

PERAMPANEL IN PLASMA BY FLUORIMETRY - FAST - Code Z79210

INTRODUCTION

The Perampanel is an antiepileptic drug that acts selectively on AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolone propionate), which have a central role in the generation and spread of epileptic seizures. As an antagonist of the AMPA receptor, Perampanel acts on seizures by blocking the effects of the neurotransmitter glutamate, the main cause of the crisis.

The Perampanel is indicated for the adjunctive treatment of partial seizures with or without secondary generalization, and for the adjunctive treatment of primary generalized tonic-clonic seizures in patients 12 years of age. Treatment of the epileptic patients requires a multidisciplinary medical expertise, pharmacological, psychological and social. In this context, therefore, drug therapy and the subsequent monitoring play a decisive role in the immediate seizure control and in medium and long-term prevention of relapse.

Currently, thanks to the monitoring of plasma concentrations of antiepileptic drugs, is in many cases possible to model therapy to individual patients. This method allows to determine the serum concentration and / or plasma by HPLC-fluorimetry of the Perampanel.

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This product fulfills all the requirements of Directive 98/79/EC and 332/00 on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 002	Perampanel in plasma by Fluorimetry - FAST	11/2024
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TECHNICAL FEATURES

Principle of the Method :

The Perampanel is isolated by treating the plasma or serum with an appropriate deproteinizing. After the addition of a stabilizer, the solution is directly injected into HPLC.

Recovery : >98% $\pm$ 1,4

Sensitivity : 0,003 ug/ml

Minimum concentration analyzable : 0,01 ug/ml

Linearity : 0,01 – 8 ug/ml

Accuracy intra serie (relative error %):

Ci	Cs
0,05 ug/ml	0,8 ug/ml
5,4%	3,3%

Accuracy inter serie (relative error %):

Ci	Cs
0,05 ug/ml	0,8 ug/ml
7,8%	4,1%

Reproducibility intra serie (coefficient of variation %):

Cl	Cm	Ch
0,01 ug/ml	0,2 ug/ml	1,5 ug/ml
5,8%	5,7%	3,9%

Reproducibility inter serie (coefficient of variation %):

Cl	Cm	Ch
0,01 ug/ml	0,2 ug/ml	1,5 ug/ml
12,6%	6,1%	4,7%

Coefficient of correlation R² + Dev Std 0,9973 $\pm$ 0,0006

Normal Range in plasma : 0,1 – 1 ug/ml

Components of the kit :

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

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

Reagent B – Stabilizing Solution, **1 x 30 ml**

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

Calibrator lyophil. in plasma, **1 x 1 ml**

Code Z79216 (packed separately – see data sheets)

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

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 5 μ l
Fluorimetric Detector λ_{Ex} = 290 nm λ_{Em} = 430 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 :

- 950 µl of H₂O HPLC grade
- 50 µl of **Reagent C - Test Solution**

Vortex for 10 sec.

INJECTION :

- Inject 5 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 : Sample Preparation

Dispense in a 1,5 ml vial with cap:

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

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

STEP 2 : Centrifuge at 14.000 rpm for 10 minutes.

STEP 3 : Pipette in a tube 100 µl of clear surnatant.

- Add at each tube 300 µl of **Reagent B – Stabilization Sol.**

Vortex for 10 sec.

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

INJECTION :

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

PERAMPANEL - Warnings

REAGENT C: TEST SOLUTION

PERAMPANEL	about 0,25 mg/l
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FLUORIMETRIC DETECTOR PARAMETERS

λ_{Ex}	290 nm
λ_{Em}	430 nm
GAIN	0,001 AUFS
INTEGRATION TIME	10 sec.

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 : Acetonitrile (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 : Acetonitrile (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 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	5 μ l
Recommended Flow	1,0 ml/min
Pressure	About 96 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z79216	Calibrator for Perampanel in plasma	4 x 1 ml
Z79219	Control for Perampanel in plasma – Levels 1 and 2	2 x 5 x 1 ml
Z699975902	POROSHELL 120 EC-C18Analytical Column (4,6 x 50 mm, 2.7 μ m)	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 μ l	1 x 100 Pcs
S51820717	Caps for glass vials with reduced volume from 1,5 ml to 15 μ l	1 x 100 Pcs

PERAMPANEL IN PLASMA FAST

(Reference Chromatograms)

