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DEVELOPMENT AND VALIDATION OF RPHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TRIHEXYPHENIDYL HCL AND TRIFLUOPERAZINE

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ABSTRACT

A new HPLC method was developed for simultaneous estimation of Trihexyphenidyl HCL & Trifluoperazine. Quantitative HPLC was performed with Hitachi L2130 with D2000 Elite Software with UV-Visible Detector, L-2400 PUMP, Develosil, C-18, ODS (150mm*4.6mmØ) column was used in the study. The mobile phase of water: Methanol: Acetonitrile (20:25:55) were used in this study. The conditions optimized were: flow rate (1 ml/minute), wavelength (210 nm) and run time was 13 min, column temperature was maintained at 25^0C. Retention time was found to be 2.75 min for Trihexyphenidyl Hcl & 6.57 for Trifluoperazine. The linearity was found to be in the concentration range of 5-25µg/ml for Trihexyphenidyl Hcl & 20-60µg/ml for Trifluoperazine. Results of analysis were validated statistically and by recovery studies. The recovery studies with 99.18 % were indicative of the accuracy of proposed method. The precision was calculated as repeatability, inter and intraday variation (%RSD) for the drugs.

Key words: Trihexyphenidyl HCL, Trifluoperazine, Method validation, ACN, Precision.

INTRODUCTION

It is necessary to find the content of each drug [1] either in bulk or single or combined dosage forms for purity testing. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of the pure substance in the drug and its formulations. The scope of developing and validating an analytical method [2] is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation [3]. According to the literature survey [4-9], it was found that few analytical methods such as (RP-HPLC, HPLC, UV-Visible analysis and LC-MS) were reported for the estimation of Trihexyphenidyl Hcl & Trifluoperazine. The objective of the proposed method is to develop simple and accurate methods for the determination of Trihexyphenidyl Hcl & Trifluoperazine by RP-HPLC [10] method in pharmaceutical dosage forms. Trihexyphenidyl [11] (also known as benzhexol and trihex, is an antiparkinsonian agent of the antimuscarinic class. It has been in clinical usage for decades.

The drug is available as the hydrochloride salt. Trihexyphenidyl alters unusual nerve impulses and relaxes stiff muscles. Trihexyphenidyl is used to treat the stiffness, tremors, spasms, and poor muscle control of Parkinson's disease. Trifluoperazine [12] is an anti-psychotic medication in a group of drugs called phenothiazines. It works by changing the action of chemicals in your brain. Trifluoperazine is used to treat anxiety or psychotic disorders such as schizophrenia.

MATERIALS AND METHODS

Instruments and Reagents

Preparation of mobile phase

Mobile phase was prepared by taking Water: Methanol: Acetonitrile by adjusting pH 3.7 with OPA (50:50). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

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Preparation of Standard Drug Solutions

The API mixture of Trihexyphenidyl Hcl and Trifluoperazine were taken in a ratio of 1:2 and stock solution is prepared. The resultant solution was filtered through a 0.45 μm membrane filter and degassed under ultrasonic bath prior to use

Preparation of Sample Solutions

5 ml of stock solution was pipetted out into 10 ml volumetric flask and volume was made up to the mark with methanol. The resultant solution was filtered through a 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure

Method Validation

As per the ICH guidelines [13] the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantisation.

Preparation of Calibration Curves

Standard solutions of Trihexyphenidyl Hcl in the concentration range of 5μg/ml to 25μg/ml were obtained by transferring (0.5, 1, 1.5, 2, 2.5ml) of Trihexyphenidyl Hcl stock solution (100 ppm) to the series of 10 ml volumetric flasks and standard solutions of Trifluoperazine in the concentration range of 20 μg/ml to 60 μg/ml were obtained by transferring (2, 3, 4, 5, 6) with methanol. The solutions were filtered through a 0.45 μm membrane filter and degassed under ultrasonic bath prior to use. The solutions were injected into HPLC system [14]. The run time was 10 min and the peak areas were measured.

