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Formulation and evaluation of fixed dose combination of sustain release Glipizide and conventional release of Telmisartan drug bi-layered tablet with guggul as binding agent.

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ABSTRACT

The emerging new fixed dose combination of Glipizide (GLZ) as sustained release and Telmisartan (TEL) as immediate release were formulated as a bilayer matrix tablet using guggul. Guggul used as a binding agent with three different concentrations (70%, 80%, and 90%; F1-F6) was used for the preparation of first layer of tablet containing glipizide. The conventional tablet of telmisartan was prepared as a second layer. Prepared bilayer tablets were studied for the in vitro release study carried out at pH 1.2 for first two hours and then at pH 6.8 for 6 hours using USP dissolution apparatus II (Basket). All the evaluation tests were found to be within the acceptance limits specified in Indian Pharmacopeia. The assay and dissolution study of tablets were done by HPLC which was developed and validated according to the ICH Q2 (B) guidelines. The contents of assay of the tablets were found to be 90.92% - 103.84% for F2-F5. The release of glipizide from the tablet was extended up to 8 h. Tablet containing 2.5 mg of glipizide with 80% of guggul release of drug after 8 h was found to be 82.36% and tablet containing 5 mg of glipizide with 90% guggul release of glipizide after 8 h was found to be 74.67%. The release pattern of each formulation (F2-F5) was fitted into the dissolution kinetics models and all the formulations were best fitted in the Higuchi model kinetic. From the results, we have concluded that the guggul act as a binding agent as well as rate retarding agent.

Keywords: Commiphora Mukul, Fixed dose combination, Glipizide, Guggul, High Pressure Liquid Chromatography, Method development, Telmisartan, Validation.

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INTRODUCTION

Cardiovascular diseases and diabetes are the most common diseases, which require constant monitoring. The heart pumps blood into the arteries (blood vessels), which carry the blood throughout the body. High blood pressure, also called hypertension, is dangerous because it makes the heart work harder to pump blood to the body and it contributes to hardening of the arteries or atherosclerosis, leads to heart failure[1-2]. Diabetes mellitus is another major health care problem not only in the developing countries but also in the most developed countries. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia glycosuria, negative nitrogen balance sometime ketonemia. Insulin deficiency is due to functional disorder of the pancreas[3,4]. There is an increase in the patients suffering from these diseases because of many reasons like change of life style, lack of exercise, sedentary movements and food habits etc[5].

Now a day's most developed and developing countries move towards combination therapy for treatment of various diseases and disorders requiring long term therapy such as hypertension and diabetes. People suffering from these diseases have to take multiple drugs and dosage, which may result in irregular intake. Combination therapies have various advantages over monotherapy such as problem of dose dependent side effects being minimized. A low-dose combination of two different agents reduces the dose-related risk; the addition of one agent may counteract some deleterious effects of the other, minimizes the clinical and metabolic effects that occur with maximal dosage of individual component of the combined tablet and thus dosage of the single component can be produced[6]. Development and production of quality bi-layer tablets needs to be carried out using a purpose built tablet press to overcome common bi-layer problems, such as layer-separation, insufficient hardness, inaccurate individual layer weight control, cross-contamination between the layers, reduced yield, etc. Using a modified rotary tablet press is the best approach in producing a quality bi-layer tablet under GMP-conditions[7]. Fixed dose combinations (FDC) are rapidly growing acceptance with physician as an effective treatment option to improve the patient compliance and another reason to go for FDC is the involvement of reduction of drug cost[8-10].

Glipizide (GLZ) is an oral hypoglycemic agent belongs to sulfonylurea; it is rapidly absorbed and completely metabolized. GLZ Figure 1a, is chemically described - N[2(4{[(cyclohexylcarbamoyl) amino]sulfonyl} phenyl)ethyl]-5-methylpyrazine-2-carboxamide (MW 445.535). Sulfonylureas likely to bind with ATP-sensitive potassium-channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the

membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis of insulin[11-13]. Telmisartan (TEL) is a potent, long-lasting, nonpeptide antagonist of the angiotensin II (AT1) receptor that is indicated for the treatment of essential hypertension. It is freely soluble in methanol, insoluble in water and chemically Figure 1b, 4[(1,4-dimethyl-2-propyl(2,6-bi-1H-benzimidazol]-1-yl)methyl] [1,1-biphenyl]-2-carboxylic acid .TEL selectively inhibits stimulation of the AT1 receptor by angiotensin II without affecting other receptor systems involved in cardiovascular regulation[14-16].

Our study, describes the preparation of a fixed dose combination of sustain layer of GLZ and immediate layer of TEL by using guggul as binder, to target multiple therapeutic activities which increases patient compliance remarkably by targeting hypertension and diabetes, which leads to atherosclerosis. This type of drug delivery has proved to provide a solution to several problems encountered in the repeated administration of such drugs, an attempt was made to design and evaluate bilayer tablets of GLZ sustain release and TEL as immediate release. The developed formulation was evaluated, physicochemical characterstics, and drug release profile, simultaneously studied the effect of guggul as the rate controlling excipient. The drug releases from the tablets were analyzed by using HPLC method. This is new combination developed by us, there is a no validated HPLC method is available from literature to simultaneous determination of GLZ and TEL till the date. Therefore, we developed a new RP-HPLC method for this combination (GLZ and TEL) and which was validated according to the ICH guidelines Q2B[17]. This validated method was employed for analysis of dissolution sample and amount of drug (assay) present in the prepared formulations.

