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Research Article

EXTRACTIVE VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF RAMIPRIL IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A simple, sensitive, rapid and accurate extractive visible spectrophotometric method has been developed for the determination of Ramipril in pure and in pharmaceutical formulations. The method is based on the formation of orange red colored chloroform extractable ion-pair complex between the basic nitrogen of the drug and acidic dye Tropaelin ooo (TPOOO) in the presence of 0.1M HCl with an absorption maximum of 489.0 nm.The conditions necessary for the assaying the drug are established. The calibration graph is linear over the concentration range of 2-10µg/ml. The proposed method is applied to commercial available tablets or capsules and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The method offers the advantages of rapidity, simplicity and sensitivity and low cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

Key words: ACE inhibitor, Beer's Law, Chloroform, Extraction Spectrophotometry, Tropaeolin.

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].

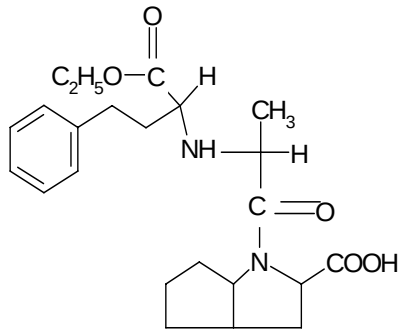

Fig. 1: Showing the chemical structure of RAM

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], Spectrofluorometry [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 a relatively simple, sensitive, validated and inexpensive extractive 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 acidic dye Tropaeolin ooo in the presence of 0.1M HCl. The method can be extended for the routine quality control analysis of pharmaceutical products containing RAM.

As the extraction spectrophotometric procedures are popular for their sensitivity and selectivity in the assay of drugs, the acid dye technique [34] was therefore, utilized in the present work for the estimation of RAM.

The present paper describes simple and sensitive extraction visible spectrophotometric method for the determination of RAM, based on its tendency to form chloroform extractable ion-association complex with acidic dye belonging to Azo (monoazo) category dye TP000 (CI No. 15510) under experimental conditions by exploiting the basic nature of the drug molecule.

MATERIALS & METHODS (EXPERIMENTAL)

A Systronics UV/Visible spectrophotometer model -2203 with10mm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade. Tropaeolin 000 (Fluka, 0.2%, $5.7x10^{-3}$M prepared by dissolving 200mg of Tropaeolin 000 in 100ml distilled water and subsequently washed with chloroform to remove chloroform soluble impurities), 0.1M HCl (prepared by diluting 8.7ml of Con. Hydrochloric acid to 1000ml with distilled water and standardized) were prepared.

Standard solution:

The standard stock solution (1mg/ml) of RAM was prepared by dissolving 100mg of RAM initially in 10ml of 0.1M sodium hydroxide, followed by dilution to 100 ml with distilled water. The working standard solution of RAM (100µg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent.

Sample solution:

About 20 tablets were pulverized and the powder equivalent to 100mg 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 the standard RAM solution (0.5ml-2.5ml, 100µg/ml) were placed in a series of 125ml separating funnels. A volume of 6.0ml of 0.1M HCl and 2.0ml of TPOOO were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0ml with distilled water. Then 10.0ml of chloroform was added to each funnel, and the contents were shaken for 2 minutes.

The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at 489.0nm (Fig. 2 showing absorption spectra) against a reagent blank within the stability period (5minutes to 1hour). The amount of drug was computed from its calibration graph (Fig-3 showing Beer's Law plot).

