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Simultaneous determination of ramipril, ramiprilat and telmisartan in human plasma using liquid chromatography tandem mass spectrometry

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ABSTRACT

A rapid and sensitive liquid chromatography tandem mass spectrometry method has been developed and validated for the simultaneous determination of ramipril, ramiprilat and telmisartan in human plasma. The solid-phase extraction technique was used for the extraction of ramipril, ramiprilat and telmisartan from human plasma. Trandolaprilat and hydrochlorothiazide were used as the internal standards (ISs). Chromatography was performed on a Hypurity C18, 5 μm, 50 mm × 4.6 mm column, with the mobile phase consisting of ammonium acetate and acetonitrile (in a 20:80 ratio), followed by detection using mass spectrometry. The method involves a simple reversed isocratic chromatography condition and mass spectrometry detection, which enables detection at sub-nanogram levels. The method was validated and the lower limit of quantification for ramipril, ramiprilat and telmisartan was found to be 0.1 ng mL^{-1}, 0.1 ng mL^{-1} and 2 ng mL^{-1}, respectively. The mean recovery for ramipril, ramiprilat and telmisartan ranged from 90.1 to 104.1%. This method increased the sensitivity and selectivity; resulting in high-throughput analysis of ramipril, ramiprilat and telmisartan using two different ISs in a single experiment for bioequivalence studies, with a chromatographic run time of 1.5 min only.

1. Introduction

The rennin–angiotensin system acts through two factors, i.e. angiotensin-converting enzyme, which converts angiotensin I to angiotensin II, and angiotensin receptors I and II to maintain volume homeostasis, control blood pressure and prevent ischemia. Therefore, controlling both the factors simultaneously provides effective blood pressure control and reduces the risk of cardiovascular events.

Ramipril and ramiprilat compete with angiotensin I and block the conversion of angiotensin I to angiotensin II. Angiotensin II contracts the muscles of most arteries in the body, including the heart, thereby narrowing the arteries and elevating the blood pressure [1,2]. Ramipril is chemically designated as (2S,3aS,6aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3a,4,5,6,6a-hexahydro-2H-cyclopenta[d]pyrrole-2-carboxylic acid.

Telmisartan, 4-((2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl) methyl) biphenyl-2-carboxylic acid, blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as the vascular smooth muscle and the adrenal gland [3,4]. Both telmisartan and ramipril were shown to be efficacious in reducing cardiovascular risk from myocardial infarction (MI). Fixed dose combination (FDC) of telmisartan 40 mg and ramipril 5 mg brings about significant reductions in the systolic and diastolic blood pressure as well as urine albumin excretion [5,6].

A number of methods have been reported for the simultaneous determination of ramipril and ramiprilat, including liquid chromatography with tandem mass spectrometric detection (LC–MS/MS) using liquid–liquid extraction [7], GC–MS using derivatisation technique [8] and high-performance liquid chromatography (HPLC) [9,10]. Although the above methods are fast and robust, they require a large number of complicated steps for sample pretreatment. Further, LC/APCI–MS with online sample preparation [11], LC–ESI–MS/MS [12] and several other bioanalytical methods using different detectors [13–15] have been developed for the determination of telmisartan.

Information from the literature reveals that the methods used for the determination of ramipril, ramiprilat and telmisertan are suitable for the determination of a single analyte or for an analyte with a metabolite, but are not suitable for their simultaneous determination in human plasma. Although Tapadiya et al.

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[16–18] developed a method for the simultaneous determination of ramipril, hydrochlorothiazide and telmisartan by UV/vis detection, this method is however limited to the pharmaceutical preparation but is not suitable for pharmacokinetic analysis. Thus, a single bioanalytical method for the determination of ramipril, ramiprilat and telmisartan for routine therapeutic monitoring is required.

LC–MS/MS was demonstrated to be superior to all the abovementioned techniques in terms of sensitivity, selectivity, simplicity and analysis throughput [19–22]. This paper describes the UPLC™ technology coupled with triple quadrupole tandem mass spectrometry that has been applied to the analysis for the simultaneous determination of ramipril, ramiprilat and telmisartan using trandolaprilat and hydrochlorothiazide as the internal standards (ISs). Two ISs were chosen in order to reduce the error while calculating the concentration, as the ranges of ramipril, ramiprilat and telmisertan are different. The use of solid phase extraction technique (SPE) using polymer cartridges DVB LP 30 mg 1 cm^3 from Orochem Technology Inc. (IL, USA) reduced the background noise produced by electrospray ionization (ESI), enabling us to develop a single and more sensitive method for ramipril, ramiprilat and telmisartan with a high sample throughput due to the short chromatographic condition and simple sample preparation.

2. Materials and methods

2.1. Chemicals and reagents

Ramipril, ramiprilat, telmisartan, trandolaprilat and hydrochlorothiazide standards (purity > 99.8%) were obtained from Hetero Drug (Hyderabad, India). Tri-potassium salts of ethylenediaminetetraacetic acid (K_3EDTA) plasma of healthy volunteers was obtained from West Cost Blood Bank, Mumbai (India). Acetonitrile (HPLC grade) and methanol were obtained from JT Baker, Germany. A Milli-Q water (Millipore Co., MA, USA) purification system was used to obtain purified water for the HPLC analysis.

