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SPECTROPHOTOMETRIC DETERMINATION OF IRBESARTAN, LOSARTAN, ATENOLOL AND HYDROCHLOROTHIAZIDE IN BULK AND DOSAGE FORMS

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ABSTRACT

Two simple and sensitive spectrophotometric methods are described for determination of irbesartan, losartan, hydrochorothiazide and atenolol in bulk and tablet forms. Method (I) depends on formation of the colored chromogen by condensation reaction between irbesartan, losartan and atenolol and vanillin in acidic conditions and the product was measured at λ_{max} 546,552 and 560 nm for irbesartan, losartan and atenolol, respectively. Under the indicated conditions, this method was linear over the concentration range of 40-240 µg/ml, 80-240and 40-200 µg/ml for irbesartan, losartan and atenolol, respectively. In method (II), 1, 2-Naphthoquinone-4-sulphonate sodium reacts with irbesartan, losartan and hydrochlorothiazide through nucleophilic substitution reaction producing orange colored product in alkaline medium showing maximum absorption at λ_{max} 465 nm for irbesartan, losartan and hydrochlorothiazide where the method was linear over the concentration range of 1-6 µg/ml, 0.2-1 µg/ml and 0.25-1.25 µg/ml for irbesartan, losartan and hydrochlorothiazide, respectively. The methods were statistically applied for the determination of drugs in both bulk and tablet forms. Results were compared with reference methods and no statistically difference was obtained.

KEYWORDS

Irbesartan, Losartan, Atenolol, Hydrochlorothiazide, Vanillin and 1, 2-Naphthoquinone-4-sulphonate sodium.

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INTRODUCTION

Irbesartan and losartan chemically are2-butyl-3-((2′-[1H-tetrazol-5-yl]biphenyl) 4-yl methyl)-1, 3-diazaspiro [4, 4] non-1-en-4-one[1] and potassium 5-(4′-((2-butyl-4-chloro-5(hydroxyl methyl)-1H-imidazol-1-yl)methyl)biphenyl-2-yl)tetrazol-1-ide,respectively[2]. They are angiotensin II receptor antagonists that affect rennin angiotensin system and used in treatment of hypertension[3,4]. Several

methods have been developed for determination of both drugs including UV spectrophorometric method[5,6], spectrofluorimetry7, high performance liquid chromatography (HPLC)[8,9], microemulsion liquid chromatography[10] and charge-transfer complexes of losartan potassium[11].

On the other hand, atenolol chemically is 2-[4-[(2RS)-2-hydroxy-3-[(1-methylethyl) amino] propoxy]phenyl acetamide[12], adrenergic beta blocker and used as antihypertensive agent[13], several methods have been developed for determination of atenolol, including UV spectrophotometric methods[14,15], high performance liquid chromatography (HPLC)[16,17], novel FT-IR spectroscopy[18], and gas chromatography-mass spectrometry[19]. Another drug, hydrochlorothiazide which is chemically6-Chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide1,1dioxide[20], is used for the congestive heart failure and chronic renal failure by reducing sodium (Na) reabsorption in the distal convulated tubule[21], several methods have been developed for its determination including UV spectrophotometric methods[22,23], reversed phase high performance liquid chromatography (RP-HPLC)[24,25], TLC and HPLC with on-line wavelength switching methods[26], diffuse reflectance spectroscopy[27], continuous wavelet transformation[28], and multivariate calibration methods[29].

Vanillin has been utilized as a chromogenic reagent for the spectrophotometric determination of a few compounds of pharmaceutical interest such as metoclopramide[30], zolmitriptan[31], metronidazole[32]. Also 1, 2-Naphthoquinone-4-sulphonate sodium (NQS) has been used asa chromogenic reagent for the spectrophotometric determination of many pharmaceutical drugs containing either primary or secondary amines such as aminophylline[33], paroxetine[34], fluvoxamine[35], and amantadine[36]. The aim of this work was to develop a method that could be used for the determination of irbesartan, losartan, atenolol and hydrochlorothiazide in bulk and tablet dosage forms by condensation reaction between these drugs and two reagents. These reactions give colored product that can be measured at different wavelengths.

MATERIAL AND METHOD

Apparatus

Labomed® *Spectro UV-VIS Double Beam (UVD-2950)* Spectrophotometer with matched 1cm quartz cells connected to Windows compatible computer using UV Win 5Software v5.0.5.

Material and Reagents

All chemicals used are of analytical reagent grade**.**

-Irbesartan was provided by Sigma Company, Egypt. Kansartan® tablets, labeled to contain 150 mg of irbesartan, batch No. 25368 (Chemipharm, Egypt).

- Losartan was provided by Eipico Company, Egypt. Cozaar® tablets, labeled to contain 50 mg of losartan, batch No.19460 (Merk sharp Dohme).

-Atenolol was provided by Sigma Company, Egypt. Ateno® tablets, labeled to contain 50mg of atenolol, batch No.1504784 (Eipico, Egypt).

-Hydrochlorothiazide was provided by Global Nabi Company, Egypt. Hydretic® tablets, labeled to contain 12.5 mg of hydrochlorothiazide, batch No.HE56263 (Eipico, Egypt).

-Vanillin was purchased from EL Nasr Pharmaceutical Chemicals Company, batch No. FVA-1332911:2011/1).

-The solution was prepared by dissolving 4gm.of vanillin 100 ml absolute methanol (99.8%).

-Sulphuric acid was purchased from El Nasr Pharmaceutical Chemicals Company, batch No.2354117:2012/3).

-1, 2-Naphthoquinone-4-sulphonate (NQS)0.3 %(w/v) was purchased from Fisher Scientific U.K. Limited, U.K. 0.3 gm. of NQS was accurately weighed transferred into a 100 ml calibrated flask, dissolved in 20 ml distilled water, and make up the volume up to the mark with bidi stilled water to obtain a solution of0.3 % (w/v). The solution was freshly prepared and protected from light during the use.

-Sodium hydroxide was provided by Elgomhouria Chemicals Company, batch No.1001/120). 9×10^{-2} M of sodium hydroxide was prepared in 100 ml

volumetric flask and made up to the mark with distilled water.

GENERAL PROCEDURES

Preparation of standard drug solutions for methods I

Stock solutions of Irbesartan, Losartan and Atenolol were prepared by dissolving 200,200 and 100 mg of pure drugs, respectively in 100 ml in volumetric flask in methanol.

Preparation of standard drug solutions for methods II

Stock solutions of Irbesartan, Losartan and Hydrochlorothiazide were prepared by dissolving 20, 20 and 50 mg of pure drugs, respectively in 100 ml in volumetric flask with methanol. Working solution of losartan and hydrochlorothiazide (0.02 mg/ml and 0.025 mg/ml) were prepared by further dilution of stock solution with methanol.

PROCEDURES

Method I (vanillin)

To a series of 10 ml calibrated flasks, an increasing volume covering the concentration range (40-240) µg/ml of irbesartan solution,(80-240)µg/ml of losartan and (40-200)µg/ml of atenolol were transferred, followed by addition of 1.2ml of 4% vanillin in case of (irbesartan and atenolol), 1.4ml in case of (losartan) and 2.5ml of H_2SO_4 in case of irbesartan, 3ml in case of losartan and 2ml in case of atenolol with occasional shaking and heated on a water bath at 60°C in case of (irbesartan and losartan) and at room temperature in case of atenolol for 25,20 and 5 minutes for irbesartan, losartan and atenolol, respectively and cooled to room temperature, finally the volume was brought up to mark with absolute methanol and the resulting solutions were measured at λmax 546,552 and 560 nm for irbesartan, losartan and atenolol, respectively against the blank, Figure No.2.

Method II (1, 2-Naphthoquinone-4-sulphonate)

Accurately measured aliquots of standard solutions containing 1-6 µg/ml of irbesartan, 0.2-1 µg/ml of losartan and 0.25-1.25µg/ml of hydrochlorothiazide were transferred into a series of 10ml volumetric flasks. In case of irbesartan to each flask, 1.2ml of 0.09 M NaOH and 1.4ml of NQS were added and mixed well, in case of losartan to each flask, 1.2ml of 0.09 M NaOH and 1ml of NQS were added and mixed well and in case of hydrochlorothiazide to each flask, 1ml of 0.09 M NaOH and 1ml of NQS were added and mixed well. The reaction solutions were allowed to proceed at room temperature for 10 minutes in case of (irbesartan, losartan) and 5 minutes in case of hydrochlorothiazide, finally the volume was brought up to mark with bidistilled water. The resulting solutions were measured at λmax 465 nm for the three drugs against the blank as depicted in Figure No.3.

PHARMACEUTICAL PREPARATION

For Kansartan tablets

Twenty tablets of Kansartan tablets were weighed and finely powdered. An accurately amounts of the powder equivalent to the concentration of irbesartan in method I and II were extracted with 10ml methanol three times. The filtrates were collected and transferred to 100 ml volumetric flasks and completed to the mark with methanol. Aliquots from these solutions equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

For Cozaar tablets

Twenty tablets of Cozaar tablets were weighed and finely powdered. An accurately amounts of the powder equivalent to the concentration of losartanin method I and II were extracted with 10ml methanol three times. The filtrates were collected and transferred to 100 ml volumetric flasks and completed to the mark with methanol. Aliquots from these solutions equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

For Ateno tablets

Twenty tablets of Ateno tablets were weighed and finely powdered. An accurately amounts of the powder equivalent to the concentration of atenolol in method I were extracted with 10ml methanol

three times. The filtrates were collected and transferred to 100 ml volumetric flasks and completed to the mark with methanol. Aliquots from these solutions equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

For Hydretic tablets

Twenty tablets of Hydretic tablets were weighed and finely powdered. An accurately amounts of the powder equivalent to the concentration of hydrochlorothiazide in method II were extracted with 10ml methanol three times. The filtrates were collected and transferred to 100 ml volumetric flasks and completed to the mark with methanol. Aliquots from these solutions equivalent to those in authentic samples were used for the application of the proposed methods applying standard addition techniques.

