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Estimation of Rabeprazole Sodium and Itopride Hydrochloride in Tablet Dosage Form Using Reverse Phase High Performance Liquid Chromatography

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Abstract: A reversed phase high performance liquid chromatography (RP-HPLC) method was developed, validated and used for the quantitative determination of rabeprazole sodium (RP) and itopride hydrochloride (IH), from its tablet dosage form. Chromatographic separation was performed on a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm), with a mobile phase comprising of a mixture of 50 mM ammonium acetate buffer and methanol (20:80v/v), pH 4.5 adjusted with acetic acid, at a flow rate of 1.3 mL/min with detection at 286 nm. Separation was completed in less than 10 min. As per International Conference on Harmonization (ICH) guidelines the method was validated for linearity, accuracy, precision, limit of quantitation and limit of detection. Linearity of RP was found to be in the range of 37.5-375 µg/mL and IH was found to be in the range of 5-50 µg/mL. The correlation coefficients were 0.9997 and 0.9995 for RB and IH respectively. The accuracy of the developed method was found to be 98.6-100.7 for RP and 99.42 -100.81 for IH. The experiment shows the developed method is free from interference of excipients. It indicates the developed RP-HPLC method is simple, linear, precise and accurate and it can be conveniently adopted for the routine quality control analysis of the tablet dosage form.

Keywords: RP-HPLC, Rabaprazole sodium , Itopride hydrochloride, Tablet.

Introduction

Rabeprazole sodium is chemically (RP) 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridil] -methyl] sulfinyl]-1*H*- benzimidazole[1] (Figure 1). It has been taken orally to treat gastric acid secretion by inhibiting the parietal cell H+/K+ ATP pump and used in short term treatment in healing and symptomatic relief of duodenal ulcers and erosive or ulcerative gastro esophageal reflux disease (GERD); long- term treatment of pathological hypersecretory conditions, including Zollinger-Ellison syndrome and in combination with amoxicillin and clarithromycin to eradicate helicobacter pylori .

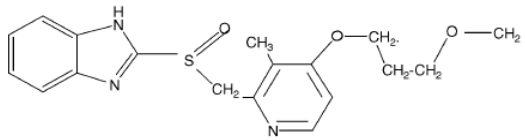

Figure 1. Structure of rabeprazole

Itopride hydrochloride (IH) is chemically *N*-[[4-(2-dimethylaminoethoxy) phenyl] methyl] -3, 4- dimethoxy-benzamide hydrochloride[2] (Figure 2) used as a prokinetic agent. It acts orally by increasing the acetylcholine concentration, by inhibiting dopamine D2 receptors and acetylcholine esterase. Higher acetylcholine concentration increases the GI peristalsis, increase the lower esophageal sphincter pressure, stimulate the gastric motility, accelerate the gastric empting and improve the gastro duodenal co-ordination

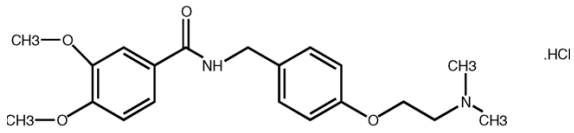

Figure 2. Structure of itopride hydrochloride

The literature survey revealed that several methods have been reported for the individual estimation of rabeprazole sodium and itopride hydrochloride and in combination with other drugs by UV[3-7], HPLC[8-18] and HPTLC[19-22]. However there is no method reported for the simultaneous analysis of the same combination by HPLC. In the present investigation, an economical, precise, accurate reversed phase HPLC method, using a PDA detector, has been developed for the simultaneous quantitative determination of RP and IH from the tablet dosage form.

Experimental

Bulk drugs RP and IH were procured from Micro labs Ltd, Bangalore, India. Methanol (HPLC grade, purity 99.80%), acetic acid (AR grade, purity 93.00%) and ammonium acetate (AR grade, purity 99.50%) were all procured from Qualigens Fine Chemicals (Mumbai, India). A commercial pharmaceutical preparation (Rablet-IT from the Lupin Pharmaceuticals Mumbai) was used. Its labeled content was RP 20 mg and IH 150 mg.

