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Simultaneous UV-Spectrophotometric determination and validation of Diclofenac Sodium and Rabeprazole Sodium using Hydrotropic agents in its tablet Dosage Form

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Abstract

A novel, simple, safe, sensitive, and economical method of spectrophotometric estimation in UV region has been developed using 5M urea solution as hydrotropic solubilizing agent for the quantitative determination of Rabeprazole sodium and Diclofenac sodium. Two methods are described for the simultaneous determination of Rabeprazole sodium and Diclofenac sodium in combined pharmaceutical tablets. Urea did not show any absorbance above 225nm, there was no interference during estimation of Diclofenac and Rabeprazole. The first method is simultaneous estimation using vierodt's equation where absorption maxima found at 285nm and 276nm for Rabeprazole and Diclofenac, respective'ly. The second method is based on Q-Absorption ratio method using two wavelengths, at 281nm (Isobestic point) and 276nm (λ_{max} for Diclofenac). Two methods follow Beer's linearity in the range of 4-28µg/ml and 5-25µg/ml with correlation coefficient r^2 of 0.999 and 0.998 for Rabeprazole and Diclofenac, respectively. According to ICH norms the parameters linearity, precision, accuracy, limit of detection, and limit of quantification were studied, the results of analysis were validated statistically and by recovery studies. The proposed methods were simple, cost effective and were successfully applied to the determination of these drugs in quality control of combined pharmaceutical dosage

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Key words:
Diclofenac sodium, Rabeprazole sodium, Simultaneous equation, Q-Absorption ratio method, urea, hydrotropic agents.

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Introduction

The term hydrotropy has been used to designate the increase in solubility of various substances due to the

presence of large amounts of additives. Various hydrotropic agents have been used to increase the aqueous solubility of large number of drugs. Hydrotropic solution may be a proper choice instead of using residual toxicity organic solvents like acetonitrile, methanol, dimethyl formamide and chloroform which cause residual toxicity. Hydrotropic agents like sodium benzoate, sodium salicylate, urea, nicotinamide, sodium glycinate, sodium ascorbate, and niacinamide, have been employed to increase the aqueous solubility's of large number of water insoluble drugs[1-13]. Chemically Diclofenac sodium is, sodium (2-(2, 6-dichloroanilino) phenyl) acetic acid, used as analgesic and anti-inflammatory drug used in the treatment of rheumatoid arthritis, osteoarthritis and alkylosing spondylotis and also for a variety of non-rheumatic inflammatory conditions. Chemically Rabeprazole sodium is 2-([4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulfinyl]-1H benzimidazole sodium salt is an anti-ulcer drug in the class of proton pump inhibitors that reduce the production of acid by blocking the enzyme (hydrogen-potassium adenosine triphosphatase) in parietal cells and used to treat duodenal ulcers and erosive or ulcerative gastro esophageal reflux disease. Diclofenac sodium is official in IP, BP and USP, literature survey reveals HPLC[14-15], spectrophotometry[16-17], and fluorimetry[18-19] methods for determination of diclofenac sodium. Rabeprazole was not official in any pharmacopoeia, HPLC[20-21], LC-MS[22], and spectrophotometric[23-24] methods have been reported for the rabeprazole sodium. Diclofenac-Rabeprazole mixture is not yet official in any pharmacopoeia, there was no official methods for the estimation of both the drugs, HPLC method for the simultaneous estimation of rabeprazole and diclofenac sodium is reported. Diclofenac sodium alone has been reported to be estimated by using hydrotropic agents[25-26], to our knowledge there was no simultaneous spectrophotometric estimation using hydrotropic agents have been described for the simultaneous estimation of both drugs in tablets. In this report two methods based on simultaneous equation and Q-Absorption ratio methods have been used to determine the diclofenac sodium and rabeprazole sodium. The following methods were validated according to ICH norms.

Experimental

Chemicals and reagents

Rabeprazole and Diclofenac were standardized by official method reported in Indian Pharmacopoeia and the purity of the sample was found to be 99.1% and 98.9% for rabeprazole and diclofenac, respectively. Urea used is of analytical reagent grade and water used was doubly distilled from all glass apparatus.

Instrumentation

Spectrophotometric analysis was performed on a- using a 1cm quartz cell and band pass of 2nm. The instruments setting were 200-400nm for normal wavelength range.

