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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TRIFLUOPERAZINE HCl AND TRIHEXYPHENIDYL HCl IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

RP-HPLC method in which determination of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride was carried out by reverse phase C-18 column (Inertsil ODS-3,250*4.6mm) using a mobile phase consisting of acetonitrile: water: triethyelamine (68: 31.8: 0.2v/v) with pH 4 adjusted by using ortho-phosphoric acid. The mobile phase was pumped at rate of 1.0 ml/min and the detection was carried out at 210 nm. The linearity was found in the range of 10-150 µg/ml and 4-60 µg/ml with regression coefficient (r=0.999 for both). The peaks obtained were sharp having clear baselines separation with a retention time of 2.78±5.68 and 2.31 ± 4.72 min for Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride respectively.

Keywords: Trifluoperazine hydrochloride, Trihexyphenidyl hydrochloride, RP-HPLC, Validation.

INTRODUCTION

Trihexyphenidyl hydrochloride is a selective M1 muscarinic acetylcholine receptor antagonist. It is able to discriminate between the M1 (cortical or neuronal) and the peripheral muscarinic subtypes (cardiac and glandular). Trihexyphenidyl hydrochloride partially blocks cholinergic activity in the CNS, which is responsible for the symptoms of Parkinson's disease. It is also thought to increase the availability of dopamine, a brain chemical that is critical in the initiation and smooth control of voluntary muscle movement. It is rapidly absorbed from the gastrointestinal tract [1].

Trihexyphenidyl is an anticholinergic used in the symptomatic treatment of all etiologic groups of parkinsonism and drug induced extrapyramidal reactions (except tardive dyskinesia). Trihexyphenidyl possesses both anticholinergic and antihistaminic effects, although only the former has been established as therapeutically significant in the management of parkinsonism [2].

Minor side effects, such as dryness of the mouth, blurring of vision, dizziness, mild nausea or nervousness. Isolated instances of suppurative parotitis secondary to excessive dryness of the mouth, skin rashes, dilatation of the colon, paralytic ileus, and certain psychiatric manifestations such as delusions and hallucinations, plus one doubtful case of paranoia all of which may occur with any of the atropine-like drugs, have been rarely reported with Trihexyphenidyl hydrochloride [3,4].

Trihexyphenidyl has been reported as a drug of abuse, and while this is uncommon it may be prudent to be cautious in prescribing this drug to patients with a history of drug addiction. The drug has euphoriant and aphrodisiac properties and is smoked, insufflated, swallowed, or dissolved under the tongue and has enhanced activity when injected [5].

MATERIALS AND METHODS

Chemicals and Reagents

Samples of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride were confirmed by spectral characterization and SOR (specific optical rotation) from Process Research Department of Emergent Laboratories

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Ltd, Hyderabad, India. HPLC-grade Acetonitrile from Rankem, Sodium dihydrogen orthophosphate dihydrate (AR grade). ACS Grade Triethyelamine, and Ortho-phosphoric acid from Sigma-Aldrich, India, was procured.

