

ESTIMATION OF TROXIPIDE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

A simple, precise, rapid and accurate Reverse Phase HPLC method was developed for the estimation of Troxipide in tablet dosage form. An Inertsil ODS-3V, 250x4.6 mm, column with 5 µm particle size and the mobile phase consisting of 0.03M Ammonium Acetate in water pH: 3.2 adjusted with Acetonitrile (50:50). Acetonitrile in the isocratic mode was used. The flow rate was 0.8 ml/min and the effluents were monitored at 212 nm. The retention time was 3.046 min. The detector response was linear in the concentration of 10-120 mcg/mL for Troxipide. The respective linear regression equation being $Y (-105593.8824) = 65647.112x + 45974.1647$ for Troxipide. The Limit of Detection (LOD) and The Limit of Quantification (LOQ) were 1 mcg/mL and 3 mcg/mL respectively for Troxipide. The percentage assay of Troxipide was 98.17 %. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Troxipide in bulk drug and in its pharmaceutical dosage forms.

Keywords: Troxipide, RP-HPLC, Estimation, and Tablets

INTRODUCTION

Troxipide, chemically is 3, 4, 5-Trimethoxy-N-(piperidin-3-yl)benzamide (Figure 1). The Empirical formula is $C_{15}H_{22}N_2O_4$ and the Molecular weight is 294.35 g/mol. It is used in the treatment of gastroesophageal reflux disease. Troxipide is a novel gastro protective agent with antiulcer, anti-inflammatory and mucus secreting properties¹. Troxipide has cytoprotective properties on the gastric mucosa. Troxipide is a new gastric cytoprotective agent, which neither inhibits acid secretion nor has acid neutralizing activity, but has been clinically proven to heal gastritis and gastric ulcers.²⁻⁴. It has been postulated that Troxipide's mucosal protective effect in gastric ulcer and gastritis is exerted via the inhibition of inflammatory responses and neutrophil-mediated⁵ mucosal injury. As neutrophils are thought to be involved in the pathogenesis of H. pylori-mediated mucosal injury, it is plausible that Troxipide may also be able to mediate gastric mucosal damage due to H. pylori⁶. Troxipide promotes ulcer repair by increasing collagen regeneration⁷ of the ulcer base and causes healing of peptic ulcer. Literature survey reveals a few chromatographic methods to determine the Troxipide in tablet dosage form and in biological fluids. So far, no assay methods by liquid chromatography were reported for the estimation of Troxipide in pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Troxipide in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reverse-phase HPLC method for the estimation of Troxipide in bulk drug samples and in pharmaceutical dosage form.

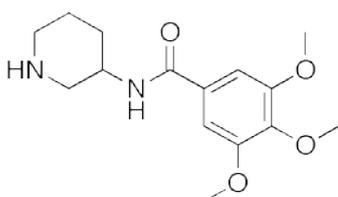


Fig. 1: Troxipide

MATERIALS AND METHODS

Troxipide was obtained as a gift sample from Zuventus Healthcare Limited, Mumbai. Acetonitrile and water used were of HPLC grade (Qualigens). Commercially available tablets Troxip[®] 100 mg, Zuventus Healthcare Limited were procured from local market.

Instrument

Quantitative HPLC was performed on Liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µl, and 2693 pump. An Inertsil ODS-3V, (250x4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

HPLC Conditions

The contents of the mobile phase were 0.03M Ammonium Acetate in water pH: 3.2 adjusted with Acetonitrile (50:50). Acetonitrile in the isocratic mode has been used. They were filtered before use, through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 0.8 ml/min. The run time was set at 30.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 212 nm.

Preparation of Standard Stock solution

A standard stock solution of the drug was prepared by dissolving 100 mg of Troxipide in 100 ml volumetric flask containing 30 ml of diluents 0.03M Ammonium Acetate in water pH: 3.2 adjusted with Acetonitrile (50:50), sonicated for about 15 min and then made up to 100 ml with Acetonitrile to get standard stock solution of 1 mg/mL of Troxipide.

Working Standard solution

5 ml of the above stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent, Acetonitrile to get a concentration of 100 µg/ml of Troxipide.

Preparation of Sample solution

Twenty tablets (Troxip[®] 100mg, Zuventus Healthcare Limited, and Mumbai) were weighed, and then powdered. A sample of the powdered tablet, equivalent to mixture containing concentration of 100 mg/ml of Troxipide active ingredient, was mixed with 30 ml of Acetonitrile as diluent in 50 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding methanol up to 100 ml to obtain a stock solution each of 1 mg/ml of Troxipide. 5ml of the above sample stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent to get a concentration of each 100 µg/ml of Troxipide.

Linearity

Aliquot of standard Troxipide stock solution was taken in a different 10 ml volumetric flask and diluted up to the mark with the mobile phase such that the final concentration of Troxipide was in the range of 10-120 µg/ml. Each of the drug solution (20 µL) was injected three times into the column, and the peak area and retention time were recorded. Evaluation was performed with PDA detector at 212 nm and a calibration graph was obtained by plotting peak area versus concentration of Troxipide (Figure 2). The plot of peak areas of sample against respective concentration of Troxipide was found to be linear in the range of 10-120 µg/ml with correlation coefficient of 1.000. Linear regression least square fit data obtained from the measurements are given in Table I. The respective linear regression equation being $Y (-105593.8824) = 65647.112x + 45974.1647$ for Troxipide. The regression characteristics, such as slope, intercept, and %RSD was calculated for this method and given in Table I.

