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Stability indicating simultaneous validation of hydrochlorothiazide, dihydralazine, and propranolol with thestudy of forced degradation behavior through RP-HPLC in thepharmaceutical dosage form

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ABSTRACT

For the instantaneous assay of Hydrochlorothiazide, Dihydralazine and Propranolol in tablet, an easy, accurate and truthful RP-HPLC method have been developed as well as validated. Isocratic RP-HPLC method was developed on BDS hypersil C18, (250mm×4.6mm internal diameter, 5µ particle size) using mobile phase as 0.05M Potassium Dihydrogenortho Phosphate (pH-6.0): Acetonitrile (40:60v/v) at a 1.0 mL/min flow rate than at 215 nm detection was carried out using tunable absorbance detector (Waters 486). Study of forced degradation study was carried out through acid degradation, base degradation, thermal degradation, oxidation of the drug. The validation of method was carried out to observe accuracy, precision, linearity, in addition to robustness. The method was found to be linear in the concentration range of 10-30μg/mL with correlation coefficient of 0.9996 for Hydrochlorothiazide,12.5-37.5μg/mL with correlation coefficient of 0.9986 for Dihydralazine, and 20-60μg/mLwith correlation coefficient of 0.9997 for Propranolol. Stress studies produce degradation products which did not hinder with the detection of Hydrochlorothiazide, Dihydralazine and Propranolol; therefore, the assay can be stability indicating.

Keywords: HPLC, Hydrochlorothiazide, Dihydralazine, Propranolol, Validation, Forced degradation.

Introduction

Hydrochlorothiazide's empirical formula is $C_7H_8CLN_3O_4S_2$and its IUPAC name is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1λ⁶,2,4-benzothiadiazine-7-sulfonamide. Figure 1demonstrates Hydro chloro thiazide chemical structure. Hydrochlorothiazide is a thiazide class of diuretic frequently considered the ideal associate of this class. It decreases the electrolytes reabsorption from the renal tubules in the kidney. This lead to increased electrolytes such as potassium, chloride, sodium, as well as magnesium.

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It has been used in the numerousillnesses treatment like hypertension, diabetes, hypoparathyroidism, and edema. Dihydralazine's empirical formula is $C_8H_{10}N_6$ and its IUPAC name is 4-Hydrazinyl-1-hydrazinylidene-1,2-dihydrophthalazine. Figure 2 shows chemical structure of Dihydralazine. Dihydralazine is a drug-related to the treatment of hypertension.

It relates to the class of hydrazinophthalazine and almost same effects to hydralazine. Dihydralazine is a vasodilator with direct action which acts principally on the arterioles. It decreases blood pressure and peripheral resistance, therefore, increasing cardiac output. It also has a tendency toprogresscerebral and renal flow of blood and its effect on diastolic pressure is additionally noticeable compared to systolic pressure. Propranolol's empirical formula is $C_{16}H_{21}NO_2$and its IUPAC name is 1-(naphthalen1yloxy)-3-[(propane-2-yr)amino]propan-2-ol.Figure 3 shows the chemical structure of Propranolol. Propranolol is a non-selective β-

adrenergic antagonist with no intrinsic sympathomimetic activity. It is used in the hypertension management, phaeochromocytoma, thyrotoxicosis, angina pectoris, myocardial infarction, and cardiac arrhythmias.

Literature survey reveals that quantitative analysis of Hydrochlorothiazide, Dihydralazine, and Propranolol have been done separately or in acombination of two and in acombination of other drugs but no any method is stated for the simultaneous valuation of Hydrochlorothiazide, Dihydralazine and Propranolol in the combined type of dosage form. The present study involved the development and validation of anRP-HPLC method for the estimation of Hydrochlorothiazide, Dihydralazine and Propranolol in combined pharmaceutical dosage form (tablet) and their forced degradation study[1-5].

Material and Methods

Instruments

The liquid chromatographic system consists of Waters series M510 equipped with a tunable absorbance detector (Waters 486), HPLC pump (Waters 510), and manual injector rheodyne valve with 20 μL fixed loop. The analytes were observed at 215 nm. Chromatographic analysis was performed on Thermo scientific BDS hypersil C18, (250mm × 4.6mm internal diameter, 5µ particle size). By using the citizen electronic balance all the chemicals and drugs were weighed. Chemiline India pH meter and Toshcon Ultrasonicator was used.

Chemicals and reagents

Acetonitrile was of HPLC grade obtained from Merck Ltd., Mumbai. Water was of HPLC grade prepared by triple distillation method. Potassium Dihydrogen Phosphate, Sodium Hydroxide (NaOH), Hydrogen Peroxide (H_2O_2) and Hydrochloric Acid (HCl) were of AR grade and were obtained from Merck, Mumbai India. Hydrochlorothiazide reference standard was procured from Oasis Laboratories. Dihydralazine and Propranolol reference standards were procured from Gitar Laboratories. Beptazine H tablets containing 20mg of Hydrochlorothiazide, 25mg of Dihydralazine and 40mg of Propranolol manufactured by MM labs were procured from local market.

HPLC Conditions

The mobile phase consisted of 0.05M Potassium Dihydrogen Ortho Phosphate (pH-6.0): Acetonitrile (40:60v/v).The mobile phase was freshly prepared, and it was sonicated by using Toshcon Ultrasonicator for 5 min before use. BDS hypersil C18, (250mm×4.6mm internal diameter, 5µ particle size) was used and it was equilibrated for at least half an hour with the mobile phase flowing through the system. The HPLC system and column were kept at ambient temperature. The eluent was monitored by UV detection at 215 nm. The analysis was done at aflowrate of 1.0ml/min with the20µl volume of injection. All data were analyzed by using Empower 3 software.

