**FORMULATION DEVELOPMENT AND EVALUATION OF TOPICAL NANOEMULGEL OF CRISABOROLE**

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**ABSTRACT**

Topical Nanoemulgel usage has expanded in recent years because to the preparation's better patient acceptance due to its non-greasy, convenient Spread ability, easy administration, and good therapeutic and safety profile. Nanoemulgel has a strong potential of being the primary topical delivery route for lipophilic medications in the future, despite several challenges. Amongst all the formulations, Nanoemulsion loaded emulgel prepared with the tween 80, Almond Oil was found to be better with the drug diffusion. Zeta potential of optimized formulation (Nanoemulsion) -32 mV which indicates the thermodynamic instability of the dispersion. The percentage of drug content was found to be in 64-96% hence, uniformity of content was maintained. When the improved formulation was compared to the marketed formulation for in-vitro drug release, it was discovered that the optimized batch (F1) had regulated release in 24 hours. In addition, the formulation was launched in 12 hours. The formulation followed the Higuchi Kinetic model of drug release. Accelerated stability study showed no significant change in the formulation upto 3 months. Finally, it can be summarized and concluded that the Nanoemulsion loaded emulgel of Crisaborole can be one of the promising tool in controlling the drug release via. Percutaneous mechanism for effective and longer treatment required for fungal infections with increased stability.

**KEYWORDS:** Crisaborole, Topical Emulgel, Atopic Dermatitis, Nanoemulsion.

**INTRODUCTION**

Atopic dermatitis (eczema) is a condition that makes your skin red and itchy. It's common in children but can occur at any age. Atopic dermatitis is long lasting (chronic) and tends to flare periodically. It may be accompanied by asthma or hay fever. No cure has been found for atopic dermatitis. But treatments and self-care measures can relieve itching and prevent new outbreaks. For example, it helps to avoid harsh soaps, moisturize your skin regularly, and apply medicated creams or ointments. [34]

 Thus, the goal of the current study was to create Crisaborole (2% w/w) in the form of an emulgel that contained the drug's nanoemulsion.



**Figure 1: Structure of Crisaborole**

 Crisaborole is a novel oxaborole approved by FDA on December 14, 2016 as Eucrisa, a topical treatment of for mild to moderate atopic dermatitis. This non-steroidal agent is efficacious in improving disease severity, reducing the risk of infection and reducing the signs and symptoms in patients 2 years old and older. It reduces the local inflammation in the skin and prevents further exacerbation of the disease with a good safety profile. Its structure contains a boron atom, which facilitates skin penetration and binding to the bimetal center of the phosphodiesterase 4 enzyme. It is currently under development as topical treatment of psoriasis. [34,36]

**MATERIALS AND METHODOLOGY**

**Materials**

Crisaborole was purchased from Pharmatech solutions, Almond Oil (Research -lab Fine Chem Industry, Mumbai), Propylene glycol and Tween 80 also from Research -lab Fine Chem Industry. Carbopol 934 was from Molychem, Mumbai. All other reagents used were of analytical grade.

**Methodology**

High pressure homogenization methods are used for the formulation of nanoemulgel. There are three steps involved in the formulation of nanoemulgel which are given follows.

1. Preparation of Nanoemulsion
2. Preparation of hydrogel and
3. Finally, nanoemulgel will be produced by the incorporation of Nanoemulsion into gel with continuous stirring.

**Table 1: Composition of Nanoemulsion formulation**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Formulation code** | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F8** | **F9** |
| **Ingredients** | **%** |
| **Crisaborole (w/w)** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Almond Oil (v/v)** | 3 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 1 |
| **Tween 80 (v/v)** | 5.25 | 4.50 | 3.75 | 5.25 | 4.50 | 3.75 | 5.25 | 4.50 | 3.75 |
| **Propylene glycol (v/v)** | 1.75 | 1.50 | 1.25 | 1.75 | 1.50 | 1.25 | 1.75 | 1.50 | 1.25 |
| **Methyl Paraben (w/w)** | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| **Propyl Paraben (w/w)** | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| **BHT** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **Water Q.S (v/v)** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**METHOD OF PREPARATION FOR NANOEMULSION:**

**Preparation of aqueous phase ‘A’**: A precisely weighed amount of Propylene glycol was added to distilled water. (800c).

