Development and validation of an LC-MS/MS method with a broad linear dynamic range utilizing natural isotopologue transitions for quantification of tivozanib

Maaike Bruin
PhD student

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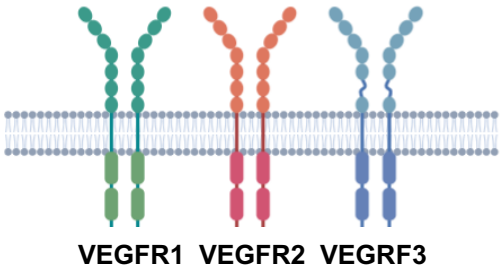
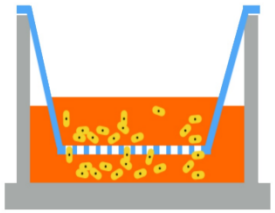
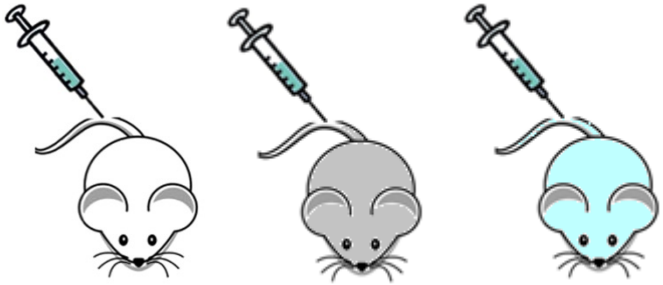
BACKGROUND



- Tivozanib is a tyrosine kinase inhibitor
- Approved for the treatment of advanced renal cell carcinoma
- Pre-clinical experiments were conducted to study the effects of several drug transporters on the pharmacokinetics of tivozanib

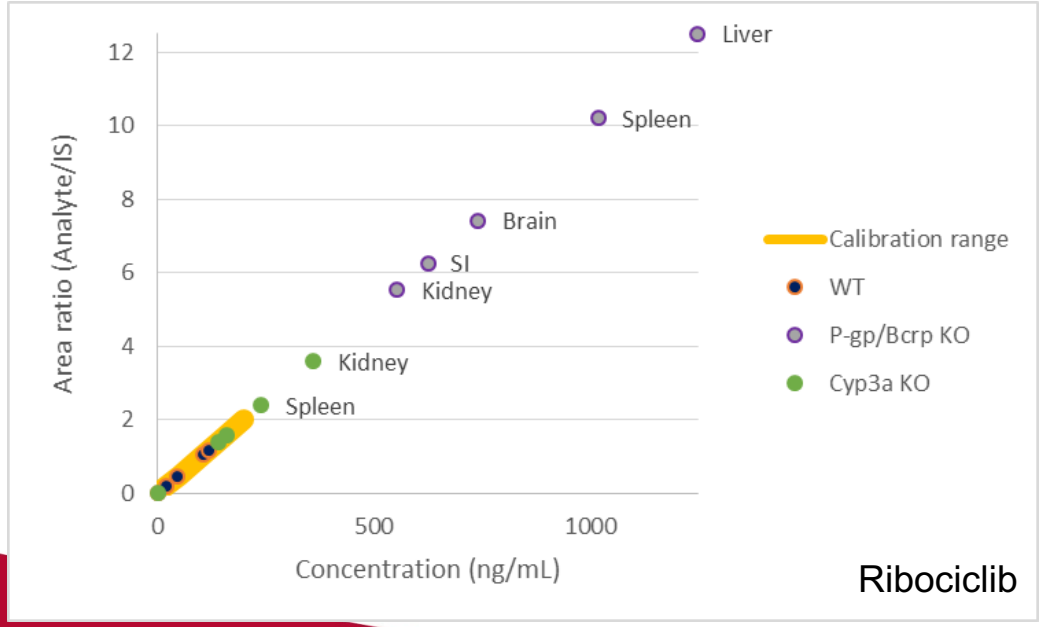




→ LC-MS/MS assay is pivotal to measure concentrations

BACKGROUND

- Difficult to predict drug concentrations for first studies in any species
- Challenging to determine the concentration range of the LC-MS/MS method



