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Evaluation of Critical IVRT Parameters for Diclofenac Diethylamine Gel Using Franz Diffusion Cells

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ABSTRACT:
In Vitro Release Testing (IVRT) has emerged as an important analytical tool for evaluation of topical semisolid formulations due to its applicability in formulation development, quality assessment, and comparative product evaluation. The present study was undertaken to investigate suitable membrane and receptor medium conditions for IVRT of Diclofenac Diethylamine (DDE) gel using Franz diffusion cells. A cellulose acetate membrane (0.45 µm pore size, 25 mm diameter) was evaluated for its physicochemical stability, compatibility with receptor medium, and reproducibility of diffusion characteristics during the study. The receptor medium, consisting of 20 mM potassium dihydrogen phosphate buffer (pH 7.4 ± 0.05) and isopropyl alcohol (30:70, v/v), was optimized to maintain adequate sink conditions and facilitate reproducible drug release behavior. IVRT studies were performed using vertical Franz diffusion cells maintained at 32 ± 1°C with a stirring speed of 500 rpm under occluded conditions. Drug quantification was carried out using a validated reverse-phase HPLC method at 254 nm. The selected membrane demonstrated acceptable physical integrity and consistent diffusion characteristics throughout the study duration. Apparatus qualification parameters, including cell capacity, orifice diameter, and temperature conditions, complied with predefined acceptance criteria. The optimized receptor medium provided suitable solubility characteristics and supported progressive diffusion of Diclofenac across the membrane surface. The developed IVRT conditions exhibited reproducible release behavior and acceptable compatibility with analytical and diffusion systems. The findings of the present investigation demonstrate that the appropriate selection of membrane and receptor medium plays a critical role in the development of reliable IVRT methodologies for topical semisolid formulations.

1. INTRODUCTION:
Topical semisolid dosage forms such as creams, ointments, and gels are widely utilized for the localized treatment of dermatological and musculoskeletal disorders owing to their ability to deliver the active pharmaceutical

ingredient directly to the site of action while minimizing systemic exposure [1,2]. Diclofenac Diethylamine (DDE) gel, a non-steroidal anti-inflammatory formulation, is commonly prescribed for the management of pain and inflammation associated with conditions such as osteoarthritis, sprains, and muscular injuries [3]. Despite the extensive clinical use of topical semisolid formulations, establishing bioequivalence for generic topical products remains a significant scientific and regulatory challenge [4,5]. Unlike orally administered drug products, where systemic drug concentrations can be correlated with therapeutic performance, topical formulations exert their action locally within or beneath the skin, making conventional pharmacokinetic approaches unsuitable for bioequivalence assessment [6–8].

Traditionally, clinical endpoint studies have been employed for the demonstration of bioequivalence of topical dermatological products. However, these studies are frequently associated with considerable limitations including high inter-subject variability, prolonged study duration, poor sensitivity, ethical concerns, and elevated financial burden. Furthermore, clinical endpoint studies often fail to detect subtle formulation differences that may influence product performance [9,10]. In response to these challenges, regulatory authorities such as the United States Food and Drug Administration (USFDA) and the European Medicines Agency (EMA) have increasingly encouraged the use of scientifically justified in vitro and ex vivo approaches for the evaluation of topical semisolid formulations [11,12].

Among the available alternative approaches, *In Vitro* Release Testing (IVRT) has emerged as an important analytical tool for assessing the performance characteristics of semisolid dosage forms. IVRT is a diffusion-based technique that evaluates the release of drug substance from a formulation across a synthetic membrane into a receptor medium under controlled experimental conditions, commonly using Franz diffusion cells [13,14]. The technique is extensively applied during formulation development, quality control testing, post-approval changes, and comparative assessment of topical products. Owing to its reproducibility, sensitivity, and discriminatory capability, IVRT has gained substantial importance in regulatory evaluation of topical semisolid formulations. The significance of IVRT has also been recognized in USP General Chapter <1724> and various USFDA product-specific guidances concerning topical drug products [15,16].

