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DESIGN, OPTIMIZATION, AND IN VITRO BIOEQUIVALENCE EVALUATION OF A GLUCOCORTICOID-CONTAINING NASAL SPRAY FOR ALLERGIC RHINITIS: A QUALITY BY DESIGN-BASED DEVELOPMENT STRATEGY

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ABSTRACT

The aim of this study was to design, optimize, and evaluate the in vitro bioequivalence of a glucocorticoid-containing nasal spray for the treatment of allergic rhinitis, following a Quality by Design (QbD)-based development strategy. A systematic experimental design approach (Plackett–Burman design) was employed to identify critical formulation and process parameters influencing product quality. Beclomethasone dipropionate was selected as the active pharmaceutical ingredient, and benzalkonium chloride and phenyl ethyl alcohol was used as preservative. The optimized formulation was subjected to comprehensive characterization including physicochemical tests, rheological evaluation, and scale-up feasibility. In vitro bioequivalence was assessed in accordance with FDA guidelines, using spray pattern, plume geometry, droplet size distribution, aerodynamic particle size distribution, and single actuation content tests. The optimized formulation demonstrated consistent physicochemical properties, acceptable rheological behavior, and robustness under stability studies. In vitro equivalence with the reference product was confirmed for

critical attributes, including spray performance and droplet size distribution, with population bioequivalence (PBE) criteria being met. The formulation showed favorable stability, preservative efficacy and reproducibility upon scale-up. A QbD-driven development strategy enabled the successful formulation of a bioequivalent beclomethasone dipropionate nasal spray. This approach demonstrates the effectiveness of QbD principles in ensuring product quality and regulatory compliance for locally acting nasal formulations.

KEYWORDS: Beclomethasone Dipropionate, Quality by Design (QbD), Nasal Spray, In Vitro Bioequivalence, Optimization, Allergic rhinitis.

1. INTRODUCTION

Allergic rhinitis (AR) is a prevalent chronic inflammatory disorder of the upper respiratory tract, affecting up to 30% of the global population and leading to a substantial reduction in quality of life.[1] Intranasal corticosteroids (INCS) remain the mainstay of therapy due to their potent anti-inflammatory activity and local efficacy. Among these, beclomethasone dipropionate has been widely used owing to its high glucocorticoid receptor affinity, prolonged nasal mucosal retention, and favorable safety profile.[2,3,4]

Nasal spray formulations present several advantages including ease of administration, rapid onset of action, and avoidance of hepatic first-pass metabolism.[5] However, their performance strongly depends on formulation composition, device characteristics, and patient usage technique. Achieving bioequivalence between test and reference nasal sprays poses significant challenges, as systemic pharmacokinetic studies are often insufficient to ensure comparable local delivery. For this reason, regulatory agencies such as the European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) recommend an in vitro equivalence-based approach supported by multiple critical quality attributes (CQAs), including droplet size distribution (DSD), plume geometry, spray pattern, single actuation content (SCU), and aerodynamic particle size distribution.[6,7,8,9]

Quality by Design (QbD) has emerged as a systematic framework to enhance product and process understanding while ensuring robust pharmaceutical development. By defining the Quality Target Product Profile (QTPP), identifying CQAs, and applying risk assessment and design of experiments (DoE), QbD enables the establishment of a design space and control strategy that ensure consistent product quality throughout the lifecycle. For nasal spray

products, integrating QbD principles can provide significant advantages in formulation optimization, manufacturing robustness, and regulatory compliance.[10]

The present study aimed to design and optimize a beclomethasone dipropionate-containing nasal spray using a QbD-driven development strategy. A Plackett–Burman experimental design was implemented to screen critical factors affecting product performance. The optimized formulation was evaluated through a series of in vitro equivalence tests according to EMA guidelines, with direct comparison to the reference product. The outcomes of this work provide evidence for the successful application of QbD in the development of locally acting nasal sprays and contribute to the broader understanding of bioequivalence assessment for non-oral drug delivery systems.

