Research Paper | Medical Science

Development And Validation of Rp-Hplc Method for Determination of Related Substances of Dimethyl Fumarate Drug Product

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ABSTRACT

The present study describes a simple and stability-indicating reverse phase high-performance liquid chromatography (RP-HPLC) method for the quantification of the related substances of dimethyl fumarate drug product. Successful separation of the fumarate esters, monomethyl fumarate, dimethyl maleate from dimethyl fumarate and other related substances were achieved on a Symmetry shield RP 18 (250 × 4.6 mm, 5 µm) and UV detector at 210 nm, 1.0 mL/min as a flow rate, and 10 µL as an injection volume. For the RP- HPLC method, pH 3.4 phosphate buffer and methanol was used as mobile phases at ratio of 75:25 and the column temperature was 40 °C. Percentage recovery obtained in the range of 96.1 - 102.3 % andthe method is linear for fumarate acid, monomethyl fumarate, dimethyl maleate and dimethyl fumarate for specified concentration range with coefficient of variation (r) not less than 0.99. Acid, base, peroxide, heat and photolytic degradation was carried in drug product. The proposed RP- HPLC method was found to be specific, linear, precise, accurate and robust.

KEYWORDS Dimethyl fumarate, Related susbtances, Fumaric acid, Dimethyl maleate, Monomethyl maleate, RP-HPLC method

INTRODUCTION

Dimethyl fumarate (DMF) is the methyl ester of fumaric acid. DMF was initially recognized as a very effective hypoxic cell radiosensitizer[1]. Dimethyl fumarate has been found to be an allergic sensitizer at very low concentrations, producing eczema that is difficult to treat. Concentrations as low as 1 ppm may produce allergic reactions[2]. Later Fumaric acid ester (FAE) therapy which is already licensed in Germany has proved to be safe and effective in patients with severe psoriasis vulgaris. This treatment was introduced nearly 30 years ago, but is only now gaining renewed interest among dermatologists. [3].

The US FDA on March 27, 2013 approved Tecfidera (dimethyl fumarate) capsules to treat adults with relapsing forms of multiple sclerosis (MS) [3].

Organic impurities in drug substances can arise during the manufacturing process and storage. Thus, the acceptance limits are based on pharmaceutical studies or known safety data[4]. Dimethyl fumarate is rapidly hydrolyzed to monomethyl fumarate and fumaric acid where the former monomethyl fumarate is regarded as the active metabolite. Fumaric acid and monomethyl fumarate are generated during manufacturing process and during storage as well whereas Dimethyl maleate is a process impurity. Several methods have been reported for the analysis of Dimethyl fumarate and other fumaric acid esters separately [6,7,8]. However, no combined validated stability-indicating reversed phase HPLC (RP- HPLC) method has been used for the separation and quantitative analysis of fumaric acid esters in its pharmaceutical forms. In this study, a rapid and validated RP-HPLC method was developed to separate fumaric acid, monomethyl fumarate, dimethyl maleate and dimethyl fumarate. The limit of detection (LOD), limit of quantification (LOQ) and sensitivity of the method was tested in accordance with ICH Q2 guidelines for analytical method validation.

2. Experimental and Methods

2.1 Reagents, Materials and Instrumentation

Methanol, HCl and potassium dihydrogen orthophosphate was obtained from Merck. All other chemicals of analytical grade were gained from local sources. The instrument used was Agilent 1200 series HPLC system consisting of a pump, a UV detector and empower3 data analysis software. Waters symmetry shield RP 18 HPLC columns (250×4.6 mm i.d., 5 µm particle sizes) were used for the analysis.

2.2 Preparation of standard solution

25 mg of DMF was weighed and dissolved in 10 mL of methanol by sonication and further diluted with 0.1 N HCl to obtain 50 mL of solution. 5 mL of the above solution was diluted to 50 mL with 0.1 N HCl and 5 mL of this solution was further diluted to 100 mL with 0.1 N HCl.

2.3 Preparation of test solution

Capsule powder equivalent to 50 mg of DMF was weighed from twenty capsules after uniform powdering. 20 mL methanol, 20 mL 0.1 N HCl was added and sonicated for 10 min and the volume was made up to 100 ml using 0.1 N HCl to achieve a concentration of 500 µg/ml of DMF. The solution was centrifuged for 10 minutes at 10000 rpm and filtered through 0.45 micron PVDF membrane filter.

