In Vitro Release Testing (IVRT) of Topical Hydrocortisone Acetate Creams: A Novel Approach Using Positive and Negative Controls

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ABSTRACT

The objective was to develop and validate an in vitro release testing (IVRT) method to assess the release of hydrocortisone acetate (HCA) from five topical formulations. A marketed generic cream containing 1% HCA was used as the reference product. Vertical diffusion cells (VDCs) were used to assess and compare the release rates of HCA from cream formulations containing 0.5%, 1%, and 1.5% HCA. The study describes a novel approach to test the discriminatory power by including both positive and negative controls to declare pharmaceutical equivalence or inequivalence. The validated method was found to be sensitive, linear, precise, reproducible, robust, and selective for the analysis of HCA from topical cream products. Equivalence or inequivalence was established based on SUPAC-SS acceptance criteria using a 90% CI with limits of 75–133.33%. The IVRT method was shown to have discriminatory ability to appropriately measure significant differences in drug release from various cream formulations. This approach also provides useful information for the future development of acceptable IVRT methods to assess topical dosage forms for local action containing different drugs.

INTRODUCTION

Since an in vitro release rate can reflect numerous and combined effects of several physical and chemical parameters, including solubility, particle size of the active pharmaceutical ingredient (API) and rheological properties of the dosage form, in vitro release testing (IVRT) has been recommended by the United States Food and Drug Administration (US FDA) as a test to assess pharmaceutical equivalence/inequivalence between pre- and post-approval product changes (1, 2). The utility of IVRT was extended in 2012 when the US FDA published a draft guidance for assessing bioequivalence (BE) of acyclovir topical ointment, which was the first such publication recommending IVRT for use as a waiver of BE studies for a locally acting topical product (3). This particular guidance provided useful and promising information for the future application of IVRT as a method for assessment of the sameness of topical formulations intended for local action. Subsequently, the US FDA has published several draft guidances using in vitro methods for biowaivers for topical products (3–8). In addition, a recent draft guideline published by the European Medicines Agency (EMA) also makes provision to use IVRT for the approval of generic products (9). However, a comprehensive validation of the IVRT method is imperative to ensure that the resulting method has the requisite attributes of sensitivity, precision, selectivity, and reproducibility necessary to detect differences relating to qualitative (Q1) and quantitative (Q2) properties and the microstructure and arrangement of matter (Q3) between products (10, 11).

In light of the above, an IVRT method was developed and validated to assess creams containing 1% hydrocortisone acetate (HCA). Two marketed products containing 1% HCA and three additional creams specifically manufactured to contain 0.5%, 1%, and 1.5% HCA were studied. A positive control was included to ensure that the method had the necessary capability to confirm sameness, and negative controls ensured the method had the requisite discriminatory power to detect significant differences.

MATERIALS AND METHODS

Chemicals and Formulations

Hydrocortisone acetate (HCA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC) grade acetonitrile was obtained from Romil Ltd (200 UV ROMIL-SpS Super Purity Solvent, Waterbeach, Cambridge, UK) and ethanol (95%) was purchased from Merck Laboratories (Wadeville, Gauteng, South Africa). The water used for chromatography was...
initially purified by reverse osmosis followed by filtration through a Milli-Q system (Millipore, MA, USA); the water purification system consisted of a Milli-Q Academic A10 with a Quantum EX Ultrapure Organex Cartridge equipped with Q-GARD 1 Progard pretreatment packs. Mylocort cream (Aspen Pharmacare Holdings Limited, South Africa, batch 116740) and Biocort cream (Akacia Al Pharm, South Africa, batch H24), which are approved, commercially available HCA products, were purchased from local pharmacies. Topical products containing HCA were specially manufactured for use as test products and were identified as cream A (1%), cream B (0.5%), and cream C (1.5%).

Reversed-Phase (RP) High-Performance Liquid Chromatography (HPLC)

The HPLC system consisted of a Waters Alliance Model 2690 separations module equipped with a 2996 photodiode array (PDA) detector and Empower 3 chromatography data software (Waters Corp., CT, USA). The chromatographic separation was achieved using a Luna C18(2) column (5 μm silica, 150 × 4.6 mm internal diameter; Phenomenex, Inc., CA, USA), and the injection volume was 10 μL. The concentrations of HCA were determined by reversed-phase high-performance liquid chromatography (RP-HPLC) using a mobile phase of acetonitrile and water (50/50 v/v, adjusted with 0.1% phosphoric acid) pumped at a flow rate of 0.8 mL/min, and the eluate was monitored at a wavelength of 242 nm. Samples were injected at ambient temperature during analysis.

