Scientific Validation: A Case Study for Quantification of Gadolinium in Multiple Tissue Types Using One Tissue as the Calibration and QC Matrix

ARCINOVA & GBCA Consortium

Outline

- Background
- Scientific Validation Strategy
- Validation Tests and Results
- Discussion

BACKGROUND

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

Drug Safety Communications

FDA identifies no harmful effects to date with brain retention of gadolinium-based contrast agents for MRIs; review to continue

This is an update to the FDA Drug Safety Communication: FDA evaluating the risk of brain deposits with repeated use of gadolinium-based contrast agents for magnetic resonance imaging (MRI) issued on July 27, 2015.

- Manufacturers of gadolinium based contrast agents (GBCA) to conduct human and animal studies to further assess the safety of these contrast agents
- GBCA Consortium formed to address concerns (Bayer, Bracco Imaging, GE Healthcare & Guerbet)

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Bioanalytical requirements

- Regulatory Compliance
  - GLP
- Samples
  - Two species (Mouse & Non-Human Primate)
  - Multiple tissues (n=6)
- Methodology
  - Samples are nitric acid tissue digests
  - Elemental analysis (Gd) using Agilent ICPMS instrumentation (7700/7900/8900 models)

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Elemental Analysis by ICP/MS

- High temperature plasma ion source decomposes, atomises sample matrix and forms ions
- Uses quadruploe mass analyzer to separate ions – unit mass resolution
- Gadolinum is exogenous so Calibration and QCs can be prepared in control biological matrices with no interference from endogenous Gd
- For some exogenous elements risk of contamination from sample collection devices is important to assess

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SCIENTIFIC VALIDATION STRATEGY

- Approach based on 2x EBF White papers :

  Recommendations from the European Bioanalysis Forum on method establishment for tissue homogenates

  Philip Timmerman, Nathalie Mokrzycki, Pascal Delrat, Marc De Meulder, Eva Erbach, Irene Lentheric, Morna McIntosh, Pawel Dzygiel. Bioanalysis, 2014,Vol. 6, No. 12, Pages 1647-1656

  Tiered approach into practice : scientific validation for chromatography based assays in early development – a recommendation from the European Bioanalysis Forum

  Philip Timmerman, Stephen White, Stuart McDougall, Morten A Kall, John Smeraglia, Marianne Scheel Fjording and Magnis Knutsson, Bioanalysis, 2015, Vol.7, No. 18, Pages 2387-2398

- Additional considerations
  - ICPMS vs Chromatographic MS methods
  - FDA expect 4-6-15 acceptance paradigm (based on their feedback)
  - Ethical

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Scientific Validation Overview

Tests in primary matrix (mouse liver digest)

- Specificity/Selectivity
- Accuracy and Precision at robustness
- Autosampler carry-over
- Dilution
- Room temperature stability
- Frozen stability
- Freeze/thaw stability

Other tests

- Assay suitability for other mouse tissues
- Assay suitability for NHP liver

Scientific Validation Set Up

- Calibrations and QCs prepared in primary matrix (mouse liver digest)
- Additional QCs prepared in other mouse tissues at QCL and QCH
  - Brain
  - Femur
  - Kidney
  - Skin
  - Spleen

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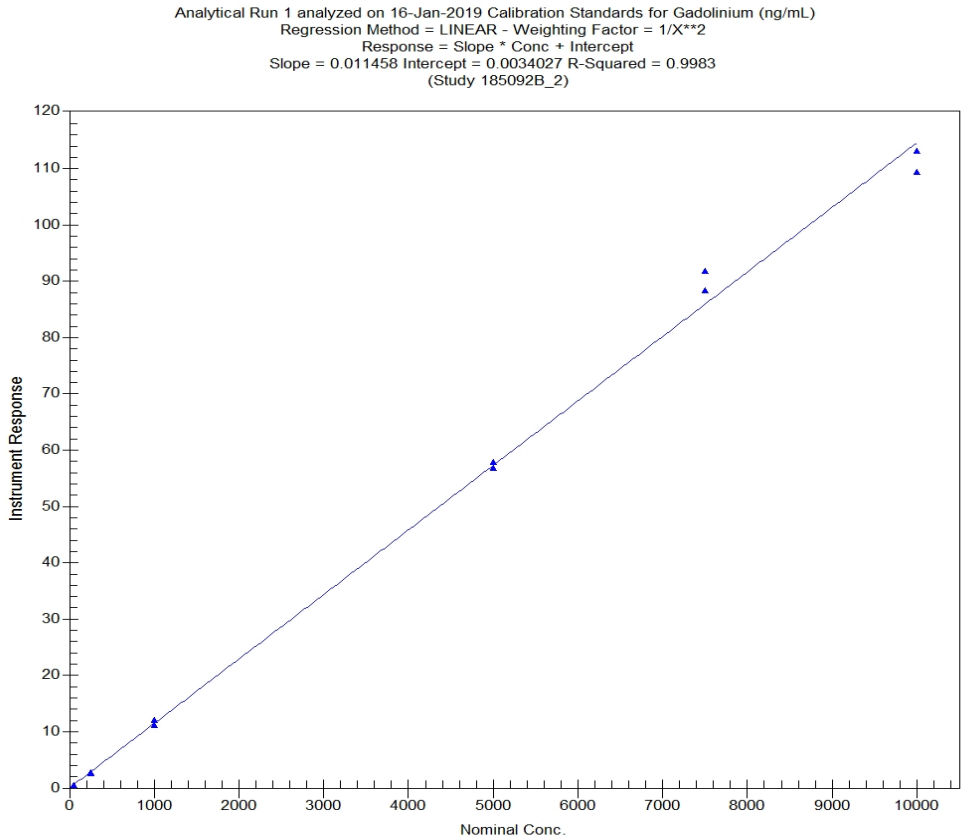
Mouse Calibration Line



