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Development and Validation of a Flame Atomic Absorption Spectrometry Method for Determining Cobalt in Mineral Premix for Animal Feed

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ABSTRACT

Cobalt is an essential trace element in animal feed mineral premixes, and its concentration must be precisely controlled to ensure product quality and safety. A flame atomic absorption spectrometry (FAAS) method was developed and validated for the determination of cobalt in mineral premixes, with particular focus on optimizing the sample digestion procedure. The optimized digestion protocol combined dry ashing at 600 °C for 4 hours with wet digestion using concentrated HNO_3 and H_2SO_4 in a 3:1 ratio. Validation parameters included linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, repeatability, intermediate precision, and robustness. The calibration curve demonstrated strong linearity across the tested concentration range, with a correlation coefficient of 0.9996. The limit of detection (LOD) and limit of quantification (LOQ) were 0.0817 mg/L and 0.1366 mg/L, respectively. Recovery values ranged from 84.74% to 92.86%, and repeatability yielded a relative standard deviation (RSD) of 4.69%, meeting the acceptance criterion established by the Horwitz equation. Intermediate precision and robustness assessments revealed no significant differences between analysts or between furnace temperatures of 600 °C and 650 °C. These results indicate that the optimized digestion-FAAS method is reliable and suitable for routine cobalt determination in animal feed mineral premixes.

Keywords: animal feed, cobalt, flame atomic absorption spectrometry, method validation, mineral premix

1. Introduction

Feed is a single food ingredient or a mixture of both processed and unprocessed ingredients given for life sustainability, to meet the nutritional needs, growth, and breeding of livestock [1]. When the requirement of feed is insufficient, or the feed quality is poor, it can cause low livestock production and disruption to the animal's body. One of the ingredients contained in feed is mineral premix [2]. Mineral premix is one of the additional ingredients mixed in feed to provide a source of minerals for livestock. This is due to the fact that livestock cannot make their own minerals, so they should be provided with their food in the proper ratio and sufficient quantities**.** One of the important minerals in animal feed is cobalt, which is used to synthesize vitamin B12 (cobalamin). Cobalt is needed by enzymes as coenzymes that function to bind substrate molecules [3]. If the levels of cobalt are used in excessive amounts, it can cause toxin effect [4].

Cobalt is a mineral element that is needed in very small quantities, namely 100 mg per kg of dry matter [5]. Cobalt in livestock, especially ruminants, functions to synthesize water-soluble vitamin B12 in the stomach, especially the rumen [6], known as cobaltamines [7]. Microorganisms in the rumen can

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synthesize Vit B12 if the cobalt content in the rumen fluid is greater than 0.5 mg/ml. If the concentration is lacking, the synthesis will be inhibited and will lead to blood and tissue disorders in cattle [8]. Therefore, feed is usually supplemented with an additive substance that can improve the quality of nutrients in feed.

Co concentration in mineral premix can be determined by AAS [9]. The sample preparation stage in determining minerals can be carried out using dry digestion, wet digestion, or microwave-assisted acid digestion methods [10]. Dry digestion has the advantage of being a simple technique and not requiring chemicals. Wet digestion has several advantages, including higher time efficiency, use of few reagents, and little loss of mineral components [11]. The digestion method in this research comes from a modified method that is different from the standard AOAC 999.10 (2012) method. The modified method uses a combination of dry digestion and wet digestion. These differences consist of the length of digestion time, temperature, and volume of reagent used (Concentrated HNO_3 : Concentrated H_2SO_4). If there are differences in the digestion procedure, it is necessary to carry out a validation process. The validation parameters carried out include linearity, instrument detection limit, quantitation limit, accuracy, repeatability, intermediate precision, and robustness [12].

Many studies have shown that determining the amount of cobalt in feed and mineral premixes is done using various types of advanced equipment and methods. Inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are commonly used because they can detect very small amounts of elements and can measure many elements at the same time. However, these methods need costly equipment and high operational expenses, limiting their applicability in routine analytical laboratories. Atomic absorption spectrometry (AAS) is still one of the most common methods used to detect metals in small amounts because it is easy to use, accurate, and cost-effective [13]. The innovative aspect of this study is the development of an improved sample decomposition approach using a combination of dry and wet methods. The modified method was developed by reducing the furnace temperature and adjusting the acid ratio.

