



# **SIMULTANEOUS ESTIMATION OF TRIFLUOPERAZINE HYDROCHLORIDE AND TRIHEXYPHENIDYL HYDROCHLORIDE IN COMBINED TABLET DOSAGE FORM BY RP-HPLC METHOD**

**SUNIL L. DABHADE, A. SATISHKUMAR SHETTY\*, MANZOOR  
AHMED, B. GOPINATH, B. K. SRIDHAR and MITHUN L. SUREJA**

Department of Pharmaceutical Analysis, National College of Pharmacy,  
SHIMOGA – 577201 (K.S.) INDIA

## **ABSTRACT**

A new reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the simultaneous estimation of trifluoperazine hydrochloride and trihexyphenidyl hydrochloride in combined dosage form. A Inertsil ods-3 C-18 column having dimensions of 250 × 4.6 mm and particle size of 5 µm, with mobile phase containing a mixture of acetonitrile : water : triethylamine in the ratio of (68 : 31.8 : 0.2 v/v) was used. The pH of mobile phase was adjusted to 4.0 with orthophosphoric acid. The flow rate was 1 mL/min and the column effluents were monitored at 210 nm. The retention time for trifluoperazine hydrochloride and trihexyphenidyl hydrochloride was found to be 2.76 and 2.3 min, respectively. The proposed method was validated in terms of linearity, accuracy, precision, limit of detection, limit of quantitation and robustness. The method was found to be linear in the range of 10-150 µg/mL and 4-60 µg/mL for trifluoperazine hydrochloride and trihexyphenidyl hydrochloride, with regression coefficient  $r = 0.999$ , and  $r = 0.999$ , respectively.

**Key words:** Trifluoperazine, hydrochloride, Trihexyphenidyl hydrochloride, RP-HPLC.

## **INTRODUCTION**

Trifluoperazine (TFP) is chemically a 10-[3-(4-methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)phenothiazine hydrochloride and it blocks postsynaptic mesolimbic dopaminergic D<sub>1</sub> and D<sub>2</sub> receptors in the brain<sup>1-4</sup>. Spectroscopic<sup>5</sup>, HPTLC<sup>6</sup> and RP-HPLC<sup>7</sup>

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\* Author for correspondence; Ph.: (M) 094491-00007; E-mail : drskshetty@rediffmail.com.

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

Trihexyphenidyl hydrochloride (THP); 1-cyclohexyl-1-phenyl-3-piperidin-1-ylpropan-1-ol hydrochloride is selective M<sub>1</sub> muscarinic acetylcholine receptor antagonist<sup>1-3,8</sup>. Various methods such as LC-MS<sup>9</sup>, RP-HPLC<sup>10</sup> and spectrophotometric method<sup>11</sup> 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 reverse phase high performance liquid chromatography 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, India. Acetonitrile (HPLC grade), water (HPLC grade), triethylamine and orthophosphoric acid were of AR grade. The market formulation of this combination (Label claim: TFP 5 mg, and THP 2 mg), Triazine-H tablets (Sun Pharmaceuticals Ltd.) was purchased from the local market.

### **Instrumentation**

A Water HPLC 2695 separation module with Water 2996-Photodiode array detector and Inertsil ods-3 C-18 column having dimensions of 250 × 4.6 mm and particle size of 5 μm was used.

