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Validated RP-HPLC Method for Simultaneous Estimation of Perindropil and Indapamide In Tablet Dosage Form

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ABSTRACT

Rapid and accurate High performance liquid chromatography method is described for Simultaneous estimation of Perindropil and Indapamide from the combination tablet dosage form. The separation of two drugs was achieved on Phenomenax (C_{18}) (4.6mm x 100mm, 3.5 μm) column. The mobile phase consists of Acetonitrile : Buffer Orthophosphoric acid 0.1% in the ratio of 40:60. The detection was carried out at a wavelength 230 nm. The method was validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution. The linear ranges for Perindropil and Indapamide were 8-40 μg/mL, 2.5- 12.5 μg/mL respectively with good recoveries i.e. 100.5% to 100.3%.

Keywords: Perindropil, Indapamide , High performance liquid chromatography.

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INTRODUCTION

Perindropil and Indapamide are available in tablet dosage form in the ratio of 1:3.2. Chemically Perindropil is 2S,3αS,7αS)-1-[(S)-1-carboxy butyl] alanyl] hexahydro-2-indolinecarboxylic acid, 1-ethyl este has anti hypertensive activity. Indapamide is 4-chloro-(2-methyl-2,3-dihydroindole) 3-sulphomoyl-benzamide has anti hypertensive agent & Diuretic. Perindropil and Indapamide is not official in any pharmacopoeias. Literature survey reveals UV[1], HPLC[2.3] methods for analysis of Perindropil and UV[4], HPLC[5,6]. There are some reported methods for analysis of both drugs in combination[7,8]. This paper presents simple, rapid, accurate and economical methods for simultaneous analysis of Perindropil and Indapamide in tablet dosage form.

MATERIALS AND METHOD

Instrument

The HPLC system used was WATERS equipped with UV detector model no 2310. The chromatogram was recorded at and peaks quantified by means of PC based N 2000 chromatographic system software.

Solvents used

Acetonitrile and Water for buffer HPLC grade from as a solvent in the study and obtained from MERCK Company, Mumbai.

Preparation Standard Stock Solutions

Stock solution was prepared by transferring 40 mg of Perindopril and 12.5 mg of Indapamide in 50 ml volumetric flask. It was sonicated to dissolve it completely and made volume up to the mark with the same diluent. From the above stock solution, 5 ml of the solution was pipette into a 25 ml volumetric flask and diluted up to the mark with diluent. From this, 1.5 ml of the solution was pipetted into another 10ml volumetric flask and diluted up to the mark with diluent.

Sample preparation

Powder equivalent to 40 mg of Perindopril was weighed and transfer into 50 ml volumetric flask, It was sonicated to dissolve it completely and made volume up to the mark with the same diluent. From the above stock solution, 5 ml of the solution was pipetted into a 25 ml volumetric flask and diluted up to the mark with diluent. From this, 1.5 ml of the solution was pipetted into another 10 ml volumetric flask and diluted up to the mark with diluent.

Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a reverse phase Phenomenax (C_{18}) (4.6mm x 100mm, 3.5µm) column. Mobile phase was made up of acetonitrile: buffer orthophosphoric acid 0.1% (Take 1000 ml of HPLC grade water and 1 ml orthophosphoric acid) in a ratio of 40:60. The mobile phase was filtered, degassed before use. The flow rate was adjusted to 0.8 ml/min. the detector wavelength was set at 230 nm. The injector volume of the standard and sample was 20µl.

Method validation

The method was validated as per International Conference on Harmonization (ICH) guidelines[9,10].

System Suitability Tests (SST)

Once a method or system has been validated the task becomes one of routinely checking the suitability of the system to perform within the validated limits. The simplest form of an HPLC system suitability test involves a comparison of the chromatogram trace with a standard trace. This allows a comparison of the peak shape, peak width, and baseline resolution. Alternatively these parameters can be calculated experimentally to provide a quantitative system suitability test report such as number of theoretical plates (efficiency), Capacity factor, Separation (relative retention), Resolution, Tailing factor. These are measured on a peak or peaks of known retention time and peak width.

Linearity

Linearity of the method was determined by mean of calibration graph using an increasing amount of each analyst. Linearity was evaluated by visual inspection of a calibration graph. At least three concentration levels were tested in agreement to ICH. The slope, intercept was reported as required by ICH. LOD and LOQ were estimated from the standard deviation of the and the slope of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard deviation of the intercepts of the regression lines done in the range of the detection limit.

Accuracy

The accuracy of the method was measured by recovery studies and ascertained by standard addition method. A known amount of pure drug at three different levels i.e. 80 %, 100 %, and 120 % was added to pre-analyzed sample solutions and total concentration was determined using the proposed method.

