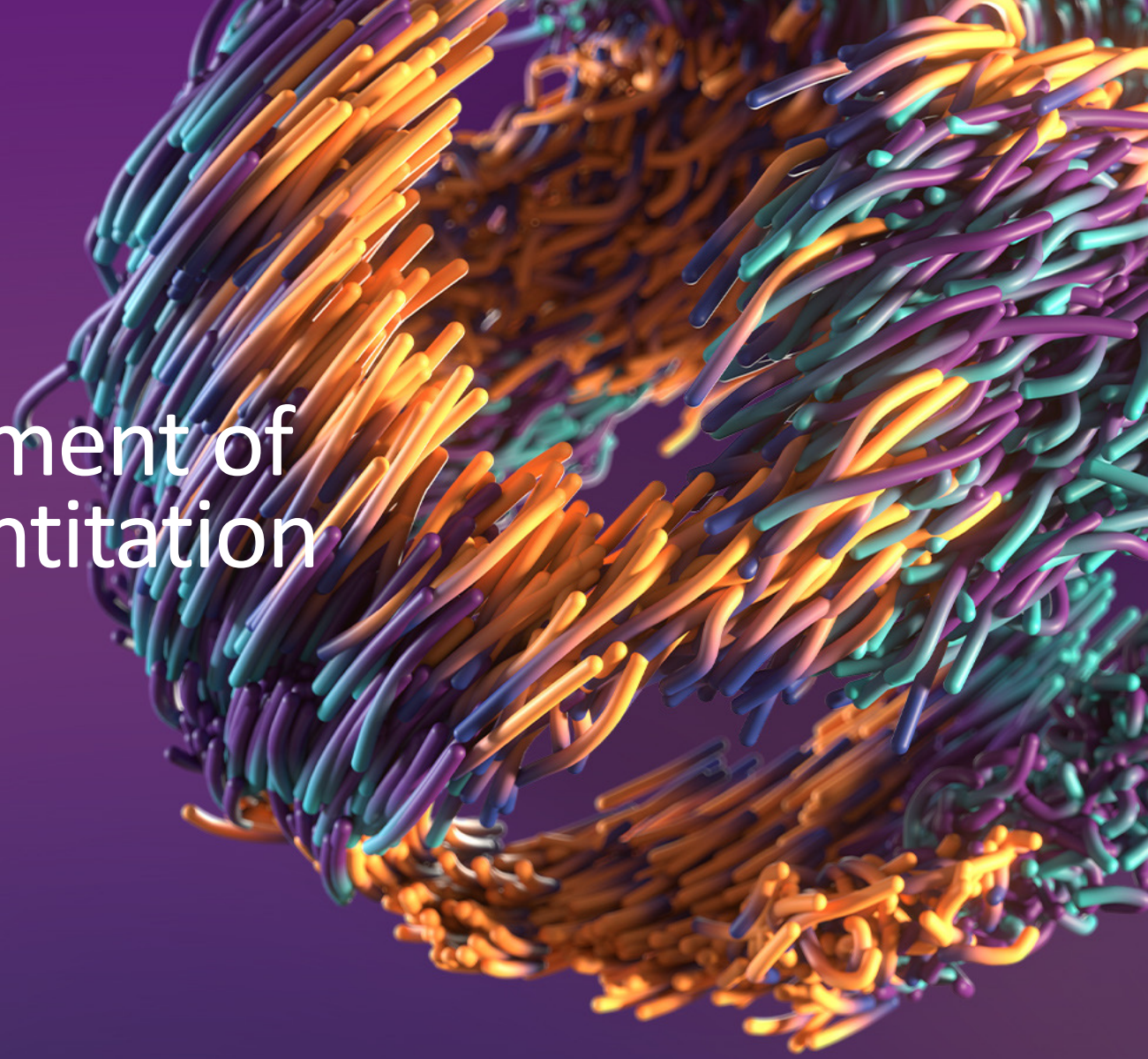
byondis

Challenges in the development of an LC-MS method for quantitation of sMET in human plasma



