ANALYTICAL METHOD DEVELOPMENT &VALIDATION OF LEFAMULIN IN BULK DRUG & DOSAGE FORM BY RP-HPLC METHOD.

Mr. Dashpute Sanket N.[1*], Mrs. Dr. P. B. Jadhav[1], Dr. R.S Kalkotwar[1]

Abstract:
Attempts were made to develop RP-HPLC method for simultaneous estimation of Lefamulin from Tablet. For the RP - Agilent Tech. Gradient System with Auto injector, UV (DAD) & Gradient Detector Reverse Phase (Waters) C18 column (4.6mm x 100mm; 2µm), a 20µl injection loop and UV730D Absorbance detector and running chemstation 10.1 software. Methanol: water (0.05%OPA), (50:50) v/v, pH 3. was used as the mobile phase for the method. The detection wavelength was 278 nm and flow rate was 1 ml/min. In the developed method, the retention time of Lefamulin were found to be be 3.513 min. The developed method was validated according to the ICH guidelines. The linearity, accuracy, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. So the proposed methods can be used for the routine quality control analysis Lefamulin in bulk drug as well as in formulations.

Keywords: LEFAMULIN, RP-HPLC, Analysis, UV-Spectroscopy.

*S.N.D. College of pharmacy, Babhulgoan, Yeola Dist- Nashik 423401 Department of Quality Assurance

***Corresponding Author ;** Mr. Dashpute Sanket N
*S.N.D. College of pharmacy, Babhulgoan, Yeola Dist- Nashik 423401 Department of Quality Assurance
Contact No. 9405359775, Email: sdashpute352@gmail.com

DOI: 10.31838/ecb/2023.12.5.490

INTRODUTION:

"LEFAMULIN"

STRUCTURE:

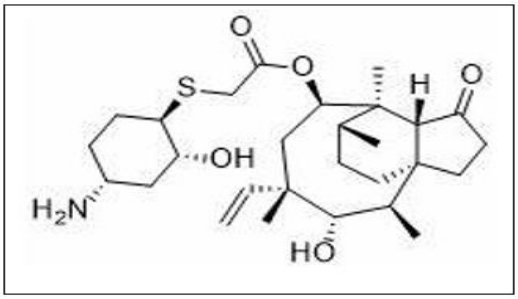


Fig No.1: Structure of Lefamulin.

Lefamulin, sold under the brand name Xenleta, is an antibiotic medication used it to treat adults with community-acquired bacterial pneumonia. It is taken by mouth or by injection into a vein.

Relatively common side effects include diarrhea, nausea, pain at the site of injection, and liver inflammation. It is a pleuromutilin antibiotic that inhibits the large subunit of bacterial ribosomes.

Table 1: Drug Profile

Molecular Formula	$C_{28}H_{45}NO_5S$
IUPAC Name	[(1S,2R,3S,4S,6R,7R,8R,14R)-4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl-9-oxo-6-tricyclo[5.4.3.0]tetradecanyl]2-[(1R,2R,4R)-4-amino-2-hydroxycyclohexyl]sulfanylacetate
Molecular weight	507.7 g/mol
Appearance	White, Crystalline powder
Solubility	soluble in ethanol, DMSO
Category	Anti-infective Agents

MECHANISM OF ACTION:

Lefamulin inhibits prokaryotic ribosomal protein synthesis via its binding to the peptidyl transferase center (PTC) of the ribosomal bacterial 50S subunit. It inhibits protein translation through binding to both the A and P sites of the PTC via four hydrogen bonds, resulting in the interruption of peptide bond formation. Lefamulin's tricyclic mutilin core is the common moiety for binding of all members of

its drug class, the pleuromutilins. Although the tricyclic motilin core doesn't form any hydrogen bonds with the PTC nucleotides, it is stabilized or anchored by hydrophobic and Van der Waals interactions. Lefamulin exerts a selective inhibition of protein translation in eukaryotes, however, does not affect ribosomal translation of eukaryotes. Lefamulin demonstrates a unique induced-fit type of action that closes the binding pocket within a ribosome, conferring close contact of the drug to its target, therefore improving therapeutic efficacy. Because of its mechanism of action that differs from that of other antimicrobials, cross-resistance to other antibiotic classes is less likely. [1-5]

PHARMACOKINETIES:

ADME:

ABSORPTION:

In a pharmacokinetic study of healthy subjects, lefamulin was rapidly absorbed after oral administration. The median Tmax was measured at 1.00 h for the intravenous preparation and 1.76 h for the tablet preparation. At steady-state doses, the Cmax of oral lefamulin is 37.1 mcg/mL. The AUC at steady-state concentrations of this drug is 49.2 mcg·h/mL. The estimated bioavailability of the oral tablets is 25%. Clinical studies have found that the AUC of lefamulin is decreased by about 10-28% in the fed state. To optimize absorption, this drug should be administered a minimum of 1 hour before a meal or, at minimum, 2 hours after a meal with water.

VOLUME OF DISTRIBUTION

The average volume of distribution of lefamulin is 86.1 L in patients with community-acquired bacterial pneumonia, but can range from 34.2 to 153 L. During clinical studies, lefamulin has been shown to significantly concentrate in the lung tissue, likely increasing its effectiveness in treating pneumonia. After lefamulin is administered, penetration into various tissues is observed, and is about 6 times greater in concentration in the fluid of the pulmonary epithelium, when compared with concentrations in the plasma. Animal studies demonstrate that lefamulin crosses the placenta.

PROTEIN BINDING

The average plasma protein binding of lefamulin is between 94.8 to 97.1% in healthy adults. A systematic review identifies the plasma protein binding at 80-87%.[6] Metabolism CYP3A4 is the main enzyme responsible for the metabolism of lefamulin.

ROUTE OF ELIMINATION

Lefamulin is largely excreted by the gastrointestinal tract and about 14% excreted by the kidneys. In healthy adult volunteers during clinical trials, a radiolabeled dose of lefamulin was administered. The total radioactivity found to be excreted in the feces was 77.3% on average with 4.2% to 9.1% as unchanged drug when the drug was administered via the intravenous route. A total radioactivity of 88.5% was measured in the feces with 7.8-24.8% as unchanged drug after a dose administered via the oral route. In the urine, it was found to be 15.5% with 9.6-14.1% excretd as unchanged drug after an intravenous dose and 5.3% after an oral dose.

HALF-LIFE

The average elimination half-life of lefamulin is about 8 hours in patients diagnosed with community-acquired bacterial pneumonia. One pharmacokinetic study of healthy volunteers revealed a mean half-life of 13.2 hours after an intravenous infusion of lefamulin.

CLEARANCE

The total body clearance of lefamulin has been determined to range from 2.94 to 30.0 L/h after an injected dose.

Side effects

Pain
Itching
Burning
Swelling
a lump at the injection site
Headache
Trouble sleeping

EXPERIMENTAL WORK:

SELECTION AND PROCUREMENT OF DRUG:

DRUG SAMPLE SUPPLIER:

Table 2: Drug and Drug Supplier

Name of Drug	Drug Supplier
Lefamulin	Swapnroop drug and pharmaceutical

LIST OF REAGENTS & CHEMICALS USED:

Table 3: List of Reagents and Chemicals used

Sr. No.	Name of chemicals	Manufacturer.
1.	Methanol (HPLC grade)	Merck Ltd., India
2.	0.05% OPA (HPLC grade)	Merck Ltd., India
3.	water (HPLC grade)	Merck Ltd., India

SELECTION OF FORMULATION:

From the literature survey and market survey we selected Maxide formulation for work.

MARKETED PREPARATION:

Table No.4: List of brand names of combined formulations of Lefamulin

Sr. No.	Brand name	Formulation	Available Strength	Address of Manufacturer
1.	Xenleta	Tablet	Lefamulin 600 mg	Nabriya pharma

The marketed preparation was obtained from local market and is referred here after in this thesis by the name as such.

RESULT AND DISCUSSION:

1. Preliminary studies on Lefamulin

1.1. Melting point

The procured reference standard of Lefamulin were found to melt in the range of 183-186 ^{0}C respectively.

1.2. Solubility

The drug was found to be Freely soluble in Methanol & Ethanol,DMSO,Acetone.

1.3. UV Spectroscopy

UV absorption of 10 µg/mL solution of Lefamulin in methanol was generated and absorbance was taken in the range of 200-400 nm. λmax

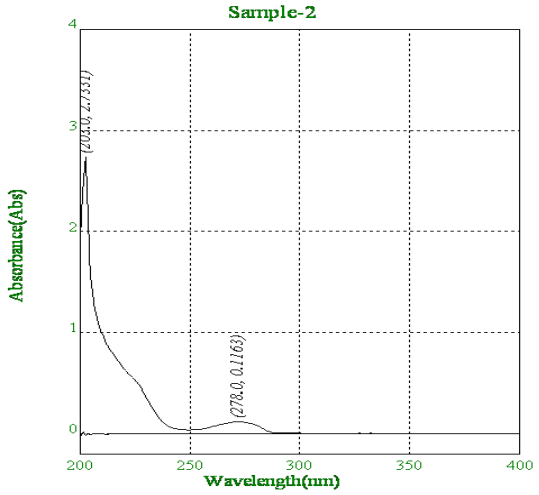


Fig. 7. Uv spectrum of Lefamulin

Standard solutions were scanned in the range of 200-400nm, against 10 ml methanol and volume make with methanol solvent system as reference Lefamulin in methanol was found to be 278nm selected wavelength is 278nm **(Figure No:7)**

1.4. Studies on the chromatographic behavior of Lefamulin.

After the selection of suitable mobile phase, it was then optimized for its reproducibility, sensitivity & accuracy. The optimized parameters for selected method are as below.

Table 9: Different Trials of Chromatographic Condition

Fig. No.	Column used	Mobile phase, Flow Rate and Wavelength	Inj. Vol.	Observation	Conclusion
1	Waters C18 (100 X 4.6mm, 2.5µ)	0.05% OPA Water+Methanol (10+90 % v/v)PH-7. Flow Rate 0.7.278 nm	20µl	Peak was obtained properly	Hence rejected
2	Waters C18 (100 X 4.6mm, 2.5µ)	Methanol + 0.05%Water (80:20 % v/v) PH-7 Flow Rate 0.7 ml., 278 nm.	20 µl	Peak was obtained properly	Hence rejected
3	Waters C18 (100 X 4.6mm, 2.5µ)	Methanol + 0.05%OPA (70:30 % v/v) PH-7 Flow Rate 0.7 ml. 278 nm.	20 µl	Peak was obtained properly	Hence rejected
4	Waters C18 (250×4.6mm, 2.5µ)	Methanol + (0.05% OPA)Water (50:50% v/v) Flow Rate 0.7 ml ,278 nm.	20 µl	Peak was obtained properly	Hence rejected
5.	Waters C18 (100 X 4.6mm, 2.5µ)	Methanol+ 0.05% (OPA) water, (50:50 % v/v) PH-3 Flow Rate 1 ml. 278 nm.	20 µl	Sharp Peaks were obtained	Hence selected

Thus, from the above, it has been observed that, using mobile phase of Methanol+0.05% (OPA)water,(50:50 % v/v),PH3.,278 nm, Flow rate 1 ml gave adequate retention at 3.513min with good peak shape (Theoretical plates Lefamulin 4855).

Chromatogram of Trial 1:

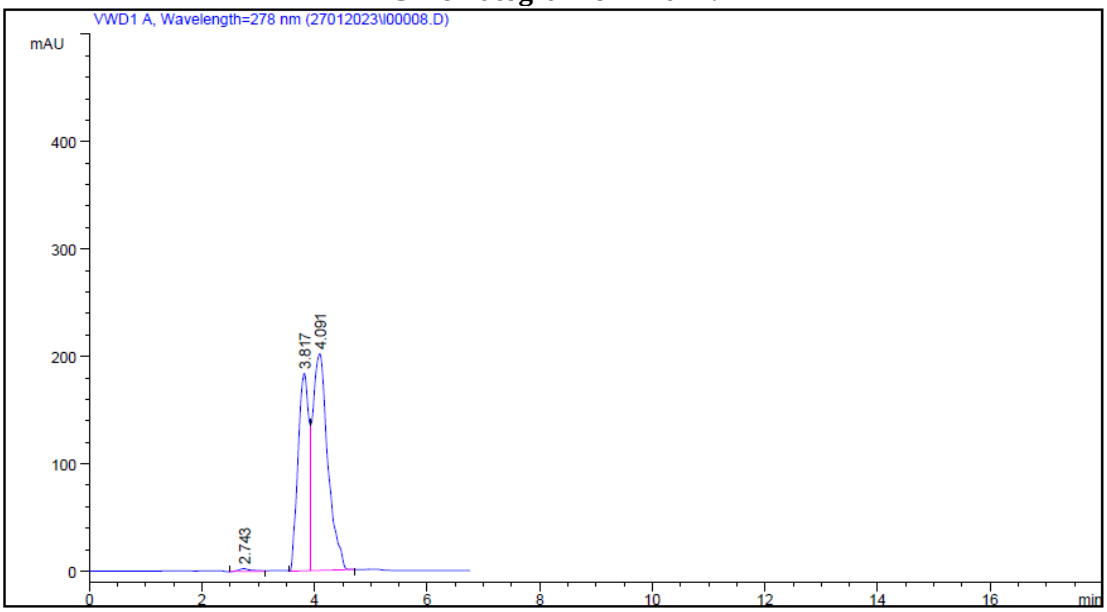


Fig No 8: Chromatogram of Trial 1

Table No 10: Result for Chromatogram of Trial 1

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.817	2447.8793	1503	1.38	0.0000
2	4.091	3784.5183	1009	0.75	2.66

Chromatogram of Trial 2:

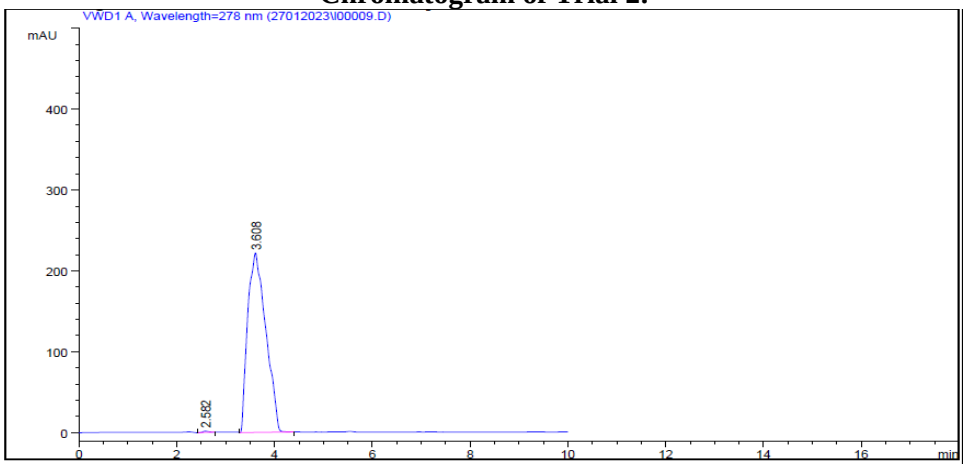


Fig No 9 : Chromatogram of Trial 2

Table No .11: Result for Chromatogram of Trial 2

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.608	5720.7763	391	0.70	0.0000

