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FORMULATION DEVELOPMENT AND EVALUATION OF FAST DISINTEGRANTS SUBLINGUAL TABLETS CONTAINING METHYLCOBALAMIN AND CHOLECALCIFEROL FOR DIABETIC NEUROPATHY

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ABSTRACT

The demand of fast disintegrating sublingual tablets has been growing during the last decade especially for geriatric and paediatrics patients because of swallowing difficulties and its characteristics for the potential emergency treatment. The aim of this study was to prepare fast disintegrating tablets of Methylcobalamin and Cholecalciferol by using different disintegrant in different concentration and to evaluate the effect of increase Methylcobalamin and Cholecalciferol load on the characteristic of fast disintegrating sublingual tablets for the treatment of diabetic neuropathy. The sublingual tablets were prepared by direct compression method using various concentration of croscarmellose sodium, sodium starch glycolate and crosprovidone, drug compatibility was confirmed by using FTIR and showed no drug–exicipient interaction, Six formulation (R1-R6) of sublingual tablets were prepared by using the various concentration of fast disintegrants were evaluated for thickness, hardness, Weight variation, friability, wetting time, water absorption ratio, disintegration time and drug content. The optimized formulation F6 with 5% croscarmellose sodium was found to be suitable which provided short wetting time 25.7sec, Water absorption ratio 21.28sec and disintegration time of 21 sec which is within pharmaceutical limits.

KEYWORDS: Methylcobalamin, Cholecalciferol, Fast disintegration, Sublingual tablets, croscarmellose sodium, *In-vitro* disintegration.

1.0 INTRODUCTION

According to 2014 statistics, diabetes affects 3.2 million people in the UK, Which equates to 60% of the population (Diabetes UK, 2015). Diabetic neuropathy is a common and type1 in type2 diabetes. Worldwide high blood glucose kills about 3.4 million people annually. Almost 80% of these deaths occur in low and middle income counties and almost half are people aged less than 70 years WHO project diabetes deaths will double between 2005 to 2030.[1] According to the International Diabetes Federation, 382 million people worldwide are currently affected by diabetes one of the leading causes of neuropathy. The distal symmetrical polyneuropathy (DSPN) is the commonest clinical form of diabetic neuropathy, affecting more than 90% of the patients.[2] There are about 60 million people with diabetes in the European region (Or) about 10.3% of men and 9.6% of women age 25 year and over. Prevalence of diabetes is increasing among all age in the European region, mostly due to increase in overweight and obesity, unhealthy diet and physical inactivity Diabetes mellitus is predicted to affects 220 million people worldwide by the year 2010 and approximately 30-60% of attain with diabetes develop long term of peripheral neuropathy and upto 10-20% of these patient experience pain.

Diabetic neuropathy is a condition that damage nerve in the body. High blood sugar level affect the nerve use glucose leading to an accumulation of sugar sorbitol as depletion of substance called Myoinositol with nerve, contributing to nerve damage. It shows the relationship between diabetes and peripheral nerve damage. The causes are probably different for different types of diabetic neuropathy. Researchers are studying how prolonged exposure to high blood glucose causes nerve damage. Diabetes gives rise to long term complication in legs, feet, blood vessels, and nerves. These result in major causes of morbidity and death from diabetes.[3]

Methylcobalamin Chemical Coα- [α- (5,6-Dimethyl-1H-benzoimidazol-1-yl)]-Coβ-methylcobamide The Methylcobalamin from of vitamin B12 is very important in the prevention of neurological disorder, as it prevents nerve damage by maintaining myelin, the fatty sheaths that cover and protect nerve endings, it is the Methylcobalamin form that is needed to protect against

central and peripheral neurological diseases. High amounts of methylcobalamin are needed to regenerate neurons and myelin sheath that protects nerve axons and peripheral nerves. Neuropathy caused by vitamin B-12 deficiency affects the balance of the patient as well as the symptoms of tingling, numbness, and weakness in the lower extremities.[4]

