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Development and Validation of Complexometric Titration Method for the Determination of Iron in Iron-Containing Liquid Dosage Forms Registered in the Republic of Armenia

V.G. Kirakosyan [1,2], A.M. Davinyan [1], A.V.Ginosyan[1,2], L.H. Varderesyan[1]

[1]Scientific Centre of Drug and Medical Technology Expertise after Academician E.Gabrielyan
49/4 Komitas av.,Yerevan, 0051, Armenia
[2]Yerevan State University
1 Alex Manoogyan Str., Yerevan, 0025, Armenia
E-mail: virb.kirakosyan@ysu.am

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Introduction

Iron containing dietary supplements and drug sare widely used for the treatment and prevention of various types of anemia, mainly caused by low levels of it in everyday food. The anemia, with its various types and difficulties, is very common nowadays, as a result we have a lot of iron containing dietary supplements and official drugs in the pharmaceutical market. Iron drugs and dietary supplements (in form of ferrous fumarate, ferrous gluconate, ferrous sulfate) come as regular, film-coated and extended release tablets, capsules and oral liquids. The availability and variety of iron containing drugs and dietary supplements have its advantages and disadvantages, first of all it is perfect for doctors and patients, because they have a relatively wide range to choose among the brands, as well as among the formulations [1,8,10]. 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, Eur. Pharm.) [2,3,6,7,12]. However, pharmacopeia monographs are not always useful. The pharmacopeia monograf"s methods for quantification of iron, in most cases are highly expensive, complicated, demanding high quality personnel and some unreachable reagents. This is a real challenge for quality control laboratory.

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.

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 [5], 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-Q^R reference water purification system.

General Procedure: 483.03 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 1 minute. Cooled to room temperature, then added 50 ml of water, shaken during 1 minute and adjusted to pH=2 with 25% ammonia solution. The obtained solution was cooled to room temperature and added to 5 mg sulfosalicylic acid. Titrate with 0.05M EDTA solution until the reddish-purple color changes to light yellow. 1 ml of 0.05 M EDTA solution is equivalent to 2.7925 mg of iron.

Results and Discussion

Although numerous highly efficient instrumental methods are now available for the determination of metal ions and other inorganic species, complexometric titrations are still widely used in routine analysis. The versatility, sensitivity, and general convenience of complexometric titrations are dependent on the correct choice of indicators for endpoint detection. [9]. One of the indicators used in complexometric titration is sulfosalicylic acid. 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 [11].

The method was validated regarding to linearity, precision, accuracy and robustness following the suggestions of the International Conference on

Harmonization (ICH) and USP [4]. 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 (5-120 mg) with R^2 equal to 1 (Table 1 and Figure 1).

Table 1

Linearity

N analysis	Weighed, $FeCl_3$ x $6H_20$, (mg)	Fe (mg)	Titrant consumption (ml)	Content %
1	24.15	5	1.78	99.41
2	48.30	10	3.60	100.53
3	96.60	20	7.15	99.83
4	144.91	30	10.70	99.60
5	193.21	40	14.30	99.83
6	241.51	50	17.90	99.97
7	289.82	60	21.50	100.06
8	338.12	70	25.00	99.73
9	386.42	80	28.65	100.00
10	434.73	90	32.25	100.06
11	483.03	100	35.75	99.83
12	531.33	110	39.40	100.02
13	579.64	120	43.00	100.06

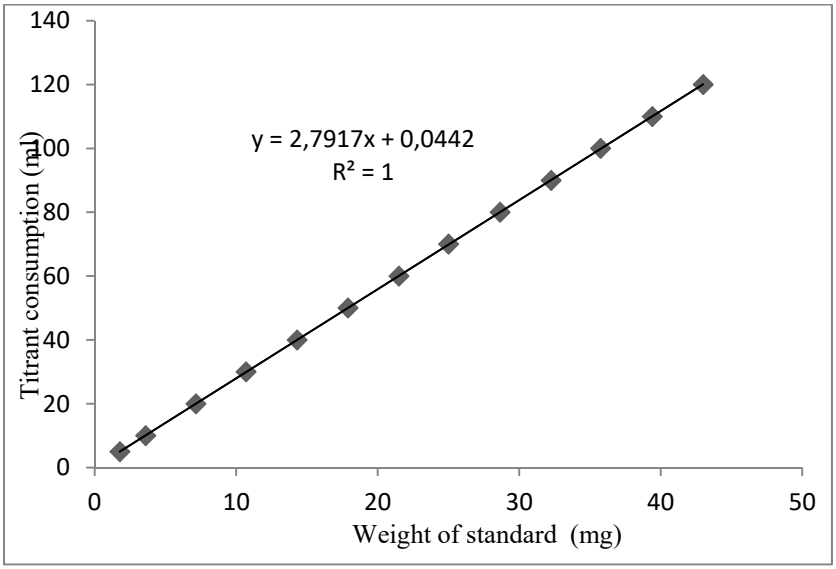

Fig. 1. Linearity curve

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 482.64 mg iron (III) chloride (99.92 mg Fe) of 6 samples of the standard solution. By consumed the volume of the titrant calculate the concentration of iron (Table 2).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 2

Precision

N analysis	Weighed (mg)	Titrant consumption (ml)	Content (%)	$\bar{x}$	*S*D	RSD
1	482.64 (99.92 mg Fe)	35.80	100.05	100.03 %	0.14	0.14 %
2		35.80	100.05			
3		35.70	99.77			
4		35.85	100.19			
5		35.80	100.05			
6		35.80	100.05			

The intermediate precision of the volumetric method was performed by analyzing the sampleson two different persons.The results were presented both separately and as the mean (Table 3,4).

