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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TRIFLUOPERIZINE HYDROCHLORIDE AND TRIHEXYPHENIDYL HYDROCHLORIDE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

A new simple, sensitive and specific spectrophotometric method has been developed for the simultaneous estimation of trifluoperazine hydrochloride and trihexyphenidyl hydrochloride in combined dosage form. The method involves solving simultaneous equation. Trifluoperazine hydrochloride has absorbance maxima at 257.5 nm in distilled water and trihexyphenidyl hydrochloride has absorbance maxima at 210 nm in distilled water.

The proposed method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. Beer's law was obeyed in the concentration range of 2-15 µg/mL and 4-35 µg/mL for trifluoperazine hydrochloride and trihexyphenidyl hydrochloride, respectively and with regression coefficient r = 0.999 for both.

Key words: Trifluoprazine, Trihexyphenidyl hydrochloride, Simultaneous equation, Multicomponent mode analysis.

INTRODUCTION

Trifluoprazine (TFP) is chemically 10-[3-(4-methylpiperazin-1-yl)propyl]-2-(trifluoromethyl) phenothiazine hydrochloride and it blocks postsynaptic mesolimbic dopaminergic D_1 and D_2 receptors in the brain[1-4]. Spectroscopic[5], HPTLC[6] and RP-HPLC[7]

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methods have been reported for the estimation of trifluoperazine individually and in combination with other drugs.

Trihexyphenidyl hydrochloride (THP) is selective M_1 muscarinic acetylcholine receptor antagonist; chemically it is 1-cyclohexyl-1-phenyl-3-piperidin-1-ylproTFP-1-ol hydrochloride[1-3,8]. Various methods such as LC-MS[9], RP-HPLC[10] and spectrophotometric method[11] have been reported for the estimation of trihexyphenidyl hydrochloride.

Literature survey reveals that no method has been reported so far for the estimation of these two drugs simultaneously in combined dosage forms. Hence, in the present study, a new spectrophotometric method was developed and validated for the simultaneous estimation of TFP and THP in combined dosage forms.

EXPERIMENTAL

Chemicals and reagents

The TFP and THP were obtained as gift samples from Microlab Ltd., Bangalore, Karnataka. Distilled water was used as solvent. The marketed formulation of this combination (Label claim: TFP 5 mg, and THP 2 mg), Triazine-H tablets (Sun Pharmaceuticals Ltd., Gujarat) were purchased from the local market.

Instrumentation

VU-visible spectrophotometer –Shimadzu 1700 with 10 mm matched quartz cell.

Preparation of stock solutions

10 mg of standard trifluoperazine hydrochloride and 10 mg of trihexyphenidyl hydrochloride were weighed accurately and transferred to two separate 100 mL volumetric flasks. Both the drugs were dissolved in 50 mL of distilled water with shaking and then volume was made up to the mark with distilled water to get standard stock solution of each drug. These stock solutions were filtered through 0.2 µm Nylon 66 (N66) 47 mm membrane filter paper to give concentration of trifluoperazine hydrochloride solution as 100 µg/mL and 40 µg/mL of trihexyphenidyl hydrochloride.

Calibration curve

For each drug, appropriate aliquots were pipetted out from each standard stock solution into a series of 100 mL volumetric flasks. The volume was made up to the mark

with mobile phase to obtain concentrations of 2, 3, 7, 9, 11, 13, 15 µg/mL of TFP and 4, 8, 12, 16, 20, 25, 30, 35 µg/mL of THP. Absorbance of the above solutions was measured at 257.5 nm and 210 nm. Overlay spectras are shown in Figures 1 and 2 for trifluoperazine hydrochloride and trihexyphenidyl hydrochloride, respectively.