Assay

20 µl of sample solution (Troxip® Tablets 100 mg, Zuventus Healthcare Limited) was injected into the injector of liquid chromatograph. The retention time was found to be 3.046 min for Troxipide. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table II.

Recovery Studies

Accuracy was determined by recovery studies of Troxipide. A known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in Table II. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solutions of Troxipide. Parameters that were studied to evaluate the suitability of the system are given in Table III.

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

The Limit of Detection (LOD) and The Limit of Quantification (LOQ) were 1 mcg and 3 mcg respectively for Troxipide. From the typical chromatogram of Troxipide as shown in Figure 2, it was found that the retention time was found to be 3.046 min. A mixture of 0.03M Ammonium Acetate in water pH: 3.2 adjusted with Acetonitrile (50:50) in the isocratic mode, was found to be the most suitable as mobile phase to obtain the peaks well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extractions were involved. A good linear relationship ($r=1.000$) was observed between the concentration range of 10-120 µg/ml for Troxipide.

Low values of standard deviation are indicative of the high precision of the method. The assay of Troxipide tablets was found to be 98.17 %. From the recovery studies it was found that about 105.63% of Troxipide was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and tablet dosage forms of Troxipide within a short analysis time.

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Table I: Linear Regression Data of Calibration curve

Parameter	Troxipide
Concentration range (µg/ml)	10-120
Slope (m)	65647.112
Intercept (b)	-105593.8824
Standard error of estimate	45975.1647
Correlation coefficient	1.000
r ²	1.000
% RSD	0.26

Table II: Assay & Recovery of Troxipide in Tablet dosage form

Tablet Formulation	Amount Claim (mg/tablet)	Amount Obtained (mg)* by proposed method	** % Recovery by the Proposed method
1	100	99.31	108.75
2	100	98.27	101.16
3	100	98.94	107.00

* Average of three determinations; ** After spiking the sample

Table III: Validation Summary: System Suitability:

Parameter	Troxipide
Theoretical Plates (N)	2087.14
Tailing factor	1.19
Retention time (Minutes)	3.046
Resolution	-----
% Peak Area	99.22
LOD (µg/ml)	1
LOQ (µg/ml)	3

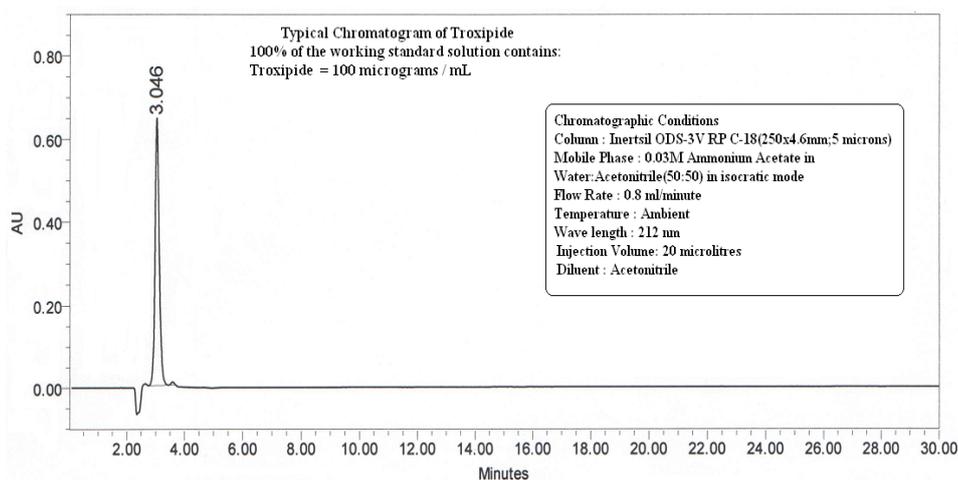


Fig. 2: Typical Chromatogram of Troxipide by RP-HPLC

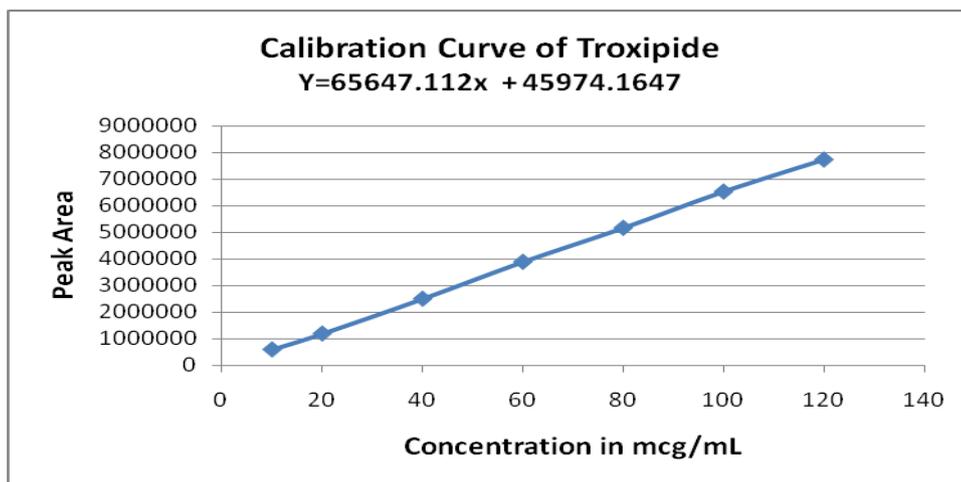


Fig. 3: Calibration Curve of Troxipide by RP-HPLC

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