

TOPIRAMATE IN PLASMA BY FLUORIMETRY – Code Z77010

BIOCHEMISTRY

Topiramate is a sulfamate substituted monosaccharide with some mechanisms of action that result in the blocking of onset of seizures: the main ones are the modulation of voltage-dependent sodium channels, potentiation of the inhibitory neurotransmitter GABA and the attenuation of activity AMPA-kainate excitatory neurotransmitter. The pharmacokinetic profile of topiramate compared with that of other antiepileptic drugs, shows a long plasma half-life, linear pharmacokinetics, predominantly by renal excretion, no significant ties to plasma proteins and lack of clinically relevant active metabolites. There is a difference between sexes in the volume of distribution: the values in females are on average 50% of those in males. This is attributed to a higher body fat in women without clinical sequelae. Food has no clinical effects on the bioavailability of topiramate.

Interference: concomitant administration of topiramate and phenytoin and / or carbamazepine resulted in a decrease in the concentration of topiramate.

Topiramate is approved for human use in both adults and pediatric patients because of its relatively broad spectrum activity against many types of seizures and minimal side effects.



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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° 009	Topiramate in plasma by Fluorimetry	06/2024
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TECHNICAL FEATURES

Principle of the Method :

This method allows to determine plasma concentrations of topiramate after SPE purification and derivatization of the plasma with a suitable reagent. After a double incubation at 50 ° C, the sample is injected into the HPLC chromatograph.

<u>Recovery :</u>	98,7 %
<u>Sensitivity :</u>	2 µg/l
<u>Dynamic Range of the Method :</u>	10 – 40.000 µg/l
<u>Reference Values:</u>	2.000 – 25.000 µg/l
<u>CV%:</u>	6,8 intra serie 6,6 inter serie

Components of the kit :

All the reagents are ready to use and stable 3 years at 2 – 8 °C. Except **Reagent G** that must be stored at – 20 °C.

Reagent A – Diluting Solution, **1 x 55 ml**

Reagent A1 – Diluting Solution for Internal Standard, **1 x 50 ml**

Reagent B – Conditioning Solution 1, **1 x 140 ml**

Reagent C – Conditioning Solution 2, **1 x 105 ml**

Reagent D – Wash Solution 1, **1 x 105 ml**

Reagent E – Wash Solution 2, **1 x 105 ml**

Reagent F – Eluting Solution, **1 x 70 ml**

Reagent G – Internal Standard Solution, **1 x 6 ml** **Store at – 20 °C**

Reagent H – Derivatization Solution, **1 x 15 ml**

Reagent I – Stabilizing Solution, **1 x 3 ml**

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

See Warnings

Plasma Calibrator, **1 x 5 ml**

Code Z77016 (packed separately – see data sheets)

Reagent M – Mobile Phase, **9 x 500 ml**

Clean up Columns, **100 pcs**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 50 µl or 100 µl
Fluorimetric Detector $\lambda_{EX}=260\text{ nm}$ $\lambda_{EM}=315\text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Whole Blood Collection Procedure:

Collect 3 ml of blood in a suitable test tube with anticoagulant Heparin.
Centrifuge at 4000 rpm for 5 minutes.
Separate the plasma and store at – 20 °C. Stable 4 weeks. It is possible to use serum (without gel) in place of plasma with Heparin.

PREANALYTICAL PROCEDURE

Preparation of Internal Standard

Dilute the **Reagent G – Internal Standard Solution** 1:100 with **Reagent A1 – Diluting Sol. for Internal Standard** preparing the volume sufficient to cover the samples of the analytic session

N.B.: this dilution is prepared fresh for each run

Preparation of Test Solution

STEP 1: In a vial of 2 ml put :

- 100 µl of **Reagent H – Derivatization Solution**
- 90 µl of Acetonitrile (not supplied in the kit)
- 40 µl of **Internal Standard Sol. diluted as stated above**
- 10 µl of **Reagent L – Test Solution**

Close the vials with caps and put on vortex for 10 sec.

STEP 2: Incubate for 15 minutes at 50 °C

STEP 3: Cooling and add 20 µl of **Reagent I – Stabilization Solution**

Close the vials with caps and put on vortex for 10 sec.

