High-Performance Liquid Chromatography and Thin-Layer Chromatography for the Simultaneous Quantitation of Rabeprazole and Mosapride in Pharmaceutical Products

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Abstract

Simple, sensitive high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) methods are developed for the quantitative estimation of rabeprazole and mosapride in their combined pharmaceutical dosage forms. In HPLC, rabeprazole and mosapride are chromatographed using 0.01M 6.5 pH ammonium acetate buffer–methanol–acetonitrile (40:20:40, v/v, pH 5.70 ± 0.02) as the mobile phase at a flow rate of 1.0 mL/min. In TLC, the mobile phase is ethyl acetate–methanol–benzene (2:0.5:2.5, v/v). Both the drugs are scanned at 276 nm. The retention times of rabeprazole and mosapride are found to be 4.93 ± 0.01 and 9.79 ± 0.02, respectively. The R_f values of rabeprazole and mosapride are found to be 0.42 ± 0.02 and 0.61 ± 0.02, respectively. The linearities of rabeprazole and mosapride are in the range of 400–2000 ng/mL and 300–1500 ng/mL, respectively, for HPLC; in TLC, the linearities of rabeprazole and mosapride are in the range of 400–1200 ng/spot and 300–900 ng/spot, respectively. The limit of detection is found to be 97.7 ng/mL for rabeprazole and 97.6 ng/mL for mosapride in HPLC; in TLC the limit of detection is found to be 132.29 ng/spot for rabeprazole and 98.25 ng/spot for mosapride. The proposed methods can be applied to the determination of rabeprazole and mosapride in combined pharmaceutical products.

Introduction

Rabeprazole (RA), 2-[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methylsulfinyl]-1H-benzimidazole, is a proton-pump inhibitor that suppress gastric acid secretion by specific inhibition of the gastric H^+, K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell.

Mosapride (MO), 4-amino-5-chloro-2-ethoxy-N-[[4-[(4-fluorophenyl) methyl]-2-morpholinyl] methyl]-benzamide, is used in reflux esophagitis and to enhance gastric motility (1–2).

Several techniques, for example spectrophotometric, liquid chromatography–tandem mass spectrometry (LC–MS–MS), high-performance thin-layer chromatography, and high-performance liquid chromatography (HPLC) have been reported in the literature for the quantitation of RA in pharmaceuticals and in biological samples (3–11).

From a literature search, the methods reported so far for the quantitation of MO in pharmaceuticals and biological samples are spectrophotometric, HPLC, and LC–MS–MS (12–16).

To our knowledge, there are no methods reported for the simultaneous quantitation of RA and MO in pharmaceutical products by HPLC and TLC methods, and the pharmacopoeias do not describe a suitable method for the concurrent determination of RA and MO in pharmaceutical formulations.

The paper describes the development of simple, sensitive, rapid, precise, reproducible, and accurate HPLC and TLC methods for the simultaneous determination of RA and MO in capsules as an alternative method.

Experimental

Reagents

RA and MO working standards were procured as gift samples from Torrent Pharmaceutical Ltd. (Ahmedabad, India). Acetonitrile, ammonium acetate, methanol (HPLC grade), ethyl acetate, benzene, and methanol (AR grade) were used for mobile phase preparation and as solvents. Silica gel 60 F_{254} TLC plates (20 × 20 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as the stationary phase in the TLC method. The commercially available combined capsules of RA (20 mg) and MO (15 mg) were procured from a local market.

Apparatus
HPLC method
A Shimadzu HPLC instrument (LC-10AT vp) equipped with

UV detector, manual injector of 20 µL loop, and Phenomenex C_{18} column (250 mm × 4.6-mm i.d., 5-µm particle size) was used, as well as a weighing balance (Sartorious CP 225 D, Mumbai, India) and a Sonicator (Frontline FS-4, Mumbai, India).

TLC method

A Camag TLC with Linomat V auto sprayer, a Camag Scanner-III, a Camag flat-bottom and twin-trough developing chamber (20 × 20 cm) and UV cabinet with dual wavelength UV lamp, Camag win-CATS software, a Hamilton syringe (100 µL), a Sartorius weighing balance, and a Sonicator (Frontline FS-4, Mumbai, India) were used during the study.

