

ANTIEPILEPTIC DRUGS 1 in serum and/or plasma by UV - FAST - CODE Z80010

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. *Phenobarbital*
2. *Carbamazepine*
3. *Phenytoin*



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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 1 in serum/plasma by UV - FAST	09/2024
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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) : 0,06 µg/ml Phenobarbital and Carbamazepine
0,1 µg/ml Phenytoin

Minimum concentration analyzable (LLOQ) : 0,2 µg/ml Phenobarbital and Carbamazepine
0,3 µg/ml Phenytoin

Linearity: 0,2 – 300 µg/ml Phenobarbital
0,2 – 200 µg/ml Carbamazepine
0,3 – 300 µg/ml Phenytoin

Therapeutic Range in serum :

<i>Phenobarbital</i>	10 – 40 µg/ml
<i>Carbamazepine</i>	6 – 12 µg/ml
<i>Phenytoin</i>	10 – 20 µg/ml

Accuracy intra serie (relative error %)
PHENOBARBITAL :

Ci	Cs
5 µg/ml	30 µg/ml
6,3%	3,6%

Accuracy inter serie (relative error %)
PHENOBARBITAL :

Ci	Cs
5 µg/ml	30 µg/ml
6,4%	4,9%

Reproducibility intra serie (coefficient of variation %)
PHENOBARBITAL :

Cl	Cm	Ch
0,2 µg/ml	15 µg/ml	80 µg/ml
6,6%	6,0%	2,1%

Reproducibility inter serie (coefficient of variation %)
PHENOBARBITAL :

Cl	Cm	Ch
0,2 µg/ml	15 µg/ml	80 µg/ml
6,5%	4,8%	2,1%

Coefficient of Correlation + Dev Std 0,9976 ± 0,0019 Phenobarbital

Accuracy intra serie (relative error %)
CARBAMAZEPINE :

Ci	Cs
3 µg/ml	15 µg/ml
8,5%	3,9%

Accuracy inter serie (relative error %)
CARBAMAZEPINE :

Ci	Cs
3 µg/ml	15 µg/ml
10,7%	4,2%

Reproducibility intra serie (coefficient of variation %)
CARBAMAZEPINE :

Cl	Cm	Ch
0,2 µg/ml	10 µg/ml	20 µg/ml
9,8%	4,8%	1,5%

Reproducibility inter serie (coefficient of variation %) CARBAMAZEPINE :

Cl	Cm	Ch
0,2 µg/ml	10 µg/ml	20 µg/ml
8,6%	4,9%	2,9%

Coefficient of Correlation + Dev Std

0,9976 ± 0,0005 Carbamazepine

Accuracy intra serie (relative error %) PHENYTOIN :

Ci	Cs
5 µg/ml	30 µg/ml
8,3%	1,9%

Accuracy inter serie (relative error %) PHENYTOIN :

Ci	Cs
5 µg/ml	30 µg/ml
10,7%	6,6%

Reproducibility intra serie (coefficient of variation %) PHENYTOIN :

Cl	Cm	Ch
0,3 µg/ml	15 µg/ml	50 µg/ml
7,4%	3,1%	1,8%

Reproducibility inter serie (coefficient of variation %) PHENYTOIN :

Cl	Cm	Ch
0,3 µg/ml	15 µg/ml	50 µg/ml
10,6%	3,0%	2,7%

Coefficient of Correlation + Dev Std

0,9953 ± 0,0030 Phenytoin

Components of the kit :

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

Reagent A – Deproteinization Solution with Internal Standard, **1 x 40 ml**

Reagent B – Stabilizing Solution, **1 x 80 ml**

Plasma Calibrator, **1 x 2 ml**

Reagent F – Test Solution, **1 x 1 ml**

Reagent M – Mobile Phase, **4 x 500 ml**

Code Z04016 (packed separately – see data sheets)
See Warnings

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10µl
Spectrophotometric Detector UV/VIS $\lambda=212$ 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.