Linearity and Range

Linearity range was found to be 5-25 μg/ml for Trihexyphenidyl Hcl and 20-60 μg/ml for Trifluoperazine. The correlation coefficient was found to be 0.999 & 0.999, the slope was found to be 14082 & 12626 and intercept were found to be 973 & 78078 for Trihexyphenidyl Hcl and Trifluoperazine respectively.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Trihexyphenidyl Hcl & Trifluoperazine were taken. From that percentage recovery values were calculated.

Precision

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Trihexyphenidyl Hcl & Trifluoperazine (API). The percent relative standard deviations were calculated for Trihexyphenidyl Hcl & Trifluoperazine. The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & (% RSD < 2%) within a day & day to day variations for Trihexyphenidyl Hcl & Trifluoperazine.

Repeatability

Repeatability was assessed using six time repetition of working concentration of THF & TFP**.**

Intra-assay & Inter-assay

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Trihexyphenidyl Hcl & Trifluoperazine revealed that the proposed method is precise.

Limit of Detection and Limit of Quantification

The LOD was found to be 0.0377 μg/ml and 0.1081 μg/ml and LOQ was found to be 0.113 μg/ml and 0.3243 μg/ml for Trihexyphenidyl Hcl and Trifluoperazine respectively which represents that sensitivity of detection [15] the method is high.

Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature ($\pm 2^0$C), Wavelength of detection (±2nm) & acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (% RSD < 2%) the developed RP-HPLC method for the analysis of Trihexyphenidyl Hcl and Trifluoperazine.

Table 1. List of Instruments

S.No.	Name of Instrument	Instrument Model	Name of Manufacturer
1	UV-Visible double beam spectrophotometer	SL159	Elico
2	HPLC(D2000 Elite Software)	L2130	Hitachi
3	Column	ODS (C_{18})	Develosil
3	Ultra sonicator	WUC 2L	Wensar
4	Electronic Balance	ATY224	shimadzu
5	P^H Analyzer	---	Elico
6	Triple Quartz Distillation Unit	---	Borosil

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Table 2. List of Reagents & Chemicals

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	----	**----**	In house laboratory.
2.	Methanol	99.9%	HPLC.	Loba Chem; Mumbai.
3.	Sodium Hydroxide	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5	Ortho phosphoric acid	99.9%	L.R	----
6	Potassium dihydrogen orthophosphate	99.9%	L.R	----

Table 3. Optimised Chromatographic conditions

Mobile phase	**Water: Methanol: acetonitrile (20:25:55)**
Wavelength	210 nm
Flow rate	1.0 ml/ min.
Run time	13 min.
Column	Develosil ODS (C_{18}) RP Column, 150 mm x 4.6 mm

Table 4. Accuracy Readings for Trihexyphenidyl HCL

Sample ID	Concentration (µg/ml)		%Recovery of	Statistical Analysis
	Pure drug	Formulation	Pure drug	
S_1 : 80 %	4	5	101.3	Mean= 100.2733%
S_2 : 80 %	4	5	99.25	S.D. = 1.025004
S_3 : 80 %	4	5	100.27	% R.S.D.= 1.02221
S_4 : 100 %	5	5	99.14	Mean= 99.18%
S_5 : 100 %	5	5	99.29	S.D. = 0.096437
S_6 : 100 %	5	5	99.11	% R.S.D.= 0.097234
S_7 : 120 %	6	5	99.21	Mean= 99.46%
S_8 : 120 %	6	5	99.54	S.D. = 0.221133
S_9 : 120 %	6	5	99.63	% R.S.D. = 0.222334

Table 5. Accuracy Readings for Trifluoperazine

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	16	20	99.13	Mean= 98.94667%
S_2 : 80 %	16	20	98.79	S.D. = 0.171561
S_3 : 80 %	16	20	98.92	% R.S.D.= 0.1733
S_4 : 100 %	20	20	99.72	Mean= 99.76%
S_5 : 100 %	20	20	99.81	S.D. = 0.045826
S_6 : 100 %	20	20	99.75	% R.S.D.= 0.0459
S_7 : 120 %	24	20	99.36	Mean= 99.37667%
S_8 : 120 %	24	20	99.28	S.D. = 0.105987
S_9 : 120 %	24	20	99.49	% R.S.D. = 0.1066

