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ORIGINAL ARTICLE

Stability-indicating HPLC–DAD methods for determination of two binary mixtures: Rabeprazole sodium–mosapride citrate and rabeprazole sodium–itopride hydrochloride

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Peak purity

Abstract Two selective stability-indicating HPLC methods are described for determination of rabeprazole sodium (RZ)–mosapride citrate (MR) and RZ–itopride hydrochloride (IO) mixtures in the presence of their ICH-stress formed degradation products. Separations were achieved on X-Bridge C18 column using two mobile phases: the first for RZ–MR mixture consisted of acetonitrile: 0.025 M KH_2PO_4 solution: TEA (30:69:1 v/v; pH 7.0); the second for RZ–IO mixture was at ratio of 25:74:1 (v/v; pH 9.25). The detection wavelength was 283 nm. The two methods were validated and validation acceptance criteria were met in all cases. Peak purity testing using contrast angle theory, relative absorbance and log *A* versus the wavelengths plots were presented. The % recoveries of the intact drugs were between 99.1% and 102.2% with RSD% values less than 1.6%. Application of the proposed HPLC methods indicated that the methods could be adopted to follow the stability of their formulations.

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1. Introduction

Rabeprazole sodium (RZ) is chemically designed as (±) sodium-2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl] methylsulfinyl]-1H-benzimidazole [1]. It is a proton pump inhibitor that covalently binds and inactivates the gastric parietal cell proton pump (H^+/K^+ ATPase) and is used in the management of acid-related disorders

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[2]. The literature reveals that several chromatographic methods have been reported for the determination of RZ in pharmaceutical dosage forms (as single component) by HPLC [3–10], stability-indicating HPLC in the presence of its degradation products [11,12] and TLC-densitometric determinations [9,13]. Several analytical methods have been described for the simultaneous determination of RZ and domperidone using HPLC method [14], with itopride using HPLC [15–19], high-performance thin layer chromatography (HPTLC) densitometry [20] and spectrophotometric methods [21,22]. A through literature search reveals that HPLC and TLC methods had been described for the simultaneous determination of RZ and mosapride in combined dosage form [23].

Mosapride citrate (MR) is chemically designed as 4-amino-5-chloro-2-ethoxy-N-{[4-(4-fluorobenzyl) morpholin-2-yl] methyl} benzamide. It is a potent gastroprokinetic agent with selectivity for 5-HT_4 receptor and is used in the treatment of gastrointestinal motility dysfunction associated with non-ulcer dyspepsia and esophagitis [1]. Reviewing the literature in hand, the reported HPLC method for the determination of MR as binary mixture with RZ [23] was described.

Itopride hydrochloride (IO) is chemically designed as *N*–[4-[2-(dimethylamino) ethoxy]-benzyl]-3,4-dimethoxy benzamide hydrochloride [1]. IO is a gastroprokinetic drug dedicated for the treatment of patients with symptomatic functional dyspepsia. Literature survey revealed that HPLC [15–19], spectrophotometric [21,22] and HPTLC [20] methods had been described for the simultaneous determination of IO with RZ in combined dosage forms and are nonstability-indicating methods. On the other hand, the investigated drugs RZ, MR and IO are not yet official in any of the pharmacopoeia.

The drug stability testing guidelines [24] require that analysis of stability samples should be performed using validated stability-indicating analytical methods. It also recommends carrying out stress testing on the drug substance to elucidate its inherent stability characteristics and hence supporting the suitability of the proposed analytical procedures [24–26] for testing drug substance or drug combination product. To the best of our knowledge, no article related to the HPLC stability-indicating method for the simultaneous determination of RZ–MR or/and RZ–IO mixtures in pharmaceutical dosage forms has ever been mentioned in the literature, which is the aim of this work. Thereafter, methods were validated according to ICH guidelines [25] and USP analytical method validation parameters [27].

Furthermore, the proposed HPLC methods were applied to the determination of RZ in its capsule dosage forms containing either MR or IO. Fig. 1 shows the structural formulae of the investigated drugs.

2. Experimental

2.1. Chemicals

RZ, kindly provided by Quimica Sintetica S.A. (Madrid, Spain), IO and MR of pharmaceutical grade, purchased from Kangnin Pharmaceutical Co. (Sanmen County, China), were certificated to contain 99.37%, 99.30% and 99.55%, respectively. Acetonitrile used was of HPLC grade (BDH, Poole, UK). Orthophosphoric acid solution (85%), potassium dihydrogen phosphate and TEA were of HPLC grade (Merck, Darmstadt, Germany). Hydrogen peroxide (30 volumes) was from Qualigens Fine Chemicals (Glaxo Ltd., England). Sodium hydroxide pellets and concentrated hydrochloric acid solution were analytical grade (Germany). HPLC water was generated in-house using a Millipore, Milli-Q reverse osmosis plus system (Bedford, MA, USA). Veloz-M® capsules labeled to contain 20 mg RZ and 15 mg MR per capsule were manufactured by Torrent® Pharmaceutical Ltd. Zorite® capsules labeled to contain 20 mg RZ and 150 mg IO per capsule were manufactured by Indoco Remedies® Ltd.

