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)

POSTER PRESENTATION at APA-2020, at Ahmedabad

Presented By:
Mr. Pritesh Contractor
Principal Scientist-Bioanalytical Research

Background

- Intravenous iron therapy is indicated for the treatment of iron deficiency.
- Several patient populations suffering from anemia
- Especially when a large loss of blood occurs
- Hemodialysis patients with chronic kidney disease
- When oral iron–supplementation is ineffective.
- Iron–carbohydrate (iron–sucrose, –gluconate or –dextran) therapies replenish depleted iron stores as iron is released from the carbohydrate complex

Challenges

- Pharmacokinetic studies of intravenous iron–sucrose are complicated by background circulating iron levels as well as the desire to differentiate and independently monitor iron–sucrose and transferrin-bound iron (TBI)
- Due to its Endogenous presence; analyte free matrix is difficult to obtain. Also, the Endogenous presence varies in individuals
- The measurement of TBI requires separating and excluding free iron and drug-derived iron from TBI.

Why ICP OES (Inductively Coupled Plasma Optical Emission Spectroscopy)

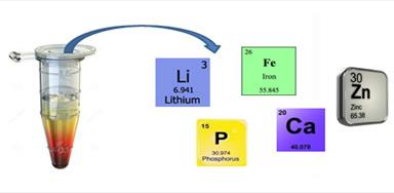
- ICP has been used in support of Environmental Protection Agency analyses and has more recently been applied for use in the pharmaceutical industry.

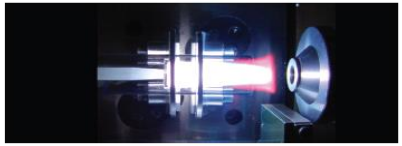
- ICP–MS and ICP–OES methods of analysis are being used to quantify the concentration of elements contained in pharmaceutical compounds and excipients used in nonclinical and clinical studies and in forensic investigations.

- 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.

ICP OES

- 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





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

Method Summary TOI & TBI

	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

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%.

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)	

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%