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Analytical Method Development And Validation Of Diroximel Fumarate In Dosage Form By Rp-Hplc

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Abstract: Diroximel Fumarate, sold under the brand name Vumerity, is a medication used for the treatment of relapsing forms of Multiple Sclerosis (MS). A Novel, Simple, Accurate, Precise method was developed for the estimation of Diroximel Fumarate in dosage form by RP-HPLC technique. Chromatographic conditions used are stationary phase Agilant C_{18} (150mm x 4.6 mm), Mobile phase was Acetonitrile: 0.05% Acetic Acid (60:40% v/v) and flow rate was maintained at 0.7 ml/min, detection wavelength was 253nm and Conditions were finalized as optimized method. Regression equation of Diroximel Fumarate is y = 81.45x + 159.0. Linearity study was carried out between 5% to 25 % levels, R2 value was found to be as 0.999. Retention time of Diroximel Fumarate was found to be 2.854 min. LOD, LOQ values obtained from regression equations of Diroximel Fumarate were 0.0498 µg/ml and 0.1510 µg/ml respectively. So it is worthwhile that, the proposed method can be successfully utilized for the routine quality control analysis Diroximel Fumarate in bulk drug as well as in formulations.

Index Terms - Diroximel Fumarate, Multiple Sclerosis, Validation, RP-HPLC, Acetonitrile and 0.05 % Acetic Acid.

I. INTRODUCTION

High-Performance Liquid Chromatography (HPLC) is a powerful, reliable, and widely accepted analytical technique used for the qualitative and quantitative analysis of pharmaceutical compounds, including active pharmaceutical ingredients (APIs), excipients, impurities, and degradation products. HPLC operates based on the principle of differential partitioning of analytes between a mobile phase and a stationary phase under high pressure, providing high resolution, sensitivity, and reproducibility. In the context of analytical method development, HPLC allows optimization of various parameters such as mobile phase composition, pH, gradient profiles, flow rate, column selection, temperature, and detection wavelength to achieve maximum separation efficiency, peak symmetry, and minimal run time. Once an HPLC method is developed, it must undergo a thorough validation process in accordance with regulatory guidelines such as those outlined by the International Council for Harmonisation (ICH Q2 (R1)), the United States Pharmacopeia (USP), and the European Medicines Agency (EMA). Method validation is essential to demonstrate that the method is suitable for its intended purpose and includes key performance characteristics such as specificity, linearity, accuracy, precision (repeatability and intermediate precision), limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability testing. Each parameter ensures the method's reliability, consistency, and reproducibility under varied conditions. HPLC's ability to handle complex mixtures and its compatibility with a range of detectors (e.g., UV-Vis, PDA, fluorescence, MS) make it indispensable in pharmaceutical development, quality control, stability testing, and regulatory submissions. As such, the development and validation of HPLC methods are critical to ensuring drug safety, efficacy, and compliance with global quality standards.

II. DRUG PROFILE

Diroximel Fumarate

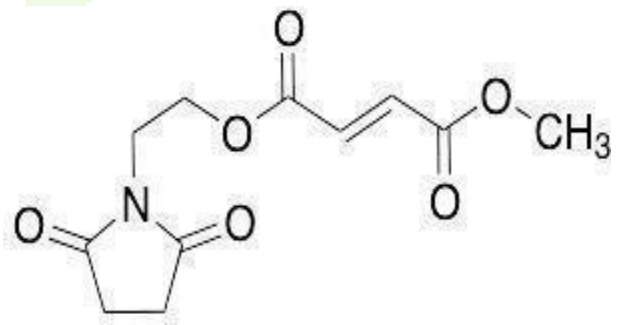


Fig.No. 1: Structure of Diroximel Fumarate.

Systematic (IUPAC) name: 4-O-[2-(2, 5-dioxopyrrolidin-1-yl) ethyl] 1-O-methyl (E)-but 2 enedioate

Molecular formula: $C_{11}H_{13}NO_6$.

Molecular Weight: 255.22 g/mol.

Solubility: Slightly soluble in Water, Ethanol, Methanol and Freely soluble in acetonitrile

Storage: Store at room temperature (20°C to 25°C / 68°F to 77°F).

Mechanism of action: Diroximel fumarate is hypothesized to regulate cell signaling pathways, causing beneficial immune and neuroprotective effects. Monomethyl fumarate (MMF) activates the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway.Nrf2 is a transcription factor that, when activated, moves into the nucleus and induces the expression of antioxidant response element (ARE) gene. 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 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. MMF also modulates immune cell activity, including reducing the number of circulating lymphocytes.

III. MATERIAL AND METHODS:

3.1 Selection and Procurement of Drug

Drug sample supplier

Table. No.1: Drug and Drug Supplier

Name of Drug	Drug Supplier
Diroximel Fumarate	RSITC, Jalgaon.

Table. No.2: List of Reagents and Chemicals

Sr. No.	Name of Chemical	Manufacturer.
1.	Acetonitrile (HPLC Grade)	Specialities Pvt. Ltd. Shiv Sagar Estate 'A' Worli, Mumbai
2.	Methanol (HPLC Grade)	Specialities Pvt. Ltd. Shiv Sagar Estate 'A' Worli, Mumbai
3.	Acetic acid (HPLC Grade)	Avantor performance material India Ltd. Thane, Maharashtra

3.2 Selection of formulation:

Marketed Preparation:

Table. No.3: List of brand names of combined formulations of Diroximel fumarate.

Sr. No.	Brand Name	Formulation	Available Strength	manufacturer
1.	VUMERITY	Capsule	231mg	Biogen Inc.

