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Liquid chromatographic-mass spectrometric method for quantitative determination of lisinopril in human plasma

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A validated liquid chromatographic-mass spectrometric (LC/MS) method for the determination of lisinopril in human plasma is presented. Enalapril was used as an internal standard. After the addition of internal standard, solid phase extraction was used as a cleaning step. To separate lisinopril and enalapril from interfering endogenous plasma substances, the analysis was performed using column switching valve. The quantitative determination was performed using selected ion monitoring (+)-electrospray LC-MS. A combination of an acidic mobile phase and a reverse phase column was used. A precision in the linear range from 10 to 500.0 ng/mL plasma, absolute recovery of 91.69% for lisinopril and 90.26% for enalapril, stability for 3.5 months at −20 °C have been achieved. Limit of quantitation (LOQ) was 10 ng/mL while limit of detection (LOD) was about 1 ng/mL.

1. Introduction

Lisinopril, (*S*)-1-[N^2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline is slowly, variably and incompletely absorbed after oral administration (Hardman et al. 1996), (Dollery et al. 1999). Lisinopril is the third oral angiotensin-converting enzyme (ACE) inhibitor used in the treatment of hypertension reducing morbidity and mortality in congestive heart failure (Parfitt et al. 1999; Pitt et al. 1994). Lisinopril was analyzed using different analytical methods including GC/MS (Leis et al. 1998, 1999), radioimmunoassay (Worland et al. 1986; Sun et al. 1991), solid-phase fluoroimmunoassay (Yuan et al. 1996), spectrofluormetric, spectrophotometric (El-Gindy et al. 2001), HPLC-UV (Kocijan et al. 2001; Wong et al. 1995). GC/MS applied two steps derivatization to make suitable for GC analysis. The HPLC methods described for analysis of lisinopril were in solid dosage form and for the measurement of lisinopril in urine (Wong et al. 1995; Bonazzi et al. 1997). The present paper describes the development of a LC-MS method for quantitation of lisinopril in human plasma and validation in terms of accuracy, precision, sensitivity, recovery, specificity and stability.

2. Investigations, results and discussion

To separate lisinopril and the internal standard enalapril from interfering endogenous plasma substances, the analysis was performed using a column switching valve. The analytes were monitored by the measurement the response of mass detection in (+)-electrospray ionization mode and single ion monitoring at 405.7 and 376.6 m/z for lisinopril and enalapril, respectively. The combination of solid phase extraction and chromatography provided a rapid assay free from interferences.

The method was evaluated in terms of linearity, accuracy, precision, sensitivity, recovery, specificity and stability. The Fig. 1 represents the liquid chromatogram of plasma sample containing lisinopril and the internal standard enalapril.

Two standard calibration curves of 8 points (non-zero standards) were prepared on 3 consecutive days. Two sets of calibration standards and five sets of 10, 30, 175, and 400 ng/mL spiked quality control samples were prepared and analyzed. The calibration curves were evaluated individually by linear regression and the concentrations of the calibration standards were back calculated. The concentrations were then normalized, by dividing by the corresponding theoretical values. The statistical parameters including the means, standard deviations, coefficient of

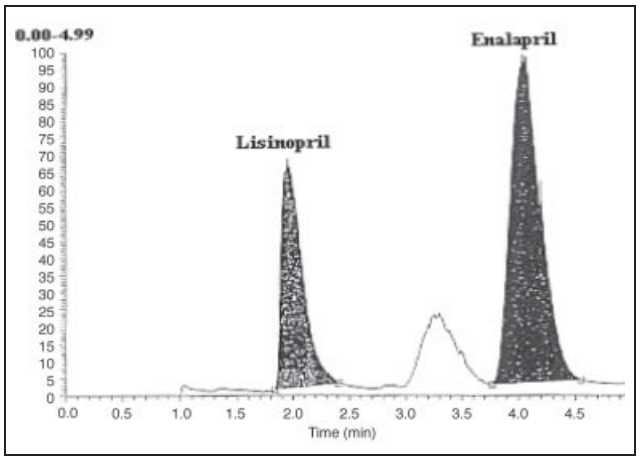

Fig.: Liquid chromatogram of a standard plasma sample containing the drug (lisinopril) at a concentration of 175 ng/ml (medium QC) and the internal standard (enalapril) at a concentration of 100 ng/ml

Table 1: Statistical analysis of back-calculated normalized lisinopril concentrations of the calibration standards in human plasma

Concentration (ng/mL)	Mean	SD	Precision as CV (%)	Accuracy (%)	RE (%)
10	1.0329	0.1425	13.80	103.29	3.29
20	0.9963	0.1199	12.03	99.63	−0.37
50	0.9849	0.1085	11.02	98.49	−1.51
100	1.0454	0.1000	9.57	104.54	4.54
150	0.9339	0.0584	6.25	93.39	−6.61
200	0.9730	0.0842	8.65	97.30	−2.70
350	0.9443	0.0676	7.16	94.43	−5.57
500	1.0359	0.0375	3.62	103.59	3.59

Table 2: Intra-day accuracy, precision and relative error of lisinopril spiked quality control samples in human plasma

Day	Theo. Conc.	Mean ng/mL	SD ng/mL	Precision as CV (%)	Accuracy (%)	RE (%)
Day 1	10	10.40	1.82	17.50	104.00	4.00
	30	30.20	3.49	11.56	100.67	0.67
	175	168.40	22.68	13.47	96.23	−3.77
	400	396.40	49.42	12.47	99.10	−0.90

variation, accuracy and relative error (%) were calculated for the back-calculated normalized concentrations of each calibration curve (Table 1). The coefficient of correlation was consistently greater than 0.9910 during the course of validation.

