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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF INDORAMIN IN PHARMACEUTICAL DOSAGE FORM

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Abstract:

In the current study, A simple, precise and economic UV and stability indicating RP- HPLC method was developed and validated for estimation of Indoramin in tablet dosage form. Inthe current study, this approach was used to estimate the Indoramin tablet formulation. The investigation was conducted using HPLC Water2469 with GL-Science, Inertsil ODS 3V C18, 5, 4.6 x 150 mm column, and UV/PDA detector with empower pro Software. Buffer and ACN, with a wavelength of 233 nm, were determined to be the most suited mobile phase. The method shows good reproducibility; moreover the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The findings in the table show that the RP-HPLC technology may be used to accurately estimate the above-mentioned medicines in theirformulation.

Keywords: Stability-indicating RP-HPLC, Indoramin, Forced degradation

1. Introduction:

Indoramin N-[1-[2-(lH-indol-3-yl)ethyl)]-4-piperidinyl]-benzamide is an alpha-1 adrenergic receptor antagonists blocking agent shown to be capable of reducing blood pressure in humans. Therefore, the development of a highly sensitive methodis necessary to study the pharmacokinetics of this drug. To date, the methods used for the analysis of this drug are gas chromatography mass spectrometry and high- performance liquid chromatography (HPLC) with fluorometric detection (1).

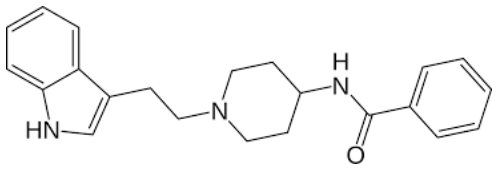


Fig. Structure of Indoramin

Category	Anti-Hypertensive Agents, Anti-Adrenergic Agent.
Chemical Name	N-{1-[2-(1H-indol-3-yl)ethyl]piperidin-4-yl}benzamide
Molecular Formula	$C_{22}H_{25}N_3O$
Molecular Weight	Kilogram per mole
Description	A white or almost white powder. It exhibits polymorphism.
Solubility	It is Slightly soluble in water; sparingly soluble in alcohol; very slightly soluble in ether; soluble in methylalcohol.
P Ka	9
Melting point	208 ° C – 210 °C

In Pharmaceutical World, an impurity is considered as any other organic materials, besides the drug substances, or ingredients, arises out of synthesis or unwanted chemicals that remains with Active Pharmaceutical Ingredient's (API's). The impurity may be developed either during formulation or upon aging of both API's and formulations. Presence of impurities in trace quantity in drug substance or drug product is inevitable. Therefore, their level should be controlled and monitored. They reinforce or diminish the pharmacological efficacy of the Active Pharmaceutical Ingredient's. [2]

ICH defines impurities profile of a drug materials is –A description of the identified and unidentified impurities, present in a new drug substance‖. For Pharmaceutical products, impurities are defined assubstance in the product that are not the API itself or the excipient used to manufacture it ‖ i.e. impurities are unwanted chemical that remains within the formulation or API in small amounts which can influence Quality, Safety and Efficacy, thereby causing serious health hazards. [3]

A simple, fast, and validated HPLC method was developed for the simultaneous quantization of five cardiovascular agents: dopamine (DPM), dobutamine (DBM), phentolamine (PTM), furosemide (FSM), and aminophylline (APL) either in infusion samples or in an injection dosage form.[4]

2. Materials and Methods

2.1 Chemicals and Reagents

Indoramin was sent from the a gift sample by Laurus Labs.pvt.Ltd. Hydrabad.RO Water and HPLC grade

Acetonitrile and Phosphoric acid 88% (Merck) Mumbai, India. 0.45 µm Millipore syringe filters(Ultipor®N66®Nylon Membrane) were from PALL Life sciences.Sodium dihydrogen phosphate, Hydrochloric Acid,Sodium Hydroxide,Peroxide Solution were from Merck life science

2.2 Instruments

HPLC (Waters 2695), UV-Spectrophotometer (Shimadzu UV-1900),Analytical balance(XPE26DR Mettler Toledo) Digital pH Meter (Thermo Scientific Orian Star A211).Sonicator (BVK interprises)

2.3 Chromatographic Equipment and Conditions

The optimized chromatographic conditions are as follows:

Column used for chromatographic separation was GL-Science, Inertsil ODS 3VC18, 5µ, 4.6 x 150mm using 5 trials. pH 3.2 Phosphate Buffer: Acetonitrile (70:30 v/v) was used asmobile phase. Water and Acetonitrile in the ratio of 80:20 v/v respectively was used as diluent. Flow rate was set to 1mL/min and injection volume to 10µl. Detection was carried out at 233 nm in UV detector at 300 C. Retention time was 3.5 min and run time was 12min.

