Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(2): 186-196

ISSN No: 0975-7384

Simultaneous estimation and validation of Rabeprazole Sodium and Diclofenac Sodium in capsule dosage form

Raj K. Prasad[a*] and Rajesh Sharma[a]

[*] *School of Pharmacy, Devi Ahilya Vishwavidyalaya, Khandwa Road, Indore, M. P., India*

Abstract

Six simple, rapid, accurate, precise and cost-effective methods, I; formation and solving of simultaneous equation method, II; absorbance ratio method, III; dual wave length method, IV; area under curve method, V; first order derivative spectrophotometry method and VI; multi-component method have been developed for simultaneous estimation of rabeprazole sodium and diclofenac sodium in capsule dosage form. Rabeprazole sodium showed absorbance maxima at 292 nm and diclofenac sodium showed at 276 nm in 0.01N NaOH solution. Beer's law was obeyed in concentration range 5-30 µg/ml for rabeprazole sodium and 5-35 µg/ml for diclofenac sodium respectively for all proposed six methods. The sampling wavelengths for method VI, selected for both the drugs were 260nm, 276nm, 286nm, 292nm and 295 nm on trial and error basis using 0.01 N NaOH solutions as solvent. All the six methods allowed rapid analysis of binary pharmaceutical formulation with accuracy. Results of analysis for six methods were tested and validated for various parameters according to ICH guidelines.

Keywords: Rabeprazole sodium; Diclofenac sodium; Dual wavelength, Simultaneous equation; Absorbance ratio; Derivative spectrophotometry.

Introduction

Rabeprazole Sodium (RS) is chemically 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1H-benzimidazole. RS is a selective and irreversible new proton pump inhibitor [1] and it has proven efficacy in healing, symptoms relief and prevention of relapse of gastric ulcer, duodenal ulcer and gasteroesophageal reflux disease [2] while diclofenac sodium (DS), or

Sodium-2-[(2, 6-dichlorophenyl) amino] phenyl] acetate, is widely used as non-steroidal anti-inflammatory agent in therapeutics, it inhibits the cyclooxygenase enzyme [3].

Literature survey revealed chromatographic methods for determination of RS in tablet dosage forms [4] and spectrophotometric determination for RS in combination with other drugs [5-6]. Stability indicating [7] and bioanalytical chromatographic methods [8] for quantitification of RS were also reported. Various spectrophotometric, fluorimetric, potentiometric and chromatographic methods [9-13] have been developed for quantification of DS.

RS is not yet official while DS is official in I.P., B.P. and U.S.P. Extensive literature survey revealed that not a single UV or HPLC method is however reported for the simultaneous analysis of RS and DS in their combined dosage form. So the need was felt to develop simple, economical, rapid, precise and accurate methods to analyze the drugs simultaneously. A successful attempt has been made to estimate the two drugs simultaneously by UV spectrophotometric analysis.

Material and Methods

Instrumentation

UV/visible double beam spectrophotometer (Shimadzu Model 1700) was employed with spectral bandwidth of 1nm and wavelength accuracy of ±0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells).

Reagents and chemicals

Analytical pure standard samples of RS and DS were supplied as gift sample by Burgeon Pharmaceutical Pvt. Ltd., Pondicherry, India and Ranbaxy Laboratories Ltd., Dewas, India respectively and used without further purification. The Pharmaceutical dosage form used in study was a Safediclo capsule (Label claim: 20 mg of RS as enteric coated pellets and 100mg of DS I.P. as sustained release pellets) manufactured by Themis Laboratories Private Limited, 22, Milestone, Patli Morh, Tarore, Bari Brahamana, Jammu and Kashmir-181 133.

