

## Application Note

# Diclofenac in Rat Plasma by LC-MS/MS

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Waters Corporation

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

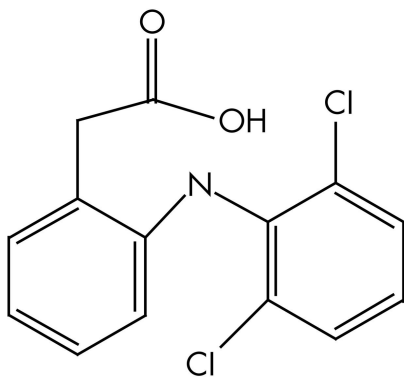
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## Abstract

This application brief highlights the analysis of diclofenac in plasma.

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## Introduction



DICLOFENAC

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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:	0.05% HCOOH
Mobile phase B:	ACN
Isocratic mobile phase composition:	60% A; 40% B
Flow rate:	0.2 mL/min
Injection volume:	50 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, 12 eV
Desolvation temperature:	350 °C
Cone gas flow:	150 L/hr

Drying gas flow: 600 L/hr

Cone voltage: 30 V

## OASIS® MAX EXTRACTION METHOD

Oasis® MAX Extraction Plate, 10 mg/96-well  
Part Number 186000375

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

### **ELUTE PLATE:**

300 µL 5% HCOOH in methanol

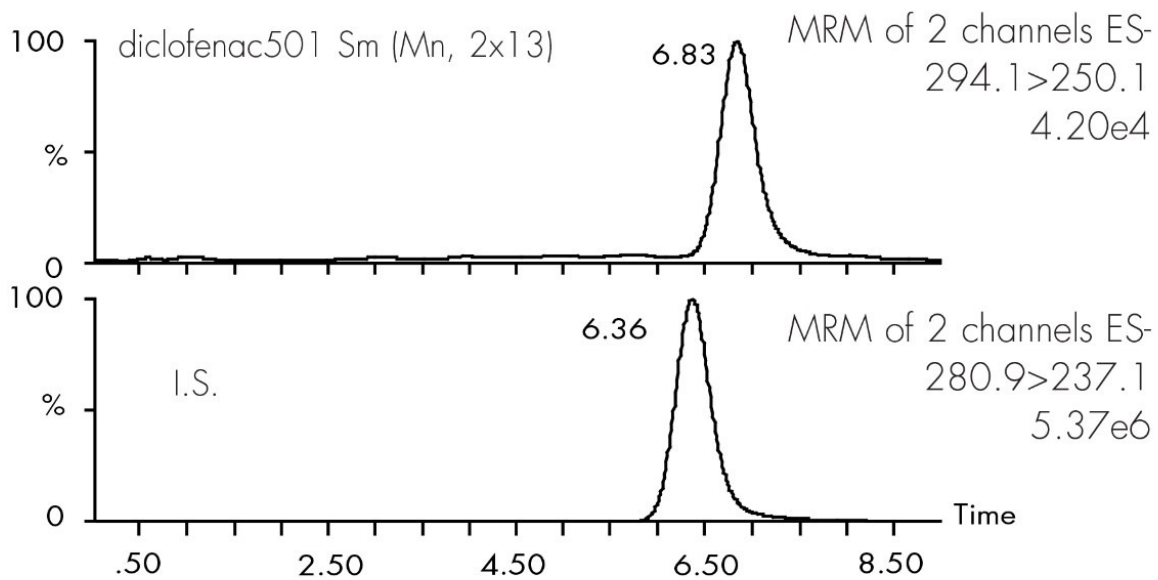
### **DILUTE:**

200 µL water

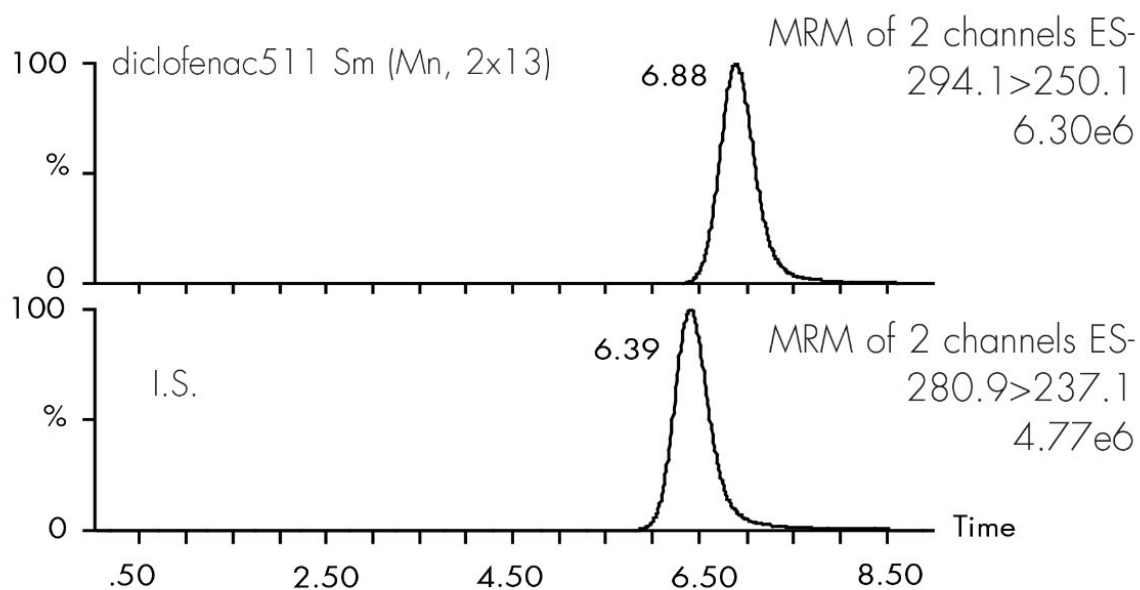
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## Results and Discussion

Spike 10 ng/mL, 40/60 ACN/H<sub>2</sub>O 0.05 % Formic Acid

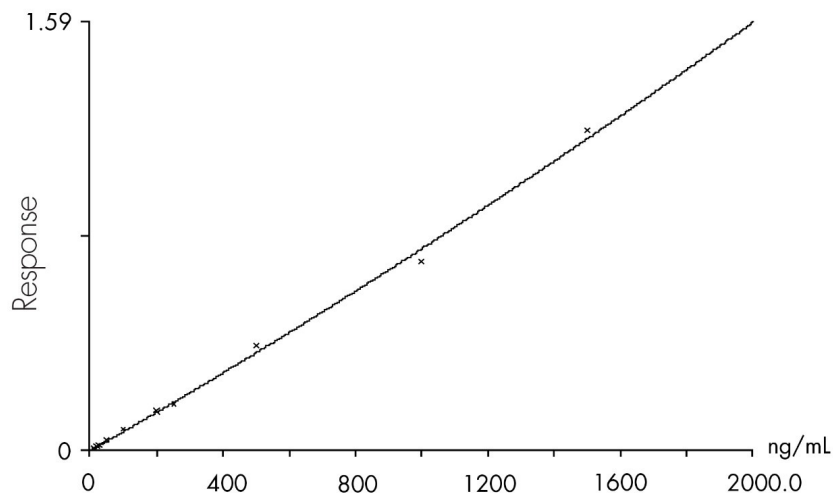


# Spike 2001 ng/mL, 40/60 ACN/H<sub>2</sub>O 0.05 % Formic Acid



DICLOFENAC (ng/mL)	mean	Standard deviation	Coefficient of variation (%)	Recovery (%)
10	10.26	0.3	3	102
25	24.06	0.83	3.5	96
100	101.68	2.69	2.7	101
250	250.05	8.13	3.3	100
500	496.41	27.09	5.5	99
1000	997.8	28.1	2.8	99
1500	1497.25	27.98	1.9	99

Compound name: Diclofenac  
Coefficient of Determination: 0.997049  
Calibration curve:  $4.68008 \times 10^{-8} x^2 + 0.000702987 x + 0.000144152$   
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

Alliance HPLC System <<https://www.waters.com/534293>>

WA31764.70, June 2003

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