Cholecalciferol Chemical (3β, 5Z, 7E) 9,10 Secocholesta 5,7,10(19)-trien3ol. Vitamin D appears to play an important role in preventing nerve damage and maintaining healthy pain receptors. In addition, it is believed that adequate vitamin D levels result in improved nerve repair and growth. Vitamin D is a fat-soluble vitamin that regulates calcium metabolism and is necessary for the calcification of bone and teeth, in addition to its role in calcium absorption, vitamin D induces the uptake of phosphate and magnesium from the intestines. When a peripheral nerve is cut, surgery to repair the damag**e** is sometimes unsuccessful and the nerve fails to recover.[5]

2.0 MATERIALS AND METHODS

2.1 Materials

Methylcobalamin (99.96% purity) was obtained from Micro-orgo chem., Worli, Mumbai. Cholecalciferol (99.96% purity) was procured from Fermeta Bio-Tech Ltd, Maharastra, India. Pearlitol flash, Sucralose were procured from Unitec Sweet Zibo, Shandong, China. Colloidal silicon dioxide was procured from Fisher Scientific, Chennai, India. Iron oxide Red was procured from Nellicon Food Dyes & Chemical, Mumbai, India. Propylene glycol, Isopropyl alcohol were obtained from Manali Petro Chemical,Chennai, India . Butylated hydroxyl toluene was procured from Thermo Fisher Scientific India, USA. Croscarmellose Sodium was obtained from Prachin Chemical, Gujarat, India. Mixed fruit flavour Firmeiche Chennai, India. Talc was obtained from Intermed Pharmaceuticals, Mumbai, India. Magnesium strearate was procured from Legend Industries, Gujarat, India. All others reagents and chemicals used were of analytical reagent grade.

2.2 Methods

2.2.2 Preformulation studies

A preformulation study is the first step in the rational development of dosage forms of a drug substance. It can be defined as phase of research and development process of physical and chemical properties of a new drug substance alone and when provide a rational for formulation design, or support the need for molecular combined with excipients, in order to develop stable, safe and effective dosage form. The overall objective of preformulation studies is to generate information useful to the formulator in developing stable and bioavailable dosage forms that can be mass produced.

A thorough understanding of physicochemical properties may ultimately modification or merely confirms that there are no significant barriers to the compounds development. The goals of the preformulation study are

- To establish the necessary physicochemical characteristics of a new drug substance.
- To determine its kinetic release rate profile.
- To establish its compatibility with different excipients.[6]

Compatibility study: The drug and the excipients chosen for the formulation were screened for compatibility by physical methods and Fourier Transform infrared spectrometric method (FTIR).

Physical Compatibility study

The physical compatibility studies were conducted to provide valuable information to the formulator in selecting the appropriate excipients for the formulation. It was done by mixing the drugs and the excipients in the ratio of 1:1 and stored in air tight containers at room temperature and at 40^0C and 75%RH. Any change in color of the physical mixture was observed visually.[7]

Fourier transform infrared spectrometry (FT-IR)

Infrared spectroscopy can be used to identify a compound and also to investigate the composition of the mixture. Pure drugs, polymers, excipients, drug excipient mixture was subjected to FTIR studies using Shimadzu FT-IR spectrometer model to investigate the Drug-excipient interactions. The IR spectra of the test samples were obtained by pressed pellet technique using potassium bromide and the ratio of sample is 1:100.[7]

2.2.3 Preparation of blends of Methylcobalamin and cholecalciferol

Step 1: Weighing and sifting

Weigh all the ingredients accurately and passed through 60 # sieve.

Step 2: Dry mixing

Methylcobalamin, cholecalciferol, Colloidal silicon dioxide, Pearlitol flash, Iron oxide red.

Step 3: Preparation of binder solution

Take weighed quantity of Propylene glycol, Butylated Hydroxy toluene and dissolve slowly with constant stirring in quantity sufficient of I.P.A.

Step 4: Granulation

Now granulate the dry mix of step 2 with binder solution with hard binding so that the granules are properly formed.

Step 5: Drying

Now dry the granules in the oven not more than 45°C to 50°C.