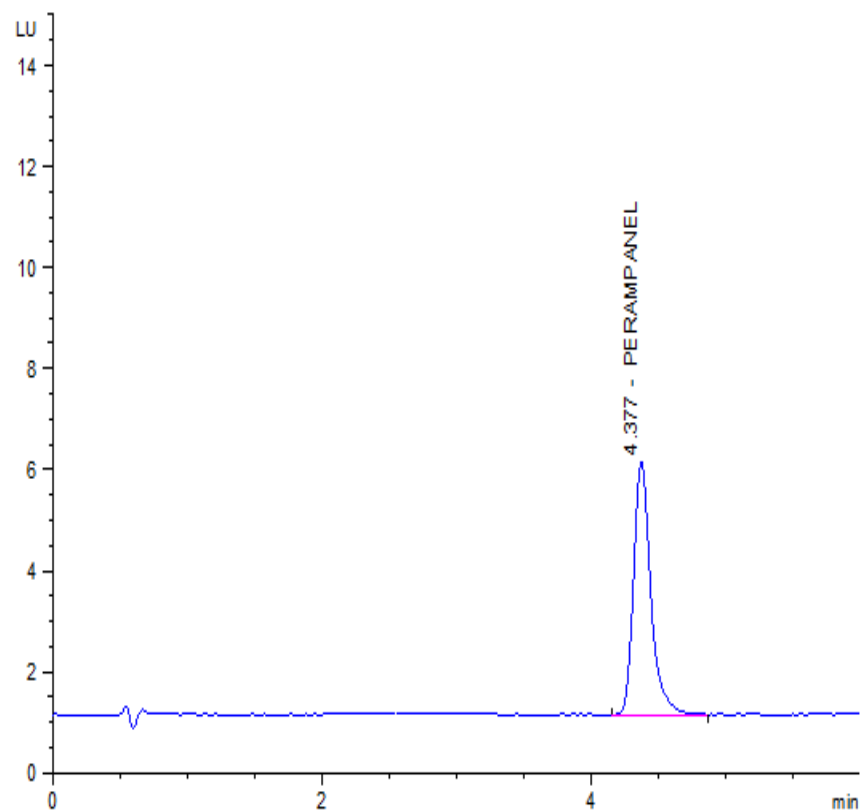


Fig. 1 :	Test Solution
	R.T. 4.37 Perampanel

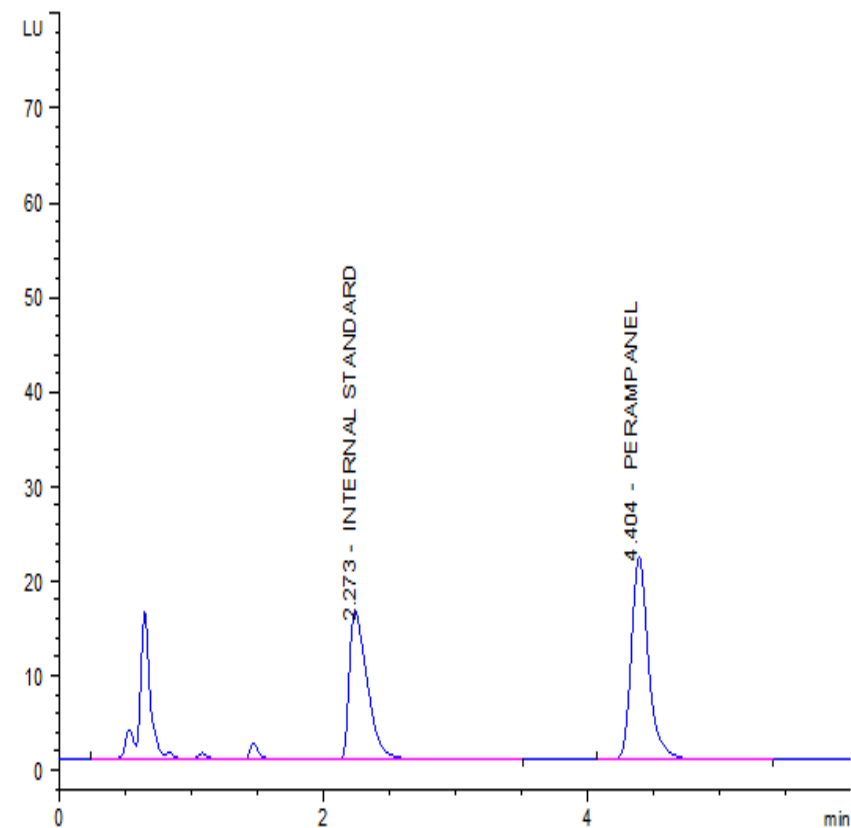


Fig. 2 :	Plasma Calibrator
	R.T. 2.27 Internal Standard
	R.T. 4.40 Perampanel 0,8 ug/ml

PERAMPANEL IN PLASMA FAST

(Reference Chromatograms)

