Journal of Chromatography B, 879 (2011) 2051–2056

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Determination of micafungin and anidulafungin in human plasma: UV- or mass spectrometric quantification?

Jens Martens-Lobenhoffer*, Victoria Rupprecht, Stefanie M. Bode-Böger

Institute of Clinical Pharmacology, Otto-von-Guericke University, Magdeburg, Germany

ARTICLE INFO

Article history:
Received 3 February 2011
Accepted 22 May 2011
Available online 30 May 2011

Keywords:
Anidulafungin
Micafungin
HPLC
UV
Mass spectrometry

ABSTRACT

Micafungin and anidulafungin are two newer antifungal drugs from the echinocandine class. They are used as monotherapy or in combination with azole-antifungal drugs. The optimized clinical treatment course for the echinocandin drugs with regard to the different infection types and patient subgroups (renal or hepatic impairment, overweight) is still under debate. Therefore, an easy and rugged assay for these two drugs is highly desirable. We here present a method for the quantification of micafungin or anidulafungin in human plasma, applying protein precipitation as sample preparation, reversed phase separation of the analytes and UV-detection and simultaneous tandem mass spectrometry. Anidulafungin served as I.S. for micafungin quantification and vice versa. The method was validated in the calibration ranges from 0.1 μg/ml to 20 μg/ml for both substances. Intra-day precision and accuracies recorded with the UV-detector were 1.80% and 2.65% for micafungin and 4.30% and 10.44% for anidulafungin at the 0.1 μg/ml level. The respective data at the 1 μg/ml level were 2.25% and −0.83% for micafungin and 4.35% and −1.85% for anidulafungin and at the 20 μg/ml level 0.97% and −2.98% for micafungin and 1.04% and 4.74% for anidulafungin, respectively. With the mass spectrometer, because of the unique properties of the analyte molecules, no acceptable validation results could be achieved. Therefore, the mass spectrometric chromatograms served only as identity confirmation of the observed UV-peaks.

1. Introduction

Micafungin and anidulafungin (chemical structures see Fig. 1) are, beside the more established caspofungin, two newer antifungal drugs from the echinocandin class. They display fungistatic activity against *Aspergillus* spp. and fungicidal activity against most *Candida* spp., including strains that are fluconazole-resistant [1]. They can be administered alone and in combination with an antifungal drug from the azole class [2,3]. After intravenous infusion, the drugs are normally well tolerated and effective. Generally, no dosage adjustments are required in patients with varying degrees of hepatic or renal impairment [4,5]. However, clinical data suggest that dose adjustments are needed for special subgroups of patients, such as infants [6], patients with severe liver dysfunction [7] or overweight or obese patients [8] to achieve effective systemic drug concentrations. Optimized drug concentrations have to avoid levels lower than the minimum inhibitory concentration (MIC) of the pathogen, as well as to high concentrations because of the so-called "Eagle effect". This is described as a paradoxical effect on the growth rates of pathogens at increasing drug concentration levels, which is reported for caspofungin and micafungin [9] and may also apply to anidulafungin. The optimized clinical treatment course for the echinocandin drugs with regard to the different infection types and patient subgroups is still under debate [10]. Thus, to facilitate dosage adjustments and to gain further insight in the clinical application of the echinocandin drugs, easy and precise methods for the determination of these drugs in human plasma are of great interest.

In the literature, some HPLC-fluorescence methods for the quantitative determination of micafungin in plasma were reported [7,11–16]. The simultaneous detection of two active metabolites of micafungin was also described [15,16]. However, because of their low concentrations in plasma, these metabolites were regarded as of no therapeutic relevance [15]. In general, there were only minor variations between these methods. Additionally, a tandem mass spectrometric method has been described in short [17], but does not give any validation data. In the case of anidulafungin, quantitative detection in plasma with HPLC UV-detection [4,18] and with mass spectrometric detection [2,19] was described. In all of the cited papers, with the exception of Zornes and Stratford [18], the focus was on clinical investigation of the echinocandin drugs and the description of the analytical method and its validation remained marginal. A dedicated publication described the simultaneous detection of micafungin and anidulafungin, together with caspo-

* Corresponding author at: Otto-von-Guericke Universität, Institut f. Klinische Pharmakologie, Leipziger Str. 44, 39120 Magdeburg, Germany.
Tel.: +49 391 671 3067; fax: +49 391 671 3062.
E-mail address: jens.martens-lobenhoffer@med.ovgu.de
(J. Martens-Lobenhoffer).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.05.033

