Chimica et Natura Acta
p-ISSN: 2355-0864 e-ISSN: 2541-2574
Homepage: http://jurnal.unpad.ac.id/jcena

Nitrogen Determination in Leaves Palm Oil using The San++ Scalar Continuous Flow Analyzer Following Wet Digestion with 50ml Dilution Sulfuric Acid: Testing and Validation

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DOI: https://doi.org/10.24198/cna.v11.n3.48210

Abstract: Nitrogen is a crucial macronutrient for the growth of oil palm plants. Insufficient nitrogen can lead to chlorosis in young leaves, causing them to turn yellow and impede growth. To determine the nitrogen content in oil palm leaves and estimate the required fertilizer amount, a test must be conducted. In this study, the Association of Analytical Communities (AOAC) standard method was employed to measure nitrogen levels. The method's performance procedure has undergone changes. The test results displayed a high correlation coefficient (r) of 99.99% and a % relative standard deviation (RSD) value of 1.8541%. The RSD value is below 2/3 of Horwitz CV, which is 7.7565. The single test accuracy was determined to be 102.01%, while the spike variation accuracy test yielded 102.66%. The limit of detection (LOD) was found to be 8.9213, and the limit of quantitation (LOQ) was determined as 29.7378. Furthermore, the instrument's limit values were established as an LOD of 8.8575 and an LOQ of 29.5250. The test results obtained meet the acceptance criteria based on AOAC.

Kata kunci: continous flow analyzer, nitrogen, palm oil, validation method, wet digestion

***Abstrak*:** *Nitrogen merupakan unsur hara makro utama yang dibutuhkan untuk pertumbuhan tanaman kelapa sawit, kekurangan nitrogen menyebabkan klorosis pada daun muda yang menguning dan menekan pertumbuhan. Pemeriksaan kadar nitrogen diperlukan untuk mengetahui kandungan nitrogen pada daun kelapa sawi, hasil penentuan ini digunakan untuk memperkirakan jumlah pupuk yang harus ditambahkan. Oleh karena itu, perlu dilakukan pengujian untuk mengetahui kandungan nitrogen pada tanaman kelapa sawit khususnya daun. Metode pengujian yang digunakan untuk mengukur kandungan nitrogen pada penelitian ini adalah metode standar dari Association of Analytical Communities (AOAC) yang telah mengalami perubahan prosedur untuk metode kinerja yang digunakan. Hasil pengujian nitrogen pada daun kelapa sawit menunjukkan koefisien korelasi (r) diperoleh sebesar 99,99% dan nilai % standar deviasi relatif (RSD) sebesar 1,8541%; nilainya di bawah 2/3 dari Horwitz CV sebesar 7,7565. Nilai akurasi uji tunggal sebesar 102,01%, dan uji akurasi variasi spike sebesar 102,66%. Batas deteksi (LOD) adalah 8,9213, dan batas kuantitasi (LOQ) adalah 29,7378. Nilai limit alat yang diperoleh adalah nilai limit of detection (LOD) sebesar 8,8575 dan limit of quantitation (LOQ) sebesar 29,5250; hasil pengujian yang diperoleh menunjukkan bahwa telah mengikuti syarat penerimaan kesesuian pengujian berdasarkan standard AOAC.*

Keywords: *continous flow analyzer**,** nitrogen, kelapa sawit, metode validasi, pengabuan basah*

INTRODUCTION

Palm oil (*Elaeis guineensis* Jacq.) is a commodity with the highest levels of production and consumption in Indonesia. In 2018, the area of oil palm plantations in Indonesia reached 14,326,350 hectares, and the most extensive total area of oil palm plantations was on the island of Sumatra, which reached 8,047,920 hectares (Umar *et al*. 2022) Indonesia is a producer and ranks number one among the world's largest palm oil exporters. The export volume of palm oil in 2017 was 29,135,179 tons, and the export value was US$ 20,802,708 (Direktorat Jenderal Perkebunan, 2018). The potential for Crude Palm Oil (CPO) production in Indonesia is quite enormous because it is used by the community as a raw material for products, especially for food needs. In addition to food needs, palm oil is also used as an industrial raw material and energy source. About

20% of palm oil production, especially its core oil, is used for non-food needs such as cosmetics, detergents, plastics, and so on (Otieno *et al*. 2016).

