STABILITY INDICATING LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF RELATED SUBSTANCES OF PERINDOPRIL ERBUMINE AND INDAPAMIDE FROM COMBINED TABLET DOSAGE FORM

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Abstract:

A stability indicating RP-HPLC method for the determination of related substances of perindopril erbumine (PDE) and indapamide (IND) from combined tablet dosage form was developed. The separation was accomplished on Inertsil ODS 3V (4.6 mm X 250 mm; particle size 5 µm) column using a mobile phase consisting of HPLC grade water (pH adjusted to 2.50±0.05 with 1:1 solution of perchloric acid) and acetonitrile in gradient elution mode. The analytes were monitored by a photo diode array (PDA) detector set at 215 nm and the flow rate was kept at 1.0 mL min^{-1}. The method was validated as per ICH guidelines for specificity, accuracy, linearity, precision, limit of detection and limit of quantitation and robustness. The method could be used for the detection of its related substances in bulk and pharmaceutical formulations of PDE and IND.

Key words – Perindopril erbumine, Indapamide, RP-HPLC, stability-indicating, validation.

Introduction

Perindopril erbumine (PDE) chemically, (2S,3∝S,7∝S)-1-[(S)-N-[(S)-1-Carboxy-butyl]alanyl]hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, compound with tert-butylamine (1:1). It is used for the treatment of patients with essential hypertension and for treatment of patients with stable coronary artery disease to reduce the risk of cardiovascular mortality or nonfatal myocardial infarction. PDE can be used with conventional treatment for management of coronary artery disease, such as antiplatelet, antihypertensive or lipid-lowering therapy. PDE may be used alone or given with other classes of antihypertensives, especially thiazide diuretics. It is available as 2, 4 & 8 mg tablets under the brand name Aceon®, marketed by Abbott Laboratories, USA[1].

Indapamide (IND) chemically, 1-(4-chloro-3-sulfamoylbenzamido)-2-methylindoline, used as anti-hypertensive/diuretic[2]. It is soluble in aqueous solutions of strong bases. It is available in 2.5 mg (Immediate release) and 1.5 mg (Slow release) tablets under the brand name Natrilix, and it is being marketed by Servier laboratories Ltd in United kingdom[3]. Les Laboratoires Servier market PDE in combination with IND in a single dosage form under the brand name Bipreterax. In combination these are available in 10/2.5mg and 5/1.25mg of PDE and IND respectively [4, 5]. The chemical structures of PDE, IND and their impurities are presented in Fig.1.

Perindopril active pharmaceutical ingredient (API) is official in British Pharmacopoeia[6]; indapamide active pharmaceutical ingredient (API) is official in British Pharmacopoeia[7] and United States Pharmacopoeia[8],

while indapamide tablets are official in British Pharmacopoeia[9] and United States Pharmacopoeia[10]. However, the combination is not official in any pharmacopoeia.

A thorough literature survey has revealed few spectrophotmetric and chromatographic methods for the simultaneous determination of PDE and IND in pharmaceutical dosage forms and biological fluids[11-19]. To the best of our knowledge, a complete validated RP-HPLC method for the simultaneous separation of PDE, IND and their related substances in pharmaceuticals was not reported. Therefore, it was thought worthwhile to develop a simple, precise, accurate reverse phase high performance liquid chromatographic method for the determination of related substances of PDE, IND in combined tablet dosage form.

EXPERIMENTAL

Chemicals & Reagents

All the reagents were of analytical-reagent grade unless stated otherwise. Milli-Q-water was used throughout the experiment. HPLC-grade acetonitrile (J.T.Baker) and perchloric acid (Merck) were used. All the solvents and solutions were filtered through a membrane filter and degassed before use.

Working standards of PDE, IND and their impurities and combined tablet dosage forms were received from the research development department of Cadila Health care Ltd, Ahmedabad, India.

