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Development and validation of stability indicating RP-HPLC method for estimation of Perindopril Erbumine and Indapamide in bulk and pharmaceutical dosage form

Dr. Tabrej Mujawar
Department of Pharmacology, Gangamai College of Pharmacy, Nagaon, Dist. Dhule, Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India
Email: tabrej_27@yahoo.com

Dr. Sufiyan Ahmad
Department of Quality Assurance, Gangamai College of Pharmacy, Nagaon, Dist. Dhule, Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India
*Corresponding author email: sufipharm1712@gmail.com

Salman Khatik
Department of Quality Assurance, Gangamai College of Pharmacy, Nagaon, Dist. Dhule, Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India
Email: khatiksalman6@gmail.com

Dr. Manoj Tare
Department of Pharmaceutics, Sitabai Thite College of Pharmacy, Shirur (Ghodnadi), Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India
Email: manojstare75@gmail.com

Dr. Dwarkadas Baheti
Department of Pharmacognosy, Sitabai Thite College of Pharmacy, Shirur (Ghodnadi), Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India
Email: dgbaheti@yahoo.com

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Asmita Gaikwad
Department of Pharmaceutical Chemistry, Sharadchandra Pawar College of Pharmacy, Otur, Tal. Junnar, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India
Email: asmitavgaikwad@gmail.com

Gauri Ghangale
Department of Pharmacognosy, Amrutvahini College of Pharmacy, Sangamner, Dist. Ahmednagar, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India
Email: gaurighangale@gmail.com

***Abstract*---**Background: Perindopril erbumine (PE) and Indapamide (IND) in combination were proven to have a synergistic antihypertensive impact when compared with the use of each component alone. Objectives: Therefore, a new simple, selective, precise, and stability indicating RP- HPLC method for analysis of Perindopril erbumine (PE) and Indapamide both in a bulk and in pharmaceutical formulation has been developed and validated. Methods: RP - HPLC method, Younglin (S.K.) Gradient System UV Detector and C_{18} column with 250mm x 4.6 mm i. d. and 5µm particle size acetonitrile 70+ water with OPA 30 was used as the mobile phase for the method. The detection wavelength was 215 nm and flow rate was 1 ml/min. Results: In the developed method, the retention time of Perindopril erbumine (PE) and Indapamide were found to be 5.4833 min and 9.4500min. The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Conclusion: Hence the method was found to be simple, accurate, precise, economic and reproducible. The forced degradation studies proved stability indicating power of the method. So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Perindopril erbumine (PE) and Indapamide in bulk drug as well as in formulations.

***Keywords*---**perindopril Erbumine, Indapamide, validation, stability, degradation, RP-HPLC

Introduction

Hypertension is one of the worldwide leading causes of mortality. It is a major adjustable risk factor for cardiovascular disease and stroke. Different classes of medicines can be used to reduce blood pressure. However, mono-therapy as a treatment for hypertension can successfully reduce blood pressure in few patients. Therefore, the majority will necessitate treatment with two or more drugs to reach normal levels. The main aim of using combination therapy is to utilize the various mechanisms of action, which have the potential to control blood pressure due to the combined effects, as well as to lower the doses of both drugs to diminish unwanted side effects (Nedogoda,S.V., 2017, Mansia,G., 2007). Perindopril erbumine (Fig. 1) is a long-acting angiotensin-converting enzyme (ACE) inhibitor. Taken once daily, Perindopril (4000 to 8000μg) is effective at controlling blood pressure in patients with mild to moderate hypertension. Patients who do not respond effectively to Perindopril as a monotherapy usually respond with the addition of a second antihypertensive agent (Todd, P. A., 1991).