2.4 High performance reverse phase development and optimization of liquid chromatography method

For method development trials, the standard solution of Indoramin was utilised to optimise the technique for determining Indoramin 20 mg. Systematic forced degradation studies were utilised to create degraded samples, which were then employed in method development experiments to optimise the method as a stability indicator.

2.4.1 PREPARATION OF SOLUTIONPreparation of Buffer solution:

Weigh and transfer 1.19 g of sodium dihydrogen phosphate in 1000 mL volumetric flask. Add 700 mL water, sonicate to dissolve and dilute up to the mark with water. Mix well and adjust pH 3.2 ± 0.05 with Phosphoric acid. Filter through 0.45µ nylon

membrane disc filter.

Preparation of Mobile phase:

Prepare mixture of Buffer pH 3.2 and Acetonitrile in the ratio of 70:30 v/v respectively, mix well.

Preparation of Diluent:

Prepare mixture of water and Acetonitrile in the ratio of 80:20 v/v respectively, mix well.

Preparation of Blank:

Use diluent as blank.

Preparation of Standard solution:

Weighed and transferred accurately about 40 mg of Indoramin working standard into 100 mLclean and dry volumetric flask. Added about 80 mL of diluent, sonicate to about 15 minutesto dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 5.0 mL of this solution to 50 mL with diluent and mix well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.(Concentration of Indoramin standard solution:40 ppm)

Preparation of Sample solution:

Weighed and transferred 5 Indoramin tablets in to 200 mL clean and dry volumetric flask. Added about 150 mL of diluent, sonicate for 30 minutes with intermittent shaking, at control room temperature and make up volume upto mark with diluent and mix. Further diluted above stock solution 4.0 mL of this solution to 50 mL volumetric flask make up with Diluent and mixed well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample. (Concentration of Sample Solution: 40 ppm)

Selection of Stationary phase:

On the basis of reversed phase HPLC mode and number of carbon present inmolecule (analyte) stationary phase with C18 bonded phase i.e.Inertsil C18 (150 mmX 4.6 mm), 5µm was selected.

Selection of Mobile Phase:

The selection of mobile phase was done after assessing the solubility of drug in different solvent as well on the basis of literature survey and finally mixture of Buffer Solution pH 3.2 and Acetonitrile was selected as a mobile phase.

Selection of Detector and Detection wavelength:

UV-visible 2487 detector was selected, as it is reliable and easy to set at the correct wavelength and 233 nm wavelengths was selected as detection wavelength.

Selection of oven temperature:

An inclusion of column temperature (30°C) minimized day to day variation ofretention time due to fluctuations in the ambient temperature; along with this peak sharpening and shortening of run time were observed.

Selection of Sample temperature:

An inclusion of sample temperature (25°C) minimized day to day variation ofretention time due to fluctuations in the ambient temperature.

3.0 RESULTS AND DISCUSSION

3.1METHOD VALIDATION

3.2.1. System suitability:

System suitability test is a pharmacopeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.

Table. system suitability test ofIndoramin

Tailing Factor	**1.0**
Theoretical plates	**16384**
Injection No.	**Area**
1	960365
2	959350
3	959981
4	960463
5	962407
6	961715
Mean	**960714**
%RSD	**0.1**

The tests were performed by collecting data from Single injection of blank (Diluent) and six replicate injections of Standard solution were injected into the chromatograph. The data obtained is summarized in above table.

Discussion:

The data demonstrates that the system suitability is within the acceptance criteria,thus thesystem is suitable.