Preparation of standard stock solution

Standard stock solution of RS and DS having concentration 100 µg/ml prepared by dissolving separately 10mg of each drug in 100 ml volumetric flask using 0.01N NaOH solution. Beer's law was obeyed in concentration range 5-30 µg/ml for rabeprazole sodium and 5-35 µg/ml for diclofenac sodium respectively for all the proposed methods. The sampling wavelengths for method VI, selected for both the drugs were 260nm, 276nm, 286nm, 292nm and 295 nm on trial and error basis using 0.01 N NaOH solutions as solvent. For method I,II,III,V and VI, seven mixed standards solutions with concentration of RS and DS in the µg/ml of 5:35, 10:30, 15:25, 20:20, 25:15, 30:10 and 35:5, for method VI seven mixed standards solutions with concentration of RS and DS in the µg/ml of 1:5 2:10, 3:15, 4:20, 5:25, 6:30 and 7:35 were prepared by diluting appropriate volumes of standard stock solutions.

METHODS

Method I: Simultaneous Equation Method

Simultaneous equation method [14] of analysis was based on the absorption of drugs (RS and DS) at the wavelength maximum of the each other. Two wavelengths were selected for the development of the simultaneous equations was 292 nm and 276 nm, λmax of RS and DS

respectively. The absorbances of both the drugs were measured at 292 nm and 276 nm. The absorptivity values E (1%, 1cm) determined for RS at 292 nm and 276 nm were 341.90 and 213.80 while respective values for DS were 252.20 and 356.50. These values were means of six estimations.

The concentration of two drugs in mixture was calculated by, using following equations

$$C_{RS} = \frac{(A_2 ay_1 - A_1 ay_2)}{(ax_2.ay_1 - ax_1.ay_2)} \quad \text{.....Eqn.1} \qquad C_{DS} = \frac{(A_1 ax_2 - A_2 ax_1)}{(ax_2.ay_1 - ax_1.ay_2)} \quad \text{.....Eqn.2}$$

Where A_1 and A_2 were the absorbances of sample at 292 nm and 276 nm respectively, a_{x1} and a_{x2} were the absorptivity E (1%, 1cm) of RS at 292 nm and 276 nm respectively; a_{y1} and a_{y2} were the absorptivity of DS at 292 nm and 276 nm respectively.

