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A High Performance Liquid Chromatographic Assay for Mosapride Citrate

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A rapid and sensitive reverse phase high performance liquid chromatographic method has been described for the estimation of mosapride citrate in bulk drug samples and pharmaceutical dosage forms using RP C-18 column. Nifedipine was used as internal standard. The mobile phase consisted of methanol and water in the ratio of 73 : 27 respectively, and was pumped at 1.3 mL/min at 40°C. The drug and internal standard were eluted at 4.11 min and 2.54 min respectively. The detection was carried out at 274 nm and the calibration curve was linear in the range of 0.01 to 20 μg/mL. The method was statistically validated for its linearity, precision and accuracy. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. The detection limit was found to be 5 ng/mL. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining mosapride citrate in bulk drug samples or in pharmaceutical formulations.

Key Words: Reverse phase HPLC; Mosapride citrate; Nifedipine; Pharmaceutical formulations.

INTRODUCTION

Gastroesophageal reflux disease (GERD) and dyspepsia are among the chronic diseases more common in Europe and North America than in Asia, but their prevalence is now increasing in many Asian countries[1]. Symptoms of GERD are recurrent retrosternal burning and regurgitation of sore or bitter fluid. Although, acid suppression with H_2 receptor antagonists or proton pump inhibitors is effective in the treatment of these conditions, it does not overcome the underlying pathological factors that allow acid, pepsin and bile to reflux into the oesophagus[2]. Gastroprokinetic agents[3] have an important role to play in conjunction with life style modifications in the short and long term medical management of GERD. Mosapride citrate, a novel and potent gastroprokinetic drug, is a selective serotonin 5-HT_4 agonist[4–6].

Chemically 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 availability of a HPLC method with high sensitivity and selectivity will be very useful for the determination of mosapride citrate in pharmaceutical formulations. The aim of this study is 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.

EXPERIMENTAL

Mosapride citrate (assigned purity 99.8%) and nifedipine (assigned purity 100.1%) were gratis samples from M/s Torrent Pharmaceuticals Ltd. Ahmedabad, India. Methanol and water used were of HPLC grade (Qualigens). The commercially available mosapride citrate tablets claimed to contain 2.5 mg or 5.0 mg of drug were procured from local market.

Quantitative HPLC was performed on a gradient high pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength 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)".

Chromatographic conditions: The contents of the mobile phase were methanol and water in the ratio of 73 : 27 v/v that were filtered before use through 0.45 μm membrane filter, degassed with a helium spurge for 15 min and pumped from the respective solvent reservoirs to the column at a flow rate of 1.3 mL/min which yielded a column back pressure of 138–140 kg/cm^2. The run time was set at 6 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)".

Procedure: About 100 mg of mosapride citrate was accurately weighed and dissolved in methanol so as to give a 1 mg/mL solution. Subsequent dilutions of this solution were made with mobile phase after addition of nefidepine as an internal standard to get concentrations of 0.01 to 20 μg/mL of mosapride citrate and 5 μg/mL of internal standard in each dilution. The standard solutions prepared, as above, were injected in triplicate into the column at flow rate of 1.3 mL/min. The ratio of drug peak area to that of internal standard was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. This regression equation was used to estimate the amount of mosapride citrate in tablet dosage forms.

Mosapride citrate solutions containing 8 μg/mL, 12 μg/mL and 20 μg/mL were subjected to the proposed HPLC analysis for finding out the intra- and inter-day variations. The recovery studies were carried out by adding known amount of mosapride citrate to the preanalysed samples and subjecting them to the proposed HPLC method.

Estimation of mosapride citrate in tablet dosage forms: Twenty tablets were weighed and crushed to fine powder. An accurately weighed portion of the powder, equivalent to 50 mg of mosapride citrate, was transferred to a 100 mL volumetric flask containing about 30 mL of methanol. The contents of the flask were sonicated to dissolve mosapride citrate, made up to volume with methanol and the resulting mixture was filtered through a 0.45- μm filter. 2 mL of this solution were added to a 100 mL volumetric flask containing a solution equivalent to 500 μg of internal standard and were made up to volume with mobile phase. This solution (20 μL) was injected in triplicate into the column. The mean values of peak area ratio of drug to internal standard of five such determinations were calculated and the drug content in the tablet was quantified using the regression equation obtained above. The same procedure was followed for the estimation of mosapride citrate in two different brands of tablet dosage forms.

RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of mosapride citrate in bulk samples or pharmaceutical dosage forms. A typical chromatogram is shown in Fig. 1. The column pressure varied from 138 to 140 kgf/cm^2. The retention times for mosapride citrate and internal standard were 4.11 min and 2.54 min respectively. Each of the samples was injected 3 times and the same retention times were observed in all cases. The ratio of peak area of mosapride citrate to peak area of internal standard for different concentrations set up as above was calculated, and the average values for 6 such determinations are shown in Table-1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient of variation (1.96%). A good linear relationship (r = 0.9999) was observed between the concentration of mosapride citrate and the ratio of peak areas of the drug to that of internal standard. The calibration graph was found to be $Y = -0.001269 + 0.62609X$ (where Y = ratio of peak area of drug to that of internal standard; X = concentration of mosapride citrate) in the range of 0.01 to 20 μg/mL. However, the lowest detection limit was found to be 5 ng/mL indicating a high sensitivity.

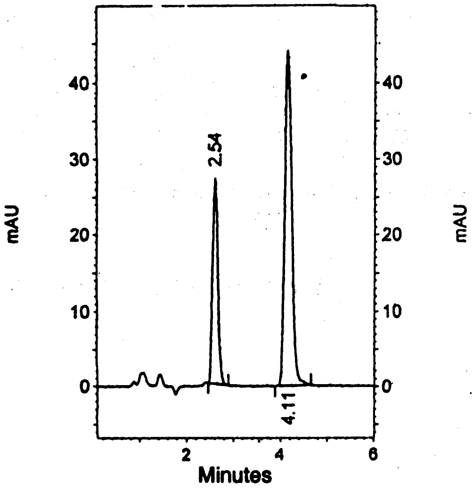

Fig. 1. Typical chromatogram of mosapride citrate.

When mosapride citrate solutions containing 8 μg/mL, 12 μg/mL and 20 μg/mL were analyzed by the proposed HPLC method for finding out intra- and inter-day variations, a low coefficient of variation was observed (Table-2). This shows that the present HPLC method is highly precise. 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 a high recovery (Table-3) of mosapride citrate (100.57 ± 0.08%) indicating that the proposed procedure for the determination of mosapride citrate in the tablet dosage forms is highly accurate.

TABLE-1
CALIBRATION OF THE HPLC METHOD

Concentration of mosapride citrate (μg/mL)	Mean peak area ratio (n = 6)	CV (%)
0.005	0.0018	0.970
0.010	0.0059	1.250
0.020	0.0124	1.390
0.040	0.0248	1.070
0.100	0.0619	1.960
0.200	0.1255	0.280
0.400	0.2463	0.042
1	0.6252	1.038
2	1.2513	1.259
4	2.4997	0.095
8	5.0118	0.776
12	7.5007	0.852
16	10.0236	1.054
20	12.5202	0.006

Regression equation (from 0.01 to 20 μg/mL).
$Y = -0.001269 + 0.62609 X$ ($r = 0.9999$)

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
8	7.98	0.93	8.04	0.87
12	12.01	0.35	12.05	1.43
20	20.06	1.44	19.99	0.75

TABLE-3
EXPERIMENTAL VALUES OBTAINED IN THE RECOVERY TEST FOR MOSAPRIDE CITRATE TABLETS BY PROPOSED 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)
4	3.991 ± 0.35	99.77 ± 1.24	4.023 ± 0.38	100.57 ± 0.08
8	8.014 ± 0.61	100.17 ± 1.01	7.998 ± 0.51	99.97 ± 1.36
12	12.107 ± 0.09	100.89 ± 0.88	12.031 ± 0.29	100.25 ± 0.76

The drug content in the tablets was quantified using the proposed analytical method. The mean content of mosapride citrate in four different tablet dosage forms is shown in Table-4. The absence of additional peaks in test solution indicates no interference of the excipients used in the tablet dosage form. The tablets were found to contain 99.8 to 100.8% of the labeled amount. The low % CV indicates the reproducibility of the assay of mosapride citrate in the tablet dosage form. In conclusion, the proposed HPLC method was found to be simple, precise, highly accurate, specific and less time consuming. Hence, it can be used for the routine determination of mosapride citrate in bulk drug samples and in its pharmaceutical formulations.

TABLE-4
MEAN (± s.d.) AMOUNT OF MOSAPRIDE CITRATE IN TABLET DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Brand of the tablet	Labelled amount of drug (mg)	Mean (± s.d.) amount found (mg) by the proposed method [n = 5]	Mean (± s.d.) % labelled amount [n = 5]
AA	2.5	2.52 ± 0.01	100.8 ± 0.19
BB	5.0	4.99 ± 0.11	99.8 ± 0.05
CC	2.5	2.51 ± 0.13	100.4 ± 0.07
DD	5.0	5.03 ± 0.42	100.6 ± 0.35

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