Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 858 (2007) 13–21
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Liquid chromatography tandem mass spectrometry method for simultaneous determination of metoprolol tartrate and ramipril in human plasma

K. Veeran Gowda [a], Uttam Mandal [a], P. Senthamil Selvan [a], W.D. Sam Solomon [a], Animesh Ghosh [a], Amlan Kanti Sarkar [a], Sangita Agarwal [a], T. Nageswar Rao [b], Tapan Kumar Pal [a,*]

[a] *Bioequivalence Study Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India*
[b] *Ranbaxy Pharmaceuticals, Canada Inc., Mississauga, ON, Canada*

Received 23 April 2007; accepted 26 July 2007
Available online 10 August 2007

Abstract

A simple, rapid, sensitive and specific liquid chromatography-tandem mass spectrometry method was developed and validated for quantification of metoprolol tartrate (MT) and ramipril, in human plasma. Both the drugs were extracted by liquid–liquid extraction with diethyl ether–dichloromethane (70:30, v/v). The chromatographic separation was performed on a reversed-phase C8 column with a mobile phase of 10 mM ammonium formate–methanol (3:97, v/v). The protonated analyte was quantitated in positive ionization by multiple reaction monitoring with a mass spectrometer. The method was validated over the concentration range of 5–500 ng/ml for metoprolol and ramipril in human plasma. The precursor to product ion transitions of *m/z* 268.0–103.10 and *m/z* 417.20–117.20 were used to measure metoprolol and ramipril, respectively.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Metoprolol tartrate; Ramipril; LC–MS/MS; Validation

1. Introduction

According to World Health Organization (WHO) estimates in 2003, about 16.7 million people around the globe die of cardiovascular diseases each year. This is over 29% of all deaths globally [1]. Cardiac attacks occur at rest or during sleep and are unpredictable. They are due to localized coronary vasospasm. There are many drugs available which are aimed at preventing and relieving coronary vasospasm. One class of drugs which aids in preventing cardiac attack is β blockers. Metoprolol belongs to the class of β blockers. This class of compounds acts by reducing cardiac work and oxygen consumption. They are effective in decreasing the frequency and severity of attacks and increasing exercise tolerance in classical angina [2].

* Corresponding author at: Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032, India. Tel.: +91 33 2414 6967; fax: +91 33 2414 6186.
E-mail addresses: mailveeran@yahoo.com (K.V. Gowda), tkpal_12@yahoo.com (T.K. Pal).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.07.047

β blockers are to be taken on a regular schedule and not on "as and when required" basis. The dose has to be individualized. Abrupt discontinuation after chronic use may precipitate severe attacks even myocardial infarction (MI). So a sustained release dosage form of any β blockers would reduce the frequency of dosing.

The angiotensin converting enzyme (ACE) inhibitors are one of the first choices of drugs in all grades of hypertension. Ramipril belongs to the class of angiotensin converting enzyme (ACE) inhibitors. Most patients require relatively low doses (2.5–10 mg/day) which are well tolerated. When used alone, they control hypertension in 50–60% of patients. When combined with a β blocker/diuretic their therapeutic efficacy extends to 90% because of supraadditive/synergistic effect. Inhibition of ACE lowers blood pressure by decreasing vasoconstriction. ACE inhibitors are the most appropriate antihypertensive in patients with diabetes, nephropathy [2].

There are various therapies available for treatment of some life threatening diseases like malaria, tuberculosis (TB), hypertension etc. Combination therapy has become the standard for

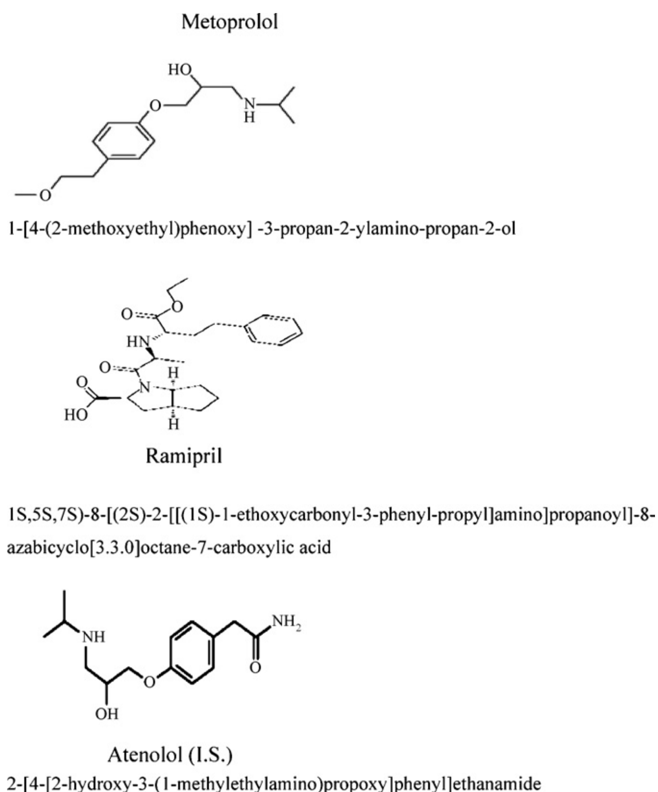

Fig. 1. Chemical structure of analysed compounds.

treating many of these diseases. In practice, a large majority of hypertensives ultimately require two or more drugs. Since blood pressure is kept up by several interrelated factors, an attempt to block one of them tends to increase compensatory activity of others. It is rational in such cases to combine drugs with different mechanisms of action or different patterns of haemodynamic effects. Drugs which increase plasma rennin activity like diuretics, vasodilators, ACE inhibitors may be combined with drugs which lower plasma rennin activity—β blockers, clonidine, methyldopa [2]. Therefore, fixed dose combinations (FDCs) remain the first choice when they are available. Co blistered combinations (CBCs) are the second choice. Single products are third but least desirable. This therapy has the benefits of slowing resistance, improving clinical outcomes, and facilitating logistics.

