

LEVETIRACETAM IN PLASMA BY UV- FAST – CODE Z84010

INTRODUCTION

Treatment of the epileptic patients requires a multidisciplinary medical knowledge, regarding i.e. pharmacology, psychology and social science. In this contest the various antiepileptic drugs (AEDs) are used to decrease the frequency and/or severity of seizures in people with epilepsy.

Monitoring of plasmatic concentrations of AEDs is important to improve patient's therapy.

Our method allows to determine in the same HPLC run the sieric and/or plasmatic concentration of the following drugs:

1. Levetiracetam



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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.

N° 002	Levetiracetam in plasma by UV - FAST	07/2024
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TECHNICAL FEATURES

Principle of the Method :

Levetiracetam is separated treating the serum/plasma with a suitable precipitant. After addition of a stabilizing Reagent the solution is directly injected into HPLC.

Recovery : >98 %

Sensitivity (LLOD): 0,75 µg/ml

Minimum concentration analyzable (LLOQ): 2 µg/ml

Linearity : 2 – 200 µg/ml

Intra-series accuracy (relative error %) :

C1	C2
7,5 µg/ml	37,5 µg/ml
7,0%	1,1%

Inter-series accuracy (relative error %) :

C1	C2
7,5 µg/ml	37,5 µg/ml
10,0%	3,5%

Reproducibility intra serie (coefficient of variation%):

C LLOQ	Cm	CUP
2 µg/ml	25 µg/ml	50 µg/ml
7,6%	3,4%	2,3%

Reproducibility inter serie (coefficient of variation%):

C LLOQ	Cm	CUP
2 µg/ml	25 µg/ml	50 µg/ml
9,8%	4,3%	3,7%

Coefficient of Correlation + DS : 0,9937 ± 0,0036

Therapeutic Range in Plasma : *Levetiracetam* 10 – 37 µg/ml

Components of the kit :

Reagent A – Deproteinization Solution, 1 x 20 ml

Reagent A1 – Internal Standard, 6 x 400 µl

Reagent B – Stabilizing Solution, 1 x 20 ml

Reagent C – Test Solution, 1 x 2 ml

Plasma Calibrator lyophil., 1 x 2 ml

Reagent M – Mobile Phase, 2 x 500 ml

All the components are ready-to-use. Stability: 3 years at 2–8 °C.

Code Z04316 (packed separately – see data sheets)

Isocratic HPLC System with loop of 10 µl

Minimum Instrumental equipment required:

Spectrophotometric Detector UV $\lambda=205\text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Whole Blood Collection Procedure:

Collect 3 ml of blood in a suitable test tube without gel and with anticoagulant Heparin.
Centrifuge at 4000 rpm for 5 minutes.
Separate the serum and store at $-20\text{ }^{\circ}\text{C}$. Stable 4 weeks.

PREANALYTICAL PROCEDURE

Pipette in a tube :

- 950 µl of H₂O HPLC grade
- 50 µl of **Reagent C - Test Solution**

INJECTION :

- Inject 5 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.

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1 : Reconstitution of Internal Standard

At each vial of **Reagent A1 – Internal Standard**, add 3 ml of **Reagent A – Deproteinization Solution** (stable 3 months at 2-8 °C)

Vortex for 10 sec.

STEP 2 : Samples Preparation

Pipette in a suitable tube with cap :

	Blank	Calibrator	Samples
Water HPLC grade	200 µl		
Calibrator		200 µl	
Samples			200 µl
Reagent A1 – Deproteinization with Internal Standard (after reconstitution STEP 1)	200 µl	200 µl	200 µl

Warning: deproteinize directly on Vortex for at least 10 seconds

STEP 3 : Centrifuge at 10000 rpm for 5 min.

STEP 4 : Pipette in a tube 200 µl of clear supernatant.

- Add 200 µl of **Reagent B – Stabilizing Sol.**

Vortex for 5 seconds

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

INJECTION :

- Inject 5 µl of this solution in the chromatographic system.

