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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF COMBINATION DRUGS TRIFLURIDINE AND TIPIRACIL IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A Rapid, simple, precise and accurate reversed phase liquid chromatographic method has been developed and validated for the stability Indicating Assay for the combination Drug Lonsurf 20 mg trifluridine (FTD) / 9 mg tipiracil **(**TPI) in Bulk and Pharmaceutical Dosage Form. The chromatographic separation was achieved on a Kromasil (250 mm × 4.6 mm, 5 µm) analytical column. A mixture of potassium dihydrogen phosphate buffer and acetonitrile in a ratio of 30:70 (pH 2.5) was used as the mobile phase, at a flow rate of 1.0 $mLmin^{-1}$ and detector wavelength at 240 nm. The retention time of Tipracil is 2.3min and for Trifluridine is 2.9min. The HPLC method was fully validated and the performance results of the proposed method were considerably satisfactory with reference to RSD values of validation parameters carried out for linearity, accuracy, precision, and robustness method precision and degradation studies. The linear dynamic range is from 50-300 ppm for trifluridine (FTD) / 22.5-135 ppm for tipiracil **(**TPI). The validated method was successfully applied to quantify the FTD and TPI in tablet form, and the corresponding recovery value was found to be 100.82 and 99.77% for both FTD and TPI. The developed method can be used for pharmaceutical dosage form and in process testing.

KEYWORDS: Trifluridine, Tipiracil, HPLC, Validation, Stability Indicating Assay

INTRODUCTION

Lonsurf is a novel oral nucleoside antitumor agent that consists of trifluridine (FTD) Figure.1 and tipiracil (TPI) Figure 2. Lonsurf is specifically indicated for patients with metastatic colorectal cancer. Trifluridine is Nucleoside Analog Antiviral a fluorinated thymidine analog with potential antineoplastic activity .Trifluridine is incorporated into DNA and inhibits thymidilate synthase, resulting in inhibition of DNA synthesis, inhibition of protein synthesis, and apoptosis. Tipiracil is a drug used in the treatment of cancer and is Tipiracil helps to maintain the blood concentration of trifluridine by inhbiting the enzyme thymidinephosphorylase which metabolizes trifluridine**.** Trifluridine (FTD) is an antineoplastic nucleoside analog discovered by Heidelberger and others at the University of Wisconsin as a drug that inhibits thymidylate synthetase (TS) similarly to existing fluoropyrimidines but exerts a growth inhibitory effect mainly by being incorporated into DNA of tumor cells. A Stability Indicating assay is reported in Literature that is An effective and sensitive stability indicating chromatographic approach based on RP-HPLC for trifluridine and tipiracil in bulk and pharmaceutical dosage form.

EXPERIMENTAL

MATERIALS

Trifluridine (FTD) and tipiracil (TPI) standard supplied by Standard Laboratories Hyderabad, India. Lonsurf containing 20 mg of FTD and 9mg of TPI were purchased from local market Vijayawada, India. Potassium dihydrogen phosphate, Hydrogen peroxide, Sodium hydroxide, HPLC methanol, Acetonitrile, orthophosphoric Acid, Distilled water, Hydrochloric acid was purchased from Merk and Rankem India. MilliQ water was used throughout the experiment.

EQUIPMENTS

Analysis was performed on a chromatographic system of Waters Alliance, with photodiode array detector. A chromatographic separation was achieved on kromasil (250 mm × 4.6 mm, 5 µm) analytical column. Data acquisition was made with Empower 2 software. The peak purity was checked with the diode array detector [DAD].

STANDARD PREPARATION

Accurately Weighed and transferred 9 mg& 20 mg of tipiracil and trifluridine working Standards into a 10ml clean dry volumetric flask respectively, add 5ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents to get a concentration of (90µg/ml tipiracil & 200µg/ml trifluridine)**.** From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluent.