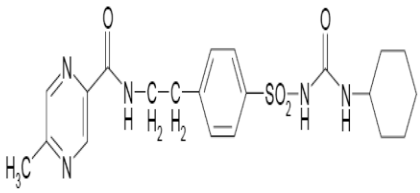

Figure.1a

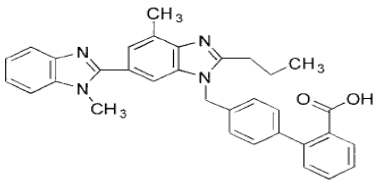

Figure.1b

Figure.1. Chemical structures of drugs used in this method. (a)Glipizide (b) Telmisartan

MATERIALS AND METHODS

Materials

The instruments employed in this study are as follows; HPLC-1260 series with auto-sampler, Agilent technology, Germany,. Dissolution apparatus- Labindia, Mumbai, India. Bi-layer compression machine with 9 stations- Karnavathi. Analytical balance- Afcoset, German. PH

meter- Systronics, Ahmadabad, India. Cyclomixer- Remi, Mumbai, India. Friability apparatus- Labindia, Mumbai, India. Melting point apparatus- Analab, Vadodara, India.

Standards and chemicals

Pure drugs Glipizide and Telmisartan are obtained from S.L.Drugs, S.R. Nagar, Hyderabad, India. Guggul a Release modifying agent was obtained from Madvik research labs. Microcrystalline cellulose, Magnesium stearate, Polyvinyl pyrollidonek-30, Ethanol, Ether, DMSO, Ethylactetate, Orthophosphoric acid, Potassium Dihydrogen phosphate, Triethylamine were obtained from S.D.Fine Chemicals Pvt Ltd. Methanol, Acetonitrile, Chloroform, Hydrochloric acid, n-hexane, Cyclohexane from Merck, Mumbai, India. HPLC grade water and distilled water was prepared by using Millipore direct Q water purification system.

Methods

Preformulation studies

Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rational development of dosage forms. Preformulation commences when a newly synthesized drug shows sufficient pharmacologic promise in animal models to warrant evaluation in human. These studies should focus on those physicochemical properties of the new compound that could affect drug performance and development of an efficacious dosage form. A thorough understanding of these properties may ultimately provide a rationale for formulation design, or support the need for molecular modification. The overall objective of preformulation testing is to generate information useful to the formulation in developing a stable and bioavailable dosage forms. The use of preformulation parameters maximizes the chances in formulating to an acceptable, safe, efficacious and stable product.

Physical compatibility:

Drug Excipient compatibility studies were carried out by mixing the drug with various excipients in different proportions was placed in a vial, rubber stopper was placed on the vial and sealed properly. Studies were carried out in glass vials at accelerated conditions, 40ºC ± 2°C / 75%RH ± 5 % RH and a storage period of 4 weeks. After storage, the samples were observed physically for discoloration and degradation[18]. Chemical compatibility of GLZ and TEL and between GLZ and TEL with excipients was carried out with IR spectroscopy by using potassium bromide (KBr) pellet method.

Determination of Bulk Density and Tapped Density:

An accurately weighed quantity of the granules/ powder (W) was carefully poured into the graduated cylinder and volume (V_0) was measured. Then the graduated cylinder was closed with lid and set into the tap density tester (USP). The density apparatus was set for 100 tabs and after that the volume (V_f) was measured and continued operation till the two consecutive readings were equal. The bulk density and the tapped density were calculated using the following formulae[19].

$$\text{Bulk density} = W/V_0 \quad \text{..... Eq (1)}$$

$$\text{Tapped density} = W/V_f \quad \text{..... Eq (2)}$$

Where, W= Weight of the powder

V_0 = Initial volume

V_f = final volume

Angle of Repose:

The accurately weighed granules were taken in a funnel. The height of the funnel was adjusted in such a manner that the tip of the funnel just touched the apex of the heap of the granules. The granules were allowed to flow through the funnel freely onto the surface. The diameter of the powder cone measured and angle of repose was calculated using the following equation [20].

$$\tan \theta = h/r \quad \text{..... Eq (3)}$$

Where, h = height of the powder heap

r= radius of the powder heap,

θ is the angle of repose.

Hausner's Ratio and Carr's index:

Hausner's Ratio indicates the flow properties of the powder and is measured by the ratio of tapped density to bulk density [21]. It is the ratio of tapped density and bulk density. Hausner found that this ratio was related to interparticle friction and, as such, could be used to predict powder flow properties. Generally a value less than 1.25 indicates good flow properties, which is equivalent to 20% of Carr's index.

$$\text{Hausner's Ratio} = \text{Tapped density/Bulk Density} \quad \text{..... Eq (4)}$$

Carr's index is measured using the values of bulk density and tapped density. The following equation is used to find the Carr's index [22].

$$\% \text{ Compressibility} = (P_t - P_O / Pt)_x 100 \quad \text{..... Eq (5)}$$

Where, P_t = Tapped density

P_O = Bulk density

FORMULATION OF BI-LAYER MATRIX TABLETS

Bilayer matrix tablets containing Glipizide and Telmisartan were prepared by wet granulation techniques using various concentrations of Guggul and other ingredients as shown in **Table. 1.**

Table.1. Quantities of excipients in different formulation (F1-F6)

