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Research Article

Formulation and Evaluation of Herbal Tablets Containing Ethanolic Extract of *Croton bonplandianus* baill –A Quality by Design (QbD) Approach

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ABSTRACT

Herbal plants have various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of plants. *Croton bonplandianus* baill belonging family of Euphorbiaceae was show antihyperlipidemic activity due to presence of various secondary metabolites such as- Flavonoids, Alkaloids, Glycosides, Tannins, Saponin and phenolic compounds. The present paper deals with formulation and evaluation of herbal tablets prepared from ethanolic extract of *Croton bonplandianus* baill stem and various excipients Polyvinylpyrrolidone (PVP) K 30, Croscarmellos sodium (CCS), Lactose, Magnesium stearate, Talk Finally, the Round and flat shaped tablets, with average weight of 600 mg were compressed, used in treatment of hyperlipidemia. The software Design Expert version 12.0.1.0 (Stat-Ease) was used for the design of the formulation. The total of 13 runs (formulations) was designed and the relationship of the dependent and independent variables was studied by gaining the surface responses, and finally, the optimized formula was achieved. Stability study of optimize formulation was done, both physically and chemically conditions, for a period of 3 months at accelerated stability conditions ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $60 \pm 5\% \text{ RH}$) and ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $75 \pm 5\% \text{ RH}$).

KEY WORDS- Flavonoids, Alkaloids, Glycosides, Tannins, Saponin Polyvinylpyrrolidone (PVP), the Croscarmellose sodium (CCS), Lactose, Magnesium stearate, Talk, etc.

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INTRODUCTION-

Plant-based traditional medicine system continues to play a vital role in the healthcare system with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care. Modern knowledge on medicinal plant research till contains at least 25% drugs and many others¹, which are synthetic

analogues, built on prototype compounds isolated from medicinal plants².

Phytomedicines are more popular than conventional synthetic drugs due to their easy availability and no side effects. *Croton bonplandianus* is widely used as traditional ayurvedic medicine all over India, South Africa, and Asia for treating different health disorders.

It is a short-height green shrub, and the whole part of the plant, including its root, has medicinal value. Different phytochemicals have been isolated from this plant, mainly alkaloids, flavonoids, saponin, tannins, glycosides, and phenols³.

Croton bonplandianus baill. (Euphorbiaceae), commonly known as “*Ban Tulsi*”, is a perennial herb found in waste lands and road side areas in India⁴. This plant is used to treat liver, Hyperlipidemia.⁵

The plant *Croton bonplandianus* baill is a heteroicous exotic weed. It is a perdurable herb and normally 30- 40 cm in height, with whorled ranches which grows in sandy clay soil, found in all situations, railway lines and roadsides, in open and follow fields in India. Due to the resemblance of the leaves and flower cymes to that of Tulsi, *Croton bonplandianus* baill is often called *Ban Tulsi* (Jungle tulsi)⁶. The parts of plant are widely used in traditional system of medicine such as hepatoprotective, swelling of the body, cure against ring worms and skin disease, antihypertensive, antioxidant, wound healing, antifungal, antimicrobial, antidiabetic, antitumor, anticancer, acute constipation, abdominal dropsy, internal abscesses, antifertility, antispasmodic, antiseptic, antidote, analgesic, repellent property against insects, nematocide, anticoronary, anti-inflammatory, larvicidal activity, antihelmentic⁷, this is also used for treatment of cholera, boils, bowel complaints, chicken pox, diarrhoea, dysentery, eye diseases, cold and coughs, epilepsy, gastric disorders, insanity, jaundice, liver complaints, scurvy, sprains, malaria, rheumatism, and so on. This plant is also considered as chologogue and purgative⁸.

QbD is described in ICH Q8 (Food and Drug Administration, 2006), Q9 (ICH, 2005) and Q10 (ICH, 2008) guidance documents and is a major shift from the traditional approach of QbD in ensuring quality control of products across the manufacturing stream⁹. QbD principles promote innovation and continuous improvement of the product. Knowledge based commercial manufacturing ensures enough regulatory flexibility for setting specifications and post approval changes. Product and process are designed using innovative risk based techniques to meet predefined quality objectives thereby satisfying the most critical patient needs and regulatory requirements at low cost¹⁰. Quality by Design (QbD) has become a new concept for development of quality pharmaceutical products, It is an essential part of the modern approach to pharmaceutical quality, QbD is a best solution to build a quality in all pharmaceutical products but it is also a major challenge to the Pharmaceutical industry whose processes are fixed in time, despite inherent process and material variability, Under this concept of QbD¹¹.

MATERIALS AND METHODS

Plant material- The plants were collected from tropical and desert areas of Jaipur, Rajasthan Plant was identified and authenticated at Botany Department of the Apex University, Jaipur.

Preparation of Extract-

In successive solvent extraction, a dried material was extracted with different solvents, starting from solvent of low polarity. The extraction was continued until the solvent in the thimble. After extraction by one solvent, material is removed from thimble, dried and again recharged, extracted with solvent of successively high polarity. Successive solvent extraction was done by using petroleum ether (60-80), Ethyl Acetate, and ethanol and purified water. The extract was filtered and concentrated. The extract was dried in vacuum drier and stored below 10°C.

Phytochemical tests of extracts

The various phytochemicals tests performed for selected plants extracts for further study are following

Test for carbohydrates:-

1. Molisch's test: To about 2ml of the sample, 2 drops of alcoholic solution of α -naphthol was added and to the mixture after being shaken well. Few drops of conc. H_2SO_4 were added along the sides of the test tube. A violet ring indicates the presence of sugars¹².
2. Fehling's test -Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.
3. Iodine test- Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate¹³.

Test for alkaloids

1. Dragendorff's test- Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendorff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids. Each extract was boiled (15 minutes) in HCl (25.0 ml, 1%). Equal volumes of the resulting suspension were filtered into two test tubes (A and B). To A, 5 drops of freshly prepared Dragendorff's reagent was added. Formation of a precipitate indicated the presence of alkaloids. To confirm the results, B was treated with saturated sodium carbonate solution until a drop of the solution turned the Universal Indicator paper blue, (pH 8- 9). The resulting solution was dissolved in $CHCl_3$ (4 ml) and allowed to stand. The aqueous layer was collected and acetic acid added to it drop wise, until the solution turned Universal Indicator paper yellow-brown (pH 5)¹⁴.

2. Hager's test –Take clean test tube and add few mL filtrate extract + 1-2 mL Hager's reagents (saturated picric acid), a creamy white precipitate indicates the test is positive¹⁴.

Tests for glycosides

- 1 Liebermann's test: - 2 ml of the organic extract was dissolved in 2 ml of chloroform and 2 ml of acetic acid was added and the solution cooled well in ice. Sulphuric acid was then added carefully. A color change from violet to blue to green indicates the presence of a

steroidal nucleus (that is, a glycone portion of glycoside).

2 Salkowski's test:- 2 ml of each extract was dissolved in 2 ml of chloroform. 2 ml of sulphuric acid was added carefully and shaken gently. A reddish brown colour indicates the presence of a steroidal ring (that is, a glycone portion of glycoside).

3 Keller-Kiliani test:- 2 ml of each extract was dissolved in 2 ml of glacial acetic acid containing one drop of FeCl_3 solution. The mixture was then poured into a test tube containing 1 ml of concentrated H_2SO_4 . A brown ring at the interphase indicates the presence of a deoxy sugar, characteristic of cardenolides¹⁴.

Tests for steroids

(i) A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid added indicates the presence of steroids.

(ii) The development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acids indicates the presence of steroids¹⁵.

Test for Phenolic compounds and Tannins

1. Gelatin test

The extract (50 mg) is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

2. Lead acetate test

The extract (50 mg) is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

3. Alkaline reagent test

An aqueous solution of the extract is treated with 10% ammonium hydroxide solution. Yellow

fluorescence indicates the presence of flavonoids.

Test for Saponins:-

The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins¹³.

