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Simultaneous Spectrophotometric Determination of Fe^{II} and Fe^{III} in Pharmaceuticals by Partial Least Squares with Chromogenic Mixed Reagents

Ali Niazi

Department of Chemistry, Faculty of Sciences, Azad University of Arak, Arak, Iran
(E-mail: ali.niazi@gmail.com)

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Simultaneous determination of Fe^{II} and Fe^{III} mixtures by spectrophotometric methods is a difficult problem in analytical chemistry because of spectral interferences. By multivariate calibration methods, such as partial least squares (PLS), it is possible to obtain a model adjusted to the concentration values of the mixtures used in the calibration range. The method is based on developing the reaction between the analytes and 1,10-phenanthroline and 5-sulfosalicylic acid as the chromogenic reagent at pH = 4.5. Experimental conditions were established so as to reduce interferences, decrease system complexity and produce a robust procedure that could be used for routine analysis. Spectra should be recorded from 5 to 10 minutes after mixing the reagents. In this study, the calibration model is based on absorption spectra in the 400–600 nm range for 34 different mixtures of Fe^{II} and Fe^{III}. Calibration matrices contained 0.1–7.0 and 0.5–14.0 μg cm^{-3} of Fe^{II} and Fe^{III}, respectively. Detection limits were 0.045 and 0.158 μg cm^{-3} for Fe^{II} and Fe^{III}, respectively. RMSEP for Fe^{II} and Fe^{III} was 0.1559 and 0.2067, respectively. The procedure was confirmed by Fe^{II} and Fe^{III} analyses in pharmaceutical products, and good reliability of the determination was proven.

Keywords:
Fe^{II}
Fe^{III}
determination
pharmaceutical
spectrophotometric
partial least squares

INTRODUCTION

Speciation studies in which two or more physicochemical forms of an element are determined have become of increased interest because the redox state of an element in solution can drastically affect its toxicity, adsorption behaviour, and transport mechanism.[1,2] For instance, Fe^{II} is required for proper transport and storage of oxygen in higher animals by means of hemoglobin and myoglobin, while its oxidized forms, metheoglobin and metmyoglobin, which contain Fe^{III}, will not bind oxygen.[3]

Iron plays an essential role in photosynthesis and is a limiting growth nutrient for photoplanktons in some open ocean regions.[4] Both Fe^{II} and Fe^{III} play important roles in the biosphere, serving as active centres of a wide range of protein oxidases, reductases and dehydrases.[5] These iron species appear together in many natural samples. On the other hand, Fe^{II} is important in the transport and storage of oxygen in higher animals by means of hemoglobin and myoglobin, while Fe^{III} will not bind to oxygen.[6] Because of this and owing to the presence of iron in environmental and biological materials, and insufficient knowledge about the role of the two oxidation states of this element, determination of both Fe^{II} and Fe^{III} is of great importance. Several techniques, such as flow injection,[7,8] electrochemistry,[9,10] chromatography,[11] atomic spectrometry,[12] and spectrophotometry,[13,14] have been used for the speciation of iron in different samples.

The spectrophotometric technique is always an acceptable alternative chemical analysis method due to its acceptable precision and accuracy, associated with its lo-

wer cost compared to other techniques. Critical points against the use of spectrophotometric procedures for the determination of metal ions in solution are the potential problems associated with chemical interferences and the colour development process, both closely related to the chemical condition of the reaction medium. In addition, almost all colour forming reagents are non-specific and only very few are species-selective. This means that separation or masking steps always have to be considered and included in these analytical procedures, making them slower and more sensitive to operational errors, which may ultimately reflect on the precision and accuracy of the method.

In fact, interest in UV-visible spectrophotometric methods has increased and been renewed through the use of signal processing and multivariate techniques[15] such as partial least squares (PLS) regression.[16–20] This tool allows simultaneous spectrophotometric determination of several elements and improves the data handling process of complex chemical systems.

