

ANTIBIOTICS IN PLASMA by LC/MS – Code LC79010

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

INTRODUCTION

Technically it defines "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 actinobacteria. 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, causing the 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 focusing particularly 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. La Ciprofloxacin is bactericidal, 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 peptoglycan 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.

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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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.

Release N° 003	Antibiotics in plasma by LC/MS	April 2013
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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 : 0,5 mg/l

Dynamic Range of the Method : 1 - 100 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.

CV%:	Daptomycin	4,0 intra serie	Daptomycin	7,1 inter serie
	Vancomycin	2,5 intra serie	Vancomycin	24,7 inter serie
	Linezolid	9,2 intra serie	Linezolid	2,6 inter serie
	Levofloxacin	5,5 intra serie	Levofloxacin	5,9 inter serie
	Ciprofloxacin	4,7 intra serie	Ciprofloxacin	9,0 inter serie
	Gentamicin	8,8 intra serie	Gentamicin	8,4 inter serie
	Streptomycin	11,0 intra serie	Streptomycin	21,5 inter serie
	Amikacin	8,4 intra serie	Amikacin	11,0 inter serie
	Teicoplanin	3,9 intra serie	Teicoplanin	4,5 inter serie

Components of the kit : All the Reagents are ready to use and stable 3 years at 2–8 °C.

Reagent A – Deproteinization Solution with Internal Standard, **1 x 30 ml**

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

Reagent C1 – Plasma Calibrator – Level 1, **1 x 2 ml** **See Warnings**

Reagent C2 – Plasma Calibrator – Level 2, **1 x 2 ml** **See Warnings**

Reagent C3 – Plasma Calibrator – Level 3, **1 x 2 ml** **See Warnings**

Reagent C4 – Plasma Calibrator – Level 4, **1 x 2 ml** **See Warnings**

Reagent C5 – Plasma Calibrator – Level 5, **1 x 2 ml** **See Warnings**

Reagent D – Diluting Solution, **1 x 20 ml**

Reagent M1 – Mobile Phase M1, **1 x 500 ml**

Reagent M2 – Mobile Phase M2, **1 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. Separate the serum and store at – 20 °C. Stable 4 weeks.

ANALYTICAL PROCEDURE FOR ANTIBIOTICS (EXCEPT TEICOPLANIN)

STEP 1 :

Pipette in eppendorf:

	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Sample	Controls
Reagent C1 – Calibrator – Level 1	100 µl						
Reagent C2 – Calibrator – Level 2		100 µl					
Reagent C3 – Calibrator – Level 3			100 µl				
Reagent C4 – Calibrator – Level 4				100 µl			
Reagent C5 – Calibrator – Level 5					100 µl		
Sample						100 µl	
Controls							100 µl
Reagent B – Stabilization Solution	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

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 200 µl of surnatant with 200 µl of **Reagent D – Diluting Solution**

INJECTION :

- Inject 10 µl of surnatant in LC system.

ANALYTICAL PROCEDURE FOR TEICOPLANIN

STEP 1 :

Pipette in eppendorf:

	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Sample	Controls
Reagent C1 – Calibrator – Level 1	100 µl						
Reagent C2 – Calibrator – Level 2		100 µl					
Reagent C3 – Calibrator – Level 3			100 µl				
Reagent C4 – Calibrator – Level 4				100 µl			
Reagent C5 – Calibrator – Level 5					100 µl		
Sample						100 µl	
Controls							100 µl
Reagent A – Deproteinization Sol + Internal Standard	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 200 µl of surnatant with 200 µl of **Reagent D – Diluting Solution**

INJECTION :

- Inject 10 µl of surnatant in LC system.

ANTIBIOTICS IN PLASMA - Warnings

REAGENTS C1-C2-C3-C4-C5 – CALIBRATOR LIOPHIL. IN PLASMA Lot. 001

ANTIBIOTICS:	Level 1 mg/L	Level 2 mg/L	Level 3 mg/L	Level 4 mg/L	Level 5 mg/L
Daptomycin	1	7,5	15	30	50
Vancomycin	1	7,5	15	30	50
Linezolid	1	7,5	15	30	50
Levofloxacin	1	7,5	15	30	50
Ciprofloxacin	1	7,5	15	30	50
Gentamicin	1	7,5	15	30	50
Streptomycin	1	7,5	15	30	50
Amikacin	1	7,5	15	30	50
Teicoplanin					
Use and Reconstitution: Calibrators are used for calibration of the LC system. This lyophilised calibrator has to be prepared like a patient sample. Add exactly 2 ml of H ₂ O HPLC grade and mix for 15 min until all material is dissolved.					
Storage and stability: 36 months if stored at 2 – 8 °C. After reconstitution 1 month if stored at 2 – 8 °C and 6 months at – 20 °C. Don't use after expiry date.					
Packaging: 5 x 1 x 2 ml					
Warning: The calibrator derives from human matrix, so it could be potentially infected. It must be handled with care.					

COLUMN CONDITIONING

Install a new analytical column GOLD 50 x 2.1 mm, 3 um. Disconnect the detector and flux a solution of H₂O : Mobile Phase B (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 H₂O : Mobile Phase A (5 : 95 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 H₂O : Mobile Phase B (5 : 95 v/v) for 15 minutes discharged. Store the column in a solution of H₂O : Mobile Phase B (5 : 95 v/v).

