VALIDATION AND FORCED STABILITY-INDICATING HPTLC METHOD FOR DETERMINATION OF TRIFLUOPERAZINE HCL

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ABSTRACT:

High performance thin layer chromatography (HPTLC) is a sophisticated instrumental technique based on the full capabilities of thin layer chromatography. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, etc. enable it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic, organic, and biomolecules.

The technique for the forced degradation was successfully applied for the development of stability indicating HPTLC method for the estimation of Trifluoperazine HCL on the plates precoated with silica gel 60 F254. The mobile phase used was ethyl acetate, methanol, and ammonia (7:3:0.1 v/v/v). The drug showed absorbance maxima at 258nm. Forced degradation study of Trifluoperazine HCL was carried out according to the international conference of harmonization (ICH) guideline Q1A (R2). The drug was subjected to acid/base hydrolysis, oxidation, thermal and photolysis degradation. There

was no interference between the drug peak and peak of degradation product; therefore, the method was found to be specific for the determination of Trifluoperazine HCL in the presence of the degradation product. Under different stressed conditions,Trifluoperazine HCL showed degradation product only under acidic hydrolysis at Rf value of 0.44 ±0.02.

KEYWORDS: Analytical Method Development, Analytical Method Validation, DoE, HPTLC, Stability Indicating Method

1 INTRODUCTION:

Phenothiazines exert photosensitization and their photodecay may occur either by a free-radicals chain process, i.e. autoxidation, and/or by involving ex-cited singlet molecular oxygen, i.e. oxygenation.[1] Tri-fluoperazine, [10(3-(4-methyl-1-piperazinyl)propyl)-2-(trifluoro methyl)-10-H-phenothiazine], is an anti-psychotic tricyclic antidepressant commonly pre-scribed for treating psychiatric disorders and core symptoms of schizophrenia. Such a drug is capable of photosensitization by both types of mechanism.[2] A number of other photodecomposition products, viz. photodegra-dates, including N-oxides, hydroxy derivatives, di-meric or polymeric products, excited monomers (excimers), in addition to the sulfoxides and sulfones, of various phenothiazines were isolated and char-acterized.[3] The molecular characteristics/phototoxicity relationship of phenothi-azines demonstrated that the tricyclic moiety is essential for the phototoxic activities. The importance of phenothiazines has prompted many researchers to establish methods for their identification and quantifi-cation. A variety of analytical procedures are based on the oxidation of the drugs, which are usually done chemically.[4-8] The versatility of liquid chromatography, especially HPTLC, in the drug assay is now very familiar because of its advantage in being a separating tool and the one most indicative of stability. Some HPTLC procedures have been described for the determination of trifluoperazines singly or with other phenothiazines.[9-10]

A comprehensive literature search revealed the lack of a suitable procedure for the determination of Trifluoperazine HCL in pharmaceutical dosage forms. The basic purpose of our work was to develop the cost-effective stability indicating HPTLC method for the determination of Trifluoperazine HCL. There after this method was validated as per ICH[50] guideline and successfully applied for the analysis of commercially available samples. In

this study we avoided the use of acetonitrile due to its scarcity in market, instead methanol was used which is readily available and environmental friendly.

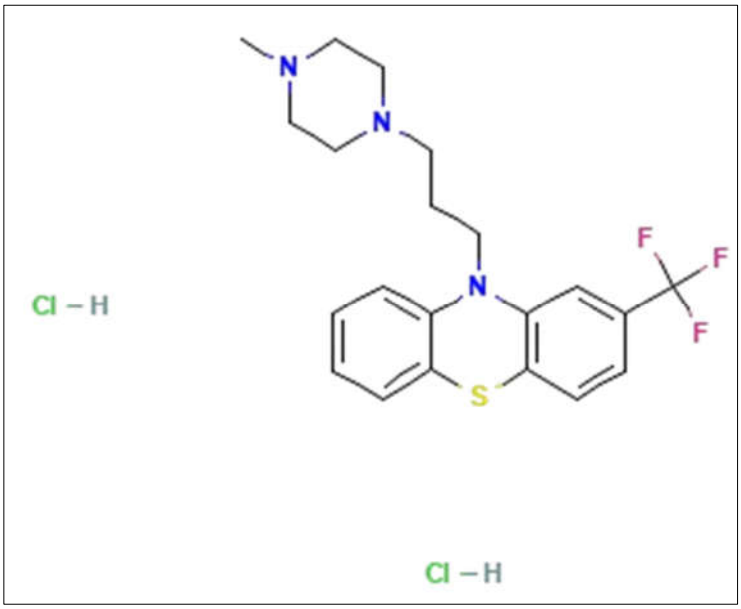


Fig.1: Structure of Trifluoperazine HCL

2 MATERIAL AND METHODS

2.1 CHEMICALS AND REAGENTS

Trifluoperazine HCL was procured from yarrow Chem. Pharmaceutical Ltd. Mumbai, India. Ethyl Acetate, Methanol and Ammonia used were analytical grade and purchased from Merck Chemicals, India. The marketed formulation of Trifluoperazine HCL (Trifluxidyl-5 tablet 5mg) was purchased from local market of Pimpri Chinchwad, Pune,, India.

