ANTIEPILEPTICS in plasma by LC-MS – Deuterated internal Std – 500 tests - Code LC05115

(Levetiracetam, Lamotrigine, Primidone, Oxcarbazepine, Carbamazepine Epoxide, 10,11-Dihydro-10-Hydroxy-Carbamazepine, Carbamazepine, Phelbamate, Desmethylsuximide, Rufinamide, Phenytoin, Methsuximide, Topiramate, Phenobarbital, Valproic Acid, Ethosuximide, Lacosamide, Zonisamide, Perampanel, Sulthiame, Pregabalin, Brivaracetam, Gabapentin, Stiripentol, Vigabatrin, Tiagabine)

INTRODUCTION

Treatment of epileptic patients requires a multidisciplinary medical knowledge, including pharmacology, psychology and social science. In this context 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 the same HPLC run to determine the sieric and/or plasmatic concentration of the following drugs: *Levetiracetam, Lamotrigine, Primidone, Oxcarbazepine, Carbamazepine Epoxide, 10,11-Dihydro-10-Hydroxy-Carbamazepine, Carbamazepine, Phelbamate, Desmethylsuximide, Rufinamide, Phenytoin, Methsuximide, Topiramate, Phenobarbital, Valproic Acid, Ethosuximide, Lacosamide, Zonisamide, Perampanel, Sulthiame, Pregabalin, Brivaracetam, Gabapentin, Stiripentol, Vigabatrin, Tiagabine.*

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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.

TECHNICAL FEATURES

Principle of the Method :

Antiepileptics are separated by treating the serum/plasma with a suitable precipitant. After addition of a stabilization Reagent the solution is directly injected into HPLC.

Recovery: 83 - 102% (according to analyte)

Sensitivity (LLOD):	**Levetiracetam**	0,05 µg/ml
	Lamotrigine	0,015 µg/ml
	Primidone	0,015 µg/ml
	Oxcarbazepine	0,015 µg/ml
	Carbamazepine Epoxide	0,03 µg/ml
	10,11-Dihydro-10-Hydroxy-Carbamazepine	0,07 µg/ml
	Carbamazepine	0,005 µg/ml
	Phelbamate	0,05 µg/ml
	Rufinamide	0,015 mg/l
	Desmethylsuximide	0,1 mg/l
	Phenytoin	0,03 mg/l
	Methsuximide	0,1 mg/l
	Topiramate	0,03 mg/l
	Phenobarbital	0,3 µg/ml
	Valproic Acid	0,3 mg/l
	Ethosuximide	5,0 µg/ml
	Lacosamide	0,015 mg/l
	Zonisamide	0,05 µg/ml
	Perampanel	0,001 µg/ml
	Brivaracetam	0,004 mg/l
	Sulthiame	0,04 mg/l
	Stiripentol	0,62 mg/l
	Gabapentin	0,01 mg/l
	Tiagabine	0,01 mg/l
	Pregabalin	0,01 mg/l
	Vigabatrin	0,02 mg/l

Minimun Concentration analyzable (LLOQ):	**Levetiracetam**	0,2 µg/ml
	Lamotrigine	0,05 µg/ml
	Primidone	0,05 µg/ml
	Oxcarbazepine	0,05 µg/ml
	Carbamazepine Epoxide	0,1 µg/ml
	10,11-Dihydro-10-Hydroxy-Carbamazepine	0,1 µg/ml
	Carbamazepine	0,02 µg/ml
	Phelbamate	0,2 µg/ml
	Rufinamide	0,05 mg/l
	Desmethylsuximide	0,3 mg/l
	Phenytoin	0,1 mg/l
	Methsuximide	0,5 mg/l
	Topiramate	0,1 mg/l
	Phenobarbital	1 µg/ml
	Valproic Acid	1 mg/l
	Ethosuximide	20 µg/ml
	Lacosamide	0,05 mg/l
	Zonisamide	0,2 µg/ml
	Perampanel	0,003 µg/ml
	Brivaracetam	0,0125 mg/l
	Sulthiame	0,125 mg/l
	Stiripentol	0,1875 mg/l
	Gabapentin	0,03125 mg/l
	Tiagabine	0,00375 mg/l
	Pregabalin	0,03 mg/l
	Vigabatrin	0,0625 mg/l

Therapeutic range :	**Levetiracetam**	10 – 37 µg/ml
	Lamotrigine	3 – 14 µg/ml
	Primidone	5 – 12 mg/l
	Oxcarbazepine	0 – 4 mg/l
	Carbamazepine Epoxide	2 – 4 mg/l
	10,11-Dihydro-10-Hydroxy-Carbamazepine	3 – 40 µg/ml
	Carbamazepine	6 – 12 mg/l
	Phelbamate	10 – 80 mg/l
	Rufinamide	1 – 50 mg/l
	Desmethylsuximide	10 – 40 mg/l
	Phenytoin	10 – 20 mg/l
	Methsuximide	10 – 40 mg/l
	Topiramate	2 – 25 mg/l
	Phenobarbital	10 – 40 mg/l
	Valproic Acid	40 – 100 mg/l
	Ethosuximide	40 – 100 µg/ml
	Lacosamide	1 – 10 mg/l
	Zonisamide	10 – 38 µg/ml
	Perampanel	0,1 – 1 µg/ml
	Brivaracetam	2 – 10 mg/l
	Sulthiame	/
	Stiripentol	/
	Gabapentin	/
	Tiagabine	/
	Pregabalin	/
	Vigabatrin	/

Linearity :	**Levetiracetam**	0,2 – 400 µg/ml
	Lamotrigine	0,05 – 50 µg/ml
	Primidone	0,05 – 100 µg/ml
	Oxcarbazepine	0,05 – 100 µg/ml
	Carbamazepine Epoxide	0,1 – 100 µg/ml
	10,11-Dihydro-10-Hydroxy-Carbamazepine	0,1 – 100 µg/ml
	Carbamazepine	0,02 – 30 µg/ml
	Phelbamate	0,2 – 400 µg/ml
	Rufinamide	0,05 – 100 mg/l
	Desmethylsuximide	0,3 – 150 mg/l
	Phenytoin	0,1 – 100 mg/l
	Methsuximide	0,5 – 100 mg/l
	Topiramate	0,1 – 100 mg/l
	Phenobarbital	1 – 100 µg/ml
	Valproic Acid	1 – 400 mg/l
	Ethosuximide	20 – 150 µg/ml
	Lacosamide	0,05 – 50 mg/l
	Zonisamide	0,2 – 300 µg/ml
	Perampanel	0,003 – 5 µg/ml
	Brivaracetam	0,0125 – 10 mg/l
	Sulthiame	0,125 – 30 mg/l
	Stiripentol	0,1875 – 60 mg/l
	Gabapentin	0,03125 – 50 mg/l
	Tiagabine	0,0375 – 3 mg/l
	Pregabalin	0,03 – 25 mg/l
	Vigabatrin	0,0625 – 90 mg/l

Accuracy intra serie (relative error %) Levetiracetam:

Ci	Cs
5 µg/ml	30 µg/ml
5,7%	4,2%

Accuracy inter serie (relative error %) Levetiracetam:

Ci	Cs
5 µg/ml	30 µg/ml
6,1%	4,9%

Reproducibility intra serie (coefficient of variation %) Levetiracetam:

Cl	Cm	Cs
0,2 µg/ml	0,15 µg/ml	50 µg/ml
2,1%	1,1%	0,8%

Reproducibility inter serie (coefficient of variation %) Levetiracetam:

Cl	Cm	Cs
0,2 µg/ml	0,15 µg/ml	50 µg/ml
3,2%	1,5%	1,0%

Coefficient of correlation R^2 + Dev Std 0,9945 ± 0,0009 Levetiracetam

Accuracy intra serie (relative error %) Lamotrigine:

Ci	Cs
1 µg/ml	10 µg/ml
3,2%	2,9%

Accuracy inter serie (relative error %) Lamotrigine:

Ci	Cs
1 µg/ml	10 µg/ml
4,9%	5,7%

Reproducibility intra serie (coefficient of variation %) Lamotrigine:

Cl	Cm	Cs
0,05 µg/ml	5 µg/ml	10 µg/ml
2,6%	1,2%	0,9%

Reproducibility intra serie (coefficient of variation %) Lamotrigine:

Cl	Cm	Cs
0,05 µg/ml	5 µg/ml	10 µg/ml
3,2%	3,8%	2,1%

Coefficient of correlation R^2 + Dev Std 0,9972 ± 0,0020 Lamotrigine

Accuracy intra serie (relative error %) Primidone:

Ci	Cs
3 µg/ml	10 µg/ml
5,7%	3,8%

Accuracy inter serie (relative error %) Primidone:

Ci	Cs
3 µg/ml	10 µg/ml
6,6%	4,3%

Reproducibility intra serie (coefficient of variation %) Primidone:

Cl	Cm	Cs
0,05 µg/ml	5 µg/ml	20 µg/ml
4,5%	2,2%	2,1%

Reproducibility inter serie (coefficient of variation %) Primidone:

Cl	Cm	Cs
0,05 µg/ml	5 µg/ml	20 µg/ml
5,5%	3,2%	3,1%

Coefficient of correlation R^2 + Dev Std 0,9989 ± 0,0008 Primidone

Accuracy intra serie (relative error %) Oxcarbazepine:

Ci	Cs
1 µg/ml	9 µg/ml
2,9%	2,1%

Accuracy inter serie (relative error %) Oxcarbazepine:

Ci	Cs
1 µg/ml	9 µg/ml
3,9%	2,4%

Reproducibility intra serie (coefficient of variation %) Oxcarbazepine:

Cl	Cm	Cs
0,05 µg/ml	18 µg/ml	100 µg/ml
3,6%	3,3%	3,2%

Reproducibility inter serie (coefficient of variation %) Oxcarbazepine:

Cl	Cm	Cs
0,05 µg/ml	18 µg/ml	100 µg/ml
3,6%	3,3%	3,2%

Coefficient of correlation R^2 + Dev Std 0,9952 ± 0,0012 Oxcarbazepine

Accuracy intra serie (relative error %) Carbamazepine Epoxide:

Ci	Cs
1 µg/ml	9 µg/ml
4,5%	3,9%

Accuracy inter serie (relative error %) Carbamazepine Epoxide:

Ci	Cs
1 µg/ml	9 µg/ml
5,2%	4,8%

Reproducibility intra serie (coefficient of variation %) Carbamazepine Epoxide:

Cl	Cm	Cs
0,1 µg/ml	3 µg/ml	18 µg/ml
3,2%	3,7%	3,8%

Reproducibility inter serie (coefficient of variation %) Carbamazepine Epoxide:

Cl	Cm	Cs
0,1 µg/ml	3 µg/ml	18 µg/ml
5,2%	4,7%	4,2%

Coefficient of correlation R^2 + Dev Std 0,9960 ± 0,0015 Carbamazepine Epoxide

Accuracy intra serie (relative error %) 10,11-Dihydro-11-Hydroxycarbamazepine:

