APPLICATION INFO

DETERMINATION OF CARBAMAZEPINE IN SERUM BY XLC-MS/MS USING 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.

Carbamazepine an anticonvulsant medication is used to treat certain types of seizures in patients with epilepsy. It is also used to treat trigeminal neuralgia, a condition that causes facial nerve pain. Brand names are: Carbatrol; Tegretol; Tegretol-XR. Carbamazepine is usually measured in Human Serum.

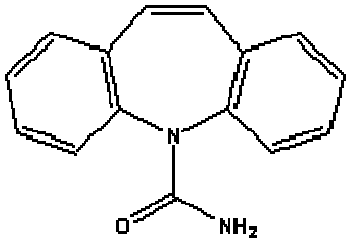

Figure 1: Carbamazepine

- CAS#00298-46-4
- C15H12N2O
- Mw 236.27
- Physical Properties:
  - Water solubility 17.7 mg/L
  - Log P (Octanol-water) 2.45
  - pKa dissociation constant 13.9

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

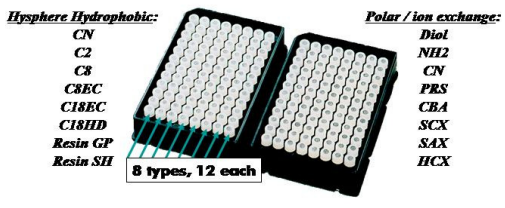

Figure 2: Method Development Cartridge Tray

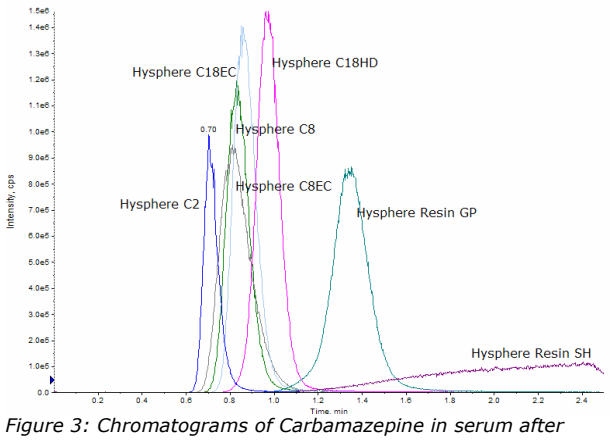

Figure 3: Chromatograms of Carbamazepine in serum after sorbent screening using the HyShpere Hydrophobic MD tray.

From figure 3 can be derived that C18HD gives the highest signal and also the best peak shape using the standard Symbiosis™ XLC protocol. The protocol using C18HD was further optimized by changing the wash conditions (see figures 4 and 5).

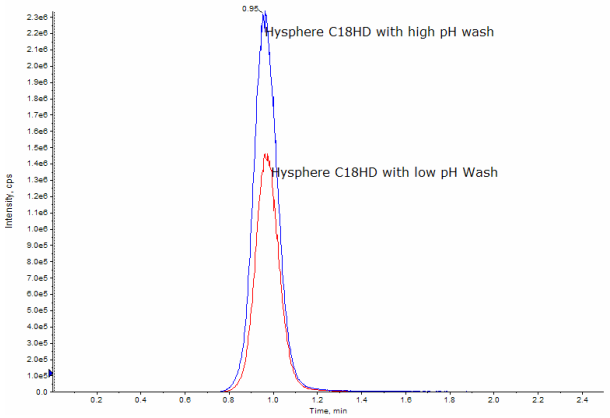

Figure 4: Optimizing pH during wash.

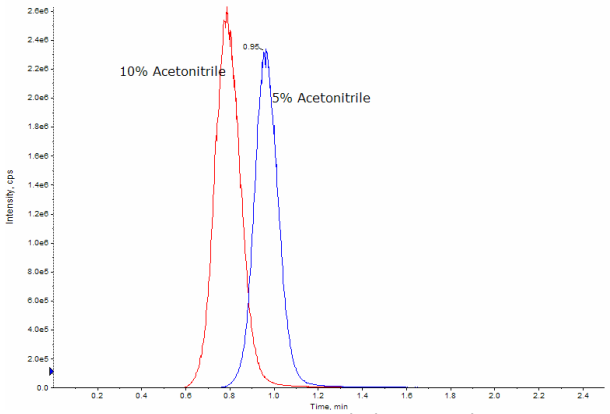

Figure 5: Optimizing organic strength during wash

After optimizing the wash protocol a recovery >95% was calculated from the peak area of a neat solution and serum sample (figure 6).

