

VALPROIC ACID IN PLASMA by UV - FAST – Code Z05310

BIOCHEMISTRY

Valproic acid (VPA) is an anticonvulsant widely used both in the therapy of different forms of generalized epilepsy in both partial epilepsies, in particular those idiopathic. It is also used in some mood disorders (mania, bipolar disorder). The VPA acts through multiple mechanisms, not fully understood, but this complexity is probably the basis of the broad spectrum of action. It is rapidly absorbed from the intestinal tract and binds almost completely to plasma proteins. The bioavailability of sodium valproate is greater than 90% after oral or intravenous administration. The blood peak is reached after 1-4 hours in relation to the formulation. Steady-state plasma concentrations are attained rapidly (3-4 days) after oral administration, with intravenous form steady-state can be reached in a few minutes. The half-life is approximately 8-20 hours. In children it is usually shorter. At the level of interactions, replacing phenytoin in plasma proteins and inhibits the metabolism of several drugs, such as phenobarbital, phenytoin, carbamazepine.

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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		06/2024
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TECHNICAL FEATURES

Principle of the Method :

The VPA is extracted from plasma after appropriate deproteinization. Following addition of HPLC grade water, the solution is injected into the chromatographic system.

Recovery : 93%

Sensitivity (LLOD) : 3,33 µg/ml

Minimum concentration analyzable (LLOQ): 10 µg/ml

Linearity : 10 – 1.000 µg/ml

Reference Values : 40 – 100 µg/ml

**Accuracy intra serie
(Relative error %) :**

Ci	Cs
25 µg/ml	100 µg/ml
13,74%	3,72%

**Accuracy inter serie
(Relative error %) :**

Ci	Cs
25 µg/ml	100 µg/ml
8,06%	3,21%

**Reproducibility intra serie
(Coefficient of variation %) :**

C LLOQ	Cm	CA
10 µg/ml	50 µg/ml	200 µg/ml
7,06%	4,86%	2,19%

**Reproducibility inter serie
(Coefficient of variation %) :**

C LLOQ	Cm	CA
10 µg/ml	50 µg/ml	200 µg/ml
9,32%	4,35%	2,86%

Coefficient of Correlation R2 + DEV. STD: 0,9972 ± 0,0016

Components of the kit :

All the Reagents are stable 3 years at 2–8 °C.

Reagent A – Deproteinization Solution with Internal Standard, **1 x 30 ml**

Reagent B – Test Solution, **1 x 5 ml**

See Warnings

Plasma Calibrator Iyophil., **1 x 1 ml**

Code Z05116 (packed separately – see data sheets)

Reagent M – Mobile Phase, **2 x 500 ml**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 10 μ l
Spectrophotometric Detector UV/VIS $\lambda=215$ 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 Phase :

If not is analyzed immediately, freezing the plasma at -20° C. Stable 1 month.

PREANALYTICAL PROCEDURE

Test Solution Preparation

Pipette in a tube :

- 900 µl of H₂O HPLC grade
- 100 µl of **Reagent B** - Test Solution

NOTE : Before starting the analytical session is advisable to inject 10 µl of this solution into HPLC to identify the retention time of Valproic Acid that must be similar to that shown in fig.1. If the test was successful, proceed to assay. If not, check the functionality of the analytical system.

Important: This test thus obtained should not be used as a calibrator

ANALYTICAL PROCEDURE

STEP 1 : Sample, Calibrator and Controls Preparation

Dispense in a tube:

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

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

STEP 2 : Centrifuge at 10.000 -14.000 rpm for 5 minutes

STEP 3 : Take 200 µl of supernatant and add 200 µl of water HPLC grade

Vortex for 10 sec.

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

INJECTION :

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

VALPROIC ACID - Avvertenze

REAGENT B : TEST SOLUTION

VALPROIC ACID	About 10.000 µg/ml
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SPECTROPHOTOMETRIC DETECTOR PARAMETERS

λ	215 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec

HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase Poroshell 120 EC-C18 (50 mm X 4,6 mm, 2,7 µm), the use of Javelin Colfilter (cod. S90199511) is obligatory.

HPLC COLUMN CONDITIONING

Install a new analytical column Poroshell 120 EC-C18 (50 mm x 4,6 mm, 2,7 µm). Disconnect the detector and filter 30 ml of H₂O : Acetonitrile (20 : 80 v/v) set flow at 1,0 ml / min. **Don't recycle the washing solutions.** Flux 30 ml of H₂O : Acetonitrile (80 : 20 v/v) set flow at 1,0 ml / min. **Don't recycle the washing solutions.** Finally inject the Chemical Standard and verify the quality of the HPLC run.

It is 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 AND INJECTION NEEDLE CLEANING

Disconnect the detector. Flux 15 ml of H₂O : Acetonitrile (20 : 80 v/v) set flow at 1 ml/min. Flux a solution of H₂O : Acetonitrile (70 : 30 v/v) for 30 minutes and discharge. When you re-use the column, flux 30 ml of H₂O before a new conditioning with the Mobile Phase. Store the column in a solution of H₂O : Acetonitrile (70 : 30 v/v). Wash the injection needle with a solution of H₂O : Acetonitrile/Methanol/Ethanol (80 : 20 v/v).