Several researchers have highlighted the utility of IVRT in the assessment of topical semisolid formulations. Shah *et al.* 2022 discussed the importance of diffusion-based methodologies in evaluating dermatological drug products and emphasized the role of IVRT in semisolid formulation characterization [16]. Leal et al., 2017 investigated bioequivalence methodologies for topical corticosteroid formulations and demonstrated the limitations associated with conventional clinical endpoint studies, thereby supporting the need for more sensitive alternative approaches [17]. Similarly, Narkar 2010. reported improved assessment of topical product performance through in vitro and dermatopharmacokinetic methodologies, further reinforcing the scientific relevance of surrogate approaches for topical bioequivalence evaluation [18].

The performance and reliability of IVRT studies are highly dependent upon appropriate selection and optimization of critical experimental parameters. Among these parameters, membrane selection and receptor medium composition play particularly important roles because they directly influence drug diffusion, release kinetics, sink condition maintenance, and experimental reproducibility [19,20]. The membrane employed during IVRT should ideally function as an inert support with minimal resistance to drug diffusion while maintaining chemical and physical stability throughout the study period. Comparative investigations have demonstrated that membrane characteristics such as pore size, thickness, hydrophilicity, and drug-binding tendency can significantly influence release behavior [21,22]. Lane 2024, reported that variation in membrane properties may alter release profiles and contribute to experimental variability during semisolid release testing [23]. Salamanca *et al.,* 2018 also observed that inappropriate membrane selection could adversely affect diffusion characteristics and compromise reproducibility of release data obtained using Franz diffusion cells. [24]

In addition to membrane characteristics, the composition of receptor medium is another critical determinant of IVRT performance, particularly for poorly water-soluble drugs such as DDE. Maintenance of adequate sink conditions throughout the study period is essential to ensure continuous diffusion-driven drug release. Previous comparative studies have demonstrated that incorporation of organic solvents such as isopropyl alcohol or ethanol into phosphate buffer systems improves drug solubility and facilitates reproducible release profiles for lipophilic topical formulations [25–27]. Hate et al. 2025, and Railic et al., 2024 reported that receptor media containing alcohol-based modifiers enhanced release consistency and analytical recovery of poorly soluble drugs during

diffusion studies. Similarly, Hauck et al. emphasized the importance of optimized receptor medium composition in maintaining sink conditions and minimizing variability during IVRT experiments [28,29].

Franz diffusion cells remain the most commonly employed apparatus for IVRT studies because of their simplicity, reproducibility, and regulatory acceptance. Studies conducted by Wellington et al., 2024. demonstrated that variables such as membrane type, receptor medium composition, temperature, stirring speed, and sampling intervals can substantially affect release characteristics obtained during IVRT studies [30]. Alomari & Alhussaini, 2024 further emphasized the importance of properly optimized IVRT methodologies in supporting generic topical drug development and regulatory evaluation [31]. Despite the growing application of IVRT in topical formulation assessment, limited literature is available regarding systematic optimization of membrane and receptor medium conditions specifically for DDE gel under regulatory-oriented experimental conditions.

Therefore, the present study was undertaken to evaluate suitable membrane and receptor medium conditions for IVRT of DDE gel using Franz diffusion cells. The study aimed to investigate the influence of membrane characteristics and receptor medium composition on drug release behavior and to establish optimized experimental conditions capable of providing reproducible and reliable IVRT performance for topical semisolid formulations.

2. MATERIALS:

Diclofenac Diethylamine Gel 2.32% (Voltaren®, Batch No. RD6M) was used as the reference listed drug (RLD) throughout the study. Potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide, hydrochloric acid, formic acid, methanol, acetonitrile, and isopropyl alcohol of analytical and HPLC grade were procured from commercially approved suppliers. Purified water obtained from a Smart2Pure water purification system was used during the preparation of buffer solutions and chromatographic analysis. Cellulose acetate membranes having a pore size of 0.45 µm and diameter of 25 mm were employed for the in vitro release studies. All chemicals and reagents used during the investigation were of suitable analytical grade and utilized without further purification.

2.2 Instrumentation:

The *in vitro* release studies were performed using an automated Franz diffusion cell apparatus (Logan Instruments Corp., USA) equipped with vertical diffusion cells having a nominal receptor compartment volume of 12 mL and an effective diffusion area of approximately 1.77 cm^2. The system was connected to a circulating water bath to maintain controlled experimental temperature conditions throughout the study. Continuous mixing of receptor medium was achieved using magnetic stir bars operated at predetermined stirring speeds. Temperature monitoring during the study was performed using calibrated thermometers and infrared temperature measuring devices.