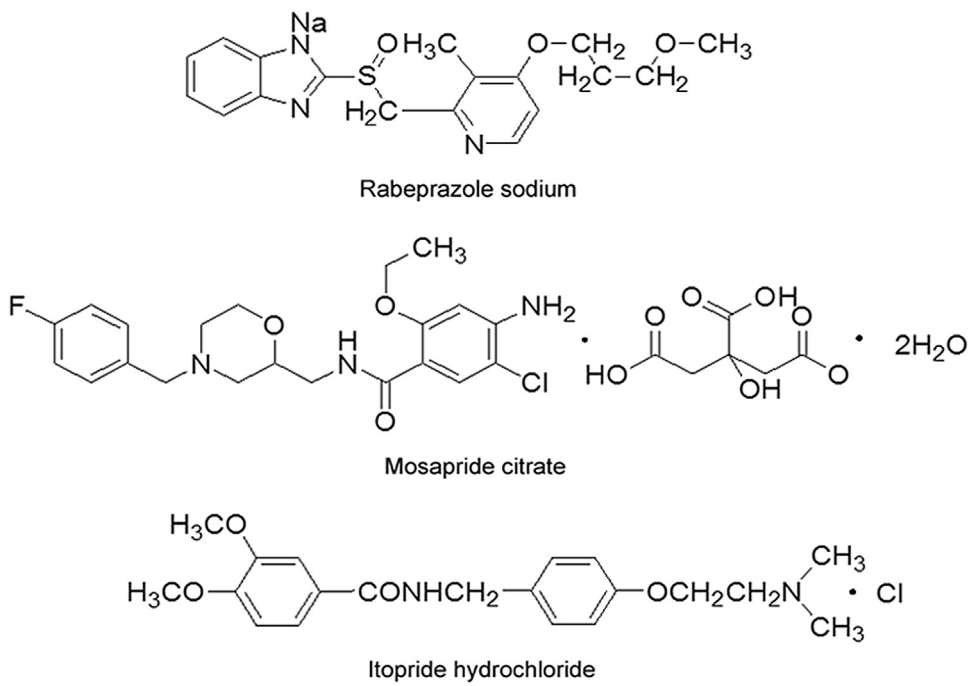

Fig. 1 Structural formulae of the investigated drugs.

2.2. Instrumentation

The HPLC system consisted of Waters Alliance solvent management system 2695, a photodiode array detector (DAD) 2998, thermostatically controlled column apartment and an auto-sampler with a 250 µL loop. The control of HPLC system and data processing were performed using Empower® version 2 Software (All Waters, Milford, MA, USA). pH measurements were carried out using Metrohm pH meter 744 (Metrohm Ltd. CH 9101, Herisau, Switzerland). Degradation experiments in acid, alkaline and neutral conditions were performed using a water bath (model DB28120-26, Thermolyne, Iowa, USA). Dry air oven (Postfach 102, GmbH Binder, Tuttlingen, Germany) was used to study the effect of dry heat.

2.3. Chromatographic conditions

The chromatographic separations were achieved on X-Bridge (Waters, Milford, MA, USA) C18 column (150 mm × 4.6 mm i.d.), 3.5 µm particle size, 135 Å pore diameter and 185 m^2/g surface area. Two mobile phases were used: the first for RZ–MR mixture consisted of acetonitrile: 0.025 M KH_2PO_4 solution: TEA (30:69:1, v/v) adjusted to pH 7.0 using orthophosphoric acid solution; the second for RZ–IO mixture consisted of acetonitrile: 0.025 M KH_2PO_4 solution: TEA (25:74:1, v/v) adjusted to pH 9.25. The mobile phases were filtered through 0.45 µm membrane filter and degassed ultrasonically before use. The elution was performed in an isocratic mode with a flow rate at 2 mL/min and column temperature maintained at 40 °C. The detector was set at wavelength range of 200–400 nm with sampling rate at 10 points/s and spectral resolution 1.2 nm. The HPLC chromatograms were recorded at 283 nm. The UV spectra (spectrograms) were collected at different time intervals across the peak elution time and were smoothed at level 5 and their derivative spectra at level 9. The purity parameters included 100% active peak region and auto-threshold with non-purity pass level. DAD library search was set at threshold degree 10.0 and level 3 in depth. A search threshold criterion of the noise due to instrument and solvent was at angle 1.0. The retention time pre-search was at ±5% (t_r window %). The wavelength pre-search (window %) was at ±1 nm.

2.4. Generation of stress samples for development of stability-indicating methods

Forced degradation of each of drug substances and their binary mixtures (i.e., RZ–MR or RZ–IO) was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions.

Thermal and photo-degradation of drug substances and drug mixtures was carried out in solid state. For thermal stress, samples of drug substances and drug mixture were placed in a temperature-controlled oven at 60 °C for 8 h. For photolytic stress, samples of drug substances and drug product, in solid state, were irradiated with UV radiation having peak intensities at 254 and 366 nm for 8 h.

For hydrolytic and oxidative degradation, solutions were prepared by dissolving drug substance or drug mixture in acetonitrile: water (1:1 as diluent) and later diluted with 0.01 M hydrochloric acid, 0.5 M sodium hydroxide and 0.3% hydrogen peroxide solution to achieve a concentration of 1000 µg/mL for each of RZ and MR or RZ and IO mixtures, respectively. During the initial forced degradation experiments, it was observed that acid hydrolysis was a fast reaction for RZ and almost complete degradation occurred within 1 h at ambient temperature when 0.1 M hydrochloric acid was used. Thus, in later experiment, acid hydrolysis of drug substance and drug mixtures in solution state was conducted using 0.01 M hydrochloric acid solutions at ambient temperature for 1 h, neutralized using 0.05 M sodium hydroxide and kept in a cool dark place immediately. Alkaline and neutral hydrolysis was conducted at 60 °C for 8 h in water. For oxidative stress, sample solutions of drug substance and drug mixtures in 0.3% hydrogen peroxide were kept in dark at ambient temperature for 24 h. The sample solutions for acid/alkaline hydrolysis and oxidation were diluted with mobile phase to get solutions containing 200 µg/mL for RZ and MR or RZ and IO mixtures, respectively. All of the above-diluted solutions were filtered through 0.45 µm PVDF membrane filter and then 10 µL aliquots were injected into HPLC system.

2.5. Optimization of the stability-indicating HPLC methods

Studies were carried out first on samples of different stress conditions for each drug individually and later on resolution of drug and degradation mixtures were studied in a mixture of solutions in which decomposition was observed. Finally, resolution of both intact drugs and their corresponding stress-formed degradation products mixture was achieved.