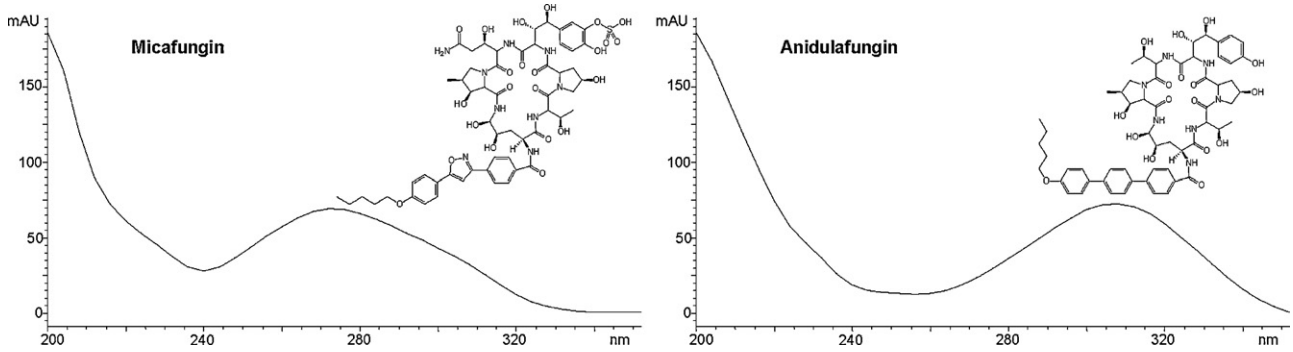

Fig. 1. Chemical structures and UV-spectra of micafungin and anidulafungin.

fungin and various azole antifungal drugs, utilizing HPLC–mass spectrometry [20]. However, this method suffered from the conflictive molecular nature of caspofungin (basic) and micafungin (acidic). Thus, the authors were able to optimize the chromatographic method only for caspofungin and the neutral anidulafungin, whereas micafungin resulted in distorted and broad peaks. This problem was avoided by Decosterd et al. [21] by excluding micafungin in their UPLC–mass spectrometric multiplex method for the quantification of anidulafungin and caspofungin together with various azol-antifungal drugs. In the analytical methodology of echinocandines, the choice of the I.S.'s proved to be difficult. Either the I.S.'s had no structural similarity to the analyte [2,21] or were custom synthesized analogs, which were not commercially available [4,7,12–15,17–20]. This makes it difficult to establish such a method outside a dedicated clinical study. For an everyday rugged routine analytical method, an easily available I.S. with similar physicochemical properties would be much more desirable.

Here, we described the development and validation of a method for the quantitative detection of micafungin or anidulafungin in human plasma, which is easy, precise and accurate. In the case of the quantification of micafungin, anidulafungin served as I.S., and vice versa. All other method parameters were identical for both substances. The method featured sample preparation by protein precipitation and chromatogragphic separation on a reversed phase column. We compared the performance characteristics of UV-detection and tandem mass spectrometric detection to investigate their respective pros and cons in everyday laboratory routine.

2. Materials and methods

2.1. Instrumentation

The HPLC system consisted of an Agilent 1100 system (Waldbronn, Germany) comprising a binary pump, an autosampler, a thermostatted column compartment and a diode array UV–vis detector. The analytical column was a Zorbax Eclipse XDB-C18 150 mm × 2.1 mm with 3.5 μm particle size (Agilent Technologies, Böblingen, Germany), protected by a SecurityGuard system (Phenomenex, Aschaffenburg, Germany) equipped with a 4 mm × 2 mm C18 filter insert. The mass spectrometric detection was performed on a Thermo Fisher Scientific TSQ Discovery Max triple quadrupole mass spectrometer (San Jose, CA, USA), equipped with an ESI ion source.

2.2. Chemicals

Reference substance of micafungin (Lot No. 122320KA, purity >98.5%, potency = 93.3%) was a kind gift of Astellas Pharma Inc. (Ibaraki, Japan) whereas reference substance of anidulafungin (Lot No. PF-3910960-0002, purity >98%, potency = 82.4%) was a kind gift of Pfizer Inc. (Groton, CT, USA). Ultra pure water was produced in our laboratory by a Barnstead EASYpure UV-system (Werner, Leverkusen, Germany). Drug free human plasma was obtained from the blood bank of the University Hospital Magdeburg (Germany). All other chemicals were of analytical grade or better.

2.3. Patient samples

Patient samples were obtained in the course of therapeutic drug monitoring during standard antifugal therapy with micafungin or anidulafungin, respectively. Steady state dosing was 100 mg/day for micafungin as well as for anidulafungin in all subjects studied. Blood samples were drawn into vacuum tubes without additives 30 min after the end of the infusion (C_{max}) and immediately prior to the next application (C_{min}). The blood samples were allowed to clot for 30 min and blood cells were separated by centrifugation at 1400 × *g* for 10 min. The serum samples were immediately frozen at −80 °C until analysis.

2.4. Stock solutions of micafungin and anidulafungin

Stock solutions were prepared as following: 5.34 mg of micafungin reference substance or 6.07 mg of anidulafungin reference substance were dissolved in 10 ml methanol/water 50/50 (v/v), respectively. The achieved concentrations in these stock solutions were 500 μg/ml in both cases. The stock solutions were stored at −80 °C until usage.