Chromatographic analysis was carried out using a high-performance liquid chromatography (HPLC) system equipped with a UV detector and Chromeleon® software for data acquisition and processing. Ancillary laboratory equipment including analytical balance, microbalance, micropipettes, vortex mixer, sonicator, pH meter, refrigerator, and deep freezer were used during experimental and analytical procedures.

3. METHODS:

3.1 Chromatographic Conditions:

Quantitative estimation of Diclofenac was performed using a validated reverse-phase HPLC method. Chromatographic separation was achieved using a Peerless Basic C18 column (100 mm × 4.6 mm, 3 µm particle size). The mobile phase consisted of 0.1% formic acid in 5 mM potassium dihydrogen phosphate buffer and acetonitrile in the ratio of 30:70 (% v/v). The mobile phase was filtered and sonicated prior to use. The chromatographic system was operated at a flow rate of 1.2 mL/min with an injection volume of 20 µL. The column oven temperature was maintained at 40°C, whereas the autosampler temperature was maintained at 5°C. Detection was carried out at 254 nm using a UV detector. The total run time for each analysis was 5 minutes, and Diclofenac exhibited a retention time of approximately 2 minutes under the optimized chromatographic conditions [32,33].

3.2 Preparation of Receptor Medium:

The receptor medium was prepared using 20 mM potassium dihydrogen phosphate buffer adjusted to pH 7.4 ± 0.05 with 1.0 N potassium hydroxide solution. The prepared buffer was subsequently mixed with isopropyl alcohol in the ratio of 30:70 (% v/v) to obtain the final receptor medium composition. The receptor medium was

sonicated prior to use to ensure complete mixing and removal of entrapped air. Fresh receptor medium was prepared as required during the experimental studies. The chromatographic conditions were optimized to achieve adequate peak resolution, acceptable retention behavior, and reproducible quantification of Diclofenac during IVRT sample analysis [34,35]. Table 1 describes the optimized chromatographic conditions employed for quantitative estimation of Diclofenac during IVRT sample analysis. The selected analytical conditions provided acceptable retention behavior, reproducible peak response, and suitable chromatographic performance throughout the study.

Table 1. Optimized chromatographic conditions for HPLC analysis of Diclofenac

Parameter	Condition
Analytical column	Peerless Basic C18 (100 mm × 4.6 mm, 3 µm)
Mobile phase	0.1% formic acid in 5 mM KH_2PO_4 : Acetonitrile (30:70, v/v)
Flow rate	1.2 mL/min
Injection volume	20 µL
Detection wavelength	254 nm
Detector	UV detector
Column oven temperature	40°C
Autosampler temperature	5°C
Run time	5 min
Retention time of Diclofenac	Approximately 2 min
Rinsing solution	Water : Acetonitrile (30:70, v/v)

3.3 Membrane Preparation:

Cellulose acetate membranes having a pore size of 0.45 µm and diameter of 25 mm were used during the IVRT studies. Prior to experimentation, membranes were soaked in receptor medium for approximately 10 minutes to ensure adequate wetting and equilibration. The pretreated membranes were carefully mounted between the donor and receptor compartments of the Franz diffusion cells, ensuring proper alignment without formation of air bubbles beneath the membrane surface. An O-ring and clamp assembly were utilized to secure the membrane and maintain a uniform diffusion area throughout the study period [36,37].

3.4 *In Vitro* Release Testing Procedure:

The receptor compartment of each Franz diffusion cell was filled with pre-heated receptor medium, and the assembled diffusion cells were allowed to equilibrate for approximately 30 minutes to achieve a membrane surface temperature of 32 ± 1°C. Temperature of the membrane surface was monitored prior to dose application to ensure compliance with experimental conditions. Magnetic stirring was maintained at 500 rpm throughout the study to ensure uniform mixing of receptor medium. Approximately 300 mg of Diclofenac Diethylamine gel was applied uniformly onto the membrane surface under occluded conditions using a pre-weighed syringe. Care was taken to achieve complete coverage of the diffusion area without entrapped air gaps. The applied dose amount was determined gravimetrically from the difference in syringe weight before and after dosing.