Table 3

Intermediate precision

A analyst

N	Weighed, mg ($FeCl_3$ x 6 H_20)	Fe (mg)	Titrant consumption (ml)	Content (%)	$\bar{x}$	*S*D	RSD
1	480.02	99.38	35.50	99.75	99.96 %	0.12	0.12 %
2	479.26	99.22	35.50	99.91			
3	478.27	99.01	35.45	99.98			
4	483.82	100.16	35.90	100.09			
5	482.56	99.90	35.80	100.07			
6	483.25	100.04	35.80	99.93			

Table 4

Intermediate precision

B analyst

N	Weighed, mg ($FeCl_3$ x 6 H_20)	Fe (mg)	Titrant consump-tion (ml)	Content (%)	$\bar{x}$	*S*D	RSD
1	483.50	100.09	35.85	100.02	99.96 %	0.049	0.05 %
2	484.44	100.29	35.90	99.96			
3	483.53	100.10	35.85	100.01			
4	483.25	100.04	35.80	99.93			
5	483.09	100.01	35.80	99.96			
6	484.09	100.22	35.85	99.89			

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 5).

Table 5

Accuracy

N analysis	Weighed, $FeCl_3$ x $6H_20$ (mg)	Titrant consumption (ml)	Recovered analyte (mg)	Content (%)	$\bar{x}$	RSD (%)
1	386.30 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	483.00 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	580.00 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		

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 complexim**e**tric method titration used to quantify of iron in Ferrum Lek 50 mg/5 ml sirup (density 1.015 g/ml), can serve as a confirmation of robustness

results of studies carried out by varying the volume of the analyte (Table 6). The content of the analyte in the sample is calculated according to the following formula:

$$\text{Fe (mg/ml)} = \frac{V_{titrant\ consumption} \cdot K \cdot 2.7925 \cdot \rho \cdot 1\,\text{ml}}{m}$$

V – titrant consumption (ml), K – correction factor, 2.7925 mg – 1 ml of 0.05M EDTA solution is equivalent to 2.7925 mg of iron, ρ – drug density (g/ml), m – sample size (g).

Table 6

Robustness

N	Initial amount of Ferrum Lek (g)	Fe (mg)	Titrant consumption (ml)	Content (%)	RSD (%)
1	1.010	10.1	3.60	101.02	0.15
2	0.995	9.95	3.55	101.12	
3	1.012	10.12	3.60	100.83	

Conclusion

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

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Разработка и валидация метода комплексометрического титрования для определения количества железа в железосодержащих жидких лекарственных формах, зарегистрированных в Республике Армения

В.Г. Киракосян, А.М. Давинян, А.В.Гиносян, Л.А. Вардересян

Разработанный метод был валидным, поскольку он успешно удовлетворял желаемым критериям руководящих принципов Международной конференции по гармонизации (ICH), линейности, правильности, прецизионности и устойчивости. Метод экономичен и не требует большого количества времени, по сравнению с другими известными аналитическими методами. Таким образом, этот метод подходит для рутинного анализа

определения железа в жидких лекарственных формах без вмешательства вспомогательных веществ.

Հայաստանի Հանրապետությունում գրանցված երկաթ պարունակող հեղուկ դեղաձևերում երկաթի քանակության որոշման կոմպլեքսաչափական եղանակի մշակում և վալիդացում

Վ.Գ. Կիրակոսյան, Ա.Մ. Դավինյան, Ա.Վ.Գինոսյան, Լ.Հ. Վարդերեսյան

Մշակված մեթոդը համարվում է վալիդացված, քանի որ այն հաջողությամբ բավարարել է Ներդաշնակեցման միջազգային համաժողովի (ICH) ուղեցույցի չափանիշներին՝ գծայնությամբ, ճշտությամբ, ճշգրտությամբ և կայունությամբ: Մեթոդը տնտեսապես մատչելի է և երկարատև ժամանակ չի պահանջում այլ հայտնի վերլուծական մեթոդների համեմատությամբ: Այսպիսով, այս մեթոդը հարմար է հեղուկ դեղաձևերում երկաթի քանակական որոշման ամենօրյա վերլուծություններում, որի ընթացքը չեն խոչընդոտում նրանցում առկա օժանդակ նյութերը:

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