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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD DIROXIMEL FUMARATE IN BULK DRUG AND IT'S DOSAGE FORM

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Abstract:
A Diroximel Fumarate is clinically used in combination in the treatment of anti-inflammatory agent. The present work deals with the RP-HPLC methods for Diroximel Fumarate in pharmaceutical formulation. attempts were made to develop rp-hplc method for estimation of diroximel fumarate bulk drug and formulation For the rp hplc method, agilent (s.k) gradient system uv detector and C18 column with 250mm x4.6 mm i.d and 5µm particle size methanol: water (0.05% Formic Acid) (90:10v/v) ph 3 was used as the mobile phase for the method. the detection wavelength was 255 nm and flow rate was 1.0 ml/min. in the developed method, the retention time of diroximel fumarate were found to be 3.78 min. the developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis diroximel fumarate in bulk drug as well as in formulations..

Keywords: *Diroximel Fumarate, RP-HPLC, Accuracy.*

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1. INTRODUCTION:

PHARMACEUTICAL ANALYSIS:

Pharmaceutical analysis may be defined as a branch of practical chemistry which deals with the resolution, purification, identification, and determination of a given sample of a medicine or a pharmaceutical as well as the detection and estimation of impurities that may be present in it. The most common techniques used in analytical chemistry are the following

Types of Analysis:

Qualitative analysis (Identification)

Quantitative analysis (Estimation)

Qualitative analysis (Identification)

It is performed to the established composition of natural and synthetic substances. The qualitative analysis deals with the identification of elements, ions or compounds present in the sample.

Quantitative analysis (Estimation)

Quantitative analysis deals with the determination if how much of one or more constituent are present. These techniques are mainly used to quantify components or substances in the sample.

Types of analytical techniques

A) Spectral Methods:-The spectral techniques measure electromagnetic radiation, which is either absorbed or emitted by sample. For example: UV visible spectroscopy, I. R. spectroscopy, N.M.R., E.S.R. spectroscopy, flame photometry, flourimetry etc.

b) Titration Methods:- It is a common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of an identified analytic.

For example: Acid-Base Titration, Redox Titration, Precipitation Titration, and Complexometric Titration.

c) Electro Analytical Methods: - Electro analytical methods involve the measurement of current, voltage or resistance. For example: Potentiometric, Conductometry, Aerometry etc.

d) Titration Methods:- It is a common laboratory method of quantitative chemical analysis that is used to determine the unknown concentration of an identified analytic.

For example: Acid-Base Titration, Redox Titration, Precipitation Titration, and Complexometric Titration.

e) Gravimetric analysis:- It is a process of isolating and weighing an element or a compound of an element in the pure form. It is quantitative determination of an analytic based on the mass of solid.

TGA: It is a measurement of amount and rate of change in the weight of a substance with respect to a temperature and time in a controlled environment

DTA: In Differential Thermal Analysis, the temperature difference that develops between a sample and an inert reference material is measured, when both are subjected to identical heat - treatments.

f) Radiochemical methods: - Radiochemistry is defined as "the chemical study of radioactive elements, both natural and artificial, and their use in the study of chemical processes"

g) Microscopy: - Microscopy is the technical field of using microscopes to view objects and areas of objects that cannot be seen with the naked eye (objects that are not within the resolution range of the normal eye).

For Example: optical, electron, and scanning probe microscopy.

It is define as the technique of separation of a mixture of components into an individual component through equilibrium distribution between two phases.

h) Chromatographic methods: - It is define as the technique of separation of a mixture of components into an individual components through equilibrium distribution between two phases..

2. DRUG PROFILES

Diroximel Fumarate:

Molecular Formula: C11H13NO6

Molecular weight: 255.22 g/mol

Structure:

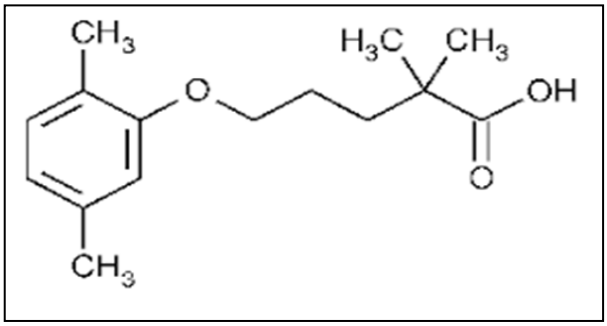

Fig. No.1: Chemical Structure of Diroximel Fumarate

Description: white to almost white solid.

IUPAC Name: 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid

Mechanism of action:

Currently, the mechanism of action of this drug in MS is not fully understood. Diroximel fumarate is hypothesized to regulate cell signaling pathways, causing beneficial immune and neuroprotective effects. Monomethyl fumarate (MMF) is the active metabolite of diroximel fumarate, and activates the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway in humans. This pathway occurs as a response to oxidative stress in cells.

In addition to the above, MMF is a nicotinic acid receptor agonist in the laboratory setting. The relevance of this finding to the treatment of MS is unknown currently. The mechanism by which this drug leads to less gastrointestinal effects is purported to be due to its lack of a methanol leaving group in its chemical structure, and substitution with inert 2-hydroxyethyl succinimide.

3. MATERIALS AND METHODS:

Chemicals used:

In method development and validation of preservatives following chemicals and reagents were used. .

Table 1: List of Reagents and Chemicals used

Ingredients	Grade	Suppliers
Diroximel Fumarate	API	R.S.I.T.C Jalgaon.
Formic Acid	HPLC	Avantor Performance material India Ltd. Thane, Maharashtra
Methanol	HPLC	Merck Specialities Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai
Water	HPLC	Merck Specialities Pvt. Ltd.Shiv Sager Estate 'A' Worli, Mumbai

HPLC:

Selection of Analytical Technique

HPLC was selected as analytical technique for estimation of Diroximel Fumarate

Instruments:

The analysis of the drug was carried out on Agilent (S.K.) Gradient System UV Detector. Equiped with Reverse Phase (Grace) C18 column (4.6mm x 250mm; 5µm), a SP930Dpump, a 20µl injection loop and UV730D Absorbance detector and running Chemstation..

Table .2: chromatographic conditions (HPLC) details used during method Development

1.	HPLC	Agilent (S.K)Gradient System
2.	Software	Chemstation
3.	Column	(Agilent) C18 column (4.6mm x 250mm)
4.	Particle size packing	5 µm
5.	Stationary phase	C-18 (Agilent)
6.	Mobile Phase	MEOH : Water (0.05% OPA)
7.	Detection Wavelength	220 nm
8.	Flow rate	0.8 ml/min
9.	Temperature	25° C (Ambient)
10.	Sample size	20 µl
11.	pH	3.
12.	Run Time	15 min
13.	Filter paper	0.45 µm

4. EXPERIMENTAL WORK

METHOD DEVELOPMENT OF HPLC:

❖ **List of Mobile Phase**

Table .3: Selection of mobile Phase

Sr.no	Mobile Phase
1.	[80% MEOH +20% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 255 nm (column 250mm X 4.6, 5 µm)
2.	[70% MEOH +30% Water (OPA pH 3.2 adjust with TEA) Flow 0.8 ml/min abs at 255 nm (column 250mm X 4.6, 5 µm)
3	[90 % MEOH + 10 % Water (formic acid pH 3.3) Flow 1.0 ml/min abs at 255 nm (column 250mm X 4.6, 5 µm)

Selection of wavelength by UV-Visible Spectrophotometry: -

Preparation of standard stock solution: -

- **Diroximel Fumarate standard stock solution: (Stock II)**

An accurately weighed quantity, 10 mg of Diroximel Fumarate (DF) was dissolved in methanol in 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 1000 µg/ml.