**Preparation of Oil phase ‘B’**: A weighed amount of Almond Oil and tween 80 were mixed together in a heated condition, then a weighed amount of Crisaborole was added, followed by the addition of methyl paraben, propyl paraben, and BHT.

**Incorporation of solution ‘A’ in dispersion ‘B’**: Both the phases were mixed properly with the help of High-pressure Homogenizer maintaining the respective rpm.

**Preparation of gel**

**Table 2: Composition of gel**

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Ingredients (% w/w)** | **Quantity** |
| 1 | Carbopol 934 | 1% |
| 2 | Triethanolamine | 0.1% |
| 3 | Water (q.s.) | 100 |

The weighed quantity of carbopol 934 was mixed in distilled water (400c) further addition of triethanolamine to maintain the desired pH range of the solution. The uniformity in the stirring was maintained and then the gel was kept in the refrigerator for 24 hrs.

**Preparation of Emulgel:**

Further incorporation of 20 % nanoemulsion was incorporated to obtain 100 % (w/w) emulgel which contains 2% of drug.

**Filling to container:**

The formulation was transferred into previously cleaned and dry containers.

**EVALUATION OF NANOEMULSION**

***Scanning Electron Microscopy***

Scanning electron microscopy (SEM) may be used to analyze the morphology of a nanoemulsion. A three-dimensional picture of the particle is produced using SEM. The samples are inspected at various magnifications and an appropriate accelerating voltage, typically 20 kV. [4]

***Particle Size Analysis***

Developed Nanoemulsion's hydrodynamic particle size has to be examined. In most cases, the dynamic light scattering method for measuring particles and further particle size distribution is utilized with nanoemulsions. [4]

***Zeta potential measurements***

Zetasizer hsa 3000 (Malvern Instrument Ltd., UK) was used to calculate the zeta potential for nanoemulsion. Results were obtained after samples were put in transparent disposable zeta cells. Before each experiment, cuvettes were cleaned with methanol and rinsed with the sample to be analyzed before adding the fresh sample. [5]

**EVALUATION OF NANOEMULGEL**

***Physical Appearance***

Visual examination of the produced nanoemulgel formulations was done to check for color, homogeneity, consistency, and pH. [4]

***Determination of pH***

With the use of a digital pH meter, the formulation's pH was determined. The pH meter electrode was cleaned with distilled water before being put into the mixture to test the pH. [6, 7]

***Measurement of viscosity***

A Brookfield Viscometer (RVDV-I Prime, Brookfield Engineering Laboratories, USA) with spindle 63 was used to determine the viscosity of the prepared batches. The viscosity-to-be-determined formulation was added to the beaker and left to settle for 30 minutes at the assay temperature (25±1ºC) before testing. [6, 7]

***Drug content study***

A drug content analysis was carried out to assess the amount of drug contained in a certain quantity of the formulation. 1 g of the formulation was placed in a 10 ml volumetric flask.

Methanol was added to it, and it was shaken thoroughly to make up the volume. The Volumetric flask was maintained for 2 hours and well mixed in a shaker. The solution was passed through filter paper and filtered the mixer before measuring absorbance at 252 nm with a spectrophotometer. [6, 7, 9]

***In-vitro Drug release study***

Emulgel in-vitro drug release investigations were conducted on Diffusion cells employing egg membrane. This was gently clamped to one end of the dialysis cell's hollow glass tube. On the surface of the egg membrane dialysis membrane, 1gm of emulgel was placed. The receptor chamber was filled with freshly produced PBS solution (pH 7.4). The total amount of gel used to solubilize the medication in the tube. The magnetic stirrer was used to agitate the receptor chamber. After proper dilutions, samples (1ml aliquots) were collected and tested for drug content using a UV visible spectrophotometer at 252 nm. [6, 7, 10]