→ A broad range is desirable to prevent sample dilution and re-analysis

- Easy and time-saving
- Limited amount of sample from pre-clinical studies

Figure provided by Alejandra Martinez

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AIM

To develop and validate an LC-MS/MS assay with a broad linear dynamic range to quantify tivozanib in various matrices

1. Development
2. Validation
3. Application

- Pre-treatment
- Liquid chromatography
- Mass spectrometry

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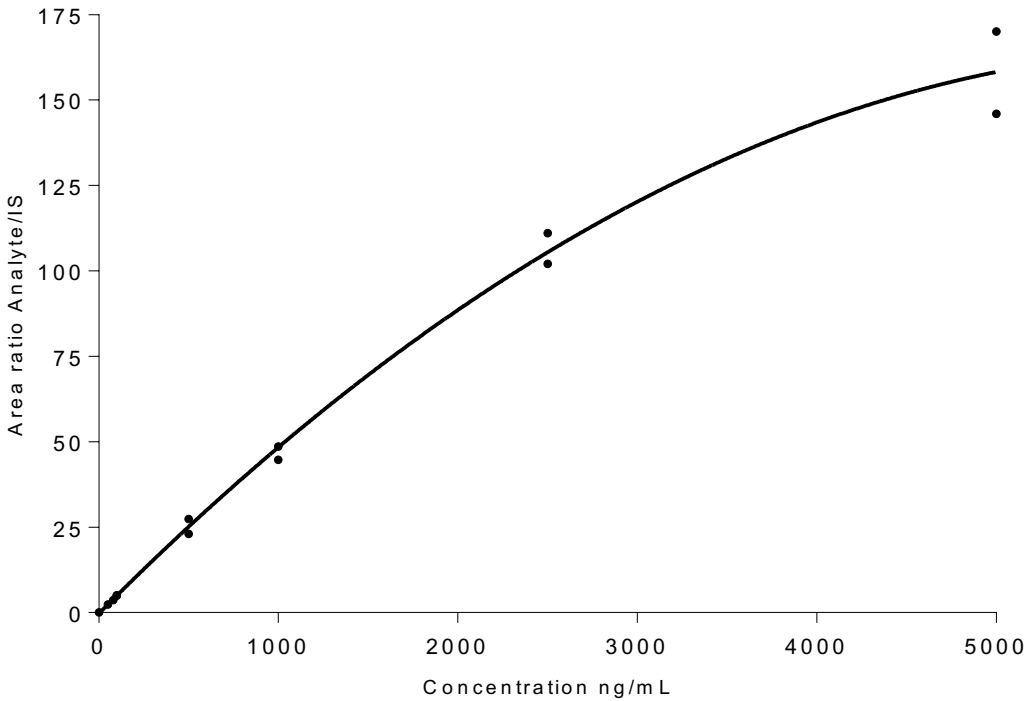
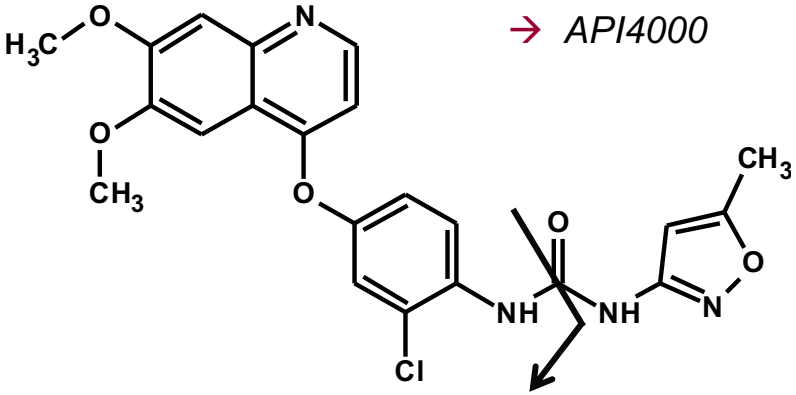
1 Development





