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Applikationsbericht

Trimethoprim in Rat Plasma

Waters Corporation



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

Abstract

This application brief demonstrates the analysis of Trimethoprim in Rat Plasma using Symmetry Columns.

Introduction

The compound analyzed in this study is Trimethoprim.

TRIMETHOPRIM

Experimental

HPLC Method

Column:

XTerra MS C_{18} 2.1 x 30 mm, 3.5 μm

Part number:	186000398	
Mobile phase A:	1.0% NH4OH	
Mobile phase B:	ACN	
Isocratic mobile phase composition:	40% A; 60% B	
Flow rate:	0.2 mL/min	
Injection volume:	30 μL	
Detection:	MS ESI+	
Instrument:	Alliance 2790, Micromass Quattro Ultima	
Ion source:	ESI+	
Ion source: Source temperature:	ESI+ 150 °C	
Source temperature:	150 °C	
Source temperature: Gas cell:	150 °C 1.5e ⁻³ mbar, 25 eV	
Source temperature: Gas cell: Desolvation temperature:	150 °C 1.5e ⁻³ mbar, 25 eV 350 °C	
Source temperature: Gas cell: Desolvation temperature: Cone gas flow:	150 °C 1.5e ⁻³ mbar, 25 eV 350 °C 150 L/hr	

OASIS® MCX EXTRACTION METHOD

Oasis® MCX Extraction Plate, 10 mg/96-well Part Number 186000259

CENTRIFUGE:

25 mL of EDTA rat plasma at 10 000 (RPM)

SPIKE:

5 mL of centrifuged plasma with drug (max 5% organic load) Add 100 μ L H_3PO_4

CONDITION PLATE:

500 µL methanol followed with 500 µL water

LOAD PLATE:

500 µL spiked rat plasma

WASH PLATE:

500 µL 2% HCl in water

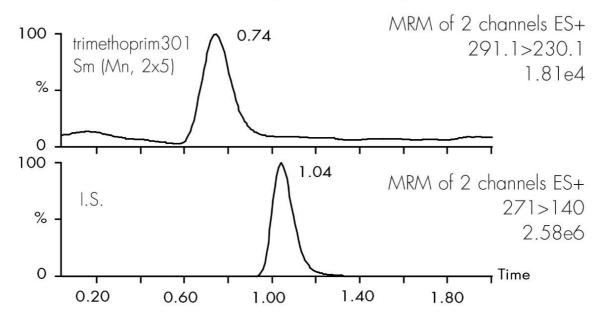
ELUTE PLATE:

 $300~\mu L~5\%~NH_{4}OH~in~methanol$

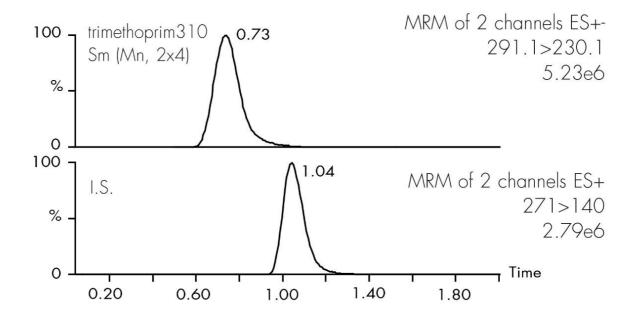
DILUTE:

200 µL water

Spike 1 ng/mL, 60/40 ACN/H₂O 1.0 % NH₄OH



Spike 250 ng/mL, 60/40 ACN/ H_2 O 1.0 % NH_4 OH



TRIMETHOPRIM (ng/mL)	Mean	Standard deviation	Coefficient of variation (%)	Recovery (%)
1	1.013	0.039	3.9	101
2.5	2.54	0.061	2.4	101
5	4.86	0.18	3.8	97
10	10.015	0.18	1.8	100
20	20.31	0.3	1.5	101
25	24.64	0.76	3.1	98
50	51.62	1.1	2.1	103
100	96.95	0.98	1	96
200	204.13	5.22	2.6	102
250	247.42	4.93	2	98

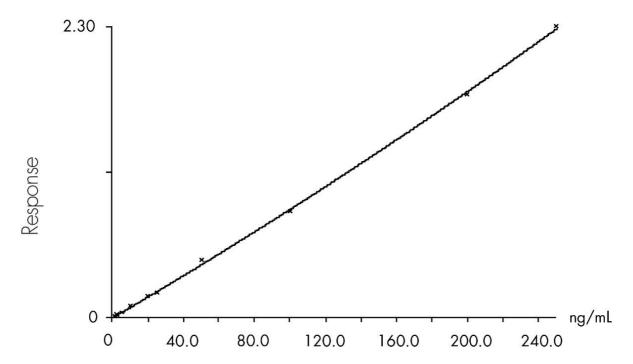
Compound name: Trimethoprim

Coefficient of Determination: 0.999608

Calibration curve: $3.83074e-6* x^2 + 0.00818910* x + 0.000423977$

Response type: Internal Std (Ref 1), Area* (IS Conc./IS Area)

Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x2, Axis trans: None



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