The increasing public demand for palm oil products has increased palm oil production in Indonesia yearly (Direktorat Jenderal Perkebunan, 2018). The uneven distribution of rainfall, especially in the interior of Indonesia, is a contributing factor affecting oil palm plant production. The other most important contributing factor limiting oil palm yields is fertilization. Fertilization aims to ensure the adequacy and balance of plant nutrients so that the growth of seedlings is maximized (Albari *et al*. 2018). The need for nutrients for oil palm plants in each growth phase differs. The amount of nutrients added through fertilizer must consider nutrient losses due to leaching, evaporation, and the soil's physical and chemical properties. Sudradjat *et al*. (2014) reported that seedlings that grew well had nutrient levels of N, P, K, Mg, and Ca in the vegetative organs of plants, which were 1,27%, 0,14%, 1,48%, and 0,21%, respectively (Sudradjat *et al*. 2014).

Previous research conducted by (Arifin *et al*. 2022) regarding the interaction on fertilization shows that the application of nitrogen fertilizers has an earlier effect on tree and fruit quality compared to the number of leaves. A deficiency of one of these nutrients will inhibit vegetative growth and decrease oil palm production. Nitrogen deficiency is usually recognized first by a pale green or yellowish-green color, especially in grasses, and premature necrosis of older leaves, starting at the shoots and spreading along the veins towards the neck of the stem and leaf margins (Goswami & Awasthi 2021). Nitrogen deficiency causes chlorosis of young leaves to turn yellow and depressed growth (Albari *et al*. 2018). Therefore, providing of nitrogen nutrients to oil palm plants needs to be done.

Nitrogen in plants functions in protein formation, chlorophyll synthesis, and metabolic processes (Albari *et al.* 2018). Nitrogen composes important organic compounds such as amino acids, proteins and nucleic acids (Arifin *et al*. 2022). Another function of nitrogen for plants is to increase plant growth, can nourish leaf growth, increase protein levels in plant bodies, improve the quality of leaf-producing plants and increase the proliferation of microorganisms in the soil (Yuliani *et al*. 2017). Unfortunately, the cost of fertilization for high mature oil palms is very expensive, where fertilization accounts for more than 50%-70% of maintenance costs and 25% of all production costs (Ningsih *et al*. 2015). Therefore, to optimize yields and reduce fertilization costs in oil palm plants.

Examination of nitrogen levels is necessary to determine the nitrogen content in the leaves of the oil palm; the results of this determination are used to estimate how much fertilizer should be added to each oil palm plant so that it does not experience excess or deficiency of nitrogen and saves the cost of fertilization needed by the palm oil plants.

The analytical method applied and carried out to measure nitrogen content in this study is a standard method from the Association of Analytical Communities (AOAC), which has undergone changes in the procedure for the performance method used. The technique used in this study uses the wet digestion method, this method is often used to test the content of macro metals and micro metals in plants, but there has been no research that has pushed the nitrogen content using the wet digestion method in oil palm leaves. The nitrogen testing method on oil palm leaves needs to be validated first to determine whether or not the procedure can be used in dealing with the testing problems encountered so that valid results and good performance data can be obtained. The results of this validation depend on the conditions and competence of personnel and the capabilities of different equipment. The parameters specified in the method validation are linearity, precision, accuracy, limit of detection (LOD), and limitation of quantification (LOQ).

MATERIAL AND METHODS

Instrumentation

The equipment used is the Digestion Block Gerhardt KBL 40S which consists of 40 tubes used for cooking samples, Destruction tubes, Analytical balances, Measuring flasks, Measuring pipettes, Measuring cups, Dispensette 10 ml, Beaker Glass, Diluter Hamilton Beach 503 A is used to dilute the solution extract, Vortex Maxi Mix II used to homogenize the solution, test tubes, reagent bottles, and The San++ Scalar Continuous Flow Analyzer Tool used to analyze the nitrogen content in oil palm leaves.

Chemical

Selenium Black pa, Sodium Sulphate Dehydrate pa, Sulfuric Acid 95% pa, purchased from Merck (Darmstadt, Germany) was used for the preparation of kjedhal extract solution. Brij 35% pa, Scalar Hydrochloric Acid 37% pa, Potassium Sodium Tartrate Tetrahydrate, Salicylic Acid Extra Pure DAB, Sodium Dicloroisocyanurate Dihydrate, Sodium Hydroxide pa, Sodium Nitroprusside Dihydrate GR ACS, Sodium Salicylate pa, Trisodium Citrate Dehydrate pa, purchased from Merck (Darmstadt, Germany) is used to make solutions for the measurement needs of macro-nitrogen nutrients.

