SDC2: Bioanalytical methods

The bioanalytical data presented were obtained by inductively coupled plasma mass spectrometry (ICP-MS) of total gadolinium (Gd). Analyses and method validation were conducted in compliance with the relevant US and EU guidelines[1,2]. The performance parameters for the bioanalytical methods used are shown in Table S1 and Table S2. As there is no known metabolic degradation of gadoquatrane, total Gd concentrations represent unchanged gadoquatrane.

For samples collected in the main part of the study, the same ICP-MS method was used as in prior clinical studies with gadoquatrane[3,4] ("Method 1"). For samples collected 1, 3, and 6 months after the injection – these samples contained only trace amounts of Gd – this method was slightly modified ("Method 2") to increase its sensitivity. The major difference between the two methods is the increased sample volume as well as the decreased sample digestion volume. Together, the two methods cover the complete concentration range of interest. A successful cross-validation of both methods was performed using one concentration level in the overlapping concentration area of the working range with six replicates on three separate occasions..

Gd was determined in 573 plasma samples after digestion in 1% TMAH/1% EDTA containing europium internal standard followed by ICP-MS detection using an Agilent 7700x instrument. Plasma and urine were stored at or below -20 °C and analyzed within 397 days (plasma) and 384 days (urine) after sample collection.

Table S1: Performance of ICP-MS methods for the quantification of gadolinium in human plasma

Calibration standards	**Method 1**	**Method 2**
Calibration range (LLOQ to ULOQ)	0.0318 to 317.97 µmol Gd/L	0.000636 to 0.1272 µmol Gd/L
Mean inter-assay accuracy of back-calculated concentrations (except LLOQ)	-2.12% to 1.11%	-1.56% to 1.26%
Precision (except LLOQ)	≤6.53%	≤6.25%.
Accuracy at the LLOQ	-0.618%	16.7%
Precision at the LLOQ	9.18%	14.3%
Quality control samples		
Concentration range	0.0636 to 254.37 µmol Gd/L	0.0016 to 0.0954 µmol Gd/L
Accuracy	-4.43% to -1.76%	-5.65% to 7.56%
Precision	5.56% to 29.4%	5.49% to 7.31%.

Abbreviations: ICP-MS, inductively coupled plasma mass spectrometry; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.

Table S2: Performance of ICP-MS methods for the quantification of gadolinium in human urine

Calibration standards	**Method 1**	**Method 2**
Calibration range (LLOQ to ULOQ)	0.0636 to 317.97 µmol Gd/L	0.000636 to 0.1272 µmol Gd/L
Mean inter-assay accuracy of back-calculated concentrations (except LLOQ)	-2.06% to 3.71%	-1.25% to 1.27%
Precision (except LLOQ)	≤7.14%	≤6.35%.
Accuracy at the LLOQ	-2.51%	-5.65%
Precision at the LLOQ	10.5%,	16.7%,
Quality control samples		
Concentration range	0.1272 to 254.37 µmol Gd/L	0.0016 to 0.0954 µmol Gd/L
Accuracy	-0.912% to 3.00%	0.640% to 1.58%
Precision	9.95% to 14.8%.	1.35% to 12.5%.

Abbreviations: ICP-MS, inductively coupled plasma mass spectrometry; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.

References:

1. European Medicines Agency (EMA). Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2**. 2011. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Accessed May 8, 2024.
2. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). Bioanalytical method validation - guidance for industry: May 2018, Biopharmaceutics. 2018. Available at: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Accessed May 8, 2024.
3. He X, Matsuki S, Li K, et al. Pharmacokinetics, safety, and tolerability of the novel tetrameric gadolinium-based MRI contrast agent gadoquatrane in healthy Chinese and Japanese men: Two randomized dose-escalation studies including concentration-QTc modeling. *Eur J Pharm Sci*. 2024:106749.
4. Hofmann BM, Riecke K, Klein S, et al. Pharmacokinetics, safety, and tolerability of the novel tetrameric, high-relaxivity, macrocyclic gadolinium-based contrast agent gadoquatrane in healthy adults. *Invest Radiol*. 2024;59(2):140-149.