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Original Article

Analytical method development and validation of simultaneous estimation of rabeprazole, pantoprazole, and itopride by reverse-phase high-performance liquid chromatography

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ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form
28 May 2014
Accepted 30 May 2014
Available online 28 October 2014

Keywords:
Itopride
Pantoprazole
Rabeprazole
RP-HPLC

ABSTRACT

A simple, selective, rapid, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of rabeprazole (RP), pantoprazole (PP), and itopride (IP) has been developed. The compounds were well separated on a Phenomenex C_{18} (Luna) column (250 mm × 4.6 mm, dp = 5 μm) with C_{18} guard column (4 mm × 3 mm × 5 μm) with a mobile phase consisting of buffer containing 10 mM potassium dihydrogen orthophosphate (adjusted to pH 6.8): acetonitrile (70:30 v/v) at a flow rate of 1.0 mL/min and ultraviolet detection at 288 nm. The retention time of RP, PP, and IP were 5.35, 7.92, and 11.16 minutes, respectively. Validation of the proposed method was carried out according to International Conference on Harmonisation (ICH) guidelines. Linearity range was obtained for RP, PP, and IP over the concentration range of 2.5–25, 1–30, and 3–35 μg/mL and the r^2 values were 0.994, 0.978, and 0.991, respectively. The calculated limit of detection (LOD) values were 1, 0.3, and 1 μg/mL and limit of quantitation (LOQ) values were 2.5, 1, and 3 μg/mL for RP, PP, and IP correspondingly. Thus, the current study showed that the developed reverse-phase liquid chromatography method is sensitive and selective for the estimation of RP, PP, and IP in combined dosage form.

1. Introduction

Rabeprazole (RP), [2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1*H*-benzoimidazole] (Fig. 1), is used for the treatment of severe gastroesophageal reflux disease (GERD) and peptic ulcer. Pantoprazole (PP), [6-(difluoromethoxy)-2-[[(3, 4-dimethoxy-2-pyri-dinyl)-methyl] sulfinyl] 1-benzoimidazole] (Fig. 1), is used for the treatment of erosive esophagitis associated with GERD. Both RP and PP are proton pump inhibitors and they inhibit gastric acid secretion by targeting the gastric acid pump H^+ K^+ adenosine

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http://dx.doi.org/10.1016/j.jfda.2014.05.003

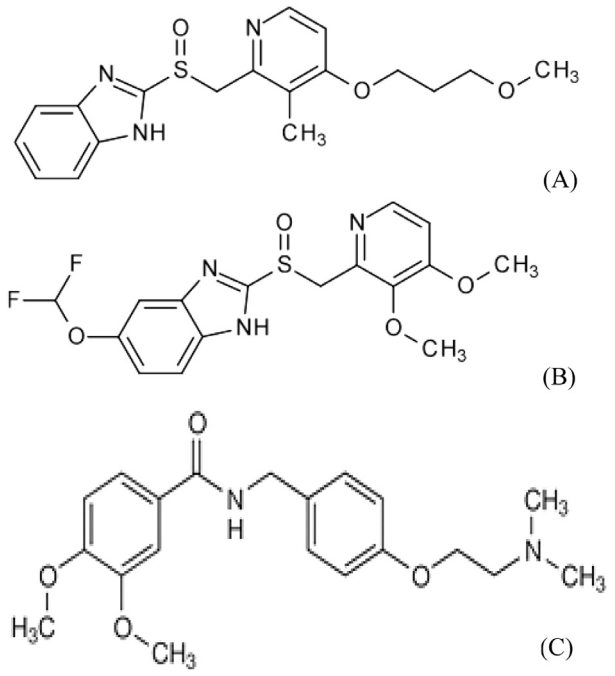

Fig. 1 – Chemical structures of (A) rabeprazole, (B) pantoprazole, and (C) itopride.

triphosphatase of the parietal cell. Proton pump inhibitors also are effective in treating patients with Zollinger-Ellison syndrome [1]. Itopride (IP), [N-[4-[2-[dimethyl amino ethoxy] phenyl] methyl]-3,4-dimethoxybenzamide (Figure 1), inhibits the dopamine D_2 receptor at the parasympathetic nerve ends and increases the release of acetylcholine, thereby increasing the esophageal and gastrointestinal motility. It also exerts an antiemetic action [2].

