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Determination of Ramipril in Pharmaceutical Dosage Forms by Reversed-Phase Liquid Chromatography

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A rapid and sensitive HPLC method was developed for the estimation of ramipril in pharmaceutical dosage forms. Ramipril was chromatographed on a reverse phase C18 column, mobile phase consisting of 0.05 M KH_2PO_4, acetonitrile and methanol in the ratio of 40 : 40 : 20. The mobile phase was pumped at a flow rate of 1.0 mL/min and the eluent was monitored at 216 nm. The calibration curve was linear in the range of 0.5–50 μg/mL. The results of the study show that the present HPLC method is simple, precise, specific, less time consuming and accurate for the estimation of ramipril in pharmaceutical dosage forms.

Key Words: Ramipril, Dosage forms, Reverse-phase HPLC.

INTRODUCTION

Ramipril (RAM) is a 2-aza-bicyclo [3,3,0]-octane-carboxylic acid derivative chemically (2s, 3as, 6as)-1[(S)-N-[(S)-1-carboxy-3-phenyl propyl]alanyl]octahydrocyclopenta [b]pyrrole-2-carboxylic acid, 1-ethyl ester. Ramipril inhibits angiotensin-converting enzyme (ACE) in human subjects and animals. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin-I to the vasoconstrictor substance, angiotensin-II. Angiotensin-I also stimulates aldosterone secretion by the adrenal cortex. Inhibition of ACE results in decreased plasma angiotensin-II, which leads to decreased vasopressor activity and to decreased aldosterone secretion. The later decrease may result in a small increase of serum potassium.

Literature survey reveals that few HPLC[1–6], spectrophotometric methods[7–9], gas chromatography[10], atomic absorption spectroscopy[11], radio immuno assay[12], potentiometric methods[13], TLC[14] and LCMS[15, 16] were reported for its analytical monitoring in either biological fluids or formulations. In this attempt the author has developed a sensitive and precise HPLC method for the determination of RAM in bulk samples and pharmaceutical formulations by using a C18 column

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[(Kromosil BDS C-18 (250 × 4.6) mm, packed with 5 micron)] mobile phase combination [0.05 M KH_2PO_4 : acetonitrile : methanol (pH adjusted to 3.0 with orthophosphoric acid) 40 : 40 : 20].

EXPERIMENTAL

Quantitative HPLC was performed on a gradient HPLC waters with Shimadzu 10AT vp series HPLC pump, SIL 10AD vp series auto sampler equipped with a 20 µL sample loop and SPD 10A vp dual absorbance detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software. Kromosil BDS C-18 (250 × 4.6 mm, packed with 5 micron) column was used for the separation.

Reagents used: Acetonitrile HPLC grade (E. Merck), potassum dihydrogen phosphate Excelar grade (Qualigens), orthophosphoric acid Excelar grade (Qualigens) and methanol HPLC grade (E. Merck) and used as supplied.

Mobile phase and stationary phase: A mixture of 0.05 M KH_2PO_4 : acetonitrile : methanol (40 : 40 : 20, v/v), adjusted to pH 3.0 with orthophosphoric acid was used as a mobile phase. A Kromosil BDS C-18 column (250 × 4.6 mm, packed with 5 µm) was used as a stationary phase.

Standard stock solution: Standard stock solution was prepared by dissolving accurately weighed 20 mg of RAM in 100 mL of methanol (0.2 mg/mL).

Working standard solution: A 5 mL volume of standard stock solution was diluted to 50 mL with mobile phase. This solution was used as working standard for assay analysis.

Sample solutions

Capsules: Twenty capsules were weighed and crushed to a fine powder. An accurately weighed portion of this powder equivalent to 25 mg of RAM was taken in a 50 mL volumetric flask; about 30 mL of methanol was added to it and the flask was kept in an ultrasonic bath for 10 min. The solution was then diluted to 50 mL with methanol. This solution was then centrifuged; 2 mL of the supernatant solution was diluted to 50 mL with mobile phase and used for the analysis.

Calibration: Aliquots of standard stock solution of RAM were taken in different standard volumetric flasks and diluted with mobile phase to obtain the final concentrations of RAM in the range 0.5–50 µg/mL. Each prepared solution was injected into the chromatograph. The evaluation of RAM was performed with UV detector at 216 nm. Peak areas were recorded for all the chromatograms. Calibration curve was constructed by plotting peak areas (Y-axis) against the amount of drug in µg/mL (X-axis) and the linear relationship was evaluated by calculation of regression line by the method of least squares.

