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Design and Development of combination therapy for treatment of Multiple sclerosis

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ABSTRACT:

Combination therapies are recent trends used to treat critical diseases based on the synergistic effects. Present study is designed to develop fixed dose combination of two drugs to treat multiple sclerosis with reduced side effect and more effectively. Fixed dose combination was designed as capsules in tablets. Capsules are filled with delayed release tablets and immediate release blend that required for immediate action by achieving blood level immediately and delayed release tablets to add on effect. Teriflunomide and Dimethyl Fumarate were selected to treat multiple sclerosis in which Teriflunomide was designed for immediate action and Dimethyl Fumarate for delayed action maintain optimum plasma levels of both drugs at a time and for a prolonged period. Wet granulation process was adopted for immediate release formulation whereas direct compression process was selected for delayed release layer with optimized excipients concentrations. Formulation variables for immediate release layer include sodium starch glycolate as super disintegrant and hydroxy propyl cellulose as binder. Methacrylic acid copolymer is used as delayed release polymer in Dimethyl Fumarate Tablets for delayed action. Physico-chemical parameters like weight variation, lock length, disintegration time and in-vitro dissolution of capsules were evaluated.

KEY WORDS: Combination therapy, delayed release, multiple sclerosis, Sodium starch glycolate, Methacrylic acid.

INTRODUCTION:

Multiple sclerosis (MS) is the most common neurological disorder diagnosed in young adults. Multiple Sclerosis is a disease of the central nervous system(CNS) that damages the protective insulation (known as “myelin”) surrounding the nerves and may also damage the nerves as well within the CNS. As a result, messages from the brain and spinal cord may short circuit, causing reduced or lost bodily function. The term multiple sclerosis refers to multiple areas of scarring (sclerosis) scattered through the brain and spinal cord**.** [1-5]

Three typical patterns of Multiple Sclerosis can be [6]

1. Relapsing-remitting Multiple Sclerosis
2. Secondary-progressive Multiple Sclerosis
3. Primary-progressive Multiple Sclerosis

Common symptoms include weakness or in-coordination of the limbs, Impaired balance or instability walking, Sensory disturbances, Blurred or double vision, Impaired urinary or sexual function, Cognitive dysfunction such as impaired memory or concentration.[6]

Teriflunomide is the active metabolite of leflunomide, a drug with immunosuppressant and anti-inflammatory properties for the use in rheumatoid arthritis. Teriflunomide exerts its biological function through inhibition of dihydroorotate dehydrogenase (DHODH), a key mitochondrial enzyme in de-novo pyrimidine synthesis pathway required by rapidly dividing cells such as proliferating B and T cells. Teriflunomide is a safe drug with only mild to moderate treatment adverse events (AE)[7-8]

Dimethyl Fumarate (DMF) has been shown to deeply impact MRI disease activity measures proving a promising therapeutic agent for Multiple Sclerosis treatment. DMF enables activation of nuclear factor E2 (erythroid derived 2)-related factor-2 (NRF2) which in turn promotes transcription of many genes involved in the antioxidative stress cell machinery.[9]

MATERIALS AND METHODS:

Materials:

Teriflunomide and Dimethyl Fumarate were gift sample from SPS Pharmaceuticals, Mumbai. Lactose monohydrate, maize starch, Microcrystalline cellulose, Hydroxy propyl cellulose, sodium starch glycolate, Methacrylic acid co-polymer, Croscarmellose sodium, colloidal silicon dioxide, Talc, Magnesium stearate, titanium dioxide, Polysorbate 80, Triethyl citrate were gift sample from pharmaceutical vendors.

Methods:

Preparation of Standard Curve for Teriflunomide

100mg of Teriflunomide was weighed and transferred to 100ml of volumetric flask containing 50ml of 0.1 N HCl and sonicated for 30 minutes and volume was made up to 100ml (1mg/ml). Then the stock solution was diluted to get 2 -10 ppm solution and absorbance was measured at 220nm.Standard curve was obtained by plotting concentration vs mean peak area.

Preparation of Standard curve for Dimethyl Fumarate

100 mg of Dimethyl Fumarate was accurately weighed and transferred to 100 mL volumetric flask. The drug was dissolved in diluents and volume was made up to mark (50 ppm). Stock solution was further diluted to get concentrations of 10 to 70 ppm. Solutions of different concentration were injected and area was calculated. Standard curve was obtained by plotting concentration vs. mean peak area.

