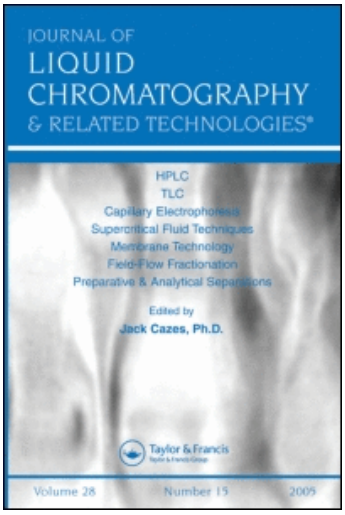

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A RAPID HPLC-DAD METHOD FOR SEPARATION AND DETERMINATION OF OMEPRAZOLE EXTRACTED FROM HUMAN PLASMA

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ABSTRACT

A high-performance liquid chromatographic (HPLC) method for the determination of omeprazole in human plasma is described. Omeprazole and the internal standard (H168/24) were extracted from plasma samples by solid phase extraction (SPE) using a polymeric sorbent-based cartridge. The separation was accomplished under reversed phase conditions using an Eclipse XDB-C8 Rapid Resolution (4.6 × 50 mm) column.

The mobile phase consisted of 23% acetonitrile and 77% of 30.4mM Na_2HPO_4 and 1.76mM KH_2PO_4 solution, pH 8.4, in which a gradient elution was used to linearly change solvent composition to 33% acetonitrile and 67% phosphate buffer during the first minute. Absorbance was monitored at 302 nm for omeprazole and at 294 nm for the internal standard and the total analysis time was 4 minutes.

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The lower limit of quantitation was 10 ng/mL and the calibration function is linear to 2000ng/mL. This method has been shown to be appropriate for pharmacokinetic studies involving children.

INTRODUCTION

Omeprazole is a potent (H^+, K^+)-ATPase (proton pump) inhibitor, which has been demonstrated, to be effective in the treatment of gastric acid related diseases; duodenal and gastric ulcers, reflux esophagitis, and Zollinger-Ellison syndrome.[1] This drug is metabolised principally by CYP2C19 to generate 5'-hydroxy-omeprazole (OH-OPZ),[2] and a minor pathway, through CYP3A4 enzymes generates omeprazole-sulphone (OPZ-SO_2).[3] CYP2C19 is known to present genetic polymorphism and individuals are classified as being either extensive metabolisers (EM) or poor metabolisers (PM).

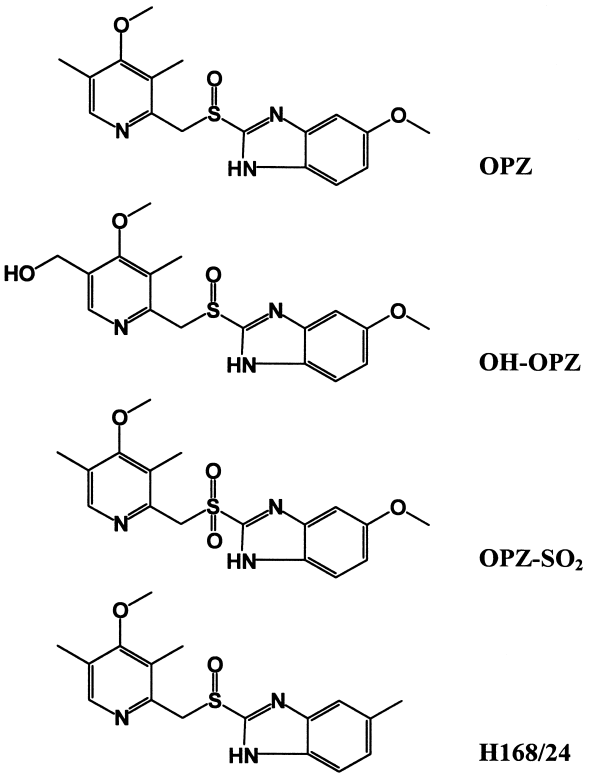

Figure 1. Structural formulae of omeprazole, 5'-hydroxyomeprazole, omeprazole sulfone, and internal standard (H168/24).

