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Short communication

A rapid HPLC assay for voriconazole in human plasma

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Abstract

This report describes a simple, rapid and reproducible method with a calibration range of 0.2–10 μg ml^{-1} voriconazole in human plasma which is more appropriate for routine clinical use than the authors previously published method. The method utilises protein precipitation with acetonitrile as the only sample preparation involved prior to reverse phase HPLC. No internal standard was required. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Voriconazole; Reverse phase chromatography; Protein precipitation; Human plasma

1. Introduction

Voriconazole ((2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1*H*-1,2,4-triazol-1-yl)-2-butan-2-ol) (Fig. 1) is a novel broad spectrum antifungal agent which includes cidality against *Aspergillus* species [1,2].

An existing assay method [3] with a calibration range of 10–3000 ng ml^{-1} voriconazole in human plasma has been successfully applied to the analysis of samples from several phase I clinical trials. However, this method is based on size exclusion chromatography coupled on-line with a reverse phase HPLC system with column switching and is therefore complex. The sensitivity of this method enabled the detailed pharmacokinetics of voriconazole to be determined.

This report describes a simple, rapid and reproducible method with a calibration range of 0.2–10

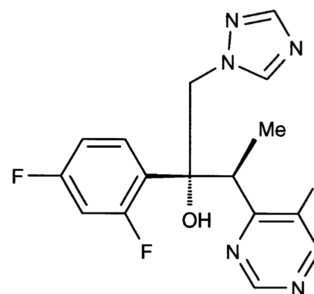

Fig. 1. Structure of voriconazole.

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μg ml^{-1} voriconazole in human plasma which is more appropriate for routine clinical use. The method utilises protein precipitation with acetonitrile as the only sample preparation involved prior to reverse phase HPLC. No internal standard was required.

2. Experimental

2.1. Chemicals

Acetonitrile and methanol (both Super Purity Solvent grade) were purchased from Romil Chemicals (Loughborough, UK). Ammonia (30%) and ammonium dihydrogen orthophosphate (both Analar grade) were purchased from Merck (BDH) (Lutterworth, UK). Ultra-pure water was provided by a Milli-Q-Plus water purification unit (Millipore, Watford, UK). Voriconazole was supplied by the Pharmacy, Pfizer Central Research.

2.2. Instrumentation

The HPLC system was constructed from the following components: a Shimadzu LC-6A pump, SPD-6A variable wavelength UV detector set at 255 nm (Dyson Instruments, Hetton, UK), and either a Shimadzu SIL-6B autoinjector with SCL-6B controller or a Gilson 232 autoinjector (Anachem, Luton, UK). Data collection and processing was carried out by a Multichrom 2.1 chromatography data system (VG Data System, Fisons Instruments, Loughborough, UK).

The analytical column was Kromasil C18, 5 μm, 250 × 4.6 mm (Hichrom, Reading, UK) with a 10 × 3.2 mm guard cartridge (Hichrom, Reading, UK) packed with the same material. The mobile phase was acetonitrile-ammonium phosphate buffer (pH 6.0; 0.04 M) (1:1 v/v) and was degassed by filtration through a 0.45 μm nylon filter under vacuum. The flow rate was 0.8 ml min^{-1} and all chromatography was carried out at ambient temperature (~ 21°C).

2.3. Sample preparation

Plasma samples were thawed and then centrifuged at 1700 × g for 5 min. An aliquot (0.5 ml) was pipetted into a 5 ml polypropylene tube (Sarstedt, Leicester, UK) and acetonitrile (0.8 ml) added. The mixture was vortex mixed briefly and after standing for 10 min at room temperature the mixture was centrifuged at 1200 × g for 5 min. Then 0.15 ml of the supernatant was transferred to a 0.3 ml polypropylene autosampler vial (Phase Separations, Queensferry, UK) and 0.1 ml injected into the HPLC system.

Stock solutions of voriconazole (~ 1 mg ml^{-1}) were prepared in methanol and diluted in ammonium phosphate buffer (pH 6.0; 0.04 M) to give 100 μg ml^{-1}. Standards were prepared by adding the diluted voriconazole solution (1–50 μl) to appropriate volumes of plasma to give a total volume of 0.5 ml and concentrations of 0.2, 0.6, 2, 4, 6, 8 and 10 μg ml^{-1}. Calibration curves were constructed by plotting the peak area of voriconazole against concentration using a weighted ($1/Y^2$) least squares regression in the Multichrom data system.

