Lowering the LC-MS/MS Assay Quantitation Limit to 50 pg/mL for Ranibizumab in Human Plasma after Intravitreal Administration by Using SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready

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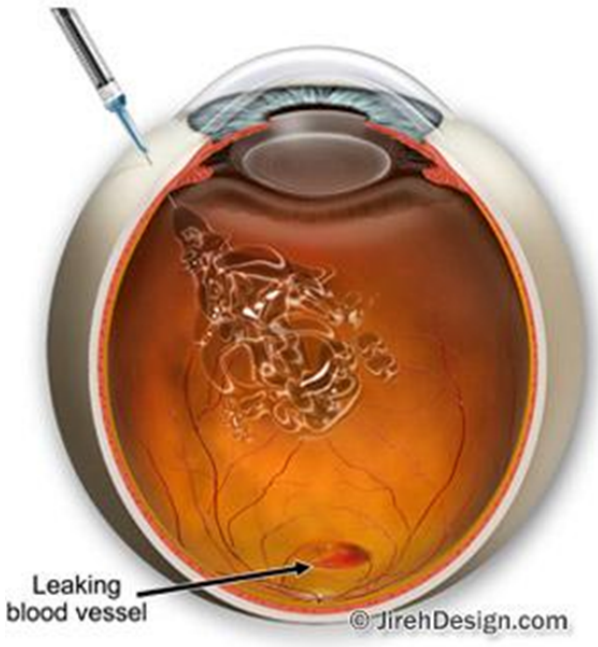
HELPING DELIVER LIFE-CHANGING THERAPIES

Outline

1. Background
2. Methods / Techniques
3. Comparison of Platforms
   - a. Validation Data
   - b. Chromatograms
4. Conclusions

https://eyewire.news/articles/lucentis-05-mg-prefilled-syringe-now-available-to-order-in-the-us/

Ranibizumab

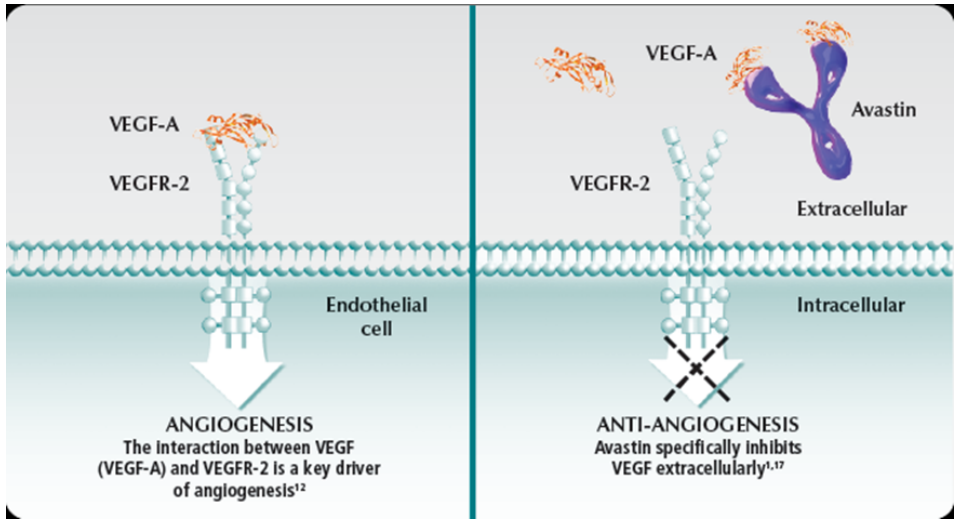


https://maculacenter.com/eye-procedures/avastin/

- Trade name: Lucentis (Genentech)
- Approved by the FDA in 2006 to treat wet Age-related Macular Degeneration (AMD)
  - Subsequently approved for the treatment of diabetic retinopathy, diabetic macular edema, macular edema following retinal vein occlusion, and myopic choroidal neovascularization
- Intravitreal Administration
- Humanized Fab Biologic Drug, produced from mouse spleen cells immunized with VEGF-A
  - ~ 48,000 Da

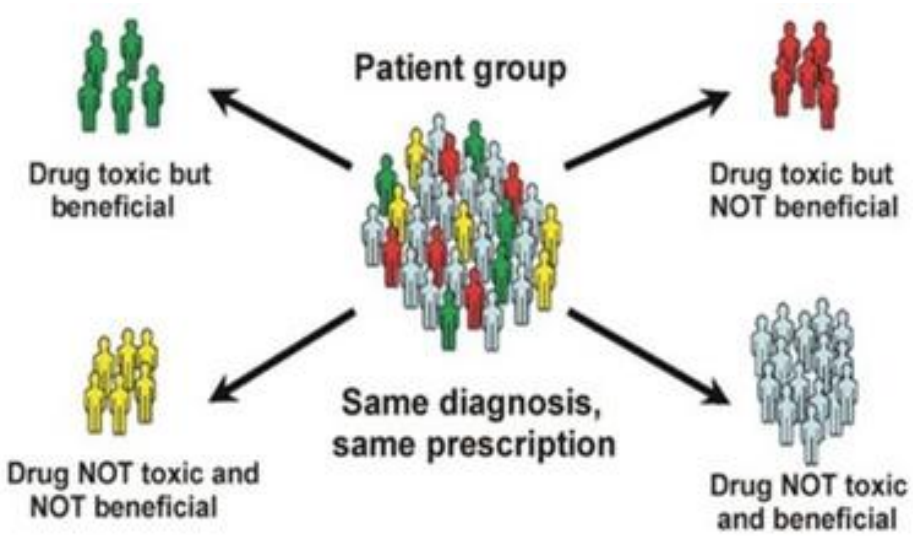
VEGF-A Inhibitors

- Presence of VEGF-A triggers production of new blood vessels
- Patients with AMD have increased VEGF-A, which leads to overproduction of abnormal leaky blood vessels, resulting in blindness
- Blocks angiogenesis by binding to (and therefore inhibiting) VEGF-A