RESULTS AND DISCUSSION

Method I

Enamines are formed by a condensation reaction of secondary amine and an aldehyde or ketone in the presence of an acid catalyst, Figure No.6 is an example of the possible reaction between irbesartan or losartan and vanillin. Another supplementary Figure No.1 is showing a proposed condensation reaction between atenolol and vanillin. The proposed method is based on the formation of chromogenic enamine between the secondary amine group of the three drugs and aldehyde group of vanillin. The colored enamines exhibits λmax at 546nm or 552nm or 560nm for irbesartan, losartan and atenolol, respectively. The reagent blank showed a negligible absorbance at these wavelengths.

Factors for method I (vanillin)

Effect of the reagent concentration

The concentration of reagent was studied.1.2ml of 4%*w/v* vanillin solution was used as optimum concentration incase of irbesartan and atenolol and 1.4ml in case of losartan as shown in Figure No.8.

Effect of acid volume

Concentrated sulphuric acid was used because the reaction was very slow in dilute acid medium. Best results were obtained on using2.5 ml, 3 ml and 2 ml of acid in case of irbesartan, losartan and atenolol, respectively, Figure No.9.

Effect of time

The effect of time was studied by following the colour developments at room temperature incase of atenolol for 5 min. It was found that maximum absorption was obtained at25 and 20 minutes for irbesartan and losartan, respectively after heating in a water bath at (60°C) as depicted in Figure No.10.

Stiochiometry of the reaction

The molar ratio of the reagent and the three dugs in the reaction was studied. The molar ratio was found to be 1:1 (drug: reagent) as seen in Figure No.11.

Method II

The NQS reagent reacts with Irbesartan, Losartan and Hydrochlorothiazide contain of secondary amino group by nucleophilic substitution of the sulfonic acid group of 1, 2-Naphthoquinone-4-sulfonic acid in alkaline medium, forming an orange colored product exhibiting λmax at 465 nm for the three drugs is shown in Figure No.7. Another supplementary Figure No.2 is showing a proposed condensation reaction between hydrochlorothiazide and NQS.

Factors for method II (NQS)

Effect of the reagent concentration

The concentration of reagent was studied .1.4 ml of 0.3%*w/v* NQ Solution was used as optimum concentration in case of irbesartan and 1 ml in case of losartan and atenolol as shown in Figure No.12.

Effect of alkalinity

Alkaline medium was necessary to activate substitution reaction. Different bases were examined such as sodium hydroxide and potassium hydroxide to obtain high sensitivity. Best results were obtained on using 1.2 ml in case of (irbesartan, losartan) and 1 ml in case of atenolol of 0.09 M NaOH as depicted in Figure No.13.

Effects of temperature and reaction time

Carrying out the reaction at room temperature and at varying elevated temperature. The results

revealed that the absorbance reached maximum after leaving the solution 10 min in case of (irbesartan, losartan) and 5 min in case of (losartan) at room temperature, Figure No.14.

Stiochiometry of the reaction

The molar ratio of the reagent and the three dugs in the reaction was studied. The molar ratio was found to be 1:1 (drug: reagent) in case of (irbesartan, losartan) and 1:2 (drug: reagent) in case of (hydrochlorothiazide) as depicted in Figure No.15.

Method validation

The validity of the proposed methods was tested regarding linearity, range, limits of detection, limits of quantification, accuracy, precision, robustness and specificity according to ICH recommendations [37].

Linearity and range

The calibration graphs obtained by plotting the values of the absorbance versus the final concentrations (µg/ml) were found to be rectilinear over the concentration ranges cited in the Table No.1, Figures No.4 and 5.

The calibration graph is described by the equation Y=a+bX

(Where Y= absorbance, a=intercept, b=slope and X =concentration in $\mu g.ml^{-1}$).

Correlation coefficient, intercept and slope for the calibration data are summarized in Table No.1.

LIMITS OF DETECTION AND LIMITS OF QUANTIFICATION

The limit of detection (LOD) was determined by evaluating the lowest concentrations of the analyte that can be detected according to the following equation: LOD = 3.3 S /K

The limit of quantification (LOQ) was determined by establishing the lowest concentrations that can be quantified according to the following equation:$LOQ = 10\,S/K$

Where S is the standard deviation of the three replicate determination values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely, the slope of calibration graph. The results are summarized in Table No.2 and 3.

Accuracy and precision

Accuracy was evaluated as percentage relative error between the measured concentration for irbesartan, losartan, atenolol and hydrochlorothiazide. The accuracy of the proposed methods was checked by performing recovery experiments through standard addition technique and results are shown in Table No.4 and 5.

The precision of the method was calculated in term of intermediate precision (intraday and interday). Three different concentrations five times of drugs were analyzed during same day (intra-day precision) and each five days (inter-day precision). The standard analytical errors, relative standard deviations (RSD) and recoveries obtained by the proposed method were found to be acceptable. The results are summarized in Table No.6.

Robustness

Method robustness was tested by making small changes in method variables such as change reagent volume (±0.05 ml), change in acid volume (±0.05 ml), change in base volume (±0.05 ml) and reaction time (±2 min). The results are listed in Table No.7.

Analysis of proposed and reference methods of irbesartan, losartan, atenolol and hydrochlorothiazide

The proposed methods were applied to the analysis of the drugs and the results were statistically compared with reference methods[38-41] for irbesartan, losartan, atenolol and hydrochlorothiazide respectively, by calculating Student's t-and F-values. The evaluated t-and F-values were less than the tabulated values at the 95 % confidence level and the results listed in Table No.8 are showing that there is no statistical significance between the proposed and reference methods.

Table No.1: Analytical parameters for the determination of irbesartan, losartan, atenolol and hydrochlorothiazide using vanillin and NQS as reagents

Parameters	Vanillin (Method I)			NQS (Method II)		
	Irbesartan	Losartan	Atenolol	Irbesartan	Losartan	Hydrochlorothiazide
λmax, nm	546 nm	552 nm	560 nm	465 nm	465 nm	465 nm
Volume of conc H_2SO_4, ml	2.5 ml	3 ml	2 ml	---	---	---
Volume of 0.09 M NaOH, ml	---	---	---	1.2 ml	1.2 ml	1 ml
Volume of vanillin(4g%), ml	1.2 ml	1.4 ml	1.2 ml	---	---	---
Volume of NQS(0.3 g%), ml	---	---	---	1.4 ml	1 ml	1 ml
Time	25 min	20 min	5 min	10 min	10 min	5 min
Temperature	(60°C)	(60°C)	(25°C)	(25°C)	(25°C)	(25°C)
Beer's law limits, µg/ml	40-240 µg/ml	80-240 µg/ml	40-200 µg/ml	1-6 µg/ml	02-1 µg/ml	0.25-1.25 µg/ml
Regression equation*	y=0.0048x +0.0356	y=0.0038x -0.0996	y=0.002x -0.011	y=0.140x +0.156	y=0.464x +0.036	y=0.6152x +0.021
Slope(b)	0.0048	0.0038	0.002	0.140	0.464	0.6152
Intercept(a)	0.0356	0.0996	0.011	0.156	0.0306	0.021
Correlation Coefficient	0.9999	0.999	0.999	0.9998	0.9998	0.9998

Table No.2: Results of the analysis for determination of irbesartan, losartan and atenolol using vanillin method

Vanillin									
Parameters	Irbesartan			Losartan			Atenolol		
	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %	Taken µg/ml	Found µg/ml	Recovery %
	40	39.45	98.64	80	79.10	98.88	40	39.31	98.27
	80	80.91	101.14	120	120.42	100.35	80	80.68	100.86
	120	120.91	100.76	160	160.94	100.59	120	121.03	100.86
	160	159.25	99.53	200	199.63	99.81	160	160.34	100.21
	200	200.70	100.35	240	239.36	99.73	200	200.68	100.34
	240	240.5	100.208	-	-	-	-	-	-
Mean recovery*	-	-	100.108	-	-	99.875	-	-	100.112
SD	-	-	0.899	-	-	0.661	-	-	1.067
RSD	-	-	0.898	-	-	0.662	-	-	1.066
SE	-	-	0.367	-	-	0.295	-	-	0.477
Variance	-	-	1.009	-	-	0.437	-	-	1.140
Slope	-	-	0.0048	-	-	0.0037	-	-	0.0029
LOD.	-	-	11.757	-	-	23.887	-	-	11.979
LOQ.	-	-	39.191	-	-	79.623	-	-	39.930
Sandell's Sensitivity(S.S)***	-	-	0.068	-	-	0.117	-	-	0.205
Apparent** Molar absorptivity $L.Mol^{-1}.cm^{-1}$	-	-	2214.9	-	-	1299.673	-	-	739.7926

* Average of three different experiments.

