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Development and validation of HPLC method for the simultaneous determination of Perindopril and Indapamide in pharmaceutical formulations

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ABSTRACT

A simple isocratic HPLC method has been developed and subsequently validated for simultaneous determination of Perindopril and Indapamide in pharmaceutical dosage forms. The method employs an Inertsil ODS C18 column, 5 µ, 250 mm x 4.60mm id with flow rate of 1.5 ml/min using UV detection at 215nm. The separation was carried out using a mobile phase consisting of potassium di hydrogen phosphate buffer having pH 5 and acetonitrile in the ratio of 60:40 respectively. The retention time for Perindopril and Indapamide was found to be 2.85 min and 3.85 min respectively. A linear response was observed over the concentration range of 5.0-40.0 µg / ml and 2.5 - 15.0 µg / ml for the assay of Perindopril and Indapamide with correlation coefficient of 0.9992 and 0.9994, respectively. The results of analysis were validated stastically and by recovery studies. Hence the proposed method was found to be accurate, precise, reproducible and specific and can be used for simultaneous analysis of these drugs in tablet formulations.

KEYWORDS

HPLC;
Simultaneous determination;
Perindopril;
Indapamide.

INTRODUCTION

Perindopril erbumine (PE), [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; 2- methylpropan 2-amine, Figure 1 is a white crystalline powder freely soluble in water, alcohol and chloroform is used in the treatement of hypertension and heart failure[1]. Perindoprilate (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.

Indapamide (ID),4-chloro-N-(2,3-dihydro-2-methyl-1H-indol-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

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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) acts directly on the kidney and enhances the excretion of sodium, chloride and water by interfering with transport of sodium ions across renal tubular epithelium[3].

Fixed dose combination containing PE (2mg / 4mg) and ID (0.625 mg/1.25 mg) are available in market as tablets. PE is official in B.P[4], USP[5] and E.P[6] Tablets are not official in any of the pharmacopoeias. ID tablets are official in I.P[7], B.P[8] and U.S.P[9].

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 (spectrofluorimetry, densitometry, HPTLC, colorimetry, electrochemical methods, HPLC methods) alone and in combination with other drugs, but the simultaneous estimation of these drugs from their combination tablets were very few[10-12]. Therefore, it was thought worthwhile to develop a simple, precise and accurate RP-HPLC method for the simultaneous determination of PE and ID in tablets. The new method was validated as per ICH guidelines to confirm the reproducibility and wide applicability of the method.

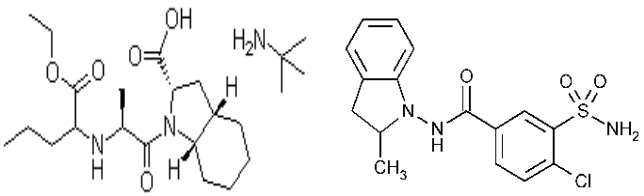

Figure 1 : The structural formulae for the studied drugs.

EXPERIMENTAL

Apparatus

The HPLC chromatograms were obtained using a Shimadzu instrument, Model LC-10 AD VP, equipped with a variable wavelength UV-visible detector, Model SPD-10 AD VP, Degasser Model DGU-12 A and a 20-µl volume Rheodyne injector. Inertsil ODS C18 (5µm, 250mm x 4.6 mm I.D) column was used as a stationary phase.

Materials

Samples

Perindopril erbumine and indapamide were kindly supplied by Servier Laboratories S.A.E, 6th October City Egypt. Their purities were found to be 99.4 ± 0.76 and 99.75 ± 0.63, respectively, according to their official procedures[4,13].

Bipreterax® tablets (Servier Laboratories S.A.E, 6th October City, Egypt); batch NO. 701050., claimed to contain 4 mg perindopril erbumine and 1.25 mg indapamide per tablet, respectively.

Chemicals

All chemicals and reagents were of pure analytical grade.

De-ionized water, acetonitrile, 0.01 M phosphate buffer and o-phosphoric acid (E-Merck, Darmstadt, Germany) were of HPLC grade.

Standard solutions

Perindopril stock solution (0.1 mg.ml^{-1})

Accurately weighed 10.0 mg of PE powder was transferred into 100-ml measuring flask. 20.0 ml of methanol was added to the flask, sonicated for a while for dissolving the drug. The flask was made up to 100 ml with methanol so as to get a concentration of 100 µg/ml.

Indapamide stock solution (0.1 mg.ml^{-1})

Accurately weighed 10.0 mg of ID powder was transferred into 100-ml measuring flask then the preparation was completed as mentioned before.

