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Research Paper

Reliability and Reproducibility of Vertical Diffusion Cells for Determining Release Rates from Semisolid Dosage Forms

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Purpose. USP has formed Advisory Panels to ensure the integrity of laboratory procedures for non-oral routes of administration and expects that the panels will recommend performance tests (performance qualification, PQ) for these dosage forms as well as performance verification tests (PVT) for those PQ tests. An integral part of PQ is PVT, in which a standard formulation is first tested in a metrologically sound collaborative study to set acceptance criteria. Individual laboratories can then test the performance of their product by comparing their results to those obtained from the USP collaborative study. These studies are guided by metrological principles, e.g., those of the International Organization for Standardization (ISO) 43-1, which succinctly states that "one of the main uses of proficiency testing schemes is to assess laboratories' ability to perform tests competently."
Materials and methods. Four laboratories conducted two collaborative studies to determine the reliability and reproducibility—understood in metrological terms—of release rates from semisolid dosage forms using the vertical diffusion cell (VDC).
Results. The experiments reported here from the second study found that the major contributor to variability is the interlaboratory component that may include intermediate precision considerations other than analyst. Because all laboratories used the same model equipment, one might expect that the observed reproducibility CV was lower than if the laboratories used different models or equipment made by different manufacturers. Also, more variability was observed with the creams than the other dosage forms.
Conclusions. The results from the preliminary collaborative study found inconsistency among the laboratories. After operator training, the results from the second study were more consistent, suggesting the initial results were associated with variations among the laboratories in performing the methods and procedures and conducting the protocols. Those results emphasize that although the *in vitro* release procedure is simple and reproducible, training is needed. The data presented suggest that testing of *in vitro* release by VDCs should be considered as a PVT for topical semisolid dosage forms. Thus, a standard semisolid product is needed, along with a means for setting acceptance criteria. The SUPAC-SS Guidance may be helpful in the latter regard.

KEY WORDS: Franz Cell; performance qualification; performance verification test; semisolid dosage forms.

INTRODUCTION

Many elements are involved in ensuring the integrity of laboratory procedures, with a focus on installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). The US Pharmacopeia (USP) provides reference standards for PQ for some procedures, most notably tablets for dissolution testing. USP has formed Advisory Panels for non-oral routes of administration (i.e., inhalation, mucosal, parenteral, and topical), and expects that those panels will recommend performance tests for dosage forms used via those routes as well as performance verification tests (PVT) for those performance tests (1). Acceptance criteria for a PVT are established by USP based on collaborative studies. When supplied with a technical data sheet and troubleshooting guide, USP's reference standards for PVT can be used by first parties (manufacturers), second parties (purchasers), and third parties (independent or governmental laboratories) to determine whether results obtained in their laboratory are similar to those of the USP collaborative study (2,3). Beyond PQ, the general approach is that of proficiency testing in which a single laboratory assesses its capability relative to the laboratories in the USP collaborative study. As the introduction to ISO 43-1 succinctly states, "One of the main uses of proficiency testing schemes is to assess laboratories' ability to perform tests competently" (4).

Since the inception of *in vitro* release testing for semisolid dosage forms using diffusion cell testing methods, several diffusion cell types and designs have been applied.

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The vertical diffusion cell (VDC) design, or "Franz Cell," has emerged as the most popular design for testing *in vitro* release of topical semisolid dosage forms (5,6). In 1997, the FDA released the SUPAC-SS Guidance (7). The Guidance recommends *in vitro* diffusion cell (VDC) testing for semisolid dosage forms when manufacturing or material changes have been made to an approved topical dosage form. If any of a variety of manufacturing or material changes occurs, the Guidance requires *in vitro* release test using a VDC to compare the pre- and postchange release rates of the product.

