UV-SPECTROPHOTOMETRIC AND RP-HPLC METHODS FOR THE ESTIMATION OF TROXERUTIN IN BULK AND TABLET FORMULATION

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ABSTRACT

Troxerutin is used as an anti-coagulant drug for the treatment of Hemiplegia, Aphasia, Cardiac stem arteriosclerosis, etc. The aim of the present study is to develop a new analytical method for the estimation of Troxerutin in bulk and in tablet dosage form. Spectroscopic method (method-1) and RP-HPLC (method-2) method have been developed for the quantification of Troxerutin in bulk and in the formulation. These methods are simple, cost effective, accurate and precise. In method-1, Troxerutin showed maximum absorbance at 348 nm in 0.1M acetic acid which is selected as solvent for our analysis based on its stability. Beer's law obeyed in the concentration range of 5-40 mcg / ml. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.2628 and 0.7966 mcg/ml respectively. In RP-HPLC (method-2), the mobile phase selected is 20 mM Phosphate buffer (pH-8): Acetonitrile: Methanol in the ratio 60:25:15%v/v. The flow rate was 1 ml/min. The linearity range was found to be 5-25 mcg /ml. The formulation OXERUTE was selected for analysis and the amount present was found to be 101.69 % and 100.98% for method 1 and 2 respectively. Both the methods are validated as per ICH guidelines. The methods were found to be simple, accurate, Precise and rapid.

Keywords: Troxerutin, Hemiplegia, Aphasia, Arteriosclerosis, RP-HPLC.

INTRODUCTION

Troxerutin is chemically 2-[3,4-bis (2-hydroxyethoxy)phenyl] -5-hydroxy-7- (2 hydroxyethoxy)-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R, 6 S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one[1]. Its Molecular formula is $C_{33}H_{42}O_{19}$ and possessing a molecular weight of 742.675180 g/mol. It is Soluble in aqueous solvents and insoluble in organic solvents. Troxerutin is used as an anti-clotting drug for the treatment of hemiplegia, aphasia, cardiac stem, arteriosclerosis etc. [2]. The structural formula of Troxerutin is given below.

Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development in the recent era. Standard analytical procedure for newer drugs or formulation may not be available in pharmacopoeia hence, it is essential to develop newer analytical methods, which are accurate, precise, and specific, linear, simple and rapid [3]. From the extensive literature survey, it was revealed that there were a very few methods reported for the estimation of Troxerutin from plasma and for Pharmaceuticals dosage forms. Therefore, here an attempt was made to develop simple, cost, effective, accurate,

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precise, sensitive and specific methods for the analysis.

MATERIAL AND METHODS
Reagents and Chemicals
Acetonitrile (HPLC grade), 0.1 M Sodium hydroxide (AR grade), 0.1 M acetic acid, Methanol (HPLC grade), Water for HPLC, Distilled water, were purchased from Qualigens India Pvt. Limited and Loba Chemie India Limited. Troxerutin was obtained as a souvenir sample from Fourrts India (P) Ltd., Kelambakkam, Chennai.

Formulation Brand OXERUTE (Fourrts India Pvt. Limited) containing Troxerutin equivalent to 1000 mg was purchased from a Local Pharmacy.

Instrumentation
Shimadzu AUX- 220 Digital balance, Shimadzu-1700 Double Beam- UV- Visible spectrophotometer consists of halogen lamp and deuterium lamp as light source, with silicon photo diode detector. Shimadzu HPLC system consists of deuterium arc lamp as light source, equipped with UV detector, and C18 Column (150 mm × 4.6 mm i.d., 5 μ) was used for the analysis. Sonicator – Sonica ultrasonic cleaner – model 2200 MH, Micropipette.

