22nd International REID Bioanalysis Forum

Recent directions in LC-MS adoption for large molecule bioanalysis

Waters – Vision of the Future
MS detection
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http://dmpk.waters.com/en

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Outline

- The trend: novel, eclectic modalities

- Peptide & protein bioanalysis workflows

- Insight into adoption of LC-MS for large molecule bioanalysis

- Protein quantification using the surrogate peptide approach

  - Trastuzumab quantification case study: Tandem MS *versus* HRMS

- "Intact level" Trastuzumab quantification proof of principle
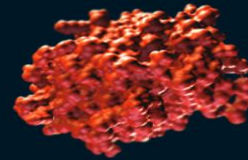
- Summary

- Future perspectives

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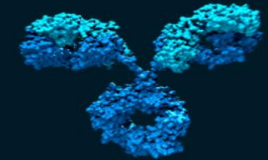## theShape
OF DRUGS TO COME

Protein engineers are using the building blocks nature provides to design an ever-expanding toolkit of new drug modalities to fight disease.
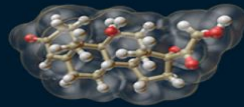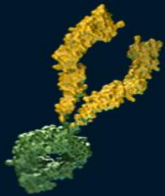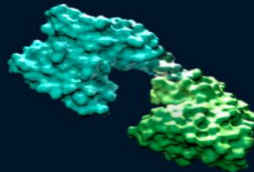
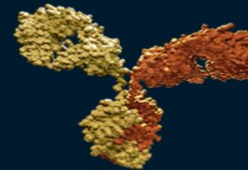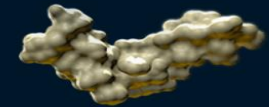SMALL MOLECULES

