IJPSR (2015), Vol. 6, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 December, 2014; received in revised form, 10 March, 2015; accepted, 26 April, 2015; published 01 August, 2015

STABILITY INDICATING ISOCRATIC RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR INDAPAMIDE AND PERINDOPRIL ERBUMINE IN PURE AND ITS COMBINED TABLET DOSAGE FORM

B. Siva Sai kiran[1], G. Raveendra Babu [*2], M. Venkta Kumari[1] and G. Vijaya Kumar [3]

Department of Pharmaceutical Analysis [1], Daggubati Chenchuramaiah Memorial Pharmacy College, Inkollu, 532167, Andhra Pradesh, India
Department of Pharmaceutical Analysis [2], Department of Pharmacology [3], Alluri Krishna Rao and Govindamma College of Pharmacy, Nallajerla, 534112, Andhra Pradesh, India.

Keywords:

HPLC, Stability, Indapamide, Perindopril, Assay, Linearity, Precision

Correspondence to Author:
G. Raveendra Babu
Assistant Professor
Department of Pharmaceutical, Analysis and Quality Assurance, Alluri Krishna Rao and Govindamma College of Pharmacy, Nallajerla, West Godavari, Pincode-534112, Andhra Pradesh, India

E-mail:g_raveendra@yahoo.com

ABSTRACT: A reverse phase liquid chromatography method (RP-HPLC) was developed to estimate the amount of Indapmide and Perindopril in bulk and its pharmaceutical formulations. A Waters Alliance HPLC system equipped with auto sampler, UV-Visible detector and YMC Column (150 x 4.6mm, 3µ particle size) were used for the quantification of the drugs. Separation was carried out by using potassium dihydrogen phosphate buffer of pH 2.5 and acetonitrile in the ratio 60:40 v/v as mobile phase at a flow rate of 1 ml/min and the detection was carried out at a wavelength of 230 nm. The retention time, tailing factor and USP theoretical plates were found to be 2.5 min., 1.38 and 2551 for Indapamide and 4.18., 1.75 and 2778 for Perindopril respectively. The area of the peak was proportional to the concentration of the drug in the range 15-35, 48-112 µg/ml of Indapmide and Perindopril. The values of LOD and LOQ for Indapmide and Perindopril were found to be 0.07, 27.87 and 0.23, 84.47 µg/ml respectively. The mean recoveries of the substances were found to be 99.23 and 100.28 %. The bulk active pharmaceutical ingredient was subjected to thermal, hydrolytic (acidic and basic) and oxidative stress conditions and stressed samples were analyzed by the proposed method. The developed method was 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.

INTRODUCTION: Indapamide **Fig. 1** is a thiazide diuretic drug and chemically termed as 4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl) -3-sulfamoyl-benzamide, which is available generically as 1.25 mg and 2.5 mg non-scored tablets for oral administration.

It is used for hypertension, congestive heart failure. The empirical formula for Indapamide is $C_{16}H_{16}CIN_3O_3S$ and its molecular weight is 365.83 [1-2].

It is used for treatment of high blood pressure, heart failure and the empirical formula for Perindopril is $C_{19}H_{32}N_2O_5$ and its molecular weight is 368.468 [3-4]. The literature survey revealed that few analytical methods made to estimation of Indapamide and Perindopril as individuals and in combination with other drugs such as spectrophotometric [5-7], HPLC [8-15] and HPTLC[16-17] methods have been reported for the determination of Indapamide and Perindopril.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(8).3428-38

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(8).3428-38

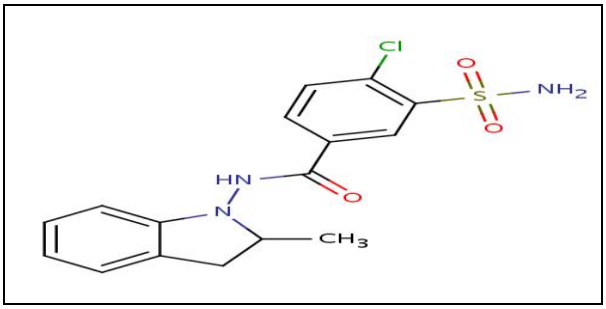


FIG. 1: CHEMICAL STRUCTURE OF INDAPAMIDE

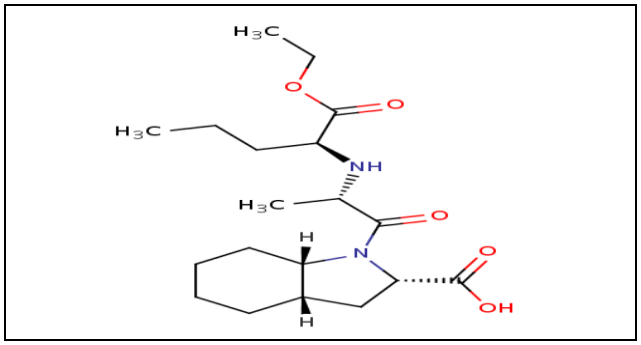


FIG. 2: CHEMICAL STRUCTURE OF PERINDOPRIL

MATERIALS AND METHODS:

Chemicals and Reagents Used: The working standards of Indopamide and Perindopril were provided as gift sample from SPECTRUM LABS, HYDERABAD, INDIA. Water [HPLC Grade], Methanol [HPLC Grade], Acetonitrile [HPLC Grade], Indopamide and Perindopril [Working standards], Orthophosphoric Acid & Sodium Dihydrogen Ortho Phosphate all the chemicals were procured from QUALIGENS FINE CHEMICALS LTD., MUMBAI, INDIA. NaOH procured from S D FINE- CHEM LIMITED & H2O2 procured from ALPHA PHARMA LIMITED for the study and the tablets were collected from the Local market.