In one of our studies, we had formulated a bilayer tablet containing metoprolol tartrate and ramipril as a fixed dose combination product. So an analytical method for the simultaneous determination of both the analytes in plasma was necessary. Fig. 1 shows the chemical structure of Metoprolol, ramipril and atenolol (IS) used in this study.

Literature survey reveals few analytical methods for the determination of ramipril viz. voltammetry [3], capillary electrophoresis [4], GC–MS [5], HPLC [6–8], LC–MS/MS [9], radioimmunoassay [10].

Determination of metoprolol, viz HPLC [11–16], LC–MS [17–20], GC–MS [21–22] has been reported. Enantiomers of metoprolol have also been determined by HPLC [23–25]. Usually in a clinical study, large numbers of samples are collected. So a rapid and reliable assay method is essential to analyse such huge pool of samples in a very short time. An ideal method should have simple sample preparation, fast on-column separation and sensitive and specific detection. Liquid chromatography coupled with mass spectrometry (LC–MS/MS) is one such efficient analytical tool which meets most of the above needs [26] particularly in simultaneous analysis of fixed dose combination dosage forms. LC–MS/MS method facilitates analysing large samples in a very short period of time. Previously reported methods either had long retention time (10–15 min) or suffered from low sensitivity and in some cases required large sample injection volumes (100 μl). There are various methods reported for the determination of MT and ramipril separately. To the best of our knowledge, there is no method reported in the literature for the simultaneous determination of these analytes. Hence the main objective of this work was to develop a simple, sensitive rapid and reliable mass spectrometry (LC–MS/MS) method for the simultaneous quantification of metoprolol and ramipril in human plasma.

2. Experimental

2.1. Chemicals and reagents

Metoprolol tartrate was obtained from Aurobindo laboratories, (Hyderabad, India) Ramipril was supplied by Aristo Pharmaceuticals (Mandideep, India). HPLC grade ammonium formate and methanol were purchased from Merck (Mumbai). HPLC grade water generated from Milli Q water purification system was used throughout the analysis.

2.2. Apparatus

The LC system used was a Shimadzu series LC 20 AT pump, SPD 20A Ultra Violet/Visible detector, CTO 10 AS VP column oven, SIL 20 AC auto-sampler (Kyoto, Japan). The mass spectrometer system used was a API 2000 triple quadrupole mass spectrometer (MDS Sciex, Canada) equipped with electrospray ionization (ESI) source. Data acquisition was performed with Analyst 1.4.1. software. Chromatographic separation was achieved on a C8 column, 50 mm × 3 mm i.d., 3 μm (Phenomenex, USA).

2.3. Chromatographic conditions

The chromatographic analysis was carried out at ambient temperature. Mobile phase used for separation of the analytes was methanol:10 milli molar ammonium formate buffer (97:3, v/v). The flow rate was set at 1 ml/min. The injection volume was 20 μl and the total run time was 5 min. The column was maintained at ambient temperature (23 °C) whilst the autosampler temperature was set at 10 °C.

2.4. Mass spectrometry

Electrospray ionization (ESI) with multiple reaction monitoring (MRM) was used to acquire the mass spectra of the

Table 1
Main working parameters for mass spectrometry

Parameter	Value
Curtain gas (psi)	10.00
Ion spray voltage (V)	5500.00
Source gas temperature (°C)	500.00
Ion source gas 1 (psi)	30.00
Ion source gas 2 (psi)	60.00
Collision associated dissociation (CAD gas)	6.00

Parameter	Metoprolol Tartrate	Ramipril	Atenolol (IS)
Declustering potential (V)	26.00	20.00	28.00
Focusing potential (V)	394.00	375.00	395.00
Entrance potential (V)	10.00	10.00	10.00
Collision energy (V)	49.00	55.00	35.00
Collision cell exit potential (V)	4.00	3.00	5.00
Ion transition *m*/*z*	268.0 → 103.10	417.20 → 117.20	267.10 → 145.10

compounds. Ions were measured in positive ionization mode. The tuning parameters were optimized by injecting 100 ng/ml of standard solution containing all three drugs at 20 μl/min by means of an external syringe pump directly connected to the mass spectrometer. The turbo ion spray source temperature was set at 500 °C and the turbo ion spray voltage was set at 5500 V. The nebulizer gas (GS1), the turbo ionspray gas (GS2) and the curtain gas values were set at 30, 60 and 10 units respectively. The collision associated dissociation (CAD) gas value was fixed at 6 (arbitary units). Optimized values of compound related parameters and source gas parameters are summarized in Table 1.

2.5. Standard solutions

Separate solutions containing 1 mg/ml of MT, ramipril and IS were prepared using water, water and methanol (50:50 v/v), and methanol for the three drugs, respectively. These solutions were further diluted suitably with the diluents to obtain a stock solution of 10 μg/ml. The stock solutions prepared for the drugs were diluted further to obtain seven working solutions for calibration standards. All solutions were stored at 2–8 °C.

