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ZINC QUANTIFICATION IN SELECTED PHARMACEUTICAL PRODUCTS BY TWO ANALYTICAL METHODS

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ABSTRACT. The aim of this study was to develop two methods that can be used to determine the concentrations of zinc in some pharmaceutical formulations. The first proposed method follows the formation of a red complex between dithizone and zinc which can be spectrophotometrically determined at 516 nm. The second method is based on the detection of zinc through atomic absorption spectrometry, at 213.857 nm, after wet digestion of the samples. The wet ashing method was performed using a mixture of concentrated nitric acid and 30 % hydrogen peroxide (8:2). Both methods were evaluated in terms of linearity, precision (repeatability and intermediate precision), recovery, limit of detection and limit of quantification. The obtained RSD values for the analyzed performance parameters were smaller than the maximum limits recommended by the international standards, therefore the proposed methods can be successfully applied for the determination of zinc in pharmaceutical formulations.

Keywords: *UV-Vis spectrophotometry, FAAS, zinc-dithizone complex, method validation*

INTRODUCTION

Nowadays, the use of dietary supplements based on vitamins, micro and macro elements is widespread. Both young and older people use these preparations in order to compensate for deficiencies in their diet. They are recommended both in some treatments and prophylactically. The use of these supplements has grown in recent years and continues to grow according to a study conducted in 2007 [1].

Zinc is the second transition metal after iron in terms of importance therefore its level in the human body is essential to a healthy growth and development [2, 3]. This metal is an essential constituent for many biological processes catalysed by metalloenzymes (over 300 enzymes), zinc being involved in lipid, protein and carbohydrate metabolisms [4]. So maintaining

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an optimal level of zinc in the body is very important. For this purpose, continuous monitoring of the concentration of zinc in dietary supplements is of great importance. This can be achieved through the development of simple methods for rapid and accurate determination of this metal. Numerous analytical methods have been used for this purpose and among them we mention: spectrophotometry [5], spectrofluorimetry [6], voltammetry [7], chromatography [8], chemiluminescence [9], capillary electrophoresis [10], atomic absorption spectrometry [11], inductively coupled plasma mass spectrometry [12]. Although some of these methods offer very good precision and accuracy, they require expensive and demanding instruments. Therefore, in this study we used UV-Vis spectrophotometry (Method 1) and Flame Atomic Absorption Spectrometry (FAAS) (Method 2) because they are simple, rapid and their use involves minimal resources. The FAAS method relies on a specific light wavelength which is absorbed by the zinc atoms whereas the spectrophotometric method makes use of ligands which bind to zinc in order to produce a coloured complex [12-14].

This paper presents the determination of zinc content in 3 pharmaceutical preparations from Romanian market. The proposed methods were compared in terms of sensitivity, accuracy and applicability.

RESULTS AND DISCUSSION

The reaction between zinc and alkaline dithizone is presented in figure 1, when a red complex (soluble in chloroform) is formed. In order to optimize the spectrophotometric method, different volumes of 0.01% dithizone were used (0.5 - 2 ml). It was observed that at levels higher than 1 ml, the colour of the complex is masked by the colour of dithizone.

Therefore, different amounts of alkaline ammonium citrate solution were used, establishing the optimum volume at 2 ml.

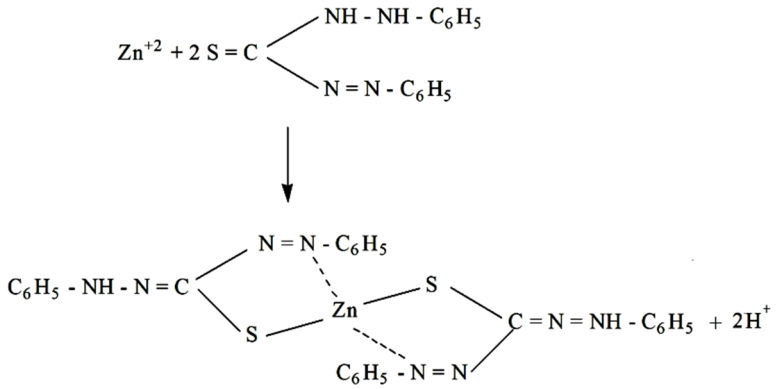

Figure 1. Reaction of zinc with dithizone [15]

Analytical validation

The methods proposed by us were validated according to the ICH recommendations for validation of analytical procedures [16].

