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Original Article

METHOTREXATE FAST DISINTEGRATING TABLET AS A DOSAGE FORM FOR DYSPHAGIA PATIENTS

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ABSTRACT

Objective: The objective of the present study was to develop, optimize and evaluate fast disintegrating tablet (FDT) of methotrexate (MTX) as a dosage form for dysphagia patients with a view to enhance patient compliance and safety.

Methods: FDT was prepared by direct compression technique and effect of incorporating major excipients such as Croscarmellose Sodium (CCS), Crospovidone (CP), Sodium Starch Glycolate (SSG), neusilin and magnesium stearate on various parameters were evaluated. The blends prepared were evaluated for pre and post compression evaluations. The optimized FDT was assessed for various studies such as FT-IR, Differential Scanning Calorimeter (DSC), X-Ray Diffraction (XRD), *in vitro* disintegration, dissolution, *in vivo* pharmacokinetic and stability studies.

Results: The optimized blend mixture had good flow and compressible property; hence tablets produced were of uniform weight with acceptable weight variation. The drug content assessed by HPLC was within limits indicating drug had uniformly distributed in the tablets. Tablet produced can withstand abrasion during handling, packaging and shipment as tablet had optimum hardness and least friability. There was no drug-excipient incompatibility as confirmed by FT-IR and DSC studies. Change in crystalline nature of the drug after tableting was confirmed by XRD studies. FDT prepared using CP showed quick disintegration with excellent dissolution rate profile and exhibited satisfactory stability at normal temperature. *In vivo* studies of FDTs (test) compared with drug solution (control) showed no statistically significant difference in pharmacokinetic parameters.

Conclusion: The study indicated that MTX FDT disintegrates rapidly in seconds and hence is a promising dosage form for dysphagia patients.

Keywords: Fast disintegrating tablets, Dysphagia, Head and neck cancer, Methotrexate, Geriatric patients.

INTRODUCTION

Amongst the various routes of drug delivery, oral route is perhaps the most preferred and acceptable from patient compliance aspects. However, certain conventional oral dosage forms such as tablets, capsules and liquids are found to be difficult to swallow in case of dysphagic patients and hence tend to withdraw the medication which leads to improper cure of the disease.

Dysphagia, derived from the Greek *phagein*, meaning "to eat". Dysphagia is a clinical syndrome resulting from a biomechanical disorder which is defined as “an inability to swallow, or a sensation that solids or liquids do not pass easily from the mouth to the stomach”. Dysphagia can occur at any age but are most prevalent in elderly individuals and is a growing healthcare concern as geriatric population expands. An estimated 35% of the general population, and an additional 30 – 40% of elderly institutionalized patients and 18 - 22% of all persons in long-term care facilities, suffer from dysphagia [1]. It causes significant morbidity and even mortality. It is a common problem that lowers quality of life for the elderly patients. Dysphagia causes potentially compromising nutritional status, complicating the administration of solid medications [2].

To ensure safety during oral administration, patients with dysphagia require an appropriate oral dosage form or modification of the dosage form. Crushing tablets and opening capsules are the main alterations of dosage forms and account for up to one third of oral drug administrations in long-term nursing homes. However, crushing of enteric-coated or sustained release tablets can lead to adverse events (*e. g*., choking episodes, adverse drug reactions resulting from the immediate release of drug from a sustained-release product, refusal to take medications) [3]. Drugs with teratogenic, carcinogenic or cytotoxic properties such as antineoplastics should not be crushed or opened due to its harmful effects.

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death [4]. Higher incidence of non-communicable diseases, especially cancer is associated with percentage of aged population of a country [5]. It is predicted that the elderly population of India shall be among the highest in the world by the year 2025, i. e. 177 million (80 % of them residing in rural areas) [6]. Dysphagia is common in patients with advanced HNC [1]. The incidence of head and neck cancer increases with age, especially after 50 years of age [7]. Over 200,000 cases of head and neck cancer occur each year in India verses 30,000 for the US [8]. Methotrexate (formerly Amethopterin) is an antimetabolite used in the treatment of many neoplastic diseases, one such is HNC. MTX remains the standard of therapy for patients with recurrent or metastatic disease. It has been given in the dosage range of 25 – 50 mg/m^2 [9-10]. MTX is available in the market as 2.5, 5, 10 and 15 mg tablets strengths. Due to increased frequency of administration to achieve the desired dose required to treat HNC, the conventional tablet may be difficult to swallow by dysphagic patients which results in withdrawal of the medication. Hence the objective of the present research was to develop fast disintegrating tablet of MTX that can be easily taken by patients with dysphagia thereby improving patient compliance.

Fast disintegrating tablets are also called as orodispersible tablets, quick-disintegrating tablets, mouth-dissolving tablets, orally disintegrating tablets, fast dissolving tablets, rapid-dissolving tablets (mentioned as ‘tablets’ which is nothing but FDT in our article). The United States Food and Drug Administration define FDT as ‘a solid dosage form containing medicinal substance or active ingredient which disintegrates rapidly usually within a matter of seconds when placed upon the tongue’. The disintegration time for FDTs generally ranges from several seconds to about a minute [11].

These tablets display a fast and spontaneous de-aggregation in the mouth, soon after the contact with saliva, though they can be handled or extracted from the package without alteration. The active agent can thus rapidly dissolve in the saliva and be absorbed through membrane it encounters, during deglutition, unless it is protected from pre-gastric absorption [12]. The present investigation was focused on screening of formulation variables

such as superdisintegrants (CCS, CP and SSG) and other excipients (neusilin and magnesium stearate) to obtain MTX FDT which possess quick disintegration time usually in matter of seconds and satisfying all other evaluation parameters.