Results and discussion

3.1 Optimization of chromatographic conditions

Few HPLC columns such as octadecyldimethyl-silane (C18) and octyldimethylsilane (C8), polar group embedded C18, phenyl stationary phases with changing buffer pH, organic modifiers were used, since the objective of the method is to quantify Fumaric acid, Monomethylfumarate, Dimethyl maleate in Dimethyl fumarate drug the major focus. Separation of these components their peak shape and interference from blank sample were monitored in all trials.

Retaining and separating fumaric acid from diluents peaks has been a challenge in the method development because conventional C18 and C8 columns were poor in retaining fumaric acid. The peaks were eventually separated from each other and DMF using Symmetry shield RP 18 HPLC column (250 mm × 4.6 mm, 5 µm). The chromatograms recorded using these columns are presented in **Figure 1**.

In alkaline condition DMF readily hydrolysis in to fumaric acid and monomethyl fumarate hence alkaline diluents and mobile phases are avoided. A asymmetry fumaric acid peak was observed at a pH of 2.4 and 4.4, hence the method was developed with buffer pH 3.4 prepared using potassium dihydrogen phosphate as mobile phase A, methanol as mobile phase B. The binary pumps were programmed as initial isocratic elu-

tion at a ratio of 60:40 up to 25 minutes, linear gradient at the ratios of 20:80 up to 30 minutes, continued until 34 minutes, 75:25 up to 37 minutes, continued until 40 minutes. 0.1 N HCl was used as diluents to avoid hydrolysis of fumaric acid.

A better symmetric peak of fumaric acid was obtained when the column temperature was 40 . The optimized wavelength was 210 nm based on the UV spectrum of DMF and the spectra are presented in **Figure 2**. The resolution was achieved using different test mobile phases and columns which are mentioned in **Table 1**. The relative retention times of fumaric aicd, monomethyl fumarate, dimethyl maleate and DMF were about 0.16, 0.31, 0.46 and 1.0 respectively. The tailing factor of each component was not more than 1.0. The components were spectrally pure as calculated by the software Empower 3 chromatographic data management system.

For the RP-HPLC, pH 3.4 potassium dihydrogen phosphate buffer as mobile phase A and methanol as mobile phase B was used and the column temperature was maintained at 40 . The wavelength was set at 210 nm using a Symmetry shield RP 18 HPLC column (250 mm × 4.6 mm, the diameter of the particle was 5μm). The flow rate was 1.0 mL and the injection volume was 10 μL.

Figure 1. Sample spiked with Impurities

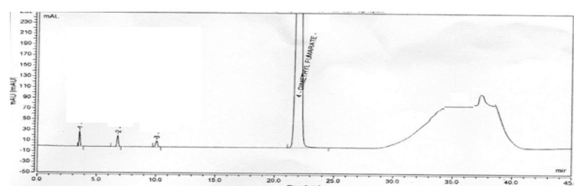

Figure 2. UV Spectrum of DMF and its related substances

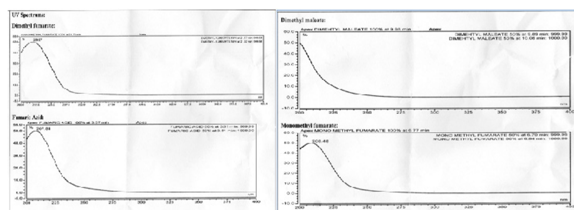

Table1. The system suitability parameters using different test mobile phase and different columns.

Mobile phase A	Ratio	Column	Fumaric acid		Resolution between	Diluent interference
			Rt	Tailing factor	Monomethyl fumarate and Dimethyl maleate	
pH 3.15 phosphate buffer	75:25	YMC pack ODS (250 x 4.6 mm), 5μ	2.312	1.81	2.1	-
pH 3.15 phosphate buffer	75:25	Symmetry shield RP 18 (250 x 4.6 mm), 5μ	3.502	1.03	2.8	-
pH 2.5 phosphate buffer	75:25	Symmetry shield RP 18 (250 x 4.6 mm), 5μ	4.067	1.02	2.8	Diluent peak merges at Dimethyl maleate peak
pH 4.4 phosphate buffer	75:25	Symmetry shield RP 18 (250 x 4.6 mm), 5μ	3.121	1.87	2.9	-
pH 3.15 phosphate buffer	80:20	Symmetry shield RP 18 (250 x 4.6 mm), 5μ	3.913	1.71	2.8	-
pH 3.15 phosphate buffer	70:30	Symmetry shield RP 18 (250 x 4.6 mm), 5μ	2.432	1.12	1.7	diluent peak merges at Fumaric acid peak

Table 2. Linearity, LOD, LOQ and accuracy data of DMF and its related substances.

