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Development and Validation of RP-HPLC Method for the Simultaneous Determination of Rabeprazole Sodium and Itopride Hydrochloride in Solid Dosage Form

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Abstract: A simple, sensitive, precise, accurate, rapid and reproducible reverse phase high performance liquid chromatographic procedure is developed for simultaneous determination of rabeprazole sodium and itopride hydrochloride in solid dosage form. The mobile phase used was a combination of acetonitrile: buffer (35:65 v/v) and the pH was adjusted to 7.0 ± 0.1 by addition of triethylamine. The detection of the capsule dosage form was carried out at 266 nm and a flow rate employed was 1 mL/min. Linearity was obtained in the concentration range of 2 to 16 µg/mL of rabeprazole sodium and 5 to 55 µg/mL of itopride hydrochloride with a correlation coefficient of 0.9992 and 0.9996 respectively. The results of the analysis were validated statistically and recovery studies confirmed the accuracy of the proposed method.

Keywords: Rabeprazole sodium, Itopride hydrochloride, RP-HPLC, Simultaneous estimation.

Introduction

Rabeprazole sodium(RS) is chemically known as 2-({[4-(3-methoxy propoxy)-3-methyl-2-pyridinyl]-methyl}sulfinyl)-1*H*-benzimidazole sodium salt (Figure 1)[1]. It is a latest proton pump inhibitor, which suppresses gastric acid secretion by inhibiting the gastric H^+ K^+ ATPase at the secretary surface of the gastric parietal cell[2]. Literature survey revealed that various analytical methods such as estimation of rabeprazole sodium in tablet dosage form[3], simultaneous determination of rabeprazole and domperidone in pharmaceutical dosage

form[4], development and validation of a dissolution test for rabeprazole sodium[5], liquid chromatographic/mass spectrometric assay of rabeprazole in dog plasma[6], quantitation of citalopram hydrobromide, donepezil hydrochloride and rabeprazole sodium[7] have been reported.

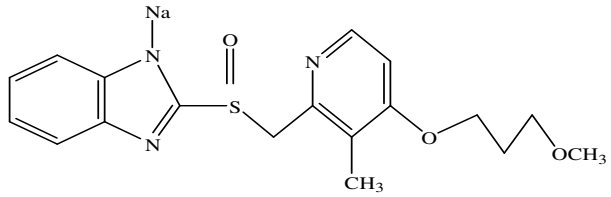

Figure 1. Structure of rabeprazole sodium.

Itopride hydrochloride (IH) is chemically known as *N*–[4-[2-(dimethylamino)ethoxy]-benzyl]-3,4-dimethoxybenzamidehydrochloride (Figure 2)[8]. Itoride hydrochloride (IH) is a gastroprokinetic agent, which increases the release of acetylcholine (Ach) through Dopamine D2 receptor antagonistic action and inhibits decomposing of released Ach through its acetylcholinesterase inhibitory action, resulting in enhancement of gastrointestinal motility[8]. Few methods were reported for the estimation of itopride hydrochloride such as itopride hydrochloride and its metabolites in human serum and urine[9], quantitation of itopride in human serum[10], determination of itopride hydrochloride in presence of its degradation product[11] have been reported.

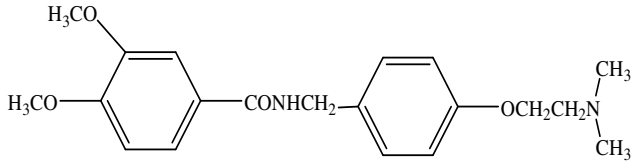

Figure 2. Structure of itopride hydrochloride.

There is no HPLC method reported yet for simultaneous estimation of rabeprazole sodium (RS) and itopride hydrochloride (IH) in combined dosage forms. Therefore, it was thought to develop a simple, fast, accurate reproducible and economical method for the simultaneous estimation of rabeprazole sodium in combination with itopride hydrochloride in solid dosage form.

