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Compatibility Study and Solubility Enhancement of Febuxostat Using Box Behnken Design

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ABSTRACT

Compatibility study (based on microenvironmental pH and isothermal stress testing) of Febuxostat was carried out with selected excipients (Microcrystalline cellulose (MCC), Mannitol, Lactose, Hydroxypropyl-β-cyclodextrin (HP-β-CD), Polyethylene glycol(PEG) 6000, Polyvinyl pyrollidoneK30, Eudragit EPO, Sodium starch glycollate, Croscarmellose sodium (CCMC), Sodium lauryl sulphate, Magnesium stearate (MgS), Sodium steryl fumarate, Aerosil 200 and Purified talc) using FTIR and HPLC. Among them, Aerosil 200, MgS, Purified talc, Lactose and MCC were selected for the formulation of Febuxostat tablet. Three different polymers viz. PEG 6000, HP-β-CD and CCMC were selected as independent variables to enhance the dissolution rate by their complexation. Fifteen formulations obtained from Box Behnken design (BBD) (Minitab 16) were prepared through kneading method. Contour plot suggested CCMC (13.01mg) and HP-β-CD (65.45mg) excluding PEG 6000 for optimized formulation. Drug release profile of optimized formulation compared separately with formulation without filler, without polymer, physical mixture and a marketed product using similarity (fs) and dissimilarity (fd) factors showed similarity with marketed product. Similarly, similarity and dissimilarity factors for formulation without filler and optimized formulation was obtained within the range (fs= 82.34 and fd=5.42) indicating that the filler does not have any effect on the drug release. fs and fd for formulation without polymer and physical mixture lied outside the range suggesting the importance of the polymer complexation in the formulation. An accurate, simple, precise and robust reversed-phase liquid chromatographic method was developed for the estimation of Febuxostat. Furthermore, solid state characterization evaluated by FTIR showed that complexation between the polymers has occurred in the optimized formulation.

Keywords: Compatibility Study, Solubility Enhancement, Febuxostat, Box Behnken Design, Complexation

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INTRODUCTION

Febuxostat is a novel, orally administered, potent, non–purine, Xanthine oxidase inhibitor used in the management of hyperuricaemia in patients with gout & chronic tophaceous gout. It is approved by the European Commission for the treatment of chronic hyperuricaemia in conditions where urate deposition has already occurred (including a history or presence of tophus and/or gouty arthritis) (Pascual et al., 2009)[1]. It is Biopharmaceutical Classification System (BCS) class II compound. The drug is freely soluble in dimethylformamide, soluble in dimethylsulfoxide, sparingly soluble in ethanol, slightly soluble in methanol and acetonitrile and practically insoluble in water (Pandey et al., 2012)[2].

Excipients, although pharmacologically inert, can initiate, propagate or participate in chemical or physical interaction and can affect the chemical nature, stability and bioavailability of drugs and, consequently, their therapeutic efficacy and safety (Bharate et al., 2010)[3]. Thus, before developing the formulation it is important to know whether the excipients used in the formulation are compatible with the drug or not. Different approaches have been suggested and practiced to evaluate the compatibility issues. Design of compatibility studies involves the use of mixtures of drug with one or more excipients, forming binary or multicomponent drug–excipient mixtures (Narang et al., 2009)[4]. The study is commonly carried out by exposing the mixture on the stress conditions like elevated temperature, high humidity or by subjecting to the mechanical and oxidative stress. Samples stored in various stress conditions are subjected to qualitative and quantitative analysis. Generally, compatibility studies include visual inspection for colour changes, compactness and tablet integrity. In additional to this, quantitative chemical analysis can be carried out using UV-visible spectroscopy and High Performance Liquid Chromatography (HPLC) for monitoring drug degradation.

After determining the compatible excipients, formulations can be developed and optimized using experimental designs such as Central Composite design (CCD) or Box Behnken design (BBD). However, owing to the poor solubility of the drug, different approaches have been investigated and followed. Rapid disintegrating tablet of Febuxostat was developed by preparing solid inclusion complex of drug with Beta Cyclodextrin (β-CD) and Hydroxypropyl Beta Cyclodextrin (HP-β-CD) and characterized by Fourier Transform Infrared spectrophotometry (FTIR), Differential Scanning Calorimetry (DSC) and Powder X-ray Diffractometry (PXRD) studies (Kuchekar et al., 2013)[5]. Similarly, an optimized formulation of Febuxostat gastroretentive floating matrix tablet was developed from matrices by solid dispersion technique. Solubility of Febuxostat was enhanced

by solid dispersion using PEG 6000 as a hydrophilic carrier following the fusion technique (Kadam, 2012)[6]. The purpose of the study is to determine compatible excipients for Febuxostat tablet with enhancement in drug dissolution profile by kneading method.

MATERIALS AND METHOD

Materials

Febuxostat along with its reference standard and other excipients namely, MCC, Mannitol, Lactose, HP-β-CD, PEG 6000, PVP, Eudragit EPO, SSG, CCMC, SLS, MgS Sodium steryl fumarate, Colloidal silicon dioxide and Purified talc were received from Deurali Janta Pharmaceuticals Pvt. Ltd, Dhapasi, Kathmandu, Nepal as gift samples. One of the marketed products from India was purchased from retail pharmacy and was used as reference product for dissolution study.

Analytical method

A reversed-phase liquid chromatographic method was developed for the quantitative analysis of Febuxostat. The chromatographic condition is summarized in Table 1. The proposed method developed was validated using parameters viz. specificity, linearity, accuracy, precision, robustness, limit of detection and quantification for Febuxostat.

Table 1: Chromatographic Condition

S.No.	Parameter	Condition
1	Column	Inertsil C18, 150mm × 4.6mm
2	Mobile Phase	A mixture of 0.047M potassium dihydrogen orthophosphate and acetonitrile (1:1 v/v)
3	Flow Rate	1 ml/min
4	Column Temperature	35°C
5	Detection wavelength	260nm
6	Injection Volume	20µl

Preparation of Febuxostat standard and sample solution

Standard Solution Preparation

The standard stock solution of 500 ng/ml was prepared with mobile phase. Then, stock solution (2 ml) was further diluted to 20 ml (50 ng/ml).

