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Method Development and Validation for Quantification of Propranolol HCl in Pharmaceutical Dosage form by RP-UPLC

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Abstract: Objective: to develop and validate a new very rapid, sensitive, reverse phase Ultra Performance Liquid Chromatography (RP-UPLC) technique for the estimation of Propranolol Hydrochloride in dosage form, as there is no official monograph & no analytical method by UPLC. Methodology: Chromatographic separation was achieved on a Waters Acquity BEH C_{18} column (30 x 2.1 mm, 1.7µm) using a gradient method with mobile phase composed of trifluoroacetic acid (0.1%) and acetonitrile in the ratio 80:20 v/v. The flow rate was 0.3 ml/min, temperature of the column was maintained at ambient and detection was made at 230 nm. The run time was as short as 2.5 min. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness. Results: The developed method was linear for Propranolol Hydrochloride from 10 - 50 µg/ml and the linear regression obtained was > 0.999. Precision, evaluated by intra- and inter-day assays had relative standard deviation (R.S.D) values within 0.72 %. Recovery data were in the range 96.70 to 98.72%**.** Conclusion: The method is precise, accurate, linear, robust and fast. The short retention time of 0.98 min allows the analysis of a large number of samples in a short period of time and, therefore, should be cost-effective for routine analysis in the pharmaceutical industry.

Keywords: Propranolol hydrochloride, Ultra Performance Liquid Chromatographic, Method development and Validation.

Introduction

Today's pharmaceutical industries are looking for new ways to cut cost and shorten time for development of drugs while at the same time improving the quality of their products and analytical laboratories are not exception in this trend. Though high-performance liquid chromatography (HPLC) is a well-established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients (API's) and dosage forms, it is often a slow technique because of the complexity of some of the samples, it could still be improved. Ultra Performance Liquid Chromatography (UPLC) is comes from HPLC. The underlying principles of this evolution are governed by the Van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency).

$$H = A + \frac{B}{v} + Cv$$

Where A, B and C are constants
v is the linear velocity, the carrier gas flow rate.

The A term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform.

The B term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by *v*.
The C term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus this term is proportional to *v*.
UPLC improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particles and saves time and reduces solvent consumption**.**

In the present work, this technology has been applied to the method development and validation study of related substance and assay determination of Propranolol Hydrochloride.[1]

Propranolol[2] (INN) is a sympatholytic non-selective beta blocker. Sympatholytics are used to treat hypertension, anxiety and panic. Propranolol is available in generic form as Propranolol Hydrochloride marketed in India under brand names like Ciplar and Ciplar LA by Cipla, also other brands from AstraZeneca and Wyeth under brand names Inderal, Inderal LA, Avlocardyl, Deralin, Dociton, Inderalici, InnoPran XL, Sumial, Anaprilinum, Bedranol SR (Sandoz). It is first-line treatment for hypertension and blocks the action of epinephrine and norepinephrine on both β_1- and β_2-adrenergic receptors. It has little intrinsic sympathomimetic activity (ISA) but has strong membrane stabilizing activity (only at high blood concentrations, e.g. overdosage).

The literature provides some of the references on the estimation of Propranolol Hydrochloride include HPLC[3-5], RP-HPLC[6-9] as alone or in combination with other drugs. The reported HPLC methods are more time consuming, complex mobile phase mixtures, use high flow rate of analysis, lack of sensitivity and peak symmetry. There is only one method for estimation of Propranolol Hydrochloride includes UPLC[10] as in combination with other drugs. It is, therefore, felt necessary to develop a new rapid method for the determination of Propranolol Hydrochloride by UPLC method. Hence a reproducible RP UPLC method was developed for the quantitative determination of Propranolol Hydrochloride tablets by using Waters Acquity BEH C_{18} column (30 x 2.1 mm, 1.7µm) UPLC column. The proposed method was validated as per the guidelines suggested by ICH.[11]

Experimental Details

Materials and Reagents

Propranolol Hydrochloride working standard was procured from Sigma Aldrich, India. Commercially available Propranolol Hydrochloride purchased from local pharmacy. Methanol, Acetonitrile and Trifluoroacetic acid HPLC grade were obtained from Sigma Aldrich, India. Water was prepared by using Millipore Milli-Q water purification system.