Apparatus and Chromatographic Conditions:

Equipment: High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.

UV/VIS spectrophotometer: UV probe (Shimadzu) EV-100

PH meter: ELICO

Weighing machine: SP-202

Temperature: Ambient

Column: YMC Column (150 x 4.6mm, 3µ particle size)

Phosphate Buffer: 2.5milligrams of Sodium Dihydrogen Ortho Phosphate in 1000 ml Water [HPLC Grade] pH adjusted with Orthophosphoric Acid.

PH: 2.5

Mobile phase: Phosphate Buffer: Acetonitrile (60: 40v/v)

Flow rate: 1 ml per min

Wavelength: 230nm

Injection volume: 20µl

Run time: 10min.

Preparation of Phosphate buffer:

The buffer solution was prepared by dissolving accurately weighed 1.1503g of ammonium dihydrogen orthophosphate and transferred into a clean and dry 1000ml volumetric flask, dissolved and diluted with 1000ml water [HPLC Grade]. The final pH of the buffer was adjusted to 2.5 by using orthophospharic Acid.

Preparation of mobile phase:

The Mobile Phase was prepared by mixing 600 ml (60%) of the above buffer and 400 ml of acetonitrile [HPLC Grade] (40%) and degaussed in an ultrasonic water bath for 15 minutes. Then the resultant solution was filtered through 0.45 µ filter under vacuum filtration.

Preparation of the Indopamide and Perindopril Standard & Sample Solution:

Standard & Sample Solution: The stock solution was prepared by weighing accurately 12.5 mg of Indapamide & 40mg of Perindopril and transferred into a clean and dry 50 ml volumetric flask. About 30 ml of diluent was added and sonicated and the volume was made up to the mark with the same diluent. From the above prepared Stock solution pipette out 5ml & 5ml of solution and transferred into a clean and dry 50ml volumetric flask, the diluent was added up to the mark to get final concentration.

Preparation of Sample Solution:

The stock solution was prepared by weighing accurately 24.5 mg of Indapamide and Perindopril and transferred into a clean and dry 50 ml volumetric flask. About 30 ml of diluent was added and sonicated. The volume was made up to the mark with the same diluent. From the above prepared Stock solution pipette out 5ml & 5ml of solution and transferred into a clean and dry 50ml volumetric flask, the diluent was added up to the mark to get final concentration. The standard and sample solutions were injected five times and the peak areas were recorded. The men and percentage relative standard deviation was calculated from the peak areas.

System Suitability:

The Tailing factor for the peaks due to Indapamide and Perindopril in Standard solution should not be more than 1.5. The Theoretical plates for the Indapamide and Perindopril peaks in Standard solution should not be less than 2000. The system suitability of the method was checked by injecting five different preparations of the Indapamide and Perindopril standard. The parameters of system suitability were checked.

Assay calculation for Indapamide and Perindopril:

$$\text{Assay percentage} = \frac{A_t}{A_s} \times \frac{W_s}{D_s} \times \frac{D_T}{W_t} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

A_t = average area counts of sample preparation.

As = average area counts of standard preparation.

W = Weight of working standard taken in mg.

P = Percentage purity of working standard

LC= Label claim

D_T = Dilution factor of test sample.

System Suitability Results for Indapamide:

The Tailing factor obtained from the standard injection was **1.38.**

The Theoretical Plates obtained from the standard injection was **2551.**

Assay Result for Indapamide:

Assay of Indapamide is 98.07

System Suitability Results for Perindopril:

The Tailing factor obtained from the standard injection was **1.75.**

The Theoretical Plates obtained from the standard injection was **2775.**

Assay Result for Perindopril:

Assay of Perindopril is 101.57 **Fig.3** and **Fig. 4.**

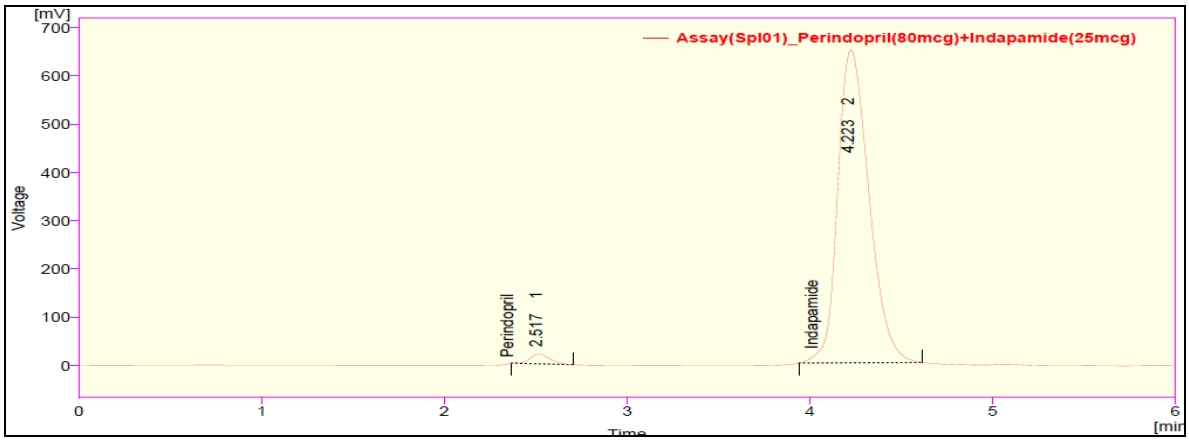


FIG. 3: STANDARD CHROMATOGRAM OF INDAPAMIDE AND PERINDOPRIL

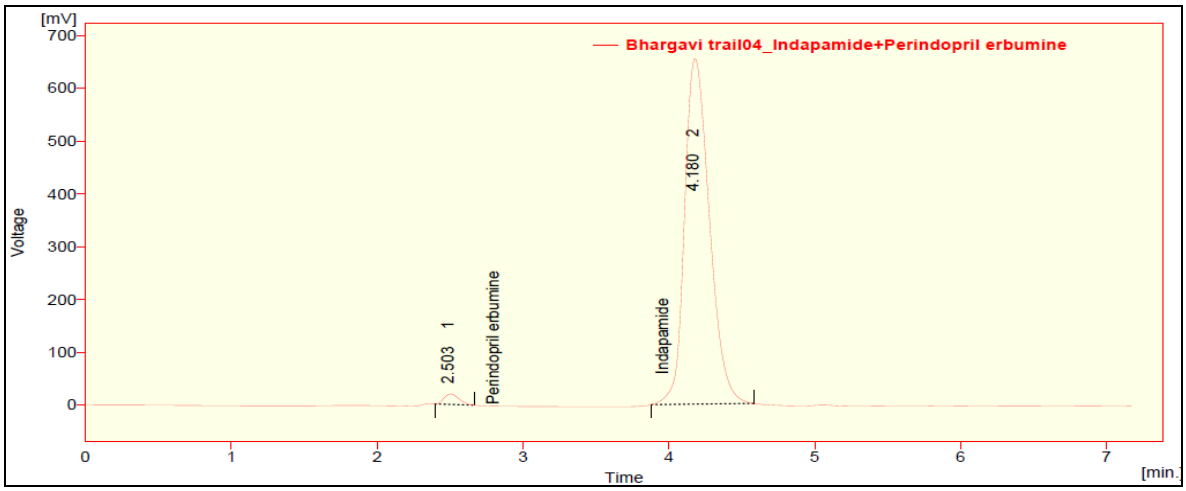
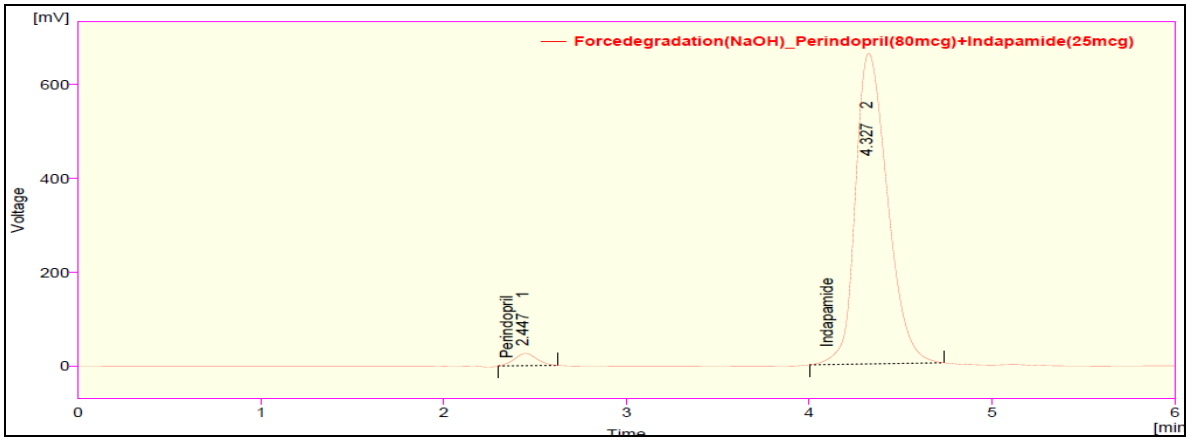


FIG. 4: SAMPLE CHROMATOGRAM OF INDAPAMIDE AND PERINDOPRIL



Validation Development: [18-19]

Precision: It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as a percentage of the relative standard deviation (% RSD). The standard solution was injected for five times and measured the area for all five injections in HPLC. The percentage Relative Standard Deviation for the area of five injections was found to be within the specified limits **Table 1** and **Table 2.**

TABLE 1: PRECISION STUDY DATA OF INDAPAMIDE

Injection	Area
Injection-1	7761.64
Injection-2	7795.27
Injection-3	7823.27
Injection-4	7876.64
Injection-5	7810.70
Injection-6	7739.84
Average	7801.23
Standard Deviation	48.23
%RSD	0.62

TABLE 2: PRECISION STUDY DATA OF PERINDOPRIL

Injection	Area
Injection-1	135.68
Injection-2	136.62
Injection-3	137.86
Injection-4	135.90
Injection-5	136.89
Injection-6	140.46
Average	137.24
Standard Deviation	1.76
%RSD	1.28

Intermediate Precision:

To evaluate the intermediate Precision was performed on a different day by using a different make column of the same dimensions. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits **Table 3** and **Table 4**.

