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Research Article

Stability Indicating UV Spectrophotometric Assay Method Development for Simultaneous Determination of Ranitidine and Dicyclomine in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, accurate, economic and reproducible ultraviolet spectrophotometric method for the simultaneous estimation of ranitidine and dicyclomine has been developed and validated. The method was involved the measurement of absorbance at two wavelengths 325 nm and 218 nm for ranitidine and dicyclomine respectively by using methanol as solvent. The method was found to be linear in the range of 3-18 µg/ml for ranitidine and 1-6 µg/ml for dicyclomine respectively. The method was validated in terms of linearity, accuracy, precision, limit of detection and limit of quantitation as per ICH guidelines. The method has good reproducibility with % RSD less than two. Thus, proposed method can be successfully applied for ranitidine and dicyclomine in routine analysis work.

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Key-words: Ranitidine, dicyclomine, UV spectrophotometric method, validation, forced degradation.

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INTRODUCTION

Chemically, ranitidine (RANI) is N-[2-[[[5-[(dimethylamino) methyl] furan-2-yl] methyl] thio] ethyl] -N-methyl-2-nitroethene-1,1-diamine (figure- 1a). It is official in Indian Pharmacopeia and British Pharmacopeia.[1-2] RANI is a histamine H_2 receptor antagonist and used as antiulcer agent to treat erosive esophagitis, benign gastric ulcer, duodenal ulcer gastroesophageal reflux disease and pathological hypersecretory conditions.[3]

Chemically, dicyclomine, also known as dicycloverine (DICY) is 2-(diethylamino) ethyl bicyclohexyl – l – carboxylate (figure- 1b). It is official in Indian Pharmacopeia and British Pharmacopeia.[1-2] It is an antispasmodic and anticholinergic agent, acts through muscarinic receptors like M_1 and M_2 rather than M_3 and M_4 receptors and used for its spasmolytic effect on various smooth muscle spasms, particularly those associated with the gastrointestinal tract. It is also useful in dysmenorrhea, pylorospasm, and biliary dysfunction.[3]

The literature survey reveals that number of methods such as spectrophotometric, HPTLC, HPLC, potentiometric, spectroflurometric are reported for the estimation of RANI from its formulation or biological fluids in single or combination with other drugs. Similarly number of methods such as spectrophotometric, spectrofluorometric, RP-HPLC, voltametric are reported for the estimation of DICY from its formulation or biological fluids in single or combination with other drugs. This paper is in continuation with our work [17-22] where we studied spectrophotometric method for single or multicomponent drugs. There was no any method reported for the simultaneous estimation of RANI and DICY from their combined dosage form. So, present study was aimed to develop and validate stability indicating spectrophotometric assay method for simultaneous estimation of RANI and DICY in bulk and combined tablet dosage form which would be simple, sensitive, cost effective and easily adopted for analysis of finished product.

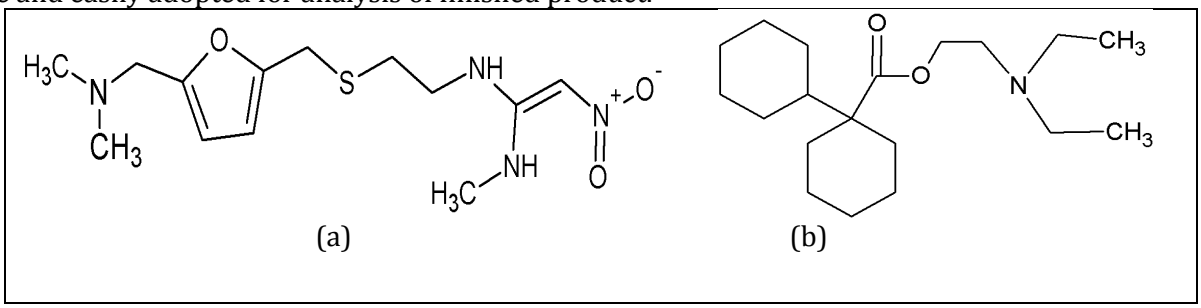


Figure 1: Chemical structure of (a) RANI (b) DICY

MATERIALS AND METHODS

Instrumentation

The Spectrophotometric measurements were carried out using UV-visible double beam spectrophotometer (Model: UV-1800, Shimadzu Corp., Japan) with 2 nm spectral bandwidth using 1 cm matched quartz cells used for development of analytical method over the range of 200-400 nm. Data acquisition was made by using computer operated software UV-probe version 2.33.

