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Validation of the quantification of zinc as active ingredient in pharmaceutical supplementations using flame atomic absorption spectrophotometer

Osamah M.A. Shahlol[1], Khawla I. Salem[2], Salha N. Alkateb[3]

[1,2]Faculty of oil and Gas, Engineering & Renewable Energy Zawia University

[3]Faculty of Science and Medical Technology - Tripoli

الملخص

يتم الآن تحديد كمية الزنك من خلال مطيافية الامتصاص الذري (AAS) ومطيافية الفلورسنت ومطيافية البلازما المقترنة بالحث (ICP) . تعد مطيافية الامتصاص الذري باللهب (FAAS) تقنية مفضلة أكثر لتحديد المعادن الثقيلة نظرا لدقتها وحساسيتها ودقتها وسهولة استخدامها وانخفاض تكلفة التحليل. تعتبر الطرق التحليلية بالغة الأهمية في ضمان استيفاء جوانب جودة المنتج. لذلك، لا يمكن تحقيق الجودة إلا إذا خضعت الطريقة التحليلية لإجراء التحقق. يعد التحقق من صحة الطريقة التحليلية نهجا رسميا متسقا ومسجلا لتقييم قدرة الطريقة التحليلية على تقديم نتائج جديرة بالثقة ودقيقة وقابلة للتكرار. تعمل مبادئ المجلس الدولي للتوافق (ICH) كمعيار عالمي لـ كل من الهيئات التنظيمية وشركات الأدوية. الهدف من هذا العمل هو التحقق من صحة مطيافية الامتصاص الذري باللهب (FAAS) للقياس الكمي للزنك في المستحضرات الصيدلانية وفقا لإرشادات ICH Q2 (R1) من حيث حد الكشف (LOD) وحد القياس الكمي (LOQ) والدقة والخطية والضبط. تم إجراء تحليل الزنك باستخدام مطيافية الامتصاص الذري باللهب من خلال نطاق التركيز 0.20–0.75 جزء في المليون من(II) Zn ، وكان منحنى المعايرة الخطي يملك معامل انحدار (R^2) بقيمة 0.997. تم تحقيق دقة كافية للطريقة من نسبة استرداد الزنك، والتي تتراوح من 100.15 إلى 101.21٪. مما يدل على أن الطريقة قابلة للتكرار. وجد أن حد الكشف LOQ وحد القياس الكمي LOD هما 0.131 و 0.043 جزء في المليون على التوالي. يمكن استخدام الطريقة المقترحة لتقدير الزنك في الأقراص التجارية، وفقا للتحقق الإحصائي للبيانات.

Abstract

Zinc is now quantified by atomic absorption spectrometry (AAS), spectrofluorimetry, and inductively coupled plasma spectroscopy (ICP). Flame atomic absorption spectrometry (FAAS) is a more preferable technology for determining trace metals because of its accuracy, sensitivity, precision, easiness, and lower cost per analysis. Analytical methods are critical in ensuring that product quality aspects are fulfilled. So, quality can only be attained if the analytical method undergoes a validation procedure. Analytical method validation is a formalized, consistent, and recorded approach for evaluating the ability of an analytical method to deliver trustworthy, accurate, and repeatable results. The International Council for Harmonization (ICH) principles serve as a global standard for both regulatory agencies and the drug companies. The goal of this work is to validate Flame atomic absorption spectrometry (FAAS) for quantitative measurement of zinc in pharmaceutical formulations according to the ICH Q2 (R1) guideline in terms of limit of detection (LOD), limit of quantification (LOQ), precision, linearity and accuracy. Analyses of zinc samples using FAAS were performed

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through the concentration range 0.20–0.75 ppm Zn(II), and the linear calibration curve had 0.997 as regression coefficient (R^2). Adequate accuracy of the method was achieved from zinc recovery%, ranging from 100.15 to 101.21%. The (%RSD) with intra and inter-day precision was less than 1%, demonstrating that the method was repeatable. The LOQ and LOD were found to be 0.131 and 0.043 ppm, respectively. The proposed method can be used to estimate zinc in commercial tablets, according to statistical validation of the data.