PREANALYTICAL PROCEDURE

Pipette in a tube :

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

INJECTION :

- Inject 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

Dispense in a tube :

	Calibrator	Sample
Calibrator	200 µl	
Sample		200 µl
Reagent A – Deproteinization Solution	400 µl	400 µl

Warning: deproteinize directly on Vortex for at least 10 seconds

STEP 2 : Centrifuge at 14.000 rpm for 10 minutes

STEP 3 : Pipette in a tube 400 µl of clear supernatant

STEP 4 : Add 400 µl of **Reagent B – Stabilizing Solution**

Vortex for 10 seconds

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

INJECTION :

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

ANTIEPILEPTICS 1 - FAST - Warnings

REAGENT F: TEST SOLUTION

Phenobarbital	About 52,4 µg/ml
Phenytoin	About 8,4 µg/ml
Carbamazepine	About 5,0 µg/ml

SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	212 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 seconds

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 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 µl
FLOW	1,0 ml/min
PRESSURE	About 150 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z04016	Calibrator in plasma for Antiepileptics 1	4 x 2 ml
Z04019	Control in plasma for Antiepileptics 1 – Levels 1 and 2	2 x 5 x 2 ml
Z699975902	POROSHELL 120 EC-C18 Analytical Column (4,6 x 50 mm, 2,7 µm)	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 IN SERUM OR PLASMA (Reference Chromatograms)

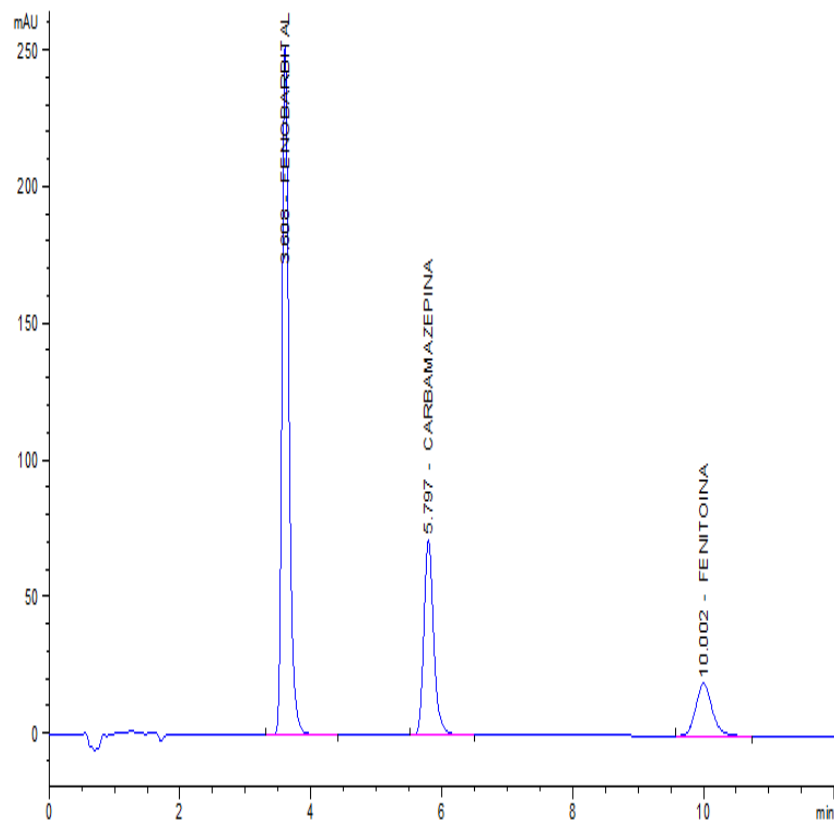


Fig. 1:	Test Solution
R.T. 3.60	Phenobarbital
R.T. 5.79	Carbamazepine
R.T. 10.00	Phenytoin

