International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 3, 2011

Research Article

A RUGGED AND ECONOMIC METHOD FOR THE ESTIMATION OF QUINAPRIL AND ITS METABOLITE IN HUMAN SERUM BY LCMS/MS DETECTION FOR CLINICAL TRIALS

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Received: 13 March 2011, Revised and Accepted: 11 April 2011

ABSTRACT

Quinapril and its metabolite- Quinaprilat were extracted from an aliquot of human serum using solid phase extraction method, then injected into a liquid chromatograph, equipped with mass spectrometry detector. Internal standard method was used for quantitation of Quinapril, and it metabolite from human serum. Linear regression with $1/X^2$ weighting was performed to determine the concentration of the drug from serum .Ramipril was used as internal standard. A common solid phase extraction procedure for the isolation of drug and its metabolite was developed from serum samples. The samples were analyzed on API 3200 Triple quadrapole mass spectrometer using Chromolith, RP-18e column in atmospheric pressure electro spray ionization. The mobile phase composition was an isocratic mixture of 0.01% Ammonia in water: acetonitrile (30:70 %v/v). The method was validated over a linear range of 10 – 1000 ng/mL and the limit of quantification was 10 ng/mL. Recoveries were observed above 70% for all the three analytes. The storage stability of Quality control samples was investigated under various conditions

Keywords: Quinapril , Quinaprilat, LC-MS-MS , Pharmacokinetic studies.

INTRODUCTION

Quinapril hydrochloride is chemically described as [3S-[2[R*(R*)], 3R*]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxo-propyl]-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid, mono-hydrochloride is a prodrug. It belongs to the angiotensin-converting enzyme (ACE) inhibitor class of medications It is used for the treatment of hypertension and congestive heart failure[1]. Quinapril is a prodrug which is hydrolyzed to the pharmacologically active metabolite, Quinaprilat [2].

Quinapril alone (without Quinaprilat) has been assayed by ion-pair high-performance liquid chromatography (HPLC) with UV detection in the pharmaceutical form [3].

Quantification of Quinapril and Quinaprilat in human plasma has been accomplished by various techniques including HPLC with fluorescence or radiochemical detection [4] and by gas chromatography (GC) with negative-ion chemical ionization mass spectrometry (MS) or electron-capture detection (ECD) [5].

Another method showed estimation of Quinapril as well as its metabolite from human plasma using high-performance liquid chromatography with ultraviolet detection [6]. One more method showed the detection of Quinapril and Quinaprilat from human plasma using ultra performance liquid chromatography coupled with mass spectrometry[7]. Following the similar method for using mass spectrometer as a detector, we have developed a method for the estimation of Quinapril and its metabolite from human serum.

The proposed LC-MS/MS method utilizes a simple and less time consuming SPE procedure for sample extraction and allows the simultaneous determination of these drugs at low concentration levels.

In this article, we have validated a simple and selective high performance liquid chromatography couples with mass spectrometry method for the detection of Quinapril, and Quinaprilat from human serum rather then plasma as per USFDA guidelines [8]. Ramipril was used as internal standard.

Quinapril and its metabolite- Quinaprilat were extracted from an aliquot of human serum using solid phase extraction method and then injected into a liquid chromatograph, equipped with mass spectrometry detector. Internal standard method was used for quantitation of Quinapril, and Quinaprilat. Linear regression with $1/X^2$ weighting was performed to determine the concentration of the drug from serum samples. All regressions and figures presented in this validation report were generated by analyst software version 1.4.1.

MATERIALS AND METHOD

Chemicals and reagents

All reference standards were purchased from Synfine Research, Canada or Sigma Aldrich, USA. Ammonia, Acetonitrile, methanol, formic acid and Orthophosphoric acid were obtained from Qualigens (Worli, Mumbai) India. De-ionized water was prepared on Milliq Laboratory Plant (Millipore, Bedford. USA). Organic solvents and reagents used were of analytical grade. HLB 30mg 1cc solid phase extraction cartridge (Oasis, Waters, USA) was used for sample clean up procedure.

