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**RESEARCH ARTICLE**

# STABILITY INDICATING VALIDATED DISSOLUTION METHOD FOR DETERMINATION OF PROPRANALOL AND HYDRALAZINE BY MULTICOMPONENTMODE METHOD AND SECOND ORDER DERIVATIVE METHOD

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**ABSTRACT**

The aim of this work was development and validation of a dissolutionmethod for Propranolol and Hydralazine (Carbetazine Tablets). The dissolution established conditions were 900 mL of 0.1M HCl (pH 1.0) as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method the absorbance of solution were recorded at288.20nm and 259.20nm for Propranolol and Hydralazinemixture for Multicomponent Mode methodand at 221.8nmand 243.36nm for Propranolol and Hydralazine respectively for Second order derivative method .Ahead of the results it can be concluded that the method developed consists in an efficient alternative for assays of dissolution for tablets.



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## INTRODUCTION

Propranolol hydrochloride (PRP)chemically is (RS)-1-[(1-methylethyl) amino]-3-(naphthalene-1-yloxy) propan-2-olhydrochioride [Barar, 2009] and chemical structure of PRP is given in the Fig. 1.The exact mode of hypertensive action is including an effect on the CNS, an adrenergic neuron blocking effect, an antirenin effect and the resetting of the baroreceptors. The cardiac output falls, and on prolonged use an initial rise in TPR is followed by a fall. Propranolol has appreciable antirenin activity and its response is good in moderate hypertensive with normal or high Propranolol, whereas it is poor if the is low [Indian Pharmacopeia, 1996]. Propranolol has been lately employed in the management of malignant hypertensive emergencies. Hydralazine (HCZ) chemically is phthalazin-1-ylhydrazine hydrochloride [Barar, 2009]. Chemical structure of HCZ is given in the fig. 2.Hydralazine directly dilates the arteriole, reducing the TPR. It seems to exert a more favorable effect on the diastolic BP than on the systolic BP, as it affects the precapillary resistance vessels much more than the post capillary capacitance vessels. Hydralazine reflex stimulates the heart, causing tachycardia, increased cardiac output and blood flow[Indian Pharmacopeia, 1996]. Literature survey revealed that various analytical technique such as spectrophotometric technique[Chapke and Game, 2013; Hapse et al., 2012; Sahu and Patel, 2006; Adegoke, 2008; de AssisGonsalves, 2011]. Several methods based on separation technique including HPTLC[Bhavar and Chatpalliwar, 2008; Patilet al., 2012; Shahet al., 2007], and HPLC[Srikanthet al., 2012; El-Saharty, 2003; Tulja Rani et al., 2011] have been reported. No single method is available for this combination by using mobile phase as methanol: ortho phosphoric acid (60:40v/v). The present work therefore emphasizes on the quantitative estimation of PRP and HCZ in synthetic mixture by HPLC. This method was validated as per the International Conference on Harmonization (ICH) guidelines [ICH, Q2A1994; ICH, Q2B1996].

## MATERIALS AND METHODS

Gift sample of Propranolol was obtained from Flamigo Private Ltd., Nanded. And Gift sample of Hydralazine was obtained from Alkem laboratories limited, taloja MIDC, Navi Mumbai. Formulations of Propranolol and Hydralazine are purchased from local market (Carbetazine).

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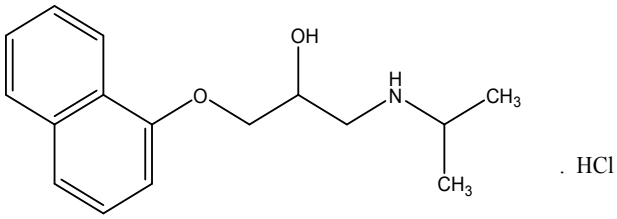