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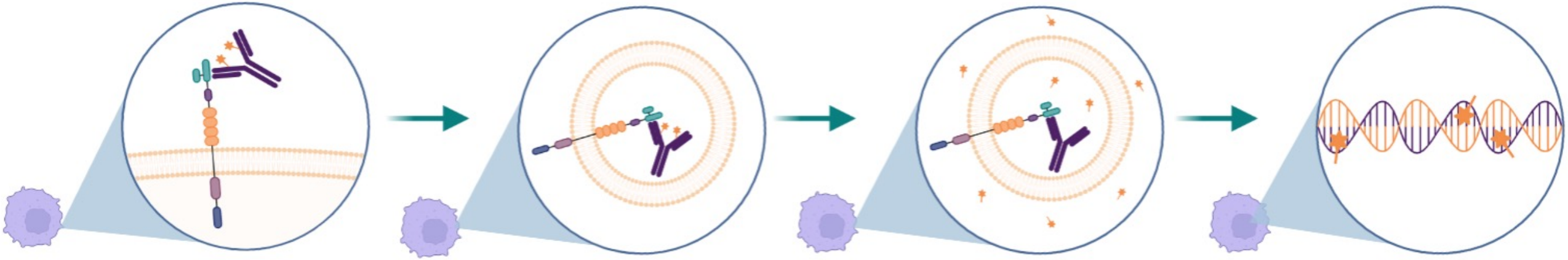
BYON3521: an anti-cancer ADC

- *c-MET over-expressed on surface of various solid cancers*
- *BYON3521 binds to c-MET*
- *Cytotoxin cleaved proteolytically after ADC internalization*

→*Induces tumor cell death*

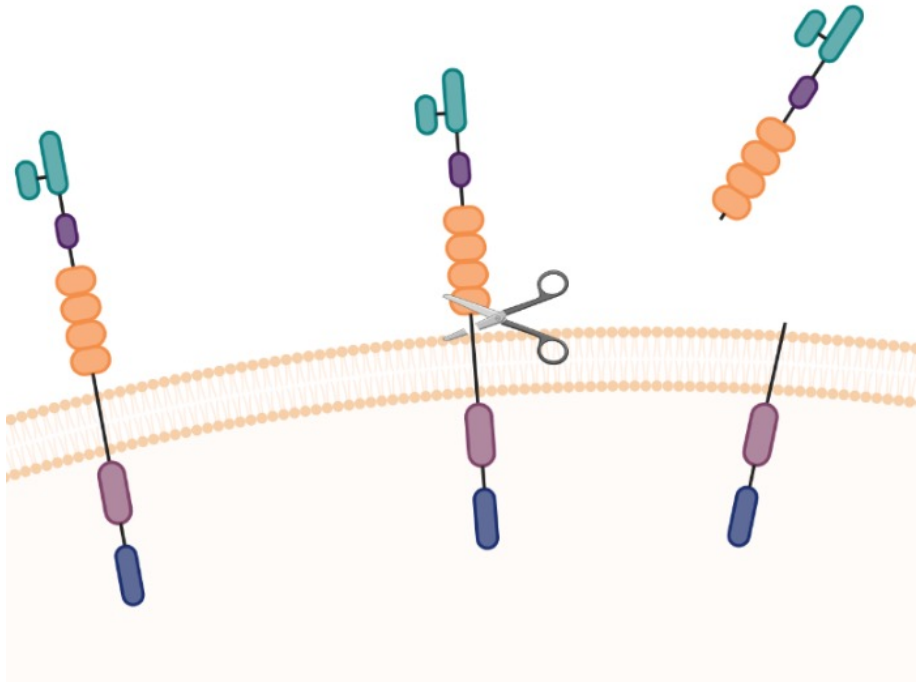


Soluble c-MET (sMET)

- Extracellular c-MET cleavage forms shed MET (sMET)

