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WHITE PAPER: USE OF IN VITRO RELEASE TESTING (IVRT) FOR SEMISOLID TOPICAL FORMULATIONS

INTRODUCTION:

In vitro release test (IVRT) has increasingly garnered attention and weight in product development as well as regulatory assessment of complex drug products such as nano emulsions, suspensions, multivesicular liposomes, and microspheres, as IVRT provides key information about the quality and performance of drug products. An ideal IVRT method should correlate the changes in the critical quality attributes (CQAs) of the drug product directly to the drug's release characteristics, and thus provide valuable information to ensure batch-to-batch consistency in quality, facilitate assessment of post-approval changes, and assist with product comparison to support determination of bioequivalence.

IVRT is one of the methods listed in the literature for characterizing topical, semisolid dosage forms. This test does not necessarily correlate to the in vivo performance or bioavailability; however, it gives insight into product performance and changes in the product's performance due to formulation characteristics.

Importance of IVRT study

- To assess the changes in composition on the rate of release
- Effect of viscosity changes on the rate of release
- To assess the changes in process parameters
- Screening formulations before clinical PK or bioequivalence studies
- Compare batch formulations to assess scale-up and post-approval changes
- Waive clinical endpoint studies for certain generic formulations in certain cases

Figure-1: IVRT instrument -Teledyne Hanson Phoenix Dry Heat Diffusion Testing System and Dry Heat Diffusion Cell