Ci	Cs
5 µg/ml	30 µg/ml
3,2%	1,9%

Accuracy inter serie (relative error %) 10,11-Dihydro-11-Hydroxycarbamazepine:

Ci	Cs
5 µg/ml	30 µg/ml
3,6%	2,2%

Reproducibility intra serie (coefficient of variation %) 10,11-Dihydro-11-Hydroxycarbamazepine:

Cl	Cm	Cs
0,05 µg/ml	15 µg/ml	30 µg/ml
2,7%	3,4%	3,2%

Reproducibility inter serie (coefficient of variation %) 10,11-Dihydro-11-Hydroxycarbamazepine:

Cl	Cm	Cs
0,05 µg/ml	15 µg/ml	30 µg/ml
4,7%	5,7%	4,1%

Coefficient of correlation R^2 + Dev Std 0,9937 ± 0,009 10,11-Dihydro-11-Hydroxycarbamazepine

Accuracy intra serie (relative error %) Carbamazepine:

Ci	Cs
5 µg/ml	15 µg/ml
5,1%	3,8%

Accuracy inter serie (relative error %) Carbamazepine:

Ci	Cs
5 µg/ml	15 µg/ml
5,7%	4,2%

Reproducibility intra serie (coefficient of variation %) Carbamazepine:

Cl	Cm	Cs
0,02 µg/ml	10 µg/ml	20 µg/ml
1,7%	2,0%	1,3%

Reproducibility inter serie (coefficient of variation %) Carbamazepine:

Cl	Cm	Cs
0,02 µg/ml	10 µg/ml	20 µg/ml
2,7%	2,1%	1,5%

Coefficient of correlation R^2 + Dev Std 0,9963 ± 0,0017 Carbamazepine

Accuracy intra serie (relative error %) Phelbamate:

Ci	Cs
10 µg/ml	100 µg/ml
4,1%	3,8%

Accuracy inter serie (relative error %) Phelbamate:

Ci	Cs
10 µg/ml	100 µg/ml
4,7%	4,5%

Reproducibility intra serie (coefficient of variation %) Phelbamate:

Cl	Cm	Cs
0,2 µg/ml	50 µg/ml	100 µg/ml
2,1%	2,0%	1,5%

Reproducibility inter serie (coefficient of variation %) Phelbamate:

Cl	Cm	Cs
0,2 µg/ml	50 µg/ml	100 µg/ml
3,1%	4,9%	2,8%

Coefficient of correlation R^2 + Dev Std 0,9976 ± 0,0016 Phelbamate

Accuracy intra serie (relative error %) Rufinamide:

Ci	Cs
10 mg/l	30 mg/l
4,2%	5,8%

Accuracy inter serie (relative error %) Rufinamide:

Ci	Cs
10 mg/l	30 mg/l
4,9%	5,8%

Reproducibility intra serie (coefficient of variation %) Rufinamide:

Cl	Cm	Cs
0,05 mg/l	20 mg/l	50 mg/l
2,4%	1,7%	1,5%

Reproducibility inter serie (coefficient of variation %) Rufinamide:

Cl	Cm	Cs
0,05 mg/l	20 mg/l	50 mg/l
4,1%	3,9%	3,2%

Coefficient of correlation R^2 + Dev Std 0,9989 ± 0,0008 Rufinamide

Accuracy intra serie (relative error %) Desmethylsuximide:

Ci	Cs
5 mg/l	30 mg/l
3,5%	2,3%

Accuracy inter serie (relative error %) Desmethylsuximide:

Ci	Cs
5 mg/l	30 mg/l
5,3%	3,8%

Reproducibility intra serie (coefficient of variation %) Desmethylsuximide:

Cl	Cm	Cs
0,3 mg/l	15 mg/l	40 mg/l
3,8%	2,6%	1,1%

Reproducibility inter serie (coefficient of variation %) Desmethylsuximide:

Cl	Cm	Cs
0,3 mg/l	15 mg/l	40 mg/l
4,1%	3,5%	1,9%

Coefficient of correlation R^2 + Dev Std 0,9975 ± 0,0012 Desmethylsuximide

Accuracy intra serie (relative error %) Phenytoin:

Ci	Cs
5 mg/l	20 mg/l
5,4%	2,8%

Accuracy inter serie (relative error %) Phenytoin:

Ci	Cs
5 mg/l	20 mg/l
6,1%	4,1%

Reproducibility intra serie (coefficient of variation %) Phenytoin:

Cl	Cm	Cs
0,1 mg/l	10 mg/l	30 mg/l
2,6%	1,4%	1,6%

Reproducibility inter serie (coefficient of variation %) Phenytoin:

Cl	Cm	Cs
0,1 mg/l	10 mg/l	30 mg/l
5,2%	3,9%	3,7%

Coefficient of correlation R^2 + Dev Std 0,9963 ± 0,0008 Phenytoin

Accuracy intra serie (relative error %) Methsuximide:

Ci	Cs
5 mg/l	30 mg/l
6,1%	4,3%

Accuracy inter serie (relative error %) Methsuximide:

Ci	Cs
5 mg/l	30 mg/l
7,4%	5,2%

Reproducibility intra serie (coefficient of variation %) Methsuximide:

Cl	Cm	Cs
0,5 mg/l	15 mg/l	50 mg/l
4,9%	4,8%	4,7%

Reproducibility inter serie (coefficient of variation %) Methsuximide:

Cl	Cm	Cs
0,5 mg/l	15 mg/l	50 mg/l
5,7%	5,2%	5,1%

Coefficient of correlation R^2 + Dev Std 0,9987 + 0,0017 Methsuximide

Accuracy intra serie (relative error %) Topiramate:

Ci	Cs
5 mg/l	25 mg/l
4,7%	3,1%

Accuracy inter serie (relative error %) Topiramate:

Ci	Cs
5 mg/l	25 mg/l
5,8%	4,2%

Reproducibility intra serie (coefficient of variation %) Topiramate:

Cl	Cm	Cs
0,1 mg/l	10 mg/l	50 mg/l
2,2%	1,7%	1,9%

Reproducibility inter serie (coefficient of variation %) Topiramate:

Cl	Cm	Cs
0,1 mg/l	10 mg/l	50 mg/l
5,2%	4,9%	4,2%

Coefficient of correlation R^2 + Dev Std 0,9965 $\pm$ 0,0013 Topiramate

Accuracy intra serie (relative error %) Phenobarbital:

Ci	Cs
10 µg/ml	40 µg/ml
3,3%	1,4%

Accuracy inter serie (relative error %) Phenobarbital:

Ci	Cs
10 µg/ml	40 µg/ml
4,7%	1,8%

Reproducibility intra serie (coefficient of variation %) Phenobarbital:

Cl	Cm	Cs
1 µg/ml	20 µg/ml	60 µg/ml
3,1%	3,6%	3,6%

Reproducibility inter serie (coefficient of variation %) Phenobarbital:

Cl	Cm	Cs
1 µg/ml	20 µg/ml	60 µg/ml
5,7%	5,0%	5,2%

Coefficient of correlation R^2 + Dev Std 0,9982 + 0,0015 Phenobarbital

Accuracy intra serie (relative error %) Valproic Acid:

Ci	Cs
20 mg/l	80 mg/l
2,9%	1,8%

Accuracy inter serie (relative error %) Valproic Acid:

Ci	Cs
20 mg/l	80 mg/l
4,4%	2,1%

Reproducibility intra serie (coefficient of variation %) Valproic Acid:

Cl	Cm	Cs
1 mg/l	40 mg/l	160 mg/l
2,2%	2,4%	1,7%

Reproducibility inter serie (coefficient of variation %) Valproic Acid:

Cl	Cm	Cs
1 mg/l	40 mg/l	160 mg/l
4,9%	4,3%	3,1%

Coefficient of correlation R^2 + Dev Std 0,9987 $\pm$ 0,0014 Valproic Acid

Accuracy intra serie (relative error %) Ethosuximide:

Ci	Cs
20 µg/ml	80 µg/ml
3,7%	2,9%

Accuracy inter serie (relative error %) Ethosuximide:

Ci	Cs
20 µg/ml	80 µg/ml
3,9%	2,9%

Reproducibility intra serie (coefficient of variation %) Ethosuximide:

Cl	Cm	Cs
1 µg/ml	40 µg/ml	100 µg/ml
4,1%	3,0%	2,5%

Reproducibility inter serie (coefficient of variation %) Ethosuximide:

Cl	Cm	Cs
1 µg/ml	40 µg/ml	100 µg/ml
6,1%	5,9%	3,1%

Coefficient of correlation R^2 + Dev Std 0,9988 $\pm$ 0,0018 Ethosuximide

Accuracy intra serie (relative error %) Lacosamide:

Ci	Cs
2 mg/l	10 mg/l
6,3%	3,9%

Accuracy inter serie (relative error %) Lacosamide:

Ci	Cs
2 mg/l	10 mg/l
6,3%	3,9%

Reproducibility intra serie (coefficient of variation %) Lacosamide:

Cl	Cm	Cs
0,05 mg/l	5 mg/l	25 mg/l
4,5%	2,8%	2,7%

Reproducibility inter serie (coefficient of variation %) Lacosamide:

Cl	Cm	Cs
0,05 mg/l	5 mg/l	25 mg/l
5,2%	5,0%	4,9%

Coefficient of correlation R^2 + Dev Std 0,9977 ± 0,0010 Lacosamide

Accuracy intra serie (relative error %) Zonisamide:

Ci	Cs
5 µg/ml	25 µg/ml
3,9%	2,2%

Accuracy inter serie (relative error %) Zonisamide:

Ci	Cs
5 µg/ml	25 µg/ml
5,4%	4,5%

Reproducibility intra serie (coefficient of variation %) Zonisamide:

Cl	Cm	Cs
0,2 µg/ml	15 µg/ml	50 µg/ml
4,0%	3,7%	2,5%

Reproducibility inter serie (coefficient of variation %) Zonisamide:

Cl	Cm	Cs
0,2 µg/ml	15 µg/ml	50 µg/ml
7,5%	6,7%	3,1%

Coefficient of correlation R^2 + Dev Std 0,9979 ± 0,0012 Zonisamide

Accuracy intra serie (relative error %) Perampanel:

Ci	Cs
0,1 µg/ml	1 µg/ml
0,9%	0,6%

Accuracy inter serie (relative error %) Perampanel:

Ci	Cs
0,1 µg/ml	1 µg/ml
2,3%	1,5%

Reproducibility intra serie (coefficient of variation %) Perampanel:

Cl	Cm	Cs
0,003 µg/ml	0,5 µg/ml	2,5 µg/ml
1,1%	1,0%	1,5%

Reproducibility inter serie (coefficient of variation %) Perampanel:

Cl	Cm	Cs
0,003 µg/ml	0,5 µg/ml	2,5 µg/ml
2,0%	1,8%	1,9%

Coefficient of correlation R^2 + Dev Std 0,9990 ± 0,0003 Perampanel

Accuracy intra serie (relative error %) Brivaracetam:

Ci	Cs
1 mg/L	4 mg/L
3,0%	3,3%

Accuracy inter serie (relative error %) Brivaracetam:

Ci	Cs
1 mg/L	4 mg/L
7,4%	6,0%

Reproducibility intra serie (coefficient of variation %) Brivaracetam:

Cl	Cm	Cs
0,25 mg/L	2,5 mg/L	6,25 mg/L
2,2%	3,7%	3,2%

Reproducibility inter serie (coefficient of variation %) Brivaracetam:

Cl	Cm	Cs
0,25 mg/L	2,5 mg/L	6,25 mg/L
8,6%	4,4%	4,6%

Coefficient of correlation R^2 + Dev Std 0,9923 ± 0,0022 Brivaracetam

Accuracy intra serie (relative error %) Sulthiame:

Ci	Cs
2 mg/L	8 mg/L
6,7%	4,1%

Accuracy inter serie (relative error %) Sulthiame:

Ci	Cs
2 mg/L	8 mg/L
7,2%	3,8%

Reproducibility intra serie (coefficient of variation %) Sulthiame:

Cl	Cm	Cs
0,5 mg/L	5 mg/L	12,5 mg/L
2,1%	6,6%	2,4%

Reproducibility inter serie (coefficient of variation %) Sulthiame:

Cl	Cm	Cs
0,5 mg/L	5 mg/L	12,5 mg/L
13,1%	7,4%	3,1%

Coefficient of correlation R^2 + Dev Std 0,9951 ± 0,003 Sulthiame

Accuracy intra serie (relative error %) Gabapentin:

Ci	Cs
2,5 mg/L	10 mg/L
2,8%	6,3%

Accuracy inter serie (relative error %) Gabapentin:

Ci	Cs
2,5 mg/L	10 mg/L
3,4%	5,7%

Reproducibility intra serie (coefficient of variation %) Gabapentin:

Cl	Cm	Cs
0,625 mg/L	6,25 mg/L	15,6 mg/L
6,1%	6,2%	6,4%

Reproducibility inter serie (coefficient of variation %) Gabapentin:

Cl	Cm	Cs
0,625 mg/L	6,25 mg/L	15,6 mg/L
13,6%	5,4%	5,7%

Coefficient of correlation R^2 + Dev Std 0,9950 ± 0,001 Gabapentin

Accuracy intra serie (relative error %) Pregabalin:

Ci	Cs
1,2 mg/L	4,8 mg/L
2,3%	3,6%

Accuracy inter serie (relative error %) Pregabalin:

Ci	Cs
1,2 mg/L	4,8 mg/L
2,8%	4,2%

Reproducibility intra serie (coefficient of variation %) Pregabalin:

Cl	Cm	Cs
0,3 mg/L	3 mg/L	7,5 mg/L
4,2%	3,3%	3,5%

Reproducibility inter serie (coefficient of variation %) Pregabalin:

Cl	Cm	Cs
0,3 mg/L	3 mg/L	7,5 mg/L
9,5%	3,7%	3,0%

Coefficient of correlation R^2 + Dev Std 0,9961 ± 0,0006 Pregabalin

Accuracy intra serie (relative error %) Stiripentol:

Ci	Cs
3 mg/L	12 mg/L
2,8%	1,6%

Accuracy inter serie (relative error %) Stiripentol:

Ci	Cs
3 mg/L	12 mg/L
5,2%	9,3%

Reproducibility intra serie (coefficient of variation %) Stiripentol:

Cl	Cm	Cs
0,75 mg/L	7,5 mg/L	18,7 mg/L
7,1%	3,4%	3,8%

Reproducibility inter serie (coefficient of variation %) Stiripentol:

Cl	Cm	Cs
0,75 mg/L	7,5 mg/L	18,7 mg/L
13,0%	4,8%	7,7%

Coefficient of correlation R^2 + Dev Std 0,9983 ± 0,001 Stiripentol

Accuracy intra serie (relative error %) Tiagabine:

Ci	Cs
0,3 mg/L	1,2 mg/L
2,3%	1,9%

Accuracy inter serie (relative error %) Tiagabine:

Ci	Cs
0,3 mg/L	1,2 mg/L
2,6%	4,8%

Reproducibility intra serie (coefficient of variation %) Tiagabine:

Cl	Cm	Cs
0,075 mg/L	0,75 mg/L	1,875 mg/L
5,4%	2,7%	4,0%

Reproducibility inter serie (coefficient of variation %) Tiagabine:

Cl	Cm	Cs
0,075 mg/L	0,75 mg/L	1,875 mg/L
4,8%	4,4%	5,9%

Coefficient of correlation R^2 + Dev Std 0,9964 ± 0,0016 Tiagabine

Accuracy intra serie (relative error %) Vigabatrin:

Ci	Cs
5 mg/L	20 mg/L
5,0%	6,3%

Accuracy inter serie (relative error %) Vigabatrin:

Ci	Cs
5 mg/L	20 mg/L
6,8%	7,1%

Reproducibility intra serie (coefficient of variation %) Vigabatrin:

Cl	Cm	Cs
1,25 mg/L	12,5 mg/L	31,25 mg/L
2,2%	1,4%	3,1%

Reproducibility inter serie (coefficient of variation %) Vigabatrin:

Cl	Cm	Cs
1,25 mg/L	12,5 mg/L	31,25 mg/L
4,9%	5,7%	6,4%

Coefficient of correlation R^2 + Dev Std 0,9960 ± 0,0027 Vigabatrin

Components of the kit (500 tests):

All reagents are ready to use and stable 3 years at 2 - 8 °C, except **Reagent A.**

Reagent A – Deproteinization Solution with Internal Standards, **1 x 250 ml** **Store at -20 °C**

Reagent B – Stabilization Solution, **1 x 250 ml**

Reagent C – Buffer Solution, **1 x 15 ml**

Plasma Calibrator – Level 0, 3 x 1 ml	**Code LC04916 (packed separately – see data sheet)**
Plasma Calibrator – Level 1, 3 x 1 ml	**Code LC04916 (packed separately – see data sheet**
Plasma Calibrator – Level 2, 3 x 1 ml	**Code LC04916 (packed separately – see data sheet**
Plasma Calibrator – Level 3, 3 x 1 ml	**Code LC04916 (packed separately – see data sheet**
Plasma Calibrator – Level 4, 3 x 1 ml	**Code LC04916 (packed separately – see data sheet**
Plasma Calibrator – Level 5, 3 x 1 ml	**Code LC04916 (packed separately – see data sheet**
Plasma Calibrator – Level 6, 3 x 1 ml	**Code LC04916 (packed separately – see data sheet**

Reagent M1 – Mobile Phase A, **4 x 500 ml**

Reagent M2 – Mobile Phase B, **4 x 500 ml**

Minimum Instrumental equipment required:

LC/MS System with triple quadrupole
MRM Work Mode, positive/ negative ESI
Operational Computer

Optional Equipment:

Autosampler.

Blood Collection Procedure:

Take 3 ml of whole blood in a test tube without gel or with EDTA as anticoagulant. Centrifuge at 4000 rpm for 5 minutes. Separate the serum and store at – 20 °C. Stable 4 weeks.

ANALYTICAL PROCEDURE

STEP 1 : Sample Preparation

Dispense in an eppendorf:

	Calibrator	Sample	Control
Calibrator	50 µl		
Sample		50 µl	
Control			50 µl
Reagent C – Buffer Solution	25 µl	25 µl	25 µl

Waiting 3 minutes

Dispense in an eppendorf:

	Calibrator	Sample	Control
Calibrator buffered as in STEP 1	75 µl		
Sample buffered as in STEP 1		75 µl	
Control buffered as in STEP 1			75 µl
Reagent A – Deproteinization Sol. with Internal Standards	500 µl	500 µl	500 µl

Warning: deproteinize directly on Vortex for at least 20 sec.

STEP 2 : Incubate at 2-8 °C for 15 minutes.

STEP 3 : Centrifuge at 13.000 rpm for 15 minutes

STEP 4 : Put 50 µl of surnatant in a glass vial of 2 ml.

- Add 450 µl of **Reagent B** – Stabilization Solution (or an appropriate volume for the instrument sensitivity)

Vortex for 10 seconds

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

INJECTION :

- Inject 1-10 µl of the eluate into the chromatographic system.

ANTIEPILEPTICS - Warnings

COLUMN CONDITIONING

Install a new analytical column Poroshell EC-C18 50 x 2,1 mm – 1,9 um, thermostated at 50°c. Disconnect the detector and flux a solution of Mobile Phase M1 : Mobile Phase M2 (20 : 80 v/v) set flow at 500 ul / minute. Condition further on the column with a solution of Mobile Phase M1 : Mobile Phase M2 (90 : 10 v/v) set flow at 500 ul / minute. **Don't recycle the washing solutions**. Condition further on the column with a solution of Mobile Phase M1 set flow at 500 ul / minute for 10 minutes.

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

Wash with a solution containing Mobile Phase M1: M2 (30: 70 v / v) at a flow of 500 ul / minute for 10 minutes. The column must be stored in a solution containing Mobile Phase M1: M2 (30: 70 v / v).

WASH AUTOSAMPLER SYRINGE

Wash the syringe, before every analytical session, with a solution of Methanol at 75%.

