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Analytical Method Development and Validation of Perindopril and Amlodipine as Multicomponent Formulation by HPLC Method

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Abstract: The work aims to develop and validate a simple, precise and accurate HPLC method per ICH guidelines. The linearity, specificity, Precision, and accuracy were within limits specified by the ICH guidelines. Hence the proposed system was found to be robust and precise. The objective of this study was to validate the simultaneous assay of Perindopril and Amlodipine by using High-Performance Liquid Chromatography (HPLC). Perindopril and Amlodipine were estimated with C18 Column (2.5 cm × 4.6 mm and five µm particle size) with a VWD detector. The mobile phase consists of Buffer Solution and Acetonitrile 55:45 v/v with a flow rate of 1 ml/min. The volume injected was 20 µL. The detection wavelength was 215 nm, respectively. The retention time of Perindopril and Amlodipine was found to be 3.40 min and 4.73 min. The system suitability and all essential validation parameters were meticulously completed. The LOD and LOQ for Perindopril were found to be 2.62 µg/ml and 7.95 µg/ml, respectively, and for Amlodipine, 1.06 µg/ml and 3.22 µg/ml, respectively. The analytical curve was linear (R2 = 0.999). The system exhibits sufficient accuracy with a relative standard deviation under 2%. The method showed good duplicability and recovery with % RSD less than 2%. The linearity, specificity, Precision, and accuracy were within limits specified by the ICH guidelines. Hence the proposed system was found to be robust and precise. The method has to be such that it takes less time development and the best accurate and complete results have been obtained. Hence the proposed method for simultaneous estimation of Perindopril and Amlodipine by HPLC was found to be robust and precise.

Keywords: Perindopril, Amlodipine, HPLC, ICH guideline, LOD, LOQ.

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1. INTRODUCTION

The science that deals with the identification and quantification of the components of material systems are called Analytical Science. It is so called because the process of determining the level of any or all ingredients in a material system is called Analysis. It involves both physical and chemical processes. The chemical process is called chemical analysis or, more broadly, Analytical Chemistry[2]. Analytical chemistry is "the science of inventing and applying the concepts, principles and strategies for measuring the characteristics of chemical systems and species." Analytical procedures can be classified in two ways: first, in terms of the goal of analysis and second, like the method used. The purpose of the analysis and classification can be based on whether the analysis is qualitative or quantitative. Analytical techniques are essential in maintaining and assuring the quality of substance and are critical components of quality assurance and quality control[35]. There are many reasons for the need to validate analytical procedures. Among them are regulatory requirements, sound science and quality control requirements. The Code of Federal Regulation (CFR) explicitly states that "the accuracy, specificity, sensitivity and reproducibility of test methods employed by the firm shall be established and documented." Of Course, as scientists, we would want to apply sound science to demonstrate that the analytical method used had demonstrated accuracy, sensitivity, specificity, and reproducibility. Analytical methods used to be validated, verified or revalidated in the following instances[6-8]:

- Before initial use in routine testing.
- When transferred to another laboratory
- Whenever the conditions or method parameters for which the method has been validated change (for example, an instrument with different characteristics or a sample with a different matrix), the difference is outside the original scope of the method.

The work aims to develop and validate a simple, precise and accurate HPLC method per ICH guidelines. The linearity, specificity, precision, and accuracy were within limits specified by the ICH guidelines. Hence the proposed system was found to be robust and precise. The analytical method should be[33, 34]

1. Most productive, economical and convenient,
2. As accurate and precise as required,
3. As simple as possible,
4. Most specific

Perindopril (Fig.1) is a non-sulfhydryl angiotensin-converting enzyme (ACE) inhibitor with antihypertensive activity. Its chemical name is (2S, 3aS, 7aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxopentan-2-yl] amino] propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid. It has the molecular formula of $C_{19}H_{32}N_2O_5$, and its molecular weight is 368.5 g/mol. Perindopril is an angiotensin-converting enzyme (ACE) inhibitor used in the therapy of hypertension and stable coronary artery disease. Perindopril is associated with a low rate of transient serum aminotransferase elevations and has been linked to rare acute liver injury [1, 3].