Selection and preparation of hydrotropic solvent

From the solubility studies 5M urea solution was selected as hydrotropic agent for spectrophotometric analysis of rabeprazole and diclofenac, respectively in their tablet formulations, 5M urea solution was prepared by accurately weighing 15g in to a 50ml volumetric flask and the volume was made up to 50ml with distilled water.

Preparation of stock and standards for linearity

Stock solutions were prepared by dissolving rabeprazole sodium and diclofenac sodium in 5M urea as hydrotropic solubilizing agent separately to obtain a concentration of 1mg/1ml for each compound. The standard solutions for calibration curve were prepared by dilution of the stock solutions in distilled water to reach concentration ranges of 4-

28 µg/ml and 5-25µg/ml for rabeprazole sodium and diclofenac sodium, respectively.

Preparation of sample stock solution

Ten tablets containing rabeprazole sodium and diclofenac sodium as active ingredients were weighed and finely powdered in a glass mortar. A portion of the powder equivalent to about 10mg of rabeprazole sodium was weighed accurately, transferred to a 10ml volumetric flask and either suspended in 5M urea then shake for some time and the mixture was sonicated for 30min then flask was completed to volume with the same solvent. Then the mixtures was filtered, the filtered solution was diluted to get a concentration of 10µg/ml with distilled water.

UV-spectrophotometric methods

Simultaneous equation method

This method of analysis was based on the absorption of drugs rabeprazole sodium and diclofenac sodium at the wavelength maxima of each other. Two wavelengths were selected for the development of the simultaneous equations at 285nm and 276nm (fig 1&2) for Rabeprazole and Diclofenac respectively. The absorptivity values E (1%, 1cm) were determined for two drugs at all selected wavelengths. The concentration of two drugs in mixture was calculated by using following equations.

$$C_X = (A_2\ ay_1 - A_1\ ay_2)/(ax_2\ ay_1 - ax_1\ ay_2)$$

$$C_Y = (A_1\ ax_2 - A_2\ ax_1)/(ax_2\ ay_1 - ax_1\ ay_2)$$

Where, C_x and C_y are the concentrations of rabeprazole and diclofenac respectively in mixture and in sample solutions. A_1 and A_2 are the absorbencies of sample at 285nm and 276nm, respectively, ax_1 and ax_2 are the absorptivity of rabeprazole at 285nm and 276nm. All standard and sample solutions absorbance was measured at 285nm and 275nm with their respective blanks.

Q-Absorption ratio method

This method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbance at any two wavelengths is a constant value, independent of concentration and path length. The solutions of 25µg/ml and 24µg/ml for diclofenac sodium and rabeprazole were scanned in the wavelength range of 400 to 200nm to obtain overlain spectra (fig 5). Two wavelengths, 281nm as iso absorptive point and 276nm (λ_{max} of diclofenac sodium) were selected for the formation of Q-absorbance ratio equation. The calibration curves were determined in the concentration range of 5-25µg/ml and 4-28µgml for diclofenac sodium and rabeprazole sodium respectively. The absorptivity coefficients of each drug at both the wavelengths were determined. The concentration of the individual components, Cx and Cy can be calculated by using the following equations.

$$C_X = (Q_M - Q_Y/Q_X - Q_Y)\ \times (A_1/ax_1)$$

$$C_Y = (Q_M - Q_X/Q_Y - Q_X) \times (A_2/ay_1)$$

Where A_1 and A_2 are absorbance of sample solution at iso-absorptive point 281nm and 276nm λ_{max} of diclofenac sodium respectively, ax_1 and ax_2 are the absorptivities of the rabeprazole at 281nm and 276nm respectively and ay_1 and ay_2 are the absorptivities of the diclofenac at 281nm and 276nm respectively.

Validation of UV- visible spectrophotometric methods

Linearity and range

Five aliquots of each drug solutions were taken from standard stock solution and transferred to 10ml volumetric flask to get a final concentration of 5, 10, 15, 20 and 25 µg/ml of diclofenac and 4, 8, 12, 16 and 20, 24 and 28µg/ml of rabeprazole and the volume was completed with the distilled water and each flask content was measured to determine the absorbance at all the selected wavelength. For simultaneous equation method the absorbance of all standard solutions were measured at 276nm and 285nm, the calibration curves of absorbance vs. concentration was plotted and correlation coefficient and regression line equations for both rabeprazole and diclofenac sodium were determined. For Q-Absorption ratio method the wave lengths selected were 281nm (iso

absorptive point) and 276nm (λ_{max} of diclofenac). The absorbance at these two wavelengths for all standard solutions of both rabeprazole and diclofenac were measured and the calibration curves and linear regression equation of rabeprazole and diclofenac at 281nm and 276nm were determined.

