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# Impact of shelf-life on the pharmaceutical equivalence assessment of a hydrocortisone semisolid W/O emulsion: Evaluation of rheological, structural, and release properties

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ABSTRACT

Developing generic topical medicines is particularly challenging because of pharmaceutical equivalence criteria at microstructure level. The EMA guideline on bioequivalence of topical products introduced flexibility, acknowledging the high variability inherent to topical formulations. This study evaluated the evolution of rheological parameters (yield stress, viscous modulus in LVR, elastic modulus in LVR, zero shear viscosity and viscosity at 100 $s^{-1}$), droplet size, and hydrocortisone release in 5 batches of a commercial semisolid W/O hydrocortisone buteprate cream over the 3-year shelf-life. Despite high intrinsically variability, rheological parameters remained stable over time. In contrast, droplet size and release parameters fluctuated over time, although without a distinct temporal pattern. All rheological parameters met equivalence as per the EMA guideline requirements, while droplet size and release parameters only did so in some comparisons. Principal Component Analysis revealed that rheological parameters were strongly interrelated but minimally correlated with release behavior, suggesting that rheological parameters could be redundant and have limited influence on drug release. Overall, the findings indicate that EMA's equivalence criteria with extended limits better reflect the natural variability of dispersed systems, although limits may need to be further broadened for some topical products. The results warrant further research to clarify the role of rheology in topical bioequivalence assessment.

## 1. Introduction

The topical route has emerged as one of the fastest-growing routes of drug administration in recent years, due to several advantages: (i) the ease of application and avoidance of inherent limitations of parenteral or oral administration, making it suitable for a diverse patient population; (ii) reduced systemic adverse effects, due to minimal systemic drug exposure; and, (iii) when systemic effects are required, avoidance of physiological processes such as the hepatic first-pass effect, thereby enhancing drug bioavailability.

The development of generic formulations for this route of administration is challenging. A generic product (test product; TP) must demonstrate:

- Pharmaceutical equivalence (PE): same identical active pharmaceutical ingredient (API) in the same dose and dosage form as the reference product (RP).
- Bioequivalence (BE) established through comparative pharmacokinetic, pharmacodynamic, clinical, or in vitro studies with RP.

Currently, comparative clinical endpoint trials are used to establish BE for most dermatological drug products.

The EMA published in 2024 a guideline on quality and equivalence of locally applied, locally acting cutaneous products (EMA, 2024),

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including guidance to support a claim of therapeutic equivalence with a RP through tests that replace clinical assessment. In the context of the guidance, to be considered PE, TP and RP must demonstrate same qualitative composition (Q1), similar quantitative composition (Q2) and the same or at least sufficiently similar arrangement of matter (Q3). Demonstration of Q3 includes comparison of both physical properties as well as product performance (in vitro drug release, IVRT).

The draft version of this guideline established a very narrow limit of 10% for the 90% confidence interval (CI) of the difference of means between the TP and the RP, which needs to be met for all quantitative parameters characterizing the microstructure and performance (assuming normal data distribution)(2). Many studies demonstrated that some of these parameters change throughout the shelf-life of a topical product, compromising the equivalence decision (Mangas-Sanjuán et al., 2019; Mañez-Asensi et al., 2024; Miranda et al., 2023; Miranda et al., 2020; Miranda et al., 2022; Navarro-Pujol et al., 2021; Ocaña et al., 2020; Pleguezuelos-Villa et al., 2019; Xu et al., 2020). When comparing batches of different manufacturing date within the self-life, changes in the mean value of the parameters can lead to differences in the mean TP/RP ratio value, potentially shifting the 90% CI of the ratio of means outside the limit set by the regulation, even if it is narrow in value. Moreover, combination of batches with different manufacturing dates results in an increased parameter variability, resulting in a 90% CI of the ratio of means exceeding the 10% limit. The final guideline considers a log-normal distribution of data and the possibility of extending the 90% CI limit from 10% to 20% depending on the variability of the parameter evaluated in the RP, using scale-averaged equivalence (EMA, 2024).

While it remains to be clarified which rheological parameters actually characterize the physical structure of a topical formulation and affect the API release, the EMA guideline suggests the determination of a large number of rheological parameters.

Rheological properties are key for a product's quality and performance, as they determine long-term physical stability, ease of application, patient acceptability, API release and API permeation through skin (Badruddoza et al., 2023; Barreiro-Iglesias et al., 2001; Pleguezuelos-Villa et al., 2019; Simões et al., 2020). While the effect of gelling agents on the release of the API has been extensively investigated, inconsistency on which rheological parameters determine API release exists. An increase in the concentration of gelling agent was associated with an increase in the complex modulus G*, resulting in a more rigid structure, and in a reduced release flux of the API (Badruddoza et al., 2024). Several studies have demonstrated that an increase in a formulation's rigidity is associated with diminished API release and, to a lesser extent, a diminished skin permeation (Badruddoza et al., 2023; Nguyen et al., 2015). Contrarily, another study showed that the deposition of imiquimod in skin correlated more with the water content of the formulation than with the degree of structuring of the system, as the deposition in both epidermis and dermis increased as the ratio elastic/viscous moduli (G'/G") increased until it reaches a point where it decreased (Telò et al., 2017). Another research that focused on the dynamic viscosity of the hydrogels tested ($\eta_{10\ s^{-1}}$) showed that drug penetration was largely unaffected by this parameter, suggesting that viscosity can be adapted to develop a formulation with appropriate application characteristics, without concerns on drug permeation (Binder et al., 2019).

