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Research Paper

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KEYWORDS : RP-HPLC, Ramipril, Amlodipine, chromatoghraphy.

RP-HPLC method development for simultaneous determination of the drugs Ramipril and Amlodipine

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ABSTRACT

A new, simple, rapid, specific and sensitive reverse phage high performance liquid chromatographic method has been developed for estimation of Ramipril and Amlodipine. For this validation following parameters precision, accuracy, linearity, limit of detection, limit of quantification, robustness have been studied. The developed method is recommended for routine analysis since it is rapid, simple, precious and robust quantitative analytical method for simultaneous estimation of Ramipril and Amlodipine. Validation has done according to international conference for harmonization (ICH).

1. Introduction:

Pharmaceutical Analysis is defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. The term "Pharmaceutical analysis" is otherwise called quantitative pharmaceutical chemistry. Pharmaceutical analysis includes both qualitative and quantitative analysis of drugs and pharmaceutical substances starts from bulk drugs to the finished dosage forms. In the modern practice of medicine, the analytical methods are used in the analysis of chemical constituents found in human body whose altered concentrations during disease states serve as diagnostic aids and also used to analyze the medical agents and their metabolites found in biological system.

In modern practice of pharmacy it is important that pharmacists have more than an appreciative analytical methodology. The term "quality" as applied to a drug product has been defined as the sum of all factors, which contribute directly or indirectly to the safety, effectiveness and reliability of the product. These properties are built into drug products through research and during process by procedures collectively referred to as "quality control". Quality control guarantees with in reasonable limits that a drug products.

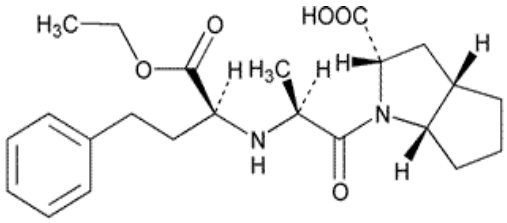

Fig:1. Structure of Ramipril

IUPAC NAME:

(2S,3aS,6aS)-1-[(2S)-2-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino}propanoyl]-octahydrocyclopenta[b]pyrrole-2-carboxylic acid

PHARMACOKINETIC DATA :

Bioavailability	:	28%
Protein binding	:	73% (ramipril)
Half-life	:	2 to 4 hours
Excretion	:	60% Renal and fecal 40%.

Ramipril, a prodrug, is converted to the active metaboliteramiprilat by liveresteraseenzymes. Ramiprilat is mostly excreted by the kidneys. The half-life of ramiprilat is variable (3–16 hours), and is prolonged by heart and liverfailure, as well as kidneyfailure.

Brand Names Available in the Market are PRILACE, RAMIPPRO, ATIACE, TRITACE.

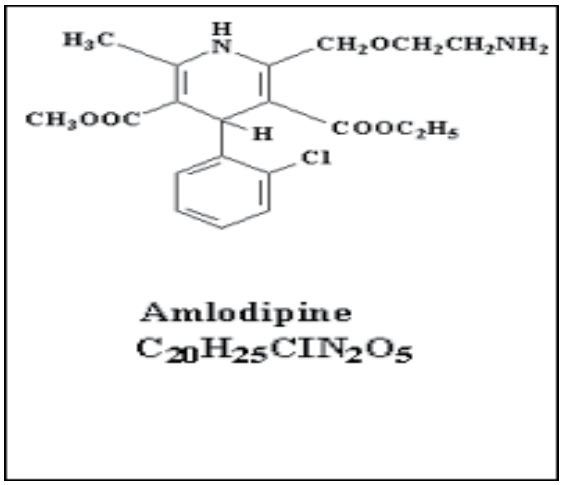

Fig:2. Structure of AMLODIPINE

IUPAC Name: (RS)-3-ehyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-clorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate.

Chemical Formula : $C_{20}H_{25}CIN_2O_5$

Amlodilpine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-chanel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

BRAND NAMES available in market are Amlopin, Amlosun, Camlodin.

PHARMACOKINETIC DATA:

Bioavalability	: 64to 90%
Metabolism	: Hepatic
Half-life	: 30 to 50 hours
Excretion	: Renal
Protein binding	: 97.5%

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METHOD VALIDATION:
Validation is documented evidence, which is completed to ensure that an analytical method is accurate, reproducible and robust over the specific range. The quality of the analytical data is a key factor in the success of a drug development program. The process of method development and validation has a direct impact on the quality of these data.

Method validation is the process to confirm that analytical procedure employed for a specific test is suitable for its intended use. Method needs to be validated or revalidated

- Whenever the conditions changes for which the method has been validated , e.g., instrument with different characteristics
- Whenever the method is changed, and the change is outside the original scope of the method.