Precision

In intraday study concentration of two drugs were calculated on the same day at an interval of one hour. In inter day study the concentration of drug contents were calculated on three different days study expresses with in laboratory variation in different days. In both intra and inter-day precision study for the methods %RSD were calculated.

Accuracy

Accuracy of the developed method was confirmed by doing recovery study as per ICH norms at three different concentration levels 80%, 100%, 120% and the values were measured at all wavelengths for rabeprazole and diclofenac sodium. This operation was done in triplicate. From the recovery study it was clear that the method is very accurate for quantitative estimation of rabeprazole and diclofenac sodium in tablet dosage forms as the statistical results were within the acceptance range.

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification of diclofenac and rabeprazole by proposed methods were determined using calibration standards.LOD and LOQ were calculated as 3.3σ/S and 10σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of response.

Results and discussions

Linearity and range

The linearity of rabeprazole sodium and diclofenac sodium were found to be in the range of 4-2µg/ml and 5-25µg/ml with correlation coefficient of 0.999 and 0.998 for rabeprazole and diclofenac, respectively for simultaneous method, for Q-Absorption ratio method linearity was found to be 4-28µg/ml with correlation coefficients of 0.999 and0.999 at 281nm and 276nm for rabeprazole sodium, the calibration data with %RSD for both the methods shown in (table-1, 2, 3, 4, 5, 6 and 10) and calibration curves are shown in (figure 3, 4, 6, 7, 8 and 9).

Precision

%RSD found for the simultaneous equation method in the range of 1.23-1.6 for rabeprazole and 1.005-1.78 for diclofenac. The %RSD found for Q-Absorption ratio method in the range of 1.02-2.05 (281nm) and 1.27-2.94 (276nm) for rabeprazole, 1.06-1.83(281nm) and 1.00-2.1(276nm) for diclofenac sodium, respectively as shown in Table 7.

Accuracy

Accuracy of the methods was confirmed by doing recovery studies from marketed formulation at three concentration levels of standard addition. The %recoveries found for the simultaneous equation method was 99.3-100.1 and 99.04-99 for rabeprazole and diclofenac simultaneously. For Q-Absorption ratio method the %recoveries found to be 99.9-100.1 and 98.8-99.1 for rabeprazole and diclofenac, respectively as shown in table 8.

Limit of Detection and Limit of Quantification

The limit of detection found to be 0.517 and 0.517 for simultaneous equation method for both rabeprazole and diclofenac sodium, respectively, the limit of quantification found to be 1.724 and 1.724 for both rabeprazole and diclofenac sodium, respectively. For Q-Absorption ratio method the limit of detection found to be 0.611 at (281nm), 0.675 at (276nm) and 0.642 at (281nm), 0.651 at (276nm) for rabeprazole and diclofenac, respectively, the limit of quantification found to be 2.172 at (281nm) and 1.923 at (276nm), 2.412 at (281nm) and 2.172 at (276nm) for both rabeprazole and diclofenac sodium as shown in table 9.

Analysis of marketed formulation (RCLONAC tablets) by UV spectrophotometric method

The percentage of rabeprazole sodium and diclofenac in the estimated formulation was found to be 98.75% and 98.62% for rabeprazole and diclofenac sodium, respectively for simultaneous equation method. For Q-Absorption ratio method the percentage of rabeprazole sodium and diclofenac in the estimated formulation was found to be 99.5 and 98.75% as shown in table 10.

Conclusions

The present paper describes application of hydrotropic solubilization phenomenon for the simultaneous estimation of rabeprazole sodium and diclofenac sodium by simultaneous equation method and Q-Absorption ratio method. Both the drugs showed good linearity and regression values for their respective wavelengths. Hence the proposed methods are new, simple, cost effective, and free from pollution. It is concluded that the described methods have the potential for the application in the quality control laboratories.