Evaluation of aceclofenac emulsions in a quality-by-design approach, showed that minor variations in critical quality attributes do not necessarily affect product performance. This underscores the need to establish scientifically justified acceptance criteria for each critical quality attribute of topical formulations. Moreover, permeation of an API through the skin depends on the characteristics of the vehicle, and many physicochemical properties of the API, including thermodynamic activity (solubility) and lipophilicity (Ilić et al., 2017).

While investigations to assess the relationship between rheological parameters and API release are warranted, a further complication during PE determination is that both the rheological parameters and API release can undergo variations throughout the shelf-life of the formulation (Mañez-Asensi et al., 2024).

The objectives of this study are (i) to analyze whether variations in rheological parameters, active ingredient release, and particle size throughout the shelf-life of a W/O hydrocortisone formulation affect the PE determination as per the EMA guideline; (ii) to determine the relationship between the evolution of the parameters assessed; and (iii) to evaluate whether a correlation exists between the evolution of the parameters analyzed and the composition of the formulation.

## 2. Material and methods

5 batches of a hydrocortisone buteprate 1 mg/g cream commercialized in the Spanish market were evaluated: Batches S002 (expiry: April 2024), S003 (expiry: May 2024), S005 (expiry: May 2024), S009 (expiry: July 2024) and S018 (expiry: December 2024). The formulation had a total shelf-life of 3 years and was an oil-external phase emulsion, containing stearyl alcohol, filmy petrolatum, liquid petrolatum, polysorbate 60, glyceryl monostearate, propylene glycol, methylparaben, butylparaben, citric acid and deionized water. The samples were stored between 20 and 25 °C.

The samples were analyzed 12, 24, and 36 months (end of shelf-life) after their manufacturing. At each analysis time, 12 replicates of each batch were evaluated to determine the parameters described below.

### 2.1. Rheological analysis

The measurements were performed with a controlled stress rheometer (Rheostress RS1 Thermo Scientific-Haake®, Karlsruhe, Germany), equipped with a thermostatic bath, using the RheoWin 4.0 software. The samples were kept at rest 600 s after loading to ensure stress relaxation and temperature equilibrium. Measurements (n = 12 replicates per batch) were performed at 20 °C (standard storage conditions) using serrated parallel plates (35 mm diameter, 1 mm gap). The below described rheological measurements were performed.

#### 2.1.1. Flow curves

Stepped flow curves were performed in controlled stress mode (60 s per step in logarithmic distribution). The shear stress interval (between 1 Pa and 400 Pa, in 20 steps) was chosen to obtain share rates ranging from very low values (approx. $10^{-5}\ s^{-1}$) to over $100\ s^{-1}$. The simplified Carreau model (Eq (1)) was successfully fitted to the viscosity values as a function of the shear rate data (Mezger, 2014) using Kaleida graph 4.03 software (Synergy Software):

$$\eta = \frac{\eta_0}{\left(1 + \left(\frac{\dot{\gamma}}{\dot{\gamma}_c}\right)^2\right)^s} \quad (1)$$

where $\eta_0$ is the zero-shear viscosity, $\dot{\gamma}_c$ is critical shear rate, and $s$ the shear thinning index related to the slope of the viscosity curve for high shear rates in double-log scale. The zero-shear viscosity ($\eta_0$) and the viscosity at $100\ s^{-1}$ ($\eta_{100}$) were calculated from the curve fits and used to compare batches.

#### 2.1.2. Small-Amplitude oscillation sweep tests

Oscillatory sweep tests were performed at 1 Hz, varying the stress amplitude from 3 to 70 Pa (20 steps in logarithmic distribution). The elastic and viscous moduli in the linear viscoelastic region (LVR) were determined by averaging the constant values of each modulus in the plateau region ($G_0$' and $G_0$"). The yield stress, $\sigma_{0,}$ was estimated as the stress value at which the G' deviated 10% respect from the constant value in plateau region of the LVR (5% tolerance).

### 2.2. Droplet size measurement

The size of the internal phase droplets was measured using an optical microscope Leica DMR (40x) image capture system DFC450C and LAS Software. Approximately 120 droplets were measured for each formulation batch and analysis time point (2 fields; 60 droplets per field).

