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UV-Vis Spectrophotometric Determination of Aluminum in Feminine Antiperspirants: Method Development and Validation

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Abstract*– Aluminum is the third most abundant metal on earth and is currently widely used in industrial, technological and medical sectors, including pharmaceuticals and personal care products. However, in recent years, several studies suggest possible health risks of aluminum, being associated with neurotoxicity, Alzheimer's disease and breast cancer. Therefore, the aim of this study was to propose, develop and validate a simple, reliable and accessible UV-VIS spectrophotometric method for the quantification of aluminum by means of the aluminum-alizarin complex. The method proved to be linear with a coefficient of determination R^2 of 0.9998, precise with a relative standard deviation (RSD) of less than 11% and accurate with recovery percentages higher than 80% and lower than 110%, as stipulated by the AOAC. In conclusion, the method proved to be simple, accessible, reliable, precise and accurate for the quantification of aluminum in antiperspirant samples.

***Keywords*— Aluminum, spectrophotometry, validation, antiperspirants, alizarin red**

I. Introduction

Aluminum is the third most abundant metal on earth, with an 8% distribution over the earth's crust [1]. Due to its great abundance, it can be found in various products for human use. Since the isolation of aluminum in its elemental form by the physicist Hans Oersted [2], until today, aluminum has played a very important role in processes related to food processing and preservation [3], as well as in the industrial, technological and medical sectors, highlighting its usefulness in various applications for health, such as medicines, cosmetics and personal care products, in which we can highlight lipsticks, antiperspirants, toothpaste, etc. [4], [5], [6]. Given the high presence of aluminum in pharmaceutical products, food and drinking water, international organizations such as the World Health Organization (WHO), Food and Drug Administration (FDA) and Scientific Committee on Consumer Safety (SCCS) began to regulate the maximum allowable concentrations of aluminum in various products. The organizations issue bulletins, opinions and, among other means, information on the safety of aluminum, the maximum permitted levels of aluminum intake in food and beverages and information on pharmaceutical products with their permitted aluminum concentrations.

In recent years, the scientific community began to question the innocuousness of aluminum with respect to human health, and several studies began to suggest the participation of aluminum in various diseases [4], such as in the generation of chromosomal instabilities [5], in in vitro models. The possible involvement of aluminum in Alzheimer's disease [7], since aluminum causes neurotoxic events, the deposition of β-amyloid and the formation of neurofibrillary tangles in the brain, together with increased inflammatory signaling [8], [9], [10]. On the other hand, the role of aluminum in breast cancer is also suspected [11], [12], because several studies showed that patients diagnosed with breast cancer possessed high aluminum concentrations in breast tissue [8], [10], [11].

Therefore, in recent years, new methods for aluminum quantification that are reliable and accurate have been developed. At present, multiple analytical methods are known to quantify aluminum, but the presence of interferences produced by the nature of the methods and the insufficiency of the detection limits are problems that still need to be improved [13].

Among the main methods of aluminum quantification are the Electronic Atomization Atomic Absorption Spectrometry (ETAAS), the Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS), the Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS), Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS) and Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-

AES) [14], [15], [16], which are precise, accurate, sensitive and linear. However, the availability of equipment makes the application of these methods not easily accessible, so it is important to develop more accessible alternatives that meet the validation guidelines of an analytical method established by the AOAC. Therefore, this research proposes to develop and validate a simple analytical method for the quantification of aluminum through the alizarin-aluminum complex by UV-Vis spectrophotometry.

II. Materials and Methods

A. Reagents and equipment

Aluminum nitrate nonahydrate, alizarin red and aluminum hydroxide P.A., were purchased from Merck (Darmstadt, Germany). Absolute ethyl alcohol was purchased from J.T. Baker (Avantor). In addition, ultrapure water (18.2 MΩ cm) obtained from an Easypure ™ II was used. Spectrophotometric readings were performed on the Cary 60 UV-VIS spectrophotometer with quartz cuvettes. Sonics Materials VCX-130PB ultrasonic processor and HETTICH EBA 20 centrifuge were used for sample preparation.