**Fig. 1. Propranolol**

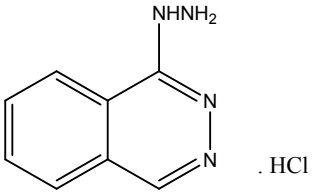


**Fig. 2. Hydralazine**

**Instrument (Instruction Manual Model TDT-06L USP Standards Dissolution test apparatus.)**

Dissolution test was performed in a ELECTROLAB (VK7025) Model (TDT-06L) (Patil et al., 2012)dissolution apparatus, multi-bath (n=6), in accordance to USP Pharmacopoeia generalmethod. The medium were vacuum degassed under in house vacuum and were maintained at 37.0 ± 0.5°C by using a thermostatic bath. A double-beam UV-Visible spectrophotometer (Model:UV 1800, Shimadzu] with a fixed slit width (2 nm)using 1.0 cm quartz cell was used for all absorbance measurements. Elico pH analyzer (Model: Elico 11610) was used to determine the pH of all solutions.

**Method for stability indicating dissolution media selection and for dissolution study**

**Stability studies**

In stability study nine dissolution media were selected and prepared such as distilled water, 0.1M HCl, and Phosphate buffer pH (6.0, 6.2, 6.4, 6.8) and as per USP guidelines [United States Pharmacopoeia XXX, 2007]. Stock solutions of PRP and HCZ were prepared by dissolving accurately weighed10 mg of both drug in 100 ml of distilled water, 0.1M HCl, and Phosphate buffer pH(6.0, 6.2 ,6.4 ,6.8) separately to obtain 100 µg/ml solutions. All the solutions were sonicated using ultrasonicater to dissolve the drug. From these solutions 1 ml was pipette out into 10 ml volumetric flask and diluted with the same solvent system up to the mark to obtain 10 µg/ml solutions. Two sets of 10 µg/ml solutions ofPRP and HCZ are prepared and stability was tested in the above prepared dissolution media at room temperature (RT) and 37$^{o}$C in an incubator (Thermo lab) for 48 hrs separately. These samples are studied at 0, 24 and 48 hrs interval by using a double-beam UV-visible spectrophotometer (shimadzu UV1800) connected to UV probe software. The λmax and absorbance value was measured for all the solutions and deviations in the values are recorded which indicates stability in 0.1MHCl respectively. These stable dissolution Medias are used for further dissolution studies of both the drugs.

**Multicomponent mode method**

The release of kinetic of Propranolol and Hydralazine from tablets was studied by conducting dissolution tests. Dissolution tests performed using USP type 2 dissolution apparatus and 900ml of 0.1N HCl at 37$^{\pm}$ 0.5$^{0}$c at 50rpm 10ml sample were withdrawn at the intervals of 5,10,15,20,25,30,35,40,45,50, 55min. Sampling was carried out and every time replaced with fresh 10ml with 0.1N HCl. The absorbance of solution were recorded at 288.20nm and 259.20nm using 0.1N HCl as blank. The dissolution studies were performed in triplicate (n=3). Overlay spectra of mixed standard is shown in Figure 3.

Following the above procedure the absorbance of solution were recorded at 288.20nm (PRP) and 259.20nm (HCZ) using 0.1N HCl as blank. The dissolution studies were performed triplicate. Result is shown in Table 1 and Figure 4.

**Derivative method**

Following the above procedure the absorbance of solution were recorded at 221.8nm(PRP) and 243.36nm(HCZ) using 0.1N HCl as blank. The dissolution studies were performed triplicate. Result shown in Table 2 and Figure 6.

**Stability indicating assay method**

**Preparation of stock solution:**

Standard stock solution of Propranolol & Hydralazine was prepared by dissolving 10mg of Propranolol & Hydralazine in 100ml of 0.1N HCl which gives 100µg/ml solution.

**Preparation of working solution:**

From the above stock solution 1ml was transferred into 10ml volumetric flask & the volume made was up to mark with 0.1N HCl to give 10µg/ml.

**Preparation of Blank solution:**

In separate 10ml volumetric flask, each containing 5ml of solvents used for degradation such as 0.1N HCl, 1N HCl, 0.1N NaoH, 1N NaoH & 3% $H_2O_2$ neutralize with solvent & Volume was made up with 0.1N HCl.