Chromatographic conditions
HPLC method

Chromatography was performed on a 250 mm × 4.6 mm i.d., 5 µm particle size, Phenomenex C_{18} (2) reversed-phase column. The mobile phase was 0.01M 6.5 pH ammonium acetate buffer–methanol–acetonitrile (30:40:30, v/v) adjusted to pH 5.70 ± 0.02 with acetic acid–ammonia. The flow rate was 1 mL/min. The mobile phase was filtered through a nylon 0.45 µm–47 mm membrane filter and degassed before use. The detection wavelength was 276 nm and injection volume was 20 µL.

TLC method

Chromatographic estimations were performed under the following conditions: stationary phase, precoated silica gel 60 F_{256} aluminium sheets (20 × 10 cm, prewashed with methanol and dried in air); chamber saturation time 45 min; temperature 25 ± 2°C; wavelength of detection 276 nm, and slit dimensions 4 × 0.1 mm.

The following spotting parameters were used: bandwidth 4 mm; space between two bands 4 mm; and spraying rate, 10 µL.

Preparation of combined standard solution of RA and MO
HPLC method

RA (20 mg) and MO (15 mg) were weighed accurately and transferred into a 50-mL volumetric flask. Methanol (25 mL) was added and sonicated for 20 min, and then diluted up to the mark with methanol. The aliquot (0.25 mL) was further diluted to 50 mL with the same solvent. The final solution contained 2000 ng of RA and 1500 ng of MO per mL of the solution.

TLC method

The RA and MO stock solution was prepared by weighing RA (20 mg) and MO (15 mg) in a 50-mL volumetric flask, and after dissolving in methanol (10 mL), it was sonicated for 20 min, and then diluted up to the mark with methanol. A sample (1.0 mL) of this solution was then placed in a 10-mL volumetric flask and diluted with the same solvent. The final solution contained 40 µg of RA and 30 µg of MO per mL of the solution.

Preparation of calibration curve
HPLC method

Calibration curves were adequately measured by plotting peak areas versus concentrations of RA and MO, and the regression equations were calculated. The calibration curve was plotted over a concentration range of 400–2000 ng/mL and 300–1500

ng/mL for RA and MO, respectively. Accurately measured standard working solutions of RA and MO (1.0, 2.0, 3.0, 4.0, and 5.0 mL) were transferred into a series of 5-mL volumetric flasks and diluted to the mark with the mobile phase. 20 µL of each solution was injected under operating conditions previously described.

TLC method

Aliquots of 10, 15, 20, 25, and 30 µL of standard solution were spotted onto precoated TLC plates using an automatic spotter under a nitrogen gas stream. The plate was dried in air and developed up to 90 mm at a constant temperature, using a mixture of ethyl acetate–methanol–benzene (2:0.5:2.5, v/v) as the mobile phase in a Camag twin-trough chamber previously saturated with mobile phase for 45 min. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 276 nm using a Camag TLC scanner 3. The calibration curves were adequately measured by plotting the peak area versus the concentration (ng/spot) corresponding to each spot.

Procedure for pharmaceutical formulation

Pellets of each of the 20 capsules were accurately weighed, powdered, and analyzed as described.

HPLC method

The mass of pellets (powder) equivalent to RA (20 mg) and MO (15 mg) was accurately weighed and mixed with methanol in a 50-mL volumetric flask, sonicated for 20 min, and the solution was filtered through Whatman filter paper No. 41 to remove any insoluble matter. The filterate (0.25 mL) was diluted to 50 mL with the same solvent. The final test solution contained 4000 ng of RA and 3000 ng of MO per mL of the solution. Accurately measured standard working solutions of RA and MO (1.0, 2.0, and 5.0 mL) were transferred into a series of 5-mL volumetric flasks and diluted to the mark with the mobile phase. A sample solution (20 µL) was injected into the instrument and chromatographed. The amounts of RA and MO present in the sample solution were determined by fitting area values of peaks corresponding to RA and MO into the equation of the line representing the calibration curve of RA and MO. All determinations were performed in triplicate.

TLC method

An amount of the pellets (powder) equivalent to RA (20 mg) and MO (15 mg) was accurately weighed and transferred into a 50-mL volumetric flask, mixed with methanol (20 mL), and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41. The filterate (1.0 mL) was further diluted to 10 mL with the same solvent. The final test solution contained 40 µg of RA and 30 µg of MO per mL of the solution. A sample solution (20, 25, and 30 µL) was applied on the TLC plate under a nitrogen gas stream using an automatic spotter. The TLC plate was developed and photometrically analyzed as described under the chromatographic conditions. The amounts of RA and MO present in the sample solution were determined by fitting the area values of peaks corresponding to RA and MO into the equation of the line representing the calibration curve of RA and MO. All determinations were performed in triplicate.

