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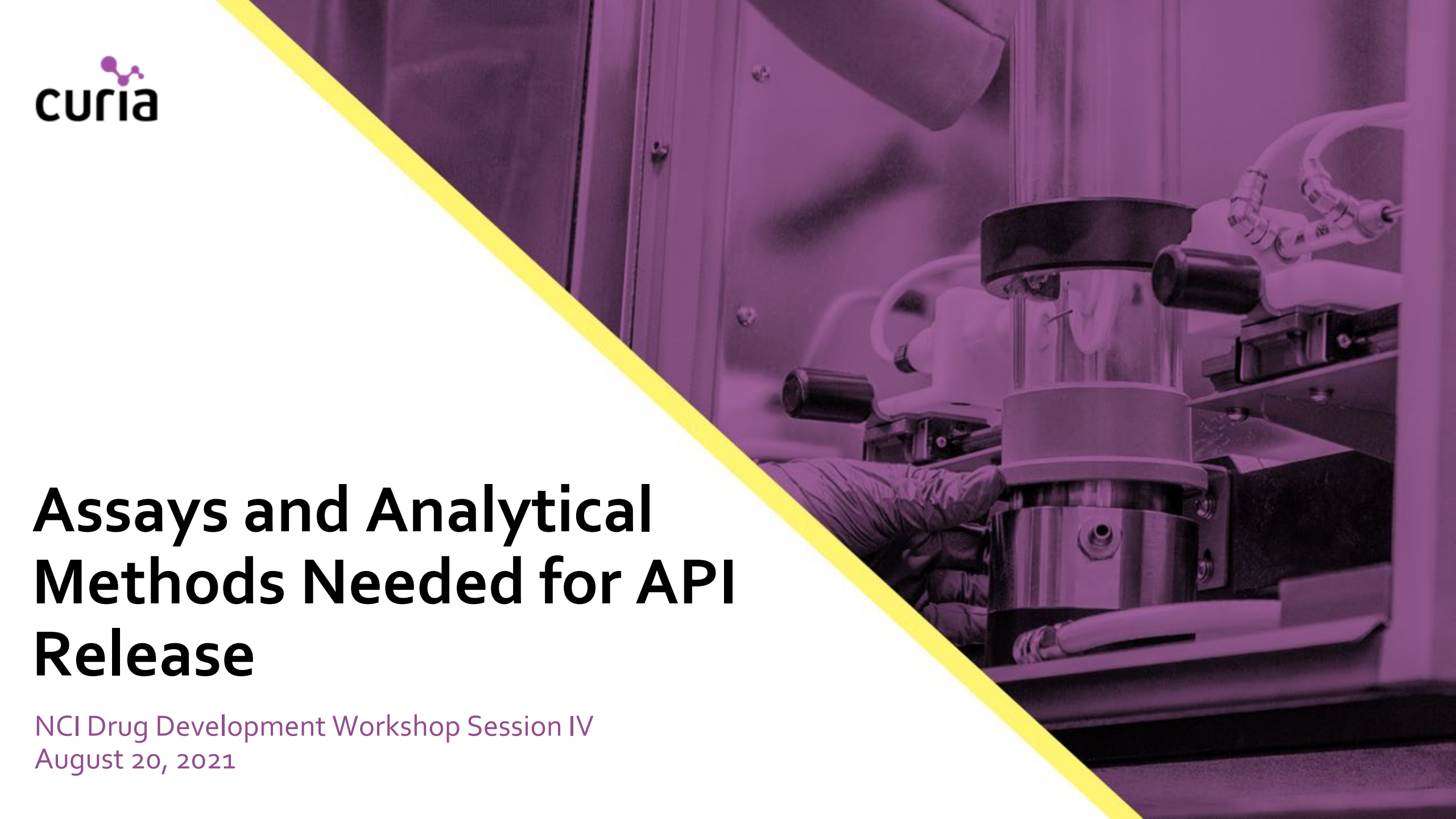