STANDARD SOLUTION AND CALIBRATION GRAPH

Standard stock solution of (90µg/ml tipiracil & 200µg/ml trifluridine)**.** Was prepared in diluent which was a mixture of Water and ACN (50:50). To study the linearity range serial dilutions were made by adding this standard stock solution in the range of 25% to150%for Tipiracil and 50% to 150% for trifluridine (90µg/ml tipiracil & 200µg/ml trifluridine)**.** A graph was plotted as concentration of drug versus peak area response. It was found to be linear. The system suitability test was performed from six replicate injections of standard solution

SAMPLE PREPARATION

10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 7ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluents. Filter through 0.45µ Nylon syringe filter.

METHOD VALIDATION

The HPLC method was validated in terms of linearity, accuracy, precision, robustness method precision and degradation studies according to ICH guidelines ICHQ1A (R2) ICHQ1B . Assay method precision was determined using five independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on different days. The accuracy of the assay method was evaluated by spiking the solution 25% to150% for Tipiracil and 50% to 150% for trifluridine (90µg/ml tipiracil & 200µg/ml trifluridine) of drug substance on placebo in the range of about. Linearity test solutions were prepared from 50-300 ppm for trifluridine(FTD) / 22.5-135 ppm for tipiracil **(**TPI). The degradation of (90µg/ml tipiracil & 200µg/ml tifluridine) and placebo was performed under different stress conditions

(hydrolysis, oxidation, and photo stability, alkaline, acidic, and thermal stress). To determine the robustness of the method, the final experimental conditions were altered and the results were examined. The flow rate was varied by (±) 0.1 $mLmin^{-1}$. the percentage of organic modifier was varied by (±) 2%. Column temperature was varied by (±) 5 °C and pH of mobile phase was varied by (±) 0.1.

RESULTS AND DISCUSSIONS

Validation of Method

Optimization of the chromatographic conditions

During the analysis of basic drugs like Lonsurf 20 mg trifluridine (FTD) / 9 mg tipiracil **(**TPI) one of the well known problem is peak tailing. Since these compounds strongly interact with polar ends of HPLC column packing materials, causing severe peak asymmetry and low separation efficiencies. High purity silica backbone and advances in bonding technology have alleviated the tailing problem of polar compounds in HPLC to a significant extent. During the optimization of the method, different columns (Discovery C18, 250 mm × 4.6 mm, 5 µm; kromacil C18 250 mm × 4.6 mm, 5 µm, Zorbax C18 250 mm × 4.6 mm, 5 µm; Symmetry C18 250 mm × 4.6 mm, 5 µm) and two organic solvents (acetonitrile and methanol) were tested. The chromatographic conditions were also optimized by using different buffers like phosphate, acetate and citrate for mobile phase preparation. After a series of screening experiments, it was concluded that0.05M potassium dihydrogen phosphate buffer pH adjusted to 2.5with orthophosphoricacid buffers gave better peak shapes than their acetate and citrate counterparts. The chromatographic separation was achieved on a Kromasil 250mm x 4.6 mm, 5µ.) Column, by using a mixture of Buffer and Acetonitrile were taken in the ratio of (30:70% v/v) as mobile phase Kromasil columns are having high pH (2–9.5) and temperature (60 °C) stability. About mobile phase, due to basic drug buffer and and acetonitrile was considered as organic solvent instead of methanol. At 25°C column temperature and pH 2.5 of mobile phase, the peak shape of trifluridine(FTD) / tipiracil **(**TPI) was found symmetrical. The flow rate kept was 1.0 mL/min to achieve adequate retention time of trifluridine(FTD) / tipiracil **(**TPI) peak.

Selectivity

Neither formulation ingredients nor degradation products interfered with quantitation of trifluridine (FTD) / tipiracil **(**TPI). All samples and placebo were analyzed using the assay chromatographic condition described. No evidence of interactive degradation products was seen during evaluation. However trifluridine (FTD) / tipiracil **(**TPI) was observed to be susceptible to acidic and Alkaline condition. Selectivity was demonstrated showing that trifluridine (FTD) / tipiracil **(**TPI) peak was free of interference of degradation products indicating that the proposed method is stability indicating.