Results and Discussion

RA and MO are soluble in methanol; therefore, methanol was selected as the solvent. The formulation was dissolved in methanol with sonication for 20 min to assure complete release of the drug from the formulation matrix.

HPLC method

The mixture of ammonium acetate buffer–methanol–acetonitrile (40:20:40, v/v) could resolve RA and MO with a better peak shape. The combination of this mobile phase offered optimum separation (RT 4.93 ± 0.01 for RA and 9.79 ± 0.02 for MO) and resolution.

The specificity (selectivity) of the RP-HPLC method was checked by a comparison of the chromatograms obtained from samples and the corresponding placebo. Additives in capsules are practically insoluble in methanol or the mobile phase, whereas the active constituents are freely soluble. The chromatograms obtained from the placebo and of samples for the capsules are shown in Figures 1 and 2, respectively. No interference from additives was obtained.

The linearity of RA and MO were in the range of 400–2000 ng/mL and 300–1500 ng/mL, respectively, with correlation coefficient more than 0.9916. The average linear regression equation was represented as $y = 217.75x - 7125$ for RA and $y = 190.19x - 920.3$ for MO, where x is the concentration of the drug and y is the peak area.

The limit of detection (LOD) and the limit of quantitation (LOQ) of the drugs were calculated using the following equations as 3.3 s/m and 10 s/m, respectively, where s is the standard deviation of the response and m is the slope of the regression equation. The LOD was found to be 97.7 ng/mL for RA and 97.6 ng/mL for MO. The LOQ was found to be 398 ng/mL for RA and 295 ng/mL for MO.

The intra- and inter-day precision was expressed as relative standard deviation (RSD). For intra-day, three replicates of RA (400–2000 ng/mL) and MO (300–1500 ng/mL) were analyzed on the same day, and these standards were analyzed in five replicates over a period of one week to establish interday precision. The results obtained from intra- and interday precision study were found to be in the range of 1.36–3.89% and 0.84–3.58%, respectively. These values indicate that the method is precise.
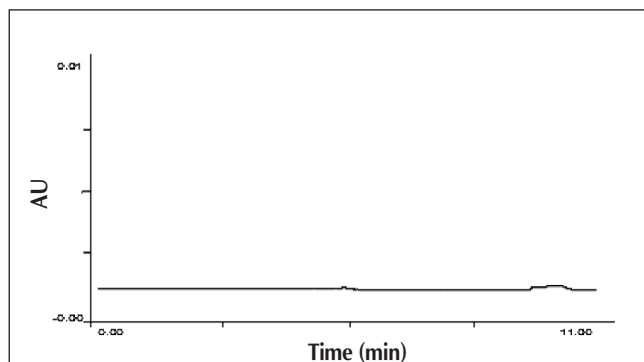
The precision of the instrument was checked by repeated injection of the same concentration (1200 µg/mL for RA and 900 ng/mL for MO) of both drugs seven times without changing the condition of the instrument; the RSD for measuring the peak area was found to be 1.76% for RA and 1.03% for MO. The % RSD for measuring the peak area was less than 2%, ensuring proper functioning of HPLC system.

The accuracy of the method was evaluated by calculating the recovery of RA and MO by the standard addition method. The percentage recovery was found to be 99.68–101.26% for RA and 99.79–99.94% for MO, ensuring that the method is accurate.

The method was found to be robust, although small deliberate changes in method conditions did not have an effect on the chromatographic behavior of the solutes. The chromatographic conditions investigated were mobile phase composition (organic modifier volume fraction, buffer pH), flow-rate, and detection wavelength. The results indicate that a minor increase or decrease in the pH (± 0.30) has no effect on the separation of solutes, but a drastic change in pH causes a large effect on the chromatographic behavior of RA and MO. Small deliberate changes in the mobile phase flow-rate (± 0.010) have no effect on the chromatographic behavior of RA and MO, although even a change of the mobile phase flow-rate (± 0.020) causes a sharp change in the retention time of the drugs used for this method. A decrease in the flow-rate is undesirable because it leads to a sudden increase in tailing of each drug peak. Alteration of the detection wavelength in the range of 270–280 nm causes a variation of peak areas, but these do not affect the chromatographic behavior of RA and MO.

