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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR AMLODIPINE BESYLATE AND LISINOPRIL IN COMBINED TABLET DOSAGE FORM BY USING SIMULTANEOUS ESTIMATION METHOD

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ABSTRACT
The purpose of this study was to develop and validate a simple, sensitive, accurate, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method for simultaneous estimation of amlodipine besylate and lisinopril in a Tablet dosage form. The chromatographic measurement was performed on a Phenomenex C18 (250 × 4.6 mm, 5um) column with an optimised Acetate buffer mobile phase: Methanol (65:35). The flow rate was one ml/min, and the detecting wavelength was 221 nm. Triethanolamine was used to adjust the pH to 5. In the concentration range of 4-20 ug/ml, amlodipine besylate and lisinopril showed a linear response of the suggested approach. The correlation coefficients ('r' values) for amlodipine besylate and lisinopril were 0.9998 and 0.9995, respectively, and the retention times were 3.279 for amlodipine besylate and 6.124 for lisinopril, respectively. The developed chromatographic technique was validated for specificity, linearity, precision, accuracy, LOD, and LOQ using ICH Q2(R1) criteria. The analysis results have been validated in accordance to ICH guidelines.

Keywords: Analytical Method Development, ICH Q2 (R1), Amlodipine Besylate, Lisinopril.

1. INTRODUCTION
Most versatile analytical approach for separating, identifying, and quantifying active components in a mixtures is high performance liquid chromatography (HPLC). In laboratories, it is the most often used analytical technique. The elements (or analytes) are dissolved in a solvent before being allowed to flow under high pressure through a chromatographic column. In the column, the mixture is separated into its constituents. The active components in a mixture are separated based on their interactions with the mobile and stationary phases. Components having a high affinity for mobile phase detect earlier, while components with a high affinity for stationary phase detect later. Because of this, HPLC obtains a level of flexibility that does not match by other chromatographic procedures, and it can separate a wide range of chemical combinations. In HPLC, high pressure is utilised to pump solvent through closed columns containing extremely minute particles, resulting in high-resolution separations. This approach can determine a wide range of organic, inorganic, and biological materials.

An HPLC system consists of a solvent reservoir, a pump, an injector, an analytical column, a detector, a recorder, and a waste reservoir. Additional key components are an inlet solvent filter, post-pump inline filter, sample filter, guard column, back pressure regulator, and solvent purging system. The pump keeps the liquid flow rate steady. The injector is used to inject the sample, which is then transferred to the column to be separated. Components of the mixture are separated and detected one by one from the column. HPLC employs either liquid-solid adsorption or liquid-liquid partition chromatography modes, as well as normal or reversed phase chromatography. Because polarity influences both adsorption and solubility, partition and adsorption chromatography are both affected by changes in solute polarity. Partition chromatography, in general, works

best for separating very polar materials, whereas adsorption chromatography works best for separating comparatively non-polar materials [1-3].

1.1. Most commonly used methods of HPLC

1.1.1. Normal phase chromatography

Mechanism involves contact of the stationary phase's polar surface with the polar areas of sample molecules causes retention. A Stationary Phase is a siloxane bonded with polar functional groups such as SiO_2, Al_2O_3, $-NH_2$, -CN, $-NO_2$, -Diol, etc. A mobile phase includes Heptane, hexane, cyclohexane, chloroform, ethyl ether, and dioxane as nonpolar solvents. Through the technique non-ionic, nonpolar, and mildly polar materials are separated. The least polar components are eluted first.

1.1.2. Reverse phase chromatography

Mechanism involves contact between the nonpolar hydrocarbon chain of the stationary phase and the nonpolar portions of the sample molecules causes retention. A stationary phase is nonpolar functional groups connected to siloxane include n-octadecyl (C18) or n-octyl (C8), ethyl, phenyl, and -(CH_2) n-diol-(CH_2) n-CN. Mobile phase is usually acetonitrile, water, methanol, or buffer (often with THF or dioxane additions) as polar solvents. Through the technique non-ionic ion-forming nonpolar to medium-polar substances (carboxylic acids, hydrocarbons) must be separated. The majority of polar components elute first [4-6].

2. DRUG PROFILE

Table 1: Drug profile For Amlodipine besylate

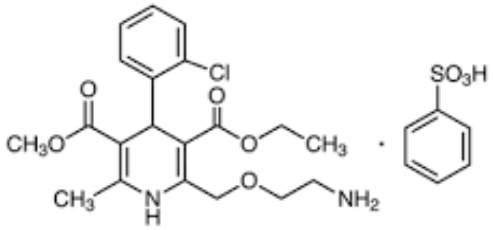
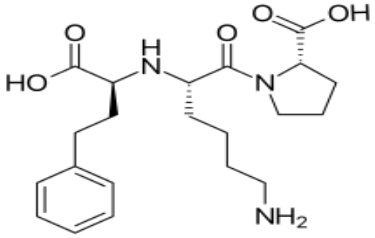
Parameter	Amlodipine besylate
Structure	
IUPAC Name	IUPAC Name 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate; benzene sulfonic acid
Molecular formula	C26H31ClN2O8S
Molecular weight	567.1
pKa	6.7
Melting point	156° C -160°C
Category	Antihypertensive
Pharmacokinetics and Pharmacodynamics	High oral bioavailability and permeability; adult dose is 5mg once daily, while children's dose is 2.5mg. Amlodipine works by inhibiting voltage-sensitive calcium channels. It acts on L-type calcium channels. Amlodipine slows conduction in the SA and AV nodes, where action potentials are generated. The propagation of SVT is dependent on slow inward Ca2+ current, which slows the heart and terminates SVT by producing partial AV blockages. It shortens the action potential plateau and lowers the force of contraction. lowers Ca2+ entry, which lowers depolarization and, as a result, reduces premature ectopic beats.

