[ APPLICATION HIGHLIGHT ]

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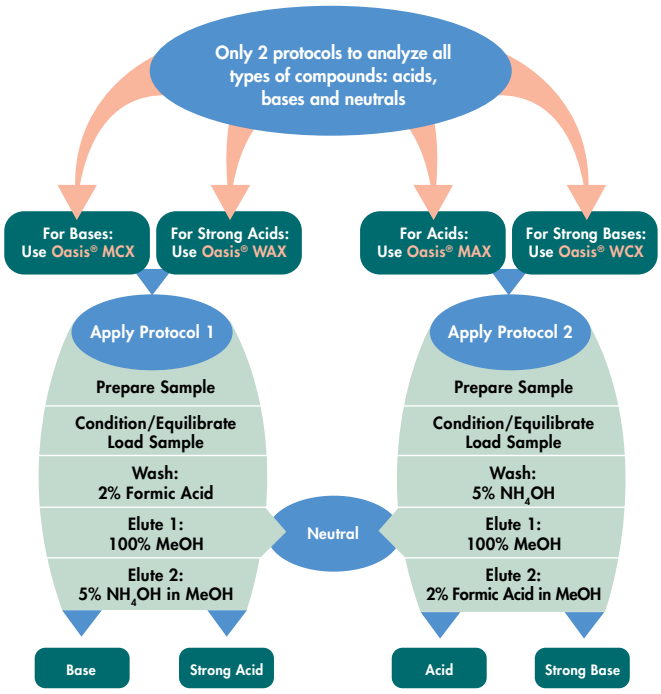
# OASIS 2X4 METHOD: PROOF OF CONCEPT

## 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.

## TEST CONDITIONS:

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 $NH_4HCO_3$ in MeOH, pH 10 |
| Flow Rate: | 0.4 mL/min |

Gradient:

| Time (min) | Profile %A | %B |
|---|---|---|
| 0 | 95 | 5 |
| 3.0 | 5 | 95 |
| 4.8 | 5 | 95 |
| 5.0 | 95 | 5 |
| 7.0 | 95 | 5 |

| | |
|---|---|
| Injection Volume: | 10.0 µL |
| Column Temperature: | Ambient |
| Detection: | UV @ 254 nm (Prednisone) |
| Instrumentation: | 2777 Sample Manager, 1525µ Binary HPLC Pump, Quattro Premier™ and 2996 PDA |

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+

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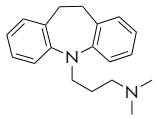
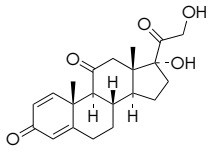
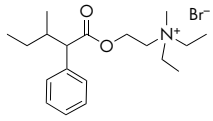
## COMPOUNDS:



