



DETERMINATION OF ROSUVASTATIN IN RAT PLASMA BY XLC-MS USING SYMBIOSIS™ PHARMA

April 2006
0053.067-01

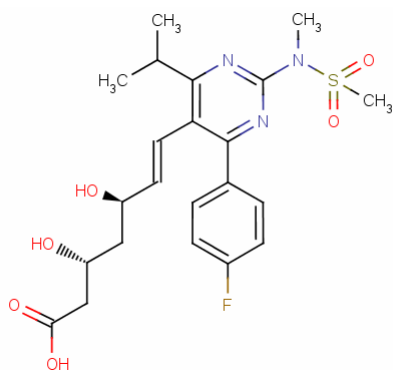
APPLICATION INFO

Introduction

Symbiosis™ Pharma is Spark Holland's unique solution for integrated online SPE-LC-MS automation (XLC-MS). The system offers large flexibility in processing different types of samples selecting one of the three fully automated operational modes LC-MS; XLC-MS; AMD (Advanced Method Development).

Rosuvastatin is a synthetic lipid-lowering agent and used with diet changes (restriction of cholesterol and fat intake) to reduce the amount of cholesterol and certain fatty substances in your blood.

Rosuvastatin is in a class of medications called HMG-CoA reductase inhibitors (statins). It works by preventing the body from forming harmful cholesterol and increasing the amount of HDL ('good cholesterol') in your blood.



Rosuvastatin, Mw = 481.5 with LogP = 2.65 (pH=2),
C₂₂H₂₈FN₃O₆S, CAS#287714-41-4,

Rosuvastatin calcium is a white amorphous powder that is sparingly soluble in water and methanol, and slightly soluble in ethanol. Rosuvastatin is a hydrophilic compound with a partition coefficient (octanol/water) of 0.13 at pH of 7.0.

It is sold under the name Crestor® by AstraZeneca Pharmaceuticals LP.



Figure 1: Symbiosis™ Pharma

Method Development

The XLC mode of Symbiosis™ Pharma in conjunction with the HySphere™ method development cartridge tray (Spark p.n. 0722.650) enables "quick sorbent screening" for the most suitable SPE cartridge and optimal clean-up conditions.

The following data was obtained in less than 1 hour using generic pre-defined SPE conditions of Symbiosis™ Pharma.

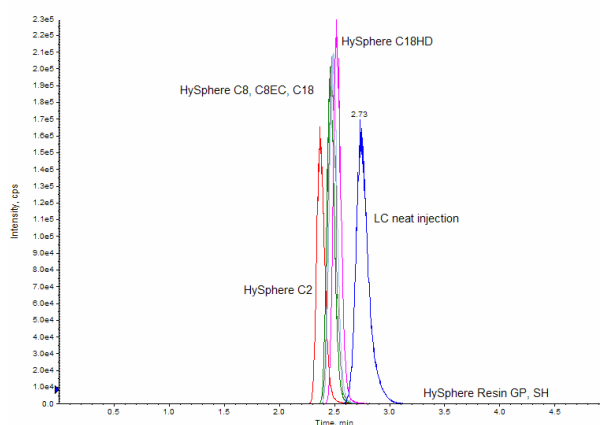


Figure 2: Chromatograms of Rosuvastatin in plasma after sorbent screening using the HySphere™ hydrophobic MD tray.

After the "quick sorbent screening" the HySphere™ C18HD cartridge gives the highest signal and also the best peak shape, as can be seen in figure 2.

Recovery compared to an LC injection area is higher than 99% for the optimized SPE method.

