Application Note 20569

LC-MS/MS Method for the Determination of Diclofenac in Human Plasma

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Key Words
SPE, SOLA, Accucore RP-MS, diclofenac, Core Enhanced Technology, solid core

Abstract
A liquid chromatography-tandem mass spectrometry method for the determination of diclofenac in human plasma was developed. Using Thermo Scientific SOLA cartridges and plates, sample preparation was fast and efficient and gave excellent recovery levels for the compound. Separation was carried out using a Thermo Fisher Scientific Accucore RP-MS column with a cycle time of 1 minute. Good chromatographic peak shape and linearity over the dynamic range 1 to 1000 ng/mL was achieved.

Introduction

SOLA™ products revolutionize solid phase extraction (SPE). This first-in-class SPE product range introduces next generation, innovative technological advancements that give unparalleled performance compared to conventional SPE, phospholipid and protein precipitation products.

This includes:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA products have significant advantages for the analyst when processing compounds in complex matrices, particularly in high-throughput, bioanalytical and clinical laboratories where reduced failure rates, higher analysis speed and lower solvent requirements are critical. The increased performance gives higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development.

Accucore™ HPLC columns use Core Enhanced Technology™ to facilitate fast and highly efficient separations. The 2.6 µm diameter particles are not totally porous but have a solid core and a porous outer layer. The optimized phase bonding creates a series of high-coverage, robust phases. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of the Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Diclofenac is a non-steroidal anti-inflammatory drug that is used in the treatment of post operative pain, rheumatoid arthritis and the chronic pain regularly associated with cancer (Figure 1). Diclofenac works by blocking the action of cyclo-oxygenase that produces prostaglandins in response to injury, which subsequently results in pain and inflammation. Diclofenac is marketed under many brand names which include, Voltarol®, Diclomax SR® and Dicloflex®.

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The extraction of diclofenac from human plasma is demonstrated in this application.

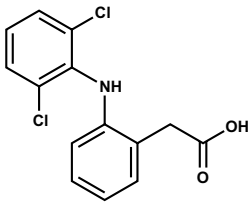

Figure 1: Diclofenac

Experimental Details

Consumables	Part Number
Fisher Scientific LCMS grade water	W/011217
Fisher Scientific LCMS grade methanol	M/4062/17
Fisher Scientific LCMS grade acetonitrile	A/0626/17
Fisher Scientific Analytical grade acetic acid	A/0360/25
Thermo Scientific National Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Sample Handling Equipment	Part Number
Thermo Scientific 96 well plate vacuum manifold	60103-351
Thermo Scientific UltraVap sample evaporator	CLS-229070

Sample Pretreatment

A standard spiking solution (diclofenac) was prepared in water. A working internal standard solution (diclofenac-d4) was also prepared in water. A 200 µL sample of blank human plasma was taken. For standards and quality control (QC) samples, 10 µL of standard spiking solution was added. For all other samples, 10 µL of water was added.

For standards and QCs, 10 µL of working internal standard solution was added; for blanks, 10 µL of water was added. Then, 200 µL of 70% formic acid in water was then added to the spiked samples and mixed well.

Sample Preparation		Part Number
Compound(s):	Diclofenac, Diclofenac–d4 (IS)	
Matrix:	Human plasma	
Cartridge/plate type:	Thermo Scientific SOLA 10 mg/1 mL	60309-001
Conditioning stage:	0.5 mL methanol then 0.5 mL water	
Application stage:	Apply all of the spiked sample	
Washing stage:	200 µL 90:10 (v/v) water / methanol	
Elution stage:	2 x 200 µL acetonitrile	
Additional stage:	Dry down under nitrogen at 40 °C and reconstitute in 100 µL 50:50 (v/v) water/ acetonitrile. Mix well.	

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Accela 600 UHPLC	
Column:	Accucore RP-MS 2.6 µm, 50 x 2.1 mm	17626-052130
Mobile phase A:	Water + 0.1% acetic acid	
Mobile phase B:	Acetonitrile + 0.1% acetic acid	
Gradient:	50%–100% B in 1 minute	
Flow rate:	0.6 mL/min	
Column temperature:	40 °C	
Injection details:	20 µL	
Injection wash solvent 1:	80:20 (v/v) water / acetonitrile	
Injection wash solvent 2:	45:45:10 (v/v/v) IPA / acetonitrile / acetone	

MS Conditions	
Instrumentation:	Thermo Scientific TSQ Vantage MS
Ionization conditions:	HESI
Polarity:	Negative
Spray voltage:	3000 V
Vaporizer temp:	300 °C
Sheath gas pressure:	65 arb
Aux gas pressure:	30 arb
Capillary temp:	210 °C
Collision pressure:	1.5 mTorr
Scan time:	0.05 s
Q1 (FWHM):	0.7
Q3 (FWHM):	0.7
Compound transitions:	Table 1