Preparation of Mobile Phase

The mobile phase was prepared by mixing 0.05M Potassium Dihydrogen Ortho Phosphate (pH-6.0) and Acetonitrile in the ratio of (40:60%v/v). The solution was then filtered through 0.45 microns membrane filter and degassed.

Preparation of 0.05M Potassium DihydrogenOrtho Phosphate (pH-6.0)

Take about 6.8gm Potassium dihydrogen orthophosphate into a 1000ml beaker. Add 800ml water and dissolve. Adjust ph6.0 of this solution with 0.1N Sodium hydroxide. Make up volume upto 1000ml with water.

Preparation of standard stock solution

A standard stock solution of Hydrochlorothiazide, Dihydralazine, and Propranolol were prepared by accurately weighing 20mg, 25mg, and 40mg respectively and dissolving them separately in 100ml with methanol to prepare asolution of 200μg/mL, 250μg/mL and 400μg/mL.

Preparation of working standard solution

Add 1ml each of standard stock solution of Hydrochlorothiazide, Dihydralazine,and Propranolol in 10 ml volumetric flask and volume make up to 10ml with methanol.

Preparation of sample stock solution

Weigh tablet powder equivalent to 20mg of Hydrochlorothiazide, 25mg of Dihydralazine and 40mg of Propranolol) into a 100ml volumetric flask. Add 60ml methanol andshake for 15 minutes. Makeupvolume with methanol upto 100ml. Filter this solution.

Preparation of sample working solution

Take 1ml from sample stock solution into a 10ml volumetric flask and makeup with mobile phase to prepare a solution of Hydrochlorothiazide 20mcg/ml, Dihydralazine 25mcg/ml and Propranolol 40mcg/ml.

Forced Degradation Study

Preparation of solution for acid degradation

The study of acid decomposition was achieved by keeping the working solution of all three drugs (1 ml) in 2 ml of 0.1N HCl for 4 hrs. After 4hrs solution neutralized with 2ml 0.1N NaOH and lastly made up to thevolume of 10 ml with mobile phase, sonicated and filtered by 0.45µm membrane filter paper then insertedinto HPLC system. Degradation samples were

prepared as ablank sample, separate standard samples and acombined sample of all three drugs.

Preparation of solution for basic degradation

The study of alkali decomposition was performed by keeping the working solution of all three drugs (1 ml) in 2 ml of 0.1N NaOH for 3.5 hrs. After 3.5 hrs solution neutralized with 2 ml of 0.1N HCl and finally made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as ablank sample, separate standard samples and acombined sample of all three drugs.

Preparation of solution for oxidative degradation

The study of oxidative decomposition was performed by keeping the working solution of all three drugs (1 ml) in 2 ml 3% H_2O_2 for 4 hrs. After 4hrs volume made up to 10 ml with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as ablank sample, separate standard samples and acombined sample of all three drugs.

Preparation of solution for thermal degradation

The study of thermal decomposition study was performed by refluxing the working solution of all three drugs (1 ml) for 3hrs at 105 ºC. After 3hrs volume made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as ablank sample, separate standard samples and acombined sample of all three drugs.

Preparation of solution for UV degradation

The study of UV degradation was performed by exposing the working solution of all three drugs (1ml) to Sunlight for 3 hours. After 3 hours volume made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as ablank sample, separate standard samples and acombined sample of all three drugs were prepared.

Determination of λ max

The UV spectra of standard stock solutions of Hydrochlorothiazide, Dihydralazine,and Propranolol were taken between the wavelength range of 200-400nm using methanol as blank. The λ max was found to be 233.73nm, 250.66nm, and 207.02nm for Hydrochlorothiazide, Dihydralazine,and Propranolol respectively. Overlay of the three spectra taken and theiso-absorptive point was selected and it was found that all three drugs show appreciable absorbance at 215 nm, so it is used for the further study.

Procedure of Analysis

1ml from Hydrochlorothiazide Standard stock solution, 1ml from Dihydralazine Standard stock solution and 1ml from Propranolol Standard stock solution were taken and volume was makeup to 10ml with Mobile phase to obtain aWorking standard solution containing Hydrochlorothiazide (20μg/mL), Dihydralazine (25μg/mL) and Propranolol (40μg/mL).

The contents of standard and sample solution were then filtered through 0.45 µm syringe filter. Chromatograms standard solution (six replicates) was recorded. A typical chromatogram of Hydrochlorothiazide, Dihydralazine,and Propranololis presented in figure 5. The retention time of Hydrochlorothiazide, Dihydralazine and Propranololwere 3.227 min, 9.097 min,and5.807 min respectively. The peak areas were measured, and the quantitation was carried out by keeping these values to the regression equation of calibration curve.

Optimized Chromatographic Condition:

Stationary phase: Thermo scientific BDS hypersil C_{18} (250mm × 4.6mm, 5µ).

Mobile phase: Potassium dihydrogen orthophosphate (pH 6.0):Acetonitrile (40:60)

Flow rate : 1.0 ml/min

Run time (min) : 11min

Detection: At 215 nm

Injection (volume): 20µl

Method validation procedure

The developed method was validated for the parameters listed in ICH guidelines [6-13]

Linearity

The method was linear in the range of 10-30μg/mL, 12.5-37.5 μg/mL and 20-60μg/mL for Hydrochlorothiazide, Dihydralazine and Propranolol respectively. The linear correlation coefficient for Hydrochlorothiazide, Dihydralazine and Propranolol were found to be 0.9996, 0.9986 and 0.9997 respectively, and are recorded in table 2, 3 and 4. Calibration curve of Hydrochlorothiazide, Dihydralazine and Propranolol was obtained by plotting the peak area ratio versus the respective concentrations (Figure 6, 7 and 8).

