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ORIGINAL ARTICLE

Method Development and Validation of Simultaneous estimation for Ramipril and Valsartan by UV-VISIBLE Spectrophotometric method

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ABSTRACT
A simple, specific, accurate UV – UV-visible spectrophotometric chromatographic method was developed for the simultaneous determination of Ramipril and Valsartan by simultaneous equation method. The regression strength of Ramipril and Valsartan over their absorbances were obtained as y=0.00917x-0.00494 and y=0.0142x-0.006 respectively with a correlation coefficient (r2) of 0.9995 for Ramipril and 0.9998 for Valsartan. The intra-day precision in addition to inter-day precision for Ramipril and its % RSD were obtained as 0.30% and 0.33% respectively. The intra-day precision in addition to inter-day precision for Valsartan and its % RSD were obtained as 0.21% and 0.26% respectively. This confirms the procedure is precise. Accuracy is determined for both drugs by spiking with 80, 100, and 120% of additional pure drugs and the % mean recovery of the Ramipril and Valsartan were obtained as 98.86 and 99.66 respectively (Table 05). The percentage purity for the assay of Ramipril and Valsartan were obtained as 99.6% and 98.92% respectively
Keywords: Ramipril, Valsartan, simultaneous estimation, method development UV-VISIBLE.

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INTRODUCTION

Analytical method development and validation are continuous and interconnected activities conducted throughout the drug development process. The practice of validation verifies that a given method measures a parameter as intended and establishes the performance limits of the measurement. Ramipril is chemically called 1-[(2S,3aS,6aS)-1-[(2S)-2-[[(1S)-1-(Ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl] octahydro-cyclopenta[b]pyrrole-2-carboxylate] |A-D-Glucopyranuronic Acid Mass is 416.518 g/mol. Ramipril is a prodrug and nonsulfhydryl angiotensin-converting enzyme (ACE) inhibitor with antihypertensive activity [1]. Ramipril is converted in the liver by de-esterification into its active form ramiprilat, which inhibits ACE, thereby blocking the conversion of angiotensin I to angiotensin II [1]. This abolishes the potent vasoconstrictive actions of angiotensin II and leads to vasodilatation. This agent also causes an increase in bradykinin levels and a decrease in angiotensin II-induced aldosterone secretion by the adrenal cortex, thereby promoting diuresis and natriuresis[2-4]. Appearance is Solid powder, Melting Point is 109°C, Refractive Index 1.731, and solubility is soluble. The structure is shown in the fig 1.

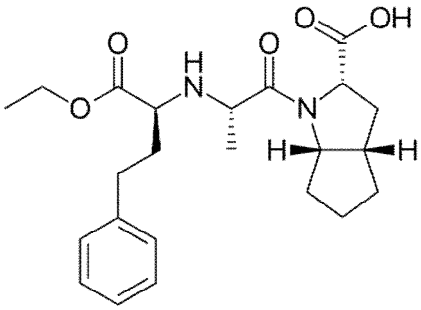

Figure 1: Structure of Ramipril

Valsartan is chemically called (2S)-3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] amino] butanoic acid[5]. Molecular weight is 435.528 g/mol Chemical name: C24H29N5O3Valsartan is an orally active nonpeptide triazole-derived antagonist of angiotensin (AT) II with antihypertensive properties[5-9]. Valsartan selectively and competitively inhibits the action of angiotensin II receptor, preventing AT II-mediated vasoconstriction, aldosterone synthesis, and secretion, and renal reabsorption of sodium, resulting in vasodilation, increased excretion of sodium and water, a reduction in plasma volume, and a reduction in blood pressure[10-14]. It appears to be a Solid powder that has a melting point of 109°C and has a Refractive Index of 1.73, Flash Point is 116-117oC. Soluble in ethanol and methanol. The structure is shown in Fig 02.

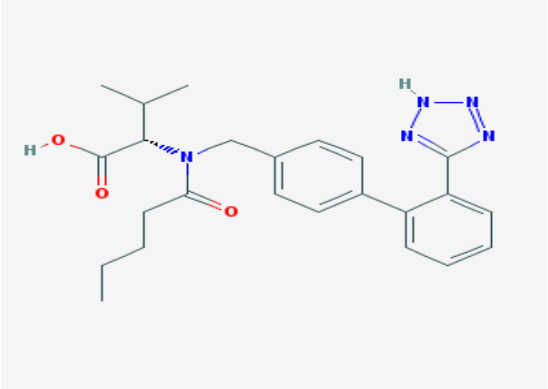

Figure 02: Structure of Valsartan

MATERIAL AND METHODS

Ramipril and Valsartan were Active pharmaceutical ingredients procured from Yarrow Chemicals, Mumbai, India. Were procured from the local pharmacy. Solvents like Acetonitrile, Methyl alcohol, Acetic acid, Ammonium acetate, and Water (Milli Q grade) were obtained from Distilled water used for the experiment.