- **Preparation of Stock Standard Solution :(Stock III) [ZIP]**

Accurately weight and transfer 10 mg Diroximel Fumarate working standard into 10 ml volumetric flask as about diluent methanolcompletely and make volume up to the mark with the same solvent to get 1000µg/ml standard (stock solution) and 15 min sonicate to dissolve it and remove the unwanted gas,further an aliquots portion of Diroximel Fumarate stock solution in ratio of 90:10were mixed in volumetric flask in 10 ml and volume was adjusted up to mark with mobile phase from the resulting solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with methanol:Water (0.05% Formic Acid), prepared in (9ml methanol: 1ml Water (0.05% Formic Acid))solvent.

HPLC used for chromatographic condition apply on the Preparation of standard solution:-

- **Preparation of std. Diroximel Fumarate solution: (Stock II)**

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

Selection of mobile phase:

Each mobile phase was vacuum degassed and filtered through 0.45µ membrane filter. The mobile phase was allowed to equilibrate until steady baseline was obtained. The standard solution containing mixture of Diroximel Fumarate was run with different individual solvents as well as combinations of solvents were tried to get a good separation and stable peak. From the various mobile phases tried, mobile phase containing methanol and Water (0.05% Formic Acid) with pH adjust (3.0)was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for Diroximel Fumarate. Chromatograms of Diroximel Fumarate are shown in **(Table No: 10)** respectively.

Procedure for calibration curve of Diroximel Fumarate:

The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. From the freshly prepared standard stock solution, pipette 10 mg Diroximel Fumarate in 10ml of volumetric flask and diluted with mobile phase. From it 0.1, 0.2, 0.3, 0.4 and 0.5ml of solution were pipette out in 10 ml volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration 10,20,30,40 and 50µg/ml of Diroximel Fumarate. Sample were injected and peaks were recorded at 255 nm as the graph plotted as concentration of drug verses peak area is depicted respectively.

Study of system suitability parameters:

The system suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from five replicate injections of standard solution.

<u>Calibration Experiment</u>:

➢ **RP-HPLC Method :**

a) Preparation of Calibration curve standard:

The above standard stock solution (10µg/ml) of Diroximel Fumaratewas diluted with mobile phase to yield five calibration curve (cc) standards with concentrations of 10,20,30,40 and 50µg/ml of Diroximel Fumarate

b) <u>Selection of detection Wavelength</u> :

Standard solutions were scanned in the range of 200-400nm, against 10 ml methanol and volume make with water solvent system as reference **and** Diroximel Fumarate were showed absorbance maxima (lamda max) at 255 nm.

c) Calibration standard drug and regression equation data :

From the standard stock solution of Diroximel Fumarate, different concentration were prepared 10-50µg/ml for Diroximel Fumarate and measured at 255 nm. The calibration curves were plotted

d) Calibration runs and regression analysis:

These calibration standard solutions were analyzed in three replicates using the under mentioned chromatographic conditions.

- Analytical column: Grace C18 Column (250mm x 4.6mm, 5µm partical size).
- Injection volume : 20µl.
- Flow rate : 1.0 ml/min.
- Mobile phase : methanol: Water (0.05% OPA) (90: 10 % V/V).
- Detection : 255 nm.

Validation of method for analysis of Diroximel Fumarate:

- The developed method was validated as per ICH guidelines.

Linearity:

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range,

- **Determination:**

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration Percentage curve fittings is calculated**.**

- **Acceptance Criteria:**

The plot should be linear passing through the origin.
Correlation Coefficient should not be less than 0.999.
The Result are shown in;

- **Preparation of standard stock solution for linearity:**

Weight of 10 mg Diroximel Fumarate was weighed and transfered to 10 mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. 0.1 ml of this solution diluted upto 10 ml volumetric flask with diluents was added to make up the volume.

- **Preparation of linearity solution:**

A series of standard preparations of working standard of were prepared.

Table 4: Table of linearity for RP-HPLC Method

Diroximel Fumarate
10
20
30
40
50

Accuracy (recovery):

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay,

- **Acceptance Criteria:**

Mean recovery should be in the range of 98-102%.
The Relative Standard Deviation should not be more than 2.0%.

- **Preparation of standard stock solution:**

10mg of Diroximel Fumarate working standards were weighed and transfered to 10 mL volumetric flask & diluent was added to make up the volume 0.1 ml of this solution diluted upto 10 ml with diluent.

- **Accuracy**

The accuracy was determined by Diroximel Fumarate (10mg of Diroximel Fumarate(50 %, 100 % and 150 % of the label claimed, respectively) to quantity equivalent to average weight of marketed Capsules. This powder mixture containing 10 mg of Diroximel Fumarate were triturated and then subjected to chromatographic analysis using the described method. The resulting mixtures were analyzed in triplicates over three days. The % recovery of added drug was taken as a measure of accuracy**.**

Table 5: Table of Accuracy for RP-HPLC Method

Sample	**Diroximel Fumarate**	
	Taken	Added
Accuracy 50%	10	5
Accuracy 100%	10	10

Repeatability:

Precision of the system was determined with the sample of RP-HPLC Method for . Six replicates of sample solution containing 10 mg of Diroximel Fumarate were injected and peak areas were

measured and %RSD was calculated is was repeated for five times

- **Application of proposed method for analysis of marketed formulation:**

Weight 10 mg of Diroximel Fumarate was weighed and transfered to 10mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. The above solution was filtered through 0.45µm membrane filter 0.1 ml of this solution diluted upto 10 ml with diluent.

Precision:

Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. Also, the results obtained were subjected to one way ANOVA and within-day mean square and between-day mean square was determined and compared using F-test.

Result of Intra day and Inter day Precision studies on RP-HPLC method for Diroximel Fumarate

Intra-day precision:

Sample solutions containing 10 mg of Diroximel Fumarate three different concentration 20 µg/ml, 30µg/ml, 40µg/ml Diroximel Fumaratewere analyzed three times on the same day and %R.S.D was calculated.

Inter-day precision:

Sample solutions containing 10mg of Diroximel Fumarate three different concentration 20µg/ml, 30µg/ml, 40µg/ml Diroximel Fumarate different days and % R.S.D was calculated. It is usually expressed as standard deviation or relative standard deviation.

- **Acceptance criteria:**

The Relative Standard Deviation should not be more than 2% for test

- **Preparation of standard stock solution:**

10 mg of Diroximel Fumarate working standards were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume. 0.1 ml of this solution diluted upto 10 ml with diluent.

