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응용 자료

Oasis 2x4 Method: Proof of Concept

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

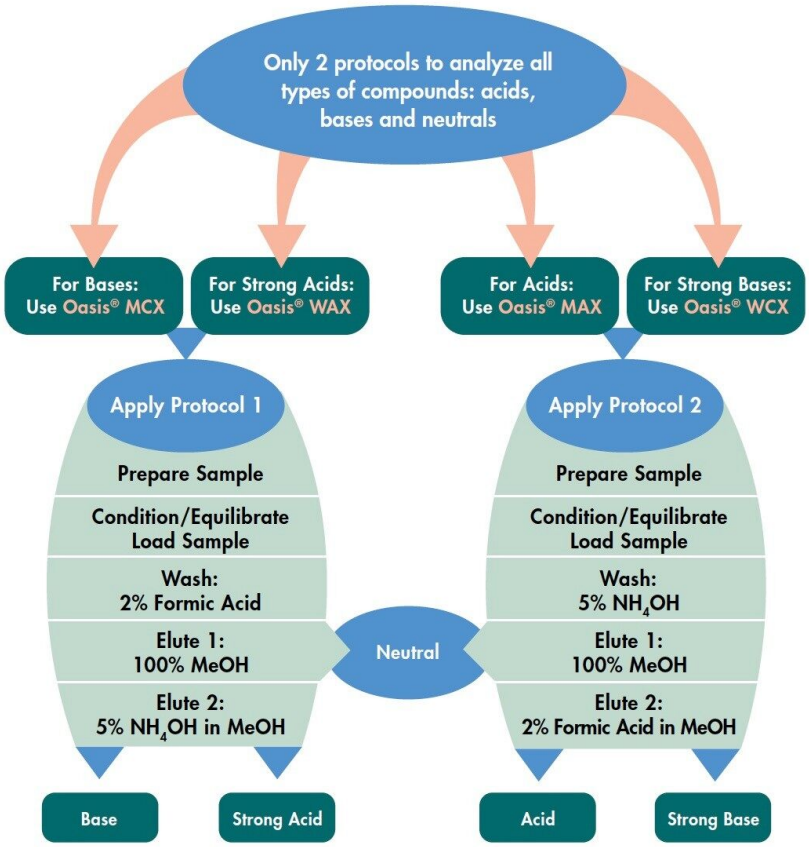
This application brief highlights Oasis 2x4 Method which is a viable approach to SPE sorbent and protocol selection.

Introduction

In order to prove that the Oasis 2x4 Method is a viable approach to SPE sorbent and protocol selection, a group of representative small molecules was spiked into rat plasma. The molecules include a base, a quaternary ammonium salt, a neutral, an acid and a strong acid. The spiked plasma samples were then extracted following the Oasis 2x4 Method. All molecules elute in the correct Elute 1 or Elute 2 fractions according to what theory predicted, proving that the method is viable.

Follow the simple steps outlined in this flow chart to achieve high recoveries and the cleanest extracts:

- Characterize your analyte [Neutral, Acid or Base, pKa].
- Select one of the four Oasis sorbents.
- Apply the indicated Protocol [1 or 2].
- Determine SPE recoveries by LC analysis.



Experimental

10 mg Oasis 96-well Plates

Protocol 1

Condition:	500 µL MeOH
Equilibrate:	500 µL H_2O
Load:	500 µL sample (250 µL plasma diluted 1:1 with 4% H_3PO_4 in H_2O)
Wash 1:	500 µL 2% HCOOH in H_2O
Elution 1:	2 x 125 µL MeOH
Elution 2:	2 x 125 µL 5% NH_4OH in MeOH
Dilution:	250 µL water

Protocol 2

Condition:	500 µL MeOH
Equilibrate:	500 µL H_2O
Load:	500 µL sample (250 µL plasma diluted 1:1 with 4% H_3PO_4 in H_2O)
Wash 1:	5% NH_4OH in H_2O
Elution 1:	2 x 125 µL MeOH

Elution 2:	2 x 125 µL 2% HCOOH in MeOH
Dilution:	5% NH_4OH in H_2O (To neutralize acid for high pH LC)
Column:	XBridge C_{18} 2.1 x 20 mm IS, 3.5 µm
Mobile Phase A:	10 mM NH_4HCO_3, pH 10
Mobile Phase B:	10 mM NH4H
Injection Volume:	10.0 µ L
Column Temperature:	Ambient
Detection:	UV @ 254 nm (Prednisone)
Instrumentation:	2777 Sample Manager, 1525µ Binary HPLC Pump, Quattro Premier and 2996 PDA

Gradient

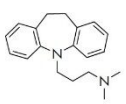
Time (min)	Profile
	%A
0	95
3.0	5
4.8	5
5.0	95