Key words: *Method validation; Zinc; Supplementation; COVID-19; Atomic absorption spectrometry.*

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was discovered and caused Coronavirus Disease (COVID-19) in late 2019. Because of the disease's high contagion, it dispersed around the planet, directing the World Health Organization (WHO) to announce it as an epidemic.

COVID-19, without a doubt, represents the most severe acute health threat to society in modern history [1]. COVID-19 pandemic has prompted a surge to find traditional, complementary, and integrative medicine (TCIM) and pharmaceutical treatments. Indirect evidence now suggests that zinc may lessen the risk, duration, and severity of SARS-CoV-2 infections, particularly in populations at risk of zinc deficiency, such as people with the chronic disease co-morbidities and the elderly [2]. SARS-CoV-2 was efficiently removed from the nasopharynx with a combination of nitazoxanide, ribavirin, and ivermectin, as well as a zinc supplement, in less time than symptomatic therapy [3].

Zinc is necessary for immunological function, tissue repair, insulin and blood pressure control, and gene expression regulation [4]. It can be supplemented as a stand-alone nutraceuticalor as part of a multi-mineral, multi-vitamin, or multi-herb product. The majority of zinc supplements are taken orally in single or divided daily dosages as a lozenge, tablet, pill, liquid, or syrup [2].

Zinc is now quantified by atomic absorption spectrometry (AAS), spectrofluorimetry, and inductively coupled plasma spectroscopy (ICP) [5-8]. Flame atomic absorption spectrometry (FAAS) is a more preferable technology for determining trace metals because of its accuracy, sensitivity, precision, easiness, and lower cost per analysis [9-11].

Analytical methods are critical in ensuring that product quality aspects are fulfilled. So, quality can only be attained if the analytical method undergoes a validation procedure. Analytical method validation is a formalized, consistent, and recorded approach for evaluating the ability of an analytical

method to deliver trustworthy, accurate, and repeatable results [12-14]. The International Council for Harmonization (ICH) principles serve as a global standard for both regulatory agencies and the drug companies [15].

The goal of this work is to validate flame atomic absorption spectrometry (FAAS) for quantitative measurement of zinc in pharmaceutical formulations according to the ICH Q2 (R1) guideline [16] in terms of limit of detection (LOD), limit of quantification (LOQ), precision, linearity and accuracy.

EXPERIMENTAL

Chemicals

Analytical grade chemicals were used as received without further purification. Nitric acid, 60% (Merck); hydrochloric acid, 30% (Merck) and zinc stock solutions of 1000 ppm were used. Standard operating solutions were made immediately before use from stock solutions. Water that has been deionized was used (Milli-System, Millipore). Before usage, all glassware (Class A) was immersed in nitric acid (1%) for 15 minutes and rinsed with deionized water.

Instrumentation

The determination of zinc was carried out by FAAS using a GBC Scientific equipment model (Savant AA), with a zinc hollow cathode lamp. CEM, MDS 2000 Microwave Digestion System was used for sample preparation. Table 1 summarizes the spectrometer's experimental parameters for the zinc determination.

Table 1. Instrumental conditions for the measurement of zinc by FAAS

Parameter	
Wavelength (nm)	213.9
Slit Width (nm)	0.5
Light source	Zinc hollow lamp
Lamp current (mA)	5.0
Flame, flow setting (l min^{-1})	Air (2), Acetylene (10)
Integration time (s)	2

Sample Preparation

Zn-tablets were crushed with a porcelain pestle and mortar. An approximately 800 mg of sample was weighed and digested in microwave with 12 ml of nitric acid (70% HNO_3) according to

the microwave program I (Table 2). The digests were completed to 100 ml using deionized water, and the resultant solutions were diluted (1 ml in 100 ml deionized water) then subjected to zinc determination by FAAS.

Table 2. Microwave parameters

	Program		
Stage	(1)	(2)	(3)
%Power	45	45	45
Pressure, PSI*	40	80	120
Time, (min)	6.00	10.00	10.00

Method validation

The method's dependability was determined by calculating multiple analytical figures of merit (ICH, 2005) [17]. Linearity, repeatability, reproducibility, accuracy, sensitivity as indicated by the limit of detection (LOD) and limit of quantification (LOQ), and measurement uncertainty are all factors to consider.