Besides AAS, cobalt can also be measured using other methods like ICP-OES, ICP-MS, UV-Vis spectrophotometry, and electrochemical techniques. ICP-based methods are very sensitive and can detect multiple elements at the same time, but spectrophotometric methods use easier equipment. However, they often need complicated chemical reactions or extra steps to prepare the sample before testing.

This study introduces an optimized and validated combined dry ashing and wet digestion procedure for determining cobalt in mineral premix using FAAS. The procedure lowers the ashing temperature and adjusts the HNO_3 to H_2SO_4 ratio, while maintaining analytical performance suitable for routine quality control.

This research aims to develop and validate a modified digestion procedure combined with flame atomic absorption spectrometry for determining cobalt in animal feed mineral premix.

2. Methods

This section describes the materials, instrumentation, digestion procedures, validation parameters, and statistical analyses used for the development and validation of the FAAS method for cobalt determination in mineral premix.

2.1. Materials

For this study, the reference material consisted of a ring test mineral premix sourced from the 2017 proficiency testing scheme, which served as a benchmark for comparing methods. The assigned cobalt level in this matrix was established at 26.00 mg/kg based on the reference protocol. To handle calibration and spiking procedures, we utilized a cobalt stock standard solution (985 mg/L, analytical grade). Additionally, the experimental work required analytical reagent-grade concentrated nitric acid (HNO_3, 65%) and sulfuric acid (H_2SO_4, 98%), with deionized water employed across all preparation steps.

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2.2. Instrumentation

Cobalt concentration was measured using a Thermo Scientific iCE 3000 Series flame atomic absorption spectrometer equipped with a cobalt hollow cathode lamp under working conditions; wavelength: 240.7 nm, spectral bandwidth: 0.2 nm, lamp mode: BGC-D2, hollow cathode lamp current: 10 mA, burner height: 7.0 mm, burner angle: 0, flame type: air-acetylene, acetylene flow rate: 1.0 L/min. Co hollow cathode lamps and glassware

2.3. Procedure

2.3.1. Standard method

Mineral premix samples with known Co content were carefully weighed 2 g in a porcelain cup then heated at a furnace temperature of 700 °C for 3 hours then added 3 mL concentrated HNO_3, 3 mL concentrated H_2SO_4, and 10 mL distilled water then heated on an electric heater with a temperature of 200 ºC for 10 minutes, then add 2 mL of concentrated HNO_3 until completely oxidized (yellow solution), then add ± 3 drops concentrated HNO_3 for up to 10 minutes, then add 5 mL of distilled water until it boils, then cool. The cooled solution was put into a 100 mL measuring flask, and distilled water was added until the calibration mark. The solution was filtered using Whatman No. 41 filter paper, and the filtrate was collected in a 100 mL Erlenmeyer flask

2.3.2. Modification method

The mineral premix sample was weighed at 2 grams, then heated in a furnace and added with concentrated HNO_3 and concentrated H_2SO_4, then heated on an electric heater at a temperature of 200 ºC for 10 minutes. Variations in the digestion process in this research data are seen in Table 1.

Table 1. Variation of the digestion procedure

Method	Ashing temperature (°C)	Ashing time (h)	Concentrated $HNO_3 : H_2SO_4$ ratio
A	700	3	3:3
B	600	4	3:3
C	600	4	3:1

After the digestion process was complete, the solution was left to reach room temperature, then transferred into a 100 mL measuring flask, diluted to volume with deionized water and homogenized, then filtered and measured using AAS.

2.3.3. Validation of Analysis Methods

Linearity: Performed by measuring the absorbance of the standard solution of mineral Co at concentrations of 0.197; 0,492; 0,985; 1,970; and 4,925 mg/L. Each standard was measured for absorbance and repeated 3 times.