GRADIENT			
Time (min)	**% M1 (PUMP A)**	**% M2 (PUMP B)**	**Flow (µl/min)**
0	100	0	500
0.53	100	0	500
0.54	87	13	500
1.80	87	13	500
1.81	79	21	500
2.80	79	21	500
3.30	50	50	500
3.80	50	50	500
3.90	20	80	500
4.20	20	80	500
4.30	5	95	500
4.80	5	95	500
4.90	100	0	500
6.00	100	0	500

ESI+ PARAMETERS (optimized on triple quadrupole Sciex 4500)

Curtain Gas (CUR): 30 Collision Gas (CAD): 10 Ion Spry Voltage: (IS): 5000 V Temperature (TEM): 650°C Ion Source Gas 1 (GS1): 60 Ion Source Gas 2 (GS2): 50

ESI- PARAMETERS (optimized on triple quadrupole Sciex 4500)

Curtain Gas (CUR): 30 Collision Gas (CAD): 10 Ion Spry Voltage: (IS): -4500 V Temperature (TEM): 650°C Ion Source Gas 1 (GS1): 60 Ion Source Gas 2 (GS2): 50

DEPROTEINIZATION SOLUTION WITH INTERNAL STANDARD:

Solution with: **Levetiracetam-*d6*, Zonisamide-*d4*, Lacosamide-*d3*, Phelbamate-*d4*, Ethosuximide-*d3*, Desmethylsuximide-*d5*. *Store at – 20 °C.***		
Ionization	**Analyte**	**Internal Standard used for the Calibration**
Positive	Levetiracetam	*Levetiracetam-d6*
	Lamotrigine	*Lamotrigine13C3-d3*
	Primidone	*Primidone-d5*
	Oxcarbazepine	*Oxcarbazepine-d4*
	Carbamazepine Epoxide	*Oxcarbazepine-d4*
	10,11-Dihydro-10-Hydroxycarbamazepine	*10,11-Dihydro-10-Hydroxycarbamazepine-d3*
	Carbamazepine	*Carbamazepine-d10*
	Phelbamate	*Phelbamate-d4*
	Rufinamide	*Zonisamide-d4*
	Phenytoin	*Carbamazepine-d10*
	Methsuximide	*Methsuximide-d5*
	Topiramate	*Topiramate-d12*
	Pregabalin	*Pregabalin-d4*
	Perampanel	*Tiagabine-d6*
	Gabapentin	*Pregabalin-d4*
	Stiripentol	*Stiripentol-d9*
	Vigabatrin	*Pregabalin-d4*
	Brivaracetam	*Levetiracetam-d6*
	Sulthiame	*Zonisamide-d4*
	Tiagabine	*Tiagabine-d6*
	Lacosamide	*Lacosamide-d3*
Negative	Phenobarbital	*Phenobarbital-d5*
	Valproic Acid	*Valproic Acid-d6*
	Desmethylsuximide	*Desmethylsuximide-d5*
	Ethosuximide	*Ethosuximide-d3*
	Zonisamide	*Zonisamide-d4*

FRAGMENTATIONS (optimized on TRIPLE QUADRUPOLE Sciex 4500)		
ANALYTE	**Q1**	**Q3**
Levetiracetam	171.1	126.1
Lamotrigine	256.1	211.1 – 159.1
Primidone	219.2	162.2 – 91.2
Oxcarbazepine	253.2	236.2 – 180.2
Carbamazepine Epoxyde	253.0	210.0 – 180.0
10,11-Dihydro-10-Hydroxycarbamazepine	255.2	237.2 – 194.1
Carbamazepine	237.2	194.2
Phelbamate	239.3	178.2
Rufinamide	239.3	127.2
Desmethylsuximide	188.0	143.0
Phenytoin	253.1	104.3 – 182.3
Methsuximide	204.1	126.2 – 79.2
Topiramate	357.1	264.1
Lacosamide	251.2	91.2 – 108.2
Phenobarbital	231.0	188.1
Valproic Acid	143.0	143.0
Ethosuximide	140.1	140.1
Zonisamide	211.0	119.0 – 147.0
Pregabalin	160.1	142.1
Gabapentin	172.1	154.1
Vigabatrin	130.1	113.1
Stiripentol	217.0	187.0
Brivaracetam	213.2	168.2
Tiagabine	376.1	247.0
Sulthiame	291.1	274.1
Perampanel	350.1	219.0
Levetiracetam-d6	177.1	132.1
Lamotrigine 13C3-d3	262.1	217.1
Primidone-d5	224.2	167.2
Oxcarbazepine-d4	257.2	240.2
10,11-Dihydro-10-Hydroxycarbamazepine-d3	258.2	240.2
Carbamazepine-d10	247.2	204.2
Phelbamate-d4	243.2	182.2
Desmethylsuximide-d5	193.0	148.0
Methsuximide-d5	209.2	124.0
Topiramate-d12	369.1	270.2
Lacosamide-d4	254.0	108.0
Phenobarbital-d5	236.0	192.9
Valproic Acid-d6	149.0	149.0
Ethosuximide-d3	143.1	143.1
Zonisamide-d4	215.0	123.0
Pregabalin-d4	164.1	146.1
Stiripentol-d9	226.0	196.0
Tiagabine-d6	382.1	253.0

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC04916	Calibrator for Antiepileptics in plasma	7 x 2 x 1 ml
LC04919	Control for Antiepileptics in plasma - Levels 1, 2 and 3	3 x 5 x 1 ml
SK05110	Starter kit for Antiepileptics	44 x 1 ml
LC05111	Mobile Phase M1	4 x 500 ml
LC05121	Mobile Phase M1	4 x 500 ml
LC05130	Deuterated Internal Standard Mix Solution in plasma	1 x 55 ml
Z699675902	Poroshell 120 EC C18 (50 x 2,1 mm –1,9 um) Analytical Column	1 Pc
S51843550	Clear Glass vials with reduced volume globet form from 15 µl to 1,5 ml	1 x 100 Pcs
S51820717	Caps for clear Glass vials with reduced volume globet form from 15 µl to 1,5 ml	1 x 100 Pcs
L-30-210	Eppendorf Tubes by 1,5 ml with caps	1 x 1000 Pcs

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

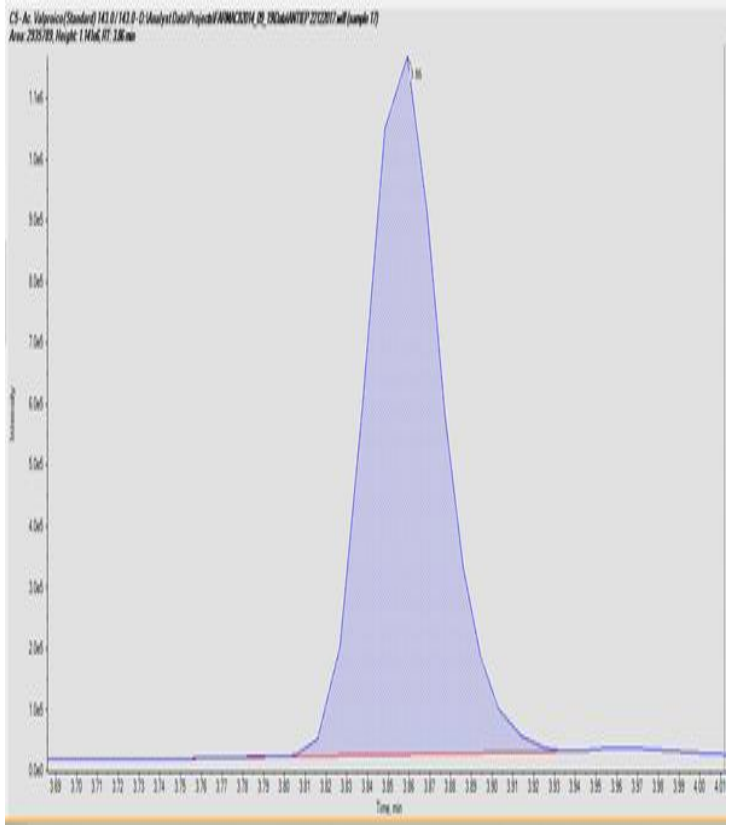


Fig. 1 :	***Plasma Calibrator***
	R.T. 3.86 Valproic Acid

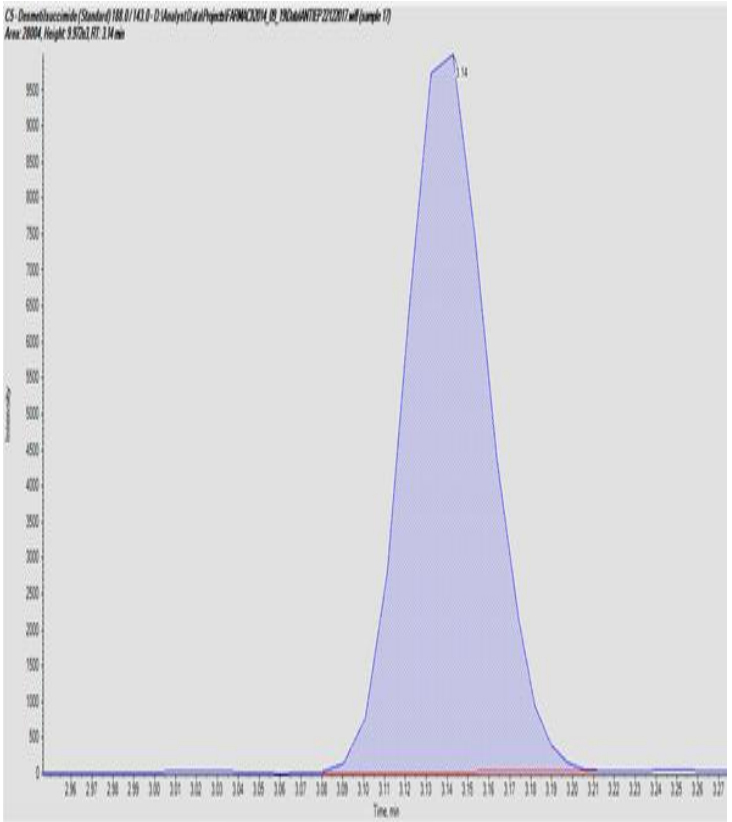
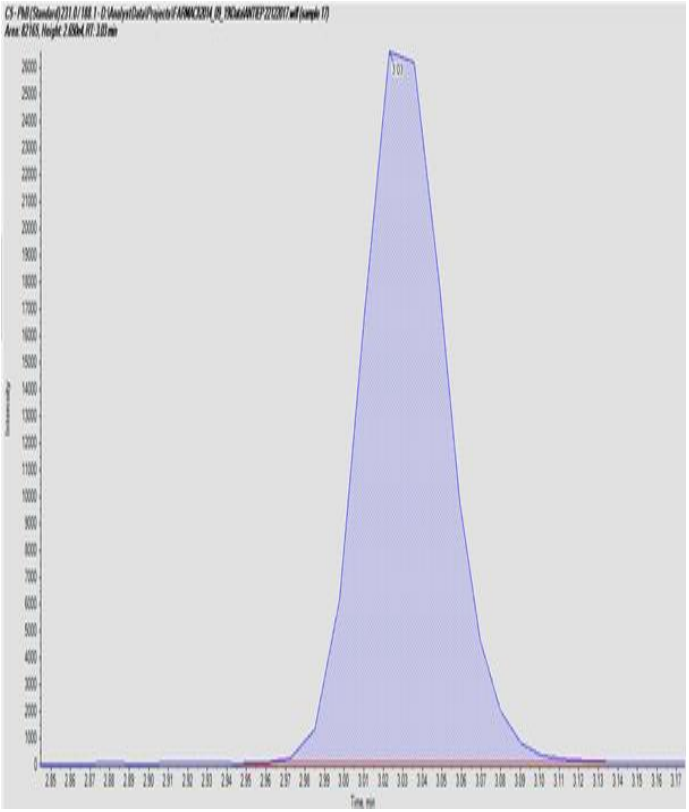


Fig. 2 :	***Plasma Calibrator***
	R.T. 3.14 Desmethylsuximide

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)



