International Journal of Pharmacy Teaching & Practices 2013, Vol.4, Issue 4, 793-796.

Development and Validation of HPLC Method for Determination of Pantoprazole in Pantoprazole Pellets

Ognjenka Rahić*, Edina Vranić, Indira Mujezin, Jasmina Hadžiabdić, Alisa Elezović
Department of Pharmaceutical Technology, Faculty of Pharmacy, Zmaja od Bosne 8, 71000 Sarajevo Bosnia and Herzegovina

Research Article

Please cite this paper as: Ognjenka Rahić*, Edina Vranić, Indira Mujezin, Jasmina Hadžiabdić, Alisa Elezović. Development and Validation of HPLC Method for Determination of Pantoprazole in Pantoprazole Pellets. IJPTP, 2013, 4(4), 793-796.

Corresponding Author:

Ognjenka Rahić
Department of Pharmaceutical Technology, Faculty of Pharmacy, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina
Email: *orahic@gmail.com*
Phone no: +38733 586 173

Abstract

Since the assay method for pantoprazole in pantoprazole pellets is not described in current pharmacopoeias (USP, BP), the aim of this work was to develop and validate a simple, precise and accurate method for determination of pantoprazole in pantoprazole pellets. Separation was achieved on a reversed-phase C_8 column (250 x 4.6 mm i.d.; 5 m) using a mixture of phosphate buffer pH 3.0 and acetonitrile as mobile phase, at a flow rate 2 ml/min and UV detection at 290 nm. The method was validated according to ICH Guidelines. Validation showed that developed method was valid and reliable for determination of active substance in pantoprazole pellets.

Keywords: *pantoprazole; pantoprazole assay; HPLC method; method validation*

Introduction

A characteristic feature of mammalian stomach is its ability to secrete acid as part of its involvement in digesting food. The acid secretory unit of the gastric mucosa is the parietal cell. Secretion of acid by gastric cells is regulated by the actions of various mediators at receptors, such as histamine agonism of H_2-receptors, gastrin activity at G-receptors and acetylcholine at muscarinic M_2-receptors[1].

Over the years, it became evident that gastric acid can lead to acid related disorders of stomach, esophagus and duodenum, such as gastritis, peptic ulcers and gastro-esophageal reflux disease. The first target in treatment of acid related diseases was the H_2-receptors. The second medicinal target was the gastric acid pump, the gastric H^+,K^+-ATPaze or proton pump.

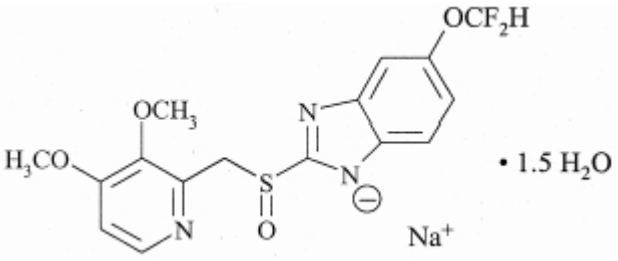
Since the proton transport by the proton pump is the final step of gastric acid secretion, it was anticipated that drugs of this type would be more effective as inhibitors of acid secretion. So this group of drugs was called Proton pump inhibitors (PPIs)[2].

The first clinically useful PPI was omeprazole, after which lansoprazole, pantoprazole and rabeprazole were developed.

Pantoprazole

Pantoprazole sodium sesquihydrate is a selective proton pump inhibitor (PPI) with prolonged effect. Like other PPIs it consists of substituted benzimidazole and pyridine moiety linked through methylenesulfinyl group[3]. It was originally synthesized as pantoprazole, but since 1986, it is synthesized as salt. This was the way to enhance solubility, stability and excipient-compatibility of the original molecule[4].

