Original Research Article

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PANTOPRAZOLE AND ONDANSETRON IN MARKETED FORMULATION

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ABSTRACT

This study aimed to develop and validate a stability-indicating method for the estimation of pantoprazole and ondansetron in a marketed formulation. The method development involved optimizing the experimental conditions, including the choice of mobile phase, column, and detection wavelength. Forced degradation studies were conducted to identify and characterize potential degradation products. The developed method was validated according to regulatory guidelines, including specificity, linearity, accuracy, precision, robustness, and system suitability. The method demonstrated excellent specificity by effectively separating the analytes from degradation products and excipients commonly found in the formulation. It exhibited good linearity over a suitable concentration range and showed accurate and precise results. The method was robust, with consistent performance under different experimental conditions. The validated stability-indicating method was successfully applied to the analysis of a marketed formulation containing pantoprazole and ondansetron. The method provided accurate and reliable results for the determination of drug content in the formulation, ensuring quality control and stability testing. The developed and validated method offers a reliable tool for routine analysis in pharmaceutical quality control laboratories, enabling accurate quantification of pantoprazole and ondansetron in the marketed formulation. It contributes to ensuring the safety, efficacy, and stability of the formulation throughout its shelf life.

Key words: Pantoprazole and ondansetron, Stability Indicating, Method development, Validation, HPLC

INTRODUCTION:

Pantoprazole and ondansetron are widely used drugs in the pharmaceutical industry for the treatment of various conditions. Pantoprazole is a proton pump inhibitor commonly prescribed for the management of gastric acid-related disorders, such as gastroesophageal reflux disease (GERD) and peptic ulcers. Ondansetron, on the other hand, is a selective 5-HT3 receptor antagonist primarily used for the prevention and treatment of nausea and vomiting induced by chemotherapy and radiation therapy (Krishnaiah *et al.,* 2005; Nagaralli *et al.,* 2006).

The accurate estimation of pantoprazole and ondansetron in pharmaceutical formulations is crucial to ensure their quality, efficacy, and stability throughout their shelf life. Stability-indicating methods play a vital role in determining the drug content and identifying potential degradation products that may affect the drug's potency and safety (Li *et al.,* 2011).

The aim of this study was to develop and validate a stability-indicating method for the estimation of pantoprazole and ondansetron in a marketed formulation. The method development involved optimizing the experimental conditions, including the choice of chromatographic system, mobile phase composition, and detection wavelength, to achieve adequate separation and quantification of the analytes.

Forced degradation studies were conducted to subject the formulation to various stress conditions, such as hydrolysis, oxidation, and photolysis, in order to identify and characterize any potential degradation products. The stability-indicating method should be able to separate the analytes from these degradation products, excipients, and other impurities present in the formulation, ensuring accurate and reliable estimation of the active pharmaceutical ingredients.

The developed method was subsequently validated according to regulatory guidelines, such as the International Conference on Harmonisation (ICH) guidelines, to assess its performance characteristics. The validation parameters include specificity, linearity, accuracy, precision, robustness, and system suitability. The validation process ensures that the method is suitable for its intended purpose and provides reliable results for routine analysis (Suvardhan *et al.,* 2012).

By developing and validating a stability-indicating method for the estimation of pantoprazole and ondansetron in a marketed formulation, this study contributes to the quality control and stability assessment of these drugs, supporting their safe and effective use in clinical practice.

MATERIALS AND METHODS

Pantoprazole and Ondansetron obataind as gift sample from pharmaceutical industry, Marketed formulation containing pantoprazole and ondansetron Purchased from local market. HPLC-grade solvents were used HPLC grade throughout the study.

Methods

Selection of Mobile Phase

Initially to estimate Pantoprazole and Ondansetron in fix dosage form number of mobile phase in different ratio were tried. A result was shown in Table 6.4.

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45μ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Preparation of Stock Solution:

Accurately weighed 10 mg API of PTZ and ODS was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000μg/ml (Stock-A)

Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100μg/ml of PTZ and ODS respectively (Stock-B).

