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Isolation and Evaluation of Disintegrant Properties of Fenugreek Seed Mucilage

Ravi Kumar*[1], Swati Patil[4] ,M. B. Patil[2], Sachin R. Patil[1], Mahesh S. Paschapur[3]
[1]Department of Pharmaceutics, K.L.E.S's College of Pharmacy, Ankola-581314, Karnataka,
[2]Department of Pharmacognosy, K.L.E.S's College of Pharmacy, Ankola-581314, Karnataka,
[3]Department of Pharmacology, K.L.E.S's College of Pharmacy, Ankola-581314, Karnataka
[4]Department of Pharmacognosy, Principal KM Kundnani college of Pharmacy, Cuffe Parade, Mumbai

Corres. e-mail: ravikumar300@gmail.com

ABSTRACT: In the present study, Polysaccharide mucilage derived from the seeds of fenugreek, *Trigonella foenum-graceum* L (family Leguminosae) was investigated as disintegrant for use in mouth dissolving tablet formulations containing metformin hydrochloride. Mucilage extracted from fenugreek seeds were subjected to toxicity studies, it showed that extracted mucilage is devoid of toxicity. Fast disintegrating tablet (FDT) of metformin HCl was formulated using different concentration (2, 4, 6 8 and 10% w/w) of natural disintegrant viz; isolated mucilage of fenugreek seed and synthetic superdisintegrants like croscarmellose sodium and were compared. Disintegration time and drug release were taken as the basis to optimize the rapidly disintegrating tablet. Prepared tablets were evaluated for thickness, hardness, friability, uniformity of weight, disintegration time, wetting time and dissolution study. The formulated tablets had good appearance and better drug release properties as compared to the marketed conventional tablets. Fenugreek mucilage in the concentration of 4 % gives shorter disintegration in 15 sec. and shows 100% drug release within 18 min. is selected as the optimized formulation (F2).Hence, the present study revealed that this natural disintegrant (fenugreek mucilage) showed better disintegrating property than the most widely used synthetic superdisintegrants like Ac-di-sol in the formulations of FDTs. Studies indicated that the extracted mucilage is a good pharmaceutical adjuvant, specifically a disintegrating agent. Optimized formulation was subjected to stability studies as per ICH guidelines at 25^0 and 65% RH, 40° and 75% RH showed insignificant change in hardness, disintegration time and *in vitro* drug release at the end of three months.
KEYWORDS: Metformin HCl, Disintegrant, Fast-dissolving tablets, Seed mucilage, pharmaceutical excipients

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentration-hyperglycemia-caused by insulin deficiency, often combined with insulin resistance[1]. Metformin HCl, an important drug of biguanide class, is currently available drug for treating hyperglycemia in (Non-Insulin Dependent Diabetes Mellitus (NIDDM); but has been associated with severe and sometimes fatal hypoglycemia and gastric disturbances like nausea, vomiting, heartburn, anorexia and increased appetite after oral therapy. Since these drugs are usually intended to be taken for a long period, patient compliance is also very important[2-3]. Metformin, which is slowly and partially absorbed by the gut, is taken in the form of oral tablets of 500 and 850mg, usually at a dose of 2g (maximum of 3g) per day. The absolute bioavailability of a 500mg immediate-release tablet is about 50 to 60%; the half-life is 2 -6h and the maximum plasma concentration is reached after 2.5h, Almost 80-100 % of the drug is excreted unchanged. In the Bioavailability Classification System (BCS), metformin is classified as a class III drug, because of its high water solubility.

Many patients, especially elderly find it difficult in swallowing tablets, capsules, fluids and thus do not comply with prescription, which results in high incidence of non-compliance oriented research has resulted in bringing out many safer and newer drug delivery systems. Rapidly disintegrating/dissolving tablet is one of such example, for the reason of rapid disintegration or even with saliva. Significance of this drug delivery system includes administration without water, accuracy of dosage, ease of portability, alternative to liquid dosage

forms, ideal for paediatric and geriatric patients and rapid onset of action[4-10].

Excipients are the additives used to convert active pharmaceutical ingredients into pharmaceutical dosage form suitable for administration to patients[11]. New and improved excipients continue to be developed to meet the needs of conventional drug delivery systems and to meet the needs of advanced tablet manufacturing.

Plant products serve as an alternative to synthetic products because of local accessibility, environment friendly nature and lower prices compared to imported synthetic products. Herbs are non-polluting renewable resources for sustainable supplies of cheaper pharmaceutical products. Today, we have a number of plant-based pharmaceutical excipients. A number of researchers have explored the utility of plant-based materials as pharmaceutical excipients[12-18]. Majority of investigations on natural polymers in drug delivery systems are centered on polysaccharides and proteins, due to their ability to produce a wide range of materials and properties based on their molecular structures[19].

Number of natural, semi synthetic and synthetic polymer materials are used in the various drug delivery systems. Recent trend towards the use of vegetable and nontoxic products demands the replacement of synthetic additives with natural one[20]. In view of the easy availability of the plant and high demand of gum through the world, the mucilage obtained from Fenugreek seeds were investigated for its application as a disintegrating agent[21-22]. The natural materials like gums and mucilages have been extensively used in the field of drug delivery for their easy availability, cost effectiveness, Eco friendliness, emollient and non irritant nature, non toxicity, capable of multitude of chemical modifications, potentially degradable and compatible due to natural origin[23-25].

Trigonella Foenum-graceum, commonly known as Fenugreek, is an herbaceous plant of the leguminous family[26] and is native to Western Asia, from where it has spread widely over Europe, the Mediterranean, and the rest of Asia. It is one of the oldest cultivated plants and has found wide applications as a food, a food additive, and as a traditional medicine in every region where it has been cultivated. The leaves and both the ripe and unripe seeds of *Trigonella Foenum-graceum* are used as vegetables. The seeds also function as a food preservative and are added to pickles, chutneys, and other similar food products[27]. The ripe seeds have numerous applications in cosmetic and traditional medicine system of India. Fenugreek has been used in treating colic flatulence, dysentery, diarrhoea, dyspepsia with loss of appetite, chronic cough, dropsy, enlargement of liver and spleen, rickets, gout, and diabetes[28]. It is also used as gastro protective, antiurolithiatic[29], diuretic, antidandruff agent, Anti-inflammatory agent and as antioxidant. The seed is stated to be a tonic. It also is used in post-natal care and to increase lactation in nursing mothers. Fenugreek seeds contain a high percentage of mucilage (a natural gummy substance present in the coatings of many seeds). Although it does not dissolve in water, mucilage forms a viscous tacky mass when exposed to fluids. Like other mucilage-containing substances, fenugreek seeds swell up and become slick when they are exposed to fluids. The resulting soft mass is not absorbed by the body, but instead passes through the intestines and triggers intestinal muscle contractions[30].

The objective of present study was to isolate and investigate the suitability of the fenugreek seed mucilage as a disintegrant to develop FDTs of the selected model drug metformin HCl. The disintegration and swelling properties of FDT were compared with widely used super disintegrant like Ac-di-sol. Metformin HCl, an antidiabetic drug, was selected as the model drug as it was widely used in the treatment of Type –II diabetes.

MATERIALS AND METHODS

Materials

Metformin HCl, talc, magnesium stearate, aspartame, aerosil were obtained from Zydus Research centre, Ahmedabad, India as gift samples. Fenugreek seeds were procured from the local market. All the other solvents, reagents and chemicals used were of either pharamcopoeial or analytical grade. Different instruments *viz*; Vernier calipers, Monsanto hardness tester, Roche friabilator and disintegration apparatus were supplied by Campbell Electronics, Mumbai. USP XXIII dissolution apparatus-2 was from Tab- Machines, Mumbai and 1601 PC Shimadzu UV Spectrophotometer from Tokyo, Japan.

Methods

Extraction of Mucilage[31]

The seeds were powdered using pestle and mortar and 100 g of the powder was extracted with hexane to remove lipophilic compounds using a soxhelet apparatus. To remove pigments and to deactivate enzyme, the defatted powder was boiled in ethanol for 20 min. This treated powder was then soaked in 10 litres water and the pH was adjusted to 3.5 using 0.5 M Hydrochloric acid. The mixture was stirred by a mechanical stirrer for 12 h and then filtered through filtration paper. The filtrate was centrifuged (5000 g) and the supernatant was concentrated in vacuum to 50% of its initial volume. The resulting solution was mixed with the same volume of 96% ethanol and stored in a refrigerator for 4 h. The precipitated mucilage was separated by centrifugation (5000 g). The collected mucilage was re-suspended in distilled water, agitated for 20 min and re-precipitated one more time to eliminate chloride ions and other impurities. Finally the residue was washed with diethyl ether and acetone and dried overnight at 45°C, resulting in an off-white powder.

