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A Novel RP-HPLC Method Development and Validation for Simultaneous Estimation of Trifluoperazine and Isopropamide in Tablet Dosage Form

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ABSTRACT

The present paper describes a reverse phase HPLC method for simultaneous estimation of Trifluoperazine and Isopropamide from their combined tablet dosage form. The proposed RP-HPLC method utilizes an Agilent Zorbax, C_{18} column (250 × 4.6 mm, 5µm particle size) using a mobile phase consisting of mixture of Buffer: Acetonitrile (80: 20 v/v) mobile phase flow rate of 0.8 ml/min; and UV detection at 227nm. The retention time for Trifluoperazine and Isopropamide were 2.4 and 3.62 minutes respectively. The method was linear in the range of 40-120µg/ml and 100-300µg/ml and correlation coefficients were 1.00 and 1.00 for Trifluoperazine and Isopropamide respectively. The limit of detection was found to be 2.963 and 2.985µg/ml for Trifluoperazine and Isopropamide respectively. Limit of quantification was found to be 9.877 and 9.9502 for Trifluoperazine and Isopropamide respectively. The percentage recoveries for Trifluoperazine and Isopropamide ranged from 100.03-100.36% and 100.00-100.07 % respectively. The percentage of RSD for precision of the method was found to be less than 2%. The proposed method can be used for the routine analysis for the estimation of these drugs in combined dosage form.

Keywords: Trifluoperazine, Isopropamide, RP-HPLC, Validation.

INTRODUCTION

Trifluoperazine (TFP), 10-[3-(4-methylpiperazin-1-yl) propyl]-2-(trifluoromethyl)-10H-phenothiazine, dihydrochloride (Fig. 1) is an anti-psychotic drug. Its molecular weight is 480.42 g/mol with an empirical formula $C_{21}H_{24}F_3N_3S$. It is potent It is believed to work dopa mine D_1 and D_2 in the mesocotical and mesolimbic pathways, relieving or schizoph renia as hallucinations, delusions etc.

3 -carbamoyl-3 - diphenylpropyl)diisopropyl methylammonium, iodide (Fig. 2) is a anticholinergic drug. Its molecular weight is 480.43 g/mol with an empirical formula $C_{23}H_{33}N_2O$. It is used in the treatment of peptic ulcers and other gastrointestinal disorders involving hyperacidity (gastrointestinal acidosishypermotility. The combination of this anti-cholinergic, anti emetic, anti-psychotic drugs are used for peptic ulcer and irritable bowel syndrome. [1-2]

The literature survey, it was found that Trifluoperazine or combination with other drugs by analytical methods reversed -liquid some

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spectrophotometric methods like mass spectrophotometry. [3-16] The HPLC method has its relative merits but the majority of them are carried out at elevated temperatures, time consuming, use relatively expensive reagents, involve extraction, use of buffer system. In this report, we present one simple, sensitive, cost-effective and accurate method for the simultaneous determination of Trifluoperazine and Isopropamide The main objective of the present study is to develop simple, sensitive, accurate and precise RPHPLC method for simultaneous estimation of TFP and IPP in bulk and tablet dosage forms. The proposed method was validated according to ICH guidelines. [17-21]

