

ANTIMYCOTICS IN PLASMA by FLUORIMETRY - FAST –
Code Z79010
(Posaconazole, Voriconazole, Itraconazole, OH-Itraconazole)

INTRODUCTION

Systemic fungal infections are the leading cause of death in patients whose immune system is compromised due to cancer or chemotherapy, organ transplantation, or by HIV-1. The fungi commonly cause superficial infections of the skin and other soft tissues. [1]

For nearly 30 years, Amphotericin B, which is known to cause significant nephrotoxicity, was the only drug available for the control of serious fungal infections. The approval of the use of imidazoles and triazoles in the late 80s and early 90s was a major advance in the ability to treat in a safe and effective way local and systemic fungal infections.

The high safety profile of triazoles in fact, in particular fluconazole, has led to their extensive use. The triazole antifungal drugs are frequently used in the prophylaxis and treatment of IFD (invasive fungal infection) [2]. There are now a number of drugs used in therapy, such as those of the group of azoles that are Posaconazole (Noxafil®), Voriconazole (Vfend®), Itraconazole (Sporanox®), Ketoconazole (Nizoral) and Fluconazole (Diflucan®), but also other types such as the 5-Flucytosine and the family of Echinocandins, such as Caspofungin, Anidulafungin and Micafungin.

Considering the different metabolism of human beings, to start systemic therapy effectively, you need a proper monitoring of the plasma levels of antifungal drugs in order to prevent the concentration during therapy may not be sufficient to prevent or exceed toxic levels.

The present method is able to determine the plasma concentration of the antifungal drugs most commonly used, using chromatographic technique HPLC-Fluorimetry.

Bibliography:

- [1] Goodman & Gilman's, The Pharmacological Basis of Therapeutics, Laurence L. Brunton, Bruce A. Chabner, Björn C. Knollmann
[2] Quantitation of Azoles and Echinocandins in Compartments of Peripheral Blood by Liquid Chromatography-Tandem Mass Spectrometry. Fedja Farowski, Oliver A. Cornely, Jörg J. Vehreschild, Pia Hartmann, Tim Bauer, Angela Steinbach, Maria J.G.T. Rüping and Carsten Müller; Antimicrob. Agents Chemother. 2010, 54(5):1815.DOI: 0.1128/AAC.01276-09 (2010)
[3] HPLC/UV or bioassay: two valid methods for posaconazole quantification in human serum samples E. Cendejas-Bueno, A. Forastiero, J. L. Rodriguez-Tudela, M. Cuenca-Estrella and A. Gomez-Lopez Servicio de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain
[4] Posaconazole: clinical pharmacology and potential for management of fungal infections. Groll AH1, Walsh TJ. Children's University Hospital, Dept. of Hematology/Oncology, Center for Bone Marrow Transplantation, Albert-Schweitzer-Strasse 33, 48149 Münster, German



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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 005	Antimycotics in plasma by Fluorimetry - FAST	07/2024
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TECHNICAL FEATURES

Principle of the Method:

Antimycotics are separated treating the serum/plasma with a suitable precipitant with internal standard and centrifugated. The solution is directly injected into HPLC.

Recovery : >99%

Sensitivity (LLOD):

Voriconazole	0,083 µg/ml
Posaconazole	0,005 µg/ml
OH-Itraconazole	0,014 µg/ml
Itraconazole	0,042 µg/ml

Minimum concentration analyzable (LLOQ):

Voriconazole	0,125 µg/ml
Posaconazole	0,016 µg/ml
OH-Itraconazole	0,042 µg/ml
Itraconazole	0,083 µg/ml

Linearity :

Voriconazole	0,125 – 50 µg/ml
Posaconazole	0,016 – 50 µg/ml
OH-Itraconazole	0,042 – 50 µg/ml
Itraconazole	0,083 – 200 µg/ml

Accuracy intra serie (relative error %)
Voriconazole:

Ci	Cs
2 µg/ml	5 µg/ml
8,1%	1,5%

Accuracy inter serie (relative error %)
Voriconazole:

