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A validated RP-HPLC method for the determination of mosapride citrate in bulk drug samples and pharmaceutical formulations

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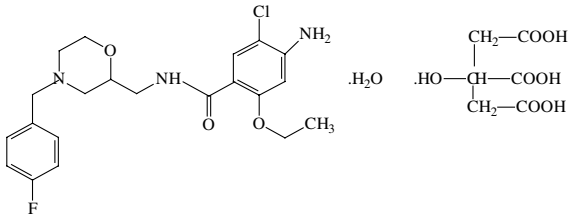
Mosapride citrate, a selective serotonin 5-HT_4 agonist, is a novel and potent gastroprokinetic drug. So far no assay procedure has been reported for the estimation of this drug either in bulk drug samples, pharmaceutical formulations or in biological samples. A rapid and sensitive high-performance liquid chromatographic method was developed for the estimation of mosapride citrate in bulk drug samples and pharmaceutical dosage forms. Risperidone was used as an internal standard (ISD). A HPLC system consisting of gradient pump, reverse phase C-18 analytical column, a variable UV-visible detector set at 274 nm and an integrator was used. The mobile phase consisted of acetonitrile: 0.02 M potassium dihydrogen phosphate buffer (pH adjusted to 4.0 with o-phosphoric acid) in the ratio of 50 : 50 (v/v), and was pumped at 1 ml/min at 40 °C. The drug and ISD were eluted at 8.10 and 2.27 min, respectively. The peak drug/ISD area ratio versus drug concentration relationship was linear ($r = 0.9998$). The method was validated for its linearity, precision and accuracy. The calibration curve was linear in the range of 0.5 to 30 μg/ml. The lower detection limit was found to be 0.23 μg/ml. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. The mean recovery of the drug from the solutions containing 2, 4 or 10 μg/ml was 101.55 ± 0.97% indicating high accuracy of the proposed HPLC method.

1. Introduction

Gastroesophageal reflux disease (GERD) and dyspepsia are common chronic diseases in Europe and North America and its prevalence is now increasing in many Asian countries [1]. Mosapride citrate, a novel and potent gastroprokinetic drug, is a selective serotonin 5-HT_4 agonist [2–4]. It exerts a more profound effect on motor functions involved in the protection against abnormal gastroesophageal reflux compared with other gastroprokinetic substances such as cisapride. It has no dopamine D_2-receptor antagonist action and does not have central action, but in contrast, it does not stimulate colonic motor hyperactivity.

Mosapride citrate is a 4-amino-5-chloro-2-ethoxy-N-[[4-[(4-fluorophenyl) methyl]-2-morpholinyl]-methyl benzamide, and is not yet official in any Pharmacopoeia.



So far no assay procedure has been reported for the determination of this drug either in bulk drug samples, pharmaceutical formulations or biological samples. The aim of this study was to develop a simple, rapid, precise and accurate reversed-phase HPLC method for the determination of mosapride citrate in bulk drug samples or in pharmaceutical dosage forms.

2. Investigations, results and discussion

To develop a rapid HPLC method for the analysis of mosapride citrate in bulk drug samples and its tablet formulations the most commonly employed RP C-18 column with UV detection was used. In order to test the column efficiency, the number of theoretical plates or plate count (N), tailing factor (T) and resolution factor (R) for the separation of mosapride citrate were calculated according to USP XXIII. The optimum N values lie in the range of 10,000 to 70,000/m. With the column used in the present study, N was found to be 27,993/m. With a well-packed column, T lies between 0.9 and 1.1. With the column used in the present study, it was found to be 0.942. For a good resolution, the value of "R" should be above 1. With the proposed HPLC method, it was found to be 12.88. Thus, the results of the validation on the column and HPLC method used in the present study demonstrate good column efficiency and system suitability and high resolution for the analysis of mosapride citrate.

