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FIRST ORDER DERIVATIVE SPECTROSCOPY METHOD FOR SIMULTANEOUS ESTIMATION OF RANITIDINE HCl AND DICYCLOMINE HCl IN ITS COMBINED DOSAGE FORM

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ABSTRACT

Simple, accurate, precise and reproducible Spectrophotometric method for the simultaneous estimation of Ranitidine Hydrochloride and Dicyclomine Hydrochloride in tablet dosage form. Method is based on UV Spectrophotometric for determination of two drug, by using Methanol as a solvent and diluted the same with 0.1N NaOH, solution. This derivative Spectrophotometric method was developed by recording the absorbance at 222.2 nm (Zero crossing point of RANTD) and at 334.9 nm (Zero crossing point of DICY). This method was validated according to ICH guideline and Linearity range, it was found to be 7.5-37.5 µg/ml and 1-5 µg/ml for RANTD and DICY, respectively. The method could be applied for determination of in its tablet dosage forms without any interference from excipients or endogenous substances. The proposed method is suitable for routine quality control analysis.

KEYWORDS: UV Spectrophotometric; derivative Spectrophotometric method, Ranitidine Hydrochloride (RANTD); Dicyclomine Hydrochloride (DICY); ICH guidelines; Validation.

1. INTRODUCTION

Ranitidine Hydrochloride N-(2-[(5-(dimethylaminomethyl) furan-2-yl) methylthio]ethyl)- N-methyl- 2- nitroethene- 1,1-diamine (Figure 1) is histamine H2 receptor antagonist. Dicyclomine Hydrochloride 2-(diethylamino) ethyl cyclohexylcyclohexane-1-carboxylate (Figure 2) is antiemetic, antimuscarinics and anticholinergic agent. Combination is frequently used in the treatmentof acute ulcer. A literature survey revealed there are many analytical methods reported for estimation of Ranitidine Hydrochloride & Dicyclomine Hydrochloride in individuals & in combination with other drugs in bulk and pharmaceutical dosage forms.

We have developed and validate specific, accurate, precise spectrophotometry for estimation of marketed drug formulations.

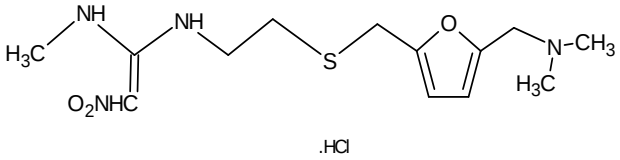
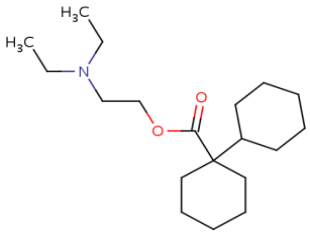


Figure 1: Ranitidine Hydrochloride.



.HCl

Figure 2: Dicyclomine Hydrochloride.

2. MATERIALS AND METHODS

2.1 MATERIALS

Standard bulk drug samples of ranitidine hydrochloride and Dicyclomine hydrochloride were provided by Yarrow chem. Mumbai Tablets of combined dosage form were procured from the local market. AR grade Methanol and NaOH were obtained form Molychem Limited, Mumbai.

2.2 INSTRUMENTATION

Spectrophotometric analysis was developed on a computer controlled Shimadzu UV-Visible spectrophotometer 1800, double beam spectrophotometer with spectral width 1 nm using 10 mm quartz cells. Absorption correction method UV spectra for the solution of RANTD and DICY were recorded in a 10 mm cell over the range 200-400 nm using methanol in the reference cell.

2.3 PREPARATION OF SOLUTIONS

2.3.1 Preparation of standard stock solution of Ranitidine Hydrochloride (RANTD)

Accurately weighed quantity of Ranitidine Hydrochloride 150 mg (equivalent to 150 mg) 100 ml was transferred into 100 ml volumetric flask. Add 60 ml methanol; sonicated for 10 min

and final volume was made 100 ml with the same to get stock solution containing 1500µg/ml of RANTD.

2.3.2 Preparation of working stock solution of RANTD

A solution of 150 µg/ml of RANTD was prepared by diluting 10 ml of standard stock solution with methanol in 100 ml volumetric flask up to the mark.