Ci	Cs
2 µg/ml	5 µg/ml
8,7%	3,8%

Reproducibility intra serie (coefficient of variation %) Voriconazole:

C LLOQ	Cm	CUP
0,125 µg/ml	3,5 µg/ml	7,5 µg/ml
6,0%	4,9%	2,5%

Reproducibility inter serie (coefficient of variation %) Voriconazole:

C LLOQ	Cm	CUP
0,125 µg/ml	3,5 µg/ml	7,5 µg/ml
6,8%	4,1%	3,9%

Accuracy intra serie (relative error %)
Posaconazole:

Ci	Cs
0,6 µg/ml	3 µg/ml
5,6%	3,8%

Accuracy inter serie (relative error %)
Posaconazole:

Ci	Cs
0,6 µg/ml	3 µg/ml
6,2%	2,7%

Reproducibility intra serie (coefficient of variation %) Posaconazole:

C LLOQ	Cm	CUP
0,016 µg/ml	2 µg/ml	5 µg/ml
6,3%	4,3%	2,2%

Reproducibility inter serie (coefficient of variation %) Posaconazole:

C LLOQ	Cm	CUP
0,016 µg/ml	2 µg/ml	5 µg/ml
6,9%	4,4%	3,2%

Accuracy intra serie (relative error %) OH-Itraconazole:

Ci	Cs
0,5 µg/ml	3 µg/ml
6,7%	2,0%

Accuracy inter serie (relative error %) OH-Itraconazole:

Ci	Cs
0,5 µg/ml	3 µg/ml
8,1%	2,1%

Reproducibility intra serie (coefficient of variation %) OH-Itraconazole:

C LLOQ	Cm	CUP
0,042 µg/ml	2 µg/ml	5 µg/ml
6,6%	4,2%	2,1%

Reproducibility inter serie (coefficient of variation %) OH-Itraconazole:

C LLOQ	Cm	CUP
0,042 µg/ml	2 µg/ml	5 µg/ml
7,9%	4,8%	3,8%

Accuracy intra serie (relative error %) Itraconazole:

Ci	Cs
0,5 µg/ml	3 µg/ml
6,1%	3,0%

Accuracy inter serie (relative error %) Itraconazole:

Ci	Cs
0,5 µg/ml	3 µg/ml
8,4%	4,5%

Reproducibility intra serie (coefficient of variation %) Itraconazole:

C LLOQ	Cm	CUP
0,083 µg/ml	2 µg/ml	5 µg/ml
4,9%	4,2%	2,4%

Reproducibility inter serie (coefficient of variation %) Itraconazole:

C LLOQ	Cm	CUP
0,083 µg/ml	2 µg/ml	5 µg/ml
7,0%	6,3%	4,5%

Coefficient of correlation R2 + Dev. std:

Voriconazole	0,9947 ± 0,0035
Posaconazole	0,9994 ± 0,0002
OH-Itraconazole	0,9993 ± 0,0002
Itraconazole	0,9973 ± 0,0019

Range of normality in blood :

Voriconazole	2 – 5,5 µg/ml
Posaconazole	0,6 – 3 µg/ml
OH-Itraconazole	0,5 – 2,9 µg/ml
Itraconazole	0,5 – 2,9 µg/ml

Bibliography: Schultz M, Schmoldt A. (2003) Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. Pharmazie 58(7): 447-74

<u>Components of the kit :</u>	All reagents are ready to use and stable 3 years at 2–8 °C.
Reagent A – Deproteinization Solution + Internal Standard, 2 x 10 ml	
Reagent B – Test Solution, 1 x 1 ml	<u>See Warnings</u>
Plasma Calibrator, 1 x 1 ml	<u>Code Z79016 (pace separately – see data sheet)</u>
Reagent M – Mobile Phase, 5 x 500 ml	

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 µl
 Fluorimetric Detector $\lambda_{EX}=256\text{ nm}$ $\lambda_{EM}=360\text{ nm}$
 Chromatograms Recorder

Optional Equipment:

Autosampler
 Operational Computer

Blood Collection Procedure:

Collect 3 ml of blood in a suitable test tube with EDTA or Heparin as anticoagulant. Centrifuge at 4000 rpm for 5 min. Plasma samples should be stored at –20° C for no longer than 4 weeks.