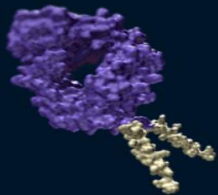THERAPEUTIC PROTEINS

MONOCLONAL ANTIBODIES
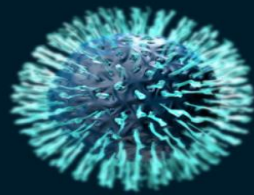
FUSION PROTEINS

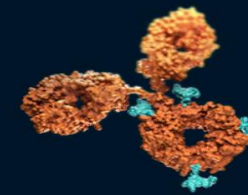BITE° ANTIBODY CONSTRUCTS

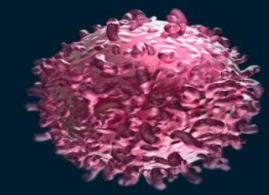BISPECIFIC ANTIBODIES

PEPTIDES

PEPTIBODIES

ONCOLYTIC IMMUNOTHERAPY VIRUSES

ANTIBODY DRUG CONJUGATES

CAR T CELLS

Peptide and Protein Bioanalysis Workflows
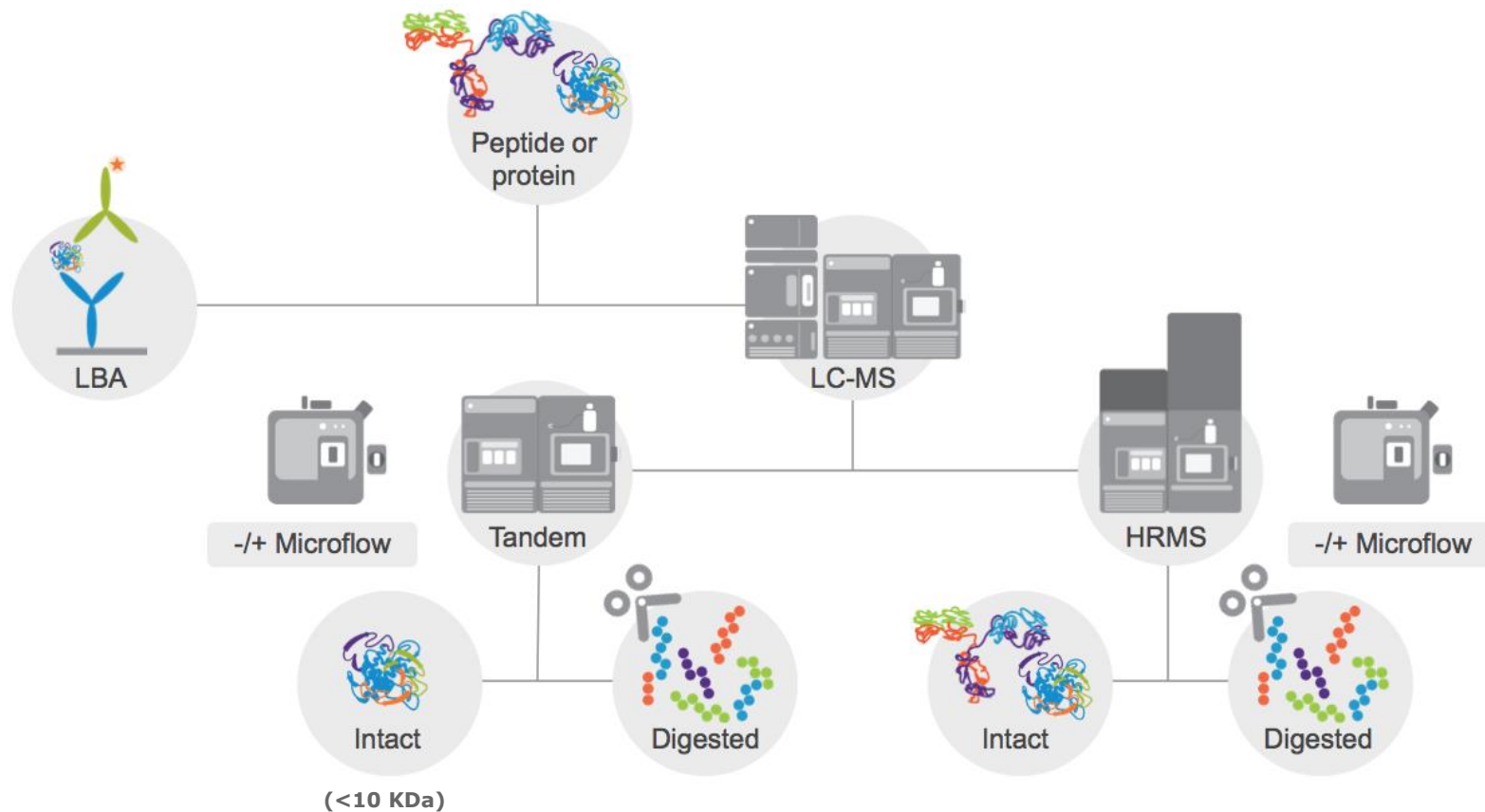
"What is the true concentration?"

Trastuzumab pharmacokinetic profile

Concentration (μg/ml)

Time after first dose (days)

- FTISATDSK — Total mAb (LC-MS/MS)
- IYPTNGYTR — Non-deamidatied mAb (LC-MS/MS)
- ELISA — Immuno-reactive mAb (ELISA)
- IYPTisoDGYTR
- IYPTDGYTR — Deamidated mAb (LC-MS/MS)
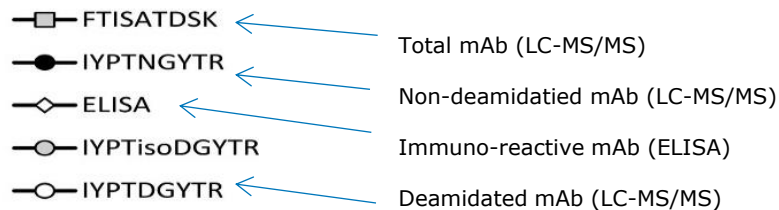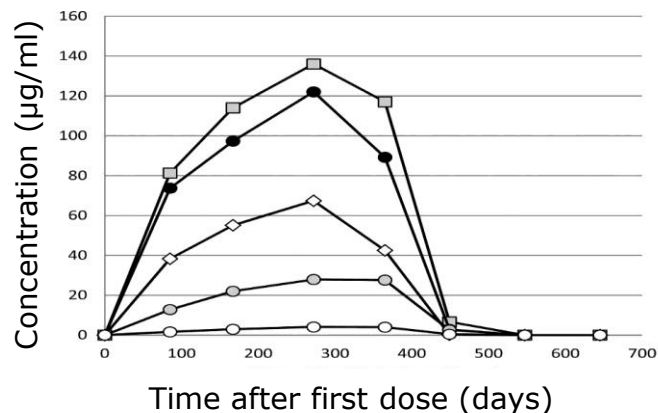
"Deamidation of Trastuzumab at the complementarity determining region (CDR) leads to the loss of recognition of the antibodies used in the ELISA assay"

