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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PERINDOPRILAND INDAPAMIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A simple, accurate and precise High Performance Liquid Chromatographic (HPLC) method was developed and validated for the simultaneous estimation of Indapamide and Perindopril in bulk and Pharmaceutical dosage forms. The method was developed using Waters HPLC system with Inertsil – C18, ODS column (250 x 4.6 mm, 5µ), Mobile phase containing Methanol and water in the ratio 80:20 v/v in isocratic elution mode at a flow rate of 1.0ml/min (load of 20µl). The detection was carried out at 210 nm. Retention time of Indapamide and Perindopril were found to be 5.828 min and 3.304 min respectively. The developed method was validated with respect to linearity, robustness, precision and accuracy. It was successfully applied for the simultaneous quantitative estimation of indapamide and perindopril in the Pharmaceutical dosage form. Results show that the retention and run time were decreased, so it is evident that the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION

Hypertension is a major risk factor for cardiovascular morbidity and mortality worldwide. Effective management often requires combination therapy targeting multiple pathophysiological mechanisms. Indapamide, a thiazide-like diuretic, lowers blood pressure by promoting renal excretion of sodium and water and exerting direct vasodilatory effects. Perindopril (13-15), an angiotensin-converting enzyme (ACE) inhibitor, reduces angiotensin II formation, leading to vasodilation, decreased aldosterone secretion, and improved cardiovascular outcomes. The combination of indapamide and perindopril offers synergistic antihypertensive effects, enhancing blood pressure control while minimizing adverse effects associated with higher doses of individual agents. This fixed-dose combination is widely used in clinical practice for the treatment of hypertension and Associated cardiovascular disorders. Pharmacological treatment strategies for hypertension frequently employ combination therapy to achieve better blood pressure control and reduce drug-related adverse effects. This approach utilizes agents acting via complementary mechanisms, improving efficacy while minimizing the required doses of individual drugs. Perindopril is a long-acting ACE inhibitor that reduces the formation of angiotensin II, a potent vasoconstrictor, thereby inducing vasodilation, decreasing aldosterone secretion, and ultimately lowering blood pressure. Beyond its antihypertensive effects,

Perindopril has been demonstrated to improve endothelial function and reduce cardiovascular events.

Indapamide, a thiazide-like diuretic, exerts its antihypertensive effect through inhibition of sodium and chloride reabsorption in the distal convoluted tubule of the nephron, promoting natriuresis and diuresis. Additionally, it has direct vasodilatory properties, which contribute to its efficacy in reducing blood pressure. The combination of Perindopril and Indapamide in fixed-dose formulations provides synergistic blood pressure lowering, improves patient compliance by reducing pill burden, and mitigates adverse effects seen with high doses of either drug alone. Quality control of pharmaceutical formulations containing multiple active pharmaceutical ingredients (APIs) requires reliable, sensitive, and validated analytical methods capable of simultaneous quantification. Existing analytical techniques for Perindopril and Indapamide include spectrophotometry, HPLC, and LC-MS/MS; however, many are complex, time-consuming, or lack validation for combined dosage forms. The objective of this study was to develop and validate a simple, precise, sensitive, and robust reversed-phase HPLC method for simultaneous estimation of Perindopril and Indapamide in pharmaceutical dosage forms, fulfilling regulatory requirements.

2. MATERIALS AND METHODS:

Chemicals and Reagents: Active pharmaceutical ingredients: Perindopril erbumine and Indapamide reference standards were procured from certified suppliers with purity ≥ 99%.

Formulations: Marketed fixed-dose combination tablets containing Perindopril and Indapamide were used for assay and method applicability studies.

Solvents and reagents: HPLC grade methanol, acetonitrile, potassium dihydrogen phosphate, and ultrapure water were used for preparation of mobile phases and solutions.

Instrumentation:

The chromatographic system consisted of a WATERS HPLC SYSTEM 2690/5 equipped with an autosampler and PDA detector. Data acquisition and processing were carried out using Empower 2 software. UV-Visible spectrophotometer with matched 10 mm quartz cells was used for preliminary absorbance studies to select optimal detection wavelength.

