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Original Article

Development of an Ion Chromatography Method for the Determination of Organic Impurities in Ferumoxytol

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ABSTRACT

Aim: To the development of an ion chromatography method for the determination of organic impurities in Ferumoxytol. **Materials and Methods:** A new ion chromatographic method was employed for the precise and suitable quantification of organic impurities in Ferumoxytol drug. Specifically, LOD, LOQ, Linearity, and Accuracy evaluations were conducted to thoroughly validate each of these areas. **Results:** All the studied carbohydrate impurities showed linear ranges within 0.0005 to 0.0030 mg/mL with the correlation coefficients higher than 0.9991. The limits of detection were all less than 0.0002 mg/mL. The limits of quantification were all less than 0.0006 mg/mL. The RSDs of the method were less than 3%. **Conclusion:** This method showed a lot of promise for precise impurity testing in Ferumoxytol drug substance, improving pharmaceutical quality assurance and making sure that patients are protected.

Keywords: Ferumoxytol, Ion Chromatography, Method Development, Organic Impurities, Pharmaceutical Analysis.

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INTRODUCTION

Ferumoxytol was an iron replacement injection which was employed to diagnosis and treats the iron deficiency anemia in adults with chronic kidney disease. It contains a specialized formulation of iron oxide nanoparticles, and emerged as a critical intervention in the management of iron deficiency anemia, particularly among individuals grappling with chronic kidney disease. Its significance in healthcare area due to its unique composition and properties. Because of its intricate molecular structure and colloidal nature, the manufacturing process of Ferumoxytol demands meticulous attention to detail and adherence to rigorous quality control protocols. Ensuring the safety and effectiveness of Ferumoxytol requirements a comprehensive evaluation of potential organic impurities that could compromise its therapeutic integrity.[1-5]

Organic impurities, originating from diverse sources such as raw materials, synthetic intermediates, or degradation byproducts, and expose a significant concern in pharmaceutical manufacturing. These impurities, if not adequately addressed, have the potential to undermine the efficacy and safety of medical interventions. Traditionally, analytical techniques like GC and HPLC have served as cornerstones for impurity analysis in pharmaceutical formulations.[6-11] However, when applied to complex formulation like Ferumoxytol, these conventional methods encounter notable limitations. Challenges including limited sensitivity, inadequate selectivity, and prolonged analysis times were the present hurdles in accurately detecting and quantifying impurities within Ferumoxytol drug substance.[12,13] In light of these challenges, the pharmaceutical industry has turned its attention toward alternative analytical approaches that promise enhanced performance and reliability.

In recent decades, Ion Chromatography (IC) has garnered significant attention owing to its unique ability to accurately detect anions across various matrices with exceptional precision. It offers several advantages including low detection limits, minimal sample and reagent usage, and swift analysis times.[14,15] Recently, a number of ion chromatographic methods were developed for the measurement of traces ions and impurities in dugs and other samples.[16-18] Presently, the primary hurdles in environmental analysis through ion chromatography revolve around the separation and determination of novel analytes at trace levels. With rapid technological advancements, new substances have

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emerged in the environment, necessitating the identification of these new analytes in conventional matrices or standard anions in novel matrices. To address this challenge, innovative stationary phases with tailored selectivity and superior efficiency of separation have been introduced.[19-24] It is crucial to underscore the importance of method validation to ensure the quality and fitness for purpose of analytical results, aligning them with the requirements of stakeholders.[24]

Ion Chromatography (IC) has emerged as a frontrunner in this regard. Leveraging the unique principle of ion exchange, IC demonstrates exceptional capabilities in separating and quantifying ions and polar molecules with heightened sensitivity and selectivity. Unlike conventional chromatographic methods, IC offers a tailored solution for the intricate analysis of ionic and polar compounds present in pharmaceutical analysis.[24-27] The main objective of this research is to harness the advantages of IC in developing a robust methodology for identifying organic impurities within Ferumoxytol drug substances. By capitalizing on IC's sensitivity, selectivity, and compatibility with aqueous samples, the aim is to transcend the limitations encountered by existing analytical approaches. Through systematic method development and comprehensive validation processes, the ultimate goal is to equip pharmaceutical laboratories with a reliable tool for ensuring the safety, efficacy, and consistency of Ferumoxytol products, thereby advancing the standards of patient care in the realm of iron deficiency anemia management.

