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Research Article

Stability Indicating RP-HPLC Method for the Estimation of Trifluoperazine Hydrochloride as API and Estimation in Tablet Dosage Forms

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ABSTRACT
A new stability indicating method was developed for the estimation of trifluoperazine HCl in API, this method was also successfully utilized for the estimation of drug in tablet dosage forms. All the methods were utilized for the degradation of drug i.e. 0.1 N HCl, 0.1 N NaOH, 3 % H_2O_2, thermal and photodegradation. Drug peak was found to be seperated from all the degradants with resolution of more then two. This method was also used for the estimation of Trifluoperazine HCl in tablet dosage forms. The method was found to be simple, precise, specific and accurate. Developed HPLC method is able to separate all degradants produce form any stress condition from drug peak by resolution of more than 2. The order of stability trifluoperazine HCl was found to be H_2O_2<UV<alkali<acid<heat. The newly developed method can be used for routine analysis for the estimation of Trifluoperazine HCl in bulk and tablet dosage form in pharmaceutical industry.

Key-words: TFH, HPLC, Forced degradation, Stability indicating HPLC

INTRODUCTION

Trifluoperazine Hydrochloride, 10-[3-(4-methylpiperazin-1-yl) propyl]-2 tirfluoro methyl phenothiazine dihydrochloride used as antipsychotic. Cream coloured fine powder from absolute alcohol, freely soluble in water, insoluble in dil base, ether and benzene[1]. MOA includes inhibiting dopamine D_2 receptors in the brain possessing α-adrenergic blocking, antiemetic and some anticholinergic activity[2].

Literature review shows that there are developed methods for including UV[2-8], Fluorometric[9,10], HPTLC[11,12], GC-MS[13,14], HPLC/DAD[15] and GLC[16] methods. The review of literature reveals that, stability indicating HPLC method for TFH has published yet. So the aim of present study is to develop and validate a stability indicating HPLC method for TFH and application of developed method for its estimation in dosage form.

MATERIAL AND METHOD

All the reagents used in the method were of analytical grade. HPLC Model JASCO equipped with Winchrom software along with UV-975 detector and PV-980 pump was used. ODS column (Agilent technology) 25×0.46 cm, 5µ pore size was used to separate trifluoperazine and its degraded components.

Standard TFH was kindly provided by Oasis Test House, Ahmedabad, manufactured by A.N. Pharma lab ltd. Formulations for the estimation were purchased from local market and of two different companies, Neocalm (Intas) and Trazine (Sun Pharma).

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Optimization of chromatographic conditions

Chromatographic conditions were optimized to methanol:buffer ratio 75:25, flow rate 1 ml/min and wavelength of 257 nm. Buffer was prepared by dissolving accurately weighed 0.340 gm of potassium dihydorgen phosphate in 250 ml of doubled distilled water, 1.5 ml of triethylamine was added and pH of the solution was adjusted to 2 with a ortho-phosphoric acid. Injection volume 20 µl and run time 15 minutes.

Table 1: Result recovery study using Neocalm (Intas)

Conc. selected (µg/ml)	Conc. before spiking (µg/ml)	Conc. of std added (µg/ml)	Conc. after spiking (µg/ml)	% Recovery
10	10.03	6	16.01	99.88
10	10.03	10	19.93	99.50
10	10.03	14	23.98	99.79

Preparation of standard solution of Trifluoperazine

Accurately weighed 25 mg of TFH was transferred to 50 ml volumetric flask, 30 ml of water added and sonicated for 1 min. volume was made up with water.

Calibration curve for trifluoperazine hydrochloride

4 ml of the standard solution of TFH was diluted to 10 ml with mobile phase. Aliquots of solution like (0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml, 1.2 ml, 1.4 ml, 1.6 ml, 1.8 ml) was diluted to 10 ml with mobile phase to prepared solution of

Table 2: Recovery study using Trazine (Sun Pharma)

Conc. selected (µg/ml)	Conc. before spiking (µg/ml)	Conc. of std added (µg/ml)	Conc. after spiking (µg/ml)	% recovery
10	10.01	6	16.03	100.12
10	10.01	10	20.00	99.95
10	10.01	14	23.98	99.88

concentration (4 µg/ml, 8 µg/ml, 12 µg/ml, 16 µg/ml, 20 µg/ml, 24 µg/ml, 28 µg/ml, 32 µg/ml, 36 µg/ml) each solution was run for 15 min.

Form the calibration curve data, linear regression equation and correlation coefficient found to be y = 937795 X – 469152 and r = 0.9994, where 'y' is area of peak and 'x' is the concentration of drug solution respectively.

