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Method for therapeutic drug monitoring of azole antifungal drugs in human serum using LC/MS/MS

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ABSTRACT

Fungal infections occur in immunocompromised patients. Azole antifungal agents are used for the prophylaxis and treatment of these infections. The interest in therapeutic drug monitoring azole agents has increased over the last few years. Inter- and intra-patient variability of pharmacokinetics, drug–drug interactions, serum concentration related toxicity and success of therapy has stressed the need of frequently therapeutic drug monitoring of the drugs, belonging to the group of azoles. Therefore a simple, rapid and flexible method of analysis is required. This method is based on the precipitation of proteins in human serum with LC/MS/MS detection. Validation was performed according to the guidelines for bioanalytical method validation of the food and drug administration agency. The four most used azole drugs can be detected in human serum within the clinical relevant serum levels with good accuracy and reproducibility at the limit of quantification. Intra- and inter-day validation demonstrated good accuracy and reproducibility. A rapid, sensitive and flexible LC/MS/MS method has been developed and validated to measure voriconazole (VRZ), fluconazole (FLZ), itraconazole (ITZ) and posaconazole (PSZ) in human serum. This new method is suitable for clinical pharmacokinetic studies and routine monitoring in daily practice.

1. Introduction

Invasive fungal infections occur especially in immunocompromised patients [1] and azole antifungal compounds (e.g. fluconazole, itraconazole, posaconazole and voriconazole) have gained a solid position in the prophylaxis and treatment of these fungal infections. Fluconazole is used for treatment and prophylaxis of candidiasis while voriconazole is mainly used for the treatment of invasive aspergillosis[2] or infections with fluconazole resistant *Candida* spp. [3]. Itraconazole and posaconazole are both active against *Candida* en *Aspergillus* spp. and have a place in the prophylaxis of fungal infections [4,5]. Posaconazole is also recommended for the treatment of zygomycosis or as salvage therapy for invasive aspergillosis [6,7].

The pharmacokinetics of azole antifungal agents show a large inter- and intraindividual variability which can be partly explained by non-linear pharmacokinetics, differences in bioavailability, drug–drug interactions and cytochrome P450 polymorphisms. For many years the clinical impact of this variability was unknown until observational studies showed a correlation between plasma concentrations of azole agents and efficacy and toxicity [5,8–14]. Therapeutic drug monitoring of azole agents may therefore be warranted [9,15–20]. In the last few years therapeutic drug monitoring of azole agents has evolved and has now reached the point of becoming daily practice dosing as the patients condition may change rapidly [21,22]. For routine monitoring of plasma levels of azole antifungal agents an universal method of analysis for high through put of samples is required. Therefore our objective was to develop a simple, rapid and flexible LC–MS/MS method of analysis for the four different azoles in human serum preferably without solid phase extraction. This method was based on the precipitation of proteins in human serum with precipitation reagent containing the internal standard and subsequently HPLC analysis and MS/MS detection of the transition ions of the azole and the internal standard.

2. Methods

2.1. Chemicals, materials and reagents

Fluconazole and voriconazole were kindly provided by Pfizer (New York, USA), posaconazole by Schering-Plough (New York, USA) and itraconazole and hydroxyitraconazole were received from Janssen-Cilag. The internal standard, cyanoimipramine, was supplied by Roche (Woerden, The Netherlands). Acetonitrile for LC/MS and water for LC/MS were provided by BioSolve BV (Valkenswaard,

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The Netherlands). Methanol Lichrosolv and the other used chemicals were of suitable analytical grade and purchased from VWR (Amsterdam, The Netherlands). The precipitation reagent consisted of 0.04 mg/L cyanoimipramine, internal standard, dissolved in a mixture of methanol and acetonitrile (4:21, v/v, respectively). Pooled human serum samples were made available according to the guidelines of the University Medical Center Groningen.