**Calculated on the basis of molecular weight of the drug

*** S.S: Sandell's sensitivity (µg/ml per 0.001 M).

Table No.3: Results of the analysis for determination of irbesartan, losartan and hydrochlorothiazide using NQS method

NQS									
Parameters	**Irbesartan**			**Losartan**			**Hydrochlorothiazide**		
	Taken µg/ml	**Found µg/ml**	**Recovery %**	**Taken µg/ml**	**Found µg/ml**	**Recovery %**	**Taken µg/ml**	**Found µg/ml**	**Recovery %**
	1	0.992	99.285	0.2	0.202	101.293	0.25	0.255	102.080
	2	2	100	0.4	0.396	99.137	0.5	0.492	98.504
	3	3.028	100.952	0.6	0.596	99.497	0.75	0.747	99.696
	4	4.064	101.607	0.8	0.806	100.754	1	1.006	100.617
	5	5.028	100.571	1	0.997	99.784	1.25	1.248	99.869
	6	5.992	99.880	-	-	-	-	-	-
Mean recovery*	-	-	100.382	-	-	100.093	-	-	100.153
SD	-	-	0.831	-	-	0.8999	-	-	1.316
RSD	-	-	0.828	-	-	0.899	-	-	1.314
SE	-	-	0.3397	-	-	0.4025	-	-	0.588
Variance	-	-	0.6922	-	-	0.8099	--	-	1.734
Slope	-	-	0.14048	-	-	0.464	-	-	0.615
LOD.	-	-	0.248	-	-	0.059	-	-	0.063
LOQ.	-	-	0.829	-	-	0.199	-	-	0.212
S.S.***	-	-	0.00297	-	-	0.0011	-	-	0.0009
Apparent Molar absorbitivity $L.Mol^{-1}.cm^{-1}$**	-	-	87521.1	-	-	231176.7	-	-	280475.1

* Average of three different experiments

**Calculated on the basis of molecular weight of the drug

*** S.S: Sandell's sensitivity (µg/ml per 0.001 M)

Table No.4: Application of standard addition technique for the determination of Kansartan®, Cozaar® and Ateno® tablets form through reaction with vanillin

Items	Kansartan ®tablets				Cozaar ®tablets				Ateno ®tablets			
	Added pure drug (µg/ml)	**Taken kansartan tablet (µg/ml)**	**Conc. found (µg/ml)**	**Recovery %**	**Added pure drug (µg/ml)**	**Taken Cozaar tablet (µg/ml)**	**Conc. found (µg/ml)**	**Recovery %**	**Added pure drug (µg/ml)**	**Taken Ateno tablet (µg/ml)**	**Conc. found (µg/ml)**	**Recovery %**
	80	0	80.125	100.156	120	0	119.105	99.254	80	0	78.8	98.5
	80	40	118.666	98.888	120	40	160.157	100.098	80	40	122.4	102
	80	80	160.333	100.208	120	80	198.578	99.289	80	80	162.4	101.5
	80	120	199.708	99.854	120	120	239.631	99.846	80	120	198.8	99.4
	80	160	239.083	99.618	120	160	278.578	99.492	80	160	241.6	100.66
Mean*	-	-	-	99.745	-	-	-	99.596	-	-	-	100.413
SD	-	-	-	0.534	-	-	-	0.366	-	-	-	1.453
RSD	-	-	-	0.536	-	-	-	0.367	-	-	-	1.447
SE	-	-	-	0.239	-	-	-	0.163	-	-	-	0.650
Variance	-	-	-	0.286	-	-	-	0.134	-	-	-	2.112

*mean of three different experiments.

Table No.5: Application of standard addition technique for the determination of Kansartan®, Cozaar® and Hydretic®tablets form through reaction with NQS

Items	Kansartan ® tablets				Cozaar ®tablets				Hydretic ® tablets			
	Added pure drug (µg/ml)	Taken kansartan tablet (µg/ml)	Conc. found (µg/ml)	Recovery %	Added pure drug (µg/ml)	Taken Cozaar tablet (µg/ml)	Conc. found (µg/ml)	Recovery %	Added pure drug (µg/ml)	Taken Ateno tablet (µg/ml)	Conc. found (µg/ml)	Recovery %
	2	0	2	100	0.4	0	0.398	99.526	0.5	0	0.5	100
	2	1	2.992	99.763	0.4	0.2	0.606	101.065	0.5	0..25	0.742	99.061
	2	2	4.049	101.241	0.4	0.4	0.805	100.650	0.5	0.5	1.019	101.936
	20	3	4.921	98.439	0.4	0.6	0.977	97.798	0.5	0.75	1.25	100
	2	4	6.035	100.591	0.4	0.8	1.212	101.025	0.5	1	1.498	99.882
Mean*	-	-	-	100.007	-	-	-	100.013	-	-	--	100.176
SD	-	-	-	1.0464	-	-	-	1.385	-	-	-	1.059
RSD	-	-	-	1.046	-	-	-	1.385	-	-	-	1.057
SE	-	-	-	0.467	-	-	-	0.619	-	-	-	0.473
Variance	-	-	-	1.094	-	-	-	2.132	-	-	-	1.122

*mean of three different experiments

Table No.6: Results of the intraday and interday precision for the determination of irbesartan, losartan, atenolol and hydrochlorothiazide using vanillin and NQS methods

	Drug	Conc.ug/ml	Intraday		Interday	
			mean ± SD	RSD	mean ± SD	RSD
Method I (Vanillin)	**Irbesartan**	40µg/ml	99.645±1.804	1.828	98.125±1.518	1.547
		80µg/ml	100.625±0.260	0.258	100.520±0.232	0.231
		120µg/ml	99.490±1.403	1.140	99.375±1.177	1.184
	Losartan	80µg/ml	98.991±0.502	0.507	98.947±0.428	0.433
		120µg/ml	100.058±0.334	0.334	99.912±0.346	0.347
		160 µg/ml	100.427±0.164	0.163	100.493±0.187	0.186
	Atenolol	40µg/ml	98.275±0.862	0.877	100.517±1.156	1.150
		80µg/ml	99.425±1.794	1.804	101.206± 1.595	0.964
		120µg/ml	99.233±1.417	1.428	100.114 ± 1.707	1.698
Method II (NQS)	**Irbesartan**	2µg/ml	100 ± 1.428	1.428	100.071±1.774	1.177
		3µg/ml	99.920±1.073	1.074	98.857±1.644	1.663
		4µg/ml	100.892±0.944	0.936	99.821±1.781	1.784
	Losartan	0.2µg/ml	100.933± 1.646	1.630	101.077±1.405	1.390
		0.4 µg/ml	98.778 ±0.662	0.629	98.814±0.481	0.487
		0.6µg/ml	98.659±0.747	0.757	98.706±0.590	0.597
	Hydrochlorothiazide	0.25µg/ml	101.934± 1.013	0.994	99.767±1.792	1.796
		0.5 µg/ml	99.391±1.066	1.072	98.640± 1.779	1.784
		0.75 µg/ml	99.871±0.337	0.338	99.502± 1.241	1.243

Table No.7: Results of the robustness for the determination of irbesartan, losartan, atenolol and hydrochlorothiazide using method I and method II

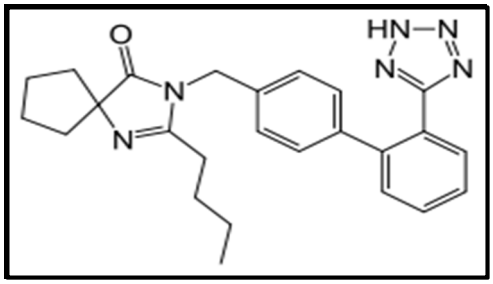
S.No	Parameters	Irbesartan	Losartan	Atenolol	Hydrochlorothiazide
1	vanillin+0.05 ml	99.879±1.042	99.656 ±0.868	99.767 ±1.238	---
2	vanillin-0.05 ml	100.109± 1.147	100.292 ± 1.077	100.456 ± 1.391	---
3	H2SO4+0.05 ml	99.671±1.264	99.436±1.245	100.008±1.064	---
4	H2SO4-0.05 ml	100.317± 1.147	100.204±0.929	100.525± 1.495	---
5	Time+2 min in method I	99.900± 1.029	99.458 ±1.204	99.905± 1.110	---
6	Time-2 min in method I	100.525 ± 1.147	100.248 ± 1.001	100.594 ± 1.608	---
7	NQS+0.05ml	99.662±1.439	100.105±1.220	---	99.985±2.019
8	NQS-0.05 ml	100.136±0.886	100.462± 0.769	---	100.593±1.372
9	NaOH+0.05ml	99.705± 1.364	100.125±1.184	---	100.040±1.922
10	NaOH-0.05 ml	100.567± 1.252	100.482± 0.794	---	100.630±1.375
11	Time+2 min in method II	99.748± 1.292	100.085 ± 1.257	---	100.077±1.860
12	Time-2 min in method II	100.783 ± 1.629	100.879± 1.253	---	100.703±1.399

Table No.8: Statistical analysis of results obtained by the proposed methods applied on irbesartan, losartan, atenolol and hydrochlorothiazide compared with reference method

