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Development of a Spectrophotometric Method Based on Ferric Hydroxamate Reaction for Determination of Ramipril in Formulations

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Abstract— A Simple, sensitive, extraction free and cost effective spectrophotometric method in visible region was developed for the estimation of Ramipril in bulk and dosage forms. The method is based on the ferric hydroxamate reaction purple red colored species is formed with hydroxylamine-ferric per chlorate reagent which exhibits maximum absorption at 530 nm. Beer's law obeyed in the concentration range of 20-60µg/ml. commercially available tablets were analyzed, the results obtained by the proposed method were in good agreement with the labeled amounts. The proposed method is reproducible with an accuracy of ±1 %. The method offers the advantages of rapidity, simplicity and sensitivity and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents. © 2011 IGJPS. All rights reserved

Keywords : ACE Inhibitor, Estimation, Hydroxylamine, Ferric Perchlorate, Visible Spectrophotometry, Tablets.

INTRODUCTION

Ramipril (RAM) (Fig.1) is highly lipophilic, long acting angiotensin-converting enzyme (ACE) inhibitor and chemically it is (2S, 3aS, 6aS)-1[(S)-N-[(S)-1-carboxy-3-phenylpropyl] alanyl] octahydrocyclopenta[b]pyrrole-2-carboxylic acid-1-ethyl ester [1].

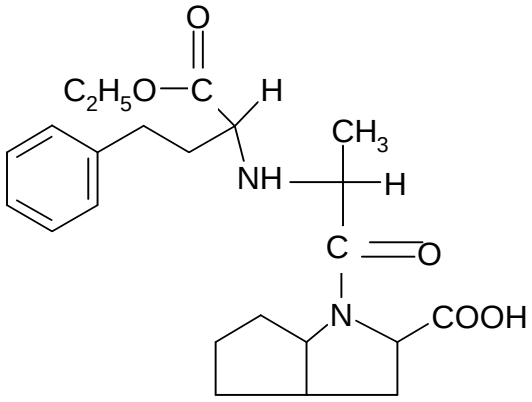


Fig.1: Showing the chemical structure of RAM

It is used in the treatment of hypertension, congestive heart failure and diabetic nephropathy with microalbuminuria. Ramipril acts as a prodrug of diacid ramiprilat. Ramipril owes its activity to ramiprilat to which it is converted after oral administration. The drug effectively reduces both supine and standing blood pressure without significant alteration in the pulse rate. RAM is official in USP and BP [2-3] which describes HPLC and potentiometric titration method for its assay in tablets. Literature survey revealed that several analytical techniques which include HPLC [4-12], HPTLC [13-14], LC-MS [15], GC [16-17], Voltametry [18], Radioimmunoassay [19], Capillary electrophoresis [20], ion selective electrode potentiometry [21-22], atomic absorption Spectrophotometry [23-24], Spectro fluorometry [25-26], visible spectrophotometric [27-32] and UV [33] have been reported for quantitative determination of Ramipril in biological fluids and pharmaceutical formulations. The main purpose of the present study was to establish relatively simple, sensitive, validated and inexpensive extraction free visible spectrophotometric method for the determination of RAM in pure form and in pharmaceutical preparations, since most of the previous methods involve critical reaction conditions or tedious sample preparations and less specificity.. So the authors have made some attempts in this direction and succeeded in developing a method based on the reaction between the drug and hydroxylamine –ferric per chlorate [34-35] reagent. The method can be extended for the routine quality control analysis of pharmaceutical products containing RAM.

MATERIALS AND METHODS

Systronics UV/Visible spectrophotometer model -2203 with10mm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade. Neutral hydroxyl amine (prepared by mixing equal volume of 12.5% solution of hydroxyl amine hydrochloride in methanol and 12.5% solution of NaOH in the same solvent and filtered. Ferric per chlorate solution (prepared by dissolving 5.0 gram of ferric per chlorate in a mixture of 10 ml of 70% per chloric acid and 10ml of water, dilute to 100ml with ethanol while cooling) were prepared.

Preparation of Standard drug solution:

The standard stock solution (1mg/ml) of RAM was prepared by dissolving 100mg of RAM in 100 ml ethanol. This solution was further diluted stepwise with the same solvent to obtain working standard solution concentration of 200µg/ml.

Preparation of Sample solution:

About 20 tablets were pulverized and the powder equivalent to 100mg of RAM was weighed, dispersed in 25ml of isopropyl alcohol (IPA), sonicated for 30minutes and filtered through whatman filter paper no.41. The filtrate was evaporated and the residue was used for the preparation of working sample solutions in the same way as under working standard solutions.

