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Research Article

Development and Validation of RP-HPLC Method for Estimation of Ramipril in Tablet Dosage Form

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ABSTRACT

The present work explains the development and validation of a simple and reliable RP-HPLC method for the quantitative determination of Ramipril (RMP). Chromatography was carried out by reversed phase technique on a Fortis C18 (100 mm × 4.6 mm; 2.5 µm particle size). The optimized mobile phase was consisted of methanol and citric acid sodium citrate buffer solution (50:50 v/v) having pH 3.0. The retention times were 3.645 min for RMP. The detection was carried out at 270 nm and a column temperature of 25°C. The method was evaluated for the various validation parameters, such as linearity, accuracy, precision, LOD, LOQ, specificity, selectivity, and sample stability. The proposed method was validated and successfully applied for the analysis of pharmaceutical formulations and laboratory prepared mixture containing Ramipril respectively.

Keywords: Ramapril; LOD; LOQ; RP-HPLC; Column temperature

INTRODUCTION

(2S, 3aS, 6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta (b) pyrrole-2-carboxylic acid Ramipril inhibits the RAAS system by binding to and inhibiting ACE there by preventing the conversion of angiotensin I to angiotensin II. As plasma levels of angiotensin II fall, less activation of the G-protein coupled receptors Angiotensin Receptor I (ATR1) and Angiotensin Receptor II (ATR2) occurs. The extent of absorption is at least 50-60%. Food decreases the rate of absorption from the GI tract without affecting the extent of absorption. The absolute bio availabilities of Ramipril and Ramipril at were 28% and 44%, respectively, when oral administration was compared to intravenous administration.

The serum concentration of Ramipril at was unchanged when capsules were opened and the contents dissolved in water, dissolved in apple juice, or suspended in apple sauce. Ramipril is a pro drug that undergoes de esterification in the liver to form Ramipril at, its active metabolite. Ramipril rapidly distributes to all tissues, with the liver, kidneys and lungs showing markedly higher concentrations of the drug than the blood. Hepatic metabolism accounts for 75% of total Ramipril metabolism. 25% of hepatic metabolism produces the active metabolite Ramipril at *via* liver esterase enzymes. 100% of renal metabolism converts Ramipril to Ramipril at. Other metabolites, diketopiperazine ester, the diketopiperazine acid, and the glucuronides of ramipril and ramipril at, are inactive.

Route of elimination 60% of the parent drug and its metabolites are eliminated in the urine with the remaining 40% eliminated in the feces. The drug eliminated in the feces represents both absorbed drug and drug eliminated through biliary excretion although the proportion of these has not been determined. Less than 2% of drug is eliminated in the urine unchanged (Figure 1). Ramipril is effective for shortening the duration of labor and it may be useful in the treatment of irritable bowel syndrome and in peptic and gastric ulcers [1].

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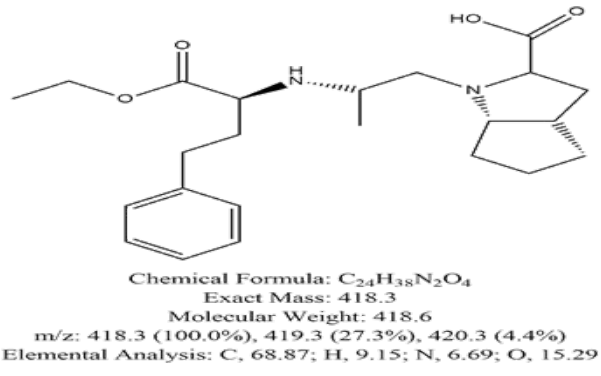

Figure 1: Ramipril.

