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Original Research Paper

Stability-Indicating High-Performance Liquid Chromatographic Method Development and Preformulation-Driven Design of Betamethasone Dipropionate Topical Systems

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Abstract -

Betamethasone dipropionate (BDP) is a potent topical corticosteroid widely used in semisolid formulations; however, its physicochemical instability, particularly its susceptibility to hydrolytic and photolytic degradation, poses significant formulation challenges. The present study aimed to develop a robust stability-indicating high-performance liquid chromatography (HPLC) method and apply it to preformulation-driven excipient compatibility studies to support rational formulation design.

A selective and reproducible HPLC method was developed and validated in accordance with regulatory guidelines, demonstrating excellent specificity, precision (%RSD < 1%), accuracy (recovery 100–102%), and linearity ($R^2 = 0.9957$). The method effectively resolved BDP from its related impurities, including structurally similar degradation products, confirming its suitability for stability assessment in complex semisolid matrices.

Forced degradation studies revealed that BDP is highly susceptible to alkaline hydrolysis (14.6%) and photolytic degradation (20.3%), but comparatively less sensitive to acidic and oxidative conditions. Excipient compatibility studies conducted under accelerated conditions (60°C for 7 and 15 days) demonstrated a significant influence of excipient type on impurity generation. Polar solvents and surfactants, including propylene glycol, polyethylene glycol derivatives, and lecithin, significantly increased impurity levels (up to 1.28%). In contrast, carbomer, poloxamer, parabens, and sorbic acid showed improved compatibility with minimal degradation.

Mechanistic analysis indicated that microenvironmental polarity and water activity play critical roles in governing BDP ester hydrolysis. These insights were translated into formulation design strategies, including conventional semisolid systems and pluronic lecithin organogels, aimed at minimizing degradation while maintaining drug release and permeation.

This study demonstrates the value of integrating analytical method development with preformulation studies to enable data-driven optimization of stable and effective topical drug delivery systems.

Keywords - Betamethasone dipropionate, Stability-indicating HPLC, Drug–excipient compatibility, Impurity profiling, Semisolid formulations, Preformulation studies, Corticosteroid stability, Pluronic lecithin organogels

1. Introduction

Betamethasone dipropionate (BDP) is a highly potent synthetic glucocorticoid widely used in topical pharmaceutical formulations to manage inflammatory and dermatological disorders. Owing to its pronounced anti-inflammatory, immunosuppressive, and vasoconstrictive effects, BDP is commonly incorporated into semisolid dosage forms such as creams, ointments, lotions, and gels. These formulations contain various functional excipients, including solvents, emulsifiers, penetration enhancers, antioxidants, and preservatives, which are critical for ensuring optimal drug release, permeation, and patient acceptability. (1-3)

However, the physicochemical stability of BDP remains a significant concern due to its steroidal backbone and ester functionalities, which are inherently susceptible to hydrolytic, oxidative, and isomerization pathways. In complex semisolid systems, drug–excipient interactions can further influence degradation behavior, potentially leading to the formation of structurally related impurities. Such degradation not only affects therapeutic

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efficacy but may also pose safety risks, thereby necessitating a comprehensive understanding of stability profiles during the early stages of formulation development. (4)

Although BDP-containing topical products are widely available, there is a paucity of systematic studies addressing the influence of excipient composition on degradation kinetics and impurity generation under accelerated conditions. In particular, limited attention has been given to preformulation-driven impurity profiling in semisolid matrices, where excipient variability can significantly alter drug stability. (5) Addressing this gap is essential to the rational design of stable, regulatory-compliant formulations.

From an analytical perspective, quantifying BDP and its related impurities in semisolid dosage forms poses significant challenges due to the complexity of the formulation matrix, potential co-elution of structurally similar degradation products, and interference from excipient components. (6) The presence of multiple closely related impurities, including positional isomers and ester analogs, further complicates chromatographic separation and accurate quantification. Therefore, the development of a robust, selective, and stability-indicating analytical method capable of resolving BDP from its degradation products and excipient interferences is critically important.

Regulatory frameworks, including ICH Q1A(R2) and Q3B guidelines, emphasize the importance of identifying and quantifying degradation products in finished dosage forms to ensure product quality throughout its shelf life. Accelerated stability studies are an effective tool for elucidating degradation pathways and predicting long-term stability within a reduced timeframe. (7)

High-performance liquid chromatography (HPLC) is widely recognized as a robust and reliable analytical technique for stability-indicating assays, particularly for corticosteroids and their related impurities. Its high sensitivity, selectivity, and reproducibility enable accurate quantification of the active pharmaceutical ingredient (API) in the presence of degradation products and formulation excipients, even within complex matrices. (4, 8)

In this study, a stability-indicating HPLC method was developed and applied to investigate the degradation behavior of BDP under short-term accelerated conditions (60°C for 7 and 15 days) in the presence of selected excipients commonly used in topical formulations. The work focuses on systematic impurity profiling, evaluation of drug–excipient compatibility, and quantification of degradation products over time. The outcomes of this study are expected to provide critical insights into excipient-induced variations in stability and support the development of robust semisolid formulations with improved quality and shelf-life performance.

Despite existing analytical and formulation approaches, few studies have systematically integrated stability-indicating analytical methods with preformulation-driven excipient selection to rationally design stable semisolid systems. (9) Therefore, the present study aims to bridge this gap by correlating impurity profiling with formulation strategy to enable data-driven optimization of topical delivery systems.

The present study aims to develop and evaluate stable topical formulations of BDP through a systematic preformulation approach. The primary objective is to investigate the impact of commonly used excipients on drug stability and impurity formation under accelerated conditions. Additionally, the study focuses on the development and validation of a robust stability-indicating HPLC method for accurate quantification of BDP and its degradation products in complex semisolid matrices. The work further seeks to assess drug–excipient compatibility, identify critical factors influencing degradation behavior, and generate scientific data to support formulation optimization, quality assurance, and regulatory compliance. This study uniquely integrates analytical method development with preformulation-driven formulation design, providing a systematic framework for stability optimization in semisolid drug delivery systems.

2. Materials, Methods, and Results

BDP and its impurities (SimSon Pharma Limited) were a kind gift from Lupin Limited, Pune. All other chemicals were of analytical grade, purchased from Loba Chem, and used without further purification. Millipore water was used to prepare samples. The research strategy was designed to systematically evaluate the stability and compatibility of BDP in semisolid topical formulations. Initially, a stability-indicating HPLC method was developed and validated to accurately quantify BDP and its related impurities. Preformulation studies were then conducted by exposing BDP to selected excipients under accelerated thermal conditions (60°C for 7 and 15 days) to assess drug–excipient interactions and impurity formation. Based on compatibility outcomes, prototype formulations were developed and screened. This integrated approach enabled correlation between excipient selection, degradation

behavior, and formulation stability, supporting the rational design of robust topical delivery systems.

