

## SPECTROPHOTOMETRIC DETERMINATION OF ANTIHYPERTENSIVE DRUGS & PHARMACEUTICALS

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### ABSTRACT

Simple, sensitive and accurate methods for quantitative determination of drugs *viz.*, Fosinopril (FSP), Ramapril (RMP) and Sildenafil Citrate (SDC) have been developed. The methods depend upon the oxidation of the drug by a known excess N-Bromosuccinimide (NBS) in Hydrochloric acid medium and subsequent determination of unreacted NBS using Rhodamine-B dye. The methods have been validated in terms of LOD, LOQ, precision accuracy, %RSD, robustness and ruggedness. Factors affecting the absorbance *viz.*, concentration of HCl and time of reaction are optimized. The effect of excipients has also been studied and found to have no effect. The calibration curves are found useful for determination of pure drug and can be applied to pharmaceuticals in bulk drug and pharmaceutical industries.

**KEYWORDS:** Spectrophotometry, Drugs, N-Bromosuccinimide, Rhodamine-B Dye, Validation.

### 1. INTRODUCTION

Fosinopril (FSP) is an angiotensin converting enzyme (ACE) inhibitor<sup>[1]</sup> used for the treatment of hypertension, alport syndrome, diabetic kidney disease and left ventricular dysfunction. Fosinopril is the only phosphinate-containing ACE inhibitor. Fosinopril is administered as a prodrug and is converted *in vivo* to the active form fosinoprilat. Fosinopril is de-esterified by the liver or gastrointestinal mucosa and converted to its active form, fosinoprilat.<sup>[2]</sup> Fosinoprilat competitively binds to ACE, preventing ACE from binding to and converting angiotensin I to angiotensin II which lowers peripheral vascular resistance and decreases blood pressure.<sup>[3]</sup> thus helping to alleviate the negative effects of all on cardiac

performance. It is chemically known as (2S, 4S) -4-cyclohexyl-1-[2-[[2-methyl-1-(propanoyloxy)propoxy]-(4-phenylbutyl) phosphoryl]acetyl]pyrrolidine-2-carboxylic acid.<sup>[4]</sup>

Unlike other ACE inhibitors that are primarily excreted by the kidneys where as fosinopril is eliminated from the body by both renal and hepatic pathways.<sup>[5]</sup> This characteristic of fosinopril makes the drug a safer choice than other ACE inhibitors for heart failure patients. Literature survey reveals that there are some reported analytical methods like simple HPLC<sup>[6]</sup>, multiwavelength UV Spectrophotometry<sup>[7]</sup>, UV method & third derivative method<sup>[8,9]</sup> and absorption spectroscopy<sup>[10]</sup> of Fosinopril in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage.

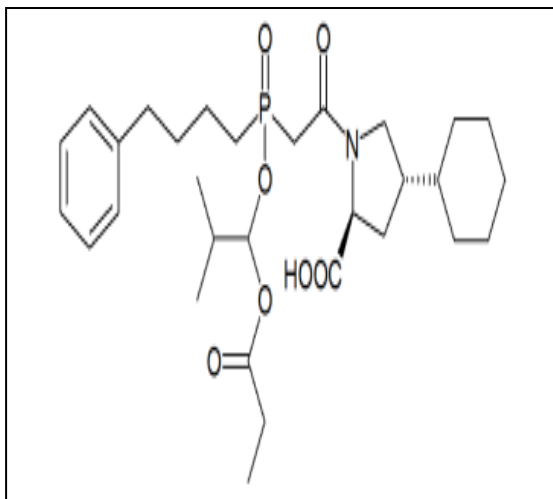
Ramipril is highly lipophilic, long acting orally active angiotensin converting enzyme (ACE) inhibitor with antihypertensive activity. An inactive prodrug, Ramipril is converted to ramiprilat in the liver and is used to treat hypertension and heart failure, to reduce proteinuria and renal disease in patients with nephropathies, to prevent stroke, myocardial infarction and cardiac death in high-risk patients. Ramiprilat, the active metabolite, competes with angiotensin I for binding at the angiotensin-converting enzyme, blocking the conversion of angiotensin I to angiotensin II.<sup>[11]</sup> As angiotensin II is a vasoconstrictor and a negative feedback mediator for renin activity, lower concentrations result in a decrease in blood pressure and an increase in plasma rennin. Ramiprilat may also act on kininase II, an enzyme identical to angiotensin-converting enzyme that degrades the vasodilator bradykinin.<sup>[12]</sup> It is chemically known as (2S, 3aS, 6aS) -1[(S)-N-[(S) -1-Carboxy-3-phenylpropyl]alanyl] octahydrocyclopenta[b] pyrrole-2-carboxylic acid, 1-ethyl ester. Literature survey reveals that few analytical methods were reported on Ramipril and in combination with other compounds viz, Electron Capture-Gas Chromatography<sup>[13]</sup>, Spectrophotometric and atomic absorption spectrometric method<sup>[14]</sup>, voltammetry<sup>[15]</sup>, capillary electrophoresis<sup>[16]</sup>, UV spectroscopy<sup>[17-20]</sup>, RP-HPLC.<sup>[21-23]</sup>

