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METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF PERINDOPRIL AND INDAPAMIDE IN BULK DRUG AND MARKETED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT
Analytical Method Development and Validation for Alogliptin and Metformin in bulk and Combine Dosage Form by RP-HPLC, New method was established for simultaneous estimation of Alogliptin and Metformin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Alogliptin and Metformin by using Symmetry C18 5µm (4.6 x 150mm), flow rate was 1.0 ml/min, mobile phase ratio was Phosphate buffer (0.02M) pH-3.8: Methanol: Acetonitrile (60:20:20%v/v), detection wavelength was 260nm. The retention times of Alogliptin and Metformin were found to be 2.324mins and 4.314mins respectively. The % purity of Alogliptin and Metformin was found to be 99.865% and 99.658% respectively. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Alogliptin and Metformin was found in concentration range of 0µg-36µg and 0µg-39µg and correlation coefficient (r2) was found to be 0.9995 and 0.9998, % recovery was found to be 100.280, %RSD for repeatability was 0.174 and 0.709, % RSD for intermediate precision was 0.093 and 0.937 respectively. The precision study was precise, robust, and repeatable. LOD value was 1.377 and 1.079, and LOQ value was 4.174 and 3.272 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Alogliptin and Metformin in API and Pharmaceutical dosage form.

KEYWORDS: Alogliptin and Metformin, Method Development, Validation, Accuracy.

INTRODUCTION
Perindopril is an ACE inhibitor prodrug used to treat hypertension, mild to moderate congestive heart failure, and to reduce cardiovascular risk in patients with hypertension or post-myocardial infarction. Perindopril[1] is a non-sulfhydryl angiotensin converting enzyme (ACE) inhibitor with antihypertensive activity. Upon hydrolysis, perindopril is converted to its active form perindoprilat, inhibiting ACE and the conversion of angiotensin I to angiotensin II; consequently, angiotensin II-mediated vasoconstriction and angiotensin II-stimulated aldosterone secretion from the adrenal cortex are inhibited and diuresis and natriuresis ensue. Perindopril[2] is a nonsulfhydryl prodrug that is metabolized via first pass effect (62%) and systemic hydrolysis (38%) to perindoprilat, its active metabolite, following oral administration. Perindoprilat lowers blood pressure by antagonizing the effect of the RAAS. The IUPAC Name of Perindopril[3] is (2S, 3aS, 7aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxopentan-2-yl] amino] propanoyl]-2, 3, 3a, 4, 5, 6, 7, 7a-octahydro indole-2-carboxylic acid. The Chemical Structure of Perindopril is following.

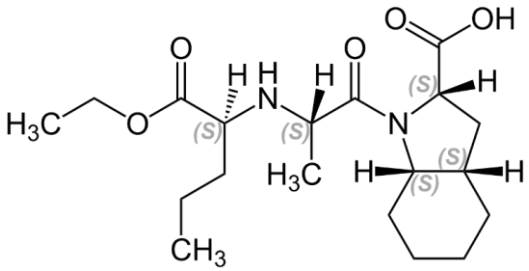

Fig-1: Chemical Structure of Perindopril.

Indapamide is a thiazide diuretic used to treat hypertension as well as edema due to congestive heart failure. Thiazide-like diuretics such as Indapamide[4] are a valuable tool for the treatment of hypertension and continue to grow in popularity, falling behind only ACE inhibitors in terms of prescription frequency. When compared to hydrochlorothiazide (another commonly prescribed diuretic), Indapamide[5] has been shown to be superior at lowering systolic blood pressure, reducing left ventricular mass index, lowering oxidative stress, inhibiting platelet aggregation, and reducing microalbuminuria associated with diabetes. Interestingly,

unlike thiazide diuretics, several sources suggest that Indapamide is not associated with glucose or lipid disturbances. Classified as a sulfonamide diuretic, Indapamide[6] is an effective antihypertensive agent and by extension, has shown efficacy in the prevention of target organ damage. Administration of Indapamide produces water and electrolyte loss, with higher doses associated with increased diuresis. Severe and clinically significant electrolyte disturbances may occur with Indapamide[6] use - for example, hypokalemia resulting from renal potassium loss may lead to QTc prolongation. Further electrolyte imbalances may occur due to renal excretion of sodium, chloride, and magnesium. The IUPAC Name of Indapamide is 4-chloro-N-(2-methyl-2, 3-dihydro indol-1-yl)-3-sulfamoyl benzamide. The Chemical Structure of Indapamide is follows.

