Int J Pharma Res Health Sci. 2019; 7 (5): 3047-3050
DOI:10.21276/ijprhs.2019.05.01
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CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Stability Indicating RP-HPLC Method for Estimation of Pantoprazole and Ondansetron in Pharmaceutical Dosage Form

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ARTICLE INFO

Received: 03 Jul 2019
Accepted: 12 Oct 2019

ABSTRACT

Introduction: A simple, sensitive, precise, and accurate reverse phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of pantoprazole and ondansetron. The combination of pantoprazole and ondansetron is used for the treatment of nausea and vomiting. **Materials and methods:** The chromatographic separation was achieved isocratically on Discovery (250 x 4.6mm, 5µ) using 0.01% potassium dihydrogen phosphate buffer (pH 5.4): acetonitrile (60:40) as mobile phase, at a flow rate 1.0mL/min. The detection was monitored at 260nm. **Results and Discussion:** The separation was achieved within 6minutes, with retention times 2.281 and 2.840minutes for pantoprazole and ondansetron, respectively. %RSD of the pantoprazole and ondansetron were and found to be 1.0 and 0.8, respectively. %Assay was obtained as 99.26% and 99.09% for pantoprazole and ondansetron, respectively. LOD, LOQ values are obtained from regression equations of pantoprazole and ondansetron were 0.10µg/mL, 0.07µg/mL and 0.32µg/mL, 0.21µg/mL respectively. Regression equation of pantoprazole is y = 6589x + 20552, and y = 16218x + 5357 for ondansetron. The analytes were subjected to degradation studies using acid, alkali, oxidative, thermal, and photodegradation. **Conclusion:** The results obtained prove that the method is reproducible and selective for the determination of pantoprazole and ondansetron. The method was validated as per ICH guidelines in terms of accuracy, precision, linearity,, and specificity.

Keywords: Pantoprazole, Ondansetron, Degradation studies, RP-HPLC, Isocratic.

1. INTRODUCTION

Pantoprazole (figure 1a) is a proton pump inhibitor drug that inhibits gastric acid secretion. It acts by controlling the final step in gastric acid production by forming a covalent bond to two sites of the (H^+, K^+) ATPase enzyme system at the secretary surface of the gastric parietal cell. This action is dose-relatedd and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus [1, 2]. Chemically Pantoprazole is 6-(Difluromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)Methylsulfinyl]-1H-

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benzimidazole. The drug is recommended for short-term treatment of erosion and ulceration of the oesophagus caused by gastro-oesophageal reflux disease [3].

Ondansetron is a selective serotonin 5-HT_3 receptor antagonist. The antiemetic activity of the drug is brought about through the inhibition of 5-HT_3 receptors. This inhibition of 5-HT_3 receptors, in turn, inhibits the visceral afferent stimulation of the vomiting center likely indirectly at the level of the area postrema as well as through direct inhibition of serotonin activity within the area postrema and the chemoreceptor trigger zone [4, 5]. Ondansetron is chemically known as 9-methyl-3-[(2-methyl-1H-imidazole-1-yl) methyl]-2, 3-dihydro-1H-carbazol-4(9H)-one [6]. The combination is available as pantoprazole 40 mg and Ondansetron 4 mg fixed dose tablet dosage form. The literature review reveals that few analytical methods like spectroscopic methods [7-10], HPLC [11-17] and HPTLC [18] method available for estimation of selected drugs individually or in combination with other drugs. The present work aims to develop a sensitive, simple, rapid, and accurate method for the simultaneous estimation of pantoprazole and ondansetron as per ICH guidelines [19].

2. EXPERIMENT

Reagents and chemicals: Pantoprazole and Ondansetron pure drugs were obtained as gift sample from Spectrum Pharma research solutions, Hyderabad. Combination Pantoprazole and Ondansetron tablets were obtained from a local pharmacy store. Acetonitrile, phosphate buffer, methanol, potassium, orthophosphoric acid and water of HPLC (High-Performance Liquid Chromatography) were obtained from Rankem, Mumbai.

Instrument: The experiment was carried on Waters 2965 equipped with auto Injector and PDA detector. The software used was Empower2. The separation was achieved by using Discovery (250 x 4.6 mm, 5µ) column and 0.01% potassium dihydrogen phosphate buffer (pH 5.4): acetonitrile (60:40) as mobile phase with 1mL/min flow rate. The detection was monitored at 260nm.

Preparation of Buffer: the Accurately weighed the amount of 0.1g of potassium dihydrogen phosphate was transferred to 1000mL volumetric flask and made t mark with water. The pH of the resulting solution was adjusted to 5.4 using dilute orthophosphoric acid.

