QPS IS YOUR LINK.

# Gadolinium (Gd) in plasma and urine: An improved highly accurate and precise ICP-MS method for supporting clinical studies with MRI contrast agents and formulations.

**Fred van Heuveln, Henri Meijering and Mark Giezen**
**QPS Netherlands B.V., Petrus Campersingel 123, 9713 AG Groningen, The Netherlands**
**Correspondence: fred.van.heuveln@qps.com TEL: +31 (0)50 3681011**

## INTRODUCTION

Gadolinium (Gd) based contrast agents (GBCA's) are widely used in MRI applications, because of the large paramagnetic moment of Gd. Since free Gadolinium ($Gd^{3+}$) may lead to serious side effects, i.e. nephrogenic systemic fibrosis (NSF), Gd is commonly administered as a chelating complex, e.g. Gd-DPTA. Recently, it is however recognized that all Gd contrast agents are still potentially toxic, because they degrade in-vivo into toxic free $Gd^{3+}$. Toxicity is determined by the amount of free $Gd^{3+}$ that is formed in vivo and thus by the stability properties of the Gd-complex in-vivo.
For an accurate and precise measurement of clinical samples to support the development of new stable contrast agents and formulations, a highly accurate and precise bioanalytical assay is required.

## OBJECTIVE

1) To develop a highly accurate and precise bioanalytical assay with a 5% criterion instead of the generally applied 15% criterion on precision and accuracy .
2) To develop an LC-ICP-MS application to enable the simultaneous analysis of free and complex bound Gd in one analytical run.

Objective 1 is presented in this poster, objective 2 is currently under investigation.

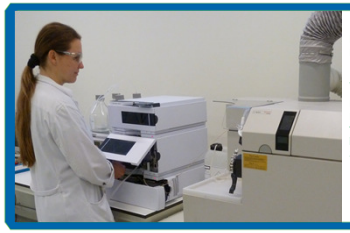


**Figure 1:**
**ICP-MS at QPS Netherlands B.V.**
**Right: Agilent 7500c ICP-MS with collision cell**
**Left: Agilent HPLC used for LC-ICP-MS. For ICP-MS only the LC autosampler is used in combination with 96 or 384 well plates .**

## EXPERIMENTAL

Gd reference standard was used for preparation of calibration and validation samples. 5µL sample and 15µL Eu (IS) were simultaneously transferred by an Agilent 1200 series autosampler, mixed and injected into the Agilent 7500C series ICP-MS using continuous flow injection (FIA), see fig 2.
Conditions are:

- mobile phase 5mM tetrabutyl-ammonium bisulfate
- flow rate 1mL/min
- ICP-MS tuned using a Gd/Eu solution (CFA, see upper part fig 2)
- Concentric nebulizer

The monitored Gd and Eu (IS) isotopes were m/z = 155, 156, 157, 158 and 160 for Gd and Eu m/z= 151 and 153, respectively.
Data acquisition was performed using Chemstation software and peak integration using Analyst software.

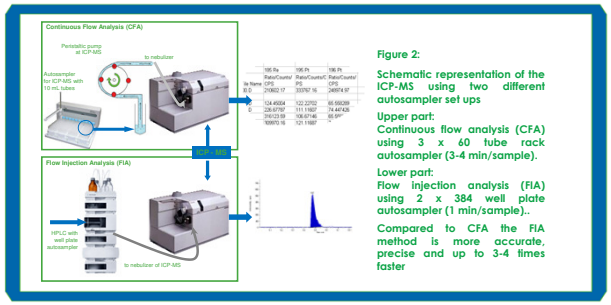



**Figure 2:**
**Schematic representation of the ICP-MS using two different autosampler set ups**
**Upper part:**
**Continuous flow analysis (CFA) using 3 x 60 tube rack autosampler (3-4 min/sample).**
**Lower part:**
**Flow injection analysis (FIA) using 2 x 384 well plate autosampler (1 min/sample)..**
**Compared to CFA the FIA method is more accurate, precise and up to 3-4 times faster**

