

ANTIMYCOTICS IN PLASMA by LC/MS - Code LC82010

(Posaconazole, Voriconazole, Itraconazole, OH-Itraconazole, 5-Flucytosine, Caspofungin, Fluconazole, Ketoconazole, Anidulafungin, Miconazole, Isavuconazole)

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 HIV-1. Fungus commonly cause superficial skin infections and other soft tissues. [1]

For nearly 30 years, Amphotericin B, which is known to cause significant nephrotoxicity, has been the only available drug for controlling serious fungal infections. Approval of the use of imidazoles and triazoles between the late 1980s and the early 1990s was a remarkable advance in the ability to safely and effectively treat local and systemic fungal infections. [2] The high safety profile of triazoles, in particular fluconazole, has led to their extensive use. Triazole antifungal drugs are frequently used for the prophylaxis and treatment of IFD (Invasive Fungal Infection) There are today many medicines 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 5-Flucytosine and the Echinocandine family, such as Caspofungin, Anidulafungin and Miconazole.

Considering the different metabolism of humans, to initiate effective systemic therapy, a proper monitoring of plasma levels of antimycotic drugs is necessary to prevent concentrations are insufficient or to prevent them from exceeding toxic levels during therapy.

This method is able to determine the plasma concentration of antimycotic drugs most commonly used by LC/ MS chromatographic technique.

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

 EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com



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 LC/MS	06/2024
--------	---------------------------------	---------

TECHNICAL FEATURES

Principle of the Method:

The plasma samples with Antimycotics are deproteinized with a suitable reagent containing the internal standard, centrifuged and directly injected into LC / MS.

Recovery :

>99%

Sensitivity (LLOD):

Voriconazole	0,003 µg/ml
Posaconazole	0,005 µg/ml
OH-Itraconazole	0,014 µg/ml
Itraconazole	0,002 µg/ml
5-Flucytosine	0,154 µg/ml
Caspofungin	0,004 µg/ml
Fluconazole	0,004 µg/ml
Ketoconazole	0,003 µg/ml
Anidulafungin	0,006 µg/ml
Micafungin	0,02 µg/ml
Isavuconazole	0,01 µg/ml

Minimum concentration analyzable (LLOQ):

Voriconazole	0,005 µg/ml
Posaconazole	0,04 µg/ml
OH-Itraconazole	0,042 µg/ml
Itraconazole	0,007 µg/ml
5-Flucytosine	0,66 µg/ml
Caspofungin	0,03 µg/ml
Fluconazole	0,02 µg/ml
Ketoconazole	0,005 µg/ml
Anidulafungin	0,06 µg/ml
Micafungin	0,125 µg/ml
Isavuconazole	0,05 µg/ml

Linearity :

Voriconazole	0.005 – 50 µg/ml
Posaconazole	0,04 – 50 µg/ml
OH-Itraconazole	0,042 – 50 µg/ml
Itraconazole	0,007 – 40 µg/ml
5-Flucytosine	0.66 - 360 µg/ml
Caspofungin	0.03 - 40 µg/ml
Fluconazole	0.02 - 45 µg/ml
Ketoconazole	0.005 - 39 µg/ml
Anidulafungin	0.06 - 50 µg/ml
Micafungin	0.125 - 50 µg/ml
Isavuconazole	0,05 – 1000 µg/ml

Accuracy intra serie (relative error %)

Voriconazole:

C1	C2
2 µg/ml	5 µg/ml
16.7%	6.0%

Accuracy inter serie (relative error %)

Voriconazole:

C1	C2
2 µg/ml	5 µg/ml
10.6%	7.1%

Reproducibility intra serie (coefficient of variation %) Voriconazole:

C LLOQ	Cm	CUP
0.005 µg/ml	0.77 µg/ml	7 µg/ml
7.6%	5.2%	3.9%

Reproducibility inter serie (coefficient of variation %) Voriconazole:

C LLOQ	Cm	CUP
0.005 µg/ml	3.5 µg/ml	7.5 µg/ml
12.4%	8.2%	8.8%

Accuracy intra serie (relative error %) Posaconazole:

C1	C2
0.6 µg/ml	3 µg/ml
8.1%	6.7%

Accuracy inter serie (relative error %) Posaconazole:

C1	C2
0.6 µg/ml	3 µg/ml
11.5%	6.5%

Reproducibility intra serie (coefficient of variation %) Posaconazole:

C LLOQ	Cm	CUP
0.04 µg/ml	0.5 µg/ml	2 µg/ml
9.9%	8.5%	2.6%

Reproducibility inter serie (coefficient of variation %) Posaconazole:

C LLOQ	Cm	CUP
0.04 µg/ml	2 µg/ml	5 µg/ml
12.8%	10.5%	8.0%

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

C1	C2
0.21 µg/ml	3.5 µg/ml
10.21%	5.0%

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

C1	C2
0.21 µg/ml	3.5 µg/ml
10.21%	5.5%

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

C LLOQ	Cm	CUP
0,007 µg/ml	0.77 µg/ml	7 µg/ml
12.5%	8.6%	4.4%

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

C LLOQ	Cm	CUP
0,007 µg/ml	0.77 µg/ml	7 µg/ml
17.8%	11.1%	9.5%

Accuracy intra serie (relative error %) Itraconazole:

C1	C2
0.21 µg/ml	3.5 µg/ml
10.6%	6.0%

Accuracy inter serie (relative error %) Itraconazole:

C1	C2
0.21 µg/ml	3.5 µg/ml
10.2%	5.5%

Reproducibility intra serie (coefficient of variation %) Itraconazole:

C LLOQ	Cm	CUP
0,007 µg/ml	0.77 µg/ml	7 µg/ml
12.5%	8.6%	4.4%

Reproducibility inter serie (coefficient of variation %) Itraconazole:

C LLOQ	Cm	CUP
0,007 µg/ml	0.77 µg/ml	7 µg/ml
17.8%	11.1%	9.5%

Accuracy intra serie (relative error %) 5-Flucytosine:

C1	C2
6 µg/ml	60 µg/ml
5.7%	4.5%

Accuracy inter serie (relative error %) 5-Flucytosine:

C1	C2
6 µg/ml	60 µg/ml
5.7%	4.4%

Reproducibility intra serie (coefficient of variation %) 5-Flucytosine:

C LLOQ	Cm	CUP
0,66 µg/ml	30 µg/ml	120 µg/ml
9.9%	8.1%	6.4%

Reproducibility inter serie (coefficient of variation %) 5-Flucytosine:

C LLOQ	Cm	CUP
0,66 µg/ml	30 µg/ml	120 µg/ml
10.8%	12.4%	8.2%

Accuracy intra serie (relative error %) Caspofungin:

C1	C2
0.5 µg/ml	7.5 µg/ml
13.7%	4.0%

Accuracy inter serie (relative error %) Caspofungin:

C1	C2
0.5 µg/ml	7.5 µg/ml
16.5%	6.6%

Reproducibility intra serie (coefficient of variation %) Caspofungin:

C LLOQ	Cm	CUP
0,03 µg/ml	1.05 µg/ml	15 µg/ml
17.4%	11.9%	7.7%

Reproducibility inter serie (coefficient of variation %) Caspofungin:

C LLOQ	Cm	CUP
0,03 µg/ml	1.05 µg/ml	15 µg/ml
17.3%	12.5%	7.8%

Accuracy intra serie (relative error %) Fluconazole:

C1	C2
0.23 µg/ml	7.5 µg/ml
4.9%	4,0%

Accuracy inter serie (relative error %) Fluconazole:

C1	C2
0.23 µg/ml	7.5 µg/ml
9.0%	3.2%

Reproducibility intra serie (coefficient of variation %) Fluconazole:

C LLOQ	Cm	CUP
0,02 µg/ml	1.05 µg/ml	15 µg/ml
12.1%	7.3%	3.1%

Reproducibility inter serie (coefficient of variation %) Fluconazole:

C LLOQ	Cm	CUP
0,02 µg/ml	1.05 µg/ml	15 µg/ml
11.5%	7.5%	3.6%

Accuracy intra serie (relative error %) Ketonazole:

C1	C2
0.5 µg/ml	3 µg/ml
8.9%	6.7%

Accuracy inter serie (relative error %) Ketonazole:

C1	C2
0.5 µg/ml	3 µg/ml
10.7%	6.0%

Reproducibility intra serie (coefficient of variation %) Ketonazole:

C LLOQ	Cm	CUP
0,005 µg/ml	1.05 µg/ml	15 µg/ml
11.9%	6.0%	4.6%

Reproducibility inter serie (coefficient of variation %) Ketonazole:

C LLOQ	Cm	CUP
0,005 µg/ml	1.05 µg/ml	15 µg/ml
12.4%	6.6%	6.0%

Accuracy intra serie (relative error %) Anidulafungin:

C1	C2
1 µg/ml	5 µg/ml
8.1%	7.0%

Accuracy inter serie (relative error %) Anidulafungin:

C1	C2
1 µg/ml	5 µg/ml
8.1%	7.2%

Reproducibility intra serie (coefficient of variation %) Anidulafungin:

C LLOQ	Cm	CUP
0,06 µg/ml	3 µg/ml	10 µg/ml
12.5%	5.9%	5.6%

Reproducibility inter serie (coefficient of variation %) Anidulafungin:

C LLOQ	Cm	CUP
0,06 µg/ml	3 µg/ml	10 µg/ml
17.6%	5.9%	5.9%

Accuracy intra serie (relative error %) Micafungin:

C1	C2
1 µg/ml	5 µg/ml
11.7%	8.9%

Accuracy inter serie (relative error %) Micafungin:

C1	C2
1 µg/ml	5 µg/ml
13.6%	9.4%

Reproducibility intra serie (coefficient of variation %) Micafungin:

C LLOQ	Cm	CUP
0.125 µg/ml	3 µg/ml	10 µg/ml
9.5%	5.2%	3.8%

Reproducibility inter serie (coefficient of variation %) Micafungin:

C LLOQ	Cm	CUP
0.125 µg/ml	3 µg/ml	10 µg/ml
14.2%	10.9%	6.9%

Accuracy intra serie (relative error %) Isavuconazole:

C1	C2
1 µg/ml	5 µg/ml
2.29%	3.72%

Accuracy inter serie (relative error %) Isavuconazole:

C1	C2
1 µg/ml	5 µg/ml
5.27%	7.95%

Reproducibility intra serie (coefficient of variation %) Isavuconazole:

C LLOQ	Cm	CUP
0.05 µg/ml	2.5 µg/ml	7.5 µg/ml
0.98%	1.82%	0.54%

Reproducibility inter serie (coefficient of variation %) Isavuconazole:

C LLOQ	Cm	CUP
0.05 µg/ml	2.5 µg/ml	7.5 µg/ml
4.29%	3.54%	3.97%

Coefficient of correlation R2 + Dev. std:

Voriconazole	0,9976 ± 0,0016
Posaconazole	0,9927 ± 0,0018
OH-Itraconazole	0,9993 ± 0,0002
Itraconazole	0,9960 ± 0,0024
5-Flucytosine	0,9966 ± 0,0007
Caspofungin	0,9971 ± 0,0012
Fluconazole	0,9988 ± 0,0004
Ketoconazole	0,9994 ± 0,0004
Anidulafungin	0,9972 ± 0,0007
Micafungin	0,9959 ± 0,0006
Isavuconazole	0,9930 ± 0,0028

Normal Range in blood :

Voriconazole	2 – 6 µg/ml
Posaconazole	> 0,7 µg/ml
OH-Itraconazole	0,5 – 2,9 µg/ml
Itraconazole	0,4 – 2 µg/ml
5-Flucytosine	20 – 50 µg/ml
Caspofungin	1 – 11 µg/ml
Fluconazole	15 – 95 µg/ml
Ketoconazole	1 – 3 µg/ml
Anidulafungin	ND
Micafungin	0,5 – 2,9 µg/ml
Isavuconazole	3 – 8 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 the reagents are ready to use and stable 3 years at 2–8 °C.
Reagent A – Deproteinization Solution + Internal Standard, 1 x 30 ml	
Calibrator lyoph. in plasma – Level 0, 1 x 1 ml	<u>Code LC82016 (packed separately – see data sheet)</u>
Calibrator lyoph. in plasma – Level 1, 1 x 1 ml	<u>Code LC82016 (packed separately – see data sheet)</u>
Calibrator lyoph. in plasma – Level 2, 1 x 1 ml	<u>Code LC82016 (packed separately – see data sheet)</u>
Calibrator lyoph. in plasma – Level 3, 1 x 1 ml	<u>Code LC82016 (packed separately – see data sheet)</u>
Calibrator lyoph. in plasma – Level 4, 1 x 1 ml	<u>Code LC82016 (packed separately – see data sheet)</u>
Calibrator lyoph. in plasma – Level 5, 1 x 1 ml	<u>Code LC82016 (packed separately – see data sheet a)</u>
Calibrator lyoph. in plasma – Level 6, 1 x 1 ml	<u>Code LC82016 (packed separately – see data sheet)</u>
Reagent M1 – Mobile Phase M1, 1 x 500 ml	
Reagent M2 – Mobile Phase M2, 1 x 500 ml	
Reagent M3 – Mobile Phase M3, 1 x 500 ml	

Minimum Instrumental equipment required:

LC/MS System with triple quadrupole
MRM Work Mode, Positive/Negative ESI
Operational computer
Chromatograms Recorder

Whole Blood Collection Procedure:

Take 3 ml of whole blood in a test tube with EDTA or Heparin as anticoagulant. Centrifuge at 4000 rpm for 5 minutes. Separate the serum and store at – 20 °C. Stable 4 weeks.

ANALYTICAL PROCEDURE

STEP 1: Preparation of Calibrator, Controls and Samples

Dispense in tubes of 1,5 ml:

	Blank	Calibrator	Control	Sample
Water HPLC grade	100 µl			
Calibrator		100 µl		
Control			100 µl	
Sample				100 µl
Reagent A – Deproteinization Sol. + Internal Std	300 µl	300 µl	300 µl	300 µl

Warning: deproteinize directly on Vortex for almost 10 seconds

STEP 2: Centrifuge at 10.000 rpm for 10 minutes.

STEP 3: Transfer the supernatant in glass vial with reduced volume.

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

INJECTION :

- Inject 10 µl of supernatant in LC system.

CAUTION: For the analysis of all Antimycotics, except for Micafungin, use Mobile Phases M1 (Float A) and M2 (Float B). For only Micafungin, use the Mobile Phase M3 (Float A) and M2 (Float B).

ANTIMYCOTICS IN PLASMA - Warnings

COLUMN CONDITIONING

Install the new analytical column **ACCUCORE C18 2.6µm, 30mm - 2.1 mm**. Thermostated at 40 °C. Disconnect the detector and flux a solution of H₂O : Acetonitrile (10 : 90 v/v) set flow at 200 µl / minute for 30 minutes. **Don't recycle the washing solutions.** Condition further on the column with a solution of H₂O : Mobile Phase M2 (20 : 80 v/v) set flow at 300 µl / minute for 15 minutes. **Don't recycle the washing solutions.** Condition further on the column with a solution of H₂O : Mobile Phase M1 (5 : 95 v/v) for 10 minutes. **IMPORTANT: FOR ANALYSIS OF MICA FUNGIN CONDIZIONATE WITH MOBILE PHASE M3 INSTEAD OF MOBILE PHASE M1.**

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 AND STORAGE

Column cleaning at the end of the session is not necessary. If the analytical sessions are not repeated the following day it is advisable to store in a solution of H₂O: Acetonitrile (5:95 v/v) at 200 µl/minute flow. The column should be stored in a solution of H₂O: Acetonitrile (5:95 v/v).

WASH AUTOSAMPLER SYRINGE

Wash the syringe, before every analytical session, with Methanol for LC/MS.

