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ORIGINAL ARTICLE / ÖZGÜN MAKALE

APPLICATION OF PLACKETT-BURMAN DESIGN FOR DEVELOPMENT AND EVALUATION OF A BETAMETHASONE SUSPENSION FOR INJECTION FORMULATION

BİR BETAMETAZON ENJEKSİYONLUK SÜSPANSİYON FORMÜLASYONUNUN GELİŞTİRİLMESİ VE DEĞERLENDİRİLMESİ İÇİN PLACKETT-BURMAN TASARIMININ UYGULANMASI

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ABSTRACT

Objective: *In this study, a quality-by-design (QbD) approach was used to develop a betamethasone suspension for injection formulation and to investigate the possible effects of formulation and process variables on the critical quality attributes (CQAs) of the formulation.*

Material and Method: *It was determined that the CQAs of the formulation were particle size distribution, viscosity, sedimentation time, density and assay of active substances and preservatives, considering the quality target product profile (QTPP). Potential risk factors that may affect the CQAs of the formulation were identified using an Ishikawa diagram, and a six-factor, two-level Plackett-Burman experimental design was used to statistically investigate the effects of selected formulation and process variables. The prepared formulations were tested, and variance and multiple linear regression analyses were performed with the acquired data.*

Result and Discussion: *As a result of the one-way analyses of variance (ANOVA) and multiple linear regression analyses, the established statistical models for the assay of methyl parahydroxybenzoate and propyl parahydroxybenzoate, and viscosity were found to be significant, the established models for other independent variables were not significant. The concentration of carmellose calcium and filter type was found to be the most significant formulation and process variables. In conclusion, this study showed that understanding the formulation and process*

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variables that may affect the CQAs of injectable suspension formulations with a QbD approach could be useful for formulation development and optimization.
Keywords: *Betamethasone, design of experiments, Plackett-Burman, quality-by-design, suspension*

ÖZ

Amaç: *Bu çalışmada, bir betametazon enjeksiyonluk süspansiyon formülasyonu geliştirmek, formülasyon ve üretim değişkenlerinin formülasyonun kritik kalite özellikleri (CQAs) üzerindeki olası etkilerini araştırmak amacıyla kalite tasarımı yaklaşımı kullanılmıştır.*
Gereç ve Yöntem: *İlk olarak hedef ürün kalite profili (QTPP) dikkate alınarak formülasyonun kritik kalite özelliklerinin partikül büyüklüğü dağılımı, viskozite, sedimentasyon süresi, yoğunluk ve etkin maddeler ile koruyucuların miktar tayini olduğu belirlenmiştir. Formülasyonun kritik kalite özelliklerini etkileyebilecek potansiyel risk faktörleri Ishikawa diagram ile tanımlanmış, seçilen formülasyon ve proses değişkenlerinin etkilerini istatistiksel olarak araştırmak için altı faktörlü, iki seviyeli bir Plackett-Burman deney tasarımı kullanılmıştır. Hazırlanan formülasyonlar test edilmiş, elde edilen veriler ile varyans ve çoklu lineer regresyon analizleri yapılmıştır.*
Sonuç ve Tartışma: *Tek yönlü varyans analizi (ANOVA) ve çoklu lineer regresyon analizleri sonucunda, metil parahidroksibenzoat miktar tayini, propil parahidroksibenzoat miktar tayini ve viskozite için kurulan istatistiksel modeller anlamlı bulunurken, diğer bağımsız değişkenler için kurulan modeller anlamlı bulunmamıştır. Karmelloz kalsiyum konsantrasyonu ve filtre tipinin en kritik formülasyon ve proses değişkenleri olduğu görülmüştür. Sonuç olarak, bu çalışma enjeksiyonluk süspansiyon formülasyonlarının kritik kalite özelliklerini etkileyebilecek formülasyon ve proses değişkenlerinin QbD yaklaşımı ile anlaşılmasının formülasyon geliştirilmesi ve optimizasyonu için fayda sağlayabileceğini göstermiştir.*
Anahtar Kelimeler: *Betametazon, deney tasarımı, kalite tasarımı, Plackett-Burman, süspansiyon*

INTRODUCTION

Betamethasone is a synthetic glucocorticoid that has anti-inflammatory, immunosuppressive and antiallergic effects in disorders of many organ systems [1-4]. Betamethasone is available in several ester forms such as dipropionate, acetate, sodium phosphate, valerate, and benzoate, and in various dosage forms such as ointment, lotion, cream, injectable suspension/solution, tablet, syrup and aerosol [5,6].