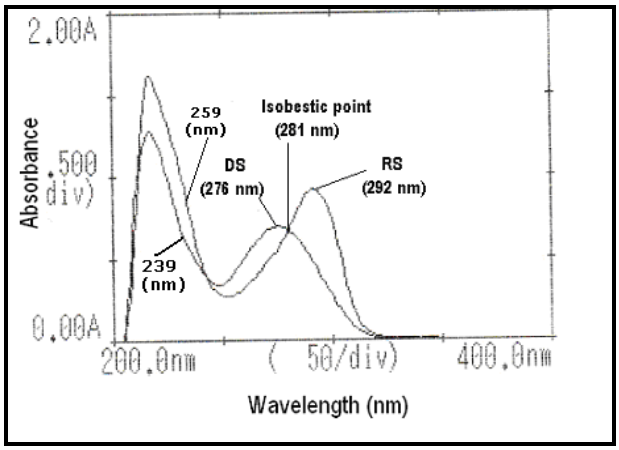


Fig.1: Overlain spectra of Rabeprazole Sodium and Diclofenac Sodium

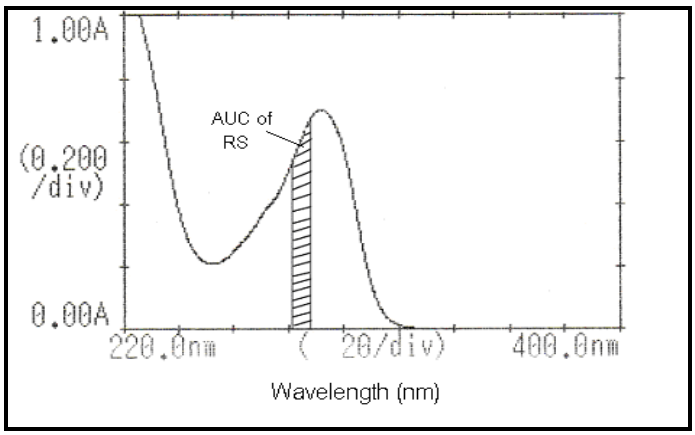


Fig. 2a: **The UV spectra of RS along with AUC range**

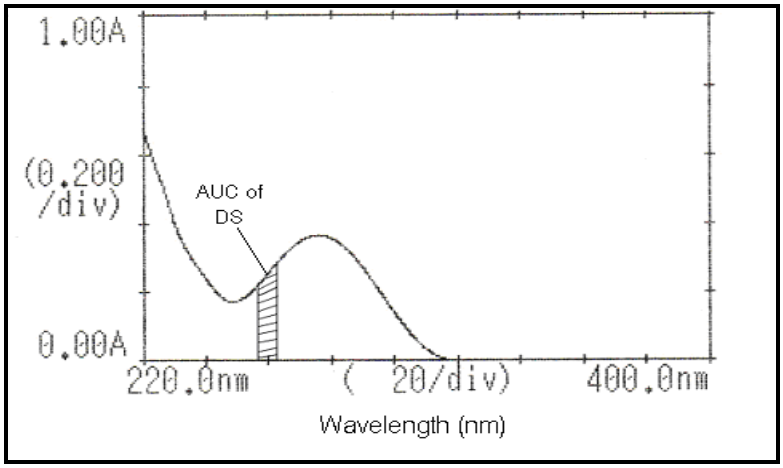


Fig. 2b: **The UV spectra of DS along with its AUC range**

Method II: Absorbance ratio method

Absorbance ratio method [15] of analysis was based on the absorbance's at two selected wavelengths, one of which is an iso-bestic point and the other being the wavelength of maximum absorption of one of the two components. From overlain spectra (Fig.1) 281 nm (Iso-bestic point) and 292 nm (λ_{max} of RS) were selected for the formation of Q absorbance equation (Eqn. 3 and 4). The absorbances at 281 nm and 292 nm for DS and RS were measured. The absorptivity values of each drug at both wavelengths were determined which was the mean of six independent values.

The absorbances and absorptivity at this wavelength were substituted in following equations to obtain the concentration of both drugs.

$$C_{RS} = \frac{(Q_M - Q_Y)}{(Q_X - Q_Y)} \cdot \frac{A_1}{ax_1} \quad \text{.....Eqn.3}$$

$$C_{DS} = \frac{(Q_M - Q_X)}{(Q_Y - Q_X)} \cdot \frac{A_1}{ax_1} \quad \text{.....Eqn.4}$$

Q_M, Q_X, and Q_Y were obtained as bellow:

$$Q_M = \frac{A_2}{A_1},\ Q_X = \frac{ax_2}{ax_1},\ Q_Y = \frac{ay_2}{ay_1}$$

Where A_1 and A_2 were the absorbance of the sample at 292 nm and 281 nm respectively, a_{X1} and a_{X2} were the absorptivity of RS at 292 nm and 281 nm respectively and a_{y1} and a_{y2} were the absorptivity of DS at 292 nm and 281 nm respectively.

Method III: Dual Wavelength Method

In this method, two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for one drug at a time. The spectrum of RS showed that the absorbance of RS is identical at 239 nm (λ_1) and 276 nm (λ_2) fig.1, so these two wavelengths were selected for the analysis of DS. All the solutions of series were scanned to ensure that absorbance difference between λ_1 and λ_2 is zero. Similarly, the DS solution was scanned to determined two wavelengths where absorbance was same. These two wavelengths were found to be 259 nm (λ_3) and 292 (λ_4) so these two wavelengths were selected for the analysis of RS Fig.1. All the solutions of this series were scanned to confirm that absorbance difference zero between λ_3 and λ_4. For RS, the

calibration curve was prepared by difference in absorbance i.e. $A_{(\lambda 3)} - A_{(\lambda 4),}$ at 259 nm and 292 nm (difference was zero for DS) plotted against the respective concentration. Similarly for DS, calibration curve prepared by plotting difference in absorbance i.e. $A_{(\lambda 1)} - A_{(\lambda 2),}$ at 239 nm and 276 nm (difference was zero for RS) against the respective concentration. Sample solutions containing DS and RS was scanned at 239 nm, 276 nm and 259 nm, 292 nm and concentration of DS and RS was calculated from their calibration curve.