### 2.3. In vitro release test

In vitro release tests (IVRT) through Tuffryn membranes (hydrophylic polyethersulfone 0.45 μm pore, 25 mm diameter, 140 μm thickness; PALL Corporation, Ann Arbor, MI, USA) were conducted according to the USP general chapter <1724>(21). Vertical Franz cells with an effective area available for diffusion of 0.79 $cm^2$ and a receptor compartment capacity of 6 mL (Vidrafoc, Barcelona, Spain) were used. The membrane's inertness to hydrocortisone and validity for these release assays were previously evaluated. Given the limited hydrocortisone water solubility, an ethanol/water (30:70) solution was used to both fill the receptor compartment and pre-soak the membranes for 30 min. The temperature was controlled at 32±1 °C at the membrane surface and the receptor solution was stirred at 200 rpm. An infinite dose (300 nominal mg) of hydrocortisone was uniformly applied in the donor compartment. Sink conditions were ensured during the assay.

6 samples per cell were collected and stored at −20 °C until analysis. Samples were taken at 0.5, 1, 2, 3, 4 and 6 h to ensure a steady state. The amount of drug released per unit membrane area was plotted versus the square root of time. The hydrocortisone release flux ($IVRT_{Flux}$) was calculated from the slope of this plot (SUPAC-SS: Nonsterile Semisolid Dosage Forms, 2025)using Microsoft Excel 365.

#### 2.3.1. Drug quantification

Hydrocortisone was quantified by high-performance liquid chromatography (HPLC) with UV detection at 245 nm. Samples were injected into a Brisa $LC^2$ C18 (Teknokroma) column (5 μm particle size, 4.6 × 150 mm). The mobile phase consisted of 65% acetate buffer (700 mM; pH 3.0) and 35% acetonitrile. The flow rate was 1 mL/min and the retention time was 4 min.

The samples' hydrocortisone concentration was calculated by interpolation in a calibration curve. The chromatographic method was validated as per ICH Q2(R2), assessing linearity, precision, accuracy, limit of detection and quantitation, specificity, interval and robustness (SUPAC-SS: Nonsterile Semisolid Dosage Forms, 2025).

### 2.4. Statistical analysis

Mean values and coefficients of variation (CV%) of each parameter were calculated. The Shapiro-Wilk test was used to determine whether the samples followed a normal distribution. Parameters not following a normal distribution were log-transformed for further comparison. The differences between batches and sampling times were calculated using an analysis of variances (ANOVA) test, followed by the post hoc Tukey test. The statistical significance level for these tests was set at $\alpha$=0.05.

The equivalence of the topical formulations was evaluated using the EMA criteria (EMA, 2024). The 90% CI of the geometric mean ratio (GMR) between the TP (longer time since manufacturing) and RP (shorter time since manufacturing) of each parameter was calculated. Parameters were considered equivalent (compliant) if the resulting 90% CI of the comparison was within the limits that apply depending on the variability of the parameter in the first-time analysis (12 months).

The overall variability between the different parameters was evaluated by a principal component analysis (PCA).

The R (V. 4.4.2) and R-Studio (V 1.4.1106) software were used.

## 3. Results and discussion

### 3.1. Parameter evolution during shelf-life

The rheological properties, the droplet size, and the active ingredient release of a hydrocortisone buteprate 1 mg/g cream were evaluated throughout shelf-life (Fig. 1 and Table 1) to assess its impact on the mean value, the dispersion and the declaration of PE. The batches used for this analysis were manufactured by the same company, with the same technical requirements and were approved for commercialization (i.e., they were considered PE).

The mean value of all parameter remained stable throughout the shelf-life (Fig. 1; Table 1).

The intra-batch and inter-batch variability for all parameters exceeded the conditions of high variability (CV >10%) in most cases. Each of the batches had an intra-batch variability of $\sigma_0$ and droplet size >20%, reaching 45% in the 12-month-old batch S018 and in the 24-month-old batches S003 and S018.

High variability has also been reported for some parameters of other semisolid formulations, regardless of their complexity. For example, O/W emulgel formulations had high variability in zero shear and 100 $s^{-1}$ viscosities, while, $G_0'$, $G_0''$, and $\sigma_0$ showed low variability (CV < 10%) (Mañez-Asensi et al., 2024; Pleguezuelos-Villa et al., 2019). A carbomer gel with dimethindene exhibited high variability in all parameters (>30% in most cases), whereas a diclofenac emulgel showed variability <10% in $G_0'$, $G_0''$, and $\sigma_0$ Miranda et al. (2022). High variability in most parameters was also reported for various O/W and W/O emulsions containing bifonazole (Miranda et al., 2023). Formulations similar to the hydrocortisone W/O emulsion evaluated in the present study, containing capsaicin (Navarro-Pujol et al., 2021), had very high intra-batch variabilities in viscosity, relative thixotropic loop area, complex modulus and delta tangent, being droplet size variability even higher than the one of hydrocortisone W/O emulsion (Table 1). Conversely, all parameters of an ointment with paraffin and petroleum jelly had a CV% <15% (Mangas-Sanjuán et al., 2019).

While these studies vary in their design and experimental set-up (with the exception of (Mañez-Asensi et al., 2024) and the present study), high variability seems to be inherent to the nature of dispersed systems, independently of their physicochemical nature, as observed for creams, gels, emulgels, W/O, and O/W emulsions. Variability of the raw materials used as excipients, which can lead to differences in the final product (Miranda et al., 2020), could be a determinant of inter-batch variability. While data to confirm this possibility are lacking, it is notable that the mean values for all rheological parameters of 1 batch (S002) were higher than those observed in other batches, (Fig. 1), similar to what had been observed for diclofenac emulgel (Mañez-Asensi et al., 2024).

