

# LC-MS/MS Method for the Determination of HCTZ and Losartan from Human Plasma Using SOLA CX

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## Abstract

Thermo Scientific SOLA CX cartridges allow for faster methods, more reproducible results and requires smaller sample volumes than conventional loose-packed SPE cartridges. Coupled with a Thermo Scientific Accucore aQ column this allows the separation of losartan and HCTZ in human plasma with good peak shape and sensitivity in two minutes.

## Introduction

SOLA™ products are 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 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 2.6µm 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. The polar functional group used to end-cap Accucore aQ provides an additional controlled interaction mechanism by which polar compounds can be retained and resolved making Accucore aQ ideal for the quantitative analysis of trace levels of polar analytes.

Hydrochlorothiazide (HCTZ) (Figure 1) is a thiazide diuretic which is commonly used in the treatment of fluid retention and hypertension by increasing sodium elimination from the body. HCTZ is on the market under a number of trade names and also through multiple generic manufacturers.

Losartan is an angiotensin II receptor antagonist also used for the treatment of hypertension. Losartan (Figure 2) has also demonstrated an ability to delay the



progression of kidney related diseases in people with type 2 diabetes and combined with HCTZ is used in the treatment of high blood pressure. The extraction of HCTZ and losartan from human plasma is demonstrated in this application.

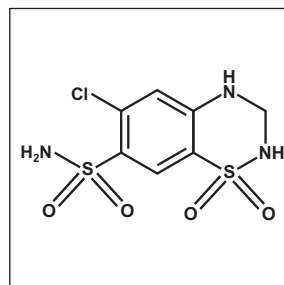


Figure 1. Hydrochlorothiazide (HCTZ)

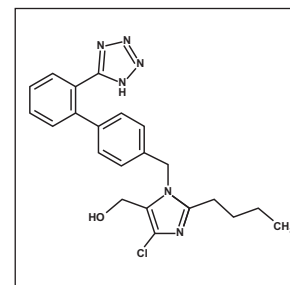


Figure 2. Losartan

## Experimental Details

Consumables	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade acetonitrile	A/0626/17
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
SPE Positive pressure manifold Thermo Fisher Scientific	60104-232
Thermo Scientific Ultra-Vap	CLS-229070

## Sample Pretreatment

Aliquot 121.4 µL of spiked plasma into a clean tube. Add 13.6 µL of standard spiking solution, and 15 µL of Internal Standard spiking solution, for blanks add methanol, mix well

## Key Words

- SPE
- SOLA CX Cartridges and Plates
- Accucore aQ
- hydrochlorothiazide (HCTZ)
- Losartan

Sample Preparation - SOLA CX		Part Number
Compound(s):	HCTZ, losartan, furosemide (IS)	
Matrix:	Human plasma	
Cartridge type:	Thermo Scientific SOLA CX 10mg/1mL	60109-002
Conditioning stage:	1 mL methanol, 1 mL water	
Application stage:	100 µL of spiked human plasma containing internal standard	
Washing stage:	200 µL water + 0.1% formic acid	
Elution stage:	200 µL acetonitrile + 3% ammonia	
Additional stage:	dry down samples and reconstitute in 100 µL of 80:20 (v/v) water / acetonitrile	

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific Accela 600	
Column:	Accucore aQ, 2.6µm 50 x 2.1mm	17326-052130
Mobile phase A:	water + 0.1% formic acid	
Mobile phase B:	acetonitrile + 0.1% formic acid	
Gradient:	20-70%B in 2 minutes	
Flow rate:	0.4 mL/min	
Column temperature:	40 °C	
Injection details:	2.5 µL	
Injection wash solvent 1:	80:20 (v/v) water / acetonitrile	
Injection wash solvent 2:	100% organic	