The approved products in injectable suspension dosage forms contain a combination of betamethasone dipropionate and betamethasone sodium phosphate, as well as a combination of betamethasone acetate and betamethasone sodium phosphate. These two combination products are indicated for the treatment of acute and chronic corticosteroid-response disorders such as rheumatoid arthritis, osteoarthritis, bursitis, ankylosing spondylitis, chronic bronchial asthma, atopic dermatitis, discoid lupus erythematosus, psoriasis, keloids, and that are administered via intramuscular, intraarticular, periarticular, intrabursal, intradermal, and intralesional injection [7,8]

Betamethasone sodium phosphate is a soluble ester of betamethasone, responsible for the immediate activity, while betamethasone dipropionate is practically insoluble in water and provides sustained activity to control symptoms over a longer period [3,7,8]. Betamethasone dipropionate is suspended in water by using a suspending agent, a viscosity increaser agent, and a surface-active agent. In addition, it is necessary to use a filter for the sterilization of the product. Therefore, several formulations and process parameters can have potential effects on the critical quality attributes (CQAs) of formulations.

As defined by the International Conference on Harmonisation (ICH), Quality-by-Design (QbD) is a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and control, based on sound science and quality risk management [7]. Design of experiments (DoE) within the scope of QbD enhances formulation development capability and speed by allowing changing of more than one factor at the same time compared to conventional experimental approaches [7,8]. Many experimental designs are used for the optimization of products and processes. Screening designs are comprised of factorial designs and Plackett-Burman design (PBD), which are used to identify the most crucial independent variables that influence the predetermined responses [9-13].

The purpose of this study is to develop a betamethasone suspension for injection formulation using a QbD approach to investigate the potential risk factors that may affect the CQAs and to understand the influences of formulation and process parameters on the CQAs of injectable suspension formulations.

MATERIAL AND METHOD

Materials

Betamethasone dipropionate and betamethasone sodium phosphate were purchased from Symbiotica (Butterworth, Pulau Pinang, Malaysia). Additionally, the following excipients were used throughout the formulation: Polysorbate 80 (Merck KGaA, Darmstadt, Germany), Macrogol (BASF, Ludwigshafen, Germany), Carmellose Sodium (Ashland, Alizay, France), Sodium Phosphate Dibasic Anhydrate (Merck KGaA, Darmstadt, Germany), Sodium Chloride (Merck KGaA, Darmstadt, Germany), Benzyl Alcohol (Merck KGaA, Darmstadt, Germany), Methyl Parahydroxybenzoate (Lanxess Distribution GmbH, Leverkusen, Germany), Propyl Parahydroxybenzoate (Lanxess Distribution GmbH, Leverkusen, Germany), Disodium Edetate (Merck KGaA, Darmstadt, Germany), Hydrochloric Acid (Concentrated) (Merck KGaA, Darmstadt, Germany). 0.2 µm pore size cellulose acetate (CA) and polytetrafluoroethylene (PTFE) filters were purchased from Sartorius GmbH (Göttingen, Germany). All other chemicals were of analytical reagent grade.

Preparation of Betamethasone Suspension for Injection Formulation

Suspension for injection formulation was prepared using an IKA RCT Basic Magnetic Stirrer (Staufen, Germany), an IKA RW20 Digital Mechanical Overhead Stirrer (Staufen, Germany), and an IKA T25 Digital Ultra-Turrax (Staufen, Germany). Briefly, methyl parahydroxybenzoate and propyl parahydroxybenzoate were dissolved in water for injections at 80-85°C. After these two excipients are completely dissolved, the solution was then cooled down to 20-25°C. Benzyl alcohol, sodium chloride, disodium edetate, macrogol, carmellose sodium, betamethasone sodium phosphate and polysorbate 80 were added to the solution, respectively after each is completely dissolved. The pH of the solution was adjusted to pH 7.0 ± 0.1 using the hydrochloric acid solution. The weight of the solution was completed to the quantity that was stated in the manufacturing batch record using water for injections. The solution was filtered using the filter specified in the experimental design. Sterile betamethasone dipropionate was added to the filtered solution and stirred for 10 min. Then, the obtained suspension was homogenised using an ultra-turrax at different speeds. The prepared suspensions were stored in glass bottles for subsequent analyses.

Risk Identification: Ishikawa Diagram

An Ishikawa diagram was established for the risk identification of the formulation and the process parameters given in the manufacturing method in section of Preparation of Betamethasone Suspension for Injection Formulation, and to understand their potential effects on the CQAs of the formulation [14].