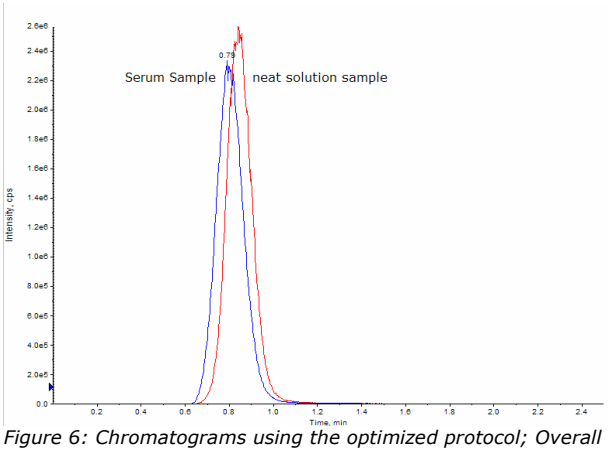

Figure 6: Chromatograms using the optimized protocol; Overall recovery >95%

XLC-MS protocol

The serum samples are processed with the developed XLC-MS method (as described below) using Symbiosis Pharma and an API-4000 System.

Figure 7: 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 parameters
- 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

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

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

Cartridge:	10x2mm HySphere C18HD (Spark Pn:0722.609)	
Solvation:	1 mL ACN	5 mL/min
Equilibration:	1 mL 10% ACN 3%NH4OH	5 mL/min
Sample Loading:	1 mL 10% ACN 3%NH4OH	2 mL/min
Washing:	1 mL 10% ACN 3%NH4OH	5 mL/min
Elution:	30 Seconds	
Clamp flush:	2 mL 90% ACN + 1 mL Water	5 mL/min

Table 2: SPE settings; Total SPE time is 2 min 30 sec.

LC conditions

Column:	Spark HySphere C18HD 10 x 2 mm. (Spark Pn:0722.609)
Mobile phase	30% ACN in 0.1% Formic Acid.
Flowrate	0.5 mL/min.

Table 3: LC conditions.

MS conditions

A Sciex API 4000 ms with a Turbo IonSpray is used.

Cur:	40.00
GS1:	45.00
GS2:	45.00
IS:	4600
TEM:	450
CAD	4

	Carbamazipine
Q1 Mass	237.04
Q3 Mass	194.20
Dwell (ms)	150
DP	71
EP	10
CE	29
CXP	16

Table 4: MS Parameters

Results

The following samples are prepared in new born calf serum. No internal standards were used.

- Calibration standards: 0.05; 0.1; 0.5; 1.0; 5.0; 10; 50; 100 ng/mL.
- QC samples: 0.5; 10; 100 ng/mL.

Chromatograms

Figures 8 and 10 are representative chromatograms of the upper and lower limits of the calibration standards indicate excellent quantitative suitability of the XLC-MS method. The carry over is calculated as <0.01% (figure 9).

