ANTIBIOTICS IN PLASMA by LC/MS – Code LC79215

(Daptomycin, Vancomycin, Streptomycin, Linezolid, Levofloxacin, Ciprofloxacin, Gentamicin, Amikacin, Teicoplanin, Meropenem, Ceftazidime)

INTRODUCTION

Technically, an "antibiotic" a substance of natural origin produced by a microorganism, able to kill another. The term in common usage today means a drug, natural or synthetic (chemotherapy) can slow or stop the proliferation of bacteria. Antibiotics are distinguished therefore bacteriostatic (ie, inhibit reproduction of the bacteria, preventing the split) and bactericidal (ie directly kill the organism). Usually have no effect against viruses, fungi and parasites, which act on other kinds of chemotherapy.

Streptomycin is a bactericidal antibiotic, the first to be discovered by a family called aminoglycosides, one of the first remedies against tuberculosis. Is obtained by attinobacteria. This drug can not be administered orally, but through regular intramuscular injections; one of its side effect is ototoxicity, which can lead to a temporary loss of hearing.

Vancomycin is a drug antibiotic produced by Streptococcus orientalis which is part, together with teicoplanin, the class of glycopeptides. Are molecules with high molecular weight, which act by inhibiting the polymerization of the wall of the peptidoglycan of Gram positive bacteria. The enterococcal endocarditis are treated with vancomycin and gentamicin. Pneumonia caused by Streptococcus pneumoniae, suspected resistance to penicillin, are treated with vancomycin combined with ceftriaxone and rifampicin. Vancomycin is also given as an alternative in patients allergic to penicillins and / or cephalosporins. In healthy dosage is a 1g every 12 hours for children are just 30 mg per kg per day.

Daptomycin is a new antibiotic lipopeptide used in the treatment of certain infections caused by Gram-positive organisms. It is a compound that exists in nature, which is located in the scavenger of the soil Streptomyces roseosporus. Its mechanism of action it could make it useful in the treatment of infections caused by multi-resistant bacteria. Daptomycin has a different mechanism of action, which is based on the interruption of many different functional aspects of the bacterial cell membrane. It seems that binds to the membrane and cause a rapid depolarization, resulting in loss of membrane potential. It thus inhibits the synthesis of proteins, DNA and RNA, causing bacterial cell death.

Gentamicin is an aminoglycoside antibiotic produced by Micromonospora purpurea, with high activity against Gram positive and Gram negative bacteria is a potent inhibitor of protein synthesis by binding tenaciously to the 30s subunit of ribosomes, with the same fragmentation and cell death, this property affects the mechanism of action of all aminoglycosides, since their operation is directly connected to its membrane permeability of each bacterium.

Linezolid is an antibiotic for hospital use active against Gram-positive aerobic and anaerobic, and in particular on MRSA (methicillin-resistant strains of Staphylococcus aureus) and VRSA (vancomycin-resistant strains of Staphylococcus).

Levofloxacin is an active ingredient which belongs to the class of second-generation fluoroquinolones.

Ciprofloxacin is the generic name of an international group of synthetic antibiotics fluoroquinolones. Ciprofloxacin is a bactericide. Its mode of action depends on blocking of bacterial DNA replication, through its binding to the enzyme DNA gyrase (a type II topoisomerase), which allows the unrolling needed to replicate a double helix of DNA. The drug has a hundred times more affinity for the bacterial DNA gyrase than that of mammals.

Teicoplanin is an antibiotic medication that belongs, along with the vancomycin, to class of glycopeptides, extracted from Actinoplanes teichomyceticus.
Are molecules with high molecular weight, which act by inhibiting the polymerization of the wall of peptoglican of gram positive bacteria. The high molecular weight does not allow such molecules to cross the outer cell membrane of Gram-negative germs, for which the teicoplanin as well as vancomycin, is ineffective against these bacteria.
However the teicoplanin has a longer half-long (45 - 70 hours) of vancomycin, and this may allow administration in a single daily dose. Furthermore, the teicoplanin can be injected either intravenously which intramuscularly.

Meropenem is a β lactam antibiotic, belonging to the class of carbapenems with a very wide spectrum of action including Gram+ and Gram-. Meropenem is useful in case of complicated skin infections caused by some strains of Staphylococcus aureus or Streptococcus pyogenes, Streptococcus agalactiae, Streptococci viridans, Enterococcus faecalis non vancomycin resistant, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Bacteroides fragilis and some species of Peptostreptococcus. It is also used in cases of complicated appendicitis caused by streptococci viridans, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacteroides fragilis, Bacteroides thetaiotaomicron and Peptostreptococcus species. Finally it is useful in the treatment of bacterial meningitis caused by Streptococcus pneumoniae, by some strains of Haemophilus influenzae and by Neisseria meningitidis.