2.6. Preparation of standards solutions for RZ–MR and RZ–IO mixtures

Stock standard solutions were prepared by dissolving each of drugs in acetonitrile: water mixture as diluents to achieve concentration of 1000 µg/mL. Aliquots were diluted with mobile phase to achieve the concentrations 200 and 150 µg/mL for RZ and MR or 20 and 150 µg/mL for RZ and IO.

2.7. Analysis of pharmaceutical preparations

Twenty capsules for Veloz-M® or Zorite® capsules were weighed; the average weight of each capsule was calculated. An amount of powdered mass equivalent to 40 mg of RZ and 30 mg of MR (for Veloz-M®) and 4 mg of RZ and 30 mg of IO (for Zorite®) was weighed and transferred to 100 mL volumetric flasks. The drugs from powder were extracted and completed to volume with diluent. To ensure complete extraction of drugs it should be sonicated for 10 min. The extract was centrifuged at 3000 rpm and the supernatant solution was filtered through 0.45 µm PVDF membrane filter. Appropriate aliquots from sample stock solution were suitably diluted with mobile phase to obtain solutions containing 200 and 150 µg/mL for RZ and MR and 20 and 150 µg/mL for RZ and IO. A 10 µL was injected into HPLC concurrently with the appropriate standard mixture solution for each of binary mixtures.

2.8. Validation procedure

The proposed HPLC–DAD method was validated according to ICH guidelines [25] and USP analytical method validation parameters [27] concerning system suitability test, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

2.8.1. System suitability test

The system suitability test parameters of the optimized method were calculated using Empower® 2 Software.

2.8.2. Specificity

The specificity of the proposed methods towards the investigated compounds was illustrated through study of resolution and capacity factors of the intact drugs, i.e., RZ and MR or RZ and IO, by analyzing the peaks with respect to each other in binary mixture and to the nearest degradation peaks, respectively. In addition, the specificity was established throughout the study of the purity and matching plots using Empower®2 Software. The spectral homogeneity and purity of the peaks due to the eluted RZ, MR and IO in their binary mixtures were also checked using the methods of relative absorption spectra (RA) and log *A* versus the wavelength plots. The absorption spectra (spectrograms) collected at different time intervals across the elution of the peaks of RZ, MR and IO were used to construct their RA spectra and log *A* versus λ plots [28,29].

2.8.3. Linearity

The linearity of the detector response with the concentrations of the investigated drugs was evaluated. Stock standard solutions were prepared at strengths 2 mg/mL of RZ, 1.5 mg/mL of MR and 15 mg/mL of IO. Dilution with the mobile phase was carried out to obtain two series of standard mixture solutions containing concentrations ranging from 50 to 400 µg/mL, 25 to 300 µg/mL of RZ and MR, 5 to 40 µg/mL, 50 to 300 µg/mL of RZ and IO, respectively. The solutions were injected in triplicate into the HPLC system. Peak areas were plotted versus the corresponding concentration to obtain calibration graphs. Regression data analysis was performed using least squares linear regression statistical analysis.

2.8.4. Accuracy

The accuracy of the methods was evaluated by spiking a mixture of stress-degraded samples with RZ–MR and RZ–IO binary mixtures at three different concentrations. The percentage recoveries of RZ and MR, and RA and IO were calculated from the difference between the peak areas of fortified and unfortified solutions. Also recovery studies were carried out by applying the standard addition method to pharmaceutical preparations i.e., Veloz-M® and Zorite® capsules.

2.8.5. Precision

For determination of repeatability, ten samples solutions were prepared at 100% level of the analytical method concentration and kept in a cool dark place. The results were expressed as RSD% for the ten determinations. The intra-day precision studies were performed by analysis of three different concentrations at 80%, 100% and 120% of the analytical concentration of the drug in triplicate ($n=3$) on the same day. The inter-day precision

Table 1 System suitability results of the proposed HPLC methods for separation of RZ in two binary mixtures.

Solution	Composition	System suitability parameters						
		Capacity factor(K′)	Resolution (Rs)[a]	Selectivity factor (α)[a]	Tailing factor	Column efficiency	RSD%[b]	
							t_r	PA[c]
RZ–MR standard mixture	RZ	3.955	–	–	1.01	8955	0.24	0.54
	MR	11.22	21.3	2.838	0.98	11,567	0.27	0.66
RZ–IO standard mixture	RZ	2.577	–	–	1.09	7048	0.77	0.62
	IO	6.272	15.3	2.375	1.07	8576	0.33	0.58
Stress-degraded RZ–MR matrix plus RZ–MR standard	Peak I	3.399	–	1.145	1.30	8253	0.33	0.77
	RZ	3.977	3.28	1.173	1.01	11,398	0.27	0.83
	Peak II	4.889	4.54	1.227	0.96	12,146	0.44	1.22
	Peak III	7.994	–	1.320	0.97	13,410	0.55	1.44
	MR	11.25	8.75	1.407	0.95	12,443	0.31	0.65
	Peak IV	15.03	8.01	1.336	0.96	14,609	0.88	1.66
Stress-degraded RZ–IOmatrix plus RZ–IO standard	Peak I	1.844	–	1.312	1.24	5788	0.55	0.96
	RZ	2.209	2.66	1.198	1.03	6423	0.44	0.66
	Peak II	3.674	12.2	1.663	1.33	55,147	0.39	1.02
	Peak III	4.332	–	1.179	1.47	6502	1.08	1.11
	IO	5.617	4.50	1.297	1.04	7422	0.64	0.82
	Peak IV	7.549	4.50	1.344	1.17	3819	0.72	0.66
Acceptance criteria		$0.5>X<15$	>2	>1	<2.0	>2000	<2.0	<2.0

[a] α and Rs are the selectivity factor and the resolution, respectively, between two consecutive peaks in the elution order.
[b] RSD % for three determinations.
[c] Peak area.

studies were done by repeating the studies on three consecutive days.

2.8.6. LOD and LOQ

ICH guideline Q2 (R1) [25] describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal-to-noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and calculated according to the $3.3\sigma/s$ and $10\sigma/s$ criteria, respectively; where σ is the standard deviation of the intercept values and s is the slope of the corresponding calibration curve.