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

3.3 Study on the selection of uv spectrum use in uv-vis spectrometer of Diroximel fumarate:

Accurately weight and transfer 5mg Diroximel fumarate working standard into 10ml volumetric flask and make volume up to the mark with the same solvent to get 500 µg/ml standard (stock solution) and 15 min sonicate to dissolve it and remove unwanted gas, and from the resulting solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with ACN.

3.4 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 Tech. Gradient System with Auto injector, UV (DAD) Detector. Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 150mm; 5 µm), a Quaternary Gradient (G130A) pump, a UV730D absorbance detector and running chemstation 10.1 software.

Stock preparation:

Stock -I: Standard Sample Preparation-

Std. Diroximel Fumarate 5 mg in 10 ml ACN= 500 µgm/ml.

Stock-II: CAP solution Preparation:-

Take 6.16gms in 10ml ACN i.e = 1000µgm/ml Diroximel fumarate

❖ **For Accuracy solution preparation**:

For accuracy solution Prepare 10 µg/ml from stock II

80 % = 0.1ml Capsule Solution and Add 8 µg/ml Stds. (0.08 ml) and make up volume 10 ml with Diluent.

100 % = 0.1ml Capsule Solution and Add 10 µg/ml Stds. (0.10 ml) and make up volume 10 ml with Diluent.

120 % = 0.1ml Capsule Solution and Add 12 µg/ml Stds. (0.12ml) and make up volume 10 ml with Diluent.

3.5 Instruments and Equipments

Table.No.4: Instrument (HPLC) Details used during Method Development

Sr. No.	Name of Instrument	Name of Company
1.	HPLC Instrument	Agilent Tech. Gradient System with Auto Injector (Chemstation 10.1 Software)
2.	UV-Spectrophotometer	Analytical Technologies Limited
3.	Column (C18)	Agilent C_{18} (150mmX 4.6mm; 5µm)
4.	pH Meter	VSI pH Meter
5.	Balance	™ High Resolution Balance
6.	Sonicator	Ultrasonic electronic instrument

3.6 High Performance Liquid Chromatography (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 Tech. Gradient System with Auto injector, UV (DAD) Detector. Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 150mm; 5µm), a Quaternary Gradient (G130A) pump, a UV730D absorbance detector and running chemstation 10.1 software.

3.7 Chromatographic conditions:

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table. No.5: Chromatography condition (HPLC) details used during method development.

1.	HPLC	Agilent Tech. Gradient System with Auto Injector
2.	Software	CHEMSTATION 10.1
3.	Column	Agilent C18 column (150mmX 4.6mm)
4.	Pump	Quaternary Gradient (G130A)
5.	Detector	UV (DAD) G13148
6.	Stationary phase	RP-C18(Agilent)
7.	Mobile phase	ACN : 0.05 Acetic Acid (60:40% v/v)
8.	Detection Wavelength	253 nm.
9.	Particle size packing	5µm
10.	Temperature	31.5°C
11.	Flow Rate	0.7ml/min
12.	Sample size	20 ml

3.8 METHOD DEVELOPMENT OF HPLC:

- **List of Mobile Phase :**

Table. No.6: Selection of Mobile Phase.

Sr. No.	Mobile Phase
Trial 1	ACN+ (0.05%) ACETIC ACID (90+10% v/v), 10MCG, wavelength 253, Flow rate 0.7ml/min, C18 (Agilent) (4.6mm x 150mm).
Trial 2	ACN+ (0.05%) ACETIC ACID (80+20% v/v), 10MCG, wavelength 253, Flow rate 0.7ml/min, C18 (Agilent) (4.6mm x 150mm).
Trial 3	ACN+ (0.05%)ACETIC ACID (70+30% v/v), 10MCG, wavelength 253, Flow rate 0.7ml/min, C18 (Agilent) (4.6mm x 150mm)
Trial 4	ACN+ (0.05%)ACETIC ACID (60+40% v/v), 10MCG, wavelength 253, Flow rate 0.7ml/min, C18 (Agilent) (4.6mm x 150mm)
Final Trial	ACN+ (0.05%)ACETIC ACID (60+40% v/v), 10MCG, wavelength 253, Flow rate 0.7ml/min, C18 (Agilent) (4.6mm x 150mm)

IV. RESULT AND DISCUSSION

4.1 Preliminary studies on Diroximel fumarate.

- **Solubility**

The drug was found to be:

- Slightly soluble in Water, Ethanol, Methanol and freely soluble in acetonitrile.
- Insoluble in ether.

- **UV Spectroscopy**

UV absorption of 10mcg solution of Diroximel Fumarate in Methanol was generated and absorbance was taken in the range of 200-400 nm. The wavelength (λ max) of Diroximel Fumarate was found to be 253nm respectively