HPLC Condition
0.1 M acetic acid was selected as a solvent for the proposed UV spectroscopic method, The absorbance was measured at 348 nm. It was operated at a temperature of 15 to 35° C. Reverse phase C18 column was employed as stationary phase with a mobile phase consisting of a mixture of 20 mm Phosphate buffer (pH - 8) : Acetonitrile: Methanol in the ratio of 60:25:15% v/v and the flow rate of the mobile phase was maintained at 1 ml/min. The column temperature was maintained at ambient. The volume of injection was 20 μl, and the eluent was detected at 254 nm.

METHOD DEVELOPMENT
1. UV Spectroscopic Method [4]
Preparation of standard stock solution
25 mg of Troxerutin was accurately weighed and transferred into 50 ml of volumetric flask and dissolved in 0.1 M acetic acid and made up to 50 ml using 0.1M acetic acid the solution was observed to contain 500 μg/ml.

Preparation of working standard solution
10 ml of the standard stock solution was pipetted into a standard flask and the volume was made up to the mark with 0.1 M acetic acid and obtained a concentration of 50 μg/ml

Quantification in formulation
Twenty tablets of formulation (Oxerute) containing 1000 mg of Troxerutin were accurately weighed and powdered. Powdered tablet equivalent to 25 mg of Troxerutin was transferred into a 100 ml volumetric flask, added 10 ml of 0.1M acetic acid and sonicated for 10 min.

Repeated the extraction consequently by five times (5×10) and made with 100 ml with 0.1 M acetic acid. The above solution was collected by filtering through whatmann filter paper No.41. The aliquots of 1 ml each was taken in to 10 ml volumetric flask and made up to mark with 0.1 M acetic acid and produced 25 μg/ml solutions. The absorbance measurements were made six times for each formulation at 348 nm. The amount of Troxerutin present in each formulation was calculated from the respective calibration curve.

2. RP – HPLC Method
Preparation of standard Troxerutin solution
An accurately weighted quantity of 25 mg of Troxerutin was dissolved in a minimum quantity of methanol and the total volume was made up to 25 ml with more amount of methanol (1000 μg/ml). Further dilution was made by diluting 2.5 ml to 25 ml with methanol and obtained 100 (g/ml solution [5-8].

Estimation of Troxerutin in tablet formulation
Twenty tablets of formulation (Oxerute containing 1000 mg of Troxerutin) were weighed accurately. The average weight was found and powdered. The tablet powder equivalent to 25 mg of Troxerutin was weighed and added a minimum quantity of methanol to dissolve the substance and the total volume was brought to 25 ml with more Methanol (1000 μg/ ml) in a 25 ml volumetric flask. The solutions were sonicated for 10 minutes, centrifuged at 100 rpm for 15 minutes and filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 2.5 ml into 25 ml volumetric flask with methanol and obtained 100 μg/ml solution. 1ml was pipetted out and transferred into six 10 ml volumetric flask individually and made up to the mark with the mobile phase. With optimized chromatographic conditions mentioned earlier, a steady baseline was recorded. After the stabilization of the baseline for about 60 minutes, six test solutions of formulation were injected after filtering through 0.2 (membrane filter and recorded the chromatogram. The concentration of each test solution was determined by using slope and intercept values from calibration graph.

Method validation
The developed method was validated according to ICH and USP guidelines for the validation of analytical procedures.

System suitability
System suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatography system. The parameters like tailing factor, asymmetry factor, the number of theoretical plates, capacity factor were calculated.

1. UV Spectroscopic Method
Linearity and calibration

To series of eight 10 ml volumetric flask, aliquots (1ml to 8 ml) were taken from the above solution and made to the mark using 0.1 M acetic acid. .The absorbance was measured at 348 nm against 0.1 M acetic acid as blank. The calibration curve was plotted in the concentration range 5 to 40 µg/ml using absorbance versus concentration.

