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Method Validation of Complexometric Titration for Determination of Iron in Iron Containing Drugs

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ABSTRACT

A simple and inexpensive titrimetric method was developed and validated according to International Conference on Harmonization (ICH) and United State Pharmacopoeia (USP). The method is based on complex formation between ferric (Fe^{3+}) ions and disodium edetate (EDTA) in strong acidic media.The endpoint of titration was determined visually. Sulfosalicylic acid was used as an indicator (color change: from dark pink to light yellow at pH = 2). The method was validated regarding to linearity, precision, accuracy and robustness giving good results.

Keywords: Iron, Titrimetric, Assay, Complexometric titration, EDTA, Sulfosalicylic acid

INTRODUCTION

Iron is a mineral that is naturally present in many foods, added to some food products, and available as a dietary supplement. Iron is an essential component of hemoglobin, an erythrocyte (red blood cell) protein that transfers oxygen from the lungs to the tissues [1]. As a component of myoglobin, another protein that provides oxygen, iron supports muscle metabolism and healthy connective tissue [2]. Iron is also necessary for physical growth, neurological development, cellular functioning, and synthesis of some hormones [2,3]. Dietary iron has two main forms: heme and nonheme [1]. Plants and iron-fortified foods contain nonheme iron only, whereas meat, seafood, and poultry contain both heme and nonheme iron [2].

Table 1 lists the current iron RDAs for non-vegetarians. The RDAs for vegetarians are 1.8 times higher than for people who eat meat. This is because heme iron from meat is more bioavailable than nonheme iron from plant-based foods, and meat, poultry, and seafood increase the absorption of non heme iron [4] (Table 1).

Table 1: Recommended Dietary Allowances (RDAs) for Iron.

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	0.27mg*	0.27mg*		
7–12 months	11 mg	11 mg		
1–3 years	7 mg	7 mg		
4–8 years	10 mg	10 mg		
9–13 years	8 mg	8 mg		
14–18 years	11 mg	15 mg	27 mg	10 mg
19–50 years	8 mg	18 mg	27 mg	9 mg
51+ years	8 mg	8 mg		

* Adequate Intake (AI)

Adults with normal intestinal function have very little risk of iron overload from dietary sources of iron [2]. However, acute intakes of more than 20 mg/kg iron from supplements or medicines can lead to gastric upset, constipation, nausea, abdominal pain, vomiting, and faintness, especially if food is not taken at the same time [2,4]. Taking supplements containing 25 mg elemental iron or more can also reduce zinc absorption and plasma zinc concentrations [3,5,6]. In severe cases (e.g., one-time ingestions of 60 mg/kg), overdoses of iron can lead to multisystem organ failure, coma, convulsions, and even death [7,8]. Tolerable upper levels of iron intake are presented in Table 2.

Table 2: Tolerable Upper Intake Levels (ULs) for Iron

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	40mg	40 mg		
7–12 months	40 mg	40 mg		
1–3 years	40 mg	40 mg		
4–8 years	40 mg	40 mg		
9–13 years	40 mg	40 mg		
14–18 years	45 mg	45 mg	45 mg	45 mg
19 + years	45 mg	45 mg	45 mg	45 mg

Although iron deficiency is the most common cause of anemia, deficiencies of other micronutrients (such as folate and vitamin B12) and other factors (such as chronic infection and inflammation) can cause different forms of anemia or contribute to their severity.

Iron deficiency (ID), with or without anemia (iron deficiency anemia or IDA), represents a major global health problem affecting more than 2 billion people worldwide, mainly because of poverty and malnutrition in developing countries. Individuals with increased requirement of the micronutrient, like preschool children, adolescents during the growth spurt, and women of childbearing age, are at the highest risk. Nonetheless, IDA is also frequent in western countries, with a prevalence ranging from 4.5 to 18% of the population, where elderly with multimorbidityand polypharmacy represent an adjunctive subcategory at high risk. IDA can be due to a wide range of different causes, which can roughly grouped into three major categories: imbalance between iron intake and iron needs, blood losses (either occult or overt), and malabsorption. The coexistence of multiple causes or predisposing factors is not uncommon in certain patients, particularly those with severe and/or recurring IDA, and in the elderly. Complex overlap of different mechanisms can occur in the individual patient. Just as an example, gastrointestinal angiodysplasia represent a relatively frequent cause of occult bleeding in the elderly, which can be difficult to diagnose when localized in the small bowel unless wireless capsule endoscopy is performed. Angiodysplasia often associate (in 20–25% of cases) with calcific aortic stenosis, giving rise to the so-called Heyde's syndrome [9].

