

Determination of quinapril and quinaprilat in human plasma by ultraperformance liquid chromatography–electrospray ionization mass spectrometry

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ABSTRACT: A novel, specific and sensitive ultraperformance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) method was developed for the simultaneous determination of quinapril and its active metabolite quinaprilat in human plasma. The method involves a simple, one-step extraction procedure coupled with an Acquity UPLC™ BEH C₁₈ column (100 × 2.1 mm, i.d., 1.7 μm) with isocratic elution at a flow-rate of 0.2 mL/min and lisinopril as the internal standard. Detection was performed on a triple-quadrupole tandem mass spectrometer in multiple reaction monitoring mode via electrospray ionization. Using 250 μL plasma, the methods were validated over the concentration range 5.010–500.374 ng/mL for quinapril and 10.012–1000 ng/mL for quinaprilat, with a lower limit of quantification of 5.010 ng/mL for quinapril and 10.012 ng/mL for quinaprilat. The intra- and inter-day precision and accuracy were within 10.0%. The recovery was 85.8, 62.6 and 61.3% for quinapril, quinaprilat and lisinopril, respectively. Total run time was 3.0 min only. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: quinapril; LC-MS/MS; human plasma; acquity; UPLC

Introduction

Quinapril HCl, 2-(2-[[1-(ethoxycarbonyl)-3-phenyl-propyl]amino]-1-oxopropyl)-1, 2, 3, 4-tetra hydro-3-isoquinoline carboxylic acid mono hydrochloric acid, is a potent orally active non-sulphydryl non-peptidic angiotensin converting enzyme (ACE) inhibitor. It is de-esterified *in vivo* to the active di-acid metabolite quinaprilat (Begg *et al.*, 1994). The full activity of quinapril depends on conversion to the active metabolite quinaprilat (Squire *et al.*, 1994).

Quinaprilat possess high affinity for ACE in the artery, heart and renal tissues, and exhibits potent and long-lasting ACE inhibitor effect, thus it is used widely in the treatment of hypertension (Yamada, 2003).

Several analytical methods for the quantitation of quinapril and quinaprilat in biological fluids have been reported, such as gas chromatography ($R_T > 15.0$ min, plasma volume ≥ 1.0 mL; Ferry *et al.*, 1987; Blum *et al.*, 1990), liquid chromatography with radioimmunoassay (Yamada *et al.*, 2003), liquid chromatography with photometric detection (limit of quantitation, LOQ = 60 ng/mL for quinapril and 50 ng/mL for quinaprilat; Prieto *et al.*, 2001), liquid chromatography with ultraviolet detection (LOQ = 10 ng/mL for quinapril and 20 ng/mL for quinaprilat, $R_T > 17.0$ min; Abbara *et al.*, 2002) and liquid chromatography with radiochemical detection, coupled to liquid scintillation (Kugler *et al.*, 1995).

However, these methods are not suitable for pharmacokinetic work, because they involve arduous sample preparation, long chromatographic run time and large plasma volumes. Moreover, no publication has described the quantitative analysis of quinapril and quinaprilat using tandem mass spectrometry. This paper reports sensitive, simple and specific method for simultaneous

determination of quinapril and quinaprilat in human plasma by solid-phase extraction and LC/MS/MS detection. The method is not only selective and sensitive but faster and simpler than any other reported methods. Lisinopril (Fig. 1) was used as an internal standard.

Experimental

Chemicals and Reagents

The working standards of quinapril, quinaprilat and lisinopril obtained from Synchron Research Services Pvt Ltd (Ahmedabad, India). High-purity water was prepared in-house using a Milli-Q water purification system obtained from Millipore (Bangalore, India). HPLC methanol and acetonitrile were purchased from Merck (Mumbai, India). Formic acid were purchased from Merck (Germany). Drug-free (blank) human plasma was obtained from Prathma laboratory (Ahmedabad, India) and was stored at -20°C prior to use.