Ingredients	F1*	F2*	F3*	F4*	F5*	F6*
First layer for GLZ						
GLZ	2.5	2.5	2.5	5	5	5
GUGGUL	140	160	180	180	160	140
Methyl carboxy cellulose	56	36	16	13.5	33.5	53.5
Magnesium stearate	1.5	1.5	1.5	1.5	1.5	1.5
Water	q.s	q.s	q.s	q.s	q.s	q.s
Second layer for TEL						
TEL	20	20	20	40	40	40
Methyl carboxy cellulose	27	27	27	7	7	7
Magnesium stearate	0.5	0.5	0.5	0.5	0.5	0.5
PVP-K30	2.5	2.5	2.5	2.5	2.5	2.5

*Quantities are mentioned in mg

Steps involved in the formulation of first layer granules:

Glipizide, Guggul powder and microcrystalline cellulose were passed through sieve #40. Sufficient purified water is added to form granulating agent. Totally six formulations were prepared with different concentrations of guggul. In F1 and F6 guggul with one concentration. In F3 and F4 guggul with one concentration. F2 and F5 with one concentration. By using water to their respective formulation wet mass is prepared. This wet mass passed through the sieve #20 to obtain the granules and the dose difference are for F1, F2, F3 is 2.5mg and for F4 ,F5, F6 is 5mg. Granules were dried in rapid dryer. Inlet air temperature was maintained at 60±5ºc, loss. After drying, these granules were passed through the sieve #40 sieve. Magnesium stearate was passed through sieve #40 and mixed with prepared granules as a gliding agent.

Steps involved in the formulation of second layer granules:

Telmisartan, microcrystalline cellulose, were passed through sieve #40. PVP K-30 is mixed with other ingredients in required concentration and purified water is added and made granules. As F1, F2, F3 with one API dose and F4, F5 and F6 with other dose. Totally six formulations were prepared two concentrations of API with PVP K-30 as binder. In F1, F2 and F3 the dose is 20 mg while in F4, F5 and F6 dose it is 40 mg. By using this binding agent to their respective formulation, wet mass is prepared. This wet mass passed through the sieve #20 to obtain the granules.

Granules were dried in rapid dryer. Inlet air temperature was maintained at 60±5ºc, loss. After drying these granules were passed through the sieve #40 sieve. Magnesium stearate was passed through #40 and mixed with prepared granules, which is used as a gliding agent.

Compression of granules:

The prepared granules were punched by using Karnavati 9 station bi-layer punching machine. By maintaining following parameters. Punch: 9mm, Shape: round shaped, Pressure: 4-5 Psi, Tooling: BB size (medium). At first, sustained release, granules of glipizide were compressed with pre compression force followed by immediate release granules of telmisartan for final compression. The prepared bilayer tablets were studied weight variation, percentage friability, thickness, hardness and in vitro drug release.

EVALUATION OF BI-LAYERED TABLETS:

The formulated bi-layered tablets were evaluated for different parameters like, weight variation, friability, hardness, drug content and dissolution (Invitro drug release from the dosage form) according to the procedures mentioned in IP and USP [23-25].

Weight variation:

The weight variation test was carried out by weighing 20 tablets individually, calculating the average weight. Then calculated the percentage (%) deviation from the average weight of each tablet, the tablets meet the USP test if no more than 2 tablets are outside the % limit and if no tablet differs by more than 2 times the % limit.

Hardness:

The hardness of a tablet is indicative of its tensile strength and is measured in terms of load/pressure required to crush it when placed on its edge. Pfizer hardness tester was used for this study and hardness of 5kg is considered minimum for uncoated tablets for mechanical stability.

Friability:

Generally, it refers to loss in weight of tablets in the containers due to removal of fine particles from their surfaces. Roche friability tester was used pre-weighed tablets are placed in the apparatus, which is given 100 revolutions after which the tablets are weighed again. The difference in the two weights represents friability. The weight loss should not be more than 1%.

Thickness:

Thickness of the tablet was determined for 20 tablets of each batch using a digital vernier scale. The average thickness was determined in mm. the tablets thickness should be controlled within a ±5% variation of the standard.

Drug content:

The amount of drug present in the prepared formulations was determined by using a validated HPLC method. Twenty tablets were weighed (individual formulations separately), finely

powdered and accurately weighed. The sample of powder equivalent to one tablet was transferred into 100 mL volumetric flask and drug was extracted from methanol. This solution was filtered through Whatmann No. 1 filter paper and the solution obtained was diluted with mobile phase, to obtain a concentration in the range of linearity previously determined.

Dissolution:

The Invitro dissolution studies were carried out using USP apparatus II (Basket) at 75 rpm. It was carried for 8 h; first 2 h in acidic media pH 1.2 and then in pH 6.8 phosphate buffer. The samples (5mL) were collected at different time intervals and made up of a suitable dilution with mobile phase, injected into the HPLC system. The concentration of each drug was calculated from the respective linearity graph.

HPLC method development and validation:

The literature search revealed that there were no HPLC methods reported for this (TEL and GLZ) combination. Hence, we developed and validated a simple RP-HPLC for simultaneous determination of TEL and GLZ.

HPLC method optimization:

TEL and GLZ are hydrophobic, almost insoluble in aqueous solutions and are freely soluble in methanol; the reversed phase chromatography was adopted. Hydrophobic C-8 a stationary phase column was tried. During the method development, top priority was given for the complete separation of TEL and GLZ. The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier, buffer used in the mobile phase and composition of mobile phase. Buffers like ammonium acetate, phosphate buffer in various strengths were tried along with methanol and acetonitrile as organic solvent. Mixtures of methanol and phosphate buffer (pH 3.5) in the various proportions were tested as mobile phase with grace Smart C-8 column and the method was optimized with the mobile phase composition of methanol: phosphate buffer pH 3.5 (70:30 % v/v).Buffer molarity of 10, 20 and 50 mM were tried. There were no significance changes in the chromatographic response and peak shape with change in buffer molarity, a buffer molarity 20mM was selected for further analysis. After several trials, the method was optimized as a mixture of 20mM potassium dihydrogen phosphate buffer (pH 3.5) and methanol (30:70 % v/v), at a flow rate of 0.8 mL/min with detection wavelength of 225 nm for 24 min run time. These chromatographic conditions achieved satisfactory system suitability parameters like, resolution (Rs), retention time (Rt), theoretical plates (TP) and tailing factor (TF) for both drugs of TEL and GLZ.