WASH AUTOSAMPLER SYRINGE

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

OPERATIONAL COMPUTER PARAMETERS

IN CONFORMITY WITH THE SPECIFICATION OF COMPUTER SOFTWARE

FLOW SET

GRADIENT			
Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	Flow (µl/min)
0	95	5	300
0.50	95	5	300
2.00	5	95	300
3.59	5	95	300
4.00	95	5	300
5.00	95	5	300

ANALITE	PARENT ION
Daptomycin	811.1
Vancomycin	725.2
Linezolid	338.0
Levofloxacin	362.0
Ciprofloxacin	332.4
Gentamicin	478.3
Streptomycin	582.0
Amikacin	586.2
Teicoplanin	940.8
Internal Standard (Diazepam)	285.4

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC79016	Calibrator in plasma for Antibiotics	5 x 1 x 2 ml
LC79017	Control in plasma for Antibiotics – Level 1	5 x 2 ml
LC79018	Control in plasma for Antibiotics – Level 2	5 x 2 ml
LC79019	Control in plasma for Antibiotics – Levels 1 and 2	2 x 5 x 2 ml
S29307U	Clear Glass Vials with reduced volume with caps	1 x 100 Pk
ZTF2500305213	Analytical Column GOLD 50 x 2.1 mm, 3 µm	1 Pk
SK79010	Starter kit for Antibiotics	1 Pk



ANTIBIOTICS IN PLASMA
(Reference Chromatograms/Spectra)

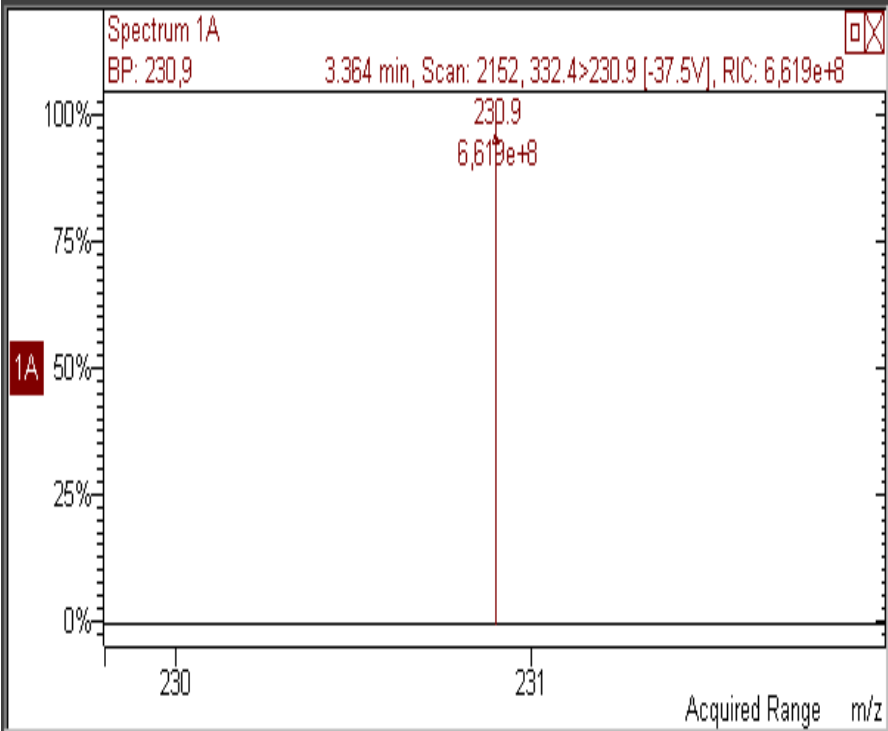
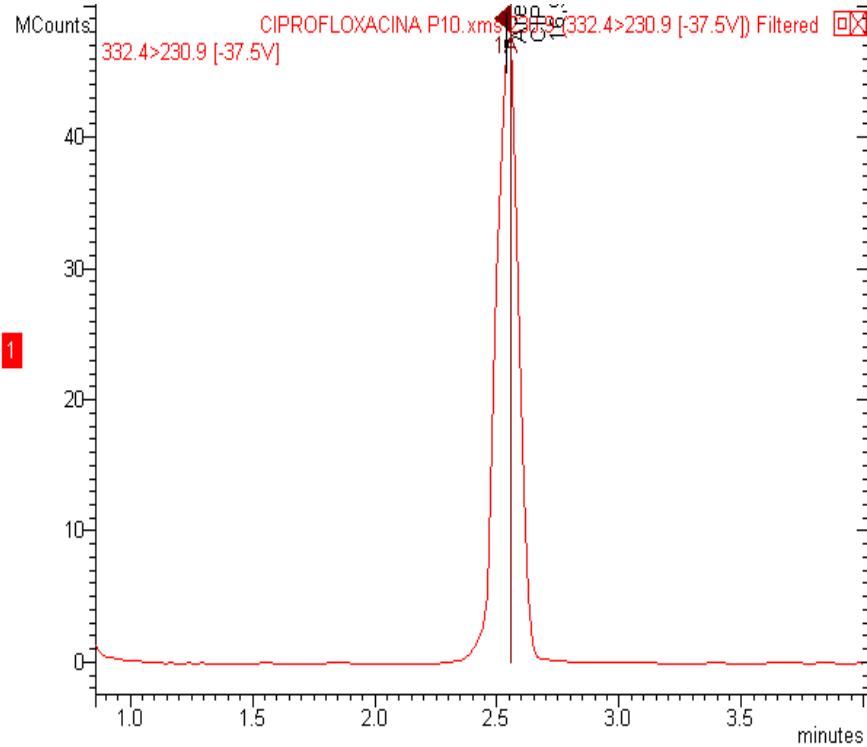