Sample Preparation

The test sample was taken from the palm leaves and was then ground using a grinder of 2 millimeters. Weigh carefully 0.3000 grams of refined palm leaves, then put them into the digestion tube, add 0.6 grams of selen mixture and some boiling stones, and 4.5 ml of concentrated H_2SO_4 pa, after stirring, heat on the digestion block heater for 60 minutes at 160^oC while

constantly shaking so that it does not foam, the heating temperature is increased to 360ºC, and the digestion tube is closed until the digestion results are white, the heating is continued for 3 hours until the digestion is complete, the digestion tube is lowered from the digestion block, and after it cools, it is added with a little pure water and allowed to stand. Cold after chilling, it is diluted up to the marked line 50 ml with pure water, shaken and centrifuged, and left overnight to obtain a clear extract. The clear section is used for the measurement of elemental nitrogen.

Reagents

Buffer Solution pH 5.2

Prepare a 1000 ml measuring cup, fill it with ± 800 ml of distilled water, then add 33 grams of Potassium Sodium Tartrate, then add 24 grams of tri-sodium citrate, stir until dissolved, followed by measuring up to 1000 ml. Sample the pH of the solution with hydrochloric acid until it reaches a pH of 5.2 ± 0.1, end with 3 ml of 35% Brij and homogenize the solution and transfer it into a 1000 ml dark reagent bottle. This solution is stable for 1 week, store in the refrigerator at 40ºC when not in use.

Sodium Salicylate Solution

Prepare a 1000 ml measuring cup, fill it with ± 50 ml of distilled water, add 25 grams of sodium hydroxide, then stir until dissolved and let stand until cold, then add distilled water up to ± 800 ml, then add 80 grams of sodium salicylate and stir until dissolved, followed by measuring until 1000 ml. This solution is stable for 1 week, store in the refrigerator at 40ºC when not used.

Sodium Nitroprusside Solution

Prepare a 1000 ml measuring cup, fill it with ± 800 ml of distilled water, add 1.0 grams of Sodium Nitroprusside, then stir until dissolved, followed by weighing up to 1000 ml, end with homogenizing the solution and transfer it to a 1000 ml dark reagent bottle. This solution is stable for 1 week, store it in the refrigerator at 40ºC when unused.

Sodium Dichloroisocyanurate Solution

Prepare a 1000 ml measuring cup, fill it with ± 800 ml of distilled water, add 2.0 grams of Sodium Dichloroisocyanurate, then stir until dissolved, followed by measuring up to 1000 ml, end with homogenizing the solution and transfer it to a 1000 ml clear reagent bottle.

Standard Stock Solution 1000 ppm N

In a 1000 ml volumetric flask, 3.819 grams of Ammonium Chloride (NH_4Cl) was dissolved with + 80 ml of pure water, diluted to the mark of 1000 ml, and shaken until homogeneous. This solution should be stored at 40ºC and stable for 1 month.

Nitrogen Macro Nutrient Measurement

Diluting the sample extract 30 times, 0.15 ml sample extract 4.350 ml deionized water into a test tube, shaken with Vortex Maxi Mix II to homogenize the extract solution, was measured with The San++ Continuous Flow Analyzer at a wavelength of 660 nm, first the instrument was calibrated with a standard series and after that the sample extract was measured. Every 19 times the measurement, the instrument is re-calibrated with a zero standard and a second standard. Standard and sample readings are recorded automatically in the computer, analysis results are printed from the computer and calculated or corrected if necessary.

Linearity

A standard series solution was prepared with a concentration of 0; 2.0; 4.0; 6.0; 8.0; and 10.0 ppm, measured with The San ++ Scalar Continuous Flow Analyzer, then a graph of the relationship between concentration and absorbance was made to find its linearity so that the correlation coefficient can be calculated. The linearity test is used to determine whether the linear region we have chosen has a good consistency, test the tool's sensitivity to that concentration, and try the accuracy of practitioners in making standards (Table 1).

Precision

This precision (repeatability) test measured standard samples and 4,00 ppm Nitrogen mother common solution for nine replications. Repetitions are carried out by the same analyst and not far apart (on the same day). The tool will be given the same sample or extract with a minimum amount of 9 models to see the results of readings and repeatability. The piece used is leaves with the same concentration of 9 extracts. The 9 extracts were weighed separately, and after consideration, the seven parts were destroyed and treated equally. This piece is done to avoid unwanted contamination and guarantee the test's precision. Repeatability is