3. Formulation Design Strategy Based on Preformulation Insights

3.1 Rational Design of Conventional Topical System (System I)

Based on the preformulation and drug–excipient compatibility studies presented in Section 6.3, a rational formulation strategy was adopted to develop a topical BDP dosage form. The design approach was guided by the observed degradation behavior of BDP in the presence of commonly used excipients under accelerated conditions.

Stability-indicating analytical methods with preformulation-driven excipient selection will be used to evaluate excipients such as isopropyl myristate, mineral oil, and cetostearyl alcohol for their dual role in enhancing dermal permeation while maintaining formulation stability. Hydrophilic polymers such as carbomer and hydroxypropyl methylcellulose (HPMC) will be prioritized as thickening agents due to their minimal contribution to degradation. (10)

The developed prototypes will undergo in vitro release testing (IVRT) to assess drug-release kinetics, followed by correlation with impurity profiles generated by the validated stability-indicating HPLC method. This integrated evaluation will enable the identification of formulations that strike an optimal balance between drug-release performance and chemical stability. (11)

Optimized formulations will be further evaluated using the vasoconstriction (VC) assay, a well-established pharmacodynamic model for topical corticosteroids, to confirm that improvements in formulation design do not compromise therapeutic efficacy.

3.2 Development of Pluronic Lecithin Organogels (System II)

In parallel with conventional formulations, an advanced delivery system based on pluronic lecithin organogels (PLO gels) was explored to address the inherent stability limitations of BDP in aqueous and semi-polar environments. (12)

Organogels are thermodynamically stable, viscoelastic systems composed of a three-dimensional network of phospholipid reverse micelles in a nonpolar organic phase. This unique microstructure provides a low-water activity environment, which is particularly advantageous for drugs such as BDP that are susceptible to hydrolytic degradation. (13)

Preformulation studies revealed that lecithin-containing systems exhibited increased impurity formation under accelerated conditions, suggesting a potential risk associated with phospholipid-mediated interactions or residual moisture. (14) In response to these findings, the formulation strategy for PLO was systematically optimized. This approach involved careful control of lecithin concentration and the use of high-purity grades to minimize degradation pathways. (15, 16) Additionally, pluronic (poloxamer) co-surfactants were incorporated to enhance the stability of the organogel network, thereby reducing dependence on elevated lecithin levels. (17) Furthermore, nonpolar solvents, such as isopropyl palmitate and isopropyl myristate, were selected to maintain a hydrophobic microenvironment, thereby improving formulation stability. (18)

Pluronics, nonionic block copolymers composed of ethylene oxide and propylene oxide units, enable the formation of stable organogels even with lower-grade lecithin, thereby enhancing formulation robustness. Their amphiphilic properties further enhance drug solubilization and controlled release while minimizing the risk of degradation. (19)

PLO-based systems are hypothesized to reduce the hydrolytic degradation of BDP by limiting its exposure to aqueous domains. In addition, these systems are expected to enhance dermal permeation through the synergistic interaction between lecithin and nonpolar solvents. Furthermore, the presence of a structured gel network is anticipated to provide sustained drug release. (12)

A systematic evaluation of PLO formulations will be conducted by varying the ratios of oil phase, lecithin, and pluronic components. The resulting systems will be characterized for rheological properties, drug release behavior (IVRT), and chemical stability using the developed HPLC method.

3.3 Integration of Analytical and Formulation Development

A key strength of the present study lies in integrating a stability-indicating analytical methodology with formulation design. The validated HPLC method enables precise quantification of BDP and its related impurities in complex semisolid matrices, thereby serving as a critical tool for screening formulation prototypes based on impurity profiles, monitoring excipient-induced degradation pathways, and establishing stability–performance relationships. This integrated approach ensures that formulation optimization is guided not only by physicochemical and performance attributes but also by regulatory-relevant considerations for impurity control. (20)

3.4 Formulation Selection Criteria

Formulation candidates will be selected using a comprehensive, multi-parameter evaluation framework encompassing chemical stability, drug-release behavior, permeation efficiency, pharmacodynamic performance, and the regulatory suitability of excipients. Chemical stability will be assessed by determining total impurity levels and characterizing degradation profiles. Drug release characteristics will be evaluated using in vitro release testing (IVRT) to ensure optimal performance, while permeation potential will be examined to confirm enhanced dermal delivery. (21) Pharmacodynamic response will be assessed through vasoconstriction assays to establish therapeutic efficacy. (22) Additionally, all excipients will be critically evaluated for regulatory acceptability to ensure compliance with established guidelines. (23) This systematic and data-driven approach is anticipated to support the development of robust topical formulations of BDP with improved stability, enhanced efficacy, and assured quality compliance.

4. Analytical Method Development

The analytical method was developed to enable selective and stability-indicating quantification of BDP in the presence of excipients and degradation products, supporting both compatibility studies and formulation screening.(8)

An assay method for quantifying BDP was developed for an in-house batch of 0.05% w/v lotion and innovator semisolid formulations. The Chromatographic Conditions are presented in Table I. The retention time for the standard BDP solution was 4.4 minutes.

The mobile phase was 40:60 (0.01% w/v orthophosphoric acid: acetonitrile, ACN), and the diluent for sample preparation was 90:10 methanol:water. All solutions were freshly prepared in MilliQ water, degassed by sonication, and filtered through a 0.45 µM membrane. (4)

Chromatograms are shown in Figures 1A and 1B.

Table I. Chromatographic Conditions for quantitative determination of BDP and impurities present

Parameters	Method development for BDP	Method Development for impurities in BDP
Column	Phenomenex Luna, Phenyl Hexyl C18, 150 x 4.6 mm, 5 µm	Phenomenex Luna, Phenyl Hexyl C18, 150 x 4.6 mm, 5 µm
Flow rate	1.5 mL/min	1.0 mL/min
Injection volume	50 µl	20 µl
Wavelength	240 nm	240 nm
Column Temperature	40°C	35°C
Run Time	15 Min	60 Min
Sample Compartment	25°C	25°C
Mobile Phase	0.01 % w/v orthophosphoric acid in acetonitrile (ACN) 40:60	Gradient flow as shown in Table II
Sample diluent	Methanol: Water 90:10	ACN: Water 70:30

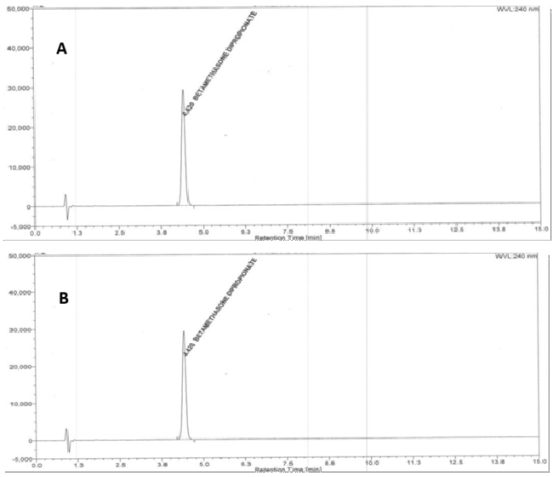


Figure 1: Chromatograms of the Betamethasone Dipropionate assay method, A. Standard, B. Test sample as per the chromatographic conditions specified in Table I.