**Acid degradation**

10 ml volume flask containing 3 ml stock solution of propranolol & hydralazine 5 ml (0.1 and 1 N HCl), was added & heated at 60$^{0}$c for 3 hours. Which was then neutralized with proper solvent and final volume made up to mark with NaOH to form solution 10µg/ml of drug stock solution?

**Alkali degradation**

10 ml volumetric flack containing3 ml stock solution of propranolol & hydralazine 5 ml (0.1 to 1N NaOH), was added & heated at 60$^{0}$c for 3 hours. Which was then neutralized with proper solvent and final volume made up to mark with 0.1 N HCl to form solution 10µg/ml of drug stock solution?

**Oxidation degradation**

10 ml volumetric flack containing, 3 ml stock solutionofpropranolol & hydralazine 5 ml 4% $H_2O_2$ was added kept in 3 hrs for room temperature and final volume made up to mark with NaOH to form solution 10µg/ml of drug stock solution.

**Table 1. Media selection of Propranolol and Hydralazine**

| Medium | 0Hour | | 24 Hour | | 48 HOUR | | % CV |
|---|---|---|---|---|---|---|---|
| | $\lambda_{max}$ | Absorbance | Wavelength(nm) | Absorbance | $\lambda_{max}$ | Absorbance | |
| Distilled water | 288.20 | 0.319 | 288.20 | 0.323 | 288.20 | 0.330 | 1.7184 |
| 0.1M HCL | 288.20 | 0.190 | 288.20 | 0.195 | 288.20 | 0.197 | 18.108 |
| Buffer (6.0) | 289.0 | 0.803 | 289.0 | 0.825 | 289.0 | 0.856 | 3.275 |
| Buffer(6.2) | 288.60 | 0.725 | 288.60 | 0.756 | 288.60 | 0.788 | 4.1650 |
| Buffer(6.4) | 288.80 | 0.755 | 288.80 | 0.768 | 288.80 | 0.789 | 2.2261 |

