

Trimethoprim in Rat Plasma

Waters Corporation



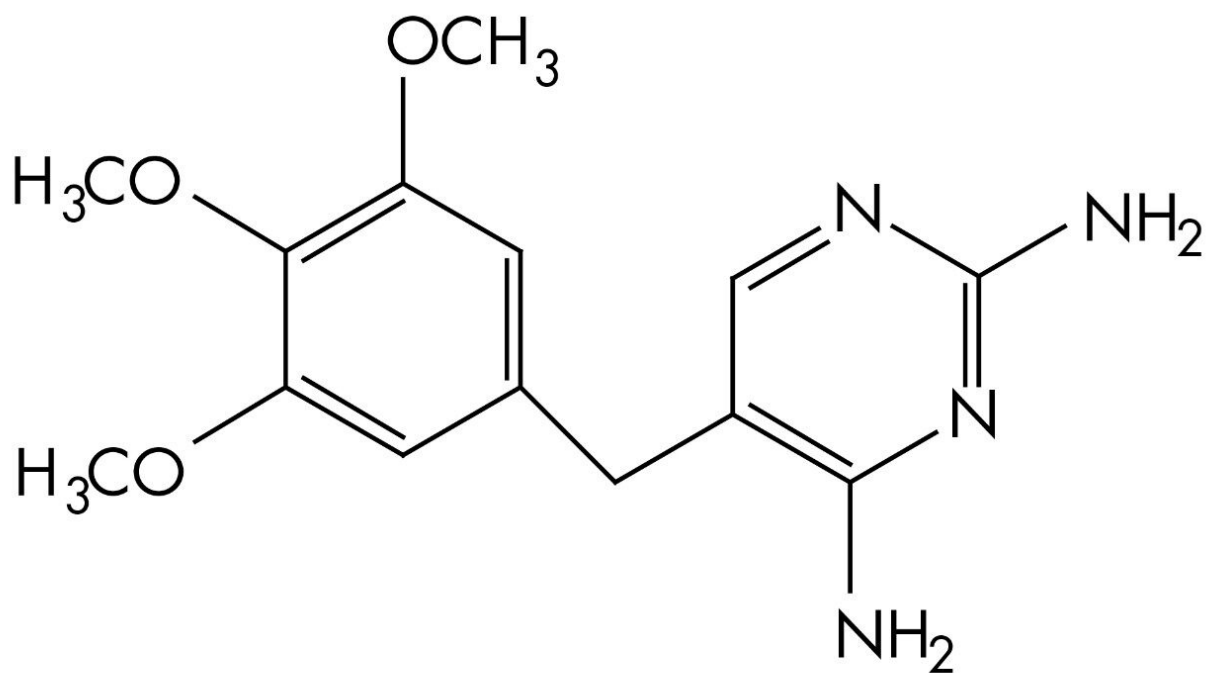
This is an Application Brief and does not contain a detailed Experimental section.

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 C₁₈ 2.1 x 30 mm, 3.5 μm

Part number:

186000398

Mobile phase A:	1.0% NH ₄ OH
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+
Source temperature:	150 °C
Gas cell:	1.5e ⁻³ mbar, 25 eV
Desolvation temperature:	350 °C
Cone gas flow:	150 L/hr
Drying gas flow:	600 L/hr
Cone voltage:	20V

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 µL H₃PO₄

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:

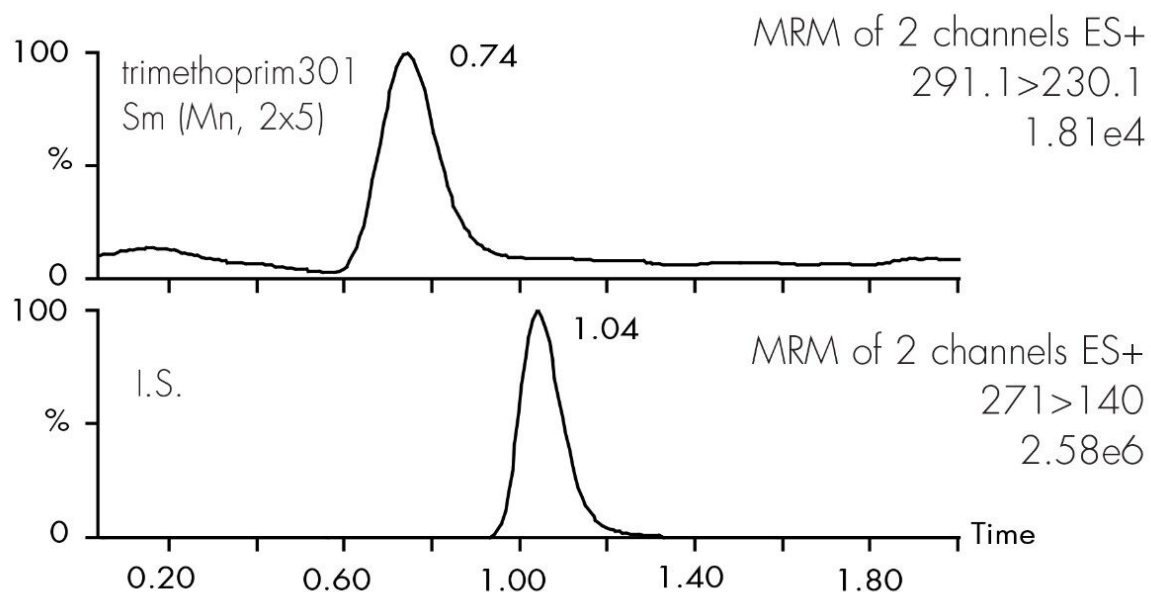
300 µL 5% NH₄OH in methanol

DILUTE:

