Application Note: ANCCSSOLARSTA

Determination of Rosuvastatin in Human Plasma by SPE-LC-MS/MS using SOLA and Accucore RP-MS column.

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Key Words

- Rosuvastatin
- SOLA Cartridges and Plates
- Solid Phase Extraction (SPE)
- Accucore RP-MS
- Tandem Mass Spectrometry
- Solid Core

Abstract

A simple, rapid and sensitive procedure for the determination of rosuvastatin in human plasma by liquid chromatography-tandem mass spectrometry was developed and evaluated. The drug was isolated from within the plasma matrix using a Thermo Scientific SOLA 96 well plate, and the components of the resultant extracts were separated on a Thermo Scientific Accucore RP-MS column under reversed-phase, gradient conditions. Detection was performed on a triple quadrupole mass spectrometer under positive polarity, heated electrospray ionisation (HESI) conditions operating in selected reaction monitoring (SRM) mode.

The analytical procedure is both accurate and precise, and is characterized by high levels of recovery and an absence of any significant matrix interfering effects.

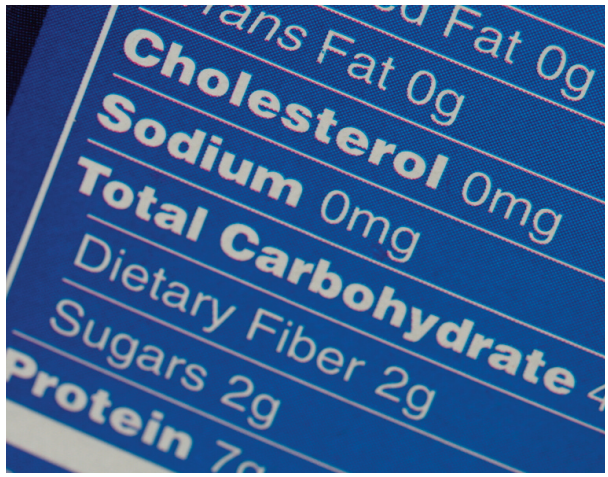
Introduction

Rosuvastatin [(3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulphonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid] is a synthetic, orally-administered member of the 'statin' class of cholesterol-lowering drugs. This particular statin is marketed by Astra Zeneca as 'Crestor'. Employed as an adjunct to dietary modification, the drug is used to treat primary hypercholesterolaemia, mixed dyslipidaemia and hypertriglyceridaemia in an attempt to reduce the risk of atherosclerosis and poor cardiovascular health.

In terms of the mechanism of its action, rosuvastatin is a selective and competitive inhibitor of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This particular enzyme catalyses the conversion of HMG-CoA to mevalonate, a precursor of cholesterol.

Accucore™ columns are packed with silica particles that have been manufactured using a core enhanced technology. These particles comprise a solid core upon which a porous layer is deposited. This configuration minimizes diffusional (mass transfer) pathways and permits the use of shorter columns and higher flow rates to achieve remarkably fast, highly efficient and highly resolved separations. In contrast to totally porous sub-2 µm particles, columns containing the solid core particles generate a competitive number of theoretical plates, but, with greatly reduced backpressures.

Accucore RP-MS columns employ an alkyl chain of an optimized length in order to provide a more effective coverage of the silica surface. The immobilised phase exhibits a slightly lower retentivity than that offered by traditional C18 phases, but, the resultant high efficiencies



and excellent peak symmetries make this the phase of choice for those chromatographic procedures which demand detection by mass spectrometry.

SOLA™ is a revolutionary new solid phase extraction (SPE) product range. This first in class SPE product range introduces next-generation, innovative, technological advancements which offer unparalleled performance in comparison to conventional SPE and products intended for the precipitation of proteins and phospholipids. The benefits of using SOLA products include: (1) higher levels of reproducibility, (2) improvements in the cleanliness of extracts, (3) reduction in requirements for both sample and solvent, and, (4) increases in procedural sensitivity.