STEP 4: Incubate for 5 minutes at 50 °C

STEP 5: Cooling and add 200 µl of H₂O HPLC grade

Vortex for 10 sec.

NOTE : Before starting the analytical session it would be better to inject 20 - 50 µl of this solution in HPLC to identify the time of retention of Topiramate, that must be similar to that shown in Fig. 1. If the Test has given positive result, proceed with the analytical session. If it wouldn't be so, verify the functionality of the analytical system.

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1 : Preparation of Samples, Standard and Controls

Dispense in a tube :

	Calibrator	Control	Sample
Calibrator	500 µl		
Control		500 µl	
Sample			500 µl
Reagent A – Diluting Solution	500 µl	500 µl	500 µl

Vortex for 10 sec.

STEP 2 : Conditioning of Clean-up Columns

Prepare Clean up columns per each Sample, Calibrator and Controls

- Condition clean up columns by fluxing 1 ml of **Reagent B – Conditioning Sol. 1** *don't let the columns be dry!*
- Condition clean up columns by fluxing 1 ml of **Reagent C – Conditioning Sol. 2** *don't let the columns be dry!*

Discharge the percolated liquid

STEP 3 : Sample loading

- Dispense 1,0 ml of sample, previously prepared, into clean up columns
Discharge the percolated liquid, if needed a vacuum system could be used for percolation, at speed of 1 ml/min (drop by drop).

STEP 4 : Washing

- Wash with 1 ml of **Reagent D – Wash Solution 1** *Don't let the columns be dry!*
- Wash with 1 ml of **Reagent E – Wash Solution 2** *Let dry with a light flux of air! Discharge the percolated liquid*

STEP 5 : Eluting

Elute with 0,5 ml of **Reagent F – Eluting Solution** *Let dry with a light flux of air!*

Vortex for 10 sec.

N.B.: at this step, the sample is stable 2 weeks at - 20 °C

Dilute the **Reagent G – Internal Standard Solution** 1:100 with **Reagent A1 – Diluting Sol. for Internal Standard** preparing the volume sufficient to cover the samples of the analytic session

N.B.: this dilution is prepared fresh for each run

STEP 6: In a vial of 2 ml put :

- 100 µl of eluate obtained in STEP 5
- 40 µl of Internal Standard Sol. diluted as stated above
- 100 µl of **Reagent H – Derivatization Solution**

Close the vials with caps and put on vortex for 10 sec.

STEP 7: Incubate for 15 minutes at 50 °C

STEP 8: Cooling and add 20 µl of **Reagent I – Stabilizing Solution**

Close the vials with caps and put on vortex for 10 sec.

STEP 9: Incubate for 5 minutes at 50 °C

STEP 10: Cooling and add 200 µl of Water HPLC grade

Vortex for 10 sec.

N.B.: at this step, the sample is stable 2 days at - 20 °C

INJECTION :

- Inject 20 - 50 µl of solution into the chromatographic system

TOPIRAMATE - Warnings

REAGENT L : TEST SOLUTION/CHEMICAL STANDARD

TOPIRAMATE	100.000 µg/l
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FLUORIMETRIC DETECTOR PARAMETERS

λ_{Ex}	260 nm
λ_{Em}	315 nm
GAIN	Low

ANALYTICAL COLUMN PROTECTION

To save the analytical column Hypersil Gold CN 4,6 x 150 mm, 5 µm, the use of Javelin Colfilter – Superchrom is obligatory.

COLUMN CONDITIONING

Install a <u>new</u> analytical Hypersil Gold CN Column 4,6 x 150 mm, 5 µm. Disconnect the detector and filter 30 ml of H ₂ O : Acetonitrile (20 : 80 v/v) set flow at 1 ml/min. Don't recycle the washing solutions. Condition the column with the mobile phase at a flow of 2 ml / min and discharge the first 30 ml. 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 AND INJECTION NEEDLE CLEANING

Disconnect the detector. Flux for 15 minutes H ₂ O at 2 ml/min and discharge. Flux a solution of H ₂ O : Acetonitrile (20 : 80 v/v) at 2 ml/min for 30 minute and discharge. The column must be stored in a solution of H ₂ O : Acetonitrile (20 : 80 v/v). Wash the injection needle with a solution of H ₂ O : Acetonitrile or Ethanol (80 : 20 v/v).

