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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF DIMETHYL FUMARATE AND DIMENHYDRINATE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for simultaneous estimation of Dimethyl Fumarate and Dimenhydrinate. Chromatography was performed on a Lichrospher® 100, C_8(250 mm×4.6 mm, 5µ) column using Methanol : 1% Triethylamine (40 : 60) as a mobile phase. The detection was carried out at 265 nm with a flow rate of 0.8 mL/min. The retention times were 4.3 and 5.8 minutes for Dimethyl Fumarate and Dimenhydrinate, respectively. Proposed method was validated as per ICH guidelines for linearity, accuracy, precision; specificity and robustness for estimation of Dimethyl Fumarate and Dimenhydrinate and results were found to be satisfactory. The linearity of the method was excellent over a concentration range 20-100µg/mL for Dimethyl Fumarate and 40-200µg/mL for Dimenhydrinate. The correlation coefficient was 0.9996 and 0.9992 for Dimethyl Fumarate and Dimenhydrinate, respectively. The limit of detection was 1.62µg/mL and 2.62µg/mL for Dimethyl Fumarate and Dimenhydrinate, respectively. The limit of quantitation was 4.93µg/mL and 7.95µg/mL for Dimethyl Fumarate and Dimenhydrinate, respectively. The relative standard deviation values for repeatability, intraday precision and interday precision studies were less than 2% and % recovery was 98% to 102% for both drugs. So the proposed method was found to be suitable for the routine estimation of Dimethyl Fumarate and Dimenhydrinate.

KEYWORDS: Dimethyl Fumarate, Dimenhydrinate RP-HPLC method, Validation.

INTRODUCTION

Dimethyl Fumarate is a methyl ester of fumaric acid and chemically is (E)-2-Butenedoic acid dimethyl ester. Dimethyl Fumarate was initially recognized as a very effective hypoxic cell radiosensitizer. Later, Dimethyl Fumarate was used in the treatment of psoriasis along with three other fumaric acid esters and also used to treat, necrobiosis, lipoidica, granulose annulare and sarcoidosis. Phase III clinical trails found that Dimethyl Fumarate successfully reduced relapse rate and increased time to progression of disability in Multiple Sclerosis(MS). The mechanism by which Dimethyl Fumarate is unknown. Dimethyl Fumarate and its metabolite MonomethylFumarate activates the nuclear factor (erythroid-derived 2)-like 2(Nrf2) pathway and acts as a nicotinic acid receptor agonist in vitro.[1,2]

Dimenhydrinate, is chemically 8-chloro-1, 3-dimethyl-2, 6dioxo-2, 3, 6, 7 – tetra DMFro-1H-purin-7-ide; [2(diphenylmethoxy) ethyl] dimethylazanium is used in the management of nausea and vomiting in the postoperative patient controlled analgesia. Dimenhydrinate is a competitive antagonist at the histamine H1 receptor, which is widely distributed in the human brain. Dimenhydrinate's anti-emetic effect is probably due to H1 antagonism in the vestibular system in the brain. Dimenhydrinate is also prescribed as an antipruritic drug.

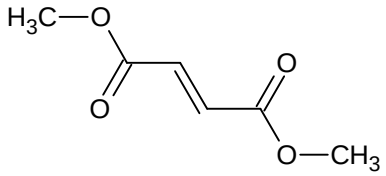
Dimethyl fumarate is used in effective treatment of Multiple Sclerosis but at the same time it is responsible for the generation of nausea, vomiting, diarrhea, itching, gastritis, gastroenteritis, leucopenia, hypersensitivity, burning sensation and flushing.

In order to minimize the adverse effect of DMF such as nausea, vomiting, itching. Dimenhydrinate can be prescribed or given in combination with Dimethyl Fumarate.

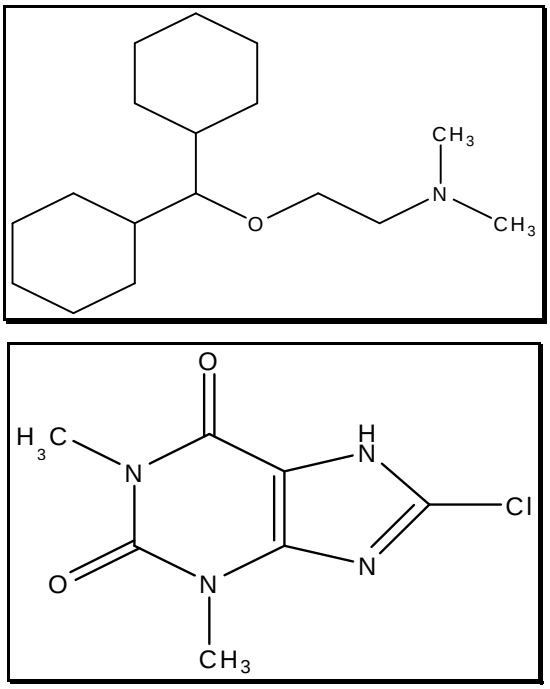
But no analytical method was found for the simultaneous estimation of Dimethyl Fumarate and Dimenhydrinate in combination by HPLC method.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS: The reference samples of Dimethyl Fumarate & Dimenhydrinate were purchased from SIGMA ALDRICH. Methanol and Triethylamine were of HPLC Grade.