Table 2: Drug Profile For Lisinopril

Parameter	Description
Name of drug	Lisinopril
Structure	
IUPAC Name	(2S)-1-[(2S)-6-Amino-2-{[(1S)-1-carboxy-3-phenylpropyl]amino}hexanoyl]-2-pyrrolidinecarboxylic acid
Molecular formula	C21H35N3O7
Molecular weight	441.5
pKa	3.17
Melting point	199 - 201°C
Category	Antihypertensive
Pharmacokinetics and Pharmacodynamics	Angiotensin-converting enzyme inhibitor (ACEI) lisinopril is used to treat hypertension, heart failure, and myocardial infarction. The only ACEIs that are approved are lisinopril. Prodrugs are not permitted. It works by inhibiting angiotensin converting enzyme and the renin angiotensin aldosterone system. ACEIs, together with thiazide diuretics or beta blockers, are often used as first-line medication in the treatment of hypertension.

3. MATERIAL AND METHODS

A Gradient HPLC (Shimadzu) with an LC-2010CHT double reciprocating pump, UV visible spectrophotometer (UV2450), and the RP-HPLC system were used for data processing. Cipla pharmaceutical Ltd, India, sent a gift sample of lisinopril (10gm) and amlodipine besylate (10gm) reference standards. Water, ethanol, acetonitrile, sodium acetate, and ortho phosphoric acid were also used.

3.1. Chromatographic condition

A PhenomenexC18 (250 4.6mm, 5micron) column was used to create the method. Methanol: Water (63:35, pH 5) was utilised as the mobile phase. The flow rate used was 1ml/min. The detection wavelength was 221 nm. Amlodipine besylate and Lisinopril had retention times of 3.136 and 6.024 seconds, respectively.

3.2. RP-HPLC method development

3.2.1. Standard Stock Solution Preparation

Two separate 100 mL volumetric flasks containing 100 mg of each of the reference standards, lisinopril and amlodipine besylate were taken. Both the drugs were dissolved in 50 mL of methanol and then sonicated to completely dissolve them. The methanol (1000 g/ml) solution was then diluted to the desired concentration. 10ml was added to 100ml of methanol for further dilution. 1ml to 10ml of methanol dilution was added.

3.2.2. Preparation of working solution

Standard stock solutions (10 g/ml) of 04, 0.8, 1.2, 1.6, and 2.0 ml were taken for each of the copmounds, lisinopril and amlodipine besylate. The capacity in a 10 ml volumetric flask with methanol was made up.The final drug concentration varied between 4 and 20 g/ml.

3.2.3. Preparation of 0.1% OPA in water

One mL of OPA was pipetted out and poured into a 100 mL volumetric flask, adjusting the volume with water to the mark. Mixed thoroughly and sonicated for 5 minutes.

3.2.4. Preparation of an 20.00 mM phosphate buffer in water

Potassium dihydrogen orthophosphate (0.0136 gm) was transferred to 1000 mL of water, then dissolved with 10M sodium hydroxide to adjust the pH to 5.

3.2.5. Preparation of 20.00 mM Acetate buffer in water

In 1000ml of water, we weighed roughly 0.027 g and 12 ml of glacial acetic acid. Diluted OPA solution was used to adjust the pH to 5 ± 0.05.

3.3. Callibration curve

Methanol was chosen as the solvent for dissolving Amlodipine besylate and Lisinopril. Amlodipine besylate and Lisinopril were spiked into the HPLC apparatus and ran in several solvent systems.

The standard solution was scanned from 200 to 400 nm. The drug's maximum absorption wavelength was established. Maximum absorbance was observed for amlodipine besylate and lisinopril at 243nm and 218nm, respectively. The wavelength chosen was 221nm. Amlodipine besylate and Lisinopril 10 ppm solutions were scanned separately between 200-400 nm. At 221nm, the isosbestic point was determined.

3.4. Analysis of the marketed formulation

A total of twenty tablets were crushed into fine powder. The tablet powder, containing 5 mg of Amlodipine besylate and 5 mg of Lisinopril, was transferred to a 100 mL clean and dry volumetric flask, dissolved in 50 mL of mobile phase, and ultra sonicated for 10 minutes. The volume was ultimately brought up to the appropriate level after the solution was filtered through a 0.45um nylon syringe filter. More dilution with mobile phase and standard stock solution was added to form a mixed sample solution containing 5mg of each drug. To achieve the appropriate final concentrations, 2 ml of above filtrate were diluted with diluent up to a level of 10 ml.