MATERIALS AND METHODS

Accurately weighed quantity (10 mg) of Ramipril was transferred to 10 ml volumetric flask, dissolved and diluted up to the mark with methanol. From this solution, 0.2 ml was diluted to 10 ml with methanol (concentration 20 µg/ml). The solution was mixed and filtered through 0.2 µ membrane filter accurately weighed quantity (404 mg) of RMP was transferred to 10 ml volumetric flask, dissolved and diluted up to the mark with methanol. From this solution, 0.2 ml was diluted to 10 ml with methanol (concentration 20 µg/ml). The solution was mixed and filtered through 0.2 µ membrane filter. 12.03 gm sodium citrate and 1.72 gm citric acid in 1000 ml water and adjust pH 3.2 with ortho phosphoric acid standard stock solution A and B were appropriately diluted with methanol to obtain final concentration of 20 µg/ml each. The diluted standard solutions were filtered through 0.2 µ membrane filter. The filtrates were injected into the HPLC system and run in different solvent systems. Mixture of solvents with varying polarity were tried in order to determine optimum chromatographic conditions for RMP. After several permutation and combination, it found that mixture 5 mM buffer solution and methanol gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase 5 mM buffer: Methanol (50:50 v/v) was selected as it gave high resolution of Ramipril with minimal. Retention time for Ramipril is 3.64 minute. 5 mM sodium citrate in citric acid buffer (5 mM) was prepared by dissolving accurately weighed quantity 12.03 g of Sodium citrate and 1.72 gm citric acid in a 1000 ml of double distilled water. Mobile phase was prepared by mixing 100 ml of 5 mM Phosphate buffer with 100.0 ml of methanol. This mobile phase was ultrasonicated for 10 minutes and then it was filtered through 0.45 µ membrane filter standard stock solution A and B were diluted separately with mobile phase to obtain final concentration of 20 µg/ml of RMP. Each solution was scanned using double beam UV visible spectrophotometer 1800 in the spectrum mode between the wavelength range of 400 nm to 200 nm and their spectra was overlaid (Tables 1 and 2). The wavelength selected was 270 nm as the drugs showed significant absorbance at this wavelength (Figures 2 and 3) [2-6].

Table 1: Optimized chromatographic conditions.

Mobile phase	**MeOH: Buffer (50:50 v/v)**
Column	C18 (FORTIS)
Flow rate	1.0 ml/min
Detection wavelength	270 nm
Injection volume	20 µL
Run time	10 minutes

Table 2: Analysis of formulation and recovery studies.

Level of recovery (%)	**Weight of tablet powder taken (g)**	**Mean peak area***	**Amount of drug added (mg)**	**Amount of drug recovered (mg)**	**% Recovery**
		RMP	**RMP**	**RMP**	**RMP**
80	0.404	1946.89	8	8.05	100.66
80	0.404	1941.38	8	8	100
100	0.404	2143.88	10	9.95	99.54
100	0.404	2138.67	10	9.9	99.04
120	0.404	2371.9	12	12.15	101.29

120	0.404	2361.83	12	12.05	100.48
				Mean	100.16
				S.D.	± 0.811
				C.V.	0.809

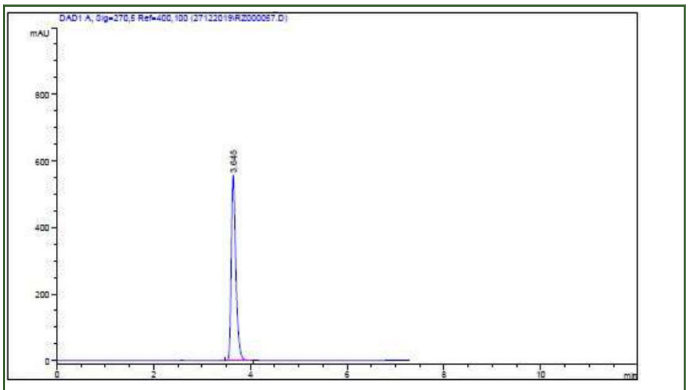

Figure 2: Typical chromatogram of Ramipril.