Equipment Used[15]:

S.No.	Name of the equipment	Company
1.	UV 3092 UV/Visible spectrophotometer with 1 cm matched Quartz cells.	LABINDIA
3.	Electronic Balance	Shimadzu BL-220H, Japan
4.	LI 120 PH Meter	Elico India.
5.	R8c Laboratory Centrifuge	Remi motors Ltd, India
6.	Vortex Mixer	Remi motors Ltd, India.
7.	Ultra-Sonicator	ILE

ANALYTICAL METHODS:

PREPARATION OF STANDARD DRUG SOLUTION [16]:

Standard stock solutions that hold Ramipril and Valsartan were prepared by dissolving 2.5 mg of Ramipril and a quantity of Valsartan equivalent to 2.5 mg separately in 20 ml of methyl alcohol. It was then

sonicated for 10 minutes and the final volume of both the solutions was made up to 50 ml with methyl alcohol to get stock solutions containing 50 microgram/ml each of Ramipril and Valsartan in two different 50 ml volumetric flasks [16].

PROCEDURE FOR DETERMINING THE SAMPLING WAVELENGTH FOR SIMULTANEOUS ANALYSIS:

The dilution of two standard drug solutions with methyl alcohol, solutions containing 10 microgram/ml of Ramipril and 10 microgram/ml of Valsartan were scanned separately in the range of 200-400 nanometers to determine the wavelength of maximum absorption for both drugs. RM and VAL showed absorbance maxima at 210 nanometers and 240nanometer respectively. (Fig. 3 & Fig. 4)[16].

SELECTION OF METHOD AND WAVELENGTH:

For the estimation of Ramipril employing 210 nanometers as analytical wavelength 240nanometer for was selected Valsartan[17].

LINEARITY:

For the estimation of Ramipril and Valsartan lambda max was found to be for Ramipril 210nanometer and Valsartan was found to be 240nanometer in methyl alcohol solvent. The linearity for both Ramipril and Valsartan in the strength range of 10-50 microgram/ml. (Table 01 and Figures 05 and 06).

PRECISION:

For the intra-day calculation of precision 0-10 hours with an interval of every two hours and interday precision of 1-6 days were chosen and readings were taken every day for Ramipril and Valsartan tabulated in table 2.

STABILITY PARAMETER:

The precise amount of tablet formulation which is equal to 40mg of Telmisartan and 5mg of Ramipril was transferred into a 100 ml standard flask and maintained under the subsequent conditions which hold Alkaline (0.1 N Sodium hydroxide), Acidic (0.1 N Hydrochloric acid) reflux for 3 hours, 3% Oxydol at 50ºC, heat (60ºC), humidity (75 percentage Relative humidity) for 24 hr and after the particular time quantity was diluted to the mark with distilled water, separated using Filter paper[18]. From this stock solution, a 5 ml portion of the filtrate was pipetted out and further diluted with distilled water in a 100 ml standard flask (10 microgram/ml) [19]. The standard stock solution of two drugs was prepared and compared against a label claim and results were tabulated in Table 03.

DETECTION LIMIT AND QUANTIFICATION LIMIT:

The detection limit (LOD) and quantification limit (LOQ) for Ramipril were verified to be 0.15 microgram/ml and 0.32 microgram/ml respectively. The detection limit (LOD) and quantification for Valsartan examined to be 0.35 microgram/ml and 0.95 microgram/ml (Table 04) respectively.

ACCURACY:

Accuracy was determined for drugs by spiking with 80, 100, and 120 percent of pure drug and the mean recovery of the Ramipril and Valsartan were to be 98.86% and 99.66% respectively (Table 05).

ASSAY:

The assays of Ramipril and Valsartan were done and their percentage purity was found to be 99.6% and 98.92% respectively.

