Determination of Pregabalin in Human Plasma by SPE-LC-MS/MS Using Thermo Scientific SOLA CX Mixed Mode Solid Phase Extraction Cartridges and a Thermo Scientific Accucore PFP HPLC Column

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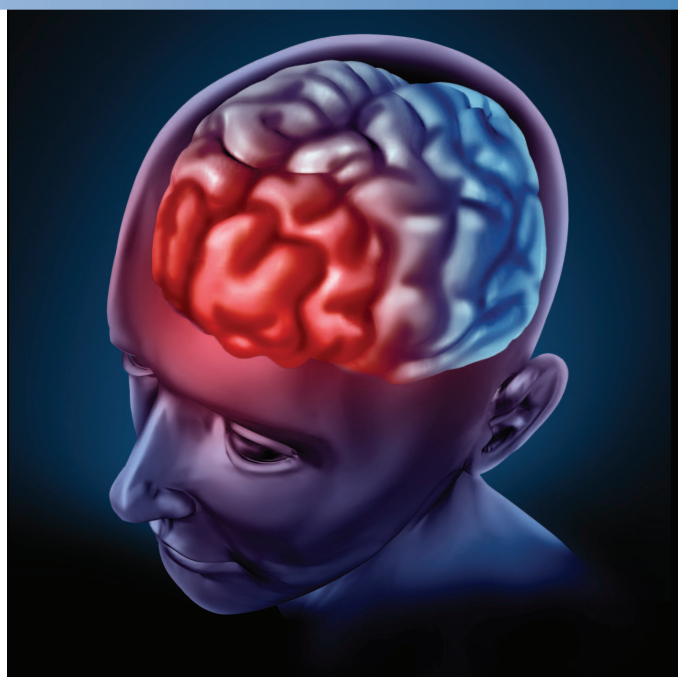
Key Words
SPE , SOLA CX, Accucore PFP, solid core, pregabalin, gabapentin

Abstract
A simple, rapid and sensitive procedure for the determination of pregabalin in human plasma by liquid chromatography-tandem mass spectrometry was developed and evaluated. The drug was isolated from a plasma matrix using SOLA CX solid phase extraction material, and the components of the resultant extracts were separated on an Accucore PFP HPLC column under reversed-phase, gradient conditions. Detection was performed on a triple quadrupole mass spectrometer using positive polarity, heated electrospray ionisation (HESI) conditions operating in selected reaction monitoring (SRM) mode.

Gabapentin was used as the internal standard. Good chromatographic peak shape and linearity over the dynamic range 1 to 250 ng/mL was achieved with excellent recovery and precision.



Introduction
SOLA™ is a revolutionary solid phase extraction (SPE) device. This first in class SPE product range introduces next generation, innovative technological advancements, giving unparalleled performance characteristics 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 has significant advantages for the analyst when processing compounds in complex matrices particularly in high throughput bioanalytical and clinical laboratories where reduced failure rate, higher analysis speed and lower solvent requirements are critical. SOLA's 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 high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The Accucore PFP phase provides extra retention for halogenated species and unique selectivity for non-halogenated compounds orthogonal selectivity to the C18 phase.

Pregabalin is used to relieve neuropathic pain (pain from damaged nerves) that can occur with diabetic patients or patients who are suffering from shingles (a painful rash that occurs after infection with the herpes zoster virus). It is also used to treat fibromyalgia (a long-lasting condition that may cause pain, muscle stiffness and tenderness, tiredness, and difficulty falling asleep or staying asleep). Pregabalin is used with other medications to treat certain types of seizures in people with epilepsy. Pregabalin is in a class of medications called anticonvulsants, and works by

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decreasing the number of pain signals that are sent out by damaged nerves in the body[1].

The purpose of this particular study is to demonstrate the effectiveness of a SOLA CX solid phase extraction material and an Accucore PFP HPLC column for the determination of pregabalin in human plasma by liquid chromatography-tandem mass spectrometry using gabapentin as an internal standard. Figure 1 shows the structures of pregabalin and gabapentin.