HPLC method validation:

The validation parameters like selectivity, linearity, sensitivity, accuracy, and stability studies were carried out according to the ICH guidelines[19]. Selectivity studied by comparing the chromatograms obtained from placebo sample with chromatogram obtained from standard drug mixture. Calibration curves were prepared by assaying standard samples containing two drugs of TEL and GLZ. The linearity of method was determined by plotting the peak area (y) of drug verses the nominal concentration (x) of drug respectively. Intra- and inter-day accuracy and precision for this method were determined at three different concentration levels on three different days, and on each day, three replicates were analyzed with independently prepared calibration curves. The accuracy and precision is expressed as percentage accuracy and relative standard deviation (% RSD) respectively and calculated by using following equations 6 and 7.

% Accuracy = [Mean observed concentration/Nominal concentration] X 100 ------- Eq. (6)

% RSD = [Standard deviation/Mean] X 100 ----------Eq. (7)

The limit of detection (LOD) and limit of quantification (LOQ) are defined as the lowest concentration giving a signal-to-noise ratio of at least 3-fold and 10-fold, respectively. The LOD and LOQ of this method were verified based on the standard deviation of response and slope by using the following equations 8 and 9.

LOD = [3.3σ]/slope ----------Eq. (8)

LOQ = [10σ]/slope----------Eq. (9)

Where, σ = standard deviation of intercept from calibration curve, Slope = Average slope of the calibration curve

The stability of the drug solution was determined for short-term by keeping at room temperature (25°C) for 24h and auto-sampler stability was determined by storing the samples in the auto-sampler for 24h. Each sample injected three times into HPLC and concentrations obtained were compared with the nominal values of the quality control (QC) samples

RESULTS AND DISCUSSIONS

Preformulation studies:

The Preformulation studies melting point of GLZ and TEL was observed as 208.7°C and 260.3°C respectively. The drug excipient compatibility was studied by mixing the specific ratio of drug and individual excipient kept at 40°C/75% RH, and then the colour change was observed in every week upto 4 weeks. There were no physical changes observed. FTIR: the chemical compatibility of drug with excipients and with other drug was studied by FTIR. IR spectra of GLZ showed peaks at: 1689(- C=O, amide), 1651 (-C=O, urea), 1528 (Ar-CH, stretching), 1443

(Ar-CH, bending), 1333 and 1159 cm^{-1}(-SO2NH). The IR spectrum of pure TEL showed a distinct absorption band for the carbonyl group C=O at 1700 cm^{-1} and the O-H band at 3100cm^{-1}. Bilayer tablet spectrum showed no extra peaks resulting in no interaction among the GLZ , TEL and with the excipients. The formulation of bi-layered tablet was made by using the guggul as binding agent. Batches of F1-F6 were prepared. The first layer was prepared with three different strengths of guggul i.e. 140mg, 160mg and 180mg as binding agent (to prolong the drug release from the tablet). The tablet blend of all the batches were evaluated for pre-copmpression and post –compression parameters.

Pre-compression evaluation:

The powder and prepared granules were evaluated for bulk density, tapped density, angle of repose and also studied for the Carr's index and Hausener's ratio for the different formulations. The results of angle of repose and compressibility index indicated that the powder flowability was good. The results of all these parameters are represented in **Table.2.**

Table.2. Results of pre-compression of granule evaluation parameters

	F1	F2	F3	F4	F5	F6
GLZ						
BD	0.41±0.002	0.38±0.001	0.41±0.001	0.47±0.005	0.40±0.001	0.42±0.007
TD	0.54±0.01	0.46±0.001	0.46±0.001	0.53±0.05	0.56±0.11	0.45±0.004
AR	30.41±0.15	31.72±0.11	30.44±0.34	28.17±0.04	31.38±0.32	29.86±0.09
CI	13.63±1.45	17.78±1.49	18.26±1.91	10.58±5.53	17.94±5.23	19.16±4.37
HR	1.15±0.01	1.15±0.01	1.20±0.01	1.16±0.005	1.35±0.02	1.15±0.003
TEL						
BD	0.396±0.01	0.476±0.02	0.384±0.002	0.458±0.005	0.456±0.002	0.464±0.01
TD	0.451±0.005	0.553±0.003	0.473±0.005	0.588±0.01	0.526±0.002	0.527±0.02
AR	27.66±0.11	28.81±0.05	29.90±0.372	27.89±0.57	29.25±0.55	30.88±0.95
CI	12.99±0.48	19.20±1.29	26.12±0.77	14.09±1.39	13.85±0.37	13.72±0.55
HR	1.16±0.02	1.11±0.05	1.23±0.007	1.17±0.002	1.23±0.012	1.28±0.05

Values are expressed in Mean±SD, BD-bulk density, TD-tap density, AR-angle of repose, CI-carr's index, HR-Hausener's ratio.