Total Hydrolysis of the Mucilage and Sugar Analysis[32]

The mucilage (0.1 g) and 25 ml 2M Hydrochloric acid were refluxed for 2 h at 100°C. The solution was then concentrated under vacuum and the excess acid was removed by repeated codistillation with distilled water. The residue was dissolved in methanol containing a few drops of water and chromatographed on precoated silica gel plates in n-buthanol:acetic acid:water (4:1:5). The

thymol-sulfuric acid reagent was used to detect sugars (5 min at 120°C).

Determination of Swelling Index[33]

The swelling index is the volume in ml occupied by 1g of drug; including any adhering mucilage after it has been swollen in an aqueous liquid for 4h .The swelling index of Fenugreek mucilage powder, was determined according to the BP method. One gram of mucilage powder was taken in a 25 ml ground glass stoppered cylinder graduated over a height of 120 to 130 mm in 0.5 divisions. To this 25 ml of water was added and this was shaken vigorously every 10 m for 1h and then allowed to stand for 24 h. The volume occupied by the disintegrating agent including adhering mucilage was measured. The Swelling index was calculated from the mean of three determinations.

Physicochemical characterization of mucilage[34-36]

The separated mucilage was evaluated for solubility, swelling index, loss on drying, ash value, microbial load, density, compressibility index and angle of repose.

Viscosity Determination

1 g of dried and finely powdered fenugreek mucilage was suspended in 75 ml of distilled water for 5 h. Distilled water added up to 100 ml to produce the concentration of 1% w/v. The mixture was homogenized by mechanical stirrer for 2 h and its viscosity determined using a Brookfield viscometer, spindle –LV2 (Brookfield LV-II, USA) at 20 rpm and 25°C.

Evaluation of Toxicity

Toxicity studies were carried out according to the method of Knudsen and Curtis[37]. The animals used in the toxicity studies were sanctioned by the Institute Animal Ethical Committee. The male albino rats of Wistar strain weighing 160-200 gm were divided into different groups comprising of six animals each. The control group received normal 0.5%CMC solution (20ml/kg i.p). The other groups received 500, 1000, 2000, 3000, 4000 and 5000 mg/kg of mucilage suspension in normal saline orally. The animals were observed continuously for the behavioral changes for the first 4 hours and then observed for mortality if any for 72 hours. Since no mortality, no toxic manifestations were observed and behavioural pattern was unaffected. In chronic toxicity studies, 22 animals were used, divided in to two groups, 6 as control and 16 as test animals. In the test group a dose of 500 mg/kg was administered daily for a period of 30 d. body weights were recorded for both the groups at an interval of 10d. And at the end of 30 days, hematological and biochemical parameters were studied in both the groups and after 30 days of chronic toxicity study the animals were scarified and subjected to histopathological studies.

Characterization of Drug and Excipients

Preformulation study on Metformin HCl

The metformin HCl was subjected to various preformulation studies like solubility, description, pH, partition coefficient, pKa values.

Drug-excipient compatibility studies

This study has been done to check whether there is any compatibility related problems are associated with drug and the excipients used for the formulation of mouth dissolving tablets. The drug and excipients must be compatible with one another to produce a product that is stable, efficacious, attractive, and easy to administer and safe. If the excipients are new and not been used in formulations containing the active substance, the compatibility studies are of paramount importance. Thermal analysis, TLC, HPLC, FTIR, can be used to investigate and predict any physicochemical interactions between components in a formulation and can therefore be applied to the selection of suitable chemically compatible excipients.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were recorded on samples prepared in potassium bromide (KBr) disks using a Shimadzu Corporation, (Tokyo, Japan) Model-1601 PC. Samples were prepared in KBr disks by means of a hydrostatic press at 6-8 tons pressure. The scanning range was 500 to 4000 cm^{-1}.

Differential Scanning Calorimetry (DSC)

DSC analysis was performed using Shimadzu DSC-60, Shimadzu Limited Japan. A 1:1 ratio of drug and excipient was weighed into aluminum crucible. And sample was analyzed by heating at a scanning rate of 20^0C over a temperature range 20^0-300^0 under nitrogen environment.

Thin Layer Chromatographic analysis (TLC)

Drug and Excipients were subjected to TLC analysis. The solvent system for TLC of Metformin HCl was glacial acetic acid: Butanol: water (10:40:50). RF values of pure drug and drug with different Excipients were calculated.

pH stability testing of the drug

Weighed quantities of the drug (100 mg) was dissolved in different media with different pH conditions like distilled water, 0.1 M hydrochloric acid (pH 1.2), Acetate buffer (pH 4.5), Phosphate buffer (pH 6.8) etc. The study was conducted for a period of one day and the samples were withdrawn at intervals of 1 hour, 4 hour and 24 hours. The samples were analyzed by HPLC and spectrometrically.

Standard Calibration Curve of Metformin HCl

Solutions ranging from 2 to 4 µg/ml were prepared in phosphate buffer (pH 6.8 fluid). Absorbance was measured for each solution at λ_{max} of 233 nm, using1601 PC Shimadzu UV Spectrophotometer. Correlation coefficient was found to be 0.9998 in phosphate buffer.

Formulation of Mouth Dissolving Tablets

Mouth dissolve tablets of Metformin HCl were prepared by the conventional direct compression technique using Fenugreek mucilage powder at concentrations of 2, 4, 6, 8 and 10 % w/w. All ingredients were passed through mesh no.60. Required quantity of each was taken for particular formulation and the blend was mixed by tumbling in a polythene bag. The composition of each formulation is given in table 1.

Evaluation of powder Blend

Pre compression parameters[37-38]

The prepared powder blend was evaluated for various parameters like bulkiness, bulk density, tapped density, angle of repose, compressibility index and Hausner ratio.
After evaluation of powder blend the tablets were compressed with Cadmach single punch compression machine using 12mm flat faced punches.

Evaluation of tablets

Post compression parameters[39-42]

After tablet compression, all the tablets were evaluated for different parameters as thickness, hardness, friability, uniformity of weight, disintegration time, water absorption ratio, wetting time, drug content. *In vitro* dissolution studies were carried out in USP dissolution test apparatus (Type 2), using simulated intestinal fluid (pH 6.8) (900ml, 37± 0.5^0C) at 50 rpm.

pH of the solution

The pH of the solution was measured using pH meter, after dissolving the tablet in around 200 ml of water.

Accelerated stability studies

Stability studies were carried out on optimized formulation as per ICH specifications. The tablets were stored at 25 ± 2 ^{o} C / 60 ± 5% RH and 40 ± 2 ^{o} C / 75 ± 5% RH for duration of three month. After an interval of one month samples were withdrawn and tested for various physical tests and *in vitro* drug release.

RESULT AND DISCUSSION

Polysaccharide mucilage derived from the seeds of fenugreek, *Trigonella foenum-graceum* L (family leguminosae) was investigated as disintegrating agent for use in moth dissolving tablet formulations containing metformin hydrochloride.

The percentage yield of the mucilage extraction from fenugreek seeds was 31% w/w. The mucilage obtained was an off white to cream color powder, and the viscosity of its 1% aqueous dispersion was 500 cP. Analysis of mucilage confirmed that the sugars existing in mucilage were mainly mannose (66.75 %), galactose (33.45%), and xylose (0.32%). The absence of sharp peak at 1700–1800 cm^{-1} in the FTIR spectrum indicates that there is no carboxyl group in the extracted sample. On the other hand, the presence of peak at 1000–1200 cm^{-1} corresponds to the presence of alcoholic group mostly secondary alcohols. These findings proved that there were no uronic sugars or esters in the structure (Figure 1). The powder was slightly soluble in water and practically insoluble in organic solvents. Swelling characteristics studies revealed that the swelling was affected by pH of the medium and powder showed good swelling ratio in distilled water. The loss on drying, ash value and microbial count were well within official limits. The compressibility index and angle of repose indicated that the powder is having good flow with moderate compressibility. The result of physicochemical characterization of fenugreek mucilage is reported in table 2.

Preformulation studies of drug

Various Preformulation studies are carried out for metformin HCl. The results are given in table 3. Results found to comply with the specification of Pharmacopoeia.

Drug Excipient Compatibility Study

Fourier Transform Infrared (FTIR) Spectroscopy

The IR spectral analysis of metformin HCl and the physical mixture of metformin HCl and other excipients are presented in Figure 2 and 3 respectively. Pure metformin HCl spectra showed principal peaks at different wave numbers corresponding to its functional groups, confirming the purity of the drug as per established standards. All the above characteristic peaks appear in the spectra of physical mixture of metformin HCl and other excipients, indicating no modification or interaction between the drug and excipients.

