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A NEW STABILITY INDICATING RP-HPLC ASSAY METHOD OF TROXIPIDE IN API's [PURE] AND DOSAGE FORMS

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ABSTRACT

A new stability indicating RP-HPLC method for determining troxipide (TXD) in pharmaceutical formulations was developed using KH_2PO_4Buffer (pH - 3.0): Acetonitrile in the ratio 65:35 v/v as mobile phase. The calibration curve was linear over the range of 2.0 - 10.0µg.mL^{-1}. The correlation coefficient, slope and intercept were found to be y = 177376x + 422755 with r^2= 0.9994 respectively. The limit of detection and limit of quantification was 0.0249µg and 0.0831µg/ml respectively. The percentage assay of dosage formulation of troxipide was 99.98%. The method was validated by determining its accuracy, precision and system suitability as per ICH norms and the results of this study revealed that the proposed RP-HPLC method is simple, rapid, precise and accurate and stability indicating, which is useful for the routine determination of troxipide in pure[API's] and in its pharmaceutical dosage form.

KEYWORDS: Troxipide, RP-HPLC, API's, and Dosage forms

INTRODUCTION

Troxipide [3,4,5-trimethoxy-N-(3-piperidyl) benzamide as seen in Fig. 1, is a novel gastric Cytoprotective agent with antiulcer, anti-inflammatory and mucus secreting properties. Its molecular formula is$C_{15}H_{22}N_2O_4$ and its molecular weight is 294.4 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 [1-10]. Troxipide has shown to inhibit neutrophil mediated inflammation and oxidative stress in addition to improving the gastric mucus composition and output. Furthermore, it has been found to increase the secretion of cytoprotective prostaglandins. The gastric mucosal metabolism and blood flow are also enhanced by troxipide.

Literature survey reveals that only one analytical method[11] has been reported for the estimation of troxipide (TXD) in dosage forms. The objective of this study was, therefore, to develop and validate, a simpler, economic, rapid, precise and accurate new RP-HPLC method with good sensitivity for quantitative analysis of troxipide(TXD) in pure and marketed formulations in accordance with International Conference on Harmonization (ICH) guidelines.

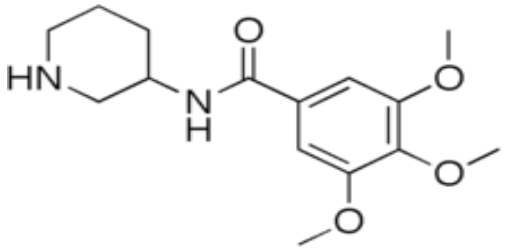

Fig.1. Structure of Troxipide

EXPERIMENTAL:

REAGENTS AND CHEMICALS: Methanol of HPLC grade was procured from E.Merck (India) Ltd, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Potassium dihydrogen phosphate Orthophosphoric acid (AR-Grade), Acetonitrile (HPLC grade), Analytical grade Hydrochloric acid, Sodium hydroxide pellets and Hydrogen peroxide solution 30% (v/v) were obtained from Ranbaxy Fine Chemicals. A reference standard of troxipide(TXD)(99.9% pure) was procured from Zeventus Health company, Mumbai. Tablets of troxipide(Troxip), with a 100mg label claim, manufactured by Zeventus Health company, Mumbai, India were procured from a local pharmacy.

HPLC INSTRUMENTATION: Chromatographic separation was performed on a LC-10AT*vp* binary pump, an SPD-M10Avp photodiode array detector and a Rheodyne manual injector model 7725i with 20µl loop (Shimadzu, Kyoto, Japan) equipped with Phenomenex C_{18} column (250 × 4.6mm I.D.,5µm) connected to a multi-instrument data acquisition and data processing system (Class-VP 6.13 SP2, Shimadzu). The eluent was monitored using PDA detector at a wavelength 240nm. The column was maintained at ambient temperature and injection volume of 20µL was used. The mobile phase was filtered through 0.45 µm filter prior to use. The chromatographic and integrated data were recorded in computer system

PREPARATION OF BUFFER SOLUTION: Dissolve 3.40 g of potassium dihydrogen phosphate in 900ml of water. Adjust to pH 3.0 with orthophosphoric acid and dilute to 1000.0 ml with water.