Assays and Analytical Methods Needed for API Release

NCI Drug Development Workshop Session IV
August 20, 2021

From Pre-Clinical to Phase I – What Do I Need for the IND from Analytical

30 Day Stability on released DP

- Requires GMP released DP
- Requires GMP released DS to make DP

Release DP batch

- Requires manufacture from released DS batch
- Requires DP specification
- Requires all DP methods in spec are GMP qualified

Specification for DP batch

- Tox package to determine qualified impurities
- In-Silico tox assessment for PGIs
- Data from previous batches to set spec on degradants

30 Day Stability on released DS

- Requires GMP released DS
- RS characterization with proof of structure

Release DS batch

- Requires manufacture under GMP
- Requires specs on API, GMP intermediates and GMP starting materials
- Requires release of all RMs, SMs, intermediates and API against specs for each of these

Specification for DS batch

- Requires GMP qualified methods for all tests listed in specs of RMs, SMs, intermediates and API
- Spec requires 3 development data from 3 development lots
- Tox package to determine qualified impurities

cGMP vs GLP

Current Good Manufacturing Practice is a special set of rules in ICH and regulatory agencies for manufacture and testing of pharmaceutical batches for human use by the Quality Unit

- Only last few steps are GMP
- All GMP materials have to be released against specifications
- All GMP test methods must be qualified/validated or verified before use for release

Good Laboratory Practice is a special set of rules for the conduct of animal trials.

- GLP testing begins with the test article which is delivered into the animal, includes testing of test article during and before animal trial to support stability, also includes testing of biological samples obtained from the subjects
- GLP test articles are made up of non-GLP (and typically non-GMP) components
- The purpose of GLP is to qualify impurities.

GLP studies are also used to identify Potential Genotoxic Impurities (PGIs)

- For non-oncology applications
- In-silico assessment of DP components and API synthesis scheme including structures of SMs, Intermediates, and API to identify any structural alerts.
- GLP Ames tests can be used to definitively test a compound to determine if it is genotoxic.
- Specifications must include special analytical testing for any PGIs deemed necessary to control down to 1.5 ug/day for lifetime exposure. This often means analytical methods down to low or sub-ppm levels in the API. ICHM7

Sample API CoA for Phase I

TEST	SPECIFICATION	RESULTS
Appearance	White to light yellow solid	White solid (TM.795)
Solubility (100 mg/mL in water)	Clear solution	Clear solution (TM.795)
pH (100mg/mL in water)	Report results	1.3 (USP<791>)
Identification: IR Spectrum (ATR) ^{1}H NMR Spectrum Retention Time by HPLC Cl: Retention Time by IC	 Conforms to structure Conforms to structure Retention time of the major peak is comparable with that of the reference standard Retention time of the major peak is comparable with that of the reference standard	 Conforms to structure (TM.41) Conforms to skeletal structure (TM.52) Retention time of the major peak is comparable with that of the reference standard (TM.05438) Retention time of the major peak is comparable with that of the reference standard (TM.05447)
Purity by HPLC (area %) Impurities by HPLC (area %) Individual Impurities (area%) Total Impurities	≥95.0% Impurity A: Report results Impurity 1 Impurity 2 Impurity 3 Impurity 4 Total Impurities 1-4: ≤3.0% Any other unknown impurity: ≤0.5% ≤5.0%	TM.05438 Purity: 98.6% 0.40% None Detected 0.07% 0.25% 0.33% 0.65% RRT Area% 0.78 < 0.05% 1.34 0.23% 4.23 0.09% 1.4%
Assay by HPLC (w/w %) w/w% free base w/w% salt on anhydrous base	 Report results 85.0 to 105.0%	TM.05438 78.6% 90.9%
Residual Impurity X by HPLC (area %)	≤2.0%	None Detected (TM.05434)
Aspartic Acid by HPLC (w/w %)	Report results	1.1% (TM.05437)
Residual acetic acid by IC	Report Results	310 ppm (TM.05447)
Chiral purity by HPLC (area %)	D-API: ≤0.3%	Not Detected (TM.05192)

Sample API CoA for Phase I cont'

