eurofins | CDMO

API DEVELOPMENT TOOL BOX

CASE STUDIES IN MULTIVARIATE PROCESS ANALYSIS

Written By Graham McGowan, Ph.D.,
CMC and Regulatory Sciences Group Leader at Eurofins CDMO Alphora Inc.

eurofins | CDMO

API/DRUG SUBSTANCE DEVELOPMENT TOOL BOX

Taking a product from a laboratory model to the commercial stage is a daunting task with many challenges, both anticipated and unexpected. The development burden is especially high for small, early-phase developers where the goal is establishment of a safe, demonstrated working model, rather than view on the long term Chemistry, Manufacturing and Controls (CMCs).

The goal of development is establishment of a process that is safe, well-designed, well-understood, well-controlled and provides products meeting specification and regulatory expectations.

Whether development is pursued in-house or at a Contract Development and Manufacturing Organization (CDMO) such as Eurofins CDMO, several tools and study stages and types are available for Drug Substance process development, and can be applied across development.

Multivariate analysis, generally defined as the measurement of the output of variation of simultaneous input variables, is one such tool.

Multivariate tools have been widely-used for many years in the non-pharma world, where continuous, non-batch-based processing is common, and in Drug Product development [1]. The tools support design of experiment 'DoE' philosophy and represent one component of a Quality by Design 'QbD' approach that focuses on understanding and control of input conditions and parameters over batch end-testing.

Despite common use in other areas, Drug Substance processes still tend towards one-variable-at-a-time OVAT, or univariate approaches and batch-based processing, where the natural tendency is to stick with what is known. This hesitancy may be amplified for complex, low volume products, and especially those in early-stage development and, or subject to short development timelines. As these products move forward the tendency may persist, potentially missing out on the benefits of more data-rich approaches.

However, as pharmaceutical products and associated processes become more complex, a move towards science-driven-, risk- and process-based thinking is being actively encouraged by regulators [2]. Further, according to the recommended lifecycle approach as exemplified in current Process Validation guidance, the data and knowledge gathered in early stages of development represent the building block for subsequent long term manufacture [3].

We at Eurofins CDMO support the application of multivariate tools as a simple and approachable step towards adoption of modern, risk-based CMC development across all stages.

---

[1] ICH Q8(R2) Pharmaceutical Development (August 2009)

[2] ICH Q11 Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological / Biological Entities) (May 2012)

[3] US FDA CDER Guidance for Industry Process Validation: General Principles and Practices (January 2011)

- In ***early-stage development*** the tools may be used for screening of route, reagents and conditions.
- When supporting ***process scale-up,*** well-placed studies can inform on optimal conditions, critical parameters, edge(s) of failure and operational envelope, as well as providing confidence in the process.
- In ***long term manufacture***, the methodology can be applied in response to process observations or drift, or to understand the impact of implementation of process improvements such as implementation of new technologies allowing for reduced residence time, or alternative input material grades and vendors.

Several software platforms are available for the design of multivariate studies; Eurofins CDMO Alphora Inc. employs the DesignExpert® by StatEase platform, and its use allows for:

- **Ease of establishment** of input study variables, for example batch parameter ranges
- **Ease of design** - reducing or removing the laborious burden of selection of factors for OVAT studies
- **Well-defined study plans**, in number and nature of trials, with well-defined target outcomes

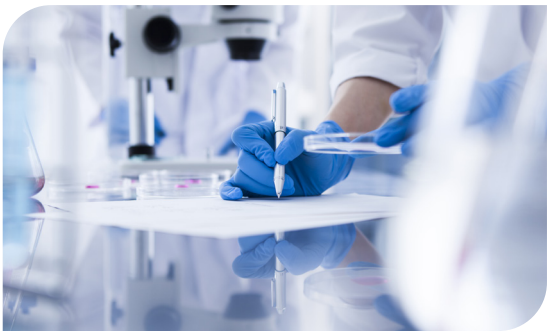
The adoption of multivariate tools allows for mapping of a broader process area than might be available by OVAT or univariate methods, or combinations of parameters that we may not otherwise 'think' ourselves into, and further, allowing for identification and visualization of global minima or maxima.

