Electronic Supplementary Material (ESI) for Analyst.

Supplementary information

Qualitative analysis of antibody-drug conjugates (ADCs): an experimental comparison of analytical techniques of cysteine-linked ADCs

Malin Källsten[1,3], Rafael Hartmann[2], Konstantin Artemenko[1], Sara Bergström Lind[1], Fredrik Lehmann[3] and Jonas Bergquist[1].
[1] Department of Chemistry-BMC, Analytical Chemistry, BMC, Uppsala University, Box 599, SE-T51 24, Uppsala, Sweden, [2] Department of Medicinal Chemistry, Organic Pharmaceutical Chemistry, BMC, Uppsala University, Box 574, SE-751 23 Uppsala, Sweden and [3] Recipharm OT Chemistry AB, Viridings allé 32b, SE-754 50, Uppsala, Sweden

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Chromatograms for HIC-UV/VIS depicting sample signal and consecutive blank runs

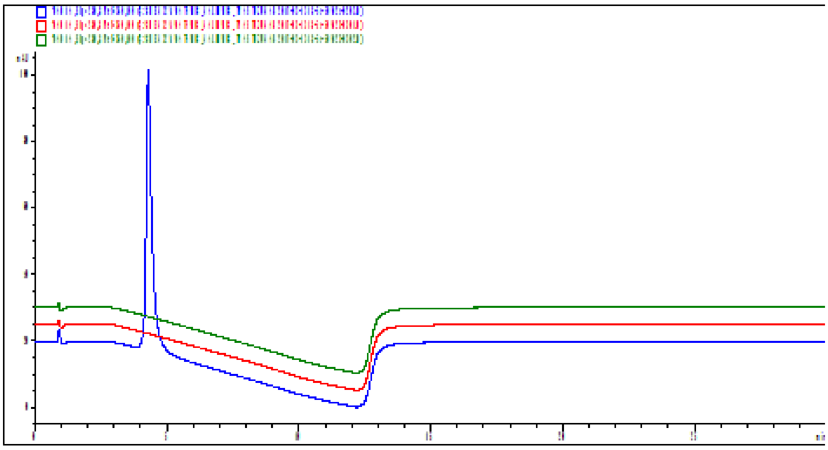


Figure S1. Chromatogram for mAb (blue) and consecutive blank runs before introduction of the next sample batch onto the column for HIC-UV/Vis.

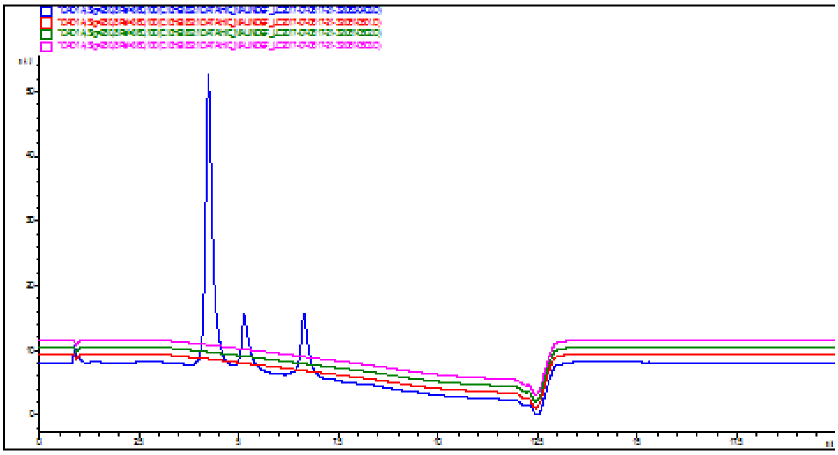


Figure S2. Chromatogram for ADC with average DAR of 1 (blue) and consecutive blank runs before introduction of the next sample batch onto the column for HIC-UV/Vis.