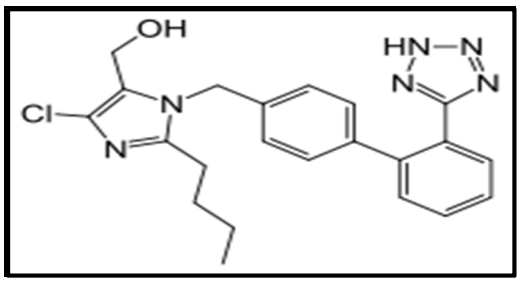
Para meters	Method I (vanillin)	Method II (NQS)	Reported method [38]	Method I (vanillin)	Method II (NQS)	Reported method [39]	Atenolol		Hydrochlorothiazide	
	Irbesaratn	Irbesartan		Losartan	Losartan		Proposed method	Reported method [40]	Proposed method	Reported method [41]
N	6	6	6	5	5	5	5	5	5	5
Mean Recovery*	100.108	100.382	99.989	99.875	100.093	100.004	100.112	99.656	100.153	100.134
SD	0.899	0.831	0.699	0.661	0.899	0.657	1.067	0.641	1.316	2.0265
RSD	0.898	0.828	0.699	0.662	0.899	0.657	1.066	0.644	1.314	2.0238
SE	0.367	0.339	0.285	0.295	0.402	0.294	0.477	0.287	0.588	0.827
Variance	1.009	0.692	0.579	0.437	0.809	0.432	1.140	0.412	1.734	4.106
Student-t**	0.255(2.228) [a]	0.886(2.228) [a]	-	0.309(2.306) [a]	0.178(2.306) [a]	-	0.819(2.306) [a]	-	0.017(2.306) [a]	-
F-test**	1.654(4.283) [b]	1.413(4.2883) [b]	-	1.012(5.05) [b]	1.872(5.05) [b]	-	2.770(5.05) [b]	-	2.370(5.05) [b]	-

*Average of three experiment

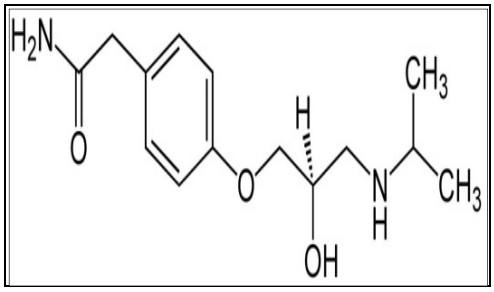
** a and b theoretical Student t-values and F-ratio at p=0.05



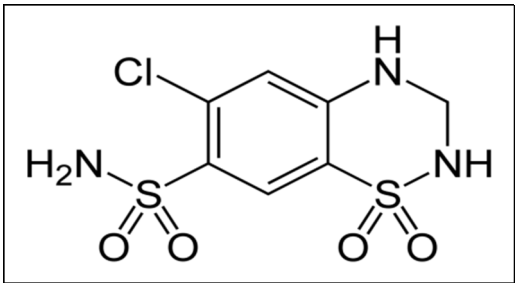
Irbesartan



Losartan



Atenolol



Hydrochlorothiazide

Figure No.1: Structure of Irbesartan, Losartan, Atenolol and Hydrochlorothiazide

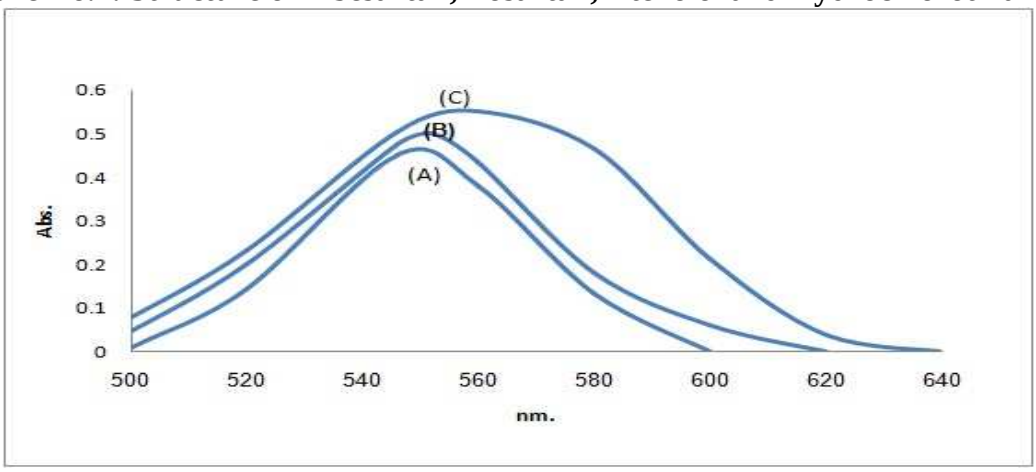


Figure No.2: Absorption spectra for the reaction between vanillin and (A) irbesartan (100µg/ml) at λmax546 nm, (B) losartan (150 µg/ml) at λmax 552 nm and (C) atenolol (200 µg/ml) at λmax at 560 nm

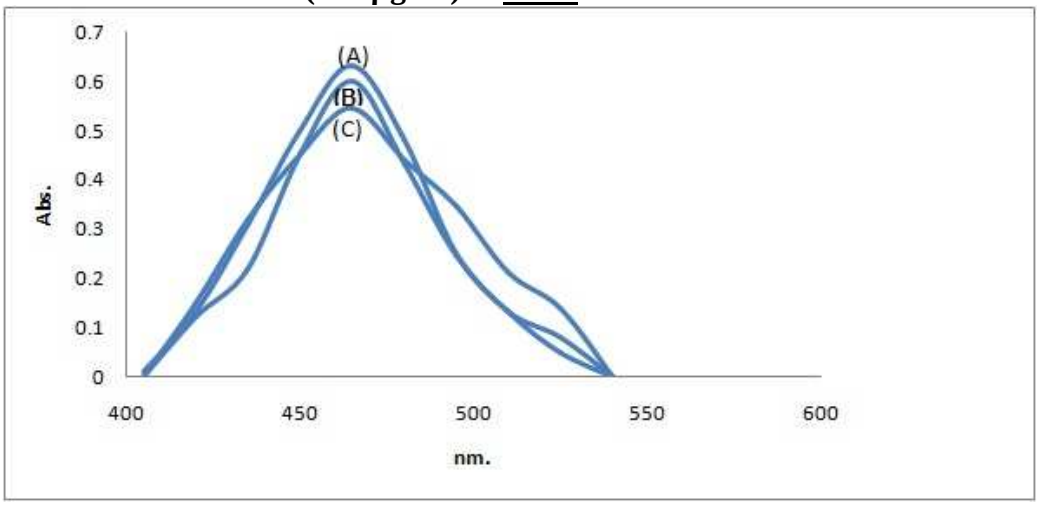


Figure No.3: Absorption spectra for the reaction between NQS and (A) irbesartan (4µg/ml),(B) losartan (1µg/ml) and (C) hydrochlorothiazide (1 µg/ml)at λmax 465 nm

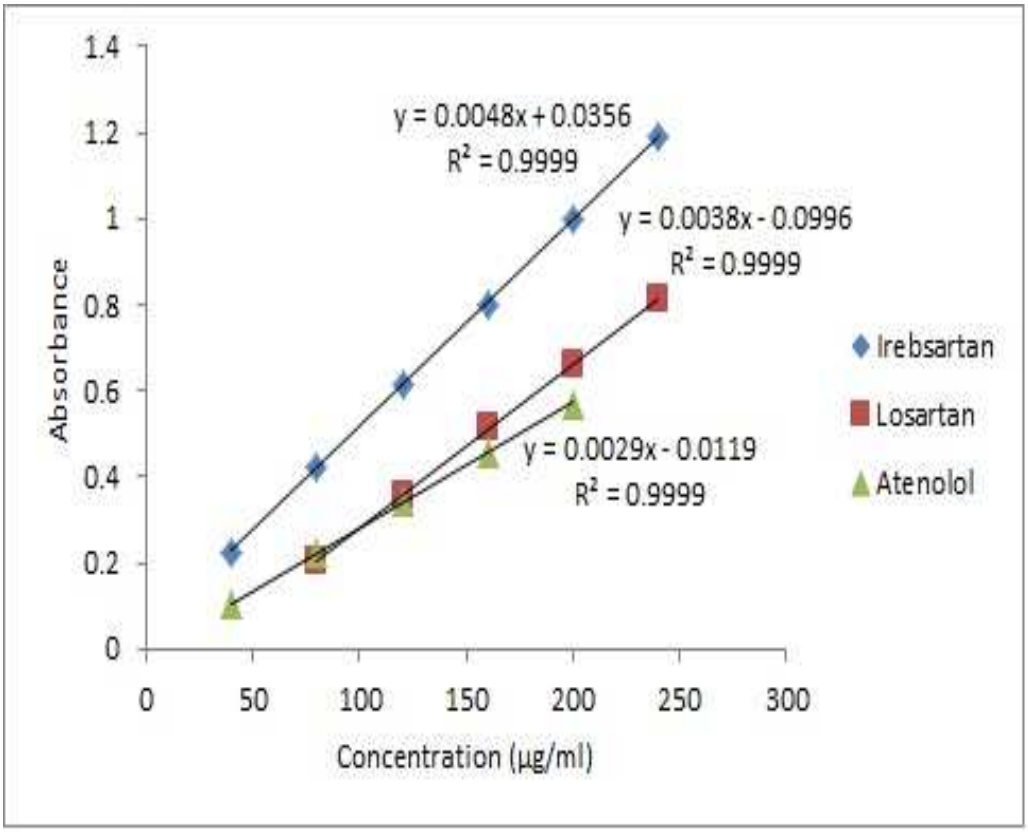


Figure No.4: Calibration curve for the reaction between vanillin and irbesartan, losartan and atenolol at λmax 546 nm, 552 nm and 560 nm, respectively

