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Research Article

METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PERINDOPRIL ERBUMINE AND INDAPAMIDE BY RP-HPLC IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

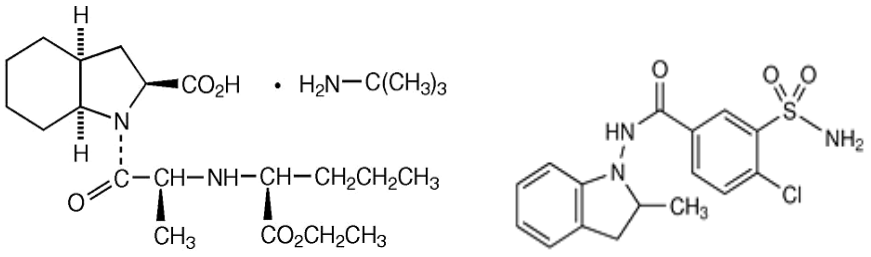
This research is concerned with the development of simple, specific, accurate and reproducible isocratic reversed phase high performance liquid chromatographic (RP-HPLC) method which is subsequently validated using ICH recommendations for the simultaneous estimation of Perindopril Erbumine (PE) and Indapamide (ID) in combined tablet dosage form. The determination was carried for a runtime of 20min at 40°C on Inertsil ODS-3V column having 250mm x 4.6mm i.d. with 5µm particle size and potassium dihydrogen phosphate buffer adjusted to pH 3.0 using ortho phosphoric acid and acetonitrile (60:40 v/v) was used as eluent at a constant flow rate of 1.0ml/min with UV detection wavelength of 215nm. The retention time of PE and ID was about 11.9 and 4.9min with correlation coefficient of 0.9992 and 0.9990 respectively. The linearity was established at 8-24µg/ml for PE and 2.5-7.5µg/ml for ID and the mean recovery for both drugs were found to be 100.3% at a load volume of 50µl.

Keywords: Perindopril Erbumine, Indapamide, RP-HPLC, Simultaneous estimation, Validation.

INTRODUCTION

Perindopril Erbumine, (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; 2-methylpropan 2-amine is a white crystalline powder freely soluble in water, alcohol and chloroform is used in the treatment of hypertension and heart failure[1]. Perindoprilat (active metabolite) lowers blood pressure by the inhibition of angiotensin converting enzyme (ACE) activity. Inhibition of ACE results in decreased plasma angiotensin II, leading to decreased vasoconstriction. PE has rapid absorption of 75% and half life is about 1-3 hours. Indapamide, 4-chloro-N (2 methyl-2,3-dihydroindol-1-yl)- 3-sulfamoyl benzamide is a white to yellow-white crystalline (tetragonal) powder, soluble in methanol, ethanol, acetic acid and ethyl acetate; very slightly soluble in ether, chloroform and benzene and practically insoluble in water is used as a diuretic. ID is the first of the new class of antihypertensive diuretics which is prescribed to treat the salt and fluid retention associated with congestive heart failure[2]. ID (thiazide like diuretic) act directly on the kidney and enhances excretion of sodium, chloride, and water by interfering with transport of sodium ions across renal tubular epithelium[3]. It also acts as a carbonic anhydrase inhibitor. ID reaches maximum plasma concentration within 2-2.5 hours and half life is about 14-18 hours. Fixed dose combination containing PE (2mg/4mg) and ID (0.625mg/1.25mg) are available in market as tablets.

Perindopril A.P.I. (active pharmaceutical ingredient) is official in B.P[4] and E.P[5]. Tablets are not official in any of the pharmacopoeias. ID tablets are official in I.P[6], B.P[7] and U.S.P[8]. An extensive survey of literature revealed the availability of several methods for the estimation of PE (immunoassay, spectrophotometric, HPLC, biosensor method, LC-MS/MS, capillary gas chromatographic method) and ID (spectrofluorometry, densitometry, HPTLC, colorimetry, electrochemical methods, HPLC) alone and in combination with other drugs, but the simultaneous estimation of these drugs from their combination tablets were very few[9, 10]. Therefore, it was thought worthwhile to develop a simple, precise and accurate RP-HPLC method for simultaneous estimation of PE and ID in tablets. The newly developed method was validated as per ICH guidelines to confirm the reproducibility and wide applicability of the method.