The approach is especially-useful for the mapping of 'black box' chemistry that is subject to complex, unknown or indiscernible interactions of input factors, fast conversions where corrective actions may not be available or where an unexpected process event may be irreversible.

Furthermore, adoption of multivariate thinking provides a spring-board to other more contemporary and encouraged tools, such as Process-Analytical Technology (PAT) and continuous manufacturing.

Steps and Case Studies

This highlight article is not intended to provide an in-depth statistical view of the use of multivariate tools as one component of Drug Substance development, but to illustrate approachable steps to adopt the methodology as one tool in the development tool-box, alongside more traditional approaches.



Case study examples are provided below to highlight the approachability and potential utility of the approach, from early-phase development, to long-term manufacture.

eurofins | CDMO

General Principles

Acknowledging the sometimes-difficult move away from OVAT / batch-based thinking for early-phase or low volume products, moving to the use of multivariate tools can be broken into a few highly-approachable steps, and the same principles can be applied for more mature processes.

The general steps are summarized below:

Stages	Description
Stage 1 **Definition of the product and its target attributes**	• Define material attributes at Drug Substance and its precursors (Starting Material(s), Intermediates(s)) • Assess the criticality of test attributes and their relationship to final Drug Substance, with focus on potential for impact to identity, potency and safety (purity / impurity content) of the Drug Substance
Stage 2 **Presentation of the process under study in its constituent parts**	• Describe the process in its constituent parts (temperature, concentration, charge levels, etc.)
Stage 3 **Assessment of and ranking of potential for impact of the constituent process parts on the product attributes**	• Assess potential for respective process components to impact downstream material attributes, with focus on critical attributes • Assessment can be based on development and batch experience, level of knowledge available for a particular operation (or absence of knowledge), sound scientific judgment or documented experience with similar operations or products
Stage 4 **Study design**	• Establish study type with rationale (for example Design-Expert®, 'Stat-Ease') • Establish study ranges with rationale
Stage 5 **Study execution**	• Execute study as defined taking care to complete all defined trials
Stage 6 **Evaluation of results and reporting**	• Assess results for individual output attributes • Look for relationships for input combinations (e.g. solvent and reagent charge; time and temperature), and overlays of multiple study relationships to identify operational ranges and design space • Document and apply any findings, e.g. into production batch instructions or specifications • Determine need for additional study as required based on outcome and, or additional development or manufacturing experience

eurofins | CDMO

Stage 1: Example criteria for assessment for material criticality at Drug Substance and precursors:

Attributes at precursors to Drug Substance [Starting Material(s), Intermediate(s) or reagent(s)]	Attributes at Drug Substance	Criteria
Critical Material Attribute (CMA)	Critical Quality Attribute (CQA)	• High, direct potential to impact Drug Product identity, safety, or potency
Potential/Tentative Critical Material Attribute (CMA*)	Potential/Tentative Critical Quality Attribute (CQA*)	• Unknown, moderate or indirect potential to impact Drug Substance identity, safety or potency, and, or: • Insufficient level of knowledge to assign criticality, and, or: • Attribute controlled by factors external to the chemical process and analytical controls (e.g. cGMP conditions, equipment cleaning procedures; engineering controls), and, or: • Attribute controlled upstream of Drug Substance but requires consideration in overall control strategy
Not critical, routine, or complementary attribute	Not critical, routine, or complementary attribute	• Low potential to impact Drug Substance identity, safety, or potency, and, or: • Attribute readily controlled by routine process and, or when all other attributes meet respective criteria, and, or: • Attribute is included for the purposes of gathering process knowledge and comparison to existing data

Stage 1: Example criticality assessment, from Starting Material to Intermediate, to Drug Substance:

Attribute	Starting Material(s)	Intermediate(s)	Drug Substance	Rationale
Description	-	-	Potential CQA*	Critical if variable or indicative of absence of control of other attributes
Identity	CMA	-	CQA	Effectively set at Starting Material, retained if all other criteria met
Assay	CMA	CMA	CQA	Typically critical throughout for impact on potency
Purity	CMA	CMA	CQA	Typically critical throughout for impact on safety
Residual solvents (From RSM)	CMA	Potential CMA*	Potential CQA*	Critical at point of introduction, use, control Potential at product if carried-over
Residual solvents (Used in Intermediate)	-	CMA	Potential CQA*	Critical at point of introduction, use, control Potential at product if carried-over
Residual solvents (Last step)	-	-	CQA	Typically critical, tested
Water content (Upstream materials)	Potential CMA*	Potential CMA*	-	Impact on stability, process performance, assay
Water content (Product)	-	-	Potential CQA*	Product-dependent
Solid form (Upstream materials)	Potential CMA*	Potential CMA*	-	Impact on stability, process performance (solubility)
Solid form (Product)	-	-	Potential CQA*	Product-dependent

Stage 2-4: Example process assessment for conversion of 'Starting Material' to 'Intermediate':

Stage	Operation	Assessment (Impact to attributes, decisions)	Proposed Study	Potential study ranges (SOP: Standard Operating Procedure)
Conversion of Starting Material to Intermediate	Charge reagent	Potential impact to assay (CMA), purity (CMA)	Multivariate study Monitor residual Starting Material, assay, purity	Range allowed by SOP
	Charge solvent			Range allowed by SOP
	Heat and agitate			Range allowed by equipment Average +/- standard deviation
	In-process control (IPC) for residual Starting Material	Potential impact to purity (CMA) Decision on further agitation or other actions		-
Quench	Adjust temperature	Potential impact to assay (CMA), purity (CMA), residual inorganic content (CMA*)	Multivariate study Monitor assay, purity, residual inorganics	Average +/- standard deviation
	Charge quench reagent			Range allowed by SOP
	Agitate			Average +/- standard deviation
	Adjust temperature and agitate			Average +/- standard deviation
	Separate phases, discard aqueous		-	-
Isolation	Concentrate to defined volume	Potential impact to assay (CMA), purity (CMA), solid form (CMA*)	OVAT study, volume Monitor purity (CMA)	Average +/- standard deviation
	Seed		OVAT study Monitor purity, form	No seeding / with seeding
	Adjust temperature and agitate		Multivariate study Monitor assay, purity, form	Averages +/- standard deviation
	Filter			
	Wash solids	Potential impact on residual water (CMA*), solvents (CMA) Decision to dry further		
	Dry solids			-
	In-process control (IPC) for residual solvent, water content, assay, purity, solid form	Decision on further drying or purification		-

Case Study #1: Multivariate Study Supporting Further Scale-Up & Process Validation

The project involves manufacture of a small volume, complex Drug Substance indicated for an ultra-rare pediatric disease managed under an accelerated approval pathway.

Although accelerated approval pathways offer hope and benefit to patients and sponsors they also require urgency and focus in program, not only in establishing the process and basic CMCs, but also in mapping what longer-term CMCs may look like and to meet regulatory expectations in multiple regions. In our experience accelerated programs require a higher development and CMC burden, earlier, when compared to more traditional programs.

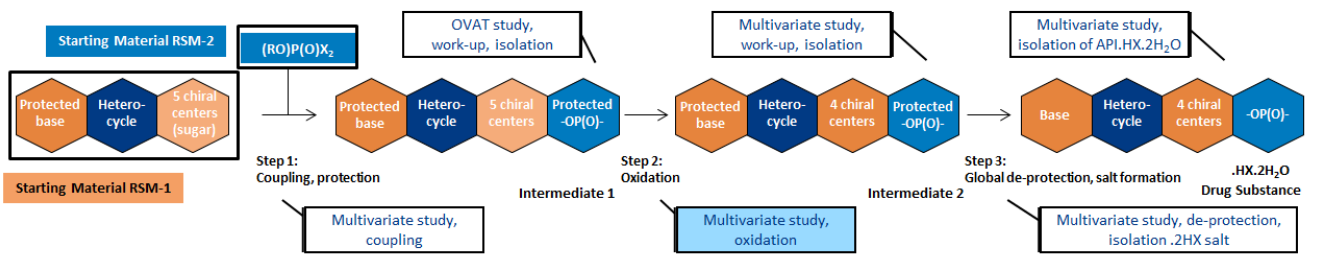
In addition to high CMC burden, the product and processes were also subject to several challenges:

- Sensitivity to oxidation, heat and pH at Intermediates and final Drug Substance
- High potential for impurity formation, with apparent close relationship to the process, with a corresponding need for a high degree of impurity structure, purge and control knowledge
- Close relationship of process operations to Drug Substance composition and solid form

Notably, each of the three manufacturing steps described rapid conversions, with limited opportunity for in-process corrective actions, and high likelihood for impurity generation.

Although initial scale-up development demonstrated that the processes could be executed as designed, process mapping was undertaken, targeting establishment of breadth of operational ranges, and any Critical Process Parameters.

Multivariate tools were applied across all steps; an example below exemplifies the approach for a rapid oxidation process described in Step 2 (of 3).



- Critical and potential Critical Material Attributes of the product, and their relationship to the Drug Substance were assessed, with total purity and impurity content identified as CMAs, and assay and solvent content as tentative CMAs
- The process was separated into its constituent parts and each component assessed for its potential to impact CMA, and potential study ranges estimated:
  - Nominal ranges and ranges allowed by company SOP or equipment constraint
  - Typical executed range, including standard deviation
  - Estimated critical ranges and reasonable range to map Proven Acceptable Range (PAR)
- Two multivariate studies were pursued:
  - Conversion: half-fractional central composite design (CCD) with five factors and four center-points
  - Isolation: full-factorial CCD with three factors and three center-points
- Study results indicated that the process was subject to broad allowable operational ranges with no apparent critical, uncontrollable or irreversible process relationships.

The study allowed for effective 'de-risking' of the process by the act of gathering and interpretation of knowledge; the outcome gave confidence that the assumed 'difficult-to-control' process actually operates within broad, readily-controllable ranges.

Example steps and content are presented below:

Stages 1-5

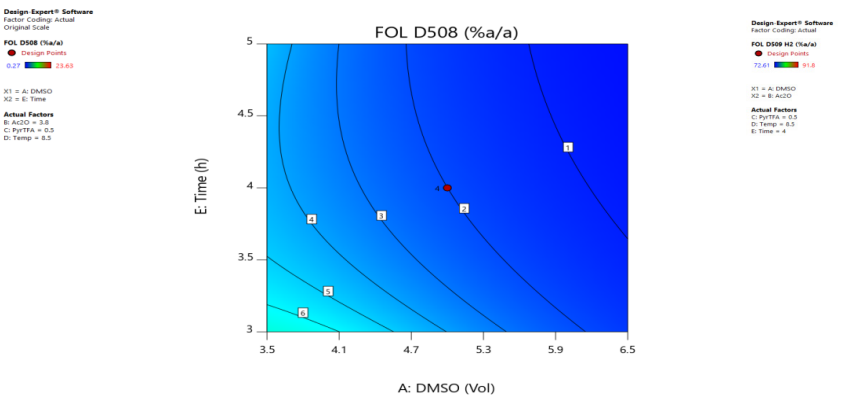
	Nominal Value	Estimate of Std. Dev.	Multiplier for Criticality	Test for Criticality	Test Range for Criticality		Suggested PAR Range		Comments
Unit Operation		**(σ)**		**(2 - 4 σ)**	**Low**	**High**	**Low**	**High**	**Suggested PAR range = x σ**
Charge DMSO (kg)	5 vol	5% of total charge	4 σ	1.0	4.0	6.0	3.5	6.5	6 σ gives PAR at reasonable range
Charge Ac_2O (kg)	3.8 eq	2% of total charge	4 σ	0.3	3.5	4.1	3.34	4.26	5 σ gives PAR at reasonable range
Charge PyrTFA (kg)	0.5 eq	2% of total charge	4 σ	0.04	0.46	0.54	0.42	0.58	2 x 4 σ gives PAR at reasonable range
Adjust Temperature (°C)	8.5 °C	1.5	2 σ	3	5.5	11.5	5	12	Set axial points at 4 σ
Agitate (h)	4 h	0.25	4 σ	1	3.0	5.0	3	5	Set axial points at 2 x criticality range