Accuracy

Accuracy of the method was calculated by recovery studies at three levels for 50%, 100%, 150% levels. The mean percentage recovery obtained for trifluridine (FTD) / tipiracil **(**TPI) was found to be in between 100.38 and 99.77% respectively.(Table1),(Table 2)

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements

obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The method precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing five replicate analyses of the same working solution. The relative standard deviation [R.S.D.)] obtained for trifluridine (FTD) / tipiracil **(**TPI) was 0.32

The intra-day precision of the developed LC method was determined by preparing the samples of the same batch. Accurately Weighed and transferred 9 mg& 20 mg of tipiracil and trifluridine working Standards into a 10ml clean dry volumetric flask respectively, add 5ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents. From the above stock solutions, 1ml was pipette out in to a 10ml volumetric flask and then make up to the final volume with diluents. The %R.S.D,% assay of the assay results was used to evaluate the method precision. The inter-day precision was also determined by the same procedure. The results indicated the good precision of the developed method.

View within Article Linearity

Linearity test solutions were prepared from 50-300 ppm for trifluridine (FTD) / 22.5-135 ppm for tipiracil **(**TPI). The correlation coefficient ('r') value for the drug was 0.999.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of pH of the mobile phase, flow rate, wavelength, column oven temperature. The standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected for every condition and % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (Table 4)

CONCLUSIONS

It can be concluded that the proposed method is fully validated and is found to be simple, sensitive, accurate, precise, reproducible, and relatively inexpensive giving an acceptable recovery of the analyte. The method was specific to drug. The advantage of this method is short retention time and Run time over the other method to gives better result. As the method separates the drug from its degradation products it can be employed as stability indicating one. The developed method can be applicable for pharmaceutical dosage forms and in process testing.

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APPENDICES

Table 1: Recovery of Tipracil Drug

Accuarcy Level	Recovery Area	% Drug Recovery
50%	768304	99.77
100%	1018586	99.52
150%	1266649	99.20

Table 2: Recovery of Trifluridine Drug

Accuarcy Level	Recovery Area	% Drug Recovery
50%	3672133	100.38
100%	4886233	100.82
150%	6099084	99.84

Table 3: Results of Forced Degradation Studies

Stress condition/Duration/solution	Degradation % Tipiracil	Degradation% Trifluridine
Oxidation(1 ml of 20% hydrogen peroxide (H2O2) for 30 min at 60°c	98.41	98.32
Acid Degradation(1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c)	95.50	95.51
Alkali Degradation (, 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0c.	97.42	97.46
Dry Heat Degradation (solid sample 105^0c for 6 hrs)	99.66	99.37
Photo Stability studies	99.16	99.18
Neutral Degradation Studies	99.24	99.32

Table 4: Results of Robustness Study

Change in Parameter	Tipiracil% RSD	Trifluridine %RSD
Flow plus (1.2 ml/min)	1.4	0.4
Flow minus(0.8 ml/min)	1.1	0.2
Column temperature (+2)	0.5	0.4
Column temperature (-2)	0.8	0.7
pH Variation (+0.2)	0.8	0.1
pH Variation (-0.2)	0.7	0.6