Fig. 1 :	Plasma Calibrator
R.T. 2.5	Ciprofloxacin 10 mg/l

Fig. 2 :	Massa Spectrum of Ciprofloxacin
TRANSITIONS: 332.4 >230.9	

ANTIBIOTICS IN PLASMA
(Reference Chromatograms/Spectra)

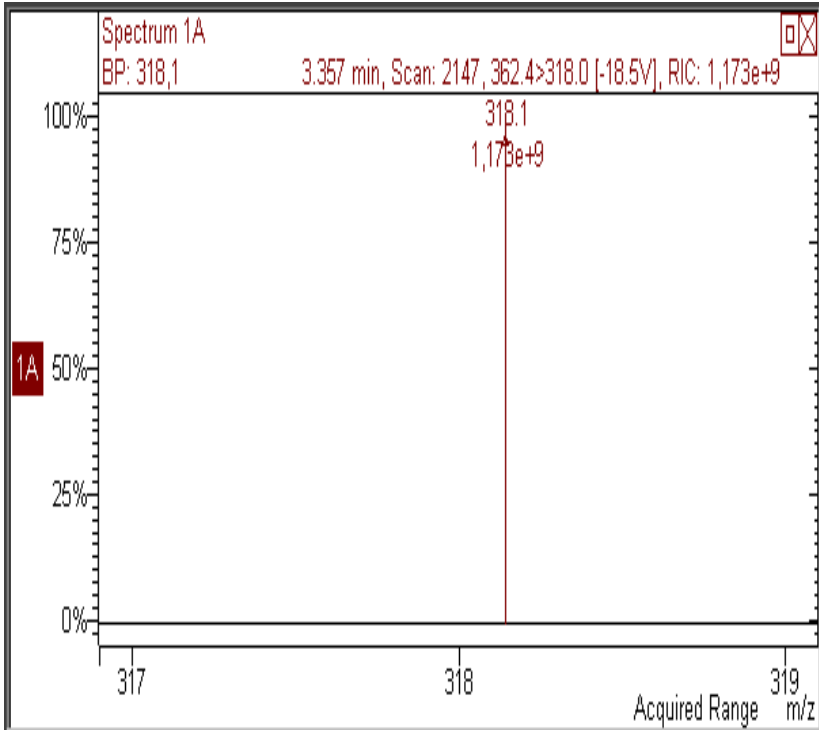
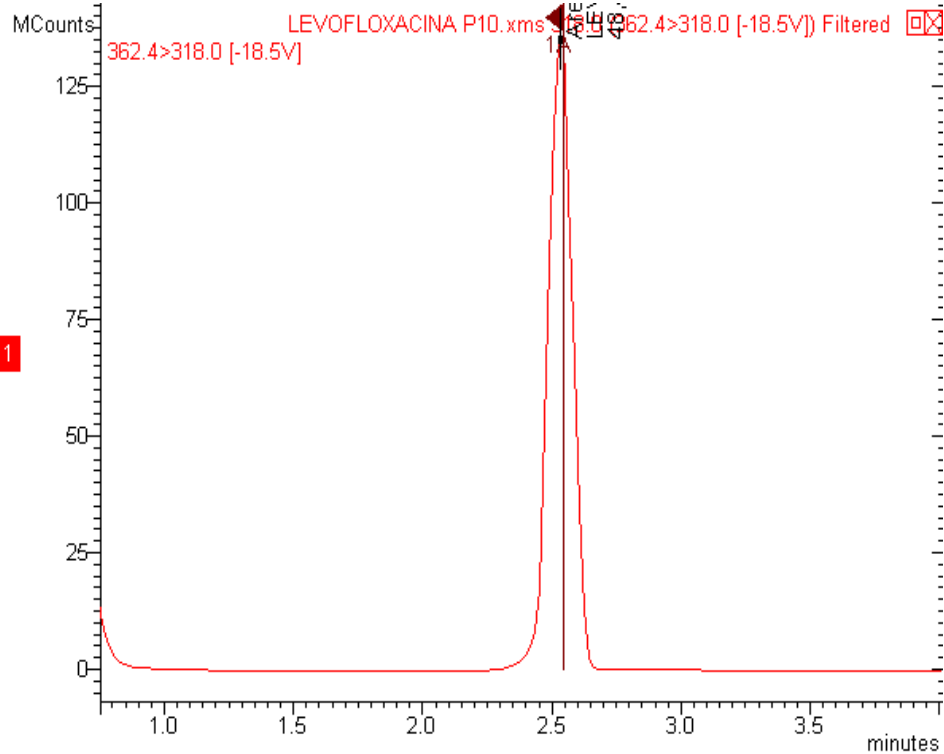


Fig. 3 :	Plasma Calibrator
R.T. 2.5	Levofloxacin 10 mg/l

Fig. 4 :	Massa Spectrum of Levofloxacin
TRANSITIONS: 362.4>318.1	

ANTIBIOTICS IN PLASMA
(Reference Chromatograms/Spectra)