Assay: Each working standard and sample solution were injected into the chromatograph and the peak areas were recorded as described in the calibration procedure. From the peak area of RAM in standard and sample solutions the amount of RAM was computed by external standard quantification.

RESULTS AND DISCUSSION

Chromatography: To develop the HPLC method for the determination of ramipril (RAM), different mobile phases were employed. Initially a mobile phase consisting of 0.05 M KH_2PO_4 : acetonitrile : methanol in the ratio of 30 : 30 : 40 were tried. Kromosil BDS-C 18, column 250 × 4.6 mm packed with 5 microns was used. Early elution with tailing of peaks was observed in the above condition. Then the composition of mobile phase was changed in the ratio of 40 : 35 : 25; under these conditions broad peak shape and pronounced tailing was observed. For the same mobile phase, the ratio of KH_2PO_4, acetonitrile and methanol was changed to 40 : 40 : 20 and used as eluent. RAM was eluted at around 4.8 min with symmetric peak shape. A typical chromatogram of RAM is shown in Fig. 1.

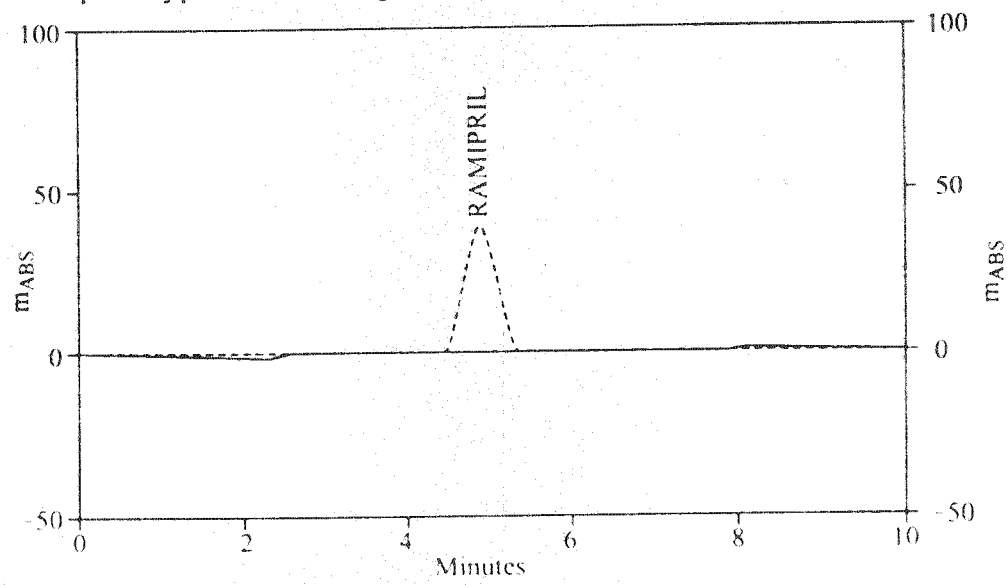

Fig. 1. Model chromatogram for Ramipril (RAM)

The wavelength of 216 nm was selected from the UV detection because at this wavelength RAM exhibits a maximum.

System suitability: To ascertain the effectiveness of system suitability test, five replicate injections of freshly prepared standard stock solution of RAM (5 µg/mL) were injected into the chromatograph and the relative standard deviation (RSD) of peak area was calculated. Observed RSD was 0.29% (USP limit is not more than 2%).

Linearity, limit of detection and limit of quantification: The plot of the peak area *vs.* the respective concentration of RAM was found to be linear in the range of 0.5–50 µg/mL. The calibration curve could be represented by the following linear regression equation:

$$Y_{RAM} = 0.951 \times 10^3 + 0.1140 \times 10^5 X \quad (r = 0.9998)$$

where Y is area, X is concentration in µg/mL and r is correlation coefficient.

The limit of detection (LOD) and the limit of quantification (LOQ) of RAM were calculated by using equations given in the International Conference on Harmonization (ICH) Guidelines[17].

The limits of detection and the limits of quantification for RAM were found to be 0.3 and 0.9 µg/mL, respectively.

