Use of Design of Experiments to define Manufacturing Process Conditions for an ADC

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Abstract

This poster describes the use of design of experiments (DOE) to understand the impact of process parameters at the reactive stages on an ADC product quality attributes. A 2^4 full factorial design was performed and the results were analysed via the MODDE software, with Drug to Antibody ratio (DAR) results shown. The software enabled identification of safe manufacturing set points for the process parameters.

Introduction

Antibody drug conjugates (ADCs) represent a significant area of growth for the biopharmaceutical market. To date, there are nine commercially approved molecules for oncology applications, with five of these molecules approved in 2019 and 2020.

ADCs combine the tumor targeting ability of a monocolonal antibody (mAb) with a cytotoxic payload. They are manufactured by attaching the monoclonal antibody to potent cytotoxic payload via a heterobifunctional linker. The process for manufacturing an ADC generally consists of concentration/diafiltration by tangential flow filtration, reaction(s), 0.2 µm sterile filtration, filling and occasionally chromatography. Frequently, the reaction step(s) of an ADC process is influenced by temperature, pH, concentration and time, requiring an understanding of the impact of these parameters on product quality. Here we describe the use of design of experiments (DOE) to understand the impact of reaction parameters on product quality. Further, the use of DOE software enabled efficient definition of reaction parameter set points and ranges to ensure process robustness while achieving target product quality.

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Material and Methods

Antibody Buffer Exchange

The antibody was first buffer exchanged from initial buffer into a suitable storage buffer by tangential flow filtration (TFF). Diafiltration of 1070mL of antibody at 29mg/mL was carried out on a Sartocon ECO Hydrosart 30kDa cassette (crossflow rate = 5.5L/min/m^2, loading 220g/m^2) with 20mM sodium acetate, pH 5.0 buffer. The resulting buffer exchanged material was formulated to 4% w/v sucrose, 20mM sodium acetate, pH 5.0 at a final concentration of 19mg/mL. Yield = 100%. SEC analysis showed no change in %monomer and aggregation levels.

Initial screening experiments

The reactive stages include antibody reduction followed by conjugation.

Initial screening experiments were conducted to establish reducing agent (TCEP) equivalence to target a DAR of ~3.9 and explore ranges for the following reduction process parameters : protein concentration, temperature, pH and reduction time. %DMA and reaction time for the conjugation reaction were also evaluated to ensure completeness of the reaction with minimal impact on aggregation.

From the screening experiments, it was determined to set TCEP equivalence to 2.25mol per mol of antibody, and to fix the following conjugation parameters : payload vcMMAE (6 mol vcMMAE per mol antibody), DMA concentration (7% v/v), conjugation time (45 min).

DOE Parameters

Factors				
Name	**Units**	**Low**	**High**	**Control Range (±)**
Protein Conc.	mg/mL	5	15	1
Temperature	°C	16	26	2
pH		6.8	7.8	0.2
Reduction Time	min	60	160	30
Response				
Name	**Abbrev.**	**Min**	**Target**	**Max**
Drug Antibody Ratio	DAR	3.4	3.9	4.4

Figure X – Table of factors used to define the Design with their corresponding Low and High values. The key response of Drug Antibody Ratio (DAR) with Min, Target and Max values. The Control Range is used in part to define a robust Set-Point.

Results

Exp No	Exp Name	Run Order	Protein Conc. (mg/mL)	Temp. (°C)	pH	Reduction time (min)	Drug Antibody Ratio
1	N1	7	5	16	6.81	61	2.83
2	N2	5	15	16	6.74	181	4.08
3	N3	15	5	26	6.81	180	3.94
4	N4	10	15	26	6.74	60	3.84
5	N5	17	5	16	7.83	180	3.97
6	N6	13	15	16	7.78	60	4.12
7	N7	19	5	26	7.83	60	3.80
8	N8	9	15	26	7.78	181	4.12
9	N9	4	10	21	7.26	120	3.92
10	N10	6	10	21	7.26	120	3.93
11	N11	14	10	21	7.26	120	3.97
12	N12	8	5	16	6.78	180	3.67
13	N13	1	15	16	6.78	60	3.49
14	N14	18	5	26	6.78	60	3.13
15	N15	16	15	26	6.78	180	4.18
16	N16	3	5	16	7.87	60	3.50
17	N17	12	15	16	7.77	180	4.20
18	N18	11	5	26	7.87	180	4.01
19	N19	2	15	26	7.77	60	4.23

Figure X – DOE parameters and results for Drug Antibody Ratio (DAR). The design was a full factorial design in 4 factors with 3 center points, 16+3 experiments. Reduction time and pH values are updated to experimentally measured values.

