

# Rp-Hplc Assay Method For Estimation Of Dimethyl Fumarate In Bulk And Capsules

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## ABSTRACT:-

A simple, sensitive and accurate gradient reverse phase high performance liquid chromatography method was developed for determination of dimethyl fumarate in formulation . RP-HPLC method has been developed and validated to live dimethyl fumarate at wavelength ( 210nm)in order to assay. The retention time of dimethyl fumarate was found to be  $3.3\pm 0.02$  min. the worth of coefficient of correlation between peak area and concentration was found to be 0.9987. The mean percent recovery of dimethyl fumarate in capsules was found within the range of 99.65 to 101.64%. The results of forced degradation studies indicated that the drug was found to be stable in basic, oxidative and thermal conditions while degraded in acidic conditions. The mixture of buffer and ACN ,in the ratio (70:30) used as a mobile phase .It are often conducted from results that the developed HPLC method is straightforward , accurate, precise and specific. Results of stress testing study revealed that the tactic is stability indicating. Thus, this method are often used for routine analysis of dimethyl fumarate capsules and check their stability.

**Keywords :**RP-HPLC, Method validation, Stability indicating assay method, Dimethyl fumarate, Simple and accurate method.

## INTRODUCTION

Dimethyl fumarate is an anti-inflammatory drug, which is chemically trans-Butenedioic acid dimethyl ester [7]. The US FDA approved Tecfidera capsules (containing 240 mg of dimethyl fumarate) on March 27, 2013. This drug is indicated for the treatment of a patient with relapsing forms of multiple sclerosis [2]. Dimethyl fumarate isn't a politician drug in any Pharmacopoeia. Literature survey revealed that some methods have been reported for the determination of dimethyl fumarate by HPLC [3-6] and hyphenated techniques such as LC-MS [1], either alone or in combination. However, there's no stability indicating HPLC assay method was reported yet for estimation of dimethyl fumarate in capsules. This paper presents an easy stability indicating RP-HPLC assay method for estimation of dimethyl fumarate in bulk and capsules which will be utilized in stability testing.

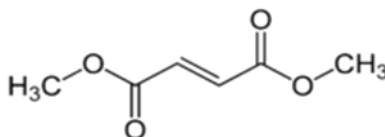


Fig. 1: Chemical structure of dimethyl fumarate

## MATERIALS AND METHODS

### Chemicals and reagents

Dimethyl Fumarate API was procured from gift sample in collage for research purpose. Capsules (containing 240 mg of dimethyl fumarate) were obtained in market brand Tecfidera capsule of Biogen

company .Methanol, Acetonitrile was obtained from Rankem Pvt. Ltd, Mumbai and Perchloric acid was obtained from Merck Specialties Pvt. Ltd, **Mumbai**.

### **Instruments**

The method was performed on, HPLC waters HPLC system with automatic injection facility and UV-Visible detection system Software –Empower. Analytical Balance Mettler Toledo XS205 and Column Symmetry Shield Inertsil ODS (150 x 4.6 mm, 5 $\mu$ ) were used for this study.

### **Preparation of standard stock solution**

100 mg of dimethyl fumarate was accurately weighed and transferred into a 100 ml volumetric flask. This drug was dissolved with 30 ml of methanol and sonicated for 15 min. Then this solution was diluted up to the mark with a diluent (Acetonitrile: buffer PH 6.8 50:50% v/v) this solution was further diluted 10 times with the same diluent.

### **Assay of capsules**

The content of twenty capsules was weighed and an average weight of a capsule was calculated. An accurately weighed amount of powder like 100 mg of drug was transferred to a 100 ml volumetric flask and 30 ml of methanol was added thereto . Then the mixture was sonicated for 15 min and diluted up to mark with the diluent. This solution was further diluted to get about 100  $\mu$ g/ml solutions with an equivalent diluent and filtered through 0.45 $\mu$  nylon membrane syringe filter before injection. This procedure was repeated in triplicate. The results of the assay of capsules are shown in table 2.

