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# Development of a Lyophilization Cycle for the Manufacture of a Phase I Intravenous Formulation of the Novel Boron-Containing Antibacterial Agent GSK2251052

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## Abstract

**Purpose:** To develop a fit-for-purpose lyophilization cycle for the manufacture of an intravenous formulation of the novel, boron-containing antibacterial agent GSK2251052 (formerly AN3365) for use in a First-in-Human (FiH) clinical trial. GSK2251052 is a leucyl-tRNA synthetase inhibitor with *in vitro* and *in vivo* activity against *Pseudomonas aeruginosa* and multidrug-resistant *Enterobacteriaceae* currently in development for the treatment of serious Gram-negative bacterial infections.

**Methods:** GSK2251052 is a small molecule with physicochemical characteristics (readily crystalline, high water solubility, excellent solid state stability, fast dissolution kinetics) suitable for development as a lyophilized powder for reconstitution. DSC studies were conducted to identify glass transition, crystallization, and eutectic melting temperatures, define drying temperatures, and assess needs for thermal treatment. Exploratory cycles were first tested in a laboratory-scale VirTis Genesis 25EL laboratory lyophilizer set in a non-sterile environment. To target the required 600 mg GSK2251052 (as HCl salt) per vial, runs were executed using 120 mg/g GSK2251052.HCl aqueous solution in a small number of 10-mL vials. To simulate the vapor load of a full lyophilizer but save on drug substance, 5% aqueous potassium chloride was used as a surrogate. Water content analyzed by Karl Fischer was used to determine the length of the drying stages. The resulting freeze-dryer cycle was then transferred to a VirTis Ultra 35EL lyophilizer in a sterile, particle-free, cGMP environment.

**Results:** The final cycle used for clinical product was derived from lab scale lyophilization runs, DSC, and KF data, and included an annealing step (-50°C to -20°C), primary drying at -10°C, and secondary drying at 30°C. To ensure cycle success while keeping with short development timelines and limited drug substance supplies, each step was then set to a conservatively long duration. The cGMP clinical batch of 2600 single-use, sterile vials for reconstitution containing 600 mg/vial GSK2251052 (HCl salt) for intravenous use was successfully manufactured, released, and shown to be stable for at least 12 months when stored at 5°C.

**Conclusion:** A fit-for-purpose lyophilization cycle was efficiently developed under significant drug substance constraints to enable the successful initiation and conduct of a FiH clinical trial by the intravenous route.

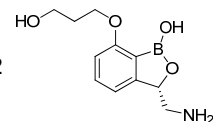


Figure 1. Structure of GSK052

## Results

### DSC Data in Support of Cycle Development

Differential Scanning Calorimetry (DSC) runs were performed to determine if a thermal treatment (annealing) step should be incorporated into the lyophilization cycle to aid crystallization in a particle free environment such as that of the sterile cGMP suite. For these experiments, 120 mg/g aqueous GSK2251052 (HCl salt) solution was analyzed. As shown on Figures 2 and 3, the crystallization temperature of 120 mg/g aqueous GSK2251052 is approximately -22°C. The absence of a glass transition and crystallization event following thermal treatment at -18°C as seen in Figure 4 indicates that an annealing step would be advantageous in facilitating crystallization.

Figure 2. Results from DSC Procedure 1, Cycle 2  
Cycle 1 - Ramp to -60°C then isothermal Cycle 2 - Ramp to 30°C

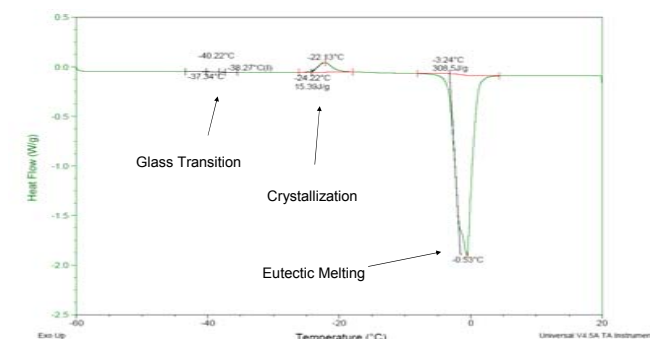


Figure 3. Results from DSC Procedure 2, Cycle 2  
Cycle 1 - Ramp to -60°C then isothermal Cycle 2 - Ramp to -18°C then isothermal

