Application of Immunicapture sample pretreatment for improved specificity and sensitivity

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Inspired by patients.
Driven by science.

Quantification of proteins

LC-MS/MS vs. LBA

LBA assays historically used for large molecule bioanalysis

- Need for specific reagents.
- Lengthy method development.
- Once established sample are cheap to run with high throughput.

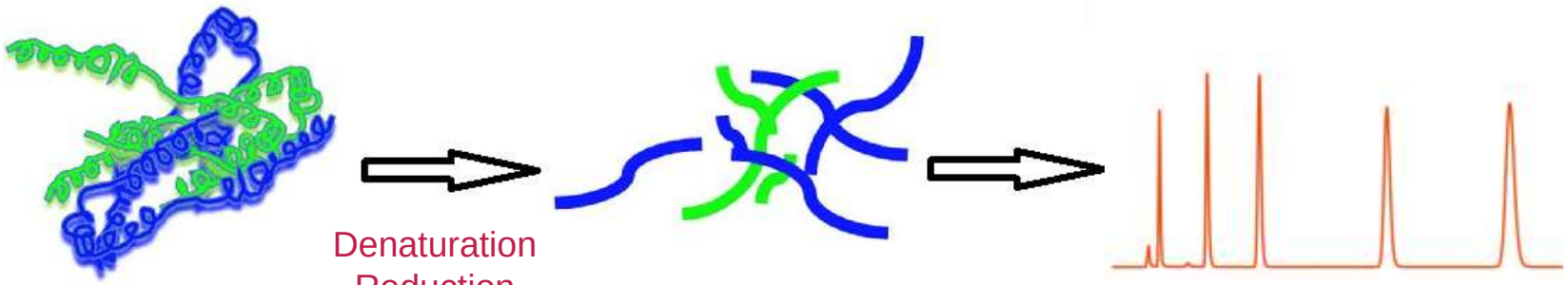
In recent years increasing use of LC-MS/MS for quantification of protein therapeutics

- No or less reagents needed.
- Fast method development.
- Relatively expensive and time consuming (digestion).
- Basic methods often have a sensitivity which is inferior to LBA assays.

Quantification of proteins

Basic methodology and sensitivity improvement options

Basic analysis methods are based upon tryptic digestion followed by analysis of a signature peptide by LC-MS/MS.



Immunocapture

SPE

2D-LC

Quantification of large molecules

Sensitivity increase in LC-MS/MS analysis

- Solid Phase Extraction
  - Little additional value in clean-up of tryptic digests, sensitivity increases limited to the degree of sample concentration.
- 2D-LC
  - Increases sensitivity by elimination of baseline noise and ionization effects. Best results obtained when orthogonal solid phases are used.
  - Once the system is set-up, relatively simple to apply.
- Immunocapture
  - Can be performed using the target or a non-interfering capture reagent (different datasets for soluble targets).
  - Theoretically should obtain data comparable to LBA.
  - Different possible techniques (beads, tips,….
  - Easily automatable, yet expensive.
  - Highest yield in sensitivity, comparable to LBA.

Test case

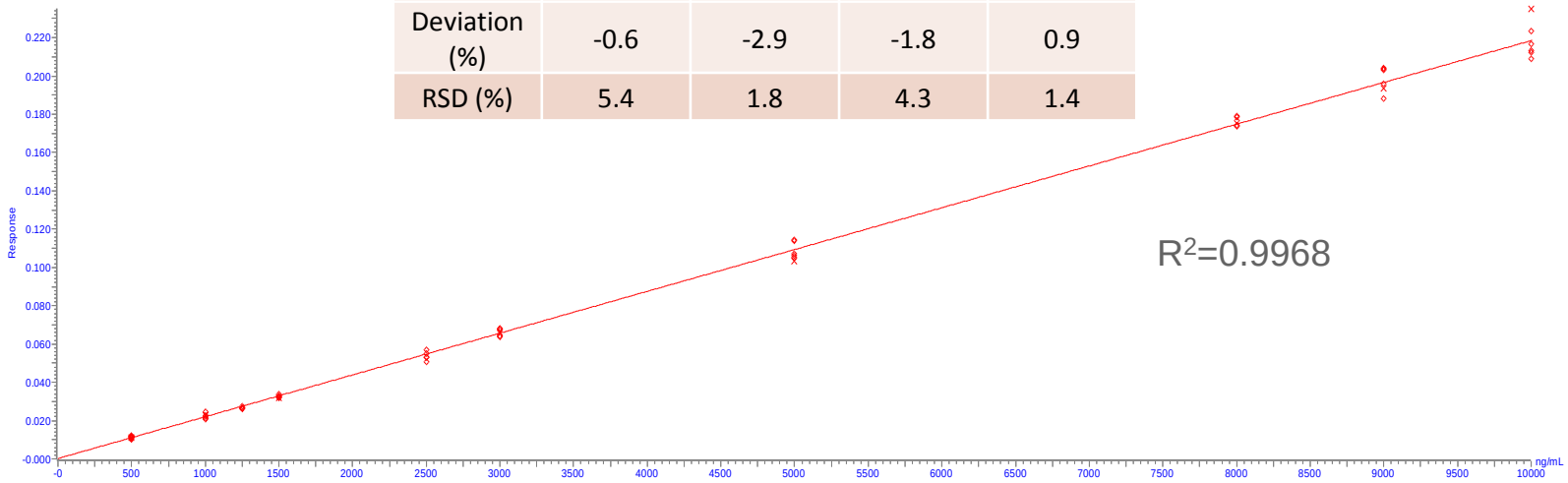
- Quantification of a protein therapeutic, Fab-PEG construct.
- Initial method developed by LBA on the MSD platform with a lower limit of quantification of 32.5ng/mL.
- LC-MS/MS methods developed and qualified with and without immunocapture.

