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MIXTURE DESIGN EXPERIMENT ON FORMULATION DEVELOPMENT AND OPTIMIZATION OF EMPAGLIFLOZIN INN TABLET A NEW MILESTONE ON IMMEDIATE RELEASE DRUG DELIVERY SYSTEM

Md. Mehdi Hasan[1]*, Saiful Islam[2], Kanchi Akter lima[1], Sharmin Akter Bristy[3], Md. Feroze Mahmud[3], Nishat Angum[3], Israt Jahan Mim[5] and Md. Yakub Hossain[4]

[1]Department of Pharmacy, Faculty of Health Science, Northern University Bangladesh, Dhaka -1205, Bangladesh.

[2]Department of Pharmacy, Atish Dipankar University of Science and Technology, Bangladesh.

[3]Department of Pharmacy. University of Asia Pacific. 74/A, Green Road, Dhaka-1205.

[4]Department of Pharmacy, State University Bangladesh. 77, Satmasjid Road Dhanmondi, Dhaka 1205, Bangladesh.

[5]Department of Pharmacy. World University of Bangladesh .3/A, Road 4, Dhaka -1205.

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***Corresponding Author**
Md. Mehdi Hasan
Department of Pharmacy, Faculty of Health science, Northern University Bangladesh, Dhaka -1205, Bangladesh.

ABSTRACT

Background & Objectives: The oral drug delivery system which includes the solid dosages form such as conventional dosages form and immediate release dosages form. The objective of this study was to formulate orodispersible tablets containing empagliflozin by direct compression method with sufficient hardness and rapid disintegration time and to study the effect of functionality differences of super-disintegrants on the tablet properties. **Methods:** The basic approach used in development immediate release solid dosages form by using superdisintegrant like sodium starch glycolate (Primogel, Explotab), Polyvinylpyrrolidone (PVP) etc. Impact of various formulation variables in extended release part was assessed usingstatistical interpretation such as analysis of variance. Which provides in instantaneous disintegration of tablet after administration. By using various techniques in can be formulate like wet granulation, direct compression etc. Prepared batches were evaluated for all pre-compression parameters and post-compression parameters. This method was found to be linear in a

concentration range of 5-30 µg/ml of the drug (r^2 = 0.999). The low value of % RSD in the precision study indicates reproducibility of the method. The low value of LOD and LOQ suggests the sensitivity of the method. Optimization was done by fitting experimental data to the software program (design expert). The design spacefor formulation variables and its influence on drug release was developed. In-vitro release data observed fromthe optimized formulation was fitted into various kinetic equations. **Results:** The results of forced degradation studies indicated that the drug was less stable in thermal and photolytic condition and degraded in acidic, basic, oxidative conditions. On the basis of formulation evaluation, batch was found to be promising formulation suitable for the immediate release of empagliflozin. **Conclusions:** Results obtained by validation studies suggested that the developed stability indicating assay method is simple, accurate, specific, sensitive and precise. Thus, this method can be used for routine analysis of empagliflozin formulation and to check the stability testing. The immediate release of empagliflozin tablets was successfully developed by employing granulation technology and formulation optimization facilitated by experimental design.

KEYWORDS: Empagliflozin, Formulation R&D, Tablets, HPLC, Stability indicating assay method.

INTRODUCTION

Quality by Design (QbD) is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. It helps to assess the critical material attributes (CMAs) and critical process parameters (CPPs) that impacting the predefined critical quality attribute (CQAs). The design space concept is introduced as "the multidimensional combination and interaction of input variables (e.g., materials attributes) and process parameters that have been demonstrated to provide assurance of quality".[1] Using this approach, it is essential to define the relationship between critical formulation/process parameters and CQAs 4.Response surface methodology (RSM) is one of the popular methods in the development and optimization of drug delivery systems. Based on the principles of design of experiments (DoE), the methodology involves theuse of various types of experimental designs, generation of polynomial mathematical relationships, and mapping of the response over the experimental domain to select the optimum formulation. Central composite design, three level factorial design, Box-Behnken design and D-optimal

design are the different types of RSM designs available for statistical optimization of the formulations.[2] Factorial design is one type of RSM design enables, all factors to be varied simultaneously, allowing quantification of the effects caused by independent variables and interactions between them. Factorial design requires fewer experimental runs, less time and thus provides a cost-effective technique than the conventional processes of formulating and optimization of dosage forms. Hence,factorial design was selected as DoE 5/ Empagliflozin[1] is a C-glycosyl compound consisting of a beta-glucosyl residue having a (4-chloro-3-{4-[(3S)-tetrahydrofuran-3-yloxy]benzyl}phenyl group at the anomeric centre.

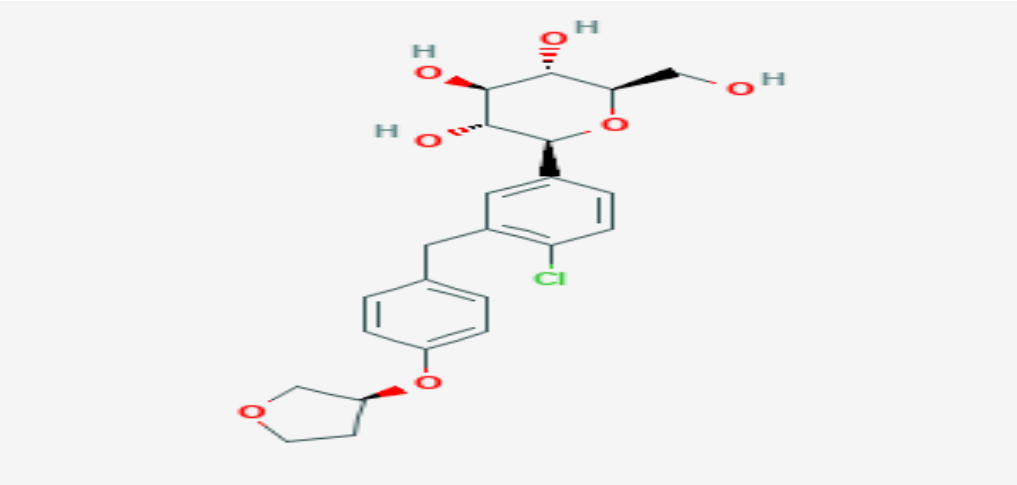


Fig: Empagliflozin.

A sodium-glucose co-transporter 2 inhibitor used as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. It has a role as a sodium-glucose transport protein subtype 2 inhibitor and a hypoglycemic agent. It is a C-glycosyl compound, an aromatic ether, a tetrahydrofuryl ether and a member of monochlorobenzenes. Empagliflozin is a sodium glucose co-transporter-2 (SGLT-2) inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type 2 diabetes. SGLT2 co-transporters are responsible for reabsorption of glucose from the glomerular filtrate in the kidney. The glucuretic effect resulting from SGLT2 inhibition reduces renal absorption and lowers the renal threshold for glucose, therefore resulting in increased glucose excretion. Additionally, it contributes to reduced hyperglycaemia and also assists weight loss and blood pressure reduction.[3]

Empagliflozin is a sodium-glucose co-transporter 2 (SGLT2) inhibitor as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. To reduce

the risk of cardiovascular death in adult patients with type 2 diabetes mellitus and established cardiovascular disease.