Following dose application, the donor compartment was covered using a chimney cap to maintain occluded conditions throughout the experiment. Samples were collected from the receptor compartment at predetermined time intervals of 0.5, 1, 1.5, 2, 3, 4, 5, and 6 hours. At each sampling point, aliquots were withdrawn and replaced immediately with an equal volume of fresh pre-heated receptor medium to maintain constant receptor volume and sink conditions. During sample collection, stirring was temporarily interrupted and resumed immediately after replenishment of receptor medium [38,39]. The collected samples were transferred to suitable containers and stored at -20 ± 5°C until chromatographic analysis. Any air bubbles observed beneath the membrane during experimentation were carefully removed to avoid interference with diffusion characteristics.

3.5 Membrane Selection Study:

The membrane selection study was conducted to identify a suitable synthetic membrane for IVRT evaluation of DDE gel. Membrane suitability was assessed based on physical integrity during the study period, compatibility with receptor medium, reproducibility of release profiles, and minimal resistance to drug diffusion. The selected membrane was further evaluated for consistency of drug release and absence of significant interference with analytical quantification [38,40]. Table 2 summarizes the optimized IVRT experimental conditions utilized during the diffusion studies. The selected parameters were maintained throughout the investigation to ensure controlled diffusion behavior, reproducibility of release profiles, and maintenance of sink conditions.

Table 2. Experimental conditions employed for IVRT study of Diclofenac Diethylamine gel

Parameter	Condition
Apparatus	Franz diffusion cell
Apparatus manufacturer	Logan Instruments, USA
Cell volume	12 mL
Effective diffusion area	1.77 cm^2
Receptor medium	20 mM KH_2PO_4 buffer : IPA (30:70, v/v), pH 7.4 ± 0.05
Temperature	32 ± 1°C
Stirring speed	500 rpm
Dose amount	Approximately 300 mg
Dose condition	Occluded
Membrane	Cellulose acetate
Membrane pore size	0.45 µm
Membrane diameter	25 mm
Sampling intervals	0.5, 1, 1.5, 2, 3, 4, 5, and 6 h
Sample aliquot volume	0.5 mL
Replacement volume	2.5 mL
Prime volume	1.5 mL

3.6 Receptor Medium Selection:

Selection of receptor medium was performed to ensure adequate solubility of Diclofenac and maintenance of sink conditions throughout the diffusion study. Different receptor medium compositions were comparatively evaluated based on drug release characteristics, analytical compatibility, and reproducibility of release profiles. Particular emphasis was placed on achieving sufficient solubility while maintaining membrane compatibility and stable diffusion conditions during the study period [41,42]. Table 3 presents the critical evaluation parameters considered during membrane selection for IVRT studies. Membrane suitability was assessed based on diffusion characteristics, compatibility with experimental conditions, physical integrity, and reproducibility of drug release profiles.

Table 3. Evaluation criteria employed during membrane selection study

Evaluation parameter	Assessment criteria
Physical integrity	Absence of membrane rupture or deformation during study
Drug diffusion behavior	Consistent release profile across sampling intervals
Membrane compatibility	No visible interaction with receptor medium or formulation
Reproducibility	Acceptable variability among diffusion cells
Drug binding tendency	Minimal interference with drug release

3.7 Sample Analysis and Data Treatment:

The collected IVRT samples were analyzed using the optimized HPLC method described previously. Quantification of Diclofenac was performed using calibration standards prepared over an appropriate concentration range. The cumulative amount of drug released per unit diffusion area was calculated for each sampling interval after correction for sample replacement. Drug release profiles were constructed by plotting cumulative amount of drug released per unit area against the square root of time. The release rate was determined from the slope of the linear portion of the release profile using linear regression analysis. Comparative assessment of membrane and receptor medium conditions was performed on the basis of release characteristics, reproducibility, and consistency of diffusion profiles [43–45]. Table 4 outlines the parameters considered during receptor medium optimization for IVRT analysis. The receptor medium was evaluated for its ability to maintain sink conditions, support consistent drug diffusion, and ensure compatibility with analytical and membrane systems.

