International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor (2018): 7.426

Determination of Iron (III) and Iron (II) from Iron Sucrose Injection and Iron Polymaltose by Ion Chromatography

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Abstract: ***An accurate, simple, reproducible, and sensitive method for the estimation of Iron(III) and Iron(II) was developed and validated. Iron(III) and Iron(II) weres separated using chelation ion chromatography technique using isocratic elution with a flow rate of 1.2mL/min. Post column derivatization with 4-(2-pyridylazo) resorcinol (PAR) was carried out to form light abosrbing complex an detected at 530nm.The mobile phase include pyridine-2,6-dicarboxylate (PDCA) which acts as chelating agent and helps to retain Iron(III) and Iron(II) on mixed mode separator column. The linearlity of method has been tested in the range of 5.0mg/L to 15mg/L of Iron(III) and 0.5mg/L to 1.5mg/L of Iron(II) and correlation cofficient (R^2) was >0.999 for both Iron(III) and Iron(II). The method was shown excellent reproducible, linear, specific, sensitivity, rugged. The Limits of Detection and Quantification have been also established for Iron(III) as 0.05mg/L & 0.1mg/L and for Iron(II) as 0.3mg/L & 0.5mg/L respectively. Hence, the validated method is easy to adapt for regular analysis.***

Keywords: Iron Sucrose, Iron Polymaltose, Post Column Reagent, Iron Speciation, Ion Chromatography, Chelation, PDCA, PAR

1. Introduction

Iron deficiency anemia (IDA) is the most extensively accepted nutritional inadequacy in pregnancy. Prophylactic oral iron is prescribed during pregnancy to meet the stretched out prerequisite. In India, ladies end up pregnant with low standard hemoglobin level bringing about high occurrence of direct to extreme anemia in pregnancy where oral iron treatment can't meet the necessity. Pregnant ladies with moderate anemia are to be treated with parenteral iron treatment[1].

Iron Sucrose injection is an iron auxiliary item for consumption, is a tanned, sterile, aqueous, complex of polynuclear iron (III)-hydroxide in sucrose for intravenous use. Iron sucrose injection has a molecular weight of around 34,000 to 60,000 Daltons. Each mL of Iron Sucrose has 20 mg elemental iron as iron sucrose in water for injection. Similarly, Iron polymaltose is a water soluble, macro-molecular complex of iron (III) hydroxide and isomaltose. It is also used in the treatment of iron-deficiency anemia.

It is very important to determine Iron(II) in these intravenous injections as Iron(II) delays or make less probable delivery to the desired transferrin site. Also, it can readily binds to bacterial siderophores, which might leads to infection. It can cause damage to cellular constituents when it reacts with peroxides with generation of highly toxic reactive oxygen species [2].

USP monograph had given limit of 0.4% of Iron(II) in Iron Sucrose injections[3], but their provided polarographic method is highly cumbersome as it utilizes dropping mercury electrode. Extra care must be taken to protect environment from mercury.

Present study provides estimation of Iron(III) and Iron(II) from Iron Sucrose and Iron Polymaltose injections using Ion Chromatography with post column reaction and detection at 530nm using VWD detector having PEEK flow cell. Column utilized is IonPac CS5A which is a cation exchange column that allows determination of transition metals with short run time[4]. This technique provides sensitive determination of Transition Metals with ease of use and minimum operational cost.

2. Experimental

2.1 Reagents and Chemicals

All chemicals used for preparation of reagents, standards and mobile phase were of analytical grade. Ultrapure deionized water (18.2 MΩ cm, Milli-Q system) was used for the preparation of mobile phase and preparation of diluent, 0.1M HCl (Trace metal grade, Fisher Chemicals, P/N A508-P1) was used as diluent for preparation of standards and samples. Ferric Nitrate Nonahydrate (Merck, CAS 7782-61-8) was used for Iron(III) standard preparation and Ferrous Sulfate Heptahydrate (S D Fine Chemicals, P/N 20112 K05) was used for Iron(II) standard preparation. Other reagents and chemicals used for preparation of Eluent and Post Column reagent are as follows:

- Pyridine-2,6-dicarboxylic acid (PDCA), (Thermo Fisher Scientific Dionex, P/N 039671)
- Potassium Hydroxide (Merck, P/N 1.93103.0521)
- Potassium Sulfate (Merck, P/N 1.93249.0521)
- Formic Acid (Sigma Aldrich, CAS 64-18-6)
- 2-Dimethylaminoethanol (S D Fine Chemicals, P/N 38190 L05)
- Liquor Ammonia (Merck, P/N 1.93100.0521)
- Sodium Bicarbonate (Merck, P/N 1.93237.0521)
- 4-(2-pyridylazo) resorcinol (PAR) (Loba Chemie, CAS 16593-B1-0)

2.2 Apparatus

The equipment used was Thermo Fisher Dionex Ion Chromatograph ICS 5000+ having AS-AP Autosampler with a 50µL loop, IonPac CS5A column (4 x 250mm) and its guard (4 x 50mm) was used. The experiment was conducted using eluent mixture of 7.0mM PDCA, 66mM Potassium Hydroxide, 5.6mM Potassium Sulfate, 74mM Formic Acid. Post column reagent (PCR)consists of mixture solution of 0.12g/L PAR, 1.0M 2-Dimethylaminoethnaol, 0.5M Ammonium Hydroxide (Liquor Ammonia), 0.3M Sodium Bicarbonate. Eluent flow rate used was 1.2ml/min and PCR flow rate was kept at 0.6ml/min. Flow rate of PCR was adjusted pneumatically (Nitrogen Pressure) by PC-10 assembly (Thermo Fisher Scientific Dionex). Outlet of column and outlet of PC-10 assembly was connected to mixing tee and outlet of mixing tee was connected to 375µL reaction coil which was then further connected to VWD (UV-Visible) Detector. Software used for data acquisition was Thermo Fisher Scientific Dionex Chromeleon (version: 6.80 SR15). Chromatograms were monitored simultaneously during analysis.

2.3 Procedure

Preparation of Eluent: - 1.16g of PDCA, 3.7g of Potassium Hydroxide, 0.976g of Potassium Sulfate and 3.4ml of Formic acid was taken in 1000mL volumetric flask and 600mlg of Deionized Water (D. I. Water) was added and sonicated to dissolve. It was make up to mark with D.I. Water. It was then filtered through 0.2µ nylon membrane filter.

Preparation of Post Column Reagent (PCR):- 0.12g of 4-(2-pyridylazo) resorcinol, 40mL of Liquor Ammonia, 89.14mL of 2-Dimethylaminoethnaol and 25.2g of Sodium Bicarbonate was taken in 1000mL volumetric flask and 600mlg of D. I. Water was added and sonicated to dissolve. It was make up to mark with D.I. Water.

Preparation of standard solutions: Certified Ferric Nitrate Nonahydrate was used for Iron(III) standard preparation and Ferrous Sulfate Heptahydrate was used for Iron(II) standard preparation. From this salt, separate 1000mg/L of Iron(III) and Iron(II) standard solution was prepared in diluent. From this 1000mg/L standard solution, 5.0, 7.5, 10.0, 12.5 and 15.0mg/L of Iron(III) and 0.5, 0.8, 1.0, 1.3 and 1.5mg/L of Iron(II) mixture was prepared for the Linearity study, and Mixture of 10.0mg/L of Iron(III) and 1.0mg/L of Iron(II) was prepared for the precision study. 0.05 and 0.1mg/L of Iron(III) were prepared from 10mg/L standard solution for limit of detection and limit of quantification respectively. Similarly, 0.3 and 0.5mg/L of Iron(II) were prepared from 10mg/L standard solution for limit of detection and limit of quantification respectively.

Sample preparation: - Iron Sucrose Injection: Around 100mg of sample was mixed with 2ml of Conc. HCl. It was sonicated for two minutes to mix effectively. It was then diluted to 100ml with D. I. Water. Further, it was filtered through 0.2u nylon membrane filter.