https://www.avastin-hcp.com/about-avastin/proposed-moa.html

Purpose of Developing a Method for Bioanalysis of Ranibizumab

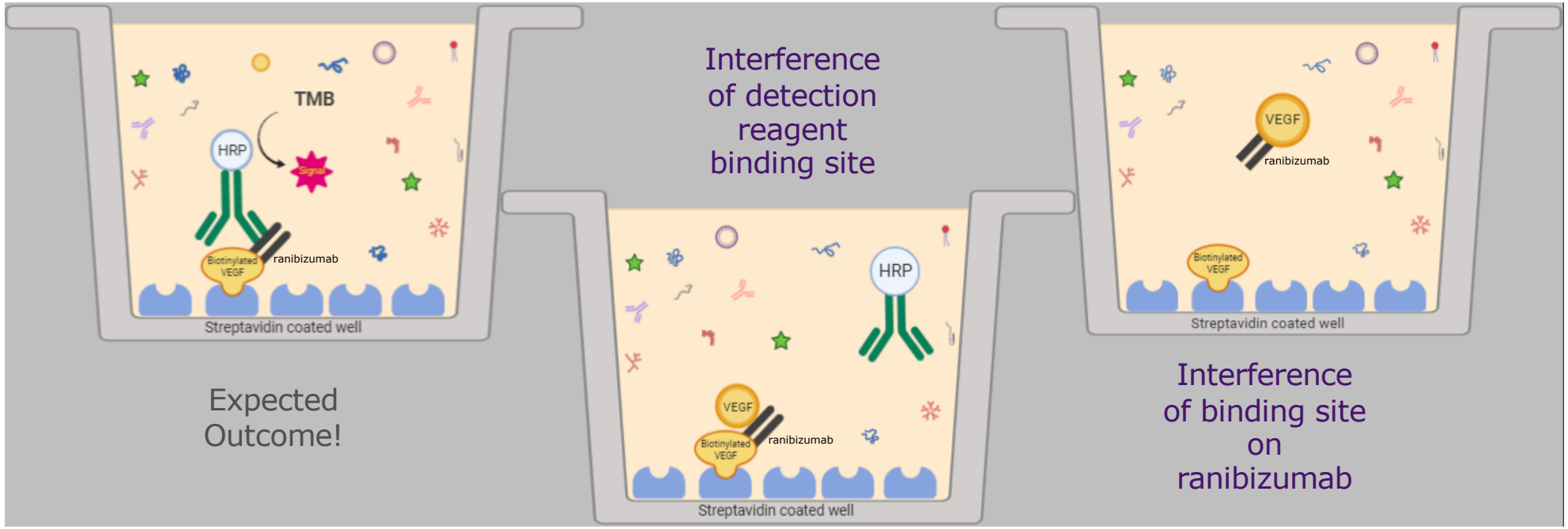


https://www.gyngroup.com/services/pharmacogenomics

- Therapeutic Drug Monitoring
  - Personalized medicine
  - Evaluation of **patient toxicity** and **efficacy** in the clinical setting
- To support future clinical trials
  - Additional indications
  - Biosimilars
    - Lucentis Patent Expiry June 2020

Bioanalysis of Proteins

- LBA - Traditional method
  - Advantages: ease-of-use, acceptance, equipment costs, throughput, sensitivity
  - Drawbacks: heavy reliance on customized special reagents, **selectivity issues & cross-reactivity** (ie ADA, VEGF)
- Use of LC-MS/MS method as an alternative or complementary technique
  - Advantages: decrease interference, increased selectivity (MS/MS MRM)