GRADIENT			
Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	Flow (ml/min)
0.5	100	0	0.5
1.00	60	40	0.5
3.00	5	95	0.5
4.00	5	95	0.5
4.01	100	0	0.5
5.00	100	0	0.5

ANALYTE	PARENT ION
5-Flucytosine	129.9
Caspofungin	547.4
Fluconazole	307
Ketoconazole	531
Itraconazole	705.1
OH-Itraconazole	721.2
Voriconazole	350
Posaconazole	701.2
Anidulafungin	1122.4
Micafungin	1268.4 (-)
Fenbuconazole	337.81
Isavuconazole	438.2 – 224.0 CE 22
Isavuconazole-D4	442.2 – 224.0 CE 22

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC82016	Calibrator in plasma for Antimycotics	7 x 2 x 1 ml
LC82019	Control in plasma for Antimycotics – Levels 1, 2 and 3	3 x 5 x 1 ml
TF17126032130	Analytical Column ACCUCORE C18 (30 mm x 2,1 mm – 2,6 µm)	1 Pc
SK82010	Starter kit for Antimycotics in plasma	13 x 1 ml
S51843550	Glass vials with reduced volume (globet form)	1 x 100 Pcs
S51820717	Caps for Glass vials with reduced volume (globet form)	1 x 100 Pcs



ANTIMYCOTICS IN PLASMA (Reference Chromatograms)

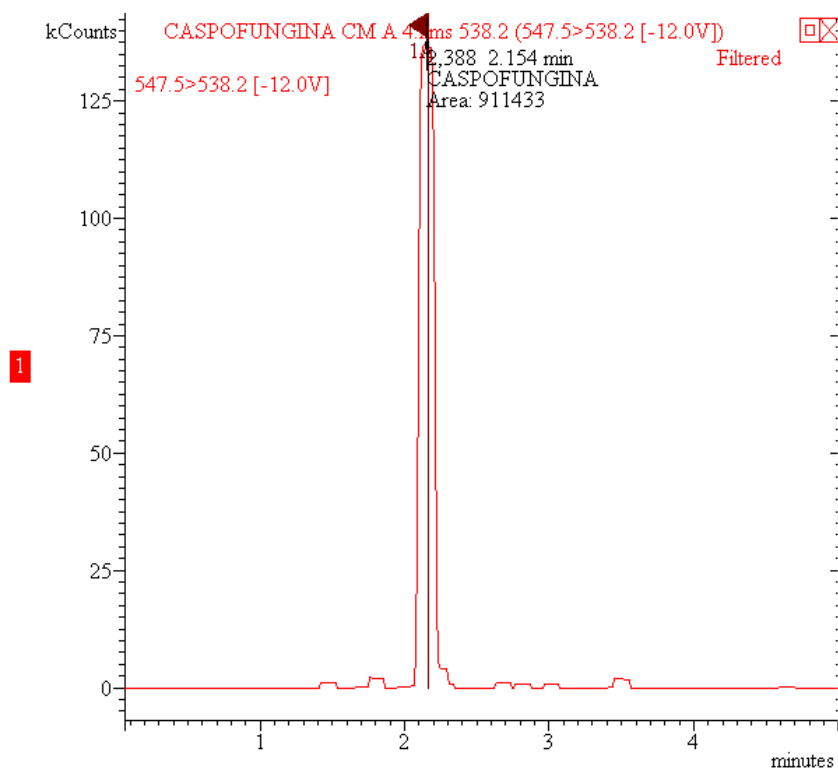


Fig. 1 : Plasma Calibrator

R.T. 2.154 Caspofungin 0,5 ug/ml

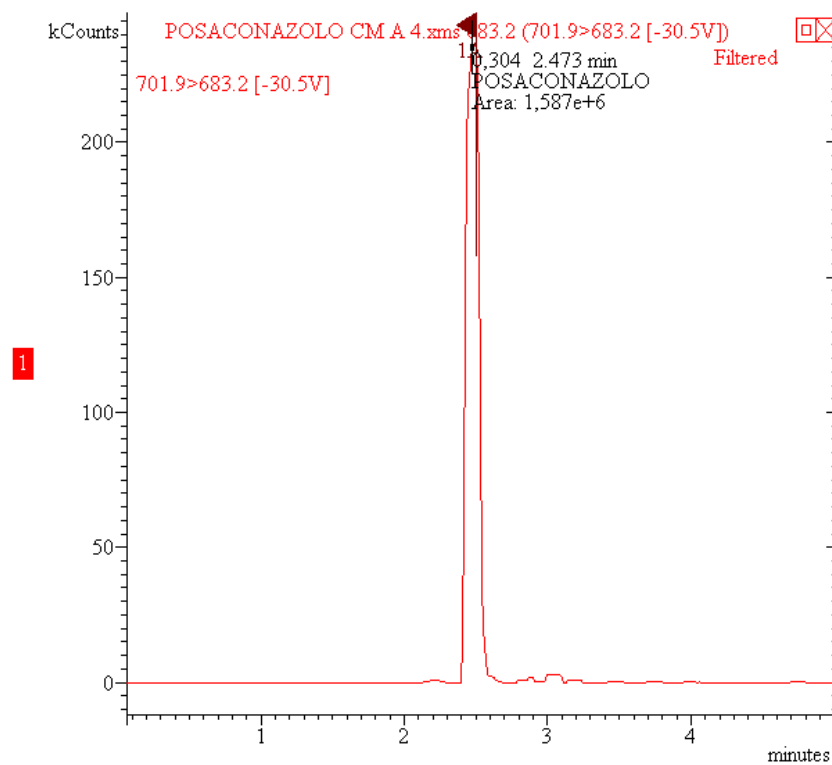


Fig. 2 : Plasma Calibrator

R.T. 2.473 Posaconazole 0,35 ug/ml

ANTIMYCOTICS IN PLASMA (Reference Chromatograms)

