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Development and Validation of Stability Indicating HPLC Method of Haloperidol and Trihexyphenidyl in Tablet Dosage Form

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ABSTRACT:

A simple, rapid, economical, precise and accurate Stability indicating RP-HPLC method for simultaneous estimation of Trihexyphenidyl and Haloperidol in their Combined Dosage Form has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Trihexyphenidyl and Haloperidol in their combined dosage form has been developed. The separation was achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer (pH 6.0): Methanol (30:70) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 235 nm. Retention time of Haloperidol and Trihexyphenidyl were found to be 3.117 min and 5.860 min, respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Haloperidol 12.5-37.5 µg/ml and for Trihexyphenidyl 5-15 µg/ml. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Trihexyphenidyl and Haloperidol in their combined dosage form. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, Considerable Degradation was found in alkaline degradation. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined dosage form.

KEY WORDS: Haloperidol, Trihexyphenidyl, Stability indicating RP-HPLC Method, Validation.

INTRODUCTION:

The Combination of Haloperidol and Trihexyphenidyl used For the treatment, control, prevention, & improvement of the following diseases, conditions and symptoms: difficulty concentrating, depressed mood, sleeping too much or not enough, anxiety, suspiciousness, delusions **Haloperidol:** Haloperidol (Fig.1) 4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one. It is used primarily to treat schizophrenia and other psychoses. **Trihexyphenidyl:** Trihexyphenidyl (Fig.2) which is chemically (RS)-1-cyclohexyl-1-phenyl-3-(1-piperidyl)propan-1-ol, Hydrochloride[4]. It is a widely used as antiparkinsonian agent of the antimuscarinic class. It has been in clinical usage for decades.

A review of the literature states that the quantity of their analytical methods is available for the estimation of its combination in the form of Haloperidol and Trihexyphenidyl. But no method has been reported for stability indicating simultaneous estimation of Haloperidol and Trihexyphenidyl in combined pharmaceutical dosage form by RP-HPLC. So it is develop stability indicating reverse phase high performance liquid chromatographic method for simultaneous estimation of Haloperidol and Trihexyphenidyl in their Combined Dosage form.Therefore, the goal of the present work is to develop simple, accurate, fast, Specialized, sensitive and selective stability indicating HPLC method for simultaneous estimation of Haloperidol and Trihexyphenidyl in their combine dosage form.

EXPERIMENTALWORK:

Material and Method:

The reference samples of Trihexyphenidyl and Haloperidol were obtain Form the Mepro pharma Pvt Ltd. Purified water was obtain from the Finar Limited., Mumbai. HPLC grade methanol and acetonitrile also obtain from the Finar Ltd., Mumbai were used for the preparing the mobile phase and the diluent. Potassium Dihydrogen ortho Phosphate and Ortho Phosphoric Acid analytical grade obtain from Ranbaxy Chemicals. Combiplan tablet a combination of Haloperidol(5mg) and Trihexyphenidyl (2mg) manufactured by Intas Pharma was purchased from local firms.

METHOD DEVELOPMENT:

Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study drug solutions of Trihexylphenidyl (10 ppm) and Haloperidol (25 ppm) were prepared in Methanol. These drug solutions were than scanned in UV region of 200-400 nm and overlay spectrums were recorded.

Selection of Mobile Phase

Trail contains various mobile phase which are considered of Methanol, Water and Acetonitrile in different proportions and different volumes at different flow rate were tried.

Optimization of flow rate

1ml/min flow rate, proved to be better than the other in terms of resolution, peak shape and shorter retention time

Chromatographic Separation:-

Standard solutions of 12.5-37.5µg/ml of Haloperidol and 5-15 µg/ml of Trihexylphenidyl were injected in column with 20 µl micro syringe. The chromatogram was run for appropriate minutes with mobile phase Buffer (pH 6.0): Methanol (40:60). The detection was carried out at wavelength 235 nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc were recorded using software.

