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Development and Validation of Absorption Correction UV-Spectrophotometry Method for Simultaneous Estimation of Ranitidine HCL and Dicyclomine Hydrochloride in Their Marketed Formulation

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ABSTRACT

A Simple, selective, precise and rapid absorption correction Spectrophotometric method has been developed and validated as per ICH guideline for the simultaneous estimation of Ranitidine Hydrochloride and Dicyclomine Hydrochloride in tablet dosage form. Method is base on UV Spectrophotometric for determination of two drug, by using Methanol as a solvent and diluted the same with 0.1N NaOH, solution. In this UV method, the two wavelength were selected, 311.4 nm and at 217 nm for RANTD and DICY, respectively. this method was validated according to ICH guideline and Linearity range, was found to be 7.5-37.5 µg/ml and 1-5 µg/ml for RANTD and DICY, respectively. The method was successfully applied to assay drugs in tablet.

Keywords :- UV Spectrophotometric absorption correction method; Ranitidine Hydrochloride (RANTD); Dicyclomine Hydrochloride (DICY); ICH guidelines; Validation.

I. 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 treatment of acute ulcer.[32-37] 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.[45-52] We have developed and validate new specific, very simple, accurate, precise spectrophotometry for estimation of marketed drug formulations.

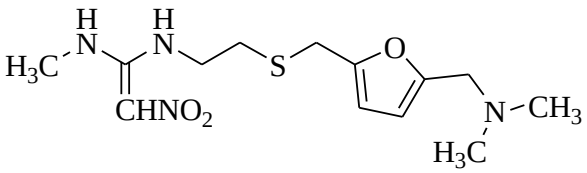

Figure 1 : Ranitidine Hydrochloride

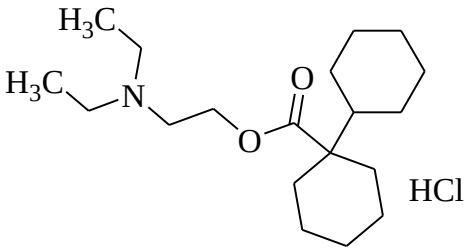

Figure 2 : Dicyclomine Hydrochloride

II. 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 from 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 Ranitidine) 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 1500 µg/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 Dicyclomine 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 1000µg/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 2 ml of standard stock of DICY in to a 100 ml volumetric flask. Dilute it to 100 ml with methanol to get 150 µg/ml of Ranitidine Hydrochloride and 20µg/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 [23-31]

2.4.1 Linearity and Range

The linearity was evaluated through a linear regression analysis. The linearity for RANTD (7.5-37.5 µg/ml) and DICY (1-5 µg/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 7.5-37.5 µg/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 (20 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N NaOH solution.

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 substances.

Take 1.0ml of working stock solution of mixture of 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 15 µg/ml RANTD and 2µg/ml DICY.

Mixed solutions containing 15µg/ml RANTD and 2µg/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 (150 µg/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.

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 30µg/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. (Table 5 & 6)

2.4.3 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

$$LOD = 3.3 \times (SD / Slope)$$

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

$$LOQ = 10 \times (SD / Slope)$$

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

2.4.4 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 pre analysed sample solution (15 µg/ml of PCM & 2 µg/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%.

2.4.5 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 150 µg/ml. and DICY 20 µg/ml.The filtrate of 5.0ml transfered in to 50 ml volumetric flask and volume was adjusted up to the mark with methanol to obtain the concentration of 15µg/ml of RANTD and 2µg/ml of DICY. (Table 11)

III. RESULT AND DISCUSSION

From the stock solutions, working standard solutions of RANTD (100 µg/ml) and DICY (20µg/ml) were prepared. By appropriate dilutions, the solutions with concentrations 7.5-37.5µg/ml (for RANTD) and 1-5µg/ml (for DICY) were prepared and scanned between 200 to 400nm.

For RANTD and DICY, analytical wavelengths of 311.4nm and 217nm were selected respectively. Absorptivity of RANTD and DICY were calculated at both the wavelengths.

The concentrations of RANTD and DICY can be calculated from following equations:

$C_{x\,(RANTD)} = A_2 / a_{x2}$

$C_{y\,(DICY)} = A_1 - a_{x1}\, C_{x\,(RANTD)} / a_{y1}$

Where, A_1= absorbance of mixture at 217nm

A_2=absorbance of mixture at 311.4nm

a_{x1}= absorptivity of RANTD at 217nm

a_{x2} = absorptivity of RANTD at 311.2nm

a_{y1}= absorptivity of DICY at 217nm

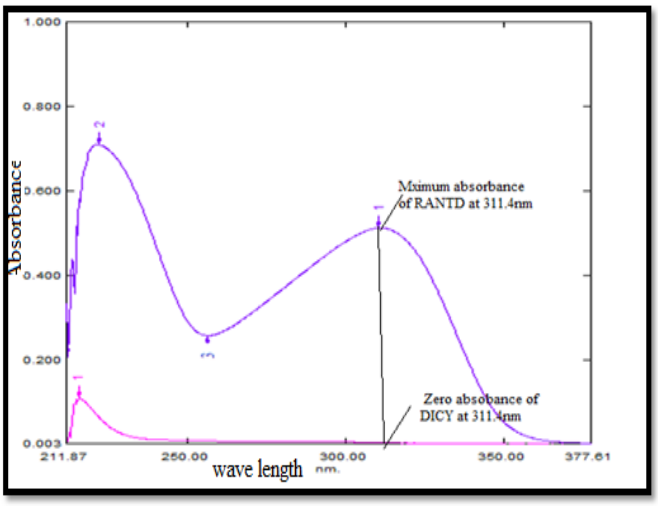

Figure 3 : Zero order overlain spectra of Ranitidine Hydrochlorideand Dicyclomine Hydrochloride

