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RP HPLC Estimation OF Atenolol and Indapamide in Bulk and Pharmaceutical Dosage Form Simultaneously

Sunitha N[1*], Hemantha N[2], Manohar Babu S[3]

[1]Associate Professor , SIMS College of Pharmacy, Mangaldas nagar, Guntur, India

[2]Student, SIMS College of Pharmacy, Mangaldas nagar, Guntur, India

[3]Professor, SIMS College of Pharmacy, Mangaldas nagar, Guntur, India

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[*]**Corresponding author:** Sunitha N. Associate Professor , SIMS College of Pharmacy, Mangaldas nagar, Guntur, India

ABSTRACT

A reserve phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Atenolol and Indapamide in bulk and its pharmaceutical formulation. Separation was achieved with anphenomenex C18 coloumn, 250mm x 4.6mm (particles with 5µm).A mixture of methanol and HPLC water (50:50)as mobile phase at a flow rate of 1 ml/min and the column temperature was maintained at 25˚c. Dual wavelength detector was performed at 231 nm with a run time of 10 minutes. The method was rapid, simple and sensitive. The described method of Atenolol and Indapamide is linear in the range of 10-50µg/ml with correlation coefficient of 0.996 for Atenolol and 0.997 for Indapamide respectively. The method enables accurate, precise and rapid analysis of Atenolol and Indapamide. It can be conveniently adopted for routine quality control analysis of bulk and pharmaceutical formulation.

Keywords: RP-HPLC , HPLC methanol ,HPLC water , phenomenex C18 column

INTRODUCTION

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs[1]. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients[2].

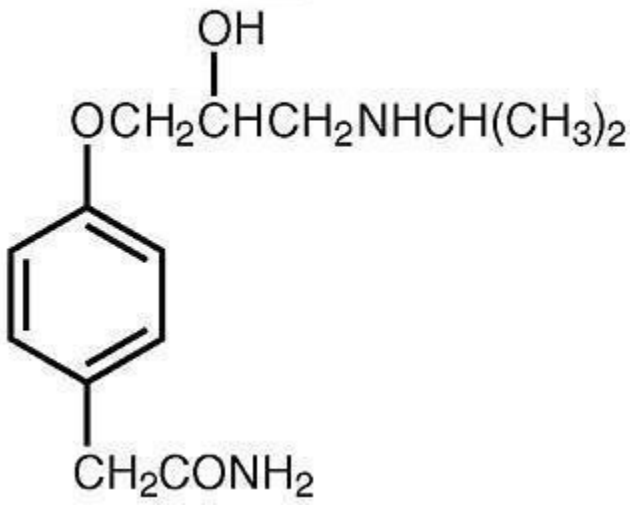
The wide variety of challenges is encountered while developing the methods for different drugs depending on its nature and properties[3]. This along with the importance of achieving the selectivity, reproducibility and accuracy of results gives an opportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry and chemical laboratories [5].

Atenolol

It is a β- blocker medication primarily used to treat high blood pressure associated with chest pain it works by blocking β1 adrenergic receptors in the heart, thus decreasing the heart rate and work load.

Figure 1. Structure of Atenolol

IUPAC Name: (rs)-4-(2-hydroxy-3-isopropyl amino propoxy) phenylacetamide



Molecular Formula: C14H22N2O3

Molecular weight: 266.3

Solubility: soluble in ethanol, sparingly soluble in water, slightly soluble in dichloromethane, practically insoluble in ether

pKa: 9.6

Category: anti- hypertensive

Dose : 50- 100mg,daily in 1or 2 doses

Description: a white powder

It contains not less than 99.0 percent and not more than101.0% of atenolol, calculated on the dried basis.

Melting point: 146-148°c or 150-152°c

Storage: store at a room temperature away from light and moisture

Uses: Atenolol is used with or without medication to treat high blood pressure(hypertension) lowering blood pressure helps to prevent strokes, heart attacks, kidney problems. This medication is also used to treat chest pain(angina) and to improve the survival after a heart stroke

Atenolol belongs to a class of drugs known as beta blockers it works by blocking the certain natural chemicals in your body such as epinephrine, on the heart and blood vessels. This effects lowers the heart rate , blood pressure, and strain on the heart

Mechanism of action: A relatively selective β1blocker having low lipid solubility. It is incompletely absorbed orally, but first pass metabolism is not significant. Because of longer duration of action, once daily dose is often sufficient. side effects related to CNS action are less likely. No deleterious effects on lipid profile have been noted.. S(-) atenolol is a pure active enantiomer is effective at half of the dose and may be better tolerated

Adverse effects: Dizziness, lightheadedness, nausea

Contraindications: overdose of atenolol leads to the very slow heart rate, severe dizziness, trouble breathing

Marketed formulations : Atenolol oral tablets

Indapamide

Indapamide is a thiazide like diuretic drug generally used in treatment of hypertension , as wellas decompensated heart failure.

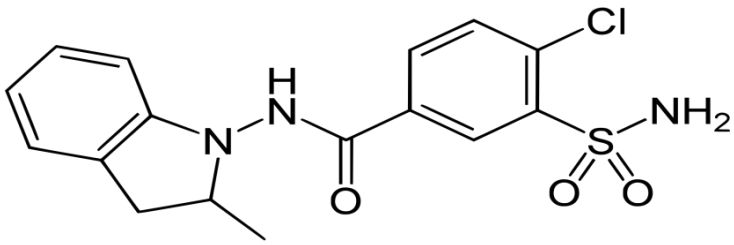


Figure 2. Structure of Indapamide

IUPAC Name: 4-chloro-N-(2-methyl-1-indonyl)-3-sulphamoylbenzamide.

Molecular Formula: C16H16ClN3O3S

Molecular weight: 365.832g/mol

Solubility: soluble in 95% ethanol very slightly soluble in ether, practically soluble insoluble in water.

Pka: 8.8

Category: Anti hypertensive , Diuretic

Description: A white to off-white, crystalline powder

Dose: 2.5mg daily

Melting point: 161°c

Storage: stored at room temperature between 59-86°F(15-30°C)away from light and moisture. Applications : it is a thiazide like diuretic .used as anti hypertensive. used to treat edema.

Mechanism of action: Indapamide blocks the slow component of delayed rectifier potassium current (IKs) without altering the rapid component (IKr) or the inward rectifier current. Specifically it blocks or antagonizes the action the proteins KCNQ1 and KCNE1. Indapamide is also thought to stimulate the synthesis of the vasodilatory hypotensive prostaglandin PGE2.

Adverse effects: Low potassium levels, fatigue, orthostatic, hypotension (blood pressure decrease on standing up), and allergic manifestations.

Contraindications : Sympathectomy , Gout , Diabetes , Low amount of sodium levels in blood , Diabetes , Severe renal impairement , Liver problems , High amount of uric acid in blood , Absence of urine formation , extreme loss of body water.

Available marketed formulations : Indapamide oral tablets.

METHOD DEVELOPMENT

Preparation of solutions for estimation of Atenolol & Indapamide

MOBILE PHASE-A

Take 80ml of HPLC grade methanol in 100ml measuring cylinder rand 20ml of HPLC grade water and filter the solution by using vaccum filtration pump and degas the solution for 20minutes

MOBILE PHASE-B

Take 50 ml of HPLC grade methanol in 100ml measuring cylinder and 50ml of HPLC grade water and adjust the pH to 2.8 with 0.1%orthophosphoric acid and degas the solution for 20minutes

Stock solution.1

Weigh 10mg of Atenolol in 10ml volumetric flask and dissolve with methanol and make upto the final volume with HPLC grade water

Stock solution.2

Weigh10mg of In dapamide in 10ml volumetric flask and dissolve with methanol and make upto the final volume with HPLC grade water

Sample solution

Pipette out 0.1 ml of sample from stock solution-1 and 0.1 ml of sample from stock solution -2andmake upto the final volume with HPLC grade methanol and HPLC grade water

Selection of wave length

The working standard solution of Atenolol and Indapamide was scanned in the u.v region and spectrum wasre corded. Methanol and water are used as blank .solutions werescannedonspectrophotometerintherangeof200-400nm.Itwasseenthatat230nmthemaximumabsorbance was found and the concentarations of Atenolol and Indapamide were calculated byusingabsorptionratiomethodat231and242nm.