MATERIALS AND METHODS

Materials

MTX was obtained procured from Sigma Aldrich (Bangalore, India). CP (Polyplasdone XL-10) and Spray Dried Mannitol (SD mannitol; Pearlitol SD 200) were obtained as a gift sample by Micro Labs (Bangalore, India), Mannitol GR was procured from Lobachemie Pvt. Ltd. (Mumbai, India), SSG and CCS was gifted by Malpe biotech Pvt. Ltd. (Pune, India). Neusilin US2 was gifted by Fuji Chemical Industry (Toyama, Japan). Sucralose was kindly gifted from J. K. Sucralose India Ltd. (Delhi, India); Strawberry flavor was procured from SK Florescences Pvt. Ltd. (Delhi, India), Magnesium stearate was purchased from SD Fine Chemicals (Mumbai, India). Acetonitrile (Merck Ltd., Mumbai, India), Methanol (Merck Ltd., Mumbai, India) and water used were of High-Performance Liquid Chromatography (HPLC) grade. All other chemicals and reagents used were of analytical grade. The integrated HPLC system (SHIMADZU LC-2010A HT, Kyoto, Japan) equipped with low pressure quaternary gradient pump along with dual wavelength UV detector (SPD-20A) and auto sampler was used for the analysis.

Method

Fast disintegrating tablets containing MTH varying concentrations of superdisintegrants and other excipients were prepared by direct compression technique. All the ingredients (excipients and drug) were properly weighed and sieved through a 40-mesh screen, except for magnesium stearate which was sieved through a 60-mesh screen. The blend was prepared by mixing the ingredients (except magnesium stearate) manually for 10 min by tumbling action in a poly-ethylene bag of suitable size. Finally, magnesium stearate was added to this blend and mixing was continued for 2 min to obtain homogenous powder mixture.

The tablets were produced by compressing the powder mixture on a ten-station rotary press (Rimek, Ahmedabad) using round convex 8 mm punches at an average hardness of 3.6 kg/cm^2. The composition of optimized FDT formulation is shown in Table 1.

Table 1: Composition of optimized methotrexate FDTs

Ingredients	F1	F2
Drug	45	90
Crospovidone	12 (4 %)	17.5 (5 %)
Neusilin (10 %)	30	35
Sucralose	5.7	5.7
Strawberry flavor	12	12
Magnesium stearate (3 %)	9	10.5
Spray dried mannitol	Make up to 300 mg	Make up to 350 mg

Evaluation of tablets

Fourier Transformer Infra Red (FT – IR) Spectroscopy

FT-IR spectroscopic studies were carried out to assess any possible drug excipient interaction. Drug:excipient mixture was mixed with Potassium bromide in the ratio of 1:100, triturated and compressed to prepare the pellet. Twenty spectral scans were acquired in the 4000 – 400 cm^{-1} range with a resolution of 4 cm^{-1} using FT-IR spectrophotometer (Shimadzu, 8400S, Japan). FT-IR spectrum of physical mixture of drug:excipient was compared with that of plain drug [13].

Methotrexate analytical method

A rapid and simple HPLC method was used for the quantification of MTX [14]. Analysis was performed on a phenomenex column luna 5µ C18 (2) 100A, (250 X 4.60 mm i. d., 5 µm particle size) at a flow rate of 1.2 ml/min with a UV detection wavelength of 302 nm. The mobile phase consisted of Acetonitrile: Solution 'A' [1:9]. Solution 'A' was prepared using 0.2 M dibasic sodium phosphate and 0.1 M citric acid (63:37), adjusted if necessary with 0.1 M citric acid or 0.2 M dibasic sodium phosphate to a pH of 6.0. The chromatographic data was processed using LC solution version 1.25 software. The results were expressed as mean of six determinations.

Preparation of stock and standard solutions

Primary stock solution for generating standard curves for MTX was prepared by dissolving the drug in mobile phase to yield concentration of 1 mg/ml. Calibration standard solutions were prepared from primary stock solution by serial dilution with mobile phase to yield final concentration of 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 µg/mL. Standard solutions were injected and analyzed with a run time of 12 min. A calibration curve was constructed by plotting the peak area on ordinate as a function of MTX concentration on abscissa. A good linear relationship was observed between the concentrations of 0.025 – 12.8 µg/mL. The method was validated as per International Conference on Harmonization (ICH) for the parameter precision and accuracy to assess reliability of the method. The overall precision of the method was expressed as % CV [100(SD/M)] and accuracy of the method expressed as percent to true value [100(M/T)], where M is the mean and SD is the standard deviation of M.

Pre and post compression evaluations

The precompression properties such as bulk and tapped density (Electrolab Density Tester ETD – 1020), true density, angle of repose, Carr's index, hausner ratio, porosity and post compression properties like hardness (Pfizer Digital Hardness tester), friability (Electrolab EF - 2 Friabilator), weight variation and content of the compressed tablets were determined as per standard procedures [15]. The results were reported as average of six determinations.

Wetting time and water absorption ratio

A piece of tissue paper (12×10.75 cm) folded twice was placed in a Petri dish (internal diameter of 9 cm) containing 10 mL of buffer solution (pH 6.8) simulated saliva and amaranth (dye). The dye solution was used to enable suitable visual end-point detection. A tablet was carefully placed on the surface of the tissue paper with the help of forceps and the time required for the dye to reach the upper surface of the tablet was recorded as wetting time. The wetted tablet was then weighed. Water absorption ratio 'R' was calculated using the equation:

$$R=100 \times (W_b-W_a)/W_a$$

Where, W_a is weight of tablet before water absorption and W_b is weight of tablet after water absorption [16-17].

In vitro disintegration time

In vitro disintegration time of the tablets was evaluated using 2 different methods [18].

A. Conventional disintegration apparatus-basket rack assembly (as mentioned in Indian pharmacopeia) was used to check the DT of the tablets. Distilled water was used as disintegrating medium. The basket-rack assembly is rigid and supports six cylindrical glass tubes. The volume of medium was 900 mL maintained at 37 ± 0.5 °C.

B. Disintegration test was conducted by placing the fast dissolving tablet in a glass cylinder fitted with 10 mesh at its base. This set up was further placed in a shaking water bath operated at 150 rpm. 1 mL of purified water maintained at 37 °C temperature was used as medium. The critical parameters of this method were the operational speed of shaking water bath and volume of the medium.

