

ANTIEPILEPTIC DRUGS 3 IN SERUM OR PLASMA BY UV - FAST
CODE Z82010

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. *Primidone*
3. *Lamotrigine*
4. *Phelbamate*
5. *10,11-Dihydro-10-hydroxycarbamazepine*
6. *Carbamazepine Epoxide*
7. *Oxcarbazepine*



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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° 004	Antiepileptics 3 in serum/plasma by UV - FAST	08/2024
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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) : 0,1 µg/ml

Minimum concentration analyzable (LLOQ) : 0,2 µg/ml

Dynamic Range : 0,2 – 200 µg/ml

Accuracy intra serie (relative error %) ETHOSUXIMIDE :

Ci	Cs
28,8 µg/ml	144,1 µg/ml
2,3%	1,0%

Accuracy inter serie (relative error %) ETHOSUXIMIDE :

Ci	Cs
28,8 µg/ml	144,1 µg/ml
9,6%	5,5%

Reproducibility intra serie (variation coefficient %) ETHOSUXIMIDE :

C LLOQ	Cm	CUP
31,62 µg/ml	67,8 µg/ml	168,8 µg/ml
6,6%	3,48%	2,3%

Reproducibility inter serie (variation coefficient %) ETHOSUXIMIDE :

C LLOQ	Cm	CUP
31,62 µg/ml	67,8 µg/ml	168,8 µg/ml
6,3%	3,8%	2,4%

Coefficient of Correlation + DS: 0,9977 ± 0,0007 Ethosuximide

Accuracy intra serie (relative error %) PRIMIDONE :

Ci	Cs
5,2 µg/ml	53,5 µg/ml
6,7%	3,8%

Accuracy inter serie (relative error %) PRIMIDONE :

Ci	Cs
5,2 µg/ml	53,5 µg/ml
6,8%	3,3%

Reproducibility intra serie (variation coefficient %) PRIMIDONE :

C LLOQ	Cm	CUP
6,1 µg/ml	13,9 µg/ml	34,3 µg/ml
5,3%	4,9%	2,1%

Reproducibility inter serie (variation coefficient %) PRIMIDONE :

C LLOQ	Cm	CUP
6,1 µg/ml	13,9 µg/ml	34,3 µg/ml
9,8%	3,4%	3,6%

Coefficient of Correlation + DS:

0,9982 ± 0,0020 Primidone

Accuracy intra serie (relative error %)
PHELBAMATE :

Ci	Cs
31,7 µg/ml	184,3 µg/ml
8,2%	5,1%

Accuracy inter serie (relative error %)
PHELBAMATE :

Ci	Cs
31,7 µg/ml	184,3 µg/ml
10,9%	5,9%

Reproducibility intra serie (variation coefficient %)
PHELBAMATE :

C LLOQ	Cm	CUP
28,8 µg/ml	70,4 µg/ml	184,9 µg/ml
6,2%	3,6%	1,7%

Reproducibility inter serie (variation coefficient %)
PHELBAMATE :

C LLOQ	Cm	CUP
28,8 µg/ml	70,4 µg/ml	184,9 µg/ml
6,6%	3,7%	2,2%

Coefficient of Correlation + DS:

0,9986 ± 0,0011 Phelbamate

Accuracy intra serie (relative error %)
LAMOTRIGINE :

Ci	Cs
3,5 µg/ml	18,1 µg/ml
5,1%	2,9%

Accuracy inter serie (relative error %)
LAMOTRIGINE :

Ci	Cs
3,5 µg/ml	18,1 µg/ml
6,3%	4,7%

Reproducibility intra serie (variation coefficient %)
LAMOTRIGINE :

C LLOQ	Cm	CUP
3,3 µg/ml	8,4 µg/ml	16,9 µg/ml
3,6%	3,5%	2,6%

Reproducibility inter serie (variation coefficient %)
LAMOTRIGINE :