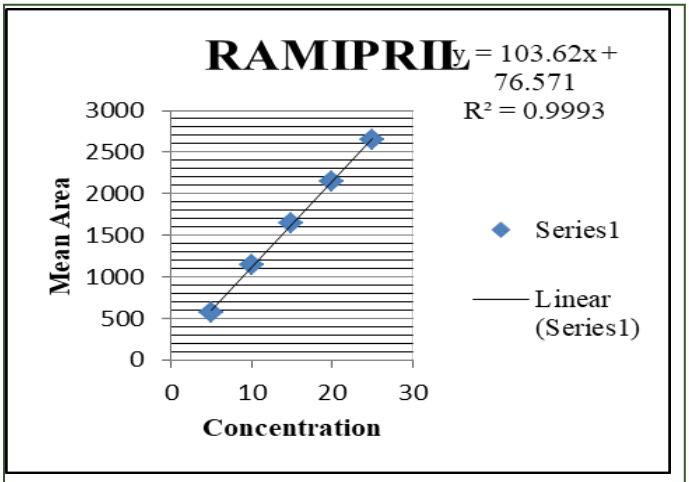

Figure 3: Curve of Ramipril.

RESULTS

Recovery studies: To study the accuracy, reproducibility and precision of the above methods, were carried out by addition of standard drug solution to pre analyzed sample at different levels. Results of recovery were found to be satisfactory and are reported in Table 2 [7,8].

Validation of proposed method

An accurately weighed quantity of pre analysed tablet powder equivalent to about 80 mg Ramipril was transferred individually in six different 50.0 ml volumetric flasks (Table 3).

Content of drug PA spl weight of std. (mg) df spl avg. weight of in sample=~~~~~×~~~~~~~~~~~~~~~~×~~~~~~~~~~~~~~~~× tablet (g) (mg/tab) PA std df std weight of tablet powder taken (g)~~~~~ (Equation 1)

Where, PA spl: Peak Area of sample; PA std: Peak area of Standard; df std: dilution factor for standard; df spl: dilution factor for sample [9-12].

Table 3: Ramipril concentration (µg/ml) *vs.* mean peak area.

Ramipril	
Concentration (µg/ml)	**Mean peak area***
0	0
5	567.21
10	1137.69
15	1649.06
20	2147.34
25	2652.8

Then 30 ml mobile phase was added to each flask and content of the flask were ultrasonicated for 20 minutes, volume was then made up to the mark with mobile phase. The solution was individually mixed and filtered through Whatman filter paper no. 42. From the filtrate, 5.0 ml solution was diluted to 50.0 ml with mobile phase. Further dilute 1.0 ml of this solution to 10

ml with mobile phase. The diluted solution was filtered through 0.2 µ membrane filter. Equal volume of standard and sample solution (20 µl) were injected (in triplicate) into the column and chromatographed using optimized chromatographic conditions. The corresponding chromatograms were recorded and area of each peak for RMP was measured at 270 nm (Table 4). Amount of RMP in sample was calculated by comparing the mean peak area for standard and sample solution by equation 1 [13-17].

Table 4: Chromatographic parameters of robustness evaluation.

Chromatographic changes			
Factor	**Conc. (µgm/ml)**	**Area**	**Retention time**
Flow rate (ml/min) ± 0.1 ml	**Conc. (µgm/ml)**	**RMP**	**RMP**
0.9	15	1838.55	5.135
1.1	15	1613.14	4.466
	Mean	1725.84	4.800
	S.D.	159.38	0.4730
Mobile phase (v/v) ± 1 ml	**Conc. (µgm/ml)**	**RMP**	**RMP**
51:49:00	15	1722.05	4.694
49:51:00	15	1724.79	4.94
	Mean	1723.42	4.797
	S.D.	1.9374	0.473
Wavelength change ± 1 nm	**Conc. (µgm/ml)**	**RMP**	**RMP**
269	15	1870.9	4.831
271	15	1779.64	4.831
	Mean	1825.27	4.831
	S.D	64.53	0

Robustness of method: To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of change in flow rate and mobile phase ratio on retention time and tailing factor were studied. The solution containing 15 µg/ml of RMP was injected (in twice) into sample injector of HPLC under the varied conditions [18-20].