Indapamide (Fig. 2) is a sulfonamide derivative pharmacologically related to thiazide diuretics. In spite of reports that proved that the antihypertensive activity of Indapamide is mainly due to its diuretic activity, few cases of diuresis were discovered with the typical antihypertensive dose of 2500μg daily. Various studies suggest that it may also lower blood pressure by lowering vascular reactivity and peripheral vascular resistance (Chaffman, M., 1984). Indapamide has been successfully reported to control mild to moderate hypertension as a monotherapy or in combination with other antihypertensive agents (De Leeuw, P. W., 2001). The gainful impacts of Indapamide and Perindopril as a combination therapy in reducing blood pressure have been demonstrated in numerous trials (Mourad, J. J., 2004). Perindopril and Indapamide as a combination therapy was proven to have a major effect on systolic blood pressure, arterial stiffness, and microcirculatory alterations (Croom, K., 2008, de Leeuw, P. W. 2011). A literature survey revealed that Perindopril is officially in the British Pharmacopoeia (British Pharmacopoeia 2007), whereas Indapamide is officially in the British Pharmacopoeia and U.S. Pharmacopeia (USP 2007). A detailed literature survey found that a number of reported methods have been constructed for the estimation of Perindopril and Indapamide individually (Abdellatef, H. E. 1998, 1999, Lin, S. J.,1996, Prameela Rani, A. 2009, Simoncic, Z., 2008, Saleh, H. M., 2001, Tarkase Kailash, N., 2012, Pannu, H., 2012, Legorburu, M. J., 1999, Hang, T. J., 2006, Gao, X., 2005).

Literature survey reveals that few methods have been reported for the estimation of PE and Indapamide in combination and individual method by using UV spectrophotometric (Erk, N. 2001, Pektaş, G., 2009), HPLC [Elshanawane, A. A., 2008], Plasma RP-HPLC (Albu, F., 2005, Medenica, M., 2007) and LC (Jain, D. S., 2006). This paper describes the validation of an analytical method with RP-HPLC analysis for the simultaneous estimation of both the drugs in bulk and combined tablet dosage form as per current ICH and FDA guideline (ICH 1995, 2000, FDA 2000). Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product. Results are applied in development of manufacturing processes, selecting proper packaging, storage conditions, product's shelf life and expiration dates. Because the distribution environment is highly variable, products must be distributed in a manner that ensures that the product quality will not be adversely affected.

The effect of possible temperature and humidity fluctuations, outside of labeled storage conditions, during transportation of drug products, can be evaluated on the basis of the stability analysis for that drug (Grimm W., 2000, Dean DA., 2000, ICH, 2003). Validation of an analytical method typically involves for linearity, accuracy, specificity, Method precision, Intermediate Precision and stability. A number of papers have been published detailing validation studies for the analytical method (Lalitha, N. A., 2009, Singh R., 2009, Kalta, R. R., 2008, Sharma, R., 2009). The purpose of this study was to develop simple, specific, sensitive, rapid, and economical and can be used for simultaneous estimation of Indapamide and Perindopril in bulk and their combined dosage form for routine analysis and stability studies.

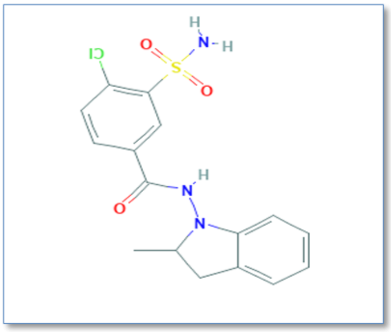

Fig. 1. Structure of perindopril

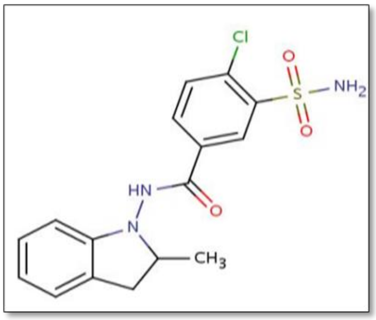

Fig. 2. Structure of Indapamide

Materials and Methods

Instrumentation and chemicals

The analysis of the drug was carried out on Young line (S. K.) gradient system UV detector. Equipped with reverse phase (Grace) C_{18} column (4.6mm x 250mm; 5µm), a SP930D pump, a 20µl injection loop and UV730D absorbance detector and running autochro-3000 software. Perindopril erbumine (PE) and Indapamide drug were procured from Sardia pharma Pvt. Ltd. Methanol, acetonitrile and potassium phosphate buffer (HPLC grade Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai), 0.45 µm filter (Millipore, Bangalore). A combination of Perindopril erbumine (1.25mg) and Indapamide (4 mg) in tablet formulation was procured from Sardiapharma brand name Coversyl plus.