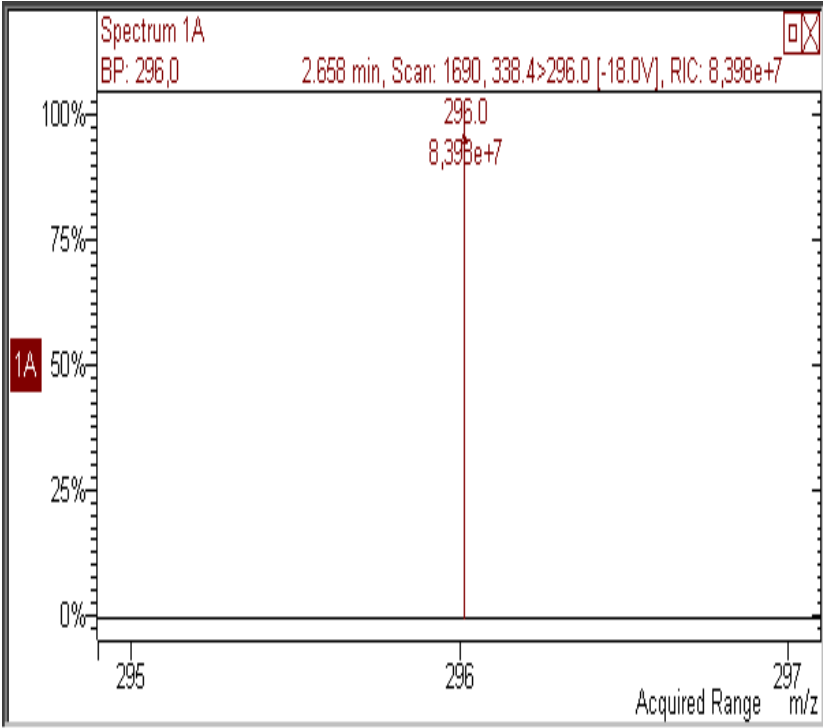
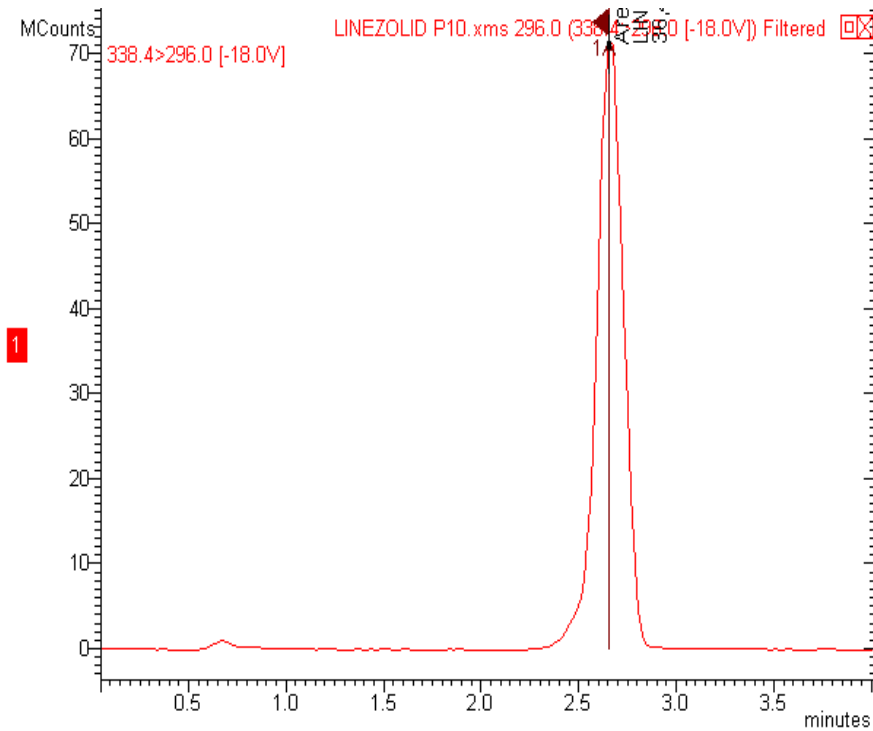


Fig. 5 :	Plasma Calibrator
R.T. 2.6	Linezolid 10 mg/l

Fig. 6 :	Massa Spectrum of Linezolid
TRANSITIONS: 338.4>296.0	

ANTIBIOTICS IN PLASMA
(Reference Chromatograms/Spectra)