Method IV: Area calculation Method (AUC)

AUC method[16] involves the calculation of integrated value of absorbance with respect to wavelength. Area calculation processing item calculates the area of bounded by the curve and horizontal axis. Here horizontal axis represents baseline.

$$(\alpha + \beta) = \int_{\lambda_2}^{\lambda_1} A d\lambda$$

Where; α = area of portion bounded by curve data and a straight line connecting the start and end point, β = area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, $\lambda_1$and λ_2 are wavelength representing start and end point of curve region. This method involved calculation in regions 288 nm to 284 nm for RS and 269 nm to 266 nm for DS respectively. These regions were selected on the basis of repeated observation that plot area calculation of pure single drug v/s concentration. The UV spectra of RS and DS along with its AUC region are shown in Fig. 2a and Fig. 2b respectively.

$$\int_{284}^{288} A d\lambda = K_1 C_1 \ldots\ldots \textbf{Eqn.5} \qquad \int_{266}^{269} A d\lambda = K_2 C_2 \ldots\ldots \textbf{Eqn.6}$$

$$\int_{284}^{288} A d\lambda = K_3 C_1 \ldots\ldots \textbf{Eqn.7} \qquad \int_{266}^{269} A d\lambda = K_4 C_2 \ldots\ldots \textbf{Eqn.}$$

Where C_1 & C_2 are concentration of RS and DS respectively in μg/ml and K_1, K_2, K_3, and K_4 are constant. Area of curve between 284 nm to 288 nm and 266 nm to 269 nm were represented by $\int_{284}^{288} A d\lambda$ and $\int_{266}^{269} A d\lambda$ for RS and DS respectively. In view of that following two final equations were developed for estimation of RS and DS.

$$\int_{284}^{288} A d\lambda = 0.0677 C_1 + 0.0959 C_2 \ldots\ldots \textbf{Eqn. 9} \qquad \int_{266}^{269} A d\lambda = 0.1683 C_1 + 0.1181 C_2 \ldots\ldots \textbf{Eqn.10}$$

Sample solutions were scanned and area was calculated with in indicated wavelength range. Concentration of both components was calculated using above-mentioned Eqn.9 and 10.

Method IV: Derivative spectrophotometric Method

In this method [17] the standard stock solution of RS and DS were scanned from 200 nm to 400 nm. The spectra obtained were derivatized in first order and then overlain spectra recorded Fig. 3. From the entire derivative spectra obtained, the wave lengths were selected in a manner such that RS had zero crossing point at 288.5 nm and DS showed a measurable dA/dλ where as the zero crossing point of DS at 275.5 nm. RS showed appreciable dA/dλ. Hence wavelengths 275.5 nm and 288.5 nm were selected as analytical wavelength for determination of RS & DS respectively. The mixed standards were scanned in the spectrum mode, derivatized in first order

with derivative interval of 5 nm and absorbances were measured at the selected wavelengths. Calibration curve for Ds (5-30µg/ml) & RS (4-24µg/ml) were plotted as dA/dλ verses concentration. By extrapolating the value of absorbances, the conc. of corresponding drugs in the sample was determined.