Step 6: Sifting and milling

Now after drying check the L.O.D. of the granules which should not be less than 1.65, and sift the granules

through 30# sieve. And mill the oversize granules using 1.0 mm screen to get the granules of desired size.

Step 7: Blending
After sifting blend the granules with extra granular ingredients Pearlitol flash, Sucralose, Croscarmellose sodium, Mixed fruit flavour Colloidal silicon dioxide and Talc for 10 minutes.

Step 8: Lubrication
Now finally lubricate the blend with magnesium stearate for 2 minutes.

Step 9: Compression
Compress the blend using 6mm standard round concave punches.

Table1: Composition Sublingual granules

S.NO	INGREDIENTS	R1	R2	R3	R4	R5	R6
				(mg/T)			
1	Methylcobalamin	2.00	2.00	2.00	2.00	2.00	2.00
2	Cholecalciferol	0.10	0.10	0.10	0.10	0.10	0.10
3	Pearlitol Flash	68.394	68.394	68.394	68.394	68.394	68.394
4	Colloidal Silicon Dioxide	4.00	4.00	4.00	4.00	4.00	4.00
5	Iron Oxide Red	0.50	0.50	0.50	0.50	0.50	0.50
6	Propylene Glycol	0.005	0.005	0.005	0.005	0.005	0.005
7	Butylated Hydroxy Toluene	0.001	0.001	0.001	0.001	0.001	0.001
8	Isopropyl Alcohol	0.025	0.025	0.025	0.025	0.025	0.025
9	Pearlitol Flash	17.50	17.50	17.50	16.50	15.50	14.50
10	Sucralose	0.50	0.50	0.50	0.50	0.50	0.50
11	Sodium Starch Glycolate	3.00	-------	-------	-------	-------	-------
12	Croscarmellose Sodium	--------	3.00	-------	4.00	5.00	6.00
13	Crospovidone	--------	--------	3.00	------	------	------
14	Mixed Fruit Flavour	0.50	0.50	0.50	0.50	0.50	0.50
15	Colloidal Silicon Dioxide	1.00	1.00	1.00	1.00	1.00	1.00
16	Purified Talc	1.00	1.00	1.00	1.00	1.00	1.00
17	Magnesium Stearate	1.50	1.50	1.50	1.50	1.50	1.50
	Total weight	100mg	100mg	100mg	100mg	100mg	100mg

2.2.4 Characterization of granules
Prior to compression, blends were evaluated for their characteristic parameters like density, bulk density, tapped density, compressibility index and Hausner's Ratio. Carr's index was calculated from the bulk and tapped densities using a digital tap density apparatus (Electrolab Ltd, India).

2.2.5 Preparation of sublingual tablets
The tablet was compressed as sublingual tablet using both Methylcobalamin and Cholecalciferol blend; it was selected from its best in characterization. Sublingual tablets were compressed by using 21 X 7.4 mm D tooling oblong shape punch in 21 station tablet compression machine (Cadmach, India).

2.2.6 Physico -chemical properties of sublingual tablets
The prepared tablets were subjected to various evaluation tests like thickness, hardness, weight variation, friability, and drug content. Thickness of the tablets was determined by using Vernier calliper. Randomly 10 tablets were selected and used for determination of thickness. It is expressed in mm. Hardness is termed as the tablet crushing strength and it is the force required to break a tablet diametrically. Hardness of tablets was measured by selecting 6 tablets randomly and the hardness of each tablet was determined using digital hardness tester (Elchem, Mumbai). The hardness was noted. The hardness is usually measured in terms of kg/cm^2. The tablet friability is a measure of loss due to abrasion. The pre weighed tablets were exposed to repeat shocks in friabilator (Esico, India) in which they are initially weighed ($W0$) and kept in a tumbling and rotating apparatus drum and were subjected to fall from 6 inches' height. After completion of 100 rotations, the tablets were reweighed (Wf) and the percent loss in weight or friability (f) was calculated by the formula given below

$$\% \text{ Friability} = \frac{\text{Initial weight of the tablets } (W0) - \text{Final weight of the tablets } (Wf)}{\text{Final weight of the tablets } (Wf)} X100$$