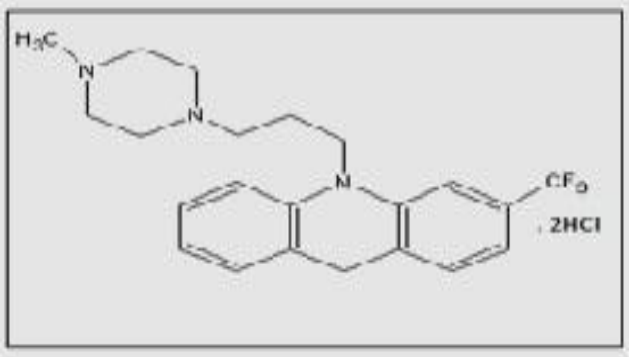


Fig 1. Trifluoperazine hydrochloride

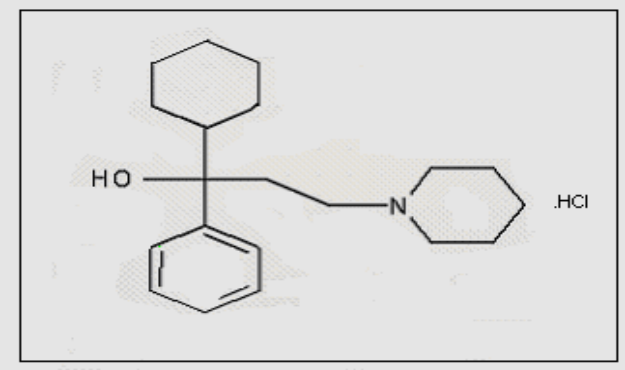


Fig 2. Trihexyphenidyl hydrochloride

Instrumentation

Chromatographic system consisted of a LC-10AT VP Shimadzu liquid chromatography with SPD-10A Shimadzu UV-Vis detector equipped with auto sampler Photodiode array detector. The data recorded using LC Solutions software. The column used was C-18 column, Inertsil ODS-3,250*4.6mm i.d., particle size 5 µm with flow rate of 1 ml / min using PDA detection at 210 nm.

Selection of mobile phase

The pure drug of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride were injected into the HPLC system and run in different solvent systems. Different mobile phases were tried in order to find the best conditions for the separation Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride. It was found that acetonitrile and water gives satisfactory results as compared to other mobile phases. This mobile phase system was tried with different proportions and using different flow rates. Finally, the optimal composition of the mobile phase was determined to be acetonitrile: water: triethyelamine (68:31.8: 0.2 v/v) with pH 4 adjusted by using ortho-phosphoric acid [6,7].

Preparation of stock, working standard solutions, and sample solution

20 mg of standard Trifluoperazine hydrochloride and 10 mg of Trihexyphenidyl hydrochloride was weighed accurately and transferred to two separate 100 ml volumetric flasks. Both the drugs were dissolved in 50 ml of mobile phase with shaking and then volume was made up to the mark with mobile phase to get 200µg/ml and 100µg/ml of standard stock solution of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride respectively. These stock solutions were filtered through 0.2 µm Nylon 66 membrane filter paper [8].

Preparation of mobile phase

The HPLC grade acetonitrile was ultrasonicated for 20 minutes on ultrasonicator and then filtered through 0.45µ m Nylon 66 membrane filter paper. Double distilled water was also ultrasonicated for 20 minutes and then filtered through 0.45µ m Nylon 66 (N66) 47mm membrane filter paper. Mobile phase was prepared by mixing 680 ml of acetonitrile with 318 ml of water. Then add 2ml triethyelamine then adjust the pH 4 by using ortho-phosphoric acid.

Selection of analytical concentration range and preparation of calibration curve for Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride

Trifluoperazine hydrochloride:

Appropriate aliquots were pipetted out from the standard stock solution (200 µ g/ml) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 10, 20, 50, 62.5, 100, 125, 150 µg/ml of Trifluoperazine hydrochloride.

Triplicate dilutions of each of the above mentioned concentrations were prepared separately and from these triplicate solutions, 20 µ l of each concentration of the drug were injected into the HPLC system two times separately and their chromatograms were recorded under the same chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

Trihexyphenidyl hydrochloride

Appropriate aliquots were pipetted out from the standard stock solution (100 µ g/ml) in to a series of 10 ml volumetric flasks. The volume was made up to the mark with the mobile phase to get a set of solutions having the concentration range, ranging from 4, 8, 20, 25, 40, 50, 60 µg/ml of Trihexyphenidyl hydrochloride.

Triplicate dilutions of each of the above mentioned concentrations were prepared separately and from these triplicate solutions, 20µl of each concentration of the drug was injected into the HPLC system two times separately and their chromatograms

were recorded under the same chromatographic conditions as described above. Peak areas were recorded for all the peaks and a standard calibration curve of AUC against concentration was plotted.

Both the drugs follow the Beer's & Lambert's law in the concentration range of 10-150 µ g/ml for Trifluoperazine hydrochloride and 4-60 µ g/ml for Trihexyphenidyl hydrochloride. The linearity of calibration curves and adherence of the system to Beer's & Lambert's law was validated by high value of correlation coefficient and less than 2% relative standard deviation (R.S.D.) for the intercept value.

Development and validation of HPLC Method

Present study was conducted to obtain a new, affordable, cost effective and convenient method for HPLC determination of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996, and Global Quality Guidelines-2002. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, and robustness [9,10].

Specificity & Selectivity

The specificity of the RP-HPLC method was determined by complete separation of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride, with parameters like retention time (t_R), resolution (R_S) and tailing factor (T_f). Here tailing factor for peaks of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride was less than 2% and resolution was also more than 1% [11].