C LLOQ	Cm	CUP
3,3 µg/ml	8,4 µg/ml	16,9 µg/ml
7,8%	4,2%	3,6%

Coefficient of Correlation + DS:

0,9982 ± 0,0008 Lamotrigine

Accuracy intra serie (relative error %) 10,11-
DIHYDRO-10-HYDROXYCARBAMAZEPINE :

Ci	Cs
2,1 µg/ml	46,1 µg/ml
8,1%	1,0%

Accuracy inter serie (relative error %) 10,11-
DIHYDRO-10-HYDROXYCARBAMAZEPINE :

Ci	Cs
2,1 µg/ml	46,1 µg/ml
8,7%	3,3%

Reproducibility intra serie (variation coefficient %)
10,11-DIHYDRO-10-HYDROXYCARBAMAZEPINE :

C LLOQ	Cm	CUP
3,1 µg/ml	21,5 µg/ml	48,8 µg/ml
8,1%	2,0%	1,9%

Reproducibility inter serie (variation coefficient %)
10,11-DIHYDRO-10-HYDROXYCARBAMAZEPINE :

C LLOQ	Cm	CUP
3,1 µg/ml	21,5 µg/ml	48,8 µg/ml
8,6%	2,3%	1,9%

Coefficient of Correlation + DS:

0,9989 ± 0,0009 10,11-Dihydro-10-Hydroxycarbamazepine

Accuracy intra serie (relative error %)
CARBAMAZEPINE EPOXIDE :

Ci	Cs
15,6 µg/ml	74 µg/ml
4,5%	2,7%

Accuracy inter serie (relative error %)
CARBAMAZEPINE EPOXIDE :

Ci	Cs
15,6 µg/ml	74 µg/ml
5,9%	3,0%

Reproducibility intra serie (variation coefficient %)
CARBAMAZEPINE EPOXIDE :

C LLOQ	Cm	CUP
17,7 µg/ml	53,4 µg/ml	89,2 µg/ml
5,1%	2,1%	0,9%

Reproducibility inter serie (variation coefficient %)
CARBAMAZEPINE EPOXIDE :

C LLOQ	Cm	CUP
17,7 µg/ml	53,4 µg/ml	89,2 µg/ml
5,8%	2,5%	1,9%

Coefficient of Correlation + DS:

0,9982 ± 0,0005 Carbamazepine Epoxide

Accuracy intra serie (relative error %)
OXCARBAZEPINE :

Ci	Cs
5,2 µg/ml	32,6 µg/ml
7,2%	1,8%

Accuracy inter serie (relative error %)
OXCARBAZEPINE :

Ci	Cs
5,2 µg/ml	32,6 µg/ml
7,3%	4,2%

Reproducibility intra serie (variation coefficient %)
OXCARBAZEPINE :

C LLOQ	Cm	CUP
5,3 µg/ml	18,3 µg/ml	43 µg/ml
4,7%	3,6%	2,3%

Reproducibility inter serie (variation coefficient %)
OXCARBAZEPINE :

C LLOQ	Cm	CUP
5,3 µg/ml	18,3 µg/ml	43 µg/ml
6,6%	4,0%	3,4%

Coefficient of Correlation + DS:

0,9984 ± 0,0014 Oxcarbazepine

Therapeutic range :

<i>Ethosuximide</i>	40 – 100	µg/ml
<i>Primidone</i>	5 – 12	µg/ml
<i>Lamotrigine</i>	3 – 14	µg/ml
<i>Phelbamate</i>	10 – 80	µg/ml
<i>10,11-Dihydro-10-hydroxycarbamazepine</i>	3 – 40	µg/ml
<i>Carbamazepine Epoxide</i>	2 – 4	µg/ml

<u>Components of the kit :</u>	All the components are ready-to-use and stable 3 years at 2-8 °C.
Reagent A – Deproteinization Solution with Internal Standard, 1 x 20 ml	
Reagent B – Stabilizing Solution, 1 x 10 ml	
Reagent D – Test Solution 7 parameters, 1 x 1 ml	<u>See Warnings</u>
Plasma Calibrator lyophil., 1 x 2 ml	<u>Code Z04716 (packed separately – see data sheet)</u>
Reagent M – Mobile Phase, 6 x 500 ml	

Minimum Instrumental equipment required:

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

Optional Equipment:

Autosampler
 Operational Computer

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.