Accuracy

The accuracy of the developed method was evaluated by recovery experiments. Working standard with a known concentration was added to the fixed concentration of the pre-analyzed Drop solution. Calculation of percentage recovery was done by comparing the area before and after the addition of

working standard. In the same way, all the3 drugs recovery was performed. The studies of recovery were achieved in triplicate and outcomes are noted in table 5.This standard addition method was performed at the level of 80%, 100%, 120% and the calculation of percent recovery was performed. Percentage recovery was within the range of 99.41 to 100.79 for Hydrochlorothiazide, 99.34 to 100.82 for Dihydralazine and 99.77 to 100.93 for Propranolol which specifies the accuracy of the method.

Precision

For the precision study, repeatability study was carried out for brief time interval under the same chromatographic condition. The sample was injected in six replicates. The peak area for all the six replicates was recorded. The mean and % relative standard deviation (%RSD) was calculated and the results are shown in table 6. The %RSD for Hydrochlorothiazide, Dihydralazine, and Propranolol were found to be 0.64%, 0.94%,and0.87 % respectively. From the obtained data the established RP-HPLC method was found to be accurate. For interday and intraday precision three different concentrations (50%, 100% and 150% of analyte) of standard solutions were injected on thesame day and three consecutive days in three replicates and results were recorded in table 7 & 8.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The standard deviation of response and calibration curve slope were used for calculation of limit of detection and quantification and results are recorded table 9. The LOD for Hydrochlorothiazide, Dihydralazine, and Propranolol was found to be 0.842µg/ml, 1.956µg/ml,and1.282µg/ml respectively. The LOQ is the lowestanalyteconcentration, which gives areply that can be preciselymeasured. The LOQ for Hydrochlorothiazide, Dihydralazine,and Propranolol was 2.551µg/ml, 5.927µg/ml,and3.886µg/ml.

Robustness

Robustness of the method was evaluated by making minorcautious variations in chromatographic conditions such asmobile phase ratio, flow rate, as well as buffer pH and the result was recorded in table 10.It was detected that there were no noticeable deviations in chromatograms and % relative standard deviation was found below 2%, which confirmed that the established RP-HPLC method is robust.

Specificity

The specificity of the proposed method is justified by the chromatograms of blank, placebo, standard and sample solutions under same chromatographic conditions shown in figure 9. The placebos did not interfere in the determination of Hydrochlorothiazide, Dihydralazine, and Propranolol in acommercial tablet. Specificity of the established method was also checked by diverse types of stress conditions (base, acid, oxidation, thermal and photolytic) to Hydrochlorothiazide, Dihydralazine, and Propranolol tablet.

Degradation Study

From the results of forced degradation studies showed that these components do not remain intact under stressed conditions and hence special storage conditions should be provided for the dosage form. The specificity studies showed that the principle peaks were well resolved (peak purity 99.99%) and free from any interference from the degradation product. The stress conditions were applied and degraded products of all three drugs are compared and showed in table 11 and chromatograms are in figure 10. It is concluded from the stress studies that substantial degradation of Hydrochlorothiazide, Dihydralazine, and Propranolol occurred in acid, basic, oxidative thermal and photolytic stress conditions. The degradation products (impurities) in addition to percent degradation under base, acid, thermal, oxidation, and photolytic stresses have unique retention times (RT) to acidic stress (9 impurities, RT: 2.563 min, 2.677 min, 3.310 min, 4.707 min, 5.720 min, 7.377 min, 7.813 min, 9.160 min and 11.263 min), basic stress (9 impurities, RT: 2.500 min, 2.637 min, 3.247 min, 4.627 min, 5.817 min, 6.850 min, 9.037 min, 10.233 min and 12.057 min), oxidative stress (10 impurities, RT: 2.457 min, 2.580 min, 3.180 min, 4.493 min, 5.973 min, 6.920 min, 7.283 min, 8.870 min, 10.573 min and 12.250 min), thermal stress (11 impurities, RT: 2.500 min, 2.633 min, 3.247 min, 4.630 min, 5.220 min, 5.817 min, 6.690 min, 7.787 min, 9.027 min, 10.867 min and 11.690 min) and photolytic stress (8 impurities, RT: 2.490 min, 2.633 min, 3.247 min, 4.267 min, 5.817 min, 7.007 min, 8.053 min and 9.027 min). Degradation studies justified the method specificity for its intended application.

Results and Discussion

For developing a novel RP-HPLC method, numerous compositions of mobile phase were tried.Anacceptable separation and good peak proportion was attained with BDS hypersil C_{18}, 250mm×4.6mm internal diameter, 5µ particle size or equivalent column and mobile phase comprising of Buffer (0.05 M potassium dihydrogen orthophosphate) pH 6.0 : Acetonitrile (40:60v/v) at a 1.0 ml/min flow rate to get improved reproducibility in addition to repeatability. Quantification was

accomplished with UV detection at 215nm based on peak area. The retention time for Hydrochlorothiazide, Dihydralazine and Propranolol were found to be 3.227 min, 9.097 min,and 5.807 min respectively.