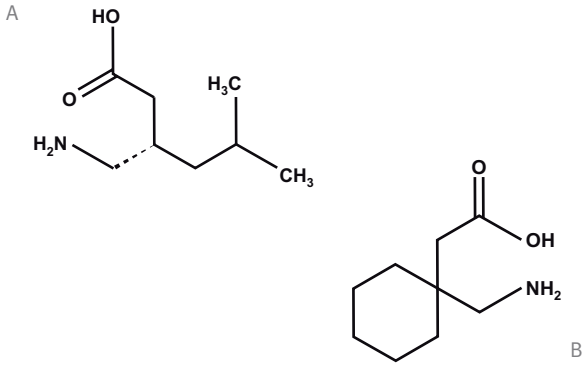

Figure 1: Structures of pregabalin A. and gabapentin (IS) B.

Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific Optima LC/MS grade methanol	A456-1
Water, from TKA Water Purification System	
Formic acid	
Pregabalin from Ind-Swift	
Gabapentin from sigma	
Human plasma with CPD	

Sample Handling Equipment	Part Number
Thermo Scientific FinnPipette (100-1000 µL)	642090
Thermo Scientific FinnPipette (10-100 µL)	4642070
Thermo Scientific FinnPipette (1-10 µL)	4642040

Vials and Closures	Part Number
Thermo Scientific Micro+™ Vial 300 µL, Fused Insert	60180-507
Thermo Scientific 9mm Screw Top Cap W/ PTFE/Silicone septa	60180-516

Solid Phase Extraction	Part Number
Thermo Scientific SOLA CX 10 mg/1 mL cartridge	60109-002

Preparation of samples:

Calibration standards:

A stock solution of pregabalin was prepared in 50:50 (v/v) methanol / water at a concentration of 1 mg/mL. Secondary standards (SS1 – SS9) were prepared by subsequent serial dilution of the pregabalin stock solution in 50:50:0.1 (v/v) methanol / water / formic acid.
A stock solution of the internal standard, gabapentin was prepared in 50:50 (v/v) methanol / water at a concentration of 0.1 mg/mL. Further dilutions, were prepared in 50:50:0.1 (v/v) methanol / water / formic acid.
Plasma spiked calibration standards of pregabalin were prepared at nine different concentration levels (1, 2, 5, 25, 75, 125, 175, 225 and 250 ng/mL) by fortification of plasma (285 µL) with 15 µL of appropriate stock standard. The internal standard (gabapentin) was added (30 µL) at the 100 ng/mL level into each of the calibrants. Standards (S1-S9) were prepared and S1 and S9 were in duplicate. The concentration range selected was determined by targeting a lower limit of quantification as 1 ng/mL (< 1% of C_{max}, 2.15 µg/mL)[2].
Further samples were prepared to allow for calculation of precision, recovery and matrix effect. A mid-range concentration was prepared six times and subjected to the same extraction procedure to determine

	precision. An extracted blank plasma sample was over spiked at a mid-range concentration and compared with the same concentration of extracted spiked plasma to calculate recovery. Matrix effects were determined by comparing the overspiked blank extract with that of the same concentration of an unextracted standard.

Extraction procedure for pregabalin using SOLA CX		**Part Number**
The extraction was carried out using a positive pressure SPE manifold		60104-236
Conditioning stage:	0.5 mL methanol	
Equilibration stage:	0.5 mL water + 1.0% formic acid	
Load:	500 µL aliquot (of 15 µL drug + 285 µL plasma + 30 µL IS + 270 µL 2.0% formic acid in water)	
Wash1:	0.5 mL of water + 1.0% formic acid	
Wash2:	0.5 mL methanol + 1.0% formic acid	
Elute:	0.5 mL methanol + 2.0% ammonium hydroxide solution	
	The eluents were evaporated to dryness under a stream of nitrogen at 40 °C and reconstituted in 50:50:0.1 (v/v) water/ methanol/formic acid (250 µL).	

Separation Conditions		**Part Number**
Instrumentation:	Separation was carried out using a Thermo Scientific Accela 1250 pump interfaced to both a Thermo Scientific Accela Open Autosampler, and a Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.	
Column:	Thermo Scientific Accucore PFP 2.6 µm, 50 mm x 2.1 mm	17426-052130
Mobile Phase A:	Water + 0.1% formic acid	
Mobile Phase B:	Methanol + 0.1% formic acid	
Gradient:	Time (min)	% B
	0	20
	0.2	20
	1.0	60
	3.0	60
	4.0	90
	4.2	20
	5.0	20
Flow rate:	0.4 mL/min	
Column temperature:	Ambient	
Detection:	MS	
Injection volume:	10 µL	
Syringe volume:	100 µL	
Loop Size:	20 µL	
Syringe flush:	Wash1: 80:20(v/v) water/methanol	
	Wash2: 50:25:25:0.1 (v/v) methanol/acetonitrile/water/formic acid	
Cool Stack temperature:	10°C	
Detection:	MS	
Column backpressure:	205 bar	
Run time:	5.0 minutes	

