# STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF PROPRANOLOL AND HYDROCHLOROTHIAZIDE IN BULK AND PHARMACUTICAL DOSAGE FORMS

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## ABSTRACT

The active pharmaceutical components of Propranolol and Hydrochlorothiazide in their bulk dosage forms were to be measured using a unique, straightforward, responsive, and stable RP-HPLC method that was being developed and gradually validated. For the quantitative determination of Propranolol and Hydrochlorothiazide, a straightforward, specific, verified, and well-defined stability that exhibits gradient RP-HPLC approach has been developed. The Waters $C_{18}$ column (150mm×4.6mm, 5mm particle size) was used for the chromatographic method, which involved isocratic elution with a mobile phase made up of orthophosphoric acid (0.1%) and acetonitrile (40:60% v/v). The instrument parameters called for a flow rate of 1 ml/min and a detection wavelength of 265 nm using the UV detector. The chromatographic method was expedited using the impurity-spiked solution. The proposed method's validity was examined in accordance with the international conference on harmonization (ICH) guidelines. The plotted calibration charts had linear regression coefficients of 0.999, indicating that their linearity was within acceptable bounds. The suggested approach is quick, easy, practical, and reasonably priced. It can be used for routine manufacturing sample analysis during stability tests and to confirm the caliber of medication samples during stability studies.

**Keywords:** Propranolol, Hydrochlorothiazide, Method development, RP-HPLC, Validation

## INTRODUCTION

Quantitative analysis identifies the species in the sample chemically. Analytical quality control of a pharmaceutical product is the only way to ensure its safety and efficacy [1-6]. Propranolol, chemically 1-[(1-methylethyl) amino]-3-(1-naphthylenyloxy)-2-propranolol, is a β-adrenergic antagonist that is non-selective and has no inherent sympathomimetic effect. Angina pectoris, myocardial infarction, phaeochromocytoma, thyrotoxicosis, hypertension, and cardiac arrhythmias are all conditions it is used to treat. There are currently several spectrometric methods available to quantify propranolol. For assaying the medication, the USP describes an HPLC method, but the Indian Pharmacopoeia describes a spectrometric method. Propranolol and other beta-blockers can be determined using HPLC methods either on its own or

in combination with other medications [7-11]. Hydrochlorothiazide is belongs to the thiazide class of diuretics chemically 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide, reduces sodium reabsorption in the distal convoluted tubule via acting on the kidneys. As a result, less water is reabsorbed from the collecting ducts, boosting urine output by increasing the osmolarity in the lumen. It is frequently used to treat hypertension, congestive heart failure, symptoms of edema, and to prevent kidney stones [12-17].

The objective of this work was to develop a simple, precise, and rapid procedure that would serve as an assay method for combination drug product of Propranolol and Hydrochlorothiazide. Hence present study aims to develop an accurate, precise, specific, linear, simple, rapid, validated and cost effective analytical method for Propranolol and Hydrochlorothiazide by RP-HPLC method. The scope of our work extends to validate for the developed method as per ICH guidelines.

## MATERIALS AND METHODS

Propranolol and Hydrochlorothiazide was obtained from Yarrow Chem Products, Mumbai, India. HPLC grade of potassium dihydrogen orthophosphate was obtained from Rankem Ltd., India and HPLC grade of Acetonitrile was obtained from Merck Private Limited, India. HPLC grade of water and ortho phosphoric acid was obtained from Rankem Ltd., India. Tablet dosage form (Ranox-40H) contains Propranolol 40mg and Hydrochlorothiazide 25mg were kindly supplied by Mylan Labs.

**Preparation of ammonium acetate buffer:** A 0.01M Potassium dihydrogen orthophosphate buffer was prepared by dissolving 1.36 gm of Potassium dihydrogen orthophosphate in 1000mL of HPLC grade water and pH was adjusted to 3.48 with orthophosphoric acid. The buffer was filtered through 0.45µm nylon membrane filter to remove all fine particles and gases.

