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Nota applicativa

# 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+ |
| 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 $H_3PO_4$

**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 µL 5% $NH_4OH$ in methanol

**DILUTE:**
200 µL water

## Results and Discussion

Spike 1 ng/mL, 60/40 ACN/$H_2O$ 1.0 % $NH_4OH$

Spike 250 ng/mL, 60/40 ACN/$H_2O$ 1.0 % $NH_4OH$

| 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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