The intra-day accuracy and precision of the assay was measured by analyzing 5 replicates of 10, 30, 175, and 400 ng/mL spiked quality controls samples of lisinopril. Intra-day accuracy of the method for lisinopril ranged from 96.23 to 104.00%, while the intra-day precision ranged from 11.56 to 17.50% at concentration of 10, 30, 175, and 400 ng/mL (Table 2).

The inter-day precision of the assay was measured by analyzing 15 replicates of 10, 30, 175, and 400 ng/mL quality controls of lisinopril obtained from day 1, 2 and 3. Inter-day accuracy of the method for lisinopril ranged from 98.73 to 106.70%, while the inter-day precision ranged from 8.92 to 12.65%, at concentration 10, 30, 175, and 400 ng/mL (Table 3).

The limit of quantitation defined as the concentration with acceptable accuracy and precision (below 15%) was 10 ng/mL plasma. While the limit of detection was about 1 ng/mL it was sufficient for bioavailability studies of lisinopril.

The percent absolute recovery was determined by measuring the absolute peak height of lisinopril and enalapril from a plasma sample prepared according to the method mentioned earlier. The absolute peak height obtained from the injection

Table 3: Inter-day accuracy, precision and relative error of the lisinopril spiked quality control samples in human plasma

Analyzed on 3 days	Measured lisinopril concentration in human plasma			
	10 ng/mL	30 ng/mL	175 ng/mL	400 ng/mL
Mean (μg/mL)	10.67	31.27	176.07	394.93
SD (μg/mL)	1.35	2.79	19.88	41.41
Precision CV (%)	12.65	8.92	11.29	10.49
Accuracy (%)	106.70	104.23	100.61	98.73
RE (%)	6.70	4.23	0.61	−1.27

Table 4: Absolute analytical recovery of lisinopril and enalapril

Conc. (ng/ml)	Normalized mean of lisinopril		Recovery (%)
	Peak in human plasma	Peak in direct injection	
30	55346	61148	90.51
175	54144	56478	95.87
400	50589	57047	88.68
Mean			91.69
	Mean peak area of enalapril		
	16831442	18647705	90.26

of the prepared plasma standards was compared to the absolute peak area of an equivalent aqueous standard, which was prepared to contain a concentration of drug and internal standard assuming 100% recovery. The percent absolute recovery of lisinopril and enalapril is shown in Table 4.

Relative recovery of lisinopril was determined by comparing the measured concentration with actual added ones using three different quality control samples 30, 175, and 400 ng/mL. The data are presented in Table 5.

The specificity of the method was determined by screening six different batches of controlled human blank plasma; which were free from interfering endogenous plasma components. This was evidenced by the lack of interfering peaks in the chromatograms of plasma samples. Solutions of commonly used drugs including: aspirin, acetaminophen, ascorbic acid, caffeine, nicotine and ibuprofen were prepared in mobile phase and then were injected to check for interference. No interferences were observed.

Testing for freeze and thaw anaylte stability was determined during freeze and thaw cycles. Fifteen replicates of each concentration of two different quality control samples 30 ng/mL and 400 ng/mL were prepared and stored at −20 °C for 24 h. All samples were thawed unassisted at room temperature. When completely thawed 5 controls of each concentration were analyzed and the rest were returned to freezer and kept frozen for 24 h. The same procedure was repeated for the

Table 5: Relative recovery of lisinopril

Actual concentration (30 ng/ml)		Actual concentration (175 ng/ml)		Actual concentration (400 ng/ml)	
Measured concentration	% Relative recovery	Measured concentration	% Relative recovery	Measured concentration	% Relative recovery
30	100.00	182	104.00	447	111.75
26	86.67	200	114.29	344	86.00
34	113.33	157	89.71	387	96.75
32	106.67	189	108.00	356	89.00
33	110.00	155	88.57	361	90.25
Mean					
	103.33		100.91		94.75

Table 6: Freeze-thaw stability of lisinopril of quality control samples 30 and 400 ng/mL

	Mean concentration of lisinopril			Mean	SD	CV%
	F.T once	F.T twice	F.T third			
Q.C 30 ng/ml	29.00	27.80	26.20	27.67	1.40	5.06
Q.C 400 ng/ml	404.00	390.80	383.40	392.73	10.44	2.66

Table 7: Sample stability after preparation of 30 and 400 ng/mL for 24 h at room temperature