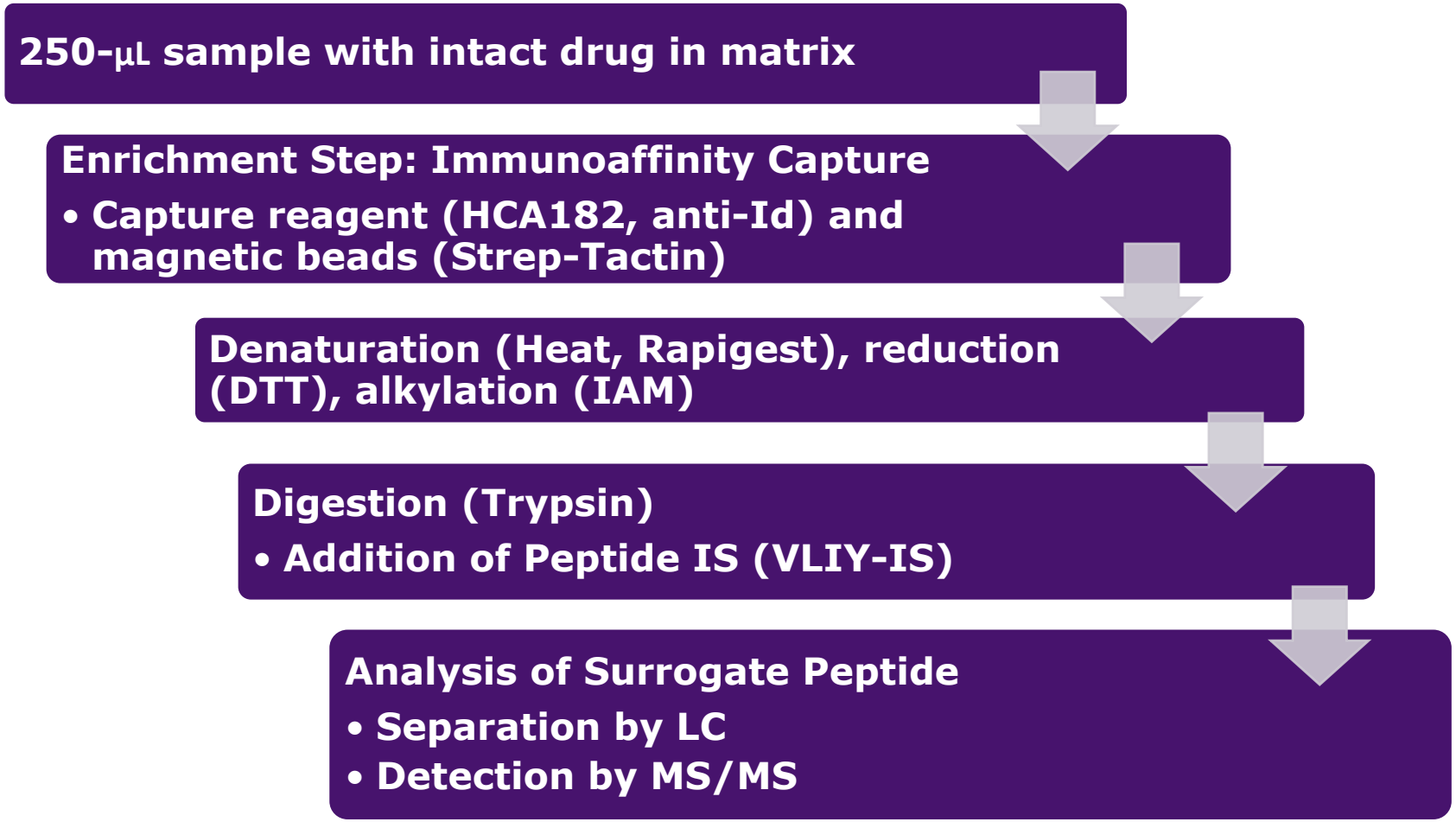


Created using Biorender Software

Overview of Comparison Studies

- Quantitation of Ranibizumab in human plasma after intravitreal administration
- Originally Fully Validated at PPD
  - SCIEX Triple Quad 6500 System
  - Calibration Range from 0.3 to 100 ng/mL
- Comparison of Mass Specs – Fit-for-Purpose Cross Platform Evaluation
  - Evaluation to lower the LLOQ using novel MS platform
  - SCIEX QTRAP 6500+ System (meant to represent the original validation conditions for new evaluation) compared to SCIEX 7500 System
  - Calibration Range from 0.05 to 100 ng/mL
  - Validation experiments: accuracy and precision, unfortified specificity, and fortified specificity

Ranibizumab Bioanalysis by LC-MS/MS Sample Extraction Prep Design



LC-MS/MS Optimized Conditions

Original validation - PPD

Analytical Column	Acquity UPLC BEH C_{18}, 130Å, 2.1mm x 50mm, 1.7µm
Guard Column	**Acquity UPLC Protein BEH C4 VanGuard Pre-column, 300Å, 2.1 mm X 5 mm, 1.7 µm**
Column Temperature	60°C
Mobile Phase A	0.1% Formic acid in Water
Mobile Phase B	0.1% Formic acid in ACN
Flow Rate	0.4 mL/min
MP Gradient (eluting pump)	**3.0 – 8.0 min 10-30%B**
Ionization Source	Positive Electrospray Ionization
Mass Spectrometer (QqQ)	**SCIEX Triple Quad™ 6500 LC-MS/MS System**
Surrogate Peptides	VLIYFTSSLHSGVPSR (quantitative) and LSCAASGYDFTHYGMNWVR (ranibizumab specific, confirmatory)
Internal Standard	VLIYF*TSSLHSGVPSR [F*=Phe(13C9,15N)]
MRM Transitions	588.3 (+3) → 719.4 (y13, +2) (analyte); 591.3 (+3) → 724.4 (y13, +2) (IS)
Calibration Range	**0.3 to 100 ng/mL**

