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A RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF S-AMLODIPINE AND LOSARTAN POTASSIUM IN COMBINED DOSAGE FORMS

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ABSTRACT

A simple, accurate, precise and sensitive analytical RP-HPLC method was developed for the determination of S-amlodipine and Losartan Potassium in tablet form. The mobile phase used was a mixture of 55 volumes of phosphate buffer ph 5.0 prepared by dissolving 0.68 g of potassium Di-hydrogen orthophosphate and 4.0 ml tri-ethylamine in 1000 ml of water, adjust to pH 5.0 with dilute Ortho phosphoric acid, 22 volumes of acetonitrile and 18 volumes of methanol. A stainless steel column of 250mm x 4.6 mm, packed with octylsilane bonded to porous silica (5µm) was used, with flow rate 1.5 ml/min, wavelength 237 nm and injection volume 20 µl. The optimized method was then validated as per ICH guidelines Q2 [R1]. The method was linear over the concentration range 80% -120%. The percentage recoveries of S-amlodipine and Losartan Potassium were 101.13% and 100.88 % respectively. The assay results obtained by two analysts using two instruments on different days had a statistical RSD less than 2%.The method was also found to be robust and rugged. All of the validation parameters were within theacceptance criteria as per the ICH guidelines. Hence the proposed method can be used for the routine control analysis of S-amlodipine and Losartan Potassium in combined dosage form.

KEYWORDS: S-amlodipine, Losartan Potassium, Analytical method validation, HPLC.

INTRODUCTION

Validation is an integral part of quality assurance; it involves the systematic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified. Validation means demonstration, by provision of

objective evidence that consistently meets its predetermined requirements. The word "validation" comes from Latin word term *valdius* meaning worth/strong, thus suggesting that something is true, useful, and reliable. The most accurate definition of validation is provided by ISO 900:2000 as the conformation, by means of a through examination and obtaining realistic and unequivocal evidences, that the procedure is effectively applicable for its intended purposes. It is the act of providing that any approach, strategy experimental procedure, process, instrumentation and room conditions selected for the method will function in a proper way under a fixed set of conditions. Besides it can be used to individually evaluate the appropriateness of these factors.

Method Validation means establishing documented evidence that a specific method and the ancillary instruments included in the method will consistently yield results that accurately reflect the quality characteristics of the product tested. It is the process of demonstrating that an analytical procedure is suitable for its intended purpose. The method validation evaluates the range and conditions of applicability, and checks if every future measurement in routine analysis will provide a concentration of the analyte close enough to the true value. In addition, it can also quantify the degree of coincidence of a measured concentration and the true value, by the calculation of the bias and the uncertainty associated with the result. Therefore, the validation verifies if the method is suitable to be used as a quality control tool and for research support. It is an essential step in method development, which must be implemented by laboratories to prove they can produce analytical data with high reliability. Method Validation is an important requirement for any package of information submitted to international regulatory agencies to support new product marketing or clinical trials applications. Analytical method should be validated, including methods published in the relevant pharmacopoeia or other organized standard references. The suitability of all test methods used should always be verified under the actual conditions of use and should be well documented.

Method development is a continuous process that progress in parallel with the evolution of the drug product. The notion of phase-appropriate method development is critical oneif time cost and efficiency are concerns. The goal and purpose of the method should reflect the phase of drug development. During early drug development, the method may focus on API behavior. They should suitable to support pre-clinical safety evolution, preformulation studies and protype product stability studies.

When there are no authoritative methods are available, new methods are being developed for analysis of novel products. To analyze the existing either pharmacopoeial or non-pharmacopoeial products novel methods are developed to reduce the cost besides time for better precision and ruggedness. These methods are optimized and validated through trial runs. Alternate methods are proposed and put into practice to replace the existing procedure in the comparative laboratory data with all available merits and demerits.

Drug profile

S-amlodipine besylate

S-amlodipine Besylate Hemi pentahydrate also known as Levamlodipine Besylate Hemi pentahydrate. It is a pharmacologically active enantiomer of amlodipine besylate. S-Amlodipine besylate hemipentahydrate is used in the treatment of Angina (heart-related chest pain) and Hypertension (high blood pressure). S-Amlodipine besylate hemipentahydrate is a dihydropyridine group of calcium channel blocker. In high blood pressure, it normalizes the blood pressure by relaxing the blood vessels to reduce the pressure on them, thereby improving the blood flow in the body. The enhanced blood flow in the body further relaxes the heart muscles by reducing the workload on the heart.It also improves the oxygen flow in the body, thereby, preventing any heart-related chestpain.