PREPARATION OF MOBILE PHASE: The mobile phase used was potassium dihydrogen phosphate buffer (pH 3.0 adjusted with orthophosphoric acid) and acetonitrile in the ratio of 65:35(v/v)

PREPARATION OF DILUENT: Mobile phase is used as diluent in the present analysis.

PREPARATION OF STANDARD SOLUTION: The standard stock solution of troxipide(TXD) was prepared by dissolving 25mg of the drug in 25mL of the methanol to get 1.0mg/mL solution. Working solutions were prepared by diluting the aliquots of stock solutions with the mobile phase to contain 2.0 – 10.0µg.mL^{-1} of standard troxipide (TXD) and injected under operating chromatographic conditions. Calibration curves were constructed by plotting peak area versus concentration of troxipide(TXD) and the regression equation were calculated.

PREPARATION OF MARKETED FORMULATIONS: For assaying marketed formulations ten tablets[Troxip;100mg label claim, manufactured by Zeventus Health company, Mumbai] purchased from local pharmacy were accurately weighed and were crushed to fine powder and an amount equivalent to 100mg of troxipide(TXD) was added into 100mL volumetric flask containing 10ml of methanol and later the final volume was made upto the mark with the diluent and was filtered through a millipore filter (0.45µ). From this suitable aliquots were pipetted, diluted with the same diluent to get concentrations 2.0 – 10.0µg.mL^{-1} of working solutions in different volumetric flasks. These flasks were kept in a constant temperature bath at 37^{o}C for half an hour with constant stirring and the contents of the flask were filtered through a millipore filter (0.45µ) and the respective solutions were injected through a rheodyne of 20µL loop to HPLC system at a flow rate of 1.0mL/min as described above((Table..5).

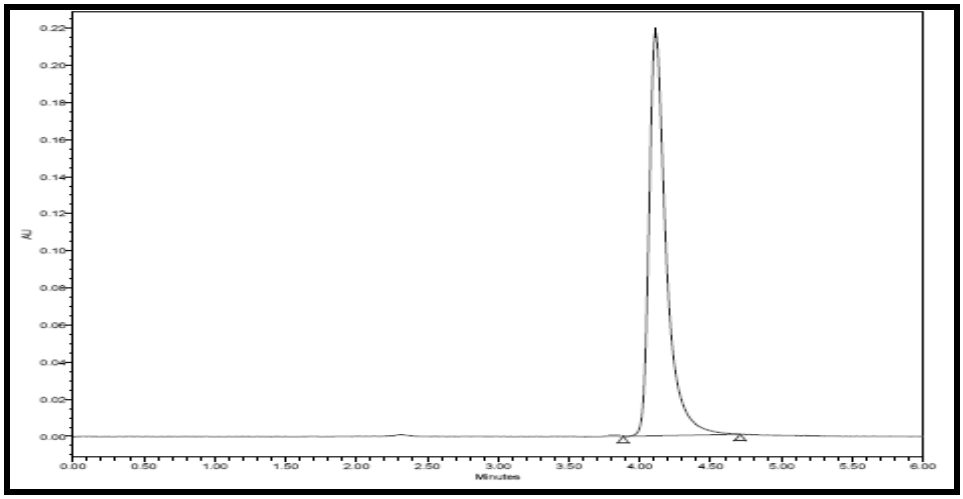

Fig.2. HPLC Chromatogram of Troxipide(TXD)

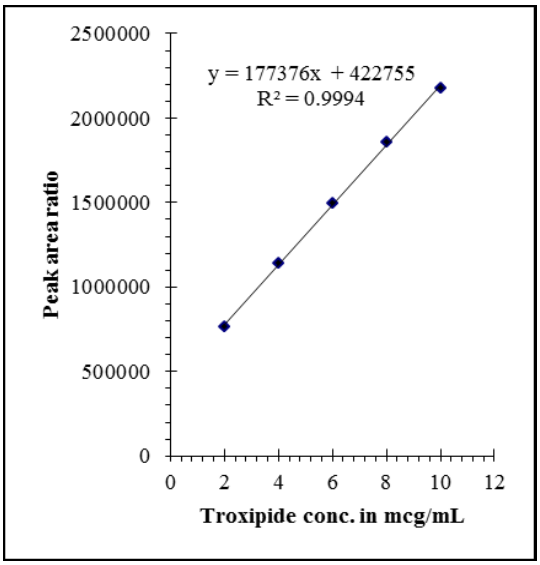

Fig.3. Linearity of Troxipide (TXD)