LC-MS/MS Optimized Conditions

For comparison studies

Analytical Column	Acquity UPLC BEH C_{18}, 130Å, 2.1mm x 50mm, 1.7µm
Column Temperature	60°C
Mobile Phase A	0.1% Formic acid in Water
Mobile Phase B	0.1% Formic acid in ACN
Flow Rate	0.4 mL/min
MP Gradient (eluting pump)	**0.5 – 12.5 min 3-21%B**
Ionization Source	Positive Electrospray Ionization
Mass Spectrometer (QqQ)	**QTRAP® 6500+ LC-MS/MS System and SCIEX 7500 System**
Surrogate Peptides	VLIYFTSSLHSGVPSR (quantitative) and LSCAASGYDFTHYGMNWVR (ranibizumab specific, confirmatory)
Internal Standard	VLIYF*TSSLHSGVPSR [F*=Phe(13C9,15N)]
MRM Transitions	588.3 (+3) → 719.4 (y13, +2) (analyte); 591.3 (+3) → 724.4 (y13, +2) (IS)
Calibration Range	**0.05 to 100 ng/mL**

Mass Spectrometer Platforms Tested

The SCIEX 7500 System contains a larger sampling orifice than the SCIEX QTRAP 6500+ System and a novel dual stage RF ion guide that incorporates a dodecapole in the first stage and new ion source, allowing for increased sensitivity.

SCIEX QTRAP 6500+ System

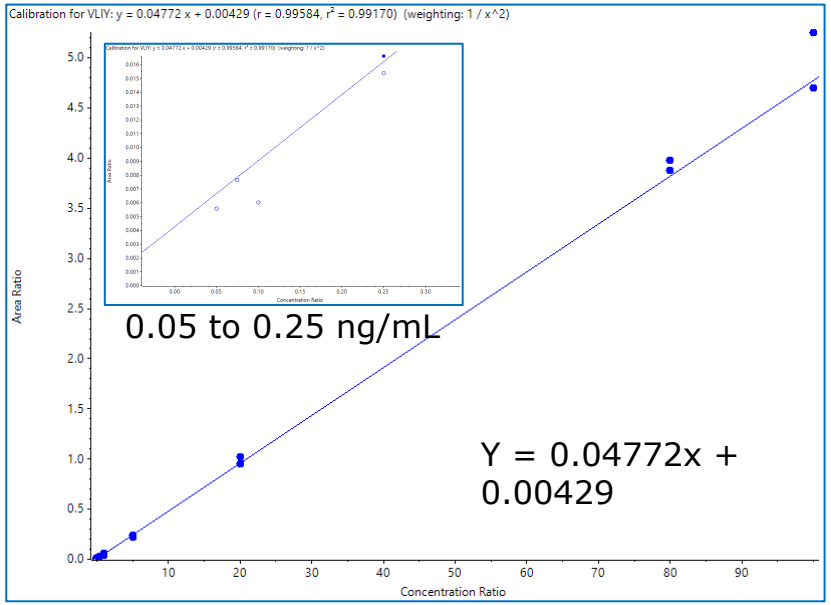
SCIEX 7500 System

Human Plasma Calibration Curves (Linear, $1/x^2$) – 0.05 to 100 ng/mL

SCIEX QTRAP 6500+ System

R^2 = 0.9891

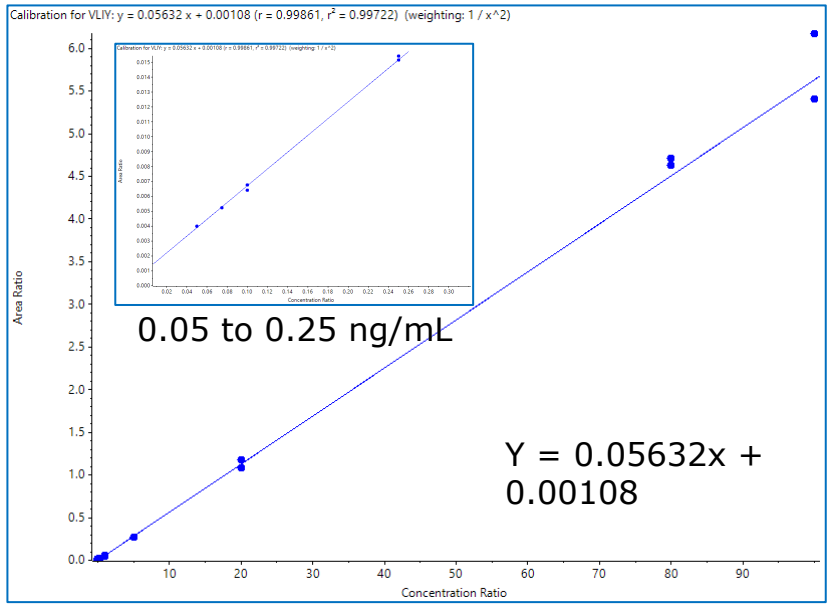
Due to sensitivity constraints, low calibration standards were deleted from the curve, resulting in LLOQ of 250 pg/mL



SCIEX 7500 System

R^2 = 0.9972

Targeted LLOQ was achieved at 50.0 pg/mL



Human Plasma Calibration Curves (Linear, $1/x^2$) – 0.05 to 100 ng/mL

SCIEX 6500+ System; R^2 = 0.9891

Nominal Conc (ng/mL)	Mean Conc n=2 (ng/mL)	% Accuracy
0.0500	N/A	N/A
0.0750	N/A	N/A
0.100	N/A	N/A
0.250	0.220	88
1.00	0.917	92
5.00	4.74	95
20.0	21.0	105
80.0	83.9	105
100	106	106