2.2 PREPARATION OF STOCK STANDARD

In a stock standard solution, methanol was used as a solvent. Stock standard solution was prepared by dissolving 10mg of Trifluoperazine HCL in 10ml volumetric flask and dissolve in methanol it becomes 1000µg/ml concentration. From 1000µg/ml concentration withdraw 1ml of solution and transfer in 10ml of volumetric flask and make volume 10ml with methanol to obtain 100µg/ml concentration.

2.3SELECTION OF DETECTION WAVELENGTH

The standard stock solution (100 µg/ml) was diluted with methanol and scanned at 200-400 nm to obtain the spectra. The drug had significant absorption at 258 nm.

3. HPTLC INSTRUMENTATION

For chromatographic measurements Camag HPTLC, Win cats 1.4.2 software, Linomat 5 applicator, twin through chamber, 100µl syringe and TLC scanner3.

3.1 Chromatographic conditions

The stock standard solution (300ng-800ng) was applied in an 8mm band on pre-coated silica gel 60 F254 (10cm × 10cm × 0.2mm thickness) plates using the Camag Linomat 5 applicator. A continuous application rate of 120nL/sec was employed and the spacing between the two bands was kept at 7.0mm. The slit dimensions were preserved at 5mm × 0.30mm. A mobile phase consisting of ethyl acetate, methanol, and ammonia (7:3:0.1 v/v/v) was employed. A 20cm x 10cm glass container with twin troughs was used for linear ascending development. The optimum chamber saturation time for the mobile phase was 20 minutes at room temperature (24 ± 2ºC), and the chromatogram formed up to 80mm in length. The created TLC plates were dried in the current air using an air dryer. The Camag TLC scanner 3 was used in absorbance mode at 258 nm for densitometric scanning. The source of radiation used for detection was a deuterium lamp.

4 Force degradation study of bulk drug

Force degradation studies were carried out under condition of acid/ base hydrolysis, oxidation, thermal and photolysis. For each study, samples were prepared as follows.

1. The blank subjected to stress in the same manner as the drug solution

2. Working standard solution of Trifluoperazine HCL subjected to stress condition.

Dry heat and photolytic degradation were carried out in solid state. 0.8µL of the resultant solution was then applied at TLC plate and densitogram was developed.

4.1 Acidic degradation

The study of acidic degradation was performed by heating the 1mL of standard stock of Trifluoperazine HCL (1000 µg/mL) with 1 mL 0.1 N Hydrochloric acid (HCl) at 30^0C for 30 mins. and mixture was neutralized with 0.1 N Sodium hydroxide (NaOH) volume was made up to 10 mL with methanol. From this stock 5µL sample was spotted and analyzed.

