Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 1313–1318

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Determination of voriconazole in human serum and plasma by micellar electrokinetic chromatography

Regula Theurillat[a], Stefan Zimmerli[b], Wolfgang Thormann[a,*]

[a] Department of Clinical Pharmacology and Visceral Research, University of Bern, Murtenstrasse 35, CH-3010 Bern, Switzerland
[b] Institute for Infectious Diseases, University of Bern, Bern, Switzerland

ARTICLE INFO

Article history:
Received 22 April 2010
Received in revised form 11 May 2010
Accepted 12 May 2010
Available online 20 May 2010

Keywords:
Capillary electrophoresis
HPLC
Therapeutic drug monitoring
Voriconazole
MEKC

ABSTRACT

The micellar electrokinetic capillary chromatography (MEKC) separation and analysis of voriconazole and UK 115794 (internal standard) were examined and an assay for determination of voriconazole in human plasma and serum was developed. The MEKC medium comprises a 2:15 (v/v) mixture of methanol and a pH 9.3 buffer composed of 5 mM $Na_2B_4O_7$, 7 mM Na_2HPO_4 and 54 mM SDS. Sample preparation is based upon liquid/liquid extraction with ethylacetate and dichloromethane (75%/25%) at physiological pH. Using this approach with 250 μl serum or plasma and reconstitution of the dried extract into 100 μl of a buffer composed of 0.5 mM $Na_2B_4O_7$ and 0.7 mM Na_2HPO_4 (pH 9.3), the detection and quantitation limits were determined to be 0.1 and 0.2 μg/ml, respectively, a sensitivity that is suitable for therapeutic drug monitoring of voriconazole (provisional therapeutic range: 1–6 μg/ml) in human plasma and serum samples. The method was validated and compared to an HPLC method, showing excellent agreement between the two for a set of 91 samples that stemmed from patients being treated with voriconazole. The MEKC assay is also demonstrated to be suitable to explore pharmacokinetic data of voriconazole.

1. Introduction

Voriconazole ((2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yl)-2-butan-2-ol, formerly known as UK-109496, for chemical structure see Fig. 1) is a recent systemic triazole drug with broad-spectrum antifungal activity which is used for prophylaxis and treatment of aspergillosis and candidiasis. It is a single stereoisomer compound which is administered orally or intravenously and represents a derivative of fluconazole. Voriconazole acts as an enzyme inhibitor blocking the synthesis of ergosterol, a constituent of fungal membranes, and thereby inhibits the growth of fungi. Voriconazole has a non-linear pharmacokinetic profile with a wide inter- and intraindividual variability. Plasma and serum levels of voriconazole vary due to gender, age, genotype and drug–drug interactions. Thus, in order to avoid drug inefficacy or toxicity, therapeutic drug monitoring (TDM) of voriconazole is often recommended. A provisional therapeutic range of 1–6 μg/ml has been suggested [1–5]. Almost no unchanged voriconazole is excreted in urine and faeces. Voriconazole is extensively metabolized via N-oxidation of the fluoropyrimidine ring (UK-121265, major metabolite formed by the liver enzymes CYP3A4, CYP2C19 and CYP2C9), hydroxylation of the fluoropyrimidine ring and methyl hydroxylation. The latter two metabolites become conjugated with glucuronic acid [6–8].

Several validated analytical methods have been developed for the analysis of voriconazole in human plasma and serum. These are based on high-performance liquid chromatography (HPLC) with UV absorbance [9–12] or mass spectrometry (MS) [13,14] detection and using protein precipitation [10,14], liquid/liquid extraction [12], solid-phase extraction [11] or column switching [9,13] for sample preparation. Voriconazole assays with UV absorption detection offer adequate sensitivity and specificity for TDM and are cheaper to operate compared to those with MS detection. Capillary electrophoresis (CE) is a complementary technique to HPLC, often providing substantially different selectivity due to the different separation mechanism involved. It can also provide higher separation efficiencies, lower sample and reagent requirements, and quicker analysis times. CE has been used extensively to analyze drugs and their metabolites in biological fluids [15–18]. To date, there have been four reports on the separation of voriconazole by CE, the first two dealing with the separation of voriconazole and its stereoisomers by cyclodextrin modified micellar electrokinetic capillary chromatography (MEKC) at pH 7.0 [19,20] and two reporting the separation of voriconazole and other antifungals by capillary zone electrophoresis using acidic buffers [21,22] where voriconazole bears a partly positive charge (pKa = 1.76 [23]). Voriconazole is neutral over the majority of the pH range and

Abbreviations: CE, capillary electrophoresis; HPLC, high-performance liquid chromatography; IST, internal standard; MEKC, micellar electrokinetic capillary chromatography; MS, mass spectrometry; TDM, therapeutic drug monitoring.

