In Vitro Release Test
A Development tool and More....

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IVRT

Traditionally, a variety of physical and chemical tests such as
- solubility,
- particle size,
- viscosity,
- form of API (crystalline or amorphous)
have been used to assure product performance for a semisolid dosage form.

More recently, In Vitro Release Test (IVRT) has provided a means of comprehensively and more directly assuring performance of a dosage form.
Semisolid Dosage Forms

1. Particle size of API
2. pH of API
3. Incorporation of API in semisolid matrix
4. Solubility of API in semisolid matrix

1. Viscosity
2. Spread ability
3. Overall pH
4. Moisture content of dosage form

1. Presence of emollients and penetration enhancers
2. Effect of excipients on release of API from matrix
3. Compatibility of excipients with API and with environmental agents such as moisture, gases, to affect the release of API
4. Manufacturing process
5. Manufacturing site
IVRT is a useful tool in

**Development phase**
Selection of appropriate clinical candidate formulation and characterization of the dosage form

**Clinical phases**
Monitoring and correlating the in vivo vs. in vitro results—further characterization of final formulation

**Post-approval**
Changes in manufacturing site, composition, manufacturing process
Dissolution and/or Drug Release

Identify Critical Manufacturing Variables

Formulation Development

Quality Control

Gauge in-vivo Performance

Product Performance Assessment

Post Approval Changes
Applications of IVRT

- Release test to support formulation development
- Release test to compare generic vs innovator formulation
- Release test to characterize clinical formulation/gather release data for the duration of clinical trial
- Validated release test to compare new and old formulations post marketing per SUPAC-SS
Considerations in Developing a Release Test Using Vertical Diffusion Cells

The Release Test Must be

- Accurate in providing same release profile from day to day for the same lot of formulation
- Discriminatory for different dosage strengths
- Sensitive to differences in excipients
- Rugged
- Robust
Introduction - Summary

- In principle, release tests are based on passive diffusion of active ingredient from the product matrix into a receiving medium.

- There are many tests/apparatuses for different types of semisolid dosage forms.

- Among these, release testing using Vertical diffusion cells is widely used for many traditional and novel topical products.
Development of IVRT to support Formulation Development

- Assay Modification
- Selection of Membrane
- Selection of Receiving Medium
IVRT for Formulation Development Support

- Early stage formulations to be tested..need to have test sensitive to discern differences between formulations with different excipients, viscosity, particle size.
- Necessary to be able to run several formulations simultaneously for good comparison..often 3-cell runs, good precision important.
- Some correlation with early pharmacological data desirable to decide on final one or two clinical candidates.
Selection of Membrane: Filter Interference

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Formulation 1 (0.02%)</th>
<th>Formulation 2 (0.02%)</th>
<th>Formulation 3 (0.05%)</th>
<th>Formulation 4 (0.05%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulosic</td>
<td>83</td>
<td>82</td>
<td>90</td>
<td>87</td>
</tr>
<tr>
<td>Fluoropore</td>
<td>89</td>
<td>86</td>
<td>97</td>
<td>90</td>
</tr>
<tr>
<td>Nylon</td>
<td>92</td>
<td>85</td>
<td>99</td>
<td>89</td>
</tr>
<tr>
<td>Polycarbonate</td>
<td>96</td>
<td>90</td>
<td>104</td>
<td>95</td>
</tr>
<tr>
<td>Supor</td>
<td>89</td>
<td>85</td>
<td>96</td>
<td>89</td>
</tr>
</tbody>
</table>
Selection of Receiving Medium: Solubility of API in Receiving Medium

<table>
<thead>
<tr>
<th>Solvent Ratio (EtOH:Aqueous*)</th>
<th>Solubility (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>Sparingly Soluble</td>
</tr>
<tr>
<td>30:70</td>
<td>6.89</td>
</tr>
<tr>
<td>40:60</td>
<td>64.09</td>
</tr>
<tr>
<td>50:50</td>
<td>216.61</td>
</tr>
</tbody>
</table>