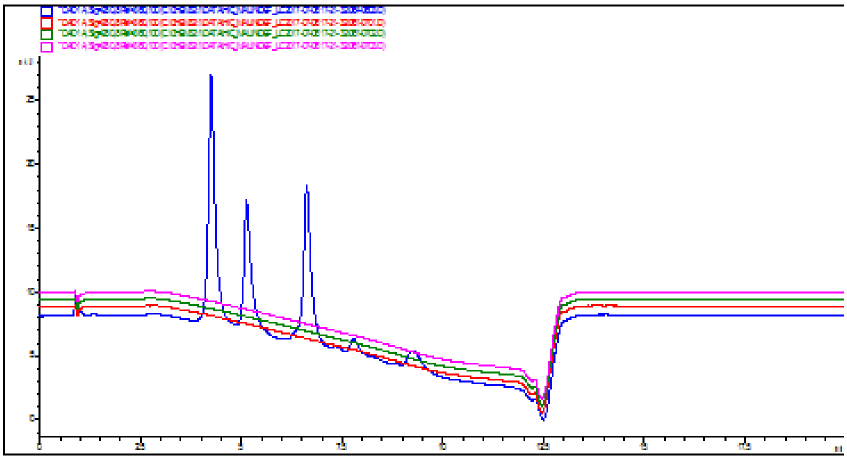


Figure S3. Chromatogram for ADC with average DAR of 2 (blue) and consecutive blank runs before introduction of the next sample batch onto the column for HIC-UV/Vis.

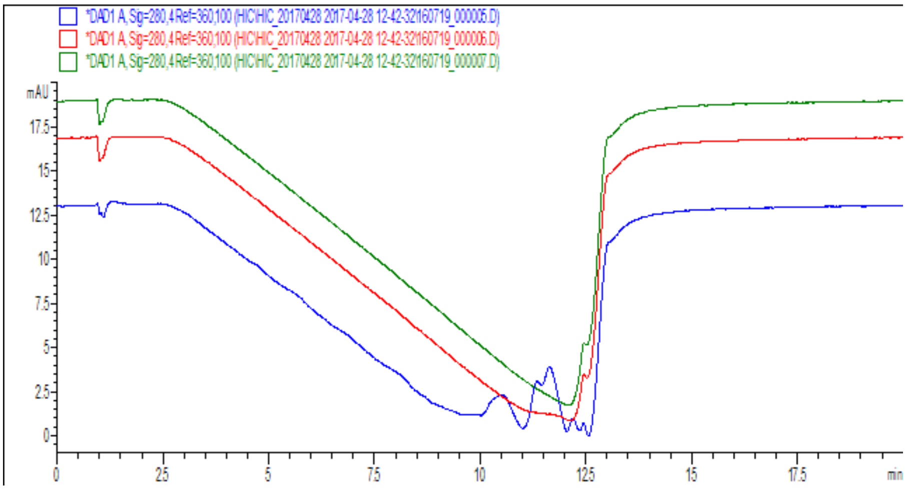


Figure S4. Chromatogram for ADC with average DAR of 6 (blue) and consecutive blank runs before introduction of the next sample batch onto the column for HIC-UV/Vis.