2.5. Calibration and quality control samples

To prepare calibration and quality control samples, 200 μl of the stock solution of micafungin or anidulafungin was diluted with 800 μl water to produce working solution A with the concentration of 100 μg/ml. A further dilution by factor 10 with water resulted in working solution B with a concentration of 10 μg/ml. By spiking 990, 980, 950 or 900 μl of drug free plasma with 10, 20, 50 or 100 μl of working solution B, respectively, and 980, 950, 900 or 800 μl drug free plasma with 20, 50 100 or 200 μl working solution A, plasma calibration samples with the concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg/ml were produced. Quality control samples were prepared in a similar way in the concentrations of 0.1, 1 and 20 μg/ml. Calibration samples and quality control samples were prepared separately for the quantification of micafungin and anidulafungin, respectively.

2.6. Sample preparation

The sample preparation for both substances was identical. To 100 μl calibration sample, quality control sample or patient sample 20 μl of the I.S. solution (anidulafungin 10 μg/ml in the case

of micafungin quantification, or micafungin 10 μg/ml in the case of anidulafungin quantification) was added. To precipitate the plasma proteins, 200 μl acetonitril was added and the samples were centrifuged at 11000 × *g* for 5 min. From the clear supernatant, 100 μl was diluted with 100 μl of HPLC mobile phase A (see next section). The prepared samples were transferred into autosampler vials with microliter inserts and were ready for injection into the HPLC system.

Initial experiments were performed with solid phase extraction on OASIS HLB mixed mode cartridges (Waters, Eschborn, Germany). The cartridges were loaded with 500 μl Plasma, washed with 1 ml water and 1 ml water:methanol 50:50 (v:v) and finally eluted with 1 ml methanol:ammonium hydroxide 98:2 (v:v).

2.7. Chromatography and detection

The mobile phase A for the HPLC separation consisted of 0.1% (w/w) ammoniumacetat in water, with the ph adjusted to 7.0 with 25% NH_3 solution. Mobile phase B was acetonitrile. The gradient elution took place at a constant flow rate of 0.35 ml/min starting with a composition of 70% mobile phase A and 30% mobile phase B. The ratio changed in 11 min to 35% A and 65% B and was held constant until run stop at 17 min. A post-run time of 4 min was necessary to re-equilibrate the system back to starting conditions. The separation took place at 35 °C and an injection volume of 25 μl was applied.

The effluent was monitored at the two UV-wavelengths of 273 nm and 306 nm, which represent the absorption maxima of micafungin and anidulafungin, respectively. Subsequently, the effluent was directed without splitting into the ESI source of the mass spectrometer. Ionization took place in positive mode, with sheath gas and auxiliary gas settings at 49 and 24 arbitrary units, respectively, and a capillary temperature of 250 °C. Micafungin and anidulafungin were monitored in the selected reaction monitoring mode with a parent-fragment ion transitions of *m/z* 1271 → 1191 and *m/z* 1271 → 1173 for micafungin and *m/z* 1141 → 1123 for anidulafungin, respectively. The resolutions of the quadrupole mass filters were set to 2.5 amu.

2.8. Stability

To assess the stability of micafungin and anidulafungin in plasma, quality control samples ($n = 6$) were measured after 3 freeze-thaw cycles and repeatedly over a time range of 24 h at room temperature. Stability of micafungin and anidulafungin in prepared samples at room temperature was tested by comparing the quantitative results or freshly prepared samples with repeated injections of the same samples after 24 h.

3. Results and discussion

3.1. Sample preparation and extraction yield

The only sample preparation step in the here presented method for the quantification of micafungin and anidulafungin from plasma was protein precipitation by the addition of acetonitril. This is also the preferred sample preparation procedure reported in the literature. Only Zornes and Stratford [18] applied SPE for extraction and cleanup. The well-known absorption problems of echinocandines [22] and our own unsuccessful initial experiences with SPE extraction of micafungin or anidulafungin, where we achieved very low extraction yields of about 40%, discouraged us from further investigation in this matter. However, the simple protein precipitation resulted in nearly quantitative extraction yields and extracts clean enough for successful quantification. For micafungin, the extraction yields at concentrations of 0.1, 1 and 20 μg/ml were 96.01 ± 12.82%, 91.63 ± 4.31% and 92.37 ± 3.27%, respectively. The analogous results for anidulafungin at the same concentrations were 93.36 ± 7.29%, 98.31 ± 12.64% and 93.44 ± 2.04%, respectively.

3.2. Chromatography and detection

The chromatographic separation of echinocandin drugs on reversed phases columns is well established. However, the chromatographic behavior of micafungin was strongly dependent on the pH of the mobile phase. At acidic pH, the sulfonic acid moiety got partially de-ionized, which resulted in longer retention times and distorted peak shapes [20]. In contrast, at neutral pH, the acidic function was fully ionized, leading to symmetric peak shapes and shorter retention times. The neutral anidulafungin was not responsive to pH changes and its retention time and peak shape remained unchanged. A gradient elution was employed to achieve good separation from endogenous substances as well as elution of both substances at reasonable retention times. Under the described conditions, the retention times of micafungin and andiulafungin were 7.8 min and 11.9 min, respectively (Figs. 2 and 3).