### **Validation of the method**

The developed chromatographic method was validated for linearity, range, accuracy, precision, robustness and specificity parameters, as per ICH guidelines [2].

#### **Linearity and range**

Working standard solutions were injected under the optimized chromatographic conditions and peak areas were calculated at 210nm. A calibration curve was plotted between areas against corresponding concentrations of the drug. Linear regression data for calibration curve was shown in fig. 3. The range of solution has been decided according to Karl Pearson's correlation coefficient.

#### **Precision**

Repeatability study was carried out with six replicates and intermediate precision studies were carried out with three concentrations of dimethyl fumarate with three replicates. The values of % relative standard deviation (% RSD) of precision study are shown in table 3.

#### **Accuracy**

The accuracy of the tactic decided by calculating percent recovery of the drug by standard addition method. Percent recovery of dimethyl fumarate was determined at three different levels 50, 100 and 150% of the target concentration in triplicate. The results of accuracy study are shown in table 4.

#### **Robustness**

Robustness of the optimized method was studied by changing flow rate ( $\pm$ 0.2 ml/min), change in wavelength ( $\pm$ 2 nm) and change in mobile phase composition ( $\pm$ 5%) during analysis. The sample was injected in triplicate for every condition and cumulative % RSD was calculated for each condition is shown in table 5.

### **Specificity**

Blank (diluent), standard, sample and identification solutions (spiked with a fumaric acid and monomethyl fumarate impurities) were injected to HPLC. The results (retention time, purity angle and purity threshold) obtained by this study is summarized in table 6.

The chromatograms obtained by this study are presented in fig. 4(a),(b) and (c).

## Forced degradation studies

To evaluate stability, dimethyl fumarate was subjected to force degradation conditions (acid, base, neutral hydrolysis and oxidation as well as heat) as per international conference on harmonization (ICH) guidelines [3-5]. No treatment sample was prepared similarly as assay procedure for capsules. The chromatogram of no treatment sample is presented in fig. 5(a).

### Acid hydrolysis

An accurately weighed amount of capsule's powder equivalent to 100 mg of dimethyl fumarate was transferred to a 100 ml volumetric flask. Then 5 ml of 0.1N HCl was added and refluxed at 80 °C for 1 h [13]. This solution was neutralized by adding 5 ml of 0.1 N NaOH. Methanol (30 ml) was added to this mixture and sonicated for 15 min. Then volume was made up to the mark with diluent. The resultant solution was further diluted, filtered and analyzed using HPLC. The chromatogram obtained by acid hydrolysis is given in fig. 5(b).

### Alkaline hydrolysis

An accurately weighed amount of capsule's powder equivalent to 100 mg of dimethyl fumarate was transferred to a 100 ml volumetric flask. Then 5 ml of 0.1N NaOH was added and refluxed at 80 °C for 1 h [14]. This solution was neutralized by adding 5 ml of 0.1 N HCl. Methanol (30 ml) was added to this mixture and sonicated for 15 min. Then volume makeup, further dilution, filtration and analysis was done similar to acid hydrolysis. The chromatogram obtained by alkaline hydrolysis is given in fig. 5(c).

### Oxidative degradation

An accurately weighed amount of capsule's powder equivalent to 100 mg of dimethyl fumarate was transferred to a 100 ml volumetric flask. Then 5 ml of 3% H<sub>2</sub>O<sub>2</sub> was added and refluxed at 80 °C for 1 h. Methanol (30 ml) was added to this mixture and sonicated for 15 min. Then volume makeup, further dilution, filtration and analysis were done similar to acid hydrolysis. The chromatogram obtained by oxidative degradation is given in fig. 5(d).

### Thermal degradation

An accurately weighed amount of powder equivalent to 100 mg of drug was taken and kept in oven for 1 h 105 °C. Sample was transferred to 100 ml volumetric flask and 30 ml of methanol was added to it. Then the mixture was sonicated for 15 min. the sample was allowed to cool at room temperature. Then volume make up, further dilution, filtration and analysis was done similar to acid hydrolysis. The chromatogram obtained by thermal degradation is given in fig. 5(e).