Chromatographic interferences :

The drugs Zonisamide and Lacosamide elute almost at the Primidone peak but the integration is still executable.

PREANALYTICAL PROCEDURE

Pipette in a tube :

- 950 µl of H₂O HPLC grade
- 50 µl of **Reagent D** - Test Solution 7 parameters

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. **The same procedure for Reagent E, F and G.**

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1 : Samples Preparation

Dispense in a 1,5 ml vial with cap :

	Blank	Calibrator	Control	Sample
HPLC grade water	100 µl			
Calibrator		100 µl		
Control			100 µl	
Sample				100 µl
Reagent A – Deproteinization Sol. + Internal Standard	200 µl	200 µl	200 µl	200 µl

Warning: deproteinize directly on Vortex for at least 10 seconds

STEP 2 : Centrifuge at 10.000 rpm for 10 minutes

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

STEP 4 : Add 100 µl of **Reagent B – Stabilizing Solution**

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.

ANTIEPILEPTICS 3 - Warnings

REAGENT D: TEST SOLUTION 7 PARAMETERS

Ethosuximide	613,0 µg/ml
Primidone	76,0 µg/ml
Lamotrigine	63,0 µg/ml
Phelbamate	375,0 µg/ml
10,11-Dihydro-10-hydroxycarbamazepine	45,0 µg/ml
Carbamazepine Epoxide	51,0 µg/ml
Oxcarbazepine	260,0 µg/ml

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.

HPLC COLUMN CONDITIONING

Install a new analytical C₁₈ 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.
It's NOT possible to make analysis at recycling phase. If room temperature is > 20 °C tore 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 µl
FLOW	1 ml/min.
PRESSURE	About 80 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z04716	Calibrator for Antiepileptics 3 in plasma and/or in serum	4 x 2 ml
Z04719	Control for Antiepileptics 3 in plasma and/or in serum, Levels 1 and 2	2 x 5 x 2 ml
Z82011	Mobile Phase	4 x 500 ml
Z699975902	POROSHELL 120 EC-C18 (4,6 x 50 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 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 3 IN SERUM OR PLASMA (Reference Chromatograms)