2.6. Calibration curves

A seven point standard calibration solutions of MT and ramipril was prepared by spiking blank plasma with appropriate amounts of analytes and IS (100 ng/ml). Standard curves were prepared in human plasma to yield final concentrations of 5, 10, 25, 50, 100, 250 and 500 ng/ml for both the analytes. Three quality control (QC) samples were prepared at three concentration levels of 20, 250 and 400 ng/ml for both the analytes. Calibration curves were plotted with peak area ratio of drug and IS on *Y*-axis and concentration on *X*- axis.

2.7. Sample preparation and extraction

Liquid–liquid extraction procedure was used for the extraction of the drug from the plasma. Calibration standards, quality control samples were treated with 3 ml mixture containing diethyl ether and dichloromethane (70:30, v/v). Fifty microlitres of internal standard (100 ng/ml) were added to each plasma sample (0.25 ml) and vortex mixed for 10 min followed by centrifugation for another 10 min. The organic layer containing the analytes was separated, transferred to a separate test tube and evaporated to dryness under a stream of N_2 at 40 °C. The residue obtained on drying was reconstituted with the 250 μl of mobile phase. The reconstituted sample was transferred to an auto sampler vial and injected into the liquid chromatography mass spectrometry (LC–MS/MS) system.

2.8. Validation

The accuracy, sensitivity, precision, stability, recovery, reproducibility and reliability of the analytical method was confirmed by validation in accordance with the USFDA guidelines [27].

2.8.1. Linearity and LLOQ

To establish linearity, a series of calibration standards were prepared by adding a known concentration of MT, Ramipril and IS to drug free human plasma and analysed. The lowest concentration on the standard curve with detector response five times greater than the drug free (blank) human plasma was considered as the LLOQ. The analyte peak in LLOQ sample should be identifiable, discrete and reproducible with a precision of 20% and an accuracy of 80–120%.

2.8.2. Specificity

Specificity is the ability of an analytical method to differentiate and quantify the analyte in presence of other components in the sample. The specificity of the method was evaluated by screening six lots of blank plasma. These lots were spiked with known concentration of analytes along with IS at low, medium and high concentrations. The spiked samples were analysed after extraction to confirm lack of interference and absence of lot-to-lot variation.

2.8.3. Accuracy and precision

Intra day precision and accuracy of the assay was evaluated by running three validation batches on three separate days. Each batch consisted of one set of calibration standards and five replicates of quality control (QC) samples at low, medium and high concentration. The inter day precision and accuracy was also assessed in similar manner. A comparison was made between the obtained values and the experimental values. Precision was expressed as percentage of relative standard deviation (%R.S.D.). The mean value of accuracy should be within 15% of the actual value except at LLOQ, where it should not deviate by more than 20%. The precision determined at each concentration level should not exceed 15% of R.S.D. except for the LLOQ, where it should not exceed 20% of R.S.D.

2.8.4. Extraction recovery and matrix effect

The extraction recovery of the analytes from the plasma was evaluated by comparing the mean detector responses of three replicates of processed QC samples at low, medium high concentrations to the detector responses of standard solutions of same concentration. Recovery of an analyte need not be 100%, but the extent of recovery of analyte and the IS should be consistent, precise and reproducible [27].

Endogenous matrix components may change the efficiency of droplet formation or droplet evaporation, which in turn affects the amount of charged ion in the gas phase that ultimately reaches the detector [28–30]. Two sets of samples were prepared by directly spiking the analytes into reconstitution solution with and without the presence of residue extracted from control plasma. Ion suppression was assessed at three QC sample concentrations by comparing the mean analyte peak areas obtained from these two sets of testing samples. The matrix effect (ME) was calculated by using the equation:

$$\mathrm{ME} = \frac{A_{\mathrm{ep}}}{A_{\mathrm{ns}}} \times 100$$

where A_{ep} and A_{ns} represent the analyte peak area of the extracted plasma residue and the neat solution, respectively.

2.8.5. Stability

2.8.5.1. Long and short term stability. Three aliquots of each low and high QC samples were kept in deep freezer at −20 °C ± 5 °C for 1 month. The samples were processed and analysed and the concentrations obtained were compared with the actual value of QC samples to determine the long-term stability of analyte in human plasma.

Three aliquots each of high and low unprocessed QC samples were kept at ambient temperature (20–30 °C) for 8 h in order to establish the short-term stability of the analytes. The samples were analysed and the concentrations obtained were compared with the actual values of QC samples. Samples were concluded stable if the %R.S.D. of the stability samples was within ±15% of the actual value.

2.8.5.2. Post preparative stability. Three aliquots each of high and low QC samples were stored at 10 °C in an auto sampler for 24 h, analysed and the concentrations were compared with the actual values. Stability was concluded when the %R.S.D. was within ±15% of the actual value.

2.8.5.3. Stock solution stability. Separate standard stock solutions containing 500 ng/ml of Ramipril, MT, IS were prepared and stored at 2–8 °C for 30 days. The response obtained from the three drugs was calculated and compared with that of the freshly prepared solutions of the same concentration with an acceptable limit of ±2% [31].

2.8.5.4. Freeze–thaw stability. The stability of the analytes after three freeze and thaw cycles was determined at low, high QC samples. The samples were stored at −20 °C for 24 h and thawed unassisted at room temperature. After completely thawing, the samples were refrozen for 12–24 h. After three freeze–thaw cycles, the concentration of the samples were analysed.

