

ANTIEPILEPTIC DRUGS 6 IN PLASMA BY UV DETECTION – FAST -
CODE Z87010

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 sieriic and/or plasmatic concentration of the following drugs:

1. *Ethosuximide*
2. *Zonisamide*
3. *Lacosamide*
4. *Primidone*
5. *Lamotrigine*
6. *10,11-Dihydro-10-Hydroxycarbamazepine*



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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	Antiepileptics Drugs 6 in plasma by UV - FAST	07/2024
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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 stabilizing Reagent the solution is directly injected into HPLC.

Recovery : >98 %

Sensitivity (LLOD) :

0,66 mg/l	Ethosuximide
0,06 mg/l	Zonisamide
0,06 mg/l	Lacosamide
0,06 mg/l	Primidone
0,06 mg/l	Lamotrigine
0,06 mg/l	10,11-Dihydro-10-Hydroxycarbamazepine

Minimum concentration analyzable (LLOQ):

2,0 mg/l	Ethosuximide
0,2 mg/l	Zonisamide
0,2 mg/l	Lacosamide
0,2 mg/l	Primidone
0,2 mg/l	Lamotrigine
0,2 mg/l	10,11-Dihydro-10-Hydroxycarbamazepine

Linearity:

2 - 200 mg/l	Ethosuximide
0,2 - 200 mg/l	Zonisamide
0,2 - 200 mg/l	Lacosamide
0,2 - 200 mg/l	Primidone
0,2 - 200 mg/l	Lamotrigine
0,2 - 200 mg/l	10,11-Dihydro-10-Hydroxycarbamazepine

Accuracy intra serie (relative error %)
Ethosuximide:

Ci	Cs
15 mg/l	60 mg/l
6,64%	3,18%

Accuracy inter serie (relative error %)
Ethosuximide:

Ci	Cs
15 mg/l	60 mg/l
11,22%	3,68%

Reproducibility intra serie (coefficient of variation %) Ethosuximide:

CI	Cm	Cs
2 mg/l	30 mg/l	100 mg/l
11,01%	7,75%	2,87%

Reproducibility inter serie (coefficient of variation %) Ethosuximide:

CI	Cm	Cs
2 mg/l	30 mg/l	100 mg/l
10,19%	8,48%	4,72%

Coefficient of correlation R² + Dev Std 0,9950 ± 0,0030 Ethosuximide

Accuracy intra serie (relative error %)
Zonisamide:

Ci	Cs
6,25 mg/l	25 mg/l
5,64%	1,81%

Accuracy inter serie (relative error %)
Zonisamide:

Ci	Cs
6,25 mg/l	25 mg/l
7,43%	3,66%

Reproducibility intra serie (coefficient of variation %) Zonisamide:

CI	Cm	Cs
0,2 mg/l	12,5 mg/l	50 mg/l
8,96%	4,07%	2,45%

Reproducibility inter serie (coefficient of variation %) Zonisamide:

CI	Cm	Cs
0,2 mg/l	12,5 mg/l	50 mg/l
11,86%	5,15%	4,42%

Coefficient of correlation R² + Dev Std

0,9963 ± 0,0021 Zonisamide

Accuracy intra serie (relative error %) Lacosamide:

Ci	Cs
3,125 mg/l	12,5 mg/l
9,92%	8,08%

Accuracy inter serie (relative error %) Lacosamide:

Ci	Cs
3,125 mg/l	12,5 mg/l
6,66%	5,32%

Reproducibility intra serie (coefficient of variation %) Lacosamide:

CI	Cm	Cs
0,2 mg/l	6,25 mg/l	25 mg/l
4,49%	2,17%	1,89%

Reproducibility inter serie (coefficient of variation %) Lacosamide:

CI	Cm	Cs
0,2 mg/l	6,25 mg/l	25 mg/l
8,31%	6,86%	2,79%

Coefficient of correlation R² + Dev Std

0,9970 ± 0,0026 Lacosamide

Accuracy intra serie (relative error %) Primidone:

Ci	Cs
3,125 mg/l	12,5 mg/l
4,55%	3,31%

Accuracy inter serie (relative error %) Primidone:

Ci	Cs
3,125 mg/l	12,5 mg/l
3,97%	3,04%

Reproducibility intra serie (coefficient of variation %) Primidone:

CI	Cm	Cs
0,2 mg/l	6,25 mg/l	25 mg/l
6,46%	2,47%	1,4%

Reproducibility inter serie (coefficient of variation %) Primidone:

CI	Cm	Cs
0,2 mg/l	6,25 mg/l	25 mg/l
13,42%	6,82%	2,66%

Coefficient of correlation R² + Dev Std

0,9970 ± 0,0017 Primidone

Accuracy intra serie (relative error %) Lamotrigine:

Ci	Cs
3,125 mg/l	12,5 mg/l
3,76%	1,16%

Accuracy inter serie (relative error %) Lamotrigine:

Ci	Cs
3,125 mg/l	12,5 mg/l
3,67%	2,07%

Reproducibility intra serie (coefficient of variation %) Lamotrigine:

Cl	Cm	Cs
0,2 mg/l	6,25 mg/l	25 mg/l
4,07%	1,88%	1,96%

Reproducibility inter serie (coefficient of variation %) Lamotrigine:

Cl	Cm	Cs
0,2 mg/l	6,25 mg/l	25 mg/l
12,05%	8,8%	2,57%

Coefficient of correlation R² + Dev Std

0,9970 ± 0,0010 Lamotrigine

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

Ci	Cs
6,25 mg/l	25 mg/l
4,26%	2,21%

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

Ci	Cs
6,25 mg/l	25 mg/l
3,73%	2,55%

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

Cl	Cm	Cs
0,2 mg/l	12,5 mg/l	50 mg/l
2,03%	3,07%	1,92%

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

Cl	Cm	Cs
0,2 mg/l	12,5 mg/l	50 mg/l
9,18%	7,38%	6,47%

Coefficient of correlation R² + Dev Std

0,9910 ± 0,0053 10,11-Dihydro-10-Hydroxycarbamazepine

Therapeutic range :

Ethosuximide	40 – 100 mg/l
Zonisamide	10 – 38 mg/l
Lacosamide	1 – 10 mg/l
Primidone	5 – 12 mg/l
Lamotrigine	3 – 14 mg/l
10,11-Dihydro-10-Hydroxycarbamazepine	3 – 40 mg/l

Components of the kit :

All the components are ready-to-use and stable 3 years at 2-8 °C.

Reagent A – Deproteinization Solution, 1 x 30 ml

Reagent B – Stabilizing Solution, 1 x 10 ml

Reagent C – Test Solution, 1 x 2 ml

See Warnings

Plasma Calibrator lyoph., 1 x 2 ml

Code Z87016 (packed separately – see data sheet)

Reagent M – Mobile Phase, 3 x 500 ml

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 µl
Spectrophotometric Detector UV/VIS λ=205 nm
Chromatograms Recorder

Blood Collection Procedure:

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

PREANALYTICAL PROCEDURE

Pipette in a tube :

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

Vortex for 20 sec.

INJECTION :

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

Verify that the Test Solution has retention time similar to fig. below. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

ANALYTICAL PROCEDURE

STEP 1 : Samples Preparation

Dispense in a 1,5 ml vial with cap

	Blank	Calibrator	Sample	Control
Blank	100 µl			
Calibrator		100 µl		
Sample			100 µl	
Control				100 µl
Reagent A – Deproteinization Sol. + Internal Standard	300 µl	300 µl	300 µl	300 µl

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

STEP 2 : Centrifuge at 14.000 rpm for 10 minutes.

STEP 3 : Pipette in a tube 100 µl of clear supernatant.

- Add 100 µl of **Reagent B – Stabilizing Sol.**

Vortex for 5 sec.

N.B.: at this step, the sample is stable 1 day at 2-8 °C

INJECTION :

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

ANTIEPILEPTICS 6 - Warnings

REAGENT C: TEST SOLUTION

ANTIEPILEPTICS :	About
Ethosuximide	400 mg/l
Zonisamide	200 mg/l
Lacosamide	200 mg/l
Primidone	200 mg/l
Lamotrigine	200 mg/l
10,11-Dihydro-10-Hydroxycarbamazepine	200 mg/l

SPECTROFOTOMETRIC DETECTOR PARAMETERS

λ	205 nm
GAIN	0,001 AUFS

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL 120 EC C-18 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 C₁₈ Reversed-Phase column POROSHELL 120 EC-C18 (50 x 4,6 mm, 2,7 μ m).
Disconnect the detector and filter 30 ml of **H₂O : Acetonitrile (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 : Acetonitrile (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.
It is possible to make analysis at recycling phase, providing that you filter the same mobile phase with a filter of 0,22 μ before any analytical run. 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).**

INJECTION NEEDLE WASHING

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

HPLC PARAMETERS

LOOP	10 μ l
FLOW	1,0 ml/min.
PRESSURE	About 115 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z87016	Calibrator for Antiepileptics 6 in serum and/or in plasma	4 x 2 ml
Z87019	Control for Antiepileptics 6 in serum and/or in plasma – Level 1 and 2	2 x 5 x 2 ml
Z699975902	Poroshell 120-EC-C 18 Analytical Column (50 x 4,6mm –2,7 um)	1 Pc
S90199511	Javelin Colfilter	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs

ANTIEPILEPTICS 6 IN PLASMA (Reference Chromatograms)

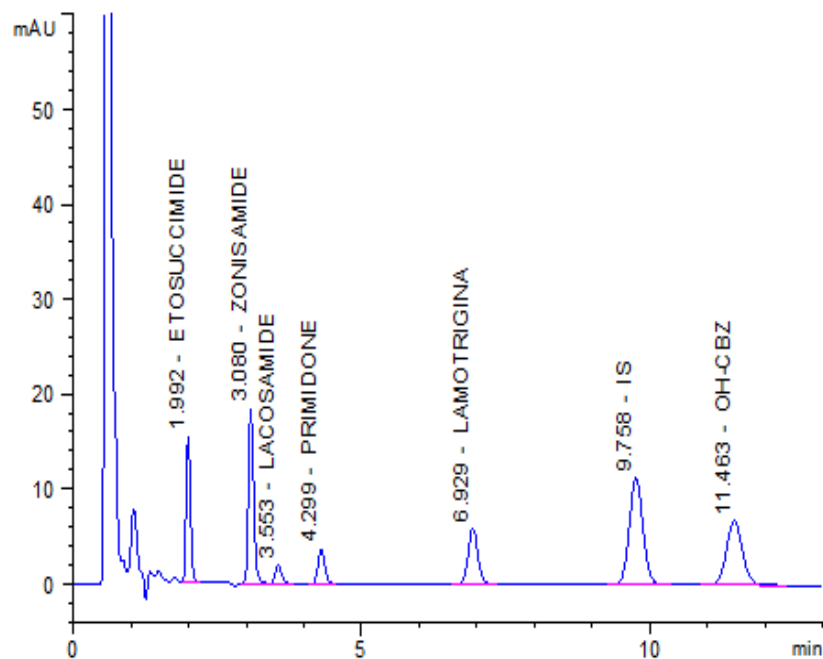


Fig. 1 :	Plasma Calibrator
	R.T. 1.99 Ethosuximide
	R.T. 3.08 Zonisamide
	R.T. 3.55 Lacosamide
	R.T. 4.29 Primidone
	R.T. 6.92 Lamotrigine
	R.T. 9.75 Internal Standard
	R.T. 11.46 10,11-Dihydro-11-Hydroxycarbamazepine

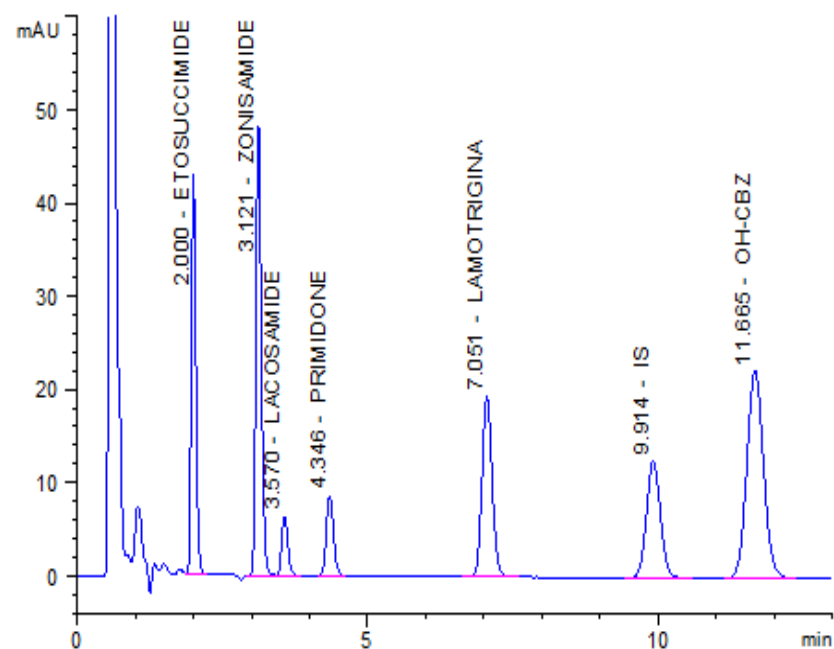


Fig. 2 :	Plasma Control – Level 2
	R.T. 2.00 Ethosuximide
	R.T. 3.12 Zonisamide
	R.T. 3.57 Lacosamide
	R.T. 4.34 Primidone
	R.T. 7.05 Lamotrigine
	R.T. 9.91 Internal Standard
	R.T. 11.66 10,11-Dihydro-11-Hydroxycarbamazepine