Preparation of Teriflunomide Immediate release Blend:

Immediate release blend containing Teriflunomide with other inactive excipients were formulated according to below formula using fluid bed granulation process.

Table 1: Formulation trails for Immediate Release Layer Blend Preparation

Sr. No.	B. No.	T1	T2	T3	T4	T5
	Ingredients	**%w/w**				
Intra granular Part:						
1	Teriflunomide	9.33	9.33	9.33	9.33	9.33
2	Lactose monohydrate	45.67	45.67	45.67	45.67	45.67
3	Maize starch	25.33	25.33	25.33	25.30	25.33
4	Sodium starch glycolate	2.50	2.50	1.50	1.50	1.50
Granulating Agent						
5	Hydroxy propyl cellulose	2.33	1.50	1.50	1.50	1.00
6	Purified water	q.s.	q.s.	q.s.	q.s.	q.s.
Extra granular Part						
7	Microcrystalline Cellulose	9.00	9.80	8.30	7.32	8.80
8	Sodium starch glycolate	5.00	5.00	7.50	9.00	7.50
9	Colloidal Silicon dioxide	0.55	0.55	0.55	0.55	0.55
10	Magnesium stearate	0.33	0.33	0.33	0.33	0.33

Manufacturing process for Immediate release Blend:

Teriflunomide, Lactose monohydrate, Maize starch, Sodium starch glycolate were co-sifted through 40mesh sieve. Co-sifted material was dry mixed in Fluid bed granulator and granulated using hydroxy propyl cellulose dispersed in water. Granulated material was unloaded from FBP and passed through #30 mesh sieve and loaded in an octagonal blender. Extra granular material except magnesium stearate was passed through #30 mesh and mixed for 10 minutes in blender. Magnesium stearate was sifted through #60 mesh sieve and mixed with pre-lubricated blend for 5 minutes in blender.

Preparation Dimethyl Fumarate Delayed release Tablets:

Delayed release tablets containing Dimethyl Fumarate was prepared by direct compression method and enteric

coating polymer Eudragit L_{100-55} as delayed release polymer.

Table 2: Formulation trails for delayed release tablets

Sr. No.	Batch No / Ingredients	D1 %w/w	D2	D3	D4	D5
1	Dimethyl Fumarate	30.0	30.0	30.0	30.0	30.0
2	Silicified Microcrystalline Cellulose	64.50	63.75	62.25	61.75	61.75
3	Croscarmellose sodium	3.75	4.5	6.0	5.25	5.25
4	Colloidal silicon dioxide	0.5	0.5	0.5	0.5	0.5
5	Talc	0.75	0.75	0.75	0.75	0.75
6	Magnesium stearate	0.5	0.5	0.5	0.5	0.5
Coating						
7	Methacrylic acid copolymer Eudragit L100-55	95.9	95.9	95.9	95.9	95.9
8	Triethyl citrate	2.89	2.89	2.89	2.89	2.89
9	Talc	0.85	0.85	0.85	0.85	0.85
10	Polysorbate 80	0.35	0.35	0.35	0.35	0.35
11	Isopropyl Alcohol	q.s.	q.s.	q.s.	q.s.	q.s.
12	Purified Water	q.s.	q.s.	q.s.	q.s.	q.s.
Coating percentage over core		9.0	9.0	10.0	10.50	11.50

Manufacturing process for delayed release tablets:

Dimethyl Fumarate, Microcrystalline cellulose, Croscarmellose sodium, colloidal silicon dioxide and talc were passed through #40mesh sieve. Sifted material was loaded into a blender and mixed for 10 minutes. Magnesium stearate was sifted through #60mesh sieve and mixed with pre-lubricated blend for 5 minutes in blender. Lubricated blend was compressed to tablets. Core tablets were enteric coated using coating solution.

Blend Properties Characterization:

Determination of Angle of Repose:

10 g granules were loaded on funnel with 6mm orifice and allowed to fall at once on the flat surface to form a heap. The height of the heap was measured. The diameter of the heap was measured at three different ends and the average was taken. The angle of repose is defined as,

$$\tan \theta = h/r$$

Where, θ = angle of repose, h = height of the heap and r = radius of the heap.