2. MATERIALS AND METHODS

1.1.Materials

Beclomethasone dipropionate was obtained from Symbiotica (Malaysia). Benzalkonium chloride, phenyl ethyl alcohol, microcrystalline cellulose, and other excipients were of pharmaceutical grade and used as received. HPLC-grade acetonitrile, methanol, and water were employed for chromatographic analysis. All reagents and solvents were of analytical grade. The reference product was procured from the Europe market for comparative studies.

1.2.Methods

1.2.1.Characterization of the Active Pharmaceutical Ingredient (API)

Beclomethasone dipropionate was characterized using Fourier-transform infrared spectroscopy (FT-IR), X-ray powder diffraction (XRPD), and differential scanning calorimetry (DSC). Particle size distribution was determined using laser diffraction. Identity, purity, and assay were confirmed by a validated high-performance liquid chromatography (HPLC) method.

1.2.2.HPLC Method Development and Validation

An HPLC method was developed for the simultaneous determination of Beclomethasone dipropionate, phenyl ethyl alcohol and benzalkonium chloride. Method validation was performed according to ICH Q2(R1) guidelines, including assessments of specificity, linearity, accuracy, precision, robustness, and solution stability.

Table 1: Experimental design matrix prepared by using Plackett-Burman experimental design for Beclomethasone Dipropionate and Benzalkonium Chloride Assay-Robustness.

Injection No.	Wavelength (nm)	Buffer Ratio at Mobile Phase (%)	Organic Phase Ratio at Mobile Phase (%)	Temperature (°C)	Flow Rate (mL/min)
1	213	65.5	35.5	43	1.95
2	217	65.5	34.5	43	2.05
3	217	64.5	34.5	37	2.05
4	213	64.5	35.5	43	2.05
5	213	65.5	35.5	37	2.05
6	217	65.5	34.5	43	1.95
7	213	64.5	34.5	43	2.05
8	217	64.5	35.5	37	1.95
9	213	64.5	34.5	37	1.95
10	217	65.5	35.5	37	2.05
11	213	65.5	34.5	37	1.95
12	217	64.5	35.5	43	1.95

Table 2: Experimental design matrix prepared by using Plackett-Burman experimental design for phenylethyl alcohol Assay-Robustness.

Injection No.	Wavelength (nm)	Mobile Phase B Ratio (%)	Temperature (°C)	Flow Rate (mL/min)
1	213	24.5	43	1.6
2	217	24.5	37	1.4
3	217	24.5	43	1.4
4	213	25.5	43	1.4
5	213	25.5	37	1.4
6	217	25.5	37	1.6
7	213	24.5	37	1.6
8	217	24.5	43	1.6
9	213	25.5	43	1.6
10	213	24.5	37	1.4
11	217	25.5	37	1.6
12	217	25.5	43	1.4

1.2.3. Formulation Development

A Quality by Design (QbD) approach was employed. The Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs) were identified. A Plackett–Burman design was used to screen critical formulation and process parameters, including viscosity, droplet size distribution, and suspension stability. Formulations were prepared as aqueous suspensions and filled into nasal spray devices under controlled conditions.

Table 3: Beclomethasone dipropionate 100 µg Nasal Spray, Suspension: Plackett-Burman Experimental Design Matrix and Independent Variables – Formulation Parameters.

Formulation No.	Ratio of Microcrystalline Cellulose to Sodium Carmellose (% w/w)	Polysorbate 20 Content (% w/w)
F01	1.6	0.007
F02	1	0.0035
F03	1	0.0035
F04	1.6	0.007
F05	1	0.0035
F06	1.6	0.0035
F07	1.6	0.007
F08	1.6	0.0035
F09	1	0.007
F10	1.6	0.0035
F11	1	0.007
F12	1	0.007

Table 4: Beclomethasone dipropionate 100 µg Nasal Spray, Suspension: Plackett-Burman Experimental Design Matrix and Independent Variables – Process Parameters.