Reagents and materials

Working standard of ranitidine and dicyclomine were procured as a gift sample from Orchev Pharma Pvt. Ltd. Rajkot, Gujrat, India and Wockhardt Pvt. Ltd Aurangabad, Maharashtra, India respectively. Marketed formulation RANILA-SPAS (Ranitidine - 150 mg, Dicyclomine - 10 mg) of Akums drugs & Pharma Ltd, was purchased from local market. All chemicals and solvents of AR grade were purchased from S. D. Fine Chem Ltd., Mumbai, India.

Selection of the solvent

The solubility of drug is an basic criterion for selection of the solvent. Methanol was selected as solvent system for both the drugs for developing analytical method and this selection was made after assessing the solubility of RANI and DICY in different solvent.

Preparation of standard stock solution

Accurately weighed quantity of RANI (10 mg) and DICY (10 mg) were transferred into two separate 100 ml volumetric flasks and volume was made up the mark with methanol to get 100 µg/ml. The stock solution was sonicated for 15 min.

Preparation of working standard solution

From the standard stock solution, 1 ml of each RANI and DICY was transferred to separate 10 ml volumetric flasks and the volume was made up to the mark with methanol to get 10 µg/ml solution of RANI and DICY respectively.

Selection of Analytical Wavelengths

The working standard solutions containing 10 µg/ml of RANI and 10 µg/ml of DICY were scanned separately in the range of 200 - 400 nm for absorbance maxima using methanol as blank. RANI shows maximum absorbance at 325 nm while DICY at 218 nm. From the overlay spectra of both the drugs wavelength selected for quantification were 325 nm and 218 nm for RANI and DICY respectively. The absorption spectrum was obtained for RANI, DICY and their overlain spectra are shown in figure 2 and 3 respectively.

Preparation of Calibration curve

Appropriate aliquotes of RANI and DICY from stock solution further diluted with methanol to obtain 3, 6, 9, 12, 15, 18 µg/ml and 1, 2, 3, 4, 5, 6 µg/ml concentration of RANI and DICY respectively. The absorbance of solution was measured at 325 nm and 218 nm and the calibration curves were plotted for these concentrations against absorbance value obtained at respective λ_{max} and they are shown in figure 4 respectively and data is reported in table 1.

Table 1: Linearity study data of RANI and DICY

Sr. No.	RANI		DICY	
	Conc. (µg/ml)	Abs. at 325 nm	Conc. (µg/ml)	Abs. at 218 nm
01.	3	0.185	1	0.415
02.	6	0.408	2	0.422
03.	9	0.603	3	0.428
04.	12	0.794	4	0.434
05.	15	0.957	5	0.441
06.	18	1.139	6	0.447

Analysis of marketed formulation

Twenty tablets of marketed formulation RANILA-SPAS were accurately weighed and average weight was calculated. Then these tablets were crushed to fine powder and quantity equivalent to 150 mg of RANI and 10 mg of DICY were weighed and transferred into 100 ml volumetric flask, then volume was made up to the mark with methanol and this mixture was sonicated for about 20 min. After sonication, it was filtered through Whatmann filter paper and the filtrate was further diluted with methanol to get a final concentration of 15 µg/ml and 1 µg/ml of RANI and DICY respectively. The absorbance of the solutions were measured at 325 nm and 218 nm respectively. The analysis procedure was repeated six times with tablet formulation and result is shown in table 3.

