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Uhplc Method Development, Validation And Forced Degradation Study For Simultaneous Estimation Of Phentermine And Topiramate In Bulk And Tablet Dosage Form

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ABSTRACT

A novel approach was used to develop and validate a rapid, specific, accurate and precise Ultra Performance Liquid Chromatographic (UHPLC) method for the simultaneous determination of Phentermine and Topiramate in pharmaceutical dosage forms. The chromatographic separation was achieved on Aquity UPLC BEH C8 100 x 4.6 mm, 2.7µm, column using a mobile phase ammonium acetate Buffer PH 4.5:Acetonitrile: Methanol in the ratio of 60:20:20. The flow rate was 0.5 mL min−1 and the detection wavelength was 263 nm. The limit of detection (LOD) for phentermine and Topiramate was 0.46 and 1.97µg mL−1, respectively. The limit of quantification (LOQ) for phentermine and Topiramate was 1.5 and 6.51µg mL−1, respectively. This method was validated with respect to linearity, accuracy, precision, specificity, and robustness. The method was also found to be stability-indicating.

Key words: Phentermine and Topiramate; UHPLC; Forced Degradation method; ICH Validation.

1 INTRODUCTION

Phentermine /topiramate, sold under the brand name Qsymia, is a combination drug of phentermine and topiramate used to treat obesity. It is used together with dietary changes and exercise. The complete information with data supplemented from the literature that there are only few methods like HPLC, and UV-VIS spectroscopy methods were available till date. Hence it felt necessary to establish a new, easier, accurate, precise, stability indicating method to enhance the sensitivity and to reduce the time and consumption of solvents, which can be easily applicable for routine drug performance evaluations.

Phentermine IUPAC Name-2-methyl-1-phenylpropan-2-amine
Topiramate IUPAC Name-2,3:4,5-Bis-O-(1-methylethylidene)-β-D-fructopyranose sulfamate

Phentermine structure **Topiramate structure**

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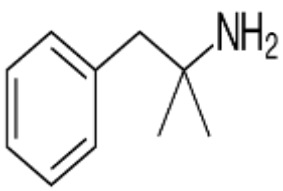
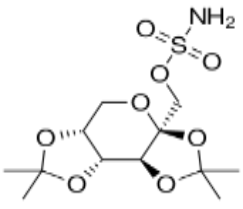




2 MATERIALS & METHOD

Instrumentation

Waters-Acquity UPLC of waters make is used which contains binary mode gradient pump system, Auto sampler, temperature-controlled column oven compartment and PDA detector for detection. Empower 2 software was used as interphase. Dissolution profiling was performed using Distek dissolution Apparatus type II system. Acquity Ethylene Bridged Hybrid technology (BEH) C18 (100*4.6mm &2.7µm) column was used.

Chemicals

Phentermine and topiramate pure substances, qsymia tablets from vivus, Inc. were taken from sun Pharma. Ultra-pure Acetonitrile, methanol were taken from Merck chemicals. Ultrapure water has taken from Evoqua water purifier.

METHODOLOGY

Preparation of standard solution
Weigh accurately 15mg of PHENTERMINE and 92 mg of TOPIRAMATE in 100 ml of volumetric flask and dissolve in 70ml of mobile phase and make up the volume with mobile phase. From above stock solution 15µg/ml of PHENTERMINE and 92µg/ml of TOPIRAMATE is prepared by diluting 5ml to 50ml with mobile phase respectively.

Preparation of sample solution:
Accurately weighed and transferred 20tablets into mortar and pestle, crushed in fine powder then weighed powder equivalent to 15mg of PHENTERMINE and 92 mg of TOPIRAMATE transferred in to a 100mL volumetric flask then added 70mL of mobile phase, sonicated for 30min then made volume up to mark and mixed well. From above stock solution 15µg/ml of PHENTERMINE and 92µg/ml of TOPIRAMATE is prepared by diluting 5ml to 50ml with mobile phase respectively. From above solution 200µg/ml of PHENTERMINE and 50µg/ml of TOPIRAMATE is prepared by diluting 5ml to 100ml with mobile phase respectively.