LEVETIRACETAM - Warnings

REAGENT C: TEST SOLUTION

LEVETIRACETAM	500 µg/ml
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SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	205 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

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

Install a <u>new</u> analytical C ₁₈ Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 µm). Disconnect the detector and filter 30 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. And it is possible to make analysis at recycling phase, as long as you have the foresight to filter the same mobile phase with 0.22 µ filter before each analytical run. The signal that the mobile phase must be changed is given by the significant increase of the background noise of the detector Fluorimetric. 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) .
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INJECTION NEEDLE WASHING

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

LOOP	10 µl
FLOW	1,0 ml/min.
PRESSURE	About 150 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z04316	Calibrator for Levetiracetam in plasma	4 x 2 ml
Z04319	Control for Levetiracetam in plasma – Levels 1 and 2	2 x 5 x 2 ml
Z84011	Mobile Phase	4 x 500 ml
Z699975902	Poroshell 120 EC C18 (50 x 4,6 mm –2,7 µm) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
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

LEVETIRACETAM IN PLASMA

(Reference Chromatograms)

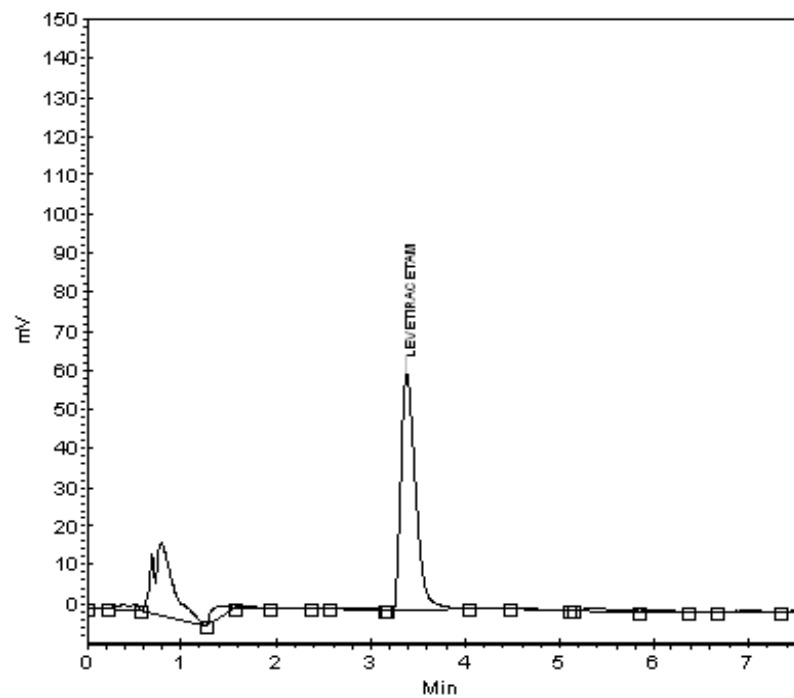


Fig. 1 :	Test Solution
	R.T. 3.39 Levetiracetam

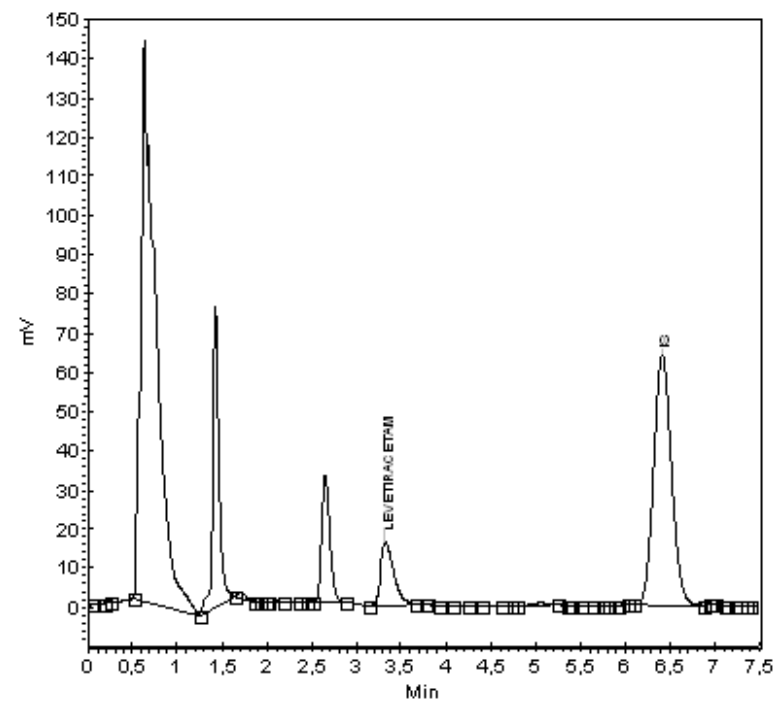


Fig. 2 :	Plasma Calibrator
R.T. 3.33 Levetiracetam	23,9 µg/ml
R.T. 6.42 Internal Standard	