Instrumentation

The chromatographic system consist of LC-2010HT (Shimadzu, Japan) equipped with SIL-HTc autosampler, DGU-14 A Vacuum Degasser, LC-10-VP Pump, CTO-20 A Column Oven. Mass Spectrometric analysis were conducted using API 3200 Q-trap Triple quadrapole instrument (Applied Biosystem, Sciex, Concord, Canada), equipped with a pneumatically assisted APCI(heated nebulizer) and ESI (electro spray) ionization source, which was operated in negative mode.. The whole system was controlled using Analyst software version 1.4.1(Applied-Biosystem-Sciex, Concord, Canada). HLB 30mg 1cc solid phase extraction cartridge (Oasis, Waters, USA) was used for sample clean up solid phase extraction procedure.

Liquid chromatography and mass spectrometry conditions

Chromatographic separation was achieved on Merck Chromolith, RP-18e (100*3.0 mm) analytical column maintained at 25°C temperature. Mobile phase composition was a mixture of 0.01% Ammonia in 1000 ml deionized water and acetonitrile with ratio of 35:65 %v/v. Flow rate was maintained at 0.6 mL min^{-1}. The run time was about 2.5 minutes and the retention time of Quinapril, Quinaprilat, Ramipril as well as internal standard were about 2.1 , 2.1 and 2.2 minutes.

Mobile phase was introduced in to the mass spectrometer via the ESI source operating in the positive ion mode under multiple reaction monitoring conditions (MRM). Quantitation was performed using selective ion Monitoring (SIM) mode at m/z 439.21, 411.31 and 417.29 for Quinapril, Quinaprilat and Ramipril respectively. Nitrogen was used as the nebulizing and curtain gas. Fragmentation was achieved with nitrogen. Dwell for each transition was 300 msec. The temperature was 700°C and the resolution was set as unit and ion spray voltage was set at -4500 volts.

Stock solution, calibration standard solutions and quality control standard solutions

Stock solution of Quinapril and Quinaprilat was prepared by accurately weighing and dissolving respective reference standards in methanol to give the final concentration of 100 µg/mL of each. Stock solution of internal standard i.e. Ramipril was obtained in methanol at a concentration of 1500 ng/mL and was used directly for serum sample preparation. Stock solution of Quinapril and Quinaprilat was further diluted with methanol to give serial concentrations of 200, 400, 1000, 2000, 4000, 8000, 12000, 16000, 20000 ng/mL to form working solution of calibration standards. Quality control standard solutions of Quinapril and Quinaprilat were prepared in methanol at concentration of 600, 6000 and 18000 ng/mL. Working solution of analytes as well as internal standard was stored at 4°C.

Sample preparation procedure

A common procedure for the isolation of Quinapril and Quinaprilat from serum samples prior to LC/MS/MS was developed. For analysis of Quinapril and Quinaprilat, 25 µL of Ramipril, 1500 ng/mL and 475µL of 10% aqueous ortho phosphoric acid solution were added to 500 µL human serum. The mixture was vortexed for several seconds. Conditioned the Water's Oasis, HLB 30 mg 1cc C_{18} cartridge with 1 mL methanol followed by equilibration with 1 mL water. 1 mL of serum sample was loaded, washed with 1 mL of 2% methanol and 1 mL water. Eluted with 1000 µL of methanol (100%).

Method validation [8]

The method was validated for specificity, linearity, precision, accuracy, recovery and stability.

Specificity

Specificity was determined by analyzing six different lots to check interference at the retention time of Quinapril, Quinaprilat and Ramipril.

Linearity

Linearity of calibration standards (n=9) for all three analytes were assessed by plotting peak area ratio (y) of analyte to internal standard against the concentration (x) of analytes. The calibration curves were constructed by weighted ($1/x^2$) least square linear regression.

Precision and accuracy

To determine intraday precision, replicate analysis of quality control sample was performed on the same day. The run consisted of one set of calibration standards and five replicates each of low, middle and high concentration quality control sample. The inter-day precision was accessed by analysis of batches on different days. Precision was expressed as % CV.