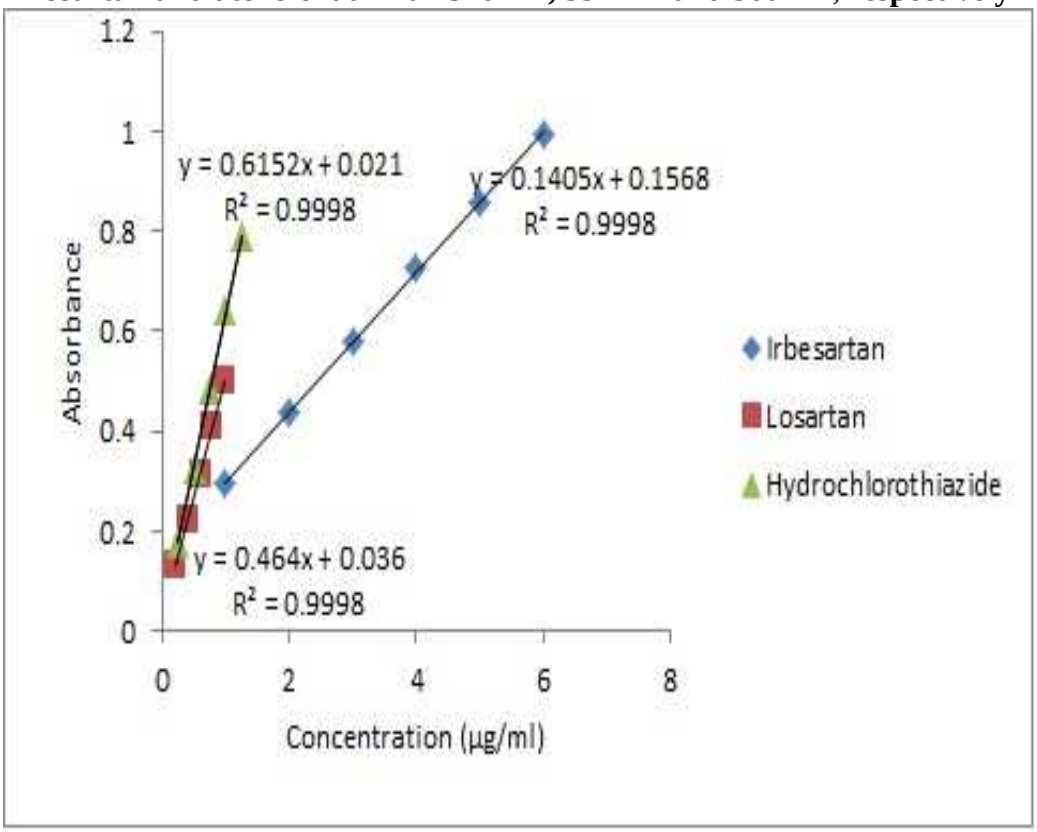


Figure No.5: Calibration curve for the reaction between NQS and Irbesartan, losartan and hydrochlorothiazide at λmax 465 nm

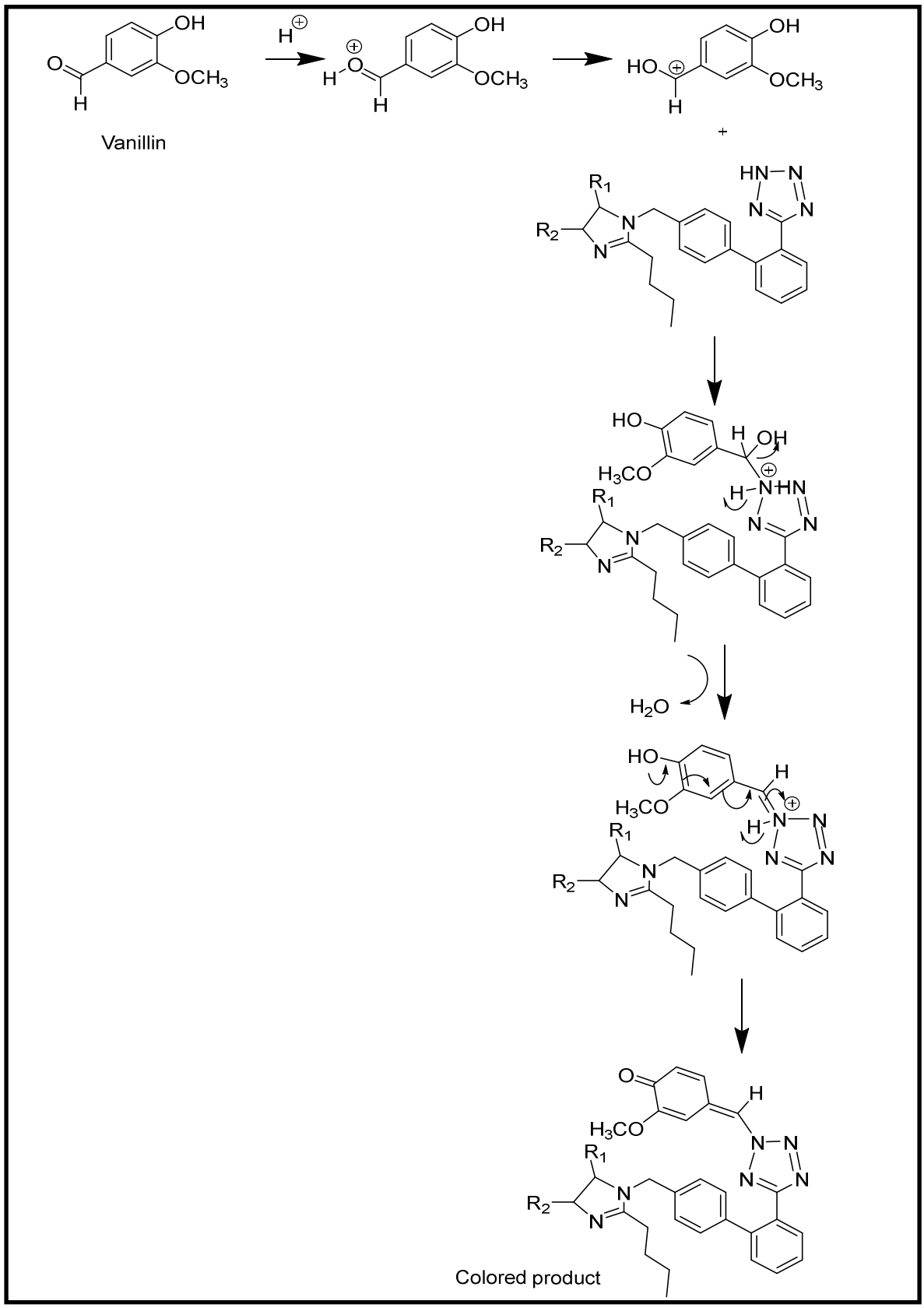


Figure No.6: Proposed reaction mechanism between vanillin and irbesartan, losartan

***R_1 = (C=O in case of irbesartan), (CH_2OH in case of losartan)**

***R_2 = (Cyclopentane ring in case of irbesartan), (Cl in case of losartan)**

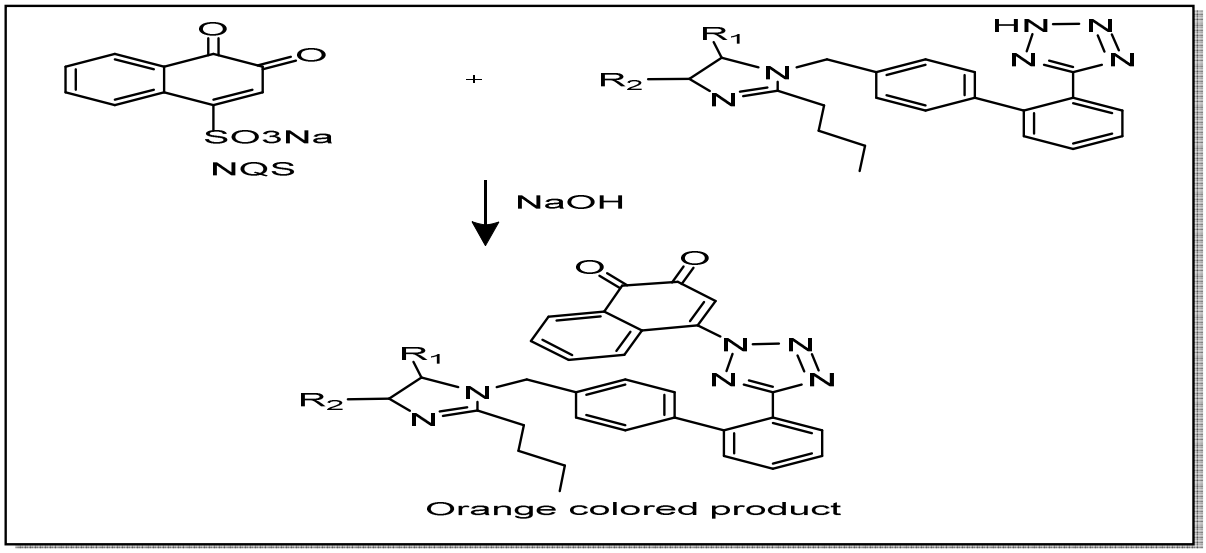


Figure No.7: Proposed reaction mechanism between NQS and irbesartan, losartan
***R_1 = (C=O in case of irbesartan), (CH_2OH in case of losartan)**
***R_2 = (Cyclopentane ring in case of irbesartan), (Cl in case of losartan)**