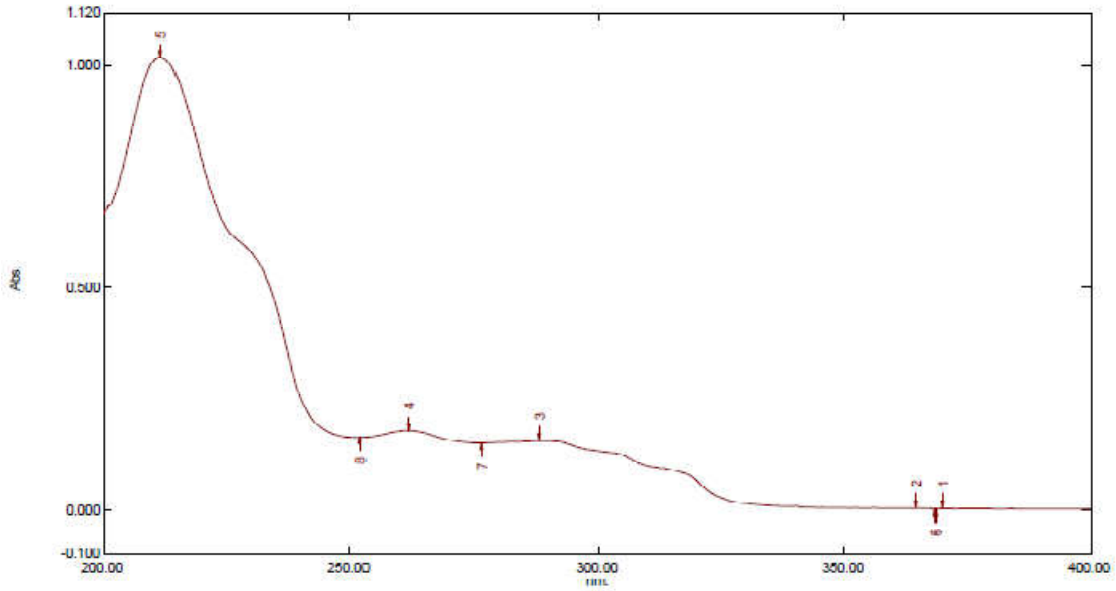


**Figure 3. Spectrum of Mixture by Multicomponent Mode method**

**Table 2.Calculation by Multicomponent Mode method**

| S.No. | SamplingTime (Min) | Absorbance | | PercentageReleased(%) | |
|---|---|---|---|---|---|
| | | PRP(288.20nm) | HCZ(259.20nm) | PRP | HCZ |
| 1 | 5 | 0.838 | 0.707 | 42.5 | 36.56 |
| 2 | 10 | 1.0944 | 0.938 | 55.5 | 48.5 |
| 3 | 15 | 1.232 | 1.081 | 62.5 | 55.9 |
| 4 | 20 | 1.452 | 1.211 | 73.65 | 62.6 |
| 5 | 25 | 1.676 | 1.328 | 85 | 68.66 |
| 6 | 30 | 1.962 | 1.462 | 99.5 | 75.56 |
| 7 | 35 | 1.972 | 1.636 | 100 | 84.56 |
| 8 | 40 | 1.932 | 1.752 | 98 | 90.56 |
| 9 | 45 | -- | 1.920 | -- | 99.23 |
| 10 | 50 | -- | 1.935 | -- | 100 |
| 11 | 55 | -- | 1.867 | -- | 96.5 |

