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A review of analytical method development and validation of ferrous ascorbate and folic acid as nutritional supplements

Afroz Patan◆, P. Indumathy and T. Seema

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram-600117, Chennai, Tamil Nadu, India

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Abstract

Anemia is the term for a low red blood cell count. Anemia is defined as a low hemoglobin or hematocrit in a routine blood test. The main protein found in your red blood cells is known as hemoglobin. It distributes oxygen throughout the whole body. If, you have anemia, you will also have low hemoglobin levels. Ferrous ascorbate and folic acid are a combination of two nutritional supplements that aid in the body's replenishment of essential nutrient stores. Folic acid is a form of vitamin B. Red blood cell synthesis, which is responsible for distributing oxygen throughout the body, is dependent upon it. Because of its importance in the brain development of the developing fetus, it is also vital during pregnancy. Ferrous ascorbate aids in cell growth and proliferation, transports and uses oxygen more easily, and catalyzes several biochemical processes in our body. This enhances the synthesis of hemoglobin and red blood cells. This review based on ferrous ascorbate and folic acid makes it possible to regularly analyze sacubitril and valsartan in bulk and pharmaceutical dose form using a novel approach that can be developed, validated, and compared with the current method in future research. Promising results are obtained from the statistical analysis of the development and validation process's successful outcomes in this review.

1. Introduction

Anaemia is a global health issue. Anaemia has a very high prevalence in India. Due to hemoglobin concentrations below suggested levels, the World Health Organization estimated that two billion people are anemic. The combination of ferrous ascorbate and folic acid under the brand “MYMIFER”. Dietary iron deficiency, infectious diseases that reduce red blood cell counts (RBCs), deficiencies in other essential micronutrients like folic acid, vitamin B_{12}, and retinol, or genetic disorders affecting RBCs like thalassemia are the main causes of anemia (Srivalli *et al*., 2017). Pregnant women are particularly susceptible to iron deficiency and iron deficiency anemia because they need more iron during their pregnancy. The average prevalence of iron-deficiency anemia in pregnant women is estimated to be between 35 and 75% in developing countries and 18% in industrialized countries. In Central Asia, the prevalence is very high, with 87% of cases reported in India. It is estimated that iron deficiency causes anemia is 90% of cases in India (Jyotsna Sharma *et al*., 2022). For intestinal absorption, iron must be in its ferrous state, which is the physiological form. Iron in the ferric is converted to insoluble ferric hydroxide in the small intestine’s alkaline pH, where it can only be absorbed to a limited extent. For the intestinal mucosa to absorb trivalent iron, ferric species must be reduced to ferrous iron, which is then transferred into the enterocytes through the membrane. This results in the generation of free radicals. The lowering effect of ascorbate that guards against cellular damage from free radicals is an additional benefit of ferrous ascorbate (Narendra Malhotra *et al*., 2021; Vimal Raj and Sumithra, 2023).

Corresponding author: Ms. Afroz Patan

Assistant Professor, Department of Pharmaceutical Chemistry and Analysis School of Pharmaceutical Sciences Vels Institute of Science, Technology and Advanced Studies (VISTAS) Pallavaram-600117, Chennai, Tamil Nadu, India

E-mail: afroz.sps@velsuniv.ac.in

Tel.: +91-9176080692

2. Drug profile

2.1 Ferrous ascorbate (vitamin C)

L-ascorbic acid, another name for vitamin C, is a member of the class of vitamins known as water-soluble vitamins. The 5(R)-5-[(1S)-1, 2-dihydroxyethyl] is its systematic name. 2-hydroxy-3,4-furanone(2(5H)) L-ascorbic acid (AA) and its oxidized product, dehydro-L-ascorbic acid (DHAA), are found in food naturally. Vitamin C must be consumed by humans through food. Certain other mammals possess the capacity to generate their vitamin C through the utilization of the enzyme L-gulono-γ-lactone oxidase, which converts it from glucose. Isoascorbic acid (IAA), the stereoisomer of L-ascorbic acid, is exclusively synthesized. The oxidized product of IAA is known as dehydroascorbic acid (DHIAA) (Jessy van Wyka *et al*., 2005).

2.1.1 Structure

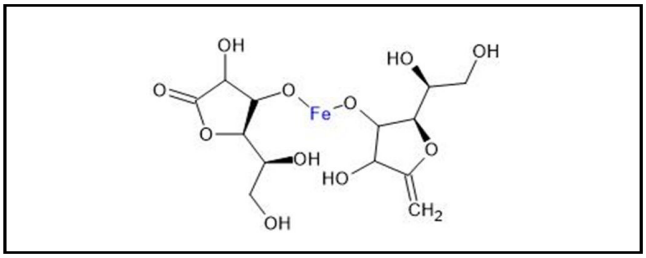

Figure 1: Chemical structure of ferrous ascorbate.