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride at different concentrations level were used for this purpose. Before injection of the solutions, the column was equilibrated for at least 30 min with the mobile phase. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy

Recovery studies were carried out by applying the method to drug sample present in tablet dosage form to which known amount of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride, corresponding to 80%, 100% and 120% of label claim was added (standard addition method). The concentration of the sample mixture was determined as per the procedure given for the tablet formulation by determining AUC at selected analytical wavelength 210 nm. The variation of the results within the same day was analyzed and statistically validated.

Precision

The precision of the method was determined by repeatability (intraday) and intermediate precision (inter-day) study. Repeatability was determined by performing four repeated analysis of the standard solutions of Trihexyphenidyl hydrochloride (40µg/ml) and Trifluoperazine hydrochloride (100µg/ml) on the same day, under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis of previous standard solutions on three different days (inter-day) in the same laboratory. The relative standard deviation (% RSD) was determined in order to assess the precision of the method. The procedure for the preparation of solution for the determination of precision was same as explained in the analysis of tablet formulation.

Reproducibility

Reproducibility expresses the precision between laboratories. It is assessed by means of inter laboratory trial. It should be considered in case of standardization of an analytical procedure. The area under curve of the sample mixture was measured by another analyst at selected analytical wavelength210 nm under the same chromatographic condition as described above. The results obtained were evaluated using t-test to verify their reproducibility.

Robustness

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in method parameters. The solution containing Trifluoperazine hydrochloride 100µ g/ml and 40µ g/ml Trihexyphenidyl hydrochloride was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate, percentage of acetonitrile in the mobile phase and column temperature.

System suitability

The system suitability was assessed by six replicate analysis of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride at a 100% level to verify the resolution and reproducibility of the chromatographic system adequate for the analysis to be done. This method was evaluated by analyzing the repeatability of retention time, peak area for both Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride.

Table 1. Statistical data of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride at 210 nm by RP–HPLC method

Parameter	TFP at 210 nm	THP at 210 nm
Linear Range (µg/ml)	10-150	4-60
Slope	26960	13795
Intercept	13786	-1072
Limit of Detection (µg/ml)	0.00187	0.0046
Limit of Quantification (µg/ml)	0.0056	0.014

Table 2. Linear regression data for Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride

Component	Linear range (µg/ml)	Slope	Intercept	Regression coefficient (r)
TFP	10-150	26960	13786	0.999
THP	4-60	5517	1072	0.999

Table 3. Statistical validation of linear regression data of Trifluoperazine hydrochloride

Parameter	Mean*	Standard Deviation*	Co-efficient of Variation*
Slope	26961.3	2.3	0.0086
Intercept	14750.3	836.48	5.67
Regression coefficient (r)	0.999	0	0

Table 4. Statistical validation of linear regression data of Trihexyphenidyl hydrochloride

Parameter	Mean*	Standard Deviation*	Co-efficient of Variation*
Slope	5494.667	20.40	0.37
Intercept	1347	374.1	27.77285
Regression coefficient (r)	1347	0	0

**n = 6*

Table 5. Determination of Accuracy of Trifluoperazine Hydrochloride and Trihexyphenidyl Hydrochloride

Level of % recovery	Amount present (mg/tab)		Total amount recovered (mg)		% Recovery		Mean*		Standard Deviation*		Co-efficient of Variation*	
	TFP	**THP**	**TFP**	**THP**	**TFP**	**THP**	**TFP**	**THP**	**TFP**	**THP**	**TFP**	**THP**
80%	5	2	9.00	3.54	100.01	98.35	100.2	98.8	0.24	0.24	0.20	0.21
	5	2	8.98	3.55	99.79	98.72						
	5	2	9.02	3.55	100.26	98.68						
100%	5	2	10.05	3.94	100.5	98.48	100.17	98.82	0.58	0.31	0.58	0.32
	5	2	10.05	3.96	100.5	99.1						
	5	2	9.95	3.95	99.5	98.87						
120%	5	2	10.94	4.36	99.43	99.2	99.05	98.4	0.43	0.65	0.34	0.66
	5	2	10.87	4.36	98.79	99.03						
	5	2	10.88	4.31	98.92	98						