3.2.2 Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution, impurity Solution, placebo solution andsamplesolution. The data obtained is summarized in Table

Table. Specificity (Identification andInterference)

Solution	**Retention time (min)**
Blank solution	NA
Standard solution	3.480
Sample solution	3.450

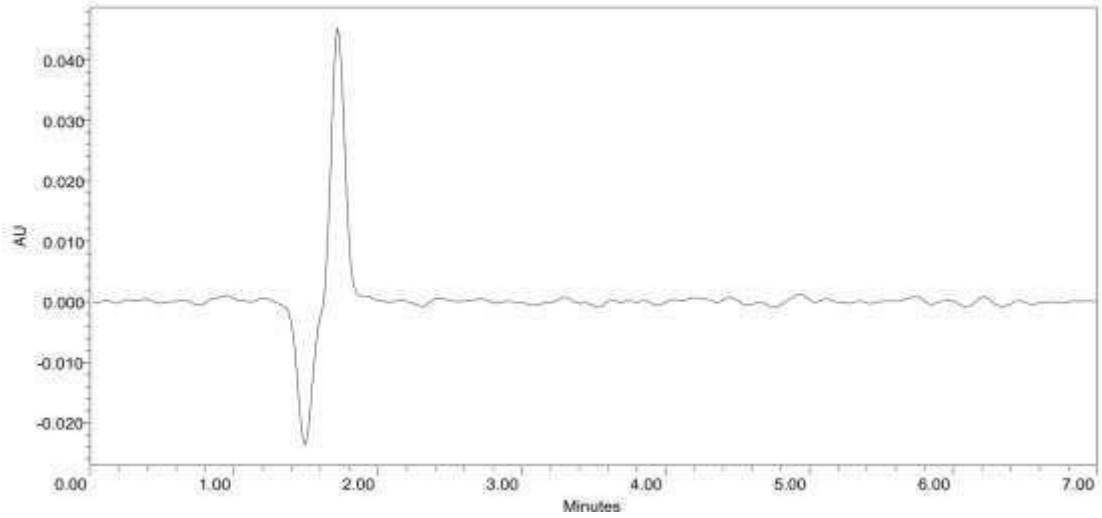


Fig . Chromatogram of Blank

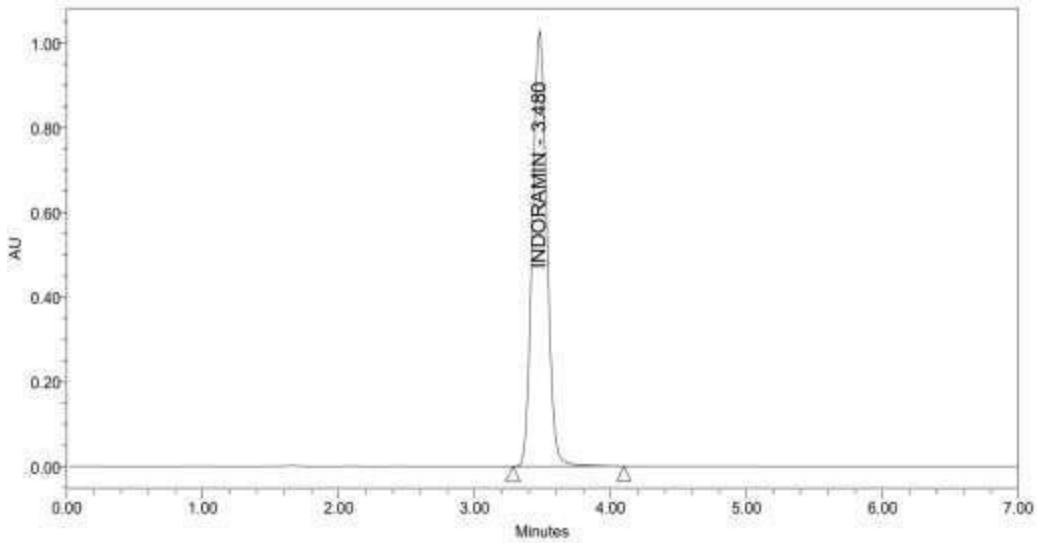


Fig .ChromatogramofStandard

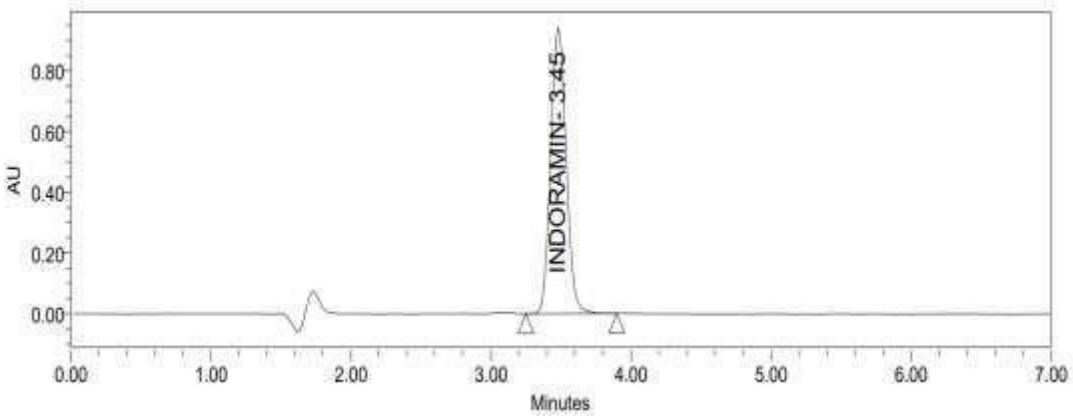


Fig .Chromatogram of Sample

Discussion:

The data demonstrates that retention time in standard and sample is same for Indoraminpeak.

The data demonstrates that there is no interference in retention time of Indoramin peak in sample solution.

