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FORMULATION BY DESIGN APPROACH FOR EFFERVESCENT GRANULES OF VITAMIN C USING STATISTICAL OPTIMIZATION METHODOLOGIES

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ABSTRACT

Introduction: Ascorbic acid (vitamin C), is a potent antioxidant, and the human body cannot synthesis it, so ascorbic acid can be obtained from external sources as food and pharmaceutical products. **Objective:** This study was using formulation by design (FbD) concept to prepare effervescent granules of vitamin C with high quality and more patient compliance in less effort and cost. **Methods:** A 2^3 factorial design was used to study the physical and chemical characteristics of the granules (disintegration time (DT), taste of product (TP), carbon dioxide amount (CO_2), and pH of the solution). Also the effects of and interaction between selected independent variables (citric acid, tartaric acid, and sodium bicarbonate) evaluated, while, their concentrations were optimized by application of optimization model methodology for factorial design. Formulations were prepared by non-aqueous wet granulation method. **Results**: From the results of designed batches, the disintegration time ranged from 33±2.7 to 153±3.3sec while the amount of carbon dioxide ranged from 0.360±0.004gm to 1,512±0.002gm also the pH range was from 2.9±0.09 to 6.42±0.07 but the taste of product was coded numerically in proportional with acceptability from 1 to 5. According to the experimental design, both of studied independent variables have significant effect in determining the physicochemical characteristics of effervescent granules. **Conclusion:** FbD concept effectively was utilized for the development of vitamin C effervescent granules as per modern quality practices used in pharmaceutical industries.

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INTRODUCTION

Ascorbic acid (vitamin C) is a water soluble vitamin and possesses a potent antioxidant activity. Vitamin C presents naturally in Citrus and most of fruits and vegetables. It has many functions like protects against respiratory tract infections and reduces risk for cardiovascular diseases and even Cancer. Moreover, the processes of collagen synthesis and iron absorption are enhanced in presence of vitamin C. on the other hand, when no intake of vitamin C, deficiency vitamin C diseases will occur that named scurvy, the scurvy manifestations are bleeding gums and increased hemorrhage potential due to blood capillaries fragility, in addition to tired feeling and psychological issues like depression, hysteria, and social introversion [1,2]. Today, Oral administrated drugs occupied the majority of manufactured medicines in the world especially solid dosage forms, and oral delivery of drug is the most used route of administration among all the routes that were utilized for the systemic delivery of drugs via diverse pharmaceutical products of different dosage forms. The ease of administration and greater stability are the most reasons that made orally taken drugs more popular than liquid and semi-solid forms [3,4].

Effervescent granules are one of the solid dosage forms that is taken orally, and noticeably the effervescent preparations are becoming increasingly due to many advantages such as good stability, quickly dissolve, masking of unpleasant taste and ease administration with highly compliance in patients with difficulty in swallowing of pills and tablets, moreover the bioavailability of low absorbed drugs can be increased. However, the disadvantages are needing controlled preparation and packaging area to protect from moisture and light that makes the medicine more expensive. The major of effervescent products are analgesics, antacids, supplements and flu-cough formulations [4,5].

Experimental design is statistical experiments aim to improve the quality of product so is named Quality by Design (QbD) and one of the modern approach that is involved in the formulation of a pharmaceutical dosage forms (FbD). Design of experiment (DOE) is an optimization technique meant for products and/or processes, and developed to evaluate all the potential influential factors simultaneously, systemically and quickly. On the other hand, the traditional approach of optimizing a formulation or process basically consists studying the influence of one variable at a time (OVAT), whereas keeping all others at constant. Therefore, Design of experiment (DOE) has being applied widely in product and process optimization and validation to gain a high-quality product in shorter time with using minimum trails [5,6]. The above shows the importance of vitamin C for health and due to the most of naturally occurring species that consist vitamin C are seasonal and difficult to stay for long time. Therefore, vitamin C has been formulated as pharmaceutical dosage form such as effervescent granules and tablets that already has been available in the market many years ago. However, as mentioned before the optimization process in formulation development was OVAT in past time but the aim of this study use the FbD concept for development and optimization of vitamin C effervescent granules that makes understanding and treating the formula statistically with high accuracy and precision to obtain the desirable properties of the product in less time and effort and the dose of vitamin C intended to develop was 1000mg.