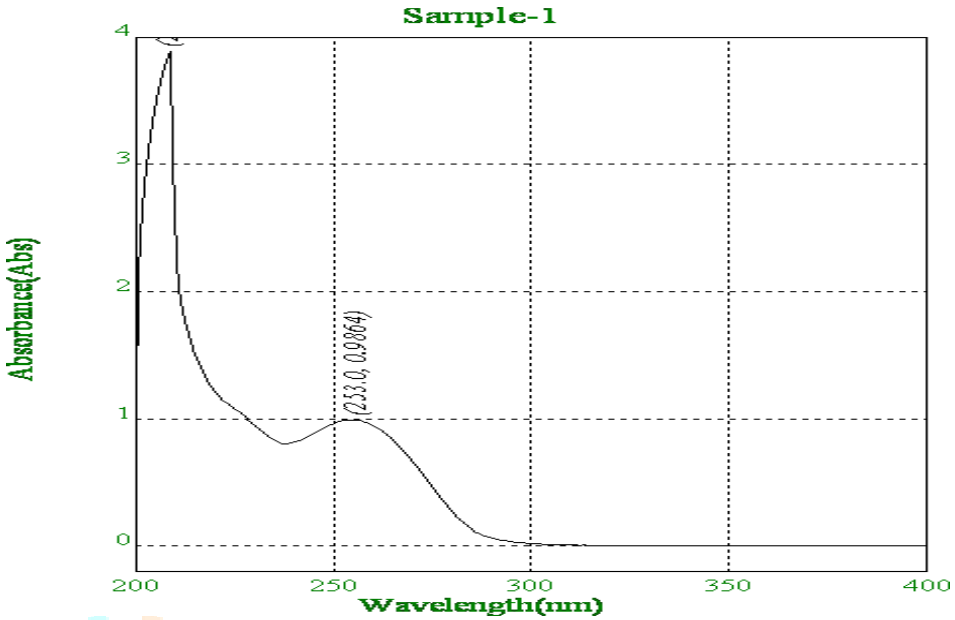


Fig.No.2: UV Spectrum of Diroximel Fumarate

4.2 METHOD DEVELOPMENT OF HPLC :

Trial-1:

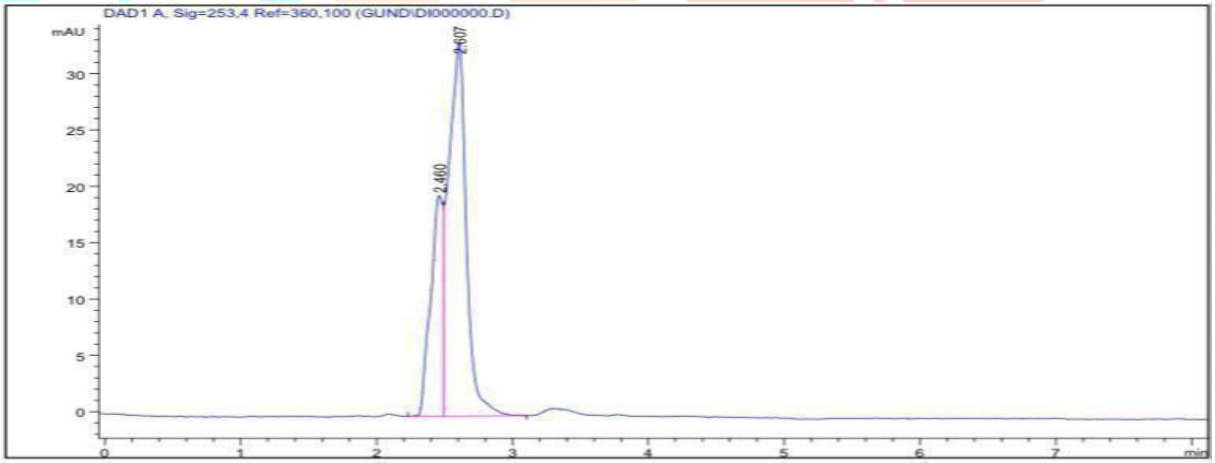


Fig.No.3: Chromatogram of Trial-1

Table.No.7: Trial-1 of chromatogram of Diroximel fumarate

No.	RT(min)	Area (mAU*s)	Area%	Theoretical plate
1.	2.607	304.30	100%	1342

Observation: Peak was eluted so method was rejected.

Trial-2:

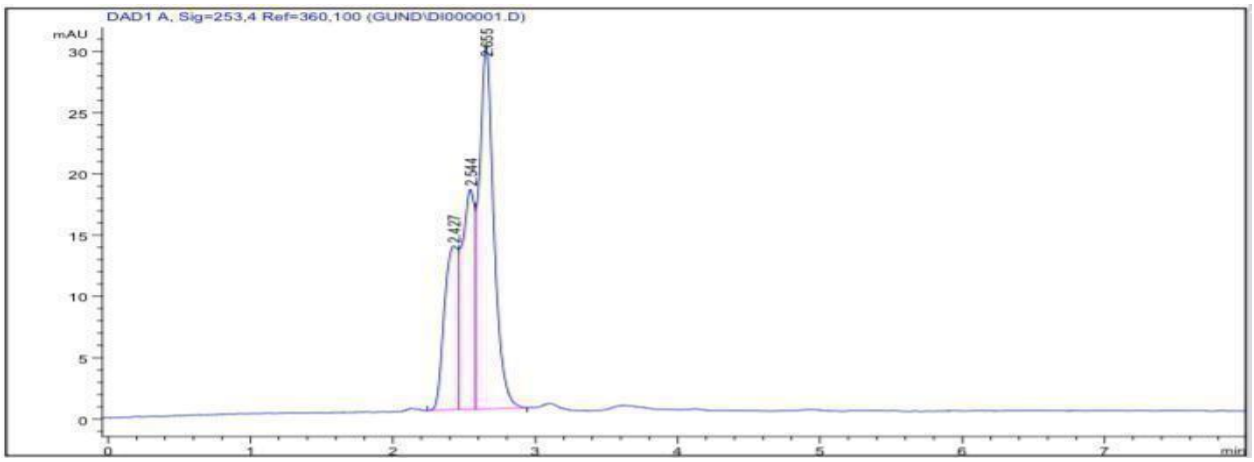


Fig.No.4: Chromatogram of Trial-2

Table.No.8: Trial-2 of chromatogram of Diroximel fumarate

No.	RT(min)	Area (mAU*s)	Area%	Theoretical plate
2.	2.427	86.488	100%	2769

Observation: Peak was eluted so method was rejected

Trial-3:

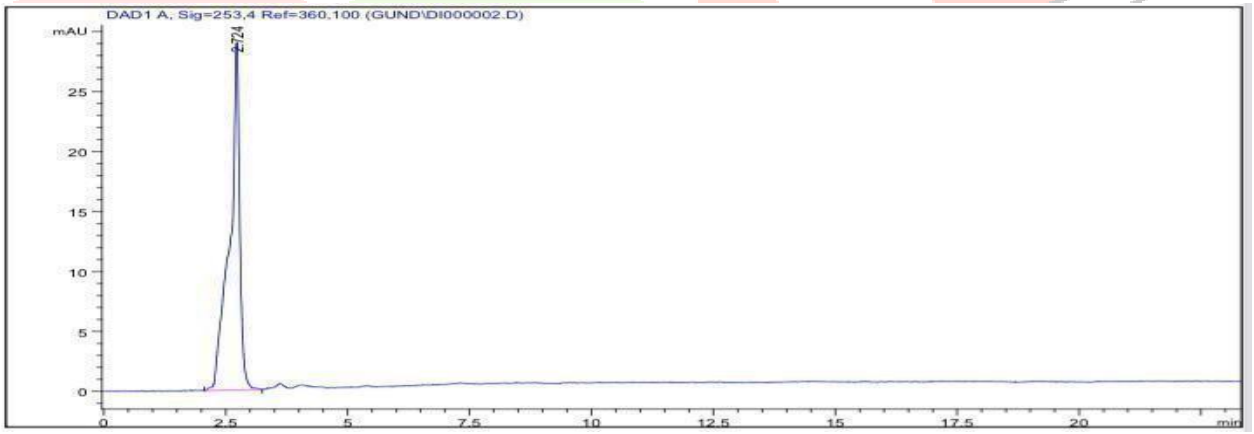


Fig.No.5: Chromatogram of Trial-3

Table. No.9: Trial-3 of Chromatogram of Diroximel fumarate

No.	RT(min)	Area (mAU*s)	Area%	Theoretical plate
3.	2.724	416.25	100%	2118

Observation: Peak eluted and Sharp peak were not obtained so method was rejected.

Trial-4:

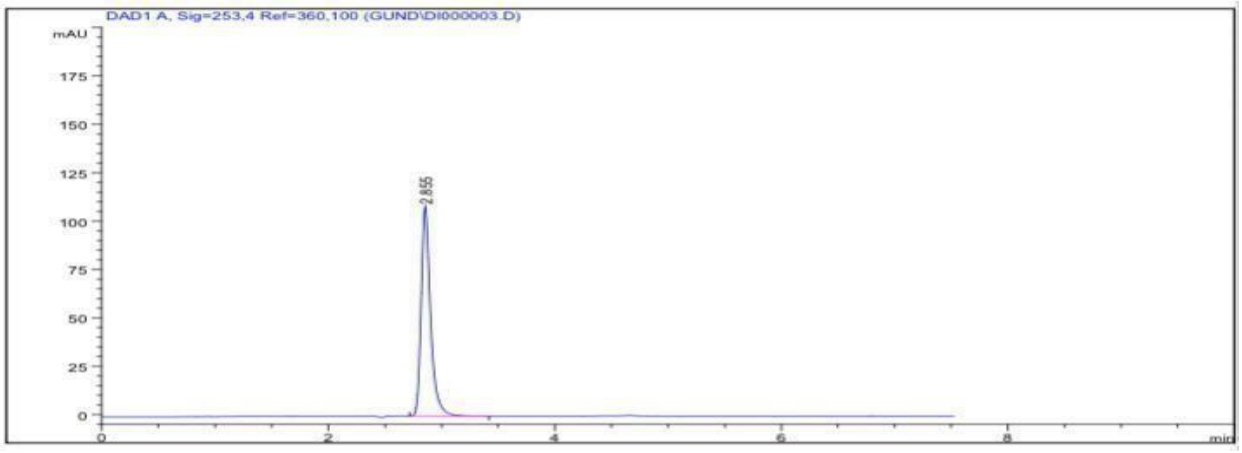


Fig.No.6: Chromatogram of Trial-4

Table.No.10: Trial-4 of Chromatogram of Diroximel fumarate

No.	RT(min)	Area (mAU*s)	Area%	Theoretical plate
4.	2.855	623.33	100%	6604

Observation: Peak eluted and Sharp peak were not obtained so method was rejected.

Trial-5:

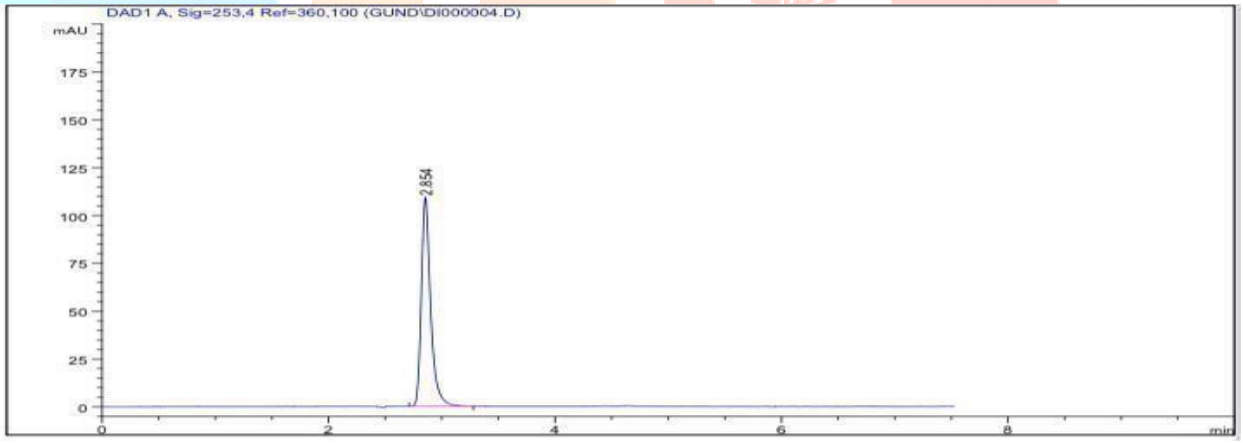


Fig.No.7: Chromatogram of Trial-5

Table.No.11: Trial-5: Chromatogram of Diroximel fumarate

No.	RT(min)	Area (mAU*s)	Area%	Theoretical plate
5.	2.854	622.33	100%	6484

Observation: Sharp and well resolved peak were obtained, very good symmetry and theoretical plates satisfactory result was found, so method was selected.

4.3 RP-HPLC Method:

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentration in the range 5-25µg/ml for Diroximel Fumarate (Table No:15) depict the calibration data of Diroximel Fumarate. The respective linear equation for Diroximel Fumarate was y = 81.45x +159.0.

Where x is the concentration and y is area of peak. The co-relation co-efficient was 0.999. The calibration curve of Diroximel Fumarate is Depicted in (Fig.No.13)

Table.No.12: 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 peak Area
		1	2			
RP-HPLC Method	5	568.58	564.74	566.660	2.72	0.48
	10	974.08	971.77	972.925	1.36	0.17
	15	1369.03	1368.33	1368.680	0.49	0.04
	20	1812.66	1813.73	1813.195	0.76	0.04
	25	2182.58	2182.58	2182.985	0.57	0.03
Equation				y =81.45x +159.0		
R^2				0.999		

The RP-HPLC Method for the respective linear equation for Diroximel Fumarate was y = 81.45x +159.0.Where x is the concentration and y is area of peak. The co-relation co-efficient was 0.999.

4.4 Analytical of Method Validation:

- **Linearity:**

From Diroximel Fumarate standard solution, different working standard solution (5-25 µg/ml) were prepared in mobile phase 20µl of sample solution was injected into the chromatographic system using mixed volume loop injector chromatograms were recorded.

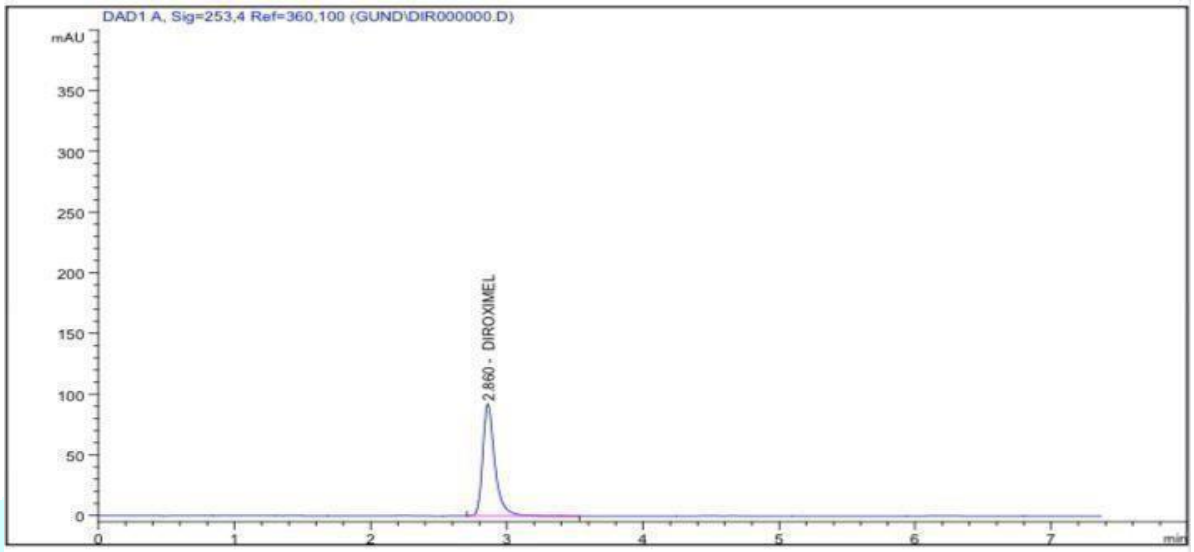


Fig.No.8: Chromatogram of Linearity

- **Accuracy:**

Recovery studies were performed to validate the accuracy of developed method. To pre anlayzed tablet solution, a definite concentration of standard drug (80%, 100 % and 120%) was added and then its recovery was analyzed and Statistical validation of recovery studies shown respectively.