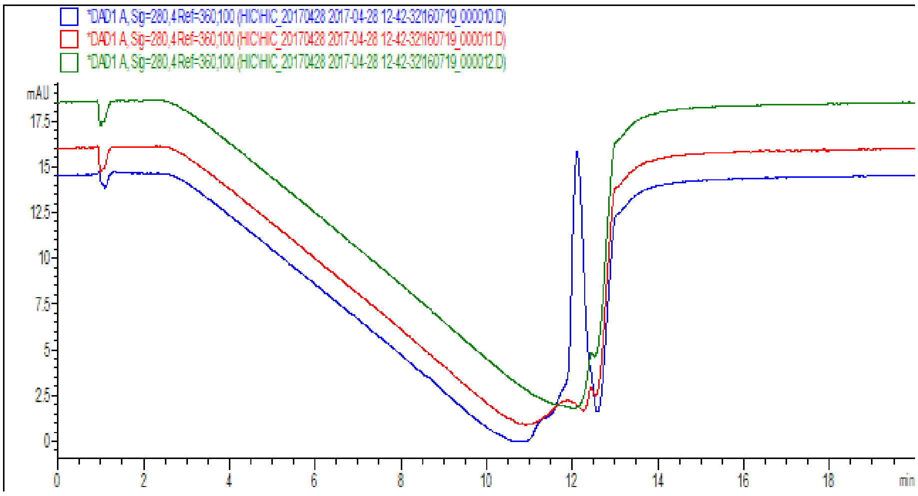


Figure S5. Chromatogram for ADC with average DAR of 8 (blue) and consecutive blank runs before introduction of the next sample batch onto the column for HIC-UV/Vis.

Chromatograms for RPLC-MS QTOF depicting sample signal and consecutive blank runs

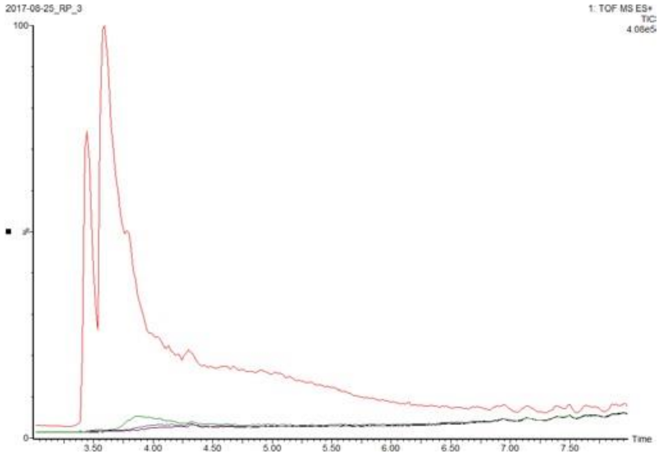


Figure S6. TIC chromatogram for mAb (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (QToF).

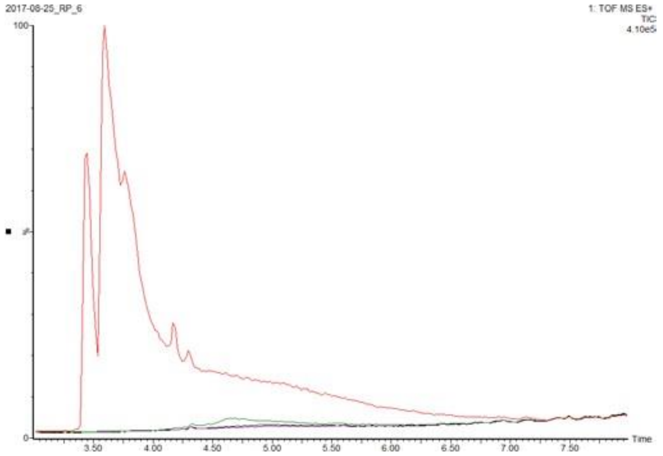


Figure S7. TIC chromatogram for ADC with average DAR of 1 (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (QToF).

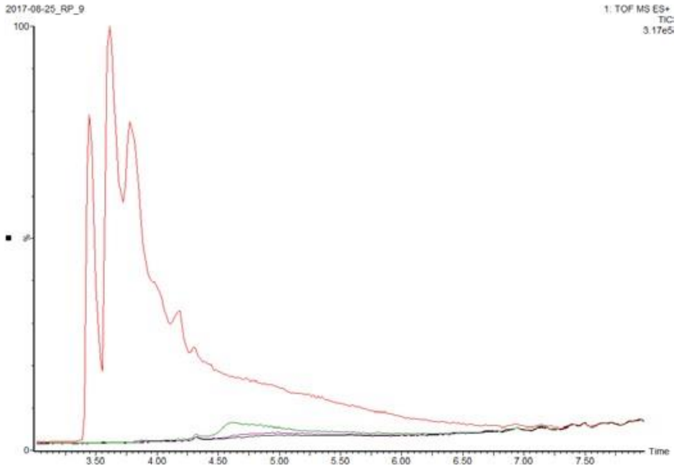


Figure S8. TIC chromatogram for ADC with average DAR of 2 (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (QToF).

