International Journal of Research in
Pharmaceutical Chemistry and Analysis

Stability indicating method development and validation for the determination of haloperidol and benzhexol by RP-HPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Haloperidol and Benzhexol in Tablet dosage form. Chromatogram was run through Kromasil (250mm 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrile and methanol in the ratio of 48:52 was pumped through column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. Optimized wavelength for Haloperidol and Benzhexol was 220nm. Retention time of Haloperidol and Benzhexol were found to be 2.415 min and 2.820min. %RSD of the Haloperidol and Benzhexol were and found to be 0.6 and 0.2 respectively. %Recover was Obtained as 98.92% and 99.60% for Haloperidol and Benzhexol. LOD, LOQ values were obtained from regression equations of Haloperidol and Benzhexol were 0.42ppm, 1.27ppm and 0.04ppm, 0.14ppm respectively. Regression equation of Haloperidol is $y = 24009x + 38704$, and of Benzhexol is $y = 40558x + 2880$. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries

Keywords: benzhexol; haloperidol; RP-HPLC.

ISSN: Awaiting
Research Article

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Article Info

Received on: 12-05-2019
Revised on: 03-06-2019
Accepted on: 11-06-2019

DOI: https://doi.org/10.33974/ijrpca.v1i2.77

INTRODUCTION

The phenomenal growth in chromatography is largely due to the introduction of the versatile technique called high-pressure liquid chromatography, which is frequently called high-performance liquid chromatography. Both terms can be abbreviated as HPLC. High-pressure liquid-solid chromatography (HPLC) is rapidly becoming the method of choice for separations and analysis in many areas. Most of the samples that are dissolved can be separated on some type of HPLC column[4]. Haloperidol is a psychotropic agent indicated for the treatment of schizophrenia. It also exerts sedative and antiemetic activity. Haloperidol principal pharmacological effects are similar to those of piperazine-derivative phenothiazines. The drug has action at all levels of the central nervous system-primarily at subcortical levels-as well as on multiple organ systems. Haloperidol has strong antiadrenergic and weaker peripheral anticholinergic activity; ganglionic blocking action is relatively slight. It also possesses slight antihistaminic and antiserotonin activity[9]. Trihexyphenidyl is an anticholinergic used in the symptomatic treatment of all etiologic groups of parkinsonism and drug induced extrapyramidal reactions (except tardive dyskinesia). Trihexyphenidyl possesses both anticholinergic and antihistaminic effects, although only the former has been established as therapeutically significant in the management of parkinsonism.

Literature survey reveals that no analytical method has been reported for the estimation of Haloperidol and Benzhexol. So, in the present study an attempt have been made to develop simple reverse phase high performance liquid chromatographic methods for quantitative estimation of Haloperidol and Benzhexol in bulk drug and pharmaceutical formulations. It was thought meaningful to develop simple, precise, rapid, efficient, reliable and economical RP- HPLC in its pharmaceutical dosage form. An attempt has been made to develop and validate all methods to ensure their accuracy, precision, repeatability and other analytical method validation parameters as mentioned in the ICH guidelines[20].

MATERIALS AND METHODS

Chemicals and Reagents: The chemical and materials used for the present study are HPLC grade. Methanol, acetonitrile and mili Q water, Orthophosphoric acid are procured from Rankem, cipla private Ltd., India is used.

Instrumentation: HPLC system operated in isocratic mode was used for the present work. It was equipped with 210nm. A Discovery 250 C18 column (250 x 4.6 mm, 5 μm particles) was used as stationary phase. A 10μL Hamilton syringe was used for injecting the samples. De- gassing of the mobile phase was done by using an sonicator (Ultrasonic Inc.PTE008). A AFCOSET(ER_200A) used for weighing the materials. Chromatograms were recorded and integrated using Empower software.

Optimal chromatographic conditions: The optimal chromatographic conditions used for HP and TXH were: the mobile phase containing OPA: Acetonitrile : in the ratio of 48:52%,210nm detection wavelength; Kromasil C18 column (250 mm x 4.6 mm, 5 μm) in isocratic mode; 1.0 ml/mn flow rate; 1-8 pH of the mobile phase, Temperature was maintained at 30c. The active pharmaceutical ingredient (API) concentrations used for HP and TXH were 25,50,75,100,125,150μg/mL, and 5,10,15,20,25,30 μg/mL, respectively. The runtime was 10 minutes and the samples were injected using a 10μL injector.

