APPLICATION INFO

DETERMINATION OF QUINAPRIL AND TWO METABOLITES BY TANDEM ONLINE SPE USING SYMBIOSIS™ PHARMA

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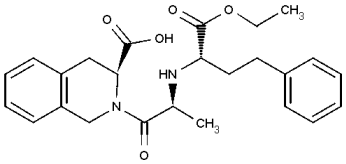
0053.063-01

Introduction

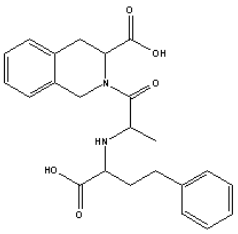
Symbiosis™ Pharma is Spark Holland's unique solution for integrated online SPE-LC-MS automation (XLC-MS). The system offers large flexibility in processing different types of samples selecting one of the three fully automated operational modes LC-MS; XLC-MS; AMD (Advanced Method Development).

This application info describes how Quinapril is analyzed together with two of its most common metabolites; Quinaprilat and Hydrochlorothiazide (HCT) using a tandem SPE setup in the Symbiosis Pharma.
For Quinapril and Quinaprilat as internal standard Benazepril is used and for HCT Hydroflumethiazide is selected as internal standard.

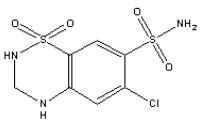


Quinapril (MW = 438.53, LogP = 3.72, $C_{25}H_{30}N_2O_5$, pKa = 3.2 and 5.5, CAS: 85441-61-8, Solubility: 0.1 mg/L)

Metabolites:



Quinaprilat (LogP = 3.2)



Hydrochlorothiazide (LogP = 0.5)

Quinapril belongs in a class of drugs called Angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors are used for treating high blood pressure and heart failure and for preventing kidney failure due to hypertension and diabetes. Angiotensin converting enzyme play an important role in the production of Angiotensin II.

Angiotensin II contracts the muscles of the arteries in the heart and the rest of the body, narrowing the arteries and thereby elevating blood pressure. ACE inhibitors such as Quinapril lower blood pressure by inhibiting the formation of Angiotensin II, thereby relaxing the arterial muscles and enlarging the arteries. Quinapril was approved by the FDA in November, 1991.

Method Development

Figure 1: Symbiosis™ Pharma System

The AMD mode of Symbiosis Pharma in conjunction with the HySphere method development cartridge tray (Spark PN 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 predefined SPE conditions of the Symbiosis Pharma.

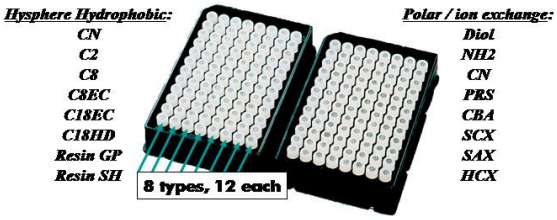


Figure 2: Method Development Cartridge Tray

Eight HySphere reversed Phase cartridges were tested for the recovery of Quinapril, Quinaprilat and Hydrochlorothiazide. The results of this quick sorbent screening are presented in the chromatograms in figure 3 and 4.

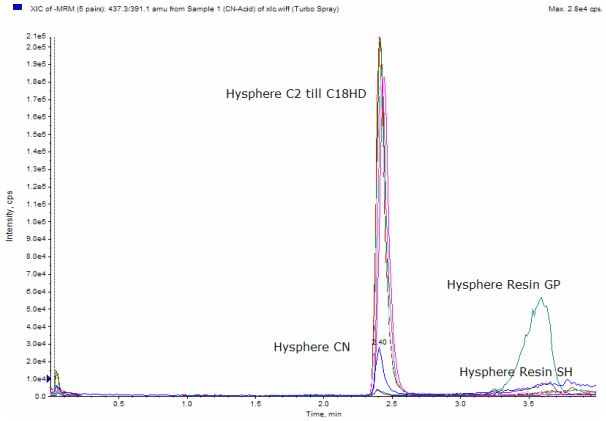


Figure 3: Chromatograms of Quinapril in serum after sorbent screening using HySphere hydrophobic MD tray.