### Stability of analytical solutions

The standard and sample solutions were kept at bench top and in stability chamber at 15 °C for 46 h and injected from time to time on to the HPLC. The data obtained are summarized in table 7.

**Filter compatibility study** Unfiltered and filtered standard solutions (by PVDF, Nylon, and PTFE) were injected to HPLC system. Sample solutions were centrifuged at 5000 rpm for 10 min, filtered similarly as standard solutions and injected to the HPLC system. The data obtained by this study is summarized in table 8.

## RESULTS AND DISCUSSION

### Optimization of chromatographic conditions

UV spectrum of dimethyl fumarate showed the utmost absorbance of the drug was found at 210 nm. Hence, 210 nm wavelength was selected for UV detection. Initially, various chromatographic conditions were tried so as to get better separation characteristics, by changing the composition of various mobile phases. Finally, mobile phase consists of acetonitrile: potassium dihydrogen phosphate buffer pH 6.8 (50:50% v/v) was selected at a rate of flow of 1.0 ml/min and UV detection (210 nm). the worth of retention time of drug was found to be 3.46 min, indicated that the tactic is rapid. The chromatogram of dimethyl fumarate is shown in fig. 2. The optimized chromatographic conditions and system suitability parameters are mentioned in table 1.

### Assay of capsules formulation

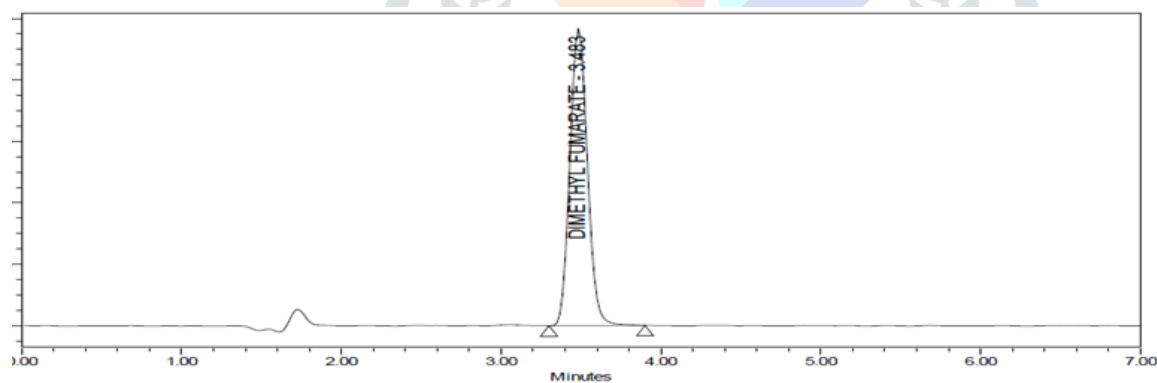
The value of deviation drug within the capsules was found to be 100.1 % (table 2), which was within acceptance criteria.

Precision

The method is precise and therefore the % RSD values were within an appropriate limit.

**Table 1: Optimized chromatographic conditions and system suitability parameters**

Parameters	Details
Mobile phase	Acetonitrile: Potassium dihydrogen phosphate buffer pH 6.8 (50:50% v/v)
Column	GL Science, Inertsil ODS, 150 x 4.6 mm, 5 $\mu$ .
Flow rate	1.0 ml/min
Detection	210 nm
Injection volume	5 $\mu$ l
Run time	7 min
Retention time	3.3+0.02 min
Diluent	Acetonitrile: (Potassium dihydrogen phosphate) buffer pH 6.8 (50:50% v/v)
Tailing fact	1.25



**Fig. 2: Chromatogram of dimethyl fumarate**

**Table 2: Results of assay of dimethyl fumarate**

S. No.	Sample solution concentration ( $\mu$ g/ml)	Area	Amount of drug estimated mean $\pm$ %RSD*
1	100	8549821	
2	100	8569754	100.1 $\pm$ 0.12
3	100	8567736	

