APPLICATION INFO

DETERMINATION OF SALBUTAMOL IN SERUM BY XLC-MS/MS USING THE SYMBIOSIS™ PHARMA

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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 will present a study that demonstrates the capabilities of the AMD-mode to speed-up method development. The presented results were obtained within 2 days and show a XLC-MS protocol that generates acceptable accuracy, precision and linearity over the calibration curve.

Salbutamol (also known as Albuterol) relaxes the muscles in the airways to improve breathing and is therefore often prescribed to patients from bronchospasm. Salbutamol is analyzed in human serum.

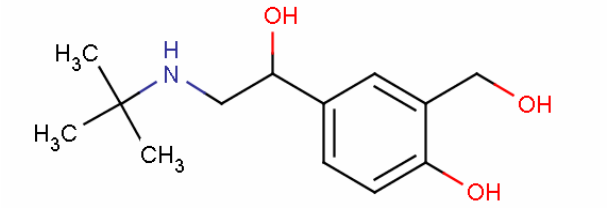

Figure 1 Salbutamol

- CAS#018559-94-9
- C13H15O3N
- Mw 239.32
- Physical Properties:
  - Water solubility 14.9 g/L
  - Log P (Octanol-water) -0.69
  - pKa dissociation constant 9.4

Method Development

The AMD mode of Symbiosis™ Pharma in conjunction with the HySphere method development cartridge tray enables "quick sorbent screening" for most suitable SPE cartridge and optimal wash conditions for clean-up. The tray holds 8 most common SPE sorbents. The following data was obtained in less than 1 hour using generic pre-defined SPE conditions of the Symbiosis™ Pharma.

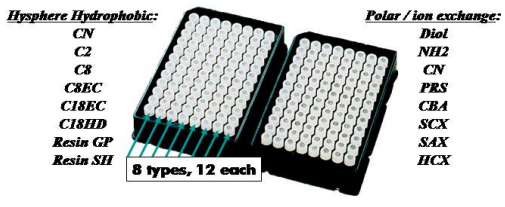

Figure 2: Method Development Cartridge Tray

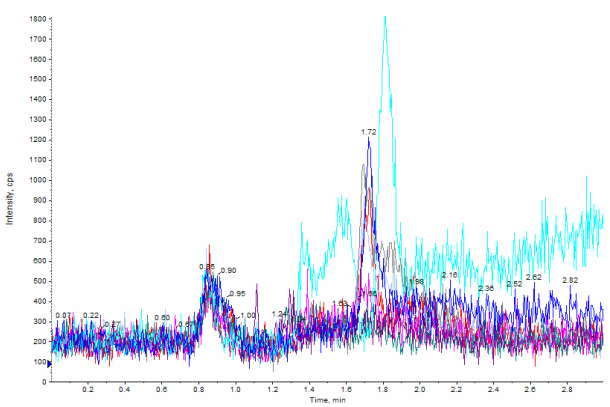

Figure 3: Chromatograms of Salbutamol in serum during sorbent screening using the HySphere hydrophobic MD tray

From Figure 3 can be derived that none of the 8 most common hydrophobic SPE sorbents gave reasonable recovery. To improve recovery an ion-exchange sorbent was explored. To elute the compounds of an ion-exchange sorbents stronger solvents are required. An integrated make-up flow (see figure 4) in the Symbiosis Pharma dilute the organic eluens just before the LC column and allows the compounds to focus on the top of the analytical column.

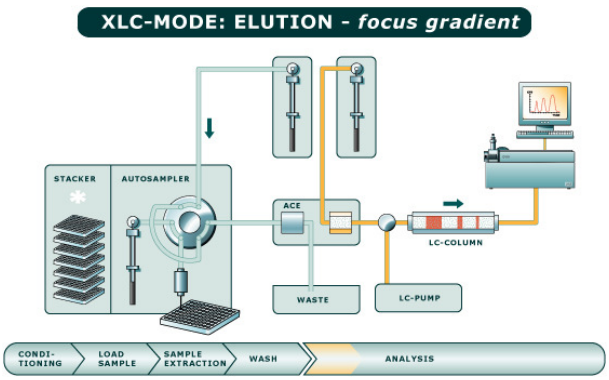

Figure 4: Schematic overview of flow path during peak focusing

To enable ion-exchange the secondary amine of Salbutamol was protonated using Formic Acid (pH 3.5). An OASIS MCX sorbent based on Cat-ion-exchange was tested for recovery (figure 4).

The overall recovery >90% was calculated from the LC run of a neat plasma injection and a XLC run of a serum sample (figure 5).