MATERIALS AND METHODS

Reagents and Chemicals

All chemicals utilized in the preparation of reagents, mobile phase and standards were of analytical grade. Sodium hydroxide pellets (Merck, CAS 1310-73-2) were employed for the mobile phase preparation. Ultrapure deionized water (18.3 MΩ cm, Milli-Q system) or Milli-Q water was utilized for both mobile phase preparation and as a diluent for standards and samples. Ferumoxytol (CAS 722492-56-0) was utilized for the preparation of sample solutions. The other reagents and chemicals used for the preparation of standard stock solutions include: Fructose (Sigma Aldrich, CAS 57-48-7), Glucose (Sigma Aldrich, CAS 50-99-7), Sucrose (Sigma Aldrich, CAS 57-50-1), and Maltose (Merck, CAS 6363-53-7).

Apparatus

A Thermo Fisher Dionex Ion Chromatograph ICS 5000 with an ECD detector was the apparatus utilized, Dionex Carbopac PAl, (4x250 mm) column connected in Guard column Dionex Carbopac PAI Guard (4x50 mm) with flow rate of 0.8 mL/min or run time of 40 min. The experiment was conducted using AS-AP sampler with push partial injection mode, partial cut volume of 10 and temperature control of 10°C. The research was done by utilizing eluent mixture of Sodium hydroxide of IC grade or Milli-Q water. Dilution mix properties such as draw speed was 60 µL/s and dispense speed was 30 µL/s. In EDetl Options of IntAmp heaving Amperometry cell and Channels (ED_l, ED_1 _total) on, data collection rate set at 20 Hz, References electrode of the equipment made of AgCl and the waveform selector made of gold, Carbo, quad.

Procedure

Preparation of Mobile Phase

Dissolve 4.0 g of sodium hydroxide pellets in 2000 mL of water. Filter the solution through a 0.22 µm or finer porosity membrane and this solution will be degasified.

Standard Stock Solution Preparation

To prepare the 0.2 mg/mL diluent stock solution of Sucrose, Glucose and Fructose and Maltose.

Standard solution Preparation

Transfer 1 mL of each standard stock solution-I into separate 100 mL volumetric flasks and dilute to volume with the diluent.

Preparation of System Suitability Solution

Accurately weigh and transfer about 40 mg of Ferumoxytol sample into a 100 mL volumetric flask. Add approximately 25 mL of diluent, then add 1 mL of standard stock solution, dilute to volume with the diluent, mix, and filter with a 0.22 PVDF filter.

Preparation of Sample Solution

Accurately weigh and transfer about 40 mg of Ferumoxytol sample into a 100 mL volumetric flask. Add approximately 25 mL of diluent, mix, dilute to volume with the diluent, and filter with a 0.22 PVDF filter.

RESULTS

Specificity

Ion chromatography is a most sensitive precise method than the other chromatographic methods because it is depends on the single interaction and can also detect the ions in trace quantities. The determination of organic impurities in Ferumoxytol demonstrated selectivity and specificity for carbohydrates such as glucose, fructose, sucrose, and maltose. Comprehensive details regarding specific data for blank are tabulated in Table 1. Each of the four selected carbohydrate impurities exhibited clear resolution from one another, with no interference observed either from the blank or from their respective drug substances. The retention times observed for each carbohydrate were as follows: 5.8 min for glucose, 6.3 min for fructose, 7.6 min for sucrose, and 21.1 min for maltose. The injection volume utilized was 10.0 µL, with a dilution factor of 1.00, and the total run time was 40 min. Comprehensive details regarding glucose, fructose, sucrose,

and maltose are tabulated in Table 2, while chromatograms illustrating their separation are depicted in Figure 1.