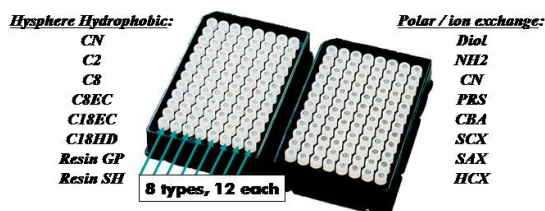


Figure 3: Method development cartridge tray

For more information visit our website:
[HTTP://WWW.SPARKHOLLAND.COM](http://www.sparkholland.com)

Spark
HOLLAND

XLC-MS Protocol

Autosampler conditions

25 µL of sample is injected using a µL Pick-up (no sample loss) autosampler configuration.
 Washing is performed with two wash solvents;
 Wash solvent 1: 1% NH4OH in water, pH=11
 Wash solvent 2: 50% ACN with 0.1% Formic Acid.

Table 1: Autosampler wash routine.

Wash solvent	Wash volume	Valve wash
1	700 µL	no
2	700 µL	no
1	700 µL	yes
2	700 µL	yes
1	1500 µL	yes

SPE conditions

Cartridge:	10 x 2 mm HySphere C18HD (Spark PN:0722.609)	
Solvation:	1 mL ACN	5 mL/min
Equilibration:	1 mL 5% ACN in 0.1 % FA	5 mL/min
Sample Loading:	1 mL 5% ACN in 0.1 % FA	2 mL/min
Washing:	1 mL 5% ACN in 0.1 % FA	5 mL/min
Elution	1 min. with LC gradient	LC flow
Matrix:	Rat plasma	

LC conditions

Column:	Phenomenex Luna C18(2) 4.6x50mm
Mobile phase A:	0.2% Formic acid in water
Mobile phase B:	0.2% Formic acid in MeOH

Table 2: LC gradient

Time (mm:ss)	Flow (mL/min.)	A (%)	B (%)
00:00	0.25	40	60
00:05	0.25	40	60
02:05	0.25	10	90
02:35	0.25	10	90
03:00	0.25	40	60
05:00	0.25	40	60

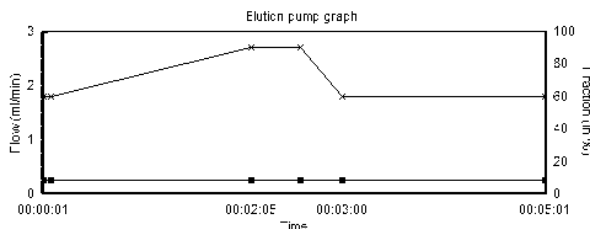


Figure 6: LC gradient

MS conditions

A Sciex API 3000 with a Turbo IonSpray (ESI pos) is used.

Table 3: MS parameters

NEB	15
CUR	12
IS	5000
TEM	400
CAD	6
GAS	7000

Table 4: Compound dependable MS settings

	Rosuvastatin	Rosuvastatin D6
Q1 mass	482.2	488.2
Q3 mass	258.1	264.1
Dwell time	150	150
ESI	positive	positive
DP	56	56
FP	350	350
EP	10	10
CE	47	47
CXP	18	18

Result

The following samples are prepared in Rat plasma with internal standard Rosuvastatin D6.

- Calibration standards: 1.0; 2.5; 10; 50; 100; 500; 1000 ng/mL
- QC samples: 1; 50; 500 ng/mL

Chromatograms

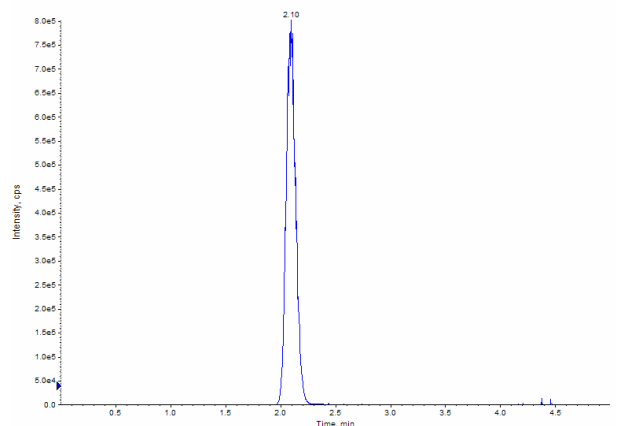


Figure 7: Chromatogram representing 1000 ng/mL Rosuvastatin in rat plasma.