In vitro release testing is thus a candidate performance verification test for semisolid dosage forms (8). To understand the reliability and reproducibility of the *in vitro* test using the VDC, the USP Biopharmaceutics Expert Committee requested additional information in the form of a collaborative study. USP conducted two collaborative studies, a preliminary study that is reported in summary only and a second, more comprehensive study that is reported here in detail. The objectives of the collaborative studies were to evaluate the reliability (intralaboratory) and reproducibility (interlaboratory) of the VDC system for *in vitro* drug release determination for semisolid dosage forms and to develop a procedure that could be used as an official pharmacopeial test.

MATERIALS AND METHODS

Equipment

The VDC design features a donor compartment at the top of the assembly and a receptor compartment directly below. The two compartments are separated by a synthetic membrane that is not intended to be a barrier but rather an avenue through which drug diffusion takes place. The membrane is also intended to be a support for the test product, ensuring that the product remains in place with constant and consistent contact with media in the receptor compartment.

This type of cell is commonly used for testing penetration properties of a drug product and for determining *in vitro* release rates of topical semisolid drug products such as creams, gels, and ointments. The cell is made of clear glass and uses a clamp to secure the donor side of the cell to the receptor side. The clamp ensures that all components, including the membrane, remain in place during the test. A glass disk is used to support the dosage wafer and to facilitate viewing the donor material during the test. The cell is temperature-controlled at 32°C *via* a water jacket and bath circulator. The alignment ring ensures that the donor and receptor orifices are accurately aligned. The sampling and replacement ports have Luer connections that facilitate the collection of sample and media replacement, respectively. In addition, a bubble trap is incorporated in the replacement port to remove bubbles that may be inadvertently introduced by the sampling process. A magnetic stirrer rotates the helix and magnet to keep the receptor media stirred and homogeneous. Tests using the VDC are usually conducted with groups of six cells. All laboratories in these studies used vertical diffusion cell equipment manufactured by Hanson Research (Model 58-6 M with 7-ml VDC).

Test Procedures

The same test procedures were used for both collaborative studies. For testing hydrocortisone, and betamethasone cream and gel, the membranes (Pall HT-450, Tuffryn Membrane Filter, 25-mm diameter, 0.45 μm) were presaturated in a 15% solution of Ethomeen in isopropyl myristate (IPM) for 30 min and placed on the dosage wafer (6). The receptor media was 70% water and 30% ethyl alcohol. For testing betamethasone ointment, the membranes were saturated in IPM for 30 min. The receptor media was 85% ethyl alcohol, 10% IPM, and 5% water. In both cases, the dosage wafers were inverted, and then filled with test product (approximately 300 mg of drug). In the studies, the 300-mg dose was defined as an infinite dose for the tests. The dosage wafer was then placed on a cell filled with receptor media that had come to temperature (32°C). The glass disk, alignment ring, and clamp were applied to the cell assembly, and stirring was initiated (time zero).

Samples were collected at 1, 2, 3, 4, and 6 h. The VDC was equipped with a check valve attached to the replacement port and a sampling port cannula to aid in the manual sampling process (9). The check valve prevents replacement media from flowing back out once the syringe is removed. The sampling process introduces approximately 1 ml of replacement media through the check valve and into the replacement port while concurrently forcing sample out of the sampling port for collection. Approximately one-half (0.5 ml) of the sample was used to rinse the sample port and cannula. The other half was retained for HPLC analysis. The sampling process was performed with the stirrer off. The HPLC peak area data for each sample time point were used to calculate the amounts released ($\mu g/cm^2$) for each VDC.

All samples were analyzed using reversed-phase HPLC with the following chromatographic conditions:

– Hydrocortisone cream: wavelength: 242 nm; flow rate: 1.0 ml/min.; injection volume: 10 μl; run time: 10 min; column: 50-mm×3.9-mm Symmetry C-18, 5μm; and mobile phase: 20/80 acetonitrile (ACN)/H_2O.

