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Research Article

Development and Validation of Spectrophotometric Method for Analysis of Topiramate

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ABSTRACT

Selective, inexpensive and validated method was developed and optimized for determination of antiepileptic drug, Topiramate in drug substance and drug product as well as in the presence of pyridine. The method is based on the reaction of the primary amino group of Topiramate with Ninhydrin reagent in ethanolic medium in the presence of 10% pyridine solution. The colored product was measured at 568 nm. The linearity range was found to be 50 – 300 µg mL-1 with mean recovery 98-102%. All variables affecting the reaction conditions were thoroughly studied. The results were found to agree statistically, the method was validated according to the ICH guidelines. The proposed method is practical and valuable for their in quality control laboratories for analysis of Topiramate.

Key-words: Topiramate, pyridine, Ninhydrin, Drug product

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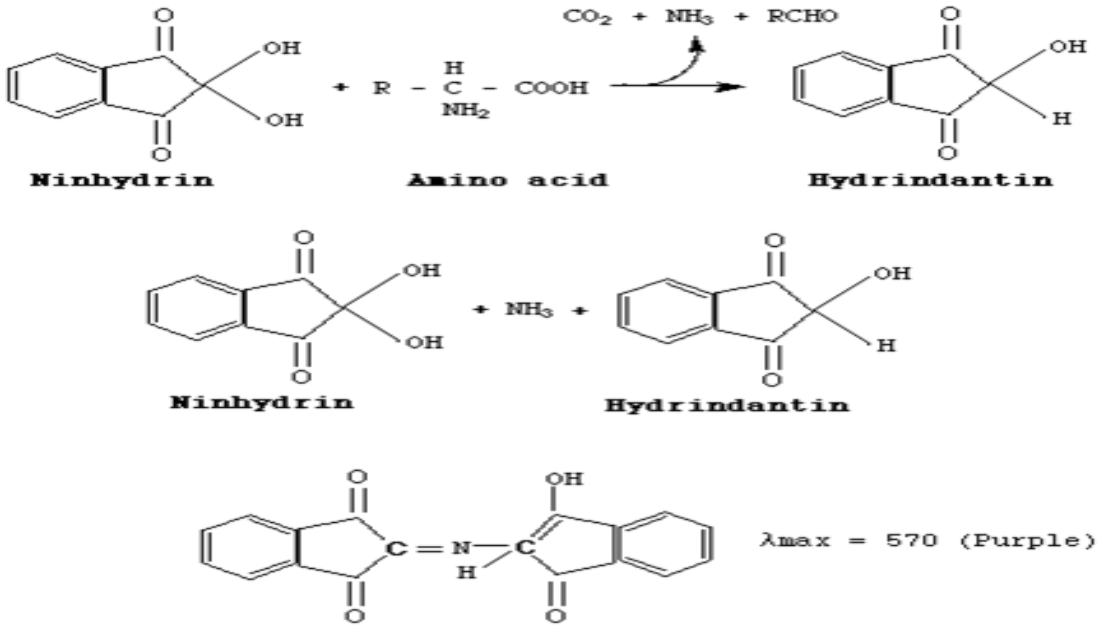
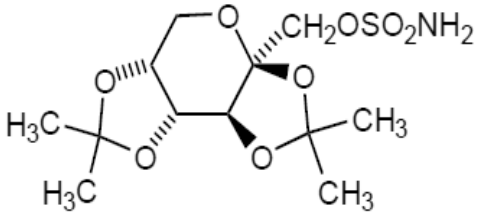
INTRODUCTION