MATERIALS AND METHOD

Materials

The active ingredient vitamin C (CSPC Weisheng Pharmaceutical Shijiazhuang Co. Ltd) and the excipients anhydrous citric acid (Weifang ensign industry Co. Ltd), tartaric acid (Yantai Taroke Bioengineering Co. Ltd), sodium bicarbonate (TOSOH Manufacturing Co. Ltd), sodium saccharin (Tianjin Changjie Chemical Co. Ltd), aspartame(Ajinomoto Aspartame), sunset yellow (Senset Colors Inc.), orange flavor (Aromsa), and absolute ethanol were received as a gift sample from Modern and global Pharma companies for manufacturing of medicines. Other used reagents in this study were of analytical grade.

Pre-formulation studies

Determination of melting point

The melting point for both of Ascorbic acid and excipients were determined according to British pharmacopeia 2017 method 1.

Fourier transform infrared spectroscopy FTIR

FTIR spectroscopy was utilized on each samples to determine the structure of Ascorbic acid as alone and with other used excipients and to identify the presence of specific functional groups in a sample. The ascorbic acid-excipients interaction was examined by using the results of spectra (**figure 1**) [7]

Preparation of Vitamin C granules

The effervescent granules have been prepared by wet non-aqueous granulation method in witch absolute ethanol was used.

Vitamin C tartaric acid, calcium carbonate, sodium bicarbonate, aspartame, sodium saccharin and sunset yellow were weighted and triturated geometrically and sufficiently blended with absolute ethanol to obtain a wet mass, then this dough mass was passed through sieve No. 18. The resulted granules were dried in an oven at 35°± 5°C until the moisture content was below 0.3%. Precisely, the amount of ethanol was taken and the granulation step was timed. The eight products were prepared according to full 2^3 factorial design that shown in **table 1**.

FbD based statistical optimization of effervescent formulation

For the optimization of effervescent formulation, Minitab® (version 18) software was used to design experimental recipe for statistical optimization. Statistical analysis of experimental data was performed using this software as well. Excipients under study were added to the formulation according to the experimental recipes developed by Minitab® software while concentrations of rest of the components were similar.

Factorial experimental design

Effervescent granules of vitamin C were made according to a full 2^3 factorial design. An amount of anhydrous citric acid-(X1), tartaric acid-(X2), and sodium bicarbonate-(X3) were selected as independent variables. The formulation compositions and the effervescent mixture composition s are listed in **table 1**. The responses evaluated were the disintegration time (DT), pH value, amount of carbon dioxide (CO_2), and taste of product (TP). Levels of independent variables and dependent variables (response) were listed in **table 2**. The best fitting mathematical model was selected for each response. The model predictor equations were estimated by stepwise multiple regression analysis. Validation of the model was performed by analysis of variance with a significance level of 0.05 and by the coefficient of determination (R^2). All statistical calculations and graphic plots were performed using the Minitab® version 18 software.

Physical and chemical characterization

Disintegration time

About one dose of each vitamin C effervescent granules batch was placed in 200ml of water at 25C°. the granules were considered disintegrated when completely dispersed fragments were gained and the liberation of gas stopped. This test was performed according to the British pharmacopeia 2017. [8]

Amount of Carbon Dioxide

This present study determined the amount of carbon dioxide by using method developed by G. Rajalakshmi *et al*. In which solution of 10% sulfuric acid was prepared in purified water. Then to the previously weighted beaker 250 about 100ml of prepared sulfuric acid solution was taken. One dose of vitamin C granules were added in a beaker and the granules were observed for complete release of carbon dioxide from the granules. Again weight of the beaker was taken and the difference in weight before and after release of carbon dioxide shows the amount of carbon dioxide generated. [9-10]

pH of the solution

About one dose of each vitamin C effervescent granules batch was placed in 200ml of water at 25C°. The effervescent granules were kept in the beaker which allowed it completely to dissolve. The pH was measured using digital pH apparatus (Model: Metrohm, Make: Herisau). [10]

Taste of product

The taste for each experimental batch was evaluated by giving about one dose of product for every 40 volunteers [11]. The expressions of volunteers about the tasting were recorded, then each expression was coded proportionally based on acceptability feelings by numerical code from 1 – 5 as it illustrated in **table 5**.