Linearity

A graph of 'concentration' versus 'absorbance' was plotted at the observed absorbance at 213.9 nm wavelength is used to assess linearity. Calibration curve was prepared ranging from 0.20 to 0.75 ppm (0.20, 0.30, 0.40, 0.50, 0.60 and 0.75 ppm) with each concentration was measured three times. The coefficient of correlation (R^2) was calculated using regression analysis with the help of slope and intercept data.

Limit of detection (LOD) and Limit of quantification (LOQ)

In quantitative analysis, the limit of detection (LOD) and limit of quantification (LOQ) are two key factors. The United States Pharmacopeia defines LOD as the minimum concentration of the analyte that can be detected but not quantified. Under the specified experimental conditions, the LOQ is the minimum concentration in a sample that can be measured with an acceptable level of accuracy and precision [18-22]. Equations (1) and (2) were used to calculate LOD and LOQ values [20, 21]:

$$\text{LOD} = 3 \text{ x } \frac{\text{the standard deviation of the response}}{\text{Slope of the calibration curve}} \quad (1)$$

$$\text{LOQ} = 10 \text{ x } \frac{\text{the standard deviation of the response}}{\text{Slope of the calibration curve}} \quad (2)$$

Precision

The precision is a measure of how closely the mutually independent test results agree, and it's usually expressed in terms of standard deviation. When the analytical technique is applied, precision is caused by random errors that arise throughout the measuring procedure [23]. Repeatability, intermediate precision and reproducibility are distinguished in term of precision [24]. Six repeated concentrations of 10 ppm on the same day and two different days were analyzed for repeatability and intermediate precision. The percentage relative standard deviation (% RSD) was calculated using equation (3):

$$\% \text{ Relative standard deviation} = \frac{Standard deviation}{Mean} \times 100 \qquad (3)$$

Accuracy

Accuracy refers to the level of agreement between the measured and true values. Determination of accuracy (recovery, % R) was done by preparing three samples, spiked with three zinc different concentrations of 80%, 100% and 120% with three times replication [15]. Equation (4) was used to calculate recovery, % R.

$$\text{Recovery, \%R} = \frac{\text{average experimental measured}}{\text{theoretical amount spiked}} \times 100 \qquad (4)$$

RESULTS AND DISCUSSION

Linearity and range

Linearity is seen in the zinc concentration range of 0.20 to 0.75 ppm. Zinc demonstrated strong linearity after least square analysis, with a coefficient of determination (R^2) of 0.9970, indicating excellent calibration curve linearity (Table 3). The analytical response was linear over the zinc concentration range (0.20-0.75 ppm), since the obtained R^2 value was greater than 0.995 [14]. The calibration plot of zinc concentrations and their associated absorbance values is shown in Fig.1.

Table 3. Linearity data for analysis of zinc using FAAS.

Concentration of zinc standard (ppm)	Mean absorbance	Standard deviation	Relative standard deviation RSD%
0.20	0.0754	0.0003	0.3978
0.30	0.1108	0.0006	0.5511
0.40	0.1419	0.0006	0.4304
0.50	0.1743	0.0002	0.0994
0.60	0.2006	0.0011	0.5328
0.75	0.2417	0.0013	0.5205
Slope	0.3013		
intercept	0.0194		
R^2	0.9970		

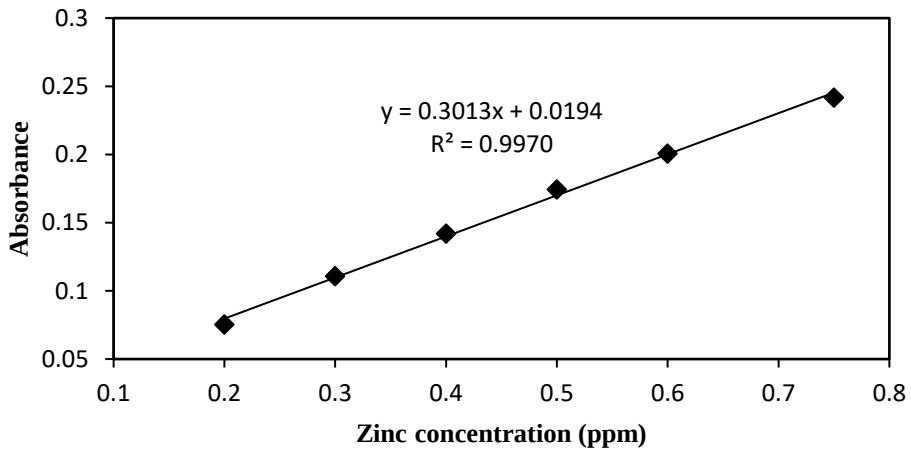

Fig.1. Calibration curve of zinc using FAAS.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ of zinc were determined using the slope of the calibration curve to be 0.043 ppm and 0.131 ppm, respectively. The LOD and LOQ data revealed that the approach was highly sensitive when it came to estimating zinc.

