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Extractive colorimetric determination of iron(III) in pharmaceutical formulations by means of trifluoroacetylacetone

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Summary

A colorimetric procedure for the determination of iron(III) in pharmaceutical formulations is proposed. It is based on the complexation reaction between the metal ion and trifluoroacetylacetone (Htfa) and on the extraction in *n*-hexane of the complex $Fe(tfa)_3$ formed. This complex is intensely coloured and allows the assay of iron in the range 1.5–30 ppm. The optimal experimental conditions were: pH = 1, [Htfa]/[Fe] = 1000, time of heating = 50 min at 60°C, λ = 435 nm. The method, applied to various pharmaceutical preparations, proved suitable, in terms of accuracy and precision, for iron(III) determination.

Introduction

Iron is an essential constituent of the human body, where it is mainly present in the form of haemoglobin and in the form of enzymes responsible for oxidative processes involving living tissues. Iron-containing pharmaceutical formulations are commonly used in the treatment or prophylaxis of iron-deficiency anaemias (Baran, 1988). The most common products administered by mouth in the cure of these diseases contain ferrous salts which, however, produce as side effects gastro-intestinal irritation and abdominal pain (Martindale, 1989). In order to limit such effects, the usage of ferric complexes has been introduced; iron is delivered slowly from these complexes so that irritating concentrations are never present and the gradual reduction to the ferrous form prevents a quick absorption of the latter. The increasing interest in iron(III) formulations prompted us to develop a new and direct method of analysis of this ion. In fact the official texts, FU IX, BP (1988) and DAB 9, report the determination of ferrous salts by means of a titrimetric procedure with cerium (IV); on the other hand, USP XXI and AOAC (1984) prescribe only one colorimetric method for iron(III), based on the use of the reagent α,α'-dipyridyl, which forms a complex with Fe(II), obtained from the latter via reduction with ascorbic acid.

The present investigation concerns the implementation of a colorimetric method for the determination of iron in the higher oxidation state. As specific reagent 1,1,1-trifluoroacetylacetone

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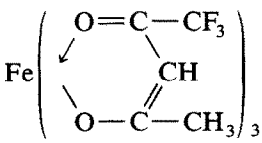
(Htfa) was chosen, since it forms with Fe(III) a stable and deeply coloured complex (I), ferric trifluoroacetylacetonate, $Fe(tfa)_3$.



(I)

Materials and Methods

Apparatus

A double-beam spectrophotometer (model Uvidec-610 Jasco, Japan), microanalytical balance (type 5 Mettler, Switzerland) and pH meter (model 325 Amel, Italy) were used.

Materials

All reagents were of analytical grade (C. Erba, Italy) and demineralized water was used. The examined pharmaceutical preparations, commercially available, were the following: ferric sodium gluconate (injections), I; ferric saccharate (injections), II; Fe-chondroitin sulphuric acid (capsules), III; extractive ferritin (elixirs), IV; ferric-protein succinylate (tablets), V.

Procedures

(a) Synthesis of $Fe(tfa)_3$ (Berg, 1960) A volume of 10 ml of 10% $Fe(NO_3)_3 \cdot 9H_2O$ aqueous solution, 10 ml of 0.7 M Htfa alcoholic solution and 0.6 g of CH_3COONa, previously dissolved in 10 ml of water, were mixed and refluxed at 60 °C for 2 h. The obtained complex was separated, purified by repeated crystallization from ethanol, and finally desiccated. The melting point was 117 °C, according to the literature.

(b) Calibration curve for $Fe(tfa)_3$ A stock solution of $Fe(tfa)_3$ (2.148×10^{-3} M, corresponding to 120 ppm Fe) was prepared by dissolving 0.2765 g of the ferric complex in 250 ml of *n*-hexane. The standard solutions (1.5–30 ppm Fe) were prepared by successive dilution. The absorbance values of standard solutions were recorded at 435 nm, using *n*-hexane as blank, and plotted vs Fe concentrations (Fig. 1).

(c) Analytical procedure A volume of 10 ml of a $Fe(NO_3)_3$ solution of the appropriate concentration was poured into a flask containing 4 ml of Htfa-hexane solution (0.6 M) and NaCl (2%). HCl (2 N) was added to obtain a reaction mixture of pH 1. The mixture was refluxed at 60 °C for 50 min and the organic layer was carefully separated, diluted to 10 ml and utilized for spectrometric measurements at 435 nm. A blank was obtained in the same manner, using H_2O instead of $Fe(NO_3)_3$ solutions.

(d) Calibration curve for Fe(III) A stock solution of Fe(III) at 1000 ppm was obtained by dissolving 0.7234 g of $Fe(NO_3)_3 \cdot 9H_2O$ in 100 ml of distilled water. Standard solutions (1.5–30 ppm Fe) were prepared by successive dilutions and subjected to procedure (c). The absorbance values were plotted vs Fe concentration (ppm) (Fig. 1).