HPLC PARAMETERS

LOOP	10 µl
FLOW	1,0 ml/min
PRESSURE	About 120 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z05116	Calibrator in plasma for Valproic Acid	4 x 2 ml
Z05119	Control in plasma for Valproic Acid – Levels 1 and 2	2 x 5 x 2 ml
Z699975902	Poroshell 120 EC-C18 Analytical Column (50 x 4,6mm -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



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VALPROIC ACID IN PLASMA (Reference Chromatograms)

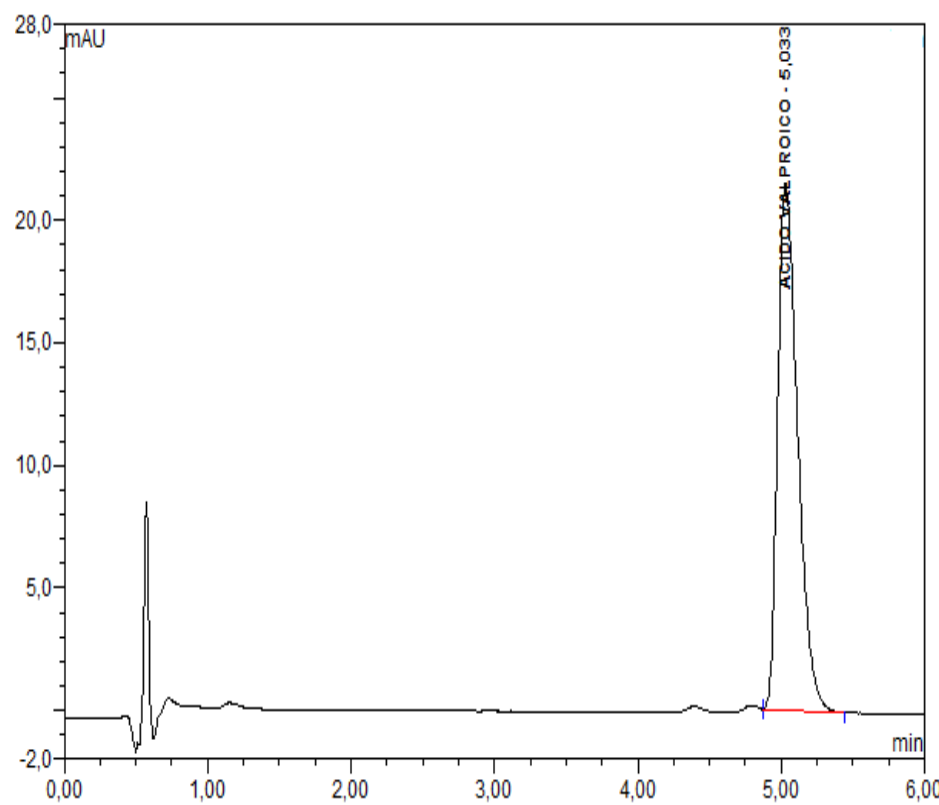


Fig. 1	Test Solution
	R.T. 5.0 Valproic Acid

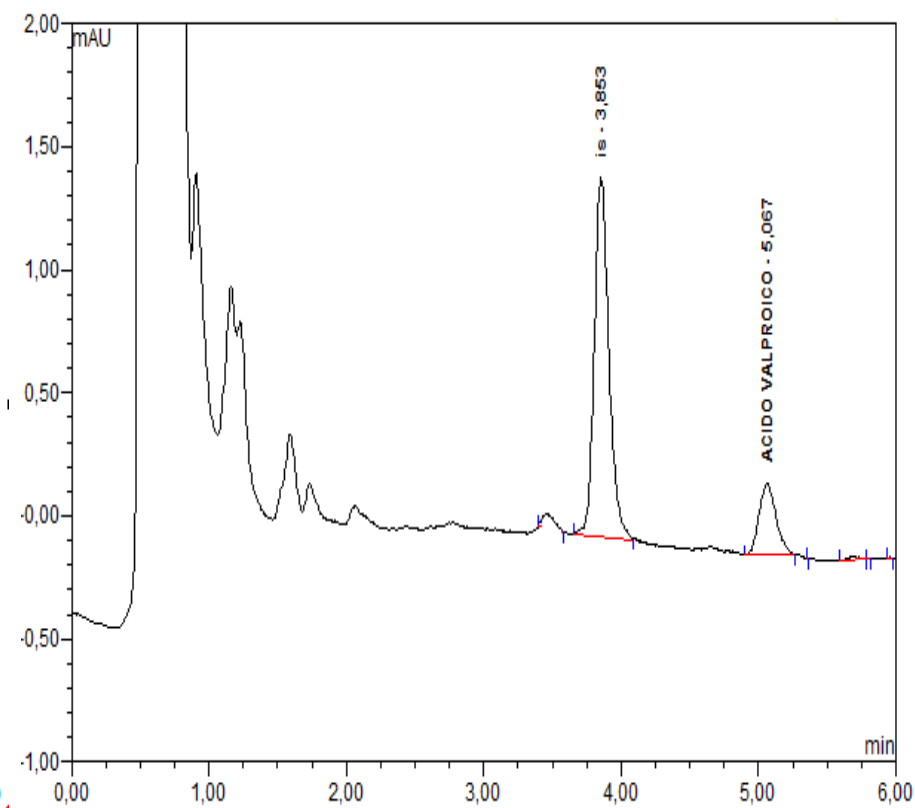


Fig. 2	Plasma Calibrator lyophil.
	R.T. 3.8 Internal Standard
	R.T. 5.0 Valproic Acid 125,9 ug/ml