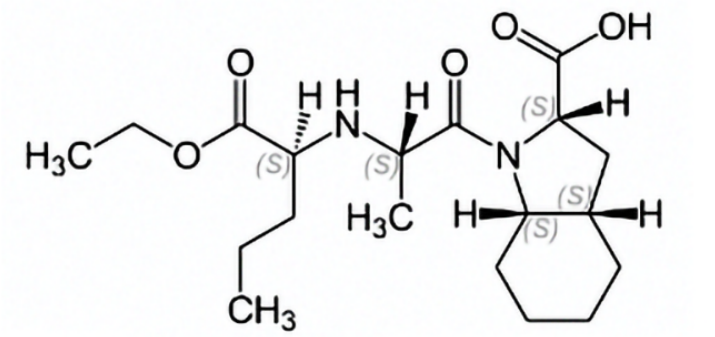

Fig 1: Chemical structure of Perindopril

Amlodipine (Fig.2) is a fully substituted dialkyl 1, 4-dihydropyridine-3,5-dicarboxylate derivative, which is used for the treatment of hypertension, chronic stable angina and confirmed or suspected vasospastic angina. Initially approved by the FDA in 1987, Amlodipine is a popular antihypertensive drug belonging to the group of drugs called dihydropyridine calcium channel blockers. Its chemical name is 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate. It has the molecular formula of $C_{20}H_{25}ClN_2O_5$, and its molecular weight is 408.9 g/mol. Amlodipine is commonly used to treat high blood pressure and angina [1, 3].

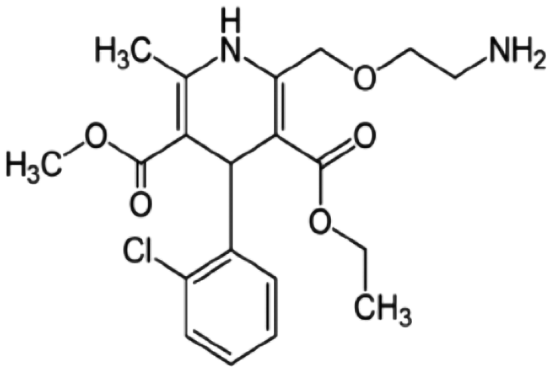

Fig 2: Chemical structure of Amlodipine

2. MATERIALS AND METHODS

2.1 Instrument

The drug analysis was carried out on Agilent (Series1260) Infinity II with Auto-injector and UV detector with EZ-chrome software and equipped with Reverse Phase (Agilent) C18 Column (Intersil ODS 2, 25 cm × 4.6 mm, five µm) at 60°C temperature.

2.2 Chemicals and Reagents

Merck Limited, India obtained water, Acetonitrile and Buffer solution of HPLC grade.

2.3 Chromatographic Condition

C18 Column (Intersil ODS 2, 25cm × 4.6mm, 5µm) was used for chromatographic separation at a detection wavelength of 215 nm using a flow rate of 1 ml/min. The mobile phase used was buffer Solution: Acetonitrile in the ratio of 55:45 V/V. HPLC conditions are given in Table 1.

Table 1: HPLC Condition

Sr. No	Parameter	Condition
1	HPLC	Agilent 1260 Infinity II
2	Column	(Inertsil) C18 column (4.6 mm × 250 mm, 5 µ m).
3	Detector	215 nm
4	Injection volume	20 µ L
5	Flow rate	1 ml/min
6	Temperature	Ambient
7	Run time	15 min

2.4 Buffer Preparation

Weigh 1.46g of Sodium Heptane Sulphonate Monohydrate into 1000 mL of water and mix.

2.5 Mobile Phase

The mixture of Buffer Solution: Acetonitrile in the ratio of 55:45 v/v.

2.6 Diluent

The mixture of Buffer: Acetonitrile in the ratio of (55:45), Adjust pH 2 with Perchloric acid.

