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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF HALOPERIDOL AND BENZHEXOL IN BULK AND TABLET DOSAGE FORM

Pharmacy

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ABSTRACT

The proposed study development and validation of stability indicating RP-HPLC method has been developed for simultaneous estimation of Haloperidol and Benzhexol in bulk and tablet dosage forms. The developed new method was a sensitive, precise and accurate RP-HPLC method for estimation of Haloperidol and Benzhexol. An isocratic RP-HPLC system was used for analysis of samples at 300C column oven temperature. The chromatographic separation was achieved on Kromasil C18, 5 micron column using 0.1% perchloric acid and acetonitrile 50:50 as mobile phase at a flow rate of 1 ml/min. The injection volume was 10 µl and the total run time was set as 10 mins. The detection of analytes was carried out at 210 nm using PDA detector. The developed method was validated for linearity, precision, accuracy and forced degradation studies as per ICH guidelines. The results demonstrated that the method was suitable for quality control analysis of combination of haloperidol and benzhexol both in bulk and tablet dosage forms.

KEYWORDS

RP-HPLC, Haloperidol, Benzhexol, Method development, Validation

INTRODUCTION:

Haloperidol [1-7] is a phenyl-piperidinyl-butyrophenone that is used primarily to treat schizophrenia and other psychoses. Chemically, Haloperidol is 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl) butan-1-one [figure 2.1]. Haloperidol is a psychotropic agent indicated for the treatment of schizophrenia. It also exerts sedative and antiemetic activity. Benzhexol [8-13] is an anti cholinergic used in the symptomatic treatment of all etiologic groups of Parkinsonism and drug induced extrapyramidal reactions (except tardive dyskinesia). Benzhexol possesses both anti cholinergic and antihistaminic effects, although only the former has been established as therapeutically significant in the management of Parkinsonism. Chemically it is 1-cyclohexyl-1-phenyl-3-(piperidin-1-yl) propan-1-ol. Most of the Pharmaceutical industries utilize sophisticated equipments like HPLC, HPTLC and LC-MS for qualitative analysis of various drugs. These equipments also used in analyzing the raw material to ensure the obtained product is pure and also to determine how much amount of the drug is present in the final product.

Among all analytical methods, the modern method of choice for drug analysis is Chromatographic technique which requires highly sophisticated equipment, trained personal, high purity chemicals and proper maintenance. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is assuming one of the best analytical equipment for various categories of pharmaceutical drugs. An extreme literature survey revealed that very few analytical methods have been reported for Haloperidol, Benzhexol in individual and combination and other drugs. Therefore it was thought of interest in development and validating a new and advanced sensitive, specific, precise, accurate stability indicating RP-HPLC method for simultaneous estimation of Haloperidol and Benzhexol in bulk drug and in pharmaceutical dosage form.

MATERIALS AND METHODS:

Drugs and chemicals: Haloperidol and Benzhexol were obtained as gift sample from Spectrum Pharma Research laboratory in Hyderabad. Tablets (Hexidol Forte, Torrent Pharmaceuticals Ltd, Secundrabad, Telangana, India.) containing Haloperidol-10 mg and Benzhexol-2 mg Marketed formulation was purchased from local market. Acetonitrile, Water HPLC grade were obtained from Merck. Mumbai, India and Potassium dihydrogen ortho phosphate, Triethylamine from RANKEM, Mumbai, India. All solvents used in this work are HPLC grade.

Instrument: A Waters 2695 RP-HPLC separation module (Waters Corporation, Milford, USA) equipped with PDA detector having back pressure 5000psi, automatic injector and Kromasil-250x4.6mm, 5µ. Single pan Balance (Mettler Toledo), Control Dynamics PH meter (Mettler Toledo), Sonicator (Labindia Instruments).

Chromatographic conditions: An isocratic RP-HPLC system was used for analysis of samples at 30 °C column oven temperature. The chromatographic separation was achieved on Kromasil-250x4.6mm, 5µ column using (0.1%) Perchloric acid and Acetonitrile 50:50 %v/v as mobile phase at a flow rate of 1ml/min. The injection volume was 10 µl and the total runtime was set as 10min. The determination of analytes was carried out at 210nm using PDA detector.