Empagliflozin is not an official drug in any Pharmacopoeia. Literature survey reveals that only one marketed formulation of Empagliflozin is available which is very costly and cannot afford to poor patients. Therefore, a cost-effective formulation as compared to the marketed formulation is developed. Some methods have been reported for their determination of Empagliflozin by UV, HPLC[4] and hyphenated techniques such as UPLC–MS/MS,[5] LCMS,[6] GCMS,[7] either alone or in combination. This paper presents formulation development of Empagliflozin as well as development and validation of stability indicating assay method by using the RP-HPLC technique.The pediatrics and geriatrics patients are of particular concern. To overcome this, orodispersible tablets have been developed. Orodispersible tablets dissolve completely and rapidly. Orodispersible tablets are considered as one of the novel solid dosage forms which turn immediately into liquid in less than a minute and release their drug into the mouth after taking into the mouth and touching saliva. These tablets have had a huge improvement in recent years due to high patient compliance and ease of administration.

The common anti-diabetic drug Empagliflozin shows bad dissolution and tableting behavior due to its hydrophobic structure. Additionally its high co hesivity results in low flowability. Another problem in its manufacturing is its high tendency of sticking to the punches. There are three methods of tablet manufacturing with the choice depending upon the dose and the drug's physical properties, such as, compressibility and flow of the blend. Direct compression is a process by which tablets are compressed directly from mixtures of the drug and excipients, without any preliminary treatment. A simple formula is considered to be composed of an active ingredient, a diluent and a lubricant .

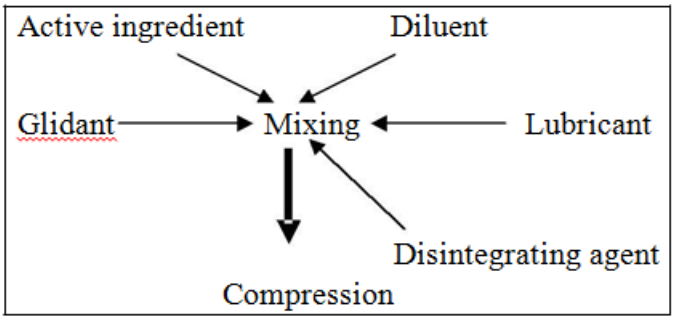
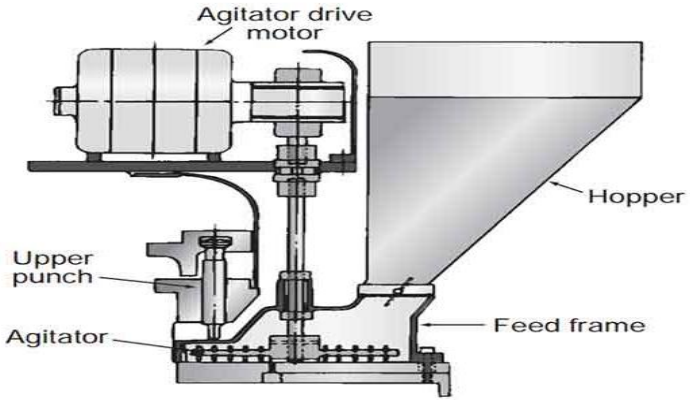


Fig. 1: The direct compression process of tablet manufacturing (Armstrong, 2002).

Tablet manufacturing by direct compression has increased steadily over the years. It offers advantages over the other manufacturing processes for tablets, such as wet granulation and provides high efficiency.[8] As direct compression is more economic, reducing the cycle time and straight forward in terms of good manufacturing practice requirements. On the other hand wet granulation not only increases the cycle time, but also has certain limits imposed by thermol ability and moisture sensitivity of the active. So pharmaceutical industry is now focusing increasingly on this process.[9] The unnecessary exposure of any drug to moisture and heat can never be justified. Tablets produced by direct compression method give lower microbial levels than those prepared by the wet granulation method. The compaction process exerts lethal effect on the survival of microorganisms. The tablets prepared by direct compression disintegrate into API particles instead of granules that directly come into contact with the dissolution fluid and exhibit a comparatively faster dissolution. The serious limitation of direct compression is the use of more than 30% of the dr.ug in the formulation, mainly for drugs that present low flow ability and segregation.

Now days, most of experimentation of tablet formulation development is still performed by changing the levels of each variable (factor) at a time, in an unsystematic way, keeping all other variables constant in order to study the effects of that specific variable on the formulation.[10]

The plan of present research is to develop a cost effective Ibuprofen 200mg tablet by direct compression method. The aim is to cut down the time required for disintegration of the tablets in small intestine. Secondarily, direct compression is being studied for its simplicity, cost effectiveness and for its comparatively shorter process. Thus nine different formulations were designed to obtain best optimized product.



Induced die feeder, Ansel's pharmaceutical dosage Forms and Drug delivery systems

Advantages of direct compression technology

The adoption of direct compression technology is based on the following advantages or benefits

1. Direct compression method requires fewer processing steps (unit operations) and less equipment. Therefore, the method is potentially less expensive than other methods used in tablet manufacture.
2. Tablet manufacture can be carried out without the involvement of moisture and heat. Hence, product stability is almost guaranteed.
3. Some direct compressible excipients possess inherent disintegration properties e.g., microcrystalline cellulose.
4. Tablets produced by direct compression method generally show faster dissolution times than those prepared by wet granulation. This is because tablets manufactured by direct compression method disintegrate into primary particle state unlike those manufactured by wet granulation method which breaks down into granules and finally into primary particle state.
5. Changes in dissolution profile are less likely to occur in tablets manufactured by direct compression (if stored for a long time) than in those prepared by wet granulation.
6. Because direct compression excipients have a relatively high binding capacity, the pressure required to manufacture the desired hardness is, in general, less with direct compression vehicles than with conventional granulations, resulting in both higher production rates and longer machine life.
7. Lubrication is performed in the same vessel as powder mixing, thereby reducing both transfer losses and contamination of equipment.

MATERIALS AND METHODS

Materials

Commercially available tablets of Empagliflozin were procured from local market and Empagliflozin API was obtained from an approved supplier Shandong Haohong Biotechnology Co; Ltd, Shandong, China. All excipients were obtained from Active Fine Chemical Ltd., Dhaka, Bangladesh. HPLC grade solvents used in this study were obtained from Active Fine Chemical Ltd., Dhaka, Bangladesh.