* Corresponding author. Tel.: +41 31 632 3288; fax: +41 31 632 4997.
E-mail address: wolfgang.thormann@ikp.unibe.ch (W. Thormann).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.05.015

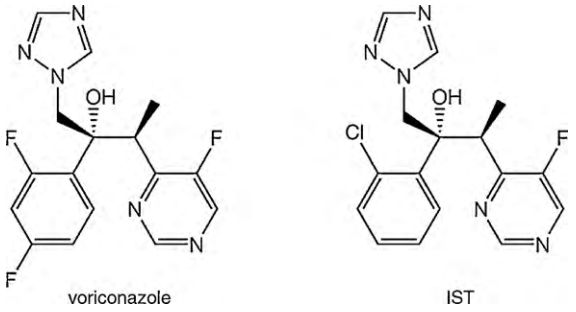

Fig. 1. Chemical structures of voriconazole and the internal standard.

is moderately hydrophobic (octanol/water partition coefficient: log P = 1.8 [6]). Thus, it is an ideal candidate for MEKC with dodecylsulfate micelles. MEKC comprises two distinct phases which migrate at different velocities thereby permitting chromatographic separations of neutral and charged compounds in a capillary configuration with a longitudinal electric field [15,24,25]. No CE-based assay for voriconazole in plasma or serum has been described thus far. However, MEKC has been used to determine other antifungal drugs in serum and plasma, including fluconazole [26], itraconazole [27] and flucytosine [28], as well as many other compounds and some of their metabolites [15–18,29,30]. MEKC analyses are often quicker than those by HPLC and in some cases can minimize or even eliminate sample pretreatment [28,29].

Described in this paper are (i) the separation of voriconazole and UK 115794 (used as internal standard (IST), for structure see Fig. 1) by MEKC containing sodium dodecylsulfate (SDS) and (ii) a validated MEKC assay for the determination of voriconazole in human serum and plasma. Sample pretreatment involves liquid–liquid extraction with a mixture of ethylacetate and dichloromethane. Patient data obtained with the MEKC assay are compared to those from the HPLC method used for TDM in our departmental drug assay laboratory.

2. Materials and methods

2.1. Drugs, chemicals, origin of samples and solutions

Voriconazole and UK 115794 (IST) were kindly obtained from Pfizer (Zürich, Switzerland). Sodium tetraborate (borax) was obtained from Hänseler (Herisau, Switzerland), Na_2HPO_4 was from Merck (Darmstadt, Germany), SDS and ethylacetate were from Fluka (Buchs, Switzerland) and acetonitrile, methanol and dichloromethane were from Prolabo (Leuven, Belgium). Patient plasma and serum samples were from the departmental drug assay laboratory where they were received for TDM of voriconazole by HPLC. The serum samples used to evaluate the pharmacokinetics after a single p.o. dose of 400 mg voriconazole (two 200 mg tablets of Vfend, Pfizer) stemmed from a healthy volunteer with a body mass of 73 kg who gave his consent and whose blood samples were withdrawn over a 24 h period. Blank bovine plasma was obtained from the local abattoir and was used for the preparation of calibration and control samples. Stock solutions of 1000 μg/ml of voriconazole and IST were prepared in methanol, and diluted with water as required. Calibrator and control samples were prepared with bovine plasma that was spiked with aliquots of diluted voriconazole stock solutions.

2.2. Capillary electrophoresis

If not stated otherwise, MEKC experiments were performed on a Beckman P/ACE 5510 CE system (Beckman Coulter, Fullerton, USA) with a Beckman P/ACE station version 1.1 used for data acquisition and using fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 75 μm I.D. with a length of 37.0 cm (30.0 cm to detector) and a voltage of 17–18 kV (current of about 62–65 μA for the pH 9.3 buffer). Sample was injected for 5 s at low pressure (about 0.8 cm sample plug length). Detection was performed using UV absorbance at 200 nm with the capillary temperature set at 20 °C. After method development, selected experiments were also made on a P800 capillary electrophoresis analyzer (Beckman Coulter) employing a 75 μm I.D. capillary of 40 cm total length (30.0 cm to detector), an injection of 5 s at 0.5 psi, a voltage of 18 kV and a capillary cartridge temperature of 25 °C. If not stated otherwise, the separation medium comprised a 2:15 (v/v) mixture of methanol and a buffer composed of 5 mM $Na_2B_4O_7$, 7 mM Na_2HPO_4 (pH 9.3) and 54 mM SDS. New capillaries were etched with 1 M NaOH for 30 min. Capillaries were conditioned daily by sequential rinsing with 1 M NaOH, 0.1 mM NaOH, water and buffer (5 min each). Between separations, the capillary was rinsed with 0.1 M NaOH and water (0.5 min each) followed by buffer for 1 min. At the end of a measuring day, the capillary was rinsed with water for 10 min.

2.3. Sample pretreatment and quantification

For liquid/liquid extraction, 250 μl of sample (blank, spiked bovine plasma, and patient samples) was mixed with 20 μl of 40 μg/ml IST solution and 1.25 ml of a mixture of ethylacetate and dichloromethane (75%/25%) in a 2 ml Eppendorf vial, shaken for 10 min on a laboratory shaker (dial 6, Gerhardt LS 20, Königswinter, Germany), and centrifuged at 15,000 × g for 5 min. The clear supernatant was decanted into a new Eppendorf vial, evaporated under a gentle air flow at 45 °C and reconstituted in 100 μl of a buffer composed of 0.5 mM $Na_2B_4O_7$ and 0.7 mM Na_2HPO_4 (pH 9.3).