Table 1. Standard series 0 -10 ppm N

Series of (concentration)	1	2	3	4	5
ml stock solution 1000 ppm N	0.2	0.4	0.6	0.8	1
ml of rinse solution	99.8	99.6	99.4	99.2	99
ppm standard series N	2	4	6	8	10

obtained by finding the average value and standard deviation (standard so that the relative standard deviation can be calculated) (Yao *et al*. 2021). Precision Test is a test that aims to measure the stability of the tool against a sample being tested. The results were then processed to determine the % RSD compared with the Horwitz CV value. The following equations 2 and 3 define the precision value (Riyanto 2014):

$$SD = \sqrt{\frac{\sum (Xi - \bar{X})^2}{n-1}} \quad (1)$$

$$\%RSD = x\ 100\%\ \frac{SD}{X} \quad (2)$$

$$CV\ Horwitz = 21 - 0.5 \log C \quad (3)$$

Information:
Xi = measurement of the test sample
$\bar{X}$ = average
n = number of measurements
SD = standard deviation
C = concentration fraction

Accuracy

The accuracy test was carried out by pre-determining the Nitrogen content in the standard sample of leaves with as many as 3 replications. Spikes were carried out with a concentration of 4.0 ppm for 7 repetitions, and variations of spikes were carried out with a concentration of 0.50; 0.60; 0.70; 0.80; 0.90 ppm for 5 repetitions and 9 repetitions at a concentration of 0.70 ppm, then measured using The San++ Scalar Continuous Flow Analyzer. The accuracy test is used to find out whether the results of the analysis we are doing can we trust the results. Accuracy tests are also used to see whether or not disturbances occur during the analysis process. Usually, the accuracy test is carried out simultaneously with calculating the recovery value from the analysis (Umar *et al*. 2022). Meanwhile, the recovery calculation is taken from pure samples in the accuracy analysis with at least 3 extracts as a benchmark. The accuracy of the analysis results is an essential factor in determining the systematic error in the test (Kumar & Misra 2020). The accuracy of the analysis results can be determined by comparing them with other methods, using certified reference material (CRM), or adding standards (Yang *et al*. 2020). Percent recovery (% recovery) derived from the added analyte shows the accuracy value (Sujito *et al*. 2022). While the result of the comparison between the acquisition value and the actual value is the percentage recovery value. The following is the formula for calculating % recovery (Riyanto 2014) (Table 2).

Limit of Detection

The detection limit test is carried out to measure how small an instrument can measure a concentration or to determine the sophistication of the instrument for the analytical method used (Munteanu & Apetrei 2021). The test was carried out by measuring 10 standard solutions with the lowest concentration values. The measurement results must be the same as the b value in the linear line equation. The results are then processed to obtain the average and standard deviation (SD). The results are calculated LOD and LOQ values. The determination of LOD and LOQ can be determined by the following equations 5 and 6 (Faridah *et al*. 2020):

$$Sy/x = \sqrt{\frac{\sum (yc - yi)^2}{n-2}} \quad (4)$$

$$LOD = \frac{3 \times Sy/x}{slope} \quad (5)$$

$$LOQ = \frac{10 \times Sy/x}{slope} \quad (6)$$

Information:
yi: Measured standard absorbance
yc: Theoretical standard absorbance
n: Standard amount

$$\%\ recovery = \frac{sample\ concentration + spike - sample\ concentration}{spike\ concentration} \times 100\% \quad (1)$$

Table 2. % recovery value based on sample concentration (Riyanto 2014)

Analytes in the sample matrix	Recovery received (%)
10 < A < 100 (%)	98 – 102
1 < A < 10 (%)	97 – 103
0.1 < A < 1 (%)	95 – 105
0.1 x 10-2 < A < 0.1(%)	90 – 107
100 ppb < A < 1 ppm	80 – 110
10 ppb < A < 100 ppb	60 – 115
1 ppb < A < 10 ppb	40 – 120