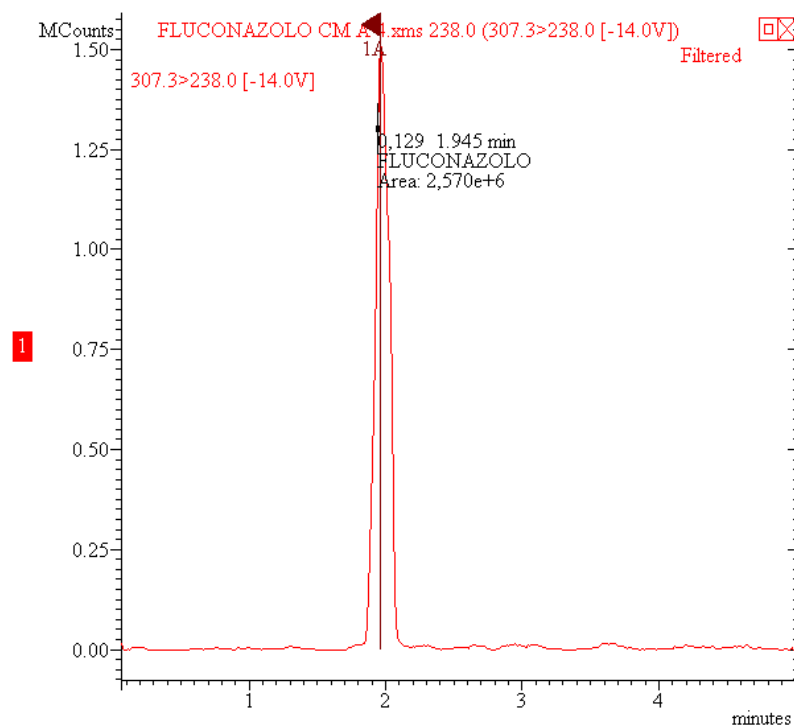


Fig. 3 :	Plasma Calibrator
R.T. 1.945	Fluconazole 0,5 ug/ml

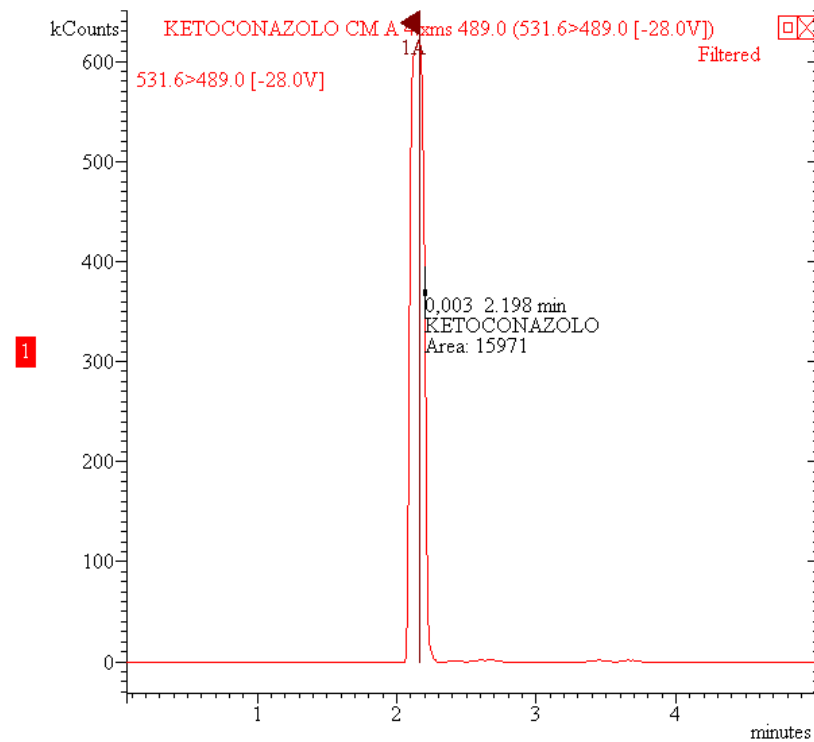


Fig. 4 :	Plasma Calibrator
R.T. 2.198	Ketoconazole 0,5 ug/ml

ANTIMYCOTICS IN PLASMA (Reference Chromatograms)

