Use of Alkaline Mobile Phase to Achieve Good Peak Shape in The Rapid LC-MS/MS Analysis of Lisinopril in Human Plasma

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Introduction

Lisinopril (LIS) is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension, congestive heart failure, and heart attacks and was the 3rd most commonly prescribed drug in 2016. Experience with ACE inhibitors and literature review indicated that *cis/trans*- isomerization of the proline moiety would significantly affect peak symmetry. Indeed, the broad peak shape observed under acidic conditions that promoted retention of the polar zwitterionic compound was unacceptable, *as illustrated in Figures 3a-d.*

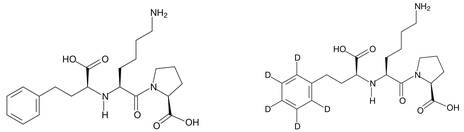
Needs:

- High-throughput plasma method with a simple processing approach
- Injection cycle ≤ 3 minutes
- LLOQ of 0.5 ng/mL in human plasma

Challenges:

- Multifunctional analyte, 2 acidic and 2 basic groups
- Very polar and poorly retained by reversed phase (RP) chromatography
- Poor peak shape

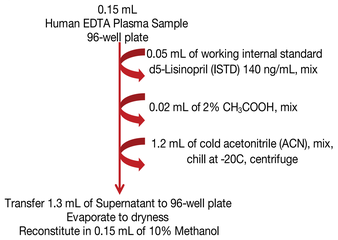
Figure 1. Chemical Structures



Lisinopril
405.5 Da

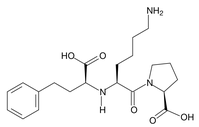
d_5-Lisinopril
410.5 Da

Sample Processing

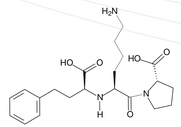


The peak splitting observed in lisinopril chromatography (and that of other proline containing ACE inhibitors and peptides) is elucidated as slow rotational kinetics around the proline peptide bond. Rotation is slow due to relative rigidity of the peptide bond, and stabilization of the major *trans* isomer by hydrogen bonding between the peptide carbonyl (O) and proline carboxylic acid (H)[1].

Figure 2. Lisinopril Rotational Isomers

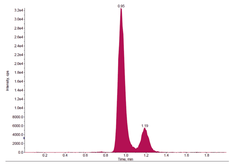


trans-LIS



cis-LIS

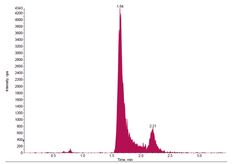
Figure 3 a-d. Peak tailing and splitting observed in method development, flow rate 0.5 mL/minute



a) Zorbax Bonus-RP, 50 x 2.1 mm, 3.5 µm

5:95:1 ACN:H_2O:HCOOH

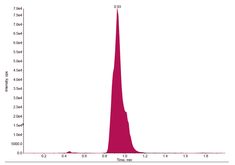
Temperature: Ambient, k'~3



b) Waters, XBridge® C18, 50 x 2.1 mm, 3.5 µm

10:90:1 ACN:H_2O:HCOOH

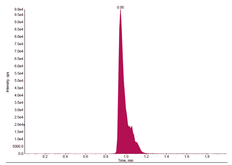
Temperature: 50°C, k'~6



c) Waters, XBridge® C18, 50 x 2.1 mm, 3.5 µm

20:80:2:0.04 ACN:H_2O:HCOOH:HFBA

Temperature: 50°C, k'~3



d) Waters, CSH Phenyl-Hexyl, 5um, 2.1x100 mm

10:90 ACN:10mM CH_3COONH_4 pH 5.0

Temperature: 50°C, k'~1

Validated Analytical Conditions

Waters ACQUITY UPLC® Binary Solvent Manager

- Mobile Phase A: 50 mM CH_3COONH_4, pH 9.3
- Mobile Phase B: 60:40 MeOH:ACN
- Column: Waters, ACQUITYUPLC® BEH C18, 50 x 2.1 mm, 1.7 µm, Temperature: 50 °C
- Flow Rate:0.6 mL/minute
- Linear Gradient Program:

Steps	Total time (min)	MPH A%	MPH B%
1	0.00	96	4
2	1.50	89	11
3	1.51	10	90
4	1.90	10	90
5	1.91	96	4
6	2.40	96	4

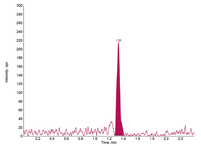
Waters ACQUITY UPLC® I-Class System

- Injection Volume: 20uL
- Sample Manager Wash: 50:50 ACN:H_2O
- Purge: 100% H_2O
- Temperature: 4°C

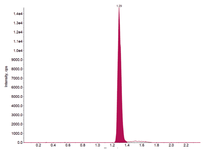
DETECTOR: AB SCIEX API 4000, ESI Negative Mode

Ions monitored (m/z)	Precursor		Product	Dwell time (msec)
Lisinopril:	404.3	–>	114.0	200
d_5-Lisinopril (IS):	409.3	–>	114.0	100

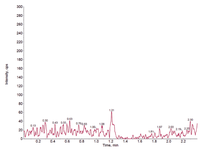
- A post column divert valve is used to keep the interface clean.
- An extra pump introduces 10:90 ACN:H_2O at 0.3 mL/min during diversion.
- Final Chromatography:



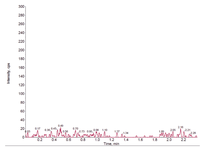
LIS 0.5 ng/mL



ISTD 47ng/mL



LIS 0 ng/mL



ISTD 0 ng/mL

Results

Table 1. Inter-Batch Precision and Accuracy for Lisinopril in Human Plasma (EDTA)

	LLOQ QC 0.500 ng/mL	QC A 1.50 ng/mL	QC B 7.00 ng/mL	QC C 75.0 ng/mL
Inter-Batch Mean	0.454	1.44	6.82	74.7
Inter-Batch % CV	14.6	7.6	2.4	1.6
Inter-Batch % Bias	-9.2	-4.0	-2.6	-0.4
n	24	30	24	30

Table 2. Back-Calculated Calibration Curve Standard Concentrations of Precision and Accuracy Batches for Lisinopril in Human Plasma (EDTA)

Batch	STD B 0.500 ng/mL	STD C 1.00 ng/mL	STD D 2.00 ng/mL	STD E 5.00 ng/mL	STD F 10.0 ng/mL	STD G 20.0 ng/mL	STD H 40.0 ng/mL	STD I 80.0 ng/mL	STD J 100 ng/mL
18	0.519	0.916	2.02	4.96	10.3	20.2	39.7	79.7	101
19	0.518	0.967	1.86	4.83	10.1	20.5	42.2	79.9	101
21	0.499	1.02	1.95	5.00	9.45	21.3	40.3	77.5	102
Mean	0.512	0.968	1.94	4.93	9.95	20.7	40.7	79.0	101
SD	0.0113	0.0520	0.0802	0.0889	0.444	0.569	1.31	1.33	0.577
% CV	2.2	5.4	4.1	1.8	4.5	2.7	3.2	1.7	0.6
% Bias	2.4	-3.2	-3.0	-1.4	-0.5	3.5	1.8	-1.3	1.0
n	3	3	3	3	3	3	3	3	3

Table. 3 Matrix Factor for Lisinopril in Human Plasma (EDTA) – Low QC Concentration

	Lot	QC A LIS Area	MF	ISTD Area	MF	Normalized MF	Unextracted Low QC	Unextracted ISTD
	1	4236	0.918	137674	0.950	0.966	4489	138370
	2	4199	0.910	126253	0.871	1.04	4493	146677
	3	3728	0.808	124621	0.860	0.939	4707	146684
	4	4136	0.896	135074	0.932	0.962	4455	147492
	5	4240	0.919	130208	0.898	1.02	4687	147680
	6	3952	0.856	128729	0.888	0.964	4866	148866
Mean						0.983	4614	144962
% CV						4.2		
n						6		

Matrix Effect data (Table 4) was generated by spiking ten lots of plasma at the LLOQ and high QC concentrations.

Freeze/thaw and short term room temperature stability testing were combined (Table 5) using LLOQ and Dilution QCs. The samples remained at room temperature for 27 hrs during one of six freeze-thaw cycles. The cumulative time spent at room temperature during six cycles totaled 54 hrs.

Hemolyzed (Table 6) and lipemic (Table 7) evaluations were performed at the LLOQ and high QC concentrations in 3 separate lots of plasma.

Table 4. Matrix Effect Data

Validation Matrix Effect Data	LLOQ QC 0.500 ng/mL	QC C 75.0 ng/mL
Mean	0.438	75.8
% CV	13.5	2.1
% Theoretical	87.6	101.1
n	10	10

Table 5. Freeze/Thaw-Short Term Stability

Combined Freeze/Thaw- Short Term	LLOQ QC 0.500 ng/mL	QC D 200 ng/mL DF=5
Mean	0.406	201
% CV	9.7	2.7
% Theoretical	81.2	100.5
n	6	6

Table 6. Hemolyzed Determination

Hemolyzed (5% Whole Blood) Data	LLOQ 0.500 ng/mL	QC C 75.0 ng/mL
Mean	0.578	74.7
% CV	9.3	6.1
% Theoretical	115.6	99.6
n	3	3

Table 7. Lipemic Determination

Validation Lipemic Data	LLOQ 0.500 ng/mL	QC C 75.0 ng/mL
Mean	0.525	76.3
% CV	5.8	1.1
% Theoretical	105.0	101.7
n	3	3

Recovery was 57% across the analytical range.

Conclusions

The challenge of maintaining peak shape for an analyte prone to rotational isomerization was ensured using an alkaline mobile phase at elevated temperature. It is possible, but unproven, that ionization of the proline carboxylate precludes the stabilization of the *trans* isomer through hydrogen bonding thus facilitating rapid rotation at high temperature.

A reliable and reproducible method for Lisinopril was developed and validated in K2EDTA plasma in conformance with the European Medicines Agency Guideline on Bioanalytical Method Validation (2009) and the US FDA Guidance on Bioanalytical Method Validation (May 2001). The method met the internal goals of simple sample processing with a fast analysis time at the required sensitivity. Incurred sample reproducibility using the method was 98.6% in application.

Acknowledgements

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Reference

1. Bouabdallah, S., et al. Study of a Conformational Equilibrium of Lisinopril by HPLC, NMR, and DFT; International Journal of Analytical Chemistry, Volume 2014, Article ID 494719, 8 pages

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