Differential Scanning Calorimetry (DSC)

The DSC analysis (Figure 4) of pure metformin HCl showed a characteristic, sharp endotherm peak at 226°C corresponding to its melting point and indicates the crystalline nature of the drug. The DSC analysis of physical mixture of drug and excipients (figure 5) revealed negligible change in the melting point of metformin HCl in the presence excipients, indicating no modification or interaction between the drug and excipients.

Thin Layer Chromatographic analysis

The R_f value of pure metformin HCl was found to be 0.92. In the presence of excipients, the
R_f value of the drug was unchanged and found to be 0.92.

pH stability testing of the drug

The pH stability studies of Metformin HCl were carried out in different pH media by afore mentioned procedure. It was found that the drug (Metformin HCl) was stable for a period of one day in all pH conditions. The results are shown in figure 6 to11.

Toxicity study of isolated mucilage

To determine the safety level of extracted mucilage, acute and chronic toxicity studies were carried out. In acute toxicity study no mortality was observed even at 5000mg/kg of fenugreek mucilage on oral administration and all animals were found to be normal during and at the end of the observation period of three days. Food and water consumption also did not differ significantly and there was no change in general behavior or other physiological activities of the animals in both control and treated groups.

To assess the suitability of mucilage for the oral delivery we have recorded the body weight profile for the animals during the chronic toxicity studies at regular intervals of 10 days. It was found that the body weight of both control and treatment group and the rate of increase in body weight were comparable. Hence, it could be inferred that chronic administration of the gum might not influence either the food intake or growth. Biochemical and hematological parameters were determined at the end of 30 days of continuous administration of mucilage suspension and the biochemical and hematological parameters were found to be comparable to that of normal mice. The results are shown in table 4 and 5.

Histological examination of the main organs like liver, kidney, heart and brain were carried out at the end of 30days of chronic toxicity study. From this study it was revealed that there was no sign of pathological changes in both control and in treatment group. The results are shown in figure 12.

Precompression parameters of powder blend

Since, the flow properties of the powder mixture are important for the uniformity of mass of the tablets, the flow of the powder mixture was analyzed before compression to tablets. Low Hausner ratio, compressibility index and angle of repose values indicated a fairly good flowability of powder mixture. The values of pre-compression parameters evaluated were within prescribed limits and indicated good free flowing property (Table 6).

Post compression parameters of fast dissolving tablets

The data obtained for post compression parameters such as hardness, friability, weight variation, uniformity of content, thickness, are shown in Table 7. The hardness was found to be in the range of 2.8±0.12 to 3.2±0.14 kg/cm^2 in all the formulations indicating good mechanical strength with an ability to withstand physical and mechanical stress conditions while handling. In all the formulations the friability value is less than 1% and meets the IP (Indian Pharmacopoeia) limits. All the tablets passed weight variation test as the % weight variation was within the pharmacopoeial limits. The weight of all the tablets was found to be uniform with low standard deviation values indicating efficient mixing of drug, disintegrants and excipients. The percentage drug content of all the tablets was found to be between 98.12 ± 0.04% and 101.91 ± 0.01 % of metformin HCl. pH of the solution of all the tablets was found to be between 6.3 to 7.7, which suggest that the tablets can be conveniently administered orally and will not cause any discomfort.

The separated mucilage was evaluated for its performance as disintegrant in tablets at various concentrations (2, 4, 6, 8, 10 %w/w) and the optimum concentration found was 4 %. Its performance was compared with cross carmellose sodium at optimum concentration (8%) and it was found better than cross carmellose sodium in tablet formulations with less disintegration time (15 s) compared to that of cross carmellose sodium (28 s).

It was observed that the increased concentration of cross carmellose sodium, decreases disintegration time and optimized the drug release. Cross carmellose sodium in the concentration of 8% acts as potential disintigrant and disintegrates the tablet within 28s fulfilling the criteria of mouth dissolving tablet. Cross carmellose sodium when comes in contact with water it quickly wicks water into the tablet through capillary action to create internal pressure that disintegrates the tablet. On the other hand it was observed that the increased concentration of fenugreek mucilage decreases disintegration time and wetting time upto 4% concentration in the tablet, but further increase in the concentration of mucilage showed increase in disintegration time and wetting time. The mucilage of fenugreek showed very high percentage of swelling index as compared to the other disintegrating agents. This rapid disintegration of the FDTs was due to the penetration of saliva into the pores of the tablet, which lead to the swelling of mucilage to create enough hydrodynamic pressure for quick and complete disintegration of the tablet.

Since the dissolution process of a tablet depends upon the wetting followed by disintegration of the tablet, the measurement of wetting time may be used as another confirmative test for the evaluation of dispersible tablets. Wetting times decreased with increase in the level of croscarmellose (2 to 10 %w/w). A significant decrease in the wetting times is seen with increase in the level of fenugreek mucilage (2 to 4%). The comparison of disintegration time *in vitro*, disintegration time in oral cavity and wetting time for various formulations are given in figure 13.

All designed formulations using fenugreek mucilage powder cross carmellose sodium showed rapid dissolution and percent cumulative drug release (%CDR) at the end of 20 minutes was 75-98 %. Results are as shown in figure 14.

From drug release it was observed that increase in concentration of fenugreek mucilage increases the drug release upto 4% concentration in the tablet, but further increase in the concentration of fenugreek mucilage does not show any increase in the dissolution rate.

From drug release it was observed that increase in concentration of crosscarmellose sodium increases the drug release at 8 % concentration in the tablet, but below or above this concentration of crosscarmellose sodium does not show any increase in the dissolution rate. Therefore formulation F2 having disintegrant fenugreek mucilage in the concentration of 4% was selected as the optimized formulation.

Dissolution profile of the optimized formulation (F2) was compared with the marketed formulation of Metformin HCl (Glyciphage®). Marketed formulation of metformin releases 100% drug in 35 minutes. Where as optimized formulation F2 released 100% drug in 18 minutes shows its superiority over marketed formulation (figure 15).

The formulations containing fenugreek mucilage has shown lower water absorption capacity than that containing crosscarmellose sodium. With the same disintegrant, there was a linear increase in water uptake with increase in concentration of superdisintegrants. The results are shown in figure 16.

The optimized formulation F2 was kept at real time (25 ± 2^o C / 60 ± 5% RH) and accelerated (40±2 ^{o}/75±5% RH) storage conditions for a period of 3 months. After stability test period, tablets were analyzed for drug content, hardness, friability, *in vitro* release and disintegration tests. Stability studies result showed that there was no significant change in hardness, friability, drug content, and dissolution profile of formulation F2 (figure 17). The formulation was stable under accelerated conditions of temperature and humidity.

CONCLUSION

This study has demonstrated the potential of fenugreek seed mucilage to act as a disintegrant in mouth dissolving formulation as it shows better disintegrating property (4%w/w) than the most widely used synthetic super disintegrants like cross carmellose sodium. From this entire study we can conclude that fenugreek seed mucilage can be used as disintegrants in the formulation of fast/dispersible tablets, since the primary ingredients are in expensive, devoid of toxicity, biocompatible, biodegradable and easy to manufacture, they can be used in place of currently marketed superdisintegrants. Direct compression method (by the addition of superdisintegrants) was found to be the best approach in the formulation of fast dissolving tablet. The prepared tablet also gives benefit in terms of patient compliance, rapid onset of action, increased bio-availability, low side effect and good stability which make these tablets popular as a dosage form for the treatment of diabetes.

