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Development of New Visible Spectrophotometric Assay for Ramipril estimation in Bulk and Formulations using Quinone as Chromogenic reagent

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Abstract: A simple, sensitive and economical visible spectrophotometric method was described for the estimation of Ramipril in bulk and dosage forms. This method is based on the interaction of N-alkyl vinyl amine formed from the condensation of the free secondary amine group in the drug and acetaldehyde with p-chloranil to give vinyl amino substituted quinone. The blue colored product exhibits an absorption maximum at 665nm in dioxane. 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 method offers the advantages of rapidity, simplicity, sensitivity and normal cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

Keywords: ACE Inhibitor, TQ, Acetaldehyde, Assay, Spectrophotometer, Validation.

INTRODUCTION

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

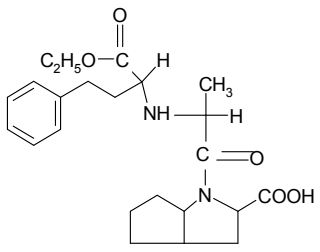

Fig.1: Showing the chemical structure of RAM

It is used in the treatment of hypertension, congestive heart failure and diabetic nephropathy with micro albuminuria. RAM acts as a pro drug of diacid ramiprilat. RAM owes its activity to ramiprilat to which it is converted after oral administration. Ramipril and ramiprilat compete with angiotensin I and block the conversion of angiotensin I to angiotensin II. Angiotensin II contracts the muscles of most arteries in the body, including the heart, thereby narrowing the arteries and elevating the blood pressure. The drug effectively reduces both supine and standing blood pressure without significant alteration in the pulse rate. RAM is official in BP[2] and USP[3] which describes potentiometric titration and HPLC 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 [27-33], UV [34] and conductometric[35] have been reported for quantitative determination of RAM in biological fluids and pharmaceutical formulations.

A direct chemical analysis based on the reactivity of the intact molecule without cleavage is not frequently encountered. The methods that are based on the charge-transfer complexation are usually rapid and simple to perform π- acceptors (quinones such as 2, 3, 5, 6,-tetrachloro-p-benzoquinone) (TQ) are known to yield charge-transfer complexes with a variety of electron donors. The present work describes an improved direct simple analytical procedure that can be applied to quality control laboratories for the analysis of pharmaceutical products containing RAM.

MATERIALS AND METHODS

(EXPERIMENTAL)

Systronics UV/Visible spectrophotometer model -2203 with10mm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade. Chloranil (TQ, BDH, 0.1%, $4.067x10^{-3}$M prepared by dissolving 100mg of chloranil in 100ml of 1,4-dioxane) was prepared and acetaldehyde(Qualigen) used directly.

Preparation of Standard drug solution:
The standard stock solution (1mg/ml) of RAM was prepared by initially dissolving 100mg of RAM in 10 ml DMF and the volume was brought to 100 ml with1, 4-dioxane. The working standard solution of RAM (200µg/ml) was obtained by appropriately diluting the standard stock solution by using the same solvent.

Preparation of Sample solution: About 20 tablets or capsules were weighted to get the average tablet or capsule weight and pulverized. The powder equivalent to 100 mg of RAM was weighed, dispersed in 25ml of Isopropyl alcohol, sonicated for 30 minutes and filtered through Whatman filter paper No 41. The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

Assay:
Aliquots of working standard RAM drug solution (200µg/ml) such as 1.0, 1.5, 2.0, 2.5 ml and 3.0ml were taken separately in a series of 10ml calibrated tubes .Then 0.5ml of acetaldehyde and 2ml chloranil ($4.067x10^{-3}$M) were added successively and shaken for 2 minutes and kept aside for 15 minutes at room temperature and made up to the mark with 1, 4-dioxane and sonicated for 1 min. The violet colored species was obtained and it was stable for 1 hour. The absorbance of the colored species was measured at 665 nm against the reagent blank (Fig.2). The calibration graph was constructed by plotting the drug concentration versus absorbance (Fig.3).

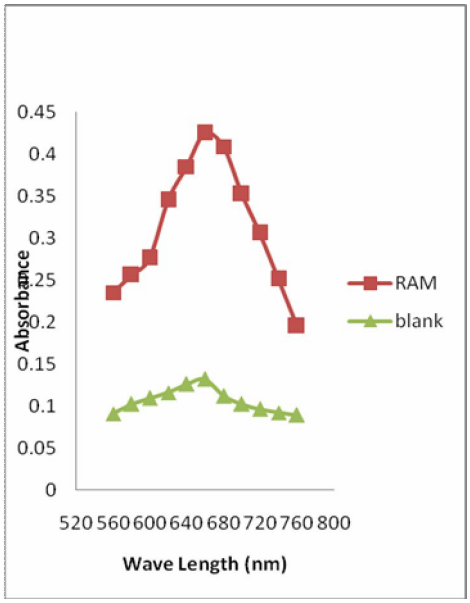

Fig.2 Determination of λ_{max} (TQ-ACD)