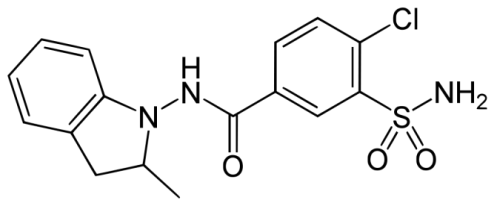

Fig-2: Chemical Structure of Indapamide.

EXPERIMENTAL

Materials and Method

Drugs, chemicals and solvents: Perindopril and Indapamide in API were kindly given by Advanced Analytical Research and Training Institute, Gujarat. All the chemicals and solvents used were of analytical grade.

Instruments

The analysis was performed on Agilent HPLC, fitted with an isocratic pump PDA detector and Altima C18 (4.6×150mm, 5.0 µm) using Methanol: TEA Buffer pH 4.5: Acetonitrile in the ratio of 50:25:25% v/v/v with flow rate of 1.0ml/min. The injection volume was 10µl. The chromatographic run time was adjusted to 7 min. The wavelength of the detector was set at 225 nm for analysis of the drugs.

Preparation of Standard Solution

Accurately weigh and transfer 10 mg of Perindopril and Indapamide working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Perindopril and 0.375ml of the Indapamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions[7] and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.[20-21]

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 50:25:25 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Altima C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of Triethylamine (TEA) buffer (pH-4.5)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the p^H 4.5. Filter and sonicate the solution by vaccum filtration[8] and ultrasonication.

Preparation of mobile phase

Accurately measured 400 ml (40%) of Methanol, 200 ml of Triethylamine buffer (20%) and 400 ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

System Suitability

Accurately weigh and transfer 10 mg of Perindopril and 10mg of Indapamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Perindopril and 0.375ml of the Indapamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY

Preparation of Standard Solution

Accurately weigh and transfer 10mg of Perindopril and 10mg of Indapamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Perindopril and 0.375ml of the Indapamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution
Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Perindopril and Indapamide sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of the above Perindopril and 0.375ml of the Indapamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity
Accurately weigh and transfer 10 mg of Perindopril and 10mg of Indapamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I (5 ppm of Perindopril & 12.5ppm of Indapamide)
Pipette out 0.05ml of Perindopril and 0.125ml of Indapamide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (10 ppm of Perindopril & 25ppm of Indapamide)
Pipette out 0.1ml of Perindopril and 0.25ml of Indapamide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (15 ppm of Perindopril & 37.5ppm of Indapamide)
Pipette out 0.15 ml of Perindopril and 0.375ml of Indapamide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20 ppm of Perindopril & 50ppm of Indapamide)
Pipette out 0.2 ml of Perindopril and 0.5ml of Indapamide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (25 ppm of Perindopril & 62.5ppm of Indapamide)
Pipette out 0.25ml of Perindopril and 0.625ml of Indapamide stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure
Inject each level into the chromatographic system[8] and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION
Repeatability
Preparation of Perindopril and Indapamide Product Solution for Precision
Accurately weigh and transfer 10 mg of Perindopril and 10mg of Indapamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1ml of the above Perindopril and 0.375ml of the Indapamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision
To evaluate the intermediate precision[9] (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure
Day 1
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Day 2
The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy
For preparation of 50% Standard stock solution
Accurately weigh and transfer 10 mg of Perindopril and 10mg of Indapamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.075ml of the above Perindopril and 0.187ml of the Indapamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution
Accurately weigh and transfer 10 mg of Perindopril and 10mg of Indapamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.15ml of the above Perindopril and 0.375ml of the Indapamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution
Accurately weigh and transfer 10 mg of Perindopril and 10mg of Indapamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.225ml of Perindopril and 0.56ml of Indapamide from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure
Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions.[10] Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Perindopril and Indapamide and calculate the individual recovery and mean recovery values.[11]