Test for Flavonoids:-

1. **Shinoda test:** To 1ml of the extract, add 8 – 10 drops of concentrate HCl and a pinch of magnesium powder or filing. Boil for 10 to 15 minutes and cool. A red coloration indicates the presence of flavonoids¹⁴.

2. To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

3. **Alkaline reagent test:-** 1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl) An intense yellow colour, becomes colourless on addition of diluted acid.

4. **Shibata's reaction/Cyanidin test:-** 1gm Aq. extract + dissolved in 1-2 mL 50% methanol by heating + metal magnesium + 5-6 drops of conc. HCl A red colour {flavonols}, orange colour {flavones}¹²

Test for Triterpinoides:-

Salkowski's test: - Filtrate + few drops of conc. H_2SO_4 (Shaken well and allowed to stand) Golden yellow layer (at the bottom)¹⁴.

Gums and mucilage Test:-

1. **Spot test:** - small quantity of the extract was taken and pressed between 2 filter papers. The appearance of spots indicates presence of oils

2. **Saponification test:** - To the extract, few drops of 0.5N alcoholic KOH and few drops of phenolphthalein were added. This mixture was heated for about 2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils or fats¹².

Table 1. – Show Results of Phytochemical investigations of the extracts of Stem of *Croton bonplandianus* baill plant.

S.No.	Phyto-constituents	Petroleum Ether (60-80 °C) (Extract)	Ethyl Acetate (Extract)	Ethanol (Extract)	Purified Water (Extract)
1.	Flavonoids	-	-	+++	+
2.	Alkaloids	-	-	++	+
3.	Glycosides	-	-	++	+
4.	Steroids	-	+	++	-
5.	Phenolic compounds and Tannins	-	-	++	++
6.	Saponin	-	-	+++	+
7.	Carbohydrates	-	+	-	+
8.	Triterpenoids	-	-	++	+
9.	Fixed oils and Fats	+	++	-	-
10.	Gum and Mucilage	-	+	+	+

‘+’ and ‘-’ indicate the presence and absence of the compounds respectively. ‘+ +’ indicates moderate degree of presence and ‘+ + +’ indicates the high degree of the presence.

Formulation of Tablets

The full factorial design was applied to the formulation design of the herbal tablets. The three different factors are evaluated in this design at two different levels. The

three independent variables selected are PVP K30, CCS and lactose. There are two different levels for the selected variables as low and high, and they are coded as – 1, and + 1, respectively. The responses are considered

as dependent variables, and they are friability and disintegration time of the designed and formulated herbal tablets. The software Design Expert version 12.0.1.0 (Stat-Ease) was used for the design of the formulation. The total of 13 runs (formulations) was designed and the relationship of the dependent and independent variables was studied by gaining the surface responses, and finally, the significant model was achieved.

Steps in Tablet formulations^{16,17}

This procedure use for the optimize formula of tablets.

- First all herbal ingredients were accurately weighed and passed through sieve separately. Due to hygroscopicity of extract was triturated with accurately weighed quantity of anhydrous lactose (dry mixture)
- Made translucent granulating paste with required quantity of water and starch.

- The remaining herbal ingredients i.e., *Croton bonplandianus* baill (stem) Ethanolic Extract along with Croscarmellose sodium were mixed with this granulating paste until a wet coherent mass was formed.
- The wet coherent mass was then passed through sieve 10 to form granules. The granules were kept in oven at 40-45° until the granules were properly dried.
- After proper drying the granules were passed through sieve no. 22 mesh superimposed on sieve no. 44 mesh to separate granules from fines.
- The granules were then mixed thoroughly with the accurately weighed quantity of talc and magnesium stearate
- Finally, the Round and flat shaped tablets, with average weight of 600 mg were compressed by using single tablet Punch machine, press with a die size of 6 mm.

Table-2 Composition of Formulation

Formulation code	Factor 1 Polyvinylpyrrolidone (PVP) K 30 (mg)	Factor 2 Croscarmellose sodium (CCS) (mg)	Factor 3 Lactose (mg)	Magnesium Stearate (%)	<i>Croton bonplandianus</i> baill(stem) Ethanolic Extract (mg)	Talk
F 1	15	20	80	0.5	400	q.s
F 2	60	30	50	0.5	400	q.s
F 3	37.5	30	20	0.5	400	q.s
F 4	37.5	10	20	0.5	400	q.s
F 5	15	30	50	0.5	400	q.s
F 6	37.5	30	80	0.5	400	q.s
F 7	37.5	10	80	0.5	400	q.s
F 8	60	20	20	0.5	400	q.s
F 9	15	20	20	0.5	400	q.s
F 10	60	20	80	0.5	400	q.s
F 11	15	10	50	0.5	400	q.s
F 12	37.5	20	50	0.5	400	q.s
F 13	60	10	50	0.5	400	q.s

Pre-Formulation Evaluation Parameters

Bulk Density.

Apparent bulk density (pb) of powder blend was determined by placing pre-sieve blend into a graduated cylinder and measuring the volume (V) and weight (M). Bulk density was calculated by using given equation¹⁴.

$$pb = M/V$$

Tapped Density.

Tapped density was determined by pouring the accurately weighed quantity of powder blend into the graduate cylinder and the volume (V) was measured. Then the graduated cylinder was closed with lid, and tapped by using bulk density apparatus till a constant volume was maintained in the cylinder. The tapped density was calculated by using given equation¹⁸.

$$pt = M/V$$

Compressibility Index (Carr's index)

Compressibility index is also known as Carr's index and can be obtained by employing the poured density and tapped density values of a material. Theoretically it can be said that the less compressible a material the more flowable it is. It can be determined by substituting the

values of poured density and tapped density in the equation given below:

$$C = (pt - pb) / pt \times 100$$

Where, pt is tapped density and pb is untapped density¹⁹.

Hausner's Ratio

Hausner's ratio is an index of powder flow and was measured by the ratio of tapped density to the bulk density.

$$\text{Hausner's ratio} = pt / pb$$

Where, pt is tapped density and pb is untapped density¹⁹.

Angle of repose: -Angle of repose was determined using funnel method. The blend was poured through a funnel that can be raised vertically until a maximum cone height (h) was obtained. Radius of the heap (r) was measured and the angle of repose (Θ) was calculated using the formula

$$\Theta = \tan^{-1} (h / r)$$

Where, Θ = Angle of repose, h = Height of the pile, r = Average radius of the powder cone²⁰

Post-compression Evaluation Colour and Appearance

The compressed tablets were examined for their colour and appearance²¹

Tablet thickness

The thickness of 5 tablets each selected at random from the formulated tablets was determined using a vernier caliper and the mean of these readings was taken as the mean tablet thickness²¹.

Hardness

Hardness also term as crushing of the tablets Tablet hardness was measured with Monsanto hardness tester. The reading at the marked scale was recorded for the pressure at which breakdown of the tablet occurs²². Hardness was expressed in kg/cm².

Weight variation test

The average weight was determined by randomly selecting and weighing 20 tablets. Each tablet was also weighed individually. The deviation from the average weight in each case was calculated and expressed as percentage. Not more than two of the tablets from the sample size ton deviate from the average weight by a greater percentage and none of the tablets deviate from than double that percentage²¹.

Friability test

This test is carried out by using Friability apparatus. The weighted tablets are placed in the apparatus and it is rotated at 25 rpm for 4 minutes. After sometimes tablets are removed out from apparatus and again they are weight. The friability is calculated by using following formula:

It should be preferably below 1.0%²³.

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

Where,

W1= weight of tablets before test,

W2 = weight of tablets after test

Disintegration Time

Disintegration is the breakdown process of the tablet into small particles. In disintegration apparatus there are 6 baskets in which one tablet each was placed and it was kept in a beaker filled with 900ml of water at maintained temperature 37±0.5°C. The time in minutes determines the disintegration of the tablet²⁴.

Stability study

The herbal tablet formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing (25°C±2°C and 60 ± 5% RH) and (40°C±2°C and 75 ±5% RH) for 3 months. The formulation was checked for Physical appearance, Hardness, friability test and disintegration test at the interval of 30, 45, 60, 90 days (3 month) months. The formulation was tested for stability under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines²⁵.