2.3.3 Preparation of standard stock solution of Dicyclomine Hydrochloride (DICY)

Accurately weighed quantity of Ranitidine Hydrochloride 100 mg was transferred into 100 ml volumetric flask. Add 60 ml methanol; sonicated for 10 min and final volume was made 100 ml with the same to get stock solution containing 1000 µg/ml of DICY.

2.3.4 Preparation of working stock solution of DICY

A solution of 20 µg/ml of DICY was prepared by diluting 2 ml of standard stock solution with methanol in 100 ml volumetric flask up to the mark.

2.3.5 Preparation of working stock solution of Mixture of RANTD and DICY

Pipette out 10 ml of standard stock solution of RANTD and 2ml of standard stock of DICY in to a 100 ml volumetric flask. Dilute it to 100 ml with methanol to get 150µg/ml of Ranitidine Hydrochloride and 20 µg/ml of Dicyclomine Hydrochloride.

2.3.6 Preparation of sample stock solution of Mixture of RANTD and DICY

Twenty tablets weight; average weight determined and crush to fine powder in a glass motar. Powder equivalent to the 15 mg of RANTD and 2 mg of DICY was weighed and transferred in to the 100 ml of volumetric flask, dissolved in 60 ml methanol and sonicate it for 15 minutes. Filter the solution through Whatman filter paper no.42 and diluted up to mark with same.

It gives the solution of RANTD 150 µg/ml. and DICY 20 µg/ml.

2.4 Validation of Proposed Method

2.4.1 Linearity and Range

The linearity was evaluated through a linear regression analysis. The linearity for RANTD (7.5-37.5µg/ml) and DICY (1-5µg/ml) was determined in terms of correlation coefficient.

Preparation of the solution for calibration curve of RANTD.

The series consisted of solutions having different concentrations of standard RANTD solution ranging from 7.5-37.5µg/ml. The solutions were prepared by pipetting out 0.5 1.0, 1.5, 2.0 and 2.5 ml of the working stock solution of RANTD (150µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N NaOH solution.

Preparation of the solution for calibration curve of DICY.

The series consisted of solutions having different concentrations of standard DICY solution ranging from 1-5µg/ml. The solutions were prepared by pipetting out 0.5 1.0, 1.5, 2.0 and 2.5 ml of the working stock solution of DICY (20µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N NaOH solution.

The amplitudes ($dA^2/d\lambda$) of first-order derivative spectra of the resulting solutions were measured at 361.2 nm and 277.4 nm for estimation of RANTD and DICY respectively against methanol as a blank. Calibration curve was prepared by plotting amplitude versus respective concentration. (Table 1 & 2)

2.4.2 Precision

Precision was considered at different levels, i.e. Method Precision, System Precision, Intraday and Interday.

Repeatability

Repeatability was studied by carrying out System precision and Method Precision.

System Precision was determined from results for six replicate of mixture of drug substance.

Take 1.0ml of working stock solution of mixture of RANTD and DICY (150µg/ml and 20 µg/ml respectively), transferred into 10 ml volumetric flask and diluted up to mark with 0.1N NaOH solution to get solution containing 15 µg/ml RANTD and 2µg/ml DICY.

Mixed solutions containing 15 µg/ml RANTD and 2 µg/ml DICY were analyzed 6 times and %RSD was calculated. (Table 3)

Method Precision was determined from results for six replicates of formulation.

Take 1.0ml of sample stock solution RANTD and DICY (150µg/ml and 20µg/ml respectively), transferred into 10 ml volumetric flask and diluted up to mark with 0.1N NaOH solution to get solution containing 15µg/ml RANTD and 2µg/ml DICY.

These solutions containing 15 µg/ml RANTD and 2 µg/ml DICY were analyzed 6 times and %RSD was calculated. (Table 4)

Intraday & Interday

Intraday precision was determined by analyzing the Combined solution containing the concentration 15, 22.5 and 30 µg/ml of RANTD and 2, 3 and 4 of DICY, for 3 times in the same day. Interday precision was determined by the same concentration of drug daily for 3 days. % RSD was calculated for both Intraday and interday. (prepared by pipetting out 1.0, 1.5, 2.0 ml of working stock solution of mixture into 10 ml volumetric flasks and diluted up to mark with 0.1 N NaOH) in triplicates analyzed three times on the same day and % RSD was calculated. (Table5&6)

LOD (Limit of Detection) and LOQ (Limit of Quantification)

The LOD is estimated from the set of 5 calibration curves used to determine method linearity. The LOD may be calculated as

LOD = 3.3 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

The LOQ is estimated from the set of 5 calibration curves used to determine method linearity. The LOQ may be calculated as

LOQ = 10 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

The values of LOD and LOQ are given in Table 7

2.4.3 Accuracy

The accuracy of the method was expressed by in term of the recovery study (80, 100, and 120%) was carried out by adding known amount of pure drug corresponding to 80, 100, and 120% to preanalysed sample solution (15 µg/ml of PCM & 2µg/ml of DICY) and the samples were reanalysed at each level 3 determination were performed (Table 8 & 9). The acceptance criteria for percent recovery are between 98 to 102%.