HPLC PARAMETERS

LOOP	50 or 100 µl
FLOW	2 ml/min
PRESSURE	About 100 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z77016	Calibrator in plasma for Topiramate	4 x 5 ml
Z77019	Control in plasma for Topiramate – Levels 1 and 2	2 x 5 x 5 ml
TF25805-154630	Hypersil Gold CN (150 x 4,6mm -5 µm) Analytical Column	1 Pc
TF88400	Javelin Colfilters - Superchrom	1 x 4 Pcs
S29057U	Glass Standard Vials of 2 ml with screw caps	1 x 100 Pcs

TOPIRAMATE IN PLASMA

(Reference Chromatograms)

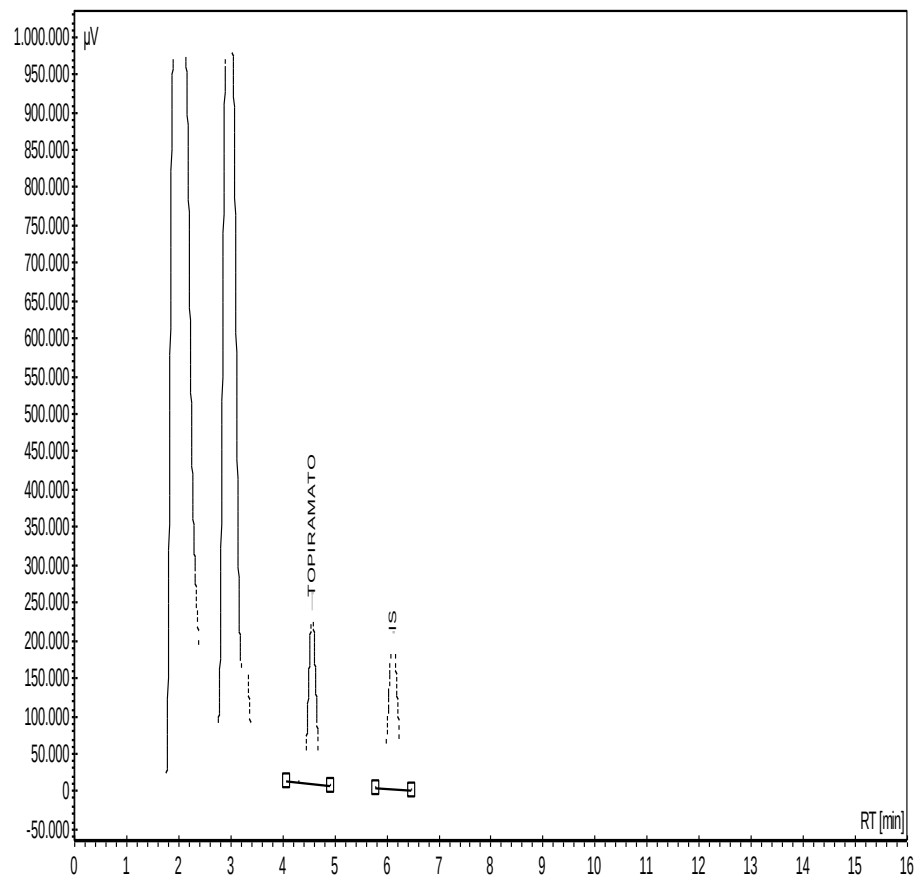


Fig. 1	Test Solution
	R.T. 4.56 Topiramate
	R.T. 6.11 Internal Standard

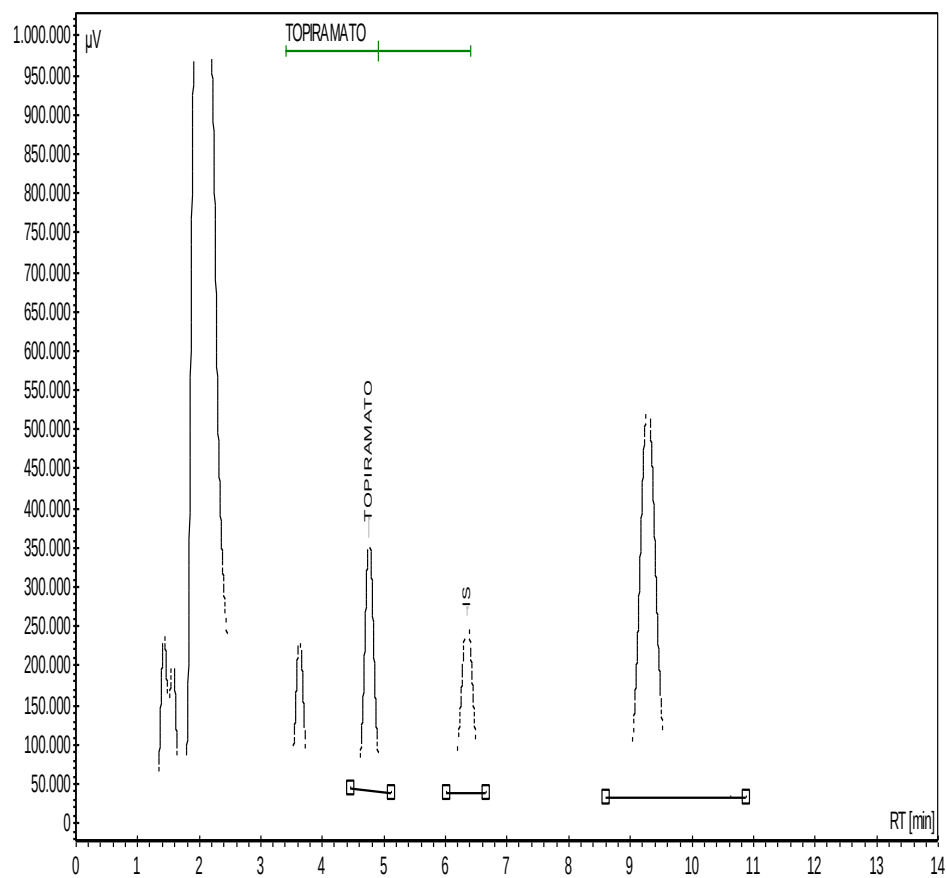


Fig. 2	Plasma Calibrator
	R.T. 4.62 Topiramate 8.800 $\mu g/l$
	R.T. 6.19 Internal Standard

TOPIRAMATE IN PLASMA (Reference Chromatograms)