Sample Solution Preparation

Twenty tablets were crushed and sample powder equivalent to 25 mg Febuxostat was taken. The dilution of the powder was done as per standard solution preparation.

Injection of Standard and sample solution

The standard and sample solutions previously filtered with 0.2µ filter and transferred in vials were

kept in rack of HPLC with 20µl injection volume setting along with other Chromatographic parameters. The peak area for Febuxostat was measured and then % assay was calculated.

Compatibility studies of Febuxostat

For the compatibility studies, MCC, Mannitol and Lactose were chosen as diluents, HP-β-CD, PEG 6000, PVPK 30 and Eudragit EPO as polymers, SSG, CCMC and SLS as disintegrants and MgS, Sodium steryl fumarate, Aerosil 200 and Purified talc as lubricants and glidants. Compatibility study was conducted through microenvironment pH testing and isothermal stress testing.

Microenvironmental pH testing

In 20 ml vial, 300 mg of Febuxostat and 300 mg of each of excipient were taken. The mixture was prepared by mixing in a vortex mixer for 4 minutes and 60µl of purified water was added to achieve water concentration of 20% w/w. This mixture was further mixed for 4 minutes in a vortex mixer in order to achieve consistent mixing. The vials were sealed properly and were stored in an oven at 50 ± 1°C for 4 weeks period. Control samples were prepared by blending drug and excipients in absence of water. The mixture was stored in refrigerator.

The microenvironmental pH of drug-excipient blend was estimated by adding 3 ml of previously boiled and cooled purified water to 600 mg blend in the vial. The suspension was mixed on a vortex mixer and the pH was recorded with a pH meter. Similarly, microenvironmental pH at zero time was also observed (Serajuddin et al., 1999)[7].

Isothermal stress testing

To 10 ml (screw capped) borosilicate glass vial (n=3), 100 mg of Febuxostat and 100 mg of selected excipients were added. To achieve water concentration of 10% w/w, 20µl of purified water was added. The mixture was mixed for 4 minutes in a vortex mixer followed by mixing with heat sealed glass capillary to achieve consistent mixing. To prevent any loss of material, the capillary was broken and left inside the vial. Vials were sealed properly and were stored in an oven set at 50 ± 1°C for 4 weeks period. Control samples were prepared by blending drug and excipients in absence of water. The mixture was stored in refrigerator (Serajuddin et al., 1999)[7]. The drug excipient blends were periodically examined for any unusual color change. After completion of 4 weeks samples were analyzed using HPLC and FTIR.

HPLC analysis

The control sample and IST samples were analyzed in HPLC method (Table 1).

FTIR analysis

Potassium bromide was oven dried and 2 mg of it was taken in an agate mortar. Similarly, 2 mg of drug excipient blend from samples was added. Proper mixing was carried out in mortar with pestle. The mixture was kept in the sample plate directly in FTIR. Thus obtained powder sample was analyzed by diffuse reflection method in FTIR in the IR range of 4000 to 400 cm^{-1} taking average of 20 consecutive scans.

Box Behnken Design

Box Behnken design (BBD) was used with three independent variables viz. PEG 6000, HP-β-CD and CCMC using Minitab 16 to get contour plots of the excipients that were significantly affecting the drug release (Table2 (a) & 2 (b)).

Table 2 (a): Design Table (randomized)

Run	Blk	PEG 6000	HP-β-CD	CCMC
1	1	0	-	+
2	1	0	0	0
3	1	0	+	-
4	1	-	-	0
5	1	0	0	0
6	1	+	-	0
7	1	-	+	0
8	1	-	0	-
9	1	0	-	-
10	1	+	0	-
11	1	-	0	+
12	1	+	+	0
13	1	0	0	0
14	1	+	0	+
15	1	0	+	+

Table 2 (b): Box- Behnken Design factor for tablet formulations

Factors	Polymers	Low (-)	Medium(0)	High (+)
A	PEG 6000	0	40	80
B	HP-β-CD	0	40	80
C	CCMC	0	7	14

The +, 0 and – represent highest, medium and lowest values respectively (Table 2 (a)). With the aim of developing the formulation with improved dissolution profile, amounts of three polymers viz. PEG 6000, HP-β-CD and CCMC are taken as factors in the design of formulation of Febuxostat tablet with the compression weight of 280 mg.

Tablet preparation method

For each of fifteen formulations (F1 to F15) obtained from BBD, Febuxostat, microcrystalline

cellulose and lactose were sieved through sieve no. 80, aerosil was sieved through sieve no.40 and CCMC, talcum and MgS were sieved through sieve no. 100. PEG 6000 was milled and passed through sieve no. 80. Similarly, HP-β-CD was passed through sieve no. 80. Febuxostat, CCMC, PEG 6000 and HP-β-CD were mixed by manual tumbling in a poly bag for 10 minutes. The mixture was kneaded in mortar and pestle with ethanol and water (1:1 v/v) for 30 minutes. The kneaded mass was dried in hot air oven at 60 ± 2°C and was pulverized through sieve no. 14. Previously sieved MCC and lactose were added and was mixed by manual tumbling in a poly bag for 10 minutes. Lubricant premix was prepared by mixing talcum, aerosil and magnesium stearate in a polybag followed by sieving through sieve no. 80. The above mixture and lubricant premix were mixed for about 3 minutes. Pre-compression parameters like bulk density, tapped density and Carr's consolidation index were determined for lubricated granules. The blend was compressed using 10 station rotary tablet machine.

Similarity Factor and Dissimilarity Factor

Similarity Factor

Similarity between the two products is assessed by using similarity factor (Eq. 2.1). The similarity factor (F_s) is a logarithmic transformation of the sum-squared error of differences between the test T_j and reference products R_j over all points (Prior et al., 2004)[8].

$$F_s = 50\, X\, log\, \left\{\left[1 + \left(\frac{1}{n}\right)\sum_{j=1}^{n}(Rj - Tj)^2\right]^{-0.5} X\, 100\right\} \quad \text{.....(2.1)}$$

Where, *n* is the sampling number, R_j and T_j are the % dissolved of reference and the test products respectively at each time points *j*. f_s value higher than 50 and close to 100 show the similarity of the dissolution profiles.