The RP-HPLC method was validated according to the International Council for Harmonization (ICH) guidelines and relevant criteria as described by Tiffner et al. (10, 11).

Membrane Screening

Membrane screening was carried out using various synthetic membranes such as Magna Nylon (0.45 μm, 25 mm; GVS Life Sciences, USA), HT Tuffryn (0.45 μm, 25 mm; Pall Corporation, MI, USA), cellulose acetate (0.45 μm, 25 mm; Sartorius AG, Göttingen, Germany), fluoropore polytetrafluoroethylene (PTFE) (0.2 μm, 25 mm; Merck Millipore Ltd., Ireland), and Strat-M polyethersulfone (PES) (25 mm; Millipore). Binding of HCA was investigated on the five membranes. Each membrane was immersed in 10 mL of the test solution containing 200 μg/mL of HCA in the receptor medium of ethanol and water (55/45 v/v) at 32 ± 1 °C for 6 hours. The maximum possible concentration assuming 100% release of HCA from the applied dose into the receptor medium was 250 μg/mL. Hence, 80% of the highest possible concentration of HCA (i.e., 200 μg/mL) observed during IVRT was selected for membrane binding studies based on the assumption that the higher concentration of HCA will be able to determine which membrane may result in significant (> 5%) HCA binding. A duplicate HCA solution without the membranes was also evaluated as the control. The solutions were analyzed using RP-HPLC after 6 hours. The recovered amount of HCA was calculated relative to the control solution to determine if there was a decrease in HCA content. A decrease in the concentration of HCA in a particular solution meant that there was binding of drug to membrane, indicating the unsuitability of the membrane.

HCA Solubility and Receptor Fluid Selection

The solubility of HCA in receptor fluid was investigated using a six-cell vertical diffusion cell (VDC) assembly. HCA was dissolved in six different solutions containing varying percentages of ethanol/water, ethanol/normal saline, and ethanol/phosphate buffer at pH 5.8 (70/30 and 50/50 v/v, of each mixture). An additional receptor medium consisting of ethanol/water (55/45 v/v) was also investigated. Excess amounts (~60 mg) of HCA were weighed out and added to the respective solutions and stirred for 6 hours at ~500 rpm and left to stand overnight. These tests were performed at 32 ± 1 °C, and all the openings (cell tops and arms) were closed with Parafilm-M to prevent loss of solvent. Samples of the supernatant were withdrawn, diluted, and analyzed by RP-HPLC.

Vertical Diffusion Cell and Assembly

In vitro release studies were performed using six vertical cells (1.767 cm² diffusional surface area) mounted on a six-station diffusion apparatus equipped with individual stirrer motors, and the cells were connected to a heated water circulator (Grant Instruments Ltd, Shepreth, Cambridge, UK). The diffusion cells and apparatus were assembled with donor and receptor chambers separated by the selected synthetic membrane.

Apparatus Qualification

Factors affecting drug release were assessed to satisfy the requirements of an apparatus qualification test based on the United States Pharmacopeial Convention (USP) dissolution toolkit procedures for mechanical calibration and performance verification testing (PVT) for Apparatus 1 and 2 (1, 12, 13). Environmental conditions such as suitable working area and workbench levelness, exposure to direct sunlight and direct cooling vents, capacity of each VDC, diameter of the VDC orifice, temperature of the receptor medium, stirring speed, mass of the magnetic stirrers, and dispensed sample volume were assessed.
Performance Verification Test (PVT)
The PVT was performed using 1% hydrocortisone cream (Emo-Cort, GlaxoSmithKline Inc., ON, Canada) applied on Tuffryn membranes (13, 14). The receptor fluid consisted of ethanol and water (70/30 v/v), which was held at a constant temperature of 32 ± 1 °C and stirred at 500 rpm. The membranes were pretreated by immersion in the receptor fluid for 30 minutes. Samples of receptor medium were collected at 0.5, 1, 2, 3, 4, and 6 hours after application of 300 mg of Emo-Cort to each of the six VDCs, and the experiments were conducted in duplicate. Separation was performed using acetonitrile and water (30/70 v/v) on a Luna C18(2) column (5 μm silica, 150 × 4.6 mm internal diameter) maintained at 25 °C. The flow rate was set to 1.0 mL/min, and detection was at 254 nm.