### **Chromatographic condition**

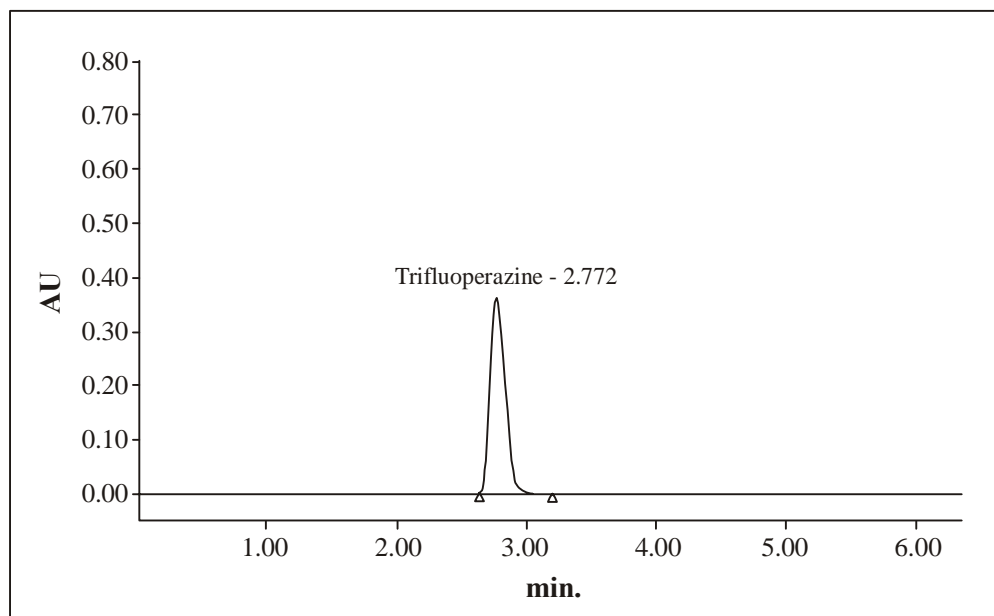
The mobile phase containing acetonitrile : water : triethylamine (68 : 31.8 : 0.2 v/v) with pH 4.0 adjusted by using ortho-phosphoric acid was selected as the optimum composition of mobile phase, because it was found that this solvent system resolved both the components ideally. The flow rate was set to 1 mL/min and UV detection was carried out at 210 nm. The mobile phase and samples were degassed by ultrasonication for 20 min and filtered through 0.45 μm Nylon 66 (N66) 47 mm membrane filter paper. All determinations were performed at constant column temperature (25<sup>0</sup>C).

### Preparation of stock solutions

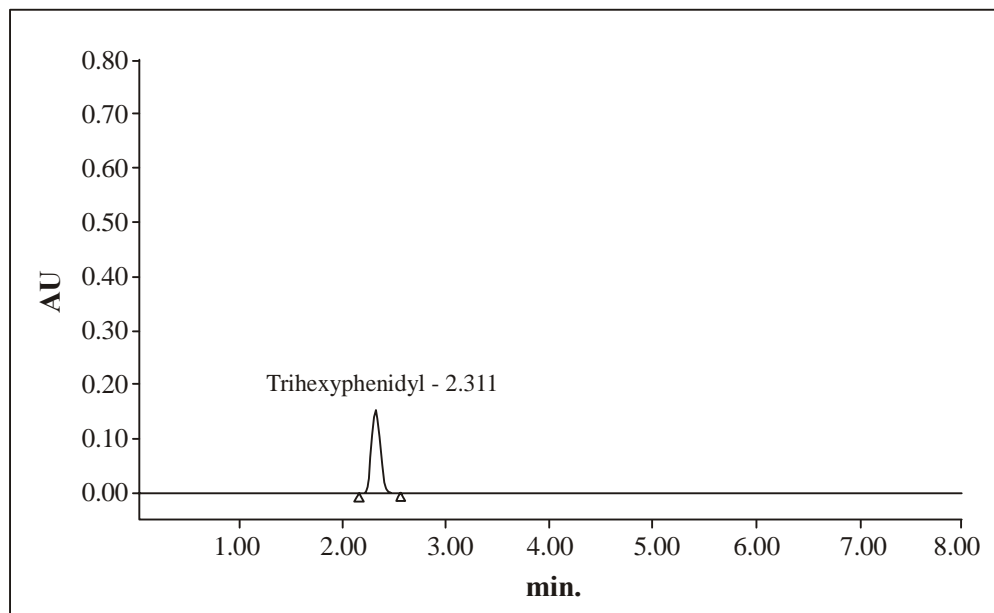
20 mg of standard trifluoperazine hydrochloride and 10 mg trihexyphenidyl hydrochloride was weighed accurately and transferred to two separate 100 mL volumetric flasks. Both the drugs were dissolved in 50 mL of mobile phase with shaking and then volume was made up to the mark with mobile phase to get standard stock solution of each drug. These stock solutions were filtered through 0.2  $\mu\text{m}$  Nylon 66 (N66) 47 mm membrane filter paper and having concentration of trifluoperazine hydrochloride as 200  $\mu\text{g/mL}$  and as 100  $\mu\text{g/mL}$  trihexyphenidyl hydrochloride.