- *Phosphate buffer pH 4.0
- Solubility of API in 30:70 EtOH:phosphate buffer is too low for sink conditions especially for doses higher than 0.05%.
- Solubility of API in 40:60 EtOH:phosphate buffer is sufficient to allow for sink conditions.
- 40:60 receiving medium was selected for next studies.
Preliminary Release Rate Test Conditions

- Preliminary IVRT conditions:
  - Heater/circulator set to 37 ± 0.05°C for vaginal application
  - Cells filled with receiving medium (ethanol:phosphate buffer pH 4.0, 40:60)
  - Membrane (polycarbonate) clamped onto bottom of each of 6 cells
  - ~300mg vaginal gel applied to each cell
  - 200µL aliquot removed at 0.5, 1, 2, 4, 6, and 24 hours and replaced with fresh medium
  - API concentration in receiving medium determined by reversed-phase HPLC

- The conditions were further optimized during method development (noted in subsequent slides)
## Preliminary Studies: Test Gel Variants

<table>
<thead>
<tr>
<th>Form. No.</th>
<th>API (%)</th>
<th>Gel Type</th>
<th>Gel Content Variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.01</td>
<td>Solution</td>
<td>PG, polymers, pH 7</td>
</tr>
<tr>
<td>B</td>
<td>0.02</td>
<td>Suspension</td>
<td>EtOH, polymers, pH 6</td>
</tr>
<tr>
<td>C</td>
<td>0.02</td>
<td>Suspension</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.05</td>
<td>Suspension</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.02</td>
<td>Solution</td>
<td>Vitamin E, TPGS, PG, polymers, pH 6</td>
</tr>
<tr>
<td>F</td>
<td>0.01</td>
<td>Solution</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>0.02</td>
<td>Solution</td>
<td>Cremophor, PG, polymers, pH 6</td>
</tr>
<tr>
<td>H</td>
<td>0.01</td>
<td>Solution</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>0.02</td>
<td>Solution</td>
<td>Tween20, PG, polymers, pH 6</td>
</tr>
<tr>
<td>K</td>
<td>0.01</td>
<td>Solution</td>
<td></td>
</tr>
</tbody>
</table>
Preliminary IVRT of Gel Variants

Average Cumulative Amount of API Released into Receiving Medium (Nylon Membrane)

Cumulative Amount of API Released

Square Root of Time (hours)

- C
- D
- E
- F
- G
- H
- J
- K
- L
- A
- B

(n) Average Cumulative Amount of API Released into Receiving Medium (Nylon Membrane)
Variables to be Evaluated for Dependence on Release Rate:

- Composition of Formulation
- Dosage form Strength
- Particle Size of API
- Stress/viscosity
- Air exposure
- Sample weight
- Consistency in release rate over time
## Release Rate Dependence on Formulation Composition

<table>
<thead>
<tr>
<th>API (% w/w)</th>
<th>Formulation</th>
<th>Release Rate ($\mu g/cm^2/hr^{0.5}$)</th>
<th>Standard Deviation</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>2% vitamin E TPGS, 28% propylene glycol, modified polymers, pH6</td>
<td>2.90</td>
<td>0.64</td>
<td>22</td>
</tr>
<tr>
<td>0.01</td>
<td>2% Tween 20, 28% propylene glycol, modified polymers, pH6</td>
<td>5.63</td>
<td>0.36</td>
<td>6.4</td>
</tr>
<tr>
<td>0.01</td>
<td>2% Cremophor, 28% propylene glycol, modified polymers, pH6</td>
<td>5.86</td>
<td>0.93</td>
<td>16</td>
</tr>
<tr>
<td>0.01</td>
<td>40% propylene glycol, modified polymers, pH7</td>
<td>6.14</td>
<td>1.2</td>
<td>19</td>
</tr>
<tr>
<td>0.025</td>
<td>2% Cremophor, 28% propylene glycol, modified polymers, pH6</td>
<td>11.7</td>
<td>1.1</td>
<td>9.3</td>
</tr>
<tr>
<td>0.025</td>
<td>2% vitamin E TPGS, 28% propylene glycol, modified polymers, pH6</td>
<td>11.9</td>
<td>1.3</td>
<td>11</td>
</tr>
<tr>
<td>0.025</td>
<td>2% Tween 20, 28% propylene glycol, modified polymers, pH6</td>
<td>13.9</td>
<td>2.0</td>
<td>15</td>
</tr>
<tr>
<td>0.025</td>
<td>vitamin E TPGS, 5% propylene glycol, modified polymers, pH6</td>
<td>14.7</td>
<td>2.3</td>
<td>16</td>
</tr>
<tr>
<td>0.025</td>
<td>4% ethanol, modified polymers, pH6</td>
<td>15.4</td>
<td>2.0</td>
<td>13</td>
</tr>
</tbody>
</table>
Release Rate Dependence on Dosage Strength