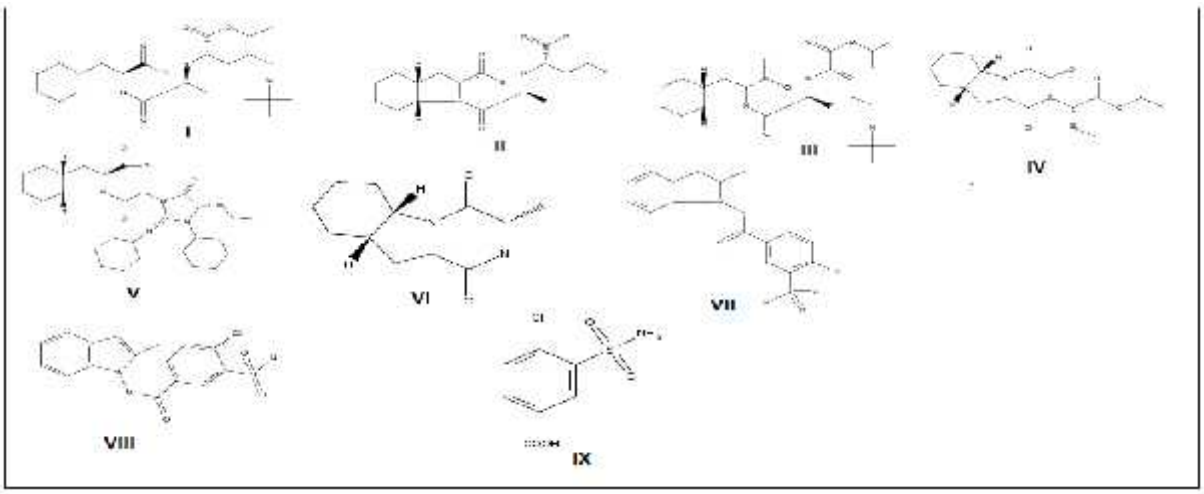

Fig.1: Chemical structures of PDE, IND and their impurities. [I. Perindopril erbumine; II. Perindopril Related compound B; III. Perindopril Related compound E; IV. Perindopril Related compound F; V. Perindopril Related compound H; VI. Perindopril Related compound K; VII. Indapamide; VIII. Dehydroindapamide(DI); IX. 4-chloro-3-sulfamoylbenzoic acid (CSBA).]

Instrumentation

The HPLC system used was of model Agilent 1200 series equipped with quaternary pump, auto sampler, thermostated column compartment and variable wavelength detector controlled by the chemstation software. The column used was Inertsil ODS 3V (250 mm X 4.6 mm, 5µm). Column temperature was maintained at 50^0C.

Chromatographic conditions

The analysis was carried out on Inertsil ODS 3V (250 mm X 4.6 mm, 5µm) column. The solvent A consisting of Milli Q water (pH adjusted to 2.50±0.05 with 1:1 solution of perchloric acid, prepared by mixing equal volumes of 70% perchloric acid and water) and solvent B of Acetonitrile (ACN) were pumped at a flow rate of 1.0 mL/min according to the linear gradient program: 0 min 10% B; 18 min 22% B; 45 min

25% B; 65 min 30% B; 90 min 70% B; 95 min 70% B; 97 min 10% B; 105 10% B. Before delivering the mobile phase into the system, it was degassed and filtered through 0.45 µm PTFE filter using vacuum. The injection volume was 20 µL and the detection was performed at 215 nm using a photo diode array (PDA) detector.

Analytical Procedures

Solutions (1.0 mg/mL) of PER and IND and their impurities (0.5 mg/mL) were prepared by dissolving known amounts of the components in diluent solvent A: solvent B (50:50 v/v). The solutions were adequately diluted to determine the accuracy, precision, linearity, and LODs and LOQs. Working solutions of 80 µg/mL of PER and 25 µg/mL of IND were prepared from the above stock solutions. Fresh solutions were prepared every day prior to use.

Sample Preparation

Twenty tablets of combined dosage form of PDE and IND were accurately weighed and grounded to fine powder. An amount equivalent to 96 mg of PDE was transferred into a 50 mL volumetric flask and about 30 mL of diluent was added, sonicated for not less than 30 min with occasional stirring and made up the volume with diluent. The above solution was filtered through 0.45 µm millipore PVDF filter.

Method Validation

The method was validated as per ICH guidelines[20] for specificity, accuracy, linearity, precision, limit of detection and limit of quantitation and robustness.