(a) Perindopril Erbumine **(b) Indapamide**

Fig. 1: Chemical structure of (a) Perindopril Erbumine and (b) Indapamide

MATERIALS AND METHOD

Instrumentation

The HPLC system from Agilent Technologies 1200 series equipped with auto injector, degasser, single head dual plunger G1311A quaternary pump for constant flow and constant pressure delivery and UV detector with deuterium lamp attached to EZ-Chrome Elite software for controlling the instrumentation as well as for data acquisition was used. UV-Visible double beam spectrophotometer from Shimadzu 2450 model with spectral slit width of 1.0nm and automatic wavelength corrections with 10mm matched quartz cells attached to UV-Probe software was used for the selection of λ_{max}. All weighing were done on electronic balance (Model: Sartorious CT-225D). Ultrasonicator (Model: Fast clean 2K911009) and pH meter (Model: Poloman LP-139S) was used for solution preparation and pH determination respectively.

Regents and Chemicals

PE and ID pure drug samples and combination tablets containing 4mg PE and 1.25mg ID manufactured by M.S.N. Laboratories Ltd.,

Formulations Division, Bollaram, Hyderabad was used. Acetonitrile (HPLC grade) and potassium dihydrogen phosphate (AR grade) from Standard Company, Hyderabad, sodium heptane sulphonic acid (HPLC grade), triethylamine and ortho-phosphoric acid (AR grade) from Rankem and Milli-Q purified water (In-house preparation) was used through out the research work.

Selection of λ_{max}

Preparation of mixed stock solution

An accurately weighed 10mg each of PE and ID were transferred into a 100ml volumetric flask. 20ml of methanol was added into it and sonicated for awhile for dissolving the drugs. The flask was made up to 100ml with methanol so as to get a concentration of 100µg/ml.

Preparation of mixed standard solution

From the stock solution, 1ml was transferred into a 20ml volumetric flask and volume was made up to mark with methanol to get a final concentration of 5µg/ml. The resulting solution containing 5µg/ml of PE and 5µg/ml of ID were scanned in UV-Visible spectrophotometer from 400-200nm to determine the wavelength of maximum absorption of both the drugs in combination. The λ_{max} of drugs in combination was found to be 215nm.

The values were shown in table no.1 and the spectra of combination of PE and ID was appended in Fig. 2.

Table 1: Observation from UV-Visible spectrophotometer

S.no.	Wavelength (NM)	Absorbance of combination
1	200	0.501
2	210	0.793
3	215	0.519
4	220	0.346
5	230	0.303
6	250	0.230
7	300	0.030
8	400	0.003

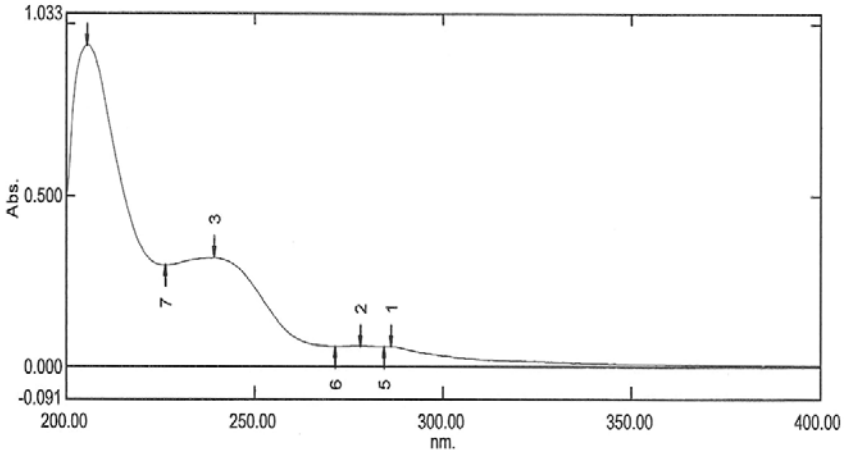


Fig. 2: Spectra for combination of Perindopril Erbumine and Indapamide

Chromatographic conditions

The stationary phase used is Inertsil ODS-3V, 5µm, 250mm x 4.6mm column maintained at 40˚C. An isocratic mobile phase constituting Phosphate Buffer which is prepared by dissolving 0.62gm of sodium heptane sulphonic acid and 1.7gm of potassium dihydrogen phosphate in 2L Milli-Q water, added 1ml triethylamine, pH adjusted to 3.0+/-0.05 with ortho-phosphoric acid which was filtered using 0.45µ filter paper, Acetonitrile in ratio 60:40(v/v), at a flow rate of 1.0ml/min was used. The mobile phase was degassed for about 15min by sonication. Samples of 50µl were injected into the HPLC system and the effluents were analysed at 215nm. For this optimized condition, runtime was 20min.

