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LC–MS/MS bioassay of four proton pump inhibitors

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ABSTRACT

A new validated bio-analytical LC–MS/MS method was developed for the simultaneous extraction and determination of four proton pump inhibitors: esomeprazole, lansoprazole, pantoprazole and rabeprazole in human plasma using escitalopram as an internal standard. The proteins in plasma samples were precipitated using acetonitrile for the extraction of analytes which is a simple economic method. The separation was accomplished using a mobile phase composed of 10 mM ammonium formate: acetonitrile: methanol (20:40:40% v/v) at a flow rate of 0.8 mL/min in isocratic mode on a reversed phase C18 INERTSIL ODS-3 (5 µm, 150 × 4.6 mm) and column temperature of 40 °C. Positive mode electrospray ionization source was used prior to multiple reaction monitoring (MRM) detection using parent and daughter ions: *m/z* 346.2 → 198.1 for esomeprazole, *m/z* 370.1 → 252 for lansoprazole, *m/z* 384.2 → 200.2 for pantoprazole, *m/z* 360.1 → 242.1 for rabeprazole and *m/z* 325.2 → 109 for escitalopram. The calibration curves were constructed, and the method was linear in the range of 20–5000 ng/mL applying weighted ($1/X^2$) linear regression coefficient for all drugs. The method was fully validated following US-FDA and EMA guidelines.

1. Introduction

Proton pump inhibitors (PPIs) are mainly used for the relief of peptic ulcer, gastro-esophageal reflux disease, *Helicobacter pylori* infection and management of hypersecretory states such as Zollinger-Ellison syndrome. Also, these drugs are used in the prevention and treatment of the gastro-intestinal lesions caused by non-steroidal anti-inflammatory drugs (NSAIDs) [1–3].

Proton pump inhibitors (PPIs) suppress gastric acid secretion by specific inhibition of the H+/K+-ATPase in the gastric parietal cell. PPIs block the final step in acid secretion in the partial cell is the ("pumping") of protons. PPIs form a covalent disulfide bond with the ATPase enzyme, leading to an irreversible inhibition of the pump. Since the inactivation of the receptor site (the ATPase in this case) is irreversible and complete, the PPIs are very potent and long-acting therapeutic entities. The ATPase is not able to recover from its irreversible interaction with the inhibitor structure and the body must synthesize new enzyme de novo which takes time. Until new protein is made, gastric acid secretion is halted [4,5]. The PPIs are more effective in the short term than the H_2-blockers in healing duodenal ulcers and erosive esophagitis and can heal esophagitis resistant to treatment with the H_2-blockers [5].

All currently FDA-approved PPIs [6] such as Esomeprazole, 6-methoxy-2-[(S)-(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-1H-benzimidazole (ESO, Fig. 1A), Lansoprazole, (RS) 2-[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl]-1H-benzimidazole (LAN, Fig. 1B), and its (*R*)-(+)-enantiomer isomer; Dexlansoprazole, Pantoprazole, 6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-1H-benzimidazole (PAN, Fig. 1C), and Rabeprazole, 2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl]-1H-benzimidazole (RAB, Fig. 1D) contain a 2-pyridyl methyl sulfinyl benzimidazole pharmacophore but with different substituents on the benzimidazole and pyridine rings [7]. Chemical structures of the cited drugs are illustrated in Fig. 1.

(Position of Fig. 1)

Literature review revealed several HPLC methods developed for the determination of PPIs either alone, in combination with other drugs or with their metabolites. These include three methods for the determination of LAN and its metabolites in plasma and urine [8–10], in addition to a reported method for the determination of LAN enantiomers in dog plasma by column-switching liquid chromatography with tandem mass spectrometry and its application to a preclinical pharmacokinetic study [11]. One method was reported for the determination of PAN in mixture with ketoprofen and valsartan in human plasma by HPLC [12], another method in mixture with ibuprofen and itopride in human plasma by HPLC using new generation core shell column [13] in addition to a reported HPTLC method for the determination of itopride, PAN, and mosapride in their formulations and spiked human

