

LAMOTRIGINE IN SERUM AND IN PLASMA BY UV – FAST - Code Z03310

BIOCHEMISTRY

Lamotrigine or 3,5-Diamino-6-(2,3Dichlorophenyl)-1,2,4-triazine is a novel anticonvulsant agent. It is partially soluble in water, with a $pK_a=5.5$. After oral administration, lamotrigine is rapidly and completely absorbed in the gastro-intestinal tract, reaching peak plasma concentrations 1.4 to 4.8 hours (T_{max}) post-dosing. Lamotrigine is approximately 55% bound to human plasma proteins. The $t_{1/2}$ average is 26 hours after single doses and about 10 % eliminated with urine.

Javad et al (1) demonstrated that the $t_{1/2}$ of lamotrigine is affected by therapeutic concentrations of phenytoin, phenobarbital, carbamazepine or primidone. These drugs cause a decrease of $t_{1/2}$ of around 50%, while valproic acid doubles it.

The concurrent administration of lamotrigine affects plasma concentrations of carbamazepine-10,11-epoxide with an increase of around 45% of this primary metabolite. The determination of Lamotrigine is recommended in patients treated with other anticonvulsant drugs in order to detect overdosages. In case of association with carbamazepine the determination of carbamazepine-10,11-epoxide is also recommended.

Bibliography:

1. Jawad S, Oxley J, Yuen WC, Richens A. - The effect of Lamotrigine, a novel anticonvulsant on interictal spikes in patients with epilepsy. Br. J. Cl. Pharmacol 1986 - 22- 191 - 3
2. Wolf P. - Lamotrigine. Preliminary clinical observations on pharmacokinetics and interactions with traditional antiepileptic drugs. J. Epilepsy 1992 - 5 - 73 – 9

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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° 003	Lamotrigine in serum and in plasma by UV – FAST	11/2025
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TECHNICAL FEATURES

Principle of the Method :

Lamotrigine is separated treating the serum/plasma with a suitable precipitant. After addition of a stabilization solution, 50 µl of the eluate is directly injected into HPLC.

Recovery : 100 %

Sensitivity (LLOD) : 0,05 mg/l

Minimum concentration analyzable (LLOQ) : 0,2 mg/l

Linearity : 0,2 – 200 mg/l

Therapeutic range: 3,0 – 14,0 mg/l

Accuracy intra serie (relative error %)

Lamotrigine :

Ci	Cs
1 µg/ml	10 µg/ml
2,65%	3,96%

Accuracy inter serie (relative error %)

Lamotrigine :

Ci	Cs
1 µg/ml	10 µg/ml
5,67%	2,95%

Reproducibility intra serie (coefficient of variation %)

Lamotrigine :

C LLOQ	Cm	CUP
0,2 µg/ml	5 µg/ml	25 µg/ml
1,86%	2,16%	6,02%

Reproducibility inter serie (coefficient of variation %)

Lamotrigine :

C LLOQ	Cm	CUP
0,2 µg/ml	5 µg/ml	25 µg/ml
2,36%	2,37%	5,61%

Coefficiente di correlazione R2 + Dev. Std

Lamotrigine 0,9955 ± 0,0021

<u>Components of the kit :</u>	All the components are ready to use. Stability: 3 years at room temperature ($\leq 20^{\circ}\text{C}$). Plasma Calibrator is stable 36 months at $2-8^{\circ}\text{C}$.
Reagent A – Deproteinization Solution with Internal Standard, 1 x 30 ml	
Reagent C – Test Solution, 1 x 2 ml	<u>Store at $2-8^{\circ}\text{C}$</u>
Plasma Calibrator , lyophil., 1 x 2 ml	<u>Code Z03016 (packed separately – see data sheets)</u>
Reagent M – Mobile Phase, 2 x 500 ml	

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of $10\ \mu\text{l}$
Spectrophotometric Detector UV/VIS $\lambda=220\ \text{nm}$
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.

Pre-analytic step:

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 : Calibrator, Samples and Controls preparation

Pipette in a tube :

	Calibrator	Control	Sample
Calibrator	200 µl		
Control		200 µl	
Sample			200 µl
Reagent A – Deproteinization Sol.	200 µl	200 µl	200 µl

Warning: deproteinize directly on Vortex for at least 10 sec.