The standard treatment of iron deficiency involves supplementation with solid or liquid iron supplement preparations; there are many official formulations (drugs), as well as wide range of dietary supplements and food additives. Official formulations usually based on a ferrous salt, such as: ferrous sulphate, ferrous fumarate, ferrous gluconate and etc. On the contrary of official formulations dietary supplements and food additives based on various concentrates of high iron containing plants and food [10]. In spite of the availability of classic recipes (drugs and dietary supplements) there are also modern and traditional achievements for iron deficiency treatment. For example, nanosized polyphosphate bodies (PPB) are potential iron supplements for iron deficiency treatment, which can be produced by microalgal cell factories [11]. Traditional medicines, such as Ayurveda, prescribe herbal formulations containing sugarcane derivatives for the management of pandu, a condition similar to IDA. This article reviews molasses, a sugar industry by-product, as a potential raw material to develop nutraceutical products for IDA. Molasses contains iron and its absorption enhancers, such as sulfur, fructose, and copper, which make it a potential dietary supplement for IDA [12].

Another problem is the determination of iron quantity in this all supplements and drugs, despite it, that some formulations have their monographs in current pharmacopeias (USP, Ph.Eur.) [13-16]. However, pharmacopeia monographs are not always useful. The pharmacopeia monograph's methods for quantification of iron, in most cases are highly expensive, complicated, demanding high quality personal and some unreachable reagents. This is a real challenge for quality control laboratory.

MATERIALS AND METHODS

Apparatus

A standard borosilburrets, pipetts, standard flasks, measuring cylinders and conical flasks are calibrated as per International Conference on Harmonization (ICH) guidelines.

Iron(III)chloride hexahydrate (Merck), Disodium EDTA was AR Grade (Merck); Sulfuric acid (Alpha Chemika), Hydrochloric acid (Alpha Chemika), Nitric acid (Carl Roth), Sulfosalicylic acid (Carl Roth), Ammonia solution 25% (Alpha Chemika), Water was taken from Milli-QR reference water purification system.

General Procedure

483.035 mg (100 mg Fe) exactly weighed iron (III) chloride was placed in a 250 ml conical flask, 5 ml of concentrated nitric acid, 2 ml of concentrated hydrochloric acid and 5 ml of water was added and heated on a plate for 3 minutes. Cooled to room temperature, then added 50 ml of water, shaken during 1 minutes. and adjusted pH 2 with 25% ammonia solution. The obtained solution was cooled to room temperature and added 5 ml of 10% sulfosalicylic acid solution. Titrate with 0.05M EDTA solution until the reddish-purplecolor changes to light yellow. 1 ml of 0.05 M EDTA solution is equivalent to 2.7925 mg of iron.

RESULTS AND DISCUSSIONS

Complexometric titrations of metal ions serve as analytical determinations of different metal ions in millimole concentrations. The substances that have a pair of unshared electrons (e.g. on N, O, S atoms in the molecule) are capable to satisfy the coordination number of the metal ions. The metal ions (in our case iron III ions)are Lewis acids (electron pair acceptor) and the complexing agent is a Lewis base (electron pair donor). Usually, the result of their interaction is a very stable chelate. A chelating agent is an organic substance that has two or more groups capable of bondingwith metal ions, forming a chelate. This type of titration is called a chelometric titration, which is a particular type of complexometric titration. There are six bonding groups in EDTA molecule. The EDTA is represented by the symbol H4Y. The four hydrogens in the formula refer to the four acidic hydrogens on the four-carboxyl groups. It is a triprotic acid. The pair of unshared electrons is located on each of the two nitrogen atoms and each of the four-carboxyl groups [17,18].

