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Analytical Method Development and Validation for The Simultaneous Estimation of Topiramate and Rufinamide for RP-HPLC Method

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Abstract

A new method was established for simultaneous estimation of Rufinamide and Topiramate by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Rufinamide and Topiramate by using Xterra C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Rufinamide and Topiramate was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100% and 100.5%, %RSD for repeatability was.0.2 and 0.4, % RSD for intermediate precision was 0.5 and 0.1 respectively.

Keywords
Rufinamide, Topiramate, RP-HPLC, Phosphate buffer, CAN.

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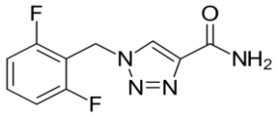
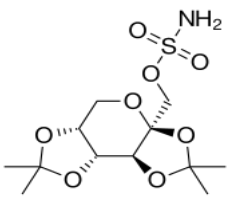
Rufinamide is an anticonvulsant medication. It is used in combination with other medication and therapy to treat Lennox–Gastaut syndrome and various other seizure disorders.



Rufinamide

Topiramate, sold under the brand name Topamax among others, is a carbonic anhydrase inhibitor medication used to treat epilepsy and prevent migraines. It has also been used in alcohol dependence.[6] For epilepsy this includes treatment for generalized or focal seizures.



Topiramate

MATERIALS AND METHOD:

Instrumentation

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited-Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000+pH meter, Weighing machine

Chemicals

Rufinamide and Topiramate, Potassium dihydrogen orthophosphate, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid.

Trial-5:(Optimized)

Chromatographic conditions:

Column : Xterra C18 5µm
Mobile phase ratio: Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v)
Detection wavelength: 255nm
Flow rate : 1ml/min
Injection volume : 20µl
Column temperature : Ambient

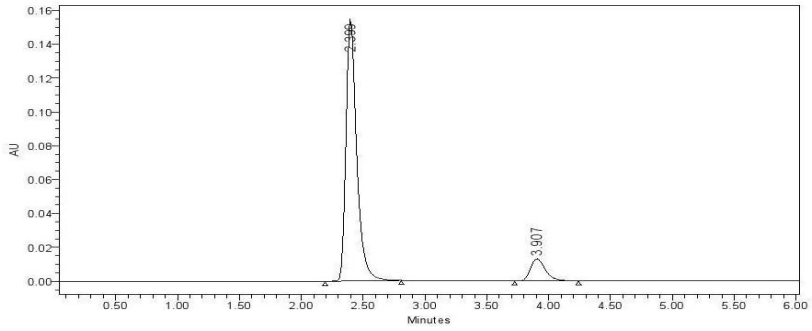


Fig. 1 Chromatogram of Trail-5

Table 1 Details of Trail-5

S.No	Peak name	Rt	Area	Height	USP Plate count	USP Tailing	USP Resolution
1	Rufinamide	2.399946	1241554	295105		1.3	8.1
2	Topiramate	3.907	1154113	23788		1.4	

Accuracy

Table 2 Accuracy results of Topiramate

%Concentration (At specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	2332744	5	5.10	101.8%	
100%	3132697	10	9.99	99.9%	100.5%
150%	3918997	15	14.9	99.1%	

Table 3 Accuracy results of Rufinamide

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovery	Mean Recovery
50%	353867	5	5.0	101.3%	
100%	473508	810	9.94	99.4%	100.5%
150%	591179	815	14.8	99.2%	

Precision

Table 4 Repeatability results of Rufinamide & Topiramate.

	Name	RT	Area	Height (µV)
1	Rufinamide	2.231	2235319	196999
2	Rufinamide	2.317	2240678	198254

3	Rufinamide	2.323	2249490	195128
4	Rufinamide	2.322	2245822	196164
5	Rufinamide	2.324	2251694	195887
Mean			2244601	
Std.Dev.			6656.8	
% RSD			0.30	

	Name	RT	Area	Height(µV)
1	Topiramate	4.304	1501417	100275
2	Topiramate	4.300	1486940	100079
3	Topiramate	4.308	1490656	98257
4	Topiramate	4.310	1487329	98165
5	Topiramate	4.314	1490384	98153
Mean			1491345	
Std.Dev.			5881.4	
% RSD			0.39	

Intermediate precision/Ruggedness

Table 5 Ruggedness results of Topiramate & Rufinamide

	Name	RT	Area	Height(µV)
1	Rufinamide	2.328	2194758	189693
2	Rufinamide	2.326	2197500	190025
3	Rufinamide	2.327	2196191	189862
4	Rufinamide	2.326	2195326	190700
5	Rufinamide	2.331	2200951	189426
Mean			2196585	
Std.Dev.			2496.0	
% RSD			0.11	

	Name	RT	Area	Height(µV)
1	Topiramate	4.335	1456296	95623
2	Topiramate	4.336	1457422	95150
3	Topiramate	4.334	1456513	95165
4	Topiramate	4.337	1454579	95298
5	Topiramate	4.340	1451483	95251
Mean			1455259	
Std.Dev.			2347.6	
% RSD			0.16	

Linearity

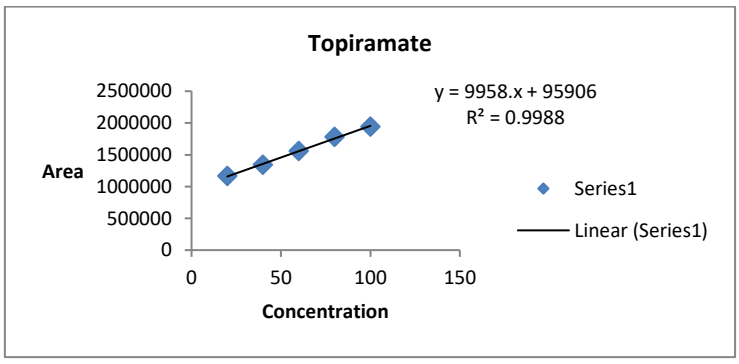


Fig 2 Calibration curve of Topiramate

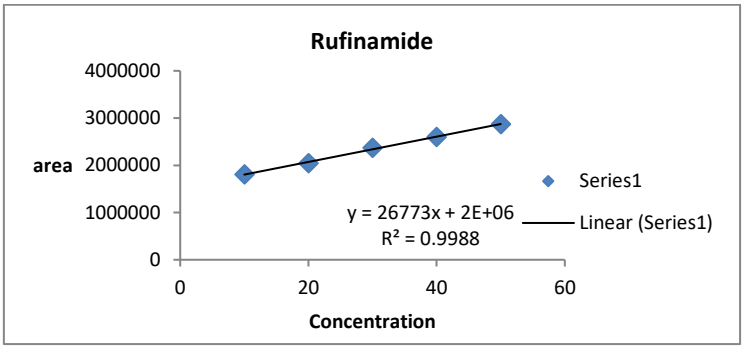


Fig.3 Calibration curve of Rufinamide

Robustness

Table 6 System suitability results For Topiramate (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	1748.5	1.22
2	1.0	1548.2	1.2
3	1.2	1948.0	1.2

Table 7 System suitability results for Rufinamide (Flow rate)

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	883.3	1.56
2	1.0	1234.0	1.1
3	1.2	969.2	1.6

Table 8 System suitability results for Topiramate (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10% More	1948.0	1.2

Table 9 System suitability results for Rufinamide (Mobile phase)

S.No	Change in Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	883.3	1.56
2	Actual	1234.0	1.1
3	10% More	969.2	1.6

SUMMARY AND CONCLUSION:

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Rufinamide and Topiramate was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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