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Quantitative analysis of the macromolecular complex of iron (III) hydroxide with polymaltose in liquid dosage forms

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Abstract

Background: It is fundamental to develop new analysis methods for proving the utmost quality of medicines containing iron compounds. This study aims to determine whether the quantity of Fe (III) in dosage forms taken for analysis is equivalent to the concentration specified by the manufacturer.
Materials and methods: The concentration of Fe (III) in dosage forms taken for the study was determined using the standard addition method at acidic and alkaline pH using the sulfosalicylic acid as reagent. The obtained data was analyzed mathematically and graphically. The iodometric method of titration was applied to verify the obtained results.
Results: According to mathematical data analysis, the concentration of Fe (III) in the Ferimax syrup was 45.00 ± 2.23 mg/5ml at acidic and 50.11 ± 0.5 mg/5ml at alkaline pH. Using the graphical method, the concentration of Fe (III) was 43.75 mg/5ml at acidic and 50.60 mg/5ml at alkaline pH. For Ferrum Lek, the Fe (III) concentration was 50.68 ± 0.73 mg/5ml and 50.60 mg/5ml at alkaline pH using the mathematical and graphical methods. The redox titration method showed that in the Ferimax syrup, the average mass of Fe (III) is 44.07±0.7 mg/5ml, and in Ferrum Lek – 50.36±0.26 mg/5ml.
Conclusions: The concentration of Fe (III) in Ferimax syrup in acidic medium was lower than the reference of the label, while in basic medium both Ferimax and Ferrum Lek syrups have the expected concentration.

Key words: photometry, iodometry, iron polymaltose, Ferimax, Ferrum Lek.

Introduction

Iron plays an important role in the vital processes of the organism like oxygen and electrons transportation, DNA and steroids synthesis. It is an important structural part of hemoglobin, myoglobin, as well as the one of many enzymes. Because of its lack, symptoms like chronic fatigue, muscular weakness, loss of concentration, decreased resistance to stress and microbial infections or skin dryness can occur in the organism [1]. Among other medications, the doctors recommend the administration of drugs containing the complex of iron (III)-hydroxide with polymaltose for relieving the symptoms mentioned above [2]. According to the National Clinical Protocol "Iron deficiency anemia in children" approved by the Ministry of Health of the Republic of Moldova, decree No. 442 of 10.04.2013 among the medications used for the treatment of iron deficiency in children is listed the complex of iron (III) hydroxide with polymaltose in the following dosage forms: syrups, solutions or tablets [3].

The active ingredient of the above-mentioned dosage forms represents polynuclear molecules of iron (III) hydroxide surrounded by the polymaltose molecules thereby forming a stable complex. This way, the unionized iron does not interact with food and does not form reactive oxygen species which could damage the membranous structures of the gastro intestinal tract [4]. The structure of the complex maximally resembles that of the natural iron compounds with ferritin, a protein occurring naturally in the human body. Due to the similarity, the complex is absorbed through an active mechanism, and thus prevents the overdosage [5].

The advantage of iron polymaltose complex is a lower adverse reaction incidence in comparison with ionized Fe^{2+} compounds such as iron sulfate [6]. Thus, the development of new analyses methods for iron (III) is of importance. Its purpose is to ensure the good quality of the dosage forms. The aim of the study further exposed consists in the elaboration of new methods of quantitative analysis of iron (III) in two liquid dosage forms applying the photometric method of standard addition using the sulfosalicylic acid as the reagent.

Material and methods

In the Moldovan pharmacies, Ferimax and Ferrum Lek are the two most frequently found iron (III) containing syrups in doses equivalent to 50 mg/5ml of elemental iron. Thereby, both of these syrups were used in the study.