Pantoprazole sodium sesquihydrate is sodium 5-(difluoromethoxy)-2-[(*RS*)-[(3,4-dimethoxypyridin-2-yl)methyl]sulfinyl]benzimidazol-1-id sesquihydrate[5]. Structural formula of pantoprazole sodium sesquihydrate is shown in Figure 1. It shows better solubility and stability in buffer media with higher pH value. It decomposes practically immediate in an acidic medium[6, 7]. So it should be, in appropriate manner, protected from gastric acid when administered.



and enantiomer

Figure 1. Structural formula of pantoprazole sodium sesquihydrate[5]

The only way pantoprazole can perform an action on the proton pump is to be systematically absorbed and then distributed back into the stomach (in its unionized form) from general circulation. Once it diffuses back from the general circulation across the

gastric membranes it encounters the acid-filled canalicular network, protonates, rearranges and bonds to proton pump. To overcome the activation and stability issues, the best approach appears to be incorporation of pantoprazole into enteric-coated dosage forms.

Pellets

Pellets are of great interest to the pharmaceutical industry for a variety of reasons. They show multiple technological, physiological and therapeutic advantages over conventional, monolithic dosage forms. Because pellets disperse freely in the gastrointestinal tract, the invariably maximize drug absorption, reduce peak plasma fluctuations, and minimize potential side effect. Pellets also reduce variations in gastric emptying rates and overall transit times, so intra- and inter- subject variability of plasma profiles are minimized. One significant advantage of pellets over monolithic dosage forms is their shape, which is ideal for film-coating[8].

Film-coating may be applied for a many reasons, such as: esthetic reasons, taste-masking reasons and stability enhancement. When formulated as controlled-release dosage forms, pellets can deliver active substance at a specific site in the gastrointestinal tract. They can also sustain the action of drugs over an extended period of time[9].

Considering characteristics of active substance and advantages of pellets as a dosage form, pantoprazole was formulated in the form of sustained release pellets. Since current Pharmacopoeias[10, 11] do not describe assay method for pantoprazole in pantoprazole pellets, the aim of this work was to develop and validate a method for determination of pantoprazole in pantoprazole sustained release pellets

Material and Method

Standard

Reference standard of pantoprazole sodium (Lot. F0H266) was used.

Reagents

Sodium hydroxide, orto-phosphoric acid, potassium dihydrogen phosphate and HPLC-grade acetonitrile was purchased from Merck KgA (Darmstadt, Germany). The water used was produced by milliQ-Gradient A10 system (Millipore, Billerica, USA).

Sample preparation

Preparation of placebo solution

A quantity of Placebo mixture equivalent to 8000 mg of pantoprazole was diluted with 200.0 ml of a mixture of sodium hydroxide solution/ACN (50:50, v/v) (*placebo stock solution*). 5 ml of *placebo stock solution* was then diluted to 500.0 ml with the same mixture of solvents.

Preparation of standard solution

Standard solution was prepared diluting 46.0 mg of pantoprazole sodium reference standard (equivalent to about 40 mg of pantoprazole) with 50.0 ml of a mixture of sodium hydroxide solution/ACN (50:50, v/v). This solution was then diluted with the same mixture of solvents, to obtain pantoprazole concentration of 0.4 mg/ml.

Preparation of test solution

An amount of pantoprazole sodium sesquihydrate (equivalent to about 162.7 mg, 200.0 mg and 244.1 mg of pantoprazole) was diluted to 500.0 ml with mixture of sodium hydroxide solution/ACN (50:50, v/v) in order to obtain test solutions of 80%, 100% and 120% of target concentration. Prior to dilution 5 ml of *placebo stock solution* was added into each test solution.

Equipment and chromatographic conditions.

The HPLC analysis and validation was performed on a HPLC system Shimadzu LC-2010A (Shimadzu, Kyoto, Japan) with a PDA detector. Data acquisition and treatment were performed with the CLASS-VP software (Shimadzu). Hypersil BDS C-8 (250mm x 4.6mm, 5□m) was used as a stationary phase. Chromatography was performed using a mixture of phosphate buffer pH 3.0/ACN (70:30, v/v) as a mobile phase, with the flow rate of 2 ml/min, and maintaining the column temperature at 30°C.

Method validation

The HPLC method was validated according to ICH Guideline Q2 (R1) [12].

Results and Discussion

Initial conditions selection

The separation method has been developed in HPLC with UV detection in order to obtain fast, simple and reliable method which will be applicable for a routine work. Chromatographic conditions were chosen, modified and adapted considering recommendations of Pharmacopoeias[11, 12] for pantoprazole sodium sesquihydrate and other dosage forms containing pantoprazole as active substance.

