

ANTITUBERCULOSIS DRUGS IN PLASMA BY LC/MS – Code LC79110
(Ethambutol, Isoniazid, Rifampicin)

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.

Ethambutol is an active ingredient that is used against tuberculosis. Antituberculous chemotherapy drug is active against mycobacteria resistant to other drugs commonly used to treat tuberculosis. The dose reaches about 15 mg / kg per day. Among the most common side effects you experience optic neuritis, peripheral neuritis, rash, thrombocytopenia, rash.

Isoniazid is the main anti-tuberculosis antibiotic, acts by inhibiting the synthesis of mycolic acids, essential components of the cell wall of the bacterium *Mycobacterium tuberculosis*. It was first synthesized in 1912 but its activities as antibiotic was discovered in 1951. It is never used alone in the treatment of tuberculosis to prevent the onset, or rapid, forms of resistance. Isoniazid also has antidepressant properties, and was one of the first antidepressants discovered.

The **Rifampicin** is a bactericidal antibiotic of the rifamycin group. It is a semi-synthetic compound derived from *Ammycolaptosis rifamycinica* (formerly known as *Ammycolaptosis Mediterranean* or *Streptomyces Mediterranean*). Rifampicin is abbreviated as RIF, RMP, RD, RA or R. It is also known by the name of rifaldazina, R / AMP and Rofact (in Canada). There are various rifamycins from which it derives, but this particular form, with a group 4-methyl-1-piperazinaminile is clinically more effective.

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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° 003	Antituberculosis drugs in plasma by LC/MS	08/2024
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TECHNICAL FEATURES

Principle of the Method:

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

Sensitivity : 0,05 mg/l for **Ethambutol** and **Isoniazid**
0,005 mg/l for **Rifampicin**

Dynamic Range of the Method : 1 - 100 mg/l

<u>Therapeutic Range :</u>	Ethambutol	n. d.
	Isoniazid	n. d.
	Rifampicin	n. d.

CV%:	Ethambutol	6.8 intra serie	Ethambutol	7.1 inter serie
	Isoniazid	7.5 intra serie	Isoniazid	8.5 inter serie
	Rifampicin	8.5 intra serie	Rifampicin	6.0 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 – Diluting Solution, **1 x 100 ml**

Plasma Calibrator – Level 0, 1 x 1 ml

Code LC79116 Packed separately – see Technical Sheets

Plasma Calibrator – Level 1, 1 x 1 ml

Code LC79116 Packed separately – see Technical Sheets

Plasma Calibrator – Level 2, 1 x 1 ml

Code LC79116 Packed separately – see Technical Sheets

Plasma Calibrator – Level 3, 1 x 1 ml

Code LC79116 Packed separately – see Technical Sheets

Plasma Calibrator – Level 4, 1 x 1 ml

Code LC79116 Packed separately – see Technical Sheets

Plasma Calibrator – Level 5, 1 x 1 ml

Code LC79116 Packed separately – see Technical Sheets

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

STEP 1 :

Pipette in eppendorf:

	Calibrator – Level 0	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Sample	Controls
Calibrator – Level 0	100 µl							
Calibrator – Level 1		100 µl						
Calibrator – Level 2			100 µl					
Calibrator – Level 3				100 µl				
Calibrator – Level 4					100 µl			
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	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: Take 100 µl of supernatant and add with 900 µl of **Reagent B – Diluting Solution**

INJECTION :

- Inject 10 µl of solution in LC system.

ANTITUBERCULOSIS DRUGS IN PLASMA - WARNINGS

COLUMN CONDITIONING

Install a new analytical column GOLD 50 x 2.1 mm, 3 μ m. Disconnect the detector and flux a solution of H₂O : Mobile Phase B (5 : 95 v/v) at flow of 300 μ l / 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 μ l / 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..

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
Ethambutol	205.0
Isoniazid	137.8
Rifampicin	823.6
Internal Standard	285.4

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC79116	Calibrator in plasma for Antituberculosis drugs	6 x 1 x 1 ml
LC79119	Control in plasma for Antituberculosis drugs – Levels 1 and 2	2 x 5 x 1 ml
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 µl	1 x 100 Pcs
ZTF2500305213	Analytical Column GOLD 50 x 2.1 mm, 3 µm	1 Pc
SK79110	Starter kit for Antituberculosis drugs	3 x 1 ml

ANTITUBERCULOSIS DRUGS IN PLASMA

(Reference Chromatograms/Spectra)

