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To Develop A New UPLC Method for Estimation of Topiramate In Pharmaceutical Dosage Forms

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Abstract

A simple and selective UPLC method is described for the determination of Topiramate Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 80 volumes of Methanol and 20 volumes of Water with detection of 276 nm. Linearity was observed in the range 50-150 µg /ml for Topiramate (r^2 =0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

Introduction

Ultra Performance Liquid Chromatography

UPLC refers to Ultra Performance Liquid Chromatography. It has Equipment that operates at high pressure than that used in HPLC & in this system uses fine particles(less than 2.5µm) & mobile phases at high linear velocities decreases the length of column, reduces solvent consumption & saves time.

According to the van Deemter equation, as the particle size decreases to less than2.5 µm, there is a significant gain in efficiency, while the efficiency does not reduce at increased flow rates or linear velocities therefore by using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC The technology takes full advantage of chromatographic principles to run separations Using columns packed with smaller particles (less than 2.5µm) and/or higher flow rates for increased speed, this gives superior resolution and sensitivity.

Principle

The UPLC is based on the principal of use of stationary phase consisting of particles less than 2.5 µm (while HPLC columns are typically filled with particles of 3 to 5 µm).

$H=A+B/v+Cv$

Where;

A, *B* and *C* are constants

v is the linear velocity, the carrier gas flow rate.

*The *A* term is independent of velocity and represents “eddy” mixing. It is smallest when the packed column particles are small and uniform.

The *B* term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by *v*.

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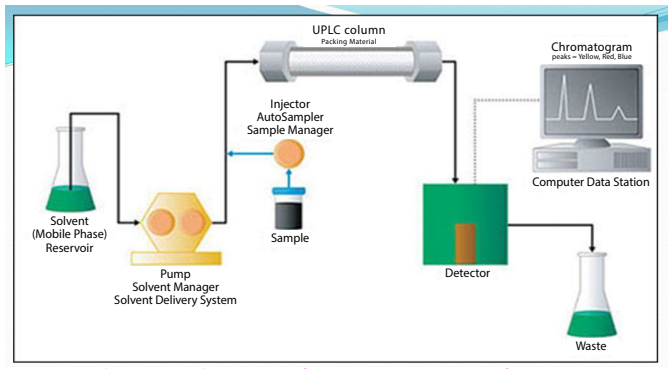


Figure 1: Schematic diagram for UPLC instrument.

* The *C* term is due to kinetic resistance to equilibrium in the separation process. Thus, term is proportional to *v.*

Advantages of UPLC

- Decreases run time and increases sensitivity
- Provides the selectivity, sensitivity, and dynamic range of LC analysis.
- Maintaining resolution performance.
- Expands scope of Multi residue Methods.
- UPLC's fast resolving power quickly quantifies related and unrelated compounds
- Faster analysis through the use of a novel separation material of very fine particle size
- Operation cost is reduced
- Less solvent consumption
- Reduces process cycle times, so that more product can be produced with existing resources

Disadvantages

- Due to increased pressure requires more maintenance and reduces the life of the columns of this type.
- So far performance similar or even higher has been demonstrated by using stationary phases of size around 2 µm without the adverse effects of high pressure.
- In addition, the phases of less than 2 µm are generally non-regenerable.

Instrumentation

The Ultra Performance Liquid Chromatography have the ability to work more efficiently with higher speed, sensitivity and resolution at a much wider range of linear velocities, flow rates and backpressures to obtain superior results Figure 1.

The Acquity UPLC system consists of

- Sample Injection
- UPLC Column
- PST Columns
- Solvent Delivery System
- The Detector