Table 6. Data showing repeatability analysis

Concentration of THP+TFP in ppm	Rt of THP	Peak area of THP	Rt of TFP	Peak area of TFP
15 +40	2.75	207895	6.57	4711296
15 +40	2.79	213452	6.48	4692550
15 +40	2.75	213152	6.57	4670347
15 +40	2.75	212339	6.57	4765505
15 +40	2.77	213412	6.57	4853049
AVG	2.762	212050	6.552	4738549
S.D.	0.017889	2365.513	0.040249	73053.1
%RSD	0.647666	1.115545	0.614304	1.541676

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Table 7. Results of intra-assay & inter-assay for Trihexyphenidyl Hcl

Conc. of Trihexyphenidyl Hcl(API) (µg/ml)	Observed Conc. of Trihexyphenidyl HCl (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.03	1.03	10.41	0.46
20	20.49	0.51	20.94	0.28
100	99.14	0.19	99.19	0.15

Table 8. Results of intra-assay & inter-assay for Trifluoperazine

Conc. of Trifluoperazine (API) (µg/ml)	Observed Conc. of Trifluoperazine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
20	20.01	0.86	20.03	0.87
30	30.02	0.30	30.03	0.32
100	99.97	0.13	99.95	0.11

Table 9. Summary of Validation Parameters by RP-HPLC Method

Validation parameters		Trihexyphenidyl Hcl & Trifluoperazine
Specificity		% interference <0.5 %
Range (µg/ml)	Linear range	5-25 µg/ml for thf & 10-60 µg/ml for tfp
	Working range	5µg/ml for thf & 20 µg/ml for tfp
Accuracy (% Recovery) 98-102%		99.18% for thf & 99.76% for tfp
Precision (% RSD)	Repeatability	0.647666 for thf & 0.614304 for tfp
	Intraday(10,20,100 µg/ml)	1.03, 0.51, 0.19 for thf & 0.86, 0.30, 0.13 for tfp
	Inter day(10,20,100 µg/ml)	0.46, 0.28, 0.15 for thf & 0.87, 0.32, 0.11 for tfp
LOD (µg/ml)		0.0377 µg/ml and 0.1081 µg/ml
LOQ (µg/ml)		0.113 µg/ml and 0.3243 µg/ml

Figure 1. Structure of Trihexyphendyl Hcl.

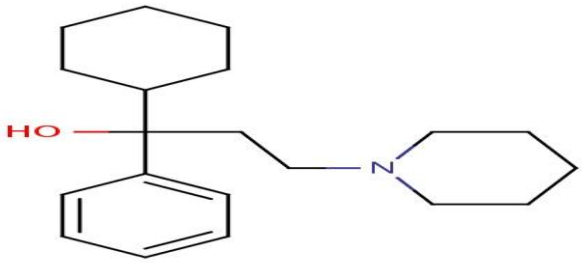

Figure 2. Structure of Trifluoperazine

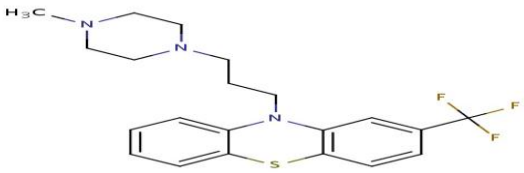

Figure 3. The chromatogram of optimized conditions Trihexyphenidyl Hcl (2.75), Trifluoperazine (6.57)