Thermogravimetric analysis (TGA) and DSC studies

TGA and DSC measurements were performed using a SDT Q600 (V20.9 Build 20). Samples (~2 mg) were placed in an aluminum crucible cell which was firmly crimped around the lid to provide an adequate seal. The analysis was done under purge of dry nitrogen gas at a flow rate of 25 ml/min. The DSC of the FDT (A) and pure drug (B) was performed by heating it from ambient temperature to 300 °C with heating rate of 10 °C/min whereas TGA was carried out for final tablet by heating it from ambient temperature to 1000 °C.

X-Ray Diffraction studies (XRD)

X - ray diffraction (XRD) analysis of tablet, in comparison with pure drug and placebo was performed using Rigaku Miniflex II desktop X-Ray diffractometer (Japan) using a monochromator addition that captures X-rays other than Cu Kα for use in analysis. The samples were scanned over a 2θ range of 10° to 60° at a scan speed of 10°/min and a step size of 0.01°.

***In vitro* dissolution studies**

Dissolution studies of the optimized tablet (F1 and F2) and plain drug (equivalent dose) were performed using USP dissolution paddle apparatus (Electrolab TDT 08L, Mumbai, India). The dissolution studies were carried out with a stirring speed of 50 RPM at 37 ± 0.5 ºC using three different buffer systems (pH 1.2, pH 4.5 acetate buffer and pH 6.8 phosphate buffer) of 900 mL. Two milliliters aliquots of dissolution media were collected at predetermined time intervals and replaced with equal volumes of respective buffer. The collected samples were filtered through 0.22 µm millipore filter and the concentration of the dissolved MTX was determined using the HPLC technique. The results were the average of six determinations. Dissolution profile of tablet formulation was compared with that of the plain drug. The data obtained were statistically analyzed using one way analysis of variance ($p < 0.05$).

***In vivo* evaluation**

Study design

The study was conducted in accordance with the principles of laboratory animal care and was approved by institutional ethics committee of the JSS College of Pharmacy, JSS University, Mysore (076/2011). Male wistar rats (weighed 240 – 260 g) used in the present experiment were housed in a room maintained on a 12 h light/dark cycle at 23 ± 2 °C with free access to food and water. The rats were anaesthetized with an intraperitoneal injection of urethane, 1 g kg^{-1}, and the jugular vein was cannulated to facilitate removal of blood sample. The rats were divided into two groups namely control and test, with twelve animals in each group. Initially, six rats in test group received FDT formulated specially for animals by converting human dose of 25 mg/m^2 to animal dose (3.92 mg/kg) whereas the standard group received MTX solution (equivalent dose as FDT for 6 animals). For the rest 6 animals in test and standard group received FDT and solution same as above with a conversion of human dose of 50 mg/m^2 to animal dose (7.842 mg/kg).

For tablet application, 50 µl aliquot of distilled water was dropped into the rat oral cavity under light ether anesthesia and then FDT tablet preparation was placed on the tongue. After ensuring disintegration of the tablet, anesthesia was discontinued. It took up to 3 – 10 s for the tablet to disintegrate completely, thus the duration of anesthesia was adjusted to 20 s. For oral administration of MTX solution (control), rats were orally using a stomach sonde needle under light ether anesthesia and the anesthesia continued for 20 s [19]. Blood samples (0.25 mL) were obtained immediately before drug administration and at pre determined intervals after dosing from jugular vein. Blood samples were collected in heparinized tubes and were centrifuged (Remi Equipments Ltd., Mumbai, India) for 5 min at 10 000 rpm at - 4 °C. Separated plasma was stored at – 50 °C until further analysis.

Bioanalysis of MTX in rat plasma

Simple and reliable bio-analytical method used to determine MTX concentration in plasma samples was carried out as given by Wang et al. [20]. MTX primary stock solution (1 mg /mL) was prepared by dissolving drug substance in 0.1 N sodium hydroxide solution. From the primary stock solution, working standard solutions were prepared in the concentration range of 0.25 - 128 µg/mL. Drug free plasma specimens were brought to room temperature prior to analysis. Briefly, 100 µl of the plasma was pipetted into series of micro-centrifuge tubes and spiked with 100 µl of different working standard solutions. The volume was made up to 2 mL with protein precipitating reagent (10 % perchloric acid; v/v) to get final concentrations of 0.0125 – 6.4 µg/mL of MTX in rat plasma. The tubes were briefly vortex-mixed for 5 min then centrifuged at 10000 rpm for 8 min. The clear supernatant liquid was directly injected into a C_{18} (2) column (phenomenex luna, 250 X 4.60 mm i. d. 5 µm particle size) to determine standard concentration of drug in the plasma. The mobile phase was composed of a mixture of 50 mM ammonium acetate buffer (pH 6.0) and methanol (77:23, v/v) with a flow rate of 1.0 ml/min. The ultraviolet absorbance of the effluent was monitored at a wavelength of 313 nm. The total run time of the method was 10.0 min. The calibration curve was constructed by plotting the peak area on ordinate as a function of MTX concentration on abscissa. A good linearity was obtained in the concentration versus area curve for standard (0.0125 – 6.4 µg/mL) from spiked plasma samples. The estimation method was validated for precision and accuracy as given under MTX analytical method. Drug from unknown plasma samples (test) withdrawn at various time intervals was extracted and analyzed as done for spiked plasma samples. By using standard calibration curve MTX concentration in the unknown plasma was determined.

Recovery of rat plasma

Plasma sample: The stock solution of MTX was added to rat plasma to yield final concentrations of 0.0125, 0.2 and 6.4 µg/mL.

Diluted sample*:* MTX stock solution was diluted using acetonitrile to obtain concentrations of 0.0125, 0.2 and 6.4 µg/mL.

Both plasma samples and the diluted solutions were processed as described earlier and the ratio of peak area (plasma sample/diluted solution) for MTX was used to calculate the % recovery in rat plasma (n = 6 for each concentrations).

Pharmacokinetic and statistical analysis

The maximum plasma concentration (C_{max}) and the time to reach peak plasma concentration (T_{max}) were obtained directly from the concentration–time data. The elimination rate constant (K_E) was obtained from terminal log-linear portion of the plasma concentration–time profile. The elimination half-life ($t_{1/2}$) was calculated from $0.693/K_E$, while the area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was obtained as $AUC_{0-t} + Ct/K_E$. The values are expressed in micromol/L. The significance of the differences observed for the mean pharmacokinetic parameters of test (MTX tablet) and control (MTX solution) was evaluated using student's *t*-test at a significance level of $P < 0.05$.