According to the ICH guidelines optimized method was authenticated. The parameters of system suitability were detected by using this optimized condition were testified. The method was found to be linear in the concentration range of 10-30 μg/mL with correlation coefficient of 0.9996 for Hydrochlorothiazide, 12.5-37.5 μg/mL with correlation coefficient of 0.9986 for Dihydralazine, and 20-60 μg/mL with correlation coefficient of 0.9997 for Propranolol. The results of recovery study (99.42% for Hydrochlorothiazide, 99.34% for Dihydralazine and 99.77% for Propranolol) suggest that the method has good recovery. The accuracy of the projected method was carried in terms of the repeatability. The low% RSD (<2) values of 0.58%, 0.44% and 0.87% variation for Hydrochlorothiazide, Dihydralazine and Propranolol, respectively, reveals that the proposed method is precise. The LOD and LOQ values for Hydrochlorothiazide were found to be 0.842µg/ml and 2.551µg/ml, for Dihydralazinewere 1.956µg/ml and 5.927µg/ml and for Propranolol were 1.282µg/ml and 3.886µg/ml. The outcomes of robustness in the current method exhibited no significant variations. The outcomes of drop analysis specified that no interference due to common excipients was detected with the developed method. Degradation studies justified the method specificity for its intended application. Consequently, the projected method can be used for routine analysis of three drugs as a combination in pharmaceutical dosage form.

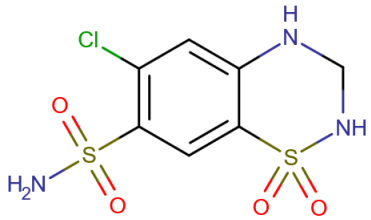


FIGURE 1: Structure of Hydrochlorothiazide

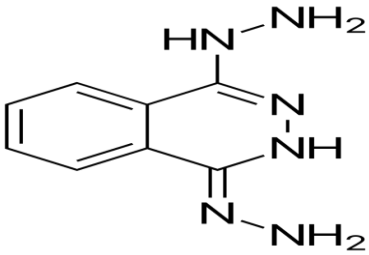


FIGURE 2: Structure of Dihydralazine

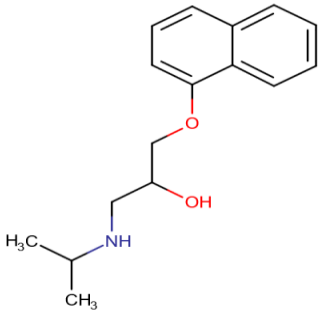


FIGURE 3: Structure of Propranolol

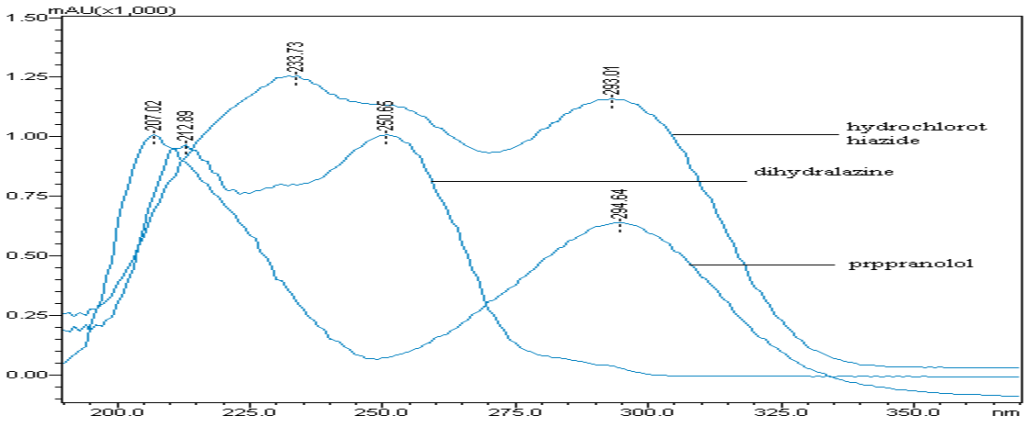


FIGURE 4: Overlay absorption spectrum for Hydrochlorothiazide, Dihydralazine and Propranolol

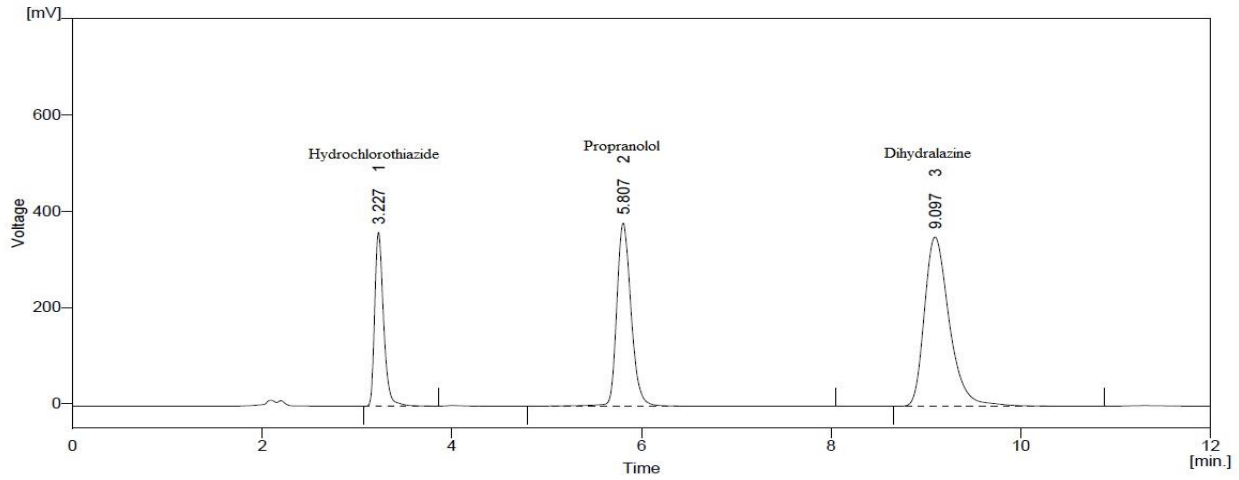


FIGURE 5: Standard Chromatograms of Hydrochlorothiazide, Dihydralazine and Propranolol.