Robustness:

The mobile phase composition was changed in (±1 ml/ min^{-1}**)** proportion and the flow rate was methanol: Water (0.05 % Formic acid) in the mobile phase composition (±1ml/ min^{-1}**)** and the change in detection wavelength (±1 ml/ min^{-1}**)** and the effect of the results were examined**.** it was performed using 20 µg/ml solution of Diroximel Fumarate in triplicate.

Detection Limit

Based on the S.D. of the response and the slope of calibration curve, the detection limit (DL) was calculated as,

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

Quantitation Limit

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as,

$$QL = \frac{10\sigma}{S}$$

Where,

σ = the S.D. of the y-intercepts of regression lines.

S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y-intercepts of regression line in calibration curve.

Analysis of marketed formulation

To determine the content of Diroximel Fumarate in marketed Capsules (label claim 10 mg of Diroximel Fumarate), 20 Capsules powder weighed in 6.03 gms and average weight of powder was calculated in 0.3015 gms. Capsules were triturated and powder equivalent to weigh in 13.05 mg the drug was extracted from the Capsules powder with 10 mL methanol. To ensure complete extraction it was sonicated for 15 min. 0.1mL of supernatant was then diluted up to 10 mL with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted**.**

Regression equation was generated using peak areas of standard solutions. Using the regression equation and peak area of the sample the amount of Diroximel Fumarate in the sample was calculated. The amount of Diroximel Fumarate per Capsules was obtained from the regression equation of the calibration curve as described in analysis of Capsules formulation.0

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5. RESULT AND DISCUSSION:

Preliminary studies on Diroximel Fumarate

Melting point

The procured reference standard of Diroximel Fumarate were found to melt in the range of 96-99^{0}C respectively.

Solubility

The drug was found to be Diroximel Fumarate slightly soluble in water, soluble in methanol DMSO, ethanol. Insoluble in ether.

UV Spectroscopy

UV absorption of 20mcg solution of Diroximel Fumarate in MEOH was generated and absorbance was taken in the range of 200-400 nm .λ max of Diroximel Fumarate was found to be 255 nm respectively.

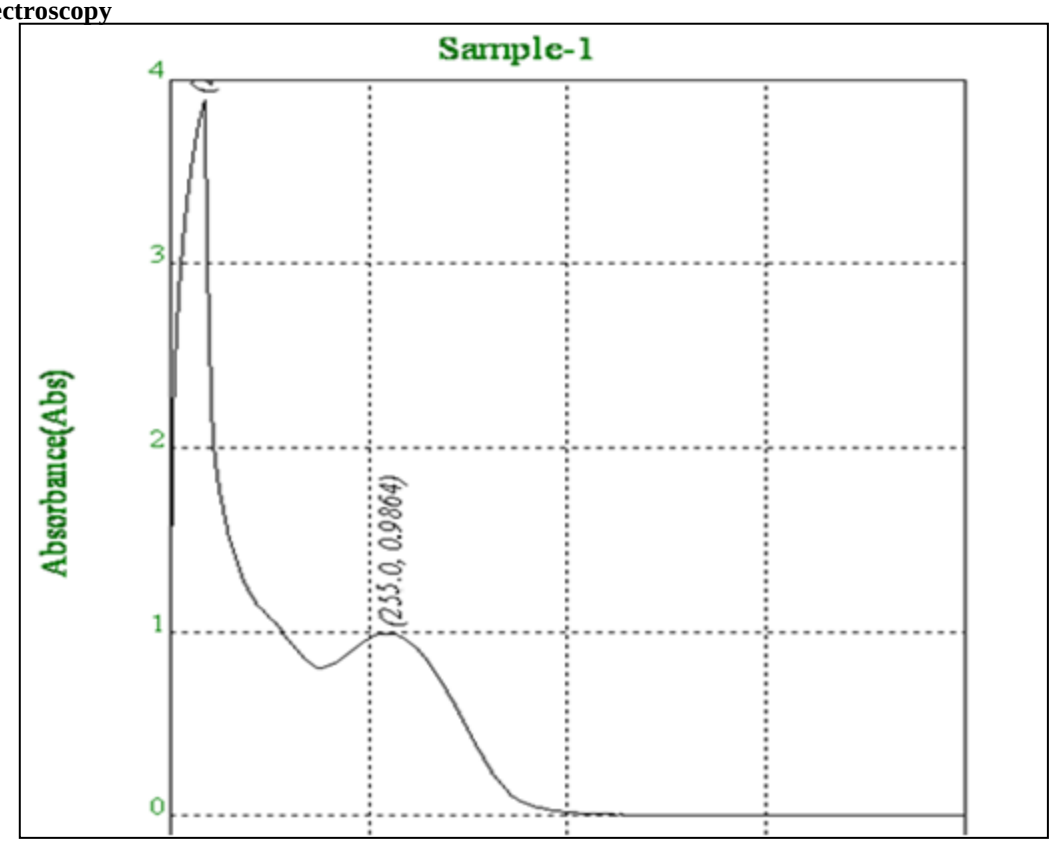

Fig No. 2: UV Spectrum of Diroximel Fumarate

Studies on the chromatographic behavior of Diroximel Fumarate

Table 6: Chromatographic behavior of Diroximel Fumaratemobile phase of various compositions.

Sr No.	Mobile Phase	Retention time	Remark
1.	[80% MEOH +20% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 255 nm (column 250mm X 4.6, 5 µm)	6.59	Broad peak is obtained (TF more than 2)
2	[70% MEOH +30% Water (OPA pH 3.2 adjust with TEA) Flow 0.8 ml/min abs at 255 nm (column 250mm X 4.6, 5 µm)	15.85	Retention time was high
3	[90 % MEOH + 10 % Water (Formic Acid pH 3.3) Flow 1.0 ml/min abs at 255 nm (column 250mm X 4.6, 5 µm)	3.83	Sharp Peak was obtained

Conclusion, from the above, it has been observed that, using mobile phase of MEOH + Water (0.1% Formic Acid) (90 + 10 % v/v)255 nm, 1.0ml,gave adequate retention time at 3.83 min. with good peak shape (Theoretical plates of Diroximel Fumarate)

Chromatogram of Trial 1:

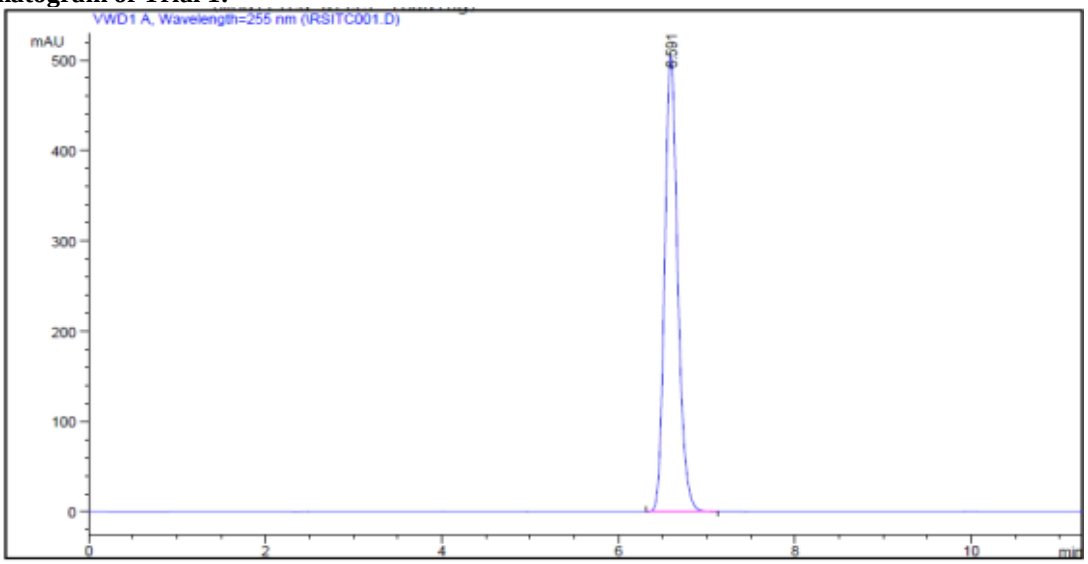

Fig.No.3: Representative Chromatogram of Diroximel Fumarate on 80% MEOH +20% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min abs at 255 nm (column 250mm X 4.6, 5 µm)

