APPLICATION INFO

# DETERMINATION OF BUPRENORPHINE IN HUMAN PLASMA 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 will demonstrate the compatibility of the Symbiosis™ Pharma to add internal standard during a XLC-MS routine with excellent accuracy, precision and linearity over the whole calibration range.

**Buprenorphine** (BUP) is a powerful semi-synthetic analgestic. BUP's molecular structure it related to Morphine, but it is estimated to be least 25 times more potent. Initially, it was available for the treatment of acute and chronic pain. (Brand names are: Temgesic®, Buprenex® and Subutex®.

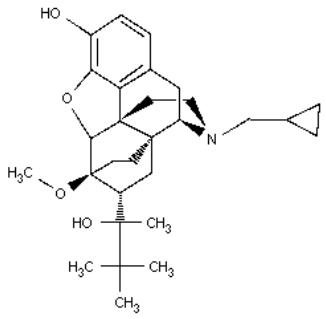


Figure 1: Buprenorphine, CAS# 52485-79-7, $C_{29}H_{41}NO_4$, Mw 467.6472. Physical properties: Water solubility 0.63 mg/L, LogP 4.98, pKa dissociation constant 8.31.

## 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 pre-defined SPE conditions of the Symbiosis™ Pharma.

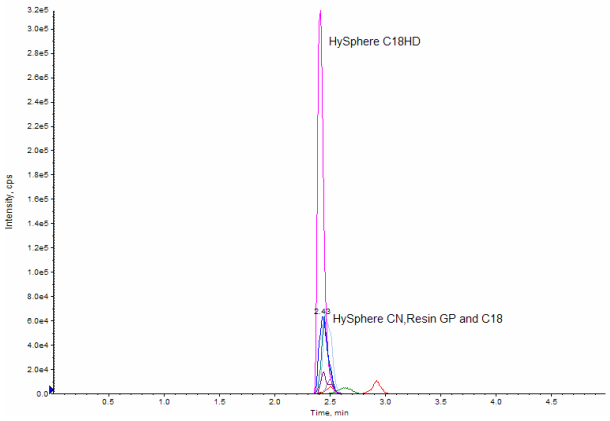


*Figure 3: Chromatogram of Buprenorphine in human plasma during sorbent screening.*

After the sorbent screening the C18HD cartridge is selected and the wash procedure is optimized.

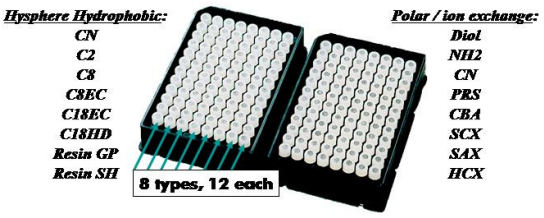


*Figure 4: Method Development Cartridge Tray*

After optimizing the wash protocol an overall recovery of >95% was calculate from the peak area of a neat solution and serum sample (figure 4)

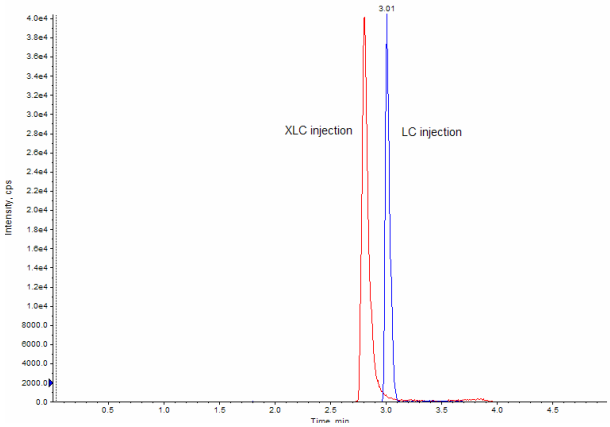


*Figure 5: Overlay of 10 ng/mL plasma XLC sample and 10 ng/mL aqueous LC sample.*

# XLC-MS Protocol

## Autosampler Conditions

30 µL sample is injected using µL pick up mode injection routine. In this injection mode the autosampler adds the internal standard (Buprenorphine D4) to sample.