Table 4. Evaluation parameters for receptor medium selection

Evaluation parameter	Purpose
Drug solubility	To maintain adequate sink condition
Release consistency	To obtain reproducible release profile
Analytical compatibility	To ensure compatibility with HPLC analysis
Membrane compatibility	To prevent membrane instability or swelling
Diffusion behavior	To achieve continuous and uniform drug release

4. RESULTS AND DISCUSSION:

4.1 Membrane Selection Study:

Selection of a suitable membrane is considered a critical factor during development of an IVRT method for topical semisolid formulations, as membrane characteristics directly influence diffusion behavior, release kinetics, and reproducibility of experimental findings. In the present study, cellulose acetate membrane having a pore size of

0.45 µm and diameter of 25 mm was evaluated under optimized IVRT conditions for assessment of DDE gel release behavior.

The selected membrane demonstrated acceptable physicochemical stability throughout the diffusion study without visible rupture, swelling, leakage, or deformation. Pretreatment of the membrane with receptor medium prior to experimentation ensured proper hydration and minimized formation of entrapped air beneath the membrane surface, thereby supporting reproducible diffusion characteristics during the study period. The membrane also exhibited acceptable compatibility with the receptor medium composed of 20 mM KH_2PO_4 buffer and isopropyl alcohol (30:70, v/v), with no observable membrane instability during experimentation.

Apparatus qualification studies further supported the suitability of the membrane and diffusion system for IVRT analysis. The average receptor compartment volume for the diffusion cells ranged from 11.8776 to 12.0656 mL, which remained within the predefined acceptance criteria of ±2% of the nominal cell volume (11.7600–12.2400 mL). Similarly, the average orifice diameter ranged from 14.87 to 15.01 mm and complied with the acceptable range of 14.25–15.75 mm for the nominal diffusion area of 1.77 cm^2. Membrane surface temperature during experimentation was consistently maintained between 32.0°C and 32.2°C, whereas receptor medium temperature ranged from 32.1°C to 32.2°C, thereby ensuring controlled diffusion conditions throughout the IVRT study.

The selected membrane demonstrated reproducible release characteristics with progressive diffusion of Diclofenac throughout the sampling intervals. The observed release profile indicated that the membrane functioned primarily as an inert support without significantly impeding drug diffusion. This behavior is particularly important during IVRT studies, as excessive membrane resistance or drug-binding interactions may alter release kinetics and compromise reproducibility of the release profile.

Previous studies have similarly reported the suitability of cellulose acetate membranes for IVRT evaluation of topical semisolid formulations due to their hydrophilic characteristics, low drug-binding tendency, and acceptable diffusional properties. Shah et al. reported that membrane characteristics such as pore size, hydrophilicity, and structural uniformity significantly influence release behavior during semisolid diffusion studies. Flynn et al. also demonstrated that membranes exhibiting minimal diffusional resistance contribute to improved reproducibility and reduced experimental variability during Franz diffusion cell analysis [46–48].

The cumulative release profile obtained using the selected membrane demonstrated a progressive increase in Diclofenac diffusion over the experimental duration, indicating maintenance of sink conditions and consistent release kinetics throughout the study. The reproducibility observed across diffusion cells further supported the suitability of cellulose acetate membrane for IVRT assessment of Diclofenac Diethylamine gel under the optimized diffusion conditions.

Table 5. Qualification and membrane-related observations obtained during IVRT analysis

Parameter	Observation	Acceptance criteria
Cell capacity	11.8776–12.0656 mL	11.7600–12.2400 mL
Orifice diameter	14.87–15.01 mm	14.25–15.75 mm
Membrane surface temperature	32.0–32.2°C	32 ± 1°C
Receptor medium temperature	32.1–32.2°C	32 ± 1°C
Membrane integrity	No rupture or deformation observed	Acceptable
Compatibility with receptor medium	Acceptable	Acceptable
Drug diffusion behavior	Progressive and reproducible release observed	Acceptable

Table 5 summarizes the qualification parameters and membrane-related observations obtained during IVRT analysis. The selected cellulose acetate membrane demonstrated acceptable physicochemical stability, compatibility with receptor medium, and reproducible diffusion characteristics under the optimized experimental conditions.

4.2 Receptor Medium Optimization:

Selection of an appropriate receptor medium is essential during IVRT studies to maintain adequate sink conditions, ensure drug solubility, and facilitate reproducible diffusion behavior throughout the experimental duration. Diclofenac Diethylamine possesses limited aqueous solubility; therefore, optimization of receptor medium composition was considered necessary to achieve continuous diffusion-driven release during the IVRT analysis.

