DETERMINATION OF AMLODIPINE IN SERUM BY XLC-MS USING SYMBIOSIS™ PHARMA

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).

Amlodipine belongs to a class of medications called calcium channel blockers. These medications block the transport of calcium into the smooth muscle cells lining the coronary arteries and other arteries of the body.
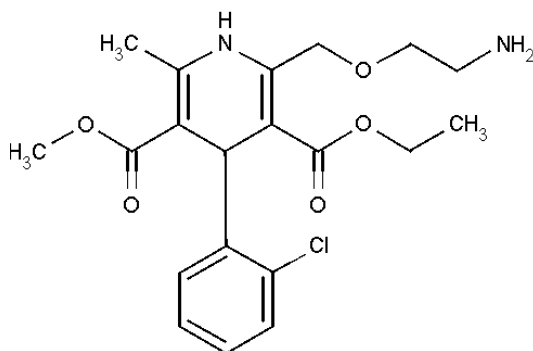

Figure 1: Amlodipine, Mw 408.3 or 567 with LogP = 3.0, $C_{20}H_{25}ClN_2O_5$, CAS#088150-42-9, Water solubility 75 mg/L

Amlodipine lowers high blood pressure (hypertension). It also and relieves different types of chest pain (angina). Generic Amlodipine is sold under the commercial name "Norvasc®".

Method Development

Figure 2: Symbiosis™ Pharma System

The AMD mode of Symbiosis Pharma in conjunction with the HySphere method development cartridge tray (Spark p.n. 0722.650) enables "quick sorbent screening" for most suitable SPE cartridge and optimal wash conditions for clean-up. The following data was obtained in less than 1 hour using generic predefined SPE conditions of the Symbiosis™ Pharma.
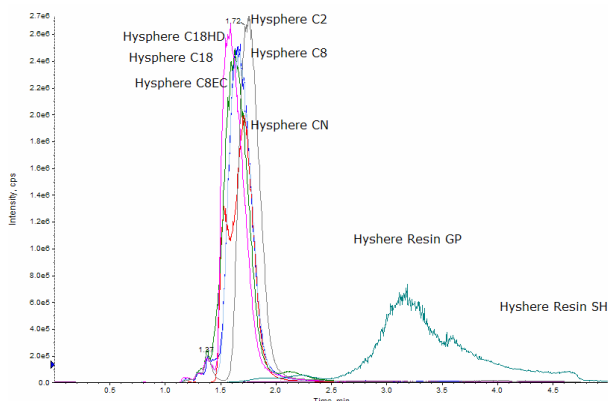

Figure 2: Chromatograms of Amlodipine in serum after sorbent screening using the HySphere hydrophobic MD tray.

From figure 2 can be derived that the HySphere C2 gives the highest signal and also the best peak shape. Recovery compared to a LC injection area is higher than 90%.
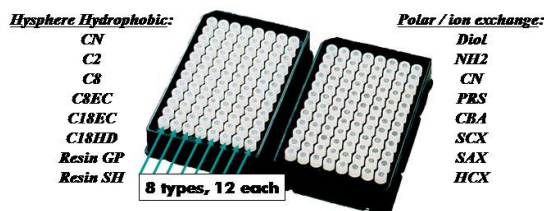

Figure 3: Method Development Cartridge Tray

XLC-MS Protocol

Autosampler Conditions

50 µL of undiluted serum is injected using a standard autosampler configuration.
Washing is performed with two wash solvents;
Wash solvent 1: 50% ACN with 0.1% Formic Acid.

Wash solvent 2: 90% CAN.

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

Table 1: Autosampler wash routine.

SPE conditions
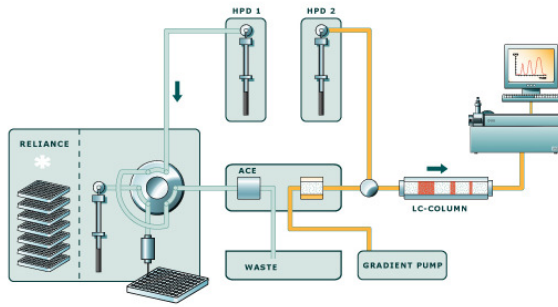
Figure 5: Schematic overview of flow path

Cartridge:	10 x 2 mm HySphere C2 (Spark PN:0722.605)	
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	4 min. with LC Gradient LC	
Matrix:	Serum	

Table 2: SPE conditions

LC conditions

Column:	Waters Nova Pak C18 3.9 mm x 50 mm
Mobile phase A:	0.1% Formic Acid in Water
Mobile phase B:	0.1% Formic acid in ACN

Time (mm:ss)	Flow (mL/min.)	A (%)	B (%)
00:00	0.25	40	60
00:05	0.25	40	60
02:25	0.25	0	100
03:00	0.25	0	100
03:30	0.25	40	60
04:00	0.25	40	60
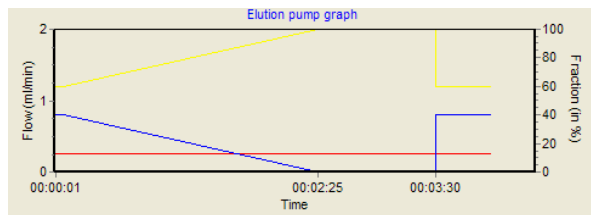
Figure 6: LC gradient

MS Conditions

A Sciex API 2000 with a Turbo IonSpray is used.