To assess the consistency of parameters over time, a comparison was performed using the ANOVA test. The analysis showed that there were no statistically significant differences in the rheological parameters due to conservation time. Although mean values of particle size showed statistically significant differences over time (Table 2), there was no trend towards an increase in droplet size due to aggregation. In the case of IVRT parameters, while significant differences were observed between the 12-month and 24-month values and the 24-month and 36-month values, these differences were not significant between the 12-month and 36-month values (Table 2). Given the lack of a consistent trend in the change over time of these 3 parameters (particle size, release flux, and accumulated amounts in receptor), those differences might be due to technical reasons. The oily nature and high viscosity of the formulation pose difficulties in handling the sample and cannot be ruled out that might have resulted in areas of the Franz's cells membrane without product, although especial attention was given to this issue. Moreover, particle sizes were measured semi-manually, introducing another potential source of variability. Altogether, this suggests that despite the differences between some readings of droplet size and

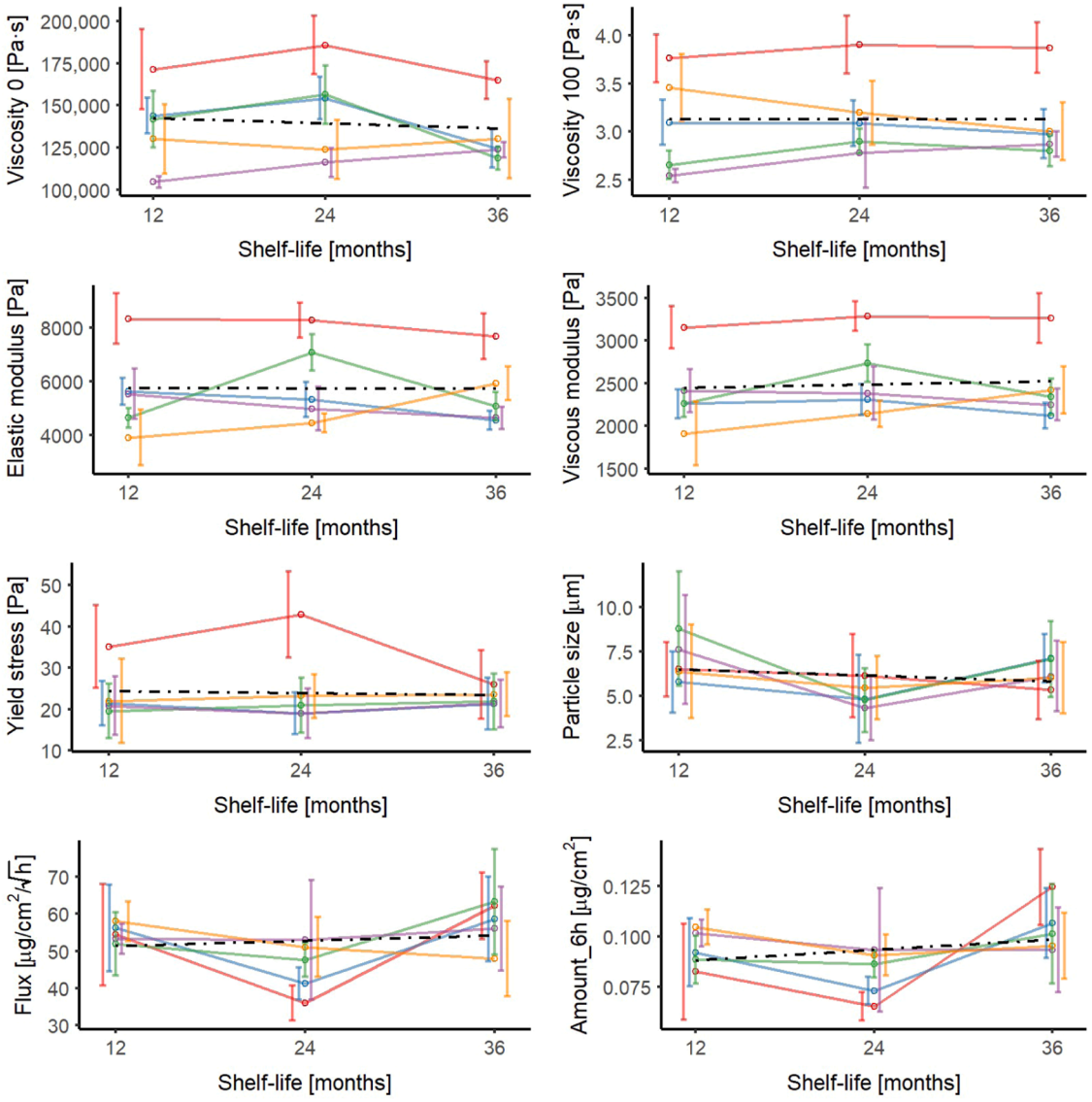


**Fig. 1.** Longitudinal evolution of parameters throughout shelf-life. The solid lines represent the mean value with standard deviation (n = 12) of each batch (red: S002, blue: S003, green: S005, purple: S009, orange: S018). The dashed black line corresponds to the mean value of all batches (n = 60).

release, none of the parameters analyzed for the hydrocortisone W/O emulsion varied during the validity period.