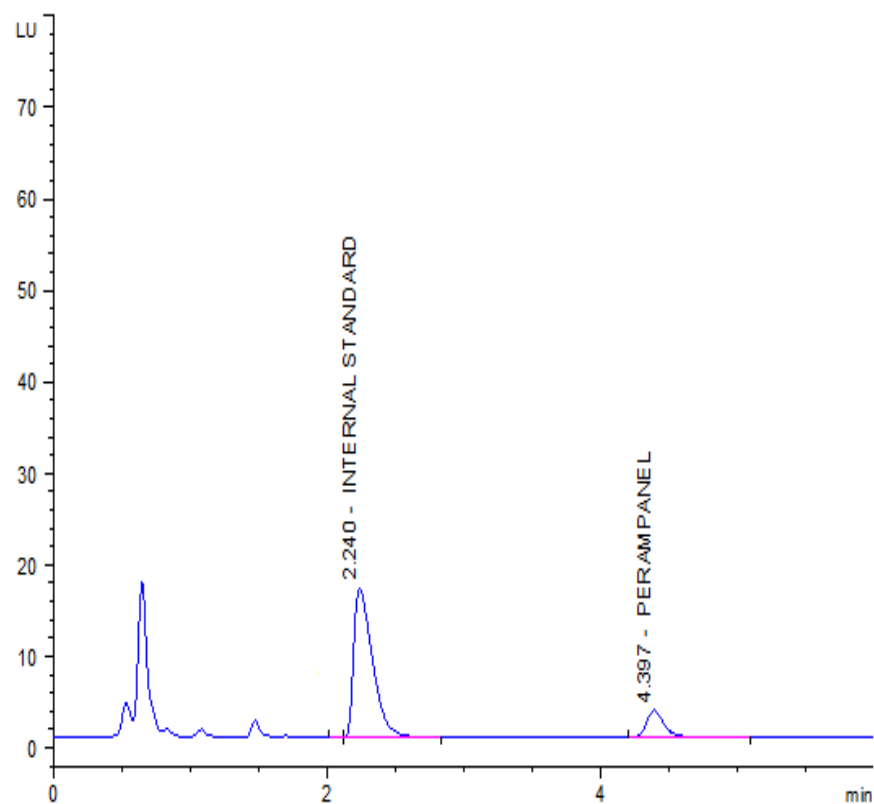


Fig. 3 :	Low Control
	R.T. 2.24 Internal Standard
	R.T. 4.39 Perampanel 0,1 ug/ml

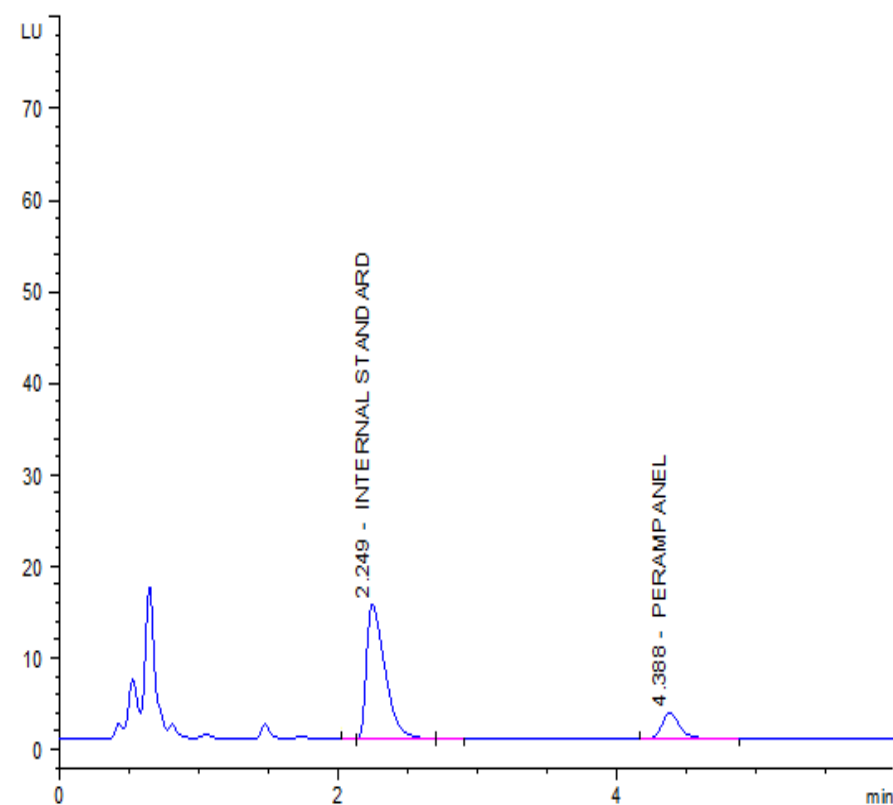


Fig. 4 :	Plasma Sample
	R.T. 2.24 Internal Standard
	R.T. 4.38 Perampanel 0,11 ug/ml