	Amlodipine
Q1 mass	409.02
Q3 mass	238.1
Dwell time	150
DP	1.00
FP	370
EP	4.5
CEP	18
CE	17
CXP	4

Table 3: Compound dependable MS settings (CUR = 20; IS = 4000; TEM = 450)

Result

The following samples are prepared in new born calf serum.

- Calibration standards:0.5; 1.0; 5.0; 10; 50; 100; 500; 1000 ng/mL
- QC samples: 1; 50; 500 ng/mL

Chromatograms

Figure 7 is a representative chromatogram of standard sample in the upper limit of the calibration curve.
The chromatogram of a blank injection immediately after the high standard is shown in figure 8.
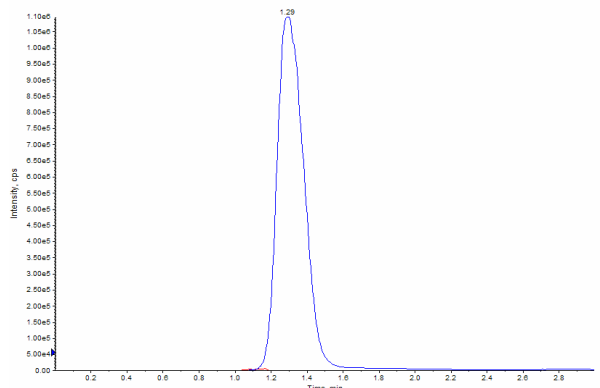The carry over is calculated as <0.02%.

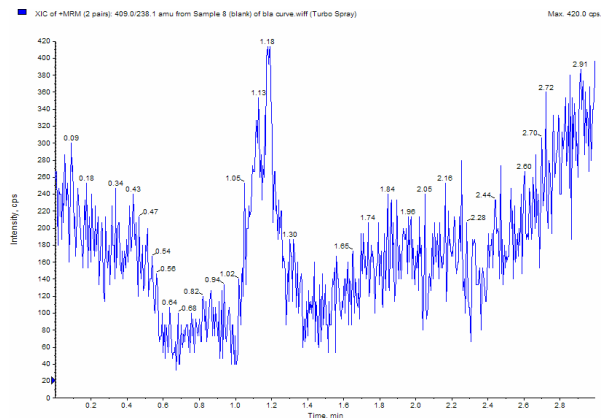*Figure 7: Chromatogram representing 1000 ng/mL Amlodipine.*

Figure 8: Chromatogram representing blank (15% of LLOQ)

Linearity, Accuracy and Precision

A calibration standard was determined by injecting a full set of calibration standards. From this set, the 1000 ng/mL calibration point is rejected, because the area lies outside the linear range of the API 2000 MS detector. For the other points a calibration curve is calculated with a R^2 0f 09995 (1/X weighting).
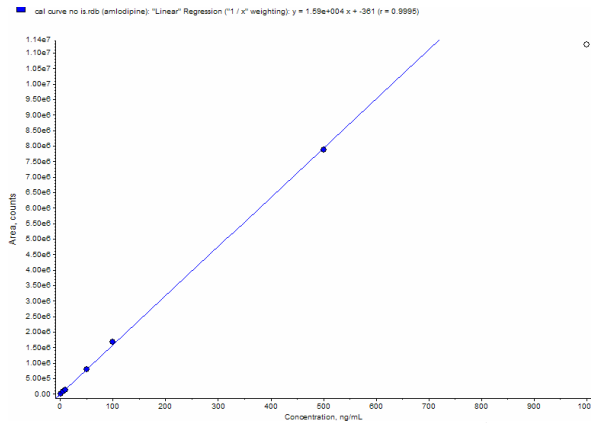

Figure 9: Calibration curve of Amlodipine R^2=0.9995

Sample (ng/mL)	Accuracy (%)
0.5	115
1	96.0
5	92.6
10	86.2
50	100
100	106
500	99.2
1000	N/A

Table 4:   Accuracy calculated from three combined sets of Calibration standards

Sample (ng/mL)	CV (%)	Accuracy (%)
QC 10	9.31	85.0
QC 50	1.12	99.9
QC 500	1.84	93.2

Table 5:   Accuracy and precision calculated from three combined sets of QC standards.

Conclusions

From this study it is concluded that within a time frame of 2 days it is possible to develop a XLC-MS method with an absolute recovery >90% and run a set of calibration standards with a linear range from 0.5 to 500 ng/mL (R^2 of 0.9995) and an accuracy between 86-115%.

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