Precision

Precision was investigated at three levels, intra-day, inter-day, and reproducibility. The intra- and inter-day variability were assessed by using standard drug solution at three different concentration. Intra-day precision was carried out by analyzing the drug solutions within same day. The inter-day precision was measured using standard solution over three consecutive days. Reproducibility of the method was determined by performing same analytical procedure at different laboratories using same experimental design.

Robustness

The robustness of the method was investigated under a variety of conditions including changes of pH of the eluent, flow rate and of buffer composition. The obtained results were compared with that of standard results.

Specificity

Specificity was studied in order to assess unequivocally an analyst in the presence of components that may be expected to be present. Specificity was confirmed by obtaining positive results (by comparison with a known reference material) from samples containing the analyst, coupled with negative results from samples which do not contain the analyst. The parameters like retention time (R_t), resolution (R_s) capacity factor, tailing factor were calculated.

RESULTS AND DISCUSSION

Method Development

Different columns containing Octyl and Octadecylsilane stationary phases were tried for the separation and resolution. It was found that Phenomenax column offered more advantages. Individual drug solution was injected into column and elution pattern of all the drugs and resolution parameters were studied. In addition to this, UV spectra of individual drugs were recorded at the wavelength from 200 to 400 nm and the response for optimization was compared. The choice of wavelength 230 nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity.

Method Validation

System Suitability

System Performance parameters of developed HPLC method were determined by injecting Standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution (R_s), retention time (R_t) were determined. The results are shown in Table 1, it indicates good performance of system.

Table 1: System Performance for Perindopril and Indapamide.

Drug substances	Retention time	Symmetry factor	No.of plates	Resolution factor
Perindopril	2.19	1.3	4520	-
Indapamide	3.5	1.0	7857	7.7

Linearity

Under the experimental conditions described above, linear calibration curves for the two drugs were obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the two drugs (y) v/s concentration (x). The linear ranges of Perindopril and Indapamide are 8-40 µg/mL, 2.5- 12.5 µg/mL respectively showed in (Table 2), (figure1 a,b & 2)