PREANALYTICAL PROCEDURE

Pipette in a tube :

- 450 µl of H₂O HPLC grade
- 50 µl of **Reagent B – Test Solution**

Vortex for 10 sec.

INJECTION :

- Inject 10 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 1. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

ANALYTICAL PROCEDURE

STEP 1: Preparation of Calibrator, Controls and Samples

Dispense in eppendorf of 1,5 ml with cap :

	Blank	Calibrator	Control	Sample
Water HPLC grade	100 µl			
Calibrator		100 µl		
Control			100 µl	
Sample				100 µl
Reagent A - Deproteinization Sol. + Internal Std	200 µl	200 µl	200 µl	200 µl

Warning: deproteinize directly on Vortex for at least 10 seconds

STEP 2: Store for 15 minutes at 2-8 °C.

STEP 3: Centrifuge at 14.000 rpm for 10 minutes.

STEP 4: Transfer all the supernatant in glass vials with reduced form.

N.B.: at this step, the sample is stable 3 days at 2-8 °C

INJECTION :

- Inject 10 µl of supernatant in HPLC system.

ANTIMYCOTICS IN PLASMA - Warnings

REAGENT B: TEST SOLUTION

VORICONAZOLE	100 µg/ml
POSACONAZOLE	10 µg/ml
ITRACONAZOLE	10 µg/ml
OH-ITRACONAZOLE	10 µg/ml

FLUORIMETRIC DETECTOR PARAMETERS

λ_{EX}	256 nm
λ_{EM}	360 nm
GAIN	0,001 AUFS

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 EC C-18 4,6 x 50 mm, 2,7 µ, the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical C₁₈ Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 µ).
Disconnect the detector and filter 0 ml of **H₂O : Methanol (20 : 80 v / v)** set flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Flux 30 ml of a solution of **H₂O : Methanol (80 : 20 v / v)** at flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1,0 ml/minute and discharge the first 20 ml. Finally inject the Chemical Standard and verify the quality of the HPLC run.
It's NOT possible to make analysis at recycling phase. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flux 15 ml of **H₂O : Acetonitrile or Methanol (80 : 20 v / v)** set flow of 1,0 ml/minute and discharge. Flux a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v)** for 30 minutes discharge it. **It is recommended to perform the washing of Analytical Column at the end of each analytical run.** Store the column in a solution of **H₂O : Acetonitrile or Methanol (30 : 70 v / v)**.

INJECTION NEEDLE WASHING

Wash with a solution of Water / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

HPLC PARAMETERS

LOOP	10 µl
LOW	1,2 ml/min
PRESSURE	Circa 140 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z79016	Calibrator in plasma for Antimycotics	4 x 1 ml
Z79019	Control in plasma for Antimycotics – Levels 1 and 2	2 x 5 x 1 ml
Z79011	Mobile Phase	4 x 500 ml
Z699975902	Analytical Column Poroshell 120-EC-C 18 (50 x 4,6mm –2,7 µm)	1 Pc
S90199511	Javelin Prefilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs
S51820717	Caps for clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs

ANTIMYCOTICS in PLASMA (Reference Chromatograms)