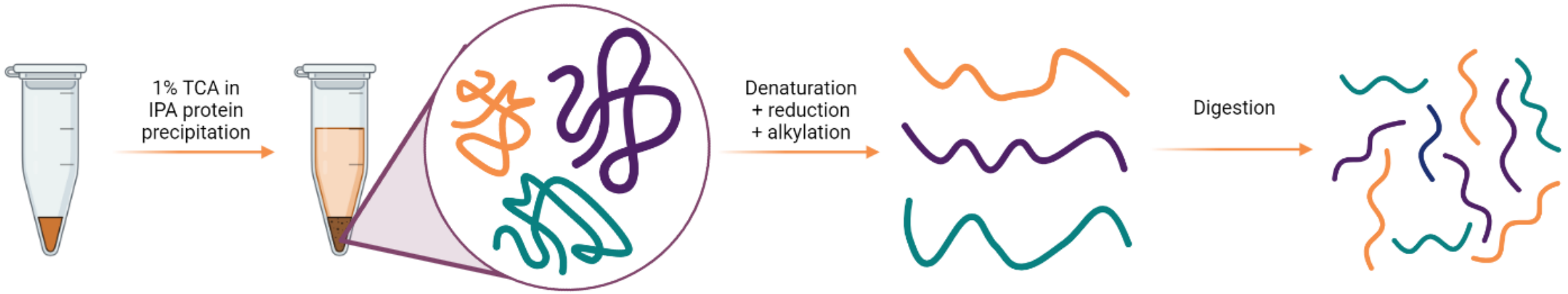
- Free sMET also binds BYON3521
  - Possibly reduced ADC efficiency
  - Interferes with in-house assays

→sMET concentration provides important information



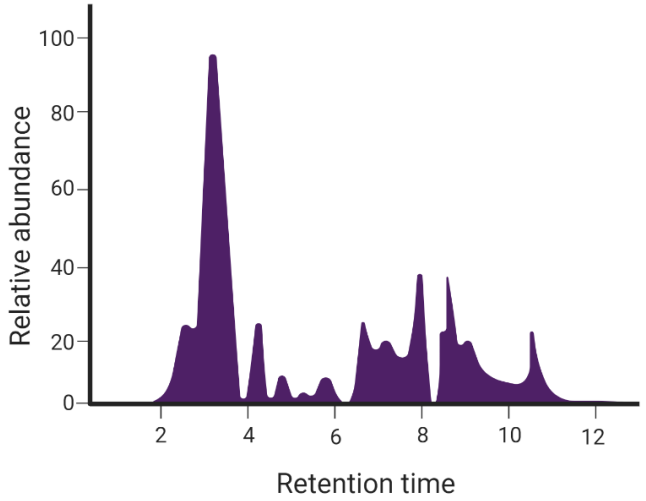
Assay set-up: bottom-up approach

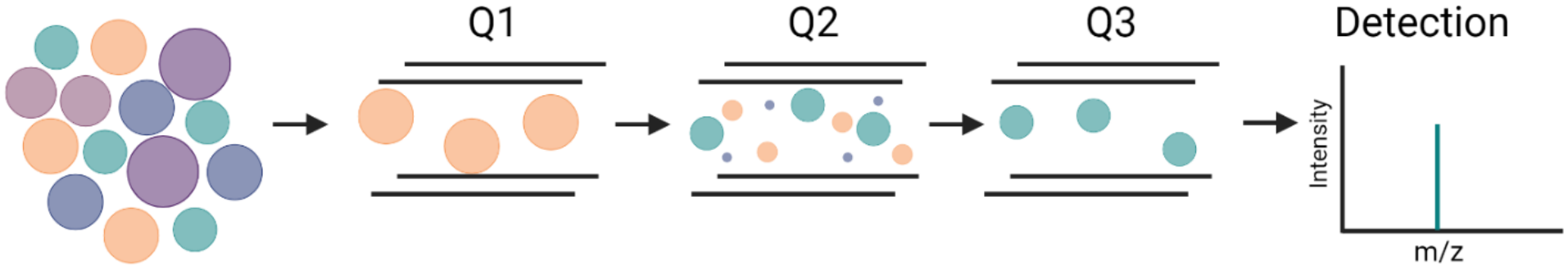
- Target matrix
  - 10µL cynomolgus monkey (preclinical studies)
  - 10µL human plasma (clinical studies)



Original LC-MS settings

- LC system: Shimadzu Exion LC
  - Reverse phase peptide separation
  - Waters BEH peptide C18 300Å, 50x2.1mm, 1.7µm
  - Eluent A: 0.1% FA in MilliQ
  - Eluent B: 0.1% FA in ACN
- MS system: Sciex TripleQuad 7500
  - MRM mode