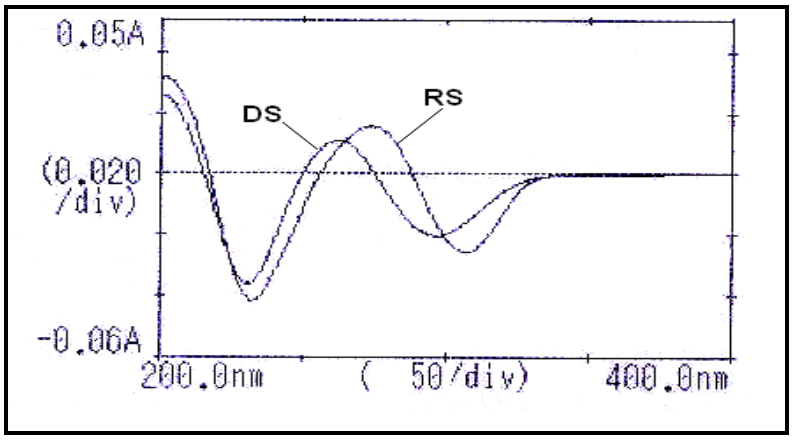
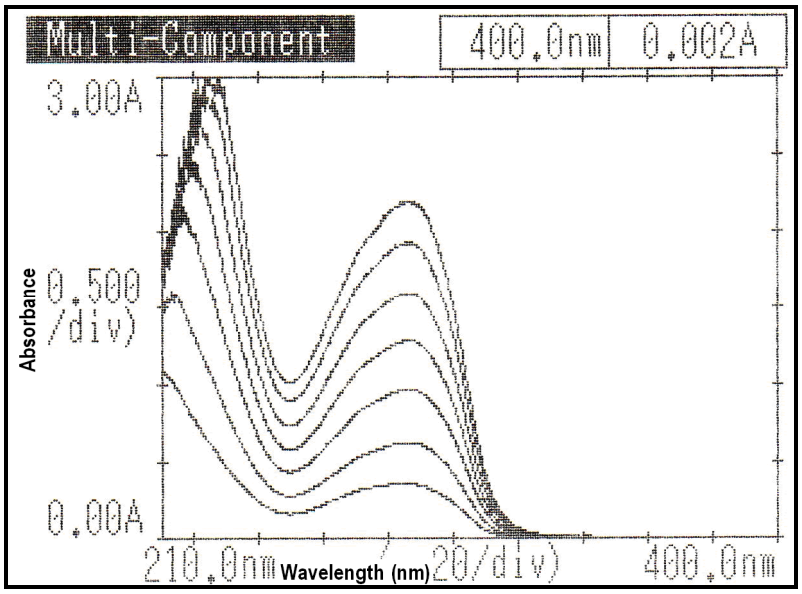


Fig. 3: First order derivative of Overlain spectra of RS and DS



.

Fig.4: Overlain spectra of mixed standards of RS and DS

Method VI: Multicomponent Method

In this method [18], the seven mixed standard solutions with concentration of RS and DS in the ratio of 1:5, 2:10, 3:15, 4:20, 5:25, 6:30 and 7:35 (mcg/ml) were prepared in 0.01N NaOH. All the mixed standard solutions were scanned over the range of 400-210 nm. In the multi-component the wavelength selected were 260nm, 276nm, 286nm, 292nm and 295nm. Sampling wavelengths were selected on trial and error basis. The concentration of individual drug was feed to the multi-component mode of the instrument. The instrument collects and compiles the

spectral data from mixed standards. Overlain spectra of mixed standards solution are given in Fig.4. Mixed standard solution of both the drug was scanned on all the selected wavelengths to study the range of Beer's Lambert' s range.

The sample solutions were scanned over the range of 400-210 nm in the multi-component mode of the instrument and concentration of each component was obtained by analysis of spectral data of sample solution with reference to that of seven mixed standards, in the terms of µg/ml.

Preparation of sample stock solution

Twenty capsules were taken, there average weight was determined, and crushed to a fine powdered, powdered equivalent to 20mg of RS & 100mg 0f DS was weight and dissolved in 100 ml of 0.01N NaOH with vigorous shaking for 15 minute. The solution was filtered through whatman filter paper No. 41 to a 100ml of volumetric flask and volume was made up to mark with 0.01N NaOH to get sample stock solution which was further diluted with 0.01N NaOH to get required concentration in linearity range. Sample solutions were scanned using proposed six methods and the results were obtained and reported in Table 1.

Validation of the developed methods

The developed methods for the simultaneous estimation of RS and DS were validated as per ICH guidelines (ICH 1996).

Linearity

For each drug**,** appropriate dilutions of standard stock solutions were assayed as per the developed methods. To establish linearity of the all proposed six methods, six separate series of solutions of RS and DS (5-30 µg/ml and 5-35 µg/ml in 0.01N NaOH) were prepared from the stock solutions and analyzed.

