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Stability Indicating Validated Dissolution Method for Determination of Propranalol and Hydralazine by Simultaneous equation method and Q-Analysis method.

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Abstract : The aim of this work was development and validation of a dissolution method 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 area of solution were recorded at288.20nm and 259.20nm for Propranolol and Hydralazine respectively for Simultaneous equation method and at 288.20nm(PRP) and 236.00nm(Isobestic point) for Q-Analysis method. Ahead of the results it can be concluded that the method developed consists in an efficient alternative for assays of dissolution for tablets.
Key Words : Dissolution, Spectroscopy, Simultaneous equation method, Q-Analysis method, Stability, alidation.

Introduction

Propranolol hydrochloride (PRP)chemically is (RS)-1-[(1-methylethyl) amino]-3-(naphthalene-1-yloxy) propan-2-olhydrochioride [1]and chemical structure of PRP is given in the fig. 1.

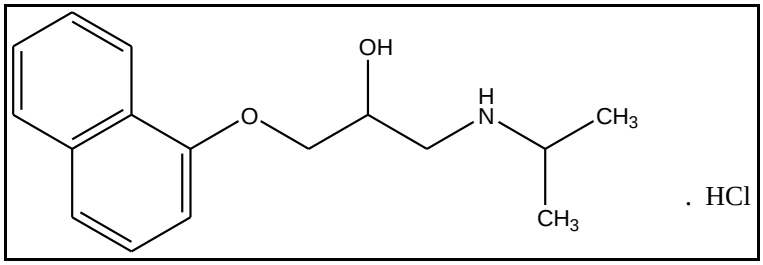

Figure I:Propranolol

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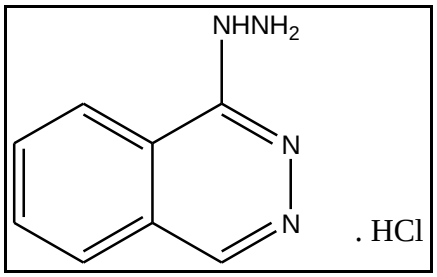

Figure II: Hydralazine

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 [2]. Propranolol has been lately employed in the management of malignant hypertensive emergencies.

Hydralazine (HCZ) chemically is phthalazin-1-ylhydrazine hydrochloride. [3] 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 [4] . Literature survey revealed that various analytical technique such as spectrophotometric technique [5-9]. Several methods based on separation technique including HPTLC [10-12], and HPLC [13-18] 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 [19-20]

Materials and Methods

Materials

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

Instrument[21]

Dissolution test was performed in a ELECTROLAB (VK7025)Model(TDT-06L)[11]dissolution apparatus, multi-bath (n=6), in accordance to USP Pharmacopoeia general method. 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

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 weighed 10 mg of both drug in 100 ml of distilled water, 0.1M HCl, and Phosphatebuffer 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 of PRP 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.

Simultaneous Equation 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 drugs standard is shown in figure 3.