Table 1: Formulation of Mouth Dissolving Tablets of Metformin HCl

INGREDIENTS (mg/each tablet)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
Metformin HCl	500	500	500	500	500	500	500	500	500	500
FGM*	12	24	36	48	60	--	--	--	--	--
Cross Carmellose Sodium	--	--	--	--	--	12	24	36	48	60
Aspartame	6	6	6	6	6	6	6	6	6	6
Magnesium Stearate	12	12	12	12	12	12	12	12	12	12
Talc	3	3	3	3	3	3	3	3	3	3
Aerosil	3	3	3	3	3	3	3	3	3	3
Flavor (Orange)	6	6	6	6	6	6	6	6	6	6
Avicel q.s.to	58	46	34	22	10	58	46	34	22	10
Total weight of tablet	600	600	600	600	600	600	600	600	600	600

FGM* Fenugreek Mucilage

Figure 1: FT-IR spectra of fenugreek mucilage powder (between 500 to 4000 cm^{-1})

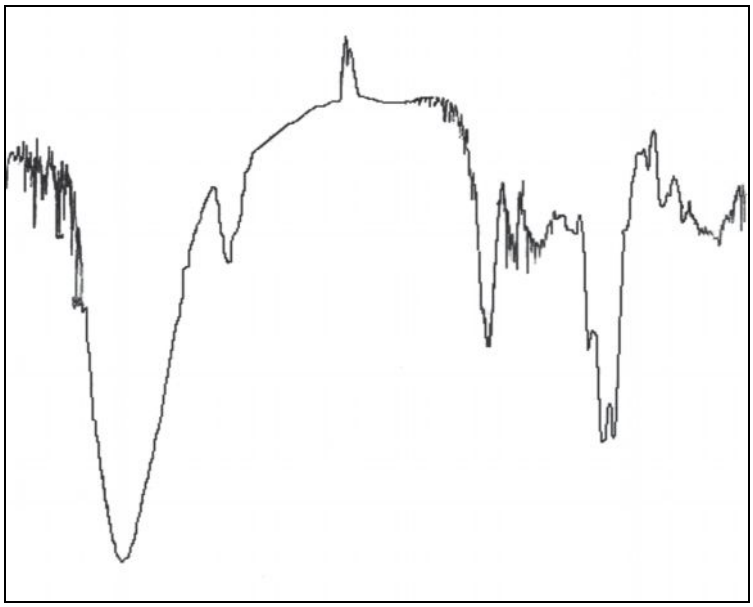


Table No 2: Physico-chemical characterization of Fenugreek seed mucilage

Parameters	Result
State	Amorphous powder
Odor	No characteristic odor
Taste	Tasteless
Color	Off white- cream yellow color
Identification	-
a. Mounted in 96% ethanol	Transparent angular masses
b. Mounted in Ruthenium red	Particles stained red
c. Mounted in iodine solution	Particles stained blue
pH (1%w/v)	5.6
Moisture content (%)	2.4
Ash value (%)	0.85
Water-soluble ash (%)	0.35
Acid insoluble ash (%)	0.21
Swelling index	-
In distil water	35
In 0.1 N HCl	10
In Phosphate Buffer pH 7.4	15
Test for carbohydrate (Mollish's test)	+
Test for tannins (Ferric chloride test)	-
Test for chloride (silver nitrate test)	-
Test for sulphate (Barium chloride test)	-
Total bacterial count	
E.coli	Absent
Salmonella typhi	Absent
S.aureus	Absent
Solubility	Slightly soluble in cold water, But quickly dissolves in warm water, forms viscous colloidal solution, insoluble in ether, acetone, chloroform, methanol, ethanol
Angle of repose	22.25^0
Bulk density	0.64
Tapped density	0.53
Compressibility Index (%)	15.20
True density	1.3g/dl
Yield (%)	31
Viscosity (1%)	500 cP
Hausner ratio	0.12

Table 3: Results of Preformulation studies of Metformin HCl

Sl.No.	Parameters	Results
1.	Description	White and crystalline powder
2.	Loss on drying	0.2
3.	pH (1% solution)	6.5
4.	Log P	-1.43
5.	pKa	11.5
6.	Solubility	
7.	In distil water	>100mg/ml
8.	In 0.1 N HCl	100mg/ml
	In pH 4.5	100mg/ml
	In pH 6.8	100mg/ml
	In pH 9.5	100mg/ml
9.	Chloroform	Practically insoluble
10.	Acetone	Practically insoluble
11.	Ether	Practically insoluble