Dissimilarity Factor

The difference factor (F_d) measures the percent error between two curves over all time points (Eq. 2.2):

$$F_d = \left[\frac{\sum_{i=1}^{n}(Rj - Tj)}{\sum_{i=1}^{n} Rj}\right] X10 \quad \text{.....(2.2)}$$

The percentage error is zero when the test and drug reference profiles are identical and increase proportionally with the dissimilarity between the two dissolution profiles. f_d values should be close to 0 to be similar. In general, the values lower than 15 show the similarity of the dissolution profiles (Prior et al., 2004).

RESULTS AND DISCUSSION

Analytical Method Validation

An accurate, simple, precise an robust reversed-phase liquid chromatographic method was developed for the estimation of Febuxostat. The tailing factor obtained from standard injection was 0.878. Similarly, theoretical plate obtained was 4220.32. As the tailing factor obtained is less than 2.0 and theoretical plate is more than 2000, it shows that the system is suitable for the analysis. Similarly, from the concentration of 1, 2.5, 5, 10 and 20 mg/L, the curve of concentration versus area was plotted. A linear equation, Y = 193354X + 70005 with 0.9998 correlation coefficient (R^2) value was found. It signifies that the method of analysis of Febuxostat by HPLC was suitable and can be performed in varying concentration within this concentration range. From 5 standard solution of Febuxostat, the % RSD for the area was 0.033. As % RSD was less than 2%, it shows that the method is precise. Similarly, with different column of same dimension used on different day gave the intermediate precision/ ruggedness of the method. The % RSD for the area of five standard injections of Febuxostat was 0.051. As % RSD was less than 2%, it shows that the method is precise.

On injecting the standard solutions with 60, 100 and 140% accuracy chromatographic column, individual recovery as well as mean recovery value was determined. All the % recovery values obtained are within the acceptable range of 98.0% to 102.0%. Similarly, mean % recovery value obtained was 99.59. As all these values are within the acceptable range, it shows that the method holds good accuracy. Moreover, to evaluate the impact on the method, changes were made in the flow rate of the mobile phase (0.9 ml/min, 1.0 ml/min and 1.1 ml/min) and detection wavelength (255nm, 260nm and 265 nm). The result shows that even on changing the flow rate and detection wavelength, the tailing factor obtained was less than 2.0 and theoretical plate was more than 2000. This proves that the method is robust even on change in the flow rate of the mobile phase and detection wavelength.

Compatibility Study

Microenvironmental pH

Microenvironmental pH of mixture of drug and excipients was studied. As compared to zero time samples, there was no significant change in pH of the mixture of control samples and stability samples (Table 3).

Isothermal stress testing

Isothermal stress testing (IST) samples and control samples were periodically observed for any physical changes. There was no change in color. After completion of four weeks, samples were analyzed using FTIR and HPLC. HPLC analysis showed that the potency of the drug in control

and IST sample were 99.79 and 99.61 % respectively. It shows that moisture and temperature have not affected the stability of Febuxostat. Furthermore there was no significant difference in the potency in control as well as IST samples (Table 3).

To confirm the results from HPLC, FTIR spectrum were observed. FTIR spectrum of Febuxostat shows prominent peaks at 2928 cm^{-1} for C-H, 2231 cm^{-1} for C≡N, 1714 cm^{-1} for C=O, 1629 cm^{-1} for C=N and 1516 cm^{-1} for C=C (Kuchekar et al., 2014). FTIR spectra of drug alone along with IST sample of drug and IST sample of drug-excipients were scanned. There was no change in the principal peak of Febuxostat in the control and IST samples. (FTIR spectra not shown). This reflects that moisture and temperature used during IST testing did not affect the stability of Febuxostat. Furthermore, the result from microenvironment pH and isothermal stress testing (Table 3 and Table 4) signifies that the excipients tested are compatible with the drug.

Formulation Development

Based on the compatibility and the availability of the excipients, MCC was selected as diluent, PEG 6000, HP-β-CD and CCMC were selected as polymers for enhancing the release profile of the drug (40 mg), MgS (3 mg), aerosil 200 (3 mg) and purified talc (4 mg) were chosen as lubricants and glidants and lactose (14 mg) was selected in the formulation to increase the compressibility of the tablet. The amount of the excipients was determined based on the Handbook of Pharmaceutical Excipients(Rowe et al., 2009)[9].

As the drug chosen has poor solubility, the formulation was developed with the intension of improving the solubility of the drug. For this purpose, the drug was kneaded with the polymers after sieving all the ingredients. Number of trials revealed that drug:each polymer must be more than 1:2 to have desired increment in dissolution rate of the drug. As the weight of the tablet would be more than 280 mg with this ratio, BBD was carried out using CCMC in addition to PEG 6000 and HP-β-CD. The amount of CCMC was determined based on Handbook of Pharmaceutical Excipients.

Box Behnken Design

Regression analysis of dissolution at 45 minutes from Minitab 16 gave the equation 3.1

$$Z = 0.0045\ P + 0.373\ H + 4.505\ C + 0.0009\ P^{*}\ P - 0.0029\ H^{*}\ H - 0.184\ C\ *\ C + 0.00008\ P\ *\ H - 0.0088\ P\ *\ C + 0.0041\ H\ *\ C + 57.2304 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

Where, Z = Drug release, P = PEG 6000, H= HP-β-CD and C = CCMC

Among 3 factors, PEG 6000 has the lowest and CCMC has the highest multiplication coefficient value (Equation 3.1). It denotes that CCMC and HP-β-CD show their effect on the drug release as

Table 3: Microenvironmental pH of Control sample and IST sample of Febuxostat Statistically,

Excipients	MCC	Mannitol	Lactose	HP-β-CD	PEG 6000	PVPK 30	EPO	SSG	CCMC	SLS	MgS	SSF	Aerosil	Talc
pH in Zero Time ± SD	4.30± 0.01	5.02± 0.02	6.50± 0.04	4.14± 0.03	4.47± 0.02	3.77± 0.04	6.53± 0.01	5.57± 0.02	5.44± 0.02	5.75± 0.01	5.46± 0.02	7.09± 0.01	4.72± 0.02	5.93± 0.03
pH in Control Sample± SD	4.32± 0.02	4.98± 0.03	6.53± 0.03	4.18± 0.03	4.42± 0.02	3.82± 0.03	6.50± 0.03	5.61± 0.01	5.39± 0.03	5.77± 0.03	5.49± 0.01	7.00± 0.06	4.75± 0.03	5.95± 0.02
pH in IST Sample± SD	4.39± 0.03	5.05± 0.05	6.59± 0.05	4.22± 0.03	4.46± 0.05	3.79± 0.03	6.56± 0.04	5.69± 0.02	5.47± 0.02	5.72± 0.03	5.56± 0.01	7.13± 0.02	4.83± 0.02	5.98± 0.01