2.8.7. Robustness

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variation in method parameters such as slight change of % organic modifier and pH of the mobile phase. Thus these values were selected one below and one above the optimized values used in the chromatographic conditions. Robustness of the proposed method was evaluated in terms of system suitability parameters of drug peak in a mixture of stress-degraded samples towards the small-intended afore-mentioned variations.

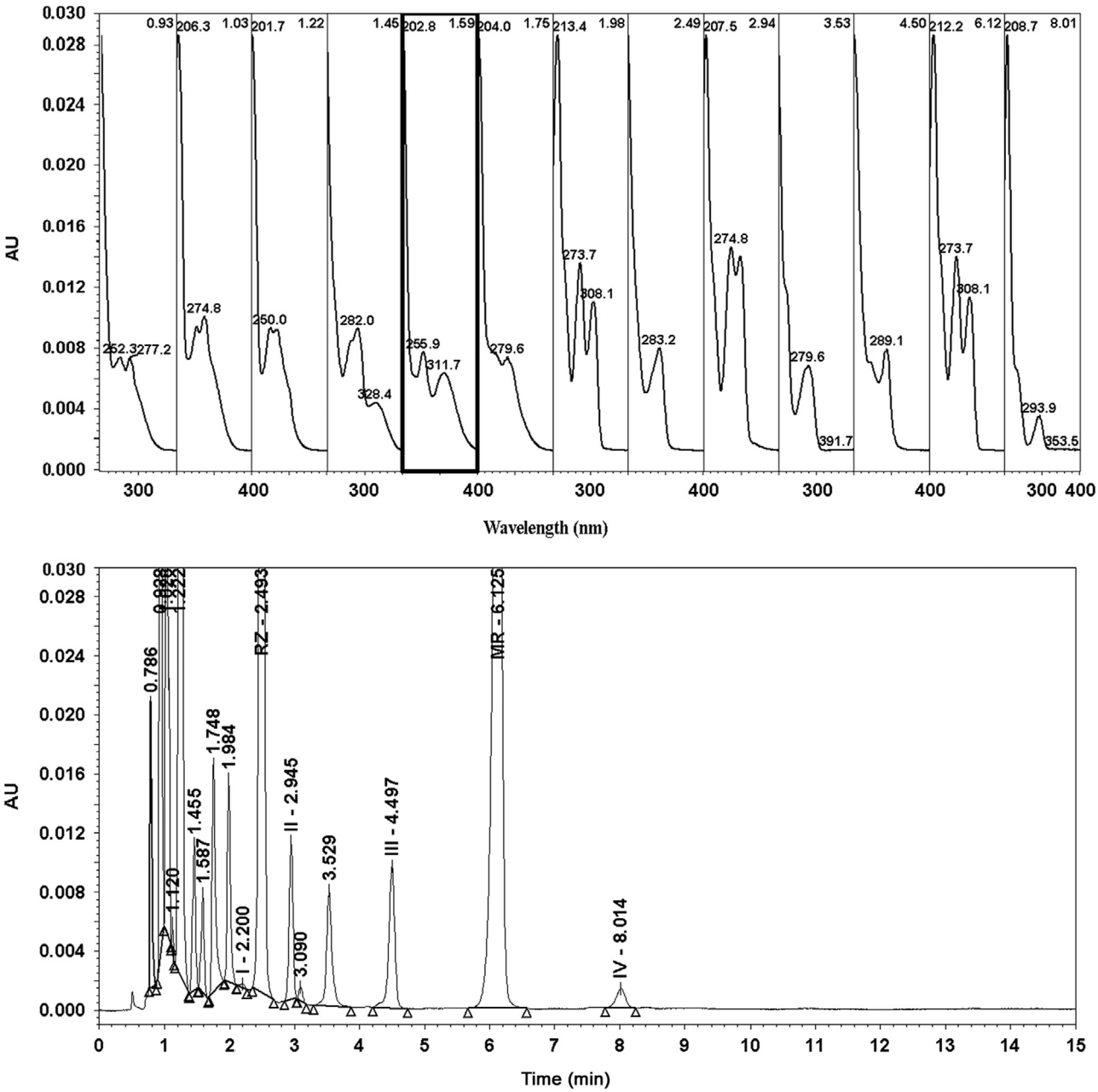

Fig. 2 Chromatogram of a mixture of stress degradation products of RZ–MR in their binary mixture and their corresponding spectrum index plots.

3. Results and discussion

3.1. Development and optimization of the HPLC methods

During the optimization of the separation method, three columns (Kromasil C18: 5 µm, 250 mm × 4.6 mm; Symmetry C18: 5 µm, 150 mm × 4.6 mm; and X-Bridge C18: 3.5 µm, 150 mm × 4.6 mm) and the mobile phase composed of acetonitrile and 0.025 M phosphate buffer solution adjusted to five different pH values (5–7.5) with and without TEA were tested. Of the stationary phases experienced, X-Bridge column was chosen and the most suitable separation factors were obtained as it is suitable for retention of basic compounds at high pH and in high percentage aqueous mobile phases. After trying several mobile phases containing acetonitrile with various buffer proportions, the mobile phase consisting of acetonitrile: 0.025 M KH_2PO_4 buffer solution: TEA (1%) was proved to be useful for better resolution and peak symmetry. To optimize this mobile phase, proportions of acetonitrile and 0.025 M KH_2PO_4 buffer were systematically changed from 50:50 whilst percentage of TEA (1% pH 6.0) remained unchanged. Higher acetonitrile ratio resulted in shorter retention times of all

