APPLICATION INFO

Determination of Chloramphenicol in eggs by XLC-MS using the Symbiosis Pharma System

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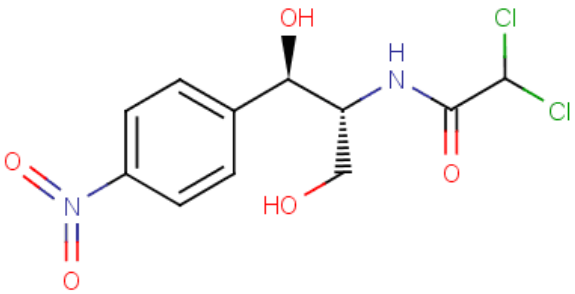
Introduction

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).

Figure 1: Symbiosis Pharma System



Chloramphenicol, Mw 323 with LogP = 1.2,
C11H12Cl2N2O5, CAS#56-75-7, pKa=11.5

It was originally isolated from a species of *Streptomyces* bacteria. Chloramphenicol's antibiotic activity results from its interference with protein synthesis in invading microbes. However, it is a very toxic substance, its most serious and potentially lethal effect being depression of red blood cell production in bone marrow; cases of leukemia were also attributed to early use of chloramphenicol. Because of its toxicity, chloramphenicol is rarely prescribed for infections that can be treated by other antibiotics.

Method Development

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.

For Chloramphenicol a new spe method is developed using the sorbent screening approach.
With this approach the 8 HySphere cartridges in the method development tray are screened. The screening is performed with an acid and a base wash. For chloramphenicol the 1 mL 5% acn in 2% NH4OH wash gave the highest recovery (lowest breakthrough) compared to a LC injection.

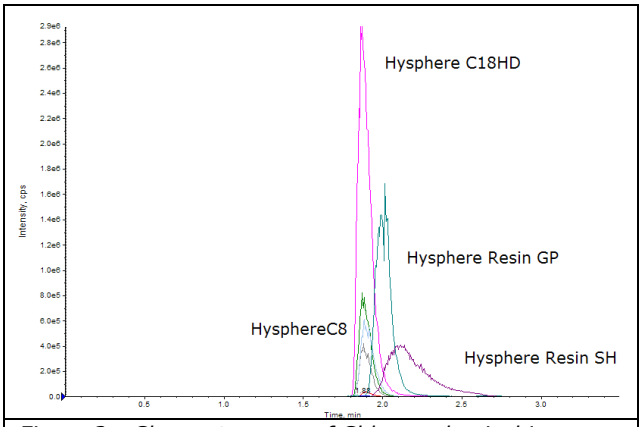


Figure 2: Chromatograms of Chloramphenicol in egg after sorbent screening using the HySphere hydrophobic MD tray and a base wash.

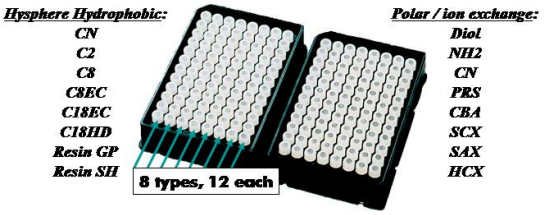


Figure 3: Method Development Cartridge Tray

XLC-MS Protocol

Autosampler Conditions

100 µL of egg sample supernatant is injected using a standard autosampler configuration. Autosampler cooling was set to 15 degrees Celsius.

Washing is performed with two wash solvents;

Wash solvent 1	40% ACN in 0.1% FA
Wash solvent 2	90% ACN

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 2 % NH4OH	5 mL/min
Sample Loading:	1 mL 5% ACN in 2 % NH4OH	2 mL/min
Washing:	1 mL 5% ACN in 2 % NH4OH	5 mL/min
Elution	1 min. with LC Gradient	
Matrix:	egg	

LC conditions

Column:	Waters Xterra MS C18 3X50 mm 5µ
Mobile phase A:	Water
Mobile phase B:	MeOH

Table 2: LC gradient

Time (mm:ss)	Flow (mL/min.)	A (%)	B (%)
00:00:01	0.50	90	10
00:00:05	0.50	90	10
00:01:05	0.50	10	90
00:01:30	0.50	10	90
00:02:00	0.50	90	10
00:03:30	0.50	90	10

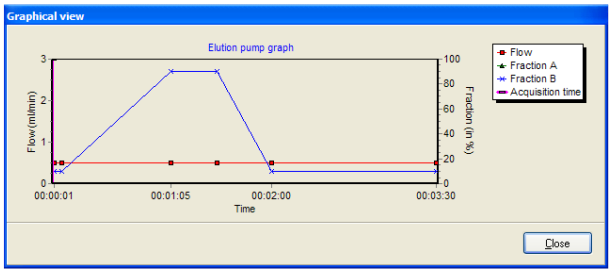


Figure 6: LC pump gradient

A 1 to 1 split is used allowing 250 µL to enter the MS.

MS Conditions

A Sciex API 3000 with a Turbo IonSpray in negative mode is used to analyze the samples.

