

## Quantitative Assessment of Daratumumab in Serum via Intact Light Chains Measurement Using Liquid Chromatography-High Resolution Mass Spectrometry: A Method suitable for Therapeutic Drug Monitoring

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### Supporting Information

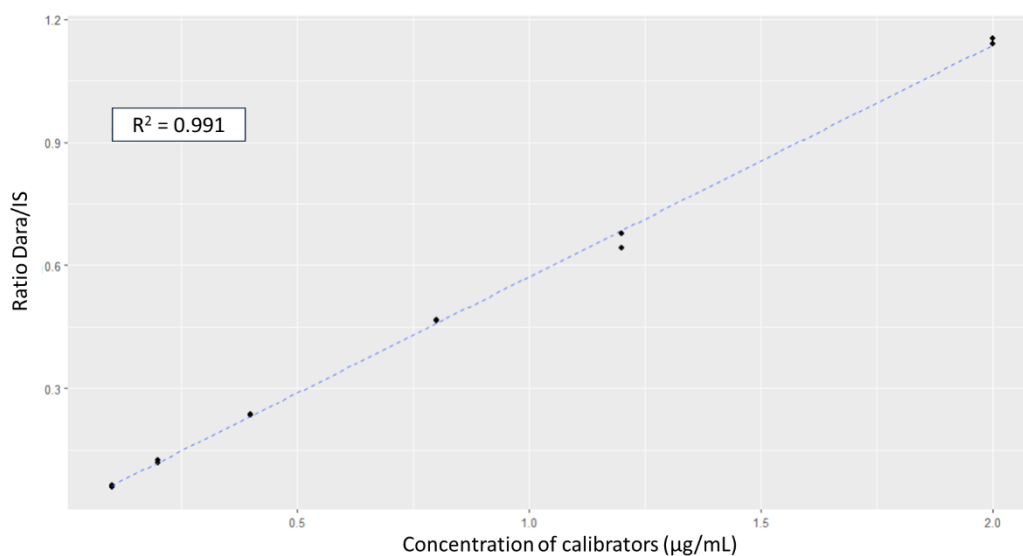


Figure S1. Representative calibration curve of Daratumumab in serum, run in duplicate.

Table S1. Method comparison of Dara quantification.

Author (year)	Matrix	Sample enrichment	Sample treatment	Trypsinization	LC	MS	Figures of merit
Our method	Human serum 20 uL	Melon gel	Denaturation Reduction	Not required	UHPLC MABPac® RP 2.1 × 50 mm 5 min gradient	OT Thermo QExactive Plus Full Scan, resolution 17500.	LLOQ=100 µg/mL RE <9% RSD <6%
Zajec et al. (2018)	Human serum 2 uL	Melon gel	Denaturation alkylation reduction	Overnight at 37 °C	nano-LC system PepMap C18 75 µmID× 250 mm, 2 µm 28 min gradient	OT Thermo Fusion Lumos Tribrid scheduled PRM	LLOQ=1 µg/mL
Noori et al. (2021)	Human serum 0.12 uL	Not reported	Denaturation alkylation reduction	Overnight at 37 °C	nano-LC system PepMap C18 75 µmID× 250 mm, 2 µm 30 min gradient	OT Thermo Fusion Lumos Tribrid scheduled PRM	LLOQ=1 µg/mL
Yamaoka et al. (2023)	Human serum 50 uL	Protein A	Denaturation alkylation reduction	120 min at 45 °C	UHPLC Inertsil Peptides C18 4 µm, 2.1×150 mm Gradient 13 min	TQ Sciex QTRAP 4500 MRM	LLOQ=50 µg/mL RE <13% RSD < 9%
Li et al. (2024)	Rat serum 10 uL	Not required	Denaturation No reduction No alkylation	30 min at 45 °C	UHPLC C18 2.1 × 150 mm, 1.7 µm Gradient 10 min	TQ Agilent 6495 MRM	LLOQ=1 µg/mL RE <15% RSD <15%

Abbreviations: DTT dithiothreitol, IAA iodoacetamide, PRM Parallel reaction monitoring, MRM Multiple reaction monitoring, SPE solid phase extraction, OT orbitrap, TQ triple quadrupole, LLOQ lower limit of quantification, UHPLC ultra high-performance liquid chromatography, RE relative error, RSD relative standard deviation.