Preparation of stock, working standard and sample solution

A stock solution each of RP and IH (100 µg/mL) was prepared by taking 10 mg of each drug, accurately weighed, in separate 100 mL volumetric flasks. They were dissolved in 25 mL of mobile phase and then the volume was made up to the mark to get 100 µg/mL. The internal standard solution was prepared by taking 10 mg of mosapride in a 100 mL standard flask. It was dissolved by adding 25 mL of mobile phase, shaken for few minutes to get a clear solution and the

final volume was made up to 100 mL. For each drug, appropriate aliquots were pipetted out from the standard stock solution into a series of 10 mL volumetric flasks to get a concentration of 37.5,75,150,225,300 and 375 µg/mL of itopride hydrochloride and 5, 10, 20, 30, 40 and 50 µg/mL rabeprazole and 20 µg/mL of mosapride (Internal Standard).

Instruments and chromatographic conditions

Chromatographic separation was performed on a Shimadzu LC-20 AT HPLC (Double pump) with Rheodyne 7725i type injector with 20 µL loop capacity and SPD M20A, Prominence Diode Array Detector. The wavelength of detection chosen was 286 nm. A reverse phase Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) was used for the analysis. The mobile phase comprising of a mixture of 50 mM ammonium acetate buffer and methanol (20:80v/v), pH 4.5 adjusted with acetic acid, at a flow rate of 1.3 mL/min. The injection volume was 20 µL.

Method validation

Every 20 µL of the working standard solution of RP in the mass concentration range of 5 to 50 µg/mL, and that for IH in the mass concentration range of 37.5 to 375 µg/mL, was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of RP and IH were obtained by plotting the peak area ratio versus the applied concentrations of RP and IH. The linear regression coefficients were found to be 0.9997 and 0.9995 for RP and IH, respectively. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 ∂/S and 10 ∂/S, respectively as per ICH guidelines[23], where ∂ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot

The instrument precision was performed by injecting 20 µL of both RP and IH (10 µg/mL and 37.5 µg/mL), in six replicates, into the chromatographic system, under optimized chromatographic conditions. Parameters evaluated were repeatability of peak response of drugs. The relative standard deviations (RSDs) of the peak area were found to be 1.12% and 1.73% for RP and IH, respectively.

Repeatability of the method was checked by injecting replicate injections of the combined solution (10 µg/mL and 37.5 µg/mL of RP and IH respectively). Variability of the method was studied by analyzing the solution on the same day (intra-day precision) and on three different days (interday precision). The results obtained for intra-day precision (RSDs) were 1.12% and 0.95%, respectively, at n = 6, for both RP and IH. The inter-day precisions (RSDs) were 0.72% and 0.45%, respectively, at n = 6, for both RP and IH.

Accuracy of the method was tested by carrying out recovery studies at three different spiked levels (50, 100% and 150%) on the basis of the label claim. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, which are listed in Table 1.

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both RP and IH from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific.

Table 1. Recovery studies of RP and IH (n=6)

Drug	Concentration of std solution used, µg/mL	Concentration of sample solution added, µg/mL	Amount found µg/mL	% Recovery	% RSD
RP	10	5	14.80	98.66	0.463
	10	10	20.14	100.7	0.634
	10	15	24.87	99.48	0.487
IH	75	37.5	112.69	100.16	0.243
	75	75	151.22	100.81	0.521
	75	112.5	186.43	99.42	0.597

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed. System suitability parameters for the method are listed in Table 2.

