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Method Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Pantoprazole and Ondansetron Hydrochloride in Bulk and in a Synthetic Mixture

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Abstract: A simple, rapid and precise reverse phase liquid chromatographic (RP-HPLC) method was developed and subsequently validated for simultaneous estimation of Pantoprazole and Ondansetron in bulk drug and in a synthetic mixture. The analysis was carried out using Hypersil ODS C18 (Hypersil ODS 250 x 4.6 mm, 5µ, Make: Thermo Scientific) prepacked column. The separation was carried out using a mobile phase containing buffer adjusted to pH 3.6 and acetonitrile (40:60 v/v), was pumped at a flow rate of 1.0 mL/min with UV-detection at 210 nm. Both the drugs were well resolved on the stationary phase and the retention times were around 3.265 minutes for Pantoprazole and 4.092 minutes for Ondansetron. The method was validated and shown to be linear for Pantoprazole and Ondansetron. The correlation coefficients for Pantoprazole and Ondansetron are 0.9995 and 0.9997 respectively.
Key words: Pantoprazole, Ondansetron, HPLC, Validation.

Introduction

Pantoprazole (PAN) is chemically known as 5-(Difluromethoxy)-2-{[3, 4-dimethoxy-2-pyridinyl] Methyl} sulfinyl] 1H-Benzimidazole (Fig. 1). It is a proton pump inhibitor, which suppresses gastric acid secretion by H+/K+-ATPase enzyme system at the secretary surface of the gastric parietal cell. This drug is used for the treatment of duodenal, gastric and oesophageal ulceration. Pantoprazole is official drug in Indian Pharmacopoeia [2], United States Pharmacopoeia [3] and British Pharmacopoeia [4].

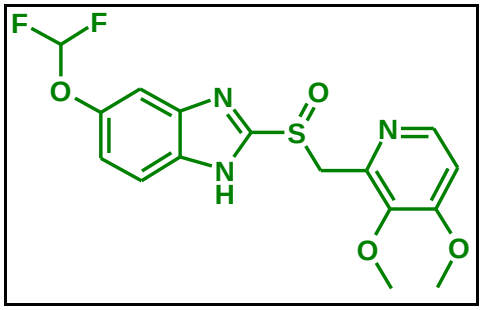


Figure 1: Chemical structure of Pantoprazole.

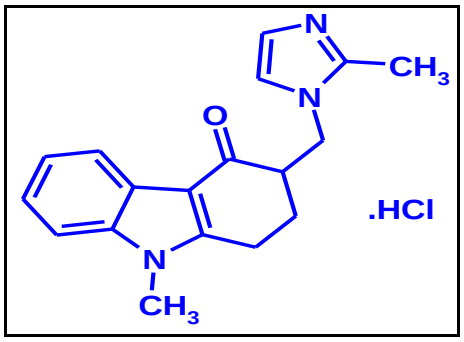


Figure 2: Chemical structure of Ondansetron hydrochloride.

Ondansetron (OND) is chemically known as 9-methyl-3-[(2-methyl-1H-imidazol-yl) methyl]-2, 3, 4, 9-tetrahydro-1H-carbazol-4-one hydrochloride dihydrate (Fig. 2). It represents the class of selective serotonin 5-HT3 antagonists which acts both, peripherally on the vague nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema in brain. It is indicated for the prevention of nausea and vomiting associated with highly emetogenic cancer chemotherapy [5]. Ondansetron is the subject of monograph in Indian Pharmacopoeia [2], United States Pharmacopoeia [3], and British Pharmacopoeia [4] . Ondansetron is used as selective 5-HT3 receptor antagonist indicated for the prevention of nausea and vomiting associated in chemotherapy. Pantoprazole is irreversibly inhibits proton pumps activity and decreases acid secretion. A combination regimen of Ondansetron plus Pantoprazole has been suggested in patients with peptic ulcer, gastro esophageal reflux disease (GERD) to prevent nausea [6].

Literature reveals that various Spectrophotometric [7-11], colorimetric [12], Thin layer chromate-graphic [13], spectroflourimetric [14] and HPLC [15-21] have been reported for the determination of PAN in Pharmaceutical preparations. Some analytical methods for the quantitative determination of OND as single or in combination with other drugs in Pharmaceutical preparations are described in literature like Spectrophotometric [22-27] and HPLC [28-31] their individual analysis, along with other combinations in pharmaceutical formulation and fluids. To the best of our knowledge no HPLC method of analysis has yet been reported for simultaneous analysis of Pantoprazole and Ondansetron. Hence, in the present communication we would like to report a simple, economic, feasible, rapid, sensitive, and validated [32] specific RP-HPLC method for the simultaneous estimation of Pantoprazole and Ondansetron in Bulk and in the synthetic mixture.