2.4. Accuracy and precision

The intra-run accuracy and precision in human plasma were assessed by performing replicate analyses of samples fortified with voriconazole at 0.05, 0.1. 0.2, 2 and 10 μg ml^{-1}. Where 0.05 and 0.1 μg ml^{-1} samples were assayed, the calibration line was extended with 0.05 and 0.1 μg ml^{-1} standards as appropriate to avoid extrapolation. The inter-run accuracy and precision were determined from the back calculated concentrations for the standards used to construct four different calibration curves in separate runs. Precision was calculated as the relative standard deviation (R.S.D.) and the accuracy (%) as 100% + 100% × (Found concentration − Prepared concentration)/Prepared concentration.

2.5. Selectivity

Six different batches of human plasma were analysed to check for interference from endoge-

Table 1
Intra-run accuracy and precision for voriconazole added to human plasma

Voriconazole concentration (μg ml^{-1})		n	Accuracy (%)	Precision (R.S.D., %)
Prepared	Found (mean)			
0.05[a]	0.0496	6	99.2	13
0.1[b]	0.0970	6	97.0	5.8
0.2	0.203	7	102	7.2
2	2.02	7	101	6.0
10	10.5	7	105	2.6

[a] Calibration line was extended by the addition of 0.1 and 0.05 μg ml^{-1} standards.
[b] Calibration line was extended by the addition of a 0.1 μg ml^{-1} standard.

nous compounds. Several drugs which may be co-administered with voriconazole were added to plasma and analysed. The drugs tested were ampicillin, aspirin, 3′-azido-3′-deoxythymidine (AZT), oxytetracycline, paracetamol, prednisolone and theophylline.

2.6. Recovery

The extraction efficiency of voriconazole from plasma was determined by comparing the peak area of voriconazole (4 μg ml^{-1}) extracted from plasma with the peak area for a similar concentration in a mixture of acetonitrile and water (8:5) injected directly to the HPLC.

3. Results and discussion

Acetonitrile precipitation of protein provided a simple method of sample preparation and has been used previously for the analysis of antifungal drugs in plasma [4,5]. Initial experiments with equal volumes of acetonitrile and plasma gave extracts which gave a rise in column back-pressure and distortion of peak shape throughout a run of approximately 70 samples. These problems were overcome by using a higher ratio of acetonitrile to plasma, 8:5, which enabled the injection of several hundred extracts per column with minimal effect on back-pressure or peak shape. Although the proportion of acetonitrile in the extracts injected was higher than in the mobile phase this had little effect on peak shape compared to injections in mobile phase. The mean ($n = 6$) recovery of voriconazole from plasma was 99% and the retention time was 7.7 min.

Calibration curves were linear over the range 0.2–10 μg ml^{-1} with mean values ($n = 4$) for slope of 48632 (S.D. 2728), intercept, 854 (S.D. 1226) and correlation coefficient, 0.998 (S.D. 0.00171). The assay was initially validated over the range 0.2–10 μg ml^{-1} and both the intra-run and inter-run accuracy values were within 6% of nominal value with precision less than 8% (Tables 1 and 2). Further validation at 0.1 and 0.05 μg ml^{-1} was performed to determine the limit of quantification (LOQ) of the assay. This is generally accepted [6–8] as a concentration at which accuracy is within $\pm 20\%$ of nominal value with an R.S.D. $\leq 20\%$. Since the accuracy and precision was within these limits at the lowest concentration tested, that concentration (0.05 μg ml^{-1}) was taken as the LOQ. At that concentration the ratio of peak height of voriconazole to noise was 10.7.

Typical chromatograms obtained for blank plasma and for plasma fortified with 0.2 and 10 μg ml^{-1} voriconazole are shown in Fig. 2A–C, respectively. The assay was selective for voriconazole as no significant peaks due to endogenous compounds were observed which would compromise the determination of voriconazole down to 0.2 μg ml^{-1} when six different samples of control plasma were analysed. Also, the compounds which may be co-administered with voriconazole did not co-chromatograph with voriconazole (potential metabolites were not checked).

