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RP-HPLC ANALYSIS FOR SIMULTANEOUS DETERMINATION OF RABEPRAZOLE SODIUM AND DOMPERIDONE FROM TABLET AND BULK DRUG BY INTERNAL STANDARD METHOD

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ABSTRACT

A specific and sensitive high-performance liquid chromatographic method for the simultaneous determination of rabeprazole sodium and domperidone in bulk drug and capsules was developed. Ranitidine hydrochloride was used as an internal standard. Separation of the drugs was carried out on the Luna C_{18} (5µ, 250 mm X 4.60 mm i.d.) at ambient temperature using a mobile phase consisting of ammonium acetate buffer pH 7.4 and acetonitrile (60:40 v/v). Flow rate was 1.0 ml/min with an average operating pressure of 158 kg/cm^2. Quantitation was achieved with UV detection at 286 nm based on peak area with linear calibration curves at concentration ranges 10-50 µg/ml and 15-75 µg/ml for rabeprazole and domperidone, respectively. The method has been successively applied to pharmaceutical formulation. No chromatographic interference from the capsule excipients was found. The method was validated in terms of precision, robustness, recovery and limit of detection and quantitation.

Key words: Rabeprazole sodium, Domperidone, HPLC

INTRODUCTION

Chemically Rabeprazole sodium (RAB) is 2-[[[4-(3-Methoxypropoxy)-3-methyl-2-pyridinyl]methyl] sulfinyl]-1 *H* – benzimidazole sodium **(Figure 1)** [1]. It is newer proton pump inhibitor; claimed to cause fastest acid suppression and to aid gastric mucin synthesis. Rabeprazole is more effective than H_2-blokers in controlling hyperactivity in Zollinger-Ellison syndrome. It is indicated for the treatment of active duodenal ulcer, active benign gastric ulcer, symptomatic erosive or ulcerative gastro-oesopgageal reflux disease (GORD). The usual dose of rabeprazole sodium for adults/elderly in active duodenal ulcer and active benign gastric ulcer is 20 mg once daily in the morning [2-3]. In the literature, colorimetric method and Few HPLC methods for determination of rabeprazole sodium from pharmaceutical formulations and biological samples have been reported [4-7]. Different methods including packed column supercritical fluid chromatography, direct injection column switching-liquid chromatography, capillary electrophoresis, voltammetric behaviour at a glassy carbon electrode have been described for the determination of rabeprazole or its sodium salt in bioequivalence study and in pharmaceutical preparations. [8-11]. Determination of rabeprazole and its active

metabolite, rabeprazole thioether in human plasma by column switching HPLC and its application to pharmacokinetic study has been reported [12].

Domperidone (DOM) is chemically 5-chloro-1-[1-[3-(2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)propyl]-piperidin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one **(Figure 1)** [13]. It is official in British pharmacopoeia and European pharmacopoeia [14, 15]. It is a peripheral dopamine receptor antagonist and shows lower ceiling antiemetic and prokinetic actions. As Domperidone crosses blood brain barrier (BBB) poorly; therefore, the extrapyrimidal side effects are rare, but hyperprolactemia can occur [16]. Several, HPLC and LC-MS methods for quantitative estimation of domperidone in human plasma and rat plasma have been reported [17-21]. Few HPLC methods for quantitative estimation of domperidone single and combination with other drugs have been reported [22-25]. Several UV- spectrophotometric methods for determination of domperidone alone and in combination with other drugs omeprazole, cinnarizine in pharmaceutical dosage forms have been reported [26-28]. Several HPTLC methods have also been reported for quantitative estimation of domperidone alone and combination with cinnarizine, ranitidine [29-32]. The present work presents, a new method for simultaneous determination of rabeprazole sodium and domperidone in capsule using reverse phase HPLC. The method is simple, reduce the duration of analysis and suitable for routine determination of two drugs.

EXPERIMENTAL

Materials: Rabeprazole sodium and domperidone obtained as gift samples from Torrent Pharmaceuticals Ltd., Ahmedabad India, were used as working standards. Methanol and Ammonium acetate were used as solvents to prepare the mobile phase. All the chemicals used were of Analytical reagent grade (S.D.Fine. Chem. Ltd., Mumbai)

Instrumentation: The HPLC system consisted of a pump LC-10AT *vp* equipped with a Rheodyne (7725 i) injection system with a 20 µL loop. Detection was accomplished with an Photodiode array detector SPD-10 A*vp*, SHIMADZU. The column used was Luna C_{18} (250 mmX 4.6 mm, 5.0µ) phenomenex, USA. Different mobile phases were tested in order to find the best conditions for separating both the drugs simultaneously. The optimal composition of mobile phase was determined to be Ammonium acetate buffer pH 7.4: Acetonitrile: (60:40, v/v). The flow rate was set to 1.0 mL min^{-1} and UV detection was carried out at 286 nm.