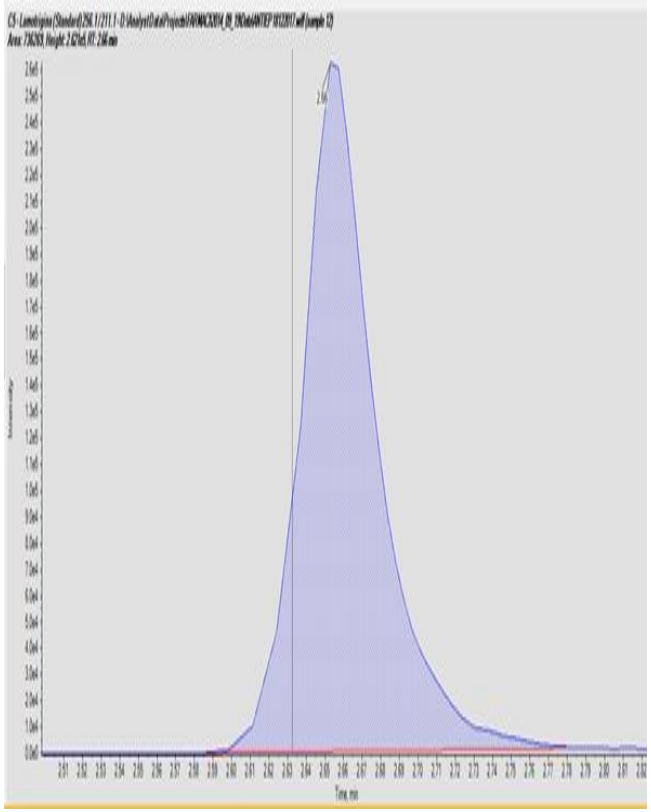


Fig. 3 :	***Plasma Calibrator***
	R.T. 3.03 Phenobarbital

Fig. 4 :	***Plasma Calibrator***
	R.T. 2.66 Lamotrigine

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

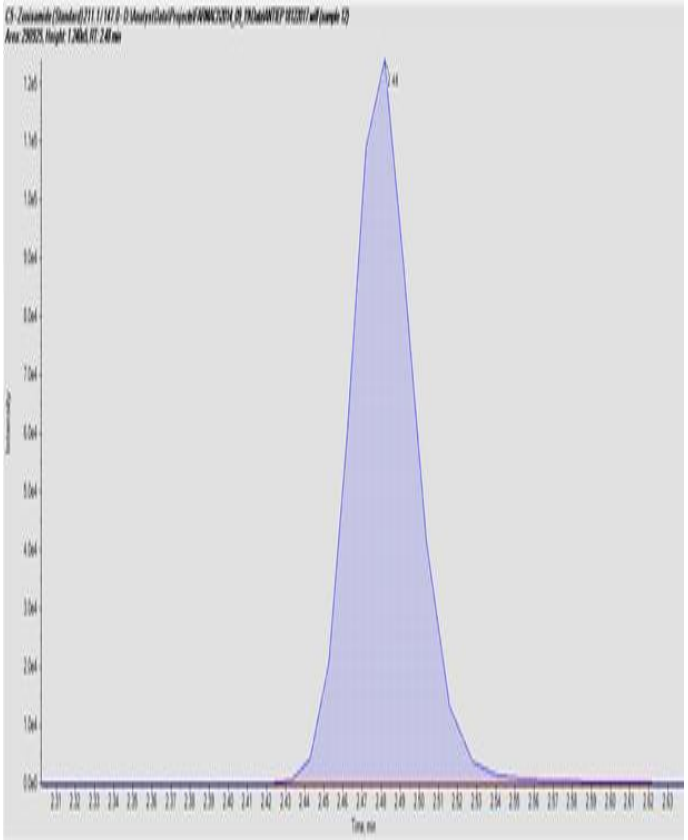


Fig. 5 :	***Plasma Calibrator***
	R.T. 2.48 Zonisamide

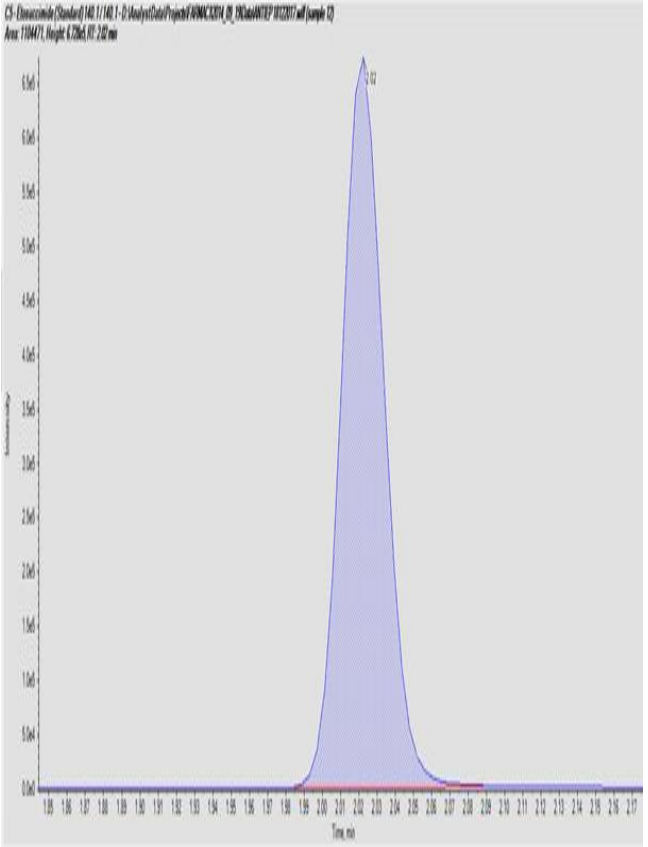


Fig. 6 :	***Plasma Calibrator***
	R.T. 2.02 Ethosuximide

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

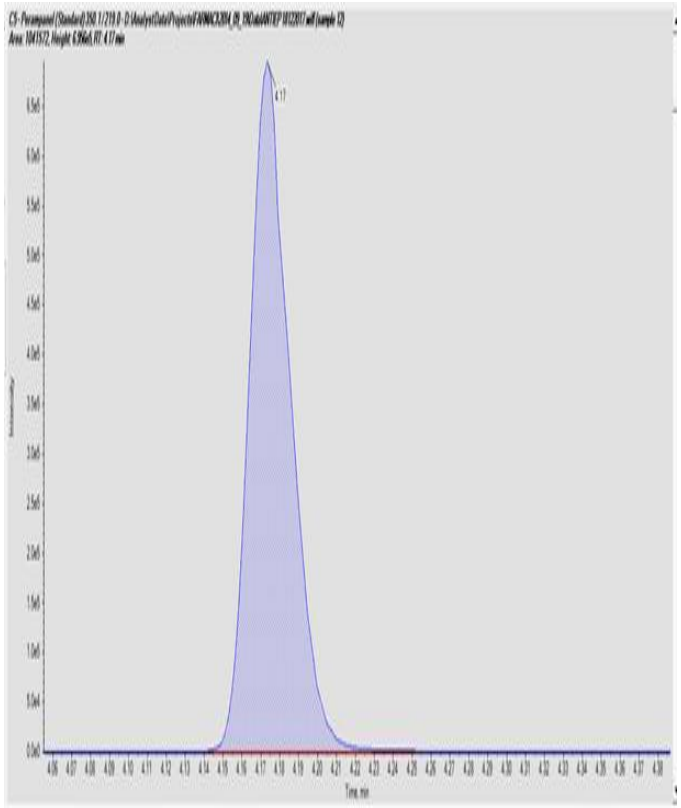


Fig. 7 :	***Plasma Calibrator***
	R.T. 4.17 Perampanel

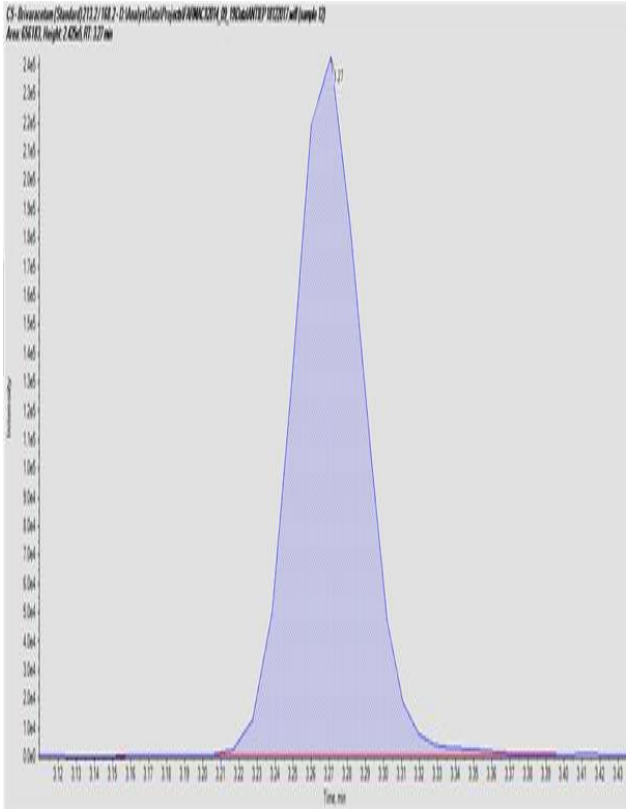


Fig. 8 :	***Plasma Calibrator***
	R.T. 3.27 Brivaracetam