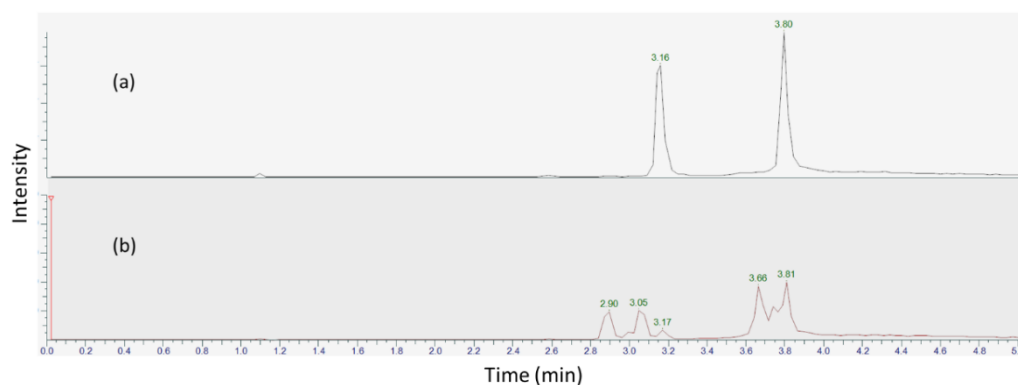


Figure S2. Effect of different reducing agents on the chromatographic separation of light and heavy chains of Dara. (a) DTT as reducing agent (Tris·HCl 0.2 M, Guanidine·HCl 12M, DTT 0.1 M, incubation for 30 min at

60 °C). (b) TCEP as reducing agent (0.05 M, used in combination with NH<sub>4</sub>HCO<sub>3</sub> 0.025 M, incubation for 30 min at 37 °C).