Table 7: Chromatogram result of Diroximel Fumarate on 80% MEOH +20% Water (pH 3.0 adjust with OPA) Flow 0.7 ml/min at 255 nm (column 250mm X 4.6, 5 µm)

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	6.591	5215.05	9979	2.82

Conclusion for rejection of trial 1:

Broad peak was obtained TF is high. (TF less than 2) Unsatisfactory result.

Chromatogram of Trial 2:

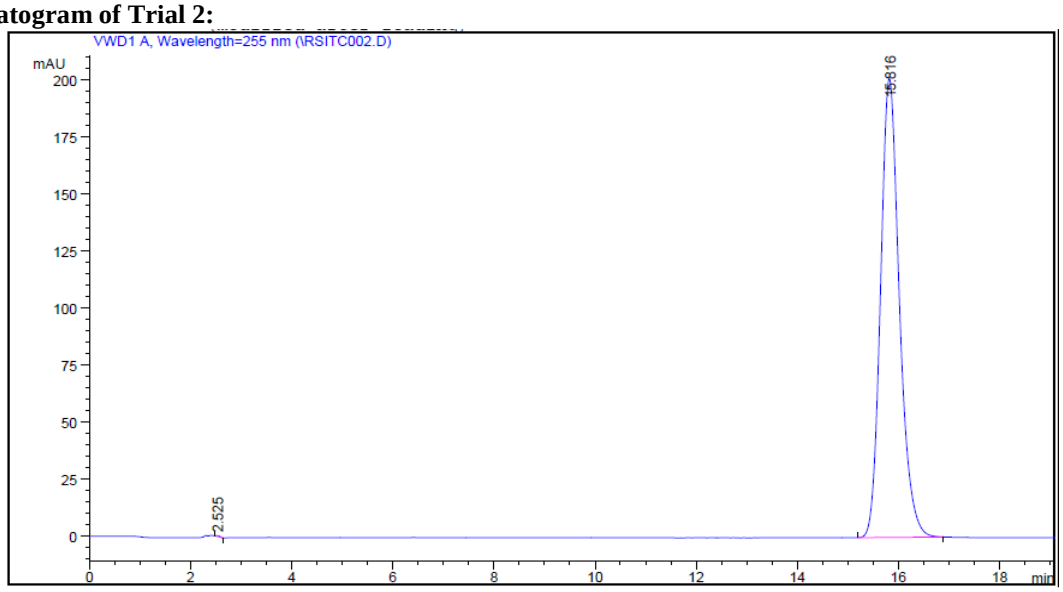

Fig.No.4: Representative Chromatogram of Diroximel Fumarate on 80% MEOH +20% Water (pH 3.0 adjust with OPA) Flow 0.8 ml/min at 255 nm (column 250mm X 4.6, 5 µm)

Table 8: Chromatogram result of Diroximel Fumarate on 80% MEOH +20% Water (pH 3.0) Flow 0.8 ml/min at 255 nm (column 250mm X 4.6, 5 µm)

Drug name	R.T	AREA	TH.PLATES	SYMM

Diroximel Fumarate	15.816	5199.21	9193	0.81

Conclusion for rejection of trial 2:
Retention time is high, unsatisfactory result.
Chromatogram of Trial 3:

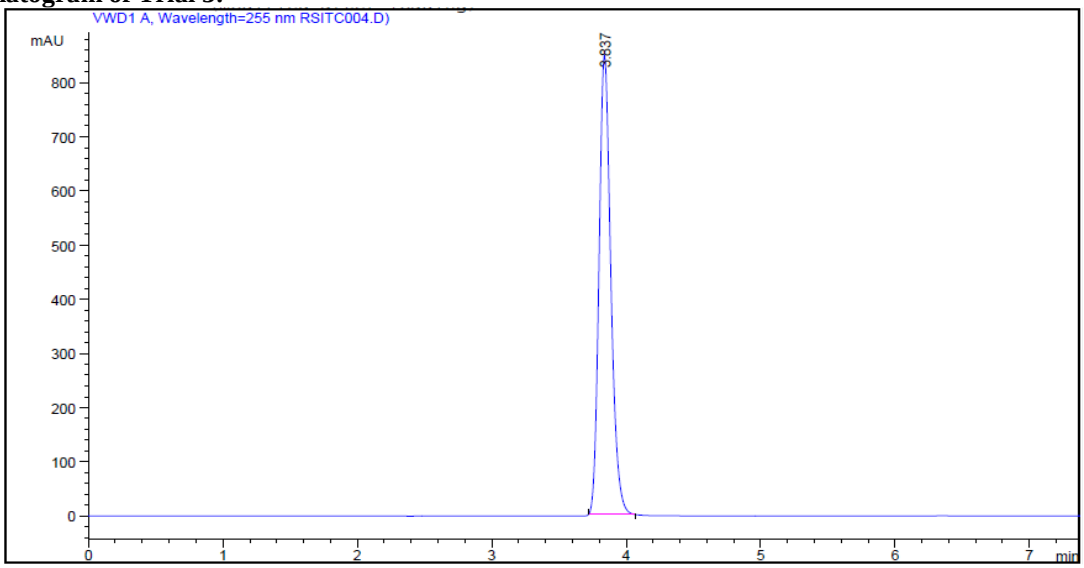

Fig. No. 5: Representative Chromatogram of Diroximel Fumarate on75% MEOH +25% Water (Formic Acid) Flow 0.7 ml/min abs at 255 nm (column 250mm X 4.6, 5 µm)

Table 9: Chromatogram result of Diroximel Fumarate on75% MEOH +25% Water (Formic Acid) Flow 0.7 ml/min abs at 255 nm (column 250mm X 4.6, 5µm)

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	3.837	5165.39	9852	0.79

Conclusion for acceptance of trial 3:
The shape of peak was obtained, be good with USP more plate count and satisfactory result, so method was selected.