RESULTS AND DISCUSSION

Physical- chemical characterization

Routine experimental studies include the utilize OVAT, whereas other variables are keeping constant. This type of strategy shows limited and sometime inaccurate information about the influence of the variables included in the study. Factorial design permits a well-planned experiment that produces more information for less effort and cost. This strategy enables the identification of the individual contribution of each variable and the potential interactions among them. [12-13]. The 2^3 factorial design was used in this current study to evaluate the feasibility of obtaining vitamin C effervescent granules and to characterize the most influential factors included in the physical and chemical properties of the formulations. All of the possible combinations of three factors and two levels were studied, leading to rise eight different formulations (**table 1**). Due to the properties of the formulations, binding agents were not necessary to utilize. The independent factors and their ranges were selected from previous tests. This present study conducted to study the proportional

variation of effervescent mixture citric acid anhydrous (X_1), tartaric acid (X_2) and sodium bicarbonate (X_3). Factorial design allows the evaluation of the studied factors and their combine interactions. The regression equation for dependent variables was made based on the observed responses for independent variables.

Table 1: Composition of vitamin C effervescent granules

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Vitamin C	1000	1000	1000	1000	1000	1000	1000	1000
Citric acid	1000	100	1000	100	100	1000	100	1000
Tartaric acid	100	1000	1000	100	100	1000	1000	100
sodium bicarbonate	1500	1500	100	1500	100	1500	100	100
Cal. Carbonate	203	203	203	203	203	203	203	203
Sod. Saccharin	15	15	15	15	15	15	15	15
Aspartam	30	30	30	30	30	30	30	30
Orange flavor	30	30	30	30	30	30	30	30
Sunset yellow	2	2	2	2	2	2	2	2
	3880	3880	3380	2980	1580	4780	2480	2480

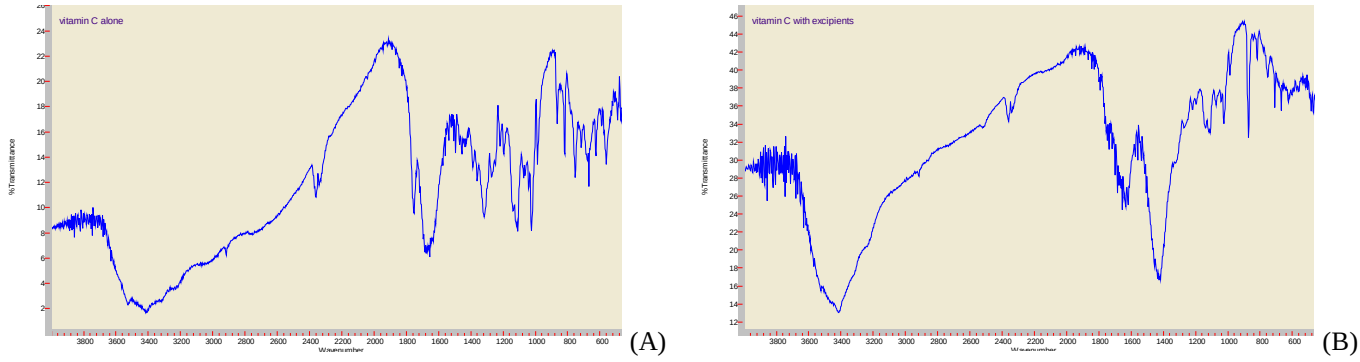
Table 2: variable and their levels in 2^3 factorial design

Independent variables			**Levels**	
			Low	**High**
X_1 = Citric acid (anhydrous) (mg)			100	1000
X_2 = Tartaric acid (mg)			100	1000
X_3 = Sodium bicarbonate (mg)			100	1500
Dependent Variables (response)				
Y_1 = Disintegration time (DT)	Y_2 = Taste of product (TP)	Y_3 = pH	Y_4 = CO_2	

Table 3: 2^3 factorial design observed responses

Code	**X1**	**X2**	**X3**	**pH**	**DT(sec.)**	**CO_2**	**TP**
F1	1000	100	1500	4.8±0.01	97.5±3.5	1.46±0.004	4
F2	100	1000	1500	4.5±0.16	153.5±3.3	1.52+0.002	3
F3	1000	1000	100	2.9±0.09	33±2.8	0.58±0.001	2
F4	100	100	1500	6.4±0.07	33±2.7	1.45±0.001	1
F5	100	100	100	4.3±0.04	40±3.1	0.52±0014	3
F6	1000	1000	1500	4.1±0.06	152.5±3.5	1.13±0.001	4
F7	100	1000	100	3.4±0.04	28±4.2	0.76±002	5
F8	1000	100	100	3.3±0.04	32±3.9	0.36±0.004	4

DT= Disintegration time , **TP**= taste of product, **X1**= citric acid, **X2**= tartaric acid, **X3**= sodium bicarbonate



(A) (B)

Figure 1: Fourier transform infrared spectrum of vitamin C alone (A) and vitamin C with excipients (B).

