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Method validation for determination of eight elements in ammi-visnaga plant using graphite-atomic absorption spectrometer in Egypt

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Abstract

In this study, we developed an analytical method for quantifying eight elements in ammi visnaga plant. The method was to quantify heavy metals including aluminum (Al), iron (Fe), chromium (Cr), selenium (Se), zinc (Zn), and potassium (K) and trace elements including copper (Cu) and manganese (Mn) using graphite atomic absorption spectroscopy technique (GFAAS). Validation of each method was performed according to ICH Q2 parameters. The methods showed linearity with R^2 of the regression equation > 0.999. Limit of detection (LOD) was calculated to be 4.7, 2.7, 0.11, 0.13, 0.022, 0.063, 0.38, and 0.03 ppb for Al, Cr, Cu, Fe, Mn, Se, K, and Zn respectively. While limit of quantification (LOQ) was calculated to be 14, 8.1, 0.34, 0.40, 0.067, 0.19, 1.2, and 0.08 ppb for Al, Cr, Cu, Fe, Mn, Se, K, and Zn respectively. The methods are found to be accurate and precise by calculating the accuracy, repeatability, and intermediate precision measurements with RSD < 2. The methods were found robust by applying small changes in digestion time and different graphite furnaces. In this work, we applied the developed methods to measure the concentration of eight elements in ammi-visnaga plant which is sold in the Egyptian market. 119, 1.79, 0.55, 0.68, 1.81, 0.97, 19.8, and 0.68 ppb were the found concentrations of Al, Cr, Cu, Fe, Mn, Se, K, and Zn in the Egyptian ammi visnaga.

Keywords: Ammi-visnaga, heavy metals, trace elements, method validation, GFAAS.

1. Introduction

The reliance on herbal medicines originated in ancient times and has continued through the years. In the recent century, the demand for consuming alternative remedies known as herbal medicines has been elevated [1]. WHO [2] reported the spread of the herbal medicine market compared to the previous decades in both developed and developing countries to prevent and treat diseases as a complementary medicine. According to the consumers, herbal medicine is affordable with less side effects, accessible, and improves health [3-5]. The recent publications focused on the extraction of main active constituents, quantification of their concentrations, and developing the method of extraction and analysis.

However, few studies have attempted to estimate the quantity of heavy metals [6, 7] and trace elements [8, 9] in medicinal plants. Those elements are necessary for several physiological processes, including metabolism, immunity, and cell function [10]. According to reports, aluminium can prevent the formation of bacteria and fungi and may even help to shield the body from the harm caused by free radicals. A daily dietary limit of 1 mg/kg of body weight for aluminium is recommended by the World

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Health Organization (WHO) as it can be also harmful in excessive concentrations [2]. Blood sugar levels are regulated by chromium, which is involved in the metabolism of glucose [2, 10]. The synthesis of red blood cells and the metabolism of iron both requires intake of copper [2, 10]. Iron, on the other hand, is a trace element that is necessary for several processes, including the transport of oxygen, the creation of energy, and immunity [10-12]. Thus, iron insufficiency is the most widespread nutrient shortage in the world, and it can result in a number of symptoms, such as exhaustion, breathlessness, and pale skin [12]. The function of potassium is to control muscular contraction, heart rate, and blood pressure. Muscle cramps, tiredness, and irregular pulse can result from potassium deficiency lack [2, 11, 12]. Selenium aids in the development of thyroid hormones, reproduction, and defense against oxidative stress [10, 11]. Manganese is necessary for the synthesis of neurotransmitters, healthy bones, and energy. Manganese deficiency results in weariness, muscle weakness, and bone pain [9, 10]. While zinc is a cofactor for numerous enzymes necessary for cell division, protein synthesis, and DNA synthesis [9, 11]. To profit from their existence and ensure their safe usage, it is crucial to comprehend the trace elements/heavy metals composition of medicinal plants. The trace elements/heavy metals found in medicinal plants should be studied and quantified. Presence of certain elements can be beneficial and can be used a remedy by itself. And importantly, if some elements are present in high concentration with prolonged usage can lead to toxicity or accumulation within the body.

Methods which can be used to detect heavy metals and trace elements in medicinal plants are atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and X-ray fluorescence spectrometry (XRF). Graphite furnace atomic absorption spectrometry (GFAAS) is one of AAS methods and is considered a sensitive detection technique [13-18].