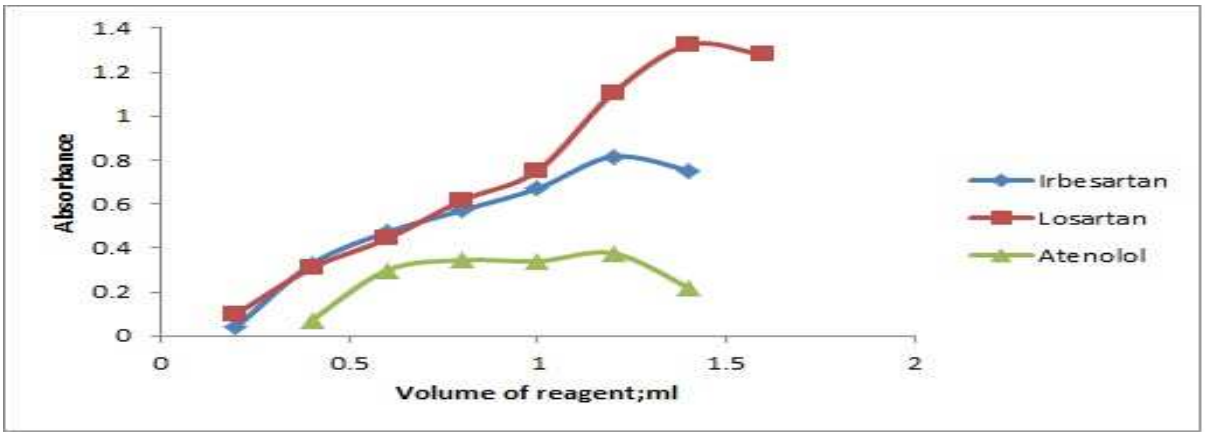


Figure No.8: The effect of vanillin volume on the reaction of vanillin with (200 µg/ml) in case of irbesartan, losartan and (100 µg/ml) in case of atenolol

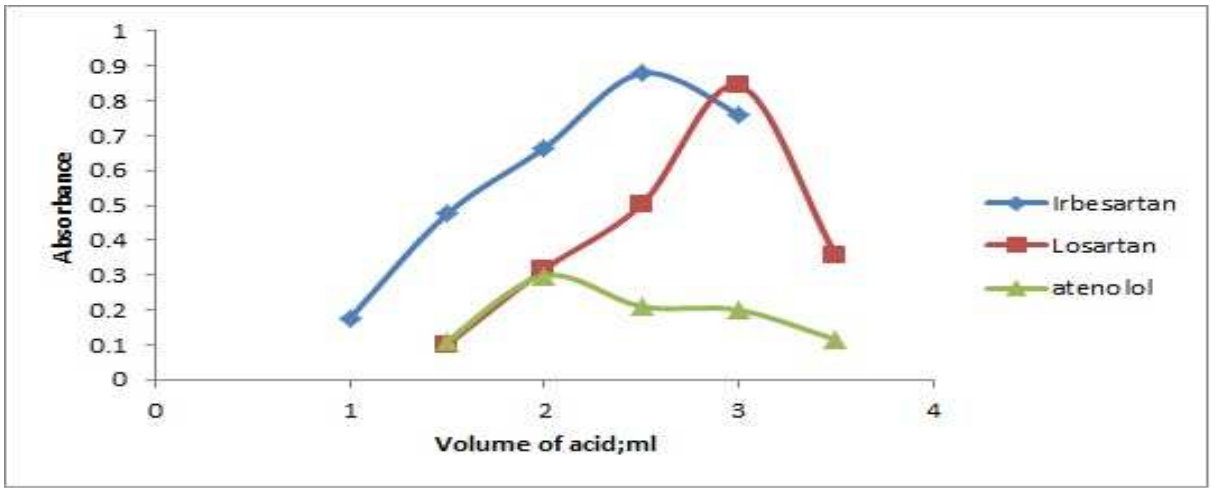


Figure No.9: The effect of acid volume on the reaction of vanillin with (200 µg/ml in case of irbesartan, losartan and (100 µg/ml) in case of atenolol

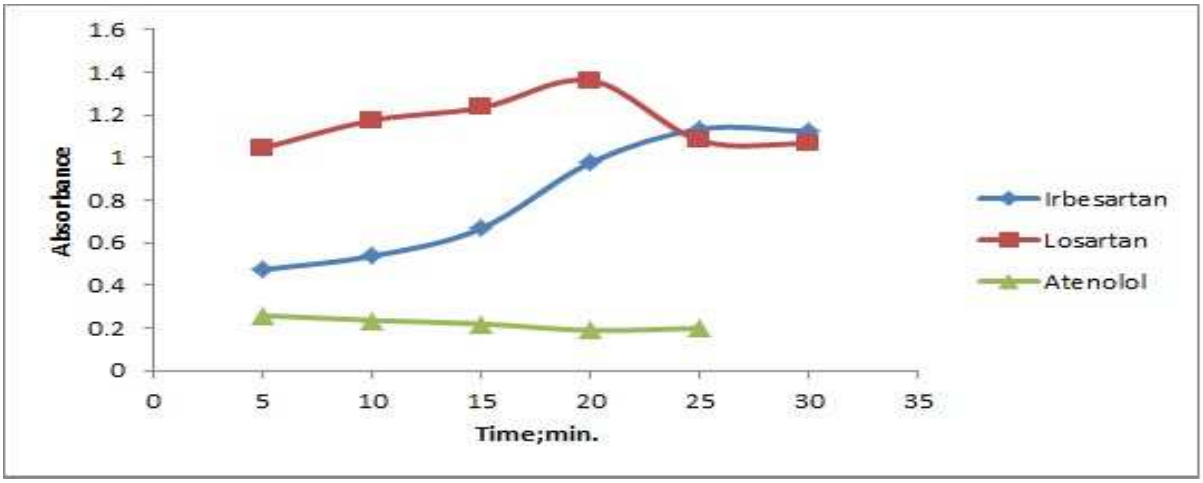


Figure No.10: The effect of heating time on the reaction of vanillin with (200 µg/ml in case of irbesartan, losartan and (100 µg/ml) in case of atenolol

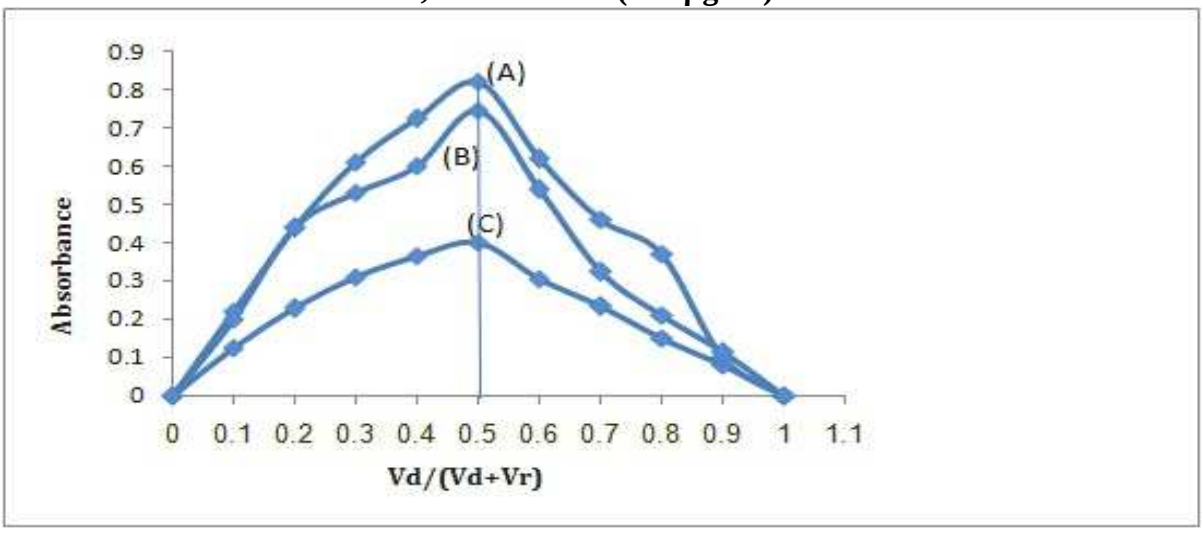


Figure No.11: Continuous variation plots for the reaction between: (A) 1×10^{-2} M of vanillin and 1×10^{-2} M of irbesartan, (B) 4×10^{-2} M of vanillin and 4×10^{-2} M of losartan and (C) 1×10^{-2} M of vanillin and 1×10^{-2} M of atenolol