As can be seen in Fig. 1, both micafungin and anidulafungin displayed distinctive UV-spectra with absorption maxima of 273 nm and 306 nm, respectively. These relatively long wavelengths enabled selective detections undisturbed from endogenous substances. In the retention time window of interest, no peaks from substances other than micafungin and anidulafungin showed up in blank samples (Fig. 2) or in patient samples (Fig. 3). However, for both substances, a smaller peak at a shorter retention times (7.2 min and 10.3 min, respectively) was detected. These peaks displayed nearly identical UV-spectra in comparison to their related substances micafungin and anidulafungin and slowly increased in intensity if the samples were left at room temperature for extended time ranges of 12 or more hours (see Section 3.5, stability). Most probably, theses peaks related to decomposition products of micafungin and anidulafungin or to diastereomeric analogs of their parent compounds.

The mass spectrometric detection and quantification of micafungin and anidulafungin held various difficulties. The molecules were large enough not only to be ionized in the ESI source to the single protonated quasimolecular ions ($[M+H]^+$, *m/z* 1270.4 and *m/z* 1141.5 for micafungin and anidulafungin, respectively) but also to the double protonated quaismolecular ions ($[M+2H]^{2+}$, *m/z* 635.7 and *m/z* 570.8, respectively). The relative intensities for the single and double protonated species were strongly dependent on the exact ESI conditions (flow rate, temperatures, pH, composition of the mobile phase at the moment of elution). Moreover, the high number of carbon atoms in the molecules (56 for micafungin and 58 for anidulafungin) led to isotope distribution pattern of about 70% intensity of the $[M+H+1]^+$ and 25% of the $[M+H+2]^+$ peaks. To avoid intensity losses, the mass resolution of the quadropoles had to be set to 2.5 amu centered on the $[M+H+1]^+$ peak of the isotope cluster. This low-resolution setting of the quadrupoles provided the full intensity of the signals but sacrifices some of the selectivity, which is normally a main feature of a mass spectrometer. As it can be seen in Figs. 2 and 3, the additional peaks from the decomposition products of micafungin and anidulafungin detected in the UV-chromatograms also showed up in the mass spectrometric chromatograms, featuring nearly identical mass spectra as their parent compounds. This fact underlined the strong relationship of the compounds. They may either be diastereomeric analogs or hydrolytic ring opening products, which may loose their additional water molecule during the ionization process inside the ion source. With respect to these additional peaks, the mass spectrometer did not provide better selectivity than the UV-detector. The small time offset in the retention times of about 0.1 min between the peaks in the UV-

