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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for the Simultaneous Estimation of Ranitidine and Dicyclomine in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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A B S T R A C T

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Ranitidine and Dicyclomine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Triethylamine Buffer pH 3.5 (75:25) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 255nm. The retention time of the Ranitidine and Dicyclomine was 2.456, 4.312 ±0.02min respectively. The method produce linear responses in the concentration range of 40-200µg/ml of Ranitidine and 1-5µg/ml of Dicyclomine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Ranitidine, Dicyclomine, RP-HPLC, Mobile phase, validation.

A R T I C L E I N F O

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1. Introduction

Ranitidine is a histamine H2-receptor antagonist similar to cimetidine and famotidine. An H2-receptor antagonist, often shortened to H2 antagonist, is a drug used to block the action of histamine on parietal cells in the stomach, decreasing acid production by these cells. These drugs are used in the treatment of dyspepsia, however their use has

waned since the advent of the more effective proton pump inhibitors. Like the H1-antihistamines, the H2 antagonists are inverse agonists rather than true receptor antagonists. Dicyclomine is an anticholinergic drug, a medication that reduces the effect of acetylcholine, a chemical released from nerves that stimulates muscles, by blocking the receptors for acetylcholine on smooth muscle (a type of muscle). It also has a direct relaxing effect on smooth muscle. Dicyclomine is used to treat or prevent spasm in the muscles of the gastrointestinal tract in the irritable bowel syndrome. In addition, Dicyclomine inhibits gastrointestinal propulsive motility and decreases gastric acid secretion and controls excessive pharyngeal, tracheal and bronchial secretions.

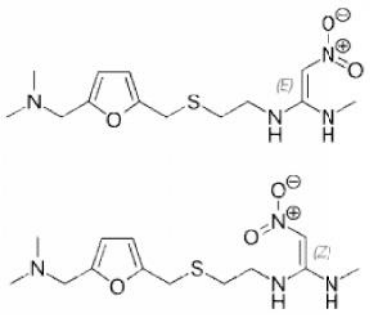

Fig 1: Structure of Ranitidine

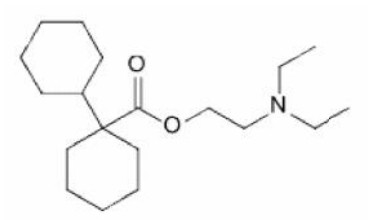

Fig 2: Structure of Dicyclomine

Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Ranitidine and Dicyclomine and single method is available for such estimation by RP-HPLC. So, I had developed new analytical method for the simultaneous estimation of Ranitidine and Dicyclomine by using RP-HPLC method for changing of few chromatographic conditions.

2. Materials and Methods

Instrument used:

The liquid chromatographic system made up of WATERS HPLC, software proving Empower 2 and it having Alliance 2695 separation module, 996 PDA detector. pH meter and Ultrasonicator was used.

Chemical and Reagents:

Working standards of Ranitidine and Dicyclomine were procured by gift sample of Sura labs, Hyderabad. HPLC grade methanol and acetonitrile were purchased from merck laboratories, Mumbai.

Optimized Chromatographic Conditions:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : 35ºC
Column : Symmetry C18 (4.6×150mm, 5µ)
Mobile phase : Methanol: Triethylamine buffer pH 4.5 (75:25v/v)
Flow rate : 1ml/min
Wavelength : 255nm
Injection volume : 10 µl
Run time : 7 min

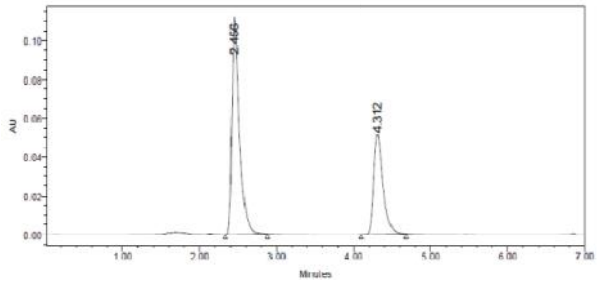

Fig 3: Optimized Chromatogram

Preparation of Triethylamine buffer (pH-4.5):

Take 6.0ml of Triethylamine in to 750ml of HPLC water in a 1000ml volumetric flask and mix well. Make up the volume up to mark with water and adjust the pH to 4.5 by using Orthophosphoric acid, filter and sonicate.

Preparation of mobile phase:

Accurately measured 750 ml (75%) of Methanol and 250 ml of Triethylamine buffer (25%) a were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Ranitidine and Dicyclomine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.03ml of Ranitidine and 1.2ml of Dicyclomine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Ranitidine and Dicyclomine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.03ml of Ranitidine and 1.2ml of Dicyclomine from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Method Validation

Method validation was done for the according to ICH guidelines Q_2 (R_1). The validation parameters like system suitability, specificity, linearity, precision, accuracy, LOD & LOQ and robustness.