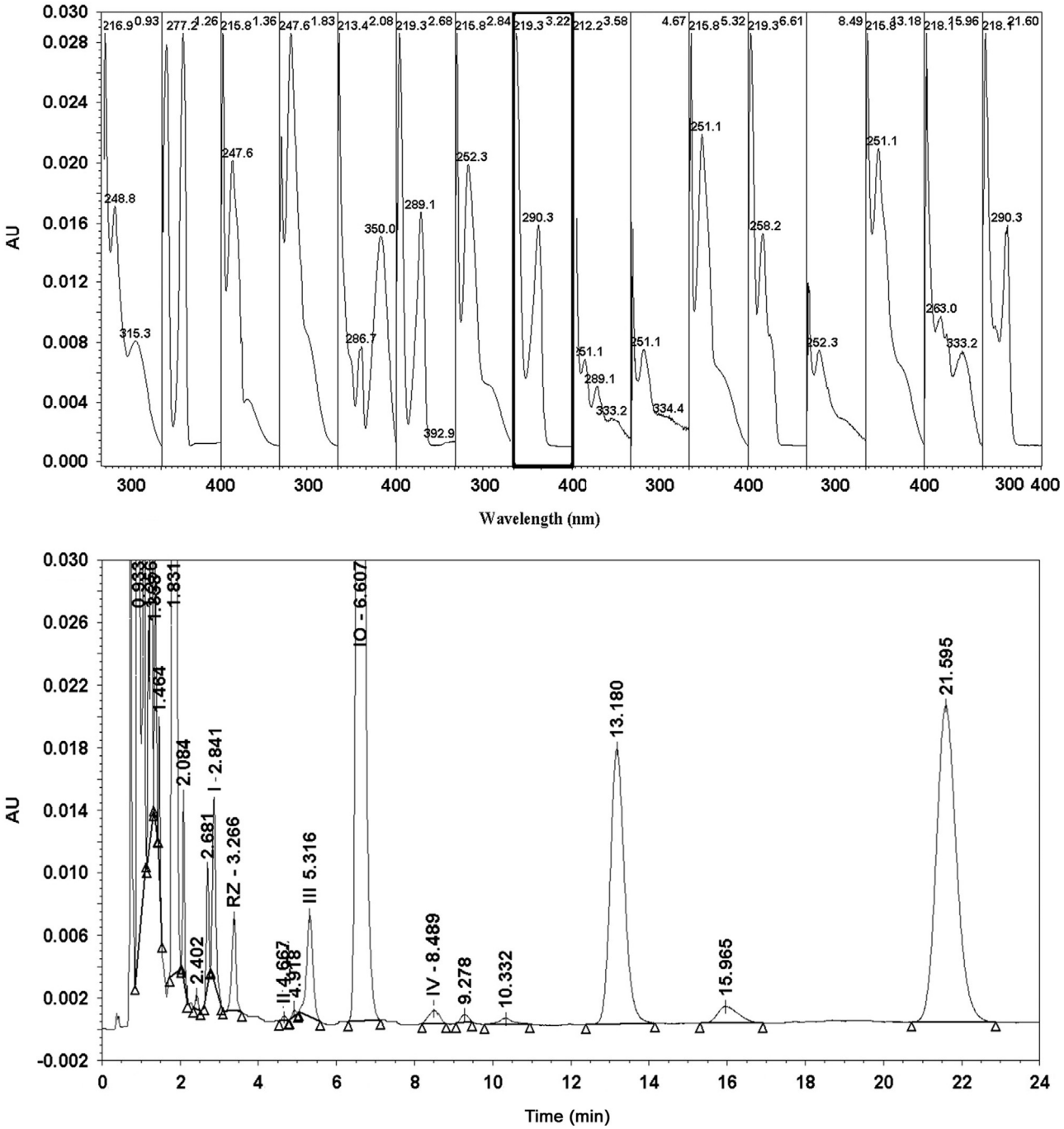

Fig. 3 Chromatogram of a mixture of stress degradation products of RZ–IO in their binary mixture and their corresponding spectrum index plots.

analytes whereas all three analytes tended to elute later with increasing ratio of buffer solution. For further optimization acetonitrile, 0.025 M KH_2PO_4 buffer solution and TEA were mixed at a ratio of 30:69:1 (v/v/v) adjusted to different pH values varied in the range of 5.0–7.5. As a result of pH screening, the optimum mobile phase was chosen as acetonitrile: 0.025 M KH_2PO_4 buffer solution: TEA (at ratio 30:69:1) adjusted to pH 7.0. The flow rate used was set to 2.0 mL/min at column temperature 40 °C. Best chromatographic results were obtained in terms of peak symmetry, resolution, selectivity and analysis time for separation of RZ and MR in the presence of their corresponding generated stress degradation products. Further modifications required for separation of RZ–IO mixture to elute IO after RZ to improve the resolution between IO and degradation products that originated from its acid degradation and to avoid the co-elution of IO with cluster of peaks due to RZ stress-generated degradation products. The mobile phase composed of acetonitrile: 0.025 M KH_2PO_4 buffer solution: TEA at a ratio of 25:74:1 adjusted to pH 9.25 was chosen to obtain best results in term of resolution and peak symmetry for separation of RZ and IO in the presence of their corresponding stress-generated degradation products. The chromatograms were recorded at 283 nm. Separation parameters are summarized in Table 1. Figs. 2 and 3 show the chromatograms and spectrum index plots of RZ–MR and RZ–IO in a mixture of their ICH-generated degradations from all their corresponding stress conditions, respectively.

3.2. Stability studies

The results in Table 2 indicated that RZ underwent extensive degradation in acid, H_2O_2 and photolysis. Moderate degradation occurred in thermal and neutral conditions. RZ was relatively stable in alkaline medium at room temperature and showed moderate degradation upon refluxed with 0.5 M NaOH at 60 °C. Meanwhile MR underwent moderate degradation in all stress conditions. Like MR, IO showed similar degradation pattern but to more extent in acid/base hydrolysis. Fig. 4 shows the chromatograms of forced degraded samples for both RZ–MR and RZ–IO mixtures in different stress conditions.

