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Analysis of omeprazole and its main metabolites by liquid chromatography using hybrid micellar mobile phases

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ABSTRACT

Omeprazole is a selective inhibitor of gastric acid secretion and is one of the most widely prescribed drugs internationally. A chromatographic procedure that uses micellar mobile phases of sodium dodecyl sulphate and propanol buffered at pH 7 and a C18 column is reported for the determination of omeprazole and its principal metabolites (omeprazole sulphone and hydroxyomeprazole) in urine and serum samples.

In this work, direct injection and UV detection set at 305 nm was used. Omeprazole and its metabolites were eluted in less than 11 min with no interference by the protein band or endogenous compounds. Adequate resolution was obtained with a chemometric approach, in which the retention factor and shape of the chromatographic peaks were taken into account. The analytical parameters including linearity ($r > 0.9998$), intra- and inter-day precision (RSD, %: 0.6–7.9 and 0.14–4.7, respectively) and robustness were studied in the validation of the method for the three compounds. The limits of detection and quantification were less than 6 and 25 ng mL^{-1}, respectively. Recoveries in micellar medium, plasma and urine matrices were in the 98–102% range. Finally, the method was successfully applied to the determination of omeprazole and its metabolites in physiological samples. Omeprazole was also analysed in pharmaceutical formulations.

1. Introduction

Omeprazole (OME) (Fig. 1a) is a proton pump inhibitor which blocks production of acid by the stomach [1]. It is a substituted benzimidazole gastric antisecretory agent. It is used in the treatment of dyspepsia, peptic ulcer disease, gastro-oesophageal reflux disease and Zollinger–Ellison syndrome, which are all caused by stomach acid. OME blocks the enzyme in the wall of the stomach that produces acid, thus decreasing its production, and allowing the stomach and oesophagus to heal.

Chemically, OME is a hydrophobic compound ($\log P_{o/w} = 2.23$) and it is a weak base whose dissociation constants are p*K*a = 4.2, 9.0 [2]. OME is a racemate (i.e. it can have equal amounts of the two *S* and *R* enantiomers). In the acidic conditions of the stomach, both are converted to achiral products, which react with the cysteine group in H^+/K^+-ATPase, thereby destroying the ability of parietal cells to produce gastric acid.

OME is combined with antibiotics in eradication therapy for Helicobacter pylori infection of the stomach. In medical practice, OME is available as tablets and capsules in strengths from 20 to 120 mg daily, and as a powder (omeprazole sodium) for intravenous injection. Most oral omeprazole preparations are enteric-coated due to rapid degradation of the drug in the acidic conditions inside the stomach.

After oral administration, OME is rapidly absorbed in the small intestine, a process that is usually completed within 3–6 h. Concomitant intake of food has no influence on bioavailability. OME is almost completely metabolised by the cytochrome P450 system, mainly in the liver. Metabolites that have been identified to date are omeprazole sulphone (OMES) (Fig. 1b) and 5-hydroxyomeprazole (HOME) (Fig. 1c), which are the most important, omeprazole sulphide, and carboxyomeprazole, and at least three other unidentified compounds which exert no significant effect on acid secretion. About 80% of an orally given dose is excreted as metabolites in urine and the remainder is found in faeces. The initial plasma concentration for toxicity is observed at 1.01 mg L^{-1} [1].

Several methods for determination of OME have been reported in pharmaceuticals and biological fluids, including high performance liquid chromatography (HPLC) employing electrochemical and coulometric detection [3,4], capillary electrophoresis [5], spectrophotometry [6], polarography [7] and voltammetry [8].

Most of the methods that have been described refer to the use of HPLC-UV and HPLC-MS systems for determination of OME alone in biological fluids [9–14]. Moreover, few publications have reported a simple HPLC method for the simultaneous determination of OME, HOME and OMES in human plasma [15–23]. HPLC methods includ-

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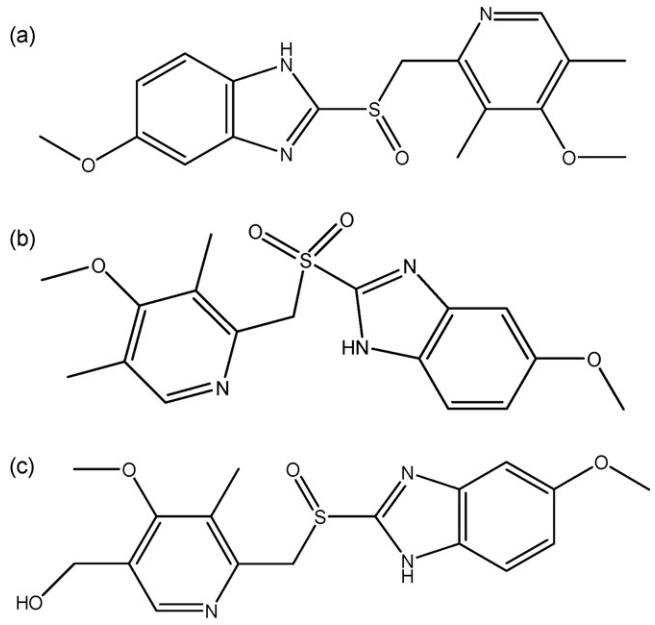

Fig. 1. Structure of omeprazole (a) and its metabolites omeprazole sulphone (b) and 5-hydroxyomeprazole (c).

ing enantioselective separation have recently become a method of choice [24–28]. Although these methods are selective, fast and sensitive, they are not suitable for routine clinical analysis because they require long-term pretreatment of the samples and expensive equipment that is not available in most control laboratories. Other investigations have focused on studying the stability and degradation of OME [29–33]. The review published by Espinosa-Bosch et al. [34] concludes that the ion-spray LC–MS–MS method has advantages in providing a shorter analytical run time, higher selectivity and a much lower limit of quantification compared with previous analytical methods.

