

## Rapid Determination of Lisinopril Level in Human Plasma by LC-MS/MS

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Received: July 24, 2019; Published: August 02, 2019

**Abstract**

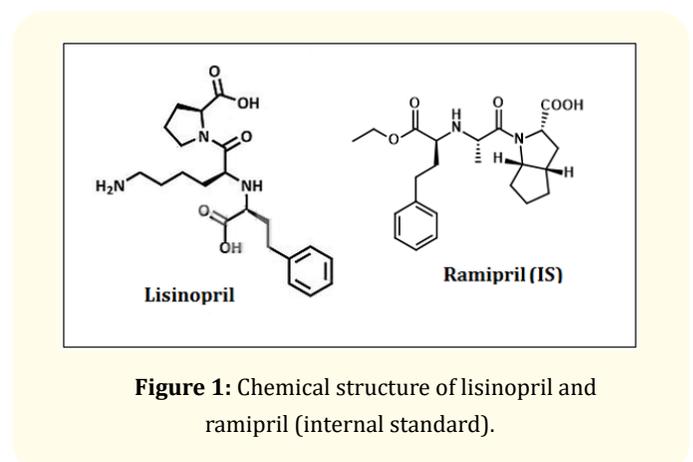
A rapid liquid chromatographic tandem mass spectrometry (LC-MS/MS) assay to measure lisinopril level in human plasma was developed and validated. 1.0 ml plasma samples containing lisinopril and 0.1 µg ramipril (as internal standard, IS) were extracted with 4 ml tert-butyl methyl ether and reconstituted in 60 µl mobile phase (5 mM ammonium formate and acetonitrile; 30:70, v:v). Analysis was performed on a reversed phase Atlantis dC18 column, the mobile phase delivered at a flow rate of 0.30 ml/minute. Mass spectrometry acquisition was performed in positive ion mode using multiple reaction monitoring transition for lisinopril and the IS (m/z: 406.3 → 246.3 and 417.4 → 234.1, respectively). Retention time of lisinopril and the IS was 1.28 and 1.86 minutes, respectively. The limit of detection of lisinopril in plasma was 0.5 ng/ml. Relationship between lisinopril level and peak area ratio of lisinopril/IS was linear ( $R^2 \geq 0.9980$ ) in the range of 1.0-200 ng/ml, intra- and inter-day coefficient of variations (CV) and bias were  $\leq 7.5\%$  and  $\leq 10.1\%$ , and  $\pm 6.2\%$  and  $\pm 6.8\%$ , respectively. Mean extraction recovery of lisinopril and the IS was 96% and 98%, respectively. Lisinopril stability in processed (24 hours at room temperature or 48 hours at  $-20^\circ\text{C}$ ) and unprocessed samples (24 hours at room temperature, 12 weeks at  $-20^\circ\text{C}$ , or three freeze-thaw cycles) was  $\geq 92\%$ .

**Keywords:** Lisinopril; Ramipril; Human Plasma; LC-MS/MS

**Introduction**

Lisinopril (CAS: 83915-83-7), chemically known as N2-[1-carboxy-3-phenylpropyl-l-lysyl] proline, is an angiotensin-converting enzyme inhibitor frequently prescribed for hypertension treatment and to minimize the risk of heart failure [1,2]. It has an absolute bioavailability of about 16% with mean peak plasma level in the range of 85.2-137.8 ng/ml, 6-8 hours after ingestion of a 20 mg therapeutic dosage [3,4]. Figure 1 depicts the chemical structures of lisinopril and ramipril, the internal standard (IS) used in the study.

Several analytical methods have been reported for determination of lisinopril level in biological fluids including radioimmunoassay [5-8], time-resolved fluoroimmunoassay [9], high performance liquid chromatography (HPLC) with ultra-violet or fluorescence detection [10,11], gas chromatography-mass spectrometry [12,13] and liquid chromatography mass spectrometry (LC-MS/MS) [14-19]. Immunoassay-based methods usually offer high sensitivity



but often suffer from cross-reactivity by related substances [20]. On the other hand, HPLC-based methods offer high selectivity but inadequate sensitivity to conduct bioequivalence/pharmacokinetic studies. Gas chromatography mass spectrometry-based methods have adequate sensitivity; however, their sample preparation in-

volves an additional derivatization step that is usually laborious and time-consuming [12,13]. LC-MS/MS assays may have a limit of quantification (LOQ) of 2.0 ng/ml [15,16] and 1.29 ng/ml [17].

