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Simultaneous Determination of Fe(II) and Fe(III) in Pharmaceutical Samples by Post-Column Derivatization/HPLC

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A post-column derivatization HPLC method with visible absorbance detection at 521 nm was modified for the simultaneous determination of Fe(II) and Fe(III) in mixtures. The method was applied to pharmaceuticals marketed in Turkey. Iron species were separated on a post-column derivatization HPLC, IonPac CS5A (4x250 mm) analytical column using a pyridine-2,6-dicarboxylic acid based eluent. The determinations of Fe(II) and Fe(III) were also realized by the most common method of FAAS, total iron determination. Detection limits (3S) were found to be 0.109 μg/L for Fe(II) and 0.217 μg/L for Fe(III), respectively. The mean recovery of the HPLC and AAS method was found to be 98-101%. This application note, by using the principles of ion exchange, describes an attractive alternative to traditional spectroscopic methods.

Key Words: HPLC, Fe(II) , Fe(III), AAS, Pharmaceuticals

Introduction

Metal ions can exist in several forms. Ion-exchange plays an important role in modern analytical chemistry. Transition metals can be separated using either anion or cation exchange chromatography, depending on the choice of complexing agent used in the eluent[1].

Iron deficiency is the most common of nutritional anemia in human beings. It is also an essential component of myoglobin, cytochomes, catalase and peroxidase. Iron deficiency can affect the metabolism in muscle independently of the effect of anemia on oxygen delivery. Its deficiency has also been associated with behavioral and learning problems in children and with abnormalities in the catecholamine metabolism[2]. Iron preparations are one of the most commonly used medicaments for the problems arising due to iron deficiency in human beings.

An adult male has a requirement of only 13 μg/kg per day (about 1 mg) whereas a menstruating female requires about 21 μg/ kg per day (about 1.4 mg). In the last two trimesters of pregnancy, requirements

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increase to about 80 μg/kg per day (5-6 mg) and the infant has similar requirements due to its rapid growth. The usual therapeutic dose of iron is about 200 mg per day (2-3 mg/kg). Children weighing 15 to 30 kg can take half the average adult dose and small children and infants can tolerate relatively larger doses (5 mg/kg)[3].

Methods used for the assay of iron in various samples include ion chromatography[4−6], UV-Vis. spectrophotometry[7−9] and atomic absorption spectrometry[10].

In this study, a simple, rapid and specific HPLC method for the determination of Fe(II) and Fe(III) is developed. The method is also selective enough for the simultaneous determination of Fe(II) and Fe(III) in various matrices. Most of the Fe preparations on the market are in the form of Fe(II). The ones in the form of Fe(III) are reduced to Fe(II) within the human organism. In order to obtain maximum efficiency in terms of bioavailability, the iron given to the metabolism should be in the form of Fe(II)[11,12]. Since Fe(II) can easily be oxidized to Fe(III) by the effect of air and light, the simultaneous determination of the Fe(II) and Fe(III) contents of the preparations becomes very important.

Experimental

Instrumention

An HP (Agilent) 1100 High Performance Liquid Chromatograph was coupled with a Dionex PC10 Post-Column Delivery (PCD) System. Fe(II) and Fe(III) were detected with an HP Variable Wavelength UV-Vis detector at 521 nm.

The IonPac CS5A analytical column (4x250 mm, sulfonic acid alkanol quaternary ammonium) and CG5A guard (4x50mm) column were used for the separation process. The nature of the cross-linked polymeric structure of the packing materials makes the CS5A column compatible with pH 0-14 eluents. An ATI-UNICAM 929 Atomic Absorption Spectrometer was also used for the determination of total Fe.

A schematic diagram of the HPLC/PCD system is given in Figure 1 and the operating conditions of the system are listed in Table 1.

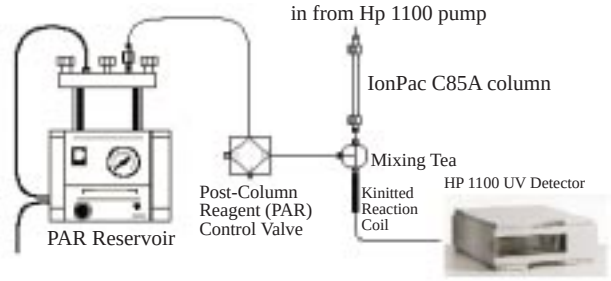

Figure 1. A schematic diagram of the HPLC/PCD system for iron separation.