ASSAY:

To aliquots of standard RAM drug solution [1.0-3.0ml, 200µg/ml] in ethanol in a series of 10 ml calibrated tubes, 0.3ml neutral hydroxylamine hydrochloride solution is added and kept in water bath at 70°c for 5 minutes. Then allowed to cool and dilute to 5ml with ferric per chlorate. Shaken for 2 minutes and kept aside for 5 minutes at room temperature and made up to the mark with ethanol. The purple colored species was obtained and it was stable for 30 minutes. The absorbance of the colored species was measured at 530 nm against the reagent blank (Fig.2 showing absorption spectra). The amount of RAM was computed from its calibration curve (Fig.3 showing Beer's law plot).

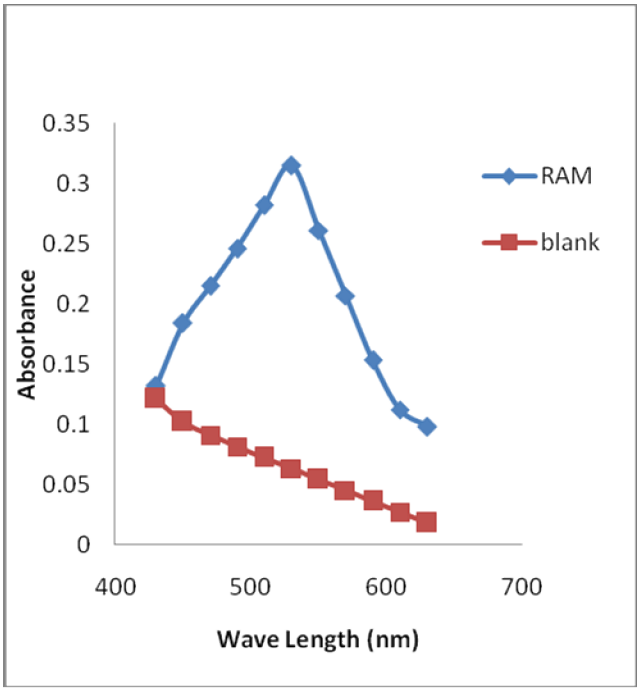


Fig.**2. Showing Absorption spectra**

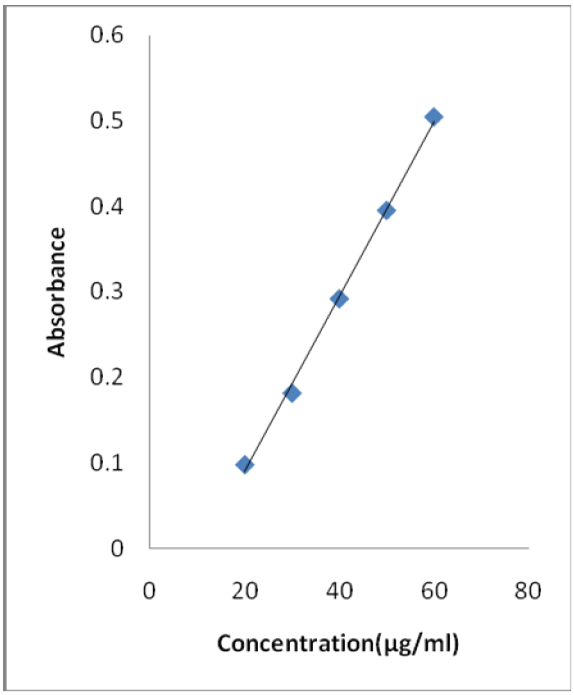


Fig.3.Showing Beer's law Plot

RESULTS & DISCUSSION

In developing this method, a systematic study of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed. The effect of various parameters such as time, volume and strength of hydroxyl amine, ferric per chlorate, stability of colored species and solvent for final dilution of the colored species were studied and the optimum conditions were established. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements containing $3/4^{th}$ of the amount of the upper Beer's law limits) were calculated and the results are summarized in table-1. Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and are shown in Table-1.

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHOD.

Parameter	Values
λ_{max} (nm)	530
Beer's law limit(µg/ml)	20-60
Sandell's sensitivity (µg/cm^2/0.001 abs. unit	0.12658
Molar absorptivity (Litre/mole/cm)	3.29051x10^3
Correlation Coefficient	0.998
Regression equation (Y)*	
Intercept (a)	-0.115
Slope(b)	0.010
%RSD	0.746
% Range of errors(95% Confidence limits)	
0.05 significance level	0.7828
0.01 significance level	1.2276

*Y = a +b x, where Y is the absorbance and x is the concentration of Ramipril in µg/ml

Commercial formulations containing RAM were successfully analyzed by the proposed method. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and f-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. These results are summarized in Table-2. The ingredients usually present in formulations of RAM did not interfere with the proposed analytical method.

