

Validation Method of Flame Atomic Absorption Spectrometry (FAAS) of Dry Ashing and Wet Ashing Method for Mineral Analysis in Isotonic Water

Metode Validasi Flame Atomic Absorption Spectrometry (FAAS) Metode Destruksi Kering dan Destruksi Basah untuk Analisis Mineral Minuman Air Isotonik

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ABSTRACT

Assay of inorganic cations in some drinks was an important quality control. Validation method of (Flame Atomic Absorption Spectrometry) FAAS provides an assurance of reliability for determination of metal Potassium (potassium) in isotonic water. Sample of isotonic water was destructed by dry ashing method and wet ashing. FAAS for determination of metals has been validated. The developed method was linear over the concentration ranges 100.0 – 300.0 µg/mL of Potassium. The correlation coefficient (r) was higher than 0.99. Limit of detection (LOD) of Potassium, was found 1.3448 µg/mL, while limit of quantitation (LOQ) was found 4.4827 µg/mL (K). Statistical analysis by independent T-test showed that the degree of confidence ($\alpha = 0.05$) got the value of the F count of 7,288 while the value of F table at 0.27 and the p-value was 0,000, which means it was smaller than $\alpha = 0.05$. The F value was more than the F table. The p-value was less than 0.05 indicates that there was no significant difference between wet ashing method and dry ashing method

Keywords: *Flame Atomic Absorption Spectrometry, dry ashing, wet ashing, method validation*

ABSTRAK

Pengujian kation anorganik dalam beberapa minuman merupakan kontrol kualitas yang penting. Metode validasi (Flame Atomic Absorption Spectrometry) FAAS memberikan jaminan keandalan untuk penentuan logam Kalium (kalium) dalam air isotonik. Sampel air isotonik dihancurkan dengan metode destruksi kering (dry ashing) dan destruksi basah (wet ashing). FAAS untuk penentuan logam telah divalidasi. Metode yang dikembangkan linier pada rentang konsentrasi 100,0 - 300,0 µg / mL Kalium. Koefisien korelasi (r) lebih tinggi dari 0,99. Batas deteksi (LOD) Kalium diperoleh 1,3448 µg / mL, sedangkan batas kuantitasi (LOQ) diperoleh 4,4827 µg / mL (K). Analisis statistik dengan independent T-test menunjukkan bahwa derajat kepercayaan ($\alpha = 0,05$) didapatkan nilai F hitung sebesar 7,288 sedangkan nilai F tabel sebesar 0,27 dan p-value sebesar 0,000 yang artinya lebih kecil dari $\alpha = 0,05$. Nilai F lebih dari F tabel. Nilai p yang lebih kecil dari 0,05 menunjukkan bahwa tidak ada perbedaan yang signifikan antara metode destruksi basah dan metode destruksi kering

Kata kunci: *Flame Atomic Absorption Spectrometry, destruksi kering, destruksi basah, validasi metode*

INTRODUCTION

Assay of inorganic cations in some drinks was an important quality control. Some cations were needed by the human body to regulate the body's fluid compartment, pH, and metabolic process, but on the other hand they could be toxic, so it should be (Green, 1996). There were several methods that could be used for the assay.

Flame absorption spectrometry (FAAS) was a very common technique for detecting metals present in isotonic drinks, such as Potassium (Ca), Magnesium (Mg), and Potassium (K) (Stewart, 1984).

In the AAS, the absorption was measured against the element of metal which was contained a free atom. The flame on Atomic Absorption Spectrometry formed an evaporation state and resulted crystal salts. Atoms absorbed electromagnetic waves from the cathode lamps and displaced into excited state, and then light intensity reduction that reaches the detector was considered as sample concentration (Christou et al, 2009). It was necessary to optimize digestion method, in order to remove interference from the matrix, and convert the analyte into a suitable form for measurement (Stewart, 1984)

Procedures of dry ashing method used furnace for maintaining temperatures 200°C until 600 °C to remove the organic matrix, then the residues were dissolved with suitable solvent. In this digestion method, the reagents that needed was low and have low blank value, but it may lead some volatilize elements and get a low recovery rate. Wet digestion method could be performed by adding strong oxidizing acids into samples and heating for decomposing the organic materials in sample (Christou et al, 2009).