2.8.5.5. Dry state stability. Three aliquots each of low and high QC samples were stored at −20 °C without reconstitution after extraction (i.e. in dry state). The samples were analysed after 24 h and a deviation of ±15% was acceptable.

3. Results and discussion

3.1. Internal standard

A stable isotope labeled analyte has to be used as an IS to deal with sample matrix effects. Since such IS is not available commercially, an alternative approach has been used. IS chosen should match the chromatographic properties, recovery and ionization properties of the analyte [32]. Atenolol was found to match these criteria and therefore was chosen as an IS. Other internal standards like nebivolol and carvedilol were also tried but were rejected because of their low recovery and inefficient extraction. Atenolol was selected because of its high recovery and also the intensity of MT and ramipril molecular ion peaks in mass spectrometric analysis remained unaffected as compared to carvedilol. Good chromatographic separation was another reason for its selection.

3.2. LC–MS/MS analysis

LC–MS/MS was employed for the simultaneous quantification of MT and ramipril in human plasma. To the best of our knowledge there is no LC–MS/MS method reported for the simultaneous determination of these drugs in human plasma. The LC–ESI-MS/MS in MRM mode provided a highly selective method for the simultaneous determination of MT and ramipril in human plasma. ESI source provided a better ionization of the compounds as compared to the atmospheric chemical ionization (APCI). The positive mode of ionization was selected because the intensity of the molecular ion peaks was more in positive mode.

Fig. 2 represents the full scan mass spectra of MT, ramipril and IS. The exact mass of MT, ramipril and IS were found to be *m/z* 268.00, 417.20 and 267.1, respectively. Fig. 3 shows the product ion mass spectra of MT, ramipril and IS, respectively. The corresponding exact mass of the fragment ions were found at *m/z* of 103.10, 117.20 and 145.10

Quantitation of analytes in human plasma was based on the detector response ratio of analytes to IS. Total run time set for the samples tested was 5 min as shown in Fig. 4. The results indicated that a run time of 2 min was sufficient for sample analysis. On repeated injection of the samples, the retention time never shifted beyond 10 to 15 s. MT, ramipril and IS were eluted at retention times of 0.80, 0.92 and 0.32 min respectively (Fig. 4). The main analytes MT and ramipril were separated with good resolution. Complete chromatographic separation of MT and IS was not achieved under the set analytical conditions. However due to high selectivity of tandem MS, complete chromatographic separation is not necessary any more [33]. The main advantage