Scheme 1: Proposal reaction between Fluorescamine and Topiramate

MATERIALS AND METHOD

DRUG INFORMATION



Scheme-2: Topiramate

A sulfamate-substituted monosaccharide (2, 3:4, 5-bis-O-(-1-methyl)-[beta]-Dfructopyranose Sulfamate) is a new second generation antiepileptic agent [1-4]. The drug is structurally different from other anticonvulsants and has been proved in partial and generalized tonic-clonic seizure [5]. Topiramate has no ultraviolet, visible or fluorescence absorption and available methods for analysis of the drug in biological fluids and pharmaceutical products, consisted of gas chromatography (GC) coupled with flame ionization (FID) or nitrogen phosphorous detection (NPD) [6-8], fluorescence polarization immunoassay [9]. HPLC methods, including, ionic chromatography [10], or using refractive index (RI) or chemiluminescent nitrogen or MS detector are described [11-13]. Q NMR stability indicating method was also reported for its determination [14]. Analysis of the drug in human plasma following derivatization with 9-fluorenylmethyl chloroformate (FMOCCl) or 4-Chloro-7-nitrobenzofurazan (NBD-Cl) using fluorescence or UV detection have been reported [15-18]. The aim of this work is to follow degradation of Top under stress conditions according to the ICH guidelines, and develop stability-indicating spectrophotometric method for determination of top in the presence of pyridine. The method is alternative and comparable in specificity and accuracy to chromatographic methods, which although highly specific and accurate, are more time consuming, performed in several steps, used environmentally hazardous solvents, and are rather expensive.

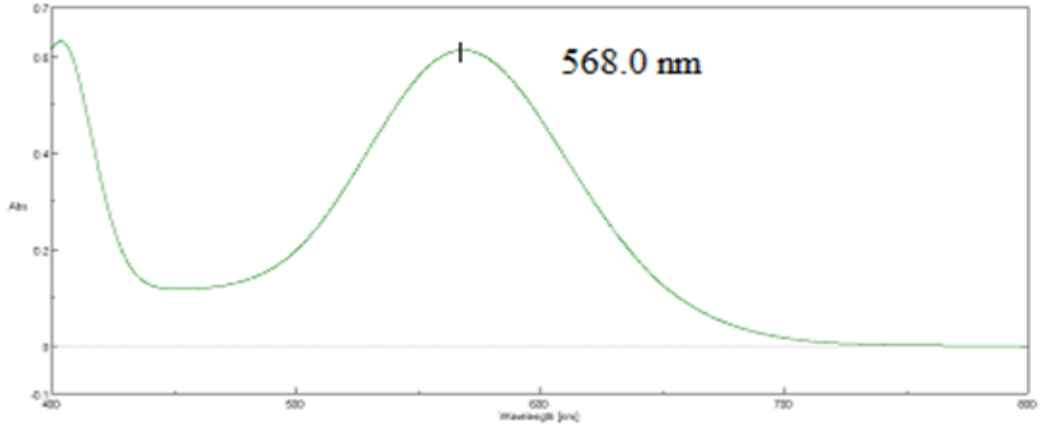


Fig-1: Excitation and emission spectra of the reaction products of Topiramate 50 µg ml-1) and its degradants (λmax 568nm)

a. Procurements of Drug sample

Table 1: Procurement of Drug Sample

Sr.No.	Drug sample*	Supplier
1.	Topiramate	Gen Pharma

*The drug samples are received with Certification of Analysis and therefore used as such without purification or analysis.

b. Reagents and Chemicals

Table 2: List of Chemicals Used

Sr.No.	Reagents/Chemical	Grade	Supplier
1.	Methanol	AR	UNIQUE
2.	Pyridine	LR	LOBA Chemicals
3.	Ninhydrin	GR	LOBA Chemicals

c. Equipments/Instruments

Table 3: List of Equipment/ Instruments used

Sr. No.	Name of Equipment /Instrument	Model /Specification	Manufacturer
1.	UV-Visible Double beam spectrophotometer	V-630 Detector-photomultiplier tube	JASCO
2.	Digital Electronic Balance	CB-50 Sensitivity- 1mg Max wt.-2g	CONTECH
3.	Ultra Sonicator	UCB-40	SPECTRALAB

Calibrated glassware was used for the study.

