

ORIGINAL RAPID UV-Vis SPECTROPHOTOMETRIC
QUANTIFICATION OF FERROUS BISGLYCINATE
IN FOOD SUPPLEMENTS

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Abstract

Ferrous bisglycinate is a salt of the glycine amino acid, and in recent years it has replaced many traditional ferrous salts used in food supplement production. The main advantage of ferrous bisglycinate is its better absorption and that at the same time it is gentle to the digestive system. Development and validation of reliable analytical methods for food supplement control is very important for improving the quality of the products on the market. The current work presents a validated UV-Vis analytical procedure for determination of the content of ferrous bisglycinate in food supplements. The described method is quick colourimetric determination and does not require complicated analytical instruments. The method is reproducible, accurate, precise and linear in a wide range from 0.15 $\mu\text{g/ml}$ to 9.10 $\mu\text{g/ml}$ with linearity coefficient 0.9999. The main problem is to convert existing traces of Fe^{3+} ions in the substance to Fe^{2+} ions and to analyse all available ferrous species. For this reason, initially we need to destroy the complex of glycine with $\text{Fe}^{2+/3+}$ ions, to reduce Fe^{3+} to Fe^{2+} , and then to form a new complex of Fe^{2+} stable at the conditions of the analytical procedure.

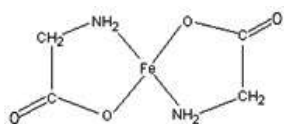
Key words: ferrous bisglycinate, UV-Vis spectroscopy, ferrous complex, ferrous ions, amino acid chelates

1. Introduction. Iron is present in the human body as a component of haemoglobin. People with iron deficiency suffer from anaemia (iron deficiency anaemia (IDA)) [1]. IDA is the most prevalent nutritional disorder worldwide. Iron deficiency anaemia during pregnancy and earlier childhood affects children's intellectual and psychomotor development [2]. The main reasons for IDA are undernutrition, diets with little meat intake, or people being exposed to infections that cause blood loss [3].

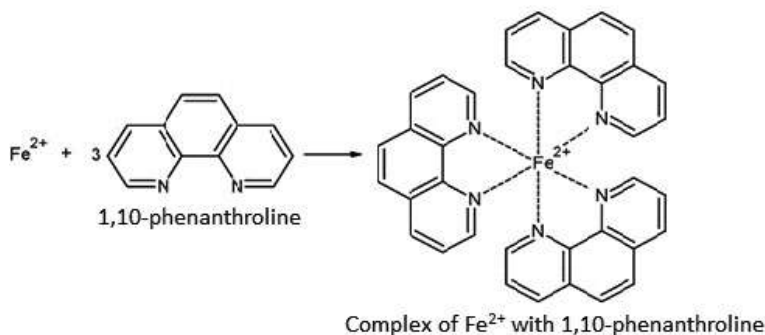
To treat iron deficiency anaemia, the first step is to treat the main cause of iron deficiency – usually doctors recommend taking iron supplements. As one of the most widespread elements on Earth, iron exists as a lot of chemical compounds, mainly salts. Iron salts are often used for treating iron deficiency: ferrous sulphate, ferrous fumarate, ferrous gluconate, and others. Very often, they have a tendency to cause gastrointestinal side effects. Polysaccharide-iron complex has a lower risk of gastrointestinal intolerance and better taste but is less effective than ferrous salts. Heme iron has a higher absorption rate but is more expensive and is not a viable option for vegetarians or vegans. According to the data in [4], iron protein succinylate may have a better tolerability and efficacy as compared to ferrous salts but is unsuitable for persons with hypersensitivity to milk protein. Carbonyl iron is inexpensive, but its efficacy and side effect benefits are unclear. Iron amino acid chelates (ferrous bisglycinate, ferric trisglycinate) are less susceptible to dietary interactions but more expensive than ferrous salts [4].

In natural compounds, iron exists in two oxidation forms, ferrous cation (Fe^{2+}) and ferric cation (Fe^{3+}). The iron contained in food, which is non-heme, is mainly in the ferric state, but the ferric state of iron is less soluble and for better absorption ferric cations must be reduced to ferrous ions. This is the reason iron food supplements are mainly ferrous and not ferric salts. Ferrous (II) preparations usually have a better bioavailability (3 to 4 times more) than the bioavailability of iron ferric preparations [5]. Formulations with iron (III)-hydroxide polymaltose complex generally show poorer bioavailability [6]. There is a need for establishing more effective oral iron preparations as the fraction of absorbed iron is insufficient and is frequently limited by side effects [4]. As a better compromising option to iron ferrous salts, ferrous bisglycinate is used. In this compound, the two-valent iron is in a complex form with glycine. The ratio of the amino acid glycine is twice the amount of Fe^{2+} ions, so it restricts unwanted reaction and is gentler to the gastrointestinal surface than ferrous salts. Each chelated Fe atom has strong chelating bonds and resists cleavage conditions in the digestive system [7]. A lot of new iron food supplements are based on ferrous bisglycinate, so it is also necessary to develop methods for analysis of this substance.