Chemically it is (4S)-2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-1, 4-dihydro-6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl 5-ethyl ester benzene sulfonate hemipentahydrate with its molecular weight 612.10 g/mol.

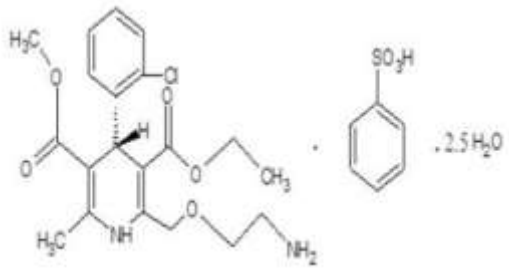

Fig. S-Amlodipine Besylate.

Losartan potassium

Losartan Potassium is the potassium salt of losartan, a non-peptide angiotensin II receptor antagonist with antihypertensive activity. Losartan selectively and competitively binds to the angiotensin II receptor (type AT1) and blocks the binding of angiotensin II to the receptor, thus promoting vasodilatation and counteracting the effects of aldosterone. Converted from

angiotensin I by angiotensin-converting enzyme (ACE), angiotensin II stimulates the adrenal cortex to synthesize and secrete aldosterone, decreasing sodium excretion and increasing potassium excretion, and actsas a vasoconstrictor in vascular smooth muscle.

Losartan potassium is chemically described as 2-butyl-4-chloro-1-[p-(o-1H- tetrazol-5-ylphenyl)benzyl]imidazole-5-metahnol monopotassium salt. Its empirical formula is $C_{22}H_{23}ClKN_6O$, and its molecular weight is 422.9.

Losartan is generally marketed as the (basic) potassium salt of the aromatized negatively charged tetrazole, called "losartan potassium". The molecule has an extended biphenyl group with a tetrazole which is being used in place of the carboxylicacid as a bioisostere.

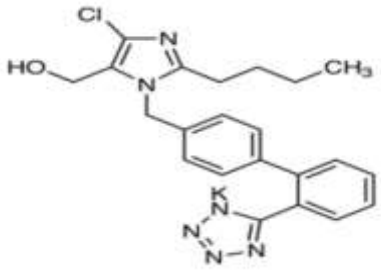

Fig. Losartan potassium.

METHODOLOGY

Chemicals and Working standard

- Sodium Acetate Anhydrous (AR Grade)
- Glacial Acetic acid(HPLC Grade)
- Potassium Dihydrogen Orthophosphate(AR Grade)
- Triethylamine (HPLC Grade)
- Phosphoric acid (HPLC Grade)
- Acetonitrile (HPLC Grade)
- Methanol (HPLC Grade)
- HPLC Grade Water
- S-Amlodipine Besylate Standard
- Losartan Potassium Standard

Instrumentation

- Prominence I (LC-2030C PLUS)

- Electronic Balance (SHIMADZU)
- Ultrasonic Sonicator (Optics Technology)
- Calibrated Glassware (Borosil)

Methodologydissolution

Determine by liquid chromatography

Apparatus:	Paddle
Medium:	900 ml of 0.01 M Sodium-Acetatesolution, adjusted to pH 4.5 with glacial acetic acid
Speed:	75 RPM
Time:	30 minutes.
Temperature:	37±0.5ºC

Standard solution

Standard Solution A: Weigh accurately about 27 mg S-*Amlodipine besylate* in 100 ml volumetric flask and make up the volume with 100 ml methanol.

Standard Solution B: Weigh accurately about 27 mg *Losartan Potassium* in 100 ml volumetric flask and make up the volume with 100 ml methanol.

Standard Solution C: Dilute 1 ml of Standard Solution A and 10 ml of Standard Solution B in 100 ml of volumetric flask and make up the volume with dissolution medium.

Test solution

Introduce 900ml of dissolution medium into the vessel of the apparatus. Warm thedissolution medium to 36.5˚-37.5°c. Place one tablet in each vessel and rotate the paddle at 75 RPM for 30 minutes. Withdraw a suitable volume of the sample and filter. Reject the first few ml of the filtrate and filter through 0.2 µm membrane filter.

Inject the standard solution. The test is not valid unless the tailing factor is not more than 2.0 The relative standard deviation for replicate injections is not more than 2.0 percent and theoretical plates is not less than 2000.