Preparation of standard solution of mixtures of Trihexyphenidyl (10 ppm) and Haloperidol (25 ppm)

(A) Trihexylphenidyl standard stock solution: (100µg/mL)

A 10 mg of Trihexylphenidyl was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase.

(B) Haloperidol standard stock solution: (250µg/mL)

A 25 mg of Haloperidol was weighed and transferred to a 100 mL volumetric flask. Volume was made up to the mark with mobile phase

(C) Preparation of standard solution of binary mixtures of Trihexylphenidyl (10µg/mL) and Haloperidol (25 µg/mL)

Take 1 mL from the Trihexylphenidyl stock solution and 1mL from Haloperidol stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

(D) Sample Stock Solution (Trihexyphenidyl HCl 10 µg/ml, Haloperidol 25 µg/ml)

Take Tablet Powder equivalent to 0.1 mg of Trihexyphenidyl HCl, and 0.25 mg of Haloperidol was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase, Shake well for 15 minutes and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

System suitability test:

These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

STABILITY STUDY:

A. Acid degradation

Acid degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and put for 4 hrs at RT. Then the volume was adjusted with diluent to get 10µg/ml for Trihexylphenidyl and 25µg/ml for Haloperidol.

B. Base degradation

Base degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 6 hrs at RT. Then the volume was adjusted with diluent

to get 10µg/ml for Trihexylphenidyl and 25µg/ml for Haloperidol.

C. Oxidative degradation

Oxidation degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and put for 3 hr at RT. Then the volume was adjusted with diluent to get 10µg/ml for Trihexylphenidyl and 25µg/ml for Haloperidol.

D. Photo Degradation

Photo degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. The flask was kept for 12 hrs under UV light. Then the volume was adjusted with diluent to get 10µg/ml for Trihexylphenidyl and 25µg/ml for Haloperidol.

E. Thermal degradation

Thermal degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. The flask was kept for 24 hrs in an oven at 80^0C temperature. Then the volume was adjusted with diluent to get 10µg/ml for Trihexylphenidyl and 25µg/ml for Haloperidol.

VALIDATION OF RP-HPLC METHOD

Specificity:

The Chromatograms of Haloperidol and Trihexyphenidyl HCL Standards and Haloperidol and Trihexyphenidyl HCL sample show no interference with the Chromatogram of Haloperidol and Trihexyphenidyl Blank, so the Developed method is Specific.

Linearity:

The linearity for Trihexylphenidyl and Haloperidol were assessed by analysis of combined standard solution in range of 5-15 µg/ml and 12.5-37.5 µg/ml respectively,

Different concentration used for the Haloperidol and Trihexyphenidyl which shown in graph.

Precision:

A. Repeatability

Standard solution containing Haloperidol (25µg/ml) and Trihexylphenidyl (10 µg/ml) was injected six times and areas of peaks were measured and %R.S.D. was calculated.

B. Intra-day precision

Standard solution containing (12.5,25,37.5µg/ml) of Haloperidol and (5,10,15 µg/ml) of Trihexylphenidyl were analyzed three times on the same day and %R.S.D was calculated.

C. Inter-day precision

Standard solution containing (12.5,25,37.5 µg/ml) of Haloperidol and (5,10,15 µg/ml) of Trihexylphenidyl were analyzed three times on the different day and %R.S.D was calculated.

Accuracy:

A. For Haloperidol

12.5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 235 nm. The amount of Haloperidol was calculated at each level and % recoveries were computed.

B. For Trihexylphenidyl

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 235 nm .The amount of Trihexylphenidyl was calculated at each level and % recoveries were computed.

Analysis of marketed formulation by developed method

Sample Stock Solution (Haloperidol 250 µg/mL, and Trihexylphenidyl 100 µg/mL):

Take Tablet powder equivalent to 25 mg of Haloperidol, and 10 mg of Trihexylphenidyl was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Working Sample Preparation (Haloperidol 25 µg/mL, and Trihexylphenidyl 10 µg/mL):

Take 1 mL from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase

Inject above Solution 20 µl for Assay Analysis.

