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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DIMETHYL FUMARATE BY RP-HPLC METHOD

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ABSTRACT:

A new, accurate, simple, precise, and specific RP-HPLC method for Dimethyl Fumarate in bulk and pharmaceutical dosage forms was developed for this study, a Shimadzu LC-2010C HT with Lab Solutions software, an Inertsil ODS-3 C18 (150x4.6 mm, 5 µ) column, and a mobile phase containing buffer: acetonitrile (50:50% v/v) were used the flow rate was kept constant at 1.0 ml/min, the column temperature was set to 35 °C, and UV detection was performed at 210 nm the studies were carried out and the method was validated in accordance with ICH guidelines dimethyl fumarate had a retention time of 3.4 minutes the correlation coefficient found between peak area and concentration was 0.9994 the average percentage of dimethyl fumarate recovery inthe percentage of capsules found ranged from 96.4 to 103.5% testing on both standard and sample solutions revealed that the method is accurate within acceptable limits the %RSD of precision determination was 2% the results of the robustness and solution stability studies were also within acceptable limits within the acceptance criteria, the proposed method demonstrated excellent system suitability, specificity, linearity and range, accuracy (recovery), precision and robustness results.

Keyword: Dimethyl Fumarate, RP-HPLC, Validation

Introduction:

Dimethyl fumarate is an anti-inflammatory. [1]It is indicated for multiple sclerosis patients with relapsing forms and is also being investigated for the treatment of psoriasis.[2] The mechanism of action of dimethyl fumarate in multiple sclerosis is not well understood. It is thoughtto involve dimethyl fumarate degradation to its active metabolite monomethyl fumarate (MMF) then MMF up-regulates the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway that is activated in response to oxidative stress. Dimethyl fumarate is marketed under the brand name Tecfidera. Dimethyl Fumarate structure shown in the **Fig1**.and the IPUAC name of dimethyl fumarate is Dimethyl (2*E*)-but-2-enedioate other name is trans-1,2-Ethylenedicarboxylic acid dimethyl ester, (E)-2-Butenedioic acid dimethyl ester.[3,6] Dimethyl fumarate as a typical of BCS ClassI drug. The molecular formula is $C_6H_8O_4$ and molecular weight is 144.127 g/mol.[7] According to the

ICH guidelines Q2 (R1)[8] the development of analytical techniques that have been validated in terms of system suitability, specificity, linearity and range, accuracy,precision, and robustness.

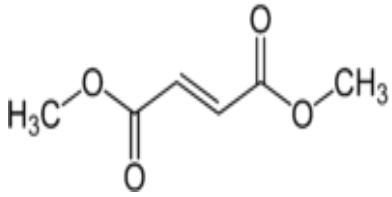

Fig. 1: Structure of Dimethyl Fumarate

Materials & Methods

Chemicals & Reagents

As a gift sample, Enaltec Research Centre, Ambernath India provided Dimethyl Fumarate API (Purity 99.2%). In an Enaltec Research Centre house, capsules (containing 240 mg of dimethyl fumarate) were obtained. Methanol, Acetonitrile, and Perchloric acid were obtained from Rankem Pvt. Ltd., Mumbai, and Merck Specialties Pvt. Ltd., Mumbai, respectively.

Instruments

For High Performance Liquid Chromatography method development and validation, a Shimadzu HPLC system (model LC-2010C HT) was used, with an Inertsil Ods-3, C18column (150 mm 4.6 mm, 5µ) and a UV/VIS detector operating at 280 nm. Data processing andevaluation were carried out using Lab Solution Software. pH meter model orion star A211 (thermo scientific, Labindia) Analytical balance is of Mettler Toledo model (XS205DU) Sonicator is of skytron and model (SI-300UC/S115B30UC) and centrifuge machine is of Eltek model (MP400) 0.45µ membrane disc filter of Millipore millex-hn is used.

Experimental works

Solvent selection

The separation will occur because the analytes have distinct chemical affinities for each of the phases. Analytes with a lower affinity for the stationary phase will elute sooner, while those witha higher affinity will elute later the solvents in the mobile phase can be changed to change the relative analyte affinity, which affects the separation's selectivity (chemical separating power) and retention time.

Selection of wavelength

A 5mg of dimethyl fumarate was weighed accurately and transferred to 50 ml volumetric flask. Accurately, 10 ml of distilled water was added to dissolve it and volume was made up to 50 ml with distilled water. Dimethyl Fumarate shows maximum absorbance at wavelength 210 nm that is 0.9471A hence 210 nm is selected as a λ max for analysis of dimethyl fumarate.