Post-Compression evaluation:

The granules were compressed by bilayer compression machine and each formulation was evaluated for various parameters like weight variation, hardness, thickness, and friability of prepared tablets. All the tablets passed weight variation test as the percent weight variation (deviation) was within the pharmacopoeial limits. Hardness were shown in the range of 4.8 –5.9 kg/cm^2 in all formulations which indicated good mechanical strength with ability to withstand physical and mechanical stress conditions while handling. In all the formulations, the friability

value was less than 1% and meets the official limit.The results of these parameters are represented in the **Table.3.**

Table.3. Results of Weight variation, Hardness, Thickness and % Friability

Batch	Weight variation	Hardness	Thickness	%Friability
F1	250.25±1.65	5.1±0.89	3.9±0.12	0.632±0.06
F2	250.4±1.87	4.88±0.30	3.72±0.10	0.655±0.153
F3	250.5±1.93	5.9±0.14	3.76±0.05	0.572±0.08
F4	250.3±1.92	5.34±0.53	3.84±0.08	0.626±0.135
F5	250.2±1.79	5.22±0.22	3.82±0.13	0.533±0.108
F6	250.3±1.89	5.74±0.52	3.78±0.16	0.477±0.141

Values are expressed as mean ± SD,

HPLC method development and validation:

A simple, sensitive HPLC method was developed and validated according to ICH guidelines.

Method optimization:

TEL and GLZ are hydrophobic, almost insoluble in aqueous solutions and are freely soluble in methanol; the reversed phase chromatography was adopted. Hydrophobic C-8 a stationary phase column was tried. During the method development, top priority was given for the complete separation of TEL and GLZ. The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier, buffer used in the mobile phase and composition of mobile phase. Buffers like ammonium acetate, phosphate buffer in various strengths were tried along with methanol and acetonitrile as organic solvent. Mixtures of methanol and phosphate buffer (pH 3.5) in the various proportions were tested as mobile phase with grace Smart C-8 column and the method was optimized with the mobile phase composition of methanol: phosphate buffer pH 3.5 (70:30 % v/v).

Buffer molarity of 10, 20 and 50 mM were tried. There were no significance changes in the chromatographic response and peak shape with change in buffer molarity, a buffer molarity 20mM was selected for further analysis. After several trials, the method was optimized as a mixture of 20mM potassium dihydrogen phosphate buffer (pH 3.5) and methanol (30:70 % v/v), at a flow rate of 0.8 mL/min with detection wavelength of 225 nm for 24 min run time. These chromatographic conditions achieved satisfactory system suitability parameters like, resolution (Rs), retention time (Rt), theoretical plates (N) and tailing factor (TF) for both drugs of TEL and GLZ. The chromatogram of standard mixture was shown in Figure.2.

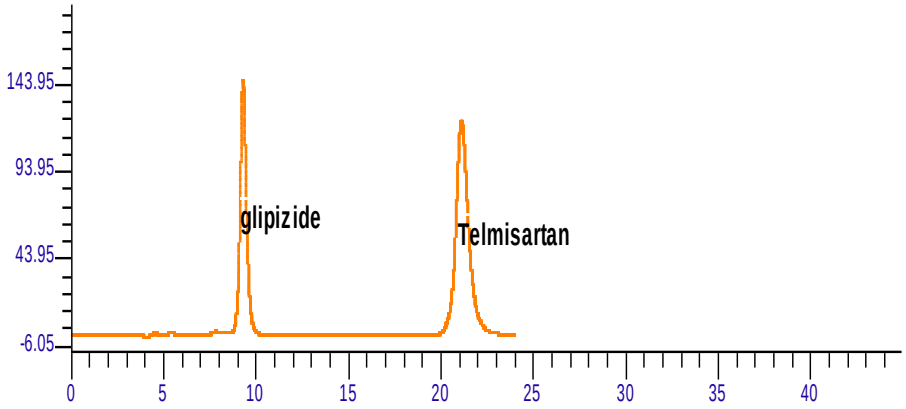

Figure.2. Standard chromatogram of TEL (21.1min) and GLZ (9.2min)

To check the system suitability, working standard mixture solution was injected fives. The retention time, tailing factor, resolution and theoretical plates for each drug were observed. Calculated % RSD of five consecutive injections for each parameter. The system suitability parameters of the present method were found to be within the acceptable limits. These results were presented in Table.4. The system suitability test confirmed that the chromatographic system was adequate for the analysis planned to be done.

Method validation:

Selectivity:

the selectivity of the method was established by checking by injecting blank sample (mobile phase) and observed the chromatogram. There was no interference found at retention times of TEL and GLZ in the placebo (blank) indicate the selectivity of the method. The carryover effect of the present method was established by using six injections of blank and an upper limit of quantification of standard mixture. These samples were analyzed alternatively to check any carryover response in the blank sample. In this study there were no such effects observed.

Table.4. System suitability parameters of GLZ and TEL

Parameters	Results				Specifications
	GLZ		TEL		
	Mean±SD	%RSD	Mean±SD	%RSD	
R_t	9.20±0.11	1.17	21.026±0.08	0.38	RSD≤2
N	4347.64±45.62	1.04	4939.33±34.42	0.69	N>2000
TF	1.21±0.009	0.784	1.14±0.02	1.75	TF≤2
Peak area	611933.3±951.74	0.155	1066876±5150.78	0.48	
R_s	13.328±0.150			1.12	R_s>2

Values expressed Mean±SD (n=5)

Linearity:

The linearity of the was evaluated by linear regression analysis, using least square method and found to be linear in the concentration range of 100-5000 ng/mL for GLZ and 50-5000ng/mL for TEL respectively. Calibration standard are prepared by spiking required volume of working standard (100µg/mL) solution into different 10mL volumetric flasks and volume made up with mobile phase to yield the concentrations of 50, 100, 200, 500, 1000, 2000 and 5000 ng/mL of TEL and GLZ. The resultant peak area of each drug was measured. Calibration curve is plotted between peak areas of drug against concentration of the drug. The linearity graph of TEL and GLZ was shown in Figure.3, and this linearity range can be useful for the determination of dissolution samples from the formulation at different time intervals. This linearity range covers the all levels of concentration of TEL and GLZ released from the tablet dosage forms.

