RESEARCH ARTICLE Am. J. PharmTech Res. 2013; 3(3) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Perindopril Erbumine and Indapamide in Combined Dosage Form

Shweta Bhadani[1]*, Mohan Sellappan[2]
1.Research Scholar, JJT University, Jhunjhunu, Rajasthan, India.
2.Saraswati Institute of Pharmaceutical Sciences, Dhanap, Ghandhinagar-382355, Gujarat, India.

ABSTRACT

The manuscript describes validated Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous estimation of Perindopril Erbumine and Indapamide in combined dosage form. The RP-HPLC separation was achieved on Intersil ODS C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) at 40^{o}C using mobile phase Acetonitrile : Acid Phthalate Buffer (pH 3.0 ± 0.05) (50 :50, v/v) at flow rate 1.0 mL/min. Quantification was achieved with photodiode array (PDA) detection at 240 nm over the concentration range of 10 - 50 µg/mL for Perindopril Erbumine and 3 - 15 µg/mL for Indapamide with mean recovery of 100.09 ± 0.52 % and 100.13 ± 0.09 % for Perindopril Erbumine and Indapamide, respectively. These methods were found to be simple, sensitive, accurate, precise and economical and applicable for the simultaneous determination of Perindopril Erbumine and Indapamide in combined dosage form.

Keywords: Perindopril Erbumine, Indapamide, RP-HPLC, Recovery.

*Corresponding Author Email: shwetapatel308@yahoo.co.in

Received 16 May 2013, Accepted 21 May 2013

Please cite this article in press as: Bhadani S. *et al.*, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Perindopril Erbumine and Indapamide in Combined Dosage Form. American Journal of PharmTech Research 2013.

INTRODUCTION

Perindopril Erbumine (PERI) is a long-acting ACE inhibitor and chemically it is tert-butylammonium(2S,3aS,7aS)-1-N-[(S)-1-ethoxycarbonylbutyl]–L-alanyl) perhydroindole -2-carboxylate (Figure 1).; $C_{19}H_{32}N_2O_5,C_4H_{11}N$.[1] Indapamide (INDA) is Indapamide is a non-thiazide sulphonamide diuretic drug and chemically it is 4-chloro-N(2-methyl-1-indolinyl)-3-sulfamoyl benzamide hemihydrates (Figure 1); $C_{16}H_{16}ClN_3O_3S$.[2]

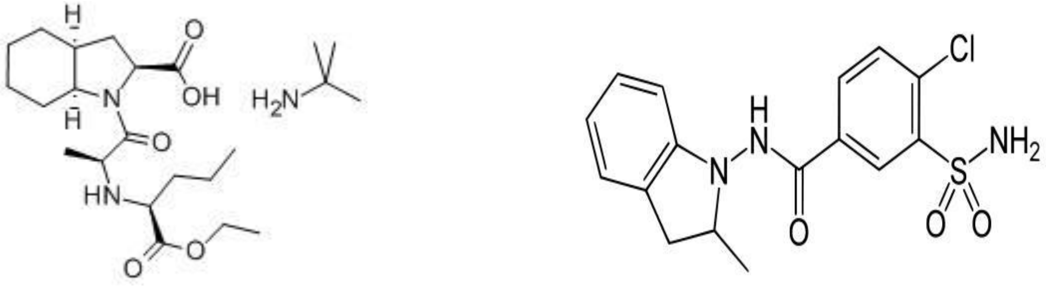

Figure 1: Structure of Perindopril Erbumine and Indapamide

PERI is official in BP[3] and INDA is official in BP[4], USP[5]. Literature survey reveals that various methods like HPLC[6-7], Flow injection analysis[8] are reported for the estimation of PERI. Literature survey also reveals various methods like Spectrophotometric[9-10], HPLC[11-12], LC-MS[13] are reported for estimation of Indapamide. The present manuscript describes simple, sensitive, accurate, precise, reproducible and economical first derivative Spectrophotometric method for the simultaneous estimation of PERI and INDA in combined dosage form. The proposed method was validated according to ICH guideline[14]. The method was found to be suitable for simultaneous and accurate determination of these drugs in tablet dosage forms without any interference from the excipients.