2.2. Instrumentation

Chromatography was performed at ambient temperature, with the mobile phase consisting of acetonitrile and 2 mM ammonium acetate (pH 3.5, 80:20, V/V). A Hypurity C18, 5 μ (50 mm × 4.6 mm, i.d.) column obtained from Thermo Hypersil, FL, USA, was used for the chromatographic separation at a flow rate of 0.5 mL/min. The mobile phase was delivered by a ultra performance liquid chromatography (UPLC) pump and the sample was injected by a UPLC autosampler (Waters® Corporation, Milford, MA, USA). Detection was performed by a Quattro Premier™ XE tandem quadrupole mass spectrometer (Waters® Corporation) fitted with an ESI source operating in a negative ion mode. A DVB LP 30 mg 1 cm^3 solid-phase extraction (SPE) cartridge for sample preparation was obtained from Orochem Technology Inc. A 48-head positive-pressure solid-phase manifold was obtained from SPEware Corporation, Baldwin Park, CA, USA.

The Quattro Premier™ XE tandem quadrupole mass spectrometer was operated at the multiple reaction monitoring (MRM) mode, monitoring the transition of molecular ions to the product ions for ramipril, m/z 415.43 → 154.97; ramiprilat 387.32 → 154.01; telmisartan 513.38 → 469.18. Fig. 1 shows a full scan mass spectrum of pure ramipril, ramiprilat and telmisartan with the most abundant product ions and the principal product ion at m/z 154.97, 154.01 and 469.18.

2.3. Preparation of standards and quality control samples

The stock solutions of ramipril, ramiprilat and telmisartan were prepared by dissolving reference standards in methanol. These stock solutions were later used to prepare the working solutions of ramipril, ramiprilat and telmisartan in methanol: Milli Q/HPLC grade water mixture (50:50, v/v) by appropriate dilution. Calibration curve standards (CS) were prepared by spiking different samples of 0.5 mL of blank human plasma, each with 25 μL working solution, in a concentration range of 0.1–25 ng mL^{-1} for ramipril and ramiprilat and 2–400 ng mL^{-1} for telmisartan. Similarly, quality control standards (QC) were prepared for the lower limit of quantification (LLOQ), low quality control (LQC), medium quality control (MQC) and high quality control (HQC). QCs were prepared on a daily basis by spiking different samples of 0.5 mL plasma, each with 25 μL of the corresponding working solution, to produce a final concentration equivalent to 0.3, 3, 15 ng mL^{-1} of ramipril, ramiprilat and 6, 100, 300 ng mL^{-1} of telmisartan, respectively. Aliquots of the QC were stored in polypropylene tubes at −20 °C for long-term stability.

The stock solutions of trandolapril and hydrochlorothiazide (HCTZ) were prepared by dissolving reference standards in methanol. These were further diluted in methanol:water (50:50, v/v) to get concentrations of 0.25 μg mL^{-1} trandolapril and 25 μg mL^{-1} HCTZ. All the stock solutions were stored at 4–8 °C for further use.

ESI was performed in the negative ion mode with a source temperature of 120 °C, desolvation temperature of 350 °C, capillary voltage of 3.00 kV, cone voltage of 12 V, cone gas flow of 68 L/h and desolvation gas flow of 650 L/h. Optimization of the triple quadrupole settings of the instrument for the detection of ramipril, ramiprilat, telmisertan and the ISs was performed by infusing a 1000 ng mL^{-1} solution of each drug dissolved in methanol:water (80:20, v/v) solution at a constant flow rate of 10 μL/min.

2.4. Extraction procedure

Plasma samples (0.5 mL) were pipetted into 2 mL Eppendorff tubes followed by the addition of 20 μL of the IS (25 μg mL^{-1} of hydrochlorothiazide and 0.25 μg mL^{-1} of trandolaprilat). The samples were vortex and mixed for 10 s. Before loading the sample, the SPE cartridges were conditioned and equilibrated with 1 mL of methanol, followed by 1 mL of water (HPLC grade), using a positive pressure manifold. The samples were loaded into the SPE cartridges and positive pressure was applied to elute the plasma. Once the plasma was eluted, the cartridges were washed with 1.5 mL of water and the remaining water was completely removed by applying pressure. The drug was eluted from the SPE cartridge with 0.5 mL of the mobile phase. The eluent was collected in 1.5 mL HPLC vials and transferred onto the autosampler for analysis

2.5. Validation

Three independent analytical batches of spiked plasma calibration standard at eight different concentrations levels ranging from 0.1 to 25 ng mL^{-1} for ramipril and ramiprilat and from 2 to 400 ng mL^{-1} for telmisartan, were prepared and analyzed. Weighted 1/(concentration)^2 linear regressions was used to construct the ramipril, ramiprilat and telmisartan calibration curves. Spiked QC samples were processed in six replicates at each concentration (0.1, 0.3, 3.0 and 15 ng mL^{-1} of ramipril and ramiprilat and 2, 6, 100 and 300 ng mL^{-1} of telmisartan) for three different analytical batch to evaluate the intra- and inter-batch assay accuracy and precision. System suitability was performed by analyzing the resolution standard (RS) containing ramipril, ramiprilat and telmisartan