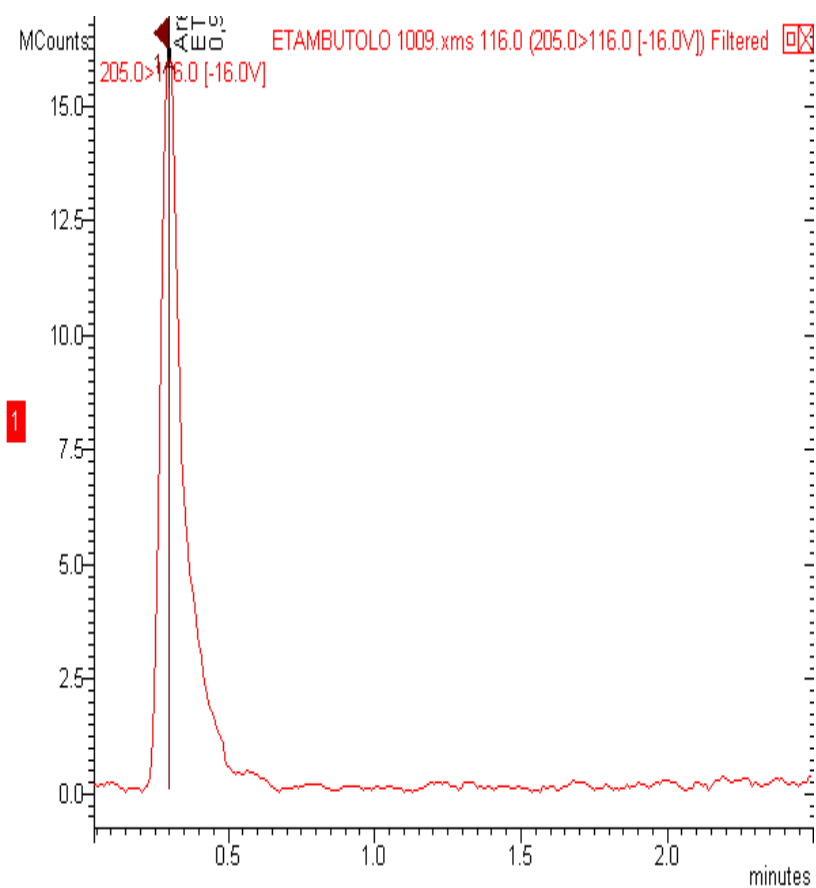


Fig. 1 :	Plasma Calibrator
R.T. 0.3	Ethambutol 10 mg/l

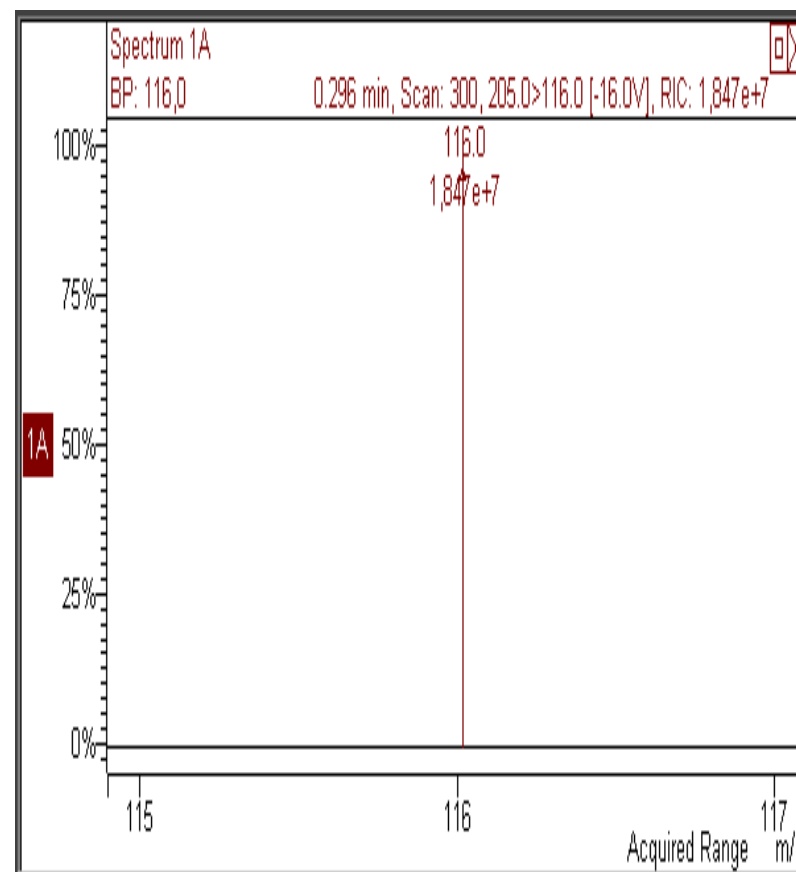


Fig. 2 :	Massa Spectrum of Ethambutol
TRANSITIONS: 205.0>116.0	

ANTITUBERCULOSIS DRUGS IN PLASMA

(Reference Chromatograms/Spectra)