Ceftazidime is a methoxyiminic cephalosporin with a very broad spectrum. The in vitro drug is active on a wide range of Gram+ and Gram- aerobes and anaerobes including enterobacter, proteus, Providencia, citrobacter serratia, pseudomonas, neisseria, legionella, clostridia, and bacterioides. The drug manages to eliminate β-lactamase-producing bacteria except for resistant meticillin. The drug is taken parenterally, has a low protein binding and is eliminated by the kidney where it comes into active form in the urine. The antibiotic quickly and in high concentrations reaches all the organs.

Antibiotics can affect the intestinal flora and giving, consequences, changes in habit, may be covered by the intake of lactic acid bacteria. Antibiotics can cause side effects like allergic reactions, due to the sensitivity of the individual towards one or more components, or high toxicity level of various districts of the organism, or even intolerance caused by interaction with other drugs. The dependence on these drugs and the abuse of them can lead to death. For this reason that is of particular importance the dosage of the latter even at low concentrations.

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CE

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.

TECHNICAL FEATURES

Principle of the Method:
The plasma samples of Antibiotics after deproteinization with a suitable reagent containing the internal standard, are centrifuged and directly injected into the LC / MS.

Sensitivity (LLOD) :

Daptomycin	0,03 mg/l
Vancomycin	0,03 mg/l
Linezolid	0,03 mg/l
Levofloxacin	0,03 mg/l
Ciprofloxacin	0,03 mg/l
Gentamicin	0,03 mg/l
Streptomycin	0,1 mg/l
Amikacin	0,03 mg/l
Teicoplanin	0,03 mg/l
Meropenem	0,003 mg/l
Ceftazidime	0,003 mg/l

Minimum concentration analyzable (LLOQ) :

Daptomycin	0,1 mg/l
Vancomycin	0,1 mg/l
Linezolid	0,1 mg/l
Levofloxacin	0,1 mg/l
Ciprofloxacin	0,1 mg/l
Gentamicin	0,1 mg/l
Streptomycin	0,3 mg/l
Amikacin	0,1 mg/l
Teicoplanin	0,1 mg/l
Meropenem	0,01 mg/l
Ceftazidime	0,01 mg/l

Daptomycin	0,1 – 50 mg/l
Vancomycin	0,1 – 50 mg/l
Linezolid	0,1 – 50 mg/l
Levofloxacin	0,1 – 50 mg/l
Ciprofloxacin	0,1 – 50 mg/l
Gentamicin	0,1 – 50 mg/l
Streptomycin	0,3 – 50 mg/l
Amikacin	0,1 – 50 mg/l
Teicoplanin	0,1 – 50 mg/l
Meropenem	0,01 – 50 mg/l
Ceftazidime	0,01 – 50 mg/l

Therapeutic Range :

Daptomycin	n. d.
Vancomycin	n. d.
Linezolid	n. d.
Levofloxacin	n. d.
Ciprofloxacin	n. d.
Gentamicin	n. d.
Streptomycin	n. d.
Amikacin	n. d.
Teicoplanin	n. d.
Meropenem	n. d.
Ceftazidime	n. d.

Accuracy intra serie (relative error %)
Daptomycin:

Cm	Cs
2,5 mg/l	10 mg/l
9,69%	6,61%

Accuracy inter serie (relative error %) Daptomycin:

Cm	Cs
2,5 mg/l	10 mg/l
8,77%	3,63%

Reproducibility intra serie (relative error %) Daptomycin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
6,64%	2,96%	1,38%

Reproducibility inter serie (coefficient of variation %) Daptomycin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
20,69%	11,10%	7,28%

Coefficient of correlation R2 + DS : 0,9954 ± 0,0024 Daptomycin

Accuracy intra serie (relative error %) Vancomycin:

Cm	Cs
2,5 mg/l	10 mg/l
8,15%	6,09%

Accuracy inter serie (relative error %) Vancomycin:

Cm	Cs
2,5 mg/l	10 mg/l
8,58%	6,56%

Reproducibility intra serie (relative error %) Vancomycin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
6,64%	3,43%	3,86%

Reproducibility inter serie (coefficient of variation %) Vancomycin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
8,09%	8,79%	6,89%

Coefficient of correlation R2 + DS : 0,9945 ± 0,0016 Vancomycin

Accuracy intra serie (relative error %) Linezolid:

Cm	Cs
2,5 mg/l	10 mg/l
10,57%	4,57%

Accuracy inter serie (relative error %) Linezolid:

Cm	Cs
2,5 mg/l	10 mg/l
8,20%	7,35%

Reproducibility intra serie (relative error %) Linezolid:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
4,41%	4,70%	2,46%

Reproducibility inter serie (coefficient of variation %) Linezolid:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
10,90%	5,38%	5,44%

Coefficient of correlation R2 + DS : 0,9961 ± 0,0013 Linezolid

Accuracy intra serie (relative error %) Levofloxacin:

Cm	Cs
2,5 mg/l	10 mg/l
6,14%	3,94%

Accuracy inter serie (relative error %) Levofloxacin:

Cm	Cs
2,5 mg/l	10 mg/l
6,30%	4,67%

Reproducibility intra serie (relative error %) Levofloxacin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
3,38%	8,76%	4,19%

Reproducibility inter serie (coefficient of variation %) Levofloxacin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
10,10%	5,77%	5,38%

Coefficient of correlation R2 + DS : 0,9975 ± 0,0006 Levofloxacin

Accuracy intra serie (relative error %) Ciprofloxacin:

Cm	Cs
2,5 ng/ml	10 ng/ml
6,14%	4,32%

Accuracy inter serie (relative error %) Ciprofloxacin:

Cm	Cs
2,5 mg/l	10 mg/l
6,53%	3,76%

Reproducibility intra serie (relative error %) Ciprofloxacin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
4,61%	5,96%	6,11%

Reproducibility inter serie (coefficient of variation %) Ciprofloxacin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
10,77%	6,55%	9,84%

Coefficient of correlation R2 + DS : 0,9964 ± 0,0017 Ciprofloxacin

Accuracy intra serie (relative error %) Gentamicin:

Cm	Cs
2,5 mg/l	10 mg/l
4,66%	7,54%

Accuracy inter serie (relative error %) Gentamicin:

Cm	Cs
2,5 mg/l	10 mg/l
10,02%	4,44%

Reproducibility intra serie (relative error %) Gentamicin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
0,38%	3,94%	3,15%

Reproducibility inter serie (coefficient of variation %) Gentamicin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
9,04%	9,16%	13,39%

Coefficient of correlation R2 + DS : 0,9972 ± 0,0008 Gentamicin

Accuracy intra serie (relative error %) Streptomycin:

Cm	Cs
2,5 mg/l	10 mg/l
12,21%	6,54%

Accuracy inter serie (relative error %) Streptomycin:

Cm	Cs
2,5 mg/l	10 mg/l
12,53%	7,01%

Reproducibility intra serie (relative error %) Streptomycin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
3,73%	3,94%	3,15%

Reproducibility inter serie (coefficient of variation %) Streptomycin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
8,35%	85,03%	91,02%

Coefficient of correlation R2 + DS : 0,9967 ± 0,0012 Streptomycin

Accuracy intra serie (relative error %) Amikacin:

Cm	Cs
2,5 mg/l	10 mg/l
9,24%	2,10%

Accuracy inter serie (relative error %) Amikacin:

Cm	Cs
2,5 mg/l	10 mg/l
6,46%	3,74%

Reproducibility intra serie (relative error %) Amikacin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
17,98%	2,45%	1,18%

Reproducibility inter serie (coefficient of variation %) Amikacin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
109,99%	52,59%	64,03%

Coefficient of correlation R2 + DS : 0,9978 ± 0,0019 Amikacin

Accuracy intra serie (relative error %) Teicoplanin:

Cm	Cs
2,5 mg/l	10 mg/l
11,84%	4,67%

Accuracy inter serie (relative error %) Teicoplanin:

Cm	Cs
2,5 mg/l	10 mg/l
11,43%	5,95%

Reproducibility intra serie (relative error %) Teicoplanin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
8,57%	3,57%	1,59%

Reproducibility inter serie (coefficient of variation %) Teicoplanin:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
7,28%	7,48%	2,10%

Coefficient of correlation R2 + DS : 0,9959 ± 0,0004 Teicoplanin

Accuracy intra serie (relative error %) Meropenem:

Cm	Cs
2,5 mg/l	10 mg/l
8,00%	5,65%

Accuracy inter serie (relative error %) Meropenem:

Cm	Cs
2,5 mg/l	10 mg/l
7,16%	4,76%

Reproducibility intra serie (relative error %) Meropenem:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
3,38%	8,49%	1,44%

Reproducibility inter serie (coefficient of variation %) Meropenem:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
3,27%	5,76%	12,66%

Coefficient of correlation R2 + DS : 0,9959 ± 0,0016 Meropenem

Accuracy intra serie (relative error %) Ceftazidime:

Cm	Cs
2,5 mg/l	10 mg/l
14,31%	4,18%

Accuracy inter serie (relative error %) Ceftazidime:

Cm	Cs
2,5 mg/l	10 mg/l
12,01%	5,04%

Reproducibility intra serie (relative error %) Ceftazidime:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
4,33%	3,05%	1,55%

Reproducibility inter serie (coefficient of variation %) Ceftazidime:

C LLOQ	Cm	Cs
0,1 mg/l	5 mg/l	25 mg/l
8,88%	4,64%	1,94%

Coefficient of correlation R2 + DS : 0,9963 ± 0,0014 Ceftazidime

Components of the kit : All the Reagents are ready to use and stable 3 years at 2–8 °C, except **Reagent A** that must stored at -20 °C.