The aims of this study was to determine the concentration of potassium (K) elements in the isotonic water sample. The proceeds digestion methods, wet ashing and dry ashing methods were compared in this research, the validation of analytical methods for determination of these metals was developed. Flame Atomic Absorption Spectroscopy for determination of Potassium (K) elements has been validated.

METHODS

Experimental Section

This experiment was performed in several steps: isotonic water sampling, validation parameters including linearity, accuracy, precision, Limit of detection (LOD), and Limit of quantitation (LOQ). The validated method used to analyze elements of Potassium in the isotonic water samples

Materials and Instrumentation

The material in this experiment used standard pure analysis, HNO₃ (pro analysis, Sigma Aldrich), KCl (pro analysis, Sigma Aldrich), H₂SO₄ (pro analysis, Sigma Aldrich) matrix of isotonic water, and distilled water. Specification of Flame Atomic Absorption Spectrometry (ZEEnit 700), light source: hollow cathode lamp for Potassium and Potassium (Lumina Tm Lamp); wavelength: 404.4 nm (Potassium) Combustion gas: Acetylene-air, speed 100 L/h.

Sample Preparation

Dry ashing method

50 ml sample entered into porcelain crucible. Inserted into the furnace for 2 hours at 550 °C for 2 hours, then sample has reached room temperature, was added 2- 4 ml of HNO₃: distilled water (1:1). Samples were destructed in the furnace for 1 hour at 500 °C. and allowed to reach room temperature, the results of the

digestion were transferred to a 100.0 ml volumetric flask quantitatively with 1% HNO₃. This solution was shaken until homogeneous and filtered with Whatman 42 filter paper (Khalid et al, 2016).

Wet ashing method

50 ml sample of isotonic water, added 24 ml HNO₃ and 12 ml H₂SO₄. The solution was placed at hotplate and heated until it is clear, colorless. After being clear, colorless, the watch glass was removed and the solution was heated until the yellow vapor disappears. Then the solution of digestion was transferred to a 100.0 mL volumetric flask quantitatively with 1% HNO₃. This solution was shaken until homogeneous and filtered with Whatman 42 filter paper (Hsiao et al, 2011).

Validation of Analysis Methods

Linearity

Instrument linearity performed by measuring the absorbance of standard solutions of minerals K (Potassium) at various concentrations. The linearity test of Potassium mineral used 100 - 300 mg/L then observed the absorbance of the five standards using FAAS. The straight-line equation ($y = bx + a$) and the correlation coefficient (r) of the absorbance concentration was determined.

Precision

This test is measuring the concentration of samples as much as seven times replication for the measurement of mineral Potassium (K). The value determined by calculating % RSD analysis. Precision is good if the RSD value analysis was less than 2%.

Accuracy

This section done by using a sample that spiked with a standard solution. The experimental concentration of spiking is the percentage of the sample concentration (80% is low), 100% is medium, and 120% is

high) which is between the linearity ranges. Added standard performed seven repetitions. Spiking was done at the beginning of the preparation before the microwave digestion. Accuracy determined by calculating percent recovery. Good accuracy is within the range of 75-120%.

LOD (Limit of Detection) and LOQ (Limit of Quantitation)

Limit of detection was determined by utilising both the measured LOD and test replicates of a sample known to contain a low concentration of analyte. Limit of quantitation was the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met (Bakircioglu et al, 2011).

RESULTS AND DISCUSSION

The method of destruction carried out in this study were the method of wet destruction (wet ashing) and dry destruction (dry ashing). Wet destruction was carried out using an open system using conventional heating. This method was cheap, widely used for routine analysis. However, this method allows of losing elements in small levels (Horwitz, 1995).

The acid combination used concentrated HNO₃: H₂SO₄ (2:1). This acid combination was relatively safe to use, its destructive power was not too large, and easy to obtain for routine analysis. Dry destruction method was carried out by high heating to remove all organic compounds, thus changing the preparation to be analyzed into ash. The temperature used is 550°C in the furnace. This method of destruction is not good for analyzing elements that have a low melting point (Laurent et al 2008).

