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Research Article

METHOD DEVELOPMENT AND VALIDATION OF HPLC FOR THE SIMULTANEOUS DETERMINATION OF DOMPERIDONE AND RABEPRAZOLE

M. Rama Chandraiah and Y.V Rami Reddy*
Department of Chemistry, Sri Venkateswara University, Tirupathi - 517 502, A. P, India.
Email:madduriswu@gmail.com

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Abstract

The use of simultaneous determination of domperidone and rabeprazole, infixed dose combination products. The absorbance values at 253.2 nm and 266.4 nm of first derivative spectrum was used for the estimation of domperidone and rabeprazole, respectively without mutual interference. This method obeyed beers law in the concentration range of 9-45 µg/ml and 6-30 µg/ml for both domperidone and rabeprazole, respectively. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method.

Keywords: Domperidone, Rabeprazole, simultaneous determination DOM, RAB.

Introduction

Domperidone (DOM) is peripheral dopamine antagonist. It is official in British Pharmacopoeia and EuropeanPharmacopoeia. Chemically it is 5-chloro-1-[1-[3-(2-oxo-2,3-dihydro-1H-benzimidazol-1-yl)propyl]-piperidin-4-yl]-1,3-dihydro-2H benzimidazol-2-one. Literature described HPLCmethod, HPTLC method, and LC-MS method, extractive spectroscopic method for its determination in plasma, serum and pharmaceutical preparations when present with other drugs.Rabeprazole (RAB) is proton pump inhibitor and is not official in any of the Pharmacopoeias and is chemically 2-({[4-(-3-mehotxypropoxy)-3-methyl-2- pyridyl] methyl} sulfinyl)-1H-benzimidazolesodium. HPLC, Capillary electrophoresis, and LC-MS method have been reported for the estimation of RAB in plasma, and in pharmaceutical preparations. HPLC and dual wavelength Spectrophotometric method were reported for the simultaneous estimation of DOM and RAB in combined dosage forms. The present paper describes a

simple, rapid, accurate and responsible method for the simultaneous estimation of DOM and RAB in bulk and dosage forms by HPLC method.

1. Materials and Methods

Both DOM and RAB were from Astron Pharmaceuticals. Sodium hydroxide AR Grade was procured from Rankem Chemicals.

1.1. Instrumentation:

Derivative spectrophotometric method has been developed for the simultaneous determination of domperidone (DOM) and rabeprazole (RAB) by using HPLC methodology. The derivative UV spectra of standard and test solutions were recorded in 1 cm quartz cells using a Shimadzu UV/Vis-1700 double beam UV/Vis spectrophotometer with a fixed slit width of 2 nm.

1.2. Chromatographic Condition:

The mobile phase was prepared by mixing sodium chlorate buffer (pH 2.5), acetonitrile and isopropyl alcohol in the ratio of 85:1:14 %.The mobile phase was filtered using 0.45 µm Nylon filter and degassed in a sonicator for 10 minutes. The flow rate was 1.5 ml.min−1.Column was maintained at 25 ºC. The injection volume to carry out the chromatography was set at 20µl. Under these conditions Domeperidone and Rabeprazole eluted at 4.6 minute. The total run time was 30 minutes.

1.3. Method Development:

The standard stock solutions of 1.5 mg/ml of domperidone and1.0 mg/ml of rabeprazole in methanol were prepared.Further dilutions were made in 0.1N NaOH to obtain concentrations ranging from 9-45 µg/ml for DOM and 6-30µg/ml for RAB.The absorbance of resulting solutions was measured at 266.4 and 253.2 nm for DOM and RAB and the calibration curves were plotted at these wavelengths. The overlain spectra DOM and RAB showed that the absorption maxima of DOM and RAB lie in close proximityand at absorption maxima of one, another exhibits substantial absorbance. This clearly indicates the existence of spectralinte rference in estimation of DOM and RAB. To over come this,spectra of these two drugs were derivatised to first order between 200-400 nm with Äë= 2 nm.The overlain first derivative spectraof DOM and RAB reveal that RAB concentration is proportional to the first

derivative signals at 266.4 nm (zero crossing point for DOM) and DOM can be estimated at 253.2 nm(zero-crossing point for RAB).

1.4. **Linearity:**

Standard stock solutions were prepared by dissolving 37.5 mg domperidone and 25 mg of rabeprazole in 25 ml volumetric flask and the volume was made up with methanol to get a concentration of 1.5 mg/ml and 1.0 mg/ml.From this, suitable dilutions were made in 0.1N NaOH to get the working standard solutions of 9-45 µg/ml for DOM and 6-30 µg/ml for RAB. The absorbances of the derivatised spectra were measured at 253.2 nm and 266.4 nm for DOM and RAB, respectively. Six replicate analysis were carried out. Absorbance Vs concentration were plotted to obtain the calibration graph. Both drugs obey the Beer.s law with the above concentration range with R2 value of 0.9993 and 09995 for DOM and RAB, respectively.

1.5. Limit of detection and limit of quantitation:

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations, intercept was calculated and the standard deviation of the y intercept was computed. From these values, the parameters Limit of Detection (LOD) and Limit of Quantitation LOD and (LOQ) were determined on the basis of response and slope of the regression equation.

1.6. Analysis of synthetic mixture of DOM and RAB

Solution in0.1N NaOH containing various proportions of DOM and RAB were prepared and their first derivative spectra were recorded. From the derivative spectra, the absorbance at 253.2 nm and 266.4 nm were noted for the estimation of DOM and RAB, respectively. From these absorbance values, the concentrations of DOM and RAB were determined using calibration graph (Table1).