**n = 3*

Table 6. Determination of inter-day precision of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride

Sl. No.	Amount present (µg/ml)		Amount obtained (µg/ml		(%)Lable claim estimated		Mean*		Standard Deviation*		Co-efficient of Variation*	
	TFP	**THP**	**THP**	**TFP**	**TFP**	**THP**	TFP	THP	TFP	THP	TFP	THP
1	100	40	99	39.24	99	98.09	99.21	98.32	0.3	0.29	0.3	0.3
2	100	40	99.47	39.25	99.47	98.13						
3	100	40	99.65	39.18	99.65	97.96						
4	100	40	99.28	39.43	99.28	98.58						
5	100	40	98.92	39.44	98.92	98.61						
6	100	40	98.96	39.42	98.96	98.56						

**n=6*

Table 7. Statistical validation data for inter-day precision

Components	Mean*	Standard Deviation*	Co-efficient of Variation*
TFP	98.98	0.41	0.41
THP	98.2	0.3	0.4

Table 8. Reproducibility results of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride at 210 nm by RP−HPLC method

Components	Analyst 1	Analyst 2	Result of t test*	Inference
TFP	2674644 ± 5895.2	2689554 ±30944.22	0.07	No significant difference
THP	5482279 ± 4531.756	5451348 ± 4383.812	0.34	No significant difference

Table 9. Robustness results for variations in flow rate (ml/min)

Method Parameter	Level	Retention Time		Tailing factor		Amount obtained %	
Flow Rate (ml/min)		TFP	THP	TFP	THP	TFP	THP
0.9	-1	3	2.5	1.3	1.3	98.96	98.78
1	0	2.78	2.31	1.2	1.1	99.28	98.58
1.1	1	2.5	2	1.4	1.2	98.92	98.61

Table 10. Robustness results for variations in amount of acetonitrile in mobile phase (v/v).

Method Parameter	Level	Retention Time		Tailing factor		Amount obtained %	
% of Acetonitrile		TFP	THP	TFP	THP	TFP	THP
67	-1	3	2.5	1.3	1.3	98.03	97.99
68	0	2.75	2.2	1.2	1.1	98.4	98.17
69	1	3	2.5	1.3	1.3	99	98.09

Table 11. Robustness results for variations in column temperature.

Method Parameter	Level	Retention Time		Tailing factor		Amount obtained %	
Columns Temperature		TFP	THP	TFP	THP	TFP	THP
26ºC	-1	2.73	2.2	1.3	1.2	99.6	97.59
27ºC	0	2.77	2.3	1.2	1.1	98.96	98.56
28ºC	1	2.73	2.26	1.3	1.2	98.93	98.27

Table 12. Statistical validation of robustness results for variations in method parameters

Method Parameters	Mean*		Standard Deviation*		Co-efficient of Variation*	
	TFP	THP	TFP	THP	TFP	THP
Flow Rate (ml/min)	2.76	2.27	0.25	0.25	9.0	11
% of Acetonitrile (v/v)	2.9	2.4	0.14	0.2	4.9	7.2
Column Temperature	2.7	2.3	0.0	0.1	0.8	2.2

Table 13. System suitability parameters of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride

Parameters	TFP	THP
Linear range (µg/ml)	10-150	4-60
Slope	26960	13795
Intercept	13786	1072
Regression coefficient (r)	0.999	0.999
Limit of Detection (µg/ml)	0.0018	0.0046
Limit of Quantification (µg/ml)	0.0056	0.014
Retention time (min)	2.78 ± 5.68	2.31 ± 4.72
Tailing factor	1.3	1.1
Resolution factor	2.7	
Capacity factor	1.3	1.3
Theoretical plate	4000	3009

Figure 3. Chromatogram for Trifluoperazine hydrochloride.