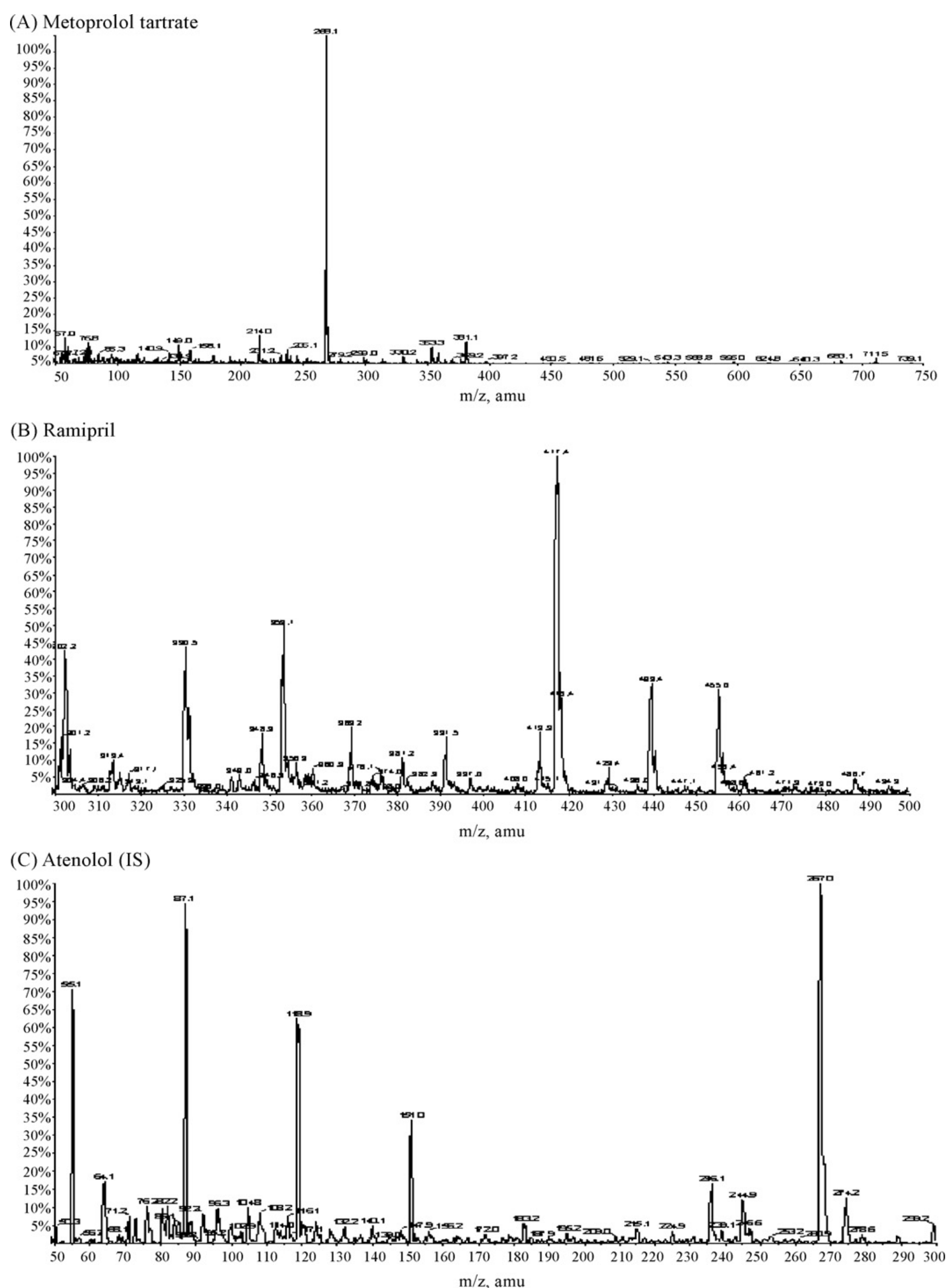

Fig. 2. LC–ESI-MS/MS full scan spectra of (A) metoprolol tartrate (500 ng/ml), (B) ramipril (500 ng/ml) and (C) atenolol (IS) (100 ng/ml).

of this method is that a relatively large number of samples can be analysed in short time thus increasing the output.

3.3. Linearity

The calibration curves were found to be linear over a range of 5–500 ng/ml for ramipril, MT. Table 2 summarizes the results of calibration. The average correlation coefficients obtained were 0.9976 and 0.9962 for MT and ramipril, respectively. The LLOQ was found to be 5 ng/ml. and LOD was found to be 1 ng/ml for both the analytes.

3.4. Specificity

No significant peaks were observed at the retention times of MT, ramipril and IS in human plasma spiked with the analytes. Representative chromatograms of blank plasma, blank plasma spiked with MT, ramipril, IS are shown in Fig. 4.

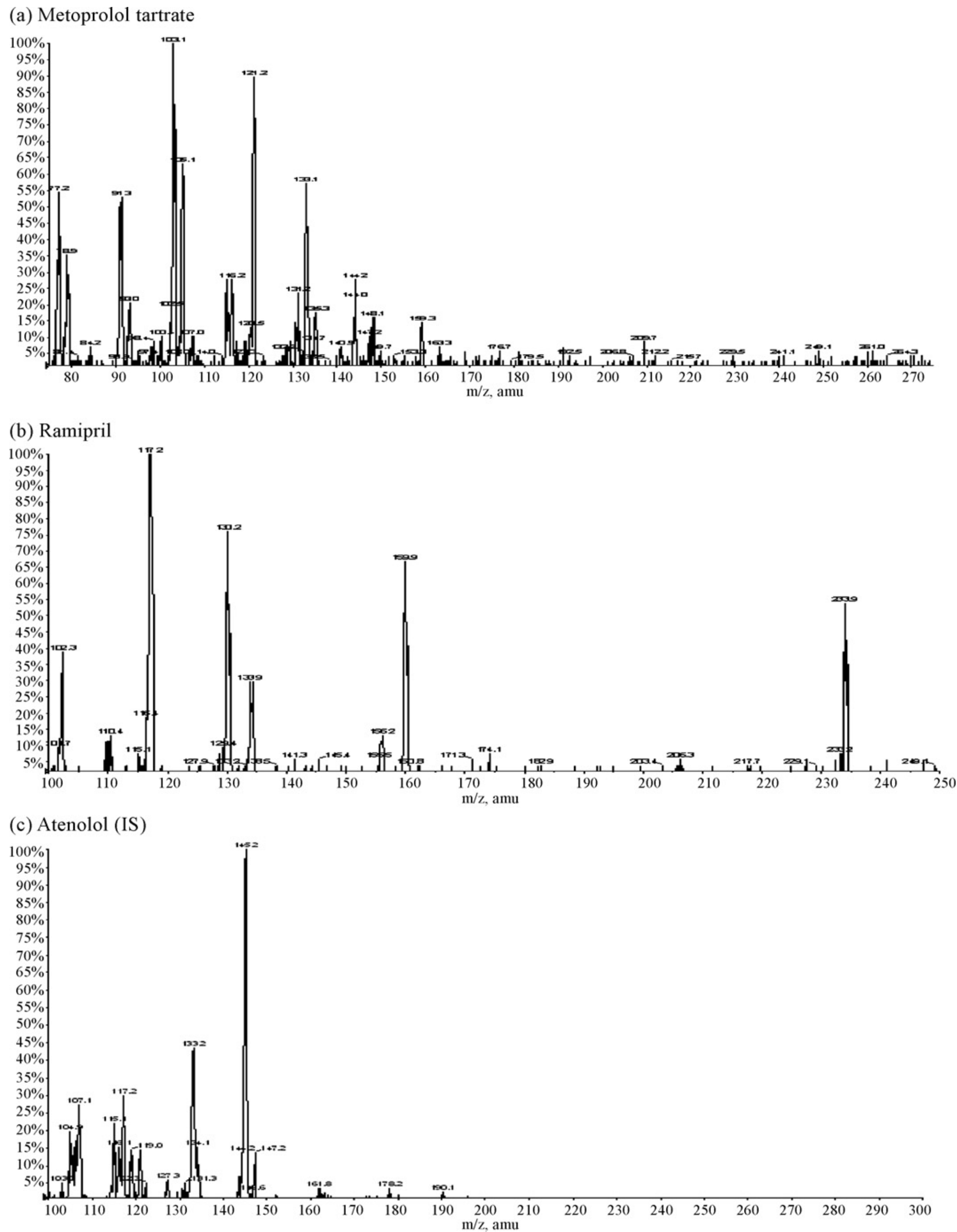

Fig. 3. Product ion mass spectra of (a) metoprolol tartrate (500 ng/ml), (b) ramipril (500 ng/ml) and (c) atenolol (IS) (100 ng/ml).