– Betamethasone dipropionate creams, gels, and ointments: wavelength: 239 nm; flow rate: 1.5 ml/min; injection volume: 50 μl; run time: 10 min; column: 300 mm×3.9 mm μBondapak C-18; and mobile phase: 60/40 ACN/H_2O.

Preliminary Collaborative Study

Four laboratories comprising a dissolution instrument manufacturer, a pharmaceutical company, FDA, and USP conducted a study using four topical semisolid products: hydrocortisone cream 1%, betamethasone dipropionate cream 0.05%, betamethasone dipropionate gel 0.05%, and betamethasone dipropionate ointment 0.05%. Semisolid dosage forms (creams, ointments, and gels) containing the same active ingredient, betamethasone dipropionate, were selected for ease of operating the analytical procedure and HPLC system. At the same time these dosage forms provided information that the procedure was workable for all types of topical semisolid dosage forms.

The release rate for each of the four products for three laboratories was compared to the corresponding rate from

the reference laboratory. Eight of 12 comparisons (one run from each of three laboratories for each of four products) passed the FDA criterion (7) of the 90% confidence interval for the ratio of medians within 75–133%.

After reviewing and discussing the results of the preliminary collaborative study, the USP Biopharmaceutics Expert Committee agreed that the failures and inconsistent results may have been due to different methods, techniques, and protocols used by the different laboratories. The Expert Committee also concluded that the best way to obtain a better understanding for the differing results and variability would be to repeat the study after first conducting training to ensure that all collaborators completely understood the methods, procedures, and protocol. Each participating laboratory sent two analysts to the reference laboratory for 1 day of training in April 2005. The training was deemed a success—many of the participants stated that they had performed some of the procedures differently and that the training showed them easier and more consistent ways of accomplishing the tasks.

Second Collaborative Study

The four laboratories in the preliminary study conducted a second study using the same four topical semisolid products, although the latter were not necessarily obtained from the manufacturer whose products were used in the first study. Only one analyst from the first study participated in the second study. All but one of the analysts in the second study had participated in the training.

The products studied were:

– Hydrocortisone Cream 1%, Pharmacia, Lot 20KJM, Exp. 10-2005 (obtained from a pharmacy and provided to all laboratories);

– Betamethasone Dipropionate Cream 0.05%, Taro, Lot 31119, Exp. 09-2006;

– Betamethasone Dipropionate Gel 0.05%, Taro, Lot 4B055, Exp. 09-2005; and

– Betamethasone Dipropionate Ointment 0.05%, Alpharma, Lot 310046, Exp. 10-2005.

For each of the four products, each laboratory was instructed to run three experiments, and each experiment consisted of results obtained from six cells. Table I shows the number of experiments performed by each laboratory. Two laboratories used two analysts for all four products. One laboratory used a single analyst for all experiments for all products. The fourth laboratory used two analysts for one product and one analyst for all experiments for the remaining three products. One laboratory conducted a complete set of experiments with each of two apparatus (same model, labeled A and B in the tables). There were no missing data.

The results obtained with hydrocortisone cream were used to verify the performance of the operating system before starting the analyses of the betamethasone products. The release rate results of laboratory C1 (reference laboratory) provided assurance that the system and analyst were ready to move forward with sample analyses. That provided assurance that the set-up of the equipment, preparation of the samples by the analyst, sample collection, and sample analyses were acceptable.

Statistical Methods

The first step was to plot the release rate as the slope of the regression of cumulative amount released versus the square root of time. This follows the FDA SUPAC-SS guidance (7). Regression analyses were performed separately for each cell for each experiment and laboratory.

The release rates (slopes) were examined by analysis of variance to estimate the components of variability. Three decisions guided the choice of a statistical model: (1) How should the use of two sets of equipment by Laboratory 1 be handled? (2) How should the presence of multiple analysts in only two laboratories (three laboratories for one compound) be handled? (3) Should cell be a fixed effect (that is, a common effect of location across laboratories), separate random effect, or left as part of the residual variability?