In this paper, we describe a simple, sensitive, and rapid LC-MS/MS assay to quantify lisinopril level in human plasma. The method was fully validated and successfully used to monitor stability of lisinopril in human plasma samples.

## Material and Methods

### Apparatus

Liquid chromatography was performed on tandem mass spectrometric (LC-MS/MS) system (Waters Corporation, Milford, MA, USA) consisting of Water Alliance 2695 separation module equipped with Quattro micro API bench-top triple quadrupole mass spectrometer interfaced with a Z-spray electrospray ionization (ESI) source. Analysis was performed on reversed phase Atlantis dC18 column (2.1 x 100 mm, 3  $\mu$ m) protected by guard column Symmetry C18 (3.9 x 20 mm, 5  $\mu$ m). Mass Lynx software (Ver 4.0) working under Microsoft Window XP professional environment was used to control the instrument parameters and for data acquisition.

### Chemical and reagents

All reagents were of analytical grade unless stated otherwise. Lisinopril, ramipril, and tert-butyl methyl ether were obtained from Sigma-Aldrich, United Kingdom. Formic acid, ammonium formate, and acetonitrile (HPLC grade) were purchased from Fisher Scientific, NJ, USA. HPLC grade water was prepared by reverse osmosis and was further purified by passing through Synergy (Millipore, Bedford, MA, USA). Drug-free human plasma was obtained from the blood bank of the King Faisal Specialist Hospital and Research Centre (KFSHRC) Riyadh, Saudi Arabia.

### Chromatographic conditions

The mobile phase consisted of 5 mM ammonium formate (pH = 4.0  $\pm$  0.1, adjusted with formic acid) and acetonitrile (30:70, v:v) and was delivered at a flow rate of 0.30 ml/minute. The analysis was carried out at room temperature under isocratic condition. Electrospray ionization (ESI) was operated in positive-ion mode at capillary and cone voltage 3.3 KV and 30 V, respectively. Nitrogen was used as nebulizing and desolvation gas at a flow rate of 50 and 600 l/hour, respectively. Argon was used as the collision gas at pressure of 1.28 x 10<sup>-3</sup> mbar. The optimum collision energy for lisinopril and the IS was 23 eV. The ion source and the desolvation temperatures were maintained at 120°C and 350°C, respectively. Lisinopril and IS were detected using positive ion multiple reaction monitoring (MRM) mode at the following transitions of mass to charge (m/z): 406.3  $\rightarrow$  246.3 and 417.4  $\rightarrow$  234.1, respectively.

### Preparation of standard and quality control samples

Stock solutions (1 mg/ml) of lisinopril and ramipril (internal standard, IS) were prepared in water. They were diluted to produce working solutions of 200 ng/ml of lisinopril in human plasma and 2.0  $\mu$ g/ml of IS in water. Calibration curve standards (eight concentrations) in the range of 1.0–200 ng/ml were prepared in human plasma. Four quality control (QC) samples (1.0, 3.0, 100, and 180 ng/ml) were also prepared in human plasma. 1.0 ml aliquots were transferred into teflon-lined, screw-capped, borosilicate glass 12 x 100 mm culture tubes (Fisher Scientific Co., Fairlawn, NJ, USA) and stored at -20°C until used.

### Sample preparation

Aliquots of 1.0 ml of blank plasma, calibration curves, or QC samples in culture tubes were allowed to equilibrate to room temperature. To each tube, 50  $\mu$ l of the IS working solution (2.0  $\mu$ g/ml) was added and the mixture was vortexed for 20 seconds. After the addition of 4 ml tert-butyl methyl ether, the mixture was vortexed again for two minutes and then centrifuged at room temperature for 10 minutes at 4700 rpm. The clear supernatant organic layer was carefully transferred into clean tube and dried under gentle stream of nitrogen at 40°C, and the residue was reconstituted in 60  $\mu$ l mobile phase and transferred to an auto-sampler vial. 10  $\mu$ l were injected into the system. The run time was 3 minutes.

### Stability studies

Three QC samples (3.0, 100, and 180 ng/ml) were used for stability studies. Five aliquots of each QC sample were extracted and immediately analyzed (baseline), five aliquots were allowed to stand on the bench-top for 24 hours at room temperature before being processed and analyzed, five aliquots were stored at -20°C for 12 weeks before being processed and analyzed, and five aliquots were processed and stored at room temperature for 24 hours or at -20°C for 48 hours before analysis. Fifteen aliquots of each QC sample were stored at -20°C for 24 hours. They were then left to completely thaw at room temperature. Five aliquots of each sample were extracted and analyzed and the rest were returned to -20°C for another 24 hours. The cycle was repeated three times.