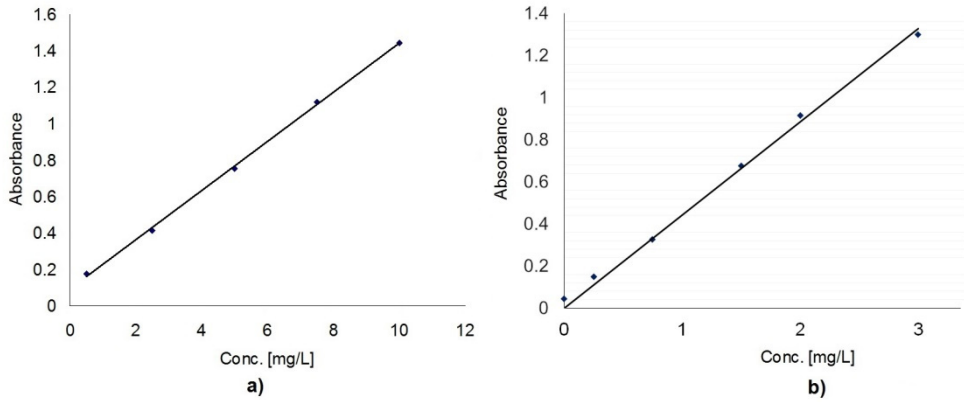

Figure 2. Calibration curves for the spectrophotometric method (a) and for the AAS method (b)

Linearity: for method 1, the results showed good relationship over the concentration range 0.5 - 10 mg/L and for method 2, the analytic response was linear in the range of 0.25 – 3 mg/L. The linear regression equations were found to be y = 1.3502x + 0.09274 for method 1 and y = 0.40915x + 0.09947 for method 2. Figure 2 presents the obtained calibration curves and Table 1 shows the statistical data regarding zinc determinations.

Table 1. Statistical data and validation parameters for zinc determination

Parameter	**Method 1**	**Method 2**
Regression analysis		
Slope	1.3502	0.4091
Intercept	0.0927	0.0994
Standard error	0.0174	0.0041
Regression coefficient (r^2)	0.9991	0.9938
Validation parameters		
Linearity (mg/L)	0.5 - 10	0.25 – 3
Limit of detection (mg/L)	0.4257	0.0078
Limit of quantification (mg/L)	1.2901	0.0238

Accuracy: this parameter represents the closeness of the obtained results to the true theoretical value [17]. The accuracy and reliability of the proposed methods were evaluated by recovery studies of standard addition method (Table 2). The lowest average recovery for all analyzed samples was 98.62 % and the highest 103.1 %, therefore, any change in the active substance concentration can be accurately determined using the proposed methods.

Table 2. Accuracy data for the proposed methods (n = 9)

Method 1			
Added conc. (mg/L)	Absorbance ± SD*	Recovered conc. (mg/L) ± SD*	% Recovery ± SD*
-	0.0015 ± 0.0001	-	-
2.500	0.4303 ± 0.0019	2.499 ± 0.100	100.0 ± 0.57
5.000	0.7631 ± 0.0033	4.976 ± 0.030	99.52 ± 0.64
7.500	1.1090 ± 0.0014	7.526 ± 0.070	100.3 ± 1.00
Method 2			
Added conc. (mg/L)	Absorbance ± SD*	Recovered conc. (mg/L) ± SD*	% Recovery ± SD*
-	0.0456 ± 0.0006	-	-
0.850	0.4424 ± 0.0070	0.838 ± 0.017	98.59 ± 2.03
1.600	0.7748 ± 0.0081	1.650 ± 0.019	103.12 ± 1.23
2.400	1.0864 ± 0.0146	2.412 ± 0.035	100.5 ± 1.48

* Standard deviation of the mean

Precision: the precision of the proposed methods was determined by studying repeatability and intermediate precision. Table 3 presents the repeatability values (expressed as % RSD), which were less than 2 for both methods [18]. These results indicate the precision under the same operating conditions over a short period of time. Intermediate precision presented in Table 4, expresses the results obtained in the same laboratory in different days. With respect to the intermediate precision study, the values for RSD were within the acceptable limits recommended by the international guidelines.

LD (Limit of detection) and LQ (Limit of Quantitation): for method 1 LD and LQ were found to be 0.4257 mg/L and 1.2901 mg/L, respectively, and for method 2, LD and LQ were found to be 0.0078 mg/L and 0.0238 mg/L, respectively.