Figure 2: FTIR Spectra of Metformin HCl

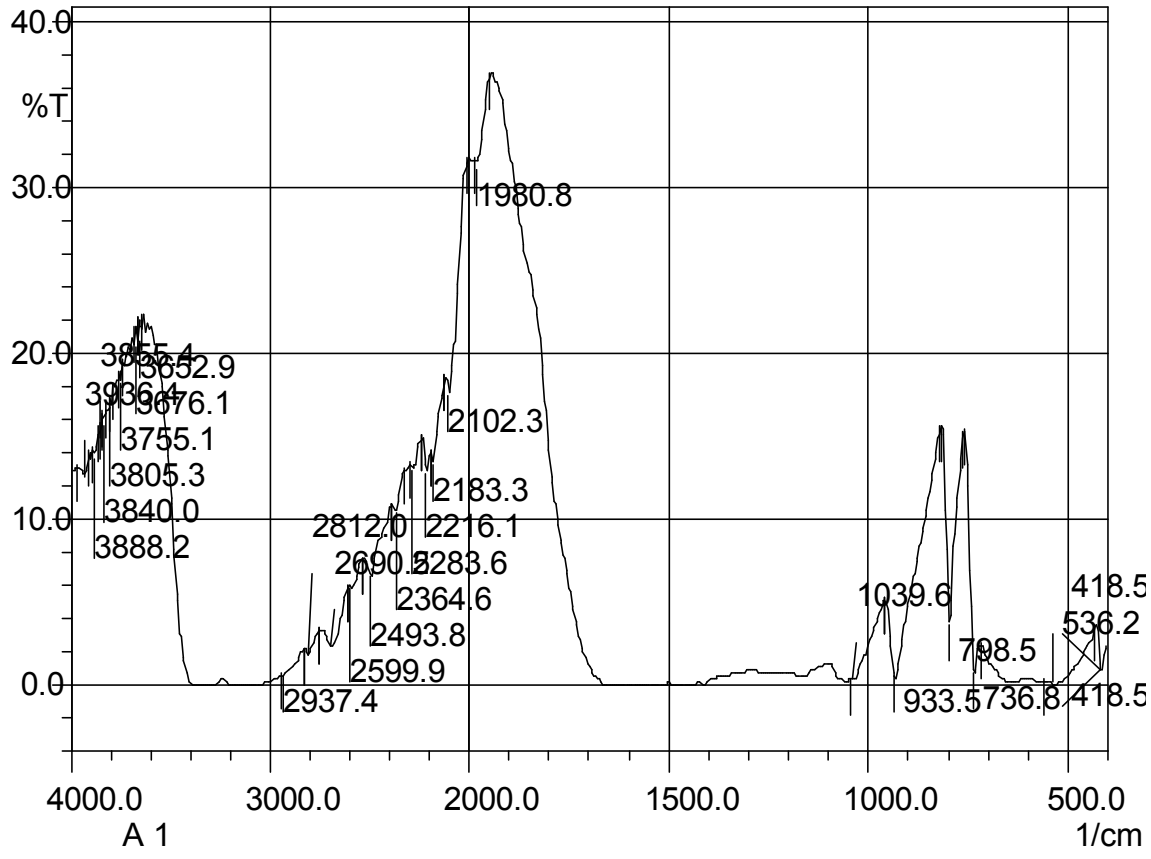


Figure 3: FTIR Spectra of physical mixture of drug and excipients

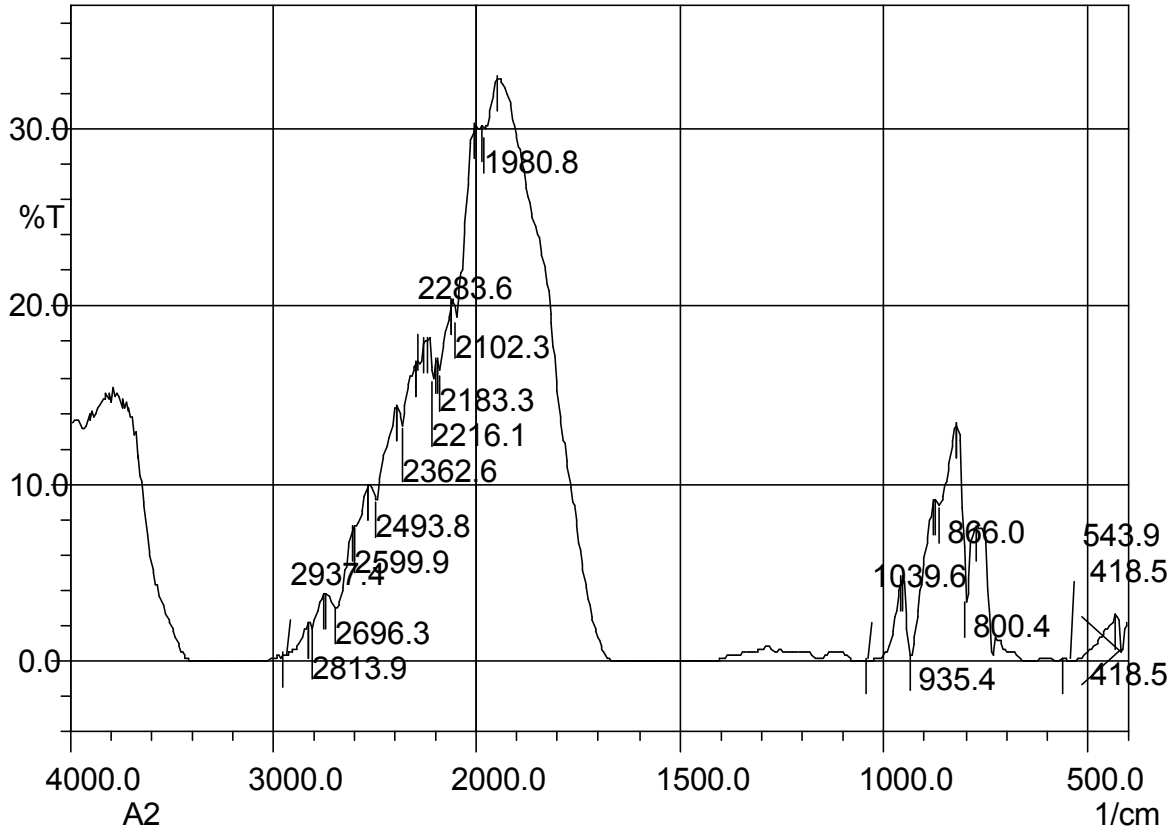


Figure 4: DSC Thermogram of Metformin HCl

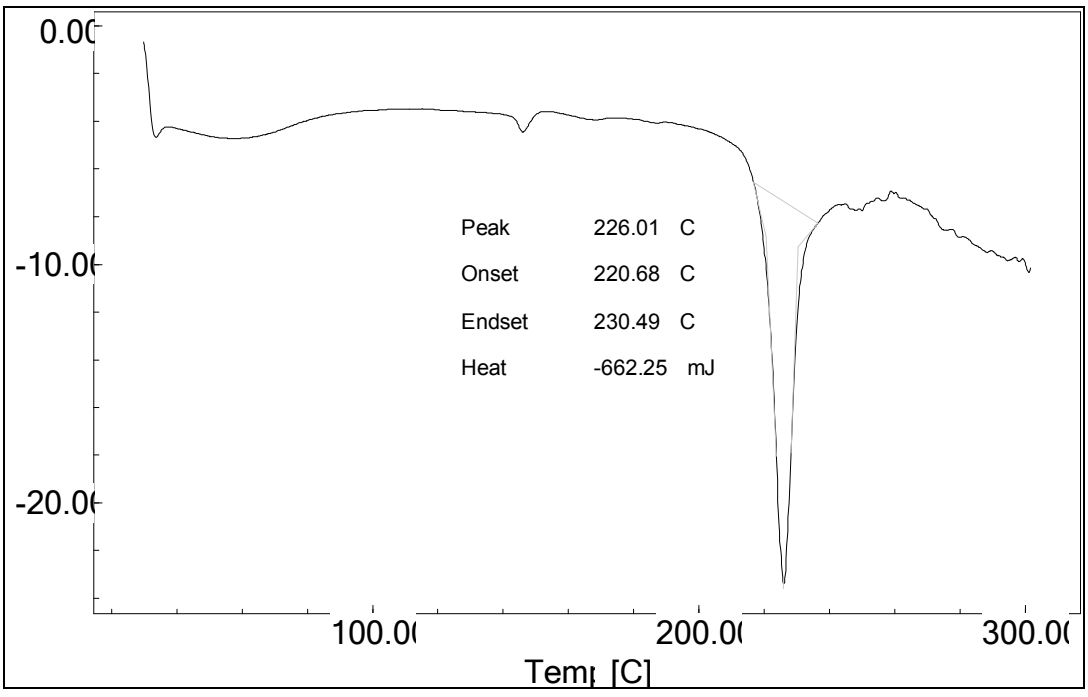


Figure 5: DSC Thermogram of physical mixture of drug and excipients

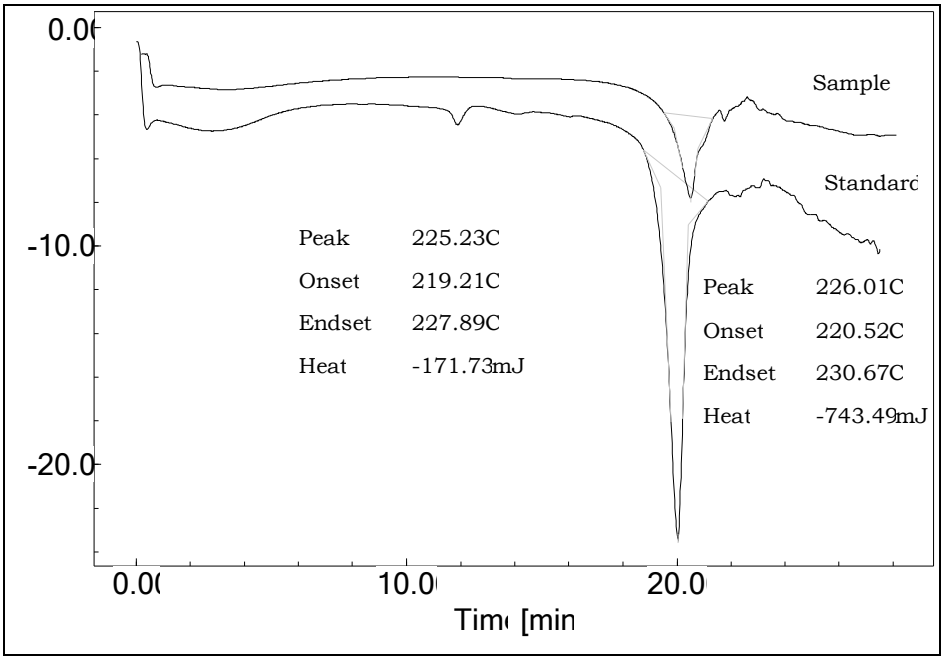


Figure 6: Chromatogram of drug in distil water at the of 1 hour

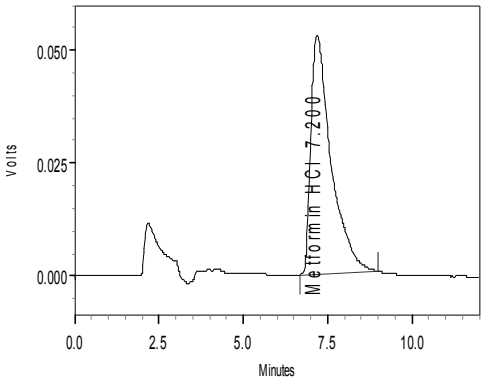


Figure7:Chromatogram of drug in distil water at the end of 24hour

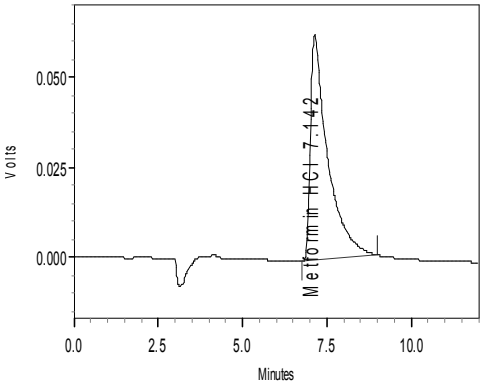


Figure 8:Chromatogram of drug in 0.1 M HCl at the end of 1 hour

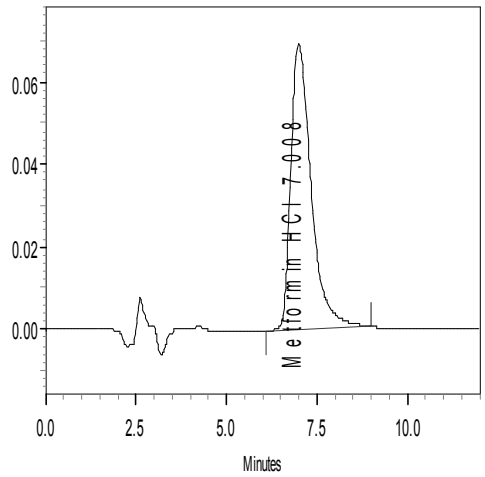


Figure 9: Chromatogram of drug in 0.1 M HCl at the end of 24 hour

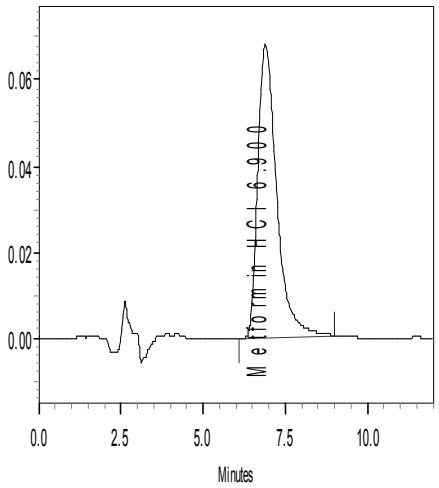


Figure 10:Chromatogram of drug in Phosphate buffer at the end of 1 hour

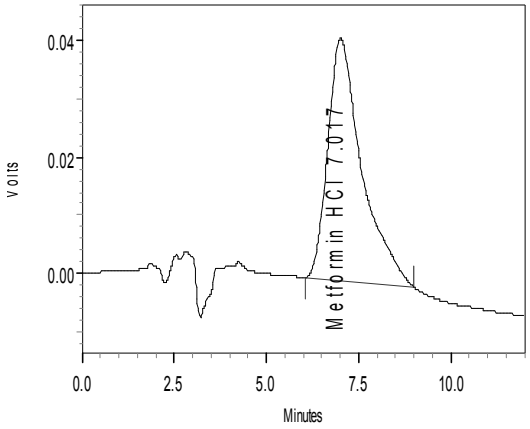


Figure 11: Chromatogram of drug in Phosphate buffer at the end of 24 hour

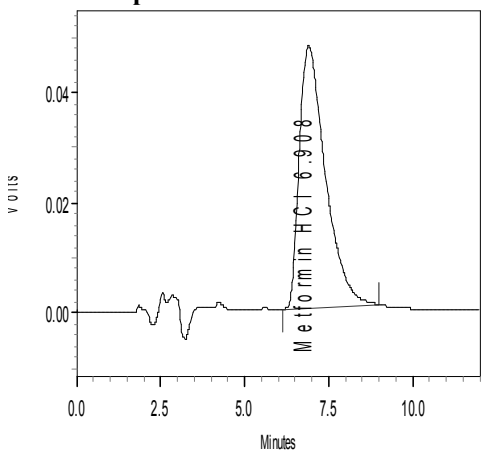


Table 4: Results of biochemical parameters in rats treated with fenugreek mucilage

Treatment	ALP (U/L)	ACP (U/L)	AST (U/L)	ALT (U/L)	Urea (U/L)	Creatinine (U/L)
Control (0.5%CMC)***	75±4.25*	35±2.95	68±3.75	60±2.16	44±1.23	0.5±0.12
Treatment (FGM)**** 500 mg/kg)	78±4.28**	35±3.00	65±3.91	63±2.56	41±2.54	0.4±0.18

*Data represents as the mean ±SD of 6 animals; **Data represents as the mean ±SD of 16 animals