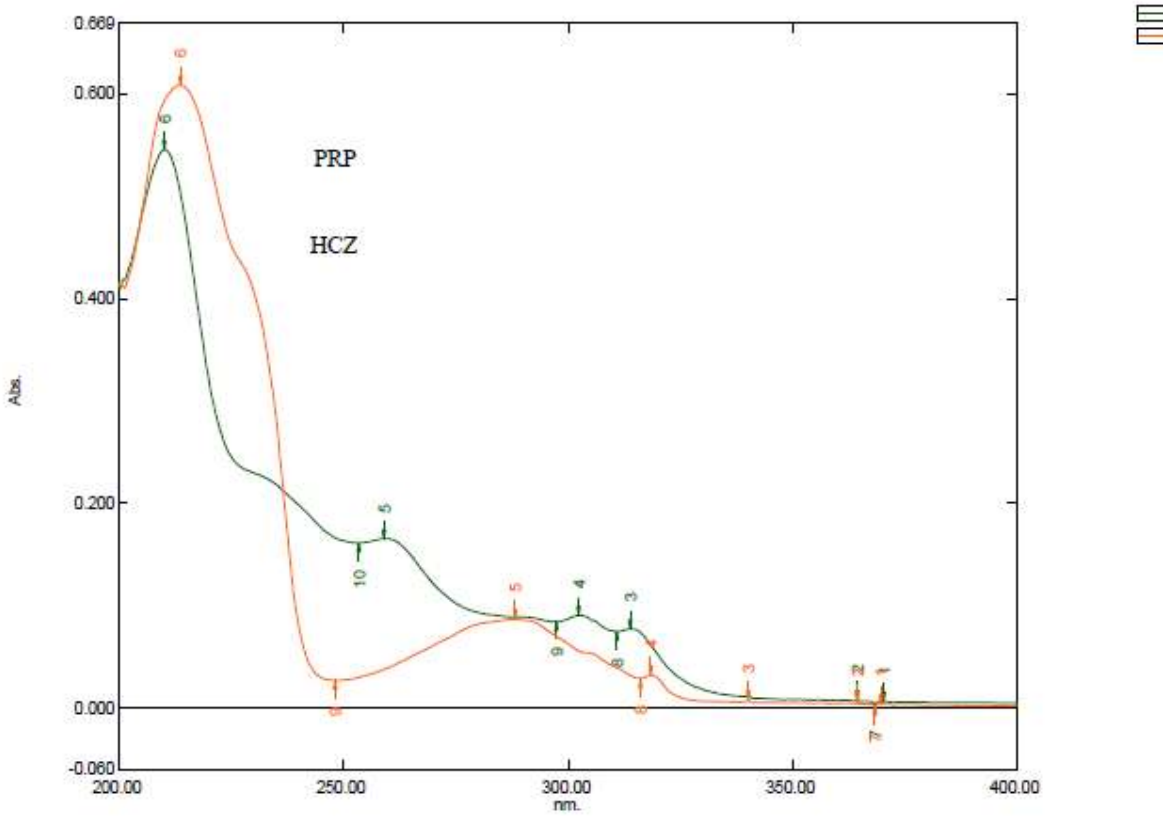

Figure III : Overlain spectra of PRP AND HCZ in 0.1N HCL.

Q-Analysis method

Following the above procedure the absorbance of solution were recorded at 288.20nm(PRP) and 236.00nm(Isobestic point) by using 0.1N HCL as blank . The dissolution studies were performed triplicate.

Method Validation

Linearity

The linearity of Propranolol response was evaluated from the range of 4-28µg 10-60µg/ml. And that for Hydralazine was 4-24µg/ml and showed a good correlation coefficient. To assess linearity, the standard curves Propranolol and Hyrdalazine were constructed by plotting concentration (µg/ml) verses area .

Precision

The precision of the method is evaluated by measuring the repeatability in two different UV Vis spectrophotometers

Recovery

The accuracy is evaluated by applying proposed method to the analysis of mixture of the tablet and with known amount of the Propranolol and Hyrdalazine working standard. Corresponding to the concentration of 80,100,and 120% which were subjected to dissolution test conditions described above.

Ruggedness

Ruggedness of the method is determined by carrying out the analysis by two different analysis and the respective dissolution values were calculated.

Stability indicating assay method

Medium	0 HOUR		24 HOUR		48 HOUR		% CV
	λ_{max}	Absorbance	Wavelength (nm)	Absorbance	λ_{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

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 containing3 ml stock solution of propranolol & hydralazine 5 ml (0.1 and 1 N Hcl) was added & heated at 60^0c 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 stocksolutionof propranolol & hydralazine 5 ml (0.1 to 1N NaOH), was added & heated at 60^0c 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 solution of propranolol& hydralazine 5 ml 4% H_2O_2 was added kept in 3 hrs for room temp.and final volume made up to mark with NaOH to form solution 10µg/ml of drug stock solution.

Thermal degradation

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

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.

