COMPARISON OF FLAME ATOMIC ABSORPTION SPECTROMETRY (FAAS) AND CERIMETRIC METHODS FOR THE DETERMINATION OF FERROUS SULFATE CONTENT OF SOME PHARMACEUTICAL PRODUCTS

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ABSTRACT

The iron content of ferrous sulphate syrup and tablet, ferroglobin, and Fefol were determined by both titration with cerium (Cerimetric method) proposed by united state pharmacopeias and flame atomic absorption spectrometry (FAAS). In FAAS both external calibration and standard addition method were used to evaluate the matrix effects. In the determination of iron content of ferrous sulphate tablet and syrup FAAS using external standard give approximately the same results as cerimetric methods of analysis. But in the case of ferroglobin syrup and Fefol capsule the external calibration results had large deviation from cerimetric method of analysis. So flame atomic absorption spectrometric involving standard addition is proposed for the analysis of iron content of these pharmaceutical products. The coefficient of variation for the FAAS determinations were in the range of 0.73-5.85 in one day and 2.39-7.51 between different days. Statistical evaluation showed good correlation between two methods.

Keywords: Titration, flame AAS, ferrous sulphate, pharmaceutical products

INTRODUCTION

Iron plays a crucial role in the body. It is important to maintain an adequate supply of iron to form hemoglobin and other molecules in the body that depend on iron to function properly. Our bodies continuously lose iron. When the body's supply of available iron is too low, iron deficiency results. Physicians may prescribe different forms of iron for patients. They rely on the amount of iron ion which is claimed on the label. So determination of the precise amount of Fe^{+2} is important. This is routinely done on the products in the pharmaceutical factory to check the correct formulation. Post marketing survival studies are also necessary to see changes in drug content in different storage conditions. So the assay of iron contents of these products is important.

Iron normally exists in solution as ferrous or ferric ions. Different methods have been used for the determination of iron in a specific oxidation state (Giokas *et al*., 2002, Harrington *et al*., 2001 and Grotti *et al*., 2003). Speciation of iron content in matrixes such as medicinal products (Yang *et al*., 2004 and Zarei *et al*., 2005) and water (Tewari *et al*., 2000 and Grotti *et al*., 2003) was also done. In most of them atomic absorption spectrometry was the method of choice for the determination of iron. In pharmaceutical products iron must be in 2+ oxidation state so antioxidant is added to stabilize the product. If this oxidation state is stable the AAS method can be used to determine the iron content. In the factory and for post marketing survival study (PMS) they used conventional spectrophotometric (Skoog 1996 and Harrington *et al*., 2001) or the cerimetric method [USP 2002, BP 2004] for the analysis of Fe^{2+} content of the products. These are accurate and precise methods of determination but when the numbers of samples are high it takes more operator time and is tiresome. Besides that the osmium tetroxide used in the cerimetric method is carcinogenic. Flame atomic absorption spectrometry (FAAS) is also an accurate and precise method of determination of metals and takes lesser operator time when the number of samples is high but it can not distinguish between the different oxidation states of metals by itself (Kenduzer *et al*., 2002 and Yan *et al*., 1999). In pharmacoepia (BP, 2004) FAAS is proposed for the determination of the dissolved total amount of tablet because of this lack of discrimination. Since antioxidant is added to prevent oxidation of iron, the aim of this investigation was to investigate if titration can be replaced by FAAS, and also if the dissolved matrix has any effect in the determination.

MATERIALS AND METHODS

Experimental

Apparatus

Flame atomic absorption spectrophotometer, CTA-3000 (Anal Tech, city UK) was used for all AAS determinations at 283 nm with an iron hallow cathode lamp (HCL).

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Table 1: Mean results for the determination of iron in ferrous sulphate syrup (50mg/5mL) and ferroglobin (10mg/5mL) by three methods (The values are the mean of four measurements).

Ferrous sulphate Syrup	Titration mg/5mL	Atomic Absorption Determination(AAS)	
		Standard Addition mg/5mL	Linear calibration mg/5mL
Excir	40.95±0.08	38.42±0.84	36.9±0.24
Pars Minoo	43.37±0.05	38.66±0.94	37.97±0.56
Ferroglobin	10.41±1.00	10.75±0.99	5.88±1.38

Table 2: Mean results for the determination of iron in ferrous sulphate tablet and FeFol capsule by three methods. Amounts on labels were 50 mg (The values are the mean of four measurements).