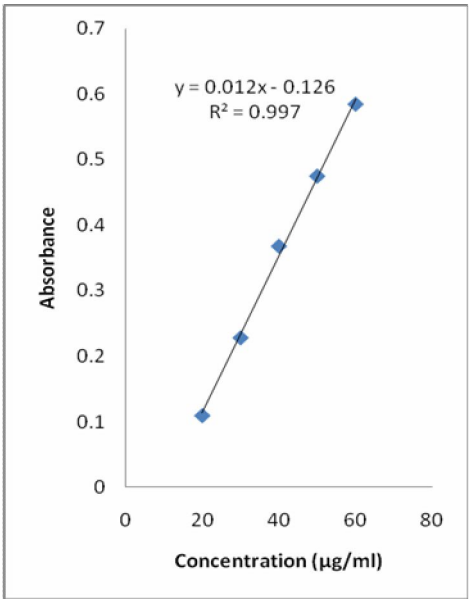

Fig.3 Beer's law plot

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

Parameter	Results
λ_{max} (nm)	665
Beer's law limit (µg/ml)	20-60
Sandell's sensitivity (µg/cm^2/0.001 abs. unit	0.10526
Molar absorptivity (Litre/mole/cm)	3956.94
Regression equation (Y)*	
Intercept (a)	-0.126
Slope(b)	0.012
Correlation coefficient	0.997
%RSD	0.747
% Range of errors (95% Confidence limits) 0.05 significance level	0.784
0.01 significance level	1.23

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

TABLE-2 ANALYSIS OF RAM IN PHARMACEUTICAL FORMULATIONS BY PROPOSED AND REFERENCE METHODS.

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	f		
TQ-ACD	Batch-1	5	4.94 ±0.032	1.79	4.39	4.92 ±0.015	98.88±0.63
	Batch-2	10	9.92 ± 0.093	1.45	1.87	9.85 ±0.068	99.24±0.93

* Different batches from two different companies.
**Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with reference method (UV). Theoretical values at 95% confidence limits t =2.57 and f = 5.05.
Recovery of 10mg added to the pre analyzed sample (average of three determinations).
Reference method (reported UV method) using methanol (λ_{max}=218 nm).

RESULTS AND DISCUSSION

In developing the method, 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 chloranil, acetaldehyde, stability of colored species and solvent for final dilution of the colored species were studied and the optimum conditions were established. The solvent for final dilution tried with different solvents such as acetinitrile, , tetrahydrofuran, ethylene glycol, dimethyl ether, 1, 4-dioxane. Among these 1, 4-dioxane was found to be superior for final dilution. It enhances not only the absorbance but also insert reproducible values for colored species. 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. 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.

Chemistry of colored species:

Henbest and his co-workers [36-37] found that many secondary or primary amines react with TQ and acetaldehyde. The N-alkyl vinyl amine obtained by condensing the amine with acetaldehyde reacts with TQ to give vinyl amino substituted quinine to give blue color. The proposed method exploits structural features aliphatic secondary amine of the RAM molecule. The nature of colored species formation with TQ-acetaldehyde reagent is initial N-alkyl vinyl amine formation with acetaldehyde then followed by formation of colored N-alkyl vinyl amino substituted quinones with TQ has been assumed in the scheme (Fig.4).

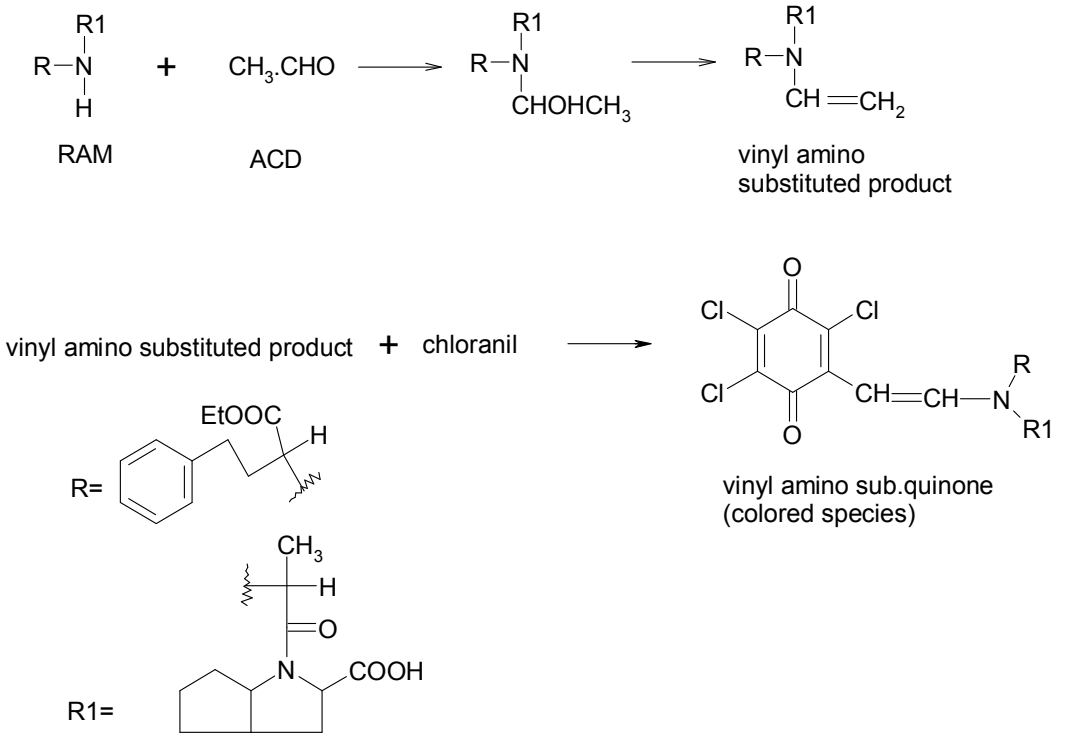

Fig.4: Scheme of the proposed analytical method

CONCLUSIONS:

The reagents utilized in the proposed method are normal cost, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed visible spectro-photometric 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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