

## Trimethoprim in Rat Plasma

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Waters Corporation



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

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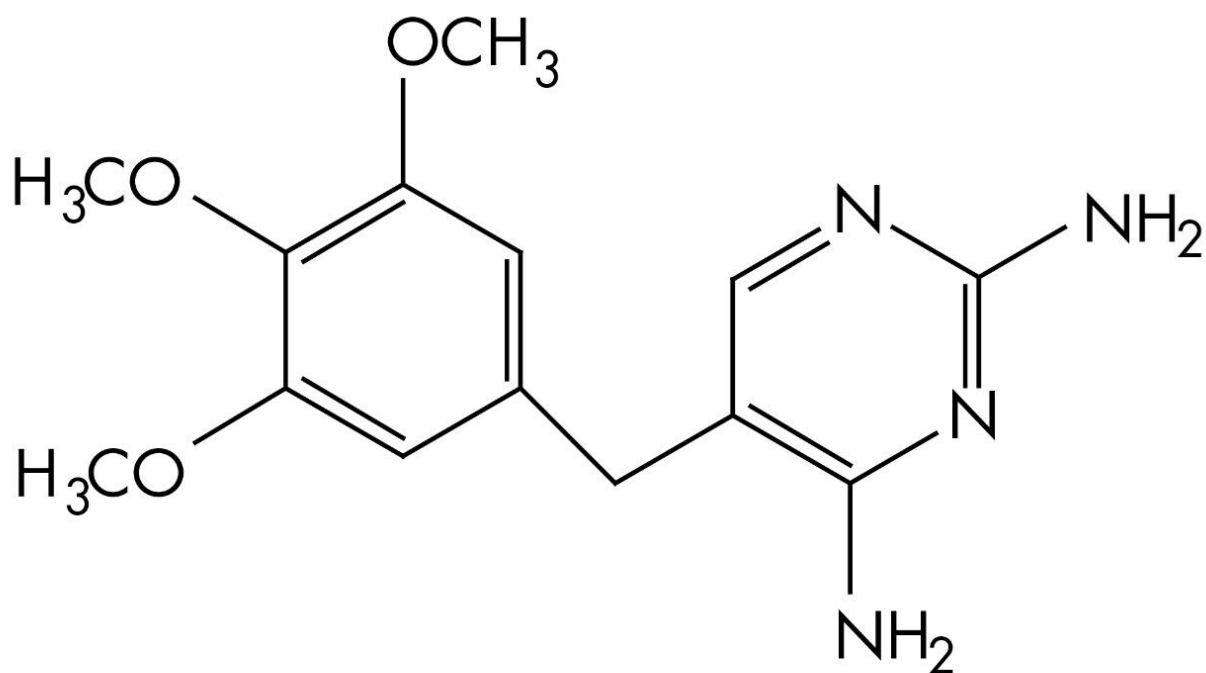
### Abstract

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

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## Introduction

The compound analyzed in this study is Trimethoprim.



# TRIMETHOPRIM

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## Experimental

### HPLC Method

Column:

XTerra MS C<sub>18</sub> 2.1 x 30 mm, 3.5 μm

Part number:	186000398
Mobile phase A:	1.0% NH <sub>4</sub> 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 <sup>-3</sup> 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 H<sub>3</sub>PO<sub>4</sub>