### Calibration curve

For each drug, appropriate aliquots were pipetted out from each standard stock solution into a series of 10 mL volumetric flasks. The volume was made up to the mark with mobile phase to obtain concentrations of 10, 20, 50, 62.5, 100, 125, 150  $\mu\text{g/mL}$  of TFP and 4, 8, 20, 25, 40, 50, 60  $\mu\text{g/mL}$  of THP. The solutions were injected in triplicates for each concentration using a 20  $\mu\text{L}$  loop system and chromatographed under the conditions as described earlier. Peak areas were recorded for all the peaks at 210 nm and a standard calibration curve of peak area against concentration was plotted. The individual chromatograms are shown in Figures 1 and 2.



**Fig. 1: RP-HPLC chromatogram of trifluoperazine hydrochloride (100  $\mu\text{g/mL}$ )**



**Fig. 2: RP-HPLC chromatogram of of trihexyphenidyl hydrochloride (50 µg/mL)**

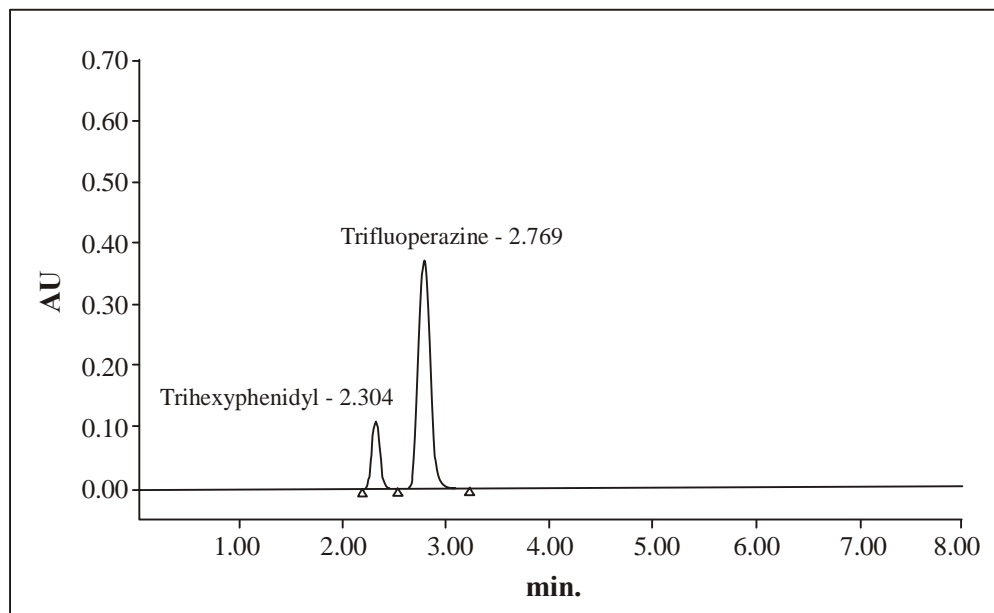
### **Analysis of tablet formulation**

Twenty tablets were weighed and their average weight was determined and these were finely powdered. The powder equivalent to 5 mg of TFP and 2 mg of THP was accurately weighed and transferred to 50 mL volumetric flask and dissolved in 25 mL mobile phase as diluent and the flask was kept in ultrasonicator for 10 min. The flask was shaken and volume was made up to the mark with mobile phase. The solution was filtered through Whatmann filter paper No. 41 and it contains final concentration of 100 µg/mL of TFP and 40 µg/mL of THP. A 20 µL volume of sample mixture was injected into the sample injector of HPLC system for six times and their chromatograms were recorded under the same chromatographic conditions as described above and is shown in Figure 3.

### **Validation method**

#### **Linearity**

The standard curve was obtained in the concentration range of 10 – 150 µg/mL for trifluoperazine hydrochloride and 4-60 µg/mL for trihexyphenidyl hydrochloride. The linearity of these methods were evaluated by linear regression analysis, using least squares method.