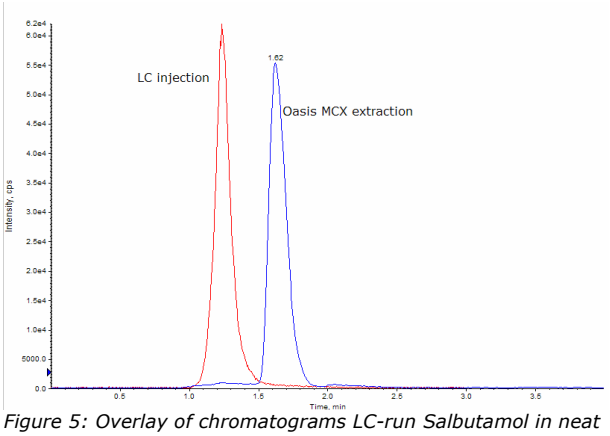

Figure 5: Overlay of chromatograms LC-run Salbutamol in neat solution and a XLC run Salbutamol in serum using OASIS MCX.

XLC–MS protocol

The plasma samples are processed with the developed XLC-MS method (as described below) using Symbiosis™ Pharma and an API-3000 System.

Figure 6: Symbiosis™ Pharma System

The XLC-MS method contains a protocol for:

- the autosampler (injection and wash routine)
- the Online SPE (extraction and clean-up)
- the LC gradient
- MS settings*

* The MS settings are stored in a separate MS acquisition file except when using the Analyst™ 1.4.1. software the XLC-MS method is then incorporated in the original Analyst acquisition method (.dam file).

Autosampler Conditions

20µL of sample is injected using the partial loop fill injection routine.
Washing is performed with two solvents:
Wash solvent1: 50% ACN with 0.1 % Formic Acid.
Wash solvent 2: 90% ACN.

Wash solvent	Wash volume
1	700 µL
2	700 µL
1	1500 µL

Table 1: wash routine autosampler.

SPE conditions

Cartridge:	10x2mm OASIS MCX (Waters Pn:186002098)	
Solvation:	1 mL ACN	5 mL/min
Equilibration:	1 mL 25% MeOH with 0.1 % Formic Acid	5 mL/min
Sample Loading:	0.5 mL 25% MeOH with 0.1 % Formic Acid	1 mL/min
Wash 1:	1 mL 25% MeOH with 0.1 % Formic Acid	5 mL/min
Wash 2:	1 mL 95% MeOH with 0.1 % Formic Acid	5 mL/min
Elution	100uL 3% NH4OH in MeOH	100 µL/min.

Table 2: SPE settings

LC conditions

Column:	Waters Xterra MS C18MS 2.1 x 50 mm. 3µ (Waters Pn:186000400)			
Mobile phase:	A: 10mM ammonium acetate (pH=9.5) B: ACN			
	Time (mm:ss)	Flow (ml/min)	A (%)	B (%)
	00:01	0.2	85	15
	00:05	0.1	85	15
	01:01	0.1	85	15
	01:05	0.2	85	15
	03:00	0.2	85	15
Run time:	3 minutes @ 0.2 mL/min			

Table 3: LC conditions

MS conditions

A Sciex API 3000 with a Turbo IonSpray is used.

Neb:	15
Cur:	10
IS:	2000
TEM:	400
CAD:	6
EP:	10

	Salbutamol	Atenolol
Q1 Mass	240.24	267.26
Q3 Mass	148.1	145.3
Dwell (ms)	150	150
DP	31	31
FP	150	150
CE	27	35
CXP	12	12

Table 4: MS parameters

Results

The following samples are prepared in new born calf serum using Atenolol (50 ng/mL) as internal standard.

- Calibration standards: 1.0; 2.0; 5.0; 10; 25; 50; 100; 250; 500 ng/mL.
- QC samples: 1.0; 250; 500 ng/mL.

Chromatograms

Figures 7 and 9 are representative chromatograms of the upper and lower limits of the calibration curve indicating the excellent quantitative suitability of XLC-MS. The carry over shown in figure 8 (blank injection) is calculated as <0.02%.

