Quantification of Drug Bound Iron, Non-Transferrin Bound Iron, Transferrin Bound Iron, and Total Iron in Serum in a Rat Pharmacokinetic Study

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Introduction

Iron is a physiologically essential element absorbed from food regulated by the enzyme, hepcidin . After absorption, iron binds to the protein, transferrin, transports iron via the ciruclation from site of absorption to site of usage. Most iron is utilized for the production of heme, a component of the oxygen carrying molecule of the blood, hemoglobin. Intravenous colloidal iron products are used to treat iron deficiency anemia in serious chronic illness particularly those requiring dialysis. Under these conditions, transferrin becomes saturated, and non-transferrin bound iron (NTBI) appears in serum. NTBI is potentially harmful due to its ability to form free radicals, thereby inducing cellular toxicity. Hence, it is very useful to monitor the real "free iron" in circulating blood to assess the possibility of toxicity. Multiple challenges arise when attempting to accurately measure NTBI, transferrin bound iron (TBI), and drug bound iron (DI) from small sample volumes. 1) For NTBI, *Kolb et al* have compared several available literature methods, of which five were based on detection of iron chelators and one assay measured the redox-active iron using the bleomycin method. There is evidence with these methods that the measure of NTBI is directly proportional to the concentration of the chelating agent or in the case of bleomycin the time at assay conditions. The result with all of these methods is over estimation of NTBI. 2) Most reported analytical methods for determination of TBI use much larger sample volumes than available in the present study. 3) No bioanalytical method has previously been reported in the literature for measurement of DI. To support the rat pharmacokinetic study, an analytical method capable of measuring the levels of NTBI, TBI, DI and total iron (TI) in low sample volume was imperative.

Objective

- **Develop a sensitive and reproducible analytical method for simultaneous quantification of NTBI, TBI, DI and Total iron (TI) with minimal sample**

Principle and Methodology

- We adopted the ultra filtration method proposed by Kolb et al (2009) with modifications in elution solvent
- No chelating agents were included as all the chelating agents have the potential to pull the iron from colloidal formulation
- Ultra filtration is the function of molecular mass and increase in molecular mass of the free charged iron leads to improved recoveries in ultra filtration step, hence sodium chloride solution was chosen as alternative

$$\mathbf{Fe} + 2\ NaCl + 2\ H_2O = \mathbf{FeCl_2} + 2\ NaOH + H_2$$

- For TBI estimation, acidified saline was used to release iron bound to transferrin which occurs at or below pH 4
- For DI estimation, the sample was treated with sodium dithionite followed by saline elution. The sodium dithionite breaks the colloidal particles to release the iron from its polysaccharide shell

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Experimental

Rat pharmacokinetic study design:

- 12 Male SD rats were given colloidal iron products at two doses (12.5 & 40 mg/kg; n=6 animals at each dose)
- Blood samples were taken at 0, 0.08, 0.25, 0.50, 1, 2, 3, 4, 8, and 24h
- Blood centrifuged at 1000 g for 10 min @ 4˚C yielded serum

Extraction/Separation Procedure: Flow chart for the sample preparation described below

- Each fraction was used for the colorimetric method proposed by Kolb et al (2009)

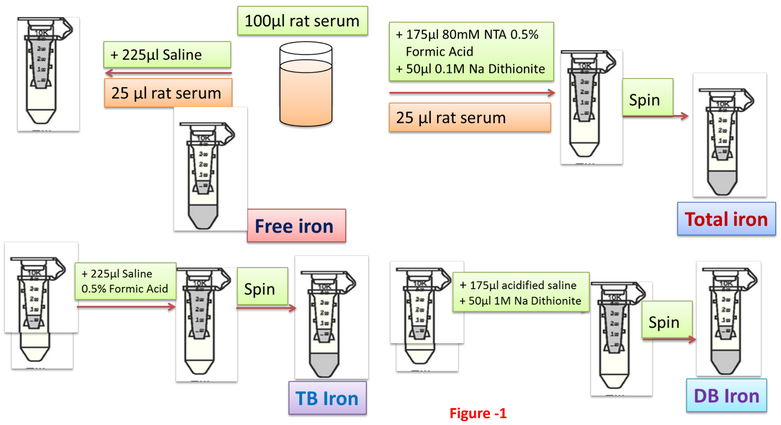


Figure -1

Results

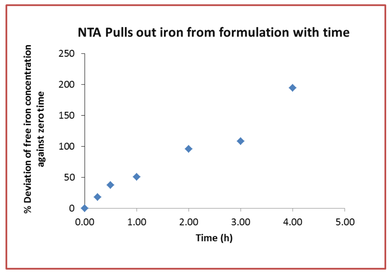
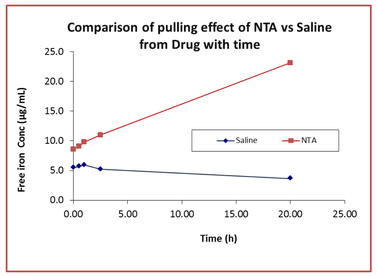