Sildenafil citrate (SDC) was patented in 1996 and launched in May 1998 as first oral drug approved by Food and Drug Administration (FDA) to treat erectile dysfunction (ED) in the United States. It is also effective for treatment of pulmonary arterial hypertension (PAH). It is a popular drug marketed as Viagra by Pfizer. Sildenafil citrate (SDC) is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazole [4,3-d] pyrimidine-5-yl)-4-ethoxyphenyl] sulfonyl]-4- methylpiperazine citrate. The mechanism of action of sildenafil involves the protection of cyclic guanosine monophosphate (cGMP) from

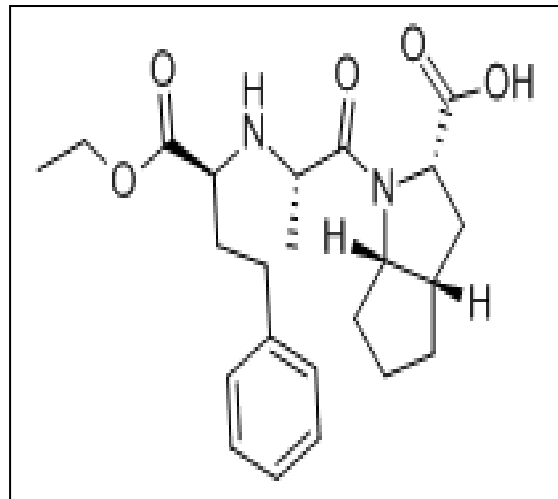
degradation by cGMP – specific phosphodiesterase type5 (PDE5) in the corpus cavernosum. Nitric oxide (NO) in the corpus cavernosum of the penis binds to guanylate cyclase receptors which results in increased levels of cGMP leading to smooth muscle relaxation (vasodilation). This smooth muscle relaxation leads to vasodilation and increased inflow of blood into the spongy tissue of the penis causing an erection.<sup>[24,25]</sup> It is eliminated predominantly by hepatic metabolism and converted to an active metabolite with properties similar to the parent Sildenafil. Sildenafil may be useful for the prevention and treatment of high-altitude pulmonary edema associated with altitude sickness such as that suffered by mountain climbers.<sup>[26]</sup> Literature survey reveals that there are some reported analytical methods like Voltammetry<sup>[27]</sup>, RP-LC method<sup>[28]</sup>, Atomic absorption spectrometric method<sup>[29]</sup>, UV spectroscopy<sup>[30,31]</sup>, HPLC<sup>[32-34]</sup> methods are reported for estimation of sildenafil citrate in bulk drug, formulations, pure active pharmaceutical ingredient and tablet dosage.

Although much work has been published on the quantification of the above drugs but the simple, sensitive spectrophotometric method using NBS as oxidizing reagent has not been reported yet. In the present communication we report the oxidation of drug using NBS as oxidant and Rhodamine-B dye as analytical reagent.