LOD and LOQ data

The Limit of Detection (LOD) for glucose was determined to be 0.0002 mg/mL. The observed average Signal-to-Noise ratio (S/N) was 1.737. Similarly, the LOD for fructose was found to be 0.0002 mg/mL, with an average S/N ratio of 1.498. For sucrose, the LOD was also determined to be 0.002 mg/mL, yielding an average S/N ratio of 0.668. Concerning the Limit of Quantification (LOQ), glucose exhibited an LOQ of 0.0002 mg/L, resulting in an observed S/N ratio of 1.946. Likewise, the LOQ for fructose was 0.0006 mg/mL, with an average S/N ratio of 1.827. Sucrose displayed an LOQ of 0.0006 mg/mL, with an average S/N ratio of 0.944, while maltose exhibited an LOQ of 0.0006 mg/mL, with an observed average S/N ratio of 0.915. Representations the results for the Limit of Detection (LOD) and Limit of Quantification (LOQ) of glucose, fructose, sucrose, and maltose are shown in Tables 3 and 4.

Linearity

The response of glucose, fructose, sucrose, and maltose was found to be linear within the concentration range of 0.0005 to 0.0030 mg/mL. Calibration curves exhibited excellent fit and significant

Table 1: Specificity data for blank.

Sl. No.	Ret. Time Min.	Peak Name	Height	Area nC* min	Real Area %	S/N
1	0.00	blank	0.000	0.000	0.00	0.00

Table 2: Specificity data for glucose, fructose, sucrose and maltose.

Sl. No.	Ret. Time Min.	Peak Name	Height	Area nC* min	Real Area %	S/N
1	5.8	Glucose	10.757	1.993	33.61	240.8
2	6.3	Fructose	9.328	1.948	32.85	209.8
3	7.6	Sucrose	4.077	1.022	17.24	87.1
4	21.1	Maltose	1.714	0.966	16.30	27.4
Total			25.876	5.929	100.00	565.105

Table 3: LOD data for glucose, fructose, sucrose and maltose.

Sl. No.	LOD parameter	Amount, mg/mL	S/N
1	Glucose	0.0002	1.73
2	Fructose	0.0002	1.49
3	Sucrose	0.0002	0.66
4	Maltose	0.0002	0.77

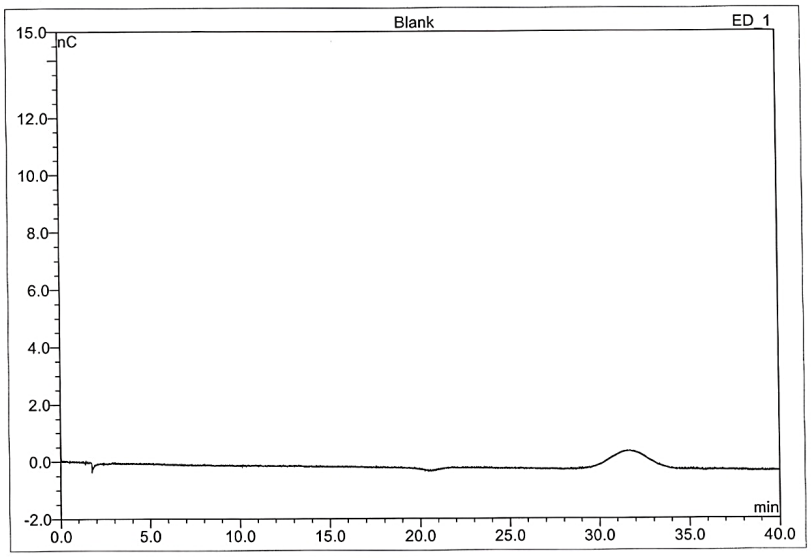

Figure 1a: Typical Blank chromatogram.

Table 4: LOQ data for glucose, fructose, sucrose and maltose.

