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Stability Indicating RP-HPLC Method For Simultaneous Determination of Perindopril and Indapamide In Pharmaceutical Dosage Form

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ABSTRACT

A simple, fast and precise reverse phase, isocratic HPLC method was developed for the separation and quantification of perindopril and indapamide in pharmaceutical dosage form. The quantification was carried out using YMC Column (150 x 4.6mm, 3µ particle size) and mobile phase comprised of ammonium dihydrogen phosphate pH 2.5 and acetonitrile in the ratio of 60:40% v/v and degassed under ultrasonication. The flow rate was 1.0 mL/min and the effluent was monitored at 230 nm. The retention time of perindopril and indapamide were 2.4 and 4.2 min respectively. The method was validated in terms of linearity, precision, accuracy and specificity. Linearity of perindopril and indapamide were in the range of 48 to 112 µg/mL and 15 to 35 µg/mL respectively. The proposed method is suitable for simultaneous determination of perindopril and indapamide in pharmaceutical dosage form.

Key words: Perindopril and Indapamide, RP-HPLC, Validation.

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INTRODUCTION

Perindopril Erbumine (PDL) 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[1]. Indapamide (IPD) is a non-thiazide sulphonamide diuretic drug and chemically it is 4-chloro-N(2-methyl-1-indolinyl)-3-sulfamoyl benzamide hemihydrates[2]. Literature survey revealed few HPLC[3-10] analytical methods for estimation of PDL and few HPLC[11-16] analytical methods IPD in combined dosage form with other drugs. Being there is no stability indicating HPLC method reported for the simultaneous estimation of two drugs PDL and IPD in a single dosage form. Hence an attempt has been made to develop a simple, precise, reliable, sensitive and selective stability indicating HPLC method for the analysis of PDL and IPD in pure samples and in combined tablet dosage form. The proposed method was validated according to ICH guidelines.

MATERIALS AND METHOD

Materials, reagents and chemicals

PDL and IPD combined dosage form tablets were purchased from local market. HPLC grade acetonitrile, methanol and analytical grade ammonium dihydrogen phosphate, was obtained from Qualigens Fine Chemicals Ltd, Mumbai. Hydrochloric acid, sodium hydroxide, hydrogen peroxide of analytical grade was obtained from Merck Chemicals Ltd, Mumbai. Milli-Q water was used throughout the experiment filtered through 0.22µ filter membrane.

Chromatographic conditions

HPLC Shimadzu Separation Module LC-20AT Prominence liquid chromatograph and UV detector was used. The output of signal was monitored and integrated using LC solutions software. YMC Column (150 × 4.6mm, 3µ particle size) was used as stationery phase. Mobile phase comprised of ammonium dihydrogen phosphate and acetonitrile in proportion of ratio 60:40% v/v. The mobile phase was mixed, filtered through 0.22µ membrane filter and degassed under ultrasonication. The mobile phase was used as diluent. Injection volume was 20µL and flow rate was 1 mL/min and run time was 6 min. The column was maintained at ambient temperature and the eluent was monitored at 230 nm.

Preparation of standard solution

Accurately weigh and transfer 50 mg of IPD and 160mg of PDL working standard into a 100 mL clean dry volumetric flask add about 50 mL of methanol and sonicate to dissolve it completely, cool the solution to room temperature and dilute to volume with methanol and used as standard

stock solution. Pipette 10 mL of standard stock solution into a 100 mL volumetric flask and dilute to volume with methanol and used as working standard solution. Working standard solution was diluted in mobile phase to contain a mixture of IPD and PDL in over the linearity range from 15 to 35 µg/mL and 48 to 112 µg/mL respectively.

Preparation of sample solution

Twenty tablets were weighed and average weight was calculated, powdered well and powder equivalent to 8 mg of PDL which contains 2.5 mg of IPD was transferred in to a clean standard flask and by using methanol drugs were extracted and filtered through 0.22µ filter. The above filtered sample solution was diluted in 10mL volumetric flask and 20µL of the sample solution was injected in to the HPLC system and peak area was noted.