Preparation of standard Indapamide solution

From the freshly prepared standard stock solution (1000 µg/ml), 0.1ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 10 µg/ml.

Preparation of standard Perindapril erbumine solution

From the freshly prepared standard stock solution (3200ug/ml), 0.1 ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10 µg/ml.

Preparation of standard Indapamide and Perindapril erbumine solution

From the freshly prepared standard stock solution (1000µg/ml), 0.1 ml stock solution was pipette out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10 µg/ml.

Selection of mobile phase

Each mobile phase was vacuum degassed and filtered through 0.45µ membrane filter. The mobile phase was allowed to equilibrate until OPA by baseline was obtained. The standard solution containing mixture of Indapamide and Perindapril erbumine was run with different individual solvents as well as combinations of solvents were tried to get a good separation and stable peak. From the various mobile phases tried, mobile phase containing Acetonitrile and Water (0.1% OPA) was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for Indapamide and Perindapril erbumine. Chromatograms of Indapamide and Perindapril erbumine are shown in (Fig. 3 and Table 1) respectively.

Chromatographic Conditions

The Chromatographic separation was carried out on Phenomenex Luna C_{18} column (4.6 x 250 mm i. d., particle size of packaging material 5 µm). Mobile phase combination is Acetonitrile : Water (70: 30) (pH adjusted to 3 with 0.1% Orthophosphoric acid) at the flow rate of 1 ml/min, Sample size 20 µl, temperature ambient and detection wavelength 215 nm.

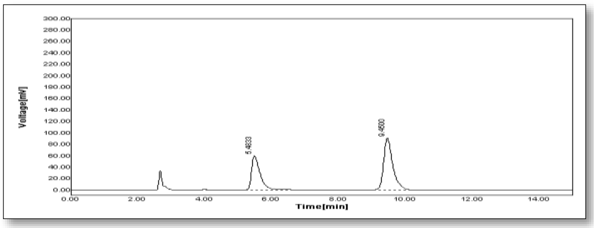

Fig. 3. Chromatogram of standard combination of IND and PE

Table 1
Details of chromatogram of standard Combination containing IND and PE

Sr. No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	2.9833	747.6716	72.57	2776.2	1.2143	0.000
2	7.3833	282.5911	27.43	6439.3	1.000	12.5714
Sum		1030.2627				

Method development and validation

Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected (Patel, K. P., 2020, Awatade, P. R., 2022, Pate, A. N., 2022).

Assay preparation for marketed formulation

For analysis of the tablet dosage form, 20 tablets were weighed individually and their average weight was determined. After that they were crushed to fine powders and powder equivalent to 12.2 mg IND and PE into 10 ml volumetric flask and diluted with 10 ml methanol, and sonicate to dissolve it completely and make volume up to the mark with diluent. The solutions were shaken vigorously for 10 min and filtered through 0.45 µm membrane filters. Further pipette 0.1ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents (10 µg/ml). The simple chromatogram of test IND and PE shown in (Fig. 4). The amounts of IND and PE per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation Analysis of marketed formulation were also % label claim was found to be 99-102% satisfactory were concluded (Table 2).

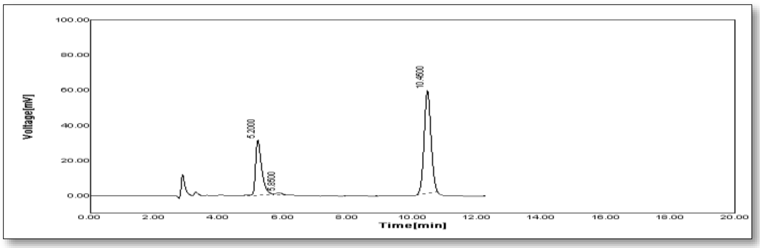

Fig. 4. Chromatogram for marketed formulation

Table 2
Analysis of marketed formulation

Assay	Drug	label claimed	Amt. found	% Label claim	S.D.	%RSD
RP-HPLC Method	IND	25	25.73	102.92	0.04	0.04
	PE	80	78.63	98.29	0.18	0.18
	IND	25	25.50	102.00	0.04	0.04
	PE	80	79.63	99.54	0.18	0.18

Results and Discussion

Linearity and Range

From IND and PE standard stock solution, different working standard solutions (50-250µg/ml) were prepared in mobile phase. Likewise from IND and PE standard stock solution different working standard solution (4-20 µg/ml) were prepared in mobile phase. 20 µl of sample solution was injected into the chromatographic System using fixed volume loop injector. Chromatograms were recorded. The area for each concentration were recorded (Table 3 and 4). The respective linear equation for IND was y = 16.234x + 7.339 and PE equation was y = 10.665x + 27.519, where x is the concentration, and y is an area of peak. The correlation coefficient was 0.998. The calibration curve of IND and PE is depicted in (Fig.5 and 6).