Reagent A – Deproteinization Solution with Internal Standards Deuterated, **1 x 30 ml** **Store at -20°C**

Reagent B – Stabilization Solution, **1 x 5 ml**

Plasma Calibrator – Level 0, 3 x 1 ml	**Code LC79016 (packed separately – see data sheet)**
Plasma Calibrator – Level 1, 3 x 1 ml	**Code LC79016 (packed separately – see data sheet)**
Plasma Calibrator – Level 2, 3 x 1 ml	**Code LC79016 (packed separately – see data sheet)**
Plasma Calibrator – Level 3, 3 x 1 ml	**Code LC79016 (packed separately – see data sheet)**
Plasma Calibrator – Level 4, 3 x 1 ml	**Code LC79016 (packed separately – see data sheet)**
Plasma Calibrator – Level 5, 3 x 1 ml	**Code LC79016 (packed separately – see data sheet)**
Plasma Calibrator – Level 6, 3 x 1 ml	**Code LC79016 (packed separately – see data sheet)**

Reagent D – Diluting Solution, **1 x 50 ml**

Reagent M1 – Mobile Phase M1, **5 x 500 ml**

Reagent M2 – Mobile Phase M2, **5 x 500 ml**

Minimum Instrumental equipment required:

LC/MS System with triple quadrupole
MRM Work Mode, positive ESI
Chromatograms Recorder

Optional Equipment:

Autosampler.
Operational Computer

Whole Blood Collection Procedure:

Take 3 ml of whole blood in a test tube without gel or with EDTA as anticoagulant. Centrifuge at 4000 rpm for 5 minutes. **Samples for Meropenem should be diluted immediately with MES 1:1 (MES = 2.4-Morpholino-etansulfonic Acid 0.1 M pH=6.5) and then frozen.** Separate the serum and store at – 20 °C. Stable 4 weeks.

ANALYTICAL PROCEDURE

Samples for Meropenem should be diluted immediately with MES 1 1 (MES = 2.4 Morpholino etansulfonic Acid 0.1 M Ph = 6.5) and then frozen. Separate the serum and store it at - 20 ° C.

STEP 1 :

Pipette in eppendorf:

	Calibrator – Level 0	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Calibrator – Level 6	Sample	Controls
Calibrator – Level 0	25 µl								
Calibrator – Level 1		25 µl							
Calibrator – Level 2			25 µl						
Calibrator – Level 3				25 µl					
Calibrator – Level 4					25 µl				
Calibrator – Level 5						25 µl			
Calibrator – Level 5							25 µl		
Sample								25 µl	
Controls									25 µl
Reagent B – Stabilization Solution	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl
Reagent A – Deproteinization Sol + Internal Standard	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl

Vortex for 20 sec.

STEP 2: Centrifuge at 14.000 rpm for 10 min.

N.B.: at this step, the sample is stable 48 hours at 2-8 °C

STEP 3: Dilute 100 µl of surnatant with 100 µl of **Reagent D –** Diluting Solution

Vortex for 20 sec.

INJECTION :

- Inject 2-10 µl of surnatant in LC system.

ANTIBIOTICS IN PLASMA - Warnings

COLUMN CONDITIONING

Install a new analytical column Polaris C18 100 x 2 mm, 3 um. Thermostated at 25°C. Disconnect the detector and flux a solution of H_2O : Mobile Phase M2 (5 : 95 v/v) at flow of 300 ul / min for 15 minutes. **Don't recycle the washing solutions**. Condition further on the column with a solution of Mobile Phase M1 : Mobile Phase M2 (95 : 5 v/v) at flow of 300 ul / min for 15 minutes.

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

Disconnect the detector and flux a solution of Mobile Phase M2 : Mobile Phase M1 (95 : 5 v/v) set flow at 300 ul/min for 15 minutes and discharged it. Store the column in a solution of Mobile Phase M2.

WASH AUTOSAMPLER SYRINGE

Wash the syringe, before every analytical session, with Methanol. Wash the autosampler needle intra - serie with Methanol.