Bulk Density (BD) and Tapped Density (TD)

The bulk density and tapped density of granules were determined separately by the cylinder method. An accurately weighed 25 g of granules were transferred to 100 mL graduated cylinder. Initial volume and final volume after 500 taps and 750 taps were noted. Calculate the bulk density and tapped density by the following formula.

BD = Mass of the granules (W) / Initial volume of the granules (V_0)

TD = Mass of the granules (W) / Tapped volume of the granules (Vf)

Carr's Index and Hausner's Ratio:

Carr's compressibility index was used to determine compressibility index. Hausner's ratio is a number that is correlated to the flowability of a powder. The formula for Carr's index and Hausner's ratio is mentioned below.

Carr's index (%) = [(TD-BD) X100] / TD

Hausner's ratio = TD / BD

Capsule filling

In capsule filling machine, Lubricated blend was loaded in a hopper and enteric coated tablets in another hopper. Capsules were filled with enteric coated tablet and immediate release lubricated blend.

Characterization of Filled Capsule:

Appearance: Capsules were observed visually for surface characteristics and appearance.

Determination of Uniformity of Weight (USP/NF):

10 capsules were taken and they were weighted together and individually by an analytical balance. The individual variations were studied from the mean weight of each set. The average weight and its related standard deviations were carried out.

Determination of Lock length (USP/NF):

Locking length was measured individually for 10 pre-weighed tablets by using a Vernier Caliper. The average locking length and its related standard deviations were evaluated

In-vitro Drug Release Study: (USP/NF-34)

For Teriflunomide: In-vitro drug release study for Teriflunomide was conducted by using a six station USP type II apparatus (Electrolab Tablet dissolution tester USP). Teriflunomide part of test formulation and its reference product (Aubagio 7 mg, USA) were analysed in 0.05M phosphate buffer as dissolution medium at 37 ± 0.5°C, 1000 mL, apparatus II (paddle) and at a rotation speed of 50 rpm. Samples at 5, 10, 15, 30, 45 and 60 min were analyzed by using HPLC.

For Dimethyl Fumarate: Enteric coated tablets of test formulation and its reference product (TECFIDERA 120mg, USA) were analysed in 0.1N HCl followed by Phosphate buffer $_{P}$H6.8 as dissolution medium at 37 ± 0.5°C, 500 mL, apparatus II (paddle) and at a rotation speed of 100 rpm. Samples at 2 hour in acid and 5, 10, 15, 30, 45 and 60 min in phosphate buffer were analyzed by using HPLC . The amount of drug present in the samples was calculated with the help of appropriate calibration curve constructed from reference standard.

RESULT & DISCUSSION:

Standard Curve of Teriflunomide:

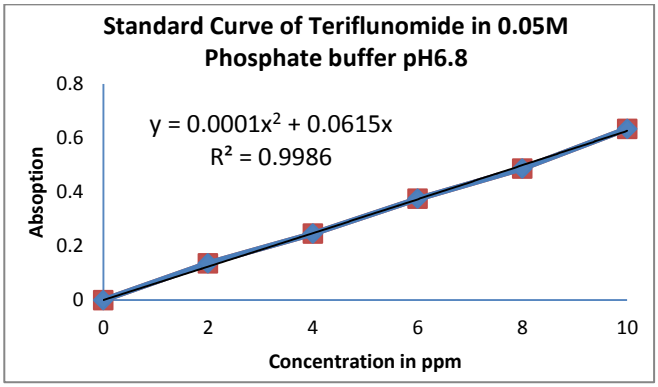

Figure 1: Standard curve of Teriflunomide in 0.05M Phosphate buffer $_{P}$H6.8

Standard Curve of Dimethyl Fumarate:

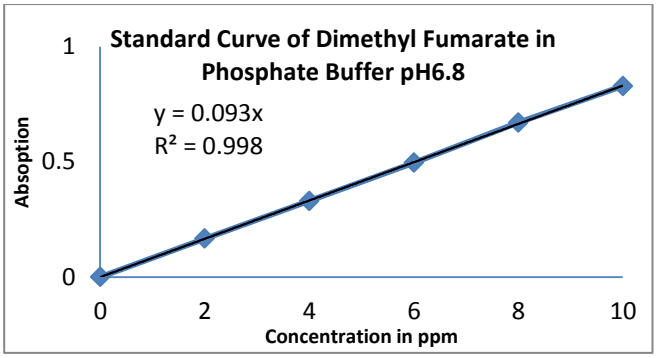

Figure 2: Standard curve of Dimethyl Fumarate in Phosphate Buffer pH6.8

Characterization of Teriflunomide Blend:

Table 3: Blend characterization of Teriflunomide Immediate release Part.