MATERIALS AND METHODS

Instrument & Apparatus

A HPLC instrument (LC-2010$C_{HT,}$ Shimadzu, Japan) equipped with a UV-Visible detector and a photodiode array detector, manual injector with 20 µL loop, Phenomenex C_{18} column (250 mm × 4.6 mm id, 5 µm particle size) and LC-solution software were used, Digital pH meter (pH 361, Systronics, Ahmedabad), Analytical balance (Sartorius balance).

Reagents and Materials

Perindopril Erbumine (PERI) and Indapamide (INDA) bulk powder was kindly gifted by Pharmaceutical Ltd, Gujarat (India), with 99.96% purity, The commercial fixed dose combination product containing 2 mg PERI and 0.625 mg INDA was procured from the local pharmacy, AR grade Methanol (S.D Fine Chemicals Ltd, Mumbai, India), HPLC grade

Acetonitrile (Finar Chemicals Ltd., Mumbai, India), HPLC grade Water (Finar Chemicals Ltd., Mumbai, India), AR grade perchloric acid (S.D Fine Chemicals Ltd, Mumbai, India), A nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India).

Chromatographic Condition

Stationary phase: Phenomenex C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) was used at 40^{o}C, Mobile Phase: Acetonitrile : Water (pH 2.8 ± 0.05) (30 :70, v/v), Flow rate: 1.0 mL/min, Detection: At 210 nm with PDA detector.

Preparation Of Solutions

Preparation of mobile phase:

All the solvents of mobile phase were filtered through nylon 0.45 µm - 47 mm membrane filter, degassed before use and were filled in neat and clean separate bottle.

Preparation of PERI and INDA mixed standard solution:

Accurately weighed PERI (32 mg) and INDA (10 mg) was transferred to a same 100 mL volumetric flask and dissolved and diluted to the mark with methanol to obtain a standard stock solutions having concentration PERI (320 µg/mL) and INDA (100 µg/mL).

Determination of analytical wavelength

The standard solution of PERI and INDA were injected under the chromatographic condition described above. Detection was carried out at different wavelength best response was achieved at 210 nm with PDA detector. So both drugs were detected at this analytical wavelength.

METHOD VALIDATION

Calibration curve (Linearity)

Calibration curves were constructed by plotting peak areas Vs concentrations of PERI and INDA, and the regression equations were calculated. Calibration curves were plotted over a concentration range of 6.4-32 µg/mL for PERI and 2-10 µg/mL for INDA. Accurately measured standard working solutions of PERI and INDA (0.2, 0.4, 0.6, 0.8 and 1.0 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µL) of each solution were injected under the operating chromatographic conditions described as above.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recovery of PERI and INDA by the standard addition method. Known amounts of standard solutions of PERI and INDA were added at 50, 100 and 150 % level to pre-quantified sample solutions of PERI and INDA (12.8 + 4.0

µg/mL). The amounts of PERI and INDA were estimated by using the regression equation of the calibration curve.

Method Precision (% Repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) standard solutions of PERI (19.2 µg/mL) and INDA (6 µg/mL) under the same chromatographic condition and measurements of peak area, retention time and tailing factor. Percentage relative standard deviation (RSD) should not be more than 2 %.

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of PERI (12.8, 19.2 and 25.6 µg/mL) and INDA (4, 6 and 8 µg/mL). The results were reported in terms of relative standard deviation (RSD).

Limit of Detection and Limit of Quantification

LOD and LOQ of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines.

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

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

Where, σ = the standard deviation of the response

S = Slope of calibration curve.

Robustness

The robustness of the method was established by introducing small changes in various parameters like mobile phase composition, pH of mobile phase and flow rate. For the same, mobile phases having different compositions, like acetonitrile-water (pH 2.80 ± 0.05) (28: 72, v/v), (32: 68, v/v) were tried and chromatograms were run. The changes made in flow rate and pH was ± 0.2 mL/min and ± 0.2 units, respectively. Robustness of the method was evaluated by calculating the % RSD values. Robustness of the method was done at different concentration levels.