2.1.2 Pharmacokinetic properties

Food ingredients and the alkaline environment of the gastro-intestinal tract can oxidize iron found in conventional ferrous salts. Ascorbate preparations maximize iron absorption through three mechanisms:

- Blocking the conversion of ferrous iron into ferric iron, which improves absorption;
- Blocking the impact of phosphates, phytates, and oxalates on iron absorption;
- Blocking the formation of insoluble iron complexes, which obstruct absorption. Certain intrinsic properties of ferrous ascorbate aid in its absorption. In aqueous solutions, ferrous ascorbate dissociates to form monomeric cations; and
- Ferrous ascorbate exhibits an ascorbate solubility-enhancing effect between pH values of 6 and 8. Twenty advance coating technology (ACT) is one of the unique manufacturing processes that gives the ferrous ascorbate chelate stability and keeps it from dissociating in increased absorption in the stomach (Narendra Malhotra *et al*., 2021).

2.1.3 Bioavailability

There is a high bioavailability of ferrous ascorbate. Iron has been absorbed at a rate of 8.3, 6.3, and 10% from ferric orthophosphate, sodium iron pyrophosphate, along ferric pyrophosphate, respectively, as well as 30.6% from ferrous ascorbate, according to a study conducted by the National Institute of Nutrition, Hyderabad, on 45 healthy male participants (Pradeep Singh *et al*., 2022). Comparably high iron absorption rates (39-43.7%) from ferrous ascorbate have been reported in several studies; in cases of iron deficiency anemia, absorption rates have been known to reach 67%. In an evaluation of the bioavailability of iron compounds, the geometric mean intake from ferrous sulfate, ferrous ammonium phosphate, along ferric pyrophosphate had been 10.4, 7.4, and 3.3%, consecutively. Because ferrous ascorbate delays or stops ferrous iron from oxidizing, it has a higher capacity to absorb iron than ferrous sulfate and has a higher oxidation resistance ferrous iron's status as an ascorbate chelate components in food and the alkaline environment of the gastrointestinal tract can oxidize iron in traditional ferrous salts (Akella Anuradha *et al*., 2023).

2.2 Folic acid vitamin B9

When we talk about "folic acid," we are talking about the synthetic form of the vitamin that's added to supplements and meals as a fortifier. All variations of the vitamin are collectively referred to as "folate". Folic acid is composed of two moiety-a para-aminobenzoylglutamate and a pterin-connected by a methylene group (Subhmalar *et al*., 2023).

2.2.1 Structure

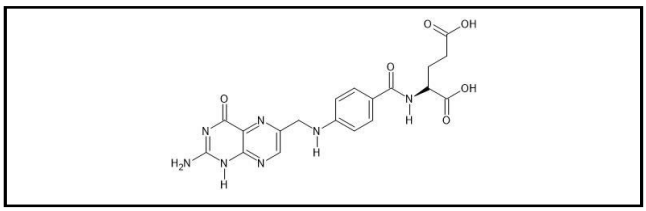

Figure 2: Chemical structure of folic acid.

2.2.2 Pharmacokinetics

2.2.2.1 Absorption

It makes sense that the amounts of absorbed and utilized folate vary amongst different vitamin chemical types and dietary sources. The most highly bioavailable form of folic acid is when it is taken as a supplement without food. Folic acid has a bioavailability that is approximately 85% higher when taken with a meal or as a dietary fortifier than when it is consumed during fasting. Natural dietary folates have approximately half the bioavailability of folic acid alone. The term "dietary folate equivalents (DFEs)" is defined as follows: One milligram of food folate and six milligrams of folic acid, either added to food or taken on its own, constitute a DFE. This definition is based on these variations in bioavailability.

2.2.2.2 Transport and cellular uptake

5-methyl THF is the primary form of folate found in blood. Less than 5% of circulating folate is transported by a variable amount that either circulates freely or is attached to a high-affinity folate binder within the serum or is linked to low-affinity protein binders such as albumin, which is responsible for roughly 50% of bound folate. Serum binders may not be physiologically significant, but in the case of a deficiency, they may regulate the distribution and excretion of folate (Natchiappan Senthilkumar *et al*., 2021). The liver is the site of maximal activity for folylpoly-g-glutamate synthase, and the liver's folate reserves account for 15-30 mg of the adult total body complement. Because of the high percentage of folate linked to proteins, which helps with cell retention, there will likely be an increase in folate (Raghavi *et al*., 2023).

2.2.2.3 Excretion

Except for a tiny amount of unabsorbed dietary folate, the gut microbiota produces the majority of the folate found in feces through vitamin biosynthesis. Under normal physiological settings, just a tiny portion (1-2%) of consumed folate is excreted through the urine. During rapid-mitotic situations like pregnancy and growth, the rate of scission of the folate molecule rises. One of the main mechanisms for the body's folate turnover may be folate scission.