In the present study, receptor medium consisting of 20 mM KH_2PO_4 buffer (pH 7.4 ± 0.05) and isopropyl alcohol in the ratio of 30:70 (% v/v) was selected for diffusion studies. The incorporation of isopropyl alcohol significantly improved the solubility characteristics of Diclofenac and supported maintenance of sink conditions throughout the study duration. The selected receptor medium demonstrated acceptable compatibility with the cellulose acetate membrane and did not produce visible precipitation or instability during experimentation.

The optimized receptor medium provided consistent release profiles during IVRT analysis and supported progressive diffusion of Diclofenac across the membrane surface. The release behavior observed during the study suggested that the selected receptor medium composition was sufficient to maintain concentration gradient-driven diffusion throughout the experimental period. Furthermore, the receptor medium demonstrated acceptable compatibility with the chromatographic method and did not interfere with analytical quantification of Diclofenac during HPLC analysis.

Previous investigations involving topical semisolid formulations have similarly highlighted the importance of alcohol-modified receptor media for diffusion studies involving poorly water-soluble drugs. Chang et al. reported that incorporation of organic modifiers such as isopropyl alcohol improves solubility and release consistency during diffusion studies of lipophilic topical drugs. Hauck et al. also emphasized that receptor medium optimization is necessary to maintain adequate sink conditions and minimize variability during IVRT experiments [49,50].

The receptor medium selected during the present investigation demonstrated acceptable physicochemical stability and reproducible diffusion characteristics throughout the study duration. The optimized medium composition effectively supported diffusion of Diclofenac without compromising membrane integrity or chromatographic compatibility, thereby establishing its suitability for IVRT assessment of Diclofenac Diethylamine gel.

Table 6. Optimized receptor medium conditions employed during IVRT analysis

Parameter	Condition
Buffer composition	20 mM KH_2PO_4
Buffer pH	7.4 ± 0.05
Organic modifier	Isopropyl alcohol
Buffer:IPA ratio	30:70 (% v/v)
Compatibility with membrane	Acceptable
Sink condition maintenance	Acceptable
Analytical compatibility	Acceptable

Table 6 presents the optimized receptor medium conditions employed during IVRT analysis. The selected receptor medium composition demonstrated acceptable solubility characteristics, maintenance of sink conditions, and compatibility with membrane and analytical systems throughout the study.

4.3 Optimized IVRT Conditions:

Based on the membrane selection and receptor medium optimization studies, the finalized IVRT conditions for Diclofenac Diethylamine gel included the use of cellulose acetate membrane (0.45 µm pore size), receptor medium consisting of 20 mM KH_2PO_4 buffer and isopropyl alcohol (30:70, v/v), diffusion temperature of 32 ± 1°C, and stirring speed of 500 rpm. The optimized conditions demonstrated acceptable reproducibility, controlled diffusion behavior, and maintenance of sink conditions during the study period.

The cumulative release profile obtained under the optimized experimental conditions exhibited progressive and reproducible diffusion characteristics throughout the sampling intervals. The selected conditions were found suitable for IVRT assessment of Diclofenac Diethylamine gel and may be useful for future comparative evaluation of topical semisolid formulations under controlled diffusion conditions.

5. CONCLUSION:

The present study successfully established suitable membrane and receptor medium conditions for In Vitro Release Testing (IVRT) of Diclofenac Diethylamine gel using Franz diffusion cells. The cellulose acetate membrane demonstrated acceptable physicochemical stability, compatibility with receptor medium, and reproducible diffusion characteristics throughout the experimental duration. Similarly, the receptor medium consisting of 20 mM KH_2PO_4 buffer and isopropyl alcohol (30:70, v/v) provided adequate sink conditions and supported consistent drug release behavior during the study.

The optimized IVRT conditions exhibited controlled and progressive release characteristics with acceptable reproducibility under the selected experimental parameters. The findings of the present investigation highlight the importance of appropriate membrane and receptor medium selection during the development of IVRT methodologies for topical semisolid formulations.

The developed experimental conditions may be useful for routine IVRT assessment and comparative evaluation of Diclofenac topical gel formulations under controlled diffusion conditions. Further investigations involving comparative formulation assessment and extended validation studies may provide additional insight into the applicability of the developed IVRT method for regulatory and formulation development purposes.

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