μ_0 = There is no significance difference in pH

μ_1= There is significance difference in pH

Level of significance: 0.05

Test Statistics: To compare two samples for means,we apply t-test

Calculated t value from t-test,

Excipients	MCC	Mannitol	Lactose	HP-β-CD	PEG 6000	PVPK30	EPO	SSG	CCMC	SLS	MgS	SSF	Aerosil	Talc
t calculated	1.57	0.14	2.00	3.00	1.50	2.33	0.01	2.00	0.25	0.20	1.86	0.38	1.75	2.33

The tabulated value of $t_{0.05}$= 12.71

Decision: As $t_{calculated} < t_{tabulated}$, null hypothesis is accepted.

Table 4: Assay of Control sample and IST sample of Febuxostat

Excipients	MCC	Mannitol	Lactose	HP-β-CD	PEG 6000	PVPK 30	EPO	SSG	CCMC	SLS	MgS	SSF	Aerosil	Talc
Potency in Control Sample± SD	99.78 ±0.18	98.76 ±1.12	98.57 ±0.11	97.88 ±0.21	98.76 ±0.45	102.74 ±0.57	99.96 ±0.75	97.59 ±0.54	98.30 ±0.56	101.41 ±1.17	100.75 ±0.54	102.75 ±0.91	99.49 ±0.59	97.74 ±0.79
Potency in IST Sample± SD	99.46 ±0.49	96.76 ±1.00	98.03 ±0.68	96.67 ±0.31	98.32 ±0.50	98.48± 0.37	97.13 ±0.72	97.16 ±0.68	97.65 ±0.50	100.79 ±1.10	99.90± 0.70	100.54 ±0.89	97.48 ±0.50	97.17 ±0.37

Statistically,

μ_0 = There is no significance difference in assay

μ_1= There is significance difference in assay

Level of significance: 0.05

Test Statistics: To compare two samples for means, we apply t-test

Calculated t value from t-test,

Excipients	MCC	Mannitol	Lactose	HP-β-CD	PEG 6000	PVPK30	EPO	SSG	CCMC	SLS	MgS	SSF	Aerosil	Talc
t calculated	2.38	2.24	6.30	4.50	6.64	0.29	1.03	12.21	6.23	3.55	0.72	1.49	1.51	8.93

The tabulated value of $t_{0.05}$= 12.71

Decision: As $t_{calculated} < t_{tabulated}$, null hypothesis is accepted.

Table 5 Comparing observed dissolution value with predicted value using F-test using regression equation

Ho: Population of observed value and predicted value from above equation have same variance
H1: Population of observed value and predicted value from above equation don't have same variance. They are different.
Level of significance :0.05
Test Statistics: To compare the differences in variance ,we apply F-test

Formulations	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**	**Optimized**
Observed Value (%)	80.57	90.67	70.45	78.59	89.96	78.98	95.12	65.12	62.36	72.49	98.16	95.99	90.59	95.61	93.29	99.21
Predicted Value (%)	80.93	90.46	70.39	79.75	90.46	80.94	93.32	67.51	58.85	73.89	96.81	95.03	90.46	93.33	97.06	100.18

F-Test Two-Sample for Variances

	Actual Value	Predicted value
Mean	84.82	84.96
Variance	149.34	148.75
Observations	16	16
df	15	15
F	1.00	
P(F<=f) one-tail	0.50	
F Critical one-tail	2.40	

The tabulated value of F0.05=2.40
Decision: As calculated F< tabulated F, null hypothesis is accepted.

compared to PEG 6000. Similarly, P value of PEG 6000, HP-β-CD and CCMC obtained from ANOVA were found to be 0.530, 0.002 and 0. P value of PEG 6000 is greater than 0.05, and that of HP-β-CD and CCMC are less than 0.05. It indicates that PEG 6000 has insignificant role in drug release in this study.

The values of independent variables, P, H and C were placed in equation 3 and predicted values of the responses were obtained as shown in Table 5. While performing F test between observed value and predicted value, as mentioned in Table 5, no significant difference was observed between observed value and predicted value at 0.05% level of significance.

Evaluation of lubricated granules

Moisture content of the granules was between 2.01(F4) and 2.71 % (F12). Similarly, bulk density and tapped density of granule were between 0.323 gm/ml (F4) and 0.588 gm/ml (F12) and 0.488 gm/ml (F8) and 0.667 gm/ml (F12) respectively. Furthermore, Carr's consolidation index was determined. Formulation F12 exhibited excellent flow property with Carr's consolidation index value of 11.84. Formulations F2, F3, F5, F6, F7, F10, F13, F14 and F15 exhibited good flow properties with the Carr's consolidation index value of 16.01, 14.97, 16.64, 16.78, 16.00, 14.54, 16.61, 14.17 and 14.36 respectively. Similarly, formulations F1 and F11 were fair passable with Carr's consolidation index value of 19.59 and 19.01 respectively. However, formulations F8 and F9 exhibited poor flow characteristic with the Carr's consolidation index value of 23.98 and 23 respectively. One of the formulations, F4, had very poor flow property with the Carr's consolidation index value (35.40).

Amount of granules obtained after kneading depends on the amount of powder present. Among 15 formulations, amount of the powder present for kneading was maximum in formulation F12. Similarly, formulations F2, F3, F5, F6, F7, F10, F13, F14 and F15 contained slightly less amount of powder as compared to formulation F12. Furthermore, formulations F1 and F11 contained less amount of powder as compared to above mentioned batches and formulations F8, F9 and F4 contained least amount of the powder while kneading. Thus, the Carr's consolidation index value shows that the flow property of the batches depends on the amount of the powder subjected to kneading and consequently granules present in the formulation.

