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

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Background:

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[1], iron–carbohydrate (iron–sucrose, –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–sucrose, most circulating iron is in the form of TBI; following intravenous iron–sucrose therapy, circulating iron is found both as a component of the dosed formulation and complexed to transferrin.

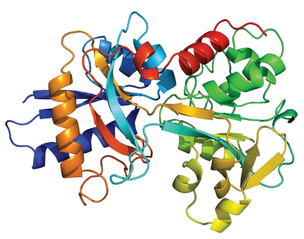
Purpose:

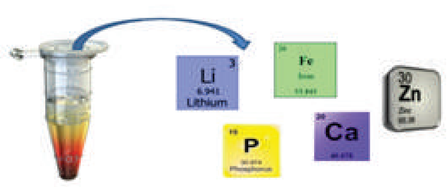
To Develop and validate the reliable method for determination of Total Iron (TOI) and Transferrin-Bound Iron (TBI) in Human Serum Samples by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The purpose of this study was to develop a robust and accurate method for the analysis of Total Iron (TOI) and Transferrin-Bound Iron (TBI) to generate pharmacokinetic data as a component of bioequivalence studies for iron–sucrose administration. A validation was conducted in accordance with the method validation protocol in compliance with the regulatory requirements.

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

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

Increasingly[2], 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:

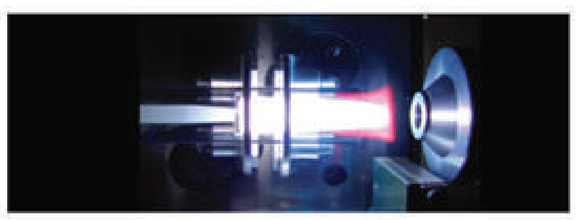
Platinum (Pt) is a component of cisplatin, carboplatin and oxiplatin: all used for oncology treatments

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

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

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

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 iron–sucrose administration. A validation was conducted in accordance with the method validation protocol in compliance with the regulatory requirements.

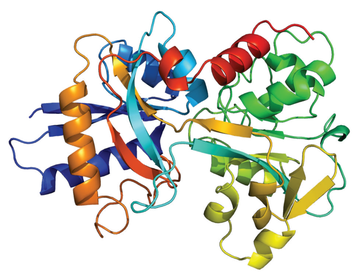
Due to its Endogenous presence; analyte free matrix is difficult to obtain. Also, the Endogenous presence varies in individuals. During method development, the measure Intensity of Endogenous drug present in serum matrix (obtain in Serum Blank samples) is subtracted from the calibration curve standards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

Solid-phase sample processing allowed the measurement of TBI. Circulating iron–sucrose 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–sucrose) 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 sucrose-bound iron for the determination of TBI. SPE columns were evaluated for their ability to remove free and sucrose-bound iron from serum. Method development concludes polyimine extraction cartridges are accurate for determination of TBI.



Sample Preparation TOI & TBI:

Calibration curve standards, Quality control samples and unknown samples (0.200mL 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 sucrose 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 No 1.0 (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	**TBI**
Sample Processing Volume	0.200mL	0.050mL
Linearity Range	0.500-60.000µg/mL	0.500 – 16.000µg/mL
Validated LLOQ	0.500 µg/mL	0.500 µg/mL
Validated LLOQ QC	1.224 µg/mL	1.970 µg/mL
Validated LQC	2.224 µg/mL	2.970 µg/mL
Validated MQC	20.724 µg/mL	6.470 µg/mL
Validated HQC	50.724 µg/mL	13.470 µg/mL
Validated AUL QC	167.397 µg/mL	87.390 µg/mL
Validated ULOQ	60.000 µg/mL	16.000 µg/mL

Stability Experiment Details TOI & TBI:

	TOI	**TBI**
Stability of Extract (SE) at Ambient Temperature	124 Hours at in Milli-Q - water	56 Hours at in Milli-Q - water
Freeze Thaw (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 (BT)	07 Hours at ambient temperature	09 Hours at ambient temperature
Auto-sampler Re-Injection Reproducibility	123 Hours at Ambient Temperature in Milli-Q Water.	69 Hours at Ambient Temperature in Milli-Q Water.
Long Term Stability of Drug in Matrix (LTM)	139 Days at -20±5°C and -78±8°C	245 Days at -20±5°C and -78±8°C
Batch Size Experiment	Total 141 samples including Calibration Curve	Total 132 samples including Calibration Curve
Dilution Integrity (DI)	10 fold, DQC: 167.397µg/mL	10 fold, DQC: 87.390µg/mL
	Amended endogenous concentration, (Dilution medium used - human Serum)	

Extraction Cartridges Efficiency Experiment:

Formulations (Iron Sucrose 100mg/5mL) spiked QCs were prepared at Higher (HQC) and Lower (LQC) QCs level to demonstrate the efficiency of extraction cartridges. Final spiked concentration of formulation was 60.0µg/mL in blank serum matrix.

These formulation spiked HQCs and LQCs (six replicates each) were processed and evaluated for its acceptance. Results are tabulated below.

	VNF LQC	**VNF HQC**
Precision (%CV)	2.09	2.55
% Mean Accuracy	105.75	103.68

Acceptance Criteria: %CV: within 15.0% and % Mean Accuracy: within ±15.00%.

Intra – Inter Precision and Accuracy TOI & TBI:

TOI	**Precision**			**% Bias**		
	RUN 1	RUN 2	RUN 3	RUN 1	RUN 2	RUN 3
Intra batch precision and % Bias (Accuracy) (HQC, MQC and LQC)	≤ 2.22	≤ 3.12	≤ 2.05	-4.65 to -2.79	-1.39 to 1.88	-2.04 to 0.40
Intra batch precision and % Bias (Accuracy) (LLOQ QC)	1.62	1.55	2.53	-4.25	-4.90	3.43
Inter batch precision and % Bias (Accuracy) (HQC, MQC and LQC)	≤ 3.30			-2.69 to -0.94		
Inter batch precision and % Bias (Accuracy) (LLOQ QC)	4.41			-1.88		

TBI	**Precision**			**% Bias**		
	RUN 1	RUN 2	RUN 3	RUN 1	RUN 2	RUN 3
Intra batch precision and % Bias (Accuracy) (HQC, MQC and LQC)	< 3.93	< 6.07	< 5.55	0.30 to 3.79	-1.82 to 2.44	0.13 to 3.36
Intra batch precision and % Bias (Accuracy) (LLOQ QC)	2.27	3.28	3.72	2.99	-0.91	-0.30
Inter batch precision and % Bias (Accuracy) (HQC, MQC and LQC)	< 4.77			-0.47 to 2.73		
Inter batch precision and % Bias (Accuracy) (LLOQ QC)	3.43			0.61		

Past Study Experience:

STUDY 01	**TOI**	**TBI**
Total No. of Sample Analyzed	6034	6034
Repeat Samples (%)	5.30%	6.56%
ISR Acceptance (%)	81.85%	86.14%

STUDY 02	**TOI**	**TBI**
Total No. of Sample Analyzed	2211	2211
Repeat Samples (%)	5.29 %	8.14 %
ISR Acceptance (%)	96.09%	94.78%

Conclusion:

Two separate Emission Spectroscopy assay has been developed to measure Total Iron (TOI) and Transferrin-Bound Iron (TBI) levels in human serum. Polyimine SPE columns effectively removed free iron and iron–sucrose from serum. Accuracy, precision and incurred sample reanalysis results indicate that the assay is reproducible and robust. The procedures have been applied to clinical analysis of pharmacokinetic studies of generic iv. iron formulations of iron–sucrose.

Reference:

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