Recovery studies

The accuracy of the proposed analytical method was determined by recovery experiments. To the pre-analyzed formulation, a known quantity of standard solution was added and the contents were mixed, finally made up to the volume with 0.1 M acetic acid, filtered and the absorbance was measured at 348 nm. The amount present was calculated from the slope and intercept. The percentage recovery was determined by using the following formula,

$$\% \text{ Recovery} = \frac{N \in xy - \in x \in y}{N \in x^2 (\in x)^2} \times 100$$

Where,

N=number of observations
X=amount added in µg
Y=amount recovered in µg/ml

Repeatability

Repeatability was done by inter-day and intra-day analysis with same sample to conform the precision of the method. The sample was three times on the same day and three times on three successive days.

Limit of detection (LOD) and limit of quantification (LOQ)

From the serial dilutions of the standard Preparation of calibration curve was constructed. Repeated for six times, and the limit of detection and limit of quantification was calculated by using the average values of the slope and standard deviation of intercept.

2. RP-HPLC
Preparation of Calibration graph

In this method, the aliquots of stock solution of Troxerutin (0.5 - 2.5 ml) were transferred into 10 ml volumetric flasks and made up to the mark with the mobile phase. A solution contains 5, 10, 15, 20, 25 µg/ ml of Troxerutin in mobile phase was injected and the chromatograms were recorded at 254 nm. It was found that the above concentration range was linear. The procedure was repeated for three times. The peak areas were plotted against concentration and the calibration curve was constructed.

Recovery studies

To ensure the reliability of the method, recovery studies were carried out by mixing a known quantity of standard drug solution with the pre-analyzed sample formulation and the content were mixed and made to the volume with mobile phase and re- analyzed by the proposed method, the percentage recovery was calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Calibration of standard was repeated for three times. The limit of detection and limit of quantification was calculated by using the average value of the slope and standard deviation of intercept. System suitability parameters such as asymmetric factor, capacity factor, and tailing factor were validated and the results are given in Table - 6.

RESULTS

Two methods were developed for the estimation of Troxerutin in pure form and in its tablet dosage form.

UV Spectrophotometric method :

Troxerutin was dissolved in 0.1 N acetic acid and made further dilution with in the 0.1 N acetic acid to get the concentration of 25 µg/ml. The solution was scanned in UV region in the wavelength range of 200-400 nm against the 0.1 M acetic acid as blank. The spectrum of Troxerutin was found and the wave length maximum was found to be 348 nm. The absorbance of the solution was measured at the selected wavelength in different time interval. It was observed that Troxerutin was stable for 3 hrs, hence this wavelength selected for analysis.

Different aliquots of Troxerutin in 0.1 N acetic acid were prepared in the concentration range of 5 to 40 µg/ml and the absorbances of solutions were measured at 348 nm. The calibration curve was plotted using concentration against absorbance. The procedure was repeated for six times and the optical parameters like correlation coefficient, slope, intercept, sandell's sensitivity, LOD and LOQ were calculated. These are shown in Table-1. The correlation coefficient value of the calibration graph was found to be 0.9996; it indicates that the concentration of Troxerutin has good linearity. The calibration graph is shown in Figure-3.

Oxerute tablets containing 1000 mg of Troxerutin were selected for the analysis. The nominal concentration of 25 µg/ml of Troxerutin was prepared and absorbance of the solution was measured at 348 nm. The amount of test solution was calculated by using slope and intercept. This procedure was repeated for six times to ensure the precision of the method. The percentage label claim of Troxerutin in the tablet formulation was found to be 101.69 %. The results were shown in Table-2.

Results of recovery studies of Troxerutin are tabulated in Table 3. Percentage relative standard deviation (% RSD) value was found to be 0.1157. The low % RSD value indicates that the method has good precision. The precision of the method was further confirmed by Inter-day

and Intra-day studies. The results of the analysis are shown in Table-4.

RP-HPLC Method

An effort has been made to identify a simple, precise, specific and accurate method for the estimation of Troxerutin in bulk and in formulation by using RP-HPLC.

