International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
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DEVELOPMENT AND VALIDATION OF UPLC METHOD FOR SIMULTANEOUS DETERMINATION OF RAMIPRIL AND VALSARTAN IN ITS PURE AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple, fast, accurate and precise method has been developed for the simultaneous determination sof Ramipril (RAM) and Valsartan (VST) from pharmaceutical formulation by UPLC. The separation was carried out on C18-BEH column using mobile phase 35:65 Phosphate buffer (0.02Mm) Ph 3.5 adjusted with O-Phosphoric acid:Acetonitrile. The retention times of Ramipril (RAM) 0.6 min, Valsartan (VST) 1.18 min. The developed method was validated as per ICH Guidelines.

KEYWORDS : Ramipril, Valsartan, HPLC, Validation

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INTRODUCTION

Valsartan [fig 1] chemically designated as (S)-3-methyl-2-[N({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)pentanamido]butanoic acid belong to angiotensin II receptor antagonist used for the treatment of hypertension**1**. A number of analytical methods have been developed for its determination in pharmaceutical formulations or in biofluids either alone or in combination with other drugs, which includesliquid chromatography-tandem mass spectrometry**2**, HPLC[3] and a spectrophotometric analysis**4**. Ramipril [fig 2], (2S,3aS,6aS)-1-[(2S)-2{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid is a prodrug which is rapidly hydrolyzed with the cleavage of an ester group through hepatic metabolism forming an active metabolite ie, ramiprilat. This prodrug itself is a poor inhibitor of angiotensin converting enzyme (ACE) but its active metabolite has a higher affinity for ACE, thus blocking the conversion of the angiotensin I to the

angiotensin II, a highly potent vasopressor activity[5,6]. The drug is officially listed in British Pharmacopoeia[7], which describes a potentiometric titration procedure for its assay in bulk and dosage forms. The determination of ramipril along with hydrochlorothiazide in binary mixture was performed by derivative compensation technique[8] as well as zero crossing derivative technique[9,10] . Few visible spectrophotometric[11] and HPLC methods have also been reported for the assay of this drug in commercial dosage forms.

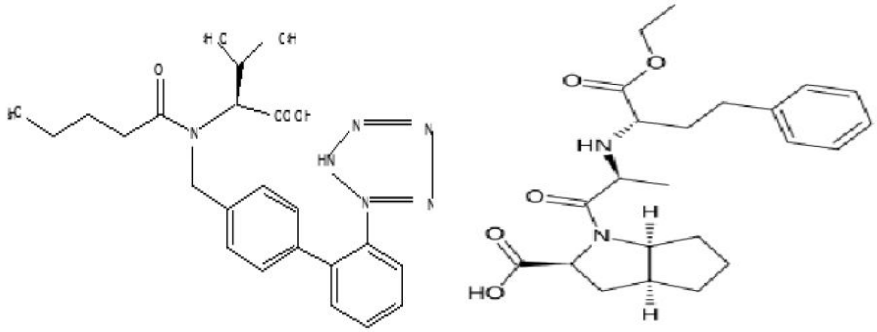


Fig no.1: structures of Ramipril and Valsartan

According to the information collected from literature there is no method reported for the simultaneous determination of valsartan and ramipril. In the present work we are therefore focused to achieve the optimum chromatographic conditions for the simultaneous determination of valsartan and ramipril in synthetic mixture. We describe a simple, sensitive and validated UPLC method with total run time less than 2.0 minutes for the simultaneous determination of valsartan and ramipril. The developed method can be applied successfully to quality control and for other analytical purposes.

1. Chemicals and Reagents

Valsartan and ramipril reference substance with claimed purity of 99.6% and 99.72% respectively were obtained from Pharma Train, Hyderabad. Acetonitrile (HPLC grade) and orthophosphoric acid (Analytical reagent grade), Phosphate buffer were purchased from Merck (Mumbai, India). All excipients used were of pharmaceutical grade. Water used was prepared in the laboratory using Milli-Q system (Millipore, USA).

2. Apparatus and Chromatographic Conditions:

UPLC apparatus consisting of Acquity Waters2996,PDA detector system equipped with A C18 BEH-column (100 × 2.1 mm id, 1.7 µm particle size) was selected. The mobile phase was composed 35:65 Phosphate buffer (0.02Mm) pH 3.5 adjusted with O-Phosphoric acid:Acetonitrile The flow rate was 0.4ml/min and the system was operated at room temperature.