Recovery

The extraction efficiency of Quinapril, Quinaprilat and Ramipril were expressed in term of recovered concentration of analyte and internal standard added to a biological matrix prior to extraction (recovery QC) versus concentration obtained with biological sample where analyte and internal standard were added following extraction. All analysis was performed in triplicate at three analyte concentrations. Percentage drug recovery with corresponding %CV was determined for each serum sample fortified with analyte. To evaluate matrix effect, blank serum was subjected to sample pretreatment described above. The resulting solution was spiked with working standard solution to prepare solutions containing analytes at three different concentrations (lower, middle and high). Matrix enhancement/suppression of ionization was evaluated by comparing the peak areas of processed spiked samples with corresponding neat standard solutions prepared in mobile phase.

Stability

The stability of Quinapril and Quinaprilat were tested by short-term stability, long-term stability and freeze-thaw stability. To test stability of these analytes in serum, six replicates of each were stored under different conditions. The short term stability tests were performed at 25^0 C temperatures for 24 hours. The autosampler stability tests were performed at 10^0 C temperatures for 48 hours. Freeze-thaw stability testing was performed for three frozen and thawed cycles. "Freezing" was performed at -20^0 C for 24 hours and "thawing" at room temperature. The results of freeze-thaw and short and long-term stability were compared with the average of intra-day calibration curves.

RESULTS

Limits of quantitation

The lower limit of quantitation i.e., lowest standard level with a coefficient of variation less than 20 % was 10 ng/mL, for Quinapril and Quinaprilat with between-batch coefficient of variation was 2.30% and 1.88% respectively and accuracy was 102.30% and 98.98% respectively.

The upper limit of quantitation for Quinapril and Quinaprilat was 1000 ng/mL with between-batch coefficient of variation was 1.27% and 2.75 % respectively and accuracy was 102.47%, and 93.06% respectively

Linearity and sensitivity

Good linearity was achieved over the concentrations in the range of 10 to 1000 ng/mL for Quinapril, and Quinaprilat. The data of linearity are listed in Table 1. The limit of quantification (LOQ) was 10 ng/mL using 500 µL of serum for Quinapril, and Quinaprilat with accuracy, precision ≤ 20%. Back calculations were made from the calibration curves to determine Quinapril, and Quinaprilat concentration of each calibration standard. The regression equations of these curves and their coefficients were calculated as follows:

Quinapril, $y = 0.0054(\pm0.0002)x + 0.0078(\pm0.0017)$, $(R = 0.9987)$;

Quinaprilat, $y = 0.0034(\pm0.0003)x + 0.0089(\pm0.0019)$, $(R = 0.9986)$;

Where y is the peak area ratio of analytes to internal standard,

X is the concentration of analytes.

Specificity

Presence of any interference from endogenous substances was estimated by analyzing human serum from six different lots of analyte (s) free human serum including hemolised and lipemic serum used for analysis .No significant interference was observed at the retention times of both analyte (s) and internal standard. Figure 1 shows the specificity of blank serum.

Precision, accuracy and recovery of method

A good precision and accuracy was observed in this method. The intra and inter-day precision and accuracies are summarized in table 2 and 3. The intra-run CV (%) were less then 6.59, and 4.59 and inter-run CV (%) were less than 7.95 and 5.44 for Quinapril and Quinaprilat respectively. The intra-run accuracies (MRE) were found to be in the range between 95.38 – 108.69 % and 88.51 to 105.52 % for Quinapril and Quinaprilat respectively and the inter-run accuracies were found in the range of 93.10 – 105.84 % and 98.62 – 102.49 % for Quinapril and Quinaprilat respectively.

Recovery

The recovery of the method was found above 70% for Quinapril and Quinaprilat respectively as shown in table no 4. The data were found satisfactory for pharmacokinetic studies.