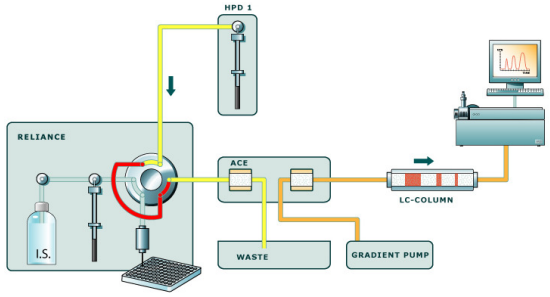


*Figure 6: Flow path of Symbiosis Pharma with I.S. addition*

Washing is performed with two wash solvents;
Wash solvent 1: 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 | 1400 µL | yes |

*Table 1: Autosampler wash routine.*

## 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 1% $NH_4OH$ pH 9.5 | 5 mL/min |
| Sample Loading: | 0.5 mL 40% ACN in 1% $NH_4OH$ pH 9.5 | 1 mL/min |
| Washing: | 1 mL 40% ACN in 1% $NH_4OH$ pH 9.5 | 5 mL/min |
| Elution | 2 min. with LC Gradient | |
| Total SPE time is 2 min 30 sec. | | |

*Table 2: SPE conditions.*

## LC conditions

| | |
|---|---|
| Column: | Waters Xterra® MS C18 2.1 X 50 mm 3.5µ |
| 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 (%) |
|---|---|---|---|
| 00:01 | 0.30 | 90 | 10 |
| 00:05 | 0.30 | 90 | 10 |
| 02:35 | 0.30 | 10 | 90 |
| 02:50 | 0.30 | 10 | 90 |
| 03:00 | 0.30 | 90 | 10 |
| 05:00 | 0.30 | 90 | 10 |

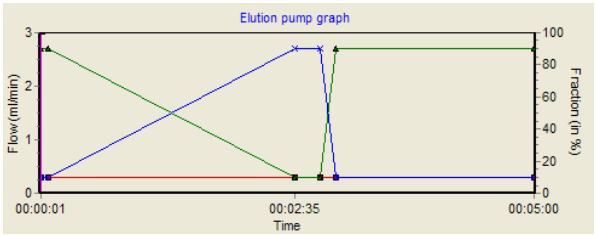


*Figure 7: LC gradient*

## MS Conditions

A Sciex API 3000 with a Turbo IonSpray in positive mode is used.

| | Buprenorphine | Buprenorphine D4 (IS) |
|---|---|---|
| Q1 mass | 468.45 | 475.45 |
| Q3 mass | 55.10 | 59.10 |
| Dwell time | 150 | 150 |
| DP | 86 | 86 |
| FP | 270 | 270 |
| CE | 103 | 103 |
| CXP | 4 | 4 |

*Table 3: Compound dependable MS settings. (MS parameters: NEB = 15; CUR = 13; IS = 5500; TEM = 400; CAD = 6; EP = 10)*

# Result

The following samples are prepared in Human Plasma. The autosampler adds the internal standard during the injection routine (Vial-to-File™ concept).

- Calibration standards: 0.1; 0.5; 1.0; 5.0; 10; 50; 100 ng/mL
- QC samples: 0.1; 1; 10; 100 ng/mL

## Chromatograms

Figure 8 and 10 are representing chromatograms of the upper and lower limits of the calibration curve. The carry-over represented in the chromatogram of the blank (figure 9) is calculated as <0.02% (< 20% of LOQ).