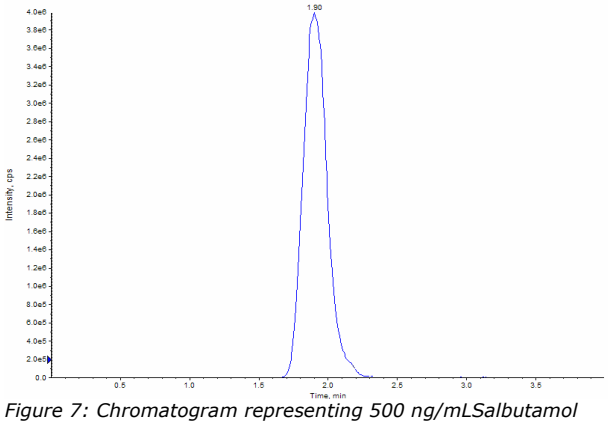

Figure 7: Chromatogram representing 500 ng/mLSalbutamol

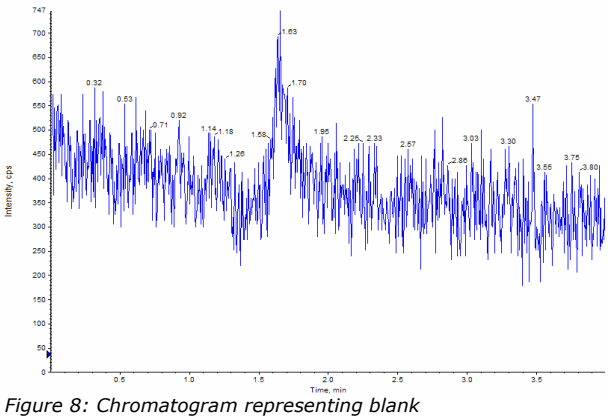

Figure 8: Chromatogram representing blank

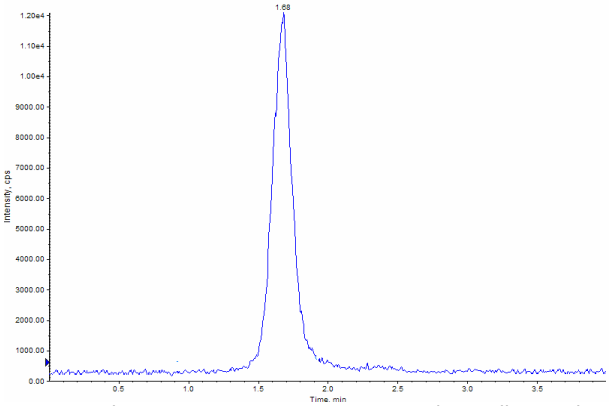

Figure 9: Chromatogram representing 1.0 ng/mL Salbutamol

Linearity, Accuracy and Precision

A calibration curve was determined by combining the results of 3 repeated injections of the full set of calibration standards.
This resulted in a R^2 of **0.997** with a 1/X weighting.

Exp. Conc. (ng/mL)	CV (%)	Accuracy (%)
1.00	6.28	118
2.00	5.37	105
5.00	2.06	94.8
10.00	6.89	96.3
25.00	3.22	92.3
50.00	2.59	89.2
100.0	6.07	99.0
250.0	5.07	101
500.0	10.4	101

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

Exp Conc. (ng/mL)	Sample Name	CV (%)	Accuracy (%)
1.0	QC 1	4.88	115
250	QC 2	7.88	103
500	QC 3	5.80	111

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

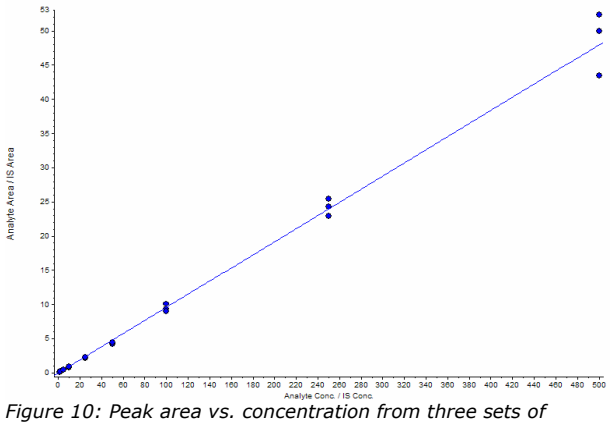

Figure 10: Peak area vs. concentration from three sets of calibration standards.

Conclusions

From this study it is concluded that within a time frame of two days it is possible to develop a Mixed Mode XLC-MS method with an absolute recovery >90%; run three sets of calibration standards with a linear range from 1-500 ng/mL (R^2 of 0.997) with accuracy between 90-105% and a precision of < 15%.
The total XLC-MS time consists of the sample preparation time + HPD focusing + LC-MS runtime. Since the sample prep + HPD focusing is executed in parallel with the LC, the total XLC-MS time is less then 4 minutes.