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UV-Visible Spectrophotometric Method Development and Validation of Assay of Iron Sucrose Injection

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ABSTRACT

A novel, safe and sensitive method of spectrophotometric estimation in UV-region has been developed for the assay of iron sucrose injection formulation. The method has been developed and validated for the assay of iron sucrose injection using concentrated hcl 37%, ammonium acetate buffer 32%, hydroxylamine Hcl10%, o-phenanthroline0.1% and water as diluents. These chemicals do not show any interference in spectrophotometric estimations. Ammonium iron III material used as standard. All the parameters of the analysis were chosen according to ICH guideline and validated statistically.

Keywords: *Spectrophotometric; Developed; Validated; Parameters; ICH*

INTRODUCTION

Now days, chronic kidney disease (CKD) iron deficiency anemia (IDA) are the most frequent forms of nutritional deficiency[1,2]. In developed countries eighteen percent prevalence of IDA has been recorded by WHO[3] which is quite different from developing countries that is about 35-75%[4]. Worldwide prevalence for anemia has been found to account for 55.9% but this prevalence is varied from urban to unurbanized countries[5]. Characteristically, anemia is defined by lowering of hemoglobin value[6]. Pallor of the skin and mucous membranes, shortness of breath, increased palpitations of heart, fatigue and indolence are most common symptoms associated with anemia[7]. Anemia is categorized into four grades on the basis of its severity (table 1)[8]. Many risk factors responsible for this nutritional deficiency[9] are marriages at very young age[10], teenage pregnancy[11], multiple pregnancies with less birth gap[12,13], low intake of folic acid and iron[14,15], high rate of prevalence for worm infections[16], eating styles(vegetarian)[17], inadequate intake of iron fortified foods(meat, fish and poultry)[18], limited eating(meal skipping)[19], having history of iron deficiency[20], persistent weight loss[21], heavy menstrual periods[22], chronic use of NSAIDS[23], frequent donation of blood[24], rigorous physical training and rapid growth[25,26], Grade 1 anemia is treated prophylactically by oral iron therapy[27], but parentral iron therapy is recommended for moderate to severe type of anemia[28].

Fig.1: chemical structure of iron sucrose

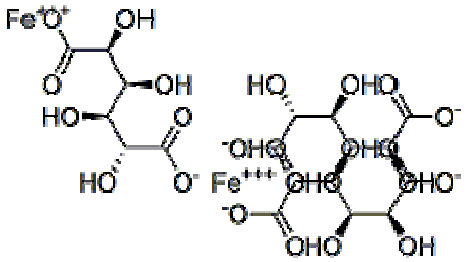


Table 1: Grading of anemia according to common terminology criteria for adverse events[8]

Mild anemia (Grade 1)	Moderate anemia (Grade 2)	Severe anemia (Grade 3)	Life threatening anemia (Grade 4)
Hgb<LLN-10.0g/dL	Hgb<10.0-8.0g/dL	Hgb <8.0g/dL	Life threatening consequences, urgent intervention is required.

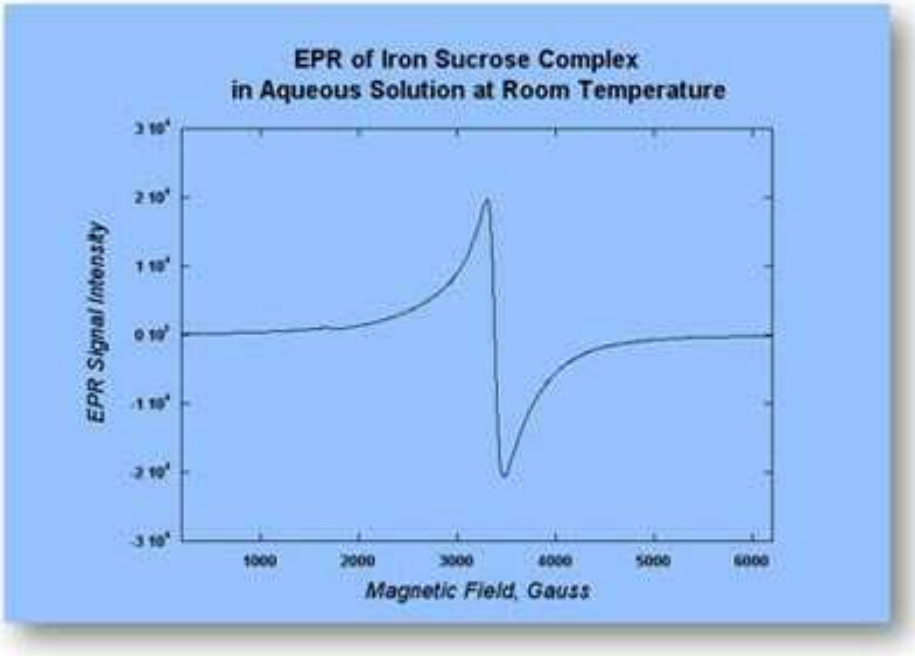
Iron sucrose (venofer)[29], Iron dextrans (cosmofer and Infed)[30], Iron carboxymaltose (Ferinject)[31] and Iron isomaltoside 1000 (Monofer)[32] are the various parentral preparations of iron to combat the serious deficiency of iron[33]. Presently, it has been proved from many studies that iron sucrose (IV) is considered to be safer than other parentral preparations in term of its lesser side effects and thus considerably increasing the hemoglobin[34], Iron sucrose injection under the trade name of Venofer is a source of elemental iron in the form of polynuclear iron(III)-hydroxide in sucrose (table 2)[35]