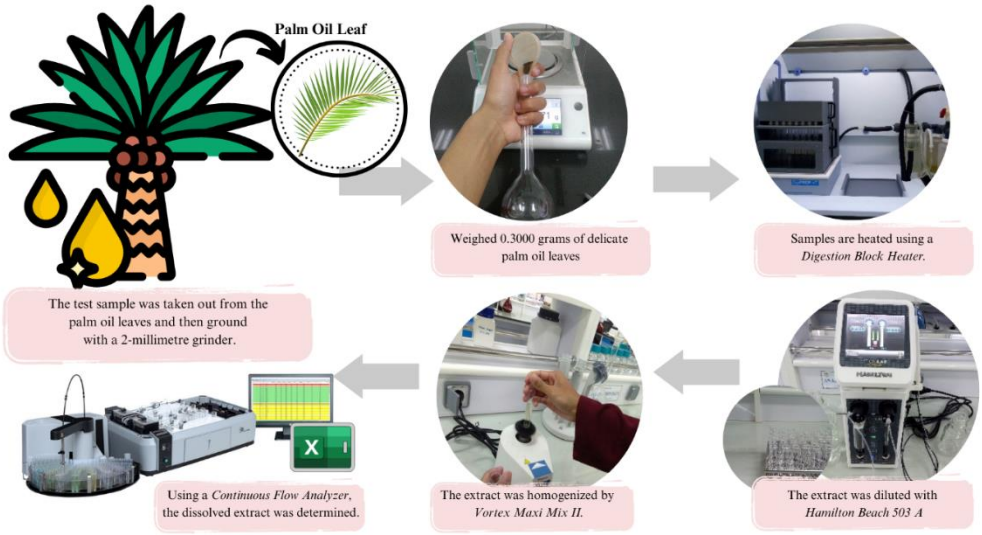

Figure 1. A schematic flowchart for sample preparation process.

Instrument Detection Limit (IDL) and Method Detection Limit (MDL)

Method detection limits are generated by processing data from blank measurements. Blank measurements were carried out at least 7 times using The San++ Scalar Continuous Flow Analyzer so that the detection limit concentration value of the method for determining nitrogen in oil palm leaves could be calculated. The instrument's detection limit is generated by processing the data from the zero standard measurements. Zero standard measurements were carried out at least 7 times using The San++ Scalar Continuous Flow Analyzer so that the value of the detection limit concentration of the instrument can be calculated from the determination of nitrogen in oil palm leaves

RESULTS AND DISCUSSIONS

Linearity

Linearity in chemical tests, in general, can be obtained from the relationship between the analyte concentration on the x-axis and the analytical signal or response on the y-axis on the calibration curve (Pagliano & Meija 2021). The linear relationship between the two axes is described by the magnitude of the correlation coefficient (r) in the linear regression equation, namely Y = ax ± b (de Higuera *et al*.,2020a). The ideal linear relationship is when the correlation coefficient value is close to one, or the b (intercept) value is zero. The value of a (slope) indicates the sensitivity or sensitivity of the analytical tool used in the analysis (de Higuera *et al*. 2020b). Linearity is a function of the analytical method that can provide a direct and appropriate response to the concentration of the analyte used in the sample. Linearity is a working range or limit when measuring analytes according to the given analyte concentration (Alladio *et al*. 2020).

The linearity of nitrogen content in leaves was tested by measuring the standard nitrogen series using The San++ Scalar Continuous Flow Analyzer with a wavelength value of 660 nm. The standard calibration series is read from one standard series, while it is read from three standard series obtained by the test results. The test results are shown in Table 3 and Figure 2.

From the measurement results, the standard series slope is 0.0744, the intercept value is 0.0051, and the

Table 3. Linearity test results for nitrogen standard series solutions

Standard	Results				
(ppm)	1	2	3	Average	Regression
0.0	-0.0001	-0.0001	0.0002	0.0000	-0.0011
2.0	0.0167	0.0169	0.0173	0.0170	0.0175
4.0	0.0356	0.0350	0.0362	0.0356	0.0361
6.0	0.0538	0.0541	0.0539	0.0539	0.0547
8.0	0.0727	0.0736	0.0726	0.0730	0.0732
10.0	0.0928	0.0924	0.0931	0.0928	0.0918

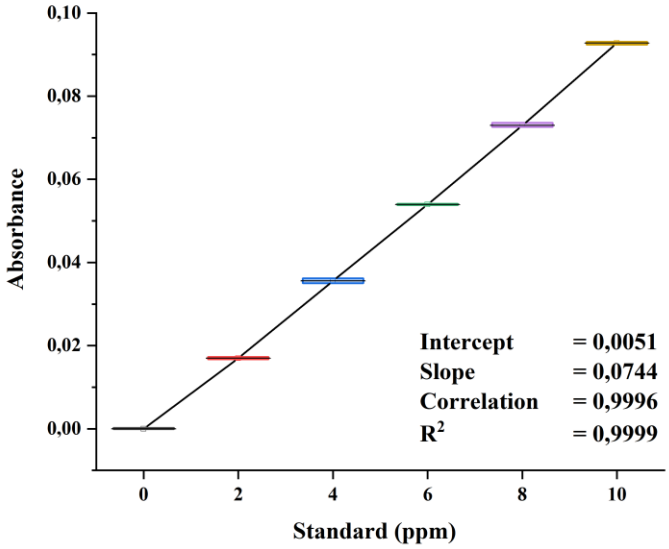

Figure 2. Nitrogen standard series solution linearity curve

correlation coefficient value is 0.9996. In addition, it is known that the slope value of the standard series as a sample is 0.0093, the intercept value obtained is -0.00123, and the correlation coefficient value (r2) is obtained at 99.99% so that this analysis method can be used and is appropriate because it shows a correlation coefficient value > 99%.