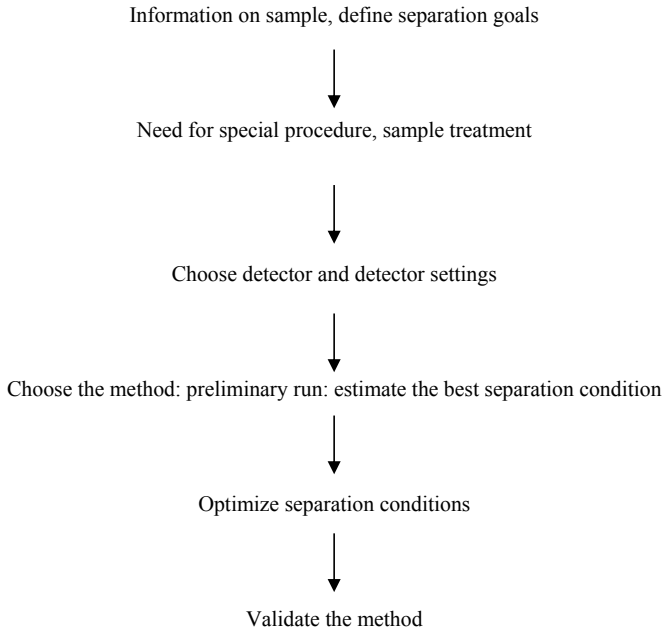
Guidelines for Analytical Method Development Figure 2.

Selection of the Chromatographic Method

The parameters that are affecting by the changes in chromatographic conditions are:

- Column efficiency (N)
- Capacity factor (K')
- Resolution factor (R_s)
- Retention Factor (R_f)
- Retention time (Rt)
- Relative retention (Rr)
- Peak asymmetry factor (As**)**

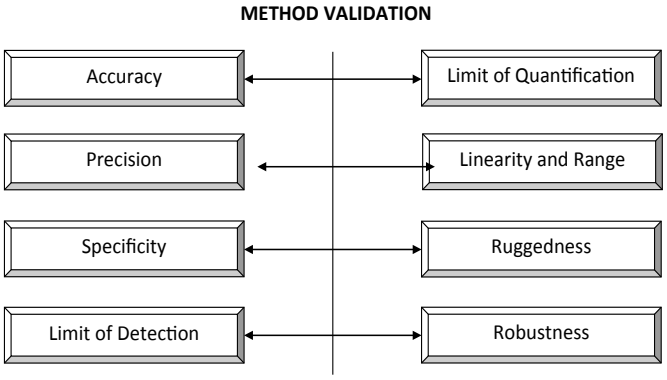
Selection of validation method



Method Development Guide Figure 2.

Guidelines for Analytical Method Validation

Method Validation



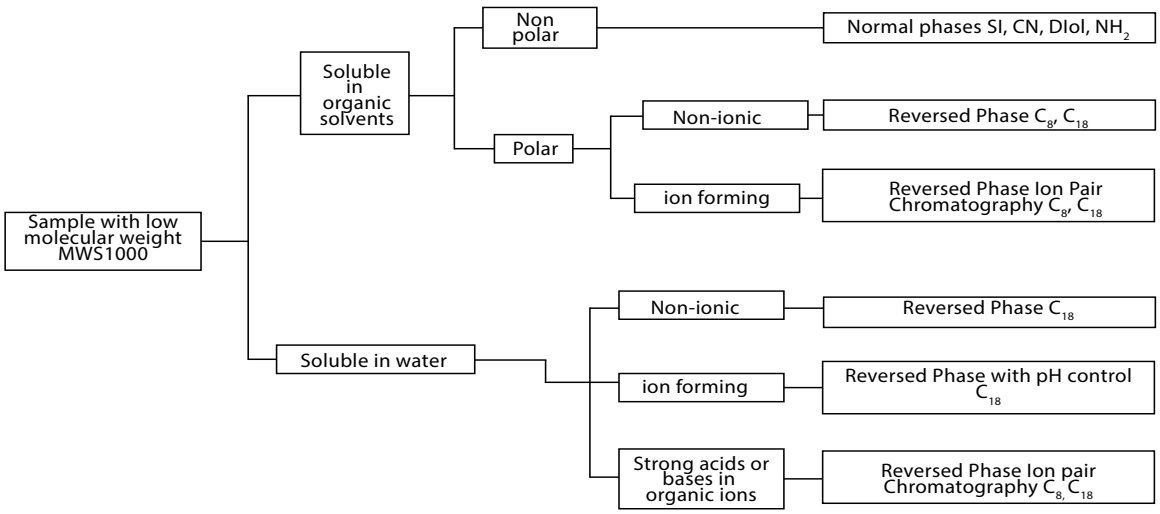


Figure 2: Guidelines for analytical method development.