FIGURES

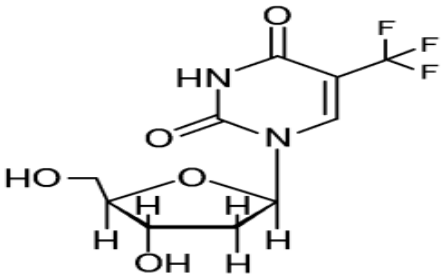

Figure 1: Structure of Trifluridine

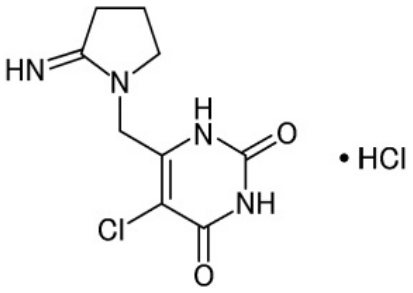

Figure 2: Structure of Tipracil Hydrochloride

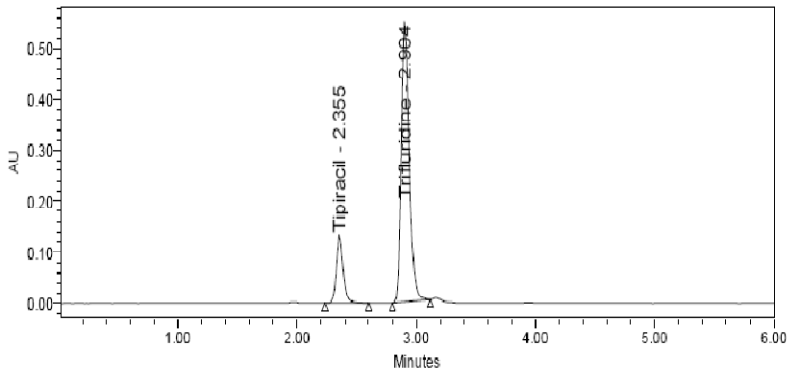

Figure 3: Standard Chromatogram FTD&TPI

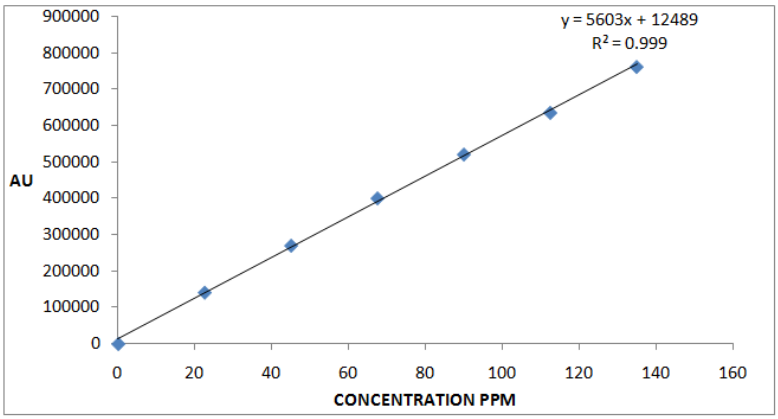

Figure 4: Linearity Plot for Tipracil