As noted, the four laboratories were not consistent in using one or two analysts for this study, which complicates determination of the variance components. Variability due to analyst should be part of intermediate precision. If variance for analyst is estimated separately, any result would be due to only a subset of the participating laboratories. If not estimated separately, that component of intermediate precision becomes part of the repeatability variability, thereby inflating that variance. Results reported here do not include the analyst. Thus, the reported repeatability variability was found as the sum of the components due to cell and experiment and is inflated by any contribution by the analyst. Any other intermediate precision components other than the analyst were not included in the experiment and are thus part of the interlaboratory variability. The total, or reproducibility variability, was found as the sum of the laboratory, experiment, and residual variance components.

Table I. Number of Experiments by Laboratory

Laboratory	Equipment[a]	Hydrocortisone Cream 1%	Betamethasone Dipropionate Cream 0.05%	Betamethasone Dipropionate Gel 0.05%	Betamethasone Dipropionate Ointment 0.05%
C1	A	3	4	3	3
C1	B	3	4	3	3
C2	A	2	1	1	1
C3	A	3	3	3	3
C4	A	3	3	3	3
TOTALS		14	15	13	13

[a] A and B are two units of the same model.

To summarize, laboratory and experiment within a laboratory were treated as nested random effects. The default variance components covariance structure was used. That analysis estimated three variance components: interlaboratory, interexperiment (intralaboratory), and residual. The analysis was performed for the slope and logarithm of the slope. The log-scale analysis yielded residuals that better approximated a normal distribution and is the analysis reported here. All results were transformed back to the original scale.

Tables II and III report summary statistics for the log slopes. The means and standard deviations were first determined for the log slopes. The averages shown in Table II are the geometric means found as the antilog of the log scale means. The percent coefficients of variation (%CVs) in Table III were found as $100\%\sqrt{\exp(S^2)-1}$ where S^2 is the variance of the log slopes. For Table IV the variances are from the analysis of variance of the log slopes. Reliability and reproducibility reported here use the International Conference on Harmonization/International Organization for Standardization/USP (ICH/ISO/USP) definition (3). The reported reliability CV was found by first summing the variance components for experiment and residual and then determining the %CV as given above. The reproducibility %CV was found by summing all three variance components and then converting to %CV as above.

All analyses were performed with SAS for Windows, Version 9.1 (SAS, Inc., Cary, NC), and using Proc Mixed with the default restricted maximum likelihood (REML) method.

RESULTS

To present a sense of the underlying data, Fig. 1 shows the average release rates by time for two experiments for hydrocortisone cream. One experiment is selected from collaborator C1 and one from collaborator C4 to highlight the differences seen in release rate (slope) and variability. The (geometric) mean averages by laboratory, equipment, and cell are shown in Table II for all four products, and %CVs are shown in Table III. The means and %CVs are each based on two to four experiments. The purpose of Tables II and III is to look for general trends, not detailed comparisons. These data support the decision to ignore equipment as a factor in the statistical model, and the three experiments using Equipment B from Laboratory C1 are treated as just another three experiments from that laboratory. Results reported for variability due to experiment thus include any contribution from differences in equipment in laboratory C1.

There is no evident cell effect in Tables II and III. For example, in Table IIa, the highest and lowest cells differ by laboratory. With no reason to treat cell as an effect that is (on average) constant across laboratories, cell was not included in the model for the primary analysis. Any effect of cell is included in the residual variability. The "Pooled" columns of Tables II and III thus combine results across cells as a summary. The pooled CVs in Table III include all the components of reliability (Table IV) except the contribution from cell. A secondary analysis of hydrocortisone data treated cell within experiment as a random effect in order

Table II. Average Release Rate ($\mu g/cm^2/min^{0.5}$) by Laboratory, Equipment, and Cell