RESULT AND DISCUSSION

In the formulation of the herbal tablets, the precompression parameters performed were angle of repose (θ), bulk density (g/cm³), tapped bulk density (g/cm³), Carr's Compressibility Index, and Hausner ratio. For the better compression of the tablet, it is important that the precompression parameters show a better response. The response reveals the flow properties, moisture content, and compressing properties. Data of the pre-compression parameter is presented in blow Table.

Table 3: Evaluation parameter of granule formulation

Formulation	Angle of repose (θ)	Bulk Density	Tapped density	Carr's index ratio	Hausner's ratio	Loss on drying
F 1	29.42±0.56	0.412±0.01	0.490±0.01	15.91±0.18	1.18±0.013	0.86
F 2	24.11±0.55	0.312±0.03	0.363±0.03	14.04±0.23	1.16±0.008	0.99
F 3	28.63±0.76	0.269±0.08	0.322±0.05	16.45±0.52	1.19±0.006	0.93
F 4	32.63±0.13	0.318±0.04	0.346±0.08	8.09±0.18	1.08±0.007	1.05
F 5	21.52±0.44	0.309±0.06	0.349±0.07	11.46±0.55	1.12±0.005	0.85
F 6	24.36±0.24	0.325±0.01	0.376±0.07	13.56±0.28	1.15±0.012	0.67
F 7	28.96±0.71	0.358±0.06	0.389±0.06	7.96±0.36	1.08±0.007	1.01
F 8	34.77±0.13	0.303±0.08	0.322±0.04	5.90±0.22	1.06±0.011	0.89
F 9	28.13±0.21	0.298±0.07	0.361±0.08	17.45±0.51	1.21±0.005	1.06
F 10	27.35±0.27	0.296±0.09	0.326±0.06	9.20±0.48	1.10±0.005	1.09
F 11	35.56±0.10	0.288±0.02	0.318±0.03	9.43±0.63	1.10±0.006	0.87
F 12	29.22±0.19	0.272±0.02	0.316±0.03	13.92±0.44	1.16±0.003	0.97
F 13	31.86±0.17	0.286±0.08	0.306±0.01	6.53±0.23	1.06±0.012	0.98

Post compression parameters are the quality control aspect of the herbal tablet. Variation in the ratio of Polyvinylpyrrolidone (PVP) K 30, Croscarmellose sodium (CCS) and lactose is responsible for the variation in response of hardness, friability, and

disintegrations. The other quality control parameters reported are thickness, hardness and weight variation. Collective results of post-compression are shown in blow Table.

Table 4: Evaluation parameter of tablet formulation

S. No	Formulation	Thickness (mm)	Hardness (kg/cm ²)	Friability (%)	Weight variation (%)	Disintegration time (min. sec)
1	F 1	5.8±0.01	5.5±0.12	0.46±0.03	600±0.88	11.16
2	F 2	5.7±0.03	5.9±0.05	0.56±0.05	600±1.54	12.36
3	F 3	6.1±0.03	8.1±0.06	0.56±0.07	598±1.29	13.25
4	F 4	6.0±0.04	5.3±0.06	0.23±0.08	599±1.57	11.33
5	F 5	6.0±0.08	5.3±0.09	0.78±0.09	600±1.87	11.1
6	F 6	5.8±0.07	5.4±0.03	0.88±0.03	600±1.97	12.32
7	F 7	5.9±0.06	8.2±0.04	0.52±0.01	601±2.01	14.28
8	F 8	5.9±0.06	7.7±0.07	0.45±0.01	603±0.75	12.08
9	F 9	6.1±0.07	6.0±0.04	0.56±0.08	602±0.67	9.35
10	F 10	6.0±0.01	6.0±0.09	0.52±0.09	601±1.87	11.11
11	F 11	6.1±0.03	5.8±0.12	0.44±0.04	600±1.16	10.18
12	F 12	6.0±0.03	5.2±0.08	0.53±0.09	601±0.99	9.48
13	F 13	6.1±0.05	8.1±0.11	0.41±0.10	599±1.33	13.45

Optimization of formulation By Design of expert (DOE) Approach

The experimental nature based on this mixture of the component has resulted in 13 separate tablet formulation batches (counting 3 centre points in construction). As indicated, numerous tablet lots were prepared and then assessed for each of the responses. The responses observed were fit to 13 runs, and it has been noted that the best fit model was the linear model for the two

dependent variables. The significance of the model with that of comparing with the other model for the analysis by analysis of variance (ANOVA). In polynomial equations, positive sign before the factor shows the linear correlation between response and factor, while the negative sign shows the inverse relation between the same. All the responses recorded for 13 runs and the relation of independent and dependent variables are presented in table

Build Information

Table 5: Build information of DOE software

File Version	12.0.1.0		
Study Type	Response Surface	Subtype	Randomized
Design Type	Box-Behnken	Runs	13
Design Model	Quadratic	Blocks	No Blocks
Build Time (ms)	1.0000		

Table 6: Coated and actual Factors of formulation

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	PVP K30	mg	Numeric	15.00	60.00	-1 ↔ 15.00	+1 ↔ 60.00	37.50	18.37
B	CCS	mg	Numeric	10.00	30.00	-1 ↔ 10.00	+1 ↔ 30.00	20.00	8.16
C	Lactose	mg	Numeric	20.00	80.00	-1 ↔ 20.00	+1 ↔ 80.00	50.00	24.49

Table 7: Formulation trials as per Box–Behnken design

Formula Tions	Factor 1 (PVP) K 30 (mg)	Factor 2 CCS (mg)	Factor 3 Lactose (mg)	<i>Croton bonplandianus</i> baill(stem) Ethanolic Extract (mg)	Magnesium Stearate (%)	Talk (mg)	Response 1 Disintegration time	Response 2 Friability test
F 1	15	20	80	400	0.5	q.s	11.16	0.46
F 2	60	30	50	400	0.5	q.s	12.36	0.56
F 3	37.5	30	20	400	0.5	q.s	13.25	0.56
F 4	37.5	10	20	400	0.5	q.s	11.33	0.23
F 5	15	30	50	400	0.5	q.s	11.1	0.78
F 6	37.5	30	80	400	0.5	q.s	12.32	0.88
F 7	37.5	10	80	400	0.5	q.s	14.28	0.52
F 8	60	20	20	400	0.5	q.s	12.08	0.45
F 9	15	20	20	400	0.5	q.s	9.35	0.56
F 10	60	20	80	400	0.5	q.s	11.11	0.52
F 11	15	10	50	400	0.5	q.s	10.18	0.44
F 12	37.5	20	50	400	0.5	q.s	9.48	0.53
F 13	60	10	50	400	0.5	q.s	13.45	0.41

Limits of Variables (Constraints)

Table 8: Variables operating range for herbal tablet formulation Constraints

Name	Goal	Lower Limit	Upper Limit	Importance
A: PVP K30	is in range	15	60	3
B: CCS	is in range	10	30	3
C: Lactose	is in range	20	80	3
Disintegration time	none	9.35	14.28	3
Friability test	none	0.23	0.88	3

Selection of working method was done on the basis of Disintegration time and Friability test of formulation. A factorial design was used to study the effect of independent variables on the dependent variables (In below table).