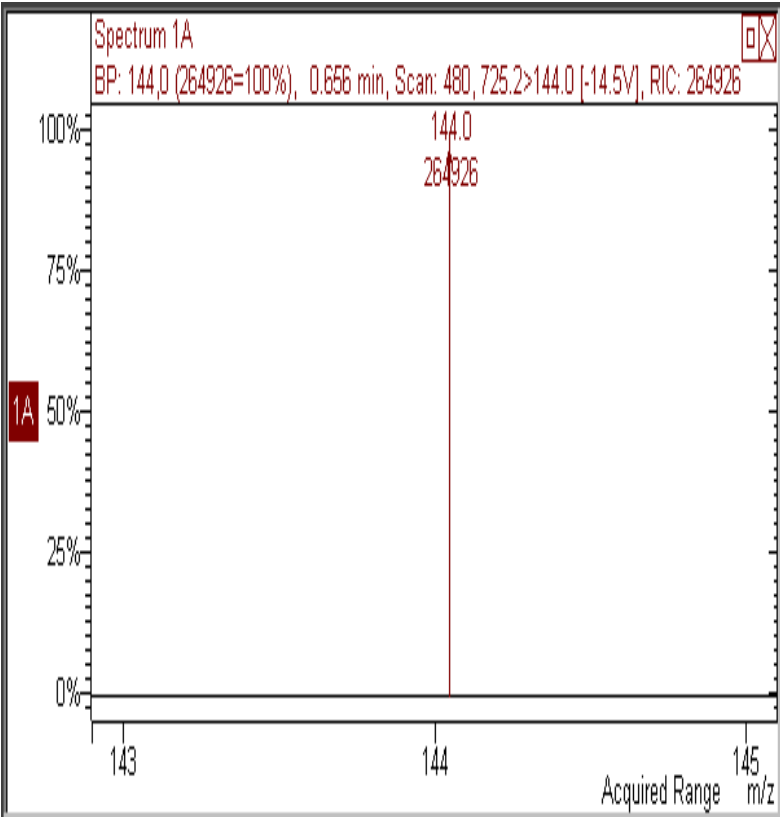
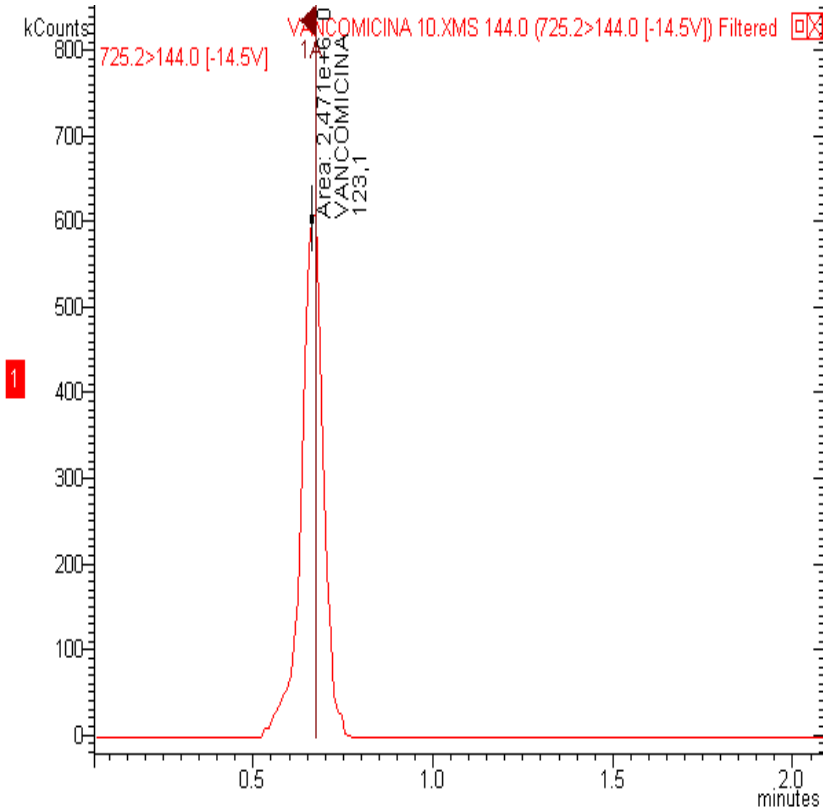


Fig. 7 :	Plasma Calibrator
R.T. 0.65	Vancomycin 10 mg/l

Fig. 8 :	Massa Spectrum of Vancomycin
TRANSITIONS: 725.2 >144.0	

ANTIBIOTICS IN PLASMA
(Reference Chromatograms/Spectra)