Stage 5

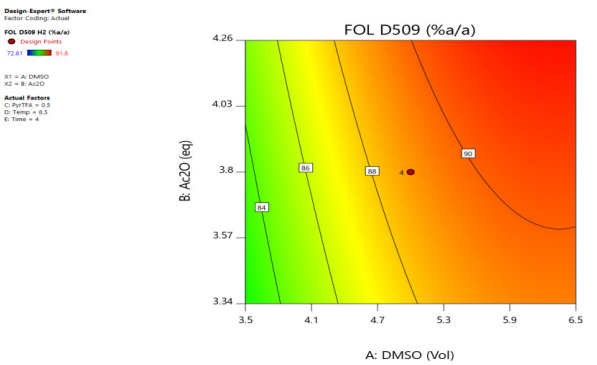
Factors and responses	Run	1	2	3	4	5	6	7	8	9	10
	Trial #	04-NWT-052	04-NWT-053	04-NWT-056	04-NWT-057	04-NWT-061	04-NWT-062	04-NWT-065	04-NWT-066	04-NWT-069	04-NWT-070
Input variables	DMSO charge, vol	6.5 (+)	6.5 (+)	5	6.5 (+)	5	5	6.5 (+)	3.5 (-)	6.5 (+)	5
	Ac_2O charge, eq	4.26 (+)	4.26 (+)	3.8	4.26 (+)	3.8	3.8	4.26 (+)	4.26 (+)	3.34 (-)	3.8
	Pyridine TFA charge, eq	0.42 (-)	0.58 (+)	0.5	0.42 (-)	0.5	0.5	0.58 (+)	0.58 (+)	0.58 (+)	0.5
	Temperature, °C	5.5 (-)	5.5 (-)	8.5	11.5 (+)	8.5	8.5	11.5 (+)	5.5 (-)	5.5(-)	8.5
	Time, h	5 (+)	3 (-)	4	3 (-)	4	4	5 (+)	5 (+)	5 (+)	4
Responses	Residual D508, % a/a	0.8	2.2	1.6	1.2	1.8	1.5	1.3	3.1	1.6	2.2
	FOL D509, % a/a	89.22	91.67	90.46	91.65	89.01	89.50	87.86	84.64	91.80	90.77
	FOL D508, % a/a	0.62	1.63	1.30	0.90	1.55	1.25	0.37	2.65	1.21	1.81
	FOL D745, % a/a	0.16	0.20	0.16	0.20	0.38	0.21	0.16	0.22	0.42	0.51
	FOL D746, % a/a	6.18	1.78	2.75	2.01	4.01	3.57	4.77	4.96	1.63	1.80
	FOL D707, % a/a	2.83	3.43	3.44	3.21	3.41	3.45	3.40	4.97	3.73	3.52
	Assay corrected yield, %	55*	52*	72	55*	70	72	52*	72	50*	71

Notes: (+): parameter varied at greater than nominal; (-): parameter varied at lower than nominal; * Output value at, or does not meet limit
DMSO = dimethyl sulfoxide; Ac_2O = acetic anhydride; TFA = trifluoroacetic acid; FOL = final organic layer; vol = volume; eq = equivalents