Table 3
Linearity data for Indapamide

Method	Conc. µg/ml	Peak area (µV. sec)		Average peak area (µV.sec)	S. D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	5	85.67	84.6	85.14	0.76	0.89
	10	172.7	170.82	171.76	1.33	0.77
	15	261.62	256.87	259.25	3.36	1.30
	20	321.96	322.89	322.43	0.66	0.20
	25	416.86	414.44	415.65	1.71	0.41
	Equation		y = 16.234x + 7.339			
	R^2		0.997			

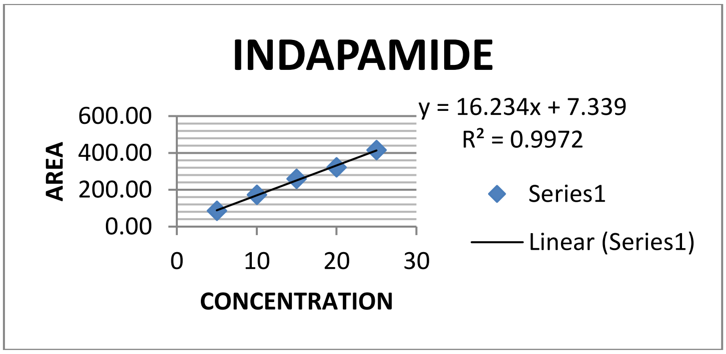

Fig. 5. Calibration curve of Indapamide

Table 4
Linearity data for Perindapril erbumine

Method	Conc. µg/ml	Peak area (µV.sec)		Average peak area (µV.sec)	S. D. of Peak Area	RSD of Peak Area
		1	2			
RP-HPLC Method	16	196.38	195.31	195.85	0.76	0.39
	32	374.28	373.59	373.94	0.49	0.13
	48	532.41	522.62	527.52	6.92	1.31
	64	731.81	723.69	727.75	5.74	0.79
	80	877.67	866.62	872.15	7.81	0.90
	Equation		y = 10.665x + 27.519			
	R^2		0.998			

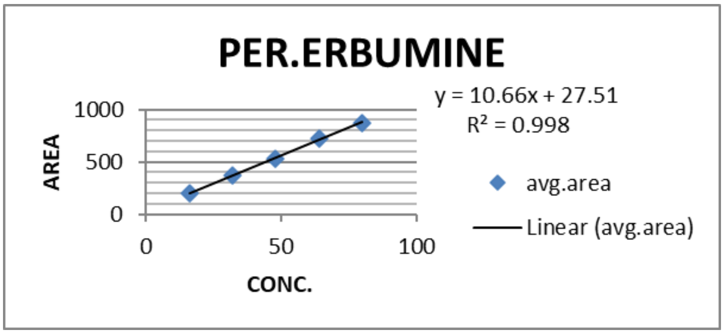

Fig. 6. Calibration curve of Perindapril erbumine

Accuracy

It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. The accuracy of the methods was determined at three different concentration levels, i.e., 80%, 100%, and 120% (Fig. 7 - 9) in triplicate for each drug as per the ICH guidelines. From the total amount of drug found, the percentage recovery was found in range of 99-101% (Table 5, 6 and 7).