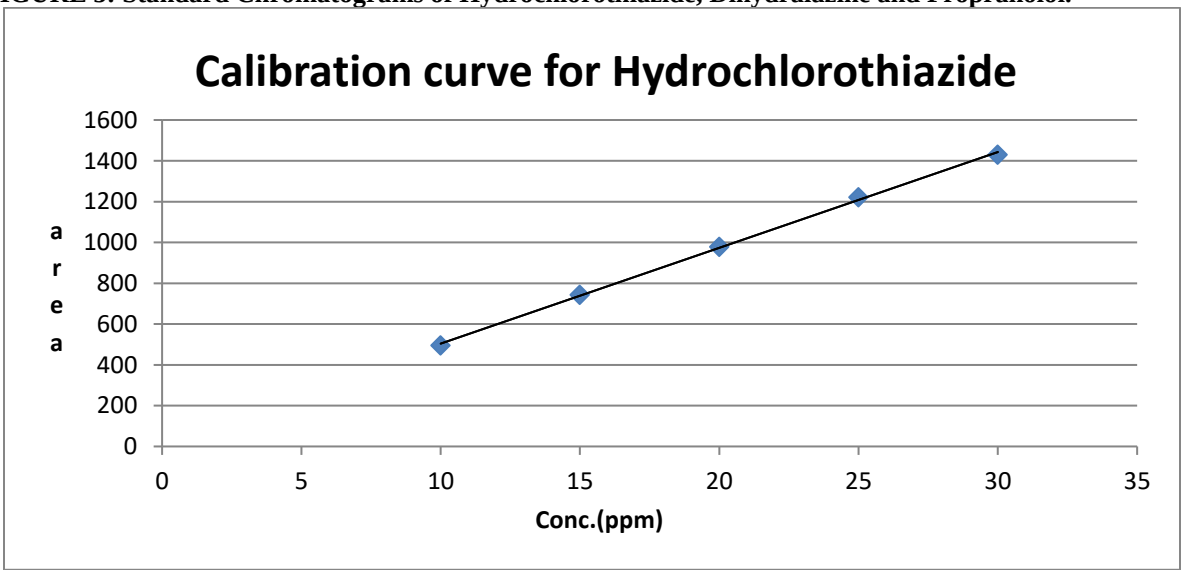


FIGURE 6: Calibration curve of Hydrochlorothiazide

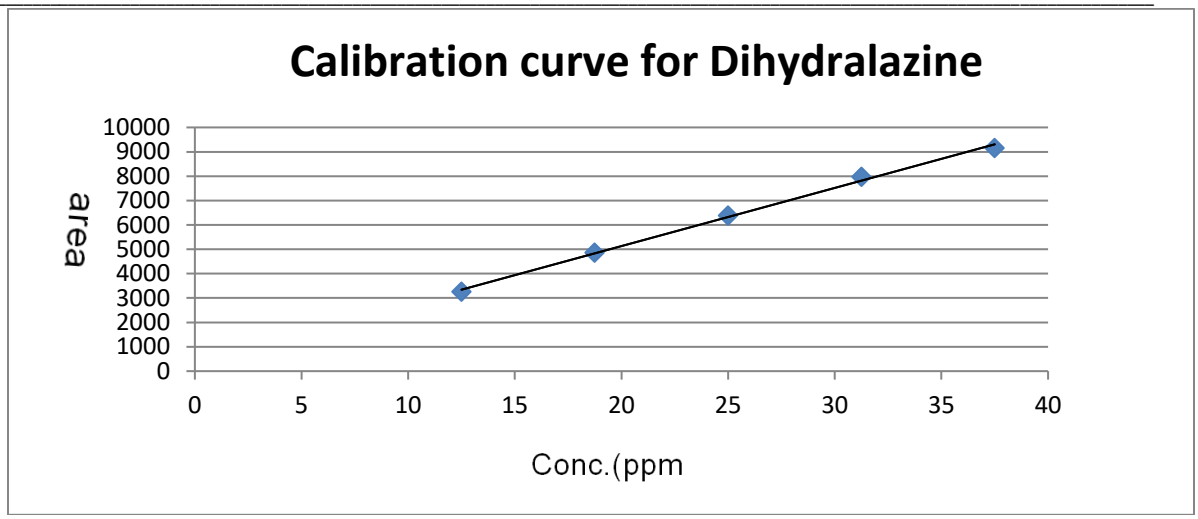


FIGURE 7: Calibration curve of Dihydralazine

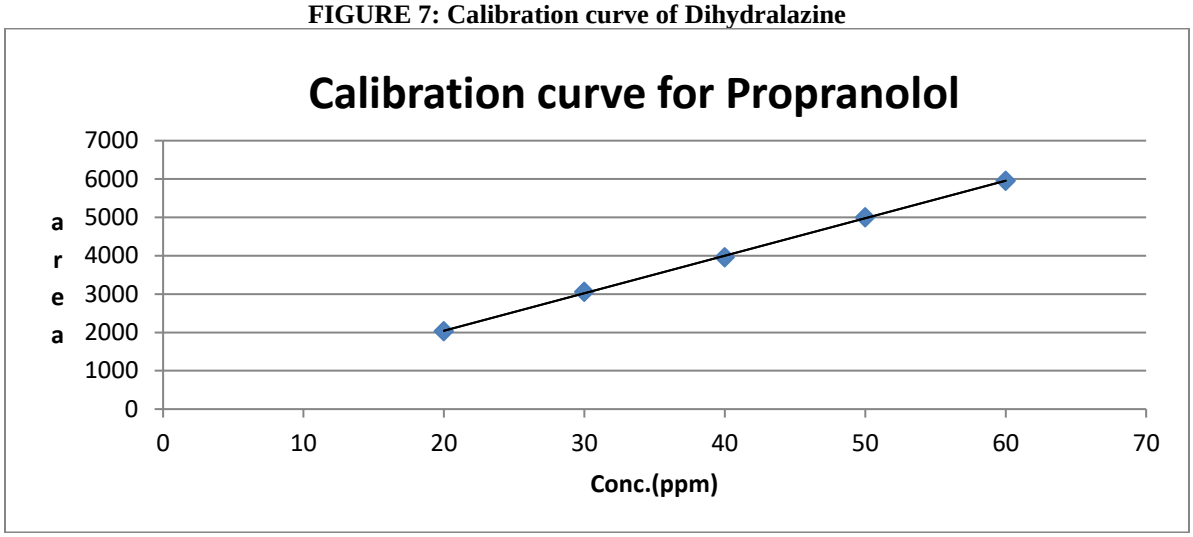
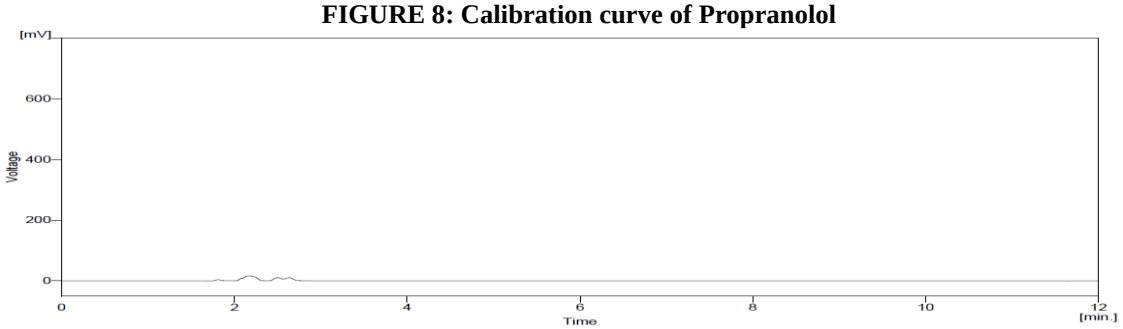