Table 1. Quality Target Product Profile (QTPP) for betamethasone suspension for injection formulation

QTPP Elements	Target
Dosage form	Injectable Suspension
Route of administration	Intramuscular, intraarticular, periarticular, intrabursal, intradermal, and intralesional injection
Dosage strength	6.43 mg/ml of Betamethasone Dipropionate, and 2.63 mg/ml of Betamethasone Sodium Phosphate
Drug product quality attributes	Physical Attributes (particle size distribution, viscosity, sedimentation time, density)
	Identification
	Assay (active substances and preservatives)
	Dissolution
	Impurities
	Microbiological quality

Based on the physicochemical characteristics as well as the in vitro dissolution characteristics of the reference product, a quality target product profile (QTPP) was defined for the betamethasone suspension for injection formulation (Table 1). According to the QTPP and prior scientific knowledge about injectable suspension formulations, particle size distribution, viscosity, sedimentation time, density, and an assay of active substances and preservatives were considered as the CQAs of the betamethasone suspense on for injection formulation (Table 2) [15-17].

Table 2. Critical Quality Attributes (CQAs) of betamethasone suspension for injection formulation

CQA	**Target**
Particle size distribution (d50)	≤ 20 µm
Viscosity	7.0 – 13.0 cP (20°C)
Sedimentation time	≤ 10 min
Density	0.9000 – 1.1000 g/ml
Assay of benzyl alcohol (BA)	90.0 – 110.0%
Assay of methyl parahydroxybenzoate (MP)	90.0 – 110.0%
Assay of propyl parahydroxybenzoate (PP)	90.0 – 110.0%
Assay of betamethasone sodium phosphate (BSP)	90.0 – 110.0%
Assay of betamethasone dipropionate (BDP)	90.0 – 110.0%

Experimental Design

The formulation and process parameters shown in the Ishikawa diagram were examined within the scope of a failure modes and effects analysis (FMEA) [18]. Macrogol type, concentration of polysorbate 80 and carmellose sodium were selected as critical formulation variables; while filter type, homogenization time and homogenization rate were selected as critical process variables. A Plackett-Burman statistical experimental design was performed to understand the effects of independent variables on the CQAs. The variable levels were chosen considering the previous experiments and prior scientific knowledge. As shown in Table 3, six independent variables were examined at two levels.

Minitab 19 (Minitab Inc.; State College, PA, USA) software was used to randomize the design matrix and for statistical analyses, and twelve experiments were prepared for six independent variables (Table 4). Multilinear regression analysis and one-way analyses of variance (ANOVA) were performed to test the significance of the model and the factor coefficients [19].

The response variables (CQAs) were particle size distribution (Y_1), viscosity (Y_2), sedimentation time (Y_3), density (Y_4), the assay of benzyl alcohol (Y_5), the assay of methyl parahydroxybenzoate (Y_6), the assay of propyl parahydroxybenzoate (Y_7), the assay of betamethasone sodium phosphate (Y_8), and the assay of betamethasone dipropionate (Y_9).

Table 3. The independent variables and their levels used in the Plackett-Burman Design

Independent Variables	**Levels**	
	Low	**High**
X_1: Macrogol Type	3350	4000
X_2: Concentration of Polysorbate 80 (mg/ml)	0.25	0.50
X_3: Concentration of Carmellose Sodium (mg/ml)	5.00	6.00
X_4: Filter Type	CA	PTFE
X_5: Homogenization Time (min)	5	10
X_6: Homogenization Speed (rpm)	3000	5000

Table 4. Plackett-Burman Design experimental matrix

Formulation Code	X_1 Macrogol Type	X_2 Concentration of Polysorbate 80 (mg/ml)	X_3 Concentration of Carmellose Sodium (mg/ml)	X_4 Filter Type	X_5 Homogenization Time (min)	X_6 Homogenization Speed (rpm)
F01	4000	0.25	6	CA	5	5000
F02	3350	0.50	5	PTFE	5	5000
F03	3350	0.50	6	PTFE	10	3000
F04	3350	0.25	6	CA	10	3000
F05	3350	0.50	6	CA	5	5000
F06	4000	0.50	5	CA	10	3000
F07	4000	0.25	6	PTFE	5	3000
F08	4000	0.50	6	PTFE	10	5000
F09	3350	0.25	5	PTFE	5	3000
F10	4000	0.25	5	PTFE	10	5000
F11	3350	0.25	5	CA	10	5000
F12	4000	0.50	5	CA	5	3000

Characterization of the Formulations

Particle Size Distribution

The particle size distribution of the suspension was measured by the laser diffraction method using a Mastersizer 3000E (Malvern Instruments Ltd., Malvern, UK) at 2000 rpm of stirring rate, 50% of ultrasound, and 10-20% of obscuration level. For this, approximately 10 ml of suspension was added directly to 600 ml of purified water as the dispersant. Measurements were performed in triplicate.