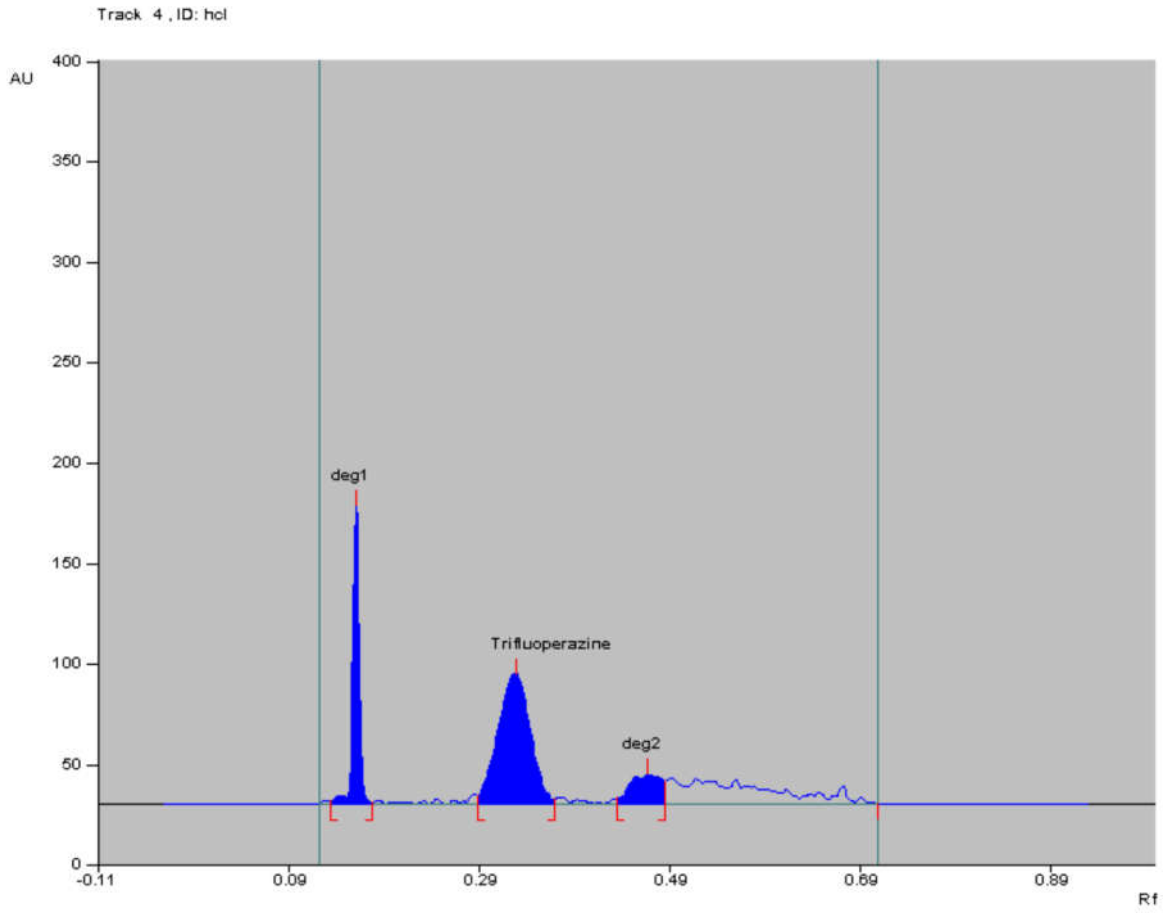


Fig.: Densitogram of HCL (Acidic) Degradation

4.2 Alkali degradation

Alkali degradation study was performed by refluxing the 1mL of standard stock of Trifluoperazine HCL (1000 µg/mL) with 1 mL 0.1 N NaOH at 30^0C for 30 mins. and mixture was neutralized with 0.1 M HCl volume was made up to 10 mL with methanol. From this stock 5µL sample was spotted and analyzed.

Oxidative degradation

Oxidative degradation study was performed by refluxing the 1 mL of standard stock of Trifluoperazine HCL (1000 µg/mL) with 1 mL 3% hydrogen peroxide (H2O2) at 30^0C for 30 mins. Volume was made up to 10 mL with methanol. From this stock 5µL sample was spotted and analyzed.

