Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 1159–1164

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

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Short communication

Validation of a spectrophotometric method for the determination of iron (III) impurities in dosage forms☆

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Received 6 June 2001; received in revised form 15 November 2001; accepted 19 November 2001

Abstract

A spectrophotometric method ($\lambda = 535$ nm) for the iron (III) impurities determination in iron protein–succinylate complex syrup using thioglycolic acid in basic ambient was proposed and validated. Assay samples were treated with 0.1 N hydrochloric acid and centrifuged to remove the interfering active drug. Linear response ($r = 0.9999$) was observed over the range of 0.005–0.2% of the iron (III) with respect to the complex nominal concentration. The accuracy could be considered very satisfactory (recovery = 97–99%). The intra-day precision (RSD) of impurity amongst six independent sample preparations, was 1.4%, and there was no significant difference between intra- and inter-day studies. Intermediate precision indicated that the assay possessed high degrees of ruggedness. The limit of quantitation was 0.005% of impurity with respect to the active drug. The results obtained for iron (III) were compared statistically with those obtained with the standard addition method by means of the Student's t-test and the variance ratio F-test; no significative difference was found. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Iron (III) impurities; Iron succinyl–protein complex; Validation; UV–Vis spectrophotometry

1. Introduction

Iron is an essential trace element, required for haemoglobin formation and the oxidative processes of living tissues. Iron preparations are employed for iron deficiency anaemia. Iron is irregularly and incompletely absorbed from the gastro-intestinal tract. The absorption is more readily affected when the iron is in the ferrous state or is part of the haem complex. The astringent action of iron preparations sometimes produces gastro-intestinal irritation and abdominal pain with nausea and vomiting when administered orally. Other gastro-intestinal effects may include either diarrhoea or constipation. These irritant side-effects are usually related to the amount of elemental iron taken rather than the type of preparation [1]. Many methods have been proposed for iron determination as active drug by spectrophotometry [2–8] and LC [9,10] in pharmaceuticals.

The aim of the present study was to propose a validated spectrophotometric method for the de-

☆ Presented at the 9th Meeting on Recent Developments in Pharmaceutical Analysis, Lipari, Aeolian Islands, Italy, June 5–8, 2001

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PII: S0731-7085(02)00170-X

termination of the iron (III) present as potential degradation product or impurity in commercial syrups containing the iron succinyl–protein complex. Iron protein–succinylate is a non-toxic therapeutic iron trivalent compound and is adsorbed by a mechanism involving digestion to release soluble, available ferric species which may be reduced at the mucosal surface to provide ferrous iron for membrane transport into enterocytes [11]. Iron (III) impurities determination is necessary for evaluating the effect of manufacturing procedure or sourcing changes on product quality. Thioglycolic acid was used in basic ambient for the determination of the iron (III) giving a highly stable red–purple complex, which was measured at a wavelength of 535 nm [12]. The major problem encountered in the analysis is the presence of the great amount of the coloured active ingredient, which could cause interference. The precipitation and removal of the iron succinyl–protein complex with 0.1 N hydrochloric acid made it the method of choice for quality control of commercial formulations. The validated method is simple, accurate and reliable for the analysis of the iron (III) impurities and also able to provide satisfactory selectivity and sensitivity.

2. Materials

2.1. Equipment

The spectrophotometric analysis were performed on a double beam spectrophotometer Jasco Uvidec-610 using 1 cm quartz cells with a slit width of 1 nm. Suitable settings were: scan speed 100 nm/min, chart speed 20 nm/cm, absorbance scale 0–0.5. Centrifugal system ALC International SrL ALC 4235 A was used.