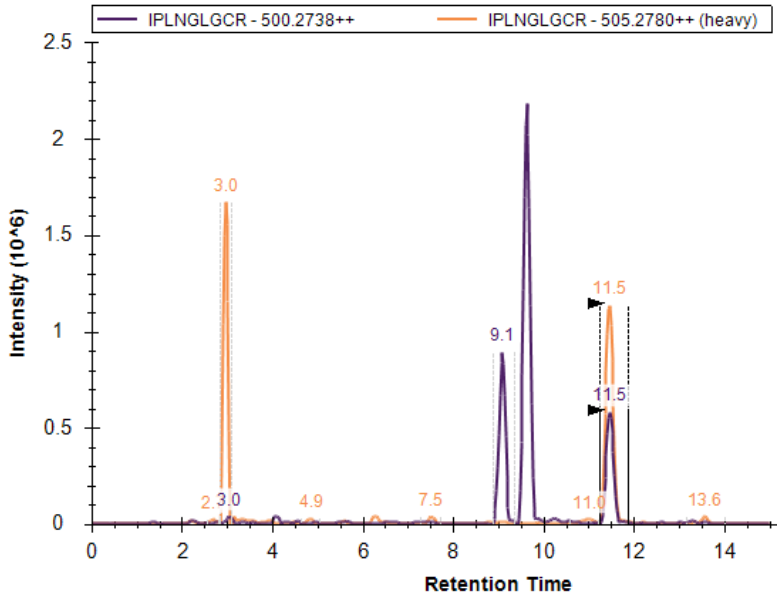
Assay parameters

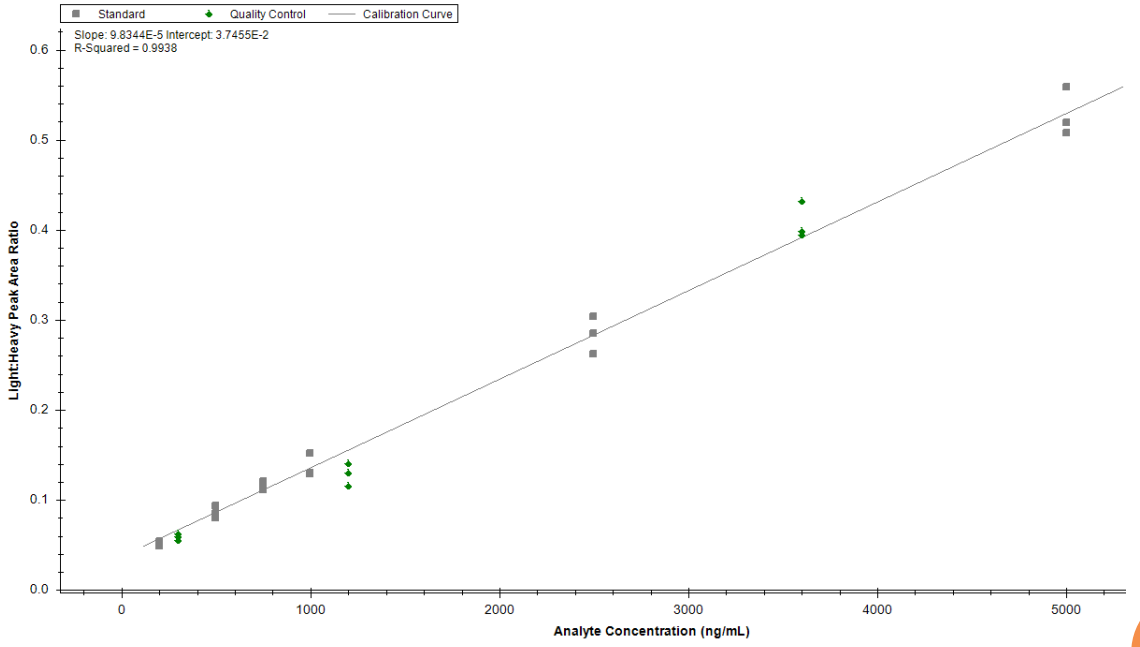
- Peptide *IPLNGLGCR (500.2738++ m/z)* selected as surrogate peptide

- Winged peptide *TKIPLNGLGCRHFQ* as internal Standard
  - *R contains $^{13}C_6$ $^{15}N_4$ isotope*
  - *Digestion sequence: IPLNGLGCR (505.2780++ m/z)*