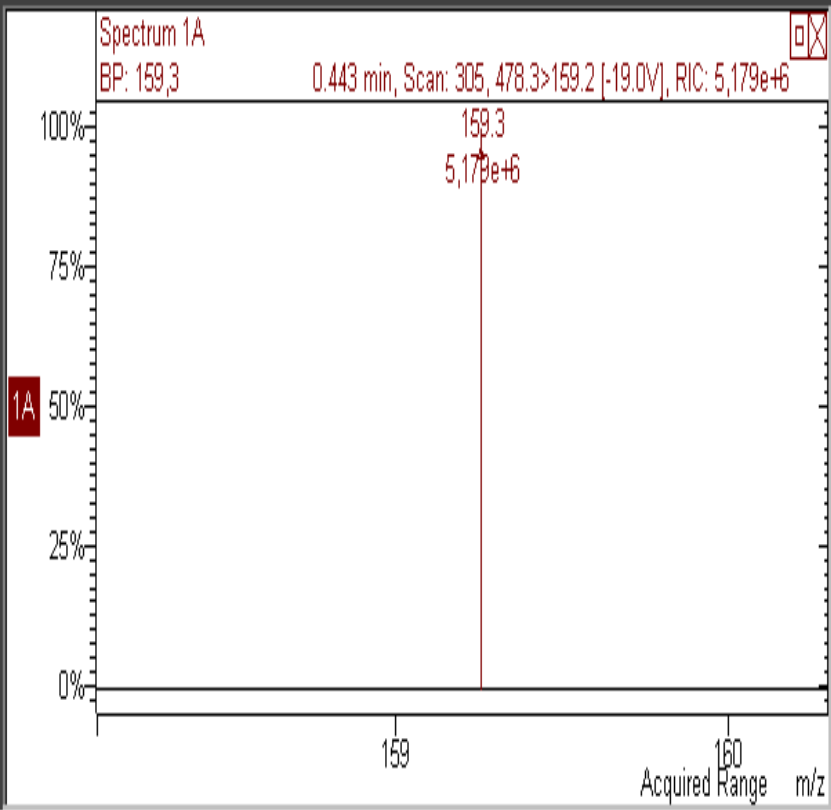
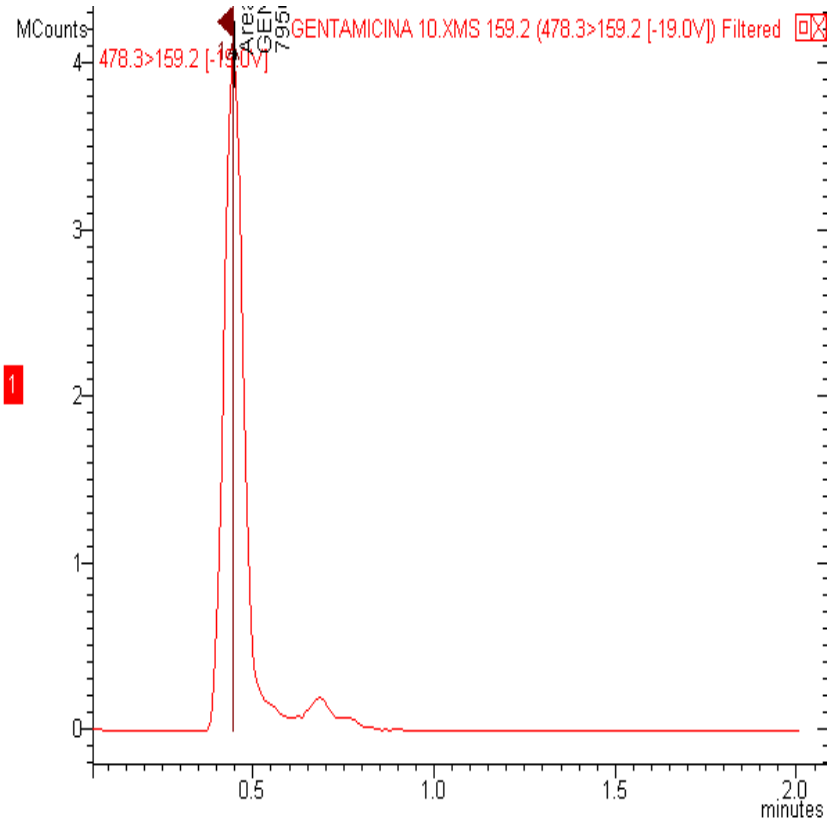


Fig. 9 :	Plasma Calibrator
R.T. 0.40	Gentamicin 10 mg/l

Fig. 10 :	Massa Spectrum of Gentamicin
TRANSITIONS: 478.3>159.3	

ANTIBIOTICS IN PLASMA
(Reference Chromatograms/Spectra)

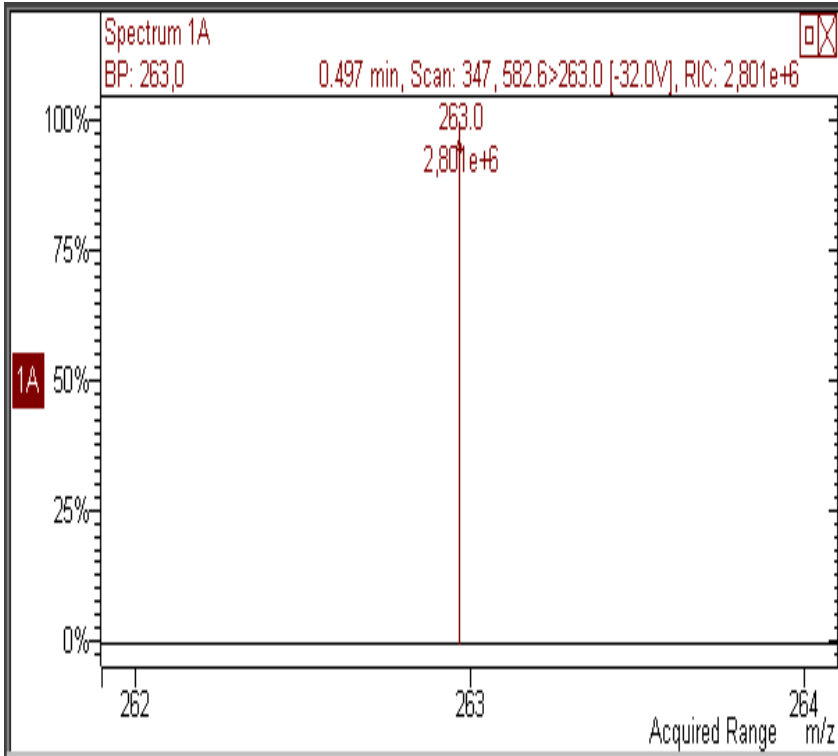
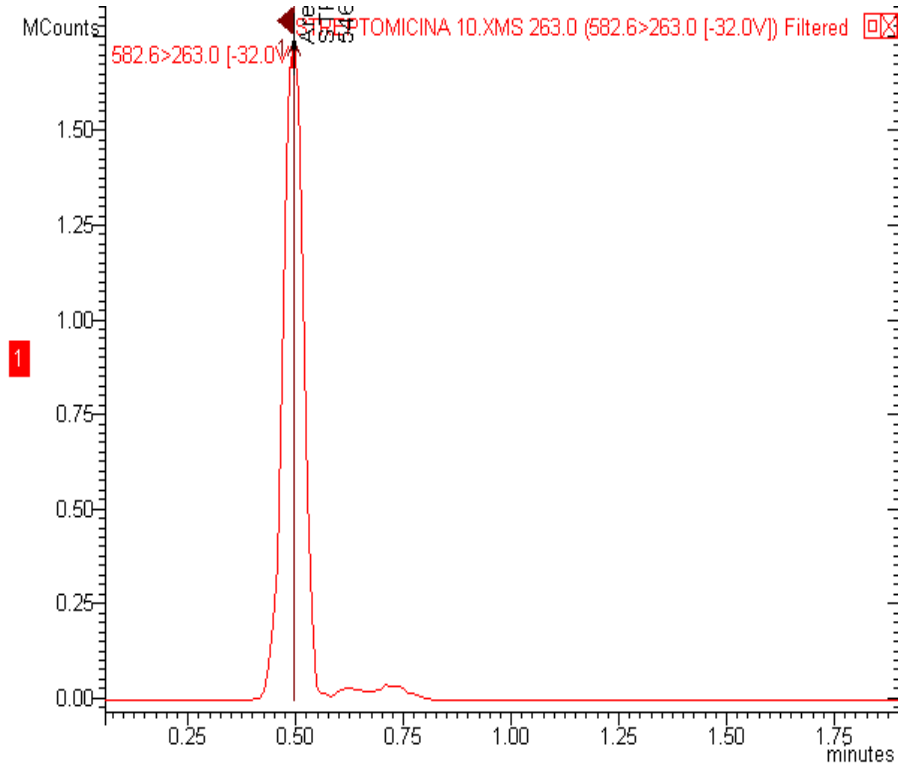