Validation of In Vitro Release Testing (IVRT) method
The IVRT system was validated by assessing membrane inertness, solubility of HCA in receptor fluid, linearity, sensitivity, specificity, selectivity, precision, robustness, and recovery in accordance with the method described by Tiffner et al. (11). A placebo cream was used for the validation of the RP-HPLC method, and Mylocort cream 1%, being a national market leader product approved in South Africa, was used as the reference HCA cream (R), because a nationally approved innovator product was not available (15). Formulations of creams containing 0.5%, 1%, and 1.5% HCA were specially manufactured for the purposes of testing the sensitivity, selectivity, and specificity of the IVRT method. Understanding and defining the release of an API from semisolid formulations is a critical aspect of product development. The objective of this project was to develop and validate an IVRT method to assess the release and diffusion of HCA from different strengths of cream formulations intended for local action and to link the differences in release rates to formulation factors.

IVRT of Creams Containing HCA
The studies were conducted in accordance with the US FDA’s scale-up and post-approval changes-semisolids (SUPAC-SS) guidance. Mylocort cream 1% (~300 mg) was applied to Tuffryn membranes mounted on each cell. The donor compartments were then covered with Parafilm-M sealing film to prevent evaporation of vehicle and ensure integrity of the formulations throughout the respective study periods. The receptor chamber was filled with 12.0 mL of ethanol/water solution (55/45 v/v) and maintained at 32 °C. The receptor solutions were continuously stirred at 500 rpm using individual magnetic stirrer bars (10 × 2.5 mm) in each VDC. Syringes (1 mL) with sampling needles were purchased from local pharmacies. An analytical balance (AE 163, Mettler Inc., Zurich, Switzerland) was used for weighing standards and samples. An electronic pipettor (Xplorer, Eppendorf AG, Hamburg, Germany) was used to transfer standard and sample solutions for dilutions. Table 1 depicts the parameters used during IVRT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Diffusional Surface Area</td>
<td>1.767 ± 0.1 cm²</td>
</tr>
<tr>
<td>Average Receptor Volume</td>
<td>12.0 ± 0.1 mL</td>
</tr>
<tr>
<td>Temperature</td>
<td>32 ± 1 °C</td>
</tr>
<tr>
<td>Membranes</td>
<td>Tuffryn</td>
</tr>
<tr>
<td>Receptor Medium</td>
<td>Ethanol/water: 55/45 v/v</td>
</tr>
<tr>
<td>Dose</td>
<td>~300 mg</td>
</tr>
<tr>
<td>Sampling Time</td>
<td>0.5, 1, 2, 3, 4, 5, and 6 h</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>200 μL</td>
</tr>
<tr>
<td>Sample Analysis</td>
<td>RP-HPLC with PDA detection</td>
</tr>
</tbody>
</table>

VDC, vertical diffusion cell; IVRT, in vitro release testing; HCA, hydrocortisone acetate; RP-HPLC, reversed-phase high-performance liquid chromatography; PDA, photo diode array

Calculation of Release Rates
Based on the periodical concentrations of HCA that were measured using RP-HPLC with PDA detection, the release rates were calculated using the Higuchi model which assumes perfect sink conditions, as depicted in the equation below. Dilution of the receptor medium due to replacement of the sampled amount was taken into account, and the concentrations of HCA in the receptor medium at different sampling times were calculated using the equation:

$$ Q_n = C_n \frac{V_c}{A_c} + \frac{V_s}{A_s} \sum_{i=1}^{n} C_{i-1}, $$

where $Q_n$ is the amount of drug released per unit area at each time ($n$) (µg/cm²); $C_n$ is concentration of drug in receptor medium at different sampling times (n) (µg/cm³); $V_c$ is volume of cell (cm³); $A_c$ is area of the orifice of cell (cm²); and $V_s$ is volume of the sample (cm³).

The release rate corresponds to the slope of the regression line of the plot of $Q_n$ versus square root of time. $Q_n$ is affected by sample volume, VDC volume, and by the diameter of the orifice of the VDC. Consequently, the dimensions of these parameters were verified during apparatus qualification.