For quantitation of voriconazole in serum and plasma, calibrators containing 0.2, 0.5, 1.0, 2.5, 5.0 and 7.5 μg/ml were prepared by dilution of the standard drug solution in blank bovine plasma. Control samples containing 0.6, 2.0 and 4.0 μg/ml voriconazole were prepared in the same way and stored at −20 °C until required. Quantitation of voriconazole was based upon multilevel internal calibration using relative peak areas, i.e. peak areas divided by detection time. The calibration range was 0.2–5 μg/ml (5 levels) and for the case of high values 0.2–7.5 μg/ml (6 levels).

2.4. HPLC of voriconazole

The HPLC data stem from the routine laboratory where TDM of voriconazole is performed under stringent quality control conditions with an assay that was developed in house. The system used comprises a Waters Alliance System with a model 2695 separations module and a model 2487 absorbance detector (Waters, Milford, MA, USA). Chromatography was performed at ambient temperature using an Nova Pack C18 4.6 mm × 150 mm, 4 μm column (Waters) using a mobile phase comprising a 35:65 (v/v) mixture of acetonitrile and water. The flow rate was 1.0 ml/min, detection times of voriconazole and IST were 6.6 and 8.1 min, respectively, the total run time was 15 min and detection occurred at 255 nm. The system was controlled and chromatograms were registered and evaluated with the Empower Software package (Waters). Sample preparation comprises protein precipitation employing 250 μl of plasma or serum, 25 μl of a 25 μg/ml UK 115794 (IST) solution, and 500 μl of acetonitrile. After vortex mixing and centrifugation at 18,000 × g for 5 min, the clear supernatant was decanted into a clean vial, evaporated at 45 °C under a gentle stream of air, reconstituted in 150 μl of water, vortex mixed and centrifuged for 2 min. The prepared sample was put in sample vial and 25 μl was injected. Calibration and control samples were prepared with BSA as described for MEKC. Quantitation of voriconazole was based upon five-level internal calibration using peak areas (calibration

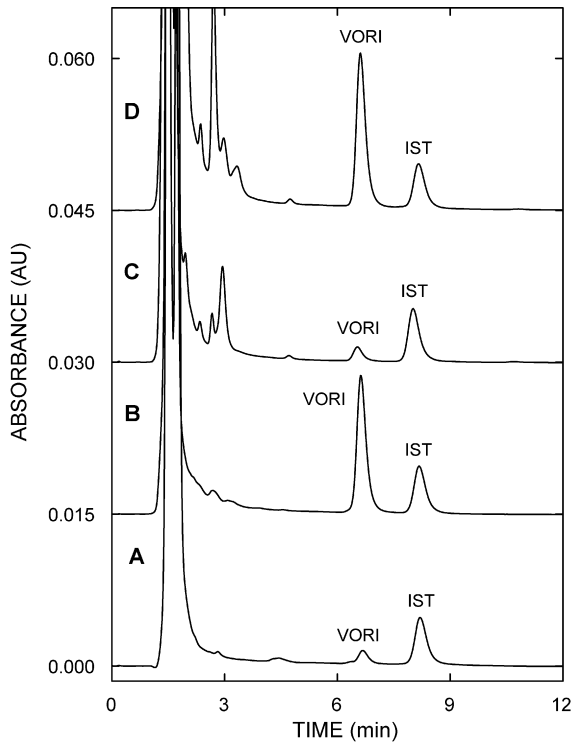

Fig. 2. Representative HPLC separations obtained with bovine plasma fortified with (A) 0.5 μg/ml and (B) 5 μg/ml voriconazole, and two patient sera containing (C) 0.45 μg/ml and (D) 6.22 μg/ml voriconazole. Other conditions as described in Section 2.4. VORI refers to voriconazole.