Analysis of Perindopril Erbumine and Indapamide In Combined Dosage Form

The response of the sample solution was measured at 210 nm under the chromatographic condition mentioned above for the quantitation of PERI and INDA. The amounts of the PERI and INDA present in the sample solution were calculated by fitting the responses into the regression equation for PERI and INDA.

RESULTS AND DISCUSSION

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for PERI and INDA was obtained with a mobile phase Acetonitrile: Water (pH 2.8 ± 0.05) (30 :70, v/v) at a flow rate of 1.0 mL/min to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 210 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Figure 2). System suitability test parameters for PERI and INDA for the proposed method are reported in Table 1.

Table 1 System Suitability Parameters of Chromatogram for Perindopril Erbumine and Indapamide

Parameters	**PERI ± RSD[a] (n = 6)**	**INDA ± RSD (n = 6)**
Retention time (min)	3.23 ± 0.30	5.20 ± 0.79
Tailing factor	1.23 ± 0.98	1.13 ± 1.18
Theoretical plates	3199 ± 0.35	4182 ± 1.00
Resolution	6.68 ± 0.024	

[a] RSD = relative standard deviation.

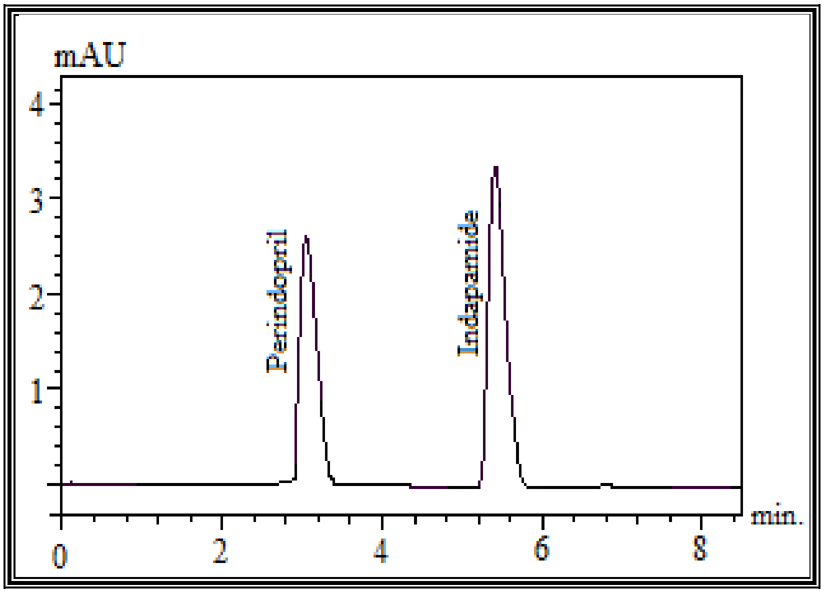

Figure 2: RP-HPLC Chromatogram of Perindopril erbumine and Indapamide

VALIDATION OF THE PROPOSED METHOD

Calibration curve (Linearity)

Linear correlation was obtained between peak areas versus concentrations in the range of 6.4-32 μg/mL for PERI and 2-10 μg/mL for INDA. Regression parameters are mentioned in Table 2.

Table 2: Regression Analysis Data and Summary of Validation Parameter for the Proposed Method

Parameters	PERI	INDA
Détection wavelength (nm)	210	210
Concentration range (µg/mL)	6.4 - 32	2 - 10
Slope	508.8	4075
Intercept	164.6	338.7
Correlation coefficient (r)	0.9995	0.9995
LOD[a] (µg/mL)	0.63	0.12
LOQ [b] (µg/mL)	1.90	0.35
% Recovery (Accuracy, n = 5)	100.04 ± 0.23	100.10 ± 0.29
Repeatability (RSD[c], n = 6), %	0.31	0.17
Precision (RSD), %	-	-
Interday (n = 6)	0.51 – 1.95	1.03 – 1.55
Intraday (n = 6)	0.34 – 1.79	0.13 – 1.07

[a] LOD = Limit of detection

[b] LOQ = Limit of quantification

[c] % RSD = Percent relative standard deviation.