4.1 Degradation Products Method for Betamethasone Dipropionate

Chromatographic conditions were set to time-programmed mode, as detailed in Table SI (Supplementary material). The retention time for BDP was 25.5 minutes. (24) Elution Orders for quantitative estimation of impurities of BDP from the standard solution are presented in Table II. Chromatograms are shown in Figures 2A and 2B.

Table II. Elution Order for quantitative estimation of impurities of BDP from the standard solution.

Name	RRT	Nature of Impurity	RF	LOQ (%)
Impurity B (Betamethasone 17-Propionate)	0.35	P&D	0.9	0.04
Impurity C (Betamethasone 21-Propionate)	0.48	P&D	0.9	0.04
Impurity D (Betamethasone 21-Acetate17-Propionate)	0.80	P&D	1.0	0.04
Betamethasone Dipropionate	1.00	P	NA	0.04
1,2 Dihydrobetamethasone Dipropionate	1.05	P	NA	NA
Impurity E (Beclomethasone Dipropionate)	1.10	P	NA	NA
Impurity H (6α-Bromobetamethasone Dipropionate)	1.26	P	NA	NA
Impurity G (Betamethasone Tripropionate)	1.30	P	NA	NA

RRT – Relative retention time, P&D – Process and Degradation, P – Process, NA –Not applicable

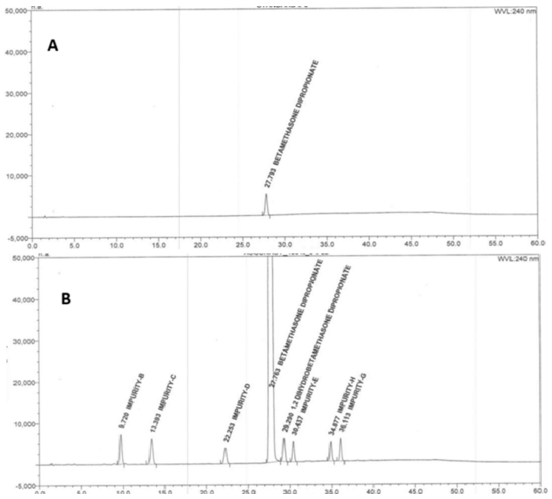


Figure 2: Chromatogram of Betamethasone Dipropionate Degradation products method specified in gradient flow: A—standard drug, B—spiked sample impurities.

5.0 Analytical method validation

5.1 Assay method validation for BDP conventional formulation (o/w lotion, B.No ACC/DES/07)

Assay quantification method of BDP developed on in-house batches of BDP formulation 0.05% and validated by HPLC.(25)

Mobile Phase - Mix methanol, water, and glacial acetic acid in a ratio of (650:350:10), sonicate, and degas. The diluent for sample preparation was methanol:water (80:20).

A standard stock solution of 500 ppm was prepared by accurately weighing 50 mg of the drug and diluting it to the desired volume. The standard stock solution was further diluted to prepare a working stock solution at 10 ppm.

Diluent injections were used as blanks, followed by standard injections. Five replicates of the same sample volume were injected. Test validation was ensured by three system suitability requirements: plate count for BDP in the standard solution must be at least 5000; tailing in the standard solution must be no more than 2.0; and relative standard deviation (RSD) for replicates must be no more than 2.0%. After ensuring system suitability, the sample preparations were performed in duplicate.

Calculation - The percentage assay of BDP is calculated by the following formula

$$\%\ Assay = \frac{At}{As} \times \frac{Wt\ of\ std\ (mg)}{200} \times \frac{2}{50} \times \frac{50}{Wt\ of\ sample\ (gm)} \times \frac{P}{LC} \times 1000$$

Where,

A_t = Average area of the BDP peak in test preparation.

A_S = Average area of BDP peak in standard preparation.

P = Potency of the BDP standard.

L.C = Label Claim of BDP, 0.5mg per gram

VALIDATION RESULTS:

5.1A: SYSTEM SUITABILITY

Study Design: Five replicate injections of the standard solution were performed as per the methodology. Theoretical plates, tailing factor, and % RSD of the peak area in replicate injections of the standard preparation were evaluated as system suitability parameters and were found to be 8307±106, 1.02±0.15, and 0.22±0.01, respectively. (26) The acceptance criteria are similar to those previously defined.

5.1B: SPECIFICITY

5.1.B.1. Interference from blank, placebo, and impurities

Study Design: Diluent blank, placebo, sample standard, and sample spiked with impurities were injected into the HPLC equipped with a DAD detector as per the methodology. The BDP peak was evaluated for chromatographic purity. (27) The results showed that no peak was observed at the BDP retention time in both the diluent blank and the placebo. A single peak was observed with the standard, and the BDP peak was recovered without interference when samples spiked with placebo were analyzed. All impurities resolved from BDP with resolution >2.0 when samples spiked with impurities were injected for analysis. Acceptance criteria were set at no more than 0.2% interference from the blank, placebo, and the impurity peak with the analyte, and peak purity should be at least 0.990/990 according to the LC solution software of the HPLC system.

5.1. B.2.Interference from degradants (Forced Degradation)

Study Design: To evaluate interference from degradants, a forced degradation study was conducted by stressing the test sample under the following maximum-stress conditions. (28)

Control samples were prepared by accurately weighing and transferring 1000 mg of the sample into a 50 mL volumetric flask. 30 mL of diluent was added, vortexed for 5 minutes, and sonicated for 20 minutes.

