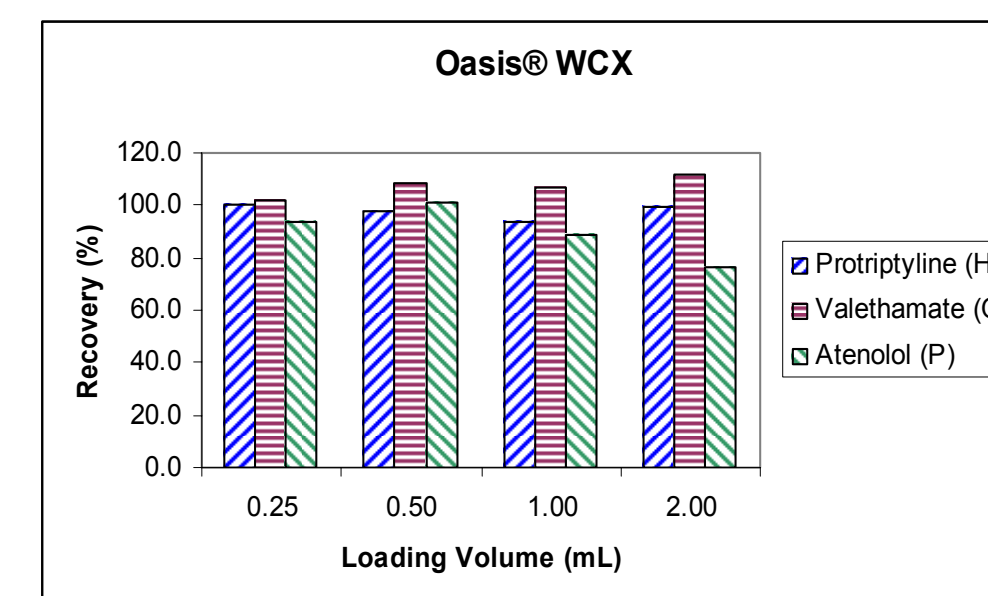


Representative LC/MS/MS data. Recovery is the SPE extraction recovery for rat plasma samples on the Oasis® WAX µElution plate. Excellent recovery is obtained for this strong acid.

Oasis® WCX versus a Silica-Based Weak Cation Exchanger 10-mg 96-well Plates

Condition: 500 µL MeOH
Equilibrate: 500 µL H₂O
Load: 0.25, 0.5, 1.0, 2.0 and 2.5 mL saline, spiked with 20 pg/µL of each analyte
Wash 1: 500 µL 25 mM phosphate buffer, pH 7
Wash 2: 500 µL MeOH
Elute: 250 µL (125 µL x 2) 2% Formic acid in MeOH
Dilute: (1) 250 µL 2% NH₄OH in H₂O for protriptyline and valethamate
(2) 750 µL 2% NH₄OH in H₂O for atenolol

OASIS® WCX VS. SILICA-BASED WCX



Comparison of the results for saline spiked with the three bases after SPE clean up with Oasis® WCX and a commercially available silica-based WCX material. Increasing amounts of spiked saline were loaded onto the sorbents. Excellent recoveries were seen for all three analytes under all loading conditions on the Oasis® WCX. However, on the silica-based WCX, the polar analyte was not retained during the load step and 80% or less SPE recoveries were observed for the quaternary amine and hydrophobic basic analytes.

CONCLUSIONS

- Oasis® WCX
 - Designed for SPE clean-up of strong bases and quaternary amines that are difficult to elute from strong cation exchange materials
- Oasis® WAX
 - Designed for SPE clean-up of strong acids that are difficult to elute from strong anion exchange materials
- Unlike silica-based SPE materials, there is no breakthrough for polar analytes.