Accuracy

Mean recoveries (% R) of zinc for three prepared samples with identical spike were 100.64 %, 101.21 % and 100.15 %, with RSDs of 1.4555, 1.1625 and 0.0190, respectively (Table 4).

Table 4. Percentage recovery (accuracy) of zinc in spiked samples

Conc., (%)	Placebo	80%	100%	120%
1st run	0	7.92	10.19	12.02
2nd run	0	8.12	10.19	12.01
3rd run	0	8.12	9.98	12.02
Average		8.05	10.12	12.02
Stand. Dev.		0.1172	0.1177	0.0023
RSD		1.4555	1.1625	0.0190
Recovery (%)		100.64	101.21	100.15

Precision

Intermediate precision includes the estimation of variations in analysis when a method is used within laboratories but on different days. The intermediate precision is assessed by analyzing two working standard solutions on two different days (interday); the RSD values obtained are given in Tables 5 and 6. The percent relative standard deviation (percent RSD) with intra- and inter-day precision was less than 1%, (0.7547 and 0.66125, respectively) demonstrating that the method was repeatable.

Table 5. Repeatability (intra-day) data for zinc analysis.

Test number	Results		average	Assay%
	1st run	2nd run		
1	10.19	10.19	10.19	101.89
2	10.11	10.11	10.11	101.12
3	9.94	10.15	10.05	100.46
4	10.15	9.94	10.04	100.44
5	10.00	10.20	10.10	101.01
6	9.97	9.97	9.97	99.66
Average				100.76
Standard Deviation				0.7605
RSD				0.7547

Table 6. Intermediate precision (inter-day) data for zinc analysis.

Test number	Results of Day 1				Results of Day 2			
	1st run	2nd run	Average	Assay%	1st run	2nd run	average	Assay%
1	10.13	10.19	10.16	101.59	10.19	9.99	10.09	100.87
2	10.21	10.11	10.16	101.62	9.91	9.91	9.91	99.10
3	9.94	10.26	10.10	101.00	9.94	9.94	9.94	99.45
4	10.15	10.00	10.07	100.73	9.94	9.94	9.94	99.42
5	10.00	10.20	10.10	101.01	10.00	10.00	10.00	99.99
6	9.97	9.98	9.97	99.71	9.97	9.97	9.97	99.66
average				100.94				99.75
Standard deviation				0.7030				0.6244
RSD				0.6965				0.6260

ANALYSIS OF MARKETED ZINC TABLETS

After digestion, the six sample solutions were tested to determine the amount of zinc in the marketed dietary supplement items. The amount of zinc in each tablet formulation and its percentage content were determined. According to the label claim, the zinc concentration determined from the mean values of six digested sample solutions was within the permissible range of 90–110 percent. The study demonstrated that the proposed method was accurate and straightforward, and that it could be used in daily routine analysis. Table 7 shows the final outcome.

Table 7. Assay data for marketed zinc tablets

Dietary supplement	Label claim (mg)	Amount found (mg) (mean ± SD)	% content (mean ± SD)
Ascozinc	20	19.78 ± 0.38	98.92 ± 1.88
Thorne	30	29.90 ± 0.92	99.77 ± 2.17

CONCLUSIONS

A method for the recovery and FAAS measurement of zinc in pharmaceuticals was developed and verified according to ICH and US Pharmacopeia criteria. The procedure is exact, concise, and dependable. This approach can be used for routine zinc analysis and pharmaceutical quality control in a variety of products that must adhere to FDA, European Commission, and other applicable requirements to assure the safety and viability of products given to consumers. The developed method

can be successfully employed for our routine determination procedure, according to the statistical report.

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