**Preparation of mobile phase:** The above prepared Potassium dihydrogen orthophosphate buffer and Acetonitrile HPLC grade were mixed in the proportion of 50:50, %v/v and was filtered through 0.45µm nylon membrane filter and degassed by sonication.

**Preparation of diluent:** Mobile phase was used as diluent.

**Preparation of standard stock solutions of Propranolol and Hydrochlorothiazide:** Standard stock solutions of Propranolol and Hydrochlorothiazide were prepared by dissolving 10mg of Propranolol and 2.5mg of Hydrochlorothiazide in 10mL of diluent into a 10mL clean dry volumetric flask and the standard solutions was filtered through 0.45 µm nylon membrane filter and degassed by sonicator to get the concentration of 1000µg/mL of Propranolol and 250µg/mL of Hydrochlorothiazide.

**Preparation of standard solutions of Propranolol and Hydrochlorothiazide for assay:** From the above standard stock solution of 1000µg/mL of Propranolol and 250µg/mL of Hydrochlorothiazide further pipette 1mL and transferred into a 10mL volumetric flask and dilute up to the mark with diluent to get the concentration of 100µg/mL of Propranolol and 25µg/mL of Hydrochlorothiazide.

**Preparation of sample solutions of Propranolol and Hydrochlorothiazide:** Twenty capsules were accurately weighed and capsule powder equivalent to 2mg of Propranolol and 0.5mg of Hydrochlorothiazide were taken into 10mL clean dry volumetric flask, diluent was added and sonicated to dissolve it completely and volume was made up to the mark with the same diluent and filtered through 0.45 µm nylon membrane filter. Further pipette out 5mL from the above Propranolol and Hydrochlorothiazide sample stock solution into a 10mL volumetric flask and diluted up to the mark with diluent to get the concentration of 100µg/mL of Propranolol and 25µg/mL of Hydrochlorothiazide. 10mL from standard and sample solution were injected into the chromatographic system and the peak areas were measured for Propranolol and Hydrochlorothiazide, and the % assay was calculated by comparing the peak area of standard and sample chromatogram.

**Table-1: Assay of marketed formulation of Propranolol and Hydrochlorothiazide**

| **Drug** | **Label Claim (mg)** | **Amount Found (mg) (n=6)** | **% Label Claim ± % RSD (n=6)** |
|---|---|---|---|
| **Propranolol** | 40 | 40.08 | 100.2± 0.72 |
| **Hydrochlorothiazide** | 25 | 25.07 | 100.28± 0.43 |

**Method Development:** To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for Propranolol and Hydrochlorothiazide were obtained with a mobile phase containing a mixture of 0.01M Potassium dihydrogen orthophosphate buffer (pH adjusted to 3.48 with orthophosphoric acid) and Acetonitrile (50:50, %v/v) was delivered at a flow rate of 1mL/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 265nm based on peak area. The retention time of Propranolol and Hydrochlorothiazide was found to be 2.989min and 2.134min respectively with resolution of 5.1. Linearity was established for Propranolol and Hydrochlorothiazide in the range of 25-150µg/mL for Propranolol and 6.25-37.5µg/mL for Hydrochlorothiazide with correlation coefficients ($r^2$=0.999) and the percentage recoveries for Propranolol are 100.34% and Hydrochlorothiazide is 100.32% respectively, which indicate accuracy of the proposed method. The % RSD values of accuracy for Propranolol and Hydrochlorothiazide were found to be < 2 %. The % RSD values of method precision are 0.9% and 1% for Propranolol and Hydrochlorothiazide respectively and % RSD values of system precision are 1% and 0.9% for Propranolol and Hydrochlorothiazide respectively. The % RSD values of intermediate precision are 0.8% and 1.4% for Propranolol and Hydrochlorothiazide respectively, reveal that the proposed method is precise. LOD values for Propranolol and Hydrochlorothiazide were found to be 0.491µg/mL and 0.03µg/mL respectively and LOQ values for Propranolol and

Hydrochlorothiazide were found to be 1.487µg/mL and 0.1µg/mL respectively. The % RSD values of robustness studies were found to be < 2% reveal that the method is robust enough [18-20].