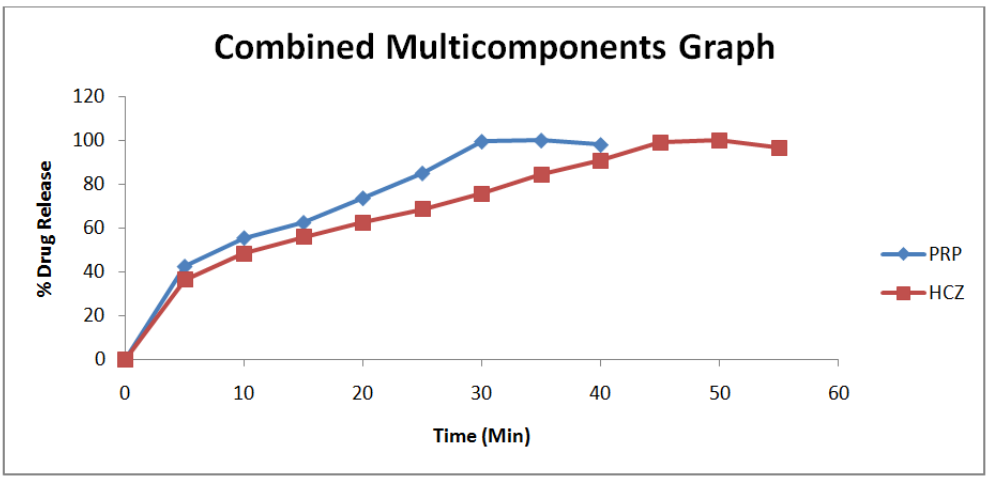


**Figure 4. Multicomponent method Graph**

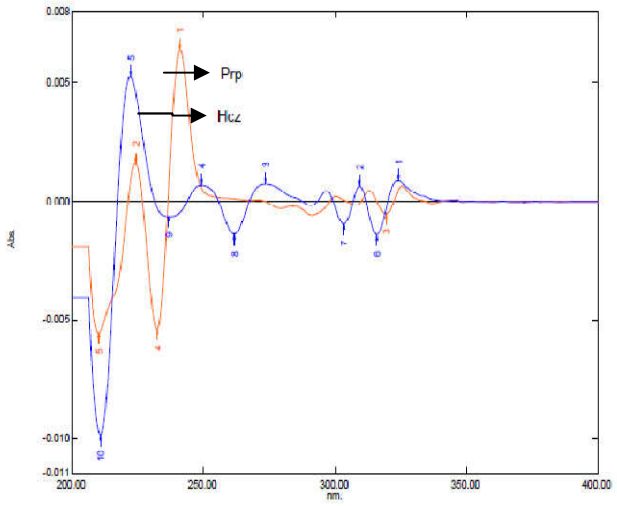


**Figure 5. Overly spectra of PRP &HCZ by Derivative method**

**Table 3. Calculation by Derivative method**

| S. No. | Sampling Time (Min) | Absorbance | | Percentage Released(%) | |
|---|---|---|---|---|---|
| | | PRP (221.8nm) | HCZ (243.36nm) | PRP | HCZ |
| 1 | 5 | 0.0124 | 0.0071 | 38.8 | 39.5 |
| 2 | 10 | 0.0171 | 0.0080 | 53.67 | 44.5 |
| 3 | 15 | 0.0238 | 0.0095 | 74.5 | 52.5 |
| 4 | 20 | 0.0262 | 0.0106 | 82 | 58.9 |
| 5 | 25 | 0.0294 | 0.0117 | 92 | 64.7 |
| 6 | 30 | 0.0318 | 0.0136 | 99.5 | 75.5 |
| 7 | 35 | 0.0320 | 0.0151 | 100 | 83.5 |
| 8 | 40 | 0.0305 | 0.0166 | 95.5 | 92 |
| 9 | 45 | -- | 0.0179 | -- | 99 |
| 10 | 50 | -- | 0.0181 | -- | 100 |
| 11 | 55 | -- | 0.0162 | -- | 90 |