Iron Carboxymaltose Injection: Around 100mg of sample was mixed with 2ml of Conc. HCl. It was sonicated for two minutes to mix effectively. It was then diluted to 100ml with D. I. Water. Further, it was filtered through 0.2u nylon membrane filter and used for injection for Iron(II) analysis. This solution was diluted 10times with diluent and used for injection for Iron(III) analysis

An Autosampler (Dionex AS-AP) was used to inject standard solution containing Iron(III) and Iron(II) into the ion chromatography system. Subsequently, the standard solution in the sample loop was transferred onto the separator column, on which Iron(III) and Iron(III) were separated. After separation on the column, Iron(III) and Iron(II) was mixed with PCR reagent in reaction coil and then were detected by VWD (UV-Visible) detector at 530nm. A sequence containing the blank, standards, samples and recovery samples were run and results were then interpreted.

Following is diagram which shows connections with various assembly of Ion Chromatography system:

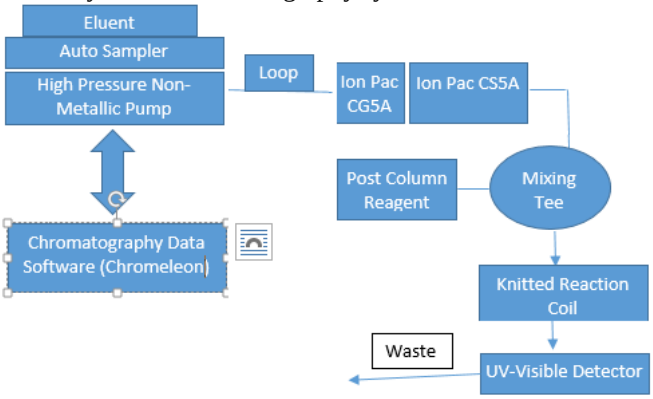


Figure 1: Ion Chromatography system schematic diagram for Iron (III) and Iron (II) analysis

3. Results and Discussions

Limit of Detection (LOD) for Iron(III) was 0.05mg/L and it was injected (n) six times and observed average signal to noise ratio (S/N) was 3.2. LOD for Iron(II) was 0.3mg/L and it was injected (n) six times and observed average signal to noise ratio (S/N) was 3.01. Limit of Quantification (LOQ) for Iron(III) was 0.1mg/L, it was injected (n) six times and observed signal to noise ratio (S/N) was 11.3. Similarly, LOQ for Iron(II) was 0.5mg/L, it was injected (n) six times and observed signal to noise ratio (S/N) was 10.1. Table 1 shows results for LOD and LOQ of Iron(III) and Iron(II)

Table 1: LOD and LOQ data for Iron(III) and Iron(II)

Iron(III)	*Amount, mg/L*	*S/N*	*% RSD (n=6)*
LOD	0.05	3.20	1.98
LOQ	0.30	11.30	1.52

Iron(II)	*Amount, mg/L*	*S/N*	*% RSD (n=6)*
LOD	*0.30*	*3.01*	*2.49*
LOQ	*0.50*	*10.1*	*1.83*

The response of Iron(III) was linear over the range of 5.0 to 15.0mg/L and of Iron(II) was linear over the range of 0.5 to

1.5mg/L. Calibration curve fits well and that is significantly linear having correlation coefficient of 0.9992 for Iron(III) and 0.9994 for Iron(II) (figure 2). This linearity study was performed for the concentration range of 5.0, 7.5, 10.0, 12.5, and 15.0mg/L for Iron(III0 and 0.5, 0.8, 1.0, 1.3 and 1.5mg/L of Iron(II). Each standard injection was repeated thrice. Therefore, number of calibration points (n) for linearity study was 15. Its data had been shown in table 2.