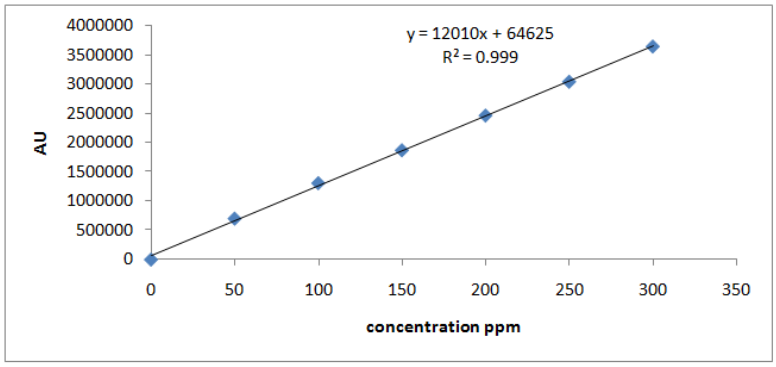

Figure 5: Linearity Plot for Trifluridine

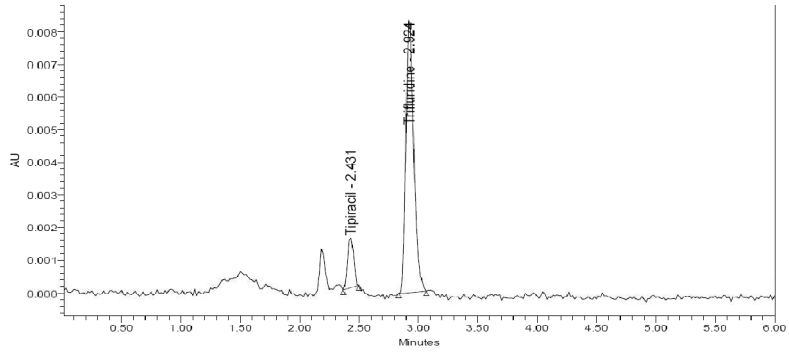

Figure 6: Chromatogram for LOD

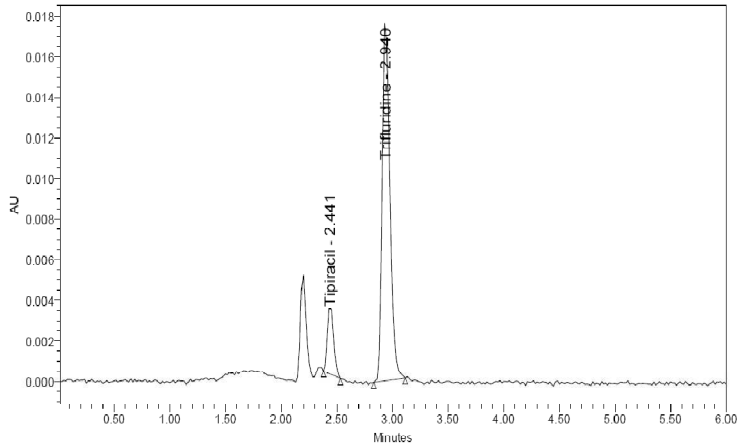

Figure 7: Chromatogram for LOQ

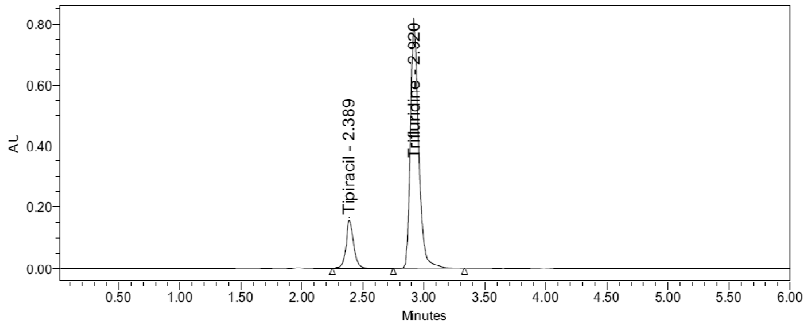

Figure 8: Chromatogram for Accuracy 50%

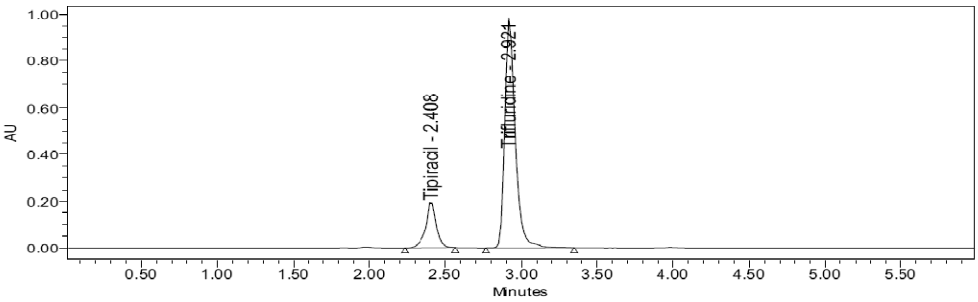