Table 2: Product description[36,37]

Trade name	Therapeutic class	Dose	Sucrose content	pH	preservative	Injection osmolarity
venofer	hematinic	5mL single dose vial(20mg/mL)	30% sucrose w/v(300mg/mL)	10.5-11.1	No	1250mOsmol/L

Elemental iron plays a very important role in performing many functions of the body such as oxygen transportation (at molecular level)[38] Synthesize hemoglobin[39] haematopoiesis[40], energy production from blood sugar[41]. Formation of physiological important heme and nonheme products[42] enzymes production[43], physical and mental growth particularly in childhood and pregnancy[44,45].

Fig.2:



Clinical significance of iron sucrose injection[37]
1. Non dialysis dependent chronic kidney disease patients receiving an erythropoietin.
2. Non-dialysis-dependent chronic kidney disease patients not receiving an erythropoietin.
3. Hemodialysis dependent chronic kidney disease patients receiving an erythropoietin.
4. Peritoneal dialysis dependent chronic kidney disease patients receiving an erythropoietin.

SPECTROSCOPY METHODS

Spectroscopy is basically a study of a relationship between matter and electromagnetic radiations[46]. At present time, this method is extensively used for the analysis of large variety of samples[47]. It is considered as one of the effective tool for structural studies either atomic or molecular[48].

Ultraviolet-Visible spectrophotometry

In order to conduct pharmaceutical analysis, UV-Visible spectrophotometry is one of the most commonly used methods[49]. Its principal of performance is usually reliant on estimation of radiation (UV/visible) absorbed by a substance of a given solution[49]. UV-visible spectrophotometer has the potential ability to measure the functional ratio of the two beams of light in the U.V-Visible region[50]. In addition, we can perform qualitative (identification of given compound) and quantitative analysis (measurement of quantity of given molecule) by spectrophotometer[51]. This spectrophotometric method is quite easy, fast, reasonably specific and appropriate for small amount of compounds[52]. In order to perform quantitative analysis, spectrophotometric technique follows one fundamental law which is known as Beer-Lambert Law[53].

Beer's law[54]

Beer's law usually explain the relationship between absorbance and concentration which means that intensity of parallel beam(monochromatic radiation) has the tendency to decrease exponentially with the number of absorption molecules.

Lambert's law[55]

This law is basically explained that intensity of parallel beam has proportional relationship with the thickness of medium.

Beer-Lambert law[56]

Beer-Lambert law is the modified form of these two fundamental laws which states that when parallel beam of monochromatic radiation is passed through a medium of uniform thickness then intensity of radiation will be decreased exponentially. Mathematical expression for this law is as:

A=a b c

Where, A=absorbance or optical density

a=absorptivity or extinction coefficient

b=path length of radiation through sample (cm)

c=concentration of solute in solution.

In above expression, both "b" and "a" are constant and so "a" has direct relationship to the concentration "c".