Preparation of Standard Solution: The stock solution of pantoprazole and ondansetron was by transferring 40mg and 4mg of drug respectively into separate 25mL volumetric flask. The final volume of 25mL was made with diluent (water: acetonitrile, 50:50 v/v). From the above each stock solution, 1mL was pipette out into a 10mL volumetric flask and then made the final volume with diluent, which results in 160µg/mL and16µg/mL of pantoprazole and ondansetron respectively.

Sample Preparation: 20 tablets, each containing 40mg of pantoprazole and 4mg of ondansetron, were weighed and crushed to a fine powder. Tablet powder weight equivalent to 40mg of pantoprazole and 4mg of Ondansetron was transferred into a 25ml volumetric flask, 20ml of diluent added and sonicated for 30 min, further, the volume made up with diluent and filtered through 0.25µ filter. From the above-prepared solution1ml was pipette out into a 10mL volumetric flask and made up to 10ml with diluent.

Method development: Different mobile phase compositions like the combination of orthophosphate buffer and potassium dihydrogen phosphate with organic solvents like methanol and acetonitrile with varied pH were tried. Trials showed a mobile phase composition of potassium dihydrogen phosphate buffer (pH 5.4): acetonitrile (60:40 v/v) with 1mL/min. Showed good peak shape and resolution. The detection was monitored at 260nm. With these conditions, the retention times of pantoprazole and Ondansetron were found to be 2.274 and 2.844minutes, respectively.

Method Validation: The method was validated as per ICH guidelines. The parameters like linearity, precision, and accuracy, limit of detection (LOD) and limit of quantitation (LOQ) were considered to validate the method. In addition, system suitability was assessed. The linearity of the method was developed by considering 6 calibration standards ranging from 32µg/mL to 240µg/mL for pantoprazole and 3.2µg/mL to 24µg/mL for ondansetron. The LOD and LOQ of the method were determined based on standard deviation of response and slope. By using working standard at three levels (50%, 100% & 150%) prepared in triplicate the accuracy of the method was determined. The measurement of accuracy is in the terms of % mean recovery. The determination of inter-day precision was done by injecting working standard (at 100% concentration level) three times a day for three days. Whereas, intra-day precision was determined by analyzing six replicate samples working standard. The precision was expressed in term of relative standard deviation (%RSD). The specificity of the method was determined by carrying stability studies, which include oxidative, acid, alkali, neutral, thermal, and photostability. The data of stress studies were given as %recovery.

3. RESULTS AND DISCUSSION

System suitability: The standard solutions of pantoprazole and ondansetron were mixed and analyzed to determine system suitability. The parameters considered were retention time, number of theoretical plates, resolution, and tailing factor. The values are presented in Table-1. No interfering peaks were found at a retention time of the analytes. The standard chromatograms were given in figure – 1 and figure -2.

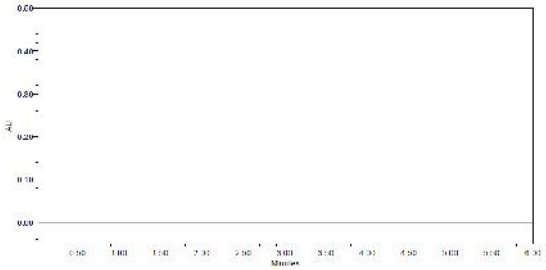

Fig 1: Chromatogram of blank

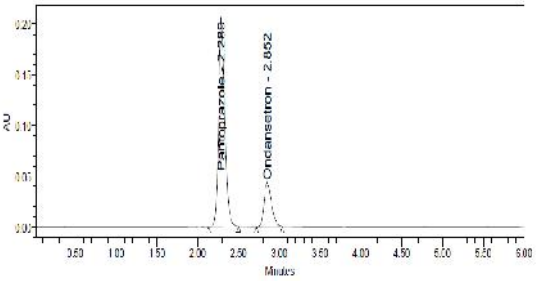

Fig 2: Typical chromatogram of pantoprazole and ondansetron

Table 1: System suitability studies of pantoprazole and ondansetron

Parameters	Pantoprazole	Ondansetron
Retention Time	2.281	2.840
No. of theoretical plates	3918 ± 63.48	4333 ± 56.67
Resolution	-	1.53
Tailing factor	1.20 ± 0.117	1.32 ± 0.027

Linearity: Six Linear concentrations of pantoprazole (32-240µg/mL) and ondansetron (3.2-24µg/mL) are prepared and injected. Regression equation of the pantoprazole and ondansetron are found to be, y = 6589x + 20552, and y = 16218x + 5357, and regression coefficient was 0.999.