Viscosity

The viscosity of the suspension was measured using a rotating viscometer (DV3T LV, Brookfield, Middleborough, United States) equipped with an enhanced UL adapter, a 0 spindle at 60-80 rotation speed at 20°C. For this, 16 ml of suspension was transferred into a sample container and the sample container temperature is adjusted to 20°C. Then, the spindle was immersed into the sample, the sample was stirred, and the apparent viscosity of the sample was then measured. Measurements were performed in triplicate.

Sedimentation Time

The sedimentation time of the suspension was measured by using a 30 ml volumetric cylinder at 20°C. For this, 20 ml of suspension was transferred into the volumetric cylinder, and the time until complete sedimentation (until forming a clear solution on the top and sediment on the bottom) was measured. Measurements were performed in triplicate.

Density

The density of the suspension was measured by using a Mettler Toledo DM-40 Density Meter (Mettler Toledo, Columbus, Ohio, United States) at 20°C. For this, approximately 10 ml of suspension was injected into the sampling unit of the density meter and the results were recorded. Measurements were performed in triplicate.

Assay of the Active Substances and Preservatives

A high-pressure liquid chromatography (HPLC) method was used for the assay of benzyl alcohol, methyl parahydroxybenzoate, propyl parahydroxybenzoate, betamethasone sodium phosphate and betamethasone dipropionate (Agilent 1260 Infinity II HPLC, Agilent, Santa Clara, California, United States). The column was a C18; 150 x 4.6 mm, 4 µm (Agilent, Zorbax Poroshell EC-120), and the detector was a DAD/UV set at 254 nm. The flow rate of the mobile phase was 1.2 ml/min at gradient

conditions. The injection volume was 10 µl, the autosampler temperature was set at 10°C and the column thermostat temperature was maintained at 45°C.

Storage Stability Study

The accelerated (40°C ± 2°C/75% ± 5% RH) and long-term (25°C ± 2°C/60% ± 5% RH) stability studies were carried out to investigate the physicochemical stability of betamethasone suspension for injection formulation for 6 months. The suspension samples were analyzed at the initial time point, 3rd and 6th months for both conditions. Particle size distribution, density, assay of BA, assay of MP, assay of PP, assay of BSP, assay of BDP and impurity analyses were conducted to evaluate the physicochemical stability.

RESULT AND DISCUSSION

Assay of the Active Substances and Preservatives

A rapid, precise, and accurate HPLC method was developed and validated for robustness, selectivity, specificity, linearity, precision, solution stability and accuracy as per the ICH Q2 (R1) guideline [20]. The system suitability parameters of the developed HPLC method are given in Table 5. The run time of the analysis was 25 min, while the retention time of benzyl alcohol, methyl parahydroxybenzoate, propyl parahydroxybenzoate, betamethasone sodium phosphate and betamethasone dipropionate were 2.6 min, 3.2 min, 8.9 min, 10 min and 16.8 min, respectively. The sample chromatogram belonging to specificity study is given in Figure 1, and the sample chromatogram obtained from the standard solution is given in Figure 2.

Figure 1. Results of specificity study: Blank solution chromatogram, placebo solution chromatogram, standard solution chromatogram, test solution chromatogram

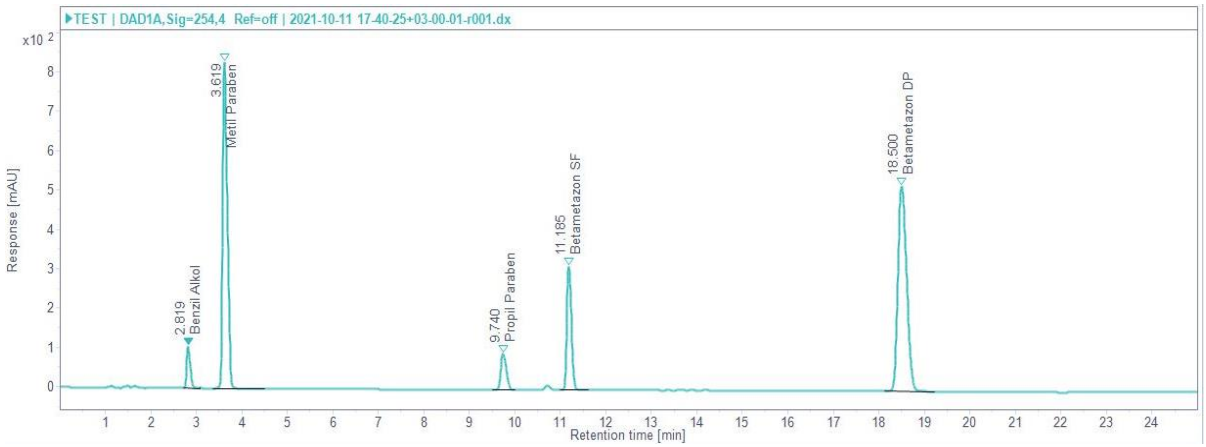

Figure 2. The sample chromatogram obtained from the standard solution and the retention time active substance and preservatives