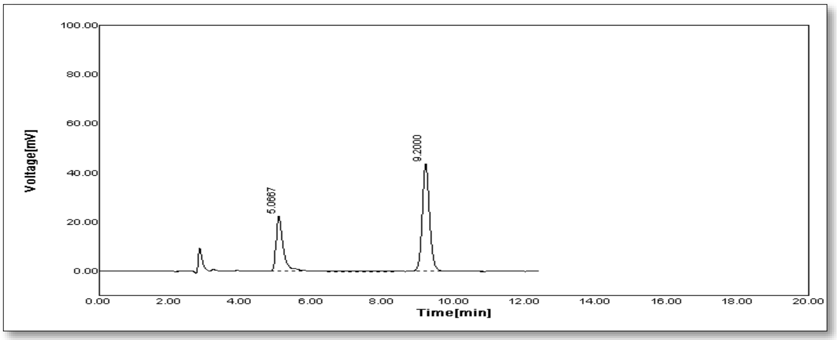

Fig. 7. Chromatogram of Accuracy 80%

Table 5
Chromatogram of Accuracy 80%

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	5.0667	322.1461	33.40	5142.4	1.7295	0.0000
2	9.2000	640.5119	66.60	11683.7	1.1557	13.2676
Sum		962.6580				

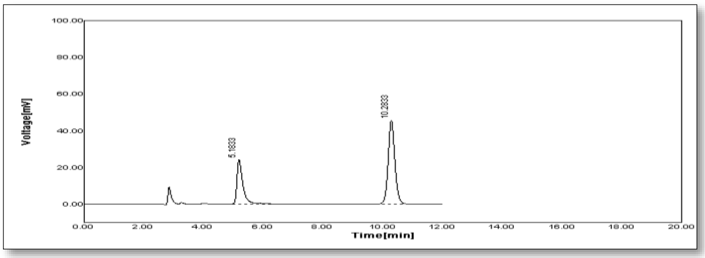

Fig. 8. Chromatogram of accuracy 100%

Table 6
Chromatogram of accuracy 100%

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	5.1833	333.7205	32.41	4962.8	1.8086	0.0000
2	10.2833	640.5105	67.59	12272.6	1.0760	15.3243
Sum		974.231				

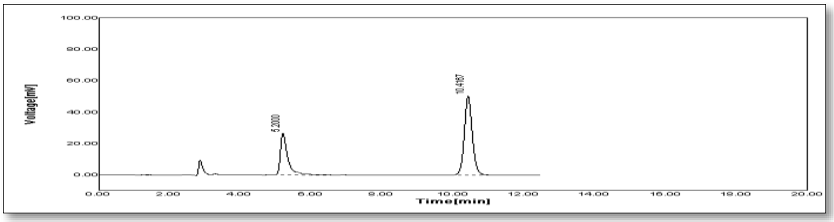

Fig. 9. Chromatogram of accuracy 120%

Table 7
Chromatogram of accuracy 120%

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	5.2000	362.8265	32.72	4352.8	1.9450	0.0000
2	10.4167	783.0782	67.28	14697.3	1.0968	15.8330
Sum		1145.9047				

Repeatability

In this studies on RP-HPLC method for IND and PE was found to be, the % RSD was less than 2%, which shows high percentage amount found in between 100% to 101% indicates the analytical method that concluded. The result shown in (Table 8).

Table 8
Repeatability studies on RP-HPLC for IND and PE

Method	Conc. of IND and PE (mg/ml)	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method	15	252.45	15.10	100.68
	15	255.35	15.28	101.87
		Mean	15.19	101.27
		SD	0.13	0.84
	48	532.71	47.24	98.41
	48	537.51	47.84	99.67
		Mean	47.54	99.04
		SD	0.42	0.89

Precision

The method was established by analyzing various standards of IND and PE. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result. The results obtained for interday and intraday variation on RP-HPLC method for IND and PE which shows the high precision % amount in between 98% to 101% indicates to analytical method were shown in the (Table 9).

Table 9
Intra day and Inter day precision studies on RP-HPLC method for IND and PE

Method	Drug	Conc. (µg/ml)	Interday Precision	
			Mean ± SD*	% Amt. Found
RP-HPLC Method	IND	10	167.91± 2.54	100.00
		15	250.75±0.71	99.93
		20	325.68±0.63	98.05
	PE	32	367.02± 1.90	99.50
		48	531.10±2.39	98.42
		64	719.45±4.57	101.41

*Mean of each 3 reading for RP-HPLC method

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The results indicate that less variability in retention time and tailing factor were observed (Table 10 and 11).