Sl. No.	LOQ parameter	Amount, mg/mL	S/N
1	Glucose	0.0006	5.94
2	Fructose	0.0006	5.82
3	Sucrose	0.0006	2.94
4	Maltose	0.0006	3.91

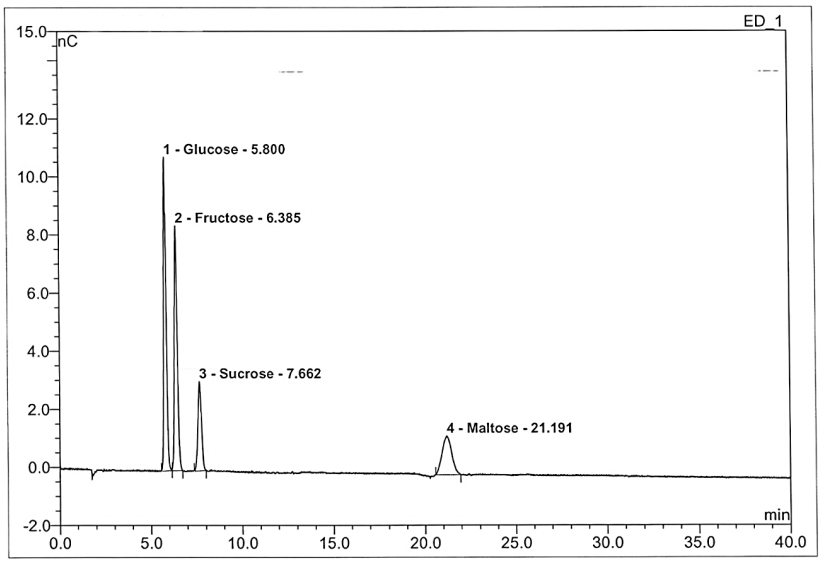

Figure 1b: Specificity chromatograms for glucose, fructose, sucrose and maltose.