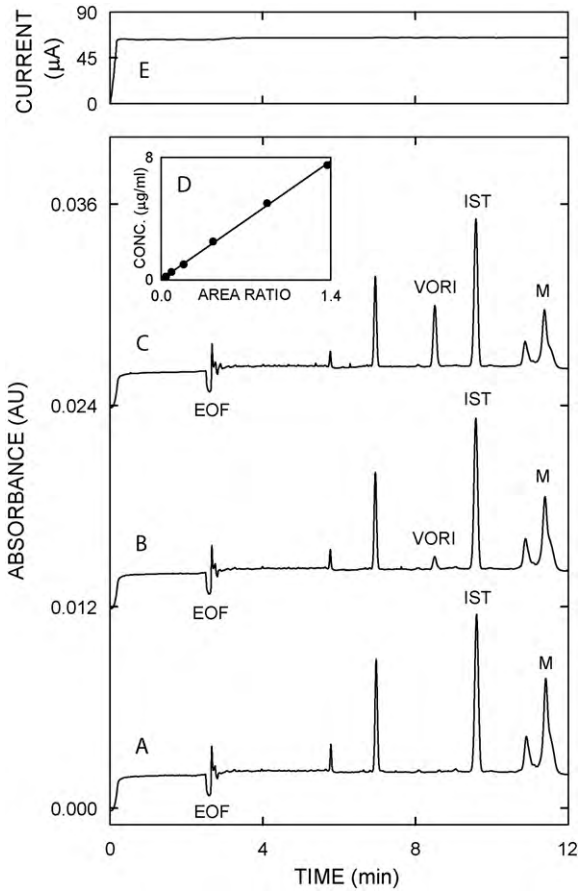

Fig. 3. Electropherograms obtained with extracts prepared from (A) bovine plasma, (B) bovine plasma spiked with 0.5 μg/ml voriconazole and (C) bovine plasma with 2.5 μg/ml voriconazole. Panels D and E depict the complete calibration graph and the temporal behavior of the current, respectively. Other conditions as described in Sections 2.2 and 2.3. VORI, EOF and M refer to voriconazole, electroosmotic flow and micelle peak, respectively.

range: 0.1–5 μg/ml). Linear calibration graphs (peak area ratio vs. concentration) were obtained ($r^2 > 0.996$, $F > 1000$) and the limit of quantification was 0.1 μg/ml. Intraday and interday precision RSD values ($n = 5$) assessed for drug levels of 0.6, 2.0 and 4.0 μg/ml were determined to be <10%. After each set of data, the column was rinsed with a 50:50 (v/v) mixture of acetonitrile and water at a flow rate of 0.5 ml/min for 1 h. Typical chromatograms obtained with two calibration and two patient samples are presented in Fig. 2.

2.5. Statistical data treatment and graphical presentations

Statistical analyses were performed using the SigmaStat for Windows version 1.0 (Jandel, Corte Madera, CA, USA). Input groups were compared with the Mann–Whitney rank sum test. Graphical presentations and non-linear regression analyses were made with SigmaPlot for Windows version 10 (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Method development and optimization

Voriconazole, a weak base with a pKa value of 1.76, is neutral over the majority of the pH range. Although it can be partly charged at low pH and thereby separated as cation [21,22], its analysis by MEKC in a dodecylsulfate-based micellar system [19,20] appeared to be more appealing to us. Preliminary experiments with standards and using a pH 9.3 buffer composed of 6 mM $Na_2B_4O_7$, 10 mM Na_2HPO_4 and 75 mM SDS, a buffer that was used previously for other MEKC drug analysis applications [15], revealed that voriconazole could be separated from the IS with almost complete resolution and the two compounds were detected shortly before the micelle peak within about 11 min (data not shown). Selectivity in MEKC is dependent on the concentration of the micelle-forming agent, the buffer pH and the use of additives, such as organic modifiers and salts [24,25]. Complete resolution and unequivocal separation of voriconazole and IST was obtained by addition of small amounts of methanol or acetonitrile (5–10% each). This, however, occurs at the expense of increased run times [15]. Acetontrile provided higher run times than methanol (about 17 vs. 15 min with a 5% content of the organic modifier). In order to avoid detection times exceeding 10 min and to keep the currents lower than 70 μA, the buffer composition was changed to 5 mM $Na_2B_4O_7$, 7 mM Na_2HPO_4 and 54 mM SDS and diluted with methanol (15 ml of buffer were combined with 2 ml methanol). With this medium comprising 11.8% methanol, voriconazole and the IST were detected after about 8.5 and 9.5 min, respectively, and the current was 62–65 μA (data not shown).

Direct serum or plasma injection as well as analysis of the supernatant obtained after protein precipitation with acetonitrile, as was used for HPLC, did not provide suitable electropherograms for the determination of voriconazole in human serum and plasma. Thus, various liquid/liquid extraction procedures based on dichloromethane and ethylacetate at physiological pH were investigated. The use of dichloromethane resulted in a broad peak making data interpretation difficult, whereas with ethylacetate a yellow insoluble precipitate was formed. Employing a mixture of ethylacetate and dichloromethane (75%/25%), however, provided nice data which allowed unambiguous detection of voriconazole and the IST in extracts prepared from 250 μl serum or plasma (Figs. 3 and 4). The recovery after sample pretreatment was determined by preparing and analyzing six bovine plasma samples