DEVELOPMENT OF ASSAY METHOD

a. **Solvent used**: Distilled water, Methanol.

b. **Reagents used**: 10% Pyridine (10ml Pyridine + Distilled Water upto 100ml)
2% Ninhydrin (2gm of Ninhydrin + Distilled Water upto 100ml)

c. **Preparation of standard stock solution**:
10 mg of TOPIRAMATE was weighed and dissolved in Distilled water and then volume was made up to 10 ml after that solution was ultrasonicated for 10 min. so as to get the concentration 1mg/ml for Topiramate.

d. **Selection of Analytical Wavelength**:

For estimation of Topiramate, such wavelength was selected where the absorbance of drug is directly proportional to the concentration of drug. Wavelength selected for estimation of Topiramate was 568 nm.

e. **Selection of analytical concentration range**:

From Standard stock solution appropriate dilution was done using distilled water. The absorbance was measured at selected wavelength and plotted against concentration. The concentration range over which the drug obeyed Beer's law was chosen. The range was found to be 50, 100 150, 250 & 300 µg/ml.

Linearity Equations are used for calculation, as shown below

f. **Equation**:

$y = 0.0019x + 0.2184$

g. **Standardization of the Colorimetric Method**:

From the standard stock solution of Topiramate, dilutions was prepared using Distilled Water to this add 1 ml of 10% Pyridine solution and 1 ml of 2 % Ninhydrin solution was added. Solutions were heated for 20 minutes in boiling water. After cooling, the spectrum was taken at fixed wavelength of 568 nm. The concentration of Topiramate was calculated by putting the value of absorbance of Drug in equation.

h. **Procedure for analysis of Tablet formulation**:

Composition: Each Tablet contains Topiramate 50 mg.

10 tablets were weighed and powdered in mortar and pestle. Quantity equivalent to 10 mg of Topiramate was weighed and transferred to 10 ml volumetric flask. The volume was made up to the mark with Methanol. The solution was filtered and clear filtrate was obtained which diluted appropriately to obtain the concentration 1000 µg/ml of Topiramate. 10% Pyridine & 2% Ninhydrin were added, solution was heated in boiling water for 20 minutes after the cooling. The absorbance was measured at the selected wavelength. The concentration of Topiramate was calculated by putting the value of absorbance of in equation 1. Result of Assay shown in Table 4.

Table 4: Analysis of tablet

Conc. of drug	Absorbance	Amt. added µg/ml	Amt. found in %
100µg	0.4254	0.4	98.95
100µg	0.4320	0.4	102.02
100µg	0.4251	0.4	98.80
100µg	0.4292	0.4	100.76
100µg	0.4329	0.4	102.05
100µg	0.4331	0.4	102.01

ANALYTICAL METHOD VALIDATION

1. **Linearity**

Suitable dilutions were made from the standard stock solution (containing 1mg/ml of Topiramate) using range of standard solution containing five different concentrations of analytes. Spectra of Five replicates per concentration were taken. The linearity of the relationship between absorbance and concentration was determined by analysing five standard solutions over the concentration range 50-300 µg/ml for Topiramate. The Linearity obtained is shown in Figure 2.

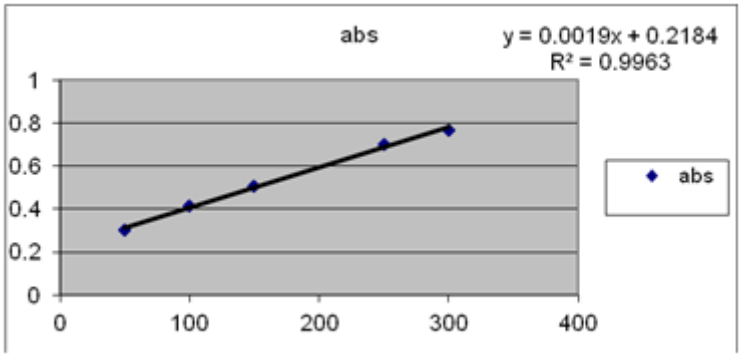


Figure 2: Linearity of Topiramate at 568 nm

2. **Accuracy**

Recovery studies were carried out by addition of known amount of working standard Topiramate at 80% and 100% and 120% level respectively to tablet formulation already analyzed. Powder weight equivalent to 10 mg of Topiramate was taken from the tablet, blend and transferred to 10 ml volumetric flask.. The volume was made up to the mark with methanol. The solution was filtered and clear filtrate was obtained. Adequate aliquot of solution was taken to six separate 10 ml volumetric flask to get concentration 1000 µg/ml of Topiramate. In three volumetric flasks, solution of working standard Topiramate added to get three above mentioned levels. The dilutions were made in Distilled Water. After addition of reagents & boiling for 20 min. cooled these solutions & absorbances were recorded at selected wavelength. The concentration of drug was then calculated by using from equation 1. The same procedure was repeated thrice to check the accuracy of the method and results are shown in Table 5.