METHOD DEVELOPMENT

For this method development, various ratios and combination of mobile phases, different stationary phases and flow rates were tried to elute the drug with better peak parameters and to provide good performance in assay. Finally, the best separation was achieved on

Mobile Phase : Ammonium acetate Buffer pH 4.5: Acetonitrile: Methanol
Ratio : 60:20:20
Column : Acquity BEH C18 (100*4.6mm &2.7µm)
Column Oven Temperature: 30°C
Wavelength : 263nm
Flow rate : 0.5ml/min

The chromatogram showed the peak with good shape, more theoretical plates and the tailing factor was also found to be within the limits. Therefore, it was finalized to be an optimized method and proceeded to carry out method validation. The standard chromatogram of phentermine and topiramate drug was presented in Figure 1.

METHOD VALIDATION

As per ICH guidelines the checked validation parameters were accuracy, precision, linearity, LOD, LOQ, Robustness, Ruggedness and Specificity.

System suitability: To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 15µg/mL of Phentermine and 92µg/mL of Topiramate were injected six times and the chromatograms were recorded for the same.
The theoretical plate number, peak asymmetry and percentage relative standard deviation obtained are within the acceptance criteria and demonstrated that the method can generate the accurate and precise results. The results were presented in Table 1.

Accuracy: It was found that the average recovery at 50%, 100%, 150% were found to be within the limits which indicated the methods Accuracy. The measured results were provided in Table 2.

Precision: The %RSD obtained was within the limits indicating the methods precision. The measured results were presented in Table 3.

Linearity: The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Phentermine and Topiramate is 0.9998 and 0.9991. The relationship between the concentration of Phentermine and Topiramate and area of Phentermine and Topiramate is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits. The obtained data was statistically analysed, and results were presented in Table 4, Table 5 and the calibration curve was depicted in Figure 2, Figure 3 for Phentermine and Topiramate respectively.

Robustness: Upon slight changes in the flow rate and temperature, the results confirmed the reliability of the method. Results were presented in Table 6.

Ruggedness: The ruggedness of the method was studied by determining the analyst to analyst variation by performing the Assay by two different analysts. Results were presented in Table 7.

Limit of Detection:

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

= (3.3)*(17578)/115569
Phentermine = 0.46µg/ml
= (3.3)* (90013.48)/138326
Topiramate =1.97µg/ml
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.
Observation**:**
The LOD for this method was found to be 0.46µg/ml for Phentermine and 1.97µg/ml for Topiramate

Limit of Quantification:

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

= (10)*(17578)/115569
Phentermine = 1.52µg/ml
= (10)* (90013.48)/138326
Topiramate =6.51µg/ml
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.
Observation**:**
The LOQ for this method was found to be 1.52µg/ml for Phentermine and 6.51µg/ml for Topiramate

Forced degradation studies:
The forced degradation results showed that, 5.2% of Phentermine degradation was achieved in Acid Degradation and 7.4% of Topiramate degradation achieved in Base degradation, Peak purity of main analyte was passed so this method proved as a stability indicating method. The standard chromatogram of phentermine and topiramate forced degradation studies was presented in Figure 4-6
The measured values are provided in Table 8.

3 RESULTS AND DISCUSSION

The present work considers the first UHPLC-UV method for simultaneous determination of Topiramate & Phentermine. Moreover, the stability of the combination was evaluated under hydrolytic, thermal, and photolytic conditions.

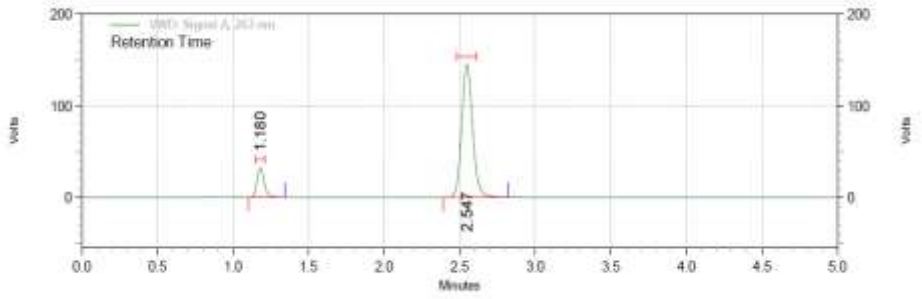


Figure 1: Chromatogram of Optimized trail

System Suitability

Table 1: System Suitability Data

S. No.	Parameters	Topiramate	Phentermine
1	Rt (min)	2.547	1.18
2	Plate count	5972	2851
3	Tailing factor	1.16	1.15
4	Peak Area (AUC)	12172340	1804611