### STRUCTURE OF DRUGS



**Fig 1. Fosinopril**



**Fig 2. Ramipril**

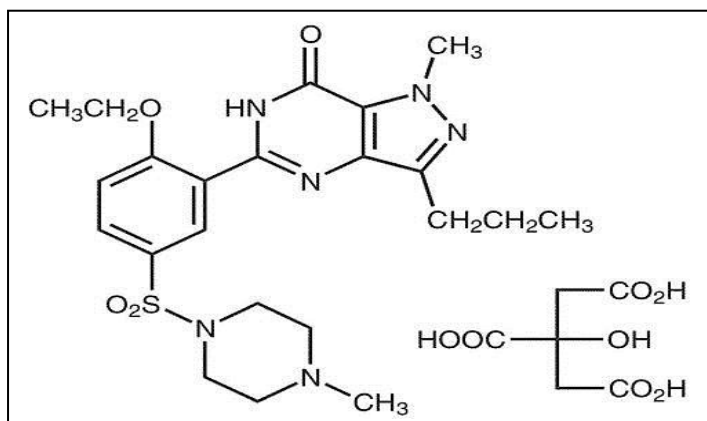


Fig.3 Sildenafil Citrate

## 2. ABOUT THE METHOD

NBS has strong oxidizing power, versatility, high oxidation potential, high stability in solution and perhaps the most important positive bromine containing organic compound, so it has been used for quantitative determination of drugs based on the oxidation of drugs. The spectrophotometric methods involved addition of excess NBS and un reacted NBS is estimated by suitable dyes, which should be oxidized by NBS *viz.*, Methyl Orange, Orange G, Safranin-O, Indigo Carmine, Metanil Yellow and Malachite Green.

Rhodamine-B dye is also suitable for estimation of unreacted NBS absorbance at 557 nm<sup>19</sup>.

## 3. EXPERIMENTAL

### 3.1 Instrumentation

The UV-VIS spectra of the study have been recorded on ELICO 210 double beam Spectrophotometer, Thermo Nicolet 1000 and also on ELICO 159 UV-VIS single beam spectrophotometers using quartz cells of 10 mm path length.

A Dhona 200 single pan electrical balance is used for weighing the samples.

### 3.2 MATERIALS AND METHODS

All reagents used were of analytical-reagent grade and distilled water was used throughout the investigation.

#### 3.2.1 N-Bromosuccinimide

0.01M N-Bromosuccinimide [1-Bromo-2,5-pyrrolidinedione], ( $C_4H_4BrNO_2$ , M.Wt. 177.98 g mol<sup>-1</sup>) solution was prepared by dissolving 1.8g of NBS (Himedia Laboratories pvt.Ltd, Mumbai) in water with the aid of heat and diluted to one liter with water and standardized

iodometrically. The solution was kept in an amber colored bottle and was diluted with distilled water appropriately to get  $70 \mu\text{g mL}^{-1}$  NBS for use in spectrophotometric method. The NBS solution was stored in a refrigerator when not in use.

### 3.2.2 Rhodamine-B dye

Aqueous solution of  $0.001M$  of Rhodamine-B dye [9-(2-Carboxyphenyl)-3,6-bis(diethylamino)xanthenium chloride was provided by s. d. Fine Chem. Ltd, Mumbai] was prepared by dissolving an appropriate weight of 50 mg in 100 ml by double distilled water and filtered using glass wool. Further the dye solution was diluted to  $50 \mu\text{g mL}^{-1}$  for use in spectrophotometric method.

### 3.2.3 Hydrochloric acid

Prepared by diluting the concentrated acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18 ) with water appropriately to get  $1 M$  acid.

**3.2.4 Preparation of drug solution** Standard drug solution ( $200 \mu\text{g mL}^{-1}$ ) was prepared by dissolving accurately weighed 20 mg drug with suitable solvent to the mark in 100 ml standard flask. The stock solutions of FSP, RMP and SDC were further diluted with the same solvent to obtain working concentrations.

## 4. PROCEDURE

Aliquots containing  $0.6-7.2 \mu\text{g mL}^{-1}$  of drug were transferred into a series of 10 ml standard flasks using a micro burette. To this, 1 mL of NBS solution ( $70 \mu\text{g mL}^{-1}$ ) was added followed by 1 mL of  $1M$  HCl and contents were mixed and the flasks were set aside for 10 min under occasional shaking. After 10 minutes, 1 mL of Rhodamine-B ( $50 \mu\text{g mL}^{-1}$ ) added to the contents. Then contents were shaken well and diluted with double distilled water up to the mark. The absorbance of each solution was measured at 557 nm against the corresponding reagent blank.

