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Development, Validation and Stability indicating RP-HPLC Method for the Analysis of Trifluoperazine Hydrochloride in API and Pharmaceutical Dosage form

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ABSTRACT

A simple, cheap, fast and accurate Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for determination of Trifluoperazine Hydrochloride and its degraded products in pharmaceutical dosage form. This method was developed by using an analytical Zodiac C18 Column (250 mmx4.6mm, 5µm) and mobile phase comprises of 70% methanol and 30% acetonitrile. The method was validated and found to be linear, selective, accurate, robust, rugged and precise. The lower Limit of Detection (LOD) and lower Limit of Quantification (LOQ) respectively were 1.50 µg/ml and 5.0 µg/ml. The methods utilized in this study are the degradation of drug i.e. aqueous, 0.1N HCl, 0.1N NaOH, 3% H_2O_2, thermal, photolytic and UV light. Developed HPLC method is able to separate all degrading products from any stress condition from drug peak by resolution of more than 2. This developed method can be used for routine analysis for the estimation of Trifluoperazine Hydrochloride in bulk and in tablet dosage form in pharmaceutical industry because it is simple, cheap and accurate.

Keywords: RP-HPLC, Trifluoperazine Hydrochloride, Validation

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INTRODUCTION

Trifluoperazine (Eskazinyl, Eskazine, Jatroneural, Modalina, Stelazine, Terfluzine, Trifluoperaz, Triftazin) systematic (IUPAC) name is 10-[3-(4-methylpiperazin-1yl) propyl]-2-(trifluoromethyl)-10H-phenothiazine (Figure 1). Molecular formula is $C_{21}H_{24}F_3N_3S \cdot 2HCl$ and molecular weight 480.42.

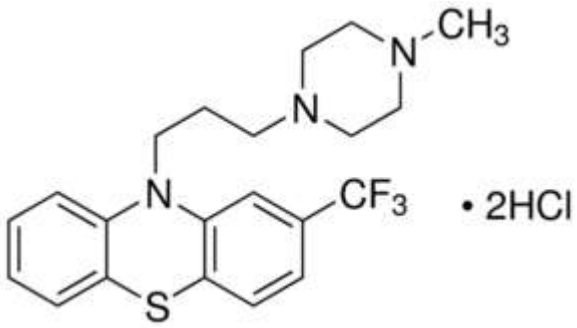


Figure 1: Structure of Trifluoperazine Hydrochloride

Trifluoperazine is a cream coloured fine powder, soluble in methanol, ethanol, and acetonitrile and partially soluble in water. Trifluoperazine is a typical antipsychotic of the phenothiazine chemical class and its derivative is intended for the management of schizophrenia and other psychotic disorders. Trifluoperazine has not been shown effective in the management of behavioural complications in patients with mental retardation.[1] Trifluoperazine has central antiadrenergic,[2] antidopaminergic,[3,4] and minimal anticholinergic effects.[5] It is believed to work by blockading dopamine D_1 and D_2 receptors in the mesocortical and mesolimbic path ways, relieving or minimizing such symptoms of schizophrenia as hallucinations, delusions and disorganized thought and speech.[6] There were HPLC methods for Trifluoperazine along other drugs like Prochlorperazine, Trihexyphenidyl HCl, Isopropamide, Chlorpromazine Hydrochloride, Fluphenazine, and Chlordiazepoxide.[7-16] But there is no method that is devoted to single drug in its pure state and also in the tablet dosage.

The aim of present work is to develop and validate a stability indicating HPLC method for Trifluoperazine Hydrochloride in its single state in the drug and application of this developed method for its estimation in dosage form.

MATERIALS AND METHOD

Chemicals and Reagents

Analytically pure sample of Trifluoperazine Hydrochloride was procured from reputed companies. Methanol and acetonitrile (HPLC grade) were purchased from Merck specialist. Ultra pure water (HPLC grade) was obtained from Fisher Scientific. Tablet formulations, Trazine were procured

from a local pharmacy with labeled amount 2 mg per tablet. Other reagents used were of analytical grade.

