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Development and Validation of New Analytical Method for Simultaneous Estimation of Domperidone and Rabeprazole in Pharmaceutical Dosage Forms

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ABSTRACT

A rapid, precise, accurate, specific and simple RP-HPLC method was developed for simultaneous estimation of Domperidone(DOM) and Rabeprazole(RAB). Separation of DOM and RAB was achieved within a single chromatographic run on a XBridge^{TM} column 5µm 4.6x250mm with UV detection at 284nm, under isocratic conditions, using Acetonitrile and Ammonium acetate buffer(P^H – 8.0±0.1) in 40:60 ratio. Validation parameters were performed to demonstrate linearity, accuracy, precision, robustness, ruggedness, LOD & LOQ in accordance to ICH guidelines. The current method demonstrates good linearity over the range of 10-50µg mL^{-1} for both DOM and RAB with intra-day and inter-day precision, expressed as the relative standard deviation (RSD), of replicates is <2.0% and accuracy in the range of 98–102%. The developed RP-HPLC method was innovation, suitable for detecting both DOM and RAB simultaneously in pure form and in pharmaceutical preparation.

Keywords: Rabeprazole, Domperidone, ICH, RP-HPLC.

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INTRODUCTION

Domperidone [1] is chemically, 6-chloro-3-[1-[3-(2-oxo-3H-benzimidazol-1-yl)propyl] piperidin-4-yl]-1H-benzimidazole-2-one. It is a synthetic benzimidazole compound that acts as a dopamine receptor antagonist. Domperidone is an antiemetic agent. Official in Indian pharmacopoeia [2] and British Pharmacopoeia [3] and standard limits - 100.5 ± 9.9%. Domperidone is a butyrophenone derivative and is a potent D_2 receptor antagonist that does not enter the CNS (Blood Brain Barrier) to a significant extent. However, it inhibits D2 Receptors in the CTZ (Chemo Trigger Zone) and causes prolactin release from the anterior pituitary. The central anti-dopminergic (D_2) action of domperidone on CTZ is clearly responsible for its antiemetic property. Literature survey reveals HPLC [4-8] methods for determination of DOM in pharmaceutical dosage forms. Rabeprazole is chemically, 2-[[[3-Methyl-4-(3-methoxypropoxy)-2-pyridinyl]methyl]sulfinyl]-1H- benzimidazole. It is a proton pump inhibitor and used for the treatment of peptic ulcer or GERD (Gastroesophageal Reflux Disease). It is not official in any pharmacopoeia. The combination of Rabeprazole and Domperidone is very useful in the treatment of patients suffering from GERD (Gastroesophageal Reflux Disease) , an open, prospective, non-comparative study was carried out among 50 adult patients of either sex attending gastroenterology OPD of a leading, tertiary-care teaching hospital in Mumbai with the clinical diagnosis of GERD. This combination may also improve the quality of life of patients suffering from GERD. [9] Literature survey reveals HPLC [10-12] methods for determination of RAB in pharmaceutical dosage forms and in human plasma. The combination of these two drugs is not official in any pharmacopeia; hence no official method available for estimation of RAB and DOM in their combined dosage forms.

MATERIALS AND METHODS

Instruments and reagents:

The Agilent 1120 Compact LC HPLC system consisting of gradient pump, rheodyne injector, UV wavelength detector, Standard cell(4MPa or 40barr) and agilent syringe was used. The separations were achieved on a XBridgeTM column 5μm 4.6x250mm with UV detection at 284nm. Analytical weighing balance (Shimadzu AUX 220) was used for weighing, sonicator (EQUITRON-230VAC, 50Hz), vaccum pump (SUPER FIT 110336 of Millipore), Millipore filtration kit (ARSONS) for solvents and sample filtration were used throughout the experiment. The EZChrome Elite software-single channel was used for acquisition, evaluation and storage of chromatographic data. Domperidone and Rabeprazole was received as gift samples from Metrochem, Jeedimetla, Hyderabad. HPLC grade Acetonitrile (Merck), Pharmaceutical formulation ROPRAZ[TM]-D SR capsules (label claim 20 mg DOM and 30 mg RAB) batch no.9744784, Mfg. Lic. No. MNB/05/101 Manufactured by Acme Formulations Private Limited, Marketed by RANBAXY laboratories limited was used in the HPLC. HPLC grade water obtained in-house by using Direct-Q®3 with pump (Elec. Ratings: 100-230V of 50-60Hz 100VA) water purification system (made in France) were used.