Accuracy

The recovery experiment was performed by the standard addition method. The recoveries obtained were 100.04 ± 0.23 % and 100.10 ± 0.29 % for PERI and INDA, respectively. The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 3.

Table 3: Recovery Data for the Proposed Method

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (µg/mL)	Mean % Recovery ± SD*
PERI	50 %	12.8	6.4	100.07 ± 0.11
	100	12.8	12.8	100.25 ± 0.65
	150 %	12.8	19.2	99.79 ± 0.57
INDA	50%	4.0	2.0	100.04 ± 0.17
	100%	4.0	4.0	99.85 ± 0.16
	150%	4.0	6.0	100.42 ± 0.56

* Mean % Recovery ± SD of five observations.

Method Precision (% Repeatability)

The RSD values for PERI and INDA were found to be 0.31 % and 0.17 %, respectively (Table 2). The RSD values were found to be <2 %, which indicates that the proposed method is repeatable.

Intermediate Precision (Reproducibility)

The low RSD values of interday (0.51 – 1.95 % and 1.03 – 1.55 %) and intraday (0.34 – 1.79 %

and 0.13 – 1.07 %) variations for PERI and INDA, respectively, reveal that the proposed method is precise (Table 2).

LOD and LOQ

LOD values for PERI and INDA were found to be 0.63 and 0.12 µg/mL, respectively and LOQ values for PERI and INDA were found to be 1.90 µg/mL and 0.35 µg/mL, respectively (Table 2). These data show that the proposed method is sensitive for the determination of PERI and INDA.

Robustness

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2 %. The low value of % RSD indicates robustness of the proposed method.

Assay of the Pharmaceutical Formulation

The proposed validated method was successfully applied to determine PERI and INDA in their tablet dosage form. The result obtained for PERI and INDA was comparable with the corresponding labeled amounts (Table 4). The RP-HPLC chromatogram for PERI and INDA in sample was recorded and is shown in Figure 3.

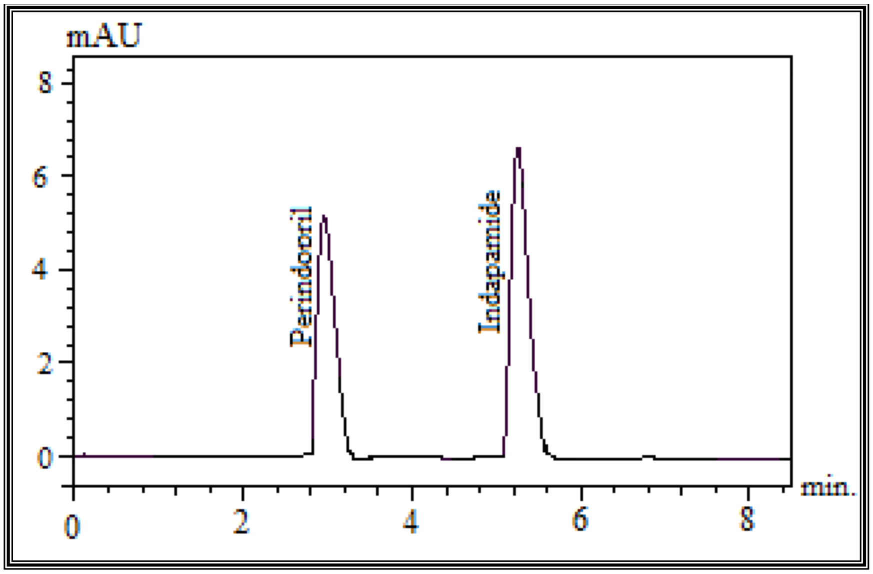

Figure 3 RP-HPLC Chromatogram of Sample Solution

Table 4 Assay Results for the Combined Dosage Form Using the Proposed Method

Sample	Label Claim (mg/tab)	Amount Found ± SD^a	% Label Claim ± SD^a(n =5)
PERI	2	1.99 ± 0.0078	99.84 ± 0.39
INDA	0.625	0.625 ± 0.0012	100.01 ± 0.20

^{a}SD = Standard deviation.

