Background: Under certain circumstances, clinicians treating patients with isavuconazole for invasive aspergillosis or mucormycosis may use therapeutic drug monitoring. However, the accuracy and reproducibility of the various assays used by different laboratories for the quantification of isavuconazole plasma concentrations have yet to be determined.

Methods: Human plasma samples spiked with known concentrations of isavuconazole were provided to 27 European laboratories that took part in a “round-robin” test (an interlaboratory test performed independently at least 2 times; 2 rounds performed in the current study). Assay methods included liquid chromatography–tandem mass spectrometry (LC-MS/MS), LC with ultraviolet detection (LC-UV), LC with fluorescence detection (LC-FL), and bioassay. The accuracy and reproducibility compared with the known concentrations for each sample in each round were compared overall, between assays, and between laboratories.

Results: Twenty-seven laboratories participated in the study (LC-MS/MS, n = 15; LC-UV, n = 9; LC-FL, n = 1; bioassay, n = 2). In round 1, for nominal concentrations of 1000, 1700, 2500, and 4000 ng/mL, the mean (SD) determined concentrations were 1007 (183), 1710 (323), 2528 (540), and 3898 (842) ng/mL, respectively. In round 2, for nominal concentrations of 1200, 1800, 2400, and 4000 ng/mL, the mean (SD) determined concentrations were 1411 (303), 2111 (409), 2789 (511), and 4723 (798) ng/mL, respectively. Over both rounds, determined concentrations were consistently within 15% of the nominal concentrations for 10 laboratories (LC-MS/MS, n = 4; LC-UV, n = 5; bioassay, n = 1) and consistently exceeded the upper 15% margin for 7 laboratories (LC-MS/MS and LC-UV, n = 3 each; LC-FL, n = 1).

Conclusions: Alignment of methodologies among laboratories may be warranted to improve the accuracy and reproducibility of therapeutic drug measurements.

Key Words: bioassay, isavuconazole, LC-FL, LC-MS/MS, LC-UV

INTRODUCTION

Triazole antifungal agents are an important class of drugs for the prophylaxis and treatment of invasive fungal infections, which are associated with high morbidity and mortality in immunocompromised patients. However, maintenance of safe and efficacious plasma or serum concentrations of these drugs can sometimes necessitate therapeutic drug monitoring (TDM). Factors inherent to a drug that dictate the need for TDM include a high level of intraindividual and interindividual variability of pharmacokinetics, a defined therapeutic range, and a narrow therapeutic window. Isavuconazole is the active moiety of the prodrug isavuconazonium sulfate, the newest available triazole antifungal agent with demonstrated efficacy and safety for the treatment of invasive aspergillosis or mucormycosis in adults. An early phase study demonstrated good predictability of the dose-exposure relationship, in a population-pharmacokinetics analysis using data from the phase IIIC.
SECURE trial in adult patients with invasive aspergillosis and other invasive mold diseases, no relationship of exposure with either efficacy or elevations/abnormalities in liver enzyme test results was found. This suggests that, in most adults, the clinical dose of isavuconazole results in exposures that are within the optimal therapeutic window, and so, routine TDM of isavuconazole may not be necessary. Nevertheless, the margins of the therapeutic window remain to be defined, and it has yet to be determined if and when TDM might be required during isavuconazole treatment.

Reliable interpretation of the results of TDM requires that the analytical assays are both accurate and reproducible. Several laboratories have published or presented methods for the analysis of isavuconazole concentrations using liquid chromatography with tandem mass spectrometry (LC-MS/MS) detection,\textsuperscript{8–12} LC with ultraviolet detection (LC-UV),\textsuperscript{13–15} or LC with fluorescence detection (LC-FL).\textsuperscript{16} However, it has not been defined how the results of such analyses compare, both with respect to the different analytical methods used and to the interlaboratory differences using the same techniques. Therefore, we conducted a round-robin exercise (interlaboratory test performed independently at least 2 times) in 27 European laboratories to assess the accuracy and reproducibility of measurements of isavuconazole concentration measurements.

MATERIALS AND METHODS

This study commenced September 2016 and completed July 2017.