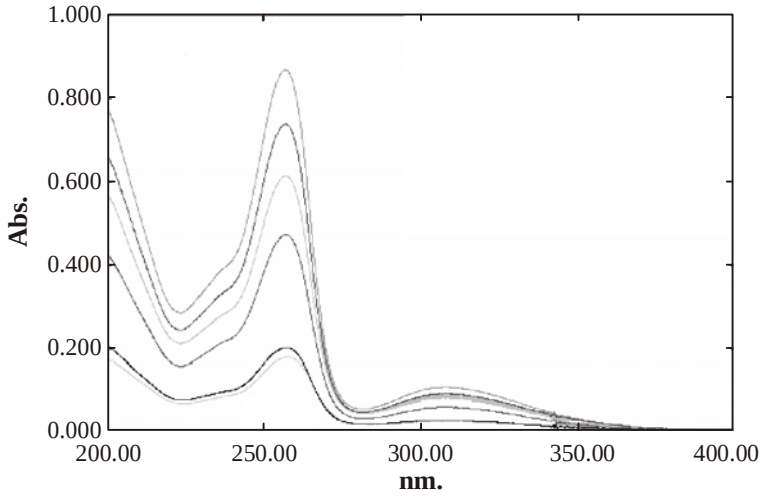

Fig. 1: Overlain spectra of TFP in the concentration range 2 - 15 µg/mL

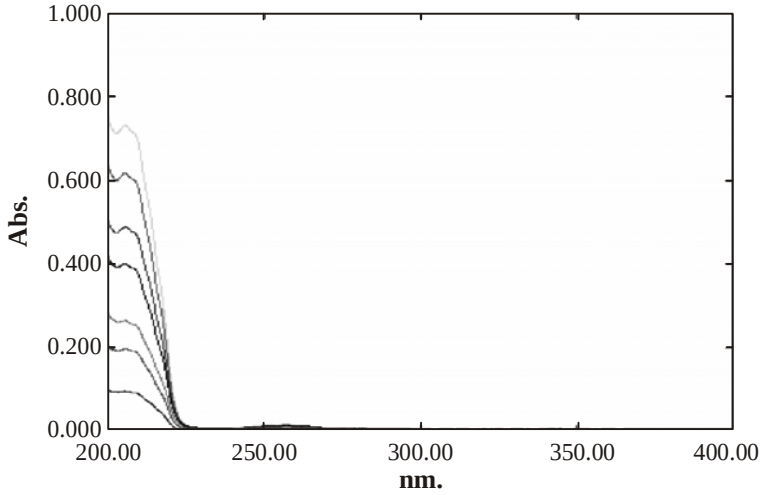

Fig. 2: Overlain spectra of THP in the concentration range 4 - 35 µg/mL

Analysis of tablet formulation

Twenty tablets of trifluoperazine hydrochloride and trihexyphenidyl hydrochloride

in combination were weighed and their average weight was determined. The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to 5 mg of trifluoperazine hydrochloride and 2 mg of trihexyphenidyl hydrochloride was weighed and transferred to 50 mL volumetric flask and dissolved in 50 mL distilled water and the content was kept in ultrasonicator for 25 min. Finally, the volume was made up to the mark with distilled water. The solution was filtered through Whatmann filter paper No. 41 and this solution was used as stock solution.

From the above stock solution, 12.5 mL of the aliquot was pipetted out and was transferred to a 100 mL volumetric flask. The volume was made up to 100 mL with distilled water to obtain a solution with final concentration of trifluoperazine hydrochloride and trihexyphenidyl hydrochloride, 12.5 µg/mL and 5 µg/mL, respectively. Six different mixtures containing trifluoperazine hydrochloride and trihexyphenidyl hydrochloride, 12.5 µg/mL and 5 µg/mL, respectively were prepared as above and absorbances of these solutions were measured at 257.5 nm and 210 nm, respectively. Concentrations of these two drugs in the sample were calculated using Eq. 1 and 2. Results are reported in Table 2.

Calculation

A set of equations were used as given below:

$$A_1 = ax_1 \times Cx + ay_1 \times Cy \quad \ldots(1)$$

$$A_2 = ax_2 \times Cx + ay_2 \times Cy \quad \ldots(2)$$

Molar absorptivity value as determined for TFP was found to be 0.67×10^4L/mol.cm. at 257.5 nm and 0.38×10^4L/mol.cm at 210 nm . Molar absorptivity values for THP at 210 nm and 257.5 nm were 0.25×10^4L/mol.cm. and 0.0055×10^4L/mol.cm, respectively. The values are shown in Table 1. The method employs solving of simultaneous equations using Cramer's rule and matrices.