The stability of standard solutions can also affect the robustness of analytical methods. The stability of the standard solutions of the drug substances used in this method was tested over a long period of time. One portion of standard solutions was kept at room temperature and another portion was stored under refrigeration at approximately 4°C, and the content of these solutions was regularly compared to that of a freshly prepared solution. No changes in drug concentrations were observed for solutions stored under refrigeration.

This method was applied to determine the content of RA and MO in three combined market samples of RA and MO capsules.
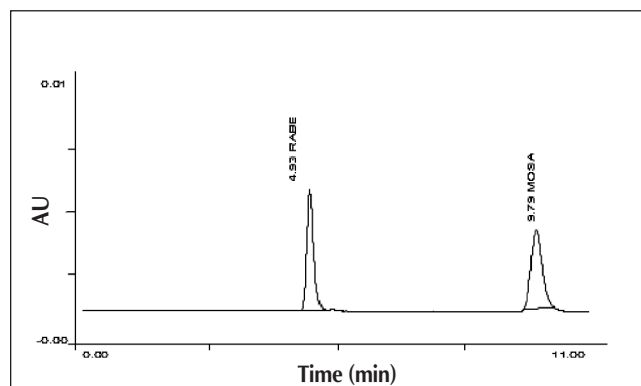

Figure 1. Chromatogram obtained during assessment of specificity study for HPLC method.

Figure 2. HPLC chromatogram of rabeprazole and mosapride with corresponding retention time at 276 nm. Chromatogram of the sample showing resolution of rabeprazole (1200 ng /mL, RT = 4.93 ± 0.01) and mosapride (900 ng/mL, RT = 9.79 ± 0.02) peaks from components of formulation matrix.

Table I. Analysis of Marketed Capsule Formulations Containing RA and MO by the Proposed HPLC and TLC Methods

Formulation	RA ± SD* (n = 5)		MO ± SD (n = 5)	
	HPLC	HPTLC	HPLC	HPTLC
A	99.26 ± 0.29	99.85 ± 0.52	100.70 ± 1.033	99.53 ± 0.26
B	99.97 ± 1.52	99.47 ± 0.37	99.25 ± 1.43	99.13 ± 0.40
C	99.94 ± 1.22	99.18 ± 0.39	99.45 ± 1.33	99.63 ± 0.12

* Mean average three determinations, SD = standard deviation.

The content and percentage of RA and MO in three market samples are presented in Table I. The result indicates that the proposed HPLC method is simple, rapid, precise, and accurate for the simultaneous estimation of RA and MO in its combined formulations.

TLC method

The mobile phase consisted of a mixture of ethyl acetate–methanol–benzene (2:0.5:2.5, v/v), which could resolve RA and MO spots with a better peak shape. The combination of this mobile phase offered optimum migration (R_f values 0.41 ± 0.02 for RA and 0.61 ± 0.03 for MO) and resolution. Even saturation of the TLC chamber with the mobile phase for 45 min assured better reproducibility and resolution.

Active constituents in capsules are freely soluble in methanol or the mobile phase, whereas additives are practically insoluble. The chromatograms obtained from placebo study and of samples for capsules are shown in Figures 3 and 4, respectively. These chromatograms reveal the selectivity of the method.