2.2. LC/MS/MS instrumentation and conditions

All experiments were performed on a Thermo Fisher (San Jose, USA) triple quadrupole LC–MS/MS with a Finnigan™ Surveyor® LC pump and a Finnigan™ Surveyor® autosampler which was set at a temperature of 20 °C. After sample preparation, 5 μL of the clear upper layer were injected on a 50 mm × 2.1 mm C_{18}, 5-μm analytic column (HyPURITY AQUASTAR, Interscience Breda, The Netherlands) for chromatographic separation. The column temperature was set at 20 °C. The mobile phase had a flow of 0.3 mL/min and consisted of an aqueous buffer (containing ammonium acetate 10 g/L, acetic acid 35 mg/L and trifluoroacetic anhydride 2 mL/L water), water and acetonitrile. The following gradient was run, time 0–2 min: 5% buffer, 95–0% water and 0–95% acetonitrile, time 2–3 min: 5% buffer and 95% acetonitrile, time 3.0–3.1 min: 5% buffer, 0–95% water and 95–0% acetonitrile, time 3.1–3.6 min: 5% buffer and 95% water. The Finnigan™ TSQ® Quantum Discovery mass selective detector was operating in electrospray positive ionization mode and performed selected reaction monitoring (SRM). The following mass parameters were used at a scan width of 0.5 *m/z*.: fluconazole *m/z* 307.2 > 219.9 (collision energy 20 eV), voriconazole *m/z* 350.0 > 281.1 (collision energy 17 eV), posaconazole *m/z* 701.3 > 683.2 (collision energy 32 eV), itraconazole *m/z* 705.2 > 392.0 (collision energy 36 eV), hydroxyitraconazole *m/z* 721.2 > 408.0 (collision energy 42 eV), and cyanoimipramine *m/z* 306.2 to *m/z* 218.0 (collision energy 39 eV). The ion source spray voltage was set at 3500 V, the sheath and auxiliary gas pressure at 35 Arbitrary units (Arb.) and 5 Arb., respectively and the capillary temperature at 350 °C. Xcalibur® software version 1.4 SR1 (Thermo Fisher, San Jose, USA) was used for peak height integration for all components.

2.3. Preparation of standard stocks and serum samples

For the preparation of the calibration standards and the quality control (QC) samples of each compound two stock solutions (A1 and B1, respectively) were prepared. Secondary working stock solutions (stock A2 and B2) were prepared by diluting stock A1 and B1 (see Table 1). Stock A1 and A2 were used for preparing the calibration samples by diluting it with pooled human serum (see Table 1). QC samples were prepared by spiking stock B1 and B2 to pooled human serum (see Table 1).

The calibration standards and QC samples were prepared on day zero and stored at −20 °C. The QC samples for determining the freeze/thaw stability were freshly prepared from stocks B1 and B2 on the first day of analysis.

2.4. Procedure of sample preparation

In a 2.0 mL autosampler vial 100 μL serum sample ((blank serum, calibration standard, Quality Control sample or patient sample), 10 μL in case of fluconazole and voriconazole) and 750 μL precipitation reagent containing the internal standard were vortexed for 1 min, stored at −20 °C for 30 min to promote protein precipitation and subsequently centrifuged at 11,000 × *g* for 5 min. From the clear upper layer 5 μL was injected onto the LC–MS/MS system.

2.5. Methodology for validation

In accordance with the Guidance for Industry Bioanalytical Method Validation of the Food and Drug Adminstration, method validation included selectivity, linearity, accuracy, precision, recovery and stability [23]. Therefore, on each analytical day, a single calibration curve was obtained and the QC samples were analyzed in fivefold. Furthermore six pools of blank human serum were analyzed in triple. The accuracy and precision were determined by analyzing QC samples on four levels in fivefold on three different days and analyzed using one-way ANOVA.

2.6. Clinical practice

At the University Medical Center voriconazole serum levels are monitored three times a week. Special attention is paid to paediatric patients with invasive fungal infections as voriconazole concentrations display a large interindividual variability in a greater extend than in adults [24,25]. Itraconazole is applied as prophylaxis for *Aspergillus* spp. in adults and children with haematological malignancies in our Hospital [5]. Routine monitoring of itraconazole concentrations is performed to evaluate absorption in those patients with mucositis [26]. In case of serum trough levels below 1 mg/L the dosage is increased and serum concentrations are evaluated. Absorption of posaconazole, which is used for (secondary) prophylaxis in specific high risk patients or as salvage therapy for invasive fungal infection in our Hospital is monitored as it needs to be administered in combination with a high fatty meal which is generally not well tolerated by critically ill patients.

2.7. Quality control program

Our laboratory participates in the international quality control program of antifungal drugs of the Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology (KKGT) [27]. The participation in the program contributes to optimization of the performance of our clinical service of therapeutic drug monitoring of azoles. Sources of error in the method of analysis, and dosage recommendations are externally evaluated [27].