2.4.4 Assay (Quantification of RANTD and DICY in tablet dosage form)

Twenty tablets weight; average weight determined and crush to fine powder in a glass motar. Powder equivalent to the 15 mg of RANTD and 2 mg of DICY was weighed and transferred in to the 100 ml of volumetric flask, dissolved in 60 ml methanol and sonicate it for 15 minutes. Filter the solution through Whatman filters paper no.42 and diluted up to mark with

same. It gives the solution of RANTD 150µg/ml. and DICY 20µg/ml. The filtrate of 5.0 ml transfered in to 50 ml volumetric flask and volume was adjusted up to the mark with methanol to obtain the concentration of 15 µg/ml of RANTD and 2 µg/ml of DICY. (Table 11)

3. RESULT AND DISCUSSION

The first order absorption spectra of RANTD and DICY solution in 0.1N NaOH are shown in (Figure 1). The spectra display overlapping in the region 200-300 nm. This makes the determination of DICY in the presence of RANTD by the conventional UV-Spectrophotometry difficult. But the determination of RANTD from 300-400 nm might be possible without interference of DICY. The derivative spectroscopy techniques were; however, chosen for the determinations of both the drugs by overcome the interference from peak overlapping and it could remove broad band contribution from the excipients.

In this first derivative spectrum the signals at 222.2 nm (Zero crossing point of RANTD) were proportional to DICY concentration and the signals at 334.9 nm (DICY reads zero) were proportional to RANTD concentration. (Figure 3)

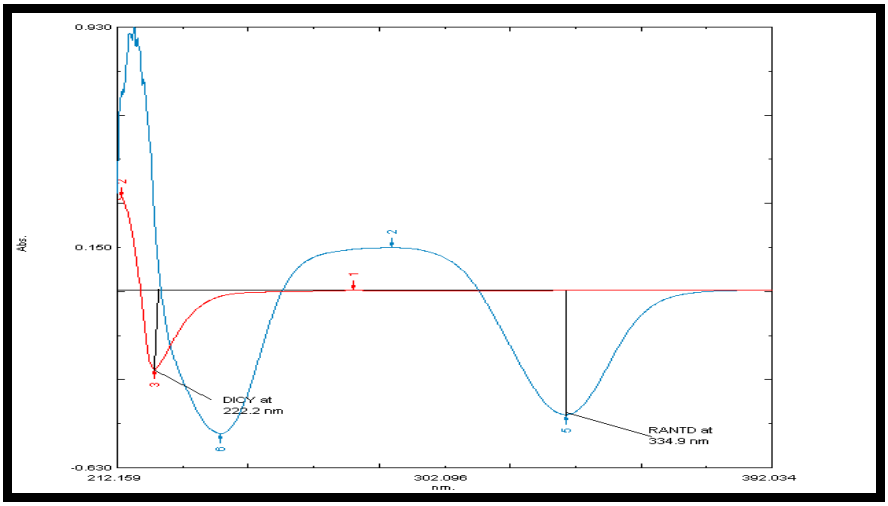


Figure 3: First-order overlain spectra of RANTD and DICY.

3.1 Linearity and Range

The linearity range for Ranitidine Hydrochloride and Dicyclomine Hydrochloride were found to be in the range of 4-24 µg/ml. (Table 1 & 2)

Correlation co-efficient for calibration curve of RANTD and DICY were found to be 0.999 and 0.999, respectively.

The regression line equation for RANTD and DICY are as following, (Figure 6.5.5 & 6.5.6)

Y_{RANTD} = 0.036x-0.05, Y_{DICY} =0.035x+0.027.