B. Preparation of the calibration curve

Concentrations of 25, 40, 55, 55, 70 and 85 ppm (mL) of aluminum nitrate, together with 0.1% alizarin red solution, were used to prepare the calibration curve. From the above solutions 500 µL were measured and diluted with $1x10^{-4}$ M aluminum hydroxide to a final volume of 5 mL. It is important to mention that the ratio of alizarin red and aluminum was 1:1. The above solutions were incubated for 30 min in darkness and room temperature. The analyses were performed in triplicate and the spectrophotometric readings were performed in triplicate at a wavelength of 615 *nm.*

C. Sample preparation

Between 0.05 g and 0.125 g of the antiperspirant cream was weighed and dissolved in 50 mL of ultrapure water. The sample was subjected to ultrasound for 10 min at 20 kHz and centrifuged for 10 min at 6000 *rpm*, this process was performed in duplicate. An aliquot of 2 mL was removed and brought to a volume of 10 mL. Finally, for reading in the spectrophotometer, 500 µL of the sample was removed and made up to a volume of 10 mL with 500 µL of 0.1 % alizarin red and $1x10^{-4}$ M sodium hydroxide. Samples were incubated for 30 min in the dark at room temperature. Reading was performed in triplicate at a wavelength of 615 *nm.*

D. Validation of the aluminum quantification method.

The validation of the method was performed according to the guidelines established by the AOAC [17].

a) Linearity

To evaluate the linearity of the method, five calibration solutions were prepared, which are described in section II.B. The samples were analyzed under established conditions. This process was carried out in triplicate and the coefficient of determination R^2 was used to evaluate the linearity parameter. With this parameter the linear regression equation was determined:

$$y = a + bx \quad (1)$$

Where "x" corresponds to the concentration of aluminum nitrate expressed in ppm, "y" is the area expressed in nm, which is obtained from the readings of the samples at established conditions, "a" corresponding to the intercept and "b" representing the slope of the line.

b) Limit of quantification and detection

To evaluate the limits of quantification (LOQ) and detection (LOD), equations 2 and 3 were used, which correspond to the minimum amount of aluminum nitrate that the method can determine but not quantify (LOD) and the minimum amount of aluminum nitrate that the method can quantify with precision and accuracy (LOQ).

$$LQ = \frac{10\ x\ \sigma}{S} \quad (2)$$

$$LD = \frac{3.3\ x\ \sigma}{S} \quad (3)$$

Where "σ" is the minimum standard deviation of the samples and "S" corresponds to the intercept of equation 1.

c) Intermediate precision

To evaluate the intermediate precision of the method, a repeatability analysis was performed, where the concentrations established in the calibration curve were used, performing a total of 5 measurements in triplicate on different days, reagents, operators and laboratory. The relative standard deviation (RSD) was determined using equation 4.

$$RSD = \frac{S_i}{\bar{x}}\ x\ 100 \quad (4)$$

Where "$\overline{x}$" is the average of the concentrations and "Si" is the standard deviation.

d) Accuracy

To evaluate the accuracy of the method, it was determined by finding the percentage of recovery (%R). For this reason, samples were prepared (section II.C.) to which a concentration of 2.5, 5.5 and 8.5 mg/L of aluminum nitrate was added, obtaining the following groups. CS: is the concentration of the sample, C_{SA}: is the concentration of the sample plus the aluminum nitrate added and C_A: is the concentration of the aluminum nitrate The formula 5 was used for the calculation.

$$\%R = \frac{C_S + C_{SA}}{C_A}\ x\ 100 \quad (1)$$

E. Statistical analysis

Statistical analysis of the calibration curve, linearity test, intermediate precision and accuracy were performed with GraphPad Prims 8 software.

III. Results and Discussion

For the determination of the wavelength for the quantification of aluminum, measuring aluminum nitrate and alizarin red, the wavelengths of 494 *nm* and 615 *nm* were used and a spectral sweep was performed obtaining a higher absorbance of the complex at 515 *nm*, but the wavelengths of 494 *nm* and 515 *nm* showed not to have an optimal linearity, unlike these wavelengths, the readings made at 615 *nm* showed to have an optimal linearity, so it was decided to work all the validation of the method at 615 *nm*

Similarly, thanks to the spectral sweep performed on the aluminum - alizarin complex, it was demonstrated that the validated method is based on the decrease of the concentration in mg/L of the alizarin red present in the medium as the concentration of aluminum nitrate increases, thus forming the aluminum - alizarin complex until the aluminum present in the solution reacts completely with the alizarin red.

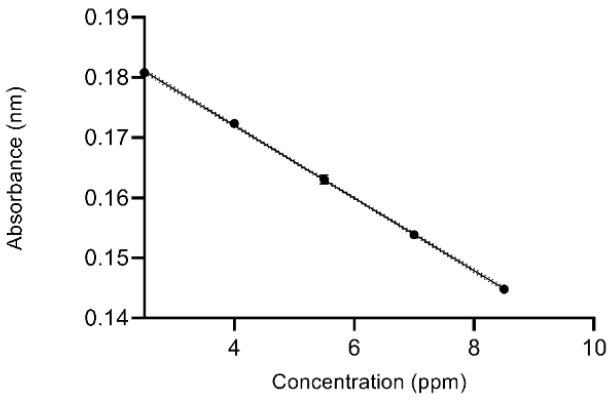

Fig. 1 Calibration curve for aluminum nitrate and alizarin red

In the evaluation of the linearity of the method, concentrations of 2.5, 4, 5.5, 7 and 8.5 mg/L of aluminum nitrate were used to make a calibration curve, which generated the necessary data to make a graph comparing the absorbance of the five different concentrations, with respect to the concentration of each one of them, obtaining a descending line as the aluminum in the reaction increases, as shown in Fig. 1. A determination coefficient R^2 of 0.9998 was obtained, which being greater than the R^2 of 0.995, demonstrates that it is a linear method between the concentrations of 2.5 and 8.5 mg/L of aluminum nitrate.