Materials and Methods

Reagents and chemicals

Methanol and Water were of HPLC grade and were purchased from Rankem, Gujarat. Acetonitrile of HPLC grade purchased from Merck, Mumbai. Orthophosphoric acid purchased from Rankem, New Delhi. Potassium dihydrogen phosphate for Chromatography purchased from Merck, Mumbai. Pantoprazole and Ondansetron were obtained from Zim Lab, Nagpur, Neon Lab LTD., Mumbai, India respectively.

Equipment and Chromatographic conditions

A high-performance liquid chromatographic system (Younglin, software: Autochro3000) equipped UV detector. All pH measurements were performed on a pH meter (Digisun Electronics, Hyderabad). Chromatographic separation was carried out at room temperature Hypersil ODS C18 (Hypersil ODS 250 x 4.6 mm, 5µ, Make: Thermo Scientific) prepacked column. For the mobile phase accurately weighed1.36gm of Potassium dihydrogen ortho phosphate was dissolved in 900ml of HPLC grade water added and sonicated to remove dissolved gases. The pH of the mobile phase was adjusted to 3.6±0.05 with dil. Orthophosphoric acid solution. The buffer solution was stirred manually to mix and finally make the volume up to 1000 mL with water. A mixture of buffer and acetonitrile in the ratio of 40:60 was prepared. Finally the mobile phase was filtered through a 0.45 µm membrane filter and degassed for 10 minutes. The injection volumes for samples and standards were 10 µl and eluted at a flow rate of 1mL/min at 30°C. The eluents were monitored at 210 nm.

Preparation of standard stock solutions

A working standard solution containing Pantoprazole and Ondansetron was prepared by weighing 40mg and 4mg of both the drugs dissolved in 20mL methanol and solution was sonicated for 10 min the volume was made up to the mark with methanol to obtain a stock solution of 1600 µg/mL of PAN and 160 µg/mL of OND respectively.

Preparation of working standard solutions

From the above stock solution of PAN and OND 1mL was pipetted out in to a 10ml volumetric flask and then made up to the final volume with diluents to get mixed standard solution containing 160µg/mL of Pantoprazole and 16µg/mL of Ondansetron. UV spectrum showing isobestic point is shown in Fig. 3.

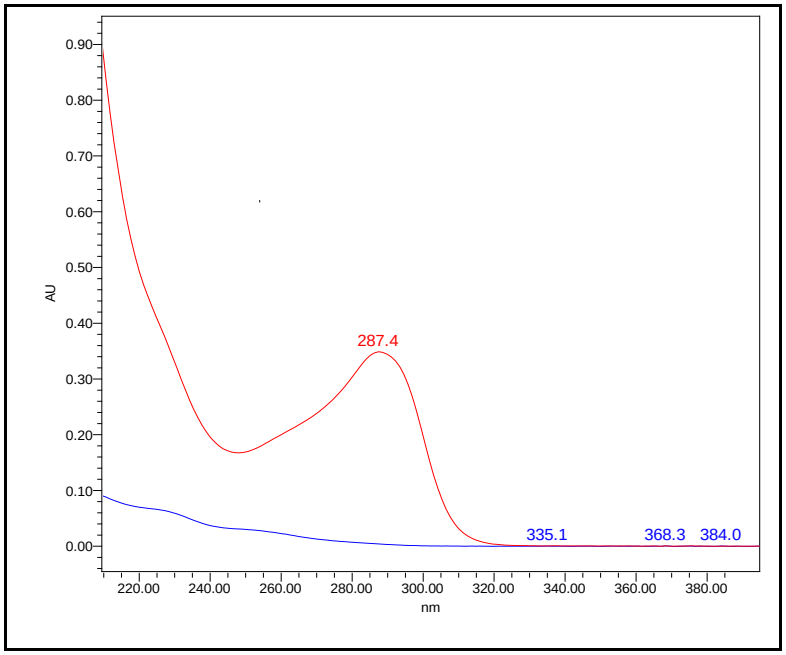


Figure 3: UV Spectrum of PAN and OND using methanol as blank.