Blend characterization of Teriflunomide Immediate release Part.

Batch No.	T1	T2	T3	T4	T5
Angle of Repose (°)	32	28	29	24	30
Bulk density (g / mL)	0.52	0.5	0.48	0.51	0.55
Tapped density (g / mL)	0.68	0.65	0.66	0.67	0.65
Carr's Index (%)	23.51	23.07	27.27	23.88	15.38
Hausner Ratio	1.31	1.30	1.38	1.31	1.18

Physico-chemical characterization of Enteric coated Dimethyl Fumarate tablets:

Table 4: Physical characterization of Dimethyl Fumarate Tablets.

Formulation	D_1	D_2	D_3	D_4	D_5
Average Weight (mg) (n=10)	112.1 ± 0.016	111.4 ± 0.012	110.3 ± 0.018	112.5 ± 0.010	110.7 ± 0.00
Mean Thickness (mm) (n=10)	3.02 ± 0.010	2.95 ± 0.005	3.05 ± 0.010	2.99 ± 0.008	3.01 ± 0.004
Mean Hardness (Kp) (N=10)	6.8 ± 0.20	7.2 ± 0.22	7.6 ± 0.10	6.9 ± 0.15	7.2 ± 0.19
Friability (% mean weight loss) (n=10)	0.26 ± 0.13	0.21 ± 0.08	0.27 ± 0.09	0.31 ± 0.12	0.30 ± 0.09

Data shown; mean ± RSD (n= number of observations).

Physico-chemical characterization of Filled Capsules:

Table 5: Physical characterization of filled capsules

Formulation	D_1	D_2	D_3	D_4	D_5
Average Weight (mg) (n=20)	1 ± 0.016	101.4 ± 0.012	101.3 ± 0.018	102.5 ± 0.010	100.7 ± 0.00
Mean fill weight (mm) (n=10)	532.6 ± 0.011	529.6 ± 0.015	534.8 ± 0.021	530.2 ± 0.020	533.4 ± 0.018
Mean Lock Length (mm) (n=10)	21.2 ± 0.010	21.2 ± 0.005	21.3 ± 0.008	21.2 ± 0.006	21.4 ± 0.004
Disintegration Time (min)(n=10)	1.25 ± 0.13	1.21 ± 0.08	1.27 ± 0.09	1.31 ± 0.12	1.30 ± 0.09

Data shown; mean ± RSD (n= number of observations).

Release profile of Teriflunomide formulation and its reference product in 0.05M Phosphate Buffer Ph6.8.

Table 6: Dissolution profiles of Teriflunomide from Capsules

Time (min)	Cumulative Amount of Drug Released in mg					
	T_1	T_2	T_3	T_4	T_5	RLD
0	0.0	0.0	0.0	0.0	0.0	0.0
5	32.1 ± 2.08	36.9 ± 2.10	38.0 ± 3.21	38.1 ± 2.58	42.1 ± 3.11	39.6 ± 2.20
10	65.9 ± 1.58	68.5 ± 2.04	69.8 ± 2.66	71.6 ± 0.99	74.2 ± 1.8	73.8 ± 1.16
15	78.9 ± 1.18	82.6 ± 1.65	82.5 ± 1.11	84.2 ± 1.25	86.5 ± 1.02	86.8 ± 1.18
30	88.9 ± 1.75	90.6 ± 1.55	90.2 ± 1.12	91.5 ± 1.48	93.6 ± 1.10	92.0 ± 1.08
45	98.0 ± 1.15	98.9 ± 1.25	99.1 ± 1.09	98.6 ± 1.19	99.9 ± 1.07	98.9 ± 1.05