Instruments

Empagliflozin tablets were formulated on single punch tablet compression machine Mini press 1 (Karnavati Engineering limited). The friability test was performed on Electrolab EF-2 friabilator USP. Disintegration test was performed on Electrolab disintegration tester ED-2L. The dissolution testing was performed on Labindia DS 8000. The method was performed on Shimadzu LC 2010 CHT, Japan having a quaternary system with automatic injection facility and UV-Visible detection system. The column used was Purospher Star RP-18e (5 µm, 250x4, 6 mm), LC solution software and Shimadzu AY-120 balance was used for this work.

Formulation of tablets

Before formulation and pre-formulation studies (organoleptic properties, solubility, and drug excipient compatibility studies) were carried out. Empagliflozin tablets were formulated by using 32factorial design as presented in table 1. Drug, binder, super-disintegrant and other excipients were weighed separately for 60 tablets per batch as per proposed formulations. The proposed formulations were coded as F1, F2, F3, F4, F5, F6, F7, F8 and F9. The amounts of drug and excipients are expressed in mg (milligram) unit. Initially, the binder, super-disintegrant and other excipients were passed through sieve no. 40. Then, Empagliflozin (API) was added, mixed properly for 5-10 min and sieved again. Blended mass was taken in the hopper and then die and punch were adjusted to get the desired weight of the tablet (100 mg). Tablets were prepared using flat face round 6.5 mm diameter punch by the direct compression process.

Table 1: Batches designed by using 32factorial design.

Ingredients	Formulation code (mg/tablet)																	
	F1	%	F2	%	F3	%	F4	%	F5	%	F6	%	F7	%	F8	%	F9	%
Empagliflozin	10.00	10	10.00	10	10.00	10.00	10.00	10.53	10.00	10.53	10.00	10.53	10.00	7.69	10.00	7.69	10.00	7.69
Lactose	20.55	20.55	25	25	20.63	20.63	30.65	32.25	27.23	28.67	21.00	22.10	42.25	32.51	28.66	22.05	-	-
Microcrystalline Cellulose	56.00	56	51.55	51.55	42.80	42.80	46.65	49.11	50.02	52.65	43.50	45.80	63.26	48.66	77.43	59.56	73.41	56.47
Polyvinyl Pyrrolidone (Povidone K-30)	3.45	3.45	3.45	3.45	-	-	1.9	2.0	2.38	2.5	1.0	-	4.50	3.46	-	-	4.50	3.46
Croscarmellose Sodium	7.50	7.5	7.50	7.5	-	-	3.8	4.0	3.33	3.5	2.00	2.10	6.5	5.0	7.8	6.0	10.0	7.69
Maize starch	-	-	-	-	20.79	20.79	-	-	-	-	15.00	15.79	-	-	-	-	28.60	22.00
Crospovidone	-	-	-	-	2.10	2.10	-	-	-	-	-	-	-	-	2.6	2.0	-	-
Magnesium Stearate	1.50	1.5	1.50	1.50	2.63	2.63	1.055	1.11	1.09	1.15	2.50	2.63	1.99	1.53	1.95	1.5	2.00	1.54
Colloidal Anhydrous Silica (Aerosil-200)	1.00	1.0	1.00	1.00	1.05	1.05	0.95	1.0	0.95	1.0	1.00	1.05	1.50	1.15	1.56	1.2	1.50	1.15
Total wt/Tablet	100 mg		100 mg		100 mg		95 mg		95 mg		95 mg		130 mg		130 mg		130 mg	

Evaluation of tablets

Appearence

Take about 20 tablets in a watch glass and observe visually with white to off white background Check the color, shape and size.

Identification

The chromatogram of the sample preparation exhibits a major peak for Empagliflozin, The Retention time of which corresponds to that exhibited in the the chromatogram of the standard Preparation as obtained in the assay.

Average weight

Weigh 20 tablets individually at random basis and record the weight.

$$\text{Average weight} = \frac{\text{Weight of 20 tablets (mg)}}{20}$$

Weight variation

Find the highest and lowest tablet weight from the average weight measurement. Calculate the positive deviation and negative deviation as follows.

$$\% \text{ of Positive deviation} = \frac{(\text{Highest tablet weight} - \text{Average weight}) \times 100}{\text{Average weight}}$$

$$\% \text{ of Negative deviation} = \frac{(\text{Lowest tablet weight} - \text{Average weight}) \times 100}{\text{Average weight}}$$

Hardness, Thickness & Diameter

Take 10 tablet and measure Hardness (in kp), Thickness (in mm) & Diameter (in mm) with LABINDA test tablet tester. Take average value of Hardness (in kp), Thickness (in mm) & Diameter (in mm) as result.

Friability

Take 20 tablets; remove any loose dust with soft brush. Weigh the tablets (W_1) and place the tablets in the drum of Friability Tester Put the tablet in Friability Tester. Run the instrument at 25 RPM for 4 minutes and remove any loose dust from the tablets as before. If no tablet cracked, split or broken, weigh the tablets (W_2). Calculate the Friability as follows.

$$\% \text{ of Friability} = \frac{(W_1 - W_2) \times 100}{W_1}$$

Disintegration time

Medium: Water

Temperature: 37.0°± 1.0°C

Fill the disintegration beaker with sufficient medium and wait to raise the temperature. Take six tablets. Introduce one tablet into each tube and add a disc to the each tube. Suspend the assembly in the beaker containing water and start the operation.

Disintegration is considered to be achieved when no residue, except fragments of tablet, remains on the screen of the test apparatus. Record the disintegration time.

Dissolution

Method: UV Spectrophotometer (UV)

Procedure

Phosphate buffer pH 6.8 preparation: Weight 6.8 gm KH_2PO_4 in 1000 ml Distilled water. Then add sufficient quantity of 2 M NaOH to adjust pH 6.8.

Dissolution condition

Dissolution medium : Phosphate Buffer pH 6.8
Apparatus : USP Type II (Paddle)
Volume : 900 ml
RPM : 75
Time : 30 min's
Vessel Temperature : 37 ± 0.5° C

Standard solution preparation

Weigh equivalent to 20 mg Empagliflozin INN working standard to a 100 ml volumetric flask, add 2 ml of Ethanol and shake to dissolved and then make volume up to 100 ml with Dissolution medium and sonicate 15 minutes dissolved. Transfer 5 ml of standard solution to a 100 ml volumetric flask with Dissolution medium.

Sample Solution preparation

Set the dissolution parameters of the instrument as mentioned above.Individually place one tablet in 6 dissolution vessel, 900 ml of dissolution medium which has been equilibrated to the temperature of 37 ± 0.5°c .Immediately start the apparatus and run for 30 minutes. At the end of specified time withdraw 15 ml solutions from zone midway between the surfaces to the dissolution medium and top of the rotating blade not less than 1 cm from the vessel wall. Filter the solution through whatman filter paper into beaker.

