International Journal of Advances in Pharmaceutical Analysis
IJAPA Vol. 4 Issue 4 (2014) 134-137

New validated RP-HPLC method for simultaneous estimation of chlorpromazine and Trihexyphenidyl HCl in tablets

N. Usha Rani*, K. Divya and G. Sahithi

Department of Pharmaceutical Analysis, Maharajah's College of Pharmacy, Vizianagaram, Andhra Pradesh, India.

Abstract

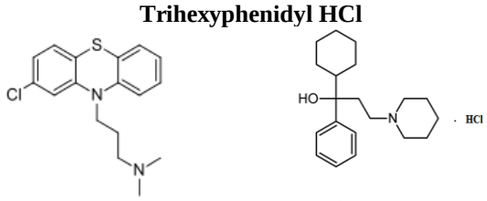
A simple, specific and precise reverse phase high performance liquid chromatographic method was developed and validated for simultaneous estimation of Chlorpromazine and Trihexyphenidyl HCl in tablets. Quantification was achieved by using a reverse-phase C18 column (Inertsil ODS 3V, 250 mm x 4.6 mm; 5µ) at 31°C. The mobile phase consisted of a mixture of ammonium acetate buffer and methanol in the ratio of 15:85 v/v at a flow rate of 1.2 mL/min. The retention times of Chlorpromazine and Trihexyphenidyl HCl were found to be 3.237 min and 5.260 min respectively. The developed method was validated as per ICH Guidelines for linearity, accuracy, precision, detection limit, quantification limit, ruggedness, robustness, specificity and system suitability. The percentage recoveries for both of the drugs from their tablets were found to be 100.34 % and 99.80 % respectively. The method may successfully be employed for the simultaneous determination of Chlorpromazine and Trihexyphenidyl HCl in pharmaceutical tablet dosage forms.

Keywords: Chlorpromazine; Trihexyphenidyl HCl; RP-HPLC; tablets.

1. Introduction

Chlorpromazine is a propylamino phenothiazine compound, chemically 3-(2-chloro-10*H*-phenothiazin -10-yl)-*N*, *N*-dimethylpropan-1-amine, soluble in methanol. It is a typical anti-psychotic drug and acts as an antagonist of dopamine-2 receptor. Trihexyphenidyl HCl is 1-cyclohexyl-1-phenyl-3-(1-piperidyl) propan-1-ol hydrochloride. The drug is available as hydrochloride salt and slightly soluble in water, soluble in alcohol and in chloroform. Trihexyphenidyl HCl binds to the M_1 muscarinic receptor and dopamine receptor. It is an anti-dyskinetic and anti-parkinson drug. The structure of a) Chlorpromazine and b) Trihexyphenidyl HCl are shown in figure 1.

Figure 1: Structure of a) Chlorpromazine and b) Trihexyphenidyl HCl



(a) **(b)**

The literature survey revealed that very few RP-HPLC[1-6] and spectroscopic[7-10] methods were reported for the simultaneous estimation of Chlorpromazine and Trihexyphenidyl HCl in formulations. The authors now propose a new validated, sensitive and reproducible HPLC method for simultaneous determination of Chlorpromazine and Trihexyphenidyl HCl. The applicability of this method in determining the drugs in commercial dosage forms were also studied.

2. Experimental

2.1 Materials

Standard Trihexyphenidyl HCl and chlorpromazine were obtained from SD fine chemicals limited (SDFCL), Hyderabad, India. Commercial formulation, Talentil T was purchased from the local market. Each tablet contains 50 mg of Chlorpromazine and 2mg of Trihexyphenidyl HCl. Other materials required were HPLC grade water, methanol and AR grade Ammonium acetate.