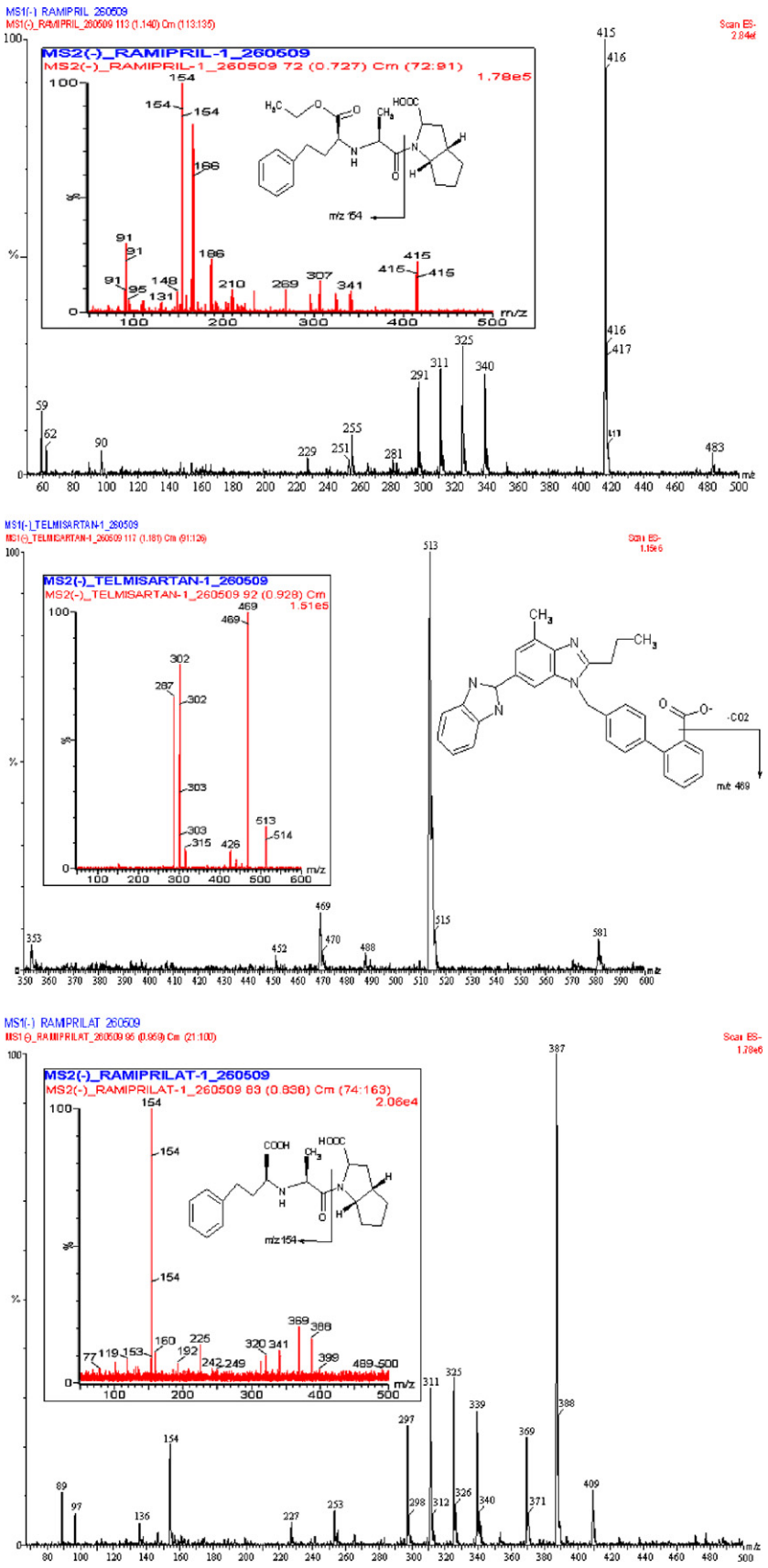

Fig. 1. Representative mass spectra of ramipril, telmisartan, ramiprilat and fragment ion.

Table 1
Back calculated concentration of ramipril, ramiprilat and telmisartan ($n = 3$).

	Concentration (ng mL^{-1})										
	STD 1 0.1	STD 2 0.2	STD 3 0.5	STD 4 1	STD 5 5	STD 6 10	STD 7 20	STD 8 25	Slope	Intercept	r^2
Ramipril											
Mean	0.094	0.214	0.525	1.113	5.094	10.503	20.437	24.828	0.0093	0.0009	0.9922
S.D.	0.004	0.006	0.032	0.066	0.181	0.758	1.628	0.366			
%CV	4.23	2.58	6.18	5.94	3.56	7.22	7.97	1.47			
%Nominal	94.37	106.83	105.07	111.27	101.88	105.03	102.19	99.31			
Ramipril											
Mean	0.099	0.205	0.472	1.044	5.017	10.537	19.443	23.696	0.0091	0.0009	0.993
S.D.	0.0062	0.0250	0.0622	0.0257	0.0466	0.2200	0.7409	0.8761			
%CV	6.31	12.18	13.18	2.46	0.93	2.09	3.81	3.70			
%Nominal	99.00	102.67	94.40	104.43	100.35	105.37	97.21	94.78			
	2	4	15	50	150	250	350	400			
Telmisartan											
Mean	1.97	4.15	14.36	52.14	153.04	244.04	325.01	415.72	0.9941	0.01	0.994
S.D.	0.035	0.113	0.247	1.61	3.405	2.175	16.271	10.177			
%CV	1.78	2.72	1.72	3.09	2.22	0.89	5.01	2.45			
%Nominal	98.5	103.75	95.73	104.28	102.03	97.62	92.86	103.93			

SD: standard deviation; n, total number of observation; STD: standard.

and the IS. Specificity was determined in six different lots of normal K_3EDTA plasma and two different lots lipimic and hemolized plasma.

Absolute recovery of the analyte was determined in normal plasma at three different concentrations (0.1, 0.3, 3.0 and 15 ng mL^{-1} of ramipril and ramiprilat and 2, 6, 100 and 300 ng mL^{-1} of telmisartan) by comparing the analyte peak areas of the extracted QC samples with the analyte peak areas of the non-extracted equivalent standard mixture representing 100% recovery. The stability of drugs in human plasma was studied by subjecting into different storage conditions at two different concentration (LQC and HQC) levels. The plasma samples were kept at room temperature for 6 h for evaluation of bench top stability and −20 °C for 45 days for long term stability. Freeze/thawed stability was also evaluated after subjecting into three cycles of freezing and thawing. The stability was evaluated by comparing with a freshly prepared calibration standard and QC samples. The analyte was considered stable in the human plasma when a percent change of ±15% of the initial concentration was found.

