Application Note: ANCCSSOLADPINE

Amlodipine in Human Plasma Using SOLA CX and Accucore RP-MS Column

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Key Words

- SOLA Cartridges and Plates
- Hypertension Drug
- Amlodipine
- Bioanalysis
- Nifedipine

Abstract

An extraction method for amlodipine from human plasma has been developed using Thermo Scientific SOLA CX well plates. The sample preparation was fast and efficient and demonstrated excellent reproducibility and accuracy. The Accucore RP-MS column was used to give a fast run time of 3.5 minutes.

Introduction

SOLA™ is a revolutionary new Solid Phase Extraction (SPE) product range. 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:

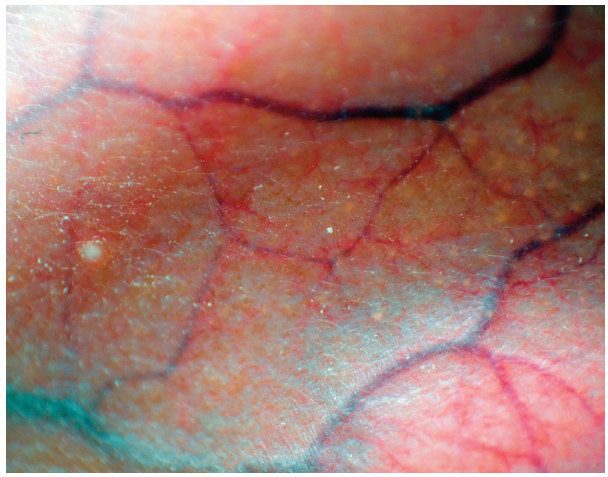
- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

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.

Accucore HPLC columns use Core Enhanced Technology to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The optimised phase bonding creates a series of high coverage, robust phases. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

Amlodipine is a drug grouped as calcium channel blockers which relaxes and widens blood vessels and improves blood flow. It is widely used to treat hypertension, chest pain (angina) and other conditions caused by coronary artery disease. Amlodipine is typically dosed at 5-10 mg tablet daily. A C_{max} value of 17.7 ng/mL has been reported for 10 mg doses [1, 2, 3].



In this application the extraction and quantification of amlodipine in human plasma are demonstrated.

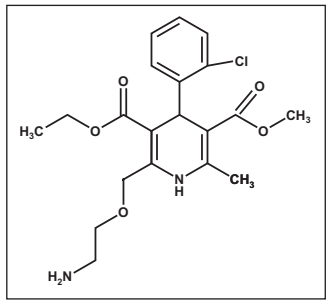


Figure1. Structure of amlodipine

Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific LC-MS grade water	W/0112/17
Fisher Scientific HPLC grade methanol	M/4056/17
Fisher Scientific LC-MS grade methanol	M/4062/17
Amlodipine besylate	
Nifedipine	
Fisher Scientific formic acid	F/1900/PB08

Sample Handling Equipment	Part Number
Finn pipettes	(Fisher Scientific part 9402151, 02-707-408, 02-707-423)
96 well manifold	60103-351
Ultra Vap, Thermo Fisher Scientific	CLS-229070

Sample and Calibration Preparation	
Compound(s):	amlodipine and nifedipine (IS)
Matrix:	human plasma

Stock Solution

1000 µg/mL stock solutions of amlodipine were prepared in LC-MS grade methanol. From a solution of 1000 µg/mL nifedipine solution made in LC-MS grade methanol, a concentration of 1600 ng/mL internal standard stock solution was prepared by diluting 16 µL with 9884 µL LC-MS grade methanol.

Calibration standards: S1-S7 calibration standards were prepared (table 1). 180 µL of plasma was spiked with 10 µL internal standards and 10 µL of amlodipine (S1-S8) solution to give 2, 5, 10, 20, 50, 85 and 100 ng/mL solution.

Standard	Amlodipine concentration required in plasma (ng/mL)	Taken spike from	Amount spiked (µL)	Amount of methanol added (µL)
S7	100	Stock	10.00	4990.00
S6	80	S8	850.00	150.00
S5	50	S8	1000.00	1000.00
S4	20	S8	200.00	800.00
S3	10	S8	100.00	900.00
S2	5	S6	100.00	900.00
S1	2	S5	100.00	900.00

Table 1. Preparation of Calibration standards.

Solid Phase Extraction - SOLA CX		Part Number
SPE cartridge:	10 mg SOLA CX 96 well plate	60309-002
Conditioning stage:	500 µL methanol	
Equilibration stage:	500 µL water with 2% formic acid	
Load:	200 µL plasma (spiked with amlodipine and IS)	
Wash 1:	200 µL water with 2% formic acid	
Wash 2:	200 µL water/methanol 70:30 (v/v) with 2% formic acid	
Elute:	200 µL methanol with 5% ammonia solution	

Extracts were dried using an UltraVap™ and reconstituted in 200 µL LC-MS grade methanol before analyzing with mass spectrometry.