Performance characteristics to be considered during the validation of a quantitative method in analysis.

Drug Profile

Drug Name: TOPIRAMATE Figure 3.

IUPAC Name

[(1R,2S,6S,9R)-4,4,11,11-tetramethyl-3,5,7,10,12-pentaoxatricyclo[7.3.0.0^{2},^{6}]dodecan-6-yl]methyl sulfamate

Categories

- Anti-Obesity Agents
- Anticonvulsants
- Carbohydrates
- Central Nervous System Agents
- Central Nervous System Depressants
- Cytochrome P-450 CYP2C19 Inhibitors

Synonyms

• 2,3:4,5-Bis-O-(1-methylethylidene)-beta-D-fructopyranose sulfamate

• 2,3:4,5-Di-O-isopropylidene-beta-D-fructopyranose sulfamate

• McN-4853

• RWJ-17021

• Tipiramate

• Tipiramato

• Topiramate

• Topiramato

• Topiramatum

Chemical Formula

$C_{12}H_{21}NO_8S$

Molecular Weight

339.362

Mechanism of Action

The precise mechanism of action of topiramate is not known. However, studies have shown that topiramate blocks the action potentials elicited repetitively by a sustained depolarization of the neurons in a time-dependent manner, suggesting a state-dependent sodium channel blocking action. Topiramate also augments the activity of the neurotransmitter gamma-aminobutyrate (GABA) at some subtypes of the $GABA_A$ receptor (controls an integral chloride channel), indicating a possible mechanism through potentiation of the activity of GABA.

Materials and Methods

Mobile Phase: A mixture of, 80 volumes of Methanol, and 20 volumes of Water. The mobile phase was sonicated for 10 min to remove gases Table1-3.

Determination of Working Wavelength (λmax)

Preparation of standard stock solution of Topiramate: 10 mg of Topiramate was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Results

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drug in methanol were

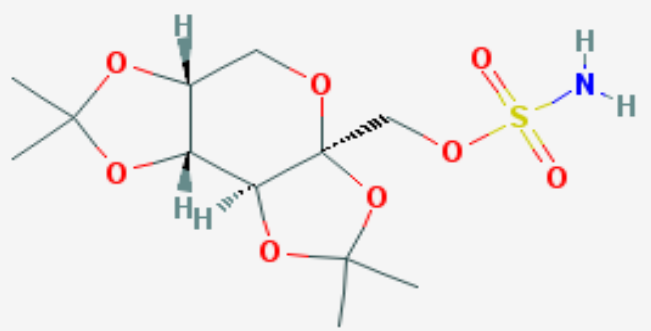


Figure 3: Chemical structure.

scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the 8.3 and The absorption curve shows characteristic absorption maxima at 276nm for Topiramate, selected as detector wavelength for the UPLC chromatographic method.

Method Development of Topiramate

Various analytical development trails has been performed by using different chemicals and reagents, organic solvents at different pH ranges and strengths in differents proportions of buffer and organic solvents to separate the peak shape. Based on the observations and conclusions obtained from the no.of chromatographic trails performed on UPLC, a particular set of chromatographic conditions were optimized to be suitable for estimation of the Topiramate in the tablets. The optimized chromatographic conditions which are found to be suitable for the estimation of Topiramate are given below.

Method Validation

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like retention times, theoretical plates, asymmetric factor were evaluated.

Assay

Preparation of standard solution: Weigh accurately 10 mg of Topiramate in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Topiramate is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Sample preparation: weigh accurately 10 Tablets (Topiramate -25 mg) weigh accurately 10 mg of Topiramate in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Topiramate is prepared by diluting 0.5ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Calculation

The amount of Topiramate present in the formulation by using the formula given below, and results shown in above table:

$$\%\,\text{Assay} = \frac{AT}{AS} X \frac{WS}{DS} X \frac{DT}{WT} X \frac{P}{100} X \frac{AW}{LC} X 100$$

Linearity and range

Preparation of standard stock solution

Standard stock solutions of TOPIRAMATE were prepared by dissolving 100 mg of TOPIRAMATE in 100 mL of Diluent. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug.