The retention time (t_R) of the mosapride citrate reference substance and the internal standard risperidone (ISD) were 8.10 and 2.27 min, respectively. (Fig.).

The retention times for both the drug and ISD were highly precise. According to the literature [5], the optimum capacity factor (k) lies in the range of 2–10. The value of k for mosapride citrate was found to be 4.4 indicating that the drug was well retained in the column during separation.

The calibration curve for mosapride citrate was constructed by plotting the ratio of peak area of mosapride citrate to peak area of internal standard (Y) against the

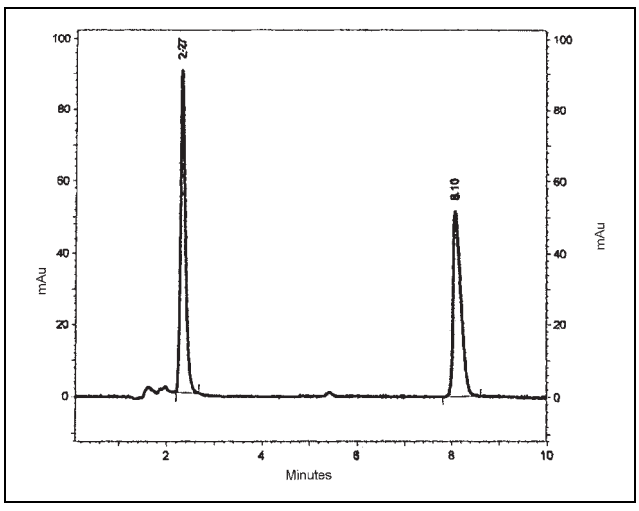


Fig.: Model chromatogram for mosapride citrate

Table 1: Calibration of the proposed HPLC method

Concentration of mosapride citrate (μg/ml)	Mean peak-area ratio (n = 6)	% CV
0.5	0.0512	0.28
1	0.1033	0.01
2	0.1947	1.02
5	0.4921	0.75
10	1.0143	0.23
15	1.5399	0.08
20	2.1102	0.73
25	2.5794	0.95
30	3.1268	1.22

$Y = -0.01342 + 0.10444\ X$ ($r = 0.99987$)

Table 2: Inter- and intra-day precision for mosapride citrate assay in pharmaceutical dosage forms by the proposed HPLC method

Concentration of mosapride citrate (μg/ml)	Observed concentration of mosapride citrate (μg/ml)			
	Intra-day		Inter-day	
	Mean (n = 5)	% CV	Mean (n = 5)	% CV
5	5.04	1.04	4.99	0.77
10	10.01	0.89	9.95	1.52
20	20.20	1.29	20.10	0.94

Table 3: Regression characteristics of the proposed HPLC method

Parameter	Value
Standard deviation of slope (S_b)	1.369×10^{-3}
Standard deviation of intercept (S_a)	7.804×10^{-3}
Standard error of estimation (S_e)	9.588×10^{-3}
Relative standard deviation (%)*	0.9592
% Range of error at 95% confidence limit	0.8020
% Range of error at 99% confidence limit	1.1866
Detection limit (μg/ml)	0.2305

* Six replicates

Table 4: Mean (± s.d.) amount of mosapride citrate in tablet dosage forms by proposed HPLC method

Brand of the tablet	Labelled amount (mg)	Observed amount (mg) (n = 3)	Purity (%)
AX	5.0	4.99 ± 0.65	99.81 ± 0.52
AY	2.5	2.48 ± 0.74	99.20 ± 0.27
BX	5.0	5.06 ± 0.15	101.34 ± 0.48
BY	2.5	2.52 ± 0.48	100.84 ± 0.19

concentration (X). A correlation coefficient of 0.99987 was determined, the representative linear regression equation being $Y = -0.01342 + 0.10444\ X$. The method was validated for its intra- and inter-day precision. In the range of 0.5–30 μg/ml, the coefficients of variation (CV), on the basis of the peak area ratios for five replicate injections, were found to be between 0.01% and 1.22% (Table 1). The inter-day assay precision (3 days, n = 5) was expressed as % CV and ranged between 0.11% and 1.52% (Table 2). Regression characteristics like standard deviation of slope (S_b), standard deviation on intercept (S_a), standard error of estimation (S_e), % RSD, % range of error (0.05 and 0.01 level) and detection limit were calculated for this method and shown in Table 3.