Fig. 11 :	Plasma Calibrator
R.T. 0.50	Streptomycin 10 mg/l

Fig. 12 :	Massa Spectrum of Streptomycin
TRANSITIONS: 582.6>263.0	

ANTIBIOTICS IN PLASMA
(Reference Chromatograms/Spectra)

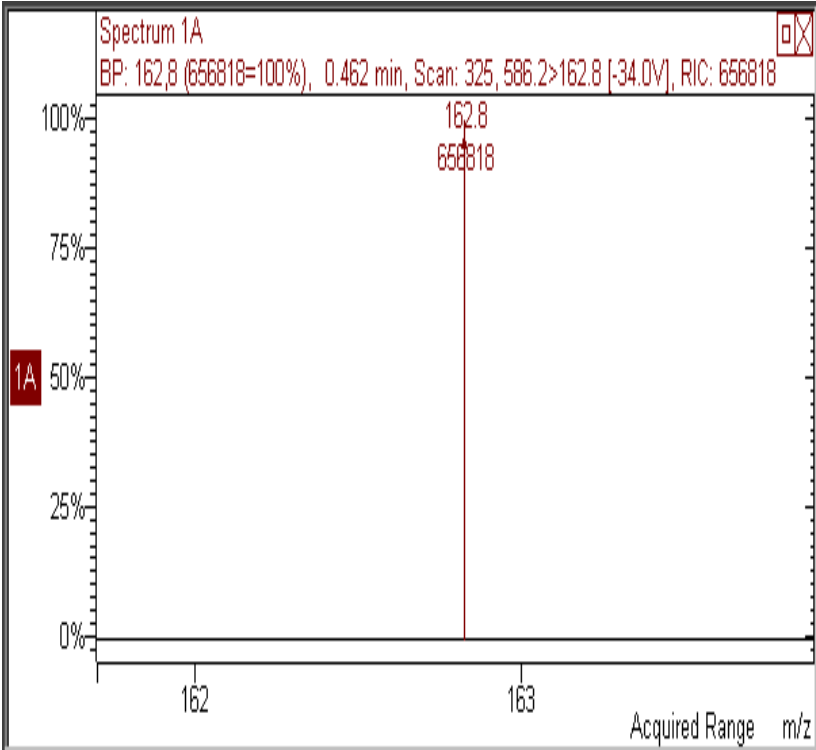
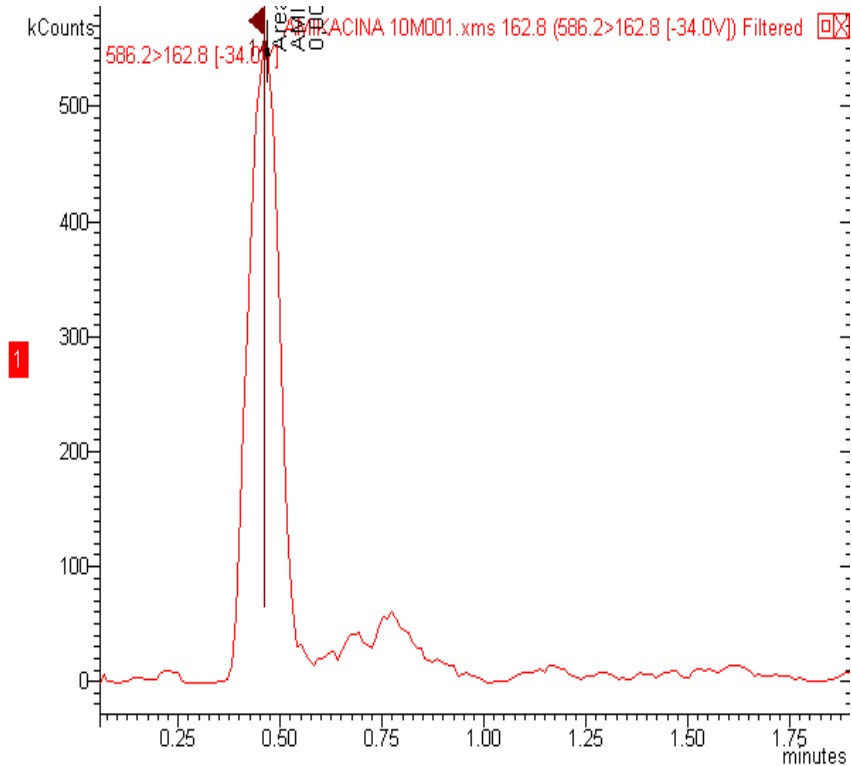