LC-MS/MS (Tandem MS or HRMS) can provide novel & insightful information, in support of PK studies, that can supplement or replace LBA and is recognized by regulatory bodies

Digested Protein Bioanalysis:
- Tandem MS or HRMS

WORKFLOW

- Optimize / fine-tune MRM transitions
- Protein-level clean-up
- Protein digestion
- Peptide SPE clean-up
- LC-MS
- Data processing
- Results
- Identify unique peptides and transitions

Trastuzumab (Herceptin)

Fab

antigen binding region

Glycosylation at Asn297

Fc

IgG

Monoclonal antibody (~150,000 Da)
- Approved in **1998** in US
- Treatment for HER2 positive breast cancer
- Designed to bind to HER2
  - Activates cells of the immune system
  - Stops HER2 producing signal for tumor cell growth
- Major side effect: heart failure
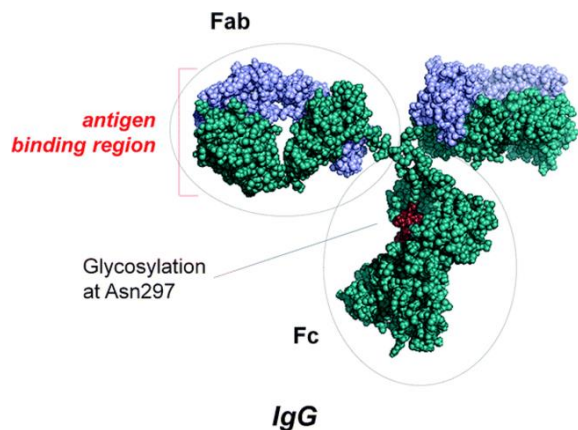
Administration: IV, every 3 weeks
Half-life: ~26 days
Dose:
  - initial 8 mg/kg, maintain 6 mg/kg
  - blood volume 75 mL/kg
  - circulating conc ~ 6 mg/75 mL = 80 µg/mL
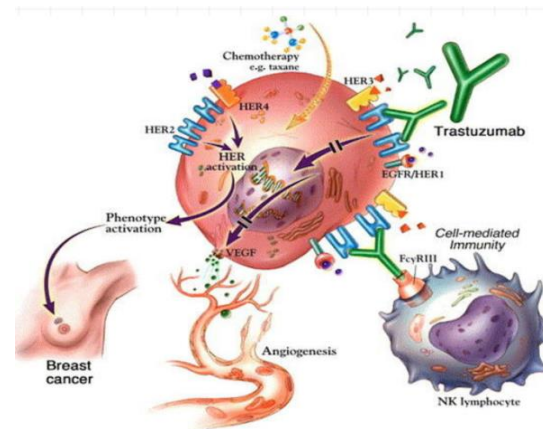
Trastuzumab Quantification via Surrogate Peptide Approach, Sample Preparation

Trastuzumab
(Infliximab as ISTD)

Rat Plasma (50 µL)

Affinity Purification
(Protein A)

Protein Digestion
(ProteinWorks eXpress Digest Kit)

90 µL final volume

Peptide Purification
(ProteinWorks µElution SPE Clean-Up)