STEP 2 : Centrifuge at 14.000 rpm for 5 min.

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

Vortex for 10 sec.

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 IN SERUM AND IN PLASMA BY UV – FAST - Warnings

REAGENT C : TEST SOLUTION

LAMOTRIGINE	About 85,6 mg/l
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SPECTROFOTOMETRIC DETECTOR PARAMETERS

λ	220 nm
GAIN	0,001 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 H ₂ O : Acetonitrile or Methanol (20 : 80 v/v) solution and subsequently 30 ml of H ₂ O : 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 H ₂ O : Acetonitrile (20 : 80 v/v) set flow at 1,4 ml/min and discharge. Store the column in a solution of H ₂ O : Acetonitrile (20 : 80 v/v). It is recommended to wash the column at the end of each analytical session.
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INJECTION NEEDLE WASHING

Flux 15 ml of a solution of H ₂ O : Acetonitrile (80 : 20 v/v) set flow at 1,0 ml/min.

HPLC PARAMETERS

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

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z03016	Calibrator for Lamotrigine in serum and/or in plasma	4 x 2 ml
Z03019	Control for Lamotrigine in serum and/or in plasma - Levels 1 and 2	2 x 5 x 2 ml
Z33011	Mobile Phase	4 x 500 ml
Z699975902	Poroshell C 18 (150 x 4,6mm -4 μ m) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S29057U	Standard glass vials of 2 ml with screw cap	1 x 100 Pcs

LAMOTRIGINE in PLASMA and/or SERUM
(Reference Chromatograms)