3.Preparation of standard and sample solution:

Standard Solution Preparation: Accurately weigh and transfer 5 mg of Ramipril and 80mg of Valsartan working standard into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.4ml of Ramipril & Valsartan the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation: Accurately weigh and transfer 251.6 mg of Ramipril and Valsartan Tablet powder into a 100ml clean dry volumetric flask add about 70ml of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) .Further pipette 0.4ml of Ramipril & Valsartan the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

3. Linearity:

The linearity of the method was established by spiking a series of standard mixtures of Ramipril (1-5µg/ml), Valsartan (16-48µg/ml) .Above solutions are injected onto the UPLC system. Construct the Calibration curves for standard solutions by plotting their response ratios (ratios of the peak area of the analytes) against their respective

concentrations. Linear regression was applied and slope (a), intercept (b), correlation coefficient (r) and standard error (Es) were determined.

Accuracy:

Accuracy was determined in terms of percent recovery. Sample solution spiked with the analytes at three different concentration levels i.e 50%.100%,150% . Another set of standard mixtures at the same concentration levels was also prepared with the diluents. Sample and standard solutions are injected onto the UPLC system in triplicate. Percentage recoveries of Ramipril and Valsartan were calculated using the following formula

Amount found:

$$\frac{sample\ area}{standard\ area} * \frac{standard\ conc}{sample\ conc} * \frac{standard\ purity\ of\ the\ drug}{100} * \frac{anerage\ weight\ of\ the\ drug\ taken}{label\ claim} * 100$$

Percentage recovery= $\frac{amount\ found}{amount\ of\ drug\ added} * 100$

Precision:

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility). In order to determine injection repeatability, samples spiked with 3ppm of Ramipril and 48ppm of Valsartan were injected 5 times into HPLC system and repeatability of the retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained.

Limit of detection & Limit of quantification:

Detection and quantification limits were determined through dilution method using S/N approach by injecting a 5µl sample. LOD was considered as the minimum concentration with a signal to noise ratio of at least three (S/N≈3), while LOQ was taken as a minimum concentration with a signal to noise ratio of at least ten (S/N≈10).

Robustness:

The robustness of the developed method was investigated by evaluating the influence of small deliberate variations in procedure variables like flow rate (±5%) and change in wave length (±5nm).

Ruggedness:

The ruggedness of the method was investigated by evaluating the influence of different analyst, different time intervals..

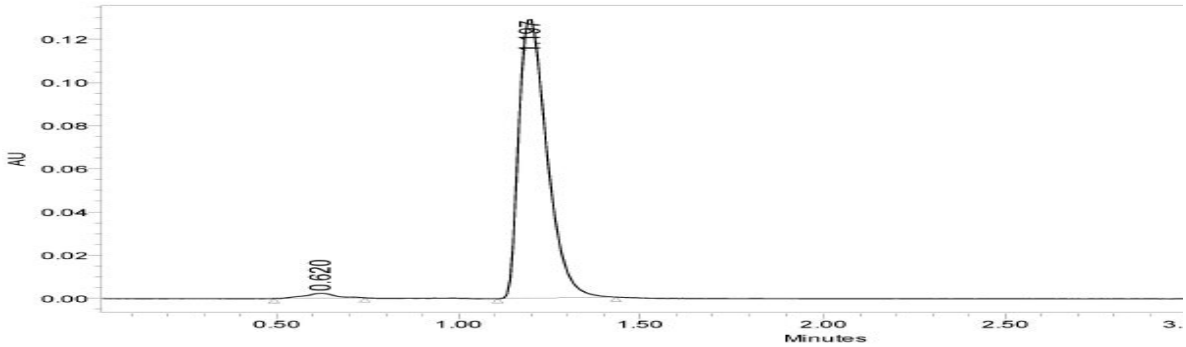
Stability:

The stability studies of Ramipril and Valsrartan samples were carried out over a period of 48 h at 25 ^{0}C (room temperature under laboratory light), 2–8^{0}C (refrigerator) and standard solutions for one month at 2–8^{0}C.

RESULTS AND DISCUSSION:

3.1.Sample preparation:

Several organic solvents were tried for the preparation of stock solutions of all analytes. Methanol was selected due to greater solubility of analytes in it. Ramipril and Valsartan is highly soluble in methanol; So methanol is used for good solubility.



Method validation:

3.2.1. Precission:

Precision data representing both repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility) are summarized in Tables 1 and 2, respectively. The %RSD values for both intra-day and inter-days were less than 2.0%, which indicates that the proposed method is precise.