RESULTS AND DISCUSSION

Method Development

The main objective of the chromatographic method is to achieve the separation of perindopril erbumine impurities from PDE, Indapamide impurities from Indapamide and also major degradation products formed under varied stress conditions. In our preliminary experiments, PDE, IND and their impurities were subjected to separation by RP-HPLC, on different commercial C18 columns: (i) Symmetry C18 (250 mmX4.6 mm) 5 µm (Waters, USA), (ii) Kromasil KR100-C18 (250 mmX4.6 mm) 5 µm (Eka Chemicals AB, Sweden), and (iii) Inertsil ODS 3V (250 mmX4.6 mm) 5 µm(GL Sciences, Japan). The compounds were well separated with good peak shapes (minimum tailing) allowing accurate determination of all the compounds when Inertsil ODS 3V was used. So it was chosen for further development. Water with ACN and methanol as organic modifiers was used as mobile phase. Broad peaks and tailing were observed for PDE, IND and their impurities. So to improve the peak shapes, the pH of the water was adjusted to 2.50±0.05 with 1:1 solution of perchloric acid in water. As the compounds were having varying polarities, a gradient method was tried. The separation of PER, IND and their impurities was shown in Fig 2.

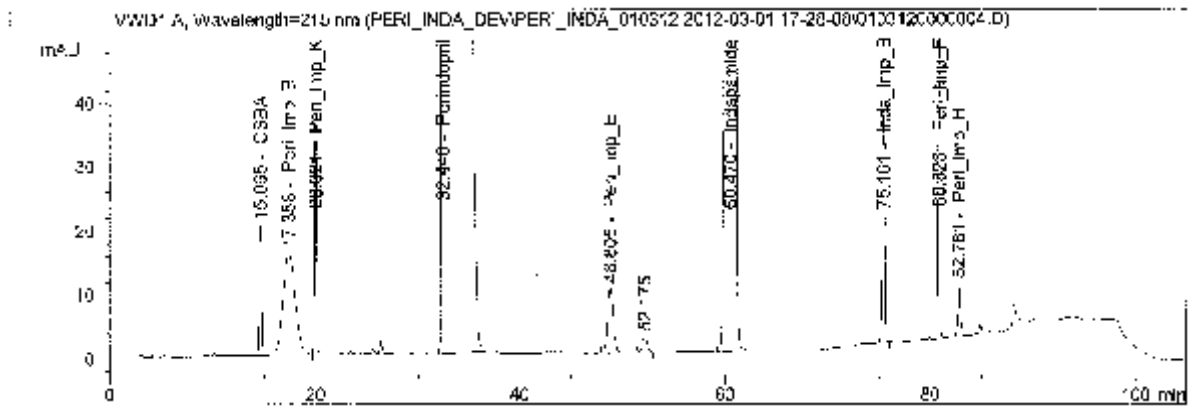

Fig 2: Chromatogram of PER, IND spiked with their impurities

Method Validation

The developed RP-HPLC method was validated in terms of specificity, accuracy, linearity, precision, limit of detection and limit of quantitation and robustness.

System Suitability

The system suitability was conducted using 0.2 % w/w of all the impurities spiked to PER and IND standard solution and evaluated by making five replicate injections. Tailing factor and resolution were calculated. Good resolution was observed between all the analytes and it is not less than 2.72 (Table 1).

Table 1: System suitability results

Sample	RT[a)]±SD	RRT	Rs	As	%RSD of peak area
CSBA	15.06±0.13	0.46[#]	-	1.01	0.6
Peri_imp B	17.36±0.17	0.52[#]	2.72	1.08	0.7
Peri_imp K	20.02±0.15	0.62[#]	3.15	1.02	0.6
PER	32.44±0.23	1.00[#]	33.8	1.18	0.4
Peri_imp E	48.80±0.18	0.82[$]	45.2	1.08	0.8
IND	60.47± 0.35	1.00[$]	33.2	1.11	0.3
Inda_impB	75.16± 0.24	1.24[$]	37.4	1.04	0.9
Peri_imp F	80.82± 0.12	1.41[$]	12.5	1.03	0.6
Peri_imp H	82.78± 0.13	1.43[$]	3.84	1.02	0.8

RT: Retention time; RRT: relative retention time; Rs: Resolution; As: tailing factor; SD: standard deviation; [a)] Average of three determinations; [#]Calculated against PER; [$]Calculated against IND.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity was demonstrated by studying the interference from placebo and degradants. Interference of placebo with analyte and known impurities was studied by injecting placebo and sample solution spiked with impurities. The impurity peaks were well separated and there were no co-

elution of these impurity peaks with that of analyte as well as placebo peaks. The peak purity of PER. IND and known individual impurities were found to be more than 996.