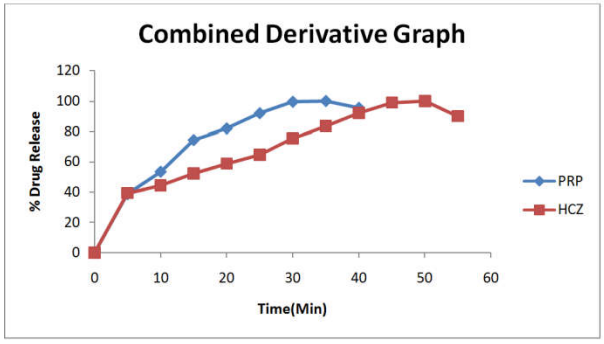


**Figure 6. Derivative method Graph**

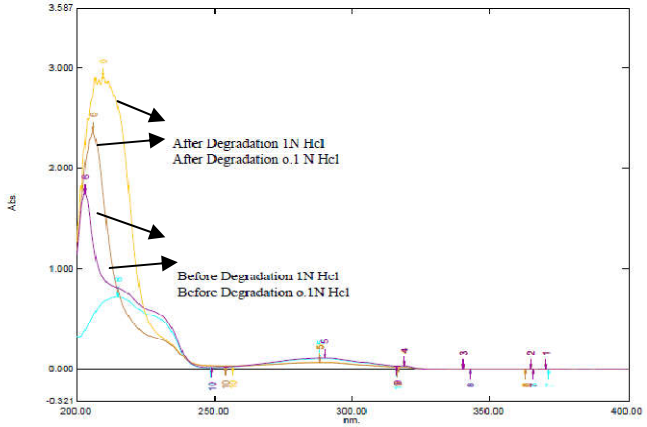


**Figure 7. Acid degradation of PRP**

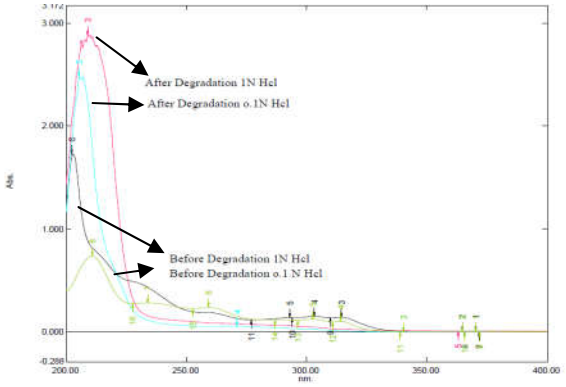


**Figure 8. Acid degradation of HCZ**

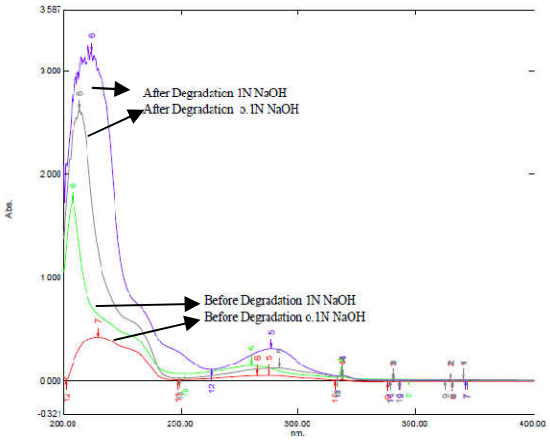


**Figure 9. Alkali degradation of PRP**

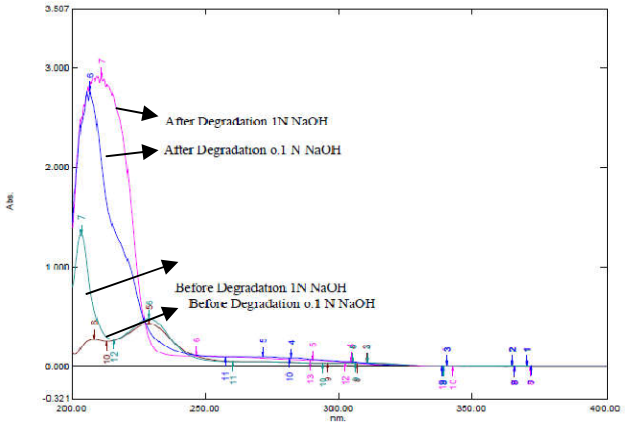


**Figure 10. Alkali degradation of HCZ**

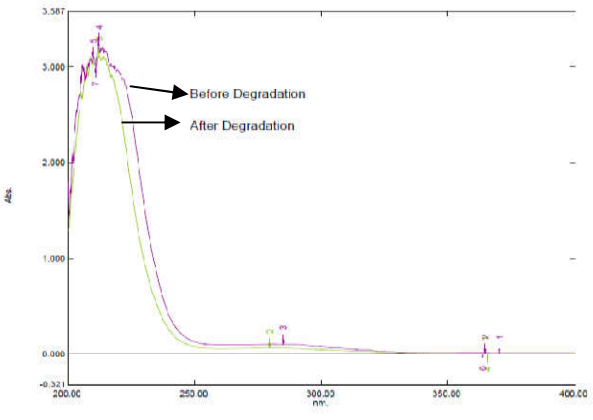


**Figure 11. Oxidation degradation PRP**

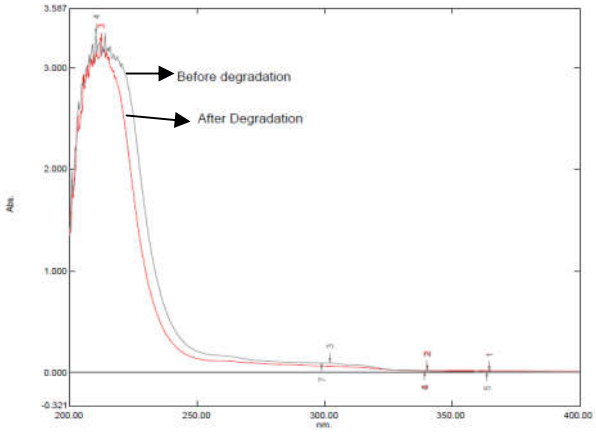


**Figure 12. Oxidation degradation HCZ**

## Thermal degradation

50mg of PRP &HCZ was weighted & kept in the oven & temperature was maintained at $80^0$c for 3hrs from this 1 mg exposedof PRP &HCZ was transferred in 100ml volumetric flack and final volume made upto 0.1N HCl