It has been demonstrated that the ratio of omeprazole to 5'-hydroxyomprazole, determined in plasma samples 2h after the administration of the drug, could be used to assign this phenotype.[4,5] For this purpose, it is important to be able to simultaneously determine omeprazole and its major metabolite, 5'-hydroxyomprazole, in plasma samples. Furthermore, the determination of omeprazole, and its major metabolites, OH-OPZ and OPZ-SO_2, confers a maximum of information on the pharmacokinetics and metabolism of this drug.

Several HPLC methods have been reported for the determination of both omeprazole and 5'-hydroxyomeprazole in human plasma. However, in most of these studies, the liquid-liquid extraction is applied.[6–9] Liquid-liquid extractions are gradually being replaced by solid phase extraction (SPE) because this latter method is faster, more straight forward, solvent saving, avoids the manipulation of toxic solvents, and is often more reproducible. One recent article describes a (SPE) method for the determination of both substances by HPLC-MS-MS.[10] Although the HPLC-MS-MS method generates good results, this apparatus is extremely expensive and is not widespread. A simple HPLC apparatus coupled with a diode array detector (DAD) is common in most laboratories and a technique developed on such a system could be of better use.

The purpose of this paper is to describe an HPLC method for the determination of OPZ in human plasma using a SPE technique, that is applied to a DAD detector. The DAD enables detection of 2 wavelengths simultaneously. Therefore, the sensitivity of detection of the internal standard is not limited by its absorption at 302 nm (maximum absorbance of omeprazole).

EXPERIMENTAL

Equipment

The method was developed on an 1100 Series HPLC System (Agilent Technologies, Kirkland, QC, Canada). This system is composed of a quaternary pump, a vacuum degasser, a standard autosampler, a thermostatted column compartment, and a diode array detector. The data analysis was performed using the HP ChemStation software, version A.06.03 (Hewlett Packard, Kirkland, Qc, Canada).

Reagents

Omeprazole, 5'-hydroxyomeprazole and internal standard (H168/24) were provided by Astra Hässle (Mölnal, Sueden). For the mobile phase, acetonitrile was purchased from V.W.R. Scientific Ltee (Montreal, Quebec, Canada), HPLC water was from Moquin scientific Inc. (Lachenaie, Quebec, Canada), potassium

dihydrogen orthophosphate and di-sodium hydrogen orthophosphate were from BDH (Toronto, On, Canada). Oasis SPE columns (60 mg sorbent bed) were purchased from Waters (Milford, MA, USA). Solvents and chemicals were all of analytical grade.

Chromatographic Conditions

The mobile phase consisted of 23% acetonitrile and 77% phosphate buffer pH 8.4 (30.4mM Na_2HPO_4 and 1.76mM KH_2PO_4 aqueous solution), in which a gradient elution was used to linearly change solvent composition to 33% acetonitrile during the first minute. The composition was kept constant thereafter at 33% acetonitrile and 67% phosphate buffer.

The system was allowed to stabilize at 23% acetonitrile for 3 minutes prior to each run. The mobile phase was filtered through a 0.45 μm membrane from Gelma Sciences (Ann Arbor, Michigan, USA) and delivered at a flow rate of 1mL/min. Separation was carried out at 25°C on an Eclipse XDB-C8 Rapid Resolution 4.6 x 50 mm, 3.5 μm particle size column, coupled with a Zorbax XDB-C18 4.6 x 12.5 mm, 3.5 μm guard column (Hewlett Packard, Kirkland, Canada).