To evaluate the interference from degradants, a forced degradation study was carried out by stressing APIs, placebo and formulation under acid, alkali, peroxide, thermal, UV and moisture. The stress conditions employed for degradation study of PDE and IND include light exposure, dry heat (105°C), acid hydrolysis, base hydrolysis , water hydrolysis, oxidation (3% H2O2) and moisture (2-3 drops of water was added to the tablets and kept at 40^0C/75% RH for 4 days). For light studies, the monitoring period was 10 days whereas for heat, acid, base and water hydrolysis it was 48 h. Oxidation was carried out for 24 h. All the stressed samples were chromatographed into HPLC system equipped with diode array detector. The peak purity of analyte was evaluated. The peak purity of analyte peaks found to be more than 990 under each of the stress condition. All the major degradants were separated from the analyte, impurity and placebo peaks. The results obtained were shown in Table 2.

Table 2: Forced degradation Data

Impurity	% of impurity	Peak Purity	
		PDE	IND
Acid hydrolysis			
Indapamide Impurity-B	0.16	1000	997
CSBA	1.1		
Perindopril Impurity-B	6.7		
Perindopril Impurity-E	0.08		
Maximum individual unknown impurity	0.45		
Total impurities	9.4		
Alkali hydrolysis			
Indapamide Impurity-B	0.09	1000	996
CSBA	0.03		
Perindopril Impurity-B	0.88		
Perindopril Impurity-E	0.07		
Maximum individual unknown impurity	0.20		
Total impurities	1.8		
Peroxide oxidation			
Indapamide Impurity-B	0.28	1000	995
CSBA	0.07		
Perindopril Impurity-B	0.05		
Perindopril Impurity-E	0.06		
Maximum individual unknown impurity	8.3		
Total impurities	11.5		
Thermal degradation			
Indapamide Impurity-B	0.11	1000	995
CSBA	BQL		

Perindopril Impurity-B	0.22		
Perindopril Impurity-E	0.07		
Perindopril Impurity-K	BQL		
Perindopril Impurity-F	2.8		
Maximum individual unknown impurity	0.15		
Total impurities	3.75		
Humidity degradation			
Indapamide Impurity-B	0.05	1000	994
Perindopril Impurity-B	0.18		
Perindopril Impurity-E	0.07		
Perindopril Impurity-F	0.15		
Maximum individual unknown impurity	0.06		
Total impurities	0.60		
UV light degradation			
Indapamide Impurity-B	0.13	1000	994
CSBA	0.03		
Perindopril Impurity-F	BQL		
Perindopril Impurity-E	0.06		
Maximum individual unknown impurity	0.07		
Total impurities	0.47		

Accuracy

The accuracy of the assay method was demonstrated by preparing recovery samples at the level of 50 %, 100 % and 150 % of target concentration. The recovery samples were prepared in triplicate in each level. The accuracy of the related substance method was demonstrated by preparing recovery samples (i.e. spiking sample with known quantities of related impurities) at the level of LOQ, 50 %, 100 % and 150 %. The recovery samples were prepared in triplicate at each level. The above samples were chromatographed and the percentage recovery for the amount added was estimated. The results were shown in Table 3.

Table 3: Accuracy Data

Amount added	**% Recovery (Average of three determinations)**								
	PER	**IND**	**CSBA**	**Peri_ imp B**	**Peri_ imp K**	**Peri_ imp E**	**Inda_ impB**	**Peri_ imp F**	**Peri_ imp H**
LOQ	-	-	101.6	101.9	101.6	102.8	103.9	102.2	102.4
50%	100.5	99.2	101.1	101.5	99.5	98.6	97.5	101.6	101.6
100%	100.4	101.4	100.3	95.6	96.6	99.2	100.2	99.2	98.2
150%	100.8	100.4	101.5	96.2	97.8	100.5	100.8	101.8	99.5

Precision

Method precision for assay was demonstrated carrying out six independent assays of test sample and %RSD of six assays was found to be 0.58 and 0.41 for PER and IND respectively. **(Table 4A)**.