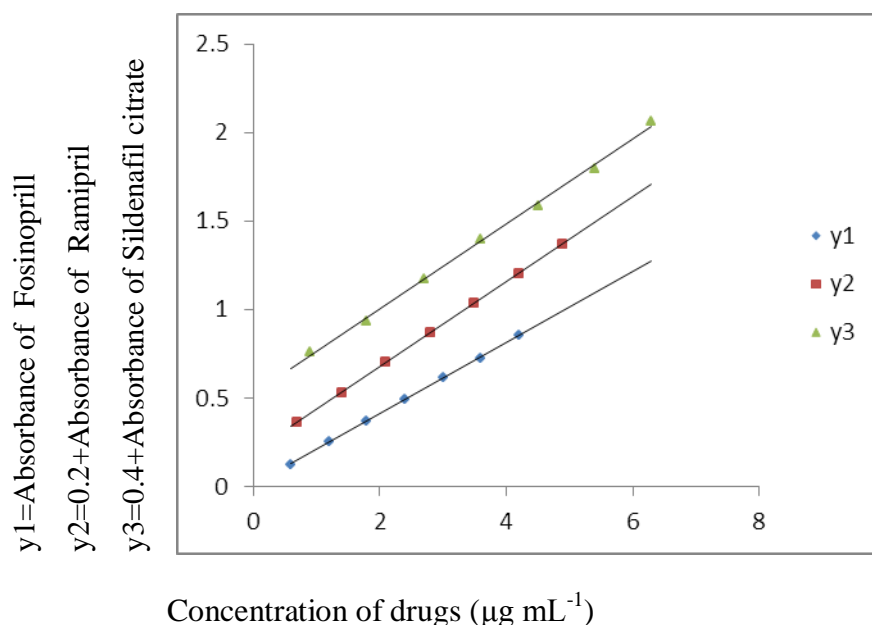
## 5. ASSAY OF PURE DRUG SAMPLE

To test the accuracy and precision of the methods developed, pure sample solutions containing drug in the Beer's Law limit were chosen. For this study  $0.6-4.2 \mu\text{g mL}^{-1}$  of FSP,  $0.7-4.9 \mu\text{g mL}^{-1}$  of RMP,  $1.2-7.2 \mu\text{g mL}^{-1}$  of SDC has been taken. To each of the solution 1 mL of  $70 \mu\text{g mL}^{-1}$  of NBS, 1 ml of  $1 M$  of HCl were added and the un reacted NBS is analyzed as described above using Rhodamine-B dye. Calibration curves were constructed

for all the drugs by plotting the absorbance versus the concentration of drugs. The absorbance data was collected for six replicate experiments and absorbance to concentration ratio called the relative response was determined. The relative responses between 95% to 105% of average are only considered for construction of the Calibration curves.

### 5.1 Procedure For Assay Of Pure Drug

Sample solutions of each drug in the beer's law limits were chosen and recovery experiments were performed to check the accuracy and precision. The concentration chosen and % of recovery are tabulated in table2, for this purpose standard deviation method also adapted. Excellent recovery and %RSD being less than 2 speaks about the precision and accuracy of the method.



**Fig.5 Calibration curves of drugs FSP, RMP and SDC.**

## 6. PROCEDURE FOR ANALYSIS OF TABLETS

### 6.1 Fosinopril

For the analysis of pharmaceutical formulations two tablets (Monopril – 20 mg) were weighed accurately and grounded. A quantity equivalent to 10mg of Fosinopril was weighed accurately, transferred into a 100 mL calibrated flask and the volume was finally diluted to the mark with double distilled water, mixed well and filtered using a Whatman No. 42 filter paper. It was used as stock sample solution and was further diluted with water to get working standard solution.

## 6.2 Ramipril

Ten tablets (RMP-2.5mg) were crushed to a fine powder and the powder equivalent to 10 mg of Ramipril was weighed accurately, transferred into a 100 mL calibrated flask, dissolved in sufficient quantity of methanol, sonicated for 10 min and the volume was finally diluted to the mark with methanol. This solution was mixed well and filtered through Whatman filter paper No. 42. It was used as stock sample solution and was further diluted with the same solvent to get working standard solution.