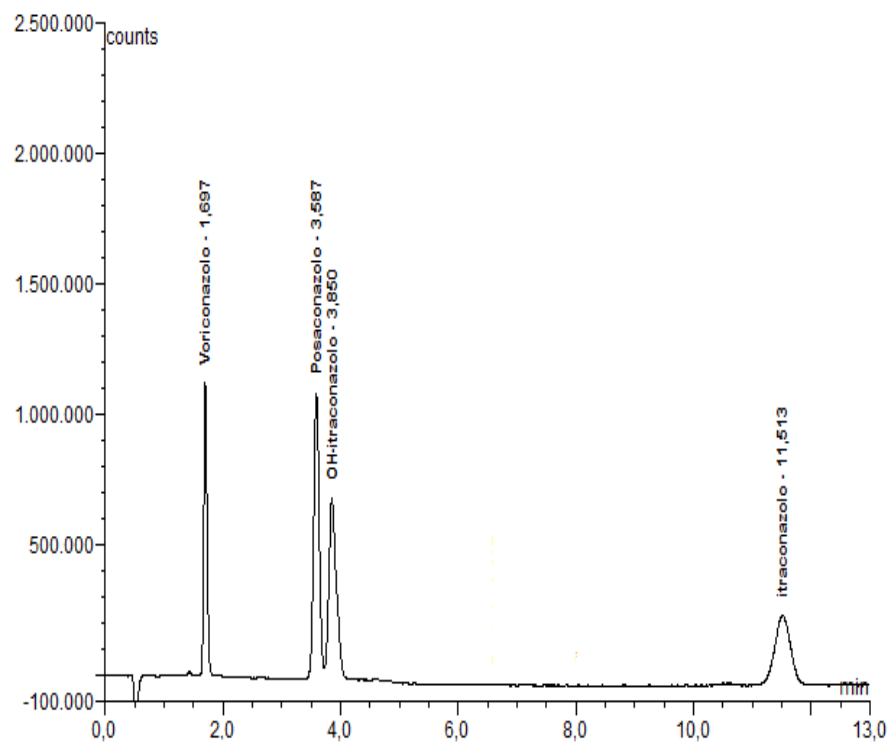


Fig. 1 :	Test Solution
R.T. 1.69	Voriconazole
R.T. 3.58	Posaconazole
R.T. 3.85	OH-Itraconazole
R.T. 11.51	Itraconazole

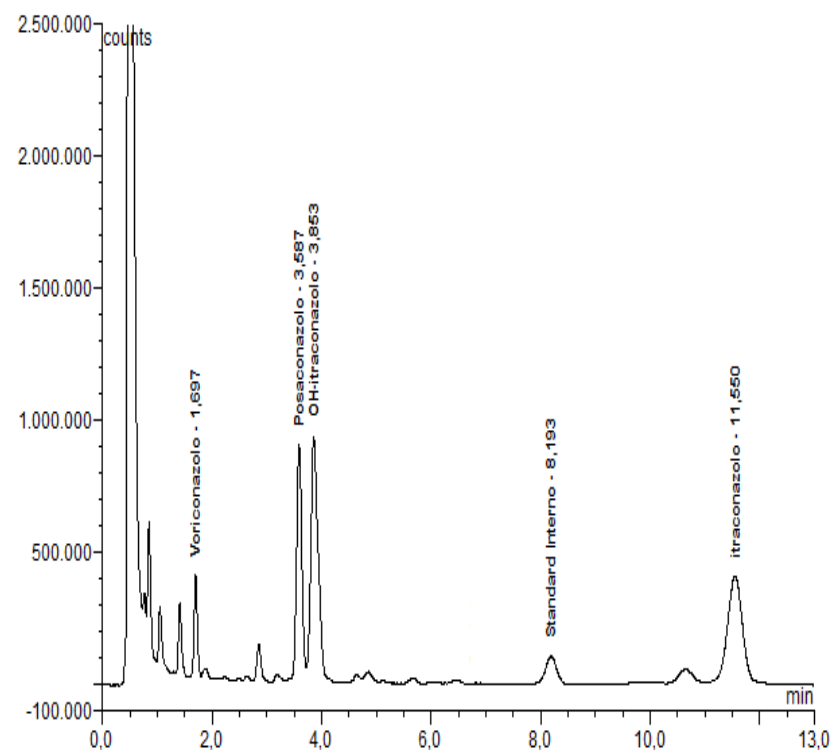


Fig. 2 :	Plasma Calibrator		
R.T.	1.69	Voriconazole	11,6 ug/ml
R.T.	3.58	Posaconazole	2,0 ug/ml
R.T.	3.85	OH-Itraconazole	3,2 ug/ml
R.T.	8.19	Internal Standard	
R.T.	11.55	Itraconazole	2,6 ug/ml