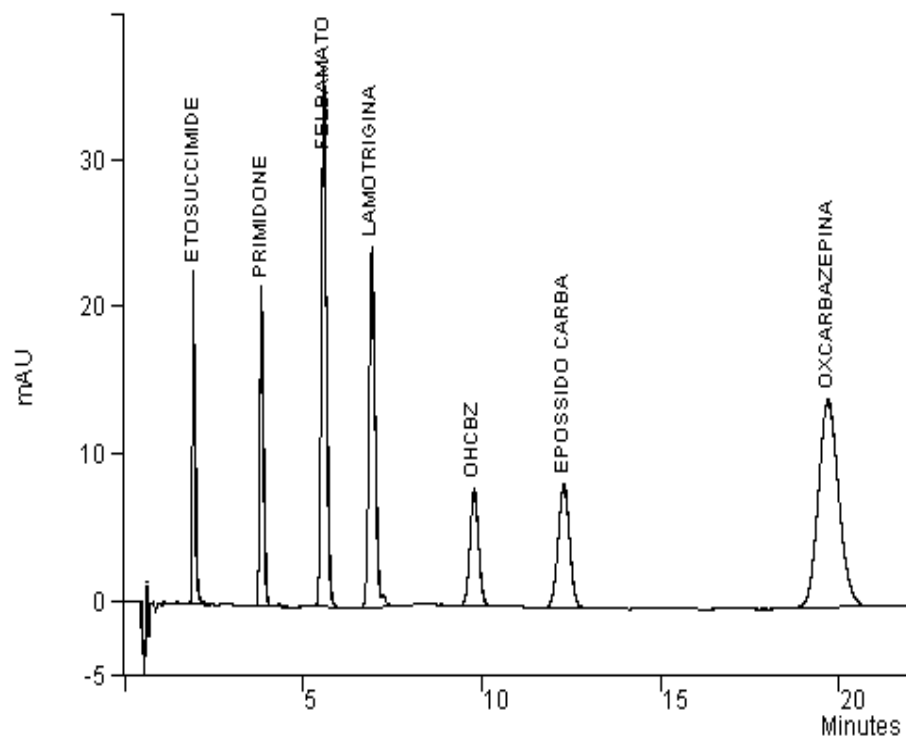


Fig. 1 :	Test Solution
	R.T. 1.90 Ethosuximide
	R.T. 3.80 Primidone
	R.T. 5.54 Phelbamate
	R.T. 6.90 Lamotrigine
	R.T. 9.76 10,11-Dihydro-10-Hydroxycarbamazepine
	R.T. 10.27 Carbamazepine Epoxide
	R.T. 19.67 Oxcarbazepine

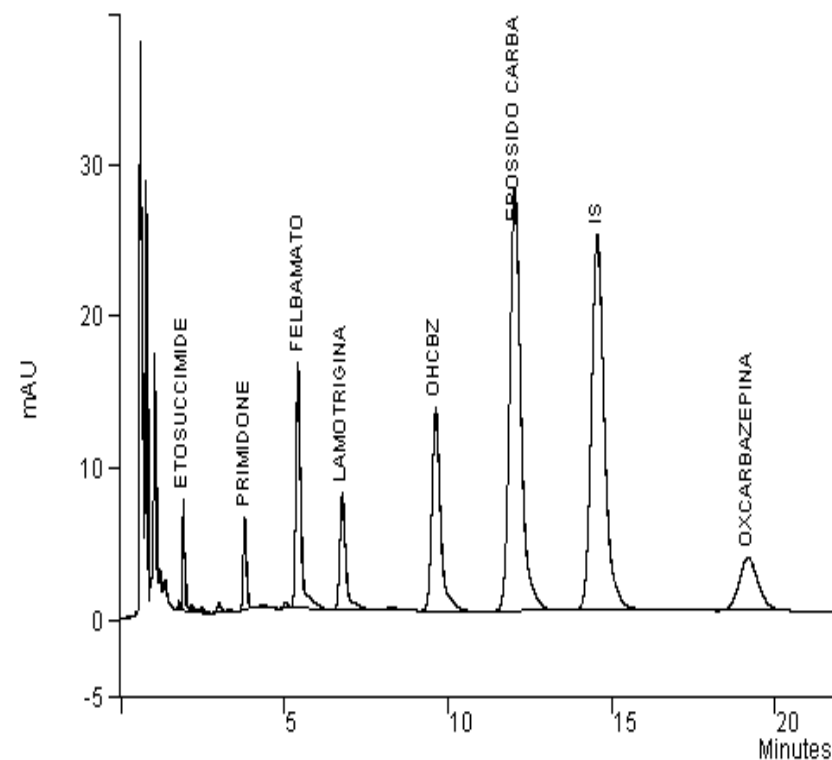


Fig. 2 :	Plasma Calibrator	
	R.T. 1.87 Ethosuximide	69,0 mg/l
	R.T. 3.75 Primidone	9,5 mg/l
	R.T. 5.38 Phelbamate	53,0 mg/l
	R.T. 6.74 Lamotrigine	8,6 mg/l
	R.T. 9.60 10,11-Dihydro-10-Hydroxycarbamazepine	24,1 mg/l
	R.T. 12.00 Carbamazepine Epoxide	48,2 mg/l
	R.T. 14.54 Internal Standard	
	R.T. 19.14 Oxcarbazepine	18,0 mg/l