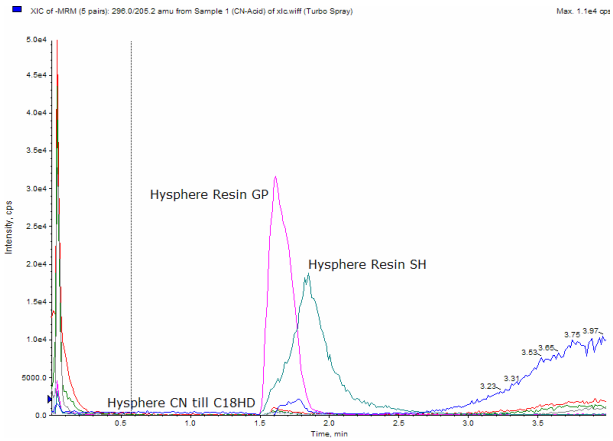


Figure 4: Chromatograms of Hydrochlorothiazide in serum after sorbent screening using the HySphere hydrophobic MD tray.

Sample Name	Quinapril (%)	Quinaprilat (%)	HCT (%)
Direct injection	100	100	100
CN-Acid	12.2	18.2	4.02
C2-Acid	93.9	101	1.24
C8-Acid	93.7	92.4	No Peak
C8(EC)- Acid	87.2	90.3	No Peak
C18-Acid	84.8	91.3	1.04
C18HD-Acid	**96.1**	**96.6**	0.74
GP-Acid	73.7	89.4	**90.1**
SH-Acid	10.9	10.4	51.4

Table 1: Recoveries of the three compounds in New born calf serum compared to a Neat solution LC run (5% ACN 0.1% FA wash)

For Quinapril and Quinaprilat; there are good recoveries on six HySphere cartridges but on the Resin GP the peak shape is very poor. For the non polar HCT only the Resin GP gives a good result. In order to extract all 3 compounds, two cartridges are placed in series. In one XLC (SPE) run.
In this application, the C18HD cartridge is placed in the left clamp and the Resin GP in the Right clamp. During XLC the two cartridges are in-line. For elution, the cartridges are eluted after each other. First the Resin GP is analyzed for 2.5 minutes and secondly the C18HD for 2.5 minutes.

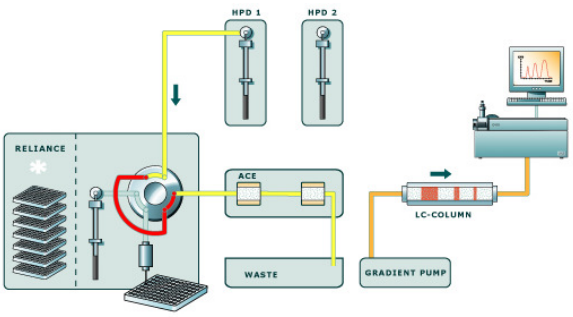


Figure 5 On-line tandem SPE configuration

XLC-MS Protocol

Autosampler Conditions

50 µL sample is injected using the partial loop fill injection routine at a temperature of 6 ºC.

Washing is performed with two wash solvents;
Wash solvent 1: 50% ACN with 1% Triethylamine (TEA) pH=11.0.
Wash solvent 2: 90% ACN.

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

Table 2: Autosampler wash routine.

SPE conditions

Cartridge 1:	10 x 2 mm C18HD (Spark PN:0722.609)	
Cartridge 2:	10 x 2 mm HySphere GP (Spark PN:0722.610)	
Solvation:	2 mL ACN	4 mL/min
Equilibration:	2 mL 5% ACN in 0.1 % FA	4 mL/min
Sample Loading:	1 mL 5% ACN in 0.1 % FA	2 mL/min
Washing:	1 mL 5% ACN in 0.1 % FA	5 mL/min
Clamp flush:	1 mL 10% ACN	5 mL/min
Elution	1 min. with LC Gradient (2X)	

Table 3: SPE settings

LC conditions

Column:	Waters Xterra MS C18 3.5 µ 50x4.6 mm (Waters PN: 186000432)
Mobile phase A:	5 mM Ammonium Formate pH = 3.5 (pH adjusted with Formic Acid)
Mobile phase B:	Methanol
Run time:	5 minutes @ 1.0 mL/min
	Equilibration time between injections is 30 sec.

