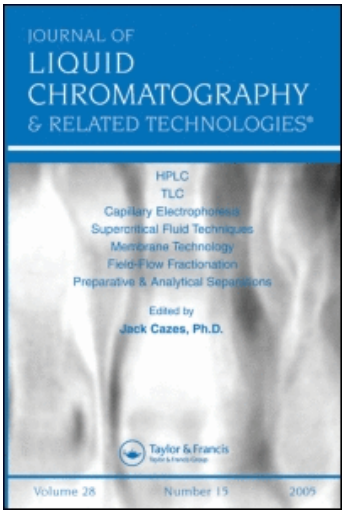

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Simple Assay for Voriconazole in Serum by HPLC

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Simple Assay for Voriconazole in Serum by HPLC

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Abstract: A simple method for analysis of the antifungal drug voriconazole in serum is described. It utilizes a short cyano cartridge column, isocratic separation, and direct serum deproteinization with a mixture of trichloroacetic acid (TCA) and methanol. The advantages of this method are the simplicity and the low concentration of the organic solvent in the mobile phase.

Keywords: Voriconazole, Antifungal, Therapeutic drug monitoring

INTRODUCTION

Voriconazole is a recent systemic broad spectrum antifungal drug indicated for the treatment of many fungal infections including invasive aspergillosis, esophageal candidiasis, and fungal infections caused by the *Scedosporium apiospermum* and *Fusarium* species. Fungal plasma membranes are similar to mammalian plasma membranes, but differing in having the non-polar sterol ergosterol, rather than cholesterol, as the principal sterol.[1] Voriconazole affects the formation of the fungal plasma membrane by indirectly inhibiting the biosynthesis of ergosterol, which affects the structure and fluidity of the membrane.[1] This results in plasma membrane permeability changes and inhibition of growth. Voriconazole is metabolized by the human hepatic cytochrome P450 enzymes. The drug protein binding is close to 58% and the volume of distribution at steady state for voriconazole is estimated to be

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4.6 L/kg. The pharmacokinetics of voriconazole is non-linear due to saturation of its metabolism. As a result of that, the half-life of the drug is dose dependent (~4–16 hrs).

The inter-individual variability of voriconazole pharmacokinetics is high. The maximum voriconazole plasma concentration in individual patients was ~1 to 6 μg/mL,[1] with trough levels being one third less. Increased incidences of liver function test abnormalities and visual disturbances can be associated with higher plasma concentrations and/or doses. The most frequently reported adverse events in the therapeutic trials were visual disturbances, fever, rash, vomiting, nausea, diarrhea, headache, sepsis, peripheral edema, abdominal pain, and respiratory disorder. The treatment related adverse events, which most often led to discontinuation of voriconazole therapy were elevated liver function tests, rash, and visual disturbances.

Voriconazole has been quantified in plasma by classical microbiological bioassays[2] and by liquid chromatography with ultraviolet (UV) detection,[3–6] or MS detection.[7–9] The bioassay, which measures activity rather than a quantity, is not suitable for patients receiving comedication and is inferior to chromatographic methods in terms of precision and accuracy. Capillary electrophoresis has been used for the determination of voriconazole in solutions, tablets, and in human liver microsomal extracts but not in serum.[10,11] The CE, in general, lacks the sensitivity to detect the low levels of this drug in serum.

Previous HPLC methods used solvent extraction and C_{18} long columns to study the pharmacokinetics of this drug. For routine therapeutic monitoring, a rapid and simple method, which avoids consumption of large amounts of organic solvents, is desirable. The organic solvents in addition to being expensive are very difficult to dispose of in most of the states. Cyano columns packed with small particles (<5 μm) offer several advantages for routine analysis such as speed, low pressure, good sensitivity, and low cost of the column. However, the main advantage is decreasing the organic solvent by one third to one half. The aim of this study is to check if a short CN cartridge column can give adequate separation for voriconazole in serum. Here we show by choosing the appropriate conditions of analysis such a column can be used for routine monitoring of the antifungal drug voriconazole.

EXPERIMENTAL

Chemicals

Voriconazole was a gift from Pfizer Pharmaceuticals, New York, NY. Acetonitrile (HPLC grade) was purchased from Burdick and Jackson, Muskegon, MI, methanol from J.T. Baker, Philipsburg, NJ, and phosphoric acid from Fisher Scientific, Pittsburgh, PA.

Instrument

A pump Model 110 A (Beckman Instruments, Fullerton, CA) was set at 0.9 mL/min. The detector, Model 440 (Waters Associates, Milford, MA) was set at 254 nm and 5 mAU full scale. The pump solvent was 15% acetonitrile containing 0.2% phosphoric acid and 0.1% n-butylamine. A cartridge column Nova-Pak CN-HP 100 × 3.9 mm, CN, 4 μm average particle size was used (Waters Associates).

Method

Serum 50 μL was vortex mixed with 50 μL of methanol and 50 μL trichloroacetic acid 5% and centrifuged at 13,000 × g for 40 s. An aliquot of 20 μL of the supernatant was injected on the column.

