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A high-sensitive spectrofluorimetric method for the determination of micromolar concentrations of iron(III) in bovine liver with 4-hydroxyquinoline

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Abstract

A new sensitive, rapid and accurate spectrofluorimetric method, suitable for the determination of micromolar concentrations of iron(III) in bovine liver, using 4-hydroxyquinoline (4-HQ) in an alkaline medium (KOH 2.0×10^{-2} mol/l) as fluorescent agent, is described. The fluorescence intensity of the working solutions was measured at λ_{ex} 305 nm and λ_{em} 380 nm. The observed decrease of the above mentioned intensity was mainly due to the quenching, caused by the interaction between the iron(III) to be analysed and the potentially fluorescent 4-HQ. The accuracy and the precision of the proposed method, after an experimental investigation, could be considered as very satisfactory. Potassium fluoride and triethylenetetramine solutions were successfully used for the masking of those metal cations existing in the bovine liver which interfere seriously with the determination of iron(III).

Keywords: Spectrofluorimetric determination; Iron; Liver; 4-Hydroxyquinoline

1. Introduction

Iron is a particularly essential constituent of the human body, necessary for haemoglobin formation and the oxidative processes of living tissues. As is well known, a human body of 70 kg, contains, on average, about 4.2 g (0.0752 mol) of iron, most of which is present as haemoglobin. The remainder exists in storage forms, such as ferritin or haemosiderin, in the reticuloendothelial system or as myoglobin with smaller amounts occurring in haem-containing enzymes or in plasma bound to transferrin [1,2].

In the human liver, as well as in the bone marrow, the concentration of iron is differentiated by the sex of the person (male 250 mg, female 80 mg of iron/kg of liver; male 200 mg, female 80 mg of iron/kg of bone marrow [3]).

Apart from serious and prolonged haemorrhage, iron is mainly lost from the body in the faeces, urine, skin and sweat, but the total loss is small. The preservation of homeostasis for this metal element, essential for physical well-being, is secured either by an increased consumption of iron-containing foodstuffs (pork and calf liver, broad and kidney beans, lentils, parsley, soybeans flower, etc.) or by taking suitable anti-anaemic medicinal formulations.

Many methods have been proposed for iron determination in biological samples. They focus interesting information on: (a) digestion of tissues [4,5]; (b) speciation of tissue iron [6]; (c) sampling, transport, storage, sample preparation and comparison of results obtained by different techniques [7]; (d) preconcentration of trace elements and separation of potentially interfering metal ions [8]; (e) splitting and isolating the iron from its iron-binding proteins and enzymes [9]; (f) catalytic effects of iron on the oxidation of special reagents [10]; and (g) solvent extraction of iron complexes [11]. Detection limits, recovery, accuracy, precision and agreement of the results for the reference materials with their certified values are also discussed.

The new analytical method described here is adequately simple and satisfactorily accurate. Iron was determined in the range of micromolar concentrations particularly at 3.0×10^{-6} mol of iron/l in the presence of the mineral constituents of bovine liver. On the other hand, the interfering effects of some foreign metal ions with the determination of iron were experimentally studied in detail.

2. Experimental

2.1. Apparatus

A Perkin Elmer LS-3 spectrofluorimeter, equipped with a RCA 931A photomultiplier and the proper prismatic silica

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cells of 10 mm, was used for the fluorescence measurements. A Hitachi, Model 100-80, double-beam ratio recording spectrophotometric system, matched with 10.0 mm quartz cells, was used for all absorbance measurements. A WTW, Model 537, microprocessor digital pH meter with a precision combined glass–calomel electrode E56, with a suitable sensor for the measurement of the temperature and automatic compensation, Model TFK 150, was used for all the necessary pH measurements.

2.2. Reagents and solutions

4-Hydroxyquinoline (4-HQ, CAS 611-36-9, Aldrich, No. H5.800-5, 98%) was used to prepare the following standard solution. Standard solution of 4-HQ (10^{-3} mol of 4-HQ/l in a 1.0 mol/l KOH aqueous solution) was freshly prepared. Stock (10^{-2} mol/l) and working solutions (10^{-4} mol/l) of iron(III), ($NH_4Fe(SO_4)_2 \cdot 12H_2O$, Merck, No. 3776, p.a.) were prepared and their final concentrations in Fe(III) was confirmed spectrophotometrically [12].