FIGURE 8: Calibration curve of Propranolol

(a)



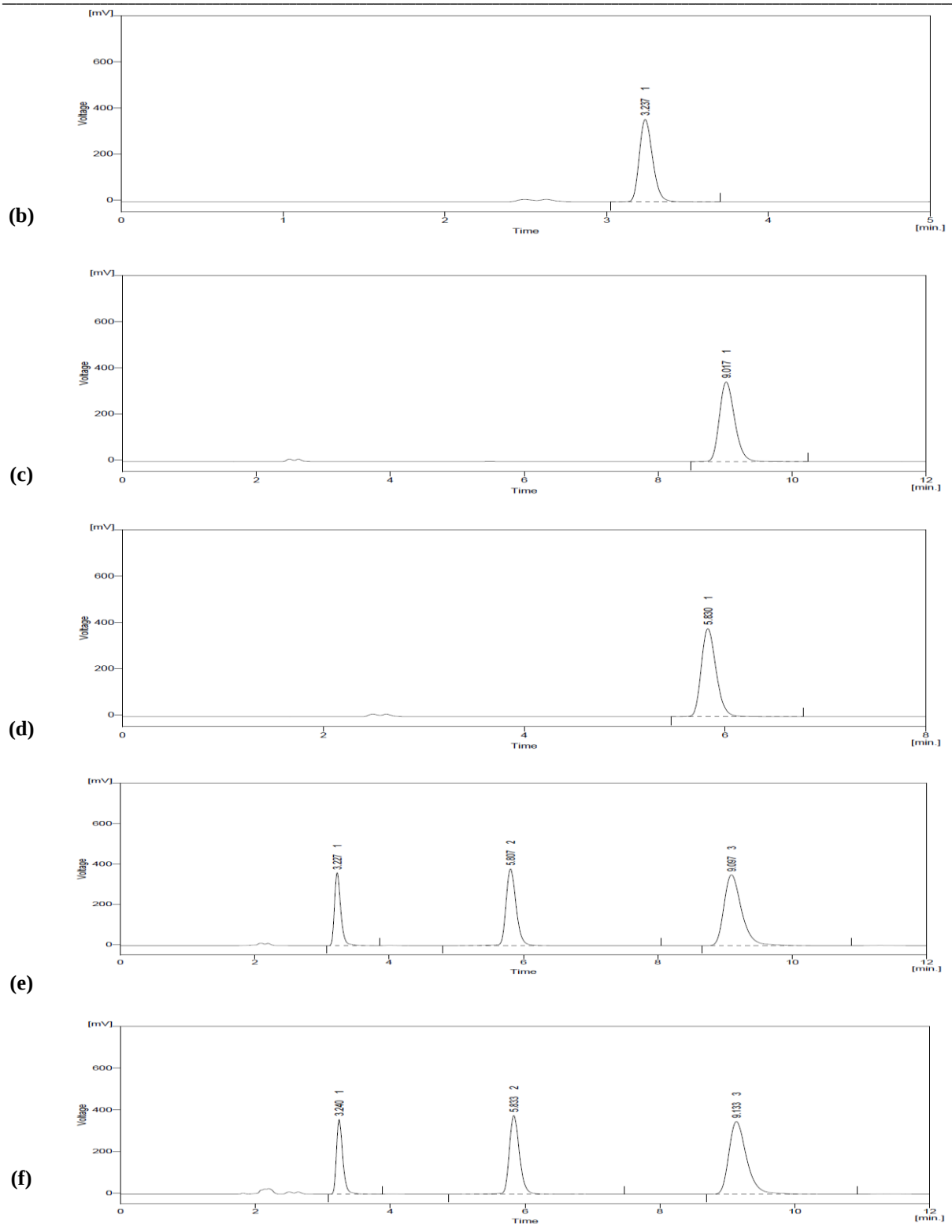


FIGURE 9: Chromatograms of (a) Blank, (b) Hydrochlorothiazide, (c) Dihydralazine,(d) Propranolol, (e) Standard mixture and (f) Sample mixture.

