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RESEARCH ARTICLE

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Derivative Spectrophotometric Method for Simultaneous Determination of Granisetron and Pantoprazole in Synthetic Mixture Patel SR*¹, Patel NK¹, Patel DB¹, Patel PA², Dr. Patel LJ³

¹Department of Pharmaceutical Sciences, Hemchandracharya North Gujarat University, Patan, Gujarat, India. ²Shree Swaminarayan Sanskar Pharmacy College, Zundal, Gujarat, India ³Shree S K Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Gujarat, India. Manuscript No: IJPRS/V1/I2/00026, Received On: 03/04/2012, Accepted On: 05/04/2012

ABSTRACT

The present work describes a First order Derivative Spectrophotometric method for simultaneous estimation of Granisetron and Pantoprazole in synthetic mixture. Method was performed on Elico's Double beam UV-Visible Spectrophotometer (SL-191) using methanol as a solvent. Absorbance were recorded at Zero Crossing Point (ZCP) of Granisetron (248 nm) and ZCP of Pantoprazole (291 nm) for all standard and sample solutions. The selected Spectrophotometric conditions were found to be effective for the determination of Granisetron and Pantoprazole from synthetic mixture in presence of common excipients without prior physical separation. Linearity was found over the range of 2-20 μ g/ml for Granisetron and over 5-100 μ g/ml for Pantoprazole. The values of Limit of Detection were found to be 0.40 μ g/ml for Granisetron and 0.62 μ g/ml for Pantoprazole. The values of Limit of use of Limit of Quantification were found to be 1.22 μ g/ml for Granisetron and 1.89 μ g/ml for Pantoprazole. The proposed method was found to be fast, simple, sensitive, accurate, precise, reproducible, robust and rugged and can be used for simultaneous analysis of these drugs in synthetic mixture.

KEYWORDS

Granisetron, Pantoprazole, Derivative Spectroscopy

INTRODUCTION

Granisetron hydrochloride is chemically 1methyl-N-((1R, 3R, 5S)-9-methyl-9-azabicyclo [3.3.1] nonan-3-yl)-1H-indazole-3carboxamide. It represents the class of selective 5-HT3 antagonists which is commonly employed as anti-emetic in combination with anti-ulcer and anti-cancer agents¹. Literature survey revealed that no method has been reported for the estimation of Granisetron in combined dosage form²⁻⁹. Second drug, Pantoprazole a sodium salt of (RS) -6- (difluoromethoxy) -2-[(3, 4 dimethoxypyridin-2-yl) methylsulfinyl] - 1H-

*Address for Correspondence: Mr. Shirish R. Patel Assistant Professor, Department of Pharmaceutical Sciences, Hemchandracharya North Gujarat University, Patan, Gujarat, India. Email: shirish_1984@rediffmail.com benzo[d]imidazole, represents the class of orally active H+ - K+ ATPase Inhibitors (Proton Pump Inhibitor) employed in the management of gastric ulcer¹⁰.



Figure: 1 Granisetron Hydrochloride



Figure: 2 Pantoprazole Sodium

Literature review did not reveal any method for simultaneous determination of Granisetron and Pantoprazole in combined mixture¹¹⁻¹⁹. So, we decided to work towards development and validation of simple, sensitive, accurate, precise, rugged and economic method for simultaneous determination of these drugs in synthetic mixture. The present work describes a validated derivative spectroscopic method for simultaneous determination of these drugs in synthetic mixture.

MATERIALS AND METHODS

Instrumentation

- A double-beam UV-Visible spectrophotometer, model SL-191 (Elico) having two matched cells with 1-cm light path
- An analytical balance (AX200, Shimadzu)
- Ultra Sonicator (Life care)
- Volumetric flasks 10ml, 50ml, 100ml Pipettes – 1ml, 5ml, 10ml, beakers, measuring cylinders.

Chemicals and Reagents

- Authentic samples of Granisetron and Pantoprazole were supplied by Torrent Research Center (Gandhinagar, India).
- Methanol (AR, Finar laboratories)

PREPARATION OF SOLUTIONS

STANDARD STOCK SOLUTIONS

Accurately weighed 10 mg of Granisetron and 10 mg of Pantoprazole were transferred to two 100 ml volumetric flasks. Drugs were dissolved in 50 ml of methanol with sonication and volumes were adjusted with methanol to prepare 100 μ g/ml standard stock solutions of both drugs.

WORKING STANDARD SOLUTIONS

Standard stock solutions (100µg/ml) of Granisetron and Pantoprazole were used as working standard solutions.