Calibration and Reagent Solutions

A stock solution of omeprazole was prepared at a concentration of 100 μg/mL by dissolving 20 mg of omeprazole in 40 mL of methanol in a 200 mL flask and by filling the flask to volume with carbonate buffer pH 11.4. Standards for calibration function in plasma (40, 10, 8, 4, 2, 1, 0.4, and 0.2 μg/mL) were prepared by serial dilution of the 100 μg/mL stock solutions. Solutions were stored at 4°C for several months without deterioration.

A single standard solution of 5'-hydroxyomeprazole was prepared in order to adequately identify its retention time and separate it from omeprazole. The quantification of this metabolite was not the aim of this study.

Analysis standards were prepared by adding 25 μL of each working standard, along with 50 μL of the internal standard solution (2 μg/mL), to 500 μL of drug-free plasma. These standards allowed calculating a calibration function in a range of concentrations from 10 to 2000 ng/mL for plasma concentrations.

Sample Preparation: Solid Phase Extraction (SPE)

The frozen plasma was first allowed to thaw at room temperature and was mixed and centrifuged. An aliquot of 500 μL of the clinical plasma was trans-

ferred to a disposable glass culture tube. To this sample was first added 50 μL of the internal standard solution, 25 μL of carbonate buffer pH 11.4, and 250 μL of phosphate buffer pH 7.4. The samples were vortexed before being loaded onto an SPE column, which had previously been conditioned by passing 1 mL of methanol followed by 1 mL of water.

The culture tubes and columns were washed with 2×375 μL of 10% methanol in water. The columns were then dried under vacuum for 15 minutes after which 2 mL of methanol was added and allowed to pass through the column without vacuum.

The eluate was recovered in a clean glass culture tube and evaporated under a stream of nitrogen. Finally, the samples were reconstituted in 250 μL of phosphate buffer pH 7.4 and a 75 μL aliquot was injected into the HPLC system for analysis.

Analytical Parameters

Extraction recoveries of omeprazole from plasma samples were evaluated by comparing peak area ratio (omeprazole/internal standard), with and without extraction. For the extracted sample, the internal standard was added after the extraction step. Plasma standard samples (10, 20, 50, 100, 200, 400, 500, 2000 ng/mL) were analysed in triplicate on three separate days. The within-day variability and inter-day variability were determined from these data.

Calibration curves were constructed by plotting peak area ratios (omeprazole/internal standard) versus standard concentrations and the best relationship was determined by linear least-squares regression analysis. Sequentially diluted solutions of omeprazole and the internal standard were injected onto the HPLC system to estimate the minimum amount detectable with a signal-to-noise ratio of 3 (limit of detection, LOD). The limit of quantification (LOQ) was set as the lowest concentration of the calibration function.

RESULTS AND DISCUSSION

The analytical method was modified from that of Lagerström et al., Kobayashi et al., and Macek et al. as they used liquid-liquid extraction from 1 mL plasma samples. Furthermore, Amantea et al. and Mihaly et al. failed to identify the 5'-hydroxyomeprazle metabolite. Our method is the only one combining a solid-phase extraction and diode array detection for the determination of omeprazole and its separation from the major metabolites, 5'-hydroxy-omeprazole and omeprazole sulfone.

Representative chromatograms of extracts obtained from drug-free plasma, plasma spiked with 200 ng/mL each of omeprazole and the internal standard, and

a subject's plasma sample in which omeprazole, its metabolites and the internal standard are apparent, are presented in Figure 2. The total run time was 4 minutes and showed good resolution of omeprazole from its metabolites.