3.3. Validation of the developed stability-indicating methods

3.3.1. System suitability test

The capacity factors (K') of the investigated drugs were $3 < K' < 12$ and the resolution between their peaks and the closest peak was higher than 2.5. The plate count was more than 5000 and their symmetry factors were in between 0.94 and 1.03. The results are summarized in Table 1.

3.3.2. Stability/specificity

Specificity can be described as the capability of the method to accurately measure the response of the two analyzed compounds

Table 2 Summary of forced degradation studies for two binary mixtures (RZ–MR and RZ–IO).

Composition	Stress condition	Recovery of intact drug (%)
RZ–MR binary mixture I		
RZ	Control (no degradation)	99.60
	Acid hydrolysis (0.5 M HCl at 60 °C)	2.66
	Acid hydrolysis (0.1 M HCl at room temperature for 1 h)	21.78
	Acid hydrolysis 0.01 M HCl at room temperature for 1 h)	52.99
	Basic hydrolysis (0.5 M NaOH at 60 °C for 8 h)	89.56
	Oxidation (0.3% H_2O_2 in dark for 24 h)	72.98
	Thermal decomposition (at 80 °C for 8 h)	88.56
	Photodecomposition under UV for 8 h	76.99
MR	Control (no degradation)	99.71
	Acid hydrolysis (0.5 M HCl at 80 °C for 8 h)	84.99
	Basic hydrolysis (0.5 M NaOH at 80 °C for 8 h)	96.98
	Oxidation(0.3% H_2O_2 in dark for 24 h)	67.67
	Thermal decomposition (at 80 °C for 8 h)	97.70
	Photodecomposition under UV for 8 h	68.99
RZ–IO binary mixture II		
RZ	Control (no degradation)	99.17
	Acid hydrolysis (0.5 M HCl at 60 °C)	2.78
	Acid hydrolysis (0.1 M HCl at room temperature for 1 h)	22.54
	Acid hydrolysis 0.01 M HCl at room temperature for 1 h)	44.77
	Basic hydrolysis (0.5 M NaOH at 60 °C for 8 h)	86.81
	Oxidation (0.3% H_2O_2)	70.11
	Thermal decomposition (at 80 °C for 8 h)	89.88
	Photodecomposition under direct daylight	84.99
IO	Control (no degradation)	99.44
	Acid hydrolysis (0.5 M HCl at 80 °C for 8 h)	72.77
	Basic hydrolysis (0.5 M NaOH at 80 °C for 8 h)	90.99
	Oxidation(0.3% H_2O_2 in dark for 24 h)	85.99
	Thermal decomposition (at 80 °C for 8 h)	96.66
	Photodecomposition under direct UV for 8 h	90.77

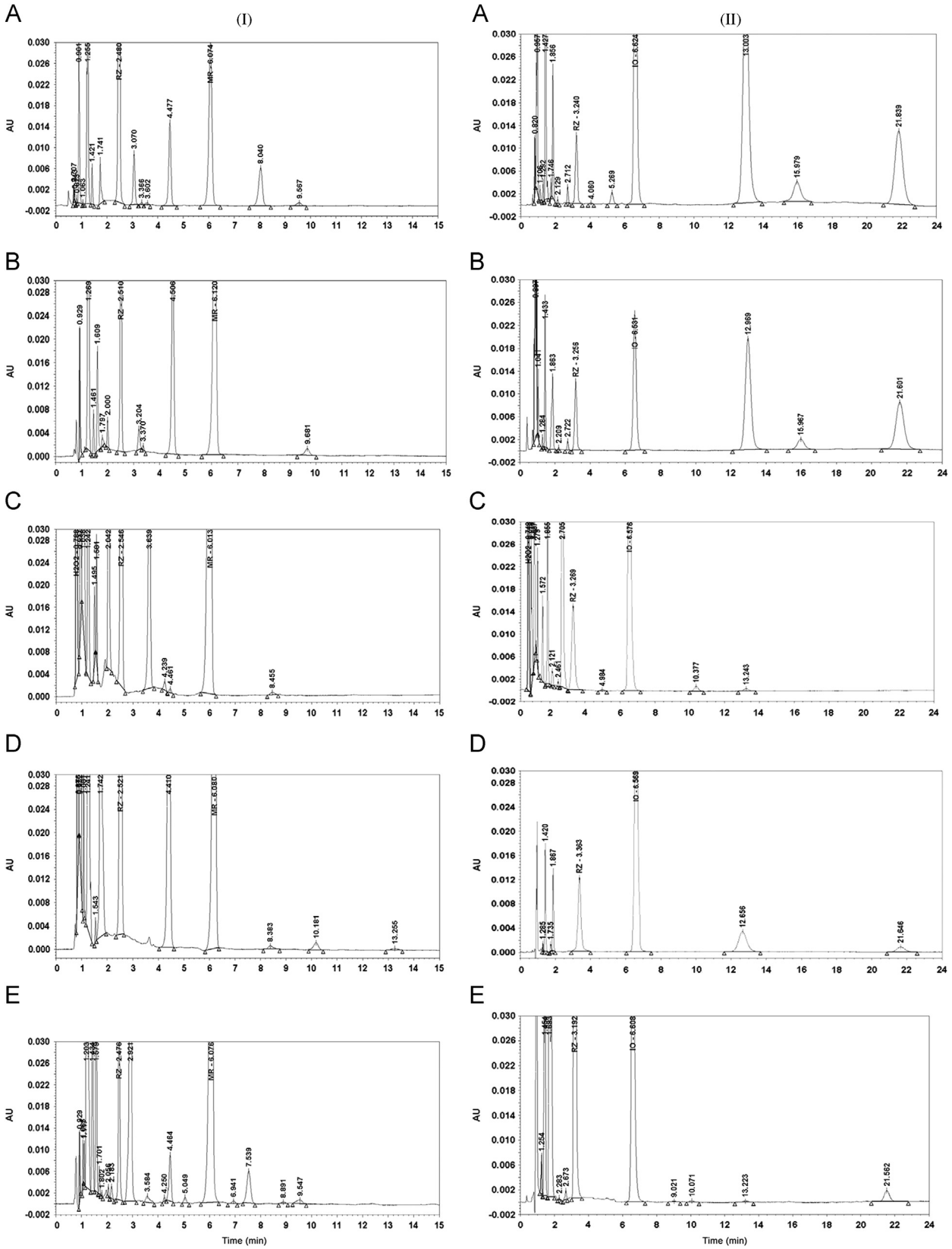

Fig. 4 Chromatograms of mixture of acid (A), alkaline (B), oxidation (C), neutral (D) and photo induced (E) degradation of RZ and MR in their mixture (I) and RZ and IO in their mixture (II).