Table 1: Absorptivity values for TFP and THP

Concentration (µg/mL)		Absorptivity at 257.5 nm		Absorptivity at 210 nm	
TFP	**THP**	**TFP**	**THP**	**TFP**	**THP**
2	4	655.00	12.50	380.00	247.50
3	8	666.67	7.50	380.00	242.50

Cont…

Concentration (µg/mL)		Absorptivity at 257.5 nm		Absorptivity at 210 nm	
TFP	**THP**	**TFP**	**THP**	**TFP**	**THP**
5	12	678.00	5.00	390.00	244.17
7	16	702.86	4.38	381.43	246.25
9	20	668.89	0.55	388.89	245.50
11	25	674.55	6.00	380.00	240.80
13	30	667.69	4.33	385.38	240.33
15	35	666.67	4.29	394.00	239.14
Mean		672.54	5.57	384.96	243.27

Validation method

Linearity

The standard curve was obtained in the concentration range of 2 – 15 µg/mL for trifluoperazine hydrochloride and 4-35 µg/mL for trihexyphenidyl hydrochloride. The linearity of these methods was evaluated by linear regression analysis, using least squares method.

Precision

Procedure for determination of intra-day precision

In intra-day precision, the sample mixture containing 12.5 µg/mL of trifluoperazine hydrochloride and 5 µg/mL of trihexyphenidyl hydrochloride was analyzed six times at different time intervals in the same day.

Procedure for determination of inter-day precision

In inter-day precision, a set of six sample mixtures containing 12.5 µg/mL of trifluoperazine hydrochloride and 5 µg/mL of trihexyphenidyl hydrochloride were prepared and analyzed on different days. The variation of the results on different days was analyzed and statistically validated.

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). After the addition of the standards, the contents were analyzed by the same procedure used for tablet analysis.

RESULTS AND DISCUSSION

The proposed chromatographic conditions were found to be satisfactory for the determination of TFP and THP in combined dosage form. The results of the assay of the marketed formulation are presented in Table 2. The method was validated statistically and validation parameters are summarized in Table 3 and 4.

Table 2: Assay results of TFP and THP in combined dosage form

Drug	Label claim	% Drug found ± SD*	RSD (%)*
Trifluoperazine hydrochloride	5 mg	4.94 ± 0.02	0.43
Trihexyphenidyl hydrochloride	2 mg	1.97 ± 0.01	0.69

*n = 6, SD; Standard deviation, RSD; Relative standard deviation

Table 3: Precision

Drug	Concentration (µg/mL)	Intra-day		Inter-day	
		Measured concentration µg/mL ± SD	% C.V.	Measured concentration µg/mL ± SD	% C.V.
Trifluoperazine hydrochloride	12.5	12.35 ± 0.1	0.82	12.35 ± 0.07	0.57
Trihexyphenidyl hydrochloride	5	4.95 ± 0.02	0.53	4.9 ± 0.02	0.55

Table 4: Accuracy studies

Level of % recovery	Amount present (mg/tab)		Amount of standard drug added (mg)		Amount recovered (mg Mean ± S.D.) (N=3)		Mean ± S.D of % recovery	
	TFP	THP	TFP	THP	TFP	THP	TFP	THP
80 %	5	2	4	1.6	8.9 ± 0.01	3.58 ± 0.01	99.53 ± 0.06	99.55 ± 0.4
100 %	5	2	5	2	9.93 ± 0.03	3.99 ± 0.01	99.33 ± 0.3	99.76 ± 0.21
120 %	5	2	6	2.4	10.97 ± 0.01	4.38 ± 0.01	99.77 ± 0.08	99.57 ± 0.16

Method validation

The developed analytical method was subjected to validation as per the ICH guidelines[19].

Linearity

Linearity was established by least square regression analysis of the calibration curve. The linearity range for the TFP and THP was found to be 3-15 µg/mL and 4-35 µg/mL, respectively. The regression coefficient were found to be r = 0.999 for TFP and THP; both. The linearity graph are shown in Figures 3, 4 and 5.

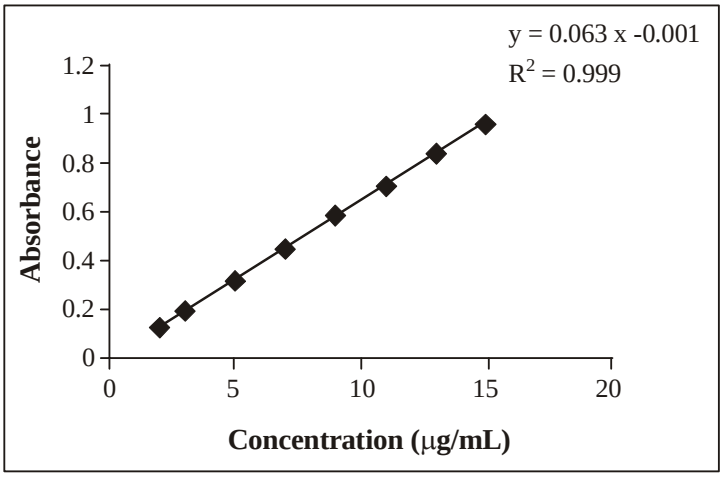

Fig. 3: Calibration curve for TFP at 257.5 nm in distilled water by simultaneous equation method