An AcOH (6.10×10^{-2} mol/l)–AcONa (1.39×10^{-1} mol/l) buffer solution (pH 5.0 $\pm$ 0.1 at 25°C) was prepared and used in the spectrophotometric confirmation of iron(III) concentration.

A reference standard solution of bovine liver sample (Lot No. 1577a, National Bureau of Standards, NBS), was prepared as described below and used in order to certify the accuracy and the precision of the proposed analytical method.

Water, freshly double-distilled from a borosilicate autostill (Jencons Ltd.) apparatus, was utilized throughout. All the other mineral and chelate reagents, as well as the organic solvents employed were of analytical grade.

2.3. Ashing of the bovine liver sample

About 0.0125 g of the dried (according to the instructions of the accompanying NBS certificate) bovine liver reference standard, accurately weighed, was quantitatively transferred into a clean porcelain crucible. Then, 12.0 ml of concentrated nitric acid (14.0 mol/l) was added to dissolve the resulting residue. The nitrate solution formed was warmed to about 90°C on an electric hot plate and under continuous magnetic stirring. Finally, an aqueous solution of H_2O_2 (30% v/v) was carefully added in 4.0 ml aliquots at intervals of 10 min, until all the black particles were digested and disappeared. The solution was evaporated to dryness and 5.00 ml of water was added under continuous stirring to completely dissolve the residual matter (reference solutions).

2.4. Calibration curve

Into a set of six 5.0 ml calibration flasks (nos. 1–6), were pipetted in order: 2.50 ml of potassium fluoride 1.0 mol/l solution, 0.50 ml of triethylenetetramine (trien) 10^{-2} mol/l solution, x ml ($x = 0.25$, 0.30, 0.35, 0.40, 0.45 and 0.50) aliquots of iron(III) working (10^{-4} mol/l) solution, and y ml ($y = 0.25$, 0.30, 0.35, 0.40, 0.45 and 0.50) aliquots of potassium hydroxide 10^{-2} mol/l solution. Then the volume of the content of each calibration flask was diluted up to about 4.90 ml with water, and 100 μl of the standard solution (1.0×10^{-3} mol/l) of 4-HQ was pipetted at 1-min intervals in each one of the nos. 1–6 calibration flasks and the content was filled up to volume with water and vortex-mixed.

After 20 min, the fluorescence of each solution (nos. 1–6) formed, was measured at 1 min intervals at $\lambda_{em} = 380$ nm ($\lambda_{ex} = 305$ nm).

The traced calibration curve is shown in Fig. 1. The optimum range of the calibration curve is extended between 5 and 10 μmol of iron(III)/l. The linearity of the calibration adequately covers the necessities of the present method at least for the range of concentration examined, although the working curve can be extended many times more using relatively larger concentrations of 4-HQ. In trace analysis, the important point is the size of the sample used. The smaller it is, the more attractive and notable the method. All the analytical characteristics are summarized in Table 1.

2.5. Measurement of the reference solution

Into a set of three 5.0 ml calibration flasks (nos. 7–9), were pipetted in order: 1.350 ml of reference solution (see Section 2.3), 0.250 ml of iron(III) working (10^{-4} mol/l) solution, 0.250 ml of potassium hydroxide 10^{-2} mol/l solution, 2.50 ml of potassium fluoride 1.0 mol/l solution, 0.500 ml of trien 10^{-2} mol/l solution and 0.050 ml of water. Into each one of the three calibration flasks (nos. 7–9), 100 μl of the standard solution of 4-HQ (1.0×10^{-3} mol/l) were pipetted at 1 min intervals. The content of the calibration flasks (nos. 7–9) was mixed well by vortexing and after 20 min the fluorescence of each solution formed was measured at 1 min intervals at $\lambda_{em} = 380$ nm ($\lambda_{ex} = 305$ nm). The addition of the constant iron amount (0.250 ml of 10^{-4} mol/