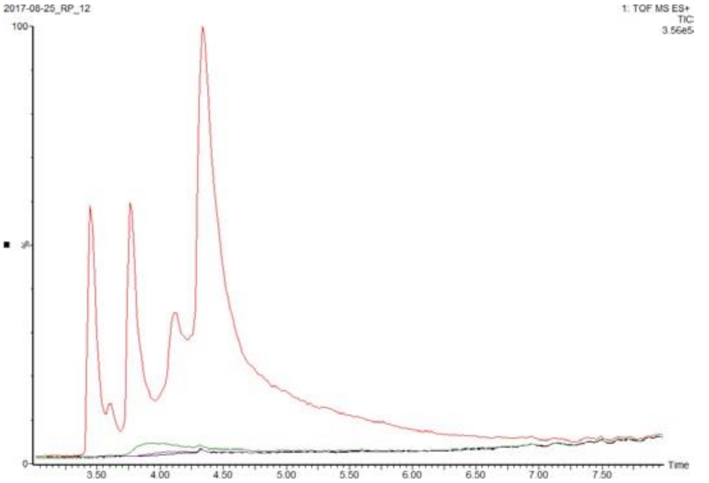


Figure S9. TIC chromatogram for ADC with average DAR of 6 (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (QToF).

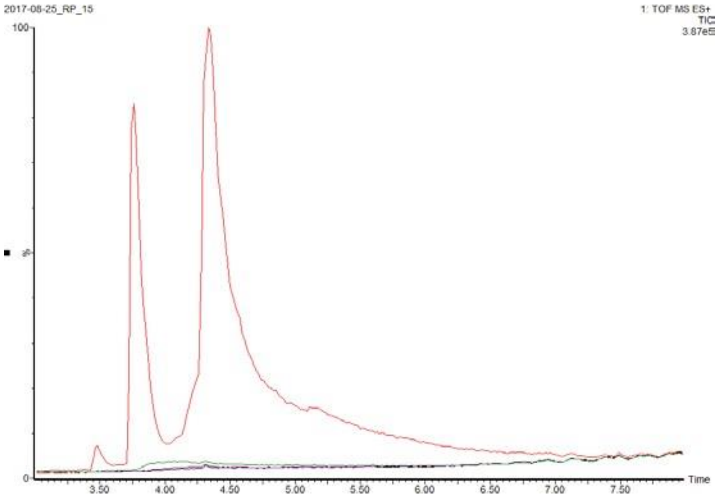


Figure S10. TIC chromatogram for ADC with average DAR of 8 (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (QToF).

Chromatograms for RPLC-MS Orbitrap depicting sample signal and consecutive blank runs

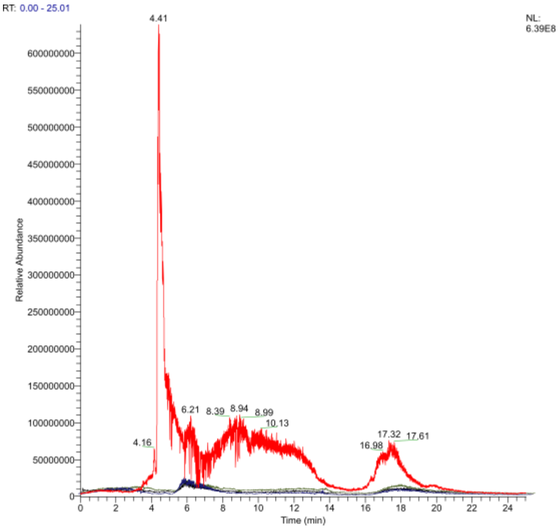


Figure S11. TIC chromatogram for mAb (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (Orbitrap).