SOLA products have significant advantages for the analyst when isolating compounds from complex matrices, particularly in high throughput bioanalytical and clinical laboratories where higher speed of analysis and the ability to use smaller quantities of sample and solvent are critical. The increased performance of SOLA products gives greater confidence in the accuracy of analytical results and lowers cost without compromising ease of use or requiring complex method development.

A number of researchers has reported the measurement of rosuvastatin in human plasma and pharmaceutical formulations [1,2]. Typical chromatographic approaches include separation using C18 phases (via hydrophobic interactions) and retention of the ionized molecule via an ion-exchange mechanism.

The purpose of this particular study is to demonstrate the effectiveness of a combination of SOLA products (reversed phase) solid phase extraction material and an Accucore RP-MS column for the determination of rosuvastatin in human plasma by liquid chromatography-tandem mass spectrometry.

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Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific Water (LC-MS grade)	W/0112/17
Fisher Scientific Methanol (LC-MS grade)	M/4062/17
Fisher Scientific Formic acid ('Optima', 90 %, LC-MS grade)	A117-50
Fisher Scientific Propan-2-ol (HPLC grade, 99.5+ %)	P/7507/17
Fisher Scientific Acetonitrile (LC-MS grade)	A/0638/17
Human plasma (Supplied by Seralab, lithium heparin as anticoagulant)	
Fisher Scientific Acetone (AR grade, 99.8+ %)	A/0600/15

Sample Handling Equipment	Part Number
Fisher Scientific Finnpipette F2 pipettor kit 10 µL - 100 µL, 100 µL - 1000 µL, 1 mL – 10 mL	PMP-020-220F
Fisher Scientific Finntip pipette tips, 10 µL	PMP-107-110W
Fisher Scientific Finntip pipette tips, 200 µL	PMP-107-600F
Fisher Scientific Finntip pipette tips, 1000 µL	PMP-103-206K
Fisher Scientific Finntip pipette tips, 10 mL	PMP-107-040R
Thermo Scientific borosilicate glass vials (2 mL, 12 mm x 32 mm) with 8 mm black screw cap fitted with a silicone/PTFE seal	60180-600

Solid Phase Extraction Hardware

Thermo Scientific positive pressure SPE manifold (capable of processing 96-well microplates).
Solvent evaporation system (capable of processing 96-well microplates).

SPE Apparatus	Part Number
SOLA 96-well plate (10 mg/2 mL)	60309-001

Calibration Standards

A primary standard of rosuvastatin was prepared in 1:4 (v/v) MeOH-water at a concentration of 10^5 ng/mL. Secondary standards (SS1 – SS8) were prepared by subsequent serial dilution of the primary standard in 1:4 (v/v) MeOH-water.

A primary standard of d_6-rosuvastatin, to be used as internal standard, was prepared in 1:4 (v/v) MeOH/water at a concentration of 5000 ng/mL.

Calibration standards of rosuvastatin were prepared at eight different levels of concentration (i.e., 1, 2, 10, 50, 250, 500, 900 and 1000 ng/mL) by fortification of the plasma matrix (100 µL) with a measured quantity (10 µL) of appropriate stock standard. The internal standard (d_6-rosuvastatin) was added at the 500 ng/mL level into each of the calibrants.

Standards S1 and S8 were prepared in duplicate whilst the remaining standards (S2 – S7) were prepared in singlet.

Quality Control (QC) Standards

QC standards of rosuvastatin were prepared in replicate (n = 6) at three levels of concentration (i.e., 3, 400 and 750 ng/mL) by fortification of the plasma matrix (100 µL) with a measured quantity (10 µL) of appropriate spiking solution. The internal standard (d_6-rosuvastatin) was added at 500 ng/mL.

QC Standards for Post-Extraction Fortification (i.e., 'overspiking')

Unfortified plasma samples (n = 9) were extracted in triplicate and fortified post extraction at three levels of concentration, i.e., 1, 400 and 750 ng/mL.