For weight variation test individual weight of 20 tablets was taken; then their average weight and their mean and standard deviation were calculated and compared with the standards. The weight of the tablet being made is measured to ensure that it contains predetermined amount of drug. The percentage deviation of tablets was calculated and compared with the USP official limits given below.[8]

USP limits for weight variation

Average weight of a tablet	Limits
130 mg or less	±10%
>130 mg and <324 mg	±7.5%
more than 324	±5%

2.2.7 Drug content

Methylcobalamin: Twenty tablets are selected randomly and grounded. The equivalent of 30mg of Methylcobalamin was weighed, 250 ml volumetric flask, add 50 ml water and shake well for 10 minutes. Make up the volume with water and mix. The absorbance of the resulting solution was measured at 522 nm taking water as blank using UV- visible Spectrophotometer. The concentration was obtained from the calibration graph.[9]

Cholecalciferol: Twenty tablets are selected randomly and grounded. The equivalent of 20 mg of Cholecalciferol was weighed.A100 ml volumetric flask, dissolved in 20 ml of Acetonitrile and made upto volume with mobile phase. The solution was filter and 5ml of the filter was diluted with 100ml of mobile phase. From the above solution 5 ml of the filtrate was again diluted with 100 ml of mobile phase solution. The absorbance of the resulting solution was measured at HPLC as blank using in mobile phase.[10]

Chromatographic conditions

Apparatus: High Performance Liquid Chromatography
Column: 250 mm x 4.6 mm, 5µm, C18,
Flow Rate: 1.0 ml/min
Temp: 250º C
Injected Volume: 20 µl
Detector: HPLC 254nm
Retention time: Cholecalciferol

3.0 RESULTS AND DISCUSSION

Methylcobalamin therapy improved peripheral symptoms. Effects on vibration perception and electrophysiological measures were not consistent. With both the vitamin B12 combination and pure methylcobalamin, symptomatic relief was greater than changes in electrophysiological results. However, more high-quality, double-blind randomized controlled trials are needed to confirm the effects of vitamin B12 on diabetic neuropathy. Vitamin B12 replacement has been shown to cause symptomatic improvement among patients with severe diabetic neuropathy.[11]

Vitamin D receptors exist in the central and peripheral nervous system. Vitamin D has been found recently to play a role in nerve growth and maintenance as it is a potent inducer of neurotrophins and neurotransmitters.[12] The most important and studied Neurotransmitter is NGF, which is a neurotrophic protein required for the development and survival of sympathetic and sensory neurones in the periphery and cholinergic neurones in the central nervous system (CNS).[13] Low vitamin D levels appear to be related to neuropathy and other neurodegenerative disorders.

The results of both physical and chemical compatibility studies shown there is no interaction between the drug and exicipent which indicates that both drug and excipients were compatible with each other and stable at different temperature and humidity.

Estimation of Pre and post compression parameters in tablet is most important and the results were given Table 2 and Table 3, 4**.** Evaluation of these properties will results the characteristics of powder ingredients before punch as a tablet and also reflect their withstanding capacity during transportation, content uniformity and drug release. The obtained result from pre-compression evaluations shown that the prepared powders were having well in their flow properties and within the accepted limits with low standard deviation and the result of post compression studies such as content uniformity, hardness, friability and weight variation shown within the official limits. It indicates all the prepared tablets were well in their physico-chemical properties.

Table 2: Pre compression study on drug and formulated blends

Formulation	Bulk Density g/cm^3	Tapped Density g/cm^3	Compressibility index (%)	Hausner's Ratio	Angle Of Repose (Degree)
R1	0.52	0.7042	26.15	1.3542	43º53"
R2	0.54	0.7366	26.07	1.3528	38º37"
R3	0.55	0.7221	23.00	1.2987	31º21"
R4	0.55	0.7118	25.25	1.3379	30º20"
R5	0.53	0.6578	23.98	1.3156	26º75"
R6	0.54	0.7142	24.39	1.3225	26º50"