Mass Tivozanib: 454
Q1 mass: 455
Q3 mass: 341

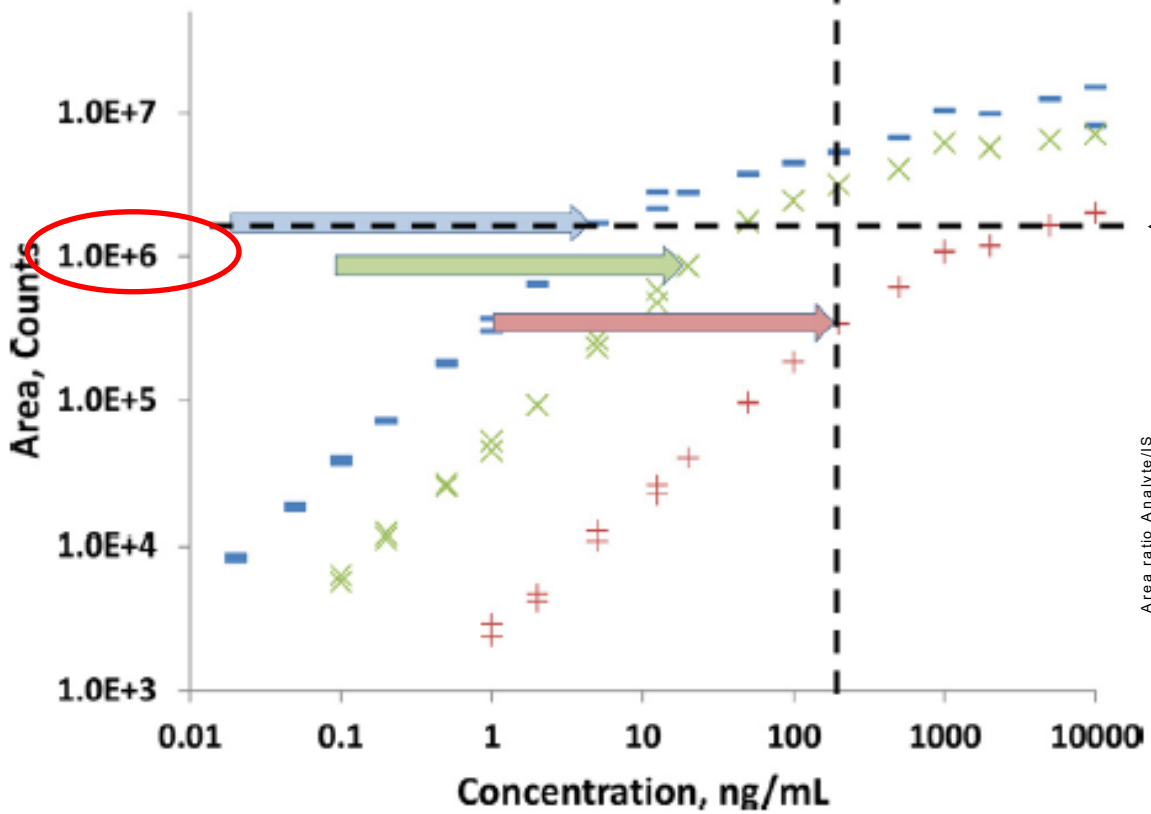
→Non-linear calibration curve 0.5-5000 ng/mL

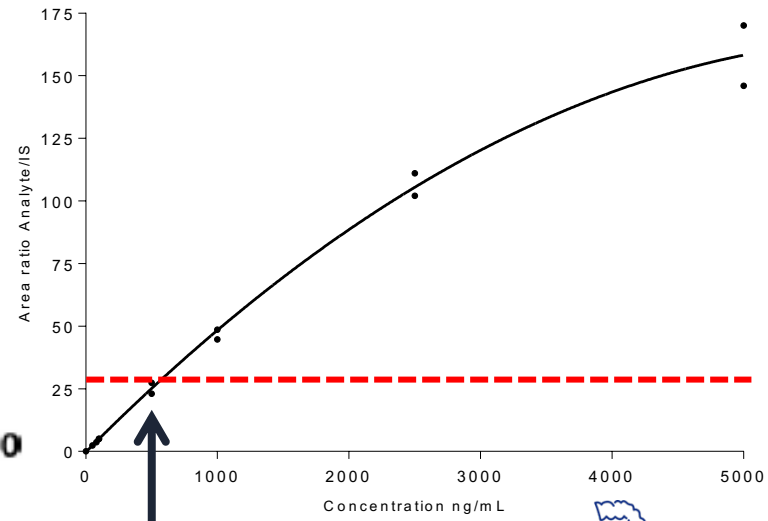
1 Development

How do we obtain a linear calibration curve with a broad range from 0.5-5000 ng/mL?