Preparation of mobile phase: The mobile phase consists of mixture of 0.05mM Ammonium acetate buffer (Buffer pH adjusted to 7.4 using 0.1 N sodium hydroxide) and acetonitrile in the ratio 60:40 (v/v). The mobile phase filtered through a 0.45 µm membrane filter and degassed prior to use.

Preparation of standard solutions: Stock standard solution was prepared by dissolving 0.10g of RAB and 0.15g of DOM in 100 ml methanol. The stock solution was protected from light by using amber colored volumetric flasks and keeping the solution at room temperature. The standard solutions were prepared by dilution of the stock solution with methanol to reach a concentration range 10-50 µg/ml and 15-75 µg/ml for RAB and DOM, respectively and each concentration contains 20 µg mL^{-1} of ranitidine hydrochloride (RAN) as an internal standard. The injections of 20µl were made three times for each concentration for RAB and DOM, respectively and chromatographed under the conditions descried above. The peak area ratios of analyte to internal standard were plotted against the corresponding drug concentrations to obtain the calibration graphs.

Preparation of sample solutions: To determine the content of RAB and DOM simultaneously in conventional capsules (label claim: 20mg RAB and 30mg DOM per capsule), the contents of twenty capsule were weighed, their mean weight determined and they were finely powdered. The powder equivalent to 20mg of RAB and 30mg DOM was weighed and transferred into a 100ml volumetric flask containing 50ml methanol, sonicated for 30 min and diluted to 100ml with methanol. The resultant solution was centrifuged for 5 min and then filtered through whatman filter paper no. 41. Appropriate aliquots were subjected to the above methods and the amount of rabeprazole sodium and DOM were determined.

Method validation [33-47]: The method was validated in compliance with ICH guidelines for the following parameters.

Precision: Precision studies were performed by using standard solutions containing both the drugs with the concentrations of drugs covering the entire calibration range. The precision of the method in terms of intra-day variation (%RSD) was determined, by analyzing RAB and DOM standard drug solutions in the calibration range three times on the same day. Inter-day precision (%RSD) was assessed by analyzing the standard drug solutions within the

calibration range on three different days over a period of one week.

Limit of detection and limit of quantitation: The limit of detection (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The limit of quantitation (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assay for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. The sensitivity of measurement of RAB and DOM by the use of the proposed method was estimated in terms of the LOQ and LOD. The LOQ and LOD were calculated by the use of the equation LOD = 3 X N/B and LOQ = 10 X N/B, where N is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

Recovery studies: Recovery studies were carried out by applying the method to drug sample to which known amount of standard RAB and DOM corresponding to 80, 100 and 120% level was added. At each level of the amount three determinations were performed and the results obtained were compared with expected results.

Specificity: Specificity is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. Complete separation and resolution of RAB, DOM and internal standard ranitidine hydrochloride, with good peak shapes and without any apparent shoulders confirms specificity of the method.

Ruggedness: The ruggedness of the HPLC method was evaluated by carrying out the analysis of the sample solution using the same chromatographic system and the same column by different analyst.

Stability of stock solution: Standard solution containing RAB (20 µg/mL) and DOM (30 µg/mL) with RAN (IS) (20 µg/mL) was prepared from stock solution and stored at room temp for 48 h. Then, solution was injected into HPLC system at time interval 3, 24 and 48 h.

System Suitability Test: The system suitability of the HPLC method was determined by the complete separation of RAB and DOM along with other parameter like retention time (t_R), Capacity factor (k), tailing/asymmetrical factor (t) etc.