Table.1:Optimized chromatographic conditions

Chromatographic parameters	PEAK HPLC
Elution	Isocratic
Mobile phase	KH_2PO_4Buffer(pH - 3.0):Acetonitrile in the ratio 65:35 v/v
Column	Phenomenex C_{18} column (250 × 4.6mm I.D.,5µm)
Flow rate	1.0mL / min
Detection	UV at 240nm
Injection volume	20 µL
Temperature	Ambient
Retention time	4.117 min
Run time	6minutes
Area	1854235
Theoretical plates	6328
Pressure	17-20 Mpa

Table.2: Regression analysis of the proposed RP-HPLC Method

PARAMETERS	VALUES
Calibration ranges (µg.mL^{-1})	2.0 – 10.0
Slope	177376
Intercept	422755
Correlation coefficient (r^2)	0.9994
Regression	177376x + 422755
LOD	0.0249
LOQ	0.0831

Table.3: Precision data

Day (Conc 8.0µg.mL^{-1})	Precision Area Mean	R.T.
Day- 1	1854235	4.117
Day-2	1876854	4.115
Day- 1	1878935	4.114
Day-2	1887902	4.110
Day- 1	1856746	4.107
Day-2	1850973	4.098
Average	1867607	4.110
S.D	15486.63	0.006
%R.S.D	0.829	0.169

Average of six determinations

Table.4: Recovery studies of the Proposed RP-HPLC method

Labled amount µg.mL^{-1}	Amount added µg.mL^{-1}	Total amount µg.mL^{-1}	Amount found µg.mL^{-1}	% of Recovery
10	2	12	11.95	99.58
10	4	14	13.94	99.57
10	6	16	15.98	99.87

All the values are the averages of three determinations

Table.5: Recovery studies of tablet containing Troxipide (TXD)

Pharmaceutical formulation	Amount of Troxipide (TXD)		% Recovery
	Labeled	*Found	
TROXIP(Tablet)	100.0 mg	99.98 mg	99.98

*Average of three determinations

FORCED DEGRADATION STUDIES: Forced degradation studies was performed for the selected drug (troxipide) by subjecting the above said drug to acidic, alkaline, oxidizing, thermal and photolytic conditions. For this studies powdered tablets, equivalent to 50mg of troxipide (TXD) was transferred into a 250ml round bottomed flask and were subjected to the given below degradation conditions. After subjecting to different degradation treatments, the stress content solutions were allowed to equilibrate to room temperature and diluted with the diluent and was injected into the column and the respective chromatograms were recorded and evaluated.

i.Acidic condition: In acidic degradation troxipide (TXD) was heated under reflux with 1.0N HCl at 80°C for 1hr and the mixture was neutralized with 1 N NaOH solution. In acidic degradation, it was found that troxipide (TXD) was not degraded.

ii.Alkaline condition: In alkaline degradation troxipide (TXD) was treated with 0.1N NaOH at room temperature for 100mins and the mixture was neutralized with 0.1N HCl solution. No degradation was found in alkali condition.

iii.Oxidative condition: In oxidative degradation troxipide (TXD) was heated under reflux with 30% hydrogen peroxide at 80°C for 1hr. In oxidative degradation, it was found that the drug was not degraded.

iv.Photolytic condition: Photolytic degradation was performed by exposing the drug content in sunlight for 72hrs. It was observed that troxipide (TXD) was not degraded in photolytic condition and the response factor (peak area ratio of standard peak area) of the standard solution and sample solution were calculated.