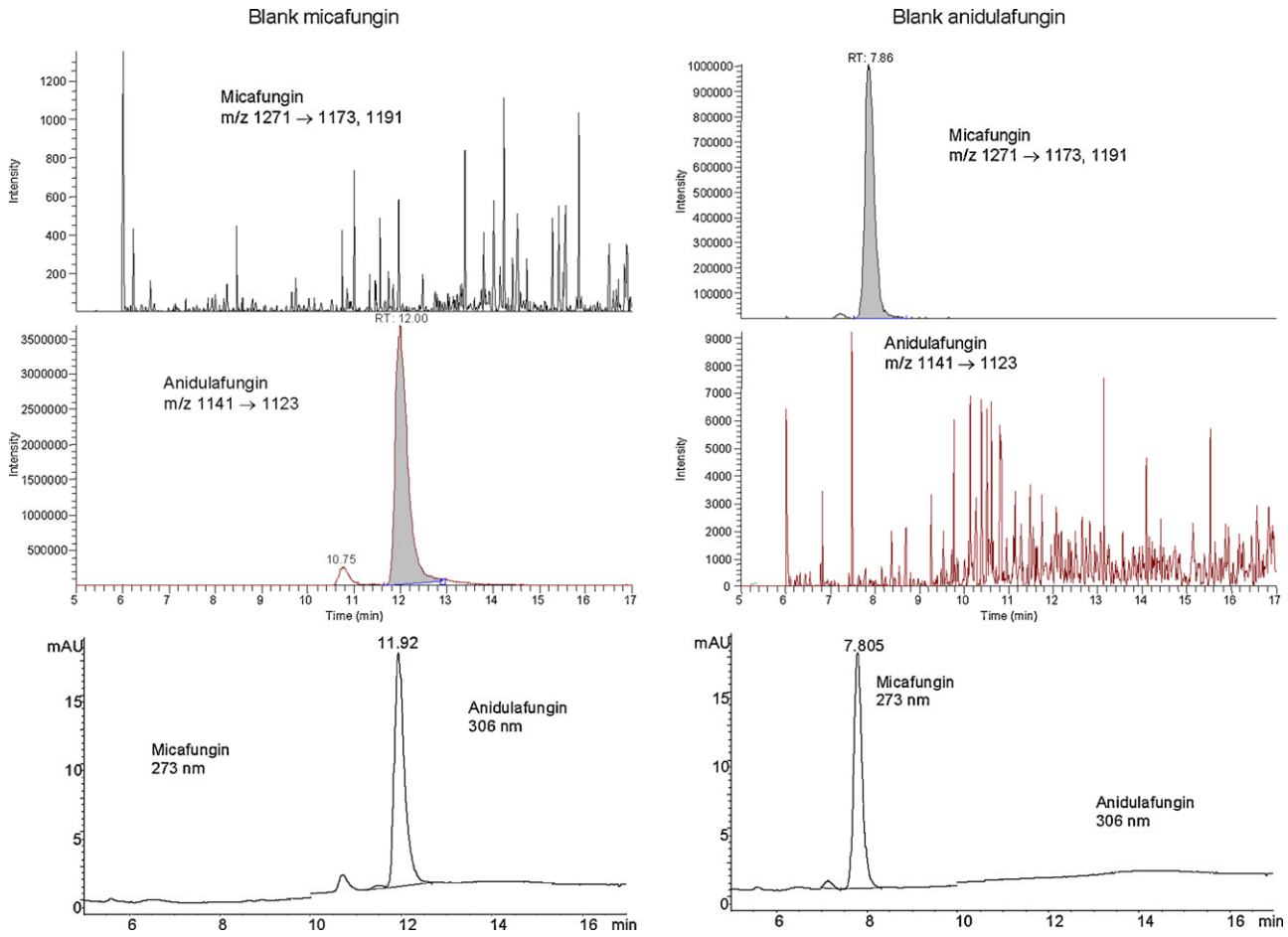

Fig. 2. Left 3 panels: chromatograms of a blank sample for micafungin; right 3 panels: chromatograms of a blank sample for anidulafungin. Panles from top to bottom: mass spectrometric trace of micafungin, mass spectrometric trace of anidulafungin and UV-chromatogram.

chromatogram and the MS/MS-chromatogram resulted from the additional tubing between the UV-detector and the mass spectrometer.

3.3. Calibration

From the UV-data, the $1/y$ weighed least square calibration functions were linear for both micafungin and anidulafungin in the range of 0.1–20 μg/ml. The regression parameters were $r = 0.9994$, slope = 0.0451 and an insignificant intercept ($p = 0.252$) for micafungin and $r = 0.9990$, slope = 0.0556 and an insignificant intercept ($p = 0.210$) for anidulafungin, respectively. The insignificant intercepts of the calibration functions confirmed that no absorption or other losses of the analytes occurred during the preparation of the calibration samples or during the sample preparation process. This is in contrast to the analogous echinocandine caspofungin, where strong absorption effects must be prevented by the addition of albumin or formic acid to each solution or HPLC mobile phase [22].

Unfortunately, the respective calibration functions using the mass spectrometer were not linear. For both substances, at higher concentrations the response was lower than expected by a linear function. Best fits were achieved using a quadratic polynomial function. The calibration fits resulted in the parameters $y = 0.0003x^2 + 0.015x$ for micafungin and $y = -0.0153x^2 + 0.5867x$ for anidulafungin, respectively. Such non-linear calibration functions pointed to problems in the ionization process in the ESI source such as self-limiting ionization efficiency or non-constant ratios of the single and double protonated quasimolecular ions with respect to analyte concentrations. With non-linear calibration functions it was very difficult to achieve acceptable performances regarding precision and accuracy, so further efforts to validate the quantification of micafungin and anidulafungin with the mass spectrometer were abandoned.

3.4. Precision and accuracy

In Table 1, the precision and accuracy data produced by the UV-detector are summarized. As can be seen, all parameters were inside the requirements issued by the US Federal Drug Administration for biological method validation. Using the mass spectrometer for quantification, far inferior results were achieved, especially the accuracy deviated from the expected results up to 50% (data not shown). These results reflected the problems with the non-linear and unstable calibration functions described in Section 3.3. Thus, the mass spectrometric detection proved unfit to produce valid quantitative measurements for micafungin or anidulafungin.

3.5. Stability

The stability of micafungin and anidulafungin was assessed with respect to the requirements of therapeutic drug monitoring. In plasma samples, the stability at room temperature and at −20 °C was investigated. Stored at room temperature for 24 h, the declines in concentrations were 24.4% ($p = 0.001$) for micafungin and 18.9% ($p < 0.001$) for anidulafungin, respectively. At −20 °C, no significant