Ammi-visnaga was cultivated in ancient times at the Nile Delta. Ammi-visnaga known as Khella was a remedy that the ancient Egyptians used to treat kidney stones, vitiligo [19], angina, and bladder stones [20, 21]. Ammi-visnaga is currently grown in a variety of places, including Egypt, Sudan, Morocco, Argentina, Chile, the USA, and many more nations [21]. Khellin, visnagin [21-23], and furanocoumarins [23, 24] are only a few of the plant's active ingredients. These substances have a range of pharmacological actions, such as the ability to relax smooth muscles [21], dilate blood vessels [21], cytotoxic activity [17, 24, 25] and have anti-inflammatory [26] and anti-microbial [27] properties. Ammi-visnaga has been reported to have hepatoprotective [21], antispasmodic [21] , anti-diabetic [28], and antioxidant properties [28, 29]. Recent publication evident their treatment of nephrolithiasis [30, 31], urolithiasis [32], and hypertriglyceridemia [32].

Trace elements and heavy metals in ammi-visnaga cultivated in Turkey has been quantified by Karahan et al. [33]. Benabderrahmane et al. quantified the trace elements in the ammi-visnaga from the Meknes region, Morocco [34] and Ebrahim et al quantified heavy metals of the Sudanese origin [35]. In this work we investigate the concentration of eight elements (Al, Cr, Cu, Fe, Mn, Se, K, and Zn) in the ammi-visnaga plant cultivated in Egypt. The method of analysis was GFAAS which is specific for heavy metal/trace elements analysis. The developed method enabled the quantification of the eight trace elements in very low concentrations of part per billion (ppb).

2. Materials and Methodology

2.1. Materials

All of the chemicals, solvents, and standards were acquired from Sigma Aldrich Co. in Germany and are of analytical grade. Standard solutions were prepared and diluted using ultra-pure water. The ammi-visnaga plant was purchased from an Egyptian local market.

2.2. Sample preparation

Two grams were accurately weighted of the powdered plant in a crucible. The powder was ignited at 150°C for 35 min. in a muffle furnace until complete ashing. 10 mL of 10 % HNO_3 was added to the ash and boiled for two min. The obtained solution was filtered and was then diluted with ultra-pure water to a volume of 100 mL. Dilution was applied when required, a blank solution was prepared similarly without adding the plant powder. Each element was analyzed using D2 lamp selective to each element using atomic absorption spectroscopy (Shimadzu AA6800 connected with graphite furnace using graphite tube (normal/GFA-EX7, Shimadzu)

and auto sampler) using argon gas. Absorption spectrum was plotted for each element, the wavelength corresponds to the maximum absorbance (λ_{max}) was selected to be detection wavelength. Presented in Table 1 the detection wavelength used for each element.

Table 1: Wavelength (nm) used for detection of each element in their analysis using GFAAS.

Element	Detection wavelength (nm)
Al	309.3
Cr	324.8
Fe	248.4
K	266.5
Mn	279.5
Se	196.03
Zn	213.9

2.3. Standard solutions preparation

Stock solutions of 500 ppb for aluminum (Al) and 1000 ppb for chromium (Cr), copper (Cu), iron (Fe), potassium (K), manganese (Mn), selenium (Se), and zinc (Zn) were prepared by accurately weighting equivalent amount and quantitatively transfer to 100-mL volumetric flask.

2.4. Data analysis

Calibration curves were constructed for each element as response (absorbance) vs. concentration. Method validation parameters according to ICH Q2 were applied, where linearity, range, R^2 (R-squared or coefficient of determination), slope and intercept of the calibration curve, Accurate calculations were made for the limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision (repeatability and intermediate precision).

3. Results and Discussion

3.1. Linearity

Linearity for the eight elements was demonstrated by plotting the calibration curve (Figure 1) as absorbance vs. five different standard concentrations, measured in triplicate. Aluminum (Al) ranged from 25.0 to 62.5 ppb, Cr and Fe ranged from 5.00 to 12.5 ppb, Cu ranged from 2.50 to 6.25 ppb, K ranged from 0.50 to 1.25 ppb, Mn ranged from 1.00 to 2.50 ppb, Se ranged from 10.0 to 25.0 ppb, and Zn ranged from 0.50 to 1.25 ppb. Linearity showed a coefficient correlation (R^2) more or equal to 0.9997 which is higher than the required 0.995 [36] that indicates linearity. Which reflects the linearity of the developed method. Table 2 presents the linear regression analysis: linearity range, slope, intercept, and R^2 of the eight elements.