Reference (unextracted) Standards

Reference standards (which were not subject to extraction) were prepared in replicate (n=6) at three different levels of concentration by the introduction of a measured quantity (10 µl) of appropriate spiking solution ($[Ros.]_{QCLLOQ}$ = 10, $[Ros.]_{QCMED}$ = 4000, $[Ros.]_{QCHIGH}$ = 7500 ng/mL) and internal standard (d_6-rosuvastatin, 10 µL, $[d_6\text{-Ros.}] = 10^4$ ng/mL) into 1:4 (v/v) MeOH-water (180 µl).

Blank Samples

Double blank samples were prepared in duplicate by the introduction of water (20 µL) into plasma (100 µL). These were unfortified with respect to both rosuvastatin and its deuterated analogue.

Zero Blank Sample

The 'zero blank' comprised plasma (100 µL) spiked with internal standard only.

Isolation of Rosuvastatin using SOLA (Extraction Procedure)

The extraction was carried out using a positive pressure SPE manifold capable of processing 96-well microplates.

- Condition SOLA with MeOH (1.0 mL at 1.0 mL/minute)
- Condition SOLA with water (1.0 mL at 1.0 mL/minute)
- Load sample (100 µL) and allow to permeate SOLA material under gravity
- Wash with 0.1 % (v/v) formic acid (500 µL at 1.0 mL/minute)
- Wash with 10 % (v/v) MeOH (500 µL at 1.0 mL/minute)
- Elute with 90 % (v/v) MeOH (2 x 200 µL at 1.0 mL/minute)

The methanolic extracts were evaporated to dryness under a stream of nitrogen at 40 °C and reconstituted in 1:4 (v/v) MeOH-water (200 µL).

Instrumentation

Separation was carried out using a Thermo Scientific Accela 600 pump interfaced to both a Thermo Scientific Accela Open Autosampler, and, a Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

Chromatographic Conditions		Part Number
Column:	Thermo Scientific Accucore RP-MS 2.6 µm, 50 x 2.1 mm	17626-052130
Guard column:	Accucore Defender (RP-MS) guard column 2.6 µm, 10 x 2.1 mm	17626-012105
Uniguard direct connection guard cartridge holder (2.1 mm I.D)		852-00
Mobile phase:		
(A)	Water + formic acid at 0.1 % (v/v)	
(B)	MeOH + formic acid at 0.1 % (v/v)	
Gradient:		
	Time (min)	% B
	0	5
	0.1	5
	1.0	95
	1.60	95
	1.62	5
	3.60	5
Flow rate:	0.75 mL/min	
Column temperature:	60 °C	
Detection:	MS	
Injection volume:	15 µL	
Syringe volume:	100 µL	
Syringe flush:	Strong solvent - 9:9:2 (v/v) MeCN/^{i}PrOH/acetone Weak solvent - 1:4 (v/v) MeCN-water Autosampler temperature - 10 °C	
Run time:	3.60 minutes	

Mass spectrometric conditions

Ionisation parameters:

Heated electrospray operating in positive polarity mode (HESI-2 probe)	
Spray voltage:	3000 V
Vaporiser temperature:	475 °C
Sheath gas pressure:	65
Ion sweep gas pressure:	0.5
Capillary temperature:	300 °C
Declustering voltage:	0 V
Collision pressure:	1.5 mTorr

MS acquisition parameters:

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

Compound	[M+1] m/z	Product m/z	Collision energy	S-Lens
Rosuvastatin	482.30	258.15	30	124
d_6-rosuvastatin	488.22	264.17	31	124

Scan type:	SRM
Peak width:	Q1 - 0.7 (FWHM)
	Q3 - 0.7 (FWHM)
Scan width:	0.2 m/z
Scan time:	0.2 s
Divert valve:	

Divert time (min)	State
0.00	Inject/waste
0.78	Load/detector
2.00	Inject/waste

MS acquisition time: 3.60 minutes

Data Processing

All data were processed using Thermo Scientific LCQuan (v. 2.6) software.

Algorithm for integration - ICIS

Results

Linearity of response

The relationship between analytical response and concentration was investigated over the range 1-1000 ng/ mL.