RESULT AND DISCUSSION

Linearity: Linearity was checked by preparing standard solutions of both RAB and DOM at five different concentration levels in the same volumetric flasks using their respective stock solutions. The calibration curves of RAB and DOM were drawn in the concentration range of 10-50µg/ml and 15-75µg/ml respectively. The regression equation, for calibration curve of RAB is y= 0.0569 x + 0.0135 and for DOM it is y=0.0422x +0.1127. The results of the linearity studies are as shown in **Table 1.**

Assay of RAB and DOM: Sample solutions of RAB and DOM were prepared in methanol. The working concentration for the determination of the assay of both drugs was 20 and 30 µg/ml for RAB and DOM respectively. The concentration of internal standard was maintained at 20µg/ml in each combined solution of RAB and DOM that were used for quantitative studies. The results of the assay are as shown in **Table 2.**

Precision: The precision of the method in terms of intra-day variation (%RSD) was determined by analyzing RAB and DOM standard solutions (10-50 µg/ml and 15-75 µg/ml) three times on the same day. Inter-day precision (%RSD) was assessed by analyzing these solutions (10-50 and 15-75 µg/ml) on three different days over a period of one week. The % RSD values depicted in **Table 3** shows that proposed method provides acceptable intra-day and inter-day variation of rabeprazole and DOM.

Recovery studies: The proposed method when used for extraction and subsequent estimation of RAB and DOM from pharmaceutical dosage form after spiking with additional drug afforded recovery of 98-102% and mean recovery for RAB and DOM from marketed formulation are listed in **Table 4.**

Ruggedness: The ruggedness of the proposed method was evaluated by performing the determinations by two different analysts, the assay results (n=5) were found to give 99.56 %, 98.55 % of RAB and 100.70 %, 99.77 % of DOM. The results are shown in **Table 5.**

Specificity: The mobile phase designed for the method resolved both the drugs very efficiently; shown in the **Figure 2.** The t_R value of RAB and DOM was found to be 6.35 and 10.13, respectively.

The typical absorption spectrum of RAB and DOM is shown in **Figure 3.**

Stability of stock solution: Standard solution of RAB (20 µg/mL) and DOM (30 µg/mL) with RAN (IS) (20 µg/mL) was prepared from stock solution and stored at room temp for 48 h. Then, solution was injected into HPLC system at time interval 3, 24 and 48 h. The results of the stability studies are indicated in **Table 6.**

System Suitability Tests: The System Suitability Tests of the HPLC method was determined by the complete separation of RAB and DOM along with other parameters like retention time (t_r), capacity factor (k), tailing /asymmetrical factor(t) etc. The specificity of the HPLC method is illustrated in **Figure 2** where complete separation for RAB and DOM was noticed in presence of tablet excipients. The average retention time of RAB and DOM were found to be 6.35±0.02 and 10.13±0.08 min, rspectively for six replicates. The peaks obtained were sharp and have clear baseline separation. The values of system suitability tests are shown in **Table 7.**

Table 1. Linearity data

Prameters	RAB	DOM
Beer's law limit (µg/ml)	10-50	15-75
Correlation Coefficient	0.9996	0.9997
Y-Intercept (A)	0.0135	0.1127
Slope(B)	0.0569	0.0422

Table 2. Results of the estimation of RAB and DOM in capsule

Prameters	RAB	DOM
Labeled Claim (mg)	20	30
Amount found ±SD (n = 5)	20.14± 0.209	29.94± 0.444
% RSD	1.04	1.48

Table 3. Intra-day and inter-day precision of RAB and DOM

Drug	Concentration [µg/mL]	Intra-day precision		Inter-day precision	
		Concentration found [µg/mL] Mean ± S. D.	% RSD [n = 3]	Concentration found [µg/mL] Mean ± S. D.	% RSD [n = 3]
RAB	20	19.78 ± 0.16	0.821	19.89 ± 0.10	0.539
	30	29.96 ± 0.25	0.855	30.09 ± 0.29	0.979
	40	40.23 ± 0.39	0.973	40.74 ± 0.28	0.700
DOM	30	29.83 ± 0.29	0.982	30.16 ± 0.17	0.578
	45	44.87 ± 0.43	0.969	44.87 ± 0.37	0.830
	60	60.39 ± 0.45	0.757	60.67 ± 0.19	0.324

Table 4. Results from recovery studies.