Effect of Disintegration time

The effervescent granules of vitamin C resulted from all formulations were found to conform the pharmacopoeial requirements related disintegration time (**table 3**). Based on the analysis of variance for factorial regression displayed this response was affected by all the independent variables studied in this research particularly the sodium bicarbonate gave the strongest influence, due to the P < 0.05 (**table 4**). Conversely, interaction of citric acid by tartaric acid (AB) showed the smallest effect. While, the main effect for each variable on the mean of disintegration time illustrated in the **figure 2,** by comparing the slopes of the lines indicated individually both variables produced longer disintegration time whenever concentration of citric acid, tartaric acid, and sodium bicarbonate transfer from low level to high level but the effect for citric acid was less than effects of tartaric acid and sodium bicarbonate [14]. Additionally, the interaction between these factors was statistically significant as shown in Pareto chart by reference line **figure 3**.

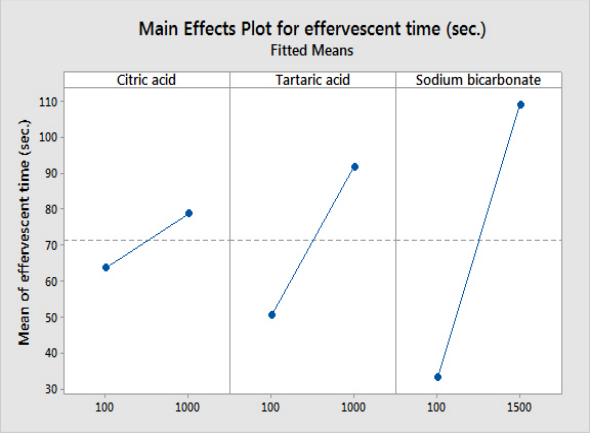


Figure 2. Main effects plot for effervescent time

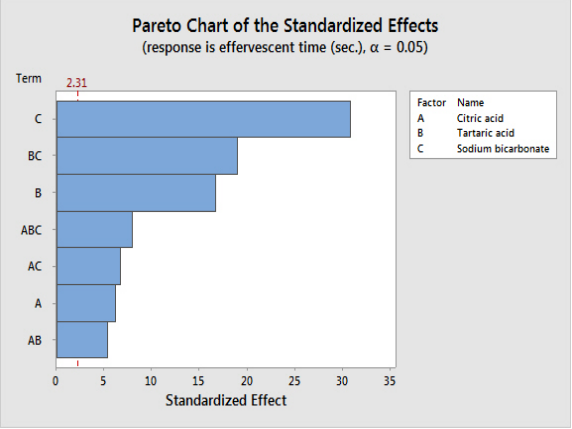


Figure 3. Effects Pareto for effervescent time

Carbon dioxide amount

The amount of carbon dioxide ranged from 0.360 gm to 1,52 gm for all the formulations F1 to F8 **table 3**. From the analysis of variance for factorial regression revealed both citric acid (X_1), tartaric acid (X_2), and sodium bicarbonate (X_3) variable had a significant effect (P < 0.05, **table 4**) on the amount of carbon dioxide. As shown on Pareto chart the largest effect was for sodium bicarbonate. While the effect for citric acid by sodium bicarbonate (AC) was the smallest. In contrast, the main effect for each variable was the high concentration of sodium bicarbonate and tartaric acid produced more carbon dioxide than low concentration but the magnitude of effect for sodium bicarbonate was greater than tartaric acid. Similar results obtained by Amit A. Patel *et. Al.* Reversely, the citric acid at low concentration produced more carbon dioxide than high concentration **figure 4**. As well as, the interaction between these three factors was statistically significant as revealed in Pareto chart **figure 5**.

pH measurement

The pH value for all experiment ranged from 2.83 to 6.42 as displayed on (**table 3**) and the statistical analysis of variance for DOE showed significant effect and interaction (P < 0.05) for the three studied variables X_1, X_2, and X_3 on the pH value, and the strongest effect was for sodium bicarbonate followed by tartaric acid then citric acid **figure 6**. Whereas, the mean value of pH decreased as the concentration of citric acid and tartaric acid transferred from 100mg to 1000mg due to an acidity of solution increased, and the pH increased as concentration of sodium bicarbonate transfer from 100mg to 1500mg because the alkalinity increased as shown on **figure 7**.