***Release kinetics of selected formulation***

The cumulative release data were fitted to models representing Zero order (cumulative% drug release v/s. time), First order (log cumulative% drug retained v/s. time), and Higuchi model (cumulative% drug retained v/s. square root of time) to investigate the drug release kinetics and mechanism. [6, 7]

***Accelerated stability studies of Emulgel [3, 5]****:*

Guidelines govern the conduct of stability studies. For three months, the organized emulgels were filled in aluminum collapsible tubes (5 g) and subjected to strength leans at 5°C, 25°C/60% RH, 30°C/65% RH, and 40°C/75% RH and 60 ± 2°. Tests were drawn back at 15-day intervals and inspected for physical appearance, pH, rheological characteristics, and medicinal content. [11,12]

**RESULT AND DISCUSSION**

***Determination of (λ max) of Crisaborole in Methanol:***

The UV spectrum of Crisaborole solution scanned between 400-200 nm using UV spectrophotometer exhibited wavelength of absorbance maxima at 252 nm.

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**Figure 2: Ultraviolet Spectra of Crisaborole in Methanol**

***Calibration of Crisaborole in Methanol***

Crisaborole calibration curve was produced in methanol because Crisaborole is soluble in methanol. The drug solution in methanol was highly transparent and easily analyzed using a UV-visible spectrophotometer. The calibration curve was determined to be linear in the concentration range of 2-10 µg/ml, as shown in the table below. [14]

**Table 3: Calibration Curve of Crisaborole in Methanol**

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Conc.(ppm)** | **Absorbance** |
| 1 | 2 | 0.2286 |
| 2 | 4 | 0.4184 |
| 3 | 6 | 0.6116 |
| 4 | 8 | 0.8015 |
| 5 | 10 | 0.9731 |

**Figure 3: Calibration curve of Crisaborole in Methanol**

***Solubility study of drug in different oils:***

**Table 4: Solubility of Crisaborole in different oils:**

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Oils** | **Solubility** |
| 1 | Castor oil | 12.19 |
| 2 | Oleic acid | 18.38 |
| 3 | **Almond oil** | **29.20** |
| 4 | Liquid paraffin | 8.19 |
| 5 | Isopropyl myristate | 22.16 |

***Solubility determination of Crisaborole in surfactants and co-surfactant***

**Table 5: Solubility of Crisaborole in different surfactants and cosurfactant**

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Excipients** | **Solubility (mg/ml)** |
| 1 | Tween 20 | 17.28 |
| 2 | Span 20 | 9.46 |
| **3** | **Tween 80** | **38.25** |
| 4 | Span 80 | 31.44 |
| **5** | **Propylene glycol** | **36.02** |

***Fourier Transform Infrared Spectroscopy***

The FTIR spectrum of Crisaborole has been shown in below figure. The major peaks observed, and corresponding functional groups are given in below Table. The spectrum shows characteristic peaks for Crisaborole. [16,18]

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**Figure 4: Representative IR spectrum of Crisaborole**

The absorption bands shown by Crisaborole are characteristics of the groups present in its molecular structure. The presence of absorption bands corresponding to the functional groups present in the structure of Crisaborole confirms the identification and purity of the Crisaborole sample used in the study. [19,20]

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**Figure 5: FTIR of Physical mixture**

**Table 6: Interpretation of FTIR Spectrum of physical mixture**

|  |  |
| --- | --- |
| **Functional Group** | **Peaks** |
| **Pure Drug** | **Physical Mixture** |
| O-H Stretch | **Yes** | **Yes** |
| C-H Stretch | **Yes** | **Yes** |
| C=N | **Yes** | **Yes** |
| C=C | **Yes** | **Yes** |