Evaluation of Tablet

Compressed tablets of all formulation had weight within acceptable limit. The average weight, hardness, friability, DT and assay of each formulation are given in Table 6.

Table 6: Physico-mechanical properties of formulated tablets

Formulation No.	Avg Wt.(mg) ± SD	Avg. Hardness ± SD	Friability (%)	DT (minutes)	Assay (%)
F1	283.25 ± 3.09	7.06 ± 0.81	0.21	6	98.39
F2	279.80 ± 2.74	6.76 ± 0.92	0.15	6	99.33
F3	276.80 ± 2.56	6.34 ± 0.67	0.15	9	100.64
F4	276.10 ± 4.02	5.53 ± 0.90	0.48	6	103.55
F5	276.15 ± 2.86	6.75 ± 0.90	0.20	6	102.88
F6	278.45 ± 2.84	6.46 ± 0.87	0.19	7	101.11
F7	280.00 ± 2.79	6.46 ± 0.74	0.16	5	99.94
F8	285.25 ± 3.35	6.48 ± 0.60	0.28	9	97.03
F9	287.75 ± 3.73	6.85 ± 0.90	0.32	8	97.47
F10	277.70 ± 2.71	6.45 ± 0.85	0.14	8	102.75
F11	276.35 ± 3.11	6.87 ± 0.71	0.19	1	102.31
F12	280.25 ± 2.08	7.07 ± 0.92	0.11	6	101.12
F13	278.85 ± 2.85	6.35 ± 0.84	0.17	4	102.37
F14	279.65 ± 2.22	6.94 ± 0.94	0.09	4	101.50
F15	281.55 ± 2.19	7.00 ± 0.69	0.16	5	100.09
Optimized	275.95 ± 2.19	6.56 ± 0.84	0.21	1	102.75

***In-Vitro* Dissolution Study**

There was increment in cumulative percentage drug release at 45 minutes compared to 15 and 30 minutes. The lowest and highest cumulative percentage drug release were shown by F9 (62.36 %) and F11 (98.17 %) respectively. Formulation F11 showing highest cumulative percentage drug release contains medium amount of HP-β-CD and highest amount of CCMC. However, Formulation F9 showing lowest cumulative percentage drug release contains medium amount of only PEG 6000. Furthermore, formulations F2, F5, F7, F11, F12, F13, F14 and F15 showing cumulative percentage drug release profile inbetween F11 and F9 contains HP-β-CD and CCMC in the formulations (Figure. 1(a), 1(b) & 1(c)). These results suggest that CCMC and HP-β-CD are playing major role in drug release as compared to PEG 6000.

Formulation Optimization

Based on the dissolution data of 15 formulations at 45 minutes from BBD, contour plots were obtained using Minitab 16 (Figure 2(a), 2(b) & 2(c)).

Contour plots showed that CCMC has maximum role in enhancement of the drug release as compared to other polymers. Besides CCMC, HP-β-CD has shown positive effect in the dissolution profile of the drug (Figure. 2 (a), 2 (b) &.2 (c)). However, the contour plot shows that PEG 6000 has not got role in enhancement of drug release profile. The amount of the polymers

that should be added in the optimized formulation also supports the same. The amount of HP-β-CD and CCMC that should be added in the final optimized formulation as given by response surface optimizer are 65.45 and 13.01 mg per tablet respectively. Although, solubility of Febuxostat was enhanced by solid dispersion using PEG 6000 through the fusion technique (Kadam, 2012), in this study dissolution profile of Febuxostat was not improved by using PEG 6000 where kneading method was chosen instead of fusion technique. The reason behind this may be the change in method of solid dispersion.

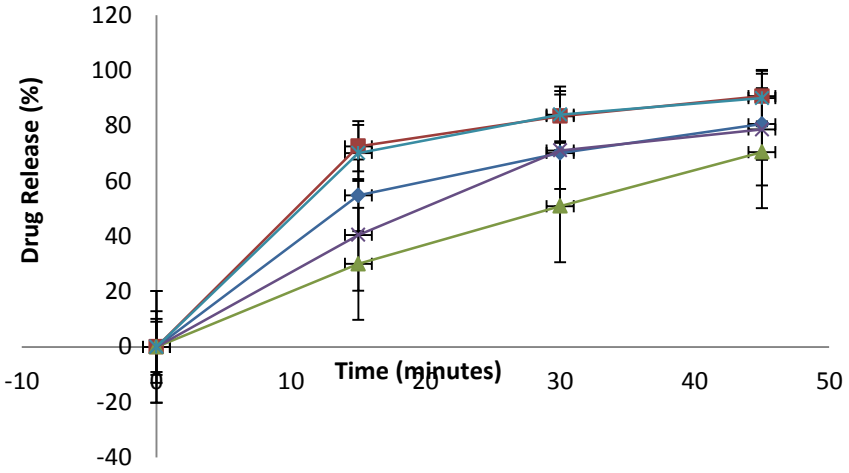

Figure 1 (a): Cumulative % drug release vs. time plot (F1- F5)

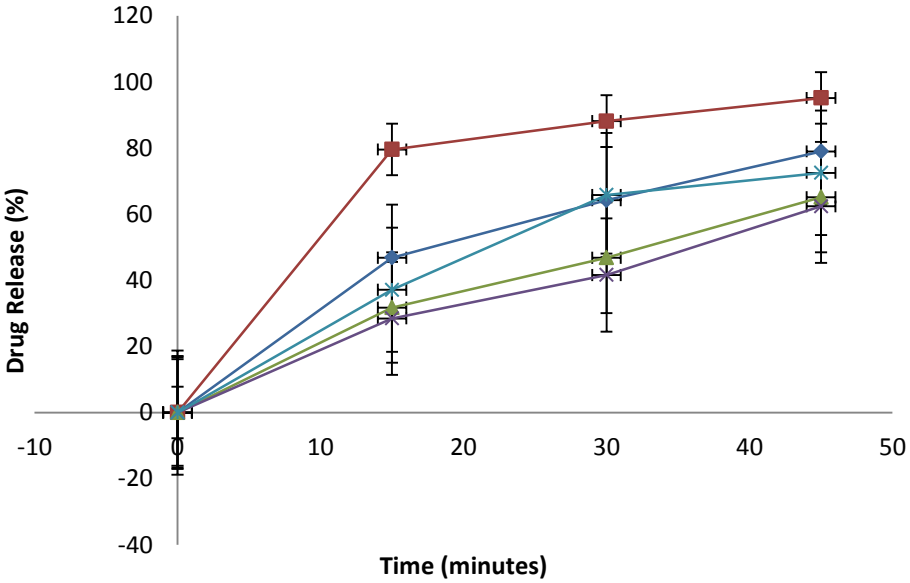

Figure 1 (b): Cumulative % drug release vs. time plot (F6 - F10)