Table 5: Recovery studies of Topiramate

Level of Recovery	Conc. of drug (µg/ml)	Amount Added (µg/ml)	Absorbance	Amount Recovered µg/ml	% Recovery
80	0.32	0.4	0.5613	0.321	80.47
80	0.32	0.4	0.5595	0.318	79.50
80	0.32	0.4	0.5599	0.319	79.75
100	0.4	0.4	0.6004	0.404	101.00
100	0.4	0.4	0.5998	0.402	100.50
100	0.4	0.4	0.5988	0.400	100.00
120	0.48	0.4	0.6365	0.480	120.00
120	0.48	0.4	0.6366	0.480	120.00
120	0.48	0.4	0.6370	0.481	120.25

Table 6: Statistical treatment of Recovery data for Topiramate

Parameters	Level of recovery		
	80%	100%	120%
Concentration added (µg/ml)	0.32	0.4	0.48
Mean of Absorbance	0.5602	0.5996	0.6367
Mean Conc. Recovered (µg/ml)	0.319	0.402	0.480
Mean % recovery	79.90	100.66	120.15
% RSD	0.4785	0.4975	0.1201

3. Precision

System Precision

For checking system precision std. solution of 10µg/ml Topiramate was prepared and six replicates were taken and % RSD were calculated and results are indicated in Table 7.

Table 7: System precision for Topiramate

Replicate	Absorbance of Topiramate
1	0.4383
2	0.4256
3	0.4204
4	0.4185
5	0.4130
6	0.4231
Mean	0.42315
SD	0.008576
% RSD	1.99

Intra and Interday precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra- day studies, 3 repeated solution of standard and sample solution was made in a day and percentage RSD were calculated.

The result obtained for intra- day variations are shown in Table 8.

Table8: Intra-day variations for Topiramate

Conc. (µg/ml)	Absorbance			Mean	Std dev	% RSD
	Trial 1	Trial 2	Trial 3			
100	0.4886	0.4658	0.4557	0.4767	0.00182	0.3826
150	0.4919	0.4779	0.4740	0.4812	0.00941	1.9550
250	0.5855	0.5782	0.5722	0.5786	0.00666	1.1509

In the inter day variation studies, 3 repeated solution of standard and sample solution was made on 3 consecutive days and percentage RSD were calculated & The results are shown in table 9.

Table 9: Percentage RSD for Topiramate

Conc. (µg/ml)	Absorbance			Mean	Std dev	% RSD
	Day 1	Day 2	Day 3			
100	0.4923	0.4687	0.4476	0.4695	0.00476	0.476
150	0.5019	0.4799	0.4777	0.5547	0.00133	0.275
250	0.5995	0.5812	0.5811	0.5872	0.00105	0.180

4. Limit of Detection (LOD)

LOD is calculated from the formula: -

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

S = slope of the calibration curve

σ = Standard dev. of the response

Topiramate: 1.823 µg/ ml

5. Limit of Quantification (LOQ)

LOQ is calculated from the formula: -

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

σ = Standard dev. of the response
S = slope of the calibration

Topiramate: 5.52 µg/ ml

6. Range

Topiramate: 50-300 µg/ ml

7. **Robustness**

Wavelength of detection was changed by 2nm and % relative difference was calculated.