## 6.3 Sildenafil Citrate

Two tablets (sildenafil Tablets, VEGRO-100mg) were weighed accurately, crushed to a fine powder, the powder equivalent to 10mg of Sildenafil citrate was weighed accurately, transferred to 100 ml volumetric flask, dissolved in sufficient quantity of methanol, sonicated for 10 min and the volume was finally diluted to the mark with methanol. This solution was mixed well and filtered through Whatman filter paper No. 42. It was used as stock sample solution and was further diluted with the same solvent to get working standard solution.

## 7. METHOD OF VALIDATION

The each method developed for quantification of drugs has been validated in terms of precision, accuracy, limit of detection, limit of quantification, linearity, selectivity and ruggedness. Absorbance-concentration curves were drawn, fixed time method was used to assess the recovery of the drug. To assess the precision each experiment was repeated at least 6 times, accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery, RSD being less than 2 for each drug demonstrates accuracy and precision of the methods. [Table2].

As mentioned earlier limit of detection is the minimum limit that can be detected but not necessarily quantified is determined for each drug.

LOD is determined from the standard deviation of y-intercepts of regression lines of replicate determinations.

$$\text{LOD} = 3.3 s_a / S$$

Where  $s_a$  = standard deviation of intercept (n=6)

S = slope of linearity plot LOQ the minimum concentration of analyst using calibration curve is also determined.  $\text{LOQ} = 10s_a/S$ .



Limits of linearity of calibration curves [Fig. 4] are mentioned in the under the title Beer's law limit. To test the selectivity known excipients of each drug are added to the pure drug sample and recovery experiments were performed. Ruggedness is resistance of method for a small change in variables like instrument, analyst or both to test the Ruggedness of the method absorbance data was collected using 3 different instruments and 2 analysts no significant changes were observed either by change of instrument or analyst hence the method may be taken as robust.

## 8. FACTORS EFFECTING ABSORBANCE

**8.1 Effect of acid:** To study the effect of acid, different types of acids were examined (HCl, H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub> and CH<sub>3</sub>COOH) to achieve maximum yield of redox reaction. The results indicated that the Hydrochloric acid was the preferable acid with NBS as oxidant.

**8.2 Effect of acid concentration:** To study the effect of acid concentration different concentrations of HCl were examined. The reaction was performed in a series of 10 ml volumetric flask containing 1.2 µg mL<sup>-1</sup> of the cited drugs, different volumes (0.5–2.5 mL) of 0.5 M, 1.0 M, 1.5 M, 2.0 M, 2.5 M HCl and 1 ml of NBS (70 µg mL<sup>-1</sup>) were added. After 10 min of time, 1ml of Rhodamine-B (50 µg mL<sup>-1</sup>) dye and water added upto the mark. It was found that the maximum absorbance was obtained with 1mL of 1M HCl. Above this volume, the absorbance decreased therefore, a volume of 1 mL of 1 M HCl was used for all measurements.

**8.3 Effect of time:** In order to obtain the highest and most stable absorbance with the effect of time on the oxidation reaction of drugs were catalyzed by the time periods ranging for 2.5-20 minutes. The time required to complete the reaction and maximum absorbance was obtained after 10 min.

**8.4 Effect of sequence of addition:** Drug-acid-NBS-dye is optimum sequence of addition and other sequences gave lower absorbance values under same experimental conditions.

## 9. ANALYSIS OF PHARMACEUTICALS

To the test the applicability of the method developed solution of pharmaceutical tablets solutions containing drug in the Beer's Law limit were chosen. To assess the precision each tablet analysis was repeated at least 6 times, accuracy is estimated in terms of percent recovery and percent RSD. Excellent percent recovery, RSD being less than 2 for each drug



demonstrates applicability of the methods for pharmaceutical analysis [Table 3]. The excellent recovery studies indicate that methods developed can be applied to pharmaceutical analysis without hesitation.