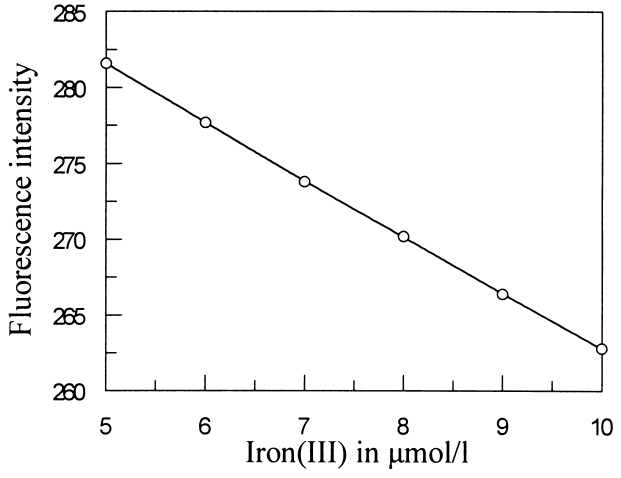

Fig. 1. Calibration curve for the spectrofluorimetric determination of micromolar concentrations of iron(III). Concentration of 4-HQ: 2.0×10^{-5} mol/l. $\lambda_{ex} = 305$ nm; $\lambda_{em} = 380$ nm.

Table 1
Experimental data and analytical parameters of the proposed method

Characteristics	Numerical data
λ_{ex}, λ_{em} (nm)	305, 380
Regression line equation	$F = aC + b$
a (slope) ± SD[a]	− 3.75 ± 0.03
b (intercept)	300.2
Correlation coefficient (r)	0.9997
Number of determinations (n)	6
RSD (%)[b]	0.80
Standard analytical error	0.0122
Confidence limits ($P = 0.05$; $(n - 1) = 5$)	− 3.75 ± 0.0315
Optimum concentration range (μmol of Fe(III)/l)	5.0–10.0

[a] SD, standard deviation.
[b] RSD, relative standard deviation.

l) in the sample was made to balance the iron concentration at the beginning of the calibration curve. Otherwise, larger samples had to be used to obtain their analyte concentrations from the working curve, but in this case the likelihood of matrix effects, which lead to interference errors, is substantial. If the value of a sample lies out of the linear range, the measurement is repeated using less reference solution.

2.6. Calculation of iron in bovine liver

The concentration of iron in the calibration flasks (nos. 7–9) was easily determined in triplicate, by means of the calibration curve (Fig. 1). Obviously, the concentration of iron determined in each calibration flask is the sum of iron contained in the reference solution plus an extra quantity (5.0 μmol/l) of this metal included in 0.250 ml of the iron(III) working (10^{-4} mol/l) solution, added into each calibration flask (nos. 7–9).

3. Results and discussion

3.1. Fluorescence spectra

Fig. 2 shows the fluorescence excitation and emission spectra, either of a 20 μmol of pure 4-HQ/l in a 2.0×10^{-2} mol KOH/l solution (curve a, excitation, λ_{em} 380 nm; curve b, emission, λ_{ex} 305 nm) or of a mixture of pure 4-HQ solution (20 μmol of 4-HQ/l) and 5.0 μmol of Fe(III)/l, where (curve a′ excitation, λ_{em} 380 nm; curve b′, emission, λ_{ex} 305 nm). The observed difference between the excitation (a,a′) and, mainly, the emission (b,b′) spectra is the prerequisite support for the experimental application of this new analytical method.

3.2. Effect of pH on the fluorescence of 4-HQ

The emission spectrum of the molecule of 4-HQ is related to dissociation of the phenolic hydrogen. The fluorescence intensity of the chelate and fluorescent agent 4-HQ, depends

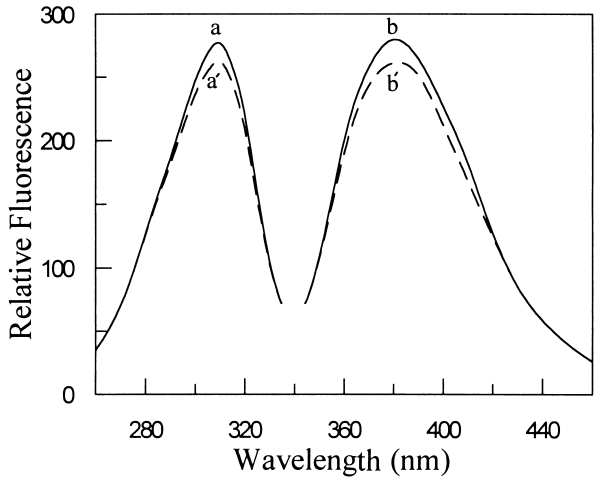

Fig. 2. Fluorescence spectra of 4-HQ (2.0×10^{-5} mol/l in a KOH 2.0×10^{-2} mol/l solution). (a,a′) Excitation (λ_{em} 380 nm); (b,b′) emission (λ_{ex} 305 nm); (a,b) in the absence of iron; (a′,b′) in the presence of 5.0 μmol Fe(III)/l.

on the pH of the solution measured, as shown in Fig. 3. In both cases no fluorescence is observed below pH 10.