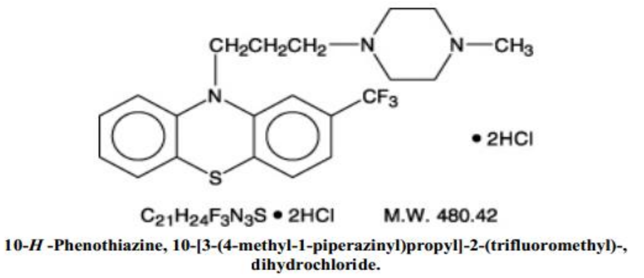


Fig. 1: Chemical Structure for Trifluoperazine

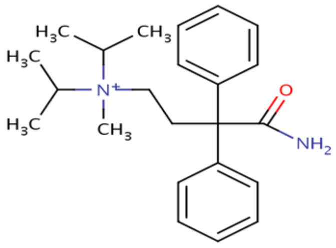


Fig. 2: Chemical Structure for Isopropamide

MATERIALS AND METHODS

Chemical and reagent: Trifluoperazine and Isopropamide were obtained as gift samples from Rainbow pharma training lab Ltd, Hyderabad. Trifluoperazine and Isopropamide combined dosage form tablets were purchased from local market. HPLC grade water, acetonitrile, methanol and analytical grade potassium dihydrogen phosphate, orthophosphoric acid was obtained from Qualigens Fine Chemicals Ltd, Mumbai. Milli-Q water was used throughout the experiment dispensed through 0.22µ filter of the Milli-Q water purification system from Merck Millipore.

Equipment: pH of the mobile phase was checked on a pH/ion analyser (Lab India, PHAN, India). The HPLC system employed in method development and assay method validation was Waters 515pump, Detector 2487 and N2000 chromatographic software as data integrator. The ultra-sonicator of ENERTECH (SE60US) used for the removal of dissolved gases in solvent and mobile phases.

Optimized chromatographic condition: The chromatographic separation was achieved on Agilent Zorbax, C_{18} column (250 × 4.6 mm, 5µm particle size) using a mobile phase consisting of mixture of Buffer: Acetonitrile (80: 20 v/v). All reagents were filtered through the 0.45µm filter paper and sonicated before use. The injection volume was 20µl the UV-Vis detector was set at the wavelength 227nm. The assay was performed at 25°C and the flow rate was fixed at 0.8 ml/min.

Preparation of Buffer: The pH of 800 mL of Milli Q HPLC grade water adjusted to 6.0 with o-phosphoric acid.

Preparation of Mobile Phase: A mixture of above prepared buffer 800 mL (80%), 200 mL of HPLC grade Acetonitrile (20%) mixed and degassed in ultrasonic water bath for 5 minutes. The mobile phase was filtered through 0.45µ filter under vacuum.

Diluent Preparation: A mixture of HPLC grade Acetonitrile and Water mixed in 20: 80 ratio was prepared degassed in ultrasonic water bath for 5 minutes and filtered through 0.45µ filter under vacuum, used for the solvent of standard and sample preparation.

Preparation of Standard Solution: Accurately weighed and transferred 20 mg of Trifluoperazine and 50 mg of Isopropamide working standard into a 50 ml clean dry volumetric flask and added about 35 ml of diluents. It was sonicated to dissolve completely and made volume up to the mark with the same diluents (Stock solution) (400, 1000µg/mL). From this, 5 ml of the solution was pipetted into another 25 ml volumetric flask and diluted up to the mark with diluents (80, 200µg/mL).

Preparation of Sample Solution: The marketed tablet Gastabid containing TFP and IPP in ratio of 2 mg: 5mg. Accurately weighed and transferred fine tablet powder equivalent to 2 mg of TFP into a 25 ml clean dry volumetric flask and added about 15 mL of diluents. It was sonicated to dissolve completely and made volume up to the mark with the same diluents. The resulting solution has TFP and IPP concentration 80, 200µg/ml respectively.

Procedure: 20µL of the standard and sample solutions were injected into the chromatographic system and areas for the TFP and IPP peaks were measured.

Method Development and Validation of HPLC Method: The proposed analytical method was validated according to ICH guidelines with respect to certain parameters such as accuracy, precision, linearity, specificity and system suitability.

System suitability: System suitability test was carried out on freshly prepared standard solution of TFP and IPP and it was calculated by injecting solution and the values of different system suitability parameter were recorded.

Accuracy: Accuracy was determined in terms of percentage recovery the accuracy study was performed for 50%, 100% and 150 % for TFP and IPP. Standard and sample solutions are injected into HPLC system in triplicate and percentage recoveries of TFP and IPP were calculated. The area of every level was used for calculation of % recovery.