The solution of 10 μg/ml of Troxerutin in mobile phase (20 mM Phosphate buffer pH-8: Acetonitrile: Methanol) was prepared and the solution was scanned in the range of 200-400 nm. At 254 nm, the drug showed maximum absorbance with 2 hours stability. Hence this was selected as a detection wavelength. Quantification of Troxerutin was done by the external standard calibration method.

The linearity was done by using an external standard calibration method with the optimized chromatographic conditions, stock solutions of Troxerutin were prepared by using Methanol and various concentrations were prepared in the range of 5 – 25 μg/ml of Troxerutin in the mobile phase. 20 μl of each solution was injected individually. The chromatograms were recorded at 254 nm.

The calibration curve was plotted using concentration against peak area. The procedure was repeated for three times. The correlation co-efficient value was found to be 0.9999 indicates that the concentration of Troxerutin has good linearity. The calibration graph is shown in Figure 4. The Optical characteristics of Troxerutin are shown in Table-5. The limit of detection

and the limit of quantification were determined by using slope and Standard Deviation. The LOD and LOQ were calculated.

The tablet formulation (Oxerute) was selected for the analysis. From the calibration curve, the nominal concentration (10 μg/ml) was prepared. 20 μl of formulation was injected and the chromatograms were recorded. The percentage of Troxerutin present in formulation was found to be 100.98%. The precision of the method was confirmed by repeatable injection of the formulation for six times. The percentage RSD value was found to be 0.1615. This indicates that the method has good precision. The values are shown in Table-7.

Accuracy was confirmed by recovery studies by adding a known amount of pure drug to the previously analyzed formulation and the mixture was re-analyzed by the proposed method and their chromatograms were recorded. The percentage recovery of Troxerutin present in formulation was found to be 105.11%. The values are given in the Table 8. The percentage RSD value was found to be 1.5876. It was extremely low when compared to the normal value. The high percentage recovery indicates that there is no interference produced due to the excipients used in formulation. Hence, the developed method was found to be accurate.

All the above parameters combined with simplicity and easy of operation ensures that the application of the proposed method for the assay of drug in pharmaceutical dosage forms.

Table 1. Optical Characteristics of Troxerutin - UV Spectrocopy Method-B

S. No.	PARAMETERS	METHOD- B
1	λ max (nm)	348 nm
2	Beer's law limit (μg/ml)	5-40
3	Sandell's sensitivity (mg/cm²/0.001 A.U)	0.044339959
4	Correlation coefficient (r)	0.99961
5	Regression equation (y=mx+c)	Y=0.02239921x +0.011566785
6	Slope (m)	0.02239921
7	Intercept (c)	0.011566785
8	LOD (μg/ml)	0.26288401
9	LOQ (μg/ml)	0.796618211
10	Standard Error of Mean	0.009149002

Table 2. Quantification of Formulation (Oxerute) by UV Method

S.No.	Labelled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained (%)	Percentage Found	S.D.	%R.S.D.	S.E.
1	1000	1021.8	102.18				
2	1000	1016.3	101.63				
3	1000	1012.7	101.27	101.69%	0.1177	0.1157	0.0480
4	1000	1020.0	102.00				
5	1000	1016.3	101.63				
6	1000	1014.5	101.45				

Table 3. Recovery studies for Formulation (Oxerute) by UV Spectroscopic Method

S.No.	Amount present (μg/ml)	Amount Added (μg/ml)	Amount Estimated (μg/ml)	Amount Recovered (μg/ml)	Percentage Recovered (%)	Average Percentage Recovery
1	2.54	2.5	5.0272	2.4818	99.27	
2	2.53	7.5	10.1818	7.6454	101.93	
3	2.55	12.5	15.0336	12.4836	99.86	100.57%
4	2.54	17.5	20.2090	17.6681	100.96	
5	2.54	22.5	25.2363	22.6909	100.84	