LEVETIRACETAM IN PLASMA

(Reference Chromatograms)

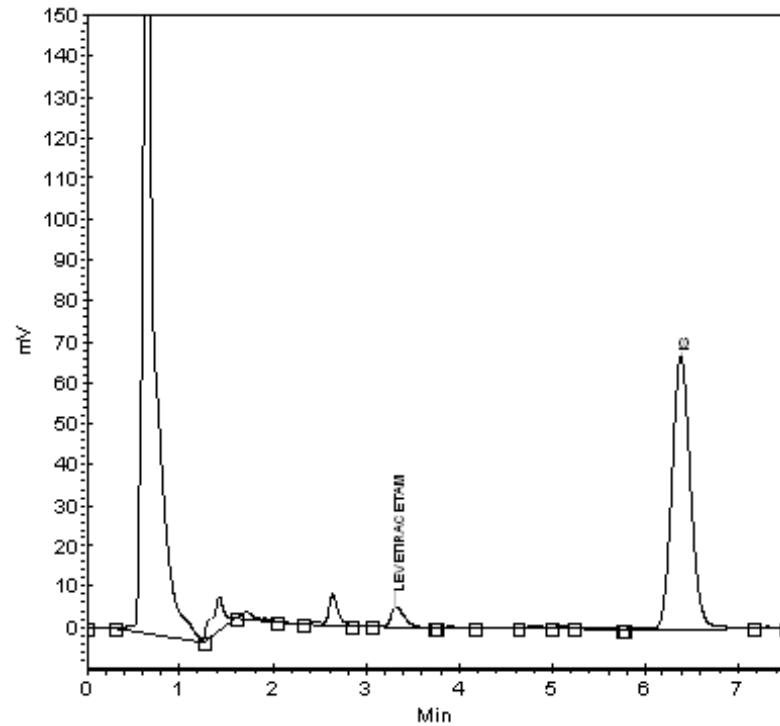


Fig. 3 :	Plasma Control level 1
R.T. 3.32	Levetiracetam 6,8 µg/ml
R.T. 6.42	Internal Standard

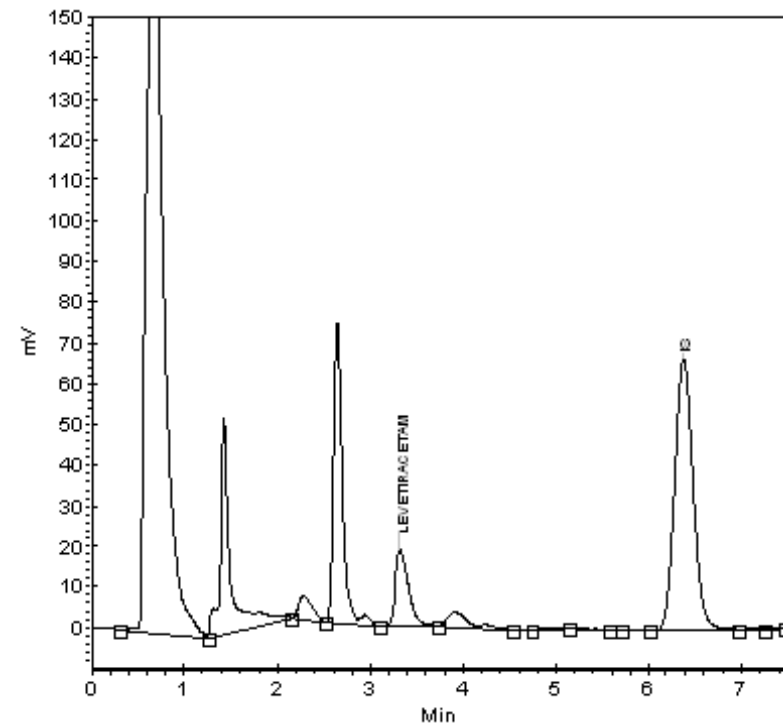


Fig. 4 :	Plasma Sample
R.T. 3.33	Levetiracetam 28,0 µg/ml
R.T. 6.38	Internal Standard