LC-MS/MS quantification

Without sample pretreatment

- 10 µl of sample
- Tryptic digestion
- LC-MS/MS analysis

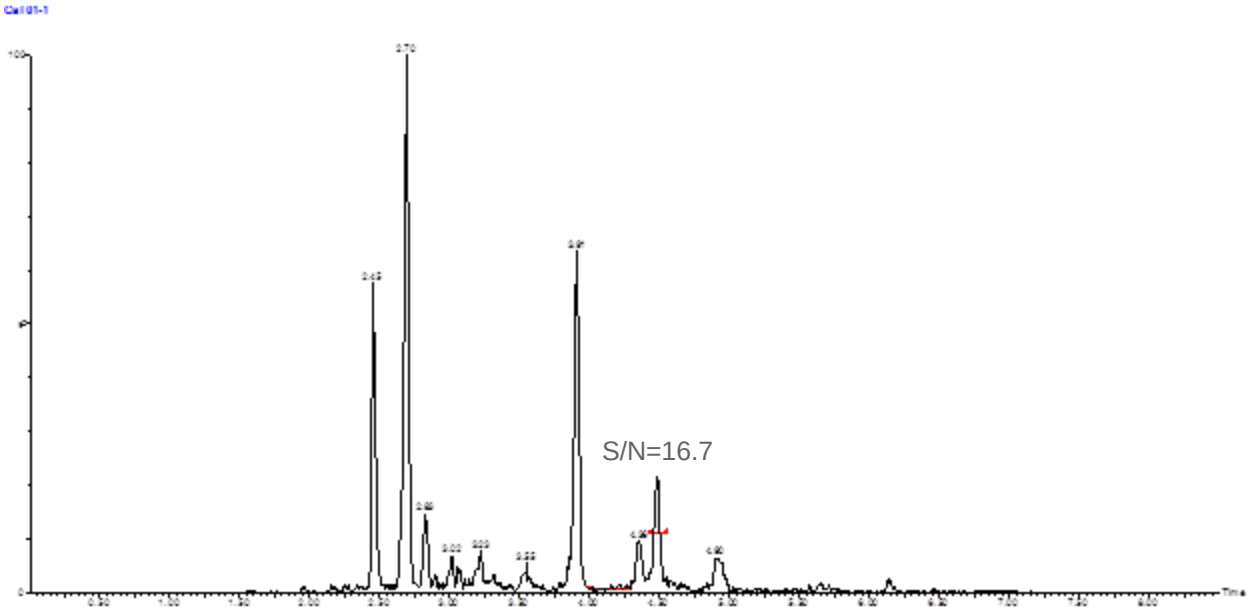
	QC LLOQ	QC Low	QC Mid	QC High
Nominal Conc.	500	1250	2500	8000
	527	1247	2317	7965
	493	1202	2423	7949
	482	1218	2502	8193
	462	1216	2432	8079
	520	1187	2602	8177
Mean	497	1214	2455	8073
Deviation (%)	-0.6	-2.9	-1.8	0.9
RSD (%)	5.4	1.8	4.3	1.4