Comparative IVRT of HCA from Five Topical Cream Products
The validated IVRT method for the analysis of topical
HCA creams was applied to commercially available creams as well as a formulated 1% cream. These creams were assessed in accordance with SUPAC-SS guidance published by the US FDA (1). Two cream products containing 1% HCA, Mylocort and Biocort creams, which are commercially available in South Africa, were included in this investigation. To test if the IVRT method had the necessary ability to determine both sameness and differences, Mylocort was compared to itself as the positive control, and three creams specifically manufactured to contain 1%, 0.5%, and 1.5% HCA (creams A, B, and C, respectively) were also included in these studies. The creams containing 0.5% and 1.5% HCA were used in the validation and served as negative controls. Pairwise comparisons of the release rates between the two commercially available products and between the specially manufactured cream containing 1% HCA versus Mylocort were determined.

**Statistical Analysis**
The statistical approach that was used to perform the sameness test is described in the USP general chapter <1724> (16). This test is based on the Wilcoxon Rank Sum/Mann-Whitney test, which requires computation of a 90% confidence interval (CI) (17). The release rates of the reference formulation (R) and each of the HCA test formulations (T1–T5) were used to calculate T/R ratios. A total of 36 T/R ratios were calculated using a combination of the six release rates of T1–T5 and R. A list of the 36 T/R ratios was sorted from the lowest to the highest number. The 90% CI was subsequently determined from the list of T/R ratios, whereby the 8th and the 29th ratio was set as the lower and upper limit, respectively. The predetermined criterion for equivalence is that the range of the 90% CI should be within 75%–133.33%.

**RESULTS AND DISCUSSION**

Validation of The HPLC Method and Qualification of the IVRT System
All validation parameters for both the HPLC method and IVRT system successfully met the predefined acceptance criteria described by Tiffner et al., as follows (11):

- **Concentration range** - 5, 10, 50, 100, and 200 µg/mL were used as calibrators
- **Selectivity** - differences in retention times with and without the matrix were less than 10%
- **Specificity** - the placebo extract did not show any interfering peaks
- **Linearity** - $R^2 \geq 0.999$

- **Accuracy** – Coefficient of variation (CV) less than 5% using at 20, 100, and 180 µg/mL
- **Inter- and intra-run precision and robustness** - CV less than 5%
- **Sample stability** - benchtop (24 ± 1 °C), HPLC sample tray (21 °C), and refrigerator (4 ± 1 °C) for 9 days
- **Lower limit of quantification (LLOQ) and limit of detection (LOD)** - 5 and 0.24 µg/mL, respectively

Membrane Screening and Receptor Fluid Selection
The inertness of various membranes was investigated. Average percentage recoveries of 99.8%, 98.6%, 99.2%, 98.6%, and 99.2% for Tuffryn, cellulose acetate, fluoropore, nylon, and Strat-M, respectively, indicate that the above-mentioned membranes exhibit low HCA binding capacity. The membranes are therefore confirmed to be inert and do not act as a rate-limiting barrier for HCA. Because of its higher percentage recovery and availability, Tuffryn was chosen as the membrane of choice for this study. Solubility studies were conducted in triplicate. The receptor medium that had the most favorable outcome was ethanol:water (55/45 v/v), where the mean HCA solubility was 4447.4 µg/mL (CV = 6.38%). The latter concentration was more than 10 times the maximum expected concentration, as recommended (18).

Performance Verification Test
Using a commercially procured 1% hydrocortisone cream (Emo-Cort), marketed in Canada, the performance of the IVRT system was tested and the results obtained are summarized in Table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acceptance Criteria</th>
<th>Results</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Run Variability</td>
<td>Intra-run CV for Run 1 (n = 6): &lt; 15%</td>
<td>13.83%</td>
<td>Pass</td>
</tr>
<tr>
<td></td>
<td>Intra-run CV for Run 2 (n = 6): &lt; 15%</td>
<td>12.08%</td>
<td>Pass</td>
</tr>
<tr>
<td>Inter-Run Variability</td>
<td>Inter-run CV for both runs (n = 12): &lt; 15%</td>
<td>11.33%</td>
<td>Pass</td>
</tr>
<tr>
<td>Product Sameness Test</td>
<td>90% CI: 75%–133%</td>
<td>89.60%–121.63%</td>
<td>Pass</td>
</tr>
</tbody>
</table>