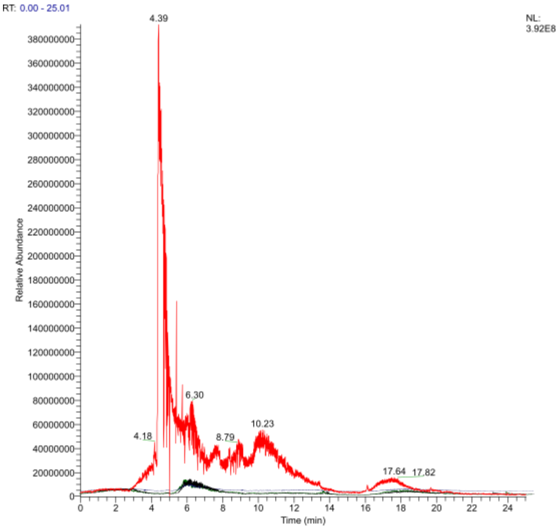


Figure S12. TIC chromatogram for ADC with average DAR of 1 (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (Orbitrap).

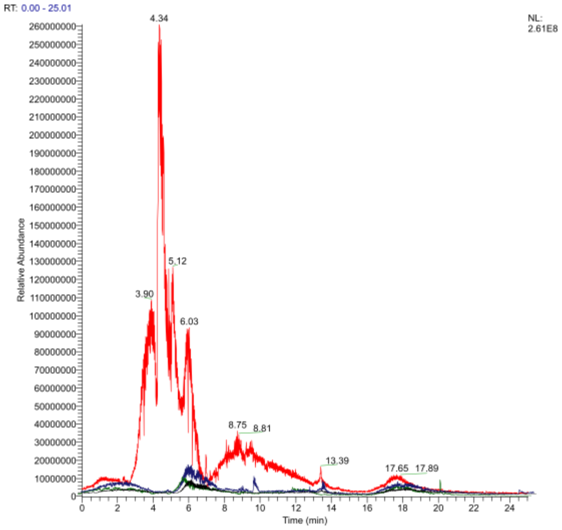


Figure S13. TIC chromatogram for ADC with average DAR of 2 (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (Orbitrap).

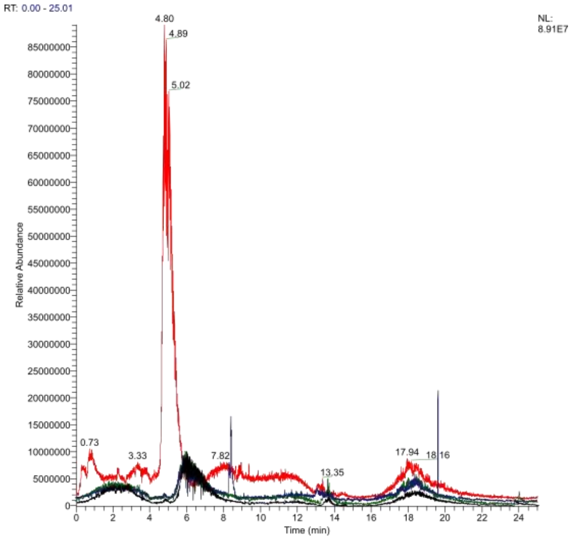


Figure S14. TIC chromatogram for ADC with average DAR of 6 (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (Orbitrap).