The linear correlation between peak area and compound concentration was checked for each component. Data for solutions of different concentrations in the range 400–1200 ng/spot for RA and 300–900 ng/spot for MO were collected and analyzed. The average linear regression equation was represented as $y = 4.2249x – 1421.1$ for RA and $y = 5.0799x – 865.69$ for MO, where x is the concentration of the drug and y is the peak area. The correlation coefficient (r) was 0.9906 for RA and 0.9959 for MO.
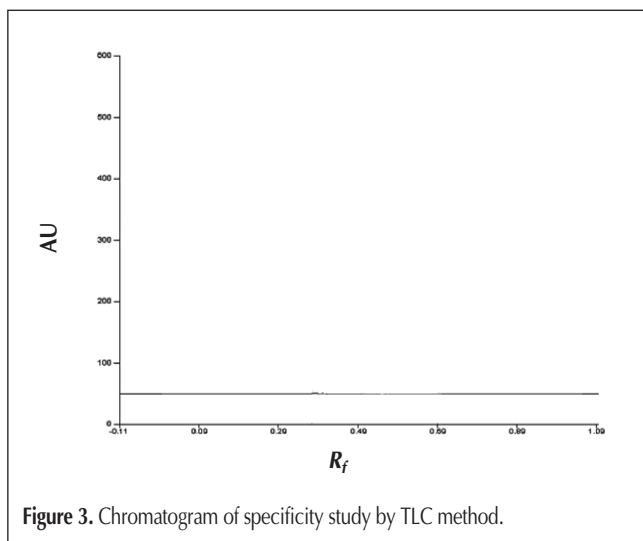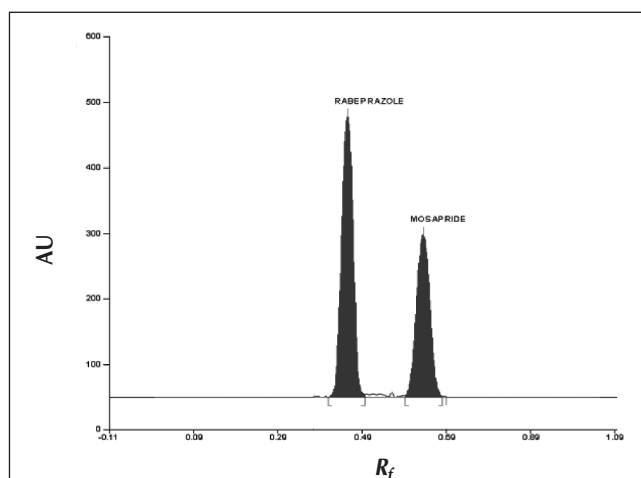
The LOD and LOQ were calculated in accordance with the 3.3 s/m and 10 s/m criteria, respectively, where s is the standard deviation of the peak area for the sample, and m is the slope of the corresponding calibration plot, determined from a linearity investigation. The LOD was found to be 132.19 ng/spot for RA and 98.25 ng/spot for MO, while the LOQ was found to be 398.96 ng/spot for RA and 297.80 ng/spot for MO.

The intraday precision (% RSD) was determined for standard RA (400–1200 ng/spot) and MO (300–900 ng/spot) 3 times on the same day, and interday precision (% RSD) was determined for standard RA (400–1200 ng/spot) and MO (300–900 ng/spot) 5 times over a period of one week. Intra- and inter-day coefficient variations (CV) for both drugs were found to be in the range of 0.30–3.67% and 1.03–3.58%, respectively. These values indicate that the method is precise.

A system suitability test was used to verify that the resolution and repeatability of the system were adequate for the analysis intended. This test was performed by repeated scanning ($n = 7$) of the same spot (1000 ng/spot for RA and 750 ng/spot for MO) of both drugs with same parameters of the instrument (e.g., detection wavelength, spotting rate, syringe, size and position of plate); the % CV for measuring the peak area was 1.58% and 0.94% for RA and MO, respectively. All of these values were within the acceptable range.

The standard addition method was used to determine the accuracy of the method. The results obtained from the determination of accuracy were 98.23–99.96% for RA and 98.05–99.93% for MO. The recovery of the TLC method was good.

The method was found to be robust, and small deliberate changes in method conditions did not have an effect on the chromatographic behavior of the solutes. The chromatographic conditions investigated were mobile phase composition (organic modifier volume fraction, pH), spotting-rate, and detection

Figure 3. Chromatogram of specificity study by TLC method.

Figure 4. Chromatogram of rabeprazole and mosapride from a capsule formulation by TLC method with corresponding R_f at 276 nm. Chromatogram of the sample showing resolution of rabeprazole (800 ng/spot, R_f = 0.42 ± 0.02) and mosapride (600 ng/spot, R_f = 0.61 ± 0.04) peaks from components of formulation matrix.

wavelength. The results indicate that changing the pH (± 0.03) has no effect on the chromatographic behavior of RA and MO. A drastic change in pH causes a sharp decrease in the R_f, and an increase in the tailing of drugs used for this method has been reported. Alteration of the detection wavelength in the range of 270–280 nm causes a variation of peak areas but these do not affect the chromatographic behavior of RA and MO. Changing the spotting rate does not affect the chromatographic behavior of the drugs.

Because the stability of standard solutions can also affect the robustness of analytical methods, the stability of the standard solutions of the drug substances used in this method was tested over a long period of time. One portion of a standard solution was kept at room temperature and another portion was stored under refrigeration at approximately 4°C, and the content of these solutions was regularly compared with that of a freshly prepared solution. No changes in drug concentrations were observed for solutions stored under refrigeration.