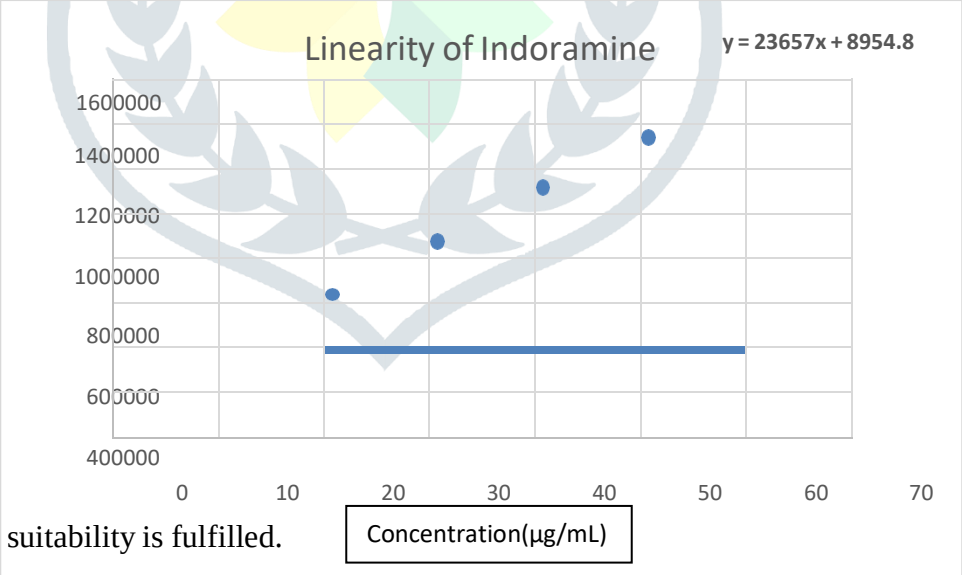
3.2.3 Linearity

Linearity was evaluated in the range of 50 % to 150 % of Indoramin for working concentration. The working concentration of Indoramin in solution is 40 µg/mL. The data summarized in Table.

Table. Linearity of Indoramin

Level (%)	Concentration w.r.t sample (µg/mL)	Peak Area Injection - 1	Peak Area Injection - 2	Mean Peak Area
50	20	479238	478626	478932
75	30	718984	718657	718821
100	40	957684	958352	958018
125	50	1196850	1199985	1198418
150	60	1419358	1424608	1421983
Correlation Co-coefficient (R)				**0.9998**
Intercept				**8954.8**
Slope of regression line				**23262**
%Y- intercept				**0.93**

Fig . Linearity plot of Indoramin



Discussion:

The data shows that system suitability is fulfilled.