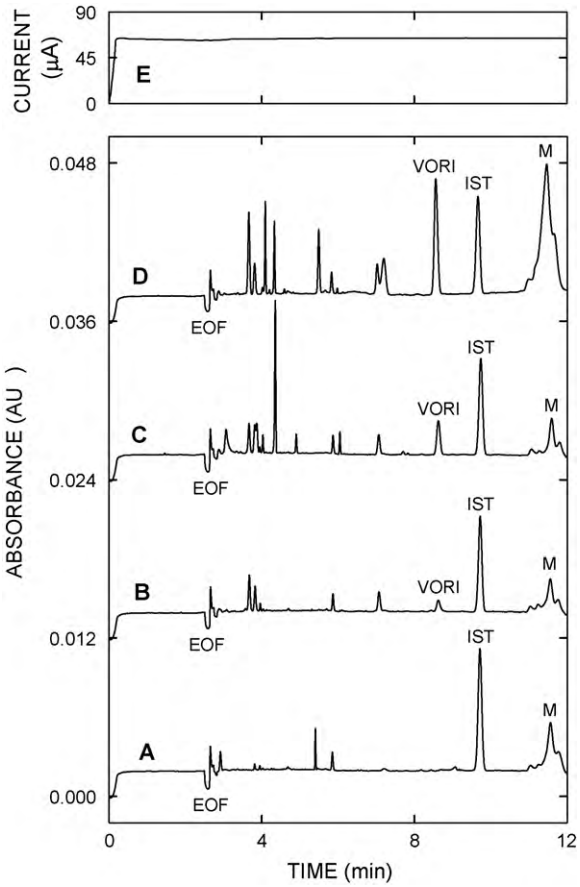

Fig. 4. Electropherograms obtained with extracts prepared from four patient samples containing (A) 0 μg/ml, (B) 0.72 μg/ml, (C) 1.99 μg/ml and (D) 6.59 μg/ml voriconazole. Panel E depicts the temporal behavior of the current for analysis of graph D. Other conditions as for Fig. 3.

spiked with 2.5 μg/ml of voriconazole and 2 μg/ml IST and comparing relative MEKC peak areas (peak areas divided by the detection time) after extraction with those obtained by injection of equal amounts of the compounds dissolved in the sample matrix composed of 0.5 mM $Na_2B_4O_7$ and 0.7 mM Na_2HPO_4 (pH 9.3). For voriconazole and the IST, recoveries were determined to be 75.7% and 71.7%, respectively. Using the extraction procedure described in Section 2.3, the limit of quantitation for voriconazole was 0.2 μg/ml (lowest calibrator concentration) and the limit of detection was 0.1 μg/ml.

Comparison of the data presented in Figs. 3 and 4 reveals that extracts from patient samples exhibit more additional peaks in the electropherograms compared to those obtained with spiked bovine plasma. Furthermore, patient samples in which no or small amounts of voriconazole were detected (graphs A and B of Fig. 4), typically revealed fewer peaks than those of patient samples with higher amounts of voriconazole (graphs C and D of Fig. 4). Analysis of a drug free serum of a healthy volunteer also showed fewer peaks (data not shown). The additional peaks found in extracts of patient samples are detected before voriconazole and could stem from voriconazole metabolites as well as from other administered drugs and their metabolites. No efforts were undertaken to identify additional peaks in the electropherograms.

Assay development was executed on the P/ACE 5510 CE system. With minor adaptation of selected instrumental conditions (see Section 2.2), comparable data were obtained with the PA800 instrument (data not shown). This became important to investigate as the P/ACE 5510 CE system is no longer available.

Table 1
Typical calibration data for voriconazole in spiked blank bovine plasma ($n = 5$)[a].

Property	Mean	SD	Range	RSD (%)
Slope	5.315	0.2385	4.991–5.581	4.49
y-Intercept	0.0221	0.0225	0.0030–0.0594	101.79
r^2	0.9995	0.0005	0.9986–0.9999	0.05
F value	15816.3	18056.1	2217.9–45861.5	114.16

[a] Voriconazole concentration and peak area ratio were taken as x- and y-axis, respectively.

3.2. MEKC assay characterization

Analysis of bovine plasma fortified with 0.2–7.5 μg/ml voriconazole provided linear calibrations graphs (inset Fig. 3, Table 1) with r^2 values between 0.9986 and 0.9999 (mean: 0.9995, $n = 5$). Mean values (RSD) for slopes and y-intercepts were determined to be 5.315 (4.49%, $n = 5$) and 0.0221 (101.8%, $n = 5$), respectively (Table 1). The repeatability of the MEKC method was evaluated by analyzing three control samples containing voriconazole levels of 0.60, 2.00 and 4.00 μg/ml, samples which were prepared independently of those used for calibration. Intraday ($n = 5$) and interday ($n = 5$) variation of detection times was less than 0.8% and 2.0% RSD, respectively, for both voriconazole and the IST. Typical precision and accuracy data for the three spiked samples are shown in Table 2. Intraday and interday precision was found to be <1.70% and <5.60%, respectively. Deviation from spiked level was not larger than 6%. Comparable data were obtained with the PA800.

3.3. Application of MEKC to patient samples and comparison with HPLC data

Electropherograms monitored for four patient samples are depicted in Fig. 4. The patient samples of panels B and D of Fig. 4 were identical to those of the HPLC data shown in panels C and D, respectively, of Fig. 2. During a period of 6 months, a total of 101 patient samples were analyzed by MEKC and the voriconazole blood levels were compared to those obtained by HPLC. With the two methods, no voriconazole could be found in nine samples, whereas one patient plasma revealed drug levels way above the calibration ranges of the two assays (13.2 and 14.6 μg/ml with MEKC and HPLC, respectively). The mean ± SD (median, range) of the remaining 91 voriconazole levels determined by MEKC and HPLC were determined to be 2.39 ± 2.10 (1.67, 0.11–7.50) μg/ml and 2.27 ± 1.99 (1.74, 0.15–7.07) μg/ml, respectively. Comparison of the two sets of data with the Mann–Whitney rank sum test revealed no statistically significant difference between the two input groups ($P = 0.704$). The mean voriconazole level determined in our samples was found to be within the provisional therapeutic range of 1–6 μg/ml.

