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Validation of an In Vitro Release Test (IVRT) for Acyclovir Creams Including Apparatus and Laboratory Qualification

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Introduction

In vitro release tests (IVRT) using vertical diffusion cells (VDCs) is a commonly used method to determine the release of active pharmaceutical ingredients out of its formulations. But according to our knowledge, no comprehensive and meaningful qualification and validation of the IVRT with its associated parameters has been published until now.

The aim of our work was the qualification of the apparatus and the laboratory performing the IVRT study and the validation of the HPLC analytical method used to assay the IVRT samples and the validation of the IVRT method itself for a 5% acyclovir cream. The qualified and validated methods were subsequently used for a pairwise comparisons of a 5% reference acyclovir cream with six different topical test products also containing 5% acylcovir in a comparative IVRT study.

Material and Methods

IVRT method and VDC apparatus
(Hanson Research Corporation, USA):

- 6 VDCs in parallel
- VDC Volume: 12 mL
- Orifice: 15 mm
- Stirring speed: 600 rpm
- Receptor medium temperature: 32°C
- Nominal amount applied: 300 mg
- Tuffryn® membrane (pore size: 0.45 µm)
- Receptor medium: 0.9% sodium chloride solution
- Sampling times: 0.5, 1, 2, 3, 4, 5 and 6 hours
- Analytical method: HPLC-UV
- Receptor sample volume: 500 µL sample aliquots

Figure 1: *VDC System*

Apparatus Qualification: We evaluated capacity and diameter of the VDCs, as well as temperature of the receptor medium, stirring speed, dispensed sampling volume and environmental conditions.

Laboratory Qualification: A pairwise comparison of two IVRT runs dosed with hydrocortisone cream was conducted according to USP General Chapter <1724>. Intra-run and inter-run variability was evaluated.

HPLC-UV Analytical Method Validation: HPLC-UV sample analysis was conducted for acyclovir according to ICH Guidelines (specificity, selectivity, linearity, accuracy, precision, robustness, stability, and lower and upper limit of quantification).

IVRT Method Validation: We evaluated membrane inertness (binding), acyclovir solubility in receptor medium as well as linearity, precision, reproducibility, recovery, and robustness of the IVRT method.

The following product were used for method validation:

- Zovirax cream 5%- purchased
- Acyclovir cream 2.5 % - prepared in house
- Acyclovir cream 5.0% - prepared in-house
- Acyclovir cream 10.0% - prepared in-house

The comparative IVRT study was conducted according to the USP general chapter <1724>. For statistical comparison of the acyclovir release rates between the reference product R and six test products (P1-P6) were performed using either Wilcoxon Rank Sum or Mann-Whitney tests.

Results

Apparatus Qualification

5 of the 6 assessed parameters were successfully validated. Parameter 1 – the capacity of the VDC cell – was 9.77 ± 0.13 mL instead of the nominal 12 mL. The measured volume of 9.77 mL was used for further calculations.

Table 1: *Results apparatus qualification*

Parameter	Passed
P1: Capacity of the cells	✗
P2: Diameter of the orifice of the cell	✓
P3: Temperature of the receptor medium	✓
P4: Speed of the magnetic stirrer	✓
P5: Dispensed sampling volume	✓
P6: Environmental conditions	✓

Laboratory Qualification

Table 2: *Results for laboratory qualification*

Parameter	Acceptance Criteria	Passed
Intra-run variability	Intra-run CV for the first run (n=6 VDCs) <6.80%	✓
	Intra-run CV for the second run (n=6 VDCs) <10.20%	✓
Inter-run variability	Inter-run CV for both runs (n=12 VDCs) <12.70%	✓
Product sameness testing	The 90% confidence interval falls within the limits of 75.00% and 133.33%	✓

IVRT Method Validation

Table 3: *Results for IVRT method validation*

Parameter	Acceptance Criteria	Passed
Membrane Inertness	No acyclovir binding to the membrane: Recovery of 105.5%	✓
Receptor Medium Solubility	Solubility was > 10 times higher than the maximum acyclovir concentration in the receptor medium observed during the IVRT study	✓
Linearity	Lowest R^2 value was 0.97, no outlier	✓
Precision and Reproducibility	Inter-run variability was 5.8%; intra-run variability 4.4%	✓
Sensitivity	The mean release rate increased with increasing acyclovir concentration: 278.97 µg/cm²/h1/2 (2.5% cream) < 440.45 µg/cm²/h1/2 (5% cream) < 605.61 µg/cm²/h1/2 (10% cream)	✓
Specificity	Linear regression model (release rate versus product concentration) R^2 =0.943	✓
Selectivity	IVRT method accurately identified in-equivalent and equivalent acyclovir products	✓
Robustness	Release rate for temperature and stirring speed variation deviated < 15%	✓
Recovery	< 10%; no excessive acyclovir depletion	✓

HPLC-UV Analytical method Validation

All validated parameters including specificity, selectivity, linearity, accuracy, precision, robustness, and stability as well as the determination of the LLOQ and LOD passed the validation.

Comparative IVRT study

None of the six test products showed an equivalent release rate compared to the reference product (Figure 2). Statistical evaluation showed that none of the computed confidence intervals for the five comparisons lay within the limits of 75% and 133.33%.

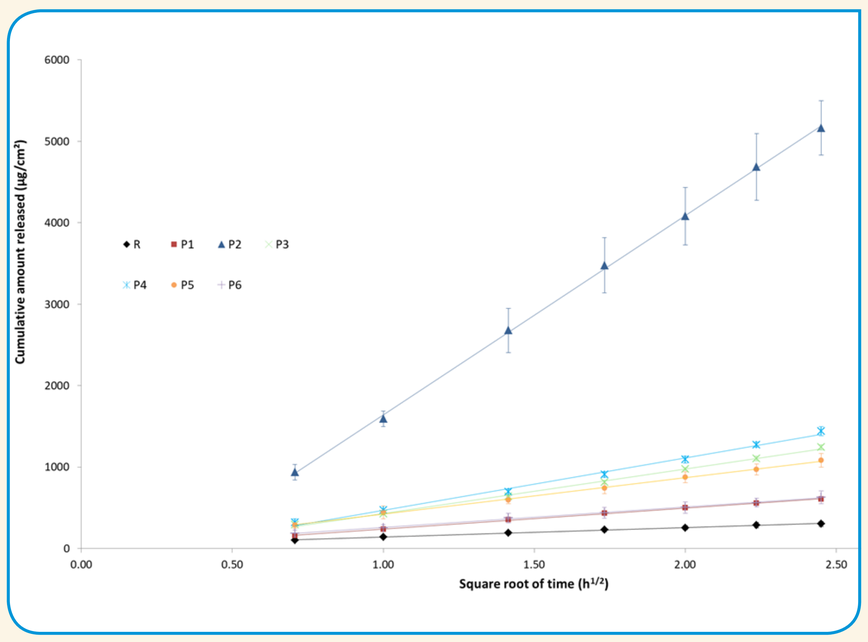

Figure 2: *Release rate curves for the comparative IVRT study*

Conclusion

- **The routine implementation of an apparatus qualification and a method validation supports the quality and reproducibility of IVRT studies.**
- **This IVRT study demonstrated that a validated IVRT method is an effective tool to detect differences in release rates of the API and to evaluate formulations.**