Precision: Express the closeness of agreement between the series of measurement obtained from multiple sampling of same homogeneous sample under the prescribed conditions. Method precision was determined in terms of repeatability (injection and analysis). In order to determine precision, six independent sample solution preparations from a single lot of formulation 80µg/ml for TFP and 200µg/ml for IPP were injected in to HPLC system, the retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained which are found to be within the specified limits.

Linearity: Express ability to attain test results where directly proportional to the concentration of analyte in the sample. The linearity of the method was established by a spiking a series of sample mixtures of TFP and IPP, the solutions of five different concentration levels 40-120µg/ml (TFP) and 100-300µg/ml (IPP) are injected into the HPLC system. Construct the calibration curves for the standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations linear regression was applied and slope, intercept, and correlation coefficient-R^2 were determined.

Sensitivity: The Limit of Detection (LOD) was determined as lowest concentration giving response and Limit of Quantification (LOQ) was determined as the lowest concentration analyzed with accuracy method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method.

Specificity: The specificity was carried out to determine whether there are any interference of any impurities (presence of components may be unexpected to present) in retention time of analytical peaks.

Robustness: Robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (± 6.6%),change in mobile phase composition (± 2.0%) change in column temperature (± 5°C). The robustness was performed for the flow rate variations from 0.7 ml/min to 0.9 ml/min.

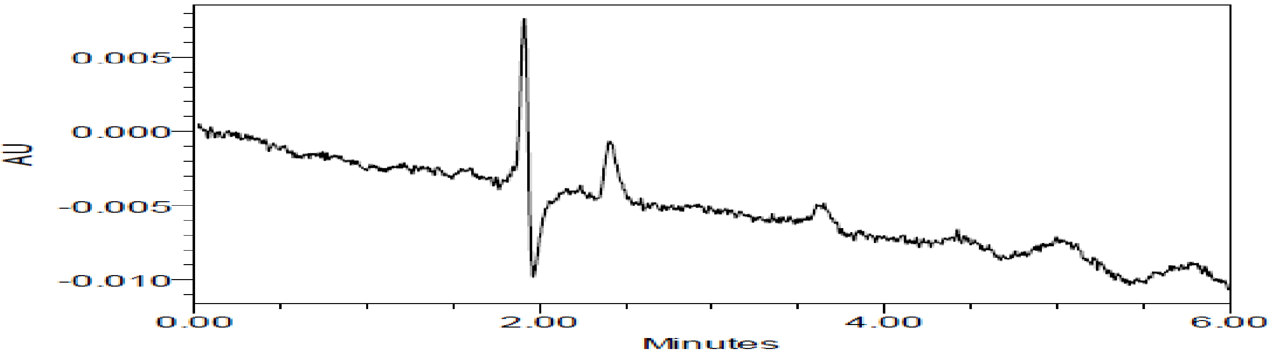
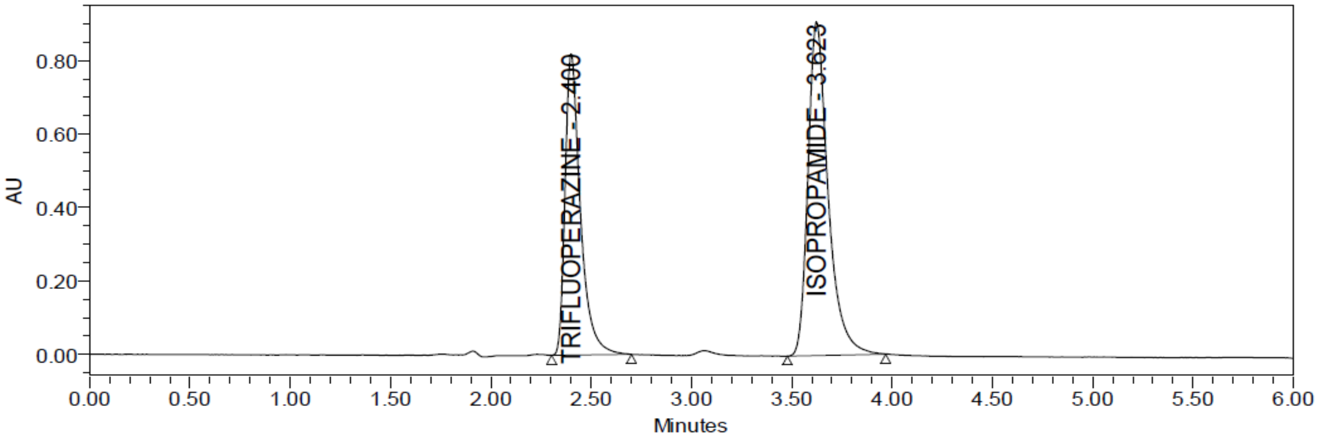