Chromatographic Conditions

Chromatography separation was performed on waters Acquity UPLC with photodiode array detector. The output signal was monitored and processed using Mass-Lynx software. The chromatographic column was Waters Acquity BEH C_{18} column (30 x 2.1 mm, 1.7µm). The mobile phase of trifluoroacetic acid (0.1%) and acetonitrile in the ratio 80:20 v/v at a flow rate of 0.3 ml/min. The detection was monitored at the Wavelength of 230 nm. The injection volume was 1.0 µL and the chromatographic runtime of 2.5 min was found. **Table 1**.

Table 1: Optimised Chromatographic Conditions

Parameter Optimized	Optimized Condition
Instrument (UPLC)	Waters ACQUITY
Column	Waters ACQUITY BEH C18 (2.1 x 50 mm, 1.7µm)
Mode	Gradient
Mobile phase	Trifluoroacetic acid : Acetonitrile
Column Oven	35 ºC
Auto sampler Temperature (^oC)	15 ℃
Flow rate	1.0 mL/min
Detector	Photodiode array
Temperature	Ambient room temperature

Detection wavelength	230nm
Injection volume	1µl
Retention time (RT)	0.98 ± 0.05 min
Run time	2.50min

Preparation of solutions

Preparation of Buffer: Pippette out 500 µl Trifluoroacetic acid in 500 ml Milli-Q water, mixed well, filter and degassed.

Preparation of Mobile Phase: Mixed well Buffer (Trifluoroacetic acid): Acetonitrile in a ratio (80:20) and filtered through 0.2µ 6, 6 Nylon membrane filter paper and degassed.

Preparation of the Propranolol Hydrochloride Standard & Sample Solution:

Standard Solution Preparation

Weighed accurately and transferred about 40.00 mg of Propranolol Hydrochloride standard in a 50.0 ml volumetric flask. Added a little quantity of diluent (Methanol) to dissolve, sonicated and degassed. Make the volume upto 50ml and sonicate. Further diluted 1.00 ml of the above solution to 10.00 ml with diluent.

Sample Solution Preparation

Each10 tablets containing 40mg of Propranolol Hydrochloride were weighed and powdered. To prepare 100µg/ml concentration of sample solution, a quantity of powder equivalent to 40 mg (216 mg) was weighed approximately and transferred to a 50 ml dried volumetric flask. The sample was initially dissolved in diluent (Methanol) and sonicated for 15 min. The volume was made upto 50ml and filtered through 0.2µm Nylon filters. Then 5 ml of this solution was further diluted to 10.00 ml with diluent to get the final concentration of 100µg/ml.

Results and Discussions

Method Development

Different chromatographic conditions were experimented to achieve efficiency of the chromatographic system. Parameters such as mobile phase selection, wavelength of detection, column selection, column temperature optimization. Several proportions of buffer and solvents were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time and run time were the major tasks while developing the method.

The typical chromatogram obtained for Propranolol Hydrochloride final UPLC conditions are depicted in (**Fig. 1 and 2).**

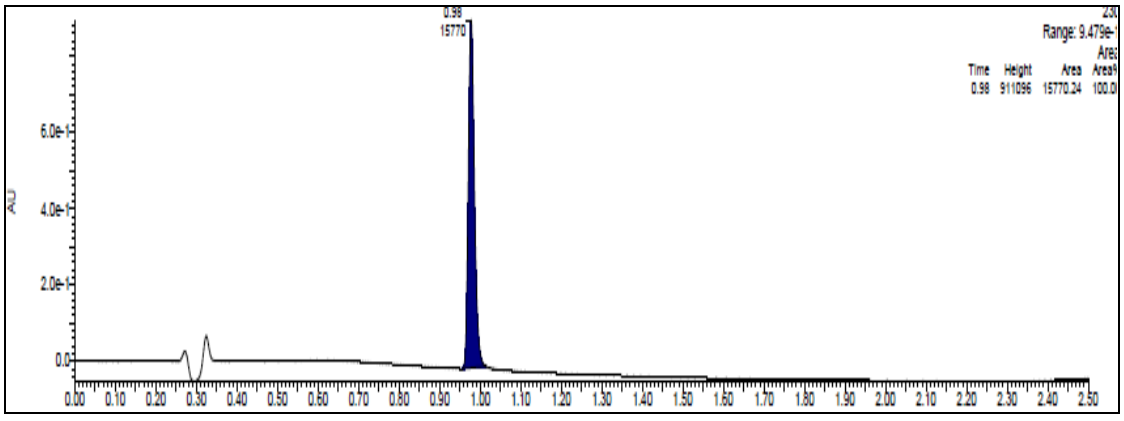

Fig.1: Chromatogram of Standard Propranolol Hydrochloride.