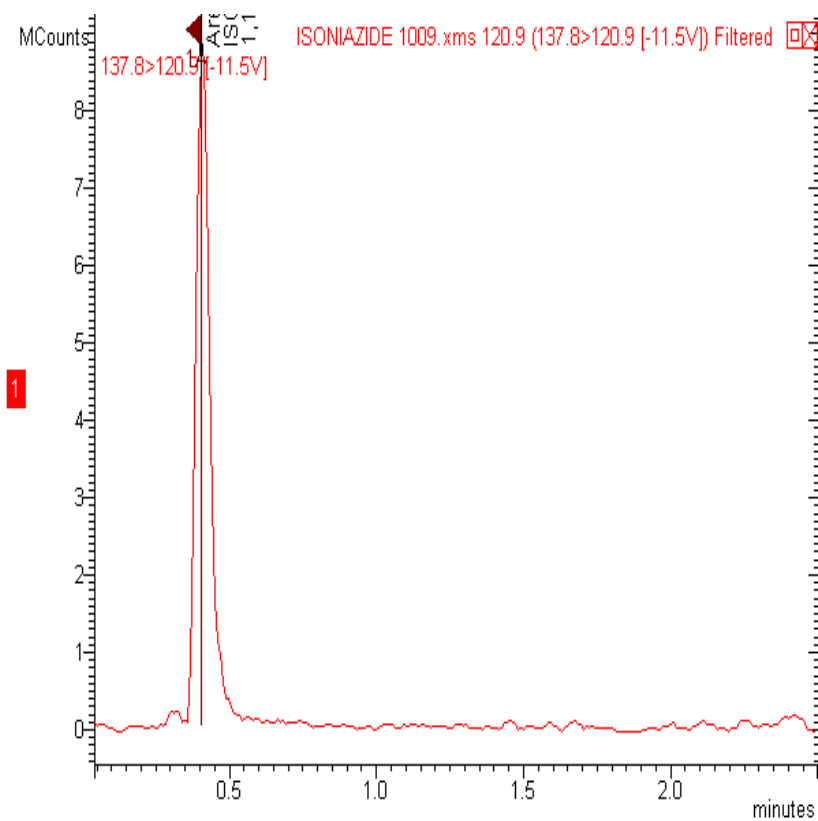


Fig. 3 :	Plasma Calibrator
R.T. 0.4	Isoniazid 10 mg/l

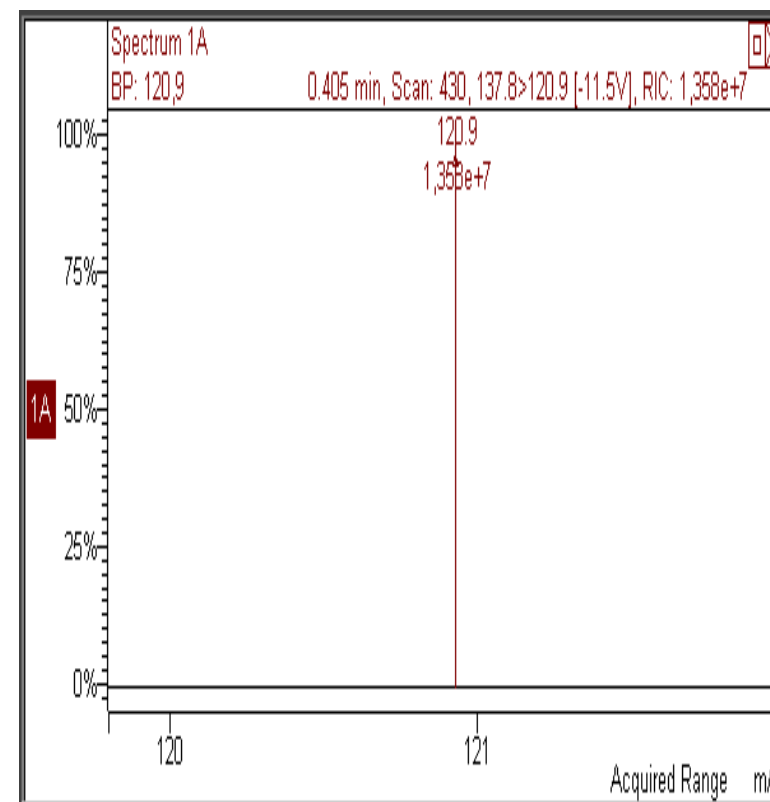


Fig. 4 :	Massa Spectrum of Isoniazid
TRANSITIONS: 137.8>120.9	

ANTITUBERCULOSIS DRUGS IN PLASMA

(Reference Chromatograms/Spectra)