Table 2: Optical characteristics and other parameters

Parameters	RANI	DICY
λ_{max} wavelength (nm)	325	218
Linearity range (µg/ml)	3-18	1-6
Limit of detection (µg/ml)	0.0531	0.549
Limit of quantitation (µg/ml)	0.1609	1.665
$y = mx + c$	-	-
Slope (m)	0.062	0.006
Intercept (c)	0.020	0.408
Regression coefficient (R^2)	0.997	0.999

Table 3: Results of analysis of marketed formulation

Drug	Label claim (mg)	Amount found (mg)*	% Label claim*	SD	% RSD
RANI	150	149.65	99.77	0.2221	0.2226
DICY	10	9.98	99.80	0.2629	0.2634

** Indicates average of six determinations*

Analytical method validation

Linearity

Both drugs followed the Beer-Lamberts law and the response was determined to be linear over the range of 3-18 µg/ml and 1-6 µg/ml for RANI and DICY respectively. The proposed method was evaluated by its regression coefficient (r^2) value which is calculated by statistical method. The regression coefficients (r2) were found to be 0.997 and 0.999 for RANI and DICY respectively. The optical characteristics and other parameters are shown in table 2.

Table 4: Results for intra-day and inter-day precision

Drug	Intra-day precision				Inter-day precision		
	% Found*	SD	% RSD		% Found*	SD	% RSD
RANI	99.87	0.1096	0.1098		99.87	0.1096	0.1100
DICY	99.84	0.2771	0.2775		99.68	0.2771	0.2780

** Indicates average of three determinations*

Table 5: Results of recovery study

Drug	Amount present	Amount added	Amount found*	% Recovery*	SD	% RSD
RANI	150	120	270.32	100.12	1.210	1.2086
	150	150	300.18	100.06	0.2847	0.2846
	150	180	329.37	99.81	0.1950	0.1953
DICY	10	8	17.96	99.76	0.470	0.471
	10	10	19.98	99.91	0.7179	0.7185
	10	12	22.02	100.07	0.5986	0.5982

** Indicates average of three determinations*

Table 6: LOD & LOQ

Parameters	RANI	DICY
LOD (µg/ml)	0.0531	0.549
LOQ (µg/ml)	0.1609	1.665

Precision

The precision study was carried out by intraday and interday variation studies. The working solution of samples were analysed in triplicate in a day for intraday precision while the working solution of samples were analysed on three different days for interday precision study. The results of precision study are reported in terms of percentage relative standard deviation (% RSD). In precision study 15 µg/ml of RANI and 1 µg/ml of DICY solution were prepared. Precision data is shown in table 4.

Accuracy

To ascertain the accuracy of the proposed method, recovery studies were carried out at three different levels (80%, 100% and 120%) by standard addition method as per ICH guidelines. By adding different concentrations of pure drug in the pre-analyzed powder of tablet formulation and analysis was carried out at each level and % recovery, standard deviation (SD), % RSD was calculated. The recovery study results of RANI and DICY are shown in the table 5.

LOD and LOQ

ICH guideline describes several approaches to determine the detection and quantitation limits. These include visual evaluation, signal to noise ratio and the use of standard deviation of the response and the slope of the calibration curve. In the present study, the LOD and LOQ were based on the third approach and calculated with use of the following equations:

$$LOD = \frac{3.3\sigma}{S} \qquad LOQ = \frac{10\sigma}{S}$$

Where σ is the standard deviation of the peak areas of the drugs, taken as a measure of noise, and S is the slope of the corresponding calibration curve. The values are mentioned in Table 6.

FORCED DEGRADATION STUDIES

Stress degradation studies were performed to check the stability of the RANI and DICY in different conditions like acid, base, neutral hydrolysis, thermal, photolytic and oxidative degradation. Standard stock solution of RANI and DICY was prepared in methanol. Summary of forced degradation study of RANI and DICY is shown in Table. 7

Acid Hydrolysis

To perform acid hydrolysis, add 10 ml of stock solution of both drugs in two separate 100 ml volumetric flask then added 10 ml of 0.1 M HCl and kept at room temperature for 6 hrs, then neutralized with 10 ml of 0.1 M NaOH and volume was made up to 100 ml with methanol and further diluted to form (15 µg/ml for RANI and 1 µg/ml for DICY) and absorbance's were measured at 325 nm for RANI and 218 nm for DICY. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