Table 10
Robustness study of Indapamide

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.9ml	20	422.69±4.16	0.98
Chromatogram of flow change 1.1 ml	20	303.27±4.29	1.42
Chromatogram of comp change 71 ACN+29 WATER	20	346.02±4.03	1.16
Chromatogram of comp change 69ACN+ 31 WATER	20	397.62±3.38	0.85
Chromatogram of comp change wavelength change 214nm	20	348.91±1.99	0.57
Chromatogram of comp change wavelength change 216nm	20	335.74±3.50	1.04

Table 11
Robustness study of Perindapril erbumine

Parameters	Conc. (µg/ml)	Amount of detected (mean ±SD)	% RSD
Chromatogram of flow change 0.9ml	64	836.89±4.76	0.57
Chromatogram of flow change 1.1 ml	64	679.85±0.74	0.11
Chromatogram of comp change 71 ACN+29 WATER	64	741.03±6.54	0.88
Chromatogram of comp change 69ACN+ 31 WATER	64	758.46±2.64	0.35
Chromatogram of comp change wavelength change 214nm	64	767.40±10.06	1.31
Chromatogram of comp change wavelength change 216nm	64	734.10±4.07	0.55

Forced Degradation studies

Forced degradation study was performed to evaluate the stability of the developed method using the stress conditions like exposure of sample solution to acid (0.1 N HCl), base (0.1N NaOH), Hydrogen peroxide (H_2O_2) and Neutral. Investigation was done for the degradation products.

Procedure for IND and PE degradation

Acid hydrolysis Degradation

The acid hydrolysis performed using 0.1N HCl at 70 °C for 1hr and 3hr for both PE and IND indicated degradation. The major degradation products for PE and IND were observed at relative retention time (RRT) & 3rd hr min. These impurities were also detected in the combination of PE and IND. (Fig. 10, 11 and Table 12, 13).

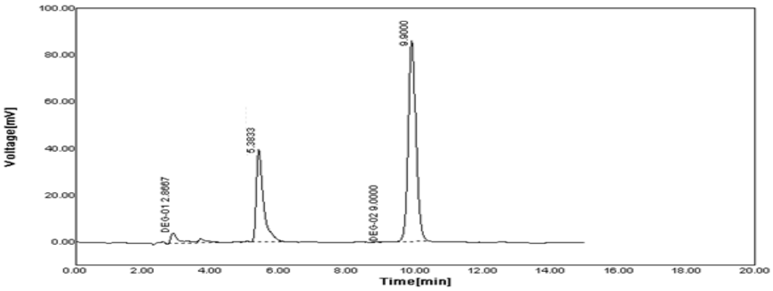

Fig. 10. Chromatogram of Acid hydrolysis PE and IND AFTER 60 MIN

Table 12
Chromatogram of Acid hydrolysis PE and IND AFTER 60 MIN

Sr. No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1.	2.8667	103.0901	5.18	1674.3	1.9137	0.0000
2.	5.3833	569.5366	28.60	4840.5	2.0638	8.5349
3.	9.0000	5.3233	0.27	4570.7	1.0640	8.5907
4.	9.9000	1313.5210	65.96	11678.8	1.1055	2.0024
Sum		1991.4709				

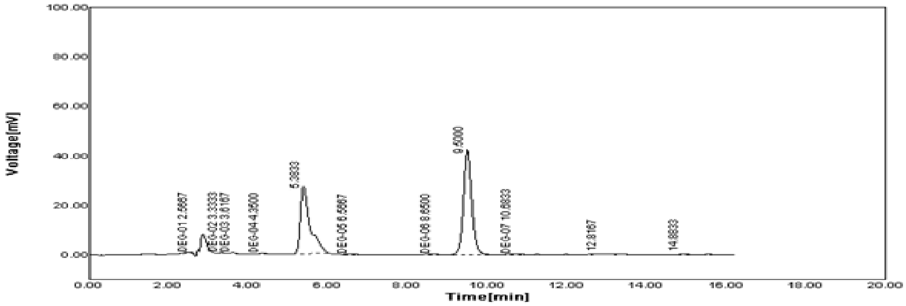

Fig. 10. Chromatogram of acidic degradation of PE and IND after 3 hrs.