Preparation of Samples

All samples were prepared by Basilea Pharmaceutica (Basel, Switzerland). A 5-mg/mL stock solution was prepared by dissolving 7.102 mg of isavuconazole in 1.284 mL of dimethyl sulfoxide (DMSO). The stock solution was then diluted to prepare spiking solutions of 100,000, 170,000, 250,000, 400,000 ng/mL (round 1) or 120,000, 180,000, 240,000, 400,000 ng/mL (round 2) in a 1/1 solution of DMSO/acetonitrile + 0.05% trifluoroacetic acid (DMSO/ACN/TFA). Samples for the round robin were prepared by addition of 5 μL of the spiking solutions to 495-μL samples of human plasma containing K3-EDTA as anticoagulant. Final concentrations of samples used were based on the clinically relevant range of plasma concentrations for isavuconazole. Concentrations in round 1 were as follows: sample 1, 1000 ng/mL; sample 2, 1700 ng/mL; sample 3, 2500 ng/mL; and sample 4, 4000 ng/mL. Concentrations in round 2 were as follows: sample 1, 1200 ng/mL; sample 2, 1800 ng/mL; sample 3, 2400 ng/mL; and sample 4, 4000 ng/mL. For each round, all plasma samples of a given concentration were mixed together and re-aliquoted before distribution, such that each laboratory effectively received the same sample.

Laboratories and Testing

European laboratories with the facilities for and expertise in drug-concentration assays using LC-MS/MS, LC-UV, LC-FL, or bioassays based on diffusion of the drug into inoculated and incubated media were identified and invited to participate in the study. All laboratories included in the study were required to have developed and validated the assays used in this study before inclusion. Participating laboratories, listed in Supplemental Digital Content 1 (see Table S1, http://links.lww.com/TDM/A319), were provided with samples in the first and/or second round of the test. Details of some of the analytical methods used have been published previously,\textsuperscript{8–10,13,15,16} others were adapted from methods established for the detection of other triazole antifungal agents\textsuperscript{18–20} or used/adopted a commercially available assay.\textsuperscript{21} All samples for each round were also tested by the Basilea Pharmaceutica bioanalytical laboratory using LC-MS/MS. Details of the methods used by Basilea Pharmaceutica and as provided by each laboratory are included in the Supplemental Digital Content 1 (see Methods, http://links.lww.com/TDM/A319). Results from Basilea Pharmaceutica were included in statistical analyses but were not considered when comparing different methods or laboratories.

Statistics

To determine the accuracy and precision of measurements made in each round, the mean, standard deviation (SD), and bias (percent deviation from nominal concentration) were calculated for all samples and for each assay method. The results were assessed with respect to the European Medicines Agency\textsuperscript{22} and the US Food and Drug Administration\textsuperscript{23} guideline requirements of accuracy (bias) and precision (relative SD) within a 15% limit.

RESULTS

In total, 34 laboratories in Germany, United Kingdom, Netherlands, Italy, France, Austria, Switzerland, and Denmark were invited to participate in the round-robin test. Of these, 27 laboratories provided results for the determination of isavuconazole concentrations in human plasma (see Table S1, Supplemental Digital Content 1, http://links.lww.com/TDM/A319) round 1, laboratories 1–10; and round 2, laboratories 11–27. The methods used for the determination were LC-MS/MS (round 1: laboratories 1–5; round 2: laboratories 11–20), LC-UV (round 1: laboratories 6–8; round 2: laboratories 21–26), bioassays (round 1: laboratories 9, 10; round 2, laboratory 10), and LC-FL (round 2: laboratory 27).

In round 1, the mean of determined values for the assessments from all laboratories for each of the samples with nominal concentrations of 1000, 1700, 2500, and 4000 ng/mL were within the 15% margins, although the SDs extended beyond those margins (Fig. 1). The overall biases at each of these concentrations were 0.7%, 0.6%, 1.1%, and −2.5%, respectively. Among all 40 samples assessed by the 10 laboratories, 29 determined concentrations were within 15% of the nominal concentrations (acceptance criterion), 7 were above the upper 15% margin, and 4 were below the lower 15% margin.

The results were also assessed as a function of the analytic method. For LC-MS/MS (laboratories 1–5), the mean of determined values for each nominal concentration was again within the 15% margins, although the SDs exceeded the upper margin at all tested concentrations (Fig. 2). Determined values from 2 laboratories were consistently within the 15% margins and those from 1 laboratory consistently...
exceeded the upper 15% margin. The overall biases at each of the tested concentrations (1000–4000 ng/mL) were 4.3%, 7.8%, 9.5%, and 6.9%, respectively. In total, 13/20 determined concentrations were within the 15% margins. The remaining 7/20 concentrations exceeded the upper 15% margin, including 1 sample for laboratory 2, 2 samples for laboratory 4, and all samples for laboratory 5. Determined values from the 3 laboratories that used LC-UV were consistently within 15% of the nominal concentrations (Fig. 2). The overall biases at each of the tested concentrations were 7.3%, 3.9%, 7.5%, and 0.2%, respectively. Of the 2 laboratories that used bioassays, all values determined from laboratory 9 were within 15% of the nominal concentrations, whereas those from laboratory 10 were all well below the lower 15% margin for all samples (overall bias at each concentration, -20%, -26.2%, -33.6%, and -34.5%, respectively) (Fig. 2).