STRUCTURE OF DIMETHYL FUMARATE



STRUCTURE OF DIMENHYDRINATE

INSTRUMENT AND CHROMATOGRAPHY CONDITION

The High Performance Liquid Chromatography consisted of SHIMADZU-SPD-20A prominence auto sampler fitted with UV Visible detector (SPD-20A) with SHIMADZU-LC-20AT pump. The chromatogram was recorded using LC Solution software. The Chromatographic separation was achieved by using Lichrospher® 100, C_8(250 mm×4.6 mm, 5µ) as stationary phase and mobile phase consists of Methanol: 1% Triethylamine (40 : 60) as a mobile phase. The detection was carried out at 265 nm with a flow rate of 0.8 mL/min.

PREPARATION OF MOBILE PHASE

Volume of 400mL HPLC grade Methanol, Volume of 600mL of 1% Triethylamine was mixed, filtered with 0.45 µ filter paper and sonicated for 20 mins. Mobile phase was used as diluent.

Diluent Preparation: Mobile phase is used as diluent.

PREPARATION OF STANDARD SOLUTION

Stock solutions of standard drugs DMF and DMN were prepared by weighing accurately 20 mg of DMF and 40 mg of DMN into a 100 mL clean dry volumetric flask. About 70 mL of the mobile phase was added and sonicated to dissolve the drugs completely. The volume was made upto 100 mL with the mobile phase and filtered through 0.45 µm membrane filter. From the above prepared standard stock solution, 1 mL was taken to 10 mL volumetric flask and the volume was made up with the mobile phase to obtain a concentration of 20µg/mL and 40µg/mL for DMF and DMN respectively.

PREPARATION OF WORKING STANDARD SOLUTION OF DMF & DMN

Twenty punched tablets were weighed and powdered. Powder weight equivalent to 20 mg of DMF and 40 mg of DMN was weighed and transferred into a 100 mL clean dry volumetric flask. About 70 mL of the mobile phase was added and sonicated to dissolve the drugs completely. The volume was made upto 100 mL with the mobile phase and filtered through 0.45 µm membrane filter. From the above prepared standard stock solution, 1 mL was taken to 10 mL volumetric flask and the volume was made up with the mobile phase to obtain a concentration of 20µg/mL and 40µg/mL for DMF and DMN respectively.

DETERMINATION

Wavelength for detection was selected by examining the resulted solution that consists of DMF & DMN (10 µg/mL) in SHIMADZU UV- Spectrometer (UV- 1800) instrument. The maximum absorbance for DMF & DMN was observed at 265 nm and hence 265 nm was selected as wavelength of detection.

METHOD VALIDATION

The proposed method was validated in compliance with ICH guidelines for linearity, accuracy, precision, specificity, robustness, and system suitability parameters by the following procedures.

LINEARITY

Linearity of developed HPLC method was studied by obtaining calibration curves of DMF, DMN at six different concentration levels in triplicate ranging from 20-100 µg/mL for DMF, and 40-200 µg/mL for DMN. Table 1 shows the linearity data of DMF and DMN. The linearity regression coefficient (R2) values were found to be 0.9996 and 0.9992 for DMF & DMN each. Linearity equation obtained for DMF & DMN were y = 48024x – 76365, and y =

25282x – 81003 respectively. Figure 3 and 4 shows calibration curves for DMF & DMN respectively. High level of correlation coefficient indicates good linearity.