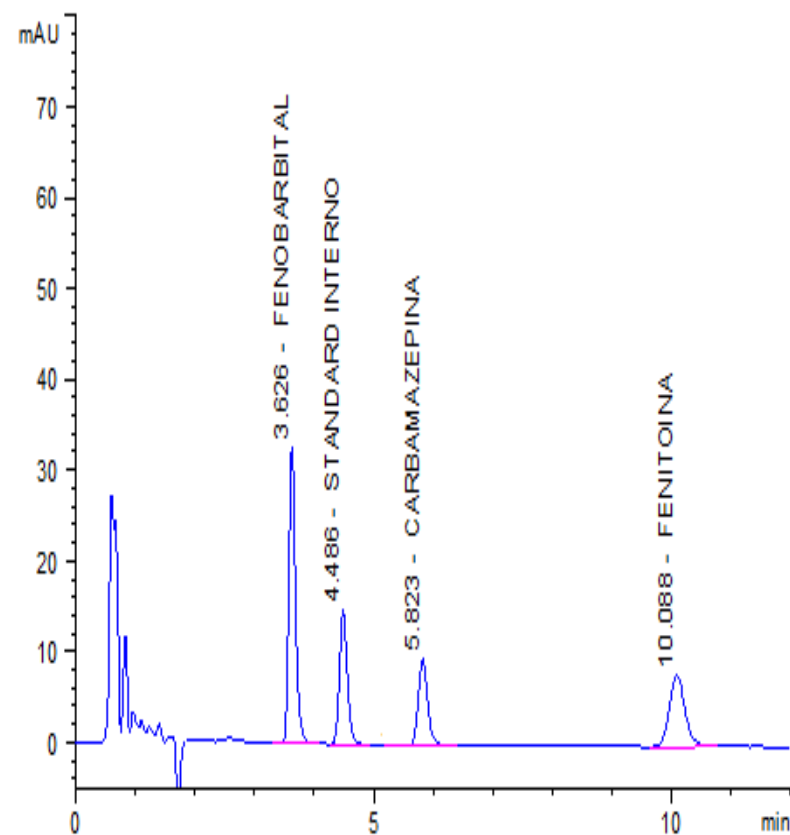


Fig. 2:	Plasma Calibrator	
R.T. 3.62	Phenobarbital	39,3 µg/ml
R.T. 4.48	Internal Standard	
R.T. 5.82	Carbamazepine	8,3 µg/ml
R.T. 10.08	Phenytoin	21,3 µg/ml

ANTIEPILEPTICS IN SERUM OR PLASMA (Reference Chromatograms)

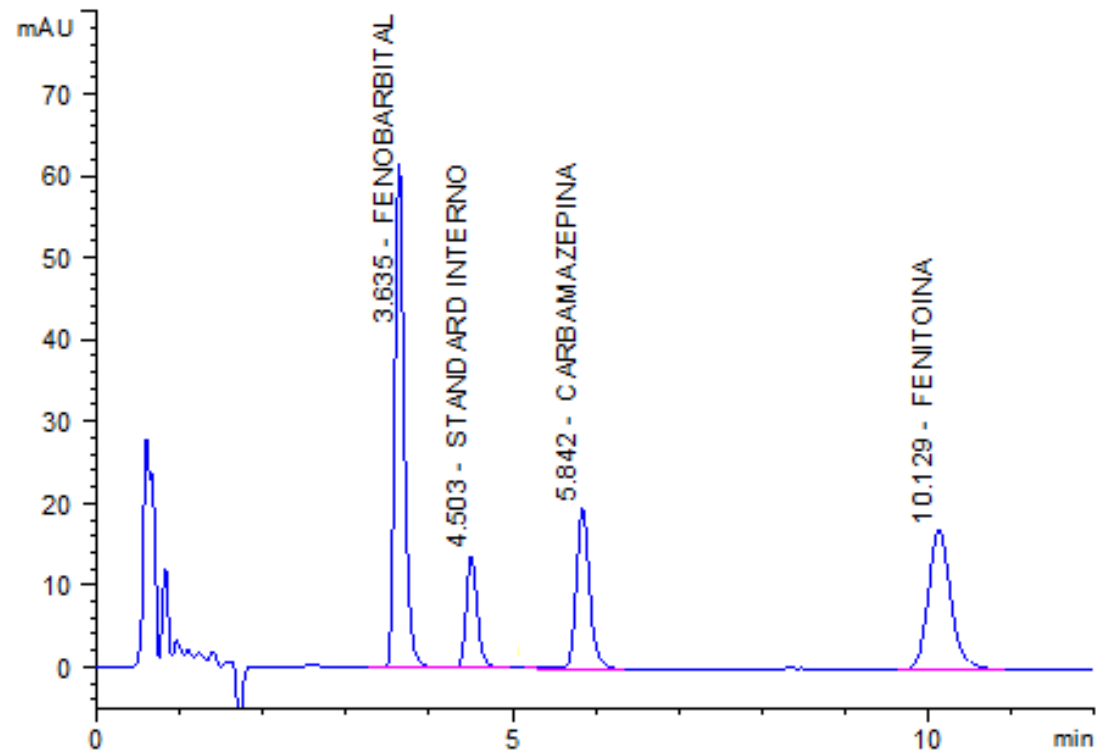


Fig. 3:	Low Control		
	R.T. 3.63	Phenobarbital	74,1 µg/ml
	R.T. 4.50	Internal Standard	
	R.T. 5.84	Carbamazepine	16,3 µg/ml
	R.T. 10.12	Phenytoin	46,2 µg/ml