The value is represented as a mean±%RSD of 3 observations

**Table 3: Repeatability and intermediate precision for dimethyl fumarate**

Precision	Concentration of drug (µg/ml)	Mean area±SD*	% RSD
Repeatability (n=6)	100	8705641±68889	0.82
Intra-day (n=3)	75	6377573.3±9957	1.57
	100	8329129.3±7385	0.88
	125	108269176±3335	0.30
Inter-day (n=3)	75	6377573.3±15357	0.24
	100	8368268.3±168167	2.00
	125	10979590.3±592188	0.5

Each value is represented as a mean±SD of n observations. SD: standard deviation, %RSD: Percent relative standard deviation.

### Accuracy

The values of percent recovery of the developed method (table 4) were found in acceptance criteria. Results of accuracy studies of the method were found satisfactory as the average mean % recovery±RSD was 100.5±0.56 %. Therefore, this method is accurate.

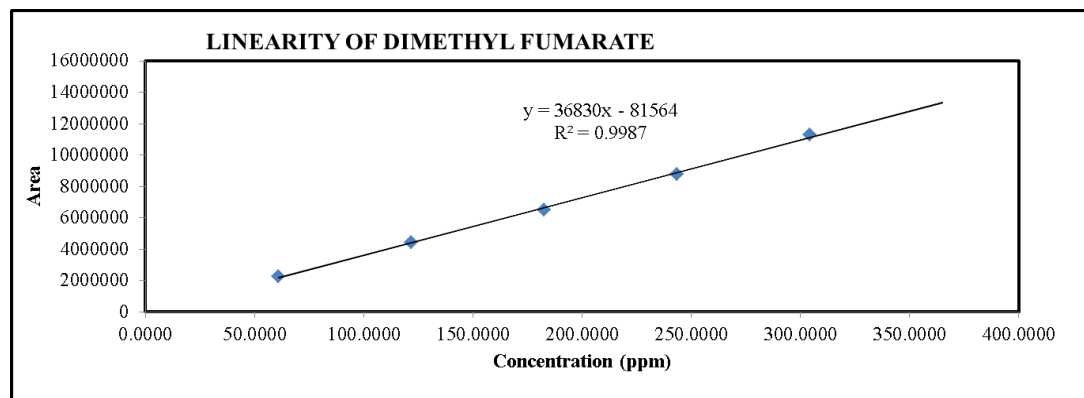
### Linearity and range

The value of correlation coefficient for dimethyl fumarate (fig. 3) demonstrated the good relationship between peak areas and concentrations. Therefore, the developed method was found to be linear in the concentration range of 25-150 µg/ml.

**Table 4: Recovery study for dimethyl fumarate**

Level %	Amount taken (µg/ml)	% recovery*	Mean %recovery±RSD
50	15	99.96 98.30 101.00	100.3±0.65
100	20	99.40 101.07 98.48	99.55±0.32
150	25	101.0 103.17 103.38	103.64±0.70

Percent recovery was in triplicate, % recovery: Percent recovery, %RSD: Percent relative standard deviation.



**Fig. 3: Calibration curve of dimethyl fumarate**

### Robustness

The value of % RSD was found to be within acceptance criteria which showed the reliability of the method.

### Specificity

The results of specificity study are shown in fig. 4 (a), (b) and (c). The obtained chromatograms showed that there no interfering peak was observed of blank, sample and standard solution at the retention time of dimethyl fumarate. Purity angle was observed that less than purity threshold for all peak observed. The value of retention time of dimethyl fumarate for standard solution and sample was same, however, the retention time of expected components was observed at different values. All these parameters indicated the specificity of the method.