**Method Validation:** The developed method for the simultaneous estimation of Propranolol and Hydrochlorothiazide was validated as per the ICH guidelines for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, robustness, limit of detection (LOD) and limit of quantitation (LOQ) [21].

**Optimized method:**

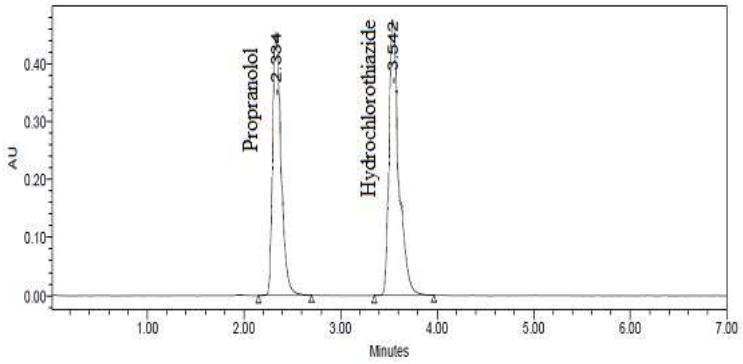


**Fig.1: Optimized Chromatogram**

**Table-2: Optimized chromatographic conditions**

| | |
|---|---|
| Mobile phase | OPA buffer:Acetonitrile (50:50 v/v) |
| Column | Kromasil C18 (4.6 x 250 mm, 5 µm) |
| Flow rate | 1.0 ml/min |
| Column & Sample temperature | Room temperature (20-25$^0$C) |
| Wavelength | 265 nm |
| Injection volume | 10 µl |
| Run time | 7 minutes |
| Retention time | 2.334 min for Propranolol<br>& 3.542 min for Hydrochlorothiazide |

**Preparation of samples for Assay**

**Standard sample:** Standard stock solutions of Propranolol and Hydrochlorothiazide (µg/ml) were prepared by dissolving 25mg of Propranolol and 25mg of Hydrochlorothiazide dissolved in sufficient mobile phase. After that filtered the solution using 0.45 micron syringe filter and sonicated for 5 min and dilute to 100 ml with mobile phase. Further dilutions made by adding 1 ml of stock solution to 10 ml of mobile phase (1000µg/ml of Propranolol and 1000µg/ml of Hydrochlorothiazide).

**Preparation of samples for Assay**

1g of dry powder (for injection) was weighed and transferred to 500 ml volumetric flask, to this 5 ml of acetonitrile was added and sonicated. Volume was made upto 500 ml with diluents and filtered through 0.45 µm or finer porosity membrane filter (1000µg/ml of Propranolol and

1000µg/ml of Hydrochlorothiazide).

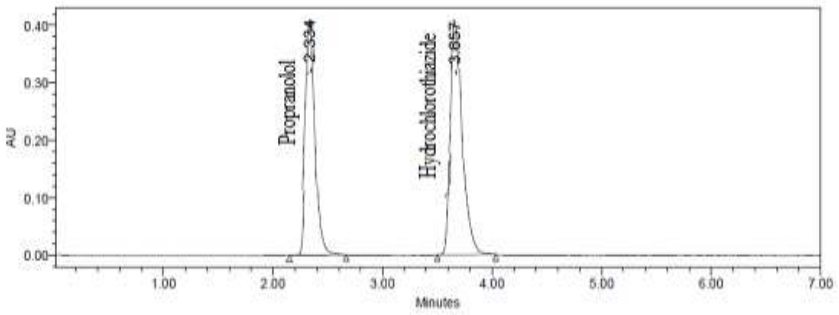


**Fig.2: Chromatogram of Assay sample preparation-1**

**Table-3: Assay of Tablet Formulation**

| S. No. | Propranolol %Assay | Hydrochlorothiazide %Assay |
|---|---|---|
| 1 | 98.84 | 99.75 |
| 2 | 99.24 | 99.40 |
| 3 | 98.93 | 100.11 |
| 4 | 99.28 | 99.80 |
| 5 | 100.14 | 99.26 |
| 6 | 99.53 | 99.52 |
| Avg | 99.33 | 99.64 |
| Std. Dev | 0.47 | 0.3091 |
| %RSD | 0.5 | 0.3 |

## RESULTS AND DISCUSSION

### System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated. The % RSD for the retention times and peak area of Propranolol and Hydrochlorothiazide were found to be less than 2%.