Table 4. Interday and Intraday analysis of formulation (Oxerute) by UV Spectroscopic method

S.No.	Labelled Amount (mg/tab)	Percentage Obtained		S.D.		% R.S.D.	
		Intra Day	Inter Day	Intra Day	Inter Day	Intra Day	InterDay
1	1000	101.14%	101.02%				
2	1000	101.08%	101.63%	0.1382	0.1721	0.1361	0.1700
3	1000	101.26%	101.02%				
	Mean	101.16%	101.22%				

Table 5. Optical Charecterstics of Troxerutin by RP-HPLC Method

PARAMETERS	TROXERUTIN
λ max (nm)	254
Beer's law limit (μg/ml)	5-25
Correlation Co- efficient (r)	0.9999
Slope (m)	283114.3
Intercept (c)	295440.6
LOD (μg/ml)	0.217545455
LOQ (μg/ml)	0.659228651
Standard Error	24844.65642

Table 6. System Suitability Parameters for the Optimized Chromatogram by RP – HPLC Method

PARAMETERS	TROXERUTIN
Tailing Factor	1.64
Asymmetric factor	2.00
Capacity factor	0.35

Table 7. Assay of Commercial Formulation (Oxerute) by RP-HPLC method

Sample No.	Labeled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained	Average(%)	S.D.(+/-)	% R.S.D.	S.E.
1	1000	1007.4	100.74				
2	1000	1012.5	101.25				
3	1000	1009.5	100.95	100.98%	0.1631	0.1615	0.0665
4	1000	1006.3	100.63				
5	1000	1010.1	101.01				
6	1000	1013.1	101.31				

Table 8. Recovery studies of Formulation – Troxerutin by RP-HPLC method

S.No	Amount Present (μg/ml)	Amount Added (μg/ml)	Amount Estimated (μg/ml)	Amount Recovered (μg/ml)	Percentage Recovery (%)	S.D.	% R.S.D.	S.E.
1.	5.04	5	10.4633	5.4633	104.63			
2	5.04	10	15.5710	5.55710	105.31			
3	5.04	15	20.2774	5.2774	101.58	1.6687	1.5876	0.9621

Figure 1. Chemical structure of Troxerutin
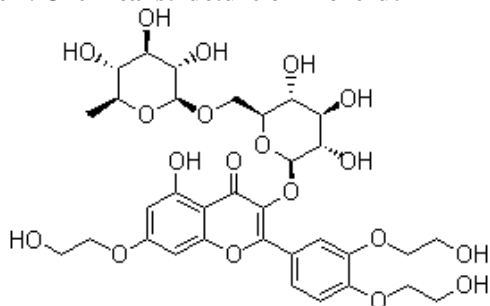
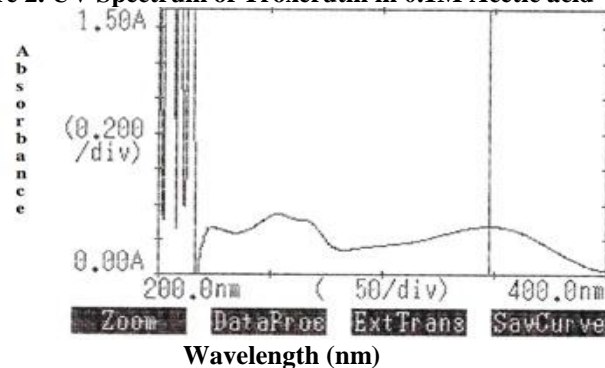**Figure 2. UV Spectrum of Troxerutin in 0.1M Acetic acid**

Wavelength (nm)