Retention times for 5'-hydroxyomeprazole, omeprazole and the internal standard were 1.8 min, 2.98 min, and 3.7 min, respectively. The peak apparent at

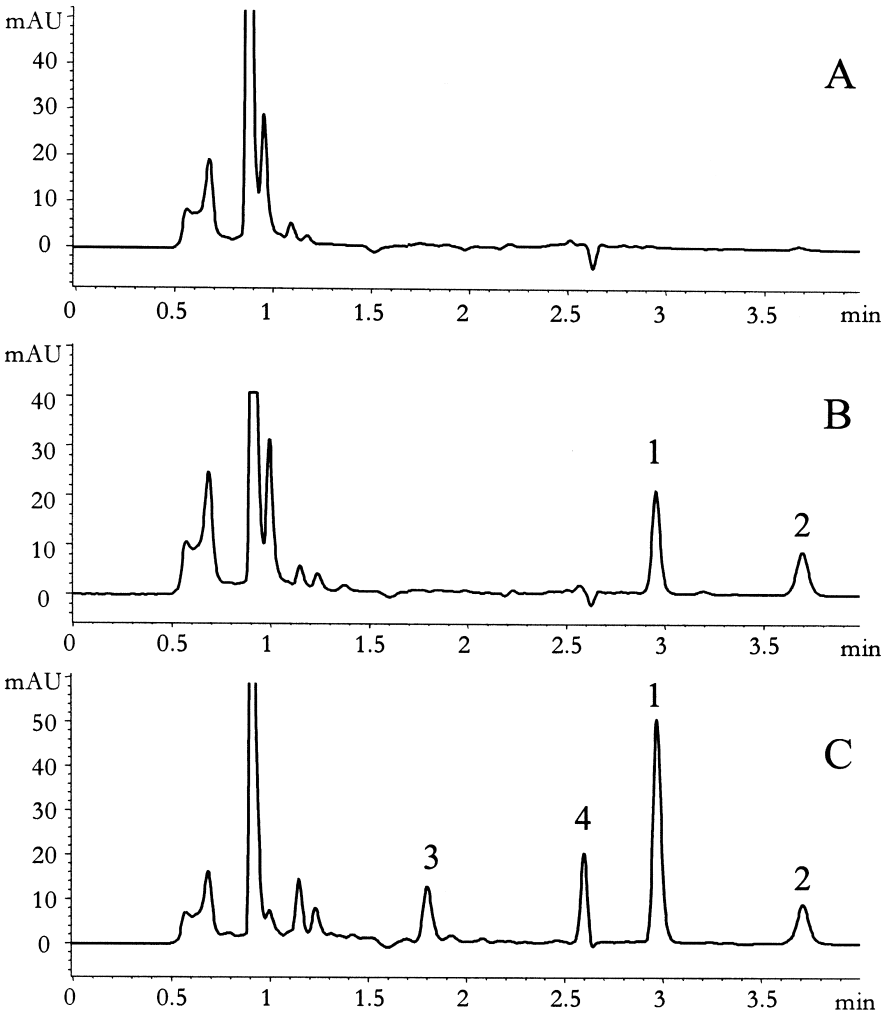

Figure 2. Chromatograms monitored at 302 nm of (A) blank pooled human plasma, (B) pooled human plasma spiked with omeprazole and internal standard at concentration of 200ng/mL, and (C) subject's plasma 3h after oral administration of 20mg omeprazole. 1 = OPZ, 2 = H168/24, 3 = OH-OPZ, and 4 = OPZ-SO_2.

2.6 min is identified as the sulfone metabolite of omeprazole; however, its quantification is not the concern of this article. The identification of this metabolite was based on its appearance as a quantitatively major metabolite product, as well as similarity of relative retention times to previously published reports in which reference standards of omeprazole sulfone were available.[6,11]

The calibration curve of omeprazole responded to the following equation: $y = 0.0066\ x - 0.0168$ $(R^2 = 0.999)$. The detector response showed good linearity for quantification of omeprazole in the studied range of concentration. The LOD was evaluated at 3 ng/mL, while the LOQ was set at 10 ng/mL. For the within-day assay variability (n=3), the coefficient of variation (C.V.%) was always lower than 1% over the entire range of concentrations. For the inter-day variability (n=3), C.V.% was always lower than 5% over the entire range of concentration.