Table 3. Results of repeatability study (n = 6)

No	Absorbance	
	Method 1	**Method 2**
1.	0.7519	0.7713
2.	0.7498	0.7718
3.	0.7510	0.7716
4.	0.7508	0.7696
5.	0.7522	0.7856
6.	0.7518	0.7771
Average	0.751	0.774
SD*	0.001	0.006
% RSD**	0.119	0.775

* Standard deviation of the mean
** Relative standard deviation

Table 4. Intermediate precision study (n = 3)

	Concentration (mg/L)			Mean (mg/L) ± SD*	% RSD**
	Day 1	Day 2	Day 3		
Method 1	2.50	2.49	2.48	2.49 ± 0.00	0.33
	4.95	4.96	4.96	4.96 ± 0.00	0.10
	7.59	7.55	7.43	7.52 ± 0.07	0.90
Method 2	0.86	0.85	0.82	0.84 ± 0.02	2.02
	1.61	1.59	1.67	1.62 ± 0.03	2.09
	2.41	2.37	2.42	2.40 ± 0.02	0.90

* Standard deviation of the mean
** Relative standard deviation

Application of the proposed methods for pharmaceutical formulations

Both methods have been applied for the determination of zinc in 3 dietary supplements containing just zinc as an active principle (tablets). Table 5 shows the average of 3 determinations for each analyzed sample, expressed in mg zinc/tablet and their recovery values. The samples were analyzed daily for three consecutive days.

Table 5. Zinc concentrations found in analyzed samples

		Method 1		Method 2	
Sample		mg found/ tablet ± SD*	% Recovery	mg found/ tablet ± SD*	% Recovery
Sample 1 15 mg zinc/tablet	Day 1	14.86 ± 0.72	99.06	14.94 ± 0.13	99.60
	Day 2	15.20 ± 0.22	101.33	14.77 ± 0.19	98.46
	Day 3	14.92 ± 0.68	99.46	14.90 ± 0.19	99.33
Sample 2 10 mg zinc/tablet	Day 1	9.92 ± 0. 82	99.20	9.92 ± 0.04	99.20
	Day 2	9.97 ± 0.75	99.70	9.92 ± 0.08	99.20
	Day 3	10.11 ± 0.90	101.1	9.51 ± 0.13	95.10
Sample 1 5 mg zinc/tablet	Day 1	5.09 ± 0.54	101.8	5.12 ± 0.05	102.4
	Day 2	5.01 ± 0.39	100.2	5.04 ± 0.07	100.8
	Day 3	4.98 ± 0.61	99.60	5.09 ± 0.09	101.8

* Standard deviation of the mean

The obtained results are in accordance with the limits imposed by the British Pharmacopoeia ed. 2013, Tablets Monograph [19] and the European Pharmacopoeia 8.0 [20].

CONCLUSIONS

A possible element of originality presented in this paper could be represented by the development, validation and application of a HR-CS-AAS method for the determination of zinc in dietary supplements which contain only zinc as an active principle.

Both methods are precise, simple, however each has its advantages and disadvantages. While the used spectrophotometric method is time consuming and needs more reagents, the FAAS is more sensitive, faster and uses smaller quantities of reagents. On the other hand, the UV-Vis method is cheaper and doesn't require sophisticated equipment. In conclusion, it has been demonstrated that the proposed methods can be successfully used for the determination of zinc in pharmaceutical preparations containing zinc as an active substance.

EXPERIMENTAL SECTION

Reagents

Zinc sulphate ($ZnSO_4$ $7H_2O$) and dibasic ammonium citrate were purchased from Chimopar SA, Romania; Dithizone was supplied by Sigma Aldrich and Chloroform, 65% Nitric acid, 30% Hydrogen peroxide were purchased

from Chemical Company, Romania. The calibration curve for Method 2 was prepared using an ICP multi-element standard solution VIII (0.1 mg/ml) which was supplied by Merck, Germany. All used reagents were of analytical grade. Double distilled water (DDW) was used throughout the experiment.

The analyzed pharmaceutical products were purchased from the local pharmacies in city of Iasi, Romania, between January and March 2014. The samples consisted of 3 types of tablets containing 15 mg zinc/tablet, 10 mg zinc/tablet and 5 mg zinc/tablet.

Preparation of reagent solutions

Dithizone solution 0.1 %: 0.1 g dithizone were dissolved in 100 ml chloroform and kept in brown bottles for one week;

Dithizone solution 0.01 %: 10 ml dithizone solution 0.1 % were mixed with 50 ml ammoniac 250 g/l in a separating funnel and stirred. The chloroformic layer was removed and the extraction was repeated 4 times, each time with 10 ml chloroform. The aqueous layer was filtered and introduced in another separating funnel along with 100 ml chloroform and hydrochloric acid 1/1 until pH = 1. The mixture was vigorously shaken after which the chloroformic layer was separated, washed with DDW several times and filtered through anhydrous sodium sulphate. This solution was prepared when needed.

Alkaline ammonium citrate solution: 25 g of dibasic ammonium citrate were dissolved in 50 ml DDW after which 50 ml of 28 % ammonium hydroxide were added.

Stock solution: zinc sulphate is highly soluble in water, but for a greater stability of the solution, HCl 0.1 M was used. 0.0879 g of $ZnSO_4$ $7H_2O$ were dissolved in 100 ml HCl 0.1 mol/L in order to obtain a concentration of 200 mg/L. After preparation, the stock solution was stored at low temperature.