Table I: Calibration curve of PRP

Conc. µg/ml	Absorbance PRP(λmax=288.20)
4	0.076
8	0.169
12	0.249
16	0.344
20	0.402
24	0.486
28	0.574

Table II: Calibration curve of HCZ

Conc. µg/ml	Absorbance HCZ(λmax=259.20)
4	0.155
8	0.345
12	0.495
16	0.659
20	0.784
24	0.928

Table III: Calculation by simultaneous equation method

SR NO.	Sampling Time (Min)	Absorbance		PercentageReleased(%)	
		PRP(288.20nm)	HCZ(259.20nm)	PRP	HCZ
1	5	0.094	0.199	35	37.8
2	10	0.132	0.229	48.9	43.5
3	15	0.163	0.292	60.5	55.5
4	20	0.198	0.339	73.5	64.5
5	25	0.230	0.366	85.5	69.55
6	30	0.268	0.404	99.5	76.77
7	35	0.270	0.455	100	86.5
8	40	0.264	0.498	98	94.5
9	45	--	0.522	--	99.09
10	50	--	0.527	--	100
11	55	--	0.500	--	95

Table IV: Calculation by Q-Analysis method

SR NO.	Sampling Time (Min)	Absorbance		Percentage Released(%)	
		PRP(288.20nm)	HCZ(236.00nm)	PRP	HCZ
1	5	0.0791	0.0805	35	37.8
2	10	0.1243	0.0926	55	43.5
3	15	0.1695	0.1182	75	55.5
4	20	0.1898	0.1326	84	62.3
5	25	0.2079	0.1459	92	68.5
6	30	0.2248	0.1563	99.5	73.4
7	35	0.2214	0.1757	98	82.5
8	40	0.2034	0.1968	90	92.4
9	45	--	0.2113	--	99.23
10	50	--	0.213	--	100
11	55	--	0.2023	--	95