TABLE 3: INTERMEDIATE PRESICION STUDY DATA OF INDAPAMIDE

Injection	Area
Injection-1	7829.61
Injection-2	8109.04
Injection-3	7932.71
Injection-4	7838.78
Injection-5	8023.42
Injection-6	7738.46
Average	7969.46
Standard Deviation	197.58
%RSD	2.48

TABLE 4: INTERMEDIATE PRESICION STUDY DATA OF PERINDOPRIL

Injection	Area
Injection-1	197.77
Injection-2	232.24
Injection-3	230.34
Injection-4	198.27
Injection-5	198.67
Injection-6	230.23
Average	245.01
Standard Deviation	24.37
%RSD	11.34

Accuracy: It is defined as an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and value found. The standard solutions of concentrations 25µg/ml, 30µg/ml, 35µg/ml of Indapamide and 80µg/ml, 96µg/ml, 112µg/ml of Perindopril were injected into chromatography system. The amount found and amount added for Indapamide and Perindopril were calculated and the individual

recovery and mean recovery values also calculated **Table 5** and **Table 6**.

TABLE 5: ACCURACY STUDY DATA OF INDAPAMIDE

%Concentration (at Specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
80%	7873.27	5	4.96	99.23	
100%	9100.13	10	10.64	100.69	99.68
120%	11199.1	15	14.89	99.14	

TABLE 6: ACCURACY STUDY DATA OF PERINDOPRIL

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	%Recovery	Mean Recovery
80%	178.43	5	5.01	100.28	
100%	233.13	10	9.81	98.13	98.98
120%	276.94	15	14.77	98.53	

Linearity:

It is defined as an analytical method constitutes its ability to elicit test results which are directly proportional to the concentration of the analyte in the sample. Different levels of solution were prepared and injected to the chromatographic system and the peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The calibration curve was represented in **Fig. 5** and **Fig. 6** and **Table 7** and **Table 8.**

TABLE 7: LINEARITY OF INDAPAMIDE

S.No	Linearity level	Concentration	Area
1	I	15	4220.62
2	II	20	6287.14
3	III	25	7531.77
4	IV	30	9683.10
5	V	35	11235.85
Corelation Coeficient			0.994

TABLE 8: LINEARITY OF PERINDOPRIL

S.No	Linerity level	Concentration	Area
1	I	48	103.17
2	II	64	142.34
3	III	80	198.41
4	IV	96	240.91
5	V	112	293.54
Corelation Coeficient			0.997

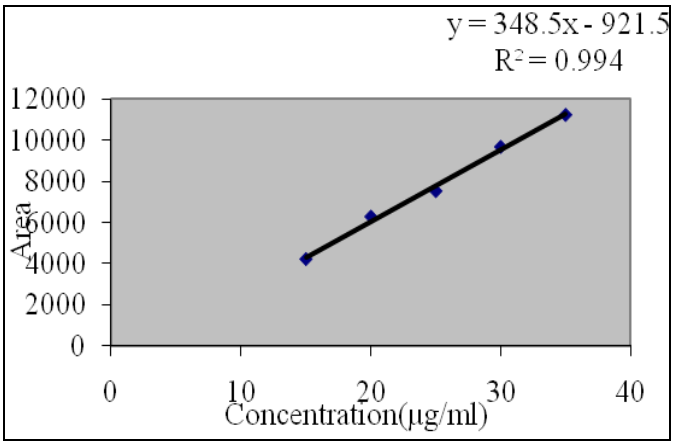


FIG. 5: LINEARITY OF INDAPAMIDE

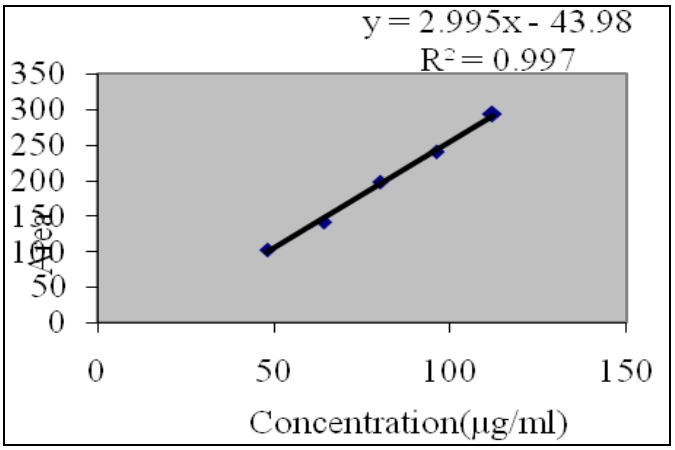


FIG. 6: LINEARITY OF PERINDOPRIL

Limit of Detection:
The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantities as an exact value.

Limit of Detection for the drugs Indapamide and Perindopril:
Limit of detection is the lowest concentration of the substance that can be detected, not necessarily quantified by the method. Calibration curve was repeated for 5 times and Intercepts was calculated the standard deviation (SD). The Limit of Detection was calculated by using the formula.

$$LOD = 3.3\ \sigma / s$$

Where,
σ = standard deviation of intercepts of calibration curves
s = mean of slopes of the calibration curves

Limit of Quantification:
It is defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions.