TQ-XS   and   G2-XS

Same LC (ACQUITY UPLC)
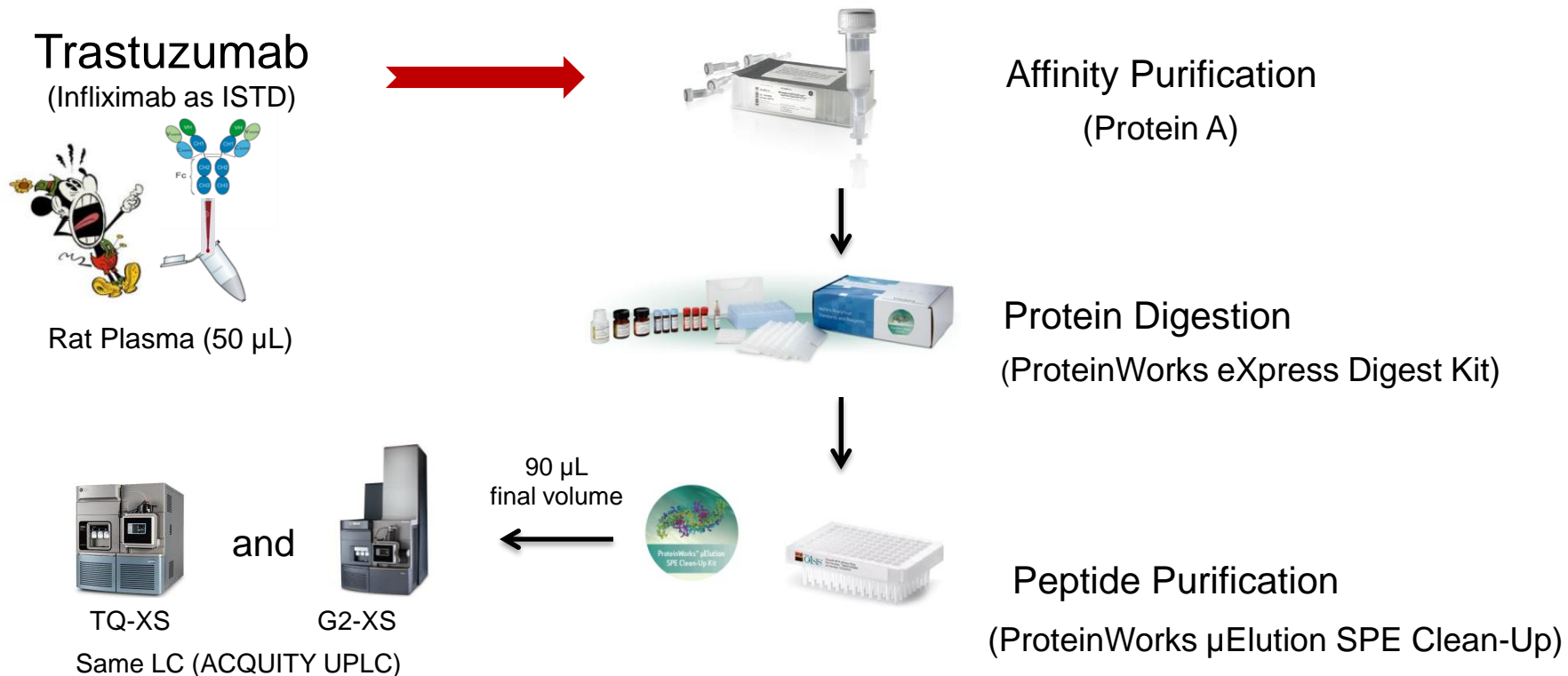
Representative Trastuzumab Chromatograms

TQ-XS (MRM)
(485.2 > 721.4, CE = 15)

FTISADTSK

G2-XS (Tof-MRM)
(485.2 > 721.373, CE=16)