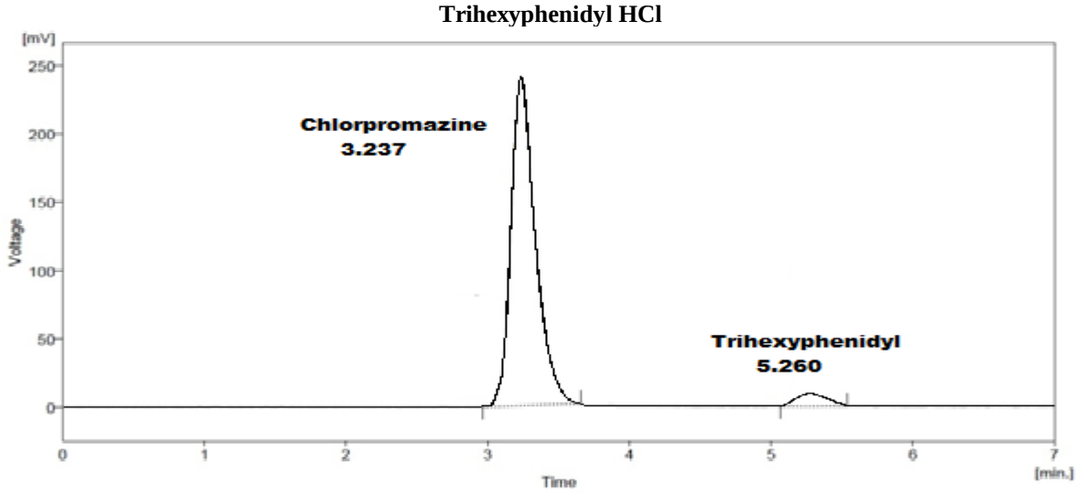
2.2 Instrumentation

A Shimadzu LC 20-AT VP high performance liquid chromatographic instrument with spin chrome software and an inertsil ODS (250 mm x 4.6 mm; 5µ) column was used for separation. The detection was done using an UV – VIS SPD 20A Detector.

2.3 Optimized Chromatographic Conditions

An inertsil ODS, C18 (250 mm x 4.6 mm; 5µ) column was used for the analytical separation. The mobile phase consisted of a mixture of 0.02M ammonium acetate buffer (pH 6) and methanol in the ratio of 15:85 v/v with an isocratic elution program. The flow rate was adjusted to 1.2 mL/min and the injection volume was set at 20 µL with a detection wavelength of 215 nm. The separation of Chlorpromazine and Trihexyphenidyl HCl under optimised conditions is shown in figure 2.

Fig 2: Typical HPLC Chromatogram corresponding to mixed standard solution of Chlorpromazine and Trihexyphenidyl HCl



Corresponding Author*: nusharani.au@gmail.com

2.4 Preparation of Standard solutions:

2.4.1 Chlorpromazine stock solution:

50 mg of Chlorpromazine was accurately weighed and dissolved in 10mL of the mobile phase. After sonication for 15 min, the volume was made upto 50mL with the mobile phase to get 1 mg/ mL solution.

2.4.2 Chlorpromazine working standard solutions:

The Chlorpromazine stock solution was diluted suitably with mobile phase to get the working standard solutions of concentrations ranging from 60 to 140 µg/mL. 20 µL of each of the dilutions was injected 5 times into the column and the chromatograms were recorded.

2.4.3 Trihexyphenidyl HCl stock solution:

ihexyphenidyl HCl equivalent to 2 mg of Trihexyphenidyl was weighed accurately and dissolved in 10 mL of the mobile phase. After sonication for 15 min, the volume was made up to the mark with sufficient volume of mobile phase in a 50 mL volumetric flask to get a 40 µg/mL solution.

2.4.4 Trihexyphenidyl HCl working standard solutions:

The Trihexyphenidyl HCl stock solution was diluted suitably with mobile phase to get the working standard solutions of concentrations ranging from 2.4 to 5.6 µg/mL. 20 µL of each of the dilutions was injected 5 times into the column and the chromatograms were recorded.

2.4.5 Preparation of mixed standard stock solution:

Chlorpromazine (100 mg) and Trihexyphenidyl HCl (Equivalent to 4 mg of Trihexyphenidyl) were weighed accurately into a 100 mL volumetric flask, dissolved in 20mL of diluent, sonicated for 15 min and the volume was finally made up with the mobile phase.