^{b}*n* = Number of determinations.

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 6.4-32 µg/mL for PERI and 2-10 µg/mL for INDA with co-efficient of correlation, (r) = 0.9995 and (r) = 0.9995 for PERI and INDA, respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the PERI and INDA in combined dosage form without any interference of the excipients.

REFERENCES

1. O'Neil MJ, Smith A, Heckelman PE, Kinneary JF. The Merk Index, an Encyclopedia of Chemicals, Drugs and Biologicals. 12th ed. Merck & Co. Inc., White House Station, New Jersey; 1996; 1234.
2. O'Neil MJ, Smith A, Heckelman PE, Kinneary JF. The Merk Index, an Encyclopedia of Chemicals, Drugs and Biologicals. 12th ed. Merck & Co. Inc., White House Station, New Jersey; 1996; 848.
3. British Pharmacopoeia. British Pharmacopoeial Commission office, London, U.K.; 2007; vol. II; 1609 – 11.
4. British Pharmacopoeia. British Pharmacopoeial Commission office, London, U.K.; 2007; vol. II; 1078, 2665.
5. The United States of Pharmacopoeia-30/ National Formulary-25. Asian ed. United States Pharmacopoeial Convention, Inc., Rockville MD; 2007; vol. II; 2340 - 41.
6. Harlikar JN, Amlani AM. Simultaneous Determination of Perindopril, Indapamide, Ramipril, Trandolapril in Pharmaceutical formulations using Reverse Phase Liquid Chromatography. Res J Chem Environ 2003;7 (4).
7. Chaudhary AB, Patel RK, Chaudhari SA. Determination of Losartan Potassium and Perindopril Erbumine in Tablet Formulations by Reversed-Phase HPLC. Int J ChemTech Res 2010;2(2):1141-6.
8. Stefan RI, Staden J, Mulaudzi L, Hassan Y. On-line simultaneous determination of *S*- and *R*-perindopril using amperometric biosensors as detectors in flow systems. Anal Chim Acta 2002;467:189–95.
9. Fernandesa N, Nimdeoa MS, Choudharia VP, Kulkarnia RR, Pandea VV, Nikaljeb AG. Dual wavelength and simultaneous equation spectrophotometric methods for estimation

of atenolol and indapamide in their combined dosage form. Int J Chem Sci 2008;6(1): 29-35.

10. Pawar PV, Gaikwad PD, Bankar VH, Pawar SP. Development and validation of UV-spectrophotometric method for simultaneous estimation of Atenolol and Indapamide in bulk and tablet dosage form. IJPT 2010;2(4): 876-85.
11. Legorburu MJ, Alonso RM, Jiménez RM, Ortiz E. Quantitative Determination of Indapamide in Pharmaceuticals and Urine by High-Performance Liquid Chromatography with Amperometric Detection. J Chromatogr Sci 1999;37:283-7.
12. Munot NM, Ghante MR, Deshpande AS, Gaikwad PV. Development and validation of RP-HPLC method for simultaneous estimation of Indapamide and Telmisartan. JPR, 2010;3(12):2941-3.
13. Chen WD, Liang Y, Zhang H, Li H, Xiong Y, Wang GJ. Simple, sensitive and rapid LC-MS method for the quantitation of indapamide in human plasma-- application to pharmacokinetic studies. J Chromatogr B Analyt Technol Biomed Life Sci 2006;842(1):58-63.
14. International Conference on Harmonization (ICH), Topic Q2R1, Validation of Analytical Methods: Methodology, Yokohama, Japan. 2005.