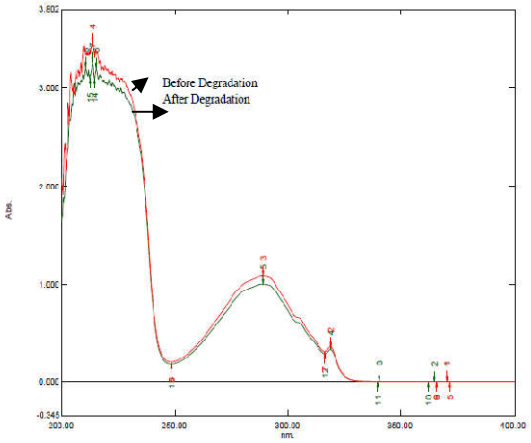


**Figure 13. Thermal Degradation of PRP**

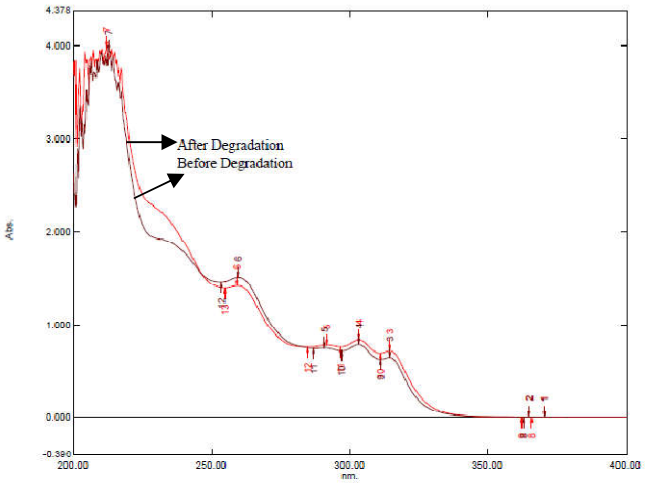


**Figure 14. Thermal Degradation HCZ**

## Photolytic Degradation

50mg of PRP & HCZ was exposed in sunlight & degradation drug not achieved. From this 1mg exposed PRP & HCZ was transferred in 100ml volumetric flack and final volume made with 0.1N Hcl