<u>Acid Degradation(5N HCl/60°C 3hrs):</u>

Accurately, 30 mL of the control samples were treated separately with 1.0 mL of 5N HCl and heated in a water bath at 60°C for 3 hours. Neutralized with 5N NaOH, cooled, centrifuged at 5000 RPM for 10 min, filtered through a 0.2 µm nylon filter, and suitably diluted with diluent. (24)

Alkali Degradation (5N NaOH/60°C 30minutes):
Similarly, 30 mL of the control samples were separately treated with 1.0 mL of 5N NaOH and heated in a water bath at 60°C for 30 minutes. Neutralized with 5N HCl, cooled, centrifuged at 5000 RPM for 10 min, and filtered through a 0.2 µm nylon filter, and suitably diluted with diluent. (29)
Oxidative Degradation (30%H_2O_2 40°C 3hrs):
Similarly, 30 mL of the control samples were treated separately with 1.0 mL of 30% H2O2 and incubated in a water bath maintained at 40°C for 3 hours, then cooled, centrifuged at 5000 RPM for 10 min, and filtered through a 0.2 µm nylon filter and suitably diluted with diluent. (30)
Solid-state thermal degradation (60°C/7 days) :
Weighed accurately and transferred about 1000 mg of sample into a 50mL volumetric flask, then heated in an oven at 60°C for 7 days. Prepared the sample as per the control sample. (31)
Photolytic degradation (1.2 million lux hours at 200-watt hours/square meter UV energy) :
Weighed accurately and transferred about 1000 mg of the sample into a 50 mL volumetric flask, then kept it in a photo-stability chamber and exposed it to light at 1.2 million lux-hrs and 200-watt-hr/sqmtr UV energy. Prepared the sample as per the control sample. (32)
Solid state Humidity degradation (40°C/75%RH for 7 Days) :
Weighed accurately and transferred about 1000 mg of sample into a 50 mL volumetric flask, then exposed it to 40°C and 75% RH for 7 days. The sample was prepared as per the control sample. (33)
Prepared the placebo simultaneously for each stress condition, in the same manner as the sample.
Results:
The peak purity of the BDP was quantitatively evaluated using the developed chromatographic method at each condition. The peak purity match value indicates that the analyte peak is homogeneous and there are no co-eluting peaks. The percentage degradation of the analyte is tabulated in Table III.

Table III: Percentage degradation of BDP

Conditions	BDP Percentage degradation
Acid (1mL 5N HCl/60°C 3Hrs)	0.2±0.04
Alkali (1mL 5N NaOH/60°C 30Mins.)	14.6±2.13
Peroxide (1mL 30% H_2O_2/40°C 3Hrs)	1.0±0.54
Thermal (60°C, 7 Days)	5.5±0.87
Photolytic (1.2 million lux hours at 200-watt hours/square meter ultraviolet energy)	20.3±5.21
Humidity (40°C/75%RH, 7 days)	1.3±0.54

5.1C: PRECISION
5.1. C.1. System precision
Study Design: The BDP standard solution was injected into the chromatograph in six replicates. %RSD for the area of replicate injections was evaluated. (34)
Results: The area responses for BDP, along with % RSD, were 767786±0.22. Results are presented in Table SII (supplementary material).
Acceptance criteria: The % RSD of the area of the BDP peak should not exceed 2.0.
5.1. C.2. Method precision
Study Design: Six test samples were analyzed according to the methodology, representing a single batch. Method precision was evaluated by computing the percentage relative standard deviation of the assay results, which was 101.2±0.24%. (35) The results are tabulated in Table SIII (supplementary material).
Acceptance criteria: % RSD for assay of six preparations should not be more than 2.0

5.1. C.3. Intermediate precision (Ruggedness)
Study Design: Six test samples were analyzed per the methodology, representing a single batch, on different days using different instruments and columns. Method ruggedness was evaluated by computing the relative standard deviation (RSD) and cumulative % RSD of the assay results from the precision and ruggedness study, yielding 101.5±0.69%. (36) The results are tabulated in Table SIV (supplementary material), and the compiled data for method precision and intermediate precision are presented in Table IV.

Table IV: Compiled data method precision and intermediate precision

Sample No.	Method Precision	Intermediate Precision
1	101.0	100.6
2	100.9	100.9
3	101.4	101.2
4	101.1	101.9
5	101.5	102.1
6	101.0	102.3
Average	101.2	101.5
% RSD	0.24	0.69
Cumulative %RSD		0.52

Acceptance criteria: first, the % RSD for the assay of six preparations should not exceed 2.0. and second, cumulative % RSD for the assay of twelve preparations (of method and intermediate precision) should not be more than 2.0

5.1. C.4. Precision at Different Levels

Study Design: Precision at different levels is assessed by analyzing the standard at 50%, 100%, and 150% of the working concentration (50 ppm) in six replicates. (37)

Results: Assay and % RSD are 383168, 757334, 1146609, and 0.49, 0.27, 0.25, respectively.

The area response is tabulated in Table SV (Supplementary Material).

Acceptance criteria: % RSD at each level should not be more than 2.0%

5.1.D. LINEARITY

Study Design: The linearity of BDP at 10 ppm (50%-150% of the working concentration) was assessed. (38)

Results: The average area responses against the corresponding concentration are tabulated in Table V, and linearity was found to be y = 73829x + 11401, and the regression coefficient was found to be 0.9976

Acceptance criteria: The squared correlation coefficient should not be less than 0.997

Table V: Linearity of analytical method

Concentration in ppm	Average area response
0	0
5.178	382517
5.585	610636
10.356	779760
12.528	923402
15.535	1150783
Slope	73829
Intercept	11401
CC*	0.9976
Squared CC*	0.9957
Residual sum of square	146782744.3

CC* : Correlation coefficient

5.1.E. ACCURACY

Study Design: The accuracy of the analytical method was demonstrated by analyzing recovery samples spiked with known quantities of drug at 50%, 100%, and 150% of the targeted concentration relative to placebo. (39)

Results: The results for % Recovery of BDP are tabulated in Table SVI (Supplementary Material). The precision at these recovery levels is tabulated in Table SVII (Supplementary Material).

Acceptance criteria: The mean % recovery at each level should be between 95.0 and 103.0. Results were to be between 100.1% and 102.0% recovery.

5.1.F. RANGE

Based on linearity, precision at different levels, and accuracy, the analytical method's range is set at 50% to 150% of the test concentration. (40)

5.1. G. SOLUTION STABILITY

Study Design: Standard and sample were prepared as per the methodology and stored at 25°C. Moreover, injections were made at regular intervals for 48 hours using the same mobile phase. The percentage difference of the analyte peak area was calculated. (41)

Results: Changes in response over time are calculated as percentage differences relative to the initial value and are tabulated in Tables SVIII and SIX in the supplementary material for the standard and the sample, respectively.

Acceptance criteria: The % area difference should not exceed ±2 relative to the initial value.