RESULT AND DISCUSSION:

Table 1: Results of System Suitability Parameter

Parameters	Haloperidol	Trihexylphenidyl
Retention Time	3.117	5.860
Theoretical Plates	5381	3596
Asymmetry	1.227	1.214

Resolution	9.783

Table 2: Haloperidol and Trihexyphenidyl HCl standard for stability

Drugs	Area
Haloperidol	547.462
Trihexylphenidyl	1195.939

Table 3: Haloperidol % Degradation

Haloperidol				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**		**Area**
Acid	464.456	15.162	Acid	464.456
Base	392.834	28.245	Base	392.834
Thermal	383.478	29.953	Thermal	383.478
Oxidation	443.618	18.968	Oxidation	443.618
Photo	415.070	24.183	Photo	415.070

Table 4: Trihexyphenidyl HCl % Degradation

Trihexyphenidyl HCl				
Parameter	**Standard**		**Sample**	
	Area	**%Degradation**		**Area**
Acid	866.431	27.552	Acid	866.431
Base	845.363	29.314	Base	845.363
Thermal	826.503	30.891	Thermal	826.503
Oxidation	1030.823	13.806	Oxidation	1030.823
Photo	1049.922	12.209	Photo	1049.922

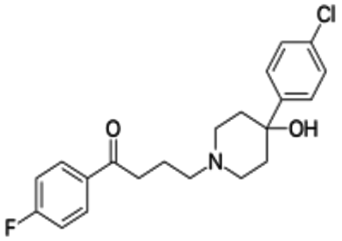

Figure 1 Haloperidol

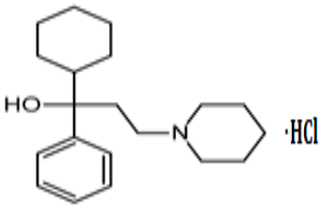

Figure 2 Trihexyphenidyl HCL

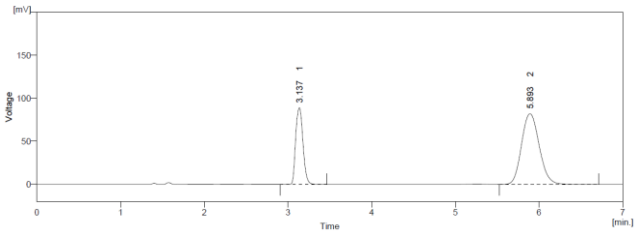

Figure 3 HPLC Chromatogram of Trihexylphenidyl 10ppm and Haloperidol 25ppm in Buffer, pH 6.0: Methanol (30:70) (Final)

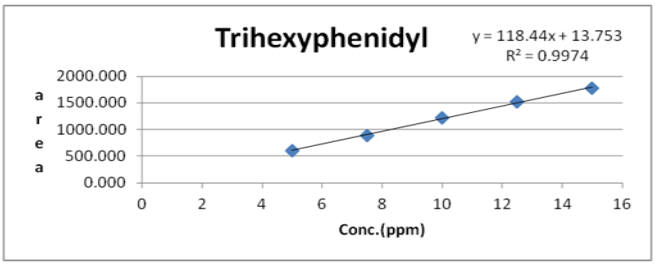

Figure 4 Calibration Curve of Trihexylphenidyl (5-15 µg/ml)

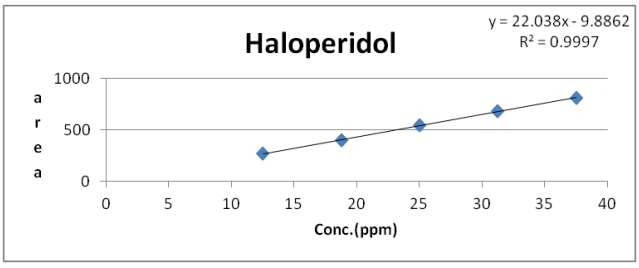

Figure 5 Calibration Curve of Haloperidol (12.5-37.5 µg/ml)