SAMPLE SOLUTION

Accurately weighed 30 mg of Granisetron, 400 mg of Pantoprazole, 10 mg of sodium starch

glycolate, 10 mg of titanium dioxide and 100 mg of direct compressible starch were mixed well to prepare synthetic mixture. Quantity of powder equivalent to 3 mg of Granisetron and 40 mg of Pantoprazole was weighed accurately and transferred in a 100 ml measuring flask and dissolved in methanol with sonication for 20 minutes. The solution was filtered through whatman filter paper no. 41. The filtrate was collected in a 100 ml volumetric flask and diluted to the mark with methanol to obtain solution containing 30 μ g/ml of Granisetron and 400 μ g/ml of Pantoprazole.

SELECTIONOFWAVELENGTHSOFMAXIMUMABSORBANCEANDZCPFORGRANISETRONANDPANTOPRAZOLEAND

Working standard solutions were scanned in the range of 200 nm to 400 nm against methanol as a blank. Maximum absorbance was obtained at 248 nm and 291 nm and ZCP were found at 248 nm and 291 nm for Granisetron and Pantoprazole, respectively (figure 3 and 4).

LINEARITY AND RANGE

Accurately measured working standard solutions of Granisetron (0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 ml) and Pantoprazole (0.5, 1.0, 1.5, 2.0, 3.0, 5.0 and 10 ml) were transferred to separate10 ml volumetric flasks and diluted up to the mark with methanol. Absorbance were measured at ZCP of Granisetron (248 nm) and Pantoprazole (291 ZCP of nm) for Pantoprazole and Granisetron, respectively using methanol as a blank. Calibration curves were constructed by plotting absorbance versus concentrations of working standard solutions. Each reading was average of three Calibration determinations. curves were plotted over concentration range 2-20 µg/ml for Granisetron and 5-100 µg/ml for Pantoprazole.

ACCURACY AND PRECISION

Recovery experiments were carried out in triplicate by spiking previously analyzed samples of synthetic mixture (4 μ g/ml for

Granisetron and 10 μ g/ml for Pantoprazole) with three different concentrations of standard solutions (2, 4, 6 μ g/ml for Granisetron and 5, 10, 15 μ g/ml for Pantoprazole). Precision was determined in terms of method precision and intermediate precision.

ESTIMATION OF GRANISETRON AND PANTOPRAZOLE IN SYNTHETIC MIXTURE

Test solutions from synthetic mixture containing Granisetron (3.0, 4.5 and 6 μ g/ml) and Pantoprazole (40, 60 and 80 μ g/ml) were prepared and absorbance was recorded at 248 nm and 291 nm. Concentrations of both drugs were calculated from corresponding calibration curves.

RESULTS AND DISCUSSION

Direct UV-absorption measurements were found to be inapplicable to the analysis of Granisetron and Pantoprazole in binary mixtures because of the spectral interference. Derivative spectrophotometry is a favourable technique to solve this problem.

SPECTRAL CHARACTERISTIC OF GRANISETRON AND PANTOPRAZOLE

Granisetron and Pantoprazole working standard solutions (1 ml of each) were separately transferred into 10 ml volumetric flasks and diluted to volume with methanol. Absorbance values were measured in first derivative spectra at 248 nm and 291 nm for Pantoprazole and Granisetron, respectively. First derivative spectra of Granisetron and Pantoprazole are shown in figure 3 and 4.



Figure: 3 First order spectra of Granisetron



Figure: 4 first order spectra of Pantoprazole

A linear correlation was obtained between peak amplitude and the corresponding concentration in the range of 2–20 μ g/ml for Granisetron (figure 5), from which the linear regression equation was computed and found to be:

Y = 0.0139x + 0.00002, R2 = 0.9961

Where Y is peak amplitude at 291 nm, x is the concentration in μ g/ml and R2 is the correlation coefficient.



Figure: 5 Calibration curve of Granisetron

A linear correlation was obtained between peak amplitude and the corresponding concentration in the range of $5-100 \mu g/ml$ for Pantoprazole (figure 6), from which the linear regression equation was computed and found to be:

Y = 0.0129x + 0.0148, R2 = 0.9999

Where Y is peak amplitude at 248 nm, x is the concentration in μ g/ml and R2 is the correlation coefficient.



Figure: 6 Calibration curve of Pantoprazole

VALIDATION^{20, 21}

LINEARITY AND RANGE

The results show that an excellent correlation existed between the absorbance and concentration of analyte. Calibration curves that were constructed were linear over the concentration range 2-20 µg/ml for Granisetron and 5-100 µg/ml for Pantoprazole. Each concentration was repeated three times. The linearity of the calibration curves and adherence of the system to Beer's law were validated by high values of the correlation coefficient (table 3).

ACCURACY

Accuracy of the method was assured by use of the standard addition technique involving analysis of synthetic mixture samples containing 3 mg of Granisetron and 40 mg of Pantoprazole, to which authentic drugs were added in 50, 100 and 150 % proportions. The resulting mixtures were assayed, and the results obtained for both drugs were compared to those expected. Good recoveries with the standard addition method (table 1) prove the accuracy of the proposed method.