5.1. H. ROBUSTNESS

Study Design: The robustness of the method was determined for the assay of BDP under the following variable conditions:

1. Change in wavelength: 254±3 nm
2. Change in Column temperature: 30±5 °C
3. Change in Flow rate 1±0.1 mL/min

Results: The results are tabulated in Table SX (Supplementary Material) for system suitability and in Table VI for assay(42)

Table VI: Robustness data for assay value of BDP

Robustness Parameter	% Mean Assay of Method & Intermediate	% Assay of Robustness	Difference	Remark

	Precision			
Robustness_Low_Wavelength	101.3	101.2	0.1	Robust
Robustness_High_Wavelength		101.3	0.0	Robust
Robustness_Low_Flow		101.5	-0.2	Robust
Robustness_High_Flow		101.4	-0.1	Robust
Robustness_Low_Temperature		100.8	0.5	Robust
Robustness_High_Temperature		101.1	0.2	Robust

Acceptance criteria: System suitability should be demonstrated for each variable, and for each variable, the analyte assay should be NMT ± 2% of the method's mean assay value for method precision and intermediate precision, or the result should fall within the method's precision and intermediate precision ranges.

5.1. I. FILTER INTERFERENCE

Study Design:

The sample solution was prepared and centrifuged; simultaneously, the samples were filtered through 0.45 (nylon), 0.45 (PVDF), and 0.45 (Teflon) filters in triplicate. All the filtered and Unfiltered samples were injected into the HPLC as per the method. (43)

Results: The filter's compatibility and equivalence were determined. The results are given in Table VII.

Acceptance criteria: The area difference of the filtered solution should not differ by more than ±2.0% from the unfiltered solution.

Table VII: Filter Interference

Sample	Observed area of BDP	% Area difference of BDP from the centrifuged sample
Centrifuged	775438	NA
Nylon-1	771058	0.6
Nylon-2	774322	0.1
Nylon-3	775050	0.1
Teflon-1	772051	0.4
Teflon-2	775230	0.0
Teflon-3	773558	0.2
PVDF-1	775799	0.0
PVDF-2	778494	-0.4
PVDF-3	779737	-0.6

6. Discussions

6.1 HPLC Method Performance

A selective and reproducible HPLC method was successfully developed to quantify BDP and its related impurities in semisolid formulations. The optimized chromatographic conditions enabled effective separation of BDP from multiple structurally related impurities, including positional isomers and ester analogs. (44)

The method demonstrated excellent system suitability, with theoretical plates exceeding 8000 and a tailing factor close to unity, indicating efficient column performance. The retention time of BDP (~25.5 min) ensured adequate resolution from early- and late-eluting impurities, confirming the suitability of the phenyl-hexyl stationary phase for steroidal compounds. (45)

The gradient impurity profiling method further enabled the separation of critical degradation products, such as betamethasone 17-propionate and 21-propionate, highlighting its ability to resolve closely related species in a complex matrix. This analytical performance formed the basis for subsequent compatibility and degradation studies. (39)

6.2 Forced Degradation Behavior

Acceptance criteria: Peak purity should not be less than 0.990/990, as determined by the LC solution software of the HPLC system.

Forced degradation studies revealed distinct degradation pathways for BDP under various stress conditions. Alkaline degradation reached 14.6%, indicating pronounced susceptibility to ester hydrolysis. Photolytic degradation exhibited the greatest extent of degradation (20.3%), highlighting the drug's sensitivity to light-induced structural alterations. Thermal degradation accounted for 5.5% of the total, suggesting moderate instability at elevated temperatures. In contrast, minimal degradation was observed under acidic and oxidative conditions, demonstrating BDP's relative stability in these environments.

Collectively, these findings confirm that hydrolytic and photolytic pathways represent the predominant mechanisms of degradation. These findings underscore the critical importance of appropriate

excipient selection and rational formulation design to enhance BDP stability and mitigate degradation-related risks.

6.3 Excipient Compatibility Analysis

Preformulation studies of betamethasone dipropionate (BDP) demonstrated a significant influence of excipient type on impurity generation under accelerated stability conditions. Excipient compatibility studies were performed using varying concentrations of commonly used formulation components, and the results are presented in Table VIII.

Preformulation studies demonstrated a significant influence of excipient type on impurity generation under accelerated stability conditions. Excipients such as propylene glycol, PEG-based surfactants (including PEG stearates and polyoxyethylene derivatives), and lecithin were identified as high-risk components, exhibiting substantial increases in total impurities, reaching up to approximately 1.28%. This behavior suggests their potential role in promoting hydrolytic degradation, possibly due to increased formulation polarity or elevated microenvironmental water activity. (46)

In contrast, excipients such as mineral oil and hydroxypropyl methyl cellulose (HPMC) were associated with a moderate risk, as they exhibited unexpected impurity elevations under specific conditions, indicating condition-dependent stability concerns. (47)

Conversely, carbomer, poloxamer F127, parabens, and sorbic acid demonstrated relatively low risk, maintaining impurity levels below approximately 0.65%. These findings indicate improved compatibility with the active pharmaceutical ingredient, supporting their suitability for formulation development with enhanced stability profiles. (48)

Table VIII. Drug–excipient compatibility study of betamethasone dipropionate under accelerated conditions (60°C for 7 and 15 days)

									API Source	Symbiotech Pharma Ltd	
Sr. No	Sample	Condition	Impurity-B	Impurity-C	Impurity-D	1,2-Dihydrobetamethasone dipropionate	Impurity-E	Impurity-G	Impurity-H	Single Max Imp	Total Imp
1	BETAMETHASONE DIPROPIONATE	Initial	0.15	0.19	0.00	NA	NA	0.15	NA	0.00	0.49
		7 Days_60 °C	0.18	0.18	0.00	NA	NA	0.15	NA	0.14	0.65
		15 Days_60 °C	0.19	0.21	0.04	NA	NA	0.14	NA	0.18	0.76
2	BETAMETHASONE DIPROPIONATE+ PEG 100 STEARATE/ GLYCERYL STEARATE	Initial	0.16	0.19	0.00	NA	NA	0.14	NA	0.00	0.49
		7 Days_60 °C	0.25	0.22	0.10	NA	NA	0.14	NA	0.14	0.85
		15 Days_60 °C	0.31	0.28	0.14	NA	NA	0.14	NA	0.20	1.07
3	BETAMETHASONE DIPROPIONATE+PROPYLENE GLYCOL	Initial	0.16	0.17	0.00	NA	NA	0.14	NA	0.04	0.51
		7 Days_60 °C	0.29	0.25	0.17	NA	NA	0.14	NA	0.15	1.00
		15 Days_60 °C	0.34	0.31	0.22	NA	NA	0.14	NA	0.25	1.28
4	BETAMETHASONE DIPROPIONATE+MINERAL OIL	Initial	0.16	0.19	0.00	NA	NA	0.14	NA	0.00	0.49
		7 Days_60 °C	0.27	0.22	0.00	NA	NA	0.14	NA	0.06	0.69
		15 Days_60 °C	0.31	0.80	0.00	NA	NA	0.14	NA	0.17	1.44
5	BETAMETHASONE DIPROPIONATE+CORBOMER C980	Initial	0.15	0.16	0.00	NA	NA	0.14	NA	0.00	0.45
		7 Days_60 °C	0.22	0.17	0.00	NA	NA	0.14	NA	0.00	0.53