Table 9: Independent variables

S. No.	Coding	Variables
1.	X1	A: Polyvinylpyrrolidone (PVP) K 30 (mg)
2.	X2	B: Croscarmellose sodium (CCS) (mg)
3.	X3	C: Lactose (mg)

Table 10: Dependent variables

S. No	Coding	Variables
1.	Y1	Disintegration time
2.	Y2	Friability test

Fit Summary

Table 11: Response 1: Disintegration time

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	0.3766	0.0390	-0.4103	
2FI	0.4349	0.0561	-0.8656	
Quadratic	0.0173	0.9104		Suggested
Cubic				Aliased

ANOVA for Quadratic model

Table 12: Response 1: Disintegration time ANOVA for Quadratic model

Response 1: Disintegration timeSource	Sum of Squares	Mean Square	F-value	p-value	
Model	26.35	2.93	14.54	0.0248	significant
A-PVP K30	6.50	6.50	32.28	0.0108	
B-CCS	0.0055	0.0055	0.0274	0.8791	
C-Lactose	1.02	1.02	5.08	0.1096	
AB	1.01	1.01	5.02	0.1110	
AC	1.93	1.93	9.60	0.0534	
BC	3.76	3.76	18.69	0.0228	
A ²	0.1020	0.1020	0.5067	0.5279	
B ²	9.90	9.90	49.18	0.0060	
C ²	3.48	3.48	17.28	0.0253	
Residual	0.6040	0.2013			
Cor Total	26.95				

Factor coding is Coded.

Sum of squares is Type III - Partial

The Model F-value of 14.54 implies the model is significant. There is only a 2.48% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, BC, B², C² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve suggested model.

Table 13: Coefficients in Terms of Coded Factors

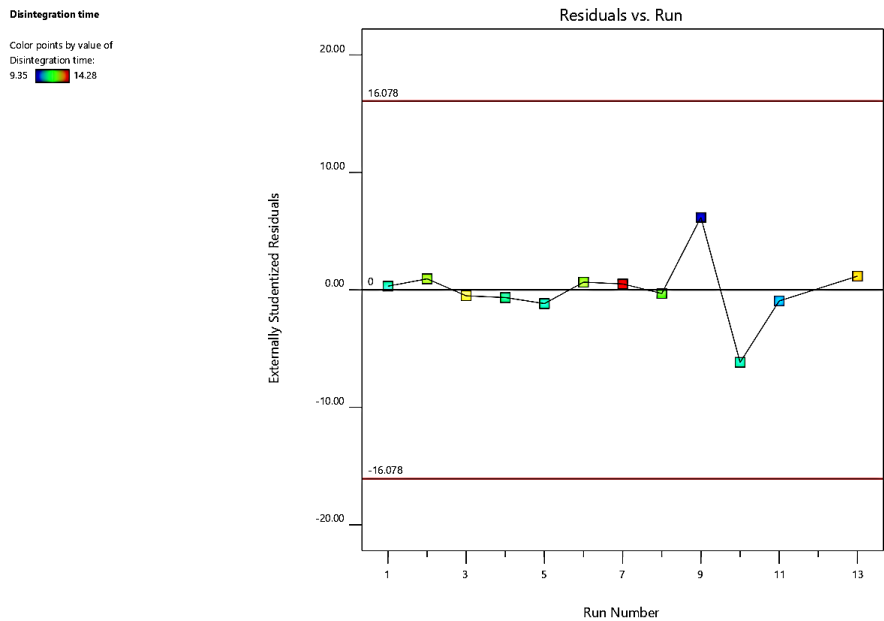
Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High	VIF
Intercept	9.48	0.4487	8.05	10.91	
A-PVP K30	0.9012	0.1586	0.3964	1.41	1.0000
B-CCS	-0.0263	0.1586	-0.5311	0.4786	1.0000
C-Lactose	0.3575	0.1586	-0.1474	0.8624	1.0000
AB	-0.5025	0.2243	-1.22	0.2115	1.0000
AC	-0.6950	0.2243	-1.41	0.0190	1.0000
BC	-0.9700	0.2243	-1.68	-0.2560	1.0000
A²	0.2113	0.2968	-0.7332	1.16	1.35
B²	2.08	0.2968	1.14	3.03	1.35
C²	1.23	0.2968	0.2893	2.18	1.35

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

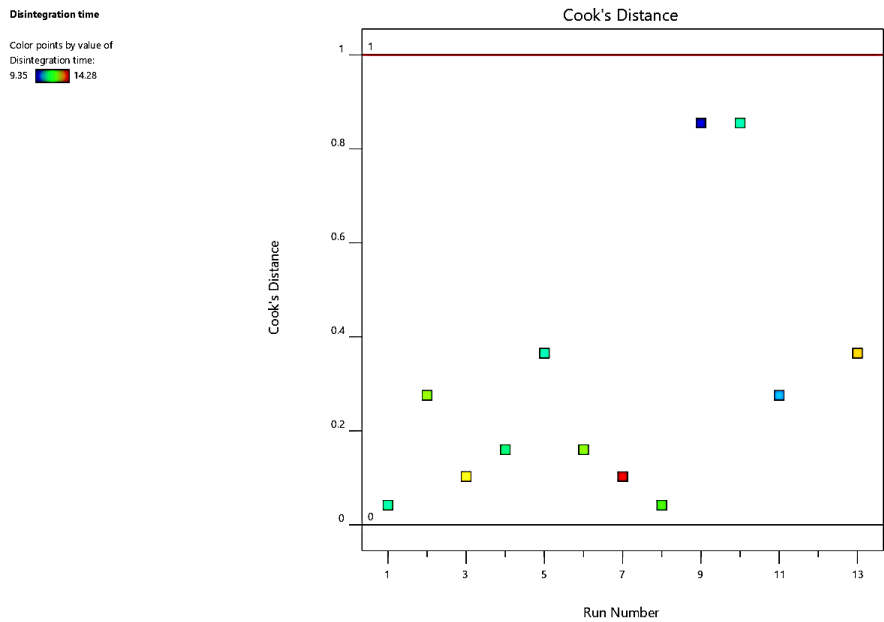
The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients

Final Equation in Terms of Coded Factors

Disintegration time = +9.48+ 0.9012 A -0.0263 B +0.3575 C -0.5025 AB -0.6950 AC -0.9700 BC +0.2113 A² +2.08 B² +1.23 C²



Graph 1: residual vs run of Disintegration time dependent variable



Graph-2 Distance of formulation
Predicted and actual value of Disintegration time (All formulation)

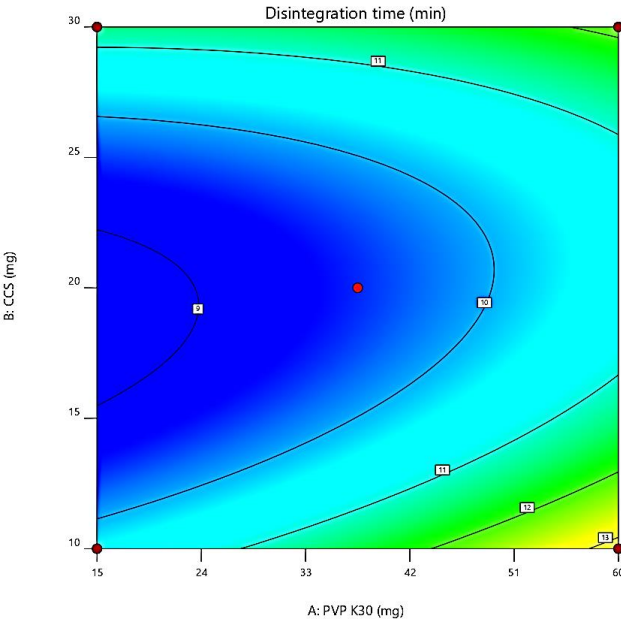
Table 14: Predicted and actual value of Disintegration time

Formulation codes	Actual Value	Predicted Value
F 1	11.16	11.08
F 2	12.36	12.15
F 3	13.25	13.38
F 4	11.33	11.49
F 5	11.10	11.35
F 6	12.32	12.16
F 7	14.28	14.15
F 8	12.08	12.16

Formulation and Evaluation of Herbal Tablets Containing Ethanolic Extract of *Croton bonplandianus* baill –A Quality by Design (QbD) Approach