Procedure— Determine the amount of Empagliflozin INN equivalent dissolved by using UV absorption at wavelength maximum absorbance at 277 nm on filtrate portion of Standard and Sample Solution.

Calculate % of content Empagliflozin INN using following formula –

$$\frac{AT}{AS} \times \frac{Ws}{100} \times \frac{5}{100} \times \frac{900}{LC} \times P$$

A_T = Absorbance of sample preparation of Empagliflozin

A_s = Absorbance of standard preparation Empagliflozin

W_s = Weight of standard preparation of Empagliflozin

P = Standard potency of Empagliflozin

LC = Label claim

Dissolution

Method: High performance liquid chromatography (HPLC)

Buffer preparation: Weight 1.36 gm KH_2PO_4 in 1000 ml Distilled water. Then add sufficient quantity of Orthophosphoric Acid (H_3PO_4) to adjust pH 4.0.

Mobile phase: Methanol: Buffer = 60: 40

Procedure

Phosphate Buffer pH 6.8 preparation: Weight 6.8 gm KH_2PO_4 in 1000 ml Distilled water. Then add sufficient quantity of 2 M NaOH to adjust pH 6.8.

Dissolution condition

Dissolution Medium : Phosphate Buffer pH 6.8

Apparatus : USP Type II (Paddle)

Volume : 900 ml

RPM : 75

Time : 30 min's

Vessel Temperature : 37 ± 0.5° C

Standard solution preparation

Weigh equivalent to 20 mg Empagliflozin INN working standard to a 100 ml volumetric flask, add 3 ml of Ethanol and shake to dissolved and then make volume up to 100 ml with Dissolution medium and sonicate 15 minutes dissolved. Transfer 5 ml of standard solution to a 100 ml volumetric flask with Dissolution medium.

Sample solution preparation

Set the dissolution parameters of the instrument as mentioned above. Individually place one tablet in 6 dissolution vessels, 900 ml of dissolution medium which has been equilibrated to the temperature of 37 ± 0.5°c .Immediately start the apparatus and run for 30 minutes. At the end of specified time withdraw 15 ml solutions from zone midway between the surfaces to

the dissolution medium and top of the rotating blade not less than 1 cm from the vessel wall. Filter the solution through whatman filter paper into beaker.

Chromatographic condition

System: Gradient

Column: C_{18} column, (250 mm × 4.6 mm; 5 µm)

Column Temperature: 30 ^{0}C

Flow rate: 1.2 ml / min.

Detection: Spectrophotometer at 277 nm

Injection volume: 100 µl

Run time: 10.0 minutes

Retention time: 5.0 minutes (approximately)

Procedure— separately injects equal volumes (about 100 µL) of the standard preparation and the dissolution preparation of sample solution into the chromatograph, record the chromatograms, and measure the area responses for the major peaks.

Calculate % of content Empagliflozin INN using following formula –

$$\frac{AT}{AS} \times \frac{Ws}{100} \times \frac{5}{100} \times \frac{900}{LC} \times P$$

A_T = Area of sample preparation of Empagliflozin

A_s = Area of standard preparation Empagliflozin

W_s = Weight of standard preparation of Empagliflozin

P = Standard potency of Empagliflozin

LC = Label claim

Assay

Method UV spectrophotometer (UV)

Apparatus

1) Volumetric Flask (1000 ml, 100 ml, 50 ml)
2) Cylinder 100 ml
3) Funnel & whatman Filter Paper
4) Glass Vial (sample holder)
5) Pipette (2 ml, 5 ml, 10 ml)

Reagents: Ethanol

Diluent: Ethanol

Standard solution preparation

Accurately weigh and transfer about equivalent to 20 mg of working standard of Empagliflozin INN into 100 ml volumetric flask. Add 30 ml of Diluent & sonicat for 10 minutes and volume then up to the mark 100 ml with Diluent and sonicat 2 minutes.

Sample Solution preparation

Ten tablet were weight accurately average weight was calculate and crush the tablet into powder well. Accurately transfer and weight the powder equivalent to 20 mg of Empagliflozin to a 100 ml volumetric flask. Add 30 ml of Diluent & sonicat for 15 minutes and volume then up to the mark 100 ml with Diluent and sonicat 2 minutes. Filter the solution through whatman filter paper into beaker.

Procedure— Determine the amount of Empagliflozin INN equivalent dissolved by using UV absorption at wavelength maximum absorbance at 277 nm on filtrate portion of Standard and Sample Solution.

Calculate % of content Empagliflozin INN using following formula –

$$\frac{AT}{AS} \times \frac{Ws}{Wu} \times \frac{Ps}{100} \times \frac{Av.wt}{LC} \times 100$$

A_T = Absorbance of sample preparation of Empagliflozin

A_s = Absorbance of Standard Preparation Empagliflozin

W_s = Weight of Standard Preparation of Empagliflozin

W_u = Weight of sample Empagliflozin

P_s = Standard potency of Empagliflozin

LC= Label claim

Alternative Method: High Performance Liquid Chromatography (HPLC)

Apparatus

1) Volumetric Flask (1000 ml, 100 ml, 50 ml)
2) Cylinder 100 ml
3) Funnel & whatman Filter Paper
4) Glass Vial (sample holder)
5) Pipette (2 ml, 5 ml, 10 ml)

Reagents

1) Distilled Water
2) Methanol

Procedure

Buffer preparation: Weight 1.36 gm KH_2PO_4 in 1000 ml Distilled water. Then add sufficient quantity of Orthophosphoric Acid (H_3PO_4) to adjust pH 4.0.

Mobile Phase: Methanol: Buffer = 60: 40

Diluent: Mobile Phase

Standard solution preparation

Accurately weigh and transfer about equivalent to 20 mg of working standard of Empagliflozin INN into 100 ml volumetric flask. Add 30 ml of Diluent & sonicat for 10 minutes and volume then up to the mark 100 ml with Diluent and sonicat 2 minutes.

Sample solution preparation

Ten tablet were weight accurately average weight was calculate and crush the tablet into powder well. Accurately transfer and weight the powder equivalent to 20 mg of Empagliflozin to a 100 ml volumetric flask. Add 30 ml of Diluent & sonicat for 15 minutes and volume then up to the mark 100 ml with Diluent and sonicat 2 minutes. Filter the solution through whatman filter paper into beaker.

Chromatographic condition

System: Gradient

Column: C_{18} column, (250 mm × 4.6 mm; 5 µm)

Column temperature: 30 ^{0}C

Flow rate: 1.2 ml / min.

Detection: Spectrophotometer at 277 nm

Injection volume: 50 µl

Run time: 10.0 minutes

Retention time: 5.0 minutes (approximately)

System suitability

Separately inject the equal volume of standard solution of six replicate injection into the equilibrate HPLC system & calculate RSD. The RSD for six replicate injections should not be more than 2.0%. Then inject Sample solution & record the chromatogram.