2.7 Preparation of Standard Solution for Perindopril and Amlodipine

Weighed accurately about 250mg of Perindopril and 15mg of Amlodipine standard, transferred into 100mL of the volumetric flask, added about 50mL of diluent, shook to dissolve, and volume was made up to mark with diluent. Table

3. RESULTS

3.1 Method Validation

Method Validation constitutes an essential part of any analytical method. In recent years, trials have been developed to harmonize pharmaceutical regulatory requirements in the United States, Europe and Japan. The FDA method validation draft guidance and USP refer to ICH guidelines. Therefore, as part of method validation as per ICH guidelines, Linearity, Accuracy, Precision, Robustness and Specificity Studies, these parameters were studied [6-7].

3.2 Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be

present. Typically, these might include impurities, degradants, matrices, etc. The procedure used to demonstrate specificity will depend on the intended objective of the analytical system [7-11]. This definition has the following implications

- Identification: To ensure the title of an analyte.
- Purity Tests: To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e., related substance test, heavy metals, residual solvent content, etc.
- Assay: To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

3.3 *Specificity for Assay*

In the case of assay, specificity must be studied for blank/diluent, placebo (in case of finished products such as tablets/capsules/creams etc. and impurity and degradation products.

3.4 *Blank Interference*

In the case of chromatographic techniques, such as HPLC, the diluent interference may not be removed entirely just by doing auto-zero. The component or impurities present in the diluent may be retained on the Column and interfere with the analyte. Therefore, a study to confirm the interference of blank was carried out. Inject diluent and evaluate interference at the RT of API and impurities. The chromatography of the blank solution (Fig.3) showed no peaks at the retention time of Perindopril and Amlodipine peaks.

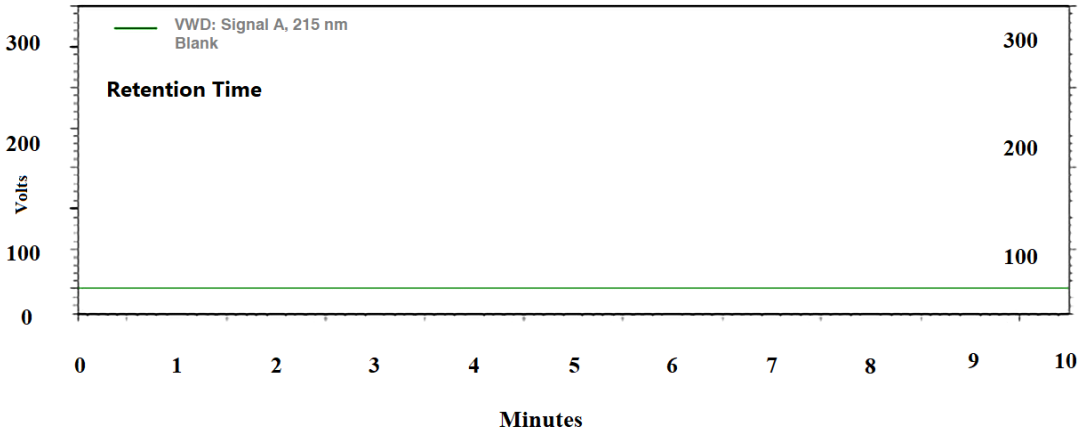

Fig 3: Blank Solution

3.5 *Placebo Solution*

Evaluation of placebo interference is applicable only for drug products such as tablets, capsules etc. Placebo consists of all excipients present in the drug product. Prepare the placebo solution by the procedure, weight 710.0mg of placebo solution diluted in 100 ml of the mobile phase. Analyze placebo solutions and evaluate interference. The results are shown in Table 2 and Figure 4.