TEST	SPECIFICATION	RESULTS
Residual Solvents		TM.05466*
	Ethanol: <5000 ppm	<5000 ppm(120ppm)
	2-Propanol: <13800 ppm	<13800 ppm (Not Detected)
	Tert-Butyl methyl ether: <5000 ppm	<5000 ppm(Not Detected)
	Ethyl acetate : <5000 ppm	<5000 ppm(660 ppm)
	Isopropyl acetate : <5000 ppm	<5000 ppm(400 ppm)
	1-Butanol: <5000 ppm	<5000 ppm(Not Detected)
	Toluene : <890 ppm	<890 ppm(Not Detected)
	Dimethylfomamide (DMF): <880 ppm	<880 ppm(Not Detected)
Residual Triethylamine by GC	<5000ppm	TM.05467 *
		<5000ppm(Not Detected)
Water Content by Karl Fisher	Report Results	3.8% (TM.05446)
Differential Scanning Calorimetry	Report Results	TM.3155
		Endo peak: 81.7 °C
		Exo peak: 143.5°C
Residue on Ignition	Report Results	1.3% (USP <281>)
Microbial Testing		USP<61>
Total Aerobic Count (TAMC)	NMT 10^3 cfu/g	< 10 CFU/g
Total Yeast and Mold (TYMC)	NMT 10^2 cfu/g	< 10 CFU/g
		(SGS Life Science Services, Lincolnshire, IL)
Microbial Testing		USP<62>
Escherichia coli	Absent in 1 g	Absent
Salmonella species	Absent in 1 g	Absent
Pseudomonas aeruginosa	Absent in 1 g	Absent
Staphylococcus aureus	Absent in 1 g	Absent
		(SGS Life Science Services, Lincolnshire, IL)
Bacterial Endotoxins	<0.02 EU/mg	<0.01 EU/mg (USP<85>)
		(SGS Life Science Services, Lincolnshire, IL)

Sample API CoA for Phase I cont'

TEST	SPECIFICATION	RESULTS
Elemental Impurities by ICP-MS	Cd ≤0.2 ppm Pb ≤0.5 ppm As ≤1.5 ppm Hg ≤0.3 ppm Pd ≤13.7 ppm	ATP-000793 Element / Concentration(ppm) Cd < 0.1 Pb < 0.25 As < 0.75 Hg < 0.15 Pd 5 (Intertek, Whitehouse, NJ)

**** TM.05466 and TM.05467 are limit-test methods. Values in parentheses are approximations.***

Storage/Special Handling: Store at -20 ± 5 °C

Related Substance (U)HPLC Method

This is in many ways the most important method, requiring the most development and qualification/validation

- Typically used for assay and purity (with external standard of known potency)
- Needs to be stability indicating (forced degradation recommended)
- Since impurity profile and qualified minors come from GLP Tox, we recommend same method be used for testing API used to make GLP Tox test article and later GMP batches

Related substance method should be qualified for sensitivity, selectivity, linearity, precision, accuracy, solution stability for early phase, add intermediate precision and robustness for late phase

- Screen mobile phases and stationary phases for best separation with no on-column degradation
- Screen diluents for maximum solution stability
- For early phase, typically long column, long run, shorten later in development if necessary

GLP tox study will prove that impurities in the GLP tox batch are not toxic to animals, this is the basis of specifications for levels of qualified impurities.

- API for GLP tox test article should be dirty
- No new impurities in GMP not in tox lot

Sample DP CoA for Phase I

Attribute	Method	Limit	Analysis Date	Result	Pass/Fail
Appearance	Visual	Off-white to pale yellow lyophilized cake		Pale yellow lyophilized cake	Pass
Reconstituted appearance	Visual	Pale yellow clear solution, essentially free from visible particulates		Pale yellow clear solution, essentially free from visible particulates	Pass
Reconstitution time	Visual	< 120 seconds		15 seconds	Pass
Volume of Injection	USP <1>	Report mL / vial		1.9 mL / vial	Pass
Residual Solvent Acetonitrile	GEN-CMC-AM-0027	< 10 µg ACN / mg EC1456		< 0.61 µg ACN / mg EC1456	Pass
Residual Water	KF oven method XXXXX-CMC-AM-0004 USP <921>	≤ 6.0%		1.8 %	Pass
pH	EQS-0005 USP <791>	4.3 – 6.3		5.4	Pass
Osmolality	Freezing point depression USP <785> SOP-EQS-0013	300 ± 100 mOsm		303 mOsm	Pass
Sterility	USP <71>	Pass (No growth observed)		No growth. Meets the requirements of USP <71>	Pass
Bacterial Endotoxins	USP <85>	≤ 4.1 EU / mg		Beginning <1.00 EU/mg Middle <1.00 EU/mg End <1.00 EU/mg	Pass
Particulate Matter	USP <788>	≥10µm: ≤ 6000 particles / vial ≥25µm: ≤ 600 particles / vial		≥10µm: 45 particles / vial ≥25µm: 2 particles / vial	Pass

