

Analytical Method Risk Assessment

Project ID:	XX
API:	ABC-XYZ
Step:	API
Project Code:	1234
Analytical Method	ABC-XYZ LC assay Impurity
Date for QRA Round 1	xx/xx/xxx
Date for QRA Round 2	xx/xx/xxx
Analytical Target Profile	<i>Selective, precise, accurate and robust method</i> around expected specification limits

Objective:

This method risk assessment is performed before registrational stability or process validation campaign and considered as a final risk assessment document.

The objective is to:

1. Identify key method parameters which could significantly affect method performance per specification requirements
2. Develop analytical control strategy for risk reduction and risk acceptance (including timeline)

Path forward:

The outcome of the risk assessment will be used as a basis to perform TT risk assessment.

Team Members

Team Member	Name
Step analyst / method author	B/CD
AL	EF
Analytical SME	AB/CD/EF/GH

6Ms	Sub-categories		Comments	Comments from SME
Method				
	Diluent	Organic solvent	Acetonitrile	
		Buffer/additive, concentration, pH	100 mM Potassium Formate 8.412 g of potassium formate and 5.0 mL formic acid in 1L water, buffer pH should be 3.5, not controlled by pH meter	buffer pH robustness, check +/- large weight and volume to identify boundary for pH change which could lead to failure
		Composition	Buffer:H2O:MeCN 25:25:50	
	Working concentration		0.25 mg/mL ABC-XYZ	rxn IPC at 30C, in DMSO, solution which might contains small amount of K2CO3 precipitation. IPC2 and IPC3 both solution in Ethylacetate. IPC8 is final dry cake. No concern on IPC sample preparation
	Sample and std weight and range		25 mg	Keep sample in saturated conditions, and then handle to see any problem
	Sample and std volume		100 mL	
	Extraction	Sonication/shake/centrifuge	in draft method "Use sonication if necessary"	see line 13
		Settings: water level, shaker speed, centrifuge RPM	Not controlled	
		Duration	not specified	Study ABC-XYZ solubility in diluent, check B hygroscopicity by DVS, check sample handling after exposure to high humidity
	Solution storage condition	RT vs fridge, protected from light	not specified	see line 13
	Stability time		not known	see line 13
	Quality of instructions		needs to be more specific	see line 13
Material				
	Analyte	Polymorph	Crystalline (Form A)	Slightly hygroscopic, see line 13
		Particle size	not controlled	
		Solubility	Not available	see line 13
		Solid state stability	water change from 0.29 to 0.86% over 6 month, rest no change hold time stability with PPQ batches being planned	
		Solution stability	stable at 5C, ambient not known	see line 13
		Hygroscopicity	slightly hygroscopic	see line 13
		Static	no issue identified	
		Safety	high band material	
	Critical / Key impurities		Five specified impurities (semi quantitative ref std available at Carbogen), eight additional identified impurities (comparison ref std available at Carbogen)	
	Reference standard quality	Source and purity	normal	
	Water/buffer/solvent	Source and purity	potassium formate is hygroscopic, formic acid needs to be pure	potassium weighing accuracy. check potassium formate hygroscopicity, formic acid hygroscopicity, check its effect on pH accuracy
Machine				
	Balance	Precision (5-place vs 6-place)	no issue identified	
		Daily calibration procedure	no issue identified	
	pH meter	Reference electrode type	Not Applicable	
		Daily calibration procedure	Not Applicable	
	Automatic pipettor		Not Applicable	
	Volumetric pipettes/ flasks/cylinders	Precision and accuracy change due to age?	no issue identified	
		Cleaning procedure	no issue identified	
	Sonicator		no issue identified	
	Shaker		Not Applicable	
	Automatic pipettor		Not Applicable	
	Glassware including vials		no issue identified	
	Sample contact surface	spatula/weighing paper, boat	no issue identified	
	Cross-contamination	such as spatula and glassware, especially for GTIs	no issue identified	
Manpower				
	Weighing skills and practice	One weighing	band 5 material	
	General wet chemistry skills	Glass pipette use, volumetric flask QS	no issue identified	
		Sample transfer	no issue identified	
		Manual shake	no issue identified	
	Other unit operation skills		Not Applicable	
	Specific skills/experience		Not Applicable	
Mother Nature				
	Lab temperature	Control and variability	Ambient lab temperature	
	Lab RH	Control and variability	Facility control	ABC-XYZ is slightly hygroscopic, see line 13
	Instrument location ('noise' influenced)	e.g., AC unit cause instrument vibration	no issue identified	
			high band material	
Measurement				
	Replicate strategy	How many preps for std and samples?	Duplicate for assay and impurities.	