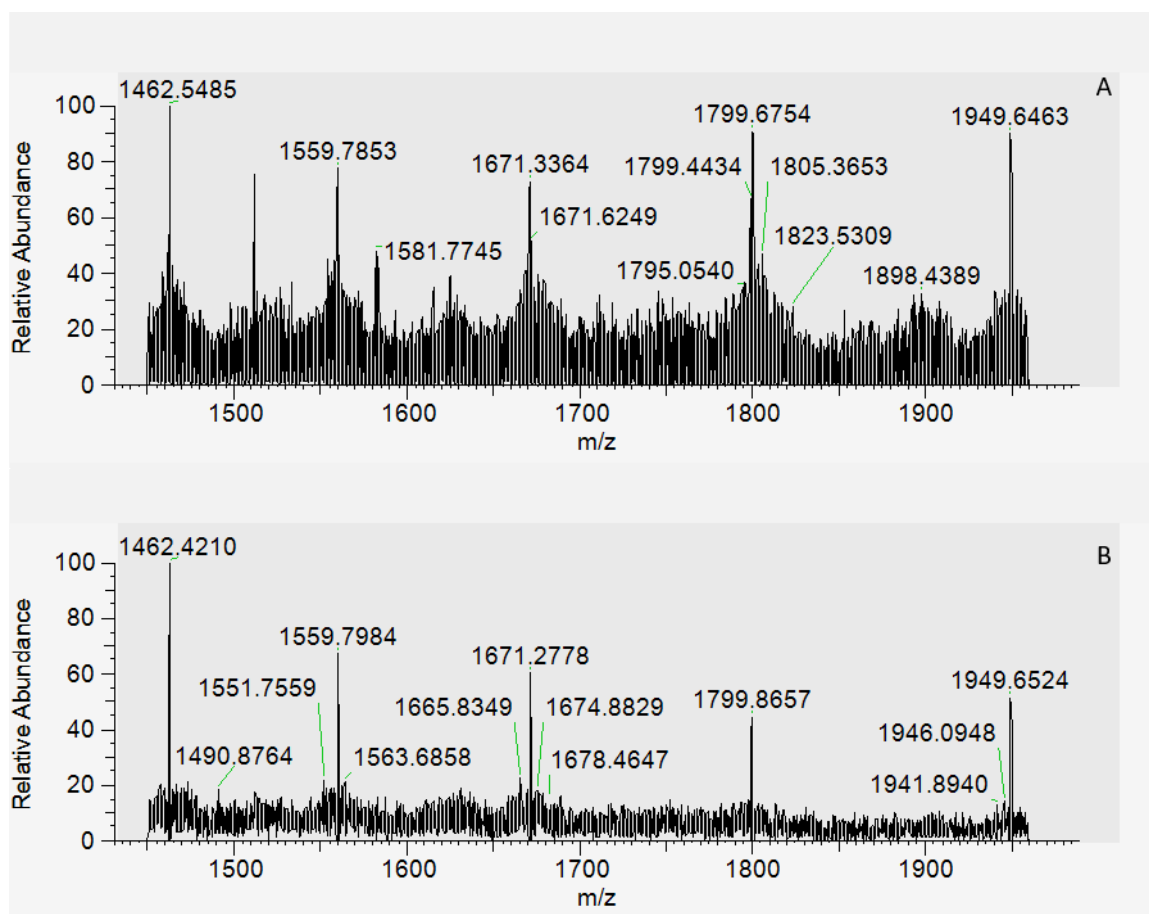


Figure S3. Dara light chain MS spectrum evaluated at 140000 (A) and 17500 (B) resolution in LLOQ serum sample. The mean S/N for the charge states was 2.5 in A and 5 in B.

Table S2. Linearity of Dara calibration curve over 7 days

Day	Intercept*	Slope*	R <sup>2</sup>
1	3.237×10 <sup>-3</sup>	5.551×10 <sup>-1</sup>	0.991
2	4.629×10 <sup>-3</sup>	5.701×10 <sup>-1</sup>	0.996
3	6.545×10 <sup>-3</sup>	4.704×10 <sup>-1</sup>	0.996
4	5.356×10 <sup>-3</sup>	5.300×10 <sup>-1</sup>	0.995
5	8.589×10 <sup>-3</sup>	5.381×10 <sup>-1</sup>	0.993
6	2.625×10 <sup>-3</sup>	5.872×10 <sup>-1</sup>	0.992
7	6.933×10 <sup>-3</sup>	5.788×10 <sup>-1</sup>	0.994

\*Obtained using 1/x<sup>2</sup> weighting factor.

Generic expression for the calibration curve: Ratio Dara/IS = Intercept + Slope × Concentration.

Table S3. Accuracy and Precision for Daratumumab calibrators

	<b>Cal 1 100 µg/mL</b>	<b>Cal 2 200 µg/mL</b>	<b>Cal 3 400 µg/mL</b>	<b>Cal 4 800 µg/mL</b>	<b>Cal 5 1200 µg/mL</b>	<b>Cal 6 2000 µ g/mL</b>
<b>Day 1</b>	100	211	425	815	1213	1880
	94	192	425	809	1210	1855
<b>Day 2</b>	94	200	408	809	1120	1994
	102	211	405	812	1181	2014
<b>Day 3</b>	99	203	416	810	1198	1901
	99	191	420	818	1202	1932
<b>Day 4</b>	99	201	418	824	1191	1936
	99	195	424	813	1191	1881
<b>Day 5</b>	98	195	429	796	1152	1916
	100	196	435	818	1187	1965
<b>Day 6</b>	95	202	407	786	1158	2094
	101	207	436	800	1148	1880
<b>Day 7</b>	95	201	410	828	1161	1943
	98	209	423	827	1167	1899
<b>Mean ± SD µg/mL</b>	<b>98 ± 3</b>	<b>201 ± 7</b>	<b>420 ± 10</b>	<b>812 ± 12</b>	<b>1177 ± 27</b>	<b>1935 ± 64</b>
<b>RSD</b>	<b>2.6</b>	<b>3.3</b>	<b>2.4</b>	<b>1.4</b>	<b>2.3</b>	<b>3.3</b>
<b>RE</b>	<b>-2.0</b>	<b>0.5</b>	<b>5.0</b>	<b>1.5</b>	<b>-1.9</b>	<b>-3.3</b>

Table S4. Intra-day Accuracy and Precision for Daratumumab.