Mass Spectrometry Conditions

Instrumentation:	Thermo Scientific TSQ Vantage

Ionisation parameters	
Ion Source Type	HESI-2
Polarity	Positive
Spray voltage	3500 V
Vaporizer Temperature (°C)	150
Sheath Gas Pressure (Arb)	50
Ion Sweep Gas Pressure (Arb)	0
Auxiliary Gas Pressure (Arb)	20
Capillary Temperature (°C)	250
Declustering Voltage	0 V
Collision pressure (mTorr)	1.5

Table 1: TSQ Vantage Ionisation Parameters

MS Acquisition Parameters

Quantification was performed by selected reaction monitoring (SRM) using the precursor-to-product combinations shown below:

Compound	Precursor m/z	Product m/z	Collision energy	S-Lens
Pregabalin	160.1	55.1	22	47
Gabapentin	172.1	95.1	22	73

Table 2: TSQ Vantage Acquisition Parameters

Scan type:	SRM
Peak width:	Q1 - 0.7 (FWHM) Q3 - 0.7 (FWHM)
Scan width:	0.02 m/z
Scan time:	0.1 s

Divert Valve

	Divert time (min)	State
	0.00	Inject/waste
	1.40	Load/detector
	3.40	Inject/waste
MS acquisition time:	5.0 minutes	

Data Processing

All data were processed using Thermo Scientific LCQuan (v. 2.6) software. Algorithm for integration - ICIS

Results and Discussion

The retention times of pregabalin and gabapentin are 2.26 and 2.17minutes respectively

Linearity

A graphical plot of relative response (A_{STD}/A_{ISTD}) as a function of the concentration of pregabalin is shown in Figure 2, with the calibration data summarised in Table 3.

A typical chromatogram of pregabalin at the LLOQ (1ng/mL, S/N ratio =198) is shown in Figure 3.

The analytical response was found to be linear (using a 1/x weighted regression algorithm) with a coefficient of determination (r^2) of 0.999 in the range 1 - 250 ng/mL.