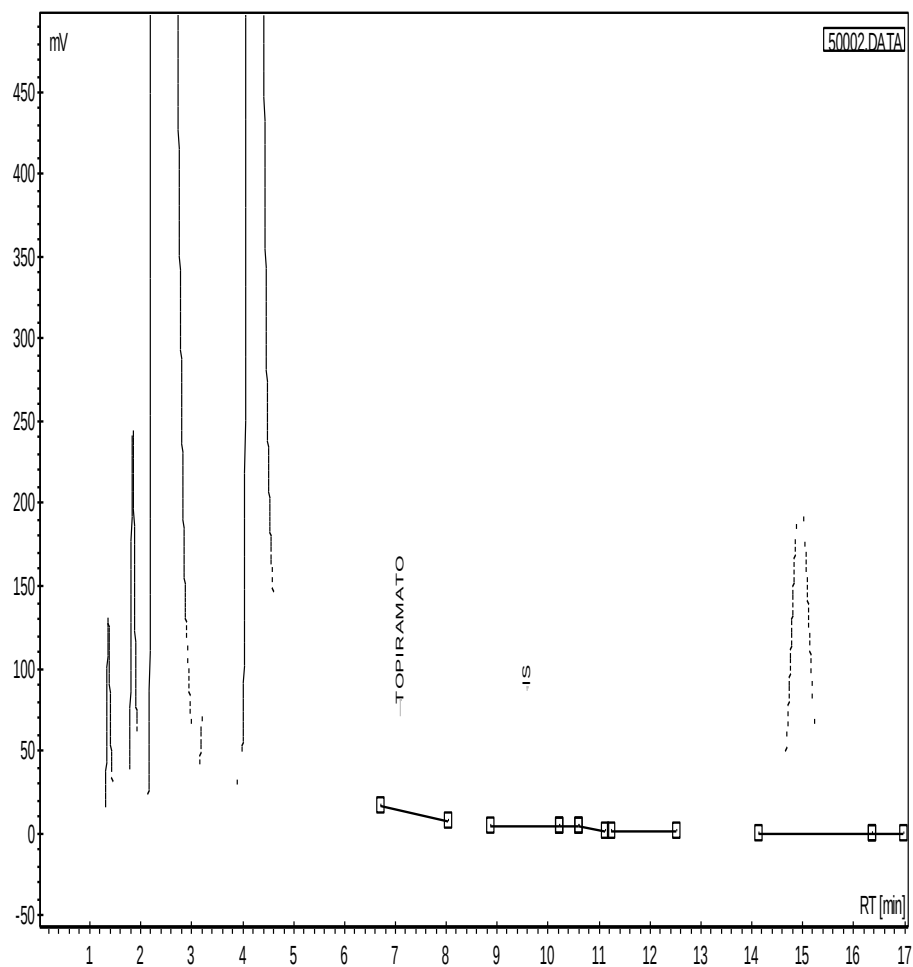


Fig. 3	Plasma Control level 2
	R.T. 4.60 Topiramate 17.400 µg/l
	R.T. 6.10 Internal Standard

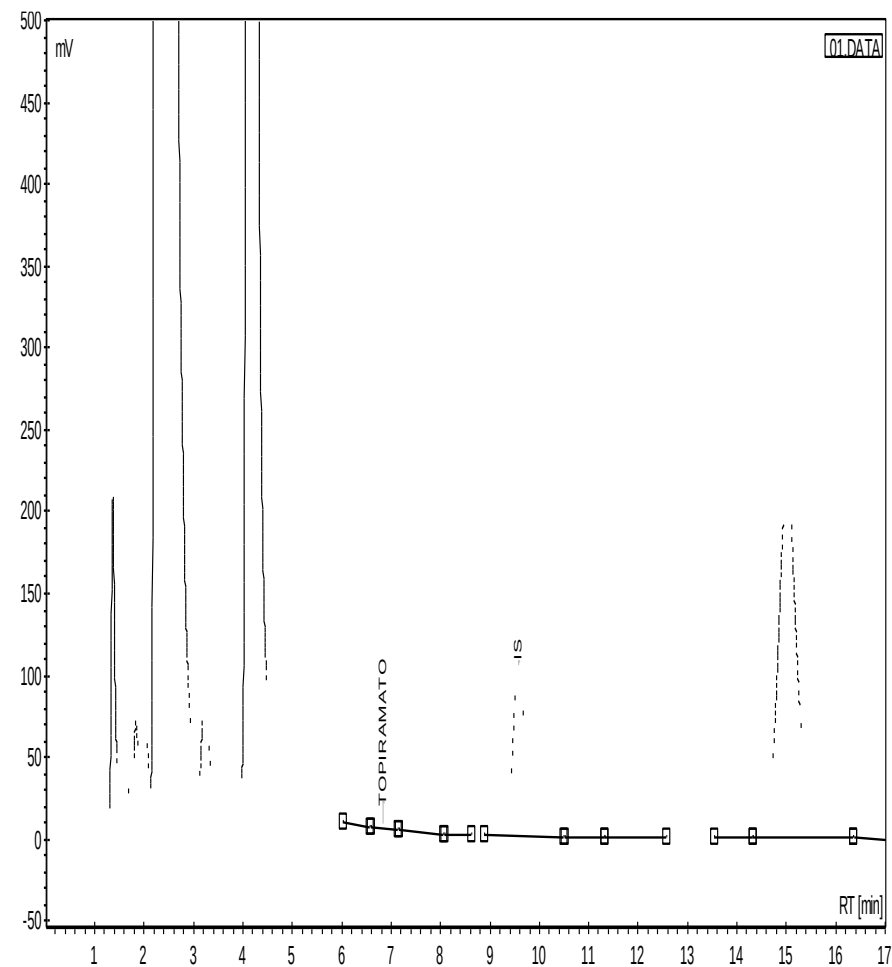


Fig. 4	Plasma Sample
	R.T. 4.69 Topiramate 6.490 µg/l
	R.T. 6.27 Internal Standard