## Development of a new workflow for multiple attribute methodology (MAM) of an antibody drug conjugate (ADC)

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### INTRODUCTION

- There are a variety of post-translational modifications (PTMs) known to occur in monoclonal antibody and ADC biotherapeutics during the manufacturing, formulation, and storage process
- Monitoring these quality attributes is of major interest because of the potential impact on a product's safety and efficacy
- Peptide mapping analysis using liquid chromatography mass spectrometry (LC-MS) based detection is commonly used for the identification and the relative quantification of attributes such as e.g. oxidation and deamidation
- However, establishing a workflow for streamlined data analysis can be challenging for stability and especially forced degradation studies with increasing numbers of samples.

### **MATERIALS AND METHODS**

For forced degradation assessment ADC samples underwent thermal, mechanical and chemical stresses. All samples, including controls, were reduced, alkylated and enzymatically digested. Obtained peptides were separated on a reversed-phase C18 column using a high-flow LC setup (ExionLC<sup>™</sup> System). MS detection was carried out on a quadrupole-timeof-flight instrument (SCIEX X500B QTOF System) using data-dependent acquisition. Subsequent data analysis was performed using SCIEX OS Software 1.7 in a new streamlined way combining a high level of automation for calculations, ease of review, and verification of the results.

Row	ıs	Group	Name	Chemical Formula		Adduct/Ch	Precursor (Q1) Mass (Da)	XIC Width (ppm)	Retention Time Mode	Retention Time (min)	Experiment Index
15		LC	SGT	SGTASWCLLNNPYPR	` 005	[M+2H]2+	899.94326	12.00076	RT value	57.70	1 +TOF MS (250 - 1800)
16		LC	SGTASWICLLINNFYPR		5	[M+3H]3+	600.29793	11.99404	RT value	57.70	1 +TOF MS (250 - 1800)
17	<b>m</b>	LC	SGTASWCLLNNFYPR		50.005 <mark></mark>	[M+2H]2+	899.94326	12.00076	RT value	62.67	1 +TOF MS (250 - 1800)
18		LC	SGTASWCLLNNFYPR		58.005 <mark></mark>	[M+3H]3+	600.29793	11.99404	RT value	62.67	1 +TOF MS (250 - 1800)
19		LC	SGTASWICLLNNFYPR		58.005 <mark></mark>	[M+2H]2+	899.94326	12.00076	RT value	63.94	1 +TOF MS (250 - 1800)
20		LC	SGTASWICLLNNFYPR		58.005 <mark></mark>	[M+3H]3+	600.29793	11.99404	RT value	64.02	1 +TOF MS (250 - 1800)
21		LC	VONALQSGNSQESVTE		005K	[M+3H]3+	1207.24129	11.99429	RT value	43.00	1 +TOF MS (250 - 1800)
22		LC	VDNALQSGNSQESVTE		005K	[M+4H]4+	905.68279	12.00199	RT value	43.00	1 +TOF MS (250 - 1800)
23		LC	VONALQSSNSQESVTE		QD3K	[M+5H]S+	724.74769	12.00418	RT value	43.04	1 +TOF MS (250 - 1800)
24		LC	VDNALQSSNSQESVTE		005K <mark></mark>	[M+6H]6+	604.12429	12.00084	RT value	43.10	1 +TOF MS (250 - 1800)
25		LC	VDNALQSSNSQESVTE		005X	[M+3H]3+	1207.24129	11.99429	RT value	42.02	1 +TOF MS (250 - 1800)
26	1	LC	VDNALQSSNSQESVTE		005K <mark></mark>	[M+4H]4+	905.68279	12.00199	RT value	42.02	1 +TOF MS (250 - 1800)
27		LC	VDNALQSSNSQESVTE		0034 <mark></mark>	[M+3H]3+	1207.24129	11.99429	RT value	43.29	1 +TOF MS (250 - 1800)
28		LC	VDNALQSSNSQESVTE		0058	[M+4H]4+	905.68279	12.00199	RT value	43.29	1 +TOF MS (250 - 1800)
29		HC	EVQLQESSPGLMOPGG			[M+3H]3+	1344.98719	12.00011	RT value	67.27	1 +TOF MS (250 - 1800)
30		HC	EVQLQESSPELVIPOE		Ç	[M+4H]4+	1008.99221	12.00207	RT value	67.25	1 +TOF MS (250 - 1800)
31		HC	EVQLQESSPGLMORGG			[M+5H]5+	807.39522	12.00156	RT value	67.25	1 +TOF MS (250 - 1800)
32	1	HC	EVQLQESSPELVIPEE			[M+6H]6+	672.99723	5.36406	RT value	67.25	1 +TOF MS (250 - 1800)
33		HC	EVQLQESSPGLVIPGG		s s s 5	[M+3H]3+	1350.31883	11.99717	RT value	63.57	1 +TOF MS (250 - 1800)
	,								1.100 ·		

Figure 1. Summary of quality attributes used for tracking purposes. Oxidation, deamidation and isoaspartate formation were defined to be monitored as part of the degradation assessment. Up to four charge states from each peptides were considered in quantification.