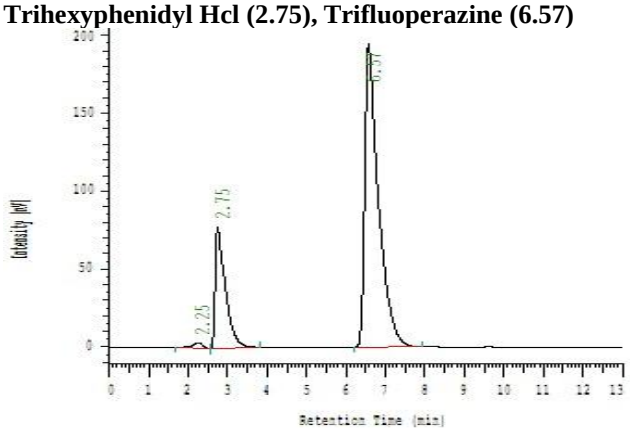

Figure 4. Calibration Curve for Trihexyphenidyl Hcl

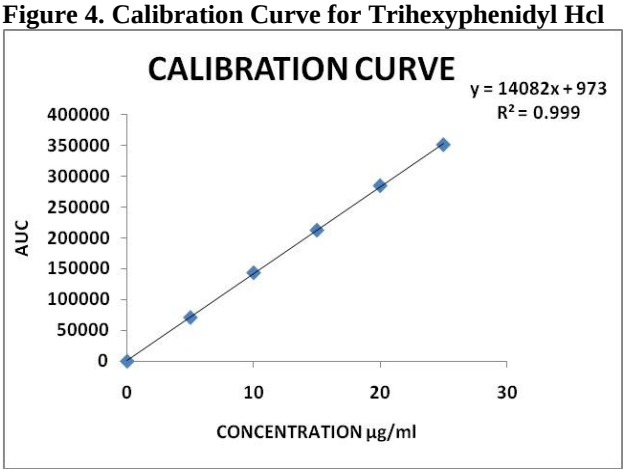

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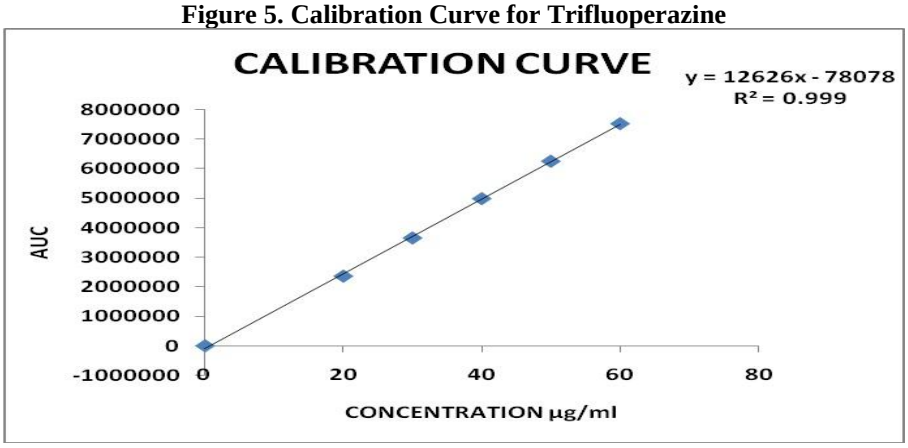
Figure 5. Calibration Curve for Trifluoperazine



DISCUSSION AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Trihexyphenidyl Hcl & Trifluoperazine different chromatographic conditions were applied & the results observed are presented. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns [16] are available, but here Develosil, C-18, ODS (150mm*4.6mmØ) column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluents for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, DMSO, acetonitrile, water, 1M NaOH, IM Hcl). The drugs were found to be highly soluble in methanol. Drugs were sparingly soluble in acetonitrile. Using these solvents with appropriate composition newer methods can be developed and validated. The result shows the developed method is yet another suitable method which can help in the analysis of Trihexyphenidyl Hcl & Trifluoperazine in different formulations. The precision of the method was demonstrated by intra-day and inter- day variation studies.

For intra - day studies the drug having concentration value 10%, 20 % & 100% of the target concentration (n = 3), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days. Data were subjected to statistical treatment for the calculation of SD and %RSD. The values of %RSD were 1.03, 0.51, 0.19 for Thf & 0.86, 0.30, 0.13 for Tfp for intra-day studies. The values for inter-day studies were 0.46, 0.28, 0.15 for thf & 0.87, 0.32, 0.11 for tfp respectively. This shows that values are not more than 2%, indicates that the developed method is precise.

The proposed method is simple, sensitive and reproducible and hence can be used in routine for determination of Trihexyphenidyl Hcl & Trifluoperazine in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision.

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