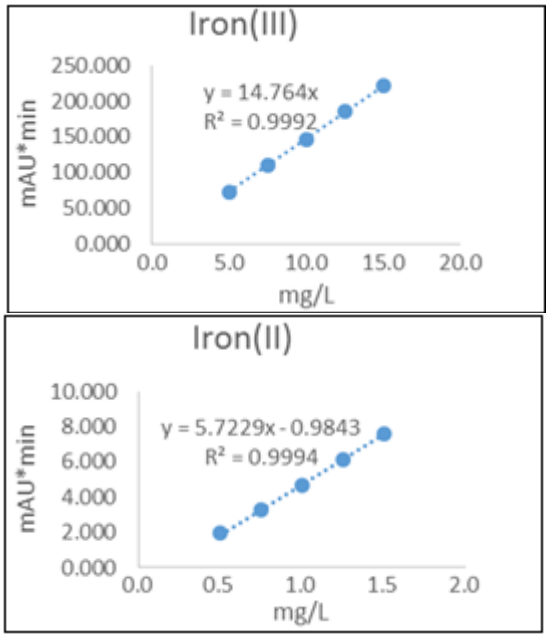
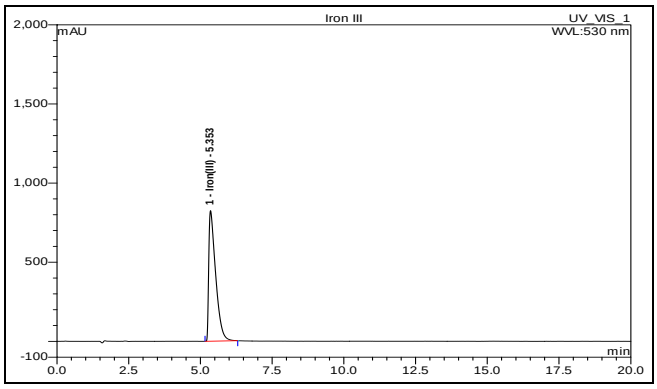


Figure 2: Linearity plot for Iron (III) and Iron(II)

Table 2: Linearity data for Iron(III) and Iron(II)

Analyte	*Points*	*Corr. Coeff.*	*Offset*	*Slope*
Iron(III)	15	0.99992	0	14.76
Iron(II)	15	0.9994	0.98	5.72

Method specificity was also done with separate injection of Iron(III) (10mg/L) and Iron(II) (1.0mg/L). Its chromatograms was shown in figure 3. When individually Iron(II) was injected, trace peak of Iron(III) was also observed which indicates conversion of Iron(II) to Iron(III) due to oxidation, but when mixture of Iron(III) and Iron(II) was injected, interconversion diminishes or stops.



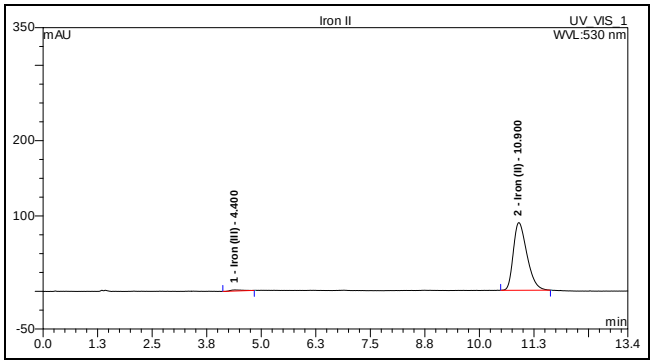


Figure 3: Specificity chromatograms for Iron(III) (10mg/L) and Iron(II) (10.0mg/L).

Replicate injections of Iron(III) and Iron(II) were done and their percent relative standard deviation for peak area was 0.77% and 1.12% respectively. Table 3 shows results for its precision study.

Table 3: Precision data for Iron(III) and Iron(II)

Analyte	*Amount, mg/L*	*% RSD (n=6)*
Iron(III)	10.0	0.77
Iron(II)	1.0	1.12

Chromatogram for Iron(III) and Iron(II) standard mixture for six consecutive injections is shown in figure 4.