Standard preparations

Working standard solution

An accurately weighed 32.0mg of PE working standard and 10.0mg of ID working standard was transferred into 200ml volumetric flask, 120ml mobile phase was added and sonicated for 10min for dissolving the drugs and volume was made up with mobile phase. Further 5 ml of the filtrate was diluted to 50 ml with mobile phase.

Sample preparation for tablet analysis

Five tablets were transferred into 250ml volumetric flask; 150ml of the mobile phase was added and sonicated to dissolve the tablets. Then volume was made up with mobile phase. Solution was filtered through 0.45µ nylon filter. Further 5ml of the filtrate was diluted to 25ml with mobile phase. 50µl of the blank, placebo, standard and sample solution were injected separately into the chromatographic system and chromatograms were recorded and the peak areas were measured. A typical chromatogram of PE and ID was appended in Fig. 3.

Method Validation

Collection and evaluation in a documented form of repetitive evidence obtained according to agreed protocols to provide high degree of assurance that the procedure under consideration will consistently produce results meeting its pre-determined specifications or quality attributes[11]. The newly developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) parameters like system suitability, linearity and range, precision (repeatability), intermediate precision (ruggedness), specificity, accuracy and robustness[12-14].

System suitability testing

A standard solution was prepared using PE and ID working standard as per the test method and was injected six times into the HPLC system. The parameters like theoretical plates, tailing factor and USP resolution for the standard solutions were calculated and the values were given in table no. 2.

Table 2: System suitability studies

S.no.	Parameters	PE[a]	ID[b]
1	Theoretical Plates	5661	3306
2	Tailing Factor	1.20	0.98
3	USP Resolution	10.1	

a, b = mean of 6 readings

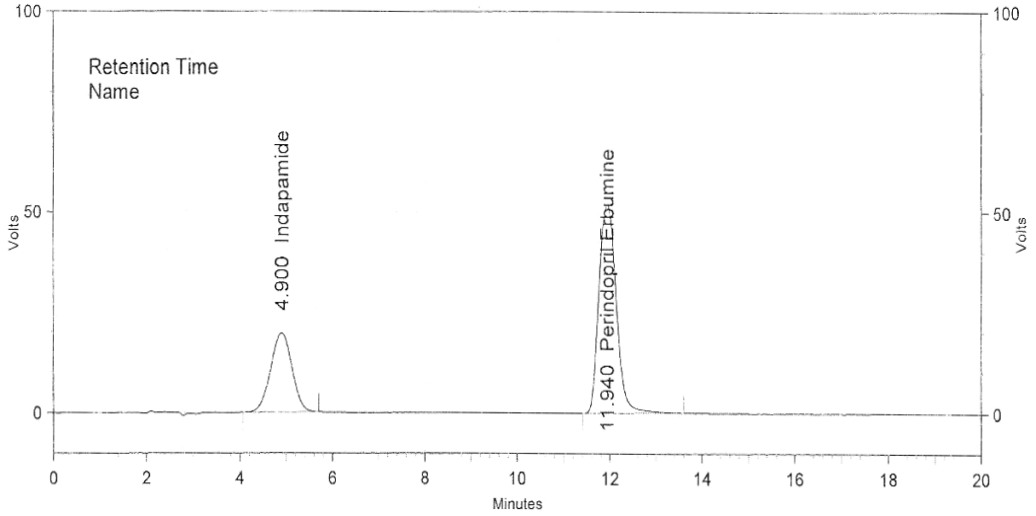


Fig. 3: A typical chromatogram of Indapamide and Perindopril Erbumine

Specificity (Sensitivity/Selectivity)

Specificity was performed to detect the presence of interference peak (blank and placebo peaks) at the retention time of the analyte peak.

The interference of placebo was detected by preparing samples by taking the placebo equivalent to about the weight in portion of test preparation as per the test method and were injected into the HPLC system. The interference of blank was detected by injecting mobile phase as per the test method.

Precision

System precision & Method precision (Repeatability)

System precision was determined by estimating the %RSD of the peak area for five replicate injections of the standard solution. Method precision was determined by preparing six samples as per the test method representing a single batch. The assay of these samples was determined and the precision of the method was evaluated by computing the %RSD. The values were given in table no. 3.

Table 3: Precision studies (Repeatability)

S. no.	System precision		Method precision	
	Standard area		% Assay	
	PE	ID	PE	ID
1	2854103	1345646	97.6	97.8
2	2853353	1343898	98.5	98.7
3	2855676	1344266	99.2	99.7
4	2854881	1341866	100.1	99.9
5	2858023	1343680	97.6	98.8
6	-	-	97.4	98.8
Mean	2855207	1343871	98.4	98.95
sd	1796.68	1356.82	1.08	0.76

SD = Standard Deviation RSD = Relative Standard Deviation

Intermediate precision (Ruggedness)

The ruggedness of the test method was determined by carrying out precision study in six replicates of assay on a single batch sample in different days by two different analysts, on two different columns and on two different instruments. The difference in the average assay of method precision and intermediate precision for PE and ID is 0.80 and 1.10 respectively which is not more than the limit 2. The values were given in table no. 4.