Variations throughout the shelf-life of a product might be due to the onset of coalescence, as suggested by the increase in the particle size, associated with a decrease in both $\eta_0$ and $\eta_{100}$, and, to an even higher extent, the $IVRT_{Flux}$ and $A_{6h}$ throughout the shelf-life of diclofenac emulgel (Mañez-Asensi et al., 2024). Other studies observing variations in rheological parameters throughout a product's shelf-life, have not or could not associate these variations with changes in API release. Viscosity, yield stress, and complex modulus values of a capsaicin W/O emulsions decreased over a 10-months period, but this study did not evaluate API release (Navarro-Pujol et al., 2021). Moreover, changes in the rheological parameters of various bifonazole creams could not be associated with changes in API release (Miranda et al., 2023)

### 3.2. Impact of time in the equivalence test proposed by the EMA

The intra-batch equivalence over time was evaluated using the 90% CI limit established in the final EMA guidance (EMA, 2024). As shown in Table 2, all rheological parameters met the limits for the 90% CIs, while particle size, $IVRT_{Flux}$, and $A_{6h}$ showed equivalence in only some of the comparisons. Particle size was considered equivalent in 2 of the 3 comparisons, while $IVRT_{Flux}$ and $A_{6h}$ were considered equivalent only in 1 comparison.

While the draft of the EMA guideline established a fixed limit of 10% in the 90% CI to consider equivalence (EMA, 2018), the final guideline version extends the limits from 90–111% (for RP parameters with a CV ≤10%) to up to 80–125% for parameters with higher CV, with scaling for intermediate situations ([U, L] = exp [±k·sR], where U is the upper limit of the acceptance range, L is the lower limit of the acceptance range, k is the regulatory constant set to 1.056, and sR is the standard deviation of the log-transformed values of the reference product parameters (EMA, 2024).

The suitability of the revised EMA equivalence criteria was assessed by reevaluating the equivalence of parameters over time within the same diclofenac emulgel product, originally evaluated as per the EMA draft criteria (Mañez-Asensi et al., 2024). Only the parameters $\eta_0$ and droplet size showed a CV > 10% in the first time point of analysis, allowing for an extension of the 90% CI limits. However, this extension of limits would not change the equivalence decision for any of these parameters.

The demonstration of equivalence of all rheological parameters as

**Table 1**
Mean value of parameters and intra-batch (n = 12) and inter-batch (n = 5) of indicated parameters, expressed as variation coefficient in percentage (CV%), in samples analyzed 12, 24, and 36 months after manufacturing. $\eta_0$: Zero-Shear viscosity, $\eta_{100}$: viscosity at 100 $s^{-1}$, $G_0$': elastic modulus in LVR, $G_0$'': viscous modulus in LVR, $\sigma_0$: yield stress, IVRT: in vitro release test, $A_{6h}$: total amount released at the end of 6 h IVRT.