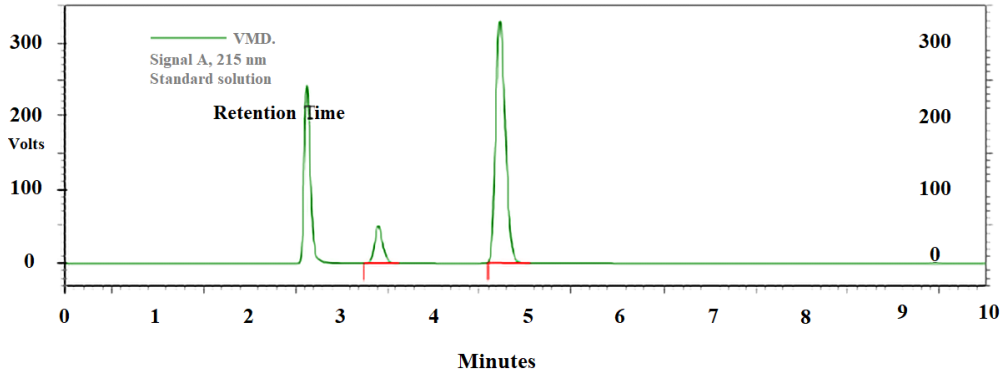

Fig 4: Placebo + Drug Substance

Table 2: Drug Substance in Placebo Solution

Sr. No	Drug	Retention Time	Area	Asymmetry
1	PERINDOPRIL	3.41	5256810	1.19
2	AMLODIPINE	4.71	40687302	1.20

3.6 *Table Linearity*

A linear relationship should be evaluated across the range of the analytical procedure. For example, it may be demonstrated directly on the active substance (by diluting a standard stock solution) and separately weighing synthetic mixtures of the product components using the proposed procedure. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration. Calibration curves were plotted with observed peak areas against concentration to obtain the calibration curve and correlation coefficients. Characteristics parameters for

regression equation (y= mx + c) of the method and this parameter were used to confirm the excellent linearity of the way. The results are shown in Figures 5 and 6.

3.7 Accuracy

Accuracy is the nearness of a measured value to the actual or accepted value. The accuracy of an analytical method indicates the similarity of agreement between the matter, which is taken either as a traditional value or a proved reference value and the value identified. The accuracy was determined by Perindopril and Amlodipine (equivalent to 250mg of Perindopril and 15mg of Amlodipine) (50%, 100% and 150% of the label claimed, respectively) to quantity equivalent to the average weight of marketed tablets. The resulting mixtures were analyzed in triplicates over three days. The % recovery of the added drug was taken as a measure of accuracy. The results are shown in Tables 3 and 4.