Accuracy: Carried out using a sample of mineral premix spiked with a standard solution of Co. The added spiking concentration was 25% (low concentration), 50% (medium concentration), and 75% (high concentration). Accuracy testing was carried out three times, and the recovery (%) was calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ): Sensitivity of an instrument can be analysed by determining the two parameters as follows: Limit of Detection LOD) and Limit of Quantification (LOQ). A standard solution of Co 0.049 mg/L that had been prepared by adding concentrated H_2SO_4 98% (w/b) as much as 1 mL and concentrated HNO_3 65% (b/b) to the extent of 3 mL was put into a 100 mL measuring flask; deionized water was added to the mark and homogenized. The absorbance of the solution was measured using AAS at a wavelength of 240.7 nm for 9 repetitions. To evaluate the instrumental limit of detection (LOD) and limit of quantification (LOQ), we performed nine consecutive measurements of a cobalt standard solution at a low concentration. The calculations were based on the formulas:

LOD = mean + 3SD
LOQ = mean + 10SD

Precision: The precision of the assay was determined by repeatability and intermediate precision. Repeatability: The sample solution without *Co spiking* was measured for absorption at a wavelength of 240.7 nm for nine replicates using AAS.

Intermediate Precision: The sample solution without spiking was measured for absorption at a wavelength of 240.7 nm as many as nine replicates by different analysts and on different days using AAS.

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Robustness: The way the sample solution was made and same as the repeatability test, except that the furnace temperature was originally 600 °C for 4 hours and was changed to 650 °C for 4 hours. The absorption of the solution was measured at a wavelength of 240.7 nm for eight repetitions using AAS.

3. Results And Discussion

3.1. Optimization of Digestion Procedure

The optimization of the digestion procedure was performed by comparing three digestion conditions. The results of the cobalt mineral analysis can be seen in Table 2.

Table 2. Result of cobalt mineral analysis on mineral premixes

Repeat	Modification method (mg/kg)			Standard method (mg/kg)
	A	B	C	
1	20.28	23.31	25.55	26.00
2	21.10	22.36	25.55	
3	18.65	24.30	24.34	
Average	20.01±1.25	23.32±0.97	25.15±0.70	
Agreement with reference method	71-81%	86-93%	94-98%	

Based on Table 2, the mineral *premix ringtest* sample in method A obtained lower accuracy results compared to other methods. This can be caused by a less-than-perfect ashing process so that not all inorganic substances are fully formed. In method B, modifications were made, namely by changing the furnace temperature to 600 °C for 4 hours with an acid ratio of concentrated HNO_3:concentrated H_2SO_4 3:3 mL, but gave results that did not meet the requirements. Modification at the measured level of C, namely *a trial* at 600 °C for 4 hours with an acid ratio of concentrated HNO_3:concentrated H_2SO_4 3:1, obtained results with high accuracy, so the method validation process was carried out on the type C modification method. Mineral premixes usually include many important elements like iron, zinc, copper, manganese, and cobalt. Comprehensive analysis of different elements can be done with methods like ICP-OES or ICP-MS. But this study looked specifically at measuring cobalt using AAS because it's a good choice for everyday lab work.

To check if the differences between the three digestion methods were really important, a one-way analysis of variance (ANOVA) was done. The ANOVA test found that the calculated F value (20.45) was higher than the critical F value (5.14) with a p-value of 0.002 ($p < 0.05$), which means at least one of the cobalt levels measured using the three different digestion methods was significantly different from each other. Further analysis using the Tukey test obtained the following results (Table 3).

Table 3. Tukey test analysis

Factor	N	Mean	Grouping	
C	3	25.147	A	A
B	3	23.323	A	A
A	3	20.010		B

Method C = group A; Method B = group A; Method A = group B. Methods that do not share the same letter are significantly different.

This result shows that it can be seen that methods B and C use the same heating temperature and heating time, with a difference in the ratio of HNO3:H2SO4 solvents. This means that the difference in acid ratio does not have a significant effect on the results of measuring Cobalt levels. Meanwhile, in method A, the Cobalt content is significantly different compared to methods B and C, as method A uses different temperature and heating time. Therefore, it can be concluded that the difference in temperature and heating time has a significant effect.