Method Validation

The developed analytical method was further subjected to validation in accordance to the ICH guidelines. The parameters evaluated were linearity, sensitivity, system suitability, precision, accuracy, robustness and stability. Coefficients of variation and relative errors <2% were considered acceptable.

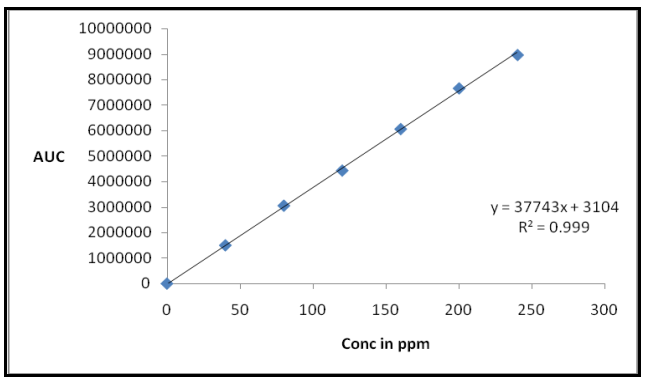
Linearity

In order to check the linearity for the developed method, solutions of six different concentrations ranging from 40-240µg / mL were prepared for Pantoprazole and 4-24µg / mL for Ondansetron, respectively. The Chromatograms peak areas were recorded and calibration curve was plotted of peak area against concentration of drug. The chromatograms were recorded and the peak areas are given in Table 1.

Table 1: Linearity data for Pantoprazole and Ondansetron

Pantoprazole conc. (µg/mL)	Mean Peak area of Pantoprazole	Ondansetron conc. (µg/mL)	Mean Peak area of Ondansetron
40	1504977	4	189264
80	3061979	8	385891
120	4440266	12	573739
160	6071599	16	780202
200	7668744	20	949462
240	8978377	24	189264

A linear relationship between areas versus concentrations was observed in the above mentioned linearity range. This range was selected as the linear range for the development of the analytical method, for the estimation of PAN and OND. The calibration curves for both drugs given in Fig. 4 and Fig. 5.



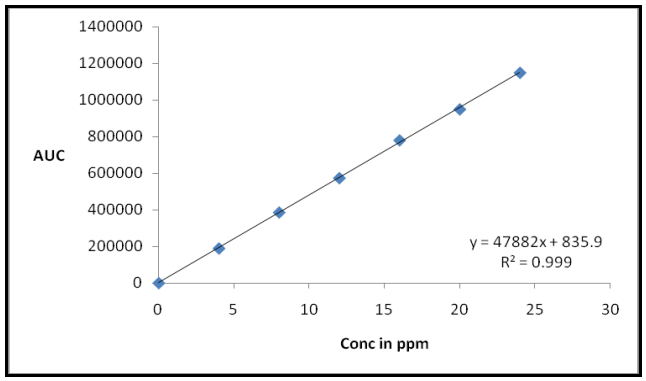


Figure 4: Calibration curve of Pantoprazole at 210 nm. **Figure 5:** Calibration curve of Ondansetron at 210 nm.

Sensitivity

The sensitivity of the measurement of PAN and OND using the proposed method was estimated as the limit of quantification (LOQ) and the lowest concentration detected under these chromatographic conditions as the limit of detection (LOD). The LOD and LOQ were calculated by using the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S, where σ was the standard deviation of the peak areas of the drug (n = 6), and S was the slope of the corresponding calibration plot. The limits of detection and quantification for PAN were 0.27 µg/mL and 0.82 µg/mL, respectively, and those for OND were 0.05 µg/mL and 0.17 µg/ mL, respectively. The results are shown in Table 2.

Table 2: Spectral and statistical data for determination of Pantoprazole and Ondansetron by proposed RP-HPLC method.

ANALYTE			
Pantoprazole		**Ondansetron**	
Absorption maxima, λ (nm)	210	Absorption maxima, λ (nm)	210
Linearity range (µg/mL)	40-240	Linearity range (µg/mL)	4-24
Coefficient of determination (r^2)	0.9995	Coefficient of determination (r^2)	0.9997
Regression equation (Y^a)	y = 37743x + 3104	Regression equation (Y^a)	y = 47882x+ 835.93
Slope (b)	37743	Slope (b)	47882
Intercept (a)	3104	Intercept (a)	835.93
Limit of detection, LOD (µg/mL)	0.27	Limit of detection, LOD (µg/mL)	0.05
Limit of quantitation, LOQ(µg/mL)	0.82	Limit of quantitation, LOQ(µg/mL)	0.17

[a]$Y = mx + c$, where x is the concentration (µg/mL).