Blank Rat Plasma - Rep 2

0.050 µg/mL

0.025 µg/mL

0.010 µg/mL

LOQ

Blank
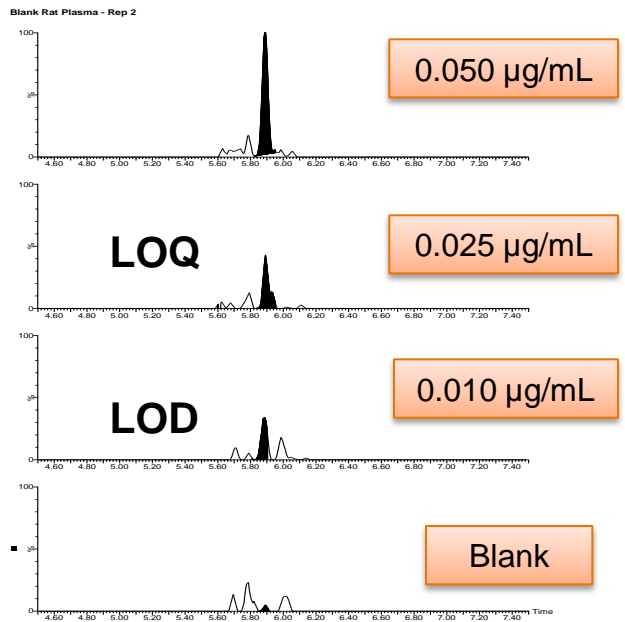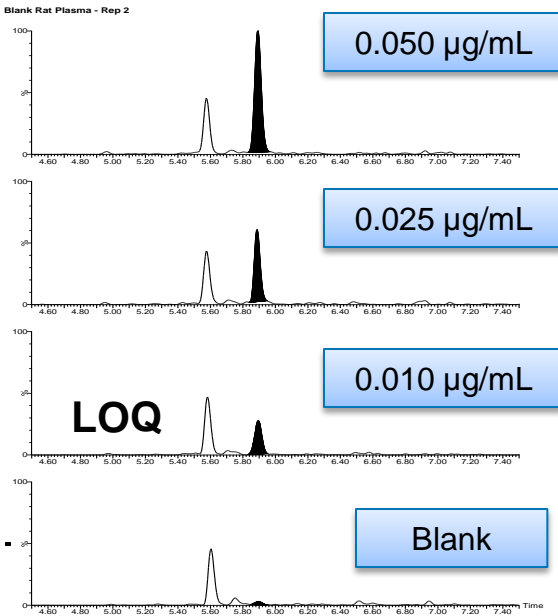
Blank Rat Plasma - Rep 2

0.050 µg/mL

LOQ

0.025 µg/mL

LOD

0.010 µg/mL
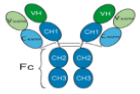
Blank

Trastuzumab Peptide Level Quantification

- Comparing TQ-XS and G2-XS
  - Optimization produced same transition using similar collision energy (CE)
  - ≥ 4 linear dynamic range on both platforms
- In general, TQ-XS is 2X more sensitive than G2-XS QTof
  - LOQs 0.01-0.025 µg/mL (TQ-XS) vs. 0.025-0.050 µg/mL (G2-XS)

	FTISADTSK		DTYIHWVR	
	Xevo TQXS Tandem MS	Xevo G2XS QTof	Xevo TQXS Tandem MS	Xevo G2XS QTof
Transition (CE)	485.2 > 721.4 (CE=15)	485.2 > 721.373 (CE=16)	545.3 > 597.3 (CE=24)	545.3 > 597.326 (CE=23)
LOD (µg/mL)	0.005	0.010	0.025	0.050
LLOQ (µg/mL)	0.010	0.025	0.025	0.050
Curve (µg/mL)	0.010 – 250	0.025 - 500	0.025 – 500	0.050 - 500
Log_{10} Range	4.4	4.3	4.3	4
Linear Fit (R^2)	0.988	0.991	0.992	0.995
% Accuracy Range	85.0 – 111.6	89.2 - 114.4	89.0 – 108.3	95.5 - 105.6
Weighting	$1/X^2$	$1/X^2$	$1/X^2$	$1/X^2$