3. Results

3.1. Method development

Fluconazole and voriconazole cause of very high detector response and to avoid detector overload it was decided to take 10 μL serum sample (calibration standard, Quality Control sample or patient sample) for the analysis of these two compounds.

3.2. Selectivity and interference

The selectivity of this method was evaluated by analyzing six lots of pooled human serum in comparison with LLOQ samples. There were no peaks observed in any of the pooled human serum samples at the retention time of the azoles respectively the internal standard. No ion suppression was observed by analyzing six lots of pooled human serum and simultaneously direct infusion of a stock solution containing fluconazole or voriconazole or posaconazole or itraconazole-hydroxyitraconazole and cyanoimipramine by a syringe pump.

3.3. Linearity, accuracy and precision

All calibration curves were linear by using a weight factor of $1/x$ over a range of 0.5–200 mg/L for fluconazole, 0.05–10 mg/L

Table 1
Concentrations of stock solutions, calibration standards and QC samples.

Compound	Stock solution (A1 and B1)	Working stock solutions (A2 and B2)	Calibration standards (mg/L serum)	QC samples (mg/L serum)			
				LLOQ	LOW	MED	HIGH
Fluconazole	4000 mg/L methanol	80 mg/L methanol	0.5; 1.5; 2.5; 5.0; 25; 50; 75; 100 and 200	0.5	5.0	40	80
Voriconazole	250 mg/L methanol	25 mg/L methanol	0.1; 0.25; 0.5; 1.0; 2.5; 5 and 10	0.1	0.25	2.5	5.0
Posaconazole	400 mg/L methanol:water (50:50, v/v)	40 mg/L methanol:water (50:50, v/v)	0.1; 0.2; 0.5; 1.0; 2.0; 4.0; 8.0 and 10.0	0.1	0.5	2.5	5.0
Itraconazole	200 mg/L methanol	80 mg/L methanol	0.1; 0.25; 0.5; 1.0; 2.0; 3.0; 4.0 and 5.0	0.1	0.5	1.0	2.0
Hydroxyitraconazole	200 mg/L methanol	80 mg/L methanol	0.1; 0.25; 0.5; 1.0; 2.0; 3.0; 4.0 and 5.0	0.1	0.5	1.0	2.0

LLOQ, lower limit of quantification; MED, medium.

Table 2
Results of accuracy and precision.

	QC levels of FLU				QC levels of VRZ				QC levels of PSZ				QC levels of ITZ (+ HITZ)			
	LLOQ	LOW	MED	HIGH	LLOQ	LOW	MED	HIGH	LLOQ	LOW	MED	HIGH	LLOQ	LOW	MED	HIGH
Mean concentration (mg/L)	0.53	5.05	42.2	83.3	0.10	0.26	2.52	5.05	0.11	0.53	2.58	4.98	0.11 (0.10)	0.53 (0.53)	1.07 (1.06)	2.06 (2.05)
Bias (%)	7.1	1.3	5.8	4.4	2.1	2.3	0.1	0.2	7.9	9.9	6.3	2.4	4.7 (2.8)	6.0 (5.1)	6.7 (5.8)	3.0 (2.2)
Within-run CV (%)	3.5	4.3	4.7	9.2	7.8	4.2	1.9	1.9	5.2	3.2	1.3	1.8	5.2 (6.1)	1.8 (2.0)	2.2 (2.2)	4.6 (3.7)
Between-run CV (%)	4.3	0.8	0	7.8	0.0	2.1	3.1	1.0	0.0	1.5	0.1	0.9	0.0 (0.0)	0.0 (0.4)	0.4 (0.0)	0.0 (0.0)

QC, quality control; FLU, fluconazole; VRZ, voriconazole; PSZ, posaconazole; ITZ, itraconazole; HITZ, hydroxyitraconazole; LLOQ, lower limit of quantification; MED, medium.

Table 3
Interassay variability of the calibration curves.

Component	Slope ± SD	Intercept ± SD	Correlation coefficient
Fluconazole	0.0267 ± 0.001	0.0031 ± 0.006	0.9955
Voriconazole	0.000026 ± 0.000000351	0.000216 ± 0.000408	0.9980
Itraconazole	0.764 ± 0.00792	0.0192 ± 0.00742	0.9988
Hydroxyitraconazole	0.214 ± 0.00150	0.00358 ± 0.00142	0.9995
Posaconazole	0.422 ± 0.00341	0.0110 ± 0.00419	0.9992

for voriconazole, 0.1–5 mg/L for itraconazole and hydroxyitraconazole and 0.1–10 mg/L for posaconazole. The results of accuracy and precision are listed in Table 2. The interassay variability of the calibration curves is shown in Table 3.

