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A NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TRIFLUOPERAZINE AND CHLORDIAZEPOXIDE IN A TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, accurate, specific and sensitive reverse phase-HPLC method has been developed and validated for the simultaneous estimation of Trifluoperazine and Chlordiazepoxide in pharmaceutical dosage form. The chromatographic separation was performed on InertsilODS-3V C18 Column (150mm×4.6mm, 5µm particle size) using a mobile phase of Mixed phosphate buffer: Acetonitrile (55:45 v/v), at a flow rate of 1.0ml/min at an ambient temperature with the detection wave length at 252nm. The retention times of Trifluoperazine and Chlordiazepoxide were 5.001 min and 3.058 min respectively. The linearity was performed in the concentration range of 2.5-15µg/ml (Trifluoperazine) and 2.5-15µg/ml (Chlordiazepoxide) with a correlation coefficient of 0.9997 and 0.9983 for Trifluoperazine and Chlordiazepoxide respectively. The percentage purity of Trifluoperazine and Chlordiazepoxide was found to be 99.46 and 99.13% w/v respectively. The proposed method has been validated for specificity, linearity, precision, accuracy and robustness were within the acceptance limit according to ICH guidelines and the developed method was successfully employed for routine quality control analysis in the combined pharmaceutical dosage forms.

Key words: Trifluoperazine, Chlordiazepoxide, RP-HPLC, Validation.

INTRODUCTION

Trifluoperazine is chemically 10-[3-(4-methyl piperazine-1-yl)propyl]-2-(trifluoromethyl)-10H-phenothiazine[1], its molecular weight is 407.496g/mol with an empirical formula $C_{21}H_{24}F_3N_3S$.It acts as anti-psychotic agent, it blocks postsynaptic mesolimbic dopaminergic D1 and D2 receptors in the brain; depresses the release of hypothalamic and hypophyseal hormones and is believed to depress the reticular activating system thus affecting basal metabolism, body temperature, wakefulness, vasomotor tone, emesis[2] and also it acts as anti-emetic, dopamine antagonist, antipsychotic [3-7]. The structure of Trifluoperazine is shown in Fig.1.

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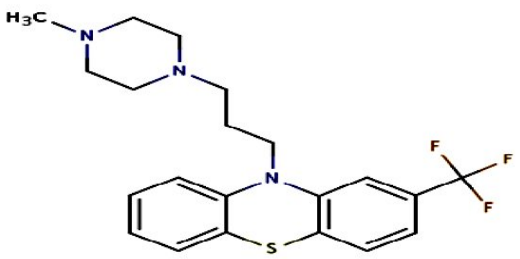


Fig1: Chemical Structure of Trifluoperazine

Chlordiazepoxide is chemically 7-chloro-2-Methylamino-5-phenyl-3H-1,4- benzodiazepine-4-oxide[6], its molecular weight is 299.80g/mol with an empirical formula$C_{16}H_{14}ClN_3O$. It was the first benzodiazepine to be used clinically with general properties similar to those of diazepam and used in the short-term treatment of anxiety disorders and insomnia [7]. The structure of Chlordiazepoxide is shown in Fig.2.

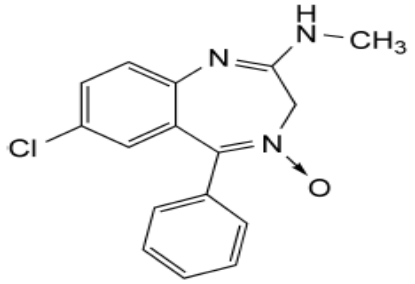


Fig2: Chemical Structure of Chlordiazepoxide

The combination of these both Trifluoperazine and Chlordiazepoxide drugs were used for the treatment of anxiety, acute alcohol withdrawal, agitation or tension, increase activity of the inhibitory transmitter GABA in different parts of CNS; they also produce antipsychotic, muscle relaxant and anticonvulsant activity. Literature survey reveals that few Spectrophotometric methods[8], Colorimetric method[9], HPLC methods[10-11], and has been reported for the estimation of Trifluoperazine and Chlordiazepoxide. The aim of the present study is to develop a simple, precise and accurate reversed-phase HPLC method for the estimation of Trifluoperazine and Chlordiazepoxide in pharmaceutical dosage form as per ICH guide lines.