### Method validation

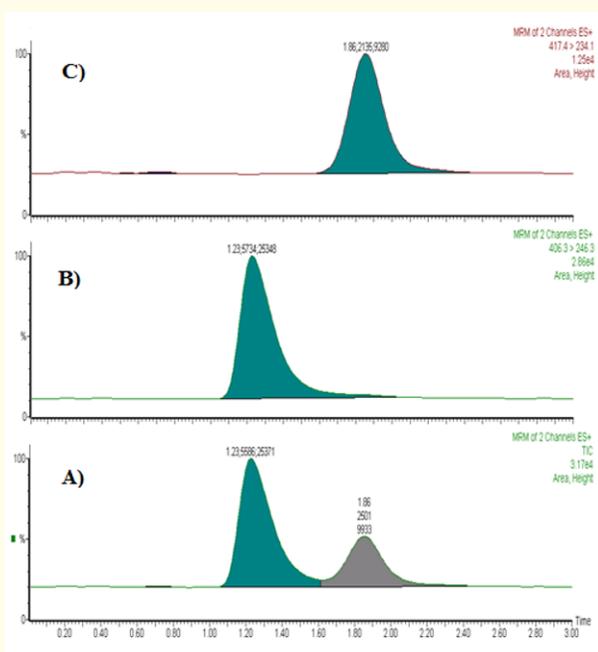
The LCMS/MS method was validated according to standard procedures described in the US Food and Drug Administration (FDA) bioanalytical method validation guidance [21]. The validation parameter included specificity, linearity, accuracy, precision, recovery, and stability.

## Results and Discussion

### Optimization of chromatographic conditions

Optimal experimental conditions consisted of a mobile phase composed of 5.0 mM ammonium formate (pH = 4.0 $\pm$ 0.1, adjusted

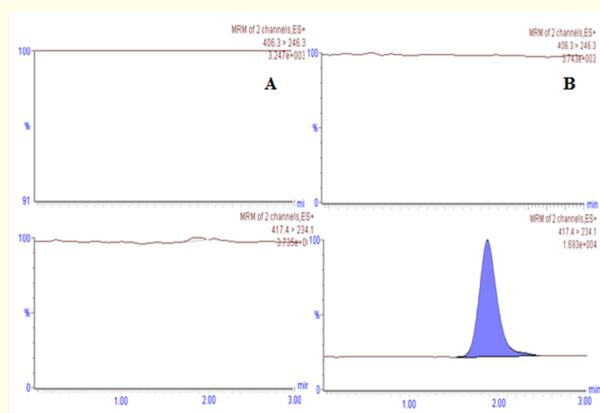
with formic acid) and acetonitrile (30:70, v:v) and delivered at a flow rate of 0.3 ml/minutes. Under these conditions lisinopril and IS were well separated and detected within a 3.0 minutes run. The retention times of the lisinopril and the IS were around 1.28 and 1.86 minutes, respectively. The electrospray ionization source was operated at an optimum capillary voltage of 3.3 KV and cone voltage of 30 V. The optimum collision energy for lisinopril and the IS was 23 eV. The ion source and the desolvation temperatures were maintained at 120°C and 350°C, respectively. Figure 2 depicts a representative total ion current (TIC) and multiple reaction monitoring (MRM) chromatograms of a standard mixture containing 200 ng/ml lisinopril and 100 ng/ml ramipril (IS) extracted from plasma.



**Figure 2:** Total ion current and multiple reaction monitoring chromatograms of a standard mixture containing lisinopril and ramipril. A) standard mixture containing (B) lisinopril (200 ng/ml) and ramipril (C) (100 ng/ml).

**Specificity**

We screened six batches of blank human plasma and nine frequently used medications (acetaminophen, ascorbic acid, aspirin, caffeine, diclofenac, ibuprofen, nicotinic acid, omeprazole, and ranitidine) for potential interference. No interference was found in plasma and none of the drugs co-eluted with lisinopril or the IS. Figure 3 depicts multiple reaction monitoring chromatograms of blank and IS spiked plasma.



**Figure 3:** Multiple reaction monitoring chromatograms of blank and IS-spiked human plasma. A) Blank plasma and B) IS-spiked plasma.