However, a red color appears just as iron and 4-HQ are mixed even in strongly acid solutions. In addition, potentiometric titration results of 4-HQ with potassium hydroxide in the absence and presence of Fe(III) are shown in Fig. 4. The low pH region of curve (c) for the protonated 4-hydroxyquinoline (4-H_2Q^+) is higher than the corresponding region of the system Fe(III) + 4-H_2Q^+ (curve e) or the system HNO_3 + Fe(III) (curve d). The low pH regions of the curves (d) and (e) completely coincide. The small difference in the pH scale between (c) and (d) or (e) is expected because of the relatively high dissociation constant of 4-H_2O^+ ($pK_a = 2.34$) [13]. Curves (a) and (b) are given to allow a proper comparison. These remarks show that the hydrogen assigned to the protonation of the pyridyl group

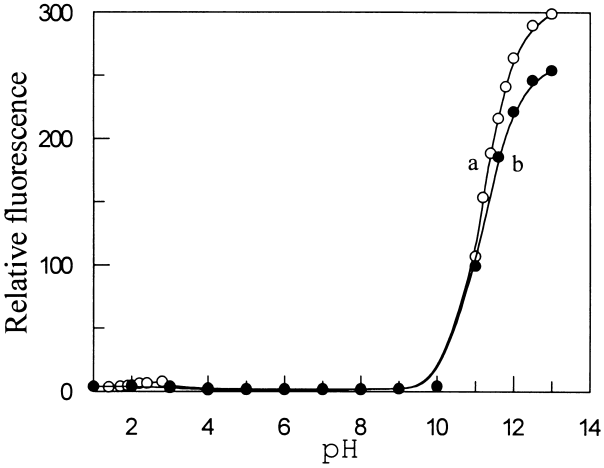

Fig. 3. Fluorescence variation of 4-HQ with pH. (a, or ○) 4-HQ: 20.0 μmol/l; (b, or ●) 4-HQ: 20.0 μmol/l + Fe(III): 20.0 μmol/l; λ_{ex} = 305 nm; λ_{em} = 380 nm.

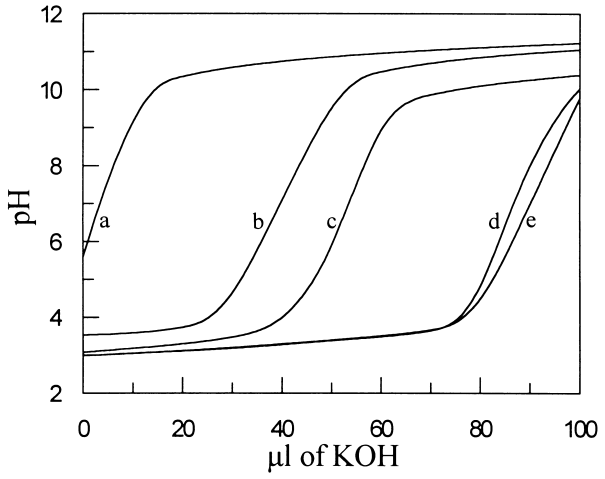

Fig. 4. Potentiometric titration curves at 25°C aqueous solutions with 1 mol/l KOH. (a) Free 4-HQ 10^{-3} mol/l; (b) free Fe(III) 2.5×10^{-4} mol/l; (c) 4-HQ 10^{-3} mol/l + HNO_3 10^{-3} mol/l; (d) Fe(III) 2.5×10^{-4} mol/l + HNO_3 10^{-3} mol/l; (e) 4-HQ 10^{-3} mol/l + HNO_3 10^{-3} mol/l + Fe(III) 2.5×10^{-4} mol/l.

is fully replaced by Fe(III) and that the preferred coordination site of 4-HQ, especially for Fe(III), is the pyridine ring nitrogen.

Variation of the fluorescence intensity of 4-HQ in connection with the concentration of Fe(III) is shown in Figs. 5 and 1. A decrease of light less than 2% is due to absorption of the sample; a percent transmittance from 99.3 to 98.3 is noticed for Fe(III) concentrations from 2.5×10^{-6} to 5×10^{-6} M, respectively (Fig. 6b,c). However, this fact does not downgrade the value of the method and no essential errors result because the light absorption is approximately proportional to the Fe(III) concentrations added. Therefore, the total fluorescence decrease of 4-HQ in the presence of Fe(III) is predominantly due to quenching and secondary to absorption by the sample matrix.