Method validation

Selectivity

Selectivity of method was tested with placebo solution and standard solution. Selectivity of method was determined through the % interference, which represents the ratio of peak areas of placebo and standard, expressed as percent. Acceptable level of interference was predetermined to be equal or less than 2.0%. It can be concluded that method has good selectivity, since placebo showed 0% interference. Chromatograms of placebo and standard solution are presented in Figure 2 and Figure 3, respectively.

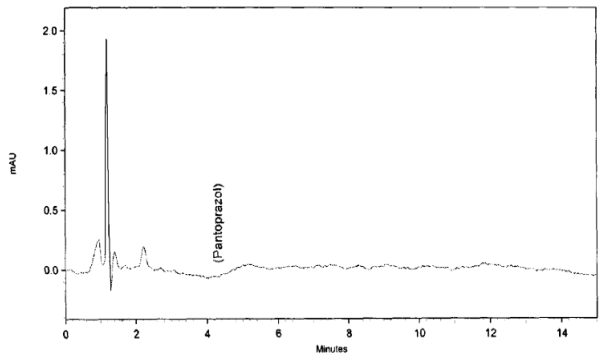


Figure 2. A typical chromatogram of placebo solution

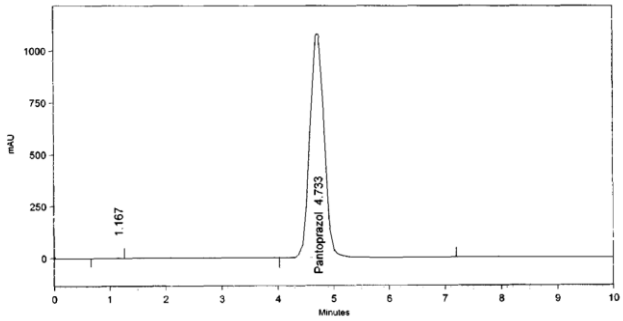


Figure 3. A typical chromatogram of pantoprazole standard solution

Accuracy

Accuracy of the method was tested with test solutions having three different concentrations (80%, 100% and 120% of target concentration). All solutions were prepared in triplicate and injected in chromatograph. The results are shown in Table 1.

Table 1. Accuracy (% recovery) of pantoprazole in pantoprazole pellets at three concentration levels

Pantoprazole concentration (mg/ml)	Accuracy (% recovery)				RSD (%)
	Sample 1	Sample 2	Sample 3	Mean	
0.32	99.75	100.23	99.61	99.86	0.33
0.40	99.81	99.12	99.28	99.40	0.36
0.48	99.67	98.43	98.27	98.79	0.78

Precision

Six different test solutions having target concentration of 0.4 mg/ml of active substance was prepared and injected into the chromatograph. The results of precision of method for determination of pantoprazole in pantoprazole pellets are given in Table 2.

Table 2. Precision of HPLC method for determination of pantoprazole in pantoprazole pellets

Pantoprazole concentration (mg/ml)	Precision (% recovery)						Mean	RSD (%)
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6		
0.40	98.98	98.15	99.67	99.72	98.10	99.41	99.01	0.74

Linearity

The linearity of the relationship between the concentration and response (peak area) was investigated using a sequence of standard and test solutions covering the range of 50% to 150% of the target concentration. Results are shown in Figure 4. Validation results were computed using MS Excel. The linearity of the results is expressed by the coefficient of correlation (r^2). For both, standard and test, the relationship was linear as the r^2 value for standard was 0.9995 and 0.9999 for test solution.

