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Research Article

DEVELOPMENT AND VALIDATION OF RAMIPRIL ESTIMATION FROM CAPSULES USING VISIBLE SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, rapid, sensitive, extraction free and cost effective visible spectrophotometric method has been developed for the determination of Ramipril in bulk and capsule formulations. The proposed method is based on the formation of yellowish brown coloured species by the drug with Folin reagent and exhibits λ_{max} at 456.5 nm. The calibration graph is linear over the concentration range of 16-48µg/ml with Molar absorptivity of $4.98783X10^3$ l/mol/cm and Sandell's sensitivity of 0.08351 µg/cm^2 0.001 abs. unit. The proposed method is applied to commercial available capsules and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. The method is applied successfully for the estimation of the Ramipril in capsule formulations without the interference of excipients.

Keywords: ACE inhibitor, Beer's Law, Estimation, Folin reagent, visible Spectrophotometry.

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] octahydro cyclopenta [b]pyrrole-2-carboxylic acid-1-ethyl ester[1]. 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. RAM is official in USP[2] and BP[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 visible spectrophotometric method for the determination of RAM in pure form and in pharmaceutical dosage forms, since most of the previous methods found to be relatively complicated and expensive. So the authors have made some attempts in this direction and succeeded in developing a method based on the reaction between the drug and Folin reagent (Sodium salt of 1, 2-Naphthaquinone 4-sulphonic acid-NQS)[34]. In this method, yellowish brown colored species (N-alkyl amino napthaquinone) was formed by replacement of the sulphonate group of the napthaquinone sulphonic acid by a secondary amino group of drug. The method can be extended for the routine assay of RAM formulations.

MATERIALS AND METHODS

A Systronics UV/Visible spectrophotometer model -2203 with10mm matched quartz cells was used for all spectral measurements. A Systronics µ- pH meter model-362 was used for pH measurements.

All the chemicals used were of analytical grade. Folin reagent (NQS) solution (Loba, 0.5%, $1.92x10^{-2}$M prepared by dissolving 500mg of NQS in 100 ml of distilled water), pH 8.0 buffer solution (prepared by mixing 30ml of potassium hydrogen phosphate (0.067M) and 970ml of disodium hydrogen phosphate (0.067M) and the pH of the solution was adjusted to 8.0) were prepared.

Standard solution: The standard stock solution (1mg/ml) of RAM was prepared by dissolving 100mg of RAM initially in 10 ml of methanol and then followed by dilution to 100ml with distilled water. The working standard solution of RAM (400µg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent.

Sample solution

Twenty capsules were emptied, pulverized and an amount of powder equivalent to 100mg of RAM was weighed, dispersed in 25ml of IPA, sonicated for 30 minutes and filtered through Whatman filter paper No 41.The filtrate was evaporated and the residue was dissolved as under standard solution preparation.

Assay

Aliquots of the standard RAM solution (1.0ml-3.0ml, 400µg/ml) were placed in series of 25ml calibrated tubes. Then 1.0ml of Folin reagent ($1.092x10^{-2}$M), 5.0ml of pH 8.0 buffer were added, the volume was adjusted to 7.5ml with distilled water in each tube and kept aside for 15 minutes at laboratory temperature for full color development .Then the volume was made up to 25ml with distilled water and mixed well. The absorbance was measured at 456.5 nm (Fig.2 Showing absorption spectra) against the reagent blank within stability period 30 minutes. The amount of drug was computed from its calibration graph (Fig.3 Showing Beer's Law plot).

RESULTS AND DISCUSSIONS

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 Folin reagent and pH buffer solution 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 3/4th 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 (Sb), standard deviation of intercept (Sa), standard error of estimation (Se), % range of error (0.05 and 0.01 confidence limits) were calculated using – MS Excel-2007. These results are shown in Table-1.