6.4 Mechanistic Interpretation of Excipient Effects

The observed degradation trends can be mechanistically rationalized based on the physicochemical interactions between the drug and formulation components. Polar solvents, such as propylene glycol, enhance water activity within the system, thereby facilitating ester hydrolysis. Polyethylene glycol (PEG)-based surfactants may further accelerate degradation through microenvironmental solubilization and potential nucleophilic interactions. Similarly, lecithin-containing systems can contribute to instability by introducing residual moisture and enabling catalytic interactions involving phospholipids. In contrast, hydrophobic excipients, such as mineral oil and isopropyl myristate, tend to reduce hydrolytic exposure; however, they may also influence drug partitioning within the formulation matrix. Collectively, these findings underscore the critical roles of microenvironmental polarity and excipient reactivity in governing BDP stability. (49) These mechanistic insights provide a scientific basis for excipient selection and highlight the need to integrate analytical stability assessment into formulation design.

6.5 Implications for Formulation Design

The findings from excipient compatibility and degradation studies directly informed the formulation design strategy. (50)Excipients associated with increased impurity generation, such as propylene glycol and PEG-based surfactants, were either minimized or strategically controlled in formulation development. Conversely, excipients

demonstrating improved compatibility, including carbomer, poloxamer, and parabens, were prioritized.

These results highlight the importance of microenvironmental control in stabilizing ester-containing corticosteroids. The formulation approaches described in Section 3 were therefore designed to minimize hydrolytic degradation while maintaining optimal drug release and dermal permeation. This integrated strategy ensures that formulation optimization is guided by both performance and stability considerations.

7. Conclusion

The present study successfully established a robust stability-indicating HPLC method for quantifying BDP and its related impurities in semisolid formulations. The method demonstrated excellent analytical performance and was effectively applied to evaluate drug–excipient compatibility under accelerated conditions.

Preformulation studies revealed that excipient selection plays a decisive role in governing degradation behavior, with polar solvents and surfactants significantly accelerating impurity formation. In contrast, hydrophobic and polymeric excipients exhibited improved compatibility.

The integration of analytical insights into formulation design enabled the development of a rational, data-driven strategy to optimize topical BDP systems. The findings provide a strong foundation for designing stable, effective, and regulatory-compliant formulations, with potential applicability to other ester-containing corticosteroids. This study highlights the importance of integrating analytical method development with formulation science to achieve stability-driven product optimization in topical drug delivery systems. This integrated analytical formulation approach provides a scalable framework for stability-driven design of topical drug delivery systems and can be extended to other labile corticosteroids.

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8. **References**

1. Tieu TM, Huynh VH, Nguyen TD, Pham DD. Formulation of metered-dose inhalers containing beclomethasone dipropionate using quality by design approach. Drug development and industrial pharmacy. 2026;52(4):780-92.

2. Yamamoto Y, Hanai A, Onuki Y, Fujii M, Onishi Y, Fukami T, et al. Mixtures of betamethasone butyrate propionate ointments and heparinoid oil-based cream: Physical stability evaluation. European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences. 2018;124:199-207.

3. Zatz JL, Varsano J, Shah VP. In vitro release of betamethasone dipropionate from petrolatum-based ointments. Pharmaceutical development and technology. 1996;1(3):293-8.

4. Marianni B, Polonini H. Compatibility of 17-α-Estradiol, Betamethasone, Finasteride, Melatonin, Prednicarbate, and Spironolactone in TrichoFoam™ - a Foaming Vehicle for Personalized Alopecia Treatments. International journal of pharmaceutical compounding. 2024;28(6):520-9.

5. da Silva JD, Viana GM, de Souza LG, Abrahim-Vieira B, de Souza AMT, Anselmo CS, et al. Identification and Characterization of Perampanel Degradation Products: Stability-Indicating HPLC and In Silico Toxicological Assessment. ACS omega. 2026;11(11):18115-24.

6. Muchakayala SK, Pavithra K, Katari NK, Marisetti VM, Dongala T, Vegesna RVK. Development and validation of a RP-UPLC method for the determination of betamethasone dipropionate impurities in topical formulations using a multivariate central composite design. Analytical methods : advancing methods and applications. 2021;13(33):3705-23.

7. Chawathe A, Sharma N. Investigation of the stability profile of therapeutic α-MSH analogue: Insights from liquid chromatography-high resolution mass spectrometry analysis of afamelanotide. Journal of pharmaceutical and biomedical analysis. 2026;272:117362.

8. Murali Krishnam Raju P, Shyamala P, Venkata Narayana B, Dantuluri H, Bhupatiraju RV. A fast, validated UPLC method coupled with PDA-QDa detectors for impurity profiling in betamethasone acetate and betamethasone phosphate injectable suspension and isolation, identification, characterization of two thermal impurities. Annales pharmaceutiques francaises. 2022;80(6):837-52.

9. Ferreira-Nunes R, Ferreira LA, Gratieri T, Cunha-Filho M, Gelfuso GM. Stability-indicating analytical method of quantifying spironolactone and canrenone in dermatological formulations and iontophoretic skin permeation experiments.