Instrumentation and optimization of chromatographic conditions

Chromatography was performed using PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system, Rheodyne manual sample injector with switch (77251), Analytical Zodiac C18 column (250 mm x 4.6 mm, 5µm), Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was employed. Pump mode is Isocratic and the pH was maintained at 5.1. The mobile phase was mixture of methanol and acetonitrile in the ratio of 70:30 v/v .The flow rate was 1.0 ml/min and run time was 10min. The injection volume was 20µl and a chromatographic peak was detected by UV detector at 220nm.

Preparation of Standard and Stock solutions for Validation

Preparation of standard solution

10mg of drug was into a 10ml volumetric flask. Methanol was used to dissolve the drug and it is dissolved completely then final volume was made up to 10ml with same diluents. 1000µg/ml stock solution was obtained. 1ml of the above stock solution was pipette out into a 10ml volumetric flask and diluted up to the mark with diluents.

Preparation of sample solution

Tablets of drug Trifluoperazine Hydrochloride (Trazine – 10mg) were grounded to fine powdered material and powder equivalent to 10mg of drug was taken into a 10 ml of volumetric flask containing 10ml of methanol and was shaken to dissolve the drug and then filtered through Nylon membrane filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 1000µg/ml.

Method Validation

Specificity

Specificity of proposed method was determined by checking blank and retention time of Trifluoperazine peak. Identification of Trifluoperazine peak in sample solution was confirmed by comparing retention time of Trifluoperazine peak with retention time of standard solution of Trifluoperazine.

Linearity

Linearity of the method was evaluated by using 6 linearity solutions of different concentrations. Accurately measured aliquots of solution standard were taken in six different 100 ml volumetric flask and diluted up to the mark with the mobile phase such that the final concentrations of

Trifluoperazine were 25.0 µg/ml, 50.0 µg/ml, 75.0 µg/ml, 100.0 µg/ml, 125.0 µg/ml, 150.0 µg/ml. A 20.0 µl aliquot of each linearity solution was injected in Triplicate.[17]

Accuracy

The accuracy of the method was determined by calculating recoveries of Trifluoperazine by the standard addition method. Known amount of standard solution of Trifluoperazine was spiked into three different levels (50%, 100% and 150% of sample concentrations) and prepared three spiked samples of each level (Total 9 determinations as per ICH guideline). These spiked samples were analyzed against solution standard and the amount of Trifluoperazine recovered in three different levels was calculated.

Intra-day Precision

For the intra-day precision, 100 µg/ml Trifluoperazine Hydrochloride was analyzed at six different intervals of time on a particular day and each time, a triplicate determination was made. The standard deviation relative standard deviation was then calculated

Inter-day Precision

For the inter-day precision, 100 µg/ml Trifluoperazine Hydrochloride was analyzed on the next day to obtain six different determinations. The standard deviation and relative standard deviation was calculated.

Robustness

Robustness of method is its ability to remain unaffected by small changes in method parameters. Robustness of proposed method was demonstrated by making slight difference in method parameters like flow rate (±0.1), detection wave length (±5nm) and mobile phase composition (±5%).

System suitability tests

A chromatogram was obtained for 100µg/ml of Trifluoperazine hydrochloride compared with a standard chromatogram obtained for the same concentration for peak width, shape and base line resolution.

Forced Degradation

Degradation test is performed by incubating the standard for 48 hours in the following different conditions.

Light (Normal and UV light)

To demonstrate the degradation of the sample, kept in open petri dish at laboratory light and UV light. Check the sample at 24 hours exposed sample at laboratory light and UV light. Prepare

sample solutions of the above, inject once again in the chromatographic conditions. Evaluate the degradants in chromatogram and compare to initial values.