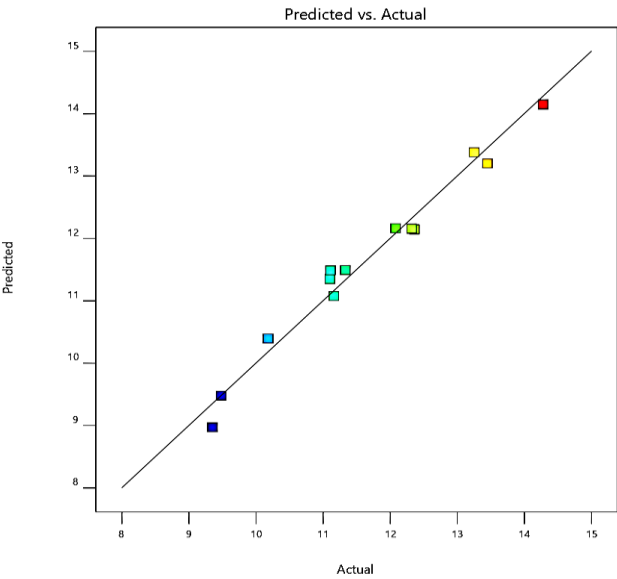
F 9	9.35	8.97
F 10	11.11	11.49
F 11	10.18	10.40
F 12	9.48	9.48
F 13	13.45	13.20

Factor Coding- Actual
● Design Points
9.35 14.28
X1 = A: PVP K30
X2 = B: CCS
Actual factor
C: Lactose = 50



Graph 3: Response surface plot of Disintegration time dependent variable

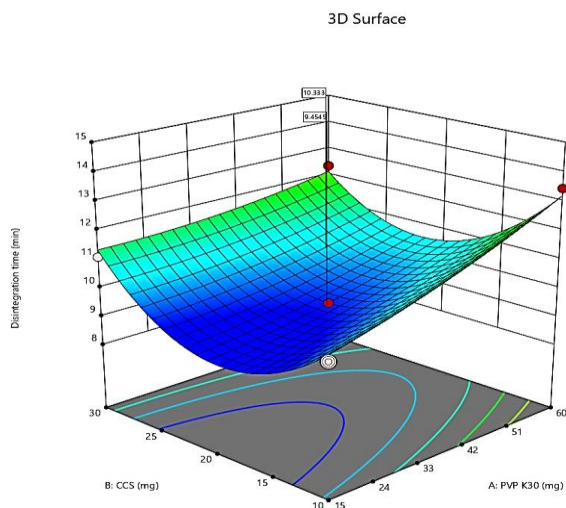
Disintegration time
Color points by value of
Disintegration time:
9.35 14.28



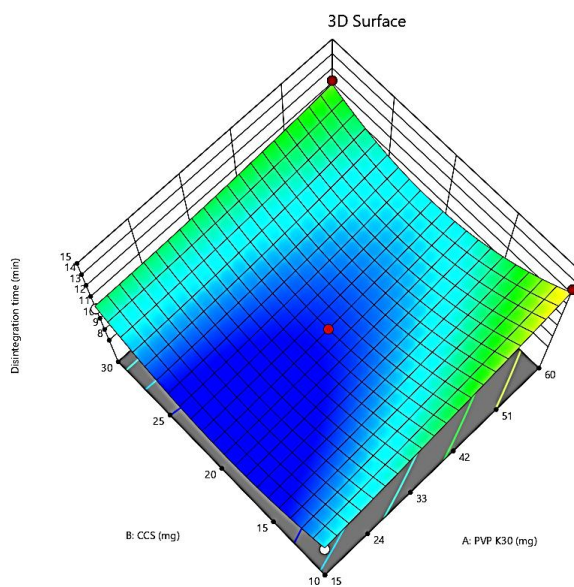
Graph 4: predicted vs actual value of Disintegration time dependent variable

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Factor Coding: Actual
 Design Points:
 ● Above Surface
 ○ Below Surface
 9.35 14.28
 Disintegration time (min) = 10.18
 Std # 1 Run # 11
 X1 = A: PVP K30 = 15
 X2 = B: CCS = 10
Actual Factor
 C: Lactose = 50

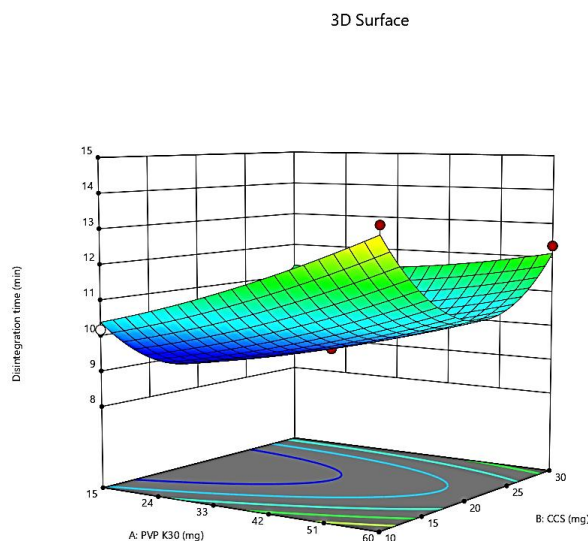


Factor Coding: Actual
 Design Points:
 ● Above Surface
 ○ Below Surface
 9.35 14.28
 X1 = A: PVP K30
 X2 = B: CCS
Actual Factor
 C: Lactose = 50



Formulation and Evaluation of Herbal Tablets Containing Ethanolic Extract of *Croton bonplandianus* baill –A Quality by Design (QbD) Approach

Factor Coding: Actual
Design Points:
● Above Surface
○ Below Surface
9.35 14.28
X1 = A: PVP K30
X2 = B: CCS
Actual Factor
C: Lactose = 50



Factor Coding: Actual
Design Points:
● Above Surface
○ Below Surface
9.35 14.28
X1 = A: PVP K30
X2 = B: CCS
Actual Factor
C: Lactose = 50

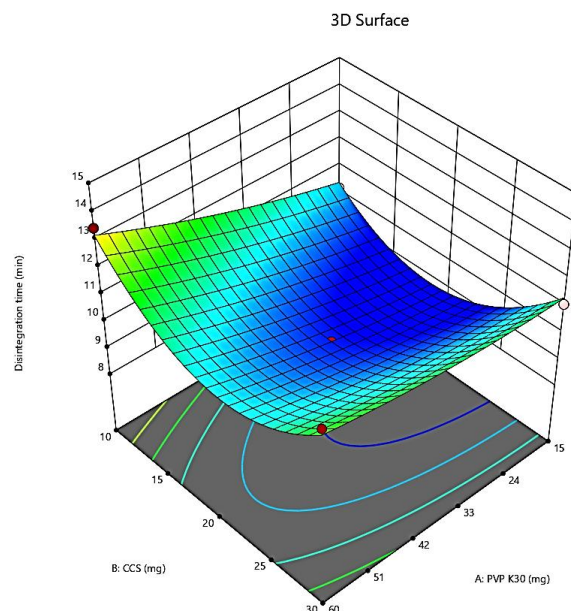


Figure 1: Response surface plot showing combined effect of PVP K 30 and CCS on Disintegration time of tablet formulation

The results of formulations as per the design when fitted into various models, a linear model was found to be significant for Disintegration time with F value of 14.54 and P value 0.0248. The model equation is as follows:
Disintegration time = $++9.48+ 0.9012 A -0.0263 B$

$+0.3575 C -0.5025 AB -0.6950 AC -0.9700 BC +0.2113 A^2 +2.08 B^2 +1.23 C^2$. The effect of both the factors A and B can be explained with the help of the 3D response surface plot as shown in above Figure.

Effect of formulation variables on Friability

Table 15: Response 2: Friability test (Fit Summary) Fit Summary

Response 2: Friability test

Source	Sequential p-value	Adjusted R ²	Predicted R ²	
Linear	0.0064	0.6392	0.4027	Suggested
2FI	0.7047	0.5645	-0.2164	
Quadratic	0.9493	0.2146		
Cubic				Aliased

ANOVA for Linear model

Table16: Response 2: (ANOVA Linear model)

Response 2: Friability test

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	0.2273	3	0.0758	8.09	0.0064	Significant
A-PVP K30	0.0113	1	0.0113	1.20	0.3017	
B-CCS	0.1740	1	0.1740	18.57	0.0020	
C-Lactose	0.0421	1	0.0421	4.49	0.0632	
Residual	0.0843	9	0.0094			
Cor Total	0.3117	12				

Factor coding is Coded.