System suitability: The standard solution was injected for five times and measured the area for all five injections in

HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were shown in table 1.

Specificity: In specificity studies standard and sample solutions were compared to the placebo and then observe the interference. There is no interference was observed in the test samples. In ability to elict the clear and plain analyte.

Linearity:

Preparation of Level – I (40ppm of Ranitidine & 1ppm of Dicyclomine): Pipette out 0.4ml of Ranitidine and 0.01 ml of Dicyclomine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (80ppm of Ranitidine & 2ppm of Dicyclomine): Pipette out 0.8ml of Ranitidine and 0.02 ml of Dicyclomine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (120ppm of Ranitidine & 3ppm of Dicyclomine): Pipette out 1.2ml of Ranitidine and 0.03ml of Dicyclomine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (160ppm of Ranitidine & 4ppm of Dicyclomine): Pipette out 1.6ml of Ranitidine and 0.04ml of Dicyclomine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (200ppm of Ranitidine & 5ppm of Dicyclomine): Pipette out 2ml of Ranitidine and 0.05ml of Dicyclomine stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results were shown in table 3 &4 , fig 4&5.

Precision: Intraday and intermediate precision was carried.

Repeatability:

The standard solution was injected for five times and measured the area for all five injections in within the day. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Accuracy:

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Ranitidine and Dicyclomine and calculate the individual recovery and mean recovery values. The data was shown in table 7&8.

Robustness: Robustness was done for the changing of chromatographic parameters like flow rate and change of organic mobile phase composition.

Effect of Variation of flow conditions: The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. Methanol: Triethylamine Buffer was taken in the ratio and 70:30, 80:20 instead (75:25), remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

3. Results and Discussion

Specificity: The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Ranitidine and Dicyclomine in drug product. The % purity of Ranitidine and Dicyclomine in pharmaceutical dosage form was found to be 99.9%.