Experimental

Pharmaceutical grade rabeprazole sodium (Batch No.: SSOL/RAB-Na/015/06, Mfg.Date: Aug-2006, Exp.Date: Jul-2010) and itopride hydrochloride (Batch No. : BIF-004R, Mfg.Date: Aug-2006, Exp. Date: Jul-2010) were supplied as gift sample by Burgeon Pharmaceuticals Pvt.Ltd, Pondicherry (India). The capsule dosage form (Rabium plus, Batch No.: RP-021) was procured from the local market (Label claim: 20 mg of rabeprazole sodium and 150 mg of itopride hydrochloride) marketed by Intus Pharmaceuticals Ltd., Matoda 382210, Ahmedabad, India. The chemicals used- Acetonitrile, water, triethylamine and potassium dihydrogen orthophosphate were of HPLC grade and obtained from Spectrochem, Mumbai, India.

Equipments

Shimadzu HPLC (LC-10 AT *VP*) system; LC system used consist of pump(Model SHIMADZU; LC- 10 AT VP) with universal loop injector(Rheodyne 7725 i) of injection capacity 20 µL. Detector consists of photodiode array detector SPD-10 AVP, SHIMADZU; the reverse phase column used was Luna C_{18} (5µM, 25 cm×4.6 mm *i.d*) phenomenex, USA, at ambient temperature.

Preparation and selection of mobile phase

The preliminary isocratic studies on a reverse phase C18 column with different mobile phase combination of acetonitrile and phosphate buffer pH 7.0±0.1 were studied for simultaneous separation of both the drugs. The optimal composition of mobile phase determined to be acetonitrile:buffer (35:65 v/v) and the pH was adjusted to 7.0±0.1 by addition of triethylamine and was filtered through 0.2 micron membrane filter.

Preparation of standard and sample solutions

RS and IH (10 mg each) were weighed accurately and transferred to separate 100 mL volumetric flasks. Both drugs were dissolved in 100 mL mobile phase to prepare standard stock solution of 100 µg/mL. for analysis of capsule dosage form (Label claim: 20 mg of rabeprazole sodium and 150 mg of itopride hydrochloride); twenty capsules were weighed, with and without shell, their average weight was determined and the content was finely powdered and powder equivalent to weight of one capsule was transferred to a 100 mL volumetric flask and dissolved in 50 mL mobile phase. The solution was shaken vigorously for 15 min and filtered through whatman # 41 filter paper.

Preparation of calibration curves

Solutions of both drugs having different concentrations were prepared by dilution of the standard solutions. These solutions (20 µL) were chromatographed and peak areas were measured. Peak areas were then plotted against the respective concentrations for both RS and IH. From the plots it was found that the linear range of RS was between 2 and 16 µg/mL whereas that for IH was between 5 and 55 µg/mL. Unknown assay samples were quantified by reference to these calibration plots.

Analysis of solid dosage form

Six replicates of the required dilutions were prepared from capsule stock solution and sonicated for 10 min. These solutions (20 µL) were injected for quantitative analysis. The amounts of RS and IH per capsule were calculated by extrapolating the peak area from the calibration plot. Results of analysis are reported in Table 1.

Table 1. Studies of rabeprazole sodium and itopride hydrochloride in marketed formulation.

Drug	Label claim, mg/cap (n=6)	Amount found, mg	% of drug content	S.D.	% COV	S.E.
RS	20	20.356	101.70	0.586	0.583	0.239
IH	150	150.453	100.30	0.694	0.693	0.283

S.D.: Standard deviation, COV: Coefficient of variance, S.E.: Standard error.

Recovery studies

To perform the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method. The results of the analysis are reported in Table 2.

Table 2. Results from the recovery study.

Drug	Amount taken, µg mL^{-1}	Amount added at %	µg mL^{-1}	% Recovery±S.D	%COV
RS	3	80	2.4	100.26±0.176	0.071
		100	3	101.74±0.218	0.089
		120	3.6	102.24±0.515	0.210
IH	22.5	80	18	100.42±0.140	0.057
		100	22.5	101.25±0.236	0.096
		120	27	102.39±0.157	0.064

S.D.: Standard deviation, COV: Coefficient of variance.

Results and Discussion

The HPLC method was found to be simple, accurate, economic and rapid for routine simultaneous estimation of rabeprazole sodium and itopride hydrochloride in combined capsule dosage form at 266 nm. The regression: 0.9992 and 0.9996, intercept: 47245 and 228514 and slope: 23525 and 27245 were found to be for rabeprazole sodium and itopride hydrochloride respectively. Results are reported in Table 3.

Table 3. Results from the study of linearity.