2.2. Reagents and solutions

Iron succinyl–protein complex was obtained thanks to Italfarmaco (Cinisello Balsamo, Mi, Italy). Thioglycolic acid (mercaptoacetic acid) was purchased from Riedel-deHaën. $NH_4Fe(SO_4)_2 \cdot 12H_2O$, 28% NH_4OH, 96% H_2SO_4 and citric acid anhydrous and other chemicals were obtained from Carlo Erba reagents (Italy). Stock solutions of $NH_4Fe(SO_4)_2$ equivalent to 200 μg/ml Fe (III) were freshly prepared dissolving the salt in water containing 4.8% H_2SO_4; then, these solutions were diluted with water to obtain the standard solutions. The H_2SO_4 (98 g/l), NH_4OH (not less than 170 g/l and not more than 180 g/l) and citric acid (200 g/l) solutions were prepared in agreement with F.U. X Ed. [13]. The placebo and the reconstituted syrup were prepared according to the manufacturing formula [14].

2.3. General procedure

A 10 ml aliquot of the working Fe (III) solution was transferred into 20-ml volumetric flask and 2 ml of citric acid solution and 0.1 ml of thioglycolic acid were added. After accurate mixing and alkalization with 2.0 ml of NH_4OH (diluted as described in F.U. X Ed. [13]), the solution was diluted to volume with water. The adsorbance of the resulting solutions was measured after 15 min at 535 nm against water.

2.4. Calibration graph

Drug concentrations in the working standard solutions chosen for the calibration curve, obtained under the conditions described in Section 2.3, were 0.25, 0.5, 1.0, 2.0, 5.0, 10 μg/ml and for the standard addition method 2.0, 3.0, 5.0, 8.0, 10 μg/ml.

2.5. Analysis of Fe (III) impurities in syrups

A variety of syrups (Ferrofolin®, Rekord Ferro, Legofer 40®, Proteoferrina®40, Folinemic Ferro) containing the iron succinyl–protein complex and available in the market was analysed as follows.

2.5.1. Sample preparation

A aliquot of syrup, equivalent to about 500 mg of iron protein–succinylate, was transferred into a 80-ml test tube and 10 ml 0.1 N HCl were added in order to assure the iron protein complex precipitation. After centrifugation for 7 min at

2000 rpm, the mixture was filtered and the surplus was washed twice with 4 ml of water, centrifuged and filtered through a paper filter. Subsequently the resulting clear and colourless solution were transferred quantitatively into a 100-ml volumetric flask and diluted to volume with water. Suitable aliquot (10 ml) of this solution (sample solution) was taken for the analysis of Fe (III) as described in Section 2.3 and the impurity content in each samples was directly determined by comparison with an appropriate standard solution (0.5 μg/ml) and the standard addition method.

By the standard addition method different volumes (1, 1.5, 2.5, 4, 5 ml) of a stock Fe (III) solution (200 μg/ml) were added to the sample solution in the same solvent prior to diluted up to volume of 100 ml. The Fe (III) adsorbance was plotted against the corresponding amounts added to obtain a standard addition calibration graph. The x-intercept, was then used for calculating the content of impurity in the samples analysed.

3. Results and discussion

The iron protein–succinylate is insoluble under the acid conditions present in the stomach, thus avoiding the release of large concentrations of potentially aggressive iron ions into the small intestine [11,15,16]. On the basis of the drug insolubility in acid ambient, in order to determine the Fe (III) impurities in syrup without interference's, the iron succinyl–protein complex was precipitated with 0.1 N HCl and removed completely by centrifugation and filtration. Then, the reaction employing thioglycolic acid in basic solution [11] was applied. In particular, to achieve optimum conditions the effect of the volume of NH_4OH diluted on the reaction was investigated. In the pH range 10.1 ± 0.5, obtained using 1.0–2.5 ml of NH_4OH diluted, both standard solutions and sample solutions showed no significant difference for 7 h.

The performance of the spectrophotometric method was evaluated with respect to selectivity, linearity, accuracy, precision, limits of quantitation and detection.

3.1. Selectivity

The shape of UV–Vis absorption is an useful parameter of the Fe (III) impurity identification. The absorption spectrum of a Fe (III) standard solution in the range of 370–700 nm has been recorded in comparison with the placebo syrup and the reagent blank solutions, prepared under the same conditions and treated similarly. The spectrum of Fe (III) shows maxima and at the same time constant absorbance in the visible region ($\varepsilon_{535} = 4260$/M cm), while any significant adsorption of the placebo and the reagent blank was observed (Fig. 1).