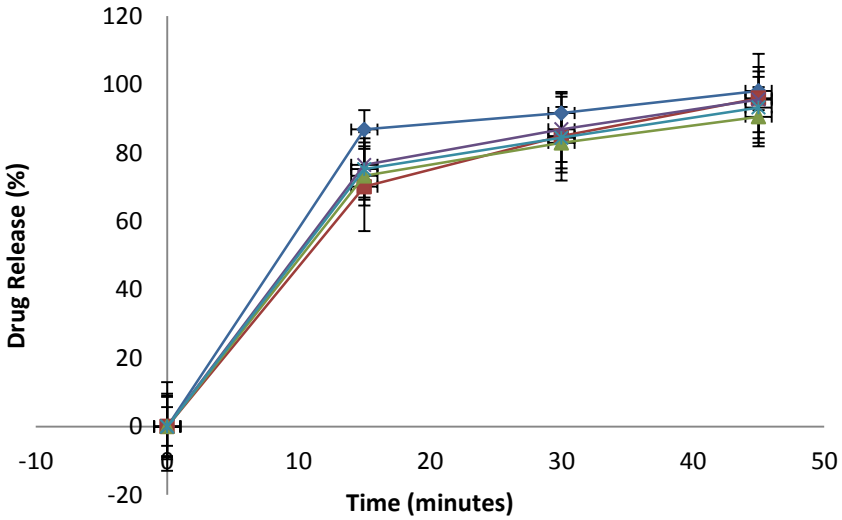

Figure 1 (c): Cumulative % drug release vs. time plot (F11 - F15)