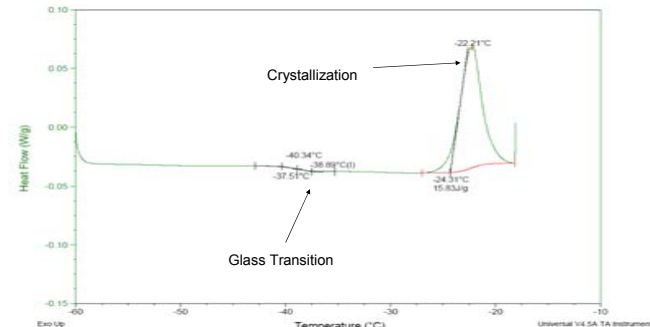
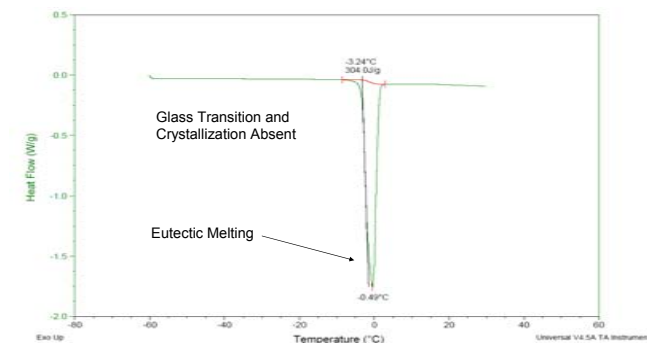


Figure 4. Results from DSC Procedure 2, Cycle 4  
Cycle 3 - Ramp to -60°C then isothermal Cycle 4 - Ramp to 30°C



### Pilot Scale Work

Pilot scale lyophilization trials were run in a VirTis Genesis 25EL laboratory scale lyophilizer with a capacity of 880 10-mL vials. Due to drug substance constraints, each run was executed with 16 to 20 vials containing 5-mL of 120 mg/g GSK2251052 (as HCl salt) aqueous solution - using an approximate total of ~2 g of drug substance. For each run, the active vials were distributed throughout the lyophilizer. The lyophilizer was then filled to capacity with vials containing 5-mL of 5% aqueous potassium chloride, which served as surrogates to simulate the vapor load of a full lyophilizer.

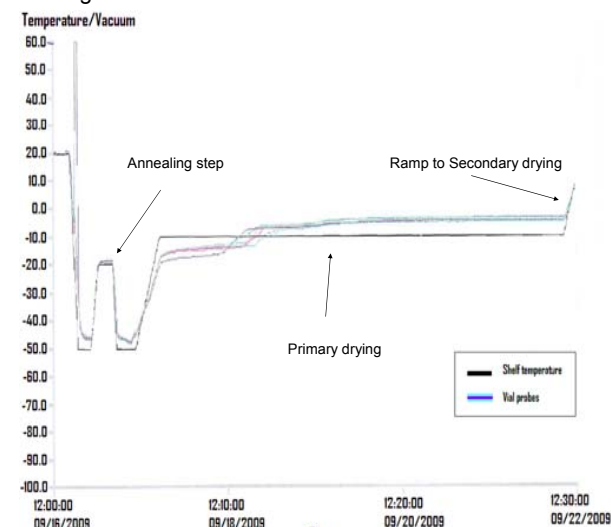
Karl Fischer analysis was used to determine the effectiveness of primary drying by analyzing vials that were freeze-dried using a partial cycle that incorporated thermal treatment and primary drying, but was arrested prior to secondary drying. These results were instrumental in determining the effectiveness of the primary drying phase, and the set points for the secondary drying phase.