A calibration line was constructed using data derived from extracted matrix-fortified samples at eight levels of concentration (excluding blanks).

Calibration lines were run at the beginning and end of the analytical sequence with the bottom and top standards being extracted in duplicate in both lines. All data were used to assess the degree of linearity.

A graphical plot of relative response (A_{STD}/A_{ISTD}) as a function of the concentration of rosuvastatin is shown in Figure 1. Calibration data are summarised in Table 1.

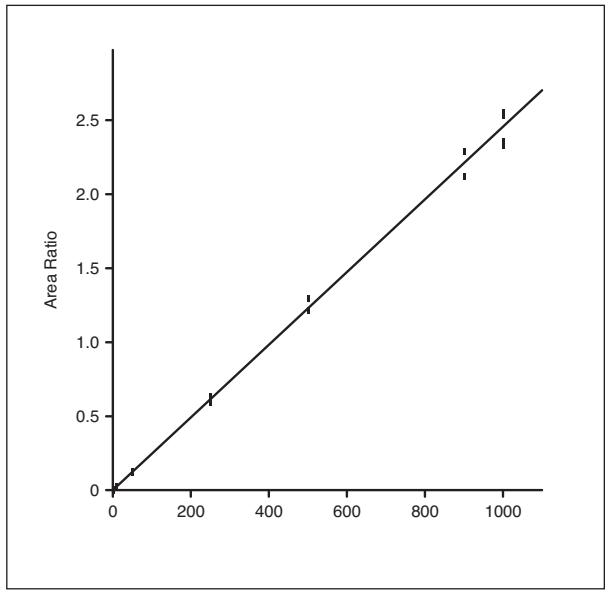

Figure 1. Linearity of response over the dynamic range 1 – 1000 ng/mL

Standard Ref.	Nominal [Rosuvastatin] ng/mL	Calculated [Rosuvastatin] ng/mL	Relative error %
S1	1	0.983	-1.7
S1	1	0.976	-2.4
S2	2	1.884	-5.8
S3	10	9.997	0.0
S4	50	53.115	6.2
S5	250	258.476	3.4
S6	500	527.678	5.5
S7	900	930.568	3.4
S8	1000	1033.005	3.3
S8	1000	1040.001	4.0
S1	1	1.007	0.7
S1	1	1.107	10.7
S2	2	1.897	-5.2
S3	10	9.669	-3.3
S4	50	49.231	-1.5
S5	250	242.090	-3.2
S6	500	495.248	-1.0
S7	900	862.270	-4.2
S8	1000	948.781	-5.1
S8	1000	960.017	-4.0

Table 1. Linearity of response for the determination of rosuvastatin in human plasma

The analytical response was found to be linear (using a 1/x weighted regression algorithm) with a coefficient of determination (r^2) of 0.9984 in the range 1 - 1000 ng/mL.

Assessment of accuracy and precision

Procedural accuracy and precision were evaluated by replicate (n = 6) examination of extracted QC samples at three levels of concentration. A summary of the results is shown in Table 2.

The accuracy and precision of the analytical procedure were found to fall comfortably within the limits of acceptance generally applied to bioanalytical methods.

QC Ref.	Nominal [Rosuvastatin] ng/mL	Calculated [Rosuvastatin] ng/mL	Relative error %	Mean [Rosuvastatin] ng/mL	Std. Dev.	% RSD
QCLOW	3	3.292	9.7	3.120	0.1799	5.77
		3.167	5.6			
		3.348	11.6			
		3.014	0.5			
		3.017	0.6			
		2.883	-3.9			
QCMED	400	425.764	6.4	416.481	20.2603	4.86
		428.366	7.1			
		422.164	5.5			
		375.644	-6.1			
		419.784	4.9			
		427.163	6.8			
QCHIGH	750	765.300	2.0	776.153	14.4699	1.86
		785.145	4.7			
		776.872	3.6			
		765.021	2.0			
		800.296	6.7			
		764.282	1.9			

Table 2. Accuracy and precision data for the determination of rosuvastatin in human plasma

Evaluation of recovery

The recovery of analyte was assessed by comparison of the measured concentrations of rosuvastatin in matrix-extracted QC samples with those concentrations found in post-extraction spiked samples which had been fortified at the same level. See Table 3.