Chromatogram of Trial 3:

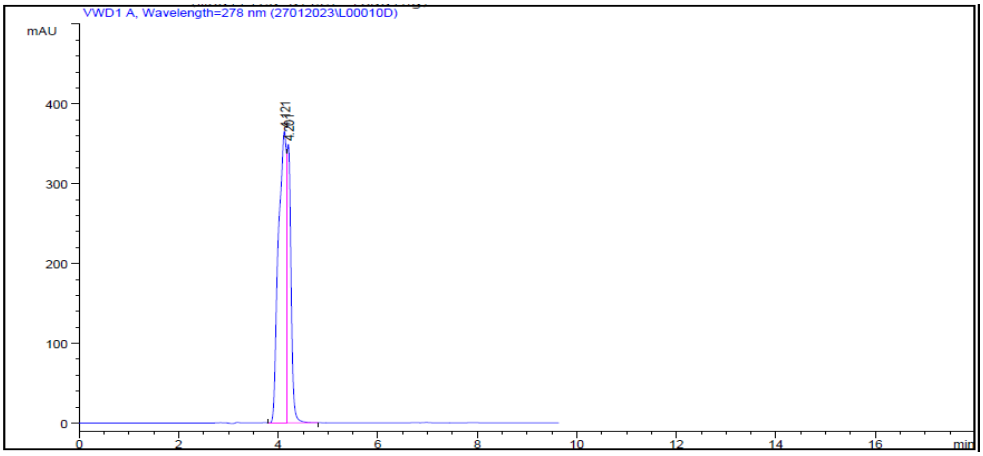


Fig No 10 : Chromatogram of Trial 3

Table No .12: Result for Chromatogram of Trial 3

RT[min]	Area[mV*s]	TP	TF	Resolution
4.121	3999.7426	2433	3.09	0.0000
4.201	1982.1810	12809	0.43	0.33

Chromatogram of Trial 4:

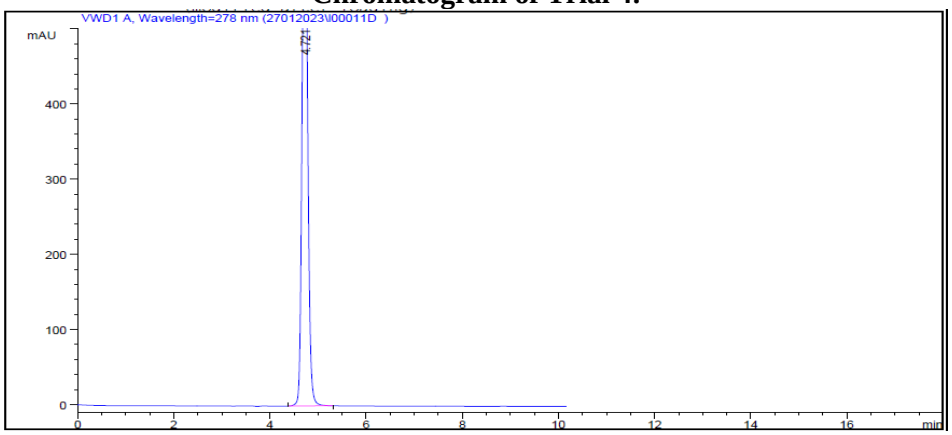


Fig No 11: Chromatogram of Trial 4

Table No .13: Result for Chromatogram of Trial 4

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.721	6129.0136	9359	0.87	0.0000

Chromatogram of Final Trial 5:

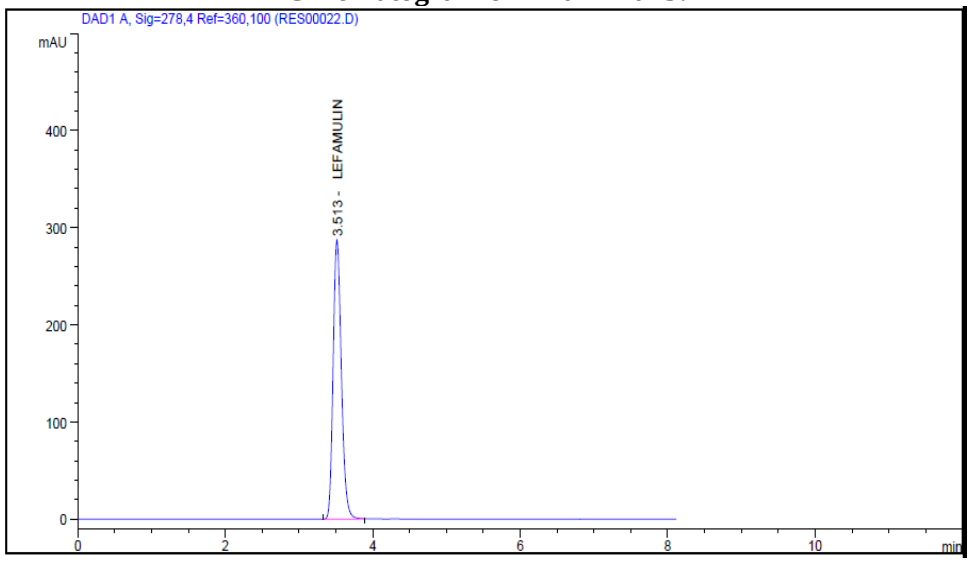


Fig No 12: Chromatogram of Final Trial 5

Table No .14: Result for Chromatogram of final Trial- 5

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.513	2229.6420	4855	0.79	0.0000

The final chromatographic conditions selected were as follow:

Analytical column : Waters C_{18} Column (100mm x 4.6mm, 2.5µm particle size).
Injection volume : 20µl
Flow rate : 1 ml/min
Mobile phase : Methanol :(0.05%OPA) water (50: 50 % V/V)
Detection : 278 nm
Run Time : 15 min

Preparation of Standard chromatogram of Lefamulin

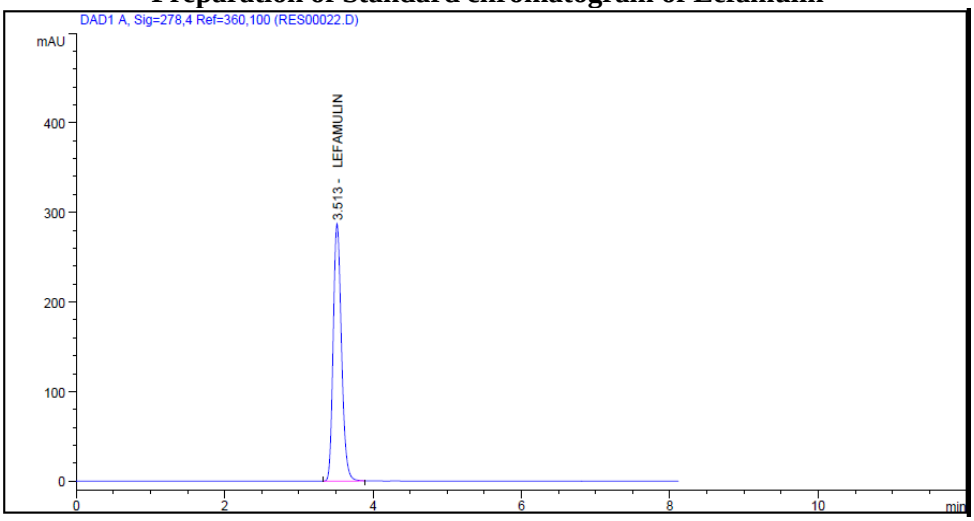


Fig No.13: Chromatogram of standard Lefamulin

Table No 15: Result for standard Chromatogram of Lefamulin

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.513	2229.6420	4855	0.79	0.0000

2. Analytical of Method Validation:

1. Linearity:

From Lefamulin standard stock solution, different working standard solution (5-25µg/m l) were prepared in mobile phase 20 µl of sample solution was injected into the chromatographic system using mixed volume loop injector. Chroma to grams were recorded. The area for each concentration were recorded (**Table No. 16**). The Calibration curves are shown in [**Fig. No.24**.]