### Forced degradation studies

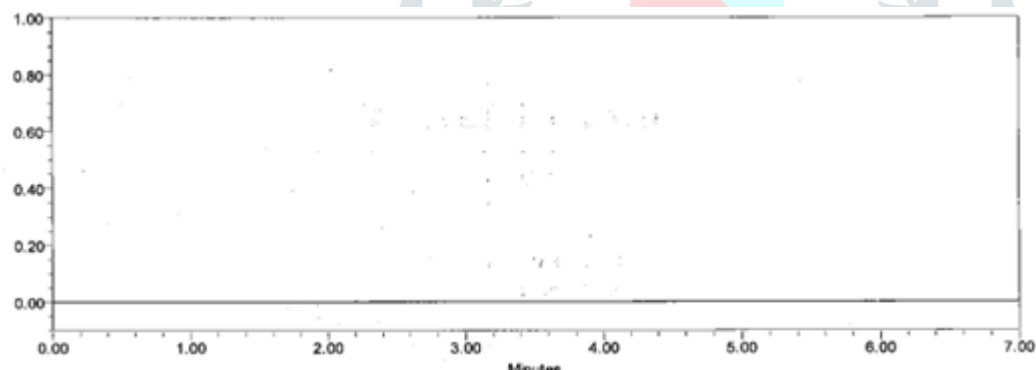
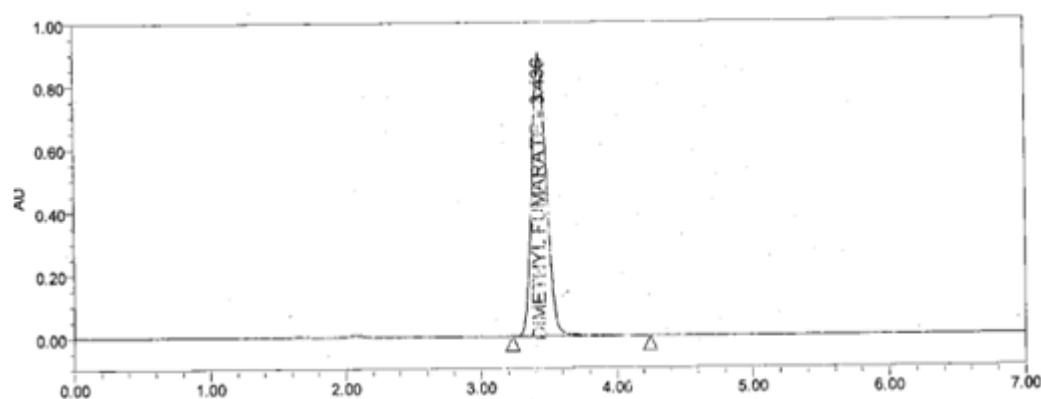
Chromatograms obtained under different stress conditions like acidic, alkaline hydrolysis, oxidative, thermal degradation are presented in fig no. 5(a), 5(b), 5(c), 5(d) and 5(e).

**Table 5: Robustness study for dimethyl fumarate**

Parameters	% RSD
<b>A: Change in flow rate (<math>\pm 0.2</math> ml/min)</b>	
0.8 ml/min	0.11%
1 ml/min	0.14%
1.2 ml/min	0.18%
<b>B: Change in Mobile Phase (<math>\pm 5\%</math>)</b>	
Buffer: ACN (55:45) v/v	0.29%
Buffer: ACN (50:50) v/v	0.15%
Buffer: ACN (45:55) v/v	0.07%
<b>C: Change in wavelength (<math>\pm 2</math> nm)</b>	
208 nm	0.35%
210 nm	0.12%
212 nm	0.25%

**Table 6: Specificity study for dimethyl fumarate**

	Component	Retention time (min)	Purity angle	Puritythreshold
1. Blank	Dimethyl fumarate	No --- ---		
2. Dimethyl fumarate standard .	Dimethyl fumarate	3.436	0.190	0.268
3. Identification solution	Fumaric acid	1.637	6.709	14.58
4. Identification solution monomethyl Fumarate.	Monomethyl fumarate	2.078	0.841	1.156
5 .Sample 240 mg	Dimethyl fumarate	3.432	0.199	0.273
6. Spiked sample 240	Dimethyl fumarate	1.645	0.121	10.084
7. Fumaric acid	Monomethyl fumarate	2.079	10.520	12.774