FLOW SET

GRADIENT			
Time (min)	**% M1 (PUMP A)**	**% M2 (PUMP B)**	**Flow (µl/min)**
0	98	2	300
0.60	98	2	300
7.00	20	80	300
7.10	2	98	300
9.00	2	98	300
9.10	98	2	300
13.00	98	2	300

Analyte	Q1	Q3	Internal Standard
Amikacin (+)	586.4	264.1	Apramycin
Streptomycin (+)	582.3	263.3	Apramycin
Gentamicin (+)	478.04	157.2	Apramycin
Daptomycin (+)	811.3	341.3	Daptomycin-D5
Vancomycin (+)	725.3	144.1	Meropenem-D6
Teicoplanin (-)	938.6	678.0	Daptomycin-D5
Levofloxacin (+)	362.1	318.1	Levofloxacin-D8
Ciprofloxacin (+)	332.2	314.3	Ciprofloxacin-D8
Linezolid (+)	338.3	296.3	Levofloxacin-D8
Meropenem (+)	384.3	141.2	Meropenem-D6
Ceftazidime (+)	547.2	468.0	Meropenem-D6
Meropenem-D6 (+)	390.1	147.2	-
Ciprofloxacin-D8 (+)	340.2	235.1	-
Levofloxacin-D8 (+)	370.1	265.1	-
Daptomycin-D5 (+)	822.7	317.2	-
Apramycin (+)	540.4	133.0	-

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC79016	Calibrator in plasma for Antibiotics	7 x 2 x 1 ml
LC79017	Control in plasma for Antibiotics – Level 1	5 x 1 ml
LC79018	Control in plasma for Antibiotics – Level 2	5 x 1 ml
LC79013	Control in plasma for Antibiotics – Level 3	5 x 1 ml
LC79019	Control in plasma for Antibiotics – Levels 1, 2 and 3	3 x 5 x 1 ml
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
ZA2001100X020	Analytical Column Polaris C18 (100 x 2 mm, 3 um)	1 Pc
SK79210	Starter kit for Antibiotics	1 Pc

ANTIBIOTICS IN PLASMA

(Reference Chromatograms)

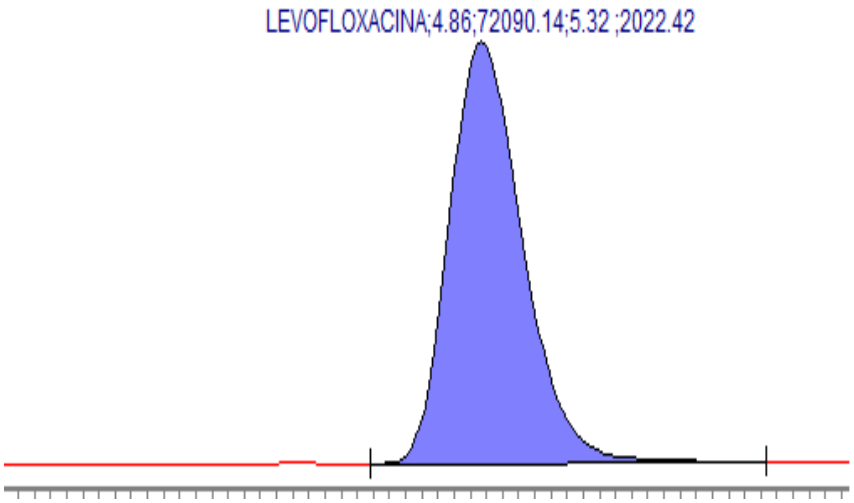


Fig. 1 :	**Plasma Calibrator**
R.T. 4.86	Levofloxacin

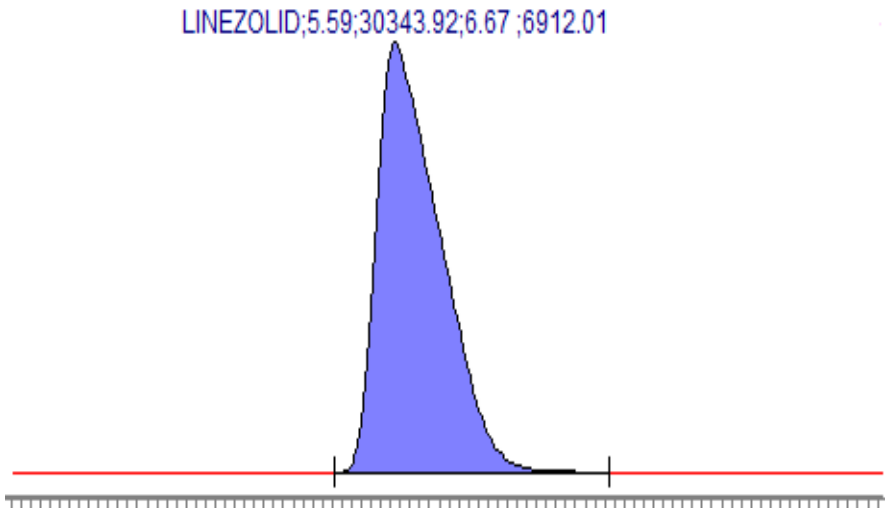


Fig. 2 :	**Plasma Calibrator**
R.T. 5.59	Linezolid

ANTIBIOTICS IN PLASMA

(Reference Chromatograms)