Thermal

The sample was kept in a petri dish and keep in oven at 80°C for 24 hours. After 24 hours exposure of the samples, prepare sample solution and inject for HPLC. Evaluate the degradedness in chromatogram and compare to initial values

Acid

Take 25mg sample in 20 ml of 0.1 N hydrochloric acid solutions. After 24hours take 5 ml of acid hydrolyzed sample solution in 25 ml volumetric flask and neutralize with 5 ml of 0.1 N sodium hydroxide solutions and make up with diluents. The above solutions inject once after system suitability solution and evaluate the degradants in chromatogram and compare with standard values

Base

Take 25mg sample in 20 ml of 0.1 N sodium hydroxide solutions. After 48 hours take 5 ml of base hydrolyzed sample solution in 25 ml volumetric flask and neutralize with 5 ml of 0.1 N hydrochloric acid solutions and make up with diluents. The above solution was injected once after system suitability solution and evaluated the degradants in chromatogram and compare with and without base hydrolysis values.

Hydrogen Peroxide

Prepare 25mg sample in 20 ml of 3% hydrogen peroxide. After 24hours take 5 ml of oxidized sample solution in 25 ml volumetric flask and make up with diluents. The above solution was injected once after system suitability solution and evaluated the degradants in chromatogram and compare with and without oxidized (initial) values.

Aqueous

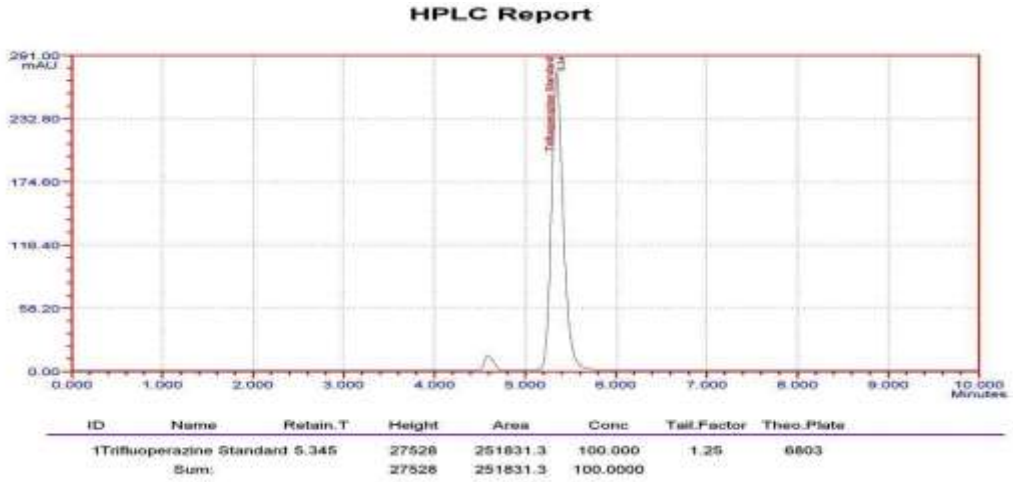
Take 25mg sample in 20 ml of aqueous solution. After 48 hours take 5 ml of sample solution in 25 ml volumetric flask and make up with diluents. The above solution was injected once after system suitability solution and evaluated the degradants in chromatogram and compare with and without aqueous values.

