TOPIRAMATE IN PLASMA BY FLUORIMETRY – Code Z77010

BIOCHEMISTRY

Topiramate is a sulfamate substituted monosaccharide with some mechanisms of action that result in the blocking onset 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. The 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.

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.

| Release N° 003 | Topiramate in plasma by Fluorimetry | June 2012 |
**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.

**Recovery:** 98,7 %

**Sensitivity:** 2 µg/l

**Dynamic Range of the Method:** 10 – 40,000 µg/l

**Reference Values:** 5,000 – 20,000 µg/l

**CV%:**
- 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 105 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 **Stored at – 20 °C**
- **Reagent H** – Derivatization Solution, 1 x 15 ml
- **Reagent I** – Stabilization Solution, 1 x 3 ml
- **Reagent L** – Test Solution, 1 x 5 ml **See Warnings**
- **Reagent N** – Plasma Calibrator, 1 x 5 ml **See Warnings**
- **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 $\lambda_{EX}=260$ nm $\lambda_{EM}=315$ 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 800 µl of Water HPLC grade

Vortex for 10 sec.

NOTE: Before starting the analytical session it would be better to inject 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!

Release N° 003 Topiramate in plasma by Fluorimetry June 2012
ANALYTICAL PROCEDURE

STEP 1: Preparation of Samples, Standard and Controls

Dispense in a tube:

<table>
<thead>
<tr>
<th></th>
<th>Calibrator</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent N – Plasma Calibrator</td>
<td>1000 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1000 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>1000 µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent A – Diluting Solution</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
</tbody>
</table>

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 2.0 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 percolated liquid  
  *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** – Stabilization 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 800 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 50 µl of solution into the chromatographic system
**TOPIRAMATE - Warnings**

**REAGENT L : TEST SOLUTION/CHEMICAL STANDARD**

| TOPIRAMATE | 100.000 µg/l |

**REAGENT N: PLASMA CALIBRATOR LYOPHIL. Lot.001**

| LEVETIRACETAM | 8.800 µg/l |

**Use and Reconstitution:** Calibrators are used for calibration of the HPLC system. This lyophilised calibrator has to be prepared like a patient sample. Add exactly 5.0 ml HPLC water to the vial and mix for 15 min. When all material is dissolved, the solution is ready to use.

**Stability:** 36 months if stored at 2-8 °C. After reconstitution the stability of the analytes is at least 1 month at +4°C and at least 6 months at -20° C. Don’t use after expiry date.

**Packaging:** 1 x 5 ml

**Warning:** The calibrator derives from human plasma, so it could be potentially infected. It must be handled with care.

**FLUORIMETRIC DETECTOR PARAMETERS**

| \(\lambda_{\text{Ex}}\) | 260 nm |
| \(\lambda_{\text{Em}}\) | 315 nm |
| GAIN | Low |

**ANALYTICAL COLUMN PROTECTION**

To save the analytical column Hypersil Gold CN 4.6 x 150 mm, 5 µ, the use of Javelin Colfilter – Superchrom is obligatory.

**COLUMN CONDITIONING**

Install a new analytical Hypersil Gold CN Column 4.6 x 150 mm, 5 µ. Disconnect the detector and filter 30 ml of H₂O : 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 H₂O at 2 ml/min and discharge. Flux a solution of H₂O : Acetonitrile ( 20 : 80 v/v ) at 2 ml/min for 30 minute and discharge. The column must be stored in a solution of H₂O : Acetonitrile or Ethanol ( 80 : 20 v/v ). Wash the injection needle with a solution of H₂O : Acetonitrile.

**HPLC PARAMETERS**

| LOOP | 50 or 100 µl |
| FLOW | 2 ml/min |
| PRESSURE | About 100 bar |
# ACCESSORIES AND CONSUMABLES

<table>
<thead>
<tr>
<th>CODE</th>
<th>DESCRIPTION</th>
<th>PACKAGING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z77016</td>
<td>Calibrator in plasma for Topiramate</td>
<td>4 x 5 ml</td>
</tr>
<tr>
<td>Z77017</td>
<td>Control in plasma for Topiramate – Level 1</td>
<td>5 x 5 ml</td>
</tr>
<tr>
<td>Z77018</td>
<td>Control in plasma for Topiramate – Level 2</td>
<td>5 x 5 ml</td>
</tr>
<tr>
<td>Z77019</td>
<td>Control in plasma for Topiramate – Levels 1 and 2</td>
<td>2 x 5 x 5 ml</td>
</tr>
<tr>
<td>TF25805-154630</td>
<td>Hypersil Gold CN Analytical Column (150 x 4.6mm -5 um)</td>
<td>1 PK</td>
</tr>
<tr>
<td>TF88400</td>
<td>Javelin Colfilter - Superchrom</td>
<td>1 x 4 PK</td>
</tr>
<tr>
<td>S29057U</td>
<td>Glass Standard Vials of 2 ml with screw caps</td>
<td>1 x 100 PK</td>
</tr>
</tbody>
</table>
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 µg/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