LC-MS/MS quantification

Without sample pretreatment

LLOQ calibration sample at 500ng/mL

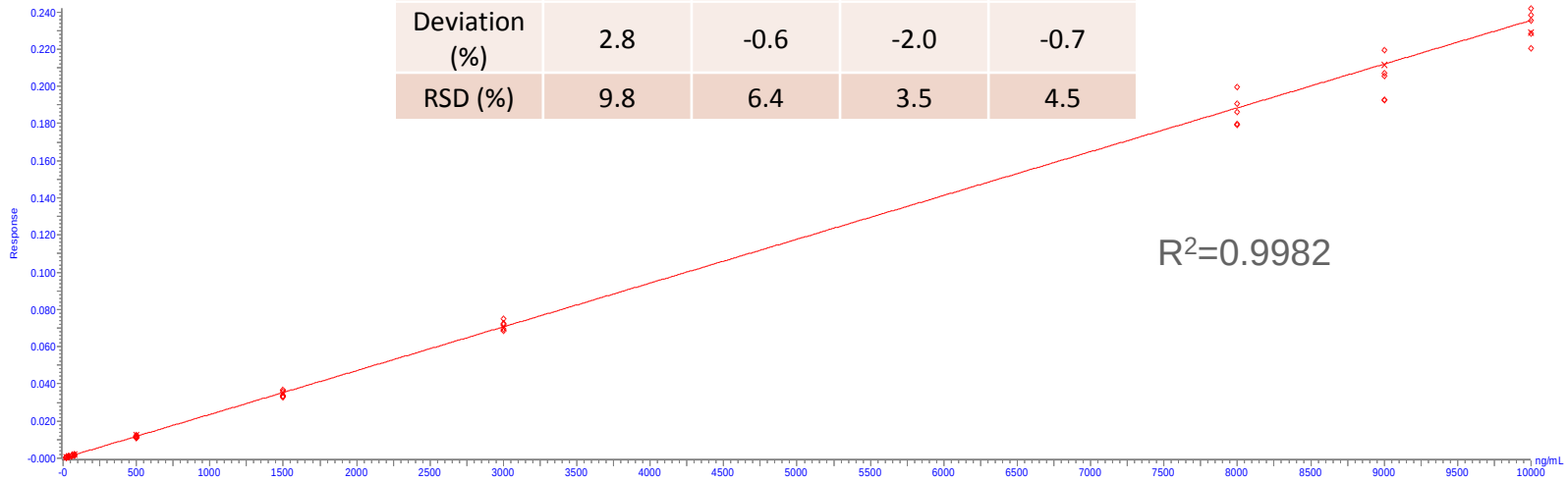


LC-MS/MS quantification

With sample pretreatment (immunocapture with target)

- 20 µl of sample
- Immunocapture
- Tryptic digestion
- LC-MS/MS analysis

	QC LLOQ	QC Low	QC Mid	QC High
Nominal Conc.	20	60	500	8000
	18.8	56.4	509	7905
	23.0	59.5	462	7637
	18.2	56.1	488	8467
	21.7	65.4	496	7613
	21.1	60.7	495	8100
Mean	21	60	490	7944
Deviation (%)	2.8	-0.6	-2.0	-0.7
RSD (%)	9.8	6.4	3.5	4.5