3. Results and discussion

3.1. Method development

The much higher selectivity of MS/MS detection allowed the development of a very specific and rapid method for the determination of ramipril, ramiprilat and telmisartan. Linearity was tested in the concentration range of 0.1–25 ng mL^{-1} for ramipril and ramiprilat and 2–400 ng mL^{-1} for telmisartan. However, the concentration of the lowest calibration standard for ramiprilat can be quantified up to 50 pg mL^{-1}, with a signal to noise ratio of more than 5. Results of the back-calculated concentrations from three calibration curves for ramipril, ramiprilat and telmisartan using LC–MS/MS are tabulated in Table 1. The table also shows the accuracy and precision for each point. The results obtained were within the acceptance criteria of no more than 20% deviation at the LLOQ and no more than 15% deviation for the standards above this point (LLOQ).

Sample clean up is one of the important components in any bioanalysis using LC–MS/MS technique. A polymer based sorbent which is water-wettable DVB LP 30 mg 1 cm^3 was investigated and found to be more rugged and robust since it does not require negative effect of drying enabling to improve selectivity and improved recovery compared to C18 silica base SPE cartridge. No endogenous substances were detected, which significantly interfered with the quantification of ramipril, ramiprilat and telmisartan. Even though there is an improvement in sensitivity and selectivity but still the aim of the researchers is to reduce the chromatographic run time that allows increase in the sample throughput. Short columns used for fast analysis are susceptible to dead volume. Care was taken by replacing the 0.010 in. internal diameter (ID) which is commonly used with smaller ID (<0.007 in.) tubing length to a minimum. The retention times for ramipril, ramiprilat, telmisartan, trandolaprilat and hydrochlorothiazide were found to be within a min. The total chromatography run time of 1.5 min made it possible to analyze a large number of samples in a batch. Figs. 2 and 3 show representative chromatograms of ramipril, ramiprilat and telmisartan at concentrations of 0.1 and 2 ng mL^{-1} in plasma along with the IS. A representative chromatogram of the extracted blank plasma of ramipril, ramiprilat, telmisartan and IS is presented in Fig. 4. This assay method was also employed to analyze plasma samples containing ramipril, ramiprilat and telmisartan from 12 healthy male volunteers after administrating single doses of 5 mg ramipril and 40 mg telmisartan each.

3.2. Precision and accuracy

The inter-batch precision and accuracy were determined from three analytical batches by analyzing spiked QC samples. The intra-batch precision and accuracy of the assay were measured by analyzing six spiked samples of ramipril, ramiprilat and telmisartan at each QC level (0.1, 0.3, 3.0 and 15 ng mL^{-1} of ramipril and ramiprilat and 2, 6, 100 and 300 ng mL^{-1} of telmisartan). Intra day and inter-day precision ranged from 2.29 to 8.56% and 3.82 to 12.07% for ramipril, 2.78 to 3.43% and 3.86 to 11.84% for ramiprilat and 3.88 to 9.9% and 3.53 to 9.14% while accuracy, expressed as % nominal was within 96.69 to 13.93% and 99.0 to 108.63% for ramipril, 98.7 to 105.2% and 97.33 to 106.67% for ramiprilat and 92.2 to 105.12% and 103.50 to 109.76% respectively, as given in Table 2.

3.3. Stability

The stability of the analytes in human plasma under different temperatures and times as well as the stability of the analytes in the stock solution were evaluated. For short-term stability (bench top) determination, the stored plasma aliquots were thawed and kept at room temperature for around 6 h. The samples were then