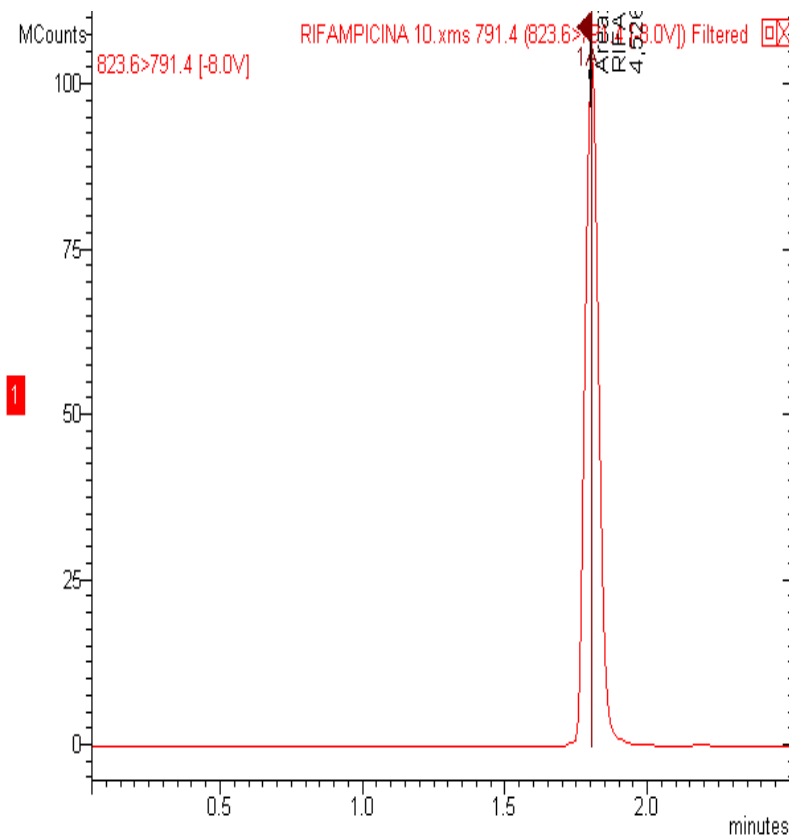


Fig. 5 :	Plasma Calibrator
R.T. 1.8	Rifampicin 10 mg/l

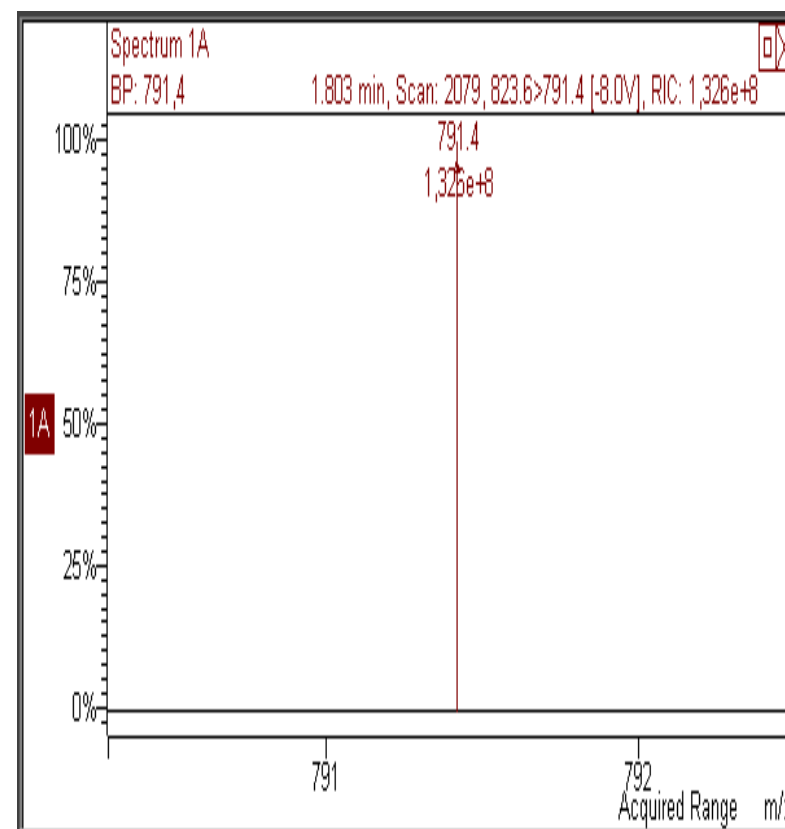


Fig. 6 :	Massa Spectrum of Rifampicin
TRANSITIONS: 823.6>791.4	