Table 5. The system suitability parameters of the developed HPLC method

System Suitability Parameters	BA	MP	PP	BSP	BDP
Retention time (min) [a]	2.7 (0.1%)	3.4 (0.1%)	9.5 (0.2%)	11.0 (0.2%)	18.3 (0.1%)
Capacity factor [b]	2.3	3.2	10.9	12.8	21.9
Resolution [c]	-	5.1	32.8	7.6	28.11
Theoretical plate numbers	45643	48676	205983	409555	306674
Peak asymmetry (%10)	1.1	1.2	1.2	1.2	1.1

BA: Benzyl Alcohol, MP: Methyl Parahydroxybenzoate, PP: Propyl Parahydroxybenzoate, BSP: Betamethasone Sodium Phosphate, BDP: Betamethasone Dipropionate.
[a]The values given in blankets were RSD % of retention times (n=10), [b]Dead retention time was found with the injection of uracil at the same conditions: t_0 = 0.8 min. [c]Values are resolution between adjacent peaks.

Risk Identification: Ishikawa Diagram

An Ishikawa diagram was used for identifying the risks and to examine their potential effects on the CQAs of the formulation. Three formulation variables and three process variables that may have an impact on the CQAs of the formulation were identified using the Ishikawa diagram, which are the concentration of carmellose sodium, macrogol molecular weight, concentration of polysorbate 80, filter type, homogenization time, homogenization speed, and are given in red in Figure 3. The influence of these variables was investigated within the context of a follow-up Plackett-Burman experimental design.

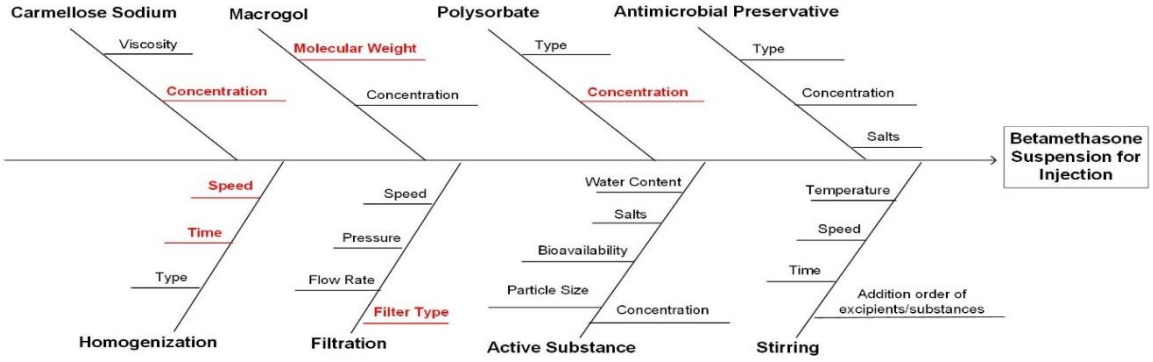

Figure 3. An Ishikawa diagram showing formulation and process variables which may have an impact on the CQAs of the formulation

Experimental Design

A Plackett-Burman design was used to establish an appropriate screening strategy for the CQAs of betamethasone suspension for injection formulation. Twelve experiments were run to screen the effects of the formulation (X_1: Macrogol Type, X_2: Concentration of Polysorbate 80 (mg/ml), X_3: Concentration of Carmellose Sodium (mg/ml)), and process variables (X_4: Filter Type, X_5: Homogenization Time (min), X_6: Homogenization Speed (rpm)) that were identified using the Ishikawa diagram. The twelve formulations that were tested and observed response variables are given in Table 6.

Table 6. Observed response variables through the Plackett-Burman Design

Formulation Code	Y_1: Particle size distribution (d50) (µm)	Y_2: Viscosity (cP)	Y_3: Sedimentation Time (min)	Y_4: Density (g/ml)	Y_5: Assay (%) of BA	Y_6: Assay (%) of MP	Y_7: Assay (%) of PP	Y_8: Assay (%) of BSP	Y_9: Assay (%) of BDP
F01	8.1	12.5	12.4	1.0118	96.50	93.70	82.80	101.40	99.45
F02	8.2	10.7	9.2	1.0111	96.30	96.50	93.20	100.20	101.20
F03	8.7	11.5	12.6	1.0106	97.10	97.60	94.80	100.40	98.80
F04	8.7	12.3	9.5	1.0110	96.90	91.60	75.00	99.70	100.20
F05	8.7	12	10.4	1.0111	95.40	90.00	76.40	99.20	99.20
F06	8.6	10.9	11.2	1.0111	97.80	91.20	79.80	98.90	98.00
F07	8.3	11.9	12.2	1.0110	98.90	97.70	92.80	98.80	100.50
F08	8.2	12	13.5	1.0116	98.60	98.60	97.10	98.50	98.40
F09	8.3	10.5	12.7	1.0110	98.80	99.50	96.00	100.30	100.60
F10	8.3	10.9	11.2	1.0110	96.80	98.00	95.00	98.50	100.00
F11	8	10.7	9.5	1.0098	98.10	93.60	83.70	97.40	96.90
F12	8.3	11.7	12.8	1.0110	97.20	93.80	86.00	96.70	98.80