Robustness
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

For preparation of Standard solution
Accurately weigh and transfer 10 mg of Perindopril and 10mg of Indapamide working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.15ml of the above Perindopril and 0.375ml of the Indapamide stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition
The sample was analyzed by variation of mobile phase i.e. Methanol: TEA Buffer: Acetonitrile was taken in the ratio and 40: 40:20, 60:10:30 instead (50:25:25), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Development of Method

Optimized Chromatogram (Standard)

Mobile phase : Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25)
Column : Altima C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 225 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 7 minutes

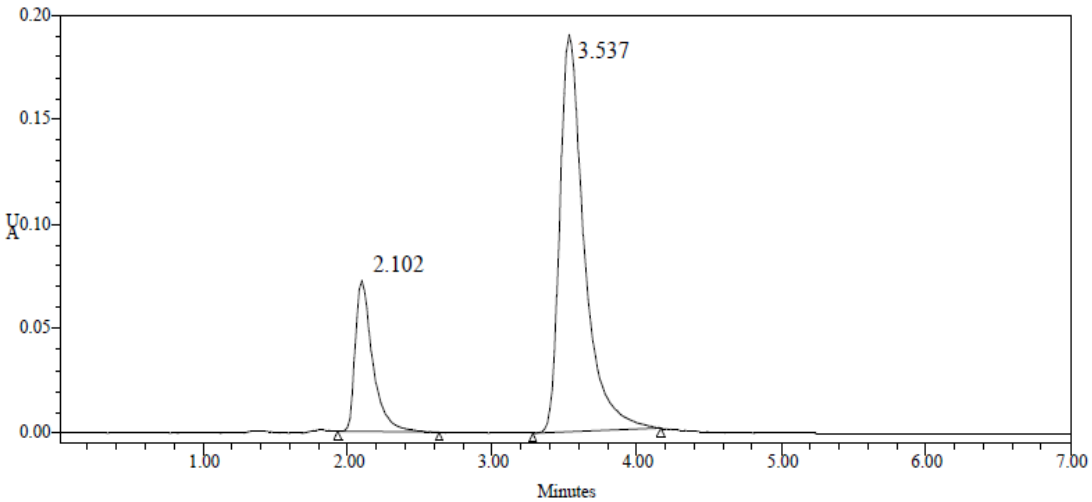

Fig-3: Optimized Chromatographic Condition.

Method Validation
Validation is a fundamental piece of value affirmation; it includes the deliberate study of frameworks, offices and procedures went for figuring out if they perform their planned capacities sufficiently and reliably as determined.

System Suitability
As indicated by the USP, framework suitability tests are a fundamental piece of chromatographic routines. These tests are utilized to confirm that the determination and reproducibility[12] of the framework are sufficient for the examination to be performed.

Table 1: Results of System Suitability for Perindopril.

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Perindopril	2.117	608452	71498	5643	1.9
2	Perindopril	2.118	606820	126412	5432	1.6
3	Perindopril	2.116	608452	126471	5123	1.6
4	Perindopril	2.109	595267	129859	5207	1.7
5	Perindopril	2.102	596608	124691	5481	1.6
Mean			**603119.8**			
Std. Dev			**6607.31**			
% RSD			**1.09**			

Table 2: Results of System Suitability for Indapamide.

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Indapamide	3.547	2234724	188631	5043	1.2	2.07
2	Indapamide	3.539	2240080	2614821	5432	1.4	2.05
3	Indapamide	3.547	2234724	2321451	5987	1.5	2.0
4	Indapamide	3.565	2204466	2324710	5845	1.6	2.01
5	Indapamide	3.537	2209574	2531247	5371	1.6	2.01
Mean			**2224714**				
Std. Dev			**16399.05**				
% RSD			**0.73**				

Specificity

The ICH documents define specificity[13] as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitates Perindopril and Indapamide in drug product.

$$\%\ \text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Perindopril and Indapamide in pharmaceutical dosage form was found to be 99.6%.