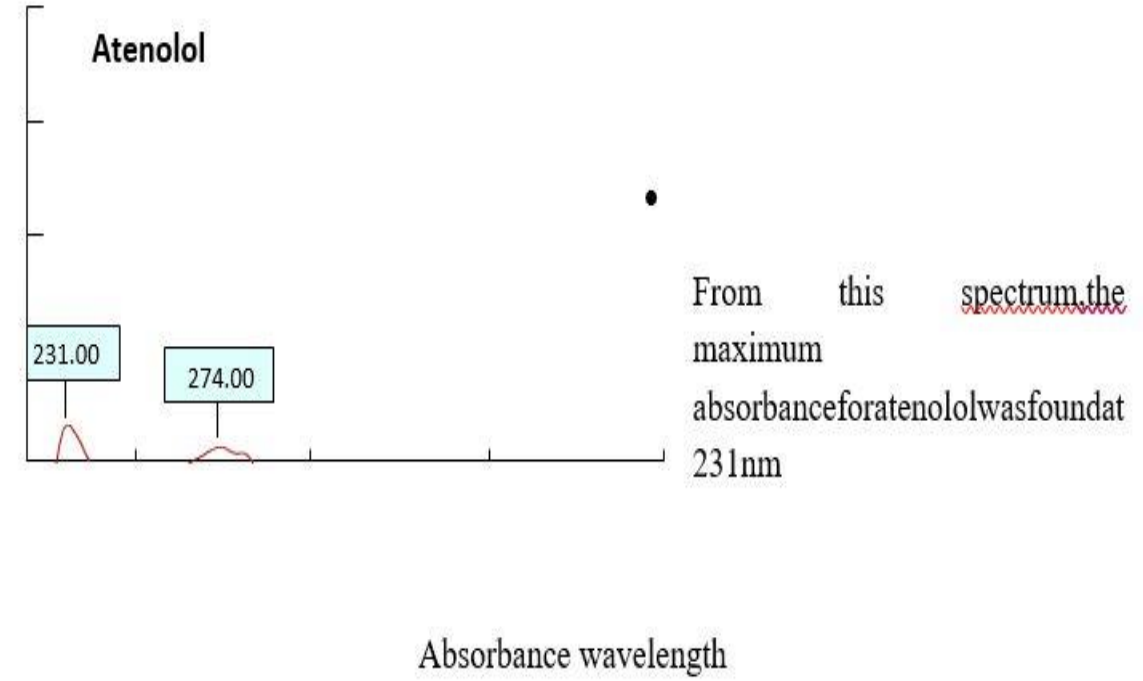


Figure 3. Absorbance spectrum of Atenolol

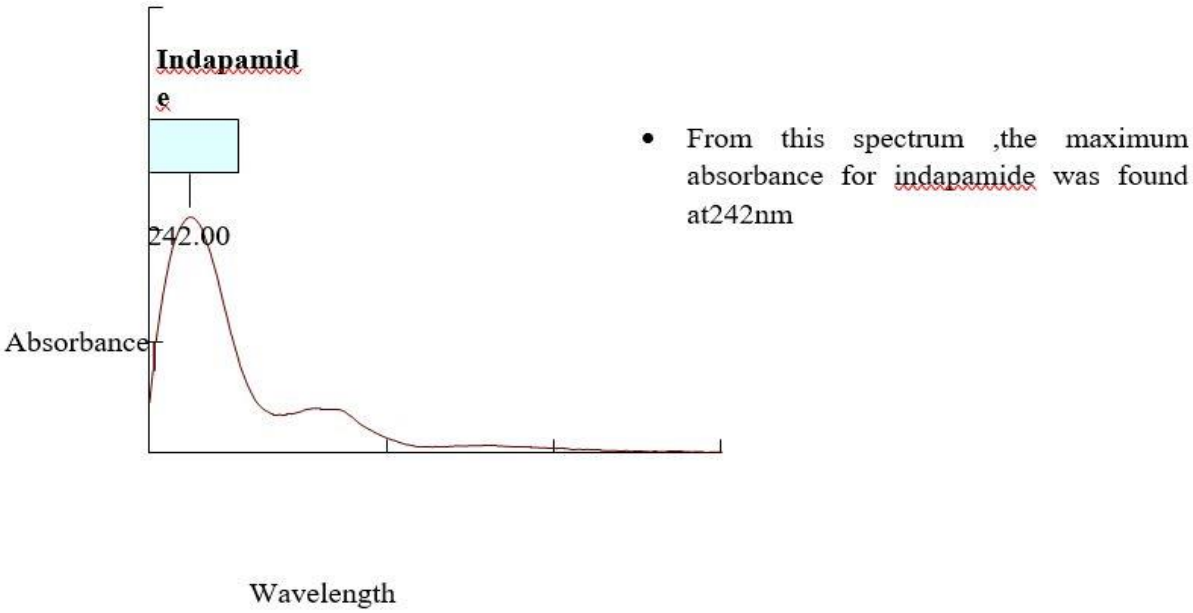


Figure 4. Absorbance spectrum of Indapamide

TEST(atenolol+indapamide)