**Fig. 4 (a): Chromatogram of blank solution****ig. 4 (b): Chromatogram of standard solution**

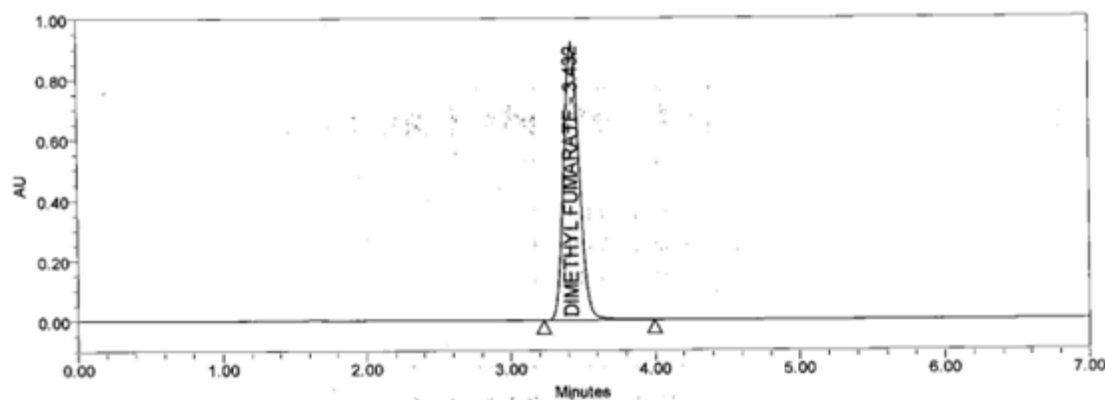


Fig. 5(a)

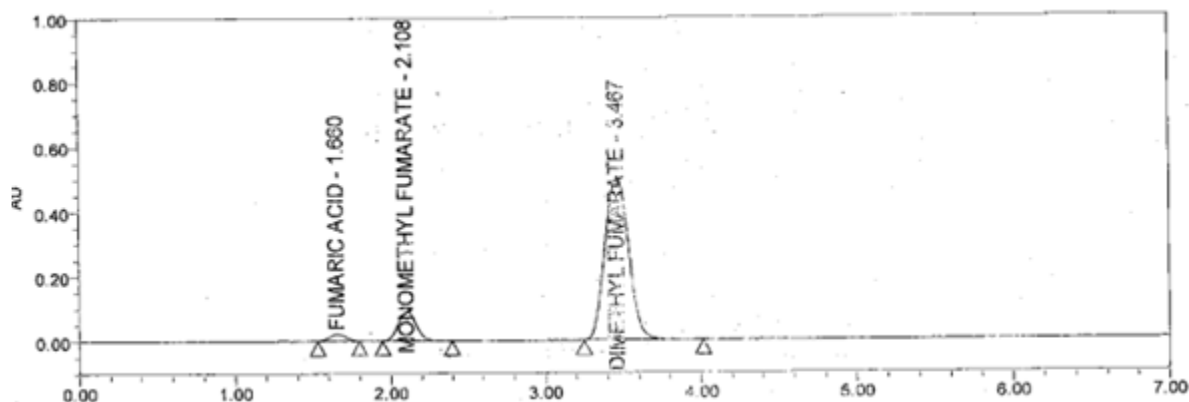


Fig. 5(b)