RESULTS AND DISCUSSION

COMBINATION OF RAMIPRIL AND VALSARTAN:

The extent of Ramipril and other Valsartan bulk samples and their tablet forms were determined by simultaneous equation method by using a UV Spectrophotometer. The regression strength of Ramipril and Valsartan over their absorbances were obtained as y=0.00917x-0.00494 and y=0.0142x-0.006 respectively with a correlation coefficient (r2) of 0.9995 for Ramipril and 0.9998 for Valsartan. The intra-day precision in addition to inter-day precision for Ramipril and its % RSD were obtained as 0.30% and 0.33% respectively. The intra-day precision in addition to inter-day precision for Valsartan and% RSD were obtained as 0.21% and 0.26% respectively [20]. This confirms the procedure is precise. Accuracy is determined for both drugs by spiking with 80, 100, and 120% of additional pure drugs and the % mean recovery of the Ramipril and Valsartan were obtained as 98.86 and 99.66 respectively (Table 05). The percentage purity for the assay of Ramipril and Valsartan were obtained as 99.6% and 98.92% respectively (Table 06). The assay result shows that the methodology was selective for the evaluation of Ramipril and Valsartan without hindering the inactive substance used in the tablet dosage form.

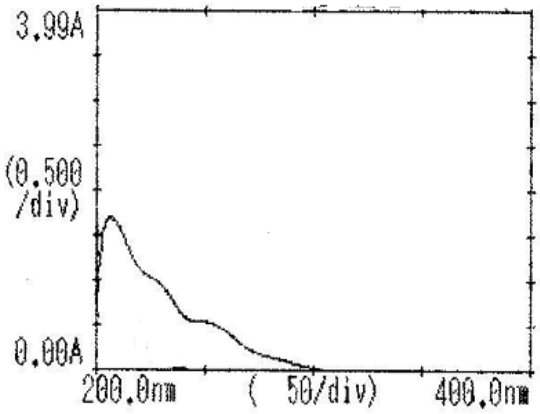

Figure 03*:* Chromatogram of Ramipril

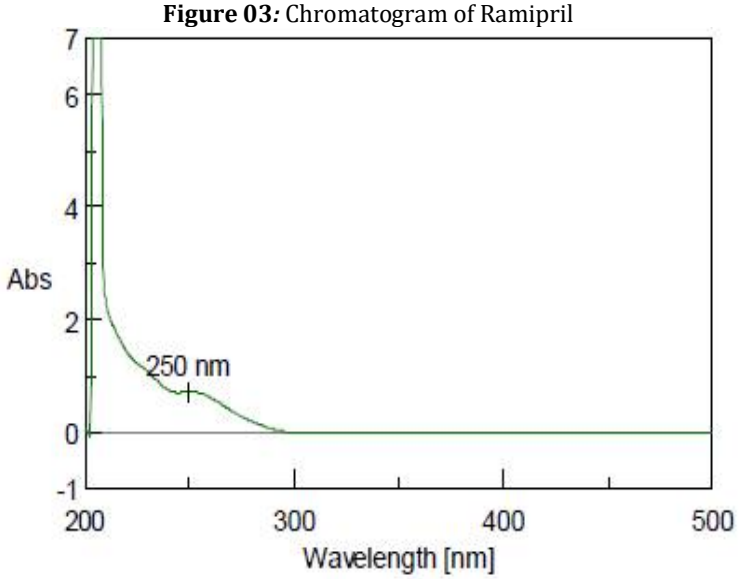

Figure 04*:* Chromatogram of Valsartan

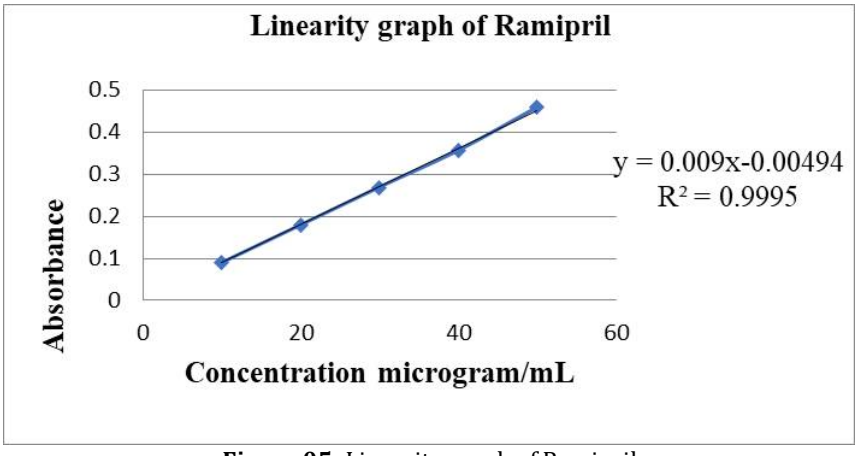

Figure 05: Linearity graph of Ramipril

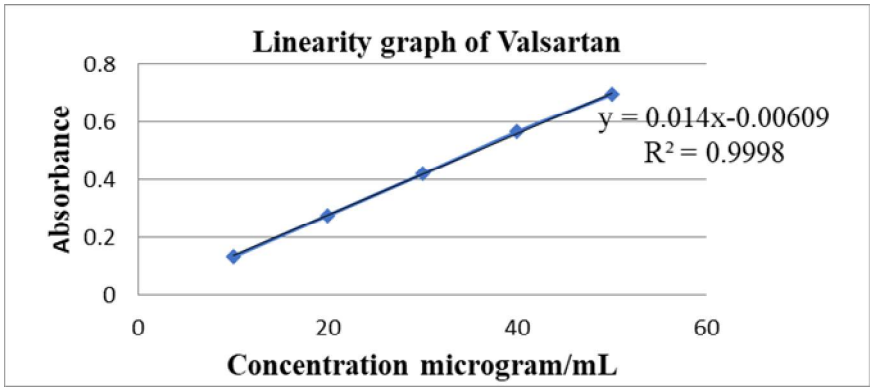

Figure 06: Linearity graph of Valsartan

Table 01: Linearity data's for Ramipril and Valsartan

S. No.	Concentration (µg/mL)	Ramipril Absorbance	Valsartan Absorbance
1	10	0.0890	0.1342