RZ–MR and RZ–IO in their binary mixture with no interferences originating from sample matrix. High percentage recovery observed with assay samples of pharmaceutical dosage forms, including standard addition experiments, indicates that the proposed methods were not affected by interferences from mixture of stress-degraded samples and excipients used in formulations. The resolution factor for the drug peaks in the mixture of degradation solutions was >3 from the nearest resolving degradation peaks as in Table 1. DAD also supported the specificity of the method and provided evidence for the homogeneity of the peaks of analytes as the purity angles were always found much less than the threshold limit in all stressed samples. In addition, the observation that the wavelengths of derivative optima (first, second, third and fourth) of the spectrograms of the separated peaks obtained by chromatography of the test solutions were identical to those of corresponding standard was considered as evidence confirming the identity of the investigated compounds [28,29]. The derivative spectra were super-imposable whenever overlaid, showing that there were no other co-eluting peaks. Furthermore, the spectral homogeneity and purity of the peaks were confirmed using RA spectra and log A versus the wavelength plots [28,29] constructed from the data collected from the spectrograms of each peak. The superimpose of the relative absorption spectra and the traces of log A versus the wavelengths plots with those of corresponding standard for each peak proved the absence of interference as shown in Fig. 5.

3.3.3. *Linearity*

A linear simple regression by the least squares method was applied. The correlation coefficiencies (r) were found to be greater than 0.999 in all instances. Table 3 shows calibration characteristics for RZ–MR and RZ–IO binary mixtures.

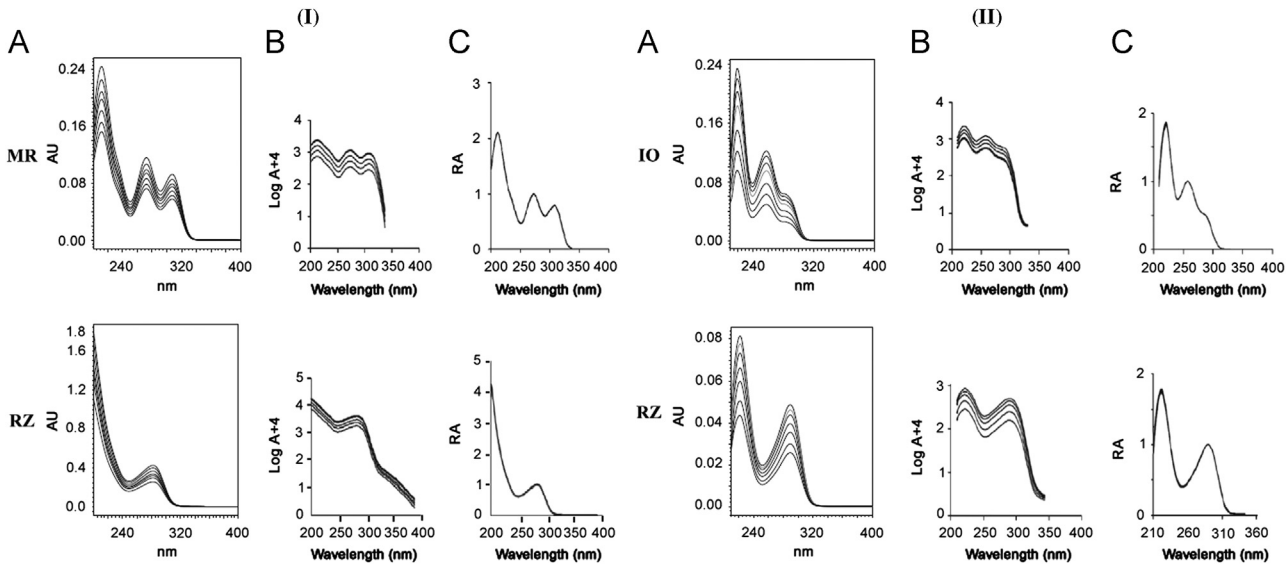

Fig. 5 The spectrograms (A), their log A (B) and their relative absorption (RA) (C) spectra versus the wavelength plots of RZ–MR peaks in their ICH-stress formed degradation (I) and RZ–IO peaks in their ICH-stress formed degradation (II).

Table 3 Analytical data and regression characteristics of rabeprazole sodium, mosapride citrate and itopride hydrochloride.

Parameters	RZ[a]	MR[a]	RZ[b]	IO[b]
Calibration range (μg/mL)	50–400	50–300	5–40	50–300
Detection limit (μg/mL)	0.415	0.442	0.357	0.204
Quantitation limit (μg/mL)	1.257	1.339	1.071	0.612
Regression equation (Y)[c]				
Slope (b)	12,065.8	7950.2	13397.6	5503.4
SD of the slope (S_b)	77.94	64.09	74.38	25.58
RSD of the slope (%)	0.646	0.806	0.555	0.465
Confidence limit of the slope[d]	11849.4–12282.2	7772.2–8127.1	13191.1–13604.1	4852.1–6154.7
Intercept (a)	5613.13	16331.85	1654.47	25762.98
SD of the intercept (S_a)	1515.74	1063.84	1449.37	340.18
Confidence limit of the intercept	−3035.5–4553.2	−32662.1–17127.5	−3308–1736.7	24474.8–27051.2
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9996

[a]Using the HPLC method for RZ–MR mixture.
[b]Using the HPLC method for RZ–IO mixture.
[c]$Y=a+bC$; where C is the concentration in μg/mL and Y is the peak area.
[d]95% confidence limit.