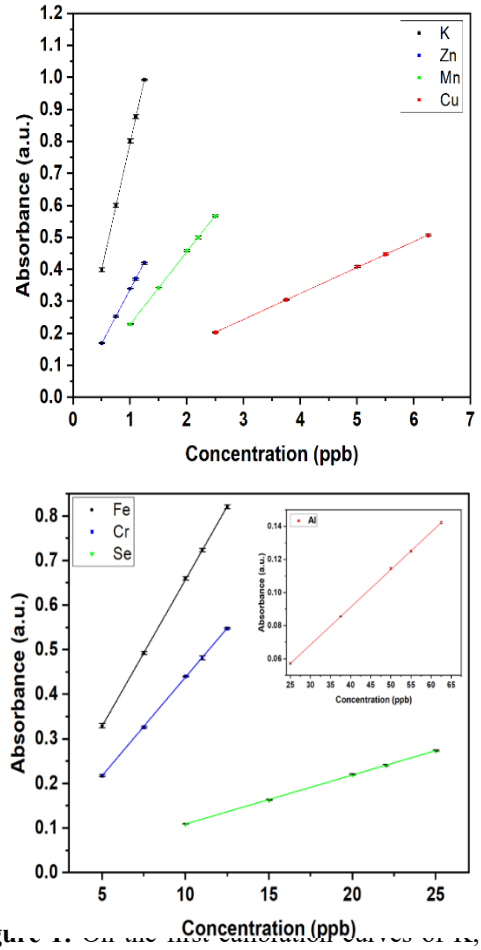

Figure 1: On the first calibration curves of K, Zn, Mn, and Cu. On the second calibration curves of Fe, Cr, Se, and Al.

Table 2: Presents the results of the linear regression analysis of each element: (linearity range, slope, intercept and R^2)

	Linearity range (ppb)	Slope	Intercept	R^2
Al	25.0 – 62.5	0.0023	0.0004	0.9999
Cr	5.00 – 12.5	0.044	-0.0032	0.9999
Cu	2.50 – 6.25	0.081	0.0013	0.9998
Fe	5.00 – 12.5	0.066	0.0014	0.9999
K	0.50 – 1.25	0.79	0.0052	0.9999
Mn	1.00 – 2.50	0.23	0.0033	0.9997
Se	10.0 – 25.0	0.011	-0.0008	0.9999
Zn	0.50 – 1.25	0.33	0.0025	0.9998

3.2. LOD and LOQ

Limit of detection (LOD) and limit of quantitation (LOQ) were established in order to assess the method's sensitivity. While LOQ is the smallest amount to be quantified and it is within linearity, LOD is the minimum amount or concentration that can be identified but not quantified and differs from background signal (blank). The equations to determine LOD and LOQ, 3.3 δ/S and 10 δ/S, respectively, are produced from the regression analysis of each element and are shown in Table 3. Where δ is the standard deviation of intercept and S is the slope of the established approach.

LOD was 4.7, 2.7, 0.11, 0.13, 0.022, 0.063, 0.38, and 0.03 ppb for Al, Cr, Cu, Fe, Mn, Se, K, and Zn respectively. LOQ was 14, 8.1, 0.34, 0.40, 0.067, 0.19, 1.2, and 0.08 ppb for Al, Cr, Cu, Fe, Mn, Se, K, and Zn respectively. The results showed the sensitivity of the developed methods for each element with very low concentrations to be detected and quantified.

3.3. *Accuracy*

The accuracy measurement, which assesses the extent to which the agreements resemble the actual value, is one of the most significant aspects of ICH standards. By assessing the recovery % of three different concentrations other than the concentrations used to construct the calibration curve, accuracy is determined. The three concentrations were spiked and measured in triplicate, the %R (percent recovery) was calculated as (measured concentrated/actual concentration) X 100. The spiked concentrations were in the range of 75 – 125 % of the working range. Accuracy is presented as mean of the %R ± SD. As presented in Table 3, 99.25 ± 0.46, 98.25 ± 0.98, 100.79 ± 0.57, 101.0 ± 1.1, 100.5 ± 0.77, 100.7 ± 0.66, 99.86 ± 0.51, and 101.1 ± 0.55 were the mean ± SD of % R for Al, Cr, Cu, Fe, Mn, Se, K, and Zn respectively. The calculated values lie within the 98 – 102 % value, demonstrating the developed methods are accurate according to ICH guidelines.

Table 3: LOD, LOQ of each element calculated as 3.3 δ/S and 10 δ/S respectively, and accuracy results for each element represented as mean ± SD of the % R of spiked three different concentrations (75-125%) in triplicate.