Precision

The accuracy test measured standard samples, which were destroyed with 98% H_2SO_4 solution. Then, 50 ppm of mother liquor (Standard Stock Solution N) was added for spiking. Accuracy testing was performed with 5 variations; each spike was replicated 5 times, and the third spike was replicated 10 times. The addition of the counted spikes is done with care. Each variation of the experiment then takes 3 data to be processed in calculating the percent recovery (% recovery) (Table 4).

Accuracy

Single Accuracy Test

A single accuracy test is usually carried out by adding spikes to the sample with a concentration in the range of 50-100% of the sample with an extracted amount of 7 or more. This single accuracy test can also see the extract's precision value because the extracts being analyzed should have the same concentration (Table 5).

Staged Accuracy Test

A step-by-step accuracy test was carried out by weighing the sample and giving a series of 5 spikes, three triple in concentrations between 50-100%. This test aims only to test the instrument's sensitivity to the sample's concentration so that truly accurate data is produced (Table 6, Figure 3).

The average % recovery value for a single accuracy test obtained from the test results was 102.01%, and the gradual accuracy test with spike variations was 102.66%. Based on the % recovery value obtained, it shows a good % recovery to meet the requirements, namely in the 80-120 % (Reich *et al*. 2002). However, several % recovery values were also found in the measurements, which did not meet the requirements; this was due to inaccuracies in the spiking process, not optimal in sample destruction, and sample dilution.

Method detection limit

Method detection limits are generated by processing data from blank measurements. Blank measurements were carried out at least 7 repetitions using The San++Scalar Continuous Flow Analyzer so that the detection limit concentration value of the method of determination of leaf nitrogen could be calculated. The detection limit test is carried out to measure how small an instrument can measure a concentration or to determine the sophistication of the instrument for the analytical method used (Munteanu & Apetrei 2021).

The limit value of the detection method indicates the minor concentration of analyte that can be determined with precision and accuracy according to the range of values specified in the agreed or specified method (Umar *et al*. 2022). Based on the calculation results, the Method Detection Limit (LOD) value is 8.9213, and the Quantitative Limit (LOQ) is 29.7378 (Table 7).

Table 4. The results of determining the accuracy (% recovery) using a variety of spike solutions.

Sample Code	**Sample+Spike concentration from standard curve (ppm)**
Sample + Spike 1	8,20
Sample + Spike 2	8,22
Sample + Spike 3	8.05
Sample + Spike 4	8,17
Sample + Spike 5	8,47
Sample + Spike 6	8,43
Sample + Spike 7	8.50
Sample + Spike 8	8.30
Sample + Spike 9	8.39
Means	8.30
Standard Deviation (Standard Deviation or S)	0.1539
Variance	0.0237
Relative standard deviation (RSD)	0.0185
Coefficient of variance (CV in %) or %RSD	1.8541
Concentration	8.30
C =	0.0000083
Log C =	-5.0807
0.5*log C	-2.5404
1-0.5 Log C	3.54037
CV Horwitz Reproducibility (%)	11.6348
CV Horwitz Repeatability (%)-2/3 CV	7.7565

Table 5. The results of determining the accuracy (% recovery) using spike solution. Sv= Standard Spike Volume (ml) Sd= Added Spike Density (ppm), Abs= Sample absorbance + Spike of standard curve, Ca= Sample concentration+Spike from standard curve (ppm).

Sample Code	**Sample Weight (g)**	**Sv**	**Sa**	**Abs**	**Ca**	**(%) Recovery**
Sample + Spike 1	0.3000	0.70	32.663	0.0729	8.20	97.15
Sample + Spike 2	0.3000	0.70	32.663	0.0730	8.22	97.77
Sample + Spike 3	0.3000	0.70	32.663	0.0752	8.47	105,42
Sample + Spike 4	0.3000	0.70	32.663	0.0749	8.43	104,2
Sample + Spike 5	0.3000	0.70	32.663	0.0755	8.50	106,34
Sample + Spike 6	0.3000	0.70	32.663	0.0738	8.30	100.22
Sample + Spike 7	0.3000	0.70	32.663	0.0745	8.39	102.97
Average					**8.36**	**102.01**

Table 6. The results of determining the accuracy (% recovery) using spike solution. Sv= Standard Spike Volume (ml) Sd= Standard Density (Spike) added (ppm), Abs= Sample absorbance + Spike from standard curve, Ca= Sample concentration+Spike from standard curve (ppm).