3.4. Recovery

The recovery was calculated by comparison the peak height of LOW, MED and HIGH control samples in serum ($n = 5$) with spiked control samples prepared in a mixture of methanol and acetonitrile (4:21, v/v) corresponding to the same concentrations ($n = 5$). The recovery for the LOW, MED and HIGH control samples and internal standard are shown in Table 4.

3.5. Stability and dilution integrity

After 2–3 cycles of freeze–thaw the concentration of the azoles was measured as percentage of the mean concentration of the fresh prepared samples for QC-level LOW, MED and HIGH. In addition the stability of the azoles in human serum was measured after 36 h at +4 °C, at room temperature and, after sample preparation, in the autosampler. The results are displayed in Table 5.

Table 4
Recovery (%).

	QC levels			Cyanoimipramine
	LOW	MED	HIGH	
Fluconazole	76.1	83.2	93.4	104.4
Voriconazole	81.5	82.5	82.9	104.4
Posaconazole	94.8	95.6	94.0	104.9
Itraconazole	88.1	109.4	91.1	105.8
Hydroxyitraconazole	90.9	99.1	82.7	105.8

QC, quality control; MED, medium.

3.6. Clinical practice

This method of analysis for therapeutic drug monitoring of azole antifungal agents has been used for two years now in our hospital. We observed that patients with haematological malignancies presenting multiple increased liver enzyme values of three to five times the upper level of normal are 'at risk' of developing high voriconazole trough levels. It appeared that the voriconazole elimination half-life was extreme prolonged, which suggest that routine monitoring of voriconazole levels after 3 days of therapy is necessary to prevent voriconazole adverse effects caused by toxic levels [28].

Drug–drug interactions with azole agents are frequently observed. Guidelines on the clinical management of these drug–drug interactions are helpful to guide the therapeutic drug monitoring of azole antifungal agents [29]. However, the extent of these interactions can be more profound in critically ill patients than in healthy volunteers. We observed that a dosage increase of voriconazole was not sufficient to compensate for phenytoin-induced metabolism compared to healthy volunteers [30]. This resulted in the change of clinical management of patients with haematological maligancies needing prophylaxis for busulphan-induced seizures.

Serum level guided dosing of voriconazole in children was very useful as doses had to be increased to great magnitude in order to result in measurable serum levels [31].

Routine monitoring of posaconazole let to the observation that co-administration of omeprazole resulted in a significant decrease

Table 5
Sample stability.

	FLZ			VRZ			PSZ			ITZ (+ HITZ)		
	LOW	MED	HIGH	LOW	MED	HIGH	LOW	MED	HIGH	LOW	MED	HIGH
4 °C												
h	168	168	168	72	72	72	72	72	72	72 (72)	72 (72)	72 (72)
Bias (%)	−1.8	2.5	6.5	1.3	−4.1	−3.6	9.0	4.8	2.0	3.3 (2.2)	5.9 (5.8)	1.7 (2.5)
RT												
h	168	168	168	72	72	72	72	72	72	72 (72)	72 (72)	72 (72)
Bias (%)	2.3	7.6	5.6	0.7	−4.4	−2.3	9.1	6.8	4.1	2.9 (3.3)	5.7 (5.7)	1.7 (3.1)
Freeze/thaw												
Cycles	2	2	2	3	3	3	2	2	2	3 (3)	3 (3)	3 (3)
Bias (%)	3.7	5.5	8.8	2.6	−4.4	−3.3	9.1	2.4	3.8	4.2 (3.3)	8.5 (6.2)	1.1 (1.1)
Autosampler												
h			48			72			120			72 (72)
Bias (%)			6.1			−4.7			8.7			3.1 (2.4)

RT, room temperature; QC, quality control; FLU, fluconazole; VRZ, voriconazole; PSZ, posaconazole; ITZ, itraconazole; HITZ, hydroxyitraconazole; LLOQ, lower limit of quantification; MED, medium.

of posaconazole serum trough concentrations based on a reduced bioavailability due to increased pH of gastrointestinal fluids [32].

3.7. Quality control program

The participation in the quality control program in 2008 for all four azole compounds passed the requirements, as defined by the program regulations, for all analyzed samples using the described methods of analysis. Samples with a low concentration had a median value of 101% (interquartile range 94.8–101%) of the theoretical value and samples with a high concentration had a median value of 98% (interquartile range 92.3–101.3%) of the theoretical value.