CMC; Carboxy methyl cellulose; FGM* ; fenugreek mucilage

Table 5: Results of Hematological changes observed in rats during and after treatment of FGM for 30 days

Treatment	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb (g/dl)	N	L	E
Control (0.5% CMC)	4.1±0.07*	6950±0.25	12.75±0.15	10±0.18	90± 0.12	0±0.00
Test(FGM) 500 mg/kg)	4.2±0.08**	7100±0.18	13.25± 0.12	13±0.14	86± 0.18	1± 0.32

*Data represents as the mean ±SD of 6 animals; **Data represents as the mean ±SD of 16 animals

Table 6: Results of blend properties of metformin HCl.

Formulation code	**Angle of repose(°)***	**Bulk density (gm/cm^3)***	**Tapped density (gm/cm^3)***	**Carr's index (%)***	**Hausner ratio (H_R)***	**Bulkiness (cc/g)***
F1	28.1±0.01	0.57±0.02	0.71±0.03	19.0±0.01	1.24±0.02	1.75±0.02
F2	26.3±0.02	0.55±0.03	0.67±0.02	16.9±0.03	1.22±0.03	1.79±0.03
F3	27.6±0.04	0.55±0.01	0.70±0.02	19.9±0.04	1.27±0.02	1.82±0.03
F4	26.9±0.05	0.54±0.03	0.73±0.03	21.5±0.02	1.35±0.04	1.72±0.20.01
F5	30±0.02	0.53±0.01	0.67±0.01	20.8±0.02	1.26±0.05	1.89±0.02
F6	28.0±0.03	0.57±0.01	0.74±0.02	23.1±0.01	1.29±0.02	1.75±0.04
F7	32.6±0.01	0.56±0.02	0.74±0.02	23.7±0.01	1.30±0.04	1.79±0.05
F8	27.3±0.02	0.54±0.04	0.73±0.03	22.8±0.01	1.32±0.01	1.75±0.01
F9	27.9±0.03	0.55±0.02	0.72±0.01	18.7±0.02	1.24±0.01	1.75±0.01
F10	26.3±0.04	0.57±0.03	0.67±0.02	19.9±0.01	1.24±0.01	1.72±0.02

*All values are expressed as mean ± SD, n=3.

Figure12: Histological sections of vital organs after treatment of FGM for 30 days