In digestion with HNO₃: H₂SO₄ (2:1), HNO₃ broken down organic matter but could not reach sufficient temperature to broken it down. H₂SO₄ would produce smoke when heated because some H₂SO₄ was broken down into H₂O and SO₃ gases. At low temperatures down below 200 ° C, the entire SO₃ exists as H₂SO₄. Above 500 ° C, almost entirely become SO₃. H₂O was retained in SO₃ liquids and gases formed would fill the vessel, then the solution was in higher temperature, and destroyed the remaining organic matter (Laurent et al 2008).

Specificity

The wavelength used in qualitative and quantitative observations of Potassium ions (K+) was 239.9 nm at concentrations of 2.0 -800.0ppm concentration. Another alternative wavelength of 422.7 was used at a smaller sample level of 0.01-3.0 ppm. The

wavelength for each element was specific. Optimum conditions were obtained by optimizing several selectivity parameters, the composition of the fuel-oxidant gas used, the flow rate of the gas, and the height of the burner Inthe observation of potassium ion analyte used acetylene-air which has a flame temperature of 2100-2400 o C with a gas flow rate of 60 cm/s and a burner height of 7.0 cm.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD values indicate the minimum level of FAAS can detect Potassium (K+) ions at a level of 1.3448 ppm. While the LOQ value indicates a minimum limit of levels that can be quantitatively detected by FAAS at 4.4827 ppm levels, table 1, below showed the absorbance of potassium (K) standard.

Table 1. Absorbance of Potassium standard solution for LOD and LOQ

Concentration (ppm)	Absorbance
100, 7820	0.1075
80, 6256	0.08591
60, 4692	0.06399
40, 3128	0.04343
20, 1564	0.02105

From the table 1, the regression equation between levels and absorbance:

$$y = 0.001015 x + 0.004455$$

$$r = 0.9988$$

LOD and LOQ, results:

$$\begin{aligned} \text{LOD} &= \frac{3 \times \text{SD}}{S} \\ &= \frac{3 \times 0,000455}{0,001015} = 1,3448 \text{ ppm} \end{aligned}$$

$$\begin{aligned} \text{LOQ} &= \frac{10 \times \text{SD}}{S} \\ &= \frac{10 \times 0,000455}{0,001015} = 4,4827 \text{ ppm} \end{aligned}$$

Based on the LOD calculation, the smallest amount of Potassium in a sample that can be detected successively was 1.3448 ppm.

At these concentrations, the analyte cannot be quantified in the right value. Whereas for LOQ, the smallest amount of Potassium analyte obtained in a sample that can be determined quantitatively was 4.4827 ppm.

Linierity

From table 2 obtained the correlation coefficient value, r was 0.9991 while the r table of 95% degree of confidence (α= 0.05) was 0.7990, showing a linear correlation between levels with absorbance because r count> r table, the value of Vxo obtained was 1.56 % smaller than the required value of 5% (Bakircioglu, 2011).

Linearity calculations using linear regression give at value of 81.0922 while the t table of 95% confidence level ($\alpha = 0.05$) was 3.3117, showing a linear correlation between levels with absorbance due to t

count > t table. The p-value = 0.000 (<0.05), shows a linear correlation between levels and absorbance. Equation of lines obtained. $Y = 0.001023x + 0.0002442$

Table 2. Linearity of Potassium (K +) ion standard solution

Concentration (ppm)	Absorbance
100,6730	0.1014
151,0095	0.1531
201,3460	0.2116
251, 6825	0.2545
302.0190	0.3082

Precision

Precision of Potassium Ion (K +) from Wet Destruction

From the results of the determination of Potassium Ion (K +) levels in the sample obtained was show in the table 3

below. The result of CV values was 0.196% less than 2% meet the applicable requirements (Bakircioglu, 2011). It can be concluded that the instrument has a repeatable reading of the absorbance of the standard solution of K⁺ 140,1797 ppm.