Base Hydrolysis

To perform base hydrolysis, add 10 ml of stock solution of both drugs in two separate 100 ml volumetric flask then added 10 ml of 0.1 M NaOH and kept at room temperature for 6 hrs, then neutralized with 10 ml of 0.1 M HCl and volume was made up to 100 ml with methanol and further diluted to form (15 µg/ml for RANI and 1 µg/ml for DICY) and absorbance's were measured at 325 nm for RANI and 218 nm for DICY. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

Neutral Hydrolysis

To perform neutral hydrolysis, add 10 ml of stock solution of both drugs in two separate 100 ml volumetric flask then added 10 ml of distilled water and kept at room temperature for 6 hrs, then volume was made up to 100 ml with methanol and further diluted to form (15 µg/ml for RANI and 1 µg/ml for DICY) and absorbance's were measured at 325 nm for RANI and 218 nm for DICY. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

Oxidative degradation

To perform oxidative degradation, add 10 ml of stock solution of both drugs in two separate 100 ml volumetric flask then added 10 ml of 3% H_2O_2 and kept at room temperature for 6 hrs, then volume was made up to 100 ml with methanol and further diluted to form (15 µg/ml for RANI and 1 µg/ml for DICY) and absorbance's were measured at 325 nm for RANI and 218 nm for DICY. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

Photolysis

Pure drugs were exposed to UV radiations for 6 hrs and samples were withdrawn at interval of 1, 2, 3, 6 hrs. The samples after exposure to light were diluted with methanol to get 15 µg/ml for RANI and 1 µg/ml for DICY

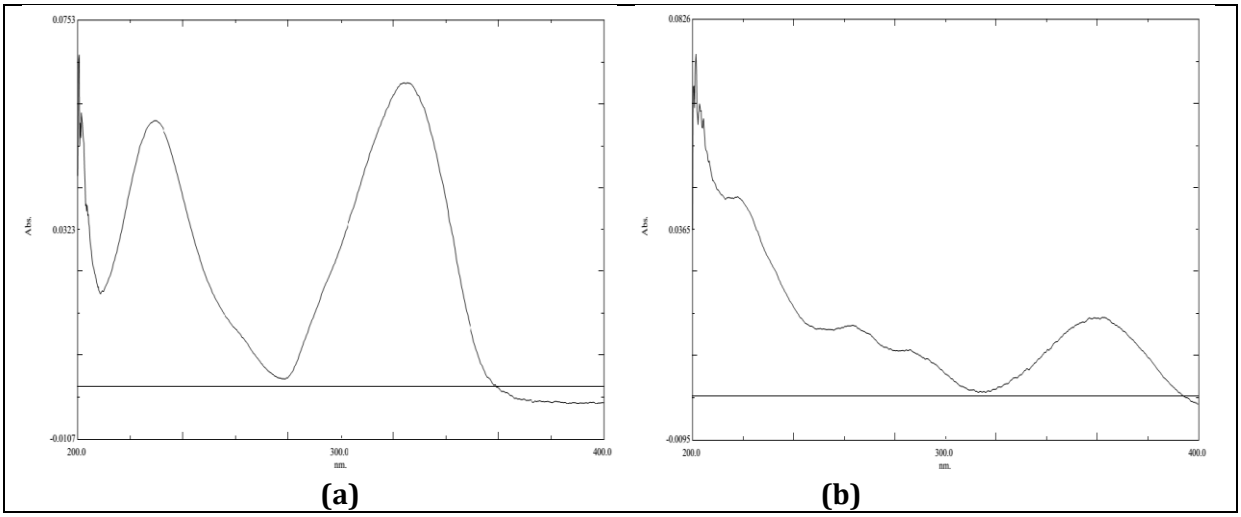
and absorbance's were measured at 325 nm for RANI and 218 nm for DICY. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

Thermal degradation

Thermal degradation was carried out by exposing pure drugs to dry heat at 80ºC for 6 hrs and samples were withdrawn at interval of 1, 2, 3, 6 hrs. The samples after exposure to heat were prepared and diluted with methanol to get 15 µg/ml for RANI and 1 µg/ml for DICY and absorbance's were measured at 325 nm for RANI and 218 nm for DICY. Finally, absorbances of sample were compared with standard absorbance and percent degradation was calculated.