Acceptance criteria

1. The % RSD for the retention times of Phentermine andTopiramate Peaks from 6 replicate injections of each Standard solution should be not more than 2.0
2. The % RSD for the peak area responses of Phentermine andTopiramate peaks from 6 replicate injections of each standard solution should be not more than 2.0%.
3. The number of theoretical plates (N) for the Phentermine andTopiramatepeaks is not less than 2000.
4. The Tailing factor (TP) for the Phentermine andTopiramate peak is not more than 2.0.

Result

The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

Recovery

Table 2: Topiramate & Phentermine Recovery Data

Accuracy level	Mean Recovery Topiramate	Mean Recovery Phentermine
50%	100.5%	100.4%
100%	98.9%	98.4%
150%	99.6%	100.3%

Acceptance criteria: The % recovery of Phentermine and Topiramate should lie between 98% and 102%.

Precision

Table 3: Results for Method precision of Phentermine and Topiramate

Injection	Phentermine %Assay	Topiramate %Assay
1	**99.8**	**101.2**
2	**99.7**	**100.3**
3	**99.6**	**101.1**
4	**100.1**	**101.3**
5	**100.2**	**100.1**
6	**99.5**	**100.4**
Average	**99.82**	**100.7**
SD	**0.3**	**0.5**
%RSD	**0.3**	**0.5**

Observation

Test results for Phentermine and Topiramate are showing that the %RSD of Assay results are within limits. %Assay of PhentermineTopiramate were 90.0 to 110.0% The results were shown in table Table 3.

Linearity

Table 4: Linearity Data of Phentermine

S.No.	Conc.(µg/ml)	Area
1	7.5	915544
2	12.0	1408678
3	15.0	1759330
4	18.0	2098777
5	22.5	2650410

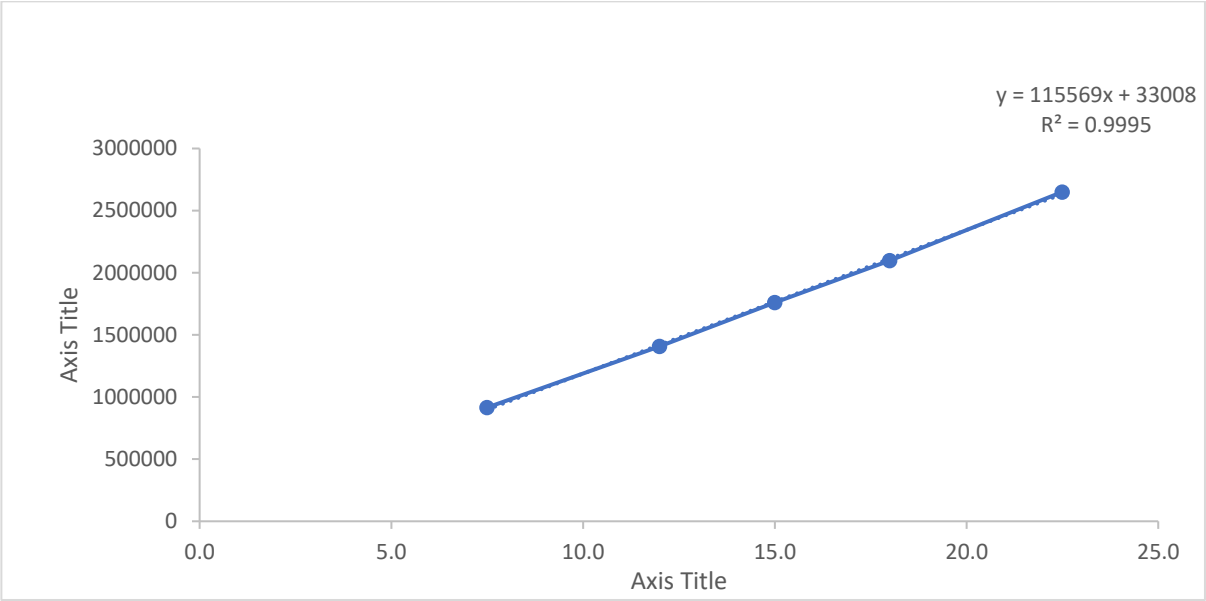
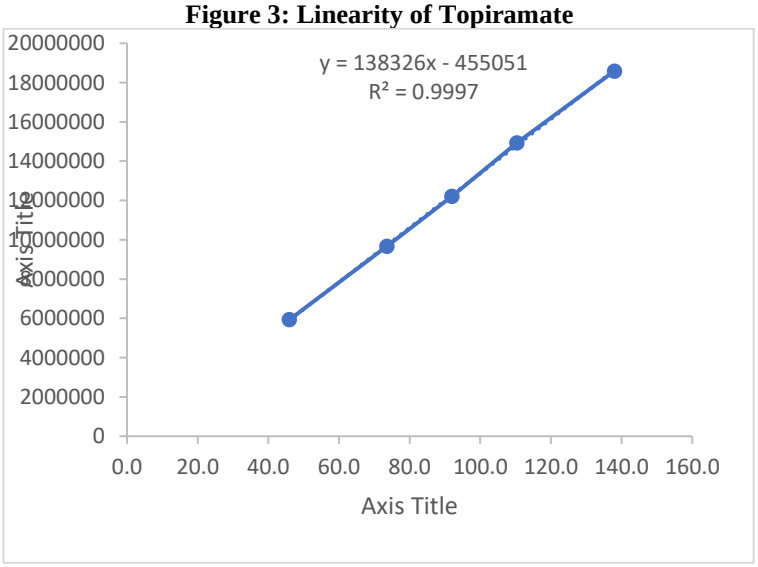