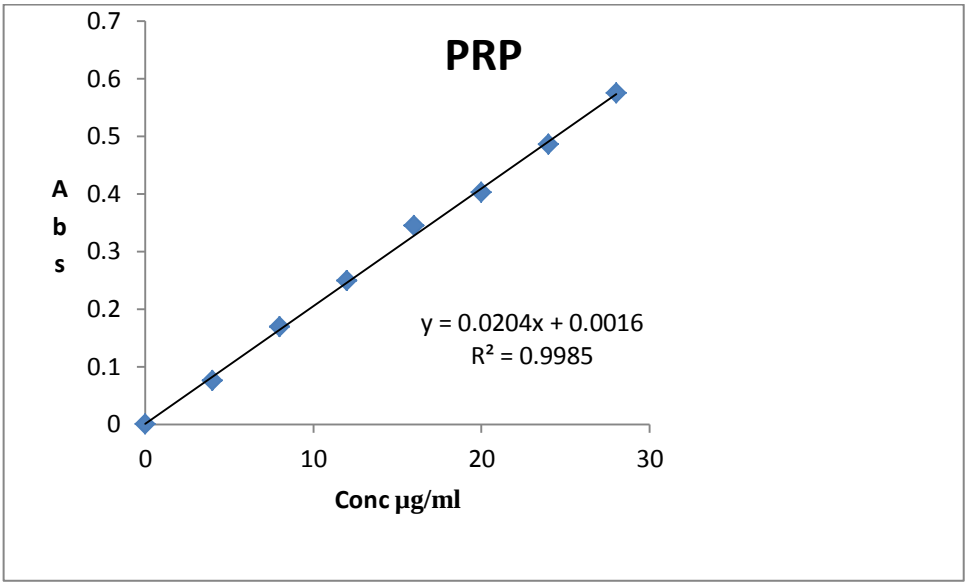

Figure IV: Calibration curve of PRP

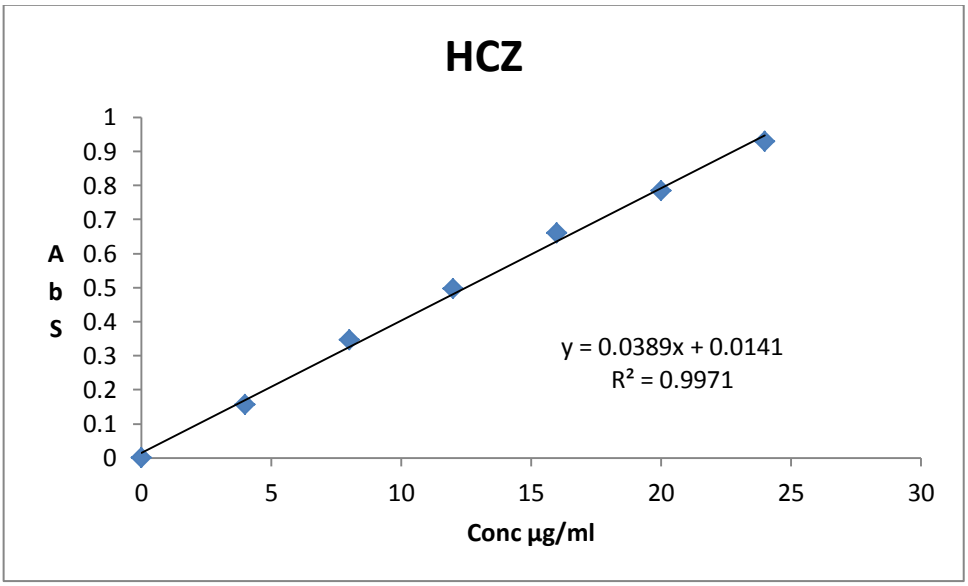

Figure V: Calibration curve of HCZ

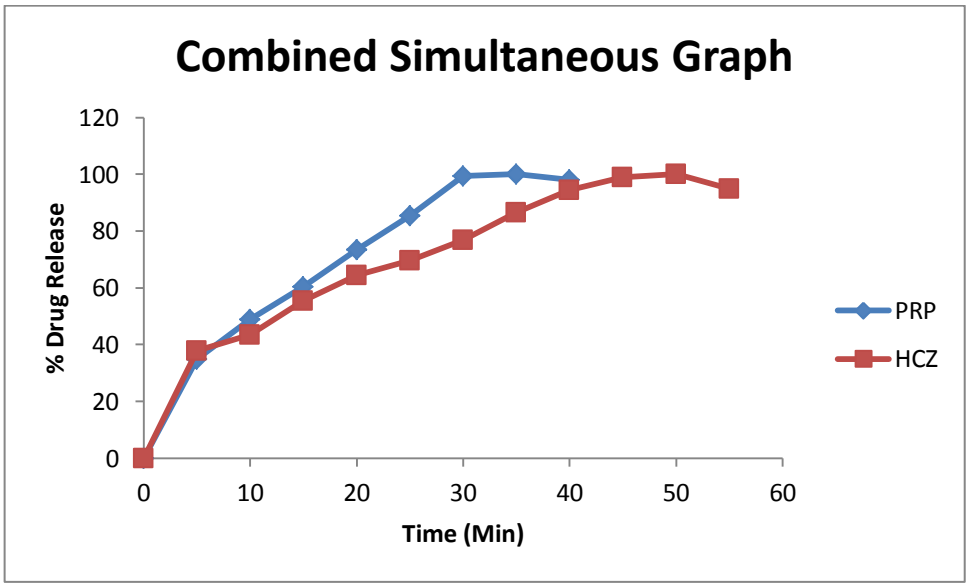

Figure VI:Simultaneous equation Graph

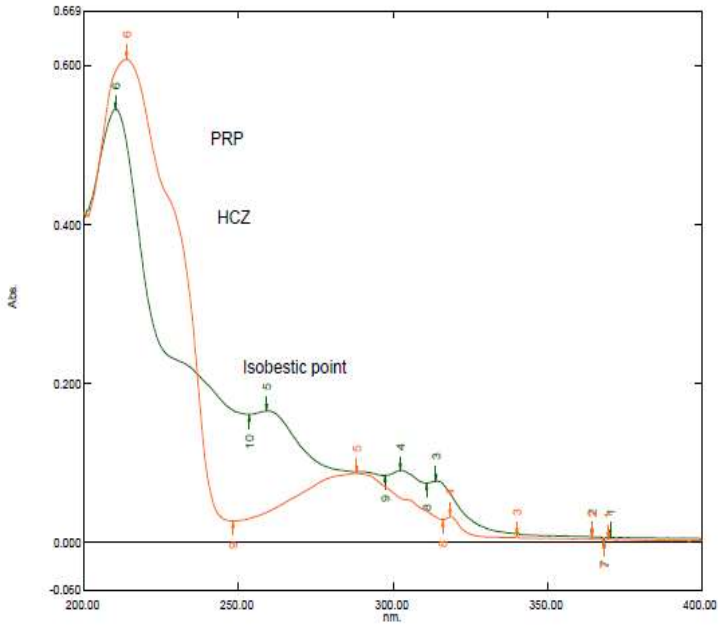

Figure VII: Overly spectra of PRP & HCZ by Q-Analysis method

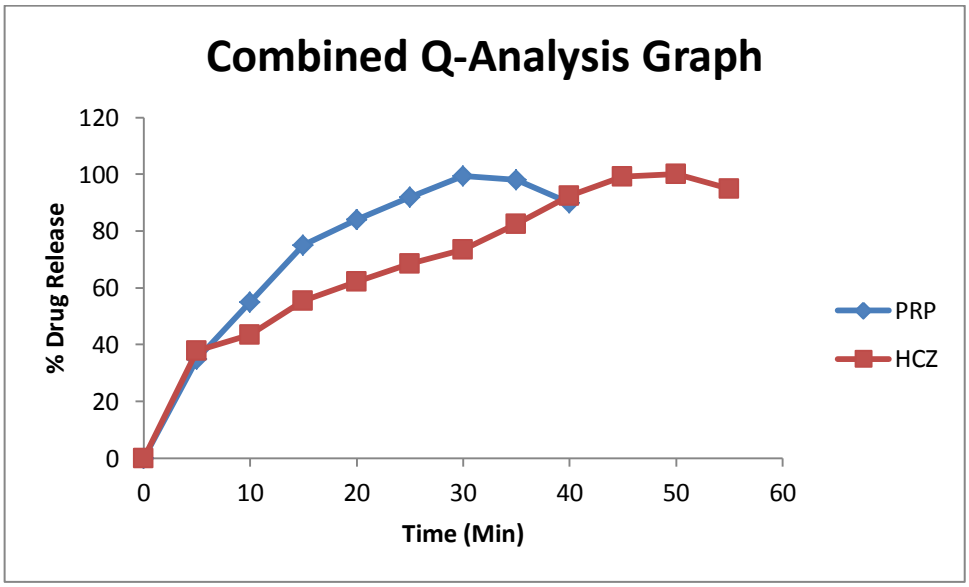

Figure VIII:Q-Analysis method Graph

Conclusion

Two dissolution methods Simultaneous equation method, Q-Analysis methods, have been developed for determination of Propranolol and Hyrdalazine in bulk & tablet dosage form. From the statistical result, it can be concluded that two methods are accurate, precise, robust and reproducible. The conditions that allowed the dissolution determination are 900 mL of 0.1 M HCl at 37.0 ± 0.5 ºC, paddle apparatus, 50 rpm stirring speed and filtration with 0.45 µ cellulose acetate membrane filters. In these conditions, Propranolol and Hyrdalazine stability is good. The percent drug delivery is higher than 90% in 40 minutes for both drugs in evaluated products. Therefore, the proposed method was successfully applied and suggested for the quality control studies of Propranolol and Hydralazine pharmaceutical dosage forms contributing to assure the therapeutic efficacy of the drug.

In forced degradation studies. Propranolol and Hydralazine is less degraded in Acid condition than Alkali condition(0.1N HCL & 0.1N NaoH).PRP is less degrade in Acid degradation than alkali degradation (1HCL & 1N NaoH). HCZ is more degraded in Acid degradation than alkali condition (1HCL & 1N NaoH).PRP is 2O.79% degrade & HCZ is 31.76% degrade in oxidative degradation (3%). PRP is 7.18% degrade &HCZ is 3.14% degradein thermal degradation. In photolytic degradation, degraded of PRP is 27.35%and HCZ is65.72% is degraded.

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