**EVALUATION OF NANOEMULSION**

***Scanning Electron Microscopy***

The micrograph exhibited some nanoemulsion agglomeration, which might be related to the evaporation of water contained in the formulation during sample preparation prior to SEM examination. The particle size of the optimized batch's nanoemulsion was determined to be 100 nm. It is observed that when the concentration of Almond Oil increases with the speed of the homogenizer, the particle size decreases. [28-30]



**Figure 8: Scanning Electron Microscopy**

***Particle size and polydispersibility index***

The particle size of the optimized batch's nanoemulsion was determined to be 100 nm. It is observed that when the concentration of Almond Oil increases with the speed of the homogenizer, the particle size decreases.

**Table 8: Size distribution and PDI**

|  |  |  |
| --- | --- | --- |
| **Formulation code** | **Particle size (nm)** | **PDI** |
| Optimized Batch (F1) | 100 | 0.194 |

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**Figure 9: Particle Size of Optimized Formulation**

***Zeta Potential***

According to the ICH recommendations for stability tests of different pharmaceutical formulations, zeta potential indicates the stability of the (colloidal dispersion) nanoemulsion under stress testing conditions. The zeta potential is altered by particle size; the lowest particle size in nano size, i.e., 50, exhibits -32 mV. The zeta potential implies thermodynamic instability of the dispersion.

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**Figure 10: Zeta Potential of Optimized formulation**

**EVALUATION OF NANOEMULGEL**

***Physical Appearance***

The emulgel formulation's physical characteristics were determined to be transparent, homogenous, and consistent. [18]

***pH***

pH of various emulgel is shown in the following table 9 which was found to be in range of 5.31 to 5.75 pH values indicate the suitability of emulgel for topical application, so as to minimize discomfort or irritation due to acidic pH and microbial growth due to basic pH.

***Viscosity***

The viscosity values of formulations are shown in the Table 10.

***Spreadability***

Spreadability and emulgel viscosity exhibit an inverse connection. The spreadability of Formulation F1 is 17.77 gm.cm/sec, which is the formulation's ideal viscosity. Spreadability is shown in Table 11.

***Drug Content***

Table No. 12 displays the formulation's medication composition. All produced emulgel formulations were found to have a medication content that ranged from 64 to 96%.

***In-vitro drug release***

The in-vitro release of Crisaborole from its various emulgel formula are represented in Table 13.

**Table 9: pH values of formulation**

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Formulation code** | **Observed pH (± SD)** |
| 1 | F1 | 5.60±0.025 |
| 2 | F2 | 5.75±0.018 |
| 3 | F3 | 5.55±0.011 |
| 4 | F4 | 5.45±0.011 |
| 5 | F5 |  5.43±0.0158 |
| 6 | F6 | 5.33±0.011 |
| 7 | F7 | 5.31±0.005 |
| 8 | F8 | 5.40±0.018 |
| 9 | F9 | 5.53±0.026 |

**Table 10: Viscosity of formulations**

|  |  |
| --- | --- |
| **Rpm** | **Viscosity (cP) at Room Temperature** |
| **Formulation Code** |
| **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F8** | **F9** |
| 10 | 14960 | 13450 | 14500 | 13750 | 12500 | 13500 | 14500 | 13500 | 12000 |
| 20 | 14200 | 12390 | 14000 | 13400 | 12250 | 12440 | 14250 | 12500 | 11709 |
| 30 | 13050 | 12050 | 13445 | 12350 | 11200 | 12203 | 13900 | 12000 | 10500 |
| 40 | 13000 | 11500 | 12230 | 12010 | 11000 | 11253 | 12750 | 11500 | 9850 |
| 50 | 12350 | 10420 | 11520 | 11250 | 10950 | 10504 | 12520 | 11200 | 9230 |