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Abbreviations used: ACE, angiotensin converting enzyme.

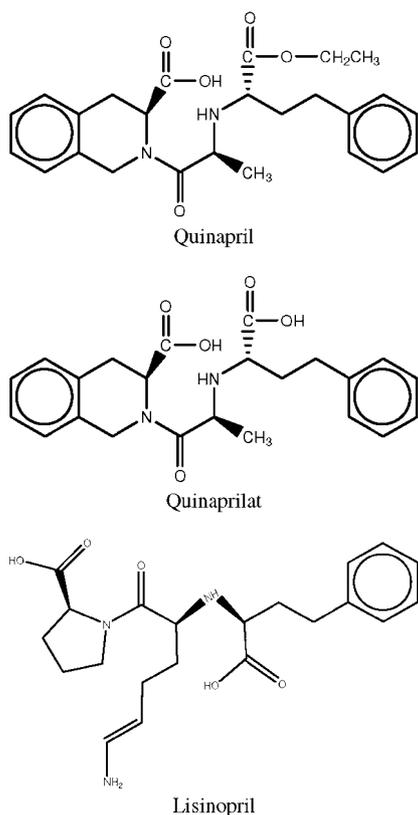


Figure 1. Chemical structures of quinapril, quinaprilat and lisinopril.

Calibration Curves

The stock solution of quinapril, quinaprilat and internal standard (lisinopril) were prepared individually in methanol at a free base concentration of 1 mg/mL. Secondary standard solutions were prepared by further diluting stock solution with methanol:water (1:1 v/v) to get a combined intermediate stock solution of 250 µg/mL for quinapril and 500 µg/mL for quinaprilat. Working standard solutions were prepared from secondary standard solution by dilution with water:methanol (1:1, v/v). These diluted working standard solutions were used to prepare the calibration curve and quality control samples. An eight-point standard calibration curve for quinapril and quinaprilat was prepared by spiking 250 µL blank plasma with 10 µL of the appropriate working standard solution. The calibration curve range was 5.010–500.374 ng/mL for quinapril and 10.012–1000 ng/mL for quinaprilat. Quality control samples were prepared at three concentration levels of 15.035 ng/mL (low), 225.027 ng/mL for (medium) and 400.049 ng/mL (high) for quinapril and 25.031 ng/mL (low), 450.00 ng/mL (medium) and 800.00 ng/mL (high) for quinaprilat.

Sample Preparation

A 250 µL aliquot of human plasma sample was mixed with 25 µL of internal standard working solution (125 µg/mL of lisinopril). To this 250 µL of 0.1% formic acid was added after vortex mixing for 10 s. The sample mixture was loaded onto an Oasis HLB extraction cartridge (30 mg/cm³) that was pre-conditioned with 2.0 mL of methanol followed by 1.0 mL 1% formic acid. The extraction cartridge was washed with 1.0 mL of 1% formic acid. Quin-

april, quinaprilat and lisinopril were eluted with 0.5 mL of mobile phase 10 µL of the extract were injected in to the LCMS/MS system.

Instrumentation

The chromatography was performed on an Acquity UPLC system (Waters Corp., Milford, MA, USA) with cooling auto-sampler and column oven enabling temperature control of the analytical column. An Acquity UPLC™ BEH C₁₈ column (100 mm × 2.1 mm, 1.7 µm; Waters Corp., Milford, MA, USA) was employed. The column temperature was maintained at 45°C and chromatographic separation was achieved using mobile phase consisting of acetonitrile–water–formic acid (70:30:0.1, v/v/v), delivered with a flow rate of 0.2 mL/min. The auto-sampler was maintained at 10°C and the injection volume was 10 µL. Total run time for each sample analysis was 3.0 min.