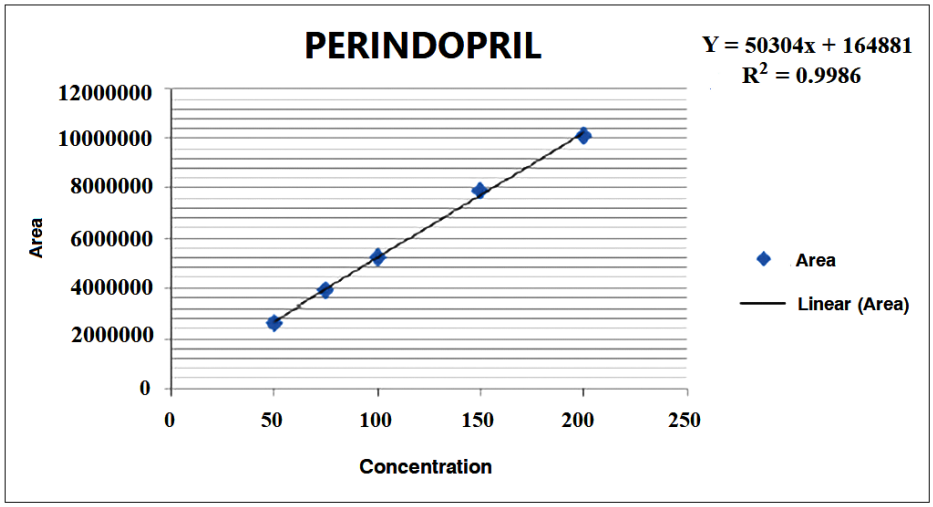

Fig 5: Calibration curved of Perindopril

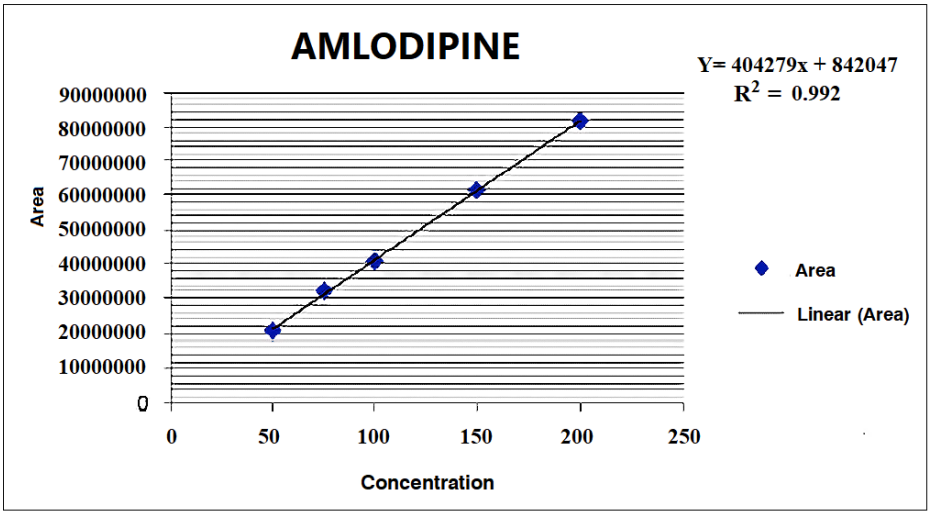

Fig 6: Calibration curved of Amlodipine

Table 3: Bracketing Standard and % Accuracy Against Average

Bracketing Std.	Retention Time	Area	Asymmetry	%Recovery
PERINDOPRIL	3.41	5257182	1.19	100.0
AMLODIPINE	4.72	40827512	1.20	100.0

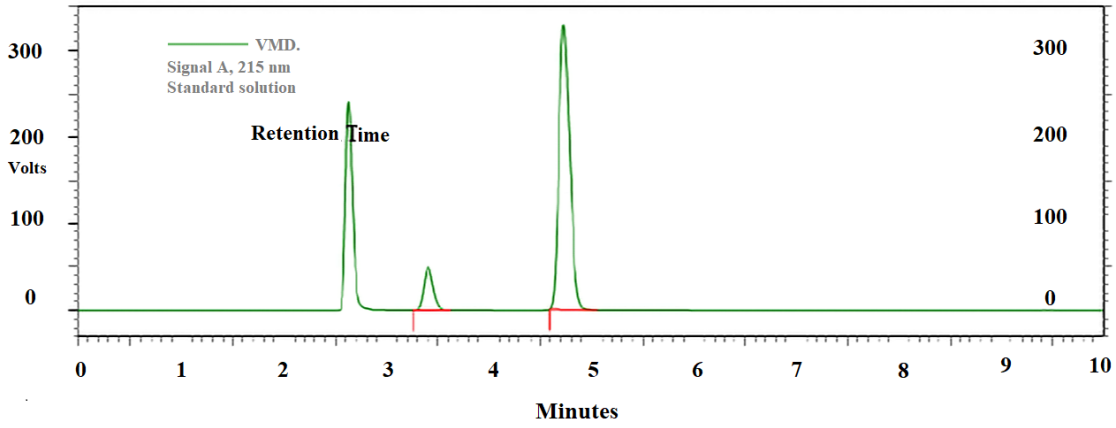

Fig 7: Bracketing Standard

Table 4: Statistical Validation of Recovery Studies Amlodipine and Perindopril

Method	Level of Recovery (%)	Mean % Recovery		Standard Deviation*		%RSD	
		AMLO	PERI	AMLO	PERI	AMLO	PERI
RP-HPLC Method	50%	100.0	99.00	0.7071	0.44	0.7	0.4
	100%	100.0	99.0	0.7	0.44	0.7	0.4
	150%	100	99.03	0.70	0.44	0.7	0.4

Denotes an average of three determinations for RP-HPLC

Table 5: Calibration Standard Solution (Perindopril + Amlodipine)

Calibration Standard	Retention Time		Area		Asymmetry	
	Perindopril	Amlodipine	Perindopril	Amlodipine	Perindopril	Amlodipine
1	3.40	4.73	5257508	40822464	1.18	1.20
2	3.40	4.73	5265399	40863928	1.17	1.22
3	3.41	4.73	5258419	40860167	1.15	1.21
4	3.40	4.73	5255627	40773646	1.17	1.21
5	3.40	4.73	5257438	40815591	1.14	1.21
Average	3.40	4.73	5258878.20	40827159	1.16	1.21
SD	0.0045	0.0000	3783.3062	36949.2566	0.0164	0.0071
%RSD	0.1	0.0	0.1	0.1	1.4	0.6

3.8 Robustness

The Robustness of a method is its ability to remain unaffected by small, deliberate changes in parameters. Therefore, the effect of changes in mobile phase composition ±1 ml/min^{-1}, wavelength ±1 ml/min^{-1} and flow rate ±1 ml/min^{-1} on retention time and tailing factor of drug peak was studied. The results of robustness studies are shown in table 7.