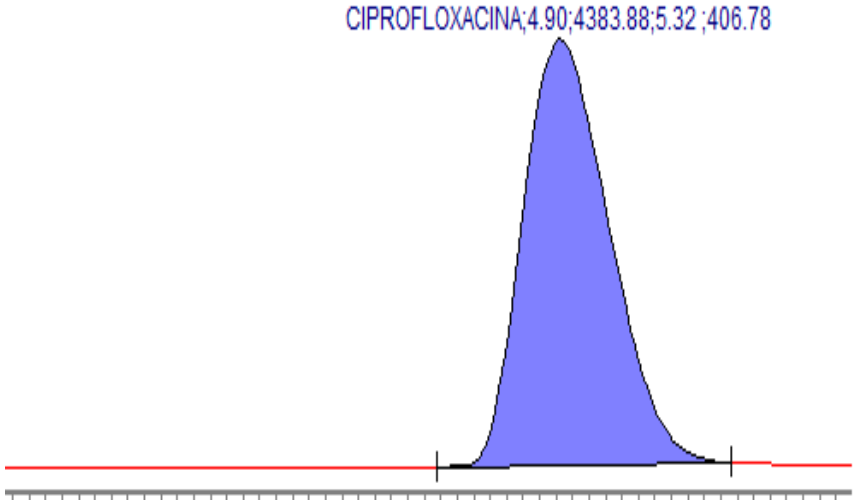


Fig. 3 :	**Plasma Calibrator**
R.T. 4.90	Ciprofloxacin

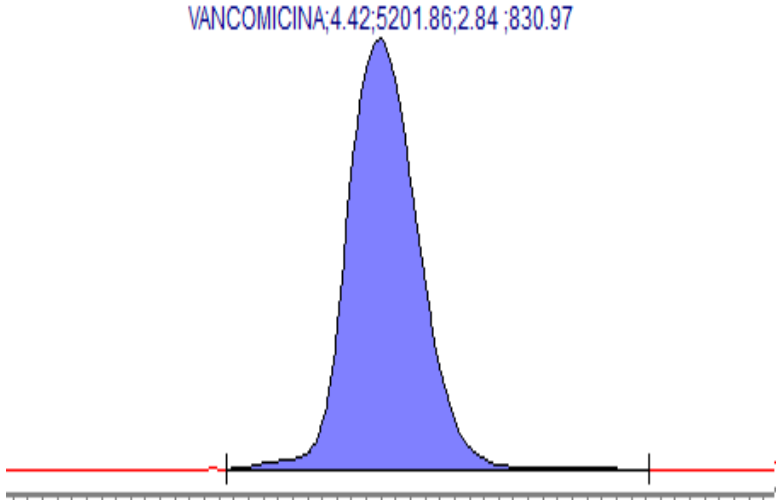


Fig. 4 :	**Plasma Calibrator**
R.T. 4.42	Vancomycin

ANTIBIOTICS IN PLASMA

(Reference Chromatograms)