Table 2. System suitability & Validation parameters for RP-HPLC

Validation Parameters	RP	IH
Linearity range, µg / mL	37.5 - 375	5-50
r	0.9997	0.9995
LOD, ng /mL	10	2.5
LOQ, ng /mL	25	5
Intra day, % RSD*	1.1273	0.9557
Inter day, % RSD*	0.7253	0.4527
Repeatability, % RSD*	1.4820	0.9568
Accuracy	98-100 %	99 – 100%
Peak purity index	1.0000	1.0000
Resolution factor(R_s)	7.3	-
Asymmetry factor(A_s)	0.95	
No.of theoritical plates(N)	3532	4666
Capacity factor (K')	0.601	-
High equivalent to theoritical plates(HETP)	33.23	43.21
Tailing factor	1.423	1.327
Seletivity factor(α)	3.639	

* *Each value is a mean of six observations*

Analysis of formulation

Twenty tablets of RP and IH in combination were weighed, their average weight was determined, and finally they were crushed to a fine powder. The tablet powder equivalent to 20 mg of RP and 150 mg of IH was weighed and transferred to a 100 mL volumetric flask, first dissolved in 50 mL of mobile phase and then the volume was made up to the mark with the mobile phase. The content was ultrasonicated for 30 min for complete dissolution. The solution was then filtered using whatman filter paper no 41. The selection of the mixed sample solution for analysis was carried out by the optimization of various dilutions of the tablet dosage form, considering the label claim. The mixed sample solution of 10 µg/mL of RP and 37.5 µg/mL of IH which was falling in the Beer's-Lamberts range with 20 µg/mL internal standard, showed good results and was selected for the entire analysis. The results of tablet analysis (n = 6) were found to be 99.20% with ±0.25% standard deviation (SD) and 99.52%

with ± 0.36% SD for RP and IH respectively. From the typical chromatogram of IH, RP and mosapride (Internal standard) (Figure 3), it was found that the retention time of IH was 2.6 min, RP was 4.2 min and mosapride was 6.9 min, which were well resolved peaks with a resolution factor of 7.3 and 8.3. The results are shown in Table 3.

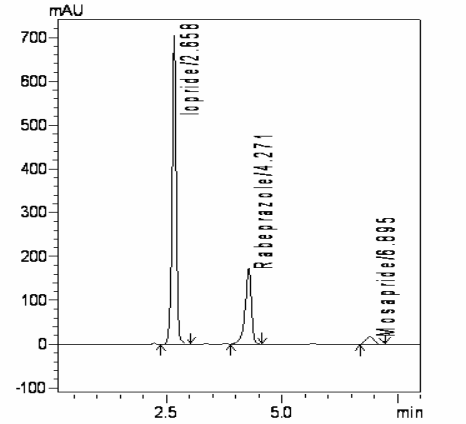

Figure 3. Chromatogram of tablet solution

Table 3. Analysis of Formulation

Formulation	Drug	Label Claim, mg/tablet	Concentration Taken for analysis, µg/mL	Amount Found, µg/mL	% Recovery (n=6)
	Rabeprazole	20	10	9.92	99.20
Rablet IT	Itopride	150	37.5	37.3	99.52

Conclusion

The developed method was validated in terms of accuracy, repeatability and precision. A good linear relationship was observed for RP and IH in the concentration ranges of 5-50 µg/mL and 37.5-375 µg/mL respectively. The correlation coefficient for RP was found to be 0.9997 and that for IH was 0.9995. The inter-day and intra-day precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of rabeprazole sodium and itopride hydrochloride estimated in the tablet dosage form were free from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise and accurate, and could be conveniently adopted for the routine quality control analysis of RP and IH, simultaneously, from its pharmaceutical formulation and bulk drug.