Procedure— separately injects equal volumes (about 50 µL) of the standard preparation and the assay preparation of sample solution into the chromatograph, record the chromatograms, and measure the area responses for the major peaks.

Calculate % of content Empagliflozin INN using following formula –

$$\frac{AT}{AS} \times \frac{Ws}{Wu} \times \frac{Ps}{100} \times \frac{Av.wt}{LC} \times 100$$

A_T = Area of sample Preparation of Empagliflozin

A_s = Area of Standard Preparation Empagliflozin

W_s = Weight of Standard Preparation of Empagliflozin

W_u = Weight of sample Empagliflozin

P_s = Standard potency of Empagliflozin

LC= Label claim

***In vitro* dissolution study**

Dissolution studies of each batch were conducted according to USP apparatus II paddle method with 900 ml phosphate buffer (pH 6.8) at 37 °C and 75 rpm. After 5, 10, 20, 30, 45 and 60 min interval samples (10 ml each) were withdrawn from the dissolution medium and replaced with fresh medium to maintain a constant volume.[11] The samples were filtered through a 0.45µ membrane filter. Then samples were diluted to a suitable concentration with phosphate buffer (pH 6.8). Then, the solution was injected into HPLC system. The cumulative percentage of drug release was calculated.[12-15]

Assay of tablets

Twenty tablets were weighed and average weight was calculated. These tablets were crushed and powdered in a glass mortar. The tablet powder equivalent to the average weight of Empagliflozin was accurately weighed, transferred to a 50 ml of volumetric flask and diluted up to mark with water: acetonitrile (60:40v/v). The solution was filtered through Whatman filter paper no.. This solution was further diluted to obtain 30 µg/ml with diluent and the sample solution was injected into HPLC system. This procedure was repeated in triplicate.

Forced degradation studies

To evaluate stability, Empagliflozin was subjected to force degradation under the condition of acid, base, neutral hydrolysis and oxidation as per international conference on harmonization (ICH) guidelines.[16-19]

Acid hydrolysis

100 mg of Empagliflozin was weighed accurately and transferred to 100 ml volumetric flask containing 100 ml of 0.1N hydrochloric acid (HCl). This mixture was refluxed at 80 °C. After 2 h, 5 ml of refluxed sample was withdrawn and neutralized with 5 ml of 0.1 N sodium hydroxide. This solution was further diluted 10 times with mobile phase to obtain a concentration of 100 µg/ml. The chromatogram obtained after 2 h of acid hydrolysis.

Alkaline hydrolysis

100 mg of Empagliflozin was weighed accurately and transferred to 100 ml volumetric flask containing 100 ml of 0.1N sodium hydroxide (NaOH). This mixture was refluxed at 80 °C. After 2 h, 5 ml of refluxed sample was withdrawn and neutralized with 5 ml of 0.1 N hydrochloric acid. This solution was further diluted 10 times with mobile phase to obtain a concentration of 100 µg/ml. The chromatogram obtained after 2 h of alkali hydrolysis.

Oxidative degradation

100 mg of Empagliflozin was weighed accurately and transferred to 100 ml volumetric flask containing 100 ml of 3% hydrogen peroxide (H2O2). This mixture was refluxed at 80 °C. After 2 h, 5 ml of refluxed sample was withdrawn. This solution was further diluted 10 times with mobile phase to obtain a concentration of 100 µg/ml. The chromatogram obtained after 2 h of oxidative degradation.

Thermal degradation

100 mg of Empagliflozin IR tablet powder sample was weighed and transferred into 100 ml volumetric flask. The contents were refluxed as such on a water bath previously maintained at 80^{o} C for 2 h. The sample was allowed to cool to room temperature and then the volume was made up to the mark with mobile phase and mixed well. The solution was filtered through a 0.45µ syringe filter and analyzed. The chromatogram obtained after 2 h of thermal degradation.

Photolytic degradation

Photolytic degradation of the drug was carried out by exposure of about 100 mg of Empagliflozin IR tablet powder sample to UV radiation for 12 h. Then the sample was transferred into 100 ml volumetric flask. The sample was allowed to cool to room temperature and then the volume was made up to the mark with mobile phase and mixed well. The solution was filtered through a 0.45µ syringe filter and analyzed. The chromatogram obtained after 12 h of photolytic degradation.

Validation of the method

The developed chromatographic method was validated for system suitability, linearity, range, accuracy, precision, LOD-LOQ and robustness parameters as per ICH guidelines.[20-23]

Linearity and Range

Working standard solutions were injected in the range of 5-30 µg/ml under the optimized chromatographic conditions and peak areas were calculated at 280 nm. The calibration curve was plotted between areas against concentrations of the drug. Linear regression data, as well as calibration curve.

Precision

Repeatability study was carried out with six replicates and intermediate precision studies were carried out with three concentrations of Empagliflozin with three replicates. The values of % relative standard deviation (% RSD) of precision study are shown in table.

Accuracy

The accuracy of the method was determined by calculating percent recovery of the drug by standard addition method. Percent recovery of Empagliflozin was determined at three different level 80%, 100%, and 120% of the target concentration in triplicate. The results of accuracy study are shown in table.

Robustness

Robustness of the optimized method was studied by changing flow rate (±0.1 ml/min), change in wavelength (±1 nm) and change in mobile phase composition (±5%) during analysis. The sample was injected in triplicate for every condition and % RSD was calculated for each condition is shown in table

Limit of detection (LOD) and limit of quantitation (LOQ)

Five sets of concentrations were prepared between 5-30 µg/ml and the corresponding areas of these sets were measured. Calibration curves were plotted for each set. The standard deviation of the y-intercept and average slope of the calibration curve was used to calculate LOD and LOQ using following formulae. LOD=3.3×SDS LOQ=10×SDS

Where SD is the standard deviation of y-intercepts of the calibration curves; S is the mean slope of six calibration curves.

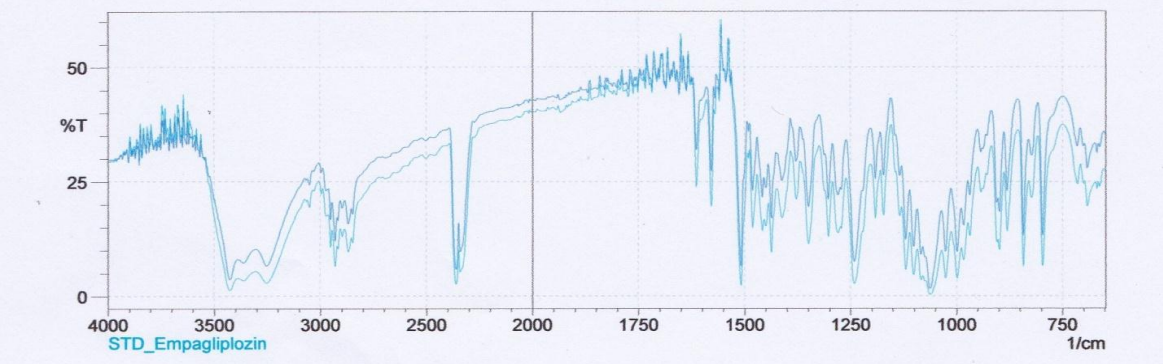


Fig. 2: IR spectrum of empagliflozin.

