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A Stability Indicating Assay Method For Propranolol Tablets By High Performance Liquid Chromatography For Stability Studies

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ABSTRACT

A simple and stability indicating HPLC assay procedure had been developed and validated for propranolol tablets stability samples. The mobile phase consisted of buffer(5.0 g Triethylamine /1000ml water pH 4.0 by HCOOH): acetonitrile:in the ratio of (70:30) isocratic elution is carried out under ambient condition at flow rate of 1.5 ml min^{-1} and detector was set at 225 nm. The column selected was Luna, C18, 5μm packing, 4.6 mm x 250 mm and injection volume was 20 μl. The procedure separated propranolol and potential degradation product. The retention time of propranolol is 8.5 min and asymmetry was 1.55. The instrument precision obtained was 0.19 %.The procedure provided a linear response in the range of 50 – 150 % of target concentration (r = 1.000). Forced degradation study shows, response of main drug is reduced in acid, alkali and peroxide degradation. The method was validated for accuracy, robustness and solution stability was obtained up to 27 hrs.

KEYWORDS

Propranolol;
Cardiovascular drug;
Force degradation;
Validation;
Solution Stability;
Stability samples.

INTRODUCTION

Propranolol, the prototype of the beta-adrenergic receptor antagonists, is a competitive, nonselective beta-blocker. propranolol competes with sympathomimetic neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation. This results in a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension[1]. Propranolol is chemically 1-(1-methylethylamino)-3-naphthalen-1-yloxy-propan-2-ol (Figure 1). Literature survey reveals that it is official in U.S.P[2], B.P[3] and several techniques using fluorescence detector[4-5], spectrophotometry[6], capillary zone electrophoresis[7] and HPLC[8-10] have been reported for estimation of propranolol in pharmaceutical formulation and in biological samples. In USP HPLC method suggested for tablets requires costly reagents, whereas in BP it is by U.V-Vis spectrophotometric method.

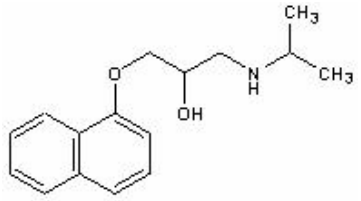

Figure 1 : Chemical structure of Propranolol

Hence to overcome the problem for estimation of Propranolol in stability samples where large numbers of sample is to be quantified on regular basis (as HPLC method suggested in USP is not cost effective and spectrophotometric method suggested in B.P is not stability indicating). In the present study we had reported a simple, rapid, accurate and cost effective stability-indicating HPLC assay procedure that can quantify Propranolol in stability samples and routine analysis.

EXPERIMENTAL

The separation was carried out under isocratic condition with mobile phase prepared by mixing buffer(5.0 g Triethylamine /1000ml water pH 4.0 by HCOOH): acetonitrile in the ratio of (70:30) at a flow rate of 1.5 ml min^{-1} with UV detection at 225 nm. The column temperature was ambient and an injecton volume of 20µ1 was used. A Luna C18 column, 5µ, 250 X 4.6 mm was used. A working standard solution containing 100 µg ml^{-1}. Propranolol was prepared by dissolving propranolol reference standard in mobile phase. A blend of propranolol tablets equivalent to 10 mg of propranolol is transferred to 100 ml volumetric flask. 20 ml of mobile phase was added and sonicated for 5 minutes with immediate shaking and diluted with mobile phase to volume and mix. This solution was centrifuged at about 2000 RPM for 10 minute, and upper clear solution was used for injection. Method validation was performed as per USP 27-NF22 [11]. The following validation parameters were addressed: specificity, precision, linearity, accuracy and solution stability of propranolol in mobile phase.

Specificity

Stress testing of the drug substance can help in identifying the likely degradation products, which in

TABLE 1 : Summary of Stress testing conditions for propranolol

S.No	Degradation	Conditions	Figure No.
1.	Add	2 ml, 2 M HCl and heated at 70°C for 1 hr.	2A
2.	Alkali	2 ml, 2 M NaOH and heated at 70°C for 1 hr.	2B
3.	Peroxide	1 ml, 30% H_2O_2 and heated at 70°C for 1hr.	2C
4.	Thermal	Heated at 70°C for 1hr.	2D
5.	Sunlight	Exposed for 2 hrs.	2E

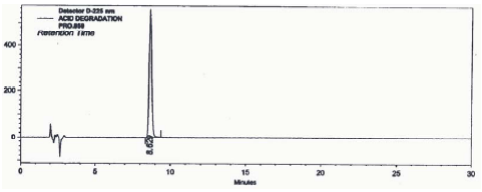

Figure : 2 (A)

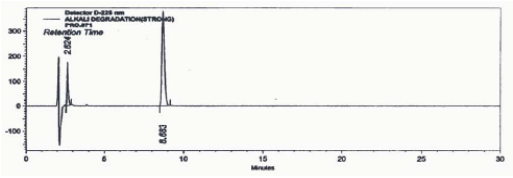

Figure : 2 (B)

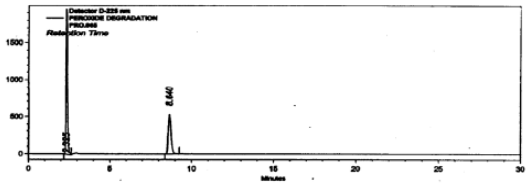

Figure : 2 (C)