Table 7: Results of forced degradation study

Sr. No.	Condition	% Degradation		% Assay	
		RANI	DICY	RANI	DICY
01.	Acid hydrolysis	39.09	15.60	60.91	84.4
02.	Base hydrolysis	8.40	9.92	91.6	90.08
03.	Neutral hydrolysis	50.24	7.21	49.76	92.79
04.	Oxidative degradation	37.93	9.56	62.07	90.44
05.	Photolytic degradation	40.02	9.14	59.98	90.86
06.	Thermal degradation	38.50	4.08	61.5	95.92



(a) **(b)**

Figure 2: Absorption spectrum of (a) RANI (b) DICY

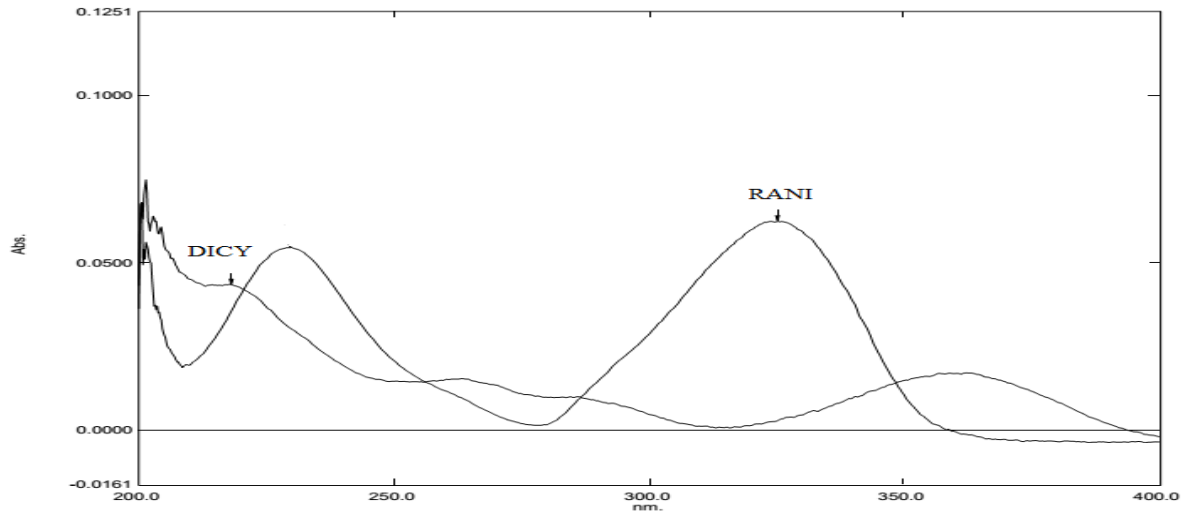


Figure 3: Overlain spectrum of RANI and DICY

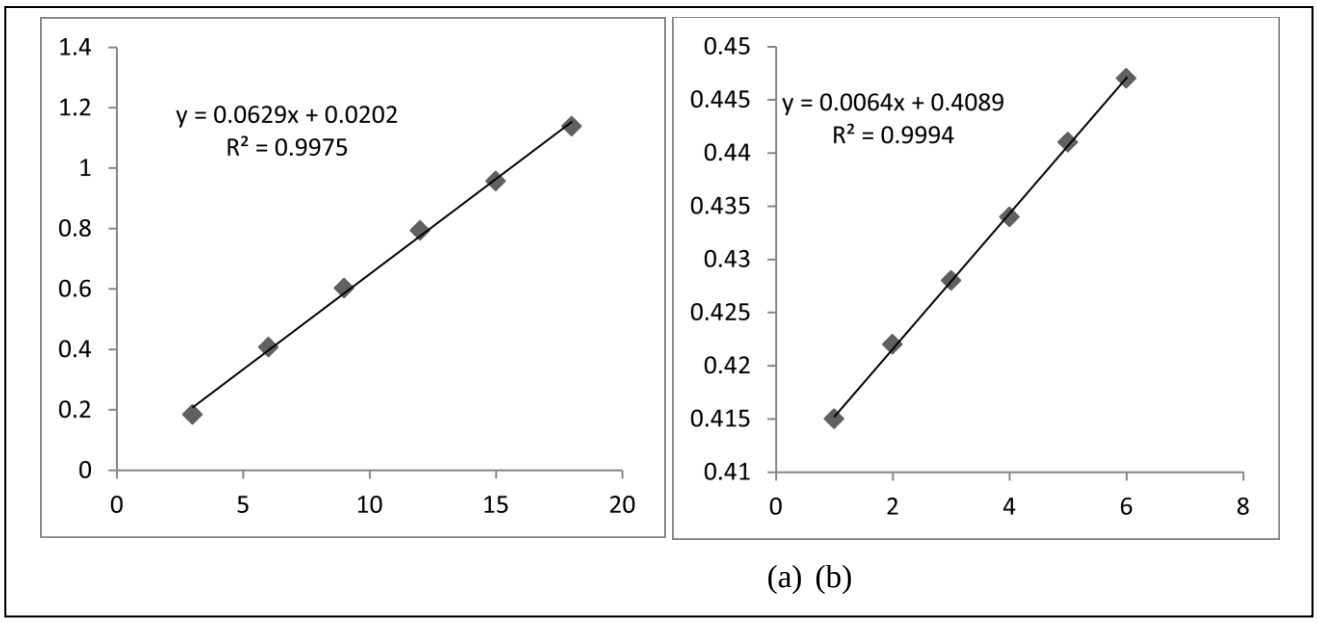


Figure 4: (a) Calibration curve of RANI (b) Calibration curve of DICY

RESULTS AND DISCUSSION

The validated stability indicating spectrophotometric method for estimation of degradation behavior of ranitidine and dicyclomine in tablet formulation has been developed using methanol as solvent. The wavelength (λ_{max}) was found to be 325 nm for RANI and 218 nm for DICY respectively. RANI and DICY follow Beer's law in the employed concentration ranges of 3 - 18 µg/ml and 1 - 6 µg/ml. Mean assay values in RANILA-SPAS were found to be 99.77 and 99.80 for RANI and DICY respectively. The precision was calculated as repeatability, inter and intraday variations and % RSD was found to be less than two. The accuracy of method was determined by recovery studies. Pure RANI and DICY were added to the pre-analyzed tablet powder at three different levels i.e. 80, 100 & 120% of label claims as per the ICH guidelines. Three