GfMs	Sub-categories	Comments	Comments from SME		
Method	Diluent	Composition Organic solvent Buffer/additive, concentration, pH Solvent mismatch	Buffer:H2O:MeCN 25:25:50 Acetonitrile 100 mM potassium formate 8.412 g of potassium formate and 5.0 ml formic acid in 1L water, buffer pH should be 3.5, not controlled by pH meter No issue identified		
	Mobile phases	Composition Binary vs tertiary Solvent type Buffer type Buffer concentration/ion strength pH Volume of each preparation Mobile phase use day - TFA and ammonium evaporation Bacteria Growth	MPA: Buffer:H2O:MeCN 25:45:30 MPB: Buffer:MeCN 25:75 Binary MeCN 100 mM potassium formate 100 mM 3.5, not measured, controlled by acid/base ratio 1 L (scalable) no issue identified no issue identified	robustness not fully evaluated, buffer precipitation is not a concern. Study MP composition on separation study buffer concentration on separation, identify critical pairs separation sensitive to pH, study pH effect on separation, and identify critical pairs TFA is not used in this method. Formic acid is in this method. Add composition of needle wash and etc..	
	Needle/sample wash solvents	Seal wash solvent	no issue identified no issue identified		
	Isocratic run	%B Late eluting/carry over Peak shape	Not Applicable Not Applicable Not Applicable		
	Gradient run	Initial %B Initial hold time Gradient slope %B at the end Final hold time Re-equilibration time	0% 0 2 step gradient 100% 1 minutes 5.9 minutes	check dwell volume difference effect on late eluter, e.g., trimer	
		Multiple step gradient Location of peaks vs gradient change points	0/20/26/27min; 0/40/100/100 %B ~ 1 minutes	robustness not fully evaluated; gradient ends at 75% organic, run to 90% organic and hold for longer time to check if there is any late eluters	
	Run time	Flow rate	33 minutes no issue identified no issue identified		
		Friction heating Temp gradient inside column Pressure and closeness to instrument Max	no issue identified no issue identified ~ 300 bar	no pressure buildup during column use	
	Column	Stationary phase Separation mode Particle size Support type - silica, hybrid and others Support structure - fully porous vs pore-shell Column length Column internal diameter On-column degradation Column limits on pressure/temperature/pH Lifetime/age/history	ACE Excel 3 C18-AR, 4.6 x 150 mm, 3 µm, P/N EXL-119-1546U Reverse Phase 3 µm Silica Fully porous 150 mm 4.6 mm short column life observed short column life observed short column life observed	slightly change in selectivity was observed, column life needs further evaluation. Suggest to run column screen to identify alternative column/condition see line 37 see line 37 see line 37	
	Column temperature	Sample temperature	30 °C Ambient only	column temp affects critical pair separation, additional study with simulation should be done. See SSP line 13	
	Injection volume	Detection	10 µL Detector type - UV/FL/CAD/MS UV Wavelength Bandwidth Reference channel Sampling rate Time constant Linearity	10 µL no interfering system peak observed check bandwidth effect None > 0.05min no issue identified	
	System suitability test	Tests and criteria	Not set	Need to better understand method robustness on how to set SST	
	Material	Water		Milli-Q	
		Organic Solvent	Grade and vendor	Acetonitrile (HPLC grade; Sigma-Aldrich)	
		Additive	Grade and vendor	Not evaluated	
		Column	Vendor Batch to batch variability	ACE limited column packing lot available for evaluation	
	Machine	HPLC Instrument	Vendor HPLC vs UHPLC Binary vs quaternary Dwell volume Mixer size Extra column volume Sample wetting material type - injector/tubing/flow cell injector type - loop vs FTN Drawing rate/loop size Mobile phase heating type - active vs passive Flow cell volume Path length Tubing ID/material/length Age of instrument	Agilent 1260/1290 and Waters H-Class no issue identified not evaluated not evaluated no issue identified no issue identified no issue identified FTN recommended no issue identified not evaluated not evaluated not evaluated not evaluated not evaluated	severe peak tailing of those quaternary amine impurities were observed on some HPLC instruments compare binary vs quaternary LC loop injection not evaluated
		pH meter	Reference electrode type Daily calibration procedure	Not Applicable Not Applicable	
		manpower	Analyst HPLC skills and knowledge	Column installation	no issue identified
			Instrument setup procedure		Low risk
			Sample cooling time		no issue identified
			Instrument service and PV		no issue identified
		mother nature	Lab temperature	Control and variability	Ambient
			Lab RH	Control and variability	Ambient
			Instrument location ('noise' influenced)	e.g., AC unit cause instrument vibration	Low risk
Measurement		Injection sequence		no issue identified	
		Bracketing		no issue identified	
		Chromatography software	Different integration parameter	no issue identified	
		Integration parameter	Skimming, apex vs traditional	no issue identified	
		Calibration	Internal vs external Single point as multiple, through zero or not	External Single point through zero (Proportional response)	
		Calibration curve	S1 only or both	S1	
		Method reporting convention / specification		Typical	
		Empower custom Field		Typical	

