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Research Article

ISOCRATIC RP-HPLC-UV METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF *HYDROCHLOROTHIAZIDE AND RAMIPRIL* IN TABLET DOSAGE FORM AND BULK DURG

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ABSTRACT

Introduction: A simple, specific, sensitive and reverse phase high performance liquid chromatographic (HPLC) by using simple iscrotic instrument, method has been developed and subsequently validated for simultaneous determination of Hydrochlorothiazide, Ramipril in combination. **Methods:** The separation was carried out using mobile phase consisting of 0.1mM sodium dihydrogen phosphate buffer and acetonitrile with pH 3.0 adjusted with ortho phosphoric acid and flow rate was set on 1.0mlmin^{-1}. The column used C_{18} reversed phased (4.6×250mm, 5.0µm), using UV detection 215nm. **Results:** The retention time for Hydrochlorothiazide and Ramipril was found 3.0371 and 3.553 mins respectively. **Conclusion:** The method developed was validated by using data elements Linearity, Accuracy, Precision, Assay, LOD, LOQ, and Specificity were evaluated, which remained within acceptable limits. The method has been successfully applied to assess the assay of solid dosage formulations.

Keywords: Hydrochlorothiazide; Liquid chromatography, Ramipril, Solid dosage formulation, Validation.

INTRODUCTION

Hypertension is a disease characterised by abnormally which the blood pressure in the arteries is elevated. It is classified as either primary or secondary. About 90-95% of cases are termed "primary(idiopathic) hypertension", which refers to high blood pressure for which no exact cause can be found the remaining 5-10% of secondary hypertension cases are caused by another condition that affect the renal, vascular lesions, or endocrine system. Blood pressure is classified based on two types of measurements; the 140mmHg systolic and 90mmHg diastolic blood pressure is the blood pressures expressed as a ratio such as '120 over 80'(120/80)mmHg though risk appears to increase even above(120/80)mmHg. Systolic blood pressure is the pressure in vessels during a heartbeat. Diastolic blood pressure between heartbeats. A combination of hydrochlorothiazide and ramipril in the form of tablet or capsule is widely used because in certain cases, a single ACE inhibitor drug does not respond sufficiently to reduce hypertension, then we used as combined dosage forms with other specific classes of drug compounds such as diuretics to moderate to severe hypertension not controlled by a single antihypertensive agent and also in older patient who have low Renin levels.

Ramipril,2-[N-[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl)]-L-alanyl]-(1S,3S,5S)-2-azabicyclo[3-3-0]octane carboxylic acid (fig-1a) as a distinctive feature of this long acting angiotensinconverting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure as well as reduce the degradation of bradykinin. Its acting by lower the production of angiotensin II, therefore relaxing arterial muscles while at the same time enlarging the arteries, allowing the heart to pump blood more easily, and increasing blood flow due to more blood being pumped into and through larger passageways.

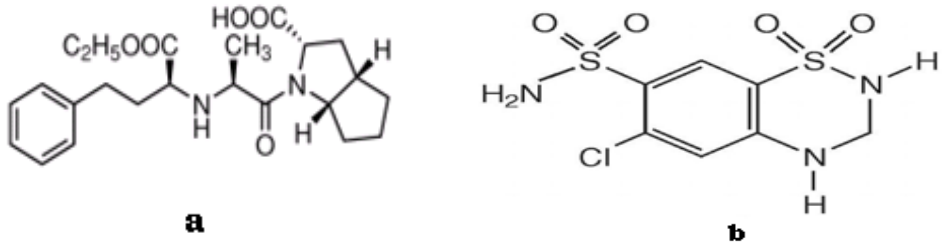


Fig. 1: **Chemical structures of (a) Ramipril (b) Hydrochlorothiazide**

Hydrochlorothiazide, 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzo-thiadiazine-7-Sulphonamide 1, 1-dioxide (Fig.1b), is a first line diuretic drug, which inhibits active chloride reabsorption at the early distal tubule via the Na-Cl co-transporter as well as inhibiting the ability to retain water by kidney, resulting in an increase in the excretion of sodium, chloride, and water

There are few analytical methods available for the quantization of Ramipril and Hydrochlorothiazide individually. The purpose of this study was the development of a simple isocratic HPLC method with ultraviolet detection for Ramipril and Hydrochlorothiazide assay in tablets and validates the same as per the ICH guidelines [1-9].