Application of quantitative chemometric methods, particularly partial least squares, to multivariate chemical data is becoming more widespread owing to the availability of digitized spectroscopic data and commercial software for laboratory computers. Each method needs a calibration step, where the relationship between the spectra and the component concentration is deduced from a set of reference samples, followed by a prediction step in which the results of the calibration are used to determine the component concentrations from the sample spectrum. The theory of PLS have been discussed by several workers[21,22] as well as its application in spectrometry.[23–25] In addition, several multicomponent determinations based on the application of these methods to spectrophotometric data have been reported.[26–33]

In this paper, a method for simultaneous spectrophotometric determination of Fe^{II} and Fe^{III} in pharmaceutical products using a partial least squares regression is proposed. The method is based on the reaction between the analytes and 1,10-phenanthroline and 5-sulfosalicylic acid as the chromogenic reagent at pH = 4.50. The aim of this work is to propose a partial least squares method to resolve binary mixtures of Fe^{II} and Fe^{III} in pharmaceutical products without prior separation.

Generally, for the evaluation of the predictive ability of a multivariate calibration model, the root mean square error of prediction (RMSEP) and the relative standard error of prediction (RSEP) can be used:[34]

$$\mathrm{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n} (y_{\mathrm{pred}} - y_{\mathrm{obs}})^2}{n}}$$

$$\mathrm{RSEP}/\% = 100 \times \sqrt{\frac{\sum_{i=1}^{n} (y_{\mathrm{pred}} - y_{\mathrm{obs}})^2}{\sum (y_{\mathrm{obs}})^2}}$$

where y_{pred} is the predicted concentration in the sample, y_{obs} is the observed value of the concentration in the sample and n is the number of samples in the validation set.

EXPERIMENTAL

Reagents and Standard Solutions

All the chemicals used were of analytical reagent grade, sub-boiling, distilled water was used throughout. Stock solutions of Fe^{II} and Fe^{III} were prepared by dissolving $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and $Fe(NO_3)_3 \cdot 9H_2O$ in water, respectively. A stock 1,10-phenanthroline solution (0.02 mol dm^{-3}) in ethanol and stock 5-sulfosalicylic acid solution (0.02 mol dm^{-3}) were prepared by dissolving solid reagent in ethanol. Buffer solution (pH = 4.5) was prepared using potassium hydrogen phthalate and hydrochloride acid with appropriate concentrations.[35] All solutions were freshly prepared daily.

Instrumentation and Software

A Perkin Elmer (Lambda 25) spectrophotometer controlled by a computer and equipped with a 1-cm path length quartz cell was used for UV-vis spectra acquisition. Spectra were acquired between 400 and 600 nm (1 nm resolution). A HORIBA M-12 pH-meter furnished with a combined glass-saturated calomel electrode was calibrated with at least two buffer solutions at pH = 3.00 and 9.00.

Data were treated in an AMD 2000 XP (256 Mb RAM) microcomputer using MATLAB software, version 6.5 (The MathWorks). PLS calculus were carried out in the 'PLS Toolbox', version 2.0 (Eigenvector Technologies).

Procedure

All standards and synthetic samples were 0.003 mol dm^{-3} with respect to 1,10-phenanthroline and 5-sulfosalicylic acid, and appropriate amounts of Fe^{II} and Fe^{III} solutions were added (with a standard microsyringe) to a 10 ml volumetric flask and made up to the mark with deionized water (final pH = 4.5). Absorption spectra were recorded between 400 and 600 nm against a blank of potassium hydrogen phthalate buffer. The spectral region between 400 and 600 nm, which implies working with 200 experimental points per spectra (the spectra are digitized each 1.0 nm), was selected for analysis, because this is the zone with the maximum spectral information among the mixture components of interest. The spectra should be recorded from 5 to 10 minutes after mixing the reagents. All absorption data were preprocessed by standard mean-centering and scaling.

Analysis of Pharmaceutical Products

The proposed method was applied to the analysis of Fe^{II} and Fe^{III} in pharmaceutical products. Pharmaceutical tablet and capsule solutions were prepared by dissolving each tablet or capsule in 10 ml of 0.4 mol dm^{-3} sulphuric acid, heating it in a water bath (70 °C) to completely dissolve. The

solution was then filtered with a filter paper (Whatman No. 1), and the filtrate was diluted with water into a 1000-ml volumetric flask. Then, an aliquot of the solution was diluted to 250 ml with water, and iron contents were measured by the proposed method.

For oral iron drop pharmaceutical samples, 1.0 ml of the sample was diluted with water in a 250-ml volumetric flask, and then 1.0 ml of the diluted solution was diluted again with water to 100 ml. Then, the iron content was measured according to the recommended procedure.