The data shows that the response is found to be linear % Limit of Y- Intercept is within ±2.0% of the corresponding Y-co-ordinate of the working level.

3.2.4 Accuracy (Recovery):

Evaluated accuracy from 50% to 150% of Indoramin oral solution, working concentrationlevel. Each level prepared in triplicates.

Table 20: % Recovery for Indoramin

% Level	Conc.	Area	Mean Area	% Recovery	Mean% Recovery
50 %	20	468981	469251	99.0	99.2
		469520			
	20	472368	472486	99.3	
		472604			
	20	471616	471258	99.3	
		470900			
	40	957268	957204	100.1	100.8
100%		957139			
	40	952684	952361	100.6	
		952037			
	40	957236	957268	100.8	
		957300			
150%	60	1423568	1421709	100.0	99.4
		1419850			
	60	1412683	1410654	99.3	
		1408625			
	60	1400036	1404856	99.0	
		1409675			

Discussion:

The data shows that the Mean recovery for 50% to 150% is in the range of 99.0%-100.8%and individual recovery for 50% to 150% is in the range of 99.0% - 100.8%.

3.2.5 Precision

3.2.5.1 System Precision

Single injection of Blank (Diluent) and six replicate injections of Standard solution were injected into the chromatographic system. The data obtained is summarized in below Table.

Table . System precision

Sr. No.	Area
1	963287
2	959900
3	961257

4	960680
5	961648
6	960847
Mean	**961286**
%RSD	**0.1**

Discussion:

Observed %RSD for six replicate of standard injections meet the system suitabilityrequirement, hence system is precise

3.2.5.2 Method Precision (Repeatability)

Single injection of blank (Diluent), Standard solution (six replicates) and sample solution (sixpreparations) was injected on the system.

Table . Method precision

Sampleset	**Taken sample(mg)**	**Area**	**% Assay**
Set-1	5.0	954367	99.8
Set-2	5.0	958036	100.2
Set-3	5.0	954064	99.7
Set-4	5.0	958687	100.2
Set-5	5.0	953691	99.7
Set-6	5.0	953026	99.6
		Mean	99.9
		% RSD	0.3

Discussion:

The data shows that system suitability is fulfilled.

The data shows that % RSD for % Assay is within the acceptance criteria and hence themethod is precise.

3.2.5.3 Intermediate Precision (Ruggedness)

six independent sample preparations were prepared on different day and by different analystand injected on the HPLC.

Table . Intermediate Precision

Parameter	**Method Precision (Analyst-I)**	**Intermediate Precision (Analyst-II)**
HPLC NO.	**AD/HPLC-022**	**AD/HPLC-008**
Column No.	**C18-011**	**C18-055**
Sample No.	**%Assay**	
1	99.8	99.1
2	100.2	99.5
3	99.7	99.6
4	100.2	100.0
5	99.7	99.7
6	99.6	99.4
Mean	**99.9**	**100.0**
Absolute Mean difference % assay	**0.4**	

Discussion:

The data shows that system suitability is fulfilled.

The data shows that % Assay is of six samples is not more than 2.0

The data shows that % Assay is within the acceptance criteria and hence the method isrugged.

3.2.6 Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table . Robustness for Indoramin

Change in parameter	Condition	Area	% Assay	Absolute difference of % Assay
Control	As per method	956387	100.4	NA
Change in flow rate1.0	0.9 ml/min	956058	100.4	0
ml/min (±0.1 ml/min)	1.1ml/min	954697	100.2	-0.2
e in wavelength (±2 nm)	235 nm	951036	99.8	-0.7
	231 nm	959687	100.7	0.3

e incolumn temperature (±5 ˚c)	35 c	957039	100.5	0.1
	25 ˚c	956358	100.4	0

Discussion:

System suitability criteria were fulfilled.

The difference of % assay value in each modified condition is within acceptance criteria.