Sum of squares is Type III - Partial

The Model F-value of 8.09 implies the model is significant. There is only a 0.64% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve suggested model.

Table 17: Coefficients in Terms of Coded Factors

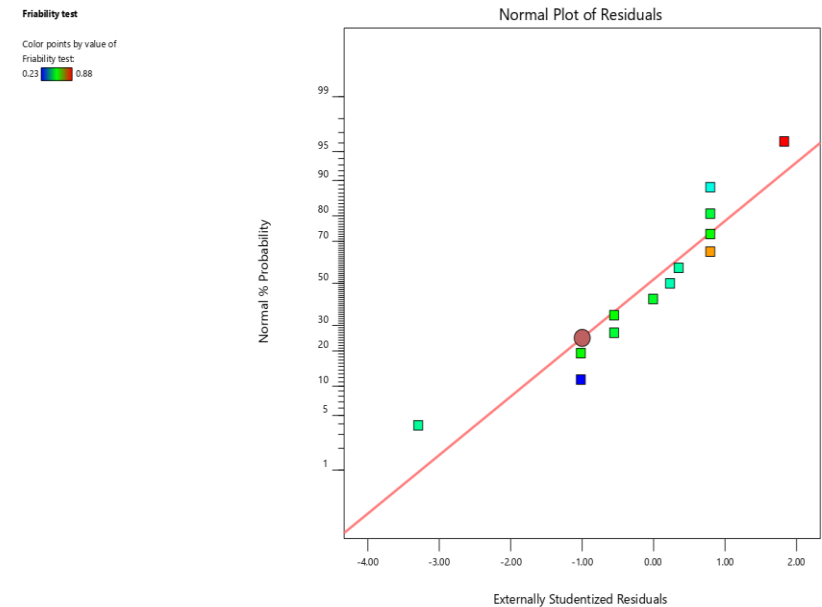
Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	0.5308	1	0.0268	0.4700	0.5915	
A-PVP K30	-0.0375	1	0.0342	-0.1149	0.0399	1.0000
B-CCS	0.1475	1	0.0342	0.0701	0.2249	1.0000
C-Lactose	0.0725	1	0.0342	-0.0049	0.1499	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

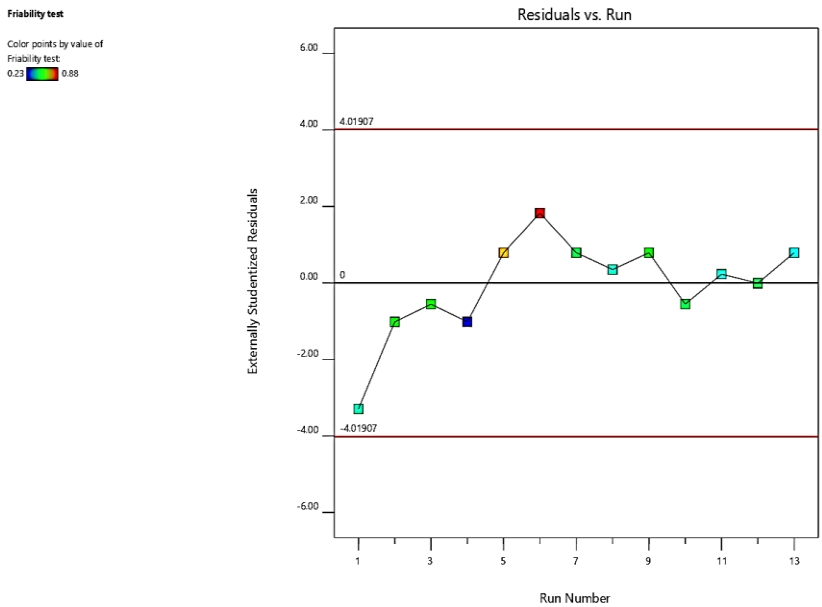
The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Coded Factors

Friability test = +0.5308 -0.0375 A +0.1475 B +0.0725



Graph 5: normal plot of residual

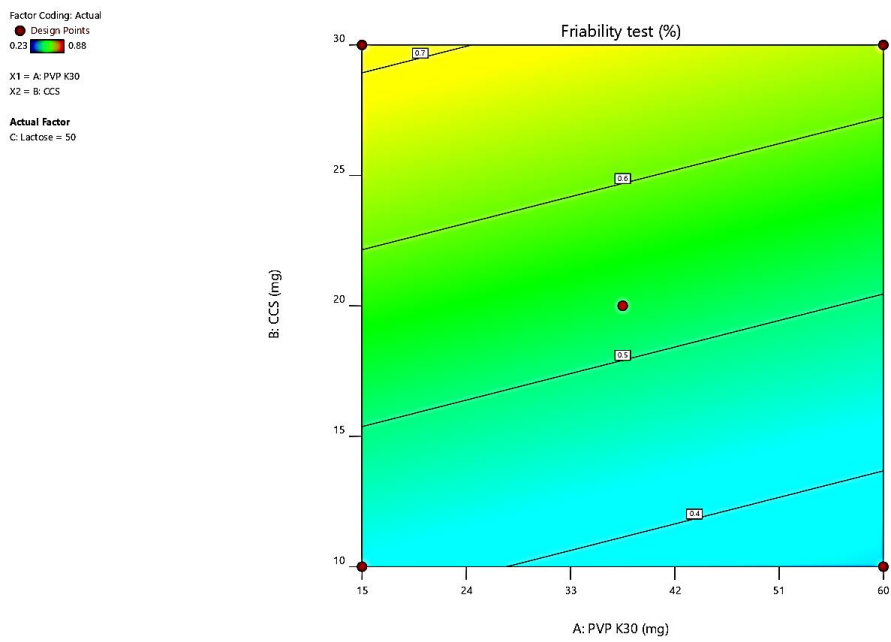


Graph 6: residual vs run of Friability test dependent variable

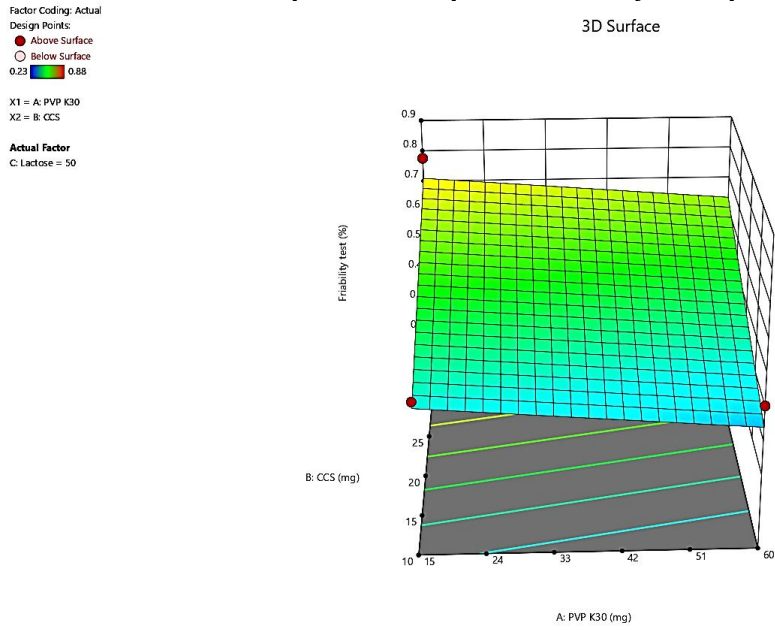
Predicted and actual value of Friability test (All formulation)