PURPOSE OF VALIDATION:
1. Enable the scientists to communicate scientifically and effectively on technical matter.
2. Setting the standards of evaluation procedures for checking compliance and taking remedial action..
3. As quality of the product cannot always be assured by routine quality control because of testing of statistically insignificant number of samples.
4. Retrospective validation is useful for trend comparison of results compliance to CGMP/CGLP.
5. Closure interaction with Pharmacopoeial forum to address analytical problems.
6. Depending on the use of the assay, different parameters will have to be measured during the assay validation. ICH and several regulatory bodies andPharmacopoeia have published information on the validation of analytical procedures.

Criteria for Validation of the Method

CHARACTERISTICS	ACCEPTABLE RANGE
Accuracy	Recovery (98-102%)
Precision, Reputability	RSD < 2%
Intermediate precision	RSD < 2%
Specificity	No Interference
LOD	S/N > 2 or 3
LOQ	S/N > 10
Linearity	Correlation Coefficient(r)>0.99
Range	80-120%
Stability	>24h or >12h

Table: 1. Criteria of Validation

2. Material and Methods:
Quantitative HPLC was performed on an isocratic LC – 10AT VP SHIMADZU High- Pressure Liquid Chromatographic instrument for the analysis. The instrument is provided with solvent delivery module with UV-visible detector SHIMADZU SPD-10A, ODS Reverse phase column (250 X 4.6mm). A 20 ml Hamilton injecting syringe and window based spinchrome software was used for its semi automatic operation, recording and analysis. A sartorious electronic balance was used for weighing the materials.

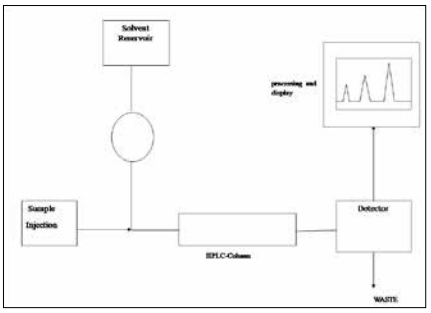

Fig:3. Flow Chart of HPLC
REAGENTS:
a. Milli-Q-water
b. Ortho Phosphoric Acid AR Grade
c. Acetonitrile HPLC Grade
d. Methanol HPLC Grade
e. PotassiumDihydrogenPhosphate AR GRADE

Calculation for Assay %:

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times 100$$

Where:
AT = average area counts of sample preparation.
As= average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = LABEL CLAIM mg/ml.

3. Results and Discussion:
PRECISION:

	PRECISION		RUGGEDNESS	
	Ramipril	Amlodipine	Ramipril	Amlodipine
Average	5040174	918296	4940174	908296
Standard Deviation	0.0	0.0	0.00	0.00
%RSD	0.00	0.00	0.00	0.00

Table: 2. Precision and Ruggedness of Ramipril and Amlodopine

Acceptance Criteria:
The % RSD for the area of five standard injections results should not be more than 2%.

INTERMEDIATE PRECISION/RUGGEDNESS:
ACCEPTANCE CRITERIA:
The % RSD for the area of five sample injections results should not be more than 2%.

ACCURACY:
The accuracy results for Ramipril and Amlodipine:

To perform the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method. The results of the analysis are reported in Table:3.

	Ramipril				Amlodipine			
%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found(mg)	% Recovery	Area	Amount Added (mg)	Amount Found (mg)	% Recovery
50%	577014	6.15	6.27	102.0%	3120009	6.07	6.17	101.7%
100%	918123	10.0	9.98	99.8%	5040174	10.0	9.97	99.8%
150%	1288229	14.3	14.0	98.0%	7087906	14.3	14.0	98.1%

Table: 3. RECOVERY STUDIES FOR RAMIPRIL AND AMLODIPINE

Mean recovery: 99.99%.
Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0.

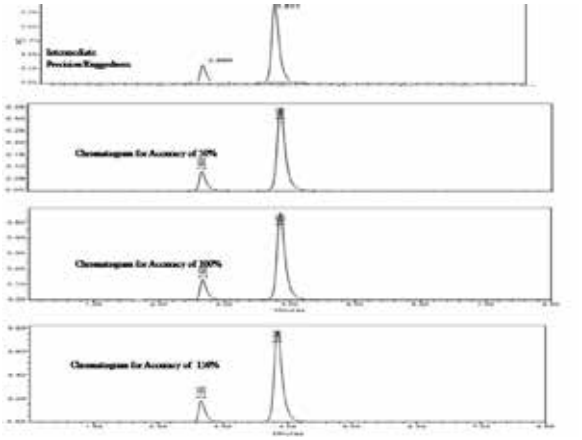

Fig:3 Chromatogram of Intermediate Precision/Ruggedness and Accuracy

LINEARITY:

S. No	Linearity Level	Ramipril		Amlodipine:	
		Concentration	Area	Concentration	Area
1	I	20ppm	2602344	40ppm	1387035
2	II	30ppm	3914138	60ppm	2106996
3	III	40ppm	5291958	80ppm	2882231
4	IV	50ppm	6385532	100ppm	3470152
5	V	60ppm	7730420	120ppm	4180508
Correlation Coefficient 0.999					

Table: 4. Linearity of Ramipril and Amlodipine
Acceptance Criteria:
Correlation coefficient should be not less than 0.999

LIMIT OF DETECTION(LOD):

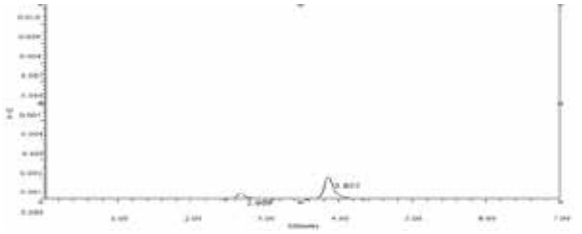

Fig:4. Limit of Detection for Ramipril and Amlodipine

	Name	Retention Time (min)	Area (V*sec)	Height (V)
1	Ramipril	2.669	932	131
2	Amlodipine	3.855	1195	133

Table:5.LOD Area and Height of Ramipril and Amlodipine.

Calculation of S/N Ratio:
Ramipril:
Average Baseline Noise obtained from Blank : 44 µV

Signal Obtained from LOD solution (0.75% of target assay concentration) : 131 µV S/N = 131/44 = 2.97

Amlodipine:
Average Baseline Noise obtained from Blank : 44 µV

Signal Obtained from LOD solution (0.01% of target assay concentration): 133 µV

S/N = 133/44 = 3.0

Acceptance Criteria:S/N Ratio value shall be 3 for LOD solution.

LIMIT OF QUANTIFICATION(LOQ):

	Name	Retention Time (min)	Area (V*sec)	Height (V)
1	Ramipril	2.669	3083	433
2	Amlodipine	3.855	3935	438

TABLE:6. LOQ Area and Height of Ramipril and Amlodipine.

Calculation of S/N Ratio:

Ramipril:
Average Baseline Noise obtained from Blank : 44 µV
Signal Obtained from LOQ solution (2.5% of target assay concentration) : 433µV S/N = 433/44 = 9.84

Amlodipine:
Average Baseline Noise obtained from Blank : 44 µV
Signal Obtained from LOQ solution (0.06% of target assay concentration) : 438µV S/N = 438/44= 9.95

Acceptance Criteria:S/N Ratio value shall be 10 for LOQ solution.

ROBUSTNESS:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

The flow rate was varied at 0.5ml/min to 0.7 ml/min.

a) Standard solution 80ppm of Ramipril& 40ppm of Amlodipinewas prepared and analysed using the varied flow rates along with method flow rate. On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.The method is robust only in less flow condition.

SYSTEM SUITABILITY:

S.No	Flow Rate (ml/min)	Ramipril		Amlodipine	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.5	3550.1	1.5	4675.7	1.4
2	0.6	3550.1	1.5	4675.7	1.4
3	0.7	3415.2	1.5	4085.2	1.4

Table: 7. System Suitability for Ramipril and Amlodipine
* Results for actual flow (0.6ml/min) have been considered from Assay standard.

b). The Organic composition in the Mobile phase was varied from 70% to 65%.

Standard solution 80 µg/ml ofRamipril&40 µg/ml ofAmlodipine have been was prepared And analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

The results are summarized on evaluation of the above results, it can be concluded that the variation in 10% Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±1.

	Name	Retention Time (min)	Area (V*sec)	Height (V)	USP Plate Count	USP Tailing
1	Ramipril	2.862	918193	128125	3550.1	1.5
2	Amlodipine	4.184	5040124	562145	4675.7	1.4

Table: 8. Plate Counting for Ramipril and Amlodipine
The above measured all parameters are optimized to analyze the drugs simultaneously. All the three components of the dosage form were identified by comparison of retention times obtained from sample and standard solutions. The work was

performed in an air-conditioned room maintained at 25 ± 2°C. Column chemistry, solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The number of plates (N) is a measure of column efficiency; which shows the high separation efficiency of the column used.

CONCLUSION

- The evaluation of obtained values suggests that the proposed HPLC methods provide simple, precise, rapid and robust quantitative analytical method for simultaneous estimation of Ramipril and Amlodipine in tablet dosage form.
- The run time was short i.e 7min which enables rapid quantitation of many samples in routine and quality control analysis of formulations.
- The mobile phase is simple to prepare and economical. After validating proposed method as per ICH guidelines and correlating obtained values with the standard values, satisfactory results were obtained.
- Hence, the method easily and conveniently adopted for routine estimation of Ramipril and Amlodipine in tablet dosage form.

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