Figure 2: Linearity graph of Phentermine

Table 5: Linearity Data of Topiramate

S.No.	Conc.(µg/ml)	Area
1	46.0	5936430
2	73.6	9676207
3	92.0	12208643
4	110.4	14940526
5	138.0	18592839

Figure 3: Linearity of Topiramate



Acceptance criteria

The relationship between the mix of Phentermine and Topiramate and area of Phentermine and Topiramate out to be linear in the pre defined range and the correlation should not be less than 0.99.

Observation: The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Phentermine and Topiramate is 0.9998 and 0.9991. The relationship between the concentration of Phentermine and Topiramate and area of Phentermine and Topiramate is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Robustness

Table 6: Robustness Data of Topiramate & Phentermine

Chromatographic Changes		Theoretical Plates		Tailing factor			
		Phentermine	Topiramate	Phentermine	Topiramate	Resolution	%RSD for 05 Replicate injections
Temperature (°C)	25°c	2660	5134	1.20	1.21	11.8	0.3
	35°C	2675	5323	1.19	1.25	11.8	0.4
Flow rate mL/min	0.4	2425	4984	1.20	1.16	11.4	0.2
	0.6	2933	5579	1.23	1.20	12.2	0.4

Ruggedness

Table 7: Results for Ruggedness

Phentermine	%Assay	Topiramate	%Assay
Analyst 01	99.8	Analyst 01	99.9
Analyst 02	99.4	Analyst 02	100.1
%RSD	0.10	%RSD	0.17

Observation: From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

Forced degradation studies

Table 8: Forced Degradation Studies Data for Topiramate & Phentermine

Injection		PHENTERMINE	TOPIRAMATE
	Condition	%Assay	%Assay
1	Thermal	100.1	101.2
2	Photolytic	99.4	100.1
3	Acid Hydrolysis	94.8	100.0
4	Base Hydrolysis	99.7	92.6
5	Peroxide Hydrolysis	100.3	98.9