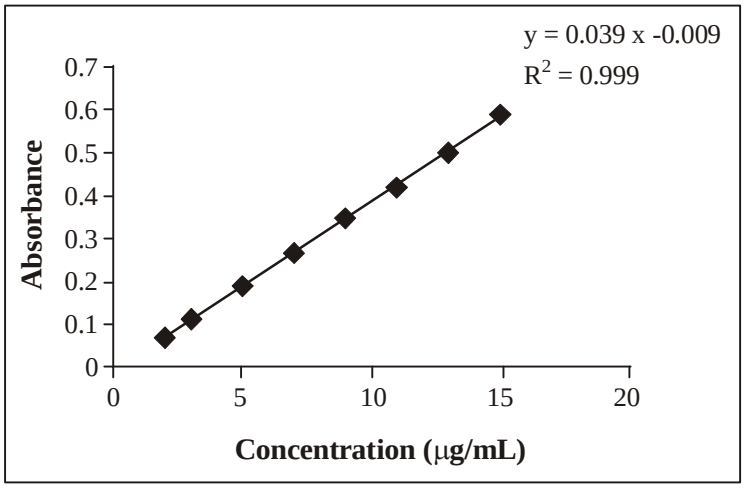

Fig. 4: Calibration curve for TFP at 210 nm in distilled water by simultaneous equation method

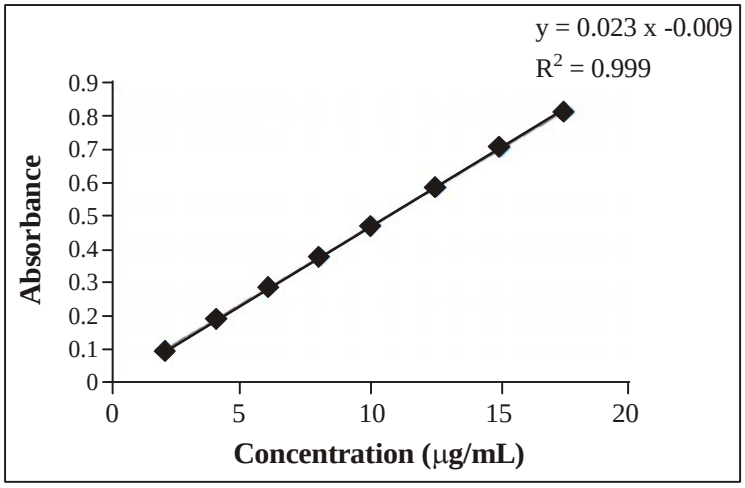

Fig. 5: Calibration curve for THP at 210 nm in distilled water by simultaneous equation method

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined based on the standard deviation of response and slope of calibration curve. LOD and LOQ were found to be 0.0018 and 0.0056 for TFP and 0.0046 and 0.014 for THP, respectively.

Precision

For intra-day studies, six concentrations were analyzed on the same day and for inter-day studies, six concentrations were analyzed on three days. The data showed that RSD was found to less than 2 % for both; intra-day and inter-day studies, which shows that the method is precise. Results are reported in Table 3.

Accuracy

Recovery studies were performed to determine the accuracy of the method. Recovery experiments were performed at three levels, in which the preanalyzed sample solutions were spiked with trifluoperazine hydrochloride and trihexyphenidyl hydrochloride at 80 %, 100 % and 120 % of the label claim. Three replicate samples of each concentration levels were prepared and the percentage recovery at each level was determined. Results are reported in Table 4.

CONCLUSION

A newly developed spectrometric method can be used for routine analysis as a method for the simultaneous estimation of trifluoperazine hydrochloride and trihexyphenidyl hydrochloride in pharmaceutical dosage form. The method was validated and found to be simple, accurate and precise. Statistical analysis of the developed method has been carried out, which shows good accuracy and precision.

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