Apparatus

For the UV-Vis spectrophotometric method a Jasco V 530 double beam UV-Vis spectrophotometer was used. All the measurements were made in 1.0 cm quartz cells at a scan speed of 1000 nm min^{-1} and a scan range of 400-800 nm, fixed slit width of 2 nm.

For the FAAS method a high resolution continuum source flame atomic absorption spectrometer (ContrAA 300, Analytic Jena, Germany) was used. The working conditions consisted of: a mixture of air and acetylene as fuel with a flow of 50 L/h, the burner high was 6 mm and the flame burner was 100 mm in length. The device is equipped with a detector CCD (Charge Coupled Device). In HR-CS-AAS, the background correction is carried out based on various algorithms that are implemented in the software of the atomic absorption spectrometer [21]. The instrumental parameters were optimized in accordance with manufacturer's recommendations.

The procedure of the methods

Method 1: from each prepared solution, a volume of 1 ml was brought to a separating funnel. 2 ml of alkaline ammonium citrate solution and 1 ml of 0.01% dithizone were added and repeated extractions with 5 mL chloroform were performed until the colour of the formed complex disappeared. The absorbance was determined at 516 nm against a blank containing dithizone.

Method 2: the mixture was treated with 8 ml 65% nitric acid and 2 ml 30% hydrogen peroxide in Erlenmeyer flasks. The mixture was subjected to reflux boiling for 4 hours at 140°C using a heating plate. After cooling, the content of the beakers were quantitatively transferred into 100 ml volumetric flasks. The volumes were made up with DDW and filtered. The obtained solutions were analyzed for zinc at 213.857 nm using a flame atomic absorption spectrometer.

Method validation

The method was validated in terms of linearity, accuracy, precision (repeatability and intermediate precision), limit of detection, limit of quantitation.

Linearity: to establish the linearity of the proposed methods, five dilutions were prepared from the stock solution (0.5 mg/L, 2.5 mg/L, 5.0 mg/L, 7.5 mg/L and 10 mg/L for Method 1). For Method 2, another five dilutions were prepared from the ICP multi-element standard solution (0.25 mg/L, 0.75 mg/L, 1.5 mg/L, 2 mg/L and 3 mg/L). The calibration curves were constructed as concentration *vs.* absorbance (Figs. 2 and 3).

Accuracy: the standard addition method was performed. To determine the accuracy of the spectrophotometric method, 3 concentration levels were used (2.5 mg/L, 5.0 mg/L and 7.5 mg/L) and for each level 3 determinations were performed; the concentrations were calculated using the calibration curve equation. In order to check if the FAAS method is accurate, the samples were spiked with known concentrations of analyte (0.85 mg/L, 1.6 mg/L, 2.4 mg/L) and then wet digested and analyzed.

Precision: precision of the method was evaluated as repeatability (intraday variation) and intermediate precision (interday variation). The repeatability studies were carried out by analyzing samples containing 5 mg/L concentration (method 1) and 1.6 mg/L concentration (method 2) for six times in the same day. Intermediate precision was determined by analyzing three concentrations (2.5 mg/L, 5.0 mg/L and 7.5 mg/L) for method 1 and (0.85 mg/L, 1.6 mg/L, 2.4 mg/L) for method 2, daily for three days.

Detection limit and quantitation limit: the limit of detection and limit of quantitation for zinc by method 1 was calculated using the calibration curves. LD and LQ were calculated as $3.3 \cdot \sigma/S$ and $10 \cdot \sigma/S$, respectively, where σ is the standard deviation of y-intercept of the regression equation and S is the slope

of the calibration curve. For method 2, LD and LQ were calculated as 3.3·σ/S and 10·σ/S, where σ represents the standard deviation of 11 blank determinations and S is the slope of the calibration curve.

Application of the proposed method for pharmaceutical formulations

Prior to analysis, 20 tablets were weighed, their average mass was calculated, after which they were manually grinded using a mortar and pestle, homogenized and sieved through a 1 mm pore diameter plastic sieve.

Quantities of homogenized powder equivalent to 15, 10, 5 mg zinc/tablet were weighed and analyzed according to the proposed methods. For method 1, the mixed powder was quantitatively transferred with HCl 0.1 mol/L in 100 ml volumetric flasks, stirred on a magnetic stirrer for 20 minutes, filtered through Whatman paper and then filled to 100 ml with DDW. Samples were labeled: sample 1 (15 mg zinc/tablet), sample 2 (10 mg zinc/tablet), sample 3 (5 mg zinc/tablet). The concentrations of zinc were determined in solution after suitable dilutions to fit the linear ranges.

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