RESULTS AND DISCUSSION

Because of the absence of any chromophoric group in the Topiramate molecule, it has no absorption in the ultraviolet visible region, and it has no native fluorescence as well. Therefore, direct spectrophotometric determination of Topiramate was not possible. Therefore, derivatization of Topiramate was attempted in the present study for the development of spectrophotometric method for its determination, Ninhydrin was used as chromogenic reagent for primary and secondary amines [20- 23], however, and its reaction with Topiramate has not been investigated yet. Therefore, the present study was devoted to explore Ninhydrin as a derivatizing reagent in the development of spectrophotometric method for the determination of Topiramate in tablets. Our preliminary experiments in investigating the reaction between Topiramate and each of Ninhydrin revealed that Ninhydrin-derivative was found to have maximum absorption at 568 nm. Figure 3. Shows the absorption spectra of Ninhydrin-Topiramate reaction product. The following sections describe the optimization of the assay variables and validation for the performance of spectrophotometric method.

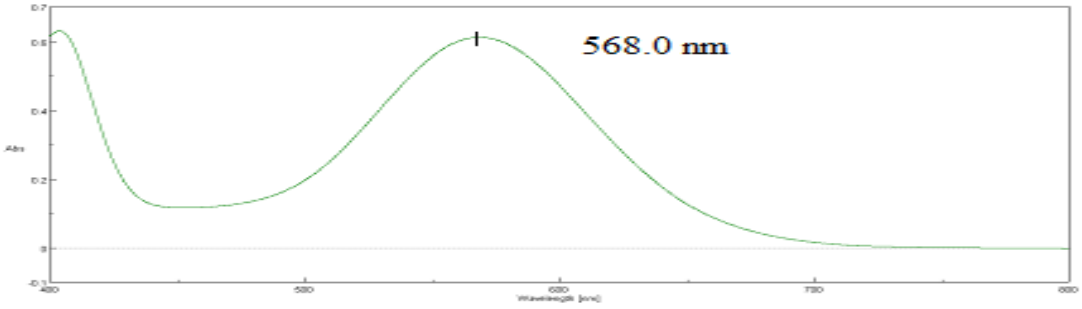


Fig-3: Zero order absorption spectra of Topiramate--Ninhydrin reaction products 50 µg ml-1

Method Development:

Optimization of Spectrophotometric Procedure: The factors affecting the derivatization reaction (the concentrations of Ninhydrin and concentration of pyridine, heating time, and the diluting solvents) were investigated by altering each variable in a turn while keeping the others constant. The studying of Ninhydrin concentrations revealed that the reaction was dependent on Ninhydrin reagent. The maximum absorption intensity was attained when the concentration of Ninhydrin was 1 mL of 2.0 % (w/v) in the final solution. The results of investigating the effect of different concentrations of pyridine on the reaction revealed that the optimum concentration was 10%. The effect of heating time on the absorption intensity was studied by carrying out the reaction at 100◦C for different time intervals the highest absorption was obtained after 20 minutes.

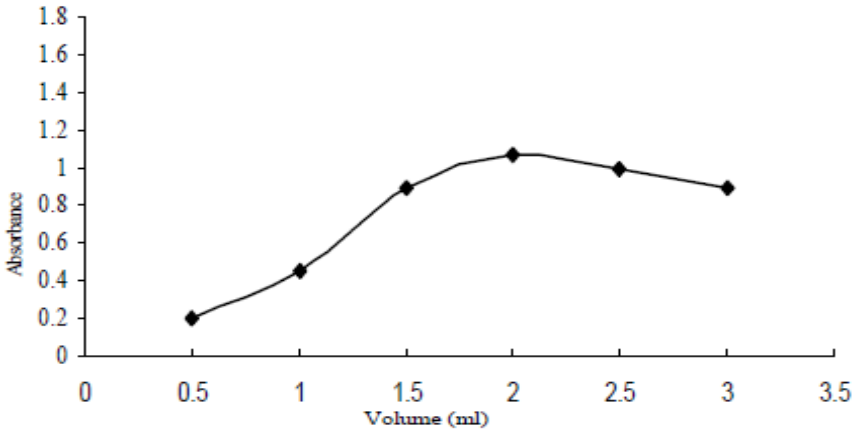


Fig-4: Effect of different volume of Ninhydrin (2.0% w/v) on the formation of colored reaction product with Topiramate (50 µg ml-1).