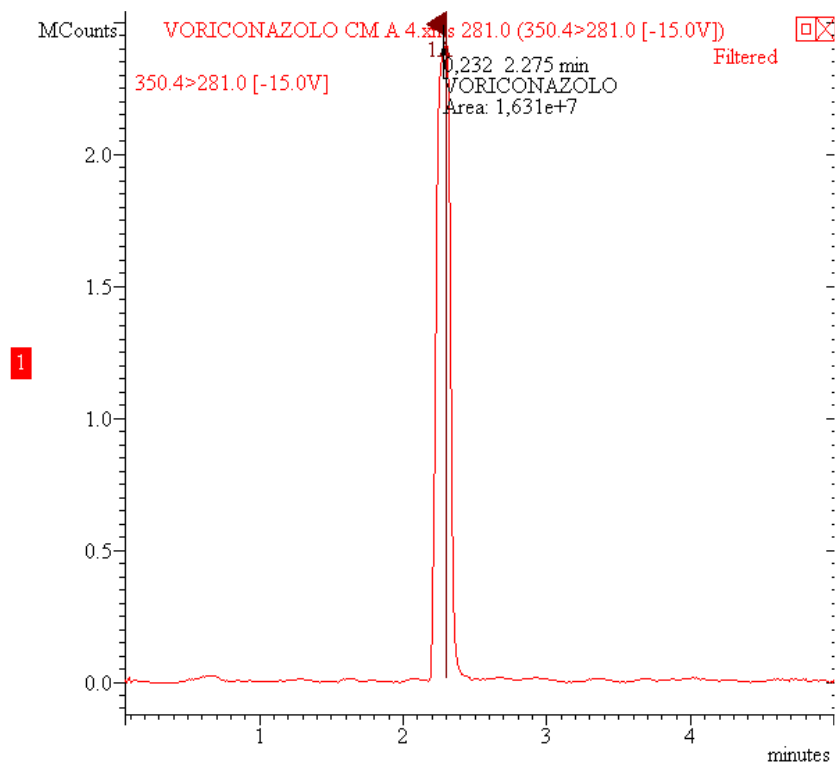


Fig. 5 :	Plasma Calibrator
R.T. 2.275 Voriconazole	0,5 ug/ml

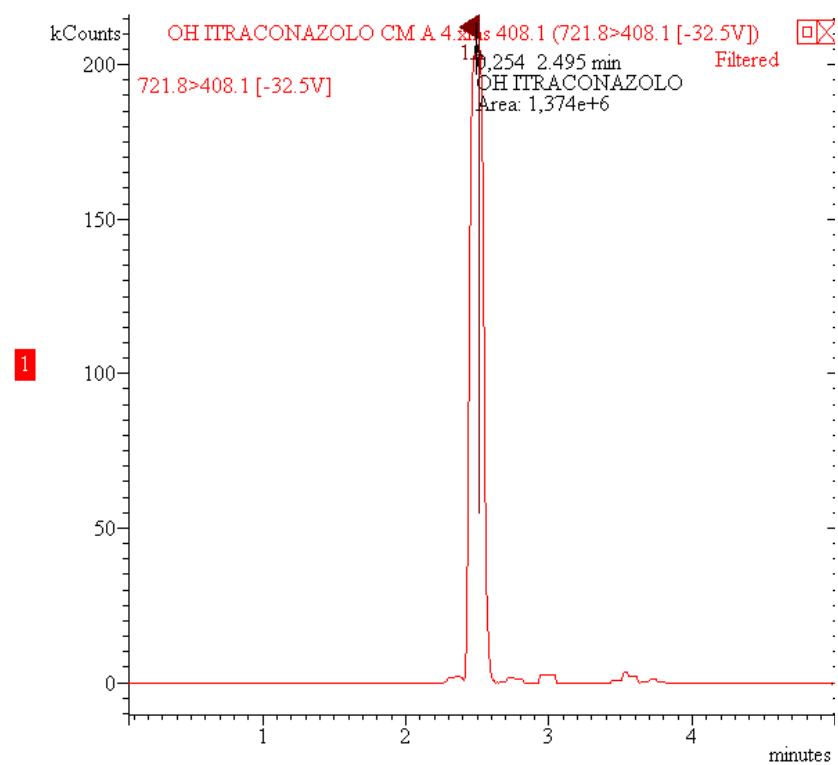


Fig. 6 :	Plasma Calibrator
R.T. 2.495 OH-Itraconazole	0,5 ug/ml

ANTIMYCOTICS IN PLASMA (Reference Chromatograms)