Sulfosalicylic acid is used as an indicator. Interaction of sulfosalicylic acid with ferric (Fe^{3+}) ions, depending on the acid-base composition of reaction medium, could lead to the formation of three complex compounds of various colors and compositions. At pH of 1.8–2.5 it becomes reddish-purple, at 4–8 - red, and at 8–11 - yellow. This method of determination of iron is also used in the determination of Ferrous (Fe^{2+}). Ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions are two oxidation states of iron that are easily interchangeable [19].

The method was validated regarding to linearity, precision and accuracy, following the suggestions of the International Conference on Harmonization (ICH) and USP [20].

The relationship between tested analyte (different weights) and response (burette reading) is expressed by linearity; which measured by regression coefficient (R^2). Following ICH guidance it must be of value less than one. In this context, the linearity calculated in range (40-120)mg with R^2 equal to 1.(Table 3 and Figure 1).

Table 3: Linearity.

N analysis	Weighed (FeCl3 x 6H20) (mg)	Fe (mg)	Titrant consumption (ml)	Content (%)
1	193.214	40	14.30	99.83
2	241.517	50	17.90	99.97
3	289.821	60	21.50	100.06
4	338.125	70	25.00	99.73
5	386.428	80	28.65	100.00
6	434.732	90	32.25	100.06
7	483.035	100	35.75	99.83
8	531.339	110	39.40	100.02
9	579.642	120	43.00	100.06

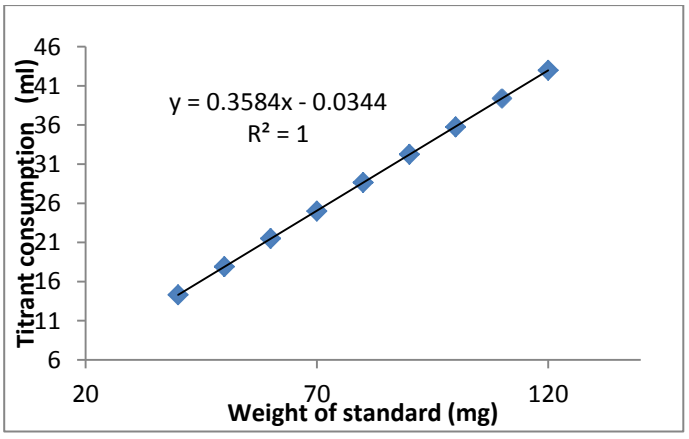

Figure 1: Linearity curve

The precision is usually expressed as the RSD of a series of measurements. It expresses the proximity of compliance between a series of assessments obtained from multiple sampling of the aforementioned homogeneous specimen under the prescribed conditions.

Titrate 483.035 mg iron (III) chloride (100 mg Fe) of 6 samples of the standard solution. By consumed the volume of the titrant calculate the concentration of iron (Table 4). The convergence of the method is assessed by the standard deviation (SD) and the relative standard deviation (RSD) for 6 standard solution samples. Acceptance criterion: RSD ≤ 2.0%.

Table 4: Precision.

N analysis	Weighed (mg)	Titrant consumption (ml)	Content (%)	$\bar{x}$	SD	RSD (%)
1	483.035 (100 mg Fe)	35.80	99.97 %	99.97 %	0.09	0.09%
2		35.80	99.97%			
3		35.75	99.83%			
4		35.85	100.11%			
5		35.80	99.97%			
6		35.80	99.97%			

The intermediate precision of the volumetricmethod was performed by analyzing the sampleson two different persons.The results were presented both separately andas the mean. (Tables 5 & 6)

A analyst

Table 5: Intermediate precision.