MATERIALS AND METHODS

Instrumental and analytical conditions:

Reagents and chemicals:

The pharmaceutical drug samples Trifluoperazine and Chlordiazepoxide were obtained as an all the chemicals used of HPLC grade such as Mixed Phosphate Buffer was obtained from Rankem (RFCL Limited)Manufacturers and Acetonitrile was purchased from Thermo fischer scientific India Pvt. Ltd, used as a mobile phase. Water used in the buffer preparation was freshly prepared from Milli-Q.

Equipment:

A Waters e2695 gradient system with Empower-2 software and 2489 UV/Vis detector is the most sensitive and versatile dual wave length absorbance detector was used. It was manufactured by the company Waters, Alliance, Japan. Intelligent LC pump with sampler programmed at 20µL capacity per injection was used.

Chromatographic conditions:

The column used was IntersilODS-3V C18 Column (150mm×4.6mm, 5µm particle size) was used for analytical separation. The mobile phase consisted of an aqueous solution of Mixed phosphate buffer and Acetonitrile in the ratio of (55:45%v/v). The flow was adjusted to 1.0ml/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 252nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL capacity.

Preparation of Analytical solutions:

Preparation of Mixed Phosphate buffer solution:

A weighed quantity of 3.25g of potassium dihydrogen phosphate and 0.69g of di potassium hydrogen phosphate taken in a 1000ml beaker. Adjust pH to 3 with orthophosphoric acid. The mixture is sonicated and filtered through 0.45µ membrane filter.

Preparation of Mobile phase (diluent):

Mix a mixture of above buffer 550 ml (55%), 450 ml of Acetonitrile (HPLC grade-45%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Preparation of standard stock solution:

Preparation of the individual Trifluoperazine standard preparation

The standard stock solution was prepared by dissolving 2mg of standard drug of Trifluoperazine in 50ml volumetric flask to which add 40ml of mobile phase [Mixed phosphate buffer: Acetonitrile (55:45,v/v)] and sonicated for about 10 min then the final volume was made upto 50 ml with the mobile phase and shaken then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration.

Preparation of the individual Chlordiazepoxide standard preparation

The standard stock solution was prepared by dissolving 10mg of standard drug of Chlordiazepoxide in 50ml volumetric flask to which add 40ml of mobile phase [Mixed phosphate buffer: Acetonitrile (55:45,v/v)] and sonicated for about 10 min then the final volume was made upto 50 ml with the mobile phase and shaken then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration.

Preparation of standard solution:

Pipette out 1ml from the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent. Then the concentration of Trifluoperazine and Chlordiazepoxide was found to be 4ppm and 20ppm respectively.

Preparation of sample solution (Marketed formulation):

10 tablets were weighed and the average weight (128.3mg) was calculated and the sample weight observed is 134.6mg which is having an equivalent to 2mg of Trifluoperazine and 10mg of Chlordiazepoxide, hence 134.6mg of powder (sample) is taken in to 50ml volumetric flask and add 10ml of mobile phase sonicated for about 10 min and finally make up the volume to 50ml with mobile phase and shaken then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration levels.

Method Development and Validation of HPLC:

The suggested analytical method was validated according to ICH guidelines with respect to certain parameters such as specificity, linearity, precision, accuracy, robustness and system suitability.

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 peak. Forced degradation studies are carried out by using 0.1M HCl, 0.1M NaOH, heat and U.V light.

Linearity:

Express ability to obtain 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 Trifluoperazine and Chlordiazepoxide, the solutions of six different concentration levels 1-6µg/ml (Trifluoperazine) and 5-30µg/ml (Chlordiazepoxide) are injected in to 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-a, intercept-b, correlation coefficient-R^2 and standard error (Er) were determined.

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 both in terms of repeatability (injection and analysis) and intermediate precision/Ruggedness (It shows the degree of reproducibility of test results obtained by analysing the sample under variety of normal test conditions such as analyst, instruments). In order to determine precision, six independent sample solution preparations from a single lot of formulation 4µg/ml for Trifluoperazine and 20µg/ml for Chlordiazepoxide was 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.