Concentration, µg/ mL	Mean ± S.D.	% COV	S.E.
RS			
2	2.1656 ± 0.026	1.200	0.489
6	6.2974 ± 0.064	1.016	0.414
10	10.9544 ± 0.059	0.538	0.219
14	14.1185 ± 0.084	0.594	0.242
16	16.0526 ± 0.077	0.479	0.195
Slope	23525	-	-
Y intercept	47245	-	-
Correlation coefficient	0.9992	-	-
IH			
5	5.1408 ± 0.0475	0.923	0.376
20	20.0162 ± 0.0858	0.428	0.174
30	30.2945 ± 0.0965	0.318	0.130
40	39.9792 ± 0.0897	0.224	0.091
50	50.2859 ± 0.1243	0.247	0.100
55	55.0449 ± 0.1193	0.216	0.088
Slope	27245	-	-
Y intercept	228214	-	-
Correlation coefficient	0.9996	-	-

Recovery was in the range of 100.42-102.39% and shows the high precision of the developed method (Table 4). The pH optimization is a key factor in proposed method because rabeprazole sodium is rapidly degrades in acidic medium where as itopride (polar) have decrease in retention in low pH may be due to the ionization of itopride hydrochloride at low pH. HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Amongst the various mobile phases used, acetonitrile: buffer in (35:65 v/v) and the pH was adjusted to 7.0±0.1 by addition of triethylamine was found robust with 1 mL/min. flow rate. Mobile phase and flow rate selection was based on peak parameters such as height, tailing, theoretical plates, capacity factor, run time, resolutions *etc*.

Table 4. Intra-day and inter-day precision of HPLC study.

Normal concentration, µg/ mL	Intra-day (n=6)		Inter-day (n=6)	
	Mean ±S.D.	Precision (%COV)	Mean ± S.D.	Precision (% COV)
RS				
3	3.046± 0.018	0.590	2.941 ± 0.026	0.884
5	5.098 ± 0.046	0.902	4.987 ± 0.039	0.782
7	7.258 ± 0.078	1.107	6.989 ± 0.092	1.316
IH				
22.5	22.565 ± 0.085	0.376	22.215 ± 0.146	0.657
37.5	37.608 ± 0.170	0.452	37.349 ± 0.290	0.776
52.5	52.712± 0.280	0.531	52.164± 0.591	1.132

S.D.: Standard deviation, COV: Coefficient of variance.

A typical chromatogram of rabeprazole sodium and itopride hydrochloride is shown in Figure 3. The optimum wavelength for detection was 266 nm at which detector response was obtained best. The average retention time for rabeprazole sodium and itopride hydrochloride was found to be 8.01 ±0.05 min. and 3.82±0.05 min. respectively. According to USP XXIV (621)[12] system suitability tests are an integral part of chromatographic method. They are used to verify reproducibility of the chromatographic system. To ascertain its effectiveness system suitability tests were carried out and its results are shown in Table 5.

Table 5. System suitability study.

Property (n=5)	RS	IH
t_R	8.01	3.82
T_f	0.99	1.27
k'	0.44	1.37
N	9208	4814
Rs	2.05	5.31

t_R: Retention time, T_f: Tailing factor, k': Capacity factor, N: Number of theoretical plates, Rs: Resolution factor.

Hence it can be concluded that the developed RP- HPLC method is an accurate, precise and robust method and can be employed successfully for the estimation of RS and IH in bulk and formulation.

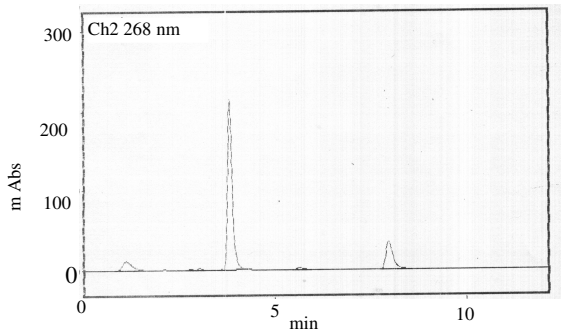

Figure 3. Typical chromatogram of itopride hydrochloride and rabeprazole sodium in marketed formulation.