Limit of Quantification for the drugs Indapamide and Perindopril:
Limit of Quantification is the lowest concentration of the substance that can be estimated quantitatively. Calibration curve was repeated for 5 times and Intercepts was calculated the standard deviation (SD). The Limit of Detection was calculated by using the formula.

$$LOQ = 10\ \sigma / s$$

Where,
σ = standard deviation of intercepts of calibration curves
s = mean of slopes of the calibration curves

Robustness: The Robustness of an analytical method are a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Effect of variation of Flow rate: The Standard solution of Indapamide and Perindopril was prepared and a study was conducted to analyze the effect of variation in flow rate by injecting 0.8 and 1.2ml/min. An evaluation of the above results, it was concluded that the variation in flow rate does not affect the method significantly. Hence it was indicated that the method was robust even with a change in the flow rate **Table 9** and **Table 10.**

TABLE 9: SUMMARY OF ROBUSTNESS (CHANGE IN FLOW RATE) FOR INDAPAMIDE

S.No	Flow Rate (ml /min)	System suitability Results USP Plate Count	USP Tailing
1	0.8 ml	2056	1.6
2	1.2 ml	2139	1.5

TAB 10: SUMMARY OF ROBUSTNESS (CHANGE IN FLOW RATE) FOR PERINDOPRIL

S.No	Flow Rate (ml /min)	System suitability Results USP Plate Count	USP Tailing
1	0.8 ml	2049	1.7
2	1.2 ml	2150	1.3

Effect of variation of the wavelength: The Standard solution for the drug Indapamide and Perindopril was prepared and analyzed by the effect of variation in wavelength at 228nm and 232 nm in HPLC. The using the varied mobile phase composition along with the actual mobile phase composition. An evaluation of the above results, it was concluded that the variation in 10% Organic composition in the mobile phase does not affect the method significantly. Hence it was indicated that the method was robust even with a change in the Mobile phase ±10 **Table 11** and **Table 12.**

TAB 11: SUMMARY OF ROBUSTNESS (CHANGE IN WAVE LENGTH) FOR INDAPAMIDE

S.No	Wave Length (nm)	System suitability Results USP Plate Count	USP Tailing
1	228 nm	2190	1.5
2	238 nm	2200	1.4

TAB 12: SUMMARY OF ROBUSTNESS (CHANGE IN WAVE LENGTH) FOR PERINDOPRIL

S.No	Wave Length (nm)	System suitability Results USP Plate Count	USP Tailing
1	228 nm	2180	1.4
2	238 nm	2192	1.3

Degradation Studies[20-21]:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The method is capable of detecting the loss in content of the active component and subsequent increase in degradation products. The aim of the work was to perform the stress degradation studies on the Indapamide and Perindopril using the proposed method. The results were summarized in **Table 13** and **Table 14.**

TABLE 13: FORCED DEGRADATION DATA FOR INDAPAMIDE

Sl. No	Degradation Studies	Retention Time	Area	Height	Purity Angle	Purity Threshold
1	Acid degradation	4.32	8140.73	6978	0.63	1.58
2	Alkali degradation	4.21	8080.17	5853	0.61	1.56
3	Oxidative degradation	4.18	7781.24	5573	0.62	1.58
4	Dry heat degradation	4.31	8321.67	6894	0.57	1.62
5	Photo stability degradation	4.31	8381.20	6976	0.53	1.62

TABLE 14: FORCED DEGRADATION DATA FOR PERINDOPRIL

Sl. No	Degradation Studies	Retention Time	Area	Height	Purity Angle	Purity Threshold
1	Acid degradation	2.44	230.32	182	0.59	1.19
2	Alkali degradation	2.74	223.18	179	0.60	1.11
3	Oxidative degradation	2.50	143.59	175	0.61	1.52
4	Dry heat degradation	2.44	244.31	193	0.52	1.21
5	Photostability degradation	2.543	206.36	152	0.54	1.28

Acid degradation studies:

1.5 ml & 0.5 ml of Indapamide and Perindopril stock solution was prepared and taken in clean, dry 10ml volumetric flask refluxed for 1day at 60^0C in which 4 ml of 0.1N HCl was added. The resultant solution was diluted to obtain 10µg/ml solutions and 10µl was injected into the system and the chromatograms were recorded to assess the stability of the sample.

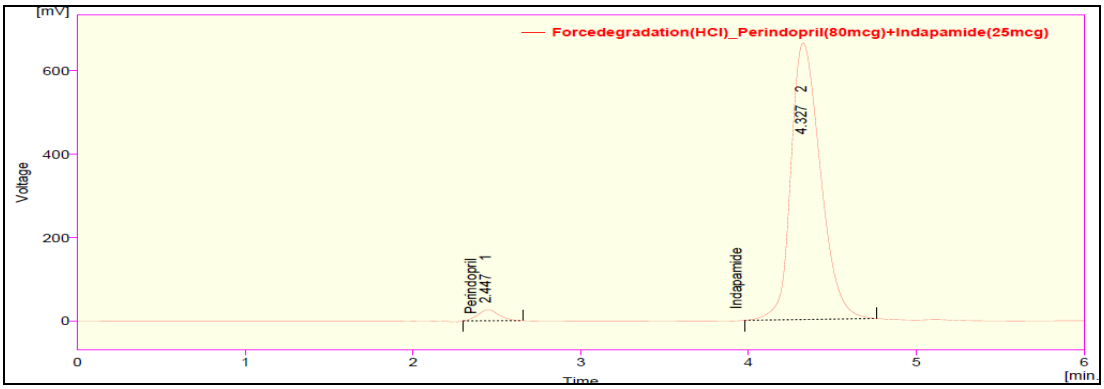


FIG. 7: ACID HYDROLYSIS OF INDAPAMIDE AND PERINDOPRIL

Alkali degradation studies: 1.5 ml & 0.5 ml of Indapamide and Perindopril stock solution was prepared and taken in clean, dry 10ml volumetric flask refluxed for 1day at 60^0C in which 4 ml of 0.1N Na OH was added. The resultant solution was diluted to obtain 10 µg/ml solutions and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of the sample.

