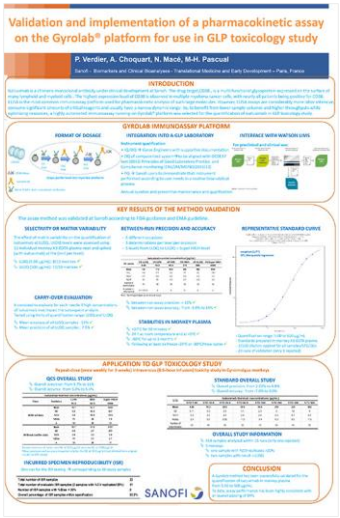
GYROS PROTEIN Technologies

# Validation and implementation of a pharmacokinetic assay on the Gyrolab™ platform for use in GLP toxicology study

## *Case Study*




*Case study based on a poster by P. Verdier, A Choquart, N. Macé, and M-H Pascual, Biomarkers and Clinical Bioanalyses, Translational Medicine and Early Development, Sanofi, Paris, France. The poster was presented at the European Gyrolab Seminar in Paris, June 2017.*

**Assay developers at Sanofi needed an alternative to ELISA for PK analysis of their monoclonal antibody isatuximab that was less labor intensive, consumed less reagents and samples, and provided a broader dynamic range. They therefore decided to develop and test a Gyrolab™ assay, which was validated for the quantification of isatuximab in monkey plasma, with a dynamic range of 5–500 µg/mL. Assay performance in a GLP toxicology study carried out in cynomolgus monkeys was highly consistent, with an overall pass rate of 94%.**

### Isatuximab for targeting multiple myeloma

Isatuximab, a chimeric monoclonal antibody under clinical development at Sanofi, targets CD38, which is a multi-functional cell-surface glycoprotein expressed in many lymphoid and myeloid cells. CD38 is highly expressed in multiple myeloma cancer cells, with nearly all patients being positive for CD38.

### Integration of Gyrolab system and development of a Gyrolab assay

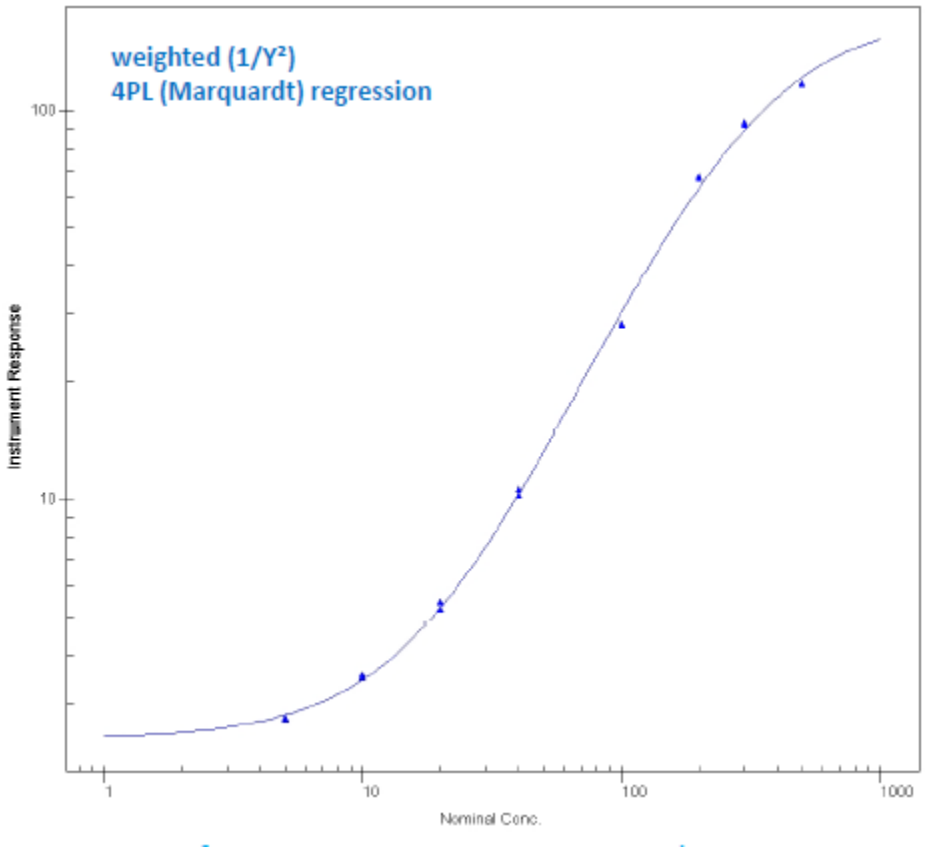
The Gyrolab system was integrated into their GLP laboratory through a rigorous procedure that included:

- IQ/OQ performed by Gyros Protein Technologies' service engineers,
- OQ of the computerized system to be aligned with OECD Number 17 Advisory Document, and
- PQ involving demonstration by Sanofi users that the instrument performed according to their needs in a routine bioanalytical process

The assay employed a biotinylated rabbit anti-isatuximab antibody as capture reagent and CD38 protein labeled with Alexa Fluor® 647 as detection reagent.

## Assay performance

Broad dynamic range




*Standards prepared in monkey K3-EDTA plasma showed that the assay provided a quantification range of 5–500 µg/mL. All samples, standards and QCs were diluted 1:100.*

Low matrix effect

To assess the effect of matrix variability, the assay was used to measure isatuximab at the Lower and Upper Limits of Quantitation (LLOQ and ULOQ, resp.) using ten individual monkey K3-EDTA plasma neat and spiked with isatuximab.
The assay passed the test, with 8/10 samples passing at LLOQ (5 µg/mL) and 10/10 at ULOQ (500 µg/mL).

No significant carryover

Testing indicated that carryover was minimal, with mean accuracy of all LLOQ samples of 15% and mean precision of 7.5%.

High accuracy and precision between runs

The assay was tested on six occasions, each including three determinations of six concentration levels, from LLOQ to ULOQ, and also a super high level. The between-run precision was < 10% and the between-run accuracy was in the range -3.6–16%.

Good stability

The samples were stable for 30 minutes at +37 °C, 24 h at room temperature and +5 °C, 3 months at -80 °C, and after six -20 °C or -80 °C freeze/thaw cycles.

Successful application to GLP toxicology study

The validated assay was used to analyze samples taken in a toxicity study involving three weekly intravenous doses. The overall precision was determined to be 3.7–11% and the overall accuracy, 5–5.4%. Standards over the dynamic range gave precision of 2.0–4.8% and accuracy of -7.6–8.0%.

Incurred specimen reproducibility (ISR) judged from one run of 33 samples was 93.4% and of the 414 samples analyzed during a total of 16 runs, only one was rejected. Only three re-assays were required, with one sample giving CV >20% for replicates and two samples with results over the ULOQ.