Table 4: Precision studies (Ruggedness)

S. No.	Analyst 1, Day 1 % Assay		Analyst 2, Day 2 % Assay	
	PE	ID	PE	ID
1	97.6	97.8	98.7	99.8
2	98.5	98.7	99.2	100.0
3	99.2	99.7	99.4	100.3
4	100.1	99.6	99.3	100.1
5	97.6	99.9	99.2	100.1
6	97.4	98.8	99.2	100.0
Mean	98.4	98.9	99.2	100.0
SD	1.08	0.76	0.24	0.16
%RSD	1.10	0.77	0.24	0.16

Accuracy (Recovery/Trueness)

The accuracy of the test method was determined by preparing recovery samples (spiking placebo with known quantities of PE and ID standard) at the level of 50%, 100% and 150% of targeted concentration. The recovery samples were prepared in triplicate at each level. The samples at different levels were chromatographed and the percentage recovery for the amount added was estimated. The precision of the recovery at each level was determined by computing the %RSD of triplicate recovery results. The values were given in table no. 5.

Table 5: Recovery studies (Accuracy)

Sl. No.	Spike Level(%)	Amount Added(PPM)	Amount[c] Recovered(PPM)	Mean %Recovery	%RSD
PE	50	8.02	8.10	101.03	0.40
	100	16.05	16.28	101.43	0.11
	150	24.07	23.68	98.33	0.16
ID	50	2.50	2.53	101.33	0.23
	100	5.00	5.07	101.47	0.11
	150	7.50	7.37	98.30	0.18

c = mean of 3 readings

Linearity

The linearity of detector response for PE and ID was determined by preparing a series of solution of PE and ID working standards over the range of 50% to 150% of targeted concentration. These solutions were injected into the chromatographic system and response area was recorded. The regression equation for PE and ID were found to be y = 173435x + 18215 and y = 255134x + 12587 with correlation coefficient 0.9992 and 0.9990, respectively. A linearity plot for PE and ID was shown in Fig. 4 and 5, and the correlation co-efficient was evaluated. The values were given in table no. 6.

Table 6: Linearity studies

Linearity Level (%)	PE		ID	
	Conc.	Area[d]	Conc.	AREA[E]
50	8	1420548	2.50	660434
75	12	2067402	3.75	957100
100	16	2871717	5.00	1328724
125	20	3475847	6.25	1598430
150	24	4148600	7.00	1909182
R^2	y = 173435x + 18215		y = 255134x + 12587	
m	173435		255134	
c	18215		12587	
r	0.9992		0.9990	

d, e = mean of 3 readings, CONC. = Concentration in ppm, R^2 = Regression Equation, m = Slope, c = Intercept, r = Correlation Coefficient