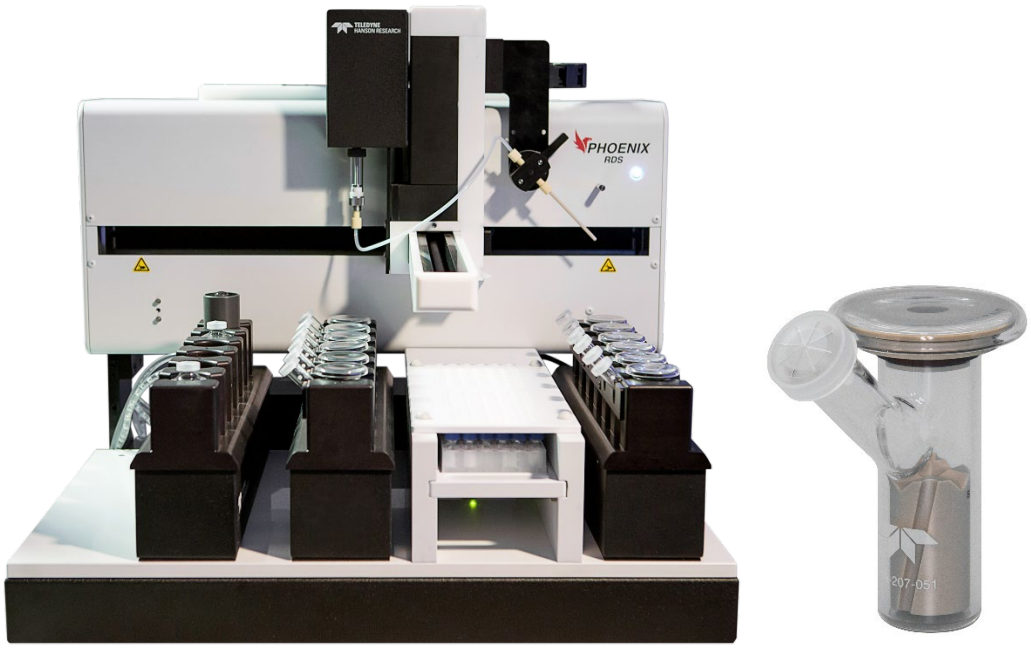
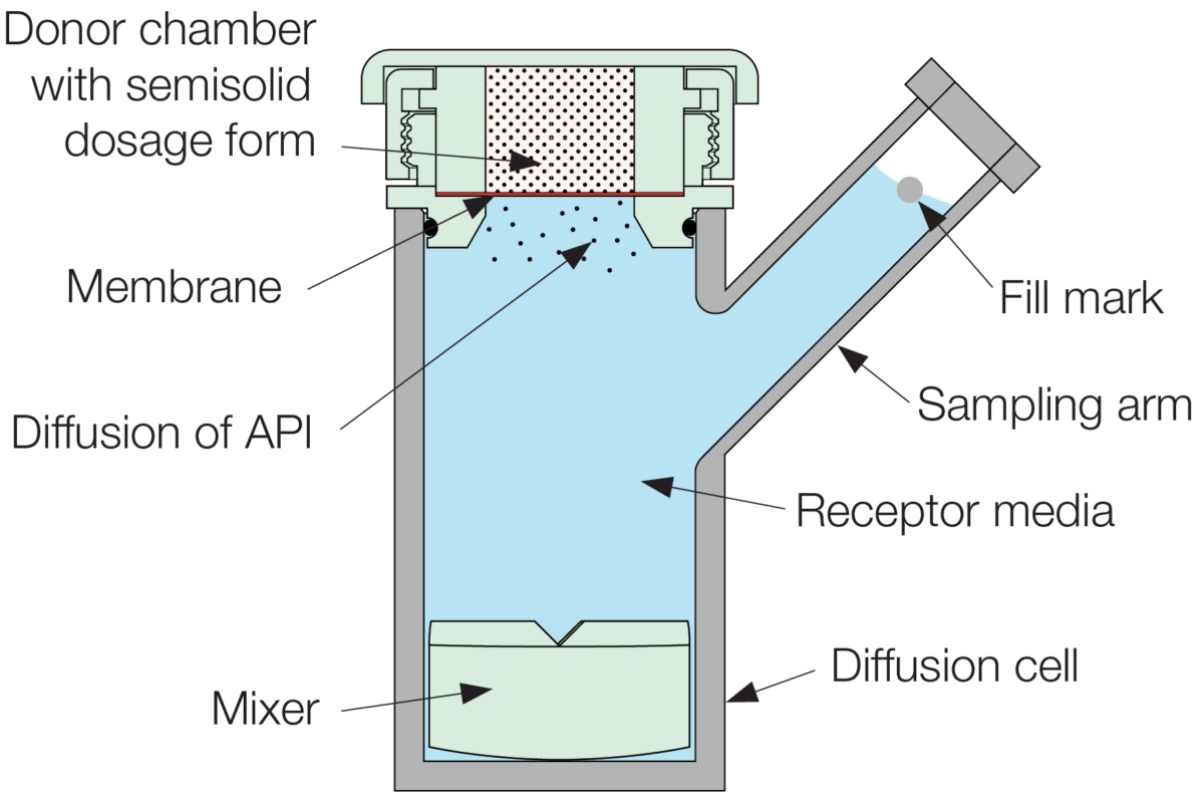


Figure-2: Parts of IVRT instrument



PROJECT HIGHLIGHTS

- Selection of suitable analytical technique for the In-Vitro Release Test.
- Optimization of sample preparation procedure
- Successful Resolution of many challenges faced during method development.
- Analytical method validation

COMPLEXITY OF ANALYTICAL METHOD DEVELOPMENT

Physical and Chemical properties of the drug substance were tabulated and evaluated. IVRT plays a very important role in evaluating any post-approval change in process that can impact product quality and performance. Automating immersion cell systems is relatively easier. In this study, a method has been developed that can detect even minor changes that may occur in the formulation and production process and the accuracy, repeatability and selectivity of the method have been verified. During the method development the following steps were considered and optimised for further studies.