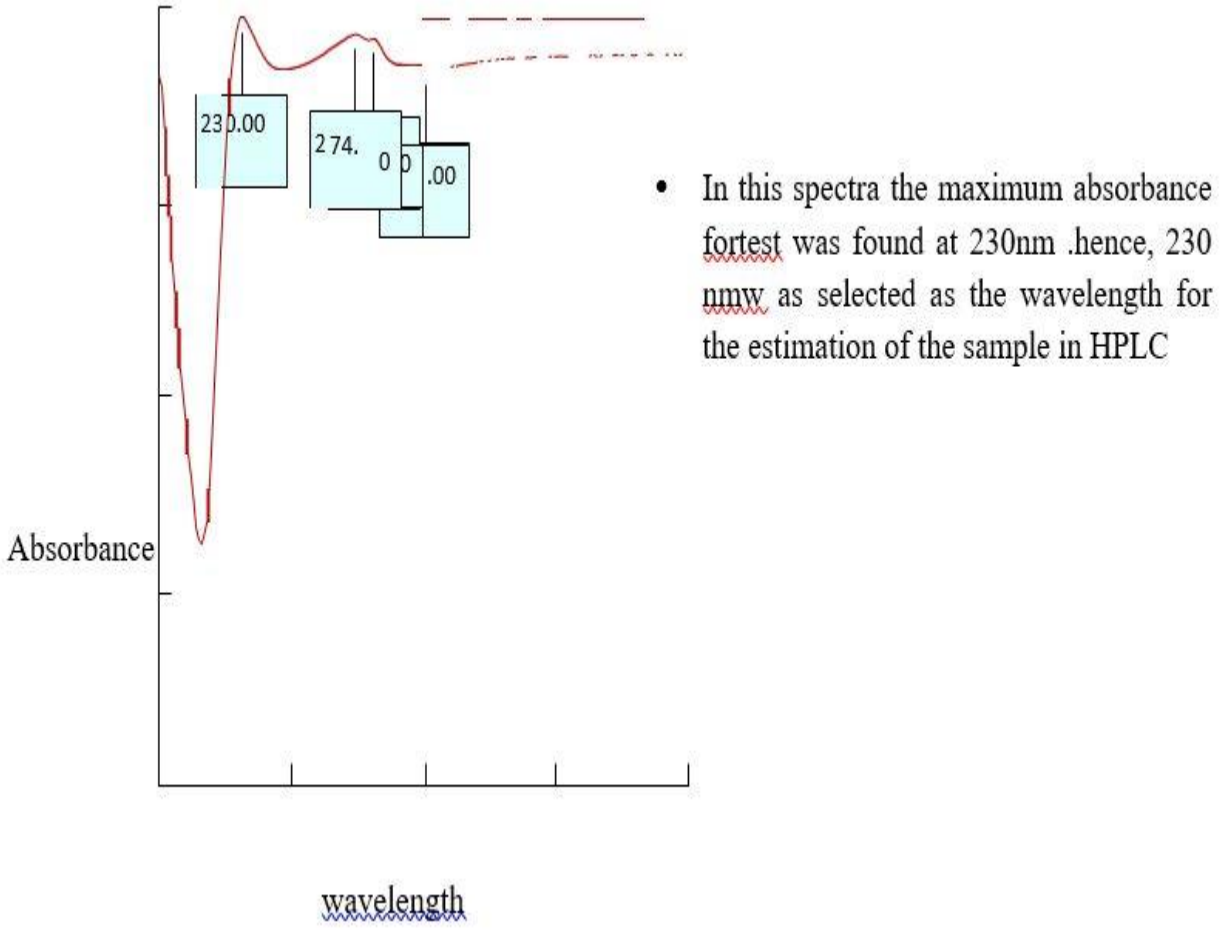


Figure 5. Absorbance spectrum of test

METHOD VALIDATION

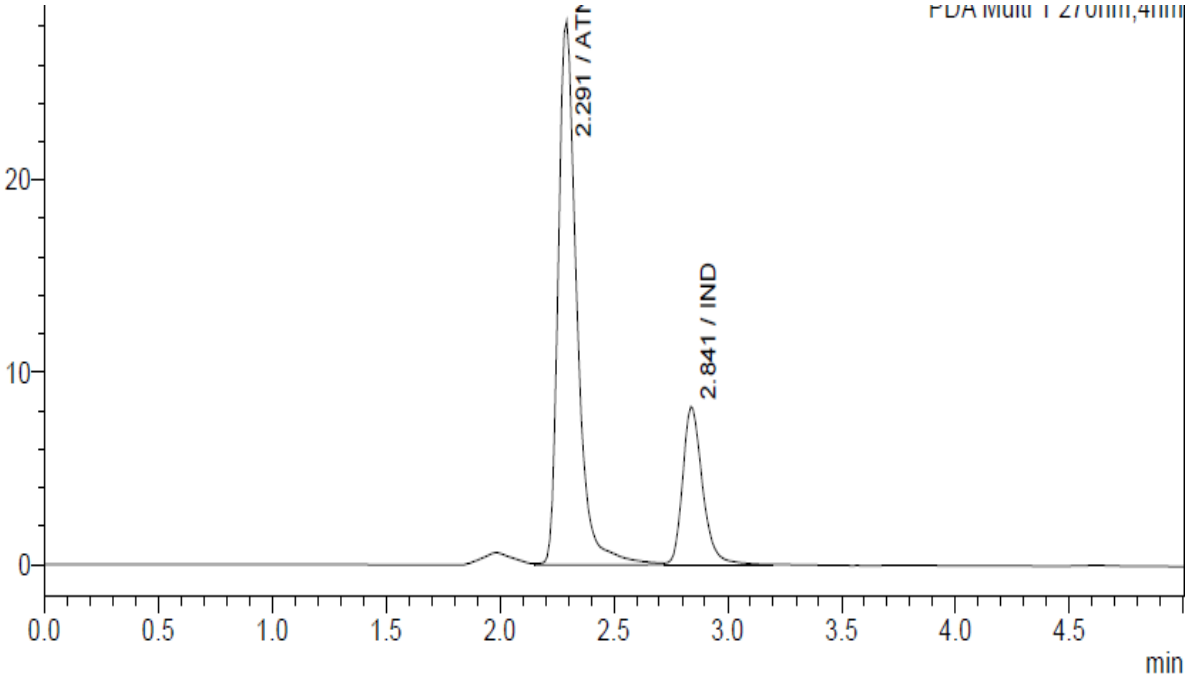


Figure 6. Standard chromatogram

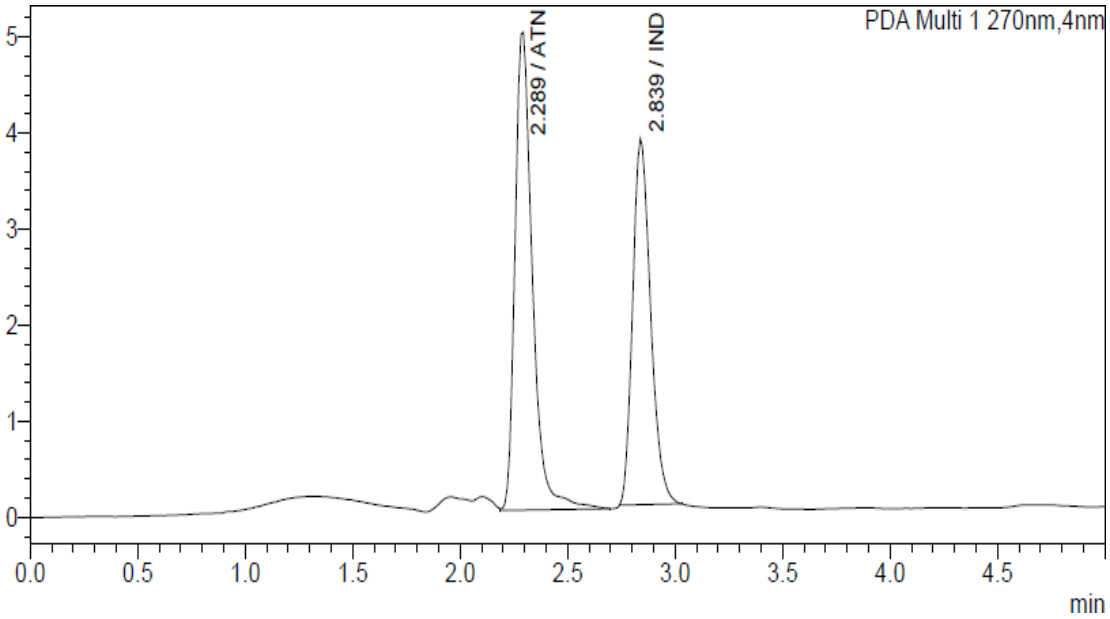


Figure 7. Test Chromatogram

System suitability

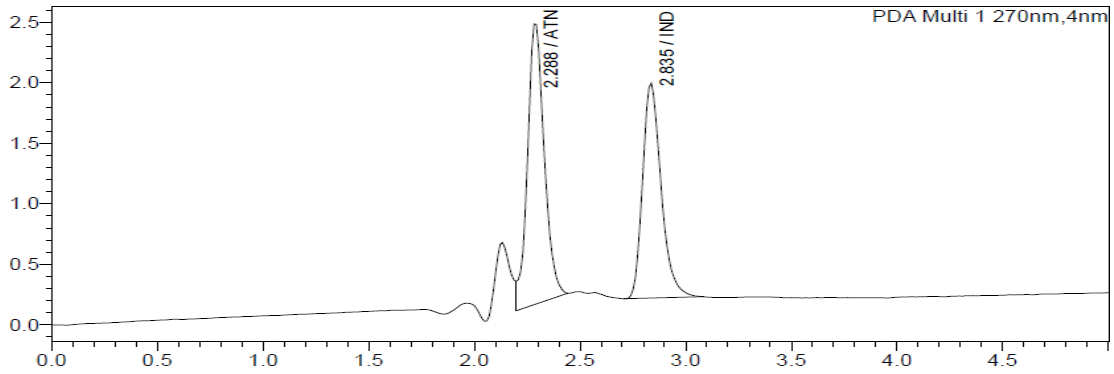


Figure 8. Chromatogram of system suitability-1