The level of analyte recovery (99.3 %) and the precision (% RSD = 4.88) between replicates demonstrate that both the efficiency of the extraction procedure and its repeatability are substantially more than satisfactory.

QC Ref.	Nominal [Rosuvastatin] ng/mL	Mean calculated [Rosuvastatin] ng/mL		Mean Recovery %	Std. Dev.	% RSD
		Pre-extracted fortified plasma samples	Post-extracted fortified plasma samples			
QCMED	400	416.481	419.560	99.3	4.84	4.88

Table 3. Recovery data for the determination of rosuvastatin in human plasma

Statistical assessment based upon data derived from the replicate examination of both pre- (n = 6) and post-extracted (n = 3) plasma samples.

Evaluation of matrix effect

The existence of a matrix effect was determined by comparison of the measured concentrations of rosuvastatin in post-extracted fortified plasma samples with those concentrations found in the reference standards that had been fortified at the same level. The results, summarised in Table 4, indicate that there is no significant matrix effect.

QC Ref.	Nominal [Rosuvastatin] ng/mL	Mean calculated [Rosuvastatin] ng/mL		Matrix effect (mean) %	Std. Dev.	% RSD
		Post-extracted fortified plasma samples	Reference standard (unextracted)			
LLOQ	1	1.024	1.047	97.8	3.13	3.20
QCMED	400	419.560	399.733	105.0	3.91	3.72
QCHIGH	750	764.577	726.117	105.3	2.00	1.90

Table 4. Effect of matrix on the determination of rosuvastatin in human plasma

Statistical assessment based upon data derived from the replicate examination of both post-extracted plasma samples (n = 3) and reference standards (n = 6).

Specificity and sensitivity

SRM chromatograms derived from the examination of the unfortified and fortified plasma samples are shown in Figures 2 and 3.

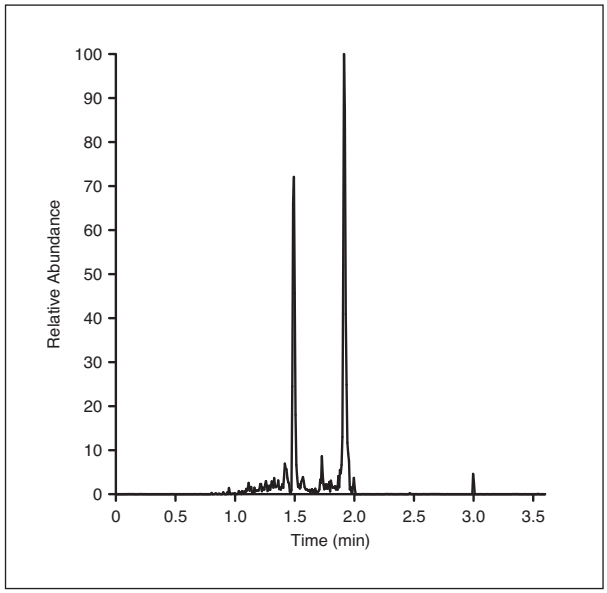

Figure 2. SRM derived from examination of extracted standard S1 (1 ng/mL)

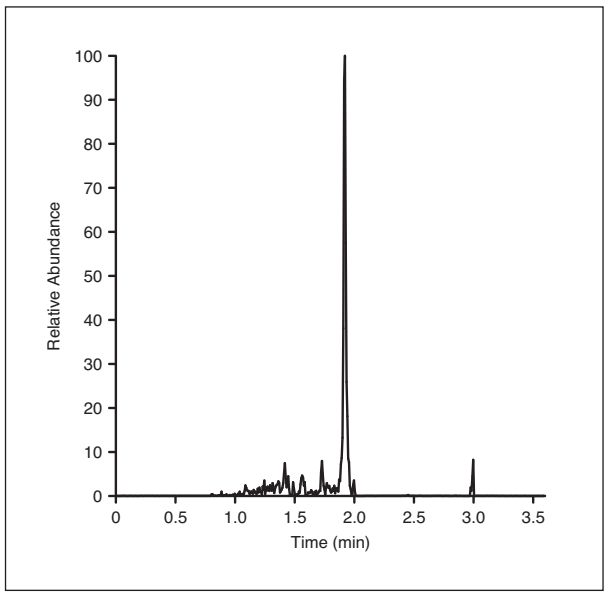

Figure 3. SRM derived from examination of an unfortified, extracted (blank) plasma sample