VALIDATION

Specificity:

Specificity determination was carried out using commonly used excipients 50 % (180 mg) lactose, 7 % (18 mg) magnesium stearate, and 15 % (37 mg) starch and no peak appeared at RT 7.34 of drug peak indicates indicates the developed method is specific.

Precision:

Repeatability studies was observed by injecting six replicate samples RSD was found 0.328 and 0.180 for area and retention time respectively and both were <1. Method was also validated interms of interday and interaday precision. RSD for intra day was 0.236–0.406 and for inter day 0.247–0.558 less then 2 showing that method is precise.

Accuracy:

Results of analysis, obtained in three experiments with API and different tablet dosage forms, had good agreement with the labeled amount of the drug. In three different 50 ml volumetric flasks, 25 ml of the pre-analyzed tablet solution (2 mg/ml) was taken and it was labeled as f1, f2, f3. And in f1, f2, f3. 3 ml, 5 ml, 7 ml of standard solution (10 mg/ml) added respectively and volume was made up to 50 ml with water, 4 ml of this solution was diluted to 10 ml with mobile phase, 1 ml of this solution was diluted to 10 ml with mobile phase finally 5 ml of this solution was diluted to 10 ml with mobile phase.

Recovery % was found 99.50% – 100.12%, which was within limit 98.00 %– 102.00 %.

Linearity:

Linearity was observed between 4-36 µg/ml. The correlation coefficient was 0.9994. The slop and intercept were found to be 937795 and 469152 respectively.

Range:

Working range was found to be 0.60 i.e LOQ to 36 µg/ml. linearity range was found to be 4-36 µg/ml. Target range found to be 16, 20 and 24 µg/ml and target concentration was found to be 20 µg/ml.

Limit of detection and limit and quantitation:

LOD and LOQ were found to be 0.20 and 0.60 µg/ml respectively.

Ruggedness:

Ruggedness test was determined by varying flow rate and mobile phase composition, flow rate was varied by 10% and mobile phase composition was varied at 5% result of analysis is given in Table 3 and 4. During ruggedness test it was found that on variation of flow rate at 10%, RSD of peak area was found 1.414 and on variation of mobile phase composition at 5%, RSD of peak area was found 0.483 which was <2.

System suitability:

RSD of RT and area of six replicate samples was found to be 0.180 and 0.328 which is less than 1. TF was found to be 1.03 and theoretical plates 6600.

ESTIMATION OF TIRFLUOPERAZINE HYDROCHLORIDE

As an API

50 mg of the sample and standard was taken in a 50 ml volumetric flask. 25 ml of water was added and sonicated for 1 minute. 4 ml of above solution was diluted to 10 ml with mobile phase. Again 1 ml of this solution was diluted to 10 ml with mobile phase.

Concentration was found to be 99.89 % of trifluoperazine HCl.

In Tablets Dosage Form

Twenty tablets (of same respective batch number) of two pharmaceutical companies were taken and average weight was determined. These tablets were powdered and triturated well. A quantity of powder equivalent to 50 mg of trifluoperazine hydrochloride was transferred to 50 ml volumetric flask, and mixed with 30 ml of water and solution was sonicated for 10 minutes there after volume was made up to 50 ml with water. The solution was filtered through syringe filter of 0.45 µ. 4 ml of this filtrate was transferred to 10 ml volumetric flasks and volume was made up with mobile phase. 1 ml of this solution was diluted to 10 ml with mobile phase. Same procedure was used to prepare standard solution.

STABILITY INDICATING STUDIES

Attempt was made to decompose 10-30% of the drug by exposing drug to stress conditions. The tolerable pH range of column is 2.5-8.5 therefore higher alkaline stress

Table 3: Result of ruggedness test by varying flow rate

Flow rate ml/min	Trial 1	Trial 2	Trial 3	Mean	SD	RSD
0.9	18954569	18775426	18642546			
1.0	18512365	18543654	18478496	18506187	261709	1.414
1.1	18206598	18196565	18245463			

Table 4: Results by varying mobile phase composition (Methanol: Buffer)

Ratio (v:v)	Trial 1	Trial 2	Trial 3	Mean	SD	RSD
72 : 28	18456952	18496532	18524789			
75 : 25	18685494	18653254	18602548	18600388	89909	0.483
78 : 22	18685854	18701542	18596524			

Table 5: Analysis of marketed product by developed method

Brand name	Label claim (mg)	Conc. found (mg)	Mean ± SD	% Drug Found	Mean % ± SD	RSD
Neocalm (Intas)	10	10.01 10.02 9.92 10.00	9.9875 ± 0.045	100.1 100.2 99.2 100	99.875 ± 0.457	0.457
Trazine (Sun)	10	10.03 10.01 10.01 10.04	10.0225 ± 0.015	100.3 100.1 100.1 100.4	100.225 ± 0.15	0.149

Table 5: Overall summary of the stability studies of TFH.