Table 1: Table shows intermediate precision, accuracy and linear regression parameters of Quinapril and Quinaprilat determination in human serum by LC-MS-MS detection

Added Concentration (ng/mL)		Mean measured concentration (n = 5) (ng/mL)		Precision (RSD,%)	Accuracy (%)
Quinapril					
10		10.23		2.3	102.3
20		16.69		12.75	83.45
50		51.85		0.62	103.71
100		95.6		2.18	95.6
200		203.04		1.49	101.52
400		389.77		1.84	97.44
600		586.65		4.61	97.77
800		836		1.86	104.5
1000		1024.68		1.27	102.47
Calibration curve					
Slope	0.0054 ± 0.0002				
Intercept	0.0078 ± 0.0017	Correlation Coefficient	0.9987		
Quinaprilat					
10		9.9		1.88	98.98
20		21.36		11.81	106.78
50		50.24		4.36	100.47
100		100.32		1.86	100.32
200		214.54		1.93	107.27
400		406.78		2.15	101.69
600		596.19		3.9	99.36
800		781.17		1.74	97.65
1000		930.61		2.75	93.06
Calibration curve					
Slope	0.0034 ± 0.0003				
Intercept	0.0089 ± 0.0019	Correlation Coefficient	0.9986		

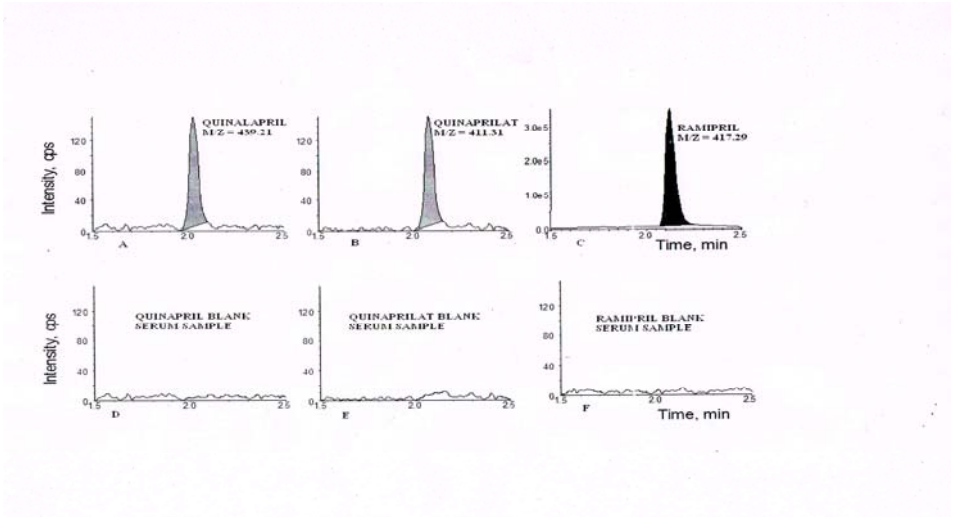

Fig. 1: It shows that A, B and C indicates typical chromatogram of blank serum spiked with Quinapril, Quinaprilat and Ramipril. D, E and F indicates typical chromatogram of blank serum

Table 2: Table shows intra- run precision and accuracy for Quinapril and Quinaprilat of QC (n = 5)

Added concentration (ng/mL)	Mean measured concentration (ng/mL)	standard deviation	CV (%)	Accuracy (%)
Quinapril				
30.00	28.62	1.89	6.59	95.38
300.00	326.06	13.71	4.20	108.69
900.00	881.81	23.25	2.64	97.98
Quinaprilat				
30.00	31.56	1.00	4.54	105.52
300.00	319.04	13.24	4.38	100.29
900.00	796.56	8.18	1.03	88.51

Table 3: Table shows inter- run precision and accuracy for Quinapril and Quinaprilat of QC (n = 30)

Added concentration (ng/mL)	Mean measured concentration (ng/mL)	standard deviation	CV (%)	Accuracy (%)
Quinapril				
30.00	27.93	2.22	7.95	93.10
300.00	317.53	14.16	4.46	105.84
900.00	904.85	8.92	0.99	100.54
Quinaprilat				
30.00	30/75	1.67	5.44	102.49
300.00	295.86	12.51	4.23	98.62
900.00	904.60	14.98	1.66	100.51