Table 4 Recovery data for rabeprazole, mosapride and itopride in a mixture of their ICH-stress formed degradation products.

Matrix	% Targeting concentration	Added (μg/mL)			Mean[b] % recovery (RSD%)		
		RZ	MR	IO	RZ	MR	IO
Mixture of stress-degraded samples for RZ–MR mixture	80	160[a]	120	(–)[c]	100.52 (1.28)	101.34 (0.82)	(–)
	100	200	150	(–)	101.39 (0.85)	100.77 (0.98)	(–)
	120	240	180	(–)	102.21 (0.89)	99.88 (1.06)	(–)
Mixture of stress-degraded samples for RZ–IO mixture	80	16	(–)	120	99.87 (0.94)	(–)	99.08 (1.14)
	100	20	(–)	150	100.90 (1.11)	(–)	102.95 (1.53)
	120	24	(–)	180	99.66 (0.92)	(–)	99.58 (1.15)

[a]% of targeting concentration of intact drug presented as % of the method concentration.
[b]Mean of three determinations.
[c](–) means it is not a component of formulation.

Table 5 Determination of intact drugs in their pharmaceutical preperations.

Pharmaceutical preparation	Batch identity symbol[a]	Mean % found (RSD%)[b]		
		RZ	MR	IO
RZ–MR capsule	BT I	98.28 (0.88)	99.51 (0.78)	(–)[c]
	BT II	99.55 (1.62)	100.10 (1.29)	(–)
	BT III	97.81 (0.96)	102.29 (1.59)	(–)
RZ–IO capsule	BT I	98.21 (0.32)	(–)	99.51 (1.08)
	BT II	99.04 (0.48)	(–)	99.94 (0.99)
	BT III	99.89 (0.69)	(–)	101.88 (1.05)

[a]BT I, II, III refers to the three batches tested.
[b]Mean and RSD % for three determinations.
[c](–) means it is not a component of formulation.

3.3.4. *Accuracy*

The mean percentage recoveries obtained after six repeated experiments were found between 99.50% and 103.0%. The RSD values were less than1.65% (Table 4) indicating that the results are accurate and precise and there is no interference from the common excipients used in the pharmaceutical dosage forms.

3.3.5. *Precision*

The developed methods were found to be precise as the RSD values for repeatability and intermediate precision studies were less than 2.0%.

3.3.6. *LOD and LOQ*

The LOD and LOQ values of the developed method are presented in Table 3.

3.3.7. *Robustness*

The results of robustness studies proved that slight but deliberate changes of the optimized chromatographic parameters would affect neither the retention of the compounds, as indicated by their capacity factors (k'), nor the resolution between any two consecutive peaks indicating that the proposed methods are robust.

3.4. *Application to pharmaceutical preparations*

The proposed validated stability-indicating HPLC methods were applied to the determination of RZ in two binary mixtures, Veloz-M® (RZ and MR) and Zorite® (RZ and IO) capsules. Table 5 shows the mean percentage drugs found and the RSD% values indicating that the proposed validated stability-indicating HPLC methods could be adopted for the selective determination of the investigated drugs in their pharmaceutical preparations without interference from either their corresponding degradation products formed under ICH-recommended stress conditions and co-

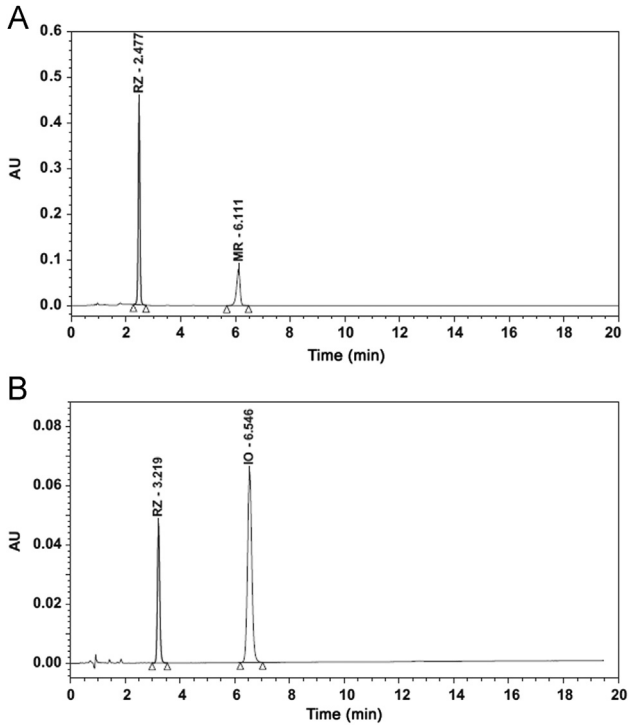

Fig. 6 (A) Representative chromatogram of test RZ–MR capsules solution labeled to contain 200 μg/mL of RZ and 150 μg/mL of MR, (B) Representative chromatogram of test RZ–IO capsules solution labeled to contain 20 μg/mL of RZ and 150 μg/mL of IO.

formulated adjuvants. Representative chromatograms are illustrated in Fig. 6.

4. Conclusion

Based on the peak purity results, obtained from the analysis of forced degraded samples using described methods, it can be concluded that there is no other co-eluting peak with the main peaks and the methods are specific for the estimation of RZ in two binary mixtures containing MR and IO in the presence of their corresponding stress-generated degradation products. The methods have linear response in stated range and are accurate and precise. Though no attempt was made to identify the degradation products, described methods can be used as stability-indicating methods for assay of RZ in its two combination drug products. It can be concluded that the proposed methods have a great promise as rapid analytical tools for the simultaneous estimation of RZ–MR and RZ–IO in their combined pharmaceutical formulations, especially for quality control laboratories.

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