Accuracy 80%

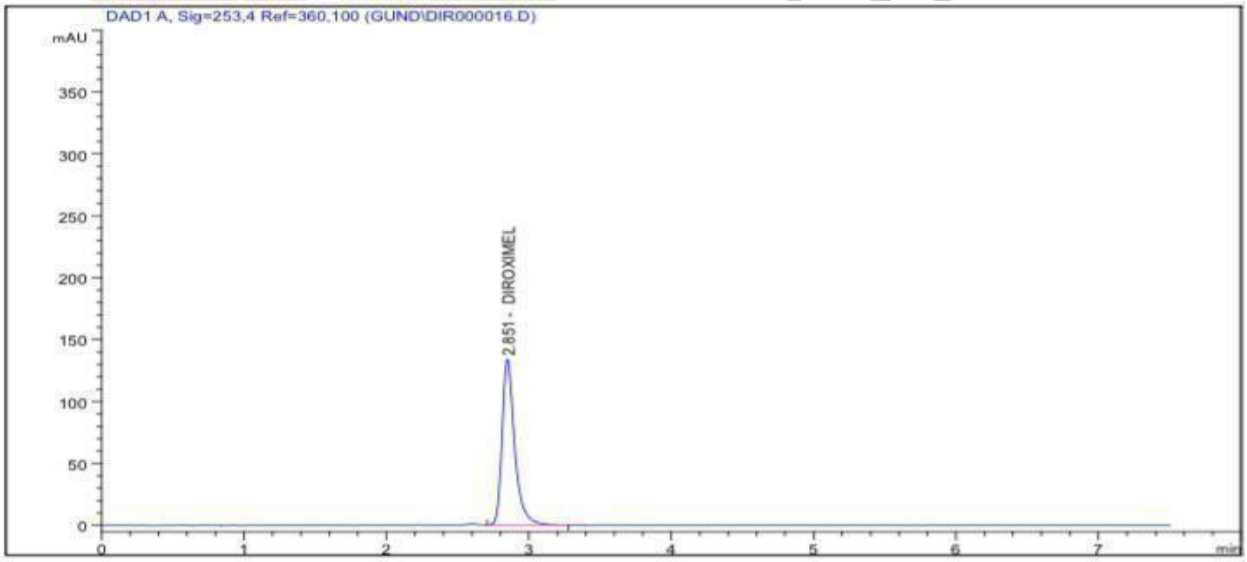


Fig.No.9: Chromatogram of Accuracy (80%)

- **System Suitability Parameters (Repeatability):**

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of Diroximel fumarate system suitability parameter were studied. The result is shown respectively.

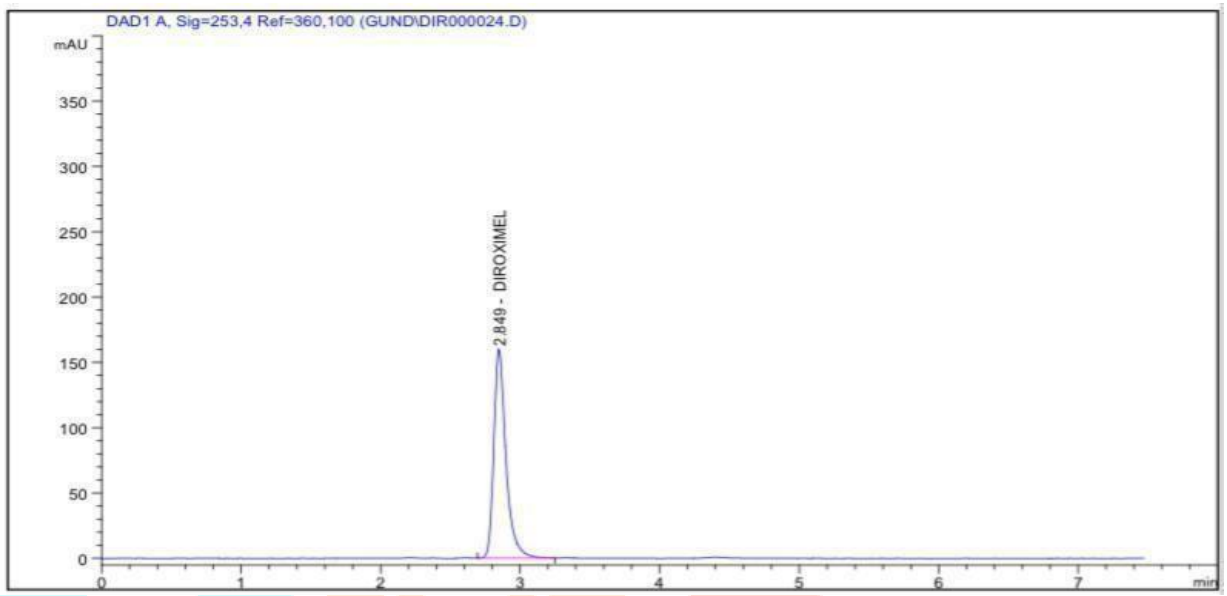


Fig.No.10: Chromatogram of System Suitability (Repeatability).

- **Precision:**

The method was established by analyzing various replicates standards of Diroximel Fumarate. All the solution was analyzed thrice in order to record any intra-day and inter-day variation in the result that concluded. The result obtained for intraday is shown respectively.