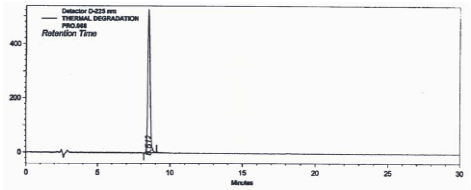

Figure : 2 (D)

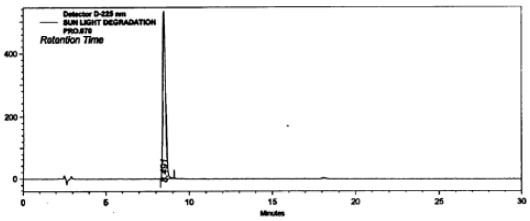

Figure : 2 (E)

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turn help's to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedure used [12].stress testing is done by exposing the propranolol to following conditions (TABLE 1).

The result obtained from degradation study shows peak purity of propranolol is 100 % as calculated by PDA detector, proving that no degradation product is interfering with the main peak.(Figure 2A-E).The % residual drug was calculated in comparison with the standard, which is 88.6,61.1,86.4,85.0 and 86.9 % for acid, alkali, peroxide, thermal and sunlight degradation respectively.

Precision

The system precision was determined by performing six replicate injections of standard solutions, the % RSD of 0.19 is obtained .The method precision was determined by performing six consecutive assays of propranolol by preparing six independent samples, the % label claim of 100.43 was obtained (TABLE -2).

Accuracy

The accuracy was evaluated by the recovery of propranolol at three different levels (80,100 and 120%) using three preparations for each level tested three times. The mean recovery data for each level is within accepted values (101.4, 100.7 and 99.4 respectively).Therefore, these results indicated a good accuracy of the method for propranolol. The mean recovery is 100.5 % (TABLE 2) and % RSD is 1.03.

TABLE 2 : Summary of the performance parameters of the HPLC procedure for propranolol tablets.

S.no	Parameters	Observed value
1.	System suitability	
	a. Theoretical plates	8134
	b. Tailing Factor	1.31
2.	Instrument Precision	RSD 0.19%
3.	Method Precision	Label Claim 100.43%
4.	Linearity and range	Correlation coefficient(r) = 1.0000
5.	Accuracy	Mean recovery 100.51%
6.	Specificity	Peak Purity of propranolol peak after degradation is 100%.
7.	Robustness	Difference from original condition 0.15%
8.	Solution stability	27 hrs

Linearity

The linearity of detector response for propranolol standard was determined by preparing and injecting solutions in the concentration range of 50 – 150 µg/ml (50-150 % of assay conc.) of propranolol standard. A calibration curve was constructed using characteristic parameters for regression equation (y = a + bx) of the HPLC method obtained by least squares treatment of the results confirmed the good linearity of the method developed.(TABLE 2).

Robustness

The robustness study helps us in demonstrating that transferring the methodology can be done successfully or not. In this study we had compared the results between normal operating conditions and by deliberately changing certain parameters like changing analyst, instrument, column(Inertsil, C 18,250 *4.6 mm,5µ).The result obtained shows that by changing deliberately some internal and external parameters of the method does not influence the results obtained.(TABLE 2).

Solution stability

The solution stability study was performed by injecting a standard solution in duplicate at different time intervals, the peak areas were compared with the initial areas, it was found that there was no significant changes in the peak areas up to 27 hrs. Hence the solution is not needed to be injected immediately.(TABLE 2).

RESULT AND DISCUSSION

There was no cost effective method reported as per our knowledge which can be used on regular basis for quantification of propranolol specially when many samples are to be analysed, during long term and short term stability studies. We used previously reported data such as Pka value of propranolol is 9.5[13], so a acidic pH of 4.0 is selected for ionization of propranolol and various trials of organic phase acetonitrile is tried to get desired retention time, so that all degradation products are well separated and

peak shape of propranolol has good asymmetry. Detector wavelength is selected at 225 nm as propranolol has dual lambda max in u.v region, at 225 and 290 nm[14], as the mobile phase selected has cut-off value below 200 nm, hence lower wavelength is selected to detect large number of impurities.

CONCLUSION

A stability – indicating, rapid, cost effective and reliable HPLC assay method was developed for the assay of propranolol tablets useful for long term and short term stability samples. This chromatographic assay fulfilled all the requirements such as specificity, precision, accuracy, linearity, robustness and solution stability up to 27 hrs. The peak shape obtained though out the study shows asymmetry of 1.31 as well as the retention time of the propranolol is 8.5(±0.1) min, showing a good column life and fast analysis of large numbers of samples in short time period, therefore this method is suitable for routine sample analysis and preferably for samples with short term stability and long term stability studies.

Supplementary informations

The HPLC system used for this study is shimadzu LC-10ATvp solvent delivery pump with SIL-10Avp autoinjector, shimadzu SPD – M10Avp detector (photo diode array), Pentium 4 computer with class VP data integrating software. HPLC grade acetonitrile and Analytical grade Triethylamine, formic acid was purchased from Merck India and National chemicals Ltd respectively. High quality pure water was prepared by using Millipore milli Q Plus purification system.

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