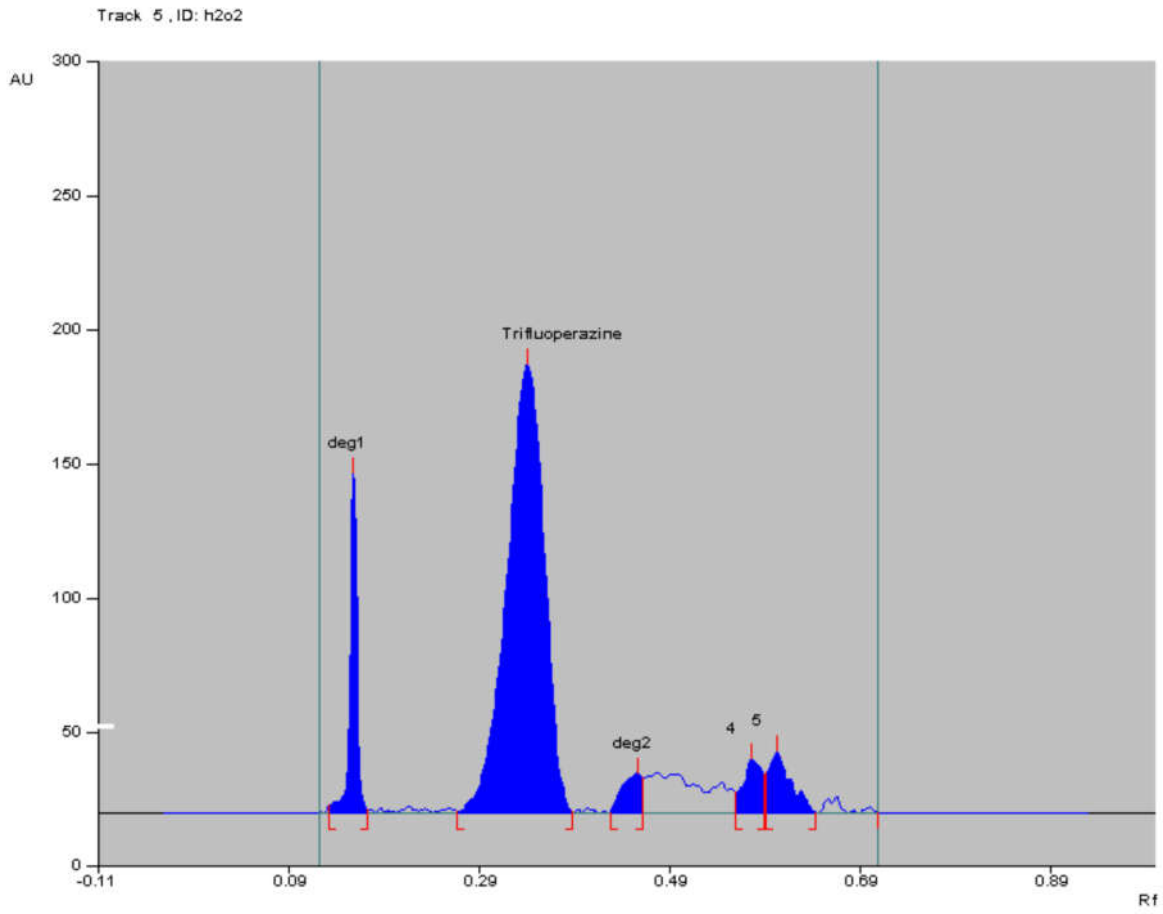


Fig.: Densitogram of H_2O_2 Degradation

4.3 Linearity

The linearity was accessed by plotting calibration curve of Trifluoperazine HCL. For these, six different concentrations of Trifluoperazine HCL like 100, 200, 300, 400, 500 and 600 ng/band were applied on plate. The calibration curve was obtained by plotting area Vs. concentration.

4.4 Precision

Intraday precision was performed by taking three different concentrations (80, 100, 120 ng/band) in the triplicates and were analyzed within a day with same operator and with same equipment. Interday precision was determined by analyzing three different concentrations (80, 100, 120 ng/band) on different day within same laboratory conditions.

4.5 Accuracy

Accuracy was determined by standard additional methods. The study was determined by spiking known amount of standard stock to the test solution prepared from tablet formulation at three different spiking level 80%, 100%, 120% of target concentration. At each level, three determinations were analyzed. The concentration of each drug was determined using line equations obtained from calibration curve.

4.6 Limit of Detection (LOD) & Limit of Quantitation (LOQ):

The linearly increasing concentration of Trifluoperazine HCL was injected and the obtained areas were plotted against respective concentration to get predication linearity plot. The LOD concentration (in µg/mL) is 3.3 times ratio of steyx and slope of the prediction calibration plot while LOQ concentration (in µg/mL) is 10 times the ratio of steyx and slope of the prediction calibration plot, which meets the criteria defined by ICH guidelines.

LOD was calculated using the following formula, LOD = 3.3 σ/S

Where **σ** is the standard deviation of the response and **S** is the slope of the calibration curve.

LOQ was calculated using the following formula, LOQ = 10 σ/S

Where **σ** is the standard deviation of the response and **S** is the slope of the calibration curve.

4.7 Robustness

The robustness study was performed by influencing small but deliberate variations like change in saturation time in the chromatographic conditions.

4.8 Specificity

Specificity refers to the ability to analyze a drug clearly in the presence of constituents that are expected to be present. Impurities, degradants, a matrix, excipients, and additional substances are typical examples. The method's specificity was determined by testing for excipient interference with the analyte.