Condition	Time	% degradation	Retention time of degradent
Acid, 0.1 N HCl	24 hr ambient temperature	0.0 %	
	4 hr reflux	39.22 %	3.40, 4.84
	3 hr reflux	31.73 %	4.89
	2 hr reflux	20.38 %	4.81
	1.5 hr reflux	14.48 %	4.81
Alkali, 0.1 N NaOH	24 hr ambient temperature	100 %	3.78
	8 hr ambient temperature	31.25 %	3.78
	5 hr ambient temperature	17.00 %	3.78
	3 hr ambient temperature	11.16 %	3.79
	2 hr ambient temperature	4.98 %	3.79
3% hydrogen peroxide	24 hr ambient temperature	100%	3.91
	5 hr ambient temperature	82.13 %	3.91
	4 hr ambient temperature	59.39 %	3.90
	3 hr ambient temperature	45.44 %	3.18, 3.95
	0.5 hr ambient temperature	18.86 %	3.11, 3.96
Exposed to UV radiation	5 hr uv exposer	26.11 %	3.12, 4.48
	4 hr uv exposer	22.78 %	4.46
	3 hr uv exposer	19.09 %	3.19, 4.48
	1.5 hr uv exposer	8.67 %	3.21, 4.44
	1 hr uv exposer	5.95 %	4.46, 5.18
Exposed to sunlight	2 hr Exposed to sunlight	5.19 %	4.44
Dry heat (at 105 °C)	8 hr	4.99 %	3.04
	16 hr	11.92 %	3.04
	20 hr	14.82 %	3.05
Wet heat	refluxed for 5 hr	16.64 %	3.03, 3.37

conditions cannot be used. Results of the degradation of Trifluoperazine HCl is presented in Table 5.

Acidic condition

25 mg drug was dissolved with 2 ml of water and 5 ml of 0.1 N hydrochloric acid was added was refluxed for 1.5 hrs. The chromatogram obtained is presented in figure 2.

Alkaline condition

25 mg of drug was dissolved in 2 ml of water and 5 ml 0.1 N NaOH was added and kept at ambient temperature for 5 hr. The chromatogram obtained is presented in figure 3.

Oxidation condition

Drug was exposed to oxidation by H_2O_2 solution. 25 mg of drug was dissolved in 2 ml of water and 2 ml of 3% hydrogen peroxide was added and kept for 30 min. The chromatogram obtained is presented in figure 4.

Exposure to UV radiation

25 mg of drug was dissolved in 50ml of water and 4 ml of this solution was diluted to 10 ml with mobile phase and exposed to UV for 5 hours. The chromatogram obtained is presented in figure 5.

Exposure to sunlight

Solution of same concentration used for UV degradation was exposed to sunlight radiation for 2 hr. The chromatogram obtained is presented in figure 6.

Thermal Degradation

Dry heat

1 gm Drug was transferred to crucible and kept in oven for 20 hr. The chromatogram obtained is presented in figure 7.

Wet heat

25 mg drug was diluted with 40 ml of water and refluxed for 5 hr. The chromatogram obtained is presented in figure 8.

CONCLUSION

It is reveal from this study that the methods are simple, precise, specific and accurate. The newly developed methods can be used for routine analysis for the estimation

Table 6: Summary of validation parameter

Validation parameter	Acceptance criteria	Result
Accuracy	98-102%	99.50% – 100.12%
Precision	%RSD≤2	intra day 0.236 – 0.406% inter day 0.247 - 0.558 %
Linearity range(µg/ml)		4-36 µg/ml
R	> 0.999	0.9994
LOD		0.20 µg/ml
LOQ		0.60 µg/ml
Ruggedness	%RSD≤2	RSD of peak area0.180 RSD of R.T. 0.328
System suitability		
a.Tailing factor	TF≤2	1.04
b.Theoretical plates	N>2000	6600

of trifluoperazine HCl in bulk and tablet dosage form in pharmaceutical industry.

The recovery studies were carried out at three levels 80, 100 and 120%. Good recoveries were obtained with %RSD lower than 2%. % recovery varies from 99.50 to 100.12% and it was within the desirable confidence interval of 98-102%, it can be said that the proposed method is accurate.

The order of stability trifluoperazine HCl was found to be H_2O_2<UV<alkali<acid<heat. Developed HPLC method is able to separate all degradants produce form any stress condition from drug peak by resolution of more than 2.

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