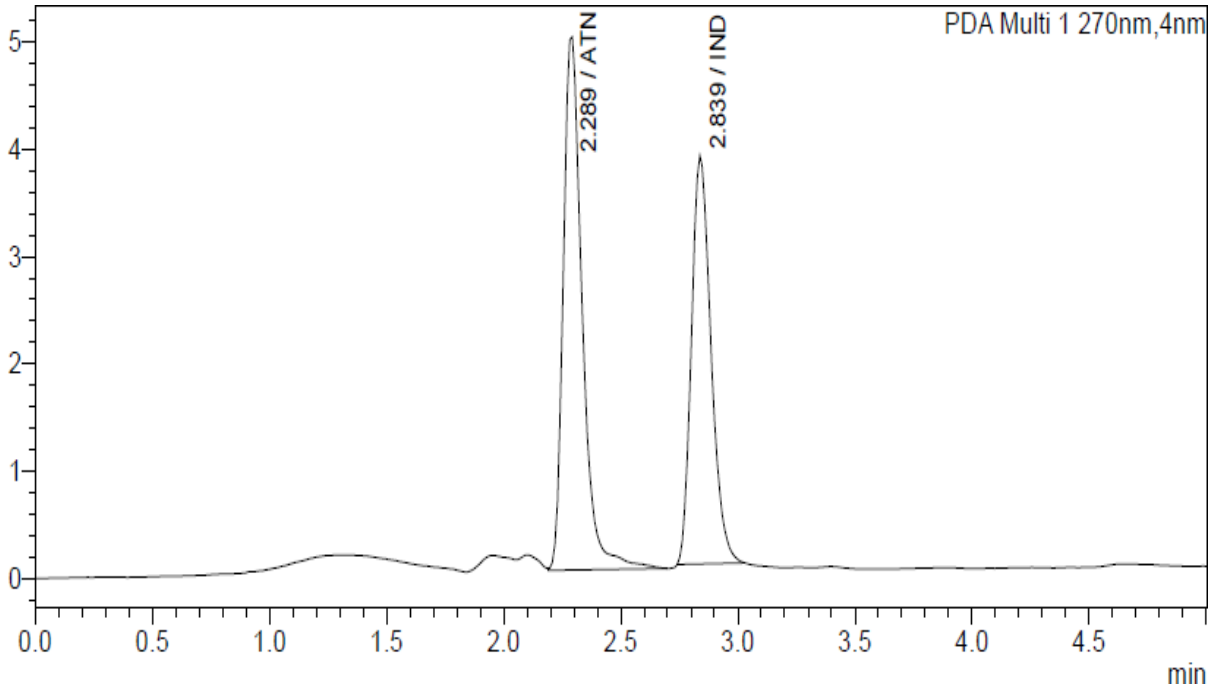


Figure 9. Chromatogramofsystemsuitability-2

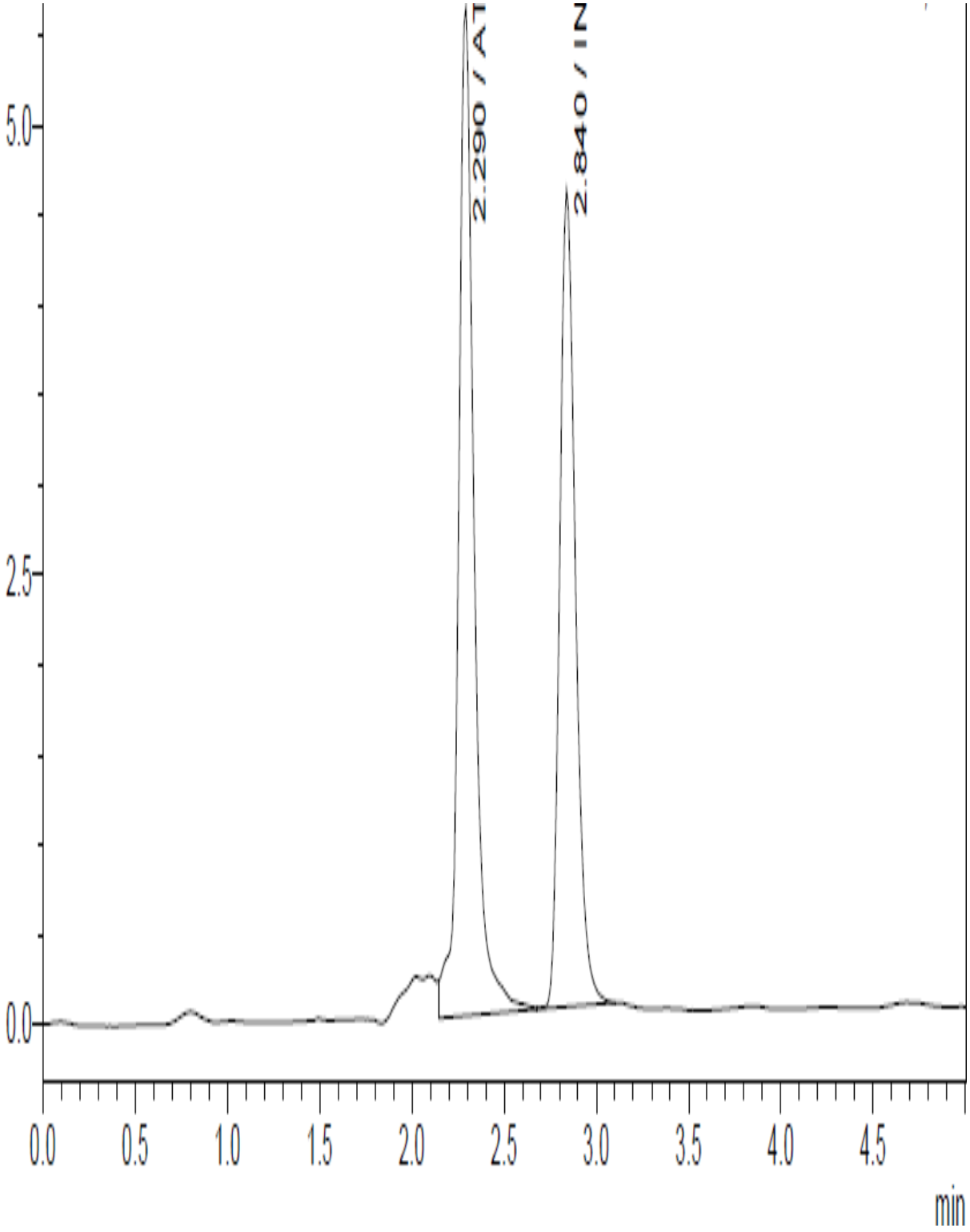

2.290 / AT
2.840 / NZ
5.0
2.5
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
min

Figure 10. Chromatogramofsystemsuitability-3

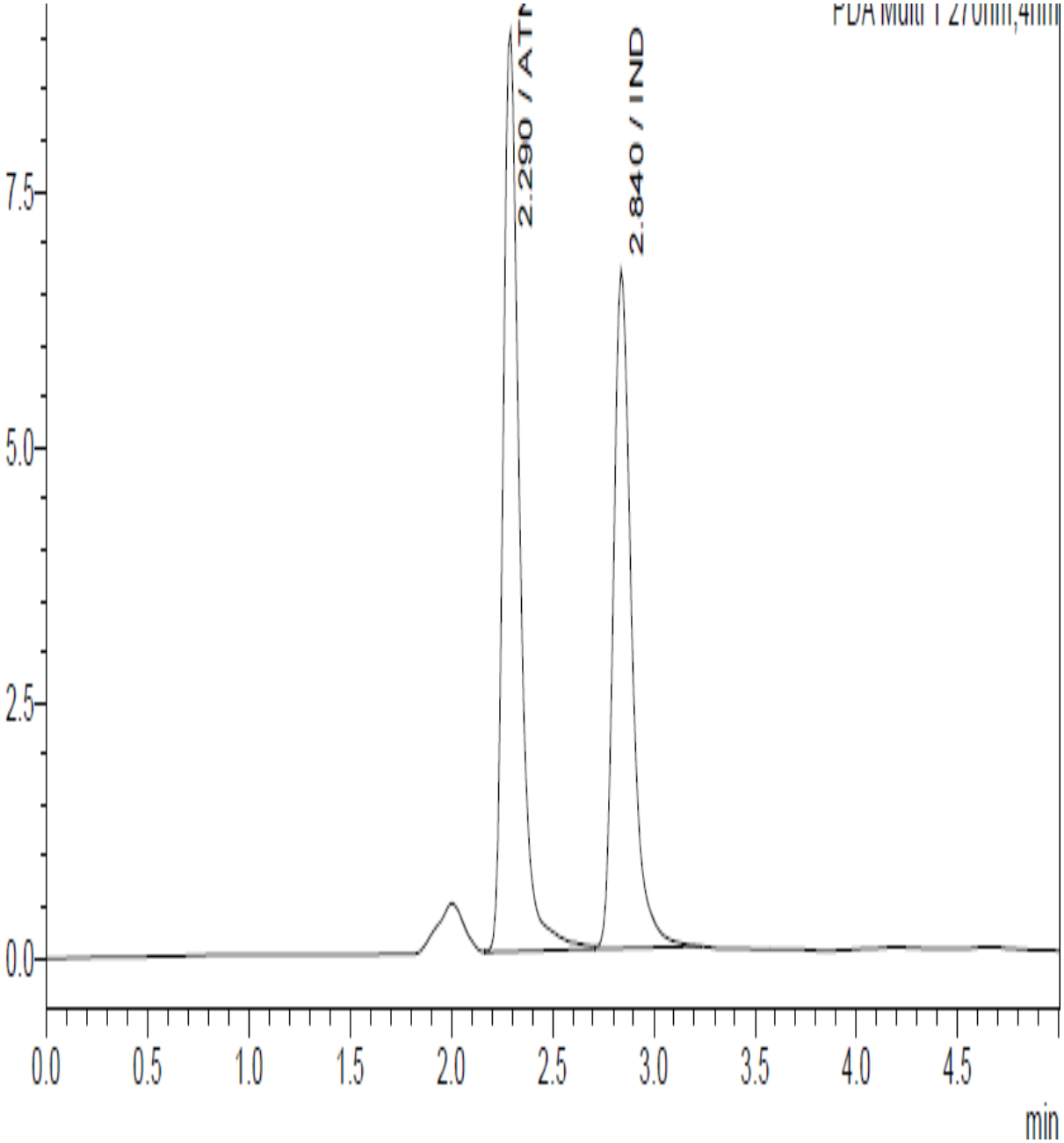


Figure 11. Chromatogramofsystemsuitability-4

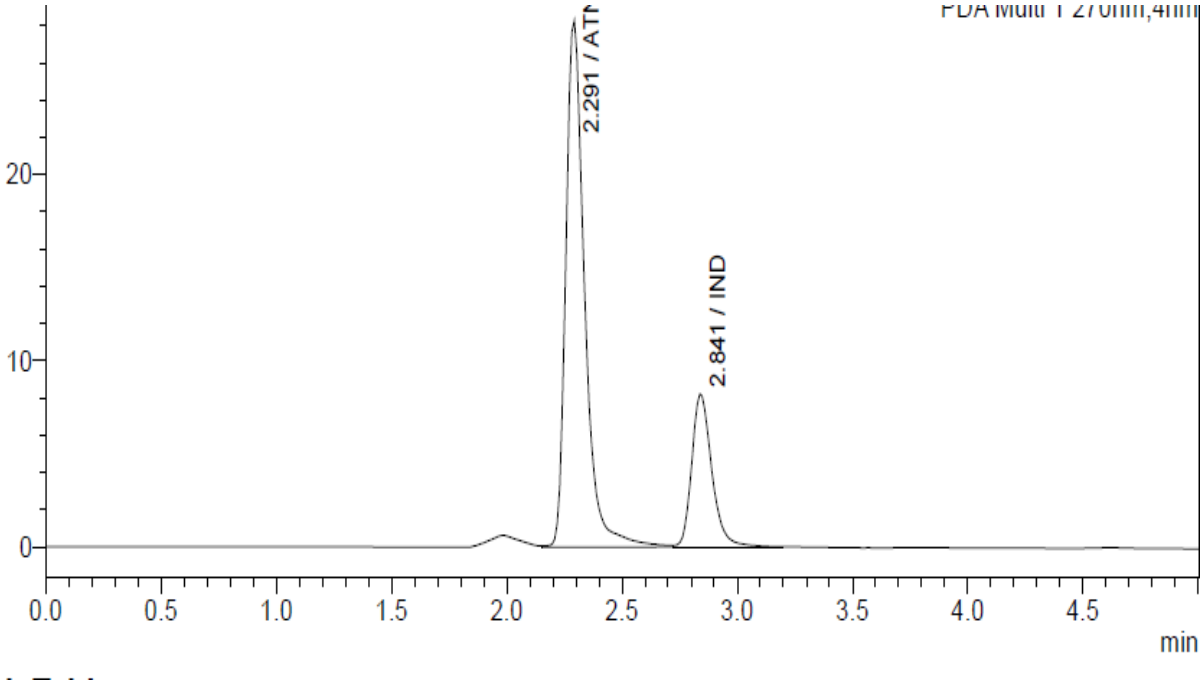


Figure 12. Chromatogram of system suitability

Table 1. Results of system suitability

	INJECTION	ATENOLOLPEAK AREA	INDAPAMIDE PEAKAREA
100µg/ml	1	21919186	23812770
	2	21820187	23812769
	3	21988086	23811758
	4	21873276	23912599
	5	21869121	23822669
STATISTICALDATA	**MEAN**	21894061.2	2383451.3
	STD	63176.04	43877.824
	%RSD	0.28853	1.84
	TAILING FACTOR	1.2	0.9
	PLATE COUNT	8256	5634