BA: Benzyl Alcohol, MP: Methyl Parahydroxybenzoate, PP: Propyl Parahydroxybenzoate, BSP: Betamethasone Sodium Phosphate, BDP: Betamethasone Dipropionate.

As a result of the ANOVA and multiple linear regression analyses, the established statistical models for viscosity (Y_2), the assay of MP (Y_6) and the assay of PP (Y_7) as response variables were found to be significant ($p < 0.05$) while the established models for other independent variables, particle size distribution (Y_1), sedimentation time (Y_3), density (Y_4), the assay of BA (Y_5), the assay of BSP (Y_8) and the assay of BDP (Y_9), were not statistically significant ($p > 0.05$) (Table 7). After a multiple linear regression analysis of the data, the following polynomial equations were constructed to describe the quantitative impact of the independent variables on the responses (Equations (1-9)).

$$\text{Particle size distribution (µm)}(Y_1) = 7.517 - 0.0667\,X_1 + 0.667\,X_2 + 0.167\,X_3 + 0.0333\,X_4 + 0.0200\,X_5 - 0.000117\,X_6 \quad (1)$$

$$\text{Viscosity (cP)}(Y_2) = 5.48 + 0.1833\,X_1 - 0.000\,X_2 + 1.133\,X_3 + 0.2167\,X_4 - 0.0333\,X_5 - 0.000000\,X_6 \quad (2)$$

$$\text{Sedimentation Time (min)}(Y_3) = 9.37 + 0.783\,X_1 + 1.47\,X_2 + 0.667\,X_3 - 0.467\,X_4 - 0.073\,X_5 - 0.000400\,X_6 \quad (3)$$

Density (g/ml) (Y_4)= 1.000910 + 0.000242 X_1 + 0.00060 X_2 + 0.000350 X_3 - 0.000042 X_4 - 0.000063 X_5 + 0.000000 X_6 (4)

Assay of BA (%) (Y_5)= 99.38 + 0.333 X_1 - 1.87 X_2 - 0.133 X_3 - 0.317 X_4 + 0.100 X_5 - 0.000350 X_6 (5)

Assay of MP (%) (Y_6)= 100.35 + 0.350 X_1 - 4.27 X_2 - 0.567 X_3 - 2.833 X_4 - 0.020 X_5 - 0.000083 X_6 (6)

Assay of PP (%) (Y_7)= 100 + 1.20 X_1 + 1.33 X_2 - 2.47 X_3 - 7.10 X_4 - 0.060 X_5+0.00032 X_6 (7)

Assay of BSP (%) (Y_8)= 94.88 - 0.367 X_1 - 1.47 X_2 + 1.000X_3 - 0.283 X_4 - 0.107 X_5 + 0.000033 X_6 (8)

Assay of BDP (%) (Y_9)= 101.63 - 0.146 X_1 - 2.17 X_2 + 0.175 X_3 - 0.579 X_4 - 0.248 X_5 - 0.000146 X_6 (9)

Table 7. Statistical analysis of response variables of Plackett-Burman Design

Independent Variables	Y_1: **Particle size distribution (d50) (µm)**	Y_2: **Viscosity (cP)**	Y_3: **Sedimentation Time (min)**	Y_4: **Density (g/ml)**	Y_5: **Assay (%) of BA**	Y_6: **Assay (%) of MP**	Y_7: **Assay (%) of PP**	Y_8: **Assay (%) of BSP**	Y_9: **Assay (%) of BDP**
	p Value	*p Value*	*p Value*	*p Value*	*p Value*	*p Value*	*p Value*	*p Value*	*p Value*
β_0: Constant	0.342	0.012	0.451	0.467	0.622	0.024	0.020	0.819	0.408
X_1: Macrogol Type	0.340	0.081	0.116	0.139	0.336	0.470	0.315	0.460	0.680
X_2: Concentration of Polysorbate 80 (mg/ml)	0.245	1.000	0.676	0.608	0.489	0.287	0.883	0.706	0.454
X_3: Concentration of Carmellose Sodium (mg/ml)	0.245	0.001	0.456	0.258	0.840	0.555	0.303	0.325	0.804
X_4: Filter Type	0.621	0.049	0.310	0.774	0.358	0.001	0.001	0.563	0.143
X_5: Homogenization Time (min)	0.465	0.367	0.676	0.301	0.461	0.915	0.894	0.586	0.122
X_6: Homogenization Speed (rpm)	0.124	1.000	0.377	0.689	0.314	0.860	0.780	0.945	0.680
Model (ANOVA)	0.342	0.012	0.451	0.467	0.622	0.024	0.020	0.819	0.408
R^2	0.6400	0.9205	0.5784	0.5699	0.4818	0.8947	0.9027	0.3521	0.6026