replicate analyses were carried out at each level. The mean recovery was found to be 100.12%, 100.06%, 99.81% and 99.76%, 99.91%, 100.07% for RANI and DICY in RANILA-SPAS samples respectively. It indicating that the method has required accuracy and there was no interference with API by excipients present in tablets. The RSD value is below 2% indicated that the method has required precision. The LOD and LOQ values of RANI and DICY were found to be 0.0531 and 0.1609 µg/ml and 0.549 and 1.665 µg/ml respectively. The forced degradation studies showed that RANI and DICY undergoes degradation in acidic, alkaline, neutral, photolytic and thermal condition and the percentage degradation was found to be 39.09, 8.40, 50.24, 37.93, 40.02, 38.50 %
And 15.60, 9.92, 7.21, 9.56, 9.14, 4.08 % for RANI and DICY respectively.

CONCLUSION

The developed spectrophotometric method is simple, sensitive and rapid for estimation of RANI and DICY in bulk and pharmaceutical tablet formulation and does not involve the use of complex instruments. The developed method was validated as per ICH guidelines and the standard deviation and % RSD calculated for the proposed method are within limits, indicating high degree of precision of the method. The performed recovery studies indicate the method to be accurate. Hence, the developed method was applied successfully for quality control and routine analysis of finished pharmaceutical dosage form.

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