4. Discussion

We developed a universal method of analysis for routine monitoring of azole antifungal drugs without time consuming sample preparation and purification or changes to the configuration of LC/MS/MS system. Our method was based on the precipitation of proteins in human serum with LC/MS/MS detection and validation was performed according to the guidelines for bioanalytical method validation of the food and drug administration agency. All compounds were detected in human serum with good accuracy and reproducibility at the limit of quantification. Intra- and inter-day validation demonstrated good accuracy and reproducibility. The method is suitable for clinical pharmacokinetic studies and routine monitoring in daily practice. To our knowledge this is the first described validation using one method of analysis for all azole compounds.

In the literature several methods of analysis of azoles in human serum have been described with conventional methods like HPLC-UV or HPLC-fluorescence using liquid–liquid extraction (LLE), solid phase extraction (SPE) but these often lack sensitivity and selectivity and sample preparation is time consuming. For high trough put analysis of large sample numbers a rapid and universal method of analysis for azole compounds is mandatory. Liquid chromatography combined with mass spectrometry has become the most suitable apparatus to comply to these requirements. Several methods have been reported based on liquid chromatography combined with mass spectrometry but these have some limitations. Single mass spectrometry lacks sensitivity and specificity compared to a tandem mass spectrometry [33]. Some of the described methods still use SPE [34–36] or LLE [37–42], which is expensive and time consuming. On-line solid phase extraction has the advantage of an increased sensitivity [43] but lacks universal application for the different antifungal agents and is therefore less suitable for routine monitoring. Also optimization of the method of analysis by different columns or great differences in mobile phase can cause a significant delay in measuring the clinical samples as stabilization of the system takes time. Only a few methods were reported based on a protein precipitation without additional purification

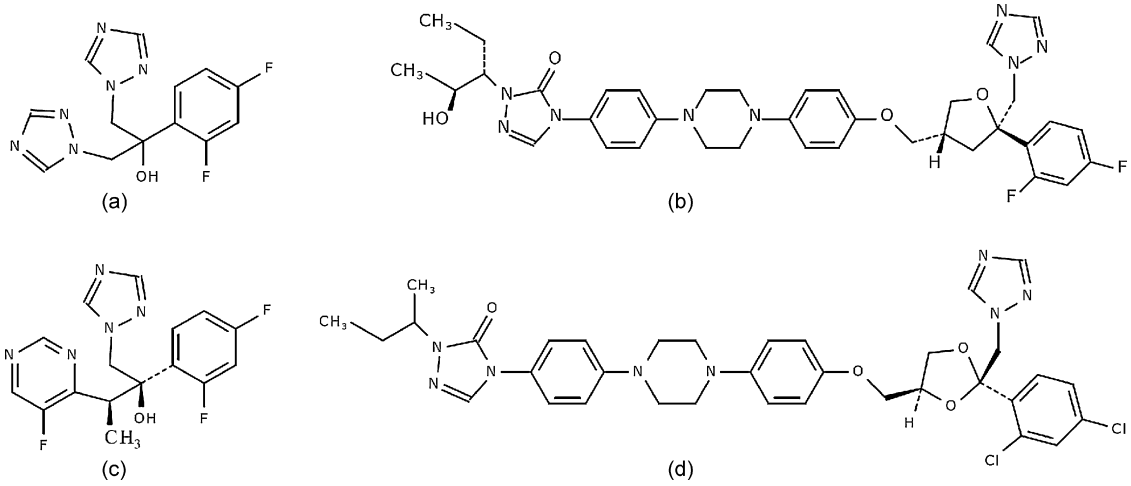

Fig. 1. Chemical structures of azoles. Structure a: fluconazole; b: posaconazole; c: voriconazole; d: itraconazole.