The HPLC method developed in the present study was used to quantify mosapride citrate in tablet dosage forms. Mosapride citrate tablets (2.5 mg and 5.0 mg) were analyzed and the results obtained are given in Table 4. The average drug content was found to be 101.34% of the labeled amount. No interfering peaks were found in the chromatogram indicating that the tablet excipients did not interfere with the estimation of the drug. Also, when a known amount of the drug solution was added to the powdered sample of the tablet dosage form and subjected to the estimation of the drug by the proposed method, there was high recovery (Table 5) of mosapride citrate (100.45 ± 0.92%) indicating that the proposed procedure for the determination of mosapride citrate in the tablet dosage forms is highly accurate. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate for the determination of mosapride citrate in pharmaceutical dosage forms.

3. Experimental

3.1. Materials

Mosapride citrate (assigned purity 99.8%) was a gift sample from M/s. Torrent Pharmaceuticals Ltd. Ahmedabad, India, whereas risperidone (assigned purity 100.1%) was from M/s. Nepal Pharmaceutical Laboratory Pvt. Ltd. Nepal. Potassium dihydrogen phosphate of analytical grade was supplied by M/s. S.D Fine-Chem Limited, Mumbai, India. Acetonitrile and water used were of HPLC grade (Qualigens). The commercially available mosapride citrate tablets claimed to contain 2.5 and 5.0 mg of drug were procured from local market.

3.2. Instrumentation

Quantitative HPLC was performed on a gradient High Pressure Liquid Chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wave length programmable UV/VIS Detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu), a disposable guard column LC-18 (Pelliguard™, LC-18,2 cm, Supelco, Inc., Bellefonte, PA.) and RP C-18 column (150 mm × 4.6 mm I.D.; particle size 5 μm) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

3.3. HPLC conditions

The contents of the mobile phase were acetonitrile and 0.02 M potassium dihydrogen phosphate (pH adjusted to 4.0 with o-phosphoric acid) in the ratio of 50 : 50 (v/v) that were filtered through a 0.45 μm membrane filter

Table 5: Experimental values obtained in the recovery test for mosapride citrate tablets by HPLC

Amount of drug added (μg) to drug solution/powdered tablet formulation	Recovery from drug solution		Recovery from powdered tablet formulation	
	Mean (± s.d.) amount (μg) found (n = 5)	Mean (± s.d.) % recovery (n = 5)	Mean (± s.d.) amount (μg) found (n = 5)	Mean (± s.d.) % recovery (n = 5)
2	2.031 ± 0.22	101.55 ± 0.97	1.994 ± 0.19	99.7 ± 1.58
4	3.998 ± 0.61	99.95 ± 1.33	4.018 ± 0.56	100.45 ± 0.92
10	10.014 ± 0.18	100.14 ± 0.85	10.031 ± 0.24	100.31 ± 1.26

before use, degassed with a helium spurge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 ml/min which yielded a column back pressure of 112–114 kg/cm^2. The run time was set at 10 min and the column temperature was maintained at 40 °C. The volume of the injection loop was 20 µl. Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems. The eluents were monitored at 274 nm and the data were acquired, stored and analyzed with the software "Class-VP series version 5.03 (Shimadzu)".

3.4. Procedure

The solutions were prepared on the weight basis and volumetric flasks were used to minimize solvent evaporation. The HPLC estimation was accomplished by an internal standard method.