IVRT methods are developed to detect differences from batch to batch, However, a sufficient IVRT method will show when any change in product occurs that may affect performance.

a. Selection of receptor media
b. Selection of membrane
c. Selection of sampling timepoints and repetition of experiment with media.
d. Test versus Reference

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A. Selection of receptor media

As pe literature "Appropriate receptor medium such as aqueous buffer for water soluble drugs or a hydro-alcoholic medium for sparingly water-soluble drugs" can be used. Hence, after establishing a basic assay method, the first task in method development is to measure the solubility of the API in several solvents ranging from aqueous solutions such as Phosphate buffer solution (PBS) to hydro-alcoholic solutions such as isopropanol/ PBS-50/50 (v/v). The intention is to identify solvents that will provide sink conditions in the IVRT receiving vessel. Sink conditions exist when a receptor medium has a relatively "high capacity to dissolve or carry away the drug" and the receptor media "exceed[s] 10% of Cs (drug solubility in the releasing matrix) at the end of the test". Usually three media, including both aqueous-based and hydro-alcoholic-based solvents, are selected for further IVRT evaluation. At a minimum, sink conditions must be maintained, and the receptor solution must be able to accommodate more than the amount of material released at the last sample point. Ideally, the receptor solution should be able to dissolve 10x the amount of material released during the test. For example, if 2 mg of product was released at the end of the test, the receptor media in the cell should be capable of holding a minimum of 2.1 mg. It would be ideal to have receptor media capable of dissolving 20 mg. Typical solvents that can be used are acetonitrile, ethanol, methanol, and isopropanol mixed with water. Typical ratios should not exceed 80/20. Solvents should be chosen based on the API solubility and chemistry.

Solubility analysis performed with different media:

Table-1: Solubility study results

S: No	Media	Solubility mg/10 mL
1	pH 7.4 phosphate buffer saline media	22.84
2	pH 3.3 phosphate buffer saline media	4.31
3	pH 3.3 phosphate buffer saline media: Ethanol (80:20)	18.05
4	pH 5.5 phosphate buffer saline media	15.69
5	pH 5.5 phosphate buffer saline media: Ethanol (80:20)	15.23
6	pH 7.4 phosphate buffer saline media: Ethanol (80:20)	18.73
7	pH 7.4 phosphate buffer saline media: Ethanol (60:40)	845.65
8	pH 7.4 phosphate buffer saline media: IPA (70:30)	169.48

An acceptable sink condition is one where the maximum concentration of the active substance in the receptor medium achieved during the experiment does not exceed 30% of its maximum solubility in the receptor medium. Sink conditions normally occur in a volume of medium that is at least 3-10 times the saturation volume. Further experiments were performed with different media to achieve the linear release and to check at least 70% of the active substance to be released. The maximum solubility was observed in receptor media pH 7.4 phosphate buffer saline: Ethanol (60:40). The Sink conditions was achieved throughout the experiment as per EU criteria.

B. Selection of membrane

The membrane should ensure that the product and the receptor medium remain separate to ensure the tested formulation remains unchanged throughout the testing period. The membrane should not be rate-limiting to active substance release. The membrane should be compatible with the drug product formulation and not bind to the active substance. There are many choices for membranes, which include recently excised tissue, tissue constructs, cadaver tissue, and synthetic

membranes. Factors influencing the selection of the proper membrane include compatibility with the test material, availability, reproducibility, cost, and, importantly, the goal of the experiment itself. Synthetic membranes vary controllably in pore size, thickness, and hydrophilicity. Since the major constituent of many semisolid products is water, hydrophilic/hydrophilized synthetic membranes are typically used. During membrane screening, usually three polymeric membranes with the same pore size are evaluated. Commonly used membranes are- Tuffryn Supor® (polysulphone), Cellulosic, Acetate Plus® (cellulose acetate), Nylon, Teflon, and Polycarbonate. Membrane selection done with two membranes with 100% sample. The 0.45 µ Polycarbonate Nuclepore membrane filter and 0.45 µ Nylon membrane filter used for the development study and the results obtained were almost similar for both the membrane. Further based on availability of membrane 0.45 µ Nylon membrane filter used for further study.

C. Selection of sampling timepoints and repetition of experiment with media

To ensure the maximum drug release, samples were collected at different time interval from 30 minutes to 6 hours using the following conditions.