Formula : Inject Standard Solution and Test solution and obtain % recovery

Recovery = (Area of test X Concentration of standard) /(Area of standard)

% Recovery = (Recovery X 100)/(Concentration of Standard)

3.5. Validation Of Rp-Hplc Method

As per ICH Q2 R1 guideline validation was performed and following parameters were performed.

3.5.1. System suitability

Data was collected from five replicate injections of the standard medication solution during the tests.

Acceptance criteria:

- Standard Chromatograms should not have a relative standard deviation of the area of analyte peaks greater than 2.0%
- Standard chromatograms should have theoretical plates of analyte peaks that are not less than 2000.
- In standard chromatograms, the analyte peak's tailing factor should not be less than 1.5.

3.5.2. Linearity

The analytical method's linearity is determined by the mathematical treatment of test data acquired from analysis of samples with analyte concentrations ranging over the claimed range. Linear graphs were obtained for the medications Lisinopril and amlodipine besylate.

3.5.3. Accuracy

The range of accuracy will be between 80% and 120% of the working concentration. The solution for each accuracy level will be prepared in three copies, with the percentage of recovery determined for each sample.

Table 3: Statistical data for assay

Sample name	Area of standard	Area of test	Standard Conc.	Test Conc.	Assay %
Amlodipine besylate	90095	90110	5ug/ml	5.0008ug/ml	100.017
Lisinopril	121465	121380	5ug/ml	4.996ug/ml	99.93

Table 4: System suitability

Name	Retention time (min)	Area	Theoretical plates	Tailing factor
Amlodipine besylate	3.297	890104	4898	1.12
Lisinopril	6.124	117666	5819	1.14

Table 5: statistical Data and result for Accuracy

Conc. Level	Test Solution	Standard solution	Mean area for Amlodipine besylate	Mean area for Lisinopril	% recovery of Amlodipine besylate	% recovery of Lisinopril
80%	5	4	10466.333	141719.3	99.88	100.231
100%	5	5	114186.333	146857.3	100.445	100.491
120%	5	6	119033.333	152083.3	101.278	100.954

3.5.4. Precision

The accuracy of an analytical process used to determine intra-day and inter-day variation. The percentage relative standard deviation (RSD) for intra-day precision was 0.6447% for amlodipine besylate and 0.288% for Lisinopril, and for inter-day precision was 0.168% for amlodipine besylate and 0.613% for Lisinopril. The obtained findings are less than 2% suggests a high level of precision.

3.5.5. Limit of Detection (LOD) Limit of Quantitation (LOQ)

The LOD and LOQ were calculated separately. LOD and LOQ for amlodipine besylate were 0.33ug/ml and 1.01ug/ml, respectively, and 0.56 ug/ml and 1.71 ug/ml for Lisinopril.

3.5.6. Robustness

The robustness of an analytical procedure assesses how unaffected it can be by tiny but intentional changes to method parameters and suggests how reliable it will be under normal conditions. The robustness study comprised making slight changes to the optimized method's parameters, such as flow rate (0.2 ml/min), wavelength (3 nm), and temperature (5°C). The retention period, theoretical plates, and tailing factor had no discernible effects.

4. RESULTS AND DISCUSSION

For the analysis, an RP-HPLC binary isocratic system was developed and validated. Phenomenex C18 (250mm x 4.6 ID, particle size: 5 micron) was used as the stationary phase. The developed approach used a mobile phase Acetate buffer: Methanol (65:35) with o-phosphoric acid, with the pH set to 5 and the flow rate set to 1 ml/min.

A solution of amlodipine besylate and Lisinopril was scanned in the wavelength ranges of 400 nm and 200 nm using a UV-visible spectrophotometer in spectrum mode. The absorbance of the medication peaks at 221 nm (Max).

The RP-HPLC approach developed for the measurement of amlodipine besylate and Lisinopril was verified in compliance with the ICH Q2 (R1) guideline. The developed method's linearity was proven throughout a concentration range of 4-20g/mL for Amlodipine besylate and Lisinopril, with a correlation coefficient of 0.999. In terms of precision and accuracy, the RSD of the approach was determined to be less than 2%. A system appropriateness test verifies that the analytical system can generate precise and reliable results. Area, the number of theoretical plates, and the tailing factor are examples of system appropriateness tests. The findings of all system suitability parameters were within acceptable ranges as defined by official guidelines.

The developed high-performance liquid chromatographic method was also examined for accuracy and precision, and it was shown to be beneficial and effective for Amlodipine besylate and Lisinopril quality control. In addition, the 10-minute analytical run time and lower solvent consumption result in a low-cost and environmentally friendly chromatographic method. As a result, the proposed methodology is quick, selective, and requires only a basic sample preparation step, and it is suitable for Amlodipine besylate and Lisinopril.

Conflict of interest

None declared

Source of funding

None declared

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