FORCED DEGRADATION:

Table. Force Degradation for Indoramin

Reagents	**Conditions**	**% Assay**	**% Degradation**	**Purity angle**	**Purity threshold**
NA	Control	99.8	NA	0.2	1.27
Acid	2 mL, 0.5 N HCl for 7 hrs.	97.4	2.4	0.2	0.8
Base	1.5 mL, 1 N NaOH for 5 hrs.	99.5	0.3	0.09	0.3
Thermal	105ºC for 24 hrs.	99.4	0.4	0.1	0.8
Oxidative	5 mL 30% H_2O_2 and heat at 50 ºC for 3 hrs.	97.8	2.0	0.05	0.2
Photo-Open	1.2 million lux hrs. and 200 watt/square	99.3	0.5	0.08	0.6

3.2.6.1 Acid Degradation:

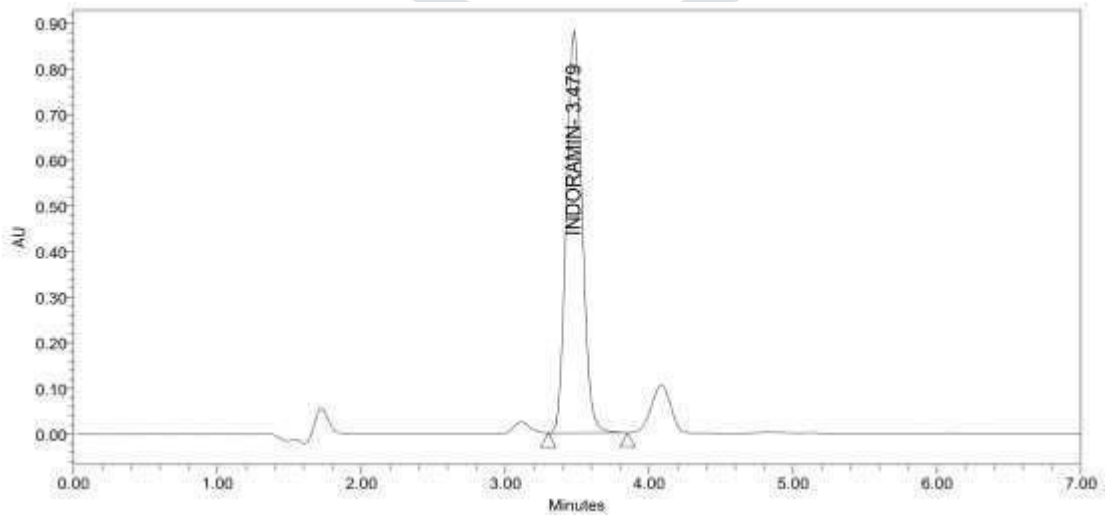


Fig. Acidic Degradation of Indoramin Tablet

3.2.6.2 Base Degradation:

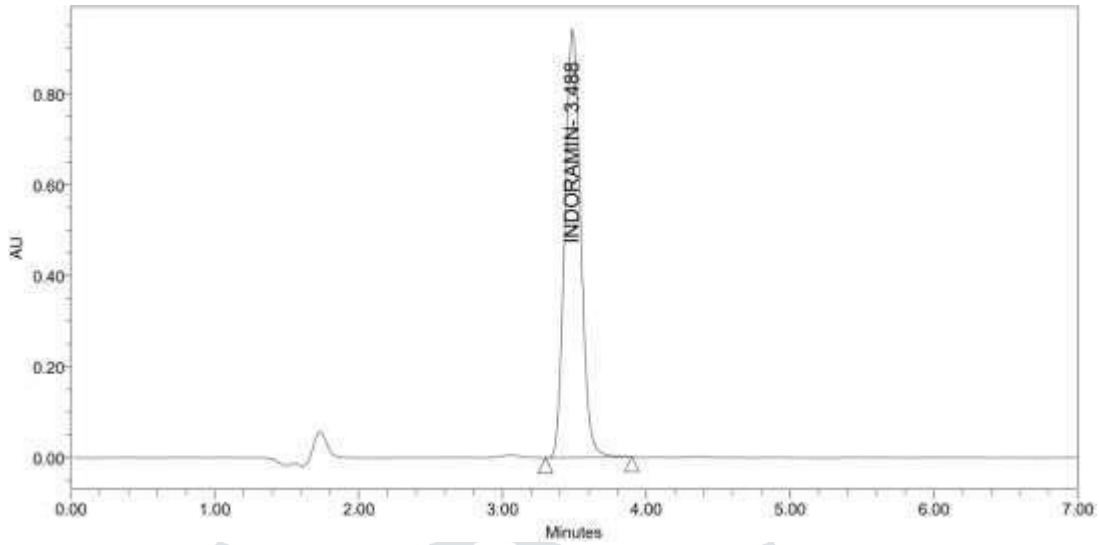


Fig. Base Degradation of Indoramin Tablet

3.2.6.3 Thermal degradation:

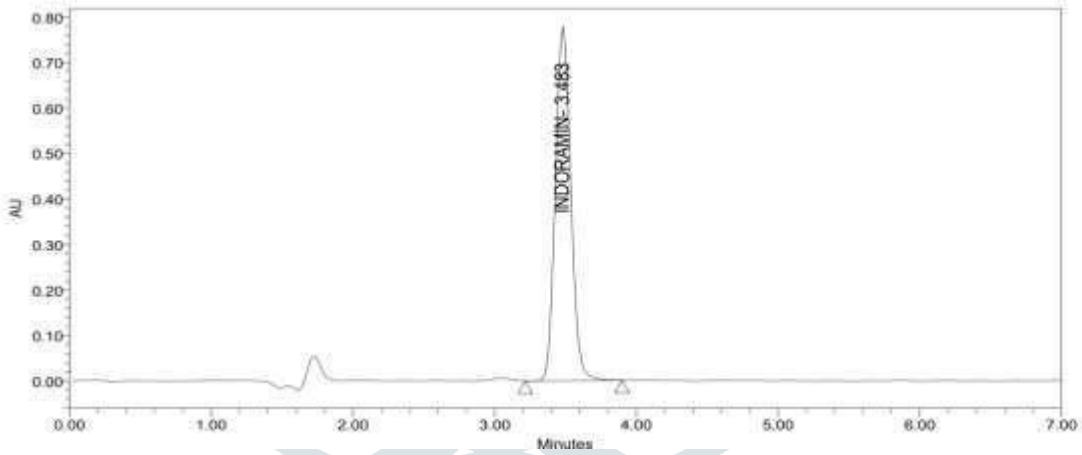


Fig. Thermal degradation of Indoramin Tablet

3.2.6.4 Oxidative Degradation:

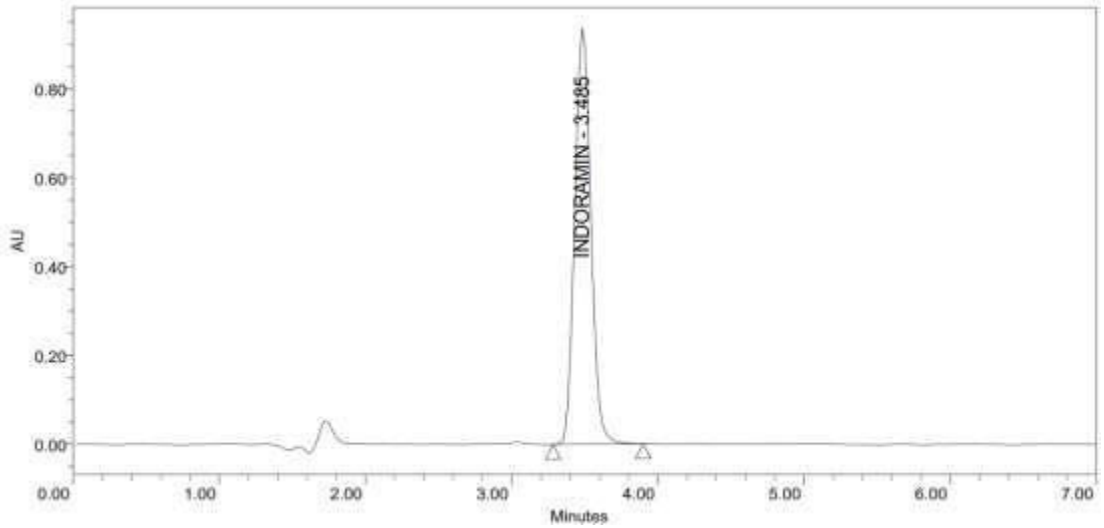


Fig. Oxidative Degradation of IndoraminTablet