Calibration experiment

- **RP-HPLC Method :**

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 10-50 µg/mL for Diroximel Fumarate (**Table No. 10)** depict the calibration data of Diroximel Fumarate The respective linear equation for RP-HPLC Diroximel Fumarate was y = 44.22 x – 15.34 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Diroximel Fumarate is depicted in (**Fig No. 6)**

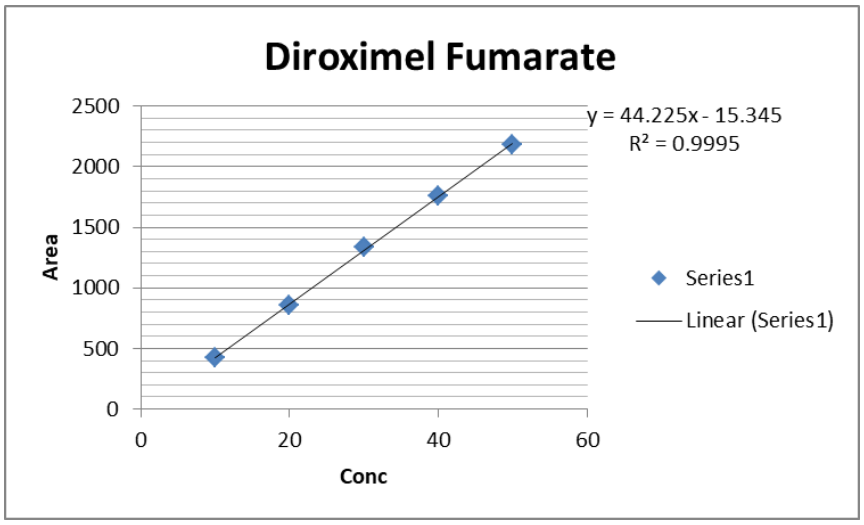

Fig.No.6: Calibration curve of Diroximel Fumarate

Table 10: Linearity data for Diroximel Fumarate

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Area
		1	2			
RP-HPLC Method	10	423.03	420.33	421.68	1.91	0.45
	20	858.11	857.42	857.77	0.49	0.06
	30	1336.86	1338.53	1337.70	1.18	0.09
	40	1758.30	1755.5	1756.90	1.98	0.11
	50	2183.34	2183.28	2183.31	0.04	0.00
	Equation		y = 44.225 x – 15.345			
	R^2		0.999			

The RP-HPLC Method for respective linear equation for Diroximel Fumarate was y = 44.225 x – 15.345 where x is the concentration and y is area of peak. The correlation coefficient was 0.999. The calibration curve of Diroximel Fumarate is depicted.

Analyticalof Method Validation:

1. Linearity:

From Diroximel Fumarate standard stock solution, different working standard solution (10-50µg/ml) were prepared in mobile phase 20 µl of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatogram was recorded. The areas of each concentration were recorded shown in (T**able 11)** the plot calibration curves ar shown in (**fig no**. 7)

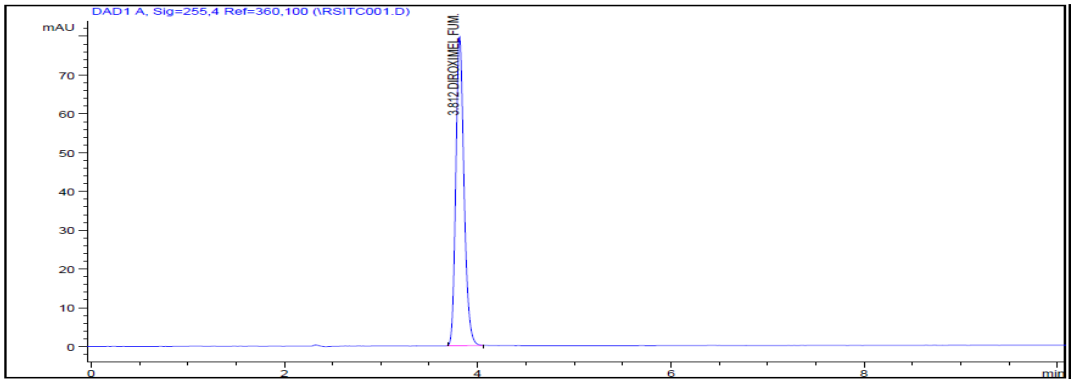

Fig.No.7. Chromatogram of linearity LIN 10mcg microgram/ml-1

Table 11: Chromatogram of linearity LIN 10 mcg microgram/ml-1

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	3.81	423.030	10189	0.79

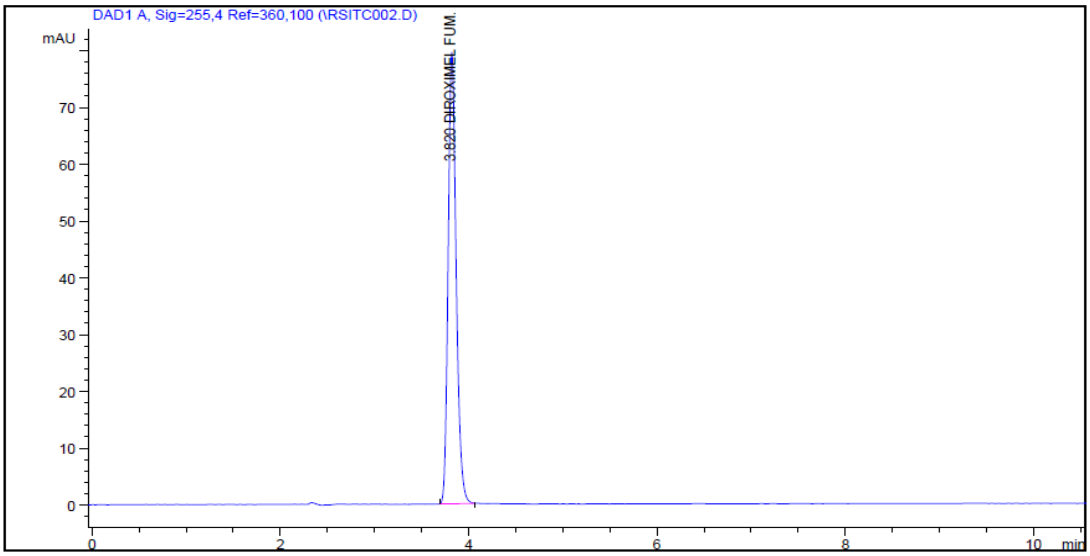

Fig.No.8. Chromatogram of linearity LIN 10 mcg microgram/ml-2

Table 12: Chromatogram of linearity LIN 10 mcg microgram/ml-2

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	3.82	420.33	10231	0.78

2. Accuracy:-

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed Capsules solution, a definite concentration of standard drug (50%, 100%, and 150%) was added and then its recovery was analyzed **(Table No.13).** Statistical validation of recovery studies shown in **(Table No. 14)**