Fig. 3: Chromatogram of blank solution



S. No	Peak name	RT	Area	USP plate count	USP tailaing	S/N	Resolution
1.	Trifluoperazine	2.400	4347057	5194	1.57	81	7.60
2.	Isopropamide	3.623	6342343	6738	1.40	201	

Fig. 4: Chromatogram of standard solution of Trifluoperazine and Isopropamide

RESULTS AND DISCUSSION

Mobile phase and flow rate selection were based on peak parameters (symmetry, tailing), run time, resolution, ease of preparation, and cost. A typical chromatogram for TFP and IPP using the Agilent Zorbax, C_{18} column (250 × 4.6 mm, 5µm particle size) with a mobile phase consisting of mixture of Buffer: Acetonitrile (80: 20 v/v) at a flow rate 0.8ml/min. The UV spectrum of both the drugs shows an absorption band at 227nm; therefore, the wavelength of detection was fixed at 227nm. In the preparation of buffer, the pH of water is adjusted to 6.00 by o-phosphoric acid, which enhance column life, is the main advantage of the proposed LC method. TFP and IPP were injected in HPLC system (Fig. 4).The retention time observed allowed rapid determination of the drug, which is important for routine analysis. System suitability was performed and peaks of both drugs are separated with good resolution (Fig. 4). No interferences from the diluents, impurities, or excipients present in pharmaceutical formulations were observed at the detection wavelength (Fig. 3). The accuracy study was performed in 50%, 100% and 150% .The percentage recovery was determined for TFP and IPP was found to be 100.15% and 100.02% presented in Table 1. The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The %Relative Standard Deviation for method precision presented in Table 2 was found to be 0.138 and 0.042. In the linearity parameter calibration curve was constructed by plotting response factor against respective concentration of TFP and IPP. The plots of peak area Vs respective concentration of TFP and IPP were found to be linear in the range of 40-80µg/ml and 100-300µg/ml with coefficient of correlation (r2) 0.999 and 1.00 for TFP and IPP respectively(Fig. 5, 6 & Table 3). The limit of detection (LOD) and limit of quantification (LOQ) were found to be 2.963µg/ml and 2.9851µg/ml for TFP and 9.877µg/ml and 9.9502µg/ml for IPP. The LOD and LOQ showed that the method is sensitive for TFP and IPP. The robustness was carried out with minor but deliberate changes in parameters i.e., column temperature, and flow rate (Table 4).

Table 1: Recovery data for accuracy parameter.

Accuracy	Trifluoperazine			Isopropamide		
Concentration	**Active drug (µg/ml)**	**Recovered Amt (µg/ml)**	**Mean Recovered**	**Active drug (µg/ml)**	**Recovered Amt (µg/ml)**	**Mean Recovered**
50%	39.642	39.784	100.36	99.704	99.704	100.00
100%	79.280	79.327	100.06	199.400	199.400	100.00
150%	118.922	118.957	100.03	299.104	299.313	100.07

Table 2: Method precision for TFP & IPP.