Time (mm:ss)	Flow (mL/min.)	A (%)	B (%)
00:00	1.0	50	50
01:00	1.0	10	90
01:30	1.0	10	90
01:45	1.0	50	50
02:30	1.0	50	50

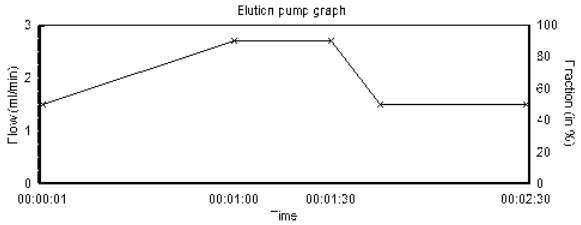


Figure 6: LC gradient

MS Conditions

An API 3000 with an ESI source in negative mode is used for analyzing the samples.

	Quinapril	Quinaprilat	Benazepril	HCT	HFT
Q1 mass	437.26	409.25	423.24	296.05	330.07
Q3 mass	391.10	175.70	174.10	205.20	238.90
Dwell time	150	150	150	150	150
DP	-21	-41	-51	-76	-76
FP	-140	-210	-220	-300	-230
CE	-12	-22	-28	-32	-34
CXP	-5	-13	-1	-11	-7
HFT = Hydroflumethiazide					

Table 4: MS settings, with the following parameters; NEB = 15, CUR = 10, IS = -4200, TEM = 400, CAD = 400.

Samples are analyzed in two MS experiment periods. In the first 2.5 minutes Hydrochlorothiazide (and I.S) is measured and in the second 2.5 minutes Quinapril and Quinaprilat (and I.S).

Results

The following samples are prepared in new born calf serum using Benazepril and Hydroflumethiazide as internal standard.

- Calibration standards: 2;5; 10; 20; 50; 100; 200; 500; 1000 and 2000 ng/mL
- QC samples; 10, 100 and 1000 ng/mL
- Reproducibility standard; 200 ng/mL

Chromatograms

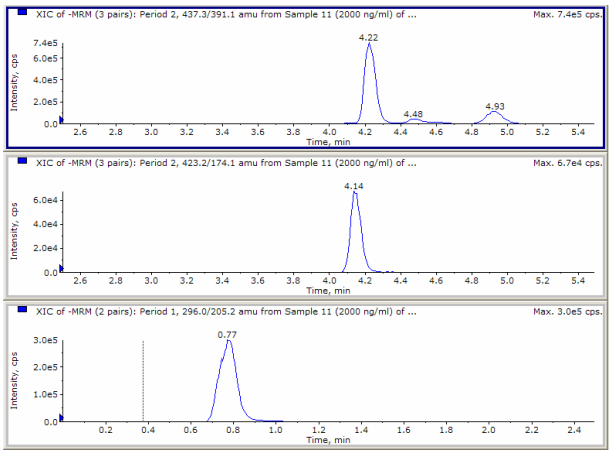


Figure 7: Example of an XLC chromatogram. 2000 ng/mL in Calf serum, 50 µL injected

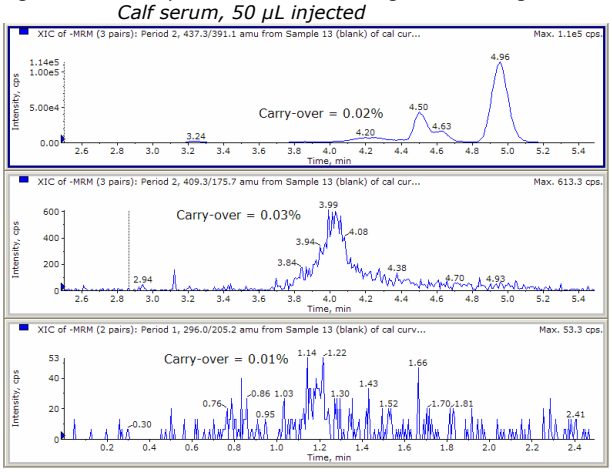


Figure 8: Carry-over after a 2000 ng/mL calf serum Std.