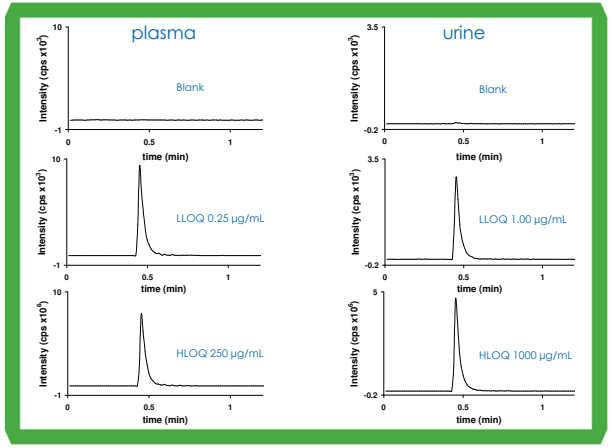
## VALIDATION SET UP

Validation of the method for the determination of total gadolinium in plasma was performed according to the EMEA / FDA guidelines.

| | |
|---|---|
| Validation design | 3 batches in 5-fold (precision and accuracy) |
| Precision (CV%) | ≤5% for LLOQ, QClow, QCmed & QChigh (n=15). |
| Accuracy (bias%) | ≤5% for LLOQ, QClow, QCmed & QChigh (n=15). |

Calibration curves, response function, selectivity, stability, dilution of samples, carry-over and matrix effect: validated according to standard criteria of international guidance (EMEA/FDA).

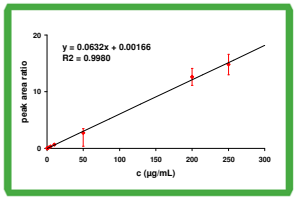
## RESULTS



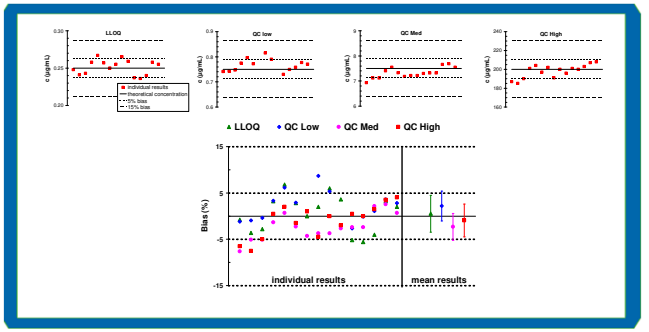

The validation results in plasma:

Selectivity: the method was selective for the determination of Gd.

Calibration curves: linear model with weighting factor 1/xx.




Precision and accuracy: the CV% of the Gd method was ≤4.0% at all concentrations. The bias was between -2.3% and 0.5%




Freezing and thawing stability: 4 freezing and thawing cycles without affecting the results
Stability of sample extracts: ambient temperature for 6 days
Bench-top stability: samples stable ambient temperature for 24 hours
Long-term stability: samples stable 4 months at -20 °C and 3 months at -70 °C, data after longer storage periods will be added
Dilution: samples can be 10-fold diluted with human plasma
Carry over: no carry over was observed during the assay
Matrix effect: no significant matrix effect was observed

*The validation of Gd in human urine is currently ongoing*

## CONCLUSIONS

An assay was developed for the determination of Gd in human plasma and urine samples with precision and accuracy <5% instead of the generally applied <15% criterion. This high precision and accuracy makes the method exceedingly suitable for application to clinical studies with Gd in contrast agents.

The excellent precision and accuracy was obtained by optimized ICP-MS parameters and conditions and by adding the internal standard through the Agilent autosampler instead of pipetting by hand.

Simultaneously determination (speciation) of free and complex bound Gd by LC-ICP-MS is currently under investigation.