Preparation of mobile phase

(Transfer accurately 2 ml of Perchloric acid 70% in 1000 ml of water, mix well. Filter through 0.45µ nylon membrane filter and degas it) Prepare a mixture of Buffer solution and Acetonitrile in the ratio of 50:50 v/v. Mix well, sonicate to degas it.

Preparation of Standard stock solution

Weigh and transfer accurately about 48.0 mg of Dimethyl Fumarate standard into 50 ml volumetricflask. Add about 15 ml of diluent-1, sonicate to dissolve, cool and dilute up to the
mark with diluent-2 and mix.

Preparation of Standard solution

Further dilute 5.0 ml of above stock solution into 20 ml of volumetric flask make up to the mark with diluent-2 and mix.

(Concentration of Dimethyl Fumarate: 240 ppm)

Preparation of sample solution

Determine the Average filled weight of 20 capsules. Weigh and transfer content of five capsules in to 500 mL volumetric flask. Add about 125 mL of diluent-1, sonicate for 40 minutes with intermittent shaking, allow it to cool and make up to volume with diluent-2 and mix.

Further dilute 10 mL of this solution to 100 mL with diluen-2 and mix. Filter the sample solution through 0.45µ Nylon membrane syringe filter. Discard first 3 or 4mL of filtrate.

(Concentration of Dimethyl Fumarate: 240 ppm)

Method validation

System Suitability

Equal volumes of Blank (Diluent-2) and Standard solution-1 (Six replicates) and Standard solution-2 (Two replicates), were injected into the HPLC.

Specificity

Specificity, standard and sample solution were prepared of 240 g/ml and injected into HPLC apparatus, where the peak area and retention time was measured.

Linearity and Range

Linearity was evaluated in the range of 25% to 150% of the working concentration level. The working concentration level of Dimethyl fumarate is about 240 ppm for Dimethyl fumarate the range is from 25% to 150% of 240 ppm.

Accuracy

Accuracy was evaluated at three levels, 25% to 150% of working concentration level for Dimethyl Fumarate Capsules. The working concentration of Dimethyl Fumarate is about 240 ppm. Each level prepared in triplicates. % Recovery was calculated by amount added and amount recovered. The mean recovery was calculated and summarized in table below.

Precision

Precision was evaluated by performing the same procedure of method precision using same lot of Dimethyl Fumarate Capsules on different day, by different analyst, on different HPLC system with different column. The reproducibility was evaluated by comparing the results obtained from ruggedness with those obtained from method precision. Six independent sample preparations for Dimethyl Fumarate Capsules was prepared and determined % Assay. The mean and % RSD calculated.

Robustness

This parameter was studied after making small, deliberate changes in the chromatographic conditions and observing the effect of these changes on the system suitability parameters by injecting standard and sample solutions. Change in chromatographic condition such as change in flow rate, change in wavelength, column temperature and the %RSD was calculated.

RESULTS AND DISCUSSION

HPLC method optimization and System suitability

Various ratios of mobile phase were tested for method optimization, such as buffer: acetonitrile (70:30) v/v and buffer: acetonitrile (60:40) v/v, Due to tailing, fronting and lack of crispness at the peak, all of these mobile phase ratios were unacceptable. Following numerous tests, the mobile phase of the ratio buffer: acetonitrile (50:50) was chosen because it produced sharp peak without tailing or fronting. The chromatogram of standard solution of dimethyl fumarate was shown in **Fig2.**Optimized
Chromatographic conditions and system suitability parameter were shown in **Table 1.**

Table1: Optimized chromatographic condition and system suitability parameter

Column	Inertsil ODS-3 C18 (150x4.6 mm, 5 µ)
Wavelength	210 nm
Column temperature	35°C
Injection volume	5 µl
Run time	7.0 min.
Flow rate	1 ml/min
Pump mode	Isocratic
Mobile phase	buffer : Acetonitrile (50:50)
Retention time	3.48 minutes
Tailing Factor	1.06
Theoretical plate	5060
Peak area	7457087