**Table 11: Spreadability values of formulation**

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Formulation code** | **Spreadability (g.cm/sec)± S.D.** |
| 1 | F1 | 17.77 ± 0.025 |
| 2 | F2 | 16 ±0.035 |
| 3 | F3 | 15.38 ±0.028 |
| 4 | F4 | 15.68 ±0.018 |
| 5 | F5 | 15.09 ±0.032 |
| 6 | F6 | 14.81± 0.012 |
| 7 | F7 | 15.53± 0.012 |
| 8 | F8 | 15.23± 0.011 |
| 9 | F9 | 15.84 ±0.018 |

**Table 12: Drug content of formulation**

|  |  |  |
| --- | --- | --- |
| **Sr. No.** | **Formulation code** | **Drug content (%)± SD** |
| 1 | F1 | 96±0.5 |
| 2 | F2 | 91.91±0.7 |
| 3 | F3 | 95±0.7 |
| 4 | F4 | 93.91±0.7 |
| 5 | F5 | 94±0.7 |
| 6 | F6 | 72±0.7 |
| 7 | F7 | 68±1.09 |
| 8 | F8 | 82±1.07 |
| 9 | F9 | 64.91±1.43 |

**Table 13: Cumulative amount of Crisaborole diffused (%) from all the emulgel formulations through egg membrane using Modified Franz diffusion cell**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Time** **hrs** | **F1** | **F2** | **F3** | **F4** | **F5** | **F6** | **F7** | **F8** | **F9** |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 9 ±0.70 | 8.17±0.76 | 7.14±0.22 | 6.25±0.071 | 6.13±0.75 | 5.34±0.82 | 5.01±0.70 | 3.41±0.85 | 2.31±0.53 |
| 2 | 17 ±0.70 | 14.08±0.73 | 12.96±0.51 | 15.22±0.24 | 11.13±0.75 | 12.96±1.06 | 16.74±0.97 | 19.15±0.75 | 16.24±0.79 |
| 3 | 25±1.07 | 24.21±0.74 | 23.01±0.16 | 22.11±0.17 | 23.12±0.74 | 19.66±0.39 | 21.30±0.81 | 18.12±0.74 | 20.11±0.74 |
| 4 | 34±1.06 | 32.65±0.38 | 31.09±0.12 | 28.23±0.79 | 26.61±0.84 | 25.66±0.64 | 23.49±0.88 | 22.44±0.31 | 20.66±0.94 |
| 5 | 41±0.70 | 40.42±0.85 | 40.97±0.52 | 35.49±0.88 | 30.99±0.50 | 32.67±0.95 | 34.69±095 | 39.45±0.69 | 39.41±0.85 |
| 6 | 50±0.53 | 48.57±0.38 | 47.87±0.47 | 45.66±0.72 | 45.35±0.83 | 40.19±0.19 | 38.09±0.75 | 35.66±0.94 | 30.11±0.74 |
| 7 | 59±1.06 | 57.45±0.31 | 55.13±0.94 | 52.79±0.99 | 48.49±0.88 | 44.09±0.10 | 41.49±0.88 | 38.09±0.73 | 37.71±0.96 |
| 8 | 68±1.07 | 65.15±0.27 | 62.14±0.16 | 60.49±0.32 | 57.18±0.76 | 54.66±1.2 | 51.78±0.66 | 48.83±1 | 49.89±1.03 |
| 12 | 78±1.41 | 72.30±0.28 | 74.25±0.32 | 64.49±0.33 | 62.16±0.75 | 58.19±0.19 | 56.99±1.07 | 54.97±1.04 | 52.31±0.81 |
| 16 | 85±1.04 | 81.89±0.50 | 73.41±0.64 | 70.89±1.05 | 68.12±0.74 | 64.69±0.45 | 61.44±0.86 | 58.10±0.73 | 54.14±0.54 |
| 24 | 96±0.66 | 90±0.38 | 87.42±0.30 | 78.94±0.50 | 72.09±0.73 | 69.99±1.0 | 65.05±0.72 | 61.19±0.76 | 58.45±1.21 |