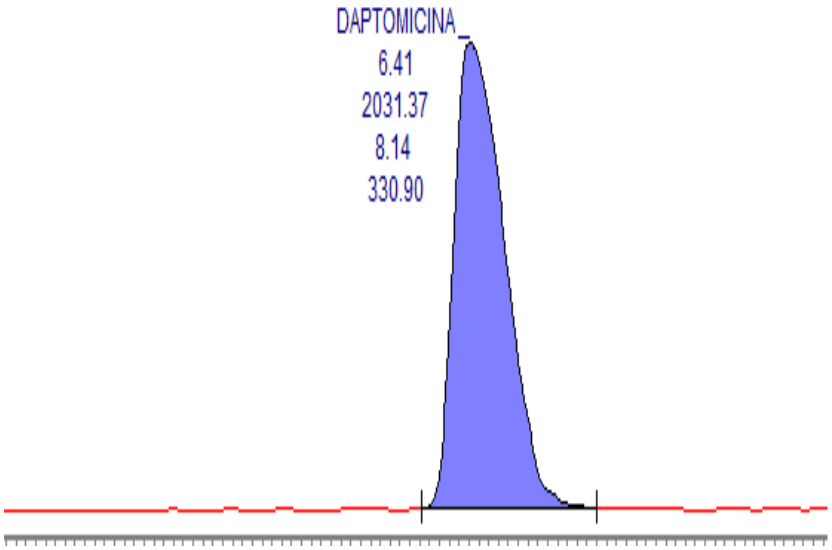


Fig. 5 :	**Plasma Calibrator**
R.T. 6.41	Daptomycin

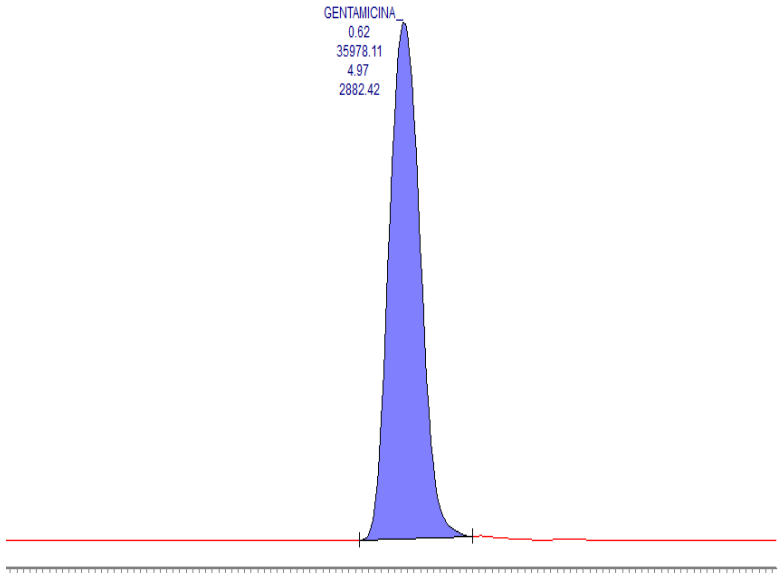


Fig. 6 :	**Plasma Calibrator**
R.T. 0.52	Gentamicin

ANTIBIOTICS IN PLASMA

(Reference Chromatograms)

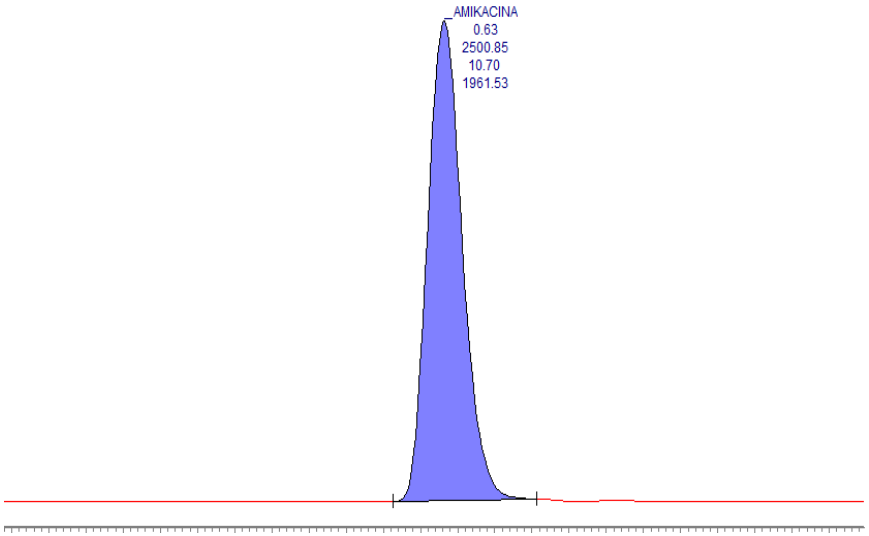


Fig. 7 :	**Plasma Calibrator**
R.T. 0.43	Amikacin

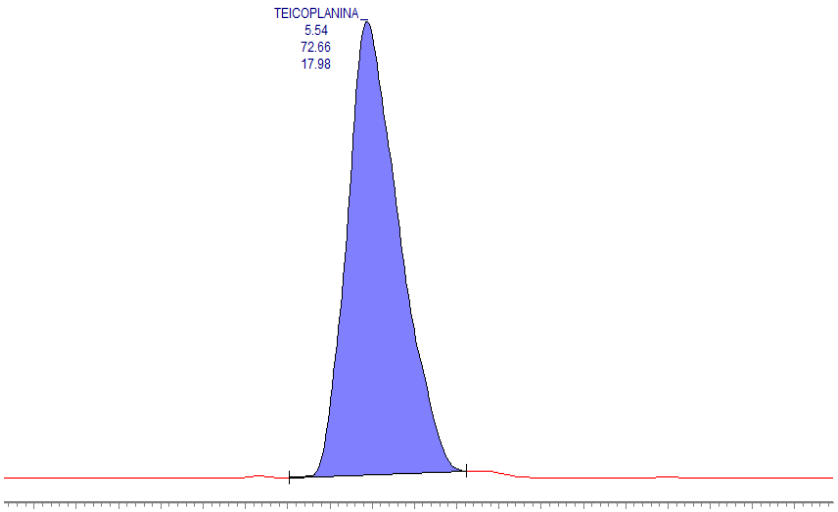
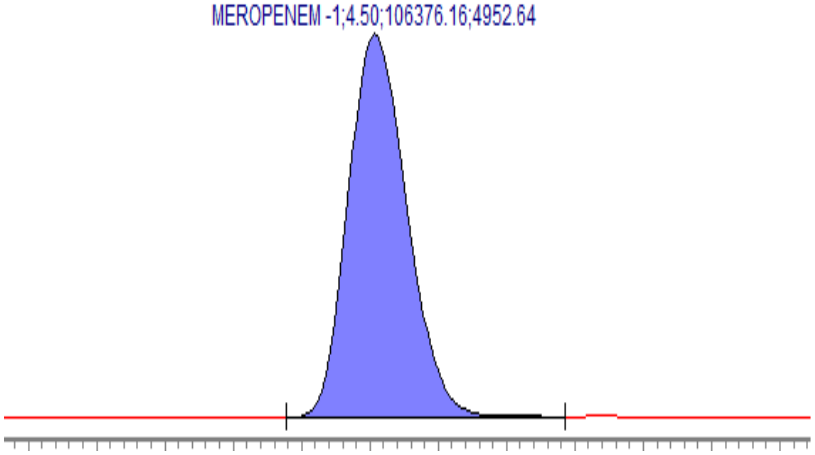