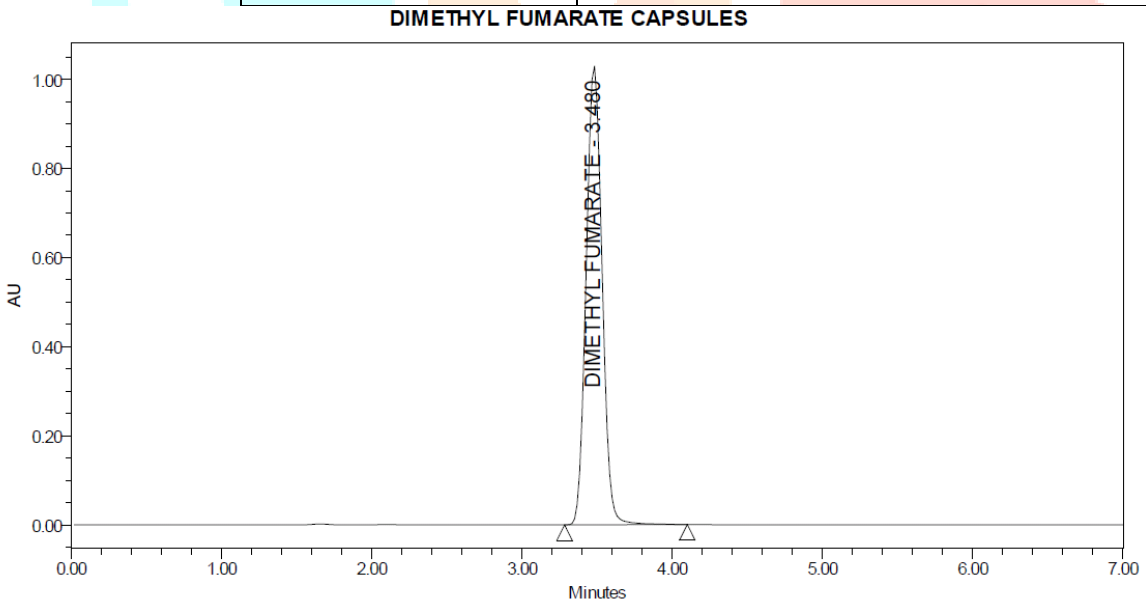

Figure 2: chromatogram of dimethyl fumarate standard

Specificity

The table 2 shows that standard solution and sample solution does not show any interference at the Retention time of Dimethyl Fumarate.

Table 2: Specificity data by HPLC method

Sr. no.	Component	*RT (min)	Purity angle	Purity threshold
1	Standard solution	3.514	0.563	1.771
2	Sample solution	3.517	0.202	0.414

Linearity and Range

In accordance with the results of the linearity investigation, the mean peak area from the HPLC was plotted against appropriate concentrations to create the calibration graph **(Fig.3)**,dimethyl fumarate has a linear relationship over the concentration range of 60, 120, 180, 240, 300, 360 µg/ml. (R^2) value was determined to be 0.9989. A linear equation, Y=33436x+321137 was derived and the result of the linearity is shown in **Table 3.**

Table 3: Linearity data by HPLC

Dimethyl Fumarate				
Level (%)	**Concentration (ppm)**	**Response**		
		1	**2**	**Mean**
25	60.00	2241653	2236189	2238921
50	120.00	4423859	4420183	4422021
75	180.00	6521387	6519677	6520532
100	240.00	8743704	8758961	87513331
125	300.00	10686331	10686284	11281712
150	360.00	12472521	12488758	12488758
SLOPE				**33463**
(r^2)				**0.9989**

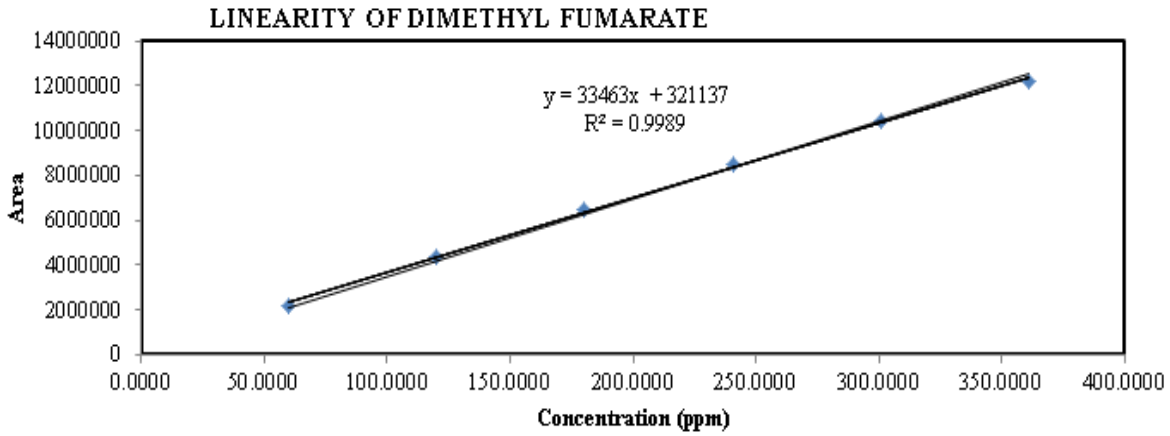

Figure 3: Linearity graph of dimethyl fumarate

Accuracy

Table 4 shows that, individual and mean recovery of each level i.e. 25 %, 100 % and 150 % is within acceptance criteria.