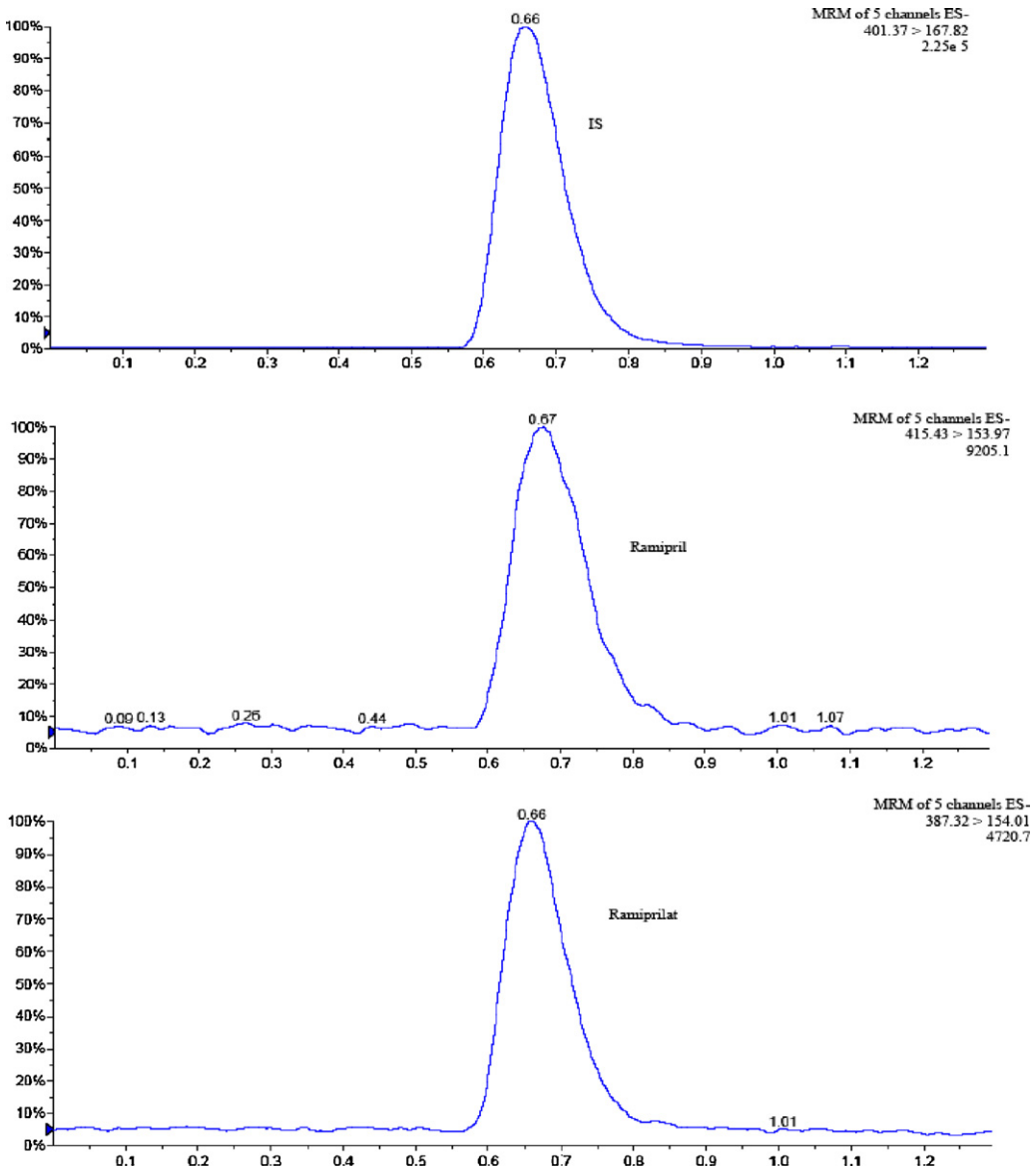

Fig. 2. Representative chromatogram of ramipril, ramiprilat at 0.1 ng mL^{-1} with trandolaprilat as IS.

Table 2
Intra day and inter day accuracy of the method for ramipril, ramiprilat and telmisartan.

Levels	Concentration added (ng mL^{-1})	Intra day				Inter day			
		n	Mean concentration found (ng mL^{-1})[a]	%Nominal	%CV	*n*	Mean concentration found (ng mL^{-1})[b]	%Nominal	%CV
Ramipril									
LLOQ	0.100	6	0.102	102.00	5.31	18	0.099	99.00	12.07
LQC	0.300	6	0.3118	103.93	3.59	18	0.286	95.33	7.99
MQC	3.000	6	2.94	98.00	8.59	18	3.19	106.33	5.23
HQC	15.000	6	14.503	96.69	2.29	18	16.295	108.63	3.82
Ramiprilat									
LLOQ	0.100	6	0.105	105.0	3.43	18	0.098	98.00	11.84
LQC	0.300	6	0.307	102.3	3.19	18	0.292	97.33	7.35
MQC	3.000	6	3.156	105.2	1.97	18	3.2	106.67	4.66
HQC	15.000	6	14.798	98.7	2.78	18	15.521	103.47	3.86
Telmisertan									
LLOQ	2.000	6	2.102	105.1	9.9	18	2.07		9.14
LQC	6.000	6	6.207	103.5	5.18	18	6.407		4.66
MQC	100.000	6	92.166	92.2	7.62	18	109.762		3.53
HQC	300.000	6	279.82	93.3	3.88	18	319.525		8.36

CV, coefficient of variance; *n*, total number of observation.
[a] Mean of 6 replicates observations at each concentration.
[b] Mean of 18 replicates observations over three different analytical batch.