Optimised Chromatographic conditions:

After several trials with the different combination and ratio of solvents, the mobile phase Acetonitrile : Ammonium acetate buffer (40:60 v/v, pH 8.0±0.1) was selected, because it was found that it ideally resolve the peaks with retention time (R_t) DOM and RAB at 4.6 and 6.0 min respectively and the same is shown in Fig Wavelength was selected by scanning all standard drugs over a wide range of wavelength 200 nm to 400 nm. Both the components show reasonably good response at 284nm.

Preparation of Standard Drug Solutions:

Accurately weighed 50 mg each of DOM and RAB in 50 mL volumetric flasks separately and dissolved in methanol and the volume were made up to the mark with the same solvent. From the above 0.1 , 0.2, 0.3, 0.4, 0.5 mL of each DOM and RAB solutions were pipette out into two separate 10 mL volumetric flasks and volume was made up to the mark with the the mobile phase (Acetonitrile:Acetate buffer = 40:60) used. This gave the concentration of 10, 20, 30, 40, 50 µg mL^{-1} of DOM and RAB. These five dilutions DOM and RAB were prepared and is estimated in HPLC.

Preparation of mobile phase:

0.02M Ammonium acetate solution was prepared by dissolving 1.54g in 1000 mL of HPLC grade water. The pH of the resulting solution was adjusted to 8.0 ± 0.1 by using 1% KOH. HPLC experiments were carried out using binary pump A containing Acetonitrile and pump B containing Ammonium acetate buffer with 40:60 ratio.

Preparation of Calibration Curves

In a series of 10 ml volumetric flask several dilutions of RAB and DOM (10-50µg/ml) were prepared using mobile phase as solvent . Each solution was injected into HPLC system and the chromatograms were recorded. The peak areas of both drugs were calculated and the respective calibration curves were plotted against ratio of area under curve and concentration of drug.

The equations of the regression lines obtained are

For RAB:
y = 56931x + 44045
R^2 = 0.999

For DOM:
 y = 56658x + 86704
R^2 = 0.999

Estimation of Rabeprazole Sodium and Domperidone in tablet:

Twenty capsules each containing 30 mg of DOM and 20 mg of RAB were weighed and powdered for further study. The powder equivalent to 30 mg of DOM and 20 mg of RAB were accurately weighed and transferred to 100 mL volumetric flask containing 50 mL of the methanol and sonicated for 10 min. The above solution was carefully filtered through Whatmann filter paper (No. 41) and the residue was washed with 3 portions of 10 mL of solvent. The volume was made up to the mark with methanol. From this solution, required dilutions for HPLC method were prepared within the linearity range using mobilephase as solvent.

Method Validation

As per the ICH guidelines, the method validation parameters checked were specific, linearity, accuracy, precision, limit of detection, limit of quantitation.

Specificity:

Specificity is the ability to asses unequivocally the analyte in the presence of components which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures. Solutions of standard and Sample are prepared as per test method and injected into the chromatographic system.

Blank interference:

A study to establish the interference of blank was conducted. Mobile phase was injected as per the test method.