The combination of PP inhibitor and IP is widely available in the market for the treatment of gastrointestinal disorders. In general, these kinds of multicomponent dosage forms are useful for effective therapy and augment patient compliance. A range of analytical techniques, such as ultraviolet (UV)-visible spectrophotometry, high-performance liquid chromatography (HPLC), high-performance thin layer chromatography (HPTLC), and liquid chromatography-mass spectrometry (LC-MS) were reported in the literature for the determination of RP [3–10], PP [11–16], and IP [17–20] in dosage forms and biological samples in separate as well as in combination. Nevertheless, there is no information on the HPLC method for the concurrent determination of these drugs in combined dosage forms. The current study describes a simple, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of RP, PP, and IP in combined dosage forms.

2. Materials and methods

2.1. Chemicals

Acetonitrile (HPLC grade), orthophosphoric acid [analytical reagent (AR) grade], sodium hydroxide (AR grade), and ammonium acetate (AR grade) were purchased from E. Merck (India) Ltd. Worli, Mumbai, India. All active pharmaceutical ingredients (APIs) RP, PP, and IP as reference standards were obtained from Medley Pharmaceuticals Limited, Mumbai, India (99.7–99.9% purity).

2.2. Equipment

Shimadzu's HPLC quaternary system with UV-visible detector (LC-10AT VP) and 7725i injector was used for method development and validation.

2.3. Method development and optimization of chromatographic conditions for separation

The chromatographic condition was optimized by using different columns, mobile phase composition, pH (6.6, 6.8, and 7.0), wavelength (285, 288, and 290), flow rate (0.9, 1.0, and 1.1), column temperature (ambient to 45°C), and injection volume (10, 20, 30, and 50 μL).

2.4. Sample preparation

2.4.1. Chemical form of the APIs

RP sodium is a white to slightly yellowish-white solid. PP sodium is a white to off-white crystalline powder. IP hydrochloride is a white crystalline solid.

2.4.2. Preparation of RP, PP, and IP stock solutions

Stock solution was prepared by weighing 10 mg each of RP, PP, and IP standards in a 10-mL volumetric flask, dissolving in mobile phase, and diluting to volume with the same mobile phase up to 10 mL and retained as stock solution. Further dilutions were made with mobile phase.

2.4.3. Preparation of RP, PP, and IP standard dilutions

One milliliter from the stock solutions of RP, PP, and IP were transferred into a 10-mL volumetric flask separately and further diluted up to 10 mL with the solvent. Then 1 mL of RP, 1.5 mL of PP, and 2 mL of IP solutions were transferred into a 10-mL volumetric flask and diluted up to 10 mL separately to attain the final concentrations of 10 μg/mL, 15 μg/mL, and 20 μg/mL of RP, PP, and IP, respectively.

2.4.4. Preparation of mixed standard solutions

From the aforementioned standard stock solution, mixed standard solution was prepared by dissolving appropriate concentration of the stocks in the mobile phase and used for the estimation of individual drugs from the combination.

2.4.5. Preparation of the sample solution

The label claim of the dosage form includes 10 mg of RP sodium, 40 mg of PP sodium, and 50 mg of IP hydrochloride.

Twenty tablets of RP, PP, and IP available as combination dosage forms were weighed and powdered. An amount of the powder equivalent to one tablet was weighed accurately and mixed with the mobile phase in a 100-mL volumetric flask, sonicated for 5 minutes and filtered through 0.2 μ membrane filter to remove insoluble matter. One milliliter of the filtrate