Preparation of Samples and Solutions

Preparation of Mobile Phase: Accurately 1ml of Perchloric acid in a 1000ml of volumetric flask adds about 900ml of milli-Q water added and degasses to sonicate and finally make up the volume with water.

Preparation of Haloperidol stock solution: Accurately Weighed and transferred 10mg of Haloperidol in to 10ml of clean dry volumetric flask, add 7ml of diluent, then sonicated for 10min and make up the volume with diluent.

Preparation of Benzhexol stock solution: Accurately weighed 2mg of Benzhexol and transferred into 10ml of clean dry volumetric flask, add 7ml of diluent, then sonicated for 10 min and make up the final volume with diluent.

Preparation of Haloperidol standard solution: From the above Haloperidol stock solution 1ml was pipette out into 10ml of clean dry volumetric flask and make up the final volume with diluent.

Preparation of Benzhexol standard solution: From the above Benzhexol stock solution 1ml was pipette out into a 10ml clean dry volumetric flask and make up the final volume with diluent.

RESULTS AND DISCUSSION

Optimized chromatographic conditions: A Reverse Phase C8 and C18 columns were tried initially to separate the analytes .After several systemic trials, a suitable C18 column was selected and good separation of the compounds was achieved with mobile phase consisting Perchloric acid and Acetonitrile in the ration of 50:50 %v/v. Finally, a simple precise, sensitive, accurate, precise and economic RP-HPLC method has been developed for performing stability studies and simultaneous estimation of Haloperidol and Benzhexol. The optimized chromatographic conditions were given in the below table.

Optimized chromatographic conditions

Parameter	Condition
RP-HPLC	Water 2695 separation module with PDA detector
Mobile phase	Perchloric acid: ACN 50:50%v/v
Column	Kromasil-250x4.6mm, 5µ column
Column Temperature	30 °C
Wavelength	210nm
Diluents	Water: ACN (50:50)
Injector volume	10µl
Flow rate	1ml/min
Runtime	7min
Retention time	Haloperidol-2.139min and Benzhexol-3.151min
Theoretical Plates	Haloperidol -2919 and Benzhexol – 7217

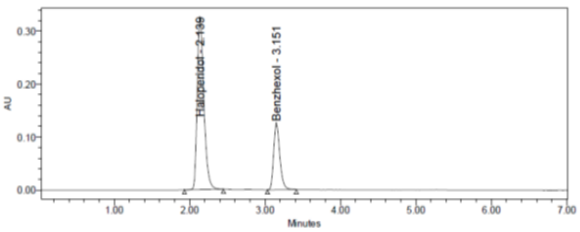


Fig 2.3: Chromatogram Of Haloperidol And Benzhexol In Api

Validation Of Proposed Stability Indicating Method: The proposed stability indicating assay method was successfully validated according to ICH guidelines. The parameters studied for validation were specificity, accuracy, linearity, precision, robustness, limit of detection, limit of quantification and system suitability.

Specificity: The specificity was successfully performed under various stress conditions like acid, base, oxidative, dry heat and photolytic and all the degraded products were separated from sample peaks. It was found that no interference of the degraded products was seen with the drug products. All Haloperidol and Benzhexol peaks were tested for purity test by comparing purity of angle and purity of threshold. This indicates that the proposed method was specific. The results of forced degradation shown in table 2.6

Table 2.6: Forced degradation studies of Haloperidol and Benzhexol

Stress condition	Haloperidol		Benzhexol	
	Purity of angle	Purity of Threshold	Purity of angle	Purity of Threshold
Acid degradation	0.272	0.345	0.136	0.339
Base degradation	0.296	0.345	0.139	0.337
Peroxide degradation	0.282	0.355	0.139	0.332
Dry heat degradation	0.258	0.276	0.130	0.330
Photolytic degradation	0.165	0.282	0.132	0.339

Accuracy: The accuracy of the proposed method was determined by standard addition method. It is the closeness of the analytical results obtained by the analysis to the true value. A known amount of standard drug was added to the fixed amount of tablet solution. Accuracy was expressed as percentage recovery. Recovery test was performed with three different concentrations i.e. 50 µg/ml, 100µg/ml and 150 µg/ml for Haloperidol and 10 µg/ml, 20 µg/ml and 30 µg/ml for Benzhexol. The % recovery results were calculated and given in table 2.7.