Table 6: Test Solution

Particulars	PERINDOPRIL		AMLODIPINE	
	Retention	Area	Retention	Area
Test Solution	3.41	5202493	4.72	40098409

Table 7: Robustness of Amlodipine and Perindopril

Parameters	Conc (µ g/ml)		SD		%RSD	
	AMLO	PERI	AMLO	PERI	AMLO	PERI
Chromatogram of flow change 0.9 ml	3.0	50	0.14	0.63	0.1	0.1
Chromatogram of flow change 1.1 ml	3.0	50	0.14	0.63	0.1	0.11
Chromatogram of comp change wavelength change 216nm	3.0	50	0.14	0.63	0.1	0.12
Chromatogram of comp change wavelength change 214nm	3.0	50	0.14	0.62	0.1	0.1
Chromatogram of Mobile Phase 56:46	3.0	50	0.14	0.63	0.1	0.1
Chromatogram of Mobile phase 54:46	3.0	50	0.14	0.62	0.1	0.1

3.9 Limit of Detection (LOD)

The smallest number of analytes that can be detected, but not always specified as an exact value, is the LOD, a specific analyte technique. For example, the limit of detection (LOD) can be calculated from the below formula, and the results are shown in Table 8.

$$LOD = 3.3\ (SD) / S$$

Where SD = Standard Deviation of the response S = Slope

3.10 *Limit of Quantification (LOQ):-*

The smallest quantity of analyte that may be quantitatively identified is the LOQ of a particular analytical process. For example, the limit of Quantification (LOQ) can be calculated from the below formula, and the results are shown in Table 8.

$$LOG = 10\ (SD) / S$$

Where SD = Standard Deviation of the response S = Slope

Table 8: Data for LOD and LOQ

Drug Name	LOD	LOQ
PERINDOPRIL	2.62 µ g/ml	7.95 µ g/ml
AMLODIPINE	1.06 µ g/ml	3.22 µ g/ml

3.11 Precision

Precision can be defined as the degree of agreement among the individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample, a more comprehensive definition proposed by the International Conference on Harmonization (ICH). It may be considered at three levels: Repeatability, Intermediate Precision and Reproducibility. It is expressed as a standard deviation or coefficient of variation. Intermediate Precision expresses within- laboratories variations, different days, different analyst and different equipment, etc. To check repeatability (Method Precision) of method for independent six different sample preparation were injected, and % RSD with six sample preparation found to be within 2.0%.

3.12 Analysis of Marketed Formulation

Ten tablets were weighted individually to analyze the tablet dosage form, and their average weight was calculated. First, the tablet was triturated to make fine powder and powder equivalent to the importance of one tablet (754.0 mg), and the volume is made up to 100ml with Acetonitrile. Then, it was sonicated for 15 minutes to dissolve it entirely and filtered through a 0.45 µm membrane filter. The analysis results for the marketed tablet formulation (Perindopril + Amlodipine) are reported in Tables 9 and 10.

Table 9: Assay for PERINDOPRIL + AMLODIPINE

Particulars	%Assay (Unrounded)		%Assay (Rounded)	
	PERINDOPRIL	AMLODIPINE	PERINDOPRIL	AMLODIPINE
Test Solution	98.928	98.215	98.9	98.2

Table 10: Analysis of Marketed Formulation

Essay	Drug	Label Claimed	Area	%Label Claim	SD	%RSD
RP-HPLC Method	AMLO	3.0	5241619	99.05	0.1414	0.1
	PERI	50	40699056	98.5	0.14	0.1
	AMLO	3.0	5141619	99.04	0.1414	0.1
	PERI	50	41690956	99.03	0.14	0.1

4. STATISTICAL ANALYSIS

Data were extracted from the open lab CDS software, and the validated Microsoft excel sheet did statistical calculations. The magnitude of per cent RSD and SD were found to be within the limit, i.e. < 2%. As per ICH guidelines.