Table no:1 precission of Ramipril and Valsartan

s.no	no. of injection	area of Ramipril	area of Valsartan
1	Injection-1	10605	699093
2	Injection-2	10603	697760
3	Injection-3	10631	699415
4	Injection-4	10680	698574
5	Injection-5	10641	693756
	Average	10632	697719.6
	S.D	31.44	2302.3
	% RSD	0.29	0.32

Table no: 2 Intraday precision(Ruggedness)

s.no	no. of injection	area of Ramipril	area of Valsartan
1	Injection-1	11449	699094
2	Injection-2	11471	693971
3	Injection-3	11479	692924
4	Injection-4	11499	698130
5	Injection-5	11456	693701
	Average	11470.8	695546
	S.D	19.72	2801.5
	% RSD	0.17	0.40

Accuracy:

Average recoveries of are 99.6%, 100%, 99.7%, respectively. The percentage recoveries of all the drugs are within the limits 99-101%. So the method is accurate presented in table no.3.

Table no : 3 Accuracy of Ramipril & Valsartan

s.no	Drug	Conc	Area	Amount added (mg)	Amount found(mg)	% recovery	Mean recovery
1	Ramipril	50%	28172	2.5	2.53	101.3%	100.4%
		100%	55485	5	4.99	99.8%	
		150%	83345	7.5	7.49	99.9%	
2	Valsartan	50%	649499	40.0	39.9	99.9%	99.5%
		100%	1294525	80.0	79.6	99.6%	
		150%	1933173	120.0	119.0	99.1%	

Linearity:

The response was found linear over a concentration range of 1-3ppm of Ramipril & 16-48ppm The correlation co-efficients for all drugs were found to be 0.999.so the method is linear presented in table no.4

Table no: 4 Linearity's of Ramipril and Valsartan

s.no	Linearity level	Ramipril		Valsartan	
		Conc	Area	Conc	Area
1	I	1ppm	4317	16ppm	351026
2	II	1.5ppm	8383	24 ppm	510933
3	III	2ppm	12684	32 ppm	696370
4	IV	2.5ppm	16054	40 ppm	831388
5	V	3ppm	20302	48 ppm	993523
Correlation Coefficient		0.999		0.999	

Limit of detection:The LOD for Ramipril and Valsartan standard solutions were found to be 0.15.µg/ml , 0.5µg/ml respectively given in figure no.3.

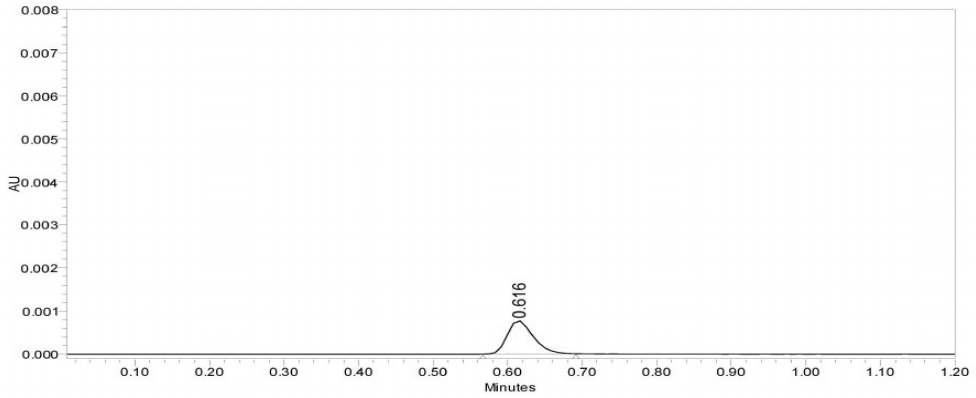


Fig no: LOD RAM

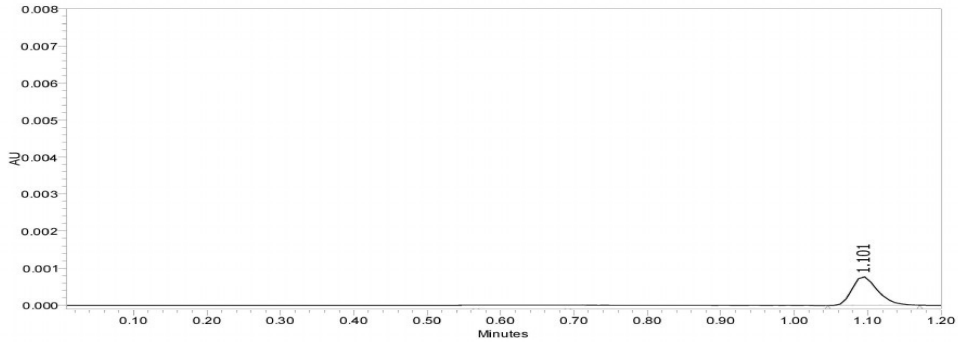


Fig no: LOD VAL

3.2.5. Limit of quantification:

The LOQ Ramipril and Valsartan standard solutions were found to be 0.5µg/ml, 0.011µg/ml respectively given in figure no.4.