Accuracy 50%

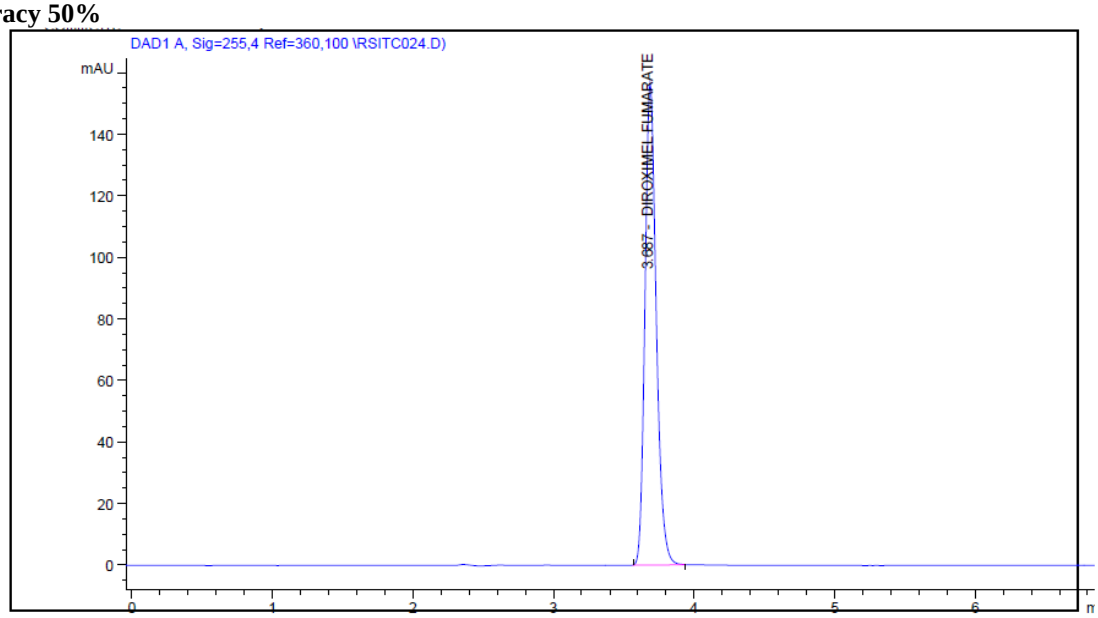

Fig. No. 9. Chromatogram of Accuracy 50%-1

Table. 13. Chromatogram of Accuracy 50% -1

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	3.687	647.25	10844	0.79

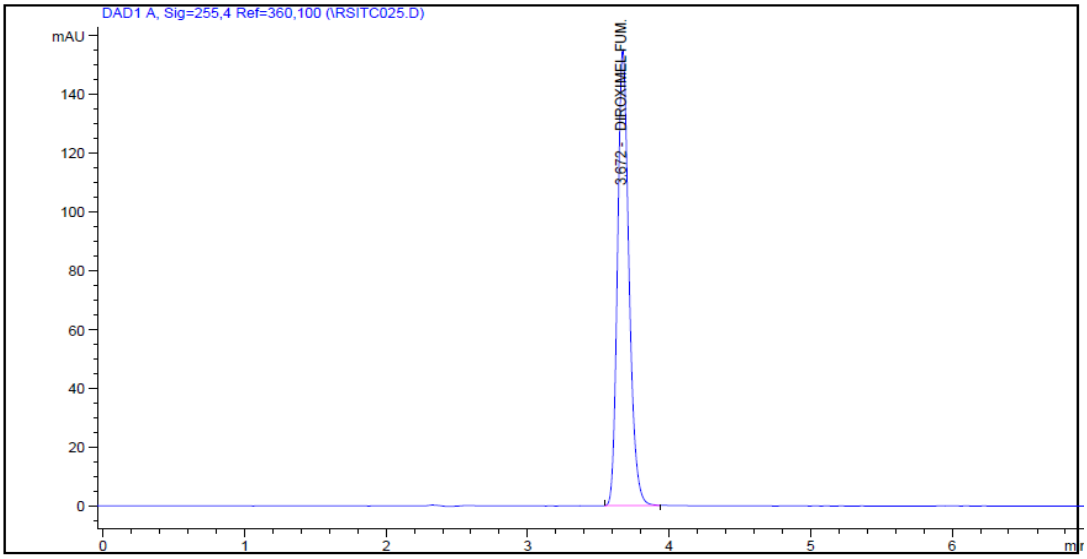

Fig.No. 10. Chromatogram of Accuracy 50%-2

Table. 14. Chromatogram of Accuracy 50% -2

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	3.67	649.09	10474	0.78

3. System suitability parameters: (Repeatability)

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Diroximel Fumarate system suitability parameters were studied. The result shown in below **(Table No.15)**