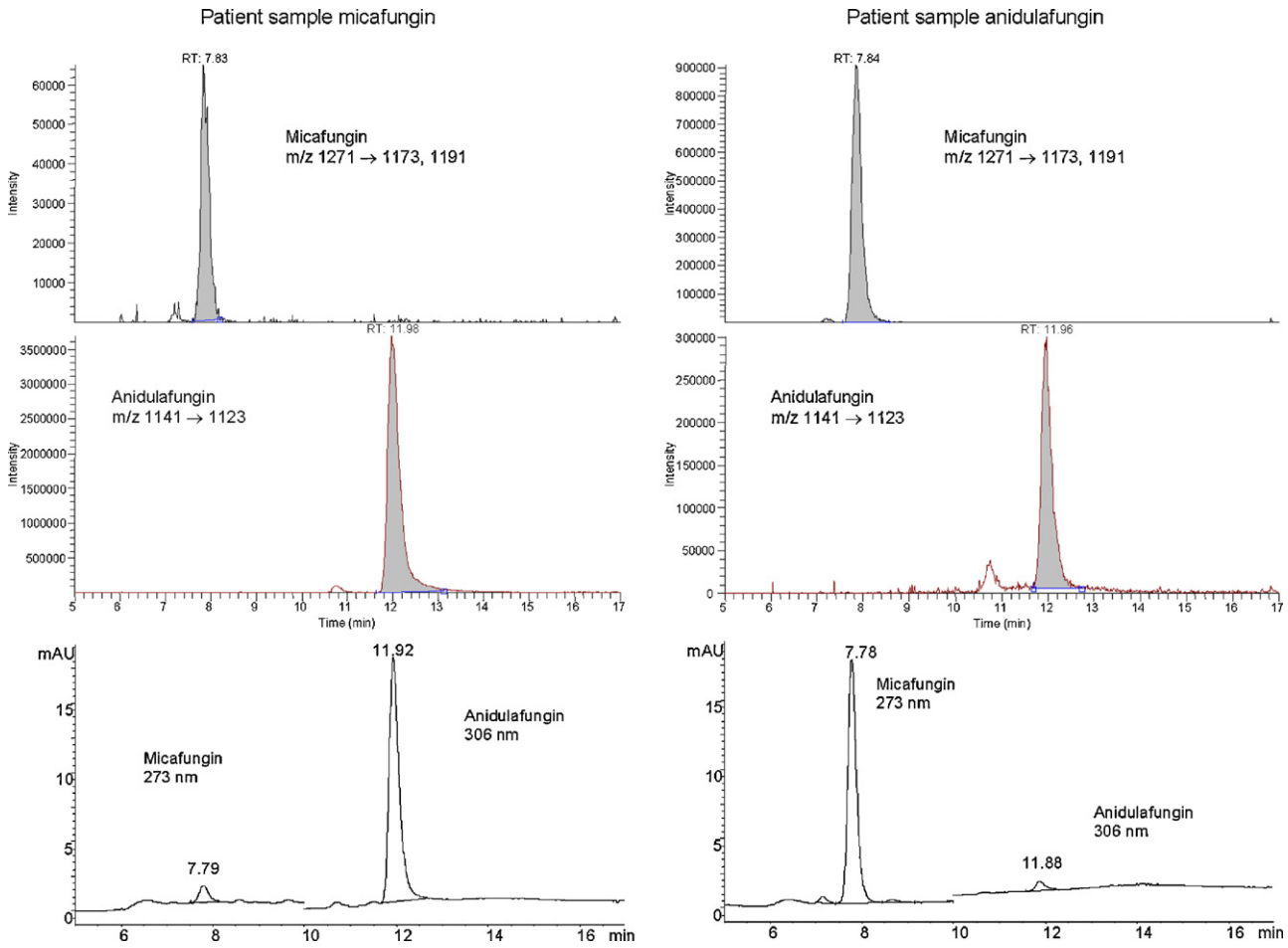

Fig. 3. Left 3 panels: chromatograms of a sample from a patient receiving micafungin 100 mg/d (anidulafungin serves as I.S.), found concentration of micafungin was 1.54 μg/ml; right 3 panels: chromatograms of a sample of a patient receiving anidulafungin 100 mg/d (micafungin serves as I.S.), found concentration of anidulafungin was 0.94 μg/ml. Panels from top to bottom: mass spectrometric trace of micafungin, mass spectrometric trace of anidulafungin and UV-chromatogram.

concentration changes could be observed during 24 h for both substances. Repeated freeze-thaw cycles also had no significant effect on the concentrations of micafungin or anidulafungin. Furthermore, long-term stability of micafungin at −20 °C has been already established [18]. Conclusively, care has to be taken that the samples arrive in the laboratory as soon as possible or that the samples are frozen during the transport.

Relevant for rugged analysis was also the stability of micafungin and anidulafungin in prepared samples on the autosampler of the HPLC-system. After 24 h, the absolute concentration of micafungin and anidulafungin declined for 6.47% and 11.89%, respectively. Since micafungin served as I.S. for anidulafungin, and vice versa, theses concentration losses over time compensated for each other to a certain degree. Hence, the calculated quantitative results showed lower deviations of −5.06% ($p = 0.09$) and 5.43% ($p = 0.11$) for micafungin and anidulafungin, respectively. Such differences could be regarded as insignificant and therefore the samples were sufficiently stable for 24 h on the autosampler.