2	20	0.1785	0.2781
3	30	0.2687	0.4213
4	40	0.3557	0.5684
5	50	0.4589	0.6998
Slope		0.00917	0.0142
Intercept		-0.00494	-0.00609
Regression Equation(y)		0.009x -0.00494	0.0142x
Correlation Coefficient		0.9995	0.9998

Table 02: Intra-day and Inter-day precision results of Ramipril and Valsartan [21]

Intra-day precision				Inter-day precision		
S. No.	Time (Hours)	Ramipril Absorbance	Valsartan Absorbance	Time (Days)	Ramipril Absorbance	Valsartan Absorbance
1	0	0.5548	0.8462	1	0.5615	0.8541
2	2	0.5397	0.8324	2	0.5554	0.8535
3	4	0.5241	0.8242	3	0.5502	0.8324
4	6	0.5154	0.8141	4	0.5414	0.8214
5	8	0.5024	0.8058	5	0.5145	0.8114
6	10	0.4999	0.7994	6	0.5262	0.8012
Mean		0.5272	0.82035	Mean	0.5415	0.829
SD		0.00163	0.00174	SD	0.00180	0.00218
%RSD		0.30918	0.2121	%RSD	0.33240	0.26296

Table 03: Stability studies parameters for Ramipril and Valsartan

Sample (treated)	Percent Label claim	
	Comparison with standard	A (1%, 1cm)
0.1 N NaOH	98.67	99.11
0.1 N HCl	95.19	95.92
60ºC for 2hr	98.79	99.01
Humidity (75% RH)	95.97	96.52

Table 04: LOD and LOQ of Ramipril and Valsartan [22]

Parameter	Ramipril measured value(µg/mL)	Valsartan measured value(µg/mL)
Limit of detection	0.15	0.35
Limit of quantification	0.32	0.95

Table 05: Recovery studies for Ramipril and Valsartan

Ramipril				**Valsartan**		
	80%	100%	120%	80%	100%	120%
Std. conc. (microgram/ml)	10	10	10	10	10	10
Conc. added (microgram/ml)	8	10	12	8	10	12
Conc. found (microgram/ml)	7.88	9.98	11.86	7.95	9.98	11.98
% Recovery	98.5	99.8	98.3	99.37	99.8	99.83
% Mean recovery	98.86			99.66		

Table 06: Assay of Ramipril and Valsartan and formulations

Formulation		**Label claim**	**Amount found**	**% Assay**
Valent R-5	Valsartan	80mg	79.14mg	98.92
	Ramipril	5mg	4.98mg	99.6