Conclusions

The proposed high-performance liquid chromatographic method has been evaluated over the linearity, precision, accuracy, specificity and proved to be convenient and effective for the quality control of rabeprazole sodium and itopride hydrochloride in given application. The proposed validated procedure was found to be precise, accurate and linear over the concentration range tested (2.0–55.0 µg/mL) with a correlation coefficient better than 0.9996. Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure and represents a good procedure of rabeprazole sodium and itopride hydrochloride determination in pharmaceutical dosage forms.

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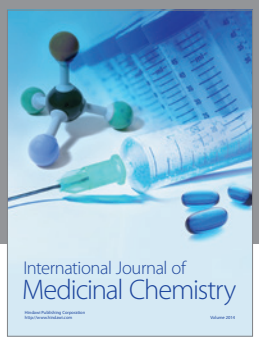

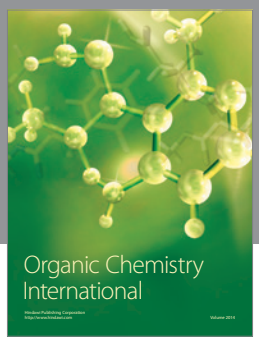

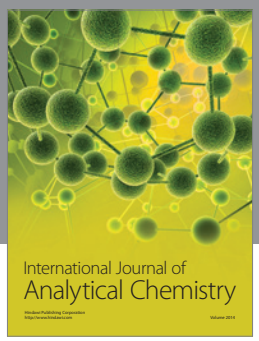

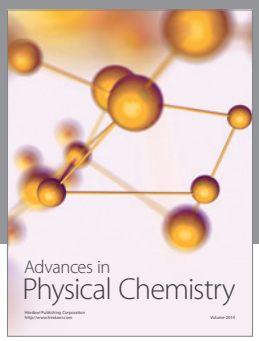

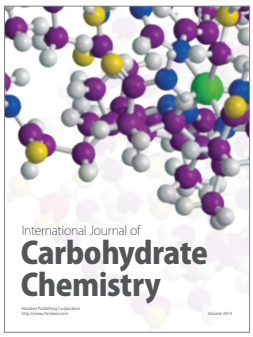

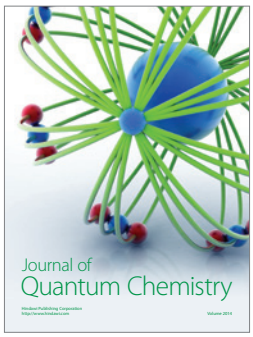

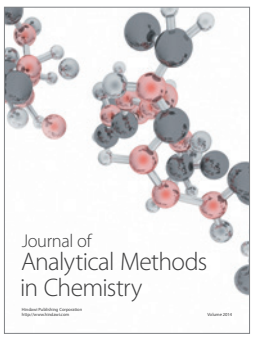

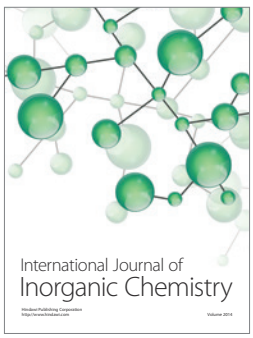

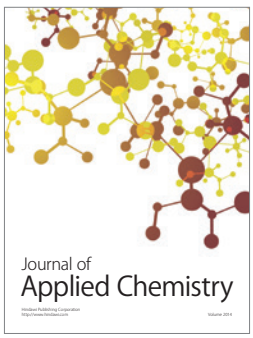

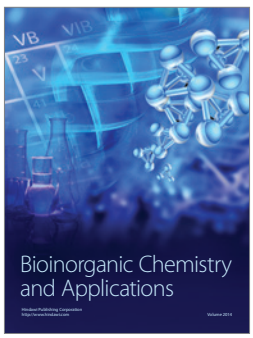

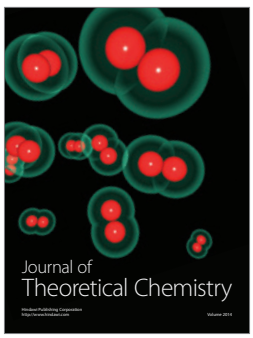

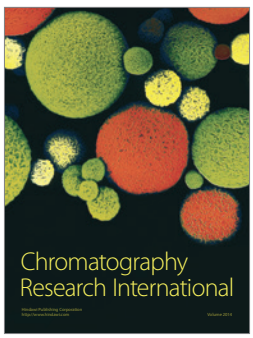

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