Taste evaluation

The obtaining data from volunteers was subjected for analysis of variance and the resulted information exhibited significant effect and interaction (p < 0.05) between the both of three variables. As it displayed on Pareto chart **figure 8,** the effect citric acid by tartaric acid interaction (AB) and citric acid by sodium bicarbonate interaction (AC) were the strongest on the taste, while the effect of citric acid alone was the smallest. On the other hand, the main effect for each variable individually was desirable taste for volunteers at high concentration of citric acid and tartaric acid than low concentration; oppositely, the taste acceptability was better at low sodium bicarbonate concentration than high concentration **figure 9**.

Table 4: results of p value and regression coefficient

Responses	p values of coefficients	
	p value	R^2
Effervescence time	0.***000***	***0.9971***
pH	0.***000***	0.9955
CO_2	0.***000***	1
Taste	0.***000***	0.9977

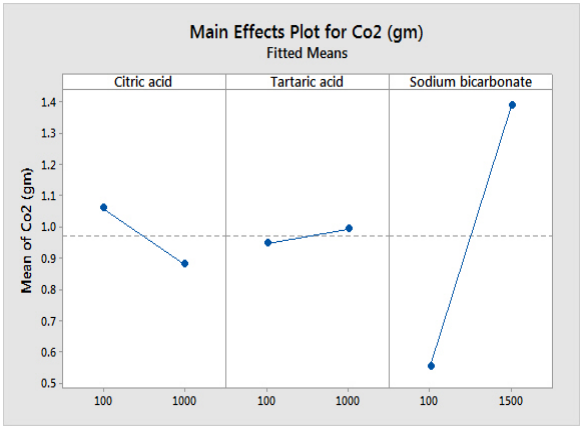


Figure 4. Main effects plot for Carbon dioxide amount

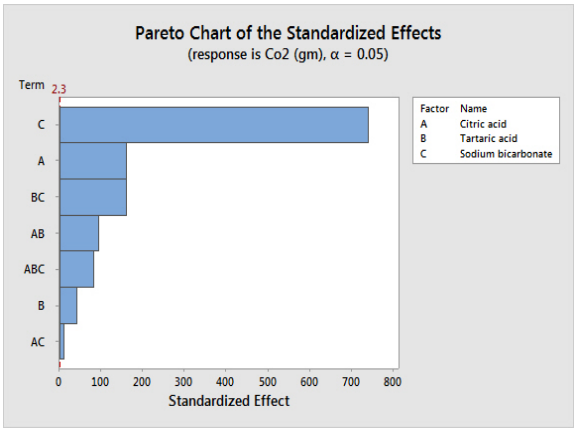


Figure 5: Effects Pareto of carbon dioxide amount

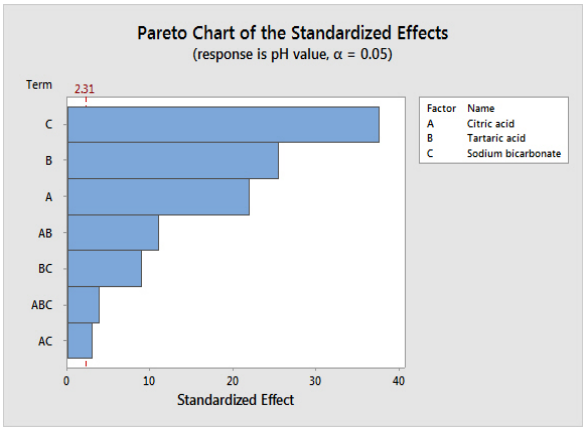


Figure 6: Effects Pareto of pH

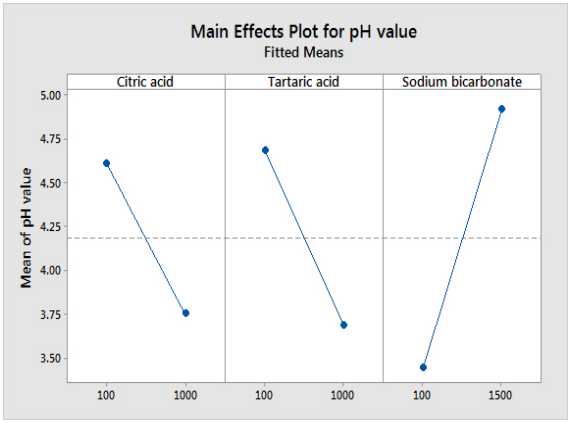


Figure 7. Main effects plot for pH

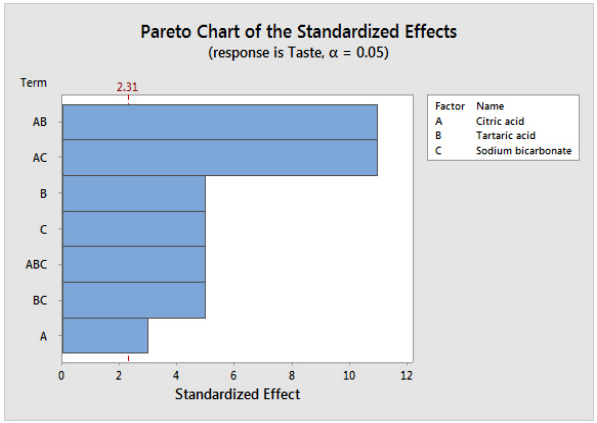


Figure 8: Effects Pareto of Taste

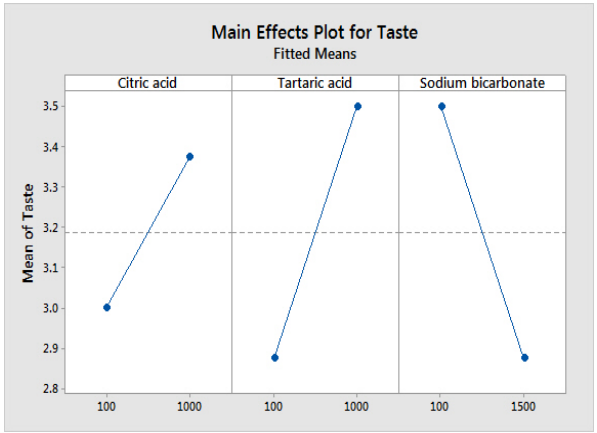


Figure 9. Main effects plot for Taste

Optimization of formula for vitamin C effervescent granules

The optimized formulation has chosen after considering the results of dependent variables of the experimental design batches. The batch with short disintegration time and moderate amount of carbon dioxide with acceptable taste and pH will be considered as optimized batch. The selected dependent variables are correlated with each other because the high amount of liberated carbon dioxide produces violent effervescence for

granules that lead to loss some of the vitamin C dose. As well, as the pH and disintegration time also highly affected by the ratio of effervescent mixture X_1, X_2, to X_3. Therefore, using the optimization model has been applied to predict the optimized formula of vitamin C effervescent granules, and the predicted amounts for optimized formula with its predicted and observed responses were showed in following **table 6**.