Accuracy and precision: To study the accuracy and the precision of the proposed methods, recovery experiments were carried out by standard addition technique. Five different levels of standards were added to preanalysed capsule samples and each level was repeated three times. The percentage recoveries were calculated and the results obtained are shown in Table-2. The percentage recovery was in the range of 100.55–100.41%. These results indicate that the method is accurate and precise and also there is no interference due to the excipients present in the formulations.

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS OF THE PROPOSED HPLC METHOD FOR RAMIPRIL

Parameter	Method
Detection wavelength (nm)	216
Linearity range (μg/mL)	0.5–50
Detection limits (μg/mL)	0.2183×15^5
Regression equation (Y = a + bC)	
Slope (b)	0.1140×15^5
Standard deviation of slope (S_b)	0.00125×15^3
Intercept (a)	0.951×15^3
Standard deviation of intercept (S_a)	0.83×10^3
Standard error of estimation (S_e)	0.791×10^3
Correlation coefficient	0.9998

TABLE-2
RESULTS OF THE RECOVERY ANALYSIS

Sample	Amount of drug from tables/suspension (mg)	Amount of drug added (mg)	Total (theoretical) amount of drug (mg)	Amount found (mg)	R.S.D. (%)	Recovery (%)
CARDIOPRIL (Capsules)	5.0	0.0	5.0	5.02	0.23	100.55
HOPECARD (Capsules)	5.0	2.5	7.5	7.47	0.54	99.89
RAMAE (Capsules)	5.0	5.0	10.0	10.03	0.86	100.30
RAMIPRES (Capsules)	5.0	7.5	12.5	12.54	0.16	100.41

Conclusions: The proposed method is simple, precise, accurate and rapid for the determination of RAM from formulations. Hence it can be easily and conveniently adopted for the routine quality control analysis.

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REFERENCES

1. H.Y. Aboul-Enein and S.A. Bakr, *Drug Dev. Ind. Pharm.*, **18**, 1013 (1992).
2. H.Y. Abou-Enein and C. Thiffault, *Anal. Lett.*, **24**, 2217 (1991).
3. M. Ito, T. Kuriki, J. Goto and T. Namabara, *J. Liq. Chromatogr.*, **13**, 991 (1990).
4. S.S. Zarapaker and S.H. Rane, *Indian Drugs*, **37**, 589 (2000).
5. D. Bonazzi, R. Gotti, V. Andrisano and V. Cavrini, *J. Pharm. Biomed. Anal.*, **16**, 431 (1997).
6. U.J. Dhorda and N.B. Shetkar, *Indian Drugs*, **36**, 638 (1999).
7. A.A. Al-Majed, F. Belal and A.A. Al-Warthan, *Spectrosc. Lett.*, **34**, 211 (2001).
8. S.M. Blaih, H.H. Abdine, F.A. El-Yazbi and R.A. Shaalam, *Spectrosc. Lett.*, **33**, 91 (2000).
9. H.E. Abdellatef, M.M. Ayad and E.A. Taha, *J. Pharm. Biomed. Anal.*, **16**, 431 (1997).
10. D. Schmidt, A. Keller and Z. Fresenius, *Anal. Chem.*, **320**, 731 (1985).
11. M.M. Ayad, A.A. Shalaby, H.E. Abdellatef and M.M. Hosny, *J. Pharm. Biomed. Anal.*, **28**, 311 (2002).
12. H.G. Eckert, G. Muenscher, R. Oekonomopulos, H. Strecker and H. Urbach Wissmann, *Arzneim-Forsch*, **377**, 788 (1985).
13. H.E. Abdellatef, M.M. Ayad and E.A. Taha, *J. Pharm. Biomed. Anal.*, **3**, 431 (1997).
14. M.B. Aleksic, D.G. Agbaba and D.M. Milojkovic-Opsenica, *Chromatographia*, **53**, S–442 (2001).
15. P.R. Tiller, L.A. Romanyshyn and U.D. Neue, *Anal. Bioanal. Chem.*, **377**, 788 (2003).
16. Z.M. Zhu and A. Vachareau, *J. Chromatogr. B*, **377**, 788 (2003).
17. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Harmonized Tripartite Guideline on Validation of Analytical Procedures: Methodology, Recommended for Adoption on September 4 at the ICH Steering Committee, IFPMA, Switzerland.

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