Results and Discussions

Method Development of Mirabegron

Trial-4: (Optimized), Trial- 4: (Optimized)

Chromatographic conditions

Mobile phase : Methanol; Water

pH : -

Ratio : 80 :20

Column : Inertsil ODS, (250×4.6× 5µ)

Wavelength : 276 nm

Flow rate: : 1ml/min

Preparation of standard solution

weigh accurately 10 mg of Topiramate in 25 ml of volumetric flask and dissolve in 25ml of mobile phase and make up the volume with mobile phase. From above stock solution 20 µg/ml of Topiramate is prepared by diluting 1.5ml to 10ml with mobile phase. This solution is used for recording chromatogram Figure 4.

Observation

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for both Topiramate
- The efficiency was more than 2000 Topiramate.
- Resolution between two peaks >1.5.
- hence this method was for optimized.

Table.1: Instruments used.

UV-Visible Spectrophotometer	Nicolet evolution 100
UV-Visible Spectrophotometer software	Vision Pro
UPLC software	Open lab EZ chrome
UPLC	Agilent Technologies
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Mettler Toledo
Syringe	Hamilton
UPLC Column	Inertsil ODS 3V(150x4.6mm) 4µm

Table 2: Reagents used.

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogen Phosphate	AR Grade
Acetonitrile	HPLC Grade
Dipotassium hydrogen phosphate	AR Grade
Orthophosphoric acid	HPLC Grade

Table 3: Drugs used.

TOPIRAMATE (API)	Gift Samples obtained from Chandra labs, Hyd.
TOPIRAMATE (25 mg) Tablet dosage form	Obtained from local pharmacy

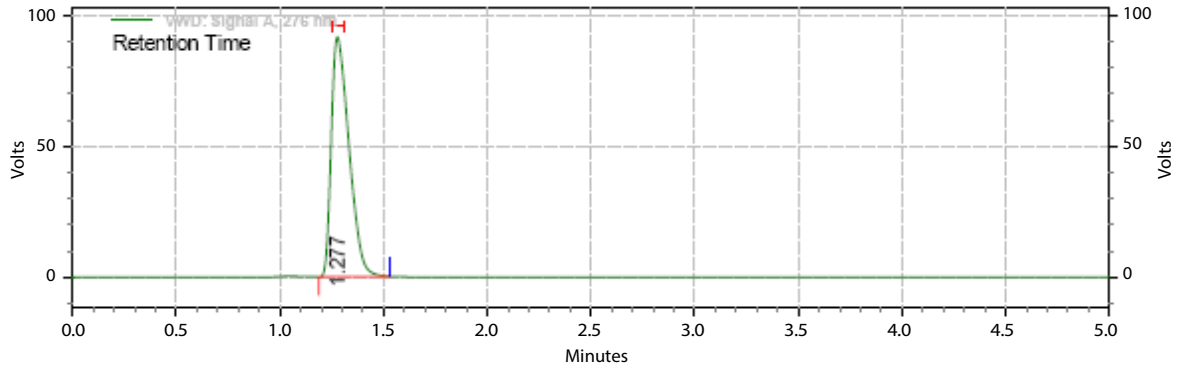


Figure 4: Chromatogram of Topiramate.

Assay: Table 5.

System suitability: Table 6.

Linearity and range: Table 7.

Linearity data of TOPIRAMATE Table 8.

Linearity graph of TOPIRAMATE Figure 5.

Accuracy: Table 9.

Method precision Table 10

Limit of Detection

$$LOD = \frac{3.3\sigma}{S}$$

= 3.3 * (15086.6)/44715

= 1.11µg/ml TOPIRAMATE

Where, σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Limit of Quantification (Loq)

$$LOQ = \frac{10\sigma}{S}$$

= 10* (15086.6)/44715

= 3.37µg/ml TOPIRAMATE

Where

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision Table 11.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts Table 12.

