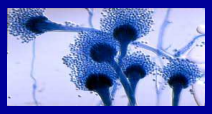
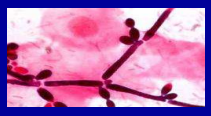
DETERMINATION OF SEVEN AZOLE ANTIFUNGALS IN HUMAN SERUM BY UHPLC-DAD





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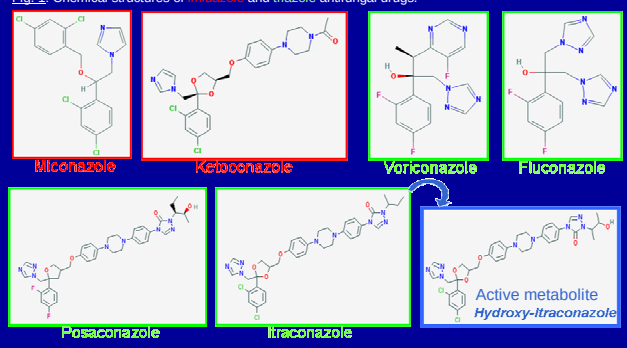
Introduction

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, **hydroxy-itraconazole**) (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.

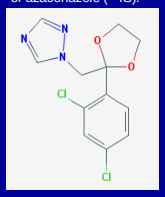
Fig. 1: Chemical structures of imidazole and triazole antifungal drugs.



Material and method

• **Sample pre-treatment**: an **internal standard** (**azaconazole** – Fig. 2) is added to 1 ml of serum sample before **liquid-liquid extraction**.

Fig. 2: Chemical structure of azaconazole (= IS).



• **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) **NH_4HCO_3** 10 M pH10

• **Flow rate**: **0,4 ml/min**

• **Injection volume**: **5 µl**

• **Quantification wavelengths**

Voriconazole	255 nm
Posaco-, (hydroxy-)itraconazole	260 nm
Fluco-, keto-, mico-, azaconazole	210 nm

Fig. 3: UHPLC-DAD system.

Fig. 4: Gradient of mobile phase.

Temps (min)	%A	%B
0	35	65
3	35	65
9	80	20
11	35	65
13	35	65

• **Validation method**: according to **the total error method approach** by using an analytical validation software (**e•noval V3.0 Arlenda®**).
→ *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 (VZ)**, posaconazole** (PZ)**, itraconazole** (IZ)**, hydroxy-itraconazole** (HIZ) **and ketoconazole** (KZ)**; from 0.3 to 10 mg/L for fluconazole** (FZ) **and from 0.1 to 10 mg/L for miconazole** (MZ) (Table 1).

Table 1: Limits of quantification (LOQ), limits of linearity (LOL) and therapeutic intervals of each azole antifungal drug.

Drugs	LOQ (mg/L)	LOL (mg/L)	Therapeutic index (mg/L)
VZ	0.05	10	1 - 6
PZ	0.05	10	1 - 6
IZ	0.05	10	0,25 - 2
IZ + HIZ	0.05	10	> 1
KZ	0.05	10	1,5 - 5
MZ	0.11	10	± 1
FZ	0.30	10	0,5 - 6

Fig. 5: Chromatogram of seven azole antifungal drugs and internal standard.

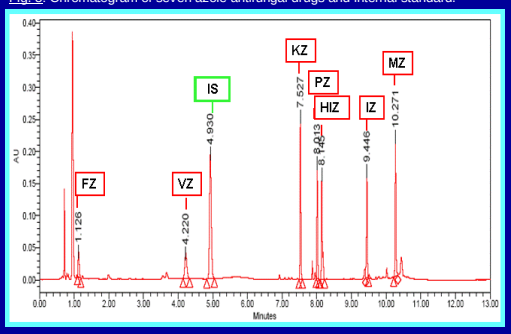
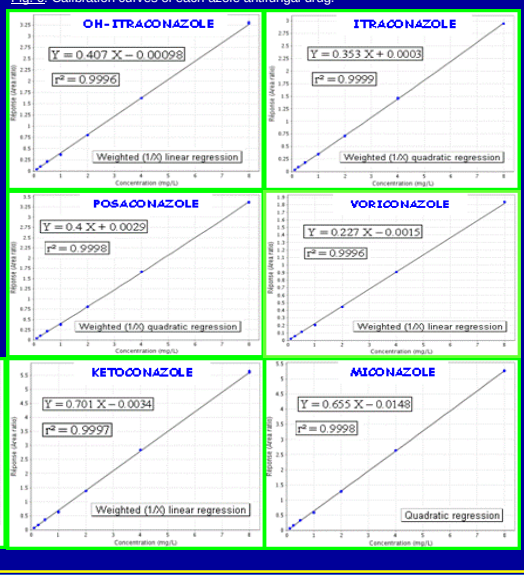


Fig. 6: Calibration curves of each azole antifungal drug.



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.**