With the data obtained from the linear regression curve and the use of equations 1, 2 and 3, it was possible to determine the limits of quantification and detection of the analytical method, which were 0.1659 mg/L and 0.0548 mg/L, respectively. Establishing that this method of aluminum quantification can accurately and precisely quantify samples from 0.1659 mg/L and above.

For the intermediate precision test, we worked with the five concentrations used in the calibration curve, as shown in Table I, which were evaluated on different days, with different reagents, operators and in different laboratories. When the data obtained for the five concentrations were processed in triplicate, an RSD% of less than 11% was obtained. This is a value established by the AOAC in the precision test according to the concentration of the analyte [17], given that our method was developed in ppm (mg/L), the maximum RSD% that could be obtained for each concentration was 11%, but as shown in Table I, all the RSD% obtained are within the values established by the AOAC, indicating that the following method has an acceptable intermediate precision.

TABLE I

Intermediate accuracy data

Concentration	I	II	III	IV	V	$\bar{x}$	Si	RSD
2.5	0.1679	0.1670	0.1681	0.1665	0.1655	0.1670	0.0011	0.63
4.0	0.1597	0.1554	0.1598	0.1585	0.1571	0.1581	0.0019	1.19
5.5	0.1518	0.1463	0.1445	0.1500	0.1473	0.1480	0.0029	1.98
7.0	0.1451	0.1382	0.1368	0.1426	0.1358	0.1397	0.0040	2.86
8.5	0.1348	0.1338	0.1264	0.1323	0.1274	0.1309	0.0038	2.90

Where: I, II, III, IV and V, were different days where the calibration curve was replicated, with different reagents, laboratories, operators.

With the data obtained in Table I, the coefficient of determination R^2 of the measurements made with different measurement conditions was found, obtaining an R^2 of 0.9991,

which indicates that the method, despite having processing conditions, continues to maintain its linearity, as shown in Fig. 2.

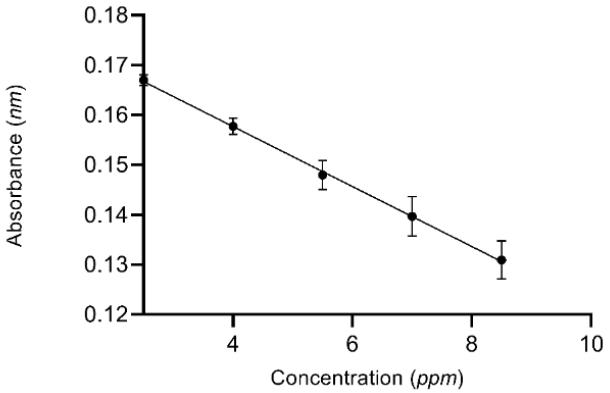

Fig. 2 Calibration curve with intermediate accuracy data

Finally, the accuracy of the method was evaluated by means of the recovery test. Table II shows the recovery percentages obtained at three different concentrations belonging to the calibration curve. These were higher than 80 and lower than 110, this being an interval established by the AOAC [17], [18]. For this reason, the method is accurate.

TABLE II

Accuracy data (Percentage of recovery)

Minimum concentration - 2.5 ppm		
	Concentration in ppm	**%R**
C_S	2.1563	88
C_{SA}	5.2188	
C_A	3.4896	
Intermediate concentration - 5.5 ppm		
	Concentration in ppm	**%R**
C_S	0.7485	92
C_{SA}	6.2281	
C_A	5.9825	
Maximum concentration - 8.5 ppm		
	Concentration in ppm	**%R**
C_S	1.7619	98
C_{SA}	10.0649	
C_A	8.5108	

Where: C_S: is the concentration of the sample, C_{SA}: is the concentration of the sample plus added aluminum nitrate and C_A: is the concentration of aluminum nitrate.

The presence of zirconium in the form of Aluminum Zirconium Tetrachlorohydrex GLY, and changes in pH at the time of forming the complex proved to be the main factors that altered the results of the samples evaluated.