Validation

The method validation was carried out as per US FDA guidelines. The method was validated for selectivity, linearity, precision, accuracy, recovery, stability and dilution integrity. Selectivity was assessed, by comparing the chromatograms of six different batches of blank plasma obtained from six different sources (or donors) with those of corresponding standard plasma samples spiked with quinapril, quinaprilat and lisinopril. Sensitivity was determined by analyzing six replicates of blank human plasma and plasma spiked with the lowest level of the calibration curve. All validation experiments were carried out at three QC levels. For the determining of intra-day accuracy and precision a replicate ($n = 6$) analysis of plasma samples was performed on the same day. The inter-day accuracy and precision were assessed by analysis of three batches on different days. The precision was expressed as the relative standard deviation (RSD%) and the accuracy as the relative error (RE%). Recovery of quinapril, quinaprilat and lisinoapril was determined by comparing the peak area of extracted analyte standard with the peak area of non-extracted standard. Dilution integrity was performed to extend the upper concentration limit with acceptable precision and accuracy. Five replicates each at a concentration of double the uppermost calibration standard were diluted 2- and 5-fold with blank plasma. The diluted samples were processed and analyzed. Process sample stability was evaluated by re-injecting the same sample with freshly spiked calibration curve and quality control samples, which (stability samples) were stored at 10°C for 24 h. Bench top stability was evaluated for 6 h and compared with freshly spiked plasma samples. The freeze–thaw stability was determined by comparing the stability samples that had been frozen and thawed three times, with freshly spiked quality control samples. Long-term stability was evaluated by analyzing at low and high quality control sampled that were stored at –20°C for 60 days together with freshly spiked calibration and quality control standards. All stability evaluations were based on back calculated concentrations.

Result and Discussion

Chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and increase the signal of analytes, as well as short run time. The presence of a small amount of formic acid in the mobile phase improved the detection of the analytes. It was found

that a mixture of water–acetonitrile–formic acid (30:70:0.1, v/v/v) could achieve this purpose and was finally adopted as the mobile phase. The use of small particles of stationary phase allowed UPLC to push the limits of peak capacity and speed of analysis (due to higher linear velocities).

Three channels were used for recording, channel 1 for quinapril with a retention time of 0.84 min, channel 2 for quinaprilat with a retention time of 0.83 min and channel 3 for lisinopril with a retention time of 0.71 min. Quinapril, quinaprilat and IS were rapidly eluted with retention time less than 2 min, and total run time was just 3.0 min per sample. This met the requirement for a high sample throughput. The resulting signal with optimized chromatography and detection parameters enabled the elimination of laborious extraction steps of evaporation of eluent and reconstitution involved in generic solid-phase extraction meth-

ods without compromising the sensitivity and reduced processing and analysis time.

Mass Spectrometry

In order to develop a method with the desired LLOQ it was necessary to use MS-MS detection, as the MS-MS method provides improved limit of detection for trace-mixture analysis (Jemal, 2000). When quinapril, quinaprilat and lisinopril were scanned directly in the mass spectrometer along with the mobile phase with positive ion interface, the full-scan spectrum was dominated by protonated molecules $[M + H]^+$ m/z 439.09, 411.15 and 406.13 for quinapril, quinaprilat and lisinopril. The mass spectra of precursor ions of quinapril, quinaprilat and lisinopril are presented in Fig. 2 and major fragment ions observed in each product