RESULTS AND DISCUSSION

Method development

To develop a simple and robust method for the simultaneous determination of PDL and IPD in combined tablet dosage form using HPLC. Solubility of standard drug was checked and methanol was chosen as the solvent. Different mobile phase compositions were pumped in to achieve the resolution of drug peaks, initial experimental conditions were column with C18 stationery phase, ammonium dihydrogen phosphate as buffer, acetonitrile as organic solvent at a flow rate of 1.0 mL/min was chosen and respective injections shown considerable resolution of drug peaks with a run time of 6 min. For better resolution the mobile phase composition was altered slightly between buffer and organic solvent acetonitrile, finally a premixed composition (60:40, v/v) of buffer and acetonitrile, pH of ammonium dihydrogen phosphate buffer adjusted to 2.5 with orthophosphoric acid was chosen as mobile phase, and the YMC Column stationery phase of particle size 3µm, 4.6×150mm was used and the runtime of the method was got minimized to 6min with better resolution, better peak shape was found with mobile phase as diluent in samples injected into chromatographic system. Injections with UV detection at a wavelength of 230nm for both drug peaks in the trail results were observed to be specific, precise and fast. The system suitability results of the method are presented in Table 1.

VALIDATION OF THE PROPOSED METHOD

Specificity

A study conducted to establish specificity of the proposed method involved comparing the chromatograms produced by sample and standard using the chromatographic conditions defined for the proposed method. The sample chromatogram showed no interference peaks at the

retention time of PDL and IPD. This indicates that diluent solution used in sample preparation do not interfere in the estimation of PDL and IPD. The chromatogram of the blank, standard and sample using the proposed method for PDL and IPD is shown in Figure. 1,2 and Figure. 3.

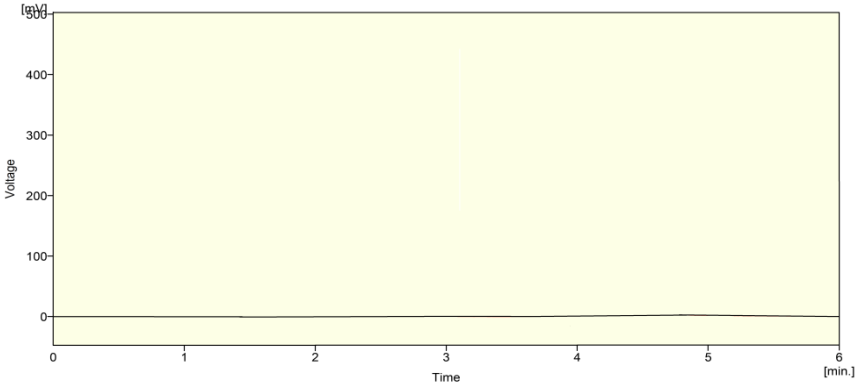

Figure. 1: Typical chromatogram showing no interference of blank for PDL and IPD.

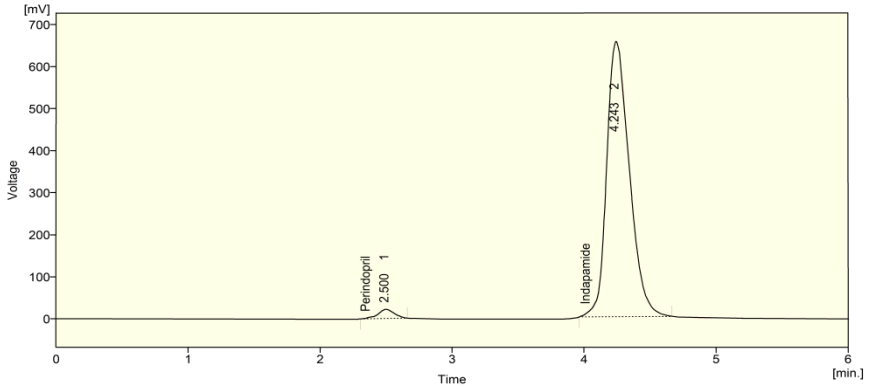

Figure. 2: Typical chromatogram of standard showing PDL and IPD.

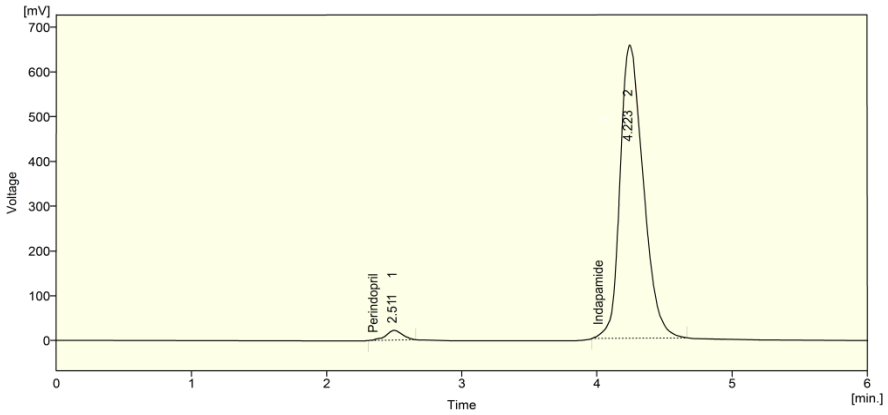

Figure. 3: Typical chromatogram of sample showing PDL and IPD.