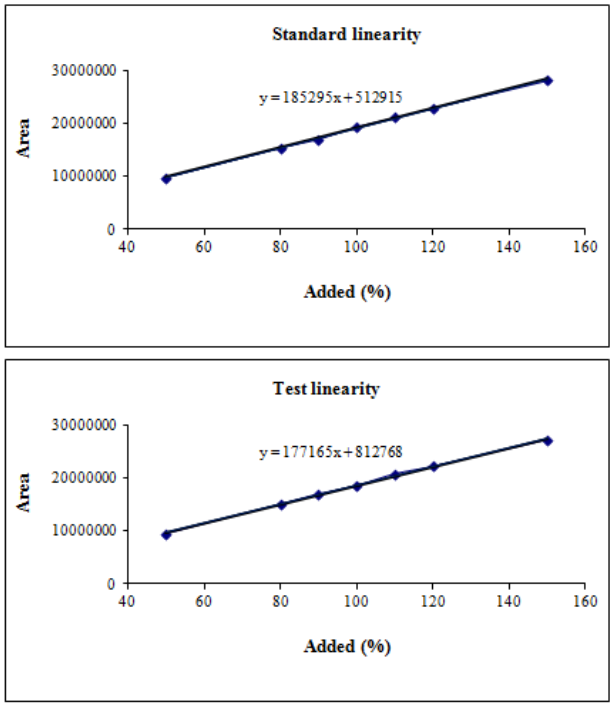


Figure 4. Linearity of HPLC method for determination of pantoprazole in pantoprazole pellets

Robustness

Robustness is the evaluation of the constancy of the results when variables inherent to the method of analysis are varied deliberately. Factors chosen for this validation were mobile phase flow rate, mobile phase ratio and pH of the buffer solution in the mobile phase. Each experiment was performed in triplicate and the mean value was used for calculations. Robustness of the method is expressed through the coefficient of variation, whose predetermined limit was equal or less than 3.0%. Results of method robustness are presented in Table 3.

Table 3. The effect of variation of method parameters on the determination of pantoprazole in pantoprazole pellets

Parameter		Recovery (%)	Coefficient of Variation (≤ 3.0%)
Flow Rate	Low Target High	99.52 99.81 99.52	0.17
Mobile phase ratio	Low Target High	100.50 99.81 100.05	0.38
pH of Buffer in Mobile phase	Low Target High	99.84 99.81 100.10	0.16

Conclusion

The analytical method described in this paper is suitable for assay of pantoprazole in pantoprazole pellets. This method has been demonstrated to have good accuracy, precision, linearity and robustness. Therefore, this method is suitable for routine analysis of pantoprazole assay in pantoprazole pellets.

References

1. Sachs G, Prinz C, Loo D, Bamberg K, Besancon M, Shin JM. Gastric acid secretion: Activation and inhibition. Yale J Biol Med. 1994 67:81-95

2. Hoogerwerf A, Pasricha PJ. Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease. in: Brunton LL. (Ed.), Goodman & Gillman's The pharmacological basis of therapeutics, 11th ed., McGraw-Hill, New York, 2005, pp967-981

3. Riley TN, DeRuiter J. Histamine and antihistaminic agents. in: Block JH, Beale JM. (Eds.), Textbook of organic medicinal and pharmaceutical chemistry, 11th ed., Lippincott Williams & Wilkins, Philadelphia, 2004, pp696-730

4. Senn-Bilfinger J, Sturm E. The development of a new proton-pump inhibitor: The case history of pantoprazole. in: Fischer J, Ganellin CR. (Eds.), Analogue-based drug discovery, 1st ed., Viley-VCH, Weinheim, 2006, pp115-136

5. European pharmacopoeia, Council of Europe, Strasbourg, 7th ed., 2010

6. Badwan AA, Nabulsi LN, Al Omari MM, Daraghmeh NH, Ashour MK, Abdoh AM, Jaber AMY. Pantoprazole sodium. in: Brittain HG. (Ed), Analytical profiles of drug substances and excipients, Academic Press. 2002, Volume 29, pp213-259

7. Kristl A. Acido-basic properties of proton pump inhibitors in aqueous solutions. Drug Dev Ind Pharm. 2009, 35:114-117

8. Ghebre-Sellassie I. Pharmaceutical pelletization technology. Marcel Dekker, New York, 1989

9. Ghebre-Sellassie I. Multiparticulate oral drug delivery. Taylor & Francis, New York, 1994

10. British pharmacopoeia, British pharmacopoeia commission, London, 2012

11. United States pharmacopoeia XXXV/National formulary XXX, The United States pharmacopoeia convention, Rockville, MD, 2012

12. International Conference on harmonization of technical requirements for registration of pharmaceuticals for human use. Validation of analytical procedures: text and methodology Q2 (R1). Current Step 4 Version, 2005

AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.