Several azole antifungal drugs (miconazole, fluconazole, ketoconazole, posaconazole, voriconazole, itraconazole and its active metabolite, hydroxy-itraconazole) are marketed in Belgium (Fig. 1) and can be administered orally or parenterally, in particular for treating invasive fungal infections. The number of these diseases is increasing and the associated mortality is a concern (1).

The determination of azole antifungals in serum can help the clinician to adjust the dose administered to patients, in order to avoid insufficient concentrations or overdose (1-3).

A method was developed and validated for the simultaneous determination of these antifungal drugs in serum utilizing ultra high-pressure liquid chromatography and diode array detection (UHPLC-DAD).

**Material and method**

- **Sample pre-treatment**: an internal standard (azaconazole – Fig. 2) is added to 1 ml of serum sample before liquid-liquid extraction.
- **Equipment**: Acquity® UPLC system (Waters Corporation®) coupled to a DAD (Fig. 3).
- **Column**: Acquity® BEH C18 (Waters Corporation®) 150 mm x 2.1 mm, 1.7 µm / T: 40°C
- **Mobile phase**: gradient mode (Fig. 4) (A) acetonitrile; (B) NH₄HCO₃ 10 M pH10
- **Flow rate**: 0.4 ml/min
- **Injection volume**: 5 µl
- **Quantification wavelengths**: Voriconazole 255 nm; Posaconazole, (hydroxy)-itraconazole 280 nm; Fluco-, keto-, mico-, azconaol 210 nm
- **Validation method**: according to the total error method approach by using an analytical validation software (e-noval V3.0 Arlena®).

The seven azole antifungals were identified together over a 13-min run time (Fig. 5).

The assay was specific and linear in ranges considered clinically satisfactory from 0.05 to 10 mg/L for voriconazole (V2), posaconazole (P2), itraconazole (I2), hydroxy-itraconazole (H2) and ketoconazole (K2); from 0.3 to 10 mg/L for fluconazole (F2) and from 0.1 to 10 mg/L for miconazole (M2) (Table 1) (2, 4).

Other analytical validation parameters were found to be very satisfactory for all drugs (trueness: < 10%; precision: < 15%; r² > 0.99).

**Discussion**

The method is successfully applied to biological samples from patients hospitalized in CHU Liège.

In the literature, it has been reported that 20% of patients treated with recommended doses of voriconazole show subtherapeutic levels (5). Assays that we performed revealed that 34% of patients treated by voriconazole had subtherapeutic serum levels. We also observed that 81% of patients treated by posaconazole had insufficient plasma concentrations (Table 2).

These preliminary results indicate the importance of the blood determination of azole antifungals for cost effective treatment of patients.

**Conclusion**

We developed and validated a simple, sensitive and selective UHPLC-DAD method for the simultaneous determination in human serum of seven azole antifungal drugs. The method is successfully applied to patient biological samples and is suitable for clinical applications, such as therapeutic drug monitoring. Since this method has been implemented, confrontation of laboratory results and clinical data has demonstrated the interest of the azole antifungal drugs determination in blood, especially for voriconazole and posaconazole.

**References**