Table 1: System suitability parameters for PDL and IPD by proposed method

Parameters	PDL	IPD
Theoretical Plates	3056	2978
Retention Time (min)	4.2	2.4
Asymmetry	1.6	1.5

Linearity

Detector response for the method determined to be linear over the concentration range of 48 to 112 µg/mL for IPD and 15 to 35 µg/mL for PDL. The calibration curve was plotted as concentration of the respective drug versus the obtained peak area at each concentration level. The linearity of the method was evaluated by linear regression analysis. The Linearity study for PDL and IPD by proposed method is given in Table 2.

Table 2. Linearity study for PDL and IPD by proposed method

S. no.	IPD		PDL	
	Concentration (µg/ml)	Peak Area	Concentration (µg/ml)	Peak Area
1	15	4220.62	48	103.17
2	20	6287.143	64	152.347
3	25	7931.778	80	198.415
4	30	9683.107	96	240.912
5	35	11535.85	112	293.54

Accuracy

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on blend collected from twenty tablets of PDL and IPD and analyzed as per the proposed method. From the data obtained, the proposed method found to be accurate. The results are summarized in Table 3.

Precision

The precision of the developed method was studied by closeness of the results obtained by following the standard procedure. For the intermediate precision, a study carried out by the different analyst working on different day. The results are showing that the proposed analytical technique has a good intermediate precision. Results are summarized in Table 3.

Table 3: Recovery studies and precision for PDL and IPD by proposed method

Drug	Labelled amount (mg/ tablet)	(%) label claim* ± S.D	% Recovery	Precision (% RSD) (n=6)
PDL	4	3.98	99.77 to 99.96	1.281
IPD	1.25	1.246	98.48 to 99.90	0.6210

Stability studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24h at room temperature. The results show that for both solutions, the retention time and peak area of PDL and IPD remained almost similar and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24h, which was sufficient to complete the whole analytical process. Further forced

degradation studies were conducted indicating the stability of proposed method. The results of the degradation studies are presented in Table 4.

Table 4: Forced degradation study results for PDL and IPD by proposed method

Stress Conditions	Degradation Time (h)	PDL			IPD		
		Peak Area	% Degradation	% Active drug remained	Peak Area	% Degradation	% Active drug remained
Standard Drug		198.42			7931.78		
Acid	1	195	1.72	98.28	7930	0.02	99.98
Base	1	191	2.23	96.26	7823	1.37	98.63
Peroxide	1	193	1.72	97.27	7901	0.39	99.61
Thermal	48	189	3.74	95.25	7832	1.26	98.74

Acid degradation sample

Twenty tablets were weighed and average weight was calculated, powdered well and powder equivalent to 8 mg of PDL which contains 2.5 mg of IPD was transferred in to a clean standard flask and by using methanol drugs were extracted and filtered through 0.45µ filter. Then add 10mL of 5N acid (Hydrochloric acid), refluxed for 60minutes at 60°C, then cooled to room temperature, neutralize with 5N base (Sodium hydroxide) and dilute to volume with methanol. Filter about 5mL of the above sample solution through 0.45µ membrane filter and further diluted by using diluent and analysed by injecting 20µL in to the HPLC system.

Base degradation sample

To the sample solution 10mL of 5N base (Sodium hydroxide) was added, refluxed for 60 minutes at 60°C, then cooled to room temperature, neutralized with 5N acid (Hydrochloric acid) and diluted to volume with methanol. Further diluted by using diluent and analysed by injecting 20µL in to the HPLC system.

Peroxide degradation sample

To the sample solution 2mL of 30% peroxide was added, refluxed for 60minutes at 60°C, then cooled to room temperature and diluted to volume with methanol. Further diluted by using diluent and analyzed by injecting 20µL in to the HPLC system.