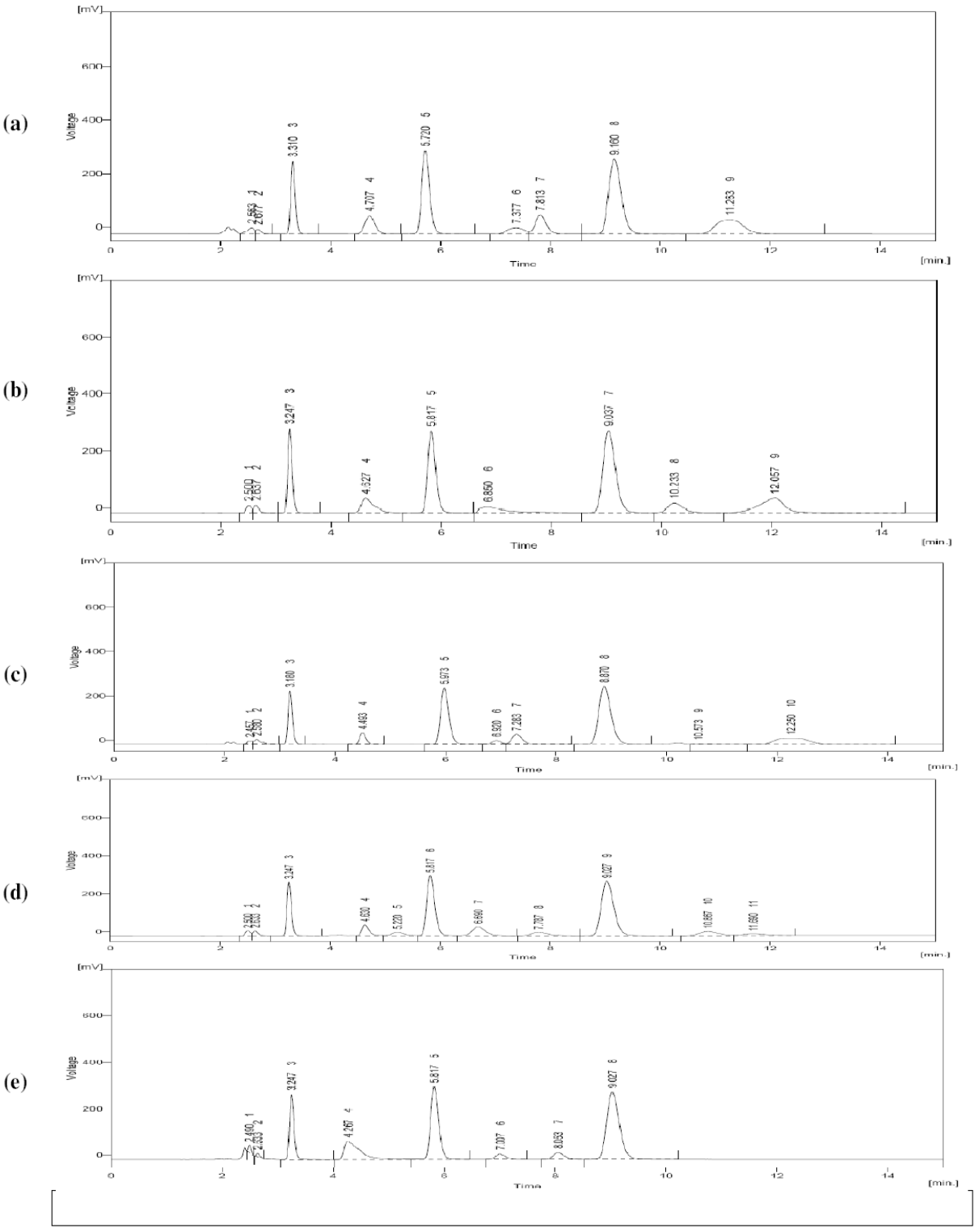


FIGURE10: Chromatograms of (a) Acid Degradation, (b) Base Degradation (c) Oxidative Degradation, (d) Thermal Degradation, (e) Photolytic Degradation

TABLE 1: System suitability of proposed method

Parameters	Hydrochlorothiazide	Propranolol	Dihydralazine
Theoretical plates	5768	7297	5989
Resolution	-	11.678	8.867
Asymmetry	1.478	1.368	1.524
Retention time	3.227 min	5.807 min	9.097 min

TABLE 2: Linearity results of Hydrochlorothiazide

Linearity Level	Concentration	Area
I	10 µg/ml	495.645
II	15 µg/ml	742.735
III	20 µg/ml	977.866
IV	25 µg/ml	1220.885
V	30 µg/ml	1430.128
Correlation coefficient		0.9996

TABLE 3: Linearity of Dihydralazine

Linearity Level	Concentration	Area
I	12.5µg/ml	3255.054
II	18.75 µg/ml	4860.674
III	25 µg/ml	6375.283
IV	31.25 µg/ml	7975.126
V	37.5 µg/ml	9153.15
Correlation coefficient		0.9986

TABLE 4: Linearity of Propranolol

Linearity Level	Concentration	Area
I	20 µg/ml	2031.625
II	30 µg/ml	3059.895
III	40 µg/ml	3953.914
IV	50 µg/ml	5002.322
V	60 µg/ml	5950.533
Correlation coefficient		0.9997