When c is in gm/100 ml, then the constant is called A (1%, 1 cm)

A=A 1% ba 1cm

Spectrophotometer can be employed for quantification of given medicinal substance by preparing its solution in blank/transparent solvent and then measuring its absorbance at appropriate wavelength[57]. This wavelength of interest is actually the maximum absorption wavelength which is denoted as λmax. In an ideal situation, optimization of accurate and precise measurements can be achieved by adjustment of concentration in such a way to give absorbance of about 0.9[58]. Measured absorbance can be slightly affected by small error in adjustment of wavelength scale[59].

Single component sample containing absorbing substances, can be assayed by taking measurement of absorbance[60]. Three standard procedures such as standard absorptivity value[61], calibration graph and single or double point standardization, are available for absorbance measurement. In the first method that is "standard absorptive value", absorptivity can be determined by taking standard A (1%, 1 cm) or E values in consideration[62]. This method has advantage in particular situation where it is rather impossible to obtain the reference sample due to some certain reasons (too expensive to afford. Second method "calibration graph method"[63] is used to plot the calibration curve by using absorbance values of standard solution(of reference substance) and sample solution at different concentrations.

This method is very helpful to determine the concentration of analyte in a given sample solution by reading the calibration graph as the conc. Matching to the absorbance of solution. Third method "single point standardization" is employed to calculate the relationship between absorbance and conc.by separately taking the absorbance of standard and sample solutions[64]. This measurement can be done by using the following mathematical expression[65].

Ctest= (Atest×Cstd)/Astd
Where
Ctest =conc in the sample solution
Cstd =concentrations standard solutions
Atest = absorbance of the sample
Astd = absorbance of the standard solutions

Methods employed for assay of substances in multi-component samples, by utilizing spectrophotometer
1. Simultaneous equation method[66]
2. Derivative spectrophotometric method[67]
3. Absorbance ratio method (Q-Absorbance method)[68]
4. Difference spectrophotometry[69]
5. Solvent extraction method[70]

METHOD VALIDATION

The term validation is usually characterized by assurance of measurement procedure[71]. It is considered as an essential part of good analytical practice (GAP)[72] by evaluating the analytical results in term of quality, reliability and consistency[73]. In order to meet the desired purpose, intended application is produced by performing the valid measurements.
Validation/revalidation of the analytical method should be performed[74,75].

- Before their introduction into routine practice.
- Whenever there are chances for either changing the conditions (e.g., an instrument with different characteristics or samples with a different matrix) or methods and that alteration is beyond the original scope.

Guidelines for validation methods are offered by following internationally established organizations.
1. American Society for Testing and Material (ASTM)[76]
2. Codex Committee on Methods of Analysis and Sampling (CCMAS)[71]
3. European Committee for Normalization (CEN)[77]
4. Cooperation on International Traceability in Analytical Chemistry (CITAC)[78]
5. European Cooperation for Accreditation (EA)[79]
6. Food and Agricultural Organization (FAO)[80]
7. United States Food and Drug Administration (FDA)[81]
8. International Conference on Harmonization (ICH)[82]

ICH Guidelines (ICH Q2R1) for Analytical Procedure and Validation[83,84]
An approach to perform the analysis is generally termed as "analytical procedure". It should suppose to explain all the crucial steps desired for each analytical test. Validation of the analytical procedure covers the sample, the reference standard, reagents preparations, use of the apparatus, generation of the calibration curve, use of the formula for the calculation, etc.

Types of Analytical Procedures to be validated[85]

Validation of analytical procedures is directed toward the four most frequent types of analytical procedures which are:

1. Identification tests[86]
2. Quantitative tests for impurities' content[87]
3. Limit tests for the control of impurities[87]
4. Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product[87].

The purpose of the analytical procedure should be intended to govern the validation characteristics which require being evaluated[87]. Characteristics of typical validation are accuracy, Precision, Repeatability, Intermediate Precision, Specificity, Detection Limit, Quantitation Limit, Linearity and Range[87].

Following are the conditions in which revalidation is quite necessary[88]

- Changes in the production of the drug substance;
- Changes in the makeup (composition)of the finished product;
- Changes in the analytical procedure

EXPERIMENTAL

Assay method of iron sucrose

Materials

Iron sucrose injection was provided by Ameer & Adnan pharmaceuticals (pvt.) ltd. Iron sucrose injection containing 20 mg elemental iron each ml. Ammonium iron III (sigma-aldrich fluka-09713), concentrated Hcl (mahakali fine chem). Ammonium acetate (akash purochem private limited, mumbai). Hydroxylamine Hcl (shandong baoyuan chemical co., ltd). o-phenanthroline (sigma-aldrich offers aldrich-131377).