Table. 3 The results of determining the K + content in the sample

Replication	Absorbance	Concentration (ppm)
1	0.14333	139, 8757
2	0.14370	140,2331
3	0.14347	140,0044
4	0.14400	140,5428
5	0.14393	140,4531
6	0.14343	139.9690
Mean		140,1797
Standard deviation (SD)		0.2745

Precision of Potassium Ion (K +) from Dry Destruction

From the results of the determination of Potassium Ion (K +) concentration in the sample obtained was show in the table 4 below. The CV values was 0,32 % less than

2% meet applicable requirements (Bakircioglu, 2011). It can be concluded that the instrument has a repeatability of reading on the absorbance of the standard solution K⁺ 138.665 ppm.

Table. 4 The results of determining the K⁺ in the sample

Replication	Absorban	Level (ppm)
1	0.14211	138,6772
2	0.14186	138,4322
3	0.14253	139.0911
4	0.14215	138,7160
5	0.14132	137.9062
6	0.14255	139.1114
Mean		138,6556
Standard deviation (SD)		0.4501

Accuracy of Potassium Ion (K⁺) Wet Digestion

Determination of accuracy and precision in the K⁺ analysis in isotonic drink samples is carried out by the standard addition method using three different levels of addition as shown in Table 5. In the

calculation of accuracy and precision above obtained % recovery 104.83 ± 2.09 %, the value of % recovery entered the requirements of 80-12% (Horwitz, 1995). RSD of 1.99% was less than the required value of 2% (Horwitz, 1995).

Table 5. Accuracy and Precision Value of Potassium Ions (K⁺)

Additions	Replication	Absorbance	Concentrations (ppm)	% Recovery	Accuracy (X ± SD)	Precision (RSD)
110,5387	1	0.27150	265,1598	105.76	104.83 ± 2.09	1.99%
	2	0.26696	260,7221	103.99		
	3	0.27009	263,7808	105.21		
140.6818	1	0.29346	286,6192	102.05		
	2	0.29521	288,3324	102.66		
	3	0.29274	285,9170	101.80		
168,8182	1	0.33949	331,6165	107.32		
	2	0.33882	330,9677	107.11		
	3	0.34030	332.4199	107.58		

Accuracy of Potassium Ion (K⁺) Dry Digestion

The determination of accuracy and precision in the K⁺ analysis in isotonic drink samples was carried out by the standard addition method as shown in Table 6. In the

calculation of the accuracy and precision above obtained % recovery of 83.45 ± 1.43 . The value of % recovery meet the requirements range of 70-110%. RSD 1.71 % was less than the required value of 2% (Horwitz, 1995).

Table 6. Accuracy and precision value of Potassium ions (K +)

Additions	Replication	Absorbance	Concentration (ppm)	% Recovery	Accuracy (X ± SD)	Precision (RSD)
110,5387	1	0.219174	214,0080	85.88	83.45 ± 1.43	1.71%
	2	0.216804	211,6906	84.95		
	3	0.215682	210,5941	84.51		
140.6818	1	0.253715	247,7722	83.70		
	2	0.254572	248,6097	83.00		
	3	0.252887	246,9622	83.41		
168,8182	1	0.258297	252,2515	82.04		
	2	0.257826	251,7903	81.89		
	3	0.257133	251,1139	81.67		

Data Analysis

Based on the data analysis using One-Way ANOVA test on the degree of confidence ($\alpha = 0.05$) got the value of the F count of 7,288 while the value of F table at 0.27 and the p-value was 0,000, which means it was smaller than $\alpha = 0.05$. The calculated of F value was greater than the F table and the value of p-value less than 0.05, it was found that there was no significant difference between wet ashing and dry ashing method.

CONCLUSIONS

Sample of isotonic water was successfully destructed by dry ashing method and wet ashing method. FAAS for determination of Potassium has been validated. The developed method was linear over the concentration ranges 100.0 – 300.0 $\mu\text{g/mL}$ of Potassium. The correlation coefficient (r) was higher than 0.99. Limit of detection (LOD) of Potassium, was found 1.3448 $\mu\text{g/mL}$, while limit of quantitation (LOQ) was found 4.4827 $\mu\text{g/mL}$ (K). There was no significant difference between dry ashing and wet ashing method, based on the data analysis using One-Way ANOVA test.

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