It is evident that the unfortified plasma sample contains an impurity (T_r = 1.92 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 (T_r = 1.49 minutes).

The SRM derived from the zero blank is shown in Figure 4. The mass spectral evidence suggests that there is no proton exchange and concomitant conversion of the deuterated to the non-deuterated form.

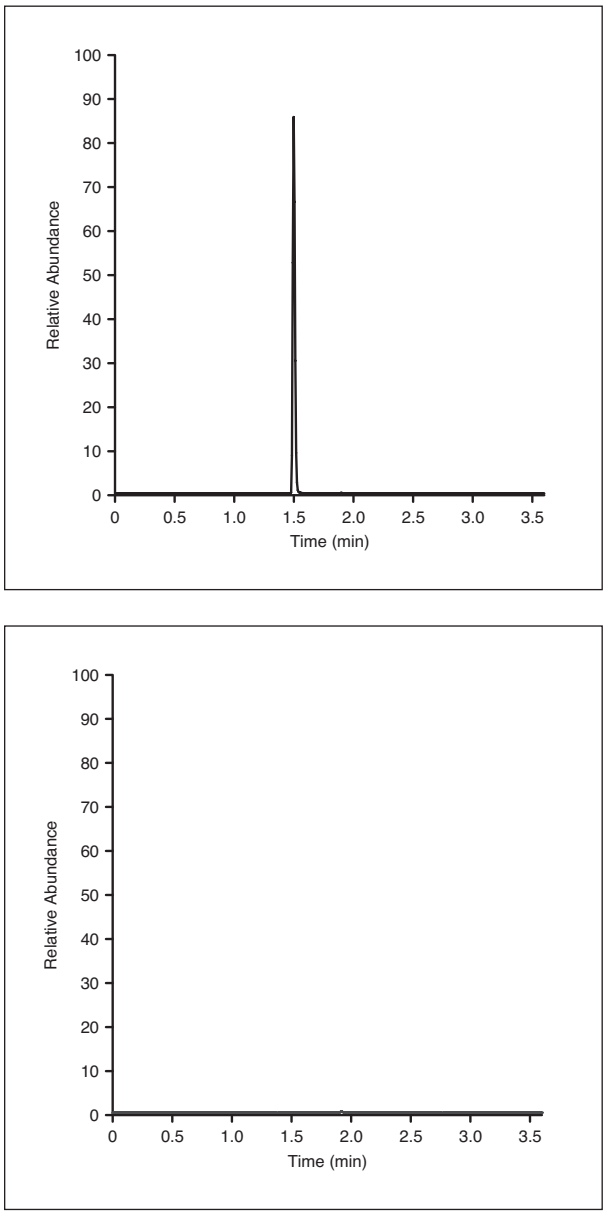

Figure 4. SRM derived from examination of the zero blank (upper trace: ^{d}6-rosuvastatin, lower trace: undeuterated analogue).

Conclusion

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

The procedure was found to exhibit good linearity (r^2 = 0.9984) for concentrations of rosuvastatin in the range 1 - 1000 ng/mL. The accuracy and precision for all samples examined were found to be < 6.1 % and < 5.8 % respectively. At a concentration of 400 ng/mL, the level of recovery of analyte (99.3 %) and its repeatability (% RSD = 4.9 %) were both found to be excellent. There was no significant matrix effect.

The performance characteristics of the method combined with its simplicity and rapidity mean that it can be adopted routinely in clinical environments.

References

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