Accuracy:

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

Robustness:

Robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (±5%) and change in wave length (±5nm). The robustness was performed for the flow rate variations from 0.9ml/min to 1.1ml/min and the method is robust only in less flow condition and even by change in the mobile phase ±1%.

System Suitability:

System suitability tests were carried out on freshly prepared standard stock solutions of Trifluoperazine and Chlordiazepoxide and it was calculated by injecting standards in six replicates at 6 minutes interval and the values were recorded.

RESULTS AND DISCUSSIONS

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of Trifluoperazine and Chlordiazepoxide. The method developed was proceeding with wavelength selection. The optimized wavelength was 252nm. In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions: The mobile phase consisted of an aqueous solution of Mixed Phosphate buffer and Acetonitrile in the ratio of 55:45% v/v and the column used was IntersilODS-3V C18 Column (150mm×4.6mm,5µm particle size). The flow rate was adjusted to 1.0ml/min. The instrument was operated at an ambient temperature. The UV detection was achieved at 252nm and purity analysis was performed over a wavelength range of 200-400nm. The injection volume was 20µL. The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak. The linearity was determined as linearity regression of the claimed analyte concentration of the range 1-6 µg/ml (Trifluoperazine) and 25-30 µg/ml (Chlordiazepoxide). The calibration curve obtained by plotting peak area versus concentration and presented in **Table 1** was linear and the correlation coefficient was found to be 0.9997 and 0.9983 for Trifluoperazine and Chlordiazepoxide respectively. The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The %Relative Standard Deviation for system precision presented in **Table 2** was found to be 0.362 and 0.425 and the % Relative Standard Deviation for method precision presented in **Table 3** was found to be 0.494 and 0.498. The % Relative Standard Deviation for ruggedness day-1 and ruggedness day-2 presented in **Table 4** was found to be 0.001, 0.001 and 0.003, 0.002 for Trifluoperazine and Chlordiazepoxide respectively. The accuracy study was performed in 50%, 100% and 150% .The percentage recovery was determined for

Trifluoperazine and Chlordiazepoxide was found to be 99.1% and 99.8% presented in **Tables 5 & 6**.The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature, and flow rate as presented in **Table 7**. Theoretical plates and tailing factor were observed and were found to be 6091.42 and 5311.82 (theoretical plates) and 1.48 and 1.12 (tailing factor) for Trifluoperazine and Chlordiazepoxide respectively.

The system suitability parameters like theoretical plates (N), tailing factor (T) were calculated and were found to be more than 2000 and not more than 2 and ascertained that proposed RP-HPLC method was accurate and precise as presented in **Table 8**.

Table No. 1: Linearity results for Trifluoperazine and Chlordiazepoxide

Trifluoperazine			Chlordiazepoxide	
S.No	Concentration(µg/ml)	Area	Concentration(µg/ml)	Area
1.	1	289760	5	3957807
2.	2	587503	10	7361399
3.	3	873352	15	10247854
4.	4	1138255	20	13275617
5.	5	1463447	25	16525102
6.	6	1736351	30	19906547