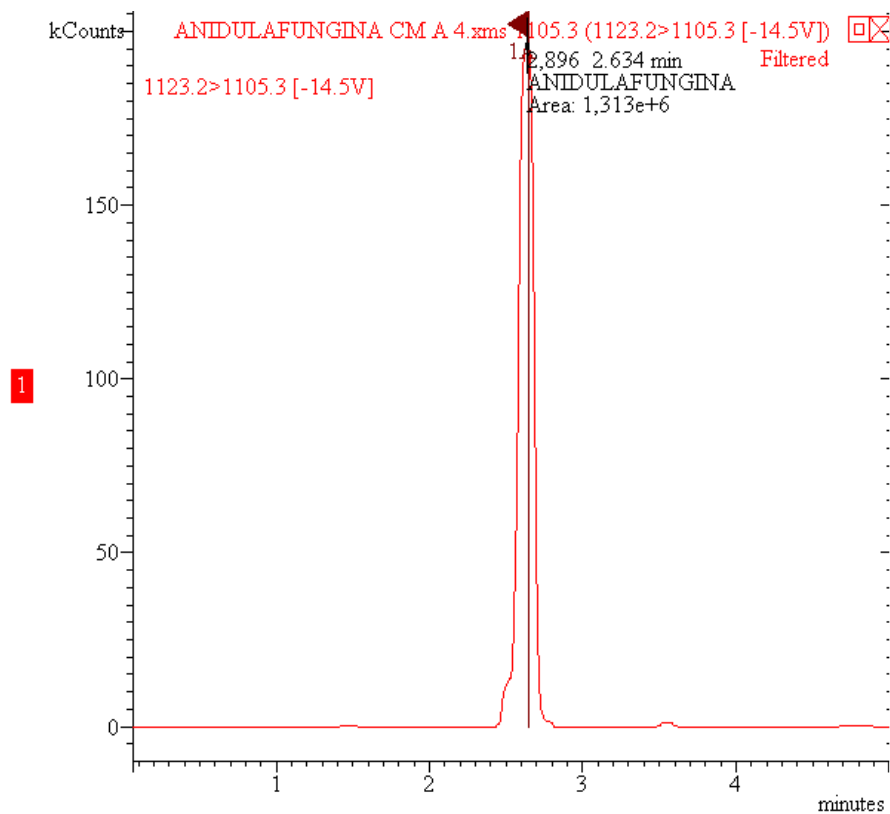


Fig. 7 : Plasma Calibrator
R.T. 2.634 Anidulafungin 2 ug/ml

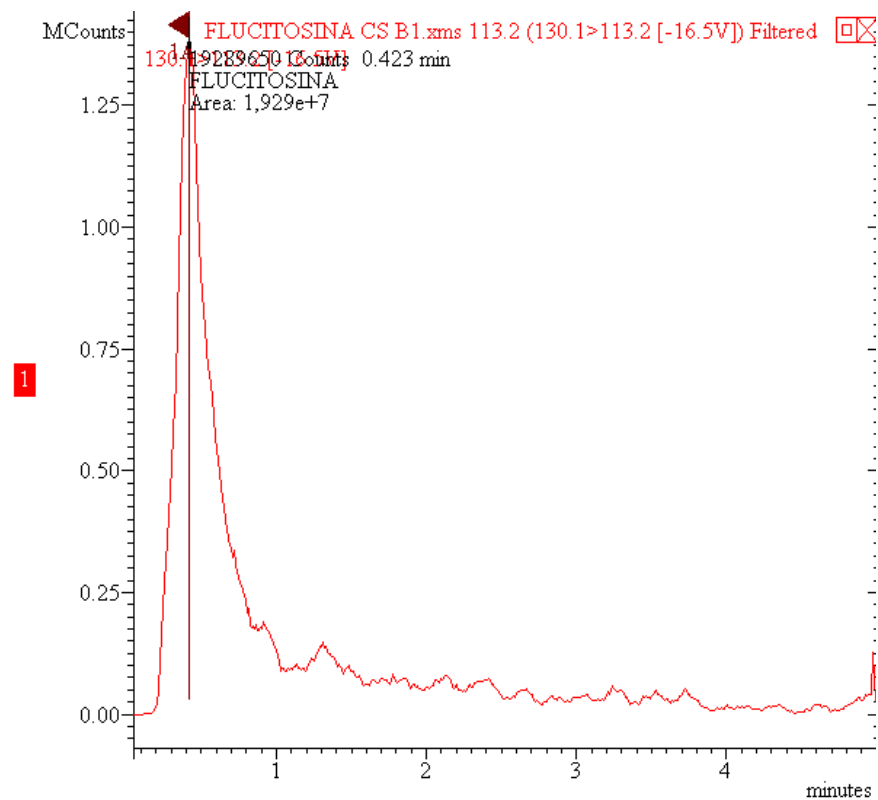


Fig. 8 : Plasma Calibrator
R.T. 0.423 5-Flucytosine 60 ug/ml

ANTIMYCOTICS IN PLASMA (Reference Chromatograms)