- ammonium iron III
- concentrated Hcl 37%
- ammonium acetate buffer 32%.(take 32g add in 100ml water)
- hydroxylamine Hcl 10% (10g in 100ml water)
- o-phenanthroline0.1% (100mg in 100ml water)

Instrumentation

UV-Visible double beam spectrophotometer with matched quartz cells (1 cm) Model: Evolution 201 Make: Thermo Scientific, 81 Wyman Street Waltham, Massachusetts, US.

PROCEDURE

Standard preparation:

Ammonium iron III were weighed and powdered. Powdered of ammonium iron III must be 173mg. weighed powder taken into 100 ml volumetric flask then 15 ml of concentrated Hcl was added and shaken well to dissolve it after that 85 ml of water was added to adjust the volume up to 100 ml. From that 10 ml of solution was withdrawn and taken in 50 ml volumetric flask. The volume was adjusted with diluents up to 50 ml.solution shown in figure 3.

Sample preparation:

Iron sucrose injection equivalent to 400mg iron sucrose taken into 100 ml volumetric flask then 15 ml of concentrated Hcl was added and shaken well to dissolve it after that 85 ml of water was added to adjust the volume up to 100 ml. From that 10 ml of solution was withdrawn and taken in 50 ml volumetric flask. The volume was adjusted with diluents up to 50 ml.Sample solution shown in figure 3.

Table 3: dilution of sample and standard with different chemicals

S. No.	ml	Blank	Sample solution	Standard solution
1	5	-	Solution of injection	Solution of ammonium iron III
2	5	Ammonium acetate	Ammonium acetate	Ammonium acetate
3	5	Hydroxylamine	Hydroxylamine	Hydroxylamine
4	1	Phenanthroline	Phenanthroline	Phenanthroline

Calculation

Standard/sample x173/100x10/50x5/25x100/1x50/10x25/5x11.6/100x5

RESULT AND DISCUSSION

Selection of wavelength

Measure the absorbance of standard solution and sample solution in UV spectrophotometer at 511nm. Iron sucrose shows λmax at 511. The proposed analytical method is simple, accurate and reproducible (Figure 6).

Method validation

Specificity: Resolution of the analyze peak from the nearest peak: Solution of each of the analyze was injected separately and their retention time is noted. The standard working solution containing a mixture of the component being analyze is also injected and each of analyze peaks is check for its resolution from the nearest.

Linearity: Six points calibration curve were obtained in a concentration range from 100-400mg for iron sucrose injection. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 2.293x-4 with correlation coefficient 0.999.

Precision: Precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per normal weight taken for analysis. Repeat the analysis four times. Calculate the % assay. The developed method was found to be precise.

Accuracy: Accuracy of the method is ascertained by standard addition method at 3 levels. Standard quantity equivalent to 50%, 100% and 125% is to be added in sample. The result shown that best recoveries (98.62-99.12%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

