S.4.4 BATCH ANALYSIS

The batch analyses of commercial scale, primary stability, and relevant clinical and toxicology drug substance batches manufactured are described herein.

Batch information (Batch No., Place of Manufacture, Date of Manufacture, Batch Size, Manufacturing Process, Use of Batch) is presented in Section 1.

Batch analyses data (data obtained from release testing) is presented in Section 2.

Relevant method history is summarized in Section 3.

1. BATCHES TESTED

The manufactured drug substance batches are categorized according to their intended use in the tables below.

Table S.4.4-1 Commercial Scale Batches of Drug Substance APItinib

Batch No.	Place of Manufacture	Date of Manufacture	Batch Size (kg)	Manufacturing Process	Use of Batch
Batch 1	Facility 2	15/8/2020	14	Process 0.1	Clinical
Batch 2		19/8/2020	32	Process 0.1	Clinical
Batch 3		24/9/2020	52	Process 0.6	Clinical / Supportive Stability
CAT1	Facility 8	2/1/2022	120	Commercial 1.0	Validation and Stability
CAT2		2/2/2022	120	Commercial 1.0	Validation and Stability
CAT3		2/3/2022	120	Commercial 1.0	Validation and Stability

Facility-2 is located at Smolecule Pharma, Omaha, NE, USA.
Facility 8 is located at Smolecule Pharma, Birmingham, AL, USA.

2. BATCH ANALYSIS DATA (TABULATED)

Batch analyses data (in tabular format) is presented herein.

Test procedure	Acceptance criteria	Method reference	Batch 1	Batch 2	Batch 3
Description	A white to brown powder	Visual inspection	Pass	Pass	Pass
Identification	Conforms with reference	Identification by IR spectroscopy	Conforms	Conforms	Conforms
Identification	Conforms with reference	Identification by HPLC	Conforms	Conforms	Conforms
Assay (on water and solvent free basis)	98% to 102% w/w	Assay by LC	94[1]	95[1]	96[1]
Related Organic impurities:					
Impurity 1	NMT 0.5% w/w	Organic impurities by LC	0.5	0.4	0.2
Impurity 2	NMT 0.5% w/w	Organic impurities by LC	0.5	0.4	0.3
Impurity 3 [2]	NMT 0.5% w/w	Organic impurities by LC	0.1	0.1	0.1
Any individual unspecified impurity	NMT 0.10% w/w	Organic impurities by LC	0.02	0.02	0.04
Total impurities	NMT 2.0% w/w	Organic impurities by LC	1.12	0.92	0.64
Mutagenic impurities					
Impurity - Mutagenic	NMT 50 ppm	ACP-5187 content by LC-MS	20	22	18
Enantiomeric purity	NLT 99.6%	LC	91.5[1]	93.5[1]	99.7[3]
Residual solvents:					
Solvent 1	NMT 0.1% w/w	Residual solvents by headspace GC	0.1	0.1	0.1
Solvent 2	NMT 2.0% w/w	Residual solvents by headspace GC	1.4	1.5	1.2
Water content	NMT 1.0%	Titration	0.8	0.8	0.8
Particle size distribution [4]					
$D_{(v, 0.9)}$	NMT 319 µm	Laser diffraction	300	250	210

Test procedure	Acceptance criteria	Method reference	Batch 1	Batch 2	Batch 3
$D_{(v, 0.5)}$	NMT 145 µm	Laser diffraction	50	80	75
$D_{(v, 0.1)}$	NMT 20 µm	Laser diffraction	5	6	7
ROI /SA	NMT 0.1% w/w	USP	0.1	0.1	0.1

[1] Batch 1, Batch 2 and Batch 3 met the clinical specification in place at time of testing; clinical specification release limit for Assay was 93% w/w; clinical specification release limit for Enantiomeric Purity was NLT 91.0%
[2] Impurity 3 was included in the release specification throughout clinical development, and no longer forms a part of a commercial release specification; see Section S.4.5 Justification of Specification and Section S.2.5 Validation (PPQ) for additional data
[3] Batch 3 did not originally meet specification criteria, and was subject to reprocessing and repurification. The updated result is presented in this table. The lot was not distributed for human use.
[4] Particle Size Distribution was included in the release specification throughout clinical development, and no longer forms a part of the commercial release specification; See Section S.4.5 Justification of Specification for data and rationale.

Test procedure	Acceptance criteria	Method reference	CAT1	CAT2	CAT3
Description	A white to brown powder	Visual inspection	Pass	Pass	Pass
Identification	Conforms with reference	Identification by IR spectroscopy	Conforms	Conforms	Conforms
Identification	Conforms with reference	Identification by HPLC	Conforms	Conforms	Conforms
Assay (on water and solvent free basis)	98% to 102% w/w	Assay by LC	98	99	99
Related Organic impurities:					
Impurity 1	NMT 0.5% w/w	Organic impurities by LC	0.4	0.4	0.5
Impurity 2	NMT 0.5% w/w	Organic impurities by LC	0.3	0.4	0.2
Impurity 3 [1]	NMT 0.5% w/w	Organic impurities by LC	ND	ND	ND
Any individual unspecified impurity	NMT 0.10% w/w	Organic impurities by LC	0.01	0.07	0.04
Total impurities	NMT 2.0% w/w	Organic impurities by LC	0.71	0.87	0.74

Test procedure	Acceptance criteria	Method reference	CAT1	CAT2	CAT3
Mutagenic impurities					
Impurity - Mutagenic	NMT 50 ppm	ACP-5187 content by LC-MS	33	31	30
Enantiomeric purity	NLT 99.6%	LC	99.9	99.9	99.8
Residual solvents:					
Solvent 1	NMT 0.1% w/w	Residual solvents by headspace GC	0.1	0.1	0.1
Solvent 2	NMT 2.0% w/w	Residual solvents by headspace GC	1.1	1.1	1.4
Water content	NMT 1.0%	USP Karl Fischer titration	0.8	0.8	0.8
Particle size distribution [2]					
$D_{(v, 0.9)}$	NMT 319 µm	Laser diffraction	150	100	50
$D_{(v, 0.5)}$	NMT 145 µm	Laser diffraction	100	40	20
$D_{(v, 0.1)}$	NMT 20 µm	Laser diffraction	8	1	2
ROI /SA	NMT 0.1% w/w	USP	0.1	0.1	0.1

[1] Impurity 3 was included in the release specification throughout clinical development, and no longer forms a part of a commercial release specification; see Section S.4.5 Justification of Specification and Section S.2.5 Validation (PPQ) for additional data
[2] Particle Size Distribution was included in the release specification throughout clinical development, and no longer forms a part of the commercial release specification; See Section S.4.5 Justification of Specification for data and rationale.

3. METHOD DEVELOPMENT HISTORY (SIGNIFICANT METHOD CHANGES MADE THROUGHOUT DEVELOPMENT)

The proposed methods for commercial use are described in the appropriate sections of this application.

This section outlines the method history that serves to contextualize batch analyses results. No significant changes have been made to the methods used during manufacturing development, except for the Water Content method as described below.

Water Content

Early in development (Facility 2), water content was measured in-house by a novel research-grade proprietary titration method. Due to issues with validating the method, and once the process was moved to Facility 8, the widely accepted compendial Karl Fischer Volumetric titration method was implemented for determination of water content.