Application Note: ANCCSSOLAEPRL

LC-MS/MS Method for the Determination of Enalapril and Enalaprilat from Human Plasma Using SOLA

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Key Words

- SPE
- SOLA Cartridges and Plates
- Hypersil GOLD
- Enalapril
- Enalaprilat

Abstract

A Liquid Chromatography tandem Mass spectrometry method for enalapril and enalaprilat from human plasma has been developed. Using Thermo Scientific SOLA cartridges, sample preparation is fast and efficient and provides excellent recovery levels for each compound. Analysis was carried out on a Thermo Scientific Hypersil GOLD 1.9µm 50 x 2.1mm column for a fast separation and a cycle time of 1 minute whilst maintaining excellent peak shape. The dynamic range was linear between 1 and 100ng/mL with a r2 of 0.9986 and 0.9974 for enalapril and enalaprilat respectively.

Introduction

SOLA™ products are a revolutionary new Solid Phase Extraction (SPE) device. This first in class SPE product range introduces next-generation, innovative technological advancements, giving unparalleled performance characteristics compared to conventional SPE, phospholipid and protein precipitation products.

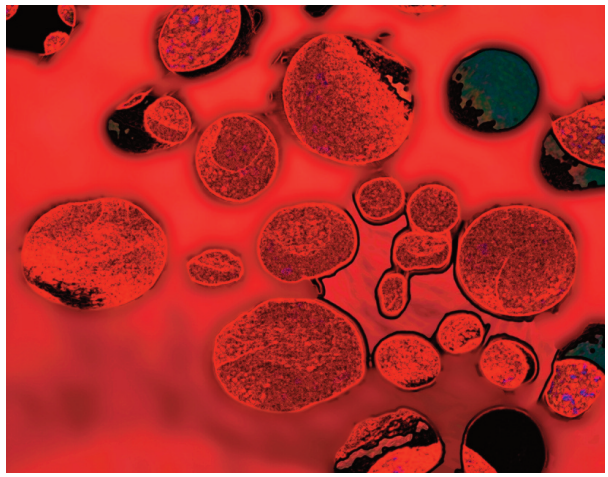
This includes:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA products have significant advantages for the analyst when processing compounds in complex matrices particularly in high throughput bioanalytical and clinical laboratories where reduced failure rate, higher analysis speed and lower sample/solvent requirements are critical.

The increased performance from SOLA products provides higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development.

Hypersil GOLD columns offer excellent peak shape. Based on highly pure silica, Hypersil GOLD™ columns provide very symmetrical peaks, even when analyzing compounds that give notoriously poor peak shape on traditional silica-based chemistries. Hypersil GOLD media provides a stationary phase with C18 selectivity and a predictable elution order, but can provide new capabilities such as improved peak shape, increased peak capacity, and greater sensitivity, especially for trace compound analysis. Obtaining symmetrical peak shapes is critical to ensuring that optimum resolution and sensitivity are achieved for basic pharmaceutical compounds. Hypersil GOLD columns use proprietary bonding technology to make certain that symmetrical peaks are obtained, producing data with the highest confidence in the accuracy and quality of results.



Enlaprilat was the first dicarboxylate-containing ACE inhibitor produced for the treatment of hypertension by preventing the restriction of blood vessels causing high blood pressure. Enalaprilat, however it is only suitable for intravenous administration due to unfavorable ionization characteristics. To overcome this, the prodrug Enalapril was developed by the esterification with ethanol of enalaprilat to produce enalapril. Enalapril can therefore be administered orally and is later metabolized in the liver to form the active component.

The extraction of enalapril and enalaprilat from human plasma is demonstrated in this application.