Range 0.0318 – 63.6 µmol/L (5-10,000 ng/mL)

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Mouse (QC) vs Mouse (Cal)

- Precision & Accuracy Data at Robustness

	LLOQ 0.0318 µmol/L	LOW 0.0636 µmol/L	LOW MID 1.59 µmol/L	MID 31.8 µmol/L	HIGH 47.7 µmol/L
Mean	0.0323	0.0670	1.58	32.6	47.8
S.D.	0.00183	0.00256	0.0349	0.981	0.731
%CV	5.67	3.82	2.21	3.01	1.53
%Bias	1.58	5.36	-0.618	2.53	0.221
n	6	6	6	6	6

- ☺ Acceptable A&P

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Mouse (QC) vs Mouse (Cal)

- Autosampler carryover

	AC-LLOQ_1 0.0318 µmol/L	AC-LLOQ_2 0.0318 µmol/L	AC-ULOQ 63.6 µmol/L	Carryover (%)
Mean	0.0334	0.0318	59.9	4.98
S.D.	0.00126	0.000883	1.45	
RSD(%)	3.78	2.78	2.42	
Accuracy	105	99.8	94.2	
n	6	6	6	

- ☺ Acceptable carry-over

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Mouse (QC) vs Mouse (Cal)

- Suitability for other mouse tissues

	Brain QCL 0.0636 µmol/L	Femur QCL 0.0636 µmol/L	Kidney QCL 0.0636 µmol/L	Skin QCL 0.0636 µmol/L	Spleen QCL 0.0636 µmol/L	Femur QCH 47.7 µmol/L	Brain QCH 47.8 µmol/L	Kidney QCH 47.8 µmol/L	Skin QCH 47.8 µmol/L	Spleen QCH 47.8 µmol/L
Mean	0.0630	0.0646	0.0634	0.0678	0.0632	48.7	48.5	48.6	52.1	48.9
S.D.	0.00248	0.00386	0.00245	0.00258	0.00334	0.895	1.34	1.33	1.92	1.31
%CV	3.94	5.98	3.86	3.81	5.29	1.84	2.76	2.74	3.69	2.68
%Bias	-0.933	1.58	-0.304	6.62	-0.618	2.11	1.55	1.76	9.09	2.39
n	12	12	12	12	12	12	12	12	12	12

- ☺ Acceptable A & P

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NHP (QC) vs Mouse (Cal)

- Precision & Accuracy Data

	LLOQ 0.0318 µmol/L	LOW 0.0636 µmol/L	LOW MID 1.59 µmol/L	MID 31.8 µmol/L	HIGH 47.7 µmol/L
Mean	0.0417	0.0619	1.56	34.0	51.3
S.D.	0.00220	0.00294	0.0247	0.619	0.960
%CV	5.27	3.30	1.58	1.82	1.87
%Bias	31.2*	-2.60	-1.60	6.80	7.47
N	6	6	6	6	6

* Confirmed on repeat

- ☺ Acceptable A&P except LLOQ ☹

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NHP (QC) vs NHP (CAL)

- Precision & Accuracy Data

	A/P LLOQ 0.0318 µmol/L	A/P LOW 0.0636 µmol/L	A/P LOW MID 1.59 µmol/L	A/P MID 31.8 µmol/L	A/P HIGH 47.7 µmol/L
Mean	0.0341	0.0625	1.56	32.2	48.8
S.D.	0.00281	0.00260	0.0424	0.890	0.988
%CV	8.24	4.16	2.72	2.76	2.03
%Bias	7.25	-1.72	-1.88	1.27	2.32
n	6	6	6	6	6

- ☺Acceptable A&P

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SUMMARY

- Multiple mouse tissues scientifically validated using liver as primary tissue and qualifying other tissues against liver calibration line
- Accuracy and precision was achieved at 4-6-15 limits
- Cross species validation with NHP liver, acceptable at all levels except at LLOQ
- Need to further investigate but can adjust calibration range if consider NHP tissue is contributing to the deviation
- This approach to validation will considerably reduce validation efforts thereby benefiting ethical considerations, time and cost savings

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Arcinova Team

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Questions ?