Intact Bioanalysis:
HRMS
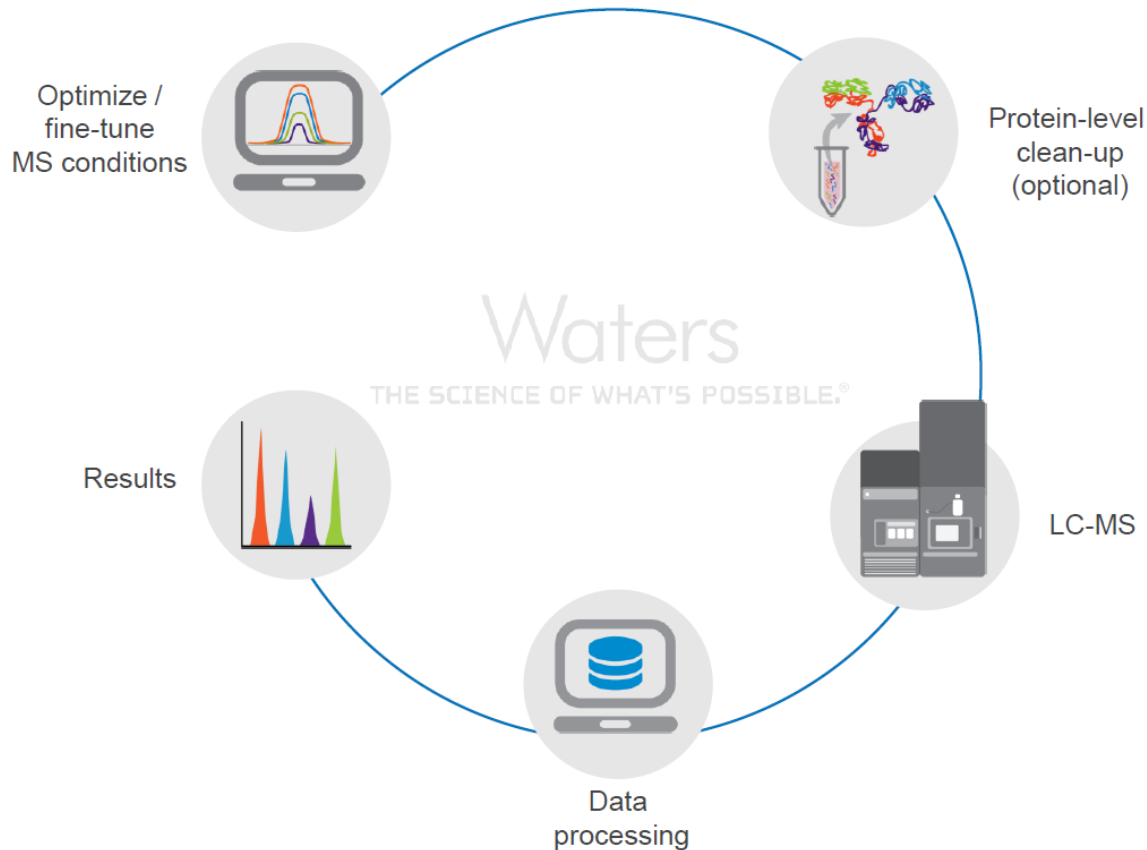
Trastuzumab in Mouse Plasma

Trastuzumab

Mouse Plasma (50 µL)

↓

Goat Anti-Human IgG FC
Magnetic bead

↓

Final volume (50 µL)

Item name: 30-35 0.2 ml/min
Channel name: 2: +2407.2496_3162.1949 : TOF MS (500-4000) 6eV ESI+

Chromatogram

Sample: T-mAb in plasma
50 µg/mL, 0.2 mL/min

Retention time [min]

7.74e3

T-mAb   MS

150 KDa (+50 charge state)
~ 3000 *m/z*
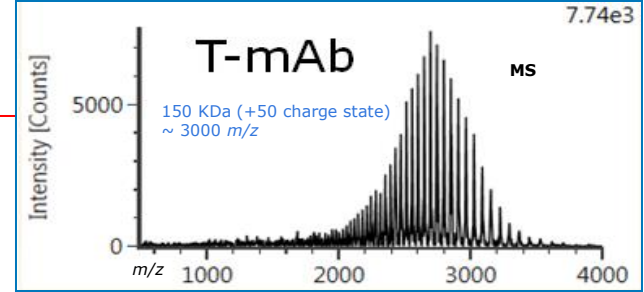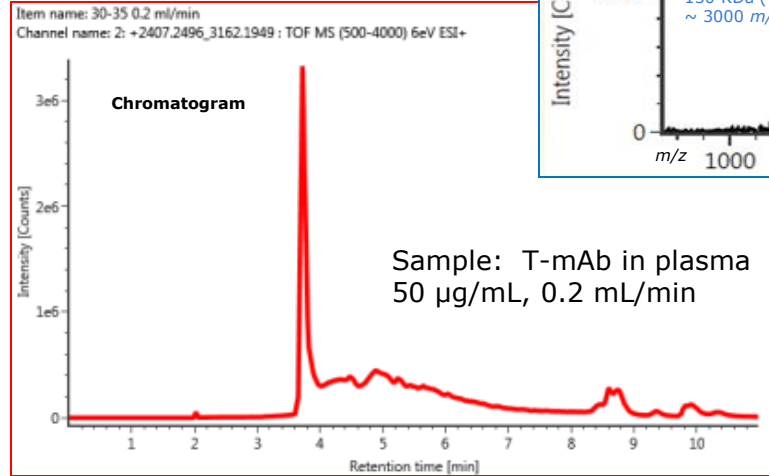
Sample preparation: can be simpler than surrogate peptide approach

