A Novel Approach for Quantitation of Total Iron and Transferrin Bound Iron in Human serum samples by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

Formulation : Ferric Carboxymaltose (750mg/15mL) Injectable / intravenous

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Overview

Intravenous iron therapy is indicated for the treatment of iron deficiency anemia in several patient populations, especially when a large loss of blood occurs, such as in haemodialysis patients with chronic kidney disease, or when oral iron–supplementation is ineffective.

Physiologically, iron–carbohydrate (iron–sucrose, iron-carboxymaltose, –gluconate or –dextran) therapies replenish depleted iron stores as iron is released from the carbohydrate complex. Once released from the dosage form, iron is taken up by plasma transferrin proteins that circulate and distribute iron throughout the body.

Pharmacokinetic studies of intravenous iron–sucrose are complicated by background cir¬culating iron levels as well as the desire to differentiate and independently monitor iron–sucrose and transferrin-bound iron (TBI). Prior to the administration of iron–formulation, most circulating iron is in the form of TBI; following intravenous iron–formulation therapy, circulating iron is found both as a component of the dosed formulation and complex to transferrin.

Purpose

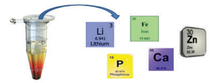
To Develop and validate the reliable method for determination of Total Iron (TOI) and Transferrin-Bound Iron (TBI) in Human Serum Samples by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The purpose of this study was to develop a robust and accurate method for the analysis of total iron and TBI to generate pharmacokinetic data as a component of bioequivalence studies for generic iv. iron formulations.

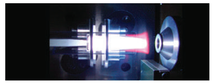
Why ICP OES (Inductively Coupled Plasma Optical Emission Spectroscopy):

For decades ICP has been used in support of Environmental Protection Agency analyses and has more recently been applied for use in the pharmaceutical industry.

Increasingly2, ICP–MS and ICP–OES methods of analysis are being used to quantify the concentration of elements contained in pharmaceutical compounds and excipients used in nonclinical and clinical studies and in forensic investigations. Validation of methods for quantification of elements in biomatrices such as whole blood, serum, plasma, urine may be performed following GLP predicate rules and bioanalytical guidances, as long as the amount of endogenous element in the matrix is determined during method development and properly accounted for during method validation.

Elemental analysis plays a role in Bio-analysis when the element is derived from the pharmaceutical being administered. Some examples of these applications are presented below:
Platinum (Pt) is a component of cisplatin, carboplatin and oxiplatin: all used for oncology treatments





(1) Iron (Fe) from Iron Sucrose, Ferumoxytol, Ferric Carboxymaltose, Sodium Ferric Gluconate and Iron Dextran

(2) Zinc (Zn) acetate for the treatment of as maintenance treatment in Wilson's disease.

(3) Lithium (Li) Carbonate indicated in the treatment of manic episodes of manic-depressive illness.

(4) Potassium (K) chloride For the treatment of patients with hypokalaemia.

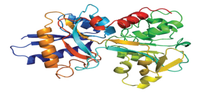
Methodology

An exclusive Emission Spectroscopy method was used to reliably measure total iron and TBI following Ferric Carboxymaltose administration. A validation was conducted in accordance with the method validation protocol in compliance with the regulatory requirements.

Due to its Endogenous presence; analyte free matrix is difficult to obtain. Also, the Endogenous presence varies in individuals. During method development, the measure Intensity of Endogenous drug present in serum matrix (obtain in Serum Blank samples) is subtracted from the calibration curve standards to construct the calibration curve and validate the curve.

Solid-phase sample processing allowed the measurement of TBI. Circulating iron–formulation could then be calculated as the difference between total iron and TBI. For the analysis of total iron (the sum of free iron, TBI and iron–associated with formulation) extensive method development was not required.

Transferrin



The predominant iron-binding plasma glycoprotein regulating and distributing circulating physiological levels of iron.

The measurement of TBI requires separating and excluding free iron and drug-derived iron from TBI. Specifically, we encountered difficulty excluding free and drug-bound iron for the determination of TBI. SPE columns were evaluated for their ability to remove free and sucrose-bound iron from serum. Finally polyimine extraction cartridges are finalized for accurate determination of TBI.

Sample Preparation TOI & TBI:

Calibration curve standards, Quality control samples and unknown samples (0.100mL for TOI and 0.050mL for TBI) were filled with internal standard (0.100mL) of Titanium and finally diluted with milli-q water as per pre-define procedure.