Drug	Initial amount [mg]	Amount added [mg]	Amount recovered Mean ± S. D. [mg] (n = 3)	% Recovery	%RSD
RAB	20	0	19.99 ± 0.12	99.96	0.61
	20	16	16.03 ± 0.10	100.21	0.66
	20	20	20.08 ± 0.08	100.40	0.28
	20	24	24.06 ± 4.06	100.25	0.84
DOM	30	0	30.10 ± 0.15	100.36	0.51
	30	24	24.39 ± 0.15	101.63	0.62
	30	30	30.60 ± 0.11	102.01	0.36
	30	36	36.68 ± 0.42	101.34	1.15

Table 5: Results of ruggedness

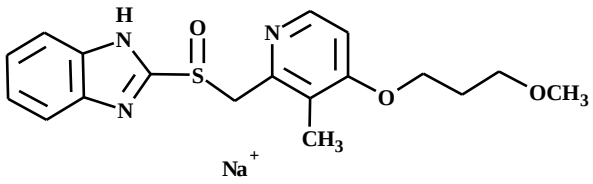
	RAB		DOM	
	Amount found [%]	%RSD (n=5)	Amount found [%]	%RSD (n=5)
Analyst I	99.56	1.67	98.55	0.48
Analyst II	100.70	0.69	99.77	0.88

Table 6: Results of stability studies

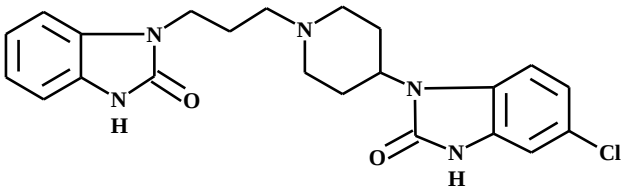
Drug	%Drug loss ± SD [%]		
	3 h	24 h	48 h
RAB	No loss	3.550 ± 0.879	5.748 ± 1.175
DOM	No loss	1.271 ± 0.690	3.474 ± 1.125

Table 7: Results of system suitability tests

System Suitability Parameters	RAB	DOM
Retention Time (t_R)	6.398	10.138
Capacity Factor (K^1)	2.03	3.80
Theoretical Plate (N)	7813	7954
Tailing Factor (As)	1.51	1.61
Resolution Factor (Rs)	9.98	10.05



Rabeprazole sodium



Domperidone

Figure 1. Structures of analytes to be analyzed

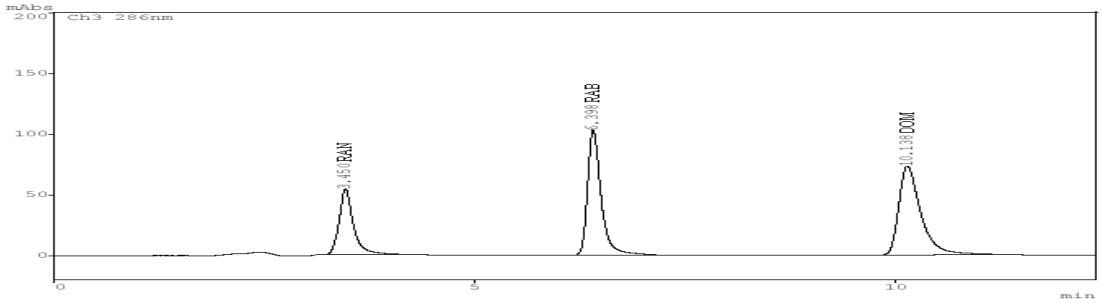


Figure 2. Chromatogram of standard RAB (20 µg/mL): R_f: 6.398, DOM (30 µg/mL): R_f: 10.138 and RAN(internal standard 20 µg/mL): R_f : 3.45, measured at 286 nm, Mobile phase: ammonium acetate buffer pH 7.4 and acetonitrile (60:40, v/v).

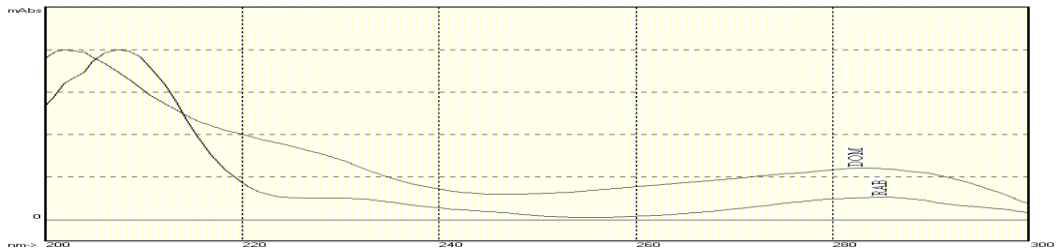


Figure 3 Typical Absorption Spectra of RAB and DOM Drug Solutions

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