Compound	Diclofenac		Diclofenac-d_4
Parent (*m/z*)	250.1		254.1
Products (*m/z*)	178.0	214.0	217.0
Collision energy (V)	25	19	21
S-lens (V)	113	113	120

Table 1: Compound transition details

Data Processing	
Software:	Thermo Scientific LCQuan

Results

Extracted diclofenac standards from human plasma gave a linear calibration curve over the dynamic range of 1 to 1000 ng/mL with an r^2 coefficient of 0.999 (Figure 2 and Table 2). The chromatography of the LOQ at 1 ng/mL is shown in Figure 3. QC samples were run in replicates of six at a concentration of 15 ng/mL. The precision of the QC level was <4.4% CV (Table 3). Overspikes were run in triplicate at a concentration of 15 ng/mL and used to calculate the percentage recovery level for diclofenac of 85.8% (Table 4).

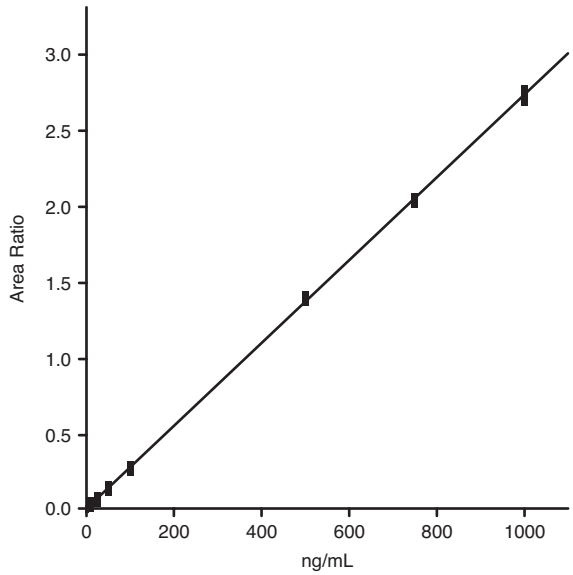

Figure 2: Diclofenac linearity over the dynamic range 1 to 1000 ng/mL

Standard	Specified Concentration (ng/mL)	Calculated Concentration	%Diff
S1	1	1.17	17
S1	1	0.906	-9
S2	10	9.36	-6
S3	25	24.8	-1
S4	50	49.7	-1
S5	100	99.0	-1
S6	500	509	2
S7	750	746	-1
S8	1000	993	-1
S8	1000	1000	0

Table 2: Accuracy data for eight extracted diclofenac standards over the linear range 1 to 1000 ng/mL

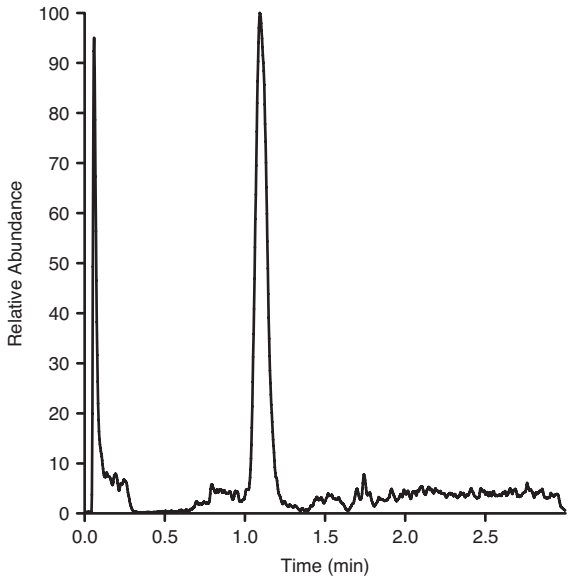

Figure 3: Representative chromatogram of diclofenac SRM, extracted from human plasma at 1 ng/mL

Standard	Concentration (ng/mL)	Average Calculated Concentration (n=6)	Average %Diff	Precision (%CV)
QCL	15	14.7	-2	4.4

Table 3: Average precision data for six replicate QCs for diclofenac

Standard	Response (n=3)	% Recovery
Average QCL area response	10629.1	86
Average overspike area response	12382.4	

Table 4: Recovery data for diclofenac

Conclusion

- SOLA SPE cartridges or plates in combination with Accucore RP-MS columns allow for a simple extraction and quantification of diclofenac from human plasma using an internal standard.
- An LOQ of 1 ng/mL for diclofenac in plasma was achieved.
- The extraction recovery was >86%.
- The method showed excellent precision with a %CV (n=6) <5%.

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