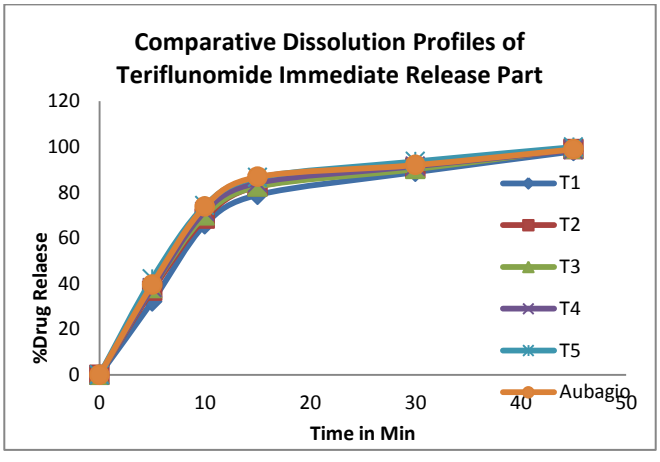

Figure 3: Graphical presentation of Teriflunomide release in Phosphate buffer pH6.8.

Release profile of Dimethyl Fumarate from delayed release tablets and Reference product in 0.01N HCl followed by Phosphate Buffer PH6.8

Table 7: Dissolution profiles of Dimethyl Fumarate

Time (min)	Cumulative amount of drug released in mg					
	0.1 N HCl					
	D_1	D_2	D_3	D_4	D_5	RLD
120	7.0 ± 0.010	7.5 ± 0.015	5.0 ± 0.018	4.0 ± 0.009	2.5 ± 0.016	2.0 ± 0.011
Phosphate Buffer $_P$H6.8						
0	0	0	0	0	0	0
5	28.6 ± 2.18	30.8 ± 2.15	32.2 ± 2.92	32.3 ± 2.21	34.7 ± 2.02	32.1 ± 2.58
10	37.9 ± 1.52	42.0 ± 1.12	43.2 ± 1.18	45.8 ± 1.04	47.5 ± 1.12	48.3 ± 1.09
15	63.1.0 ± 1.32	66.1 ± 0.99	67.5 ± 1.25	68.0 ± 1.13	69.0 ± 1.02	68.6 ± 1.25
30	75.9 ± 1.08	77.2 ± 1.14	78.0 ± 1.13	80.1 ± 1.11	80.9 ± 1.13	80.2 ± 1.19
45	84.1 ± 1.52	86.0 ± 1.01	88.1 ± 0.99	87.0 ± 1.03	87.0 ± 1.02	86.8 ± 0.88

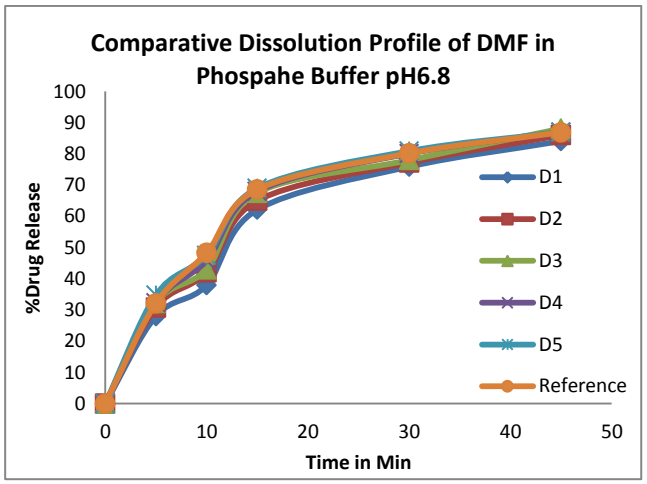

Figure 4: Graphical presentation of Dimethyl Fumarate release in phosphate Buffer PH 6.8.

CONCLUSION: Result from current experiment concluded that capsule manufactured of immediate release blend of formulation trial T_5 and delayed release of formulation D_5 were found optimized formula where the physiochemical properties of filled capsules are comparable to marketed sample. With increasing concentration of Sodium starch Glycolate, dissolution of Teriflunomide increased resulting faster systemic availability. Release of Dimethyl Fumarate from delayed release tablets depends on the concentration of Methacrylic acid co-polymer. Considering in-vitro studies, the final test formulation was similar and the dose of single dose can be reduced considering synergistic action.

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