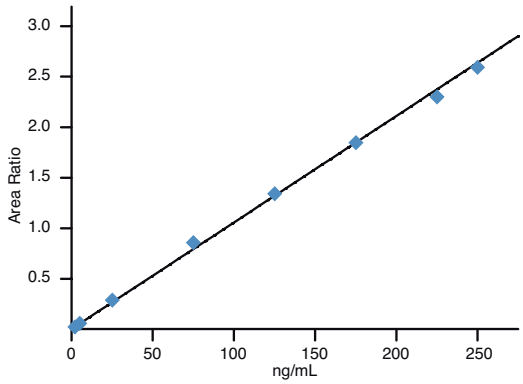

Figure 2: Linearity of response over the dynamic range 1 – 250 ng/mL

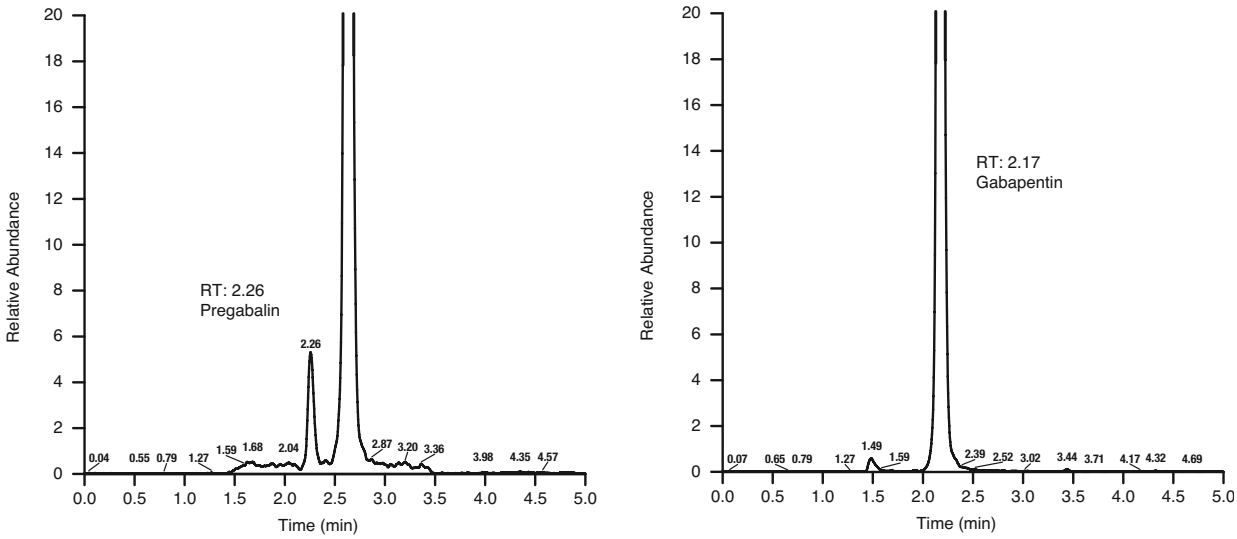

Figure 3: Representative chromatogram of pregabalin SRM, extracted from human plasma at 1.0 ng / mL (left trace) with gabapentin IS (right trace)

Standard Ref.	Nominal [pregabalin], ng/mL	Calculated [pregabalin], ng/mL	Relative error %
S1	1.0	0.9	-12.0
S2	2.0	1.9	-6.1
S3	5.0	5.2	4.4
S4	25.0	27.0	8.0
S5	75.0	81.4	8.5
S6	125.0	127.3	1.8
S7	175.0	175.1	0.1
S8	225.0	218.2	-3.0
S9	250.0	246.0	-1.6

Table 3: Linearity of response for the determination of pregabalin in human plasma

Precision

For the mid range concentration (75 ng/mL) extracted plasma samples, precision was excellent, data summarised in Table 4.

Nominal Concentration (ng/mL)	No. of samples (N)	%CV	% difference
75.0	6	2.78	-0.14 to 6.40

Table 4: Precision results for the determination of pregabalin in human plasma

Recovery

Recovery was calculated to be 91.4% at a mid range concentration (75 ng/mL).

Matrix interference was also determined. The observed value was -7.0%.

Specificity and sensitivity

SRM chromatograms derived from the examination of the extracted blank plasma and extracted spiked plasma samples are shown in Figures 3 and 4. It is evident that the unfortified plasma sample contains an endogeous peak (Tr = 2.63 minutes). However, under the adopted chromatographic conditions, the separation is sufficient to prevent any overlap of the response from this endogenous plasma species upon the principal analytical response (Tr = 2.26 minutes).

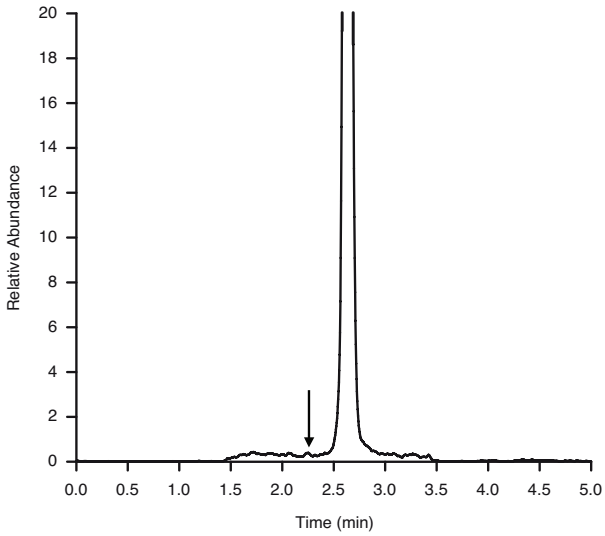

Figure 4: Representative chromatogram of a blank, extracted plasma sample

Conclusion

An analytical procedure based upon SPE-LC-MS/MS for the determination of pregabalin in human plasma was successfully developed and evaluated.

The procedure was found to exhibit good linearity (r^2 = 0.999) for concentrations of pregabalin in the range 1 - 250 ng/mL. The accuracy and precision were found to be excellent, and well within the limits of acceptance specified by the FDA[3]. The level of analyte recovery (91.4%), repeatability (% RSD = 2.78) and matrix effect (-7.0%) were found to be acceptable, demonstrating excellent recovery and minimal matrix effect. The performance characteristics of the method combined with its simplicity and rapidity mean that it can be adopted routinely in bioanalytical environments.

References

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