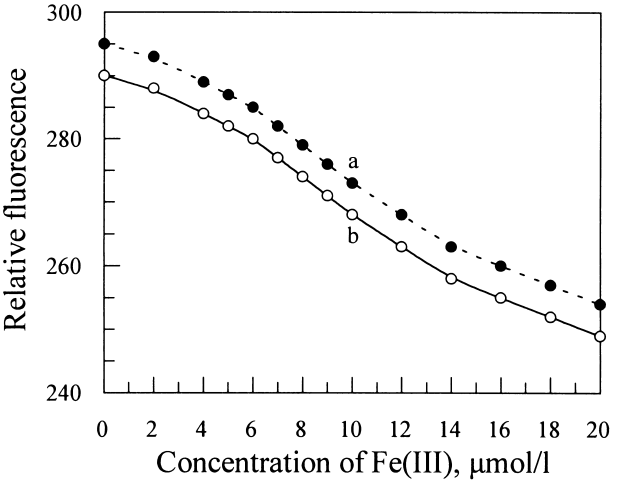

Fig. 5. Fluorescence variation of 4-HQ in connection with the concentration of iron(III) in 2.0×10^{-2} mol/l KOH. (●, a): 20 µmol 4-HQ/l + Fe(III); (○, b): 20 µmol 4-HQ/l + Fe(III) + 0.5 mol KF/l + 1.0 mmol trien/l. λ_{ex} 305 nm; λ_{em} 380 nm.

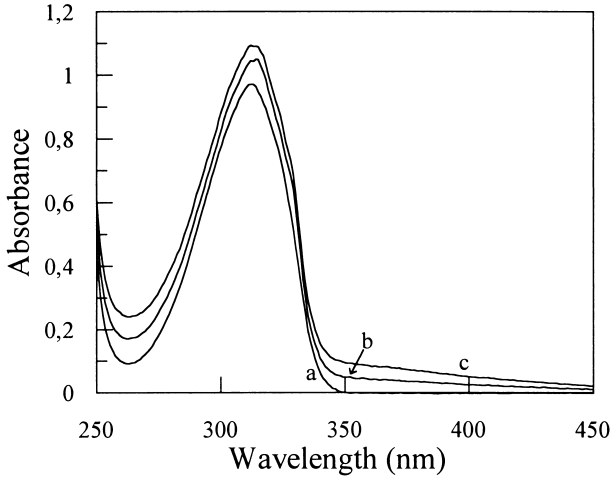

Fig. 6. Absorption spectra: (a) 4-HQ 10^{-4} mol/l in KOH 0.02 mol/l; (b) 4-HQ 10^{-4} mol/l + Fe(III) 2.5×10^{-5} mol/l in KOH 0.02 mol/l; (c) 4-HQ 10^{-4} mol/l + Fe(III) 5×10^{-5} mol/l in KOH 0.02 mol/l.

Table 2
Effect of foreign metal ions on the spectrofluorimetric determination of iron(III)[a]

Added		Concentration (µmol/l)	Deviation (%)	
Metal cation	Anion		(I)	(II)
None	Sulphate	–	–	–
Calcium (Ca^{2+})	Chloride	20	− 3.2	− 1.0
	Chloride	50	− 4.7	− 5.3
	Chloride	100	− 14.7	− 8.0
Magnesium (Mg^{2+})	Sulphate	20	− 6.2	− 3.0
	Sulphate	40	− 12.0	− 7.3
	Sulphate	100	− 18.4	− 8.7
Zinc (Zn^{2+})	Sulphate	10	− 3.7	− 1.1
	Sulphate	20	− 10.7	− 2.3
	Sulphate	30	− 17.4	− 4.8
Copper (Cu^{2+})	Chloride	2	+ 3.0	+ 1.8
	Chloride	4	+ 5.7	+ 3.4
	Chloride	10	+ 13.6	+ 6.7
Manganese (Mn^{2+})	Sulphate	0.5	+ 6.4	+ 3.3
	Sulphate	1	+ 13.4	+ 5.3
	Sulphate	2	+ 42.0	+ 7.0
Cobalt (Co^{2+})	Chloride	5	+ 3.7	+ 3.2
	Chloride	10	+ 7.9	+ 7.8
	Chloride	20	+ 16.4	+ 18.3
Cadmium (Cd^{2+})	Nitrate	10	− 1.7	− 1.6
	Nitrate	20	− 4.0	− 3.6
	Nitrate	50	− 6.4	− 4.3
Lead (Pb^{2+})	Nitrate	10	+ 5.4	+ 1.3
	Nitrate	20	+ 9.4	+ 2.0
	Nitrate	30	+ 16.1	+ 5.2
Mercury (Hg^{2+})	Sulphate	2	+ 2.3	+ 1.9
	Sulphate	5	+ 6.4	+ 2.7
	Sulphate	10	+ 13.1	+ 3.8
Molybdenum (VI) as (Mo_7O_{24})	–	10	+ 1.7	+ 1.4
	–	20	+ 6.1	+ 4.3
	–	30	+ 19.2	+ 16.0

