SPECTROPHOTOMETRIC DETERMINATION OF TOTAL IRON AND TRANSFERRIN-BOUND IRON IN HUMAN SERUM

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Intravenous iron based formulations are indicated for the treatment of severe iron deficiency anaemia. Bioequivalence studies of these formulations are complicated because of the endogenous iron level and the need to independently measure total iron and transferrin bound iron (TBI). In the present study, a selective method has been developed and validated, for use in bioequivalence studies of intravenous iron-sucrose formulations, according to the guidelines of European Medicines Agency (EMEA) and U.S. Food and Drug Administration (FDA).

For the determination of total iron, a colorimetric assay method is employed. Serum samples are diluted with saline prior to treatment with a commercial chromagen kit, consisting of reagents R1 and R2. The R1 acid dissociating reagent liberates iron from iron-sucrose and transferrin, and subsequently the R2 color reagent reacts with iron to form a blue complex. All sample pretreatment steps are performed using a robotic liquid handling system. The absorbance of the produced blue complex is measured utilizing a microplate reader-spectrophotometer with the quantitation wavelength set at 604 nm. In order to determine TBI, the diluted serum samples are initially filtered through a SPE polyimine column, to remove free and sucrose-bound iron, and the effluent is analyzed according to the procedure employed for total iron.

For the quantitation of total iron, a calibration curve is obtained (0.25 - 30.0 µg·mL⁻¹, plus endogenous iron) by using spiked serum samples and taking into account endogenous total iron levels previously determined. In a similar way, for the quantitation of TBI, a calibration curve is obtained (0.25 - 8.0 µg·mL⁻¹, plus endogenous iron). Acceptable precision and accuracy values were obtained for concentrations covering the entire calibration curve range of 0.25 - 30.0 µg·mL⁻¹ and 0.25 – 8.0 µg·mL⁻¹ for total iron and TBI respectively. The stability and dilution integrity of the samples were also found to be within the pre-defined specifications.

KEYWORDS: microplate reader-spectrophotometer, iron-sucrose, transferrin bound iron, human serum, chromagen kit, robotic liquid handling system