**Table-4: Results for system suitability of Propranolol and Hydrochlorothiazide**

| S. No. | Propranolol | | | Hydrochlorothiazide | | | Resolution |
|---|---|---|---|---|---|---|---|
| Inj | RT(min) | USP Plate Count | Tailing | RT(min) | USP Plate Count | Tailing | |
| 1 | 2.332 | 3649 | 1.42 | 3.542 | 5187 | 1.55 | 6.6 |
| 2 | 2.334 | 3519 | 1.41 | 3.582 | 5223 | 1.56 | 6.7 |
| 3 | 2.336 | 3480 | 1.40 | 3.619 | 5253 | 1.56 | 6.9 |
| 4 | 2.336 | 3554 | 1.45 | 3.633 | 5432 | 1.56 | 7.1 |
| 5 | 2.337 | 3405 | 1.45 | 3.650 | 5428 | 1.57 | 7.2 |
| 6 | 2.338 | 3379 | 1.45 | 3.652 | 5394 | 1.56 | 7.1 |

### Specificity:

Specificity is the ability of analytical method to assess unequivocally the

analyte in the presence of component that may be expected to be present, such impurities, degradation products and matrix components. The chromatograms for standards and sample are represented in Fig.3 & Fig.4. It is observed from the above data, diluent are not interfering with the Propranolol and Hydrochlorothiazide peaks.

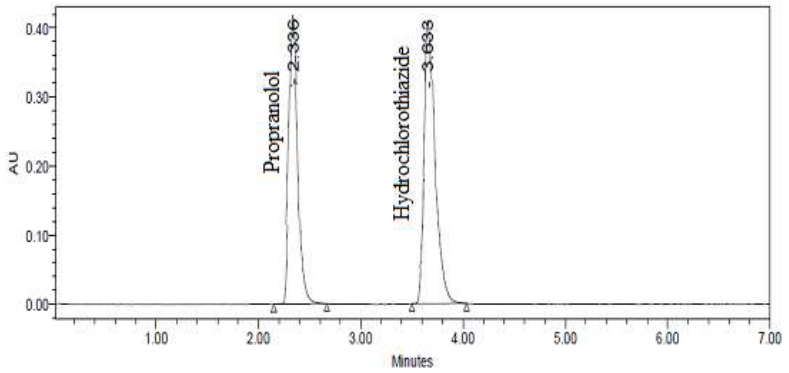


**Fig. 3: Chromatogram for Specificity of Propranolol and Hydrochlorothiazide standard**

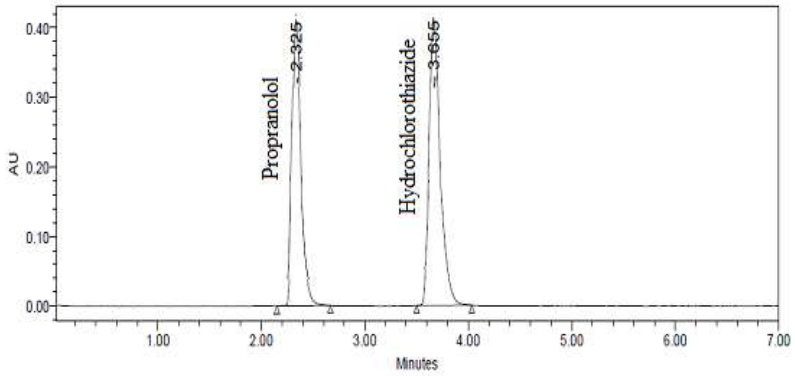


**Fig. 4: Chromatogram for Specificity of Propranolol & Hydrochlorothiazide samples**

**Linearity**

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5 ml from the stock solutions of Propranolol and Hydrochlorothiazide are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 25 ppm, 50 ppm, 75 ppm, 100 ppm, 125 ppm and 150 ppm of Propranolol and Hydrochlorothiazide. The linearity chromatograms were recorded from 25% to 150% and results were presented.