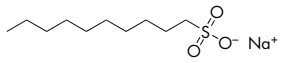
Imipramine (B)
pKa = 9.4
100 ng/mL



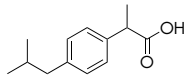
Prednisone (N)
20 µg/mL



Valethamate (QA)
pKa >12
100 ng/mL

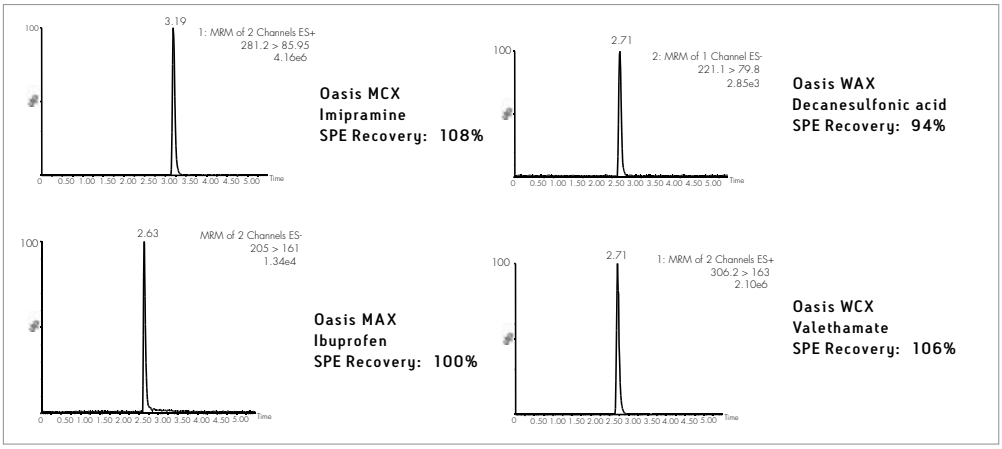


Decanesulfonic Acid (SA)
pKa <0.5
200 ng/mL

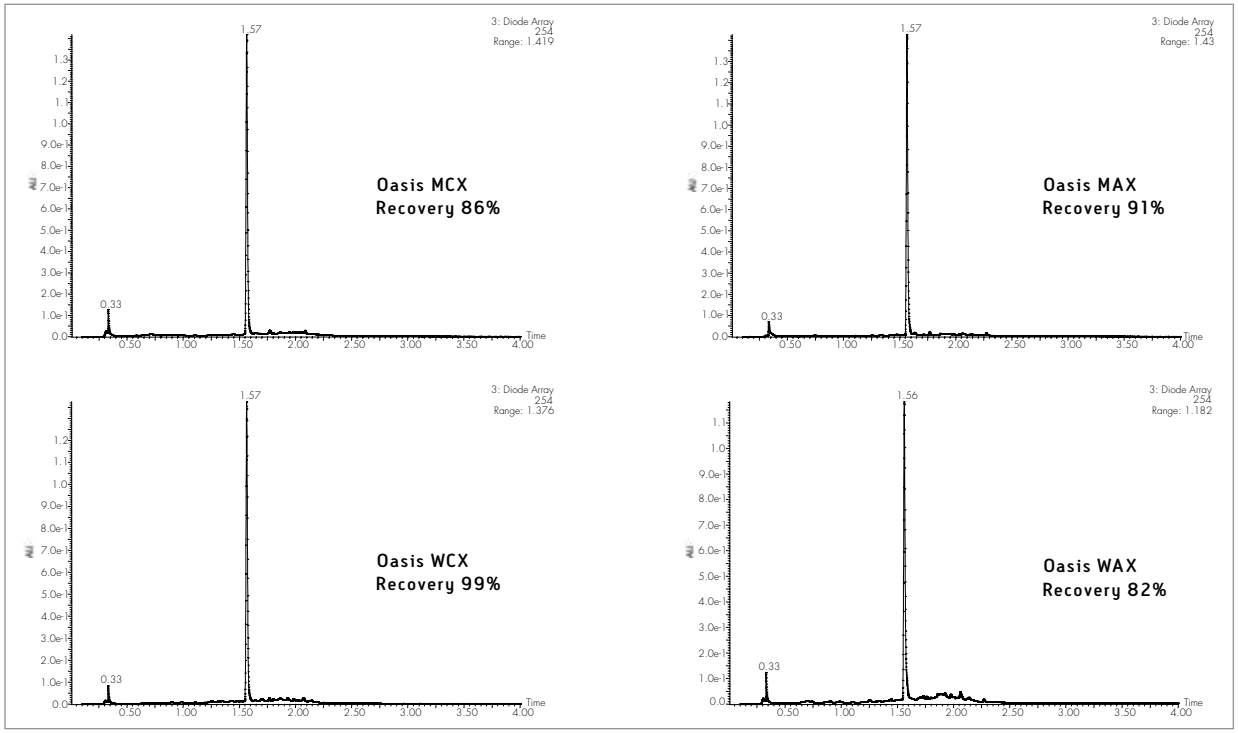


Ibuprofen (A)
pKa = 5.2
100 ng/mL

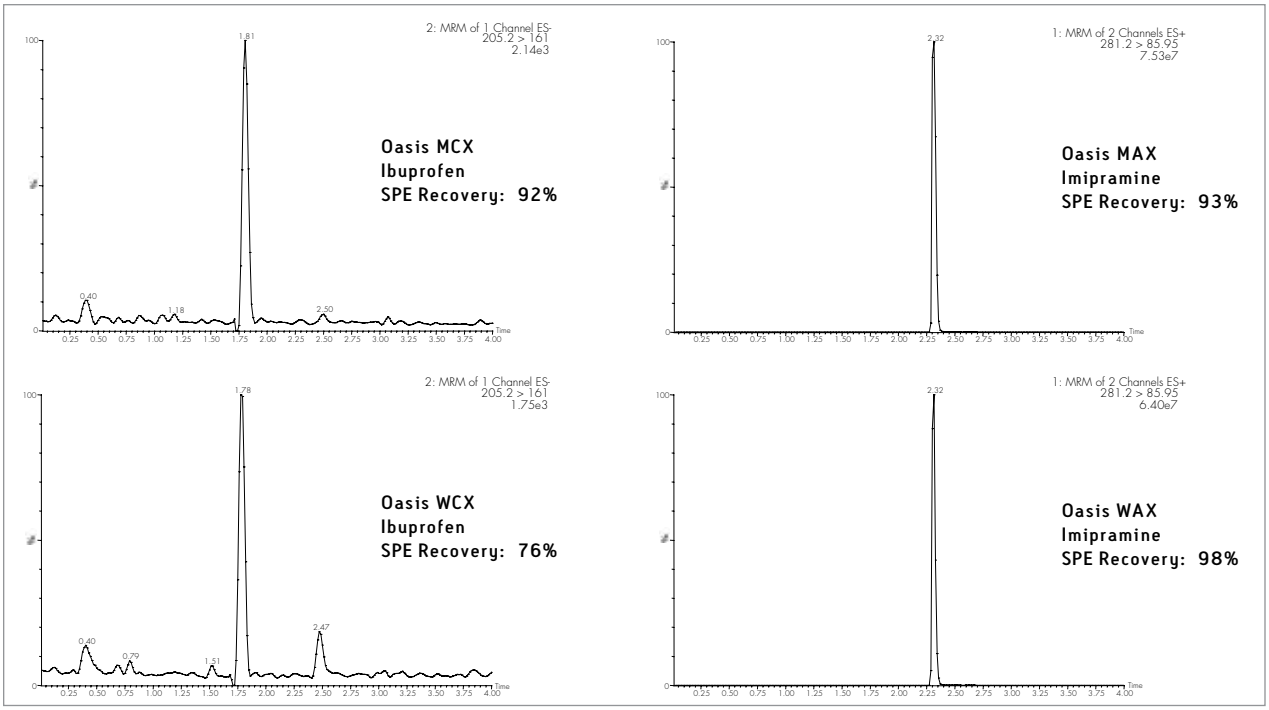
**Elute 2, Primary Analyte Data**




**Prednisone SPE Recovery Data**



Elute 1, Counter Analyte Data

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