### **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% NH<sub>4</sub>OH in methanol

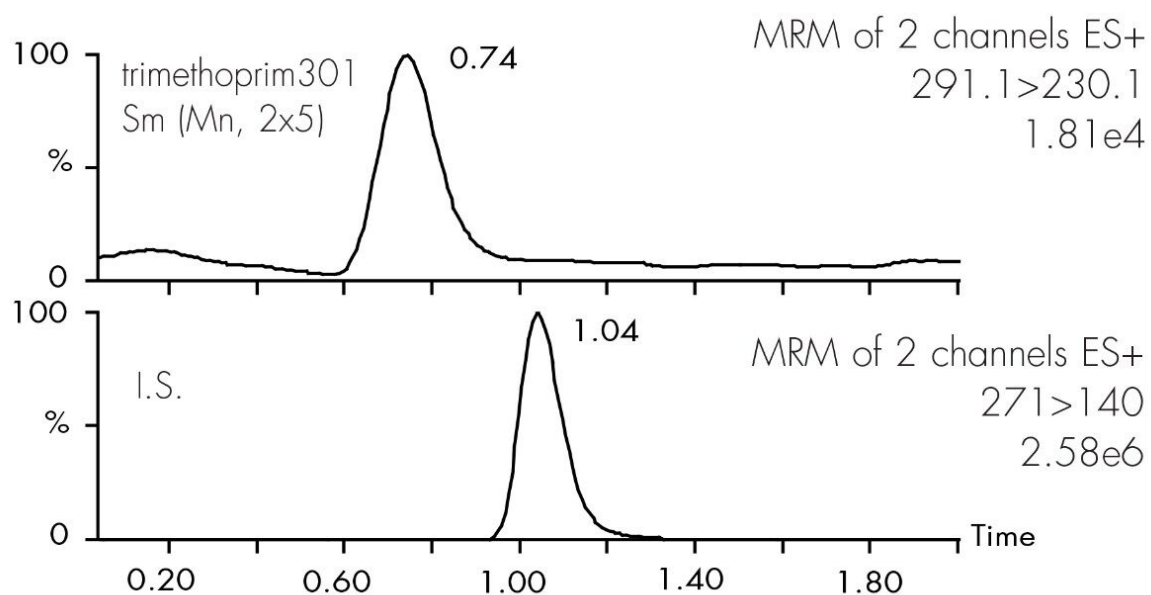
### **DILUTE:**

200 µL water

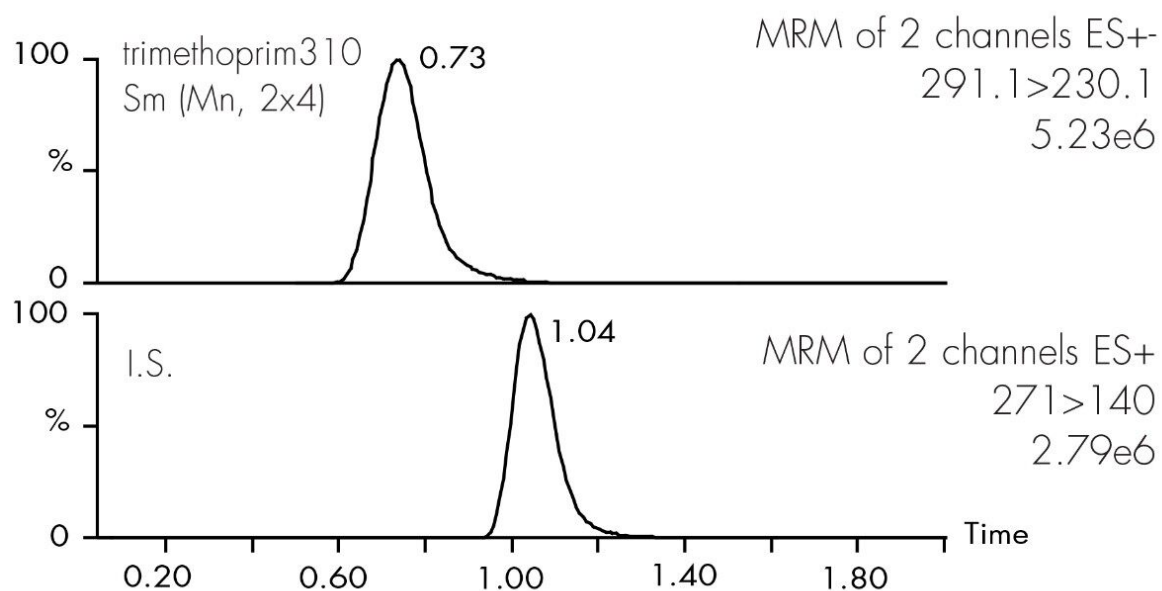
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Results and Discussion

Spike 1 ng/mL, 60/40 ACN/H<sub>2</sub>O 1.0 % NH<sub>4</sub>OH



Spike 250 ng/mL, 60/40 ACN/H<sub>2</sub>O 1.0 % NH<sub>4</sub>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

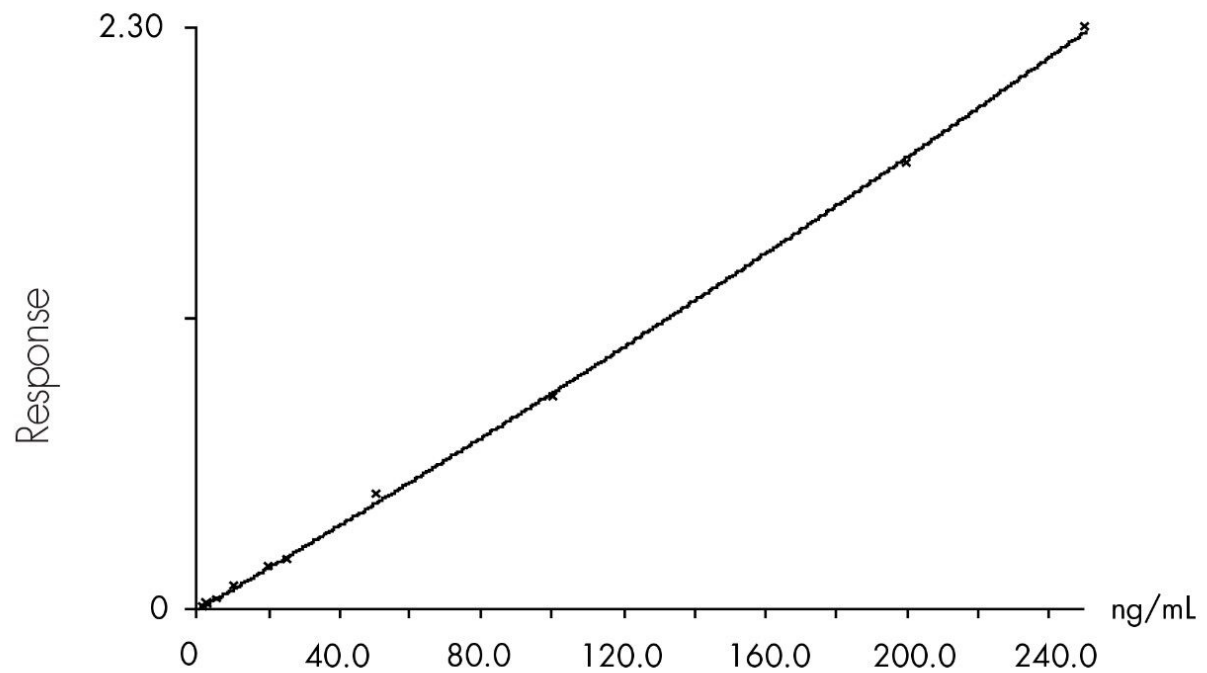
Compound name: Trimethoprim

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



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## Featured Products

WA31764.154, June 2003