2.4.6 Preparation of mixed working standard solutions:

1mLof the mixed stock solution was taken in a 10 mL volumetric flask and made upto volume with the mobile phase to get a concentration 4 µg/mL of Trihexyphenidyl HCl and 100 µg/mL of Chlorpromazine. 20 µL of this solution was injected and the chromatogram was recorded.

2.5 Preparation of mixed sample solution:

Twenty tablets were weighed accurately and crushed to fine powder. Each tablet contains 50 mg of chlorpromazine and 2 mg of Trihexyphenidyl HCl. A quantity of powder equivalent to 50 mg of Chlorpromazine and 2mg of Trihexyphenidyl HCl was weighed and dissolved in 25 mL of the mobile phase in a 50 mL volumetric flask. The volume was made up to give a concentration of 1000 µg/mL of Chlorpromazine and 40 µg/mL of Trihexyphenidyl HCl. The solution was filtered through 0.45 µ nylon membrane filter. From this filtrate, different dilutions ranging from 60-140 µg/mL of Chlorpromazine & 2.4-5.6 µg/mL of Trihexyphenidyl HCl were prepared in 10 mL volumetric flasks with the mobile phase. 20 µL of each of these solutions were injected 5 times and the chromatograms were recorded. The amount of Chlorpromazine and Trihexyphenidyl HCl present in each tablet formulation was calculated by comparing the peak area of the tablet solution with that of standard using the given formula:

$$\%\ \text{Assay} = \frac{\text{Sample Avg. peak area}}{\text{Standard Avg peak area}} \times \frac{\text{Wt. of drug (mg)}}{\text{dilution of standard}} \times \frac{\text{dilution of tablet solution}}{\text{wt. of Sample}} \times \frac{\%\ \text{Purity}}{100} \times \frac{\text{Avg. wt}}{\text{Lable Claim}} \times 100$$

2.6 System suitability parameters:

Five replicates of mixed working standard solutions were injected and the parameters like theoretical plate number (N), tailing factor (K) and resolution are calculated to check the system suitability. The results are presented in Table 1.

Table 1: System suitability test results

Sr. No	Parameters	CPZ	THP
1	Peak area (mV*min)	5835.662	65.058
2	No. of theoretical plates	4691	2857
3	Retention time (min)	3.203	5.223
4	Asymmetry	1.55	1.00

3. Results and Discussion

The chromatographic conditions were optimised to develop RP-HPLC method for simultaneous determination of Chlorpromazine and Trihexyphenidyl HCl with adequate resolution and rapid analysis time.

3.1 Method Validation:

The analytical method was developed and validated according to ICH guidelines. Analytical variable parameters such as linearity, precision, accuracy, specificity and system suitability were tested using the optimized chromatographic conditions and instruments.

3.1.1 Linearity:

Mixed standard stock solution was suitably diluted with the mobile phase to obtain the concentrations ranging from 60-140 µg/mL of Chlorpromazine & 2.4-5.6 µg/mL of Trihexyphenidyl HCl. The solutions were filtered through 0.45 µ nylon membrane filter paper and 20 µL of each of the solutions were injected and the chromatograms were recorded. A good linear relationship (R^2= 0.997 for Chlorpromazine and R^2= 0.998 for Trihexyphenidyl HCl) was observed between the concentrations of the drugs and their corresponding peak areas. The results of linearity studies are shown in Table 2.

Table 2: Linearity study data for CPZ and THP

Sr. No	Drugs	Slope	Intercept	Correlation coefficient
1	Chlorpromazine	37.877	636.88	0.997
2	Trihexyphenidyl	9.851	2.881	0.998

3.1.2 Accuracy:

The accuracy studies were performed on 80 %, 100 % and 120 % of the analytical method target concentrations of Chlorpromazine and Trihexyphenidyl HCl. Standard and sample preparations were injected into HPLC system and three determinants for each concentration level were obtained. The percentage recoveries of Chlorpromazine and Trihexyphenidyl HCl were calculated using standard at the same concentration at each concentration level. The results are presented in Table 3.