MATERIALS AND METHODS

Apparatus

Instrument used in present study was resolution of 1nm and 0.5mm slit width Shimadzu HPLC equipped with pump model LC-10AT and SPD-10A UV detector. ODS column hypersil, 250× 4.6 mm, 5 µm of thermo Electron Corporation.

Chemicals & reagents

All solvents were of HPLC grade and all reagents were of analytical grade. Sodiumdihydrogenphosphate, were obtained from SD Fine

Chemicals (India). Acetonitrile, ortho-phosphoric acid, Methanol was obtained from Rankem (India). Water was purified with Milli-Q Plus, Millipore System (USA). All solvents and solutions were filtered through a membrane filter (Millipore Millex -HV filter units, 0.45 µm pore size; nylon) and degassed before use. All solutions were profiteered before injecting into HPLC system using Millipore milex hydrophilic PTFE unit filter of 0.45 µm pore size.

Marketed formulation

Ramipril-H (THEMIS MEDICARE Ltd. Mumbai, India) was taken for study which contains Ramipril-2.5mg, Hydrochlorothiazide-12.5mg.

Preparation of the standard solutions

The stock standard solution of ramipril and hydrochlorothiazide was prepared with methanol to a concentration of 1000 µg mL-1 and stored at 4^{0}C under refrigeration. The six standard solutions from 20 to 120 µg mL-1 (20, 40, 60, 80, 100, 120 µg mL-1) in methanol were made by a serial dilution for Ramipril and hydrochlorothiazide respectively.

Sample preparation

The average tablet mass was calculated from the mass of tablets of Ramipril-H (12.5 mg Hydrochlorothiazide, 2.5mg ramipril tablet, which was composed of Ramipril and Hydrochlorothiazide and some excipients). They were then finely ground, homogenized and portion of the powder was weighed accurately, transferred into a 100 mL brown measuring flask and diluted to scale with methanol. The mixture was sonicated for at least 15 min to aid dissolution and then filtered through a Whatman no 42 paper. An appropriate volume of filtrate was diluted further with methanol so that the concentration of Ramipril and Hydrochlorothiazide in the final solution was within the working range and then analyzed by HPLC [10-16].

Table 1: Linearity of Ramipril and Hydrochlorothiazide by RP-HPLC method

Method	Range (µgml^{-1})	LR[a]	R	LOD (µgml^{-1})	LOQ (µgml^{-1})
Rp-Hplc **HCTZ**	20-120	Y = 23.63x+16.18	0.999	0.644	1.951
Rp-Hplc **RP**	20-120	Y = 1.851x-17.71	0.999	1.415	4.288

[a]Based on three calibration curves, LR:Linear regression, R: Coefficient of correlation, y: peak area, x: ramipril concentration and hydrochlorothiazide concentration. LOD: Limit of detection, LOQ: Limit of quantification

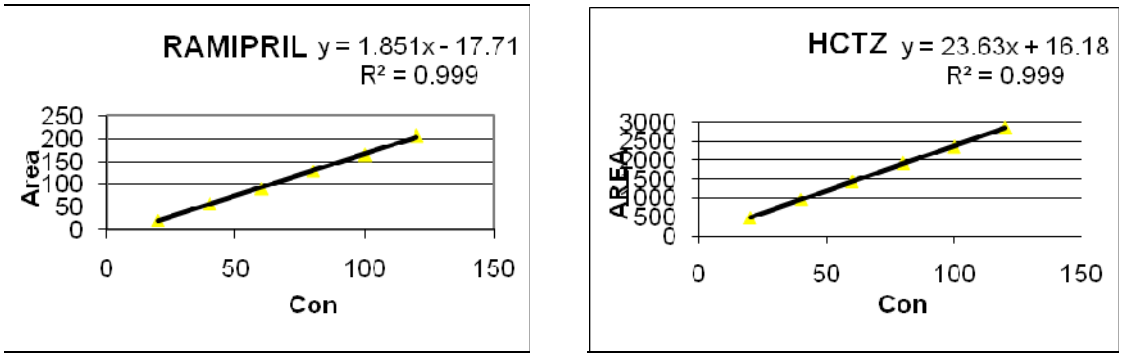


Fig. 2: calibration curves graph Ramipril and Hydrochlorothiazide

Table 2: System suitability of Ramipril and Hydrochlorothiazide by RP-HPLC method