Table 4A: Precision data for assay method

Sample	% Assay PER	% Assay PED
1	101	100
2	99.2	99.5
3	99.4	99.1
4	100	99.9
5	98.9	99.1
6	99.5	99.2
Average	99.6	99.5
%RSD	0.58	0.41

Method precision of the related substances method was demonstrated by preparing six samples as per the test method, in which the known impurities (CSBA, Peri_imp B, Peri_imp K, Peri_imp E, Inda_imp B, Peri_imp F and Peri_imp H) were spiked at 0.2 % level. The impurities were quantified for each of these samples. The precision of the method was evaluated by computing the %RSD for the content of each of individual impurity. The % RSD was found to be below 1.21 for all the impurities. The results were shown in **Table 4B.**

Table 4B : **Precision data of Related substance method**

Sample	CSBA	Peri_impB	Peri_impK	Peri_impE	Inda_impB	Peri_impF	Peri_impH
1	0.198	0.205	0.201	0.195	0.231	0.199	0.192
2	0.197	0.207	0.199	0.196	0.225	0.201	0.197
3	0.193	0.204	0.198	0.198	0.224	0.201	0.196
4	0.197	0.207	0.2	0.197	0.228	0.199	0.197
5	0.198	0.209	0.198	0.194	0.225	0.204	0.194
6	0.194	0.21	0.204	0.192	0.227	0.197	0.194
Mean	0.196	0.207	0.2	0.195	0.227	0.2	0.195
%RSD	1.09	1.10	1.14	1.11	1.14	1.20	1.02

Linearity

Linearity for the related substances method was obtained over the calibration ranges tested *i.e*. LOQ, 40%, 80%, 100%, 120% and 150% of the specification limit. Similarly, the linearity of PER and IND was studied by preparing standard solutions over the range of 25 % to 150 % of target concentration. These solutions were injected into the system and the data were subjected to statistical analysis using a linear regression model, correlation coefficients for PER, IND and all the impurities were greater than 0.9997.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LODs and LOQs represent the concentration of the analyte that would yield S/Ns of 3 and 10 for LOD and for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by injecting blank samples and calculating the S/N for each compound by injecting a series of solutions until the S/N ratio of 3 for LOD and 10 for LOQ. The results are given in **Table 5.**

Table 5: LOD and LOQ results for Impurities

S.No	Sample	LOD(µg/mL)	LOQ(µg/mL)
1	CSBA	0.115	0.320
2	Peri_imp B	0.265	0.780
3	Peri_imp K	0.255	0.610
4	Peri_imp E	0.220	0.560
5	Inda_impB	0.125	0.310
6	Peri_imp F	0.245	0.480
7	Peri_imp H	0.230	0.460

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered. The flow rate was altered by 0.2 units, column temperature was altered by 5 units, organic phase ratio of mobile phase was altered by 2 units and pH was altered by 0.2 units. When the chromatographic conditions flow rate, mobile phase composition, pH were deliberately varied and the resolution between any two peaks is greater than 2.0 and tailing factor of any peak is not more than 1.2.

Stability of analytical solution

The solution state stability in the assay method was demonstrated by leaving the test solutions of sample at vial thermostat temperature (i.e., 5°C) for 2 days. The solution thus prepared was chromatographed for every 12 hours interval up to 48 hours. The % RSD of assay of PER and IND during the said period was within 1%.

The solution stability of in the related substance method was demonstrated by leaving sample solution spiked with 0.2 % of known impurities at vial thermostat temperature (i.e., 5°C) for 2 days. Content of impurities were checked for every 12 hours interval up to 48 hours. No significant change was observed in the impurity content during the said period. Hence the solutions were stable for at least 48 hours in the developed method.

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Conclusion

A new, accurate and selective gradient RP-HPLC method was proposed for the simultaneous determination of PDE, IND and their impurities in combined dosage form and validated as per the ICH guidelines. The method was found to be simple, selective, precise, accurate and robust. Therefore, this method can be used as routine testing as well as stability analysis of PDE and IND in synthetic mixtures and combined dosage form. All statistical results were within the acceptance criteria.

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