Q.C Sample		Initially Analyzed conc. (ng/mL)	Analyzed conc. 24 h R.T	Stability (%)
30 ng/mL	Mean	30.61	28.40	93.02
	SD	2.70	2.07	5.12
	CV %	8.82	7.28	5.50
400 ng/mL	Mean	407.20	388.82	95.52
	SD	19.00	18.19	3.17
	CV %	4.66	4.67	3.32

Table 8: Long-term stability (freezer storage stability)

Q.C Sample		Analyzed conc. after 1 month (ng/mL)	Analyzed conc. after 3.5 months (ng/mL)
30 ng/mL	Mean	26.80	28.20
	SD	1.30	1.79
	CV %	4.85	6.35
400 ng/mL	Mean	385.20	378.00
	SD	19.54	19.48
	CV %	5.07	5.15

remaining controls for testing cycle 2 and cycle 3 (Table 6).
On a validation day ten samples of each concentration of two different quality control samples 30 and 400 ng/mL were prepared as described in section 3.3.3.
The supernatants of samples were pooled. Five samples of each concentration were analyzed immediately after preparation, another five samples of each concentration were stored at room temperature for 24 h. Results are presented in Table 7.
Ten replicates of each concentration of the following quality control samples: 30 and 400 ng/mL were prepared and stored at −20 °C. Five samples of each concentration were prepared as described in section 3.3. and analyzed at the end of the first month. The other 5 samples of each concentration were prepared and analyzed at the end of the three and half months (Table 8).

3. Experimental

3.1. Equipment

The LC-MS equipment used was consisted of ThermoQuest Finnigan AQA, a single quadrople, equipped with (+)-electronspray ionization (ESI) interface, probe temperature set at 300 °C and ionization voltage at 20 V, TSP P2000 pump, SN4000 System Controller, AS3000 autosampler, PR500-100-01 (switch valve) Lab PRP Rheodyne. Symmetry, Waters C18 (5 μm) (150 × 3.9 mm) column. AQA LC-MS adapts Xcaliber, Software Finnigan. Selected ion monitoring (SIM) has been applied on the following ions: 405.7 m/z for lisinopril and 376.6 m/z for enalapril.

3.2. Reagents

Lisinopril was kindly donated from West-Ward, USA and enalapril was kindly donated by Hikma Pharmaceutical, Jordan. Human blood plasma was obtained from the National Center of Blood Transfusion, Amman, Jordan. Methanol HPLC grade was obtained from Merck, Germany. Acetic acid was obtained from Panreac, Spain. Solid phase extraction cartridges (SPE), Oasis HLP 1 cc (30 mg) were obtained from Waters, USA. Deionized water was prepared in our laboratories using Easy pure RO and Easy pure UV system, Parnstead Thermolyne, USA.

3.3. Analytical procedure

3.3.1. Preparation of stock solutions

Standard stock solutions of lisinopril and enalapril were prepared by dissolving 100 mg of each in a 100 mL volumetric flask using de-ionized water to produce a concentration of 1 mg/mL. The standard working solutions were prepared by diluting the standard stock solution of lisinopril 200 folds and enalapril by 2000 folds with de-ionized water to produce a concentration of 5 μg/mL for lisinopril and 0.5 μg/mL for enalapril.

3.3.2. Preparation of calibration samples

The calibration plasma samples were prepared in 10 mL volumetric flasks as blank, zero standard, 10, 20, 50, 100, 150, 200, 350, and 500.0 ng/mL. Each volumetric flask was vortexed for 5 min and the content of each was split to several 10 mL glass tubes containing 0.5 mL plasma. The quality control samples were prepared in the following concentration: 10, 30, 175, and 400 ng/mL in 25 mL volumetric flask. Each volumetric flask was vortexed and split into 10 mL glass tubes containing 0.5 mL plasma.

3.3.3. Sample preparation

100 μL of internal standard working solution was added to 0.50 mL plasma sample (standard sample, control sample or volunteer sample) and vortexed for 30 s. Solid phase extraction technique was used as follows: 1 mL of methanol was added to SPE column for conditioning, 1 mL of de-ionized water was added for equilibrium, 0.5 mL of plasma sample with 100 μL of internal standard was loaded, 0.5 mL of de-ionized water was added for washing and finally 1 mL of methanol was added for elution. The eluated solution was evaporated to dryness at 50 °C under N_2 gas, then reconstituted with 120 μL of mobile phase and transferred to a 250 μL micro glass insert tube, centrifuged for 5 min at 13000 rpm. 50 μL aliquot sample was injected and chromatographed using Symmetry C18 (5 μm) (150 × 3.9 mm) column.

3.3.4. Chromatographic conditions

Chromatographic condition was a combination of switching valve and C18 column using the switching time events:

Switching (time events):
From 0.00 to 1.00 mi (to waste).
From 1.00 to 5.00 min (to column and mass detector).

The mobile phase used consisted of 50% methanol and 50% of 1% acetic acid in the isocratic mode. The injection volume was 50 μL and the flow rate was 0.5 mL/min.

3.3.5. Standardization and calculation

The standard calibration curve lines were shown to be linear in the range from 10 to 500 ng/mL for lisinopril in human plasma. Best-fit calibration lines of peak area ratios (peak area analyte/peak area internal standard) versus concentration were determined by single-level calibration curve.

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