**Table-5: Linearity results of Propranolol & Hydrochlorothiazide**

| S. No. | Concentration (µg/ml) | Peak area of Propranolol | Peak area of Hydrochlorothiazide |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 25 | 637769 | 853665 |
| 3 | 50 | 1248415 | 1652244 |
| 4 | 75 | 1759937 | 2462178 |
| 5 | 100 | 2367487 | 3263508 |
| 6 | 125 | 3017833 | 4144622 |

| | | | |
|---|---|---|---|
| 7 | 150 | 3547775 | 4928266 |
| Slope | | 23604 | 32826 |
| Intercept | | 26760 | 10143 |
| Regression Equation(y) | | y = 23604x + 26760 | y = 32826x + 10143 |
| Correlation Coefficient | | 0.997 | 0.999 |

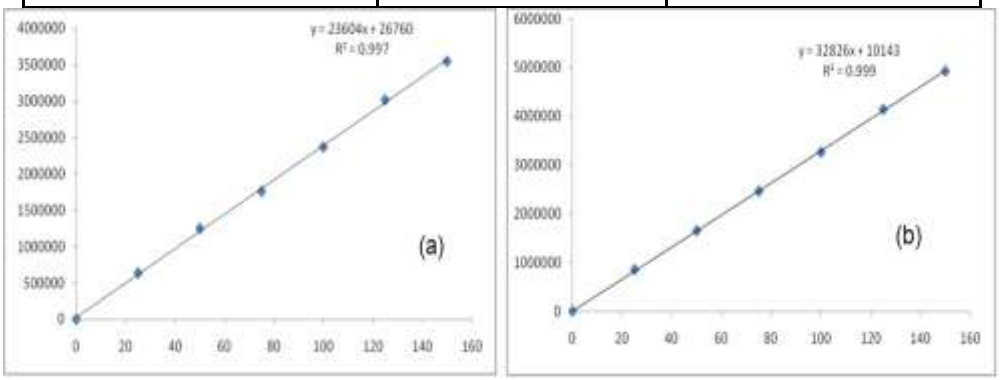


**Fig. 5: Linearity graph of Propranolol (a) and Hydrochlorothiazide (b)**

**Precision**

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.6% and 0.8% respectively for Propranolol and Hydrochlorothiazide. As the limit of Precision was less than "2" the system precision was passed in this method. The results were furnished in Table-6 and chromatogram was represented in Fig.6.

**Table-6: System precision table of Propranolol and Hydrochlorothiazide**

| S. No. | Area of Propranolol | Area of Hydrochlorothiazide |
|---|---|---|
| 1. | 2310034 | 3227462 |
| 2. | 2332829 | 3211818 |
| 3. | 2342734 | 3233800 |
| 4. | 2330070 | 3204141 |
| 5. | 2329810 | 3243356 |
| 6. | 2309148 | 3279245 |
| Mean | 2325771 | 3233304 |
| S.D | 13389.0 | 26676.3 |
| %RSD | 0.6 | 0.8 |

