**ORIGINAL ARTICLE**

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

Hamed M. El-Fatatry\(^a\), Mokhtar M. Mabrouk\(^a\), Ismail I. Hewala\(^b\), Ehab H. Emam\(^c,\ast\)

\(^a\)Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Tanta, Tanta, Egypt

\(^b\)Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Alexandria, Egypt

\(^c\)Quality Sector, Alexandria Company for Pharmaceuticals, Alexandria 21521, Egypt

Received 7 May 2013; accepted 17 September 2013
Available online 23 September 2013

**KEYWORDS**

Rabeprazole sodium; Mosapride citrate; Itopride hydrochloride; Stability-indicating HPLC–DAD; 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\(_2\)PO\(_4\) 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.

\(\text{© 2013 Xi'an Jiaotong University. Production and hosting by Elsevier B.V.}
\)

**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.
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 spectro-photometric 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-HT4 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 certified 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-MCapsules labeled to contain 20 mg RZ and 15 mg MR per capsule were manufactured by Torrent Pharmaceuticals Ltd. ZoriteCapsules 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.](image-url)
2.2. Instrumentation

The HPLC system consisted of Waters Alliance solvent management system 2695, a photodiode array detector (DAD) 2998, thermostatically controlled column compartment 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, Tuttingen, 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 m²/g surface area. Two mobile phases were used: the first for RZ–MR mixture consisted of acetonitrile: 0.025 M KH₂PO₄ 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 KH₂PO₄ 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 performed using a purity parameters [27] concerning system suitability test, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

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 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.

2.7. 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 |
| RZ–MR standard mixture       | RZ          | 3.955             | –               | –               | 1.01          | 8955              | 0.24  |
|                              | MR          | 11.22             | 21.3            | 2.838           | 0.98          | 11,567            | 0.27  |
|                              | RZ          | 2.577             | –               | –               | 1.09          | 7048              | 0.77  |
|                              | IO          | 6.272             | 15.3            | 2.375           | 1.07          | 8576              | 0.33  |
| Stress-degraded RZ–MR matrix plus RZ–MR standard | Peak I  | 3.399             | –               | 1.145           | 1.30          | 8253              | 0.33  |
|                              | RZ          | 3.977             | 3.28            | 1.173           | 1.01          | 11,398            | 0.27  |
|                              | Peak II     | 4.889             | 4.54            | 1.227           | 0.96          | 12,146            | 0.44  |
|                              | Peak III    | 7.994             | –               | 1.320           | 0.97          | 13,410            | 0.55  |
|                              | MR          | 11.25             | 8.75            | 1.407           | 0.95          | 12,443            | 0.31  |
|                              | Peak IV     | 15.03             | 8.01            | 1.336           | 0.96          | 14,609            | 0.88  |
| Stress-degraded RZ–IO matrix plus RZ–IO standard | Peak I  | 1.844             | –               | 1.312           | 1.24          | 5788              | 0.55  |
|                              | RZ          | 2.209             | 2.66            | 1.198           | 1.03          | 6423              | 0.44  |
|                              | Peak II     | 3.674             | 12.2            | 1.663           | 1.33          | 55,147            | 0.39  |
|                              | Peak III    | 4.332             | –               | 1.179           | 1.47          | 6502              | 1.08  |
|                              | IO          | 5.617             | 4.50            | 1.297           | 1.04          | 7422              | 0.64  |
|                              | Peak IV     | 7.549             | 4.50            | 1.344           | 1.17          | 3819              | 0.72  |

Acceptance criteria: 0.5 > X < 15, > 2, > 1, < 2.0, > 2000, < 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 \(\sigma\) 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 KH₂PO₄ 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 KH₂PO₄ 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$_2$PO$_4$ 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$_2$PO$_4$ 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. For further optimization acetonitrile, 0.025 M KH$_2$PO$_4$ 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$_2$PO$_4$ 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$_2$PO$_4$ 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$_2$O$_2$ 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

| Composition | Stress condition | Recovery of intact drug (%) |
|-------------|------------------|-----------------------------|
| RZ–MR binary mixture I | Control (no degradation) | 99.60 |
| RZ | 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$_2$O$_2$ 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 |
| | 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$_2$O$_2$ 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 | Control (no degradation) | 99.17 |
| RZ | Acid hydrolysis (0.5 M HCl at 60°C) | 2.78 |
| | Acid hydrolysis (0.1 M HCl at room temperature for 1 h) | 22.44 |
| | 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$_2$O$_2$) | 70.11 |
| | Thermal decomposition (at 80°C for 8 h) | 89.88 |
| | Photodecomposition under direct daylight | 84.99 |
| | Control (no degradation) | 99.44 |
| IO | 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$_2$O$_2$ 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 coefficients (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](image)