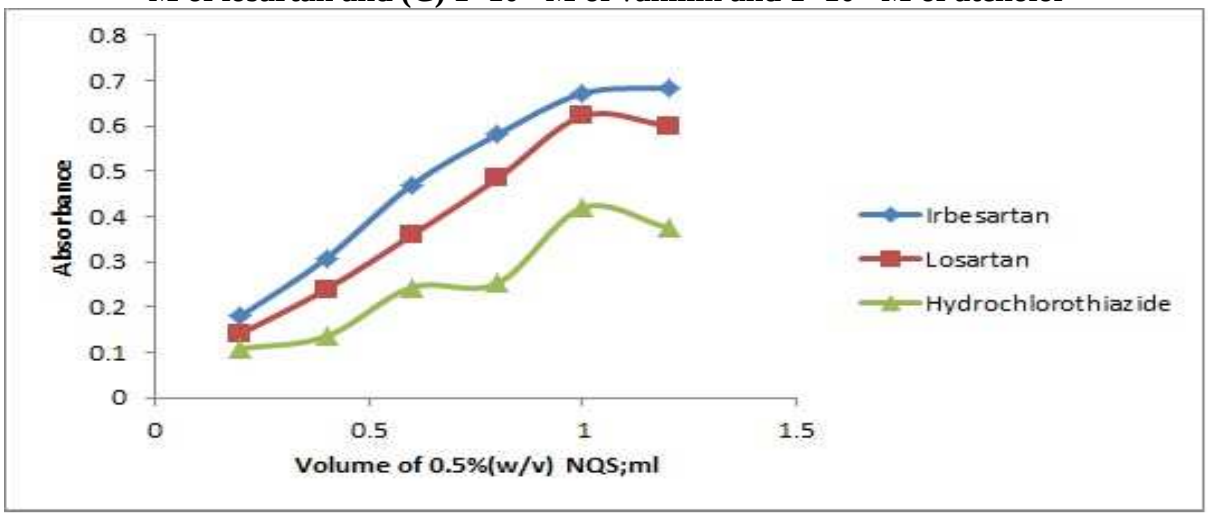


Figure No.12: The effect of NQS volume on the reaction of NQS with 20 µg/ml, 2µg/ml and 2.5 µg/ml of irbesartan, losartan and hydrochlorothiazide, respectively

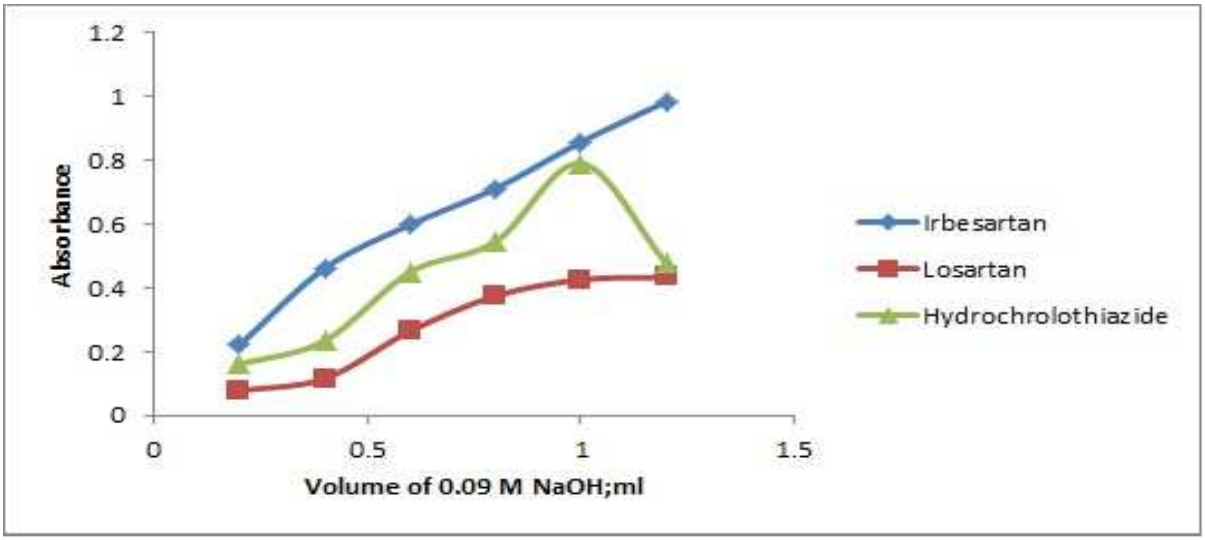


Figure No.13: The effect of sodium hydroxide volume on the reaction of NQS with 20 µg/ml, 2µg/ml and 2.5 µg/ml of irbesartan, losartan and hydrochlorothiazide, respectively

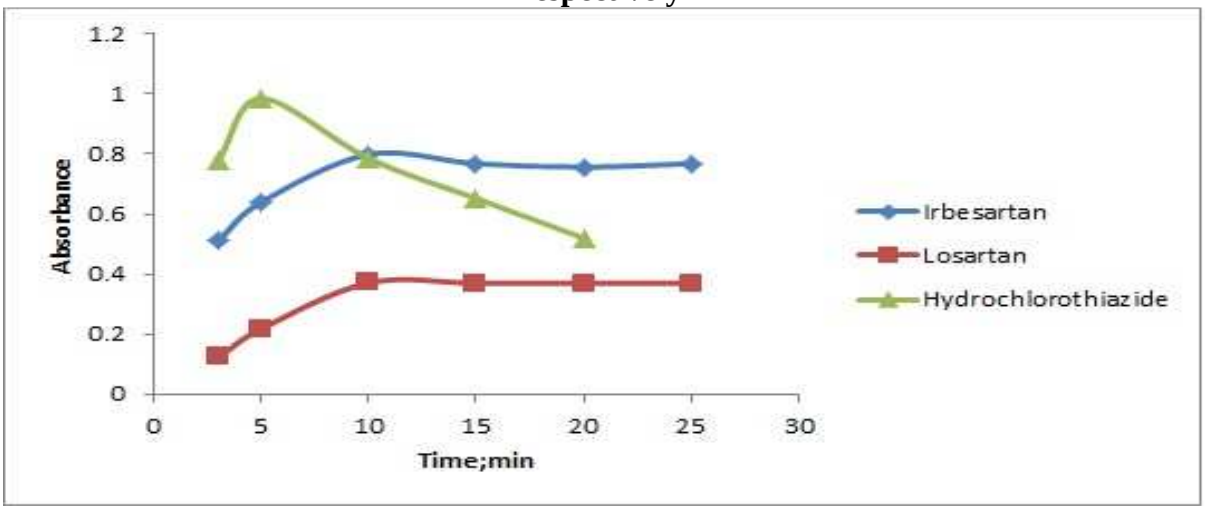


Figure No.14: The effect of the time on the reaction of NQS with 20 µg/ml, 2µg/ml and 2.5 µg/ml of irbesartan, losartan and hydrochlorothiazide, respectively

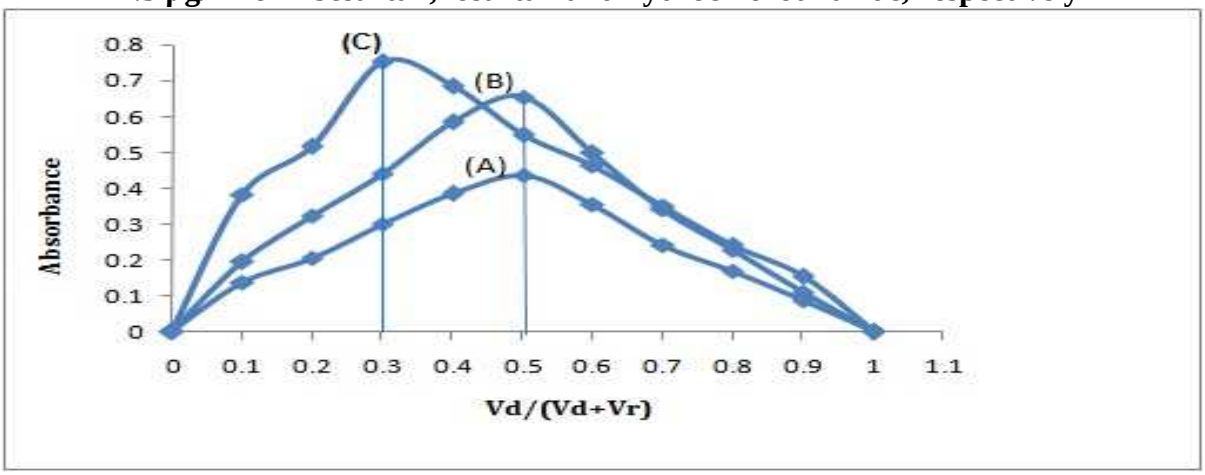


Figure No.15: Continuous variation plots for the reaction between: 1×10^{-2} M of NQS and (A)1×10^{-2} M of irbesartan, (B)1×10^{-2} M of losartan and (C)1×10^{-2} M of hydrochlorothiazide

