A New Method for the Determination of Total Serum Iron (TSI) for Pharmacokinetics (PK) and Bioequivalence (BE) Assessment of Ferumoxytol Formulations in Human

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Purpose
The PK and BE assessment of Ferumoxytol is a complex process due to the ubiquity of endogenous iron and the complexity of its metabolism and specific physicochemical properties. A spectrophotometric method was developed and validated to specifically measure TSI in human serum for PK and BE assessment.

Methods
First, iron is gently released from both Ferumoxytol carbohydrate protective shell and transferrin, the primary iron transport protein in the systemic circulation, by acid treatment. Conditions were optimized to obtain ~100% recovery of iron. At this stage, the plate is read at 595 nm to determine background noise of the assay. Then, a spectrophotometric reagent is added and the resulting mixture is incubated before the reagent/iron complex is measured at 595 nm. The final response is background-subtracted prior to performing data regression. Selectivity, precision and accuracy, freeze-thaw, bench-top, whole blood and long-term stabilities, dilutional linearity validation tests were successfully performed and clinical samples analyzed to ascertain the overall performance of the assay.

Results
Endogenous iron levels were determined in individual and in pooled serum lots prior to prepare quality control samples. Serum iron concentrations were back-calculated from a saline calibration curve over the 50 to 2,500 µg/dL range with a linear drug/response relationship (y=a*x + b; 1/y2 weighing; slope % CV= 1.1, N=6). Between-run precision and accuracy provided excellent % CV (1.0 - 6.7), % Bias (94.6 – 104.4) and % total error (2.5 – 12.1). Stabilities were successfully assessed: 2.4 hrs in whole blood at room temperature (rt), 19.4 hr bench-top at rt, 3 freeze-thaw cycles at -20°C and 31 days long-term at -20°C, as well as dilutional linearity (5X, 20X and 40 X). In-study validation data showed that 98% of incurred sample reanalysis results meet acceptance criteria with 87.2% showing a % difference lower than ±5% with original values.

Conclusion
This assay is highly precise, accurate and robust and meets regulated bioanalysis method validation requirements. It has been used for PK or BE assessment of Ferumoxytol intra-venous formulations in human.