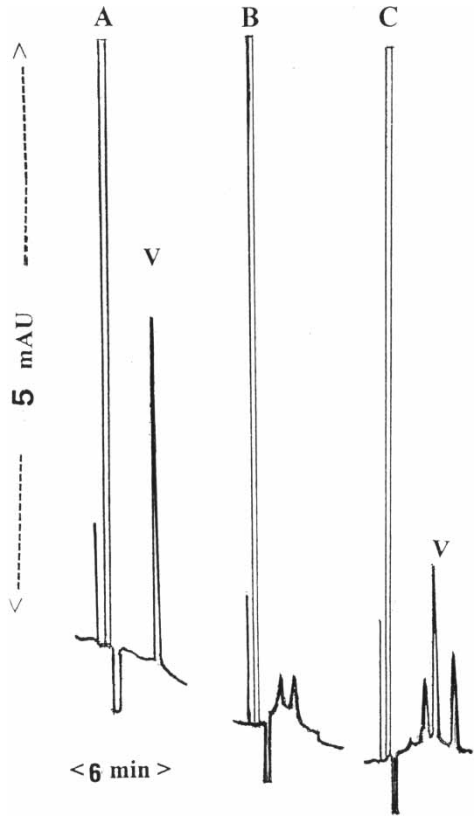

Figure 1. A-standard, 5 mg/L of voriconazole (V); B-serum from a patient not receiving the drug; and C-a serum of a patient receiving the drug (2.5 mg/L).

RESULTS AND DISCUSSION

Voriconazole is characterized by a non-linear metabolic elimination and a high bioavailability when given orally. In addition to that, the drug exposure increases with repeated administration. Thus, monitoring serum level is important to ensure adequate dosing while avoiding toxicity.

Figure 1 shows that the drug can be detected after simple deproteinization by methanol-acetonitrile with rapid elution of about 6 min. The chromatograms are relatively clean without interferences, provided the pH of the eluting solvent is kept below pH 2.5; above this pH several unknown compounds interfered with analysis. Because the chromatograms are relatively clean, and in order to keep the method very simple, we did not see any practical advantage from using an internal standard.

The analysis was linear between 0.4–10.0 mg/L ($r = 0.998$) covering the therapeutic range (2–6 mg/<L). The RSD was 2.8 ($n = 10$). The minimum detection level (3X baseline noise) was 0.3 mg/L. The average of recovery of 2.5 mg/L added to serum and deproteinized with two volumes of acetonitrile only was 74.5%, while the recovery based on deproteinization with one volume of TCA 5% and one volume of methanol was 93.4% ($n = 4$). The low recovery in acetonitrile reflects the adsorption and trapping of the drug in the precipitated proteins, especially considering the pH of serum is ~7.4. The change in peak height and k′ is illustrated in Figure 2. As the pH is decreased, the peak height is increased, while the k′ decreases. Because the pKa of this compound is below 2,[12] adjusting the pH of the solvent below 2 is not practical for the sake of the column stability. Also because

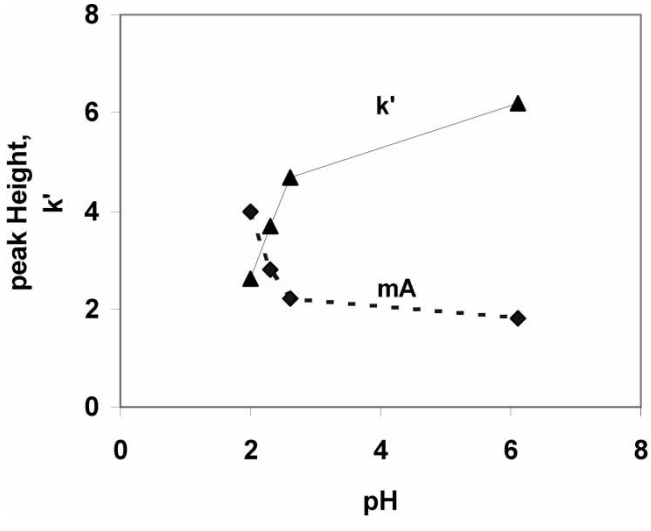

Figure 2. Capacity factor (k′) and peak height (mA) as function of the solvent pH at acetonitrile concentration of 25% in phosphate buffer 10 mmol/L.

of this low pKa the analysis of this compound is difficult by CE. In general, CN columns have the reputation of not lasting for very long. However, our experience with this particular column demonstrates that it is very stable and lasts for few hundreds of injections.

The average level of voriconazole in 15 patients on this drug was 2.6 ± 1.7, (range 0.9–7.5) mg/L, similar to previous studies.[1] We did not see any interference in these patients, provided the pH is low and the k′ is kept >5. For conservation purposes, the solvent can be recycled a few times without any problems.

CONCLUSIONS

This work illustrates that a small CN cartridge column can be used to measure voriconazole in serum without interference. Proper choice of the column, mobile phase, k′ factor, pH and the type of deproteinization solvent, all lead to a simplified assay suitable for routine analysis. The overall advantages are: speed, simplicity, and low consumption of acetonitrile.

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