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Simultaneous Determination and Validation of Ramipril And Hydrochlorthiazide by RP-HPLC Method

Madhavi.A*, A.Sathishkumar Shetty

National College Of Pharmacy, Shivamogga, Bengalore, India

Abstract : RP-HPLC method in which simultaneous determination of Ramipril and Hydrochlorthiazide, in bulk and combined formulations was carried on a reverse phase C-18 column using a mobile phase consisting of Orthophosphate buffer: acetonitrile(60:40v/v) with a neutral pH. The mobile phase was pumped at a rate of 0.8ml/min and the UV detection was carried out at 294 nm. The linearity was found to be in the range of 50-150 µg/ml and 50-150µg/ml with regression coefficient 1, for Ramipril and Hydrochlorthiazide respectively. The peaks obtained were sharp having clear baseline separation with a retention time of 2.4 min and 1.4 min for Ramipril and Hydrochlorthiazide respectively.
Key words : Ramipril, Hydrochlorthiazide, RP-HPLC.

Introduction:

Ramipril is chemically(2s,3as,6as)-1-[(s)-N-[(s)-1-carboxy-3-phenyl Propyl] alanyl] octahydro cyclopenta [b]pyrrole-2- Carboxylic acid-1-ethyl ester 2,4,7-triamino,6- phenylpteridine has molecular formula $C_{23}H_{32}N_2O_5$ and molecular weight416.511g/mol[1-6].Ramipril is an angiotensin-converting enzyme(ACE) inhibitorused to treat high blood pressure (hypertension), congestive heart failure and chronic renal failure. Ramipril inhibits angiotensin-converting enzyme (ACE) and reduces the angiotensin II levels which leads to the reduction of aldosterone secretion thus lowering of blood pressure.

Aldosterone is a hormone produced by the adrenal glands to help regulate the salt and water balance in your body.[7-15]

Hydrochlorthiazide is chemically 6-chloro-3,4-dihydro-2H-1,2,4, benzothiadiazine- 7-sulphonamide 1,1dioxide. It belong to thiazide diuretics, widely used in treatment of hypertension and edema associated with mild to moderate congestive heart failure[1-6]. It increases the rate of urine excretion by the kidneys through decreased tubular reabsorption of sodium and chloride ions and by increasing osmotic transport of water to the renal tubules, which in turn lowers cardiac output and blood pressure. Ramipril shows an additive effect in reducing the systolic and diastolic blood pressure when combined with Hydrochlorthiazide. In monotherapy non responders, the combination improves response and blood pressure normalization rates compared with monotherapy. In addition to providing enhanced blood pressure reduction and control, the combination of Ramipril and Hydrochlorthiazide is safe well tolerated with the lower incident of adverse effects compared to that observed in the monotherapy.[7-15]

On literature survey it was found that Ramipril and Hydrochlorthiazide has been estimated independently and in combination with the other drugs by several Spectrophotometric and Bioanalytical methods. However no method could be found for simultaneous estimation of Ramipril and Hydrochlorthiazide in bulk and pharmaceutical formulation and also no method is available for such estimation

in the Pharmacopoeias. A successful attempt has been made for simultaneous determination of RAM and HCZ in combined dosage form. Therefore, it was thought worthwhile to developed simple precise, accurate and reliable RP-HPLC method for simultaneous estimation of both the drug in combined dosage form.[16-28]

Experimental:

Chemicals and Reagents: Ramipril and Hydrochlorthiazide was provided by Sun Pharmaceutical industries(Bengalore , India). The commercially available tablets formulation Altace HCT was used for quantitative determination. Potassium dihydrogen orthophosphate was of analytical grade. Acetonitrile was HPLC grade purchased from Merck.Chem.Ltd., Mumbai.

Instrumentation:

The HPLC WAS MODEL SHIMADZU lc-2010 composing quaternary pump,autosampler,mobile phase degaser,heated column thermostat,and variable uv detector. The mobile phase contained 0.01M potassium dihydrogen orthophosphate-acetonitrile 960:40) and flow rate was maintained at 0.8 ml/min. Chromatographic seperations were performed at ambient temperature on a c18 column and the injection volume was 10µl.

Standard stock solution:Standard stock solution containing Ramipril and Hydrochlorthiazide by weighing 10 mg each of standard Ramipril and Hydrochlorthiazide and transferred into two separate volumetric flasks. Both of drugs were dissolved in 50 ml of mobile phase with shaking and then volume was made up to the mark with mobile phase with shaking then volume was was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of each drug. These stock solutions were filtered through 0.45 µm Nylon 47mm membrane filter paper.