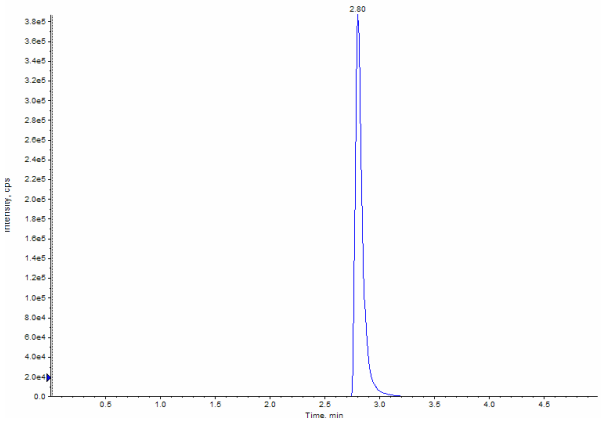


*Figure 8: Chromatogram representing 100 ng/mL Burpenorphine.*

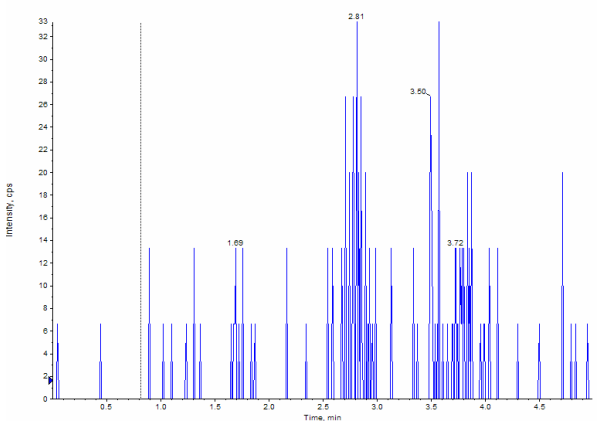


*Figure 9: Chromatogram representing a blank after 100 ng/mL.*

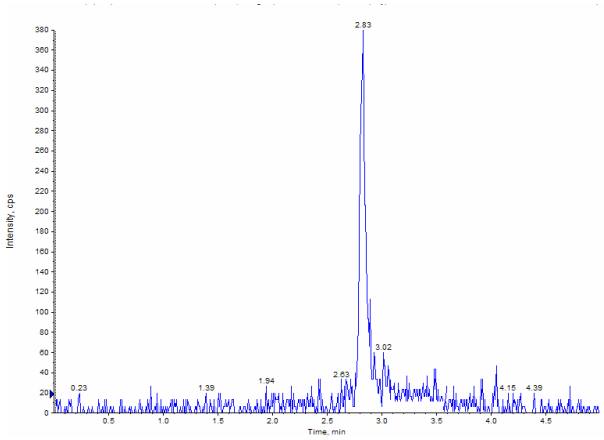


*Figure 10: Chromatogram representing 0.1 ng/mL Burpenorphine.*

## Linearity, Accuracy and Precision

A calibration curve was determined by combining the results of 6 repeated injections of a full set of calibration standards. This resulted in a $R^2$ of 0.997 with 1/X Weighting.

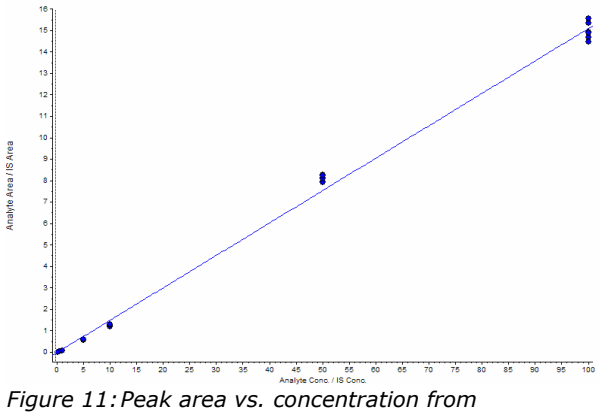


*Figure 11: Peak area vs. concentration from Buprenorphine (corrected for Internal Standard) from the 6 combined set of calibration standards.*

| Sample (ng/mL) | CV (%) | Accuracy (%) |
|---|---|---|
| 0.1 | 4.78 | 105 |
| 1.0 | 1.92 | 93.4 |
| 10 | 1.13 | 89.1 |
| 100 | 2.84 | 100 |

*Table 4: Accuracy and Precision calculated from 4 QC series*

## Reproducibility

In this application, the internal standard is added by the autosampler.
In determine the reproducibility, the area of the internals standard of 105 samples are displayed in figure 12. The calculated %RSD of the internal standard is 3.9%

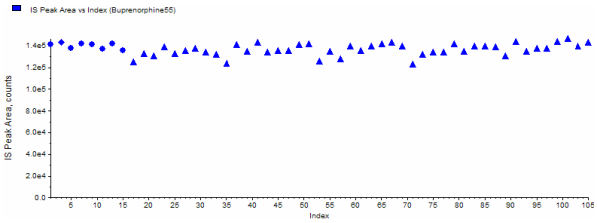


*Figure 12: Peak Area of internal standard in plasma RSD = 3.9%*

## 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 of >95%, and run 6 sets of calibration standards with a linear range from 0.1-100 ng/mL ($R^2$ = 0.998 with a 1/X weighting). The developed method has an accuracy between 85-105% and a precision of <5% CV.
Furthermore it shows that the Reliance™ autosampler in Symbiosis™ Pharma is able to add internal standards with an excellent reproducibility of 3.9% RSD.

## 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.

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