RESULTS AND DISCUSSION

Selection of the Optimum Chemical Condition

A commonly used chelator for Fe^{II} determination is 1,10-phenanthroline. An orange-red complex with stoichiometry of $(1,10\text{-phenanthroline})_3\text{-}Fe^{I}$ and an absorption band with a maximum at about 510 nm results. 5-Sulfosalicylic acid also forms a complex with Fe^{III} at pH = 4.5. The difference between the λ_{max} values of Fe^{II} and Fe^{III} complexes with 1,10-phenanthroline and 5-sulfosalicylic acid, respectively, provides suitable selectively for simultaneous determination of the two oxidation states of iron in the presence of each other.

Figure 1 shows the absorption spectra in aqueous solution of individual metal complexes and of their mixture at pH = 4.5. With the aim of investigating the possibility of determining Fe^{II} and Fe^{III} in mixtures, optimum working conditions were studied under the conditions previously established for each iron species. Effects of 1,10-phenanthroline and 5-sulfosalicylic acid concentration were also investigated; the reagent concentration of 3×10^{-3} mol dm^{-3} was chosen for both because it ensures a sufficient reagent excess. A potassium hydrogen phthalate buffer solution of pH = 4.5 was selected. In order to select the optimum pH value at which the minimum overlap occurs, the influence of the medium pH on the absorption spectra of metal ions was studied over the pH range 2.0–6.0. As absorbance spectra of these complexes are stable between 5 to 10 minutes after mixing the reagents, their absorbances were recorded from 5 to 10 minutes.

Individual calibration curves were constructed with several points, as shown in Figure 2, as absorbance *vs.* metal ion concentration in the range of 0.1–7.0 and 0.5–14.0 μg cm^{-3} for Fe^{II} and Fe^{III}, respectively. The detection limits were 0.045 and 0.158 μg cm^{-3} for Fe^{II} and Fe^{III}, respectively. The wavelengths used to generate calibration curves were 512 and 480 nm for Fe^{II} and Fe^{III}, respectively. Linear regression results, line equations and R^2 are also shown in Figure 2.

Several different approaches have been reported for determination of the detection limit in multivariate calibration procedures. Some authors are still applying the univariate definition of this parameter to evaluate it in a multivariate procedure.[36] In this paper, the detection limit was calculated from the univariate definition as described by Toribio *et al.*[37] and Ketterer *et al.*[38] The absorbance for three blank solutions was obtained from 400 to 600 nm. From PLS modeling for each element, the predicted concentrations were calculated. The standard deviation of predicted concentrations for each cation was calculated (S_b). Then, three times S_b for each element was taken as the detection limit. The detection limits for Fe^{II} and Fe^{III} were 0.045 and 0.158 μg cm^{-3}, respectively.

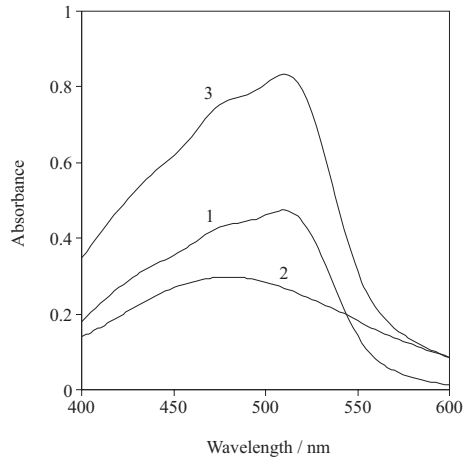

Figure 1. Absorbance spectra of Fe^{II} (3.0 μg cm^{-3}) and Fe^{III} (7.5 μg cm^{-3}) complexes with 1,10-phenanthroline and 5-sulfosalicylic acid at pH = 4.5 (curve 1 and curve 2, respectively) and their mixture (curve 3). Concentrations of 1,10-phenanthroline and 5-sulfosalicylic acid are 0.003 mol dm^{-3}.