Component	Linear Equation	Correlation co-efficient	Concentration range (μg/mL)	LOD	LOQ	% Recovery (average of 3)
Fumaric acid	Y=74849 X + 1385	1.000	0.145 μg/mL – 3.76 μg/mL	0.145 μg/mL	0.044 μg/mL	at LOQ = 96.1 at 3.75 μg/mL= 102.3 at 7.5 μg/mL= 99.2 at 10.1 μg/mL= 97.4
Monomethyl fumarate	Y=68118 X + 379.3	1.000	0.18 μg/mL – 3.73 μg/mL	0.180 μg/mL	0.054 μg/mL	at LOQ = 96.7 at 3.75 μg/mL= 99.7 at 7.5 μg/mL= 99.2 at 10.1 μg/mL= 96.9
Dimethyl maleate	Y=22585 X + 246.39	1.000	0.145 μg/mL – 3.75 μg/mL	0.145 μg/mL	0.044 μg/mL	at LOQ = 96.9 at 3.75 μg/mL= 100.2 at 7.5 μg/mL= 99.4 at 10.1 μg/mL= 99.1
Dimethyl fumarate	Y=68905 X + 968.47	1.000	0.131 μg/mL – 3.72 μg/mL	0.131 μg/mL	0.039 μg/mL	at LOQ = 97.9 at 3.75 μg/mL= 100.2 at 7.5 μg/mL= 99.3 at 10.1 μg/mL= 97.1

3.2 Method validation

3.2.1 Specificity

Blank and placebo was injected to evaluate specificity.No interference due to blank and placebo were observed at retention time of DMF and its related substances.

To demonstrate the stability indicating nature of the method, forced degradation has been carried out in acid (0.5 N HCl, 60°C, 1 hour), base (0.05 N NaOH, RT, 15 mins), oxidation (10 % H_2O_2, RT, 1 hour), photolytic degradation (under UV, white florescent light), humidity (80% relative humidity for 72 hours) and thermal (at 50°C for 24 hours). In alkaline condition 36.12 % of DMF degraded generating 29.14 % ofmonomethyl fumarate and 6.98 % of fumaric acid. No degrada-

tion products were observed in any of the other degradation conditions.

The entire peaks were found to be specific and spectrally pure as calculated by the software Empower 3 chromatographic data management system.

3.2.2 Precision

Consistency in repeated response by the chromatographic system and sample preparation procedure was evaluated by injecting six replicate preparations of sample solution spiked with impurities at 0.2 % of test concentration and 5 µg/mL of DMF in diluent. The % RSD is tabulated in **Table 3**.

Table 3: % RSD of DMF and its related substance

S.No	Component	% RSD
	Fumaric acid	3.2
	Monomethyl fumarate	1.4
	Dimethyl maleate	2.1
	Dimethyl fumarate	2.6

3.2.3 Linearity

To evaluate the linearity in detector response the components were injected from LOQ concentration to 3.75 µg/ mL concentration and the correlation coefficient was found to be not more than 0.99. The response factor was calculated from the slope of impurities and DMF linearity curve, except fumaric acid (RRF 0.30) all other impurities have RRF between 0.90 to 1.10

3.2.4 Limit of detection and limit of quantification

The Limit of detection (LOD) and limit of quantification (LOQ) were determined from the signal to noise ratio of the peaks obtained from series of low concentration solutions. The LOD and LOQ values are depicted in **Table 2.**

3.2.5 Accuracy

Accuracy was demonstrated by spiking impurity solution at LOQ, 50 %, 100 % and 150 % of 0.5 % to the test concentration.The % recovery was calculated from the amount added and amount found. The results are tabulated in **Table 2.**

3.2.6 Robustness

Robustness of the method was evaluated by injecting test material spiked with impurities and the specificity of fumaric acid. Tailing factor of fumaric acid and resolution between monomethyl fumarate and dimethyl maleate was monitored. The method was found to be robust for the below mentioned conditions.

1. ± 0.2 mL flow rate

2. ± 0.2 units pH of buffer

3. ± 5 °C column temperature

4. ± 2 % difference in gradient concentration.

4. Conclusions

The RP-HPLC method developed for the estimation of fumaric acid, monomethyl fumarate and dimethyl maleate in the DMF drug product is very useful tool for monitoring the quality of DMF and its pharmaceutical forms. The method was found to be specific, precise, robust, linear and accurate. The method can be used for checking the quality of the manufactured capsules as well as for stability studies of the pharmaceutical capsules.

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