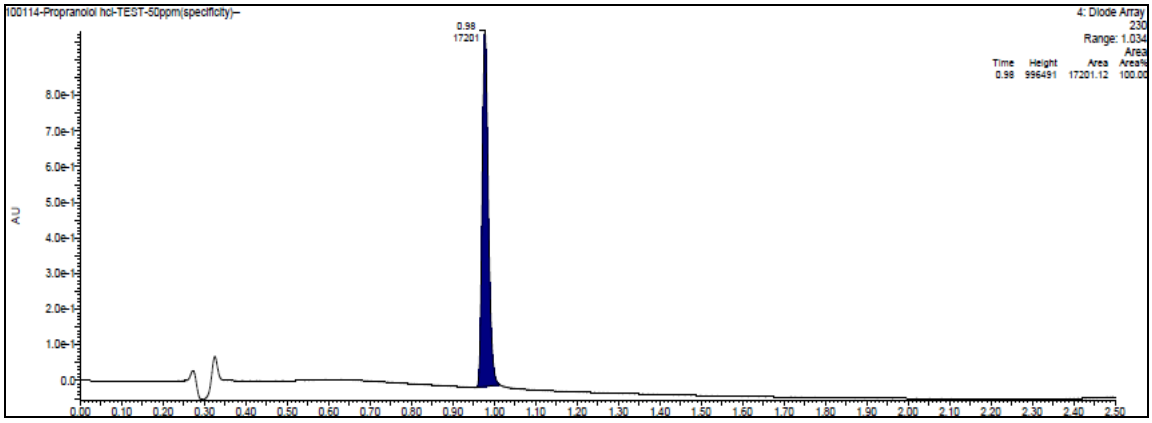

Fig.2: Chromatogram of Sample Propranolol Hydrochloride.

Table 2: Results of Validation Parameters with acceptance criteria

Parameter	Experiment	Discussion	Acceptance criteria
Specificity	Blank interference System suitability	1) No interference was observed at retention time of Propranolol Hydrochloride. 2) The relative standard deviation for six replicate measurements of peak area response of standard preparation was found to be **0.64.** 3) The relative standard deviation for retention time of six replicate injections of standard preparation was found to be 0.00.	1) No interference should be observed at retention time of Propranolol Hydrochloride. 2) The relative standard deviation for six replicate measurements of peak area response of standard preparation should be not more than 2.0%. 3) The relative standard deviation for retention time of six replicate injections of peak should not be more than 1.0%.
Linearity	1)Coefficient of correlation (r) 2) Y intercept ratio	**0.999** **1.87**	≥ 0.99 ± 2.0
Precision	1) Precision of the system	1) The relative standard deviation for six replicate measurements of peak area response of standard preparation was found to be **0.51.** 2) The relative standard deviation for retention time of six replicate injections of standard preparation was found to be **0.53.**	1) The relative standard deviation for six replicate measurements of peak area response of standard preparation should be not more than 2.0%. 2) The relative standard deviation for retention time of six replicate injections of peak should not be more than 1.0%.
	2) Precision of six replicate samples prepared.	1) The relative standard deviation for six replicate preparations was found to be **0.72.**	1) The relative standard deviation for six replicate preparations should be not more than 2.0%.
Reproducibility (Intermediate Precision)	Analyst to analyst	The %RSD for % assay obtained from 6 precision samples was found to be **0.26.**	The %RSD for % assay obtained from 6 precision samples should be not more than 2.0%.
Accuracy	Recovery study	The individual recoveries were found to be between **96.70 to 98.72%.**	All the individual recoveries should be within 95.0% to 105.0%

Solution stability	Stability of the solution at a predefined set time interval.	1) The %Cumulative RSD for standard area and % assay of sample at initial hr and at predetermined time intervals at room temperature was found to be **1.29.** 2) The pattern of chromatography remained same throughout solution stability study.	1) The Cumulative RSD for standard area and % assay of sample at initial hr and at predetermined time intervals should be not more than 2.0% 2) The pattern of chromatography should remain same throughout solution stability study.
Robustness	1] Change in flow of mobile phase from 0.3 ml/min to 0.2 ml/min and from 0.3 ml/min to 0.4 ml/min	The Cumulative RSD for % assay obtained from precision and robustness study was found **1.01.**	The Cumulative RSD for % assay obtained from precision and robustness study should be not more than 2.0%.
	2] Change in auto sampler temperature from 35 ± 5℃	The Cumulative RSD for % assay obtained from precision and robustness study was found **1.24.**	The Cumulative RSD for % assay obtained from precision and robustness study should be not more than 2.0%.
	3] Change in Buffers conc. from 0.1% ± 10%	The Cumulative RSD for % assay obtained from precision and robustness study was found to be **1.13.**	The Cumulative RSD for % assay obtained from precision and robustness study should be not more than 2.0%.