PVT, performance verification test; CV, coefficient of variation; CI, confidence interval

Validation of IVRT Method
In accordance with the SUPAC-SS guidance, 18 resulting release rates for each VDC were calculated using linear regression (1). If the release of HCA from its formulation follows the Higuchi equation, the amount released per unit area should be linear with respect to the square...
root of time. An $R^2$ value greater than 0.9 was considered acceptable to demonstrate linearity. The mean release rate and the variance components (inter-run and intra-run) were used to calculate the CV. The resultant 18 slopes from three runs (n = 6) were used to calculate intra-run variability, and the mean release rate from each run (n = 3) was used to calculate inter-run variability. A CV of less than 15% indicated acceptable precision and reproducibility (11). Inter-run and inter-run variability were 2.64 and 0.30, with mean release rate of 28.33 and 28.11 mg/cm$^2$/min$^{1/2}$, respectively. Consequently, the intra-run and inter-run CVs were 5.73% and 1.96%, respectively. The CV values were less than 15%; therefore, acceptable precision and reproducibility of the method was confirmed, as illustrated in Figure 1.

Specificity was determined by evaluating whether the change in the HCA release rate was directly proportional to the three levels of HCA concentrations of the test creams. The IVRT method successfully distinguished between the three different products as depicted by Figure 2. A linear relationship between HCA concentration and release rate was evident with an $R^2$ value of 0.9427 (Fig. 2).

Three IVRT runs, with minor deviations from the method parameters, were performed to demonstrate robustness of the method. Two temperature variations of $-2 \, ^{\circ}C$ and $+2 \, ^{\circ}C$ were evaluated and compared to the nominal temperature of $32 \pm 1 \, ^{\circ}C$. The third run was done by a different analyst at the nominal temperature. The

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**Table 3. Comparative IVRT of Five Topical Cream Products Containing HCA (n = 6)**

<table>
<thead>
<tr>
<th>Pairwise Comparison</th>
<th>90% CI</th>
<th>Decision</th>
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<tbody>
<tr>
<td>Positive Control</td>
<td></td>
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</tr>
<tr>
<td>Mylocort vs Mylocort</td>
<td>97.84–110.22</td>
<td>Pass</td>
</tr>
<tr>
<td>Negative Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mylocort vs Cream B</td>
<td>44.54–51.29</td>
<td>Fail</td>
</tr>
<tr>
<td>Mylocort vs Cream C</td>
<td>65.04–71.07</td>
<td>Fail</td>
</tr>
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**Assessment of Product Sameness**

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<tr>
<td>Mylocort vs Biocort</td>
<td>89.70–110.10</td>
</tr>
<tr>
<td>Mylocort vs Cream A</td>
<td>60.69–69.11</td>
</tr>
<tr>
<td>Biocort vs Cream A</td>
<td>62.42–71.81</td>
</tr>
</tbody>
</table>

Note - Mylocort: Market formulation containing 1% HCA (Aspen Pharmacare Holdings Ltd, South Africa, batch 116740); Biocort cream: Market formulation containing 1% HCA (Akacia AI Pharm, South Africa, batch H24); Creams A, B, and C: Test formulations containing 1%, 0.5%, and 1.5% HCA, respectively. IVRT, in vitro release testing; HCA, hydrocortisone acetate; CI, confidence interval.

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Sensitivity was evaluated by determining the effect of changing the concentration of HCA in the cream formulations on HCA release rates. The method was considered sensitive as the mean HCA release rate from the 0.5% HCA test cream was lower than that of the 1% HCA test cream and the mean release rate from the 1% HCA test cream was lower than that of the 1.5% HCA test cream, respectively. The mean ± SD release rates (n = 6) from the runs with 0.5%, 1%, and 1.5% HCA creams increased with increasing HCA concentration: $13.72 \pm 8.71$, $<18.30 \pm 19.06$, and $<30.63 \pm 16.69 \mu g/cm^2/min^{1/2}$, respectively.