Acknowledgement

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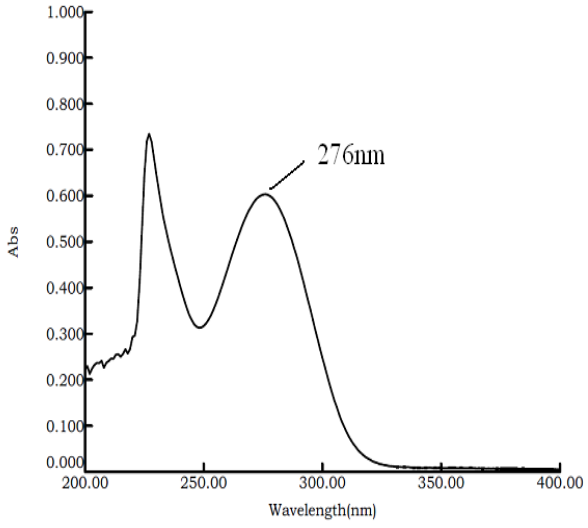


Fig-1: Absorption maxima of Diclofenac

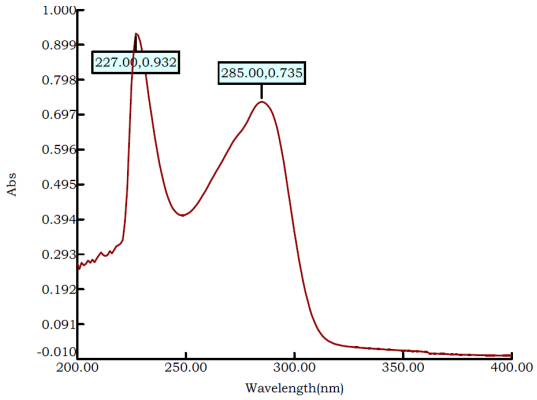


Fig-2: Absorption maxima of rabeprazole sodium

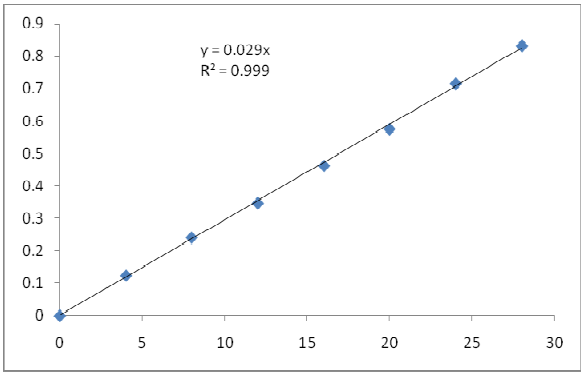


Figure3: Calibration curve and linear regression equation for rabeprazole sodium at285nm

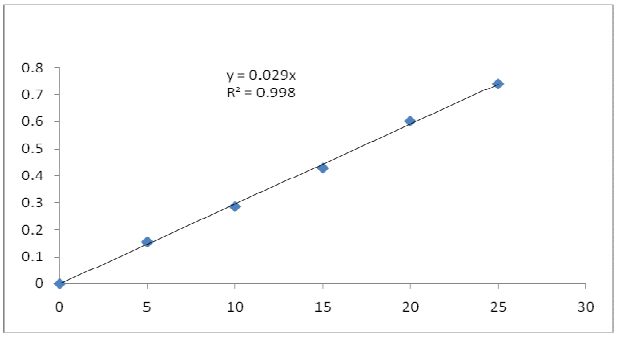
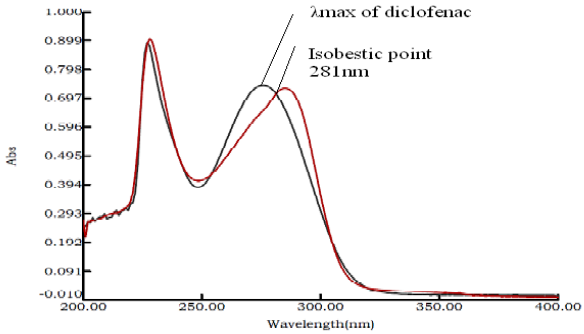


Figure4: Calibration curve and linear regression equation for diclofenac sodium at 276nm.



