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Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Haloperidol and Trihexyphenidyl in API and Combined Tablet Dosage Form

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Trihexyphenidyl and Haloperidol, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Altima C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 225 nm. The retention time of the Trihexyphenidyl and Haloperidol was 2.102, 3.537±0.02min respectively. The method produce linear responses in the concentration range of 15-75ppm of Trihexyphenidyland 37.5-187.5ppm of Haloperidol. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of pharmaceutical formulations.

Key words: trihexyphenidyl; haloperidol, RP-HPLC; validation; estimation

1. INTRODUCTION

Haloperidol is an antidyskinetic and antipsycotic drug whose IUPAC name is 1- cyclohexyl-1-phenyl-3-(1-piperidyl)-1-propanol1. Molecular formula of Haloperidol is C21H23ClFNO2; the molecular weight is 375.864; the structural formula is shown below (GK Anthony *et al*., 2014).

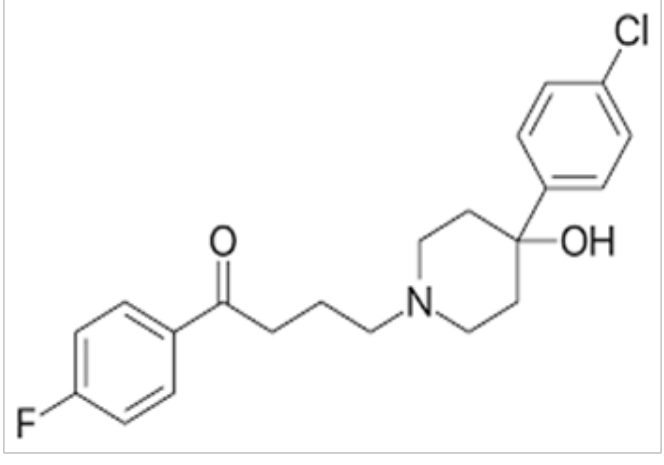

Fig. 1: Structure of Haloperidol

Trihexyphenidyl also known as benzhexol and trihex, is an antiparkinsonian, anticholinergic and antihistaminic drug. It is used for the symptomatic treatment of Parkinson's disease and to treat extrapyramidal side effects occurring during antipsychotic treatment. It reduces the frequency and duration of oculogyric crises as well as of dyskinetic movements and spastic contractions (P. Subbareddy and TE Divakar, 2016).TrihexyphenidylHCl IUPAC name is 1-cyclohexyl-1-phenyl-3-(1-piperidyl) propan-1-ol hydrochloride (N Usha Rani *et al*., 2014) The molecular formula of TXH is C20H31NO.HCl; the

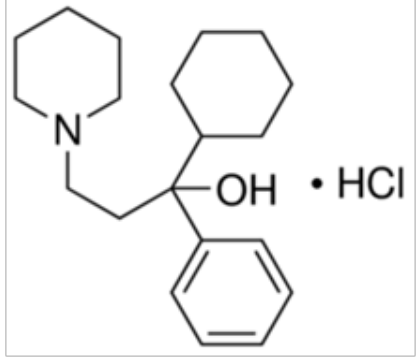

Fig. 2: Structure of Trihexyphenidyl

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molecular weight is 337.93 g/mol; the structural formula is give below (Rudra Raju *et al*., 2014).

Several works are available for simultaneous estimation of Haloperidol and Trihexyphenidyl (Rudra Raju *et al*., 2014; AA Borkar *et al*., 2009). The aim of present work is to develop and validate a simple RP-HPLC method for simultaneous estimation of Haloperidol and Trihexyphenidyl.

2. MATERIAL AND METHOD

The drug samples Haloperidol and Trihexyphenidyl were obtained from Sura Labs., Hyderabad. The solvents used were of HPLC grade methanol and water from Merck Co, Mumbai.