CONCLUSION

The proposed spectrophotometric methods provided simple, sensitive, specific and analytical procedures for determination of the drugs either in pure forms or in their tablet. The satisfactory sensitivity and reproducibility as well as the simplicity, make the proposed method suitable for routine analysis in quality control laboratories.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Patel K R, Patel S A, Darji V C, Sonpal R N. Simultaneous spectrophotometric estimation of irbesartan and hydrochlorothiazide in tablets, *International Research Journal of Pharmacy*, 2(3), 2011, 202-207.
2. The British pharmacopoeia, *H M Stationary Office, London,* 1(2), 2013, 1355-1357.
3. Tulja R G, Gowri S D, Shireesha M, Satyanarayana B. Spectrophotometric method for determination of angiotensin-II receptor antagonist in bulk and pharmaceutical dosage forms, *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(1), 2011, 198-202.
4. Priyanka R P, Sachin U R, Dhabale P, Burade K. Simultaneous UV spectrophotometric method for estimation of losartan potssium and amlodipine besylate in tablet dosage form, *Asian Journal Research Chemistry*, 2(2), 2009, 183-187.
5. Anupama B, Abhinav K, Suri N B, Surendra A. UV spectrophotometric method for irbesartan, *International Journal of Research in Pharmacy and Chemistry*, 2(1), 2012, 20-21.
6. Srinath N, Anil K A, Rama D, Krishna P V. Estimation of irbesartan in bulk and dosage forms by new simple UV spectrophotometry using hydrotropic technique, *Pharmaceutica Analytica Acta*, 4(8), 2013, 1-3.
7. Farouk M, Abd El-Aziz O, Hemdan A, Shehata M. Simple novel spectrophotometric and spectrofluorimetric methods for determination of some anti-hypertensive drugs, *Journal of American Science*, 7(1), 2011, 300-312.
8. Tangri P, Singh P, Lakshmayya, Mukhopadhyay S, Tangri S. Development and validation of UV spectrophotometric method for the estimation of losartan bulk drug and pharmaceutical formulation, *International Research Journal of Pharmacy*, 3(5), 2012, 391-393.
9. Krystyna C S and Aleksander P M. Identification and determination of selected angiotensin II receptor antagonist group drugs by HPLC method, *Acta Poloniae Pharmaceutical Drug Research*, 68(6), 2011, 831-837.
10. Sathe S R and Bari S B. Simultaneous analysis of losartan potassium, atenolol, and hydrochlorothiazide in bulk and in tablets by high performance thin-layer chromategraphy with UV absorption densitometry, *Acta Chromatographica*, 19(2), 2007, 270-278.
11. Ibrahim A D. Analytical study for the charge-transfer complexes of losartan potassium, *Analytica Chimica Acta*, 549(1-2), 2005, 212-220.
12. Nagaraja S K, Chakravarthi I E. A UV-visible spectrophotometric determination of atenolol in pharmaceutical formulations, *International Journal Scientific Research*, 2(3), 2013, 31-32.
13. Lalitha K V, Kiranjyoth I R, Padma B. UV spectrophotometric method development and validation for the determination of atenolol and losartan potassium by Q-analysis, *International Bulletin of Drug Research*, 3(4), 2013, 54-62.

14. Pawar P V, Gaikwad P D, Bankar V H, Pawar S B. Development and validation of UV-spectrophotometric method for simultaneous estimation of atenolol and indapamide in bulk and tablet dosage form, *International Journal of Pharmacy and Technology*, 2(4), 2010, 876-885.
15. Shelke O S, Sable K S, Neharkar V S, Mathdevru B V. Development and validation of a UV spectrophotometric method for the simultaneous determination of nifedipine and atenolol in combined dosage form, *International Research Journal of Pharmacy* , 3(4), 2012, 360-364.
16. Bhaskara B L, Anil KS, Anil K. Afacile and rapid HPLC method for determination of atenolol in pharmaceutical formulations, *Asian Journal of Applied Sciences*, 4(3), 2011, 306-313.
17. Bhusari V K and Dhaneshwar S. Validated HPLC method for simultaneous quantitation of amlodipine besylate, atenolol and aspirin in bulk drug and formulation, *Journal of Pharmaceutical and Biomedical Sciences*, 17(17), 2012, 1-5.
18. Gireesh K, Chanti N M, Padma Y, Venkata R M, Madhu M, Gopinath C. Novel FT-IR spectroscopic method for the quantitation of atenolol in bulk and tablet formulation, *Journal of Global Trends in Pharmaceutical Sciences*, 5(3), 2014, 1750-1755.
19. Bilal Y and Sakir A. Determination of atenolol in human urine by gas chromatography–mass spectrometry method, *Journal of Chromatographic Science*, 49(5), 2011, 365-369.
20. Patel H M, Pancholi S S, Jivani N P. UV spectrophotometric method for simultaneous estimation of carvedilol and hydrochlorothiazide in bulk and pharmaceutical dosage form by simultaneous equation method, *Inventi Rapid: Pharm Analysis and Quality Assurance*, 2013(1), 2013, 49-56.
21. Manzoor A, Nadeem J, Satishkumar S A. Simultaneous estimation of atenolol and hydrochlorothiazide in combined dosage form by UV-spectrophotometric methods, *Acta Chimica. Pharmaceutica. Indica*, 2(3), 2012, 134-142.
22. Vrushali T, Vijaya V, Ujjawala K, Shashikant D. Novel UV spectrophotometric methods for estimation of ramipril and hydrochloro thiazide by simultaneous equation and area under curve method, *International Journal of Applied Pharmaceutics*, 2(4), 2010, 20-22.
23. Nikam M B, Harshad D, Aniket A, Kondawar M S. Imultaneous estimation of valsartan, amlodipine besylate and hydrochlorothiazide by first order derivative UV spectrophotometric method, *International Journal of Pharmacy and Technology*, 2(3), 2010, 642-650.
24. Savita S Y and Janhavi R R. RP-HPLC method for simultaneous estimation of losartan, hydrochlorothiazide and amlodipine in tablet dosage form, *Asian Journal of Pharmaceutical and Clinical Research*, 7(1), 2014, 137-140.
25. Napa D and Sockalingam A. Validated liquid chromatographic method for the estimation of antihypertensive mixture in pharmaceutical dosage forms, *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(6), 2014, 1916-1927.
26. Hany W D, Said A H, Maissa Y S, Badr A E. Rapid and sensitive TLC and HPLC with on-line wavelength switching methods for simultaneous quantitation of amlodipine, valsartan and hydrochlorothiazide in pharmaceutical dosage forms, *International Journal of Pharma and Bio Sciences*, 4(1), 2013, 345-356.
27. Gotardo M A, Pezza L, Pezza H R. Determination of hydrochlorothiazide in pharmaceutical formulations by diffuse

reflectance spectroscopy, *Eclética Química*, 30(2), 2005, 17-24.

28. Mahmoud R S, Khadijeh M, Atieh J. Simultaneous spectrophotometric determination of triamterene and hydrochlorothiazide in Triamterene-H tablets using continuous wavelet transformation, *Journal of Applied Chemical Researches*, 4(14), 2010, 61-67.
29. Sule D, Ozlem A D, Burge A, Abdurrezzak E B. Spectrophotometric multicomponent resolution of a tablet formulation containing lisinopril and hydrochlorothiazide by multivariate calibration methods, spectrophotometric multicomponent resolution of a tablet formulation containing lisinopril and hydrochlorothiazide by multivariate calibration methods, *Asian Journal of Chemistry*, 25(2), 2013, 999-1002.
30. Zenita D O, Basavaiah K, Vinay K B, Revanasiddappa H D. Sensitive spectrophotometric determination of metoclopramide hydrochloride in dosage forms and spiked human urine using vanillin, *Arabian Journal of Chemistry*, 25(3-4), 2011, 631-7.
31. Kudige N P, Kanakapura B, Madihalli S R. Spectrophotometric determination of zolmitriptan in bulk drug and pharmaceuticals using vanillin as a reagent, *International Scholarly Research Notices Analytical Chemistry*, 5(1), 2013, 1-7.
32. Siddappa K, Mallikarjun M, Reddy P T, Tambe M. Spectrophotometric determination of metronidazole through schiffs base system using vanillin and PDAB reagents in pharmaceutical preparations, *Ecletica Quimica*, 33(4), 2008, 41-46.
33. Li Q and Zhang H. A novel spectrophotometric method for the determination of aminophylline in pharmaceutical samples in the presence of methanol, Spectrochimica, *Acta Part A: Molecular and Biomolecular Spectroscopy*, 70(2), 2008, 284-289.
34. Darwish I A, Abdine H H, Amer S M, Al-Rayes L I. Simple spectrophotometric method for determination of paroxetine in tablets using NQS as chromogenic reagent, *International Journal of analytical chemistry*, 257306(1), 2009, 1-9.
35. Darwish I A, Abdine H H, Amer S M, Al-Rayes L I. Spectrophotometric study for the reaction between fluvoxamine and 1,2-naphthoquinone-4-sulphonate: Kinetic, mechanism and use for determination of fluvoxamine in its dosage forms, *Spectrochimica Acta Part A : Molecular and Biomolecular Spectroscopy*, 72(4), 2009, 897-902.
36. Mahmoud A M, Khalil N Y, Darwish I A, Aboul-Fadl T. Selective spectrophotometric and spectrofluorometric methods for the determination of amantadine hydrochloride in capsules and plasma via derivatization with 1,2-Naphthoquinone-4-sulphonate, *International Journal of Analytical Chemistry*, 2009, 1-8.
37. International Conference On Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, *Validation of Analytical Procedures, London*, 2005.
38. Amit A, Anita S, Suman M, Asati K C. Quantitative analysis method development and validation for irbesartan in bulk drug by ultraviolet spectroscopy, *Journal of Advanced Pharmacy Education and Research*, 4(1), 2014, 101-105.
39. Tarkase K N, Suryawanshi S S, Joshi R S. Simultaneous derivative spectrophotometric determination and validation of losartan potassium in pharmaceutical dosage forms, *International Journal of Pharmaceutical Sciences Review and Research*, 13(2), 2012, 31-35.
40. Charde M S, Welankiwar A S, Chakole R D. Simultaneous estimation of atenolol and

chlorthalidone in combine tablet dosage form by absorption ratio method using UV-VIS spectrophotometry, *International Journal of Advances in Pharmaceutics*, 3(1), 2014, 2320-4923.

41. Shailesh G, Arvind U, Sheetal P, Poonam S, Bhavika C, Vaishali K. Development and validation of UV spectrophotometric method for hydrochlorothiazide in bulk drug using mixed hydrotropic solubilisation, *International journal of pharmaceutical research and development*, 6(04), 2014, 167-172.

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