Laboratory glassware and instruments: volumetric flasks of different capacities, automatic pipettes from brand DAC-pette with volumes ranging between 100 – 1000 µl and 1000 – 5000 µl. In the titrimetric method the titrant volume measurement is a 2.0 ml microburette, while for photometric method the solution absorbance was determined by means of photoelectrocolorimeter KFK-2MP [КФК-2МП] at wavelengths of 400 and 490 nm using cuvettes of 1 cm

path length. The molecular absorption spectra were measured using the Agilent 5483 spectrophotometer. The experimental data obtained was statistically analyzed.

Standard solutions: Mohr Salt $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$ is the initial reagent used in photometric method which was recrystallized from distillated water. It was used for preparing the standard solution [7]. A probe of this salt with the mass equal to 0.17553 g was transferred to 500 ml volumetric flask and mixed with 50 ml of distillated water, 2.5 ml of 1 mol/l of H_2SO_4 and 0.5 ml of concentrated HNO_3 which was added for oxidizing Fe (II) to Fe (III). The solution was further heated up to the boiling point until its color changed to yellow. After cooling, the solution was diluted with distilled water till mark and was homogenized. The molar concentration of Fe (III) in the standard solution was $4.4762 \cdot 10^{-4}$ mol/l and the concentration of Fe (III) was 0.05 mg/ml.

Sulfosalicylic acid with the mass fraction of 10% was prepared using the following compound $C_6H_6O_6S \cdot 2H_2O$. The $\omega(NH_3)$ = 10% solution was prepared by diluting the 25% ammonium solution.

The iodometric method for oxidant titration was also used in the study for quantitative determination of Fe (III) in studied pharmaceutical forms, in which was used $Na_2S_2O_3$ solution. This solution was prepared from fixanal, using boiled purified water and cooled to room temperature.

The solution with $c(Na_2S_2O_3)$=0.01 mol/l was prepared by dilution also using boiled purified water and cooled to room temperature. The standardization was performed using $c(1/6KIO_3)$=0,01 mol/l standard solution which was prepared from a probe of weighed accurately KIO_3 (chemically pure). The ω(KI)=10% solution was made from a chemically pure probe of KI and the 0,5% starch solution was prepared as usual.

Sample preparation: A 50 ml volumetric flask plugged with a cap was preventively weighed. After adding 0.9 ml of syrup using an automated pipette the volumetric flask was weighed with its plug cap again and the mass of the syrup sample was determined by difference. After, 2-3 ml of distillated water and 2.0 ml of 1 mol/l of H_2SO_4 were added to the flask and it was placed in a 100°C heated water bath. The dark brown color of the sample solution disappeared after 1.5 – 2.0 minutes and changed to light yellow color for each syrup. After cooling the solutions were brought to volume with purified water, and homogenized. The sample solution of Ferrum Lek had a light-yellow tint and the one of Ferimax a light purple tone. Distillated water was added up to the graduation marking.

Fe (III) quantitative analysis procedure: The quantitative analysis of Fe (III) from the sample solutions was performed using the photometric method of standard addition having sulfosalicylic acid as the reagent [8,]. The same volume of the sample solution was added to a few 50 ml volumetric flasks. Starting the second volumetric flask, increasing quantities (ml) of standard Fe (III) solution and 4 ml of 10% sulfosalicylic acid were successively added. For creating a basic pH, 4.0 ml of 10 % ammonium solution was additionally mixed. In the end, distillated water was added up to 50 ml and the formed solution was left for 10 minutes before measuring the absorbance. Afterwards, their absorbances were measured at the respective wavelength.

The iron (III) in the slightly acidified solutions to be analyzed of the studied syrups was determined quantitatively also by iodometric method of oxidants dosage, according to the following procedure. In a titration beaker for each analysis different, but exactly measured volumes of syrup solution to be analyzed by means of automatic pipette was added. Later 5-6 ml of solution with ω (KI) = 10% was added and the solution obtained was left in rest for 5 minutes. After this, the iodine released in an amount equivalent to the Fe (III) content in the solution to be analyzed was titrated with standardized $Na_2S_2O_3$ solution. To the end of the titration in the titration beaker was added 6-8 drops of starch solution and the titration was continued until the blue color of the solution appeared.