Experimental Details

Consumables	Part Number
Fisher Scientific LCMS grade water	W/011217
Fisher Scientific LCMS grade methanol	M/4062/17
Fisher Scientific Analytical grade formic acid	F/1900/PB08
NSC Mass Spec Certified 2 mL clear vial with blue bonded PTFE silicone cap	MSCERT4000-34W

Sample Handling Equipment	Part Number
96 well plate vacuum manifold, Thermo Fisher Scientific	60103-351
Ultra vap, Thermo Fisher Scientific	CLS-229070

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Sample Pretreatment
Prepare a mixed standard spiking solutions in methanol (enalapril and enalaprilat)
Prepare a working internal standard solution in water (benazepril)
Take 200 µL of blank human plasma or sample
For standards and quality control (QC) samples add 10µL of standard spiking solution for all other samples add 10µL of methanol
For standards and QC's and unkown's add 10 µL of working internal standard solution for blanks add 10 µL of water
Add 4 µL of formic acid
Mix well

Sample Preparation - SOLA		Part Number
Compound(s):	enalapril, enalaprilat, benazepril (IS)	
Matrix:	Human plasma	
Cartridge type:	Thermo Scientific SOLA 10 mg/1 mL	60109-001
Conditioning stage:	1 mL methanol, 1 mL water	
Application stage:	Load all sample and allow to flow under gravity	
Washing stage:	200 µL 0.1 % formic acid in water	
Elution stage:	2 x 200 µK 2 % ammonia solution in methanol	
Additional stage:	Dry down and reconstitute in 200 µL 90:10 (v/v) water/ methanol. Sonicate for 5 minutes.	

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Accela 600	
Column:	Hypersil GOLD, 1.9 µm, 50 x 2.1 mm	25002-052130
Mobile phase A:	water + 0.1 % formic acid	
Mobile phase B:	acetonitrile + 0.1 % formic acid	
Gradient:	10-100 %B in 1 minute	
Flow rate:	0.6 mL/min	
Column temperature:	70 °C	
Injection details:	2.5 µL	
Injection wash solvent 1:	80:20 (v/v) water / acetonitrile	
Injection wash solvent 2:	100 % organic	

Ionization conditions	HESI
Polarity	Positive
Spray voltage (V)	3000
Vaporizer temp (°C)	317
Sheath gas pressure (Arb)	52
Aux gas pressure (Arb)	43
Capillary temp (°C)	370
Collision pressure(mTorr)	1.5
Scan time (s)	0.02
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7

Table 1. TSQ Vantage™ conditions

Compound	Enalapril	Enalaprilat	Benazepril
Parent (m/z)	377.250	349.220	425.260
Products (m/z)	234.190	206.150	351.230
Collision energy (eV)	16	17	19
S-lens(Arb)	85	80	93

Table 2. Compound transition details

Data Processing	
Software:	Thermo Scientific LC QUAN

Results

Discussion

Extracted enalapril standards from human plasma were shown to be linear over the dynamic range of 1 and 100ng/mL with an r^2 correlation of 0.9986 using SOLA cartridges (Figure. 4). QC samples were run in replicates of six at a concentration of 50ng/mL. Precision for each QC level were < 6.6% CV (Table 2). Overspikes were run in duplicate at a concentration of 50ng/mL and used to calculate the percentage recovery level for enalapril of 81% (Table 3). No carryover was observed for enalapril (Table 4).

Extracted enalaprilat standards from human plasma were linear over the dynamic range of 1 and 100ng/mL with an r^2 correlation of 0.9974 using SOLA cartridges (Figure 5). QC samples were run in replicates of six at a concentration of 50ng/mL. Precision for each QC level were < 6.6% CV (Table 2). Overspikes were run in duplicate at a concentration of 50ng/mL and used to calculate the percentage recovery level for enalaprilat of 85% (Table 3). No carryover was observed for enalapril (Table 4).

Extracted benazepril from human plasma was used as an internal standard in replicates of six QC's and duplicate overspikes to calculate recovery. The calculated percentage recovery level for benazepril was 87% (Table 3). No carryover was observed for benazepril (Table 4).