5 Analysis of marketed formulation

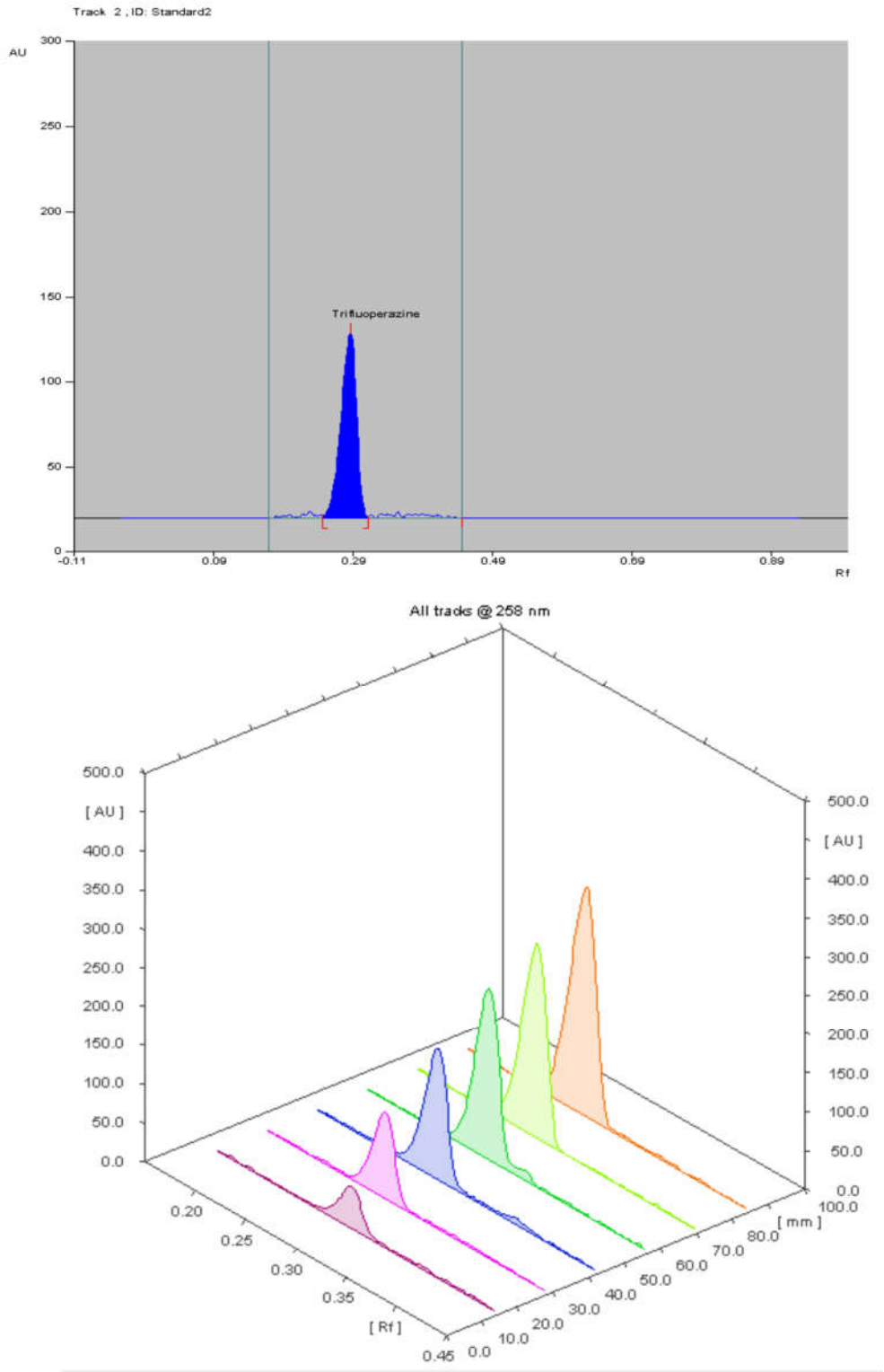
For the estimation of tablet dosage form, the TRIFLUXIDYL-5 tablet 5mg was used. Twenty tablets were weighed and average weight was calculated. Then the tablets were triturated and powder equivalent to 10mg was weighed and transferred to 10ml volumetric flask and diluted with 5ml methanol. Then it was sonicated for 5 minutes and this solution filtered through Whatman filter paper. Volume was adjusted up to 10ml to obtain 1000µg/ml. From this solution again accurate 1ml solution transfer in 10ml of volumetric flask and volume adjusted up to 10ml by methanol to obtain concentration 100µg/ml and from this solution dilutions were made according to respective method and percentage amount was calculated.

6 RESULTS AND DISCUSSION

6.1 Development of optimum mobile phase

The developed TLC procedure was optimized for analysis and quantification of Trifluoperazine HCL. TLC method was optimized with varying mobile phase ratios ethyl acetate: methanol; ammonia. The mobile phase ethyl acetate: methanol: ammonia with

ratio (7: 3: 0.1) gave excellent resolution, dense, compact and well separated spots of Trifluoperazine HCL as well as a sharp peak at Rf were found to be 0.40 for Trifluoperazine HCL (Fig.2).