[a] Iron(III) concentration: 8.0 µmol/l; 4-HQ: 20.0 µmol/l; KOH: 2.0×10^{-2} mol/l; (I) No masking; (II) 0.5 mol/l potassium fluoride + 1.0 mmol/l trien.

Table 3
Accuracy and precision of the new analytical method

Fe(III) in μmol/l		RSD (%)	SAE[b]	Confidence limits	Relative error (%)
Added	Found ± SD[a]		(SD/$\sqrt{n}$)	$P = 0.05$; $(n-1) = 3$	$[100x_i - \hat{x})/\hat{x}]$[c]
5.00	4.96 ± 0.012	0.24	0.0060	4.96 ± 0.019	− 0.80
7.00	7.04 ± 0.071	1.01	0.0355	7.04 ± 0.113	+ 0.57
10.00	9.97 ± 0.044	0.44	0.0220	9.97 ± 0.070	− 0.30

[a] SD, standard deviation (four determinations)
.[b] SAE, standard analytical error.
[c] $\hat{x}$, added values; x_i, mean values of quadruplicate solutions calculated from the fluorescence using the calibration equation in Table 1.

Table 4
Application of the new analytical method for the determination of iron(III) in bovine liver

Fe(III) in μg/g of liver		RSD (%)	SAE[b]	Confidence limits
Amount in NBS liver Lot No. 1577	Found ± SD[a]		(SD/$\sqrt{n}$)	$P = 0.05$; $(n-1) = 3$
270.0	265.0 ± 2.59	0.98	1.295	265.0 ± 4.120

[b] SAE, standard analytical error.
[a] SD, standard deviation (four determinations).

3.3. Masking of interfering metal ions

Various reagents were tested for masking other metal cations, mainly those co-existing with iron(III) in bovine liver. Better results were obtained using a combination system of 0.5 mol/l potassium fluoride and 1.0×10^{-3} mol/l triethylenetetramine (trien) [14].

The use of these masking agents must be put carefully into practice, since the sensitivity of the method described could be seriously diminished using larger concentrations of masking agents. The results of the application of the masking agents of foreign metal cations on the determination of iron(III), as well as the percentage deviation on the recovery of this metal cation (Fe(III)) are presented in Table 2.

3.4. Accuracy and precision

The accuracy and the precision of this new analytical method was confirmed with solutions of three different concentrations of iron(III) analysed in quadruplicate. The results of this investigation are summarized in Table 3. The mean relative standard deviation (RSD%), the standard analytical error (SAE), the calculated confidence limits and the relative errors were considered to be satisfactory for the range of quantities of iron(III) examined.

3.5. Analytical application of the new analytical method for the determination of iron(III) in bovine liver

The new analytical method presented here was applied for the spectrofluorimetric determination of iron(III) in bovine liver, as described in Section 2. The results, as well as the analytical parameters of this determination are presented in Table 4.

4. Conclusion

In this method only a necessary wet ashing procedure was utilized to liquefy the reference sample. No separation step was applied to remove coexisting interfering metal ions; alternately a small quantity of bovine liver was used to prevent the occurrence of interference errors from the sample matrix. Thus, the analytical process includes fewer operational steps, is clearly simpler, induces less experimental errors and is more accessible and preferable to the analyst. Furthermore, trace metal analysis in biological tissues is undoubtedly a relatively difficult task. It provides an excellent tool for the analyst who can efficiently determine other samples of the same or lower complexity.

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