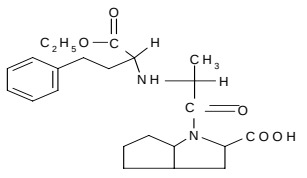

Fig. 1: Showing the chemical structure of RAM

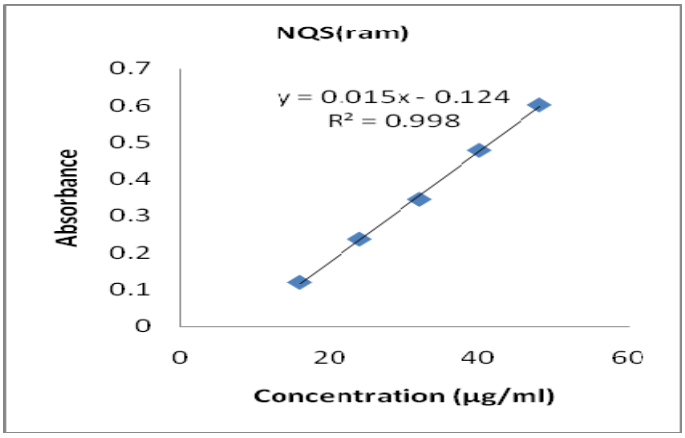

Fig. 3: showing Beer's law plot

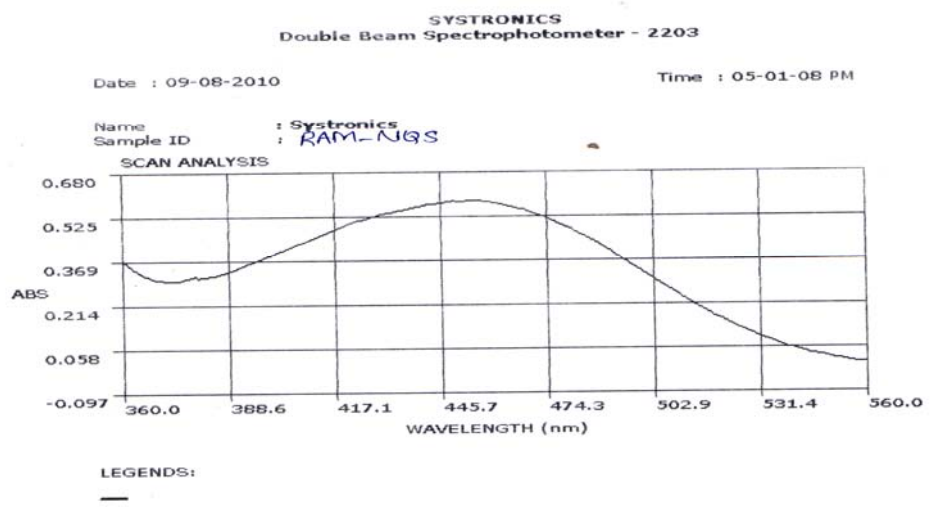

Fig. 2: Showing the absorption spectra of RAM – NQS

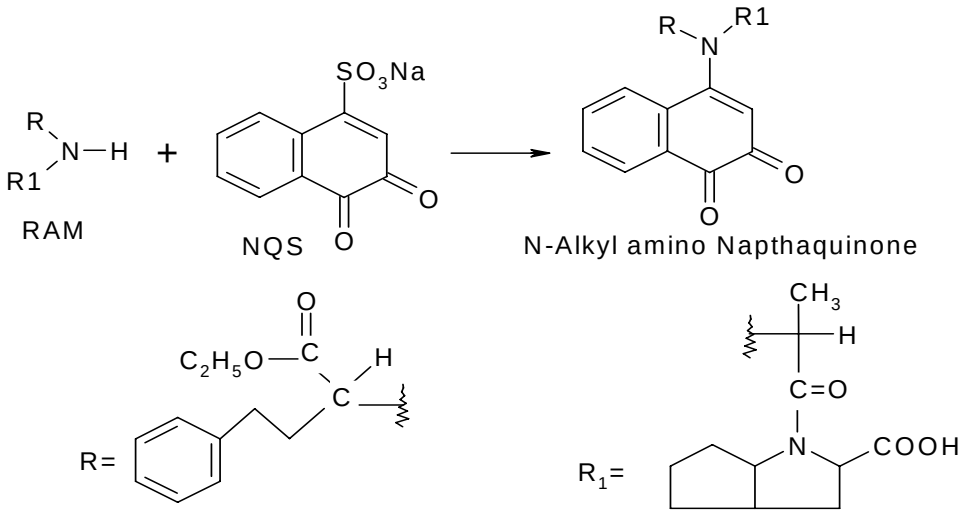

Fig. 4: Showing the scheme

Commercial formulations containing RAM were successfully analyzed by the proposed method. The values obtained by the proposed and reference method (reported UV in methanol λ_{max}=218nm) 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.

Chemistry of colored species

In the present investigation, the presence of aliphatic secondary amino group of RAM permits the development of visible spectrophotometric method for its determination through the nucleophillic substitution reaction with folin reagent. The formation of colored species with this reagent may be assigned through above analogy as shown in Scheme (Fig.4).