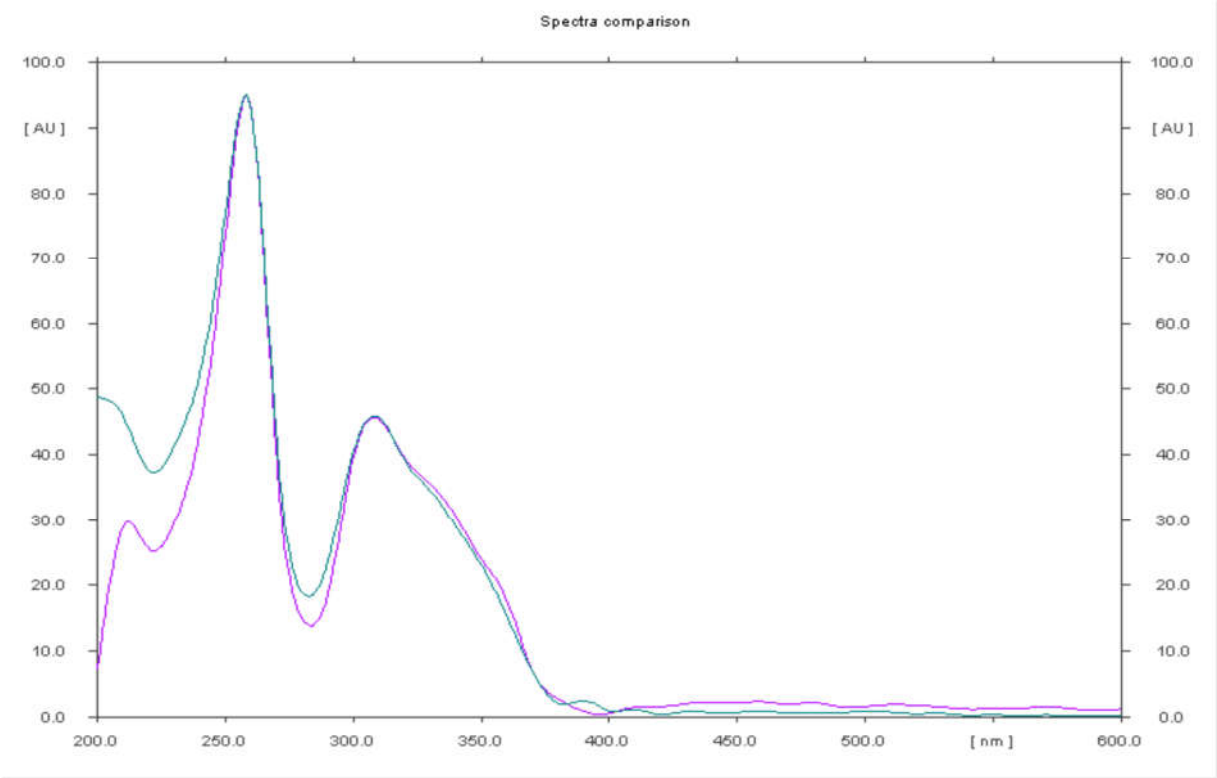


Fig.2. A) A typical TLC Chromatogram of Trifluoperazine HCL (Rf = 0.40) B) 3D overlay of HPTLC densitogram of calibration bands of Trifluoperazine HCL & spectral comparison of Trifluoperazine HCL standard & test preparations.

In this work λmax was found to be 258nm which was selected as the detection wavelength.

The calibration curve was found to be linear over the range of 300-800ng/band for Trifluoperazine HCL