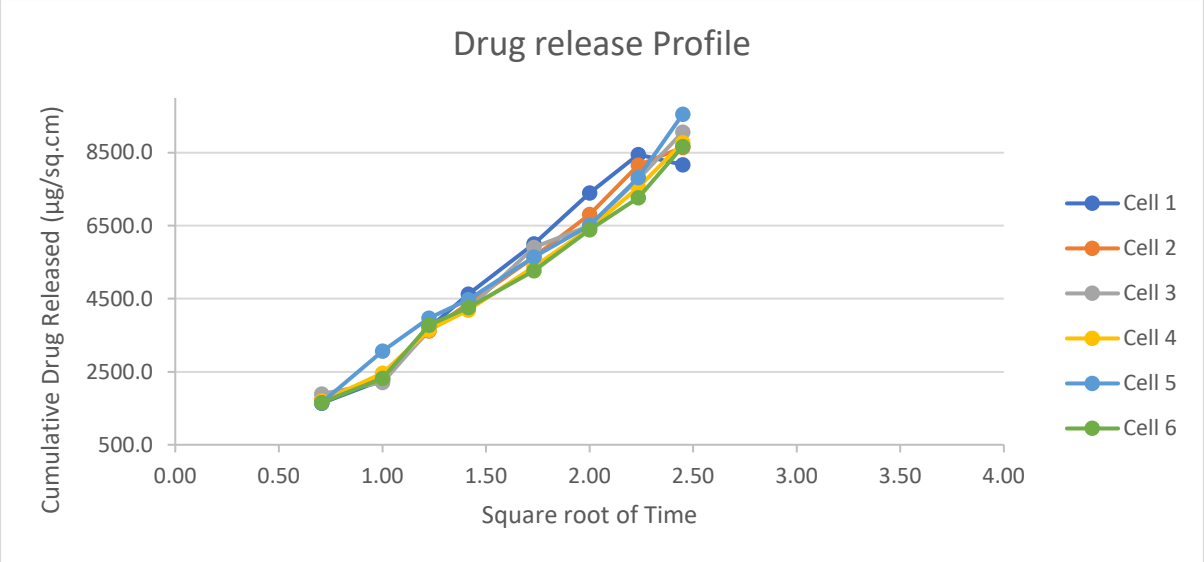
Dose amount	About 250 mg
Membrane	Nylon (0.45 micron)
Formulations used	100% Test (Batch Number 2189)
PDC Cell details	10 mL having orifice surface area of 0.64 cm^2
Receptor Media	Phosphate buffered saline pH 7.4: Ethanol (60:40 v/v)
Sample withdrawal	350 µL
Replacement Volume	350 µL partial replacement
Time points	0.50 hr, 1.00 hr, 1.50 hrs, 2.00 hrs, 3.00 hrs, 4.00 hrs, 5.00 hrs, 6.00 hrs.
Linearity range	1.25 µg/mL to 625 µg/mL

The % RSD & Regression were found within acceptance criteria. The slopes for all cells were found within the acceptance criteria. Release of time points after 6 hours were saturated. To avoid the evaporation of receptor media capped all the cell arm. The observed results for the studied time points is also linear, and release is fine. The results are mentioned below.

Table-2: IVRT drug release profile of test product

Diffusion Study Cumulative drug released/Diffused (µg/cm^2) for in house batch									
Time (Hrs)	**Square Root of Time**	**Cell 1**	**Cell 2**	**Cell 3**	**Cell 4**	**Cell 5**	**Cell 6**	**Average**	**% RSD**
0.5	0.71	1640.7	1731.2	1895.0	1694.3	1658.6	1658.6	1713.1	5.5
1	1.00	2278.8	2322.8	2203.9	2462.2	3064.1	2322.8	2442.5	12.9
1.5	1.22	3701.0	3621.2	3645.0	3643.3	3972.5	3777.0	3726.7	3.6
2	1.41	4622.9	4372.1	4219.7	4186.3	4476.7	4258.7	4356.0	3.9
3	1.73	6004.9	5642.4	5910.3	5384.6	5644.2	5262.8	5641.5	5.1
4	2.00	7401.3	6814.5	6504.0	6423.0	6509.3	6386.8	6673.2	5.8
5	2.24	8450.8	8163.3	7779.0	7546.9	7815.8	7263.5	7836.5	5.4
6	2.45	8165.6	8647.2	9067.5	8772.5	9551.7	8669.7	8812.4	5.3
Slope (vs. √Rt of Time)		**4240.1**	**4205.5**	**4193.3**	**4013.5**	**4167.8**	**3919.2**	**4114.1**	**3.1**
R-Squared (vs. √Rt of Time)		**0.98**	**0.99**	**0.98**	**0.99**	**0.98**	**0.99**	**1.00**	**0.7**