RESULTS AND DISCUSSION

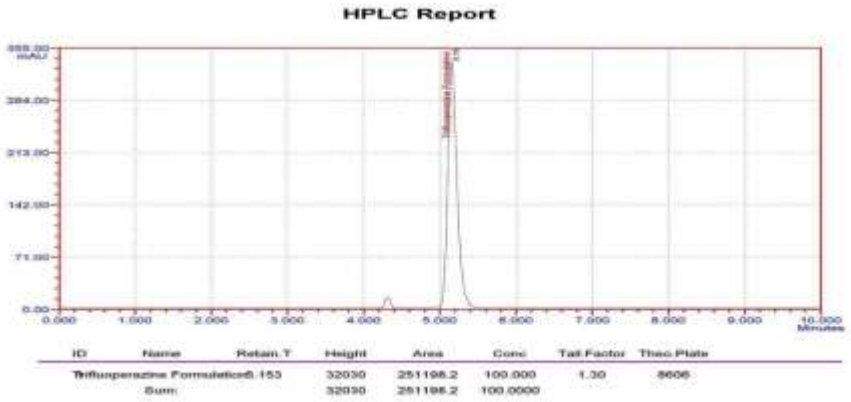
To optimize the chromatographic parameters in this method, several mobile phase compositions were tried. A satisfactory separation, good peak symmetry and to achieve good retention time was obtained with mobile phase consisting a mixture of methanol and acetonitrile in the ratio of 70:30

v/v with a flow rate of 1.0 ml/min. The suitability of the mobile phase was decided on the basis of the sensitivity of the assay, time required for the analysis, ease of preparation and use readily available cost effective solvents. The composition of methanol and acetonitrile in the ratio of 70:30 gave the good results. The proposed method was validated as per ICH guide lines with respect to specificity, linearity, accuracy, precision and robustness. All results of validation parameters meet the limits of ICH guide lines. Chromatograms of blank, Trifluoperazine pure drug and Trifluoperazine tablet form are shown in Figure 2.

Chromatogram of Blank



Chromatogram of Standard solution of Trifluoperazine Hydrochloride



Chromatogram of Trifluoperazine Hydrochloride Tablet

Figure 2 : HPLC Chromatograms of blank and Trifluoperazine Hydrochloride

Specificity

It was observed that there was no interference from blank and at the retention time of Trifluoperazine peak. Retention time of Trifluoperazine peak in sample solution matches the retention time of Trifluoperazine peak in standard solution. These results indicate that proposed method gives uniform and pure peak of Trifluoperazine.

System Suitability:

The system suitability was determined by injecting six replicates of the TFP standard solutions (100µg/ml) and analyzing for its retention time, peak area, theoretical plates, plates per meter, height equivalent to theoretical plate, and peak asymmetry. The system suitability results revealed %RSD for all the parameters. As shown in Table 1, the proposed method meets the accepted requirements.

Table 1: System Suitability

Parameter	Mean Value	%RSD
Retention Time (min)	5.147	0.377
Peak area	254782.91	1.1443
Tailing factor	1.245	1.31
Theoretical plates	7673.16	0.365

Linearity

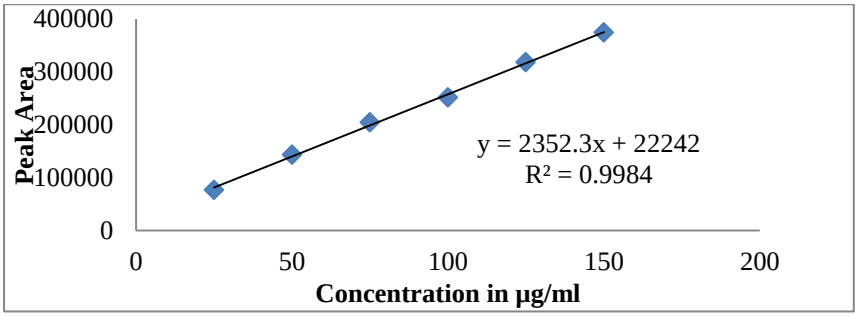


Figure 3: Calibration curve of Trifluoperazine

A calibration curve was obtained by plotting area response versus concentration. Correlation coefficient obtained from graph was 0.998. Linearity curve of Trifluoperazine is shown in Figure 3.

Accuracy

The percentage recoveries of Trifluoperazine Hydrochloride from tablet samples were calculated. Recovery ranged between 98.61% and 101.19%. Recovery values were calculated and the results were shown in Table 2.