(e) Application to pharmaceutical preparations Injections (I,II): A sample of 2 ml of injection I or II was diluted with H_2O to a concentration of 10 ppm Fe; then procedure (c) was followed.

Capsules (III): For the preparation of these samples, 20 capsules were accurately mixed; an aliquot of the powder (110 mg corresponding to 10 mg Fe(III) declared) was weighed and dissolved in water; this solution was diluted to obtain a concentration of 10 ppm Fe, then procedure (c) was followed.

Formulations with complex organic matrix (IV,V): The application of procedure (c) to

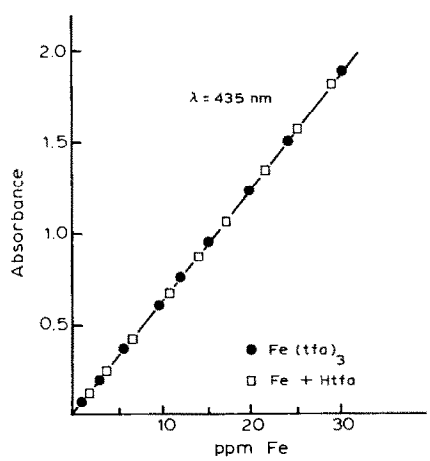


Fig. 1. Calibration graphs for iron(III).

pharmaceutical formulations with complex organic matrix resulted in exceedingly low recovery values; this problem was overcome by preliminary calcination of the samples.

Elixir (IV): As concerns product IV, 5 ml of elixir, corresponding to 10 mg of Fe declared, were evaporated and calcined. The residuum was treated with conc. HNO_3, evaporated and treated with conc. HCl, when heated; it was then successively diluted with H_2O to obtain a 10 ppm Fe solution, which was subjected to procedure (c).

Tablets (V): As concerns product V, 20 chewable tablets were accurately minced and mixed. A portion of the powder (1 g corresponding to 10 mg of Fe declared) was calcined in a porcelain crucible; then the same procedure as for IV was followed.

(f) Dipyridyl method (AOAC, 1984): The analysis is performed by reducing Fe(III) to Fe(II) with ascorbic acid and complexing Fe(II) with α,α'-dipyridyl at pH 4.5. After heating for 1 h on a steam bath, the absorbance values were measured at 532 nm ($\epsilon = 8.7 \times 10^2$). Samples of the pharmaceutical preparations were dissolved in dilute HCl or water and diluted to suitable concentration (30–40 ppm Fe). For preparations IV and V, it was necessary to apply to the samples a preliminary calcination (as reported in e).

Results and Discussion

The complexes formed by metallic ions and β-diketones have been the subject of several investigations. In particular, the chemical and physicochemical properties of the complexes of selected metals with Htfa have been studied and the feasibility of their chromatographic determination connected with their stability and volatility has been ascertained (see, for instance, Lee and Burrell, 1972). Among the complexes studied, the ferric trifluoroacetylacetonate is peculiar in its intense colour, which has suggested its possible determination by means of a colorimetric procedure. This is particularly interesting since for this particular complex the sensitivity of the chromatographic determination has proven very poor (Morie and Sweet, 1966).

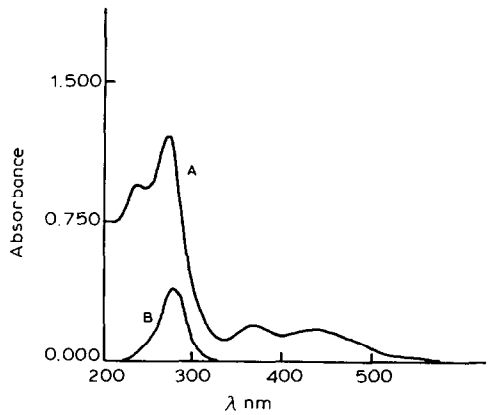


Fig. 2. Absorption spectra of *n*-hexane solution of $Fe(tfa)_3$ 5.4×10^{-5} M (3 ppm Fe) (A) and of an *n*-hexane solution of Htfa 5.4×10^{-5} M (B).

Samples of freshly prepared $Fe(tfa)_3$ in *n*-hexane present an absorption spectrum (Fig. 2A) with two bands in the visible region ($\lambda_{max} = 369$ nm, $\epsilon = 3.66 \times 10^3$ l mol^{-1} cm^{-1}; $\lambda_{max} = 435$ nm, $\epsilon = 3.48 \times 10^3$ l mol^{-1} cm^{-1}) and two in the ultraviolet region ($\lambda_{max} = 238$ nm, $\epsilon = 1.8 \times 10^4$ l mol^{-1} cm^{-1}; $\lambda_{max} = 275$ nm, $\epsilon = 2.2 \times 10^4$ l mol^{-1} cm^{-1}). The absorption spectrum of Htfa in *n*-hexane (Fig. 2B) presents one band in the UV region ($\lambda_{max} = 282$ nm, $\epsilon = 7.36 \times 10^3$ l mol^{-1} cm^{-1}).