Formulation No.	Beclomethasone Homogenization Time (min)	Homogenization Time of Microcrystalline Cellulose and Sodium Carmellose (min)	Beclomethasone Homogenization Speed (rpm)	Final Homogenization Time (min)	Final Homogenization Speed (rpm)	Waiting Period (days)
F01	5	10	4000	5	10000	1
F02	5	10	4000	5	5000	3
F03	5	30	4000	15	10000	1
F04	15	10	4000	15	5000	3
F05	15	10	5000	15	5000	1
F06	15	30	5000	5	10000	3
F07	5	30	5000	15	5000	1
F08	15	30	4000	5	5000	1
F09	15	30	4000	15	10000	1
F10	5	10	5000	15	10000	3
F11	5	30	5000	5	5000	3
F12	15	10	5000	5	10000	3

1.2.4. Scale-Up and Manufacturing Process

The optimized formulation was subjected to scale-up studies. Manufacturing involved dispersion of the API and excipients under control mixing speed, homogenization, and filling into metered-dose nasal spray pumps. Process robustness was evaluated through critical process parameter (CPP) monitoring.

1.2.5. Physicochemical Characterization

Physicochemical characterization was performed in accordance with the EMA Guideline on the Pharmaceutical Quality of Inhalation and Nasal Products (EMEA/CHMP/QWP/ 49313/2005 Corr) and as described for non-pressurized multiple-use metered-dose sprays.

1.2.6. In Vitro Bioequivalence Assessment

Bioequivalence testing is an essential step during the development of generic drugs. Regulatory agencies have drafted recommendations and guidelines to frame this step but without finding any consensus. Different methodologies are applied depending on the geographical region. For instance, in the EU, EMA recommends using average bioequivalence test (ABE), while in the USA, FDA recommends using population bioequivalence (PBE) test.

The following parameters were evaluated.

- Single Actuation Content Through Container Life
- Droplet Size Distribution by Laser Diffraction
- Drug in Small Particles/Droplets by Cascade Impactor
- Particle/Droplet Size Distribution by Cascade Impactor
- Drug Particle Size Distribution by Microscopy for Suspensions
- Spray Pattern
- Priming and Repriming

3. RESULTS AND DISCUSSION

3.1.API Characterization

Beclomethasone dipropionate was confirmed to be crystalline by XRPD and DSC analyses, consistent with literature reports. FT-IR spectra revealed characteristic peaks confirming API identity. The particle size distribution was within the expected range for nasal delivery, ensuring appropriate suspension stability.

3.2.Analytical Method Validation

The developed HPLC method showed high specificity for Beclomethasone dipropionate, phenyl ethyl alcohol and benzalkonium chloride, with no interference from excipients;

- The system suitability study confirmed the adequacy of the method. The relative standard deviation (RSD) values of the peak areas were found to be below 2.0%.

- The specificity study demonstrated no interference between the examined solutions in terms of both peaks and retention times. No peaks were observed in the mobile phase or the dilution solvent. In the placebo solutions, standard solution, and test solution, the purity of the active substance and benzalkonium chloride peaks exceeded the threshold value.
- The linearity analysis revealed that the calibration curve generated from concentration versus peak area exhibited a coefficient of determination (R^2) greater than 0.999. The regression coefficient (slope, a) was statistically significant ($p < 0.05$), whereas the intercept (b) was not significant ($p > 0.05$).
- The accuracy study demonstrated that the percent recovery results for each concentration level ranged between 98.0% and 102.0%.
- The repeatability and intermediate precision analyses revealed that the relative standard deviation (RSD) of the results for the test solution was below 2.0%. These findings confirm the precision of the method.
- The solution stability study demonstrated that both the standard and test solutions remained stable at 25°C for at least 40 hours.

Table 5: P values and regression coeffeicients of models and parameters obtained with Plackett-Burman statistical experimental design matrix for for Beclomethasone Dipropionate and Benzalkonium Chloride Assay.