Laboratory-prepared mixtures

For PE, aliquots containing 100.0, 150.0, 200.0 and 100.0 µg were transferred from its stock solution (0.1mg. ml^{-1}) into a series of 10-ml volumetric flasks. For ID, aliquots containing 25.0, 50.0, 100.0 and 100.0 µg from its stock solution (0.1mg.ml^{-1}) were added to the same flasks, completed to volume with acetonitrile and mixed well.

Chromatographic conditions

The stationary phase used is Inertsil ODS, 5µm, 250 mm x 4.6 mm column. An isocratic mobile phase containing phosphate buffer (prepared

by dissolving 1.7 gm of potassium dihydrogen phosphate in 1 L Milli-Q water, pH adjusted to 5 ± 0.05 with ortho-phosphoric acid) and acetonitrile in a ratio of 60:40 (v/v), which was filtered using 0.45 µ filter paper. The flow rate was 1.5 ml/min and the detection wavelength was 215 nm. The mobile phase was degassed for about 15 min by sonication and samples of 20µl were injected into the HPLC system.

Procedures

Construction of calibration curves

Accurately measured volumes (0.5, 1.0…..4.0 ml) of PE stock solution (0.1 $mg.ml^{-1}$) and (0.25, 0.5…..1.5 ml) of ID stock solution (0.1 $mg.ml^{-1}$) were transferred into 10 ml measuring flasks, diluted to volume with acetonitrile to get final concentrations ranged from 5.0-40.0 and 2.5-15.0 $\mu g.ml^{-1}$ for PE and ID, respectively. The prepared series of both drugs were injected into the chromatographic system and the peak area for each concentration was recorded. The relation between the concentration and the peak area was plotted for each drug and the regression equation was calculated.

Assay of laboratory-prepared mixtures

The chromatographic conditions were adopted for each laboratory-prepared mixture and the concentrations of PE and ID in these mixtures were calculated by substituting in the regression equations.

Application to pharmaceutical preparation (Bipreterax® tablets)

Ten tablets of Bipreterax® were accurately weighed and finely powdered. An amount of the powder containing 10.0 mg of either PE or ID was weighed, extracted by shaking with methanol (3 x 25 ml) in ultrasonic bath. The solution was filtered and transferred quantitatively into 100-ml volumetric flask. The volume was then completed to the mark with methanol. The necessary dilutions were made to reach concentrations of linearity as under construction of calibration curves.

Method validation

The newly developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) parameters like system suitability, linearity and range, precision, accuracy and robustness[14-16].

System suitability testing

Standard solutions were prepared using PE and ID working standards and were injected six times into the HPLC system. The parameters like theoretical plates, tailing factor, capacity factor and resolution were calculated and the values were given in TABLE 1.

TABLE 1 : The parameters required for system suitability test of the HPLC method.

Parameter	Obtained value	Reference value
Resolution (R)	2.77	R > 0.8
T (tailing factor)	ID (1.23) PE (1.14)	T = 1 for a typical symmetric peak.
α (relative retention time)	1.41	> 1
K (column capacity)	ID (6.65) PE (4.7)	1-10 acceptable
N (column efficiency)	ID (1463) PE (1444)	Increases with efficiency of the separation.
HETP	ID (0.02) PE (0.02)	The smaller the value, the higher the column efficiency.

Precision

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. 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 2.

TABLE 2 : Determination of system and method precision for both PE and ID.

Sample no.	System precision		Method precision	
	PE (Stand.Area)	ID (Stand. Area)	PE (%assay)*	ID (%assay)*
1	556054	1002683	98.4	98.1
2	555056	1018587	99.2	98.8
3	551444	1000342	100.1	99.7
4	553261	1000132	98.7	98.5
5	555800	1001779	99.6	99.2
6	557952	1009840	98.1	98.2
Mean	554928	1005561	99.02	98.75
SD	2285.01	7311.43	0.76	0.62

*** Average of three determinations.**

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Accuracy

The accuracy of the test method was determined by preparing recovery samples containing different known quantities of PE and ID standard. Each sample was prepared in a triplicate way and then all the samples were injected into the HPLC system and the percentage recovery for the amount added was estimated as shown in TABLE 3.

TABLE 3 : Determination of pure samples of PE and ID by the proposed HPLC mehod.