Stability studies

Stability studies were carried out on optimized tablets by storing them at 25 ± 2 ° C / 60 ± 5% RH and 40 ± 2 ° C / 75 ± 5% RH for 6 months in stability chambers (Thermolab humidity chambers, India). Samples were analyzed at the intervals of one month for various parameters such as hardness, drug content, disintegration time and *in vitro* drug release. The results were the average of six determinations. The content was statistically evaluated using student's *t*-test at a significance level of $P < 0.05$ whereas release studies was statistically evaluated using one way analysis of variance ($P < 0.05$).

RESULTS AND DISCUSSION

Preparation of MTX fast disintegrating tablets

Direct compression method was used because of its ease of manufacture and low cost. Formulation of FDT is a challenging task since the formulator should select raw materials which have a quick disintegration rate in the mouth and high compressibility in order to yield an adequate hardness when compressed. Preliminary trails were carried out by varying the excipients such as super disintegrants, neusilin and magnesium stearate so that it can be easily compressed by exhibiting uniform drug content, minimal weight variation, possess optimum hardness with less friability (< 1 %) to withstand mechanical resistance and more importantly the tablet should possess quick Disintegration Time (DT).

Rationale of selecting excipients

Superdisintegrants

The most important parameter that needs to be optimized in the development of FDT is the disintegration time of tablets. The fast disintegration and dissolution effect of FDTs mainly depends on the type of superdisintegrants used in the tablet formulation. One of the most desirable properties of superdisintegrants is rapid swelling without any accompanying viscosity increase (no gel formation), because high viscosity on the surface of the tablet will hinder water penetration into the tablet matrix to slow down disintegration [21]. Hence initially tablets were optimized for shorter DT using SSG (2 – 8 %), CCS (0.5 – 5 %) and CP (2 – 5 %). The concentrations of superdisintegrants were selected as per previous literature [22]. During initial studies tablets were prepared at two different strengths of 45 mg strength and 90 mg strength.

Using various types of disintegrant, tablets were prepared with a constant hardness of 3.70 ± 0.05 kg/cm^2. Disintegration study was carried out using method 'A', with 900 mL of dissolution medium. During preliminary studies, The DT for tablets (45 mg) using SSG ranged from 44.4 to 119.6 s whereas DT for CCS ranged from 24.3 to 68.5 s and DT of the tablets prepared using CP ranged from 3 to 33.1 s. In case of 90 mg tablets, DT using SSG ranged from 48.1 to 124.2 s whereas DT for CCS ranged from 26.4 to 67.3 s and DT of the tablets prepared using CP ranged from 6 sec to 35.8 s.

The difference in DT between different superdisintegrants can be attributed to the difference in nature and mechanism of individual superdisintegrants. It was found that, increase in concentration of CCS (2 % for 45 mg and 2.5 % for 90 mg) and SSG (3.5 % for 45 mg and 90 mg) up to certain concentration showed positive effect on DT i. e. DT decreased; later increase in concentration of these superdisintegrants had a negative effect on DT of the tablets. However, CP showed different behavior than CCS and SSG, there was no decrease in DT found with increase in CP. 45 mg tablets showed synergistic effect on DT up to a concentration of 4 % CP, later increase in concentration showed no significant difference in DT whereas 90 mg tablets showed increase in DT up to 5%. (Fig. not provided).

CCS, water insoluble polymeric-based material swells to a large extent when it comes in contact with water to disintegrate tablets and due to fibrous nature it allows intraparticulate, as well as extraparticulate wicking of water even at low concentration levels. However, a water soluble content of 6 % remains during processing (esterification) of CCS which tends to become viscous and adhesive when hydrated, when CCS is added at higher concentration to tablet formulations, its absorption of water might cause an increase in viscosity of the liquid within the tablet and further water penetration would be delayed. SSG mainly acts by swelling with gelling mechanism at higher concentrations, which might have formed a thick barrier to the further penetration of the disintegrating medium and hindered the disintegration or leakage of tablet contents [23]. The obtained results were similar to the finding of Pandya et al. [24] in which preparation of fast dissolve tablet of celecoxib showed decrease in DT up to certain concentration of CCS, however further increase had negative effect on DT. CP quickly wicks with minimal volume of medium to generate the volume expansion and hydrostatic pressure which results in rapid disintegration. Unlike other superdisintegrants, CP principally relies on both swelling and wicking without gel formation for disintegration [25].

On the basis of the results obtained in the preliminary screening studies, the batch containing crospovidone (4% for 45 mg and 5 % for 90 mg) showed the fastest disintegration. Hence, it was selected for further studies.

Neusilin

Neusilin® US2 is a synthetic, amorphous form of Magnesium Aluminometasilicate that can be used for both direct compression of solid dosage forms. Due to its large surface area and porous nature, US2 adsorbs high amount of water and can be mechanically compacted into high quality tablets. Neusilin® US2 improves flowability and provides sufficiently hard tablets at low compression forces [26]. Hence in the present study tablets were prepared using neusilin as disintegrant and glidant.

It was found that neusilin had positive effect on disintegration of the tablet. DT decreased by increasing the concentration of neusilin. Neusilin concentration was kept minimal, as increase in concentration resulted in ejection of the blend from the die which might be due to density of neusilin. After optimization it was found that 10 % neusilin showed good flow property with absence of ejection of blend from the die for 45 mg and 90 mg tablets.

SD Mannitol

SD Mannitol is used as diluents and/or taste improving agent for FDT because it is water-soluble exhibiting low hygroscopicity, excellent chemical stability, direct compressibility with low friability and rapid dissolution; moreover its negative heat of solution imparts a cooling effect added to the fresh feel in the mouth during disaggregation [12].

Magnesium stearate

Magnesium stearate is commonly used lubricant in tablet dosage form. Magnesium stearate can greatly increase hydrophobicity, which lowers the solvent penetration rate (speed of absorption). Hence concentration of magnesium stearate was optimized such that it there was no sticking of blends onto the punches and produced shorter DT.