3.5. Accuracy and precision

Table 3 summarizes the mean values of accuracy and precision for both intra and inter day assays. Both precision and accuracy were within the acceptable ranges for bioanalytical purpose. Intra day precision ranged from 4.89 to 8.42% for MT and 1.57 to 6.81% for ramipril. Inter day precision ranged from 7.2 to 11.11% for MT and 2.72 to 4.78% for ramipril. The percentage of accuracy was in the range of 94.40–100.03% for MT and between 96.37 and 99.15% for ramipril. The assay method demonstrated high degree of accuracy and precision.

3.6. Extraction recovery and matrix effect

Recovery results presented in Table 4 show that the maximum recovery was achieved with Atenolol (81.56%) followed by MT (77.68%). Ramipril extraction recovery ranged from 64.53 to 67.51%. The extraction recovery was found to be satisfactory as it was consistent, precise and reproducible. Thus single step liquid–liquid extraction procedure used in this method proved to be efficient and simple enough to extract three drugs (including IS) simultaneously from human plasma.

The endogenous components are mainly the cause of ion suppression effects during electrospray ionization. The extent of this

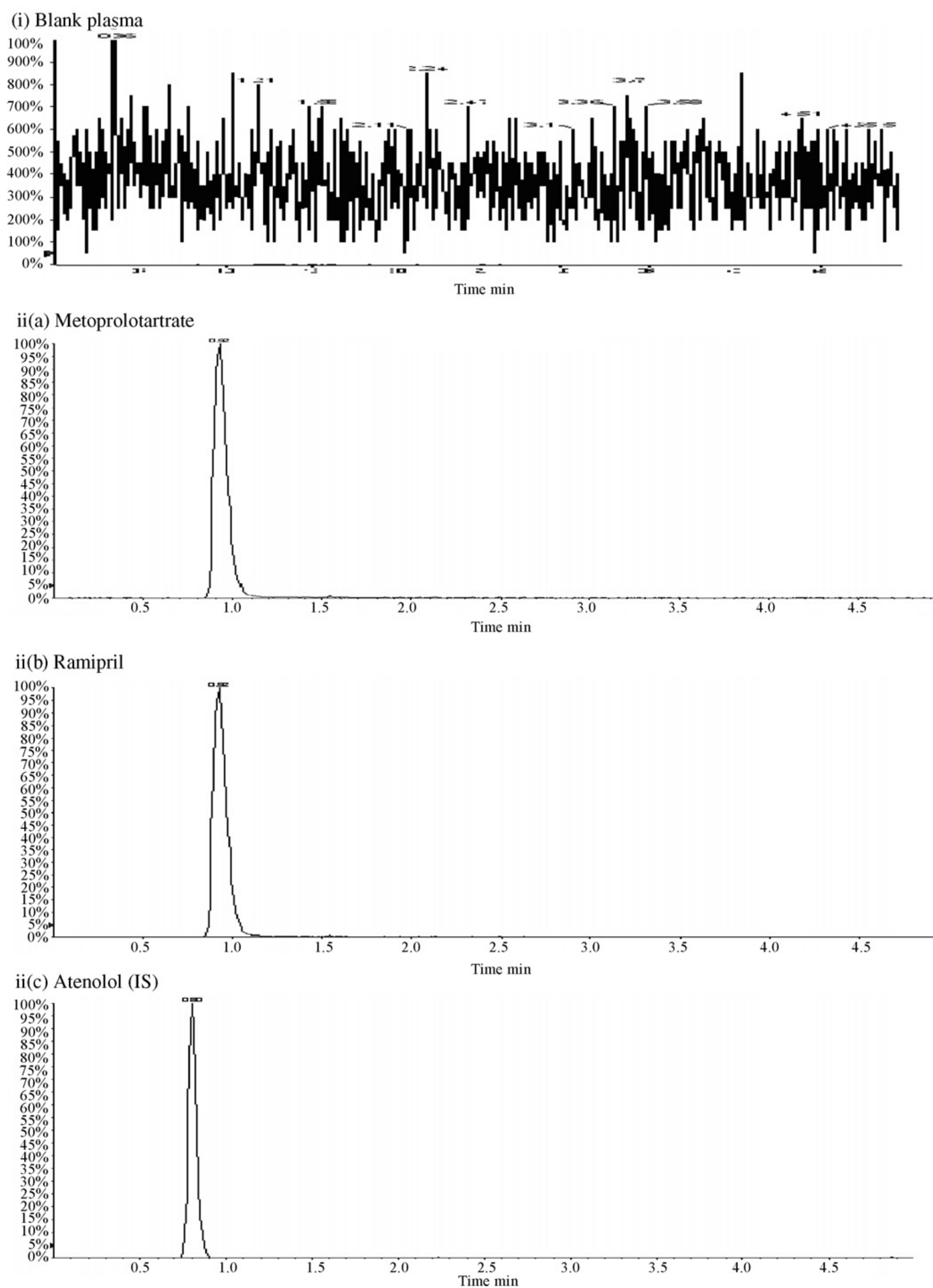

Fig. 4. Representative chromatograms of (i) blank plasma, (ii) extracted ion chromatogram of blank plasma spiked with (a) metoprolol tartrate (500 ng/ml), (b) ramipril (500 ng/ml) and (c) IS (100 ng/ml), respectively.

