



Determination of Amphetamine and Methamphetamine in Urine by XLC-MS using the Symbiosis™ Pharma System

Jan 2007
0053.062-01

APPLICATION INFO

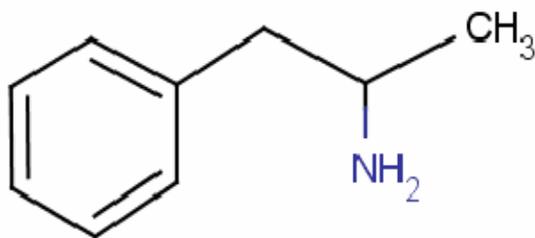
Introduction

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).

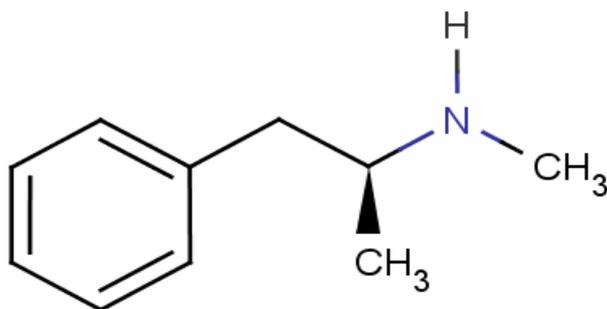
This application note shows the possibility to use mixed mode cation exchange cartridges and the optimal clean-up conditions for the determination of Amphetamine and Methamphetamine, with the use of the HPD focusing mode of Symbiosis™ Pharma and the Waters Oasis MCX cartridge.

Amphetamine (alpha-methyl-phenethylamine), also known as speed, is a synthetic stimulant used to suppress the appetite, control weight, and treat disorders including narcolepsy and Attention-deficit hyperactivity disorder (ADHD). It is also used recreationally and for performance enhancement. Amphetamine is taken orally, intranasally (snorting the powder), by needle injection, or by smoking. Abusers may become addicted quickly, needing higher doses and more often.

Methamphetamine is a very addictive stimulant drug that activates certain systems in the brain. It is chemically related to amphetamine but, at comparable doses, the effects of methamphetamine are much more potent, longer lasting, and more harmful to the central nervous system (CNS).



Amphetamine, Mw 135.27 with LogP = 1.8, C₉H₁₃N, CAS# 300-62-9



Methamphetamine, Mw 149.23 with LogP = 2.2, C₁₀H₁₅N, CAS# 537-46-2

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

Spark
HOLLAND

Method Development



Figure 1: Symbiosis Pharma System

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 most suitable SPE cartridge and optimal wash conditions for clean-up.

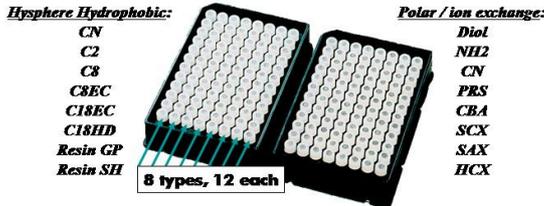


Figure 2: Method Development Cartridge Tray

None of the 8 HySphere™ hydrophobic SPE sorbents gave a reasonable recovery and peak shape. Therefore a mixed mode cation-exchange sorbent was explored. To elute the compounds of a cation-exchange sorbent a stronger solvents is required. The cartridge is eluted with a high organic solvent containing 1% NH₄OH (pH = 10). An integrated make-up flow (see figure 3) in the symbiosis™ Pharma dilutes the organic eluents before it enters the LC column and allows the compounds to focus at the top of the LC column.

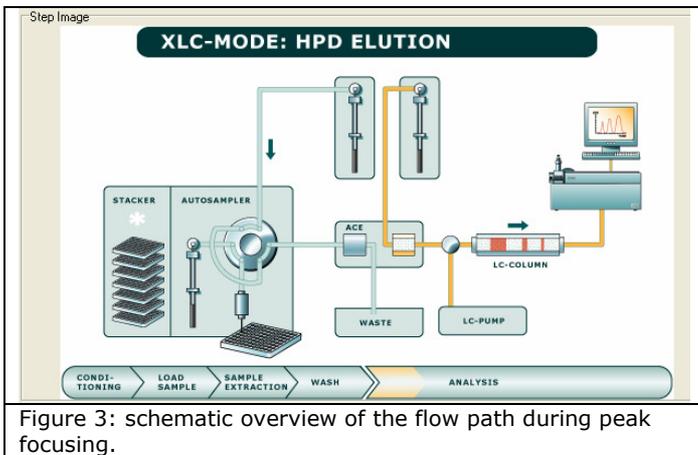


Figure 3: schematic overview of the flow path during peak focusing.