DT showed positive impact with increased concentration of magnesium stearate. DT increased from 3 to 7 s and 6 to 10 s with increased concentration of magnesium stearate from 3 to 4 % for 45 mg and 90 mg tablets respectively. Magnesium stearate decreases the wettability of the matrix and thus, may increase the DT of formulation. These results are in agreement with the results of Durig and Fassihi et al. [27], where DT increased with increase in concentration of magnesium stearate. Hence, this observation suggested the need of 3% magnesium stearate which not only circumvent lamination and capping but also favors low DT of the tablet.

Sucralose and strawberry flavor

Sucralose used in the present study is an artificial sweetener which is non-caloric, non carcinogenic and do not produce bitter after taste. Sucralose is approximately 600 times as sweet as sucrose (table sugar), twice as sweet as saccharin and 3 times as sweet as aspartame [28]. Flavor is the sensory impression of a substance, and is determined mainly by the chemical senses of taste and smell. Sucralose and strawberry flavor were incorporated to impart palatability and thereby improve patient compliance.

From the above preliminary trails, the optimized formulation for 45 mg and 90 mg are tabulated in Table 1.

FT – IR Spectroscopy

The characteristic peaks of physical mixture of drug with excipients were compared with peaks obtained from pure drug. The FT-IR spectral analysis of methotrexate (Fig. 1) showed the characteristic

peaks at 1643 cm^{-1} (-CO-NH) and 1604 cm^{-1} (C=C benzene backbone stretching), 1539, 1500 cm^{-1} (aryl systems) and 831 cm^{-1} (aromatic ring system) wave numbers [29]. Wave numbers present in the IR spectra of MTX were also found in the physical mixtures (1720 and 1604 cm^{-1}) with corresponding intensities attributing to the compatibility of drug-excipients.

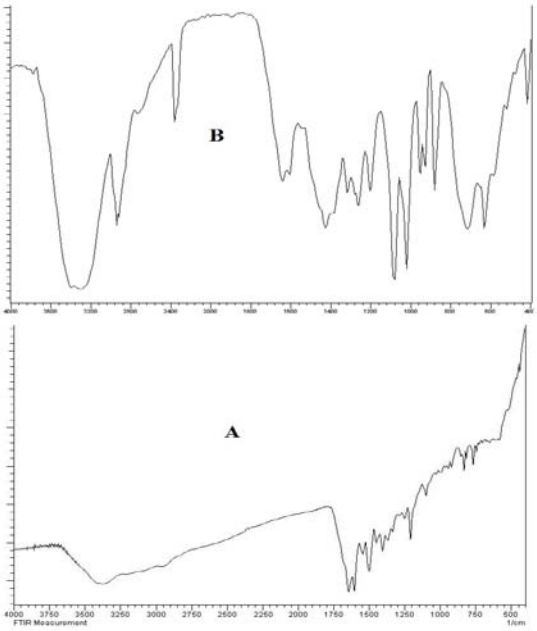

Fig. 1: FT-IR Spectra of pure Methotrexate (A) and Methotrexate-excipient physical mixture (B).

Development of MTX analytical method

A good linear relationship was observed between the standard concentration range of 0.025 – 12.8 µg/mL (r^2 = 0.9998; n = 6) with drug eluting at 6.659 ± 0.021. The accuracy at the low (0.025 µg/ml), moderate (0.4 µg/mL) and higher (12.8 µg/mL) concentrations of MTX ranged from 97.0 to 100.1 % and 95.8 to 101.3 % for intra-day and inter-day estimations respectively (n = 6 for each concentrations). The precision of the aforesaid low, moderate and higher concentration expressed as % C. V was found to be less than 7 % for intra-day assay and less than 9 % for inter-day assay indicating that % C. V was within the limits (% C. V. had to be within ± 20% for the lowest concentration and ± 15% for the upper levels).

Pre and post compression evaluations

The pre-compression and post-compression values of the optimized tablets (F1 and F2) were evaluated and values are tabulated in Table 2. Density refers to the average spatial distribution of mass in a material. Density values of the blends of F1 and F2 formulation are depicted in Table 2. Angle of repose indicates gross measurements of the flowability of powders. Flow properties of a powder are essential in determining its suitability for direct compression. Blends of formulation F1 and F2 showed excellent flow property as the values were within 30 ° [15]. The compressibility index is simple and fast method for estimating flow of the powder. As the values of carr's index increases, the flow of powder decreases. Blends showed carr's indices of ~ 14 % and hausner ratio of ~ 1.16 indicating good flow character of the granules and hence easily compressible [15]. High porosity is a critical FDT parameter because it facilitates rapid water absorption into the tablet and results in faster disintegration. The blends showed very good porosities of ~ 87 %.

The produced tablets were spherical in shape with smooth surface. Uniformity in tablet weight with acceptable weight variation (percent weight within the pharmacopeia limits of ± 7.5 % of the average weight) indicated excellent flow property of blend. Generally, the tablet dosage forms are exposed to various mechanical stress during the manufacturing steps (e. g. packaging process), shipping and handling by patients. Therefore a successful FDT must have adequate mechanical strength. The hardness of the tablets was ~ 3.6 kg/cm^2 which indicate that FDT had optimum mechanical strength. Tablet hardness is not an absolute indicator of strength. Another measure of a tablet's strength is friability.

The friability of the tablets was within the compendial limits (<1 %) indicating that tablet had good mechanical resistance [15]. The decreased friability values and mechanically strong tablets produced by FDT might also be due to the presence of crospovidone in the formulation which are highly compressible materials [30]. The drug content accessed by HPLC for the formulations was ~ 98 % indicating drug has uniformly distributed in the tablets.