Procedure

Inject Blank (50µL solvent mixture), standard solution and test solution and record the response.

Chromatographic system

- **Column:** A stainless steel column 25cm x 4.6 mm, packed with octylsilane bonded to porous silica(5µm)
- **Mobile phase:** A mixture of 55 volumes of phosphate buffer pH 5.0, prepared by dissolving 0.68 g of potassium Di-hydrogen orthophosphate and 4.0 ml tri- ethylamine in 1000 ml of water, adjust to pH 5.0 with dilute Ortho phosphoric acid, 22 volumes of acetonitrile and 18 volumes of methanol.
- **Flow rate:** 1.5 ml per minutes.
- **Wave length:** 237 nm.
- **Injection volume:** 50 µl
- **Temperature:** Ambient.

Acceptance criteria: *NLT 70% of stated amount of S-amlodipine and Losartan potassium.*

Assay

Determine by Liquid chromatography.

Standard preparation

A. Standard Solution A

Dissolve about 25 mg of S-*Amlodipine Besylate* working standard in 100 ml volumetric flask and make up the volume to 100 ml with mobile phase.

B. Standard Solution B

Dissolve about 62.5 mg of *Losartan potassium* in 50 ml of volumetric flask and makeup the volume to 50 ml with mobile phase.

C. Standard Solution C

Dilute 5 ml of Standard Solution A and 10 ml of Standard Solution B in 50 ml of volumetric flask and make up the volume with mobile phase.

Test solution

Weigh 5 intact tablets and transfer to 100ml volumetric flask. Add 10ml water and 40ml mobile phase to 100ml volumetric flask and sonicate it for 10 minutes. Cool the solution to room temperature and make up the volume to 100ml with the mobile phase. Filter the solution. Pipette 5 ml of the above solution to 50 ml with the mobile phase. Now filter it

through 0.2 µm membrane filter.

Chromatographic system

- **Column:** A stainless steel column 25cm x 4.6 mm, packed with octylsilane bonded to porous silica(5µm)
- **Mobile phase:** A mixture of 55 volumes of phosphate buffer pH 5.0, prepared by dissolving 0.68 g of potassium Di-hydrogen orthophosphate and 4.0 ml tri- ethylamine in 1000 ml of water, adjust to pH 5.0 with dilute Ortho phosphoric acid, 22 volumes of acetonitrile and 18 volumes of methanol.
- **Flow rate:** 1.5 ml per minutes.
- **Wave length:** 237 nm.
- **Injection volume:** 20 µl
- **Temperature:** Ambient.

Procedure

Separately inject 20µL of blank (mobile phase), Standard Solution C and Test Solution and record the chromatographs, measure the major peak areas.

System suitability requirements

Inject the **Standard Solution C**. The test is not valid unless the relative deviation for replicate injection for each of the peaks corresponding to *Losartan potassium*(first peak) and S-*Amlodipine* (second peak) is not more than 2.0%.The tailing factor for both peak due to *Amlodipine* and *Losartan potassium* is not more than 2.0.

Acceptance criteria: *NLT 90% and NMT 110% of the stated amount of S-amlodipine.*

Method validationspecificity

The specificity of the method is determined by checking the interference of blank and placebo with an analyte.

Blank, Standard solution, Placebo solution and Test solution were prepared. Mobile phase was used as blank. Placebo solution was preaperd by weighing 2.5g of placebo in clean and dry volumetric flask and diluted to the mark with mobile phase and heated on water bath for about 10 minutes and then filtered through 0.45 µm filters.

System suitability

System suitability tests was performed on HPLC systems to determine the accuracy and precision of the system by injecting six injections of a solution containing analyte at 100% of test concentration. % RSD of area, Resolution and Theoretical plates were analyzed.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in sample.

Linearity was determined by preparing samples of five different concentrations within the range of 80 % to 120 % of the target concentration. Each of final concentration was prepared in triplicate form. Linearity was plotted for peak area response against concentration.

Range

Range was determined by preparing the sample solutions of three different concentrations: 80%, 100% and 120%.They were then compared with the standardsolution and mean potency and the RSD% were calculated.