3.2. Linearity

The linear regression equation obtained from the linearity test is y = 0.004 + 0.058x. The intercept value (a) of 0.004 indicates that when the standard concentration of Co was 0 mg/L, the resulting absorbance was 0.004. The intercept value (a) of 0.004 shows how the instrument reacts when the amount of the substance being measured is very close to zero. This value is linked to the basic signal from the instrument and any consistent mistakes that might happen during the measurement process. A small intercept value means that the systematic error has a little effect on the analytical signal. A slope value (b) of 0.058 indicates that each unit of change in the standard concentration of Co will result in a change in absorbance of 0.058. The slope value (b) indicates the sensitivity of the curve; the larger the slope value, the higher the sensitivity curve, or a small change in analyte concentration can provide a meaningful instrument response (absorbance) [14]. The correlation coefficient (r) was 0.9996, indicating excellent linearity over the calibration range of 0.197-4.925 mg/L.

3.3. Limit of Detection and Limit of Quantification

The sensitivity of the method was evaluated by determining the LOD and LOQ. The test was carried out by measuring the smallest concentration standard as many as nine times due to the blank measurement resulting in a negative concentration. The lowest amount that can be detected is called the limit of detection, so it isn't part of the linear calibration range. In this calibration model, the zero concentration is based on the blank solution instead of using a cobalt standard solution [15]. The value has met the acceptance requirements set with a correlation coefficient value of more than 0.999 [16]. The value of the instrument detection limit and the quantification limit can be seen in Table 4.

Table 4. Limit of Detection and Quantification Limit Test Results

Information	Value
Average (mg/L)	0.0582
Standard Deviation (mg/L)	0.0078
Limit of Detection (mg/L)	0.0817
Limit of Quantification (mg/L)	0.1366

Based on Table 4, it can be seen that the detection limit value of the Co instrument was 0.0817 mg/L. The value indicates the lowest analyte concentration that can still be detected by the instrument and provides a significant response compared to blanks but does not meet the precision and accuracy requirements, meaning that the measurement below this value is instrument *noise* and cannot be trusted as a response from the analyte. The value of the Co quantification limit was 0.1366 mg/L. These values indicate that the concentration of analytes was the lowest with an acceptable level of precision and accuracy, meaning that measurements above those values have been well quantified.

3.4. Accuracy

The accuracy with the *recovery* value obtained can be seen in Table 5. It can be seen that the range of recovery values obtained from Co accuracy testing for low concentrations was (86.84-92.86) %, medium concentrations are (84.74-90.49) %, and high concentrations are (87.97-88.25) %. This value has met the acceptance requirements, namely the recovery value of (80-110)% which shows that the accuracy of Co levels in mineral premixes provides accurate results. Co recovery values that tend to be less than 100% indicate systematic errors stemming from chemical interference in AAS, including imperfect atomization and the formation of compounds that are difficult to isolate. This causes the measured sample rate to be lower than the theoretical sample rate. Although recoveries were below 100%, they remained within the acceptable range for the intended analytical purpose. This is evidenced by the test results that still meet the acceptance requirements.

Table 5. Co-metal analysis recovery test results

	Recovery (%)		
Spiking	25%	50%	75%
Repeat 1	92.86	84.74	87.97
Repeat 2	86.84	88.02	88.25
Repeat 3	90.29	90.49	88.16
average	90.00±3.02	87.75±2.88	88.13±0.14

3.5. Repeatability

Repeatability was evaluated by measuring the cobalt concentration in the mineral premix sample nine times under the same analytical conditions [17]. The results of the repeatability test can be seen in Table 6. The %RSD value obtained from the Co repeatability test was 4.69%. The value has met the acceptance requirements, i.e., %RSD < 2/3 %CV *Horwitz,* which indicates that the Co test produces data with good accuracy or low random error [18]. This result indicates acceptable repeatability and low random variation under repeatability conditions.