Average Cumulative Amount of API Released

Time (hours^{0.5})

0.025% API

0.01% API
# Release Rate Dependence on Particle Size

<table>
<thead>
<tr>
<th>Formulation Number</th>
<th>API Conc.</th>
<th>% Dose</th>
<th>Milling Type Source</th>
<th>Particle Size (d90)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-2</td>
<td>A-5</td>
<td>A-6</td>
<td></td>
</tr>
<tr>
<td>Formula 2</td>
<td>0.025%</td>
<td>0.05%</td>
<td>0.05% stressed&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;5 µm</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>0.10%</td>
<td>0.05%</td>
<td>15.1 µm</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>0.05%</td>
<td>0.05%</td>
<td>144 µm</td>
</tr>
<tr>
<td>Formula 1</td>
<td>0.025%</td>
<td>0.05%</td>
<td>.1%</td>
<td>&lt;5 µm</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>.05%</td>
<td>0.05%</td>
<td>15.1 µm</td>
</tr>
<tr>
<td></td>
<td>.05%</td>
<td>.05%</td>
<td>0.05%</td>
<td>144 µm</td>
</tr>
</tbody>
</table>

- The primary and secondary formulations are similar water-based gels that differ in solvent and preservative content. The primary formulations contained deionized water, methylparaben, propylparaben, HEC, polycarbophil, NaOH solution, propylene glycol, and vitamin E. Secondary formulations contained deionized water, sorbic acid, NaOH solution, dapivirine, HEC, Pluronic F127NF, and Klucel MF Pharm.
- Compound was stressed at 60°C for 4 weeks
- Jet micronization at Facility 1
- Jet micronization at Facility 2
Release Rate Dependence on Particle Size

Average Cumulative Amount of API Released

Square Root of Time (hours)
Release Rate Dependence on Stress-Induced Viscosity Changes

- Concentration-time curves for stressed and unstressed samples were significantly different (Wilcoxon Rank Sum/Mann-Whitney statistical rank test)
- Average viscosity of stressed samples increased to 10.8 kPa vs. 8.26 kPa for unstressed samples
- Rate of dissolution in receiving medium decreased with increase in viscosity in stressed samples
- Data suggest method can distinguish between stressed and unstressed gels that differ in small changes in viscosity

*compound was stressed at 60°C for 4 weeks*
Release Rate Dependence on Air Exposure During Testing

**Formulation 1**

- Individual Flux Values (ug/(cm²)/(time)¹/₂
- Time of exposure (minutes)

**Formulation 2**

- Individual Flux Values (ug/(cm²)/(time)¹/₂
- Time of Exposure (minutes)
Release Rate Dependence on Sample Amount

Average Slope vs. Sample Amount in mgs Used
Consistency of Release Rate Measurement Over Long Period of Time

Number of Days Over which Batches of Formulation 2 were Tested
Application of Release Rate Measurement During Stability Testing: 30 C 60% R.H.
# Rate of Release ($\mu g/cm^2/hours^{1/2}$) of Compound 1