Calibration curves:

From stock solutions the different concentrations were prepared with appropriate dilutions in the range of 50-150 µg/ml for RAM and HCZ. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area vs the drug concentration. The areas exhibited linear responses with r2=1 for RAM and r2=1 for HCZ. The results were shown in table-2

Table no. 2. System suitability parameters:

Parameters		**RAM**	**HCZ**
Linear range (µg/ml)		50-150	50-150
Slope		7885	14024
Intercept		1972	578.7
Regression coefficient (r^2)		1	1
Limit of Detection (µg/ml)		0.825	0.037
Limit of Quantification (µg/ml)		2.50	0.412
Retention time (min)		2.463	1.423
Tailing factor		1.1	1.2
Resolution factor		7.935	
Theoretical plate		4274	3668
Accuracy	MEAN±Standarddeviation		
	80%	99.78±0.92	100.23±0.18
	100%	99.51±0.89	100.17±0.16
	120%	99.11±0.50	99.74±0.52

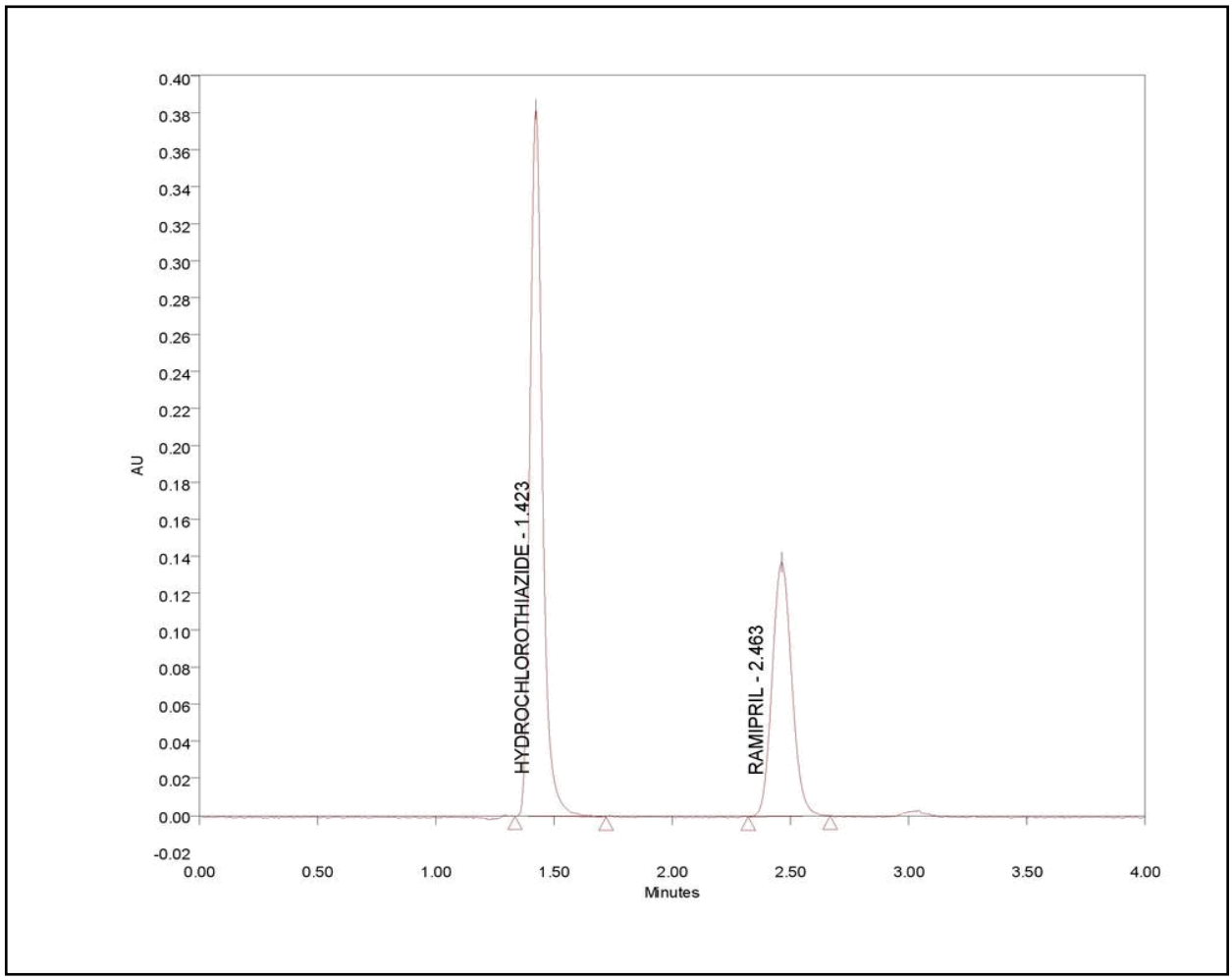

Figure-1: Chromatogram showing retention times of Ramipril and Hydrochlorthiazide respectively