PRECISION

Method precision (Repeatability)

For evaluation of method precision, solutions of 10μ g/ml of both drugs were evaluated by six replicate determinations.

Relative standard deviation (RSD) for all determinations was less than 2 % (table 2), which indicates that the proposed method is repeatable.

 Table: 1 Percentage recovery of Granisetron and Pantoprazole

Drug	Amt. taken (µg/ml)	Amt. added (µg/ml)	Amt. found (µg/ml) ± SD (n=3)	% Recove ry ± SD (n=3)
Granisetron	4	2	$\begin{array}{c} 5.99 \pm \\ 0.08 \end{array}$	99.97 ± 1.28
	4	4	8.02 ± 0.12	100.30 ± 1.47
	4	6	$\begin{array}{c} 10.02 \pm \\ 0.12 \end{array}$	100.24 ± 1.18
Pantoprazole	10	5	$\begin{array}{c} 15.19 \pm \\ 0.17 \end{array}$	101.30 ± 1.16
	10	10	20.28 ± 0. 34	101.39 ± 1.68
	10	15	$\begin{array}{c} 24.86 \pm \\ 0.49 \end{array}$	99.44 ± 1.95

Intermediate precision (Reproducibility)

For evaluation of intermediate precision, absorbances of solutions over the concentration range 2-20 μ g/ml for Granisetron and 5-100 μ g/ml for Pantoprazole were evaluated by three replicate determinations on same day and different days.

The RSD values at these concentration levels were calculated and found to be less than 2% (table 5.15). Low % RSD values of intraday and interday precision reveal that the proposed method is precise.

Table: 2 Method precision data for Granisetronand Pantoprazole

Determination	% Assay of Granisetron	% Assay of Pantoprazole	
1 st	101.52	100.83	
2 nd	100.75	103.33	
3 rd	102.29	101.67	

4 th	103.06	102.50	
5^{th}	101.52	103.33	
6 th	102.29	102.50	
Mean	101.91	102.36	
SD	0.81	0.97	
% RSD	0.79	0.95	

LIMIT OF DETECTION (LOD)

Limits of detection for both drugs were calculated theoretically as given in the text. LOD for Granisetron and Pantoprazole were found to be 0.40 μ g/ml and 0.62 μ g/ml, respectively (table 3).

LIMIT OF QUANTIFICATION (LOQ)

Limit of quantification for both drugs were calculated theoretically as given in the text. LOQ for Granisetron and Pantoprazole were found to be 1.22 μ g/ml and 1.89 μ g/ml, respectively (table 3).

Table 3: Method validation parameters for
Granisetron and Pantoprazole

Parameters	Granisetron	Pantoprazole	
Calibration range	2-20 µg/ml	5-100 µg/ml	
Detection limit	0.40 µg/ml	0.62 µg/ml	
Quantitation limit	1.22 µg/ml	1.89 µg/ml	
Slope	0.013	0.012	
Correlation coefficient	0.996	0.999	
Repeatability RSD, %	0.79	0.95	
Intraday RSD, %	1.12-1.82	0.43-1.75	
Interday RSD, %	0.56-1.60	0.23-1.43	

APPLICATION OF PROPOSED METHOD TO THE SYNTHETIC MIXTURE

The developed method was successfully applied for determination of Granisetron and Pantoprazole in synthetic mixture. Assay results were obtained in the range of 97.38-100.82 % for Granisetron and 98.96-103.13 % for Pantoprazole (table 4).

Table: 4 Assay results of Granisetron and	1
Pantoprazole in synthetic mixture	

Granisetron		Pantoprazole			
Amount taken (µg/ml)	Amount found (µg/ml) ± SD (n=3)	% Amount Found ± SD (n=3)	Amount taken (µg/ml)	Amount found (µg/ml) ± SD (n=3)	% Amount Found ± SD (n=3)
3	$\begin{array}{c} 2.97 \pm \\ 0.04 \end{array}$	99.09 ± 1.48	40	40.14 ± 0.54	100.35 ± 1.36
4.5	4.46 ± 0.08	99.11 ± 1.71	60	61.53 ± 0.34	102.55 ± 0.56
6	$\begin{array}{c} 5.92 \pm \\ 0.08 \end{array}$	98.69 ± 1.28	80	82.00 ± 0.46	102.50 ± 0.58

CONCLUSION

A validated derivative spectrophotometric method has been developed for estimation of Granisetron and Pantoprazole in synthetic mixture. The proposed method is simple, accurate, precise and specific and has the ability to determine drugs in synthetic mixture without prior physical separation. The prime importance was given to develop less time consuming and simple derivative spectrophotometric method.

Detection and quantification limits achieved, describe that the method is sensitive. High recoveries and acceptable % RSD values confirms accuracy and precision of developed method. Assay results show that the method can be successfully applied for analysis of Granisetron and Pantoprazole without any interference from commonly used excipients.

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