Table I: Recovery study of DS and RS

Method	Drug	% mean recovery	S.D.	% R.S.D.	S.E.
I	DS	98.85	0.4670	0.4725	0.2696
	RS	99.04	0.2882	0.2913	0.1663
II	DS	99.83	0.3212	0.3208	0.1854
	RS	99.75	0.3827	0.3820	0.2209
III	DS	100.21	0.4219	0.4232	0.2435
	RS	99.60	0.4135	0.4162	0.2387
IV	DS	100.14	0.4674	0.4649	0.2698
	RS	100.05	0.2000	0.1999	0.1154
V	DS	99.70	0.7000	0.6882	0.4041
	RS	99.60	0.7200	0.7221	0.4157
VI	DS	99.53	0.5492	0.5518	0.3174
	RS	99.79	0.2345	0.2350	0.1354

[b] Average of three determinations, S.D.: Standard deviation, R.S.D. : Relative standard deviation, S.E.: Standard error

Accuracy

To check the accuracy of proposed method, recovery studies were carried out from the pre-analyzed sample at three deferent level of standard addition 80%, 100% and 120% of the level claim. The results of the recovery studies are given in Table I.

Table II: Result of commercial formulation analysis

Method	Drug	Label claim (mg/capsule)	% of label claim estimated[a]	S.D.	% R.S.D	S.E.
I	DS	100	98.43	0.2291	0.2327	0.1027
	RS	20	99.34	0.5594	0.5631	0.2501
II	DS	100	99.82	0.2101	0.2100	0.0939
	RS	20	98.89	0.5271	0.5330	0.2357
III	DS	100	99.78	0.5357	0.5368	0.2395
	RS	20	99.77	0.5537	0.5549	0.2476
IV	DS	100	99.82	0.5481	0.5491	0.2454
	RS	20	99.83	0.4315	0.4322	0.1932
V	DS	100	99.54	0.2895	0.2899	0.1295
	RS	20	99.26	0.2139	0.2143	0.0956
VI	DS	100	99.59	0.4318	0.4335	0.1763
	RS	20	99.23	0.7690	0.7749	0.3139

[a] Average of six determinations, S.D.: Standard deviation, R.S.D. : Relative standard deviation, S.E.: Standard error

Table III: Interdays, intraday data of commercial samples of RS and DS and LOQ, LOD data for RS and DS

Method	Drug	% RSD Interdays	% RSD Intraday	LOD (µg/ml)	LOQ (µg/ml)
I	DS	0.5773	0.4735	0.6753	2.0465
	RS	0.2913	0.4637	0.9781	2.9640
II	DS	0.7280	0.3241	0.6572	1.9916
	RS	0.5710	0.5640	0.9781	2.9640
III	DS	0.6901	0.4245	0.1089	0.3302
	RS	0.4152	0.6853	0.1049	0.3179
IV	DS	0.2435	0.4532	0.0272	0.8270
	RS	0.1154	0.1999	0.0682	0.2079
V	DS	0.3940	0.4541	0.2068	0.6365
	RS	0.1493	0.1493	0.1153	0.3607
VI	DS	0.3145	0.4521	0.2330	0.7070
	RS	0.4215	0.5486	0.4015	1.2167

R.S.D. is relative standard deviation, LOD is least of detection, and LOQ is least of quantitation.

Precision

Repeatability

To the check of degree of repeatability of the methods, suitable statistical evaluation was carried out. Repeatability was performed for six times at all concentrations in linear range. The standard

deviation, relative standard deviation and standard error were calculated. The results of statistical evaluation are reported in Table II.

Intermediate precision (Intra-day and Inter-day precision)

The Intra and Inter-day precision was determined by assay of the sample solution on the same day and defferent days at different time intervals respectively. The results of the same are presented in Table 3.

Limit of detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of RS and DS by the proposed methods were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation. The results of the same are shown in Table 3.