Sensitivity:

The limit of detection (LOD) and limit of quantification (LOQ) are defined as the lowest concentration giving signal-to-noise ratio of at least 3:1 and 10:1, respectively. the LOD and LOQ of this method were verified based on the standard deviation of response, slope. The LOD and LOQ were found to be 1.80ng/mL and 5.46ng/mL for TEL, 23.70 ng/mL and 71.82 ng/mL for GLZ respectively.

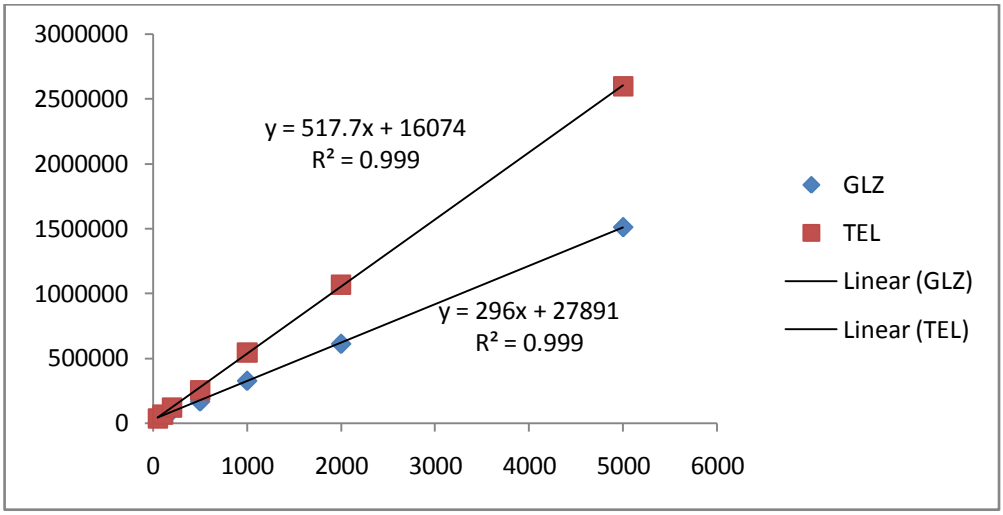

Figure.3. Linearity graph of TEL and GLZ

Table. 5. Intra-day and Inter-day precision and accuracy of GLZ and TEL

Conc. (ng/ml)	Intra-day (n=6)				Inter-day (n=9)			
	GLZ		TEL		GLZ		TEL	
	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD	Accuracy	%RSD
500	100.11± 0.38	0.38	97.41±1.57	1.61	101.46±0.38	0.38	104.75±0.91	0.87
1000	107.19±1.72	1.60	100.25±0.6	0.63	99.36±0.72	1.60	99.87±0.83	0.83
1500	102.12±1.30	1.28	102.99±1.6	1.60	102.12±1.30	1.28	103.25±1.92	1.86

Values expressed Mean±SD.

Intra-day and Inter-day precision and accuracy:

The intra- and inter-day precision and accuracy of this method was determined by analyzing replicates of quality control (QC) samples at three different concentrations on three different days. The coefficients of variation for the intra- and inter-day precision were <2%. The intra- and inter-day accuracies were found to be 97.41%-104.75% for both drugs. The data of this study was represented in Table.5, the low levels of coefficient of variation indicate the method was accurate and precise.

Robustness and Ruggedness:

Robustness of the method was done by changing slight variations in the parameters like mobile phase composition, flow rate and pH of the buffer. The method didn't show any significant change when the critical parameters were changed. The tailing factor was for the drugs were always <2 and the drugs were well separated under all the changes carried out. Ruggedness was studied along with precision and accuracy of batches where effect of column, any analyst change was observed. The observed value for precision and accuracy were within the acceptance criteria. (i.e. there is no change in the retention time, recovery and precision of the drugs), from this results we can say that the method was robust and rugged. The stability of drug was studied for the short-term and auto-sampler stability using QC samples. The samples were analyzed and compared with the freshly analyzed QC samples, no difference were found in accuracy and precision; this indicates there is no degradation of drugs during the analysis.

The HPLC method was developed validated according to the ICH guidelines, all these validation parameter values are meeting the ICH requirements. The validated HPLC method was successfully applied to the determination of amount of each drug present in the prepared belayed tablets and also applied to the drug release from the formulations (dissolution study).

Assay and in-vitro drug release from formulations:

The amount of drug present in the prepared bi-layered tablets was determined by extracting the drugs from the formulation with methanol and made suitable dilution with mobile phase, then injected six times into HPLC system. Then calculated the amount of individual drugs in different formulation from the respective calibration curve. The assay results of individual formulations were shown in Table.6. The percentage of drug content of F2-F5 were within the range of limits, found to be 90.92%-103.84% for TEL and 92.33%- 109.29% for GLZ, but F1 and F6 were out of range, found to be 136.21% and 75.96% for TEL, 275.86% and 83.36% for GLZ respectively.