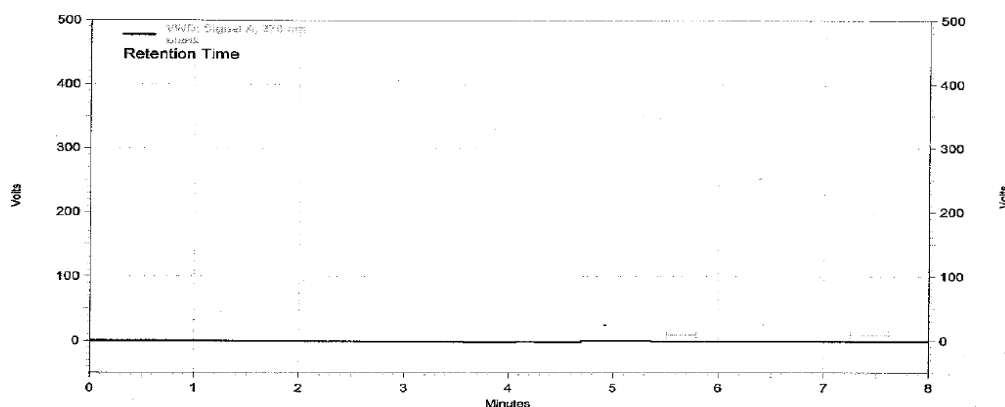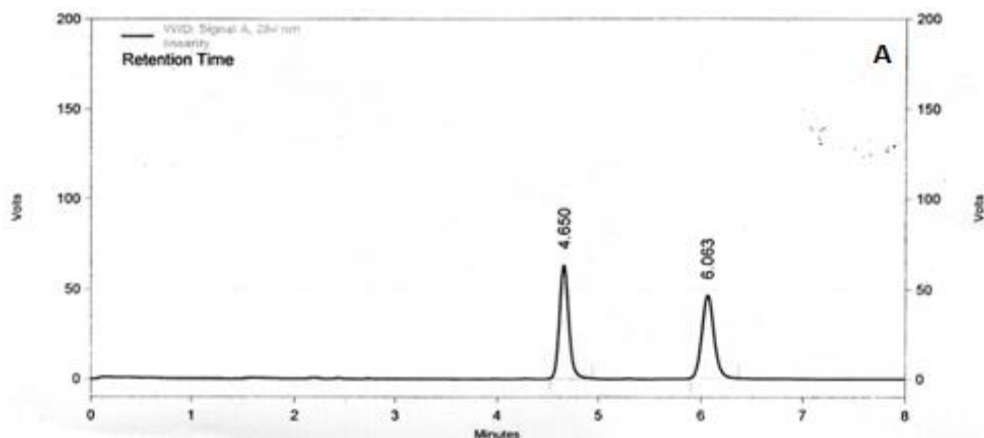Chromatogram of blank should not show any peak at the retention time of analyte peak.


Fig 4.1 Chromatogram for Blank

Fig 4.2 Chromatogram showing Retention Time (R_t) of DOM (4.667min) and mL^{-1} of RAB (6.043 min)

Linearity:

Linearity of the proposed HPLC method for determination of DOM and RAB were evaluated by analysing a series of different concentrations of standard drug. In this study five concentrations were chosen ranging between 10-50µg mL^{-1} for both DOM and RAB. Each concentration was injected three times and obtained information on variation in the peak area response of pure analytes was plotted against corresponding concentrations and result was shown in Table 5.1. The linearity of the calibration graphs was validated by the high value of correlation coefficient, slope and the intercept value was shown in Fig.5.4. The optimized method parameters are given in the Table 5.2.

RABEPRAZOLE LINEARITY

$y = 56931x + 44045$
$R^2 = 0.999$
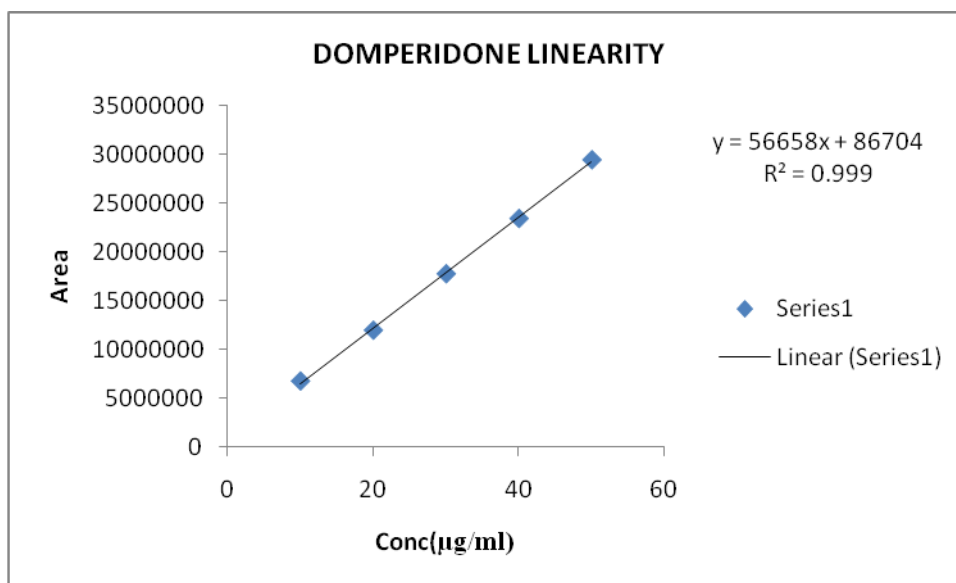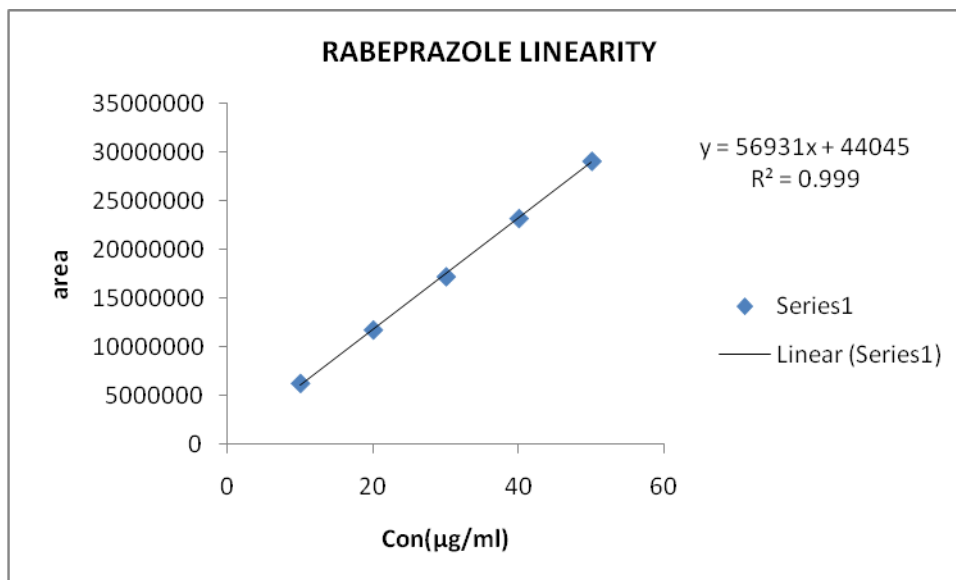
Precision:

Precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample.

Precision was demonstrated by repeatability and intermediate precision measurements of peak area and peak symmetry parameters of HPLC method for each title ingredients. The repeatability (within-day in triplicates) (Fig.5.5) and intermediate precision (for 2 days) (Fig.5.6) were carried out at five concentration levels for each compound. Triplicate injections were made and the obtained results within and between the days of trials were in acceptable range. The value of %RSD for RAB and DOM were found to be 0.5474 and 0.5904 respectively for intra-day studies. The values for inter-day studies were 0.8454 and 0.407 respectively. This shows that values are not more than 2%, indicates that the developed method is precise.

Accuracy:

Accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its linearity range. Accuracy was performed in three different levels, each level in triplicate for DOM and RAB using standards at 80%, 100% and 120% (Fig. 5.7). Each sample was analysed in triplicate for each level.

The mean recoveries were found in the range of 98.5 – 101.87 %, by which we can say the method was accurate.

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

It is calculated according to ICH recommendations where the approach is based on the signal-to-noise ratio. Chromatogram signals obtained with known low concentrations of analytes were compared with the signals of blank samples. A signal-to-noise ratio 3:1 and 10:1 was considered for calculating LOD and LOQ respectively. The values of LOD and LOQ were given in table

RESULTS AND DISCUSSION

For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for RAB and DOM in the concentration range of 10-50 µg/ml. It was also found to be accurate, precise with acceptable values of LOD and LOQ. Table 3 shows the validation parameters for the method.

Table 3: HPLC Parameters

HPLC Parameters		HPLC	
		DOM	RAB
Calibration range (µg mL^{-1})		10-50	10-50
Linearity Correlation coefficient(R^2)		0.999	0.999
Precision (%RSD)	Interday	0.52-1.04	0.06-1.03
	Intraday	0.32-1.48	0.2-1.7
Accuracy	Amount labelled (mg)	20	30
	Amount found (mg)	20.08	29.88
	%Recovery ±RSD	100.40±0.583	99.61±0.212
LOD		0.75	0.887
LOQ		2.29	2.688
Resolution		7.124	
Theoritical plates		12521	12734
Tailing factor		1.15	1.13

CONCLUSION

The method described for simultaneous estimation of Rabeprazole Sodium and Diclofenac Sodium are found to be simple, sensitive, accurate, precise, rapid, economical and rapid. Hence method could be successfully employed for routine analysis of RAB & DOM in their combined tablet dosage form.

ACKNOWLEDGEMENTS

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