Ferrous sulphate tablet	Titration mg	Atomic Absorption Determination(AAS)	
		Standard Addition mg	Linear calibration mg
Zahravi	50.26±0.52	53.25±0.95	48.8±0.71
Rozdaru	44.47±1.54	43.75±0.79	51.6±1.82
Fefol	52.18±2.19	54.43±0.61	46.07±2.90

Reagents

Ceric ammonium nitrate, mohr salt, osmium tetroxide, concentrated sulfuric and nitric acid, ferric chloride three hydrates, and all the other reagents used in this study were purchased from Merck, (Darmstad, Germany). Stock solution of 1000 μg mL^{-1} iron ions was purchased from Chem Lab N.V (UK). All solutions were prepared in doubly distilled water.

Ferrous sulphate syrup (Excir (Brojerd, Iran), Pars Minoo (Tehran, Iran)), tablet (Zahravi (Tabriz, Iran), Rozdaru (Tehran, Iran)), Ferroglobin (Vitabiotices Ltd., England), and Fefol (Smithkline&French and Smithkline & Beecham, Hertfordshire, England) with different batch numbers were purchased from local pharmacies in Ahvaz.

Preparation of samples for analysis

The samples were prepared as described in the USP (2002). In the case of capsules and tablets 8 of them were completely mixed, weighted and the USP procedure was followed. Then the solution was filtered through Wathman No. 1 filter paper. The 25 mL of filtrate were used for titration and this solution was diluted 100 times for the analysis by FAAS.

In the case of ferroglobin and ferrous sulphate syrup no special pretreatment is needed. 20 milliliters of each was directly used for titration but they were diluted for FAAS analysis in order to have the concentration in the linear range of calibration curve.

The titration procedure was performed as described in the USP. Atomic absorption determinations were performed using both external (linear) calibration curve and standard addition method.

100 μg mL^{-1} solution of iron ion was prepared by ten fold dilution of stock solution of 1000 μg mL^{-1} of each pharmaceutical product. Calibration standards of 0.5, 1, 2.5, 5, 7.5, 10 μg mL^{-1} were prepared by successive dilution of this solution (fig. 1). Solutions of 100 μg mL^{-1} iron of each iron product were prepared considering the claimed value printed on the labels by successive dilution. In the case of standard addition method 0.2 mL portions of 100 μg mL^{-1} of each drug forms were transferred to three 20 mL volumetric flasks and 0, 0.8, 1.8 mL of 10 μg mL^{-1} iron was added consequently. These solutions were diluted to the mark with distilled water. The absorbance of each solution was recorded at 283 nm. A typical standard addition curve is presented in fig. 2.

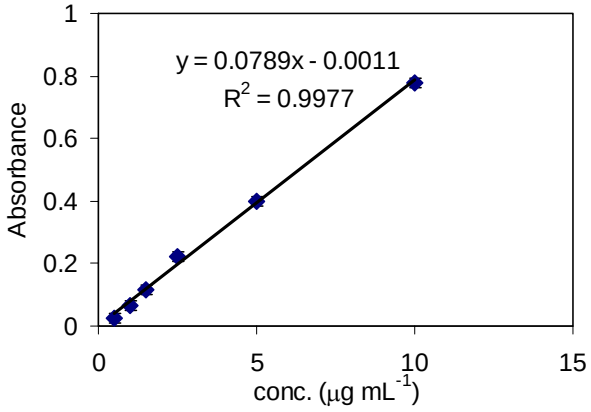

Fig. 1: typical calibration curve for iron determination by FAAS. The standard concentrations were in the range 0.5-10 μg mL^{-1}.

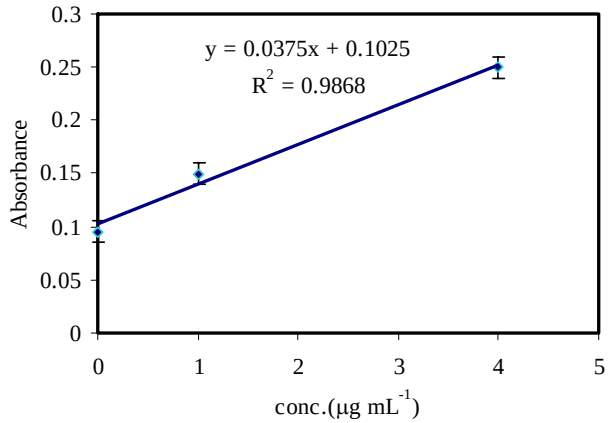

Fig. 2: Standard addition curve for the determination of iron contents of Fefol capsule.