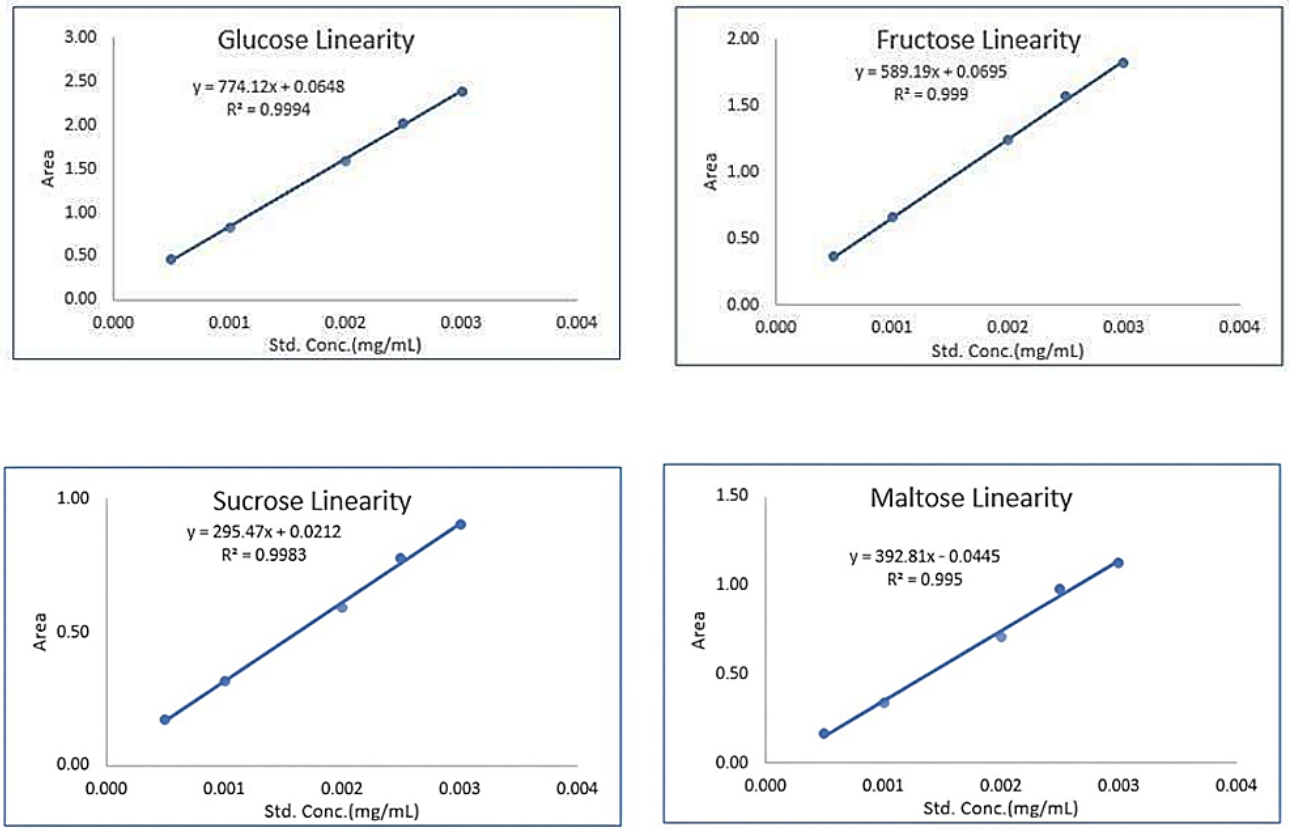

Figure 2: Linearity plot for Glucose, Fructose, Sucrose and Maltose.

linearity, with correlation coefficients of 0.9994 for glucose, 0.9990 for fructose, 0.9983 for sucrose, and 0.9950 for maltose (refer to Figure 2). This linearity assessment encompassed concentration points of 0.0005, 0.0010, 0.0020, 0.0025, and 0.0030 mg/mL for each analyte. For the linearity study, each standard injection was repeated six times. In the case of blank, sample solution, and system suitability solution, injections were performed only once. The number of calibration points (n) utilized for the linearity study was 15. Detailed data pertaining to this study are presented in Tables 5 and 6.

Accuracy

The accuracy of glucose, fructose, sucrose and maltose was determined over the range of 0.0005 to 0.0030 mg/mL. And the range of area was between 0.181 to 1.153 for glucose, 0.416 to 1.986 for fructose, 0.185 to 0.956 for sucrose and 0.174 to 1.138 for maltose. With correlation coefficients of 0.9991 for glucose, 0.9995 for fructose, 0.9983 for sucrose, and 0.9963 for maltose, the calibration curve (Figure 3) fits well and is significantly linear. The concentration ranges for glucose, fructose, sucrose, and maltose in this linearity research was 0.0005, 0.0010, 0.0020, 0.0025, and 0.0030 mg/mL. The range of area was at 0.181, 0.403, 0.742, 0.946, 1.153 for glucose, 0.416, 0.742, 1.321, 1.641 and

Table 5: Linearity data for glucose, fructose, sucrose and maltose.

Sl. No.	Analyte	Corr. Coeff.	Intercept	Slope
1	Glucose	0.9997	0.0648	774.116
2	Fructose	0.9995	0.0695	589.186
3	Sucrose	0.9992	0.0212	295.465
4	Maltose	0.9975	0.0445	392.814

Table 6: Linearity data for glucose, fructose, sucrose and maltose.

Sl. No.	Analyte	Corr. Coeff.	Intercept	Slope
1	Glucose	0.9991	0.0984	792.558
2	Fructose	0.9995	0.107	619.000
3	Sucrose	0.9983	0.0251	306.628
4	Maltose	0.9963	0.0025	373.395