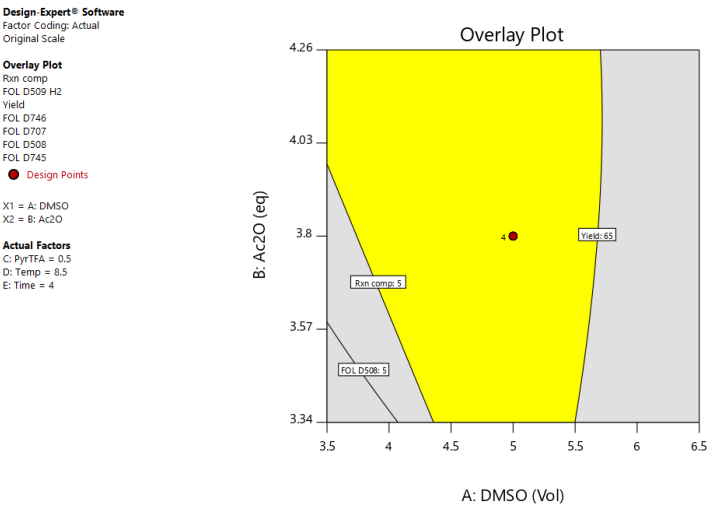
Stage 6



Time versus reagent / solvent charge



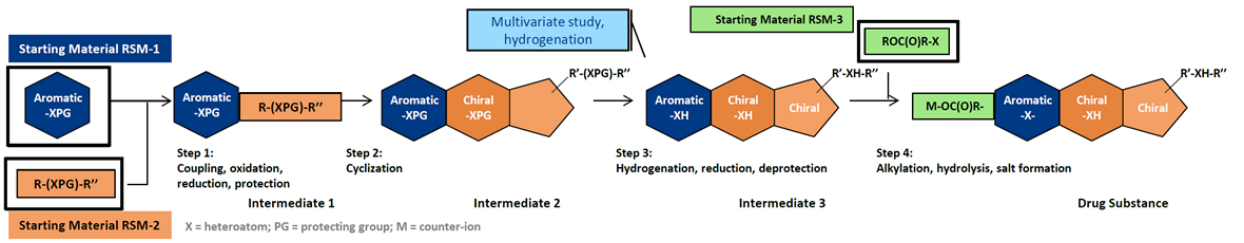
Reagent versus reagent / solvent charge



Overlay of plots for reagent charges, yield, showing broad operating range (operating range in yellow)

Case Study #2: Multivariate Study Verifying Process Sensitivity

The project involves manufacture of a complex commercially-distributed Drug Substance. The molecule contains several chiral centers, with stereochemistry installed through external auxiliary, substrate-based chiral transfer and kinetic resolution. Despite its complexity the product it is obtained in high purity with low, to non-detectable isomer content. The product is approved and commercially-distributed.



As part of a study targeting reduction of loss of solid reagents during charging in Step 3 (of 4), stage 1 of the conversion was mapped by multivariate method, targeting confirmation of sensitivity of conversion and in situ chiral purity to charge levels of the reagents.

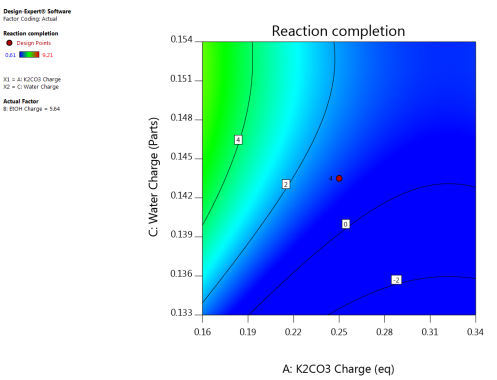
- Level of residual in-process intermediate and solution chiral purity were monitored, with level of residual precursor providing a measure of turn-over, and chiral isomer ratio mapping any sensitivity to stoichiometry.
- Study-type was established based on allowed and typical levels, with a full-fractional central composite design (CCD) selected for three reaction parameters, with four center-points over 18 trials; the study included ranges designed to provoke 'failing' outcomes and identification of any unidentified critical process parameters.
- Response surfaces were generated and overlaid to identify any critical relationships.
- Although a switch in reactivity was observed in some extreme conditions the study generally confirmed absence of sensitivity of the process to reagent charge levels across broad ranges.
- The study provided additional process knowledge as well as supporting improvement in reagent charge instructions.