1. Ionization saturation

2. Detector saturation





±500 ng/mL

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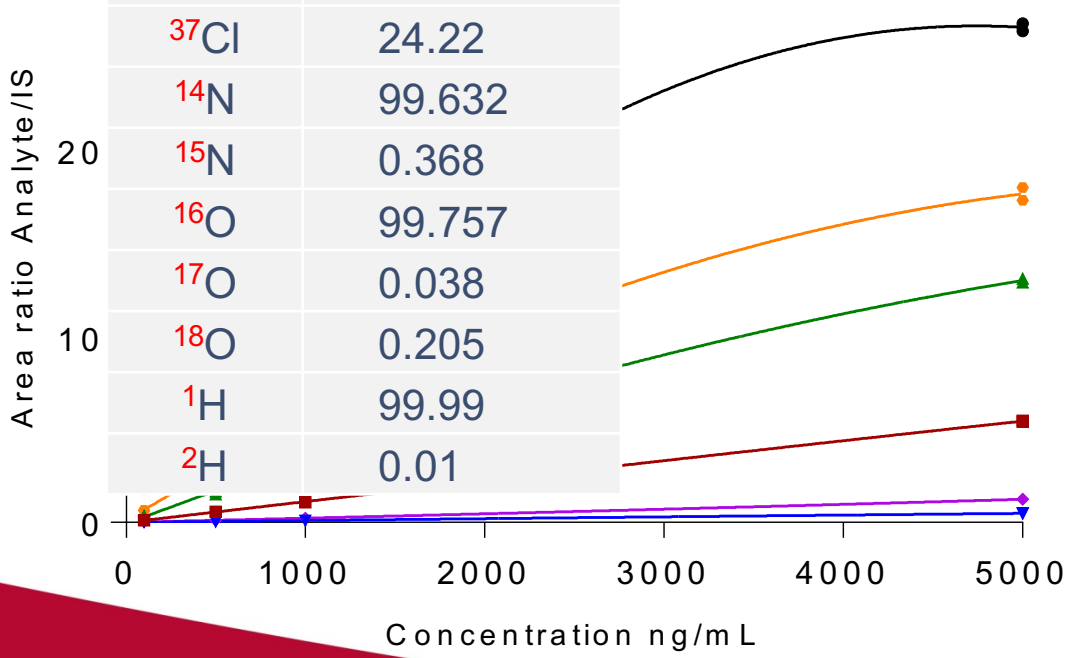
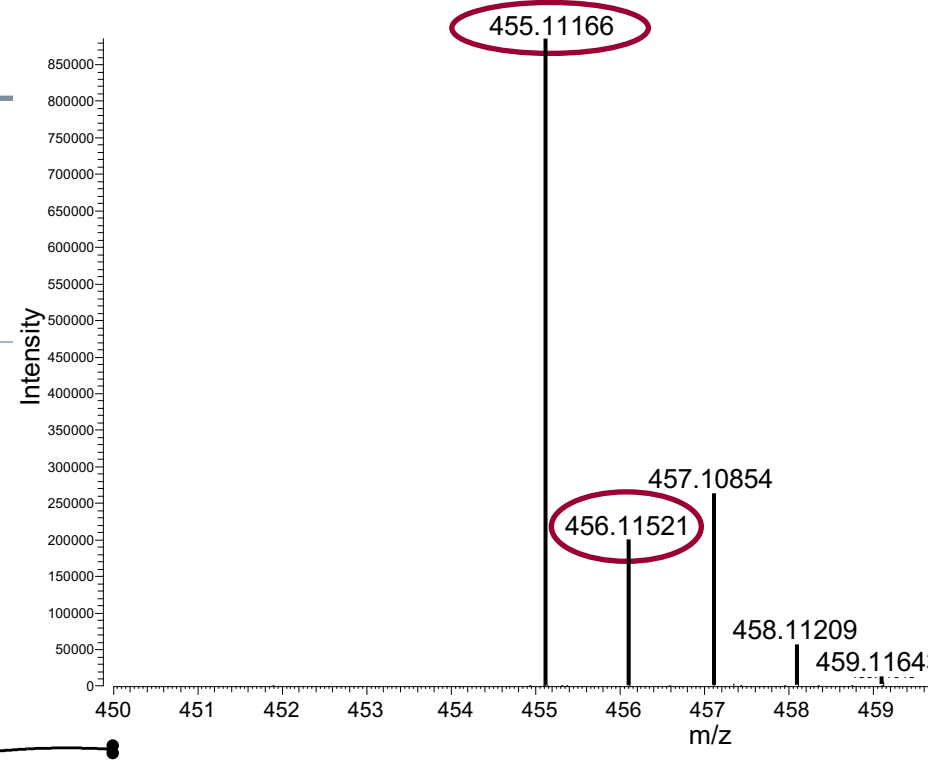
Liu H et al. *Anal Chim Acta*. 2014;850:65-70