Fig. 8 :	**Plasma Calibrator**
R.T. 5.54	Teicoplanin

ANTIBIOTICS IN PLASMA

(Reference Chromatograms)



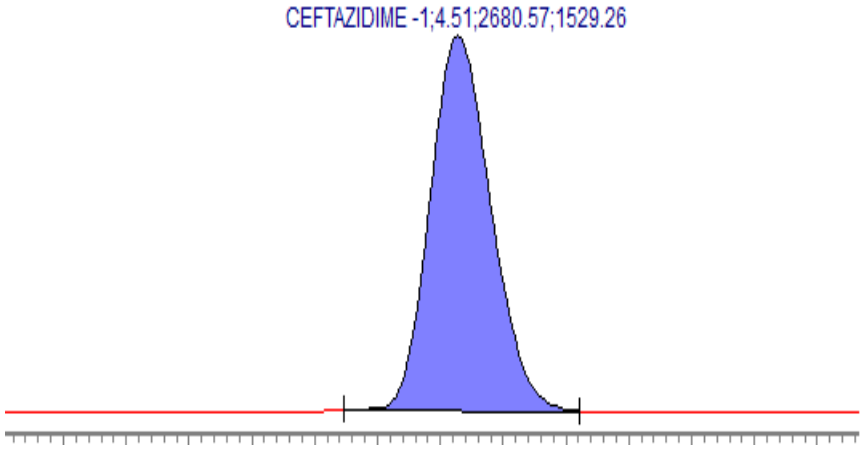


Fig. 9 :	**Plasma Calibrator**
R.T. 4.50	Meropenem

Fig. 10 :	**Plasma Calibrator**
R.T. 4.51	Ceftazidime

ANTIBIOTICS IN PLASMA

(Reference Chromatograms)

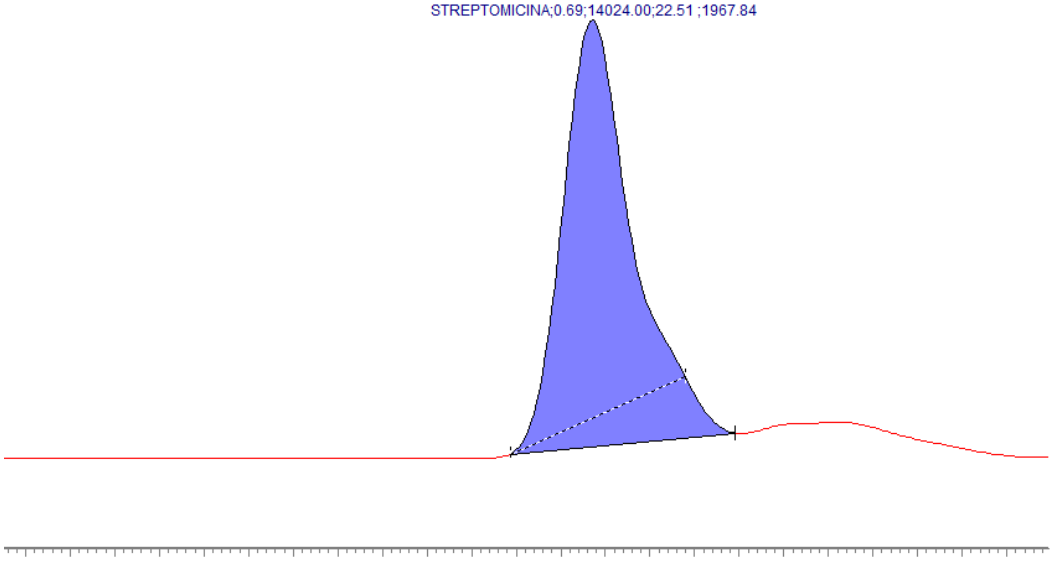


Fig. 11 :	**Plasma Calibrator**
R.T. 0.69	Streptomycin