Funding: Nil

Conflict to Interest : Nil

REFERENCES

1. Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, Ali M. (2007). Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm ;66(2):227–43.
2. Jain H.K., Agrawal R.K., (2002). Indian Journal of Pharmaceutical Sciences, 64(1), 88-71.
3. Shankar M.B., Mehta F.A., Bhatt K.K., Mehta R.S., and Geetha M., (2003). Indian Journal of Pharmaceutical Sciences, 65(2), 167-170.
4. Skoog DA, West DM, Holler FJ (1996) Fundamentals of analytical chemistry. (8thEdn), Fort Worth: Saunders College Pub.
5. Peleshok K, Piponski M, Ajie EA, Poliak O, Zarivna N, Denefil O, et al. (2021). Novel HPLC-UV method for simultaneous determination of valsartan and atenolol in a fixed dosage form; Study of green profile assessment. Farmatsiia (Sofia);68(1):43–51.
6. CPMP, (1998). Note for Guidance on Stability Testing of Existing Active Substances and Related Finished Products, Committee for Proprietary Medicinal Products, EMEA, London.
7. TPD, (1977). Stability Testing of Existing Drug Substances and Products, Therapeutic Products Directorate, Ottawa.
8. Sonia K, (2023). "Novel Validated UV Spectroscopic Method for the Analysis of Ramipril and Olmesartan Medoxomil in Drug Substance as Fixed Dosage Form". Research Journal of Pharmacy and Technology (RJPT), 16(3):1442-1446.
9. ICH, Good Manufacturing Practices for Active Pharmaceutical Ingredients, International Conference on Harmonisation, IFPMA, Geneva, 2000
10. BK, Patel DJ, Parikh KA, Nanjwade VK, Manvi FV (2011) Development and Characterization of Solid-Lipid Microparticles of Highly Insoluble Drug Sirolimus. J Bioequiv Available 3: 011-015.
11. Yi SJ, Shin HS, Yoon SH, Yu KS, Jang IJ, et al. (2011) Quantification of Ticlopidine in Human Plasma Using Protein Precipitation and Liquid Chromatography Coupled with Tandem Mass Spectrometry. J Bioanal Biomed 3: 059-063.
12. Rajender G, Narayana NGB (2010) Liquid Chromatography-Tandem Mass Spectrometry Method for Determination of Paclitaxel in Human Plasma. Pharm Anal Acta 1:101.
13. Yang G, Liu Y, Liu H, Yang C, Bai L, et al. (2010) Preparation of a Novel Emulsion-Templated MIP Monolith and its Application for on Line Assay of Nifedipine in Human Plasma. J Chromatograph Separat Techniq 1:103.
14. Jorgensen WL, Laird ER, Gushurst AJ, Fleischer JM, Gothe SA, Helson HE, et al.,(1990). Pure and Applied Chemistry, 62: 1921-1932.
15. Sadeghi S, Takjoo R, Haghgoo S. (2002). Quantitative determination of diazepam in pharmaceutical preparation by using a new extractive-spectrophotometric method. Anal Lett .35(13):2119–31.
16. Chawla, Kumar, Sahni, Mamman, Saraf SA. (2002). Development and validation of an analytical method for simultaneous estimation of diclofenac sodium and ofloxacin in bulk and ophthalmic formulations using UV–visible spectrometry. PCI- Approved-IJPSN .; 4(2):1399–402.
17. Jadhav NR, Kambar RS, Nadaf SJ. (2014). Dual wavelength spectrophotometric method for simultaneous estimation of atorvastatin calcium and felodipine from tablet dosage form. Adv Chem 1–6. https://doi.org/10.1155/2014/131974
18. Hana Tománková, Jaroslav Zýka. (1978). A study of the stability of pyrimidine series cytostatics, Ftorafur, and 5-fluorouracil: The effect of oxidation on the stability of Ftorafur and 5-fluorouracil, Microchemical journal.:400-406.
19. Worakul N, Wongpoowarak W, Boonme P. (2002). Optimization in development of acetaminophen syrup formulation. Drug Dev Ind Pharm;28(3):345–51.

20. Wang Q-Y, Dong X, Yang J, Zhen X-T, Ye L-H, Chu C, et al. (2019). Solid acids assisted matrix solid-phase dispersion microextraction of alkaloids by capillary electrophoresis coupled with quadrupole time-of-flight mass spectrometry. J Sep Sci;42(23):3579–88.
21. Zhang X, Xian Y, Li H, Huang B-X, Liang M, Chen J. (2018). Rapid determination of hexavalent chromium in textiles by a novel ammonium pyrrolidine dithiocarbamate derivatization combined with UHPLC–MS/MS. J Sep Sci;41(18):3583–9.
22. He Z, Wang J, Wang X, Dong Y. (2022). The study of the transport mechanism of isorhynchophylline in the liver. Evid Based Complement Alternat Med.22:1–8.