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

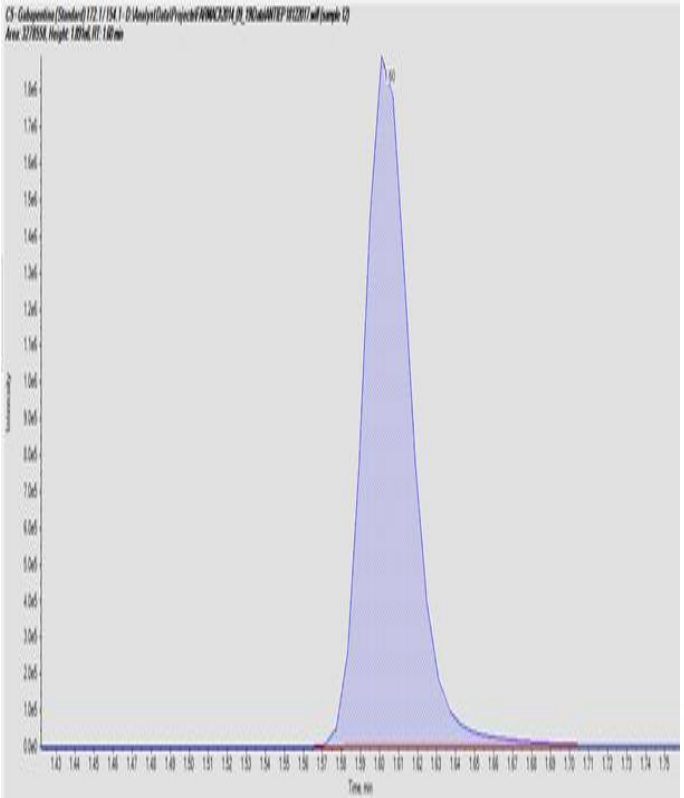


Fig. 9 :	***Plasma Calibrator***
	R.T. 1.60 Gabapentin

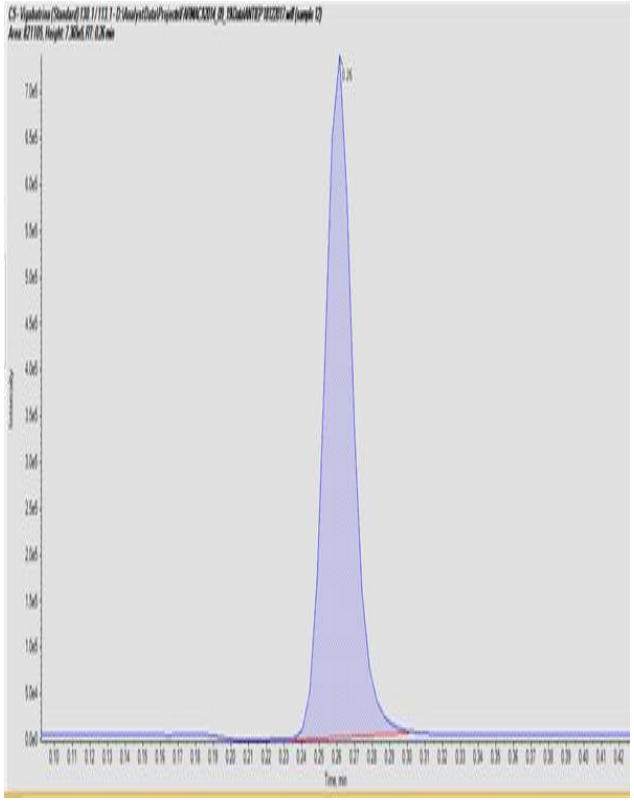


Fig. 10 :	***Plasma Calibrator***
	R.T. 0.26 Vigabatrin

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

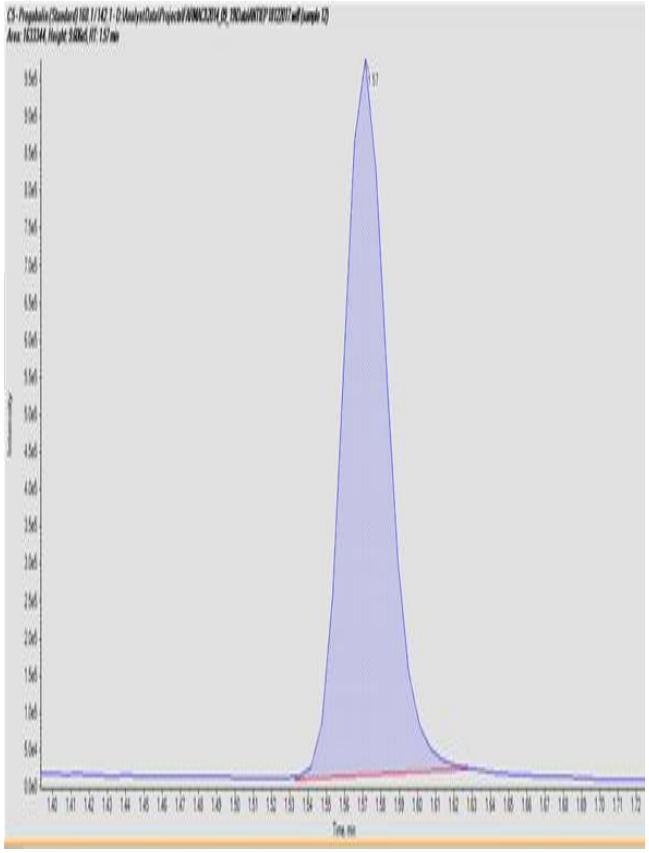


Fig. 11 :	***Plasma Calibrator***
	R.T. 1.57 Pregabalin

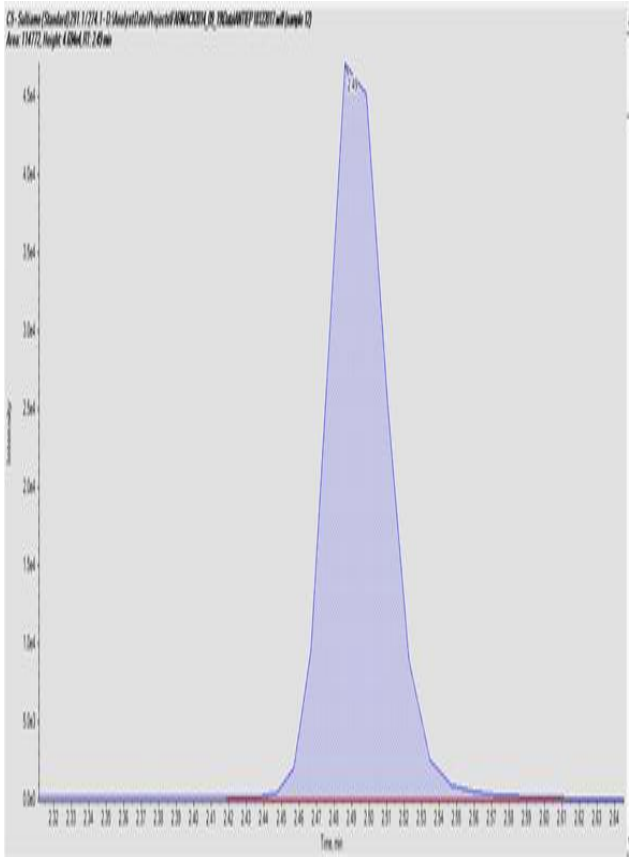


Fig. 12 :	***Plasma Calibrator***
	R.T. 2.49 Sulthiame

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

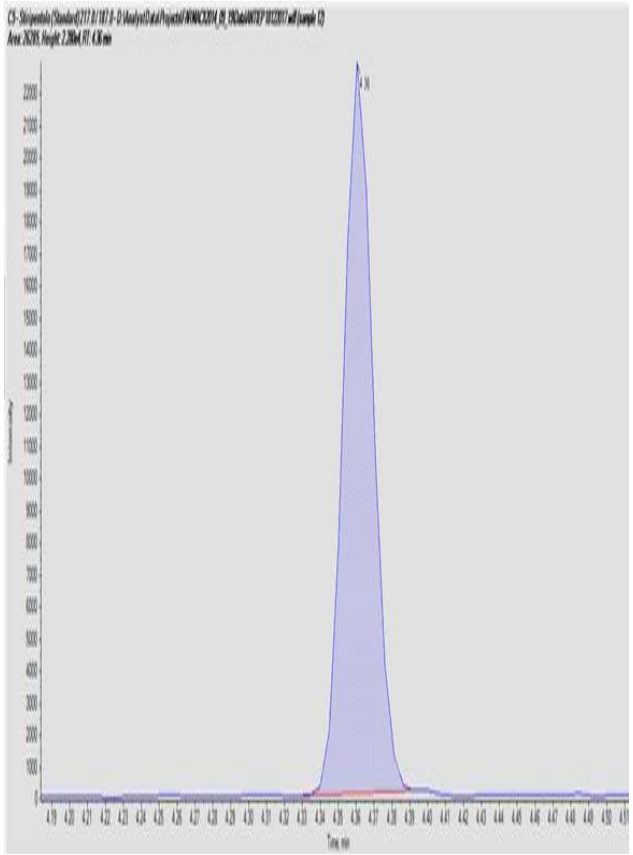


Fig. 13 :	***Plasma Calibrator***
	R.T. 4.36 Stiripentol

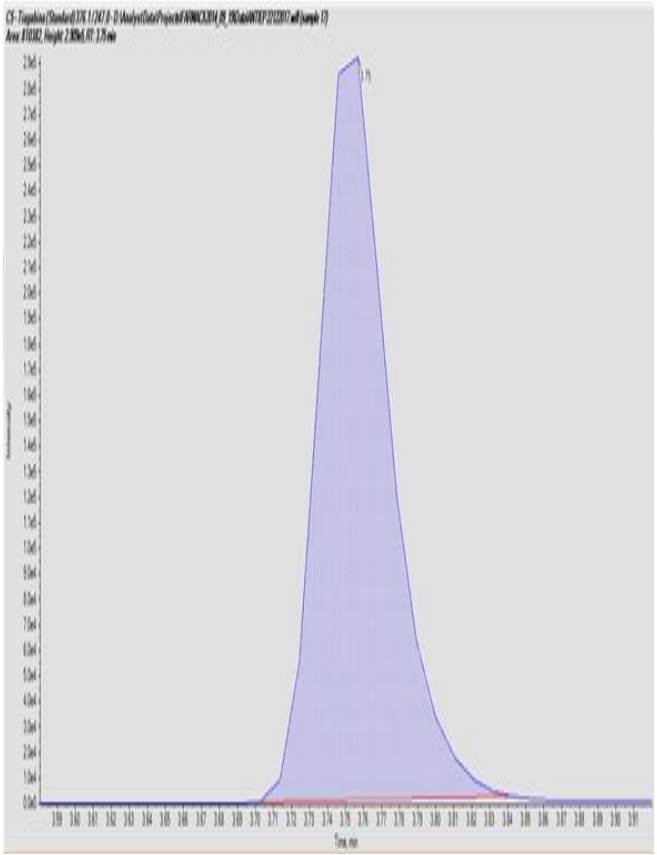