Chromatography

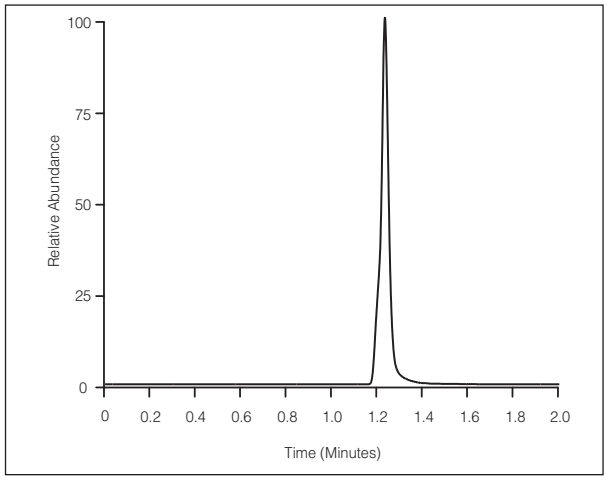

Figure 1. Representative chromatogram of enalapril SRM,extracted from human plasma at 1ng/mL

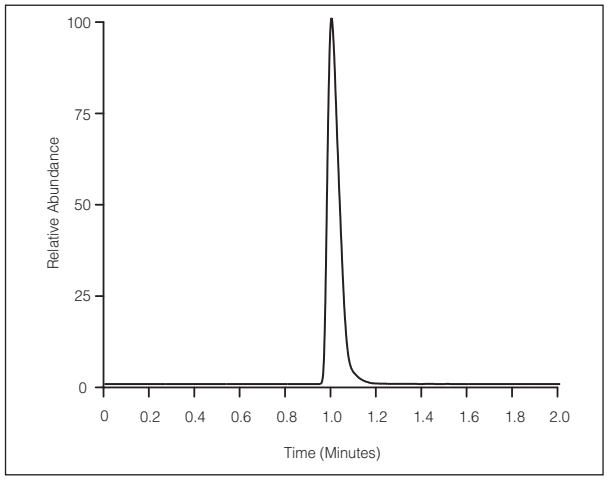

Figure 2. Representative chromatogram of enalaprilat SRM, extracted from human plasma at 1ng/mL

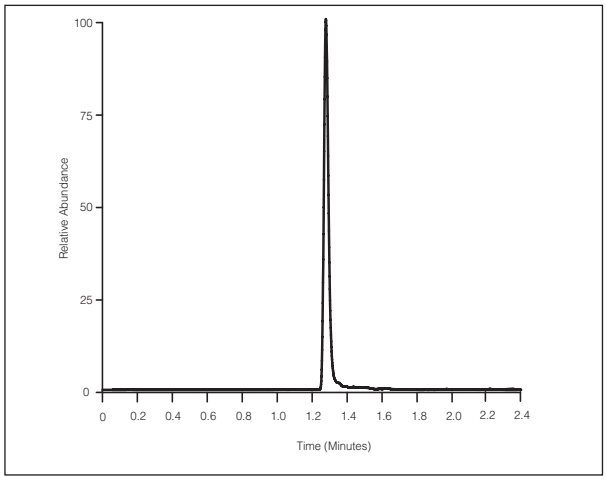

Figure 3. Representative chromatogram of benazepril SRM, extracted from human plasma at 50ng/mL