Results and discussion

Sulfosalicylic acid is used for the quantitative determination of iron (III) at acidic pH (1.8 – 2.5) forming Fe (III) mono-sulfosalicylate which has a purple color (maximum absorption at λ_{max} = 510 nm [11], or at λ_{max} = 505 nm according to [12]).

At basic pH (9.0 – 11.5) a yellow colored complex compound is formed (λ_{max} = 416 nm [11], but according to [13] the absorbance is measured at λ = 420 – 430 nm) in which the ratio between the metal and the ligand is 1:2 [11]. Fe (II) and Fe (III) can be determined separately using the photometric method at this pH as well as their total amount when they are both present in the solution [10, 13].

Preventive experiments have demonstrated that the absorption spectrum of the Ferimax syrup solution and the spectrum of a standard solution of Fe (III) with sulfosalicylic acid are identical in both the acidic and the basic media and absorb maximum electromagnetic radiation at λ_{max}=504 nm and λ_{max}=424 nm (fig. 1).

Similar spectra to those from fig. 1 which absorb maximum electromagnetic radiation at the same wavelengths in the acidic and basic media were obtained using the Fe (III) solution obtained from Ferrum Lek syrup with sulfosalicylic acid.

In the photometric method for the quantitative analysis of Fe (III) in the solutions to be analyzed, obtained from the studied pharmaceutical forms, the standard additions method was used, which was used in two variants: the calculation method and the graphical method [8, 10]. The more detailed essence of the calculation method and the final formula used to calculate the results of the analysis was previously described [10, 14]. In this report we present only the final formula after which the iron mass (m_{Fe}, mg/5ml) in the liquid pharmaceutical form was calculated by the calculation method of the standard addition:

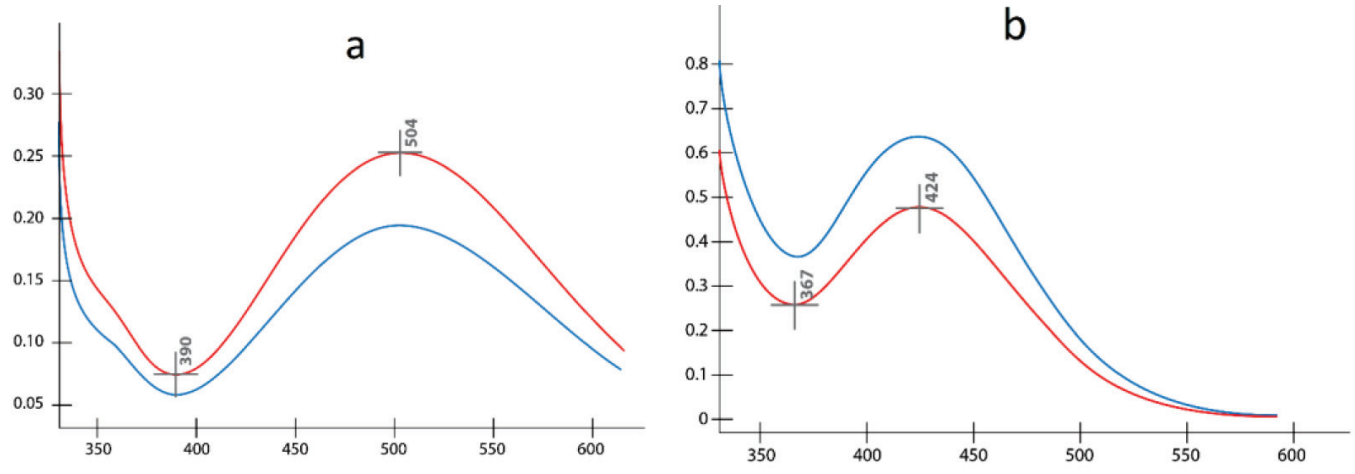

Fig. 1. Absorption spectra of the solution to be analyzed in: a – acidic medium, b – basic medium.