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Apply peak parameters to all of the components 2.4e5	23.64 Eiguro 2 Example of the	RESULTS
Minimum Peak Width 3 points 2.365 Minimum Peak Height 5000.00 2.2e5	Figure 2. Example of the	Review (Poster_ASMS.qsession)         of 99 rows       Filters: 1         Qualify for Rules Filters
S/N Integration Threshold     3     2.1e5       XIC width     0.01054     Da     2.0e5	ion chromatogram (XIC)	Sample Name         v         Component Name         v         Component Group Name           11         C04351P189
Gaussian Smooth Width     2.0     points     1.9e5       Noise Percentage     90.0     %	Integration parameters for	14         C04351P189_i         .         LC -           15         C04351P189_i         .         LC -           16         C04351P189_i         .         LC -           17         C04351P189_i         .         LC -
Baseline Subtract Window 2.00 min 1.7e5	each attribute were	17         C04351P189_1         . LC -           18         C04351P189_1         . LC -           19         C04351P189_1         . LC -           20         C04351P189_1         . LC -
Retention Time (RT)	ontimized independently	21         C04351P189_i
Expected RT 23.65 min 1.4e5	such as minimum peak	29         C04351P189_1         HC           30         C04351P189_1         HC           36         C04351P189_1         HC           37         C04351P189_1         HC
Update Expected RT No V 12e5	height, S/N threshold, XIC	38         C04351P189_          HC           40         C04351P189_          HC           41         C04351P189_          HC           42         C04351P189_          HC
Units & Calibration Defaults 1.0e5	width, peak splitting and RT	43         C04351P189_1         HC           44         C04351P189_1         HC           45         C04351P189_1         HC
Apply units and calibration parameters to all of the 9.0e4 -	window.	Apply
Concentration units 8.0e4 7.0e4		✓ Retention Time (RT)     Expected RT     60.30 min     RT Half Window     30.0 sec
Regression type Linear V 6.0e4		Update Expected RT No  Report Largest Peak Integration
Remove outliers automatically from the calibration 4.0e4		Minimum Peak Width     3     points     2.0e5       Minimum Peak Height     100.00     3     3       S/N Integration Threshold     3     1.5e5
3.0e4 - 2.0e4 -		Gaussian Smooth Width 2.0 points Noise Percentage 40.0 % 1.0e5 - Baseline Subtract Window 1.00 min
1.0e4		Peak Splitting 2 points 5,0e4 -
Apply	23.4 23.6 23.8 24.0 24.2 24.4 24.6 24.8 Time, min	0.0e0 59.0 59.5 60.0 ▼ Peak Details
		599.970 1.7 60.37
		75
<ul> <li>Accept changes and return to Calculated Columns</li> <li>X Discard</li> </ul>	<ul> <li>Accept changes and return to Flagging Rules</li> <li>X Discard</li> </ul>	70
Use the calculator to create a new formula.	Rule name RTFlag	65 -
Formula name SumArea	Flag a results column Retention Time Delta (min) 💙	<b>60</b>
	Flagging criteria Range 💙	<b>S</b> 55 -
= GETGROUP([Area], T)+GETGROUP([Area], 2)+GETGROUP([Area], 5)	Step 1: Define the values for the flagging criteria	
COUNT MAX STDEV Clear r	Alue for all components	
SUM MIN MEDIAN ( Slope	Lower limit -0.1	<b>OC</b> <sup>35</sup>
MEAN ABS MAD ) Quadratic coefficient	Upper limit 0.1	30 -
/ * - + Constant term	R  Accent changes and return to Elagging Bules  X Discard	25
Treat "N/A" values as Zero   Columns  Accuracy  Accuracy		15 -
Accuracy Acceptance	Rule name MassErrorFlag	10 - C04351
B < Accept changes and return to Calculated Columns × Discard	Flagging criteria Pango	5 - C04351P189_3 f_TRY
Use the calculator to create a new formula.	Kange	
Formula name Percentage	Step 1: Define the values for the flagging criteria	Figure 6. Oxidation c
	<ul> <li>Value for all components</li> </ul>	percentage change ac
= [Area]/[SumArea]*100	Lower limit -10	increasing stress time.
COUNT MAX STDEV Clear Regression parameters	Upper limit 10	visualization tool for ea
SUM MIN MEDIAN ( <sup>r^2</sup>	Accept changes and return to Flagging Rules X Discard	
Slope Intercept	Rule name Mod/Total Flag	CONCLUS
MEAN ABS MAD ) Quadratic coefficient	Flag a results column RatioMod 🗸	The establishe
/ * - + Constant term	Flagging criteria Vpper limit 🗸	• The establishe
Treat "N/A" values as Zero   Columns  Accuracy	Step 1: Define the values for the flagging criteria	solution for mo
Accuracy Acceptance	Alue for all components	• A very flexible
Figure 2 Upor defined coloulations for	Upper limit 5	
automatic % calculation for each		modification ind
modification An automatic calculation of	Figure 4 Flagging rules for different	range paramet
modification percentages based on extracted ion	parameters within MAM assay. To	The streamline
chromatograms (XIC) areas of modified pentides	achieve an accurate identification and high	
against all other forms of the same peptide was	throughput, attribute pass/fail criteria were	transterred to c
set up. Up to four charge states were summed to	defined for retention time (A), mass	
achieve a precise calculation (A), followed by a	accuracy (B) and modification percentage	TRADEMARKS/I
percentage calculation (B).	(C). Any attribute which fails the set criteria	The SCIEV aligned diagnostic part
	will be flagged in the assay table by a color	availability, please contact your loc

scheme, facilitating data review.

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# SANOFI



hange on the peptide DTLMISR. Metric plot of the modification cross stressed samples showing increasing levels of oxidation with The metric plot can be directly generated inside the results table as a asy and quick comparison of samples.

## SIONS

ed MAM assay represents a comprehensive onitoring product quality attributes (PQA)

and automatic calculation of the percentage of cluding visual tools (metric plot, flagging of out-ofters) speeds up the assessment of PQAs

ed workflow is compliant-ready, thus can be departments with further regulatory requirements

### CENSING

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