Forced degradation study of Ramipril

In forced degradation studies, intentional degradation was tried by exposing powdered tablet sample to following stress condition (Table 5). Add 5.0 ml of 0.1 N HCl (acid degradation), 0.1 N, NaOH (alkali degradation) and 3% H_2O_2 (oxidative degradation) respectively, (neutral degradation) containing tablet powder was kept for 15 min at room temperature (neutral degradation) forced degradation was performed in the dark to exclude the possible degradative effect of light [21-24].

Table 5: Force degradation study of Ramapril.

Sr. No.	**Stress condition**	**Concentration**	**Retention time**
		RMP	
1	Acid (0.1 N HCl)	15	4.832

2	Alkali (0.1 N NaOH)	15	4.824
3	Oxide (3% H_2O_2)	15	4.825
4	Neutral	15	4.818

DISCUSSION

Single dose tablet formulation containing Ramipril is available in market (ZIRAM) for the treatment of hypertension. A thorough literature survey revealed few spectrophotometric methods like simultaneous equation method and first derivative spectrophotometric method for simultaneous estimation of these drugs in pharmaceutical formulations. In the present work a successful attempt has been made to develop simple and specific RP-HPLC method for simultaneous estimation of Ramipril tablet formulation. As an alternative to reported methods two spectrophotometric methods, multi component mode method and area under curve methods, were also developed and validated. The multicomponent mode method has the advantage that it involves no manual calculations. The instrument directly calculates the concentrations based on standard absorptivity values thereby requiring less time for analysis. The area under curve method requires only measurement of absorbance at selected wavelengths and solving of simultaneous equations. The developed methods were statistically validated using one way ANOVA which suggested that there exist no significant difference between all methods. A RP-HPLC method has been developed and validated for simultaneous estimation of Ramipril in single dosage form. Fortis C18 (100 mm × 4.6 mm i.d.) column was used as stationary phase. After trying several permutation and combinations, it was found that mixture of methanol and sodium citrate in citric acid buffer (5 mM pH 3) gives good resolution of peaks with minimal tailing, as compared to other mobile phases. The optimum composition of the mobile phase methanol: sodium citrate in citric acid buffer (5 mM pH 3) (50:50 v/v) at 1 mL/minute flow rate was selected for the analysis. Retention time for RMP was found to be 3.64 min. Solution of RMP in appropriate dilution was scanned using double beam UV visible spectrophotometer 1800 in the spectrum mode between the wavelength range of 400 nm to 200 nm and their spectra was overlaid (Table 6). The wavelength selected was 270.0 nm as the drug was found to have significant absorbance at this wavelength.

Table 6: RP-HPLC method for simultaneous estimation of Ramipril.

Factor	Coefficient estimate	Standard df	95% CI error	95% CI low	95% CI high
Intercept	0.68	1	1.73E-03	0.67	0.68
A: Absorbance	-9.65E-04	1	2.10E-03	-5.50E-03	3.57E-03
B: Flow rate	0.024	1	2.10E-03	0.019	0.028
C: Column temp.	-4.89E-03	1	2.10E-03	-9.42E-03	-3.62E-04
AB	3.75E-03	1	2.74E-03	-2.17E-03	9.67E-03
AC	-8.75E-03	1	2.74E-03	-0.015	-2.83E-03
BC	6.25E-03	1	2.74E-03	3.30E-04	0.012

CONCLUSION

The method provides selective quantification of Ramipril this developed RP-HPLC method for estimation of Ramipril is accurate, precise, robust and specific. The method has been found to be better than previously reported method, because of its less retention time, isocratic mode and readily available mobile phase, readily available column, UV detection and better resolution of peaks. The anti-hypertensive agents of Ramipril results from to prevent cardiovascular disease in those at high risk decrease in blood pressure. Ramipril is primarily responsible for the anti-hypertensive activity.

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REFERENCES

1. Khopkar SM. Basic concepts of analytical chemistry. 1st edition. New Age International Ltd. Publishers, New Delhii, India. 1998;2:178-179.

2. Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis. 7th edition. Thomson Publications, Faridabad, India. 2017:1-3(6):145-147.
3. Mendham J, Denney RC, Barnes JD, Thomas M. Vogel's Textbook of Quantitative Analysis. 5th edition. Pearson Education, Singapore. 2003;8-9.
4. Christian GD. Analytical Chemistry. 7th edition. John Wiley and Sons. INC, New York. 1994. 2003.
5. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th edition part two. CBS Publishers and Distributors, New Delhi, India. 2002;2:275-288.
6. Sethi PD, Sethi R. HPLC High Performance Liquid Chromatography: Quantitative Analysis of Pharmaceutical Formulations. CBS Publishers and Distributors, New Delhi, India. 2001;1:3-120.
7. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis. 7th edition. CBS Publishers And Distributors, Delhi, India. 2001:7-14.
8. ICH HT. Q2A Text on Validation of Analytical Procedures. International Conference on Harmonization, Geneva. 1994;1-5.
9. ICH M. Q2B validation of analytical procedures: Methodology. International Conference on Harmonization, Geneva, Switzerland. 1996;6:1-8.
10. Singh S, Bakshi M. Stress test to determine inherent stability of drugs. Pharm Technol. 2000;4:1-4.
11. ICH O. Q1A Stability Testing of New Drug Substances and Products. International Conference on Harmonization, Geneva, Switzerland. 1993.
12. Patel JR, Pethani TM, Vachhani AN, Sheth NR, Dudhrejiya AV. Development and validation of bioanalytical method for simultaneous estimation of ramipril and hydrochlorothiazide in human plasma using liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2014;970:53-59.
13. Nagavi JB, Anantharaju PG. Analytical RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ramipril and Hydrochlorothiazide in Tablet Dosage Form. Am J PharmTech Res. 2014;4(4):2249.
14. Yadav VJ. HPLC method for determination of ramipril. Int J Pharm Boil Res. 2012;3(3):90-97.
15. Gupta VK, Jain R, Lukram O, Agarwal S, Dwivedi A. Simultaneous determination of ramipril, ramiprilat and telmisartan in human plasma using liquid chromatography tandem mass spectrometry. Talanta. 2011;83(3):709-716.
16. Yilmaz B. Determination of ramipril in pharmaceutical preparations by high performance liquid chromatography. Int J Pharm Sci Rev Res. 2010;1(1):39-42.
17. Zhu Z, Vachareau A, Neirinck L. Liquid chromatography mass spectrometry method for determination of ramipril and its active metabolite ramiprilat in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2002;779(2):297-306.
18. Jawla S, Jeyalakshmi K, Krishnamurthy T, Kumar Y. Development and validation of simultaneous HPLC method for estimation of telmisartan and ramipril in pharmaceutical formulations. Int J Pharmtech Res. 2010;2(2):1625-1633.
19. Lu XY, Shen-Tu JZ, Liu J. High performance liquid chromatography–mass spectrometric analysis of ramipril and its active metabolite ramiprilat in human serum: Application to a pharmacokinetic study in the Chinese volunteers. J Pharm Biomed Anal. 2006;40(2): 478-483.
20. Anjaneyulu N. Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lamivudine and Tenofovir Disproxil Fumerate in Combined Dosage Form. Asian J Biomed Pharm Sci. 2013;3(23):7-11.
21. Rajput PS, Kaur A, Gill NK, Mittal K, Sarma GS. Simultaneous Estimation of Ramipril and Amlodipine in Bulk and tablet Dosage form by RP-HPLC Method. J Appl Pharm Sci. 2012;2(7):160-165.
22. Patel CV, Khandhar AP, Captain AD, Patel KT. Validated Absorption Factor Spectrophotometric and Reversed Phase High Performance Liquid Chromatographic Methods for the Determination of Ramipril and Olmesartan Medoxomil in Pharmaceutical Formulations. Eurasian J Anal Chem. 2007;2(3):1306.
23. Lakshmi KS, Sivasubramanian L. A stability indicating HPLC method for the simultaneous determination of valsartan and ramipril in binary combination. J Chil Chem Soc. 2010;55(2):223-226.
24. Sweetman SC, Martindale I. The complete drug reference pharmaceutical press. London, UK. 2002.