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Diclofenac in Rat Plasma by LC-MS/MS

Waters Corporation

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

Abstract

This application brief highlights the analysis of diclofenac in plasma.

Introduction

DICLOFENAC

Experimental

HPLC Method

Column:	XTerra MS C ₁₈ 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		
lon source:	ESI		
Source temperature:	150 °C		
Gas cell:	1.5e ⁻³ 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 μ L H_3PO_4

CONDITION PLATE:

500 µL methanol followed with 500 µL water

LOAD PLATE:

500 µL spiked rat plasma

WASH PLATE:

 $500~\mu L~2~\%~NH_{\rm d}OH~in~water$

ELUTE PLATE:

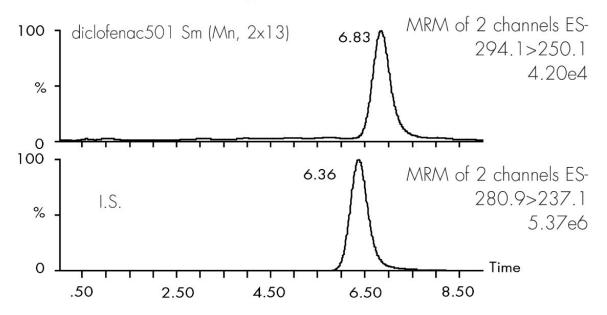
300 µL 5% HCOOH in methanol

DILUTE:

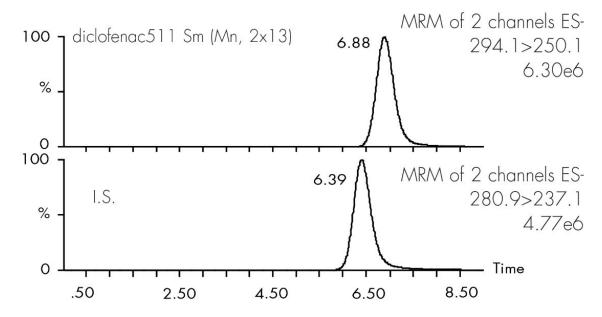
200 µL water

Results and Discussion

Spike 10 ng/mL, 40/60 ACN/ H_2 O 0.05 % Formic Acid



Spike 2001 ng/mL, 40/60 ACN/ H_2O 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

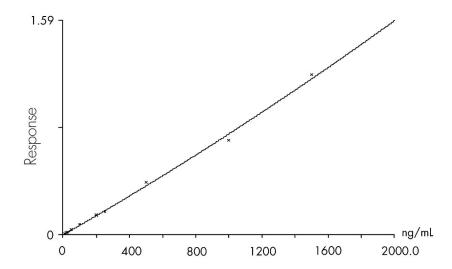
Compound name: Diclofenac

Coefficient of Determination: 0.997049

Calibration curve: $4.68008e-8*x^2 + 0.000702987*x+0.000144152$

Response type: Internal Std (Ref 1), Area* (IS Conc./IS Area)

Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x2, Axis trans: None



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

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