Linearity

A straight relationship ought to be assessed over the scope of the logical method. It is exhibited specifically on the medication substance (by weakening of a standard stock arrangement) and/or separate weighing of engineered blends of the medication item parts, utilizing the proposed technique.

Table-3: Chromatographic Data for Linearity Study of Perindopril

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	205035
66.6	10	381239
100	15	561128
133.3	20	740162
166.6	25	909922

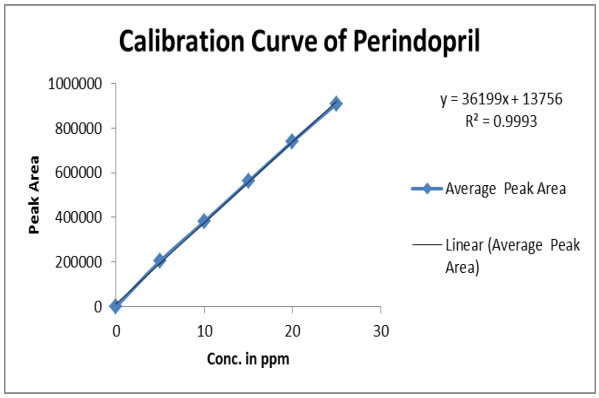

Fig-4: Calibration Graph for Perindopril.

Table-4: Chromatographic Data for Linearity Study of Indapamide

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	12.5	757881
66	12.5	757881
100	25	1458941
133	37.5	2132457
166	50	2901811

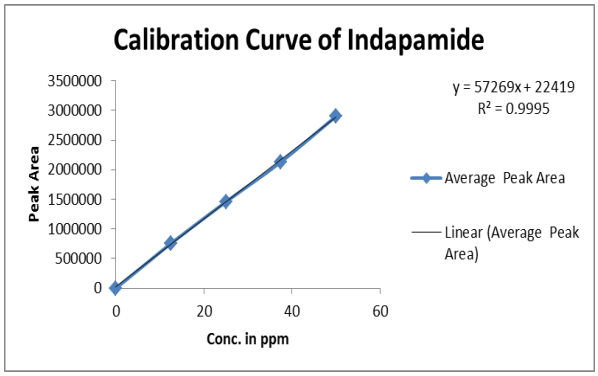

Fig-5: Calibration Graph for Indapamide.

Precision

The precision[14] of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy[15] solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-5: Results of repeatability for Perindopril.

S.No.	Name	Rt	Area	Height	USP plate Count	USP Tailing
1	Perindopril	2.108	602223	128898	2586	1.6
2	Perindopril	2.105	607748	129233	2947	1.4
3	Perindopril	2.113	607302	127409	2468	1.6
4	Perindopril	2.109	608674	127047	2146	1.9
5	Perindopril	2.109	607376	129859	2307	1.7
Mean			**606665**			
Std. Dev			**2542.3**			
% RSD			**0.42**			

Table-6: Results of method precession for Indapamide.

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Indapamide	3.552	2220333	2231111	1.6	2371
2	Indapamide	3.550	2221573	2674210	1.6	2841
3	Indapamide	3.564	2215483	2231261	1.5	2816
4	Indapamide	3.564	2217379	2421301	1.5	2872
5	Indapamide	3.565	2211255	2324710	1.6	2845
Mean			**2217205**		**1.6**	**2841**
Std. Dev			**4100.8**			
% RSD			**0.18**			

Intermediate Precision

Day 1:

Table-7: Results of Intermediate Precision for Perindopril.

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Perindopril	2.108	596608	128898	2547	1.6
2	Perindopril	2.105	598959	129233	2944	1.4
3	Perindopril	2.113	595728	127409	2361	1.6
4	Perindopril	2.109	594485	127047	2546	1.9
5	Perindopril	2.109	595267	129859	2207	1.7
6	Perindopril	2.102	596608	124691	2481	1.6
Mean			**596209**			
Std. Dev			**1718.7**			
% RSD			**0.29**			

Table-8: Results of Intermediate precision for Indapamide.