Table 2: Evaluation parameters of optimized formulations of methotrexate FDT

Evaluation parameters	45 mg dose Tablet (F1)	90 mg dose Tablet (F2)
Angle of repose (°)	26 ± 0.5	27.4 ± 0.6
Bulk density (g/mL)	0.432 ± 0.010	0.459 ± 0.008
Tapped density (g/mL)	0.502 ± 0.008	0.532 ± 0.009
True density (g/mL)	3.451 ± 0.007	3.612 ± 0.011
Hausner ratio	1.162 ± 0.009	1.159 ± 0.008
Porosity (%)	87.47 ± 0.4	87.28 ± 0.3
Carr's index (%)	13.94 ± 0.04	13.72 ± 0.05
Hardness (kg/cm^2)	3.65 ± 0.9	3.67 ± 0.6
Friability (%)	0.272 ± 0.017	0.288 ± 0.020
Drug content (%)	98.9 ± 0.5	98.5 ± 0.3
Weight variation (mg)	298.8 ± 0.8	348.3 ± 0.5
In vitro disintegration time (s)		
Method A	3 ± 1.3	4 ± 1.4
Method B	7 ± 1.2	9 ± 1.2
Wetting time (s)	9 ± 0.7	11 ± 1.1
Water absorption ratio (%)	90.5 ± 0.7	91.2 ± 0.9

Values are expressed as mean ± SD; n = 6

Wetting time and water absorption ratio

Wetting time of a dosage form is related to contact angle. The wetting time of the FDT is an important parameter which needs to be assessed to give an insight into disintegration properties of the tablets; a lower wetting time implies a quicker disintegration of the FDT. Using this test, the time required for water to penetrate the tablet completely is measured and possibly represents the time

required to disintegrate the tablet in the presence of minute volume of saliva. Wetting time for F1 and F2 (Table 2) tablets were 9 and 11 s and water absorption ratio was 90 and 91 % respectively. Water absorption ratio and wetting time, which are important criteria for FDT showed excellent results which might be due to neusilin and / or due to superdisintegrant (CP), which facilitates wicking action in bringing faster disintegration.

***In vitro* disintegration time**

According to US FDA specification, the disintegration time of FDT should not exceed 30 s [31]. Various researchers have used different methods to determine the time taken for the tablet to disintegrate. Hence an attempt was made to carryout disintegration studies by using two different methods.

F1 and F2 tablets showed DT of 3 and 4 s respectively when determined using conventional disintegration-basket rack assembly (Method 'A'). The assessment of disintegration time for the ODT is difficult using the tests for conventional method due to its rapid disintegration rate even in a small volume of water in addition to the strong agitation used during this test, and consequently, the DT obtained from the conventional disintegration tests appears not to be reflective of the DT in the human mouth. To overcome this issue, a modified disintegration test method was carried out as described by Fu et al. [32]. It was found that F1 and F2 showed DT of 7 and 9 s when determined using method 'B'. The above studies indicate no significant difference in the DT of the formulation due to change in methods (Table 2).

TGA and DSC studies

DSC thermogram of FDT (A) and pure drug (B) are presented in Fig 2. DSC thermogram of drug (B) showed a melting endothermic peak at 84.21 °C (may be hydrate peak), 178 – 191 °C (small dent with broad peak which may be due to melting of drug) and at 256.26 °C (degradation peak). The peak obtained from FDT was 82.44 °C, 175 – 190 °C and 259.46 °C indicating that drug peaks retained in the formulation hence no interaction between drug and excipients.

TGA measures the amount of weight change of a material, either as a function of increasing temperature, or isothermally as a function of time, in an atmosphere of nitrogen. The % weight change of FDT is depicted in Fig. 3. Form the fig.3 it revealed that, less than 3 % weight reduction was seen below 100 °C which may be due to evaporation of moisture; from this it was clear that the moisture content in the tablet was very low and within the acceptable USP limits [15]. A major weight loss (~ 50 %) was seen after 190 °C which may be due to melting of MTX. At 1000 °C, The 90 % weight of the FDT was lost which can be imputed due to decomposition of ingredients.

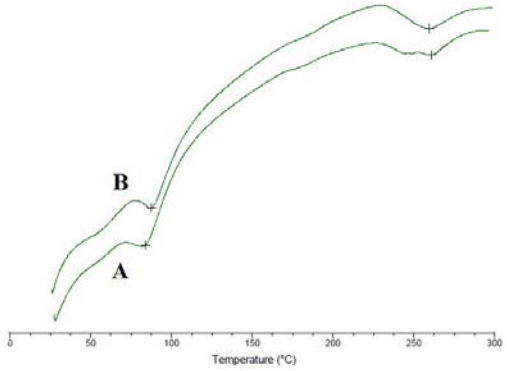

Fig. 2: DSC thermogram of MTX FDT (A) and pure drug (B) are presented in Figure 4.

XRD analysis

Crystallinity of compound is indicated by the presence of sharp peaks that remains absent in case of amorphous compounds.

X-ray diffractogram of tablet formulation, placebo tablet and pure drug were shown in Fig. 4. Diffraction pattern of pure drug showed numerous characteristic peaks at 11, 13, 18, 19 and 27° 2 θ, whereas it was absent in the diffraction spectrum of tablet. Disappearance of the intense sharp peaks indicates that MTX is amorphozised in the tablet and there could be less or no free drug (in crystalline form).

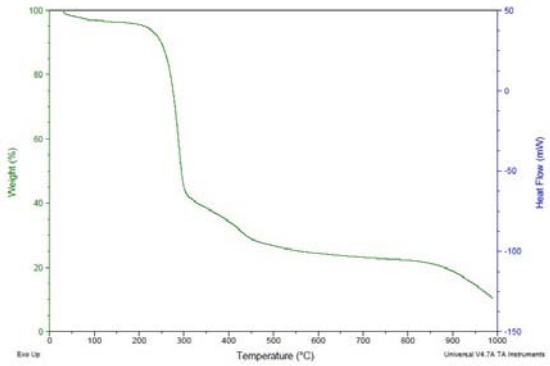

Fig. 3: TGA thermogram of optimized MTX FDT.

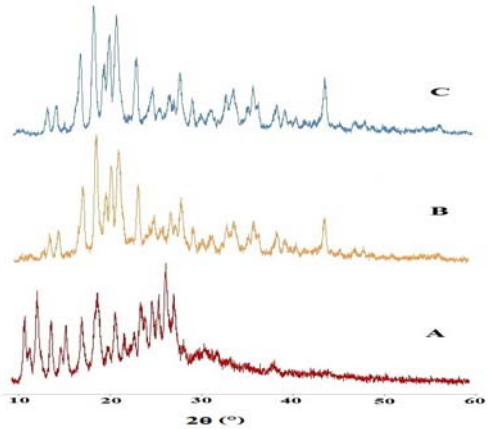

Fig. 4: X-ray diffractogram of pure drug (A), FDT formulation (B) and placebo tablet (C).