Preparation of the standard stock solution: To prepare the standard stock solution, 10 mg of HP and 2mg TXH were weighed accurately and transferred to two separate 25 ml clean dry volumetric flasks and add 5ml of diluent was added, They were sonicated for 5 minutes to dissolve completely. Sonicated for 5 minutes and made up to the final volume with diluents. A 1000 μg/mL solution of each drug was prepared. From this 0.5 mL was further diluted to 10 mL to get a stock concentration of 50 μg/mL solution for the two drugs. Required concentrations of the solutions were prepared by selective dilution.

Method development

For the method development several trials were carried out and reported. These leads to the optimized chromatographic conditions for the estimation of Haloperidol and Benzhexol in pharmaceutical dosage form. Initially various mobile phase and stationary phase were tested in an attempt to obtain the best resolution for Haloperidol and Benzhexol. The mobile phase consisting of water pH adjusted to 2 with Orthophosphoric acid, at a flow rate of 1 ml/min was chosen for method development and validation of Haloperiol and Benzhexol by RP-HPLC method.The detection was selected at 210nm, using reverse phase Discovery 250 c18 column Kromocil C18(250mm x 4.6 mm, 5m)column, the retention time of Haloperidol and Benzhexol were found to be 2.415min and 2.820min respectively. The total run time was 6 minutes. A mobile phase consisting of water pH adjusted to 2 with Orthophosphoric acid was selected to achieve maximum separation and sensitivity. The flow rate of 1 ml/min gave an optimal signal to noise ratio with reasonable separation time.

RESULTS AND DISCUSSIONS

The standard stock solution for HP and TXH was prepared with appropriate dilution. It was scanned in the wavelength region 200 nm to 400 nm using an ultraviolet (UV) - Visible spectrophotometer. The absorbance spectrum obtained was analyzed. From the spectrum of HP and TXH, 210 nm was selected as the optimum wavelength for the analysis of the binary mixture using RP-HPLC method. The absorption spectrum was shown in Figure with wavelength (nm) as X-axis and absorbance (%) as Y-axis. The measured wavelength of 210 nm was in good Results.

On the basis of our experimental results, we conclude that the UV spectrophotometric method developed for the determination of minoxidil was found to be precise, accurate and cost effective. Hence this method can be used for routine analysis of Minoxidil in bulk and pharmaceutical dosage forms.

Optimized Method

Mobile phase: Buffer and Acetonitrile were taken in the ratio of 48:52.

Chromatographic conditions

Column Used	: Kromasil-250x4.6mm, 5μ
Buffer used	: (0.1%) Ortho phosphoric acid
Mobile phase	: OPA: Acetonitrile (48:52A)
Flow rate	: 1 ml/min
Diluent	: Water: ACN (50:50)
Wavelength	: 210nm
Temperature	: 30° C

Injection Volume: 10μl

Method validation: There were nine parameters that have to be validated for the developed method in accordance with the ICH guidelines[20]. They were specificity, linearity, precision, accuracy, recovery, limit of detection, limit of quantification, robustness and ruggedness. In the present work eight out of nine parameters have been validated for both the drug.

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. normally these might including impurities, degradants, matrix, etc.An investigation of specificity should be conducted during the validation of tests, the determination of impurities and assay. The procedure used to explain specificity will depend on the intended objective of the analytical procedure.

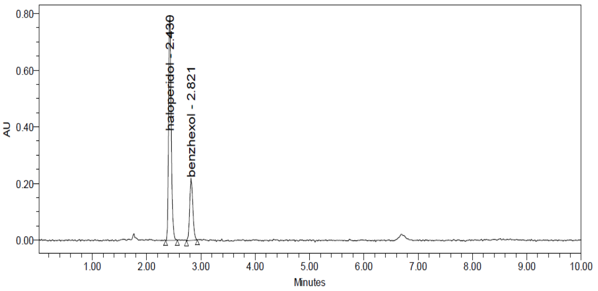

Figure 1: Optimized chromatogram of Haloperidol and Benzhexol

Linearity: Six Linear concentrations of Haloperidol (25-150ppm) and Benzhexol (5ppm to 30ppm) are prepared and Injected. Regression equation of the the Haloperidol and Benzhexol are found to be, y = 24009x + 38704 and y = 40558x + 2880.and the regression co-efficient was 0.999.