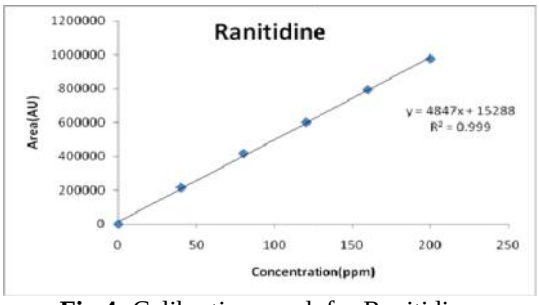

Fig 4: Calibration graph for Ranitidine

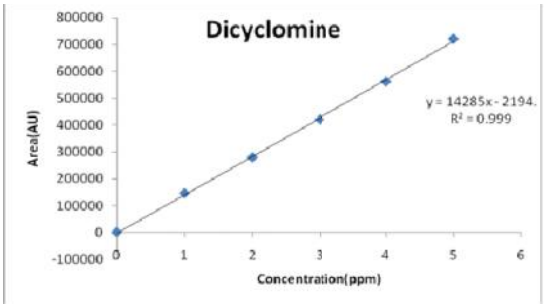

Fig 5: Calibration graph for Dicyclomine

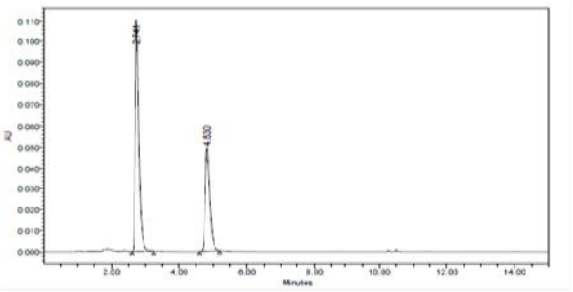

Fig 6: Chromatogram showing less flow of 0.9ml/min

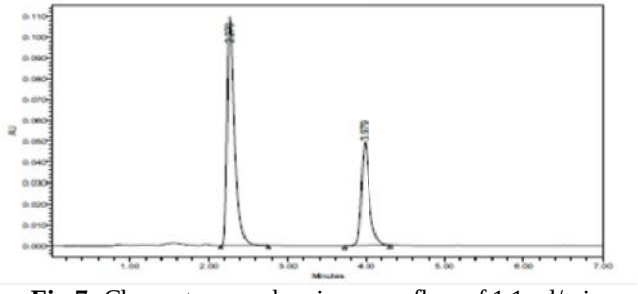

Fig 7: Chromatogram showing more flow of 1.1 ml/min

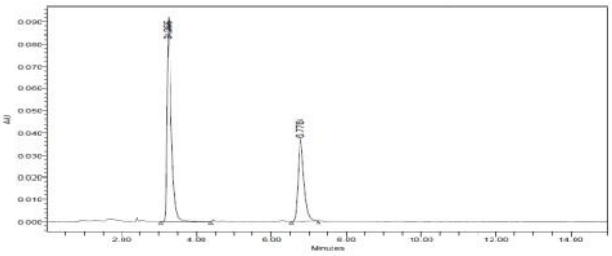

Fig 8: Chromatogram showing less organic composition

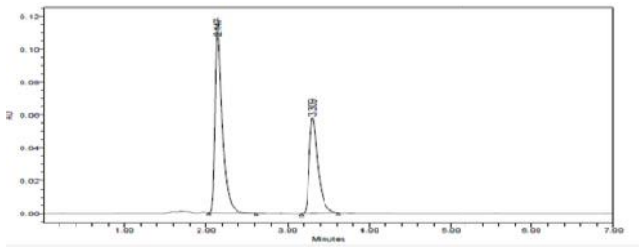

Fig 9: Chromatogram showing more organic composition

4. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ranitidineand Dicyclomine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Ranitidine and Dicyclomine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Triethylamine Buffer pH 3.5 (75:25) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed inTablesfor RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Ranitidine and Dicyclomine in bulk drug and in Pharmaceutical dosage forms.

Table 1: Results of system suitability for Ranitidine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ranitidine	2.459	602561	111160	5123	1.4
2	Ranitidine	2.466	600543	53992	5023.2	1.4
3	Ranitidine	2.472	601288	55420	5061.3	1.3
4	Ranitidine	2.452	600776	112478	5147.3	1.6
5	Ranitidine	2.450	600758	111779	5101.8	1.7
Mean			601185.2			
Std. Dev			816.3576			
% RSD			0.13			

Table 2: Results of system suitability for Dicyclomine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Dicyclomine	4.322	422674	50988	5949	1.5
2	Dicyclomine	4.323	424692	49813	5890.0	1.5
3	Dicyclomine	4.342	421255	49826	5952.5	1.4
4	Dicyclomine	4.300	415235	51804	5926.4	1.50
5	Dicyclomine	4.295	416260	51274	5898.5	1.49
Mean			420023.2			
Std. Dev			724.7845			
% RSD			0.17			

Table 3: Linearity data of Ranitidine

Concentration Level (%)	**Concentration «g/ml**	**Average Peak Area**
33.3	40	215760
66.6	80	417001
100	120	600435
133.3	160	791969
166.6	200	974736

Table 4: Linearity data of Dicyclomine

Concentration Level (%)	**Concentration «g/ml**	**Average Peak Area**
33	1	145474
66	2	279372

100	3	421045
133	4	562151
166	5	721671

Table 5: Results of Method precision for Ranitidine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ranitidine	2.453	603403	112688	5881.3	1.4
2	Ranitidine	2.455	608107	113637	5844.1	1.3
3	Ranitidine	2.453	607266	112849	5918.1	1.3
4	Ranitidine	2.452	608776	112478	5847.3	1.4
5	Ranitidine	2.450	609758	111779	5801.8	1.5
Mean			607462			
Std. Dev			2445.82			
% RSD			0.40			

Table 6: Results of method precession for Dicyclomine

S.no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dicyclomine	4.289	429183	52411	5050.9	1.49	3.2
2	Dicyclomine	4.309	416643	52475	5084.8	1.5	3.2
3	Dicyclomine	4.306	424052	51841	5000.1	1.4	3.2
4	Dicyclomine	4.300	425235	51804	5026.4	1.51	3.2
5	Dicyclomine	4.295	416260	51274	5098.5	1.51	3.2
Mean			422274.6				
Std. Dev			5646.668				
% RSD			1.3				

Table 7: Accuracy results for Ranitidine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found(ppm)	% Recovery	Mean Recovery
50%	308408	60	60	100%	100%
100%	600619	120	120	100%	
150%	894293	180	180	100%	

Table 8: Accuracy results for Dicyclomine

%Concentration(at specification Level)	Area	Amount Added(ppm)	Amount Found(ppm)	% Recovery	Mean Recovery
50%	216092	1.5	1.52	101.3	99.7%
100%	423626	3	2.9	99.3	
150%	634469.7	4.5	4.48	98.6	

Table 9: Robustness results for Ranitidine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	600122	2.456	5215	1.8
Less Flow rate of 0.9 mL/min	651206	2.741	5199	1.79
More Flow rate of 1.1 mL/min	546820	2.270	5234	1.8
Less organic phase	586420	3.266	5298	1.8
More organic phase	542813	2.147	5287	1.76

Table 10: Robustness results for Dicyclomine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	422042	4.312	5648	1.5
Less Flow rate of 0.9 mL/min	453012	4.830	5687	1.6

More Flow rate of 1.1 mL/min	398654	3.979	5602	1.5
Less organic phase	445983	3.266	5643	1.55
More organic phase	402315	2.147	5699	1.51

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