- Range: 0.200-5.00µg/mL in cyno plasma
  - Linear regression
  - 1/x weighting
  - R^2=0.9938

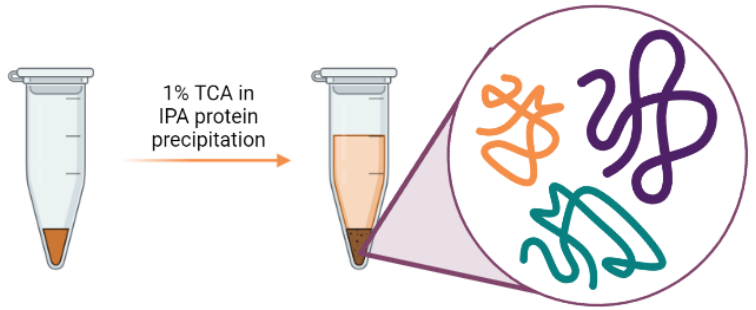
BUT: sMET is endogenously present

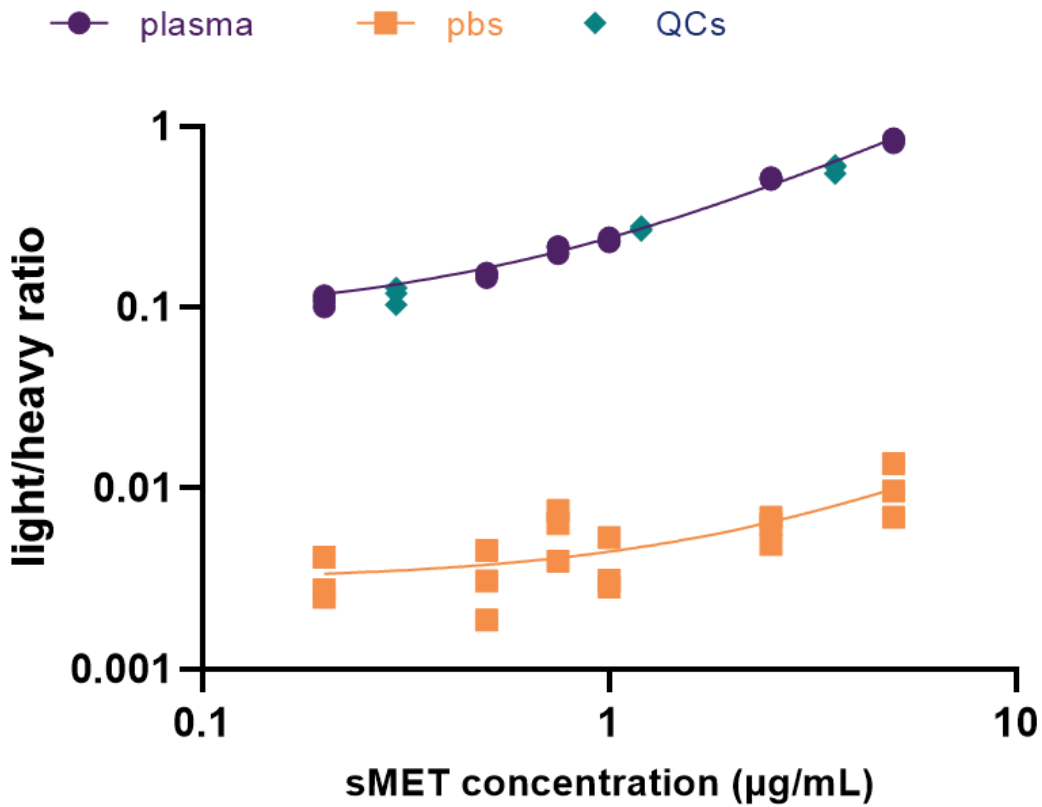




Surrogate matrix for endogenously present sMET