N	Weighed (FeCl3 x 6 H20) (mg)	Fe (mg)	Titrant consumption (ml)	Content (%)	$\bar{x}$	□D	RSD (%)
1	483.11	100.01	35.80	99.96	99.93%	0.05	0.05
2	483.75	100.14	35.85	99.97			
3	483.25	100.04	35.80	99.93			
4	483.82	100.16	35.85	99.95			
5	483.20	100.03	35.80	99.94			
6	483.01	99.99	35.75	99.84			

B analyst

Table 6: Intermediate precision.

N	Weighed (FeCl3 x 6 H20) (mg)	Fe (mg)	Titrant consumption (ml)	Content (%)	$\bar{x}$	□D	RSD (%)
1	483.50	100.09	35.85	100.02	99.92%	0.08	0.08
2	484.02	100.20	35.85	99.91			
3	483.53	100.10	35.85	100.01			
4	483.23	100.04	35.80	99.93			
5	483.09	100.01	35.75	99.82			
6	484.15	100.23	35.85	99.88			

The ratio of the differences in the relative standard deviations obtained analysts A and B is:
0.05 : 0.08= 0.625. Acceptance criterion: ≤ 5.0.

Recovery studies were carried out to evaluate the accuracy of the method, using 9 samples at three different levels (80%, 100% and 120%) from standard iron (III) chloride. (Table 7 and Figure 2)

Table 7: Accuracy.

N analysis	Weighed, FeCl3 x 6H20, (mg)	Titrant consumption (ml)	Recovered analyte (mg)	Content (%)	$\bar{x}$	RSD (%)
1	386.428 contains 80 (Fe)	28.60	79.86	99.82	99.88 %	0.10
2		28.60	79.86	99.82		
3		28.65	80.00	100		
4	483.035 contains 100 (Fe)	35.70	99.69	99.69	99.78 %	0.08
5		35.75	99.83	99.83		
6		35.75	99.83	99.83		
7	579.642 contains 120 (Fe)	43.10	120.35	100.29	100.32 %	0.06
8		43.10	120.35	100.29		
9		43.15	120.49	100.40		

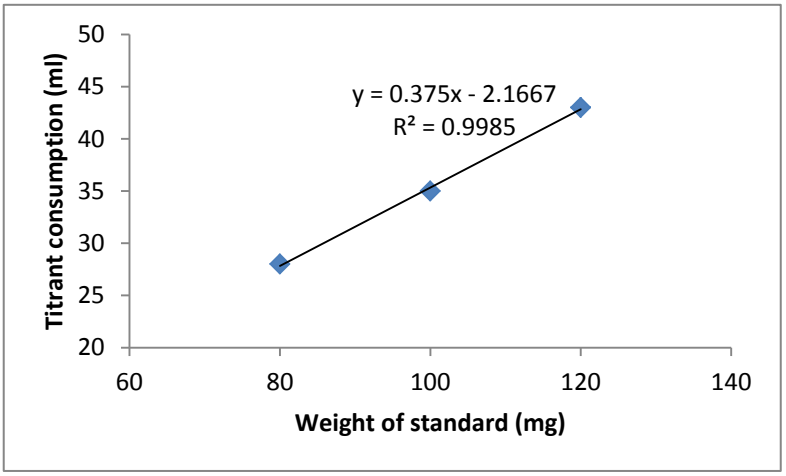

Figure 2: Accuracy curve.

Robustness is a property of the analytical method that characterizes independence of influence on the research result of deliberate changes in parameters method. Robustness depends on the type of analytical method. For the complexiметric method titration used to quantify of iron in Gino-tardiferon 80 mg drug, can serve as a confirmation of robustness results of studies carried out by varying the volume of the analyte (Table 8).

Table 8: Robustness.

N	Initial amount of Gino-Tardi-feron (mg)	Fe (mg)	Titrant consumption (ml)	Content (%)	RSD (%)
1	360.80	80.00	28.60	99.83 %	0.14
2	360.89	80.01	28.60	99.80 %	
3	362.32	80.33	28.65	99.58 %	

The volumetric method proposed is easy, sensitive and inexpensive and can consequently be applied to the determination of iron in tablets dosage form. Method validation including linearity, precision and accuracy generated acceptable results.

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