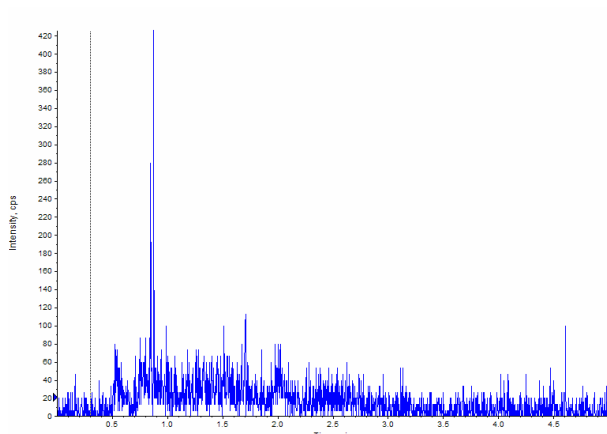


Figure 8: Chromatogram representing blank rat plasma

Linearity, Accuracy and Precision

Linearity was evaluated by injecting a full set of calibration standards. Regression analysis of the calibration data was determined, with a correlation coefficient (R) of 0.9991 and a 1/X weighting. For this data set 10 calibration curves are injected.

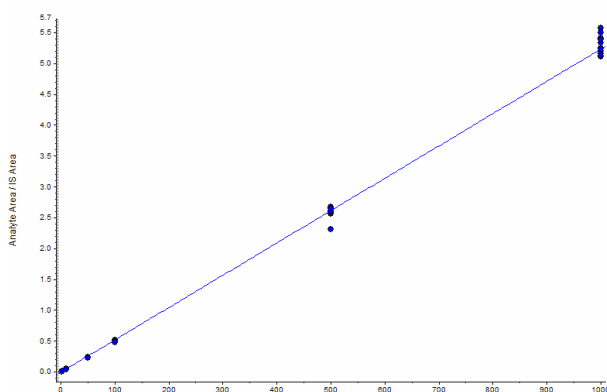


Figure 9: Calibration curve of Rosuvastatin (1 to 1000 ng/mL)

Table 5: Accuracy calculated from ten combined sets of calibration standards

Sample (ng/mL)	CV (%)	Accuracy (%)
1.0	8.25	104
2.5	2.18	92.5
10.0	3.12	88.4
50.0	2.16	94.8
100	2.32	103
5000	4.51	107
10000	2.82	109

Table 6: Accuracy and precision calculated from three combined sets of QC standards.

Sample (ng/mL)	CV (%)	Accuracy (%)
QC 1	10.1	115.0
QC 50	7.52	89.8
QC 500	6.97	96.5

Conclusions

The development of this assay on the **Symbiosis™ Pharma**, demonstrated the speed of transfer from an off-line, to an on-line XLC-MS assay, (~2 days, with optimization).

This study shows how to develop a XLC-MS method with an absolute recovery >99% and a set of calibration standards with a linear range from 1 to 1000 ng/mL (R of 0.9991) and an accuracy between 88-115%. The carry-over is less than 0.01% of the HLOQ.

About Spark

Since 1982 Spark has provided the HPLC and LC/MS markets with state-of-the-art autosamplers, column ovens and sample preparation solutions. Solid Phase Extraction with on-line elution into HPLC and LC/MS systems was pioneered by Spark and introduced in the early 90's. Spark, ISO 9001 certified, does basic research, product development, production, sales and marketing in-house, guaranteeing quality from start to finish. With 25% of the employees working in research and development Spark continues to invest in the future, making sure we can deliver the solutions you need to improve your business results. Innovation and quality are keywords when talking about our development efforts.

Spark System Solutions BV
Bendienplein 5
7815 SM Emmen,
the Netherlands

P +31 591631700
F +31 491645900
E Solutions@Sparkholland.com
W www.sparkholland.com