Table 1: Optical characteristics, precision and accuracy of proposed method

Parameter	Values
λmax (nm)	456.5
Beer's law limit(µg/ml)	16 - 48
Sandell's sensitivity (µg/cm2/0.001 abs. unit	0.083507307
Molar absorptivity (Litre/mole/cm)	4987.827
Regression equation (Y)*	
Intercept (a)	-0.124
Slope(b)	0.015
Correlation coefficient (R^2)	0.998
%RSD	0.6035
% Range of errors(95% Confidence limits)	
0.01 significance level	0.6335
significance level	0.9935

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

Table 2: Analysis of ramipril 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		
NQS	Capsule-1	5	4.96 ± 0.038	1.816	4.587	4.913 ± 0.082	99.201 ± 0.768
	Capsule-2	5	4.952 ± 0.033	2.596	4.876	4.916 ± 0.015	99.049 ± 0.664

* Capsule-1 and Capsule- 2 from two different companies (Cardiopril from Dr Reddy's and Corpril from Ranbaxy)

**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).

CONCLUSIONS

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.Moreover the method is free from interference by common additives and excipients. The proposed visible spectrophotometric method for the estimation of RAM possess 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.

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REFERENCES

1. Franz D.N., Cardiovascular Drugs (Ed: A. R. Gennaro), in Remington: The Science and Practice of Pharmacy, 19th ed., Vol. II, Mack Publishing Company, Pennsylvania, 1995, p. 951.
2. Royal Pharmaceutical Society, British Pharmacopoeia, vol. II, H. M. Stationery Office, Royal Pharmaceutical Society, London, UK, 2000, pp.1331-1333.
3. The united States Pharmacopoeia 29, NF 24, Asian Edition, Rockville, MD; United States Pharmacopoeial Convention, Inc; 2006, 1890.
4. Belal F, Al-Zaagi IA, Gadkarien EA, Abounassif MA. A stability-indicating LC method for the simultaneous determination of ramipril and hydrochlorothiazide in dosage forms. J Pharm. Biomed Anal 2001; Vol.24: 335-42.
5. Bhushan R, Gupta D, Singh SK. Liquid chromatographic separation and UV determination of certain anti hypertensive agents. Biomedical Chromatography 2005**;** Vol. 20(2): 217-24.
6. Bilal Yilmaz. The Determination of Ramipril in Pharmaceutical preparation by HPLC. Inter. J. of Pharm. Sci. Review and Res. 2010; Vol. 1(1): 39-42.
7. Aboul-Enein HY, Thiffault C. Determination of Ramipril and its precursors by RP-HPLC. Anal Lett 1991; Vol.24 (12): 2217-2224.
8. Motofumi I, Takeo K, Junichi G, Toshio N. Separation Ramipril optical isomers by HPLC. J. Liquid Chromatogr. 1990; Vol.13 (5): 991-1000.
9. Rao K.V, Vijaya kumara K, Bhanuprakash I, Prabhakar G, Begum J. The determination of Ramipril in Pharmaceutical dosage forms by Reversed Phase Liquid Chromatography. Asian J Chemistry 2006; Vol. 18: 788-92.
10. Hogan BL, Mark Williams, Anna Idiculla, Tarik Veysoglu and Ernest Parente. The development and validation of a LC method for the determination of the related substances of Ramipril in Altace capsules. J. Pharm. Biomed Anal.2000; Vol. 23(4):637-651.
11. Zarapakar SS and Rane SH. RP-HPLC determination of Ramipril and Hydrochlorothiazide in tablets. Indian Drugs 2000; vol. 37: 589-593.
12. Harlikar JN, Amlani A.M. Simultaneous Determination of Perindopril, Indapamide, Ramipril, Trandapril in Pharmaceutical formulations using RP- HPLC. Res. J. Chem. Environ 2003; vol. 7: 59- 62.
13. Patel VA, Patel PG, Chaudhary BG, Rajgor NB, Rathi SG. Development and validations of HPTLC method for the simultaneous estimation of Telmisartan and Ramipril in combined dosage form. International Journal on Pharmaceutical and Biological Research 2010; Vol. 1(1): 18-24.
14. Jadranka O, Diljana S, Mirjana A, Dusanka MO, Zivoslav T. Reversed-phase thin-layer chromatography of some angiotensin converting enzyme (ACE) inhibitors and their active metabolites. J Serbian Chem. Soc 2006; 71: 621-8.