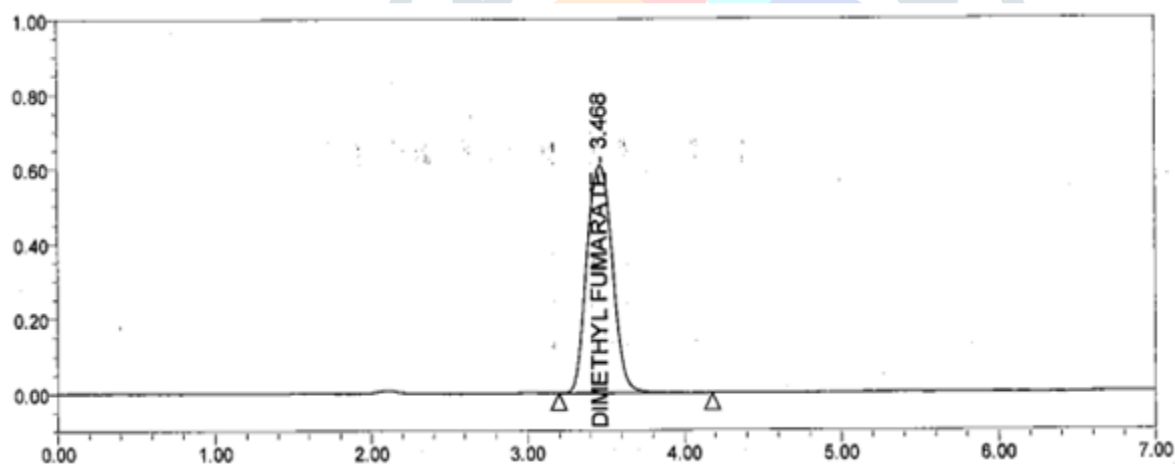


Fig. 5(c)

**Fig. 5: Typically degradation chromatograms of dimethyl fumarate; (a) in control sample; (b) in 0.1 N HCl at 80 °C after 1 h; (c) in 0.1 N NaOH at 80 °C after 1 h; (d) in 3% H<sub>2</sub>O<sub>2</sub> at 80 °C after 1 h; (e) in thermal degradation at 105 °C after 1 h**

The first chromatogram obtained by control sample [fig. 4(a)] was used for degradation of dimethyl fumarate but there was no degradation. Second chromatogram obtained by acid hydrolysis [fig.4(b)] suggested that 14.7% degradation of the drug was found, when refluxed at 80 °C for 1 h in 0.1 N HCl. The major degradation product formed was at 3.467 min retention time. This study indicates that dimethyl fumarate was not stable to acid hydrolysis. Third chromatogram obtained by alkaline hydrolysis [fig. 4(c)] indicated

that dimethyl fumarate was stable to alkaline hydrolysis when refluxed at 80 °C for 1 h in 0.1 N NaOH. The Value of % degradation was found to be 0.3% and major degradation products appeared at 3.46 min retention time. Fourth chromatogram obtained by



oxidative degradation [fig. 4(d)] suggested that 1.18% degradation was observed when refluxed with 3% H<sub>2</sub>O<sub>2</sub> at 80 °C for 1 h. The major degradation products appeared at 3.44 min retention time. Fifth chromatogram obtained by thermal degradation [fig. 4(e)]

suggested that 0.1% degradation was observed indicating that dimethyl fumarate is stable when refluxed at 105 °C for 1 h. The major degradation product was obtained at 3.45 min retention time.

### Stability of analytical solution

The stability data obtained is summarized in table 7. The values of % RSD of standard and sample solution were found within acceptance criteria.

### Filter compatibility study

Percent RSD of unfiltered and filtered standard solutions, as well as sample solutions (table8), is found within the limit. Hence, these filters are compatible.

**Table 7: Stability of analytical solutions**

	Initial	Bench top (46h)	15°C(46h)
Standard solution % RSD	0.12	1.09	0.98
Sample solution % RSD	0.14	0.67	1.24

**Table 8: Filter compatibility study of standard and sample**

Filter	Standard (%RSD)	Sample (% RSD)
Unfiltered	<b>0.58</b>	<b>Centrifuge(0.44)</b>
0.45µ Nylon Filter	1.04	0.44
0.45µ PTFE Filter	0.86	0.40
0.45µPVDF Filter	0.25	1.65

Results of method validation parameters, forced degradation, the stability of analytical solution and filter compatibility studies were found within acceptance criteria.

## CONCLUSION

The present study represents the first report for stability-indicating HPLC assay for estimation of dimethyl fumarate in bulk and capsules. The method was successfully validated as per ICH guidelines. Results of stress testing study revealed that the method is stability indicating. It can be concluded from the results that the developed method is simple, rapid, accurate, specific and precise. Thus, this method can be used for routine analysis of dimethyl fumarate API and to check the stability of capsules dosage forms.

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