Discussion

A simple and selective UPLC method is described for the determination of Topiramate Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 80 volumes of Methanol and 20 volumes of Water with detection of 276 nm. Linearity was observed in the range 50-150 µg /ml for Topiramate (r^2 =0.998) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All

Table 4: Optimized chromatographic conditions.

Mobile phase	Methanol: WATER (80:20)
Ph	-
Column	Inertsil ODS 3V column, C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25°C)
Sample temperature	Room temperature(20-25°C)
Wavelength	276
Injection volume	20 µl
Run time	6 min
Retention time	About 1.277 min for Topiramate

Table 5: Assay Results.

TOPIRAMATE		
	Standard Area	Sample Area
Injection-1	9111785	9245257
Injection-2	9128795	9272110
Injection-3	9123101	9282820
Injection-4	9101117	9257988
Injection-5	9140105	9252085
Average Area	9120980.60	9262052
Standard deviation	15086.63	
%RSD	0.2	
Assay(%purity)	101.55	

Table 6: Results for system suitability of TOPIRAMATE.

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.275	9128775	‹2000	1.82
2	1.289	9111985	‹2000	1.80
3	1.276	9108527	‹2000	1.79
4	1.278	9133128	‹2000	1.81
5	1.276	9152688	‹2000	1.80
Mean	1.28	9127020.6	-	-
SD	0.0058	17799.41	-	-
%RSD	0.45	0.20	-	-

Table 7: Linearity Preparations.

Preparations	Volume from standard stock transferred in mL	Volume made up in mL (with mobile phase)	Conc. obtained (µg/mL)
			TOPIRAMATE
Preparation 1	0.5	10	50
Preparation2	0.8	10	80
Preparation 3	1	10	100
Preparation 4	1.2	10	120
Preparation 5	1.5	10	150

Table 8: Linearity data of TOPIRAMATE.

S. No	Concentration (µg/mL)	Area
1	50	4795387
2	80	7638186
3	100	9269572
4	120	11393610
5	150	14961410

Table 9: Recovery results for TOPIRAMATE.

%Recovery	Amount present(µg/mL)	Amount found(µg/mL)*	Percent Recovery *	% Mean Recovery
50%	50	49.64	99.3	99.3
100%	100	99.04	99.0	
150%	150	149.56	99.7	

Table 10: Method precision results for TOPIRAMATE.

S. No.	RT	AREA
1	1.271	9231182
2	1.273	9241228
3	1.271	9231190
4	1.274	9231135
5	1.272	9241195
6	1.274	9231147
AVG	1.273	9234512.83
SD	0.00138	5188.82
%RSD	0.108	0.056

Table 12: Ruggedness Results of TOPIRAMATE.

TOPIRAMATE	%Assay
Analyst 01	99.75
Analyst 02	101.5
%RSD	0.27

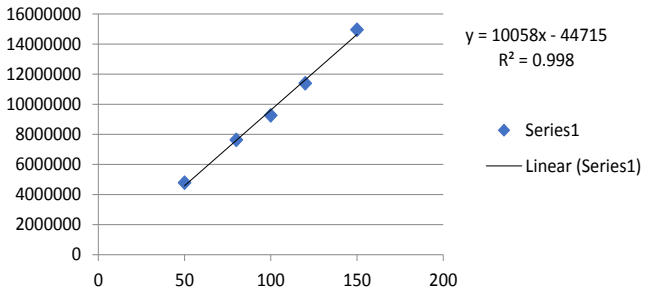


Figure 5: Linearity graph of TOPIRAMATE.

statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Conclusion

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of 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 [1-13].

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Table 11: Results for Robustness of TOPIRAMATE.

Chromatographic changes		Rt(min)	Tailing Factor	Theoretical Plates	%RSD for Standard areas
Flow rate (mL/min)	0.4	1.712	1.75	‹2000	0.12
	0.6	1.030	1.67	‹2000	0.11
Temperature (°C)	25	1.282	1.77	‹2000	0.22
	35	1.285	1.72	‹2000	0.19

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