OPTIMIZED SAMPLE PREPARATION USING A NOVEL SPE SOLUTION

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Overview

Today's high throughput bio-analytical laboratories desire high levels of reproducibility in their analytical process to have confidence and reduced time and cost spent on re-analysis. Sample preparation is a critical part of this process and is potentially an area of variability in results. The following data demonstrates the advantages of Thermo Scientific SOLA products for efficient, reproducible sample preparation from a biological matrix prior to LC-MS analysis.

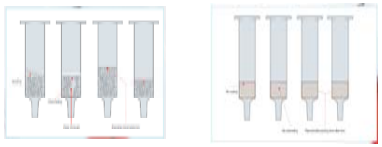
Introduction

SOLA™ products revolutionize Solid Phase Extraction (SPE). This first fritless SPE product range provides greater reproducibility with cleaner, more consistent extracts. SOLA products provide unparalleled performance characteristics compared to conventional SPE, phospholipid removal and protein precipitation products.

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

The proprietary manufacturing process involved in the production of SOLA products provides an SPE product which eliminates issues normally associated with conventional loose-packed SPE products, by combining the polyethylene frit material and media components into a uniform sorbent bed, removing the need for frits (Figure 1).

Figure 1: Comparison of traditional loose-packed SPE cartridges (left hand side) and SOLA cartridges (right hand side)



SOLA products achieve excellent recovery levels even with low volumes of extraction solvents, resulting in a more concentrated analyte and increased sensitivity. Additional cost and time saving benefits can be achieved from reduced sample dry-down time and solvent usage. These low-volume extractions would be significantly compromised when using a conventional loose-packed, low bed weight, SPE product.

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 drug for lowering cholesterol. It is marketed by Astra Zeneca as 'Crestor', and is used to treat primary hypercholesterolaemia, mixed dyslipidaemia and hypertriglyceridaemia to reduce the risk of atherosclerosis and poor cardiovascular health. It is a selective 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.

A simple, rapid and sensitive procedure for the determination of rosuvastatin in human plasma by LC-MS has been developed. The drug was isolated from plasma matrix using a SOLA 96 well plate , the resultant extracts were separated on a Thermo Scientific Accucore RP-MS column . Detection was performed on a triple quadrupole mass spectrometer.

SOLA is shown to provide excellent recovery of 93.3% and very reproducible results with a response ratio (%RSD) of 2.7% for 96 extractions performed across an entire well plate.

Methods

Sample Preparation
Compound(s): rosuvastatin, rosuvastatin-d6 (IS)
Matrix: Human plasma
Cartridge type: SOLA 10mg/2mL 96 well plate
Conditioning stage: 1mL methanol, 1mL water
Application stage: Load 100µL sample and allow to flow under gravity
Washing stage: 500µL 0.1% formic acid in water
Washing stage: 500µL 90:10 (v/v) water/ methanol
Elution stage: 2 x 200µL 10:90 (v/v) water/ methanol
Additional stage: Dry down under a stream of nitrogen at 40°c and reconstitute in 200µL 80:20 (v/v) water/ methanol. Sonicate for 5 minutes.

Liquid Chromatography
Instrumentation: Thermo Scientific Accela 600
Column: Accucore ™ RP-MS, 2.6µm, 50 x 2.1mm
Guard column: Accucore Defender™ guard, Accucore RP-MS, 2.6µm, 10 x 2.1mm p/n 17626-012105
Mobile phase A: water + 0.1% formic acid
Mobile phase B: methanol + 0.1% formic acid
Gradient: 5 to 95% B in 1.5 min. Hold at 95% B for 30 seconds.
Flow rate: 0.75mL/min
Column temperature: 60°C
Injection details: 15µL
Injection wash solvent 1: 80:20 (v/v) water / acetonitrile
Injection wash solvent 2: 100% organic

Mass Spectrometry

Instrumentation: Thermo Scientific TSQ Vantage

Table 1: TSQ Vantage™ conditions

Ionization conditions	HESI
Polarity	Positive
Spray voltage (eV)	3000
Vaporizer temp (°C)	475
Sheath gas pressure (Arb)	65
Aux gas pressure (Arb)	15
Capillary temp (°C)	300
Collision pressure(mTorr)	1.5
Scan time (s)	0.02
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7

Table 2. Compound transition details

Compound	Rosuvastatin	Rosuvastatin-d6
Parent (m/z)	399.2	405.3
Products (m/z)	134.9, 151.0	135.1, 151.0
Collision energy (eV)	15, 20	19,23
S-lens	81	89

Data Analysis

Software: Thermo Scientific LC QUAN

Results

Reproducibility
96 rosuvastatin and rosuvastatin-d6 replicates were extracted from human plasma using a SOLA 96 well plate. The %RSD for rosuvastatin was 5.4% and rosuvastatin-d6 was 3.9% (Table 3). The %RSD for the response ratio between rosuvastatin and rosuvastatin-d6 was 2.7% (Table 3).
Accucore Core Enhanced Technology columns gave a reproducible sharp peak for rosuvastatin (Figure 3).

Table 3: %RSD data for rosuvastatin, rosuvastatin-d6 and the response ratio between them

	%RSD
Rosuvastatin (Peak area of 96 replicates)	5.4
d6-Rosuvastatin (Peak area of 96 replicates)	3.9
Response Ratio (96 replicates)	2.7

Figure 2: Selected reaction monitoring chromatogram of rosuvastatin extracted from human plasma

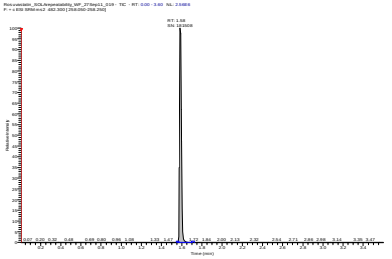
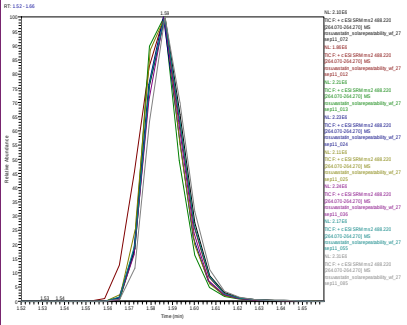


Figure 3: Overlaid chromatograms for 8 randomly chosen extracts for the 96 analyses performed.



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 4.

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

Table 4: Accuracy and precision data for the determination of rosuvastatin in human plasma

	Nominal [Rosuvastatin] ng/mL	Mean [Rosuvastatin] ng/mL	Std. Dev.	% RSD (n=6)
QCLOW	3	3.12	0.1799	5.77
QCMED	400	416.481	20.26	4.86
QCHIGH	750	776.153	14.47	1.86

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 5.

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.

Table 5: Recovery data for rosuvastatin

QC Ref.	Nominal Concentration [Rosuvastatin] ng/mL	Mean calculated [Rosuvastatin] ng/mL		Mean Recovery %	Std. Dev.	% RSD
		Pre-extracted fortified plasma samples	Post-extracted fortified plasma samples			
QC MED	400	416.481	419.56	99.3	4.84	4.88

Conclusion

SOLA products can be used to develop simple fast and reliable bioanalytical methods for the extraction of analytes from plasma matrices.

- Excellent analyte recoveries from plasma matrices can be achieved 99.3%
- Reproducible results free of matrix effects are possible with response ratio %RSD for an entire 96 well plate of 2.7%

Acknowledgements

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Additional Information

For additional information, please visit our Chromatography Resource Centre which can be found at: www.thermoscientific.com/CRC

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