<table>
<thead>
<tr>
<th>Formulation #</th>
<th>pH 3.5 Phosphate Buffer: Ethanol 65:35 v/v</th>
<th>pH 5.5 Phosphate Buffer: Ethanol 65:35 v/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0374</td>
<td>0.141</td>
</tr>
<tr>
<td>2</td>
<td>0.040</td>
<td>0.034</td>
</tr>
<tr>
<td>3</td>
<td>0.106</td>
<td>0.040</td>
</tr>
<tr>
<td>4</td>
<td>0.478</td>
<td>0.290</td>
</tr>
<tr>
<td>5</td>
<td>0.091</td>
<td>0.087</td>
</tr>
<tr>
<td>6</td>
<td>0.139</td>
<td>0.041</td>
</tr>
<tr>
<td>7</td>
<td>0.241</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>0.353</td>
<td>0.327</td>
</tr>
<tr>
<td>9</td>
<td>0.052</td>
<td>0.054</td>
</tr>
</tbody>
</table>
Sensitivity to Changes: Excipients

Formulation 2:
Twice the amount of Emollient as Formulation 1 + 3% emulsifier

Formulation 1
Sensitivity to Changes: Process

- Average Flux For Compound 1 Formulations ($\mu g/cm^2/\text{hours}^{1/2}$)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug Dissolved in</th>
<th>Flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-A</td>
<td>100% pre-dissolved in organic solvent</td>
<td>0.189</td>
</tr>
<tr>
<td>1-B</td>
<td>60% pre-dissolved in organic solvent, 40% dissolved directly into oil phase</td>
<td>0.110</td>
</tr>
<tr>
<td>2-A</td>
<td>100% dissolved in oil phase</td>
<td>0.603</td>
</tr>
<tr>
<td>2-B</td>
<td>100% pre-dissolved in organic solvent</td>
<td>1.406</td>
</tr>
</tbody>
</table>
## Sensitivity to Changes: Scale-up

- **Average Flux For Compound 1 Formulations (μg/cm²/hours\(^{1/2}\))**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Lot #</th>
<th>Avg Flux μg/cm²/hours(^{1/2})</th>
<th>Avg. Total Amt. Released after 6 hours std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A (3 kg)</td>
<td>0.347</td>
<td>1.200 0.071</td>
</tr>
<tr>
<td>1</td>
<td>B (100 kg)</td>
<td>0.406</td>
<td>1.412 0.028</td>
</tr>
<tr>
<td>2</td>
<td>A (3 kg)</td>
<td>0.054</td>
<td>0.145 0.021</td>
</tr>
<tr>
<td>2</td>
<td>B (100 kg)</td>
<td>---</td>
<td>0.020 0.013</td>
</tr>
<tr>
<td>3</td>
<td>C (3 kg)</td>
<td>0.290</td>
<td>0.855 0.140</td>
</tr>
<tr>
<td>3</td>
<td>C (100 kg)</td>
<td>0.258</td>
<td>0.799 0.085</td>
</tr>
</tbody>
</table>
Validation

- Attributes to be validated:
  - Precision
  - Accuracy/Sameness
  - Dose proportionality
  - Sensitivity to changes in
    - Excipient type
    - Amount of Excipient
    - Size of Batch
    - Method of manufacture
Validation

- Mass Balance
- Back Diffusion of Alcohol in the Dosage Form
- Dependence of Release Rate on Temperature
- Differences in Instrumentation
Average Release of Compound B Through Fluoropore Membrane into Ethanol:Water 20:80 v/v 32°C

- Day 1
- Day 2
- Day 3
- Day 4
- Day 5
- Day 6

Precision
IVRT Method Precision
Average Release of Compound 2 with Different Strengths Through Fluoropore Membrane into 20:80 ethanol: Water 20:80 v/v, 32°C

- **Compound 2 Formulation:** 200% of Label Claim
- **Compound 2 Formulation:** 50% of Label Claim
- **Compound 2 Formulation:** 100% of Label Claim
IVRT Method Ruggedness (Slope)
Comparison of Release of API from Innovator and Generic Formulation
Comparison of Innovator Vs. Generic Formulation

Cumulative Amount Released (ug) vs. SQRT Time
SUPAC-SS Guidance

- Issued in May 1997
- IVRT can be substituted for a clinical trial in certain cases

**Excipients:**

- **Level 2 change:** changes that are likely to have a significant impact on formulation quality and performance. Examples are –
  - changes in >5% but <10% of an approved excipient,
  - changes in supplier or technical grade of a structure forming excipient,
  - changes in particle size of API when it is suspension