Accuracy

Accuracy was determined by preparing spiked samples at three concentrations over the range of 80 %, 100 % and 120 % of the target concentration. Three individually prepared replicates at each concentration were analyzed. They were then compared with standard solution and then % recovery was found out.

$$\%\ Recovery = \frac{AnalyticalResult}{TrueValue} X100\%$$

Precision

The closeness among analytical results i.e. the precision of an analytical method is indicated by relative standard deviation, RSD, which is determined by the equation:

$$RSD(\%) = \frac{100}{\bar{X}}\left[\frac{\sum (X_i - \bar{X})^2}{n-1}\right]^{1/2}$$

Where,

$\bar{X}$ is the arithmetic mean of total assays or average assay value. is an individual assay value.

X_i is the total number of data, observation or measurement.

Two types of precision were performed: Repeatability and Intermediate Precision.

Repeatability

Repeatability was performed by preparing six concentration of 100% concentration of test and their mean value and % RSD were calculated.

Intermediate precision

Intermediate Precision expresses variations with laboratories, such as different days, different analysts, different equipment and so forth. Triplicate sample of 100% concentration was prepared and then carried on two different days and similarly by two analysts.

Limit of Detection & Limit of quantification: Not necessary for Assay (As per ICH guidelines)

Robustness

Robustness was performed by varying different factors. The factors that were varied are given below:

- Change in Wavelength: Analytical method was deliberately changed in wavelength by 2 nm (i.e. 237 nm to 239 nm)
- Change in Flow rate: Analytical method was deliberately changed in flow rate by 0.2 ml/min (i.e. 1.5 ml/min to 1.7 ml/min)
- Change in Column oven temperature: Analytical method was deliberately changed in oven temperature to 30^0C from Room temperature.

Solution stability

Solution Stability was determined by analysing solutions of 100% concentration test in comparison to the fresh prepared solutions and original solutions stored at room temperature in auto sampler (at least 24 h) and stored at 2 - 8 °C, in refrigerator (at least 48 hour). The mean value of the standard solutions was compared to the fresh prepared standard solutions in case of the stability of the standard solution.

RESULT AND DISCUSSION

After selecting suitable mobile phase, Column, diluents and wavelength, the chromatographic condition was selected .The Chromatographic Condition used was:

- **Column:** A stainless steel column 25cm x 4.6 mm, packed with octylsilane bonded to

porous silica(5µm)

- **Mobile phase:** A mixture of 55 volumes of phosphate buffer pH 5.0, prepared by dissolving 0.68 g of potassium Di-hydrogen orthophosphate and 4.0 ml tri- ethylamine in 1000 ml of water, adjust to pH 5.0 with dilute Ortho phosphoric acid, 22 volumes of acetonitrile and 18 volumes of methanol.
- **Flow rate:** 1.5 ml per minutes.
- **Wave length:** 237 nm.
- **Injection volume:** 20 µl
- **Temperature:** Ambient.

Method validation

The developed RP-HPLC method was validated with reference to ICH guidelines [Q2(R1)].

Specificity

No any interference of blank and placebo was seen in the principal peak of the standard and sample solution. There was no interference with the elution of analyte.

System suitability

Six replicates injection of same concentration of solution were injected. % RSD of area, resolution, theoretical plates were analyzed.

Table 1: System suitability test.

Prameters	Limit	Observation	
		S-amlodipine	Losartan Potassium
%RSD of Area	NMT 2%	0.161%	0.451%
Tailing Factor	NMT 2	1.121	1.609
Theretical Plates	NLT 2000	13232	9232

Linearity

Linearity was assessed at five different concentration. The correlation coefficient for five concentration levels was 0.997 for S-amlodipine and 0.999 for Losartan Potassium. The values found were within limit, hence the linearity meets the requirement for the assay of S-amlodipine and Losartan Potassiumin combination form of tablet. The data of Linearity were tabulated below along with their respective calibration curve.

S-amlodipine

Table 2: Linearity of S-amlodipine Besylate.

S.No.	Conc.	Area					
	%	Std 1	Std 2	Std 3	Mean	SD	RSD
1	80	497814	491308	492212	493778	3524.38	0.714
2	90	550700	555762	552066	552843	2618.84	0.474
3	100	610425	613334	606595	610118	3379.97	0.554
4	110	675388	674217	680570	676725	3380.95	0.500
5	120	718027	709986	715346	714453	4094.20	0.573

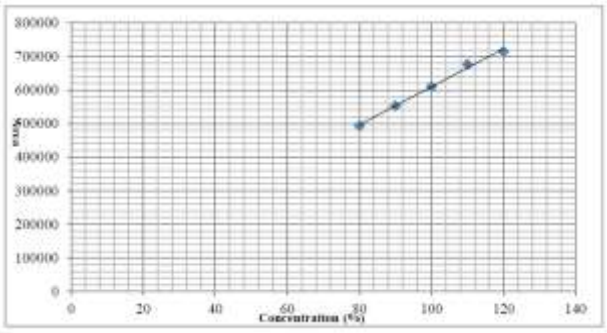

Fig. 1: Calibration curve of S-amlodipine.