Usually atomic absorption (AAS) and inductive coupled plasma (ICP) are the analytical techniques used for the determination of metal atoms or ions [8–11]; titration is also applicable [12, 13]. However, spectrophotometric methods are used very often for iron determination because of the good complexation of



Structural Formula of Ferrous Bisglycinate



Complex of Fe^{2+} with 1,10-phenanthroline

Fig. 1. Structural formula of ferrous bisglycinate and structure of a complex of Fe^{2+} with 1,10-phenanthroline

all species of Fe atoms and ions with different ligands [14–19]. In the current work, a spectrophotometric method for the determination of ferrous bisglycinate is presented with detailed explanation of every step of the development process.

2. Materials and methods. The substance ferrous bisglycinate (Fig. 1) was obtained from Dr. Paul Lohmann[®].

According to its certificate of analysis, it contained between 19–23% Fe (II) and up to 4% Fe (III). The substance ferrous bisglycinate contained about 15% citric acid and 5% water, so the total percentage of ferrous bisglycinate in the substance was about 80%. Hydroxylamine hydrochloride ReagentPlus[®] 99% and 1,10-phenanthroline $\geq 99\%$ was obtained from Sigma-Aldrich (Merck) sodium acetate anhydrous for analysis EMSURE[®] ACS, Reag. Ph Eur, and sulphuric acid 95–97% EMSURE[®] was obtained from Merck and in-house Milli Q-water was used. The analytical conditions of the UV-Vis colourimetric determination of total iron were optimised according to the maximum of the UV spectra and the best conditions to form a new complex for clear and reliable determination.

2.1. Preparation of diluents, standard and sample solutions. Diluent 1 – a solution with concentration 0.5 M of sulphuric acid and 1.2 M of sodium acetate was prepared. **Diluent 2** – Milli Q-water. The prepared solution of 1,10-phenanthroline was with concentration of 2 mg/ml. Five percent solution of hydroxylamine hydrochloride was prepared.

2.1.1. Standard solution. About 69 mg (equivalent to 15 mg of ferrous iron) were weighed and transferred to a 100 ml dried volumetric flask, 5 ml of diluent 1

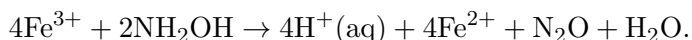
were added and dissolved, then made up to the volume with diluent 2. About 10 ml were filtered through a nylon syringe filter and the first 2 ml were rejected. Two millilitres from the filtrate were transferred to a 50 ml volumetric flask; 10 ml deionized water, 5 ml 5% solution of hydroxylamine hydrochloride and 4 ml solution of 1,10-phenanthroline with concentration 2 mg/ml were added. It was let to stay for at least 20 min, then made up to the volume with deionized water. The final concentration of the solution according to Fe^{2+} was about 6 $\mu\text{g}/\text{ml}$.

2.1.2. Placebo solution. About 180 mg excipients (microcrystalline cellulose – 170 mg, colloidal SiO_2 – 5 mg and magnesium stearate – 5 mg) were weighed and transferred to 100 ml and developed as a standard and sample solutions.

2.1.3. Sample solution. Capsule content of 20 capsules was mixed and an amount equivalent to 15 mg of ferrous iron was weighed. It was transferred to a 100 ml volumetric flask and developed as a standard solution.

The prepared solutions were measured by UV-Vis spectrophotometer EVOLUTION 300 equipped with the VISIONpro V4.20 software at visible spectra at 512 nm, where the ferrous complex has a maximum in the spectrum.

3. Results and discussion. The water solution of ferrous bisglycinate had a green-brown colour, but when 5 ml of diluent 1 were added, the solution gradually became colourless. The reason was that the pH of the solution dramatically changed from about 7.5 when the solvent was deionized water to 1.8 after adding 5 ml of diluent 1. The addition of diluent 1 destroyed the complex of ferrous iron with glycine, as at a pH range below 3 the complex is unstable [20]. Another problem is the presence of some amount of ferrous trisglycinate in the substance, whose spectrum overlaps with the spectrum of ferrous bisglycinate. Both complexes do not have equivalent colours, so their simultaneous determination is not recommended. After adding diluent 1, both complexes were destroyed and the solution gradually became transparent and the colour disappeared. The next step was the reduction of Fe^{3+} to Fe^{2+} . When 5 ml 5% solution of hydroxylamine hydrochloride were added, the available Fe^{3+} ions transformed into Fe^{2+} as follows



And the final step was adding a new ligand at a suitable concentration, so the new complex was stable at acidic pH conditions. Such ligand was 1,10-phenanthroline with concentration 2 mg/ml (Fig. 1).