The chromatogram of TEL and GLZ from the prepared bilayer tablet dosage form represented in Figure 4.

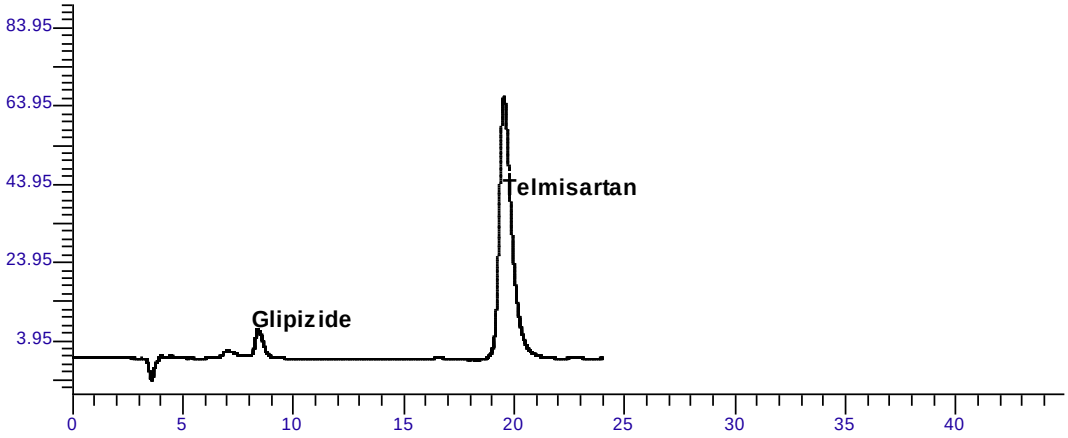

Figure.4. Chromatogram of TEL and GLZ from the formulation

Table.6. Recovery study from formulation (n=6) of GLZ and TEL:

Labeled amount (mg)			Calculated amount (mg)		Assay (%)	
GLZ	TEL	Formulation	GLZ	TEL	GLZ	TEL
		F1	6.89±0.192	27.24±0.468	275.86±7.68	136.21±2.34
2.5mg	20mg	F2	2.48±0.191	20.18±0.025	99.52±7.64	100.914±0.12
	(F1 to F3)	F3	2.43±0.047	18.18±0.020	97.20±1.90	90.925±0.10
		F4	4.61±0.008	41.53±1.095	92.33±0.16	103.84±2.73
5mg	40mg	F5	5.46±0.551	38.52±0.002	109.29±11.03	96.31±0.007
	(F4 to F6)	F6	4.16±0.001	30.38±0.170	83.36±0.03	75.96±0.42

Values are expressed in Mean ±SD

The dissolution study was carried out to check the release of drug from the formulation. From the results of assay of formulations we selected four formulations (F2-F5) out of six formulation for the dissolution study and remaining two (F1 and F6) formulations omitted from the this study as these formulations shown the assay out of range 136.2% for F1 and 75.96% for F6. The dissolution study was carried out for 8h; first 2h in acid media containing 0.1 N hydrochloric acid followed by phosphate buffer pH 6.8 for 6h. Then collected the 5ml sample at different time intervals, made a dilution as per requirement with mobile phase and injected into HPLC in triplicate. The amount of TEL and GLZ released at different time was calculated from the respective linearity graph. Then percentage of drug release and cumulative percentage drug release was calculated for each formulation for TEL and GLZ. The data of drug release from the formulations were sown in Table.7, and the graph was represented in Figure 5. The release of drug from the dosage form data of dissolution of each formulation was fitted into different kinetic models by plotting graphs by taking % release of drug vs time for zero order kinetic, log % release vs time for first order kinetic, % release vs square root of time for Higuchi model

kinetic, and log % release vs log of time for korsemeyer-peppas model kinetic. The r^2 value for four formulations (F2-F5) were found to be 0.8833-0.9658 for zero order, 0.6471-0.9321 for first order, 0.9183-0.9668 for higuchi model, and 0.836-0.9463 for korsemeyer-peppas model. The graphs of the different model were shown in Figure 6, 7, 8, and 9.

Table.7. Cumulative % drug release of TEL and GLZ from formulations (F1-F5)

Time(min)	**TEL**				**GLZ**			
	F2	F3	F4	F5	F2	F3	F4	F5
5	3.004	16.351	12.875	1.409	7.326	-	-	4.040
10	12.131	29.648	15.991	6.290	11.065	-	10.758	4.917
15	13.994	34.468	19.056	11.027	13.297	12.425	12.213	7.353
30	26.619	69.088	40.285	23.323	13.754	15.919	12.321	13.720
45	32.983	76.641	57.033	30.835	32.540	19.456	13.026	14.744
60	51.430	83.485	78.703	49.529	35.438	34.897	16.051	27.269
90	63.863	87.839	86.458	64.950	36.579	39.008	22.671	27.338
120	89.752	88.080	95.255	83.891	38.425	52.609	42.613	29.1178
180	-	-	-	-	57.705	61.884	44.478	32.625
240	-	-	-	-	61.476	75.012	44.394	57.672
300	-	-	-	-	72.464	77.238	50.296	69.377
360	-	-	-	-	73.406	77.733	52.278	82.296
420	-	-	-	-	82.287	91.078	56.388	82.336
480	-	-	-	-	82.368	93.655	74.672	92.263

To study the release pattern of drugs dosage form were fitted into four kinetic models i.e. Zero order, First order, Higuchi, and Korsemeyer-Peppas. The fitting of drug release into different kinetic models are shown in Figure. 6. All the formulations were best fitted in Higuchi model of kinetics. From the results of drug release profile indicating that the guggul acts as binding agent as well as an extending the release of drug from the prepared tablet dosage form.