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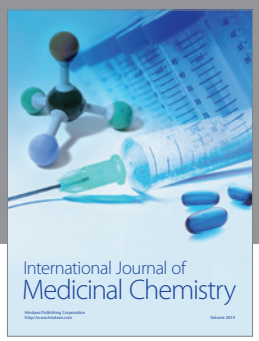

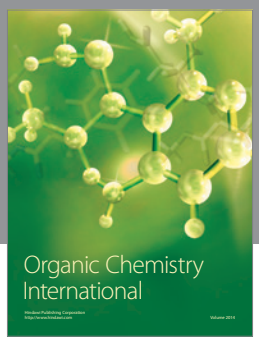

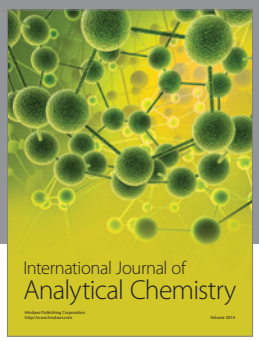

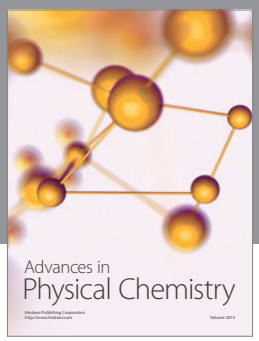

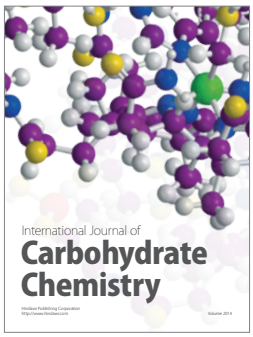

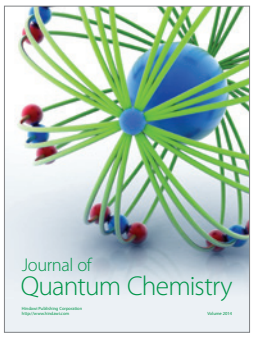

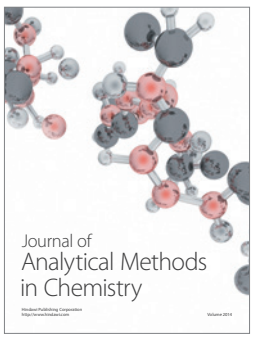

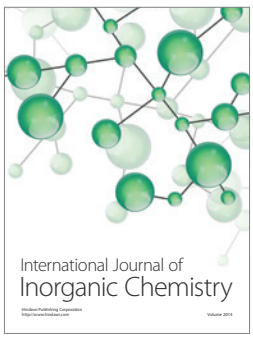

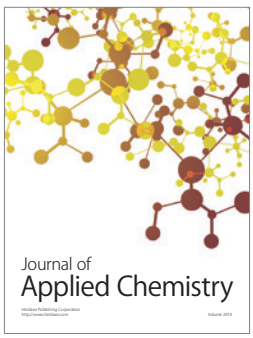

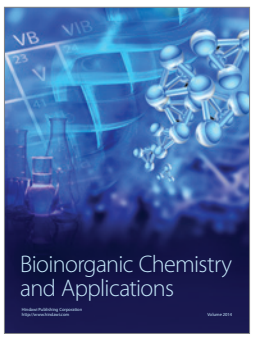

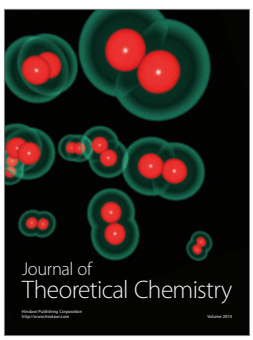

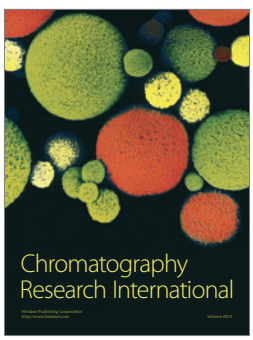

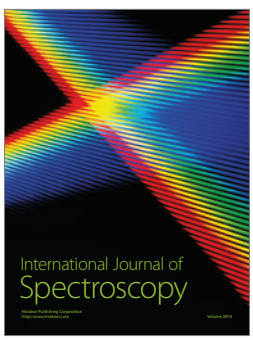

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