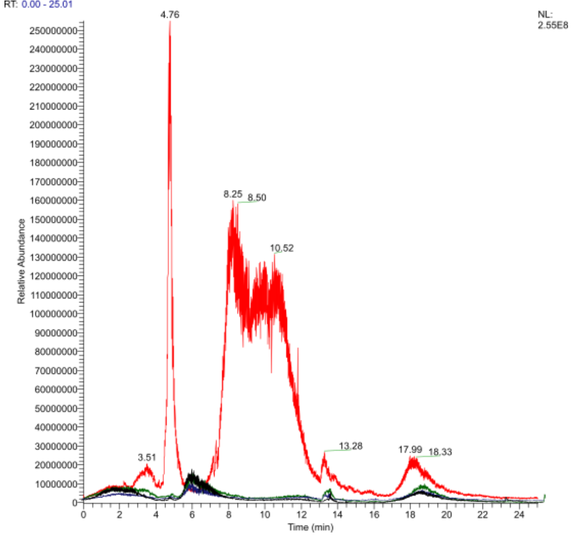
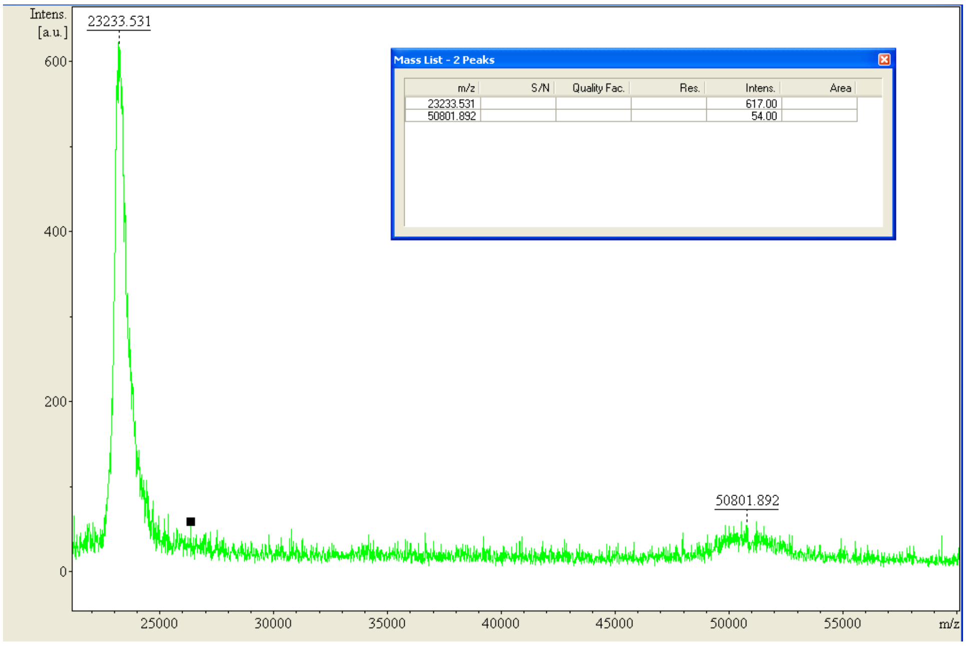


Figure S15. TIC chromatogram for ADC with average DAR of 8 (red) and consecutive blank runs before introduction of the next sample batch onto the column for RPLC-MS (Orbitrap).

Sample preparation for sample batch mAb, high DAR and max. DAR for MALDI-TOF-MS analysis

A portion of each batch (42 µg) was deglycosylated by adding 2 µL of PNGaseF (500units/mL) at 37°C and incubating for 3 hours. The interchain disulfide bonds were then reduced by addition of 4 µL of 1M DTT followed by 4 µL 550 mM iodoacetamide (IAA) for 30 and 20 min at room temperature respectively. Finally, the samples were buffer exchanged into 200mM Ammonium acetate using Biorad's Micro Bio-spin Columns with Bio-gel P-20 (40 kDa cut-off, Tris-buffer) according to the providers protocol and the sample volumes were adjusted if needed to a final concentration of 0.5 mg/mL and stored at -20°C until analysis.