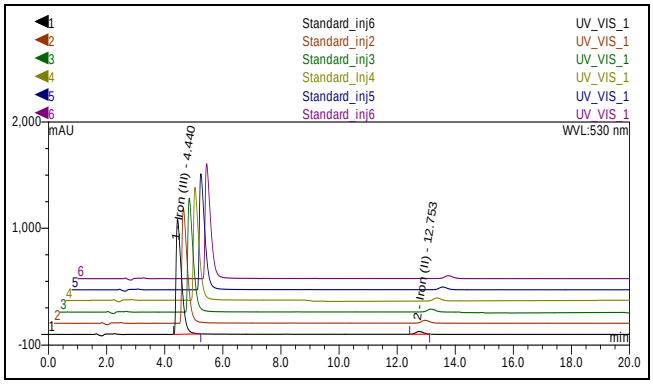


Figure 4: Standard chromatogram for Lanthanum (10mg/L)

Sample results: Samples were analyzed using the linearity calibration method. Replicate injections of same sample was also done. Its results and routine analysis sample results were shown in table 4 and table 5. As provided, Iron Sucrose Injection contains 20mg/mL of Iron as label claim. Also, as per USP monograph there is limit test for Iron(II) as not more than (NMT) 0.4% (4.0mg/mL). Similarly, Iron Polymaltose contains 50mg/ml of Iron as label claim. There is no limit guidelines for Iron(II).

Table 4: Sample precision

Analyte	*B. No.*	*Number of injections*	*% RSD*
Iron(III)	5K10447	10.0	0.96
Iron(II)	5K10447	10.0	1.24

Table 5: Routine sample analysis results

Sample	*B.No.*	*Iron(III), mg/ml*	*Iron(II), mg/ml*	*Total Iron, mg/ml*
Iron Sucrose	5K10447	17.503	2.473	19.976
Iron Sucrose	DM0917	18.225	1.799	20.204
Iron Sucrose	GPL-390/051/17	17.831	2.101	19.932
Iron Polymaltose	238062	50.023	Not Detected	50.023
Iron Polymaltose	GPL-D389/018/17	47.301	2.682	49.983

All samples were passing for its Iron(III) and Iron(II) limit. Intraday analysis of Samples was done for seven consecutive days for which they are passing its label claim limit. Sample Chromatogram was shown is figure 5 and figure 6.

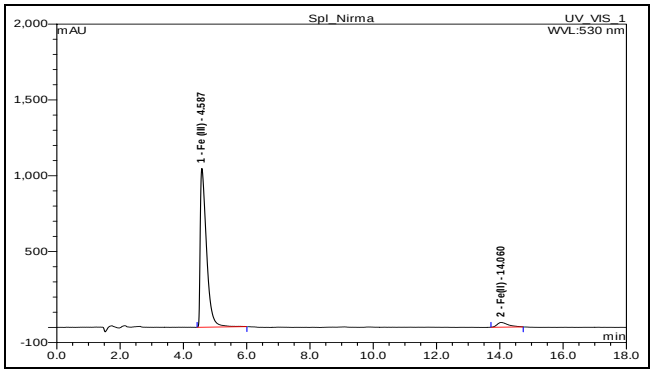


Figure 5: Iron Sucrose sample chromatogram (B. No. 5K10447) for Iron(III) and Iron(II) estimation.

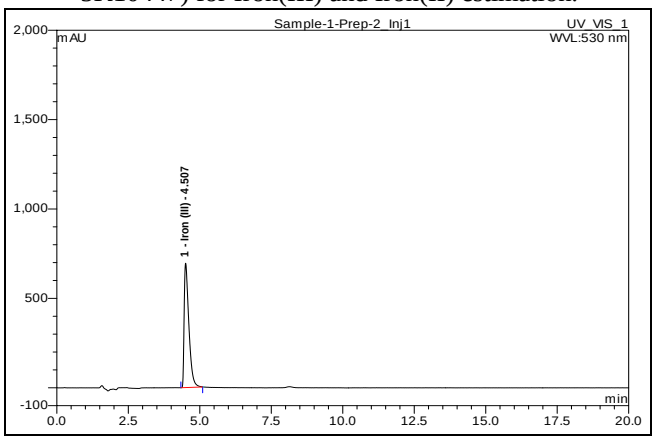


Figure 3: Iron Polymaltose sample chromatogram (B. No. 238062) for Iron(III) and Iron(II) estimation.