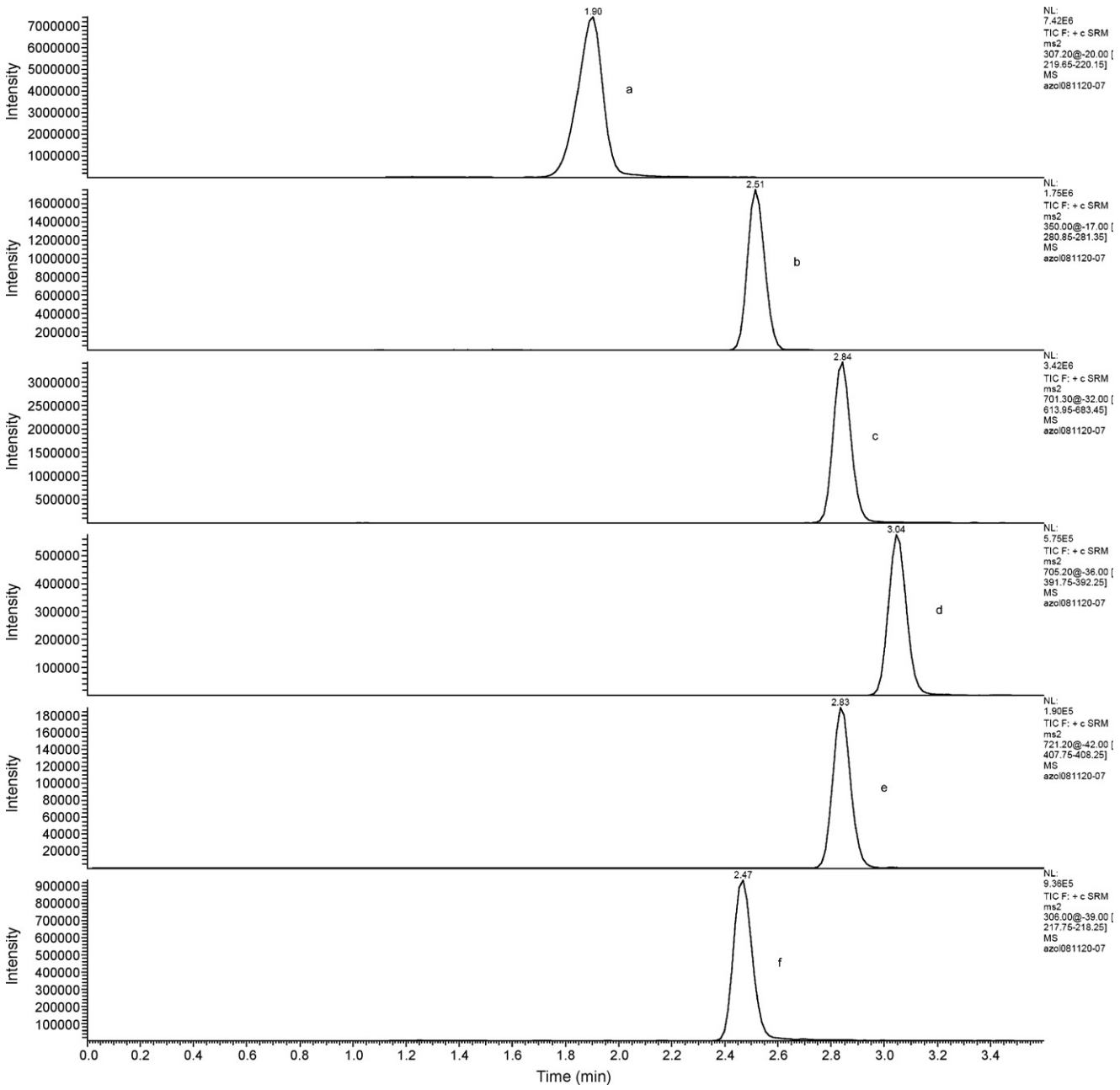

Fig. 2. Chromatogram of the azoles. Chromatogram a: fluconazole; b: voriconazole; c: posaconazole; d: itraconazole; e: hydroxyitraconazole; f: cyanoimipramine.

procedures. However, these reported methods did not cover the entire range of azoles and were limited by being only validated for measuring voriconazole in plasma of rodents [33,44] or lacked a description of the sample preparation [45]. Our method, based on precipitation of proteins in human serum, has the advantage of a simple and rapid sample preparation without further workup with LLE and SPE and is therefore easily to implement. Having the same method of analysis for the four most used azole antifungal drugs enables routine monitoring of these compounds in daily practice without additional changes in configuration of columns or fluids. The impact of therapeutic drug monitoring can increase as samples can be easily processed and results be made available at the same day. This enables hospital pharmacists and physicians on ICU's to respond rapidly to subtherapeutic or toxic concentrations of azole agents to optimize treatment of critically ill patients (Figs. 1 and 2).

5. Conclusion

In summary, a selective, linear, accurate and precise method, which can be used routinely for the determination of the four azoles in human serum, has been developed. This method is applied successfully in clinical practice. Due to the high throughput sample analysis a clinical relevant service can be offered to the physician.