| Parameter | Time (months) | Mean value (CV%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | S002 | S003 | S005 | S009 | S018 | Inter batch |
| $\eta_0$ (Pa·s) | **12** | **172,292** | **145,701** | **143,509** | **103,838** | **127,668** | **138,602** |
| | | (13.7) | (7.2) | (11.5) | (3.2) | (15.9) | (18.1) |
| | **24** | **186,150** | **152,503** | **151,530** | **116,532** | **124,763** | **146,295** |
| | | (9.3) | (8.2) | (11.3) | (7.3) | (14.0) | (18.7) |
| | **36** | **166,987** | **128,082** | **121,267** | **122,969** | **136,275** | **135,116** |
| | | (6.6) | (8.8) | (5.5) | (3.6) | (17.2) | (13.8) |
| $\eta_{100}$ (Pa·s) | **12** | **3.82** | **3.09** | **2.67** | **2.54** | **3.45** | **3.11** |
| | | (6.4) | (7.5) | (5.4) | (2.6) | (10.3) | (17.1) |
| | **24** | **3.88** | **3.15** | **2.87** | **2.66** | **3.19** | **3.15** |
| | | (7.7) | (7.5) | (4.5) | (13.5) | (10.4) | (14.6) |
| | **36** | **3.78** | **3.01** | **2.78** | **2.87** | **3.07** | **3.10** |
| | | (6.8) | (8.4) | (5.7) | (4.5) | (9.6) | (2.3) |
| $G_0$' (Pa) | **12** | **8349** | **5693** | **4767** | **5619** | **4340** | **5754** |
| | | (11.2) | (8.8) | (7.4) | (16.4) | (23.8) | (27.1) |
| | **24** | **8517** | **5258** | **7134** | **5099** | **4427** | **7316** |
| | | (7.6) | (12.5) | (9.4) | (15.8) | (7.9) | (27.7) |
| | **36** | **7802** | **4484** | **5119** | **4707** | **6019** | **5626** |
| | | (10.8) | (7.5) | (10.3) | (8.7) | (10.3) | (23.9) |
| $G_0$'' (Pa) | **12** | **3137** | **2296** | **2305** | **2450** | **2048** | **2447** |
| | | (7.9) | (7.3) | (6.9) | (10.2) | (18.1) | (16.8) |
| | **24** | **3313** | **2302** | **2772** | **2416** | **2121** | **2585** |
| | | (5.2) | (7.9) | (7.9) | (12.8) | (7.2) | (18.2) |
| | **36** | **3305** | **2110** | **2398** | **2261** | **2472** | **2509** |
| | | (8.7) | (7.0) | (8.9) | (8.2) | (11.1) | (18.5) |
| $\sigma_0$ (Pa) | **12** | **34.72** | **21.98** | **20.54** | **22.58** | **22.52** | **24.4** |
| | | (28.7) | (24.2) | (32.0) | (31.4) | (45.2) | (23.6) |
| | **24** | **41.76** | **20.72** | **23.77** | **19.21** | **21.42** | **25.3** |
| | | (24.9) | (24.4) | (27.9) | (31.4) | (31.4) | (36.6) |
| | **36** | **28.19** | **20.10** | **21.61** | **22.44** | **23.75** | **23.22** |
| | | (29.6) | (31.3) | (31.3) | (25.7) | (22.2) | (13.2) |
| Droplet size (μm) | **12** | **6.52** | **6.12** | **9.03** | **8.17** | **6.64** | **7.29** |
| | | (23.6) | (28.0) | (35.6) | (37.5) | (39.4) | (17.0) |
| | **24** | **6.70** | **5.47** | **5.09** | **4.77** | **5.68** | **5.55** |
| | | (34.8) | (45.2) | (35.3) | (38.1) | (31.4) | (13.2) |
| | **36** | **5.39** | **7.19** | **7.35** | **6.21** | **6.50** | **6.54** |
| | | (31.0) | (18.8) | (28.9) | (32.1) | (30.8) | (12.1) |
| $IVRT_{Flux}$ ($\mu g/cm^2/\sqrt{h}$) | **12** | **53.98** | **58.03** | **53.75** | **54.43** | **57.53** | **55.54** |
| | | (25.3) | (20.0) | (15.8) | (7.4) | (9.2) | (3.6) |
| | **24** | **35.95** | **40.13** | **47.69** | **49.57** | **51.37** | **44.94** |
| | | (13.2) | (10.7) | (9.6) | (32.5) | (15.6) | (14.6) |
| | **36** | **62.21** | **62.95** | **59.71** | **51.83** | **49.68** | **59.17** |
| | | (14.3) | (18.0) | (23.7) | (21.8) | (20.3) | (8.5) |
| $A_{6h}$(μg) | **12** | **0.080** | **0.093** | **0.088** | **0.100** | **0.105** | **0.093** |
| | | (29.6) | (17.9) | (13.2) | (6.8) | (8.2) | (10.2) |
| | **24** | **0.065** | **0.071** | **0.086** | **0.091** | **0.091** | **0.081** |
| | | (10.8) | (9.6) | (7.8) | (33.0) | (11.0) | (14.8) |
| | **36** | **0.124** | **0.111** | **0.110** | **0.093** | **0.092** | **0.106** |
| | | (15.1) | (15.5) | (22.2) | (22.4) | (17.6) | (11.5) |

per the revised EMA criteria in the present study, together with the studies failing to confirm intra-batch equivalence as per the draft criteria (Lourenço et al., 2024; Mangas-Sanjuán et al., 2019; Mañez-Asensi et al., 2024; Miranda et al., 2023; Miranda et al., 2022; Navarro-Pujol et al., 2021; Pleguezuelos-Villa et al., 2019), indicate that the revised criteria is better suited for topical formulations. However, given the intra-batch differences throughout the shelf-life of some complex formulations (Miranda et al., 2023; Miranda et al., 2022; Mañez-Asensi et al., 2024), the anticipated increase in variability when batches of different ages are combined, might require to further extent the 90% CI limit.

### *3.3. Principal component analysis (PCA) and stability of parameters during shelf-life*

A PCA was performed to identify relationships among parameters and understand the behavior of this formulation throughout its shelf-life (Fig. 2). A two-dimensional plot accounted for 77.88% of the total variance. The first dimension (57.7% of the total variance) positively related to droplet size (all batches, except batch S002) and positively related to rheological parameters. The second dimension (20.81% of the total variance) positively related to $IVRT_{Flux}$ and $A_{6h}$.

The rheological parameters closely related to each other, as shown by the very narrow angles between them. The 90-degree angle formed between all rheological parameters and the release parameters ($IVRT_{Flux}$ and $A_{6h}$) demonstrate the absence of a relationship between these 2, while a slight but probably irrelevant relationship between droplet size and IVRT and $A_{6h}$ was observed.