Comparison of voriconazole levels found in patient serum samples using the two methods shows excellent agreement between the two methods. Linear regression analysis of the 91 data pairs revealed a linear relationship ($r^2 = 0.9857$) described by the equa-

Table 2
Typical precision and accuracy data for determination of voriconazole in spiked blank bovine plasma.

	Added (μg/ml)	Found (μg/ml)	RSD (%)	Accuracy (%)
Intraday ($n = 5$)	4.00	4.14 ± 0.01	0.33	103.6
	2.00	1.97 ± 0.02	1.09	98.7
	0.60	0.59 ± 0.01	1.66	98.7
Interday ($n = 5$)	4.00	4.24 ± 0.14	3.26	106.0
	2.00	2.01 ± 0.07	3.41	100.4
	0.60	0.58 ± 0.03	5.51	96.3

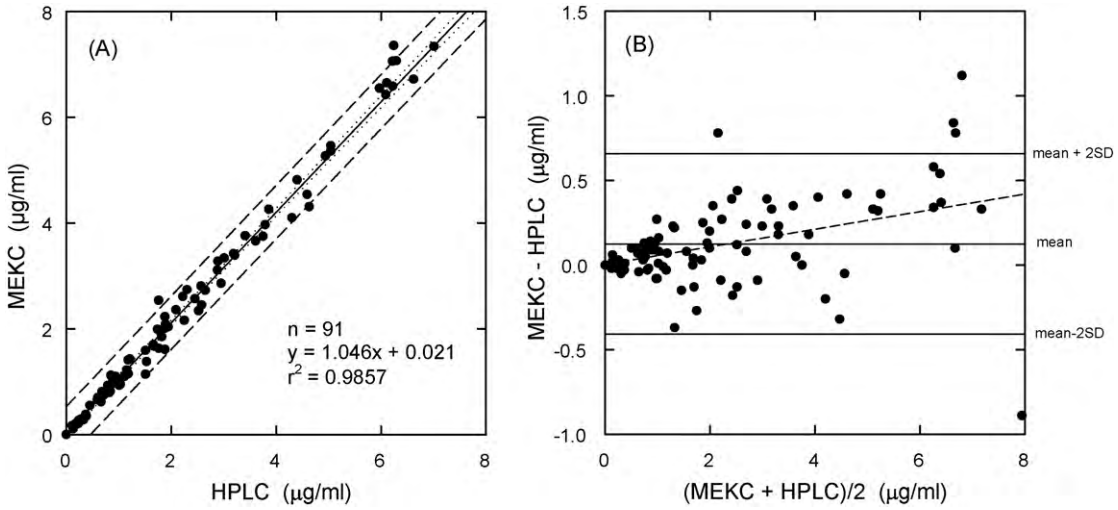

Fig. 5. (A) Comparative voriconazole drug levels and (B) bias analysis data for 91 patient samples determined by MEKC and HPLC. In panel A, the solid line represents a correlation graph determined by linear regression analysis, the broken lines describe the 95% prediction interval around the regression line and the dotted lines are the 95% confidence interval for the regression line. The data of panel B represent difference vs. mean of each data pair. The solid lines represent mean and mean ± 2 SD of the data. The broken line is a graph determined by linear regression analysis.

tion MEKC = 1.046 × HPLC + 0.021 which is very close the line of equality (Fig. 5A). Plotting the difference of the two values (MEKC–HPLC) against the mean, shown in panel B of Fig. 5, again shows excellent consistency between the two methods. The mean of the differences (SD) was 0.125 (0.267) µg/ml, indicating that, on average, the MEKC method gives slightly higher amounts than the HPLC method. This difference, however, is insignificant and has no consequences for TDM of voriconazole.

3.4. Application to pharmacokinetics of voriconazole

The data presented in Fig. 6 were determined by MEKC of blood samples that were collected during a 24 h time interval after p.o. ingestion of 400 mg voriconazole. These values were found to be in good agreement with those obtained by HPLC (MEKC = 1.012 × HPLC + 0.011, $r = 0.9976$). The highest voriconazole level (2.16 µg/ml) was found for the sample collected 1.5 h after drug intake. Both value and timing compare well with literature data [3]. The data between 1.5 and 24 h are biphasic (distribution and elimination phases, see inset in Fig. 6), which is also in agreement with the literature data [3], and were analyzed using non-linear regression analysis. Plasma concentration was fitted to a polyexponential equation

$$C_{(t)} = \sum_i Y_i e^{-\lambda_i t}$$

where $C_{(t)}$ (µg/ml) is the plasma concentration at time t; Y_i (µg/ml) is the coefficient of the ith term and λ_i (per h) is its exponent. For two-parameter ($i = 1$) and four-parameter ($i = 2$) exponential decays, regression coefficients r were determined to be 0.9884 and 0.9928, respectively. This indicates that the four-parameter equation provides a much better fit to the experimental data. This is also clearly seen with the dotted and broken line graphs presented in Fig. 6. The coefficients Y_1, λ_1, Y_2 and λ_2 of the four-parameter fit were determined to be 4.236, 0.508, 348 and 0.077, respectively. Distribution and elimination half-lives ($t_{½(d)}$ and $t_{½}$ respectively), calculated as $t_{½(d)} = 0.693/\lambda_1$ and $t_{½} = 0.693/\lambda_2$, were found to be 1.36 and 9.00 h, respectively. The use of a six-parameter equation (three terms) did not provide a better fit ($r = 0.9928$).