**Fig. 3: RP-HPLC chromatogram of a mixture of trifluoperazine (100  $\mu\text{g}/\text{mL}$ ) hydrochloride and trihexyphenidyl hydrochloride (80  $\mu\text{g}/\text{mL}$ ) in tablet formulation**

### Precision

#### Procedure for determination of intra-day precision

In intra-day precision, the sample mixture containing 100  $\mu\text{g}/\text{mL}$  of trifluoperazine hydrochloride and 40  $\mu\text{g}/\text{mL}$  of trihexyphenidyl hydrochloride was analyzed six times at different time intervals on the same day.

#### Procedure for determination of inter-day precision

In inter-day precision, a set of six sample mixtures containing 100  $\mu\text{g}/\text{mL}$  of trifluoperazine hydrochloride and 40  $\mu\text{g}/\text{mL}$  of trihexyphenidyl hydrochloride were prepared and analyzed at same time 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 transferred to 100 mL volumetric flask and dissolved in 50 mL mobile phase and the content was kept in ultrasonicator for 25 min. Finally the volume was made up to the mark with mobile phase. The solution was filtered through Whatmann filter paper No. 41. The mixed sample solutions were analyzed.

## 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 1. The method was validated statistically and validation parameters are summarized in Tables 2 and 3. The system suitability test parameters are shown in Table 4.

**Table 1: Assay results of trifluoperazine hydrochloride and trihexyphenidyl hydrochloride in combined dosage form**

Drug	Label claim	% Drug found $\pm$ SD*	RSD (%)*
Trifluoperazine hydrochloride	5 mg	98.69 $\pm$ 0.41	0.42
Trihexyphenidyl hydrochloride	2 mg	98.17 $\pm$ 0.45	0.46

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

**Table 2: Precision of proposed HPLC method**

Drug	Concentration ( $\mu\text{g/mL}$ )	Intra-day		Inter-day	
		Measured concentration $\mu\text{g/mL} \pm$ SD	% C.V.	Measured concentration $\mu\text{g/mL} \pm$ SD	% C.V.
Trifluoperazine hydrochloride	100	99.21 $\pm$ 0.30	0.30	98.98 $\pm$ 0.41	0.41
Trihexyphenidyl hydrochloride	40	39.33 $\pm$ 0.12	0.30	39.1 $\pm$ 0.1	0.4

**Table 3: Accuracy studies**

Level of % recovery	Amount present (mg/tab)		Amount of standard drug added (mg)		Mean $\pm$ S.D. amount recovered (mg) (N = 3)		Mean $\pm$ S.D % of Recovery	
	TFP	THP	TFP	THP	TFP	THP	TFP	THP
80 %	5	2	4	1.6	9 $\pm$ 0.02	3.5 $\pm$ 0.05	100 $\pm$ 0.23	98.58 $\pm$ 0.2
100 %	5	2	5	2	10.02 $\pm$ 0.6	3.9 $\pm$ 0.01	100.17 $\pm$ 05	98.82 $\pm$ 0.3
120 %	5	2	6	2.4	10.9 $\pm$ 0.04	4.34 $\pm$ 0.03	99.05 $\pm$ 0.4	98.74 $\pm$ 0.6

**Table 4: Summary of system suitability parameters of trifluoperazine hydrochloride and trihexyphenidyl hydrochloride**

Parameters	Trifluoperazine hydrochloride	Trihexyphenidyl hydrochloride
Retention time (min)	2.72	2.31
Resolution factor	2.7	
Tailing factor	1.3	1.1
Theoretical plate	4000	3009

### Method validation

The developed analytical method was subjected to validation as per the ICH guidelines<sup>19</sup>.

### Specificity

The specificity of the RP-HPLC method was determined by comparison of the chromatogram of standard solutions and sample solutions. The retention time of standard TFP and THP were compared with that of sample solution. Good correlation was obtained between the retention time of standard and sample of TFP and THP.

## Linearity

Linearity was established by least square regression analysis of the calibration curve. The linearity range for the TFP and THP were found to be 10-150  $\mu\text{g/mL}$  and 4-60  $\mu\text{g/mL}$ , respectively. Peak areas of TFP and THP were plotted against their respective concentrations and linear regression analysis was performed on the resultant curves. The regression equations were found to be:  $y = 26960x + 13795$  ( $r = 0.999$ ) for TFP and  $y = 13786x - 1072$  ( $r = 0.999$ ) for THP, respectively. The linearity graphs are presented in Figures 4 and 5.

