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Original Article

STABILITY INDICATING RP-HPLC ASSAY METHOD FOR ESTIMATION OF DIMETHYL FUMARATE IN BULK AND CAPSULES

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ABSTRACT

Objective: To develop an accurate, simple, precise and specific stability indicating RP-HPLC method for estimation of dimethyl fumarate in bulk and capsules.

Methods: An Inertsil ODS (150x4.6 mm, 5µ) column and a mobile phase containing acetonitrile: potassium dihydrogen phosphate buffer pH 6.8 (50:50% v/v) was used for this study. The flow rate was maintained at 1.0 ml/min; column temperature was fixed at 35 °C and UV detection was carried out at 210 nm**.** The forced degradation studies were performed and method was validated with as per ICH guidelines.

Results: The retention time of dimethyl fumarate was found to be 3.3±0.02 min. The value of correlation coefficient between peak area and concentration was found to be 0.9993. The mean percent recovery of dimethyl fumarate in capsules was found in 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.

Conclusion: It can be conducted from results that the developed HPLC method is simple, accurate, precise and specific. Results of stress testing study revealed that the method is stability indicating. Thus, this method can be used for routine analysis of dimethyl fumarate capsules and check their stability.

Keywords: Dimethyl fumarate, RP-HPLC, Method validation, Stability indicating assay method

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INTRODUCTION

Dimethyl fumarate is an anti-inflammatory drug, which is chemically *trans*-Butenedioic acid dimethyl ester [1]. 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 is not an official 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 [7], either alone or in combination. However, there is no stability indicating HPLC assay method was reported yet for estimation of dimethyl fumarate in capsules. This paper presents a simple stability indicating RP-HPLC assay method for estimation of dimethyl fumarate in bulk and capsules that can be used in stability testing.

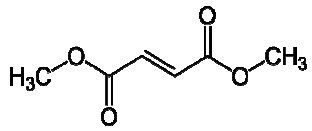

Fig. 1: Chemical structure of dimethyl fumarate

MATERIALS AND METHODS

Chemicals and reagents

Dimethyl Fumarate API was procured from Enaltec Research Centre, Ambernath, India as a gift sample. Capsules (containing 240 mg of dimethyl fumarate) were obtained in a house in Enaltec Research Centre. 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 Shimadzu LC-2010C HT HPLC system with automatic injection facility and UV-Visible detection system. Analytical Balance Mettler Toledo XS205 and Column Symmetry Shield Inertsil ODS (150 x 4.6 mm, 5µ) 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 equivalent to 100 mg of drug was transferred to a 100 ml volumetric flask and 30 ml of methanol was added to it. Then the mixture was sonicated for 15 min and diluted up to mark with the diluent. This solution was further diluted to obtain about 100 µg/ml solutions with the same diluent and filtered through 0.45µ 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 [8].

Linearity and range

Working standard solutions were injected under the optimized chromatographic conditions and peak areas were calculated at 210 nm. 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 method was determined 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 (±0.2 ml/min), change in wavelength (±2 nm) and change in mobile phase composition (±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 [9-12].

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_2O_2 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 maximum absorbance of the drug was found at 210 nm. Hence, 210 nm wavelength was selected for UV detection. Initially, various chromatographic conditions were tried in order to obtain better separation characteristics, by changing the composition of different mobile phases. Finally, mobile phase consists of acetonitrile: potassium dihydrogen phosphate buffer pH 6.8 (50:50% v/v) was selected at a flow rate of 1.0 ml/min and UV detection (210 nm). The value of retention time of drug was found to be 3.46 min, indicated that the method 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 mean % drug in the capsules was found to be 100.1 % (table 2), which was within acceptance criteria.

Precision

The method is precise and the % RSD values were within an acceptable 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, 5µ.
Flow rate	1.0 ml/min
Detection	210 nm
Injection volume	5 µl
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 factor	1.25
Theoretical plates	5857