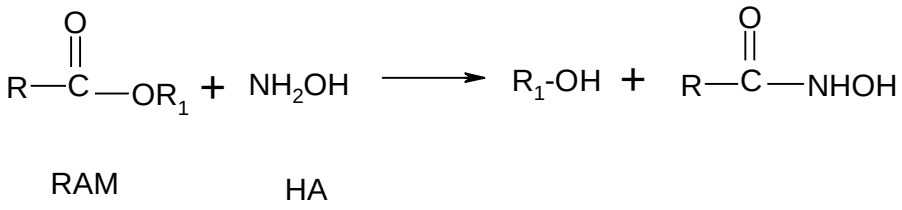
TABLE-2 ANALYSIS OF RAMIPRIL IN PHARMACEUTICAL FORMULATIONS

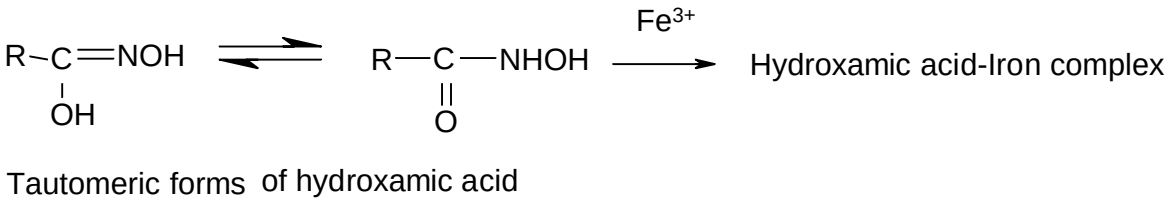
Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	f		
HA-Ferric per chlorate	Batch-1	10	9.876 ± 0.122	0.363	3.188	9.855 ±0.068	98.76 ± 1.219
	Batch-2	10	9.886 ± 0.086	0.739	3.783	9.864 ± 0.041	98.863± 0.861

* Different batches from two different companies.

**Average ± Standard deviation of six determinations, # Recovery of 10mg added to the pre analyzed sample (average of three determinations). Reference method (reported UV method) using methanol (λ_{max}=218nm).

Chemistry of colored species: Hydroxamic acids were discovered in 1869 by Lossen. Feigl and his co-workers is first introduced the use of the ferric-hydroxamic acid reaction as a spot test for compounds containing carboxylic esters. Based on it this method has been developed. In the present investigation the presence of ester group of ramipril permits for the development of visible spectrophotometric method for its determination through ferric –hydroxamic acid complex. The nature of colored species formation may be involved initially the formation of corresponding hydroxamic acid by the reaction of drug with hydroxyl amine and then followed by molecular complex with ferric ion. The formation of colored species with this reagent may be assigned through above analogy as shown in scheme (Fig.4).





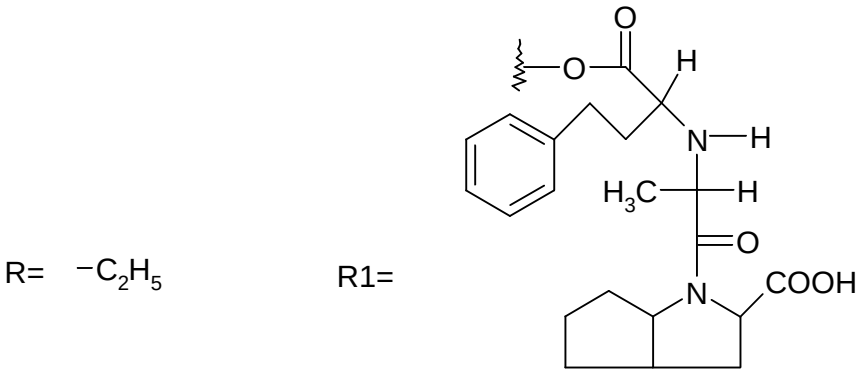


Fig.4. probable sequence reactions of the proposed method

CONCLUSION

The reagents utilized in the proposed method are cheap, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed colorimetric method possesses reasonable precision, accuracy and is simple, sensitive and can be used as alternative method to the reported ones for the routine determination of RAM depending on the need and situation.

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