Specificity

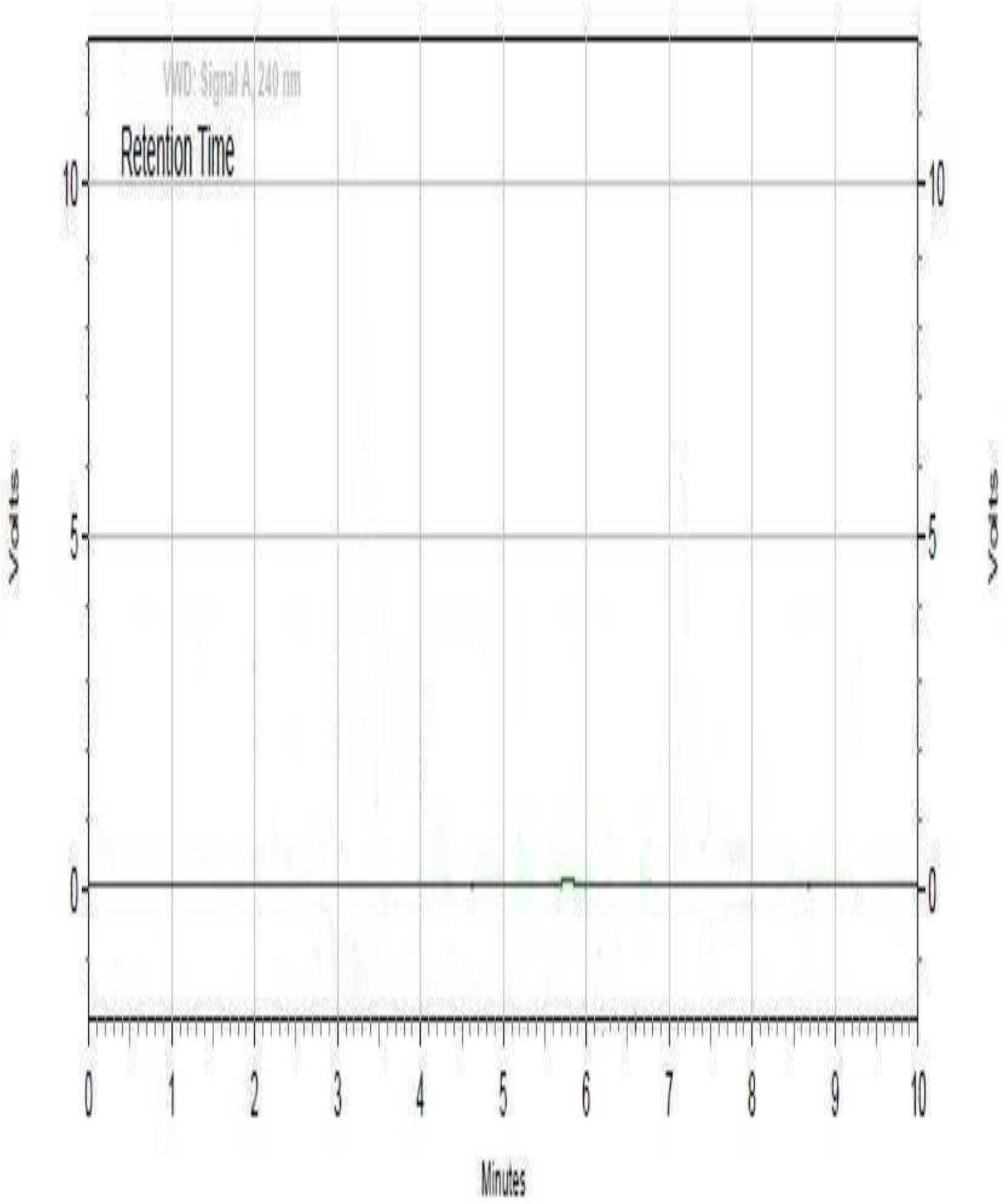


Figure 13: Blankchromatogram

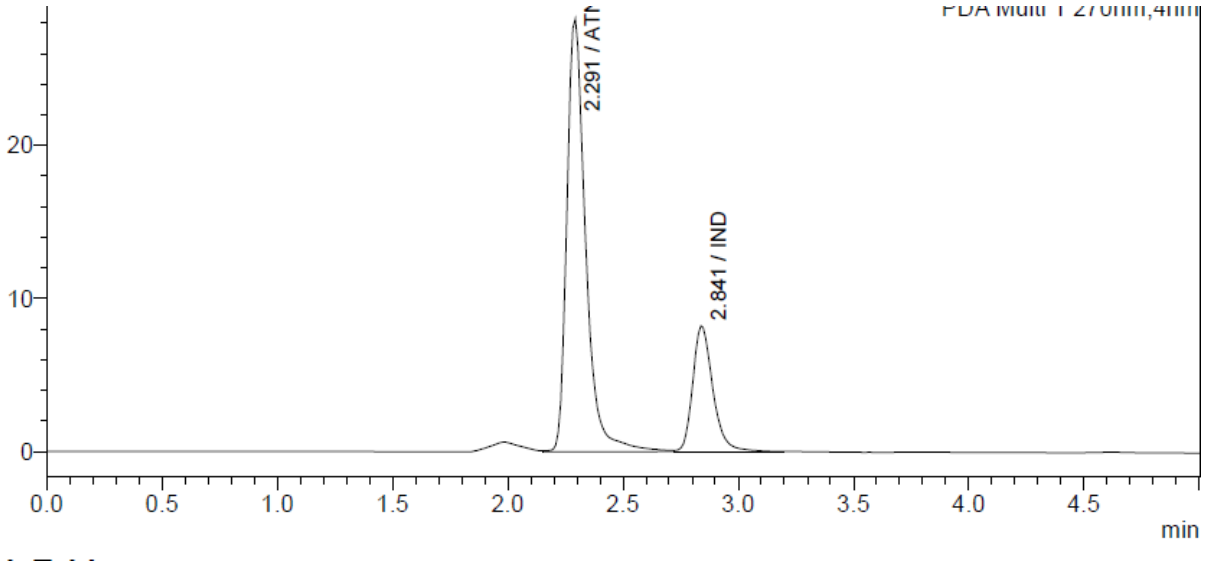


Figure 15: Typical standard chromatogram

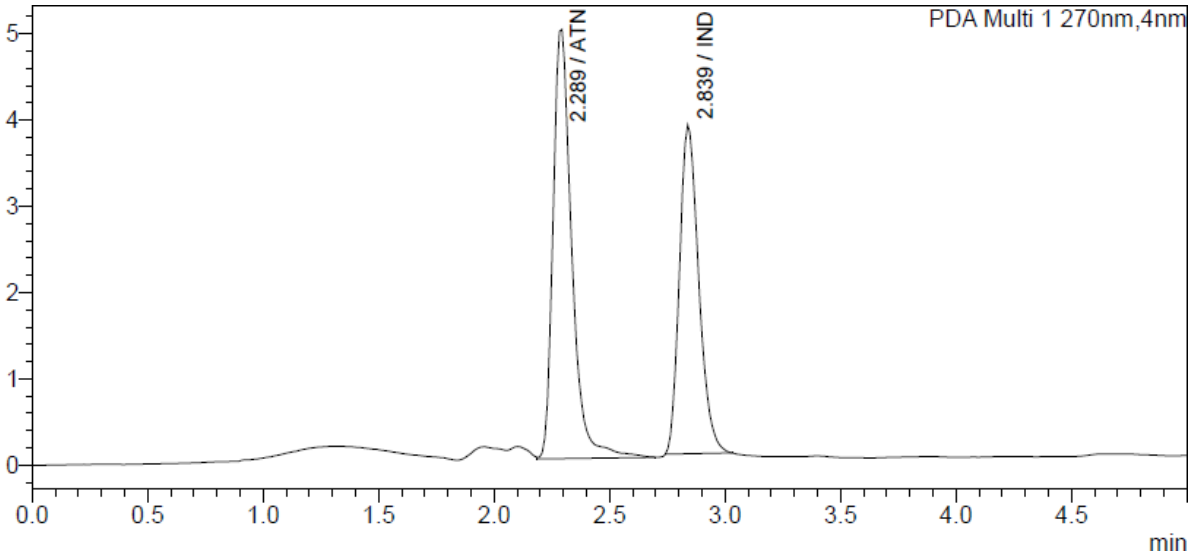


Figure 16: Typicalsamplechromatogram

Table 2: Results of specificity

Name	Retentiontime	Area	Theoretical plates	USPtailing
Standard				
Atenolol	2.291	21869121	8278	1.2
Indapamide	2.841	23822669	5672	0.9