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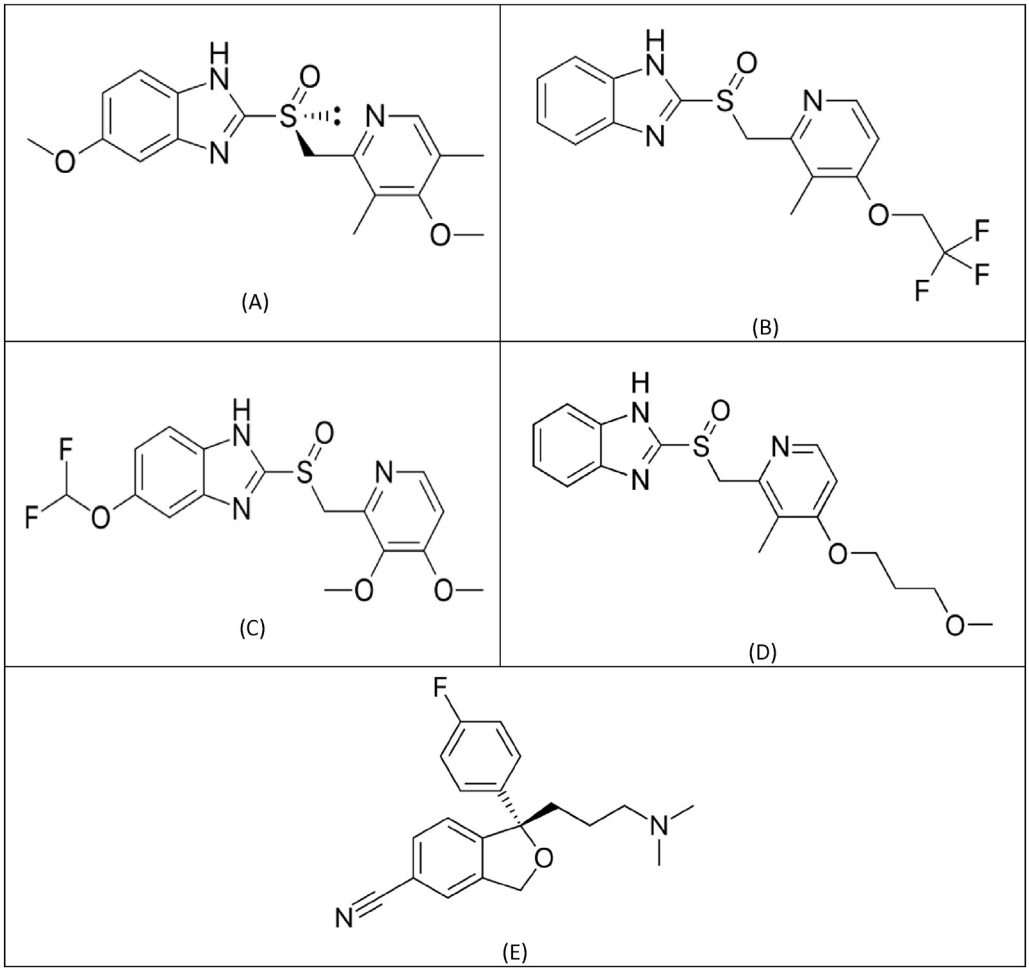

Fig. 1. Chemical structures of Esomeprazole (A), Lansoprazole (B), Pantoprazole (C), Rabeprazole (D) and Escitalopram (E).

plasma [14]. Moreover, two LC–MS/MS methods were reported for the determination of ESPO in mixture with naproxen in human plasma [15] and in presence of its two main metabolites in human, rat and dog plasma [16], one HPLC-UV method for separation and analysis of naproxen and ESO in presence of their chiral impurities: Enantiomeric purity determination in tablets [17] and another one for the estimation of co-administered ESO, leflunomide and ibuprofen in human plasma and in pharmaceutical dosage forms using micellar liquid chromatography [18]. Many methods have been reported for the determination of RAB: in presence of its active metabolite in human plasma by columns-switching HPLC [19], pharmacokinetic application [20–22] and bioequivalence study [23].

Upon literature survey, there are two HPLC-UV methods describing the simultaneous determination of lansoprazole, omeprazole, pantoprazole and rabeprazole in human plasma [24,25] but no reported method was found for the determination of the four proton pump inhibitors (ESO, LAN, PAN and RAB) in human plasma simultaneously using LC–MS/MS. Accordingly, our objective was to develop a new LC–MS/MS method for the simultaneous analysis of these four PPIs drugs. Generally, the LC–MS/MS methods are characterized by their high sensitivity and selectivity and are considered to be easier and faster to be developed compared to the conventional HPLC methods. Escitalopram was used as the internal standard (Fig. 1E). The method was fully validated following US-FDA [26] and EMA [27] guidelines.

2. Experimental

2.1. Instrumentation

The HPLC system was Agilent instrument 1260 series with vacuum degasser, mixer, auto-sampler, gradient quaternary pump and MS/MS detector (model 6410A). Agilent MassHunter Workstation software (B.03.01) was used for data acquisition. MassHunter Quantitative analysis software (B.04.00) was used for performing data. The pH was measured using a Jenway pH-meter (3505, Essex, U.K.). 0.2 µm Nylon membrane filter (Sigma-Aldrich Co., Germany) was used for mobile phase filtration. Vortex mixer (Stuart, England), cooling Centrifuge (Sigma, Germany), Vacuum concentrator (Eppendorf, Germany), and ultrasonic processor (Elma, Germany) were used.

2.2. Material and reagents

ESO, LAN, PAN, RAB and Escitalopram reference standards were kindly donated by National Quality Control Laboratory, Sana'a, Yemen. The potency was 100.10%, 99.55%, 99.7 and 99.45% for ESO, LAN, PAN and RAB, respectively, according to the manufacturer certificates of analysis. Ammonium formate was purchased from Loba Chemie, India. HPLC grade Methanol and acetonitrile were purchased from Sigma-Aldrich Co., Germany.

2.3. LC–MS/MS conditions

Chromatographic elution was carried out using reversed phase C18