Sample Area Trifluoperazine	Sample Area Isopropamide	% Assay of Trifluoperazine	% Assay of Isopropamide
4031892	6533221	99.34	99.68
4027885	6535078	99.24	99.71
4022263	6539830	99.10	99.78
4023117	6537569	99.12	99.75
4017716	6538743	98.99	99.77
4018316	6533591	99.00	99.69
Mean		99.13	99.73
Standard deviation		0.136882	0.042426
%RSD		0.138081	0.042541

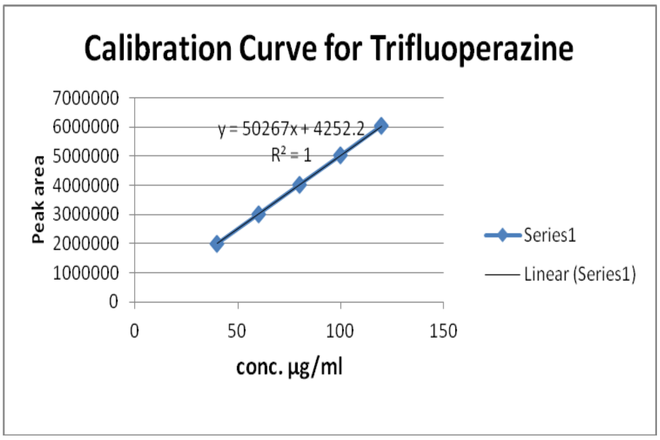


Fig. 5: Calibration Curve for Trifluoperazine

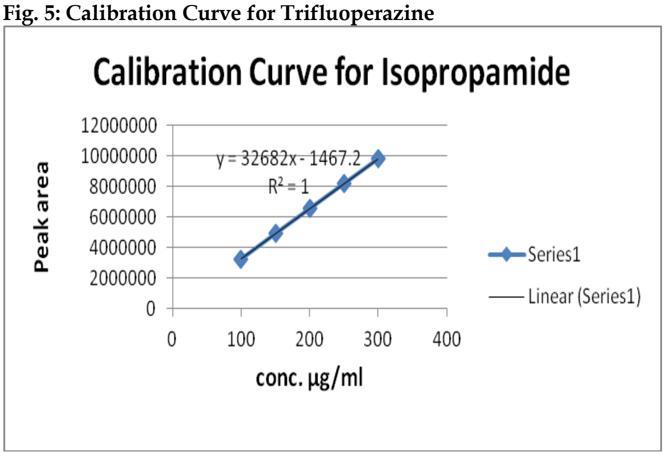


Fig. 6: Calibration Curve for Isopropamide

Table 3: Linearity results for TFP & IPP.

Trifluoperazine		Isopropamide	
Conc. (µg/ml)	**Peak area**	**Conc. (µg/ml)**	**Peak area**
40	2015622	100	3264452
60.00	3018868	150	4906640
80.00	4029855	200	6533772
100	5023776	250	8162459
120	6039844	300.00	9806943
LOD	2.963	LOD	2.9851
LOQ	9.877	LOQ	9.9502

Table 4: Robustness values for TFP & IPP.

		Trifluoperazine			Isopropamide		
Parameter		**% Assay**	**SD**	**%RSD**	**% Assay**	**SD**	**%RSD**
Flow rate (ml/min)	0.7	99.85	0.088882	0.089105	99.57	0.185562	0.186002
	0.8	99.72			99.78		
	0.9	99.68			99.94		
Mobile phase composition	78:22	99.44	0.16	0.16116	99.64	0.085049	0.085285
	80:20	99.28			99.81		
	82:12	99.12			99.72		
Column temperature	20	100.28	0.066583	0.066435	100.02	0.246847	0.246962
	25	100.15			100.16		
	30	100.24			99.68		

The developed method is fast and use simple reagents with minimal sample preparation procedure. The method was found to be precise accurate and linear for determination of Trifluoperazine and Isopropamide. All validation parameters tested were found to be within limits. The developed HPLC method is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of Trifluoperazine and Isopropamide in a combined tablet dosage form.

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