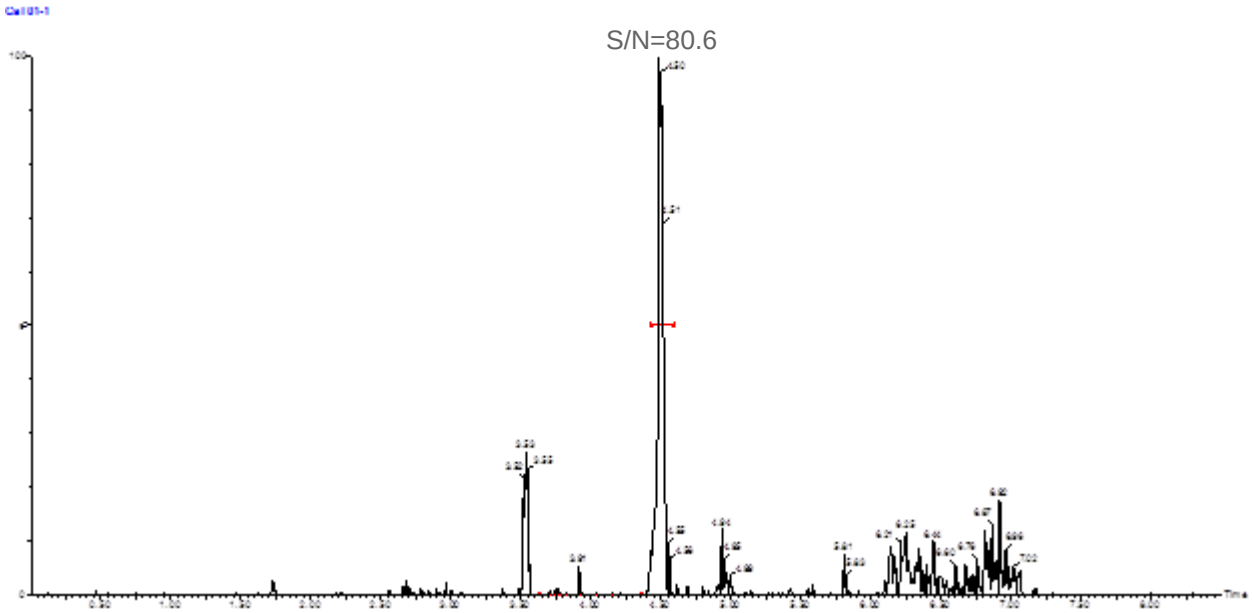


R^2=0.9982

LC-MS/MS quantification

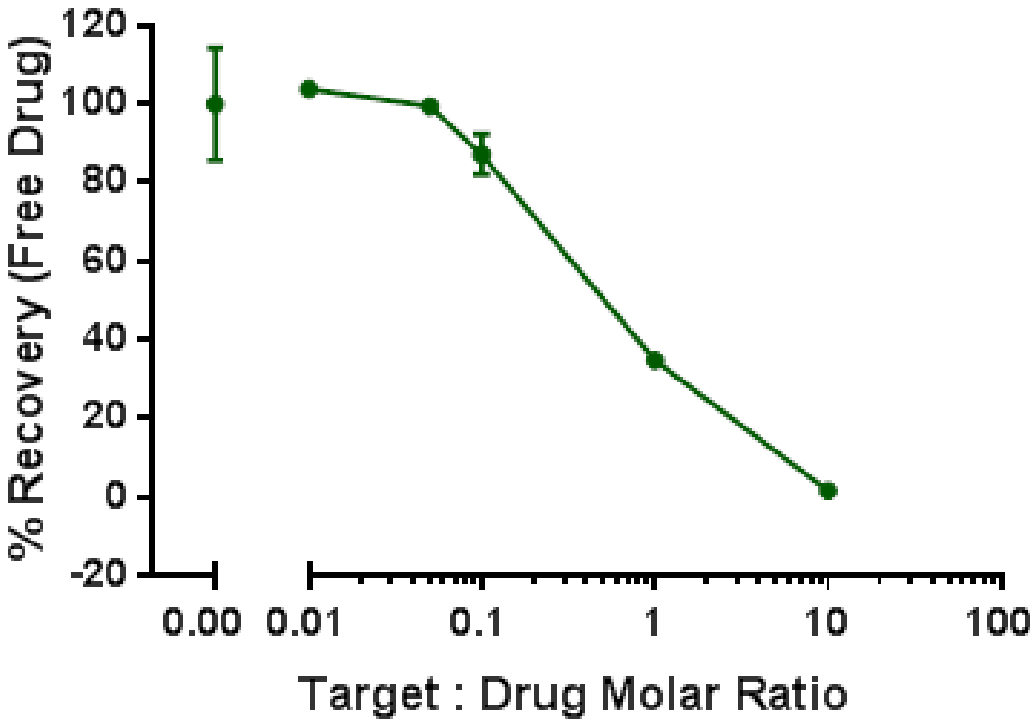
With sample pretreatment (immunocapture with target)

LLOQ calibration sample at 20.0ng/mL



What is measured in immunocapture experiments

Variable ratios of analyte and target in samples



We are measuring free drug

What are we measuring in immunocature experiments

Comparing LBA (MSD) to LC-MS/MS data in study samples

87.5% of samples meet small molecule ISR criteria!

	LBA concentration	LC-MS/MS concentration	ISR calculation (%)
sample 1	1335	1207	-10.0
sample 2	416	406	-2.5
sample 3	738	832	11.9
sample 4	239	195	-20.1
sample 5	1585	1394	-12.8
sample 6	1330	1147	-14.8
sample 7	545	523	-4.1
sample 8	2328	1872	-21.7
sample 9	333	316	-5.1
sample 10	581	509	-13.1
sample 11	997	1119	11.6
sample 12	289	315	8.6
sample 13	1386	1278	-8.1
sample 14	421	414	-1.8
sample 15	829	734	-12.1
sample 16	2349	2446	4.0
sample 17	568	522	-8.5
sample 18	528	534	1.1
sample 19	995	906	-9.4
sample 20	537	552	2.8
sample 21	255	222	-14.0
sample 22	1833	1484	-21.0
sample 23	1594	1670	4.7
sample 24	1306	1255	-4.0

Conclusion

Using immunocapture

- Methods can be developed to meet regulatory acceptance criteria (even small molecule criteria).
- Immunocapture allows for reduction of baseline noise and subsequent improvement of sensitivity (approximatly 100-fold) to match LBA methods.
- Provides results which are compatible with LBA analysis, i.e. allows for quantification of free drug.

Thanks!

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