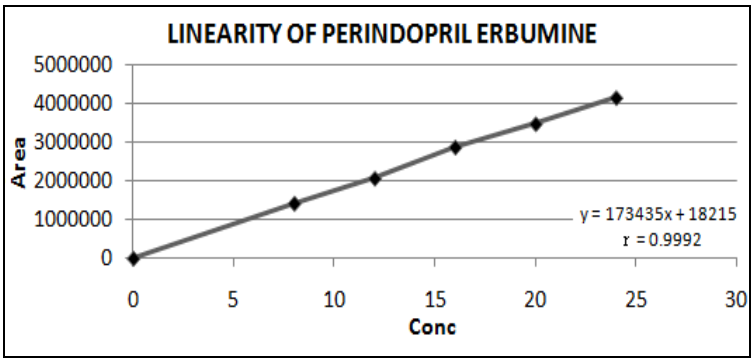


Fig. 4: Linearity plot for Perindopril Erbumine

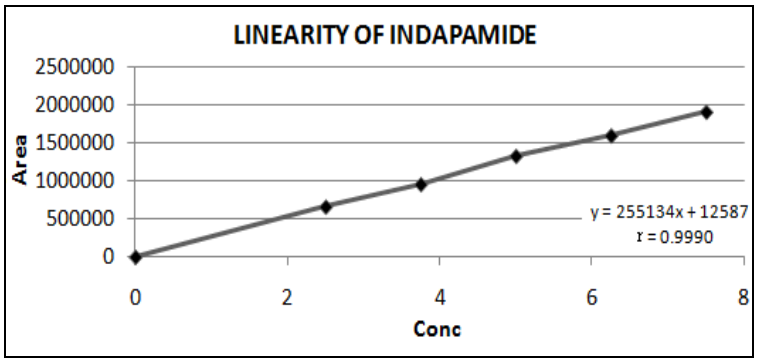


Fig. 5: Linearity plot for Indapamide

Robustness

Effect of variation in flow rate

A study was conducted to determine the effect of variation in the flow rate. Standard solution prepared as per the test method was injected into the HPLC system by keeping flow rates 0.8ml/min, 1.0ml/min and 1.2ml/min and the system suitability parameters were evaluated. The values were given in table no. 7.

Effect of variation in temperature

A study was conducted to determine the effect of variation in the temperature. Standard solution prepared as per the test method and was injected into the HPLC system at 35ºC, 40ºC and at 45ºC temperature and the system suitability parameters were evaluated. The values were given in table no. 8.

Effect of variation in filter paper

A study was conducted to determine the effect of variation in the filter paper. Standard solution prepared as per the test method was filtered through 0.45 µm membrane nylon filter paper and 0.45µm PVDF (Poly Vinyl Di Fluoride) filer paper and injected into the HPLC system. The percentage difference in assay in test solution with both the filter papers was evaluated. The values were given in table no. 9.

Table 7: Robustness (flow rate) studies

FLOW RATE	PE		ID	
(ml/min)	STD AREA[f]	TAILING[g]	STD AREA[h]	TAILING[i]
0.8	3527903	1.15	1636302	1.00
%RSD	0.22	0.48	0.06	0.44
1.0	2820019	1.15	1308778	0.99
%RSD	0.34	1.87	0.36	0.71
1.2	2334252	1.15	1082931	0.98
%RSD	0.15	0.47	0.23	0.72

Table 8: Robustness (temperature) studies

Temp.	PE		ID	
(°c)	Std Area[j]	Tailing[k]	Std Area[l]	Tailing[m]
35	2827178	1.15	1322846	0.95
%RSD	0.33	0.95	0.31	1.89
40	2823756	1.14	1313102	0.97
%RSD	0.26	0.62	0.42	1.13
45	2824695	1.14	1320815	1.01
%RSD	0.12	1.47	0.26	0.70

j, k, l, m = mean of 5 readings TEMP. = Temperature

Table 9: Robustness (filter paper) studies

S. No.	Percentage assay		
	Nylon[n]	PVDF[o]	Diff. (%)[p]
pe	99.07	98.87	0.77
id	98.63	98.83	0.27

n, o, p = mean of 3 readings, DIFF. = Difference

RESULTS AND DISCUSSION

A new RP-HPLC method was developed for the simultaneous estimation of PE and ID in tablet dosage form and validated in accordance with ICH guidelines for the parameters like system suitability, linearity and range, precision (repeatability), intermediate precision (ruggedness), specificity, accuracy and robustness. The results obtained for each of the parameters lies well within the acceptance criteria. Hence the developed method was simple, specific, linear, precise, accurate, robust and rugged and could be extensively used for the simultaneous estimation of PE and ID in tablet formulation system.

System suitability parameters proved that the proposed method suits for the simultaneous estimation of PE and ID. After various trials performed, chromatogram for PE and ID was found satisfactory on Inertsil ODS-3V, 5µm, 250mm x 4.6mm, using mobile phase composition of buffer: acetonitrile (60:40). Drug peak was found to be symmetrical as observed from asymmetry factor of 1.20 for PE and 0.98 for ID. Resolution of the proposed method was found to be satisfactory. Sensitivity of the method was good and also linearity was observed over a wide concentration range of 8-24µg/ml for PE and 2.5-7.5µg/ml for ID. Accuracy of the method was determined by recovery with spiked concentration of pure drug at five levels for PE and ID. Recovery of drug was well within the acceptance limits of 97-103%. Method was robust and rugged as observed from insignificant variation in the results of analysis on changes in flow rate, temperature, filter and analysis being performed by different analysts, in different days on different systems using different columns respectively.

CONCLUSION

From the results obtained, it was observed that the developed method was proven to be specific, precise, linear, accurate, rugged and robust and is suitable for its intended purpose. Good agreement was seen in the assay results of pharmaceutical formulation by developed method. Hence, it was concluded that the proposed method is a good approach for obtaining reliable results and found to be suitable for the routine analysis of PE and ID in pharmaceutical formulations.

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