Table 2
LOD, LOQ and calibration results

Analyte	Regression equation	R^2	LOD (ng/ml)	LOQ (ng/ml)	Range (ng/ml)
Metoprolol tartrate	$y = 0.005x - 0.002$	0.9976	1	5	5–500
Ramipril	$y = 0.0306x + 0.1419$	0.9962	1	5	5–500

Table 3
Intra- and inter-day precision and accuracy for MT and Ramipril

Analyte	QC sample (ng/ml)	Intra-day variation			Inter-day variation		
		Mean ± S.D.	R.S.D.%	Accuracy%	Mean ± S.D.	R.S.D.%	Accuracy%
MT	20	19.25 ± 1.05	5.45	96.25	18.88 ± 1.43	7.57	94.40
	250	252.11 ± 12.24	4.89	100.84	250.09 ± 18.18	7.26	100.03
	400	389.96 ± 32.83	8.42	97.49	387.11 ± 43.15	11.11	96.77
Ramipril	20	20.33 ± 0.32	1.57	101.65	19.83 ± 0.54	2.72	99.15
	250	242.56 ± 16.54	6.81	97.02	240.92 ± 11.54	4.78	96.37
	400	389.94 ± 11.96	3.06	97.48	388.44 ± 17.08	4.39	97.11

S.D.: Standard deviation; %relative standard deviation (R.S.D.) = (standard deviation/mean) × 100; (n = 5).

Table 4
Extraction recovery of metoprolol tartrate and ramipril (n = 3)

Analyte	QC sample (ng/ml)	Extraction recovery%	R.S.D.%
MT	20	75.52	11.14
	250	75.54	7.65
	400	77.68	5.88
Ramipril	20	64.53	8.64
	250	67.51	9.22
	400	66.52	6.42
Internal standard	100	81.56	4.14

% Relative standard deviation (R.S.D.) = (standard deviation/mean) × 100.

effect is mainly dependent on sample extraction procedure and is also compound dependent [34]. The results indicated that the matrix components did not alter or deteriorate the performance of the proposed method as the %R.S.D. of three QC samples was less than 7.53, 4.82 and 2.05% for MT, ramipril and IS, respectively. The matrix effect on the estimation of the analytes was found to be negligible.

3.7. Stability

Table 5 summarises the results of stability study carried out under various conditions. Both the analytes were found to be stable at ambient temperature (20–30 °C) for at least 8 h in human plasma. The percentage of accuracy obtained was more than 96.98 and 96.09% for MT and ramipril respectively. The LQC and HQC samples of both the analytes remained unaffected at −20 °C for one month. In an autosampler maintained at 10 °C, plasma samples of MT and ramipril were stable for more than 24 h. The freeze thaw stability results showed that MT and ramipril are stable for at least three freeze thaw cycles. Stability results indicated that human plasma samples could be thawed and refrozen without compromising the integrity of the samples.

Extracted and dried residues were stable for 24 h without any change in the concentration. Working solutions of MT, ramipril and IS were stable and the deviation was less than ±2%. There was no much degradation in the solutions even after 30 days.

4. Conclusion

The method described is highly specific due to the inherent selectivity of tandem mass spectrometry. The method demonstrates high throughput capability because of the short time required for analysis. Both the analytes were found to be stable in human plasma for 30 days when stored at −20 °C. A simple and convenient extraction procedure makes this method more feasible for the bioanalysis of MT and ramipril. It is expected that this method can also be applied to clinical and toxicological studies.

Table 5
Stability summary of metoprolol tartrate and ramipril (n = 3)

Stability	QC sample (ng/ml)	Metoprolol tartrate			Ramipril		
		Mean ± S.D.	R.S.D.%	Accuracy%	Mean ± S.D.	R.S.D.%	Accuracy%
Long term (30 days)	20	19.03 ± 2.04	10.71	95.18	18.95 ± 0.89	4.60	94.79
	400	384.16 ± 9.14	2.37	96.04	381.96 ± 25.14	6.58	95.49
Short term (8 h)	20	19.63 ± 1.51	7.69	98.15	19.79 ± 1.94	9.81	98.96
	400	387.92 ± 16.68	4.30	96.98	384.36 ± 35.54	9.24	96.09
Post preparative (24 h)	20	20.14 ± 0.12	0.59	100.72	19.91 ± 0.54	2.71	99.59
	400	388 ± 22.09	5.69	97.00	387.96 ± 18.89	4.86	96.99
Freeze thaw	20	19.37 ± 1.11	5.73	96.87	19.42 ± 1.11	0.57	97.13
	400	383.96 ± 10.51	2.73	95.99	383.56 ± 42.98	11.20	95.89
Dry Extract (24 h)	20	19.03 ± 0.86	4.51	95.19	19.48 ± 0.94	4.82	97.42
	400	379.36 ± 26.73	7.04	94.84	375.92 ± 31.11	8.27	93.98

%Relative standard deviation (R.S.D.) = (standard deviation/mean) × 100; S.D. = standard deviation.