To enable cationic ion-exchange the amines are protonated using Formic Acid (pH 3.5) for all SPE steps. An Oasis MCX™ sorbent based on cationic-exchange was tested for recovery. The overall recovery is higher than 90% and was calculated from the LC run of a neat injection and a XLC run of a spiked urine sample (see figure 4).

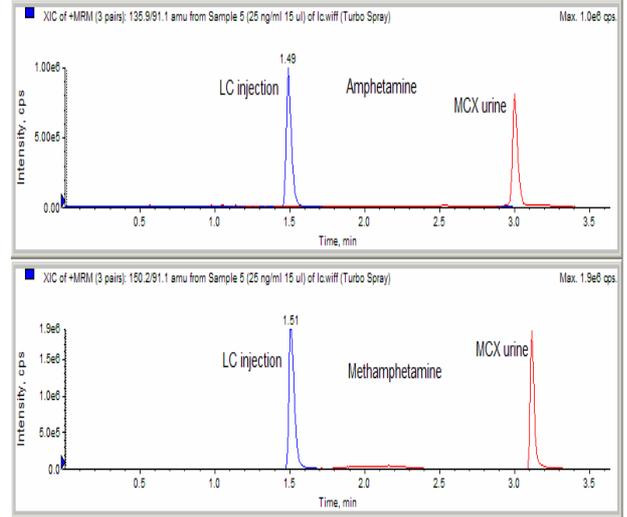


Figure 4: Chromatogram overlay of Amphetamine and Methamphetamine in urine XLC and a neat LC.

XLC-MS Protocol

Autosampler Conditions

20 µL of sample is injected using a standard autosampler configuration. Washing is performed with two wash solvents; Wash solvent 1: 50% ACN - 50% Water with 0.1% Formic Acid. Wash solvent 2: 90% ACN - 10% Water.

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	1400 µL	yes

SPE conditions

Cartridge:	10 x 1 mm Waters Oasis MCX™
Solvation:	1 mL ACN 5 mL/min
Equilibration:	1 mL 5% MeOH in 0.1 % FA 5 mL/min
Sample Loading:	1 mL 5% MeOH in 0.1 % FA 2 mL/min
Wash 1:	1 mL 5% MeOH in 0.1 % FA 5 mL/min
Wash 2:	1 mL 100% MeOH in 0.1% FA 5 mL/min
Elution	100 ul 100% MeOH in 1% NH4OH 100 uL/min
Matrix:	Diluted Urine

LC conditions

Column:	Waters Xterra C18 ms 3.9X50mm
Mobile phase A:	0.1% Formic Acid in Water
Mobile phase B:	0.1% Formic acid in ACN

Table 2: LC gradient

Time (mm:ss)	Flow (mL/min.)	A (%)	B (%)
00:00	0.9	100	0
01:01	0.9	100	0
01:05	1.0	100	100
04:00	1.0	50	50
04:30	1.0	0	100
05:00	1.0	0	100
05:30	1.0	100	0
06:00	1.0	100	0

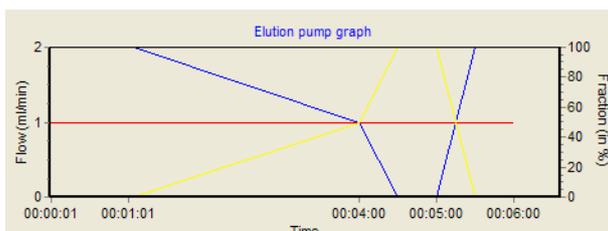


Figure 6: LC pump gradient

MS Conditions

A Sciex API 4000 with a Turbo IonSpray in positive mode is used.

Table 3: MS parameters

CUR	10
IS	1500
TEM	500
GS1	50
GS2	60
Ihe	ON
CAD	6

Table 4: Compound dependable MS settings

	Amphetamine	MethAmphetamine
Q1 mass	135.93	150.18
Q3 mass	91.10	91.10
Dwell time	150	150
ESI	Pos	Pos
DP	36	46
EP	10	10
CE	23	29
CXP	8	8

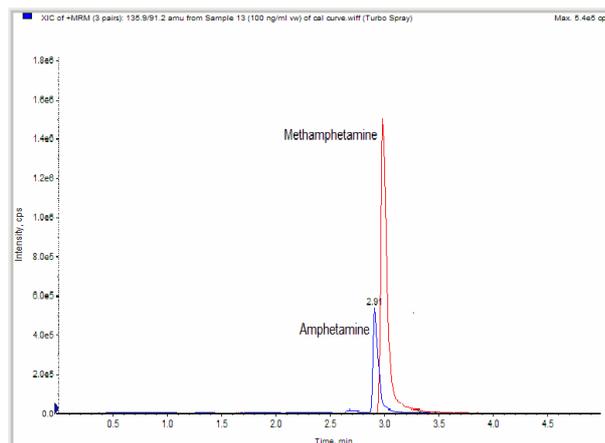


Figure 7: XLC Chromatogram representing 100 ng/mL Amphetamine and Methamphetamine in Urine.