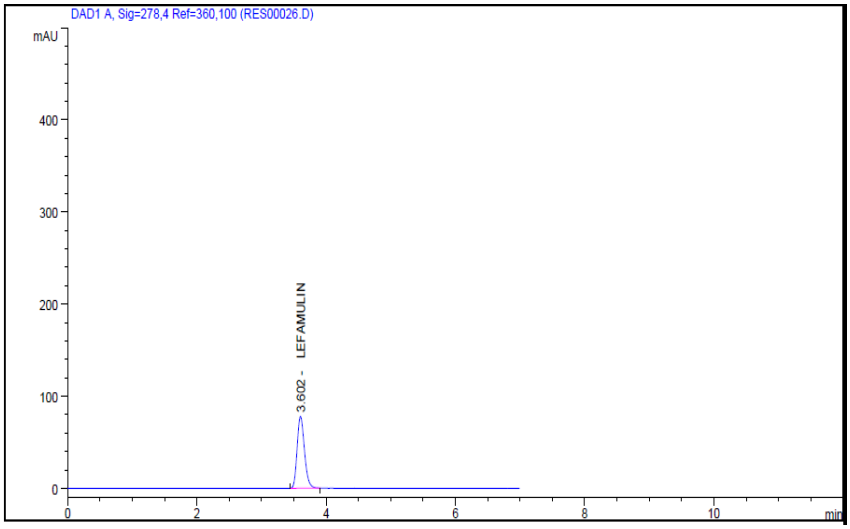


Fig.No.14.Chromatogram of linearity (5mcg)-1

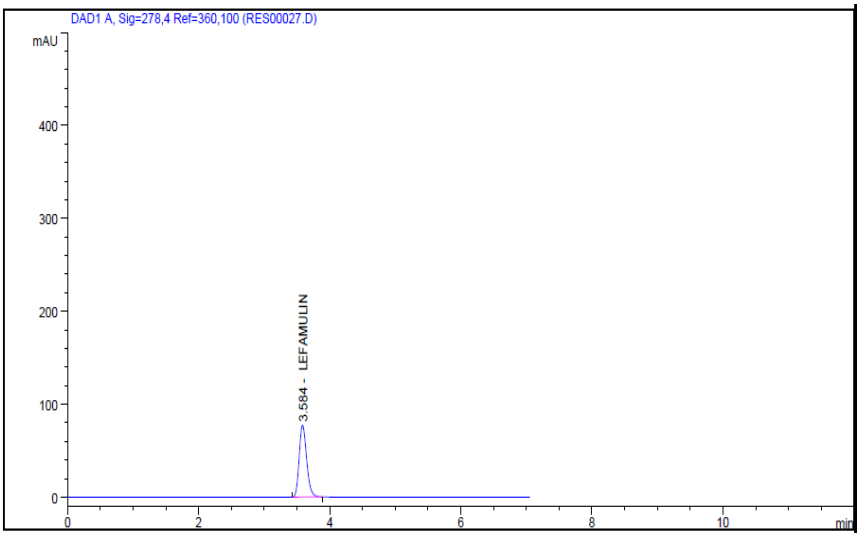


Fig.No.15.Chromatogram of linearity (5mcg)-2

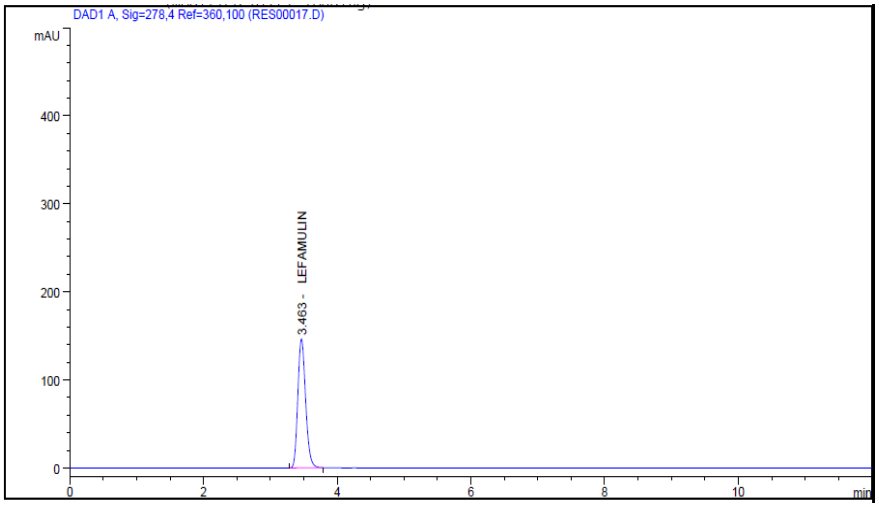


Fig.No.16.Chromatogram of linearity (10mcg)-1

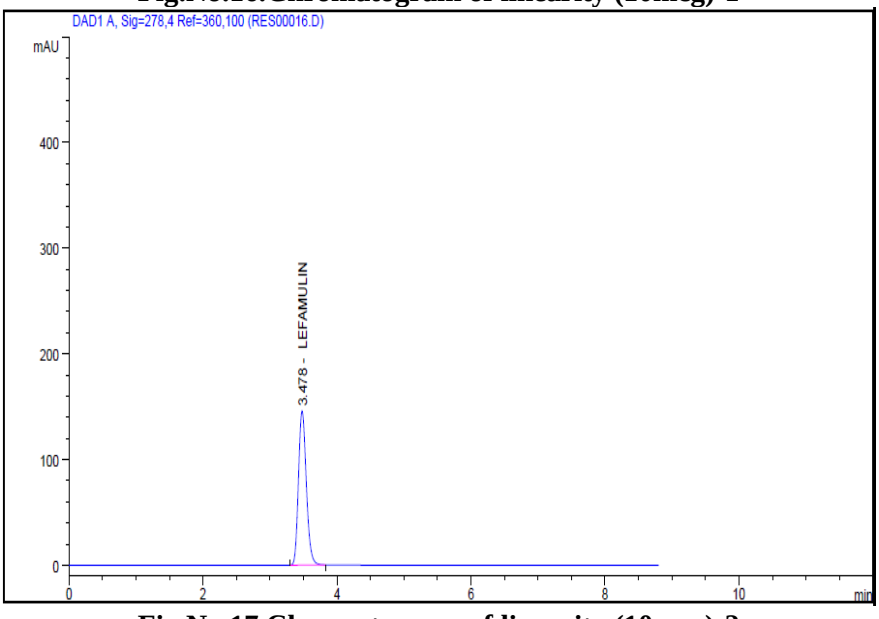


Fig.No.17.Chromatogram of linearity (10mcg)-2

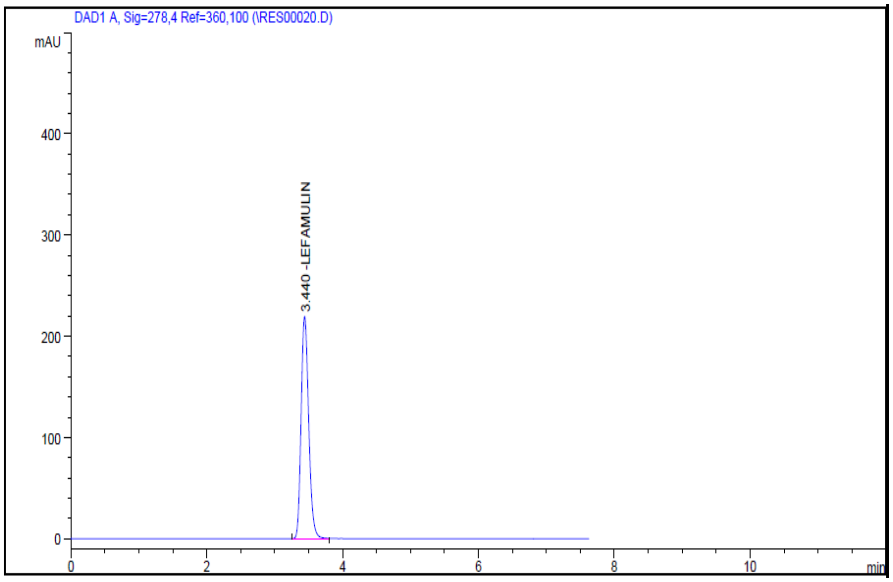


Fig.No.18.Chromatogram of linearity (15mcg)-01

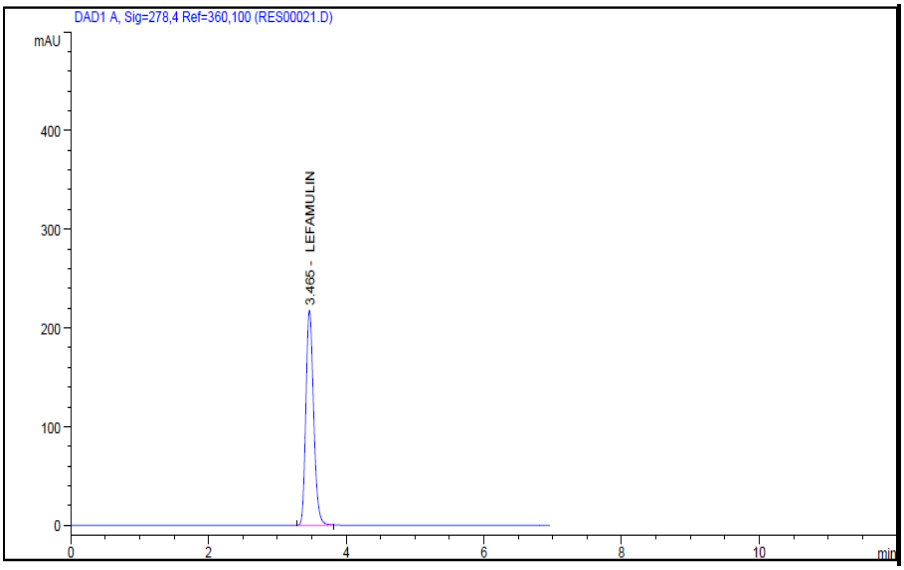


Fig.No.19. Chromatogram of linearity (15mcg)-02

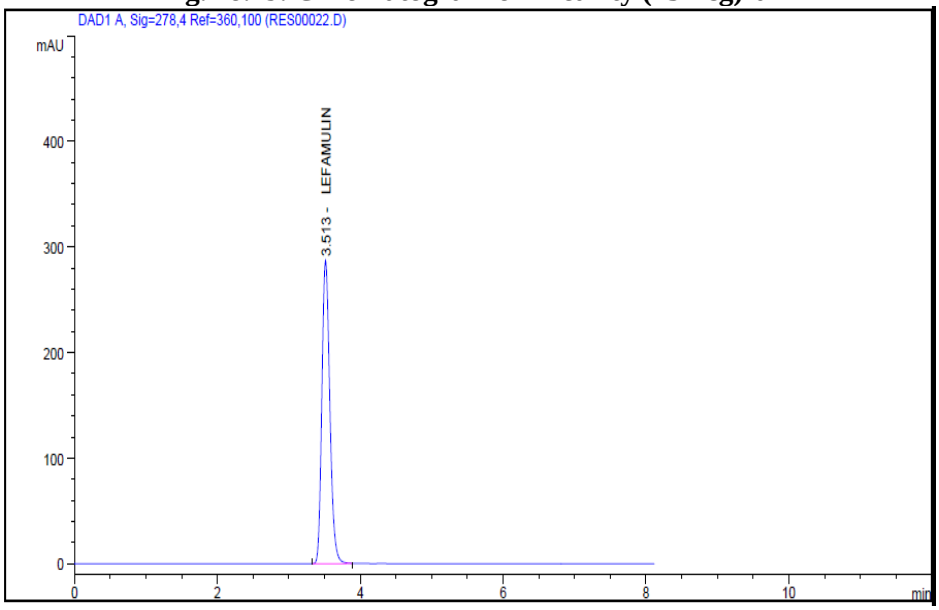


Fig.No.20. Chromatogram of linearity (20mcg)-01

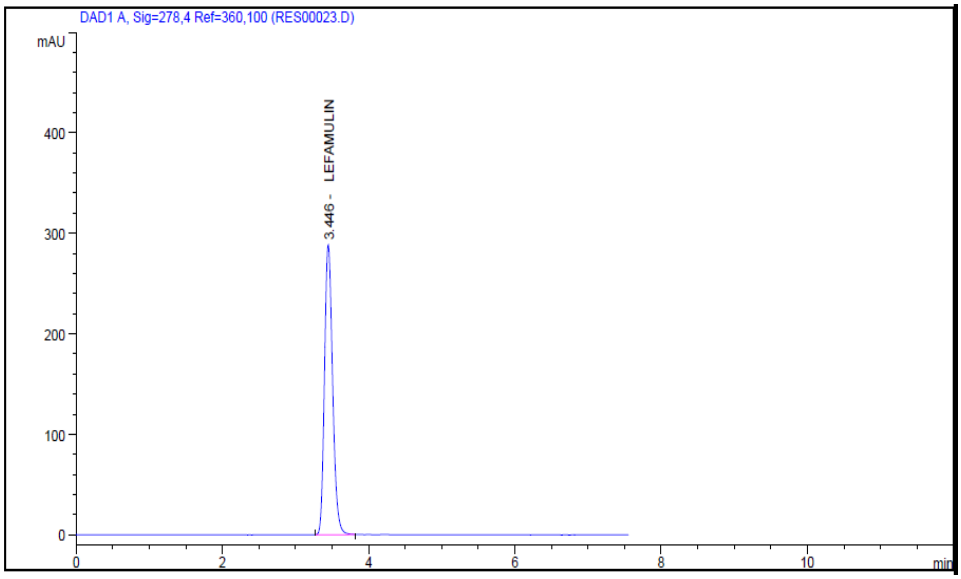


Fig.No.21. Chromatogram of linearity (20mcg)-02

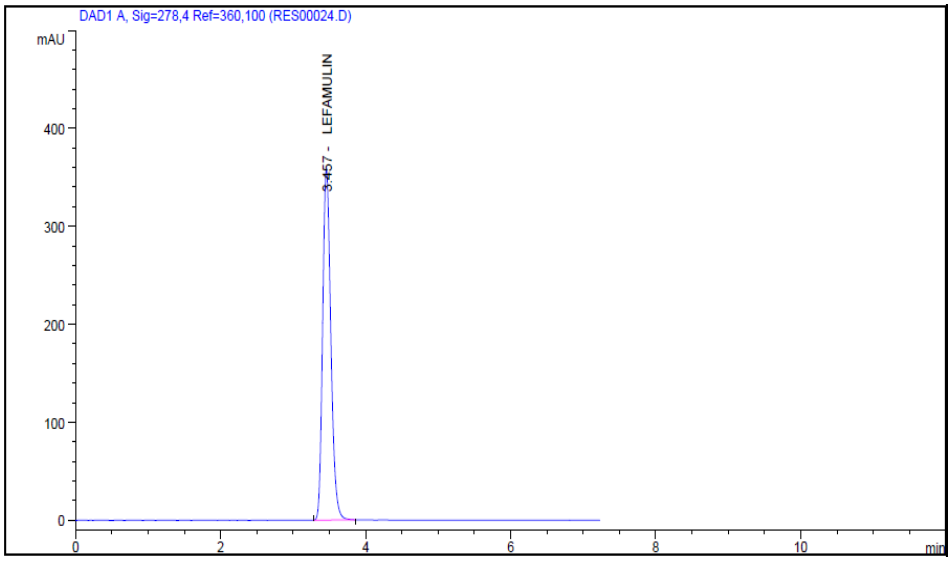


Fig.No.22. Chromatogram of linearity (25mcg)-01

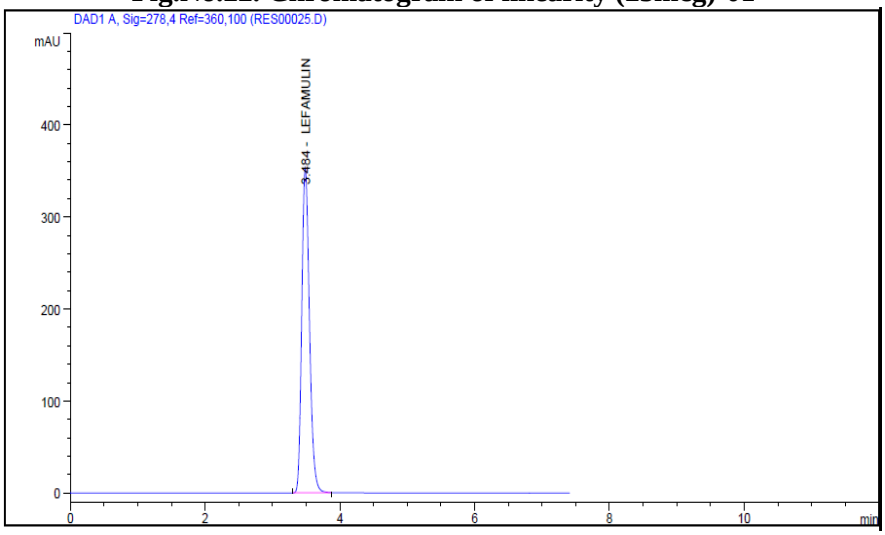


Fig.No.23. Chromatogram of linearity (25mcg)-02

Table No 16. Linearity of Lefamulin

Sr. No.	Concentration µg/ml	Area Lefamulin

1	5	626.6852
2	10	1190.0999
3	15	1701.6358
4	20	2228.1910
5	25	2816.3024

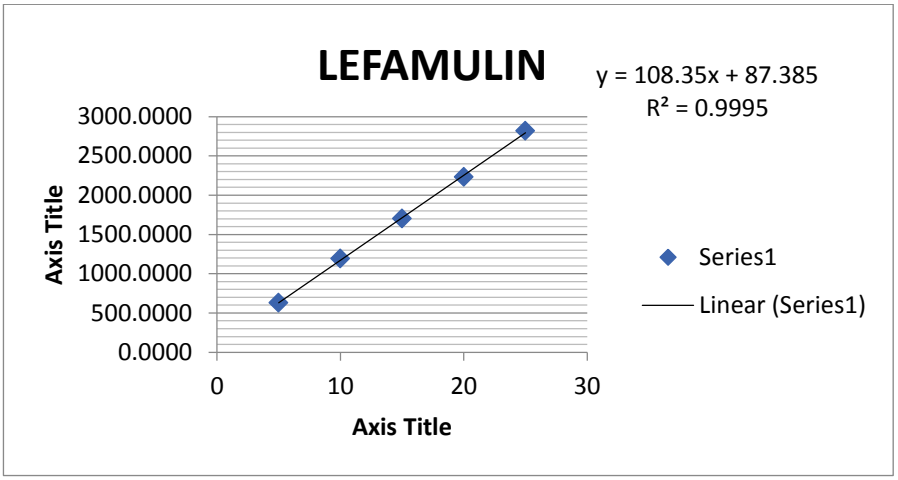


Fig.No.24. Calibration curve of Lefamulin

Table No 17. Regression equation data for Lefamulin

Regression Equation Data Y=mx+c	
Slope(m)	108.3
Intercept(c)	87.38
Correlation Coefficient	0.999

Linearity of of Lefamulin was observed in the range of 5-25µg/ml. Detection wavelength used was 278 nm.(**Table No. 16**) The calibration curve yielded correlation coefficient (r^2) 0.999 & 0.999 for Lefamulin respectively. (**Table. No. 17**)

2. Accuracy:-

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed Tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (**Table No.18**). Statistical validation of recovery studies shown in (**Table No. 19**)