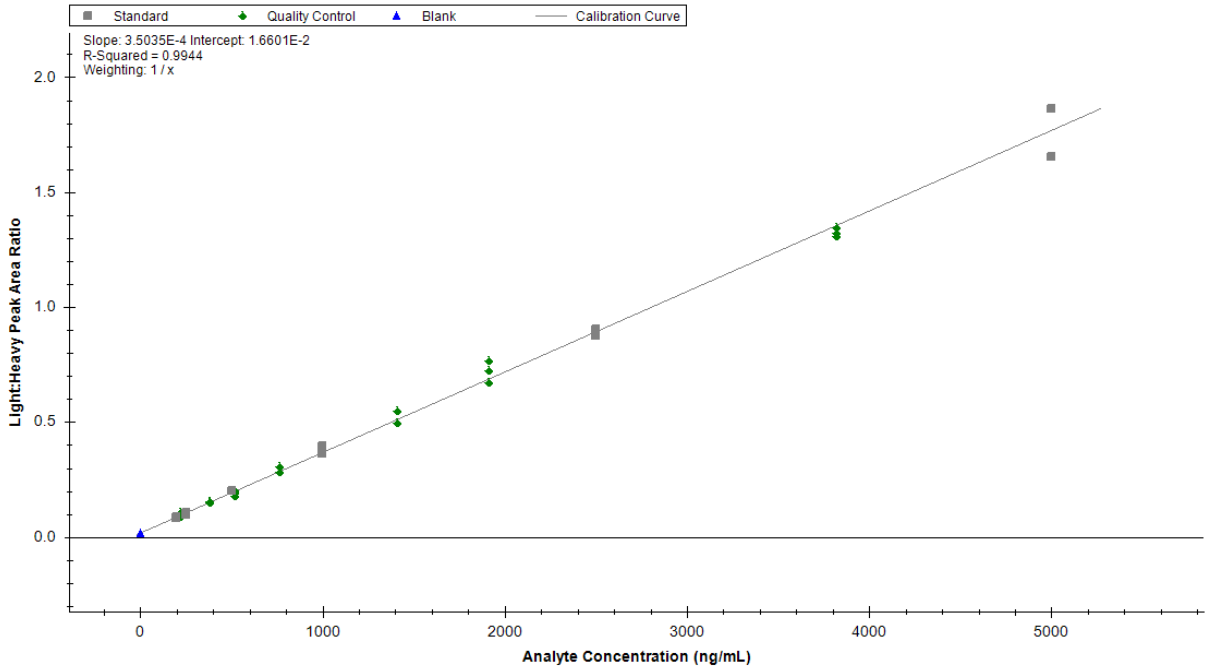
- 4% BSA in PBS standard surrogate matrix
- Problem: 1% TCA in IPA precipitates all proteins but albumin
- Other sample prep needed, or different surrogate matrix





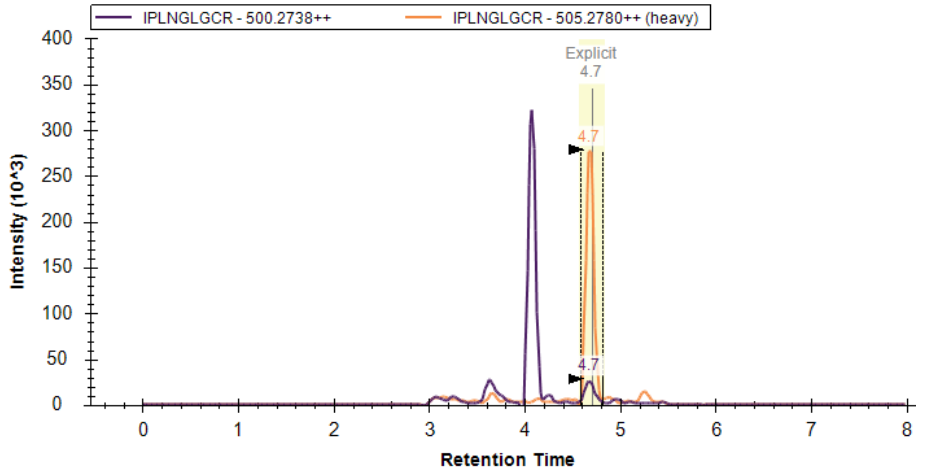
Surrogate matrix for endogenously present sMET