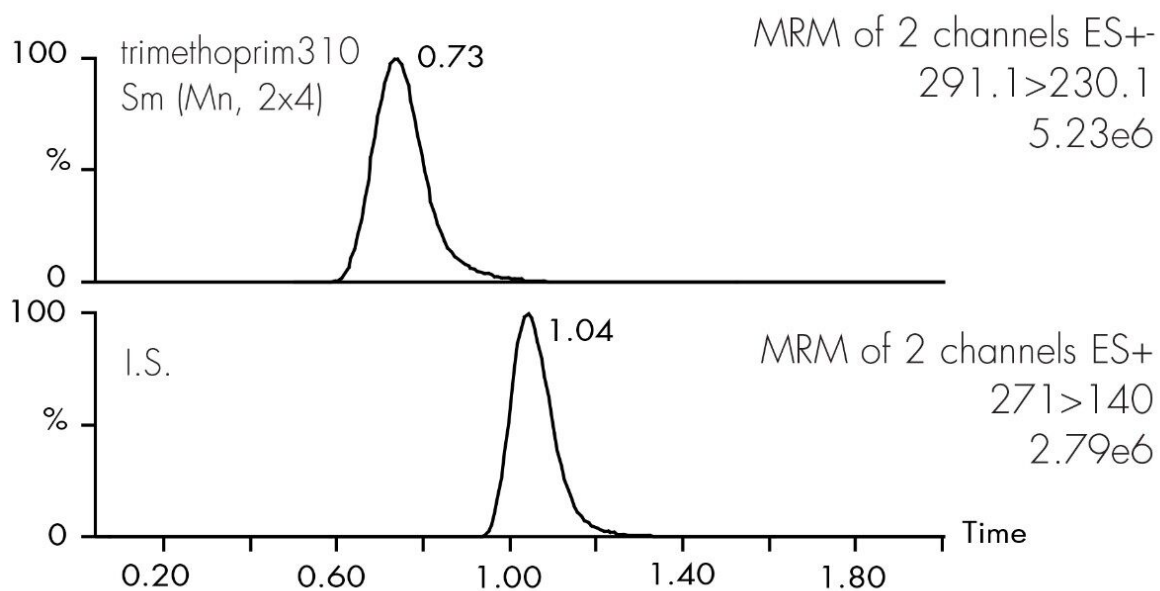
200 µL water

Results and Discussion

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



Spike 250 ng/mL, 60/40 ACN/H₂O 1.0 % NH₄OH



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

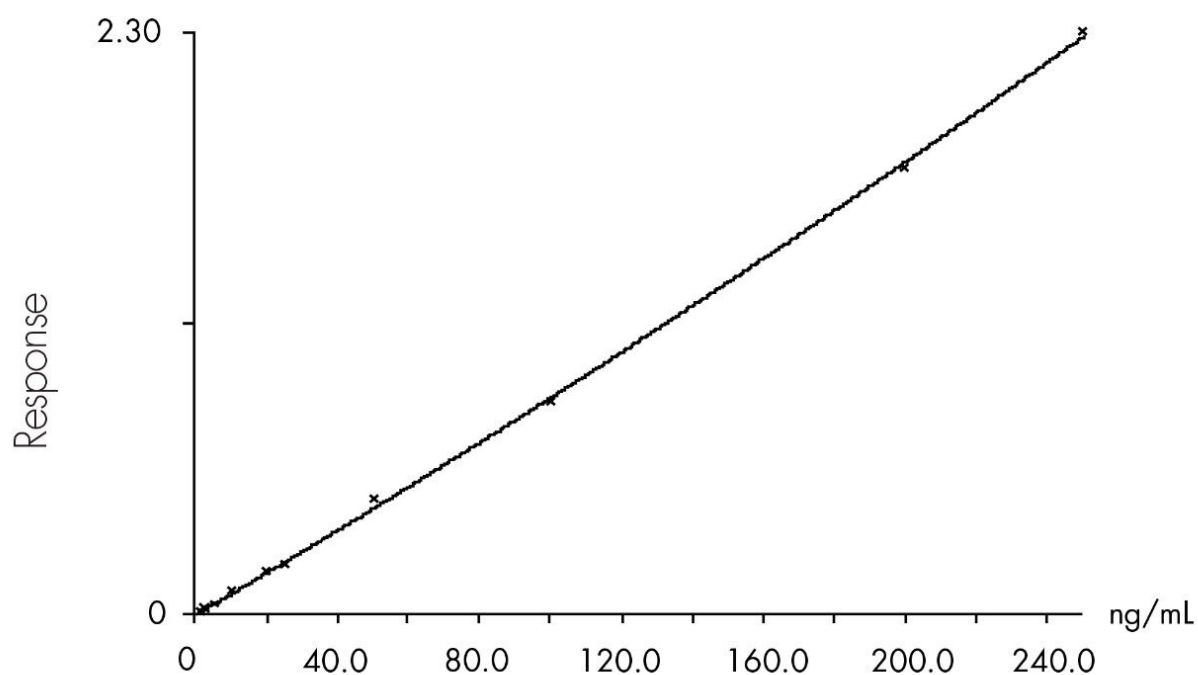
Compound name: Trimethoprim

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: $1/x^2$, Axis trans: None



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