RESULTS AND DISCUSSION

Formulation of tablets Empagliflozin was found to be soluble in acetonitrile. Drug-excipients interaction studies were performed using FTIR spectrophotometer.

The FTIR spectra for the formulation and pure drug are shown in fig. 2 and fig. 3. Characteristics peaks obtained for the pure drug correlated well with that of the formulation peaks. This indicated that the drug was compatible with the formulation components.

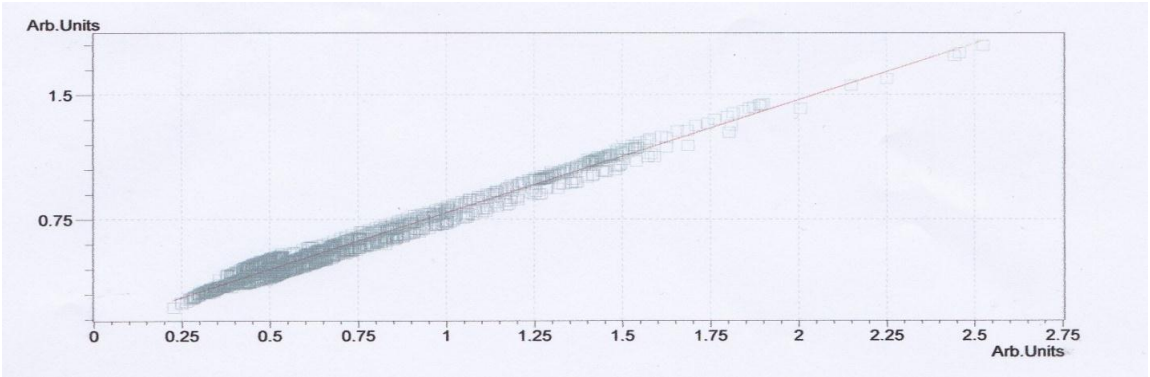


Fig. 3: IR spectrum of empagliflozin formulation.

Evaluation of powder blend

The angle of repose of all formulations was between 21.37 ° to 28.14 °, while the result of the Carr's index and Hausner ratio was between 11.71% to 20.02% and 1.03 to 1.25, respectively. Evaluation of powder blend characteristics is presented in table 3. The results indicate that the prepared powder mixtures have acceptable flow properties and compressibility. All pre-compression parameters are found to be within the acceptance criteria.

Table 2: Evaluation of powder blend characteristics

Parameters	F1	F2	F3	F4	F5	F6	F7	F8
Angle of Repose	23.05 °	22.89 °	23.49 °	21.37 °	25.55 °	25.01 °	27.07 °	27.09 °
Bulk Density	0.606	0.571	0.588	0.606	0.588	0.588	0.588	0.571
Tapped Density	0.689	0.714	0.666	0.714	0.689	0.714	0.666	0.714
Hausner's Ratio	1.13	1.25	1.13	1.17	1.17	1.21	1.03	1.25
% Carr's Index	12.04	12.02	11.71	15.12	14.65	17.64	17.97	20.02

(n=3)

Evaluation of tablets

The evaluation parameters of all formulation batches are presented in table 3. The thickness oftablets was observed in the range of 2.4±0.172 to 2.5±0.171 mm, it was found that all prepared tablets had a uniform thickness. The hardness of tablets was in the range of 3.4±0.05 to 3.7±0.10 kg/cm2, which shows sufficient mechanicalstrength. The total weight loss of the prepared tablets due to friability was found in the range of 0.50% to 0.80%, which is within acceptance criteria. The weight variation of prepared tablets was in the range of 98.43±0.61 to 100.82±0.79. The content uniformity of the prepared tablets was in the range of 93.98±1.32% to 95.25±1.12%, which reveals content uniformity. All evaluation parameters were found within the acceptance criteria.

Table 3: Evaluation of formulation batches of tablets.

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Thickness (mm)*	2.5±0.18	2.4±0.17	2.5±0.17	2.5±0.17	2.4±0.17	2.4±0.17	2.4±0.17	2.5±0.17	2.5±0.17
Weight Variation (mg)*	98.43±0.61	99.06±0.78	99.08±0.72	99.41±0.93	100.11±0.84	99.49±0.83	100.6±0.81	100.82±0.79	100.48±0.67
Hardness	3.4±0.05	3.6±0.05	3.5±0.15	3.5±0.05	3.6±0.20	3.5±0.05	3.7±0.10	3.4±0.05	3.6±0.20

kg/cm2*									
Friability (%)	0.50	0.60	0.80	0.60	0.80	0.60	0.60	0.60	0.50
Disintegratio	138±1.15	137±1.80	136±2.11	137±1.45	136±1.23	139±2.10	140±2.40	145±1.76	139±1.32
n test									
Content	94.62±0.5	94.62±1.8	94.62±1.6	94.30±0.7	93.98±1.32	94.62±1.8	95.25±1.1	94.93±0.43	95.25±1.85
uniformity	4	5	8	2		7	2		
In vitro	36.48	40.25	29.97	38.95	90.979	69.25	62.55	44.59	67.57

Dissolution

Studies

(*n=3) data represented as mean±SD

it is observed that formulation batch F5 shows maximum drug release pattern as compared to other formulations. Therefore, this batch was selected as optimized formulation batch.

Optimization of chromatographic conditions

UV spectrum of Empagliflozin showed maximum absorbance was found at 280 nm. Hence, 280 nm was selected for detection wavelength of this drug. Initially, various chromatographic conditions were tried in order to obtain better separation characteristics by changing mobile phase composition and pH[24,25]. Finally, the mobile phase containing water: acetonitrile (60:40 v/v)+0.1 % TEA was selected at 1 ml/min flow rate. The retention time of Empagliflozin was found to be 5.66 min. The chromatogram of Empagliflozin is shown in fig. 5 and optimized chromatographic conditions are mentioned in table 6.