**Limit of detection and quantification and linearity**

The limit of quantification, defined as the lowest concentration on the calibration curve that can be determined with acceptable precision and accuracy (coefficient of variation and bias ≤ 20%), was 1.0 ng/ml. The limit of detection (signal to noise-ratio ≥3) was 0.5 ng/ml. Linearity of lisinopril was evaluated by analyzing ten curves of eight (plus zero) standard concentrations prepared in human plasma. Mean (SD) of slope, intercept, and coefficient of determination (R<sup>2</sup>) of the ten curves were 0.0115 (0.0013), 0.0042 (0.0156), and 0.9980 (0.0019), respectively. The suitability of the calibration curves was confirmed by back-calculating lisinopril level from the standard curve (Table 1). All back-calculated levels were well within the acceptable limits (coefficient of variation and bias ≤15%, except LLOQ ≤20%).

Nominal Level (ng/ml)	Measured Level (ng/ml)	CV (%)	Bias (%)
	Mean (SD)		
1.0	1.15 (0.05)	3.9	14.9
5.0	5.34 (0.39)	7.3	6.7
10.0	10.49 (0.79)	7.5	4.9
20.0	20.97 (1.64)	7.8	4.7
40.0	43.66 (2.06)	5.0	2.6
80.0	79.75 (5.65)	7.1	0.0
140	138.49 (5.82)	4.2	-1.3
200	202.05 (3.21)	1.6	1.0

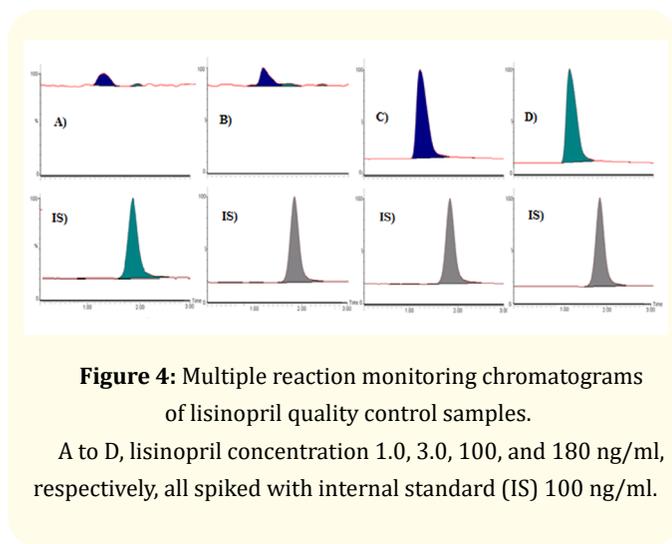
**Table 1:** Mean back-calculated lisinopril levels from ten calibration curves.

SD, standard deviation. CV, standard deviation divided by mean measured concentration x100.

Bias = (mean measured concentration – nominal concentration divided by nominal concentration) × 100.

**Precision and bias**

The intra-day and inter-day precision and bias of the method were evaluated by analyzing four QC samples concentration (1.0, 3.0, 100, and 180 ng/ml). Intra-day precision and bias (n = 10) ranged from 3.8% to 7.5% and from -5.3% to 6.2%, respectively. Inter-day precision and bias were determined over three different days (n = 20) and ranged from 3.1% to 10.1% and from -1.0% to 6.8%, respectively. The results are summarized in Table 2. Figure 4 depicts MRM chromatograms of lisinopril quality control samples extracted from plasma.



**Figure 4:** Multiple reaction monitoring chromatograms of lisinopril quality control samples.

A to D, lisinopril concentration 1.0, 3.0, 100, and 180 ng/ml, respectively, all spiked with internal standard (IS) 100 ng/ml.

Nominal level (ng/ml)	Measured level (ng/ml)	CV (%)	Bias (%)
	Mean (SD)		
Intra-day (n = 10)			
1.0	1.05 (0.06)	5.9	6.2
3.0	2.84 (0.21)	7.5	-5.3
100	96.25 (5.52)	5.7	-3.8
180	181.58 (6.93)	3.8	0.9
Inter-day (n = 20)			
1.0	1.07 (0.11)	10.1	6.8
3.0	3.18 (0.21)	6.5	6.0
100	99.91 (3.07)	3.1	-0.1
180	178.18 (6.46)	3.6	-1.0

**Table 2:** Intra- and inter-day precision and bias of lisinopril assay.

SD, standard deviation. CV, standard deviation divided by mean measured concentration x 100.