Figure-3: Drug release profile



Based on the above results, the % RSD, regression, and slopes for all the cells were found within the acceptance criteria.

IVRT DIFFUSION CELL SYSTEM PARAMETER

Based on the above trials, the following conditions were finalized for further analysis.

Parameter	Details
Receptor media	Phophate buffered saline pH 7.4: Ethanol (60:40) %v/v.
Temperature	32 ± 0.5°C
Apparatus	Hanson Teledyne Phoenix RDS or equivalent
Stirring speed	500 RPM
Dose applied	About 250mg
Orifice Surface area	0.636 cm^2
Dosage Lid cap	9mm x 4mm
Sample withdrawal	0.35 mL
Replacement volume	0.35 mL
Membrane	Nylon 0.45µm, 25mm
Sampling time points	0.5 hr, 1 hr, 1.5 hr, 2 hr,3 hr, 4 hr, 5 hr and 6 hr
Media volume	10 mL

D. Test versus Reference

Developed method was used to compare the release profile of test and reference product as per below conditions to show the formulation equivalencies.

Details of the experiment	Test_100%
Apparatus	Cell volume: 10 mL
Dose amount	About 250 mg
Orifice surface area	0.64 cm2
Stirring Speed	500 rpm
Temperature	32 ± 0.5°C
VDC Cell details	10 mL having orifice surface area of 0.64 cm2
Membrane	Nylon membrane 0.45µm, 25mm
Occluded/Non-occluded	Occluded
Receptor Media	Phosphate buffered saline pH 7.4: Ethanol (60:40)
Sample withdrawal	0.35 mL
Replacement volume	0.35 mL (Partial Volume Replacement)
Time points collected	0.00 hr, 0.50 hr, 1.00 hr, 1.50 hrs, 2.00 hrs, 3.00 hrs, 4.00 hrs, 5.00 hrs and 6.00 hrs

Table -3: IVRT drug release profile of test product and Reference product

Diffusion Study Cumulative drug released/Diffused (µg/cm²)														
Time (Hrs)	Square Root of Time	Test						RLD						
		Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6	% RSD
0.5	0.71	1640.7	1731.2	1895.0	1694.3	1658.6	1658.6	1744.7	1575.9	1538.9	1699.9	1665.3	1725.8	5.5
1	1.00	2278.8	2322.8	2203.9	2462.2	3064.1	2322.8	2482.0	2482.5	2431.7	2614.5	2591.4	2581.4	12.9
1.5	1.22	3701.0	3621.2	3645.0	3643.3	3972.5	3777.0	3217.7	3183.6	3144.3	3333.5	3322.3	3296.1	3.6
2	1.41	4622.9	4372.1	4219.7	4186.3	4476.7	4258.7	4030.0	3851.2	3806.2	3952.7	3965.2	3910.5	3.9
3	1.73	6004.9	5642.4	5910.3	5384.6	5644.2	5262.8	5098.3	4928.0	4957.4	5194.5	5342.8	5070.6	5.1
4	2.00	7401.3	6814.5	6504.0	6423.0	6509.3	6386.8	6166.5	6337.1	6199.3	6292.3	6097.4	6299.0	5.8
5	2.24	8450.8	8163.3	7779.0	7546.9	7815.8	7263.5	7840.6	8526.8	7904.8	8091.4	7626.9	8196.3	5.4
6	2.45	8165.6	8647.2	9067.5	8772.5	9551.7	8669.7	8746.8	9122.9	9308.8	9085.7	9640.3	9204.4	5.3
Slope (vs. √Rt of Time)		4240.1	4205.5	4193.3	4013.5	4167.8	3919.2	4071.5	4451.3	4373.3	4240.7	4302.8	4306.2	3.1
Average Slope		4114.1						4291						
R-Squared (vs. √Rt of Time)		0.92	0.97	0.98	0.99	0.98	0.97	0.98	0.97	0.97	0.98	0.97	0.97	0.7
Dose depletion (%)		105.3	107.6	95.8	90.3	99.1	103.9	93.2	101.0	101.5	99.1	105.1	100.4	NA