Figurs 5: overlay spectra of Diclofenac and Rabeprazole showing isoabsorptive point

Calibration curves for Q-Absorption ratio method

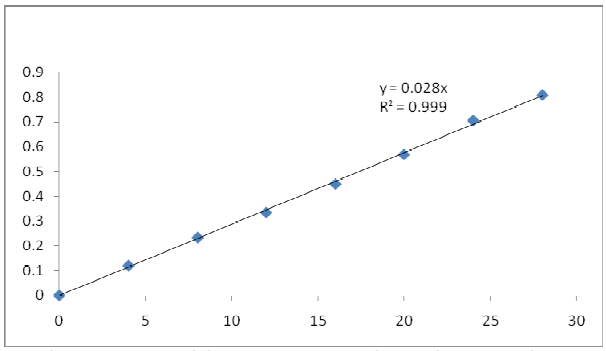


Figure 6: Calibration curve for rabeprazole at 281nm (iso-absorptive point)

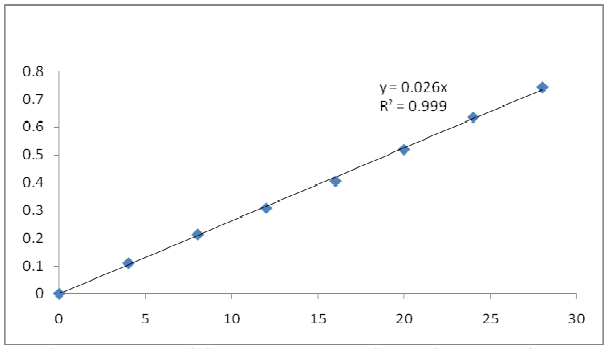


Figure 7: Calibration curve for rabeprazole at 276nm (absorption maxima of diclofenac sodium)

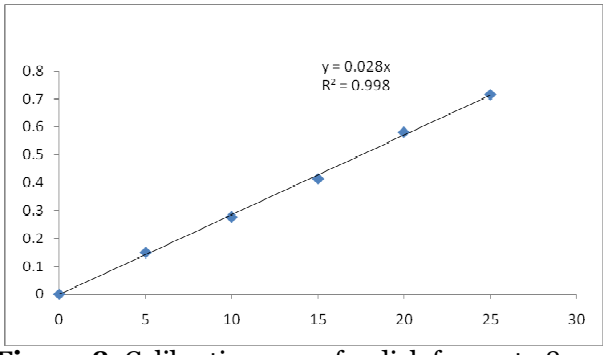


Figure 8: Calibration curve for diclofenac at 281nm (iso-absorptive point)

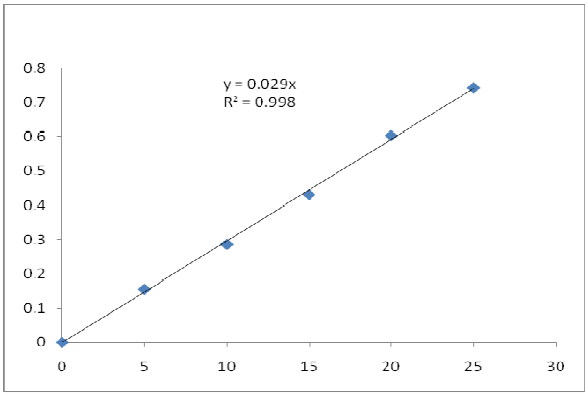


Figure 9: Calibration curve for diclofenac at 276nm (absorption maxima of diclofenac sodium)

Table 1: Calibration data of Rabeprazole sodium for simultaneous method.

S. no	Concentration (µg/ml)	Absorbance
1	4	0.124
2	8	0.241
3	12	0.347
4	16	0.462
5	20	0.575
6	24	0.715
7	28	0.832

Table 2: Calibration data of Diclofenac sodium for simultaneous method

S. no	Concentration (µg/ml)	Absorbance
1	5	0.156
2	10	0.286
3	15	0.430
4	20	0.603
5	25	0.742

Table 3: Calibration data of Rabeprazole sodium for Q-Absorption ratio method at 281nm

S. no	Concentration (µg/ml)	Absorbance
1	4	0.120
2	8	0.233
3	12	0.334
4	16	0.449
5	20	0.568
6	24	0.745
7	28	0.808

Table 4: Calibration data of Rabeprazole sodium for Q-Absorption ratio method at 276nm