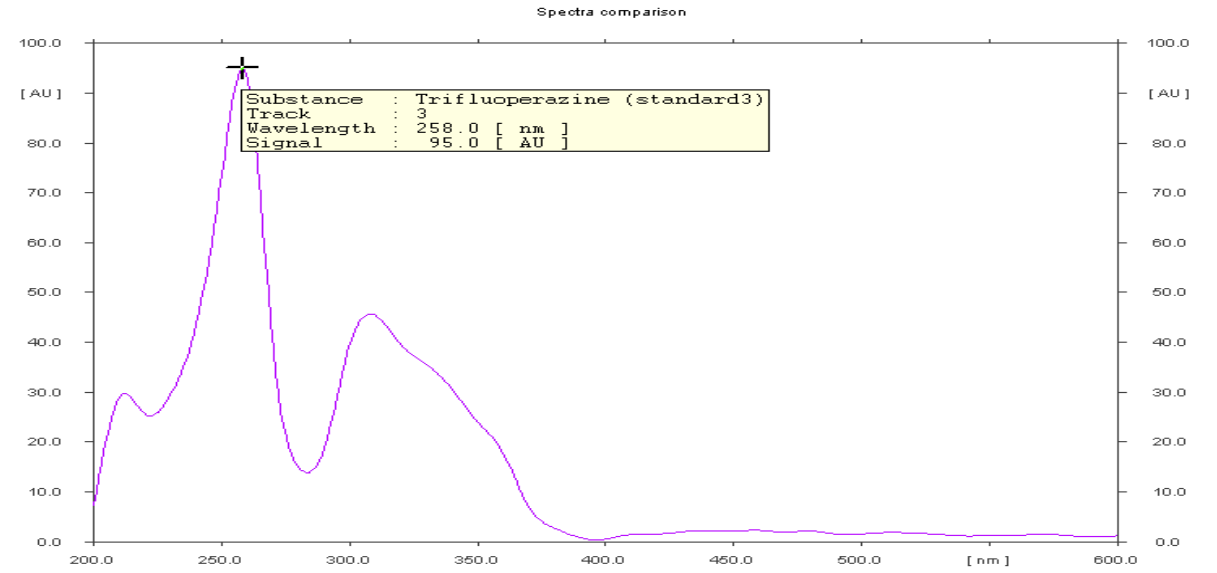


Fig. 3 UV Spectra Trifluoperazine HCL (10µg/ml)

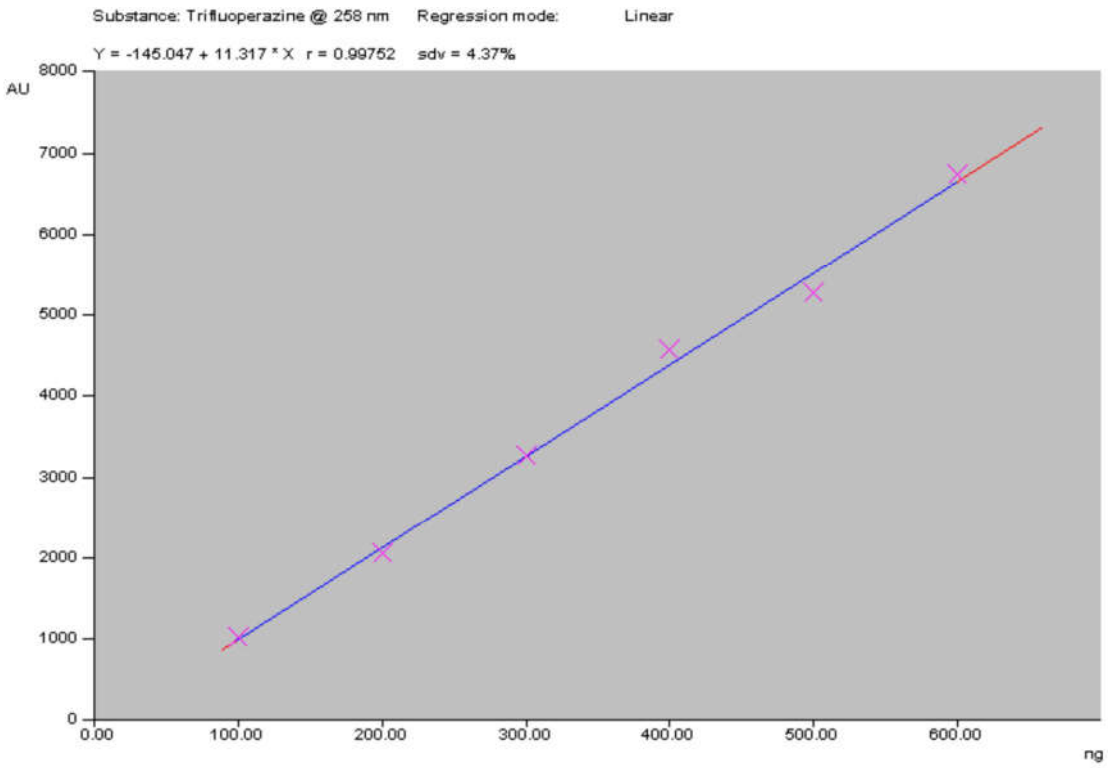


Fig. 4 Linearity Curve of Trifluoperazine HCL (300-800ng/band)

The data for regression analysis of the calibration curve are shown in table 1.

Table 1: Regression analysis data of Calibration Curve

Parameter	Trifluoperazine HCL
Wavelength (nm)	258nm
Linearity range (ng/band)	300-800
Slope	5658.4
Intercept	145.04
Correlation Coefficient	0.996

The accuracy of the method was determined by calculating recoveries which were found to be 98.78 - 99.45%. The high values indicate that method is accurate. The precision was determined by performing repeatability and reproducibility test and the RSD value

for Trifluoperazine HCL were found to be within standard limit. The low RSD values indicate that the method is precise. The detection limit for Trifluoperazine HCL was 58.035ng/band while quantitation limit was 175.86ng/band. The validation parameters are summarized in table 2.