Table 4: Accuracy data by HPLC method

Level (%)	**Theoretical concentration (µg/mL)**	**% Recovery**	**Mean recovery%**
25	59.910	103.5	103.5
	59.936	103.7	
	59.944	103.4	
100	239.598	101.0	100.8
	239.606	100.7	
	239.614	100.7	
150	359.378	96.3	96.4
	359.386	96.4	
	359.386	96.4	
Mean recovery			**100.20**
SD			**3.5837**
%RSD			**3.58**

Precision:

Six independent samples were made and injected into HPLC and the result shown in table 5

Table 5: Intermediate precision

Dimethyl fumarate		
Sample No.	**Response**	**% Assay**
1	8650446	99.4
2	8653156	99.4
3	8665615	99.5
4	8669720	99.6
5	8682624	99.7
6	8686500	99.8
Mean		**99.6**
% RSD		**0.16**

Robustness

The data shows that, cumulative %RSD of % assay in each modified condition is within acceptance criteria, when compared. Individual % Assay is within specification limit. Result is shown in Table6

Table 6: Robustness data by HPLC method

Changes in parameters	Values	Retention Time of Dimethyl fumarate	Tailing factor	Theoretical plates	% Assay
Control	As per method	3.424	1.08	5079.1	NA
Flow rate (± 0.1 mL/min)	0.9 mL/min	3.898	1.180	5628	99.0
	1.1 mL/min	3.197	1.160	4978	98.9
Column temperature (± 5°C)	30°C	3.579	1.170	5262	99.0
	40°C	3.510	1.172	5373	99.1
Change in Wavelength (± 2 nm)	208	3.517	1.170	5213	99.1
	212	3.518	1.172	5171	99.1

Conclusion

The method has been showed to be specific for determination of % Assay of Dimethyl Fumarate in Dimethyl Fumarate capsule the method has been showed to be precise, linear and accurate across the suitable analytical range. The method has been showed to be robust across the suitable analytical range the method can be used in quality control laboratory for release of production batches and stability study

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REFERENCES

1. Drug bank. Available from; https://www.drugbank.ca/drugs/ DB08908). [Last accessed on 18th Mar 2017]
2. Patient information, Tecfidera, Highlights of prescribing information, Biogen, Cambridge [Approved by the U. S. Food and Drug Administration, Revised: 1/2017].
3. Balaji J, Shri CN. Development and validation of RP-HPLC method for determination of related substances of dimethyl fumarate drug product. Indian J Res 2016;5:196-8.
4. Gennari O, Montesano D, Salzano A, Albrizio S, Grumetto L. Determination of dimethyl fumarate in desiccant and anti mould sachets by reversed-phase liquid chromatography. Biom J Res Chromatography 2011;25:1315-8.
5. Suneetha A. Role of dimethyl fumarate in oxidative stress of multiple sclerosis: a review. J Chromatography Biomed Application 2016;19:1-6.
6. Suneetha A, Rajeswari RK. Prescriptive oriented drug analysis of multiple sclerosis diseases by LC-UV in whole human blood. J Chromatographic Sci 2016;54:1-10.
7. Suneetha A, Raja K. Comparison of LC-UV and LC–MS methods for simultaneous determination of teriflunomide, dimethyl fumarate and fampridine in human plasma: application to rat pharmacokinetic study. Biomedical J Res Chromatography 2016;1:1-7.
8. ICH Harmonized-Tripartite Guidelines. Validation of Analytical Procedure: Text and Methodology. November Q2(R1); 2005.
9. ICH Harmonized-Tripartite Guidelines. Stability Testing of New Drug Substance And Products. February Q1A(R2); 2003.
10. Supriya C, Ramanjulu N, Venkatasubba N. Validated stability- indicating assay method for azathioprine in the pharmaceutical dosage form according to ICH guidelines. Int J Pharm Pharm Sci 2014;6:301-7.
11. Pratibha V, Varsha N, Soni MA. Stability indicating HPLC method for the determination if metronidazole using eco- friendly solvent as mobile phase component. Int J Pharm Pharm Sci 2013;5:496-501.
12. Jain HK, Randhe VA. Stability indicating assay method for estimation of midodrine hydrochloride in bulk and tablets. Int J Pharm Pharm Sci 2016;8:283-7.
13. Radhika S, Ragin S. Stability indicating RP-HPLC method for simultaneous estimation of dosulepin hydrochloride and methylcobalamin in tablet dosage form. Int J Applied Pharm Sci 2017;9:69-75.
14. Blessy M, Rachi P, Prajapati N. Development of forced degradation and stability indicating studies of drugs-a review. Journal of Pharma Analysis 2014;4:159-6