Laboratory	Equipment	1	2	3	4	5	6	Pooled
A. Hydrocortisone Cream 1%								
C1	B	12.8	11.7	11.2	10.9	10.5	11.2	11.4
C1	A	12.3	12.9	11.9	11.0	11.5	11.0	11.7
C2	A	12.0	11.7	11.2	11.9	11.5	12.7	11.8
C3	A	14.1	13.9	15.3	13.9	14.4	13.7	14.2
C4	A	16.2	16.3	15.5	15.4	16.2	15.4	15.8
B. Betamethasone Dipropionate Cream 0.05%								
C1	B	0.87	0.88	0.89	0.88	0.82	0.78	0.85
C1	A	0.91	0.88	0.89	0.80	0.85	0.83	0.86
C2	A	0.68	0.63	0.60	0.65	0.67	0.61	0.64
C3	A	0.82	0.88	0.86	0.83	0.83	0.88	0.85
C4	A	0.88	0.84	0.86	0.81	0.82	0.79	0.83
C. Betamethasone Dipropionate Gel 0.05%								
C1	B	1.6	1.6	1.6	1.6	1.6	1.5	1.6
C1	A	1.6	1.6	1.5	1.6	1.6	1.5	1.6
C2	A	1.5	1.5	1.4	1.4	1.5	1.2	1.4
C3	A	1.3	1.4	1.5	1.3	1.3	1.2	1.3
C4	A	1.6	1.6	1.6	1.5	1.5	1.6	1.6
D. Betamethasone Dipropionate Ointment 0.05%								
C1	B	1.9	1.8	1.9	1.7	1.8	1.8	1.8
C1	A	1.8	1.8	1.7	1.8	1.7	1.8	1.8
C2	A	2.0	2.0	1.8	1.9	1.9	1.9	1.9
C3	A	1.7	1.6	1.7	1.5	1.6	1.6	1.6
C4	A	1.9	1.9	1.8	1.8	1.8	1.8	1.8

Table III. %CV of Release Rate by Laboratory, Equipment, and Cell

Laboratory	Equipment	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	Pooled (%)
A. Hydrocortisone Cream 1%								
C1	B	6.6	6.7	3.5	3.5	4.0	1.5	4.7
C1	A	3.3	5.9	6.8	2.7	4.0	1.4	4.4
C2	A	5.1	3.8	1.7	7.6	4.4	0.4	4.5
C3	A	11.3	15.4	11.7	16.4	13.5	9.9	13.2
C4	A	17.3	12.4	12.5	9.7	11.3	8.2	12.2
B. Betamethasone Dipropionate Cream 0.05%								
C1	B	4.6	11.5	17.7	12.8	18.5	13.8	13.9
C1	A	11.9	2.7	17.0	17.5	8.9	18.5	13.9
C2	A							
C3	A	10.6	16.1	9.6	5.7	14.5	7.6	11.3
C4	A	7.5	11.8	2.8	13.1	12.7	9.9	10.3
C. Betamethasone Dipropionate Gel 0.05%								
C1	B	5.9	4.6	4.2	4.4	5.3	1.9	4.6
C1	A	1.0	0.4	5.4	5.0	2.3	2.9	3.4
C2	A							
C3	A	17.8	6.7	8.2	5.3	19.3	11.4	12.6
C4	A	4.2	0.9	5.0	9.6	2.2	3.7	5.0
D. Betamethasone Dipropionate Ointment 0.05%								
C1	B	5.8	4.9	3.1	4.5	4.9	7.8	5.4
C1	A	4.2	5.9	3.7	4.2	6.7	8.3	5.7
C2[a]	A							
C3	A	4.9	3.6	5.1	5.3	2.1	7.5	5.0
C4	A	14.0	6.8	14.0	11.5	6.7	10.6	11.0

[a] No %CV is given for betamethasone dipropionate for Laboratory C2 because there is only one datum for that laboratory.

to separate cell effects from the remainder of the residual variability. Those analyses found no separate variance component due to cell and are not reported.