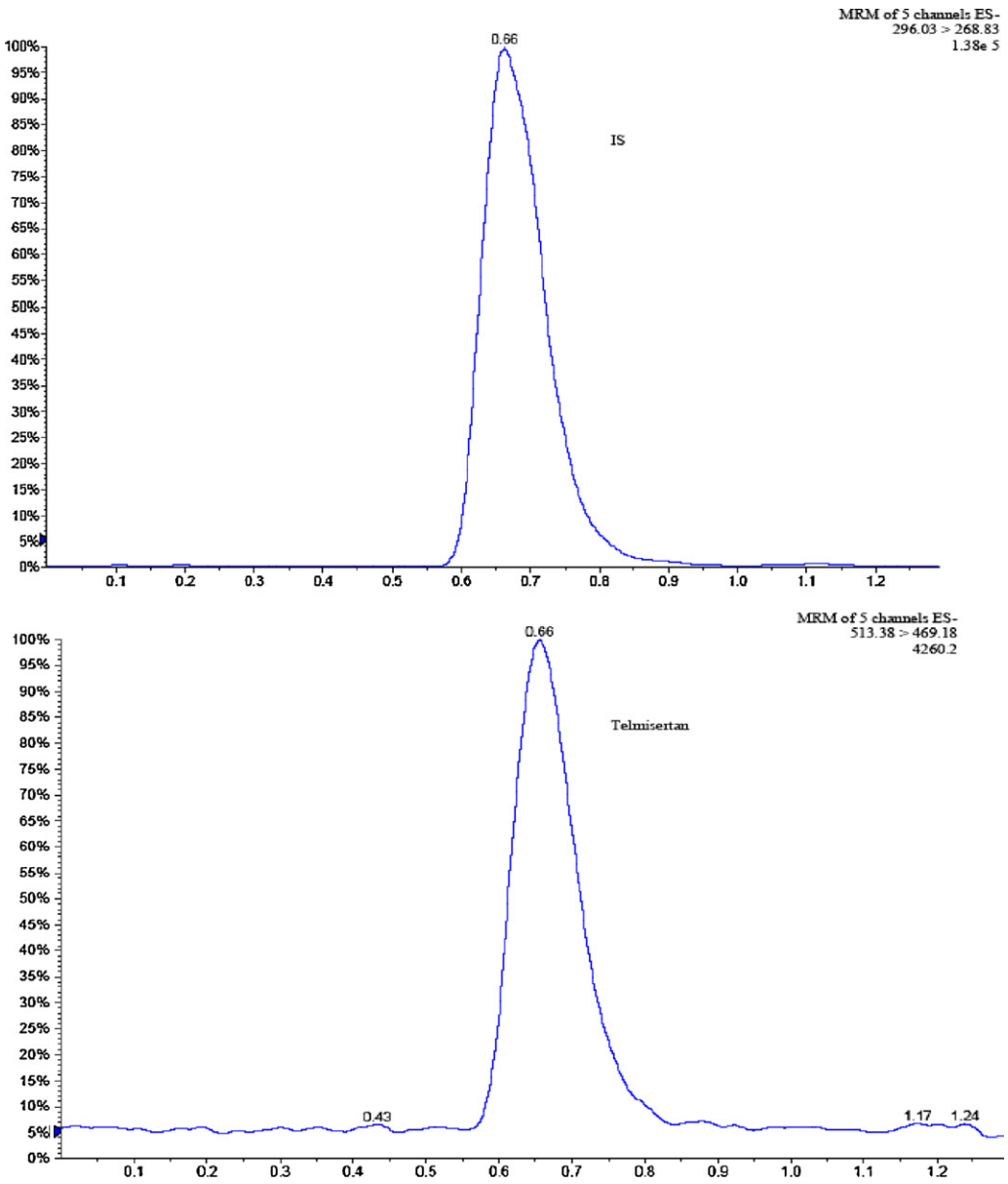

Fig. 3. Representative chromatogram of telmisartan (2 ng mL^{-1}) and hydrochlorothiazide.

extracted and analyzed according to the above-mentioned procedure. Short-term stability indicates the stability of the drug during routine experiments.

Plasma samples containing known concentrations of ramipril, ramiprilat and telmisartan were subjected to three freeze–thaw cycles to ascertain the freeze–thaw stability. The freeze–thaw stability was evaluated at the end of the third cycle by comparing with the stability of freshly prepared samples. The calculated values of this stability (test) of the samples showed no apparent changes in the concentration. Ramipril, ramiprilat and telmisartan are stable in solution (methanol) at 4–10 °C for 9 days. No degradation occurred after leaving the QC plasma samples on the bench top at room temperature over a period of 6 h. To determine the autosampler stability, QCs (LQC, MQC and HQC) samples were samples were processed and kept in the autosampler with a set temperature of 10 °C for 24 h and compared with a freshly prepared calibration standards. There was no significant difference between the observed concentrations at zero time and after 24 h at set autosampler temperature, indicating a stability of ramipril ramiprilat and telmisertan in plasma.

Long-term stability in biological matrix kept at −20 °C was assessed over a period of 45 days using two different concentrations (LQC and HQC) of ramipril, ramiprilat and telmisartan. Ramipril, ramiprilat and telmisartan were stable in plasma for at least 45 days when stored at −20 °C in polypropylene tubes.

3.4. Recovery

The absolute recoveries were evaluated for ramipril, ramiprilat and telmisartan and the ISs by comparing the peak areas of the extracted samples with the unextracted authentic standard solutions at the three QC levels of 0.3, 3.0 and 15.0 ng mL^{-1} for ramipril and ramiprilat and 6, 100 and 300 ng mL^{-1} for telmisartan. The absolute recovery determination for ramipril, ramiprilat and telmisartan was shown to be consistent, precise and reproducible. The recovery ranged from 90.1 to 104.1% at the three QC levels. Absolute analytical recoveries of the ISs were found to be 88.39 and 104.9%, respectively, for hydrochlorothiazide and trandolaprilat