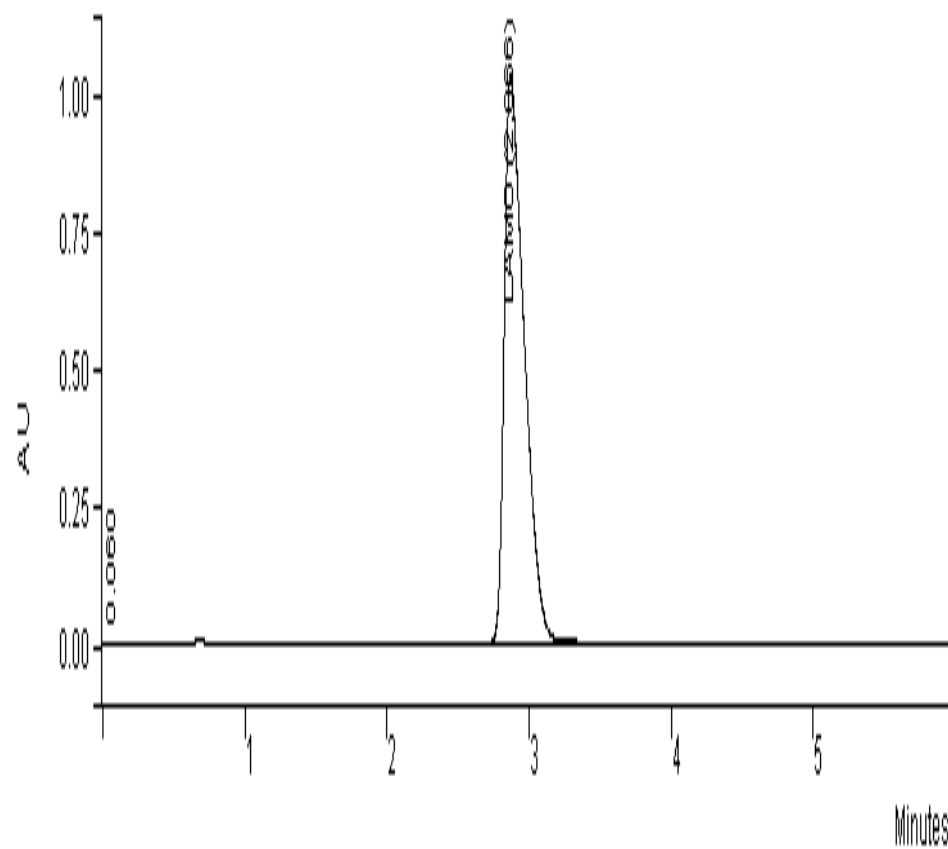


Fig. 1	Test Solution
	R.T. 2.86 Lamotrigine

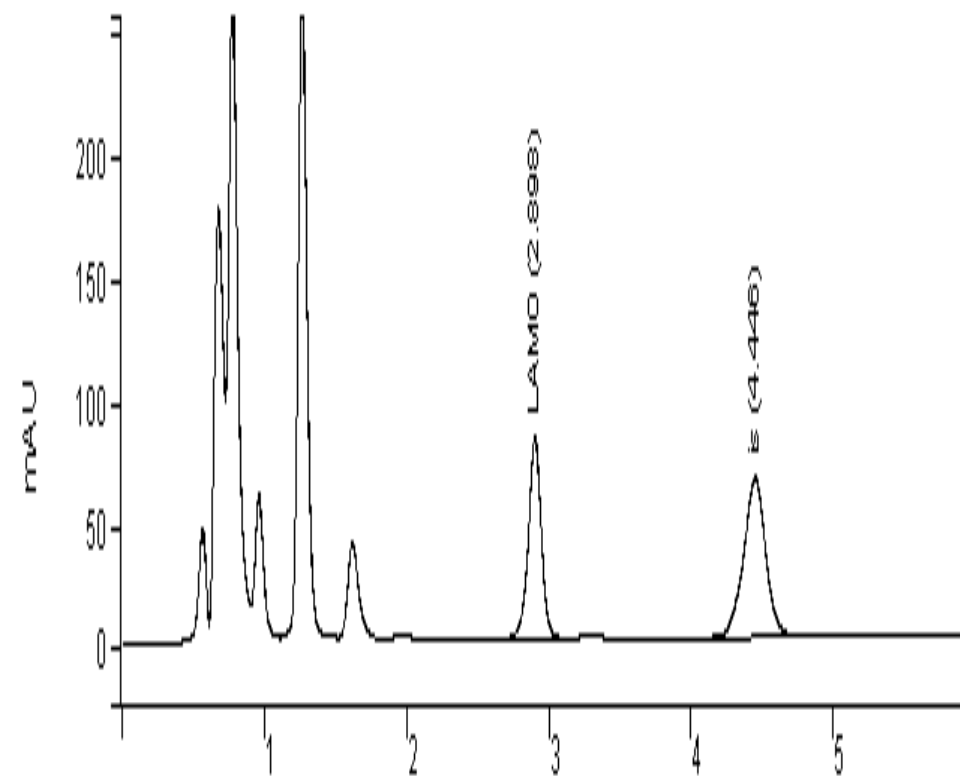


Fig. 2	Plasma Calibrator
	R.T. 2.89 Lamotrigine 25 mg/l
	R.T. 4.44 Internal Standard

LAMOTRIGINE in PLASMA and/or SERUM (Reference Chromatograms)