Table 13
Chromatogram of acidic degradation of PE and IND after 3hrs

Sr. No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	2.5667	10.3609	0.92	727.0	0.5659	0.0000
2	3.3333	1.6761	0.15	6405.1	1.0804	2.8012
3	3.6167	3.5118	0.31	2904.9	1.1559	1.3026
4	4.3500	5.0241	0.45	2519.6	2.2563	2.3846
5	5.3833	455.9061	40.42	717.7	1.9737	1.7964
6	6.5667	5.4688	0.48	3951.6	2.5007	1.9373
7	8.6500	11.7816	1.04	2960.1	0.5636	3.9575
8	9.5000	610.5621	54.13	12228.4	1.1051	1.7005
9	10.6833	3.6863	0.33	5324.3	1.0239	2.5468
10	12.8167	2.1437	0.19	14729.0	0.6286	4.2325
11	14.8833	17.7749	1.58	892.5	0.9639	1.6689
Sum		1127.8964				

Alkaline hydrolysis

The alkaline hydrolysis condition was performed using 0.1N NaOH at 70 °C for1hr and 2hr both Perindapril erbumine and Indapamide. The major degradation products for Perindapril erbumine and Indapamide were observed at for 1st hr and 3rd hr the degradation product was observed at RT. (Fig. 12, 13 and Table 14, 15).

Alkali hydrolysis degradation

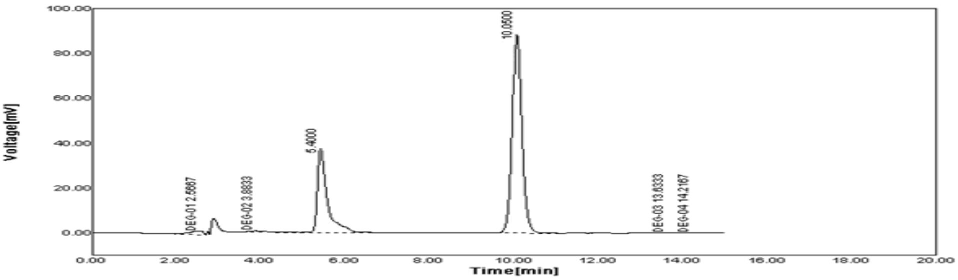

Fig. 12. Chromatogram alkali hydrolysis PE and IND 1hr

Table 14
Chromatogram alkali hydrolysis PE and IND 1hr

Sr. No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1.	2.5667	34.8622	1.71	226.7	0.5933	0.0000
2.	3.8833	22.9813	1.13	386.7	2.2482	1.9832
3.	5.4000	614.4670	30.13	3980.1	2.7403	2.6549
4.	10.0500	1362.4703	66.81	12068.4	1.1359	13.1298
5.	13.6333	2.7390	0.13	9831.0	0.8474	7.8244
6.	14.2167	1.8719	0.09	14849.2	1.1785	1.1475
Sum		2039.3916				

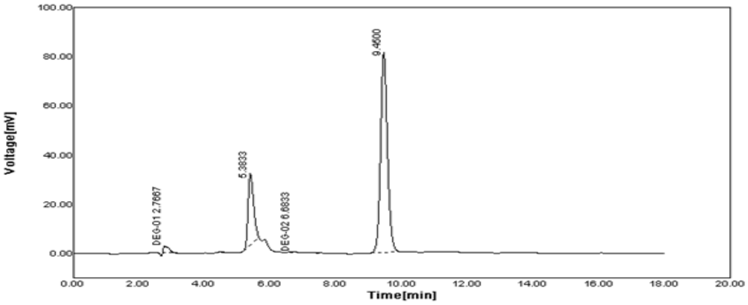

Fig. 13. Chromatogram alkali hydrolysis PE and IND after 3hr

Table 15
Chromatogram alkali hydrolysis PE and IND after 3hr

Sr. No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1.	2.7667	30.8666	1.98	1528.9	1.7465	0.0000

2.	5.3833	334.8326	21.46	6652.0	1.3319	9.5665
3.	6.6833	9.4787	0.61	2085.6	1.5338	3.0610
4.	9.4500	1185.3440	75.96	11708.6	1.1456	5.9199
Sum		1560.5219				

Hydrogen Peroxide Degradation

In the oxidation condition with 10% H2O2 for 1 hr and 3hr both IND and PE show any oxidative stress degradation peak in the chromatogram. (Fig. 14, 15 and Table 16, 17).