Bias = (mean measured concentration – nominal concentration divided by nominal concentration) × 100.

**Recovery**

The extraction recovery of lisinopril was assessed by comparing the peak area of lisinopril (5 duplicates) at four concentrations (1.0, 3.0, 100, and 180 ng/ml) spiked with blank plasma extracts (un-extracted) and extracted samples according method described. The recovery of the IS was assessed similarity at (100 ng/ml). The results are presented in Table 3. Mean recovery of lisinopril and the IS was 96% and 98%, respectively.

Level (ng/ml)	Mean peak area (SD)			*Recovery (%)	**Matrix Effect (%)
	Aqueous phase <sup>a</sup>	Un-extracted <sup>b</sup>	Extracted <sup>c</sup>		
Lisinopril 1.0	297(15)	286 (20)	268 (7)	94	3.7
3.0	743 (26)	702 (28)	678 (23)	97	5.5
100	18457(497)	17664 (234)	16610 (368)	94	4.3
180	31868(337)	30900 (852)	29997 (308)	97	3.0
IS (100)	19274 (613)	18760 (479)	18389 (548)	98	2.6

**Table 3:** Recovery and matrix effect on lisinopril and internal standard.

a) Lisinopril or ramipril solutions were prepared in water, b) Blank plasma extracts were spiked with equivalent concentration of linsopril or ramipril, c) Plasma samples extracted as described in the text.

\*Recovery = mean (n =5) peak area of lisinopril or ramipril (IS) extracted divided by un-extracted x 100.

\*\*Matrix effect = mean (n =5) peak area of lisinopril or ramipril (IS) aqueous phase minus un-extracted divided by aqueous phase x100.

### Matrix effect

Matrix effect was evaluated [22] by comparing peak area of lisinopril (5 duplicates) at four concentrations (1.0, 3.0, 100, and 180 ng/ml) spiked with blank plasma extracts (un-extracted) and aqueous sample (equivalent concentration prepared in water). Similarly matrix effect for IS was determined at concentration 100 ng/ml. Mean matrix effect was calculated as ion suppression 7.6% for lisinopril and 4.6% for the IS (Table 3).

### Stability

Lisinopril and IS stability in processed and unprocessed plasma samples was investigated using three QC concentrations (3.0, 100, and 180 ng/ml). Lisinopril in processed plasma samples was stable for at least 24 hours at room temperature ( $\geq 94\%$ ) or 48 hours at  $-20^{\circ}\text{C}$  ( $\geq 100\%$ ). Lisinopril in unprocessed samples was stable for at least 24 hours at room temperature ( $\geq 97\%$ ), 12 weeks at  $-20^{\circ}\text{C}$  ( $\geq 98\%$ ), or after three freeze-and thaw cycles ( $\geq 92\%$ ). Table 4 summarizes the results of stability studies.

Nominal Level (ng/ml)	Unprocessed		Processed		Freeze- Thaw Cycle		
	24 hrs. (RT)	12 Weeks ( $-20^{\circ}\text{C}$ )	24 hrs. (RT)	48 hrs. ( $-20^{\circ}\text{C}$ )	1	2	3
3.0	97	98	94	107	99	96	96
100	101	101	95	110	109	111	108
180	99	98	96	100	95	96	92

**Table 4:** Stability for lisinopril in processed and unprocessed human plasma.

Stability (%) = mean measured concentration (n = 5) at the indicated time divided by mean measured concentration (n = 5) at baseline x 100. Spiked plasma samples were processed and analyzed immediately (baseline, data not shown), after 24 hours at room temperature (24 hrs RT), or after freezing at  $-20^{\circ}\text{C}$  for 12 weeks (12 wks,  $-20^{\circ}\text{C}$ ), or processed and then analyzed after storing for 24 hours at room temperature (24 hrs, RT) or 48 hours at  $-20^{\circ}\text{C}$  (48 hrs,  $-20^{\circ}\text{C}$ ). Freeze-thaw (FT), samples were frozen at  $-20^{\circ}\text{C}$  and thaw at RT.

### Conclusions

The described LC-MS/MS assay for the determination of lisinopril level in human plasma is simple, precise, sensitive and accurate that makes it suitable for therapeutic drug monitoring and pharmacokinetic analysis. It requires 1.0 ml sample plasma and analysis completes within three minutes. The assay was successfully applied to assess the stability of lisinopril under various conditions encountered in the clinical laboratories.

### Conflict of Interest

None to declare.

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**Volume 3 Issue 9 September 2019**

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