Linearity, Accuracy and Precision

A calibration curve was determined by combining the results of three repeated injections of a full set of calibration standards. This resulted in a R^2 of 0.999 for Quinapril and Quinaprilat and 0.996 for Hydrochlorothiazide using a 1/X weighting.

Sample name (ng/mL)	CV (%)	Accuracy (%)
2	11.3	116
5	8.70	97.9
10	3.12	96.3
20	3.89	90.1
50	10.8	95.8
100	9.73	97.8
200	4.99	100
500	1.39	109
1000	1.39	95.0
2000	2.63	100

Table 5: Three combined calibration curves of Quinapril.

Sample Name (ng/mL)	CV (%)	Accuracy (%)
QC 10	9.14	102
QC 100	4.10	102
QC 1000	1.63	97.9

Table 6: two QC series of Quinapril (n=6)

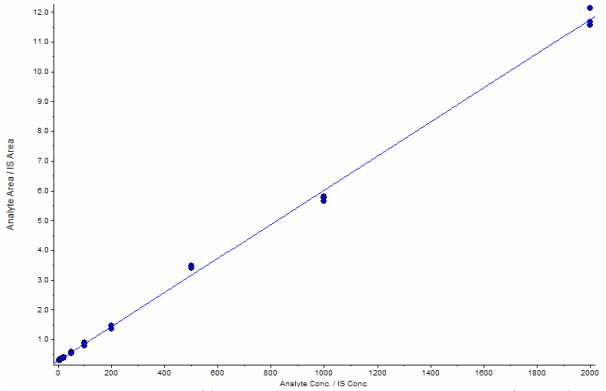


Figure 9: Quinapril/IS peak area vs. concentration from three sets of calibration standards

Sample name (ng/mL)	CV (%)	Accuracy (%)
5	1.78	97.3
10	0.24	95.4
20	2.28	97.8
50	1.45	107
100	1.81	104
200	0.78	96.1
500	1.65	106
1000	0.69	93.5
2000	1.35	101

Table 7: Three combined calibration curves of Quinaprilat (n=3)

Sample Name (ng/mL)	CV (%)	Accuracy (%)
QC 10	1.87	96.9
QC 100	2.15	103
QC 1000	1.37	97.8

Table 8: Two QC series of Quinaprilat (n=6)

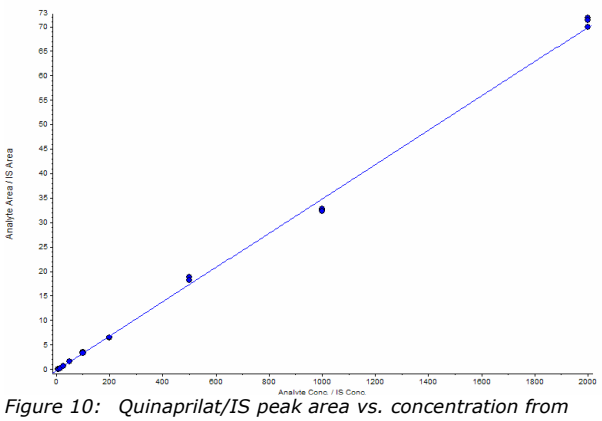


Figure 10: Quinaprilat/IS peak area vs. concentration from three sets of calibration standards

Sample (ng/mL)	CV (%)	Accuracy (%)
2	6.34	102
5	7.59	91.0
10	11.5	93.6
20	4.22	97.9
50	10.1	102
100	5.32	107
200	1.54	104
500	9.11	103
1000	6.27	101
2000	13.3	98.0

Table 9: Accuracy and precision calculated from 3 combined calibration curves of HCT

Sample (ng/mL)	CV (%)	Accuracy (%)
QC 10	1.87	96.9
QC 100	2.15	103
QC 1000	1.37	97.8