Results and Discussion

Calibration curve:

The linear regression equations obtained were; absorbance at 292 nm= [0.045×conc. in μg/ml]+0.018 (Method I and II for RS,r^2 = 0.9990), 276 nm = [0.0348×conc. in μg/ml]+0.0146 (Method I for DS, r^2 = 0.9995), 281= [0.0337×conc. in μg/ml]+0.0119 (Method II for DS, r^2 = 0.9992), A_{259}-A_{292} = [0.0259×conc. in μg/ml]+0.0064 (Method III for RS, r^2 = 0.9997) and A_{239}-A_{276} = [0.011× conc. in μg/ml]+0.0044 (Method III for DS, r^2 = 0.9996), $\int_{284}^{288} Ad\lambda$ = [0.1387× conc. in μg/ml]-0.0246 (Method IV for RS, r^2 = 0.9999); $\int_{266}^{269} Ad\lambda$ =[0.919×conc. in μg/ml]-0.0021 (Method IV for DS, r^2 = 0.9997), 275.5 nm = [0.0.0005×conc. in μg/ml]+0.0008 (Method V for RS, r^2 = 0.9994); 288.5=[0.0006×conc. in μg/ml]-0.0008 (Method IV for DS, r^2 = 0.9993) and 260nm=[0.1904×conc. in μg/ml]+0.0206 and 260nm = [0.0381×conc. in μg/ml]+0.0206 (Method VI for RS and DS respectively, r^2 = 0.9985), 276nm= [0.02834×conc. in μg/ml]+0.0417 and 276nm= [0.0567×conc. in μg/ml]+0.0417 (Method VI for RS and DS respectively, r^2 = 0.9986), 286nm = [0.03138×conc. in μg/ml]+0.0337 and 286nm = [0.0628×conc. in μg/ml]+0.0.0337 (Method VI for RS and DS respectively, r^2 = 0.9990), 292nm= [0.2983×conc. in μg/ml]+0.0243 and 292nm = [0.0597×conc. in μg/ml]+0.0243 (Method VI for RS and DS respectively, r^2 = 0.9995), 295nm = [0.2748×conc. in μg/ml]+0.0227 and 292nm= [0.055×conc. in μg/ml]+0.0227 (Method VI for RS and DS respectively, r^2 = 0.9995).

Analytical validation

Linearity

Linearity range for RS and DS estimation were found to be 5-30 μg/ml (RS) and 5-35 μg/ml (DS) at their respective selected wavelengths for all proposed methods.

Accuracy

The validity and reliability of proposed method was assessed by recovery studies by standard addition method. The means of %recovery (%RSD) were found to be low values (<2.0) for all the six proposed methods (Table I). These results revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed analytical methods.

Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicated the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation, RSD and standard error was calculated of RS and DS. The results of stastical evaluation are given Table 2. Intermediate precision study expresses within laboratory variation in different days. In intermediate precision study, %RSD values were not more than 2.0% in all the cases (Table III). RSD values found for both the analytical methods were well with in the acceptable range indicating that these all methods have excellent repeatability and intermediate precision.

LOD and LOQ

From data (standard deviation of y-intercept of regression equation and slope of calibration curve), it was possible to calculate the detection and quantitation limits. For method I, the LOD, LOQ values for RS and DS was found to be 0.9781, 2.9640 & 0.6753, 2.0465 (µg/ml) respectively; for method II, 0.9781, 2.9640 & 0.6572, 1.9916 (µg/ml) respectively; for method III, 0.1049, 0.3179 & 0.1089, 0.3302 (µg/ml) respectively; for method IV, 0.0682, 0.2079 & 0.0272 & 0.8270 (µg/ml) respectively, for method V, 0.1153, 0.3607 & 0.2068, 0.6365 (µg/ml) respectively and for method VI, 0.4015, 1.2167 & 0.2330, 0.7070 (µg/ml) respectively (Table III). These low values indicated the good sensitivity of the method proposed.