TABLE 5: Results of Accuracy

Sample	Accuracy	Standard Drug (µg/ml)	Sample Drug (µg/ml)	% of recovery	S.D.	% RSD
Hydrochlorothiazide	80%	8	10	100.79	0.372	0.369
	100%	10	10	99.42	0.581	0.585
	120%	12	10	99.92	0.964	0.965

Dihydralazine	80%	20	20	100.82	0.956	0.948
	100%	25	20	99.34	0.437	0.439
	120%	30	20	99.59	0.533	0.536
Propranolol	80%	16	20	100.93	0.706	0.699
	100%	20	20	99.77	0.872	0.874
	120%	24	20	100.36	0.979	0.976

TABLE 6: Results of Precision

Injection	Area of Hydrochlorothiazide	Area of Dihydralazine	Area of Propranolol
Injection 1	2315.318	6343.359	3960.402
Injection 2	2336.179	6346.676	3996.157
Injection 3	2329.078	6381.17	3955.152
Injection 4	2338.505	6432.34	4016.157
Injection 5	2317.699	6350.251	3928.912
Injection 6	2299.173	6250.539	3933.387
Average	2322.659	6350.723	3965.028
S.D.	14.87	59.49	34.66
% RSD	0.64	0.94	0.87

TABLE 7: Result of Interday Precision

Conc. (µg/ml)			Area			% RSD		
Hydrochlorothiazide	**Dihydralazine**	**Propranolol**	**Hydrochlorothiazide**	**Dihydralazine**	**Propranolol**	**Hydrochlorothiazide**	**Dihydralazine**	**Propranolol**
10	12.5	20	1154.135	3157.043	1971.538	1.11	1.35	1.12
20	25	40	2287.448	6270.128	3926.208	1.19	1.32	0.89
30	37.5	60	3495.995	9557.916	5946.82	0.87	1.18	1.39

TABLE 8: Result of Intraday Precision

Conc. (µg/ml)			Area			% RSD		
Hydrochlorothiazide	**Dihydralazine**	**Propranolol**	**Hydrochlorothiazide**	**Dihydralazine**	**Propranolol**	**Hydrochlorothiazide**	**Dihydralazine**	**Propranolol**
10	12.5	20	1156.495	3149.166	1972.940	0.46	1.07	0.86
20	25	40	2307.016	6303.459	3948.564	0.51	0.64	0.53
30	37.5	60	3481.917	9549.102	5936.898	0.32	0.42	0.66

TABLE 9: Results of LOD and LOQ

Parameter	Hydrochlorothiazide (µg/ml)	Dihydralazine(µg/ml)	Propranolol (µg/ml)
LOD	0.842	1.956	1.282
LOQ	2.551	5.927	3.886

TABLE 10: Results of Robustness

Condition	Variation	Average Area			% RSD		
		Hydrochlorothiazide	Dihydralazine	Propranolol	Hydrochlorothiazide	Dihydralazine	Propranolol
Flow rate	0.8 min	2515.472	6839.027	4290.518	0.46	0.58	0.72
	1.2 min	2179.172	5952.714	3717.522	0.45	0.48	0.91
Mobile phase	Buffer: Acetonitrile 42:58	2270.496	6212.749	3874.363	0.41	0.59	0.62
	Buffer: Acetonitrile 38:62	2411.253	6577.917	4147.491	0.54	1.57	0.18
pH	6.2	2260.816	6168.885	3854.81	0.29	0.89	0.27
	5.8	2387.002	6490.618	4071.495	0.64	0.98	1.12

TABLE 11: Stability study results

Type of degradation	Drug	Peak Area of Standard	Conditions	Peak area			
				Standard		Sample	
				Area	% Deg.	Area	% Deg.
Acid degradation	Hydrochlorothiazide	2343.368	4 hours at Room Temperature	1558.557	33.49	1591.252	32.10
	Dihydralazine	6420.129		4575.326	28.73	4504.732	29.83
	Propranolol	4008.286		3254.047	18.82	3130.804	21.89
Base degradation	Hydrochlorothiazide	2343.368	3.5 hours at Room Temperature	1657.651	29.26	1688.807	27.93
	Dihydralazine	6420.129		4431.61	30.97	4583.77	28.60
	Propranolol	4008.286		2970.3	25.90	2947.021	26.48
Oxidat	Hydrochlorothiazi	2343.368	4 hours at	1472.438	37.17	1341.413	42.76

ive degradation	de		Room Temperature				
	Dihydralazine	6420.129		4151.979	35.33	4068.656	36.63
	Propranolol	4008.286		2737.088	31.71	2664.427	33.53
Thermal degradation	Hydrochlorothiazide	2343.368	3 hours at 105^{0}C	1612.177	31.20	1623.205	30.73
	Dihydralazine	6420.129		4629.325	27.89	4562.647	28.93
	Propranolol	4008.286		3202.095	20.11	3229.27	19.43
Photolytic degradation	Hydrochlorothiazide	2343.368	3 hours in direct Sun light	1637.398	30.13	1619.625	30.88
	Dihydralazine	6420.129		4595.188	28.42	4630.243	27.88
	Propranolol	4008.286		3171.32	20.88	3212.114	19.86

Conclusion

A simple, precise, accurate and rapid method was established for instantaneous valuation of Hydrochlorothiazide, Dihydralazine, and Propranolol from pure and its dosage forms. The mobile phase is easy to prepare and cost-effective. The recoveries of the sample in the preparation were in good arrangement with their particular label claims. Henceforth, this method can be effortlessly and suitably accepted for routine analysis of Hydrochlorothiazide, Dihydralazine, and Propranolol in pure form and its dosage form.

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