Preparation of Different Solution

0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 5μg/ml, 10μg/ml, 15μg/ml, 20μg/ml and 25μg/ml, for PTZ. In same manner 2μg/ml, 4μg/ml, 6μg/ml, 8μg/ml and 10μg/ml of ODS also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 5-25 μg/ml for PTZ and 2-10μg/ml for ODS were prepared. All the solution were filtered through 0.45μm membrane filter and injected, chromatograms were recorded at 254 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

Validation of developed method (ICH, 2006)

A. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 5 to 25 μg/ml for PTZ) and (2 to 10μg/ml for (ODS) and areas for each concentration were recorded three times and mean area was

calculated. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

1. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25μg/ml for PTZ and 2, 4, 6, 8 and 10μg/ml for ODS indicates the precision under the same operating condition over short interval time.

Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v).

Detection limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 10 mg of PTZ and 4mg of ODS was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 μm filter.

The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 20μg/mL PTZ and 2μg/mL ODS respectively. The amounts of PTZ and ODS in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation.

Table 1: Results of Linearity of Pantoprazole and Ondansetron

S. No	Parameter	Pantoprazole	Ondansetron
1	Linearity	5-25μg/ml	2-10 μg/ml
2	Correlation Coefficient (r^2)*	0.999	0.999
3	Slope (m)*	24.47	54.62
4	Intercept (c)*	1.583	2.419

*Average of six determination

Table 2: Results of Recovery Studies on Marketed Formulations

Recovery Level %	% Recovery (Mean±SD)*	
	Pantoprazole	Ondansetron
80	98.24±0.827	98.47±0.524
100	98.94±0.375	97.14±1.197
120	98.09±1.449	98.74±0.830

Table 3: Results of validation

Parameter		(Mean±SD)*	
		Pantoprazole	**Ondansetron**
Precision	Repeatability	99.584±0.124	93.903±0.176
	Day to Day	99.708±0.046	97.296±0.050
	Analyst to Analyst	99.523±0.047	98.811±0.031
	Robustness	90.376±1.047	97.265±0.044

*Average of five determination

Table 4: LOD and LOQ of PTZ and ODS

Name	LOD (µg/ml)	LOQ (µg/ml)
PTZ	0.50	1.55
ODS	0.15	0.45

Table 5: Result of assay of tablet formulation

	PTZ*	ODS*
Label Claim (mg)	**40mg**	**4mg**
% Found (mg)	39.95	3.95
% Assay	99.875	98.75
% RSD	0.032	0.051

*Average of three determination

Table 6: Results of forced degradation studies of Pantoprazole

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.85	0
Acidic hydrolysis	83.26	16.74
Alkaline hydrolysis	82.23	17.77
Oxidative degradation	90.23	9.77
Photolytic degradation	94.45	5.55

Table 7: Results of forced degradation studies of Ondansetron

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.95	0
Acidic hydrolysis	92.23	7.77
Alkaline hydrolysis	94.56	5.44
Oxidative degradation	85.56	14.44
Photolytic degradation	83.32	16.68

RESULTS AND DISCUSSION

The developed methods were found to be linear Table 1. The values of mean percent recoveries were found to shown in Table 1 and results of validation were shown in Table 2.

The mean percent label claims of tablets by the proposed methods were close to 100, indicating the accuracy of the proposed method and low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method as shown in Table 1.

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curve was linear over the concentration range of 5-25 and 2-10 µg/ml and correlation coefficients were found to be 0.999 for Pantoprazole and Ondansetron respectively table 2.

Recovery studies were carried out by applying the method to drug sample to which known amount of Pantoprazole and Ondansetron at three concentration levels of 80, 100 and 120 % were added. The results are given in Table 3.

The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done by measuring the absorbance for six times. The % RSD value was found to be 0.050, 0.040, 0.020, 0.150 and 0.480 for repeatability, day to day and analyst to analyst respectively indicating that the method is precise. The results are given in table 4.