Key Method Variable	Risk Description	Impact on ATP and CQAs	Risk Mitigation Plan
		Accuracy, Precision, sensitivity, critical pair resolution, main peak tailing	
Buffer pH	Because of buffer pH is not measured there is a risk that buffer pH could be away from set point (3.5) resulting in peak co-elution	Critical pair resolution	study pH effect on LC separation using DryLab and Fusion Study effect of buffer/acid ratio on pH study buffer and acid hygroscopicity
Buffer concentration	Because of buffer concentration can affect main peak tailing and two quaternary amine impurity retention there is a risk that buffer concentration variation resulting in peak tailing or peak co-elution	Accuracy and Precision, and critical pair resolution, and main peak tailing	Study buffer concentration effect on LC separation using DryLab and Fusion
MP composition	Because of limited knowledge on MP composition effect on separation there is a risk that MP composition variation resulting in peak co-elution	critical pair resolution	Study MP composition on LC separation
Gradient profile	Because of limited knowledge on gradient effect on separation there is a risk that gradient variation resulting in peak co-elution	critical pair resolution	Study gradient effect on LC separation using DryLab and Fusion
column temperature	Because of limited knowledge on column temperature on separation there is a risk that column temperature variation resulting in peak co-elution	critical pair resolution	Study column temperature effect on LC separation using DryLab and Fusion
Buffer and acid grade and purity	Because buffer pH is controlled by buffer/acid ratio, there is a risk that buffer and acid quality variation resulting in buffer property change, such as pH	Accuracy and Precision	Evaluate HPLC KHCOO and formic acid from different vendors
Sample preparation	Because of limited knowledge on sample properties such as solubility, stability and hygroscopicity, there is a risk that sample preparation procedure is not robust resulting in assay error.	Accuracy and Precision	Study ABC -XYZ solubility and stability in diluent check B hygroscopicity by DVS, check sample handling after exposure to high humidity
Injection method	Because of two quaternary amine impurities there is a risk of strong interaction of quaternary amine with metal surface resulting in accuracy failure	Accuracy and sensitivity	Study method performance on LC with loop injection (classic Acquity UPLC)
LC instruments	Because of limited knowledge on two quaternary amine impurities there is a risk of peak tailing on different LC instruments resulting in separation failure	Accuracy and sensitivity	Study method performance on different LC instruments Identify root cause of quaternary peak tailing
bandwidth and sampling rate	Because of limited knowledge on detector setting on peak shape there is a risk that incorrect setting resulting in peak shape distortion	Accuracy and sensitivity	Study detector setting effect on peak shape
Column	Because of limited knowledge and small scale production there is a risk that column discontinuation or lot-to-lot variation resulting in method execution failure	All	Perform column screen to identify alternative columns
System suitability test	Because of limited knowledge on method operation space there is a risk of inappropriate SST resulting in not catching method execution failure	All	Propose appropriate SST once method operation space is well defined