1 Development

Natural abundance of isotopes

Isotope	Abundance (%)
^{12}C	**98.93**
^{13}C	**1.07**
^{35}Cl	75.78
^{37}Cl	24.22
^{14}N	99.632
^{15}N	0.368
^{16}O	99.757
^{17}O	0.038
^{18}O	0.205
^{1}H	99.99
^{2}H	0.01





✗	+ 0 + 0	**455→341**
✓	+ 1 + 0	**456→341**
✗	+ 1 + 1	**456→342**
✓	+ 2 + 0	**457→341**
✓	+ 2 + 1	**457→342**
✗	+ 2 + 2	**457→343**

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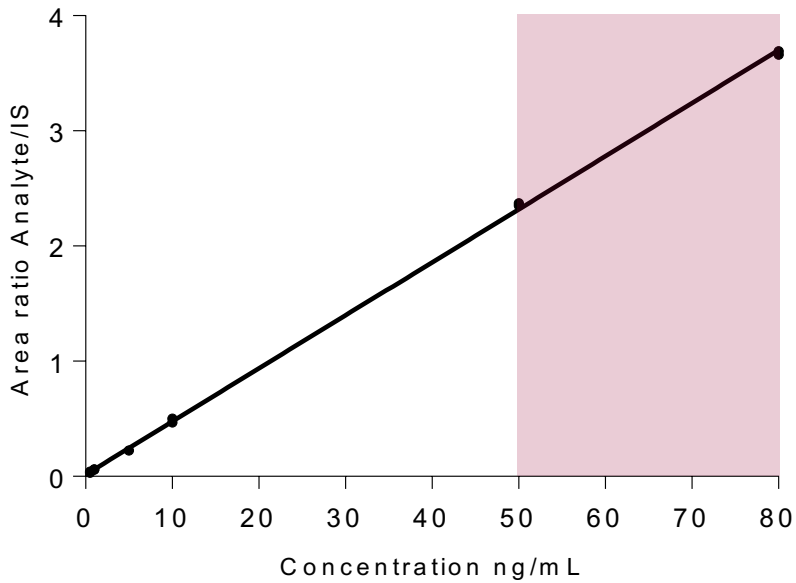
Audi, G. & Wapstra, A. H. *Nucl. Phys. A* **565**, 1–65 (1993)

