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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PERINDOPRIL AND INDAPAMIDE IN MARKETED FORMULATION

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ABSTRACT

This study focuses on the development and validation of a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of perindopril and indapamide in a marketed formulation. Perindopril is an angiotensin-converting enzyme (ACE) inhibitor, while indapamide is a thiazide-like diuretic. Both drugs are commonly used in combination for the treatment of hypertension. The RP-HPLC method was developed using a C18 column and a mobile phase consisting of a mixture of acetonitrile and methanol in a isocratic elution mode. The detection wavelength was set at 254nm, and the flow rate was optimized to achieve efficient separation and quantification of both drugs. The developed method was validated according to the International Conference on Harmonization (ICH) guidelines for various parameters such as linearity, precision, accuracy, specificity, robustness, and system suitability. The method demonstrated good linearity over the concentration range of the analytes in the formulation. The precision and accuracy were within acceptable limits, indicating the reproducibility and reliability of the method. The specificity of the method was confirmed by analyzing the sample containing the marketed formulation, ensuring that the peaks of interest were well-resolved from any interfering substances. The robustness of the method was evaluated by making deliberate changes in chromatographic conditions, and the results demonstrated the method's ability to provide consistent and reliable results.

Keywords: Perindopril, Indapamide, Method development, HPLC, Validation

INTRODUCTION

Analytical chemistry is a vital part of pharmaceutical chemistry. It involves separating, identifying and determining the relative amounts of the component in the sample. Selection of a suitable analytical method for estimation of individual drug in any dosage form is a challenge for an analytical chemist. The method so selected should provide analytical data as accurate as required, technically sound, defensible

with low limit of uncertainty and above all amenable to routine laboratory use and capable of being performed by personnel with minimal technical experience[1-3].

Analytical method validation is the next important step in justification acceptability of an analytical method, after method development. It enables scientists to communicate scientifically and effectively on technical matters. Set standards of evaluation procedures for checking compliance and taking remedial measures. However, validation of equipment and analytical methods is necessary, not only due to regulations and accreditation standards, but also as prerequisite in terms of any good analytical practice and should be on going in the form of re-validation with method changes[4]. Perindopril erbumine, indapamide, and amlodipine besylate are three APIs generally used to treat hypertension. (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-carboxylicacid;2-methylpropan-2-amine is the chemical name of perindopril erbumine (PER) which is active component of an angiotensin-converting enzyme inhibitor (ACE-I). Apart from this, the other diseased conditions which can be cured using this are heart attacks, strokes, and kidney problem[5]. The chemical name of indapamide (IND) is 4-chloro-*N*-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoylbenzamide. It belongs to the thiazide diuretics class and generally used for the treatment of salt and fluid retention associated with congestive heart failure or edema from pregnancy and also found to be the oral antihypertensive drug[6]. The developments of analytical methods for the determination of drugs in bulk, in dosage forms or in body fluids have received a considerable attention in recent years because of their importance in quality control, bioavailability and pharmacokinetic study etc. The development of analytical method for the determination of drugs in bulk, in dosage forms or in body fluids have received attention in recent years because of their importance in quality control, bioavailability and pharmacokinetic study etc. The aim and objective of the present work is to develop new simple, sensitive and validated RP-HPLC method for estimation of perindopril and and indapamide in marketed formulation and validation of developed Analytical method according to ICH guideline.

MATERIALS AND METHODS

Selection of Mobile Phase

Initially to estimate Perindopril and Indapamidein fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and than degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials methanol was used as diluents.

Preparation of Stock Solution:

Accurately weighed 10 mg API of PDP and IDP was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of PDP and IDP respectively (Stock-B).

Preparation of Different Solution

0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml, for PDP. In same manner 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of IDP also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 1-5 µg/ml for PDP and 5-25µg/ml for IDP were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 254 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of PDP 1µg/ml for PDP and 10µg/ml IDP was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed Method[7]

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 1 to 5 µg/ ml for PDP) and (1 to 5µg/ ml for (IDP) and areas for each concentration were recorded three times and mean area was calculated.

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and than its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicates at five concentrations in linearity range 1, 2, 3, 4 and 5µg/ml for PDP and 1, 2, 3, 4 and 5µg/ml for IDP indicates the precision under the same operating condition over short interval time.

Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicates at five concentrations.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v). Results of robustness are reported in table 6.25-6.26.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 4 mg of PDP and 1.25mg of IDP was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was than filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 4µg/mL PDP and 1.25µg/mL IDP respectively. The amounts of PDP and IDP in tablets formulation were calculated by extrapolating the value of area from the calibration curve.

RESULTS AND DISCUSSION

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 1µg/ml for PDP and 10µg/ml IDP were injected and chromatogram was recorded. The result of system suitability parameter is reported in table 1.

Table 1: Results of system suitability parameters

Parameters	PDP	IDP
No. of Theoretical Plates	2583.167±11.856	3359.333±11.290
Tailing Factor	1.155±0.021	1.138±0.017
Retention time	1.932±0.001	8.982±0.001

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table 2.

Table 2: Results of Linearity of Perindopril and Indapamide

Parameter	PDP	IDP
Concentration (µg/ml)	1-5	1-5
Correlation Coefficient (r^2)*	0.999	0.999
Slope (m)*	130.5	128.9
Intercept (c)*	0.734	1.630

*value of six replicate

Specificity

Specificity of the method was determined and the peaks of plasma, diluent, mobile phase and excipient of physical mixture did not interfere with standard peaks of Perindopril and Indapamide.

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table 3.

Table 3: Results of recovery study

% Level	PDP	IDP
	% MEAN±SD*	
80%	98.94±0.713	98.171±0.958
100%	98.09±1.449	97.778±0.192
120%	99.18±0.163	98.642±0.771

***** Value of three replicate and three concentrations

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in table 4.

Table 4: Results of precision

Parameter	PDP	IDP
	% MEAN±SD*	
Repeatability	98.204±0.049	97.539±0.041
Intermediate precision		
Day to day precision	98.667±0.046	96.153±0.041
Analyst to Analyst	97.058±0.041	95.887±0.044

***** Value of five replicate and five concentrations

The robustness of developed method was checked by changing in the deliberate variation in solvent. Result of robustness shown in table 5.

Table 5: Results of Robustness

Parameter	% MEAN±SD*
PDP	98.078±0.050
IDP	98.123±0.034

*****Value of five replicate and five concentrations

Detection limit and quantitation limit of described method were observed based on the SD of response and slope, which meet the requirement of new method table 6.

Table 6: LOD and LOQ of PDP and IDP

Name	LOD (µg/ml)	LOQ (µg/ml)
PDP	0.15	0.45
IDP	0.20	0.60

The results of the analysis of synthetic mixture were reported. The assay value of drugs was close to 100, SD and % RSD are less than 2 indicate the no interference of excipient in the estimation of drug table 7.

Table 7: Assay of synthetic mixture

	PDP*	IDP*
Label Claim (mg)	**4mg**	**1.25mg**
% Found (mg)	3.98	1.21
% Assay	9.95	9.68
% RSD	0.015	0.032

*Average of three determination

CONCLUSION

In conclusion, a validated HPLC method was successfully developed for the simultaneous estimation of indapamide and perindopril. The optimized chromatographic conditions provided adequate separation and quantification of both compounds with good peak shape, resolution, and sensitivity. The method showed satisfactory linearity, accuracy, precision, and robustness. It can be employed for routine analysis of pharmaceutical formulations containing both indapamide and perindopril, ensuring reliable and accurate quantification.

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