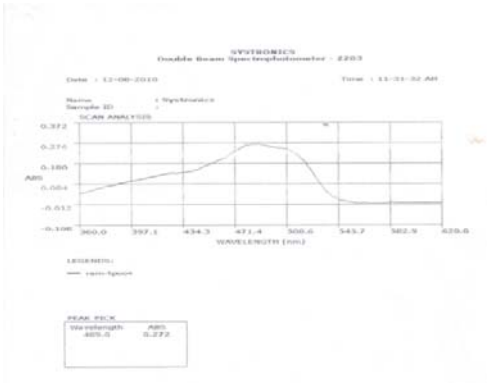

Fig. 2: Showing Absorption Spectra of RAM-TPOOO

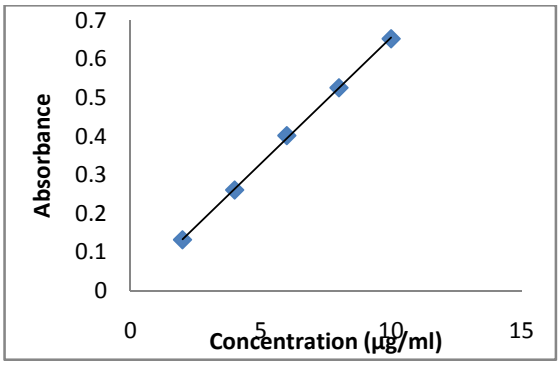

Fig. 3: Showing Calibration Graph of RAM-TPOOO

RESULTS AND DISCUSSIONS

Optimum operating conditions used in the procedure were established adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of TPOOO reagent, 0.1M HCl and solvent for final dilution of the colored species were studied.

The water immiscible solvents tested for the extraction of colored complex into organic phase include chlorobenzene, dichloromethane, carbon tetra chloride, benzene, n-butanol or chloroform. Chloroform was preferred for its selective extraction of colored drug -dye complex into organic layer from the aqueous phase. The stoichiometric ratio of the drug to dye was determined by the slope ratio method and was found to be 1:1.

The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing 3/4th of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (Sb), standard deviation of intercept (Sa), standard error of estimation (Se) 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 preanalyzed formulations at three different concentration levels (50%, 75% and 100%). These results are summarized in Table-2.

CONCLUSION

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 extractive visible spectrophotometric method is validated and possesses reasonable precision, accuracy, 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.

Chemistry of colored species:

The positively charged aliphatic secondary nitrogen of RAM molecule in acid medium is expected to attract the negatively charged part of the acidic dye TPOOO and form an ion pair held together through electrostatic attraction. Based on the analogy, the structure of ion association complex in this method is shown in the scheme (Fig-4).

Table 1: Optical Characteristics, Precision And Accuracy Of Proposed Method.

Parameter	Values
λ_{max} (nm)	489.0nm
Beer's law limit(µg/ml)	2 - 10
Sandell's sensitivity (µg/cm^2/0.001 abs. unit	0.01521
Molar absorptivity (Litre/mole/cm)	27386.2
Correlation Coefficient	0.999
Regression equation (Y)*	
Intercept (a)	+0.002
Slope(b)	0.065
%RSD	0.6502
% Range of errors(95% Confidence limits) 0.05 significance level	0.6826
0.01 significance level	1.0704

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

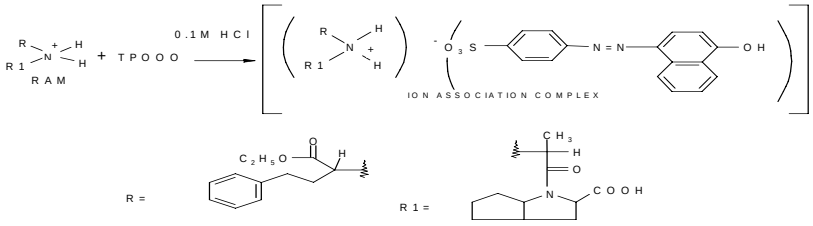

Fig. 4: Showing scheme

Table 2: Analysis Of Ramipril By Proposed And Reference Method

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	f		
TPOOO	Tablet-1	5	4.947 ± 0.0547	1.959	2.261	4.913 ±0.082	98.943 ± 1.0935
	Tablet-2	5	4.956 ± 0.0279	2.55	3.432	4.916 ± 0.015	99.125± 0.5573

* Different batches (Tablet1 &2) 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}=218nm).

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