Perindopril			Indapamide		
Taken (µg / ml)	Found* (µg / ml)	Recovery %	Taken (µg / ml)	Found* (µg / ml)	Recovery %
5.0	4.99	99.73	2.5	2.52	100.80
10.0	9.94	99.36	5.0	4.98	99.68
15.0	15.23	101.93	7.5	7.46	99.47
20.0	19.68	98.40	10.0	9.92	99.20
25.0	25.06	100.25	12.5	12.43	99.45
30.0	30.03	100.11	15.0	14.96	99.71
Mean ± S.D 99.96 ± 1.17			99.72 ± 0.56		

*** Average of three determinations.**

Specificity

The chromatographic conditions were applied for different laboratory-prepared mixtures and the concentrations of PE and ID in these mixtures were calculated by substituting in the regression equations as shown in TABLE 4.

TABLE 4 : Determination of Perindopril and Indapamide in laboratory-prepared mixtures by HPLCmethod.

Mixture no	perindopril			indapamide		
	claimed $\mu g.ml^{-1}$	found* $\mu g.ml^{-1}$	recovery %	claimed $\mu g.ml^{-1}$	found* $\mu g.ml^{-1}$	recovery %
1	10.0	10.08	100.80	2.5	2.48	99.20
2	15.0	14.92	99.47	5.0	4.95	99.00
3	20.0	19.93	99.65	10.0	10.06	100.6
4	10.0	9.96	99.60	10.0	9.95	99.50
Mean±S.D			99.89±0.61			99.56±0.72

*** Average of three determinations.**

Linearity

The linearity of the response for PE and ID was determined by preparing a series of PE and Id standard solutions. These solutions were injected into the chromatographic system and the peak area was recorded. The regression equation for PE and ID were computed and the correlation coefficient was evaluated.

Robustness

Effect of variation in pH of mobile phase

A standard solution mixture of both PE and ID was injected into the HPLC system using mobile phase of different pH values (2, 3…..6) and the system suitability parameters were estimated. The values were given in TABLE 5.

TABLE 5 : Effect of pH of mobile phase on the capacity factor, relative Retention, resolution and number of theoretical plates.

pH	capacity factor(K')		relative retention(α)	resolution (R)	number of theoreticalplates	
	ID	PE			ID	PE
2.0	3.34	2.29	1.02	1.74	1058	1048
3.0	4.96	2.87	1.12	2.30	1166	1207
4.0	5.46	3.12	1.28	2.69	1244	1381
5.0	6.65	4.70	1.41	2.77	1463	1444
6.0	5.87	3.21	1.15	1.97	1345	1409

Effect of variation in mobile phase ratio

A standard solution mixture of both PE and ID was injected into the HPLC system using mobile phase solvents of different ratios (30/70, 40/60, 50/50, 60/40 and 70/30) and the system suitability parameters were estimated. The values were given in TABLE 6.

TABLE 6 : Effect of mobile phase ratio on the capacity-factor, relative retention, resolution and number of theoretical plates.

ratio (A / B)*	capacity factor(K')		relative retention(α)	resolution (R)	number of theoreticalplates	
	ID	PE			ID	PE
30 / 70	4.92	1.27	1.26	1.20	1326	1360
40 / 60	6.65	4.70	1.41	2.77	1463	1444
50 / 50	5.42	3.24	1.37	2.31	1395	1394
60 / 40	4.33	2.13	1.13	1.97	1221	1321
70 / 30	3.76	1.91	1.08	1.77	1187	1198

*** A: acetonitrile. B: 0.01 M phosphate buffer.**

TABLE 7 : Effect of mobile phase flow rate on the capacity factor, relativeretention, resolution and number of theoretical plates.

flowrate (ml/min)	capacity factor(K')		relative retention (α)	resolution (R)	number of theoretical plates	
	ID	PE			ID	PE
1.0	5.29	3.23	1.10	1.54	1220	1237
1.2	5.77	4.08	1.29	2.40	1339	1300
1.5	6.65	4.70	1.41	2.77	1463	1444
2.0	5.43	3.94	1.17	1.97	1421	1372

Effect of variation in flow rate

A standard solution mixture of both PE and ID was injected into the HPLC system using mobile phase at different flow rate values (1, 1.2, 1.5 and 2) and the system suitability parameters were estimated. The values were given in TABLE 7.

RESULTS AND DISCUSSION

Perindopril erbumine is commercially available combined with indapamide to improve the antihypertensive effect. However, by reviewing the literature concerned with the simultaneous determination of perindopril and indapamide in their mixture, it was found that very few reports were available. Therefore, the aim of this work was to develop simple analytical methods for the simultaneous determination of PE and ID either in bulk powder or in pharmaceutical formulation.