The measurement of TBI requires separating and excluding free iron and drug-derived iron from TBI. Hence, TBI is separated by applying Solid-phase extraction with the usage of polyimine extraction cartridges. These cartridges were evaluated for their ability to remove free and drug bound iron from serum during method development and validation also.

Arrange the final prepared samples in auto-sampler and acquired by applying pre-define equipment parameters.

Method Summary TOI & TBI

Analytical Technique	Inductively coupled Plasma-Optical Emission Spectroscopy
ICP-OES	PerkinElmer Optima 8000
Auto-sampler	PerkinElmer S10 Auto-sampler
Software used	Syngestix software Version 4.5 (for analysis) and WATSON LIMS 7.3 for final regression
Iron (Fe) wavelength	259.946nm
Titanium (Ti) wavelength	334.940nm
Nebulizer	Gem Cone Low flow nebulizer
Spray Chamber	Cyclonic
Sample Flow Rate	1.20mL/min
Biological Matrix	Human Serum
Internal Standard	Titanium Standard for ICP
Quantification	Measured Peak Intensity
Regression & Equation	Linear, y = ax + b
Weighting Factor	1/X2

	TOI (µg/mL)	TBI (µg/mL)
Sample Processing Volume	**0.100mL**	**0.050mL**
Linearity Range	**0.400 - 800.000**	**0.400 - 50.000**
Validated LLOQ	0.400	0.400
Validated LLOQ QC	0.400	0.400
Validated LQC	1.200	1.200
Validated MQC	240.000	15.000
Validated HQC	600.000	37.500
Validated ULOQ	800.000	50.000
Validated AUL QC	1600.000	100.000

Stability Experiment Details TOI & TBI:

	TOI (µg/mL)	TBI (µg/mL)
Stability of Extract (SE) at Ambient Temperature	46 hours at Ambient Temperature	70 hours at Ambient Temperature
Freeze Thaw Stability (FT)	5 Cycles at freezing temperature of -20±5 °C and -78±8 °C	5 Cycles at freezing temperature of -20±5 °C and -78±8 °C
Bench Top Stability(BT)	13 hours at ambient temperature	14 hours at ambient temperature
Autosampler Re-Injection Reproducibility (ASRR)	47 hours at ambient temperature	69 hours at ambient temperature
Long Term Stability in Matrix (LTM)	50 Days at -20±5 °C and -78±8 °C	60 Days at -20±5 °C and -78±8 °C
Batch Size Experiment (BSE)	Total 154 samples including CC & QCs	Total 156 samples including CC & QCs
Dilution Integrity (DI)	DQC: 1614.973 µg/mL, 10 Fold Dilution	DQC: 117.057 µg/mL, 10 Fold Dilution

Extraction Cartridges Efficiency Experiment

Formulation spiked QCs were prepared at Higher (HQC) and Lower (LQC) QCs level to demonstrate the efficiency of extraction cartridges. Final spiked concentration of formulation is 300.000µg/mL in blank serum matrix.

Six replicates of each i.e. FCM HQC and FCM LQC were processed and evaluated for its acceptance.

ECEE	FCM HQC	FCM LQC
% Mean Accuracy	106.26	102.52
Precision (%CV)	1.23	2.20
Bench Top Stability (BT)		
% Mean Accuracy	98.97	103.57
Precision (%CV)	5.66	5.12
Freeze Thaw Stability (FT)		
% Mean Accuracy	87.74	101.77
Precision (%CV)	3.60	8.94

Conclusion

- Overall, validated methods are robust, reproducible and in compliance to regulatory requirement for endogenous molecules.
- Validated methods are ready for its application for upcoming study of Ferinject® [Ferric carboxymaltose solution for injection/infusion (50 mg iron/mL)]
- We have experience of Iron sucrose (injection 100mg/5mL) pivotal study, where the scope of study is US submission.

References

1. Melissa M et.al, Analysis of total and transferrin-bound iron in human serum for pharmacokinetic studies of iron–sucrose formulations, Bioanalysis (2011) 3(16), 1837–1846
2. Jennifer A et.al, Technical aspects of inductively coupled plasma bioanalysis techniques, Bioanalysis (2013) 5(15), 1831–1841