Table 3: Recovery study data for CPZ and THP

Sr. No	Drug	%Recovery	%RSD
1	Chlorpromazine	98.02	0.01
2	Trihexyphenidyl HCl	101.17	0.34

3.1.3 Precision:

System Precision:

System precision of the proposed method was checked by injecting five replicate preparations of the standard drug solutions of Chlorpromazine (100 µg/ml) and Trihexyphenidyl HCl (4 µg/ml). The corresponding peak areas were measured and % RSD calculated.

Method Precision:

The method precision study was performed for five replicate sample preparations of marketed formulation containing Chlorpromazine (100 µg/ml) and Trihexyphenidyl HCl (4 µg/ml). The corresponding peak areas were measured and % RSD calculated as exhibited in Table 4.

Table 4: Precision study data for CPZ and THP

Sr. No	Wt. of Sample (mg)	Peak area of Standard		Peak area of Sample		%Label claim	
		CPZ	THP	CPZ	THP	CPZ	THP
1	62.50	4318.110	48.725	4310.228	48.681	99.82	99.90
2	62.47			4310.124	48.723	99.81	100.0
3	62.65			4309.243	47.995	99.79	98.50
Mean						99.80	99.46
S.D						0.0152	0.838
%RSD						0.015	0.834

3.1.4 Specificity:

The specificity of the proposed method was determined to check whether there is any interference due to presence of excipients, impurities or other components with the retention time of analytical peaks. The HPLC chromatograms were recorded for the drug-matrix (mixture of the drug and excipient) which showed almost no interfering peaks within retention time ranges indicating that the method is quite specific.

3.1.5 Robustness:

Robustness of the developed analytical method was tested by evaluating the affect of small variations in analytical method parameters such as change in flow rate of 1.2 mL/min by ±0.2 mL/min and change in wavelength by ±2 nm. The results are shown in Table 5.

Table 5: Robustness study data for CPZ and THP

System suitability Parameters		% RSD of peak area response (n=5)		Mean tailing factor (n=5)		Mean retention time in min. (n=5)	
Variations		CPZ	THP	CPZ	THP	CPZ	THP
Change in Flow rate (mL/min)	+0.2	0.31	0.70	1.47	1.24	3.827	6.197
	0	0.017	0.043	1.55	1.00	3.237	5.260
	-0.2	0.32	0.22	0.73	0.05	2.833	4.590
Change in Wavelength (nm)	+2	0.014	0.044	1.714	1.282	3.233	5.217
	0	0.017	0.043	1.55	1.00	3.237	5.260
	-2	0.029	0.022	1.667	1.037	3.230	5.243

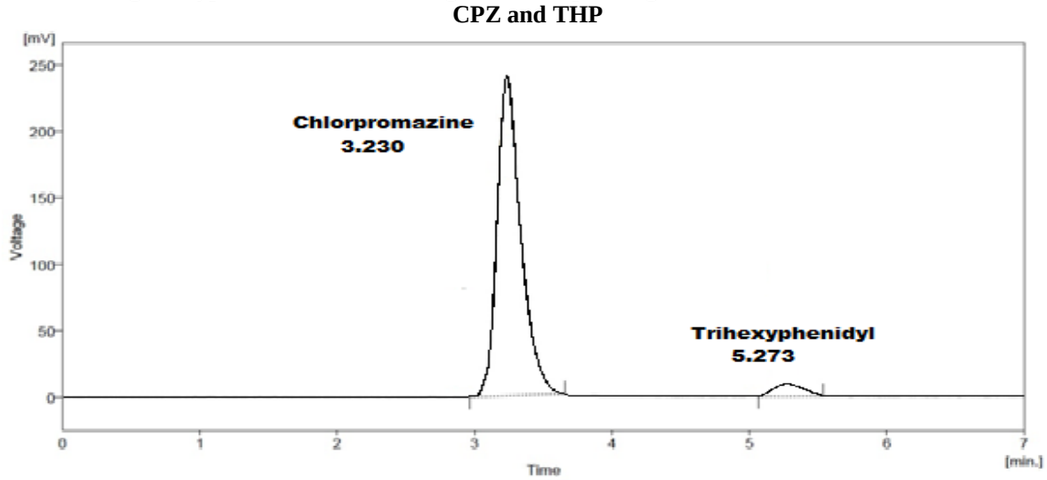
3.2 Application of the method to commercial formulation:

Twenty micro litres of each of the standard and sample solutions were injected separately. The chromatograms were recorded and the corresponding peak areas were measured. The procedure was repeated three times, individually weighing the tablet powder each time. The peak areas obtained for standard and sample were used to calculate the amount of each drug in the tablet formulation. The results obtained are shown in table 6. The chromatogram showing separation of CPZ and THP in tablet formulation is shown in figure 3.