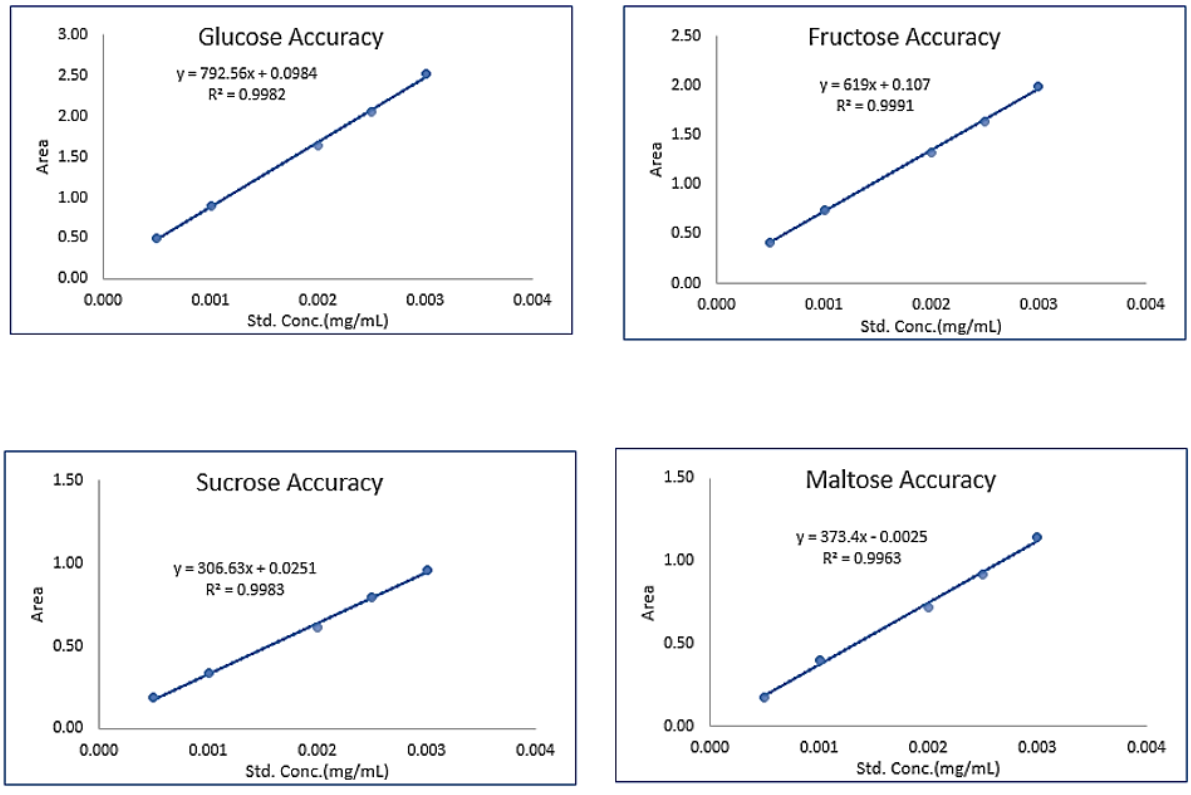

Figure 3: Accuracy plot for Glucose, Fructose, Sucrose and Maltose.

1.986 for fructose, 0.185, 0.334, 0.616, 0.794, 0.956 for sucrose and 0.174, 0.400, 0.718, 0.918 and 1.138 for maltose.

CONCLUSION

The development of an ion chromatography method to detect organic impurities in Ferumoxytol is a relevant achievement of analytical chemistry. This research has conducted comprehensive studies on the development parameters in accordance with the ICH guidelines, including specificity, sensitivity, linearity, and accuracy, through meticulous experimentation and analysis. In specificity experiments the retention times observed for each carbohydrate impurities were glucose (5.8 min), fructose, (6.3 min), sucrose (7.6 min) and maltose (21.1 min). All the studied carbohydrate impurities showed linear ranges within 0.0005 to 0.0030 mg/mL with the correlation coefficients higher than 0.9991. The limits of detection were all less than 0.002 mg/mL; the RSDs of the method were less than 5%. The limits of quantification were all less than 0.0006 mg/mL. The achieved linearity demonstrates high efficiency in producing results that are stable and repeatable over a variety of concentrations, thereby increasing the efficacy of the method in quantitative analysis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

HPLC: High performance liquid chromatography; **RSD:** Relative standard deviation; **SD:** Standard deviation; **RT:** Retention time; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **ICH:** International council of harmonization; **IC:** Ion Chromatography; **GC:** Gas Chromatography.

SUMMARY

The study aimed to establish a reliable ion chromatography method to detect organic impurities in Ferumoxytol. This research has conducted parameters in accordance with ICH guidelines, including specificity, sensitivity, linearity and accuracy. In specificity experiments chromatographic separation achieved in shorter analysis time and well separated. All the studied carbohydrate impurities shoed linear ranges within 0.0005 to 0.0030 mg/mL with the correlation coefficients higher than 0.9991. The limits of detection were all less than 0.002 mg/mL. The RSDs of the method were less than 5%. The limits of quantification were all less than 0.0006 mg/mL. The achieved linearity demonstrates high efficiency in producing results that are stable and repeatable over a variety of concentrations, thereby increasing the efficacy of the method in quantitative analysis.

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