3.6. Matrix independency

The described method was used in human plasma as well as in serum samples. Therefore, it has to be demonstrated that there were no significant differences in the chromatographic behavior and quantitative response between these two matrices. As it can be seen in Fig. 2 (plasma) and Fig. 3 (serum), there were no apparent differences in the chromatograms regarding peak shape or interfering peaks from endogenous substances as well in the UV-detector or in the mass spectrometer. To verify that the responses of micafungin and anidulafungin were matrix independent, peak areas from these substances were compared in plasma and in serum samples. As it turns out, no significant differences between plasma

Table 1
Intra- and inter-day precision and accuracy.

Analyte	Concentration (μg/ml)	Intra-day precision and accuracy				Inter-day precision and accuracy			
		n	Mean (μg/ml)	R.S.D (%)	Accuracy (%)	*n*	Mean (μg/ml)	R.S.D (%)	Accuracy (%)
Micafungin	0.1	10	0.10	1.80	2,65	5	0.11	9.27	8.09
	1.0	10	0.99	2.25	−0.83	5	0.97	3.39	−3.35
	20.0	10	19.4	0.97	−2.98	5	19.59	3.81	−2.03
Anidulafungin	0.1	10	0.11	4.30	10.44	5	0.11	8.36	12.57
	1.0	10	0.98	4.35	−1.85	5	1.04	7.45	4.40
	20.0	10	21.1	1.04	4.74	5	19.87	4.45	−0.63

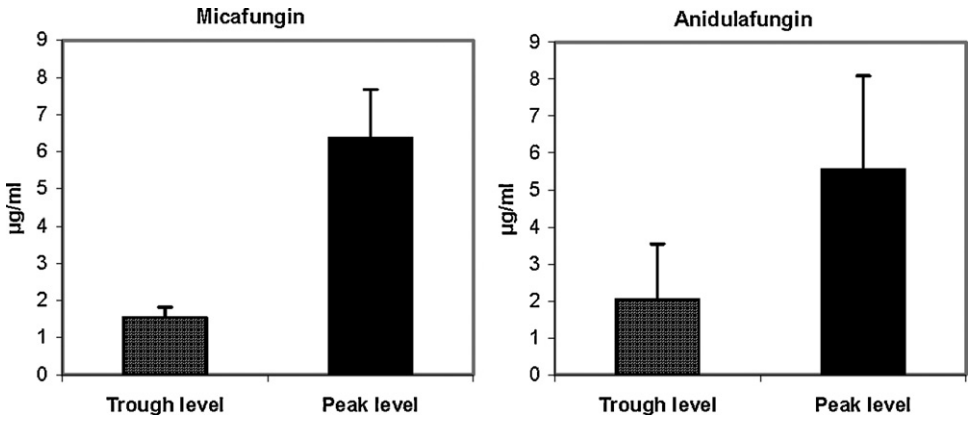

Fig. 4. Peak and trough levels of patients receiving 100 mg/day micafungin ($n = 2$) or 100 mg/day anidulafungin ($n = 5$).

and serum samples, applying an unpaired Student's *T*-test, could be observed ($n = 13$, $p = 0.191$ for micafungin and $n = 10$, $p = 0.209$ for micafungin, respectively). From these results, an independency with regard to the matrices plasma or serum could be confirmed, as it could be assumed for the stable and matrix independent UV-detector.

4. Application of the method

The here-described method has been applied for therapeutic drug monitoring of micafungin or anidulafungin in patients suffering from systemic fungal infections. In Fig. 4 the trough and peak concentration levels of some patient samples are summarized. All patients received the standard doses of 100 mg/day micafungin or anidulafungin. As it turns out, all concentrations were inside the calibration range and the concentration difference between trough and peak level were in good agreement to the reported half lives of micafungin and anidulafungin of 14.6 h and 20.8 h, respectively [13,23]. In the real patient samples, no interferences from endogenous substances or coadministered drugs were observed in the UV-chromatograms. The simultaneously recorded mass spectrometric chromatograms confirmed the identity of the chromatographic peaks (Fig. 3). Therefore, rugged analytical performance of the method was confirmed.

5. Conclusion

During the development of the method, it became increasingly apparent that a stable and precise mass spectrometric quantification of micafungin and anidulafungin would be very difficult to achieve. Especially the non-linear calibration functions and the insufficient precision and accuracy of the quality control samples made the mass spectrometric quantification invalid. On the other hand, micafungin and anidulafungin feature distinctive UV-spectra, which made selective detection and quantification with a UV-detector possible. The validation results and the experiences with real patient samples affirmed that in everyday practice the UV-detector based method is rugged, easy, precise and accurate. If a simultaneous mass spectrometric detection is affordable, it can serve as an additional tool for the affirmation of the identity of the peaks in the UV-chromatogram.

The method does not rely on difficult to obtain I.S.'s. Instead, anidulafungin serves as I.S. for micafungin and vice versa. In clinical practice, it can be excluded that both drugs are administered at the same time to the same patient, so no systematically errors in quantification should be expected. The method can be performed on a standard HPLC-system and can therefore serve as an everyday analytical tool for the monitoring of severely ill patients on antifungal therapy with echinocandine drugs.