Element	Accuracy	LOD (ppb)	LOQ (ppb)
Al	99.25 ± 0.46	4.7	14
Cr	98.25 ± 0.98	2.7	8.1
Cu	100.79 ± 0.57	0.11	0.34
Fe	101.0 ± 1.1	0.13	0.40
K	100.5 ± 0.77	0.022	0.067
Mn	100.7 ± 0.66	0.063	0.19
Se	99.86 ± 0.51	0.38	1.2
Zn	101.1 ± 0.55	0.03	0.08

3.4. Precision (repeatability and intermediate precision).

The degree to which agreements are close to the mean value is referred to as precision. The ICH states that repeatability, also known as intra-day precision, is determined by measuring three different concentrations in triplicate on the same day. While intermediate precision is calculated through the triple measurement of three concentrations by changing some variations. In this study we calculated intermediate precision by applying variations in measuring day (inter-day precision), different analyst, and different lamp. RSD (relative standard deviation) is calculated to represent the precision. RSD is calculated as the mean of % R divided by SD of %R. represented in Table 4, the RSD of

repeatability and intermediate precision of the eight elements which are less than 2 % recommendation of ICH indicating that the method is repeatable and precise.

3.5. Robustness

The developed methods were subjected to small changes in the method's optimized conditions to measure their capacity to remain unchanged. In this study, we altered two conditions: the digestion time and the graphite tube. Three digestion time (15, 30, 45 min.) and two graphite tubes (GFA-7000 and pyrolytic, Shimadzu). Each concentration was measured in triplicate and RSD was calculated to assess the robustness of the method. The calculated RSD of each method was less than 2%, indicating the robustness of the method.

3.6. Application in ammi-visnaga plant

The validation of the developed method strengths the credibility and reliability of this method to quantify accurately and precisely Al, Cr, Cu, Fe, K, Mn, Se, and Zn. The developed validated method was used to quantify the eight elements in the powdered fruits of ammi-visnaga to assess quantitatively their concentrations. The developed method showed a significant sensitivity expressed in their low LOD and LOQ. The powdered plant was collected from local Egyptian supplier (Mepaco). Concentrations of Al, Cr, Cu, Fe, Mn, Se, K, and Zn were found to be 119±1.3, 1.79±0.08, 0.55±0.05, 0.68±0.04, 1.81±0.09, 0.97±0.3, 19.8±0.2, and 0.68±0.06 ppb as represented in Figure 2. In these findings, we found ammi-visnaga are rich in aluminum which requires paying attention to the daily intake of the aluminum along with the herb.

Table 4: Precision results for each element represented as RSD, precision was measured as repeatability (intra-day precision) and intermediate precision (inter-day, analyst to analyst, and lamp to lamp).

	Repeatability (RSD)	**Intermediate precision (RSD)**		
		Day to Day	Analyst to Analyst	Lamp to lamp
Al	0.10 %	0.29 %	0.59 %	0.35 %
Cr	0.29 %	0.28 %	0.60 %	0.33 %
Cu	0.19 %	0.30 %	0.63 %	0.24 %
Fe	0.15 %	0.49 %	1.0 %	0.39 %
Mn	0.30 %	0.46 %	0.43 %	0.25 %
Se	0.27 %	0.49 %	0.58 %	0.24 %
K	0.20 %	0.68 %	0.50 %	0.34 %
Zn	0.39 %	0.55 %	0.39 %	0.25 %

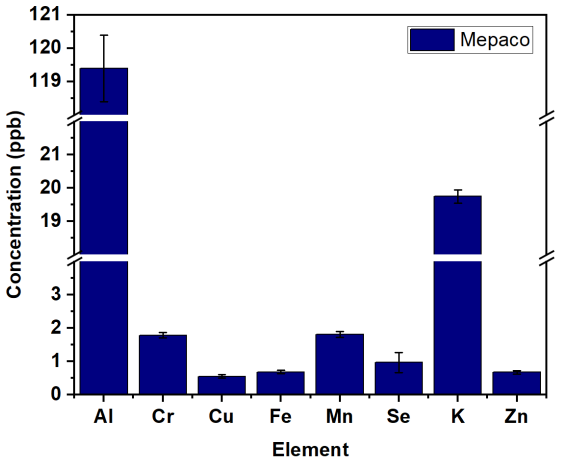

Figure 2: Found concentrations of the eight elements in the Egyptian ammi-visnaga

4. Conclusion

In this study, we developed and validated methods utilizing GFAAS for the determination of Al, Cr, Cu, Fe, K, Mn, Se, and Zn elements. Since the ammi-visnaga plant has a potent pharmacological impact in the treatment of kidney-related disorders, the major application was to highlight the levels of heavy metals and trace elements in the plant. It was crucial to carry out a thorough investigation of the plant, particularly in Egypt.

Conflict of Interest

There are no conflicts to declare.

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