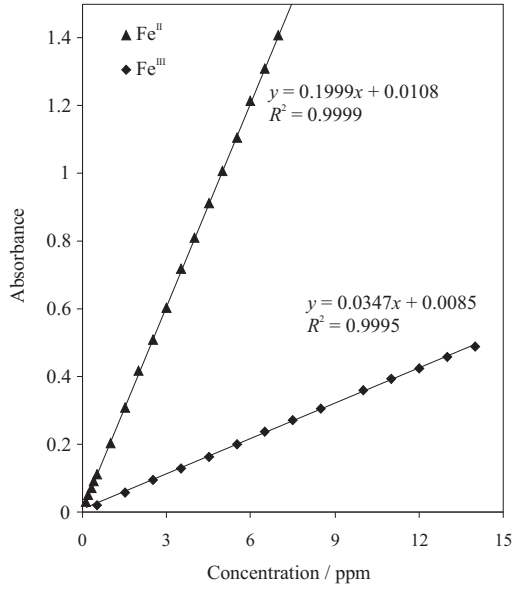

Figure 2. Analytical curve for univariate determination of Fe^{II} (λ_{max} = 512 nm) and Fe^{III} (λ_{max} = 480 nm) complexes at pH = 4.5. Concentrations of 1,10-phenanthroline and 5-sulfosalicylic acid are 0.003 mol dm^{-3}.

TABLE I. Concentration data in μg cm^{-3} of the different mixtures used in the calibration set for determination of Fe^{II} and Fe^{III} mixtures

Fe^{II}	Fe^{III}	Fe^{II}	Fe^{III}	Fe^{II}	Fe^{III}
0.15	4.50	6.70	5.90	3.00	6.90
0.30	5.70	6.70	6.70	4.20	7.80
2.70	9.70	6.90	10.30	4.70	7.80
4.80	11.10	1.50	11.90	5.20	4.80
6.50	12.15	1.85	3.00	6.50	10.40
3.40	9.00	2.05	5.00	6.50	11.90
4.50	14.00	2.05	6.50	7.00	13.60
0.75	9.45	4.85	9.20	7.00	14.00
6.20	1.25	6.90	10.50	3.70	8.60
6.90	2.70	1.70	1.70	5.60	8.80
1.75	1.10	2.50	3.05		
2.70	2.00	3.00	3.90		

Calibration and Validation

The calibration matrix was designed over the concentration ranges 0.1–7.0 and 0.5–14.0 μg cm^{-3} for Fe^{II} and Fe^{III}, respectively. According to the following basic rules, first the calibration standards should be mixtures of components in order to compensate for the effects on absorbance from the interaction between the components. Second, the peak absorbance of each standard should be less than 2.5 in the analytical wavelength range. Finally, the concentration of all the components must be independently varied within the set of standards. The calibration matrix used for the analysis is shown in Table I.

For the prediction step, 12 mixtures not included in the previous set were prepared and employed as an independent test (see Table II). To ensure that the prediction and real samples were in the subspace of the training set, the score plot of the first principal component *vs.* the second was sketched in Figure 3 and all the samples were spanned with the training set scores.

Selection of the Optimum Number of Factors

The optimum number of factors (latent variables) to be included in the calibration model was determined by computing the prediction error sum of squares (PRESS) for cross-validated models using a large number of factors (half the number of total standard + 1). The cross-validation method employed was to eliminate only one sample at a time and then PLS calibrate the remaining standard spectra.[23] By using this calibration, the concentration of the sample left out was predicted. This process was repeated until each standard was left out once.

A reasonable choice for the optimum number of factors would be that number which yielded the minimum PRESS. Since there are a finite number of samples in the training set, in many cases the minimum PRESS value causes overfitting for unknown samples that were not included in the model. A solution to this problem was suggested by Haaland *et al.*[18,19] in which the PRESS values for all previous factors are compared to the PRESS value at the minimum. The *F*-statistical test can be used to determine the significance of PRESS values greater than the minimum.

Eighteen was selected as the maximum number of factors used to calculate the optimum PRESS and the optimum numbers of factors obtained by the application of PLS models are summarized in Table III. In all in-

TABLE II. Added and found results of synthetic mixtures of Fe^{II} and Fe^{III} by using PLS

Added / μg cm^{-3}		Found / μg cm^{-3}		Recovery / %	
Fe^{II}	Fe^{III}	Fe^{II}	Fe^{III}	Fe^{II}	Fe^{III}
1.00	7.50	1.08	7.47	108.0	99.6
1.75	1.50	1.92	1.50	109.7	100.0
3.80	4.30	3.85	4.06	101.3	94.4
6.00	4.60	5.90	4.33	98.3	94.1
6.90	7.80	6.86	7.91	99.4	101.4
4.00	13.30	4.30	13.20	107.5	99.2
3.60	8.20	3.40	8.31	94.4	101.3
3.00	5.05	2.92	5.36	97.3	106.1
6.00	9.40	6.00	9.36	100.0	99.6
6.50	13.10	6.47	13.20	99.5	100.6
1.80	6.30	1.98	5.93	110.0	94.1
5.00	8.05	5.27	7.73		