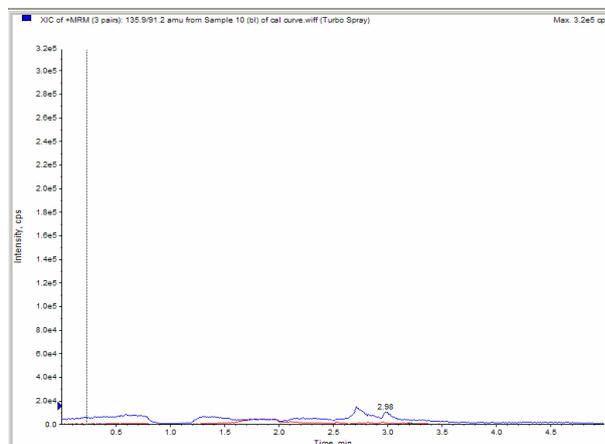


Figure 8: Chromatogram representing the blanks (less the 10% of LLOQ)

Linearity, Accuracy and Precision

Linearity was evaluated by injecting a full set of calibration standards. All points are used to determine the calibration curve. Regression analysis of the calibrated data was determined with a correlation Coefficient (r) of more the 0.996 for both compounds and a 1/X weighting.

Result

After the optimization of the SPE protocol the method was tested for reproducibility, Linearity and precision. The following samples are prepared in urine. The urine was 1 to 1 diluted with water. The calibration standard concentration is the concentration in pure urine.

- Calibration standards: 0.5; 1.0; 5.0; 10; 50; 100 ng/mL

Chromatograms

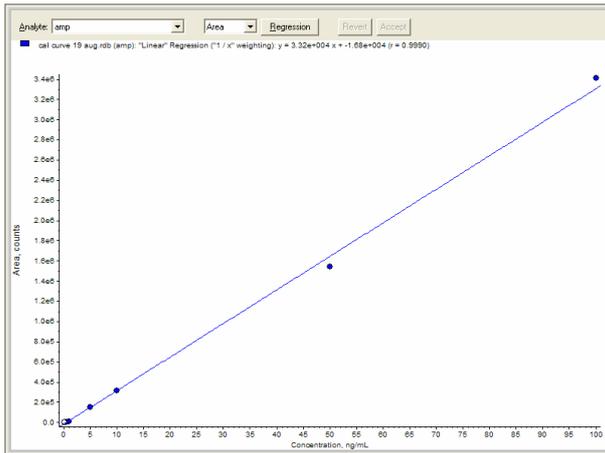


Figure 10: Calibration curve of Amphetamine R=0.999

Table 5: Accuracy calculated from three combined sets of Calibration standards

Sample (ng/mL)	Accuracy (%)
0.5	112
1	91.2
5	99.1
10	99.9
50	93.9
100	103

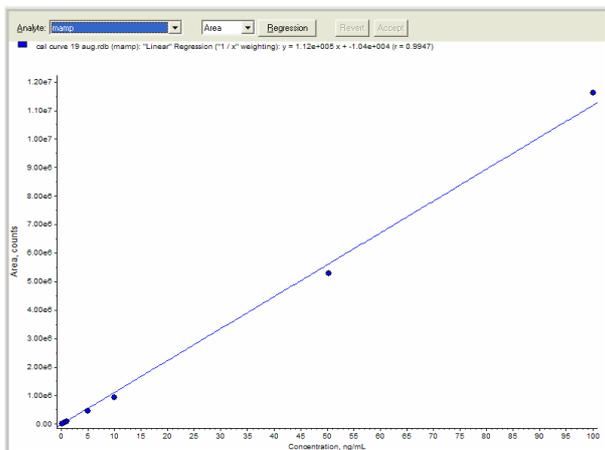


Figure 10: Calibration curve of Methamphetamine R=0.996

Table 5: Accuracy calculated from three combined sets of Calibration standards

Sample (ng/mL)	Accuracy (%)
0.5	94.6
1	90.4
5	89.6
10	88.4
50	88.6
100	108

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 a set of calibration standards with a linear range from 0.5 to 100 ng/mL (R more the 0.995) and an accuracy between 88-112%.

This study shows how to develop a XLC-MS method using a mixed mode cation exchange cartridge and the HPD focusing mode of Symbiosis™ Pharma.

The carry-over is minimized by using the valve wash option of the Reliance™ autosampler, the carry-over is reduced to levels below the detection limit.

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