**Comparative Study**

**Table 14: Cumulative drug release of formulation F1 and Marketed formulation (Eucrisa Ointment)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Time****(hours)** | **% Cumulative drug Release** **(F1 formulation)** | **Time****(hours)** | **% CDR Marketed formulation****(Eucrisa ointment)** |
| 0 | 0 | 0 | 0 |
| 1 | 9±0.70 | 1 | 8±0.77 |
| 2 | 17±0.70 | 2 | 15±0.707 |
| 3 | 25±1.07 | 3 | 24±0.70 |
| 4 | 34±1.06 | 4 | 34±0.70 |
| 5 | 41±0.07 | 5 | 42±0.72 |
| 6 | 50±0.77 | 6 | 55.57±0.91 |
| 7 | 59±0.70 | 7 | 60.45±0.83 |
| 8 | 68±0.71 | 8 | 69±0.707 |
| 12 | 78±0.70 | 9 | 76.90±705 |
| 16 | 85±0.77 | 10 | 82±0.73 |
| 24 | 96±0.71 | 12 | 92±0.76 |

**Figure 11: In-Vitro Drug release profile of optimized formulation (F1) and Marketed formulation.**

It was observed that the release of the drug from optimized (F1) emulgel formulation was higher than the commercial gel. (2 % gel). The drug release of optimised formulation shows the controlled release up to 24 hrs (96 %) and marketed formulation shows (92 %) drug release upto 12 hrs. Formulation F1 showed steady state release upto 24 hours which also indicates that this formulation would show better contact with biological membrane. The drug is entrapped in the oil phase, hence when formulation was applied on egg membrane the penetration takes place upto 24 hrs. This phenomenon of drug release also suggests that when such formulations would be applied on skin surface the drug diffusion follows mechanisms.

**Drug release Kinetics**

The drug release was studied in this work to determine the kinetics of the drug release mechanism. Figures 12 and 13 demonstrate the results for zero order model kinetics and Higuchi model kinetics, respectively. [31]

**Figure 12: Model graph for comparative evaluation of Zero order Kinetics**

**Figure 13: Model graph for comparative evaluation of Higuchi Kinetics**

**Stability study:**

The improved formulation was tested after accelerated storage and at room temperature. Stability experiments revealed that the formulation was stable at accelerated temperatures (400C± 20C, 75 % RH ± 5% RH). At room temperature, the stability of the optimized batch F1 was investigated. [19]

**Table 15: Stability Study data for F1 formulation at Accelerated condition**

**(400 C± 20 C, 75 % RH±5% RH)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sr. No.** | **Observations** | **Before Stability Testing** | **During study** |
| **3rd month** |
| **1** | **Clearity** | Translucent | Translucent |
| **2** | **pH** | 5.60±0.006 | 5.41±0.025 |
| **3** | **% Drug content** | 96±0.5 | 95.97± 0.5 |
| **4** | **Viscosity** | 14960 | 14846 | 15132±63.93 |
| 14200 | 14152 | 14886±32.67 |
| 13050 | 12948 | 13425±38.53 |
| 13000 | 12794 | 13021±27.57 |
| 12350 | 12015 | 12463±32.92 |

**CONCLUSION**

Before formulating this formulations Preformulation testing were performed for drug characterization and to analyse its purity and compatibility. Organoleptic properties, melting point, solubility testing, UV spectroscopy studies and FTIR were performed for the Crisaborole and the drug sample procured were found to be and compatible with the excipients used in formulation. The drug loaded Nanoemulsion’s were evaluated for Particle size, Poly-dispersibility index, Zeta potential and scanning electron microscopy analysis. Drug loaded emulgel were evaluated for physical appearance, pH, viscosity, spreadability, drug content, *in vitro* drug release study (diffusion study), antibacterial activity and Accelerated stability studies. After accelerated storage and room temperature, the improved formulation was assessed. Stability tests revealed that the formulation was stable at accelerated temperatures (400C±20 C, 75% RH 5% RH). At room temperature, the stability of Optimized batch F1 was studied.

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