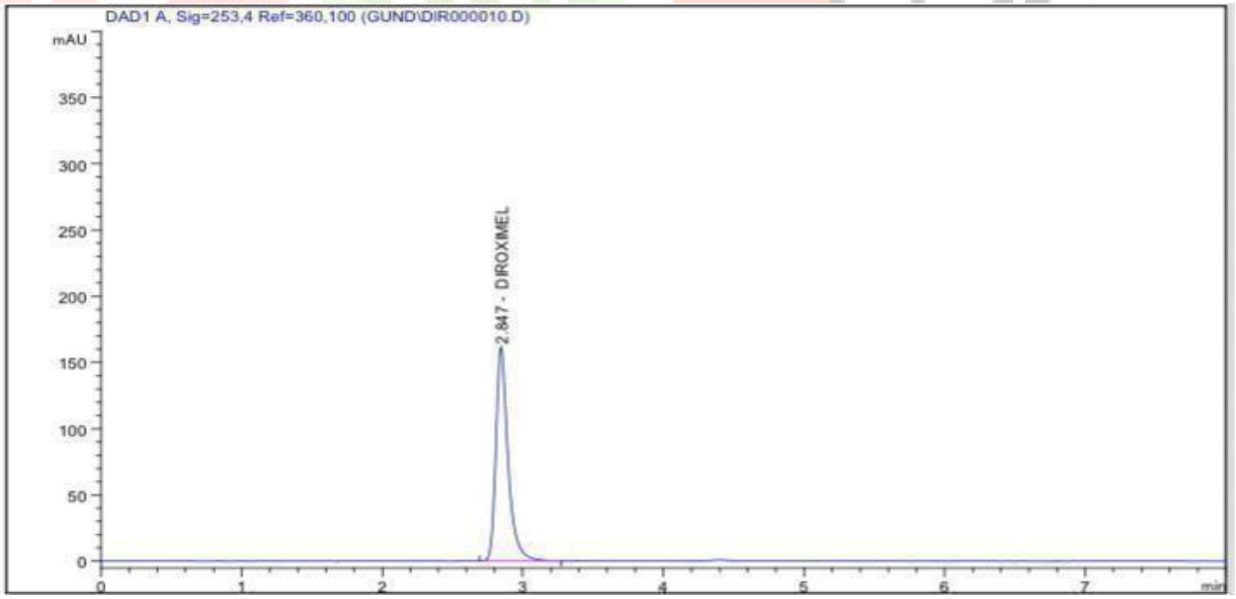


Fig.No.11: Chromatogram of Intraday precision

- **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 (±1ml/min^{-1}), and wavelength change (±l ml/min^{-1}) of optimized chromatographic condition. The Robustness parameters were also found satisfactory; hence the analytical method would be concluded.

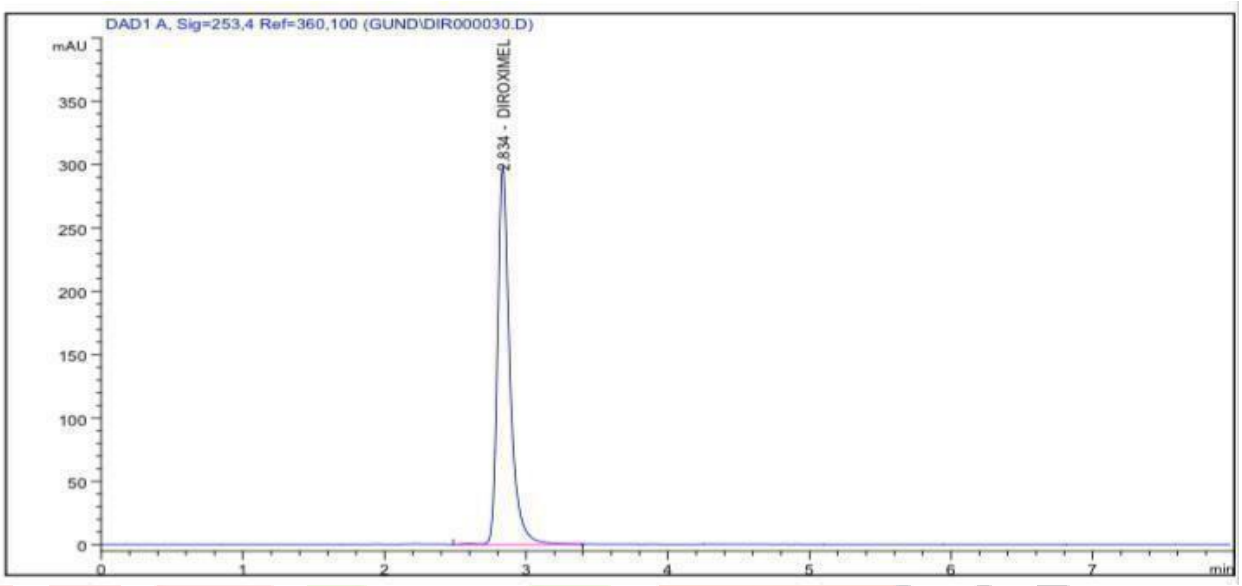


Fig.No.12: Chromatogram of Robustness: MP (61+39% v/v).

