



## 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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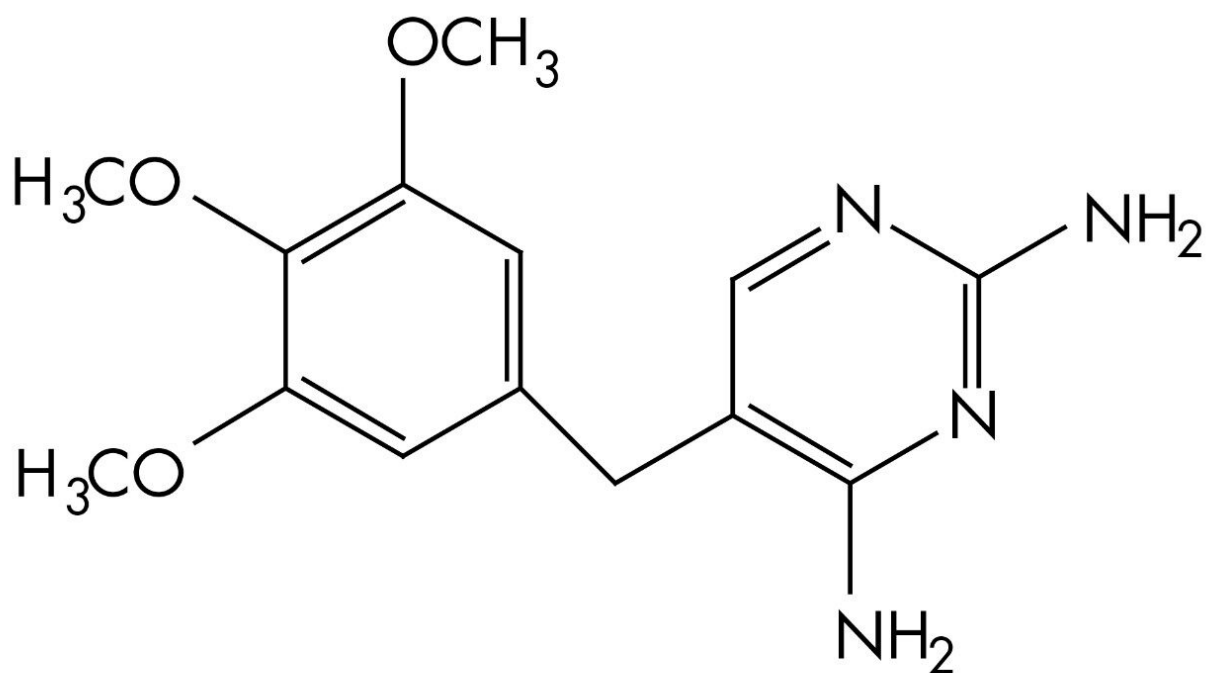
### Abstract