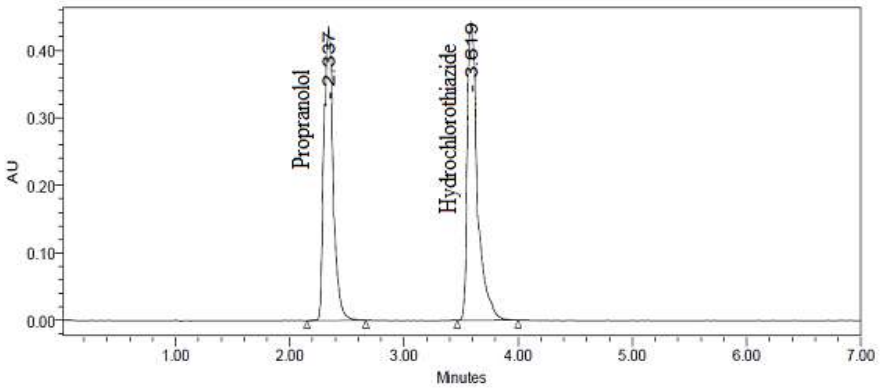


**Fig.6: System precision chromatogram**

**Repeatability:**

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.3% respectively for Propranolol and Hydrochlorothiazide. As the limit of Precision was less than "2" the system precision was passed in this method. The results were furnished in Table-7 and chromatogram was represented in Fig.7.

**Table-7: Repeatability table of Propranolol and Hydrochlorothiazide**

| S. No. | Area of Propranolol | Area of Hydrochlorothiazide |
|---|---|---|
| 1. | 2301103 | 3228291 |
| 2. | 2310442 | 3216973 |
| 3. | 2303284 | 3240102 |
| 4. | 2311248 | 3230011 |
| 5. | 2331248 | 3212531 |
| 6. | 2317132 | 3220927 |
| Mean | 2312410 | 3224806 |
| S.D | 10895.2 | 10002.8 |
| %RSD | 0.5 | 0.3 |

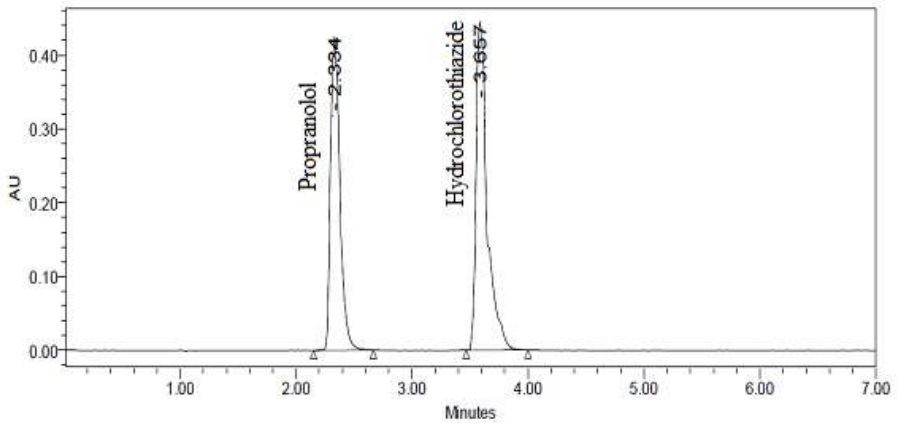


**Fig.7: Repeatability chromatogram**

**Accuracy**

Accuracy of the method was determined by recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% and 150%. The percentage mean recovery of Propranolol and Hydrochlorothiazide is 98.44 % and 98.80% respectively (Table-8 & 9).

**Table-8**: **Recovery results for Propranolol**

| % Level | Amount Spiked (µg/ml) | Amount recovered (µg/ml) | % Recovery | Mean % Recovery |
|---|---|---|---|---|
| 50% | 50 | 49.22742 | 98.45 | 98.93% |
| | 50 | 49.05724 | 98.11 | |
| | 50 | 49.45458 | 98.91 | |
| 100% | 100 | 100.5322 | 100.53 | |
| | 100 | 100.0526 | 100.05 | |
| | 100 | 98.17544 | 98.18 | |
| 150% | 150 | 147.0432 | 98.03 | |
| | 150 | 148.5476 | 99.03 | |
| | 150 | 148.59 | 99.06 | |