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Transparency declaration

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References

[1] M. Cuenca-Estrella, L. Bernal-Martinez, M.J. Buitrago, M.V. Castelli, A. Gomez-Lopez, O. Zaragoza, J.L. Rodriguez-Tudela, Int. J. Antimicrob. Agents 32 (Suppl. 2) (2008) S143.
[2] R. Herbrecht, D.W. Denning, T.F. Patterson, J.E. Bennett, R.E. Greene, J.W. Oestmann, W.V. Kern, K.A. Marr, P. Ribaud, O. Lortholary, R. Sylvester, R.H. Rubin, J.R. Wingard, P. Stark, C. Durand, D. Caillot, E. Thiel, P.H. Chandrasekar, M.R. Hodges, H.T. Schlamm, P.F. Troke, B. de Pauw, N. Engl. J. Med. 347 (2002) 408.
[3] pfizer, 2002.
[4] O.A. Cornely, J. Maertens, D.J. Winston, J. Perfect, A.J. Ullmann, T.J. Walsh, D. Helfgott, J. Holowiecki, D. Stockelberg, Y.T. Goh, M. Petrini, C. Hardalo, R. Suresh, D. ngulo-Gonzalez, N. Engl. J. Med. 356 (2007) 348.
[5] A.G. Prentice, A. Glasmacher, B. Djulbegovic, Br. J. Haematol. 132 (2006) 656.
[6] R.N. Greenberg, K. Mullane, J.A. van Burik, I. Raad, M.J. Abzug, G. Anstead, R. Herbrecht, A. Langston, K.A. Marr, G. Schiller, M. Schuster, J.R. Wingard, C.E. Gonzalez, S.G. Revankar, G. Corcoran, R.J. Kryscio, R. Hare, Antimicrob. Agents Chemother. 50 (2006) 126.
[7] T.J. Walsh, I. Raad, T.F. Patterson, P. Chandrasekar, G.R. Donowitz, R. Graybill, R.E. Greene, R. Hachem, S. Hadley, R. Herbrecht, A. Langston, A. Louie, P. Ribaud, B.H. Segal, D.A. Stevens, J.A. van Burik, C.S. White, G. Corcoran, J. Gogate, G. Krishna, L. Pedicone, C. Hardalo, J.R. Perfect, Clin. Infect. Dis. 44 (2007) 2.
[8] J.M. Poirier, G. Cheymol, Clin. Pharmacokinet. 35 (1998) 461.
[9] W.W. Hope, E.M. Billaud, J. Lestner, D.W. Denning, Curr. Opin. Infect. Dis. 21 (2008) 580.
[10] K.W. Garey, M. Rege, M.P. Pai, D.E. Mingo, K.J. Suda, R.S. Turpin, D.T. Bearden, Clin. Infect. Dis. 43 (2006) 25.
[11] A. Pascual, T. Calandra, S. Bolay, T. Buclin, J. Bille, O. Marchetti, Clin. Infect. Dis. 46 (2008) 201.
[12] J. Smith, N. Safdar, V. Knasinski, W. Simmons, S.M. Bhavnani, P.G. Ambrose, D. Andes, Antimicrob. Agents Chemother. 50 (2006) 1570.
[13] A.E. Boyd, S. Modi, S.J. Howard, C.B. Moore, B.G. Keevil, D.W. Denning, Clin. Infect. Dis. 39 (2004) 1241.
[14] A. Imhof, D.J. Schaer, U. Schanz, U. Schwarz, Swiss. Med. Wkly. 136 (2006) 739.
[15] R.J. Bruggemann, J.P. Donnelly, R.E. Aarnoutse, A. Warris, N.M. Blijlevens, J.W. Mouton, P.E. Verweij, D.M. Burger, Ther. Drug Monit. 30 (4) (2008) 403–411.
[16] S.S. Buchkowsky, N. Partovi, M.H. Ensom, Ther. Drug Monit. 27 (2005) 322.
[17] M.L. Goodwin, R.H. Drew, J. Antimicrob. Chemother. 61 (2008) 17.
[18] W.W. Hope, D.W. Denning, Intern. Med. J. 34 (2004) 147.
[19] J. Smith, D. Andes, Ther. Drug Monit. 30 (2008) 167.