2.1 Intrumentation

The HPLC system used was WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector, Altima C18 (4.6×150mm, 5µ) column.

2.2 Chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	40ºC
Column	:	Altima C18 (4.6×150mm, 5µ)
Buffer	:	Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.5. Fliter and soni cate the solution by vaccum filtration and ultra sonication.
pH	:	4.5
Mobile phase	:	Methanol: TEA buffer: ACN (50:25:25 v/v)
Flow rate	:	1ml/min
Wavelength	:	225 nm
Injection volume	:	10 µl
Run time	:	7 min

2.3 Preparation of Buffer and Mobile Phase

2.3.1 Preparation of Triethylamine (TEA) buffer (pH-4.5)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the p^H 4.5. Fliter and sonicate the solution by vaccum filtration and ultrasonication.

2.3.2 Preparation of mobile phase

Accurately measured 400 ml (40%) of Methanol, 200 ml of Triethylamine buffer (20%) and 400 ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

2.3.3 Diluent Preparation

The Mobile phase was used as the diluent.

2.3.4 Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Trihexyphenidyl and 10mg of Haloperidol working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.45ml of the above Trihexyphenidyl and 1.125ml of the Haloperidol stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

2.3.5 Preparation of Sample Solution

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Trihexyphenidyl and Haloperidol sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1.125ml of the above sample stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

3. RESULTS AND DISCUSSION

Method was validated according to ICH Guidelines in terms of linearity, range, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ) (V. Tejasvi Reddy *et al*., 2016; K Ganesh *et al*., 2016).

3.1 System Suitability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits i.e., 1.09 for Trihexyphenidyl and 0.73 for Haloperidol.

3.2 Linearity

Linearity studies were done for five different concentrations of each drug. The five different concentrations covered 15-75 µg/ml for Trihexyphenidyl and 37.5-187.5 µg/ml for Haloperidol.

The calibration curve showed good linearity in the range of 15-75 µg/ml with correlation coefficient (r2) of 0.999 and regression equation of y = 12066x +13756 for Trihexyphenidyl (Table 2). Calibration curve showed good linearity in the range of 37.5-187.5 µg/ml with correlation coefficient (r2) of 0.999 and regression equation of y = 18768x + 33265 for Haloperidol (Table 2).

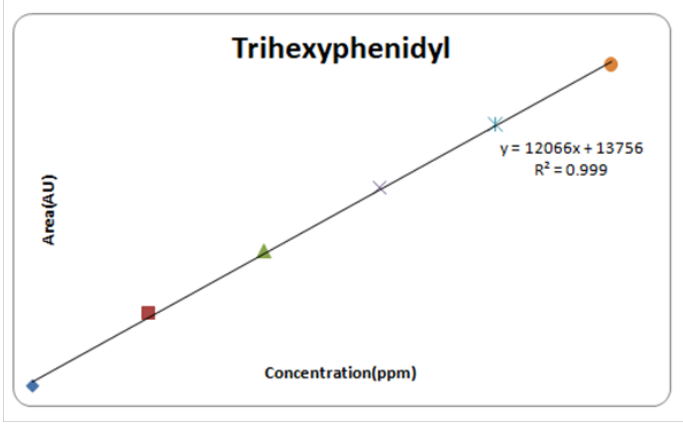

Fig.3: Calibration graph for Trihexyphenidyl

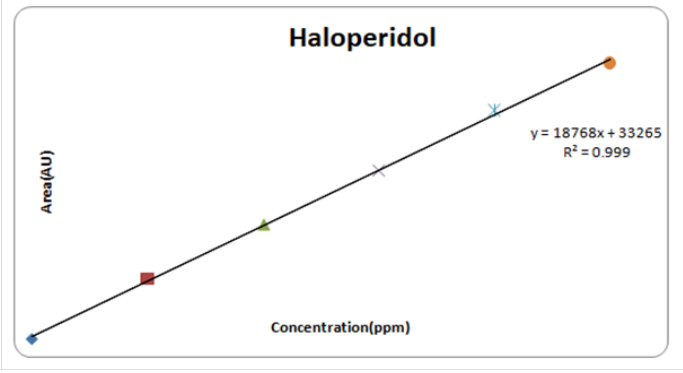

Fig. 4: Calibration graph for Haloperidol

3.3 LOD and LOQ

3.3.1 Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$LOD = 3.3 \times \sigma / s$