5. DISCUSSION

For method validation and simultaneous analysis of Perindopril and Amlodipine, various conditions, such as different columns (C18) and mobile phase mixtures, were tried. The C18 Intersil ODS Column (25cm, 4.6 mm, 5 µm) at an Ambient temperature was found to be appropriate for the separation of both drugs efficiently. Different mobile phase mixtures were tested, including Buffer solution (Sodium Heptane Sulfonate Monohydrate) and organic solvents, including Acetonitrile. Based on preliminary experiments, a mobile phase composed of acetonitrile and Buffer solution (Sodium Heptane Sulfonate Monohydrate) was selected for further analysis that showed good peak shape and resolution. Other attempts for mobile phase mixture composition showed that the mobile phase with a copy of 45% acetonitrile and 55% Buffer (Sodium Heptane Sulfonate Monohydrate) adjusted PH 2 with Perchloric acid (v/v) with the flow rate of 1mL/min exhibited the appropriate separation of peaks [28,51]. Scientifically the method was found to be sound, accurate and reproducible. The goal of this study was to validate a simple, precise and accurate HPLC method[7-12]; the retention time of Perindopril and Amlodipine was found to be 3.40 min and 4.73 min, respectively.

The respective linear equation for Perindopril was y = 50304x + 16488 and Amlodipine y = 40427x + 84204, and the correlation coefficient was 0.998 and 0.999, respectively. Perindopril and Amlodipine's calibration curve is represented in Figures 5 and 6. The accuracy of HPLC is ascertained by recovery studies performed at different concentration levels (50%, 100% and 150%).[14-18] The % recovery was found to be 99-101%, shown in table 3. The results of robustness studies are shown in table 7. Robustness was also found satisfactory; hence the analytical method would be concluded. The percentage assay of tablet formulation was found to be 98.9% for Perindopril and 98.2% for Amlodipine, respectively shown in table 9. The per cent relative standard deviation for the above was less than 2%. Therefore, the results indicate that the method is highly accurate and precise. The technique has been developed such that it took less time, and the best natural and robust results were obtained. The development and validation of a simple, precise and accurate HPLC method per ICH guidelines were successfully achieved. The chromatogram was analyzed to estimate retention time, peak area, number of theoretical plates, tailing factor etc. [11,13]. The developed method represented good resolution for both the analytes. [25,26] The Validated analytical method is simple and reproducible, which can be used in quality control departments.[22,23] The obtained results were compared with limits given in ICH guidelines Q2R1. The linearity, specificity, Precision, and accuracy were within limits specified by the ICH guidelines [12-14]. Hence the proposed system was found to be robust and precise [15-18] The scientific demonstration of the RP-HPLC strategy was approved concerning linearity, accuracy, Precision, and particularity and measurement limits. [19-23]

6. CONCLUSION

A simple, rapid and accurate method was validated for routine analysis of Perindopril and Amlodipine through an HPLC method. According to ICH recommendations, the improved technique is verified using several characteristics such as Precision, accuracy, linearity, etc. By ICH Q2 (R2) requirements, the C18 Column (Inertsil ODS) may be used to analyze Perindopril and Amlodipine with high specificity in a shorter time. The amount found from the proposed methods was in good agreement with the label claim of the formulation. The newly validated method showed acceptable Precision and accuracy, at least in the concentration range of 0.2500 to 1.0000 mg/mL for Perindopril and 0.0150 to 0.0600 mg/mL for Amlodipine. The analytical method represented good resolution for both Perindopril and Amlodipine. It is worthwhile that the proposed methods can be successfully utilized for routine quality control departments.

7. ACKNOWLEDGEMENTS

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8. AUTHORS CONTRIBUTION STATEMENT

This work was carried out in collaboration among all authors. As a result, all authors read and approved the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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