Aniline Derived Bis-Schiff Base - Analytical Reagent for the Assay of Fe (III)

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A new spectrophotometric method for the quantitative determination of Fe (III) was established based on the complexation reaction with a new bis-Schiff base, 4,4'-methylenebis-salicylidene aniline, when a stable complex with an absorption maximum at 520 nm was obtained. The conditions of the complexation reaction were established and the method was validated according to ICH guidelines in terms of linearity, accuracy, precision of the method and the limits of detection and quantification were determined. The method was applied with good results for the quantitative determination of Fe (III) in pharmaceutical products.

Keywords: spectrophotometry, bis-Schiff base, complexation, iron (III).

The biological role of metallic ions [1] and that of their ligands in some physiological and pathological processes within the biological systems can be explained through the formation of complexes [2]. Among the most important *in vitro* or *in vivo* biological ligands, there are aminoacids, Schiff bases and bis-Schiff bases, peptides, nucleotides, nucleic acids, proteins porphyrins and other multidentate organic bases [3-15].

Fe(III) can be quantitatively determined directly using a new spectrophotometric method. The optimum conditions for the complexation reaction using a type ONNO bis-Schiff base (fig. 1) have been determined by studying the following parameters: reaction *p*H, formation time, stability in time of the complex, cation/ligand combination ratio, conditional stability constant (βn) [16-19].

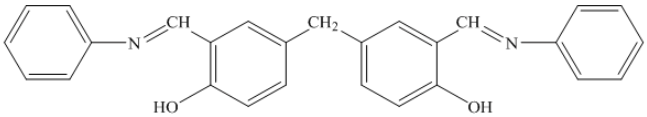

Fig. 1. Chemical structure of 4,4'-methylene bis-salicyliden aniline

Experimental part

Fe(III) ions formed a complex combination with 4,4'-methylen bis-salicylidene aniline (BSB) at *p*H = 4.5 and its absorbance measured at 520 nm was proportional to the concentration of the ions. The optimum wavelength for detection and the optimum working conditions were established. In order to evaluate the performance parameters of the method (linearity, precision and accuracy) solutions in the 5-30μg/mL Fe(III) concentration range have been used.

When establishing the optimum wavelength for the detection, 1 mL of 5-30 μg/mL Fe(III) solution was brought to *p*H 4.5 using acetate buffer, and 2 mL acetone and 1 mL BSB 1% (w/v) solution in methanol were added. After 10 min, the absorbance of the light-red complex was measured at 520 nm against the blank sample.

Method validation

In order to determine the linearity of the method 5-30 μg/mL Fe (III) solutions have been used. The obtained data was analyzed by linear regression and the calibration curve was obtained [20-22]. Detection and quantification limits were calculated using the following formulas [21]: LOD = 3·Standard error/slope and LOQ = 10·Standard error/slope.

In order to determine the precision of the method [21-24], three solutions containing 15, 20 and 25 μg/mL Fe(III) ions were used. Three assays were performed for each concentration level. The assay was performed twice in two different days in order to evaluate the intermediary precision.

In order to establish the accuracy of the method three Fe(III) solutions were used for three concentration levels: 15, 20 and 25μg/mL. For each solution, three determinations were performed [21, 23-27].

Results and discussions

The absorption spectrum of the complex (fig. 2) showed a maximum of absorbance at 520 nm.

The influence of *p*H on the complexation reaction was studied and the optimum *p*H value was established at *p*H 4.5 (fig. 3).