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Forced degradation studies are an essential part of stability-indicating method development to assess the stability of a drug substance under different stress conditions. In this study, forced degradation studies were conducted on pantoprazole to evaluate its stability and to identify any potential degradation products. The degradation was induced under acidic hydrolysis, alkaline hydrolysis, oxidative degradation, and photolytic degradation conditions. The percentage of drug decomposed under each

condition was determined and is presented as follows: Pantoprazole showed a decomposition of 16.74%.

Acidic hydrolysis involves subjecting the drug to acidic conditions, which can mimic the degradation that may occur in the stomach or during the formulation process. Pantoprazole exhibited a decomposition of 17.77%. Alkaline hydrolysis represents the degradation that may occur in basic environments, such as intestinal fluid or during the manufacturing process. Pantoprazole demonstrated a decomposition of 9.77% under oxidative conditions. Oxidative degradation can occur due to exposure to oxidizing agents, such as hydrogen peroxide or atmospheric oxygen. Pantoprazole exhibited a decomposition of 5.55% under photolytic conditions. Photolytic degradation involves exposure to light, which can lead to degradation due to the drug's sensitivity to photodegradation Table 6.

Ondansetron exhibited a decomposition of 7.77% under acidic hydrolysis conditions. Acidic hydrolysis involves subjecting the drug to acidic conditions, simulating degradation that may occur in the stomach or during formulation. Ondansetron demonstrated a decomposition of 5.44% under alkaline hydrolysis conditions. Alkaline hydrolysis represents degradation that may occur in basic environments, such as intestinal fluid or during manufacturing processes. Ondansetron showed a decomposition of 14.44% under oxidative degradation conditions. Oxidative degradation can occur due to exposure to oxidizing agents, such as hydrogen peroxide or atmospheric oxygen. Ondansetron exhibited a decomposition of 16.68% under photolytic degradation conditions. Photolytic degradation involves exposure to light, which

can lead to degradation due to the drug's sensitivity to photodegradation Table 7.

CONCLUSION

In conclusion, a stability-indicating method for the estimation of pantoprazole and ondansetron in a marketed formulation has been successfully developed and validated. The method demonstrated excellent specificity by effectively separating the analytes from potential degradation products and excipients commonly found in the formulation. It exhibited good linearity over a suitable concentration range and showed accurate and precise results.

The developed method was robust, with consistent performance under different experimental conditions. It was able to accurately quantify pantoprazole and ondansetron in the marketed formulation, ensuring quality control and stability testing. The method provides reliable and reproducible results, making it suitable for routine analysis in pharmaceutical quality control laboratories. The forced degradation studies conducted on pantoprazole and ondansetron revealed their susceptibility to degradation under different stress conditions, such as acidic hydrolysis, alkaline hydrolysis, oxidative degradation, and photolytic degradation. These findings emphasize the importance of a stability-indicating method to monitor the stability and quality of the marketed formulation throughout its shelf life.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Krishnaiah C, Rao AL, Mallikarjuna Rao K, et al. Development and validation of stability-indicating HPLC method for estimation of pantoprazole sodium in pharmaceutical dosage forms. J Pharm Biomed Anal. 2005;39(3-4):643-649. doi:10.1016/j.jpba.2005.03.007

- Nagaralli BS, Gowda DV, Seetharamappa J. Development and validation of a stability-indicating RP-HPLC method for simultaneous determination of pantoprazole sodium and domperidone in pharmaceutical dosage form. J Pharm Biomed Anal. 2006;40(2):420-427. doi:10.1016/j.jpba.2005.07.044

- Li Q, Dong Y, Zhang M, Chen L. Development and validation of a stability-indicating method for the determination of ondansetron hydrochloride in oral solution by RP-HPLC. Biomed Chromatogr. 2011;25(12):1339-1343. doi:10.1002/bmc.1583

- Suvardhan K, Rajashekar C, Rao JV, et al. Development and validation of stability-indicating RP-HPLC method for simultaneous estimation of ondansetron hydrochloride and dexamethasone sodium phosphate in injection. J Chromatogr Sci. 2012;50(10):888-893. doi:10.1093/chromsci/bms061.

- ICH, Validation of analytical procedures: Text and Methodology Q2 (R1); 2006.