Method validation: Results shown in Table 2.

Based on International Conference on Harmonization (ICH) guidelines, the method is validated with regard to system suitability, linearity, accuracy, precision, robustness and sensitivity as follows.

System suitability

System suitability test was performed every day before starting any method validation exercise. System suitability test of the UPLC system was done by giving six injection of drug dilution and there retention times were calculated.

Specificity:

The specificity of the method was determined by comparing the chromatograms obtained from the sample containing Propranolol Hydrochloride standard stock with those obtained from test sample of Propranolol Hydrochloride and blank of that. The specificity study revealed at the absence of interference of impurities with the drug since no extra peak appeared at the Retention Time of drug.

Precision

System Precision:

Six replicate injections of standard solution were given and mean of all of these values gives rise to the RSD value obtained. According to USP %RSD should not be more than 2%. If % RSD of the assay is > 2% then the developed method is not a presided method.

Method Precision:

Method precision or Intra-assay precision data are obtained by repeatedly analyzing, in one laboratory on one day, aliquots of homogeneous +sample, each of which independently prepared according to method procedure.

Intermediate Precision:

Six replicate injections of standard solution were given and mean of all of these values gives rise to the RSD value obtained. The relative standard deviation for area of six replicate injections of peak due to Propranolol Hydrochloride in the standard should not be more than 2.0%.

Linearity:

Linearity was determined by injection six replicate injections of standard solutions of Propranolol Hydrochloride to check the system suitability. The response was measured as peak area. The calibration plot was generated by replicate analysis at six concentration levels and the linear regression equation was calculated using the least square method within Microsoft Excel® program. The standard curve of Propranolol Hydrochloride shown in the (**fig.3)**

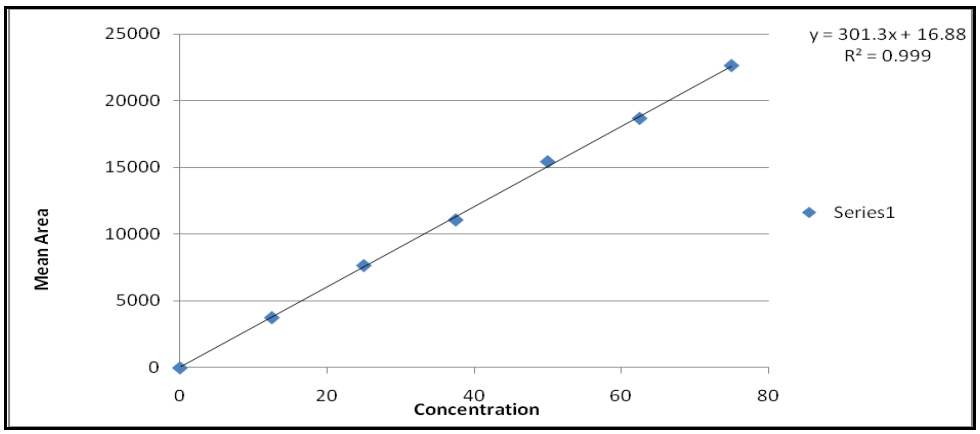

Fig.3: Standard curve of Propranolol Hydrochloride by UPLC

Robustness:

Robustness is the capacity of a method to remain unaffected by small deliberate variations in method parameters. Such as change in flow rate (±0.10 ml/min), buffer content (±10%), temperature (±5℃).

Accuracy:

The accuracy of method was determined by calculation of % recovery. Recovery is typically determined by comparing the response of the method to a reference material with the known value assigned to the material.

Stability of Solution:

A drug solution was prepared and kept at room temperature i.e. 25^{o}C for 24 hrs. After that drug solution was analyzed and it was found to be stable at room temperature.

Conclusion

The new, gradient RP-UPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. The short retention time of 0.98 min allows the analysis of a large number of samples in a short period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. It is suitable for rapid and accurate quality control of Propranolol Hydrochloride in tablet formulations.

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