VION (HRMS)

Quantification Results in Mouse Plasma
XIC of *m/z* 2907 with 2 Da window

Sample range: 0.1-50 µg/mL
Injection volume = 1 µL
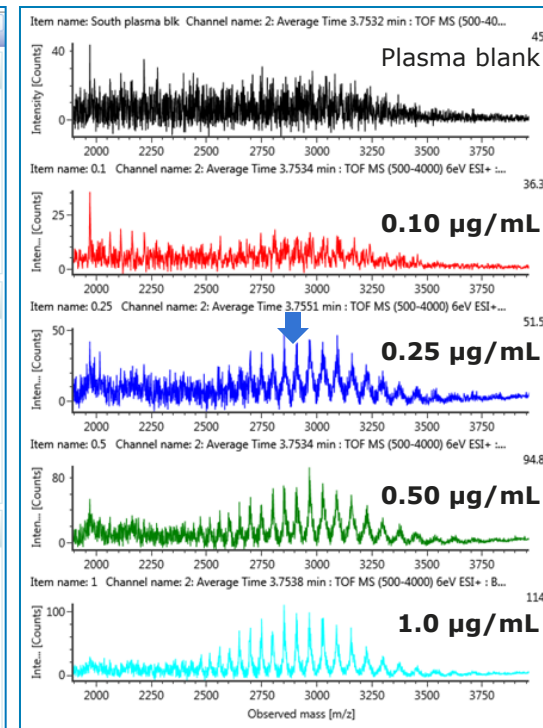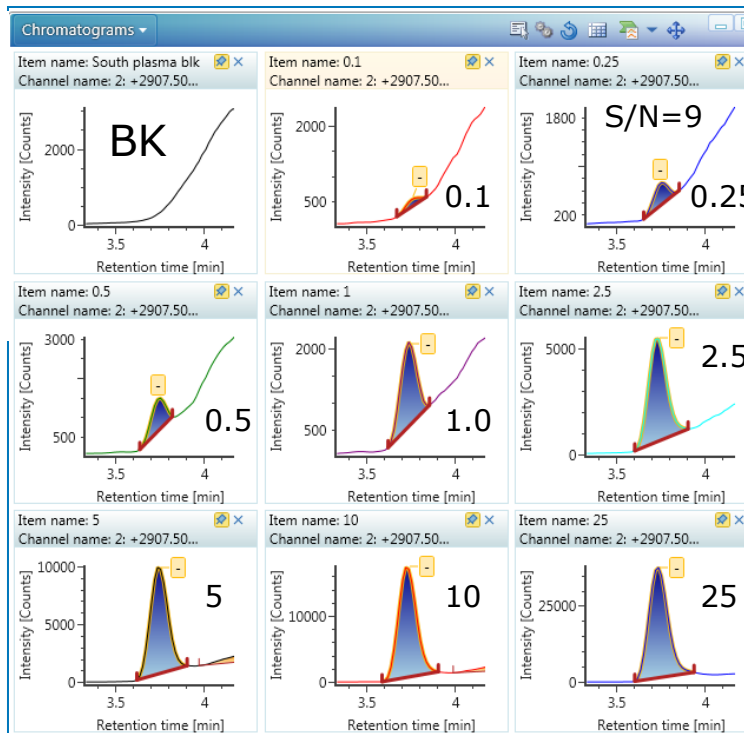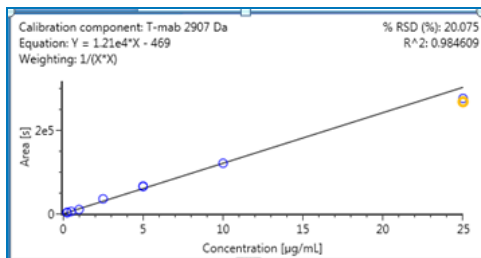
Linear range: 0.25 - 25 µg/mL
LLOQ: 0.25 µg/mL

%accuracy range: 85-118%
R² = 0.98

XIC

Spectrum

in µg/mL

Comparison of Peptide and Intact Level Quantification

- Peptide level quantification is ~5-25x more sensitive than intact trastuzumab.
- MRM based peptide quantification has wider linear dynamic range than MS scan based quantification (both for peptide and intact).