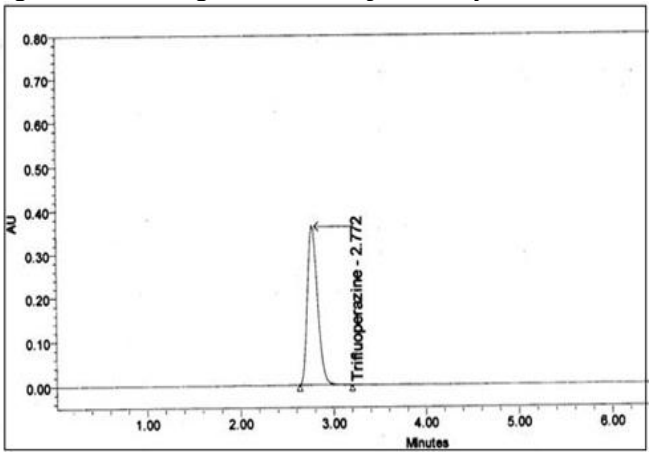


Figure 4. Calibration curve of Trifluoperazine hydrochloride at 210 nm by RP−HPLC.

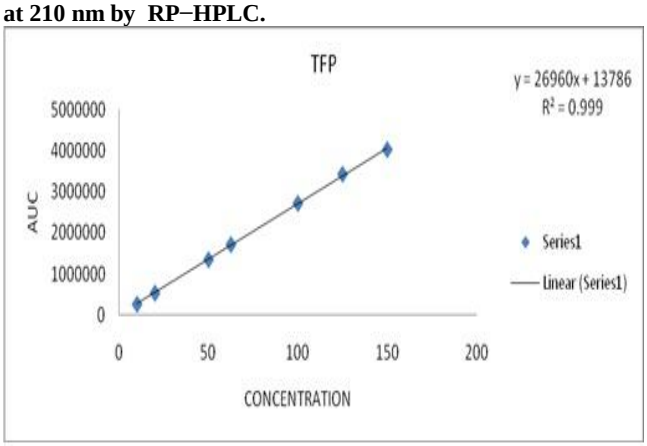


Figure 5. Chromatogram for Trihexyphenidyl hydrochloride.

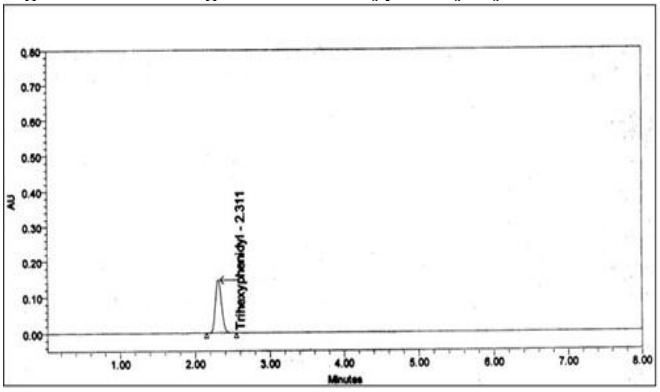


Figure 6. Calibration curve of Trihexyphenidyl hydrochloride at 210 nm by RP−HPLC method.

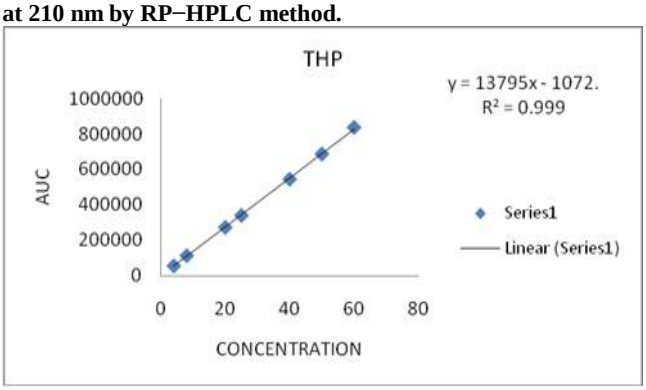
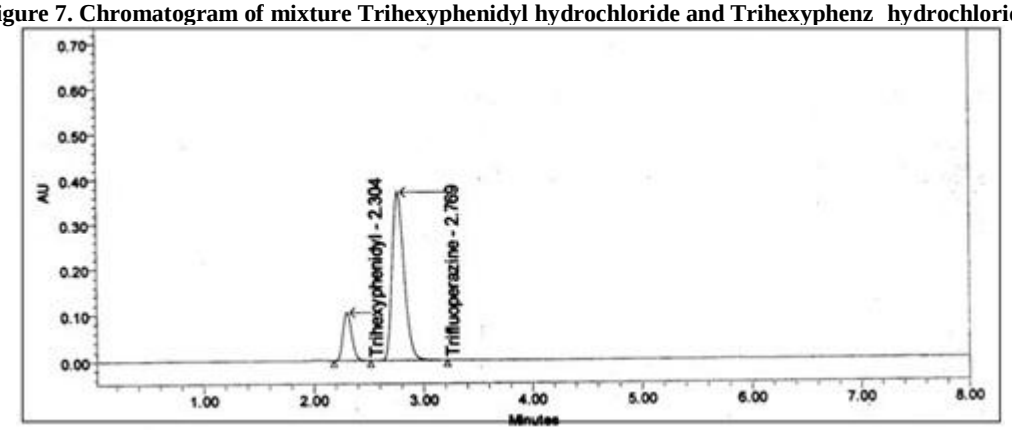


