A method was developed and validated for the simultaneous determination of 7 azole antifungal drugs in serum utilizing ultra-high-pressure liquid chromatography and diode array detection (UHPLC-DAD). These antifungals are as well imidazole drugs (miconazole and ketoconazole) as triazole drugs (fluconazole, posaconazole, voriconazole, itraconazole and its active metabolite, hydroxyitraconazole) (Fig. 1). They are marketed in Belgium and can be administered orally or parenterally.

Their determination in serum can help the clinician to adjust the dose administered to patients, in order to avoid insufficient concentrations or overdose.

**Introduction**

**Material and method**

**Equipment:** Acquity® UPLC system (Waters Corporation®) coupled to a DAD (Fig. 3).

**Mobile phase:** gradient mode (Fig. 4).

**Flow rate:** 0.4 µl/min.

**Injection volume:** 5 µl.

**Calibration standards:** in duplicate during 3 days (7 levels).

**Validation standards:** in triplicate during 3 days (8 levels).

**Results**

The seven azole antifungals were identified together over a 13-min run time (Fig. 5). All calibration curves showed good linearity ($r^2 > 0.99$) in ranges considered clinically satisfactory (Fig. 6, Table 1).

The assay was specific and linear from 0.05 to 10 mg/L for voriconazole (V), posaconazole (P), itraconazole (I), hydroxyitraconazole (H), and ketoconazole (K) from 0.3 to 10 mg/L for fluconazole (F) and from 0.1 to 10 mg/L for miconazole (M) (Table 1).

The trueness and precision values for intra- and inter-assays were lower than 10% and than 15%, respectively, for all drugs.

**Conclusion:** We developed and validated a simple, sensitive and selective UHPLC-DAD method for the simultaneous determination in human serum of seven azole antifungals. The method was successfully applied to patient samples and is suitable for clinical applications, such as therapeutic drug monitoring.