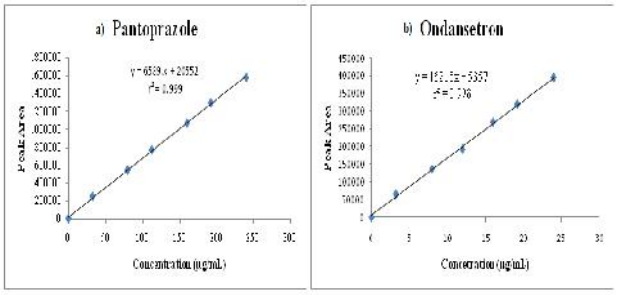

Fig 3: Calibration curve of a) pantoprazole b) ondansetron

Precision and Accuracy: The precision of the method was demonstrated by inter-day and intra-day variation studies. Six replicated injections of sample solutions were made, and the percentage RSD was calculated and represented in Table-2. From the data obtained, the developed RP-HPLC method was found to be precise. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out, and the percentage recovery and standard deviation of the percentage recovery were calculated and represented in Table-3. The high percentage of recovery indicates that the proposed method is highly accurate.

Table-2: Precision data for the proposed method

Sample	Inter-day				Intra-day			
	pantoprazole		ondansetron		pantoprazole		ondansetron	
Mean (n=6)	10928512630871092851263087				10621772702601062177270260			
SD	2827.6	396.5	2827.6	396.5	11055.4	2089.9	11055.4	2089.9
%RSD	0.3	0.2	0.3	0.2	1.0	0.8	1.0	0.8

Table-3: Accuracy data for the proposed method

Sample	Concentration (µg/ml)	Amount Recovered (µg/ml)	% Recovery	% RSD
Pantoprazole	80	79.32	99.15	0.98
	160	161.60	101.0	0.07
	240	238.07	99.20	0.06
Ondansetron	8	7.99	99.93	0.50
	16	16.11	100.71	0.88
	24	24.22	100.90	1.13

Limit of detection and limit of quantification: LOD and LOQ of the developed method were determined by based on a standard deviation of response and slope. The results were represented in Table-4.

Table 4: LOD and LOQ of pantoprazole and ondansetron

Parameter	Pantoprazole	Ondansetron
Limit of detection (LOD)	0.43	0.49
Limit of quantification (LOQ)	1.31	1.49

Robustness: Small, deliberate changes in a method like flow rate, mobile phase ratio, and temperature are made, but there were no recognized changes in the result and are within range as per the ICH guideline. The data is presented in Table-5.

Table 5: Robustness data of pantoprazole and ondansetron

Robustness condition	Pantoprazole %RSD (n=3)	Ondansetron %RSD (n=3)
Flow minus	0.6	1.4
Flow plus	0.3	0.5
Mobile phase minus	0.6	0.6
Mobile phase plus	0.6	1.3
Temperature minus	0.4	0.7
Temperature plus	0.6	1.2

Assay: Standard preparations are made from the API, and sample preparations are from the formulation. Both samples and standards are injected into six homogeneous samples. The drug in the formulation was estimated by taking the standard as the reference. The Average % Assay was calculated and found to be 99.26% and 99.09% for pantoprazole and ondansetron, respectively. The results were represented in Table-6.

Table 6: Assay results of pantoprazole and ondansetron in formulations

Formulation		Label claim	%Assay (n=6)	%RSD
Zaprol O®	Pantoprazole	40mg	99.26% ± 1.033	1.0
	Ondansetron	4mg	99.09% ± 0.766	0.8

Degradation Studies: Degradation studies were performed with the formulation, and the degraded samples were injected. Assay of the injected samples was calculated, and

all the samples passed the limits of degradation. The data was compiled in Table -7

Table 7: Degradation data of pantoprazole

Degradation	Pantoprazole		Ondansetron	
Condition	% Assay	% Degraded	% Assay	% Degraded
Acid	95.57	4.43	96.84	3.16
Alkali	96.19	3.81	97.58	2.42
Oxidation	97.78	2.22	98.52	1.48
Thermal	98.17	1.83	99.03	0.97
UV	99.18	0.82	99.25	0.75
Water	99.36	0.64	99.62	0.38

4. CONCLUSION

The present work describes the development and validation of stability, indicating RP-HPLC method for simultaneous estimation of pantoprazole and ondansetron. The developed method has an advantage over the reported method in being able to determine the selected drugs with high sensitivity, selectivity, and short analysis time using the isocratic mobile phase. The developed method was simple and economical that can be adopted in regular quality control test in Industries.

5. ACKNOWLEDGMENT

Authors are thankful for the management of V. V. Institute of Pharmaceutical Sciences for supporting to carry the present work.

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Conflict of Interest: None
Source of Funding: Nil