ANTIMYCOTICS in PLASMA (Reference Chromatograms)

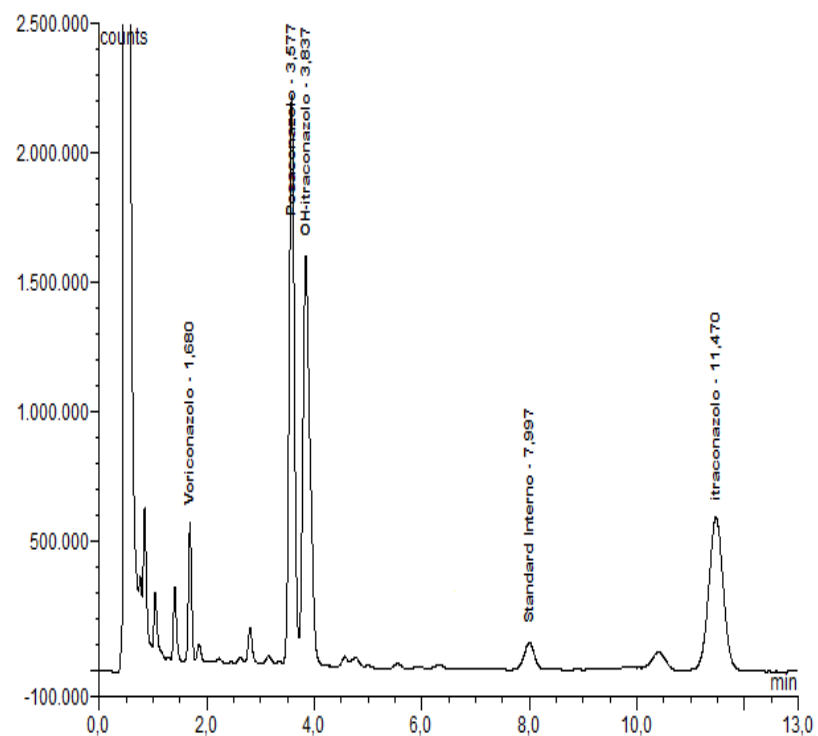


Fig. 3 : Control Level 2		
R.T.	1.68 Voriconazole	15,5 ug/ml
R.T.	3.57 Posaconazole	4,4 ug/ml
R.T.	3.83 OH-Itraconazole	5,3 ug/ml
R.T.	7.99 Internal Standard	
R.T.	11.47 Itraconazole	2,9 ug/ml