[20] S. Trifilio, G. Pennick, J. Pi, J. Zook, M. Golf, K. Kaniecki, S. Singhal, S. Williams, J. Winter, M. Tallman, L. Gordon, O. Frankfurt, A. Evens, J. Mehta, Cancer 109 (2007) 1532.
[21] T.J. Walsh, E.J. Anaissie, D.W. Denning, R. Herbrecht, D.P. Kontoyiannis, K.A. Marr, V.A. Morrison, B.H. Segal, W.J. Steinbach, D.A. Stevens, J.A. van Burik, J.R. Wingard, T.F. Patterson, Clin. Infect. Dis. 46 (2008) 327.
[22] S.W. Chapman, W.E. Dismukes, L.A. Proia, R.W. Bradsher, P.G. Pappas, M.G. Threlkeld, C.A. Kauffman, Clin. Infect. Dis. 46 (2008) 1801.
[23] U.S. Department of Healt and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM), Bioanalytical Method Validation, 2001.
[24] A.C. Pasqualotto, M. Shah, R. Wynn, D.W. Denning, Arch. Dis. Child. 93 (2008) 578.
[25] W.J. Steinbach, Pediatr. Infect. Dis. J. 24 (2005) 358.
[26] A.G. Prentice, A. Glasmacher, J. Antimicrob. Chemother. 56 (Suppl. 1) (2005) i17.
[27] R.J. Bruggemann, D.J. Touw, R.E. Aarnoutse, P.E. Verweij, D.M. Burger, Antimicrob. Agents Chemother. (2008).
[28] J.W. Alffenaar, T. de Vos, D.R. Uges, S.M. Daenen, Br. J. Clin. Pharmacol. 67 (2008) 262.
[29] R.J. Bruggemann, J.W. Alffenaar, N.M. Blijlevens, E.M. Billaud, J.G. Kosterink, P.E. Verweij, D.M. Burger, Clin. Infect. Dis. 48 (2009) 1441.
[30] J.W.C. Alffenaar, K.C.M. van der Elst, D.R.A. Uges, J.G.W. Kosterink, S.M.G.J. Daenen, Br. J. Clin. Pharmacol. 68 (3) (2009) 462–463.
[31] J.W. Alffenaar, R.A. Doedens, E. Groninger, J.G. Kosterink, Pediatr. Infect. Dis. J. 27 (2008) 189.
[32] J.W. Alffenaar, S. van Assen, T.S. van der Werf, J.G. Kosterink, D.R. Uges, Clin. Infect. Dis. 48 (2009) 839.
[33] M. Yao, L. Chen, N.R. Srinivas, J. Chromatogr. B Biomed. Sci. Appl. 752 (2001) 9.
[34] M. Vogeser, U. Spohrer, X. Schiel, Clin. Chem. Lab. Med. 41 (2003) 915.
[35] M. Vogeser, X. Schiel, U. Spohrer, Clin. Chem. Lab. Med. 43 (2005) 730.
[36] J.J. Bafeltowska, E. Buszman, Chemotherapy 51 (2005) 370.
[37] A. Carrier, J. Parent, J. Chromatogr. B Biomed. Sci. Appl. 745 (2000) 413.
[38] C. Kousoulos, G. Tsatsou, C. Apostolou, Y. Dotsikas, Y.L. Loukas, Anal. Bioanal. Chem. 384 (2006) 199.
[39] L.A. Moraes, F.E. Lerner, M.E. Moraes, M.O. Moraes, G. Corso, N.G. de, Ther. Drug Monit. 21 (1999) 200.
[40] J.X. Shen, G. Krishna, R.N. Hayes, J. Pharm. Biomed. Anal. 43 (2007) 228.
[41] J.E. Conte Jr., J.A. Golden, G. Krishna, M. McIver, E. Little, E. Zurlinden, Antimicrob. Agents Chemother. 53 (2009) 703.
[42] A. Eerkes, W.Z. Shou, W. Naidong, J. Pharm. Biomed. Anal. 31 (2003) 917.
[43] B.G. Keevil, S. Newman, S. Lockhart, S.J. Howard, C.B. Moore, D.W. Denning, Ther. Drug Monit. 26 (2004) 650.
[44] B.V. Araujo, D.J. Conrado, E.C. Palma, C.T. Dalla, J. Pharm. Biomed. Anal. 44 (2007) 985.
[45] G. Krishna, A. Sansone-Parsons, M. Martinho, B. Kantesaria, L. Pedicone, Antimicrob. Agents Chemother. 51 (2007) 812.