Characteristic	Hydrochlorothiazide	Ramipril
Capacity factor	2.9241	3.4272
Tailing factor	1.455	1.111
Theoretical plates	5787	5727
Resolution		3.010

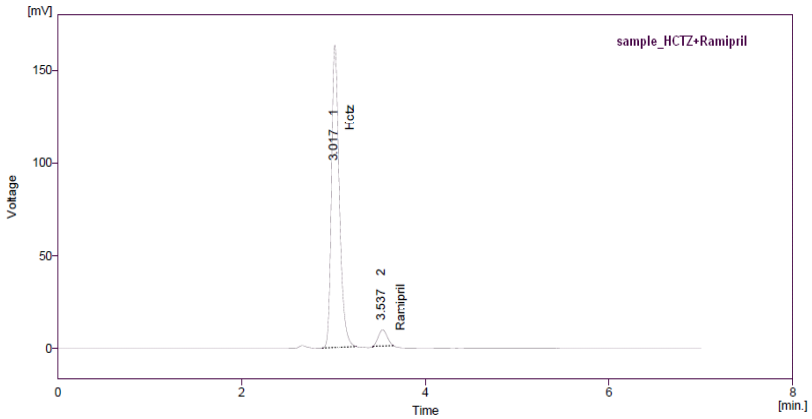
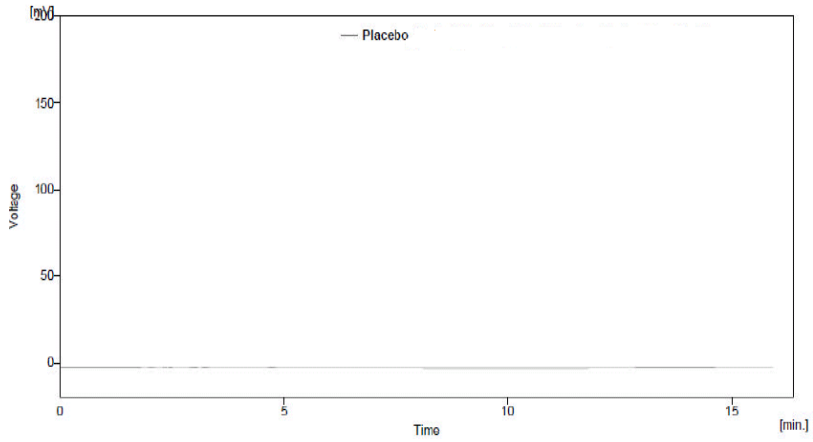


Fig. 3: Chromatogram of Hydrochlorothiazide and Ramipril.



RESULTS AND DISCUSSION

Linearity

Calibration curve was constructed for ramipril and hydrochlorothiazide standard by plotting the concentration of compound versus peak area response. Standard solutions containing 20, 40, 60, 80, 100, 120 µ g mL-1 of ramipril and hydrochlorothiazide with respectively were prepared and 10 µg L was injected into the HPLC column (Figure 2). The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method on the ordinate (Table 1).

Specificity

Specificity of the method was found out through non-interference of the blank, internal standard and mobile phase. The method showed excellent specificity with hydrochlorothiazide and Ramipril eluting at retention time (RT) 3.017and 3.5372 minutes. No interference was observed with blank, mobile phase and internal standard.

System suitability

Having optimized the efficacy of a chromatographic separation the quality of the chromatography was monitored by applying following system suitability tests: Capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor > 2.0, tailing factor ≤ 2.0 and theoretical plates > 2000. In all the analyte peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substances solution is presented in Fig 3 and table 2.

Precision

The reproducibility of the method was estimated by analysing samples. Six injections of the standard mixture were analyzed for the determination of system precision. Similarly six solutions of the individual standards were prepared and assayed for the determination of method precision the results are shown in the table 3.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplet by standard addition method at 80%, 100% and 120% concentration levels. Known amounts of standard RP and HTZ were added to the pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in table 4.