The proposed method presents a fast procedure with fewer stages of sample preparation than in similar spectrophotometric methods [17], which allows to obtain the result in 15 min with less preparative work.

Investigating the UV-Vis spectra in the interval 490–530 nm, we determined that there is a maximum absorption at 512 nm, so for further calculations we used the results at this wavelength (Fig. 2).

The method has been validated with regard to linearity, reproducibility and accuracy. The specificity of the method was proven with the investigation of

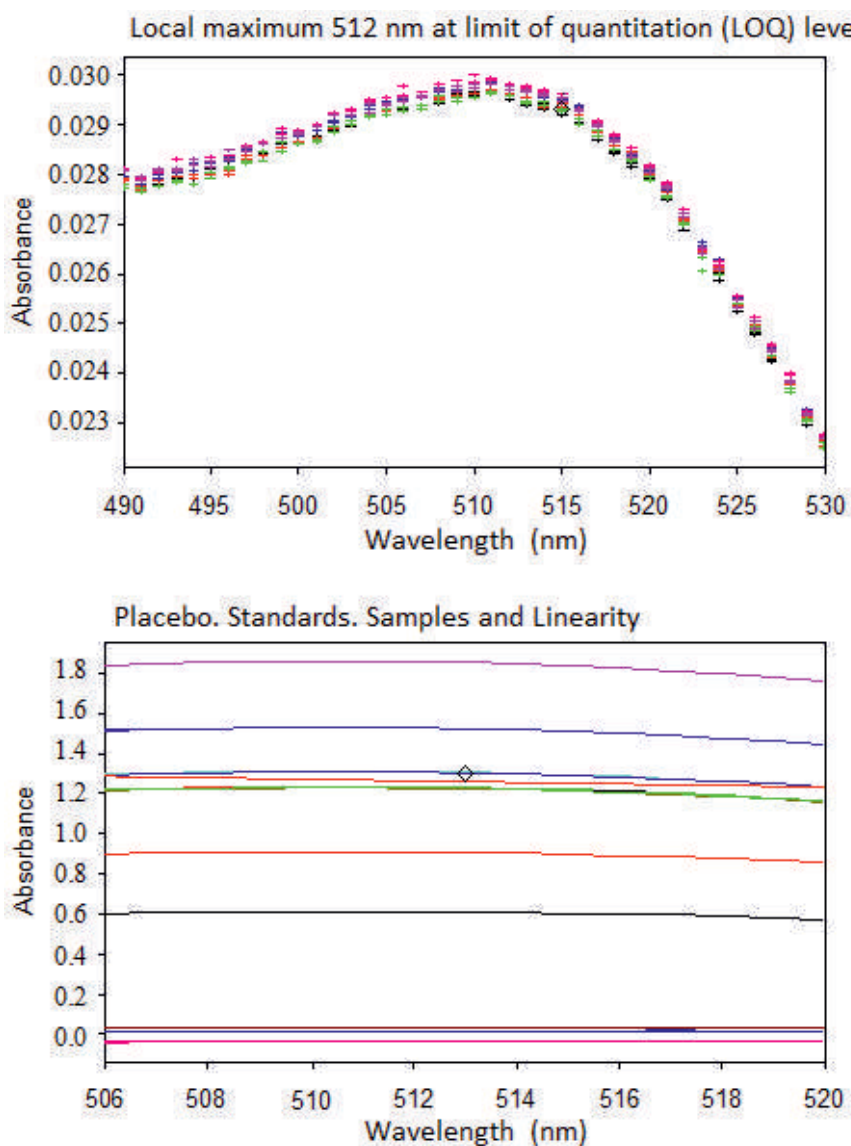


Fig. 2. Local maximum 512 nm at limit of quantitation (LOQ) level. Placebo. Standards. Samples and linearity

placebo (Fig. 2). There are no local extrema in the placebo spectra to affect the samples at wavelength 512 nm.

As the maximum in the absorption spectra was in the range of visual wavelengths, the absorption of the excipients and solvents was missing at this UV-Vis region. The limit of quantitation (LOQ) of the method was set as lowest concentration that can be determined with relative standard deviation not more than 2% (Fig. 2, Table 1).