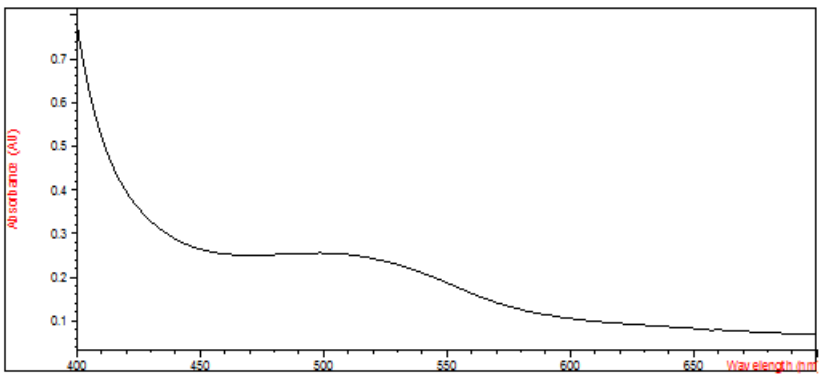

Fig. 2. Absorption spectrum of the complex

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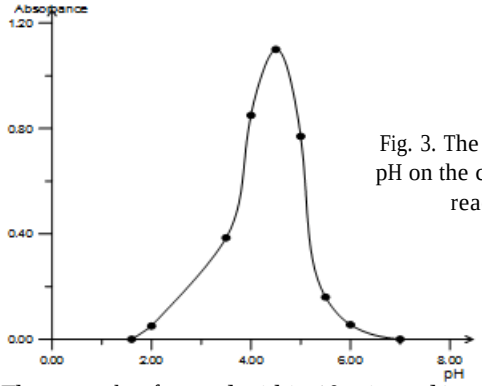

Fig. 3. The influence of pH on the complexation reaction

The complex formed within 10 min and it was stable for another 15 min (fig. 4).

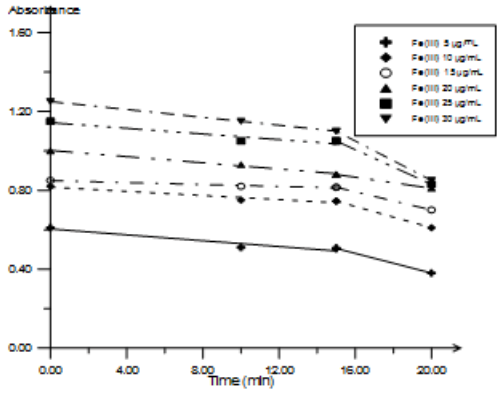

Fig. 4. Complex stability in time

The combination rate was established using the isomolar series method (fig. 5).

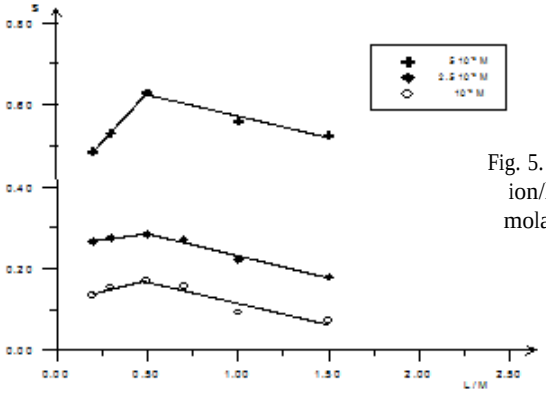

Fig. 5. Metallic ion/ligand molar ratio

The value of the stability conditional constant (ân) was established using the formula: $\beta n = (\log C_M \cdot C_L) / (\log A - n \cdot \log V)$ [22], where: C_M = molar concentration of the metallic ions (Fe(III) ions), C_L = molar concentration of the ligand (BSB), A = absorbance of the metal-ligand complex measured at 520 nm, n = M/L molar ratio; V = the volume of the solution (5 mL); ε= molar extinction coefficient = $5.99 \cdot 10^4$ $mol^{-1} \cdot L \cdot cm^{-1}$. According to the data collected, the obtained value of the stability conditional constant was $\beta n = 5.74 \cdot 10^{-5}$.

For the study of the linearity of the method, four series of Fe(III) solutions in the 5–30 μg/mL concentration range had been used. The obtained data was statistically evaluated (table 1) and the calibration curve was obtained (fig. 6).

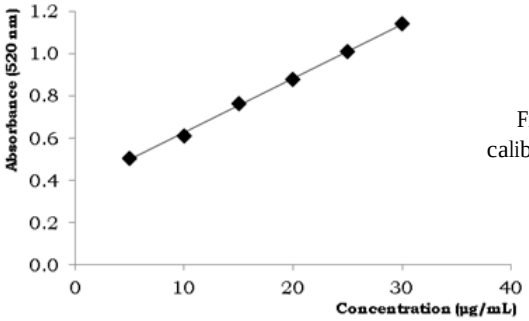

Fig. 6. The calibration curve

The detection limit (LOD) and the quantification limit (LOQ) were calculated: LOD = 0.35 μg/mL and LOQ = 12.56 μg/mL.