TABLE 1: LINEARITY DATA OF DIMETHYL FUMARATE, DIMENHYDRINATE

Dimethyl Fumarate		Dimenhydrinate	
Con. µg/mL	**Peak area**	**Con. µg/mL**	**Peak area**
20	297982	40	346137
40	1086682	80	1028370
60	1303486	120	2139540
80	3114097	160	3376873
100	4086682	200	4228370

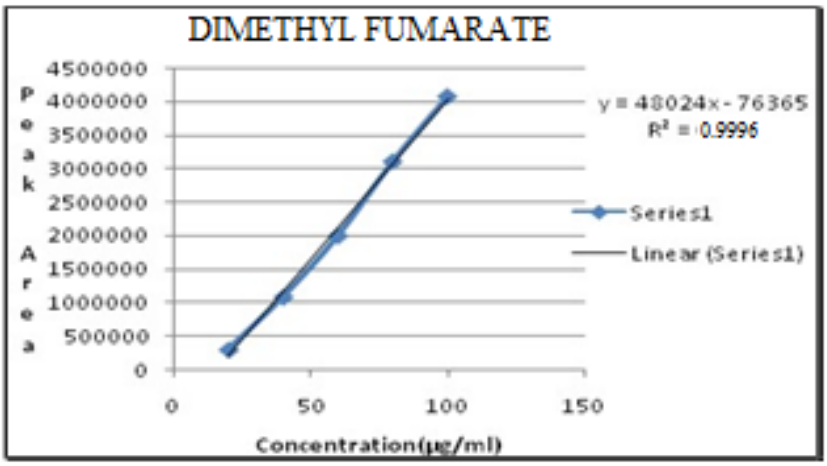


FIGURE 1: CALIBRATION CURVES FOR DIMETHYL FUMARATE.

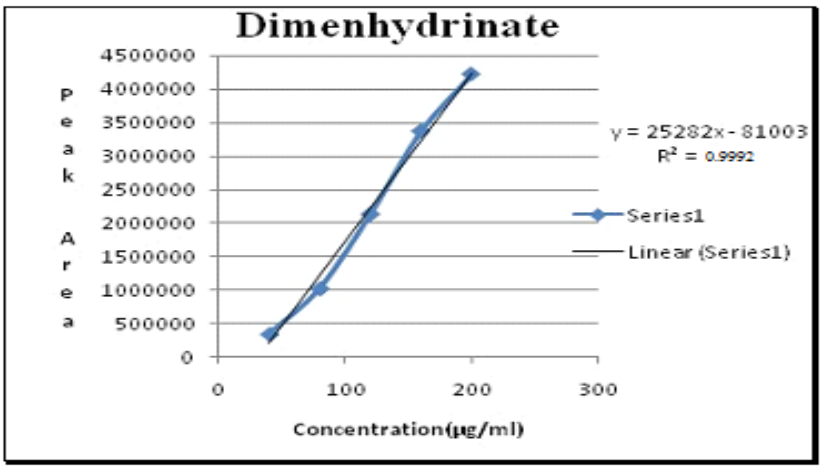


FIGURE 2: CALIBRATION CURVES FOR DIMENHYDRINATE

ACCURACY

The accuracy of the developed method was evaluated in triplicates by recovery studies at three different concentration levels of 50%, 100 %, and 150% for DMF, DMN respectively.

Known amounts of standard drug concentrations were added to the sample and peak area was determined. The mean percentage recovery values are shown in Table 2. The mean recovery of the drugs was found to be in the range of 99- 101% indicating a high degree of accuracy for the developed method.

TABLE 2: ACCURACY RESULTS.

Drug	Conc.	Amt. present	Amt. spiked	Conc. After spiking	% Mean Recovery
DMF	50%	30 µg/mL	15 µg/mL	45 µg/mL	98.33
	100%	30 µg/mL	30 µg/mL	60 µg/mL	99.85
	150%	30 µg/mL	45 µg/mL	75 µg/mL	99.97
DMN	50%	60 µg/mL	30 µg/mL	90 µg/mL	99.63
	100%	60 µg/mL	60 µg/mL	120 µg/mL	99.91
	150%	60 µg/mL	90 µg/mL	150 µg/mL	99.88

PRECISION

The precision at 20 % concentration of the assay method was evaluated by six replicate injections and measurement of peak areas by determining the % RSD of DMF, DMN. The calculated values of % RSD for DMF and DMN are mentioned in Table 3. The results indicated a high degree of repeatability.

TABLE 3: RESULTS FOR PRECISION.

Injection	Dimethyl Fumarate		Dimenhydrinate	
	RT	Area	RT	Area
1	4.3	297982	5.8	3461378
2	4.2	295763	5.8	3452469
3	4.3	293991	5.9	3462378
4	4.3	296853	5.8	3441253
5	4.2	294692	5.8	3471457
Average	2958562		3457787	
S.D	208626.8		18224	
%RSD	0.52		0.55	

SPECIFICITY

Chromatogram of only diluent was taken to check the interference of diluent with the peaks of DMF and DMN at the retention time of respective drugs. There was no peak detected at retention time of DMF 4.3 min and DMN 5.8 min. so, proposed method is specific in nature.