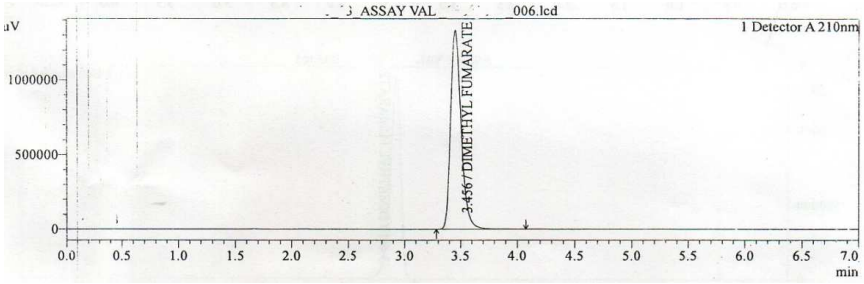

Fig. 2: Chromatogram of dimethyl fumarate

Table 2: Results of assay of dimethyl fumarate

S. No.	Sample solution concentration (µg/ml)	Area	Amount of drug estimated mean±%RSD*
1	100	8549821	
2	100	8569754	100.1±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		99.87	100.5±0.63
	15	102.00	
		98.28	
100		99.40	99.65±0.36
	20	100.07	
		99.48	
150		102.30	101.64±0.70
	25	101.17	
		101.46	

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

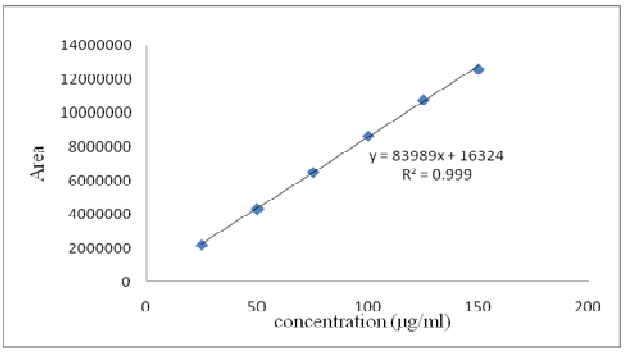

Fig. 3: Calibration curve of dimethyl fumarate

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
A: Change in flow rate (±0.2 ml/min)	
0.8 ml/min	0.13%
1 ml/min	0.11%
1.2 ml/min	0.19%
B: Change in Mobile Phase (±5%)	
Buffer: ACN (55:45) v/v	0.28%
Buffer: ACN (50:50) v/v	0.12%
Buffer: ACN (45:55) v/v	0.07%
C: Change in wavelength (±2 nm)	
208 nm	0.34%
210 nm	0.12%
212 nm	0.22%

Table 6: Specificity study for dimethyl fumarate

	Component	Retention time (min)	Purity angle	Purity threshold
Blank	Dimethyl fumarate	No	---	---
Dimethyl fumarate standard	Dimethyl fumarate	3.436	0.190	0.268
Identification solution Fumaric acid	Fumaric acid	1.637	6.709	14.58
Identification solution monomethyl Fumarate	Monomethyl fumarate	2.078	0.841	1.156
Sample 240 mg	Dimethyl fumarate	3.432	0.199	0.273
Spiked sample 240 mg	Dimethyl fumarate	3.436	0.121	10.084
	Fumaric acid	1.645	10.520	12.774
	Monomethyl fumarate	2.079	2.993	13.818