The precision of the system was assessed by performing 10 determinations of a 10 μg/mL sample, and the average value, the standard deviation and the relative standard deviation were calculated (table 2). The RSD was 1.32%, so the Fe(III) determination method using the VIS spectrophotometric method was precise. The sample concentration was calculated using the calibration curve equation. The relative standard deviation was lower than 2% (RSD = 1.46%) for each set of data. All these values confirmed that the proposed method was precise (table 2).

For the accuracy study, the concentration of the sample was calculated from the experimental value of the absorbance, using the regression curve equation (table 3). We observed that the recovery was for the studied concentration range, the mean (minimum was 98.06% and maximum was 100.66% and the relative standard deviation was under 2% (RSD = 1.30%). These values proved that the Fe(III) determination method is accurate.

The method was used for the determination of Fe(III) in effervescent tablets from a commercially available pharmaceutical product with multivitamins and multiminerals (table 4).

Nº	Concentration (μg/mL)	Absorbance (520 nm)				
		I	II	III	IV	Average
1.	5	0.51532	0.51714	0.47974	0.50779	0.50499
2.	10	0.64515	0.63546	0.54848	0.61730	0.61160
3.	15	0.75579	0.76521	0.76276	0.76305	0.76170
4.	20	0.88098	0.88025	0.87866	0.86681	0.87668
5.	25	1.04576	1.03828	0.97101	0.97905	1.00853
6.	30	1.11095	1.15601	1.14776	1.15387	1.14215
Absorbance = 0.0257 × Concentration + 0.3685 Correlation and regression coefficients: r = 0.9989, r^2 = 0.9988 Standard error = 0.003028, Intercept = 0.368496 ± 0.002068 Slope = 0.025671 ± 0.000667						

Table 1
LINEARITY OF THE METHOD

No	Absorbance	Statistical Data
1	0.61235	Mean = 0.6065
2	0.60856	SD = 0.0080
3	0.61305	RSD = 1.32%
4	0.60895	
5	0.60725	
6	0.61678	
7	0.59815	
8	0.61165	
9	0.59362	
10	0.59536	

Table 2
PRECISION OF THE SYSTEM

Fe(III) µg/mL	Method precision		Intermediate precision		Accuracy	
	Absorbance	Recovery %	Absorbance	Recovery %	Absorbance	Recovery %
15	0.75697	100.77	0.75355	99.88	0.75657	100.67
	0.74255	97.03	0.75459	100.15	0.74855	98.59
	0.74696	98.17	0.74566	97.87	0.75096	99.04
20	0.87668	98.87	0.87625	98.78	0.87658	98.85
	0.88193	99.89	0.87855	99.23	0.88096	99.70
	0.88941	101.34	0.88834	101.14	0.87341	98.23
25	0.99752	97.90	0.99584	97.64	0.99852	98.06
	1.00256	98.69	1.01895	101.24	1.01458	100.56
	1.01348	100.39	1.00421	98.24	1.00825	99.57
Statistical data	Mean Recovery = 99.23% RSD = 1.46%		Mean Recovery = 99.43% RSD = 1.30%		Mean = 99.25% Min = 98.06% Max = 100.66%	

Table 3
THE METHOD PRECISION AND ACCURACY

Pharmaceutical product	Labelled amount (µg/tablet)	Assessed amount (µg/tablet)	Recovery (%)	RSD (%)
commercially available effervescent tablets	125	123.98±1.175	99.27	1.47

Table 4
QUANTITATIVE DETERMINATION OF Fe (III) FROM EFFERVESCENT TABLETS

Conclusions

A new spectrophotometric method for the assay of Fe(III) was developed, based on Fe(III) complexation reaction with a bis-Schiff base. The complex had a maximum of absorption at 520 nm. The analyzed method has been validated, establishing the optimum wavelength of detection, the linearity (in the range of the 5-30 µg/mL, r = 0.9989), the detection limit (LOD = 0.35 µg/mL), the quantification limit (LOQ = 12.56 µg/mL), the precision of the method (RSD = 1.46%) and the accuracy (mean = 99.25%, minimum = 98.06, maximum = 100.66%).

In conclusion, the proposed method is linear, precise, accurate, simple and fast, and it was used for the quantitative assay of Fe(III) from pharmaceutical product containing the analyzed ion.

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