SCIEX 7500 System; R^2 = 0.9972

Nominal Conc (ng/mL)	Mean Conc n=2 (ng/mL)	% Accuracy
0.0500	0.0517	103
0.0750	0.0737	98
0.100	0.0978	98
0.250	0.252	101
1.00	0.970	97
5.00	4.84	97
20.0	20.1	100
80.0	82.9	104
100	103	103

Validation Results

Accuracy and precision

Criteria: %Bias (accuracy) & %CV (precision) ≤ 25% (LLOQ) / 20% (all others)

Platform		Theoretical Concentration in Human Plasma (ng/mL)				
		0.3	N/A	0.75	50.0	75.0
SCIEX Triple Quad 6500 System (org. Val)	**Intra-Mean Conc (ng/mL)**	**0.2 – 0.3**	**N/A**	**0.6 – 0.7**	**43 – 46**	**67 – 68**
	Intraday Accuracy (n=6, 3 days) (%bias)	-27 – 3	N/A	-17 – -10	-13 – -8	-10 – -9
	Intraday Precision (n=6, 3 days) (%CV)	6 – 15	N/A	4 – 8	1 – 5	2 – 5
	Inter-Mean Conc (ng/mL)	**0.266**	**N/A**	**0.651**	**44.4**	**67.6**
	Interday Accuracy (n=18, 3 days combined)	-11.4	N/A	-13.2	-11.3	-9.84
	Interday Precision (n=18, 3 days combined)	16.8	N/A	6.68	4.35	3.89

Validation Results

Accuracy and precision

Criteria: %Bias (accuracy) & %CV (precision) ≤ 25% (LLOQ) / 20% (all others)

Platform		Theoretical Concentration in Human Plasma (ng/mL)				
		0.05	0.075	0.15	50.0	75.0
QTRAP 6500+ System	**Intra-Mean Conc (ng/mL)**	**0.03 – 0.04**	**0.01 – 0.06**	**0.06 – 0.2**	**49 – 54**	**75 – 82**
	Intraday Accuracy (n=6, 3 days) (%bias)	-33 – -24	-91 – -15	-63 – 24	-1 – 7	0 – 9
	Intraday Precision (n=6, 3 days) (%CV)	22 – 105	12 – 155	4 – 60	3 – 5	3 – 7
	Inter-Mean Conc (ng/mL)	**0.0364**	**0.0406**	**0.126**	**51.5**	**78.6**
	Interday Accuracy (n=18, 3 days combined)	-27.2	-45.8	-15.9	3.08	4.76
	Interday Precision (n=18, 3 days combined)	69.2	72.6	60.5	5.56	6.42

Validation Results

Accuracy and precision

Criteria: %Bias (accuracy) & %CV (precision) ≤ 25% (LLOQ) / 20% (all others)

Platform		**Theoretical Concentration in Human Plasma (ng/mL)**				
		0.05	0.075	0.15	50.0	75.0
SCIEX 7500 System	**Intra-Mean Conc (ng/mL)**	**0.04 – 0.06**	**0.06 – 0.08**	**0.1 – 0.2**	**50 – 52**	**75 – 79**
	Intraday Accuracy (n=6, 3 days) (%bias)	-13 – 16	-25 – 1	-5 – 14	1 – 4	0 – 6
	Intraday Precision (n=6, 3 days) (%CV)	13 – 20	9 – 13	5 – 6	4 – 9	3 – 5
	Inter-Mean Conc (ng/mL)	**0.0517**	**0.0647**	**0.152**	**51.5**	**76.7**
	Interday Accuracy (n=18, 3 days combined)	3.42	-13.7	1.54	3.03	2.29
	Interday Precision (n=18, 3 days combined)	19.8	17.7	10.2	6.23	5.04

Validation Results

Specificity

Of the six individual lots and one pooled lot evaluated, no significant interfering peaks were noted in blank human plasma samples (double blanks) or blanks containing only the IS (single blanks), for specificity evaluations using either SCIEX Triple Quad 6500 System (originally); QTRAP 6500+ or 7500 System instrumentation.

Quantitation unaffected for 2/3 replicates for 5/6 individual human plasma lots evaluated, using either SCIEX Triple Quad 6500 System (0.300 ng/mL LLOQ) or 7500 System (0.075 ng/mL alternative LLOQ) instrumentation (acceptable)

	SCIEX Triple Quad 6500 System Fortified Specificity Data					
	SPF 1	SPF 2	SPF 3	SPF 4	SPF 5	SPF 6
	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
	0.274	0.333	0.301	0.294	0.309	0.300
	0.267	0.271	0.287	0.310	0.319	0.309
	0.282	0.318	0.311	0.287	0.304	0.306
Nominal Conc (ng/mL)	0.300	0.300	0.300	0.300	0.300	0.300
Low Limit	0.225	0.225	0.225	0.225	0.225	0.225
High Limit	0.375	0.375	0.375	0.375	0.375	0.375

Validation Results

Specificity

Of the six individual lots and one pooled lot evaluated, no significant interfering peaks were noted in blank human plasma samples (double blanks) or blanks containing only the IS (single blanks), for specificity evaluations using either SCIEX Triple Quad 6500 (originally); QTRAP 6500+ or 7500 System instrumentation.