Accuracy 80%

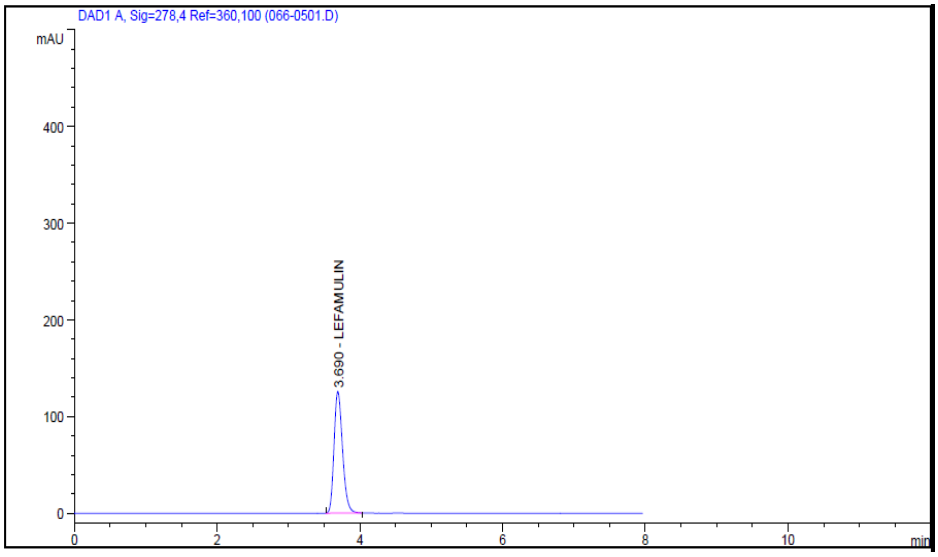


Fig.25. Chromatogram of Accuracy 80%-01

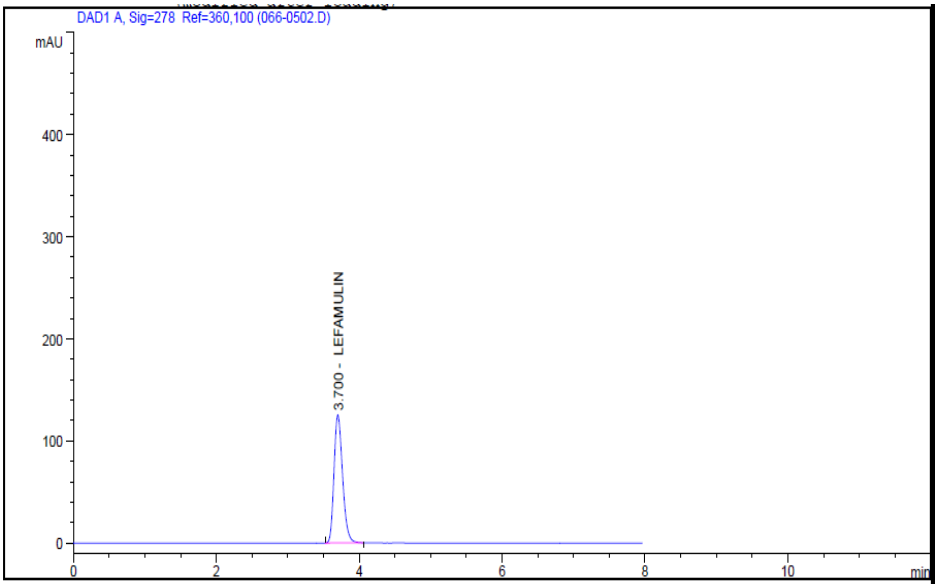


Fig.26. Chromatogram of Accuracy 80%-02

Accuracy 100%

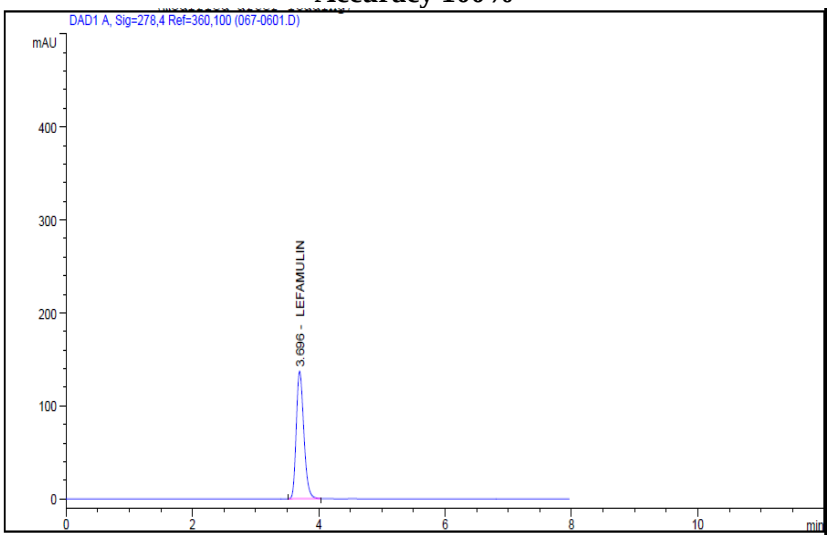


Fig.27. Chromatogram of Accuracy 100%-01

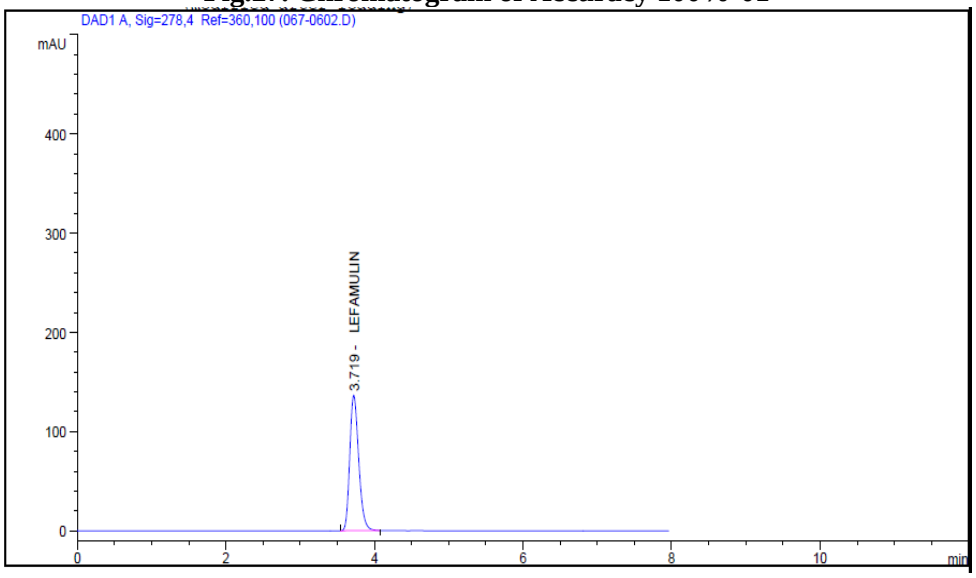


Fig.28. Chromatogram of Accuracy 100%-02

Accuracy 120%

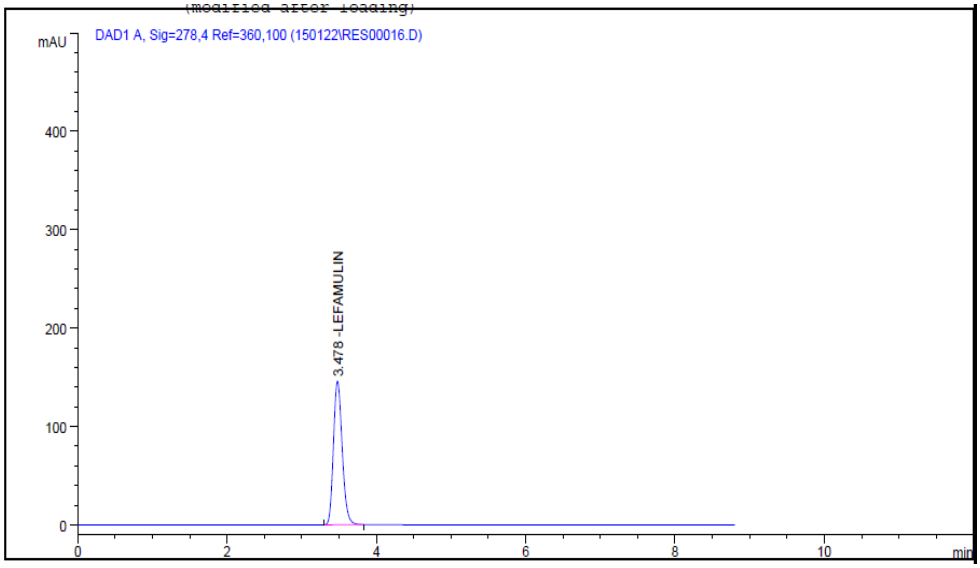


Fig.29. Chromatogram of Accuracy 120%-01

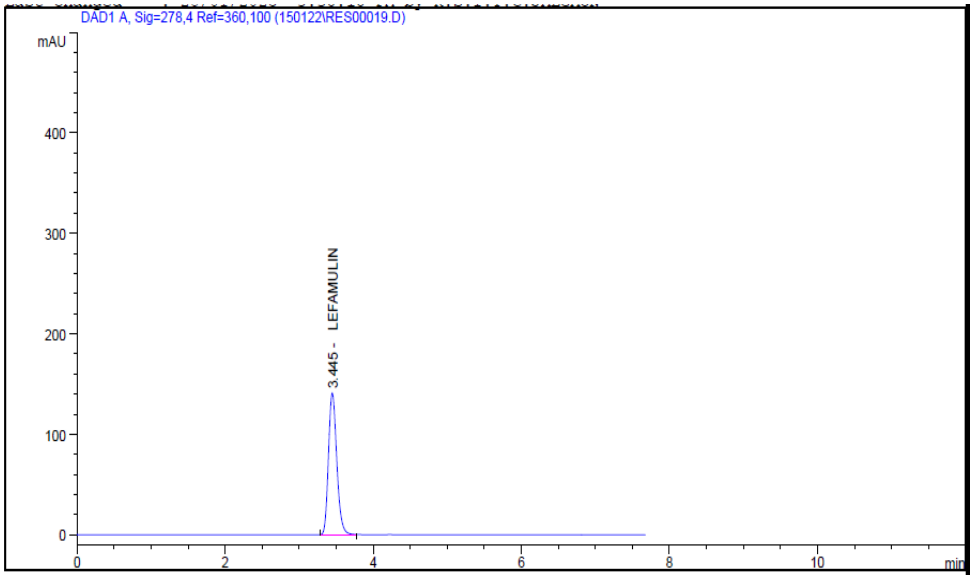


Fig.30. Chromatogram of Accuracy 120%-02

Table .18. Result of Recovery data for Lefamulin

Drug	Sr No.	Level (%)	Amt. taken (µg/ml	Amt. Added (µg/ml	Area. Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
LFM	1	80%	5	4	8.98± 0.004	3.98± 0.004	99.53±0.09
	2	100%	5	5	9.96±0.007	4.96±0.007	99.29± 0.15
	3	120%	5	6	10.95±0.01	5.95±0.012	99.17 ±0.21

***mean of each 3 reading.**

Table.19. Statistical Validation of Recovery Studies Lefamulin

Level of Recovery (%)	Mean % Recovery	Standard Deviation*	% RSD
80%	99.53	0.09	0.09
100%	99.29	0.15	0.15
120%	99.17	0.21	0.21

***Denotes average of three determinations.**

Accuracy of method is ascertained by recovery studies performed at different levels of concentrations (80%, 100% and 120%). The % recovery was found to be within 98-101% (**Table No. 18, 19**).

3. System suitability parameters :(Repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Lefamulin system suitability parameters were studied. The result shown in below **(Table No.20)**

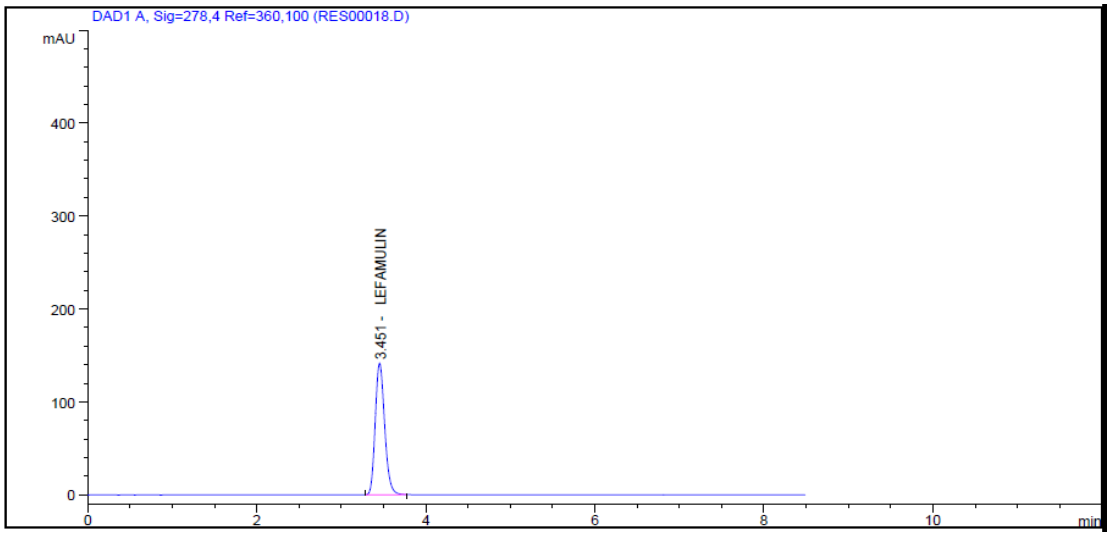


Fig No.31: Chromatogram of System suitability No- 1

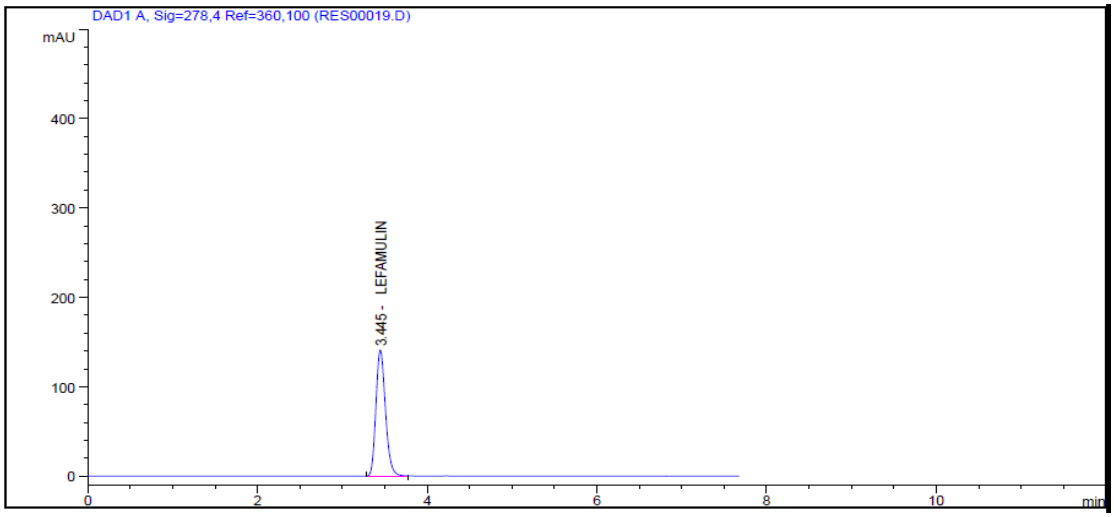


Fig No 32: Chromatogram of System suitability No- 2

RESULT FOR REPEATABILITY (SST):

Chromatogram System Suitability Results was found to be mean of five determination were also satisfactory, hence the analytical method would be concluded that result shown in (**Table No :20)**

Table No.20: Repeatability studies on Lefamulin

Sr. No.	Concentration of Lefamulin (mg/ml)	Peak area	Amount found (mg)	% Amount found
1	10	1170.954	10.0	100.08
2	10	1171.6530	10.01	100.09
		Mean	10.05	100.85
		SD	0.49	0.49
		%RSD	0.04	0.04

Repeatability studies Lefamulin was found to be ,The %RSD was less than 2, which shows high percentage amount found in between 99% to 101% indicates the analytical method that concluded .**(Table No.21)**

4. Precision:-

The method was established by analyzing various replicates standards of Lefamulin. All the solution was analyzed thrice in order to record any intra-day & inter-day variation in the result that

concluded. The result obtained for intraday is shown in**(Table No. 21)** respectively.