Chemicals

Fe(III) ion standard for AAS of 1000±0.5% mg/L in 1 M HNO_3 was provided from Fisher Scientific. $FeSO_4.7H_2O$ was from Merck Chemicals. PDCA (pyridine-2,6-dicarboxylic acid), KOH, HCOOH, K_2SO_4, PAR (4-(2-pyridylazo)resorcinol), and Postcolumn Reagent Diluent were from Dionex Co. Standard solutions were prepared from stock solutions just prior to use. Iron drugs were obdained from the market in Turkey.

Table 1. Operating conditions of the system.

HPLC / Post-Column Delivery System
Columns: Ionpac CS5A Analytical and CG5A Guard Column.
Eluent: 7.0 mM pyridine-2-6-dicarboxylic acid/66 mM potasium hydroxide/5.6 mM potasium sulfate/74 mM formic acid in 1 L de-ionized water.
(final pH: 4.2 ± 0.1)
Eluent Flow Rate: 1.2 mL/min.
Injection Volume: 100 μL.
Detector: HP 1100 VW UV-Vis Detector at 521nm.
Post-column Reagent: 0.5 mM 4-(2-pyridylazo)resorcinol (PAR) in Postcolumn Reagent Diluent.
Post-column Reagent Flow Rate: 0.6 mL/min.
Post-column Reagent Diluent 1.0 M 2-dimethylaminoethanol/0.50M aqueous ammonia/0.30M sodium bicarbonate pH: 10.4 ± 0.2.
Reaction Coil: 375μL Knitted Reaction Coil.

Chemicals

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Iron solutions: 100mg/L Fe(II) stock solution was prepared by dissolving 0.050 g $FeSO_4.7H_2O$ (Merck) in 100mL de-ionized water. Then 1.50 mL was diluted to 100mL with de-ionized water to give 1.50 mg/L Fe(II) standard; 150μL Fe(III) AAS standard was diluted to 100mL with de-ionized water to give 1.50mg/L Fe(III) standard.

De-ionized water was obtained via the MILLIPORE water system (Elix-10 followed by Milli-Q 185 Plus) and a 0.22 μm filter unit.

Methods

HPLC Procedure

Applying the AAS method, Fe(II) and Fe(III) can not be determined separately, but the total Fe content of the sample can be determined[13,14]. However, by using the proposed method, separate peaks were obtained for Fe(II) and Fe(III) on chromatogram. A chromatogram obtained from Fe(II) (prepared from $FeSO_4$) and Fe(III) (prepared from standard of AAS) standards is shown in Figure 2. The observed retention times were 5.2 min and 11.6 min for Fe(II) and Fe(III), respectively.

For this purpose, prior to application to the pharmaceutical samples, quantification was performed with synthetic samples of 1.5 ppm Fe(II) as $FeSO_4$ and 1.5 ppm Fe(III) as Fe^{3+} AAS standard, separately and together. Appropriate results were obtained and it was decided to apply the method to the pharmaceutical samples.

In this way, any sample containing Fe(II) and Fe(III) can simultaneously be determined. For the application of the method, 10 iron tablets were weighed, and finely powdered and an equivalent weight of a tablet was transferred into a 1L volumetric flask containing about 900 mL of water, and it was ultrasonicated for 30 min and diluted to 1L with water. The solution was filtered through a 0.45 μm nylon filter. From this

stock solution 1.5 ppm equivalent of iron solution was prepared. This solution was filtered before injection to HPLC.

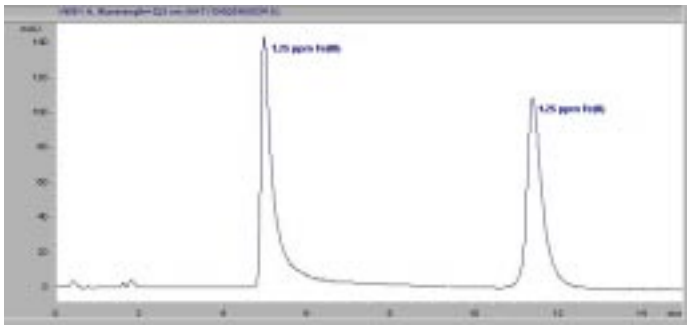

Figure 2. A typical HPLC chromatogram of Fe(II) and Fe(III) standards using the proposed method for separation.

Then 100 mM Na_2SO_3 was passed through the column for 1 h at 1.0 mL/min to eliminate any oxygen build-up on the column at the beginning of the experiment. Eluent was pumped with a flow rate of 1.2 mL/min until a stable baseline was achieved. Post-column reagent was pumped with a flow rate of 0.6 mL/min towards the mixing tee as shown in Fig. 1. A 100μL sample or standard was injected into the system. The separation of Fe(II) and Fe(III) were realized and the components reached the mixing tee. They both reacted with the post-column derivatization reagent in the knitted reaction coil to give a colored complex and were determined with a UV-Vis detector at 521 nm.