System suitability

The system suitability test is an integral part of chromatographic analysis. It is used to verify that the resolution and reproducibility of the system are adequate for the analysis. A system suitability test according to the United States Pharmacopeia Convention was performed on chromatograms obtained for standard and test solutions to check differences in the above mentioned parameters. The results obtained with six replicate injections of the standard solution are summarized in Table 3.

Table 3: System suitability parameters for Pantoprazole and Ondansetron

Parameter (*n=6)	PAN	OND
Retention time	3.265	4.092
Plate count	5390	5449
USP Tailing	1.41	1.36

***Six replicates, PAN** – Pantoprazole, **OND** – Ondansetron.

Precision

Precision was measured by the analysis of sample solutions of 6 replicates, to check the intraday and inter day variations of the method. The results are furnished in Tables 4 and 5.

Table 4: Results of the intraday precision

Pantoprazole			Ondansetron		
Conc (µg/mL)	Peak area Mean S.D (n=6)	RSD (%)	Conc (µg/mL)	Peak area Mean S.D (n=6)	RSD (%)
160	5614613	0.4	16	780202	0.8

Table 5: Results of the interday precision

Pantoprazole			Ondansetron		
Conc (µg/mL)	Peak area Mean S.D (n=6)	RSD (%)	Conc (µg/mL)	Peak area Mean S.D (n=6)	RSD (%)
160	5685785	0.5	16	696949	0.5

Accuracy

The accuracy of the method was determined by the analysis of standard additions at three levels, that is, multiple-level recovery studies. The reference standard, at three different concentrations (50, 100, and 150 %), was added to a fixed amount of the pre analyzed sample and the amounts of the drug were analyzed by the proposed method. Results from the recovery studies are given in Tables 6 and 7.

Table 6: Results of the recovery study of Pantoprazole

Amount of PAN in Sample (µg)	Total amount of PAN found (µg) Mean ± S.D	Total amount recovered (µg)	% Recovery (n=3)
50	49.85	49.85	99.70
100	99.71	99.71	99.71
150	150.18	150.18	100.12

Table 7: Results of the recovery study of Ondansetron.

Amount of OND in Sample (µg)	Total amount of OND found (µg) Mean ± S.D	Total amount recovered (µg)	% Recovery (n=3)
50	49.91	49.91	99.83
100	100.46	100.46	100.46
150	149.91	149.91	99.94

Solution Stability

The stability of PAN and OND standard solutions was determined by storing the solutions at an ambient temperature (30 ± 5°C). The solutions were checked in triplicate after three successive days of storage and the data were compared with the freshly prepared samples. In each case, it could be noticed that the solutions were stable for 48 hours, as during this time the results of PAN and OND did not decrease below 98%. This showed that were stable in standard and sample solutions for at least two days, at ambient temperature.

Robustness

The robustness of the method was determined by making slight changes in the chromatographic conditions like flow rate (±0.1), temperature (±5), and organic phase of the mobile phase (±10%). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed was robust.

Assay

The proposed method was successfully applied for the estimation of Pantoprazole and Ondansetron in bulk drug and in a synthetic mixture. Ten tablets powdered equivalent were mixed in a ratio of 40mg Pantoprazole: 4mg Ondansetron. A quantity of this synthetic mixture powder equivalent to 44 mg was taken up in a 25 mL volumetric flask, and methanol was added up to the mark. The solution was sonicated for 15 min. This solution was further diluted to obtain a concentration of 160µg/mL PAN and 16µg/mL OND. The assay results were compiled, found satisfactory and show that there is a no interference of tablet matrix with the drug and the results are summarized in Table 8, and the standard and test chromatograms are given in Fig. 6 and 7. Low % RSD shows that this method can be easily applied for the estimation of PAN and OND in bulk drug and in the synthetic mixture.

Table 8: Determination of % assay for PAN and OND.

Synthetic mixture	Drug	Label claim mg/tablet	Amount added	Conc. estimated (mg)	Mean conc. Estimated (mg)	% Assay (w/w)	% RSD
PAN+OND	PAN	40	40	40.02	40.31	100.7	0.65
				40.63			
				40.10			
				40.52			
				40.16			
				40.45			
	OND	4	4	3.98	3.98	99.5	0.45
				4			
				4.01			
				3.96			
				3.98			
				3.99			

% Assay of PAN or OND = PAN or OND Conc estimated (mg)/PAN or OND input (mg) X 100; % RSD = SD/mean X 100.