Acknowledgements

The authors are thankful to Department of Science and Technology (DST) under Pharmaceuticals Research & Development Support Fund (PRDSF), New Delhi, Govt. of India, for providing the financial assistance through their project No. VII – PRDSF/56/05 – 06 – TT.

References

[1] Website: http://www.who.int/cardiovascular_diseases/resources/ [publications/en/index.html].
[2] K.D. Tripathi (Ed.), Essentials of Medical Pharmacology, Jaypee Brothers Medical Publishers, New Delhi, 2003, p. 503.
[3] A.A. al-Majeed, F. Belal, A. Abadi, A.M. al-Obaid, Farmaco 55 (2000) 233.
[4] R. Gotti, V. Andrisano, V. Cavirni, C. Bertucci, S. Furlanetto, J. Pharm. Biomed. Anal. 22 (2000) 423.
[5] B.A. Persson, C. Fakt, M. Ervik, M. Ahnoff, J. Pharm. Biomed. Anal. 40 (2006) 794.
[6] R. Bhushan, D. Gupta, S.K. Singh, Biomed. Chromatogr. 20 (2006) 217.
[7] B.L. Hogan, M. Williams, A. Idiculla, T. Veysoglu, E. Parente, J. Pharm. Biomed. Anal. 23 (2000) 637.
[8] D. Bonazzi, R. Gotti, V. Andrisano, V. Cavirni, J. Pharm. Biomed. Anal. 16 (1997) 431.
[9] Z. Zhu, A. Vachareau, L. Neirinck, J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. 779 (2002) 297.
[10] H.G. Eckert, G. Muenscher, R. Oekonomopolous, H. Strecker, J. Urbach, H. Wissman, Arzneim. Forsch. 35 (1985) 1251.
[11] S. Albers, J.P. Elshoff, C. Volker, A. Richter, S. Laer, Biomed. Chromatogr. 19 (2005) 202.
[12] V.B. Boralli, E.B. Coelho, P.M. Cerqueria, V.L. Lanchote, J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. 823 (2005) 195.
[13] A.J. Braza, P. Modamio, C.F. Lastra, E.L. Marino, Biomed. Chromatogr. 16 (2002) 517.
[14] V.P. Ratna, E. Toropainen, A. Talvitie, S. Auriola, A. Urtti, J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. 772 (2002) 81.
[15] N.E. Basci, A. Temizer, A. Bozkurt, A. Isimer, J. Pharm. Biomed. Anal. 18 (1998) 745.
[16] F.C. Chiu, L.A. Damani, R.C. Li, B. Tomlinson, J. Chromatogr. B. Biomed. Sci. Appl. 696 (1997) 69.
[17] S.A. Wren, P. Tchelitcheff, J. Pharm. Biomed. Anal. 40 (2006) 571.
[18] R.D. Johnson, R.J. Lewis, Forensic Sci. Int. 156 (2006) 106.
[19] C. Dupuis, J.M. Gaulier, A.L. Pelissier – Aicot, P. Marquet, G. Lachatre, J. Anal. Toxicol. 28 (2004) 674.
[20] W. Naidong, W.Z. Shou, T. Addison, S. Maleki, X. Jiang, Rapid Commun. Mass Spectrom. 16 (2002) 1965.
[21] M.K. Angier, R.J. Lewis, A.K. Chaturvedi, D.V. Canfield, J. Anal. Toxicol. 29 (2005) 517.
[22] K.H. Kim, J.H. Lee, M.Y. Ko, S.P. Hong, J.R. Youm, Arch. Pharm. Res. 24 (2001) 402.
[23] V.L. Lanchote, P.S. Bonato, P.M. Cerqueira, V.A. Pereira, E.J. Cesarino, J. Chromatogr. B. Biomed. Sci. Appl. 738 (2000) 27.
[24] S.H. Jung, T.L. Pham, H.K. Lim, H.J. Kim, K.H. Kim, J.S. Kang, Arch. Pharm. Res. 23 (2000) 226.
[25] A.A. Mostafavi, R.T. Foster, Int. J. Pharm. 202 (2000) 97.
[26] K.V. Gowda, D.S. Rajan, U. Mandal, W.D. Sam Solomon, P. Senthamil Selvan, A. Bose, A.K. Sarkar, T.K. Chattaraj, T.K. Pal, Asian J. Chem. 19 (2007) 1293.
[27] Guidance for industry, Bioanalytical Method Validation. US Department of Health and Human services. Food and Drug Administration. Centre for Drug Evaluation and Research, Rockville, MD, 2001. Website: http://www.fda.gov/CDER/GUIDANCE/4252fnl.pdf.
[28] R. Bonfiglio, R.C. King, T.V. Olah, K. Merkle, Rapid Commun. Mass Spectrom. 13 (1999) 1175.
[29] T.M. Annesley, Clin. Chem. 49 (2003) 1041.
[30] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[31] S.S. Singh, K. Sharma, Anal. Chim. Acta 551 (2005) 159.
[32] N.V.S. Ramakrishna, M. Koteshwara, K.N. Vishwottam, S. Puran, S. Manoj, M. Santosh, J. Pharm. Biomed. Anal. 36 (2004) 505.
[33] Z.R. Tan, D.S. Oyuang, G. Zhou, L.S. Wang, Z. Li, D. Wang, H.H. Zhou, J. Pharm. Biomed. Anal. 42 (2006) 207.
[34] C. Kousoulos, G. Tsatsou, C. Apostolou, Y. Dotsikas, Y.L. Loukas, Anal. Bioanal. Chem. 384 (2006) 199.