Warning: the loop used is of 100 μ l

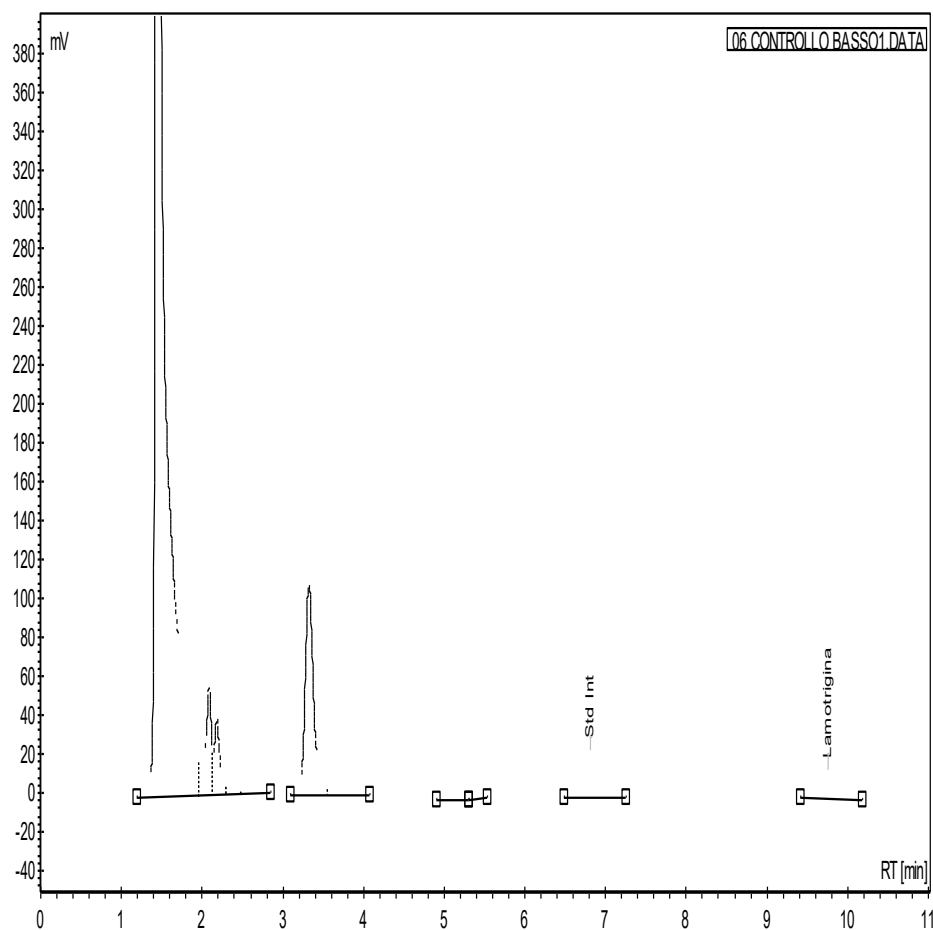


Fig. 3	Plasma Control lyophil Level 1
	R.T. 6.93 Internal Standard
	R.T. 9.87 Lamotrigine 3,50 mg/l

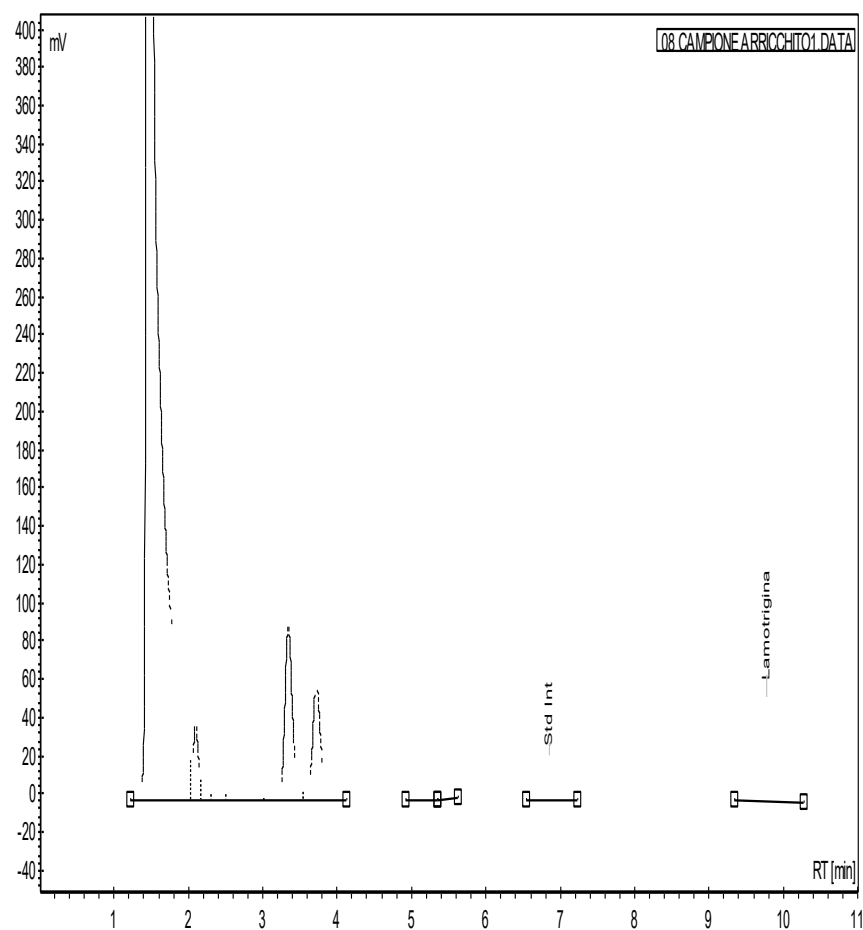


Fig. 4	Plasma Sample enriched
	R.T. 6.95 Internal Standard
	R.T. 9.90 Lamotrigine 14,40 mg/l