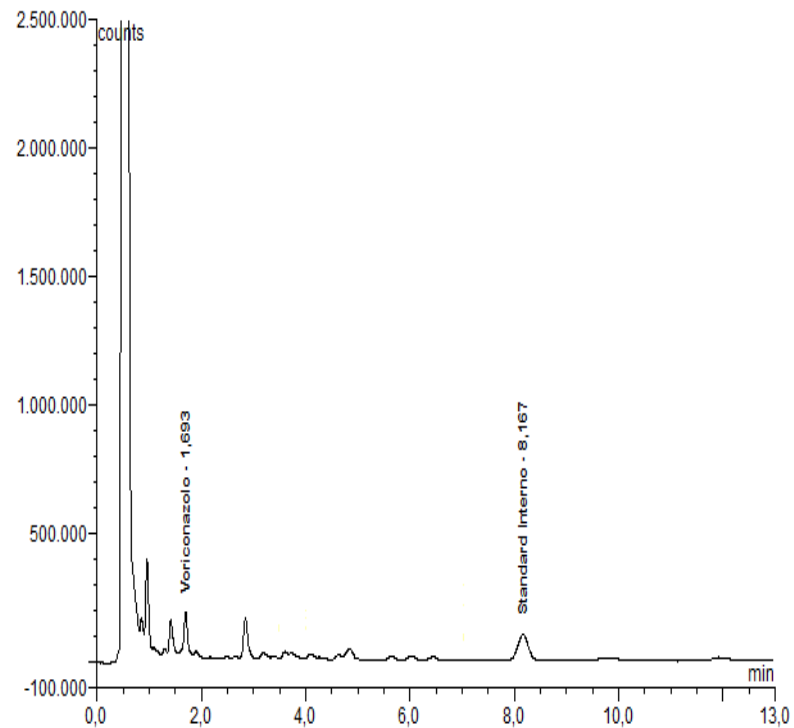


Fig. 4 : Sample of Voriconazole		
R.T.	1.69 Voriconazole	
R.T.	8.16 Internal Standard	

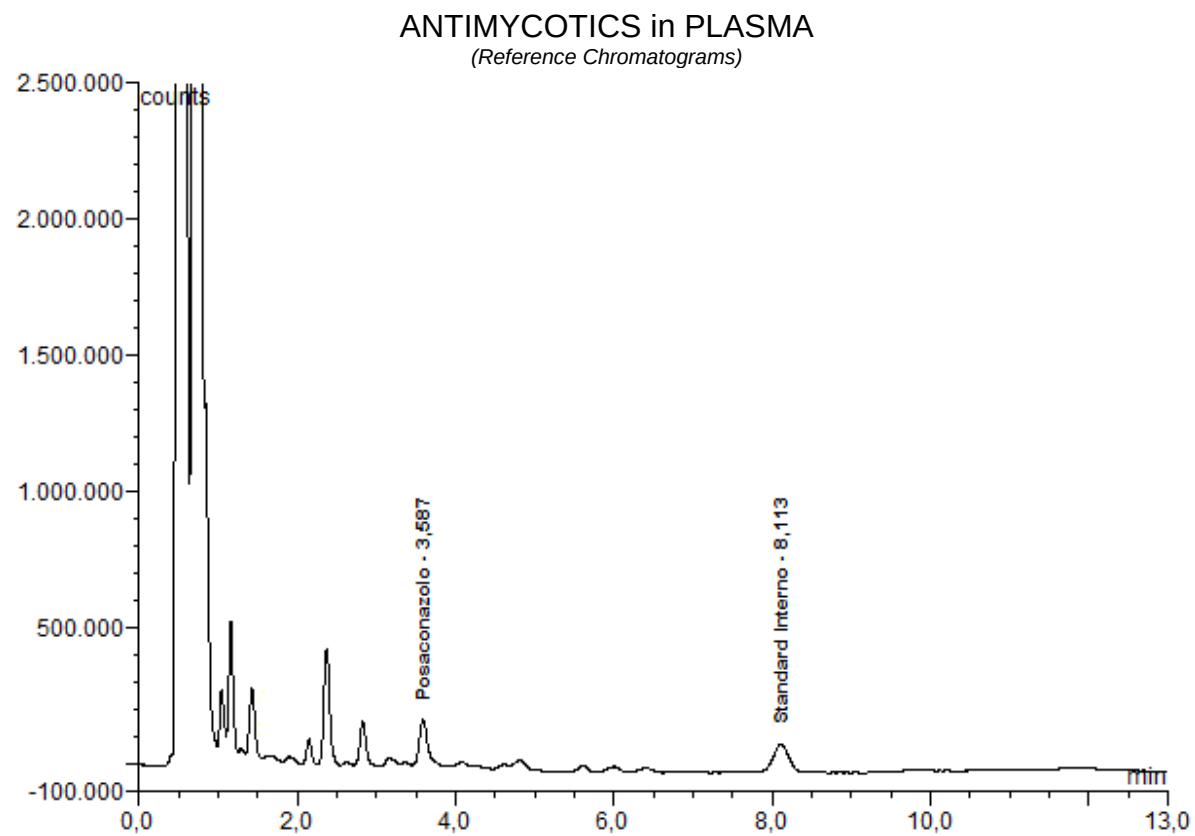


Fig. 5 :	Sample of Posaconazole
R.T. 3.58	Posaconazole
R.T. 8.11	Internal Standard