In the work done by Al-Husseini et al. [19], they validated a method of aluminum quantification with aluminum sulfate octadecahydrate and alizarin DYE, in the validation of the method they obtained a limit of quantification of 0.018 mg/L within a linearity range of 0.02 - 2 mg/L of aluminum sulfate, in addition it was suggested that the optimum pH for the formation of the aluminum - alizarin complex is in the pH range of 6 to 7.

The above statement refers to the tests performed where we verified that, at acidic or alkaline pH, the formation of the aluminum - alizarin complex was minimal and suboptimal. On the other hand, regarding the interferences of the aluminum quantification method of Al-Husseini et al. it was reported that the presence of cations such as copper, zinc and iron are interfering with the quantification and formation of the aluminum - alizarin complex.

Currently, it is known about the ability of alizarin to form complexes with metals such as zirconium, titanium, nickel, cadmium and calcium [20], [21], [22], [23], which makes the method developed and validated in the present investigation very useful for the quantification of aluminum in pharmaceutical formulations such as feminine antiperspirants containing aluminum and free of zirconium and other interfering metals.

Another research work published by Al-Husseini *et al.* [24], validated an aluminum quantification method using aluminum hydrochloride and alizarin S, obtaining a detection and quantification limit of 3.06 and 10.2 mg/L, together with a linearity range of 10 ppm up to 125 mg/L of aluminum hydrochloride by means of a diffuse reflectance spectrophotometer. Furthermore, in this study they were able to establish that a wavelength of 615 nm is optimal to evaluate the formation of the complex, considering that as aluminum increases, the concentration of alizarin decreases. The aforementioned information was corroborated in our work, since readings were taken at 494 *nm*, 515 *nm* and 615 *nm*, showing that at the lengths of 494 *nm* and 515 *nm*, the results do not present optimal linearity for the validation of the method, unlike the length of 615 nm, which proved to be linear for the quantification of aluminum using aluminum nitrate and alizarin red.

On the other hand, Supian *et al.* [25], evaluated the behavior of the aluminum - alizarin complex, making use of alizarin red and aluminum nitrate, detailing the behavior of

alizarin red. Alizarin red, being an anionic dye, can behave as a bidentate or tridentate ligand, depending directly on the pH. It is detailed that aluminum +3 reacts with the hydroxyl group of the anthraquinone ring, directly affecting positions 1 and 2 (Fig. 3), causing these auxochrome groups to affect the light absorption of the molecule, because they alter the behavior of the carbonyl group (C = O), which acts as a chromophore group. Supian and collaborators performed measurements at different wavelengths with a spectral sweep, demonstrating that alizarin red has an absorbance between 420 - 480 *nm*, but when aluminum is added, a bathochromic shift occurs, causing the wavelengths to increase, being 500 - 550 *nm.*

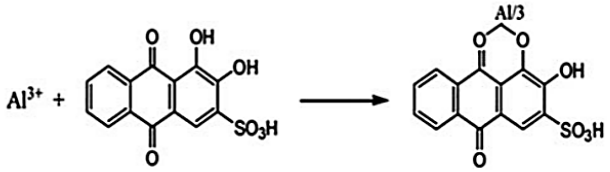

Fig. 3 Retrieved-, Quantitative determination of Al (III) ion by using Alizarin Red S including its microspheres optical sensing material [24]

When studying the formation of the complex at different pH, it was reported that alizarin red at pH between 4 and 7 favors the formation of the complex, but at pH lower than 4, the formation of the complex was almost minimal, since alizarin red is in its protonated form making the formation of the complex difficult. On the other hand, if alizarin red is at alkaline/basic pH there is a risk that aluminum will associate with free water species, and aluminum will precipitate as aluminum hydroxide.

Clearly the above mentioned explains the importance of pH in the formation of the alizarin-aluminum complex for its subsequent quantification by Spectrophometry. Therefore, comparing the results obtained by Supian et al. with the results obtained in the present research work, we can corroborate that the bathochromic shift could be evidenced and that the best formation of the complex is obtained at almost neutral pH, which can be evidenced by a slight color change from alizarin red to a reddish orange as the concentration of aluminum nitrate increased.

IV. Conclusion

An aluminum quantification method was developed for antiperspirant samples. The method was validated following the guidelines established by the AOAC, obtaining a linear method at concentrations of 2.5 and 8.5 mg/L of aluminum nitrate, with quantification and detection limits of 0.1659 mg/L and 0.0548 mg/L, respectively, proving to be precise and accurate. Resulting with a simple, fast and accessible method for the quantification of aluminum in feminine antiperspirants that are free of metals such as copper, iron, zinc, cadmium, nickel, titanium, and zirconium, being this last metal important, since most of the formulations of feminine antiperspirants have zirconium and aluminum as Aluminum Zirconium Tetrachlorohydrex GLY.

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