

**LAMOTRIGINE and 10,11-DIHYDRO-10-HYDROXYCARBAMAZEPINE IN
SERUM/PLASMA BY UV – FAST - CODE Z42110**

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. *Lamotrigine*
2. *10,11-Dihydro-10-hydroxycarbamazepine*



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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° 002	Lamotrigine and 10,11-dihydro-10-hydroxycarbamazepine by UV - FAST	06/2024
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TECHNICAL FEATURES

Principle of the Method :

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

Recovery : 100 %

Sensitivity (LLOD): Lamotrigine 0,05 µg/ml
10,11-Dihydro-10-Hydroxycarbamazepine 0,2 µg/ml

Minimum concentration analyzable (LLOQ) : Lamotrigine 0,2 µg/ml
10,11-Dihydro-10-Hydroxycarbamazepine 1 µg/ml

Linearity : Lamotrigine 0,2 – 200 µg/ml
10,11-Dihydro-10-Hydroxycarbamazepine 1 – 200 µg/ml

Accuracy intra serie (relative error %)
Lamotrigine :

C1	C2
1 µg/ml	10 µg/ml
2,82%	2,62%

Accuracy inter serie (relative error %)
Lamotrigine :

Ci	Cs
1 µg/ml	10 µg/ml
9,24%	4,04%

Reproducibility intra serie (coefficient of variation %)
Lamotrigine :

C LLOQ	Cm	Cs
0,2 µg/ml	5 µg/ml	25 µg/ml
0,83%	2,89%	1,49%

Reproducibility inter serie (coefficient of variation %)
Lamotrigine :

C LLOQ	Cm	Cs
0,2 µg/ml	5 µg/ml	25 µg/ml
8,50%	5,71%	3,72%

Coefficient of correlation R2 + Dev. Std Lamotrigine 0,9978 ± 0,0015

Accuracy intra serie (relative error %)
10,11-Dihydro-10-Hydroxycarbamazepine :

C1	C2
5 µg/ml	25 µg/ml
2,95%	1,94%

Accuracy inter serie (relative error %)
10,11-Dihydro-10-Hydroxycarbamazepine :

Ci	Cs
5 µg/ml	25 µg/ml
3,78%	2,75%

Reproducibility intra serie (coefficient of variation %)
10,11-Dihydro-10-Hydroxycarbamazepine :

C LLOQ	Cm	Cs
1 µg/ml	10 µg/ml	50 µg/ml
2,79%	3,92%	1,62%

Reproducibility inter serie (coefficient of variation %) 10,11-Dihydro-10-Hydroxycarbamazepine :

C LLOQ	Cm	Cs
1 µg/ml	10 µg/ml	50 µg/ml
5,91%	7,88%	3,70%

Coefficient of correlation R2 + Dev. Std

10,11-Dihydro-10-Hydroxycarbamazepine 0,9987 ± 0,0005

Therapeutic Range :

Lamotrigine 3 – 14 µg/ml
10,11-Dihydro-10-hydroxycarbamazepine 3 – 40 µg/ml

Components of the kit :

All the components are ready-to-use and stable 3 years at < 20 °C. Calibrators are stable 36 months at 2 – 8 °C.

Reagent A – Deproteinization Sol. with Internal Standard, **1 x 20 ml**

Reagent C – Test Solution, **1 x 2 ml**

Store at 2-8 °C

Plasma Calibrator, **1 x 2 ml**

Code Z04216 (packed separately – see data sheets)

Reagent M – Mobile Phase, **3 x 500 ml**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 5 - 10µl
Spectrophotometric Detector UV/VIS λ=220 nm
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Blood Collection Procedure:

Collect 3 ml of blood in a suitable test tube without gel and without anticoagulant. Centrifuge at 4000 rpm for 5 min.
Plasma samples should be stored at –20 °C for no longer than 4 weeks.

ATTENTION to patients treated in lamotrigine + rufinamide polypharmacy.

RUFINAMIDE EXITS ABOUT 0.2 MINUTES BEFORE LAMOTRIGIN

On some HPLC lines the separation may not be at the baseline. Usually the patient does not undergo combined dosage lamotrigine + rufinamide *, so there should be no problems.