Chromatogram of Precision:

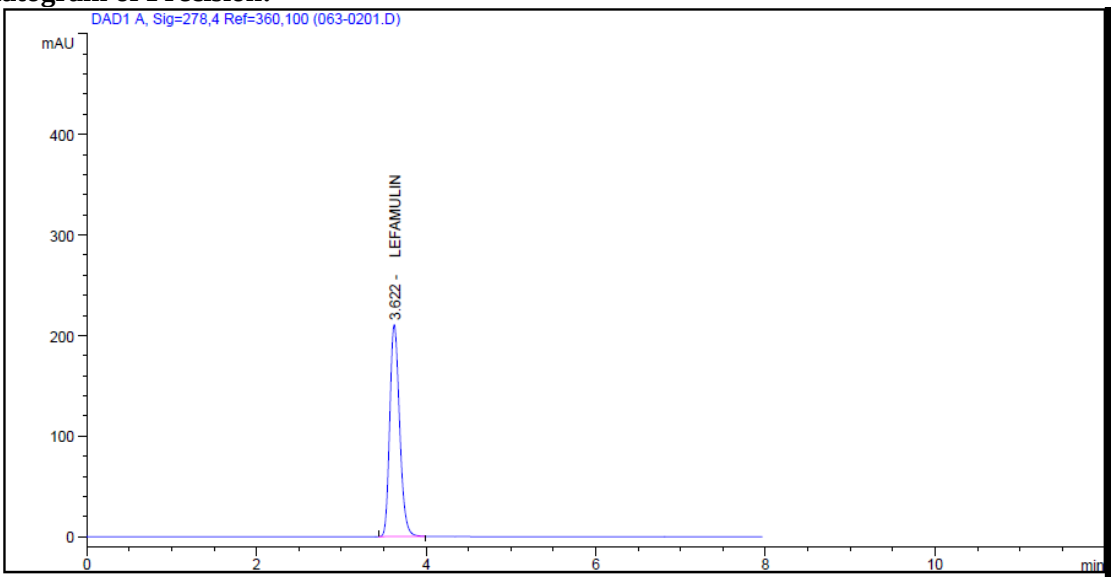


Fig No .33: Chromatogram of Precision

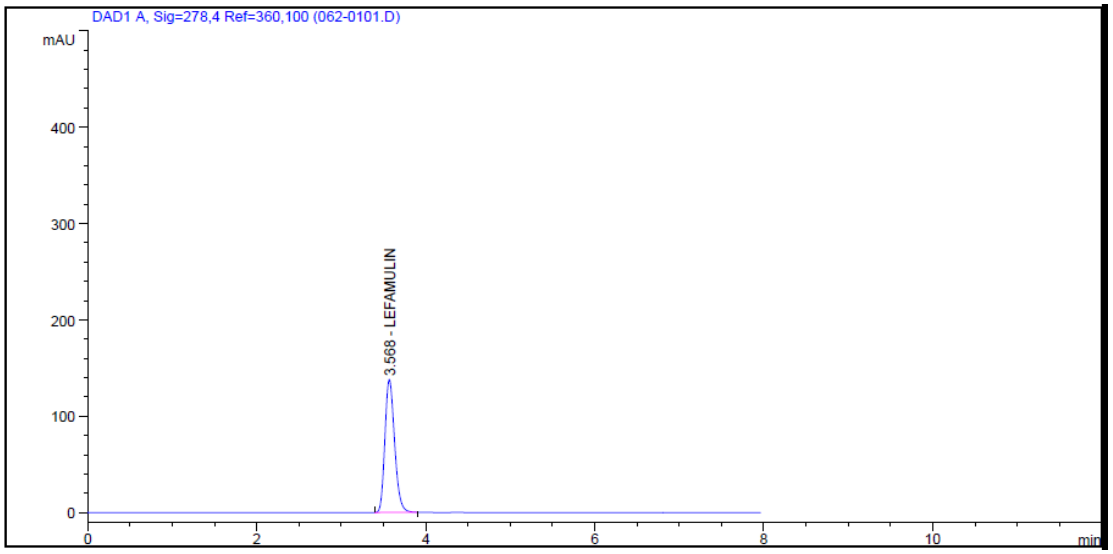


Fig No.34: Chromatogram Intra-day precision (10 mg)-01

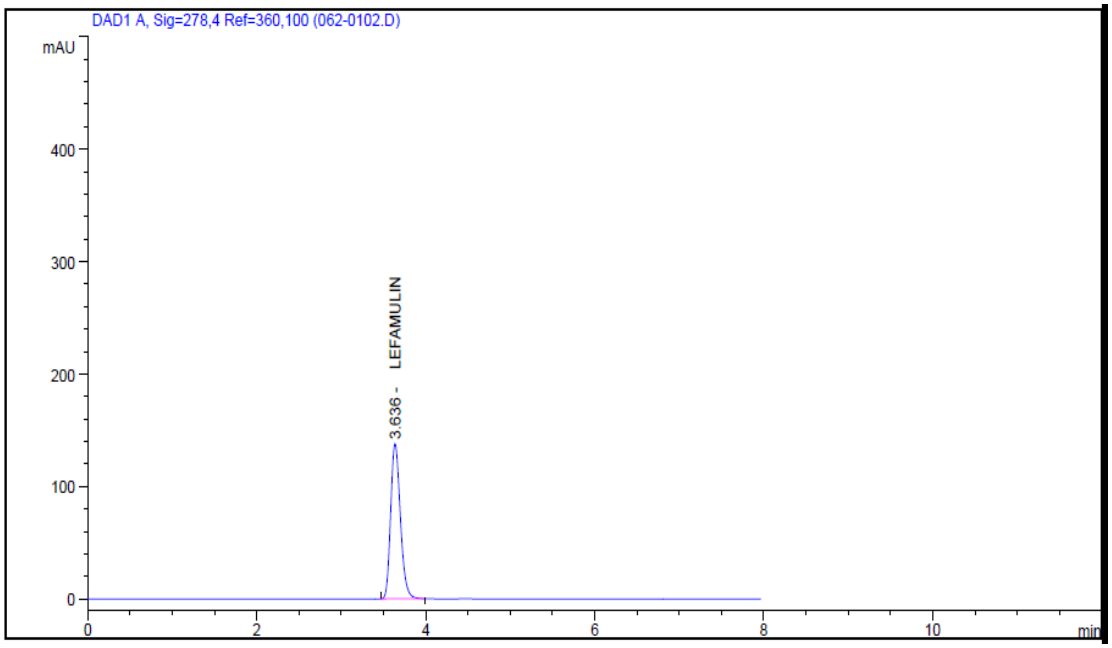


Fig No.35: Chromatogram Intra-day precision (10 mg)-02

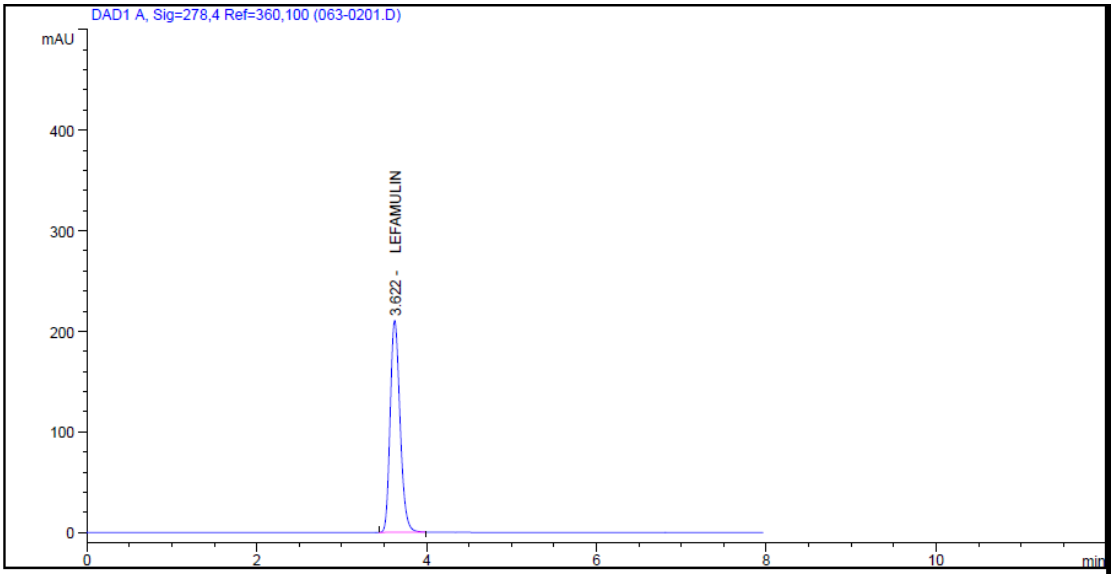


Fig No.36: Chromatogram Intra-day precision (15 mg)-01

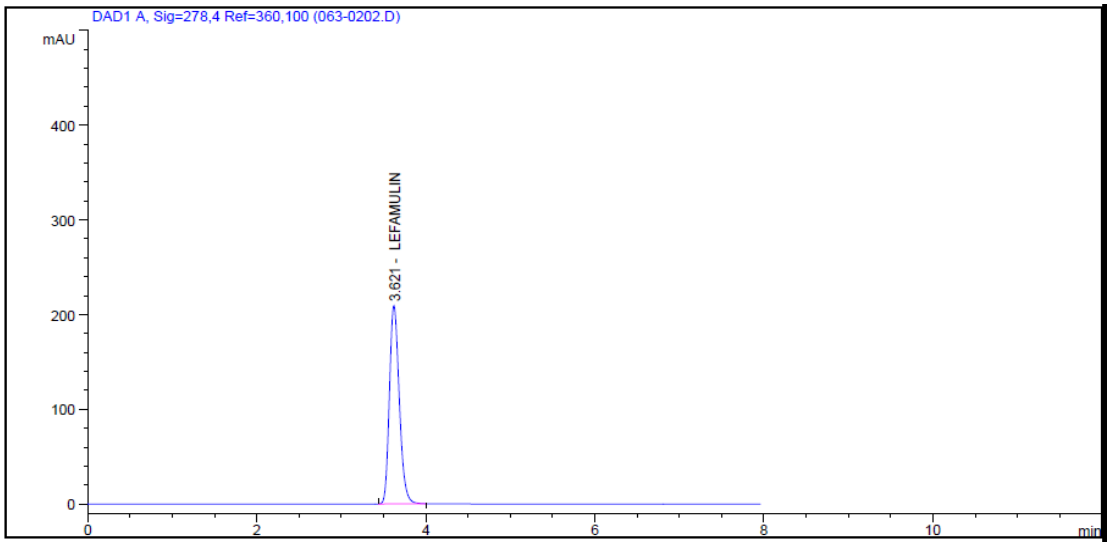


Fig No.37: Chromatogram Intra-day precision (15 mg)-02

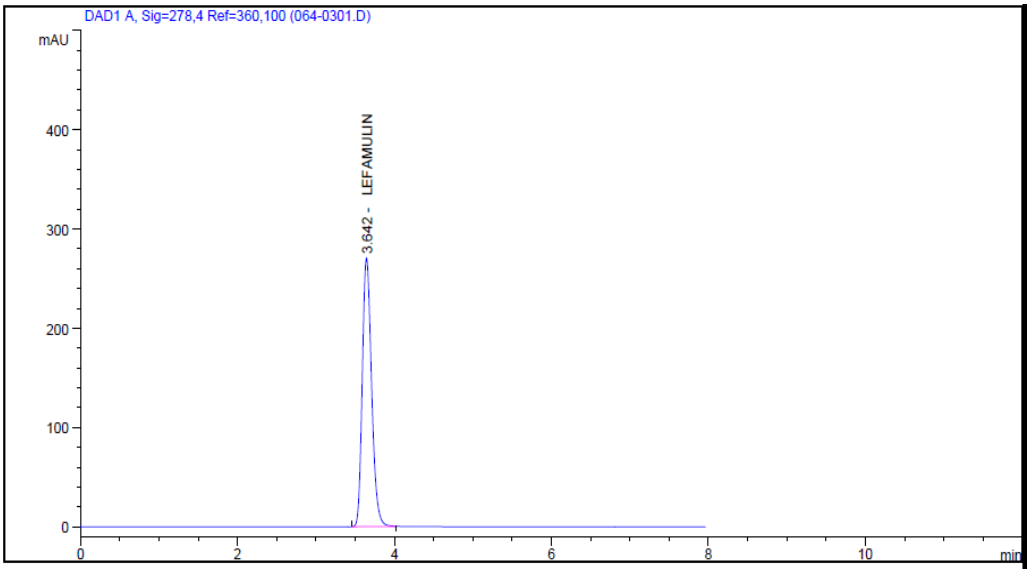


Fig No.38: Chromatogram Intra-day precision (20 mg)-01

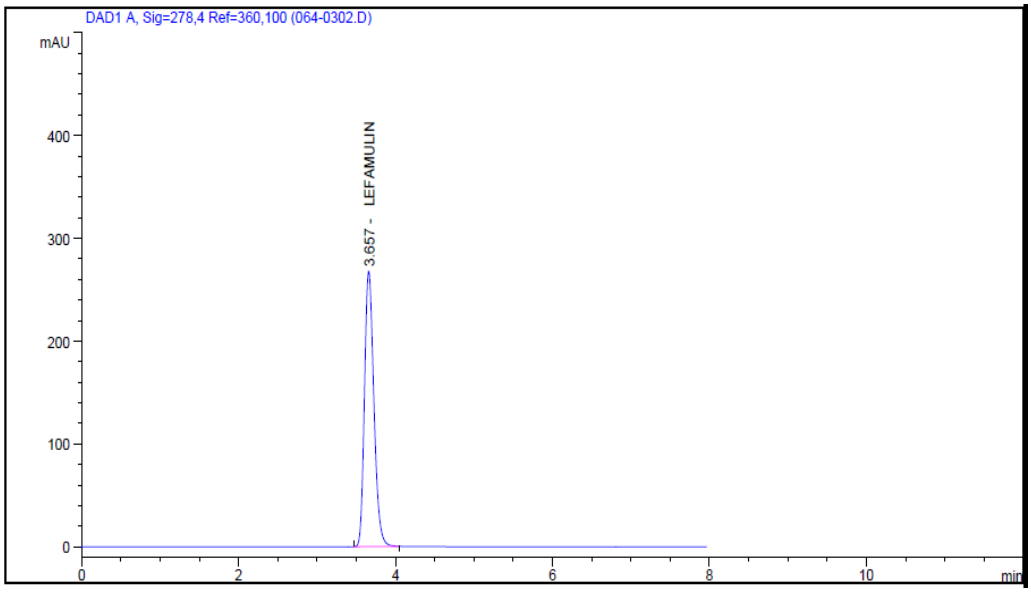


Fig No.39: Chromatogram Intra-day precision (20 mg)-02

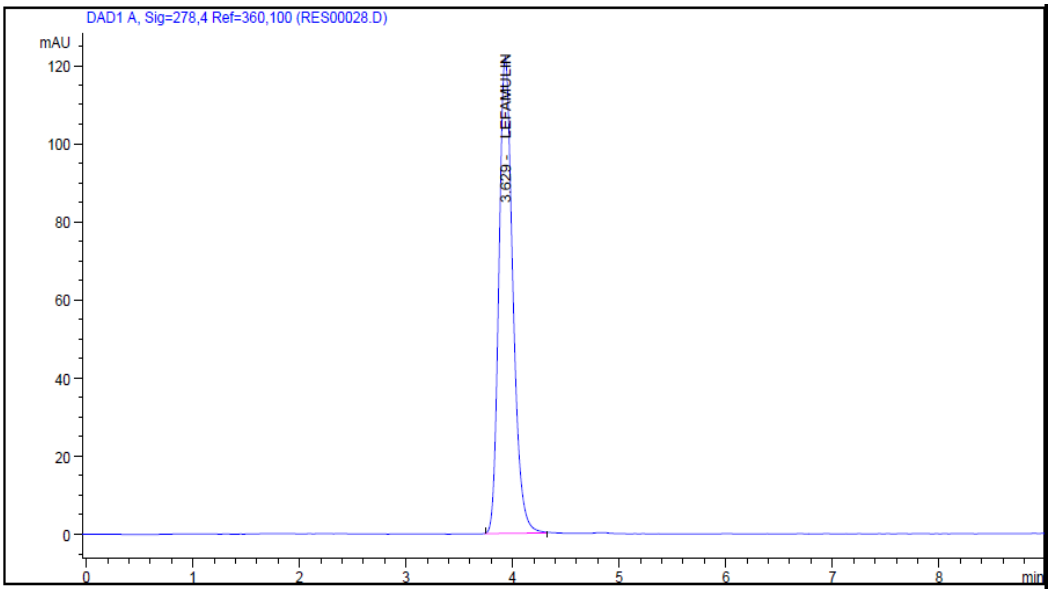


Fig No.40: Chromatogram Inter-day precision (10 mcg)-01

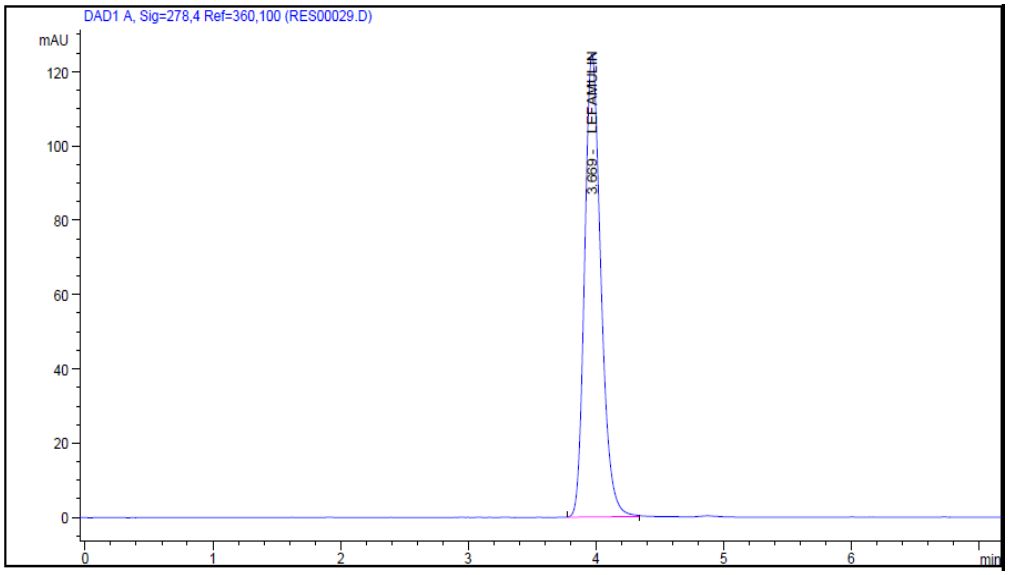


Fig No.41: Chromatogram Inter-day precision (10 mcg)-02

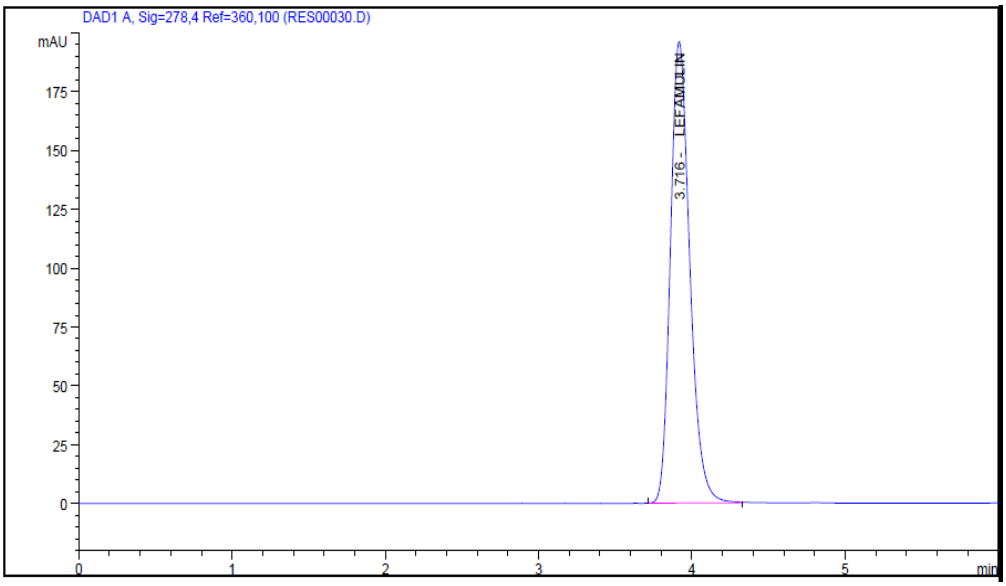