Results	Obtained p Values						R^2 Value
	Wavelength (nm)	(%) Buffer Ratio in Mobile Phase (%)	Organic Phase Ratio in Mobile Phase (%)	Temperature (°C)	Flow Rate (mL/min)	Model	Model Model
Beclomethasone Peak Symmetry	-	-	-	-	-	-	-
Benzalkonium Chloride (1) Peak Symmetry	1.000	0.445	1.000	0.050	0.017	0.081	0.7429
Benzalkonium Chloride (1) Peak Symmetry	0.017	0.097	0.097	0.004	0.004	0.004	0.9097
Amount of Beclomethasone (%)	0.127	0.809	0.127	0.253	0.253	0.228	0.6132
Amount of Benzalkonium Chloride (%)	0.468	1.000	1.000	0.805	0.624	0.957	0.1346

The main effects of chromatographic parameters on the analytical outcomes were evaluated using a Plackett-Burman experimental design. The model developed for benzalkonium chloride quantification was found to be statistically significant and demonstrated a high correlation coefficient. Although the method proved robust to minor modifications, careful control of the mobile phase composition and wavelength settings is recommended during routine analyses.

Table 6: p values and regression coefficients of models and parameters obtained with Plackett-Burman statistical experimental design matrix for Phenylethyl Alcohol Assay.

Results	Obtained p Values					R^2 Value
	Oranı (%) Mobile Phase B Ratio (%)	Wavelength (nm)	Temperature (°C)	Flow Rate (mL/min)	Model	Model
Peak Symmetry for Phenylethyl Alcohol Obtained from Standard Solution	-	-	-	-	-	-
Peak Symmetry for Phenylethyl Alcohol Obtained from Test Solution	-	-	-	-	-	-
Phenylethyl alcohol assay (%)	0.331	0.480	0.523	0.943	0.721	0.2313

When the effect of the main effect of chromatographic parameters on the results was obtained by Plackett-Burman statistical experiment design, it was observed that the established model was statistically insignificant the effect on the results. The developed analytical method has been shown to be robust against minor changes.

3.3.Formulation Development and Optimization (QbD Approach)

The QTPP was defined based on EMA guidance for nasal sprays. Critical quality attributes (CQAs) included droplet size distribution (DSD), viscosity, and delivered dose uniformity (DDU). A Plackett–Burman design identified formulation variables significantly affecting these CQAs. The optimized formulation demonstrated favorable viscosity and spray performance.

Table 7: Response variables obtained from the Plackett-Burman experimental design for Beclomethasone dipropionate 100 µg Nasal Spray, Suspension.

Formulation No	Viscosity (cP)	Droplet Size Distribution (d50) (µm)	Droplet Size Distribution (d90) (µm)	Particle Size Distribution (d10) (µm)	Particle Size Distribution (d50) (µm)	Particle Size Distribution (d90) (µm)
F01	337.5	145.9	286.9	3.2	8.2	25.7
F02	57.4	129.3	251	3.4	9.3	36.1
F03	48.9	90.2	195.1	3.2	8.4	35.0
F04	289	139.9	273.2	3.0	8.9	39.9
F05	58.5	115.4	232.7	2.9	8.5	36.3
F06	316	115.2	227.5	3.5	9.5	39.3
F07	352	109.3	216.9	3.3	9.4	45.7
F08	246	112.3	222.4	3.2	9.1	39.5
F09	59.9	96.9	202.6	3.0	8.2	34.8
F10	282.9	102.6	207.6	3.2	8.4	35.8
F11	44.1	94.8	200.1	3.2	8.4	33.6
F12	42.2	119.5	240.7	3.2	8.6	38.8