Table 2.7: % Recovery results of Haloperidol and Benzhexol

Conc.	Haloperidol			Benzhexol		
	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery
50%	50	50.52	101.04	10	10.04	100.39
	50	50.10	100.20	10	9.93	99.29
	50	49.97	99.95	10	9.95	99.50
100%	100	99.87	99.87	20	20.23	101.15
	100	102.91	102.91	20	20.18	100.89
	100	99.51	99.51	20	20.02	100.10
150%	150	150.63	100.42	30	30.33	101.11
	150	148.74	99.16	30	29.88	99.60
	150	149.61	99.74	30	30.28	100.92

Linearity: A series of six concentrations in the range of 25 to 150 µg/ml of Haloperidol and 5-30µg/ml of Benzhexol has been prepared and peak areas were recorded at 210nm. A calibration curve was plotted between peak area versus concentration of respective Haloperidol and Benzhexol and the response of the drugs were found to be linear. The linear regression equation (y=mx+c) was found to be y = 20045x + 4327. (fig 2.9) for Haloperidol and y = 34668x + 2604 (fig 2.10) for Benzhexol respectively. The linearity results were given in calibration curves in Fig 2.9 and Fig 2.10

Fig 2.9: Calibration curve of Haloperidol

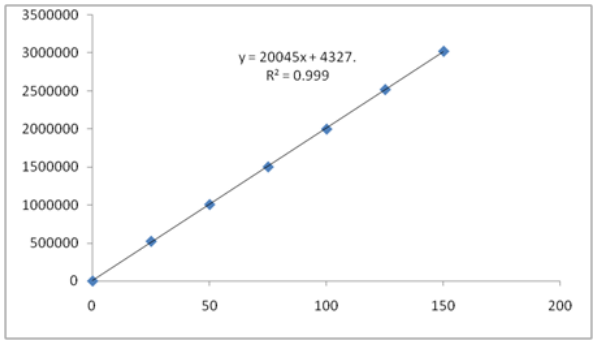
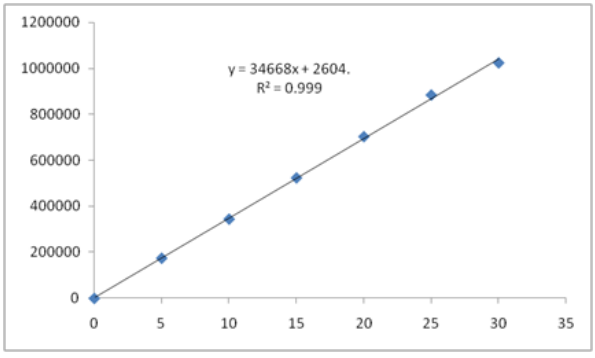


Fig 2.10: Calibration curve of Benzhexol



Application of developed method to Formulation: Analysis of marketed formulation (Hexidol Forte, Torrent Pharmaceuticals Ltd, Secundrabad, Telangana, and India) containing Haloperidol-10 mg and Benzhexol-2 mg was purchased from local market. Five tablets were weighed and average weight was calculated. Then it they were grind into fine powder and transferred to 10ml volumetric flask, 7ml of diluent was added and sonicated for 25 min; further the volume was made up with diluent. From the filtered solution, 1ml was pipette out into 10ml volumetric flask and made up to 10ml with diluent. From the solution, 10µl was injected into HPLC system and peak area was recorded (fig 2.11) with detector at 210nm. The % assay was calculated with obtained peak area of detector response. The % assay was found to be 99.79% for Haloperidol and 100.14% for Benzhexol. This indicates that developed method can be used for routine analysis. The % assay results were given below.

Table 2.20: % Assay results of Haloperidole and Benzhexol in formulation

Tablet	Drug	Dosage (mg)	Sample concentration (µg/ml)	Amount found (µg/ml)	% Assay
Hexidol Forte	Haloperidol	10	100	99.79	99.79
	Benzhexol	2	20	20.03	100.14

CONCLUSION:

The present study deals with the development of a stability indicating RP-HPLC method for estimation of combination of haloperidol and benzhexol. This study exemplifies for development of a stability indicating assay method established by following the recommendations of ICH guidelines. The proposed method was shown to be precise, accurate and selective with wide linear concentration range. The results of the present study shows that the method is suitable for the simultaneous determination of haloperidol and benzhexol in bulk and tablet dosage form.

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