#### MS Conditions

Instrumentation: Thermo Scientific TSQ Vantage

Ionization conditions	HESI
Polarity	+ Losartan / - HCTZ and Furozemide
Spray voltage (V)	3000
Vaporizer temp (°C)	300
Sheath gas pressure (Arb)	60
Ion sweep pressure	0
Aux gas pressure (Arb)	30
Capillary temp (°C)	300
Declustering voltage	0
Collision pressure(mTorr)	1.5
Cycle time (s)	0.5
Q1 (FWHM)	0.7
Q3 (FWHM)	0.7

Table 1. Vantage™ conditions

Compound	HCTZ		Losartan		Furozemide	
Parent (m/z)	295.937		423.2		329.122	
Products (m/z)	205.018	269.025	180.092	207.072	205.022	385.031
Collision energy	24	20	35	20	22	16
S-lens	98	98	91	91	104	104

Table 2. Mass-transition details

#### Data Processing

Software: Thermo Scientific LC QUAN

## Results

### Overview of Precision and Recovery

Method precision was observed to be < 6.1 % RSD for both HCTZ and losartan at both high and low QC concentrations (table 3). Recoveries were also measured at 86.4% and 65.8% for HCTZ and losartan respectively.

	HCTZ	Losartan
% RSD @ Low QC	3.3	6.1
% RSD @ High QC	1.6	4.3
Accuracy (% Difference) Low QC	11.3	7.6
Accuracy (% Difference) High QC	11.7	-2.0
% Recovery	86.4	65.8

Table 3. Recovery and Precision of HCTZ and Losartan on SOLA CX cartridges

### Hydrochlorothiazide (HCTZ)

Extracted HCTZ standards from human plasma were linear over the dynamic range between 0.5 and 500 ng/mL with an  $r^2$  of 0.9974 using SOLA CX cartridges (Figure 6). QC samples were run in triplicate at both low and high concentrations of 1.5 ng/mL and 400 ng/mL. Precision for each QC level were < 3.5% RSD (Table 5). Overspikes were run in duplicate at a concentration of 400 ng/mL and used to calculate the percentage recovery level for HCTZ of 86.4% (Table 6). No carryover was observed for HCTZ (Table 7).

### Losartan

Extracted losartan standards from human plasma were linear over the dynamic range between 0.5 and 500 ng/mL with an  $r^2$  of 0.9956 using SOLA CX cartridges (Figure 7). QC samples were run in triplicate at low and high concentrations of 1.5 ng/mL and 400 ng/mL respectively. Precision for each QC level were < 6.5% (Table 9). Overspikes were ran in duplicate at a concentration of 400 ng/mL and used to calculate the percentage recovery level for losartan of 65.8% (Table 10). An assessment of carryover was made using the response of the compounds in the blank injection which followed the top standard. Due to carryover, two blanks were analysed after the top standard had been injected (Table 11).

Sample	Calculated Concentration	% Diff
Std1_SOLA	0.537	7.4
Std2_SOLA	1.04	4.3
Std3_SOLA	2.37	-5.3
Std4_SOLA	10.1	0.86
Std5_SOLA	47.1	-5.8
Std6_SOLA	91.3	8.7
Std7_SOLA	274	9.8
Std8_SOLA	488	-2.5
Std9_SOLA2	499	0.1

Table 4. Results for standard extraction line for HCTZ using SOLA CX cartridges

Sample	Specified Concentration	Calculated Concentration	Mean % Diff	% RSD
QC low (n=3)	1.500	1.68	11.3	3.3
QC high (n=3)	400.000	447	11.7	1.6

Table 5. Results for triplicate QC extractions at two concentrations for HCTZ using SOLA CX cartridges

Average % RECOVERY (High QC)	86.4
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Table 6. Recovery data for HCTZ using SOLA CX cartridges

Area response std 1	60
20% area response std 1	12
Area response of blank after top std (9)	0
OVERALL	PASS

Table 7. Carryover data for HCTZ

Sample	Calculated Concentration (ng.mL)	% Diff
Std1_SOLA	0.596	19.1
Std1_SOLA	0.520	4.0
std2_SOLA	0.968	-3.2
Std3_SOLA	2.3	-8.1
Std4_SOLA	10.9	8.7
Std5_SOLA	23.4	-6.2
Std6_SOLA	44.3	-11.4
Std7_SOLA	87.3	-12.7
Std8_SOLA	280	11.8
Std9_SOLA	487	-2.5
Std9_SOLA2	502	0.5