Results for variance components for all four products are shown in Table IV. The major contributor to variability is the interlaboratory component that may include intermediate precision considerations other than analyst. That is particularly evident in Table IIa, which shows that Laboratories C3 and C4 have consistently higher results than Laboratories C1 and C2, and Table IIIa, in which C3 and C4 are also more variable. Because all laboratories used the same model equipment, one might expect that the observed reproducibility CV was lower than if the laboratories used different models or equipment made by different manufacturers. Also, more variability was observed with the creams than with the other dosage forms.

DISCUSSION

Because *in vitro* release (IVR) testing using the VDC system is a new method, it was important to ascertain whether different analysts/operators in different laboratories were able to carry out the test appropriately. The best way to check this was to compare the results among multiple laboratories. Two collaborative studies using VDC were conducted. The results from the preliminary study found inconsistency among the laboratories. After operator training, the results from the second study, the one reported here, were more consistent, suggesting the initial results were associated with variations among the laboratories in performing the methods and procedures and conducting the protocols. Those results emphasize that although the *in vitro* release procedure is simple and reproducible, training is needed to ensure that practitioners have a clear understanding of the methods and procedures—as is often the case when one introduces new equipment or procedures.

The preliminary study indicated a need for training and standardization to reduce interlaboratory differences. Tables II, III, IV show remaining interlaboratory differences in the second study, indicating that some remain. Taken together, this experience suggests that testing of IVR by VDCs should be considered as a PVT for topical semisolid dosage forms.

Table IV. Summary Variance Component Results (%CV)

Variance Component	Hydrocortisone Cream 1%	Betamethasone Dipropionate Cream 0.05%	Betamethasone Dipropionate Gel 0.05%	Betamethasone Dipropionate Ointment 0.05%
Laboratory	14.5	9.9	7.4	6.1
Experiment	7.4	8.1	3.4	6.1
Residual	6.2	9.2	6.6	4.4
Repeatability %CV	9.7	12.3	7.5	7.5
Reproducibility %CV	17.5	15.8	10.5	9.7