Estimation of formulation

The assay values of RS , DS for method I, II and III was found to be 99.34 %, 98.43% & 98.89%, 99.82% & 99.77%, 99.78% respectively with standard deviation<1.0 while for method IV, V, and VI, was found to be 99.83%, 99.82% & 99.26%, 99.54% and 99.23%, 99.59% respectively with standard deviation <1.0 Table I. Assay values of formulation were same as mentioned in the label claim indicating that the inference of excipient matrix is insignificant in estimation of RS and DS by all six proposed methods

Conclusion

The proposed validated six spectrophotometric methods are simple, rapid, accurate, precise and inexpensive and hence can be used for the routine analysis of RS and DS in capsule dosage forms. The sample recovery for all six methods was in good agreement with their respective label claims, which suggested non interference of formulation additives in estimation.

Acknowledgements

The authors are thankful to Burgeon Pharmaceuticals Pvt. Ltd. Pondicherry, India and Ranbaxy Laboratories Limited, Dewas, India for gift sample of pure Rabeperazole Sodium and Diclofenac Sodium Respectively and Head, School of Pharmacy for providing facilities to carry out proposed work. On of the author Mr. Raj K. Prasad is grateful to All India council of Technical education (AICTE) for providing junior research fellowship.

References

[1] The Merk Index; Merk and Co., Inc.: Whitehouse station, NJ, **2001**.
[2] C. Carswell, K.Goa, *Drugs*, **2001**, 61 (15), 2327-2356.

[3] K. D. Tripathi, Essential of Medical Pharmacology, Jaypee Brothers Medical publisher Pvt. Ltd.: New Delhi, India. **2003**, 593.
[4] M. S. Palled, M. Chatter, P. N. M. Rajesh, A. R. Bot, *Ind. J. Pharm. Scie.* **2006**, 68 (3), 403-406.
[5] C. V. Garcia, J. Sippel, M. Steppe, E. E. S. Schapoval, *Ana. Lett.*, **2006**, 39, 341-348.
[6] P. Pattanayak, R. Sharma, S. C. Chaturvedi, Anal. *Lett.* **2007**, 40, 2288-2294.
[7] A. El-Gindy, F. El-Gindy, M. M. Maher, *J. Pharm. Biomed. Anal.* **2001**, 24, 715–719.
[8] J. Haung, Q. Guo, *Rapid Comm. Mass Spectrum.* **2005,** 19 (16), 2321–2324.
[9] P. Ortega-Barbarez, A. Ruiz-Media, Fernandez-De M. L. Cordova, A. Molina-Diaz, *Anal. Sci*. **1999,** 15, 985–989.
[10] J. A. Aranciba, M. A. Boldrini, G. M. Escandar, *Talanta*, **2000**, 52 261–268.
[11] A. M. Pimenta, A. N. Araujo, M. C. B. S. M. Montenegro, *Anal. Chim. Acta*, **2002**, 470, 185–194.
[12] M. E. Abdel-Hamid, L. Novotny, H. Hamza, *J. Pharm. Biomed. Anal.*, **2001**, 24, 587–594.
[13] S. Garcia, C. Sanchez-Pedreno, I. Albero, C. Garcia. M*icrochim. Acta*, **2001**, 136, 67–71.
[14] A. H. Beckett, J. B. Stenlake, Practical Pharmaceutical Chemistry, CBS Publishers & distributors, New Delhi: Part-2, **2002**, 275-307.
[15] A. G. Davidson, A. H. Beckett, J. B. Stenlake, Practical Pharmaceutical Chemistry; CBS Publishers & Distributor: New Delhi; **2001**, 2, 284-288.
[16] H. K. Jain, R. K. Agrawal, *Ind. J. Pharm. Sci.* **2002**, 64 (1), 88-91.
[17] S. K. Jain, D. Jain, M. Tiwari, S. C. Chatutvedi, *Ind. J. Pharm. sci.* **2002**, 64 (30), 267-269
[18] S. B. Chepurwar, A. A. Shirkhedkor, S. B. Bari, S. J. Surana, *Indian Drugs*, **2006**, 43 (10), 803-807.