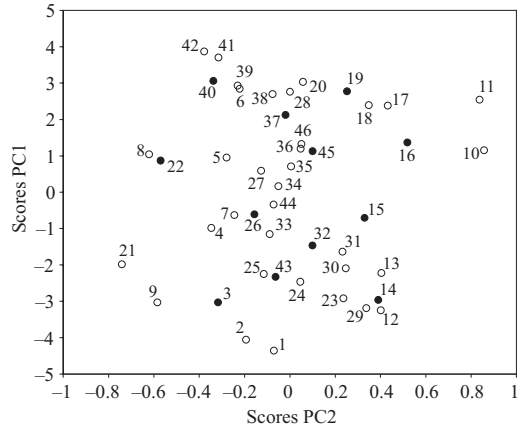

Figure 3. Plot of the first principal component *vs.* the second principal component for Fe^{II} and Fe^{III} determination. (●) Training set and (○) Problem set.

TABLE III. Statistical parameters of the optimized matrix using the PLS

	NPC[(a)]	PRESS	RMSEP	RSEP / %
Fe^{II}	2	1.0114	0.1559	1.9188
Fe^{III}	2	3.4694	0.2067	2.5560

[(a)]Number of principal components

stances, the number of factors for the first PRESS values whose *F*-ratio probability drops below 0.75 was selected as the optimum.

Fe^{II} and Fe^{III} Determination in Synthetic Mixtures

The predictive ability of the method was determined using twelve two-component metallic ion mixtures (their compositions are given in Table II). The results obtained by applying the PLS algorithm to 12 synthetic samples are listed in Table II. Table II also shows the recovery for prediction series of Fe^{II} and Fe^{III} mixtures. As can be seen, the recovery was quite acceptable. The root mean square error of prediction and relative standard error of prediction results are summarized in Table III. Plots of the predicted concentration *versus* actual values are shown in Figure 4 for iron species (line equations and R^2 values are also shown).

Analysis of Fe^{II} and Fe^{III} in Pharmaceutical Products

To evaluate the validity of the proposed method for determination of irons, a recovery study was carried out on samples to which definite amounts of Fe^{II} and Fe^{III} standards were added. The results are given in Table IV.

Effect of Foreign Ions

Interference due to several cations and anions was studied in detail. For these studies different amounts of the ionic species were added to a mixture of Fe^{II} and Fe^{III} containing 1.0 μg cm^{-3} of each. Concentrations causing changes not greater than ± 5 % in the absorption spectrum of complexes were taken as tolerated limits. The ions that interfered most strongly were Co^{2+}, Pd^{2+} and WO_4^{2-}. But there are no interferences in determination of Fe^{II} and Fe^{III} in pharmaceutical products because they do not contain any of these ions. The results are summarized in Table V. As it is shown, the method is relatively specific for Fe^{II} and Fe^{III}.

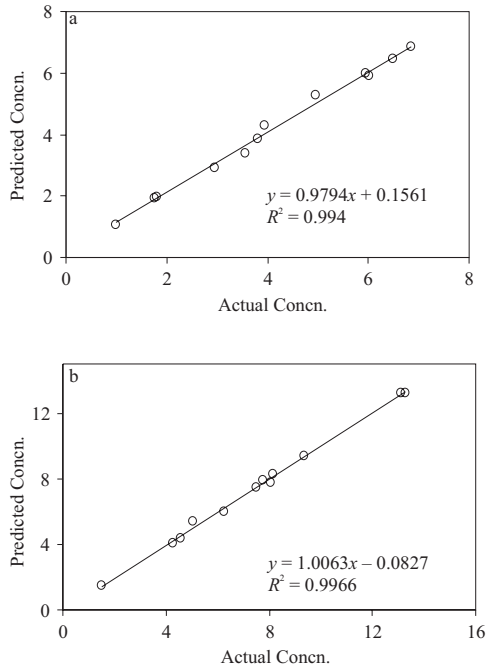

Figure 4. Plots of predicted concentration *vs.* actual concentrations for (a) Fe^{II} and (b) Fe^{III} by PLS.