This was achieved by using a reversed phase HPLC method.

Concerning the mobile phase, different systems were tried for chromatographic separation of the two components by combining homogenous design and solvent polarity optimization. Several modifications in the mobile phase compositions were performed in order to study the possibilities of improving the performance of the chromatographic system. These modifications involved the change of pH, TABLE 5 ratio of mobile phase, TABLE 6 and flow rate, TABLE 7. The best resolution was achieved when using a mobile phase consisting of acetonitrile: 0.01 M phosphate buffer (40: 60, v/ v), adjusted to pH 5 by orthophosphoric acid which gave a better resolution and sensitivity of both drugs with retention times of 2.85 and 3.85 minutes for PE and ID, respectively, Figure 2. Linear relation was obtained between peak area and the concentration of the two drugs in the range of 5.0-40.0 and 2.5-15.0 $\mu g.ml^{-1}$ for PE and ID, respectively. The linear regression equations were found to be:

Y= 13864 x + 691.8 r = 0.9990 (for PE).

Y= 80161x + 4663 r = 0.9990 (for ID).

Where Y is the area under the peak, X is the concentration in µg.ml-1 and r is the correlation coefficient.

Results obtained by applying HPLC procedure showed that PE and ID can be simultaneously analyzed in the prepared mixtures with mean percentage recoveries of 99.89 ± 0.61 and 99.56 ± 0.72, respectively, TABLE 4. The proposed method has been applied to assay PE and ID in Bipreterax® tablets. The validity of the suggested procedure was further assessed by applying the standard addition technique, TABLE 8.

TABLE 8 : Application of standard addition technique to the analysis of PE and ID in bipreterax® tablets by HPLC method.

Dosage form	Found * %	Pure added $\mu g.ml^{-1}$	Found* $\mu g.ml^{-1}$	Recovery %
Perindopril in Bipreterax®Tablets (batch no.17298)	99.52±1.16	5.0	4.97	99.40
		10.0	9.95	99.50
		15.0	14.99	99.93
Mean ± S.D				99.61±0.28
Indapamide in Bipreterax®tablets (batch no.17298)	99.55±0.94	2.5	2.47	99.06
		5.0	5.02	100.30
		7.5	7.43	99.04
Mean ± S.D				99.46±0.72

*** Average of three determinations.**

A statistical comparison of the results obtained by the proposed methods and the official method for either pure PE[4] or pure ID[13] is shown in TABLE 9. The values of the calculated t and F are less than the tabulated ones, which reveals that there is no significant difference with respect to accuracy and precision between the proposed methods and the official ones.

The results of assay validation show that the methods are accurate, precise, specific and rugged as shown in TABLE 10.

TABLE 9 : Statistical analysis of the results obtained by applying the proposed method and the official one for the analysis of pure PE and ID.

Values	HPLC		Official methods	
	ID	PE	ID [13]	PE [4]
Mean	99.96	99.72	99.56	99.34
S.D.	1.17	0.63	0.72	0.65
N	6	6	6	6
Variance	1.37	0.40	0.52	0.42
t[2.23]*	0.71	1.03	…….	…….
F[5.05]*	2.63	1.05	…….	…….

***The figures in parenthesis are the corresponding tabulated values at P=0.05.**

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TABLE 10 : Validation of the results obtained by applying the proposed Methods.

Parameter	ID	PE
LOD (μg.ml^{-1})	0.67	0.85
LOQ (μg.ml^{-1})	2.03	2.58
Range (μg.ml^{-1})	2.5-15.0	5.0-40.0
Slope	80161	13864
Intercept	4663	691.8
Mean ± S.D.	99.96±1.17	99.72±0.63
Correlation Coefficient (r)	0.9990	0.9990
RSD% [a*]	0.38-0.70	0.57-0.84
RSD% [b*]	0.81-1.12	0.62-0.93

***[a,b] Intra-day and inter-day (n=3) relative standard deviations of samples of concentrations (15.0, 20.0 μg. ml^{-1}) and (5.0, 7.5 μg. ml^{-1}) of PE and ID, respectively.**

CONCLUSION

From the results obtained, it was observed that the developed method was proven to be precise, accurate, specific, linear and robust and is suitable for the routine analysis of PE and ID in pharmaceutical formulations.

REFERENCES

[1] J.O'Neil Maryadele; Merck Index, 14th Edition, Merck, Darmstadt, 1286 **(2006)**.

[2] J.O'Neil Maryadele, Merck Index, 14th Edition, Merck, Darmstadt, 887 **(2006)**.