Sample Code	Sample Weight (g)	Sv	Sa	Abs	Ca	(%) Recovery
Sample + Spike 1	0.3000	0.50	2.3333	0.0675	7.57	109.02
Sample + Spike 1	0.3000	0.50	2.3333	0.0677	7.59	109.87
Sample + Spike 1	0.3000	0.50	2.3333	0.0684	7,67	113.30
Sample + Spike 2	0.3000	0.60	2.7997	0.0706	7,93	103.70
Sample + Spike 2	0.3000	0.60	2.7997	0.0703	7.89	102,27
Sample + Spike 2	0.3000	0.60	2.7997	0.0702	7.88	101.92
Sample + Spike 3	0.3000	0.70	3.2663	0.0729	8,20	97.15
Sample + Spike 3	0.3000	0.70	3.2663	0.0730	8,22	97.77
Sample + Spike 3	0.3000	0.70	3.2663	0.0738	8.30	100.22
Sample + Spike 4	0.3000	0.80	3.7329	0.0793	8.95	105,10
Sample + Spike 4	0.3000	0.80	3.7329	0.0775	8,74	99.48
Sample + Spike 4	0.3000	0.80	3.7329	0.0756	8.51	93.31
Sample + Spike 5	0.3000	0.90	4,1995	0.0822	9.29	101.52
Sample + Spike 5	0.3000	0.90	4,1995	0.0808	9,12	97.47
Sample + Spike 5	0.3000	0.90	4,1995	0.0811	9.16	98.42
Average						**102.66**

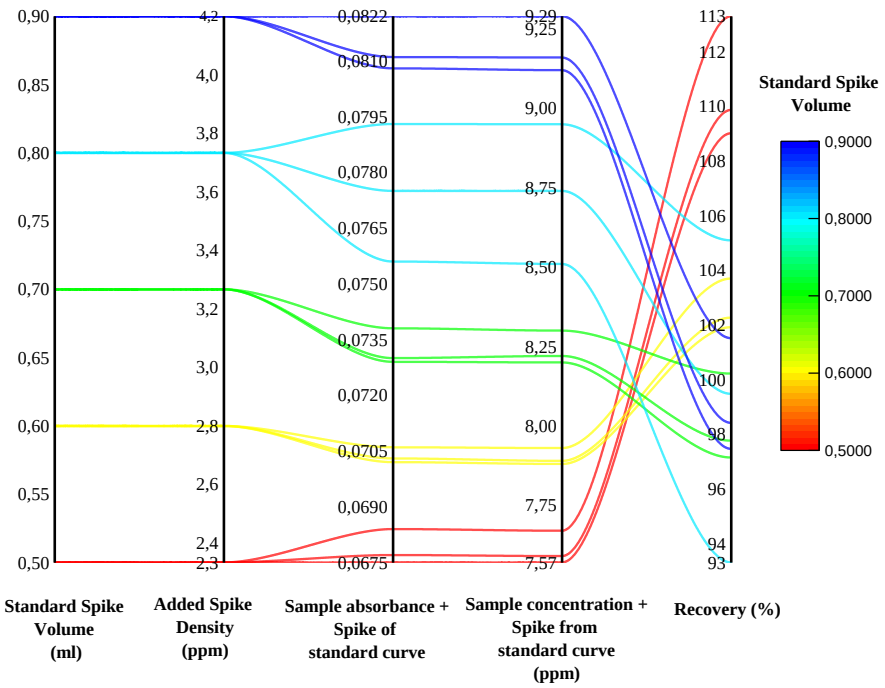

Figure 3. Parallel coordinates plot of standard spike volume (ml); added spike density (ppm); sample absorbance + spike of standard curve; sample concentration + spike from standard curve (ppm); recovery (%)

Table 7. Test Result Data of Limit Detection Method

No.	Concentration zero standard of standard curve (ppm)	Absorbance zero standard of the standard curve (Yi)	Y Count	(Y count - Yi)^2
1	0.12	-0.0001	0.0040	1.68E-05
2	0.11	-0.0002	0.0039	1.68E-05
3	0.22	0.0008	0,0048	1.60E-05
4	0.24	0.0011	0.0050	1.52E-05
5	0.17	0.0003	0.0044	1.68E-05
6	0.21	0.0007	0.0047	1.60E-05
7	0.18	0.0005	0.0045	1.60E-05
Total (Ycount-Yi)^2				1.14E-04
Total (Ycount-Yi)^2/(n-2)				2.27E-05
s(y/x)^2				0.0048
sy/x				2.9738
LOD 3xSD (IDL)				8.9213
LOQ 10xSD (IDL)				29.7378