Figure 9: Chromatogram for Accuarcy 100%

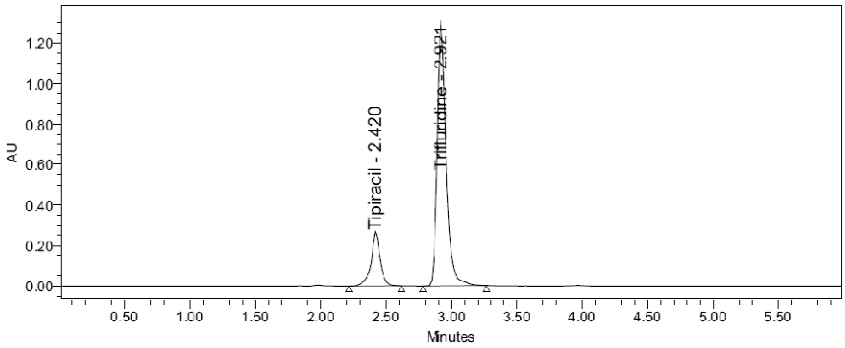

Figure 10: Chromatogram for Accuarcy 150%

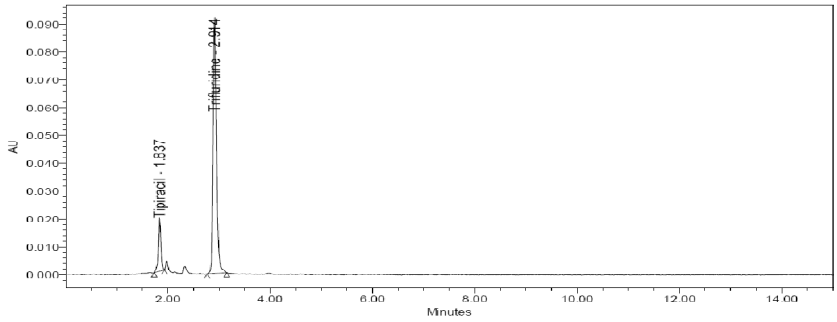

Figure 11: Chromatogram for Acid Degradation

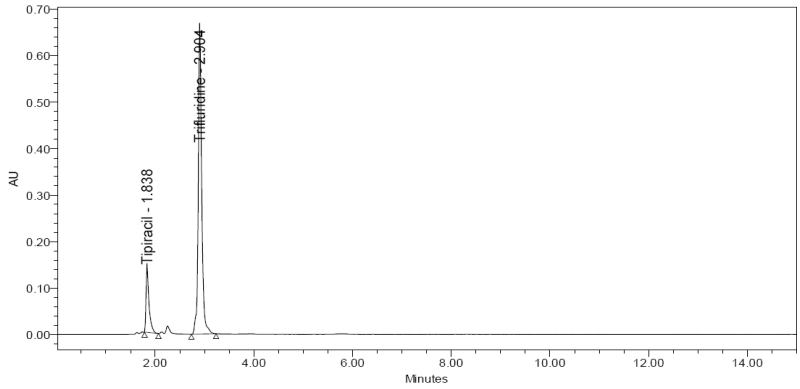

Figure 12: Chromatogram for Base Degradation