Chromatographic Conditions

- **Column:** C18 reversed-phase column (250 mm × 4.6 mm, 5 µm particle size)
- **Mobile phase:** A mixture of potassium dihydrogen phosphate buffer (pH 3.5, 0.02 M) and methanol (65:35 v/v) was optimized for adequate resolution and peak symmetry.
- **Flow rate:** 1.0 ml/min
- **Injection volume:** 20 µl
- **Detection wavelength:** 230 nm selected based on UV spectra to maximize absorbance of both drugs without interference.
- **Column temperature:** Ambient (~25°C)
- **Run time:** 10 minutes per injection to allow clear separation of peaks and column equilibration.

Preparation of Stock and Working Solutions: Stock solutions of Perindopril and Indapamide were prepared by dissolving 100 mg of each drug in 100 ml volumetric flasks with mobile phase as solvent, followed by sonication for 20 minutes to ensure complete dissolution, yielding concentrations of 1000 µg/ml. Working standard solutions were prepared by diluting 4 ml of each stock solution to 100 ml with mobile phase to yield 40 µg/ml concentrations.

Sample Preparation: Twenty tablets were weighed and finely powdered. An accurately weighed quantity equivalent to 10 mg of Perindopril and Indapamide was transferred to a 100 ml volumetric flask, dissolved in mobile phase, sonicated for 30 minutes, and filtered through a 0.45 µm membrane filter. Appropriate dilutions were prepared to bring the concentration within the linearity range.

3. METHOD VALIDATION:

The method was validated according to International Council for Harmonisation (ICH) Q2(R1) guidelines to evaluate parameters such as specificity, linearity, accuracy, precision, limit of detection, limit of quantification, and robustness.

Specificity: Specificity was demonstrated by injecting blank, placebo, standard, and sample solutions. Chromatograms were examined for absence of interfering peaks at retention times of Perindopril and Indapamide. Peak purity index was evaluated using PDA detector, confirming the absence of co-eluting impurities or excipients.

Linearity and Range: Calibration curves were constructed for seven concentration levels (20, 30, 40, 50, 60, 70, 80 µg/ml) for both drugs. Each concentration was injected in triplicate, and the mean peak area was plotted against concentration. Linear regression analysis provided correlation coefficients (r^2), slopes, and intercepts. The linearity range was found suitable for quantification of both drugs in pharmaceutical dosage forms.

Accuracy (Recovery Studies): Accuracy was assessed through recovery studies by spiking pre-analyzed samples at three concentration levels: 50%, 100%, and 150% of target concentration. Each level was analyzed in triplicate, and percentage recoveries were calculated using the formula: Percent Recovery=Amount FoundAmount Added×100\text{Percent Recovery} = \frac{\text{Amount Found}}{\text{Amount Added}} \times 100Percent Recovery=Amount AddedAmount Found×100

Recoveries between 98% and 102% with % RSD < 2% were considered acceptable.

Precision

Repeatability (Intra-day precision): Six replicate injections of standard solution (40 µg/ml) were performed, and % RSD of peak areas was calculated.

Intermediate precision (Inter-day precision): The procedure was repeated on different days and by different analysts, assessing reproducibility.

Limit of Detection (LOD) and Limit of Quantification (LOQ) LOD and LOQ were calculated using the standard deviation of the response and slope of calibration curve using the formulae:

$$LOD=3.3\times\sigma S\text{LOD} = 3.3 \times \frac{\sigma}{S}LOD=3.3\times S\sigma$$

$$LOQ=10\times\sigma S\text{LOQ} = 10 \times \frac{\sigma}{S}LOQ=10\times S\sigma$$

where σ\sigmaσ is the standard deviation of the response and SSS is the slope.

Robustness: Robustness was evaluated by deliberate variations in chromatographic parameters such as flow rate (±0.2 ml/min), mobile phase composition (±5%), and detection wavelength (±2 nm). Effects on retention time, peak area, and system suitability parameters were monitored.

4. RESULTS AND DISCUSSION

Chromatographic Performance: The optimized mobile phase provided sharp, symmetrical, and well-resolved peaks with retention times of approximately 3.8 minutes for Perindopril and 6.2 minutes for Indapamide (Figure 1). No interfering peaks were observed in blank or placebo samples, confirming the specificity of the method.