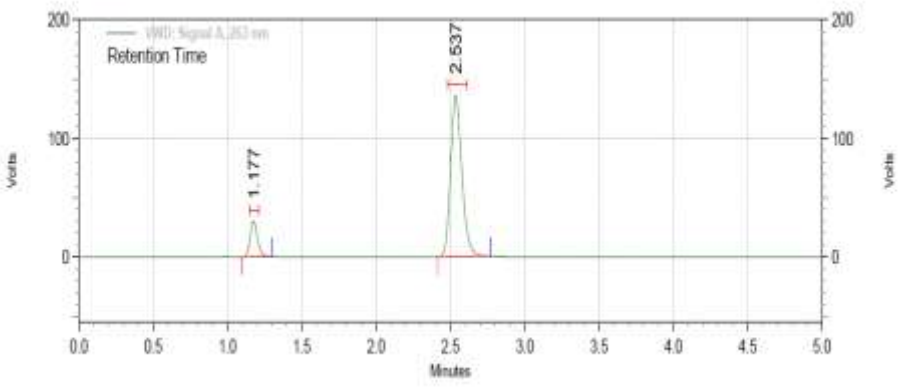


Fig:4 Chromatogram of Thermal Sample(105°C/72Hrs)

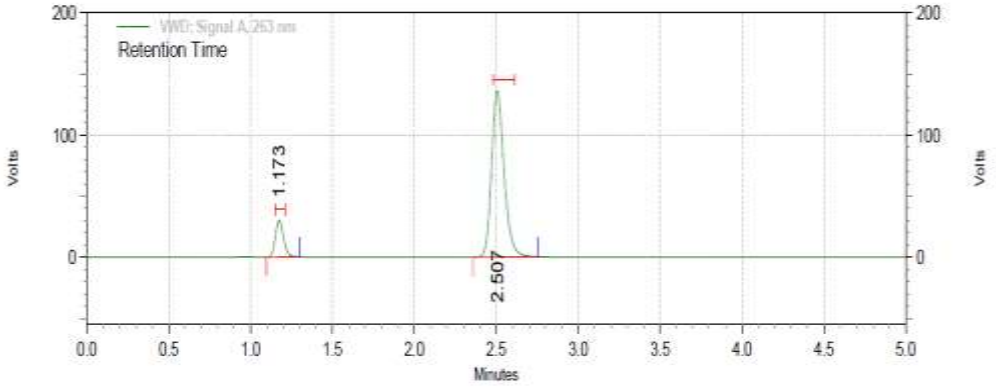


Fig:5 Chromatogram of Acid Sample preparation(5N HCl /4Hrs/60°C)

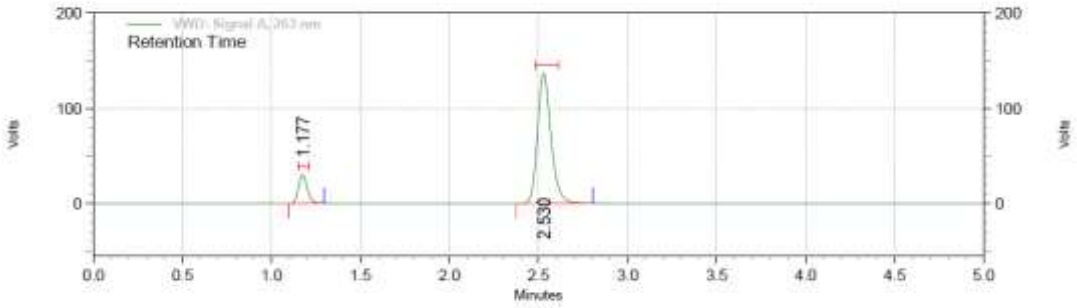


Fig :6 Chromatogram of Base Sample preparation (5N HCl /4Hrs/60°C)

Results:
In above all conditions 5.2% of Phentermine degradation was achieved in Acid Degradation and 7.4% of Topiramate degradation achieved in Base degradation, Peak purity of main analyte was passed so this method proved as a stability indicating method.

4 CONCLUSIONS

The present established stability indicating Ultra High-performance liquid chromatographic method is new, fast and easier to quantify the Phentermine and Topiramate drug with precise and accurate results. The successful separation of all the degradation products from the active pharmaceutical ingredient proved the specificity and the stability indicating nature of the developed method. In comparison to the reported methods by HPLC and UV-VIS Spectroscopy, the present developed method requires lesser analysis time and more sensitive. Thus, the shorter duration of analysis time, more sensitivity and cost effectiveness revealed that it is suitably applied for routine laboratory use.

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