1 Development

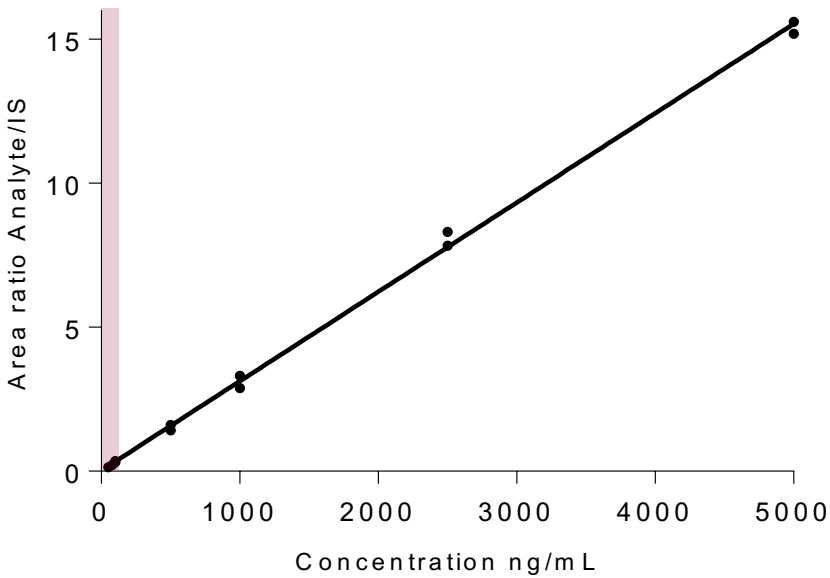
0.5-80 ng/mL

Transition: 455→341 (+0+0)



50-5000 ng/mL

Transition: 456 → 341 (+1+0)



How do we validate this?

→ 10 matrices
→ Double range

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2 Validation

FDA/EMA: *Generally a full validation should be performed for each species and matrix concerned*

- Mouse plasma
- Mouse organs
  - Brain
  - Spleen
  - Lung
  - Kidney
  - Liver
  - Small intestine
  - Small intestine content
- In vitro medium

Time consuming

Amount of blank mouse tissue and plasma is limited

Necessary?

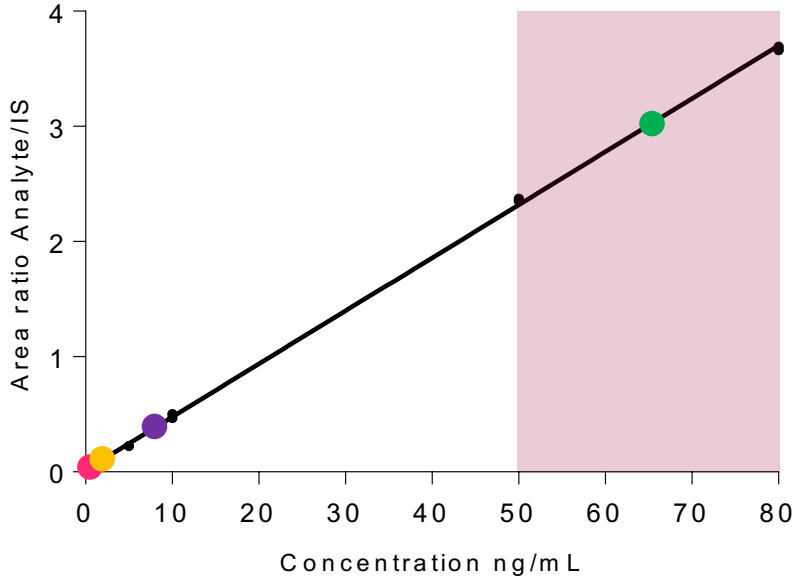
Quantification of all matrices on a human plasma calibration curve

2 Validation

ACCURACY & PRECISION

0.5-80 ng/mL

Transition: 455→341 (+0+0)

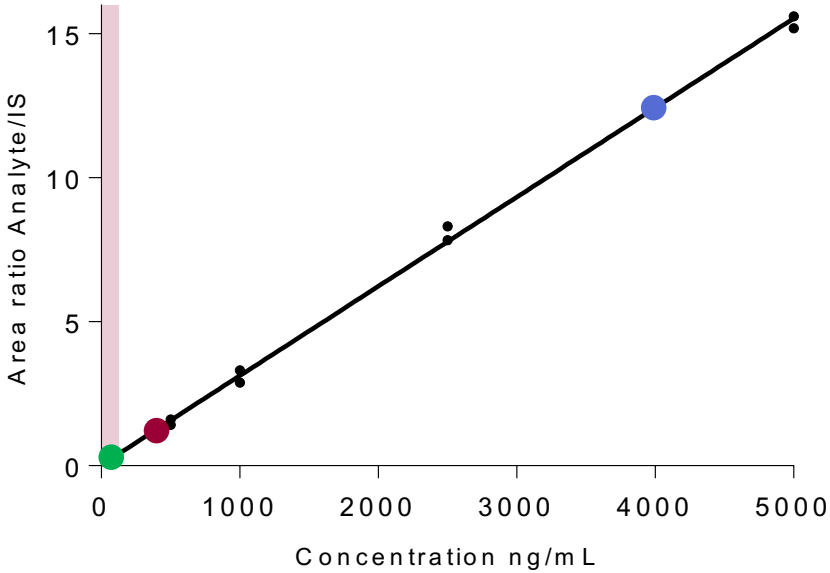


Quality control (QC) samples:

- LLOQ 0.5 ng/mL
- Intermediate 66 ng/mL

50-5000 ng/mL

Transition: 456 → 341 (+1+0)



Quality control (QC) samples:

- Intermediate 66 ng/mL
- H-High 4000 ng/mL

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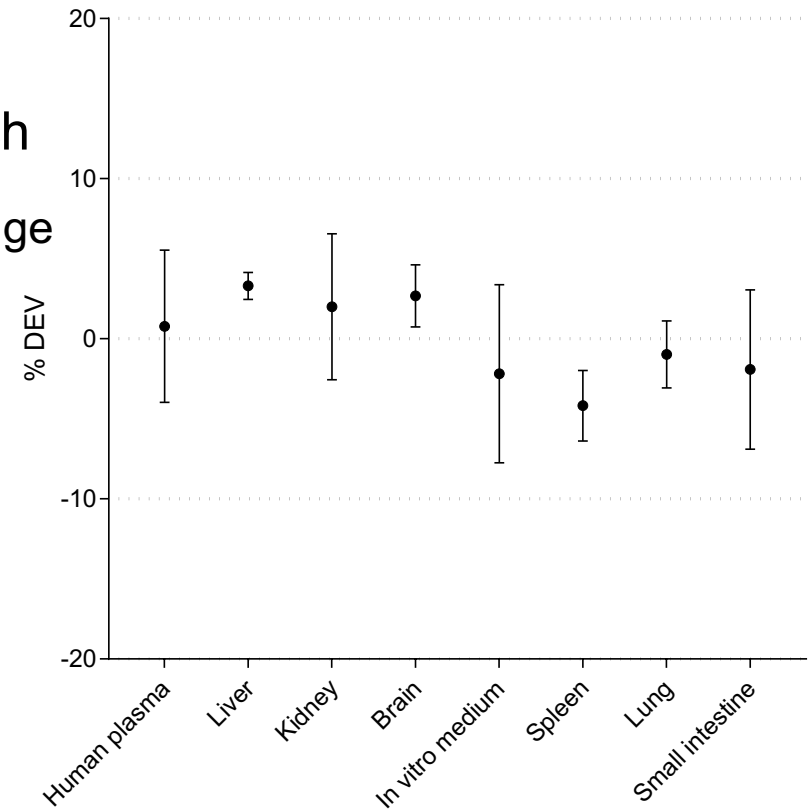
2 Validation

ACCURACY & PRECISION

Overlapping intermediate QC sample

1. To test the applicability of the approach
   - Comparison concentration low and high range
   - Staeheli et al: criteria ≤20%
2. To compare the relative precision between the two curves

Result: no difference



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Staeheli, S. N. et al. *Anal. Bioanal. Chem.* **407**, 8681–8712 (2015)