In round 2, the mean of determined values for the assessments from all laboratories slightly exceeded the upper 15% margin for each of the samples with nominal concentrations of 1200, 1800, 2400, and 4000 ng/mL (Fig. 3). The
Overall biases at each of these concentrations were considerably larger than those observed in round 1 (17.6%, 17.3%, 16.2%, and 18.1%, respectively). Among all 72 determined concentrations from the 18 laboratories, 31 were within the 15% margins, 36 exceeded the upper 15% margin, and 5 were below the lower 15% margin.

When assessed as a function of the analytic methods, the means of determined values for those using LC-MS/MS (laboratories 11–20) were slightly below the upper 15% margin (Fig. 4). Determined values from 2 laboratories were consistently within the 15% margins and those from 2 other laboratories consistently exceeded the upper 15% margin. The overall bias at each nominal concentration was again higher than observed for LC-MS/MS in round 1 (14.4%, 13.9%, 11.6%, and 12.1%, respectively). In total, 19/40 determined concentrations were within the 15% margins, 18/40 exceeded the upper 15% margin, and 3/40 were below the bottom 15% margin. Among laboratories that used LC-UV (laboratories 21–26), the mean values of determined values for all nominal concentrations exceeded the upper 15% margin (Fig. 4). Two laboratories consistently provided determined values that were within the 15% of the nominal concentrations.
concentrations, and 3 laboratories provided values that all exceeded the upper 15% margin. The overall bias at each nominal concentration was 25.6%, 24.3%, 28.6%, and 30.8%, respectively. Among the 24 total samples, 10 of the determined concentrations were within the 15% margins and 14 exceeded the upper 15% margin. For LC-FL (laboratory 27), determined values for all samples exceeded the upper 15% margin (Fig. 4). For bioassay, laboratory 10 was included again after having made technical adjustments. Although determined values for the 1200 and 2400 ng/mL nominal concentrations were each slightly below the lower 15% margin, determined values were within the 15% margins for the 1800 and 4000 ng/L nominal concentrations (Fig. 4).

DISCUSSION

In any instance for which isavuconazole TDM might be considered appropriate, its usefulness will require accurate measurement with regard to the analytic method used. In this round-robin exercise of European laboratories, 10 of the 27 laboratories included in both rounds provided determined plasma concentrations that were consistently within ±15% of

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**FIGURE 3.** Isavuconazole plasma concentrations determined by Basi-lea and all laboratories in round 2 (labs 10–27) as a function of the nominal concentrations of 1200, 1800, 2400, and 4000 ng/mL (A–D, respectively). Solid lines represent nominal concentrations; dashed lines represent ±15% margins from nominal concentrations.
the nominal concentrations. Of all total samples in both rounds, 54% (60/112) determined concentrations were within ±15% of the nominal concentrations. These data suggest that there is likely need for refinement and alignment for each of those analytical methods across the different laboratories.

It may be a concern that determined concentrations were consistently within the 15% margins stipulated in guidelines from the European Medicines Agency22 and Food and Drug Administration23 for fewer than half of the laboratories. Moreover, determined concentrations outside of those margins were far more likely to be overestimated (43/112) than underestimated (9/112). Overestimation in clinical practice might result in a decision to reduce the dose and thereby pose a risk for less efficacious treatment. From a methodological perspective, this suggests that a common systematic error across a subset of the laboratories is possible. Data regarding the assay calibrations performed were not available for all laboratories. As isavuconazole is a relatively new drug, it may be that some have not yet implemented the adjustments necessary to obtain the desired accuracy. For example, it is unclear whether all laboratories using LC controlled for possible matrix effects during
CONCLUSIONS

Taken together, the results of this study suggest that improving the accuracy and reproducibility of each method is very likely to require the standardization of methodologies across analytical laboratories. This process would require more open discussion regarding protocols and might be facilitated by implementing international standards. Ultimately, it would increase the confidence of clinicians in the event that isavuconazole TDM becomes necessary.

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