Table 3: Precision data

Parameter	Ramipril	hydrochlorothiazide
System precision(%RSD)	0.984079	0.200518
Method precision(%RSD)	0.477755	0.187311

Table 4: Recovery studies

concentrations	Ramipril	Hydrochlorothiazide
80 %	97.22834	100.0617
100%	99.22906	99.785
120%	99.45794	99.90975

*Average of 3 readings at each concentration level

LOD and LOQ Determination

Limit of detection can be calculated by using following formula,

$$LOD = 3.3\ \sigma/S$$

Limit of quantitation can be calculated based on standard deviation of the response and the slope.

$$LOQ = 10\ \sigma/S$$

Where σ = Standard deviation of the response

S = Slope of the calibration curve

The LOD and LOQ values are showed in the above **table-1**.

Table 5: Assay

Amount present (mg)		Amount found (mg)		Assay (%)	
HTCZ	Ramipril	HTCZ	Ramipril	HTCZ	Ramipril
12.5	2.5	12.4mg	2.4mg	99.4117 **%**	99.5%

Table 6: Robustness data

Variation	Retention time		Peak area		Resolution	Tailing factor		Theoretical plates	
	HCTZ	RP	HCTZ	RP		HCTZ	RP	HCTZ	RP
pH(2.5)	2.820	3.313	1819.562	124.593	2.903	1.476	1.250	5439	5026
pH(3.5)	3.243	3.810	2114.399	141.080	3.078	1.591	1.276	5828	5908
Flow(0.9ml)	3.433	4.037	2812.039	192.455	3.087	1.500	1.226	6116	5626
Flow(1.1ml)	2.693	3.163	1744.989	112.001	2.861	1.500	1.192	5350	4872

Table 7: Validation results for Ramipril and Hydrochlorothiazide assay method

S.No	Parameter	Acceptance criteria	Results obtained for RP	Results obtained for HCTZ
1.	Specificity	Non-interference of Placebo	Passing	Passing
2.	Linearity	Correlation coefficient NLT 0.995	0.999	0.999
3.	Accuracy	Recovery of spiked drug (97-103%)	Passing	Passing
4.	Precision	RSD of Standard NMT 2.0%	0.984079	0.200518
		RSD of Sample NMT 2.0%	0.477755	0.187311
5.	Robustness	RSD of Standard NMT 2.0%	passing	passing
		RSD of results between different mobile phases and buffers of different pH- NMT 2.0%		
6.	LOD& LOQ	Signal noise ratio should be more than 3:1 & 10:1	1.415 & 4.288	0.644 & 1.951
7.	System suitability	No of Theoretical plates .NLT 2000	5363	5649
		Resolution- NLT 2.0		2.997
		Tailing factor	1.233	1.545
8.	Ruggedness	RSD of Standard NMT 2.0%	passing	passing
		RSD of results between two instruments- NMT 5.0%	passing	passing
		RSD of results between two analysts- NMT 5.0%	passing	passing

Assay

The validated HPLC method was used for simultaneous determination of ramipril, and hydrochlorothiazide in their combined dosage form. In the assay experiment seven samples were weighed separately and analysed. The mean assay results, expressed as a percentage of the label claim, are listed in Table 5. The results indicate that the amount of each drug in the tablets is within the requirements of 90– 110% of the label claim.

Robustness

For the HPLC method, the robustness was determined by the analysis of the samples under a variety of conditions making small changes in the buffer pH (2.5 and 3.5), in the percentage of mobile phase component ±2.0 % (Buffer: ACN), in the flow rate (0.9and 1.1 mL min^{-1}), in the temperature conditions (35 ºC and 45 ºC) the results are shown in the table 6.

Ruggedness:

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0%, showed ruggedness of developed analytical method. The results of ruggedness were presented in **table 3**.

CONCLUSION

A simple, sensitive method has been established for isocratic separation and simultaneous determination of ramipril and hydrochlorothiazide in their combined dosage form as well as in this method proves high efficiency and good baseline factor when compared previous article. The linearity, precision, and accuracy of the method prove it is highly reproducible under quality-control conditions if the procedures described above are followed precisely.