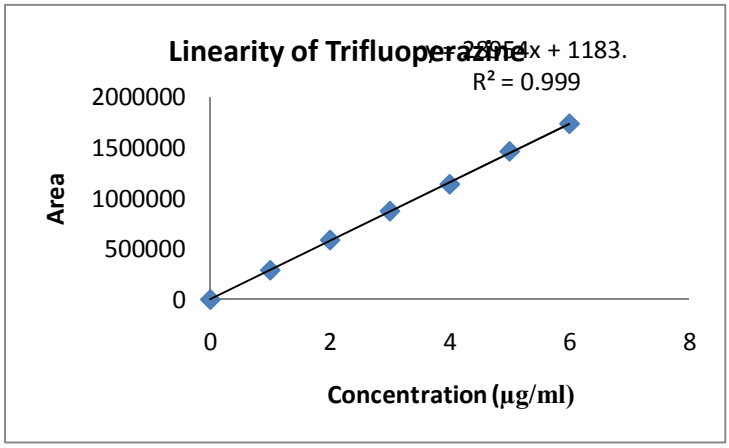


Fig3: Showing linearity for Trifluoperazine

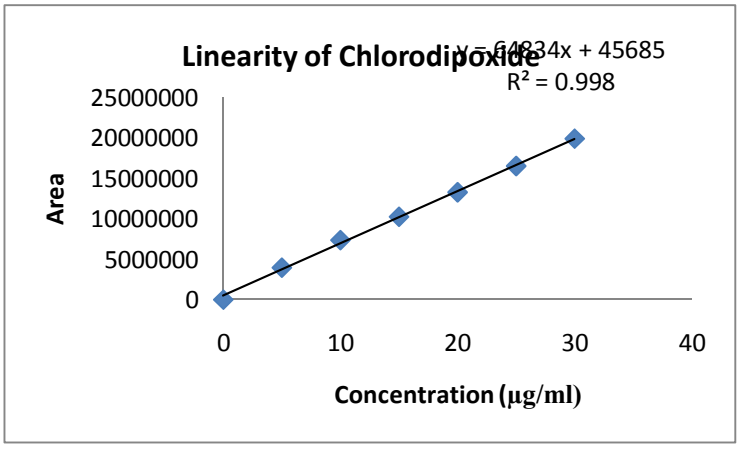


Fig4: Showing linearity for Chlordiazepoxide

Table No. 2: System Precision values for Trifluoperazine and Chlordiazepoxide

	Trifluoperazine		Chlordiazepoxide	
Injections	Rt	Area	Rt	Area
1.	5.015	1192027	3.057	14378212
2.	5.017	1189089	3.057	14279579
3.	5.014	1192020	3.058	14378212
4.	5.015	1198115	3.057	14281414
5.	5.018	1185539	3.059	14228980
6.	5.017	1194360	3.057	14276872
Avg	5.016	1191858	3.058	14303878
Std.Dev	0.0015	4314.21	0.0008	60803.28
% RSD	0.031	0.362	0.027	0.425

Table No. 3: Method Precision values for Trifluoperazine and Chlordiazepoxide

	Trifluoperazine		Chlordiazepoxide	
Injections	Rt	Area	Rt	Area
1.	5.017	1189025	3.057	14211779
2.	5.019	1183777	3.058	14279960
3.	5.017	1184535	3.057	14279960
4.	5.021	1172043	3.059	14374879
5.	5.02	1186300	3.057	14387095
6.	5.021	1182384	3.057	14374755
Avg	5.019	1183011	3.058	14318071
Std.Dev	0.0018	5840.98	0.0008	71284.53
% RSD	0.037	0.494	0.027	0.498

Table No. 4: Ruggedness values for Trifluoperazine and Chlordiazepoxide

Ruggedness-Day-1 (Intra day Precision)				
S No	Trifluoperazine		Chlordiazepoxide	
	RT	Area	RT	Area
1	5.021	1186308	3.053	14387099
2	5.023	1186317	3.055	14387122
3	5.025	1186321	3.057	14387128
4	5.027	1186325	3.059	14387135
5	5.029	1186333	3.061	14387269
6	5.031	1186345	3.063	14387299
Avg	5.026	1186325	3.058	14387175
Std Dev	0.0037	12.9	0.0037	85.56
RSD	0.074	0.001	0.122	0.001

Table No. 5: Recovery Studies for Trifluoperazine

% Concentration (at Specification level)	Area	% Recovery	Mean Recovery
50%	286607	99.8%	99.2%
100%	1138208	99.1%	
150%	1716315	99.6%	

Table No. 6: Recovery Studies for Chlordiazepoxide

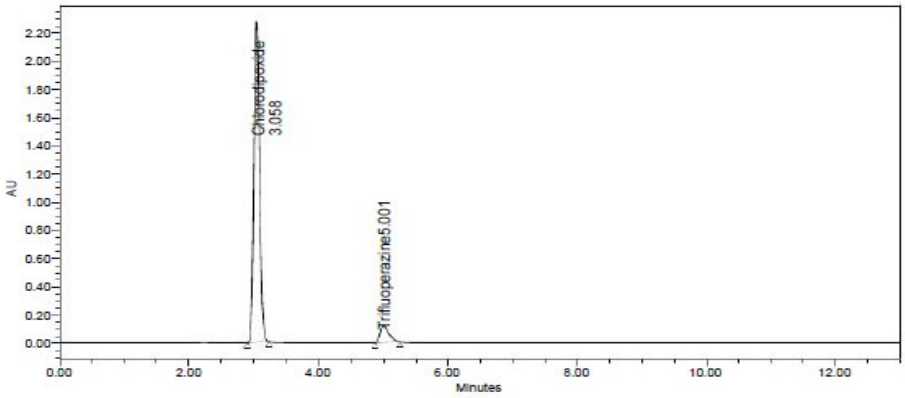
% Concentration (at Specification level)	Area	% Recovery	Mean Recovery
50%	3512793	98.8%	99.4%
100%	14188219	99.8%	
150%	21271966	99.7%	