$$m_{Fe} = \frac{A_x \cdot m_i \cdot V_i \cdot V_0 \cdot \rho \cdot 5}{(A_{x+a} - A_x) \cdot V_1 \cdot m_p} \quad (1)$$

in which:

A_x – the absorbance of the solution, in the preparation of which a certain volume of the solution to be analyzed of respective solution with the unknown Fe (III) mass;

A_{x+a} – the absorbance of the solution, in the preparation of which a certain volume of the solution to be analyzed of respective solution with the unknown Fe (III) mass, but also with the addition of a certain volume of standard Fe (III) solution;

m_i – the iron content in the initial standard solution of Fe (III), mg/ml;

V_i – volume of initial standard solution of Fe (III), which was taken for preparation of the solution with the A_{x+a} absorbance, ml.

V_0 – the capacity of the volumetric flask with the initial solution to be analyzed after decomposition of the complex of iron (III)-hydroxide with polymaltose from respective liquid pharmaceutical form taken for analysis, ml;

V_1 – the fraction of the initial Fe (III) solution to be analyzed after decomposition of the complex of iron (III)-hydroxide with polymaltose from respective liquid pharmaceutical form taken for analysis, ml.

m_p – mass of the sample solution taken for analysis, g;

ρ – the density of the syrup, g/ml.

The data obtained from the experiment, as well as the Fe (III) mass calculated using equation (1) is presented in the tables 1a, 1b and 2.

Table 1

Data for determination of Fe (III) content with sulfosalicylic acid in the Ferimax syrup by standard addition method

a – acidic pH

No.	V_1, ml	V_i, ml	m_a, mg	A_x & A_{x+a}	m_{Fe}, mg/ml
1	1.0	-	-	0.105	-
2	1.0	1.0	0.05	0.139	42.89
3	1.0	2.0	0.10	0.172	43.53
4	1.0	3.0	0.15	0.202	45.10
5	1.0	4.0	0.20	0.229	47.04
6	1.0	5.0	0.25	0.262	46.45

(V_0= 50 ml; m_i=0,05 mg/ml; m_a=m_i·V_i; m_p=1,0572 g; ρ=1,1747 g/ml; pH 2,0 – 2,1)

b – basic pH

No.	V_1, ml	V_i, ml	m_a, mg	A_x & A_{x+a}	m_{Fe}, mg/ml
1	0.5	-	-	0.082	-
2	0.5	0.5	0.025	0.123	50.00
3	0.5	1.0	0.050	0.165	49.40
4	0.5	1.5	0.075	0.204	50.41
5	0.5	2.0	0.100	0.246	50.00
6	0.5	2.5	0.125	0.287	50.00
7	0.5	3.0	0.150	0.324	50.83

(V_0= 50 ml; m_i=0,05 mg/ml; m_a=m_i·V_i; m_p=0,58155g; ρ=1,1631 g/ml)

Table 2

The experimental data for determination of Fe (III) content with sulfosalicylic acid in the Ferrum Lek syrup by standard addition method in basic medium

No.	V_1, ml	V_i, ml	m_a, mg	A_x & A_{x+a}	m_{Fe}, mg/ml
1	0.5	-	-	0.085	-
2	0.5	0.5	0.025	0.127	50.60
3	0.5	1.0	0.050	0.171	49.42
4	0.5	1.5	0.075	0.211	50.60
5	0.5	2.0	0.100	0.251	51.20
6	0.5	2.5	0.125	0.294	50.84
7	0.5	3.0	0.150	0.333	51.41

(V_0= 50 ml; m_i=0.05 mg/ml; m_a=m_i·V_i; m_p=0.60165g; ρ=1.2033 g/ml)

Data from tables 1a, 1b, 2 and the average concentration of Fe (III) (mg/5ml) having a 0.95% confidence interval were statistically analyzed. The average concentration of Fe (III) (mg/5ml) in the Ferimax syrup equals to 45.00±2.23 at acidic pH and 50.11±0.5 at basic pH (tab. 1 a and 1 b). The average concentration of Fe (III) (mg/5ml) from the Ferrum Lek syrup at basic pH is 50.68±0.73 (tab. 2).