Table 1: Analysis of Synthetic Mixtures of Domperidone and Rabeprazole.

Sample No.	**Concentration of DOM (µg/ml)**		**% Recovery**	**Concentration of RAB(µg/ml)**		**% Recovery**
	Theoretical	**Experimental**		**Theoretical**	**Experimental**	

1	9	8.72	96.8	6	5.9	100.7
2	18	17.88	99.3	12	12.15	101.6
3	27	26.70	99.7	18	17.92	98.1
4	36	36.22	100.8	24	23.69	100.3
5	45	44.06	97.9	30	30.1	98.8

1.7. Recovery studies (accuracy):

It is defined as the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It is measure of exactness of analytical method. Accuracy should be expressed as % recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals. Accuracy should be established across the specified range of the analytical procedure. It was determined by calculating the recovery of domperidone and rabeprazole by standard addition method. To the fixed amount of solution (18 µg/ml of domperidone and 18 µg/ml of rabeprazole) an increasing aliquots from working standard solution of domperidone and rabeprazole were added. The solutions were measured at 253.2 nm for domperidone and 266.4 nm for rabeprazole and % recovery of the sample were calculated (Table 2 and 3).

Table 2: Accuracy Data of Determination of Rabeprazole in the Presence of Domperidone (18ìg/ml).

Amount of Domperidone (µg/ml)	**Amount of added Rabeprazole (µg/ml)**	**Total amount found Mean ± S.D.**	**Accuracy (%)**
18	6	5.9±0.14	100.7
18	12	12.15±0.38	101.6
18	18	17.92±25	98.1
18	24	23.69±0.25	100.3
18	30	30.1±0.39	98.8

Table 3: Accuracy Data of Determination of Domperidone in the Presence of Rabeprazole (12ìg/ml).

Amount of added Rabeprazole (µg/ml)	Amount of added Domperidone (µg/ml	Total amount found Mean ± S.D.	Accuracy (%)
12	9	8.72±0.23	96.8
12	18	17.88±0.30	99.3
12	27	26.7±0.51	99.7
12	36	36.22±0.42	100.8
12	45	44.06±0.55	97.8

2. Results and Discussion

Absorption spectra of domperidone and rabeprazole showed overlapping peaks that interfere with the simultaneous determination of this formulation. Derivative spectroscopy, based on a mathematical transformation of the spectra zero-order curve into the derivative spectra, allows a fast, sensitive and precise resolution of a multicomponent mixture and overcomes the problem of overlapping of a multicomponent system. Derivative spectroscopy on the basis of zerocrossing measurements involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectra of individual components, which should be only a function of the concentration of other component. The spectroscopic parameters including derivative order, wavelength and values should be optimized to obtain maximum resolution, sensitivity and reproducibility. In this study first derivative technique was used to resolve the spectral overlapping. Zero-crossing points of 200- 300 nm is presented in. The optimums D1values without interference for domperidone and rabeprazole were 253.2 and 266.4 nm, respectively.

The linearity of the method was established form first-derivative spectra by measurement of the absorbance of standard solutions containing varying concentrations of each compound in the presence of constant concentration of the other one. The calibration curves were constructed by plotting the D1value against domperidone and rabeprazole

concentration at the zero-crossing wavelength of rabeprazole (253.2 nm) or domperidone (266.4 nm), respectively. The analytical results of synthetic mixtures obtained are summarized in Table 1. The linearity of the calibration curves and the adherence of the method to Beer.s law are validated by the high value of the correlation coefficient and the value of intercept on ordinate which is close to zero.

The limit of detection that was found to be 0.68 ìg/ml and 0.45 ìg/ml for domperidone and rabeprazole. The accuracy and precision were determined by using synthetic mixture of domperidone and rabeprazole sodium in the laboratory. The mean recoveries and SD are illustrated in Tables 2 and 3. Data of these tables showed a good accuracy and precision over the entire concentration range .The data indicate that the proposed derivative spectroscopic method is highly precise during one analysis and between different runs. The percentage of recovery in each case was calculated. The results obtained from the recoveries of both drugs (Tables 2 and3) showed excellent accuracy. The influence of excipients was studied by mixing two formulations containing 9 ìg/ml of domperidone and 6ìg/ml of rabeprazole. No interference was observed from the presence of excipient in the amounts, which are commonly present in tablet dosage forms. Study of stability of domperidone and rabeprazole in the solutions during analysis showed that analytes were stable at least for 72 h in solutions. The validation of results is summarized in Table 4.The proposed method was successfully applied to analyze preparation containing domperidone and rabeprazole.

Table 4: Validation of the Proposed Method.

.**Sample No.**	**Parameters**	**Experimental Values**	
		DOM	**DOM**
1	Precision (%C.V.) 1. Repeatability 2. Intraday precision 3. Interday precision	0.54 % 1.27-2.47% 1.90-3.25%	1.01 % 1.44-2.41% 2.23-3.46 %
2	Linearity Range	9-45 µg /ml	6-30µg/ml
3	Accuracy(%Recovery)	96.8-100.8%	98.1-101.6%
4	Limit of Detection(µg/ml)	0.68µg/ml	0.45µg/ml

5	Limit of Quantification(µg/ml)	2.00µg/ml	1.36 µg/ml
6	Correlation coefficient	0.9993	0.9995

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Corresponding Author:

Y.V Rami Reddy*

Email: madduriswu@gmail.com