Thermal degradation sample

Weigh and finely powder not fewer than 20 tablets, this powder is exposed to heat at 105°C for about 2 days. Accurately weigh and transfer a quantity of powder equivalent to 8 mg of PDL which contains 2.5 mg of IPD was transferred in to a clean standard flask and by using methanol drugs were extracted and filtered through 0.45µ filter. The above filtered sample solution was

diluted in 10mL volumetric flask and 20µL of the sample solution was injected in to the HPLC system and peak area was noted. Similarly UV-light exposure, sunlight exposure and water hydrolysis stress samples are prepared and checked for their purity by proposed method. From the above data of degradation profile it can be conclude that there is no interference found for IPD and PDL peak.

CONCLUSION

Thus the proposed stability indicating RPHPLC method for the simultaneous determination of PDL and IPD in tablet dosage form was accurate, precise, linear, reliable, simple, economic and robust. The method has several advantages, including simple mobile phase, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The method can be used for routine analysis of marketed products of PDL and IPD in combined tablet formulation.

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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. Nagalakshmi C, Prameela Rani A, Bhawani S and Bala Sekaran C. Determination of Angiotensin-Converting Enzyme Inhibitor, Perindopril Erbumine, in Bulk and Tablet Dosage Form with HPLC. American Chemical Science Journal 2012; 2: 161-176.
4. Bhadani S, Sellappan M, 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; 3: 703-711.
5. Rajesh T, Anurekha J, Deepika M, Toppo E, Multicriteria optimization methodology in development of HPLC method for simultaneous estimation of indapamide and perindopril in bulk drug and its combined dosage form, Asian Journal of Pharmaceutical and Clinical Research 2012; 5: 50-53.

6. Joseph J, Philip B, Sundarapandian M, Method development and validation for simultaneous estimation of perindopril erbumine and indapamide by RP-HPLC in pharmaceutical dosage forms, International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 3: 288-293.
7. Patel AI, Oza CK, Prajapati JP, Vyas AJ and Mehta P, RP-HPLC method for the determination of losartan potassium and perindopril erbumine in combined tablet dosage form. International Journal of Pharma and Bio Sciences 2011; 2: 709-715.
8. Ali NW, Abdelwahab NS, Zaki MM and Abdelkawy M, Validated chromatographic methods for simultaneous determination of amlodipine besylate and perindopril arginine in binary mixtures and in pharmaceutical dosage form, Chromat Separation Techniq 2012; 3: 1-5.
9. Riyaz SMD, Vasanth PM, Ramesh M, Ramesh R, Ramesh T, A sensitive RP-HPLC method development and validation for the simultaneous estimation of perindopril erbumine and amlodipine besilate in tablet dosage form, International Journal of Chemical and Life sciences 2012; 1 : 1033-1038.
10. Sahoo S, Panda PK, Mishra SK, Sahoo S, HPLC method development for simultaneous estimation of hydrochlorothiazide and perindropril in tablet dosage form, Asian Journal of Pharmaceutical and Clinical Research 2012; 5: 136-138.
11. Thulasamma P and Venkateswarlu P, RP-HPLC method for simultaneous determination of atenolol and indapamide in pharmaceutical dosage forms, human blood and milk, European Journal of Chemistry 2012; 3: 138-142.
12. Tulja Rani G, Gowri Sankar D, Kadgapathi P and Satyanarayana B, A validated RP-HPLC method for simultaneous estimation of atenolol and indapamide in pharmaceutical formulations, E-Journal of Chemistry 2011; 8: 1238-1245.
13. Karunakar N, Puranik SB, Kumar GVS, Masood MD, Sridhar KA, development and validation of RP-HPLC method for simultaneous estimation of amlodipine and indapamide in bulk and tablet dosage form, Asian Journal of Biomedical and Pharmaceutical Sciences 2012; 2: 55-59.
14. Sheth A, Patel KN, Badmanaban R, Doshi N, Shah N, Simultaneous estimation of olmesartan medoxomil and indapamide from bulk and commercial products using a validated reverse phase high performance liquid chromatographic technique, Chronicles of Young Scientists 2013;4:69-73.

15. Bhadani S, Sellappan M, 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; 3: 703-711.
16. Patel DB, Mehta FA, Bhatt KK, Simultaneous estimation of amlodipine besylate and indapamide in a pharmaceutical formulation by a high performance liquid chromatographic (RP-HPLC) method, Scientia Pharmaceutica 2012; 80: 581–590.