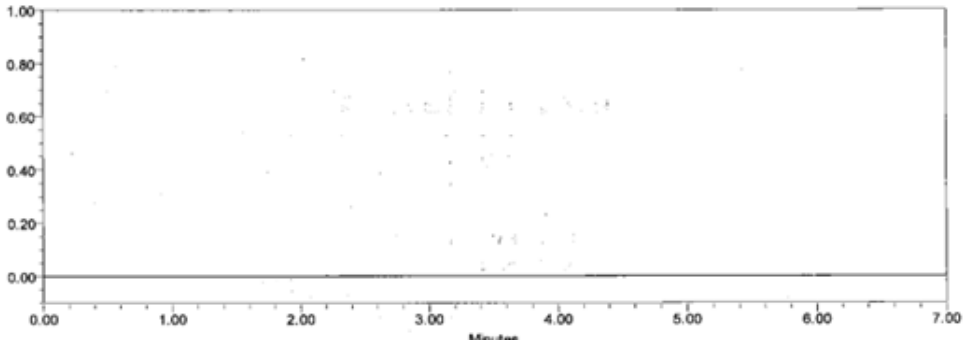

Fig. 4 (a): Chromatogram of blank solution

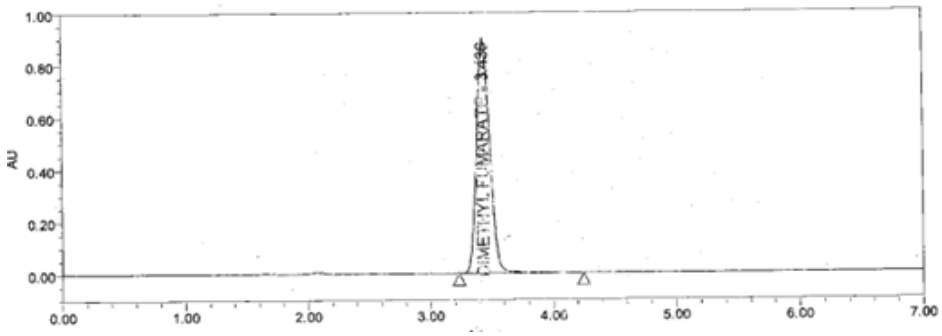

Fig. 4 (b): Chromatogram of standard solution

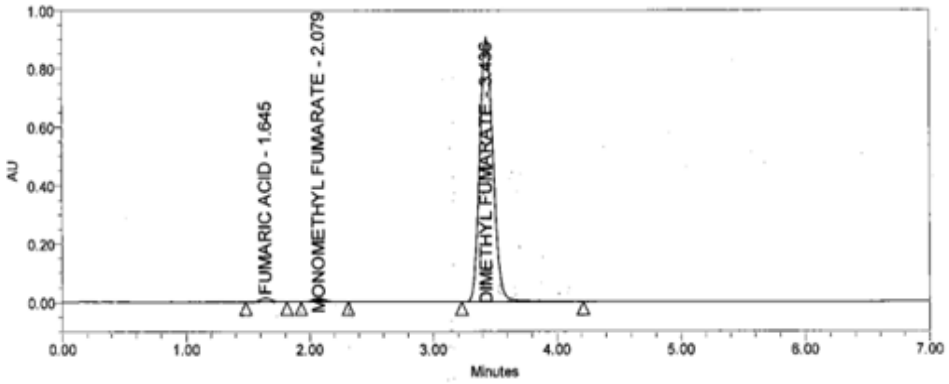

Fig. 3: (c). Chromatogram of spiked sample

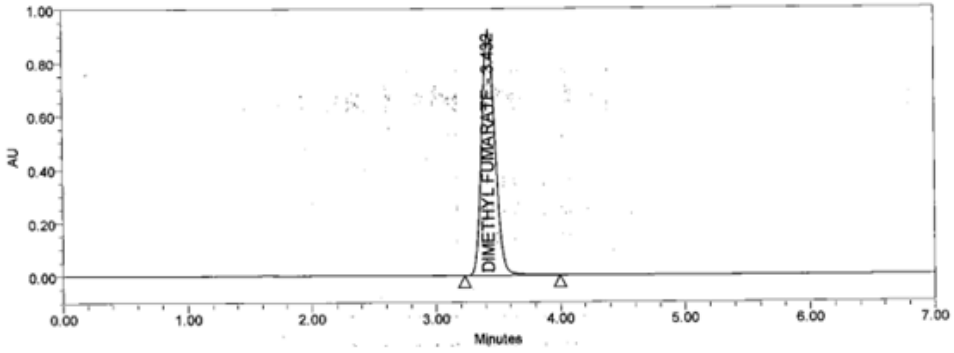

Fig. 5(a)

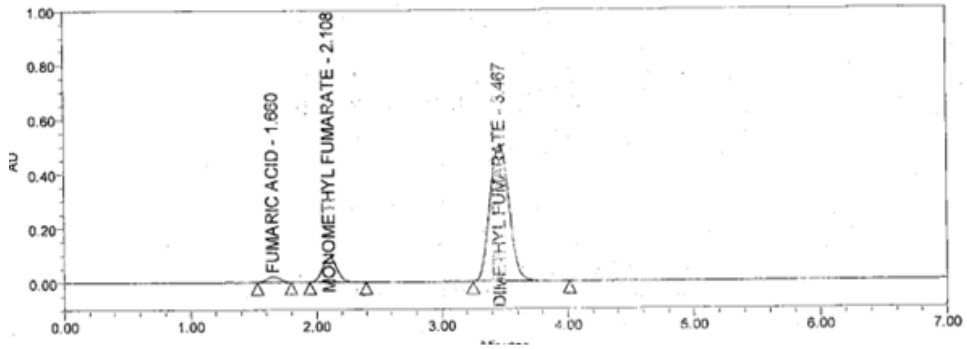

Fig. 5(b)