Fig. 14 :	***Plasma Calibrator***
	R.T. 3.75 Tiagabine

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

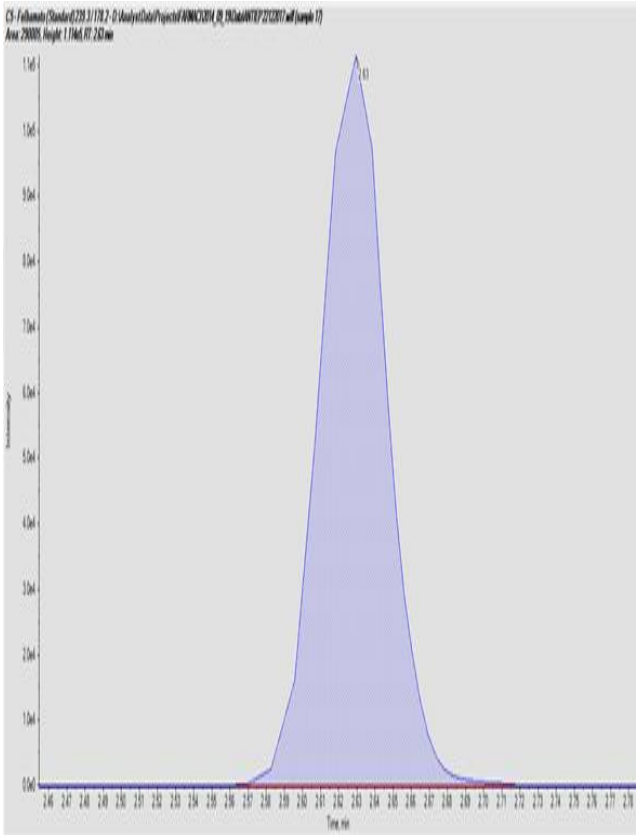


Fig. 15 :	***Plasma Calibrator***
	R.T. 2.63 Phelbamate

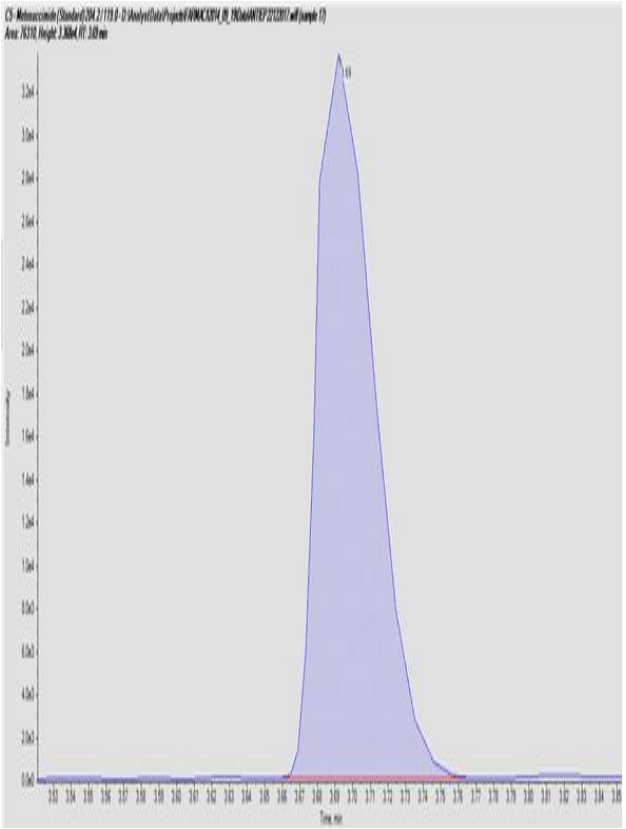


Fig. 16 :	***Plasma Calibrator***
	R.T. 3.69 Methsuximide

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

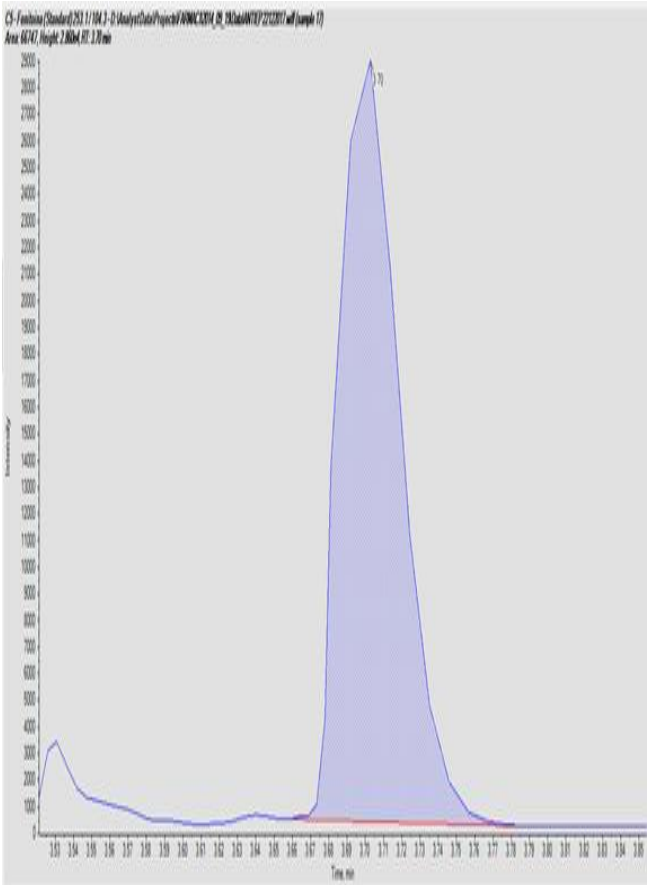


Fig. 17 :	***Plasma Calibrator***
	R.T. 3.70 Phenytoin

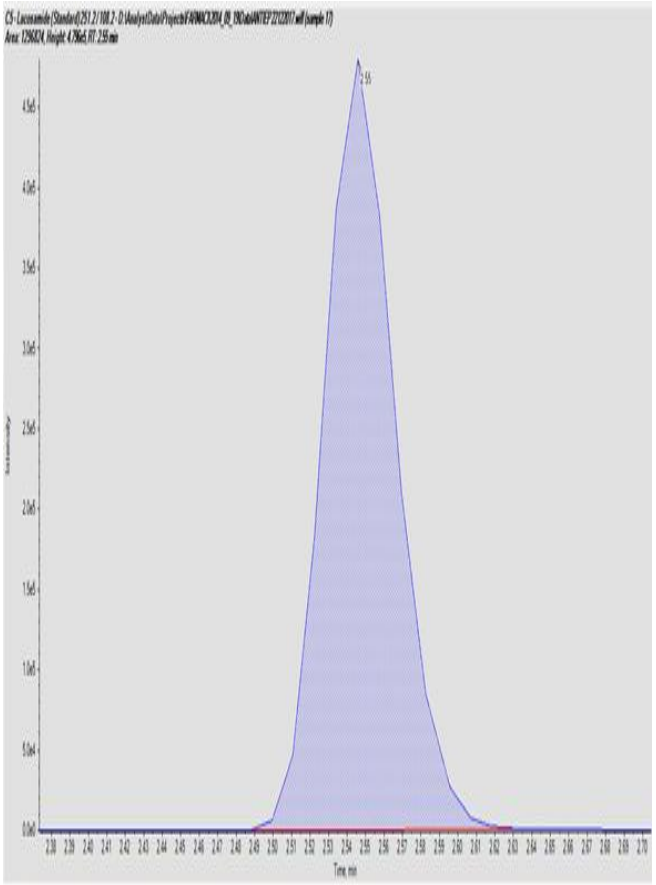
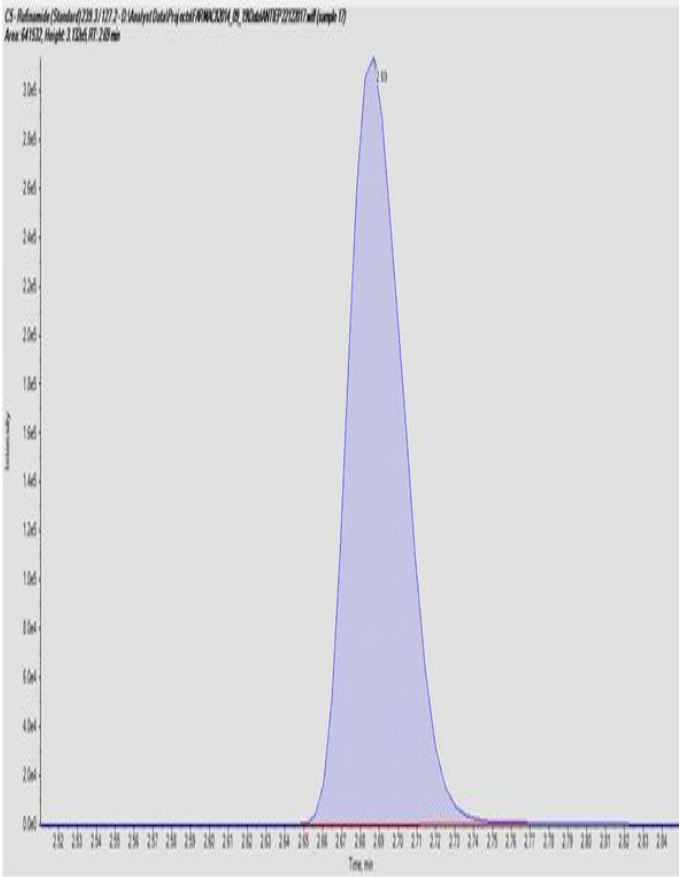


Fig. 18 :	***Plasma Calibrator***
	R.T. 2.55 Lacosamide

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)