3.1. Linearity and Range

The linearity range for Ranitidine Hydrochloride and Dicyclomine Hydrochloride were found to be in the range of 7.5-37.5 µg/ml &1-5µg/ml, respectively. (Table 6.4.1 & 6.4.2)

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 2&3)

YRANTD = 0.034x + 0.0001

Y DICY = 0.090x + 0.020

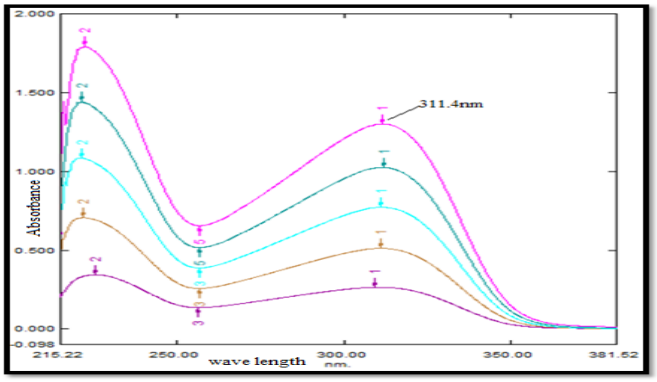

Figure 4: Linearity spectra of RANTD

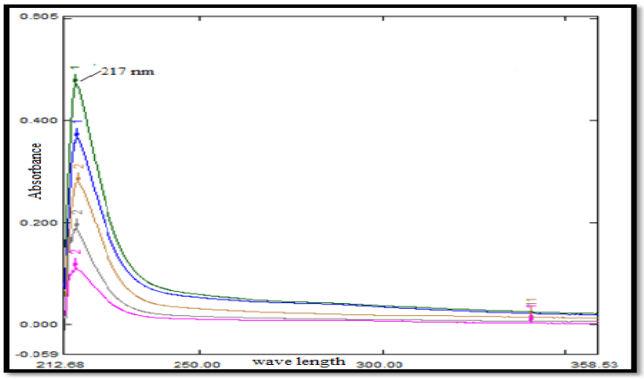

Figure 5: Linearity spectra of DICY

Table 1: Result of linearity study for standard RANTD at 311.4 nm

Sr.No.	Concentration (µg/ml)	Absorbance Mean ± S.D. (n=5)	%RSD
1	7.5	0.265±0.0006	0.22
2	15	0.512±0.0005	0.09
3	22.5	0.773±0.0005	0.07
4	30	1.025±0.0008	0.08
5	37.5	1.300±0.0010	0.08

Table 2: Result of linearity study for standard DICY at 217 nm

Sr.No.	Concentration (µg/ml)	Absorbance Mean ± S.D. (n=5)	%RSD
1	1	0.118±0.002	1.40
2	2	0.198±0.005	0.29
3	3	0.283±0.001	0.53
4	4	0.376±0.004	1.11
5	5	0.480±0.006	0.95

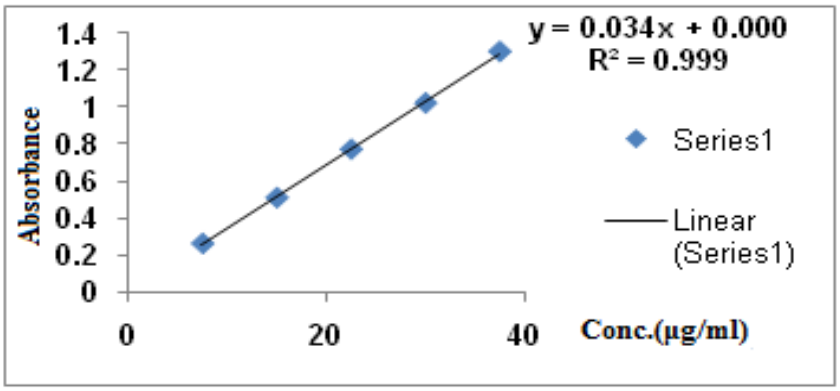

Figure 6 : Calibration curve of RANTD at 311.4 nm.