	FTISADTSK		DTYIHWVR		Intact
	Xevo TQ-XS Tandem MS	Xevo G2-XS QTof	Xevo TQ-XS Tandem MS	Xevo G2-XS QTof	Vion Qtof
LOD (µg/mL)	0.005	0.010	0.025	0.050	0.25
LLOQ (µg/mL)	0.010	0.025	0.025	0.050	0.25
Inj Vol (µL)	8	8	8	8	1
Curve (µg/mL)	0.010 – 250	0.025 - 500	0.025 – 500	0.050 - 500	0.25-25
Log_{10} Range	4.4	4.3	4.3	4	2
Linear Fit (R^2)	0.988	0.991	0.992	0.995	0.985
% Accuracy Range	85 – 112	89 - 114	89 – 108	95 - 105	85-118
Weighting	$1/X^2$	$1/X^2$	$1/X^2$	$1/X^2$	$1/X^2$

Summary

- HRMS workflows are comparable to traditional tandem LC-MS/MS
- Surrogate peptide: LOQ = 0.01-0.025 µg/mL and >4 orders linear dynamic range
- Intact Level: LOQ = 0.25 µg/mL and 2 orders linear dynamic range
- Sample preparation is especially important for intact level analysis to minimize matrix interferences
- Both peptide and intact level quantification are viable options for measuring protein levels in plasma

LC-MS quantification (Tandem MS or HRMS) using surrogate peptide or intact approaches is complementary to ligand binding assays in the context of bioanalytical strategies

Future Perspectives

Bioanalysis

The changing world of bioanalysis: summary of panel discussions

Neil Spooner*[1,2], Melanie Anderson[3], Lieve Dillen[4], Luca Ferrari[5], Martijn Hilhorst[6], Zamas Lam[7], Marco Michi[8], James Munday[9], John Smeraglia[10], Scott Summerfield[11] & Dieter Zimmer[12]

The world of bioanalysis is changing rapidly. The move toward the measurement of **How do we access the required skill sets?**

"This increase in novel pharmaceutical constructs will increasingly require bioanalysts to have a blend of analytical skills from what have previously been seen to be separate groups in most Pharma and CRO organizations…"

- Small molecule LC-MS experts will develop skills in large molecule analysis
- Those who have utilized immuno-assays will develop skills in LC-MS
- Both groups will need to develop skills in other approaches not currently commonly found in either, for example, HRMS

Bioanalysis

The heights of biopharmaceutical complexity and the current reach of analytical instrumentation

"The ever increasing complexity of intact protein biopharmaceuticals continues to require ever **increasing sensitivity, quantitative linearity and spectral resolution**"
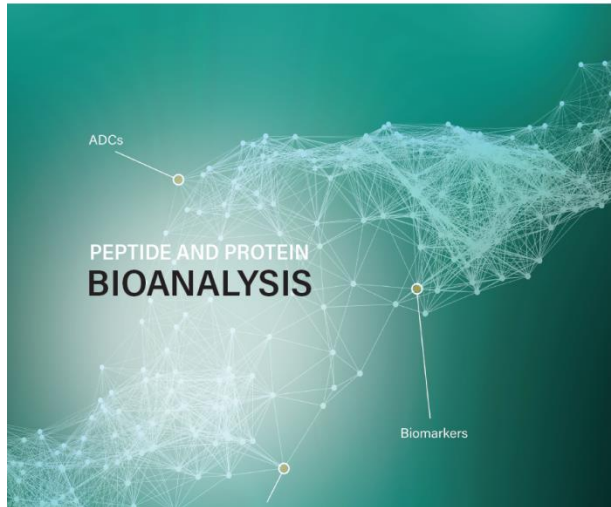
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Intact level protein quantification shows promise as seen by emerging proof of concept/principle studies

- What levels of sensitivity & dynamic range are required for "real world" PK studies in pre-clinical?

- Does there need to be universal agreement around standardized data processing routines for intact protein quantification (e.g. summed *versus* deconvolution)?

Educational content

http://dmpk.waters.com/en

http://bit.ly/2qZJZNw

Acknowledgements

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THE SCIENCE OF WHAT'S POSSIBLE.®

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THANK YOU FOR ATTENTION! ANY QUESTIONS?