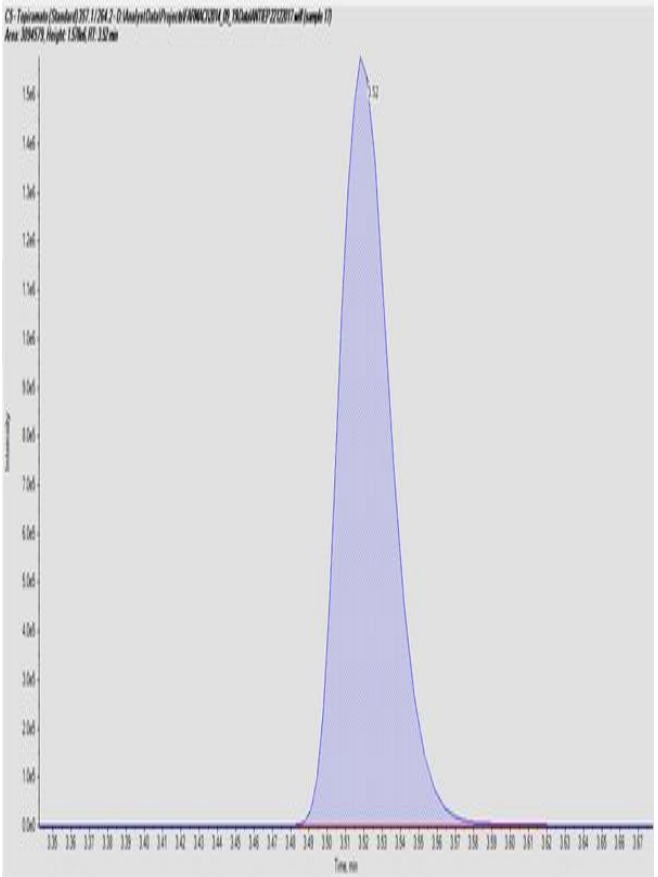


Fig. 19 :	***Plasma Calibrator***
	R.T. 2.69 Rufinamide

Fig. 20 :	***Plasma Calibrator***
	R.T. 3.52 Topiramate

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

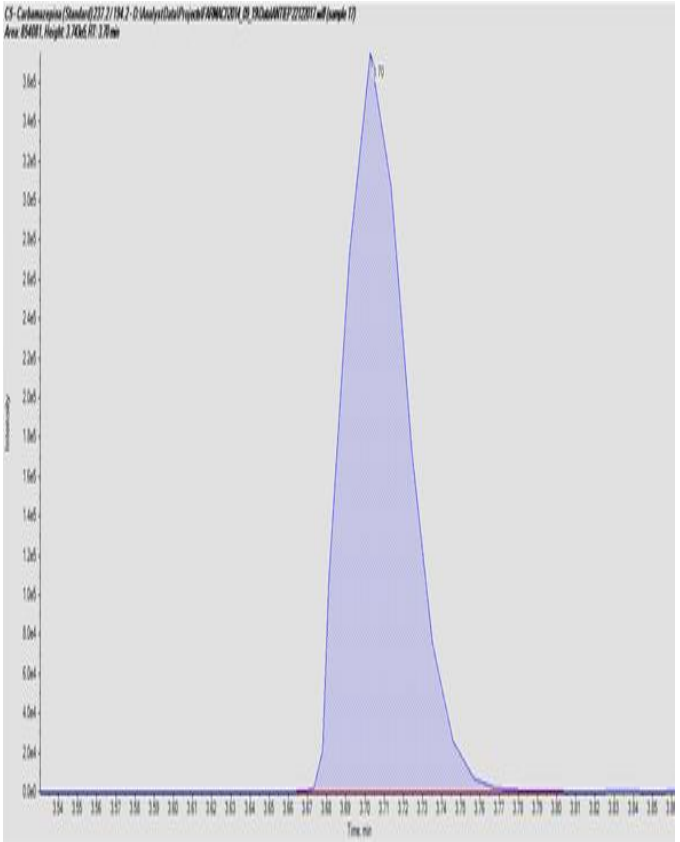


Fig. 21 :	***Plasma Calibrator***
	R.T. 3.70 Carbamazepine

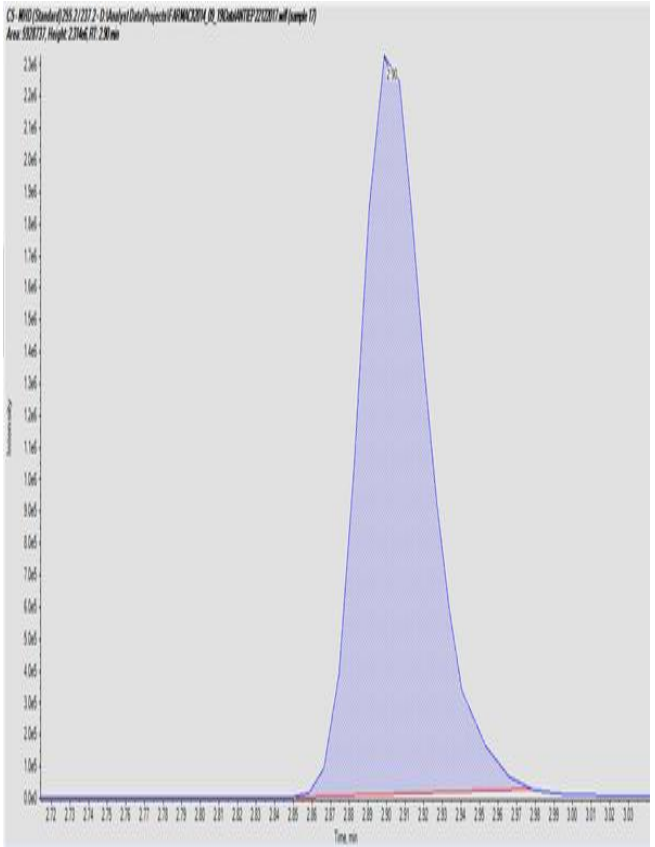


Fig. 22 :	***Plasma Calibrator***
	R.T. 2.90 10,11-Dihydro-11-Hydroxycarbamazepine

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

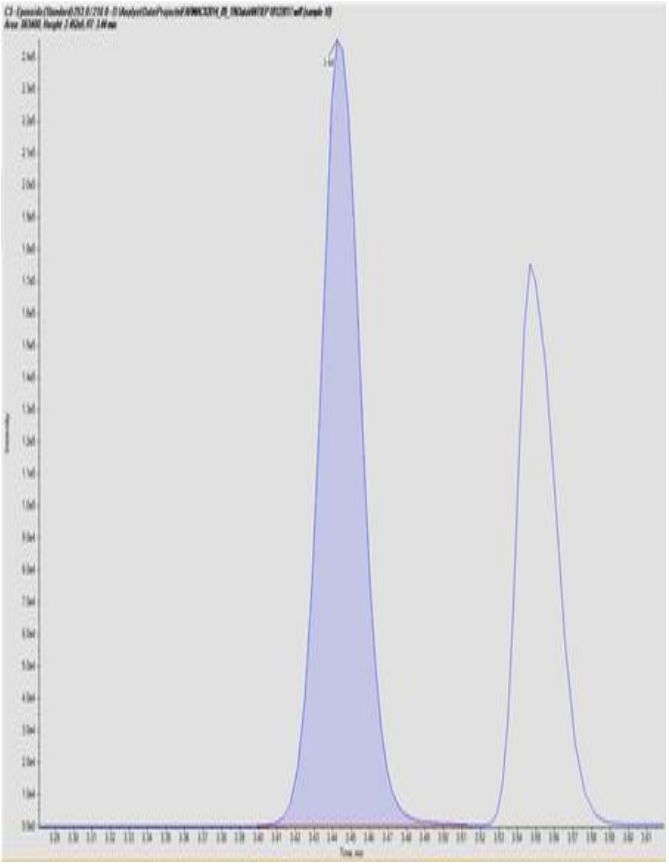


Fig. 23 :	***Plasma Calibrator***
	R.T. 3.45 Carbamazepine Epoxide

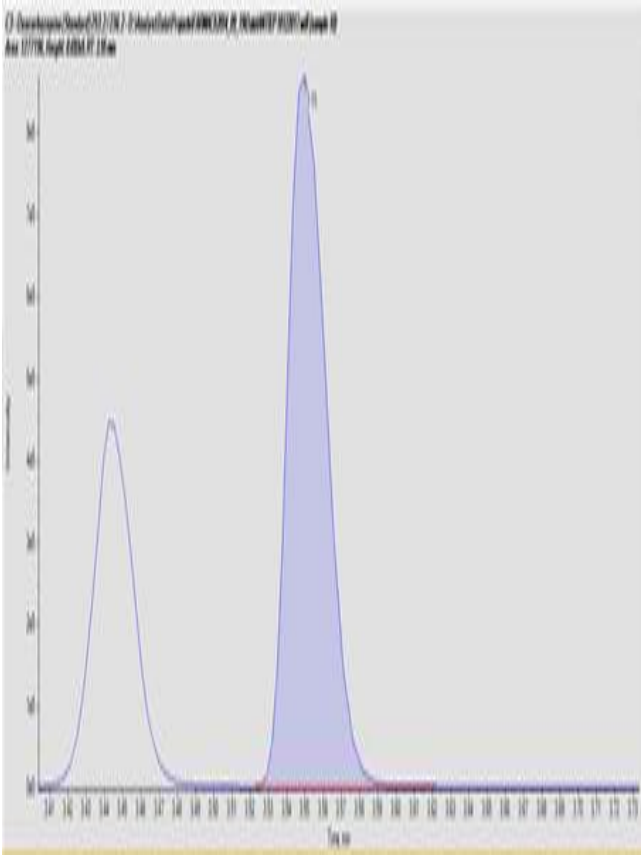


Fig. 24 :	***Plasma Calibrator***
	R.T. 3.55 Oxacarbazepine

ANTIEPILEPTICS IN PLASMA

(Reference Chromatograms)

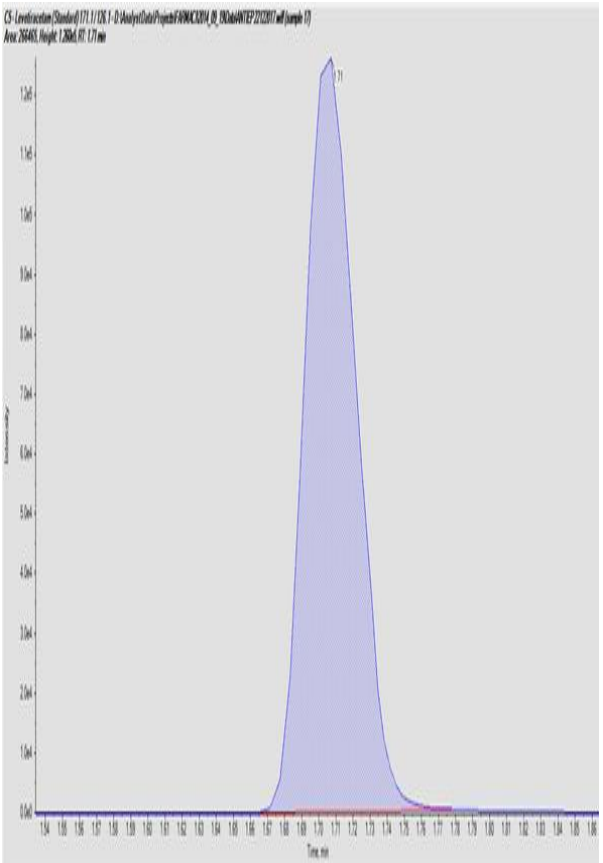


Fig. 25 :	***Plasma Calibrator***
	R.T. 1.70 Levetiracetam

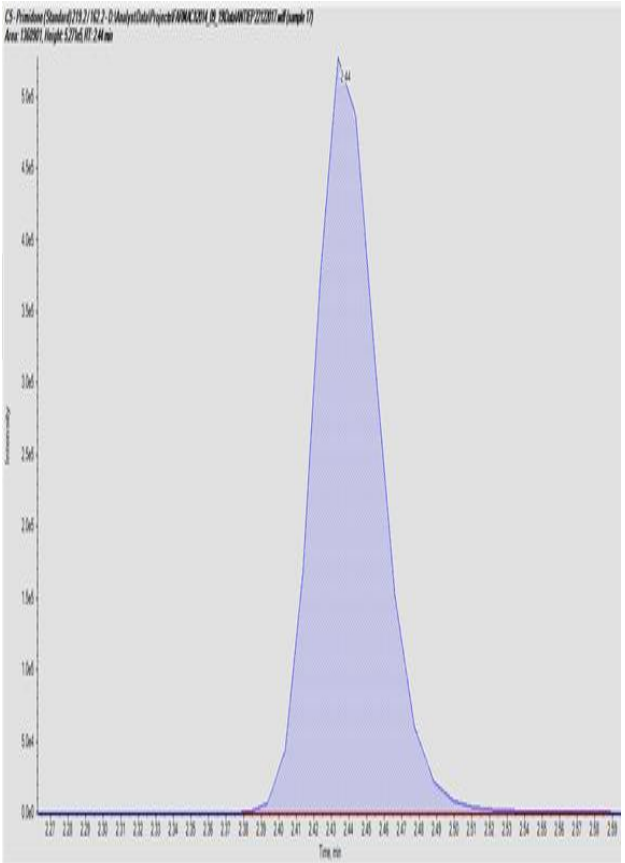


Fig. 26 :	***Plasma Calibrator***
	R.T. 2.44 Primidone