3.2. Linearity

Under the described spectrophotometric conditions a linear relationship between adsorbance and Fe (III) concentration (0.25, 0.5, 1.0, 2.0, 5.0, 10 μg/ml) was found. Least squares regression equation with correlation coefficient $r = 0.9999$ ($n = 6$), was $y = 0.03756(\pm 0.00020)x - 0.00103(\pm 0.00092)$. Moreover, in order to validate the method suitability, the Fe (III) impurities

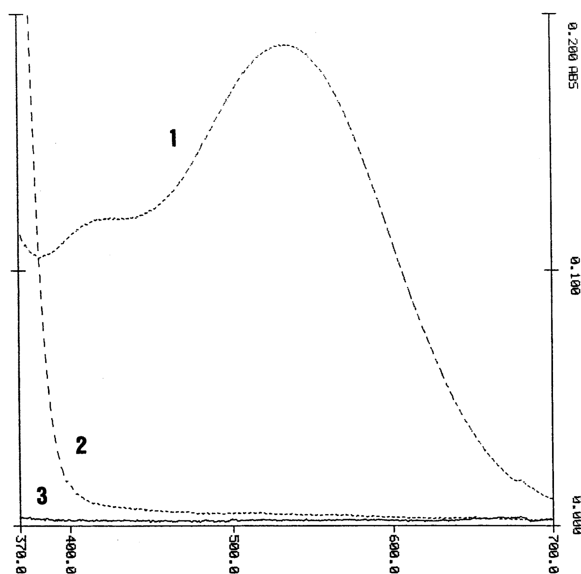

Fig. 1. Absorption spectra ($\lambda = 370$–700 nm) of the (1) iron–thioglycolic acid complex ($Fe(OH)(SCH_2COO)_2^{2-}$), (2) placebo and (3) reagent blank.

Table 1
Results of recovery assays

Amount added (μg/ml)	Amount found[a] (μg/ml)	Recovery (%)	RSD (%)
2.00	1.94 ± 0.037	97.2	1.92
5.00	4.94 ± 0.033	98.7	0.66
10.00	9.90 ± 0.055	99.0	0.56

[a] Mean value ± standard deviation ($n = 6$).

were also quantified by the standard addition method by adding increasing known amounts of pure standards (2.0, 3.0, 5.0, 8.0, 10 μg/ml) to the samples. A typical regression line obtained for the analysis of Fe (III) in a syrup sample was: $y = 0.03754(\pm 0.00058)x + 0.07137(\pm 0.00370)$; $r = 0.9996$ ($n = 5$) (x-intercept $= -1.90102$).

3.3. Limit of quantitation and detection

In this work, according with the literature [17], as regards the spectroscopic techniques or the other methods that rely upon a calibration curve for quantitative measurements, the limit of detection (LOD $= 3.3\sigma/S$, were σ is the standard deviation of the y-intercept and S is the slope of the calibration curve) was determined to be approximately 0.081 μg/ml (0.0016% of Fe (III) with respect to the iron protein–succinylate). The limit of quantitation (calculated as $10\sigma/S$ was 0.245 μg/ml (0.0049% impurity with respect to the active drug). The data exhibited a RSD of 7.8% for six analysis at the LOD level.

3.4. Accuracy

The accuracy of the method were validated by using recovery studies. For determination of the recovery, known amounts of Fe (III) were added to reconstituted syrup, after iron protein–succinylate complex precipitation, and the resulting spiked samples were analysed and compared to the known added value. All analyses were carried out in six replicate three Fe (III) concentration levels of 0.04, 0.1, 0.2% of iron protein–succinylate label claim. The recovery was in the range of 97–99% and coefficients of variation ranging from 0.56 to 1.92% (Table 1).

3.5. Precision

The intra-day precision of the method was determined, under the optimal working conditions, by triplicate absorbance measure of the six reconstituted preparations. For the determination of inter-day precision, repeat analyses of six preparations over a 7-day period was carried out. The within-day precision showed RSD of 1.4%. The between-day precision evaluated was 1.5%. Intermediate precision, as determined from six sample preparations was evaluated over 7 days by two analysts using different spectrophotometric systems. The results for both analysts were generally considered equivalent indicating high degrees of ruggedness. The RSD of each individual precision analysis was not more than 2.0%.