Data from tables 1a, 1b and 2 were used for photometric determination of Fe (III) concentration applying the graphical method of standard addition. For this, the functional dependence $A_{x+a} = f(m_a)$ was plotted, in which m_a is the mass of the addition. This dependence represents a straight line that intersects the A_x value on the Y – axis, which contains only solution to be analyzed with unknown mass of Fe (III). At the extension of these three straight lines to the intersection with the X – axis, the segments $-m_{a=}\ m_x$ were obtained [10, 14]. For Ferimax syrup in the acidic medium $\overline{m}\overline{m}_x = 0.1575$ mg was obtained, while in the basic medium for both syrups $\overline{m}\overline{m}_x = 0.0506$ mg was obtained. The mass of Fe (III) (mg/5ml) in the syrups was calculated using the following equation:

$$m_{Fe} = \frac{m_x \cdot V_0 \cdot \rho \cdot 5}{V_1 \cdot m_p} \quad (2)$$

in which all the notes see above

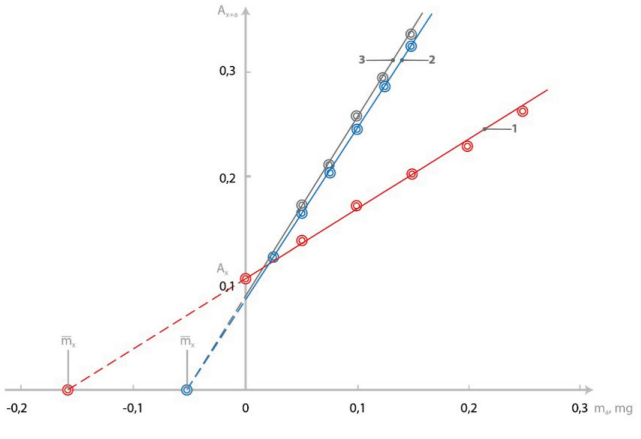

Fig. 2. The Ax+a = f (ma) dependence for photometric determination of Fe (III) with sulfosalicylic acid applying the graphical method of standard addition in:
1 – acidic medium (Ferimax), 2 – basic medium (Ferimax), 3 – basic medium (Ferrum Lek).

The data in tables 1a, 1b and 2 were used for the photometric determination of the Fe (III) mass in the solutions to be analyzed of studied syrups using equation 2 by the graphical method of the standard addition. For Ferimax syrup, the Fe (III) mass in the solution to be analyzed in the acidic medium constituted 43,75 mg/5ml and in basic media the Fe (III) mass in the solution to be analyzed in this syrup was the same as in the solution to be analyzed of Ferrum Lek syrup and constituted 50.60 mg/5ml.

The results obtained in the basic medium by the graphical method of the standard addition for the studied pharmaceutical forms fall within the limits of the acceptance of the obtained mass of Fe (III) in the solutions to be analyzed of these syrups by the photometric method of calculation in the same medium and practically correspond to the data of the manufacturing companies. However, in the basic medium with sulfosalicylic acid the photometric method determines both the Fe (II) and Fe (III), or the sum content of Fe (II) and Fe (III) in the analyzed solution [9, 10, 13]. At the same time, the quantitative determination of the Fe (III) mass in the Ferimax syrup in the acidic medium by the calculation and graphical photometric method of the standard addition showed lower results than those in the basic medium.