The recovery experiments demonstrated that the solid phase extraction is an efficient extraction procedure. Omeprazole was recovered, as an average over

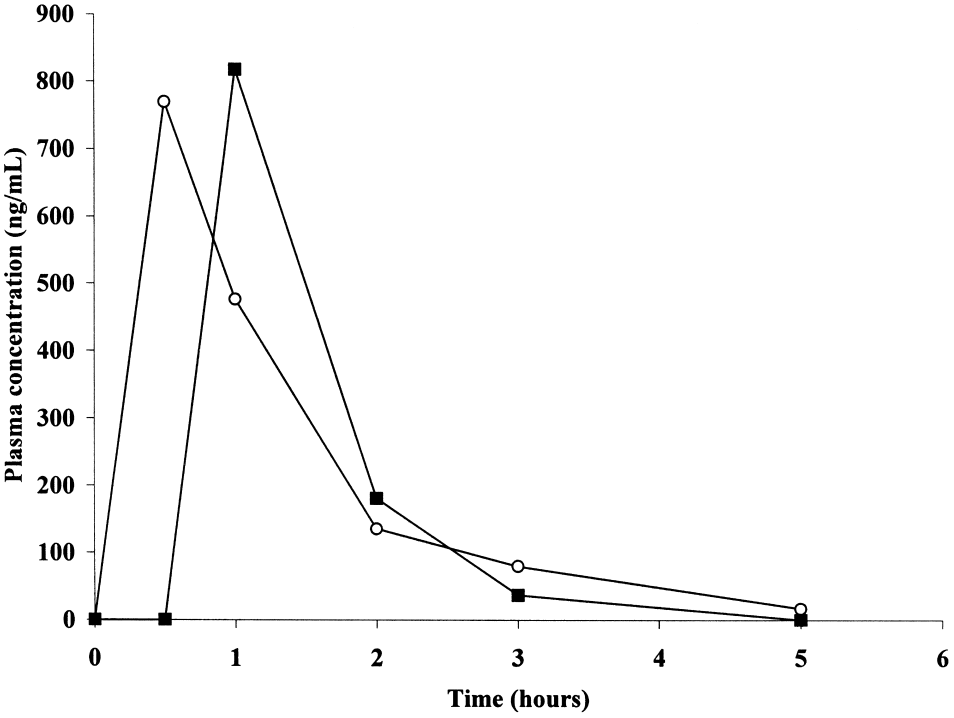

Figure 3. Plasma omeprazole concentration versus time profiles in 2 children after administration of 20mg and 15mg doses at steady state, respectively. ■ = Patient 1, age 10; ○ = Patient 2, age 5.

the entire concentration range, at 94.5 ± 5.1 %. The efficacy of the recovery was not dependent on the OPZ concentration present in the samples. The accuracy of the assay was investigated using a 300 ng/mL standard. The mean ± SD (C.V.%) was 301.74 ± 2.19 (0.73%) for this spiked plasma sample.

Application

This analytical method has been applied to clinical plasma samples collected from a pharmacokinetic study in 2 children receiving omeprazole daily. Plasma omeprazole concentrations versus time profiles from these subjects are presented in Figure 3. Patient 1 was 10 years old and received a dose of 20 mg omeprazole orally. Patient 2, 5 years old, received 15 mg omeprazole orally. 1 mL blood samples were drawn before the drug was administered and 0.5, 1, 2, 3, and 5 hours after the dose was administered. Because only a small quantity of plasma (500 μL) is necessary for this method, it is applicable for studies in pediatric populations without difficulty.

This paper describes a sensitive, specific, rapid, and solvent saving (SPE versus liquid-liquid extraction) HPLC-DAD method for the determination of omeprazole, 5'-hydroxyomeprazole, and omeprazole sulfone, and for the quantification of omeprazole in human plasma samples. This method is applicable to the determination of omeprazole quantification in the case of pharmacokinetic studies.

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