- Slope: 44351.00
- Intercept: 5652.33
- Correlation coefficient: 0.997078

Losartan potassium

Table 3: Linearity of losartan potassium.

S. No.	Conc.	Area					
	%	Std 1	Std 2	Std 3	Mean	SD	RSD
1	80	7077259	7035843	7072052	7061718	22559.14	0.319
2	90	7891098	7942388	7926758	7920081	26288.77	0.332
3	100	8802641	8810677	8707599	8773639	57333.28	0.653
4	110	9654048	9665093	9672483	9663875	9277.691	0.096
5	120	10534246	10541198	10535766	10537070	3654.844	0.035

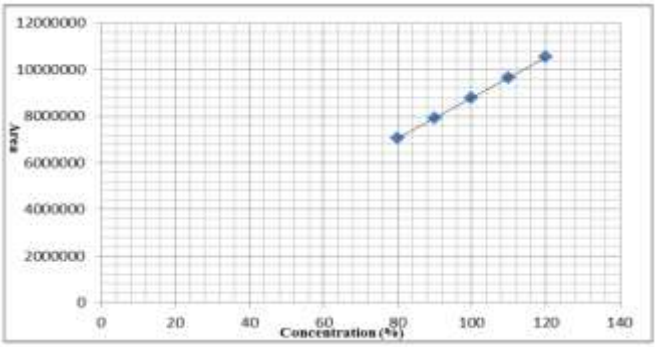

Fig. 2: Calibration curve for losartan.

- Slope: 96779.27
- Intercept: 86944.973
- correlation coefficient: 0.999970

Accuracy and Range

The content and percentage recovery of S-amlodipine and Losartan Potassium were within the limit of ± 2%. Hence the accuracy and range meets the requirement for assay of S-amlodipine and Losartan Potassium in combined dosage forms.

S-amlodipine

Table 4: % recovery of S-amlodipine.

No. of Test	Level of addition	Amount spiked (%)	% Recovery	% Recovery As per Average
Assay Test1	80%	81.28	101.60	100.09
Assay Test2		81.92	102.40	100.88
Assay Test3		81.60	102..00	100.48
Assay Test1	100%	101.20	101.20	99.69
Assay Test2		102	102	100.48
Assay Test3		101.60	101.60	100.09
Assay Test1	120%	121.44	101.20	99.69
Assay Test2		120.00	100.00	98.51
Assay Test3		121.92	101.60	100.09

Result: The accuracy is in the range of 98.51%-100.88%.

Losartan potassium

Table 5: % Recovery of losartan potassium.

No. of test	Level of addition	Amount spiked(%)	% Recovery	% Recovery As per Average
Assay Test1	80%	85.6	107.00	99.53
Assay Test2		86.20	107.75	100.23
Assay Test3		86.01	107.51	100.01
Assay Test1	100%	107.04	107.04	99.57
Assay Test2		107.32	107.32	99.83
Assay Test3		106.80	106.80	99.35
Assay Test1	120%	129.79	108.16	100.61
Assay Test2		128.68	107.23	99.75
Assay Test3		130.46	108.72	101.13

Precision

The assay results obtained by two analysts using two instruments on different days had a statistical RSD less than 2%. Hence the Intermediate precision meets the requirement forthe assay of S-amlodipine and Losartan Potassium in combination form of tablets.

S-amlodipine

Parameter	Assay% test-1	Assay% test-2	Assay% test-3	Average
Analyst-1	99.94	97.2	99.11	99.16
Analyst-2	100.56	100.69	100.65	
Instrument-1	99.66	97.35	98.4	
Instrument-2	99.78	99.92	99.84	
			RSD:	1.060

CONCLUSION

A simple, accurate, precise and sensitive analytical RP-HPLC method was developed for the determination of S-amlodipine and Losartan Potassium in tablet form. The method isolates the individual peaks of the mixture of drugs and overcomes the problem of merging and interference of mixture peaks with each other. This method was validated as per ICH guidelines for all the parameters and the results passed the criteria set forth by ICH guidelines.

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