Sample DP CoA for Phase I

Attribute	Method	Limit	Analysis Date	Result	Pass/Fail
Uniformity of Dosage Units	USP <905> Weight Variation	Passes USP requirement for acceptance value (AV ≤ 15.0)		Pass (AV = 2.7)	Pass
Identity by UPLC-MS	XXXXX-CMC-AM-0009	RT ± 2% of standard Consistent with standard $M+2H^+$ at 1313.5 ± 1.0 m/z $M+3H^+$ at 876.0 ± 1.0 m/z		RT within ± 2% of standard $M+2H^+$ at 1313.6 m/z $M+3H^+$ at 876.2 m/z	Pass
XXXXX Assay	XXXX-CMC-AM-0003	4.95 – 6.05 mg/vial		5.46 mg/vial	Pass
Purity by UPLC		≥ 94.0 %		96.4 %	Pass
Individual Specified Degradation Products by UPLC		Impurity ID / RRT / Limit Deg Prod B / 0.50 / ≤ 1.5% Deg Prod C / 0.59 / ≤ 2.5 % Deg Prod D / 0.83 / ≤ 0.80 % Deg Prod G / 1.16 / ≤ 2.5 %		Name / RRT / %Area Deg Prod B / *ND* / ND Deg Prod C / *0.58* / 0.31 Deg Prod D / *ND* / ND Deg Prod G / *1.15* / 1.2	Pass
Process Impurities		Impurity E / 0.97 Impurity H / 1.07 Report %		Impurity E / *0.96* / 0.88 Impurity H / *1.07* / 1.1	Pass
Total Degradation Products		< 6.0 %		1.6%.	Pass
Individual Unspecified Degradation Products by UPLC		No individual unspecified degradation product > 0.50% Report all degradation products ≥ 0.10%		RRT / %Area *0.35* / 0.12%	Pass

ND = Not detected at or above the reporting limit.

GMP Analytical Method Qualification/Validation

Table 1: Early Phase Validation Parameters (Phases I and II)

Validation Parameters	RSMs and Intermediates[1]	Drug Substance		
		Category I	Category II[2] (Quantitative)	Category II[4] (Limit Test)
Accuracy	-	+	+[3]	+
Precision:				
Repeatability	-	+	+[3]	+
Intermediate Precision	-	-	-	-
Specificity	+	+	+	+
Quantitation Limit (QL)	+	-	+	-
Detection Limit (DL)	+	-	+	+
Linearity	-	+	+[3]	-
Solution Stability	+	+	+[4]	+[4]

- Signifies that this parameter is not normally evaluated
+ Signifies that this parameter is normally evaluated

GMP Analytical Method Qualification/Validation cont'

Table 2: Late Phase Validation Parameters (Phase III, registration, process validation and commercial batches)

Validation Parameters	RSMs and Intermediates[1]	Drug Substance		
		Category I	Category II[2, 4] (Quantitative)	Category II (Limit Test)
Accuracy	+	+	+[3]	+
Precision:				
Repeatability	+	+	+	+
Intermediate Precision	-	+[4, 5]	+	+
Specificity	+	+	+	+
Quantitation Limit (QL)	+	-	+	-
Detection Limit (DL)	+	-	+	+
Linearity	+	+	+[3]	-
Range	+	+	+[3]	-
Solution Stability	+	+	+[4]	+[4]
Robustness	-	+	+	+

[1]Late phase RSM and Intermediate methods are typically w/w% quantitative methods with impurities quantitated by area%.