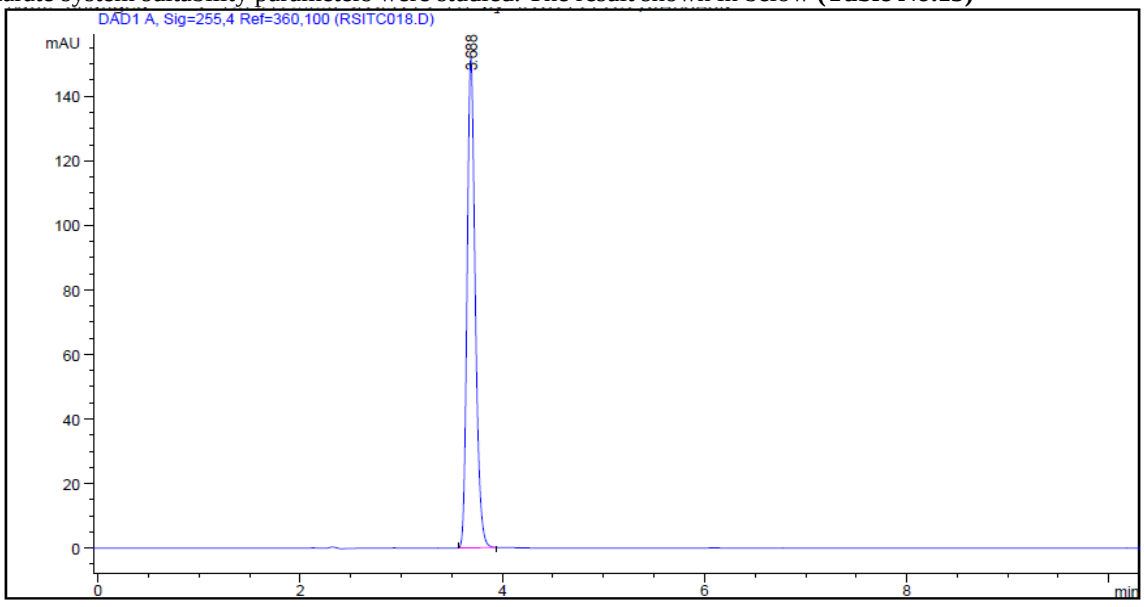

Fig No 11: Chromatogram of System suitability (20) mcg-1

Table 15: Chromatogram of System suitability (20) mcg-1

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	3.68	854.02	10532	0.79

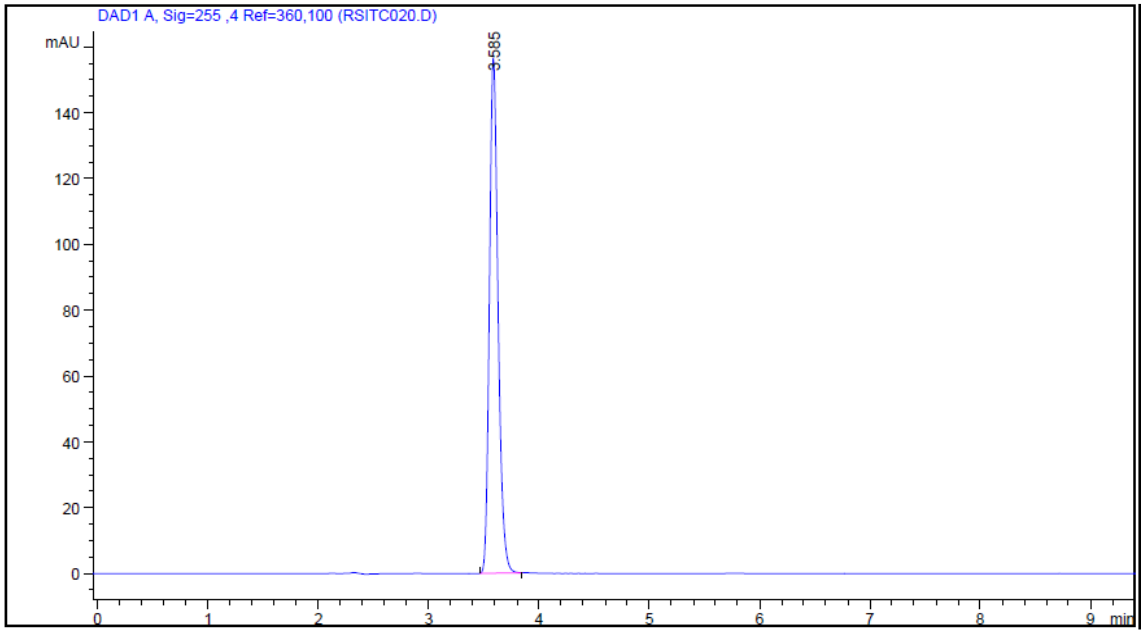

Fig No 12: Chromatogram of System suitability (20) mcg-2

Table 16: Chromatogram of System suitability (20) mcg-2

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	3.58	855.49	10534	0.77

7.3 Analysis of Capsules formulation:-

Procedure:

Weigh 20 Diroximel Fumarate Capsules and calculated the average weight, accurately weigh and transfer the sample equivalent to 13.05 mg Diroximel Fumarate into 10 ml volumetric flask .Add about 10 ml MEOH of diluent 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 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (40 µg/ml). The simple chromatogram of test Diroximel Fumarate Shown in **(Fig No: 13,14)** the amounts of Diroximel Fumarate per Capsules were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated two times with Capsules formulation. Capsules Assay for %Label claim for %RSD Calculated, Result was shown in **(Table No. 17,18)**

Brand Name: Vumerity 231 mg (BIOGEN)

Total weight of 20 Cap wt. = 6.03 Gms

Avgr Weight = 0.3015 Gms./Tab

Eq.wt for mg= 10 X 301.5 /231 = 13.05 mg

Take 13.05 mgs in 10 ml Methanol sonicate 15 min

i.e. Diroximel Fumarate 1000 µgm/ml r----- STOCK –II

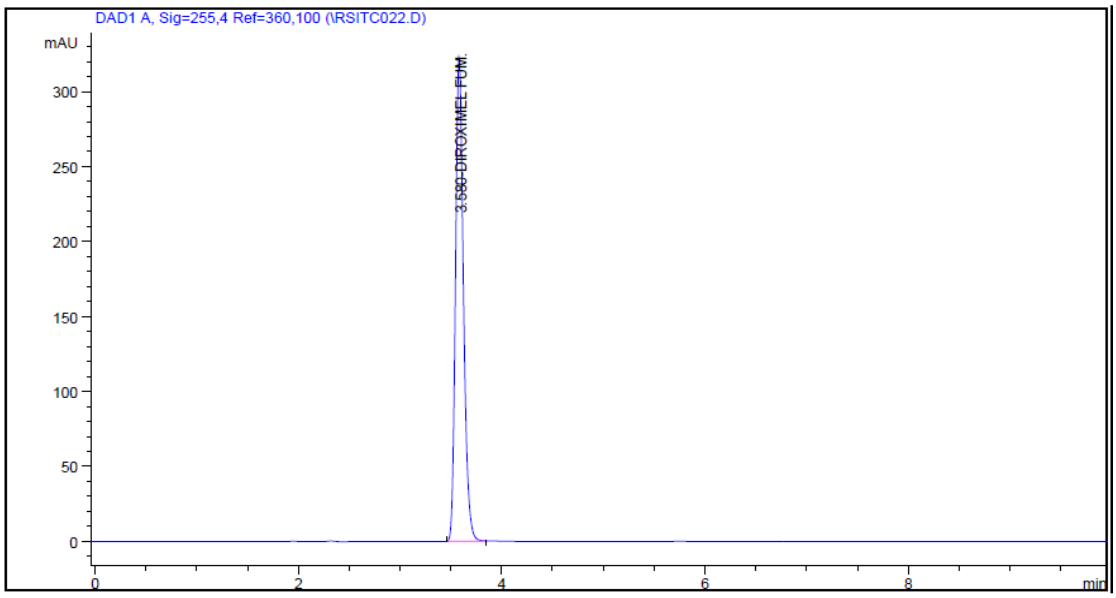

Fig No.13: Chromatogram for Marketed Formulation-1

Table. 17: Analysis of marketed formulation-1

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	3.58	1758.67	10792	0.77

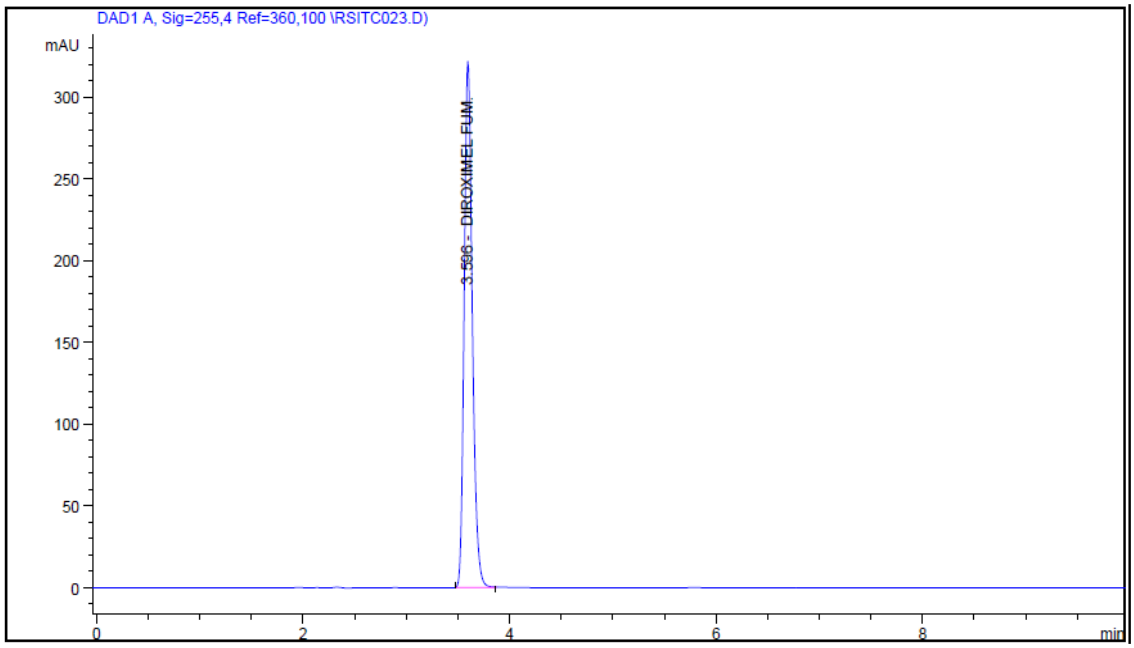

Fig No.14: Chromatogram for Marketed Formulation-2

Table.18 Analysis of marketed formulation-2

Drug name	R.T	AREA	TH.PLATES	SYMM
Diroximel Fumarate	3.59	1763.77	10595	0.78

Analysis of marketed formulation were also %Label Claim was found to be 98-101% Satisfactory are concluded**. (Table No.18)**