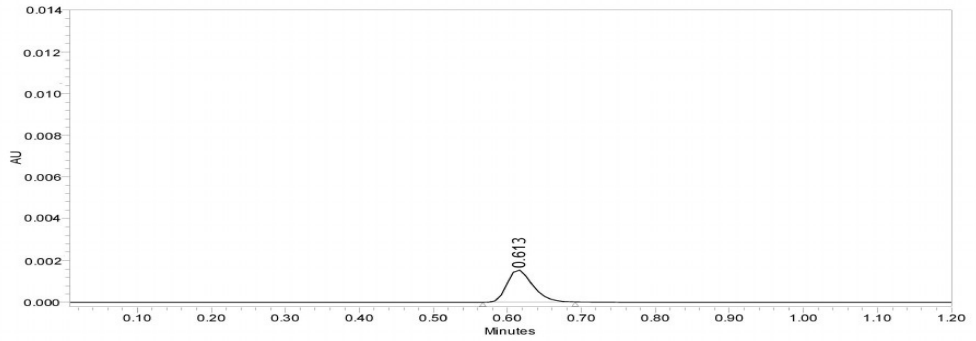


Fig no: LOQ ram

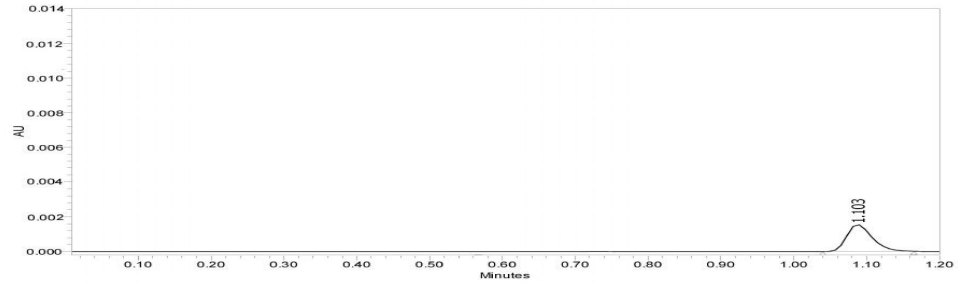


Fig no:LOQ VAL

Robustness:

Minor deliberate changes in different experimental parameters such as flow rate (±5%) and wave length (±0.1 units) did not significantly affect the recoveries, peak area and retention time of all the above drugs indicating that the proposed method is robust.

Table no 5; System suitability of Ramipril and valsartan

	Change in flow rate			Change in Organic Composition in the Mobile Phase		
DRUG	Flow rate ml/min	Retention time	USP plate count		Retention time	USP plate count
Ramipril	0.3	0.8	2286.0	10% less	0.7	2359.6
	0.4	0.6	2323.0	Actual	0.5	2323
	0.5	0.4	2147.9	10%more	0.3	2236.7
Vaisartan	0.3	1.5	2137.3	10%less	1.4	2467.8
	0.4	1.10	2161.1	Actual	1.09	2161.1
	0.5	0.9	2011.7	10% more	0.8	2381.1

Ruggedness:

The method is rugged by different analyst, different time intervals and the method did not significantly affect the recoveries, peak area and retention time of all the above drugs indicating that the proposed method is rugged.

3.2.8. Stability:

Results from the stability studies of samples and standard solutions indicated that samples were stable for 48 h when stored at room temperature (25^0C), refrigerator (2–8^0C) and while the standard solutions demonstrated stability for one month at 2–8 ^{0}C.

Table no: 6 Validation summary of Ramipril and Valsartan

S.no	Parameter	Ramipril	Valsartan	ICH acceptance limit
1	**Accuracy**	100.4%	99.5%	98-102%
2	**Precision**	0.29	0.32	%RSD < 2
3	**Correlation coefficient**	0.999	0.999	Not less than 0.999
4	**LOD**	S/N =3	S/N =3	S/N =3
5	**LOQ**	S/N =10	S/N =10	S/N =10
6	**USP Resolution**	....	23.2	Not less than 2
7	**USP Tailing**	1.0	1.5	Less than 2
8	**USP Plate count**	2323.0	2161.1	Not less than 2000

CONCLUSION:

A simple and accurate reverse phase UPLC method has been developed for the simultaneous determination of ramipril and valsartan. The method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation, selectivity and robustness. The run time of less than ten minutes allows its application for the routine determination of ramipril and valsartan. Further, the proposed UPLC method has excellent sensitivity, precision and reproducibility. Even though no attempt was made to identify the degraded products, proposed method can be used as a stability indicating method for assay of ramipril and valsartan in combined dosage forms.

AKNOWLEDGEMENT:
We are very thank full to my guide, my friends for successful completion of project work.

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