- **Level 3 change:** changes that are likely to have a significant impact on formulation quality and performance. Examples are –
  - changes in excipient beyond the ranges in level 2,
  - changes in crystalline form of API—IVRT not required but highly encouraged
Manufacturing:
• Level 2 change:
  Change in equipment to a different design or different operating principles - IVRT required

Process:
• Level 2 change:
  Process change such as rate of mixing, mixing times, rate of cooling, operating speeds, holding times outside approved application ranges - IVRT required
**Batch size:**

- **Level 2 change:**
  Changes in batch size beyond a factor of ten times the pivotal/clinical batch. IVRT required

**Manufacturing site**

- **Level 3 change:**
  Different campus—IVRT required
Implementation of SUPAC-SS Requirements

- IVRT method established during development phase
- Data is collected for one or more likely candidates for clinical formulation
- IVRT method is validated for at least one final formulation
- Upon post approval change(s), the new batch manufactured after change is compared with reference batch (manufactured before change)
First level of comparison:

- 6-cell run each with reference and test batch carried out simultaneously
- For each run, it is suggested that the experiments be run as follows:

Slopes are calculated for each cell.
- Ratios of slopes are calculated as shown in example and ranked.

Criteria: 8th and 29th ranked slope ratios should fall between 75% and 133.33%.
Example of SUPAC-SS Statistical comparison between two formulations

<table>
<thead>
<tr>
<th>Ratios</th>
<th>25.0280</th>
<th>25.2617</th>
<th>23.1360</th>
<th>29.9832</th>
<th>27.1041</th>
<th>22.7909</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.1877</td>
<td>0.85</td>
<td>0.84</td>
<td>0.92</td>
<td>0.71</td>
<td>0.78</td>
<td>0.93</td>
</tr>
<tr>
<td>20.1295</td>
<td>0.80</td>
<td>0.80</td>
<td>0.87</td>
<td>0.67</td>
<td>0.74</td>
<td>0.88</td>
</tr>
<tr>
<td>20.0860</td>
<td>0.80</td>
<td>0.80</td>
<td>0.87</td>
<td>0.67</td>
<td><strong>0.7411</strong></td>
<td><strong>0.8813</strong></td>
</tr>
<tr>
<td>19.5011</td>
<td>0.78</td>
<td>0.77</td>
<td>0.84</td>
<td>0.65</td>
<td>0.72</td>
<td>0.86</td>
</tr>
<tr>
<td>20.9997</td>
<td>0.84</td>
<td>0.83</td>
<td>0.91</td>
<td>0.70</td>
<td>0.77</td>
<td>0.92</td>
</tr>
<tr>
<td>21.7415</td>
<td>0.87</td>
<td>0.86</td>
<td>0.9397</td>
<td>0.73</td>
<td>0.80</td>
<td>0.95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ranks</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>14</td>
<td>17</td>
<td>5</td>
<td>32</td>
<td>24</td>
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</tr>
<tr>
<td>67</td>
<td>19</td>
<td>22</td>
<td>9</td>
<td>34</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>68</td>
<td>20</td>
<td>23</td>
<td>11</td>
<td>35</td>
<td><strong>29</strong></td>
<td><strong>8</strong></td>
</tr>
<tr>
<td>69</td>
<td>25</td>
<td>27</td>
<td>15</td>
<td>36</td>
<td>31</td>
<td>13</td>
</tr>
<tr>
<td>70</td>
<td>16</td>
<td>18</td>
<td>6</td>
<td>33</td>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>71</td>
<td>10</td>
<td>12</td>
<td>2</td>
<td>30</td>
<td>21</td>
<td>1</td>
</tr>
</tbody>
</table>
Second level of testing:

Failure at first level triggers the second level of comparison
- 4 additional runs of 6-cell each are carried out and slopes are computed.
- A total of 18 slopes for each batch is obtained and same T/R ratios are computed and ranked.

Criteria: 110th and 215th ranked ratios should fall within 75 to 133.33%