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REFERENCES

1. Indian Pharmacopoeia (2010). Government of India Ministry of Health and Family Welfare; Controller of Publications, Delhi, 6th ed., Vol. III, 2041-2042.
2. Shalini pachauri et al, Development & Validation of HPLC Method for Analysis of Some Antihypertensive Agents in their Pharmaceutical Dosage Forms. J. Pharm. Sci. & Res. Vol.2 (8), 2010, 459-464.
3. Zaveri Maitreyi, Khandhar Amit. Development and Validation of a RP-HPLC for the Simultaneous estimation of Atenolol and Hydrochlorothiazide in Pharmaceutical Dosage Forms. International Journal of Advances in Pharmaceutical Sciences 1(2010) 167-171.
4. Joshi S, Sharma A, Rawat MS, Bal CS. Reversed phase liquid chromatographic conditions for simultaneous determination of antihypertensive formulations. Asian J Pharm 2009;3:274-7
5. Bilal YILMAZ, Determination of Ramipril in pharmaceutical preparations by High-performance liquid chromatography. International Journal of Pharmaceutical Sciences Review and Research, Volume 1, Issue 1, March – April 2010; Article 008, pp 39-42.
6. Sunil Jawla, K Jeyalakshmi, T Krishnamurthy, Y. Kumar Development and Validation of Simultaneous HPLC method for Estimation of Telmisartan and Ramipril in Pharmaceutical Formulations. Int.J. PharmTech Res.2010, Vol.2, No.2, pp 1625-1633.
7. M. M. Baing, V. V. Vaidya , R. T. Sane, S. N. Menon, K. Dalvi; Simultaneous RP-LC Determination of Losartan Potassium, Ramipril, and Hydrochlorothiazide in Pharmaceutical Preparations, Chromatographia 2006, 64, pp 293–296 September (No. 5/6)
8. Vrushali Tambe, Vijaya Vichare, Ujjawala Kandekar And Shashikant Dhole, Novel UV Spectrophotometric Methods For Estimation of Ramipril and Hydrochlorothiazide by Simultaneous Equation And Area Under Curve Method, Int J Appl Pharm , Vol 2, Issue 4, 20-22.
9. S. Bankey, G. G Tapadiya, S. S Saboo, S. Bindaiya, Deepti Jain, S. S. Khadbadi; Simultaneous Determination of Ramipril, Hydrochlorothizide and Telmisartan by Spectrophotometry. Int.J. ChemTech Res.2009, Vol.1, No.2, pp 183-188.

10. Teli M. S, Sawant S. S., Patil A.R., Ravetkar A. S. and Shirote P. J., Stability indicating LC estimation of ramipril and hydrochlorthiazide in its bulk and tablet formulation . International Journal of Pharmacy & Life Sciences, 1(6):325-335.
11. Nafisur Rahman, Habibur Rahman, and Syed Najmul Hejaz Azmi, Kinetic Spectrophotometric Determination of Ramipril in Commercial Dosage Forms. International Journal of Biological and Life Sciences 2:1 2006, 52-58.
12. Lakshmi Narasimham Y S and Vasant D Barhate., Development And Validation Of Stability Indicating UPLC Method For The Simultaneous Determination Of Beta-Blockers And Diuretic Drugs In Pharmaceutical Dosage Forms. J. Chem. Metrol. 4:1 (2010) pp1-20.
13. YOGESH GUPTA, 222, shanti nagar, Gopal pura, Tonk road, Jaipur Rajasthan; Isocratic RP-HPLC-UV Method Development And Validation For The Simultaneous Estmation Of Ramipril And Telmisartan In Tablet Dosage Form, Asian Journal of Pharmaceutical and Clinical Research, Vol.2 Issue 4, pp104-111.
14. Ayad, M.M., Shalaby, A.A., Abdellatef, H.E., Hosny, M.M, Spectrophotometric and AAS determination of ramipril and enalapril through ternary complex formation, Journal of Pharmaceutical and Biomedical Analysis, Vol 28, No. 2, pages 311-321 (2002).
15. ICH, Q2B, Harmonized tripartite guideline, validation of analytical procedure: methodology, IFPMA, in: Proceedings of the International Conference on Harmonization, March 1996.
16. V. A. Patel, P. G. Patel, B. G. Chaudhary, N. B. Rajgor, S. G. Rathi, Development and Validation of HPTLC Method for the Simultaneous Estimation of Telmisartan and Ramipril in Combined Dosage Form, International Journal on Pharmaceutical and Biological Research, Vol. 1(1), 2010, 18-24.