Figure 7. Chromatogram of mixture Trihexyphenidyl hydrochloride and Trihexyphenz hydrochloride.



RESULTS AND DISCUSSION

The objective of the proposed work was to develop simultaneous methods for the determination of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride, and to validate the methods according to ICH guidelines and applying the same for its estimation in marketed formulations. There is no official method for the simultaneous estimation of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride in combination.

HPLC methods developed were found to be rapid, simple, precise, accurate and economic for routine estimation of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride simultaneously in commercial dosage forms.

In HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to separate title ingredients. Mobile phase and flow rate

selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time, resolution. The system with Acetonitrile: water: Triethyelamine (68:31.8: 0.2 v/v) with pH 4 adjusted by using Ortho-phosphoric acid is quite robust.

The optimum wavelength for detection was 210 nm at which better detector response for both the drugs was obtained. The average retention times for Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride was found to be 2.78 and 2.31 respectively. The calibration was linear in the concentration range of 10-150 µg/ml and 4-60 µg/ml, with regression 0.999 and 0.999, intercept 13786 and -1072 and slope 26960 and 13795 for Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride respectively. The low values of % R.S.D indicate that the method is precise and accurate. The mean recoveries were found in the range of 98 – 102 %. Sample to sample precision and accuracy were evaluated using six samples, which were prepared and analyzed on same day. Day to day variability was assessed using five concentrations analyzed on three different days over a period of three days. These results showed the accuracy and reproducibility of the assay.

Robustness of the proposed method was determined by analysis of sample at ± 1 changes in different parameter like ratio of composition of mobile phase, flow rate and column temperature using similar operational and environmental conditions, the

% R.S.D. reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and applied for analysis of the same in marketed formulations.

CONCLUSION

Trifluoperazine hydrochloride is a trifluoro-methyl phenothiazine derivative intended for the management of Schizophrenia and other psychotic disorders. Trifluoperazine hydrochloride blocks postsynaptic mesolimbic dopaminergic D1 and D2 receptors in the brain and 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, and emesis.

Trihexyphenidyl hydrochloride is an anticholinergic used in the symptomatic treatment of all etiologic groups of Parkinsonism and drug induced extrapyramidal reactions (except tardive dyskinesia). Trihexyphenidyl hydrochloride possesses both anticholinergic and antihistaminic effects, although only the former has been established as therapeutically significant in the management of Parkinsonism.

So here an attempt has been made to develop simple and accurate method for the simultaneous estimation of Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride in combined dosage form using UV-Visible spectroscopy and high performance liquid chromatography.

The working wavelength selected for Trifluoperazine hydrochloride and Trihexyphenidyl hydrochloride were 257.5 nm and 210 nm respectively, which were apart enough to be analyzed efficiently and were found to be stable in distilled water.

In Simultaneous equation method, it involves the determination of the standard absorptivity values of the drugs at the selected wavelengths and wavelength intervals. Using these standard absorptivity values, a simultaneous equation can be constructed, which can be directly used for sample measurements.

A simple, accurate and precise RP-HPLC method was successfully developed using mobile phase acetonitrile: water: triethyelamine (68:31.8: 0.2 v/v) with pH 4 adjusted by using ortho-phosphoric acid.

The peaks obtained were sharp with retention time of 2.76 min for Trifluoperazine hydrochloride and 2.31 min for Trihexyphenidyl hydrochloride. The peaks were well resolved with a resolution factor of 2.7. The method was precisely applied to the tablet formulation and the results obtained were accurate and reproducible. All the developed methods were statistically validated in terms of accuracy, precision, linearity and reproducibility.

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