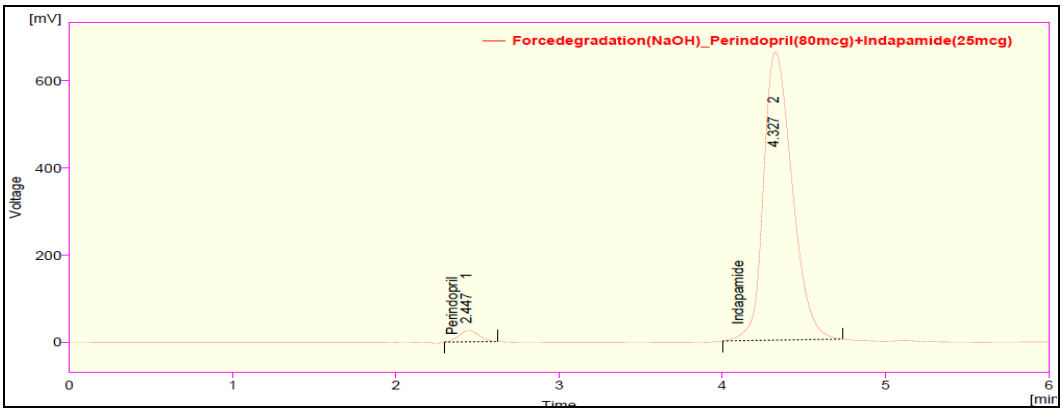


FIG. 8: ALKALINE HYDROLYSIS OF INDAPAMIDE AND PERINDOPRIL

Oxidative degradation studies:

1.5 ml & 0.5 ml of Indapamide and Perindopril stock solution was prepared and taken in clean, dry 10ml volumetric flask in which 4 ml of 20% Hydrogen Peroxide was added. Then the volumetric flask was kept at room temperature for 1 hour. The resultant solution was diluted to obtain 10 µg/ml solutions and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of the sample.

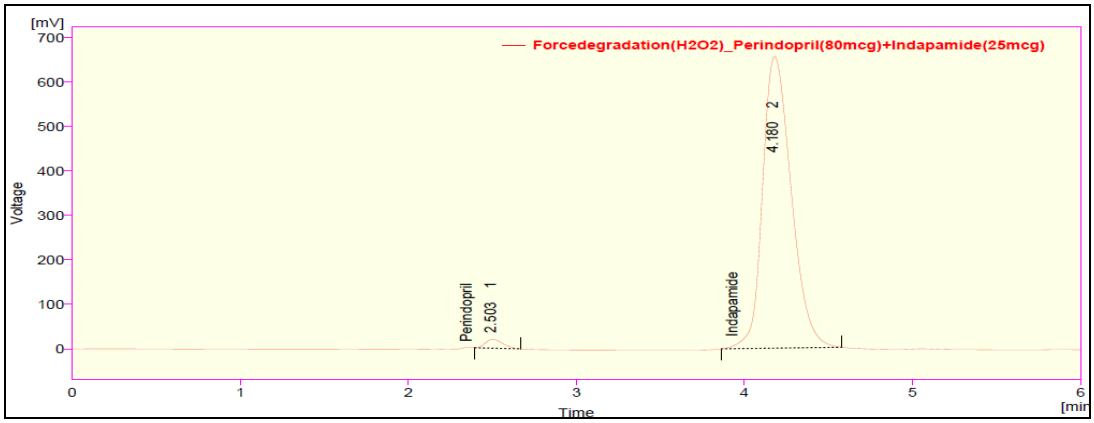


FIG. 9: OXIDATIVE HYDROLYSIS OF INDAPAMIDE AND PERINDOPRIL

Dry heat degradation studies: 1.5 ml & 0.5 ml of Indapamide and Perindopril stock solution was prepared and taken in clean, dry 10ml volumetric flask in which diluents was added. The standard drug solution was placed in an oven at 105^0C for 1 hour. The resultant solution was diluted to obtain 10 µg/ml solutions and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of the sample.

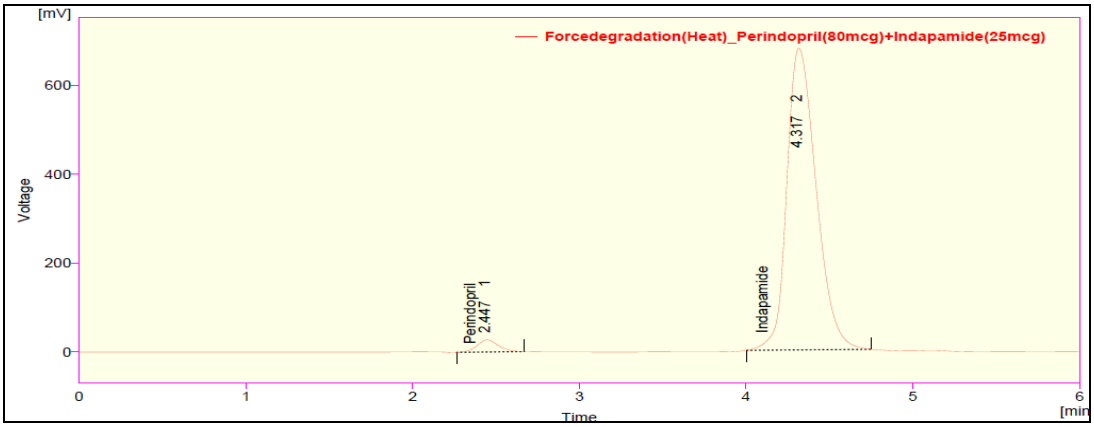


FIG. 10: THERMAL HYDROLYSIS OF INDAPAMIDE AND PERINDOPRIL

Photo stability degradation studies:
The standard Indapamide and a Perindopril drug solution was exposed to UV light by keeping the beaker in the UV chamber for 1 hour or 200 Watt hours/m^2 in photo stability chamber. The resultant solution was diluted to obtain 10 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