Table 2 : Percentage of Recovery

Level	Target in µg/ml	Amount of spiked (µg/ml)	Total in µg/ml	Amount of Recovered (µg/ml)	% Recovery
50 %	50	25	75	74.122	98.830
	50	25	75	74.457	99.276
	50	25	75	74.192	98.923
100%	50	50	100	99.377	99.377
	50	50	100	99.936	99.936
	50	50	100	98.610	98.610
150%	50	75	125	124.54	99.640
	50	75	125	126.22	100.980
	50	75	125	126.486	101.190

Intra-day and Inter-day precision

% RSD values of 6 samples (Intraday and Inter day precision samples) were found to be 1.144% and 1.225%. The closeness of % RSD values indicates that the proposed method is reproducible. Results of Intraday and Inter day precision are shown in Table 3.

Table 3 : Intra-day and Inter-day precision

S No	Amount of TFP in mg	Concentration of TFP in µg/ml	Intra-day Area	Inter-day Area
1	25	100	255439	249377
2	25	100	251507	251135
3	25	100	252838	248944
4	25	100	253026	248010
5	25	100	256441	245071
6	25	100	259444	254181
			SD =2915.69 % of RSD = 1.144	SD =3057.197 % of RSD = 1.225

LOD and LOQ

LOD and LOQ for Trifluoperazine were estimated by injecting a series of dilute solution with known concentration. The parameters LOD and LOQ were determined on the basis of peak

response and slope of the regression equation. The LOD and LOQ of the drug were found to be 1.5 µg/ml and 5.0 µg/ml respectively.

Robustness

To observe the Robustness of this method and its ability to remain unaffected by the changes in the parameters of the method, flow rate of the mobile phase, mobile phase composition and detection wave lengths are changed and results are observed. It is found out that these values indicated the robustness of the method. These results are presented in the Table 4.

Table 4: Robustness Results

S. No	Parameters changes	Change	Peak area	% Change
1	Optimized	-	251831	-
2	Wave length-change-1	215nm	256291	1.771
3	Wave length-change-2	225nm	250165	0.661
4	Mobile phase-change-1	75:25 v/v	256204	1.736
5	Mobile phase-change-2	65:35 v/v	255484	1.450
6	Flow Rate change-1	0.9ml/min	248823	1.194
7	Flow Rate change-2	1.1ml/min	250632	0.476

Forced Degradation

When the Trifluoperazine Hydrochloride was subjected to different extreme conditions like acid, base, aqueous and hydrogen peroxide treatment, thermal, light and UV light treatments, it is degraded and the number of degraded products is shown in the Table 5. The related chromatograms are shown in the Figure 4. In these forced degradation studies, it is observed that the acid degradation and peroxide degradation has shown more degradation than other degradations. Hydrogen peroxide degradation has shown 56.32463% and acid degradation exhibited 55.95975%. Minimum degradation is observed in UV with 3.042313% .

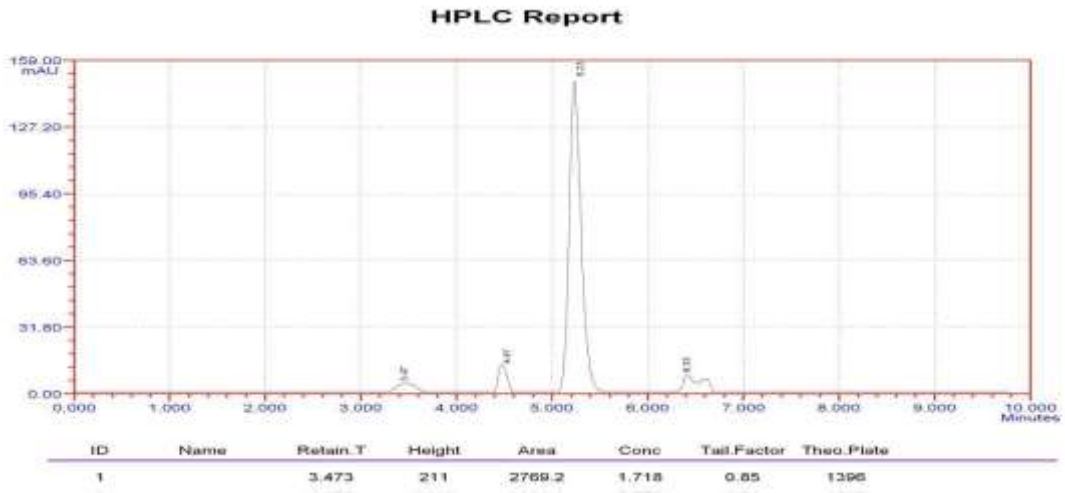
Table 5: Results of the Degradation Studies