2.2.2.4 Bioavailability

It makes sense that different food sources and vitamin chemical forms have varying amounts of absorbed and utilized folate. When taken as a supplement without food, folic acid is most bioavailable in this form (Alka Rani and Wamik Azmi, 2021). When folic acid is consumed with a meal or as a food fortifier, its bioavailability is about 85% higher than when it is consumed during fasting. With half the bioavailability of folic acid alone, natural food folates are even less bioavailable. The term "dietary folate equivalents (DFEs)" is defined as follows: One milligram of food folate and six milligrams of folic acid, either added to food or taken on its own, constitute a DFE. This definition is based on these variations in bioavailability (Dhritimoni Devi and Sumithra, 2023).

3. Method and validation of ferrous ascorbate and folic acid

Marcos Vinicius de Mouraribeiro *et al*. (2016) have created and verified a technique that uses derivative spectrophotometry to measure the amount of folic acid in various pharmaceutical formulations. Three concentration levels revealed that, within the

ANVISA-established range of 80-120%, the majority of recoveries fell between 98 and 105%. Due to spectrum overlap caused by the excipients in the formulation, values obtained in tests with the Folacin solution using the ZO technique (365.5 nm) ranged from 90 to 94% with an RSD of up to 10.3%. RSD values in the precision approach ranged from 0.2 to 4.8%, which were lower for various analysts on different days.

Christina Vakh *et al.* (2015) have been discovered for iron (II) and ascorbic acid in drugs made with the sandwich flow method. In the range of 2.0-20 mg/l and 0.5-4.0 mg/l for iron (II) and ascorbic acid, respectively, the absorbance of colored products at wavelengths of 510 nm and 512 nm, follows Beer's law. Ascorbic acid and iron (II) had LODs of 0.7 mg/l and 0.2 mg/l, respectively, according to the calibration plots based on 3 s. The repeatability of the developed method was evaluated by looking at ten matches of model solutions that included iron (II) and ascorbic acid in a range of 0.5 mg/l to 4.0 mg/l and 2 mg/l to 20 mg/l, respectively. Research has shown that the studies have revealed that the developed sandwich-style flow method yields acceptable outcomes. 3.0% was the RSD. AAS is used to measure iron, and CE is used to measure ascorbic acid. It should be noted that iron exists as Fe (II) in the presence of ascorbic acid. By contrasting the outcomes obtained from the two approaches, the accuracy of the strategy was assessed. The results show that the ascorbic acid and iron (II) concentrations measured with the reference and recommended methods do not differ.

Takreem Elkhazein *et al*. (2022) study set out to develop and validate a straightforward, precise UV-Spectrophotometric method for folic acid dosage calculation for tablets and bulk. The methodology was based on the absorbance of a folic acid solution in 0.01 M NaOH at 255 nm. The calibration curve that was generated was found to be linear ($r^2 = 0.9996$) within the focus range of 10-50 g/ml. The detection limits were 2.73 µg/ml, while the quantification was 8.27 µg/ml. The low RSD% values of the developed method were confirmed to be accurate. The recovery percentages were for the 50%, 100%, and 150% levels, the corresponding results were 98.46% ± 1.42, 100.0% ± 0.20, and 98.92% ± 1.12; n=3 for every level, indicating the procedure's accuracy and the lack of excipient interference. It was found that the two brands under investigation had folic acid content percentages of 96.59% ± 0.005 (n = 3) and 97.28% ± 0.003 (n = 3), respectively. These figures fall within the 9-110% official range.

Ahmad Farag *et al*. (2019) developed a gradient HPLC technique. In a single chromatographic run, gradient RP-HPLC was developed as a rapid, simple, and simultaneous technique to precisely and effectively identify seven water-soluble vitamins (B1, B2, B3, B6, B9, B12, and C) in a variety of nutraceutical supplements. The accuracy, sensitivity, and linearity of this method were confirmed. The quantification limits for vitamins B_9 and C were 3.225 µg/l and 10.768 µg/l respectively. For vitamin B_9 and C, the 5.117 and 1.06 were the limits of detection. The quantification and detection limits showed good linearity and good correlation coefficients (r^2). The method was found to be successful when the mean recoveries were found to be between 98 and 102%. superior to the process of extraction and separation using liquid chromatography.