Key Method Variable	Risk Description	Impact on ATP and CQAs	Analytical Control Strategy	Residual Risk
		Accuracy, Precision, sensitivity, critical pair resolution, main peak tailing		
Buffer pH	Because of buffer pH is not measured there is a risk that buffer pH could be away from set point (3.5) resulting in peak co-elution	Critical pair resolution	Control HCOOH volume and KHCOO weight within +/- 5%	Low
Buffer concentration	Because of buffer concentration can affect main peak tailing and two quaternary amine impurity retention there is a risk that buffer concentration variation resulting in peak tailing or peak co-elution	Accuracy and Precision, and critical pair resolution, and main peak tailing	Control HCOOH volume and KHCOO weight within +/- 5%	Low
MP composition	Because of limited knowledge on MP composition effect on separation there is a risk that MP composition variation resulting in peak co-elution	critical pair resolution	Simulation and OFAT lab results confirmed robustness range	Low
Gradient profile	Because of limited knowledge on gradient effect on separation there is a risk that gradient variation resulting in peak co-elution	critical pair resolution	Simulation and OFAT lab results confirmed robustness range	Low
column temperature	Because of limited knowledge on column temperature on separation there is a risk that column temperature variation resulting in peak co-elution	critical pair resolution	Simulation and OFAT lab results confirmed robustness range	Low
Buffer and acid grade and purity	Because buffer pH is controlled by buffer/acid ratio, there is a risk that buffer and acid quality variation resulting in buffer property change, such as pH	Accuracy and Precision	No impact identified to method performance	Low
Sample preparation	Because of limited knowledge on sample properties such as solubility, stability and hygroscopicity, there is a risk that sample preparation procedure is not robust resulting in assay error.	Accuracy and Precision	No impact identified to method performance	Low
Injection method	Because of two quaternary amine impurities there is a risk of strong interaction of quaternary amine with metal surface resulting in accuracy failure	Accuracy and sensitivity	No bias observed for UPLC with loop injection	Low
LC instruments	Because of limited knowledge on two quaternary amine impurities there is a risk of peak tailing on different LC instruments resulting in separation failure	Accuracy and sensitivity	Instruments verified: H-Class, AcQuity binary, 1260, 1290	Low
bandwidth and sampling rate	Because of limited knowledge on detector setting on peak shape there is a risk that incorrect setting resulting in peak shape distortion	Accuracy and sensitivity	No impact identified to method performance	Low
Column	Because of limited knowledge and small scale production there is a risk that column discontinuation or lot-to-lot variation resulting in method execution failure	All	Only one packing lot column is good for G-G dimer and Q-G dimer peaks - maintain a stock of columns and bulk at column vendor	Low
System suitability test	Because of limited knowledge on method operation space there is a risk of inappropriate SST resulting in not catching method execution failure	All	Propose appropriate SST once method operation space is well defined	Low