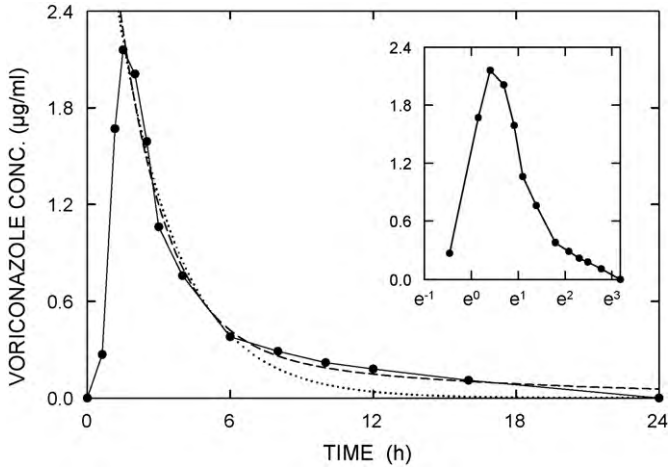

Fig. 6. Voriconazole kinetics determined by MEKC of blood samples that were collected during a 24 h time interval after p.o. ingestion of 400 mg voriconazole. Broken and dotted lines represent the four-parameter and two-parameter fits, respectively, obtained by non-linear regression analysis to the 1.5–24 h data. The inset depicts the data with a natural logarithmic scale for the x-axis.

4. Conclusions

A robust and validated MEKC method for the determination of voriconazole in human plasma and serum has been developed and applied to patient samples. The data were compared to those obtained by HPLC under TDM routine conditions. The excellent agreement between the data of the two assays indicates that the MEKC method may be suitable for routine monitoring of voriconazole levels. Both methods require sample preparation, use the same amount of sample, and have the same sample throughput (4–5 per h). CE capillaries are less expensive than HPLC columns and CE requires smaller amounts of chemicals and organic solvents, aspects which make MEKC attractive for TDM. The MEKC assay is characterized by high precision and accuracy. In the described configuration, the LOQ is 0.2 µg/ml which provides sufficient sensitivity for TDM of voriconazole with a provisional therapeutic range of 1–6 µg/ml. In addition to TDM, the assay is shown to be suitable for the exploration of the pharmacokinetics of voriconazole. It can be concluded that MEKC is an attractive alternative to HPLC for monitoring of voriconazole in human plasma and serum.

Acknowledgements

The authors acknowledge the HPLC routine analyses of voriconazole performed by Jeannine Joneli, Ursula Wanzenried and Jeannette Schiess. This work was partly supported by the Swiss National Science Foundation.