Precision values can be affected by random errors (the source of the error is uncontrollable and is not known for sure and fluctuates), such as deviations

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in electronic circuits, instrument instability, temperature variations, noise, and vibration.

Table 6. Repeatability test results

Number	Result (mg/kg)
1	25.5545
2	25.5596
3	24.3425
4	27.9461
5	24.3558
6	24.3571
7	26.7513
8	25.5596
9	25.5672
Sum	229.9936
Average	25.5548
SD	1.1985
%RSD	4.69
2/3 %CV *Horwitz*	6.55

Terms: %RSD ≤ 2/3 %CV *Horwitz*

3.6. Intermediate Precision

Intermediate precision tests are performed to ensure that in the same laboratory, a method will give the same results [19]. The intermediate precision test in this experiment was carried out by measuring samples by different analysts, with as many as nine replicates, but using the same method.

Table 7. Results of the F-test and the precision t-test between

Variable		Value
Analyst 1		
Mean (mg/kg)		25.06
SD		0.2
Analyst 2		
Mean (mg/kg)		25.00
SD		0.16
F-test	F-calculated	1.19
	F-table	3.44
	Result	1.19 < 3.34
t-test	t-calculated	0.15
	t-table	2.12
	Result	0.15 < 2.12

Acceptance conditions: F-calculated < F-table and t-calculated < t-table

The results of the intermediate precision test can be seen in Table 7. The results of the F-test and the t-test in the precision test between Co were obtained with the value of F-calculated < F-table and t-calculated < t-table, so that the variety and average of analysts 1 did not differ significantly from the variety and average of analysts 2.

Statistical evaluation via the F-test revealed that the variances between Analyst 1 and Analyst 2 were comparable, as the F-value (1.19) remained well below the F-table threshold (3.44). Furthermore, the t-test confirmed that the average values produced by both analysts shared no statistically significant differences, given that t-calculated (0.15) was lower than t-table (2.12). Taken together, these statistical findings verify the robust intermediate precision of the developed method.

3.7. Robustness

Robustness tests are conducted to see the effect of changes in the conditions of the deliberately created method on the accuracy and precision achieved [20]. To evaluate the method's robustness, the furnace temperature was deliberately adjusted from 600 °C to 650 °C, while the ashing duration was kept constant at 4 h. Variance analysis via the F-test indicated no significant divergence between the two setups, as the F-calculated value (3.32) did not exceed the F-table value (3.79). Furthermore, the t-test confirmed that the average cobalt concentration remained unaffected, with t-calculated (1.28) staying below t-table (2.14). These outcomes prove that minor fluctuations in furnace temperature do not alter the analytical performance, thereby verifying the method's robustness under these parameters. The results of the robustness test can be seen in Table 8.

Table 8. Robustness Test Results

Variable		Value
F-test	F-calculated	3.32
	F-table	3.79
	Result	3.32 < 3.79
t-test	t-calculated	1.28
	t-table	2.14
	Result	1.28 < 2.14

Acceptance conditions: F-calculated < F-table and t-calculated < t-table

3.8. Comparison with Other Analytical Methods

The comparison of the analytical performance of this proposed method for Co determination can be seen in Table 9. Table 9 shows how well the new method works compared to other methods used in studies for measuring cobalt. Plasma-based methods like ICP-OES and ICP-MS generally provide lower detection limits and broader multi-element capability. However, the FAAS method provides acceptable analytical performance for determining cobalt in mineral premix with a repeatability RSD of 4.69%, and a recovery value of 84.74–92.86%, showing that it works well for regularly testing cobalt levels in mineral premix samples. Because the compared studies involved different sample matrices, digestion procedures, unit bases, and instrumental conditions, the comparison should be interpreted qualitatively rather than as a direct ranking of analytical performance.