CONCLUSIONS

The Fe^{II}–Fe^{III} mixture is an extremely complex system due to the high spectral overlapping observed between the absorption spectra of their components. To overcome the drawback of spectral interferences, PLS multivariate calibration approaches are applied. The results obtained on the data set of the Fe^{II} and Fe^{III} mixture demonstrate

TABLE IV. PLS results applied to real matrix samples

Sample	Fe^{II} / μg cm^{-3}		Fe^{III} / μg cm^{-3}		Recovery / %		[a]R.S.D. / % (*n*=3)	
	Added	Found	Added	Found	Fe^{II}	Fe^{III}	Fe^{II}	Fe^{III}
Hematinic capsule (Zahravi Co.)	–	2.11	–	0.00	–	–	1.4	–
Haematinic capsule	2.00	4.13	2.00	2.04	101.0	102.0	1.3	1.1
Ferrosulfate tablet (Daroopaksh Co.)	–	1.05	–	0.00	–	–	1.6	–
Ferrosulfate tablet	3.00	4.08	2.00	1.94	102.7	97.0	1.4	1.3
Oral drop (Daroopaksh Co.)	–	1.12	–	0.00	–	–	1.2	–
Oral drop	1.00	2.16	1.00	0.94	104.0	94.0	1.3	1.8

[a]Relative standard deviation

TABLE V. Tolerance ratio for foreign ions in determination of 1 μg cm^{-3} Fe^{II} and Fe^{III}

Ions	Tolerance limit
Ca^{2+}, Sr^{2+}, Na^{+}, As^{3+}, Mn^{2+}, Li^{+}, Tl^{+}, Rb^{+}, Mg^{2+}, K^{+}, Mo^{4+}, Al^{3+}, Cd^{2+}, CO_3^{2-}, Br^{-}, Cl^{-}, I^{-}, SCN^{-}, CH_3COO^{-}, NO_2^{-}, $S_2O_3^{2-}$	500[(a)]
NO_3^{-}, IO_3^{-}	300
F^{-}	200
Pb^{2+}, SO_4^{2-}	100
Zn^{2+}, Ni^{2+}, Cu^{2+}	50
Cr^{3+}, CN^{-}	40
Mo^{3+}, Ba^{2+}, S^{2-}	20
Zr^{4+}, Bi^{3+}, SO_3^{2-}	15
Hg^{2+}	10
Ag^{+}	5
WO_4^{2-}	3
Co^{2+}, Pd^{2+}	1

[(a)]Maximum concentration studied

the predictive ability of the model obtained. The good agreement clearly demonstrates the utility of this procedure for simultaneous determination of Fe^{II} and Fe^{III} without tedious pretreatment in complex samples of synthetic and pharmaceutical products.

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SAŽETAK

Simultano spektrofotometrijsko određivanje Fe^{II} i Fe^{III} u farmaceutskim proizvodima smjesom kromogenih reagensa primjenom parcijalne metode najmanjih kvadrata

Ali Niazi

Simultano spektrofotometrijsko određivanje Fe^{II} i Fe^{III} u smjesama zahtjevan je analitički postupak zbog spektralnih interferencija. Do odgovarajućeg kalibracijskog modela moguće je doći primjenom parcijalne metode najmanjih kvadrata (PLS). Metoda opisana u ovom radu temelji se na reakciji između analita i 1,10-fenantrolina, odnosno 5-sulfosalicilne kiseline kao kromogenih reagensa pri pH = 4,5. Određeni su eksperimentalni uvjeti pri kojima su smanjene interferencije i kompleksnost sustava, te je razrađena robusna metoda prikladna za rutinsku analizu. Kalibracijski model temelji se na apsorpcijskim spektrima 34 različite smjese Fe^{II} i Fe^{III} u području od 400 nm do 600 nm. Uzorci korišteni za kalibraciju sadržavali su od 0,1 μg cm^{-3} do 7,0 μg cm^{-3} Fe^{II} i od 0,5 μg cm^{-3} do 14,0 μg cm^{-3} Fe^{III}. Spektri su snimani 5 do 10 minuta nakon miješanja reagensa. Granica detekcije za Fe^{II} iznosi 0,045 μg cm^{-3}, a za Fe^{III} 0,158 μg cm^{-3}. Vrijednost RMSEP za Fe^{II} iznosi 0,1559, a za Fe^{III} 0,2067. Primjenjivost i pouzdanost razrađene metode potvrđena je analizom Fe^{II} i Fe^{III} u farmaceutskim proizvodima.