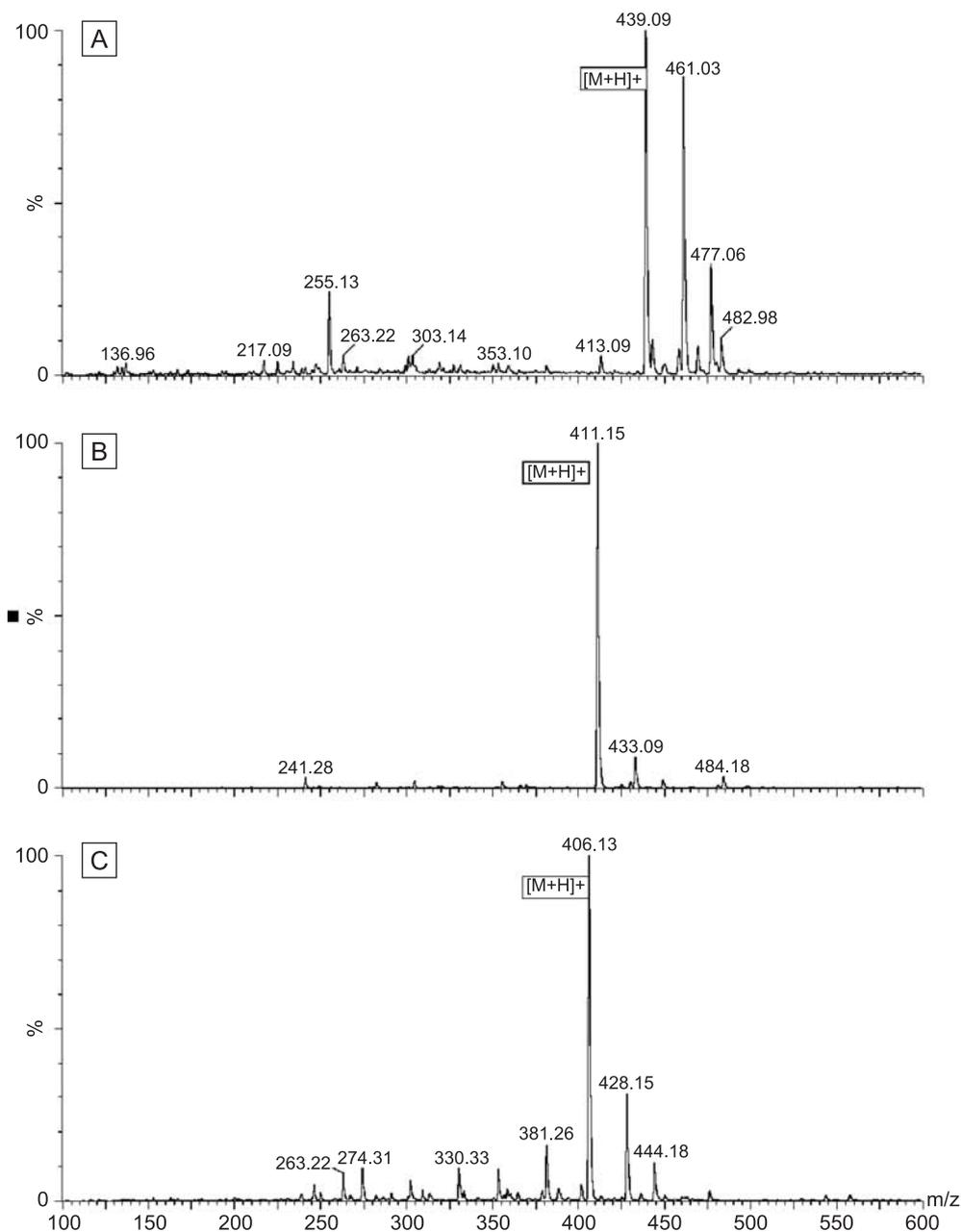


Figure 2. Electropray positive ion mass spectra of the precursor ion of (A) quinapril, (B) quinaprilat and (C) lisinopril.