For viscosity (Y_2), the two most significant variables were the amount of carmellose sodium ($p < 0.05$) and filter type ($p < 0.05$), respectively (Figure 4). The R^2 was 0.9205 indicating a good fit for the model being tested (Table 7). The individual value plot was used to detect any outliers and compare distributions, as shown in Figure 5. A visual evaluation shows that the use of PTFE filter has resulted in a slightly lower viscosity compared to CA filter with both 5% and 6% carmellose sodium concentration.

For the assay of MP (Y_6) and the assay of PP (Y_7), the significant variable was the filter type ($p < 0.05$) (Figure 6). The R^2 values were 0.8947 and 0.9027, respectively, indicating a good fit for the model being tested. The *p* values of the main effects of filter type obtained from ANOVA were both 0.001

(Table 7).

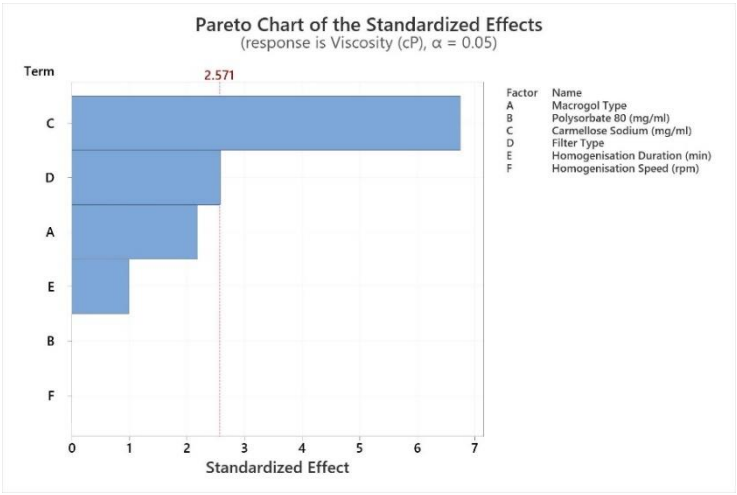

Figure 4. The Pareto chart of the independent variables showing the statistical significance of each variable on the viscosity

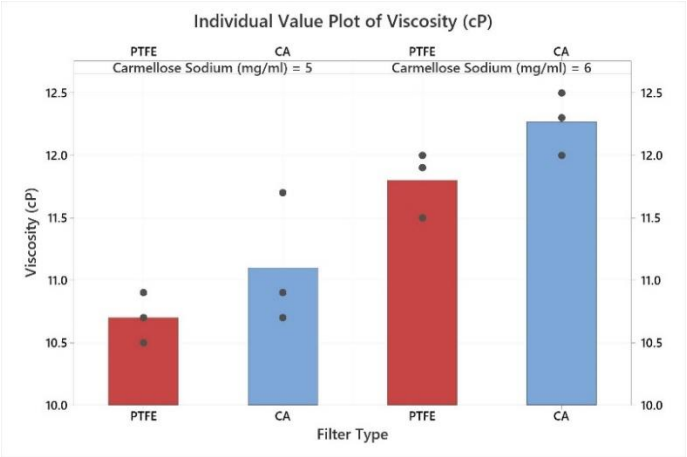

Figure 5. The individual value plots showing the effect of carmellose sodium and filter type on the viscosity