***In vitro* dissolution studies**

Dissolution of a drug substance under physiological conditions is essential for its systemic absorption. The dissolution studies of F1 and F2 FDT were conducted using bio-relevant media; simulated saliva (pH 6.8), simulated gastric fluid (0.1N HCl) and pH 4.5 acetate buffer as absorption of drug from the tablet can occur through buccal, sublingual mucosa, oropharyngeal, esophagus, and stomach. The *in vitro* dissolution profiles of F1 and F2 MTX FDT compared with plain drug are depicted in Fig. 5 and Fig. 6 respectively. Dissolution was compared with the plain drug to get an insight of release of the drug from tablets.

From the plot (Fig. 5) it was found that, ~ 95 % drug was released from F1 tablet and plain drug (equivallent dose as F1 tablet) within 240 sec at pH 6.8; whereas it took 300 and 360 s to release the drug at pH 4.5 and pH 1.2 respectively.

From the plot (Fig. 6) it was found that, ~ 95 % drug was released from F2 tablet and plain drug (equivallent dose as F2 tablet) within 360 sec at pH 6.8; whereas it took 420 and 480 s to release the drug at pH 4.5 and pH 1.2 respectively.

From the graphs it was clear that, FDT on comparision to plain drug released at similar rate, however the release profile was different between them i. e., FDT showed slightly faster release than plain

drug which may be due to amorphozisation of the drug in the tablet. However there was no statistically significant difference in release ($p < 0.05$) between tablet and plain drug at a particluar pH medium. The release of F1, F2 tablet and plain drug analyzed at different pH medium was the following assending order: 6.8 > 4.5 > 1.2.

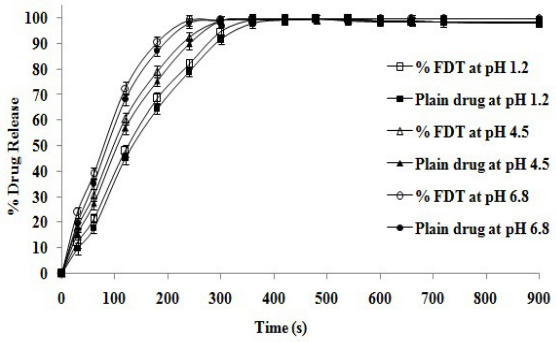

Fig. 5: *In Vitro* drug release profile of F1 FDT and plain drug (MTX) at pH 1.2, 4.5 and 6.8. Error bars indicate the standard deviation (n = 6).

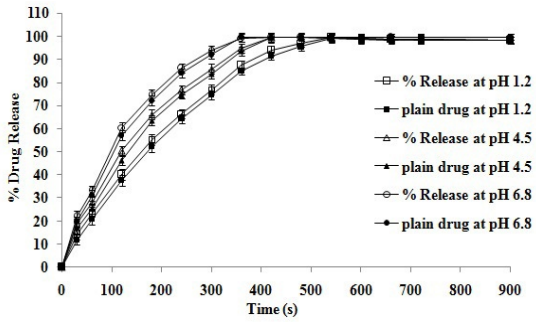

Fig. 6: *In Vitro* drug release profile of F2 FDT and plain drug (MTX) at pH 1.2, 4.5 and 6.8. Error bars indicate the standard deviation (n = 6).

In vivo evaluation

Bioanalysis of MTX in rat plasma

The bio-analytical HPLC method used for MTX estimation was rapid and simple with drug eluting at a retention time of 8.814 ± 0.024 min. A perfect linearity was observed between the concentration range of 0.0125 – 6.4 µg/mL ($r^2 = 0.9998$, n=6). The LLOQ i. e., the lowest concentration on the calibration curve that could quantify MTX with acceptable precision and accuracy was 0.0125 µg/mL. The accuracy of estimation in the seeded samples of 0.0125 µg/mL, 0.2 µg/mL and 6.4 µg/mL concentrations of MTX in the plasma were found to range from 95.1 to 101.2 % and 93.9 to 102.0 % for intra-day and inter-day estimations respectively. The precision of the aforesaid low, moderate and higher concentration seeded plasma expressed as % C. V was found to be less than 8 % for the intra-day assay and less than 10 % for the inter-day assay indicating that % C. V was within the limits. Representative chromatogram of blank rat plasma (A) and plasma peak after oral administration of MTX tablet (B) and solution (C) to rats showed well resolved peaks (Fig. 7). No interfering peaks were observed and no significant peaks were found at the retention times of the analyte in the plasma. The present method adopted a simple protein precipitation which had extraction efficiency (recovery) ranged from 85.5 – 90.05 %. Quantification of MTX carried out using previously established method produced adequate precision; Also, in our method direct deproteinization of plasma gives an extraction efficiency of more than 85 % which not only circumvents the need for internal standard but also a multi stage extraction procedure like solid phase extraction [33-34].

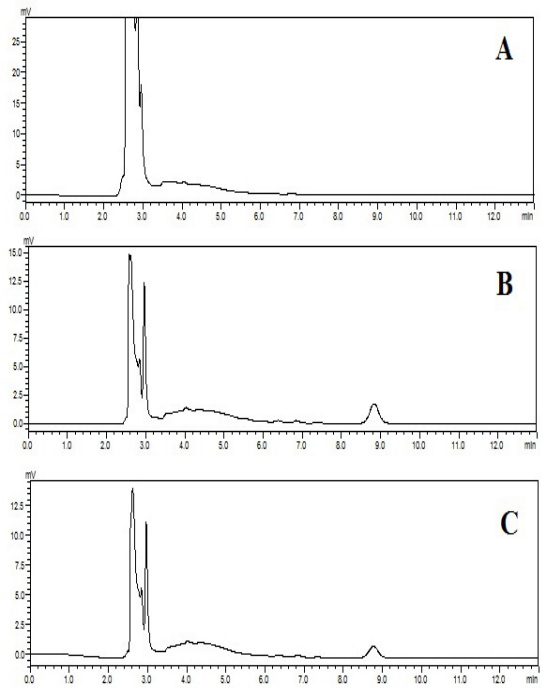

Fig. 7: Representative chromatogram of blank rat plasma(A), Typical chromatogram obtained after oral administration of MTX FDT (B) and MTX solution (C) showing well resolved peak of MTX.