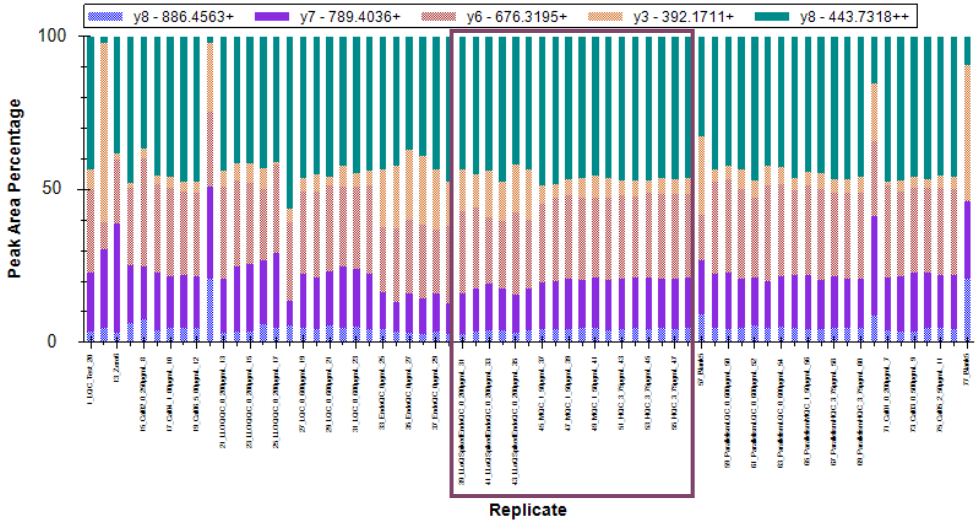
- Several plasma matrices tested in-house
- Chicken plasma shows no sMET response
- Endogenous concentration in cyno plasma:
  - 0.223µg/mL
- Assay for cynomolgus monkey validated
  - 0.200-5.00µg/mL
  - +/- 20% Bias and CV
  - Parallelism successfully evaluated
  - 2 F/T cycles + 16 hrs benchtop stability successfully evaluated



Validation in human assay: unexpected interferences

- No abnormalities observed in chromatograms
- But: increase endogenous sMET concentration observed (~0.4 µg/mL instead of ~0.2 µg/mL)

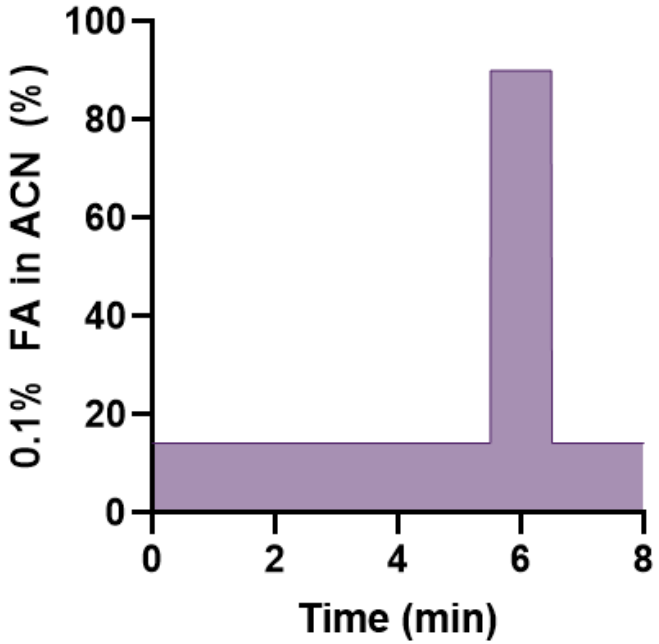




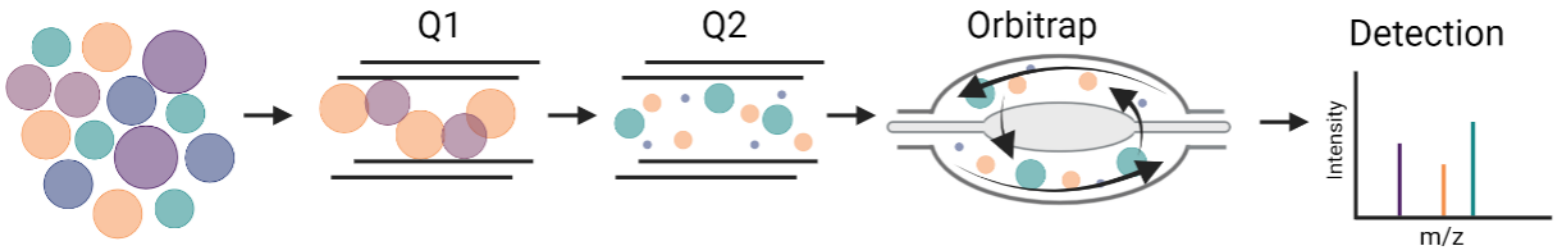
QC	Spiked concentration (µg/mL)	Concentration sMET (µg/mL)	Average bias (%)	CV (%)
LLoQ	0.200	0.200	23%	3%
L	0.600	0.600	-1%	12%
Endo	0.000	0.384	0%	5%
LLoQ Spiked	0.200	0.584	13%	2%
M	1.50	1.88	20%	2%
H	3.75	4.13	21%	2%