Chromatographic Conditions		Part Number
Instrumentation:	Thermo Scientific Accela 600 with Open Autosampler	
Column(s):	Accucore RP-MS, 2.6 µm 50 x 2.1 mm	17626-052130
Guard columns:	Accucore RP-MS, 2.6 µm 10 x 2.1 mm	17626-012105

Mobile Phase

A:	water (LC-MS grade) with 0.1% formic acid	
B:	methanol (LC-MS grade) with 0.1% formic acid	
T/min	% A	%B
0.00	95.00	5.00
2.00	5.00	95.00
2.10	95.00	5.00
3.50	95.00	5.00
Flow rate:	0.35 mL/min	
Column temperature:	40 °C	
Injection details:	5 µL	
Injection wash solvent 1:	water/acetonitrile (80:20 v/v)	
Injections wash solvent 2:	acetonitrile (100%)	

MS Conditions

Instrumentation: Thermo Scientific TSQ Vantage

Ionization conditions	HESI
Polarity	Positive
Spray voltage (eV)	3000
Vaporizer temp (°C)	350
Sheath gas pressure (Arb)	30
Aux gas pressure (Arb)	10
Capillary temp (°C)	300
Collision pressure(mTorr)	1.5
Scan time (s)	0.02
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7

Table 2. TSQ Vantage™ conditions

Compound	Amlodipine	Nifedipine
Parent (m/z)	409.30	347.10
Products (m/z)	238.09	254.14
Collision energy (eV)	5	16
S-lens(Arb)	72	68

Table 3. Compound transition details

Data Processing

Software: Thermo Scientific LC QUAN

Results

The dynamic range was shown to be linear between 2 and 100 ng/mL with a r^2 (goodness of fit) of 0.991 (figure 2). QC samples were run in replicates of 5 at a mid range concentration of 30 ng/mL and the precision for these calculated to be < 6.9% (table 2).

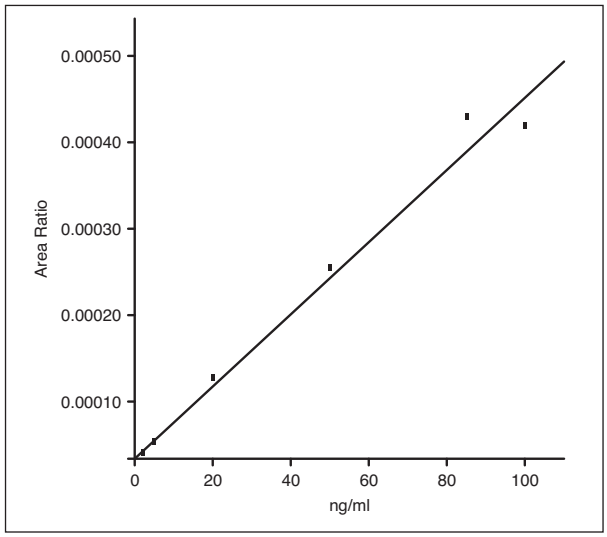


Figure 2. Extracted calibration line

Single level Standards	Actual Amount /ng.ml^{-1}	Calculated Amount /ng.ml^{-1}	Accuracy
1	30	29.1	97.0%
2	30	32.2	107.2%
3	30	30.1	100.3%
4	30	33.6	111.9%
5	30	34.1	113.7%
Mean		31.8	106.0%
RSD		6.8%	

Table 4. Determination of amlodipine in spiked plasma samples using an internal standard

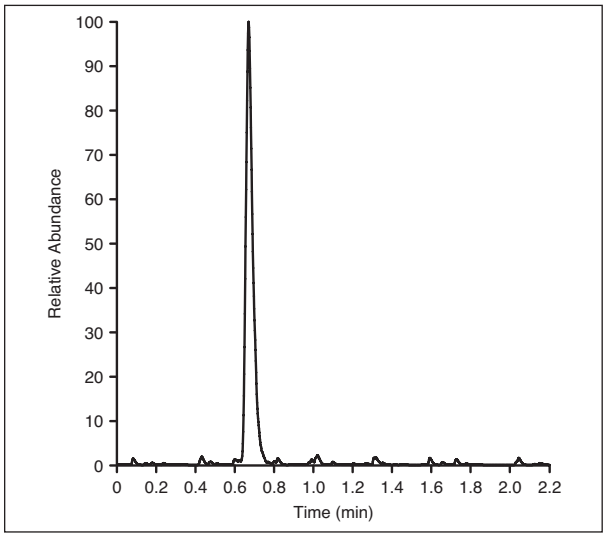


Figure 3. Chromatogram of amlodipine in extracted human plasma.

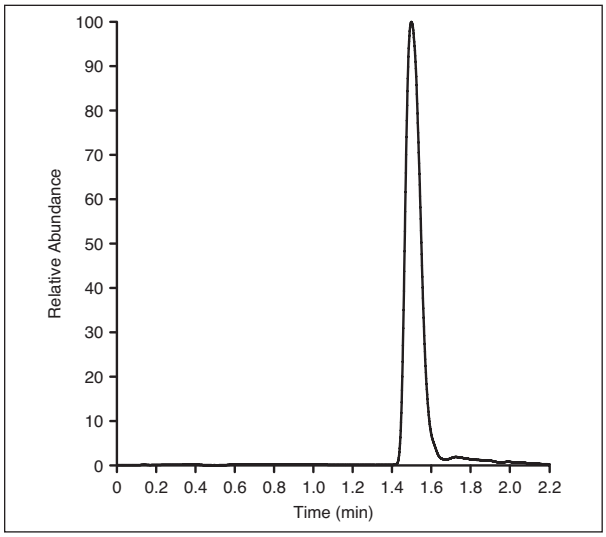


Figure 4. Chromatogram of Nifedipine (IS)

Conclusion

SOLA CX plates and Accucore RP-MS HPLC columns can be used to extract and quantify amlodipine from human plasma. In this application we have demonstrated that:

- SOLA CX plates require less elution solvent, meaning reduced solvent cost and shorter drying times.
- SOLA CX plates allow for high method accuracy and precision
- SOLA CX plates are effective in removing endogenous interferences.

References

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2. Drug Information, http://www.drugs.com/amlodipine.html, Dated 04/10/2011

3. Drug Bank, http://www.drugbank.ca/drugs/DB00381, Dated 04/10/2011

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