CEPHALEXIN ON OASIS MCX

INTRODUCTION

Cephalexin is a member of the cephalosporin class of antibiotics used for the treatment of bronchitis, pneumonia, UTI's and infections of the ears and throat. This molecule is zwitterionic, meaning it has both carboxylic acid and amine moieties. Because the molecule has ionizable functionalities that can be either positively or negatively charged, screening all 4 Oasis sorbents following the Oasis 2x4 Method is clearly the mechanism for identifying the best sorbent. Oasis MCX resulted in the best recoveries and would be used for further sample preparation method development.

TEST CONDITIONS:

Oasis MCX 10-mg plate (P/N 186000259)
Condition: 500 µL MeOH
Equilibrate: 500 µL H₂O

Load: 500 μ L (250 μ L rat plasma, diluted 1:1 with 4% H_3PO_4 in $H_2O)$

Wash 1: 500 µL 2% HCOOH Wash 2: 500 µL MeOH

Elute: $250 \mu L (125 \mu L \times 2) 5\% NH_4OH in MeOH$

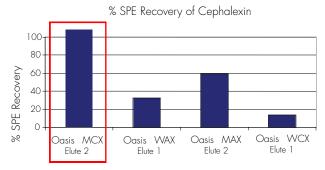
Options:

1. Dilute 250 µL H₂O with 2% FA

2. Evaporate/Reconstitute

 $3. \ \, \text{Direct inject}$

Inject: 10 μL



Clearly, Oasis MCX is the sorbent of choice.

Column: ACQUITY UPLC® BEH C_{18} 2.1 x 50 mm, 1.7 μm

 $\begin{array}{lll} \mbox{Mobile Phase A:} & 0.1\% \mbox{ HCOOH in } \mbox{H}_2\mbox{O} \\ \mbox{Mobile Phase B:} & 0.1\% \mbox{ HCOOH in MeOH} \\ \end{array}$

Flow Rate: 0.4 mL/minGradient: Time Profile (min) % A %B 98 2 initial 0.5 98 2 2.5 0 100 3.0 n 100 3.1 98 2

Injection Volume: 10.0 µL Column Temperature: 45 °C Sample Temperature: 15 °C

Sample Diluent: 50/50 water/methanol

4.0

Instrument: ACQUITY UPLC with Quattro Premier™

98

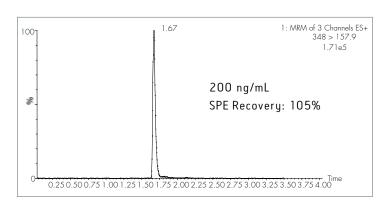
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Quattro Premier

ESI+
Capillary: 3.0 kV
Source Temp: 120 °C
Desolvation Temp: 350 °C

Cone Gas Flow: 50 L/Hr Desolvation Gas Flow: 700 L/Hr Collision Cell Pressure: 2.59 e-3 mbar

MRM transition 348->157.9 Cone Voltage 20V Collision Energy 18eV



COMPOUNDS:

Cephalexin M.W. 347.39 pKa's = 2.6 and 7.3



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