HPLC methods applied in the analysis of serum and urine usually require elaborate sample pretreatment, including the removal of interferences and extraction procedure of the analytes. Micellar liquid chromatography (MLC) is a useful technique to analyse complex matrices without the aid of extraction, which reduces the cost and the time of the analysis runs, increases sample throughput, and decreases sources of error [35]. Micelles tend to bind proteins competitively, thereby releasing protein-bound drugs and proteins, rather than precipitation into the column, they are solubilised and washed away harmlessly to elute with the solvent front. MLC has been proved to be a useful technique in the determination of diverse groups of drugs such as trazodone in serum [36], and other drug materials [37]. The retention mechanisms (i.e. solute-mobile phase and solute-stationary phase interactions) in MLC are fairly well understood, and there is a reasonable theoretical foundation on which to build. MLC is a fascinating example of the benefits of secondary equilibrium in RPLC [35]. The primary equilibrium is solute partitioning between bulk solvent (i.e. water or aqueous–organic mixture) and the stationary phase. A secondary equilibrium is established with the micelles in the mobile phase. Both equilibria are affected by a variety of factors, such as the type and concentration of surfactant and additives (e.g. salts or organic modifiers), temperature, ionic strength and pH.

In this work a sensitive method was developed and validated for the simultaneous determination of OME and its two main metabolites in biological fluids without any previous pretreatment. It was also applied to the analysis of OME in pharmaceutical formulations. The method was validated according to Food and Drug Analysis (FDA) guidelines [38].

2. Experimental

2.1. Chemicals and reagents

Omeprazole was purchased from Sigma (MO, USA). Omeprazole sulphone and 5-hydroxyomeprazole were kindly donated by AstraZeneca (Sweden). Sodium dodecyl sulphate (SDS) and sodium hydroxide were from Merck (Germany). Ultrapure water was used throughout (Millipore S.A.S., France). Sodium dihydrogen phosphate and propanol were from Scharlab (Spain). Hydrochloric acid, methanol and ethanol were from J.T. Baker (The Netherlands). The pharmaceuticals produced by Normon (Spain), Ratiopharm España (Spain) and Pensa Pharma (Spain) were purchased in a local pharmacy.

2.2. Instrumentation

The chromatographic system (Agilent Technologies, Series 1100, CA, USA) was equipped with a quaternary pump, thermostatted autosampler tray and column compartments, and a diode-array detector (range 190–700 nm). The column was a Kromasil C18 (150 mm × 4.6 mm, 5 μm particle size, Scharlab). The pH of solutions was measured with a potentiometer model GLP 22 (Crison, Spain) equipped with a combined Ag/AgCl/glass electrode. The analytical balance used was an AX105 Delta-Range (Mettler-Toledo, Switzerland). A vortex shaker and sonication unit (Selecta, Spain) were employed for sample pretreatment. A Centronic-BL centrifuge (Selecta) was used to centrifuge serum samples.

2.3. Mobile phase, standard and sample preparation

The micellar mobile phases were prepared by dissolving SDS in water and were buffered with sodium dihydrogen phosphate 0.01 M at pH 7 using sodium hydroxide 0.1 M. Finally, propanol was added to achieve the desired concentration of the organic solvent and then topped up with water to the mark on the volumetric flask. All mobile phases were filtered through 0.45 μm nylon membranes (Micron Separations, MA, USA). Typically, to prepare 500 mL of the optimum mobile phase 0.08 M SDS–10% propanol–pH 7, 11.52 g of SDS and 0.69 g of disodium hydrogenphosphate were weighted and dissolved in ultrapure water. Then the pH was fixed at 7 with NaOH 0.1 M. After this, 50 mL of 1-propanol were added to achieve the 10% concentration of the alcohol. Finally, the volumetric flask was topped up to 500 mL with ultrapure water.

A stock solution of 20 μg mL^{-1} of each compound (OME, OMES and HOME) was prepared by dissolving each of them in a few millilitres of ethanol, with the aid of an ultrasonic bath, and finally topped up with 0.1 M SDS solution at pH 9.

Blood samples were collected using a DB SST Tube (BD Vacutainer Systems, UK) and centrifuged for 5 min at 3000 rpm and a temperature of 4 °C, and then liquid was separated from precipitated protein and stored at −21 °C, it was then thawed just before use. Urine samples were collected in a Urine Collection Cup (BD Vacutainer Systems).

Urine and serum stock solutions spiked with 20 μg mL^{-1} of OME, OMES and HOME were prepared by diluting urine by a factor of 1:10 and diluting serum by a factor of 1:5, using 0.1 M SDS at pH 9. Solutions were prepared daily and protected from direct light. Typically, to prepare 20 μg mL^{-1} of OME in serum, 1 mL of serum was added to a 5 mL volumetric flask, then 1 mL of 100 μg mL^{-1} OME is added and finally the volumetric flask is filled up with SDS solution. Same procedure was followed for urine samples taking into account that the volumetric flask was 10 mL, and consequently 2 mL of 100 μg mL^{-1} OME was added.

Biological samples from patients were injected directly into the chromatographic system after the previously commented dilution