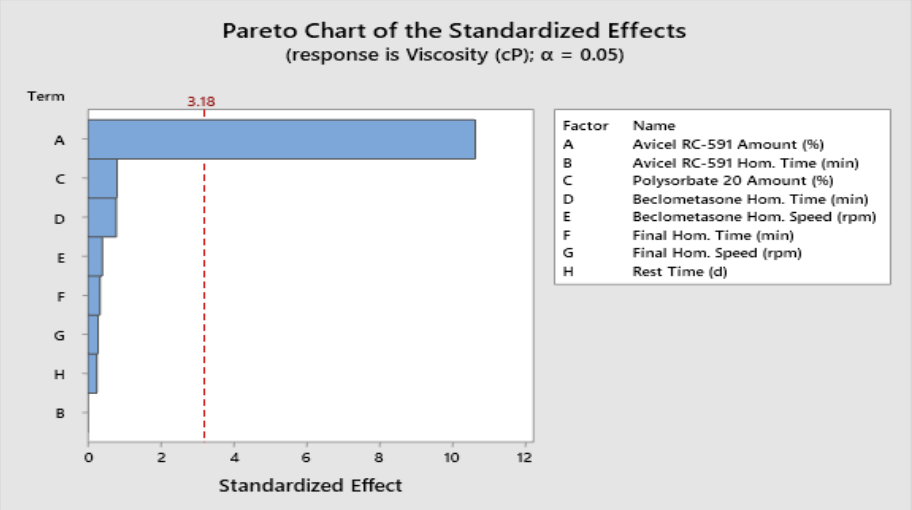

Figure 1: Pareto chart shows the main effects of independent variables.

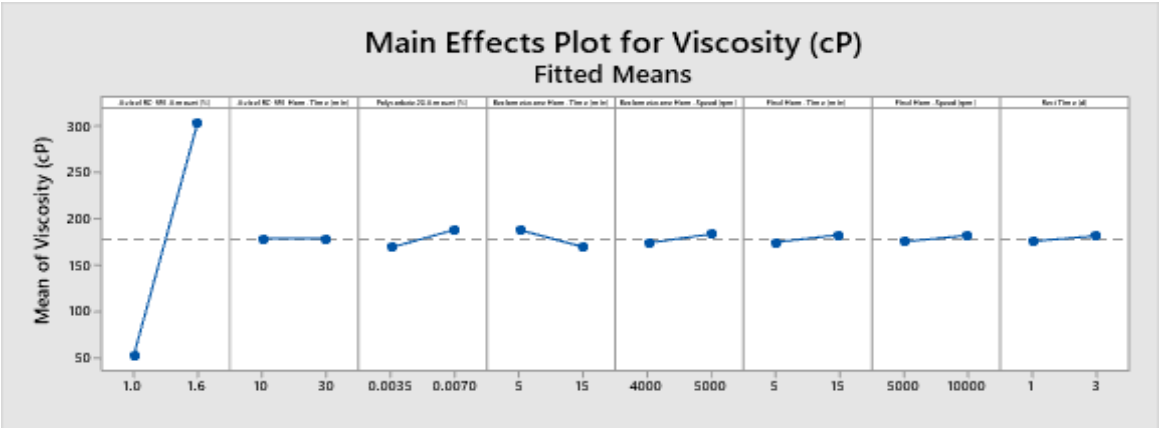

Figure 2: Main effects of the independent variables on the response variable.

An examination of the Pareto chart in Figure 1, which illustrates the first-order effects of the independent variables, shows that among the evaluated factors, the ratio of microcrystalline cellulose to sodium carmellose has a statistically significant effect on viscosity. No statistically significant influence of the other independent variables on viscosity was observed. The resulting first-order mathematical model was found to be significant ($p < 0.05$), with a coefficient of determination (R^2) of 0.9745, indicating good model fit.

Figure 2 presents the main effects of the independent variables on the response. Although only the microcrystalline cellulose–sodium carmellose ratio exhibited a statistically significant influence, the effects of the other variables are also depicted for graphical interpretation.

3.4.Physicochemical Characterization

The optimized formulation exhibited acceptable physicochemical properties consistent with nasal physiological conditions.