Other PE studies of topical products have suggested that rheological parameters may not influence the release of the active ingredient (Miranda et al., 2023; Mañez-Asensi et al., 2024; Pleguezuelos-Villa et al., 2019) The PCA of diclofenac emulgel (Mañez-Asensi et al., 2024) and of hydrocortisone W/O emulsion (Fig. 2), performed under very similar conditions, showed comparable relationships. The main difference between those PCAs is the relationship between droplet size and release parameters (IVRT, $A_{6h}$), which was only observed for diclofenac emulgel (inverse correlation). In the emulgel, the release of diclofenac

**Table 2**
90% confidence interval of the difference of means of the parameters for the comparisons done (12 vs. 24, 12 vs. 36, and 24 vs. 36 months). NC = not compliant equivalence as per the EMA criteria; C =compliant equivalence as per the EMA criteria. $\eta_0$: Zero-Shear viscosity, $\eta_{100:}$ viscosity at 100 $s^{-1}$, $G_0$': elastic modulus in LVR, $G_0$'': viscous modulus in LVR, $\sigma_0$: yield stress, IVRT: in vitro release test, $A_{6h}$: total amount released at the end of 6 h IVRT.* Statistical differences as per the ANOVA post hoc Tukey tests comparing parameters over time ($p < 0.05$).

| Parameter | Compared groups | EMA CI 90% | Final EMA criteria | Draft EMA criteria |
|---|---|---|---|---|
| **$\eta_0$** | **12 vs. 24** | 105.68 (99.53–112.20) | C | **NC** |
| | **12 vs. 36** | 98.32 (93.17–103.75) | C | C |
| | **24 vs. 36** | 93.03 (88.28–98.05) | C | **NC** |
| **$\eta_{100}$** | **12 vs. 24** | 101.18 (98.26–106.36) | C | C |
| | **12 vs. 36** | 100.09 (95.67–104.72) | C | C |
| | **24 vs. 36** | 98.93 (94.64–103.41) | C | C |
| **$G_0'$** | **12 vs. 24** | 105.89 (97.7–114.76) | C | **NC** |
| | **12 vs. 36** | 96.80 (87.57–107.01 | C | **NC** |
| | **24 vs. 36** | 93.32 (86.67–100.49) | C | **NC** |
| **$G_0$''** | **12 vs. 24** | 105.59 (100.5–111.44) | C | **NC** |
| | **12 vs. 36** | 102.50 (97.15–108.14) | C | C |
| | **24 vs. 36** | 97.07 (91.98–102.44) | C | C |
| **$\sigma_0$** | **12 vs. 24** | 102.3 (91.39–114.56) | C | **NC** |
| | **12 vs. 36** | 96.80 (87.57–107.01) | C | **NC** |
| | **24 vs. 36** | 94.60 (85.08–105.20) | C | **NC** |
| **Droplet size** | **12 vs. 24*** | **75.68 (73.12–78.32)** | **NC** | **NC** |
| | **12 vs. 36*** | 87.58 (84.78–90.48) | C | **NC** |
| | **24 vs. 36*** | 115.73 (111.97–119.63) | C | **NC** |
| **$IVRT_{Flux}$** | **12 vs. 24*** | **79.77 (74.82–85.04)** | **NC** | **NC** |
| | **12 vs. 36** | 102.02 (95.97–108.45) | C | C |
| | **24 vs. 36*** | **127.9 (118.99–137.46)** | **NC** | **NC** |
| **$A_{6h}$** | **12 vs. 24*** | **86.30 (80.52–92.48)** | **NC** | **NC** |
| | **12 vs. 36*** | 113.29 (105.99–121.08) | C | NC |
| | **24 vs. 36*** | **131.27 (122.62–140.54)** | **NC** | **NC** |

was reduced by 50% between the first months of the shelf-life and the last month of the shelf-life, while the droplet size increased remarkably in the first few months, along with a decrease in viscosity. This suggests that droplet aggregation within the diclofenac formulation caused these variations. By contrast, droplet aggregation does not seem to occur in the W/O hydrocortisone emulsion, as evidenced by the stability of particle size, viscosity, and active ingredient release throughout shelf-life. This sustained stability in droplet size throughout the shelf-life might be attributable to the excipients in the external phase of the hydrocortisone W/O emulsion. Petrolatum, sorbitan monostearate, and, especially, glycerol monostearate are fatty excipients that provide high viscosity to the formulation. The exact composition of the formulation analyzed is unknown for the authors, but based on the National Formulary of the Spanish Agency for Medicines and Health Products (AEMPS), within O/W and W/O emulsions, the proportion of petrolatum