Biomedical chromatography : BMC. 2019;33(11):e4656.
10. Polonini H, Schettino Kegele C, Koulouridas S, Augusto Leal de Oliveira M. Quality-by-Design Compounding of Semisolids Using an Electronic Mortar and Pestle Device for Compounding Pharmacies: Uniformity, Stability, and Cleaning. Pharmaceutics. 2026;18(2).
11. Habjanič N, Kerec Kos M, Kristan K. Sensitivity of Different In Vitro Performance Tests and Their In Vivo Relevance for Calcipotriol/Betamethasone Ointment. Pharmaceutical research. 2020;37(3):52.
12. Raut S, Azheruddin M, Kumar R, Singh S, Giram PS, Datta D. Lecithin Organogel: A Promising Carrier for the Treatment of Skin Diseases. ACS omega. 2024;9(9):9865-85.
13. Alsaab H, Bonam SP, Bahl D, Chowdhury P, Alexander K, Boddu SH. Organogels in Drug Delivery: A Special Emphasis on Pluronic Lecithin Organogels. Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques. 2016;19(2):252-73.
14. Weiskirchen R, Weiskirchen S, Lonardo A. Lipid droplet dynamics in metabolic regulation. RSC chemical biology. 2026.
15. Zeng T, Song F, Yang Z, Yan X, Jiang L, Li D, et al. Nanoemulsion Encapsulation of Fat-Soluble Vitamins: Advances in Technology, Bioaccessibility and Applications. Foods (Basel, Switzerland). 2026;15(1).
16. Gutiérrez-Méndez N, Chavez-Garay DR, Leal-Ramos MY. Lecithins: A comprehensive review of their properties and their use in formulating microemulsions. Journal of food biochemistry. 2022;46(7):e14157.
17. Rana H, Patel S, Thakkar V, Gandhi T. Dasatinib Cube-O-Needle hybrid system for effective and safe site-specific treatment for triple-negative breast cancer. Naunyn-Schmiedeberg's archives of pharmacology. 2026.
18. Azeem A, Khan ZI, Aqil M, Ahmad FJ, Khar RK, Talegaonkar S. Microemulsions as a surrogate carrier for dermal drug delivery. Drug development and industrial pharmacy. 2009;35(5):525-47.
19. Thakral S, Levesque C, Cung K, Li L, Mould RE, Moussa E, et al. A pharmaceutical industry survey results on exploring polysorbate alternatives in biological drug products: Poloxamer and other surfactants. Journal of pharmaceutical sciences. 2025;114(9):103917.
20. Neşetoğlu N, Ünal D. Validated GC-MS and LC-MS/MS Methods for ICH M7-Based Quantification of Alkyl Mesylate and Azido Genotoxic Impurities in Pharmaceutical Products. Biomedical chromatography : BMC. 2026;40(3):e70357.
21. Sakama R, Nagao M, Tajima M, Takano M, Yoshikawa M, Sato H, et al. Development and Stability of a New Transdermal Formulation of Pregabalin for Skin Permeation. Biological & pharmaceutical bulletin. 2025;48(11):1708-14.
22. Lehman PA, Franz TJ. Assessing topical bioavailability and bioequivalence: a comparison of the in vitro permeation test and the vasoconstrictor assay. Pharmaceutical research. 2014;31(12):3529-37.
23. Maryam GE, Nasir F, Gohar S, Pervez S, HidayatUllah K, Khalil H, et al. Enhanced topical delivery of Terbinafine HCl nanomicelles embedded in chitosan gel for improved antifungal activity. Drug development and industrial pharmacy. 2026:1-20.
24. Prakash L, Malipeddi H, Subbaiah BV, Lakka NS. Impurity profiling and a stability-indicating UPLC method development and validation for the estimation of related impurities of halobetasol propionate in halobetasol propionate 0.05% (w/w) cream. Journal of chromatographic science. 2015;53(1):112-21.
25. Solon LG, Lima IP, Nogueira FH, de Araújo JP, Vivacqua CA, Aragão CF. Development and validation of an UHPLC method for the determination of betamethasone valerate in cream, gel, ointment and lotion. Steroids. 2016;106:70-7.
26. Macartney RA, Fricker ATR, Smith AM, Fedele S, Roy I, Knowles JC. A RP-HPLC-UV method for the dual detection of fluconazole and clobetasol propionate and application to a model dual drug delivery hydrogel. Analytical methods : advancing methods and applications. 2025;17(18):3694-704.
27. Elkady EF, Fouad MA, Mozayad AN. Application of Box-Behnken experimental design and response surface methodology for selecting the optimum RP-HPLC conditions for the simultaneous determination of methocarbamol, indomethacin and betamethasone in their pharmaceutical dosage form. BMC chemistry. 2022;16(1):114.
28. Mullick P, Mutalik SP, Hegde AR, Pandey A, Jagadish PC, Kini SG, et al. Simultaneous Estimation of Apremilast and Betamethasone Dipropionate in Microsponge-Based Topical Formulation using a Stability Indicating RP-HPLC

Method: A Quality-by-Design Approach. Journal of chromatographic science. 2021;59(10):928-40.
29. Fauzee AF, Walker RB. Forced degradation studies of clobetasol 17-propionate in methanol, propylene glycol, as bulk drug and cream formulations by RP-HPLC. Journal of separation science. 2013;36(5):849-56.
30. Fu Q, Shou M, Chien D, Markovich R, Rustum AMJJop, analysis b. Development and validation of a stability-indicating RP-HPLC method for assay of betamethasone and estimation of its related compounds. 2010;51(3):617-25.
31. Pessoa DF, Marques MBF, Mussel WN, Sebastião RCJJotBCS. Thermal Decomposition Kinetics of Corticosteroids Prednisone, Betamethasone Valerate and Hydrocortisone Acetate by Applying a MLP Network. 2026;37:e-20250186.
32. Khattak S-u-R, Shaikh D, Ahmad I, Usmanghani K, Sheraz MA, Ahmed SJAP. Photodegradation and stabilization of betamethasone-17 valerate in aqueous/organic solvents and topical formulations. 2013;14(1):177-82.
33. Byrne J, Velasco-Torrijos T, Reinhardt RJJocs. An RP-HPLC method for the stability-indicating analysis of impurities of both fusidic acid and betamethasone-17-valerate in a semisolid pharmaceutical dosage form. 2015;53(9):1498-503.
34. Allahverdiyeva J, Çağlar EŞ, Saldamlı E, Gelen-Gungor D, Okur NÜ, Eroğlu İJPCJ. Development of an HPLC Method for Simultaneous Determination of Betamethasone Dipropionate and Betamethasone Sodium Phosphate in Gel Formulations. 2026:1-9.
35. Hassib ST, Mahrouse MA, Elkady EF, Sayed RMJJocs. Simultaneous HPLC determination of betamethasone esters-containing mixtures: analysis of their topical preparations. 2018;56(8):716-23.
36. Chen M-Y, Tang Y-J, Wang Y-C, Wang C-Z, Yuan C-S, Chen Y, et al. Quantitative determination of betamethasone sodium phosphate and betamethasone dipropionate in human plasma by UPLC-MS/MS and a bioequivalence study. 2016;8(17):3550-63.
37. Elkady EF, Fouad MA, Mozayad ANJBc. Application of Box-Behnken experimental design and response surface methodology for selecting the optimum RP-HPLC conditions for the simultaneous determination of methocarbamol, indomethacin and betamethasone in their pharmaceutical dosage form. 2022;16(1):114.
38. Khatawakar A, Sakat S, Inamdar P, Bagade OJs. Development and Validation of the RP-HPLC Method for the Estimation of Chlorocresol and Betamethasone Dipropionate in Semisolid Dosage Formulation. 2022;9:10.
39. Raju PMK, Shyamala P, Narayana BV, Dantuluri H, Bhupatiraju R, editors. A fast, validated UPLC method coupled with PDA-QDa detectors for impurity profiling in betamethasone acetate and betamethasone phosphate injectable suspension and isolation, identification, characterization of two thermal impurities. Annales pharmaceutiques francaises; 2022: Elsevier.
40. Chanthathamrongsiri N, Anannaweenusorn P, Chuenta W, Saraarporn S, Plianwong SJS, Engineering, Studies H. Analytical method development and validation for simultaneous determination of triamcinolone acetate, betamethasone valerate and clobetasol propionate in cream formulation by RP-HPLC. 2023:23050024-.
41. Khuyen VTK, Nhu DT, Tuan ND. A Sustainable UHPLC-PDA/QTOF-MS Approach for Comprehensive Profiling of Amoxicillin Degradation Impurities. Rapid communications in mass spectrometry : RCM. 2026;40(9):e70046.
42. Wang JW, Liu XK, Huang F, Xu YB, Huang JH, Gan WJ, et al. Integrating non-targeted metabolomics and machine learning for comprehensive phytochemical profiling and intelligent discrimination of Acorus tatarinowii and its adulterants. Journal of pharmaceutical and biomedical analysis. 2026;274:117415.
43. Canuti E. UHPLC-based C-phycocyanin quantification method to support the validation of remote sensing models for harmful cyanobacterial blooms. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2025;1265:124759.
44. Aghamirsalim M, Mobaraki M, Jabbarvand M, Sahraian A. Thermosensitive Chitosan-Hyaluronic Acid Hydrogel for Sustained Betamethasone and Levofloxacin Delivery in Corneal Wound Healing. Translational vision science & technology. 2026;15(3):2.
45. Tian X, Wang X, Fang M, Liu A, Ma F, Yu L, et al. Synergism and antagonism mechanisms of β-carotene, γ-oryzanol, and β-sitosterol on oxidative stability of walnut oil. Food chemistry. 2026;508(Pt B):148467.
46. Qu J, Wang M, Ran Y, Yang J, Hui Y, Xu H, et al. Hidden Drug-Excipient Interaction by HPLC-MS/MS Analysis: Polysorbate 80 Enhances Nimodipine Systemic Exposure Through