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

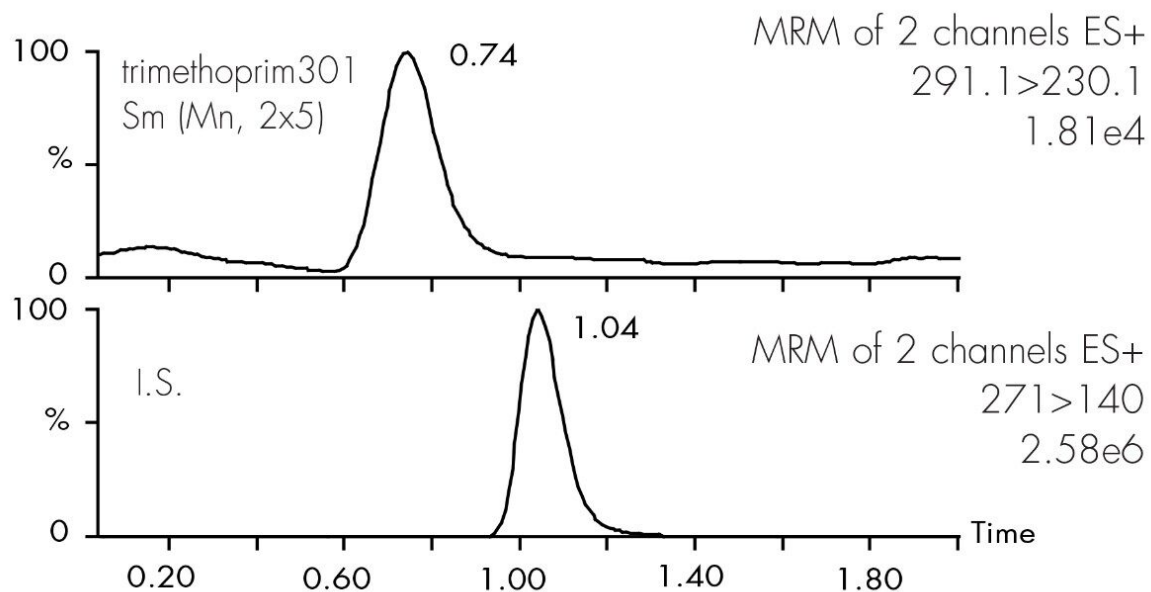
### **DILUTE:**

200 µL water

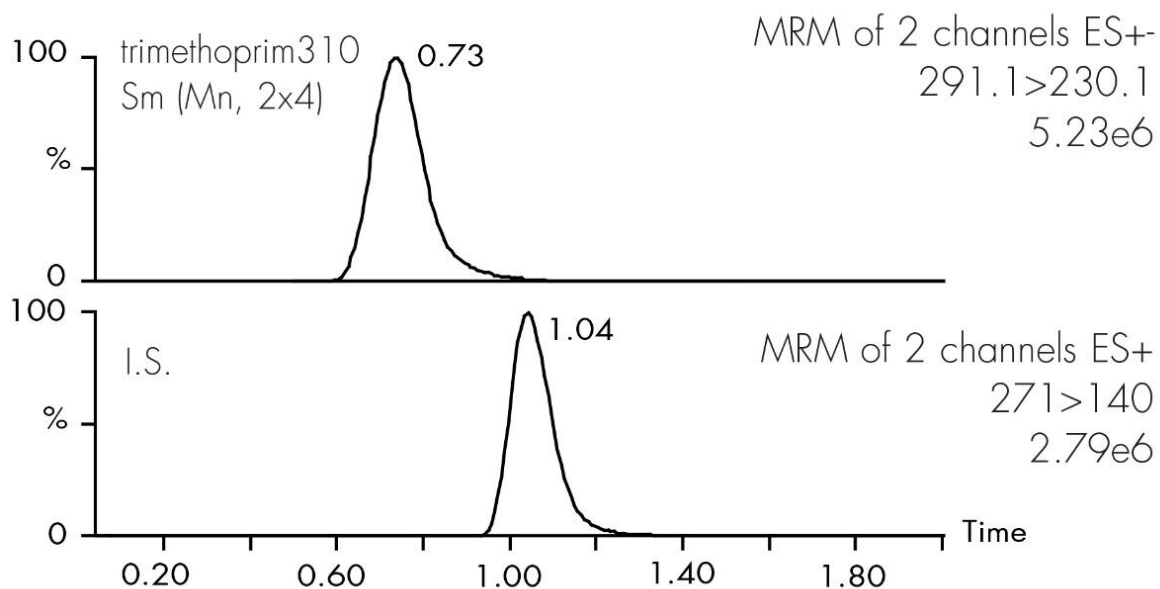
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Results and Discussion

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<br>(ng/mL) | Mean   | Standard<br>deviation | Coefficient of<br>variation (%) | Recovery<br>(%) |
|-------------------------|--------|-----------------------|---------------------------------|-----------------|
| 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              |

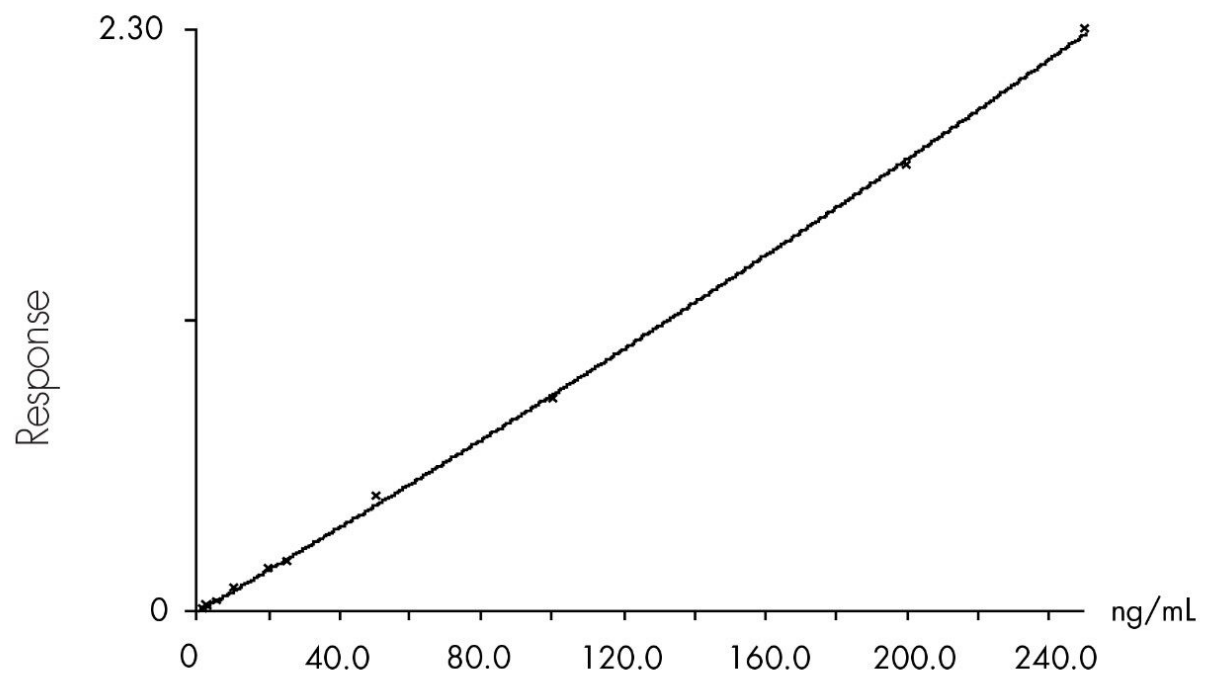
Compound name: Trimethoprim

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