The wavelength 435 nm was selected for the colorimetric determination in order to prevent interference from the complexing agent in excess (whenever needed). A calibration curve (Fig. 1; $y = 62 \times 10^{-3}x - 5.36 \times 10^{-3}$; $r = 0.9998$) was constructed using, as standard, solutions of $Fe(tfa)_3$, freshly synthesized and solubilized in *n*-hexane; the Lambert-Beer law is valid over the concentration range 1.5–30 ppm Fe. The next step toward the determination of Fe(III) consisted of reacting standard $Fe(NO_3)_3$ solutions with Htfa in *n*-hexane:

$$Fe^{3+} + 3Htfa \rightleftharpoons Fe(tfa)_3 + 3H^+ \quad (1)$$

Experimental parameters such as temperature, reagent concentration and pH were systematically examined in order to ensure total complexation and consequently quantitative extraction in *n*-hexane of the complex $Fe(tfa)_3$.

Effect of complexant concentration, temperature and pH

Tests carried out on solutions of Fe(III) 10 ppm at varying concentrations of Htfa from 0.006 to 0.6 M (see Fig. 3) show that a considerable excess of complexant is necessary to shift the equilibrium in Eqn 1 to the right. As can be seen, a molar ratio equal to or greater than 1000 between complexant and Fe(III) concentration ensures the desired total complexation.

As far as the temperature is concerned, the optimum refluxing temperature was found to be 60 °C and the duration necessary for refluxing was 50 min. The extraction tests carried out at various pH values for standard solutions of Fe(III) have shown that complete extraction occurs over the range pH 1–3.

When applying the method to the determination of Fe(III) in drugs, the lower limit of the range was chosen, along with the addition of an electrolyte (2% NaCl), since this combination of experimental parameters allows one to decompose certain Fe complexes, such as saccharate, in which the metal plays the role of active principle.

Calibration of the method

A calibration of the method proposed was effected, utilizing the optimal experimental conditions verified above, and precisely: [Htfa]/[Fe] = 1000, pH = 1, heating time = 50 min at 60 °C. The data obtained at $\lambda = 435$ nm from standard solutions of $Fe(NO_3)_3$ (Fig. 1) were found to superimpose quite well on those obtained from the complex solutions (Fig. 1), confirming that the complexation reaction and the extraction process go to completion under the conditions chosen. The precision of the method is high; reproducible results were obtained from 5 samples of 10 ppm Fe standard solutions; the relative standard deviation (RSD) was found to be 0.7%.

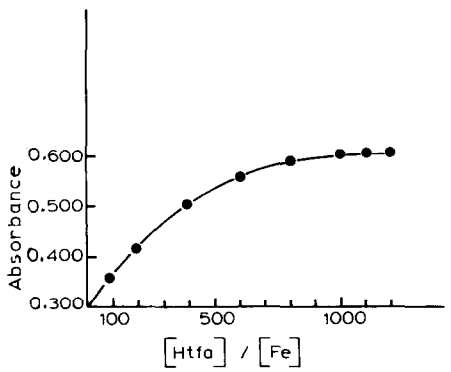


Fig. 3. Influence of the complexant concentration on the $Fe(tfa)_3$ complex formation (Fe conc. = 10 ppm, pH = 1, T = 60 °C).

TABLE 1

Analysis of Fe(III) in pharmaceutical formulations

Commercial formulations	Method with Htfa		Method with dipyridyl	
	% found of claimed ($n = 6$)	RSD % ($n = 6$)	% found of claimed ($n = 5$)	RSD % ($n = 5$)
I	100.2	1.15	100.3	1.05
II	100.1	1.20	99.9	1.10
III	100.1	0.88	99.5	1.25
IV [a]	100.5	1.75	100.4	1.80
V [a]	99.1	1.62	99.5	1.76

[a] The analysis is performed after calcination of the samples.

Stability tests

The complex $Fe(tfa)_3$ in *n*-hexane is highly stable under illumination and at room temperature. No remarkable absorbance changes were observed within 24 h.

Application to pharmaceutical formulations

The procedure was applied to various commercial formulations (capsules, tablets, injections, elixirs) containing ferric complexes. The results are reported in Table 1 as % found of claimed and RSD%. These results were compared to those obtained with the dipyridyl procedure (AOAC, 1984). The two methods appear to be equally reliable from the point of view of accuracy and precision (Table 1). One can remark, however, that our method proves superior when sensitivity is considered. Indeed, the limit of the dipyridyl method is about 6 ppm, while by our procedure a quantity of Fe as low as 1.5 ppm can be determined. The comparison is thus in favour of the direct colorimetric procedure of Fe(III) proposed here. The accuracy of the method was also verified by recovery studies, adding known amounts of Fe(III)

standard solutions to known amounts of commercial preparations I–III (% recovery = 99.8–100.2%, RSD% = 0.95; $n = 5$).

In conclusion, the method based on the complexing reaction between Htfa and Fe(III) seems to be suitable in terms of sensitivity, accuracy and precision for a satisfactory determination of iron in pharmaceutical formulations.

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