Table 8. Results for standard extraction line for losartan using SOLA CX cartridges

Sample	Specified Concentration (ng/mL)	Calculated Concentration (ng/mL)	Mean % Diff	% RSD
QC low (n=3)	1.500	1.61	11.3	7.6
QC high (n=3)	400.000	392	-2.0	4.3

Table 9. Results for triplicate QC extractions at two concentrations for losartan using SOLA CX cartridges

Average area response (QC high)	3882365
Average area response (overspike)	5900415
% RECOVERY	65.8

Table 10. Recovery data for losartan using SOLA CX cartridges

Area response std 1	4519
20% area response std 1	904
Area response of blank 1 after top std (9)	1189
Area response of blank 2 after top std (9)	0
OVERALL	PASS

Table 11. Carryover data for losartan

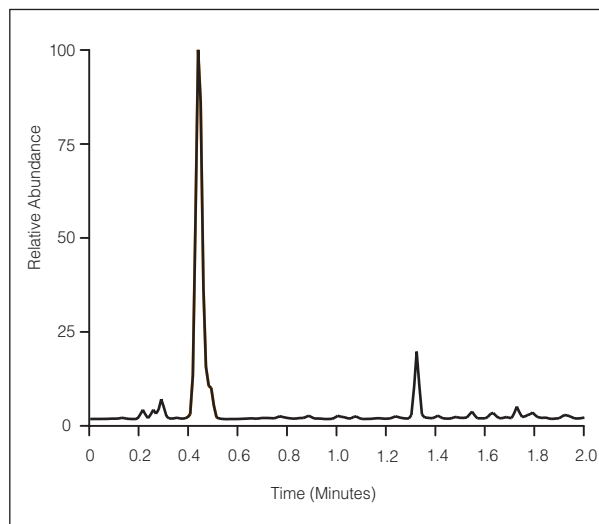


Figure 3. Extracted HCTZ from human plasma at 0.5 ng/mL using SOLA CX cartridges

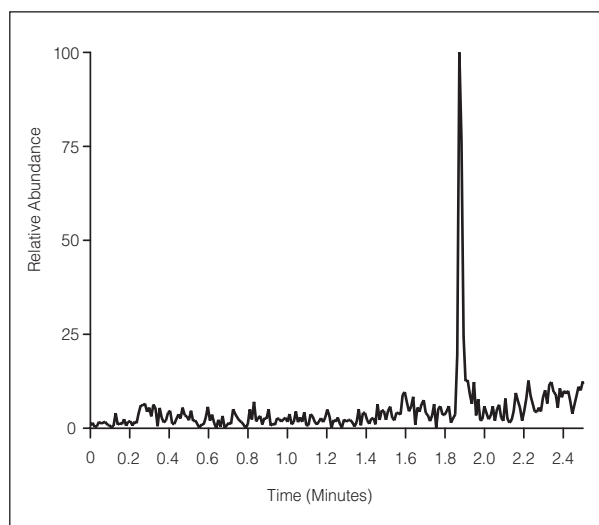


Figure 4. Extracted losartan from human plasma at 0.5 ng/mL using SOLA CX cartridges