VALPROIC ACID IN PLASMA (Reference Chromatograms)

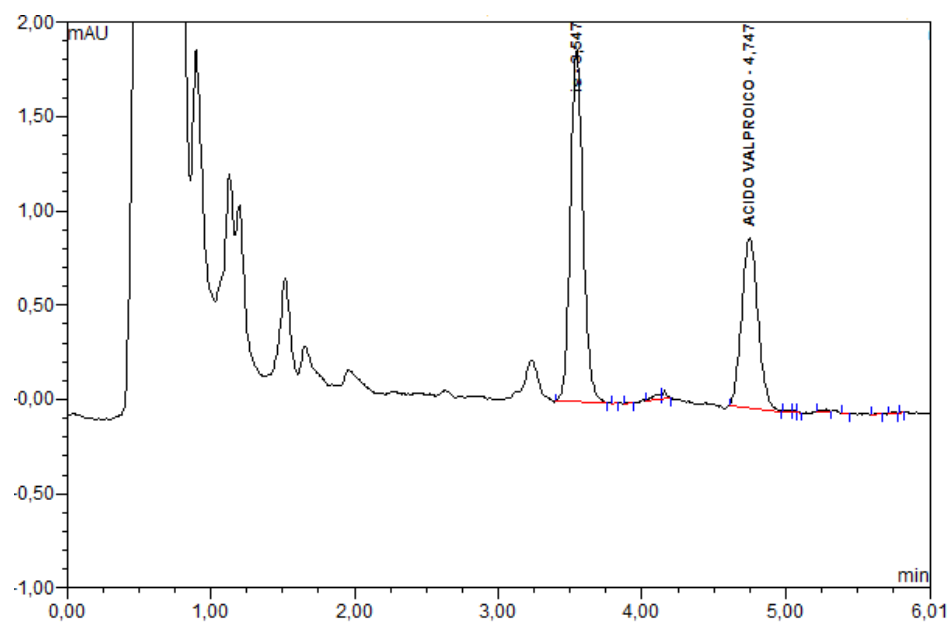


Fig. 3:	Serum Control Level 2			
	R.T.	3.54	Internal Standard	
	R.T.	4.74	Valproic Acid	233,8 µg/ml

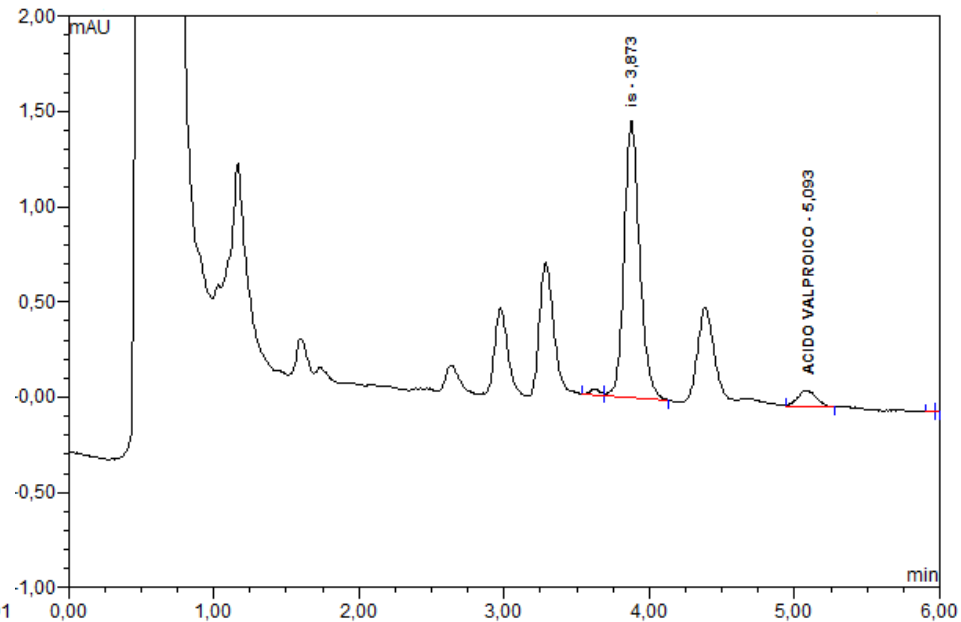


Fig. 4:	Serum Sample			
	R.T.	3.87	Internal Standard	
	R.T.	5.09	Valproic Acid	24,46 µg/ml