## 10. RESULTS AND DISCUSSION

The ability of NBS to oxidize drugs and bleach the color of Rhodamine-B dye is the basis of the indirect spectrophotometric method developed here. This method makes use of bleaching action of NBS on the dye and decolouration being caused by the oxidative destruction of the dyes. In this method the above drugs were reacted with a measured excess of NBS in acidic medium and the unreacted NBS was determined by reacting with Rhodamine-B followed by absorbance measurement at 557 nm (scheme1).

The absorbance increased linearly with increasing concentration of drug, when increasing amounts of each drug were added to a fixed amount of NBS, consumes the latter proportionally and there occurs a concomitant fall in NBS concentration. When fixed amount of the dye was added to decreasing amount of NBS, a proportional increase in the concentration of dye resulted. This was observed as a linear increase in absorbance at the respective  $\lambda_{\max}$  (557) with increasing concentration of each drug. One ml of 1M Hydrochloric acid was used in the reaction, as this acid was found suitable medium for this method and concentration was found ideal.

Drug + known excess of NBS  $\rightarrow$  oxidation product of Drug + unreacted NBS

Unreacted NBS + fixed amount of Rhodamine-B  $\rightarrow$  oxidation product of Rhodamine-B + unreacted Rhodamine-B

Unreacted Rhodamine-B measured spectrophotometrically at  $\lambda_{\max} = 557$  nm

Scheme1: Tentative reaction Scheme for the indirect determination of drug by oxidation with NBS.

## 11. ANALYTICAL DATA

A linear correlation was found between absorbance at  $\lambda_{\max}$  and concentration ranges and sensitivity parameters such as Sandal's sensitivity, detection limit and quantification limit calculated according to ICH guidelines<sup>[35]</sup> are also presented in table 1 which reveal the very high sensitivity of the methods. Regression analysis of Beer's law data using the method of

least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) is also given in table 1.

**Table 1: Analytical and Regression parameters of Spectrophotometric Method**

Name of drug Property	FSP	RMP	SDC
$\lambda_{\max}$ , nm	557	557	557
Beer's law limits ( $\mu\text{g mL}^{-1}$ )	0.6-4.2	0.7-4.9	1.2-7.2
Molar absorptivity	$1.8 \times 10^5$	$1.1 \times 10^5$	$1.2 \times 10^5$
Sandell's sensitivity ( $\mu\text{g cm}^{-2}$ )	0.003629	0.003359	0.003389
Variance ( $S_a$ ) <sup>2</sup>	0.020044	0.000348	0.00394
Limit of detection $\mu\text{g mL}^{-1}$	0.6099	0.2066	0.7007
Limit of quantification $\mu\text{g mL}^{-1}$	1.8483	0.6263	2.1234
Regression equation, Y**	$Y=0.2774x+0.0687$	$Y=0.2977x+0.0017$	$Y=0.2956x+0.0677$
Intercept, (a)	0.0687	0.0017	0.0677
Slope, (b)	0.2774	0.2977	0.2956
Correlation coefficient, (r)	0.9977	0.9997	0.9947
Standard deviation of intercept ( $S_a$ )	0.0512	0.0186	0.0627
Standard deviation of slope ( $S_b$ )	0.0312	0.0122	0.0382

\*Limit of determination as the weight in  $\mu\text{g}$  per mL of solution, which corresponds to an absorbance of  $A = 0.001$  measured in a cuvette of cross-sectional area  $1 \text{ cm}^2$  and path length of  $1 \text{ cm}$ .  $Y^{**} = a + bX$ , where  $Y$  is the absorbance and  $X$  concentration of drugs in  $\mu\text{g}$  per mL.

## 12. ACCURACY AND PRECISION

The accuracy and precision of the methods were established by analyzing the pure drug solution at 6 different levels (with working limits). The relative error (%) which is a measure of accuracy & RSD (%) a measure of precision are summarized in Table 2 and reveal the high accuracy and precision of the methods.

## 13. ROBUSTNESS AND RUGGEDNESS

To evaluate the robustness of the methods, volume of Hydrochloric acid was slightly altered. The reaction time (after adding NBS, time varied was  $10 \pm 2 \text{ min}$ ) and the time after addition of dye is slightly changed. To check the ruggedness, analysis was performed by three different analysts and on three different spectrophotometers by the same analyst.