Table 6: Results for tablet formulation study

Sr. No	Wt. of std. (mg)		Weight of sample (mg)	Peak area of std		Peak area of sample		%Label claim	
	CPZ	THP		CPZ	THP	CPZ	THP	CPZ	THP
1	50	2	62.5	4307.870	48.725	4310.228	48.681	100.5	99.90
2			62.51			4305.171	48.868	99.93	100.29
3			62.92			4334.100	48.349	100.6	99.22
Mean								100.343	99.803
S.D								0.361	0.541
%RSD								0.35	0.54

Fig 3: Typical HPLC Chromatogram corresponding to marketed formulation of CPZ and THP



4. Conclusion

The proposed RP-HPLC method is simple, sensitive, reproducible, less time consuming and is applicable for analysis of Chlorpromazine and Trihexyphenidyl HCl in bulk and in tablet dosage forms. The method was duly validated by evaluation of required parameters.

Acknowledgment

The author expresses sincere thanks to SD Fine-Chemicals Limited, Hyderabad, India for providing us gift samples and Maharajah's college of Pharmacy for providing necessary facilities for the study.

References

1. Mahadik KR, Aggarwal H, Kaul N. Development and validation of HPLC method for simultaneous estimation of Trihexyphenidyl HCl and chlorpromazine hydrochloride from tablet dosage form. *Indian drugs* 2002; 39(8): 241-245.
2. Jameel Dhabab M. Simultaneous Determination of Chlorpromazine and Trifluoperazine in Pharmaceutical Preparations Using High Performance Liquid Chromatography. *Al-Mustansiriya Journal Sciences* 2011; 22(2): 123-128.
3. Wate SP, Borkar AA. RP-HPLC Estimation of Haloperidol and Trihexyphenidyl in Tablets. *International Journal of ChemTech Research* 2009; 1(3): 675-676.
4. Srikantha Dammalapati, Ramesh Raju Rudra Raju. A RP-HPLC method for simultaneous determination of Haloperidol and Trihexyphenidy HClin tablet dosage form. *Asian Journal of Pharmaceutical and Clinical Research* 2014; 7(2): 14-18.
5. Dharmaraj Santhosam S, Kannan S. An HPLC method for the simultaneous estimation of Risperidone and Trihexyphenidy HCl from Bulk and dosage forms. *Hygeia Journal for Drugs and Medicines* 2011; 3(1): 29-33.
6. Patel D, Patel J. Development and validation of RP-HPLC method for simultaneous of Risperidone and Trihexyphenidyl HCl in tablet dosage forms. *International Journal of Pharma Sciences and Research* 2010; 4(3): 85-88.
7. Patel A, Shrivastava A, Jain A, Singh GK. Method development and validation for estimation of Trihexyphenidyl Hydrochloride in tablet dosage forms. *Asian Journal of Research Chemistry* 2009; 2(2): 104-107.
8. Roge AB, Shendarkar GR, Ghiware NB, Gond NY, Vadvalkar SM. Absorption correction method for estimation of Risperidone and Trihexyphenidyl HCl in combined tablet dosage form. *International Journal of ChemTech Research* 2013; 5(1): 121-126.
9. Wate SP, Borkar AA. Simultaneous Spectrophotometric Estimation of Haloperidol and Trihexyphenidyl in Tablets. *Indian Journal of Pharmaceutical Sciences* 2010; 72(2): 265-267.
10. Ashraf AL-Ayash S, Fadhil Jasim, Wathiq Alwan. Spectrophotometric determination of Chlorpromazine Hydrochloride in Pharmaceutical preparations. *National Journal of Chemistry* 2009; 33: 42-53.