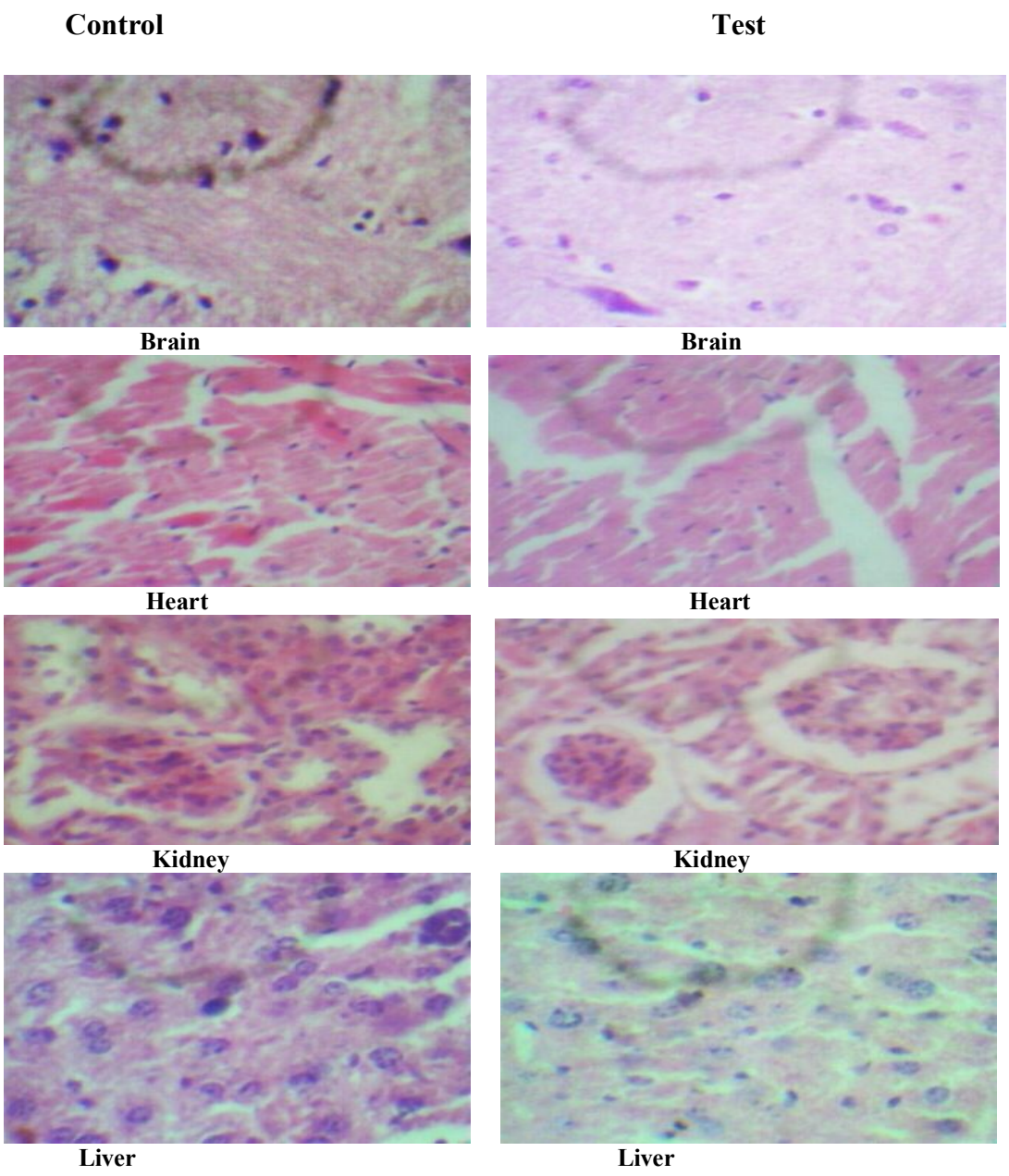


Table 7: Results of Post Compression Properties of metformin HCl Tablets

Formulation code	Thickness (mm)*	Diameter (mm)*	Hardness (kg/cm^2)*	Friability (%)***	Drug content (%)**	Weight variation (mg)**	pH of the solution
F1	4.0±0.01	12.00±0.01	2.9±0.10	0.55±0.04	99.12±0.01	599±0.01	6.3
F2	4.1±0.02	11.00±0.02	2.8±0.12	0.65±0.02	98.34±0.02	600±0.03	6.4
F3	4.2±0.01	12.00±0.03	3.2±0.14	0.33±0.03	100.12±0.04	598±0.04	6.6
F4	3.9±0.03	12.00±0.01	3.0±0.16	0.21±0.05	101.34±0.05	601±0.05	6.6
F5	3.8±0.05	11.00±0.02	2.8±0.12	0.54±0.06	98.12±0.04	602±0.06	6.5
F6	4.0±0.01	11.00±0.01	3.2±0.16	0.23±0.04	99.45±0.05	598±0.07	7.7
F7	4.1±0.02	11.00±0.04	3.1±0.14	0.24±0.05	100.43±0.06	599±0.01	7.6
F8	4.2±0.01	11.00±0.03	2.9±0.16	0.32±0.01	101.91±0.01	604±0.02	7.5
F9	4.2±0.01	12.00±0.02	3.3±0.14	0.39±0.02	100.12±0.02	598±0.03	7.4
F10	3.9±0.03	12.00±0.01	2.8±0.12	0.33±0.03	101.34±0.01	602±0.04	7.2

*All values are expressed as mean ± SE, n=5; **All values are expressed as mean ± SE, n=20; ***All values are expressed as mean ± SE, n=10.

Figure13: Comparison between disintegration time in oral cavity, wetting time and disintegration time (*in vitro*) for metformin tablets.

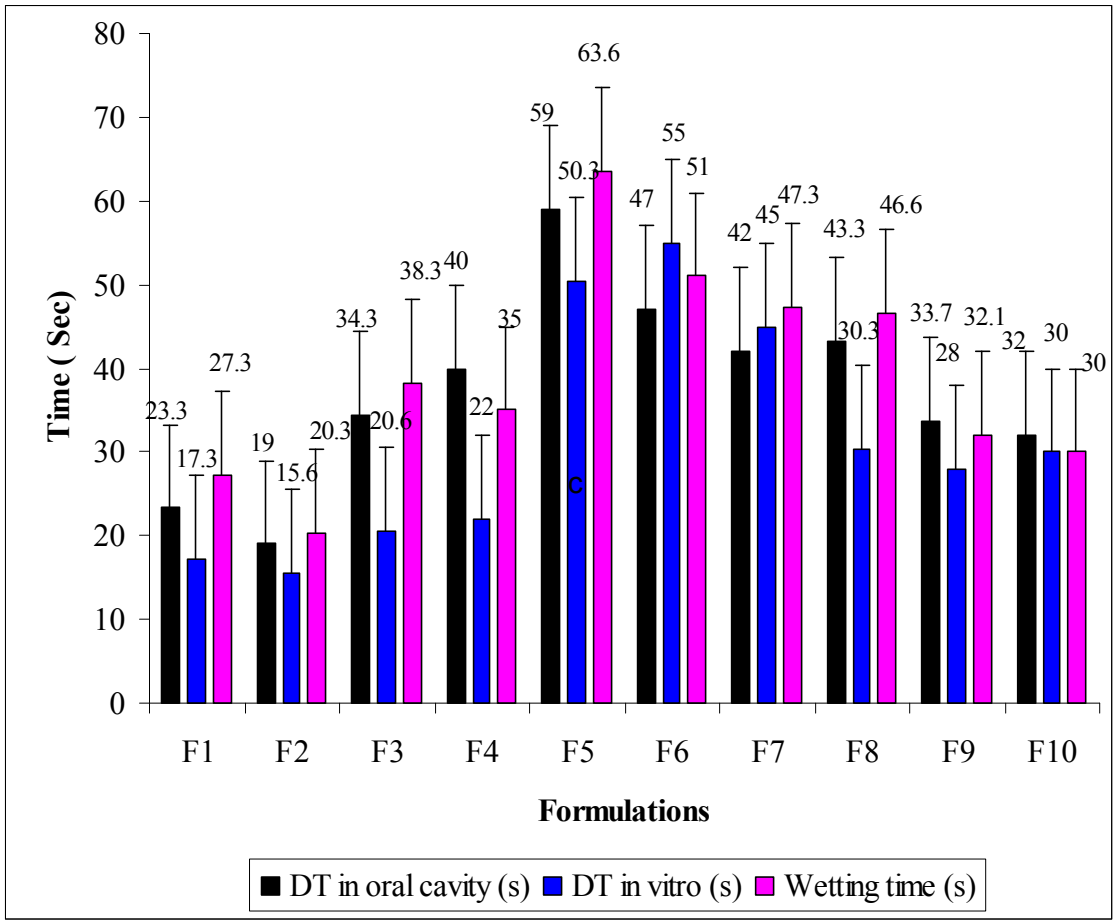


Figure 14: Comparison of dissolution profiles for various metformin HCl formulations

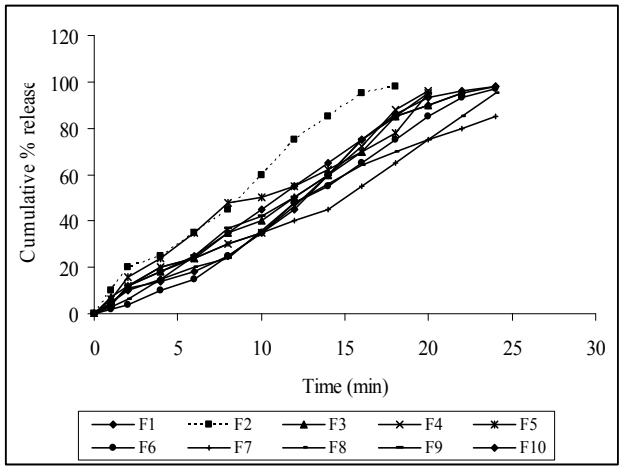


Figure 15: Comparison of dissolution profiles of optimized formulation (F2) and marketed product

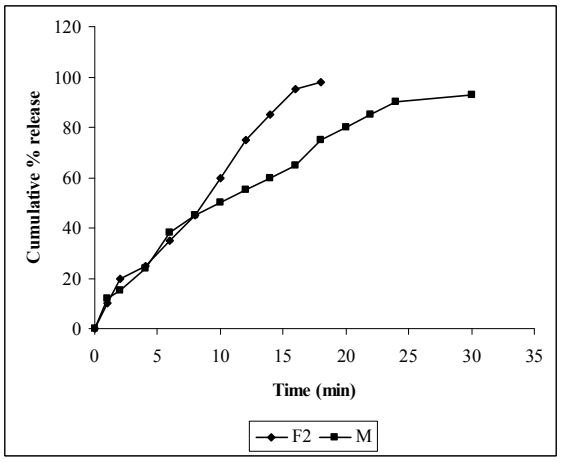


Figure 16: Moisture absorption capacity of various metformin tablets containing disintegrants

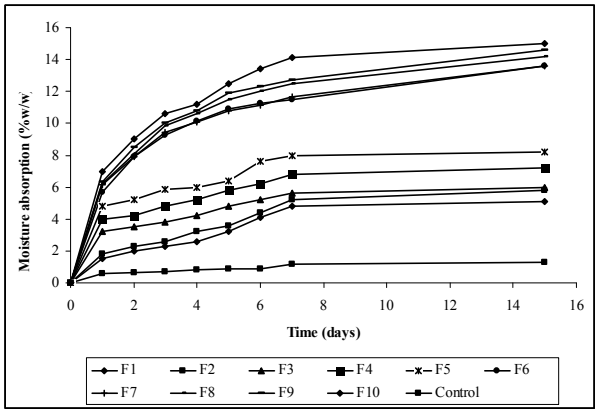
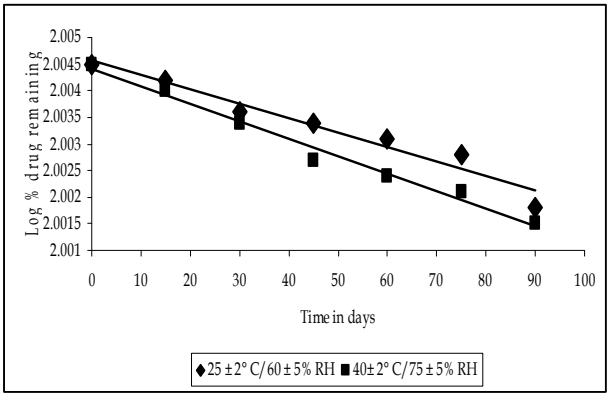