Fig No.42: Chromatogram Inter-day precision (15 mcg)-01

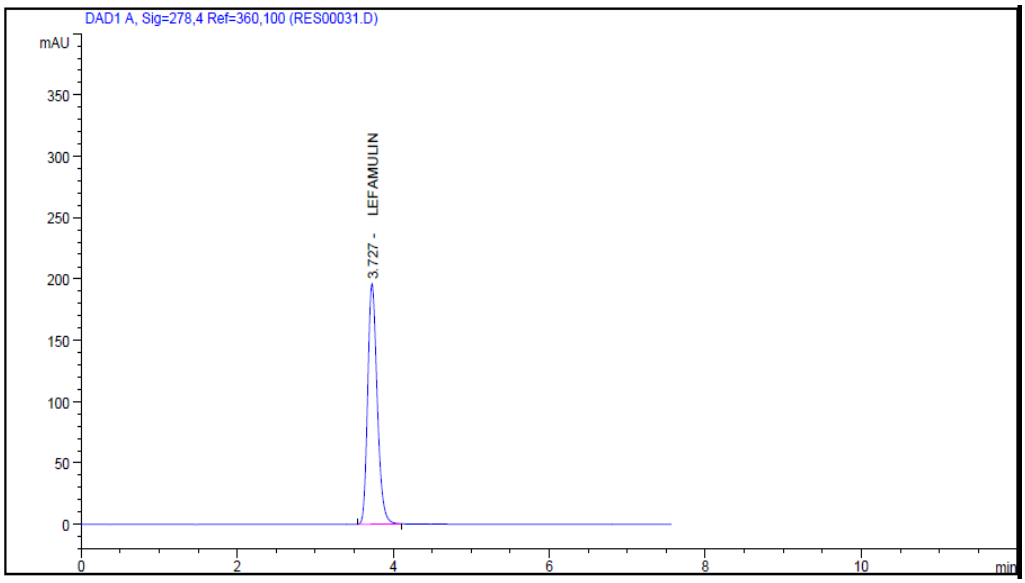


Fig No.43: Chromatogram Inter-day precision (15 mcg)-02

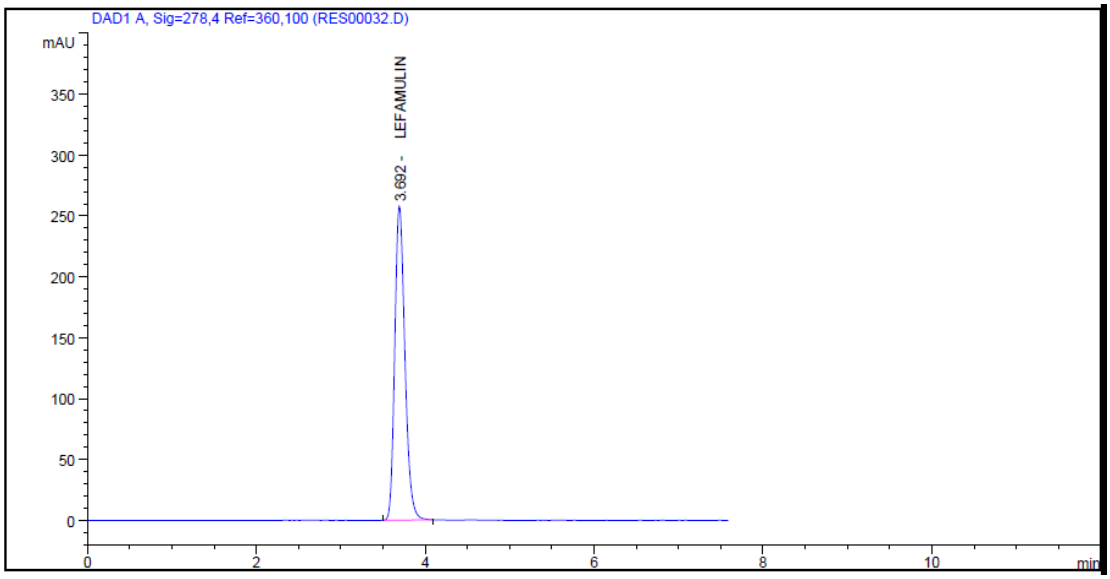


Fig No.44: Chromatogram Inter-day precision (20 mcg)-01

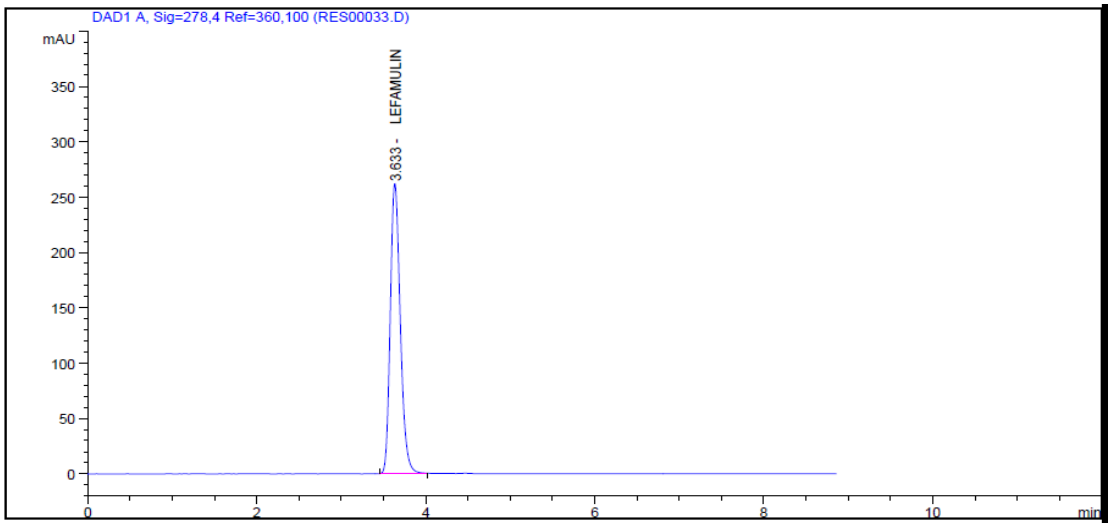


Fig No.45: Chromatogram Inter-day precision (20 mcg)-02

Table No .21: Result of Intraday and Inter day Precision for Lefamulin

Concn (µg/ml)	Intraday Precision			Interday Precision		
	Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
10	1173.55±2.46	100.29	0.21	1166.67±3.75	99.27	0.38
15	1701.55±1.04	99.36	0.06	1712.60±8.57	100.04	0.50
20	2231.36±5.55	98.98	0.25	2242.73±7.21	99.51	0.32

***Mean of each 3 reading**

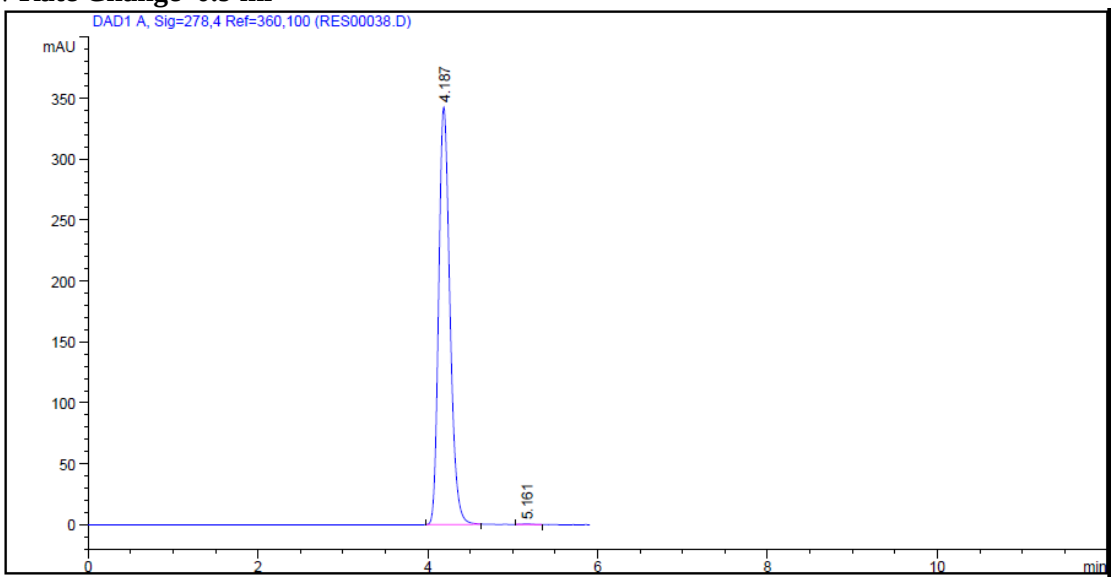
Intraday and Inter day Precision for Lefamulin which shows the high precision %amount in between 98% to 101% indicates to analytical method that concluded.