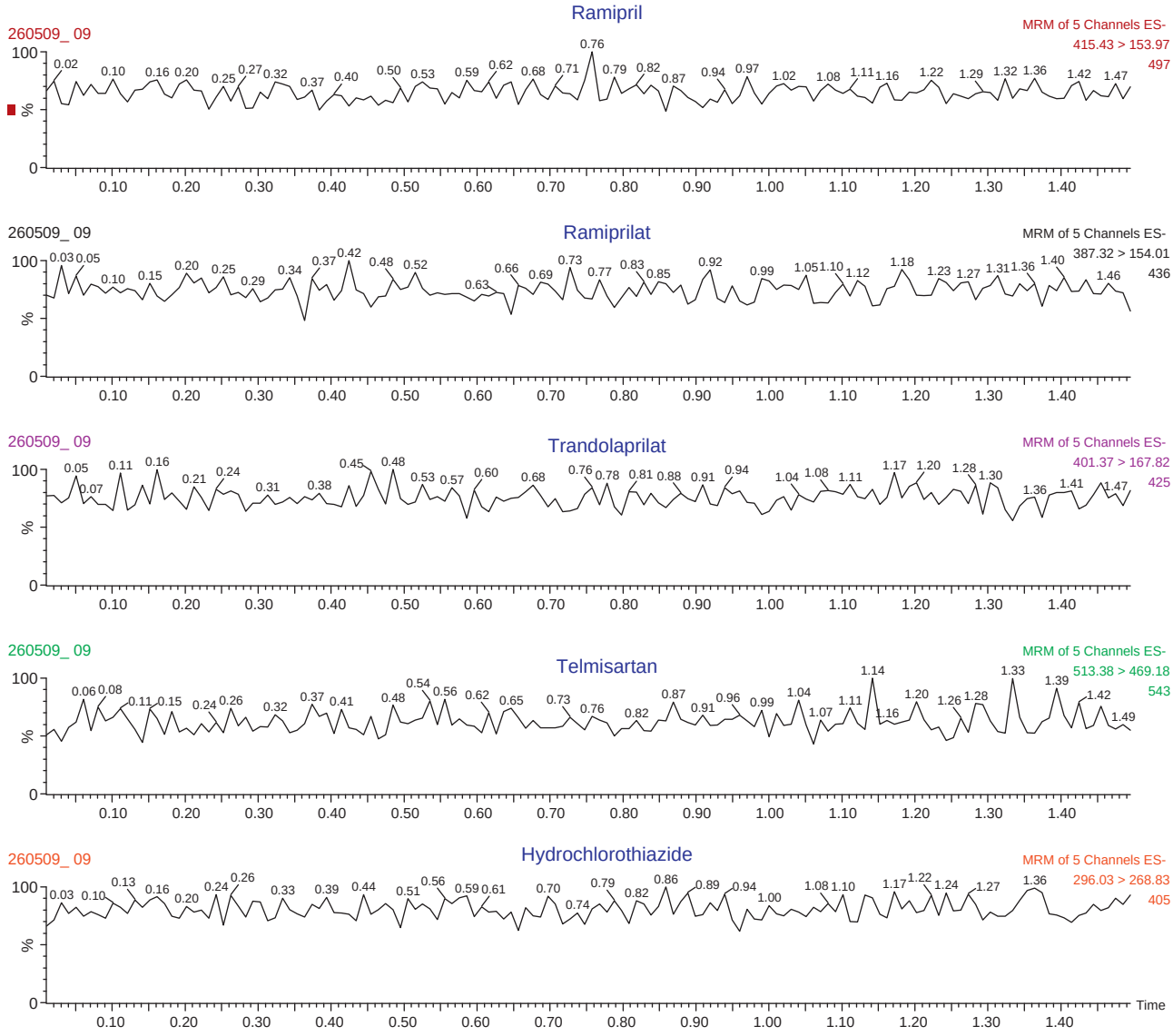

Fig. 4. Representative chromatogram of extracted blank plasma.

3.5. Matrix effect

Matrix effect was determined by IS normalization matrix factor [20] at the three concentration levels of LQC, MQC and HQC using six different plasma lots that passed the selectivity criteria. Samples were processed in triplicates at each level to ensure that the concentration was independent of variability in matrix due to its physiological nature.

The percentage coefficient of variance of all the three compounds was within the acceptance range of ≤15%. Table 3 shows the statistical data of the results.

4. Application of the method

The method was applied to the analysis of plasma samples obtained from the pharmacokinetic study. The study was conducted as a randomized, single-dose, two-treatment, two-sequence, two-way crossover study with at least 07 days washout period between each administration, in 12 Indian healthy, adult, male, human subjects under fasting condition. Each subject received an FDC of telmisartan 40 mg and ramipril 5 mg tablet of test or reference. Blood samples were collected using K_3EDTA vaccutainers at the following times: pre-dose, 0.0, 0.25, 0.5, 0.75, 1.0, 1.33, 1.67, 2.0, 2.33, 2.67, 3.0, 3.33, 4.0, 5.0 6.0,8.0,10.0,12.0,24.0,36.0, 48.0, 72.0 and 120.0 h after administration.

Table 3
Matrix effect evaluation for ramipril, ramiprilat and telmisertan.

	Peak area ratio		
	HQC	MQC	LQC
Ramipril			
Mean	2.13874	0.44300	0.03291
S.D. ±	0.02745	0.00807	0.00158
%CV	1.28	1.82	4.81
Ramiprilat			
Mean	0.8167	0.1630	0.0072
S.D. ±	0.02516	0.00984	0.00088
%CV	3.08	6.03	12.25
Telmisertan			
Mean	0.8222	1.1632	0.0071
S.D. ±	0.00979	0.01025	0.00045
%CV	1.19	0.88	6.36

Table 4
Pharmacokinetic parameters.

Analyte	Drug	Statistic	Cmax (ng mL^{-1})	Tmax (h)	Thalf (h)	AUClast (ng mL^{-1} h)	AUCtot (ng mL^{-1} h)
Ramipril	Test (T)	Mean	7.40	0.518	0.964	9.341	8.901
		RSD%	25.20	39.91	56.3	80.12	90.841
	Reference (R)	Mean	7.51	0.20	0.935	8.321	7.841
		RSD%	20.80	0.50	0.673	101.140	103.091
90% Confidence interval for the ratio of the means T/R			86.96–111.71	–	–	97.10–119.69	96.72–119.76
Ramiprilat	Test (T)	Mean	4.120	2.515	50.085	103.450	64.138
		RSD%	71.8	55.2	48.5	51.8	39.8
	Reference (R)	Mean	4.146	1.819	39.521	92.044	63.489
		RSD%	103	55.3	50.0	47.4	46.0
90% Confidence interval for the ratio of the means T/R			103.05–122.86	–	–	102.59–122.55	98.04–111.02
Telmisartan	Test (T)	Mean	348.56	1.35	11.95	1891.30	1645.20
		RSD%	39.35	52.52	28.86	17.33	22.41
	Reference (R)	Mean	367.41	1.22	11.99	1904.06	1688.19
		RSD%	37.40	45.41	23.56	17.59	21.58
90% Confidence interval for the ratio of the means T/R			84.77–104.97	–	–	94.79–103.87	91.70–103.21