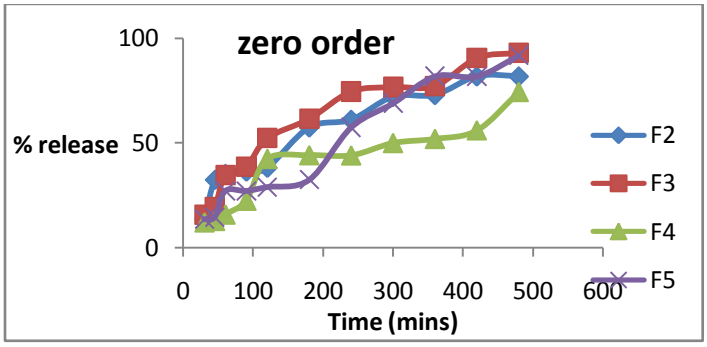

Figure 6 Zero order kinetic model of all formulation (F2-F5) of bilayer tablet of TEL and GLZ

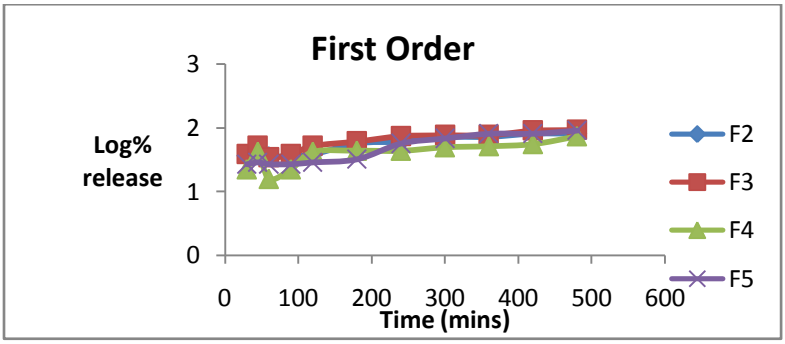

Figure 7. First order kinetic model of all the formulations of bilayer tablet of TEL and GLZ

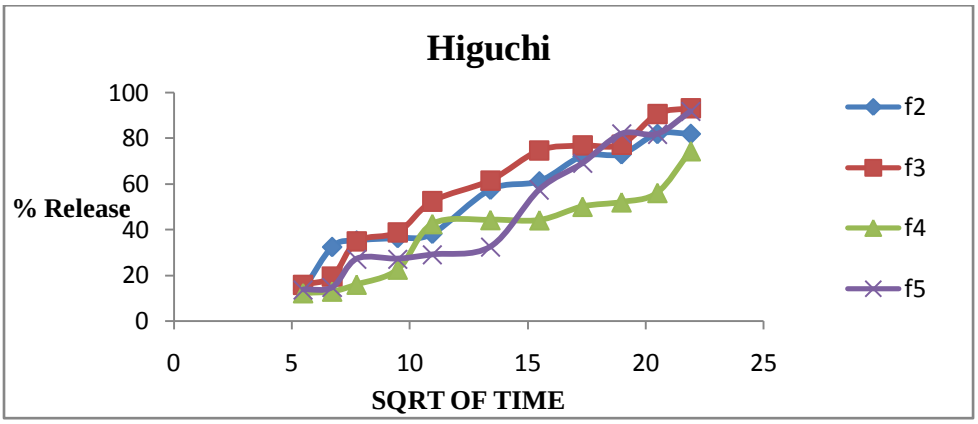

Figure 8. Higuchi kinetic model of all the formulations of bilayer tablets of TEL and GLZ.

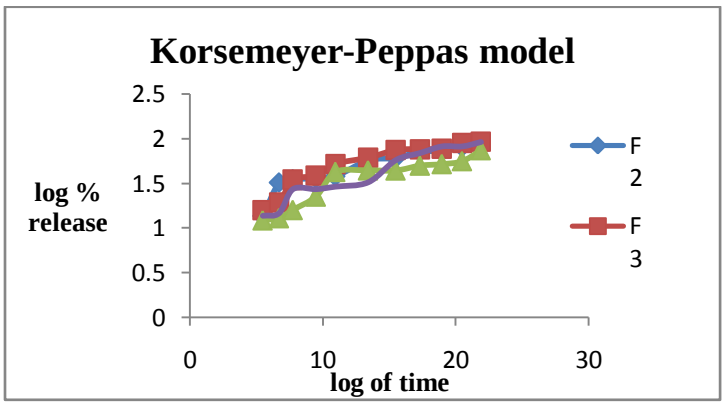

Figure 9. Korsemeyer-Peppas kinetic model of all formulation of bilayer tablets of TEL and GLZ

CONCLUSION:

The present study of the bilayer tablet of fixed dose combination of TEL and GLZ was formulated and evaluated. From the results it can be concluded that the prepared tablets were within the limits and the tablets of different concentrations of guggul (70%, 80%, 90%) were found have uniform thickness, hardness, friability and good handling property of the prepared bilayer matrix tablets. Since this work not reported earlier, we developed and validated RP-

HPLC method. All the validation parameters were in acceptable limits. Hence, this method was successfully applied for the assay and dissolution of prepared bilayered tablets. The dissolution data supporting that the guggul can acts a binding agent as well as rate retarding agent.

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