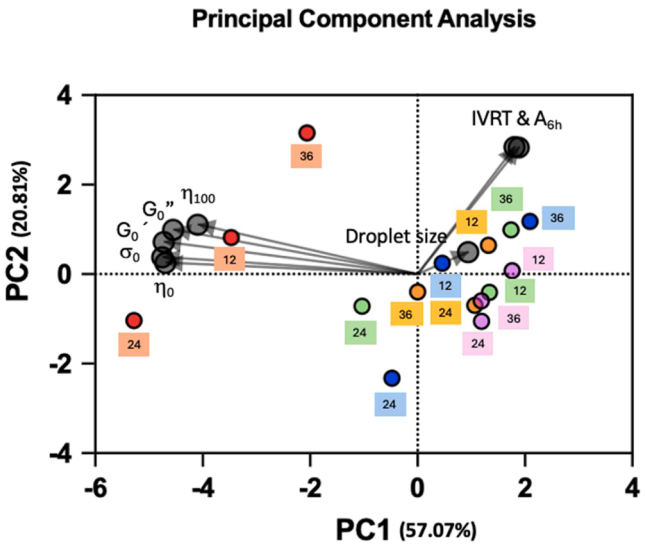


**Fig. 2.** Principal component analysis (PCA) of the parameters studied. $\eta_0$: Zero-Shear viscosity, $\eta_{100:}$ viscosity at 100 $s^{-1}$, $G_0$': elastic modulus in LVR, $G_0$'': viscous modulus in LVR, $\sigma_0$: yield stress, IVRT: in vitro release test flux, $A_{6h}$: total amount released at the end of 6 h IVRT. Red: Batch S002, Blue: Batch S003, Green: Batch S005, Purple: Batch S009, Orange: Batch S018.

ranges from 5 to 56%, of glycerol monostearate from 2.5 to 15%, and of sorbitan monostearate from 1 to 15% (Agencia Española de Medicamentos y Productos Sanitarios, 2023; Uvanesh et al., 2016).

Glycerol monostearate is a solid with a waxy consistency common excipient widely used in the pharmaceutical industry as an emulsifier, moisturizer or to increase the solubility of hydrophobic drugs (Barnes et al., 2021). In a quality by design approach study, the proportions of glycerol monostearate ranged from 5 to 20% (Simões et al., 2020). Glycerol monostearate has been classified as a "critical material attribute" (Simões et al., 2020), due to its significant influence on particle size, $IVRT_{flux}$, and rheological properties (increasing $G_0$' and $\sigma_0$ in LVR), which is consistent with stronger intermolecular interactions and greater physical stability. Along these lines, high proportions of glycerol monostearate increase viscosity and reduce interfacial tension, conferring formulations with high physical stability (Barnes et al., 2021; Simões et al., 2020; Simões et al., 2020). Accordingly, this excipient would lower droplet mobility in the formulation of the present study and, thus, the probability of coalescence. This is also consistent with the postulates of Stokes' law as reviewed in (Herbig et al., 2023), showing a relationship between a high value of $\eta_0$ and greater long-term stability of topical formulations. Since the values of both viscosity and all viscoelastic parameters are 10 to 20 times higher in the hydrocortisone W/O emulsion than in the diclofenac emulgel, altogether this would explain the greater stability of droplet size and active ingredient release throughout the shelf-life of the hydrocortisone W/O emulsion compared to the diclofenac O/W emulsion and how its physical properties protect it from coalescence.

## 4. Conclusions

This study demonstrates that the rheological parameters evaluated for the W/O hydrocortisone formulation remain stable throughout the shelf-life. While the mean value of the parameters did not change over time, they showed great intra- and inter-batch variability. Equivalence between parameters could be concluded with the extended 90% CI limits of the final EMA guideline (EMA, 2024), which would not have been met with the limits proposed in the initial draft (EMA, 2018).

Based on these results, extending the 90% CI limit is more appropriate, given the high variability inherent in complex dispersed systems. However, a wider range (e.g., 75–133% in the mean ratio) might allow

to account for differences in batch age or slight differences in the raw materials' rheological properties.

Moreover, irrespective of the extent to which the variations in rheological parameters comply with the EMA CI criteria, the PCA suggests that the rheological parameters determined may be redundant and consequently, the number of parameters to be analyzed could be diminished. Nevertheless, PCA should not be regarded as a definitive test; rather, it is merely an additional analytical tool for the study of our parameters.

Even though a weak correlation between drug release and rheological parameters has been observed, high values for both viscosities and viscoelastic moduli are associated with stable drug release rates throughout the product's shelf life. Taking into account that there are numerous other underlying factors that have the potential to influence the release of the active ingredient, further research is warranted to determine the relevance of rheological parameters on the bioequivalence of topical products.

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## CRediT authorship contribution statement

**Andreu Mañez-Asensi:** Writing – original draft, Methodology, Investigation, Formal analysis. **Mª Jesús Hernández:** Writing – review & editing, Validation, Resources, Funding acquisition, Formal analysis. **Víctor Mangas-Sanjuán:** Writing – review & editing, Formal analysis. **Ana Salvador:** Writing – review & editing, Formal analysis. **Matilde Merino-Sanjuán:** Writing – review & editing, Writing – original draft, Validation, Resources, Formal analysis, Conceptualization. **Virginia Merino:** Writing – review & editing, Writing – original draft, Validation, Resources, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare no conflicts of interest.

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## Data availability

Data will be made available on request.

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