Fig.3: solution of ammonium iron III and iron sucrose injection

Fig.4: solution of blank, standard and sample

Fig.5: FTIR spectra of iron sucrose material

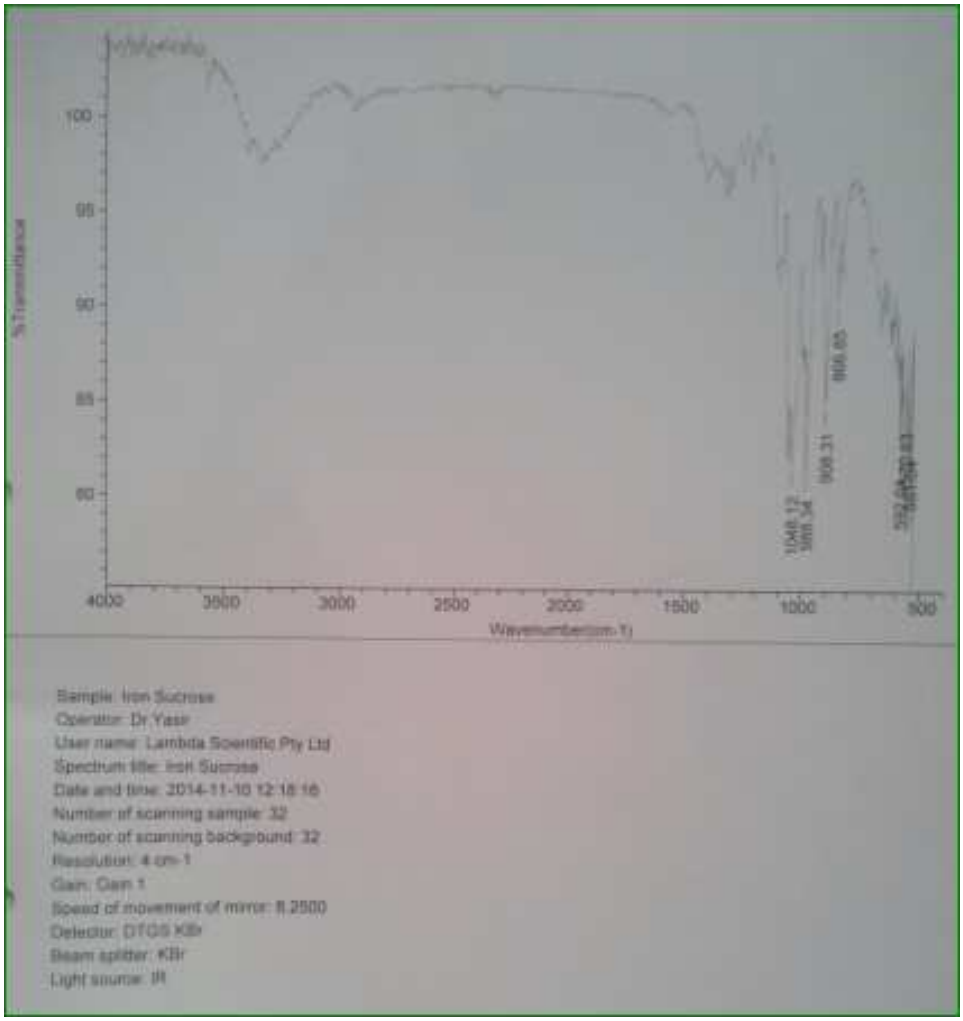


Fig.6: sample spectrum of solution

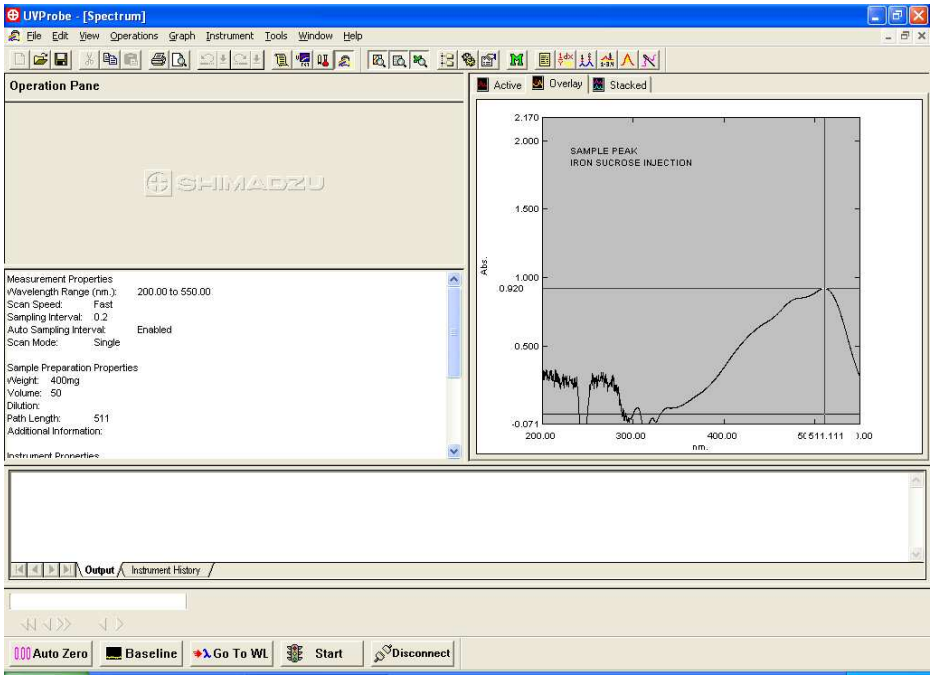


Fig.7: standard spectrum of solution

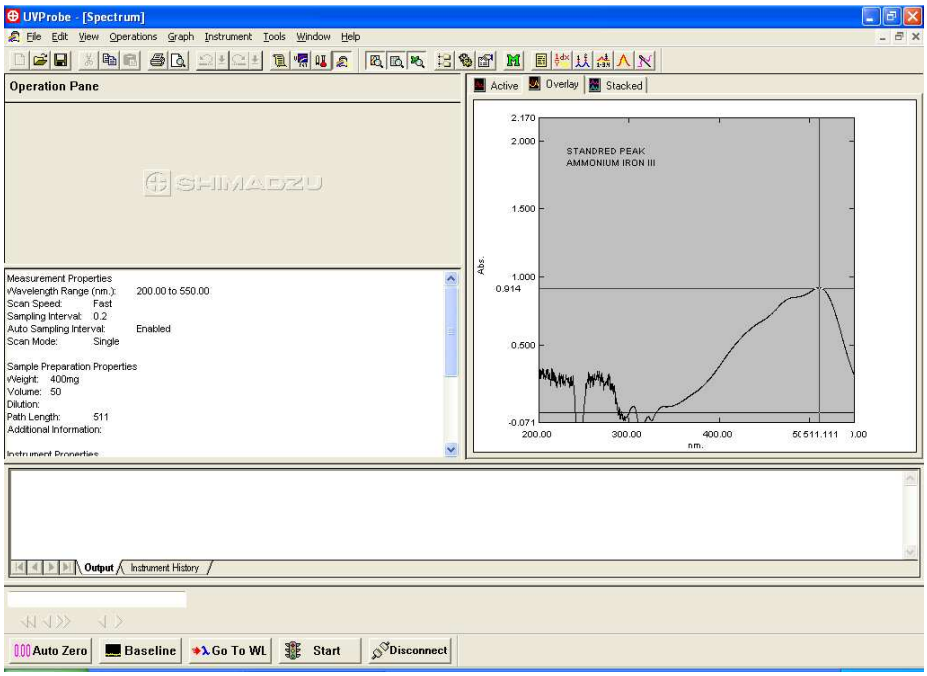


Table 4: Conc. Vs Abs. table for Linearity Study

S.No.	**Concentration of iron sucrose(mg)**	**Absorbance**
1	100	228
2	200	450
3	300	685
4	400	914

Fig.8: linearity curve of iron sucrose injection

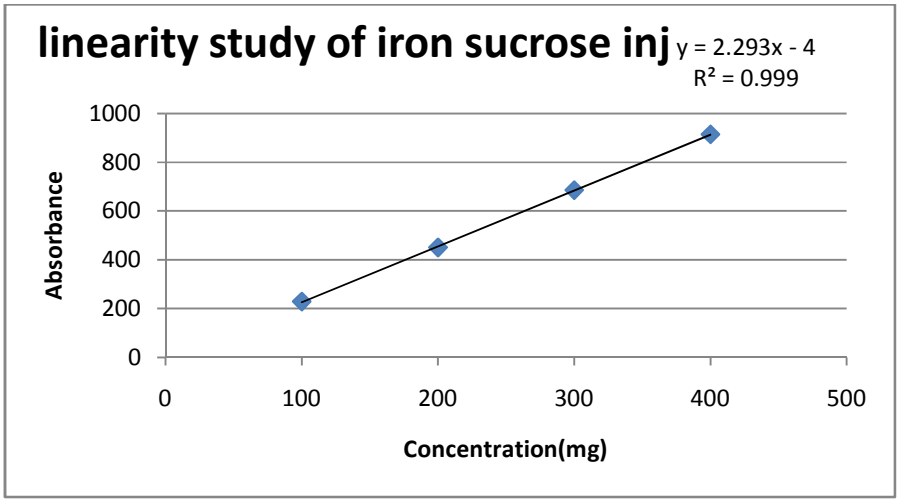


Table 5: Evaluation data of precision study

Sample no	Assay	
SET	**Intraday**	**Interday**
1	100.9	99.5
2	100.8	99.4
3	100.9	98.7
4	99.9	99.6

Table 6: Evaluation data of accuracy study

%recovery level	%recovery
50%	98.62
100%	98.55
125%	99.12

CONCLUSION

The present analytical method was validated as per ICH guideline and it meets to specific acceptance criteria. It is concluded that the analytical method was specific, precise, linear, accurate and having stability indicating characteristics. The present analytical method can be used for its intended purpose.

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