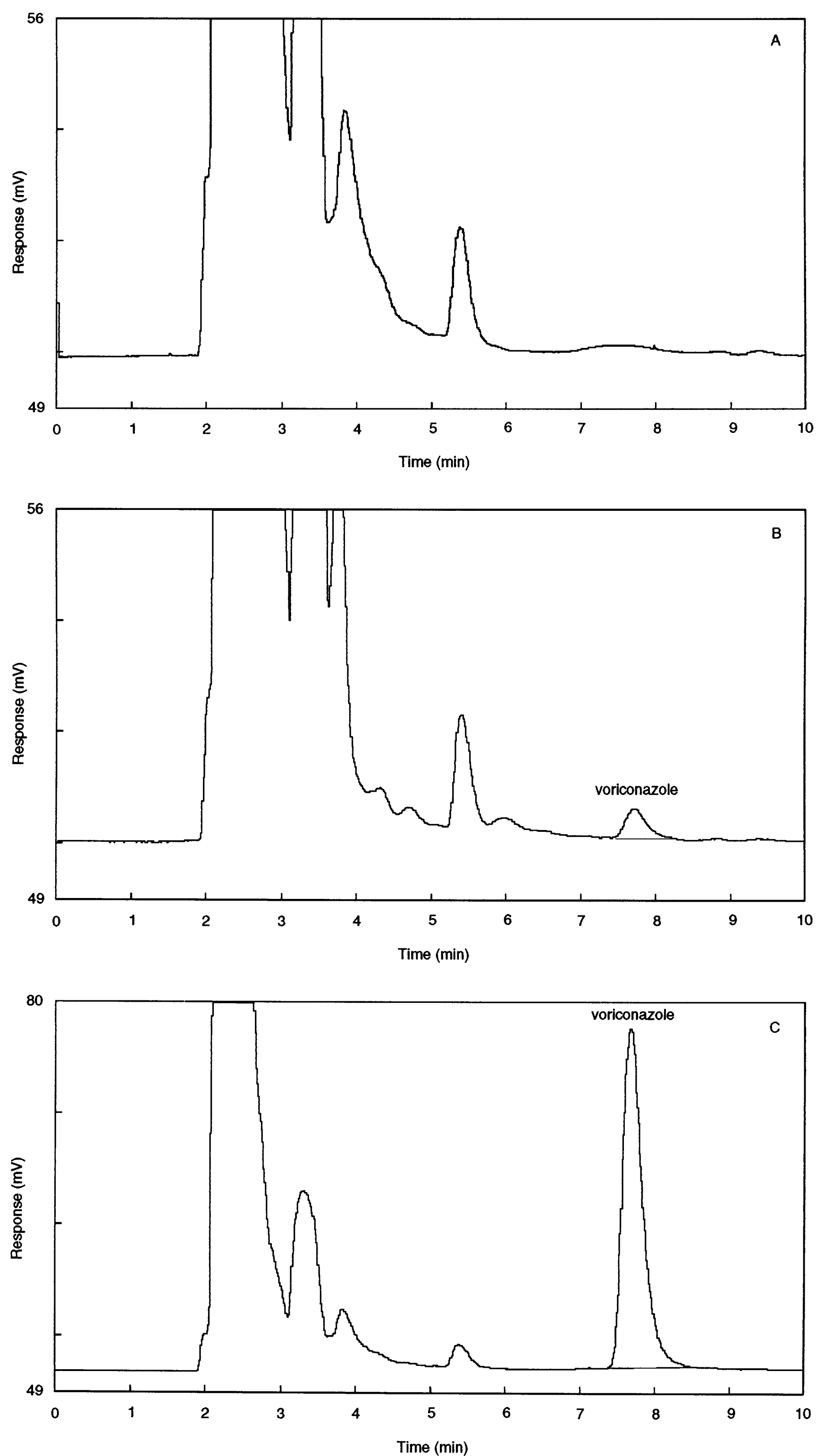

Fig. 2. Chromatograms of extracts of human plasma containing (A) no voriconazole, (B) voriconazole, 0.2 μg ml^{-1}, and (C) voriconazole, 10 μg ml^{-1}. Column was Kromasil C18, 5 μm, 250 × 4.6 mm with a 10 × 3.2 mm guard cartridge packed with the same material. Mobile phase was acetonitrile/ammonium phosphate buffer (pH 6.0; 0.04 M) (1:1 v/v), 0.8 ml min^{-1}. UV detection was at 255 nm.

Table 2
Inter-run accuracy and precision for voriconazole added to human plasma

Voriconazole concentration (μg ml^{-1})		n	Accuracy (%)	Precision (R.S.D., %)
Prepared	Found (mean)			
0.2	0.200	4	100	0.75
0.6	0.599	4	99.8	2.0
2	1.97	4	98.7	1.8
4	3.99	4	99.7	1.3
6	6.19	4	103	4.7
8	7.76	4	97.0	3.1
10	10.3	4	103	3.1

It was noticed that when the Gilson 232 autosampler was used to inject samples of buffer fortified with voriconazole, some voriconazole would be bound in the autoinjector and later released by subsequent injections of plasma extracts. The validation data demonstrate that this did not affect the assay performance when plasma samples were sequentially injected as would normally be the case. The binding was not observed with the Shimadzu autoinjector.

4. Conclusion

A simple, rapid and selective reverse phase HPLC method has been developed for the assay of voriconazole in human plasma over the range of 0.2–10 μg ml^{-1}.

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