Table 9. The comparison of the analytical performance of this proposed method for Co determination

Method	LOD	Linear range	Precision (%RSD)	Accuracy (%)	Reference
AAS	0.0817 mg/L	0.197-4.925 mg/L	4.69	84.74-92.86	This study
GF-AAS	0.005 mg/kg	0.01-2 mg/kg	<5	95-103	[21]
ICP-OES	1.9 ng/mL	0.01-100 µg/mL	<3	97-102	[22]
ICP-MS	0.002 µg/L	0.01-100 µg/L	<3	95-104	[23]

4. Conclusion

In this study, a modified digestion protocol paired with flame atomic absorption spectrometry (FAAS) was established and thoroughly validated for analyzing cobalt levels in animal feed mineral premixes. The optimized approach involves a 4 h dry ashing step at 600 °C, followed by a wet digestion phase using a 3:1 mixture of concentrated HNO_3 and H_2SO_4. The method showed excellent linearity over the concentration range of 0.197-4.925 mg/L with a correlation coefficient of 0.9996. The LOD and LOQ values were 0.0817 mg/L and 0.1366 mg/L, respectively. Analytical accuracy was supported by spike recoveries between 84.74% and 92.86%, while repeatability yielded a low RSD of 4.69%. Furthermore, intermediate precision and robustness evaluations revealed no significant deviations under the tested parameters. Consequently, this validated methodology offers a reliable and practical solution for routine cobalt monitoring in quality control labs handling animal feed premixes.

CRediT authorship contribution statement

CI: Conceptualization, Methodology. EY: Data curation, Investigation. IS: Writing – review & editing, Validation. DPN: Software, Formal analysis. S Vi: Resources, Writing – original draft. SR: Project administration, Visualization. MT: Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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References

[1] C. Marchese, R. Avolio, A. Griglione, P. Palmegiano, A. Riva, S. Gavinelli, P. Brizio, M. C. Abete, and S. Squadrone, "Cobalt in feed: A first survey in Italy," *Journal of Trace Elements in Medicine and Biology*, vol. 89, Art. no. 127637, 2025, doi: 10.1016/j.jtemb.2025.127637.

[2] B. Robert and D. V. M. Corbett, "Trace mineral nutrition in confinement dairy cattle," *Veterinary Clinics of North America: Food Animal Practice*, vol. 39, no. 3, pp. 425–438, 2023, doi: 10.1016/j.cvfa.2023.06.004.

[3] National Research Council, *Nutrient Requirements of Dairy Cattle*, 7th rev. ed.

Washington, DC, USA: National Academy Press, 2001.

[4] P. V. Postnikov, Z. G. Ordzhonikidze, V. A. Badtieva, I. A. Turin, and V. I. Pavlov, “Determination of cobalt in plasma blood samples by the ICP-MS method after oral intake of dietary supplements containing low doses of cobalt,” *Voprosy Pitaniia*, vol. 91, no. 6, pp. 92–101, 2022, doi: 10.33029/0042-8833-2022-91-6-92-101.

[5] J. R. G. Montana, F. E. Valente, A. J. Alonso, J. M. Lomillos, R. Robles, and M. E. Alonso, “Relationship between vitamin B12 and cobalt metabolism in domestic ruminants: An update,” *Animals*, vol. 10, no. 10, Art. no. 1855, 2020, doi: 10.3390/ani10101855.

[6] K. Stemme, U. Meyer, G. Flachowsky, and H. Scholz, “The influence of an increased cobalt supply to dairy cows on the vitamin B12 status of their calves,” *Journal of Animal Physiology and Animal Nutrition*, vol. 90, no. 3–4, pp. 173–176, 2006, doi: 10.1111/j.1439-0396.2005.00584.x.

[7] E. J. Underwood and N. F. Suttle, *The Mineral Nutrition of Livestock*, 3rd ed. Wallingford, UK: CABI Publishing, 2002.

[8] K. Stemme, P. Lebzien, G. Flachowsky, and H. Scholz, “The influence of an increased cobalt supply on ruminal parameters and microbial vitamin B12 synthesis in the rumen of dairy cows,” *Archives of Animal Nutrition*, vol. 62, no. 3, pp. 207–218, 2008, doi: 10.1080/17450390802027460.