S No	Condition	No additional peaks observed	Peak Area	% Obtained	% degradation
1	Standard [125µg/ml]	-	317916	100.00	0.0
2	Acidic	3	140011	44.04025	55.95975
3	Aqueous	2	246278	77.46637	22.53363
4	Base	3	252838	79.52981	20.47019
5	Light	1	252560	79.44237	20.55763
6	Peroxide	3	138851	43.67537	56.32463
7	Thermal	2	252437	79.40368	20.59632
8	UV	2	308244	96.95769	3.042313

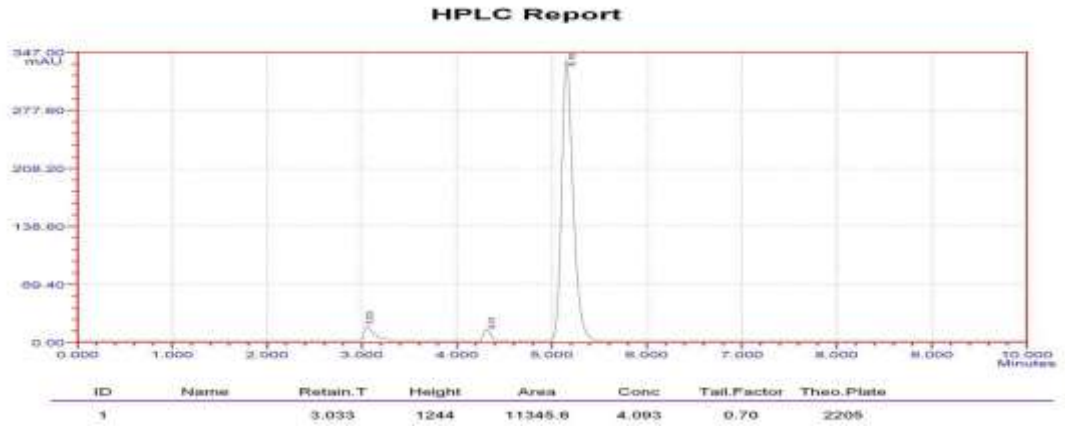
Hydrochloride sample in acidic conditions

Chromatogram of Trifluoperazine Hydrochloride sample in basic conditions

Chromatogram of Trifluoperazine Hydrochloride sample in aqueous state



Chromatogram of Trifluoperazine Hydrochloride sample with Hydrogen peroxide



Chromatogram of Trifluoperazine Hydrochloride sample in Thermal conditions

Chromatogram of Trifluoperazine Hydrochloride sample in the presence of light

Chromatogram of Trifluoperazine Hydrochloride sample in the presence of UV light

Figure 4: Forced Degradation Chromatrograms

CONCLUSION

The objective of the method was to develop and validate an analytical method for the quantization of Trifluoperazine Hydrochloride in bulk and tablet dosage form. It is accomplished by using this RP-HPLC. The assay provides a linear response across a wide range of concentrations and it utilizes a mobile phase which can be easily prepared and diluent is economic, readily available. The proposed method can be used for the quality control of Trifluoperazine Hydrochloride in bulk preparations of the drug and in pharmaceutical dosage forms.

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