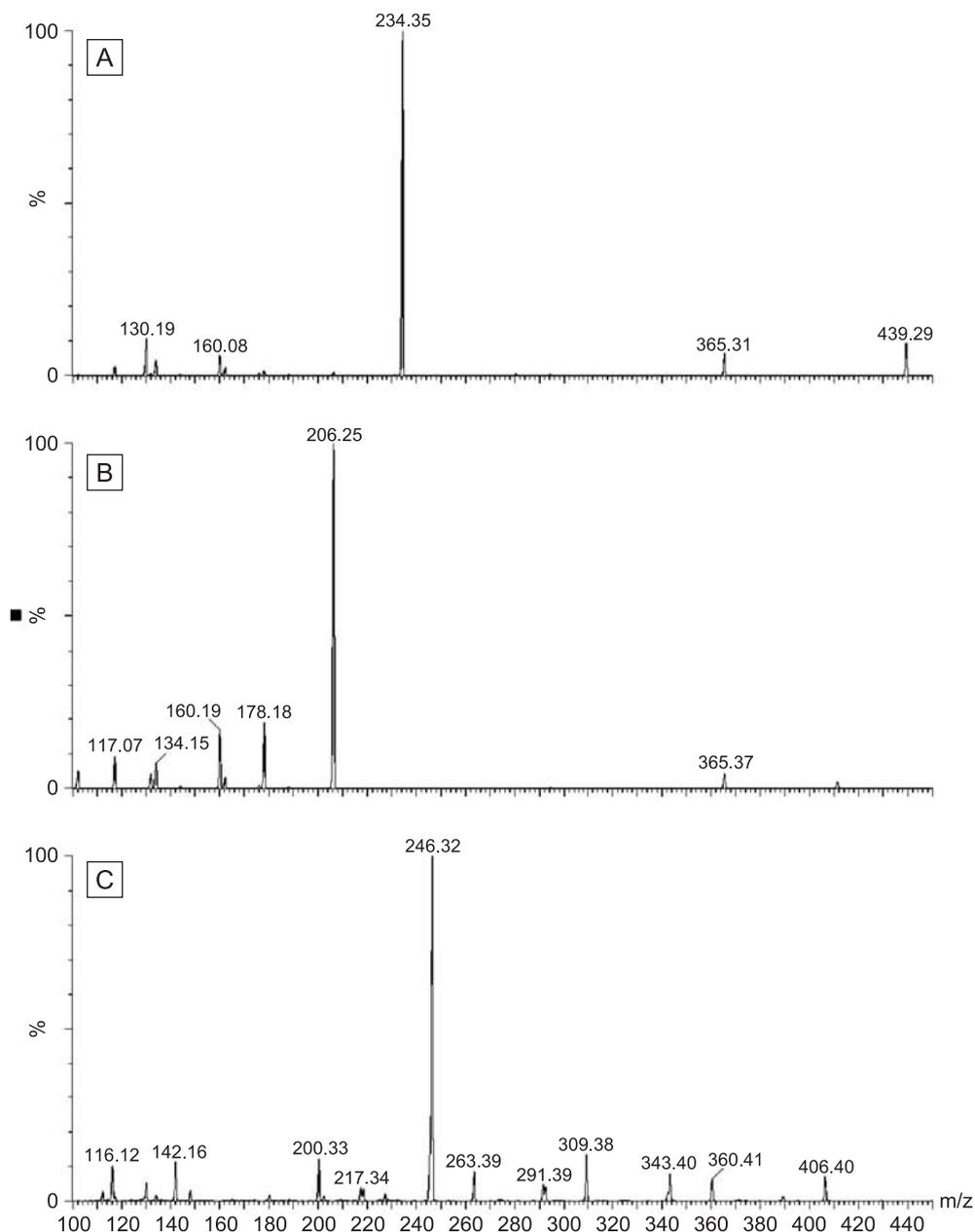


Figure 3. Electrospray product ion mass spectra for (A) quinapril, (B) quinaprilat and (C) lisinopril.

spectrum were at m/z 234.2, 206.13 and 246.18 respectively. The selected fragments of each compound, as product ions to be monitored, are indicated in Fig. 3. The impact of temperature, nebulizer gas and ESI temperature were investigated to optimize the specificity and sensitivity of m/z 234.2, 206.13 and 246.18 ions detection.