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

3.3.2 Limit of quantitation

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$LOQ = 10 \times \sigma / S$

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve

Table 1: Summary of validation parameters for the proposed method

Parameters	Trihexyphenidyl	Haloperidol
Linearity	15-75 µg/ml	37.5-187.5 µg/ml
Intercept (c)	13756	33265
Slope (m)	12066	18768
Correlation coefficient	0.999	0.999
LOD	0.8µg/ml	6.9µg/ml
LOQ	2.6µg/ml	21.1µg/ml

3.4 Precision

3.4.1 Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

3.4.2 Intermediate precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions. The %RSD for the area of six replicate injections was found to be within the specified limits.

Acceptance criteria

%RSD of Six different sample solutions should not more than 2

Table 2: Results of repeatability and intermediate precision of Trihexyphenidyl and Haloperidol

Parameters	*Mean Area*	*Standard Deviation*	*% RSD*	*Mean Area and SD (n=6)*	*Standard Deviation*	*%RSD*
		Trihexyphenidyl			Haloperidol	
Repeatability	606665	2542.3	0.42	2217205	4100.8	0.18
Intermediate precision	596209	1718.7	0.29	2205575	2899.8	0.13

3.5 Accuracy

To study accuracy of the proposed method, recovery studies were performed by spiking of standard drug solution to pre-analyzed sample at three different percentage concentrations 50, 100, 150%. The resultant solutions were then reanalyzed by the proposed method. At each percentage concentration six determinations were

Table 4: Results for robustness

Drugs	Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Trihexyphenidyl	Actual Flow rate of 1.0 mL/min	607323	2.102	5586	1.7
	Less Flow rate of 0.9 mL/min	674735	2.330	5231	1.7
	More Flow rate of 1.1 mL/min	1408920	1.950	5234	1.7
	Less organic phase	606093	2.290	5643	1.4
	More organic phase	603559	1.998	5298	1.5
Haloperidol	Actual Flow rate of 1.0 mL/min	558777	3.537	5371	1.6
	Less Flow rate of 0.9 mL/min	2505636	3.885	5324	1.7
	More Flow rate of 1.1 mL/min	1408920	3.263	5098	1.7
	Less organic phase	2239255	4.435	5239	1.2
	More organic phase	2300346	3.009	5647	1.0

performed. From the results obtained the method was found to be accurate. The percentage recovery and mean recovery were calculated and presented in table.

Table 3: Accuracy results for Trihexyphenidyl and Haloperidol

Drug	%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
Trihexyphenidyl	50%	287774	22.5	22.5	100	99.6%
	100%	551495	45	44.8	98.6	
	150%	825175	67.5	67.42	99.5	
Haloperidol	50%	1104782	56.25	56.249	100%	100%
	100%	2105321	112.5	112.48	99.9%	
	150%	3211306	168.75	168.75	100%	

3.6 Robustness

Robustness of the method was determined by changing flow conditions and variation of mobile phase organic conditions. From the results obtained the method was found to be robust.

Acceptance criteria

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

3.7 Formulation assay:

The proposed RP-HPLC method was validated by simultaneously determining Trihexyphenidyl and Haloperidol in pharmaceutical dosage form. Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Trihexyphenidyl and Haloperidol in pharmaceutical dosage form was found to be 99.6%. and 99.7% respectively.

4. CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Haloperidol and Trihexyphenidyl in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Haloperidol and Trihexyphenidyl was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.

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