A different method of analysis was used to confirm the results obtained by the photometric method. This is the iodometric titration method proposed by the Chinese Pharmacopeia for the analysis of macromolecular complex compound of $Fe(OH)_3$ with polymaltose (iron dextran) [15].

The solutions taken for analysis were the same ones used in the photometric method. In contrast to the iodometric method described in the monograph [15], $KMnO_4$ was not added before adding KI solution. In addition, the diluted titrant solution was used to titrate the iodine eliminated in an amount equivalent to the Fe (III) content in the solution taken for the titration. The results obtained are presented in tables 3 and 4.

Table 3

Iodometric determination of Fe (III) in the Ferimax syrup

No.	V_1, ml	$V(Na_2S_2O_3)$, ml	m_{Fe}, mg/5ml
1	2.0	0.550	45.03
2	2.5	0.680	44.54
3	3.0	0.805	43.94
4	3.5	0.920	43.04
5	4.0	1.090	44.62
6	4.5	1.185	43.12
7	5.0	1.350	43.18

(K=1.0554; V_0= 50 ml; m_p=1.0572g; ρ=1.1747 g/ml)

Table 4

Iodometric determination of Fe (III) in the Ferrum Lek syrup

No.	V_1, ml	$V(Na_2S_2O_3)$, ml	m_{Fe}, mg/5ml
1	3.00	0.96	50.83
2	3.50	1.11	50.38
3	4.00	1.26	50.04
4	4.25	1.34	50.08
5	4.50	1.43	50.48
6	4.75	1.51	50.50
7	5.00	1.58	50.20

(K=1.0239; V_0= 50 ml; m_p=1.0898 g; ρ=1.2109 g/ml)

The mass of Fe (III) (m_{Fe}, mg/5ml) in the respective syrup was calculated using the following equation:

$$m_{Fe} = K \cdot V(Na_2S_2O_3) \cdot 0{,}5585 \cdot \frac{V_0 \cdot \rho \cdot 5}{V_1 \cdot m_p}, \quad (3)$$

K – correction coefficient of the titrant concentration in relation to the theoretical concentration of the 0,01 N $Na_2S_2O_3$ solution;

$V(Na_2S_2O_3)$ – volume of the equivalence of the titrant, ml;

0.5585 – Fe (III) mass equivalent to 1 ml of $Na_2S_2O_3$ (c= 0.01 mol/l), mg/ml.

For more details see equation (1).

The data from tables 3 and 4 was statistically processed to obtain the mean mass of Fe (III) (mg/5ml) determined based on a 95% certitude. For the Ferimax syrup, by titration method, an average Fe (III) mass value of 44.07±0.7 mg/5ml was obtained, and for Ferrum Lek – 50.36±0.26 mg/5ml.

Conclusions

If a compound contains ions of both Fe (II) and Fe (III), through quantitative analysis using sulfosalicylic acid at acidic pH, only Fe (III) can be determined. When Fe (II) and Fe (III) are both present in a solution, the total iron amount is analyzed at basic pH using the same physico-chemical method of analysis mentioned above [8, 9, 12]. The photocolorimetric standard addition method was applied in two variants: graphic and calculation methods as well as the iodometric titration method. Two syrups produced by two different manufacturers with brand names: Ferimax and Ferrum Lek were used for the study. The results obtained after statistical analysis showed that at acidic pH the concentration of Fe (III) in Ferimax is less than 50mg/5ml which proves the presence of Fe (II) ions. While analyzing Ferrum Lek at basic pH also using the statistical methods the Fe (III) concentration was determined to be 50mg/5ml which is the same as mentioned by the manufacturer. The results obtained via the photometric method were confirmed using the iodometric titration method of oxidants, which also proved that the concentration of Fe (III) is less than 50mg/5ml in the Ferimax syrup, while the Fe (III) content in the Ferrum Lek syrup does not have any deviations from the concentration specified by the manufacturing company.

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