Selectivity

The selectivity of the method was examined by analyzing ($n = 6$) blank human plasma extract against plasma spiked with the lowest standard. As shown in Fig. 4, no significant direct interference in the blank plasma traces were observed from endogenous

substances in drug-free human plasma at the retention time of the analytes.

Linearity

Eight-point calibration curve was found linear over the concentration range of 5.010–500.374 ng/mL for quinapril and 10.012–1000 ng/mL for quinaprilat. The calibration model was selected based on the analysis of the data by linear regression with/without intercepts and weighting factors ($1/x$, $1/x^2$ and $1/\sqrt{x}$). The best linear fit and least-squares residuals for the calibration curve were achieved with a $1/x^2$ weighing factor, giving a mean linear regression equation for the calibration curve of:

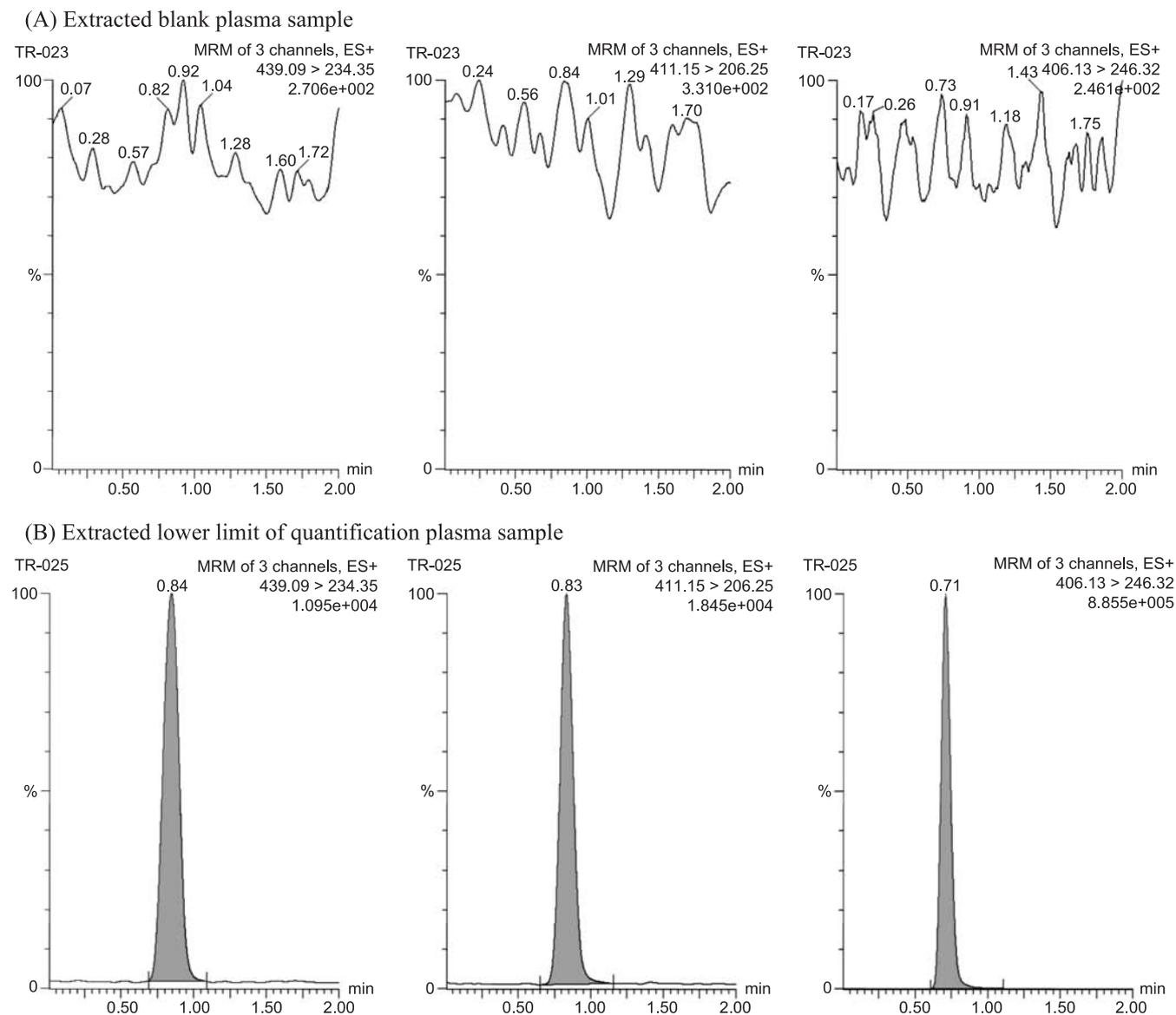


Figure 4. Representative chromatograms of (A) extracted blank plasma sample and (B) extracted lower limit of quantification plasma sample.

$y = 0.015 (\pm 0.002)x + 0.002 (\pm 0.006)$ for quinapril and $y = 0.004 (\pm 0.0008)x + 0.004 (\pm 0.006)$ for quinaprilat where y is the peak area ratio of the analyte to the IS and x is the concentration of the analyte. The mean correlation coefficient of the weighted calibration curve generated during the validation was 0.999; Table 1 summarizes the calibration curve results.

Sensitivity

The LLOQ was defined as the lowest concentration in the standard curve that can be measured with acceptable accuracy and precision, and it was found to be 5.010 ng/mL for quinapril and 10.012 ng/mL for quinaprilat. The intra-day precision at the LLOQ was 9.3 and 6.3%, and the intra-day accuracy was 6.2 and 6.2% for quinapril and quinaprilat respectively (Table 2).