**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).

| Parameters | RZ^a | MR^b | 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) | 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 | 1654.7 | 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) | 0.999 | 0.999 | 0.999 | 0.999 |

^aUsing the HPLC method for RZ–MR mixture.
^bUsing the HPLC method for RZ–IO mixture.
^cY=a+bC; where C is the concentration in µg/mL and Y is the peak area.
^d95% confidence limit.
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 than 1.65% (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$^R$ (RZ and MR) and Zorite$^R$ (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-

### 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 % recovery (RSD% ) |
|---------------------------------------------|---------------------------|---------------|-------------------------|
| Mixture of stress-degraded samples for RZ–MR mixture |                          |               |                         |
| 80                                          | 160a                      | 120 (–)       | 100.52 (1.28)           |
| 100                                         | 200                      | 150 (–)       | 101.39 (0.85)           |
| 120                                         | 240                      | 180 (–)       | 102.21 (0.89)           |
| Mixture of stress-degraded samples for RZ–IO mixture |                          |               |                         |
| 80                                          | 16                       | 120 (–)       | 99.87 (0.94)            |
| 100                                         | 20                       | 150 (–)       | 100.90 (1.11)           |
| 120                                         | 24                       | 180 (–)       | 99.66 (0.92)            |

*a% of targeting concentration of intact drug presented as % of the method concentration.
bMean 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 capsule              |                           |                         |
| BT I                       | 98.28 (0.88)              | 99.51 (0.78)            |
| 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.
concluded that the proposed methods have a great promise as rapid assay of RZ in its two combination drug products. It can be described methods can be used as stability-indicating methods for though no attempt was made to identify the degradation products, have linear response in stated range and are accurate and precise. Corresponding stress-generated degradation products. The methods 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.