## 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, five concentrations were injected into the HPLC system three times on the same day and for inter-day studies, five concentrations were injected into the HPLC system for three days. The data showed that RSD was found to be less than 2 % for both; intra-day and inter-day studies, which shows that method is precise. Results are shown in Table 3.

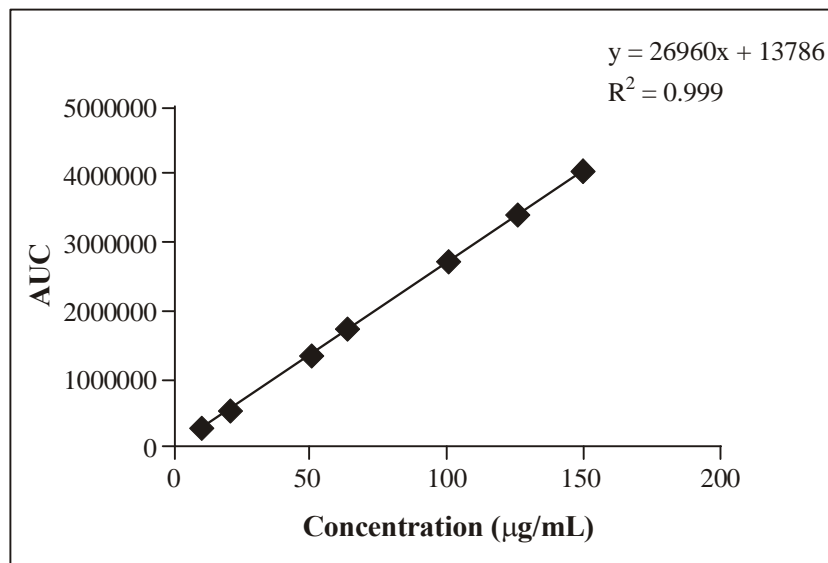
## Accuracy

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 shown in Table 4.

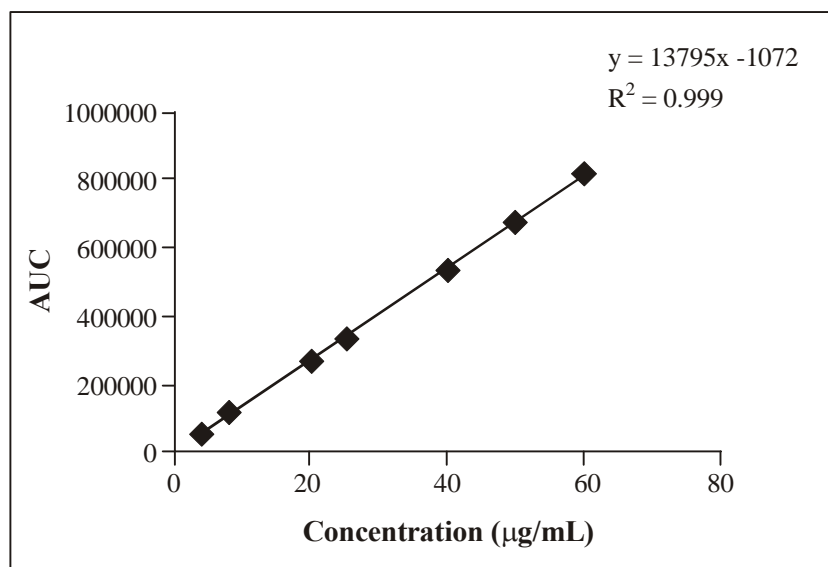
## Robustness

The robustness study was done by making small changes in the optimized method parameters like  $\pm 0.1$  change in mobile phase composition,  $\pm 0.1$  change in flow rate and  $\pm 0.1$  change in column temperature. There was no significant impact on the retention time.





**Fig. 4: Calibration curve of trifluoperazine hydrochloride at 210 nm by RP-HPLC**



**Fig. 5: Calibration curve of trihexyphenidyl hydrochloride at 210 nm by RP-HPLC**

## CONCLUSION

A newly developed RP-HPLC 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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