

ANTIEPILEPTIC DRUGS 5 IN PLASMA BY UV DETECTION – FAST -
CODE Z83110

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 spheric and/or plasmatic concentration of the following drugs:

1. *Rufinamide*
2. *Desmethyloximide*
3. *Methsuximide*
4. *Lacosamide*
5. *Phenytoin*



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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° 003	Antiepileptics Drugs 5 in plasma 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 stabilizing Reagent the solution is directly injected into HPLC.

Recovery : >98 %

Sensitivity (LLOD) :

0,33 mg/l	Rufinamide
0,083 mg/l	Desmethysuximide
0,166 mg/l	Phenytoin
0,08 mg/l	Lacosamide
0,042 mg/l	Methsuximide

Minimum concentration analyzable (LLOQ):

1,0 mg/l	Rufinamide
0,25 mg/l	Desmethysuximide
0,5 mg/l	Phenytoin
0,25 mg/l	Lacosamide
0,25 mg/l	Methsuximide

Linearity:

1,0 – 200,0 mg/l	Rufinamide
0,25 – 200,0 mg/l	Desmethysuximide
0,5 – 200,0 mg/l	Phenytoin
0,25 – 200,0 mg/l	Lacosamide
0,25 – 200,0 mg/l	Methsuximide

Accuracy intra serie (relative error %)
Rufinamide:

Ci	Cs
3,13 mg/l	40 mg/l
10,9%	1,9%

Accuracy inter serie (relative error %)
Rufinamide:

Ci	Cs
3,13 mg/l	40 mg/l
11,9%	1,9%

Reproducibility intra serie (coefficient of variation %) Rufinamide:

CLLOQ	Cm	CUP
1 mg/l	25 mg/l	75 mg/l
6,0%	2,8%	1,8%

Reproducibility inter serie (coefficient of variation %) Rufinamide:

CLLOQ	Cm	CUP
1 mg/l	25 mg/l	75 mg/l
13,0%	3,7%	2,6%

Coefficient of correlation R² + Dev Std 0,9980 ± 0,0010 Rufinamide

Accuracy intra serie (relative error %)
Desmethysuximide:

Ci	Cs
3,13 mg/l	40 mg/l
3,5%	0,5%

Accuracy inter serie (relative error %)
Desmethysuximide:

Ci	Cs
3,13 mg/l	40 mg/l
8,0%	0,0%

Reproducibility intra serie (coefficient of variation %) Desmethysuximide:

CLLOQ	Cm	CUP
0,25 mg/l	25 mg/l	75 mg/l
5,2%	2,2%	0,8%

Reproducibility inter serie (coefficient of variation %) Desmethysuximide:

CLLOQ	Cm	CUP
0,25 mg/l	25 mg/l	75 mg/l
10,0%	4,0%	2,2%

Coefficient of correlation R^2 + Dev Std

0,9970 $\pm$ 0,0018 Desmethysuximide

Accuracy intra serie (relative error %)
Phenytoin:

Ci	Cs
3,125 mg/l	40 mg/l
2,2%	0,8%

Accuracy inter serie (relative error %)
Phenytoin:

Ci	Cs
3,125 mg/l	40 mg/l
5,2%	1,2%

Reproducibility intra serie (coefficient of variation %) Phenytoin:

CLLOQ	Cm	CUP
0,5 mg/l	25 mg/l	75 mg/l
4,5%	1,9%	1,6%

Reproducibility inter serie (coefficient of variation %) Phenytoin:

CLLOQ	Cm	CUP
0,5 mg/l	25 mg/l	75 mg/l
10,0%	2,5%	1,9%

Coefficient of correlation R^2 + Dev Std

0,9980 $\pm$ 0,0010 Phenytoin

Accuracy intra serie (relative error %)
Lacosamide:

Ci	Cs
3,13 mg/l	40 mg/l
8,4%	1,2%

Accuracy inter serie (relative error %)
Lacosamide:

Ci	Cs
3,13 mg/l	40 mg/l
8,8%	1,7%

Reproducibility intra serie (coefficient of variation %) Lacosamide:

CLLOQ	Cm	CUP
0,25 mg/l	25 mg/l	75 mg/l
9,4%	2,2%	1,5%

Reproducibility inter serie (coefficient of variation %) Lacosamide:

CLLOQ	Cm	CUP
0,25 mg/l	25 mg/l	75 mg/l
10,6%	3,8%	1,8%

Coefficient of correlation R^2 + Dev Std

0,9982 $\pm$ 0,0004 Lacosamide

Accuracy intra serie (relative error %)**Methsuximide:**

Ci	Cs
3,125 mg/l	40 mg/l
7,9%	0,9%

Accuracy inter serie (relative error %)**Methsuximide:**

Ci	Cs
3,125 mg/l	40 mg/l
7,7%	1,1%

Reproducibility intra serie (coefficient of variation %) Methsuximide:

CLLOQ	Cm	CUP
0,25 mg/l	25 mg/l	75 mg/l
4,4%	2,2%	0,9%

Reproducibility inter serie (coefficient of variation %) Methsuximide:

CLLOQ	Cm	CUP
0,25 mg/l	25 mg/l	75 mg/l
7,7%	4,2%	2,2%

Coefficient of correlation R² + Dev Std

0,9960 ± 0,0017 Methsuximide

Therapeutic range :

Rufinamide	1 – 50 mg/l
Desmethyloximide	10 – 40 mg/l
Lacosamide	1 – 10 mg/l
Methsuximide	/
Phenytoin	10 – 20 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 20 ml**Reagent B** – Stabilizing Solution, 1 x 20 ml**Reagent C** – Test Solution, 1 x 2 ml**See Warnings****Plasma Calibrator lyoph.**, 1 x 2 ml**Code Z04916 (packed separately – see data sheet)****Reagent E** – Internal Standard Solution, 4 x 50 µl**Reagent M** – Mobile Phase, 4 x 500 ml**Minimum Instrumental equipment required:**Isocratic HPLC System with loop of 5 µl
Spectrophotometric Detector UV/VIS λ=210 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 :

- 800 µl of H₂O HPLC grade
- 200 µl of **Reagent C - Test Solution**

Vortex for 20 sec.

INJECTION :

- Inject 5 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 : Reconstitution of **Reagent E – Internal Standard Solution**

Add at **Reagent E – Internal Standard Solution** 5 ml of **Reagent A – Deproteinization Sol.** (Stable 1 year at 2–8 °C)

STEP 2 : Samples Preparation

Dispense in a 1,5 ml vial with cap

	Calibrator	Sample	Control
Calibrator	200 µl		
Sample		200 µl	
Control			200 µl
Reagent A – Deproteinization Sol. + Internal Standard	200 µl	200 µl	200 µl

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

STEP 3 : Wait 2 minutes and centrifuge at 14.000 rpm for 10 minutes.

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

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

Vortex for 10 sec.

N.B.: at this step, the sample is stable 7 days at 2-8 °C

INJECTION :

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

ANTIEPILEPTICS 5 - Warnings

REAGENT C: TEST SOLUTION

ANTIEPILEPTICS :	About
Rufinamide	10 mg/l
Desmethysuximide	10 mg/l
Lacosamide	10 mg/l
Methsuximide	10 mg/l
Phenytoin	10 mg/l

SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	210 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 : 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 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 **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).

PARAMETRI HPLC

LOOP	5 μ l
FLOW	1,2 ml/min.
PRESSURE	About 130 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z04916	Calibrator for Antiepileptics 5 in serum and/or in plasma	4 x 2 ml
Z04919	Control for Antiepileptics 5 in serum and/or in plasma – Level 1 and 2	2 x 5 x 2 ml
Z83111	Mobile Phase	4 x 500 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 5 IN PLASMA (Reference Chromatograms)