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Indapamide	3.552	2207732	2231134	8371	1.5	2.04
2	Indapamide	3.550	2202266	2674210	6841	1.6	2.03
3	Indapamide	3.564	2209375	2247461	7816	1.6	2.01
4	Indapamide	3.564	2204037	2454301	8872	1.6	2.05
5	Indapamide	3.565	2204466	2324710	4845	1.6	2.02
6	Indapamide	3.537	2209574	2531247	8371	1.6	2.03

Mean			**2205575**				
Std. Dev			**2899.8**				
% RSD			**0.13**				

Day 2:

Table-9: Results of Intermediate Precision Day 2 for Perindopril

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Perindopril	2.102	602155	127998	5586	1.5
2	Perindopril	2.105	603662	134844	5636	1.6
3	Perindopril	2.112	603931	161103	5432	1.6
4	Perindopril	2.113	607302	127409	5468	1.6
5	Perindopril	2.109	608674	127047	5146	1.9
6	Perindopril	2.109	607376	129859	5307	1.7
Mean			**605516.7**			
Std. Dev			**2602.622**			
% RSD			**0.42**			

Table-10: Results of Intermediate precision for Indapamide.

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Indapamide	3.537	2241579	2263528	2371	1.6	7.98
2	Indapamide	3.552	2236409	2224418	2414	1.6	6.4
3	Indapamide	3.560	2239093	2233725	2384	1.6	8.9
4	Indapamide	3.564	2215483	2231261	2816	1.5	8.3
5	Indapamide	3.564	2217379	2421301	2872	1.5	7.5
6	Indapamide	3.565	2211255	2324710	2845	1.6	5.3
Mean			**2226866**				
Std. Dev			**13567.02**				
% RSD			**0.60**				

Accuracy: Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery[16] was calculated.

Table-11: The accuracy results for Perindopril.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	287774	7.5	7.56	100.8	99.6%
100%	551495	15	14.8	98.6	
150%	825175	22.5	22.4	99.5	

Table-12: The accuracy results for Indapamide.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1104782	18.75	18.73	100%	100%
100%	2105321	37.5	37.4	99.9%	
150%	3211306	56.25	56.21	100%	

LIMIT OF DETECTION

The detection limit[17] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

Perindopril

=3.3 × 3188.4/36199

=0.2µg/ml

Indapamide

=3.3 × 39656.07/56304

=2.3µg/ml

LIMIT OF QUANTITATION

The quantitation limit[18] of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result

Perindopril

=10×3188.481242/36199
= 0.8µg/ml

Indapamide

=10 × 39656.07/56304
= 7.04µg/ml

Robustness

The robustness[19] was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Perindopril and Indapamide. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Perindopril and Indapamide were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 13: Results for Robustness of Perindopril.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	607323	2.102	5586	1.7
Less Flow rate of 0.9 mL/min	674735	2.330	5231	1.7
More Flow rate of 1.1 mL/min	1408920	1.950	5234	1.7
Less organic phase	606093	2.290	5643	1.4
More organic phase	603559	1.998	5298	1.5

Table-14: Results for Robustness of Indapamide.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	558777	3.537	5371	1.6
Less Flow rate of 0.9 mL/min	2505636	3.885	5324	1.7
More Flow rate of 1.1 mL/min	1408920	3.263	5098	1.7
Less organic phase	2239255	4.435	5239	1.2
More organic phase	2300346	3.009	5647	1.0

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Perindopril and Indapamide in bulk drug and pharmaceutical dosage forms. The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 225 nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Altima C18 (4.6×150mm, 5.0 µm) because it was giving good peak. 40 º C temperatures was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Methanol: TEA Buffer pH 4.5: Acetonitrile (50:25:25) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Perindopril and Indapamide in bulk drug and in Pharmaceutical dosage forms.

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