Rucha Petal *et al*. (2015) for the purpose of simultaneously estimating ferrous ascorbate and folic acid in their combined dosage form, they have created and verified analytical methods. A simple, accurate, and precise spectroscopic method was developed for the simultaneous determination of ferrous ascorbate and folic acid within their combination dosage form *via* a simultaneous equation as well as a first-order derivative approach. Ferrous ascorbate and folic acid absorptivity values were measured at two different wavelengths, 265 nm and 344 nm, respectively, using the simultaneous equation method. Ferrous ascorbate and folic acid both displayed zero crossing points in the first-order derivative approach, at 265.78 and 280.34 nm, respectively. For ferrous ascorbate and folic acid, the dA/dλ was measured at 280.34 and 265.78 nm, respectively, and calibration curves were displayed focusing on dA/dλ. For ferrous ascorbate, the procedure was found to be linear in the 10-80 µg/ml and 1-8 µg/ml range. Recovery studies were conducted to assess the effectiveness of the suggested strategies, and positive recovery results were obtained. The strategy proved effective in determining the concurrent dose form of folic acid and ferrous ascorbate.

Kachhawah *et al*. (2016) used reverse-phase high-performance liquid chromatography (RP-HPLC) to quantify the levels of folic acid (FLC) and ascorbic acid (ASC) in cyanobacterial metabolites. An additional expansion to the work included the addition of nutraceutical formulation. The RP-HPLC method can be used to simultaneously estimate the levels of both vitamins, ASC and FLC, in cyanobacterial metabolites and nutraceuticals, utilizing the 280 nm wavelength isosbestic point. The ICH-mandated method was chosen and verified after the system's suitability was established. It was discovered that the parameters of the established analytical technique were within allowable bounds in compliance with USP and ICH regulations. It was discovered that the retention periods for FLC and ASC were 3.892 and 2.334, respectively. It was discovered that the ASC and FLC detection and quantification limits were, respectively, 0.052 and 0.159 µg/ml and 0.087 and 0.263 µg/ml. Analytes can be extracted from the method's formulation, according to recovery tests. The procedure satisfies the validation requirements outlined in the regulations.

Vasanthraju (2017) utilizing a sensitive colorimetric as well as reverse-phase high-performance liquid chromatographic method, the quantity of ascorbic acid as well as total iron in ferrous bis-glycinate effervescent pills was estimated. The total iron was measured using a colorimetric method that involved the complexing agent analysis of 1, 10 phenanthroline at 510 nm. The verified data showed that the method's linearity was observed across a range of 0.625-3.75 µg/ml (r^2= 0.999) and that its accuracy varied between 98-102%. The RSD value for method precision was 0.49%. To sum up, the method is precise, detailed, exact, and unambiguous. The newly developed method can be used by the pharmaceutical industry to perform routine analysis of ASC and FLC. An RP-HPLC method was developed for ascorbic acid using an inertial octadecyl silyl groups column (250 × 4.6 mm; 5 µm). Both methanol (97:03) and the mobile phase consisted of concentrated potassium dihydrogen phosphate (20 mM;

pH 2.5). A 0.3M hydrochloric acid diluent containing 5% OPA was used to create an isocratic program. A photodiode array detector running at 1 milliliter per minute at 245 nm was the detection technique employed. Validated data showed that the method was linear between 4.0 and 60.0 µg/ml (r^2 = 0.999). The accuracy of the method was between 100 and 102%. The RSD for method precision was 0.8%. The developed analytical methods were validated for accuracy, precision, linearity, specificity, and system suitability by ICH guidelines Q_2 (R_1). The results were within acceptable ranges.

4. Conclusion

In conclusion, the active ingredient in ferrous ascorbate and folic acid inhibitor the mechanism of action could result the iron deficiency of anaemia. These approaches are demonstrated in this review to be both cost-effective and compliant with the validation requirements. In the future, this combination can be regularly analyzed using these techniques. Due to the way that different drugs work together to reduce side effects and lower blood pressure. Using a complementary agent instead of raising the dose of a single agent can help reduce the side effects of the individual drugs. By using a complementary agent, the side effects of individual drugs can be decreased rather than increasing the dosage of that drug. Therefore, for routine analysis of ferrous ascorbate and folic acid in bulk and pharmaceutical dose form, the suggested review analytical methods can be used**.**

Inadequate iron anemia is a nutritional condition that is most common throughout the world. IDA is associated with several adverse outcomes, including increased rates of maternal and perinatal morbidity and mortality, intrauterine growth restriction, preterm birth, postpartum hemorrhage, and their associated conditions. There are several types and combinations of iron supplements available, and the majority are reasonably priced due to their low cost. On the other hand, novel iron complexes and fixed-dose combinations with vitamins and other micronutrients are being sold more frequently, with the promise of better hematopoietic response and compliance. The goal of the current study was to review method developments that have already been implemented. In conclusion, folic acid and ferrous ascorbate are still the most cost-effective options.

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Conflicts of interest

The authors declare no conflicts of interest relevant to this article.

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