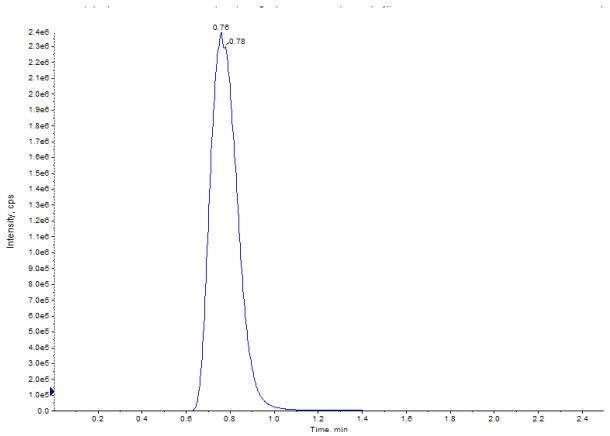

Figure 8: Chromatogram representing 100 ng/mL Carbamazepine

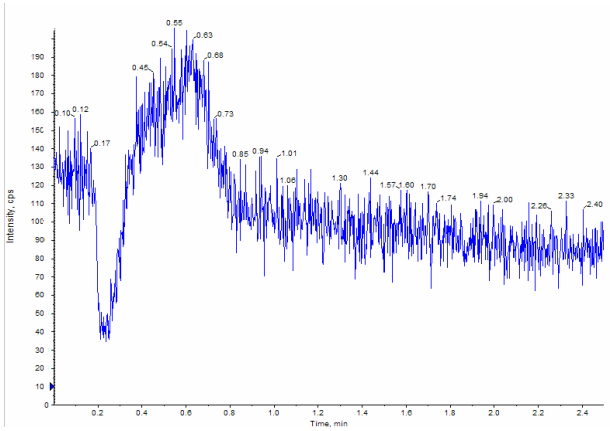

Figure 9: Chromatogram representing blanc

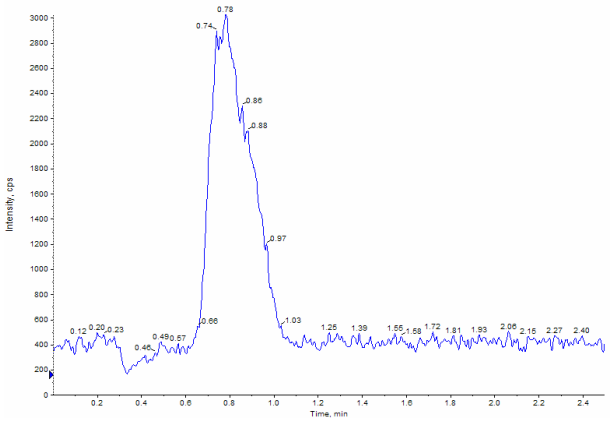

Figure 10: Chromatogram representing 0.05 ng/mL Carbamazepine

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 **0.999** with a 1/X weighting.

Exp. Conc. (ng/mL)	Sample Name	CV (%)	Accuracy (%)
0.05	0.05	10.9	118
0.10	0.1	9.81	113
0.50	0.5	4.75	88.0
1.00	1	3.90	86.6
5.00	5	3.71	95.9
10.00	10	1.43	93.9
50.00	50	1.80	107
100.00	100	2.25	97.5

Table 6: Accuracy and Precision calculated from three combined calibration standards.

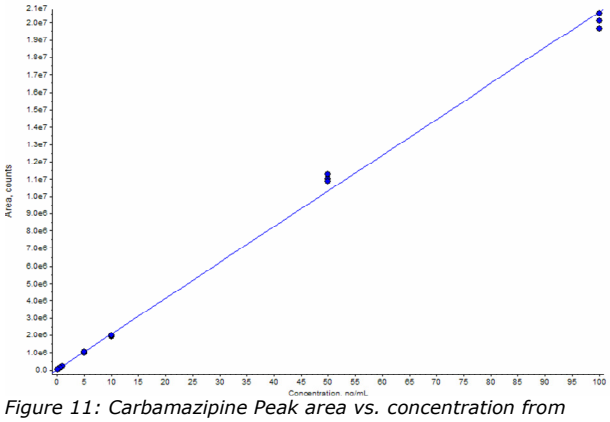

Figure 11: Carbamazipine Peak area vs. concentration from three sets of calibration standards.

Exp. Conc. (ng/mL)	Sample Name	CV (%)	Accuracy (%)
0.50	QC 0.1	2.67	86.6
10.00	QC 10	1.61	96.0
100.00	QC 100	2.85	96.1

Table 7: Accuracy and Precision calculated from three sets of QC standards

Reproducibility

To determine the reproducibility of the XLC-MS method a batch of 192 samples containing 20 ng/mL Carbamazipine in serum are processed in an overnight run. Figure 12 displays the individual peak areas uncorrected for internal standard (RSD is 2.55%).

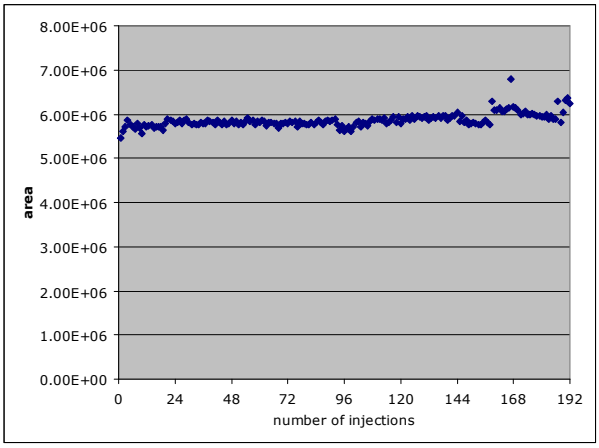

Figure 12: XLC-MS reproducibility, individual peak area of Carbamazepnine (no correction for IS) in an overnight run of 192 serum samples.

Conclusions

From this study it is concluded that within a time frame of two days it is possible to develop a XLC-MS method with an absolute recovery >95%; run three sets of calibration standards with a linear range from 0.05-100 ng/mL (R^2 of 0.999) with a accuracy between 86-118% and a precision of <15% CV; and process a batch of 192 samples with a reproducibility of 2.55% RSD.

The total XLC-MS cycle time consists of the sample preparation time + LC-MS runtime. Since the sample prep is executed in parallel with the LC, the total XLC-MS time is 2.5 minutes per sample.
The batch of 192 samples was processed in 8 hrs.

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