**Table-9**: **Recovery results for Hydrochlorothiazide**

| % Level | Amount Spiked (µg/ml) | Amount recovered (µg/ml) | % Recovery | Mean % Recovery |
|---|---|---|---|---|
| 50% | 50 | 49.82535 | 99.65 | 99.94% |
| | 50 | 49.9993 | 100 | |
| | 50 | 49.72083 | 99.44 | |
| 100% | 100 | 99.32215 | 99.32 | |

| | | | | |
|---|---|---|---|---|
| | 100 | 99.38281 | 99.38 | |
| | 100 | 100.6504 | 100.65 | |
| 150% | 150 | 151.3292 | 100.89 | |
| | 150 | 151.2022 | 100.8 | |
| | 150 | 148.9527 | 99.3 | |

**Limit of Detection:** 0.25 ml each from two standard stock solutions was pipetted out and transferred to two separate 10 ml volumetric flasks and made up with diluents. From the above solutions 0.1 ml each of Propranolol, Hydrochlorothiazide, solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluents. LOD chromatogram was represented in Fig.8. Limit of Detection was calculated by intercept method and LOD for Propranolol and Hydrochlorothiazide were found to be 0.06 µg/ml and 0.18 µg/ml respectively.

**Limit of Quantification:** 0.25 ml each from two standard stock solutions was pipetted out and transferred to two separate 10 ml volumetric flask and made up with diluent. From the above solutions 0.3 ml each of Propranolol, Hydrochlorothiazide solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluent. LOD chromatogram was represented in Fig.9. Limit of Quantification was calculated by intercept method and LOQ for Propranolol and Hydrochlorothiazide were found to be 0.19 µg/ml and 0.53 µg/ml respectively.

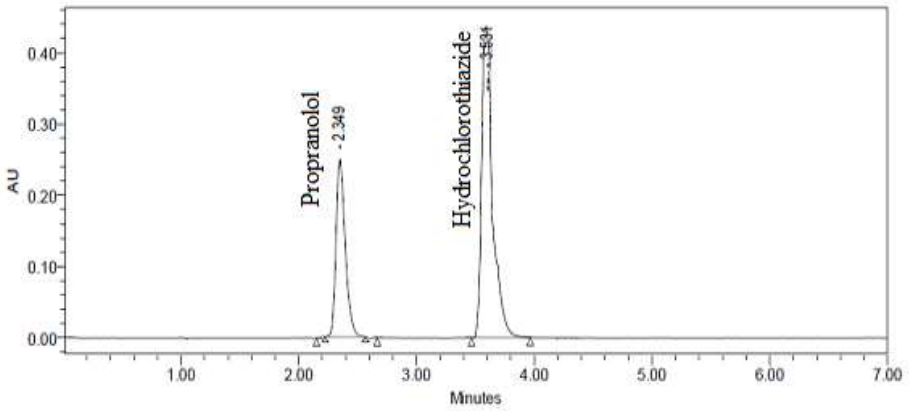


**Fig.8: LOD Chromatogram of Propranolol and Hydrochlorothiazide**

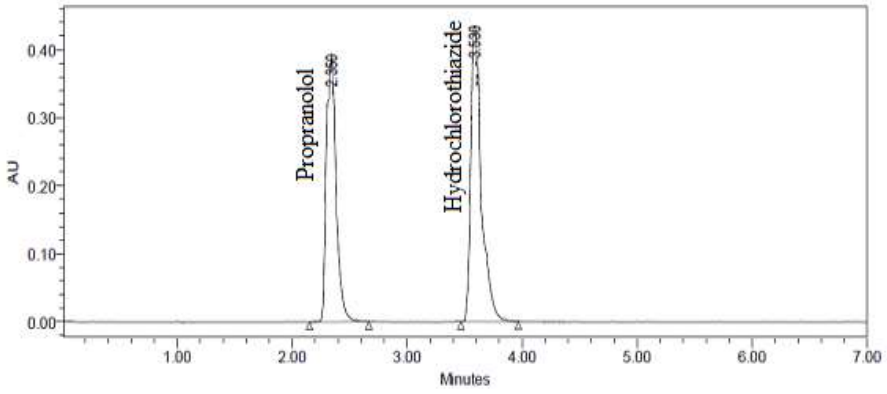


**Fig.9: LOQ Chromatogram of Propranolol and Hydrochlorothiazide**

**Robustness**

Small deliberate changes in method like flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. The data was presented in Table-10.