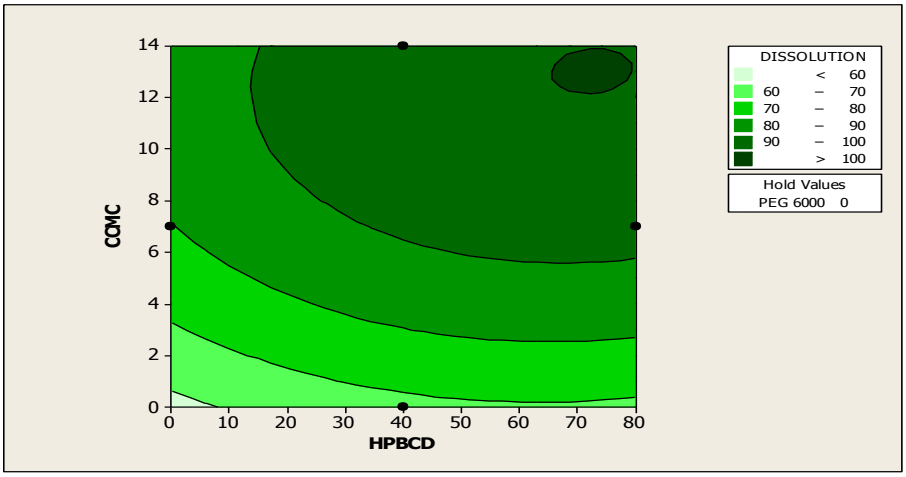

Figure 2 (a): Contour Plot of Dissolution at 45 minutes vs. CCMC and HP-β-CD

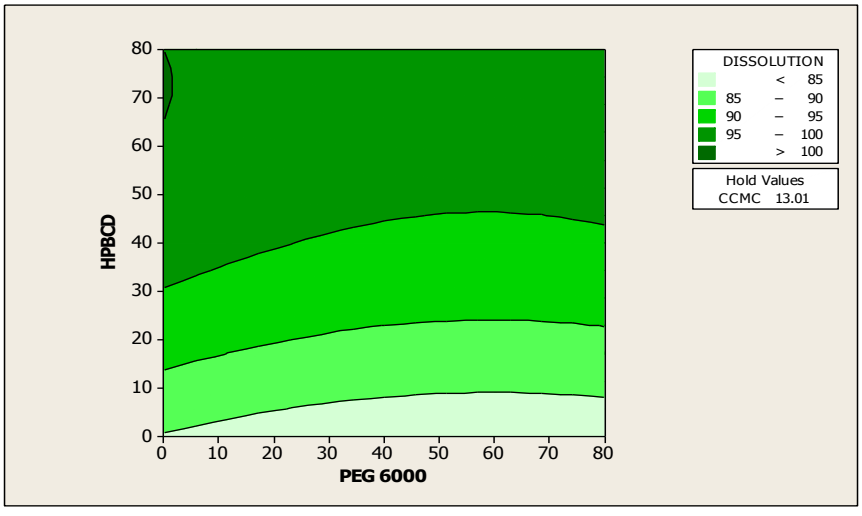

Figure 2 (b): Contour Plot of Dissolution at 45 minutes vs. HP-β-CD and PEG 6000

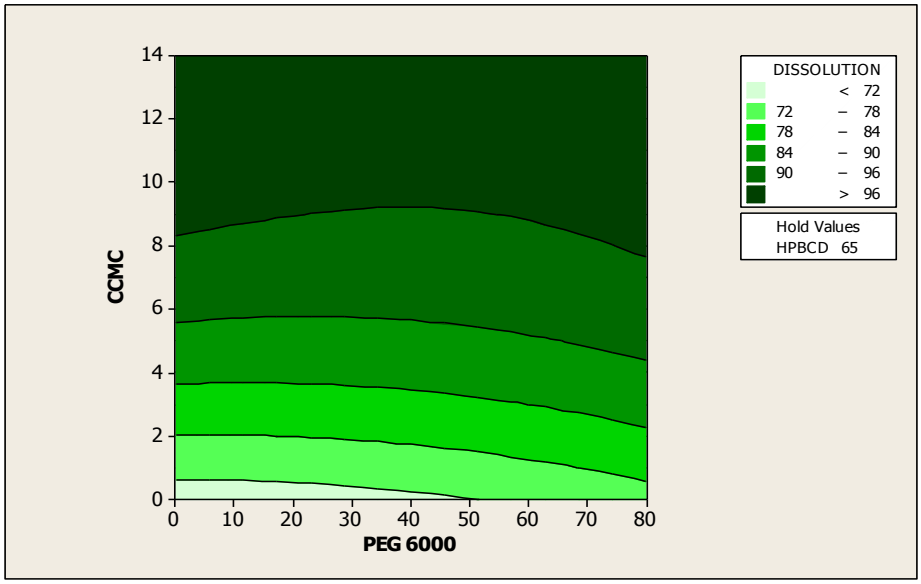

Figure 2 (c): Contour Plot of Dissolution at 45 minutes vs. CCMC and PEG 6000

Based on the amount given in response surface optimizer, the optimized formulation was prepared by kneading method. The Carr's Compressibility index obtained was 16.46 which denotes that granules for compression had good flow characteristics. Compression of the tablets was done with targeting total weight of 280 mg. The cumulative % drug release profile of the optimized formulation at 45 minutes was 99.21.

Comparison of dissolution profile with market product

A marketed product (Furic 40 mg) was purchased from the local market and the cumulative percentage drug release profile of the optimized formulation was compared with that of the marketed product. Cumulative percentage drug release shown by the market product at 15, 30 and 45 minutes were 88.82, 89.53 and 89.85 respectively.

Comparison of dissolution profile of optimized formulation, formulation without filler, formulation without polymer and physical mixture

The composition of optimized formulation, formulation without filler, formulation without polymer and physical mixture is shown in Table 7. The drug release profile of the optimized formulation was compared with that of formulation without filler, formulation without polymer and physical mixture. Cumulative percentage drug release shown by the formulation without filler at 15, 30 and 45 minutes were 85.87, 92.81 and 95.99 respectively. Similarly, cumulative percentage drug release shown by the formulation without polymer at 15, 30 and 45 minutes were 11.59, 25.66 and 34.75 respectively and those shown by physical mixture at 15, 30 and 45 minutes were 37.12, 46.12 and 54.79 respectively (Figure.3).

Table 7: Formulation details of physical mixture, formulation without polymers and formulation without filler

	Febuxostat (mg)	HP-β-CD (mg)	CCMC (mg)	Aerosil (mg)	MgS (mg)	Talcum (mg)	Lactose (mg)	MCC (mg)	Total Wt (mg)
Physical mixture	40	65.45	13.01	3	4	3	14	137.54	280
Formulation without polymers	40	0	0	3	4	3	14	216	280
Formulation without filler	40	65.45	13.01	3	4	3	14	0	142.46
Optimized formulation	40	65.45	13.01	3	4	3	14	137.54	280

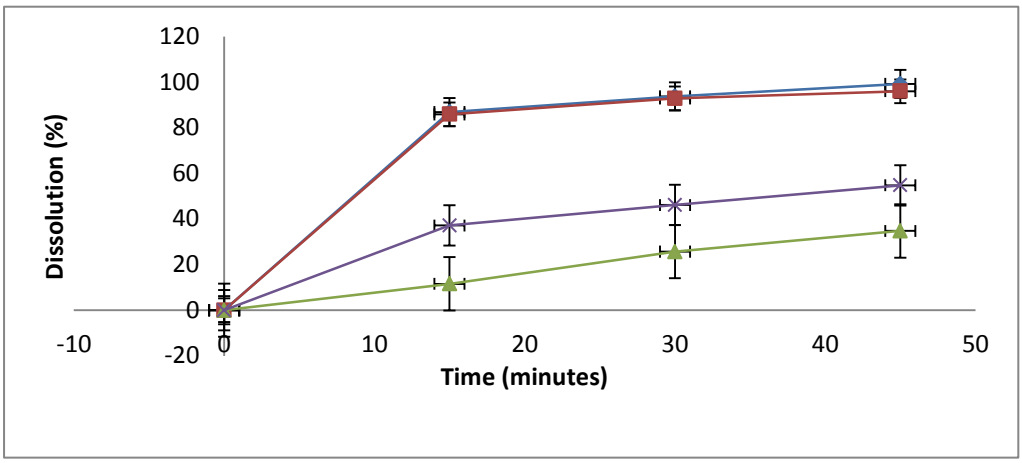

Figure 3:- Cumulative % drug release vs. time plot of optimized formulation, formulation without filler, formulation without polymer and physical mixture

The dissolution data of formulation without filler indicates that the filler has not got any effect on the drug release. However, low drug release profile of formulation without polymer and physical mixture clearly indicates the importance of the polymer complexation that has been done in the formulated batches.

Similarity and Dissimilarity Factors

The dissolution profile of optimized formulation was compared with market product, formulation without filler, formulation without polymer and physical mixture of optimized formulation. For more adequate dissolution profile comparison, similarity and dissimilarity factors were applied. Similarity and dissimilarity factors were obtained using equations 1.2 and 1.3. The values of the similarity and dissimilarity factor of market product and formulation without filler fall inside the range of 50 to 100 and 0 to 15 respectively. Similarity and dissimilarity factor obtained for market product was 60.67 and 11.71 respectively. It indicates that the drug release profile of the optimized formulation matches with that of the market product. Similarly, similarity and dissimilarity factor obtained for formulation without filler was 82.34 and 5.42 respectively. It indicates that the filler

has not got any effect on the drug release. However, similarity and dissimilarity factors for formulation without polymer was 7.92 and 224.26 and that for physical mixture was 16.24 and 152.85 respectively. It clearly indicates the importance of the polymer complexation that has been done in the formulations for the increment of drug release.

FTIR Study

FTIR spectrum of physical mixture and optimized formulation prepared by complexation are shown in Figure. 4 (a) and 4 (b) respectively.