Table 18: Predicted and actual value of Friability test

Formulation code	Actual Value	Predicted Value
F 1	0.4600	0.6408
F 2	0.5600	0.6408
F 3	0.5600	0.6058
F 4	0.2300	0.3108
F 5	0.7800	0.7158
F 6	0.8800	0.7508
F 7	0.5200	0.4558
F 8	0.4500	0.4208
F 9	0.5600	0.4958
F 10	0.5200	0.5658
F 11	0.4400	0.4208
F 12	0.5300	0.5308
F 13	0.4100	0.3458



Graph 7: counter plots of Friability test dependent variable

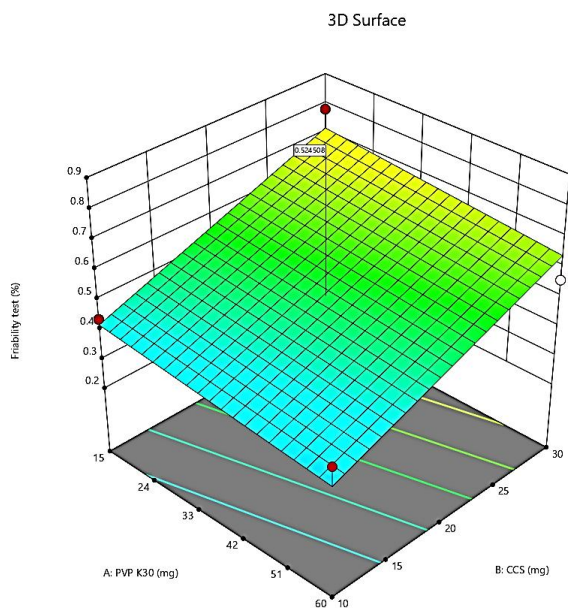


Formulation and Evaluation of Herbal Tablets Containing Ethanolic Extract of *Croton bonplandianus* baill –A Quality by Design (QbD) Approach

Factor Coding: Actual
Design Points:
● Above Surface
○ Below Surface
0.23 0.88

Fractility test (%) = 0.53
Std # 13 Run # 12:
X1 = A: PVP K30 = 37.5
X2 = B: CCS = 20

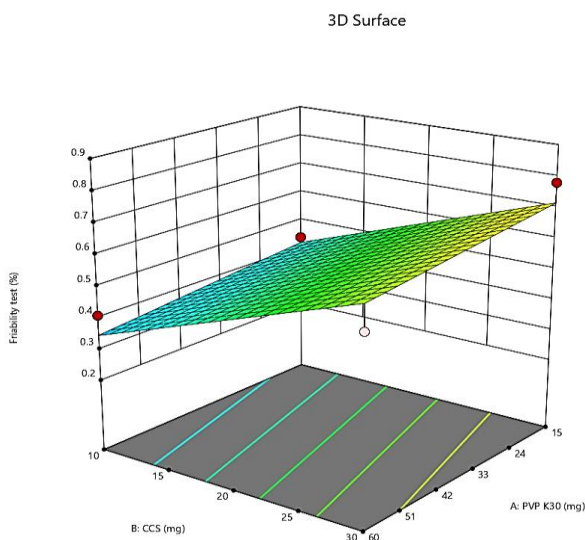
Actual Factor
C: Lactose = 50



Factor Coding: Actual
Design Points:
● Above Surface
○ Below Surface
0.23 0.88

X1 = A: PVP K30
X2 = B: CCS

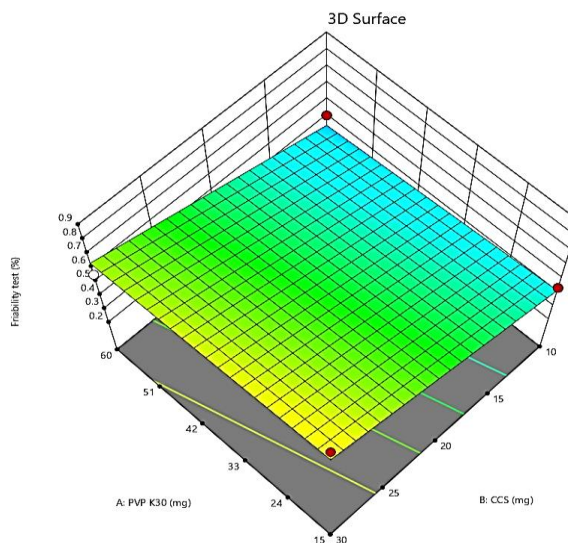
Actual Factor
C: Lactose = 50



Factor Coding: Actual
Design Points:
● Above Surface
○ Below Surface
0.23 0.88

X1 = A: PVP K30
X2 = B: CCS

Actual Factor
C: Lactose = 50

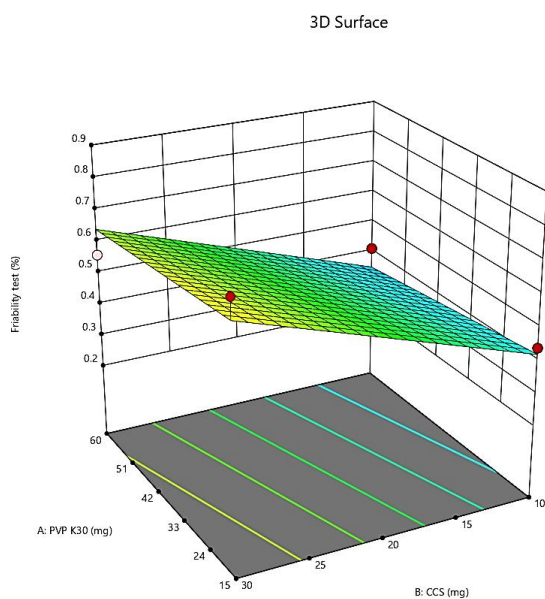


Formulation and Evaluation of Herbal Tablets Containing Ethanolic Extract of *Croton bonplandianus* baill –A Quality by Design (QbD) Approach

Factor Coding: Actual
Design Points
● Above Surface
○ Below Surface
0.23 0.88

X1 = A: PVP K30
X2 = B: CCS

Actual Factor
C: Lactose = 50



Factor Coding: Actual
Design Points
● Above Surface
○ Below Surface
0.23 0.88

Friability test (%) = 0.53
Std # 13 Run # 12
X1 = A: PVP K30 = 37.5
X2 = B: CCS = 20