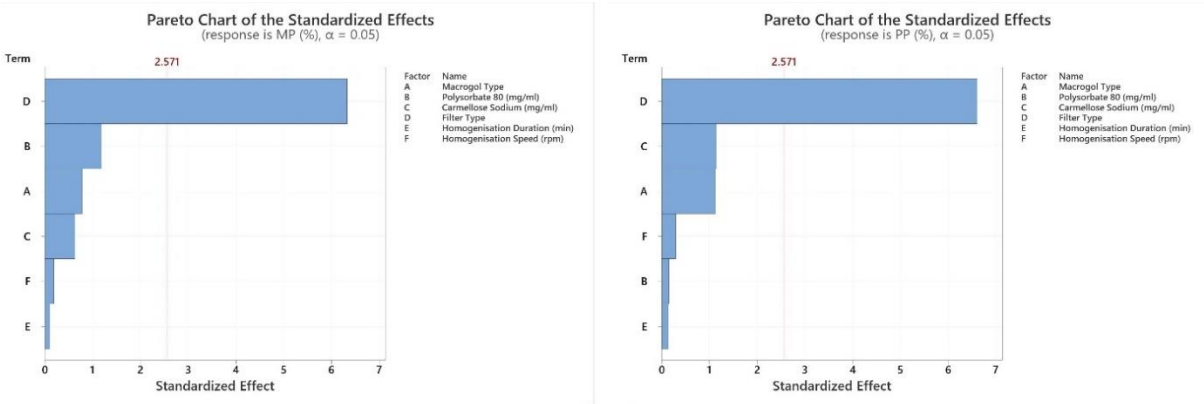

Figure 6. The Pareto chart of the independent variables showing the statistical significance of each variable on the assay of MP (left) and the assay of PP (right)

Storage Stability Study

The results of stability studies are given in Table 8. The particle size distribution, density, assay of BA, assay of BSP, assay of BDP of the formulation were found to be stable with no significant change. It can be concluded that excipient composition and manufacturing method of the optimized formulation was accurately justified. However, assay of MP and PP were decreased, when compared to the initial time point values. The decrease of the assay at the 40°C, 75% RH condition was higher than the decrease at the 25°C, 60% RH. This is thought to be related to increased reaction rates at higher temperatures and relative humidity levels. In order to understand the impact of lowered levels of antimicrobial preservatives, an Antimicrobial Effectiveness Testing (AET) was conducted according to the European Pharmacopeia. AET results showed that the lowest concentration of MP and PP obtained from stability studies provided an adequate level of antimicrobial preservation. The impurity data revealed decomposition with increased temperature and relative humidity levels, which were consistent with the stress testing carried out during analytical method development studies. These impurity results were found to be in compliance with the shelf-life specifications. All results suggested that betamethasone suspension for injection formulation had acceptable stability at accelerated and long-term conditions for at least 6 months.

Table 8. Stability results of betamethasone suspension for injection formulation at 40°C, 75% RH and 25°C, 60% RH conditions

Test	Storage Condition and Time Period				
		25°C ± 2°C/60% ± 5% RH		40°C ± 2°C/75% ± 5% RH	
	Initial	**3rd month**	**6th month**	**3rd month**	**6th month**
Density (g/ml)	1.0111	1.0075	1.0151	1.0080	1.0165
Particle size distribution (μm) (d90)	17.9	21.4	16.2	20.4	18.7
Assay of BA (%)	97.5	96.1	96.5	95.6	96.0
Assay of MP (%)	97.7	94.2	89.3	79.2	66.8
Assay of PP (%)	99.9	96.3	93.9	91.5	85.8
Assay of BSP (%)	97.8	99.8	99.1	98.7	95.1
Assay of BDP (%)	97.9	95.7	97.7	96.5	99.7
BSP Impurity (%)	0.40	0.68	0.72	1.57	2.90
BDP Impurity (%)	0.08	0.11	0.13	0.36	0.86
Total Impurity (%)	1.0	1.4	1.43	3.1	5.4

In conclusion, we investigated the effects of formulation and process variables on the CQAs of the betamethasone suspension for injection formulation using a Plackett-Burman experimental design. This study demonstrated that the filter type was the most critical process parameter for the assay of methyl parahydroxybenzoate and propyl parahydroxybenzoate in the current study. The results showed that these substances adsorbed on the PTFE filter less than the CA filter. The PTFE filter will not only reduce the extent of adsorption but also reduce the process risks. Furthermore, the concentration of carmellose sodium was the most significant formulation variable on the viscosity of the suspension. As expected, the higher concentration of carmellose sodium resulted in increased viscosity. Understanding the formulation and process parameters effects on the CQAs of suspension for injection can significantly reduce the costs of research and development due to fewer formulation trials.

AUTHOR CONTRIBUTIONS

Concept: F.Y., A.A., B.A., P.G.; Design: F.Y., A.A., B.A., P.G.; Control: F.Y., A.A., B.A., P.G., E.N.; Sources: F.Y., A.A., B.A., P.G., E.N.; Materials: F.Y., A.A., B.A., P.G.; Data Collection and/or Processing: F.Y., A.A., B.A., P.G.; Analysis and/or Interpretation: F.Y., A.A., B.A., P.G.; Literature

Review: F.Y., A.A., B.A., P.G.; Manuscript Writing: F.Y., A.A., B.A.; Critical Review: F.Y., E.N.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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