Recovery: - The sample used for recovery study was Iron Sucrose (B.No. 5K10447) (Average areas were taken for calculations). Recovery test solutions were injected in triplicate. Also for recovery study, sample was spiked with standard at three different levels as shown in below table.

Table 6: Recovery study (Iron(II) and Iron(III)) for Iron Sucrose sample (B.No. 5K10447) (n = 3)

For Iron(III)				
Recovery Level	*Target Concentration*	*Amount Added mg/L*	*Amount Recovered mg/L*	*% Recovery± Std.Dev.*
1	50%	5.00	5.13	102.60± 2.12
2	100%	10.00	10.23	102.30± 3.09
3	150%	15.00	15.22	101.47± 2.85

For Iron(II)				
Recovery Level	Target Concentration	Amount Added mg/L	Amount Recovered mg/L	% Recovery ± Std.Dev.
1	50%	0.50	0.487	97.40± 0.07
2	100%	1.00	0.986	98.60± 0.04
3	150%	1.50	1.478	98.53± 0.08

Same method was used on another Ion Chromatography instrument (ICS Aquion) with another IonPac CS5A column, for which there is no significant variation of sample results were observed.

4. Conclusions

Ion Chromarography – PCR – UV-Vis detection gives specific, sensitive and precise method for estimation of Iron(III) ad Iron(II). This present method was used for analysis of Iron-Sucrose and Iron-Polymaltose for their Iron(III) and Iron(II) content without any much pretreatment. The detection limits for Iron(III) was 0.05mg/L and for Iron(III) was 0.3mg/L. This technique is cost-effective with respect to analysis required for keeping a check on the limits of Iron(II) as provided by USP and other regulatory bodies. This method can also be useful for checking assay of Iron from these samples. This method can be further extended to Iron Carboxymaltose or any other similar sample matrix.

5. Acknowledgement

We thanks Nirma Lifesciences Pvt. Ltd,, Ahmedabad and Glenmark Research Centre, Navimumbai for providing samples to develop and validate this method.

References

[1] C. Gautam, “Iron deficiency in pregnancy and the rationality of iron supplements prescribed during pregnancy”, The Medscape Journal of Medicine, Vol. 10 No.12, pp. 283-286, 2008.
[2] A. Gupta, “Ferrous iron content of intravenous iron formulations”, Biometals, Vol. 29, pp. 411-415, 2016.
[3] Iron Sucrose Injection, The United States Pharmacopeia, 40-NF 35, First Supplement.
[4] Thermo Fisher Scientific IonPac CS5A Datasheet, https://www.thermofisher.com/order/catalog/product/046100TS, [online]
[5] Thermo Fisher Scientific IonPac CS5A manual, https://tools.thermofisher.com/content/sfs/manuals/4342-31188-06_CS5A_V17.pdf, [online]
[6] H. Manley, “Determination of iron sucrose (Venofer) or iron dextran (DexFerrum) removal by hemodialysis: and in-vitro study”, BMC Nephrology, Vol. 5 No. 1, 2004.
[7] D. Merli, “An analytical method for Fe(III) and Fe(II) determination in pharmaceutical grade iron sucrose complex and sodium ferric gluconate complex”, Journal of Pharmaceutical Analysis, Vol 2 Issue 6, 450-453, 2012.
[8] A. Pai, “Performance of Redox Active and Chelatable Iron Assays to Determine Labile Iron Release From

Intravenous Iron Formulations", Clinical and Translational Science, Vol 10, pp. 194-200, 2017.

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