Table 4: Table shows recovery of Quinapril and Quinaprilat from human serum

a. Recovery of Quinapril from human serum

	LOW QC (30 ng/mL)		MED QC (300 ng/mL)		HIGH QC (900 ng/mL)	
	Control peak response	Treated peak response	Control peak response	Treated peak response	Control peak response	Treated peak response
	221660	177923	2599761	1848458	7202064	4894286
	223122	173417	2619638	1896115	7138388	4918240
	224943	174634	2589490	1763108	7156203	5003329
	222253	195107	2599136	1914823	7191812	4995725
	218904	180685	2595161	1923683	7206972	5487674
	224543	177609	2582947	1880008	7176995	5045111
N	6.00	6.00	6.00	6.00	6.00	6.00
Mean	222570.83	179895.83	2597688.83	1871032.50	7178739.00	5057394.17
SD	2199.85	7883.99	12482.68	59256.53	27022.87	218167.78
CV (%)	0.99	4.38	0.48	3.17	0.38	4.31
Recovery	80.83		72.03		70.45	

b. Recovery of Quinaprilat from human serum

	LOW QC (30 ng/mL)		MED QC (300 ng/mL)		HIGH QC (900 ng/mL)	
	Control peak response	Treated peak response	Control peak response	Treated peak response	Control peak response	Treated peak response
	201790	155073	2022192	1263639	5097731	3391941
	204727	131254	1986888	1422701	5160529	3380274
	204881	152593	2023644	1268243	5167572	3789220
	203995	172200	2040149	1324671	5196977	3579724
	202209	135664	1994978	1237208	5201078	4078191
	202145	150298	2011766	1527860	5125453	3544886
N	6.00	6.00	6.00	6.00	6.00	6.00
Mean	203291.17	149513.67	2013269.50	1340720.33	5158223.33	3627372.67
SD	1401.63	14703.08	19710.77	113056.59	40408.66	266455.25
CV (%)	0.69	9.83	0.98	8.43	0.78	7.35
Recovery	73.55		66.59		70.32	

Table 5: Table shows stability data of Quinapril and Quinaprilat

Nominal Concentration (ng/mL)	Short term stability at about 25± 5°C for 24 hours.		Autosampler stability at about -10°C for 48 hours.		Three freeze/thaw cycles.	
	Lower Quality Control 30.00 (ng/mL)	Higher Quality control 900 (ng/mL)	Lower Quality Control 30.00 (ng/mL)	Higher Quality control 900 (ng/mL)	Lower Quality Control 30.00 (ng/mL)	Higher Quality control 900 (ng/mL)
Quinapril						
Mean found Concentration (ng/mL)	28.40	875.73	28.51	859.85	28.37	881.09
standard deviation	1.63	23.21	2.19	25.17	1.80	28.21
CV (%)	5.75	2.65	7.68	2.93	6.35	3.20
% change(bias)	1.13	-0.18	1.94	0.75	0.20	1.60
Quinaprilat						
Mean found Concentration (ng/mL)	32.85	896.58	33.62	925.46	29.05	825.44
standard deviation	1.80	62.54	0.79	65.83	1.01	26.63
CV (%)	5.47	6.98	2.35	7.11	3.49	3.23
% change(bias)	2.68	4.96	2.13	2.48	-2.48	0.95

Samples stability

Quinapril and Quinaprilat showed a good stability under the conditions used for storage and processing. The analytes were stable in human serum when stored at ambient temperature for at least 24 hours and showed good autosampler stability at 10 °C for 48 hours.. Quinapril and Quinaprilat were stable under the influence of three freeze/thaw cycles. Stability data of analytes under various storage and freeze thaw conditions were mentioned in table 5.

DISCUSSION

A convenient method for the determination of Quinapril and Quinaprilat in human serum has been developed. The analytical method was validated as per the well defined standard operation procedure of Bioanalytical laboratory. The calibration curve for the standard was linear over the range from 10 to 1000 ng/mL

ACKNOWLEDGEMENTS

I would like to thank Managing Trustee of Parul institute of Pharmacy for providing all support for carrying out this research study.

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