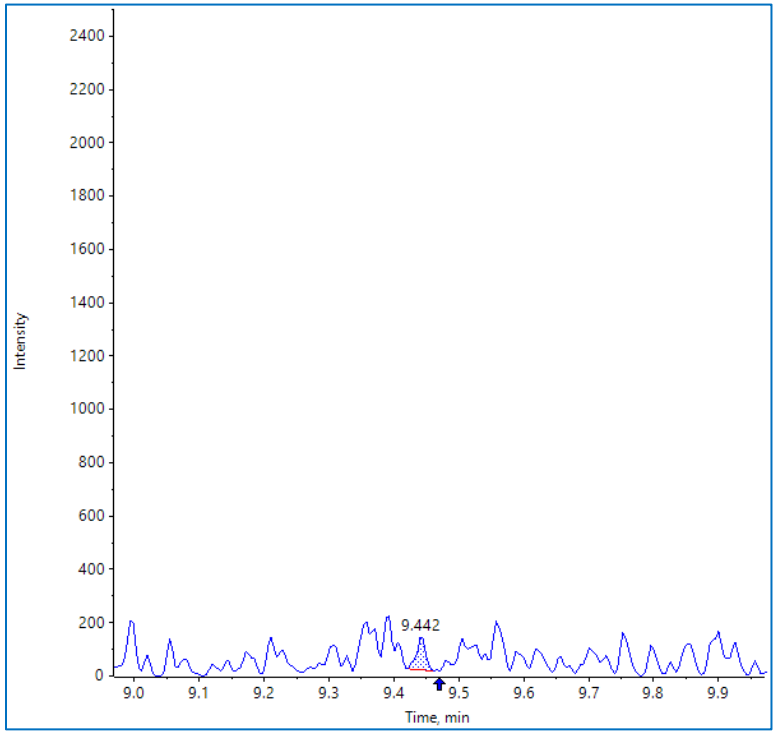
Quantitation unaffected for 2/3 replicates for 5/6 individual human plasma lots evaluated, using either SCIEX Triple Quad 6500 (0.300 ng/mL LLOQ) or 7500 System (0.075 ng/mL alternative LLOQ) instrumentation (acceptable).

	SCIEX 7500 System Fortified Specificity Data					
	SPF 1	SPF 2	SPF 3	SPF 4	SPF 5	SPF 6
	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
	0.0766	NA	0.0586	0.0325	0.0854	0.0664
	0.0814	0.0663	0.0630	0.0282	0.111	0.0653
	0.0721	0.0841	0.0715	0.0326	0.0778	0.0717
Nominal Conc (ng/mL)	0.0750	0.0750	0.0750	0.0750	0.0750	0.0750
Low Limit	0.0563	0.0563	0.0563	0.0563	0.0563	0.0563
High Limit	0.0938	0.0938	0.0938	0.0938	0.0938	0.0938