**Table 2 Determination of accuracy and precision of the methods on pure drug Samples.**

Drug	Taken (µg/ml)	Found (µg/ml)	error (%)	Recovery (%)	RSD (%)	Proposed method Mean ± SD
FSP	0.6	0.59	1.66	98.33	0.5841	98.98 ±0.5782
	1.2	1.18	0.83	99.16		
	1.8	1.81	0.55	99.44		
RMP	0.7	0.69	1.42	98.57	0.5000	99.12 ±0.4956
	1.4	1.41	0.71	99.28		
	2.1	2.09	0.47	99.52		
SDC	1.2	1.22	0.83	99.16	0.2906	99.49 ±0.2891
	2.4	2.39	0.41	99.58		
	3.6	3.59	0.21	99.72		

#### 14. APPLICATION TO FORMULATIONS

The proposed methods were applied to the determination of drugs in tablets. The results in Table 3 showed that the methods are successful for the determination of drugs and that the excipients in the dosage forms do not interfere. The results are compared to the available validated reported [10,20 & 36] methods on each drug and the results agree well with the claim and also are in agreement with the results obtained by the literature method. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed methods and the literature method at the 95 % confidence level with respect to accuracy and precision.

Recovery experiment was performed via standard addition technique to ascertain the accuracy and validity of the proposed methods. To a fixed and known amount / concentration of drug in tablet powder, pure drug was added at three levels (50, 100 and 150 % of the level present in the tablet) and the total was found by the proposed methods. Each experiment was repeated six times and the percent recovery of pure drugs added (Table 3) was within the permissible limits showing the absence interference by the inactive ingredients in the assay.

**Table 3 Results of assay of tablets by the proposed methods and statistical evaluation and recovery experiments by standard addition method.**

Tablets	Drug in tablet µg mL <sup>-1</sup>	Drug added µg mL <sup>-1</sup>	Total found µg mL <sup>-1</sup>	Error (%)	Recovery (%)	RSD (%)	Reference method Mean± SD	Proposed method Mean± SD
Monopril (FSP)	0.50	0.6	1.09	0.90	99.09	0.7763	99.68±0.859	99.42±0.7718
	0.50	1.2	1.69	0.58	99.41			
	0.50	1.8	2.30	0.00	100.00			
	0.6	0.0	0.59	1.66	98.33			
	1.2	0.0	1.19	0.83	99.16			
	1.8	0.0	1.81	0.55	100.55			

Ramipril (RMP)	0.50	0.7	1.19	0.83	99.16	0.5861	101.73 ± 0.82	99.84 ± 0.5851
	0.50	1.4	1.91	0.52	100.52			
	0.50	2.1	2.59	0.38	99.61			
	0.7	0.0	0.69	0.00	100.00			
	1.4	0.0	1.39	0.71	99.28			
	2.1	0.0	2.11	0.47	100.47			
Vergo (SDC)	0.50	1.2	1.69	0.58	99.41	0.4639	99.97 ± 0.595	99.74 ± 0.4627
	0.50	2.4	2.89	0.34	99.65			
	0.50	3.6	4.12	0.48	100.48			
	1.2	0.0	1.19	0.83	99.16			
	2.4	0.0	2.40	0.00	100.00			
	3.6	0.0	3.59	0.27	99.72			

Table 4: F-test and t-test values

	Monopril (FSP)	Ramipril (RMP)	Vergo (SDC)
<b>F-test *</b>	0.5956 (4.7571)	0.3423 (4.7571)	0.2140 (4.2839)
<b>t-test **</b>	0.1484 (3.182)	1.7318 (3.182)	0.4299 (2.447)

\*t- test and \*\*F-test values from literature.

## 15. CONCLUSION

The present study described the successful development of new, simple, sensitive, selective, accurate cost-effective and rapid spectrophotometric method for the accurate determination of the above drugs in its pharmaceutical form by using NBS as the oxidizing reagent. There is no interference from additives and excipients. The method thus can be used in the determination of these drugs in pure and pharmaceutical formulations. So, it is the good alternative to the reported methods for the determination of these drugs.

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