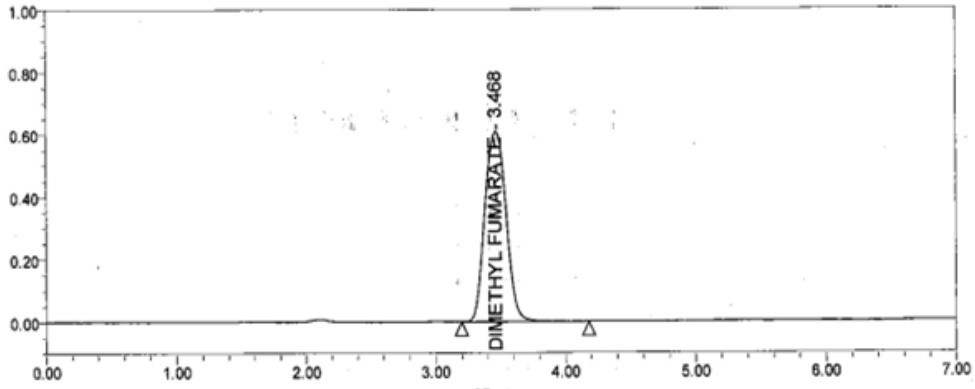

Fig. 5(c)

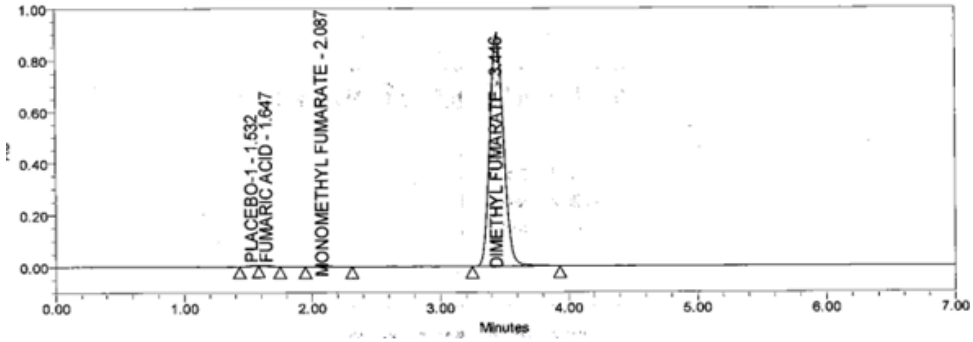

Fig. 5(d)

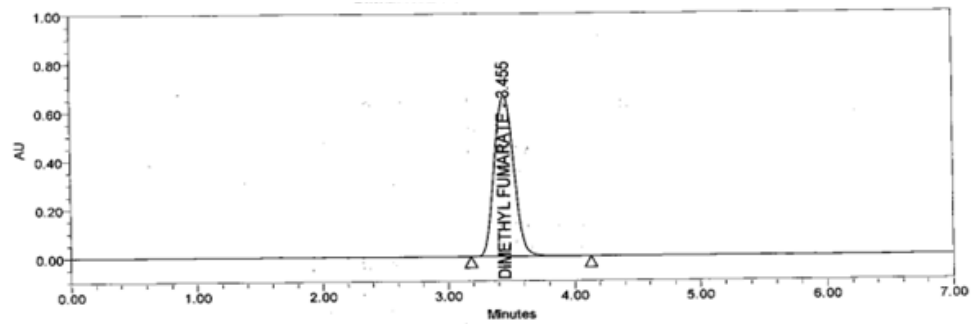

Fig. 5(e)

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_2O_2 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_2O_2 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 (table 8), is found within the limit. Hence, these filters are compatible.

Table 7: Stability of analytical solutions

	Initial	Bench top(46 h)	15 °C(46 h)
Standard solution % RSD	0.11	1.08	0.96
Sample solution % RSD	0.14	0.67	1.24

Table 8: Filter compatibility study of standard and sample

Filter	Standard (%RSD)	Sample (%RSD)
Unfiltered	0.56	Centrifuge (0.44)
0.45µ Nylon Filter	1.02	0.40
0.45µ PVDF Filter	0.80	0.39
0.45µ PTFE Filter	0.27	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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CONFLICT OF INTERESTS

Declared none

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