Precision and Accuracy

The results for intra-day and inter-day precision and accuracy for quinapril and quinaprilat in plasma quality control samples are summarized in Table 2. The intra-day precision and accuracy was ≤ 9.3 and $\leq 6.2\%$ for quinapril and ≤ 6.3 and $\leq 6.2\%$ for quinaprilat, respectively. The inter-day precision and accuracy was ≤ 7.4 and $\leq 4.3\%$ for quinapril and ≤ 5.7 and $\leq 5.5\%$ for quinaprilat, respectively.

Recovery

Five replicates at low, medium and high quality control concentration for quinapril and quinaprilat were prepared for recovery determination. The mean recovery was 85.8, 62.6 and 61.3% for quinapril, quinaprilat and lisinopril respectively.

Table 1. Precision and accuracy data of back-calculated concentration of calibration samples for quinapril and quinaprilat in human plasma

Analytes	Concentration added (ng/mL)	Concentration found (mean; ng/mL)	Precision (%)	RE (%)
Quinapril	5.010	5.37	9.75	7.19
	10.019	9.34	8.71	-6.82
	25.049	24.73	7.93	-1.29
	75.109	75.26	3.04	0.20
	250.362	238.55	2.28	-4.72
	375.355	380.13	1.83	1.27
	450.336	422.13	0.65	-6.26
	500.374	461.32	1.10	-7.80
Quinaprilat	10.012	10.12	5.81	1.12
	20.024	20.04	10.11	0.08
	50.060	50.09	8.68	0.06
	150.105	157.49	7.17	2.70
	500.350	446.35	0.83	-10.19
	750.150	707.76	0.92	-5.65
	900.000	852.04	0.96	-5.33
	1000.000	1020.40	0.40	2.24

Table 2. Precision and accuracy of the method for determining quinapril and quinaprilat concentration in plasma samples