Table 1: Linearity of Haloperidol and Benzhexol

S. NO	Haloperidol		Benzhexol	
	Conc (µg/ml)	Area	Conc (µg/ml)	Area
1	25	621246	5	203548
2	50	1292386	10	402630
3	75	1876799	15	624175
4	100	2460943	20	832366
5	125	2992636	25	992570
6	150	3631661	30	1223431

Table 2: Peak results of Method Precision

Injection	Haloperidol	Benzhexol
	Area	Area
Injection 1	2941236	937857
Injection2	2918294	934542
Injection3	2970957	937569
Injection4	2941274	939844
Injection5	2949154	936059
Injection6	2944298	939684
Average	**2944202**	**937593**
SD	**16905.4**	**2057.7**
%RSD	**0.6**	**0.2**

Accuracy: The accuracy of an analytical method is the closeness of that results obtained by that method to the true value. Accuracy may often be expressed as percent recovery by the assay of known added amount of analyte. %Recovery should be in the range of 98.0% to 102%.

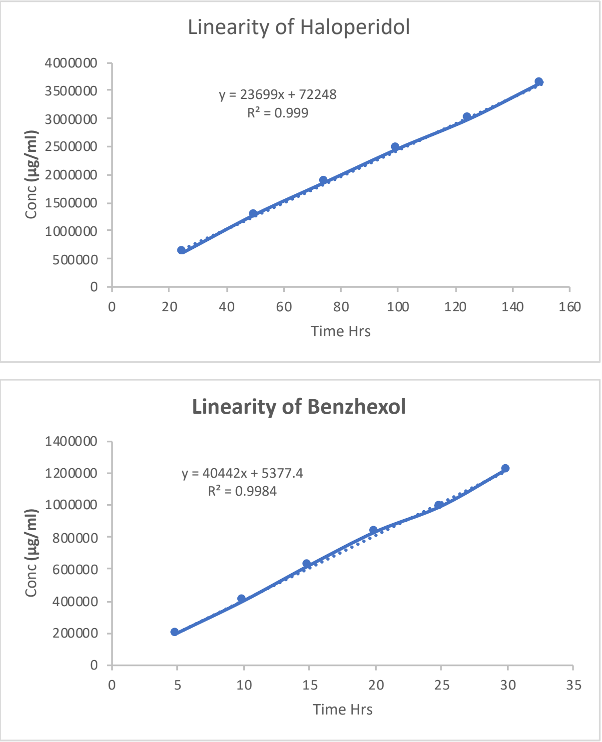

Figure 2: Linearity curve of Haloperidol and Benzhexol

Precision: The precision is procedure expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. For method precision studies, the standard solution was prepared at working concentration and analysis was carried for six injections.

Detection Limit: The detecting limit of each and every analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated under stated experimental conditions. LOD values were obtained from regression equations of Haloperidol and Benzhexol were 0.42ppm, and 0.04PPm

Quantitation Limit: The Quantification limit of individual analytical procedure is defined as the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. LOQ values were obtained from regression equations of Haloperidol and Benzhexol were 1.27ppm, and 0.14PPm.

Table 3: Results for Robustness

Parameters		Haloperidol	Benzhexol	Limit
Robustness	**FM**	**1.8**	**1.6**	%RSD NMT 2.0
	FP	1.1	0.1	
	TM	1.7	1.5	

Table 4: Accuracy peak results of Haloperidol and Benzhexol

	S.No	Spiked level of Standards	Pre analysed sample conc. (µg/ml)	Amount added (µg/ml)	Total amount found (µg/ml)	% Recovery	% Mean
Haloperidol	1	50 %	100	50	50.16	98.49	98.38
				50	50.69	98.51	
				50	50.48	98.16	
	2	100 %	100	100	98.01	98.51	98.32
				100	98.45	98.45	
				100	98.15	98.01	
	3	150 %	100	150	149.61	99.58	99.71
				150	149.65	99.77	
				150	149.67	99.79	
Benzhexol	1	50 %	20	10	9.88	98.49	98.90
				10	9.93	99.69	
				10	9.88	98.53	
	2	100 %	20	20	19.65	98.51	98.28
				20	19.62	98.51	
				20	19.68	98.51	
	3	150 %	20	30	29.87	99.77	99.25
				30	29.84	100.07	
				30	29.60	99.77	