Pharmacokinetic and statistical analysis

Plasma concentration time profile in rat plasma after oral administration of MTX (FDT as test and Solution as control) of 25 mg/m² and 50 mg/m² are depicted in Fig. 8 and 9 respectively. The pharmacokinetic parameters of MTX after single dose administration as test and control are summarized in Table 3. From the Table 3, it is clear that, T_{max} and $T_{1/2}$ were slightly lower in solution treated group than FDT treated group which might be due to immediate absorption of solubilized drug; however the FDT has to first disintegrate and then dissolve so as to get absorbed into systemic circulation. From the Fig. 9, it is clear that, there was inter-individual variability of results indicating that with the increase in dose, variation in kinetics occurred. Though there was a difference in results obtained for FDT and solution, the difference was not statistically significant ($p < 0.05$) between the groups. Based on the statistical inferences it can be concluded that MTX FDT and MTX solution exhibited comparable plasma level-time profiles.

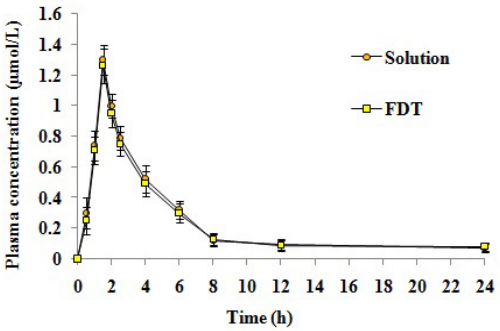

Fig. 8: Plasma concentration time profile of MTX after oral administration of F1 FDT and MTX Solution. Data represent the mean ± S. D. (n = 6).

Table 3: Pharmacokinetic parameters after oral administration of methotrexate (FDT and Solution) to rats

Parameters	Methotrexate (25 mg/m²)		Methotrexate (50 mg/m²)	
	Tablet	Solution	Tablet	Solution
C_{max} (µmol/L)	1.26 ± 0.12	1.30 ± 0.10	3.37 ± 0.3	3.53 ± 0.31
T_{max} (h)	1.86 ± 0.22	1.74 ± 0.30	2.41 ± 0.37	2.33 ± 0.51
$T_{1/2}$ (h)	2.06 ± 0.20	1.89 ± 0.18	2.93 ± 0.28	2.84 ± 0.34
AUC $_0{}^t$ (µmol. h/L)	5.36 ± 0.16	5.53 ± 0.18	14.31 ± 3.8	15.17 ± 3.3
AUC $_0{}^\infty$ (µmol. h/L)	5.60 ± 0.17	5.72 ± 0.21	14.65 ± 3.99	15.58 ± 3.7
K_e (h^{-1})	0.3356	0.3666	0.2359	0.2437

Values are expressed as mean ± SD; n = 6

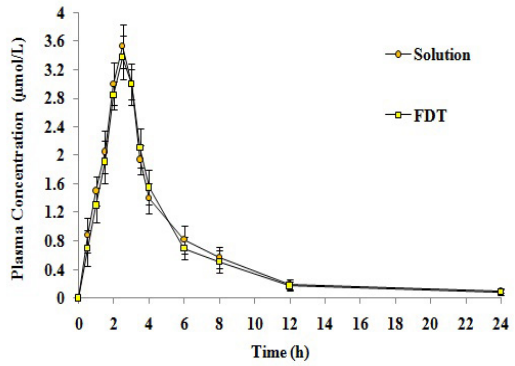

Fig. 9: Plasma concentration time profile of MTX after oral administration of F2 FDT and MTX Solution. Data represent the mean ± S. D. (n = 6).

Stability studies

When the FDT was stored at normal temperature (25 ± 2 ° C / 60 ± 5% RH) for 4 - 24 weeks; no apparent change in form, color (observed visually), hardness, content (95.8 ± 1.0 % for F1 and 95.2 ± 1.1 % for F2), DT and release was reported in F1 and F2 tablets. No statistically significant in the release was observed at various pH medium. At accelerated temperature (40 ± 2 °C/75 ± 5% % RH); the tablet tend to change its form and color (dull) at fifth month sampling. Hardness and DT decreased significantly indicating that tablets have lost their mechanical integrity. There was significant change ($p < 0.05$) in content (75.5 ± 2.0 %) and drug release observed. The overall results suggest that, MTX FDT should be stored at normal temperature (25 ± 2 ° C / 60 ± 5% RH) with proper packing.

CONCLUSION

Fast disintegrating tablets of methotrexate were successfully prepared by direct compression technique due to its superior flow properties. The produced tablet showed smooth surface without any interactions between drug and excipients as confirmed by FTIR analysis. Preliminary studies showed that a significant influence of different formulation components was observed on the tablet disintegration and dissolution with the CP (superdisintegrant), neusilin (disintegrant & glidant) and magnesium stearate (lubricant) exerting the most influence. Amongst various formulation prepared, F1 for 45mg strength (containing 4 % CP, 10 % neusilin and 3 % magnesium stearate) and F2 for 90mg strength (containing 5 % CP, 10 % neusilin and 3 % magnesium stearate) was considered optimized. F1 and F2 formulations showed satisfactory mechanical strength and resistance, quicker *in vitro* disintegration time, rapid dissolution and good stability at 25 ± 2 ° C / 60 ± 5% RH. In rats, pharmacokinetic parameters of methotrexate after oral administration showed no statistically significantly difference between the two groups (Control and Solution). The above findings suggest that the present oral FDT containing MTX rapidly disintegrates in seconds thereby reducing any potential choking hazard and also provides better acceptance of the medication which leads to proper cure of the disease especially for dysphagic and elderly patients.

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CONFLICT OF INTEREST

Authors disclose no conflict of interest.

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