Sample				
Atenolol	2.778	642715	8260	1.3
Indapamide	4.255	2748402	5660	0.9

Precision

System precision (injection repeatability)

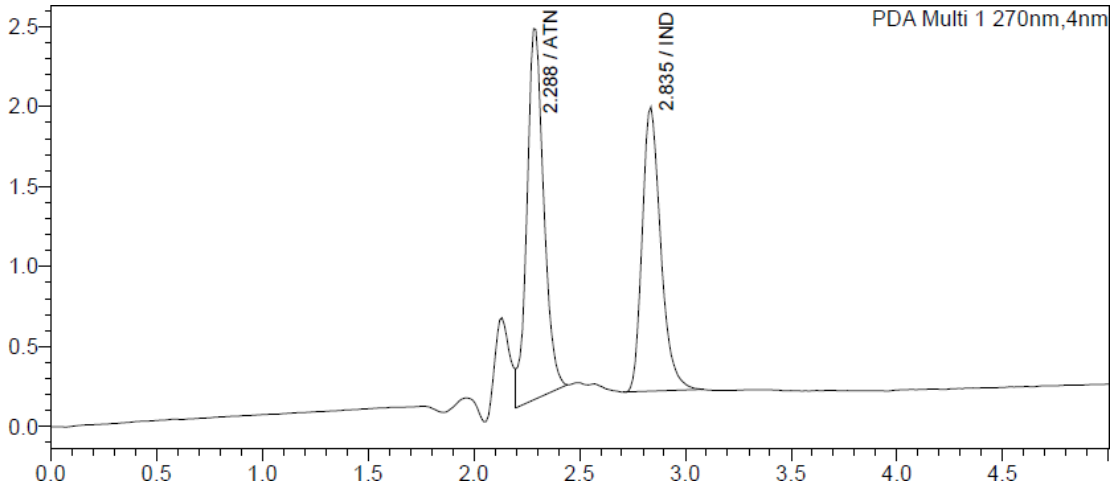


Figure 17. Systemprecisionchromatogram-1

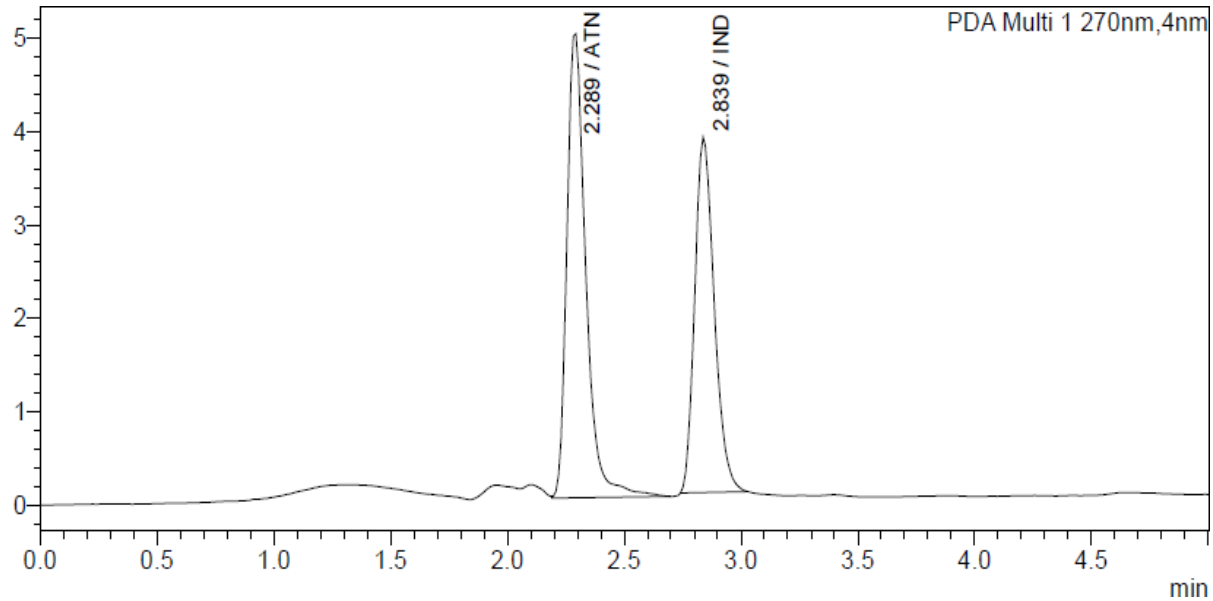


Figure 18. System precision chromatogram-2

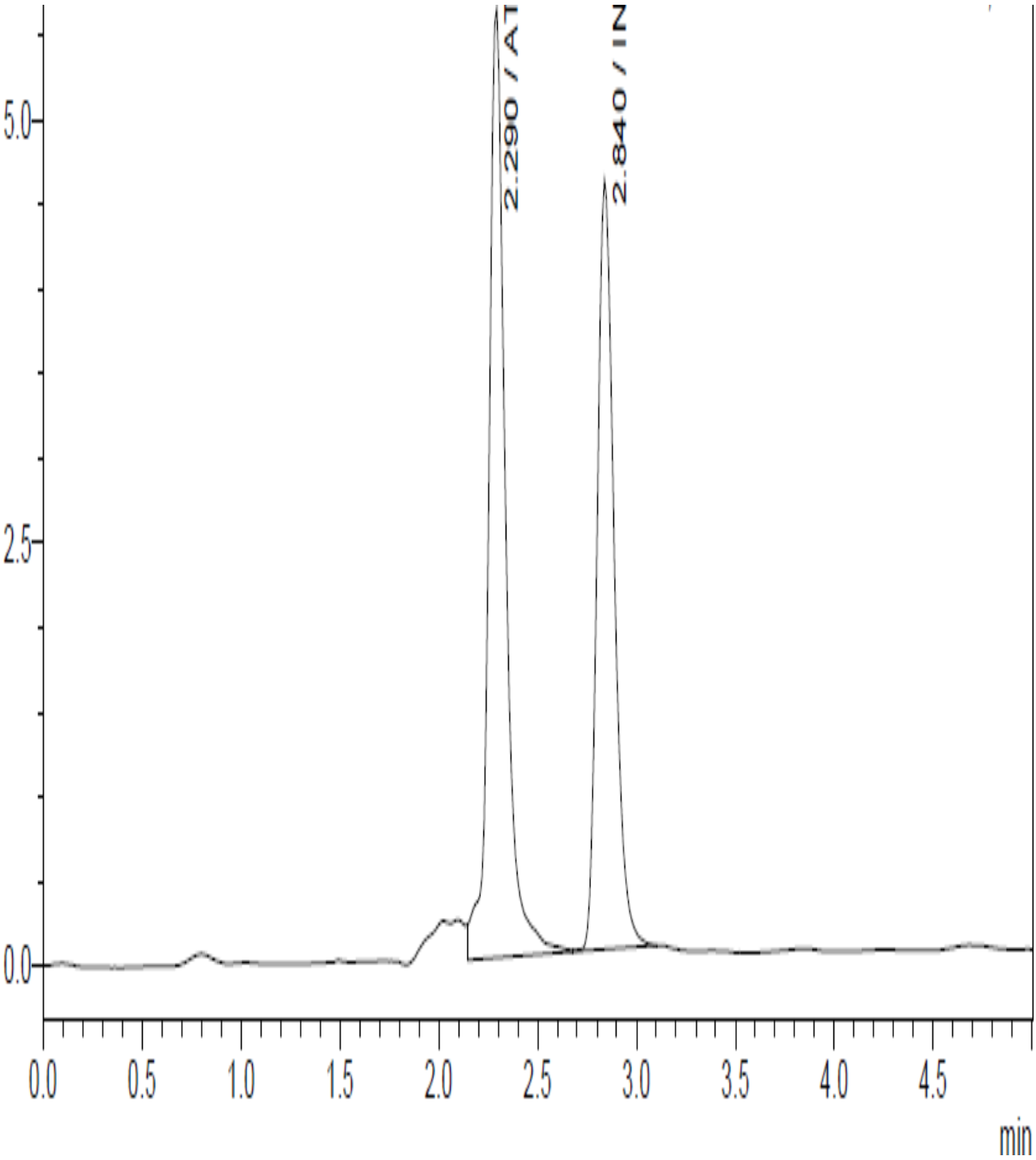


Figure 19. Systemprecisionchromatogram-3

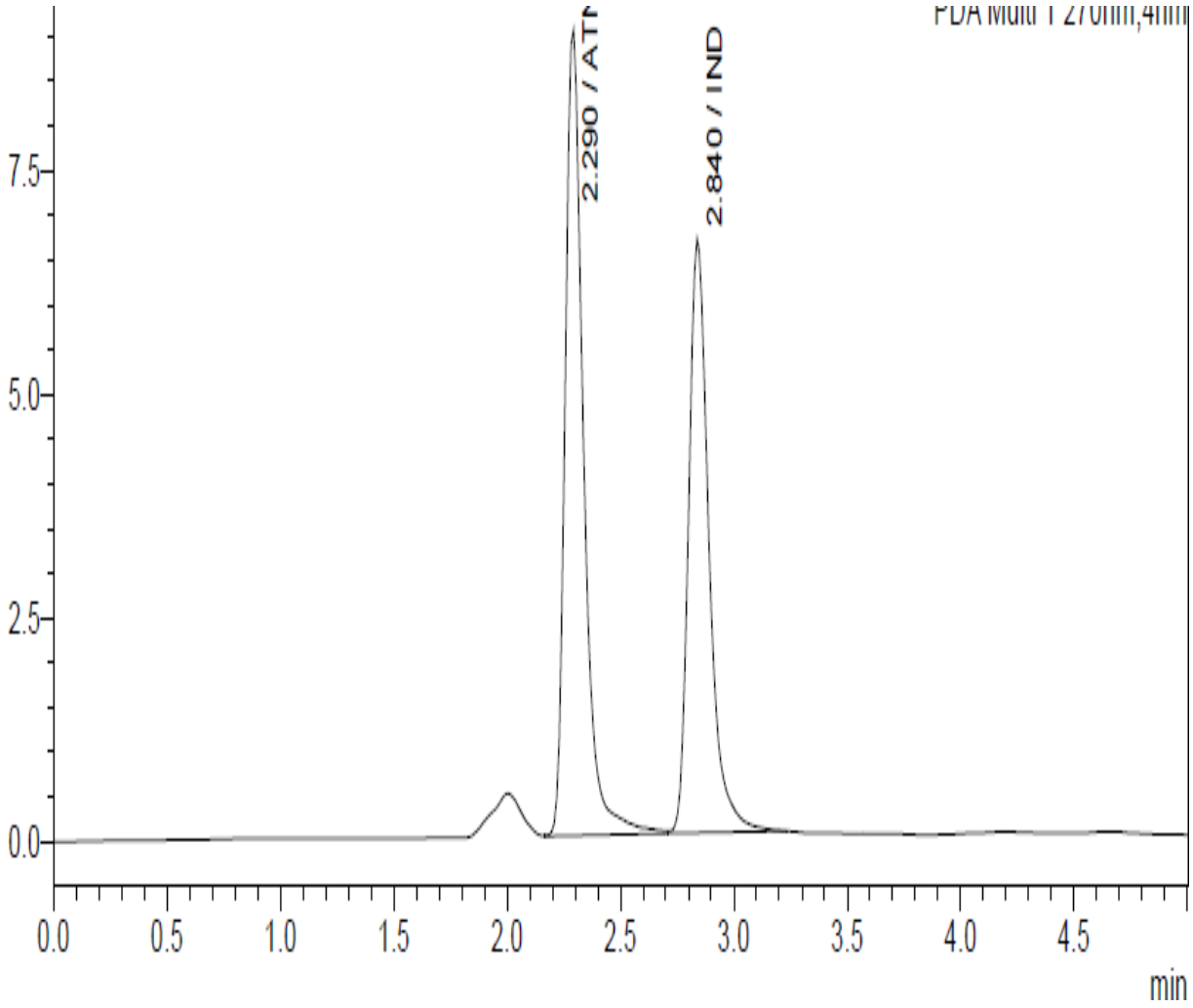


Figure 20. Systemprecisionchromatogram-4

Table 3. Results of system precision

Injections	Atenololpeakarea	Indapamidepeakarea
Injections-1	20950247	21951514
Injections-2	20675695	21714821
Injections-3	20994086	21801350
Injections-4	20918426	21892154
MEAN	20884613.4	21839959.75
STDEVIATION	142691.05	103793.07
%RSD	0.683	0.475

Method precision

Table 4. Results of Method precision

Injections	Atenololpeakarea	Indapamidepeakarea
Injections-1	20540247	20950247
Injection-2	20475695	20675695
Injections-3	20814086	20994086
Injections-4	20618426	20918426
MEAN	20612113.5	20884613.5
STANDARD DEVIATION	146750.96	142691.05
%RSD	0.711	0.683

Intermediate Precision /Ruggedness

Table 5. Results of Ruggedness

INJECTIONS	AtenololPeakarea	IndapamidePeakarea
1	21849186	23712769
2	21818276	23723969
3	21848696	23789769
4	21838276	23812569
MEAN	2183608.5	23759769
STDEV	14458.7	48916.52
%RSD	0.066	0.2058

Linearity

Table 6. Results of Ruggedness

Volume of stocktaken(ml)	Diluteto(ml)	Final conc.(µg/ml)Atenolol	Final conc.(µg/ml)Indapamide
0.1	10	10	10
0.2	10	20	20
0.3	10	30	30
0.4	10	40	40
0.5	10	50	50