Linearity: The calibration curves showed excellent linearity over the concentration range of 20-80 µg/ml with correlation coefficients (r^2) of 0.9981 for Perindopril and 0.9998 for Indapamide, indicating proportionality between peak area and concentration.

Accuracy: Recovery studies yielded results between 98.5% and 101.5% for both drugs across all spiking levels, with % RSD below 2%, confirming the method's accuracy (Table 3 & 4).

Precision: Repeatability and intermediate precision studies showed % RSD values less than 1.5% for peak area and retention times, demonstrating excellent precision and reproducibility of the assay (Table 5 & 6).

Sensitivity: LOD values of 0.8 µg/ml for Perindopril and 0.6 µg/ml for Indapamide, and LOQ values of 2.5 µg/ml and 2.0 µg/ml respectively, indicate sufficient sensitivity for routine quality control (Table 7).

Robustness: Variations in flow rate, mobile phase composition, and detection wavelength produced negligible changes in retention times and peak areas with % RSD < 2%, confirming the method's robustness (Table 8).

Assay of Marketed Formulation: The assay results for commercial tablets showed 99.2% and 98.7% content of Perindopril and Indapamide respectively, within pharmacopoeial limits (Table 9).

Table 1: Chromatographic conditions for optimized method

Parameters	Conditions
Stationary phase (column)	Inertsil - C18, ODS Column(4.6 mm× 250 , 5µ)
Mobile Phase	Methanol : Water (80:20)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	210nm
Drug RT (min)	3.304min for perindropril and 5.828min for indapamide

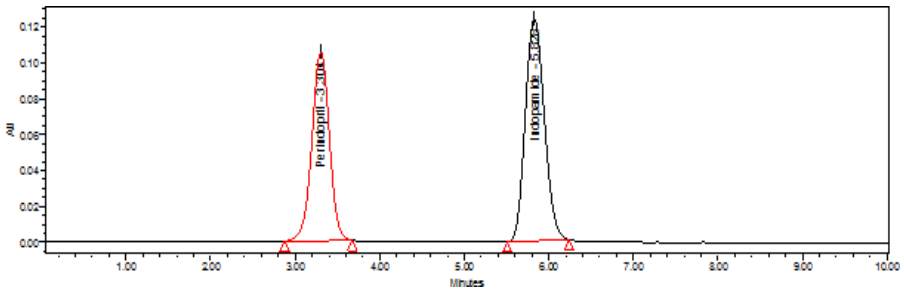

Figure 1: Optimized chromatogram

Table 2: Linearity results of perindropril and indapamide

Perindopril		Indapamide	
Conc (µg/ml)	**Peak area**	**Conc(µg/ml)**	**Peak area**
0	0	0	0
20	324151	20	603243
30	493265	30	918946
40	667312	40	1228593
50	863541	50	1519654
60	1072384	60	1813265
70	1256243	70	2098576
80	1438182	80	2429854

Figure 2: Calibration curve of Perindopril

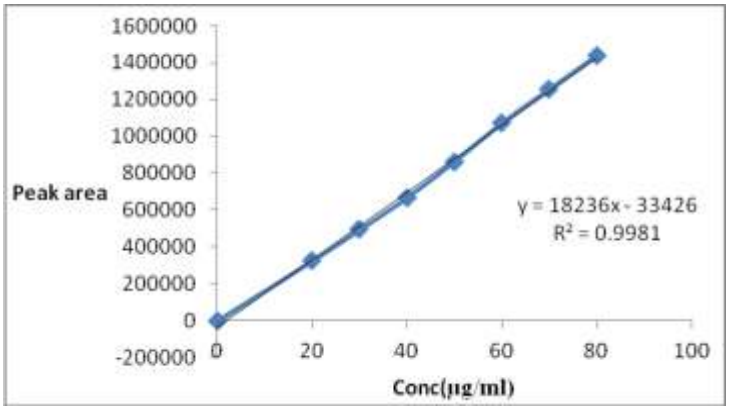

Table 3: Accuracy data of perindopril

% level	Amount spiked (µg)	Amount recovered (µg)	% Recovery	Statistical Analysis of % Recovery
50%	20	20.15	100.75	MEAN = 99.69333 %RSD = 0.92
50%		19.86	99.31	
50%		19.80	99.02	
100 %	40	39.88	99.70	MEAN = 99.83333 %RSD = 0.41
100 %		40.12	100.30	
100%		39.80	199.50	
150%	60	60.12	100.21	MEAN = 99.97333 %RSD = 0.31
150%		59.76	99.61	
150%		60.06	100.10	