6. SUMMARY AND CONCLUSION:

Simple, rapid, accurate and precise RP-HPLC method have been developed and validated for the routine analysis of Diroximel Fumarate in API and capsules dosage forms. Method is suitable for the determination of Diroximel Fumarate in formulations without any interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. 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 dosage forms.

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8. CONFLICTS OF INTEREST
Authors have no conflicts of interest to declare.

9. REFERENCES:

1. P.C. Kamboj. 'Pharmaceutical Analysis'. Vallabh Prakashan. Volumn I (2010), Page No : 1-2.
2. A. Khair. 'New Age International (P) Limited, Publisher'. Page No : 452-453.
3. A.V.Kasture, K.R.Mahadik. 'Pharmaceutical Analysis'. Nirali Prakashan. Volumn II (2011), Page No : 48-156
4. D.A Skoog, D.M.West. 'Analytical chemistry – An Introduction'. Saunder college Publishing. 6th Edition (2014). Page No 3
5. H. H .Willard et. Al. 'Instrumental Methods Of Analysis'. CBS Publishers And Distributors. 7th Edition, Page No : 513
6. D. A. Skoog, F. J. Holler. 'Instrumental Analysis'. Indian Edition, Page No : 14
7. A.V.Kasture, K.R.Mahadik. 'Pharmaceutical Analysis'. Nirali Prakashan. Volumn II (2011), Page No : 48-156
8. G.R.Chatval, A.Sharma. 'Instrumental Methods Of Chemical Analysis'. Himalaya Publication House (2004), (2) Page No : 2.624-2.639.
9. S.K.Rasool, D.V.Naik. 'RP-HPLC Method For The Estimation Of Valacyclovir In Bulk And Pharmaceutical Formulations. International Journal of Pharmacy And Pharmaceutical Sciences (2012), 4(1) Page No : 214-218.
10. Shimadzu UV-1700. Instruction Manual Operation Guide. Analytical Instrument Division, Kyoto, Japan (2001), Page No : 1-8.
11. G.R.Chatwal, S.K.Anand. 'Chromatography In Instrumental Methods Of Chemical Analysis. Himalaya Publishing House (2005), (5) Page No : 2.566. 1. P.C. Kamboj. 'Pharmaceutical Analysis'. Vallabh Prakashan. Volumn I (2010), Page No : 1-2.
12. P.Nagaraju, G.Rao. 'Role of HPLC In Pharmaceutical Analysis'. Indian Pharmacist (2003), Page No : 25-27.
13. J.Swarbrick, J.Boylan. 'Encyclopedia Of Pharmaceutical Technology'. Marcel Dekker Inc., New York (1998), (1) Page No : 217 - 224
14. S.Lindsay. 'HPLC By Open Learning, John Wiley And Sons, London (1991), Page No : 30-45.
15. P.D.Sethi. 'High Performance Liquid Chromatography Quantitative Analysis of Pharmaceutical Formulation'. CBC Publication and Distributors. 1st Edition (2001). Page No 8-10.
16. V.R.Meyer. 'Practical High Performance Liquid Chromatography. John Wiley And Sons, London (1993), (2) Page No : 26-258.
17. G.Szepei. 'HPLC In Pharmaceutical Analysis (1990), (1) Page No : 101-173.
18. Https://en.wikipedia.org/wiki/Validation(drug manufacture)#The validation process
19. M.A.Potdar. 'Pharmaceutical Quality Assurance'. Nirali Prakashan, Page No : 8.1.
20. R.Nash. 'Pharmaceutical Process Validation'. Marcel Dekkar Publication. 3rd edition (2002), Page No : 340-341.
21. R.Raghunandhan. 'Validation of Pharmacetical Process'. Pharma Times (1999), 31 (11) Page No : 61-62
22. K. D. Tripathi "Essential of Medical Pharmacology" Jaypee Publishers, 7th edition,Page no 274.

23. Douša, M., Slavíková, M., Kubelka, T., Černý, J., Gibala, P., & Zezula, J. (2018). HPLC/UV/MS method application for the separation of obeticholic acid and its related compounds in development process and quality control. Journal of Pharmaceutical and Biomedical Analysis, 149, 214-224.

24. Douša, M., Slavíková, M., Kubelka, T., Černý, J., Gibala, P., & Zezula, J. (2018). HPLC/UV/MS method application for the separation of obeticholic acid and its related compounds in development process and quality control. Journal of Pharmaceutical and Biomedical Analysis, 149, 214-224.

25. Hu, C., Li, Y., Liu, Y., Lai, Y., & Ding, L. (2022). A Sensitive HPLC–MS/MS Method for Determination of Obeticholic Acid in Human Plasma: Application to a Pharmacokinetic Study in Healthy Volunteers. Journal of Chromatographic Science, 60(6), 545-550.

26. Jyothi, R. K., Chandrasekhar, K. B., Balakrishnan, M., & Kiran, B. S. S. Stability indicating RP-HPLC method development and validation for the quantification of obeticholic acid in bulk and its pharmaceutical dosage form. Indian Journal of Pharmaceutical Education and Research.(2020) 54 (2): 344, 349.

27. Nagunuri, G. C. L., Katangoor, V., Surigilla, V., Sajja, E., Srinivasan, T. R., & Mathad, V. T. (2022). Efficient Process for Obeticholic Acid: Synthesis, Structural Assignment, and Control Strategy for Diastereoisomeric Impurities. Organic Process Research & Development, 26(12), 3265-3275.

28. Cindy Green, RAC. A Step By Step Approach to Establishing a Method Validation. Journal of Validation Technology August 2007; 13(4):p.317-323.

29. Kiranjyothi, R., Balakrishnan, M., & Chandrasekhar, K. B. (2021). Stability Indicating RP-HPLC Method Development and Validation for Estimation of Diroximel Fumarate in bulk and its dosage forms. Research Journal of Pharmacy and Technology, 14(5), 2603-2607.

30. Habib, A. A., Hammad, S. F., Amer, M. M., & Kamal, A. H. (2021). Stability indicating RP-HPLC method for determination of dimethyl fumarate in presence of its main degradation products: Application to degradation kinetics. Journal of Separation Science, 44(3), 726-734.