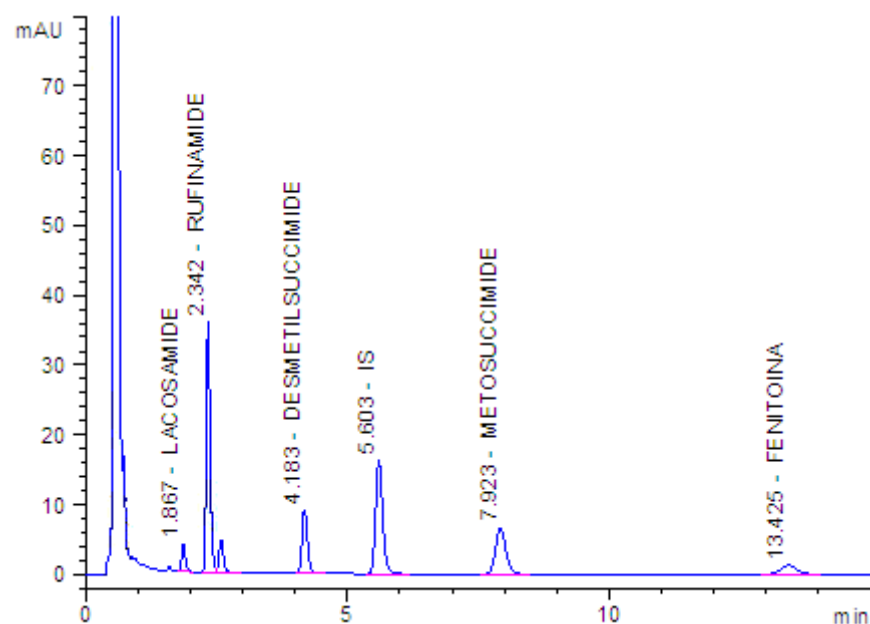


Fig. 1 :	Plasma Calibrator		
	R.T. 1.86	Lacosamide	7,5 mg/L
	R.T. 2.34	Rufinamide	45,1 mg/L
	R.T. 4.18	Desmethyloximide	29,1 mg/L
	R.T. 5.60	Internal Standard	
	R.T. 7.92	Methsuximide	19,4 mg/L
	R.T. 13.42	Phenytoin	13,7 mg/L

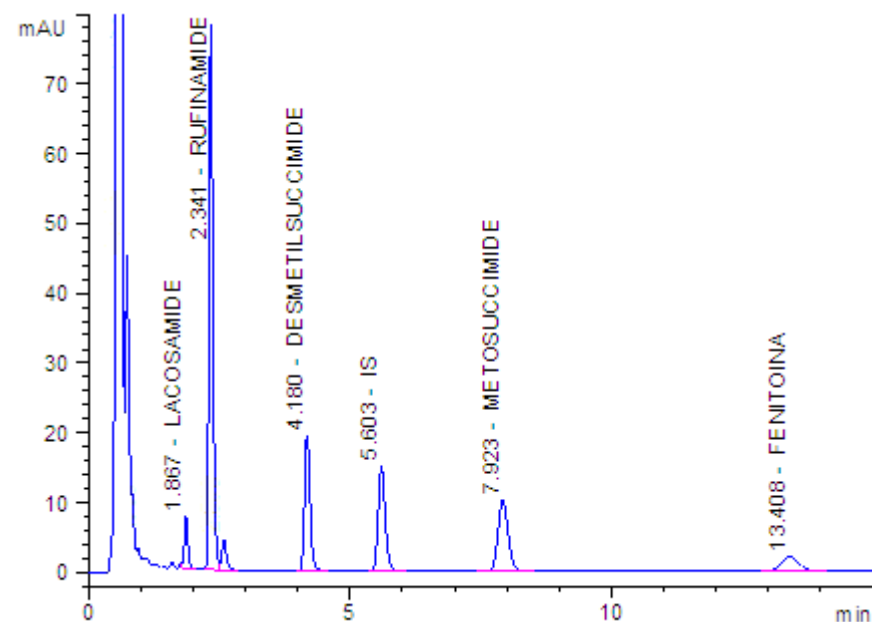


Fig. 2 :	Plasma Control – Level 2		
	R.T. 1.86	Lacosamide	16 mg/l
	R.T. 2.34	Rufinamide	90 mg/l
	R.T. 4.18	Desmethyloximide	70 mg/l
	R.T. 5.60	Internal Standard	
	R.T. 7.92	Methsuximide	40 mg/l
	R.T. 13.40	Phenytoin	32 mg/l