RESULTS AND DISCUSSION

METHOD DEVELOPMENT: The RP-HPLC procedure was optimized with a view to develop precise and stable assay method. Pure API of troxipide(TXD) was mixed in different mobile phase compositions and was injected into different C_{18} columns such as Hypersil-BDS C_{18}(5µ,250×4.6mm); ODS,C_{18},5µ,250×4.6mm and Phenomenex C_{18} column (25cm x 4.6mm i.d., 5µ). The flow rate was also varied from 0.5mL to 1.2mL.min^{-1}. Finally, Phenomenex C_{18} column (25cm x 4.6mm i.d., 5µ) with a mobile phase of a mixture of KH_2PO_4 Buffer (pH - 3.0):Acetonitrile in the ratio of 65:35v/v at a flow rate of 1.0mL.min^{-1} with UV detection at 240nm gave sharp and symmetrical peak with retention time of 4.117min for respectively (Table.1). The typical chromatogram of sample solution of troxipide is represented in Fig.2. The peak area ratio of standard and sample solutions was calculated. These assay procedures were repeated for six times and mean peak area and mean weight of standard drugs was calculated.

PROCEDURE: Subsequent dilutions of the stock solution of troxipide were made with mobile phase to get concentration of 2.0 -10.0µg/mL. The standard solutions prepared as above were injected into HPLC column with 20µL loop at a flow rate of 1.0mL.min^{-1} and the respective chromatogram was recorded as shown in Fig.2. The retention time of troxipide was found to be 4.117min. A calibration curve was constructed by plotting concentration versus peak area ratio obtained and the amount of troxipide present in sample was calculated through the standard calibration curve. This linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug versus concentration were found to be linear and the results are furnished in Table.2.

METHOD VALIDATION: The following parameter has been used to validate the developed RP-HPLC method for the estimation of troxipide (TXD) in pharmaceutical formulations.

LINEARITY AND RANGE: The linearity of the method was determined at five concentration levels ranging from 2.0 to 10.0µg.mL^{-1} for troxipide. The calibration curve was constructed by plotting response factor against concentration of drugs. The standard calibration curve was plotted and regression equation was calculated as y = 177376x + 422755with r^2= 0.9994 (Fig.2) for troxipide and the results showed that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above (Table.2). The calibration curve is shown in Fig. 3.

SENSITIVITY [LOD]: The LOD and LOQ values were 0.0249 and 0.0831 µgmL^{-1} for troxipide respectively revealing good sensitive of the proposed RP-HPLC method.

PRECISION: The precision of the method was demonstrated either by inter-day or intra-day variation studies. The author performed interday variation studies, by injecting six repeated injections of standard and sample solutions of troxipide (TXD) for three consecutive days and response factor of drug peaks and % RSD were calculated and presented in Table.3. From the data obtained, the developed RP-HPLC method was found to be precise.

ACCURACY 3[RECOVERY STUDIES]: Recovery study carried out for the drug was performed by spiking the known standard drug in powdered formulations. The assay procedure was repeated for standard and sample of troxipide six times and mean peak area ratio and concentration of drug was calculated .The percentage of individual drug found in formulation, mean, standard deviation in formulation were calculated. The results of the recovery analysis were found to be 99.57 to 99.89 (Table.4) and these results exhibited that the amount of drug were in good agreement with the label claim of the formulation(Table..5).

RUGGEDNESS AND ROBUSTNESS: The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010AHT), Agilent HPLC and Water's Breeze HPLC using different columns of similar type like ODS C_{18}, Gemini C_{18} and Hichrom-C_{18}. Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

CONCLUSIONS: The RP-HPLC method for determining troxipide (TXD) in pharmaceutical formulations was developed using KH_2PO_4Buffer (pH - 3.0): Acetonitrile in the ratio 65:35 v/v as mobile phase. The calibration curve was linear over the range of 2.0 - 10.0µg.mL^{-1}. The correlation coefficient, slope and intercept were found to be y = 177376x + 422755 with r^2= 0.9994 respectively. The proposed RP-HPLC method developed by the author is simple, economical and less time consuming than the other reported method[11]. This RP-HPLC method was validated and found specific, accurate, precise, rugged,and robust and stability indicating. The proposed RP-HPLC method developed by the author could be applied to the analysis of formulations, as no interference was observed due to excipients or other components present.

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