Table3: Post compression parameters on Sublingual tablets, Mean± S.D, n=5

Formulation	Thickness (mm)	Hardness(Kg/cm)^2	%Friability	Wetting Time (sec)	Water absorption ratio	Weight ariation (mg)
R1	3.0 ± 00	3.2± 04	0.51± 0.09	52.0±1.0	30.42±0.28	99.8
R2	3.1 ± 01	3.0± 04	0.49± 0.05	44.3±1.5	28.54±0.32	100.95
R3	3.2 ±.02	3.0± 02	0.50± 0.03	27.7±1.5	26.27±0.37	103.8
R4	3.0 ± 00	28.1 ± 04	0.53± 0.05	38.3±0.6	22.44±0.40	102.35
R5	3.0 ± 00	3.0± 00	0.51± 00.5	25.7±2.1	21.28±0.42	100.35
R6	3.1 ± 01	3.2± 01	0.55± 0.03	22.0±1.0	22.20±0.20	101.9

Table4: Post compression parameters of sublingual tablets, Drug content test

Formulation	Methylcobalamin (mg) (UV)	Cholecalciferol (IU)(HPLC)
R1	129.96	189
R2	131.18	190
R3	130.62	188
R4	130.44	190
R5	131.50	191
R6	131.32	191

The results of disintegration study on sublingual tablet shown in the Table 5 and Fig.1. It varied according to the superdisintegrants used. Formulation R5 consist of croscarmellose sodium was considered as best due to its fast release such as 21seconds compared 5 other two formulations such as 65, 45sec for sodium starch glycolate and crospovidone.

Table *5: In vitro* disintegration of sublingual tablets

Formulation	Disintegration time (sec)
R1	65±1.00
R2	43±1.5
R3	45±2.00
R4	24±2.00
R5	21±1.52
R6	19±1.73

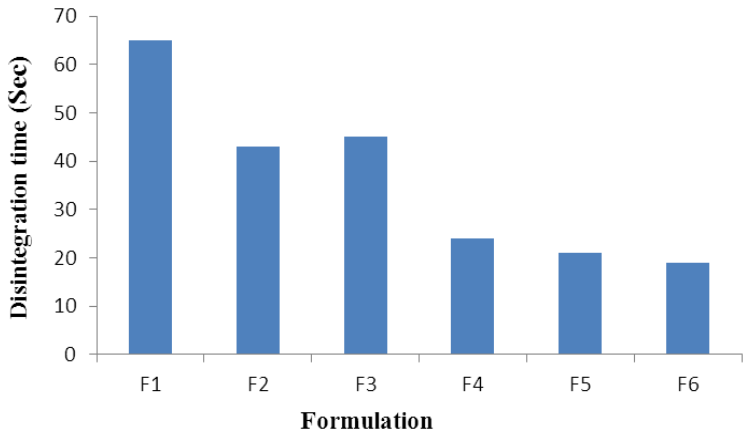

Fig.1 *In-vitro* disintegration of sublingual tablet

The sublingual fast disintegrate tablets of Methylcobalamin and Cholecalciferol were formulation using croscarmellose. It was observed that the therapeutic action of Methylcobalamin and Cholecalciferol were sublingual fast disintegration for short disintegrates time by using 5% Croscarmellose sodium in optimized batch.

The main problem observed during the first four formulations was unevenness and long fast disintegration in the reading when analysed by the disintegrated method. The next two batches were analysed using by disintegrated method. The satisfactory result was observed with the short fast disintegration.Thus, the optimized were formulated by using 5% croscarmellose sodium.

CONCLUSION

An optimized formulation of Methylcobalamin and Cholecalciferol sublingual tablets was found and prepared in this study by direct compression method. The best *in –vitro drug* disintegration formulation F5 was found to be 21±1.52 which contain the drug Methylcobalamin and cholecalciferol andcroscarmellose sodium as fast disintegratrant agent with other exicipients. The formulation F5 was found to be best among all other formulations because it has exhibited good wetting time, water absorption ratio, faster disintegration time and *in-vitro* dispersion time when compared to other formulations.

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