Utilizing a New Ion-Exchange SPE Material in Novel 96-Well Plate Formats for Plasma Sample Clean-up

Waters

Ziling Lu, Diane M. Diehl and Jeffrey R. Mazzeo, Waters Corporation, Milford, MA 01757, USA

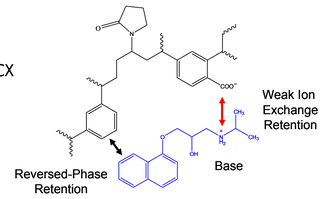
OVERVIEW

A new Oasis® solid-phase extraction (SPE) sorbent has been developed. Oasis® WCX is a mixed-mode sorbent that combines reversed-phase and Weak Cation eXchange mechanisms of retention. This material is especially useful for quaternary amines and strong bases, as well as bases that are unstable at high pH levels. This sorbent can also be used for retaining polar and hydrophobic bases, a clear advantage over silica-based WCX-type sorbents. We have developed robust SPE protocols that are highly selective and sensitive for the clean-up of bases from biological matrices such as urine and rat plasma. We can achieve excellent SPE recoveries for quaternary amines, polar and hydrophobic bases.

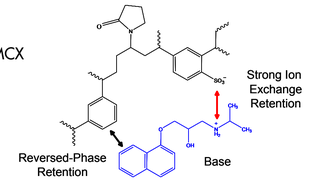
INTRODUCTION

To address the need for new SPE sorbents in the pharmaceutical, environmental and life science fields, a new weak ion-exchange SPE material was developed. This sorbent is a new addition 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 such as 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. This sorbent is ideal for the clean-up of quaternary amines from samples, as well as for all other types of basic analytes.

Oasis® WCX



Oasis® MCX



HPLC Conditions

Column: XTerra® MS C_{18} 2.1 x 20 mm *IS™*, 3.5 µm
Mobile Phase A: 10 mM NH_4HCO_3, pH 10
Mobile Phase B: MeOH with 10 mM NH_4HCO_3
Flow Rate: 0.4 mL/min
Gradient:

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

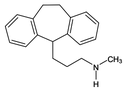
Injection Volume: 10 µL
Instrument: Waters 2777 Sample Manager and Waters 1525µ Binary HPLC Pump

MS/MS Conditions

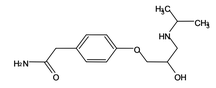
Waters Micromass® Quattro Ultima™ ESI+
Source Temp: 150 °C
Desolvation Temp: 350 °C
Cone Gas Flow: 50 L/Hr
Desolvation Gas Flow: 550 L/Hr
Collision Cell: $2.2e^{-3}$ bar (Ar gas)

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

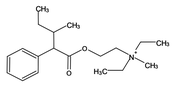
ANALYTES



Protriptyline - hydrophobic base



Atenolol - polar base

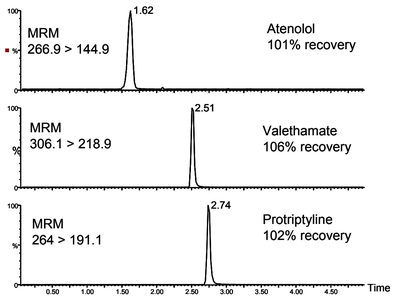


Valethamate - quaternary amine

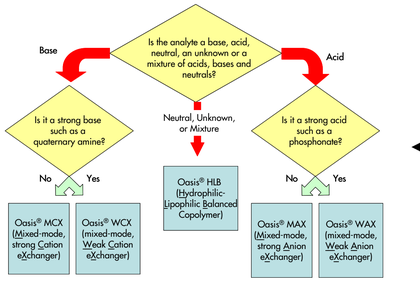
SPE METHODS

Oasis® WCX µElution Plate

Condition:	200 µL MeOH
Equilibrate:	200 µL H_2O
Load:	150 µL urine or 1:1 diluted rat plasma, spiked with10 pg/µL each analyte. To disrupt protein binding, add 2% H_3PO_4 (total sample volume) to protriptyline only
Wash 1:	200 µL 25 mM phosphate buffer in H_2O, pH 7
Wash 2:	200 µL MeOH
Elute:	50 µL (25 µL x 2) 2% FA in MeOH
Dilute:	100 µL 2% NH_4OH in H_2O
Inject:	10 µL



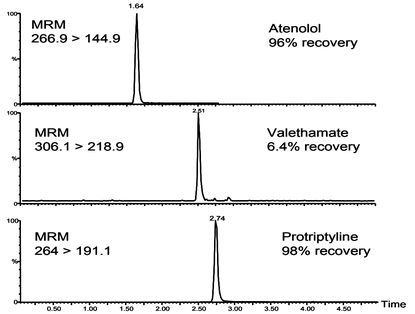
Representative LC/MS/MS data. Recoveries are the SPE recovery for rat plasma samples on the Oasis® WCX µElution plate. Excellent recoveries are obtained for all three classes of bases on this material.



Utilizing the flow chart can help to determine which Oasis® sorbent to use in SPE method development.

Oasis® MCX µElution Plate

Condition:	200 µL MeOH
Equilibrate:	200 µL H_2O
Load:	150 µL 1:1 diluted rat plasma, spiked with 10 pg/µL each analyte. To disrupt protein binding, add 2% H_3PO_4 (total sample volume)
Wash 1:	200 µL 0.1 N HCl
Wash 2:	200 µL MeOH
Elute:	50 µL (25 µL x 2) 5% NH_4OH in MeOH
Dilute:	100 µL H_2O
Inject:	10 µL

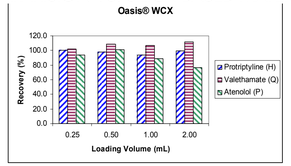


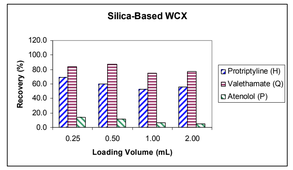
Representative LC/MS/MS data. Recoveries are the SPE recovery for rat plasma samples on the Oasis® MCX µElution plate. Excellent recoveries are obtained for both atenolol (polar base) and protriptyline (hydrophobic base). However, valethamate (quaternary amine) is retained on the sorbent.

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

Condition:	500 µL MeOH
Equilibrate:	500 µL H_2O
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 in H_2O, pH 7
Wash 2:	500 µL MeOH
Elute:	250 µL (125 µL x 2) 2% FA in MeOH
Dilute:	(1) 250 µL 2% NH_4OH in H_2O for protriptyline and valethamate (2) 750 µL 2% NH_4OH in H_2O for atenolol
Inject:	10 µL

COMPARISION VS. SILICA-BASED MATERIAL





A comparison of the results for saline spiked with the three bases after SPE clean up with the Oasis® WCX and a commercially available silica-based WCX material is shown above. 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 recoveries were observed for the quaternary amine and hydrophobic analytes.

CONCLUSIONS

The mixed-mode Oasis® WCX SPE sorbent provides a means of selective, fast and robust sample preparation with high recoveries for quaternary amine, polar, and hydrophobic basic analytes. Unlike silica-based SPE materials, there is no breakthrough for polar analytes. This material provides superior recoveries to eliminate sample breakthrough as seen on silica-based SPE materials.