T a b l e 1

Linearity of ferrous bisglycinate 0.15–9.10 µg/ml, sample precision and accuracy, repeatability and limit of quantitation

Linearity of ferrous bisglycinate 0.15–9.10 µg/ml

Number	Concentration, µg/ml	Absorption*	Standard Deviation (SD)	RSD, %
1	0.1513	0.0293	0.001	1.970
2	3.0258	0.6080	0.001	0.164
3	4.5388	0.9060	0.001	0.110
4	6.0517	1.2310	0.001	0.081
5	7.5646	1.5280	0.001	0.038
6	9.0775	1.8570	0.001	0.054

*Average value of three determinations

Sample precision and accuracy

Number	Concentration, µg/ml	Absorption	Assay, %
1	5.82	1.153	98.2
2	5.82	1.153	98.2
3	5.83	1.155	98.4
4	5.82	1.154	98.3
5	5.82	1.154	98.3
6	5.82	1.153	98.2
Average		1.1537	98.2667
Standard Deviation (SD)		0.0008	0.0817
RSD, %		0.0708	0.0831

Repeatability

Number	Concentration, µg/ml	Absorption	Average	RSD, %
1	6.05	1.220	1.2203	0.0423
2	6.05	1.220		
3	6.05	1.221		
4	6.05	1.220		
5	6.05	1.220		
6	6.05	1.221		
Standard Deviation (SD)		0.0005		

The method was linear, with a coefficient of linearity more than 0.999 ($R^2 = 0.9998$) and a relative standard deviation of all levels under 2%. The range of linearity was from LOQ to 9 µg/ml (Fig. 3, Table 1).

T a b l e 1

(continued)

Limit of quantitation			
	Absorption	Average	RSD, %
LOQ 0.15 µg/ml	0.0290	0.0293	1.7604
	0.0290		
	0.0290		
	0.0290		
	0.0300		
	0.0300		
Standard Deviation (SD)	0.0005		

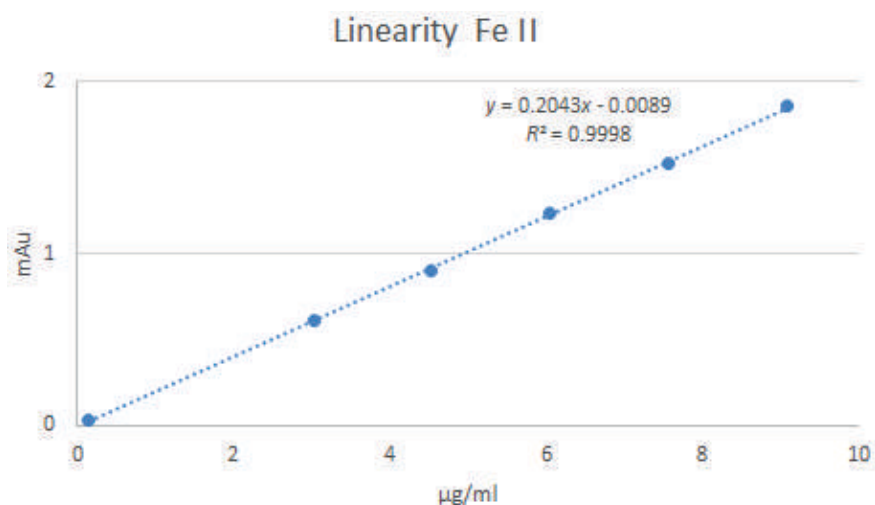


Fig. 3. Linearity

The target concentration 6 µg/ml can be determined reliably, accurately and precisely (Table 1). As acceptance criteria, the %RSD for six sample preparations was set to be $\leq 2.0\%$. The acceptance criteria for the recovery of the analytical procedure for total iron should be 97–102%.

4. Conclusions. The aim of the method presented in the current work is to aid the analysis of ferrous bisglycinate in food supplements. The method is validated according to the most important criteria of the International Conference of Harmonisations (ICH) guidelines Q2 (R1). The main goal of this method is to supply reliable, accurate and precise results and to ensure determination of Fe^{2+} . The developed analytical conditions ensure the elimination of the disturbing impact of the excipients, solvents, and the presence of other salts of Fe (III) as impurities. The transformation of the available Fe^{3+} to Fe^{2+} and the formation and the stability of the new complex with 1,10-phenanthroline, and its

absorbance at the visible part of the spectrum, are very important for avoiding the overlapping of the UV-Vis spectra at the simultaneous determination of different ferrous species. The determined sample precision of six preparations was within acceptable limits – $\leq 2.0\%$, and the recovery of total iron was over 98%, which was within the limits set (97–102%). In comparison to other similar spectrophotometric methods, the procedure described above is quicker and its quantification limit is lower due to the better selection of analytical conditions.

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