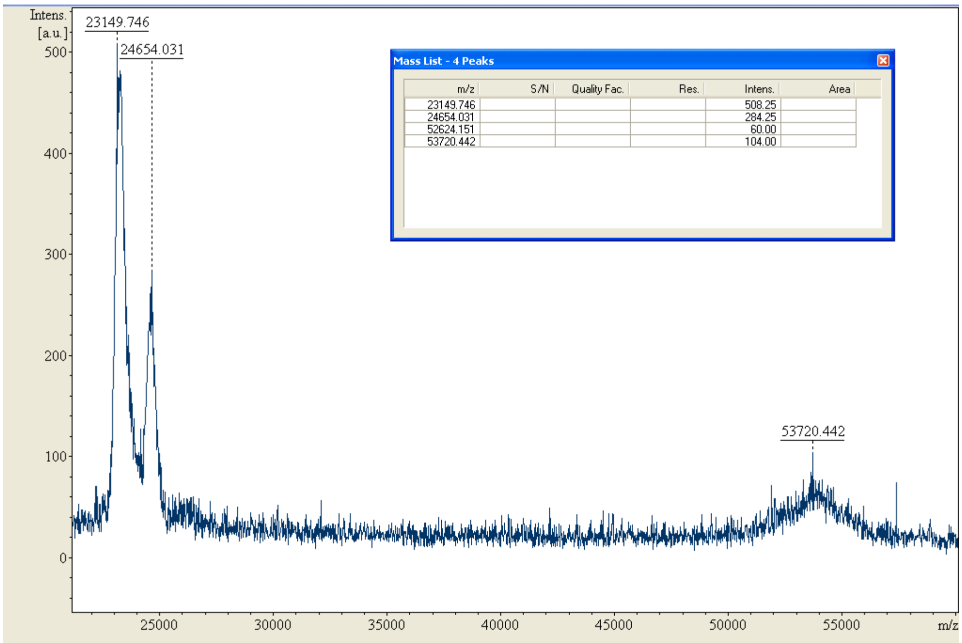
Highest spectra for mAb, DAR 6 and 8 from MALDI samples prepared according to experimental procedure in the main text



Mass List - 2 Peaks

m/z	S/N	Quality Fac.	Res.	Intens.	Area
23233.531				617.00	
50801.892				54.00	

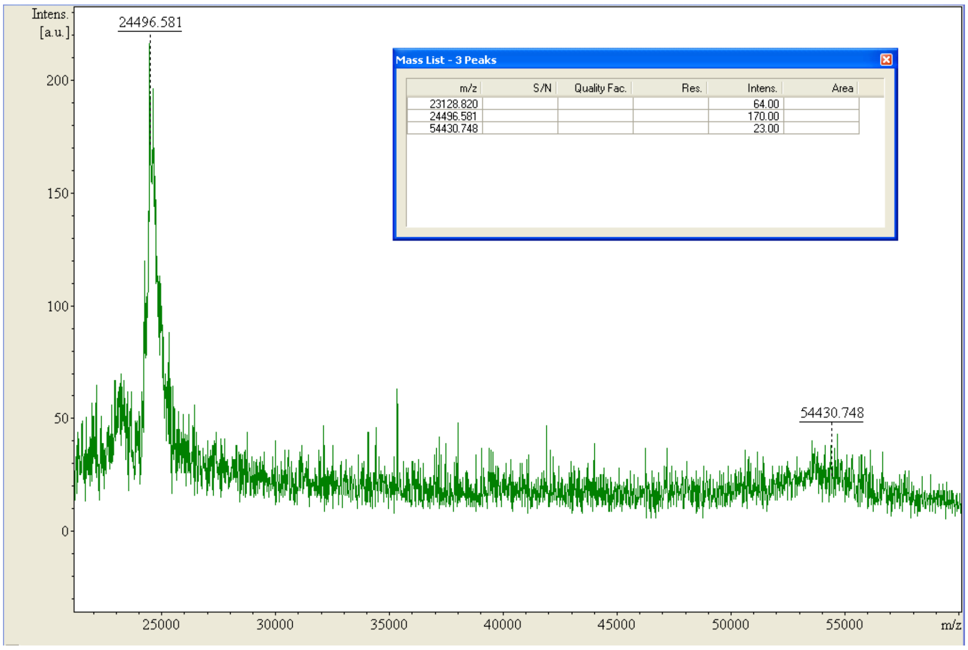
Figure S16. Highest spectra obtained for spots made from samples prepared without desalting and iodoacetamide steps for mAb.