5. Robustness:

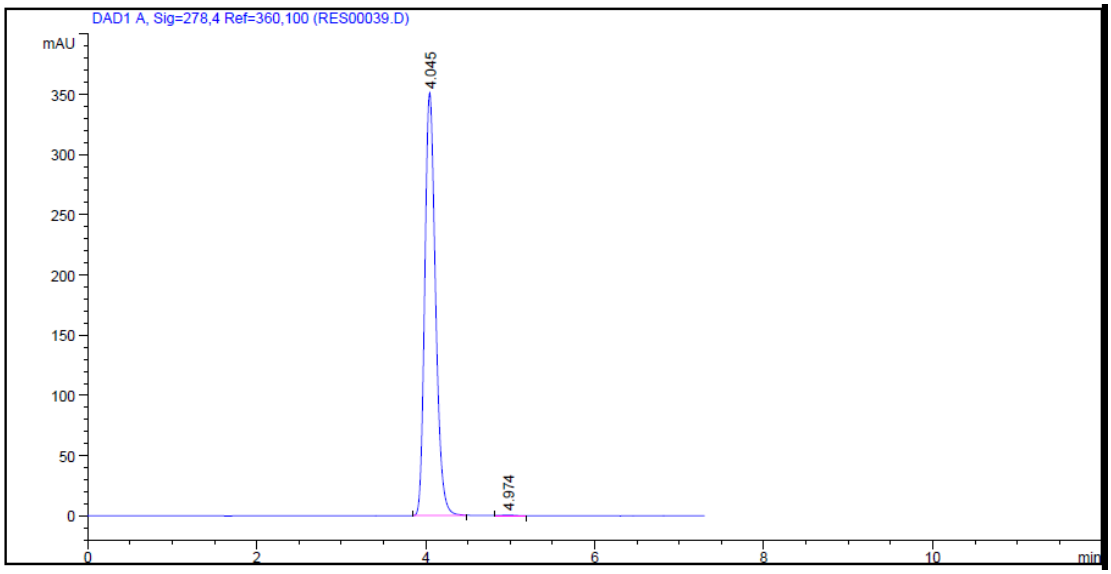
The Robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate, wavelength on retention time and tailing factor of drug peak was studied.

The mobile phase composition was changed in (±1 ml/min^{-1}**)** proportion and the flow rate was varied by of optimized chromatographic condition. The results of robustness studies are shown in **(Table No.22**).Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

1) Flow Rate Change 0.9 ml



FigNo.46. Chromatogram of Flow rate change 0.9ml-01



FigNo.47. Chromatogram of Flow rate change 0.9ml-02

2) Flow Rate Change 1.1 ml

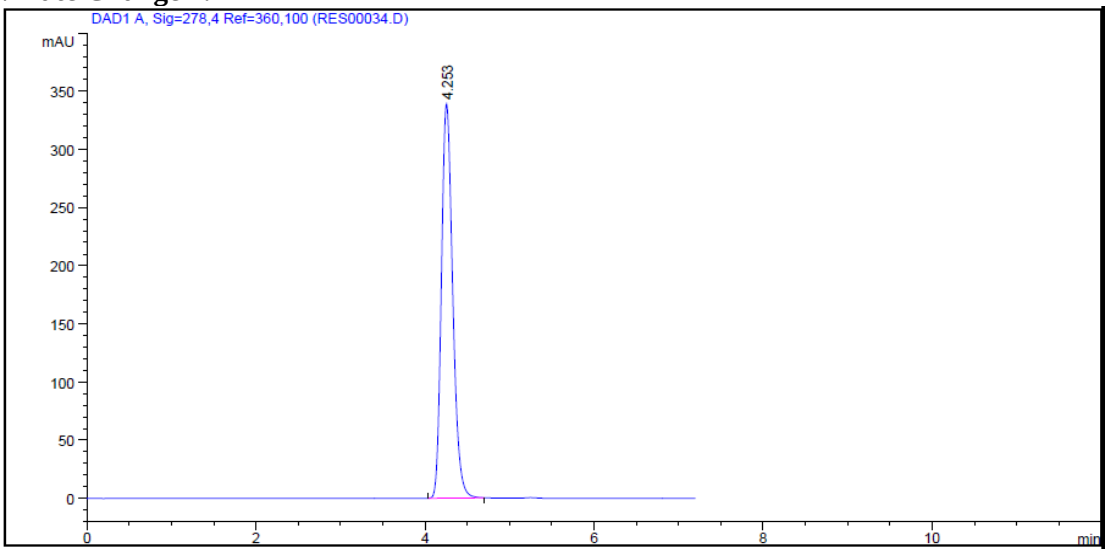


Fig No 48. Chromatogram of Flow rate change 1.1 ml-01

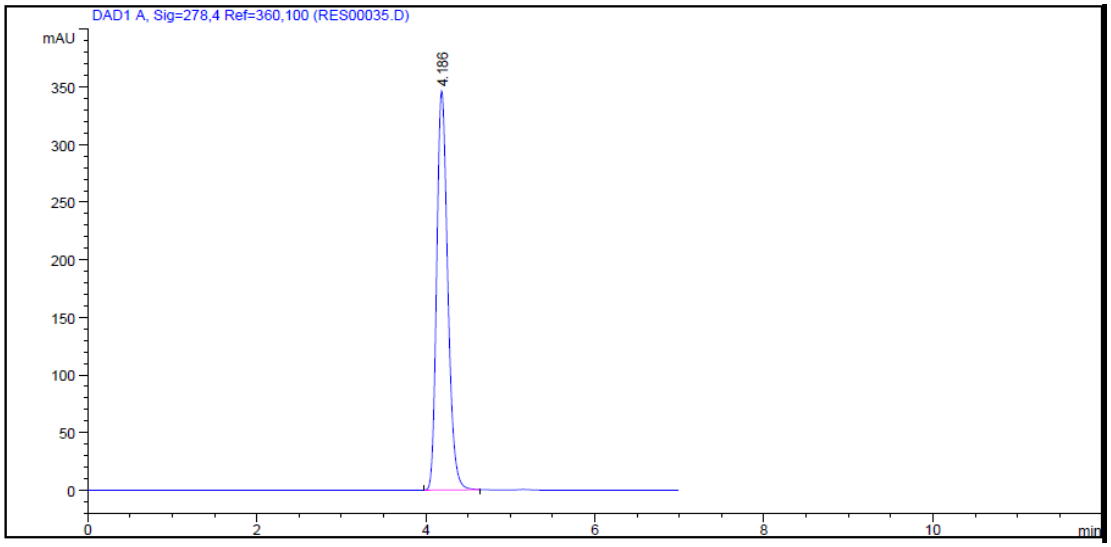


Fig No 49. Chromatogram of Flow rate change 1.1 ml-02

3) Mobile phase composition Change :49ml Meoh + 0.05%(OPA)51mlWater

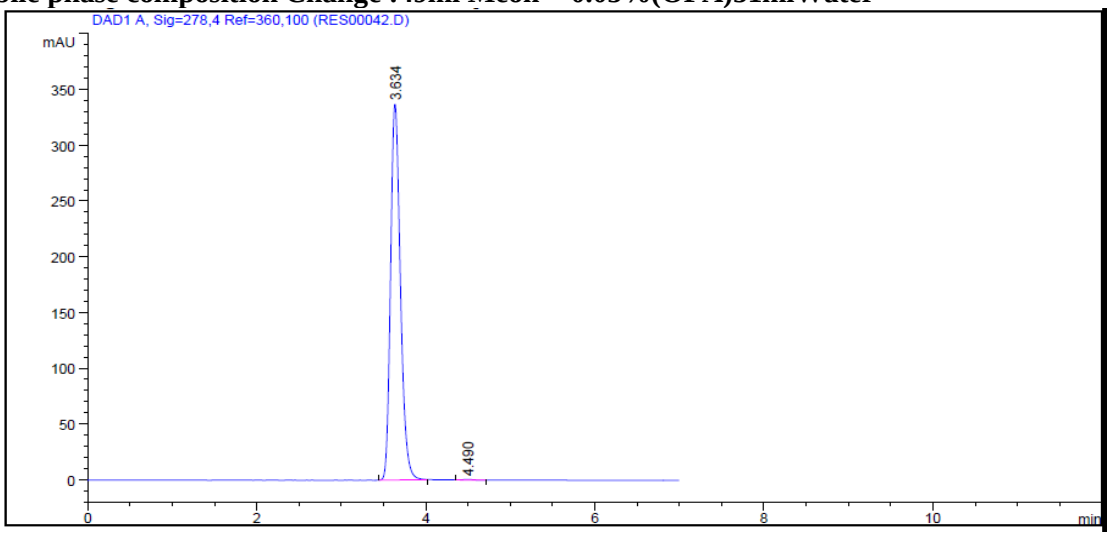


Fig No .50. Chromatogram of Mobile phase composition change 49 ml Meoh + 0.05 %(OPA) 51 ml Water

4) Mobile phase composition Change:51ml Meoh+ 0.05%(OPA)49ml Water

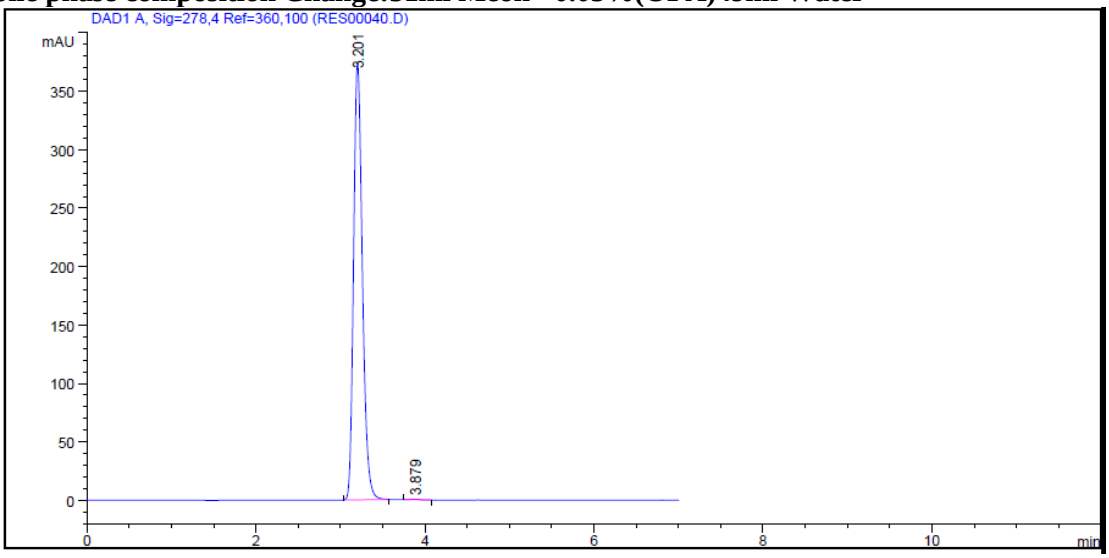


Fig No.51 Chromatogram of Mobile phase composition change 51ml Meoh + 0.05% (OPA) 49 ml Water