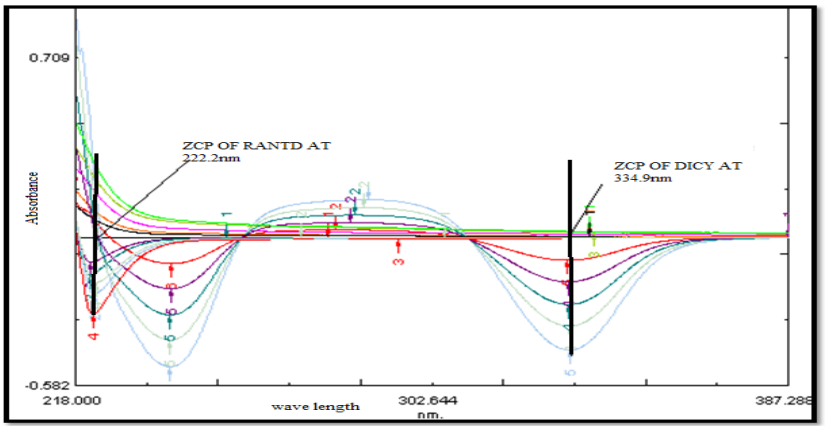


Figure 4: First-order overlay spectra of RANTD & DICY.

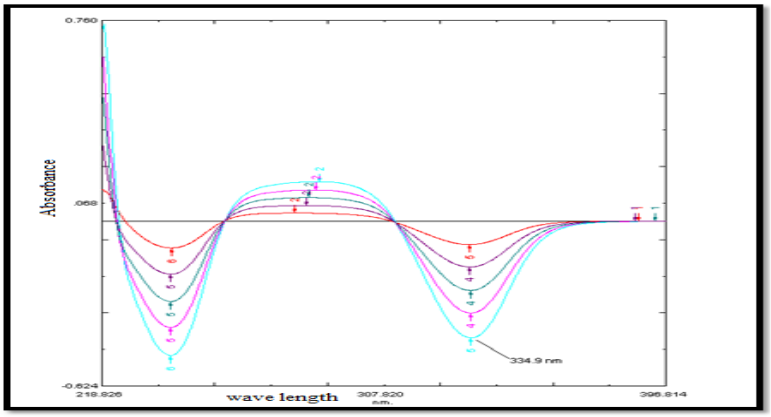


Figure 5: First-order linearity spectra of RANTD.

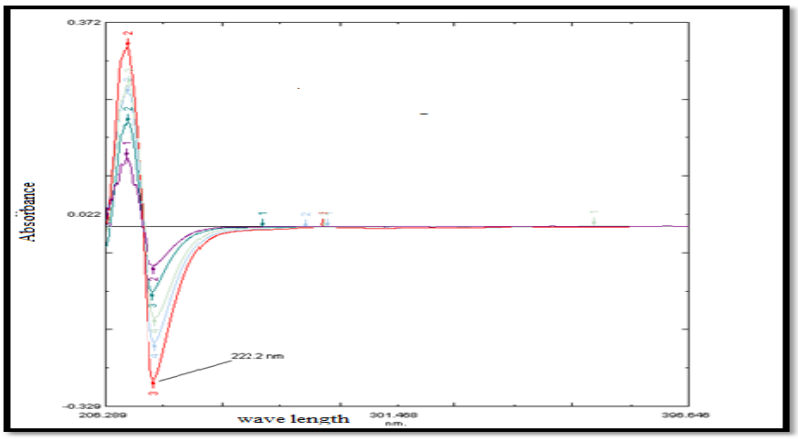


Figure 6: First-order linearity spectra of DICY.

Table 1: Result of linearity study for standard RANTD at 334.9nm.

Sr.No.	Concentration (µg/ml)	Absorbance Mean ± S.D. (n=5)	%RSD
1	7.5	0.086± 0.001	1.23
2	15	0.177±0.0008	0.45
3	22.5	0.275±0.0015	0.54
4	30	0.384±0.0021	0.52
5	37.5	0.488±0.0008	0.17

Table 2: Result of linearity study for standard DICY at 222.2 nm.