Representative Chromatograms

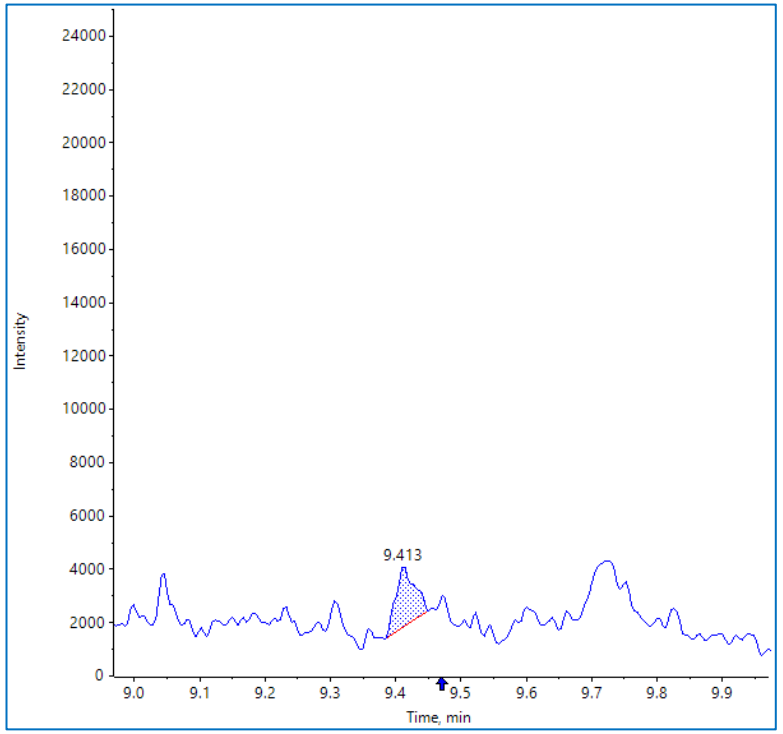
K_2EDTA human plasma blanks

SCIEX QTRAP 6500+ System



Ranibizumab Average CPS: 200

SCIEX 7500 System

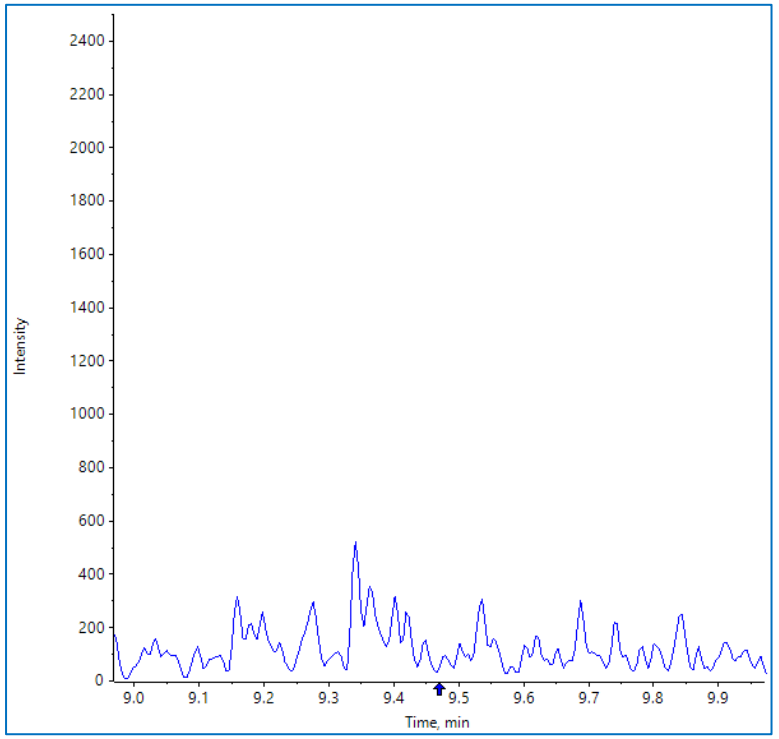


Ranibizumab Average CPS: 4000

Representative Chromatograms

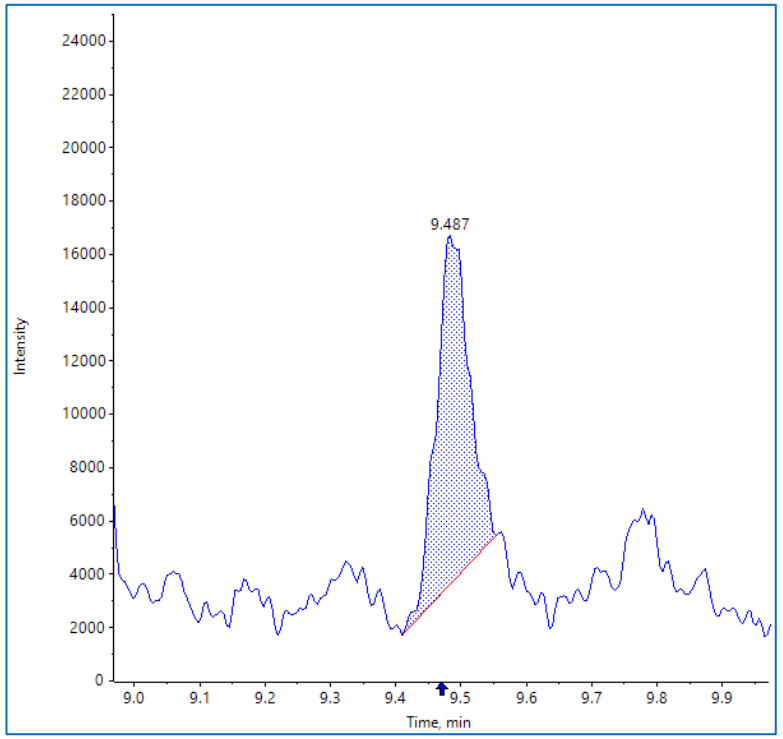
50 pg/mL ranibizumab K_2EDTA Human Plasma QC