Table 10: Accuracy and precision calculated from 2 QC series of HCT

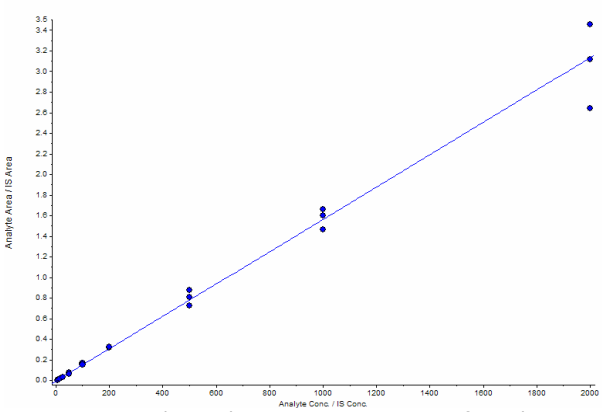


Figure 11: HCT/IS peak area vs. concentration from three sets of calibration standards

Reproducibility

The robustness of the XLC-MS/MS Method is tested by injecting fifty samples with a 200 ng/mL concentration of each compound. The peak area after each injection is plotted in figure 12-14.

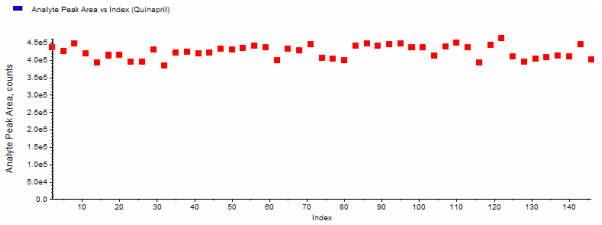


Figure 12: 50 injections of Quinapril (no I.S. compensation) CV = 4.53%

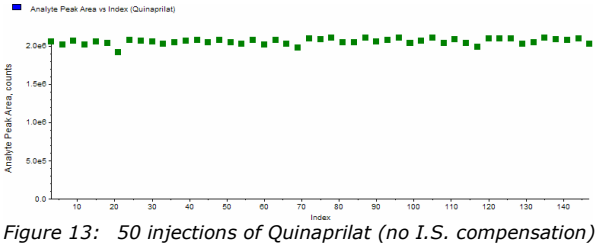


Figure 13: 50 injections of Quinaprilat (no I.S. compensation) CV = 1.84%

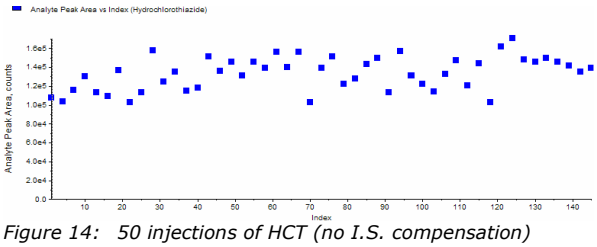


Figure 14: 50 injections of HCT (no I.S. compensation) CV = 11.23%

System Pressure

In total 160 samples are injected for this Application info; Method development, calibration curves; QC and reproducibility. The pressure increased by 3 bars only from 167 to 170 bars.

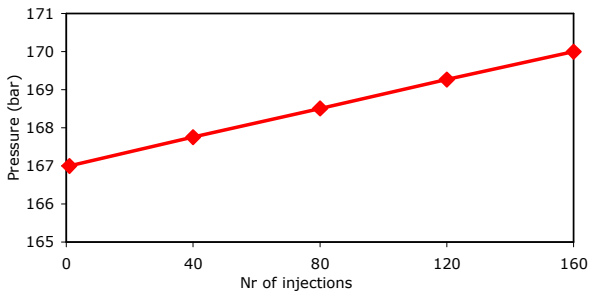


Figure 16: System pressure over 160 samples

Conclusions

From this study it is concluded that within a time frame of 1.5 days it is possible to develop a XLC-MS method and run a set of calibration standards with a linear range from 2 to 2000 ng/mL (R^2 of 0.999 for Quinapril and Quinaprilat and 0.996 for Hydrochlorothiazide) and accuracy between 91-116%.
The robustness of the developed tandem SPE method using the symbiosis™ Pharma system is shown by the excellent reproducibility of the peak heights of 50 sub sequential injections without correcting for IS the CV was <5%.

About Spark

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