Analytes	Concentration added (ng/mL)	Intra-day precision (n = 6)			Inter-day precision (n = 18)		
		Concentration found (mean; ng/mL)	Precision (%)	RE (%)	Concentration found (mean; ng/mL)	Precision (%)	RE (%)
Quinapril	5.010	4.751	9.3	-6.2	4.795	7.4	-4.3
	15.035	15.249	6.2	1.4	15.216	5.2	1.2
	225.027	225.298	5.1	0.1	222.481	4.3	-1.1
	400.049	394.634	3.8	-1.4	396.535	3.8	-0.9
Quinaprilat	10.012	10.113	5.8	-2.9	10.037	5.7	0.2
	25.031	23.469	6.1	-6.2	23.65	4.9	-5.5
	450.000	458.285	6.3	1.8	459.083	4.6	2.0
	800.000	815.339	4.7	1.9	798.492	3.9	-0.2

Dilution Integrity

The upper concentration limits can be extended to 800.098 ng/mL for quinapril and 1600 ng/mL for quinaprilat by a 2- or 5-fold dilution with human plasma with a precision of 6.2 and an accuracy of 3.4% for quinapril and quinaprilat, respectively.

Stability

The stability of the analytes in human plasma under different temperature and timing conditions was evaluated as follows and the results of the stability studies are enumerated in Table 3. QC samples were subjected to long-term storage conditions (-20°C), and to freeze-thaw stability studies. All the stability studies were conducted at two concentration levels (15.035 and 400.049 ng/mL for quinapril and 25.031 and 800.0 ng/mL for quinaprilat as low and high QC values) with five determinations for each. For process stability, the results indicated that the difference in the back-calculated concentration from time 0–24 h is 5.1 and 2.1% for quinapril and quinaprilat, respectively, which

allowed us to conclude that processed samples are stable at least for 24 h at 10°C in the auto-sampler. For bench top stability, the results allowed us to conclude that both analytes are stable for at least 6 h at room temperature in plasma samples. Freeze and thaw stability results indicated that the repeated freeze and thawing (three cycles) did not affect the stability of quinapril and quinaprilat. Long-term stability of the analytes in plasma at -20°C was found to be stable for at least 60 days at -20°C.

Conclusion

A simple, specific, rapid and sensitive UPLC-MS/MS method has been developed for the determination of quinapril and quinaprilat in human plasma and fully validated according to accepted criteria in the US FDA Guidelines (Shah *et al.*, 1991). The proposed method provided excellent specificity and reproducibility. It is concluded that this sensitive and specific method is applicable for the quantitative determination of quinapril and quinaprilat in human plasma in pharmacokinetic and bioavailability studies of quinapril.

Table 3. Stability samples result for quinapril and quinaprilat

Analytes	Stability	<i>n</i>	Spiked concentration (ng/mL)	Mean calculated comparison sample concentration (ng/mL)	Mean calculated stability sample concentration (ng/mL)	Mean percentage change	
Quinapril	Process ^a	5	15.035	14.767	14.014	-5.1	
		5	400.049	413.943	412.321	-2.9	
	Bench top ^b	5	15.035	14.062	13.619	-3.2	
		5	400.049	381.630	388.581	1.8	
	Freeze and thaw ^c	5	15.035	13.253	13.593	2.6	
		5	400.049	420.342	410.569	-2.3	
	Long-term ^d	5	15.035	15.379	14.093	-8.4	
		5	400.049	406.745	389.089	-4.3	
	Quinaprilat	Process ^a	5	25.031	25.237	24.701	-2.1
			5	800.000	832.854	817.074	-1.9
Bench top ^b		5	25.031	24.599	24.521	-0.3	
		5	800.000	731.120	756.314	2.6	
Freeze and thaw ^c		5	25.031	24.532	23.661	-3.5	
		5	800.000	821.257	803.058	-2.2	
Long-term ^d		5	25.031	25.164	24.745	-1.7	
		5	800.000	814.119	787.797	-3.2	

^a After 24 h in autosampler at 10°C; ^b after 6 h at room temperature; ^c after three freeze and thaw cycles at -20°C; ^d at -20°C for 60 days.

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