### Scale-Up and cGMP Production

Due to the short development timeline and limited drug substance, the scaled up lyophilization cycle was developed to be fit-for-purpose. As such, the focus was on insuring that the product vials produced would meet all drug product specifications and remain stable for the course of the clinical study. To that end, the cycle times for the primary and secondary drying phases were set to be conservatively long.

For the manufacture of the cGMP clinical material, the process was transferred to a sterile, particle-free facility. The lyophilizer was a VirTis Ultra 35EL and was loaded to its full capacity of 2600 10-mL vials each containing 120 mg/g aqueous GSK2251052 (as HCl salt). The resulting lyophilization cycle included an annealing step from -50°C to -20°C, followed by primary drying at -10°C for approximately 4.5 days (6658 minutes) and then secondary drying at 30°C for 12 hours. Figure 5 shows monitoring of the shelf temperature and vial probes through primary drying during cGMP production.

Figure 5. Vial and Shelf Temperature Monitoring During cGMP Production



### SAD and MAD Dosing with GSK2251052: Flexibility by Design<sup>1,2</sup>

GSK2251052 was administered in the FiH clinical trials by IV infusion. The clinical trial material, designed as single use, 600-mg GSK2251052 HCl salt lyo vials, afforded both dosing flexibility and efficient use of drug. Doses ranging from 200 to 3000 mg were administered in 200 mL of 0.9% sodium chloride for injection, USP and infused over one hour by simply adjusting the number of vials of GSK2251052 HCl salt that were reconstituted for the final delivered dose. Dosed solution stability, pH, osmolality, and compatibility with IV sets were assessed as part of the formulation development.

### Clinical Trial Material

Approximately 2600 single-use vials were produced in a single lyophilization run for use in the First-in-Human clinical trial. Each vial contained 600 mg of GSK2251052 powder (HCl salt). The drug product was a solid, white lyophilized cake intended for reconstitution in 10 mL of 0.9% sodium chloride for injection, USP. The product met all release specifications and was stable through 12 months when stored at 5°C. Table 1 shows the release and stability results.

## Conclusion

A fit-for-purpose lyophilization cycle was efficiently developed under significant drug substance and time constraints to enable the successful initiation and conduct of a FiH clinical trial by the intravenous route.

## Acknowledgments

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### References

1. LT Zane, S Shakib, R Milne, L Liu, SJ Baker, FA Heerinx. Safety, Tolerability, and Pharmacokinetics of a Novel Gram-Negative Antimicrobial, SDK2251052, in Healthy Subjects. Poster, 21<sup>st</sup> European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), May 7 - 10, 2011, Milan, Italy (available at [www.anacor.com/scientific\\_presentations.php](http://www.anacor.com/scientific_presentations.php))
2. S Yep and D Imbert. Pre-formulation of GSK2251052, A Novel Boron-Containing Small Molecule Designed with Ideal CMC Properties and In Development for the Treatment of Serious Gram Negative Bacterial Infections. Poster W4048, 2011 Annual Meeting of the American Association of Pharmaceutical Scientists (AAPS), October 23 - 27, 2011, Washington, D.C.

Table 1. Release and Stability Results cGMP Clinical Material (5°C)

Parameter	Limits	Initial	6 Month	12 Month
Appearance	White to off-white lyophilized cake	White lyophilized cake	White lyophilized cake	White lyophilized cake
Reconstitution Time (secs)	Report	35	73	47
Post-reconstitution appearance	Clear, colorless solution	Clear, colorless solution	Clear, colorless solution	Clear, colorless solution
pH	Report	3.33	3.40	3.23
Osmolality (mOsm/kg)	Report	615	595	598
Potency (Assay %)	90.0 - 110.0%	101.4%	99.7%	103.5%
Related Substances	Total <3.0%	0.08%	0.21%	0.02%
Water content	Report	0.22%	0.97%	0.55%
Particulate Matter ≥ 10µm per container	Not more than 6000 particulates	156	24	35
Particulate Matter ≥ 25µm per container	Not more than 600 particulates	2	1	4
Sterility	Conforms to USP <71>	Conforms	Not Tested	Conforms