Table 8: Physicochemical data for test and reference product.

Tests	Results					
	Test-1	**Test-2**	**Test-3**	**Reference-1**	**Reference-4**	**Reference-3**
pH	5.8	5.8	5.8	4.9	4.8	4.7
Density at 20 °C	1.02 g/mL	1.02 g/mL	1.02 g/mL	1.02 g/mL	1.02 g/mL	1.02 g/mL
Viscosity	19 cP	18 cP	17 cP	11 cP	11 cP	11 cP
Particle Size Distribution	d50: 6.2 µm	d50: 6.2 µm	d50: 6.3 µm	d50: 5.1 µm	d50: 4.8 µm	d50: 4.9 µm
Osmolality	319 mOsm/kg	327 mOsm/kg	318 mOsm/kg	306 mOsm/kg	308 mOsm/kg	311 mOsm/kg
Droplet Size Distribution	%5< 10 µm: %0 d50: 46.8 µm	%5 < 10 µm: 0 d50: 43.6 µm	%5 < 10 µm: 0 d50: 46.0 µm	%5< 10 µm: 0 d50: 44.9 µm	%5< 10 µm: 0 d50: 43.2 µm	%5< 10 µm: 0 d50: 43.7 µm

3.5.In Vitro Bioequivalence Evaluation

Statistical calculations were performed for the tests specified in the guideline.

Table 9: Bioequivalence statistical analysis results-PBE.

Test	PBE			
	σR	Ratio	Hη	Result
Single Actuation Content through Container Life (SCU)	0.071193581	1.053508654	-0.014613978	BIOEQUIVALENT
Droplet Size Distribution by Laser Diffraction-Span/30 mm	0.091559675	1.038735453	-0.02229859	BIOEQUIVALENT
Droplet Size Distribution by Laser Diffraction-Span/60 mm	0.076033158	0.970985985	-0.016857301	BIOEQUIVALENT
Droplet Size Distribution by Laser Diffraction-D50/30 mm	0.031350293	1.035237397	-0.017920919	BIOEQUIVALENT
Droplet Size Distribution by Laser Diffraction-D50/60 mm	0.029433832	1.031207742	-0.019105617	BIOEQUIVALENT
Drug in Small Particles/Droplets by Cascade Impactor	0.055605718	1.007421632	-0.0195018	BIOEQUIVALENT
Spray Pattern Dmax/30 mm	0.079301716	1.017219323	-0.014690797	BIOEQUIVALENT
Spray Pattern Dmax/60 mm	0.10086616	0.989054455	-0.016896492	BIOEQUIVALENT
Spray Pattern Ovality Ratio/30 mm	0.08331784	0.969441034	-0.015201371	BIOEQUIVALENT
Spray Pattern Ovality Ratio /60 mm	0.071185358	0.990657183	-0.017611116	BIOEQUIVALENT
Priming and Repriming-0.Day /6.Puff	0.061583729	0.995442865	-0.022505136	BIOEQUIVALENT
Priming and Repriming-15.Day /6.Puff	0.027561484	0.985595549	-0.019679174	BIOEQUIVALENT
Priming and Repriming-30.Day /6.Puff	0.061583729	0.995442865	-0.022505136	BIOEQUIVALENT

3.6.Stability and Robustness Studies

The optimized formulation maintained stability over accelerated and long-term storage conditions. No significant changes were observed in viscosity, pH, or spray performance during stability testing. Temperature cycling and robustness studies confirmed the product's resistance to physical stress.

4. CONCLUSION AND DISCUSSION

The application of QbD principles facilitated systematic identification of critical factors influencing nasal spray quality. The optimized formulation demonstrated equivalence with

the reference product across multiple in vitro tests, highlighting the robustness of the development strategy. These findings align with FDA's recommendations that weight-of-evidence from in vitro tests can sufficiently support bioequivalence of locally acting nasal sprays.

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