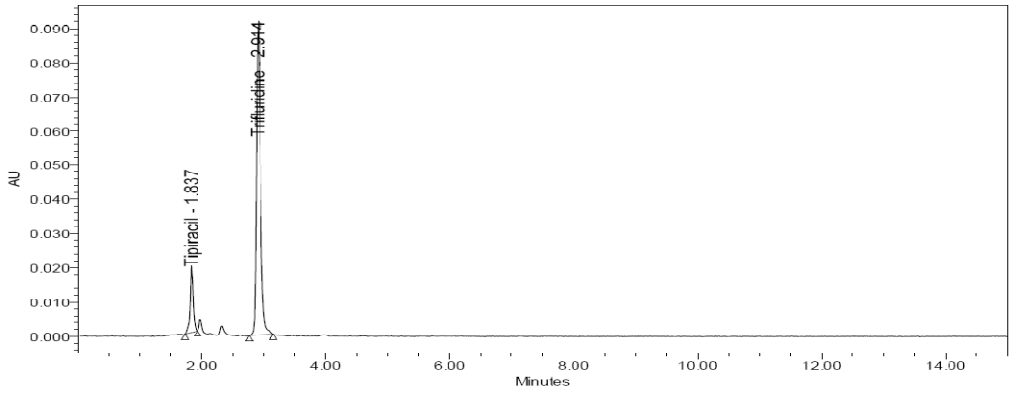

Figure 13: Chromatogram for Peroxide Degradation

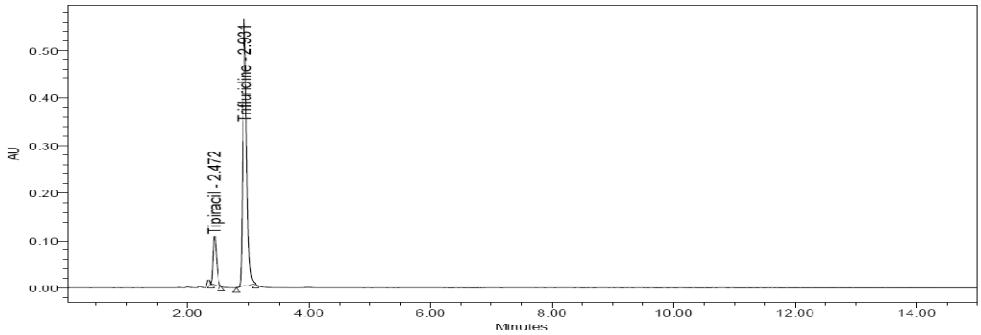

Figure 14: Chromatogram for Thermal Degradation

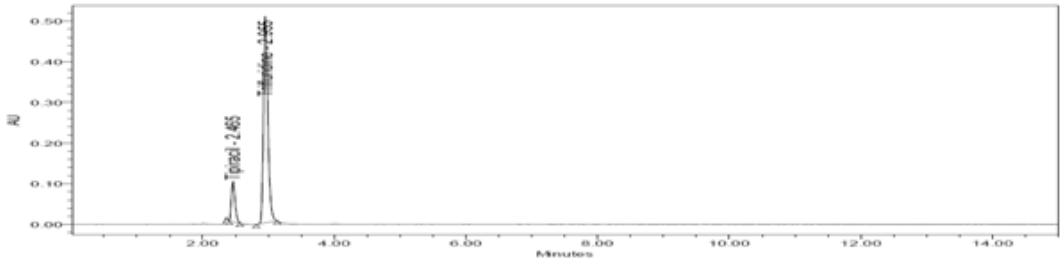

Figure 15: Chromatogram for Photolytic Degradation

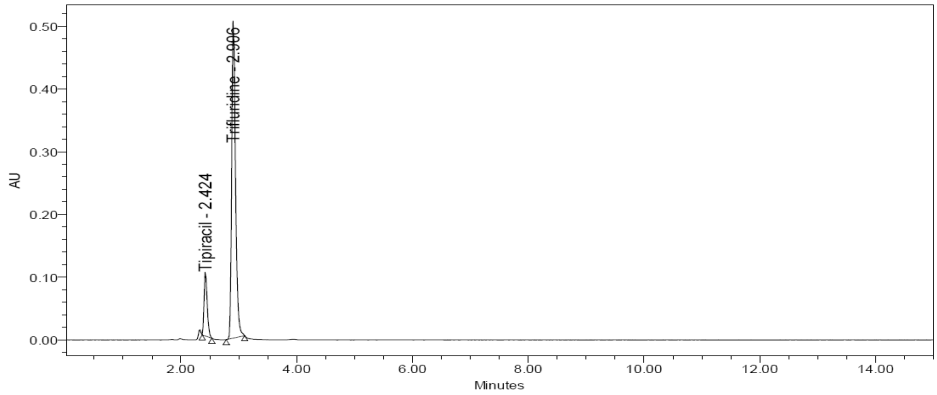

Figure 16: Chromatogram for Water Degradation