Example steps and content are presented below:

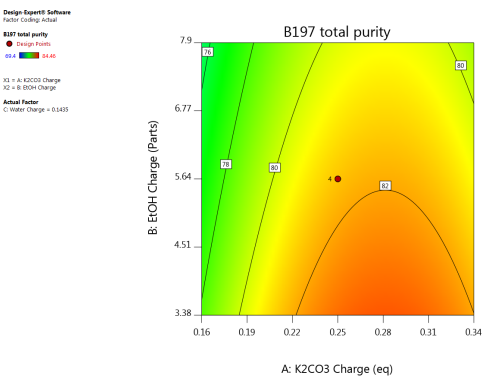
Stages 1-4

Operation / Parameter (unit)	Nominal value (range in MBR, basis)	Applied Standard Deviation (σ)	Proposed criticality study range	Proposed PAR study range	Test Range for Criticality		Test range for PAR	
					Low	High	Low	High
K_2CO_3 charge (molar equivalents)	0.25 equivalents (± 2 %)	2% of total charge	6 σ	3 x 6 σ	0.22	0.28	0.16	0.34
EtOH charge (parts, %w/w)	5.64 parts, w/w (± 5 %)	5% of total charge	4 σ	2 x 4 σ	4.51	6.77	3.38	7.90
Water content (parts, %w/w)	50-60 % wt	-	50 % weight + 10 % weight *1.5	50 % weight + 10 % weight *1.5	0.133	0.154	0.133	0.154

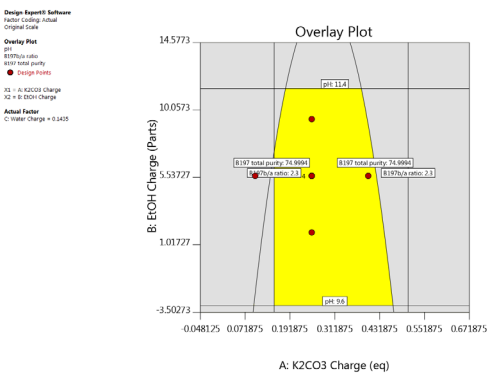
Stages 5-6



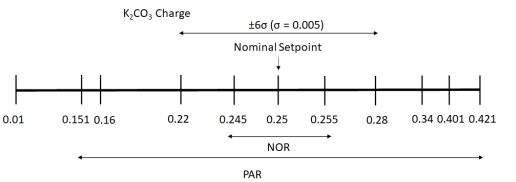
Water versus reagent charge



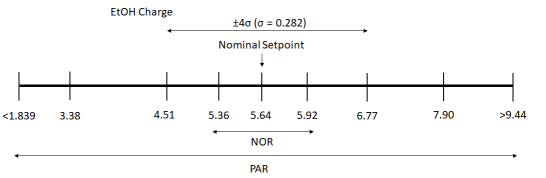
Solvent content versus reagent charges



Overlay of plots for pH, total purity and chiral isomer ratio (operating range in yellow)



Studied + established ranges, reagent charges



Studied + established ranges, solvent charge

SUMMARY

A summary of potential applications of multivariate studies in Drug Substance development have been presented.

The approaches represent a shift from univariate or one-variable-at-a-time OVAT approaches that may be instinctual to early-stage Drug Substance development.

Case studies outlining the application of multivariate studies in early-phase and later-stage programs have been presented, and indicate utility of the approach, including:

- Well-designed and well-defined approachable studies
- Process de-risking by rapid generation of data and knowledge
- Determination and visualization of process sensitivity

Contact Us

Eurofins CDMO Alphora Inc.
2395 Speakman Drive, Suite 2001
Mississauga, Ontario L5K 1B3
Canada

+1 905-403-0477
cdmo@eurofins.com
www.eurofins.com/cdmo