Figure 3. Calibration curve for Troxerutin by UV – Method

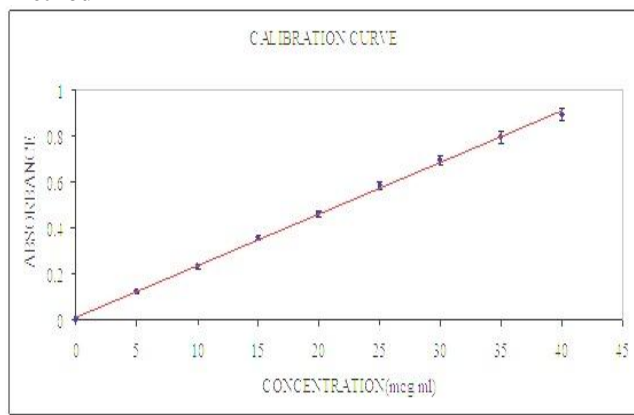**Figure 4. Calibration Curve for Troxerutin by RP-HPLC**
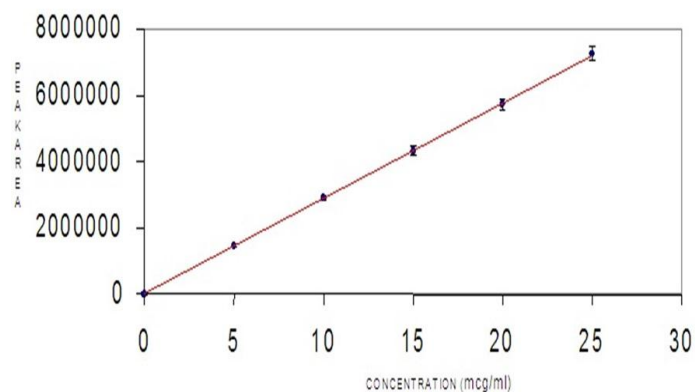
Figure 5. Linearity Chromatogram of Troxerutin (15 mcg/ml)

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Sample Name: Data File: ...2010\TROX10.DAT
Method File: TAX-10.MET
Detector: UV-VIS.   System: HPLC
Date: 11 Jan 2010   Time: 13:24:12
Run: ch1: 3
Type of Analysis : Percent On Area and Height
Report printed on : 20/1/2010 at : 10:26:32

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	5.0

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.09	27992	4335301	100.0000	100.0000	VB	0.106
		3e+04	4335301				

DISCUSSION

In recent years, HPLC has emerged as one of the most sophisticated tools for analysis. The estimation of Troxerutin is done by using UV spectroscopy and Reverse phase HPLC. In UV spectroscopy, Troxerutin showed maximum absorbance at 348 nm in 0.1M acetic acid, The percentage label claim of Troxerutin in the tablet formulation was found to be 101.69 %. Percentage relative standard deviation (% RSD) value was found to be 0.1157. The low % RSD value indicates that the method has good precision. In RP-HPLC, The employed mobile phase consists of a mixture of 20 mM Phosphate buffer (pH - 8) : Acetonitrile: Methanol in the ratio of 60:25:15% v/v. The detection is carried out using UV detector at 254 nm at a flow rate of 1 ml/min, retention time for Troxerutin was found to be 2.09 mins, Linearity range for Troxerutin was found to be 5 - 25 µg/ml.

The quantitative estimation was carried out in the tablet by using both the methods, taking a concentration of 10µg/ml. Then the quantitative results obtained were subjected to statistical validation. The values of % RSD are less than 2.0 % indicating the accuracy and precision of the developed method. The % Recovery of Troxerutin is 105.31% and the amount present in the formulation (Oxerute) was found to be 100.98% in RP-HPLC. The LOD was found to be 0.2175. The LOQ was found to be 0.6592. The suitability parameters such as Tailing factor, Asymmetry factor, Capacity factor and Resolution were in acceptable limit. The results obtained on the validation parameters met the requirements as per ICH and USP guidelines. The developed methods were found to be simple, precise, specific, linear and proportional ie. It obeys Beer-Lamberts law. Both the methods were found to have a suitable application in routine laboratory analysis with a high degree of accuracy and precision.

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