	Nominal Conc (µg/mL)	Day 1 (n=5) mean±SD %RSD, %RE	Day 2 (n=5) mean±SD %RSD, %RE	Day 3 (n=5) mean±SD %RSD, %RE
LLOQ	100	96 ± 4 3.7; -3.9	95 ± 5 5.2; -4.9	93 ± 3 3.4; -6.7
QCL	300	309 ± 13 4.3; 2.9	306.5 ± 10 3.2; 2.2	328 ± 7 2.0; 9.3
QCM	900	958 ± 55 5.8; 6.5	905.6 ± 28 3.0; 0.6	938.4 ± 10 1.0; 4.3
QCH	1500	1439 ± 38 2.6; -4.0	1417 ± 20 1.4; -5.5	1545 ± 39 2.5; 3.0

Table S5. Selectivity for Daratumumab and IS evaluated in 6 drug-free sera matrices.

	Dara %*	IS %**
<b>Matrix 1</b>	9.2	2.5
<b>Matrix 2</b>	9.6	1.9
<b>Matrix 3</b>	9.2	1.9
<b>Matrix 4</b>	7.5	1.6
<b>Matrix 5</b>	9.7	3.4
<b>Matrix 6</b>	10.3	2.2

\* Calculated as ratio of the Dara signal in matrix over the signal of Dara in the LOQ calibrator.

\*\* Calculated as ratio of the IS signal in matrix over the mean signal of IS.

Table S6. Matrix effect evaluated for Daratumumab in 6 drug-free sera matrices from healthy donors.

	Matrix 1		Matrix 2		Matrix 3		Matrix 4		Matrix 5		Matrix 6	
Actual ( $\mu\text{g/mL}$ )	QCL 300	QCH 1500	QCL 300	QCH 1500	QCL 300	QCH 1500	QCL 300	QCH 1500	QCL 300	QCH 1500	QCL 300	QCH 1500
measured	347	1541	321	1419	323	1594	325	1642	317	1544	330	1487
	338	1546	307	1425	317	1509	341	1538	332	1560	315	1494
	326	1502	309	1475	327	1546	348	1652	318	1508	333	1521
Mean $\pm$ SD $\mu\text{g/mL}$	337 $\pm$ 11	1530 $\pm$ 24	312 $\pm$ 7	1440 $\pm$ 30	322 $\pm$ 5	1550 $\pm$ 42	338 $\pm$ 12	1611 $\pm$ 63	322 $\pm$ 8	1537 $\pm$ 27	326 $\pm$ 10	1500 $\pm$ 18
%RSD	3.1	1.6	2.4	2.1	1.5	2.7	3.5	3.9	2.5	1.7	3.0	1.2
%RE	12.3	2.0	4.1	-4.0	7.3	3.3	12.7	7.4	7.5	2.5	8.7	0.0

Table S7. Stability evaluated for Daratumumab in three different conditions.

	3 days at 4 °C*		55 days 4 °C		55 days -20 °C	
Actual ( $\mu\text{g/mL}$ )	QCL 300	QCH 1500	QCL 300	QCH 1500	QCL 300	QCH 1500
measured values ( $\mu\text{g/mL}$ )	326	1439	304	1497	308	1457
	339	1492	320	1460	329	1479
	351	1656	313	1497	328	1580
Mean $\pm$ SD $\mu\text{g/mL}$	339 $\pm$ 13	1529 $\pm$ 113	312 $\pm$ 8	1485 $\pm$ 22	321 $\pm$ 12	1505 $\pm$ 66
%RSD	3.8	7.4	2.6	1.5	3.7	4.4
%RE	12.9	1.9	4.1	-1.0	7.2	0.3

\*Extracted samples in autosampler.