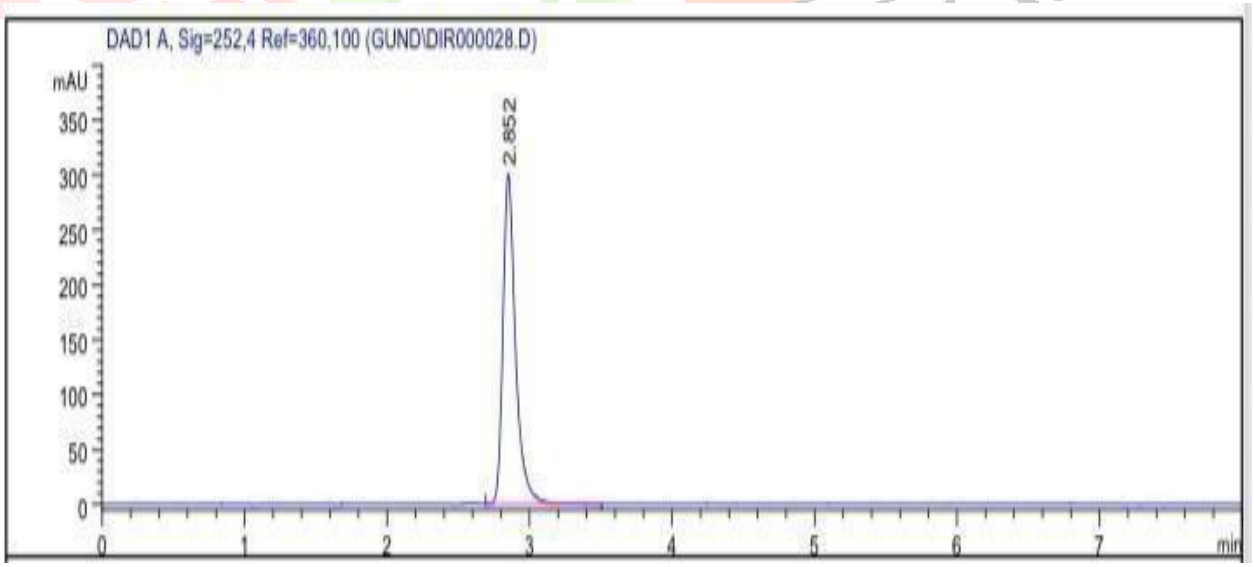
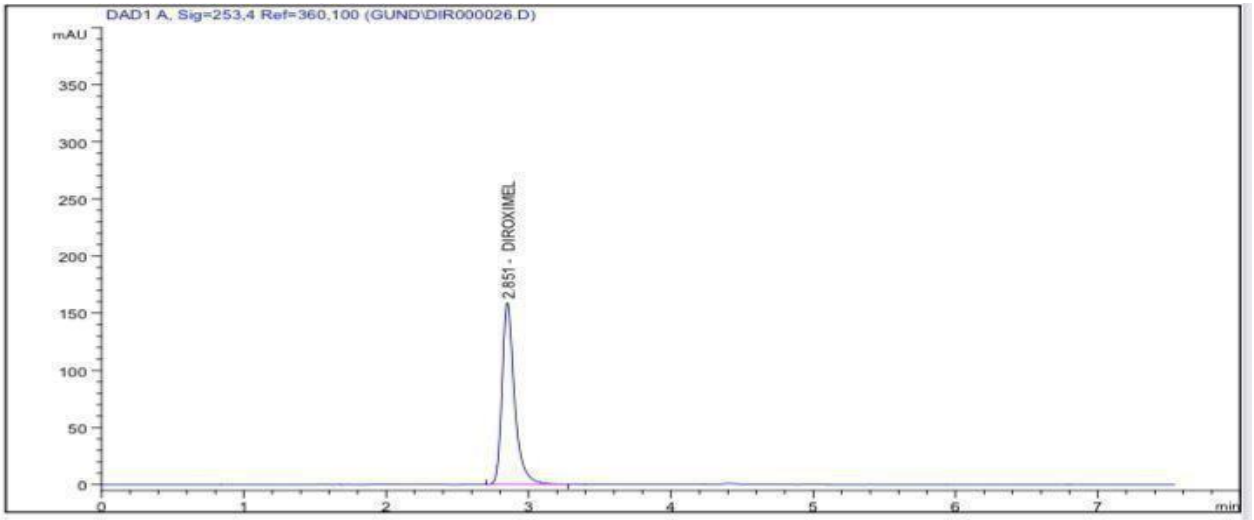


Fig.No.13: Chromatogram of Robustness: WL (252 nm)Ruggedness:

It includes different analysts, laboratories, columns, instruments, source of reagents, chemicals, solvents etc. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method. The result obtained for ruggedness is shown below.

Fig.No.14: Chromatogram of Ruggedness



- **Limit of 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 X Avd.SD/Slope

=3.3 X 1.23/81.45

=0.0498 ug/ml

- **Limit of 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 of limit (LOQ) may be expressed as:

LOQ = 10 X Avd.SD/Slope

=10 X 1.23/81.45

= 0.1510 ug/ml

V. CONCLUSION:

Different parameters were studied to create the analytical approach. For starters, the maximum absorbance of Diroximel fumarate was discovered to be 253nm. The injection volume was set at 20.0µl, which resulted in a good peak area. The Agilant C_{18} (150mm x 4.6mm) column was employed in this work, and picked a nice peak shape. The temperature of the ambient environment was determined to be adequate for the type of the medication solution. Because of the good peak area, adequate retention duration, and good resolution, the flow rate was set at 0.7 ml/min. Different mobile phase ratios were investigated, however the mobile phase with a ACN: (0.05%) Acetic acid (60:40% v/v) ratio was chosen because to its symmetrical peaks and high resolution. As a result, the planned research made use of this mobile phase

A simple, Accurate, precise method was developed for the estimation of the Diroximel fumarate in pharmaceutical dosage form. Retention time of Diroximel fumarate was found to be 2.854 min. LOD, LOQ values obtained from regression equations of Diroximel fumarate were 0.0498 µg/ml and 0.159.0 µg/ml respectively. Regression equation of Diroximel fumarate is y =81.45x + 159.0. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries. Diroximel fumarate quantification was done and confirmed thoroughly using an RP-HPLC based technique that is selective, requires minimal run time, exact, specific, robust, and cheap.

VI. Acknowledgment

The Author express their sincere gratitude to Maharashtra College of Pharmacy, Nilanga and University Libraries, and all other sources for their co-operation and advice in writing.

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