Competitive Metabolic Inhibition. Biomedical chromatography : BMC. 2026;40(2):e70330.
47. Ke X, Ping Q, Liao Z. Interconversion kinetic studies of betamethasone acetate polymorphs in water. Drug development and industrial pharmacy. 2006;32(9):1019-24.
48. Francke NM, Bunjes H. Drug localization and its effect on the physical stability of poloxamer 188-stabilized colloidal lipid emulsions. International journal of pharmaceutics. 2021;599:120394.
49. Băiţan M, Lionte M, Moisuc L, Gafiţanu E. Optimization of topical gels with betamethasone dipropionate: selection of gel forming and optimal cosolvent system. Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi. 2011;115(2):601-5.
50. Singh, A., Bhardwaj, A., & Goshain, O. (2026). Analytical quality by design (AQbD)-based development and validation of a robust RP-HPLC method for the quantitative estimation of empagliflozin in pharmaceutical dosage forms. International Journal of Research - GRANTHAALAYAH, 14(4), 134–145. https://doi.org/10.29121/granthaalayah.v14.i6.2026.7079

Supplementary Materials

Table SI. Gradient Program for quantitative estimation of impurities of BDP

Time(min)	% Buffer	% ACN
0.00	60	40
10.0	60	40
20.0	55	45
25.0	50	50
35.0	40	60
40.0	35	65
45.0	35	65
50.0	60	40
60.0	60	40

Table SII: System precision for analytical method

Injection No.	Area Response
1	765888
2	766012
3	769311
4	768006
5	770045
6	767452
Average	767786
%RSD	0.22

Table SIII: Method precision for analytical method

Sample No.	% Assay
1	101.0
2	100.9
3	101.4
4	101.1
5	101.5
6	101.0
Average	101.2
% RSD	0.24

Table SIV: Intermediate precision of analytical method

Sample No.	% Assay
1	100.6
2	100.9
3	101.2
4	101.9
5	102.1
6	102.3
Average	101.5
% RSD	0.69

Table SV: Precision at Different Levels

Sr. No.	50% Level	100% Level	150% Level
1	379776	756389	1143661
2	383339	756265	1144376
3	384162	756600	1144751
4	382445	755704	1146646
5	384496	757731	1149814
6	384789	751316	1150403
Average	383168	757334	1146609
%RSD	0.49	0.27	0.25

Table SVI: Accuracy of analytical determination of BDP

% Recovery level	S.No.	Amount Added (µg)	Amount Recovered (µg)	% Recovery
50	1	5.05	5.09	100.1
	2	5.05	5.12	101.4
	3	5.05	5.15	102.0
100	1	10.11	10.26	101.5
	2	10.11	10.20	100.9
	3	10.11	10.24	101.3
150	1	15.17	15.22	100.3
	2	15.17	15.32	101.0
	3	15.17	15.19	100.1

Table SVII: Precision at the accuracy of BDP

Sr. No.	At 50%	At 100%	At 150%
1	100.1	101.5	100.3
2	101.4	100.9	101.0
3	102.0	101.3	100.1
Average	101.2	101.2	100.5
% RSD	0.96	0.30	0.47

Table SVIII: Stability of BDP in Standard Solution.

Time in Hours	Area response	% Difference
Initial	756389	NA
6	757283	-0.1
15	753592	0.4
21	759902	-0.5
27	755734	0.1
33	763157	-0.9
39	760737	-0.6
45	764992	-1.1
51	764309	-1.0

Table SIX: Stability of BDP in Sample Solution.

Time in Hours	Area response	% Difference
Initial	774408	NA
5	774753	0
14	778023	-0.5
20	774012	0.1
26	785786	-1.5
32	779637	-0.7
38	788564	-1.8
44	787130	-1.6
50	782618	-1.1

Table SX: Robustness data for system suitability

	Theoretical Plates	Tailing Factor	% RSD of Standard peak area
Limits	NLT 5000	NMT 2.0	NMT 2.0
Robustness_Low_Wavelength	8307	1.02	0.12
Robustness_High_Wavelength	8307	1.02	0.24
Robustness_Low_Flow	8632	1.01	0.14
Robustness_High_Flow	8007	1.02	0.31
Robustness_Low_Temperature	8411	1.03	0.34
Robustness_High_Temperature	7865	1.00	0.31