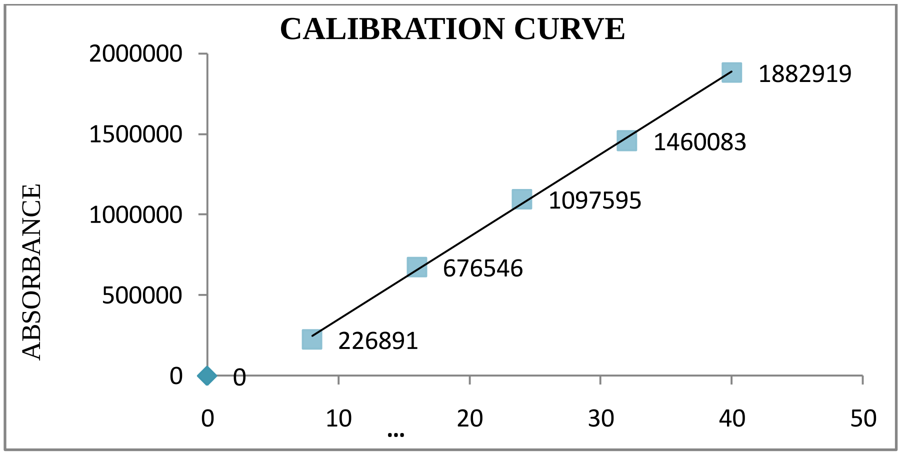

Figure 1 a: Calibrated graph of Perindopril

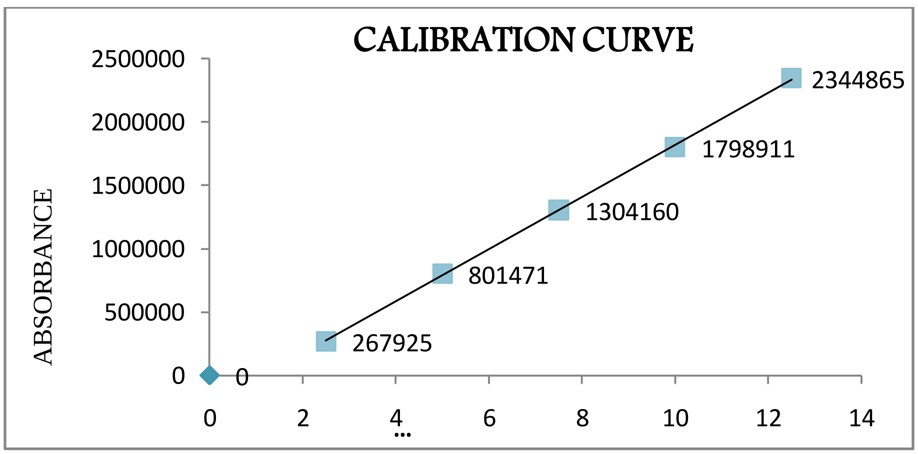

Figure 1 b: Calibrated graph of Indapamide

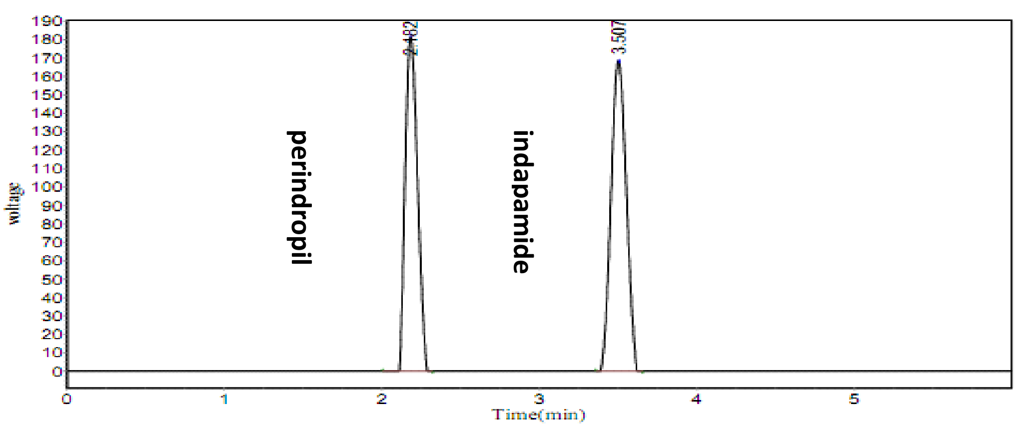

Figure 2: Chromatogram of Perindropil and Indapamide Assay

Table 2: Linearity – Regression analysis data

Parameters	Perindropil	Indapamide
Correlation coefficient (r)	0.9994	0.9998
Intercept	262.3	-1.1
Slope	3.8	10.05

Accuracy

Accuracy was determined by applying the proposed method to synthetic mixture containing known amount of each drug to 80%, 100%, and 120% of the label claim. The accuracy was then calculated as the percentage of analyze recovered by the assay.

Precision

The assay was carried out of two drugs using proposed method in six replicates. The value of relative standard deviation lie well within the limits (0.20% for Perindropil and 1.94% for Indapamide), it indicates the sample repeatability of the method enclosed in (Table 4).

Table 3: Method Precision of Perindropil and Indapamide

Injection	Peak areas of Perindropil	Peak areas of Indapamide
1	1030445	1179915
2	968130	110800
3	100121	1155515
4	1017377	1173587
5	1131363	1155984
6	251986	747247
Mean	2518159	752023
SD	5121.3	14567.6
%RSD	0.20	1.94

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:

Variation in flow rate by ± 0.1 ml/min. Variation in mobile phase.

Stability of Solution

Stock solution of sample and standard contains 24 µg/ml Perindropil, 7.5 µg/ml Indapamide. Stock solution stability was checked for 24 hrs at room temperature. The drug solution was found to be stable for the specified period.

Method Application

The validated High performance liquid chromatography method was applied to simultaneous determination of Perindropil and Indapamide. Locally available tablet dosage form contains Perindropil 1.25 mg and Indapamide 4 mg. 20 tablets were crushed and powdered, weigh powder equivalent to 40 mg of Indapamide and transfer into 50 ml volumetric flask, add 50 ml of mobile phase. It was sonicated at 35^{o}C for 6 min to dissolve completely. This solution was further diluted to get a solution having concentration of 24 µg/ml Perindropil, 7.5 µg/ml Indapamide. 20µl of this solution was injected into the chromatograph under the specified chromatographic conditions. The analyte peaks were identified by comparisons with those of respective standard for their retention time. The peak areas were used to calculate the drugs. The assay results, expressed as % of the label claim, are in (Table.4). This indicates that the amount of each drug in the product meets the requirements.

Table 4: Method application (Assay).

Peak name	Retention Time	Area	Tailing factor	Resolution
Perindropil	2.19	215561	4523	---
Indapamide	3.5	247154	7857	7.7

CONCLUSION

The proposed HPLC method provides as a fast, accurate and rugged assay with stability indicating potential for these two drugs in tablet or in solution alone. In conclusion, the developed method is strongly recommended for the assay of two drugs in the locally available pharmaceutical dosage form i.e. tablet.

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