Figure -2



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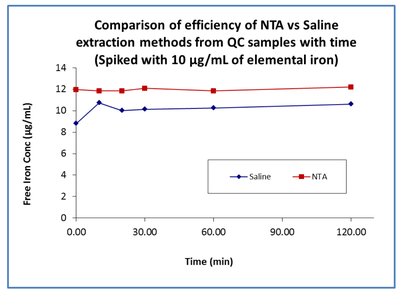

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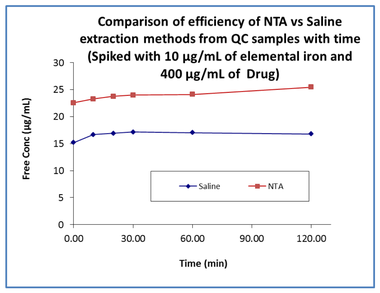


Figure -5

- NTA pulled out iron from the drug formulation over time (Fig-2)
- Saline did not acquire iron from drug for up to 24 h (Fig-3)
- Saline solution can be used as elution solvent for ultra filtration (Fig-4)
- Drug formulation contains free iron levels (1-2%) (Fig-5)

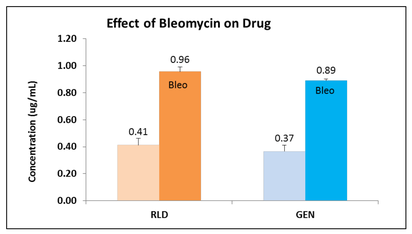


Figure -6

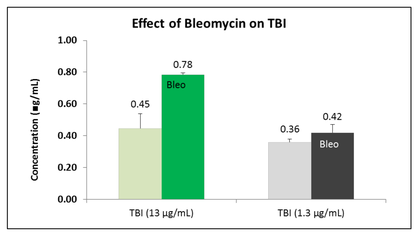


Figure -7

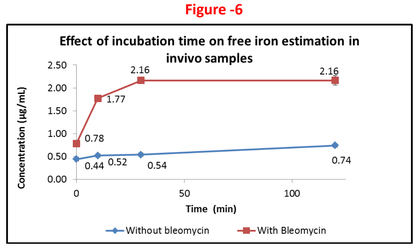
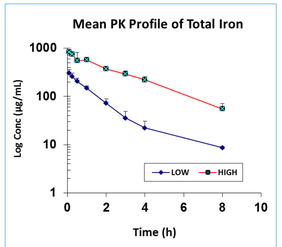
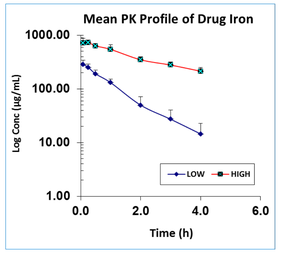
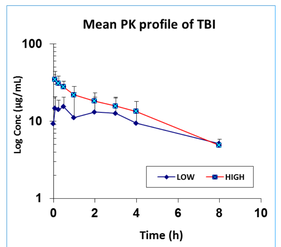


Figure -8

- Bleomycin pulls iron from the drug like other chelating agents (Fig-6)
- Bleomycin also has the potential to leach Iron from TBI (Fig-7)
- Bleomycin obtains iron from drug and TBI from in-vivo samples within 30 min
- The incubation temperature also causes leaching of free iron (Fig-8)







Parameter	40 mpk	12.5 mpk
Cl (mL/h/kg)	N/A	N/A
AUC (h*ug/mL)	2285.39 ± 329.99	405.10 ± 71.31
Vss (mL/kg)	N/A	N/A
MRT (h)	2.76 ± 0.51	1.28 ± 0.15

Parameter	40 mpk	12.5 mpk
Cl (mL/h/kg)	18.42 ± 3.03	35.67 ± 7.04
AUC (h*ug/mL)	2217.62 ± 341.00	363.32 ± 79.41
Vss (mL/kg)	52.88 ± 7.55	40.83 ± 5.87
t1/2 (h)	2.02 ± 0.35	0.81 ± 0.10

Parameter	40 mpk	12.5 mpk
Cl (mL/h/kg)	N/A	N/A
AUC (h*ug/mL)	116.72 ± 17.75	75.16 ± 28.64
Vss (mL/kg)	N/A	N/A
MRT (h)	4.26 ± 0.68	7.08 ± 3.21

Figure -9

Conclusions

- Bioanalytical method for simultaneous quantification of DI, NTBI, TBI and TI was developed and applied to a rat PK study
- The efficiency and reproducibility of the method was evaluated by analyzing individual components as well as mixed QC samples (NTBI+TBI+DI)
- The method was devoid of any chelating agent and gives confidence on measurement of free iron concentrations
- No circulating NTBI concentrations were observed at both doses
- The sensitivity of the method is as low as 0.6 µg/mL
- The sensitivity may improve significantly by switching to ICPMS detection that may require additional method development efforts

Acknowledgements

- Division of Applied Regulatory Sciences, OTS, CDER, FDA, Silver Spring, MD, USA
- Office of Generic Drugs, CDER, FDA, Silver Spring, MD, USA

References

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