Validation in human assay: QExactive+ set-up

- Vanquish Flex:
  - Reverse phase peptide separation
  - Waters BEH peptide C18 300Å, 150x2.1mm, 1.7µm
  - Eluent A: 0.1% FA in MilliQ
  - Eluent B: 0.1% FA in ACN

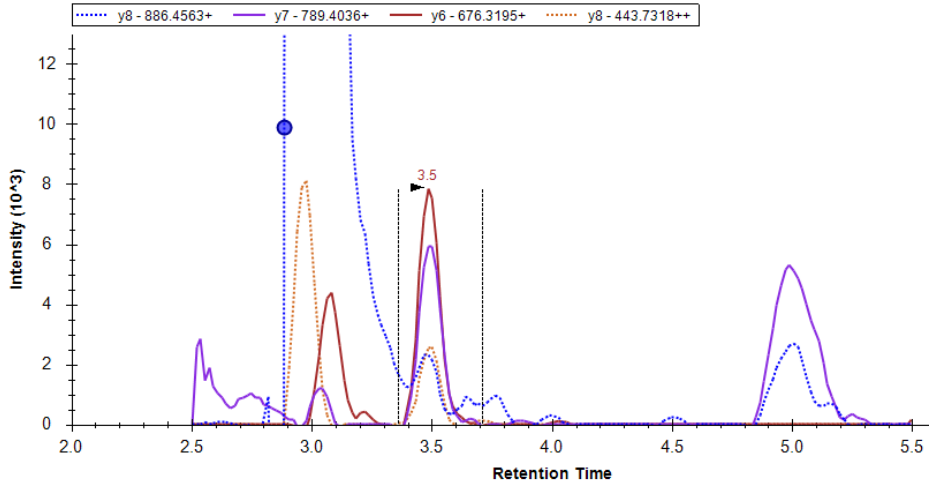


- QExactive+ MS system:
  - Data-Independent Acquisition (DIA) mode
  - 140.000Da resolution
  - Isolation window: 1.2 m/z
  - Isolation offset: 0.2 m/z



Validation in human assay: QExactive+ assay performance

- Sensitivity increased to 0.100µg/mL
- Endogenous concentration of 0.160µg/mL observed
- Assay qualifications:
  - 0.100-5.00µg/mL, linear regression, 1/x weighting
  - Parallelism, dilution integrity, selectivity, specificity, stability (sample, reinjection, bench-top, F/T, long-term) all successfully evaluated



QC	Spiked concentration (µg/mL)	Concentration sMET (µg/mL)	Average bias (%)	CV (%)
VLLoQ	0.100	0.100	16%	8%
LLoQ	0.200	0.200	15%	5%
L	0.300	0.300	15%	8%
Endo	0.000	0.159	0%	8%
VLLoQ Spiked	0.100	0.259	9%	8%
LLoQ Spiked	0.200	0.359	6%	5%
M	1.50	1.66	-7%	9%
H	3.75	3.91	-13%	6%

Conclusion

- 4% BSA in PBS is **not** suited as surrogate matrix when precipitating with 1% TCA in IPA
- Animal plasma may be better surrogate matrix than 4% BSA in PBS
- Switch to high resolution LC-MS system may be necessary to remove interfering signals
- Assay qualifications:
  - Suited for quantitation of sMET in cyno and human plasma
  - Chicken plasma as surrogate matrix
  - 0.100-5.00µg/mL
  - Currently in use in our BYON3521.001 clinical study