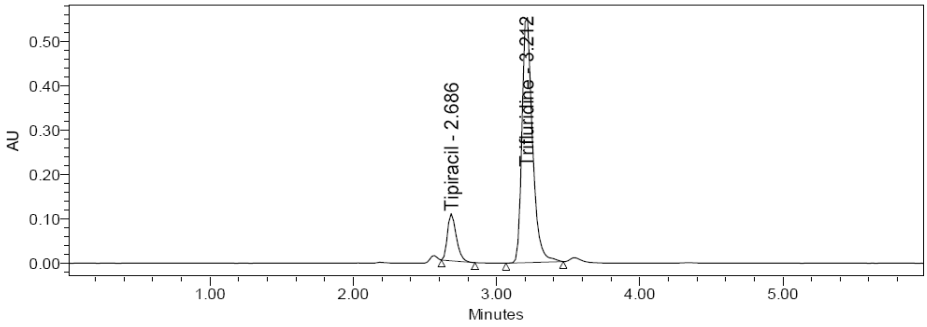

Figure 17: Chomatogram for Flow Minus

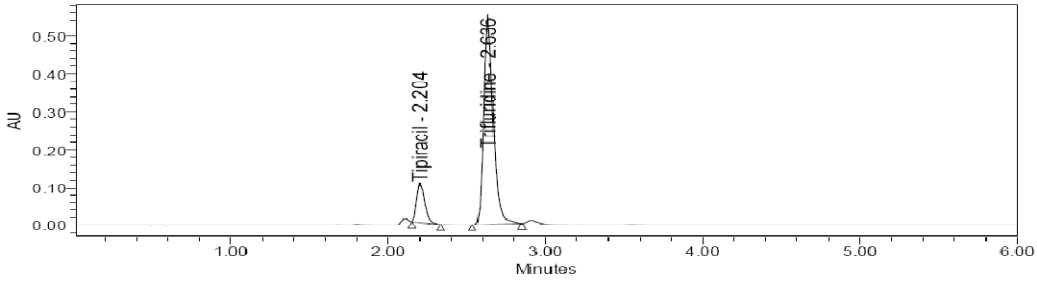

Figure 18: Chomatogram for Flow Plus

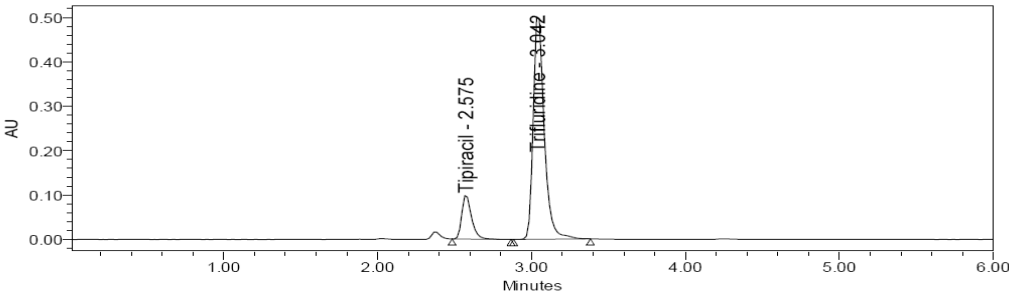

Figure 19: Chomatogram for mobile Phase Plus

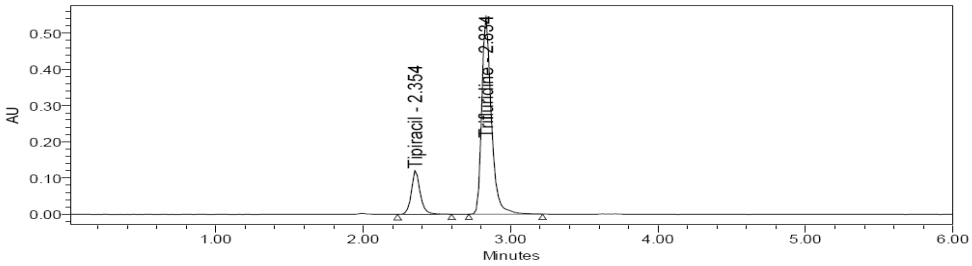

Figure 20: Chomatogram for mobile Phase Minus