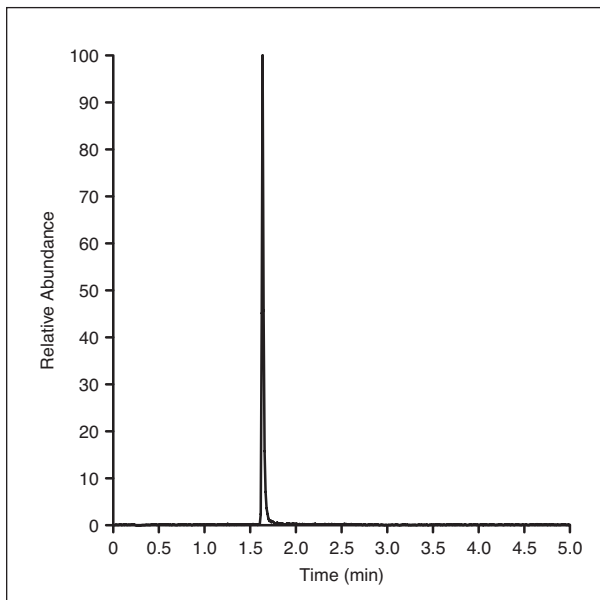


Figure 5. Extracted internal standard furoseamide at 400 ng/mL using SOLA CX cartridges

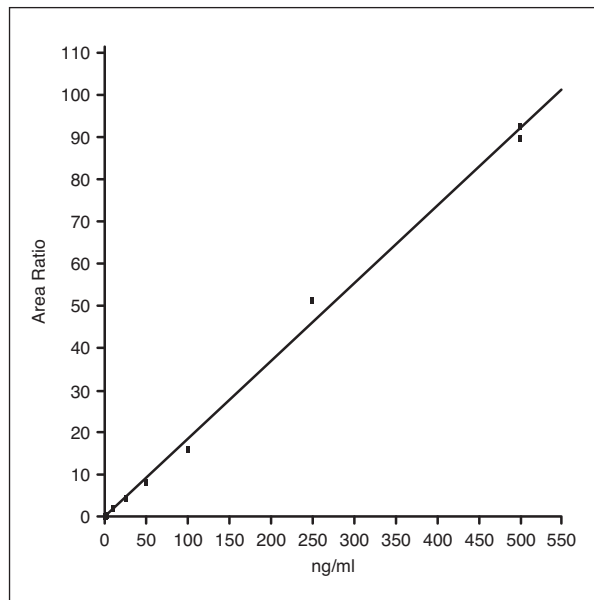


Figure 7. Linear dynamic range between 0.5 and 500 ng/mL with an  $r^2$  of 0.9956 for losartan for extracted standards using SOLA CX cartridges

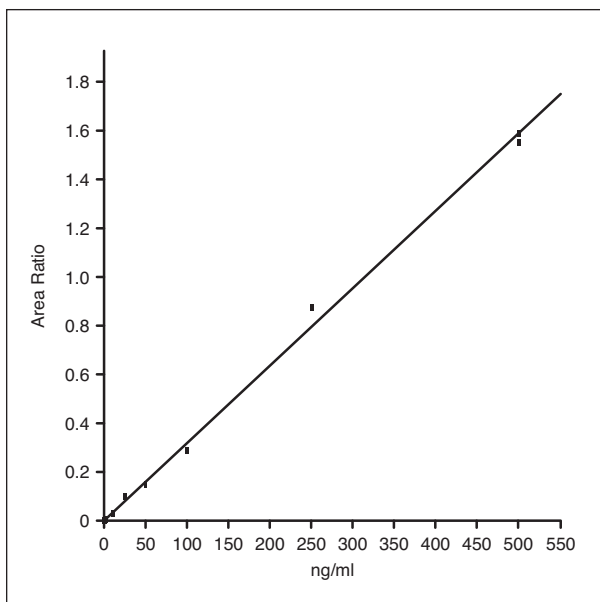


Figure 6. Linear dynamic range between 0.5 and 500 ng/mL with an  $r^2$  of 0.9974 for HCTZ for extracted standards using SOLA CX cartridges

### Conclusion

SOLA CX cartridges and Accucore aQ can be successfully used to extract and quantify HCTZ and losartan from human plasma quickly and simply. The advantages of SOLA cartridges in comparison to loose packed material ensures a reduction in elution solvent volume, hence reduced solvent costs and subsequently reduced drying times. In addition to this, good recoveries, accuracy, linearity and precision can also be achieved using SOLA cartridges, demonstrating the capabilities of the product.

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