[9] H. A. Zadeh and E. Ebrahimzadeh, “Determination of cobalt in water samples by atomic absorption spectrometry after preconcentration with a simple ionic liquid-based dispersive liquid–liquid microextraction methodology,” *Central European Journal of Chemistry*, vol. 8, no. 3, pp. 617–625, 2010, doi: 10.2478/s11532-010-0030-2.

[10] R. C. V. Costa, E. C. Ferreira, J. A. G. Neto, and A. Virgilio, “Evaluation of a cost-effective and environmentally friendly digestion method for preparing animal feed samples for elemental analysis,” *Animal Feed Science and Technology*, vol. 329, Art. no. 116511, 2025.

[11] H. Herlinawati, N. Arpi, and N. Azmi, “Comparison of wet digestion, dry ashing, and acid homogenate methods in determining Na and K in beef and chicken using flame photometer,” *Indonesian Journal of Chemical Science and Technology*, 2020.

[12] D. Singh, V. N. Singh, and S. P. Singh, “Method validation and uncertainty evaluation in trace element analysis of high-purity silver by ICP-OES,” *Analytica Chimica Acta*, vol. 1379, Art. no. 344732, 2025.

[13] D. A. Skoog, F. J. Holler, and S. R. Crouch, *Principles of Instrumental Analysis*. Boston, MA, USA: Cengage Learning, 2018.

[14] B. Magnusson and U. Örnemark, Eds., *Eurachem Guide: The Fitness for Purpose of Analytical Methods—A Laboratory Guide to Method Validation and Related Topics*, 2nd ed. Eurachem, 2014.

[15] A. Szymczycha-Madeja, M. Welna, and P. Pohl, “Elemental analysis in energy drinks by ICP-OES,” *Journal of the Brazilian Chemical Society*, 2013, doi: 10.5935/0103-5053.20130202.

[16] M. M. Hossain, A. S. Hannan, M. M. Kamal, M. A. Hossain, and S. B. Quraishi, “Appraisal and validation of a method used for detecting heavy metals in poultry feed in Bangladesh,” *Veterinary World*, vol. 15, no. 9, pp. 2217–2223, 2022, doi: 10.14202/vetworld.2022.2217-2223.

[17] Riyanto, *Validation and Verification of Test Methods: Compliant with ISO/IEC 17025 Testing and Calibration Laboratory*. Yogyakarta, Indonesia: Deepublish, 2014.

[18] Sumardi, *Validation of the Test Method*. Jakarta, Indonesia: Center for Standardization and Accreditation of the Secretariat General of the Ministry of Agriculture, 2002.

[19] W. Horwitz and R. Albert, “The Horwitz ratio (HorRat): A useful index of method performance with respect to precision,” *Journal of AOAC International*, vol. 89, no. 4, pp. 1095–1109, 2006.

[20] M. Thompson, S. L. R. Ellison, and R. Wood, “Harmonized guidelines for single-laboratory validation of methods of analysis,” *Pure and Applied Chemistry*, vol. 74, no. 5, pp. 835–855, 2002, doi: 10.1351/pac200274050835.

Volume 6, Number 1, June 2026, Page 49 - 57
e-ISSN: 2776-4508; p-ISSN: 2776-1711
Publisher : Universitas Pakuan

[21] D. V. Avila, A. R. Borges, M. G. Rodrigues, R. G. Araujo, and E. A. Passos, "Determination of Cr and Co in wet animal feeds using direct solid sample analysis by high-resolution continuum source graphite furnace atomic absorption spectrometry," *Microchemical Journal*, vol. 133, pp. 524–529, 2017, doi: 10.1016/j.microc.2017.04.028.

[22] M. Bartosiak, K. Jankowski, and J. Giersz, "Determination of cobalt species in nutritional supplements using ICP-OES after microwave-assisted extraction and solid-phase extraction," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 155, pp. 135–140, 2018, doi: 10.1016/j.jpba.2018.03.058.

[23] A. Shrivastava and V. B. Gupta, "Methods for determination of limit of detection and limit of quantitation of the analytical methods," *Chronicles of Young Scientists*, vol. 2, no. 1, pp. 21–25, 2011, doi: 10.4103/2229-5186.79345.