Sr.No.	Concentration (µg/ml)	Absorbance Mean ± S.D. (n=5)	%RSD
1	1	0.071±0.013	1.80
2	2	0.120±0.0019	1.50
3	3	0.176±0.0016	0.90
4	4	0.221±0.0025	1.13
5	5	0.284±0.0021	1.74

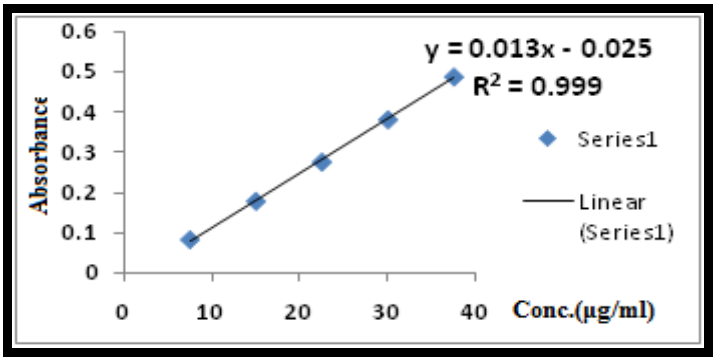


Figure 7: First-order calibration curve of RANTD at 334.9 nm.

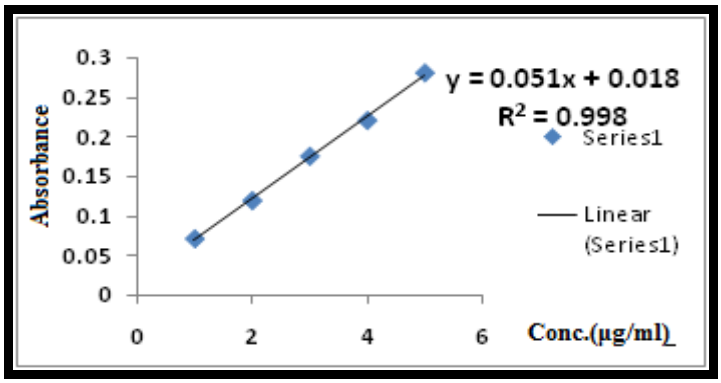


Figure 8: First-order calibration curve of DICY at 222.2 nm.

3.2 Precision

Repeatability

Result of repeatability in terms of System precision and Method Precision.

Table 3: Results of System precision for RANTD and DICY at 334.9 nm and 222.2 nm, respectively.

Conc. (µg/ml)	RANTD			Conc. (µg/ml)	DICY		
	Absorbance	Mean*± S.D.	%RSD		Absorbance	Mean*± S.D.	%RSD
15	0.179	0.179±0.0007	0.39	2	0.121	0.121± 0.0008	0.68
15	0.180			2	0.122		
15	0.179			2	0.121		
15	0.179			2	0.120		
15	0.178			2	0.120		
15	0.178			2	0.121		

***** Average of six determinations.

% RSD for System precision for combined solution of RANTD and DICY were found to be 0.39% for RANTD and 0.68% for DICY.

Table 4: Results of Method precision for RANTD and DICY at 334.9 nm and 222.2 nm, respectively.

Conc. (µg/ml)	RANTD			Conc. (µg/ml)	DICY		
	Absorbance	Mean* ± S.D.	%RSD		Absorbance	Mean* ± S.D.	%RSD
15	0.161	0.161 ±0.00073	0.43	2	0.120	0.119 ±0.0011	0.92
15	0.160			2	0.118		
15	0.161			2	0.121		
15	0.161			2	0.119		
15	0.164			2	0.119		
15	0.162			2	0.118		

***** Average of six determinations.

% RSD for System precision for combined solution of RANTD and DICY were found to be 0.43% for RANTD and 0.92% for DICY.

Percentage RSD of repeatability were <2% for both drugs, indicates that the method is precise.

Intraday Precision

Table 5: Results of **Intraday precision for RANTD and DICY at 334.9 nm and 222.2 nm, respectively.**

RANTD			DICY		
Conc. (µg/ml)	Absorbance Mean* ± S.D.	%RSD	Conc. (µg/ml)	Absorbance Mean* ± S.D.	%RSD
15	0.178±0.00057	0.32	2	0.121±0.0012	0.90
22.5	0.273±0.0028	0.73	3	0.173±0.0010	0.57
30	0.352±0.0020	0.56	4	0.213±0.0011	0.53

*Average of three determinations

The average %RSD for intraday precision was found to be 0.54% and 0.67% for RANTD and DICY, respectively.

Interday Precision

Table 6: Results of Interday precision for RANTD and DICY at 334.9 nm and 222.2 nm, respectively.