References

[1] A.J. Sucher, E.B. Chahine, H.E. Balcer, Ann. Pharmacother. 43 (2009) 1647.
[2] J.A. Dowell, J. Schranz, A. Baruch, G. Foster, J. Clin. Pharmacol. 45 (2005) 1373.
[3] J. Hiemenz, P. Cagnoni, D. Simpson, S. Devine, N. Chao, J. Keirns, W. Lau, D. Facklam, D. Buell, Antimicrob. Agents Chemother. 49 (2005) 1331.
[4] J.A. Dowell, M. Stogniew, D. Krause, B. Damle, J. Clin. Pharmacol. 47 (2007) 461.
[5] Y. Nakagawa, Y. Ichii, Y. Saeki, M. Kodama, S. Kishino, K. Suzuki, Eur. J. Drug Metab. Pharmacokinet. 33 (2008) 191.
[6] W.W. Hope, P.B. Smith, A. Arrieta, D.N. Buell, M. Roy, A. Kaibara, T.J. Walsh, M. Cohen-Wolkowiez, D.K. Benjamin Jr, Antimicrob. Agents Chemother. 54 (2010) 2633.
[7] Y. Muraki, T. Iwamoto, Y. Kagawa, H. Sakurai, M. Usui, S. Isaji, S. Uemoto, M. Okuda, Biol. Pharm. Bull. 32 (2009) 750.
[8] T. Gumbo, Curr. Opin. Infect. Dis. 20 (2007) 587.
[9] M. Fleischhacker, C. Radecke, B. Schulz, M. Ruhnke, Eur. J. Clin. Microbiol. Infect. Dis. 27 (2008) 127.
[10] M.W. Pound, M.L. Townsend, R.H. Drew, J. Antimicrob. Chemother. 65 (2010) 1108.
[11] A.H. Groll, D. Mickiene, V. Petraitis, R. Petraitiene, K.H. Ibrahim, S.C. Piscitelli, I. Bekersky, T.J. Walsh, Antimicrob. Agents Chemother. 45 (2001) 3322.
[12] G.P. Heresi, D.R. Gerstmann, M.D. Reed, J.N. van den Anker, J.L. Blumer, L. Kovanda, J.J. Keirns, D.N. Buell, G.L. Kearns, Pediatr. Infect. Dis. J. 25 (2006) 1110.
[13] M.F. Hebert, H.E. Smith, T.C. Marbury, S.K. Swan, W.B. Smith, R.W. Townsend, D. Buell, J. Keirns, I. Bekersky, J. Clin. Pharmacol. 45 (2005) 1145.
[14] S. Shimoeda, S. Ohta, H. Kobayashi, H. Saitou, A. Kubota, S. Yamato, K. Shimada, M. Sasaki, K. Kawano, Biol. Pharm. Bull. 28 (2005) 477.
[15] K. Tabata, M. Katashima, A. Kawamura, Y. Tanigawara, K. Sunagawa, Biol. Pharm. Bull. 29 (2006) 1706.
[16] Y. Yamato, H. Kaneko, K. Tanimoto, M. Katashima, K. Ishibashi, A. Kawamura, M. Terakawa, A. Kagayama, Jpn. J. Chemother. 50 (2002) 68.
[17] P.B. Smith, T.J. Walsh, W. Hope, A. Arrieta, A. Takada, L.L. Kovanda, G.L. Kearns, D. Kaufman, T. Sawamoto, D.N. Buell, D.K. Benjamin Jr, Pediatr. Infect. Dis. J. 28 (2009) 412.
[18] L.L. Zornes, R.E. Stratford, J. Chromatogr. B 695 (1997) 381.
[19] J.L. Crandon, M.A. Banevicius, A.F. Fang, P.H. Crownover, R.F. Knauft, J.S. Pope, J.H. Russomanno, E. Shore, D.P. Nicolau, J.L. Kuti, Antimicrob. Agents Chemother. 53 (2009) 5102.
[20] F. Farowski, O.A. Cornely, J.J. Vehreschild, P. Hartmann, T. Bauer, A. Steinbach, M.J. Ruping, C. Muller, Antimicrob. Agents Chemother. 54 (2010) 1815.
[21] L.A. Decosterd, B. Rochat, B. Pesse, T. Mercier, F. Tissot, N. Widmer, J. Bille, T. Calandra, B. Zanolari, O. Marchetti, Antimicrob. Agents Chemother. 54 (2010) 5303.
[22] H. Egle, R. Trittler, K. Kümmerer, Rapid Commun. Mass Spectrom. 18 (2004) 2871.
[23] D.K. Benjamin Jr, T. Driscoll, N.L. Seibel, C.E. Gonzalez, M.M. Roden, R. Kilaru, K. Clark, J.A. Dowell, J. Schranz, T.J. Walsh, Antimicrob. Agents Chemother. 50 (2006) 632.