*Rational Polytherapy with Antiepileptic Drugs, J.W. Lee and B. Dworetzky, *Pharmaceuticals* **2010**, 3, 2362-2379

PREANALYTICAL PROCEDURE

Pipette in a tube :

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

INJECTION :

- Inject 5 - 10 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 : Samples Preparation

Dispense in a suitable tube with cap:

	Blank	Calibrator	Samples
Water HPLC grade	200 µl		
Calibrator		200 µl	
Samples			200 µl
Reagent A - Deproteinization Sol. with Internal Standard	200 µl	200 µl	200 µl

Warning: deproteinize directly on Vortex for at least 10 seconds

STEP 2 : Centrifuge at 14.000 rpm for 5 minutes.

STEP 3 : Pipette in a tube 150 µl of clear supernatant

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

INJECTION :

- Inject 5 - 10 µl of this solution in the chromatographic system.

LAMOTRIGINE and 10,11-DIHYDRO-10-HYDROXYCARBAMAZEPINE - Warnings

REAGENT C: TEST SOLUTION

ANTIPILEPTICS :	
Lamotrigine	about 85,6 mg/l
10,11-Dihydro-10-hydroxycarbamazepine	about 200,0 mg/l

SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	220 nm
GAIN	0,01 AUFS
INTEGRATION TIME	10 sec.

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL C18 4,6 x 50 mm, 2,7 μ m, the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical C ₁₈ Reversed-Phase column POROSHELL C18 4,6 x 50 mm, 2,7 μ m. Disconnect the detector and filter 30 ml of H₂O : Acetonitrile or Methanol (20 : 80 v/v) solution and subsequently 30 ml of H₂O : Acetonitrile or Methanol (80 : 20 v/v) solution, set flow at 1,0 ml/min. Don't recycle the washing solutions. Condition the column with the mobile phase at a flow of 1,0 ml/min. For 15 min. Finally inject the Chemical Standard and verify the quality of the HPLC run. It is 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

Disconnect the detector. Flux 15 ml of a solution of H₂O : Acetonitrile (20 : 80 v/v) set flow at 1,4 ml/min and discharge. Store the column in a solution of H₂O : Acetonitrile (20 : 80 v/v) . It is recommended to wash the column at the end of each analytical session.

INJECTION NEEDLE WASHING

Flux 15 ml of a solution of H₂O : Acetonitrile (80 : 20 v/v) set flow at 1,0 ml/min.
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HPLC PARAMETERS

LOOP	5 - 10 μ l
FLOW	1,0 ml/min.
PRESSURE	About 130 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z04216	Calibrator for Lamotrigine and 10,11-Dihydro-10-hydroxycarbamazepine in serum and/or in plasma	4 x 2 ml
Z04219	Control for Lamotrigine and 10,11-Dihydro-10-hydroxycarbamazepine in serum and/or in plasma - Levels 1 and 2	2 x 5 x 2 ml
Z699975902	Poroshell C 18 (150 x 4,6mm -4 μ m) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 μ l	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 μ l	1 x 100 Pcs

LAMOTRIGINE and 10,11-DIHYDRO-10-HYDROXYCARBAMAZEPINE IN SERUM/PLASMA (Reference Chromatograms)