5) Wavelength Change 277 nm

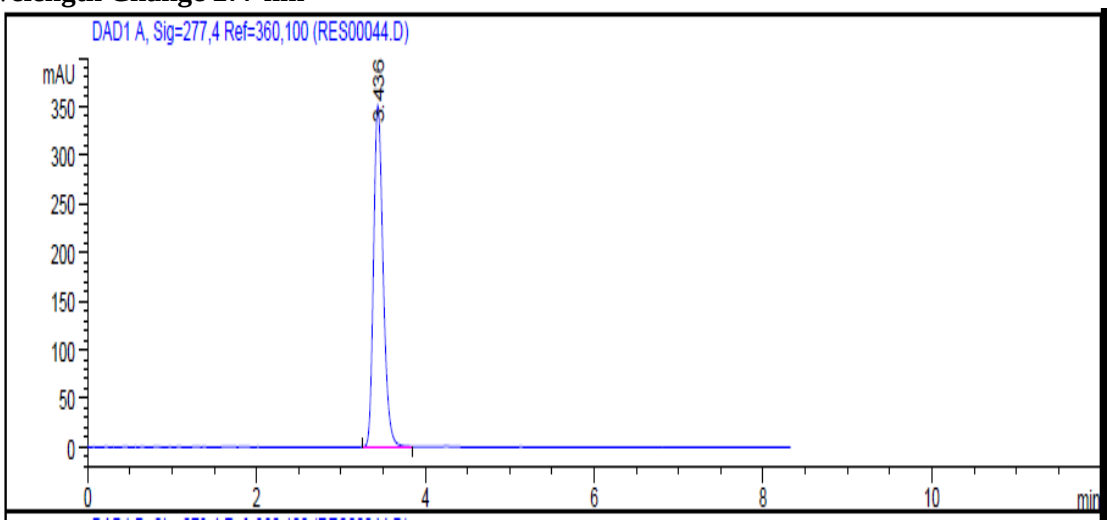


Fig. No 52: Chromatogram of comp change wavelength change 277 nm-01

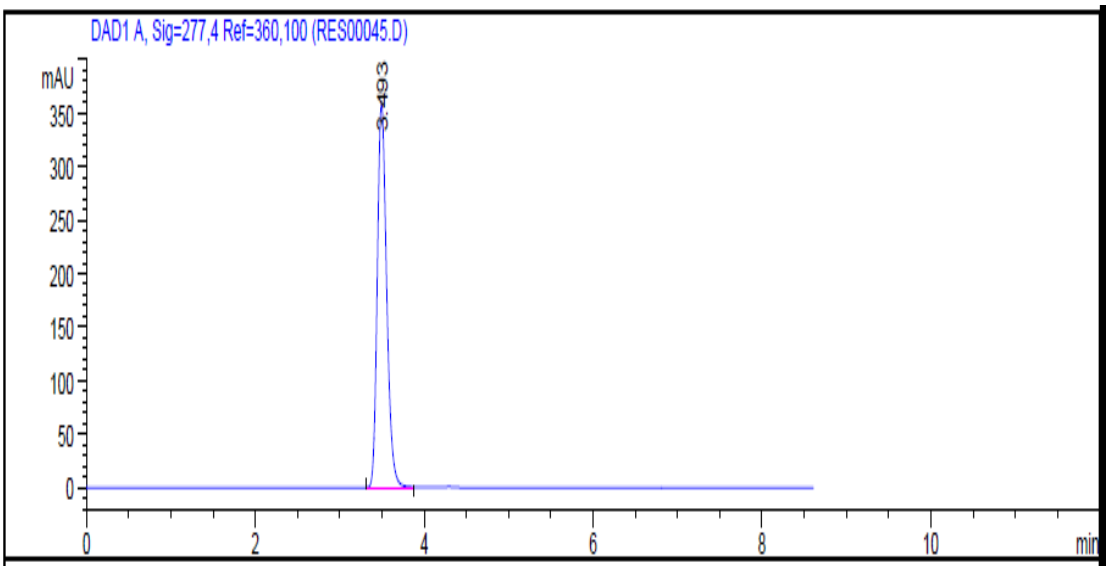


Fig. No 53: Chromatogram of comp change wavelength change 277 nm-02

6) Wavelength Change 279 nm

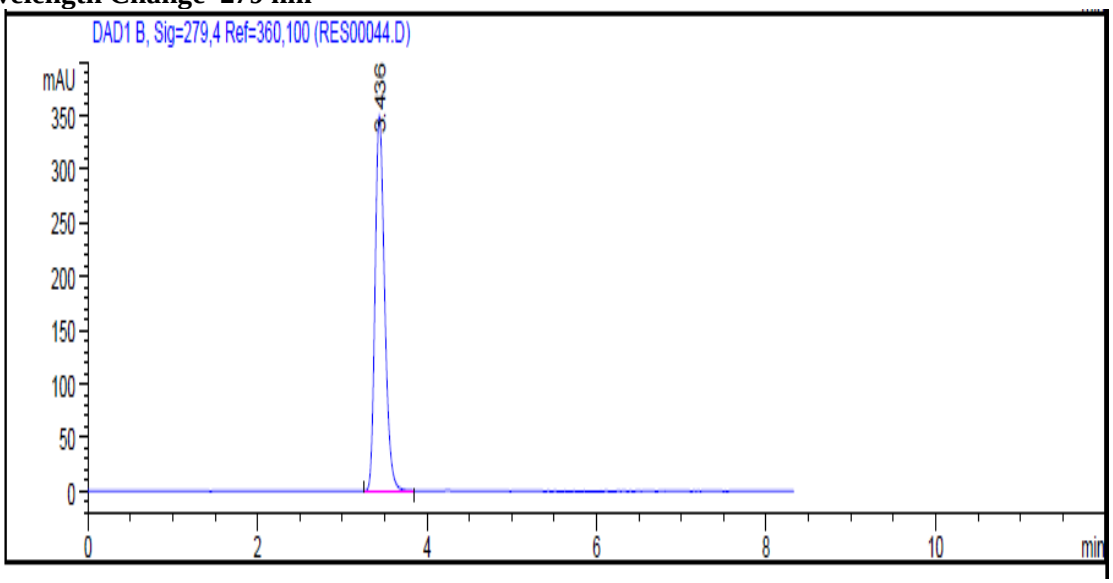


Fig. No 54: Chromatogram of comp change wavelength change 279 nm-01

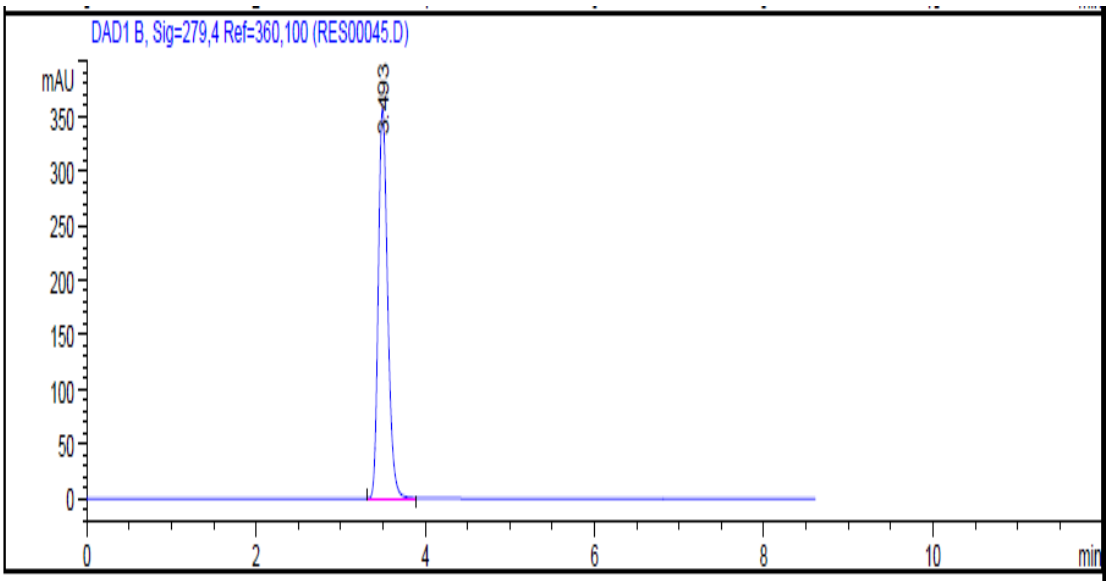


Fig. No 55: Chromatogram of comp change wavelength change 279 nm-02

Table No.22 Result of Robustness Study of Lefamulin

Parameters	Conc.(µg/ml)	Amount of detected(mean ±SD)	%RSD
Mob-phase composition(49ml+51ml) Methanol + 0.05% (OPA)water	25	2726.41±3.89	0.14
Mob-phase composition (51ml+49ml) Methanol + 0.05% (OPA)water	25	2712.04±3.05	0.11
Wavelength change 277 nm	25	2762.4±2.31	0.08
Wavelength Change 279 nm	25	2749.12±1.75	0.06
Flow rate change(0.9ml)	25	3126.34±30.48	0.97
Flow rate change(1.1ml)	25	3150.65±4.42	0.14

ROBUSTNESS STUDY OF LEFAMULIN :
The changes were did flow rate (±1 ml/ min^{-1}), PH of mobile phase composition, and Wavelength. %RSD for peak area was calculated which should be less than 2%.the result shown in analytical method that concluded. (**Table No.22)**

6. LIMIT DETECTION
The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope The limit of detection (LOD) may be expressed as:
LOD = 3.3 (SD)/S
= 3.3 X 3.21/ 108.3
= 0.097

Where, SD = Standard deviation of Y intercept
S = Slope

The LOD of Lefamulin was found to be 0.097 (µg/mL) analytical methods that concluded.

7. LIMIT QUANTIFICATION
The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope,

The quantitation limit (LOQ) may be expressed as:
LOQ = 10 (SD)/ S
=10 X 3.21 / 108.3
= 0.2963

Where, SD = Standard deviation Y intercept
S = Slope

The LOQ of Lefamulin was found to be 0.2963 (µg/mL) analytical methods that concluded.

3 Analysis Of Tablet Formulation:-
Procedure:
Weigh 20 Lefamulin Tablets and calculated the average weigh, accurately weigh and transfer the sample equivalent to 6.5 mg Lefamulin into 10 ml volumetric flask. Add about 10 ml of diluents and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.4ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (20 µg/ml). The simple chromatogram of test Lefamulin Shown in **(Fig No: 56, 57)** the amounts of Lefamulin per Tablet were calculated

by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with Tablet formulation. Tablet Assay for %Lable claim for %RSD Calculated, Result was shown in **(Table No. 23 & 24.)**

Brand Name : Xenleta-(600 mg)
Total weight of 20 Tab Powder wt. = 15.6 gms
Avg Powder Weight = 0.78 gms./Tab
Eq.Wt for 5 mg= 5 x 780/ 600 = 6.5 mg
Take 6.5 mgs in 10 ml Methanol i.e = 500 µgm/ml Lefamulin

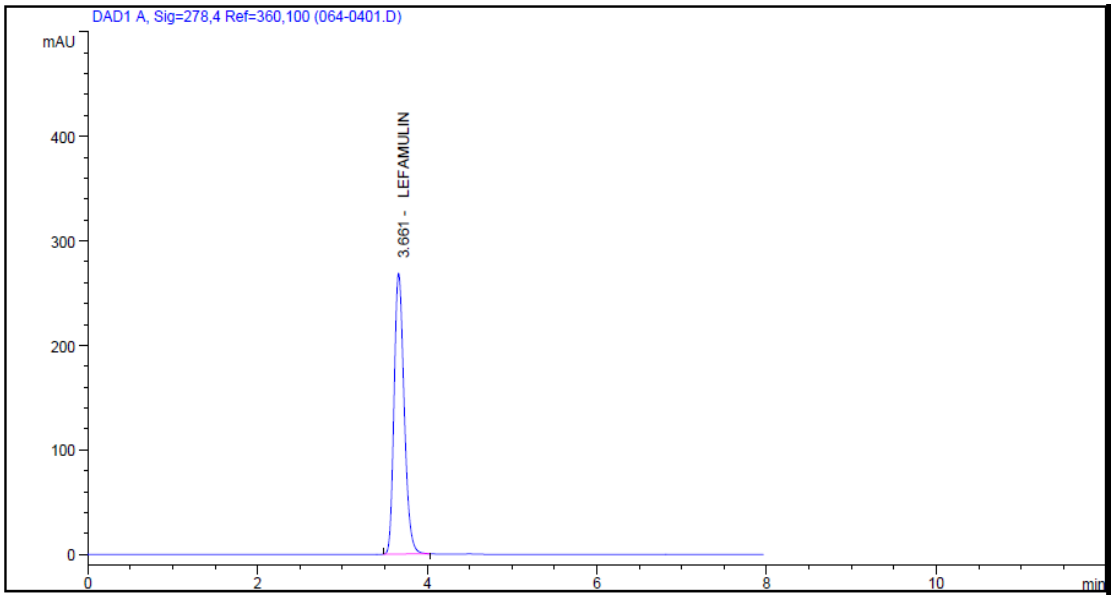


Fig No.56: Chromatogram for Marketed Formulation (20 MCG)-01

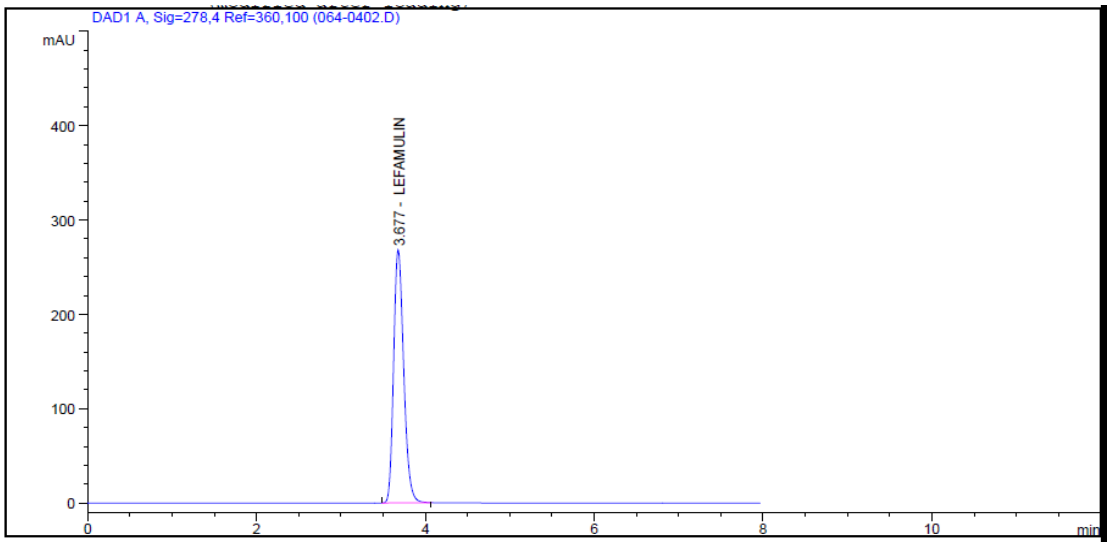


Fig No.57: Chromatogram for Marketed Formulation (20 MCG)-02

Table.23. Analysis of marketed formulation.

Sr.no	Amount present in mg	Area(I)	Amount found in mg	% Label claim
	LFM	LFM	LFM	LFM
1	20	2225.513	19.74269	98.71
2	20	2227.952	19.76521	98.83
Mean	-	2226.73	19.75	98.77
SD	-	1.725	0.016	0.080
%RsD	-	0.077	0.081	0.081

Analysis of marketed formulation were also %Lable Claim was found to be 98-101% Satisfactory are concluded. (Table No.23).

TABLET ASSAY FOR % LABLE CLAIM

Table No.24: Tablet for % Label claim

Sample	Label claimed	%Label claimed± SD	%RSD
XENLETA	Lefamulin =600mg	98.77 ± 0.08	0.081

Tablet Assay for %Lable claim for were also was found to be 100% and %RSD are less than 2 satisfactory result that concluded. (**Table No 24**)

CONCLUSION:

Lefamulin is Anti-Diuretics agents. The present work deals with "Hplc Method Development and Validation of Lefamulin in Bulk Drug and Pharmaceutical Dosage Form".

The method provides selective quantification of Lefamulin This developed RP-HPLC method for estimation of Lefamulin is accurate, precise, robust and specific. The method has been found to be better than previously reported method, because of its less retention time, isocratic mode and use of an economical and readily available mobile phase, readily available column, UV detection and better resolution of peaks.The amount found from the proposed methods was in good agreement with the label claim of the formulation. also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

REFERENCE:

1. https://www.rxlist.com/Lefamulin.
2. http://en.wikipedia.org/wiki/ Lefamulin
3. The Merck Index: An encyclopedia of chemical drugs and biological mono-1500002278.
4. https://pubchem.ncbi.nlm.nih.gov
5. www.chemicalbook.com › Chemical Book › CAS Database
6. Skoog D.A., Holler F.J. And Nieman T.A., Principles of Instrumental Analysis, 5th Ed., Thomson Brooks/Cole Asia Pvt. Ltd, Singapore, 2004, 4-7.
7. 5. Willard R.H., Merritt L.L., Dean L.A., And Settle F.A., Instrumental Methods Of Analysis, 7,C.B.S. Publishers, New Delhi, 1986, 177-178.
8. Skoog D.A., Holler F.J. And Nieman T.A., In Principles Of Instrumental Analysis, 5, Thomson Brooks/Cole, 1998, 329-335.
9. Brown R.P., Reversed-Phase High Performance Liquid Chromatography, Theory, Practice And Biomedical Applications, 1982, 10-20.
10. Settle F., In; Handbook Of Instrumental Techniques For Analytical Chemistry, Pearson Education Pte. Ltd., Delhi, 2004, 154-156.
11. Kasture A.V., Mahadik K.R. And More H.N., Instrumental Methods 14(2), Niraliprakashan, Pune, 2006,44-55.
12. Sethi, P.D., In Hplc 'High Performance Liquid Chromatography', Quantitative Analysis Of Pharmaceutical Formulations, 1, Cbs Publishers And Distributors, New Delhi, 2001, 3-72, 116-120.
13. Beckett A.H. And Stenlake J.B., In Practical Pharmaceutical Chemistry, 4(2), Cbs Publishers And Distributors, New Delhi, 2002, 275-278, 281-300.
14. Christian G.D., In; Analytical Chemistry, 6. Jhon Wiley And Sons, 2004, 1-7. 13. Connors, K.A., In; A Textbook Of Pharmaceutical Analysis, 3, Jhon Wiley And Sons, 1999, 196-198.
15. Brown R.P., Reversed-Phase High Performance Liquid Chromatography, Theory, Practice And Biomedical Applications, 1982, 10-20.
16. Saint Louis, Mo, Etats-Unis, International Symposium On High Performance Liquid Phase Separations And Related Techniques No 22, 1998, 828(1-2), 283-286.
17. Munson J.W., In; Pharmaceutical Analysis, Modern Methods, Part B, International Medical Book Distributors, Mumbai, 2001, 51-54.
18. Http://Www.Chromatography-Online.Org/Hplc.Html Accessed On 12 February 2014
19. Validation Of Analytical Procedures: Methodology, Ich Harmonized Tripartite Guidelines, November1996, 1-8.
20. Ich, Q2a, Text On Validation Of Analytical Procedures, International Conference On Harmonization, Geneva, October 1994, 1-5.
21. Ich, Q2b, Validation Of Analytical Procedures: Methodology, International Conference On Harmonization, Geneva, November 1996, 1-8.