[3] Goodman, Gilman's; The Pharmacological Basis of Therapeutics, 8th Edition, New York: McGraw Hill, 718-721 **(1992)**.

[4] British Pharmacopoeia; London: British Pharmacopoeial Commission Office, 1642 **(2010)**.

[5] United States Pharmacopoeia; 30th Edition, Rockville, MD: The United States Pharmacopeial Commission, Inc., 1418 **(2007)**.

[6] European Pharmacopoeia; 5th Edition, France: European Directorate for The Quality of Medicine Council of Europe, 2210-2211 **(2005)**.

[7] Indian Pharmacopoeia; 6th Edition, New Delhi: The Indian Pharmacopoeial Commission, 1489 **(2010)**.

[8] British Pharmacopoeia, London: British Pharmacopoeial Commission Office, 2791 **(2010)**.

[9] United States Pharmacopoeia; 30th Edition, Rockville, MD: The United States Pharmacopoeial Commission, Inc., 680-683 **(2007)**.

[10] H.Van Den Berg, G.Resplandy, A.T.H.J.De Bie, W.Floor, M.Bertrand, C.J.M.Arts; J.Pharm.Biomed.Anal., **9(7)**, 517-524 **(1991)**.

[11] H.E.Abdellatef; J.Pharm.Biomed.Anal., **17(8)**, 1267-1271 **(1998)**.

[12] N.Erk; J.Pharm.Biomed.Anal., **26(1)**, 43-52 **(2001)**.

[13] H.E.Abdellatef, M.M.Ayad, E.A.Taha, J.Pharm.Biomed.Anal., **18(6)**, 1021-1027 **(1999)**.

[14] A.Prameela Rani, C.Bala Sekaran; International Journal of PharmTech Research, **1(3)**, 575-578 **(2009)**.

[15] Ankit B.Chaudhary, Rakesh. K.Patel, Sunita A.Chaudhary; International Journal of Pharm-Tech Research, **2(2)**, 1141-1146 **(2010)**.

[16] Jogia; Khandelwal; Gandhi; Singh; Modi; J.Aoac, 108-115 **(2010)**.

[17] A.Gumieniczek, Hopkala; H.Chemical.Analysis., **43(6)**, 951-954 **(1998)**.

[18] J.F.Van Staden, R.I.Stefan, H.Aboul Enein, Y.Fresenius; Journal of Analytical Chemistry, **367(2)**, 178-180 **(2000)**.

[19] R.V.S.Nirogi, V.N.Kandikere, M.Shukla, K.Mudigonda, S.Maurya, P.Komarneni; Rapid Communications in Mass Spectrometry, **20(12)**, 1864-1870 **(2006)**.

[20] D.S.Jain, G.Subbaiah, M.Sanyal, U.C.Pande, P.Shrivastav, J.Chromatog; B.Anal.Technol.Biomed.Life Sciences, **837(1-2)**, 92-100 **(2006)**.

[21] S.J.Lin, H.L.Wu, S.H.Chen, Y.H.Wen; Analytical Letters, **29(10)**, 1751-1762 **(1996)**.

[22] C.Tsaconas, M.Devissaguet, Padieu, P.J.Biomed; J. chromatogr., **80(1-488)**, 249-265 **(1989)**.

[23] N.F.Youssef; .J.AOAC, **86(5)**, 935-940 **(2003)**.

[24] Savita S.Yadav, Janhavi R.Rao; Intern.J.Compreh.Pharm., **24(3)**, 413-9 **(2001)**.

[25] A.Radi; J.Chem.Anal., **48**, 273 **(2003)**.

[26] X.Gao, J.Chen, Ni Mei, W.Tao, W.Jiang, X.Jiang; Chromatographia, **61(11-12)**, 581-585 **(2003)**.

[27] I.Singhvi, G.Anj; Indian J.Pharm.Sci., **69(1)**, 164-165 **(2007)**.

[28] J.Joseph, B.Philip; Sundarapandian, Int.J.Pharm.Pharm.Sci., **3(4)**, 288-293 **(2011)**.

[29] United States Pharmacopoeia, 30th Edition; Rockville, MD, The United States Pharmacopeial Commission, 1006 **(2007)**.

[30] L.N.Linda; Reviewer Guidance-Validation of Chromatographic Methods, Center for Drug Evaluation and Research, 1-30 **(1994)**.

[31] ICH-Q2B; Validation of Analytical Procedures, Methodology, ICH Harmonized Tripartite Guide-