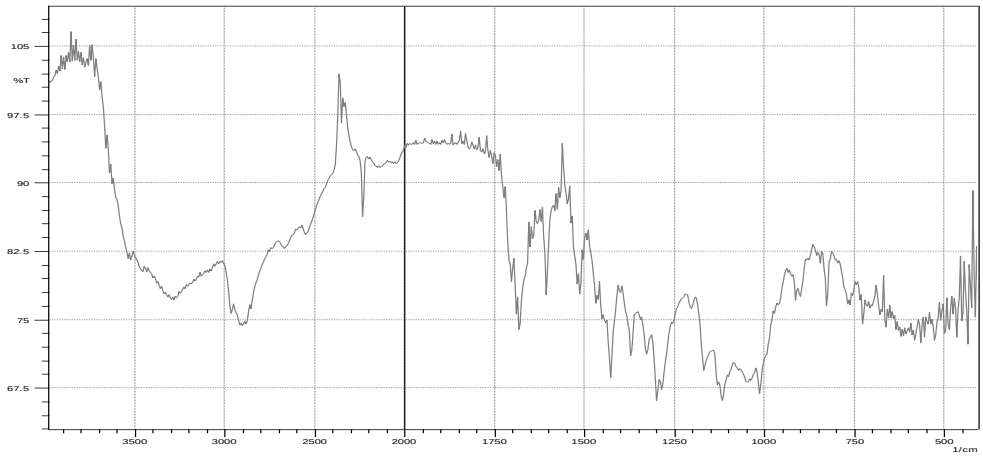

Figure 4 (a): FTIR spectrum of physical mixture

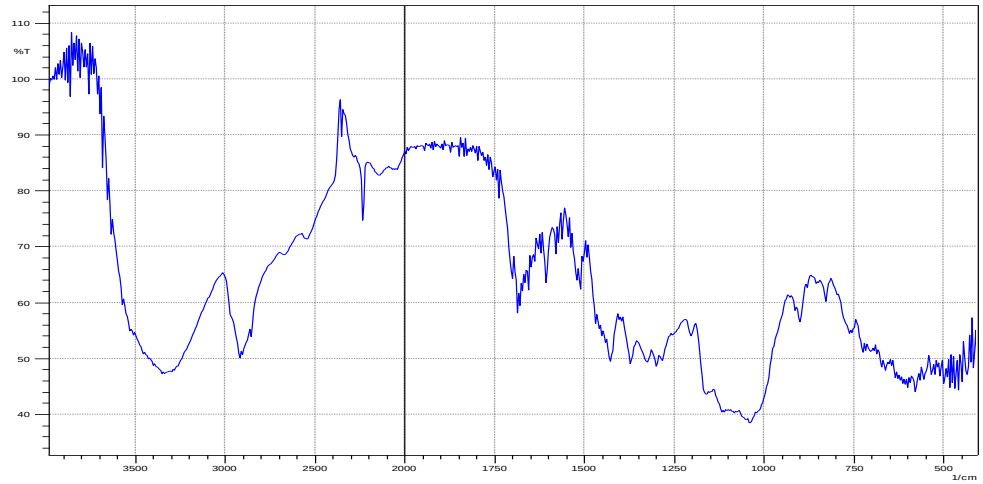

Figure. 4 (b): FTIR spectrum of optimized batch by complexation

The nonpolar isobutoxy group of the drug might have formed an inclusion complex with HP-β-CD, thus enhancing the solubility of drug in presence of CCMC. Following peaks of the drug were missing in the optimized formulation but are present in the physical mixture.

- 2961 cm^{-1} due to CH3 stretching
- 1143 cm^{-1} due to C-O-C stretching

Thus, it indicates the occurrence of complexation while formation of the optimized formulation.

SUMMARY AND CONCLUSION

In the present study, identification of compatible excipients was carried out for the development of stable febuxostat tablet with enhanced cumulative percentage drug release. Microenvironmental pH and Isothermal stress testing were carried out to evaluate the compatibility of the drug with the excipients. Different categories of excipients were selected viz. polymers, diluents, disintegrants and lubricants. Samples were tested through HPLC and FTIR analysis and compatible excipients were used in the formulation. A mixture of 0.047 M potassium dihydrogen orthophosphate and acetonitrile (1:1v/v) was used as mobile phase at flow rate of 1.0ml/min with the detector wavelength of 260 nm. The retention time of Febuxostat was found to be 4.64 minutes. The method was linear over the concentration range of 1ppm to 20 ppm. The test for accuracy of method of assay showed mean % recovery value of 99.59. For observing the robustness of the developed method, the standard solution of Febuxostat was prepared and was analyzed at different flow rates and wavelength. The results indicated that the method was robust even when flow rate and wavelength were changed.

Based on the compatibility and the availability, the excipients were chosen for the development of formulation. Complexation process was chosen for the formulation development. Three different polymers namely, polyethylene glycol 6000, HP-β-CD and CCMC were selected and the formulation was developed using Box Behnken design in Minitab 16. Fifteen formulations obtained from Box Behnken design were prepared through kneading method. Evaluation of precompression parameters of each formulation was done. Tablets were compressed with the compression weight of 280 mg and were evaluated for weight variation, drug content, hardness, friability, in-vitro disintegration time and percentage drug released. From the amount obtained from the response surface optimizer, the optimized batch was prepared. As per the optimization plot and the contour plot, PEG 6000 did not have role in enhancement of drug release profile. Thus, PEG 6000 was eliminated from the formulation and optimized formulation was formulated with CCMC and HP-β-CD. The pre-compression parameters like bulk density, tapped density and carr's index were determined. Similarly, physico-mechanical property and drug release profile of the optimized formulation was observed. Improvement was seen in the drug release profile of the tablet. In addition to this, drug release profile of the optimized formulation was compared with that of formulation without filler, formulation without polymer, physical mixture and a market product. For more adequate dissolution profile comparison, similarity and

dissimilarity factors were applied. Similarity and dissimilarity factors indicate that the drug release profile of the optimized formulation matches with that of the market product. Similarly, the value for formulation without filler was also obtained within the range indicating that the filler has not got any effect on the drug release. However, the values for formulation without polymer and physical mixture lies outside the range provided which clearly indicates the importance of the polymer and the complexation that has been done in the formulation. Furthermore, Solid state characterization was evaluated by FTIR. It showed that complexation has occurred in the optimized formulation. Hence, complexation technique can be sucessfully used for the improvement of the dissolution profile of febuxostat.

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