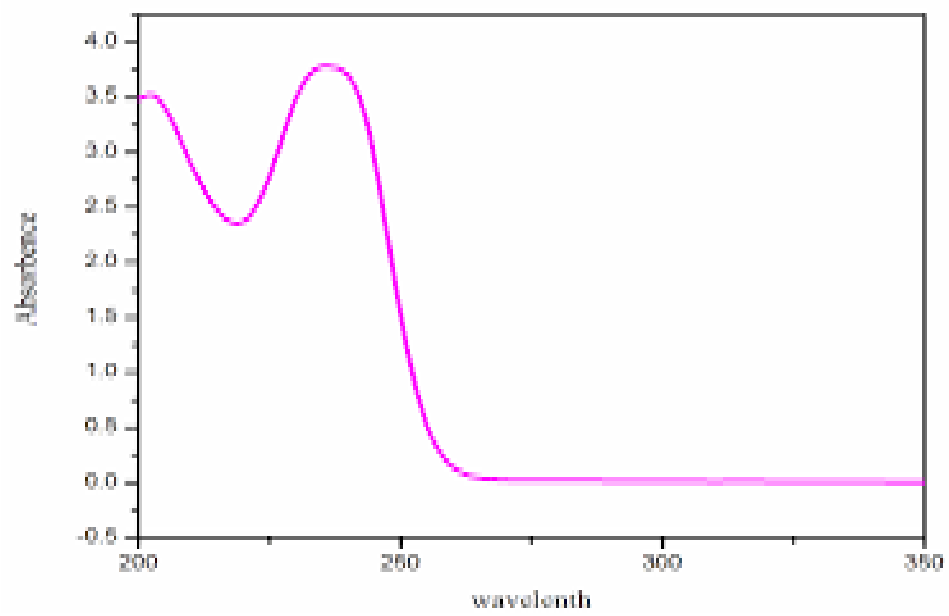


Fig: 4: UV spectrum of empagliflozin.

Assay of tablet formulation

The drug content was calculated as an average of three determinations and assay results were shown in table 7. The results were very close to the labeled value of commercial tablets. The value of mean % drug was found to be 99.31% which is within acceptance criteria.

Table 6: Optimized chromatographic conditions.

Parameters	Details
Mobile phase	Water: Acetonitrile (60:40) v/v+0.1 % TEA
Column	Purospher Star RP-18 end-capped (5 µm) Hibar 250x4, 6
Flow rate	1 ml/min
Detection	280 nm
Injection volume	20 µl
Run time	8 min
Retention time	5.66 min
Diluent	water: acetonitrile (60+40) v/v+0.1 % TEA

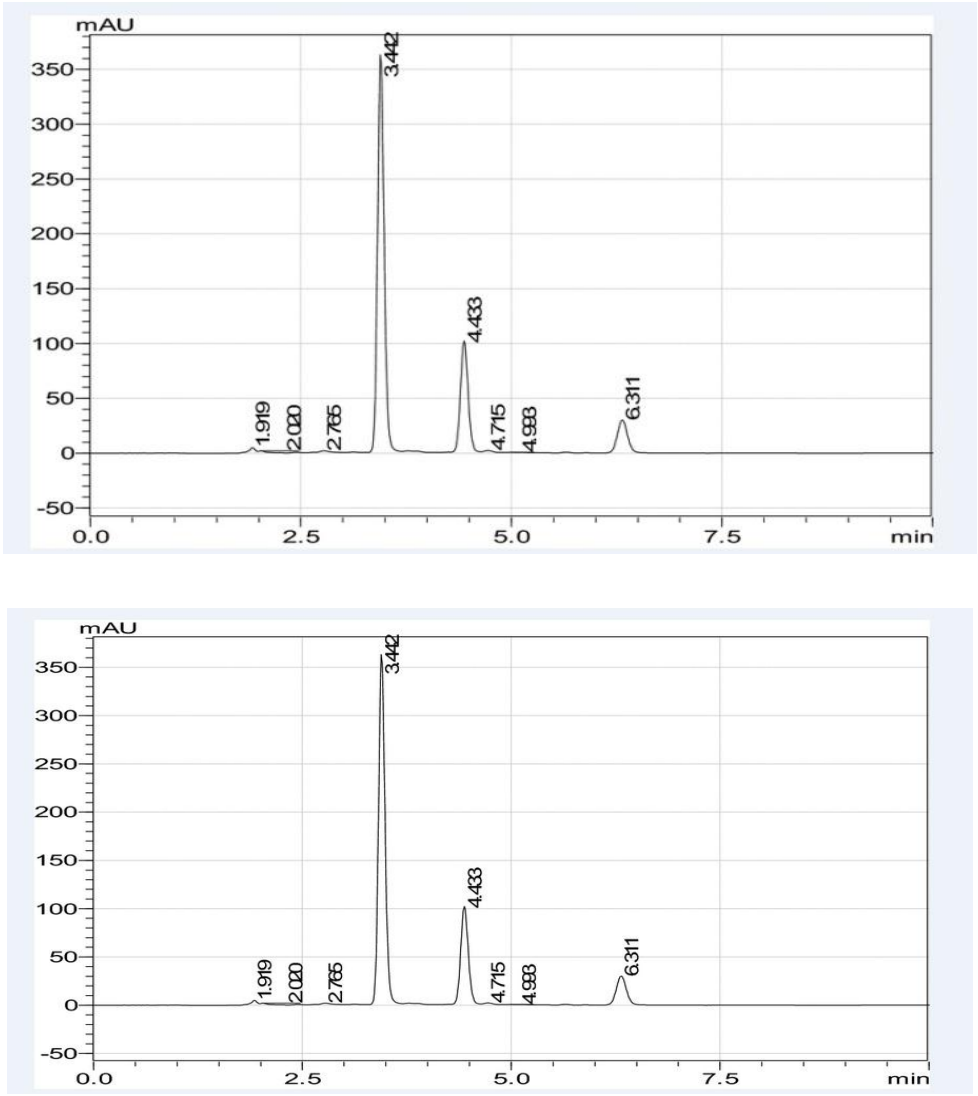


Fig. 5: Chromatogram of empagliflozin.

Table 7: Results of assay of empagliflozin

S. No.	Sample solution concentration (µg/ml)	Actual concentration found	Amount of drug estimated mean±SD*
1	30	29.81	
2	30	29.74	99.31±0.16
3	30	29.82	

*The value is represented as a mean±SD of 3 observations.

Precision

The repeatability, intra-day precision was calculated as the relative standard deviation of results from three samples, during the sameday, and the inter-day precision was studied by comparing on two different days. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2 were shown in table 8.

Table 8: Repeatability and Intermediate precision Forempagliflozin.

Precision	Concentration of drug (µg/ml)	Mean area±SD*	% RSD
Repeatability*	20	799 859±1636.10	0.37
	10	377 252±6808.76	1.80
Intra-d	20	835 741±3499.53	0.42
	30	121 438±2744.61	0.23
Inter-d	10	373 366±3209.56	0.86
	20	801 115±8040.09	0.10
	30	117 499±1936.27	0.16

*Each value is represented as a mean±SD of n observations. The value of n is 6 for repeatability study and 3 for intraday and interday precision. SD:
Standard deviation, %RSD: Percent relative standard deviation.