Analysis of Tablet formulation:

To determine the content of RAM and HCZ in tablets(Label claim 5mg RAM and 12.5 mgHCZ) the twenty tablets were weighed, their mean weight determined. And powered equivalent to 12.5 mg was transferred into a 100 mi volumetric flask and dissolved in sufficient quantity of mobilephase. The contents were ultrasonicated for 20 mins. And the volume was made up to the mark with mobile phase.Then the solution wasthen filtered through 0.2 µm Nylon 47 mm membrane filter. The solution was further diluted to get concentration 50 µg/ml of RAM and 125 µg/ml was subjected to proposed method., injected with applicable volume 10 µl, and amount of RAM and HCZ was determined.The assay procedure was repeated for five times. Chromatogram of tablet solution is shown in figure-1 and results are shown in table-1

Table no.1 Result of RAM and HCZ in marketed formulation(n=6)

Marketed Formulation	Drug	%Amount found±SD	%RSD
Altace tablet	RAM	99±01.21	1.22
	HCZ	99±1.05	1.06

Validation of HPLC method:

Specificity and Selectivity:

The specificity of the RP-HPLC method was determined by complete separation of Ramipril and Hydrochlorthiazide with parameters like retention time (t_R), resolution (R_S) and tailing factor (T_f). Here tailing

factor for peaks of Ramipril and Hydrochlorthiazide, was less than 2% and resolution was also more than 1%. The peaks obtained for Ramipril and Hydrochlorthiazide were sharp and have clear baseline separation

Precision:

Precision of the method was studied as intraday and interday precision, variations. Intra-day and Interday variation was determined by standard solutions of 50µg/ml Ramipril and 125 µg/ml Hydrochlorthiazide was analysed six times at different time intervals in the same day. In inter-day precision a set of five sample mixtures containing 50 µg/ml of Ramipril and 125 µg/ml of Hydrochlorthiazide were prepared and analyzed at same time on different days. The resuits are shown in Table-3

Table no.3. Statistical Evaluation of precision of developed method.

Drug	**Intraday**	**Interday**
RAM	**%Mean±SD**	
	100±0.08	100±0.08
HCZ	99.99±0.03	99.99±0.03

Accuracy:

The accuracy of analytical method is the closeness of the test results obtained by that method to true value. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay. Accuracy studies were performed by standard addition method at the 80,100 and 120% levels as stated in ICH guidelines. The results are shown in Table-2

Robustness:

The robustness study was done by making small changes in optimized method parameters like change in mobilephase ratio, change in flowrate and change in column temperature. There is no significant impact on retention time and tailing factor.

Ruggedness:

It should show the reliability of analysis with respect to deliberate variations in method parameters like different laboratories, different analysts. The solution containing 50µg/ml of RAM and125µg/ml of HCZ was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate and column temperature.

Conclusion:

The proposed method is simple, sensitive and reproducible and hence can be used in routine for simultaneous determination of RAM and HCZ in bulk as well as in pharmaceutical preparations. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be less than 2, which indicates the validity of method and assay results obtained by this method are in fair aggrement. The developed method can be used for routine quantitative simultaneous estimation of RAM and HCZ in multicomponent pharmaceutical preparations.

References:

1. The United States Pharmacopoeia 23 NF 18. Rockville, MD: United States Pharmacopoeial Convention, Inc; 1995. p. 1228-29, 1623.
2. URL:http://en.wikipedia.org./wiki/Ramipril.
3. Bennett, P.N. and Brown, M.J., In; Clinical Pharmacology, 9th Edn., Churchill Livingstone, Singapore, 474-81.
4. Indian Pharmacopoeia, 26th The Controller of Publication, New Delhi, Vol-I,1996,370.
5. The United States Pharmacopoeia, 26th Edn., United States Pharmacopoeial 12. Convention, Inc., Rockville, MD., 2003,909.
6. British Pharmacopoeia, Her Majesty's Stationary Office, London, Vol-I,1993,330.