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Validation

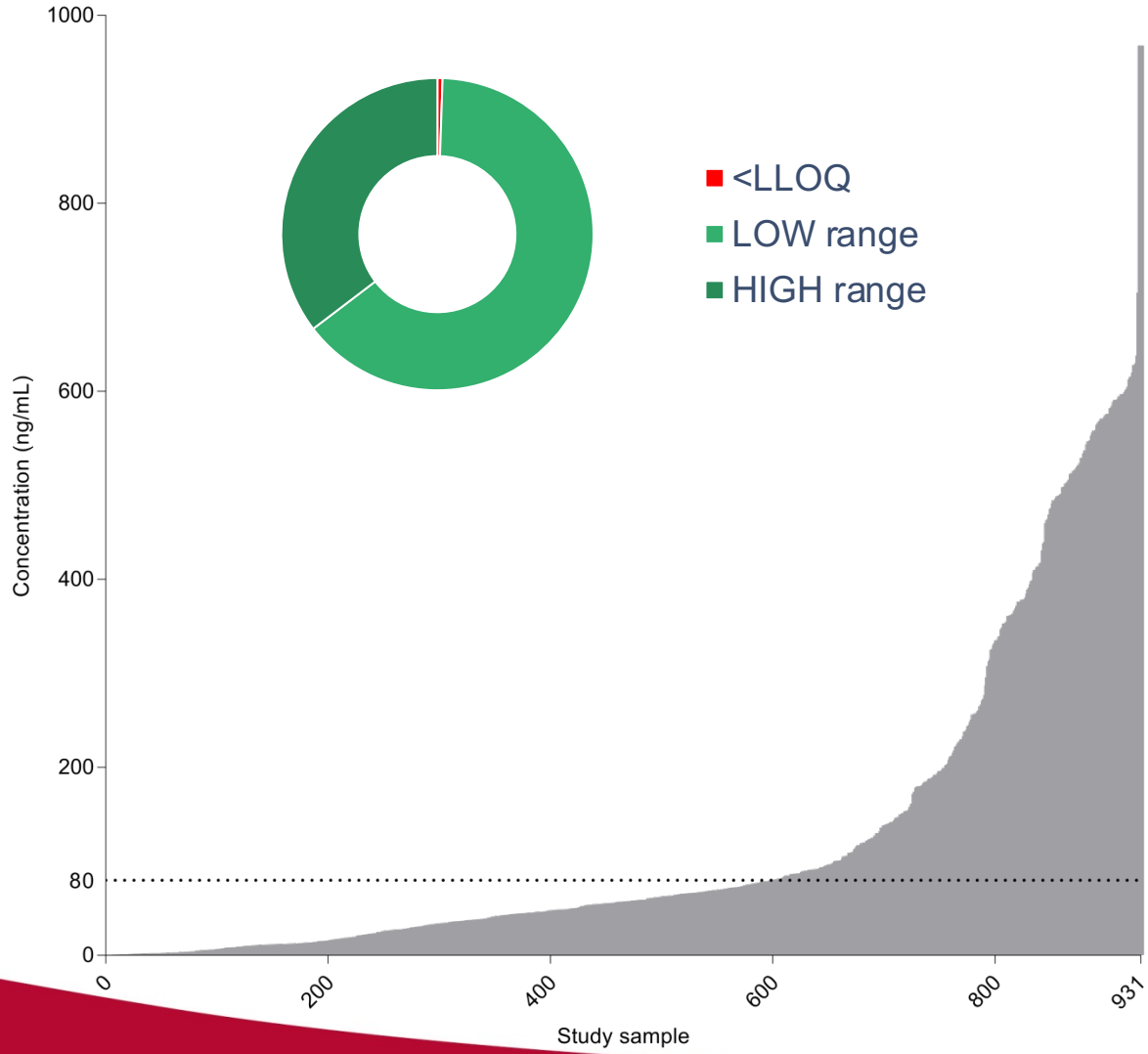
FDA/EMA GUIDELINE

Quality control (QC) samples:

• **LLOQ**	**0.5**	**ng/mL**
• L-Low	1	ng/mL
• L-Medium	8	ng/mL
• **Intermediate**	**66**	**ng/mL**
• H-Medium	400	ng/mL
• **H-High**	**4000**	**ng/mL**

	FDA/EMA	Human plasma	Organs/in vitro medium
Calibration curve	1 range 6-8 standards	2 ranges 10 standards	-
Accuracy & Precision	4 levels	6 levels	3 levels
• **Within-run**	N=5 per level	N=5 per level	N=5 per level
• **Between-run**	3 different runs	3 different runs	1 run
Stability	2 QC levels: • LOW • HIGH	1 QC level: • Intermediate	1 QC level: • Intermediate
Criteria	LLOQ ≤20% Calibration standards ≤ 15% QC-Low/Medium/High ≤ 15%	LLOQ ≤ 20% Calibration standards ≤ 15% QC-samples ≤ 15%	LLOQ ≤ 25% QC-samples ≤ 20%

3 Application



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CONCLUSIONS

- We succesfully developed an LC-MS/MS method with a broad range for the quantification of tivozanib in several matrices
- The calibration curve is linear from 0.5-5000 ng/mL using natural isotopologue transitions
- Additional experiments show the validity of this approach
- Human plasma could be used as a surrogate matrix
- The assay was succesfully applied to measure tivozanib in pre-clinical studies

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