S. no	Concentration (µg/ml)	Absorbance
1	4	0.110
2	8	0.213
3	12	0.308
4	16	0.405
5	20	0.519
6	24	0.635
7	28	0.743

Table 5: Calibration data of Diclofenac sodium for Q-Absorption ratio method at 281nm

S. no	Concentration (µg/ml)	Absorbance
1	5	0.150
2	10	0.277
3	15	0.414
4	20	0.581
5	25	0.716

Table 6: Calibration data of Diclofenac sodium for Q-Absorption ratio method at 276nm

S. no	Concentration (µg/ml)	Absorbance
1	5	0.156
2	10	0.286
3	15	0.430
4	20	0.603
5	25	0.7425

Table 7: Data showing precision of the developed method

Parameters	Drug	Concentration	1st day	2nd day	3rd day	SD	%RSD
Simultaneous equation method	RABZS	(8,12,20 µg/ml)	0.241 0.347 0.575	0.237 0.344 0.568	0.233 0.337 0.561	0.004 0.005 0.007	1.6 1.46 1.23
	DCS	(10,15,20 µg/ml)	0.286 0.430 0.603	0.281 0.424 0.598	0.275 0.419 0.591	0.005 0.005 0.006	1.78 1.17 1.005
Q-Absorption ratio method	RABZS	(8,12,20 µg/ml)	0.233 0.334 0.560 (281nm) 0.213 0.308 0.519 (276nm)	0.228 0.328 0.554 (281nm) 0.209 0.306 0.514 (276nm)	0.220 0.319 0.549 (281nm) 0.201 0.299 0.501 (276nm)	0.006 0.0075 0.0055 (281nm) 0.0061 0.0041 0.0060 (276nm)	2.53 2.29 1.02 (281nm) 2.94 1.34 1.27 (276nm)
	DCS	(10,15,20 µg/ml)	0.277 0.414 0.581 (281nm) 0.286 0.430 0.603 (276nm)	0.273 0.409 0.577 (281nm) 0.281 0.423 0.598 (276nm)	0.267 0.399 0.569 (281nm) 0.274 0.418 0.591 (276nm)	0.005 0.0076 0.0061 (281nm) 0.0060 0.0070 0.0060 (276nm)	1.83 1.86 1.06 (281nm) 2.18 1.68 1.005 (276nm)

Table 8: Data Showing Recovery of the Developed Methods

Parameters	RABZS µg/ml	DCS µg/ml	STD RABZS added µg/ml	STD DCS added µg/ml	RABZS found µg/ml	DCS found µg/ml	% RABZS recovered	%DCS recovered
Methods								
Simultaneous equation method	12	10	2.52	2.53	14.43	12.41	99.3	99.04
	12	10	5.04	5.06	17.06	14.91	100.1	99.0
Q-Absorption ratio method	12	10	2.52	2.53	14.51	12.39	99.9	98.8
	12	10	5.04	5.06	17.03	14.93	99.9	99.1

Table 9: Data showing linearity of the developed methods, LOD & LOQ

Parameters	Simultaneous equation method		Q-Absorption ratio method	
	Rabeprazole	Diclofenac	Rabeprazole	Diclofenac
λ_{max}	285nm	276nm	285nm and 281(iso-absorptive point)	276nm
Beer's-law limit(µg/ml)	4-28	5-25	4-28	5-25
Correlation coefficient	0.999	0.998	0.999(at 281nm) 0.999(at 276nm)	0.998(at 281nm) 0.998(at 276nm)
Slope	0.029	0.029	0.028(at 281nm) 0.026(at 276nm)	0.028(at 281nm) 0.029(at 276nm)
LOD	0.517	0.517	0.675 0.611	0.642 0.651
LOQ	1.724	1.724	2.172 1.923	2.142 2.172

Table-10: Results of analysis of tablet dosage forms containing rabeprazole sodium and diclofenac sodium

Methods	Simultaneous equation method		Q-Absorption ratio method	
parameters	RABZS	DCS	RABZS	DCS
Active content estimated	19.75mg	98.62mg	19.9mg	98.75mg
%Assay	98.75	98.62	99.5	98.75
SD	0.013	0.025	0.021	0.029
%RSD	1.21	1.34	1.85	1.26

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