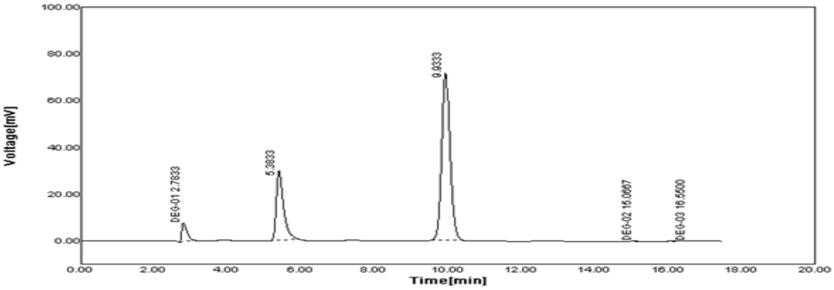

Fig. 14. Chromatogram of hydrogen peroxide PE and IND 1hr

Table 16
Chromatogram of Hydrogen Peroxide PE and IND 1hr

Sr. No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1.	2.7833	79.2976	5.00	1738.5	1.9625	0.0000
2.	5.3833	410.8277	25.93	4308.4	1.8088	8.7383
3.	9.9333	1077.0105	67.97	14201.5	1.1234	13.7571
4.	15.0667	3.9015	0.25	7502.6	0.9438	9.9754
5.	16.5500	13.5823	0.86	2184.6	0.6930	1.4046
Sum		1584.6196				

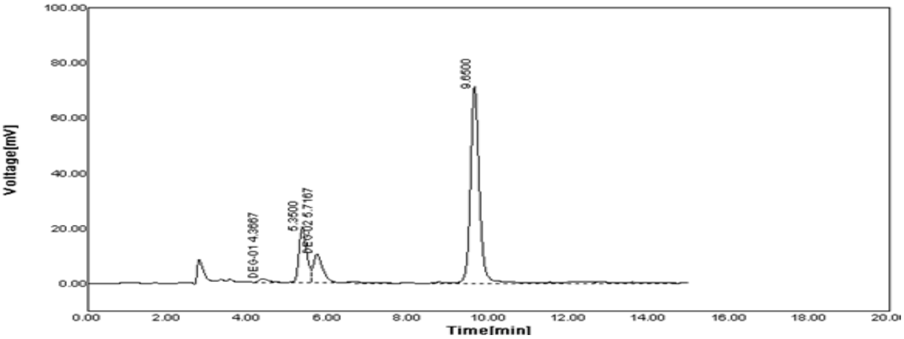

Fig. 15. Chromatogram of hydrogen peroxide PE and IND 3hr

Table 17
Chromatogram of hydrogen peroxide PE and IND 3hr

Sr. No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1.	4.3667	22.6977	1.36	1913.4	1.8606	0.0000
2.	5.3500	278.2016	16.71	4605.9	1.1436	2.7520
3.	5.7167	185.7428	11.16	3730.4	1.9702	1.0632
4.	9.6500	1178.0093	70.77	11669.1	1.1342	10.7509
Sum		1664.6512				

Neutral

There was no major degradation observed for both Perindapril erbumine and Indapamide and hence they were not sensitive to light at 70 °C for 1hr and 3hr.

Conclusion

The newly developed RP-HPLC method for determination of IND and PE in bulk sample and in pharmaceutical formulation was found to be specific, precise, accurate and robust. The proposed method was completely validated as per ICH guidelines. The method validation data showing satisfactory results for all the method parameters tested. The stability-indicating the nature of the proposed method was established by performing forced degradation, which provided degradation behavior of IND and PE under various conditions. Hence the developed HPLC method is stability-indicating and can be used for routine analysis of production samples and also to check the stability of bulk samples of IND and PE.

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Abbreviation used

HPLC: High performance liquid chromatography; UV: Ultraviolet; ICH: International Conference on Harmonization; LOQ: Limit of quantitation; LOD: Limit of detection; RSD: Relative standard deviation; RT: Retention time; OPA: Orthophosphoric acid; PE: Perindapril Erbumine; IND: Indapamide; FDA: Food and Drug Administration; SD: Standard deviation.

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