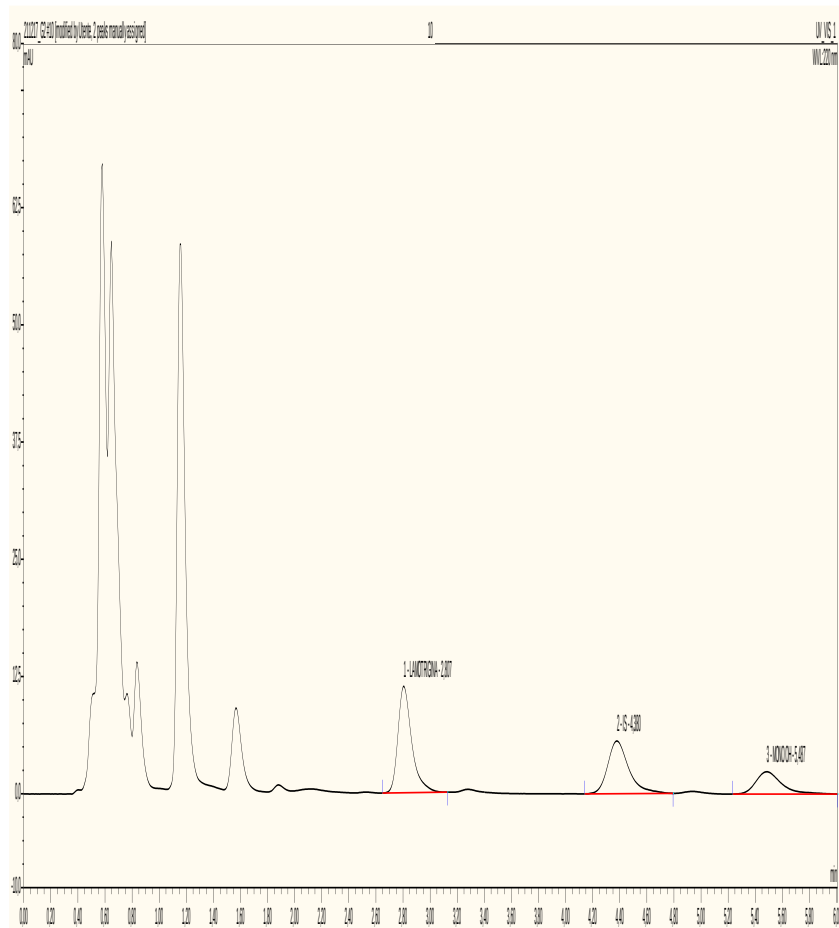


Fig. 1 :	Calibrator in plasma
R.T. 2.80	Lamotrigine 10 ug/ml
R.T. 4.38	Internal Standard
R.T. 5.48	10,11-Dihydro-10-hydroxycarbamazepine 10 ug/ml

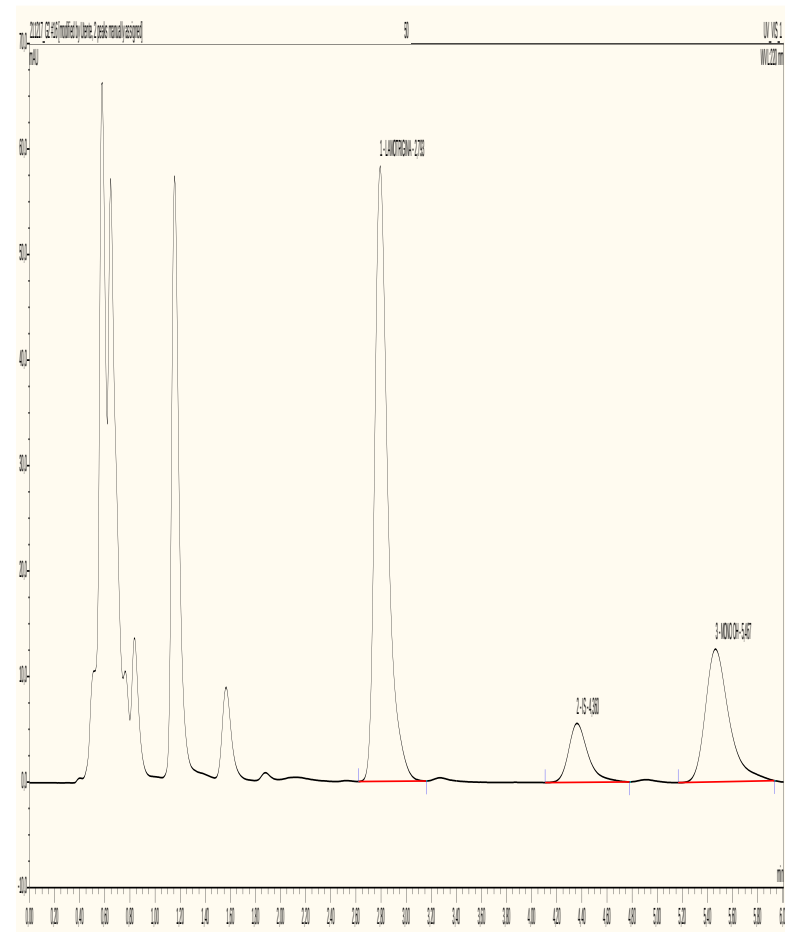


Fig. 2 :	Control in plasma Level 2
R.T. 2.79	Lamotrigine 50 ug/ml
R.T. 4.36	Internal Standard
R.T. 5.46	10,11-Dihydro-10-hydroxycarbamazepine 50 ug/ml