AAS Procedure

Pharmaceutical samples were digested via a MILESTONE MDR-1200 Microwave Furnace using concentrated HNO_3:H_2O_2 (5mL:1mL) mixture and diluted to 25 mL with deionized water.

An ATI-UNICAM Atomic Absorption Spectrophotometer equipped with a D_2 arc background correction system was used for total Fe determination at 248.3 nm, with a 12.0 mA Fe hollow cathode lamp and air/acetylene flame[13,14].

Results and Discussion

Standard Curve

Calibration standards were prepared using concentrations of 0.05, 0.2, 0.5, 1.25 and 2.5 mg/L of Fe(II) and Fe(III) in deionized water. These standards were prepared daily from the stock solution. The calibration graphs obtained for Fe(II) and Fe(III) including best fit equations are shown in Figure 3.

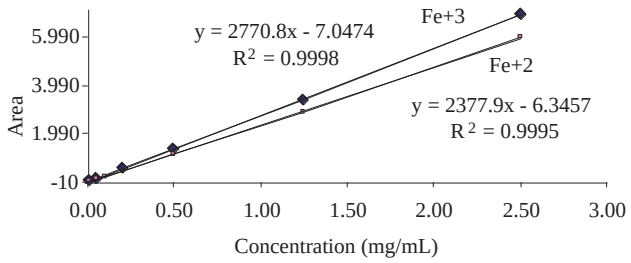

Figure 3. Calibration graphs for Fe(II) and Fe(III)

3.2. Limit of Quantitation and Limit of Detection

The limit of quantitation was determined from the iron peaks and the standard deviation of the S/N ratio. The limit of quantitation was defined as the iron concentration resulting in a height of 10 times S/N and was calculated as 0.363 μg/L and 0.723 μg/L for Fe(II) and Fe(III), respectively.

The limit of detection was defined as the concentration of iron species that produces an analytical signal equal to thrice the standard deviation of the background signal[15] and was calculated as 0.109 μg/L for Fe(II) and 0.217 μg/L for Fe(III).

The results obtained using the proposed method for the determination of Fe(II) and Fe(III) by HPLC and total Fe content by AAS in 7 different pharmaceuticals marketed in Turkey are shown in Table 2.

It is well known that speciation analysis plays a unique role in the testing of some products. A chromatographic method was developed for the simultaneous determination of Fe(II) and Fe(III). The proposed method is reproducible, rapid and simple to use in assaying pharmaceuticals for Fe(II) and Fe(III) determinations. The detection limits for Fe(II) and Fe(III) were 0.109 μg/L for Fe(II) and 0.217 μg/L for Fe(III) by using the post-column-derivativatization/HPLC technique at 521 nm. The results indicate that values obtained both from HPLC and AAS were in good agreement with the values of pharmaceuticals. The mean recovery of both methods was found to be 98 -101%.

Table 2. The results of iron determination in pharmaceuticals.

	Declared	A A S*		H P L C*		
	Amount (mg)	Found (mg)	Fe %	Found (mg)	Fe(II) %	Fe(III) %
Synthetic Fe (II)	80.50	*79.54±0.03*	98.81	*80.86±0.03*	100.44	-
Synthetic Fe(III)	100.10	*100.72±0.04*	100.62	*98.77±0.06*	-	98.67
Synthetic Mixture (Fe^{2+} *and* Fe^{3+})	80.50 Fe^{2+} 100.10 Fe^{3+}	79.68±0.04 100.28±0.04	98.98 100.18	80.71±0.05 99.96±0.07	100.26	99.86
UA	10.00 Fe^{2+}	10.10±0.04	101.00	10.03±0.09	100.30	-
TB	80.00 Fe^{2+}	79.86±0.03	99.83	80.30±0.06	100.37	-
FC	100.00 Fe^{3+}	100.77±0.06	100.77	100.30±0.02	-	100.30
SD	10.00 Fe^{2+}	10.12±0.07	101.20	10.03±0.02	100.30	-
LE	80.50 Fe^{2+}	80.66±0.08	100.02	80.74±0.07	100.30	-
FG	100.00 Fe^{2+}	100.28±0.02	100.28	102.00±0.06	102.00	-
FH	100.00 Fe^{3+}	98.00±0.06	98.00	99.80±0.07	8.80	91.00

*Results are given as $\overline{X} \pm \frac{ts}{\sqrt{N}}$ (N=4)

As can be seen from Table 2, the Fe content of a medicament containing 100 mg of Fe(III) was found to be 91% Fe(III) and 8.8% Fe(II). If a sample has both ionic form together, this method is suitable for the simultaneous determination of Fe(II) and Fe(III).

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