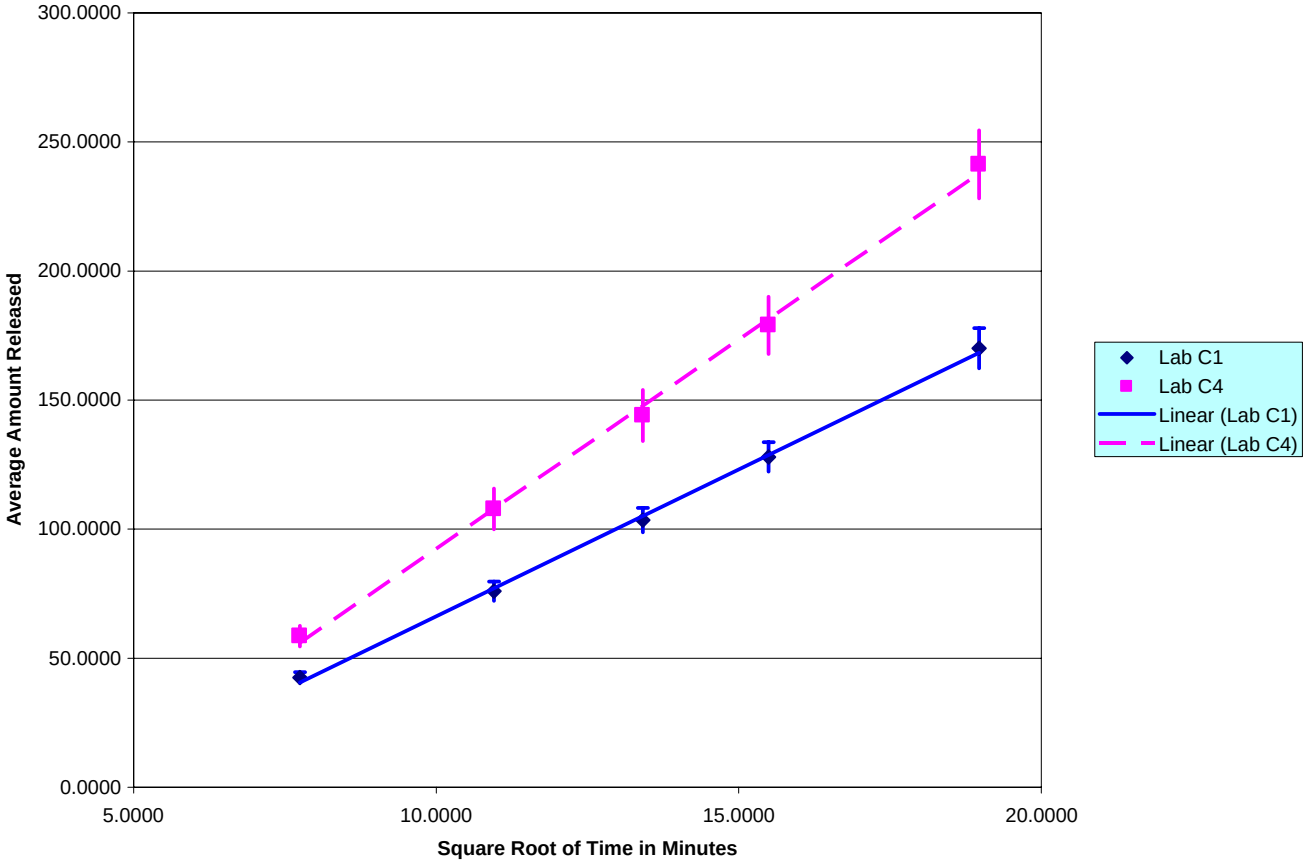

Fig. 1. Average amounts released by square root of time for two selected experiments.

With a PVT a standard formulation is first tested in a collaborative study to set acceptance criteria. Individual laboratories can then test the performance of their product by comparing their results to those obtained from the collaborative study. Thus, a standard semisolid product is needed, along with a means for setting acceptance criteria.

Development of IVR testing began by examining Hydrocortisone Cream 1%, the pioneer product from Pharmacia, more than 20 years ago. Hydrocortisone Cream 1% was selected because it is widely used and has a relatively high drug concentration (compared to other topical steroid products that contain 0.05% or lower amounts of active ingredients), which makes it reasonably easy to analyze. During several years and across different batches, the release profile and rate of Hydrocortisone Cream from Pharmacia has remained relatively constant, which indicates suitability for its use as a PVT reference standard (SW Shaw, written correspondence, 18 January 2007). The choice of hydrocortisone may not be obvious given its higher variability in testing during this study. For a PVT, however, such variability could be desirable. A standard for a PVT should be one that is sensitive to changes to the procedure, whether such changes are deliberate or not. The greater interlaboratory variability of hydrocortisone does not confirm this sensitivity but is an indicator of potential for sensitivity. One concern, however, is that because the product is a cream, its stability could be an issue, and higher variability was observed with creams compared to other dosage forms.

The next question is how to set the acceptance limits. The SUPAC-SS Guidance presented a procedure for 90% confidence interval criteria for IVR comparisons. The same principle could be utilized to ensure that the test laboratories are able to obtain the same IVR from the same batch of Hydrocortisone Cream as obtained by the collaborative study. Although the statistical details still need to be developed for this application, the Guidance provides a benchmark that can be used in the PVT.

The results from these studies will be presented to the USP Biopharmaceutics Expert Committee and the Committee's Topical/Dermal Advisory Panel charged with recommending performance tests for topical products. The Biopharmaceutics Expert Committee will then recommend to USP whether or not to use IVR using vertical diffusion cells as a performance test and whether performance verification testing should be implemented for the procedure.

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