Table 5: numerical codes for taste evaluation

Taste	code	Taste	code
Very sweet	1	Sweet/acid	4
Acid	2	Balanced/neutral	5
Sweet	3		

Table 6: composition of optimized formulation, expected observed values for response variable of ET, pH and TP

Composition			Model predicted values			Observed values		
Citric acid (anhydrous) (mg)	Tartaric acid (mg)	Sodium bicarbonate (mg)	DT (Sec)	pH	TP	DT (Sec)	pH	TP
100	1000	113*,5*	35	3*,5*	5	30	3*,37*	5

CONCLUSION

Present study may be considered as a model study to identify and understand the effect of critical formulation parameters on desired product quality attributes for selected formulation. The FbD concept was successfully applied for development and optimization of the effervescent granules. Citric acid (anhydrous), tartaric acid, and sodium bicarbonate were selected to have a most critical effect on the quality attributes, i.e., DT, pH, TP, and CO2 amount of effervescent granules. Factorial design was used to generate a highly significant statistical model, which can sufficiently describe or predict the formulation optimization of effervescent granules for described quality. Generally, the concept of FbD was effectively applied to develop an effervescent formulation by critical analysis of excipient variables. There is a highly commercial potential for this formulation so by extrapolating current research large number of various drugs can be developed like this formulation which will be getting more patient compliance. Additionally, the FbD concept was involved in this formula development therefore easily can be extrapolated to scale up for industrial level.

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Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Safwan Alagbarri contributed in the conception of this work. He also contributed in arranging materials and instruments used in the work. Abdu Faisal and Maher Al-Absi have designed and performed the studies in the laboratories. They also recorded observations, analyzed and interpreted the data of experiments. In addition, they contributed in drafting the manuscript. Mofeed Al- Nowihi has revised the manuscript. All authors read and approved the final manuscript.

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