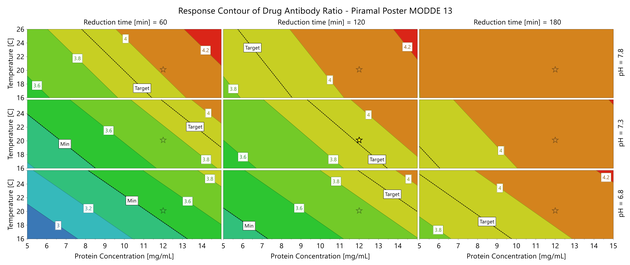

Figure X – DOE results as 4D Contour Plot. The plot displays the predicted response values for the selected response. The 9 contour plots are organized with Temperature and Protein Concentration in the inner axis with pH and Reduction Time in the outer axis. Min, Target, or Max-values are displayed inside the plot.

Conclusion

- Robust setpoints for the reactive stages of an ADC process were determined taking into account the existing manufacturing control ranges.
- This was achieved by the use of Design of Experiments, and subsequent software analysis modelling the risk of failure within the design space investigated.

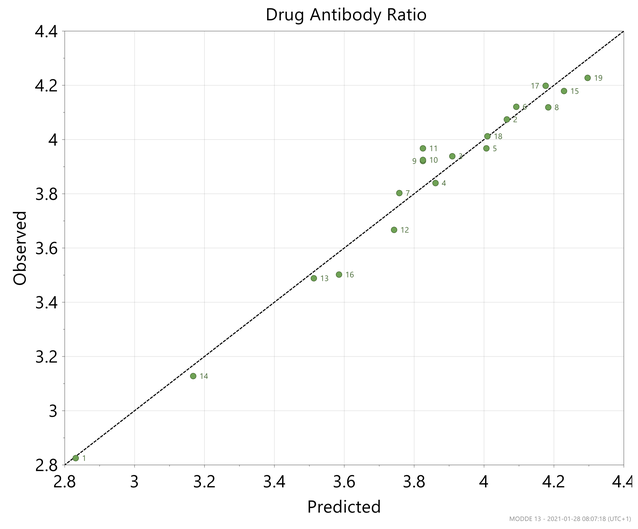

Figure X – Observed vs. Predicted Plot. Plots with points close to a straight line indicate a good model.

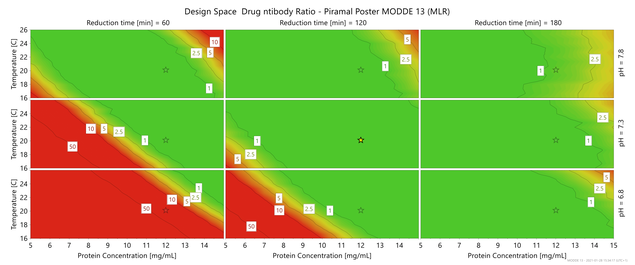

Figure X – DOE results as a 4D Design Space Plot. The plot shows the probability of failure (%) for the shown factor combinations. The robust Set-Point, yellow star, is the factor combination that is as far away from the edge of failure as possible.

The following safe manufacture setpoints were then selected:

- 12.4 ± 1 mg/mL
- pH 7.2 ± 0.2
- 20 ± 2 °C
- reduction time 115 ± 30min

Corresponding to low risk of failure.

Further Information

- If you would like further information on the CDMO services Piramal can offer, or to discuss potential projects, please contact Xavier Despinoy (xavier.despinoy@piramal.com)
- If you would like further information on the products Sartorius offer within the fields of bioconjugation and design of experiment, please contact Ian Schwartz (ian.schwartz@sartorius.com)