3.4.1. Stock solutions

Stock solutions of drug and internal standard were prepared by dissolving 100 mg of mosapride citrate and risperidone separately in 100 ml volumetric flasks containing 70 ml of methanol (HPLC grade), sonicated for about 15 min and then made up to volume with methanol. Daily working standard solutions of mosapride citrate and risperidone were prepared by suitable dilution of the stock solution with the mobile phase.

3.4.2. Calibration solutions

Ten sets of the drug solution were prepared in mobile phase containing mosapride citrate at a concentration of 0.05, 0.1, 0.5, 1, 2, 5, 10, 15, 20, 25 and 30 µg/ml along with a fixed concentration (5 µg/ml) of risperidone as internal standard. Each of these drug solutions (20 µl) was injected six times into the column, and the peak area and retention time were recorded.

3.4.3. Assay of mosapride citrate in tablets

Twenty tablets were weighed to get the average tablet weight and powdered. The sample of the powdered tablets, claimed to contain 50 mg of active ingredient, was mixed with 25 ml of methanol. This mixture was allowed to stand for 6 h with intermittent sonication to ensure complete solubility of the drug and filtered through a 0.45 µm membrane filter followed by adding methanol to get the stock solution of 1 mg/ml. An aliquot of this solution (1.0 ml) was transferred to a volumetric flask and made up to sufficient volume with the mobile phase to give an expected concentration of 10 µg/ml. All determinations were conducted in triplicate. The same procedure was used to estimate the concentration of the drug in one more commercial brand of mosapride citrate tablets of two different strenghts.

3.5. Validation

The proposed HPLC method was validated in terms of linearity, precision and accuracy. The precision (% coefficient of variation) was expressed with respect to the inter- and intra-day variation in the expected drug concentration. The accuracy was expressed in terms of percent recovery of the known amount of drug added to the known amount of drug solution.

3.5.1. Linearity

The linearity of the proposed HPLC method was determined in terms of the correlation coefficient between the concentration of the drug and its respective peak area ratio to that of internal standard. The data were subjected to regression analysis using least squares method.

3.5.2. Precision

The precision of the assay was determined in terms of the intra- and inter-day variation in the peak areas for a set of drug solutions on three different days ($n = 5$). The intra- and inter-day variation in the peak area ratio of the drug solution to that of internal standard was calculated in terms of coefficient of variation (C.V.), and was obtained by multiplying the ratio of standard deviation to the mean with 100 [C.V = (SD/mean) × 100].

3.5.3. Accuracy

The accuracy of the HPLC assay method was assessed by adding known amount (2, 4 or 10 µg/ml) of the drug to a drug solution of known concentration along with internal standard and subjecting the samples to the proposed HPLC method. Also, known amount of the drug solution (2, 4 or 10 µg/ml) was added to the volumetric flask containing the powdered sample of the tablet formulation with known amount of the drug and internal standard. The drug was estimated, as per the procedure described above, for the estimation of mosapride citrate in tablet formulations. In both the cases, the recovery studies were replicated five times. The accuracy was expressed in terms of the recovery, and was calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the percent recovery.

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References

1 Goh, K. L.; Chang, C. S.; Fock, K. M.; Ke, M.; Park, H. J.; Lam, S. K.: J. Gastroenterol. Hepatol. **15**, 230 (2000)
2 Yoshida, N.: Folia Pharmacol. Jpn. **113**, 299 (1999)
3 Yoshikawa, T.; Yoshida, N.; Mine Y.; Hosoki, K.: Jpn. J. Pharmacol. **77**, 53 (1998)
4 Sakurai-Yamashita, Y.; Takada, K.; Takemura, K.; Yamashita, K.; Enjoji, A.; Kanematsu, T.; Taniyama K.: Jpn. J. Pharmacol. **79**, 493 (1999)
5 Szepezi, G.: HPLC in Pharmaceutical Analysis, 2 Ed. p. 179–219, Vol. I, CRC Press, Boca Raton, FL 1990

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