Table 8: Repeatability data for Trihexyphenidyl

Trihexyphenidyl				
Sr No.	Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
		1205.189		

		1197.979		
		1185.789		
1.	10	1200.365	1197.924	0.597
		1203.940	±7.148	
		1194.284		

Table 8: Repeatability data for Haloperidol

Haloperidol				
Sr. No.	**Conc. (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
		538.434		
		535.215		
		534.683		
1.	25	536.292	536.010±1.884	0.352
		537.866		
		533.569		

Table 9: Intraday precision data for estimation of Trihexyphenidyl

	Trihexyphenidyl		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	597.167 ± 4.605	0.771
2	10	1203.962 ± 5.284	0.439
3	15	1806.254± 5.227	0.289

Table 10 : Intraday precision data for estimation of Haloperidol

	Haloperidol		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	12.5	267.843 ± 0.680	0.254
2	25	537.628 ± 2.649	0.493
3	37.5	805.648± 2.571	0.319

Table 11: Interday precision data for estimation of Trihexyphenidyl HCl

	Trihexyphenidyl HCl		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	592.047 ± 8.333	1.407
2	10	1204.095± 10.344	0.859
3	15	1797.959 ± 25.472	1.416

Table 12: Interday precision data for estimation of Haloperidol

	Haloperidol		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	12.5	264.996± 2.814	1.061
2	25	540.350± 0.678	0.125
3	37.5	807.216± 5.069	0.628

Table 13: Recovery data for Trihexyphenidyl HCl

SR. NO.	**Conc. Level (%)**	**Sample amount (µg/ml)**	**Amount Added (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± S.D**
1	80 %	5	4	4.016	100.388	99.021 ± 1.854
2		5	4	3.991	99.763	
3		5	4	3.876	96.911	
4	100 %	5	5	5.018	100.360	99.858 ± 0.464
5		5	5	4.988	99.761	
6		5	5	4.973	99.452	
7	120 %	5	6	5.913	98.554	98.859 ± 0.672
8		5	6	5.978	99.629	
9		5	6	5.904	98.393	

Table 14: Recovery data for Haloperidol

SR. NO.	**Conc. Level (%)**	**Sample Amount**	**Amount Added**	**Amount recovered (µg/ml)**	**% Recovery**	**% Mean Recovery ± S.D**
1	80 %	12.5	10	10.047	100.469	100.181 ± 0.703
2		12.5	10	10.070	100.695	
3		12.5	10	9.938	99.380	
4	100 %	12.5	12.5	12.556	100.447	100.249 ± 0.346
5		12.5	12.5	12.481	99.849	
6		12.5	12.5	12.556	100.450	
7	120 %	12.5	15	15.013	100.086	99.844 ± 0.210
8		12.5	15	14.958	99.722	
9		12.5	15	14.959	99.724	

Table15 : Analysis on marketed formulation

Tablet	Combidol	
Label claim	**Haloperidol (5 mg)**	**Trihexylphenidyl (2 mg)**
Assay (% of label claim*) Mean ± S. D.	97.983±0.318	97.802±0.299

6. RESULTS AND DISCUSSION:

The assay results were comparable to labeled value of each drug in Tablet dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

7. CONCLUSION:

A simple, specific, accurate and precise Stability indicating RP-HPLC method has been developed and validated as per ICH guideline for Simultaneous Estimation of Propranolol HCl and Haloperidol in their combined dosage form.Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, Specificity were tested.Observation of all these parameters leads to the point that developed Stability indicating RP-HPLC method is linear, accurate, precise, specific.It can be successfully adopted for routine quality control analysis of Propranolol HCl and Haloperidol in Combined dosage form without any interference from common excipients and impurity.This method can now transfer to utilize for routine laboratory analysis and assay of Propranolol HCl and Haloperidol in their combined dosage form.

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