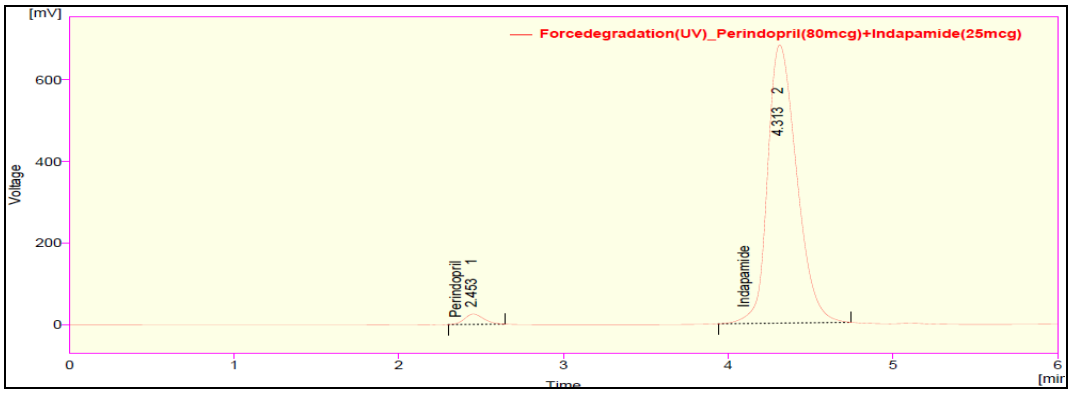


FIG. 11: NATURAL DEGRADATION OF INDAPAMIDE AND PERINDOPRIL

RESULTS & DISCUSSION:
The proposed method was simple, fast, accurate and precise method for the Quantification of drug in the Pharmaceutical dosage form, bulk drug as well as for routine analysis of Quality control. Overall the proposed method was found to be suitable and accurate for the quantitative determination of the drug in tablet dosage form. The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Indapamide and Perindopril in bulk drug and in combined dosage forms. The RP-HPLC separation was achieved on a YMC Column (150 x 4.6mm, 3µ particle size) or equivalent in an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (60%) whose pH was adjusted to 2.5 by using orthophosphoric acid & acetonitrile [HPLC Grade] (40%). The flow rate was monitored at 1 ml per min. The wavelength was selected for the detection was 230 nm. The run time was 10 min.

The retention time found for the drugs Indapamide and Perindopril were 2.5min and 4.18min respectively. It was represented in **Fig. 3.** The Precision data for the drugs Indapamide and Perindopril were represented in **Table 1 & 2**. The %RSD for sample should be NMT 2. The %RSD for the standard solution was found to be 0.62 & 1.28 for the drugs Indapamide and Perindopril respectively, which is within the limits hence the method was precise. The standard solutions of concentrations 25µg/ml, 30µg/ml, 35µg/ml of Indapamide and 80µg/ml, 96µg/ml, 112µg/ml of Perindopril were injected into chromatography system. The amount found and amount added for Indapamide and Perindopril were calculated and the individual recovery and mean recovery values also calculated **Table 5** and **Table 6**. The % recovery was found to be 99.2%- 100.6% for the drug Indapamide. The % recovery was found to be 98.5% - 100.8% for the drug Perindopril. In order to test the linearity of the method, five dilutions of the working standard solutions for the drugs Indapamide and Perindopril were prepared.

The linearity was established in the range of 15 to 35ppm for the drug Indapamide & 48 to 112 ppm for the drug Perindopril. The data were represented in **Table 7 & 8**. Each of the dilution was injected into the column and the Linearity Curve was represented in **Fig. 4 & 5**. The Correlation coefficient (R2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which was in the acceptable limit.

The limit of detection and limit of quantification of the method was calculated based on the standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD for the drugs Indapamide and Perindopril were found to be 0.07µg/ml and 2.78µg /ml respectively. The LOQ for the drugs Indapamide and Perindopril

were found to be 0.23µg/ml and 8.48µg /ml respectively. The Signal to noise ratio should be 3 for LOD. The results obtained were within the limit. The Signal to noise ratio should be 10 for LOQ solution. The results obtained were within the limit. The Robustness of the method were found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in phosphate buffer and acetonitrile in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in **Table 9 10**, 11 and 12.

In order to evaluate the stability of Indapamide and Perindopril a ability of the method to separate Indapamide and Perindopril from its degradation products, Indapamide and Perindopril was subjected to various stress conditions such as Hydrolytic degradation under acidic condition (using 0.1N HCl & 0.1 N NaOH), Hydrolytic degradation under alkaline condition (using0.1N NaOH & 0.1N HCL), Thermal induced degradation (Reflex Condition for 60 mins), Oxidative degradation (by using 20 % w/v of hydrogen peroxide). (Purity angle should be less than purity threshold. Indapamide and Perindopril peak should not have any flag in purity results table (For Waters Empower-2 software). The results were summarized in **Table 13** and **14.** The following chromatograph represents the degradation studies for the drugs of Indapamide and Perindopril, which were represented in **Fig.7, 8, 9, 10** and **11.**

CONCLUSION: The newly developed RP-HPLC method for determination of Indapamide and Perindopril 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 Indapamide and Perindopril 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 Indapamide and Perindopril.