3.2.6.5 Photo Degradation:

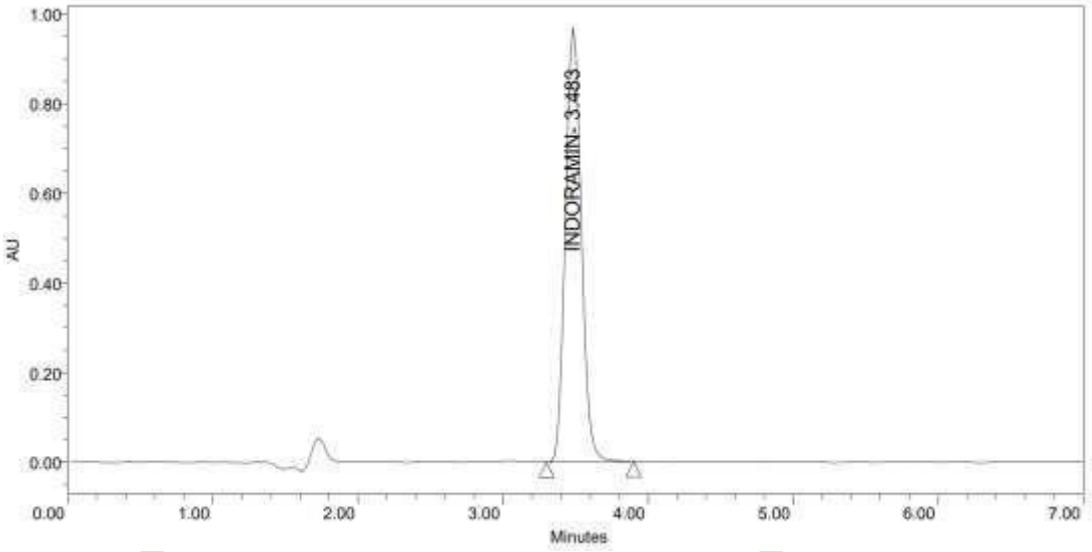


Fig. Photo Degradation of Indoramin Tablet (open)

04. SUMMARY

The results of analysis in this method were validated in terms of accuracy, precision, ruggedness, linearity. The method was found to be sensitive, reliable, reproducible, rapid andeconomic also.

Table 4.1 Summary of System suitability

Sr. No	Validation parameter		Results
1	system suitability	% RSD	0.1
		USP Tailing	1.0
		Theoretical Plates	16384
2	Specificity	**Identification**	
		R.T of Standard Solution	3.480
		R.T of Sample Solution	3.450
		Interference & Peak Purity	
		Standard Solution	Specific
		Sample Solution	Specific
3	LINEARITY	Correlation Co-coefficient (R)	0.9998
		%Y- intercept	0.93
4	Accuracy	Level 50%	99.2
		Level 100%	100.8
		Level 150%	99.4
5	Precision	Method Precision	0.3%
		Intermediate Precision	0.4%
6	Robustness		Robust

Force Degradation				
Reagents	**% Assay**	**%** difference **degradation**	**Purity Angle**	**Purity threshold**
Control	99.8	NA	0.2	1.27
Acid	97.4	2.4	0.2	0.8
Base	99.5	0.3	0.09	0.3
Thermal	99.4	0.4	0.1	0.8
Peroxide	97.8	2.0	0.005	0.2
Photo	99.3	0.5	0.08	0.6

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Conclusion:

From the studies it can be concluded that RP-HPLC technique can be successfully used forthe estimation of the Indoramin in tablet Formulations. The method shows goodreproducibility; more over the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The analysis of single dose formulation of Indoramin tablet can also besuccessfully performed by the RP-HPLC method. No interference of additives, matrix etc. is encountered in these methods. Further studies on other pharmaceutical formulations would throw more light on these studies. Suitability of these methods on biological samples needy also studies.

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