Table 3: MS parameters

CUR	10
IS	- 4500
TEM	350
NEB	10
Turbo gas 2	8000
CAD	5

Table 4: Compound dependable MS settings

	Chloramphenicol
Q1 mass	321.03
Q3 mass	152.00
Dwell time	150
DP	- 31
FP	- 220
EP	- 10
CE	- 24
CXP	- 1

Results

Sample preparation.

3 g of egg sample is homogenized in 12 ml sodium acetate buffer (pH5), incubated with β-glucuronidase (2 hours at 37°C) and then centrifuged.
The supernatant is taken through the SPE procedure described in the XLC-MS protocol section.

The following samples are spiked in egg sample supernatant.

- Calibration standards:0.0; 0.3; 0.6; 0.9 ng/mL
- QC samples: 0.3; 0.6; 0.9 ng/mL

Chromatograms

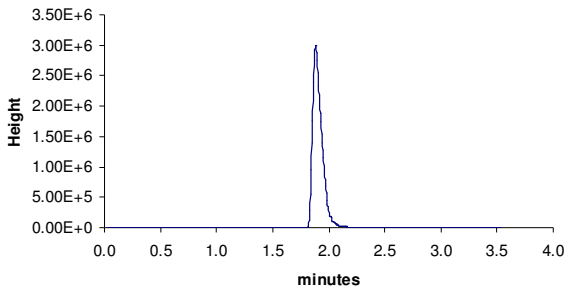


Figure 7: Chromatogram representing 1000 ng/mL Chloramphenicol.

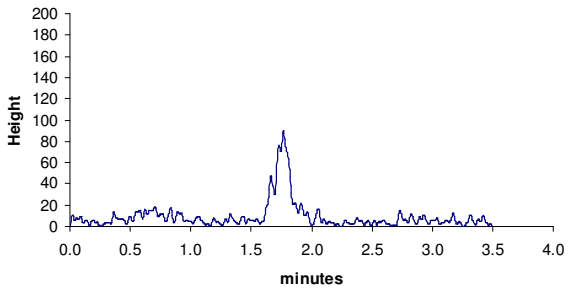


Figure 8: Chromatogram representing blank (15% of LLOQ)

Linearity, Accuracy and Precision

A calibration set of egg samples was determined by injecting a full set of calibration standards. For the calibration curve a regression is calculated of 0.999 with a 1/X weighting.

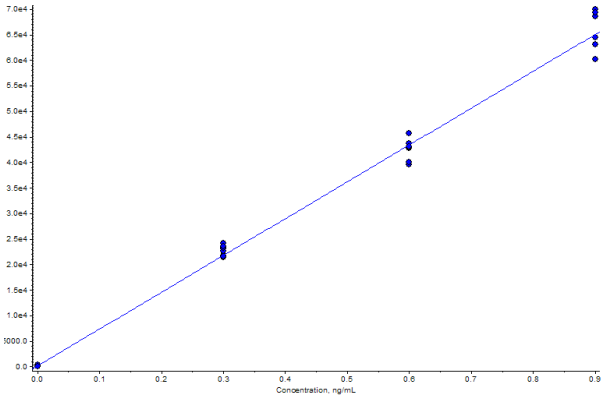


Figure 9: Calibration curve of Chloramphenicol R^2=0.999 With a 1/X weighting

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

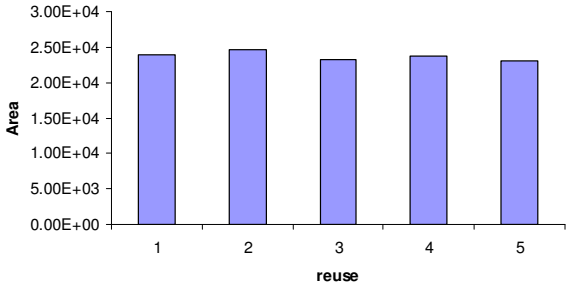
Sample (ng/mL)	CV (%)	Accuracy (%)
0.3	4.90	103
0.6	4.91	98.0
0.9	6.49	100

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

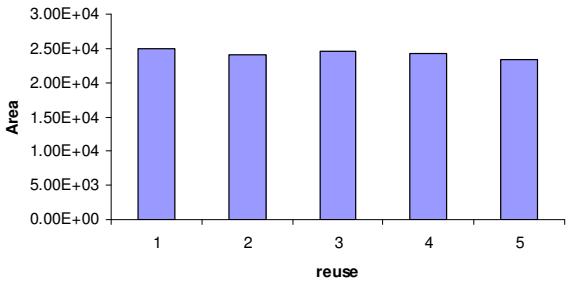
Sample (ng/mL)	CV (%)	Accuracy (%)
QC 0.3	9.31	112
QC 0.6	1.12	97.7
QC 0.9	1.84	97.1

Reuse of cartridges.

To determine the effect on performance of cartridge reuse, 2 series of 6 injections were performed using a single cartridge. There is no significant decrease in accuracy after 6x cartridge reuse.



Reuse of cartridge 1.



Reuse of cartridge 2.

Cartridge	accuracy
Cartridge 1	2.47
Cartridge 2	2.53

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 >100% and a set of calibration standards with a linear range from 0.0 to 0.9 ng/mL (R^2 0f 0.999) and an accuracy between 96-112%. This is achieved without the use of an internal standard. Carry-over is less then 15% of the LLOQ, The total XLC-MS time consist 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 4 minutes.

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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