SCIEX QTRAP 6500+ System



Ranibizumab Average CPS: 400

SCIEX 7500 System

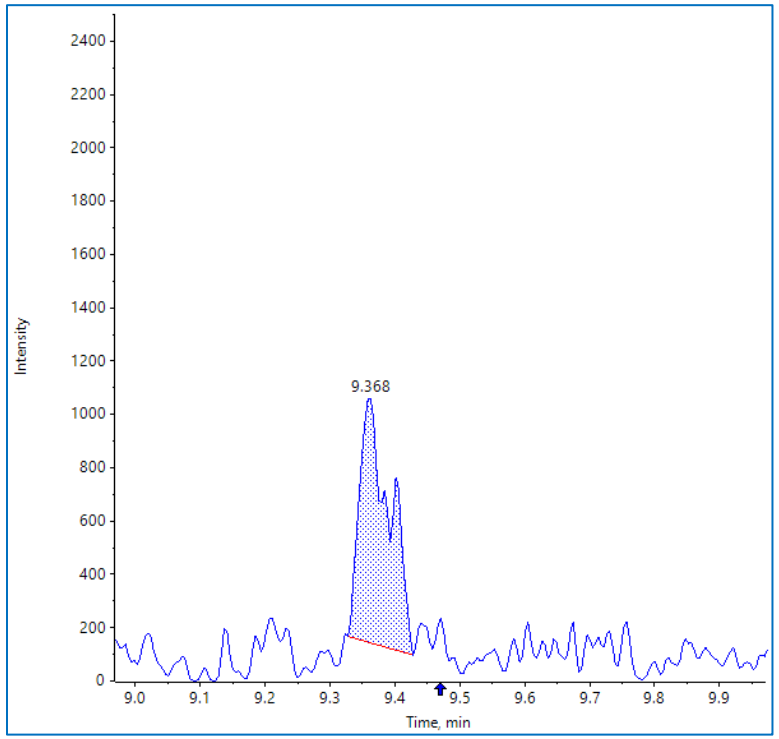


Ranibizumab Average CPS: 17000

Representative Chromatograms

250 pg/mL ranibizumab K_2EDTA Human Plasma QC

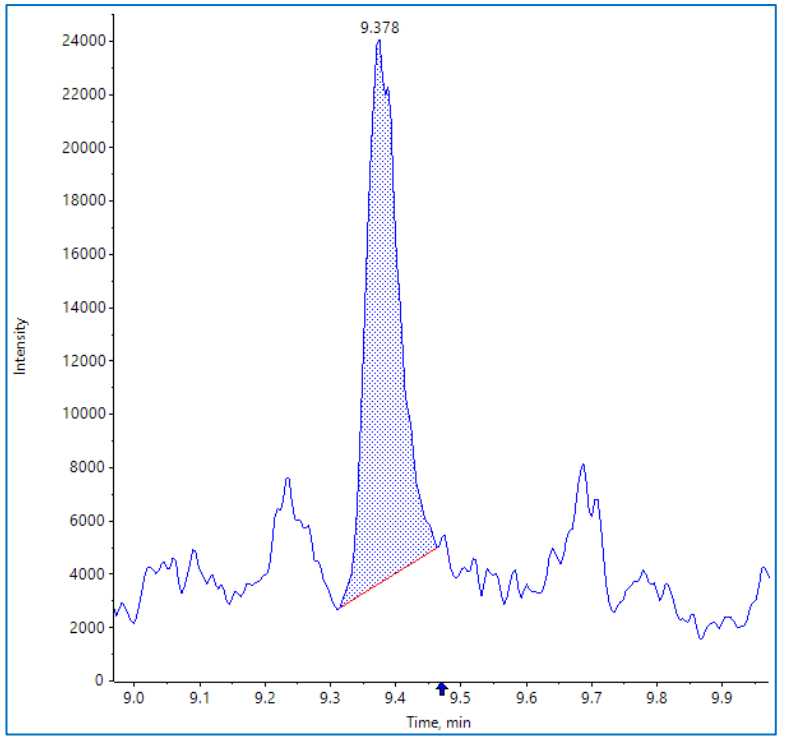
SCIEX QTRAP 6500+ System



Ranibizumab Average CPS: 1100

S/N: 7

SCIEX 7500 System



Ranibizumab Average CPS: 24000

S/N: 21

Conclusions

- Both techniques validated according the 2018 FDA Bioanalytical Method Validation Guidance
  - SCIEX Triple Quad 6500 System: 0.3 to 100 ng/mL range (previously validated)
  - SCIEX 7500 System: 0.05 to 100 ng/mL range
- Markedly higher sensitivity achieved using SCIEX 7500 System
  - 6-fold lower LLOQ
  - 3.5x higher signal-to-noise
- Both methods available to quantitatively access plasma ranibizumab drug conc. in patient samples following IVT injection; with SCIEX 7500 System reaching more clinically relevant levels!
- Future perspectives and applications to additional bioanalytical methods
  - Reduced sample aliquot volume / injection volume
    - Especially helpful for pre-clinical and pediatric
  - Reduced reagents / reduced cost
  - Ability to achieve previously unattainable LLOQs

Select References

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Acknowledgements

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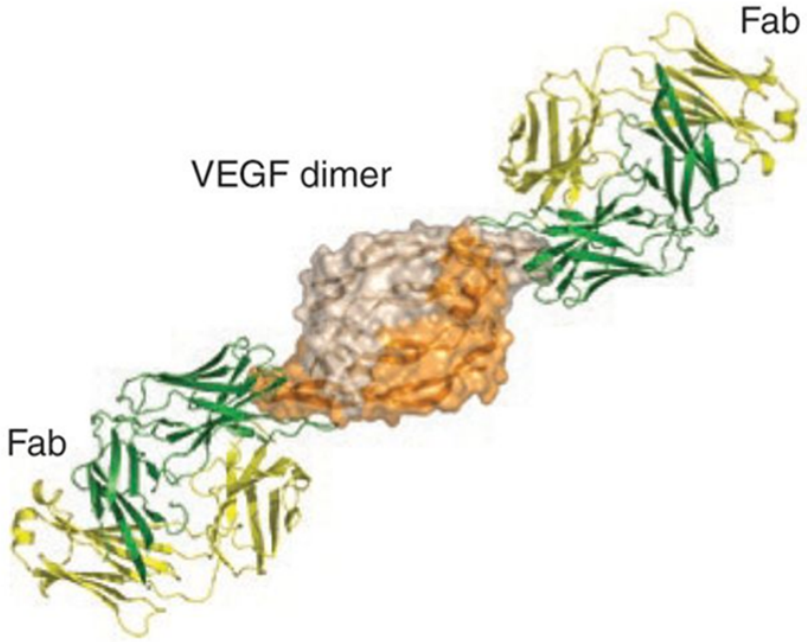
- SCIEX
  - Dr. Ian Moore

Thank you! Questions?



https://www.nature.com/articles/nm1010-1107