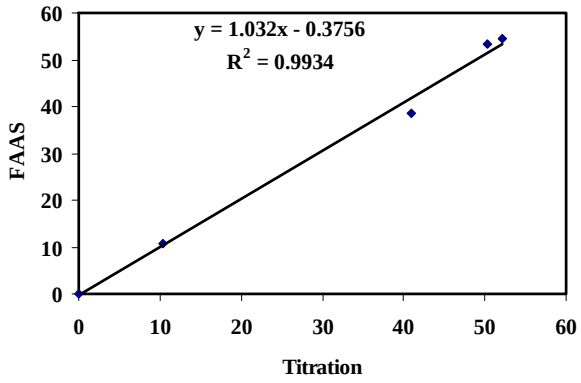

Fig. 3: Regression line for comparing titration results with those obtained by FAAS method.

RESULTS AND DISCUSSION

As mentioned before concentration of Fe^{2+} in ferrous sulphate pharmaceutical product is of vital importance in all aspect of pharmaceutical and clinical sciences. An antioxidant, usually sodium bisulphite, is added to prevent oxidation of Fe^{2+}. However it is required to check the content of Fe^{2+} now and then. The standard method of analysis mentioned in USP and BP is visible spectrophmetric and cerimetric method of analysis. In spectrometric method so many reagents must be prepared and also some reagents must be prepared daily. In post marketing survival (PMS) studies and production line where the number of samples to be analyzed is high these method takes more operator time, and is not economic. In the case of cerimetric method of analysis osmium tetroxide used in standardization of the titrant is carcinogenic. Since the antioxidant is added to the product the only form of iron is Fe^{2+}. So it was decided to evaluate the reliability of flame atomic absorption in assay of iron in these products and also the matrix effect in their FAAS determination. The mean Fe^{2+} content calculated by both methods are presented in tables 1 and 2. As it is obvious in FAAS matrixes (excipients) of samples may have some increasing or decreasing effect on the results. As it was difficult to match the matrix of standards and samples, standard addition method was also used to evaluate the matrix effect in each cases. Table 1 shows the strong effect of the matrix in the Ferroglobin syrup and no effect in ferrous sulphate tablet and syrup of Iranian company which has no other vitamins and minerals along with. Fefol which also contained folic acid showed a little matrix effect.

In order to evaluate the precision of the determination iron content of the products was determined five times in a day and five consecutive days. The coefficients of variation for between days and within days variations are reported in table 3.

Table 3 Within and between day **c**oefficients of variation for determination of iron contents of the selected products by AAS.

Products	RSD% Within days	RSD% Between days
FeFol	0.73	2.39
Tablet	5.85	7.51
Ferrous sulphate Syrup	2.70	5.99
Feroglobin	4.63	6.61

BP and USP suggest titration for assay of Fe^{2+} content of the pharmaceutical products. So this method was used as standard method and the FAAS results were compared with titration results. Student t-test was used to compare titration results with both calibration and standard addition results (table 4). As it is obvious from table 4, iron content of Ferroglobin and Fefol capsule must be determined by standard addition method. The reliability of the results was further evaluated by the regression line of FAAS against titration which had the equation of y= 1.0326x – 0.3756 with r= 0.9967 (fig. 3).

Table 4: P-value obtained by comparison of the results of cerimetric with those obtained by FAAS methods using student t-test.

Products	Linear calibration/ titration P-value	Standard addition/ titration P-value
Ferrous sulphate syrup	0.09	0.19
Ferrous sulphate tablet	0.94	0.37
Feroglobin syrup	0.0012	0.29
Fefol capsul	0.023	0.34

CONCLUSION

The reliability of atomic absorption determination of ferrous ion content in pharmaceutical products was evaluated. Cerrimetric analysis as a standard method was used to validate the flame atomic absorption results.

As statistical results (table 4) showed FAAS can be used instead of titration in all cases. In the case of ferroglobin syrup and Fefol capsules the matrix effects were so high and the standard addition method is recommended.

AAS does not by itself make any difference between oxidation states of iron so in some cases slight increase in the results is observed and the cerimetric showed the precise value of Fe^{2+}. This increase may be due to conversion of Fe^{2+} to Fe^{3+} as a result of inefficiency of antioxidant and instability of Fe^{2+} or uncertainty in the measurements. In the case of Fefol capsules a slight increase in the results is seen in table 2 which is not significant by statistical test (table 4). AAS and titration results were the same if no conversion occurred. This study showed that FAAS can be used for the determination of these products. In cases were some other nutrition is added, such as Ferroglobin Syrup and Fefol capsule, the standard addition method must be used. When ferrous sulphate plus antioxidant is in the products the external calibration curve can easily be used.

In our research group we are developing a new AAS method to determine the oxidation states of iron in the cases were the conversion between oxidation states in pharmaceutical products occurs by any reason.

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