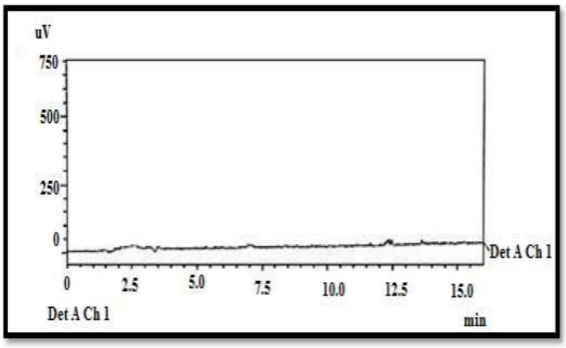


FIGURE 5: CHROMATOGRAM OF DILUENT

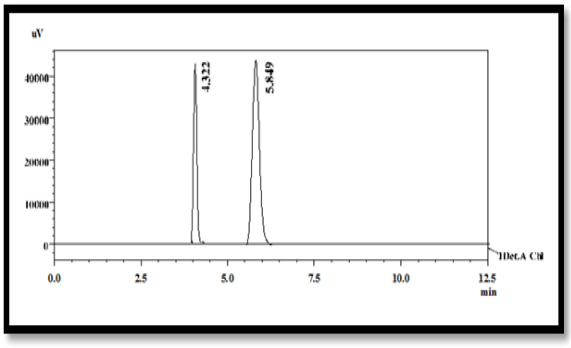


FIGURE 6: CHROMATOGRAM OF DMF & DMN

LOD and LOQ

LOD and LOQ for DMF, DMN by this method were evaluated on the basis of signal-to-noise ratio method described in ICH guidelines. A signal-to noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. A typical signal-to-noise ratio required for LOQ is 10:1. Using the proposed HPLC method, the LOD and LOQ values were calculated and are given in Table 4.

TABLE 4: LOD AND LOQ VALUES OF DMF AND DMN.

	Dimethyl Fumarate	Dimenhydrinate
LOD µg/mL	1.62µg/mL	2.62µg/Ml
LOQ µg/mL	4.93µg/mL	7.95µg/mL

ROBUSTNESS

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized parameters were made in chromatographic conditions like of flow rate and wavelength. The effect of change in flow rate and wavelength of detection on retention time and tailing factor were examined. The values obtained are mentioned in Table 5. The method was found to be unaffected by the small changes like ± 0.3 mL/min in flow-rate of mobile phase and ± 5 nm in detection wavelength.

TABLE 5: RESULTS OF ROBUSTNESS BY VARIATIONS IN FLOW RATE AND WAVELENGTH.

Parameters	Value	DMF		DMN	
		RT	TF	RT	TF
Flow rate	0.5mL/min	3.0	0.93	4.0	1.17
	1.1mL/min	5.0	0.99	5.8	1.18
Wavelength	260nm	3.2	1.0	4.0	1.2
	270nm	4.7	0.63	5.8	1.15

SYSTEM SUITABILITY

Six replicate of sample containing DMF, DMN were given to evaluate equipment, electronics, analytical operations and samples suitability. Parameters calculated for system suitability were %RSD of retention time and area, number of theoretical plates and Resolution. The results are given in Table 6.

TABLE 6: SYSTEM SUITABILITY RESULTS FOR DMF AND DMN.

SL. No	Parameters	DMF	DMN
1	Theoretical plates	5305.591	16463.92
2	Tailing factor	0.87	1.18
3	Resolution	0.000	7.27
4	Relative retention time (min)	4.3	5.8

RESULT AND DISCUSSION

Optimized chromatography condition: Chromatographic conditions were screened for mobile phase composition, mobile phase proportion and flow rate Finally, mobile phase of Methanol: 1% Triethylamine (40: 60) as a mobile phase was optimized to give symmetric peak with short runtime at UV detection wavelength of 265 nm and flow rate at 0.8mL/min was found to be appropriate with adequate separation between the two drugs. Chromatogram of DMF, DMN at optimized chromatographic condition was recorded, the runtime was 12.5 min and the retention times of DMF, DMN were found to be 4.3 and 5.8 min as shown in Figure 5, 6.

CONCLUSION

The proposed HPLC method was found to be economical, simple, sensitive, accurate, precise, specific and robust and can be used for the routine quality control analysis of DMF, DMN in bulk as well as in tablet formulation.

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