**Table-10: Robustness data of Propranolol and Hydrochlorothiazide**

| S. No. | Robustness condition | Propranolol %RSD | Hydrochlorothiazide %RSD |
|---|---|---|---|
| 1 | Flow minus (0.9 ml/min) | 1 | 1.1 |
| 2 | Flow plus (1.1 ml) | 0.9 | 0.9 |
| 3 | Mobile phase minus (65:35) | 0.8 | 0.9 |
| 4 | Mobile phase plus (75:25) | 0.3 | 0.4 |
| 5 | Temperature minus ($25^0$C) | 0.5 | 1.1 |
| 6 | Temperature plus ($30^0$C) | 1.2 | 0.7 |

**Degradation Studies:** Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

**Table-11: Degradation data of Propranolol and Hydrochlorothiazide**

| Type of degradation | Propranolol | | | Hydrochlorothiazide | | |
|---|---|---|---|---|---|---|
| | Area | %Recovered | % Degraded | Area | %Recovered | % Degraded |
| **Acid** | 2219329 | 95.33 | 4.67 | 3080901 | 95.19 | 4.81 |
| **Base** | 2269272 | 97.47 | 2.53 | 3143111 | 97.11 | 2.89 |
| **Peroxide** | 2284347 | 98.12 | 1.88 | 3181472 | 98.3 | 1.7 |
| **Thermal** | 2310694 | 99.25 | 0.75 | 3212680 | 99.26 | 0.74 |
| **UV** | 2311722 | 99.3 | 0.7 | 3219426 | 99.47 | 0.53 |
| **Water** | 2314604 | 99.42 | 0.58 | 3215168 | 99.34 | 0.66 |

**SUMMERY AND CONCLUSION**

In this scientific investigation demonstrates that the applicability of the chromatographic method to develop a new, sensitive, single RP-HPLC method for the simultaneous quantitative assay determination of two drugs Propranolol and Hydrochlorothiazide in the fixed pharmaceutical dosage form.

**Table-12: Summary of validation parameters**

| Parameters | Propranolol | Hydrochlorothiazide |
|---|---|---|
| Calibration range (μg/ ml) | 25-150 | 6.25-37.5 |
| Optimized wavelength | 265 nm | 265 nm |

| | | |
|---|---|---|
| Retention time (min.) | 2.334 | 3.542 |
| Regression equation (y) | y = 23604x + 26760 | y = 32826x + 10143 |
| Correlation coefficient( $r^2$) | 0.997 | 0.999 |
| Precision (%RSD) | 0.9 | 0.6 |
| % Recovery | 98.93 | 99.94 |
| Limit of Detection (µg/ml) | 0.06 | 0.18 |
| Limit of Quantitation (µg/ml) | 0.18 | 0.53 |

**Conclusion**

A simple, accurate, precise method was developed for the simultaneous estimation of the Propranolol and Hydrochlorothiazide in tablet dosage form. Retention time of Propranolol and Hydrochlorothiazide were found to be 2.334 min and 3.542 min. %RSD of the Propranolol and Hydrochlorothiazide were and found to be 0.9 and 0.6 respectively. %Recovery was Obtained as 98.44% and 98.81% for Propranolol and Hydrochlorothiazide. LOD, LOQ values were obtained from regression equations of Propranolol and Hydrochlorothiazide were 0.06 µg/ml, 0.19 µg/ml and 0.18 µg/ml and 0.53 µg/ml respectively. Regression equation of Propranolol is y = 23604x+26760 and of Hydrochlorothiazide is y = 32826x+10143. 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.

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