To test if the IVRT method was selective, the statistical approach for product sameness testing described in the USP General Chapter <1724> was used to compare the release rates (n = 6) of products containing 0.5%, 1%, and 1.5% HCA (16). The CI for 0.5% vs 1% and 1.5% vs 1% HCA products fell outside the limits of 75% and 133.33%; hence, the method was considered suitably selective to establish differences in release rates (Table 3).
IVRT method was considered robust to the changes in method parameters as the resulting mean release rate for the corresponding IVRT run did not deviate by more than 15% from the mean release rate for HCA from the 18 VDCs across the three IVRT runs. The results show that small differences in temperature (± 2 °C) did not result in significant differences in HCA release rates. The mean release rates calculated from the runs using different temperatures, 30 °C, 32 °C, and 34 °C, did not deviate from the nominal temperature by more than 15%. Furthermore, the IVRT performed by two different operators did not show any significant differences in HCA release rates. It can therefore be concluded that the developed IVRT method has the necessary properties to confirm robustness.

Recovery was investigated using the linearity data of three experimental runs. Approximately 300 mg of a 1% Mylocort cream was accurately weighed and applied to Tuffryn membranes on each of the six VDC cells. The mean ± SD recoveries for runs 1, 2, and 3 were found to be 25.44% ± 1.70%, 26.63% ± 0.86%, and 27.34% ± 1.56% respectively. The dose depletion of HCA was found to be within the acceptable range of ± 30%.

Accuracy was not determined to be a relevant parameter for inclusion in the validation of an IVRT method, as the rate of API release for a particular semisolid drug product would vary depending upon the parameters of the test. Therefore, there would not be a “true” release rate. Also, the amount of API in the dosage form would not be expected to completely release during the course of an IVRT, so there would be no point at which the drug release “accurately” reached 100%.

**Comparative IVRT of HCA from Five Topical Cream Products**

Table 3 depicts the results obtained when Mylocort cream was compared to itself to provide a positive control. The 90% CI which was determined as 97.84–110.22% clearly indicates that the method was able to determine sameness. Comparison of the release rates of HCA between Cream A and Mylocort clearly indicated inequivalence. Although both products contained 1% HCA, formulation differences relating to the types of excipients and amounts as well as Q3 factors likely contributed to the results. Figure 3 depicts the results of the comparison between Mylocort cream vs Biocort cream and Cream A, containing 1% HCA. The release rate of HCA from Cream A was lower than that from Mylocort cream. As depicted in Table 3, Mylocort and Biocort were found to be in vitro equivalent where the 90% CI of the pairwise comparisons were within the limits of 75%–133.33%. The specially prepared creams containing 0.5% and 1.5% HCA were found to be inequivalent to the specially prepared 1% HCA cream thereby providing further evidence that the method had the necessary discriminatory power to determine differences when assessed in accordance with the SUPAC-SS acceptance criteria. The only difference in this case can be specifically related to differences in Q2 and furthermore, also served as appropriate negative controls.

These data provide compelling evidence of the value of a validated IVRT method to determine important formulation differences and thus has the necessary ability to assess sameness and differences between creams containing HCA with a high degree of reproducibility (CVs < 15%).

**CONCLUSIONS**

A comprehensive characterization of the operational parameters of an IVRT method was performed and an IVRT method for HCA creams was qualified and validated. The validated IVRT method for the analysis of topical HCA creams was applied to commercially available creams as well as a specially formulated 1% HCA cream. These creams were assessed in accordance with the SUPAC-SS guidance published by the US FDA. Two approved and commercially available products, Mylocort and Biocort, were found to be in vitro equivalent. Positive control (Mylocort vs Mylocort) and negative controls (Cremes B and C) provided necessary evidence to confirm the discriminatory ability of the validated IVRT method to declare both equivalence and inequivalence between HCA creams. In addition, the resulting data indicated the potential of the validated IVRT method to identify differences in formulation and/or process variables (Q1/
Q2/Q3). Furthermore, the comparative data between the different strengths of specially manufactured HCA creams demonstrated that the IVRT method was able to show differences between these products which, irrefutably, are due to differences in Q2 only. The results indicate that the IVRT method was very precise and reproducible, thereby confirming its suitability to discriminate differences in release rates of HCA from topical cream formulations and its value as a useful tool in formulation development of topical products. This approach provides useful information for the future development of acceptable IVRT methods to assess topical dosage forms for local action containing different drugs.

ACKNOWLEDGMENTS
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CONFLICT OF INTEREST
The authors disclosed no conflicts of interest related to this article.

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