Three different brands of capsules were procured from a local market and this method was applied to determine the content of RA and MO. The results obtained in terms of content and percentage of RA and MO in three market samples are presented in Table I. The results indicate that a simple, sensitive, rapid, precise, reproducible, and accurate TLC method could be applied for the simultaneous quantitation of RA and MO in its combined formulations.

Comparison

The assay results for RA and MO in their combined dosage forms obtained using HPLC and TLC methods were compared by applying a paired t-test. The calculated t value 0.46 for RA and 0.23 for DOM is less than the tabulated t-value (4.60) at 95% confidence interval. Therefore, there is no significant difference in the content of RA and MO by the HPLC and TLC methods.

Conclusion

New simple, sensitive, accurate, reproducible, and precise RP-HPLC and TLC methods for the assay of rabeprazole and mosapride in pharmaceutical products have been developed and validated. These proposed methods can be used in routine pharmaceutical analysis. Apart from this, quantitation of rabeprazole and mosapride in biological fluids may be possible by using the same mobile phase as the HPLC and TLC methods described here.

References

1. S. Budavari, Ed. In *The Merck Index*, 13th ed. Merck and Co. Inc., Whitehouse Station, New Jersey, 2001, pp. 6306,8182.
2. H D. Langtry and M.I. Wilde. Proton pump inhibitors. *Drugs* **56:** 447–486 (1998).
3. J. Macek, P. Ptáek, and J. Klíma. Spectrophotometric determination of rabeprazole, lansoprazole and pantoprazole sodium via their metal chelates. *J. Chromatogr. B* **689:** 239–243 (1997).
4. A. El-Gindy, F. El-Gindy, and M.M. Maher. Spectrophotometric and chromatographic determination of rabeprazole in presence of its degradation products. *J. Pharm. Biomed. Anal.* **31:** 229–242 (2003).
5. J. Haung, Y. Xu, and Q. Guo. Development of a liquid chromatography–tandem mass spectrometry assay for quantification of rabeprazole in human plasma. *Rapid Commun. Mass Spectrum.* **19(16):** 2321–2324 (2005).
6. P.N. Presannakumaran and T. Tharu. Estimation of rabeprazole by HPTLC. *IPC* **1:** 274 (2004).
7. M. Miura, S. Sathos, H. Tada, and T. Suzuki. Determination of rabeprazole enantiomers and their metabolite by HPLC with SPE. *J. Pharm. Biomed. Anal.* **18:** 415–449 (2006).
8. H. Nakai, Y. Shimamura, T. Kanazawa, S. Yasuda, and M. Kayano. Determination of a new H(+)-K+ ATPase inhibitor (E3810) and its four metabolites in human plasma by high-performance liquid chromatography. *J. Chromatogr. B* **660:** 211 (1994).
9. T. Uno, M. Shirnizu, and K. Sugawara. Determination of rabeprazole and its active metabolite, tabeprazole thiether in human plasma by column switching HPLC and its application to pharmacokinetic study. *J. Chromatogr. B* **824:** 238–243 (2005).
10. N.V. Ramakrishna, K.N. Vishwottam, S. Wishu, M. Koteshwara, and S.S. Kumar. High performance liquid chromatography method for quantification of rabeprazole in human plasma using solid phase extraction. *J. Chromatogr. B* **816:** 209–214 (2005).
11. M. A. Mathrushri and K. Bhattmisha. Estimation of rabeprazole by high performance liquid chromatography. *Indian Drugs* **39(10):** 550–551 (2002).
12. Y. S. Krishnaiah, T.K. Murthy, and D. G. Sankar. A validated RP-HPLC method for determination of mosapride citrate in bulk drug and pharmaceutical formulation. *Pharmazie.* **57(12):** 814–816 (2002).
13. T.K. Murthy and D. G. Shankar. Determination of mosapride citrate in bulk drug and pharmaceutical dosage forms using HPLC. *Anal. Sci.* **18(11):** 1269–1271 (2002).
14. T. Morie and S. Kato. Application of LC–MS–MS for the determination of a polar impurity in mosapride. *Chem. Pharm. Bull.* **42:** 877 (1994).
15. Y. Mizuki and T. Fujii. Development and validation of LC method for determination of related substance of mosapride citrate in bulk drug and pharmaceuticals. *J. Pharm. Biomed. Anal.* **36(4):** 759–767 (2004).
16. N.V.S. Ramkrishna, S. Manoj, and D.P. Varma. Validation and application of a HPLC–tandem MS assay for mosapride in human plasma. *Biomed. Chromatogr.* **19(7):** 539–548(2004).