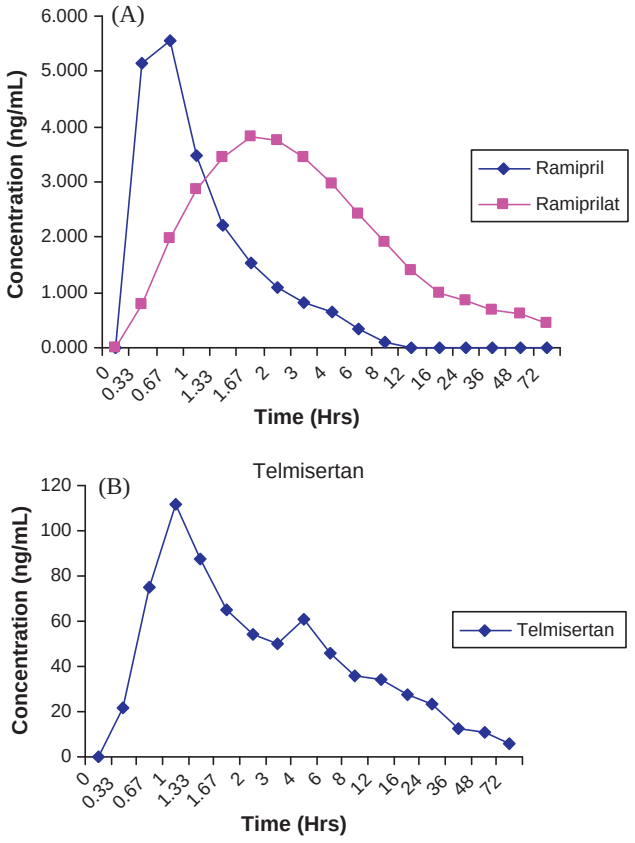

Fig. 5. Mean plasma concentration vs. time profile of ramipril, ramiprilat (A) and telmisertan (B).

Pharmacokinetic parameters were calculated from the subjects who had successfully completed periods I and II of the study. Some of the main pharmacokinetic parameters are given in Table 4. The mean plasma concentration versus time profile is shown in Fig. 5.

5. Conclusions

For the first time, a highly sensitive and selective method for the simultaneous determination of ramipril, ramiprilat and telmisartan in plasma was developed using UPLC–MS/MS with turbo-ESI. This developed assay method was used in a pharmacokinetic study in which 12 healthy male volunteers were given a fixed-dose combination of 5 mg of ramipril and 40 mg of telmisartan. The advantage of using the DVB LP polymer cartridge from Orochem Technology Inc. made it possible to detect lower concentrations. This method allows for a much higher sample throughput due to the short chromatographic time (1.5 min) and simple sample preparation. A single analytical column was used to chromatograph about 1500 extracts and ion sources, and it was not necessary to clean this column during the entire study. This validated method is an excellent analytical option for the simultaneous, rapid quantification of ramipril, ramiprilat and telmisartan in human plasma.

References

[1] G.T. Warner, C.M. Perry, Drugs 62 (2002) 1381–1405.
[2] D.J. Campbell, A. Kladis, A.M. Duncan, Hypertension 22 (1993) 513–522.
[3] B. Pitt, M.A. Konstam, Am. J. Cardiol. 82 (1998) 47S–49S.
[4] T. Unger, Am. J. Cardiol. 84 (1999) 9S–15S.
[5] A. Sharma, V. Baliga, J. Indian Med. Assoc. 196 (2008) 191–194.
[6] S.D. Jain, S. Biradar, I. Periyandavar, S.S. Sodhi, K. Anwaruddin, A. Gawde, V. Baliga, K. Gandewar, A. Desai, Curr. Ther. Res. 66 (2005) 630–642.
[7] B. Yuan, X. Wang, F. Zhang, Chromatographia 68 (2008) 533–539.
[8] B.A. Persson, C. Fakt, M. Ervik, M. Ahnoff, J. Pharm. Biomed. Anal. 40 (2006) 794–798.
[9] L.V. Allen Jr., M.L. Stiles, S.J. Prince, H.J. McLaury, M.F. Sylvestri, Am. J. Health Syst. Pharm. 52 (1995) 2433–2436.
[10] Z. Zhu, A. Vachareau, L. Neirinck, J. Chromatogr. B 779 (2002) 297–306.
[11] C. Hempen, L.G. Schwarz, U. Kunz, U. Karst, Anal. Chim. Acta 560 (2006) 41–49.
[12] J. Shen, Z. Jiao, Z.D. Li, X.J. Shi, M.K. Zhong, Pharmazie 60 (2005) 418–420.
[13] L. Gonzalez, U. Akesolo, R.M. Jimenez, R.M. Alonso, Electrophoresis 23 (2002) 997–1997.
[14] N. Torrealday, L. Gonzalez, R.M. Alonso, R.M. Jimenez, E. Ortiz Lastra, J. Pharm. Biomed. Anal. 32 (2003) 847–857.
[15] T. Yan, H. Li, L. Denga, Y. Guo, W. Yu, J. Paul Fawcett, D. Zhang, Y. Cui, J. Gu, J. Pharm. Biomed. Anal. 48 (2008) 1225–1229.
[16] V.K. Gupta, P. Kumar, Anal. Chim. Acta 389 (1999) 205–212.
[17] R.N. Goyal, V.K. Gupta, A. Sangal, N. Bachheti, Electrochem. Commun. 8 (2006) 65–70.
[18] G.G. Tapadiya, S. Bankey, S.S. Saboo, S. Bindaiya, D Jain, S.S. Khadbadi, Int. J. Chem. Tech. Res. 1 (2009) 183–188.
[19] M.J.L. de Alda, A. Gil, E. Paz, D. Barcelo, Analyst 127 (2002) 1299–1304.
[20] T. Isobe, H. Shirashi, M. Yasuda, H. Suzuki, M. Morita, J. Chromatogr. A 984 (2003) 195–202.
[21] N.C. Twaddle, M.I. Churchwell, R.R. Newbold, K.B. Delclos, D.R. Doerge, J. Chromatogr. B 793 (2003) 309–315.
[22] H.W. Zhang, J. Henion, Anal. Chem. 71 (1999) 3955–3964.