3.6. Stability

The stability of the solutions of the reconstituted syrup, spiked of 0.1% of Fe (III) with respect to the iron succinyl–protein complex and treated as described in Sample Preparation was evaluated. An initial analysis of the preparation was made before storing at room temperature. The sample solution was examined in comparison with a Fe (III) standard solution (5 μg/ml) at half hour intervals and no change in absorbance was observed for up 7 h. The stability of these solutions and the capacity of the analytical procedure to remain unaffected by the use of different quantities of NH_4OH diluted, provide an indication of the method robustness.

3.7. Analysis of Fe (III) impurities in syrups

The results of the Fe (III) impurities in commercial syrups are reported in Table 2. As it can be seen, for each sample the results obtained using the reference method were comparable with those obtained with the standard addition method. The other ingredients of the formulations did not interfere with the analysis. The Student's t-test values at a 95% confidence level for 6 degrees of freedom did not exceed the tabulated value of $t = 2.447$, indicating no significant difference between the two methods. The variance ratio F-test

values, calculated for $P = 0.05$ and $f_1 = 4$, $f_2 = 2$, did not exceed the tabulated value of $F = 19.25$, again indicating that was no significant difference between the precision of the two analytical procedures applied.

4. Conclusions

The spectrophotometric method proposed for the Fe (III) determinations, present in commercial syrups as potential degradation product or impurity, was found to be simple, rapid, specific, linear and reliable. The assay results of Fe (III) impurities in syrups agree with those obtained by the standard addition procedure. The method proved to be suitable for the quality control of pharmaceutical formulations and could also be extended for further study on the stability of iron dosage forms.

Table 2
Results from spectrophotometric analysis of Fe (III) impurities in commercial syrups

Syrup[a]	Reference method			Standard addition method			t^c	F^d
	Concentration (μg/ml)	% Found[b]	Mean (RSD%)	Concentration (μg/ml)	% Found[b]	Mean (RSD%)		
1	0.5615	0.0112	0.0111 (7.0)	0.4992	0.00998	0.0100 (3.5)	2.25	4.89
	0.6149	0.0123		0.5194	0.0104			
	0.5347	0.0107		0.4851	0.00970			
	0.5080	0.0102						
	0.5615	0.0112						
2	0.4057	0.00811	0.00757 (7.1)	0.3848	0.00770	0.00773 (3.0)	0.47	5.49
	0.3787	0.00757		0.3761	0.00752			
	0.3517	0.00703		0.3992	0.00798			
	0.3517	0.00703						
	0.4057	0.00811						
3	0.7063	0.0141	0.0140 (5.8)	0.6322	0.0126	0.0132 (4.9)	1.44	1.54
	0.7607	0.0152		0.6947	0.0139			
	0.6792	0.0136		0.6606	0.0132			
	0.7063	0.0141						
	0.6520	0.0130						
4	0.3387	0.00677	0.00677 (5.4)	0.2998	0.00600	0.00624 (3.8)	2.20	2.34
	0.3387	0.00677		0.3240	0.00648			
	0.3126	0.00625		0.3122	0.00624			
	0.3647	0.00729						
	0.3387	0.00677						
5	1.916	0.0383	0.0388 (2.2)	1.940	0.0388	0.0384 (1.0)	0.76	4.39
	1.889	0.0378		1.920	0.0384			
	1.943	0.0388		1.901	0.0380			
	1.969	0.0394						
	1.996	0.0399						

[a] Other ingredients: iron protein–succinylate, sorbitol, propylene glycol, methyl p-hydroxybenzoate sodium, propyl p-hydroxybenzoate sodium, saccharin sodium, blackberry flavour, purified water.
[b] The data are expressed as impurity percentage of protein complex claimed content.
[c] Calculated t-value; tabulated t-value = 2.477.
[d] Calculated F-value; tabulated F-value = 19.25.

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