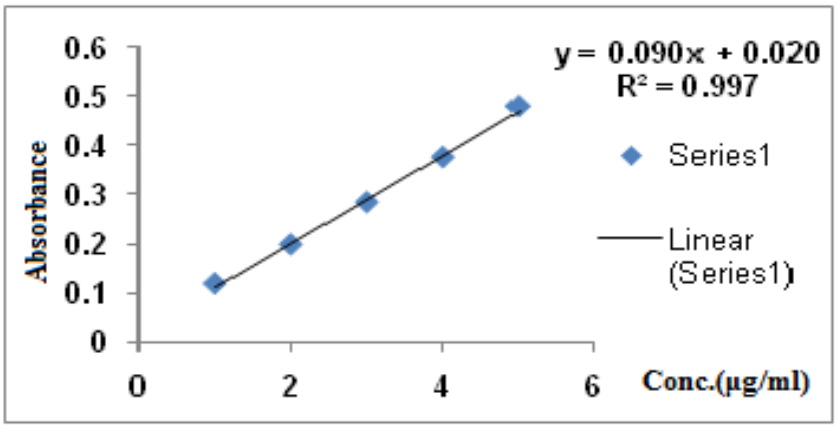

Figure 7 : Calibration curve of DICY at 217 nm.

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 311.4 nm and 217 nm, respectively

RANTD				DICY			
Conc. (µg/ml)	Absorbance	Mean* ± S.D.	%RSD	Conc. (µg/ml)	Absorbance	Mean* ± S.D.	%RSD
15	0.545	0.543± 0.0013	0.24	2	1.101	1.099± 0.0024	0.22
15	0.544			2	1.102		
15	0.544			2	1.099		
15	0.543			2	1.100		
15	0.544			2	1.098		
15	0.541			2	1.095		

* Average of six determinations.

% RSD for System precision for combined solution of RANTD and DICY were found to be 0.24% for RANTD and 0.22% for DICY.

Table 4: Results of Method precision for RANTD and DICY at 311.4 nm and 217 nm, respectively

RANTD				DICY			
Conc. (µg/ml)	Absorbance	Mean* ± S.D.	%RSD	Conc. (µg/ml)	Absorbance	Mean* ± S.D.	%RSD
15	0.493	0.493± 0.0020	0.41	2	1.023	1.021± 0.0026	0.260
15	0.491			2	1.021		
15	0.495			2	1.018		
15	0.493			2	1.023		
15	0.491			2	1.019		
15	0.496			2	1.025		

* Average of six determinations.

% RSD for System precision for combined solution of RANTD and DICY were found to be 0.41% for RANTD and 0.26% 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 311.4 nm and 217 nm, respectively

RANTD			DICY		
Conc. (µg/ml)	Absorbance Mean* ± S.D.	%RSD	Conc. (µg/ml)	Absorbance Mean* ± S.D.	%RSD
15	0.544±0.0005	0.09	2	1.097±0.0035	0.31
22.5	0.816±0.0006	0.06	3	1.448±0.0036	0.24
30	1.102±0.0005	0.04	4	1.868±0.0075	0.40

*Average of three determinations

The average %RSD for intraday precision was found to be 0.06% and 0.32% for RANTD and DICY, respectively.

Interday Precision

Table 6: Results of Interday precision for RANTD and DICY at 311.4 nm and 217 nm, respectively

RANTD			DICY		
Conc. (µg/ml)	Absorbance Mean* ± S.D.	%RSD	Conc. (µg/ml)	Absorbance Mean* ± S.D.	%RSD
15	0.486±0.0051	1.04	2	1.089±0.0037	0.34
22.5	0.778±0.0015	0.19	3	1.392±0.0098	0.70
30	1.085±0.0049	0.46	4	1.781±0.0057	0.31

*Average of three determinations

The average % RSD for interday precision was found to be 0.56% and 0.44% for RANTD and DICY, respectively.

Percentage RSD of intraday and interday precision were < 2% for both drugs, indicates that the method was 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	0.0001	0.0056
Slope	00.034	00.09
LOD (µg/ml)	0.0097	0.205
LOQ (µg/ml)	00.029	0.622

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	15.14	-	
80%	15	12	27	27.17	100.25	0.125
100%	15	15	30	29.97	98.86	0.113
120%	15	18	33	32.97	99.05	0.103

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	2.06	-	
80%	2	1.6	3.6	3.64	98.75	0.342
100%	2	2	4	4.07	100.50	0.375
120%	2	2.4	4.4	4.48	100.83	0.461

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	0.997
Precision (%RSD)	System	0.24	0.22
	Method	0.41	0.26
	Intraday	0.06	0.32
	Interday	0.56	0.446
LOD	(µg/ml)	0.0097	0.205
LOQ	(µg/ml)	0.029	0.622
Accuracy %	80%	100.25	98.75
	100%	98.86	100.50
	120%	99.05	100.83

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**	
RADIC	RANTD	DICY	RANTD	DICY	RANTD	DICY	RANTD	DICY
	15	2	15.05	1.99	100.46±0.0029	99.61±0.0020	0.298	0.297
REDEN-PLUS	15	2	14.88	2.01	99.41± 0.0041	100.4± 0.0021	0.821	0.268

* Average of three determinations.

III. CONCLUSION

A simple, accurate and rapid Absorption correction 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

IV.AKNOWLEDGEMENT

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