References

[1] S.C. Sweetman, Martindale the Complete Drug Reference, 35th ed., Pharmaceutical Press, London, 2007, pp. 1566, 1577, 1590.
[2] C.I. Carswel, K.L. Goa, Rabeprazole: an update of its use in acid-related disorders, Drugs 61 (2001) 2327–2356.
[3] B.R. Prasanna, M.S. Reddy, Development and validation of RP-HPLC for the determination of rabeprazole sodium in pharmaceutical formulations and human plasma, Asian J. Res. Chem. 2 (1) (2009) 49–51.
[4] C.V. Garcia, C.S. Paim, M. Steppe, et al., Development and validation of a dissolution test for rabeprazole sodium in coated tablets, J. Pharm. Biomed. Anal. 41 (3) (2006) 833–837.
[5] C.V. Garcia, C.S. Paim, M. Steppe, New liquid chromatographic method for determination of rabeprazole sodium in coated tablets, J. AOAC Int. 87 (4) (2004) 842–846.
[6] A. El-Gindy, F. El-Yazby, M.M. Maher, Spectrophotometric and chromatographic determination of rabeprazole in presence of its degradation products, J. Pharm. Biomed. Anal. 31 (2) (2003) 229–242.
[7] R. Shan, P. Mi-Jin, S. Hongkee, et al., Effect of pharmaceutical excipients on aqueous stability of rabeprazole sodium, Int. J. Pharm. 350 (1-2) (2008) 197–204.
[8] C.V. Garcia, N.S. Nudelman, M. Steppe, et al., Structural elucidation of rabeprazole sodium photo-degradation products, J. Pharm. Biomed. Anal. 46 (1) (2008) 88–93.
[9] A.O. Osman, Spectrofluorometry, thin layer chromatography and column high-performance liquid chromatography determination of rabeprazole sodium in the presence of its acidic and oxidized degradation products, J. AOAC Int. 92 (5) (2009) 1373–1381.
[10] S. Elumalai, K. Aher, G. Bhavar, et al., Development and validation of RP-HPLC method for determination of content uniformity of rabeprazole sodium in its tablets dosage form, J. Appl. Pharm. Sci. 6 (1) (2011) 165–170.
[11] R.D. Vasti, G.S.U. Kiran, B.V. Subbaiah, et al., Identification of degradation products in stressed tablets of Rabeprazole sodium by HPLC-hyphenated techniques, Magn. Reson. Chem. 47 (5) (2009) 443–448.
[12] B. Reguri, M. Khagga, S. Polisietty, et al., A validated stability-indicating LC method for rabeprazole sodium, Chromatographia 68 (3-4) (2008) 275–280.
[13] A.A. Shirkhedkar, S.J. Surana, Application of stability-indicating RP-TLC densitometric determination of rabeprazole sodium in bulk and pharmaceutical formulation, Eurasian J. Anal. Chem. 4 (1) (2009) 165–170.
[14] S. Sabnis., S.N.D. Dnvandev, Y.V. JadHAV, et al., Column reversed-phase high performance liquid chromatographic method for simultaneous determination of rabeprazole sodium and domperidone in combined tablet dosage form, J. AOAC Int. 91 (2) (2008) 344–348.
[15] V. Gunasekaran, S.D. Maheshwari, C. Roosevelt, et al., Validated simultaneous estimation of rabeprazole sodium and itopride hydrochloride in pure and pharmaceutical capsule formulation, Indian J. Anal. Chem. 5 (1-6) (2007) 105–117.
[16] S. Rajesh, P.M. Ganesh, C.C. Subhash, Development and validation of RP-HPLC method for the simultaneous determination of rabeprazole sodium and itopride hydrochloride in solid dosage form, E-I. Chem. 7 (3) (2010) 947–952.
[17] S. Fillaii, I. Singhvi, Quantitative estimation of itopride hydrochloride and rabeprazole sodium from capsule formulation, Indian J. Pharm. Sci. 70 (5) (2008) 658–661.
[18] D. Umanaheswari, M. Kumar, B. Jayakar, et al., Method development and validation of itopride hydrochloride and rabeprazole sodium in pharmaceutical dosage form by reversed phase high performance liquid chromatography, J. Chem. Pharm. Res. 2 (5) (2010) 399–417.
[19] B.H. Patel, B.N. Suhagia, M.M. Patel, et al., Determination of pantoprazole, rabeprazole, esomeprazole, domperidone and itopride in pharmaceutical products by reversed phase liquid chromatography using single mobile phase, Chromatographia 65 (11-12) (2007) 743–748.
[20] A. Suganthi, J. Sofiya, T.K. Ravi, Simultaneous HPTLC determination of rabeprazole and itopride hydrochloride from their combined dosage form, Indian J. Pharm. Sci. 70 (3) (2008) 366–368.
[21] S.S. Shweta, D. Nilesh, J.Y. Vijay, et al., Spectrophotometric simultaneous determination of Rabeprazole Sodium and Itopride Hydrochloride in capsule dosage form, Spectrochem. Acta 69 (3) (2008) 849–852.
[22] P. Pattanayak, R. Sharma, S.C. Chaturvedil, Simultaneous spectrophotometric estimation of rabeprazole sodium and itopride HCl, Anal. Lett. 40 (12) (2007) 2288–2294.
[23] B.H. Patel, B.N. Suhagia, M.M. Patel, et al., High-performance liquid chromatography and thin-layer chromatography for the simultaneous quantitation of rabeprazole and mosapride in pharmaceutical products, J. Chromatogr. Sci. 46 (1) (2008) 4–10.

[24] ICH, Stability Testing of New Drug Substances and Products, Q1A (R2) Geneva, 2003.

[25] ICH, Validation of Analytical Procedures, Text and Methodology, Q2 (R1), Geneva, 2005.

[26] M. Bakshi, S. Singh, Development of stability indicating assay methods, J. Pharm. Biomed. Anal. 28 (6) (2002) 1011–1040 (review).

[27] The United States Pharmacopoeia 35 and NF 35, American Pharmaceutical Association, Washington, DC, 2012.

[28] I. Hewala, H. El-Fatatre, E. Emam, et al., Development and application of a validated stability-indicating HPLC method for simultaneous determination of granisetron hydrochloride, benzyl alcohol and their main degradation products in parenteral dosage forms, Talanta 82 (1) (2010) 184–195.

[29] I. Hewala, H. El-Fatatre, E. Emam, et al., Development and application of a validated stability-indicating high-performance liquid chromatographic method using photodiode array detection for simultaneous determination of granisetron, methylparaben, propylparaben, sodium benzoate, and their main degradation products in oral pharmaceutical preparations, J. AOAC Int. 94 (5) (2011) 1447–1460.