References

[1] L.B. Johnson, C.A. Kauffman, Voriconazole: a new triazole antifungal agent, Clin. Infect. Dis. 36 (2003) 630–637.
[2] L. Purkins, N. Wood, K. Greenhalgh, M.D. Eve, S.D. Oliver, D. Nichols, The pharmacokinetics and safety of intravenous voriconazole—a novel wide-spectrum antifungal agent, Br. J. Clin. Pharmacol. 56 (2003) 2–9.
[3] L. Purkins, N. Wood, K. Greenhalgh, M.J. Allen, S.D. Oliver, Voriconazole, a novel wide-spectrum triazole: oral pharmacokinetics and safety, Br. J. Clin. Pharmacol. 56 (2003) 10–16.
[4] D. Levêque, Y. Nivoix, F. Jehl, R. Herbrecht, Clinical pharmacokinetics of voriconazole, Int. J. Antimicrob. Agents 27 (2006) 274–284.
[5] R.J.M. Brüggemann, J.P. Donnelly, R.E. Aarnoutse, A. Warris, N.M.A. Blijlevens, J.W. Mouton, P.E. Verweij, D.M. Burger, Therapeutic drug monitoring of voriconazole, Ther. Drug Monit. 30 (2008) 403–411.
[6] S.J. Roffey, S. Cole, P. Comby, D. Gibson, S.G. Jezequel, A.N.R. Nedderman, D.A. Smith, D.K. Walker, N. Wood, The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog and human, Drug Metab. Dispos. 31 (2003) 731–741.
[7] R. Hyland, B.C. Jones, D.A. Smith, Identification of the cytochrome P450 enzymes involved in the N-oxidation of voriconazole, Drug Metab. Dispos. 31 (2003) 540–547.
[8] N. Murayama, N. Imai, T. Nakane, M. Shimizu, H. Yamazaki, Roles of CYP3A4 and CYP2C19 in methyl hydroxylated and N-oxidized metabolite formation from voriconazole, a new antifungal agent, in human liver microsomes, Biochem. Pharmacol. 73 (2007) 2020–2026.
[9] D.A. Stopher, R. Gage, Determination of a new antifungal agent, voriconazole, by multidimensional liquid chromatography with direct plasma injection onto a size exclusion column, J. Chromatogr. B 691 (1997) 441–448.
[10] R. Gage, D.A. Stopher, A rapid HPLC assay for voriconazole in human plasma, J. Pharm. Biomed. Anal. 17 (1998) 1449–1453.
[11] G.J. Pennick, M. Clark, D.A. Sutton, M.G. Rinaldi, Development and validation of a high-performance liquid chromatography assay for voriconazole, Antimicrob. Agents Chemother. 47 (2003) 2348–2350.
[12] M. Wenk, A. Droll, S. Krähenbühl, Fast and reliable determination of the antifungal drug voriconazole in plasma using monolithic silica rod liquid chromatography, J. Chromatogr. B 832 (2006) 313–316.
[13] H. Egle, R. Trittler, A. König, K. Kümmerer, Fast, fully automated analysis of voriconazole from serum by LC–LC–ESI–MS–MS with parallel column-switchen technique, J. Chromatogr. B 814 (2005) 361–367.
[14] J.W.C. Alffenaar, A.M.A. Wessels, K. van Hateren, B. Greijdanus, J.G.W. Kosterink, D.R.A. Uges, Method for therapeutic drug monitoring of azole antifungal drugs in human serum using LC/MS/MS, J. Chromatogr. B 878 (2010) 39–44.
[15] W. Thormann, S. Lienhard, P. Wernly, Strategies for the monitoring of drugs in body fluids by micellar electrokinetic capillary chromatography, J. Chromatogr. 636 (1993) 137–148.
[16] W. Thormann, C.-X. Zhang, A. Schmutz, Capillary electrophoresis for drug analysis in body fluids, Ther. Drug Monit. 18 (1996) 506–520.
[17] S. Zaugg, W. Thormann, Enantioselective determination of drugs in body fluids by capillary electrophoresis, J. Chromatogr. A 875 (2000) 27–41.
[18] W. Thormann, Progress in capillary electrophoresis in therapeutic drug monitoring and clinical and forensic toxicology, Ther. Drug Monit. 24 (2002) 222–231.
[19] P.K. Owens, A.F. Fell, M.W. Coleman, J.C. Berridge, Separation of voriconazole stereoisomers by capillary electrophoresis and liquid chromatography, Enantiomer 4 (1999) 79–90.
[20] P.K. Owens, A.F. Fell, M.W. Coleman, J.C. Berridge, Complexation of voriconazole stereoisomers with neutral and anionic derivatised cyclodextrins, J. Incl. Phenom. Macrocycl. Chem. 38 (2000) 133–151.
[21] A.L. Crego, M.L. Marina, J.L. Lavandera, Optimization of the separation of a group of antifungals by capillary zone electrophoresis, J. Chromatogr. A 917 (2001) 337–345.
[22] A.L. Crego, J. Gomez, M.L. Marina, J.L. Lavandera, Application of capillary zone electrophoresis with off-line solid-phase extraction to in vitro metabolism studies of antifungals, Electrophoresis 22 (2001) 2503–2511.
[23] A.I.H. Adams, A.M. Bergold, Development and validation of a high performance liquid chromatographic method for the determination of voriconazole content in tablets, Chromatographia 62 (2005) 429–434.
[24] S. Terabe, Electrokinetic chromatography—an interface between electrophoresis and chromatography, Trends Anal. Chem. 8 (1989) 129–134.
[25] H. Nishi, S. Terabe, Application of micellar electrokinetic chromatography to the pharmaceutical analysis, Electrophoresis 11 (1990) 691–701.
[26] F. von Heeren, R. Tanner, R. Theurillat, W. Thormann, Determination of the antimycotic drug fluconazole in human plasma by micellar electrokinetic capillary chromatography with detection at 190 nm, J. Chromatogr. A 745 (1996) 165–172.
[27] M.C. Breadmore, A. Procházková, R. Theurillat, W. Thormann, Determination of itraconazole and hydroxyitraconazole in human serum and plasma by micellar electrokinetic chromatography, J. Chromatogr. A 1014 (2003) 57–70.
[28] A. Schmutz, W. Thormann, Rapid determination of the antimycotic drug flucytosine in human serum by micellar electrokinetic capillary chromatography with direct sample injection, Ther. Drug Monit. 16 (1994) 483–490.
[29] A. Schmutz, W. Thormann, Determination of phenobarbital, ethosuximide and primidone in human serum by micellar electrokinetic capillary chromatography with direct sample injection, Ther. Drug Monit. 15 (1993) 310–316.
[30] R. Kuldvee, W. Thormann, Determination of carbamazepine and carbamazepine-10,11-epoxide in human serum and plasma by micellar electrokinetic capillary chromatography in the absence of electroosmosis, Electrophoresis 22 (2001) 1345–1355.