15. Zhimeng Z, Andre V and Len N. Liquid chromatography-mass spectrometry method for determination of ramipril and its active metabolite ramiprilat in human plasma. J. Chromatography B., 2002; vol. 779(2): 297-306.
16. Maurer H.H, Kramer T, Arlt J.W. Screening for the detection of angiotensin-converting enzyme inhibitors and their metabolites and AT II Receptor Antagonists, Therap. Drug Monitor 1998; 20: 706-713.
17. Sereda K.M, Hardman T.C, Dilloway M.R, Lant A.F Development of a method for the detection of Angiotensin converting enzyme inhibitors using Electron Capture-Gas Chromatography detection. Anal Proc. 1993; 30(9): 371-372.
18. Al-Majed AA, Belal F, Abadi A, Al- Obaid AM. Voltametric study and determination of ramipril in dosage forms and biological fluids. Farmaco II, 2000; 55(3): 233-238.
19. Eckert HG, Muenscher G, Ockonomopulos R, Strecker H, Urbach J, Wissman H. A radioimmuno assay for the ACE inhibitor Ramipril and its active metabolite. Arzenein, Forsch. / Drug Research1985; Vol. 35(8): 1251-1256.
20. Hillaer S, De Grauwe K and W. Van den Bossche. Simultaneous determination of hydrochlorothiazide and several inhibitors of angiotensin-converting enzyme by capillary electrophoresis. J. Chromatography -A 2001**;** vol. 924: 439- 449.
21. Aboul-Enein HY, Stefen RI and Van Staden JF. Analysis of several angiotensin-converting enzyme inhibitors using potentiometric, enantioselective membrane electrodes. Anal Lett. 1999; vol. 32: 623-632.
22. Aboul-Enein H.Y, Bunaciu A.A, Bala C, Fleischin S. Enalapril and Ramipril selective membranes. Anal Lett.1997; 30:1999-2008.
23. Abdellatef HE, Ayad MM and Taha EA. Spectrophotometric and atomic absorption spectrophotometric determination of Ramipril and perindopril through ternary complex formation with eosin and Cu (II). J. Pharm. Biomed. Anal., 1999; Vol. 18: 1021-1027.
24. Ayad MM, Shalaby AA, Abdellatef HE and Hosny MM. Spectrophotometric and AAS determination of Ramipril and enalapril through ternary complex formation. J. Pharm. Biomed. Anal., 2002; vol. 28: 311-321.
25. Al-Majed A.A and Al-Zehouri J. Use of NBD-F for the determination of Ramipril in tablets and spiked human plasma. Farmco II, 2001; Vol. 56:291-296.
26. Hisham E. Abdellatel. Spectrophotometric and Spectro fluorimetric methods for the determination of Ramipril in its pure and dosage form. Spectro chimica Acta part A: Molecular and Bimolecular spectroscopy 2007; Vol. 66(3): 701-706.
27. Rahman N Ahmed Y and Azmi S.N.H. Kinetic spectrophotometric method for the determination of Ramipril in pharmaceutical formulations. A.A.P.S. Pharm. SciTech, 2005; Vol. 6: 543-551.
28. Blaih SM, Abdine HH, El-Yazbi FA and Shaalan RA. Spectrophotometric determination of enalapril maleate and ramipril in dosage forms. Spectroscope Lett. 2000; vol. 33: 91-102.
29. Salama FM, El-Sattar OIA, El-Aba Sawy NM and Fuad MM. Spectrophotometric determination of some ACE inhibitors through charge transfer complexes. Al Azhar J. Pharm Sci. 2001; vol. 27: 121- 132.
30. Al-Majed A.A, Belal F and Al-Warthan AA. Spectrophotometric determination of Ramipril (a novel ACE inhibitor) in dosage forms. Spectroscope Lett. 2001; Vol. 34: 211-220.
31. Rahman N, Rahman H and Azmi S.N.H. Kinetic spectrophotometric method for the determination of Ramipril in commercial dosage forms. International Journal of Biological and Medical Sciences, 2007; vol.2 (1): 52-54.
32. Singhvi I and Chaturvedi SC. Visible spectrophotometric and HPLC methods for estimation of Ramipril from capsule formulation. Indian J Pharm. Sci. 2001; Vol. 1: 69-72.
33. Bankey S, Papdiy GG, Saboo S.S,Bindaiya S, Deepti Jain, Khadbadi SS. Simultaneous determination of Ramipril , Hydrochlorothiazide and Telmisartan by UV Spectrophotometry. International Journal of Chem. Tech Research, 2009; Vol. 1(2): 183-188.
34. Rosenblatt DH, Hlinka P, Epstein J. Colorimetric determinations of aromatic and aliphatic amines. Anal Chem. 1955; Vol. 27: 1290.