Actual Factor
C: Lactose = 50

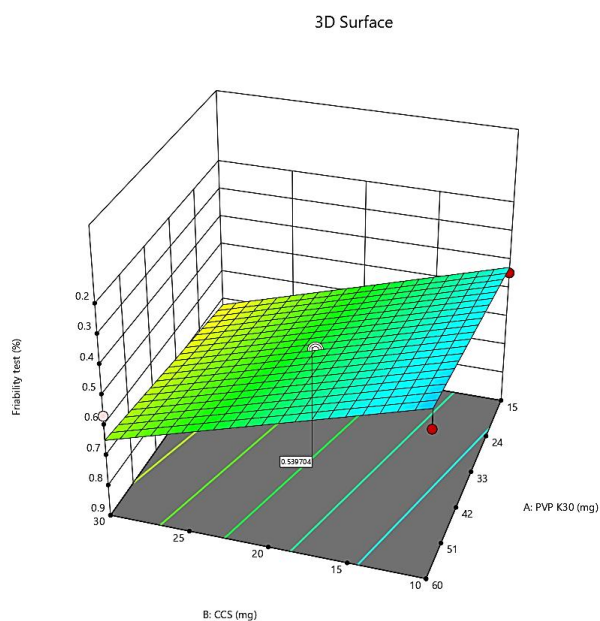
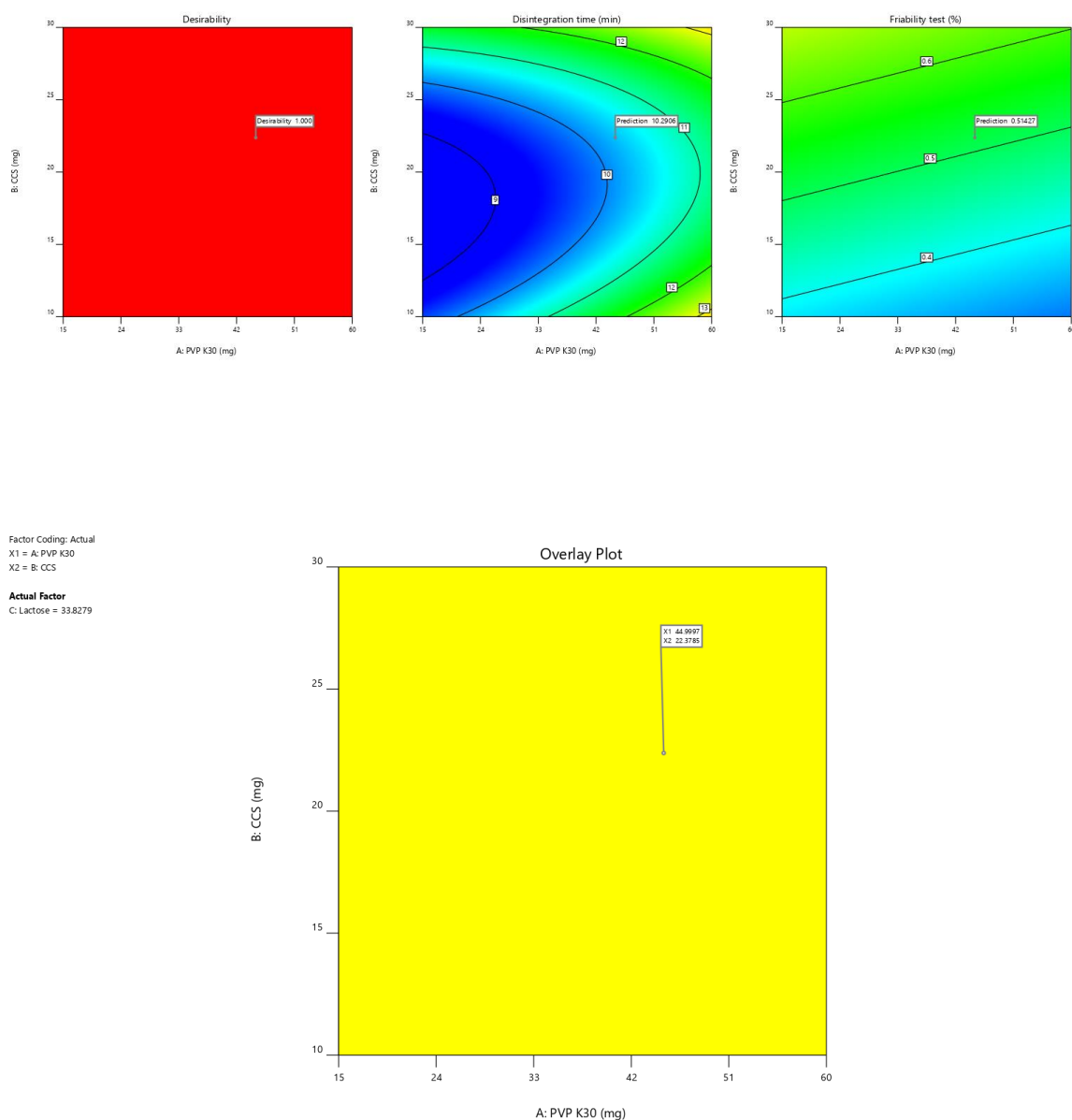


Figure 2: Response surface plot showing combined effect of (PVP) K 30and CCS on Friability test of tablet formulation

Formulation and Evaluation of Herbal Tablets Containing Ethanolic Extract of *Croton bonplandianus* baill –A Quality by Design (QbD) Approach



Graph 8: overlay plot of Friability test and disintegration time for optimization dependent variable

Optimized formula of herbal tablet formulation

Table 19: Optimized formula of tablet formulation

S. No	PVP K30(mg)	CCS(mg)	Lactose (mg)	Magnesium Stearate (%)	<i>Croton bonplandianus</i> baill(stem) Ethanolic Extract (mg)	Talk (q.s)	Disintegration time	Friability	Desirability	
1	45.000	22.379	33.828	0.5	400.000	q.s	10.291	0.514	1.000	Selected
2	37.500	10.000	80.000	0.5	400.00	q.s	14.149	0.456	1.000	
3	37.500	30.000	80.000	0.5	400.00	q.s	12.156	0.751	1.000	

The results of formulations as per the design when fitted into various models, linear model was found to be significant for Friability test with F value 8.09 and P value 0.0064. The model equation is as follows: Friability test = +0.5308 -0.0375 A +0.1475 B +0.0725. The effect of factor A, B and C can be explained with the

help of the 3D response surface plot as shown in above Figure. The results when analysed and optimized had generated numerical optimized solutions based on this experimental design. From the numerical optimization results, a solution was selected randomly, coded as

optimized formulation and considered as optimized tablet formulation.

Box-Behnken design using response surface methods was used to finish the optimization process. Excipients were considered as independent variables, along with their effects on Friability test and disintegration time of formulation in that order. In order to examine

correlations across several variables with fewer experimental runs, the Box-Behnken design was utilized. Disintegration time and friability test for formulation were taken into consideration when choosing the operating method. The impact of both independent and dependent factors was examined using a DOE full factorial approach.

Table 20: Evaluation parameter of granule

Formulation	Angle of repose (°)	Bulk density (g/cm ³)	Tapped Density (g/cm ³)	Carr's index ratio	Hausner's ratio	% of LOD
Granules	27.19	0.299	0.339	11.79	1.13	1.02

Table 21: Evaluation parameter of Optimized tablet formulation

Formulation	Hardness (kg/cm ²)	Thickness (mm)	Weight variation (%)	Disintegration time (min. sec) (Actual value)	Disintegration time (min. sec) (Predicted value)	Friability (%) (Actual value)	Friability (%) (Predicted value)
Tablet	5.4	6.0	601	10.08	10.26	0.501	0.514

STABILITY STUDY

Table 22 Stability study of herbal tablet formulation

S. No	Time (Days)	25°C±2 °C and 60 ± 5% RH			40°C±2 °C and 75 ±5% RH		
		Hardness	Disintegration time	Friability	Hardness	Disintegration time	Friability
1.	0	5.4	10.08	0.501	5.4	10.08	0.501
2.	30	5.4	10.11	0.501	5.5	10.15	0.503
3.	45	5.4	10.10	0.503	5.5	10.15	0.507
3.	60	5.2	10.09	0.500	5.7	10.18	0.502
4.	90	5.3	10.10	0.507	5.6	10.21	0.504

For a duration of three months, the formulation was shown to be physically and chemically stable at accelerated stability conditions (25°C±2°C and 60 ±5% RH) and (40°C±2°C and 75 ±5% RH). Physical appearance, the friability test, and the disintegration test studies were among the physicochemical criteria that remained relatively unchanged. Results of assay and other evaluation criteria at periodic time points of stability studies are summarized in Table. All Results were compared against final formulation of 0 days as the reference.

CONCLUSION-

The current study describes the successful implementation of a QbD approach in order to certify a good compassion of the manufacturing process, as well as to optimize the formulation of Herbal tablets. As a means of obtaining the end product with the appropriate quality. The full factorial design was applied to the formulation design of the herbal tablets. The three independent variables selected are PVP K30, CCS and lactose. There are two different levels for the selected variables as low and high, and they are coded as – 1, and + 1, respectively. The software Design Expert version 12.0.1.0 (StatEase) was used for the design of the formulation. The total of 13 runs (formulations) was designed and finally, the significant model was achieved. Tablets were prepared successfully from

ethanolic extract of *Croton bonplandianus* baill stem with appropriate ingredients by using optimized formula of tablet formulation, Stability study of optimize formulation was done, both physically and chemically, for a period of 3 months at accelerated stability conditions (25°C±2°C and 60 ± 5% RH) and (40°C±2°C and 75 ±5% RH). Results stability studies are summarized in Table-22. All Results were compared against final formulation of 0 days as the reference.

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