Generated results of test and reference products were subjected to statistical evaluation to find the sameness (Equivalency) of the formulation.

Product		Ratio (%)	90% C.I.		Equivalent
			Lower	Upper	
Test Vs Reference	Drug Release (R)	96.09	92.32	99.86	Yes
	Cumulative amount (A)	95.88	90.50	101.25	Yes

The product was developed for Europe market. Hence, EMA guideline was adopted to verify the results. As per EMA on *Draft guideline on quality and equivalence of topical products*, the 90% confidence interval for the ratio of means of the test and comparator products should be contained within the acceptance interval of 90 – 111%. The above results meet the acceptance interval and conclude the test and reference product is equivalent.

Method Validation

The purpose of validation is to demonstrate that, the test method used is suitable for its intended purpose and to establish documented evidence, which provides a high degree of assurance that the method shall yield results consistently and concurrently throughout the process and meet the predetermined quality attributes. The method was validated for the following parameters. Selectivity (Blank and Placebo Interference), specificity, linearity, precision & accuracy, recovery, membrane inertness, intermediate precision, and dose discrimination.

Summary of validation study for the IVRT method

Parameter	Results
Specificity	No significant interference was found at the retention time of analyte peak due to Diluent (Blank) and Placebo solution
Linearity	The method was linear from 1.2498 mg/mL to 624.900 mg/mL
Precision & Accuracy	The %CV and %nominal are within the limit. The between-run precision and accuracy of the method is acceptable. Correlation coefficient of Test samples is 0.99
Recovery	The % recoveries were found between 99.0%, 100.0% and 100.0% for the tested concentrations.
Membrane inertness	The analyte is stable in the presence of membrane and does not react/ bind to membrane. Nylon membrane filter 0.45 µm) is suitable.
Intermediate precision	Analytical method has acceptable level of reproducibility
Dose discrimination	The results indicate that the method is capable of distinguishing dose strength of 150%, 100% and 50% and method is discriminative.
System suitability	System suitability was established during the complete validation and results met the acceptance criteria

SUMMARY

IVRT is an efficient method for the evaluation of drug release from semisolid drug products. IVRT can be used as a method during product development and to evaluate the product quality over the period of time during shelf-life study or as a quality control test to release the batch. It is an excellent in-vitro tool to compare the test product (generics) against the reference product (Reference Listed Drug) to find the product equivalency. Recipharm had developed and validated an IVRT method as per the regulatory requirements. As a global CDMO, Recipharm is able to support various customers from different regions for the product development, analytical method development and validation activities. Phoenix RDS VDC system has provided satisfactory results to meet regulatory criteria.

About Recipharm

Recipharm is a leading contract development and manufacturing organisation (CDMO) headquartered in Stockholm, Sweden. We operate development and manufacturing facilities in France, Germany, India, Israel, Italy, Portugal, Spain, Sweden, the UK and the US and are continuing to grow and expand our offering for our customers. Employing around 9,000 people, we are focused on supporting pharmaceutical companies with our full service offering, taking products from early development through to commercial production. For over 25 years we have been there for our clients throughout the entire product lifecycle, providing pharmaceutical expertise and managing complexity, time and time again. Despite our growing global footprint, we conduct our business as we always have and continue to deliver value for money with each customer's needs firmly at the heart of all that we do. That's the Recipharm way.

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