Simultaneous Determination of Voriconazole and Posaconazole Concentrations in Human Plasma by High-Performance Liquid Chromatography

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A simple, sensitive, and selective high-performance liquid chromatographic method for the simultaneous determination of voriconazole and posaconazole concentrations in human plasma was developed and validated. Quantitative recovery following liquid-liquid extraction with diethyl ether was achieved. Linearity ranged from 0.10 to 20.0 μg/ml for voriconazole and from 0.05 to 10.0 μg/ml for posaconazole. The intra- and interday coefficients of variation were less than 8.5%, and the lower limits of quantitation were <0.05 μg/ml.

Based on the increasing number of immunosuppressed patients, a rising incidence of Aspergillus infections has been observed (15, 18). Voriconazole (VRC) and posaconazole (PSC), two broad-spectrum triazole derivatives, are the recommended antimycotics for either the treatment or the prophylaxis of invasive Aspergillus infections (4, 8). Both inhibit the cytochrome P450-dependent 14α-lanosterol demethylase, which is responsible for the synthesis of ergosterol, a key compound in the fungal cell membrane (19). VRC shows nonlinear pharmacokinetics in adults and is metabolized in the liver by CYP2C19, CYP3A4, and CYP2C9, resulting in a high interindividual variability of plasma levels (5). In contrast, PSC underlies no phase I metabolism but inhibits CYP3A4 (21). Currently, PSC is only available as an oral solution, and resorption depends strongly on gastric pH and nutrition. In order to manage possible drug interactions, to balance interindividual pharmacokinetic variability, and to ensure an effective exposure to VRC and PSC, therapeutic drug monitoring is recommended (16).

Several methods for quantitation of VRC or PSC in human plasma by high-performance liquid chromatography (HPLC) have been reported (3, 6, 7, 9–14, 17, 20). Up to now, only one HPLC assay has been published for their simultaneous determination (1). This assay uses complex compositions of extractant and eluent, as well as high volumes of eluent; requires a long period of sample preparation; and is of poor sensitivity. Therefore, the aim of this work was to develop a rapid, sensitive, and economical HPLC method for the simultaneous determination of VRC and PSC in human plasma samples.

VRC was provided by Pfizer (New York, NY) and PSC by Schering-Plough (Kenilworth, NJ). The internal standard (IS) quinoxaline and bovine serum albumin (BSA) powder were obtained from Sigma-Aldrich (Steinheim, Germany). Stock solutions of VRC (50.0 μg/ml) and PSC (25.0 μg/ml) were prepared in methanol and were diluted for the preparation of six combined working solutions (for VRC, 0.25, 0.50, 1.0, 2.0, 5.0, and 10.0 μg/ml, and for PSC, 0.125, 0.25, 0.5, 1.0, 2.5, and 5.0 μg/ml) and three combined quality control (QC) samples (for VRC, 0.50, 2.0, and 5.0 μg/ml, and for PSC, 0.50, 2.5, and 5.0 μg/ml). The IS working solution was prepared in methanol (20.0 μg/ml). Each solution was stored at −20°C. For the preparation of plasma standard samples, BSA solutions (5%, wt/vol) were spiked with VRC, PSC, and IS (0.80 μg/ml) to obtain the above-mentioned concentrations.

Five-hundred-microliter aliquots of BSA standards or plasma samples were mixed with 200 μl of 0.1 M sodium hydroxide (Merck, Darmstadt, Germany) in 10-ml glass tubes, IS (20 μl) was added, and the solutions were briefly vortexed. After the tubes were capped, samples were extracted twice with 3 ml of diethyl ether (Merck, Darmstadt, Germany) for 5 min, followed by centrifugation at 5,000 × g for 5 min. The organic layers were transferred into glass tubes and evaporated to dryness (37°C) under a gentle stream of nitrogen. The residue was dissolved with 250 μl of the mobile phase.

The HPLC system (Beckman-Coulter, Krefeld, Germany) consisted of a 126 solvent pump, a 168 UV-VIS photodiode array detector, a 508 autosampler, and 32 Karat software. A ReproSil-Pur Basic C18 column (150 mm by 2 mm by 5 μm) (Dr. Maisch GmbH, Ammerbuch, Germany) protected by a C18 guard column (4 mm by 2 mm; Phenomenex, Aschaffenburg, Germany) was used. The mobile phase consisted of 0.09 M aqueous ammonium phosphate monobasic (Riedel-de-Haën, Seelze, Germany) and acetonitrile (Merck, Darmstadt, Germany) (50%:50%, vol/vol) (pH 5.3). The flow rate was 0.2 ml/min, detection was at 260 nm, and the injected volume was 50 μl.

Representative HPLC chromatograms are shown in Fig. 1. Retention times for VRC, PSC, and IS were approximately 4.90, 14.50, and 7.50 min. No interfering endogenous peaks were detectable in the blank sample.

Linearity was evaluated over a concentration range of 0.10

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to 20.0 μg/ml for VRC and 0.05 to 10.0 μg/ml for PSC. Using the ratios of observed peak heights for each analyte and IS, the calibration curves showed a correlation coefficient ($r^2$) of 0.999 for all compounds. The limit of detection, defined as the lowest detectable concentration level resulting in a signal-to-noise ratio of three (2), was determined at 12.5 and 6.25 ng/ml for VRC and PSC, respectively. The lower limit of quantitation was established for both analytes at concentrations below 0.05 μg/ml, and the upper limits of quantitation were arbitrarily set at 50.0 and 25.0 μg/ml.

The intraday accuracy and precision of the method were determined by measuring nine replicates of each QC concentration on the same day. For interday accuracy and precision, the procedure was repeated on seven days. The intraday precision values of VRC and PSC were 2.22 to 4.24% and 2.11 to 8.46%, respectively. The interday precision values of the corresponding compounds were below 4.0% and 5.9% (Table 1).

The levels of recovery of analytes and IS were estimated by comparing the peak heights of extracted QC samples with those of unextracted standard solutions. The highest recovery values achieved were 94.4% for VRC, 101.3% for PSC, and 100.3% for IS.

To assess the selectivity of the method, drug-free BSA solutions were spiked with therapeutic concentrations of ceftazidime, ceftriaxone, ciprofloxacin, cotrimazole, cyclosporine, dimenhydrinate, levofloxacin, meropenem, metronidazole, pantoprazole, piperacillin, ranitidine, sulbactam, and vancomycin; no interference was observed.

Stability studies in spiked BSA solutions were performed with QC samples (four replicates/concentration). The analytes were stable after 7 days of storage at $-20^\circ$C, including three freeze-thaw cycles; 30 days at $-20^\circ$C; 7 days at 4°C; 24 h at room temperature; and 60 min at 56°C, with coefficients of variation for VRC and PSC of less than 7.6% and less than 3.4%, respectively.

Concentrations in plasma samples ($n = 935$) from 189 patients treated with VRC (400 mg/day) or PSC (600 to 800 mg/day) were taken as trough levels. In 53 samples, both PSC and VRC concentrations could be analyzed simultaneously. The VRC and PSC concentrations determined ranged from undetectable to 13.70 μg/ml and 6.53 μg/ml, respectively (Fig. 1).

We present a valid, selective, and reliable assay for the

![FIG. 1. Representative HPLC chromatograms (at 260 nm) of VRC, PSC, and IS in plasma samples.](http://aac.asm.org/)
simultaneous determination of VRC and PSC in human plasma. Although the two drugs are not directly combined in the treatment of fungal infections, our simple and rapid method should be very useful in a laboratory hospital as it is time and cost effective.

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