Fig. 13 :	Plasma Calibrator
R.T. 0.40	Amikacin 10 mg/l

Fig. 14 :	Massa Spectrum of Amikacin
TRANSITIONS: 586.2>162.8	

ANTIBIOTICS IN PLASMA
(Reference Chromatograms/Spectra)

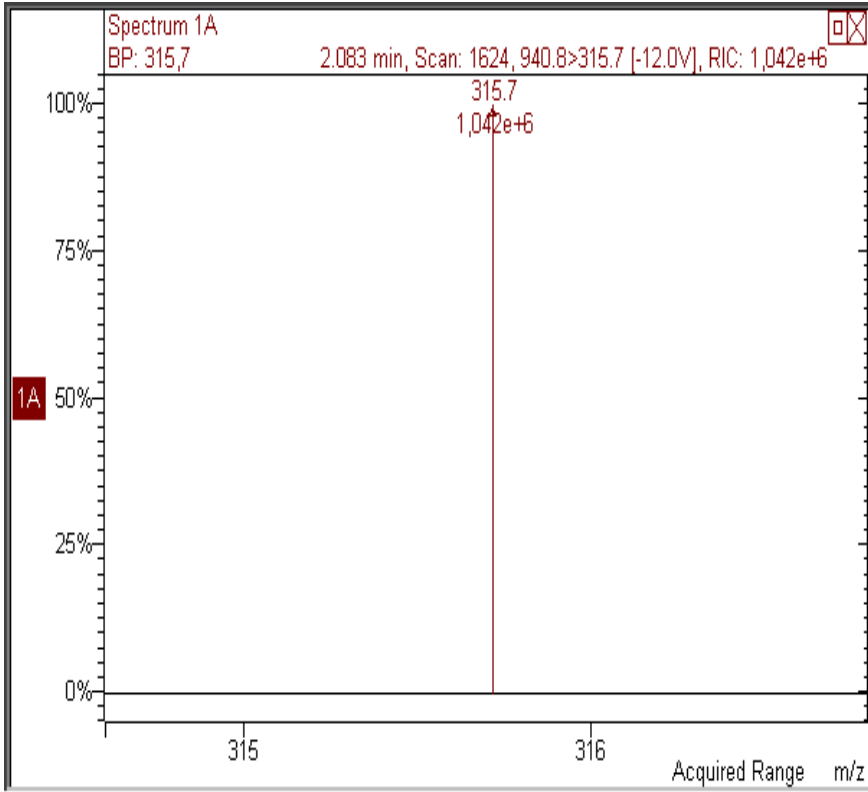
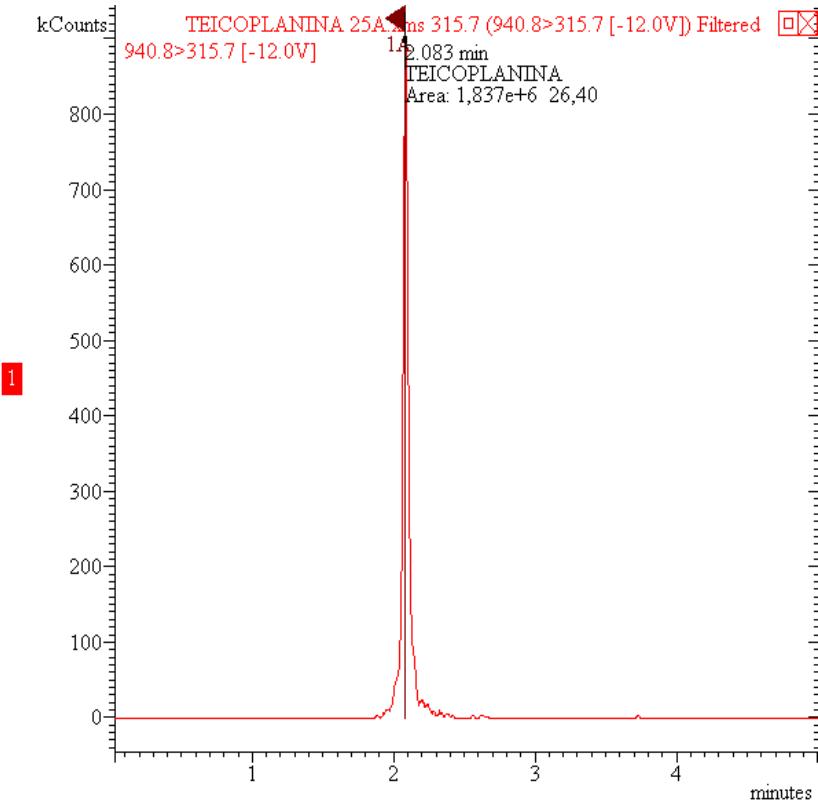


Fig. 15 :	Plasma Calibrator
R.T. 2.08	Teicoplanin 25 mg/l

Fig. 16 :	Massa Spectrum of Teicoplanin
TRANSITIONS: 940,8>315,7	