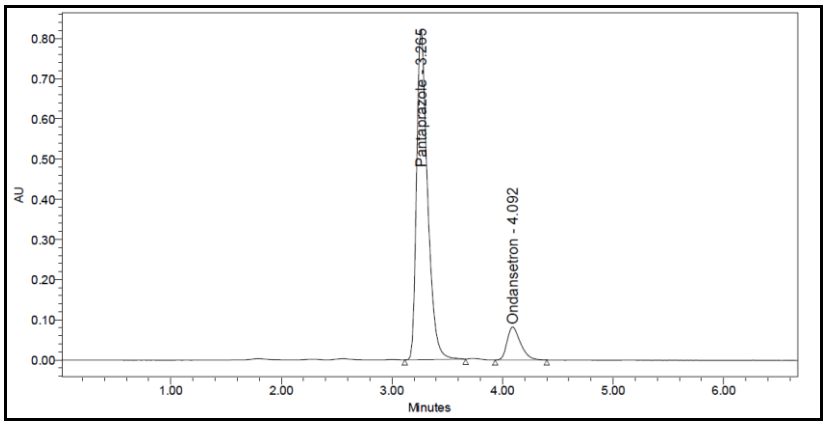


Figure 6: Chromatogram of the standard preparation of Pantoprazole and Ondansetron.

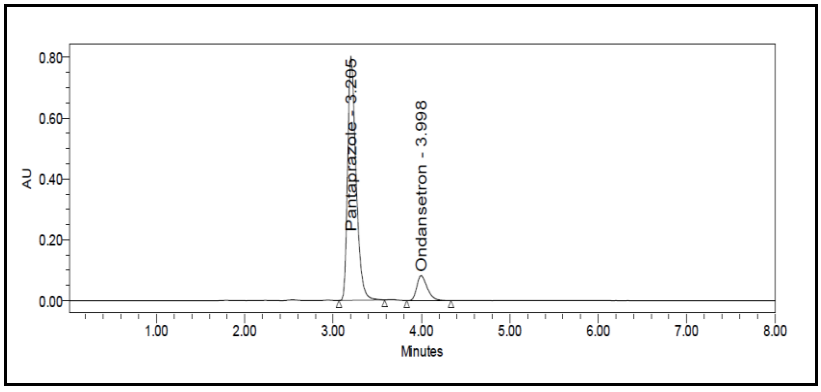


Figure 7: Chromatogram of the test sample of Pantoprazole and Ondansetron.

Results and Discussion

The RP-HPLC procedure was optimized with a view to develop an accurate and stable assay method with the pure drugs PAN and OND, in bulk drugs. Hypersil ODS C18 (Hypersil ODS 250 x 4.6 mm, 5µ, Make: Thermo Scientific) prepacked column in isocratic mode using mobile phase containing buffer and acetonitrile mobile (60:40 v/v). The flow rate was 1.0 mL/min at 30°C with UV-detection at 210 nm. Linearity was assessed by plotting concentration versus area, which is shown in Table 1, and it is linear in the range of 40-240µg / mL for Pantoprazole and 4-24µg / mL for Ondansetron, with correlation coefficients of 0.9995 and 0.9997 respectively, with a good linearity response greater than 0.999. The % recovery was found to be within limits of the acceptance criteria with a recovery range of 99. 99.70 -100.12% for Pantoprazole and 99.83 - 100.40% for Ondansetron The detection limit of the proposed method was 0.17 µg / mL and0.05µg / mL and the quantification limit was 0.82 µg / mL and0.27 µg / mL for Pantoprazole and Ondansetron respectively. A typical chromatogram of the Pantoprazole and Ondansetron standard solution of at the test level is shown in Fig 7. The assay procedures were repeated six times and the results were found to give 100.7% of PAN and99.5% of OND as shown in Table 7.

Conclusion

The proposed study describes a new and simple RP-HPLC method for the estimation of Pantoprazole and Ondansetron in bulk drug and in a synthetic mixture. The method has been validated and found to be simple, rapid, sensitive, accurate, and precise. Moreover, the lower solvent consumption along with the short analytical run time of 8 minutes leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. Therefore, the proposed method can be used for routine analysis of both drugs in the process control of bulk drug and formulated products without any interference from the excipients in laboratories and in the pharmaceutical industry.

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