Table 2: Summary of Validation Parameters

Parameters	Trifluoperazine HCL
Accuracy (%)	98.78 – 99.45
Intra-day Precision (%RSD)	0.524
Inter-day Precision (%RSD)	0.430
Specificity (%RSD)	0.441
Limit of Detection(ng/band)	0.333
Limit of Quantitation(ng/band)	1.112

The proposed method was applied to the determination of Trifluoperazine HCL in tablet dosage form. The result for Trifluoperazine HCL was comparable with the corresponding labeled amount (Table No.3).

Table 3: Assay Result of Tablet Dosage Form

Formulation	Actual Amount (mg)	Amount Found ± SD(mg)	% of Drug Found ± SD
Tablet	5	7.952± 0.117	99.40± 0.1117

The stability study revealed that Trifluoperazine HCL was stable for basic, thermal, photolytic conditions and it shows additional peak in acid stress condition. The chromatogram of the acid degraded sample for Trifluoperazine HCL showed peaks at Rf value of 0.59, 0.60 respectively (Figure 5). The areas of the degraded peaks were found to be less than the area of the standard drug concentration, indicating that Trifluoperazine HCL undergoes degradation under acidic conditions. This concluded that the drug was hydrolyzed under acidic conditions to degraded chromophoric products.

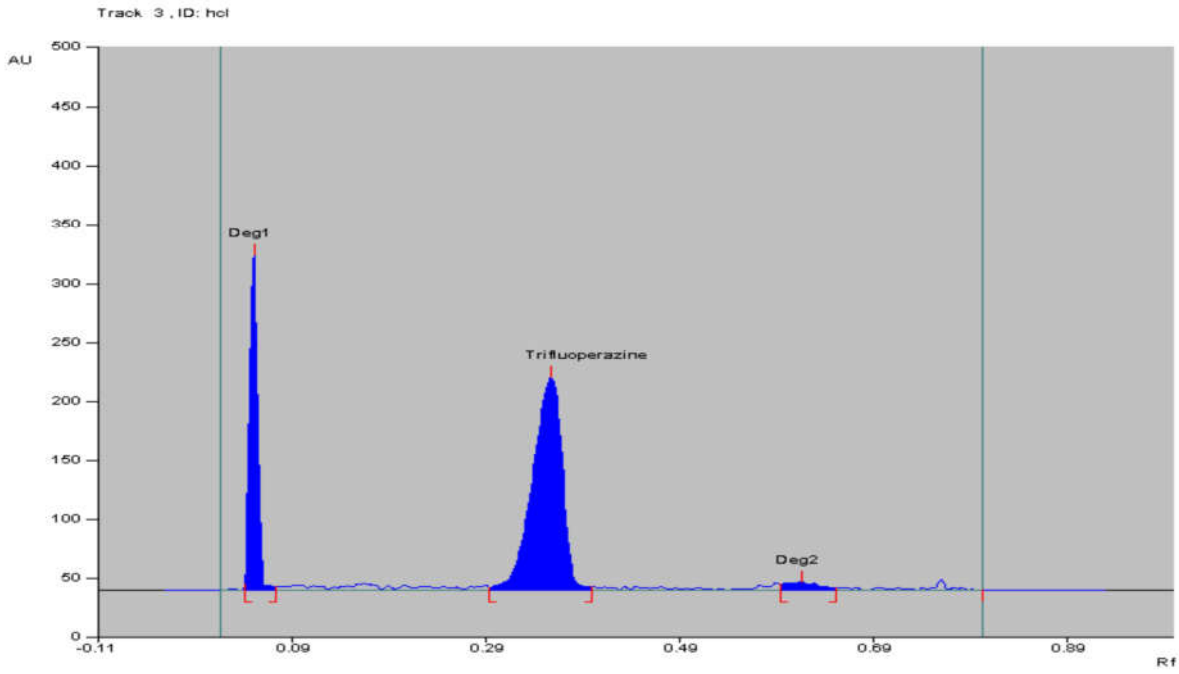


Fig. 5: Densitogram of Acidic Degradation

7 CONCLUSION

A new stability-indicating HPTLC method has been developed to estimate Trifluoperazine HCL in bulk and tablet dosage forms. The newly developed method has been evaluated and found to be simple, sensitive, precise, and robust, making it suitable for routine analysis of Trifluoperazine HCL in both bulk and pharmaceutical dose forms. The forced degradation studies were carried out by ICH standards, and the results indicated that the method was appropriate for studying the long-term stability of Trifluoperazine HCL under various conditions, including acid, basic, and oxidative. Finally, the method has been developed to be highly sensitive, simple, and cost-effective, as well as capable of separating the drug from degradation products.

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