Table No. 7: List of Robustness values for Trifluoperazine and Chlordiazepoxide

Parameters	Adjusted to	Average Area		Rt	
		Trifluoperazine	Chlordiazepoxide	Trifluoperazine	Chlordiazepoxide
Flow rate	0.9ml	1253798	15934436	5.56	3.386
	1.0ml	1173515	14283954	5.017	3.057
	1.1ml	1049598	13061927	4.587	2.788
Mobile Phase Composition (Buffer: ACN)	60:40	1167352	14427114	5.965	3.151
	55:45	1173515	14283954	5.017	3.057
	50:50	1076621	14339419	4.472	2.984

Table No 8: System Suitability Parameters for Trifluoperazine and Chlordiazepoxide

S.No	Parameters	Trifluoperazine	Chlordiazepoxide
1.	Average area	1138255	13275617
2.	Retention time	5.001	3.058
3.	Tailing factor	1.48	1.12
4.	USP Plate count	6091.42	5311.82



	Peak Name	RT	Area	Height	% Area	Resolution	USP Plate Count	Symmetry Factor
1	Chlorodipoxide	3.058	13275617	2297295	92.62		5311.82	1.12
2	Trifluoperazine	5.001	1138255	121983	7.38	9.51	6091.42	1.48

Fig5: Standard Chromatogram of Trifluoperazine and Chlordiazepoxide

SUMMARY AND CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Trifluoperazine and Chlordiazepoxide from pharmaceutical dosage form. The method was validated for parameters like specificity, linearity, accuracy, precision, robustness and system suitability values were found to be within limits. The method has significant advantages, in terms of shorter analysis time, selectivity, and accuracy than previously reported. The validation study indicates that method can be considered suitable for carrying out quality control and routine determination of Trifluoperazine and Chlordiazepoxide in bulk and pharmaceutical dosage form

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REFERENCES

1. P. Shetti and A. Venkatachalam, *E-Journal of Chemistry,* 2010; 7(1)**:** 299-313.
2. Sree Vidya Parvataneni and Pathuri Jnana Nagarjuna,*World Journal of Pharmacy And Pharmaceutical Sciences,*(2014; 3,(10), 1021-1031.
3. C.M.Bhaskar Reddy, G.V.Subba Reddy and N. Ananda Kumar Reddy, *International Journal of Scientific and Research Publications,* 2012; 2(8),1-5.
4. Donthineni Kalyan and Punna Venkateshwarlu, *Asian Journal of Pharmaceutical Research,*2015; 5(1), 7-12.
5. Jameel M. Dhabab, *Al- Mustansiriya J. Sci,* 2011; 22(2),123-128.
6. RB Saudagar, Swarnlata Saraf and S Saraf, *Indian Journal of Pharmaceutical Sciences,* 2007; 69(1), 149-152.
7. Sejal K. Patel and N. J. Patel, *Indian Journal of Pharmaceutical Sciences,*2009; 71(5), 545–547.

8. Sejal Patel, N. J. Patel, and S. A. Patel, *Indian Journal of Pharmaceutical Sciences,* 2009; 71(4),468-472.
9. Safwan Ashour and Nuha Kattan, *Journal of Pharmaceutics,* 2013; 2013; 7.
10. Sejal Patel and N. J. Patel, *Indian Journal of Pharmaceutical Sciences,*2009; 71(4), 472-476.
11. Patel, Sejal K, Patel and Natvarlal J, *An International Journal of Analytical Science,* (2010); 93,(3),904-910.

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