Table 5: Assay of Tablet

S. No.	Haloperidol %Assay	Benzhexol %Assay
1	100.12	99.07
2	98.34	98.75
3	99.12	99.31
4	99.12	99.29
5	99.38	99.10
6	99.22	98.91
AVG	99.22	99.07
STDEV	0.57	0.22
%RSD	0.57	0.22

Table 6: Degradation studies of acid results

Sl.no	PeakName	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USPP latecount	USP Tailing
1	Peak 1	1.622	231454	5.92	-	-	4366	1.4
2	Peak 2	1.880	310877	7.95	-	-	3640	1.9
3	Haloperidol	2.431	2826661	62.28	-	-	12942	1.2
4	Benzhexol	2.849	903299	23.86	5.243	5.825	12987	0.8

Table 7: Degradation studies of Base Results

Sl.no	PeakName	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USPPlatecount	USP Tailing
1	Peak 1	1.893	144143	5.65			4735	1.3
2	Peak 2	2.126	140107	5.49			8129	1.3
3	Haloperidol	2.431	2881033	67.42	0.686	0.728	12894	1.2
4	Benzhexol	2.850	918274	21.44	3.055	3.919	13779	1.2

Table 8: Degradation studies of thermal results

Sl.no	PeakName	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Platecount	USP Tailing
1	haloperidol	2.425	2943573	75.65	1.066	1.665	13171	1.3
2	benzhexol	2.819	937377	24.35	3.353	4.093	12325	1.2

Table 9: Degradation studies of water sample results

Sl.no	PeakName	RT	Area	%Area	Purity1 Angle	Purity1 Threshold	USP Platecount	USP Tailing
1	Peak 1	2.170	199333	5.81			10854	1.0
2	haloperidol	2.419	2945689	71.29			11908	1.2
3	benzhexol	2.812	940620	22.90	2.248	2.672	11205	1.2

Robustness: The robustness procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

System Suitability Testing: The system has to be tested for its suitability for the intended purpose. System suitability testing is an integral part of many analytical procedures. The tests are based on the theory that the instruments, electronics, analytical procedures and samples to be analyzed constitute an integral system that can be evaluated as such.

Parameters like plate count, tailing factors, resolution and reproducibility (% RSD retention time and area for six repetitions) are determined and compared against the specifications set for the method.

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average % assay was calculated and found to be 99.22% and 99.07% for Haloperidol and Benzhexol respectively

Degradation studies

Oxidation: To 1 ml of stock solution of Haloperidol and Benzhexol, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 600c. For HPLC study, the resultant solution was diluted to obtain 100µg/ml&20µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock s solution Haloperidol and Benzhexol, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c.The resultant solution was diluted to obtain 100µg/ml&20µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Degradation studies of acid results

Alkali Degradation Studies: To 1 ml of stock solution Haloperidol and Benzhexol, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^{0}c. The resultant solution was diluted to obtain 100µg/ml&20µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. Degradation studies of Base Results:

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 6h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml & 20µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the 1000µg/ml& &200µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/ml 20µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Degradation studies of thermal results

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 100µg/ml&20µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

CONCLUSION

The study was undertaken in order to develop and validate the analytical RP-HPLC method for estimation of Haloperidol and Benzhexol pharmaceutical formulations. Literature survey reveals no analytical methods have been reported for the estimation of Haloperidol and Benzhexol in pharmaceutical formulations. So the was developed and validated by means of accuracy, precision, linearity, LOD and LOQ and robustness as per ICH guidelines.

From this results concluded that a simple, precise, accurate and sensitive RP-HPLC method was developed for the simultaneous estimation of Haloperidol and Benzhexol pharmaceutical dosage form. This method can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Haloperidol and Benzhexol.

ACKNOWLEDGEMENT

We are thankful to Krishna teja Pharmacy College, Tirupati, for providing us necessary facilities to carry out our research work.

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