Table 8. Instrument Detection Limit Test Result Data

No.	Concentration Std. Zero of standard curve (ppm)	Absorbance zero standard of the standard curve (Yi)	Y Count	(Y Count - Yi)^2
1	0.18	0.0005	0.0045	1.60E-05
2	0.25	0.0011	0,0051	1.60E-05
3	0.25	0.0011	0,0051	1.60E-05
4	0.22	0.0008	0,0048	1.60E-05
5	0.24	0.0011	0,0050	1.52E-05
6	0.21	0.0007	0,0047	1.60E-05
7	0.17	0.0003	0,0044	1.68E-05
Total (Ycount-Yi)^2				1.12E-04
Total (Ycount-Yi)^2/(n-2)				2.24E-05
s(y/x)^2				0.0047
sy/x				2.9525
LOD 3xSD (IDL)				8.8575
LOQ 10xSD (IDL)				29.5250

Table 9. Results of validation of the wet digestion method for testing nitrogen determination in oil palm leaves

Parameter	Acceptance Standards	Results	Suitability
Linearity	>0.9900	0.9999	Accordance
Single Accuracy	%Recovery (80-120) %	102.01	Accordance
Spike Variation Accuracy (Gradually)	%Recovery (80-120) %	102.66	Accordance
Precision	%RSD < 2/3 CV Horwitz	1.8541 < 7.7565	Accordance
Limit Detection Method	LOD 3xSD (MDL)	8.9213	(N/A)
	LOQ 10xSD (MDL)	29.7378	(N/A)
Instrument Detection Limit	LOD 3xSD (IDL)	8.8575	(N/A)
	LOQ 10xSD (IDL)	29.5250	(N/A)

Instrument Detection Limit

The instrument's detection limit is generated by processing the data from the zero standard measurements. Zero standard measurements were carried out at least 7 times with repetition using The San++ Scalar Continuous Flow Analyzer so that the value of the instrument detection limit concentration can be calculated from the determination of Leaf Nitrogen.

The instrument Detection limit is a test performed to test the limit of detection that the instrument can measure. This test is carried out by testing the standard 0 sample; the Zero Standard sample is the Standard used to determine the 0 point or initial concentration on the Continuous Flow Analyzer instrument. This zero Standard was tested 7 times with a Continuous Flow Analyzer; the results were compared with the Limit Detection Method.

The Instrument's Detection Limit value indicates the minor concentration that can be measured by the instrument from the sample tested by this method. If the measured concentration is below this value, it cannot be relied upon as the measured analyte. The quantitation limit value indicates a reliable value indicating the concentration of the quantitated analyte that the instrument can measure. Based on the calculation results, the Limit of Detection (LOD) value is 8.8575 and the Limit of Quantification (LOQ) is 29.5250 (Table 8).

Method Conformity Results

The results of the validation parameter of the nitrogen testing method in leaves by wet ashing with 50 ml H_2SO_4 dilution using The San++ Scalar Continuous Flow Analyzer can be seen in Table 9.

Based on the validation results in Table 9, it shows that the validation of the nitrogen testing method in leaves using wet ashing with 50 ml dilution H_2SO_4 using the The San++ Scalar Continuous Flow Analyzer gave good results because all the parameters tested in the validation process provided values that met the acceptance requirements of each component.

CONCLUSION

Based on the validation of the research method that has been carried out, it can be concluded that the determination of nitrogen elements in oil palm leaves can be carried out using the wet digestion method using The San++ Scalar Continuous Flow Analyzer method. Repeatability as (% RSD) was 1.8541% with a value of 2/3% CV Horwitz was 7.7565%, the accuracy (% recovery) of a single test was 102,01%, and the accuracy test with spike variations was 102.66%. The method limit value (LOD) is 8.9213, and the quantization limit value (LOQ) is 8.9213. The obtained instrument limit value (LOD) is 8.8575, and the quantitation limit value (LOQ) is 29.7378. These results met the acceptance requirements so that the validation of the nitrogen test method in leaves using wet digestion with 50 ml dilution H_2SO_4 using The San++ Scalar Continuous Flow Analyzer was declared valid.

ACKNOWLEDGMENT

Research and Development Center Astra Agro Lestari supported this research. We are grateful to the Laboratory Department of Astra Agro Lestari for all the help and assistance provided.

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