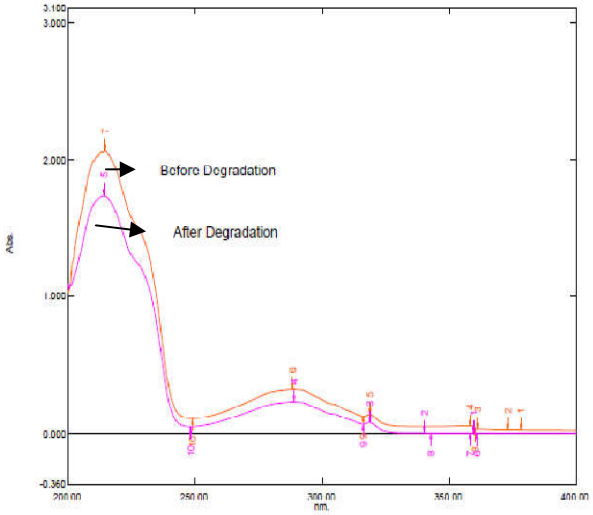


**Figure 15. Photolytic Degradaion of PRP**

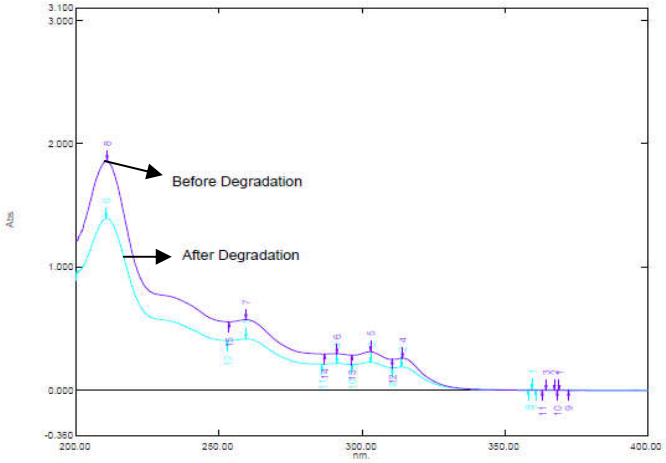


**Figure 16. Photolytic Degradaion of HCZ**

**Table 4. Validation data of developed methods**

| Method | Sample | Wavelength (nm) | Range (µg/ml) | % Recovery | Itermediate Precision |
|---|---|---|---|---|---|
| Method | PRP | 288.20 | 4-28 | 99.42±100.02 | 99.84±99.91 |
| | HCZ | 259.20 | 4-24 | 99.8±100.5 | 99.39±99.53 |
| Method | PRP | 221.8 | 4-28 | 100.03 | 99.02±99.78 |
| | HCZ | 243.36 | 4-24 | 99.40±99.55 | 99.09±99.64 |

**Table 5. Result of degradation study of PRP & HCZ at different condition in developed dissolution method**

| Name | Time | | Concentration | | % Degredation | |
|---|---|---|---|---|---|---|
| | | | PRP | HCZ | PRP | HCZ |
| Acid | 0 Min | 0.1N HCL | 0.102 | 0.237 | 00 | 00 |
| | | 1N HCL | 0.110 | 0.187 | 00 | 00 |
| | 3Hr | 0.1NHCL | 0.065 | 0.054 | 36.27 | 77.21 |
| | | 1N HCL | 0.070 | 0.087 | 36.36 | 53.47 |
| Base | 0Min | 0.1N NaoH | 0.123 | 0.100 | 00 | 00 |
| | | 1NNaoH | 0.309 | 0.099 | 00 | 00 |
| | 3Hr | 0.1N NaoH | 0.055 | 0.057 | 55.28 | 43 |
| | | 1N NaoH | 0.123 | 0.054 | 60.19 | 45.45 |
| Oxidative (3% $H_2O_2$) | 0Min | | 0.101 | 0.170 | 00 | 00 |
| | 3Hr | | 0.080 | 0.116 | 20.79 | 31.76 |
| Thermal | 0Min | | 1.085 | 1.559 | 00 | 00 |
| | 3Hr | | 1.007 | 1.510 | 7.18 | 3.14 |
| Photostability | 0Min | | 0.329 | 0.318 | 00 | 00 |
| | 7 Day | | 0.329 | 0.527 | 27.35 | 65.72 |

**Validation**

Validation is a process of establishing documentary evidence demonstrating that a procedure, process or activity carried out in production or testing maintains the desired level of compliance at all stages.

## RESULTS AND DISCUSSION

An attempt has been made to carry out the dissolution study of the marketed formulation by applying two established UV-Spectrophotometric methods for estimation of % release of the drug propranolol & hydralazine the study revealed that propranolol was released within 30 min (99.5%) & hydralazine was released within 45 min (99.09%) of the drug in 0.1N Hcl.

**Conclusion**

Two dissolution methods, Multicomponent method and Derivative method have been developed for determination of propranolol &hydralazine in bulk & tablet dosage form. From the statisticalresult, it can be concluded that two methodsare accurate, precise, robust and reproducible.In forced degradation studies. Propranolol & hydralazine is less degraded inacid condition than alkali condition(0.1N HCl& 0.1N NaoH). PRP is less degrade inacid degradation than alkali degradation (1HCl& 1N NaoH). HCZ is more degrade In Acid degradation than alkali condition (1HCl& 1N NaoH). PRP is 20.79% degrade & HCZ is 31.76% degrade in oxidative degradation (3%). PRP is 7.18% degrade &HCZ is 3.14% degrade. In thermal degradation. In photolytic degradation, degraded of PRP is 27.35% and HCZ is65.72% is degraded.

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