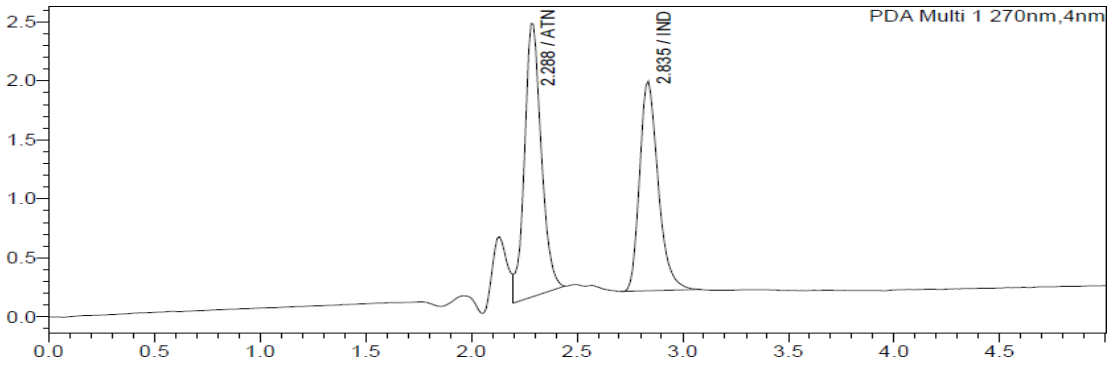


Figure 21. Chromatogramoflinearity10µg/ml

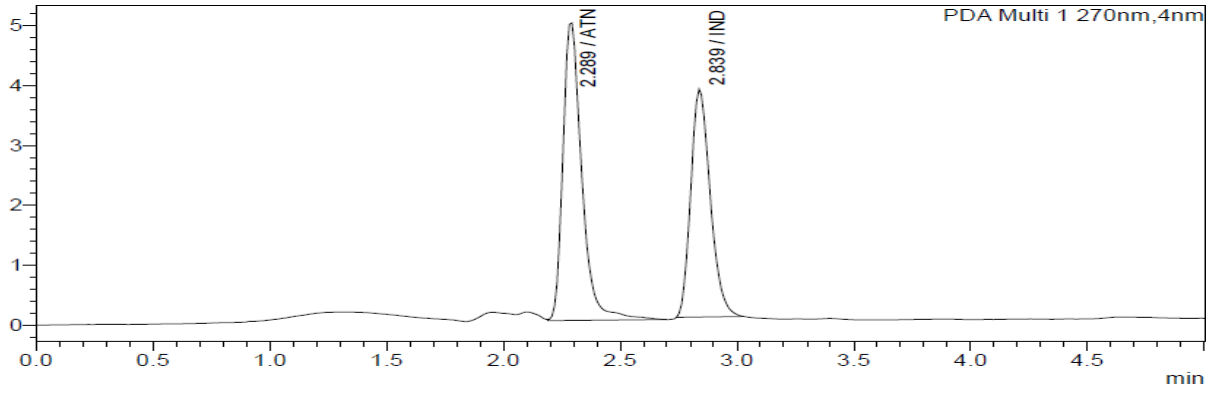


Figure 22. Chromatogram of inearity 20µg/ml

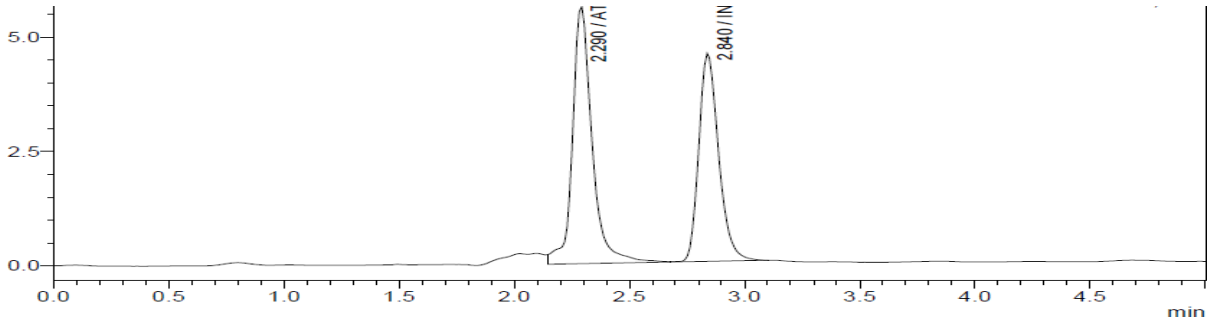


Figure 23. Chromatogram of linearity 30µg/ml

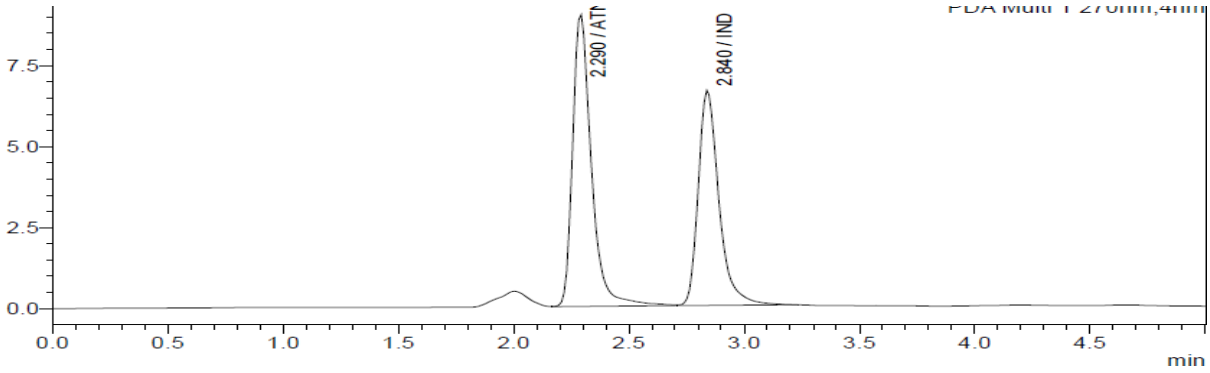


Figure 24. Chromatogram of linearity 40µg/ml

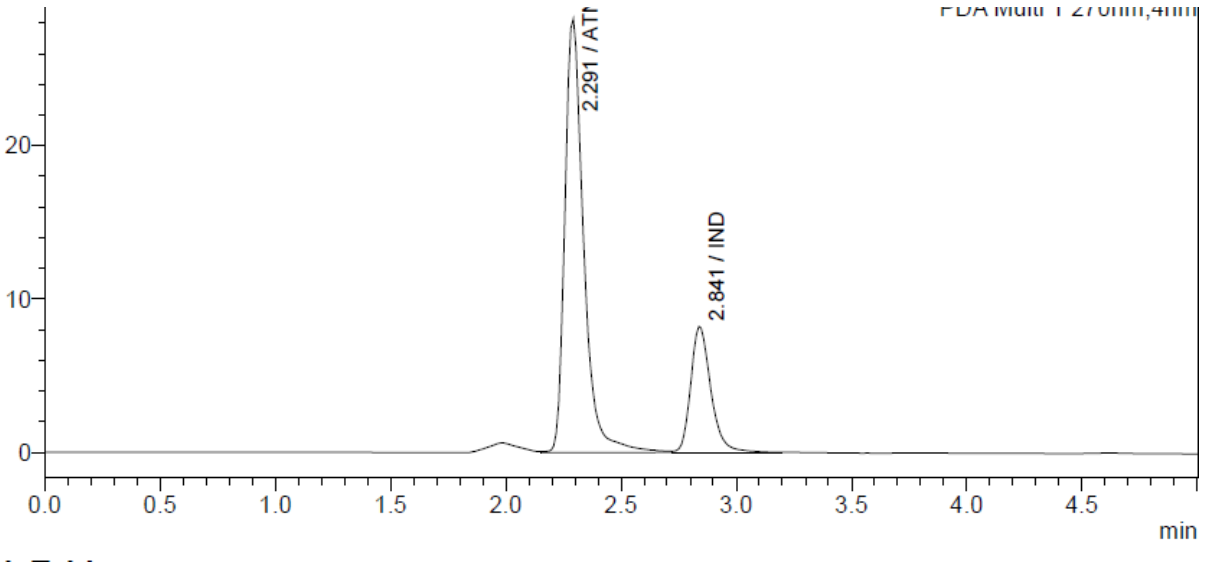


Figure 25: Chromatogram of linearity 50µg/ml

Table 7. Results of Linearity

S.No.	**Concentration(µg/ml)**	**Peakarea**
1	0	0
2	10	9152237
3	20	14605512
4	30	21849220
5	40	28339177
6	50	36304377

	Slope	70379
	Regressioncoefficient	0.996

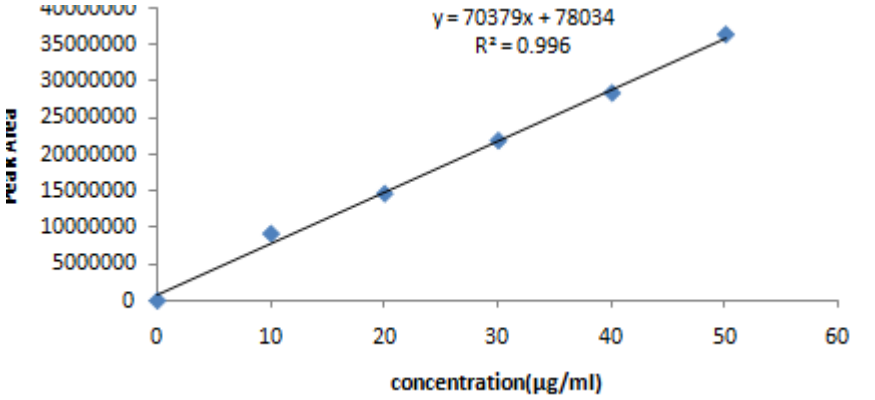


Figure 26. Linearity plot for Atenolol

Table 8. Results of Indapamide Linearity

S.No.	Conc.(µg/ml)	Peakarea
1	0	0
2	10	7349241
3	20	14785557
4	30	23712810
5	40	29613376
6	50	36553125
	Slope	73852
	Regression coefficient	0.997

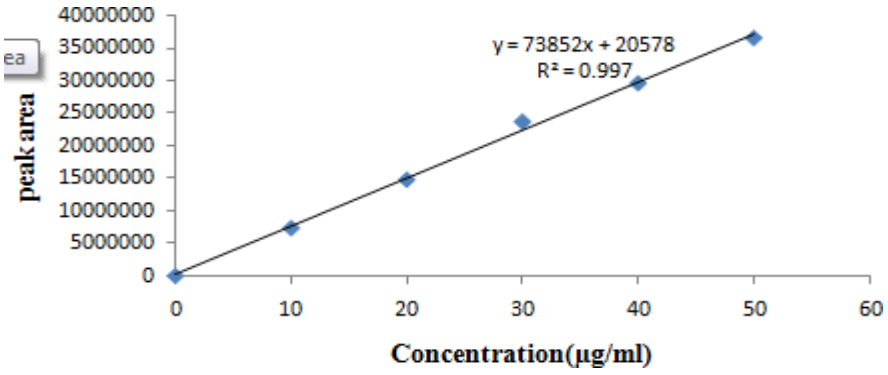


Figure 27: Linearity plot for Indapamide

Robustness

Table.9: Results of Flow rate

Concentration	Parameter	Atenolol		Indapamide	
		RT	PeakArea	RT	PeakArea
100µg/ml	Flow Rate0.9ml	4.18	22950514	7.01	24817414
		4.56	22575841	7.21	24644821
		4.89	22894918	7.12	24599350
	%RSD	**0.886**		**0.465**	
	Flow Rate1.0ml	2.29	20920514	2.84	22817414
		2.29	20645841	2.84	22644821
		2.29	20994918	2.84	22499350
	%RSD	**0.881**		**0.702**	

Table. 10: Results of wave length

Concentration	Parameter	Atenolol		Indapamide	
		RT	PeakArea	RT	PeakArea
100µg/ml		3.08		8.86	
	Wavelength225nm		20549186		21712248
		3.16	20621587	7.58	21915149
		3.88	20318481	7.83	21611419
	%RSD	**0.772**		**0.711**	
		2.29	19891486	2.84	21812248
	Wavelength230nm	2.29	19724851	2.84	21915149
		2.29	19551482	2.84	21611419
	%RSD	**0.862**		**0.709**	

SUMMARY AND CONCLUSION

The scope and objective of the present work is to optimize the chromatographic conditions to develop RP-HPLC method for the simultaneous estimation of Atenolol and Indapamide tablet dosage form and same is validated. RP-HPLC method generate large amount of quality data which serve as highly powerful and convenient analytical tool.

Literature review indicates that HPLC and UV-Spectrophotometric individual and combined methods have been reported for Atenolol and Indapamide. The developed RP-HPLC method was suitable technique for estimation of Atenolol and Indapamide in tablet pharmaceutical dosage form without any interference from other excipients. The developed method was validated as per ICH guide lines. All the parameters for drug had met the criteria of ICH guidelines for method validation. The developed method may be recommended for routine and QC

analysis of investigational drugs to provide simple, accurate and reproducible quantitative analysis. The % RSD of proposed method was found to be less than 2 % shows its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The low values of % RSD indicate the method is precise and accurate.

"From the study, we concluded that a simple, precise, accurate and economic method was developed for the routine analysis of Atenolol and Indapamide in tablet pharmaceutical dosage form".

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