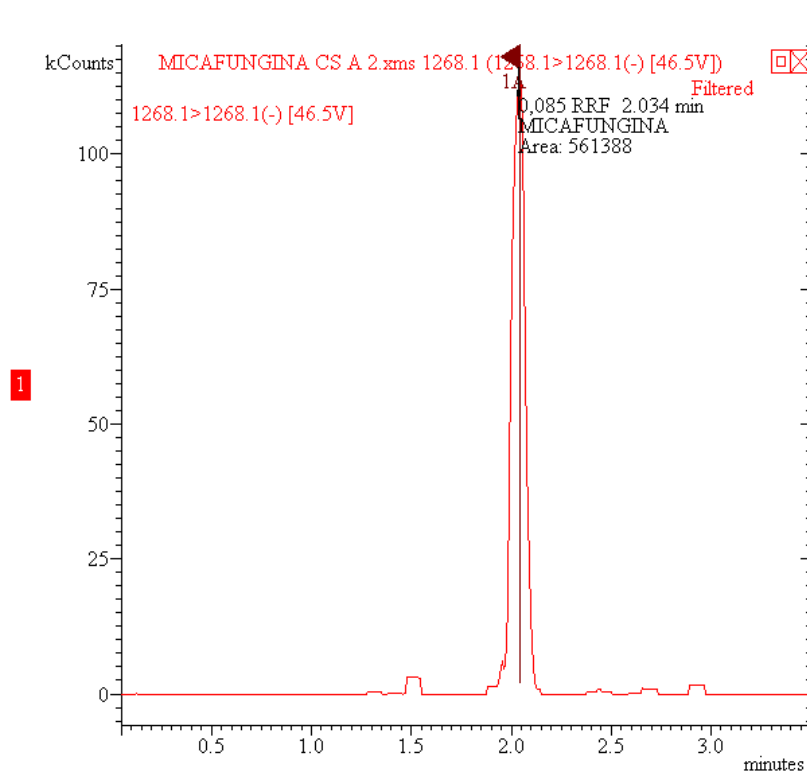


Fig. 9 : Plasma Calibrator

R.T. 2.034 Micafungin 2 ug/ml

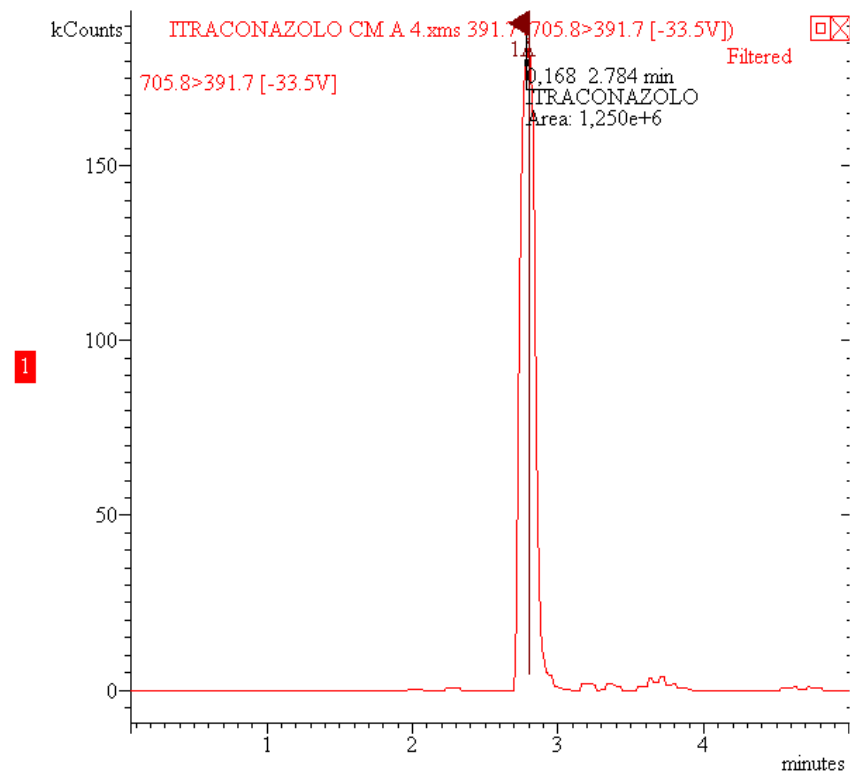


Fig. 10 : Plasma Calibrator

R.T. 2.784 Itraconazole 0,5 ug/ml

ANTIMICOTICS IN PLASMA (Reference Chromatograms)

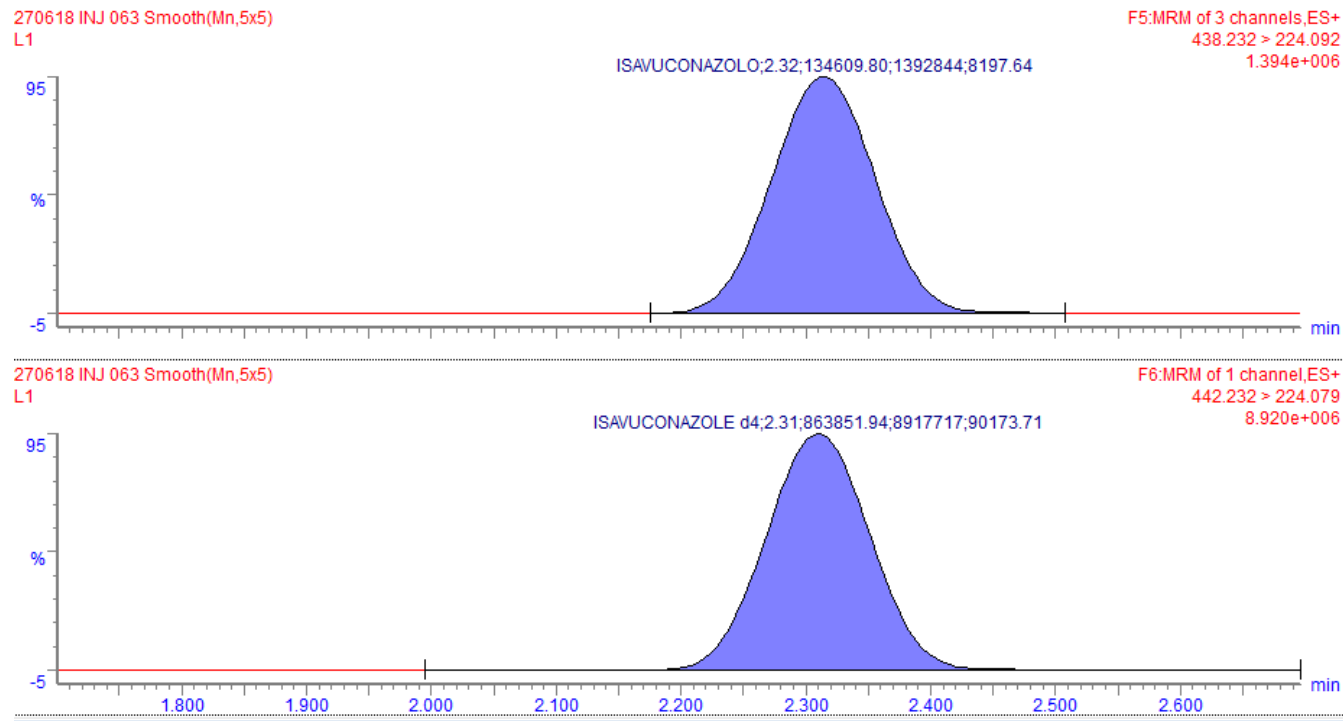


Fig. 11 :	Plasma Calibrator
R.T. 2.32	Isavuconazole 3 ug/ml
R.T. 2.31	Isavuconazole-D4