7. Connors KA. A Textbook of Pharmaceutical Analysis, 3rd ed. Delhi: Wiley intersciences Inc; 1994.
8. Parimoo P. In; Pharmaceutical Analysis, CBS publishers and distributors, New Delhi, 145.
9. Mendham j., Denny RC. And Thomas M., In; Vogel's Text Book of Quantitative Analysis, 6th ed., Pearson Education Limited, 2004:1.
10. Katzung BG., Basic and Chemical Pharmacology, 9th Edn., Mc-Graw Hill Companies, Inc.160.
11. Joel GH, Lee EL, Perry BM, Raymond WR, Alfred Cg, editor. Goodman Gilman's The pharmacological basis of Therapeutics. 9th ed. New Jersy: Mc-Graw Hill Companies;1996.
12. Christian GD. Analytical Chemistry, 6thed. John Wiley and Sons; 2004. p. 1-7.
13. Sweetmann SC. Martindale-The Complete Drug Reference. 33rd ed. London: Pharmaceutical press; 2002. p. 930-1, 966.
14. O'Neil MJ. The Merck Index-an Encyclopedia of Chemicals, Drugs and Biologicals. 13th ed. New Jersey: Merck & Co; 2001. p.1095, 8197.
15. Munson J, editor. Pharmaceutical Analysis. Vol. II, Part B, New York: Marcel Dekker Inc, 1996
16. Bilal Y. Determination of Ramipril in Pharmaceutical formulation by High Performance Liquid Chromatography. Int J Pharm Sci Review & Res 2010 Mar-Apr;1(1):39-42.
17. Srinivas Rao K, Srinivas K. RP-HPLC method for the determination of Losartan potassium and Ramipril in combined dosage form. Indian J Pharm Sci 2010;72(1):108-11.
18. Nafisur R, Habibur R, Syed NHM. Kinetic spectrophotometric determination of Ramipril in combined dosage form. Int J Bio & Lifesciences 2006;2(1):52-8.
19. Sureshkumar K, Ravikumar R, Rajshekaran A, Ravichandran V. Simultaneous spectrophotometric determination of Metoprolol tartarate in Ramipril. Digest J Nanomaterials and Biostructures 2010 Mar;5(1):173-5.
20. Patil RP, Rakesh US, Dhabale PN, Burade KB. Simultaneous UV Spectrophotometric Method for Estimation of Losartan Potassium and Amlodipine Besylate in Tablet Dosage Form. Asian J Res Chem 2009 Jan-Mar;2(10):183-7.
21. Thamake SL, Jhadav SD, Pishwaikar SA. Development and Validation of Method for Simultaneous Estimation of Atorvastatin Calcium and Ramipril in Capsule Dosage Form by First Order Derivative Spectroscopy. Asian J Res Chem 2009 Jan-Mar;2(1):52-3.
22. Abdel Razak O. Electrochemical study of Hydrochlorthiazide and its determination in urine and tablets. J. Pharm. Biomed Anal.2004,34(2),433-40.
23. Christophersen AS, Rasmussen K, Salvesen B. Determination of Hydrochlorthiazide in serum by high-pressure liquid chromatography. J Chromatogr A.1997; 132(1):91-7.
24. Hillaert S, Grauwe KD, Bossche WV. Simultaneous determination of Hydrochlorthiazide and several inhibitors of angiotensin-converting enzyme by capillary electrophoresis. J Chromatogr A.2001;924:439-49.
25. Zendelovska D, Stafilov T, Milosevski P. Development of solid phase extraction method and its application for determination of Hydrochlorthiazide in Human plasma using HPLC. J Biomed Chromatogr A. 1996;729:293-96.
26. Richter k, Oertel R, Kirch W. New sensitive method for the determination of Hydrochlorthiazide in Humanserum by high performance liquid chromatography with electrochemical detection.J Chromatogr A. 1996; 729:293-96.
27. Erk N. Simultaneous determination of Irbesartan and Hydrochlorthiazide in Human plasma by liquid chromatography. J Chromatogr B.2003;784:195-201.
28. Bhatia MS, Kaskhedikar SG, Chaturvedi SC. Simultaneous analysis of Atenelol hydrochloride, Hydrochlorthiazide and Amiloride hydrochloride from tablets. Indian Drugs, 1997; 34(10):576-79.

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