Table 4: Accuracy data of Indapamide

% level	Amount spiked (µg)	Amount recovered (µg)	% Recovery	Statistical Analysis of % Recovery
50%	20	20.04	100.2	MEAN = 100.06 %RSD = 0.18
50%		19.97	99.85	
50%		20.02	100.11	
100 %	40	40.01	100.02	MEAN = 100.04 %RSD = 0.091
100 %		40.05	100.14	
100%		39.98	99.96	
150%	60	60.08	100.14	MEAN = 100.02 %RSD = 0.09
150%		59.97	99.96	
150%		59.98	99.98	

Table 5: System Precision data of perindopril and Indapamide

S. No	Peak area	
	Perindopril	Indapamide
1	674753	1218805
2	674261	1214014
3	675298	1215474
4	679221	1227655
5	688636	1267019
Mean	678433.8	1228593
SD	6031.135	22124.07
% RSD	088897	1.800764

Table 6: Method Precision data of perindopril and Indapamide

S. No	Peak area	
	Perindopril	Indapamide
1	633495	1202110
2	635992	1203700
3	639828	1201851
4	639098	1202255
5	648289	1203283
6	631322	1203249
Mean	637312	1202687.6
SD	6988.87	771.548
% RSD	0.0891	0.1358

Table 7: LOD and LOQ data of perindopril and Indapamide

Drug name	LOD (µg/ml)	LOQ (µg/ml)
Perindopril	0.56	0.57
Indapamide	1.69	1.74

Figure 3: Calibration curve of Indapamide

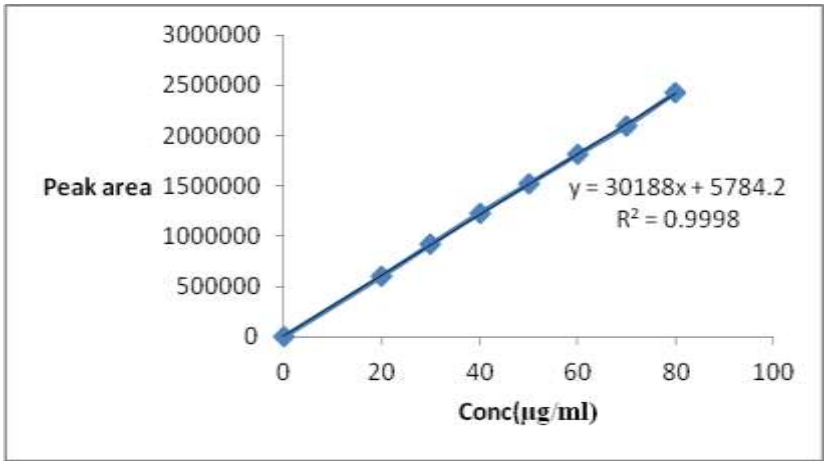

Table 8: Robustness data of perindopril and Indapamide

S. No	Drug name	Condition	Peak area	Tailing factor
1	**Perindopril**	Flow rate- 0.8 ml/min	620425	1.318
		Flow rate- 1.2 ml/min	602444	1.299
2	**Indapamide**	Flow rate- 0.8 ml/min	1273638	1.36
		Flow rate-1.2 ml/min	126277	1.287

Table 9: Assay data perindopril and indapamide

S. No	Peak area of perindopril	% Assay	Peak area of indapamide	% Assay
1	674753	100.0%	1218805	99.91%
2	674261		1214014	
3	6765298		1215474	
4	679221		1227655	
5	688636		1267019	
Mean	678433.8		1228593	
SD	6031.135		22124.07	
%RSD	0.888979		1.800764	

CONCLUSION: A novel, simple, and reliable RP-HPLC method has been successfully developed and validated for simultaneous estimation of Perindopril and Indapamide in combined dosage forms. The method complies with ICH validation criteria and offers advantages including rapid analysis, minimal sample preparation, and high sensitivity. This method is suitable for routine quality control analysis of pharmaceutical products containing these antihypertensive agents.

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