Figure 17: Stability data for optimized formulation (F2)



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REFERENCES

1. Nolte MS, Karam JH. "Pancreatic hormones and antidiabetic drugs", in Katzung BG (eds), Basic and clinical pharmacology. 8th ed., Lange Medical Books/McGraw-Hill Publishing New York, 2002; pp: 711-734.
2. Davis SN, Granner D.K. "Insulin, oral hypoglycemic agents, and the pharmacotherapy of the endocrine pancreas", in Hardman JG: Limbird LE (eds), the pharmacological basis of therapeutics. 9th ed., McGraw-Hill Co., New York, 1996; pp: 1487-1517.
3. Sweetman SC. "Martindale: the complete drug reference", 34th.ed. London: Pharmaceutical Press; 2005; pp: 2756.
4. Seager H. "Drug delivery products and the zydis fast dissolving dosage forms", J Pharm Phamacol., 1998; 50(4): 375-382.
5. Habib W, Khankari R and Hontz J. "Fast dissolving drug delivery systems critical review in therapeutics", Drug Carrier System., 2000;17(1):61-72.
6. Chang RK, Guo X, Bumside BA and Couch RA." Fast dissolving tablets", Pharm Tech., 2000; 17(1):61-72.
7. Bi YX, Sunada H, Yonezawa Y and Danjo K. "Evaluation of rapidly disintegrating tablets prepared by direct compression method", Drug Dev Ind Pharm., 1999;25(5):571-581.
8. Reddy LH, Ghosh B and Rajneesh. "Fast dissolving drug delivery system: A review of the literature", Indian J Pharm Sci., 2002; 64(4):1-3.
9. Bradoo R, Shahani S, Poojary SM, Dewwan B and Sudarshan S. "An observed blind, randomized controlled clinical trial to compare the onset of action, efficacy and safety of cetirizine conventional tablets in allergic rhinitis, cetirizine conventional tablets in allergic rhinitis", JAMA India., 2001; 4(10):27-31.
10. Mishra DN, Bindal M, Singh SK and Kumar SGV." Rapidly disintegrating oral tablets of meloxicam", Indian Drugs., 2005; 42 (10): 685-687.
11. Kibbe AH. Editor, "Handbook of pharmaceutical excipients", 3rd ed. London (UK); The Pharmaceutical Press: 2000.
12. Tripathy S, Promod K, Banthia AK. "Novel delivery system for aceclofenac", Scientific abstract, 56th Indian Pharmaceutical Congress: 2004; pp: A71.
13. Poddar SS, Saini CR, Paresh A, Singh R. "The microencapsulation of ibuprofen by gelatin-carrageenan complex coacervation", Scientific abstract, 56th Indian Pharmaceutical Congress: 2004; pp:AP111.
14. Bharadia PD, Patel MM, Patel GC, Patel GN. "A preliminary investigation on sesbania gum as a pharmaceutical excipient", Int J Pharma Excip., 2004;3:99-102
15. Srinivas K, Prakash K, Kiran HR, Prasad PM, Rao ME. "Study of *Ocimum basilicum* and Plantago *ovata* as disintegrants in the formulation of dispersible tables", Indian J Pharm Sci., 2003;65:180
16. Gilbert VL. "Tagatose, the new GRAS sweetener and health product", J Med Food., 2002;5:23-36.
17. Khanna M, Nandi RC, Singh S, Jain GK. "Standardization of pure isapgol (*Plantago ovata*) mucilage for pharmaceutical use", Indian J Pharm Sci., 1988;50:238-40.
18. Gowthamarajan K, Kulkarni GT, Muthukumar A, Mahadevan N, Samantha MK, Suresh B.

"Evaluation of fenugreek mucilage as gelling agent", Int J Pharma Excip., 2002;3:16-9.
19. Banker GS, Anderson NR. "Tablets", In: Lachman L, Lieberman HA, Kanig JL, editors, The theory and practice of industrial pharmacy, 3 rd ed., Mumbai; Varghese Publishing House, 1987; pp: 336.
20. Chien YW. "Novel drug delivery system", 2nd ed, Marcel Dekker, New York. 2005; pp: 249-267.
21. Kokate CK, Purohit AP and Gokhale SB. Pharmacognosy, Nirali Prakashan. Pune, 15th ed, 2005; pp: 98-102.
22. The wealth of India, first supplement series, volume-3: Si-Ty, New Delhi, Dr K S Krishna Marg; National institute of science communication, CSIR, 199: pp:89-137.
23. Kirtikar KR and Basu BD. "Indian medicinal plants", BLM Basu Publications Allahabad, 3rd ed ,1991: 65-67.
24. Baveja SK, Rao KV, Aroara J. "Examination of natural gums and mucilages as sustaining materials in tablet dosage forms; part-II", Indian JPharmSci., 1989; 51: 115-118.
25. Baveja SK, Rao KV, Aroara J. "Examination of natural gums and mucilages as sustaining materials in tablet dosage forms", Indian J PharmSci., 1988; 50: 89-92.
26. Trease GE, Evans MC. Editors, "Text book of Pharmacognosy", 15th ed., balliere, tindall; London: 2002.
27. Petropoulos G A. "Botany. In G. A. Petropoulus (Ed.) Fenugreek: The genus Trigonella", London: Taylor and Francis, 2002; pp: 9–17.
28. Abdul-barry JA, Abdul-Hassan IA, Al-Hakein MA. "Hypogylacemic and antihyerglycaemic effects of *Trigonella Foenum-graceum* leaf in normal and alloxan induced diabetic rats", J Ethnopharmacol., 1997; 58: 149-55.
29. Ahasn SK, Tariq M, Agell MM, Al-Yahya MA, Shah AH. "Effect of *Trigonella Foenum-graceum* and *Ammi majus* on calcium oxalate urolithiasis in rats", J Ethnopharmacol., 1989; 26: 249-54.
30. Al-Habori M A." Pharmacological properties. In G. A. Petropoulus (Ed.), *Fenugreek: The genus Trigonella*", London: Taylor and Francis, 2002; pp:162–163.
31. Karawya M S. "Mucilagenous contents of certain Egyption plants", Planta Medica., 1980; 38: 73–78.
32. Harborne J B. "Sugars and their derivatives. In, Phytochemical methods: A guide to modern techniques of plant analysis",3 ed.; London:Chapman & Hall, 1998; pp: 235–290.
33. British Pharmacopoeia Vol. II , Her Majesty's Stationery Office, London, 1988: 140.
34. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy., 24th ed. Pune; Nirali Prakashan: 2003; pp:109.
35. Indian Pharmacopoeia. 4th ed. Ministry of health and family welfare, Govt. of India, New Delhi; Controller of publications: 1996; pp: A-54.
36. British Pharmacopoeia. volume 2, 2000; pp: A-207, 210.
37. Knudsen LF, Curtiss JM. "The use of the angular formulation in biological assays", J Am Stat Soc., 1947; 42:282-96.
38. Fiese E F and Hagen T A, In; Lachman, Leon, Liberman, H.A. Kanig, J.L., Edn., "The Theory and practice of Industrial Pharmacy", 3rd Edn, varghese Publishing House, Mumbai, 1987:183.
39. Banker GS and Anderson GR. In; Lachman, Leon, Liberman, H.A. Kanig, J.L., Edn., "The Theory and practice of Industrial Pharmacy", 3rd Edn, Varghese Publishing House, Mumbai, 1987: 293.
40. The British pharmacopoeia, Department of health/ by spationary office on behalf of the medicine and health care product regulatory agency, Crown Copy Right, 5th edition. 2005:1303-1304, 2588-2589, A133.
41. The United State Pharmacopoeia 24/NF19, Asian edition, the official compendia of standard United States Pharmacopoeial Convection Inc. Rockville.1995: 1015, 1016 and 1791.
42. Qalaji-Rawas MM, Simons ER and Simons KJ. "Fast disintegrating Sublingual Tablets: Effect of Epinephrine Load on Tablet Characteristics", AAPS PharmSciTech., 2006; 7(2): E1-E7.

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