Mass List - 4 Peaks

m/z	S/N	Quality Fac.	Res.	Intens.	Area
23149.746				508.25	
24654.031				284.25	
52624.151				60.00	
53720.442				104.00	

Figure S17. Highest spectra obtained for spots made from samples prepared without desalting and iodoacetamide steps for ADC with average DAR of 6.



m/z	S/N	Quality Fac.	Res.	Intens.	Area
23128.820				64.00	
24496.581				170.00	
54430.748				23.00	

Figure S18. Highest spectra obtained for spots made from samples prepared without desalting and iodoacetamide steps for ADC with average DAR of 8.

MALDI-TOF-MS

Table S1. Average mass, theoretical mass and deviation from theoretical mass for experimentally determined masses of LC and HC with different degrees of conjugation from triplicate runs of all five batches on a MALDI-TOF-MS. Numbers marked in red indicate a visible peak in the deconvoluted spectra with questionable quality.

	Average mass (Da)					*Theoretical mass (Da)*	*Deviation from theoretical mass (Da)*				
	Un-conjugated	Low DAR	Mid DAR	High DAR	Max DAR		Un-conjugated	Low DAR	Mid DAR	High DAR	Max DAR
LC	23047	23238	23207	23031	22948	23440	-393	-202	-233	-409	-492
LC+1d		24607	24500	24378	24366	24757		-150	-257	-379	-391
HC	49730	50261	50034			49300	430	961	734		
HC+1d	51169	51459	51338			50617	552	842	721		
HC+2d				52301	52151	51934				367	217
HC+3d				53491	53463	53251				240	212

RPLC-QTOF-MS

Table S2. Average mass, theoretical mass and deviation from theoretical mass for experimentally determined masses of LC and HC with different degrees of conjugation from triplicate runs of all five batches on a RPLC-QTOF-MS.

	Average mass (Da)					*Theoretical mass (Da)*	*Deviation from theoretical mass (Da)*				
	Un-conjugated	Low DAR	Mid DAR	High DAR	Max DAR		Un-conjugated	Low DAR	Mid DAR	High DAR	Max DAR
LC	23444	23443	23443	23443		23440	4	3	3	3	
LC+1d			24760	24760	24760	24757			3	3	3
HC	49159	49159	49158			49300	-141	-141	-142		
HC+1d		50470	50473			50617		-147	-144		
HC+2d				51792		51934				-142	
HC+3d				53109	53108	53251				-142	-143

RPLC-Orbitrap-MS

Table S3. Average mass, theoretical mass and deviation from theoretical mass for experimentally determined masses of LC and HC with different degrees of conjugation from triplicate runs of all five batches on a RPLC-Orbitrap-MS. Numbers marked in red indicate a visible peak in the deconvoluted spectra with questionable quality.

	Average mass (Da)					*Theoretical mass (Da)*	*Deviation from theoretical mass (Da)*				
	Un-conjugated	Low DAR	Mid DAR	High DAR	Max DAR		Un-conjugated	Low DAR	Mid DAR	High DAR	Max DAR
LC	23456	23455	23455	23455		23440	16	15	15	15	
LC+1d		24752	24735		24773	24757		-5	-22		16
HC	49224	49230	49230			49300	-76	-70	-70		
HC+1d		50531	50549			50617		-86	-68		
HC+2d		51723	51865			51934		-211	-69		
HC+3d				53183	53185	53251				-68	-66

Mass shift from conjugation

Table S4. Theoretically and experimentally determined mass shift from conjugation of one linker and drug to the light chain and heavy chain (with or without additional conjugation) as detected by each different MS-based technique.

	MALDI-TOF-MS	*RP-MS (QToF)*	*RP-MS (Orbitrap)*	*Theoretical*
LC+1d	1319 Da	1316 Da	1319 Da	1317 Da
HC+1d	1608 Da	1313 Da	1325 Da	1317 Da
HC+2d	1289 Da	1316 Da	1318 Da	1317 Da
HC+3d	1244 Da	1316 Da	1320 Da	1317 Da