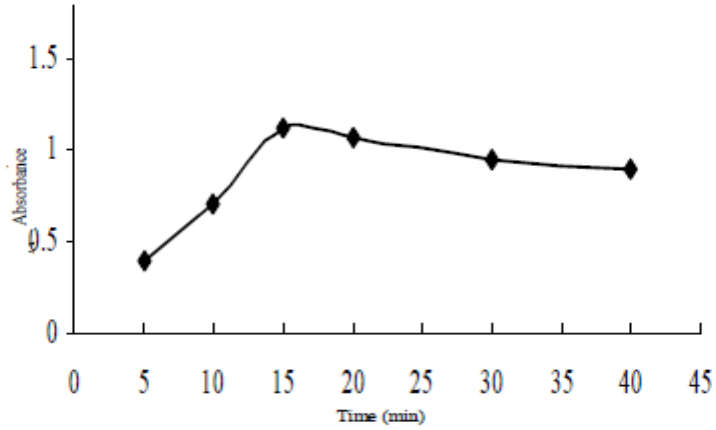


Fig-5: Effect of heating time in boiling water bath on the formation of Topiramate-Ninhydrin colored reaction product (50 µg ml -1).

Method Validation:

Linearity, Limits of Detection and Quantitation: In the proposed method, linear plots (n = 5) with good correlation coefficient (0.9963) was obtained in the concentration ranges of 50 – 300 µg ml−1 for the spectrophotometric method. The limits of detection (LOD) and quantitation (LOQ) were determined [25] using the formula LOD or LOQ =κσ/S, where κ = 3.3 for LOD and 10 for LOQ, 'σ' is the standard deviation of the intercept, and 'S' is the slope. The LOD and LOQ values were 1.823 and 5.52 µg ml−1 for the spectrophotometric method.

Table 10: Results of assay validation obtained by applying the proposed spectrophotometric method for the determination of Topiramate in drug substance.

Parameters	Spectrophotometric method
Linearity range	50-300µg/ml
LOD	1.823 µg /ml
LOQ	5.52 µg /ml
% recovery (80,100 and 120 levels)	98-102%
Precision RSD% Interday Intra-day	<2
Accuracy	80- 120

Robustness and Ruggedness: Robustness was examined by evaluating the influence of small variation of method variables, including concentration of analytical reagents and reaction time on the performance of the proposed method. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation of method variables did not significantly affect the procedures. This provided an indication for the reliability of the proposed method during its routine application for the analysis of Top. Ruggedness was also tested by applying the proposed method to the assay of Topiramate using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were found to be reproducible.
The results are shown in table 11.

Table 11: Robustness and Ruggedness for Topiramate

Conc. of drug	Wavelength at 568 nm	Wavelength at 570 nm
100 µg	0.4383	0.5602
100 µg	0.4256	0.5611
100 µg	0.4204	0.5615
100 µg	0.4185	0.5613
100 µg	0.4130	0.5612
Mean	0.42316	0.56106
Std deviation	0.009588	0.000503
% RSD	2.02	0.089

APPLICATION OF THE PROPOSED METHODS TO ANALYSIS OF TOPIRAMATE IN TABLETS

It is evident from the above-mentioned results that the proposed method gave satisfactory results with Topiramate in drug substance. Thus, its tablets were subjected to the analysis of active ingredient by the proposed method. The percentage recoveries were found to be 98-102% of claimed amount for the spectrophotometric method.

CONCLUSION

The present study described the use of Ninhydrin reagents for the development of selective, sensitive, and accurate spectrophotometric method for the determination of Topiramate in drug substance, drug product, and in the presence of pyridine (10%). To our knowledge no spectrophotometric method has been reported before for analysis of Topiramate. The proposed procedure is with comparable analytical performance devoid from any potential interference. This gives the advantage of flexibility in performing the analysis on any available instrument. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. Therefore, this method can be recommended for the routine analysis of Topiramate in quality control laboratories.

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