Time (min)	Profile
7.0	95

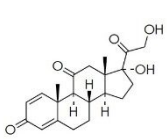
Quattro Premier

Capillary:	3.4 kV
Source Temp.:	120 °C
Desolvation Temp.:	350 °C
Cone Gas Flow:	50 L /Hr
Desolvation Gas Flow:	700 L /Hr
Collision Cell Pressure:	$2.59e^{-3}$ mbar
MRM Transitions:	Imipramine 281.2 > 85.95 ESI+ Decanesulfonic Acid 220.9 > 79.7 ESI - Ibuprofen 205.2 > 161 ESI - Valethamate 306.3 > 162.8 ESI+

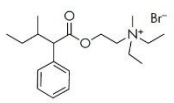
Results and Discussion



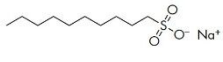
Imipramine (B)
pKa = 9.4
100 ng/mL



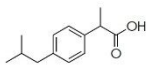
Prednisone (N)
20 µg/mL



Valethamate (QA)
pKa >12
100 ng/mL

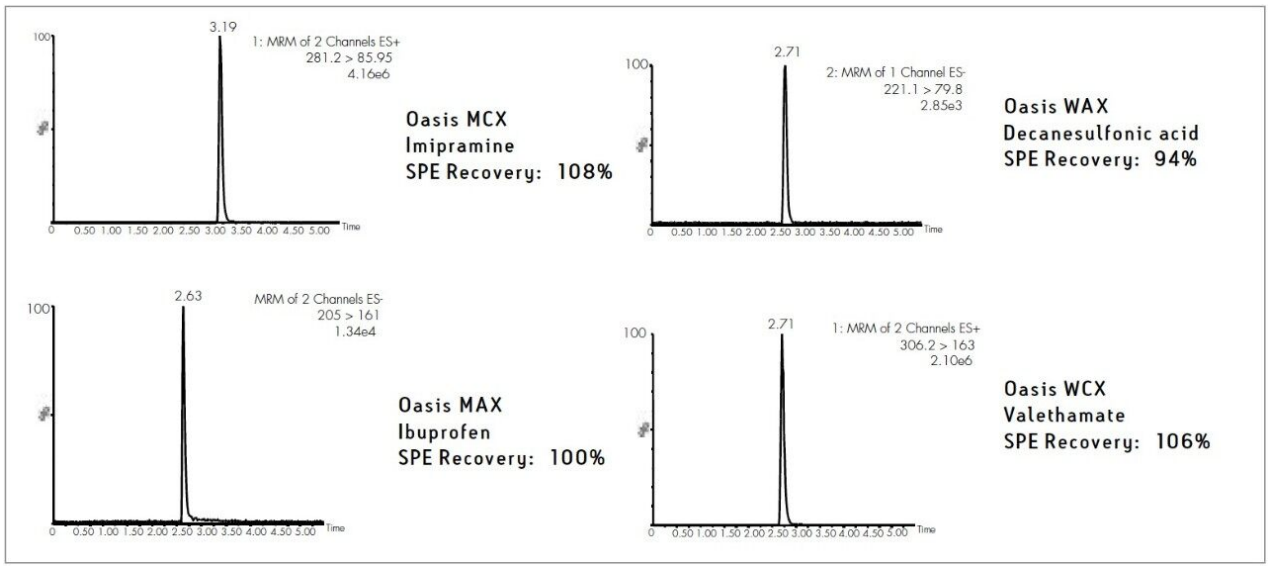


Decanesulfonic Acid (SA)
pKa <0.5
200 ng/mL

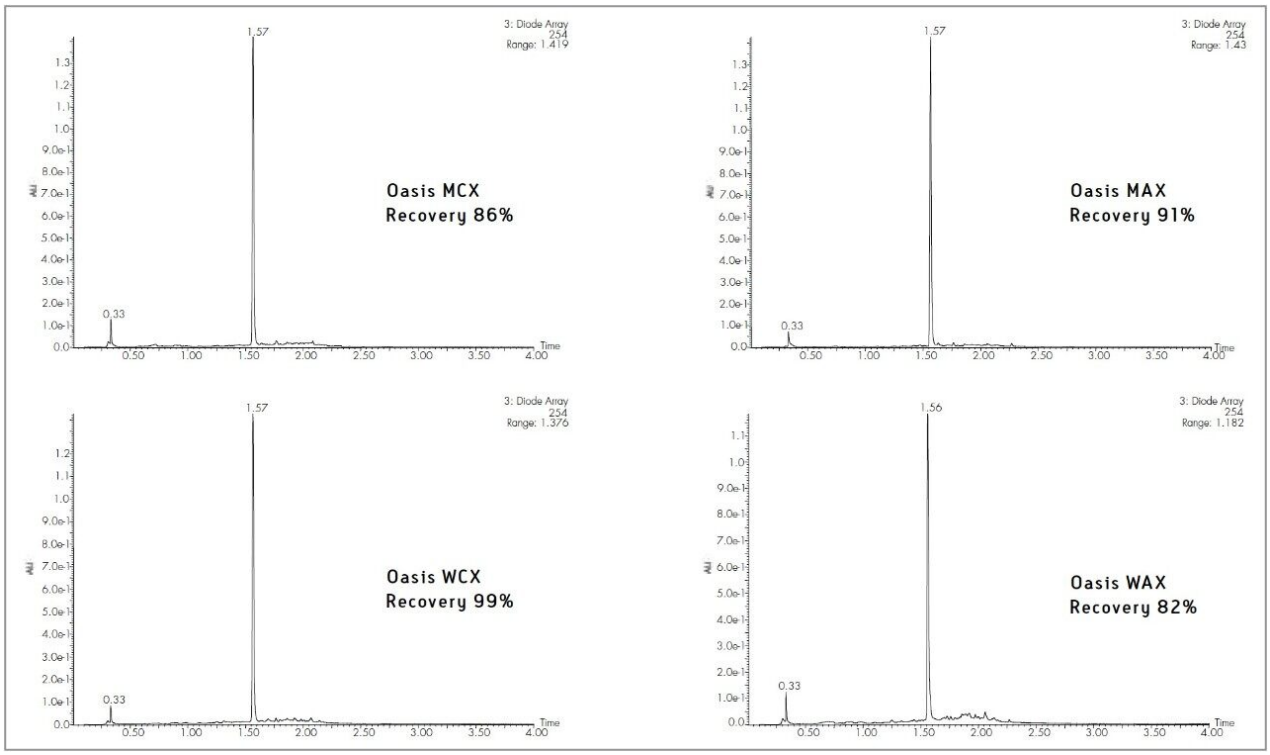


Ibuprofen (A)
pKa = 5.2
100 ng/mL

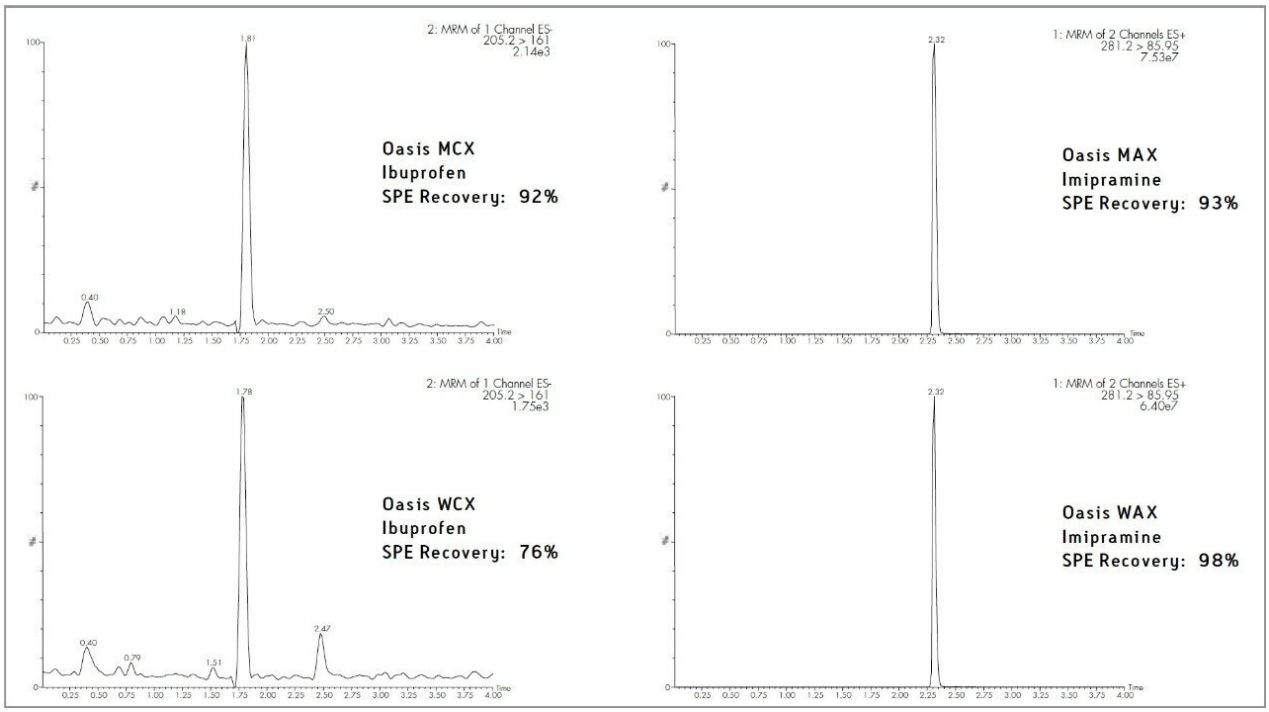
Elute 2, Primary Analyte Data



Prednisone SPE Recovery Data



Elute 1, Counter Analyte Data



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