ACKNOWLEDGEMENT: I am thankful to Spectrum labs Pvt.Ltd, Mumbai, for providing gift samples of Indapamide and Perindopril. I am glad to thanks to my colleagues, especially M.V. Nagha Bhushanam for helping me to the work done. I am also thankful to the D. C. R. M. Pharmacy College for providing facilities for performing experimental work.

REFERENCES:

1. Janega P, Kojsova S, Jendekova L, Babal P, Pechanova O: Indapamide induced prevention of myocardial fibrosis in spontaneous hypertension rats is not nitric oxide-related. Academia Scientiarum Bohemoslovaca 2007; 56 (6): 825-828.
2. Wang Z, Zhang Z, Zhang X, Fu Z: Flow-injection inhibition chemiluminescence determination of indapamide based on luminal ferricyanide reaction. Journal of Pharmaceutical and Biomedical Analysis 2004: 35 (1): 1-7.
3. Bahl VK, Jadhav UM, Thacker HP: Management of hypertension with the fixed combination of perindopril and amlodipine in daily Clinical Practice. American Journal of Cardiovascular Drugs 2009; 9 (3): 135-42.
4. Progreso Collaborative Group: Randomigita testo de perindopril-bazita sangoprem-malpliiga regimo inter 6,105 individuoj kun antaua bato au pasema iskemia atako . La Lanceto 2001; 358 (9287): 1033-1041.
5. Sharma S and Sharma MC: UV spectroscopic method for the Perindopril erbumine in pharmaceutical formulations using Indigo carmine. American, Eurasian Journal of Scientific Research 2011; 6 (4): 210-216.
6. Pillai S and Manore D: Simultaneous spectrophotometric estimation of telmisartan and indapamide in capsule dosage form. Int J Pharm Pharm Sci 2012; 4 (1): 163-166.
7. Darshana KM and Chhagan NP: Development and validation of spectrophotometric method for simultaneous estimation of Perindopril and Indapamide in combined dosage form of simultaneous equation method. Eurasian Journal of Analytical Chemistry 2011; 6(1): 46-52.
8. Dawud ER, Shakya KA: Hplc-PDA analysis of ACE inhibitors, hydrochlorothiazide and indapamide utilizing design of experiments. Arabian Journal of Chemistry 2014; 10 (52): 1-11.
9. Lakshmi RV, Sandya P, Rao CHM, Raju MDD: RP- hplc method for the simultaneous estimation of perindopril and losartan in pure form and tablet formulations. Int J Pharm Sci. Drug Res 2014; 6(1): 75-77.
10. Rao VM, Rao ND, Rao PM, Eswarudu MM: RP-hplc method development and validation of losartan potassium and perindopril erbumine in pharmaceutical dosage form. Int Res J Pharm App Sci 2013; 3 (5): 286-292.
11. Varma DPSRCHNP, Rao LA, Dinda SC: Validated stability indicating reverse phase hplc method for the

simultaneous estimation of perindopril and indapamide in pharmaceutical dosage forms. J Pharm 2013; 3 (1): 277-289.
12. Zaazaa EH, Abbas SS, Essam HAM and Mohammed G: El-Bardicy validated chromatographic methods for the determination of perindopril and amlodipine in pharmaceutical formulation in the presence of their degradation products. Journal of Chromatographic Science 2013; 51 (10): 533–543.
13. Patel BD, Mehta AF, Bhatt KK: Simultaneous Estimation of Amlodipine Besylate and Indapamide in a pharmaceutical formulation of a high performance liquid chromatography method. Scientia Pharmaceutica 2012; 80 (3): 581-90.
14. Pathak AK and Mukesh A: A selective reverse phase hplc method for simultaneous determination of perindopril and indapamide in solid dosage form and bulk drug. Journal of Pharmacy Research 2011; 4 (8): 2512-2513.
15. Joseph J, Pandian S: Simultaneous estimation of indapamide and perindopril by RP-hplc in pharmaceutical dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3 (4): 289-293.
16. Mohit GD, Kailash GB, Ashwini RM and Mrinalini CD: Simultaneous estimation of perindopril erbumine and indapamide in bulk and tablet dosage forms by hptlc. International Journal of Comprehensive Pharmacy 2011; 1 (1): 1-4.
17. Chitlange SS, Bhole RP, Wankhede SB, Sharma A: Development and validation of hplc and hptlc methods for determination of amlodipine besylate and indapamide in combined dosage form. Frontiers in Clinical Medicine 2014; 1 (3): 45-49.
18. Geneva: IFPMA; 1997. International Conference on Harmonization. Validation of Analytical Procedures: Methodology Q2B, International Conference on Harmonization 1197.
19. Geneva: IFPMA; International Conference on Harmonization. Photo stability testing of new drug substance and products Q1B, International Conference on Harmonization 1996.
20. ICH harmonized tripartite guideline. Impurities in New Drug products Q3B (R2) current step 4 versions dated 2 June 2006.
21. ICH, Q1A (R2), Stability Testing of New Drug Substances and Products, Geneva, Feb. 2003.

How to cite this article:
Sai kiran BS, Babu GR, Kumari MV and Kumar GV: Stability Indicating Isocratic RP-HPLC Method Development and Validation for Indapamide and Perindopril Erbumine in Pure and Its Combined Tablet Dosage Form. Int J Pharm Sci Res 2015; 6(8): 3428-38.doi: 10.13040/IJPSR.0975-8232.6(8).3428-38.