Accuracy

The accuracy was determined by the standard addition method. Amounts of 6; 10; 14 µg/ml of the Empagliflozin standard were added to the sample solution in which 10.0 µg/ml of the drug had been incorporated previously. The final concentrations of the fortified solutions were 16.0, 20.0 and 24.0 µg/ml of Empagliflozin. The recovery experiments were performed in triplicate for each concentration. The value of mean % recovery and % RSD (table 9) at each level was found within acceptance criteria that indicate the method is accur.

Table 9: Accuracy of empagliflozin.

Levels	Amount taken (µg/ml)	Amount found (µg/ml)	% recovery	% Mean recovery±% RSD
80%		16.36	102.2	101.25±0.17
	16	15.72	98.25	

		16.54	103.3	
100%		19.36	96.8	96.1±0.63
	20	19.15	95.75	
		19.15	95.75	
120%		27.88	92.93	93.03±0.13
	24	27.95	93.16	
		27.90	93.00	

*Percent recovery was done in triplicate, % recovery: Percent recovery, %RSD: Percent relative standard deviation

Robustness

Robustness was performed by changing various method parameters like a change in flow rate, the composition of mobile phase andchange in detection wavelength. Finally, the effect of these changes was not deliberate. The value of % RSD (table 10) was found to be within acceptance criteria which showed the reliability of the method.

Table 10: Robustness study of empagliflozin

Parameters	**% RSD**
A: Change in flow rate	1.98%
0.9 ml/min	
1 ml/min	
1.1 ml/min	
B: Change in Mobile Phase	1.64%
Water: ACN (55:45) v/v+0.1% TEA	
Water: ACN (60:40) v/v+0.1% TEA	
Water: ACN (65:35) v/v+0.1% TEA	
C: Change in wavelength	1.25%
279 nm	
280 nm	
281 nm	

*Each value is represented as % RSD of n observations. The value of n is 3 for change in flow rate, change in wavelength and change in mobile phase composition. %RSD: Percent relative standard deviation.

Limit of detection (LOD) and limit of quantitation (LOQ)

The sensitivity of measurement of Empagliflozin by use of proposed methods was estimated in terms of the limit of quantitation (LOQ) and limit of detection (LOD). The values of LOD and LOQ have been found to be 1.020µg/ml and 3.091µg/ml, respectively. These values show that method is sensitive.

Experimental design: In preliminary trials, the formulation variables in each step of the manufacturing process were evaluated for their significance by analysis of variance (ANOVA). Finally, found that the type and concentrations of ER polymer and binder concentration had a significant impact on drug release of part. The factorial design was used to evaluate the effect of independent variables (binder concentration and ER polymer concentration) on responses/dependent variables (drug release at 1h [Y1], 4 h [Y2] and 10 h [Y3]) of empagliflozin tablets. A two-factor, three-level factorial design is used for exploring quadratic response surfaces and constructing second order polynomial models with design expert (stat-ease, version-12). ANOVA (analysis of variance) is inevitably linked to experimental design, which was used to analyse the significance of the model and each selected response. It also generates polynomial equations. The response (Y1) in each trial was estimated by carrying out a multiple factorial regression analysis using the generalized

Quadratic model: $Y1 = b0 + b1X1 + b2X2 + b1b2X1X2$

Where, Y1 is the measured response associated with each factor level combination; b0 is an intercept; b1 and b2are regression coefficients computed from the observed experimental values of Y1 and X1 and X2 are the coded levels of independent variables. After fitting the response data in experimental design as in Table 1, the experimental results were analysed by ANOVA. It demonstrated the various statistical parameters such as sum of squares, F values, P values of mode lterms and correlation coefficient (R2) values. The suitability of model was authenticated by the predicted and adjusted R2 values.

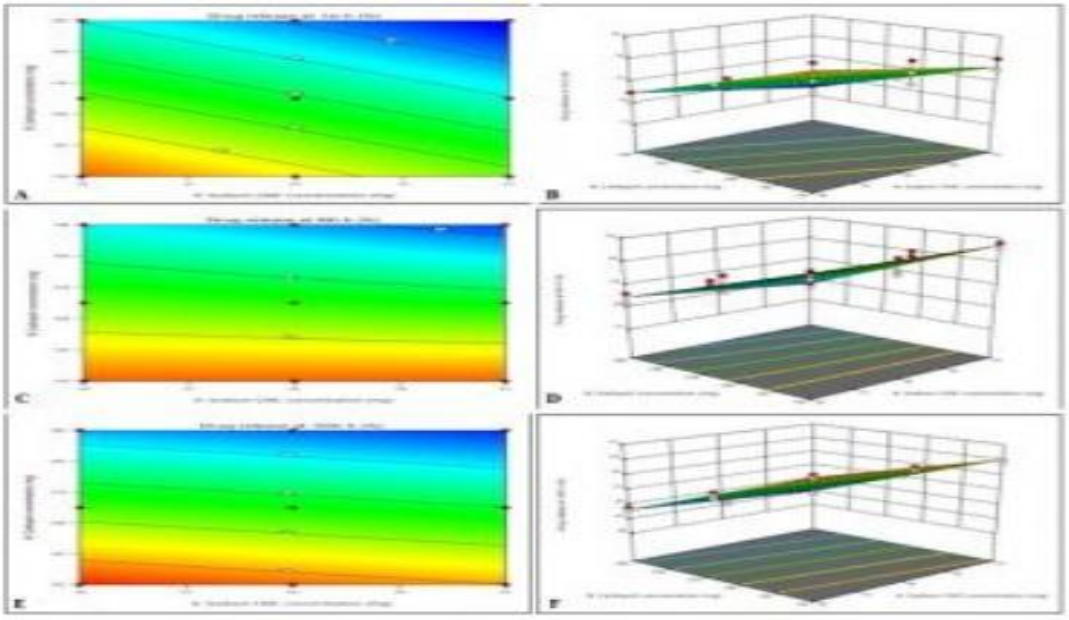


Fig: Experimental design empagliflozin INN Tablet.

CONCLUSION

Direct compression is becoming one of the most common and economical method of tablet manufacturing in the pharmaceutical industry. Although the principles governing direct compression have been well known for many years, the technique has only recently become more established as a result of the introduction of certain grades of excipients specifically designed for direct compression. A cost-effective formulation of Empagliflozin tablets was developed by 32 factorial design. Results of formulation evaluation of prepared between indicate that batch F5 is a promising formulation for the immediate release of the drug. Results obtained by validation studies suggested that the developed stability indicating assay method is simple, accurate, specific and precise. Thus, this method can be used for routine analysis of Empagliflozinformulation and to check the stability testing.

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CONFLICTS OF INTEREST

There is no conflict of interest.

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