Linearity

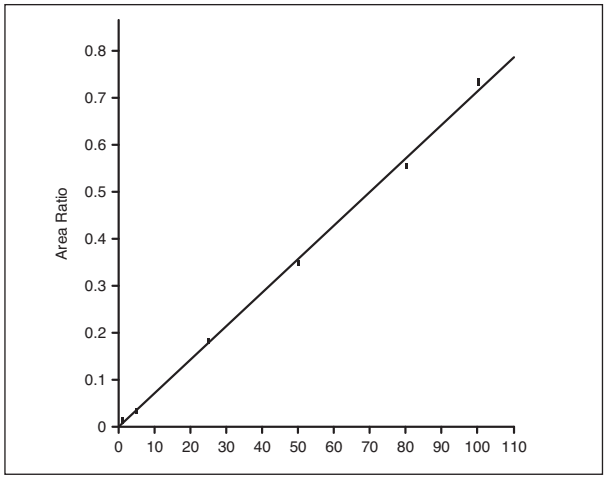

Figure 4. Enalapril linearity over the dynamic range 1-100ng/mL

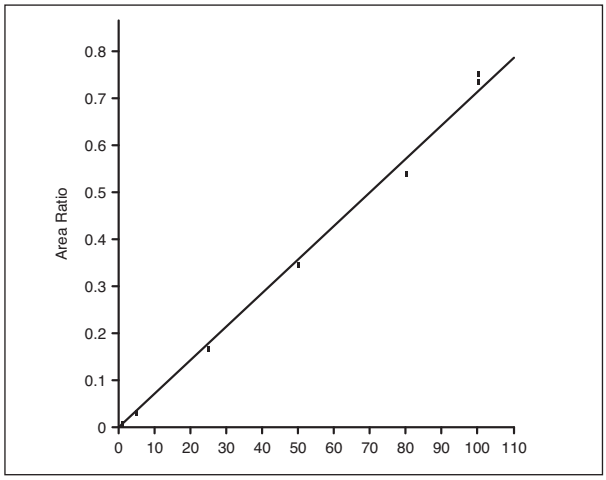

Figure 5. Enalaprilat linearity over the dynamic range 1-100ng/mL

Accuracy and precision

Standard	Analyte	Specified concentration ng/mL	Calculated Concentration ng/mL	%Diff	Mean
S1	Enalapril	1	1.05	4.6	0.0
S1	Enalapril	1	1.16	15.6	
S2	Enalapril	5	4.10	-18.0	
S3	Enalapril	25	24.84	-0.6	
S4	Enalapril	50	48.24	-3.5	
S5	Enalapril	80	77.34	-3.3	
S6	Enalapril	100	102.92	2.9	
S6	Enalapril	100	102.36	2.4	
S1	Enalaprilat	1	1.14	14.1	0.0
S1	Enalaprilat	1	1.06	6.0	
S2	Enalaprilat	5	4.33	-13.3	
S3	Enalaprilat	25	23.45	-6.2	
S4	Enalaprilat	50	48.50	-3.0	
S5	Enalaprilat	80	75.47	-5.7	
S6	Enalaprilat	100	105.21	5.2	
S6	Enalaprilat	100	102.83	2.8	

Table 1. Accuracy data for six standards over the linear range 1-100 ng/mL

Standard	Analyte	Average Calculated Concentration ng/mL	Average %Diff	Precision (%CV)
QC 50ng/mL	Enalapril	49	-1.5	6.6
QS 50ng/mL	Enalapril	53	5.9	3.5
QC 50ng/mL	Enalaprilat	46	-7.3	6.6
QS 50ng/mL	Enalaprilat	48	-4.5	0.5

Table 2. Average precision data for six replicate QC's and overspike's

Recovery

Standard	Analyte	Response	% Recovery
Average QC area response	Enalapril	540534	81
Average overspike area response	Enalapril	663794	
Average QC area response	Enalaprilat	413425	85
Average overspike area response	Enalaprilat	485681	
Average QC area response	Benazepril	1250895	87
Average overspike area response	Benazepril	1426695	

Table 3. Recovery data for enalapril, enalaprilat and benazepril

Carryover

Carryover	Enalapril	Enalaprilat	Benazepril
S1 response	9211	8078	1065361
Total carryover	1423	1576	1348
% of S1 response	15.4	19.5	0.1

Table 4. Carryover data for enalapril, enalaprilat and benazepril

Conclusion

SOLA cartridges and Hypersil GOLD allows the extraction and quantification of enalapril and enalaprilat from human plasma to be carried out simply and quickly with excellent results. The advantages of SOLA cartridges in comparison to traditional loose packed material ensures a reduction in elution solvent volume, hence reduced solvent costs and subsequently reduced drying times. In addition, greater recoveries and accuracy and comparable linearity and precision can also be achieved using SOLA cartridges, demonstrating the capabilities of the product.

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