RANTD			DICY		
Conc. (µg/ml)	Absorbance Mean* ± S.D.	%RSD	Conc. (µg/ml)	Absorbance Mean* ± S.D.	%RSD
15	0.177±0.0015	0.84	2	0.118±0.0012	1.01
22.5	0.272±0.0023	0.85	3	0.172±0.0026	1.51
30	0.336±0.0021	0.62	4	0.210±0.0025	1.19

*Average of three determinations

The average % RSD for interday precision was found to be 0.77% and 1.23% for RANTD and DICY, respectively.

Percentage RSD of intraday and interday precision were < 2% for both drugs, indicates that the method is precise.

3.3 LOD and LOQ

Calibration curve was repeated for 5 times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were measured as follows.

LOD=3.3 * SD/slope of calibration curve

LOQ=10 * SD/slope of calibration curve

SD = Standard deviation of intercepts

Table 7: Results of LOD and LOQ for RANTD and DICY.

Parameter	RANTD (µg/ml)	DICY(µg/ml)
SD of intercept	0.0026	0.0015
Slope	0.013	0.051
LOD (µg/ml)	0.66	0.102
LOQ (µg/ml)	2	0.310

3.4 Accuracy

Table 8: Results of Recovery study for RANTD.

Accuracy level	Amount of RANTD in Sample (µg/ml)	Amount of Std RANTD added (µg/ml)	Total amount of RANTD (µg/ml)	Total amount of RANTD found (µg/ml) Mean (n=3)	% Recovery \ (n=3)	%RSD
Pre analyzed	15	0	15	15.38	-	-
80%	15	12	27	27.30	99.33	0.175
100%	15	15	30	30.46	100.5	0.312
120%	15	18	33	33.53	100.8	0.421

Table 9: Results of Recovery study for DICY.

Accuracy level	Amount of DICY in Sample (µg/ml)	Amount of Std DICY added (µg/ml)	Total amount of DICY (µg/ml)	Total amount of DICY found (µg/ml) Mean (n=3)	% Recovery (n=3)	%RSD
Pre analyzed	2	0	2	2.02	-	-
80%	2	1.6	3.6	3.64	101.25	0.565
100%	2	2	4	4.01	99.50	1.450
120%	2	2.4	4.4	4.41	99.58	0.953

The data for accuracy for RANTD and DICY shown in Table 8 and 9, respectively.

Percentage recovery for RANTD was 98.86-100.25%**,** while for DICY was 98.75-100.83%. Recovery was in the range of 98-102%, indicates that method is accurate.

Table 10: Summary of all parameters.

Parameters		UV Spectrophotometry	
		RANTD	DICY
Linearity	(µg/ml)	7.5-37.5	1-5
	Regration equation	Y_{RANTD}=0.034x-0.00001	Y_{DICY} =0.090x+0.020
	R^2	0.999	0.998
Precision (%RSD)	System	0.39	0.68
	Method	0.43	0.924
	Intraday	0.54	0.67
	Interday	0.770	1.23
LOD	(µg/ml)	0.66	0.102
LOQ	(µg/ml)	2	0.310
Accuracy %	80%	99.33%	101.25%
	100%	100.50%	99.50%
	120%	100.80%	99.58%

3.5 Assay (Quantification of RANTD and DICY in tablet dosage form)

The proposed method was applied for the determination of RANTD and DICY in their combined pharmaceutical formulation and the % Recoveries confirm the suitability of the proposed method for routine determination of these components in combined formulation.

Table 11: Results of Assay for RANTD & DICY in marketed formulation.

Formulation (Tablet)	Amount of drug taken(mg)		Amount of drug found(mg)		% Assay Mean* ± SD (n=3)		%RSD	
	RANTD	DICY	RANTD	DICY	RANTD	DICY	RANTD	DICY
RADIC	15	2	14.92	1.98	99.66± 0.001	99.35± 0.0019	0.591	0.840
REDEN-PLUS	15	2	15.01	2.01	100.27± 0.002	100.16± 0.001	1.176	0.833

***** Average of three determination

4. CONCLUSION

A simple, accurate and rapid first order UV Spectrophotometric method was developed and validated for the simultaneous estimation of Ranitidine Hydrochloride and Dicyclomine Hydrochloride in their combined marketed formulation. The advantage lies in simplicity of sample preparation and the low costs of reagents used. The proposed method assured satisfactory linearity, accuracy and precision. Analysis of tablet sample containing Ranitidine Hydrochloride and Dicyclomine Hydrochloride showed no interference from the common excipients and additives. The proposed method can be easily and conveniently used for routine quality control analysis.

5. AKNOWLEDGEMENT

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