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Research Article

Validation Method on Green Analysis of Nitrite in Domestic Wastewater

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Abstract: The development and validation of methods on green analysis of nitrite in domestic wastewater has been carried out. This study is focused on the development and validation of nitrite determination using a smaller sample size with test kit nitrite from phosphoric acid, acid sulfanilamide, and N-(-naphthyl)-ethylenediamine dihydrochloride. The validation parameters studied include linearity, the limit of detection, the limit of quantification, precision, accuracy, and uncertainty. Based on the study, the green analysis procedure has good linearity with the linear regression equation of y = 2.8405x + 0.0043 with the correlation coefficient of 0.9992. Green analysis can be used detection and quantification nitrite in domestic wastewater at low concentration levels with the limit of detection of 0.01 mg/L and the limit of quantification of 0.03 mg/L. This green analysis procedure has good precision and accuracy with a % RSD of 1.61% and % recovery of 109.61%. Based on the study, the determination of nitrite with the green analysis method in domestic wastewater can be used at low concentration levels. The concentration of nitrite is 0.05 $\pm$ 0.01 mg/L for inlet sample and 0.04 $\pm$ 0.01 mg/L for outlet sample.

Keywords: nitrite, green method, validation method, linearity, LOD, LOQ, precision, accuracy, uncertainty

Introduction

Population growth has an impact on the quantity of domestic wastewater. Domestic wastewater comes from human activities that come from residences, restaurants, offices, and markets. The existence of these activities requires the domestic waste management system to prevent environmental pollution.

The Special Region of Yogyakarta is one of provinces that has the wastewaters treatment plant. The waste treatment system is carried out with a centralized system. Domestic wastewater is disposed of through a drainage pipes and streamed to the wastewater treatment plant. The wastewater treatment process is carried out by physical, chemical, and biological methods through pre-treatment, primary treatment, secondary treatment, the addition of disinfectant, and sludge treatment stage.

The water treatment process is accompanied by testing the water parameters on inlet and outlet samples. Determination of water parameters is carried out to monitor the effectiveness of the wastewater treatment process and ensure that the results of wastewater treatment are safe for the environment. Domestic wastewater has characteristics of industrial waste. Domestic wastewater contained the high levels of organic and inorganic compounds, in addition to biological contaminants. The contaminants contains in domestic wastewater are decomposed by microorganisms into compounds that can cause unpleasant odors.

One of the sources of contamination in domestic wastewater is nitrogen. Nitrogen content as nitrite ion is an important parameter in domestic wastewater quality tests, in addition to ammonium and nitrate ions. Nitrite ions pose a severe problem to human health explaining to need for fast and reliable detection of their presence in water [1]. The content of nitrite in water is very low [2]. This ion is an intermediate in the nitrification process of ammonia to nitrate and the denitrification of nitrate to nitrogen [2–6]. The nitrite ion is unstable and the concentration is low so that in water testing it is rarely not detected properly [2].

Determination of nitrite can be carried out by HPLC [7–9] with a diode array detector and ion chromatography [8–11]. The HPLC method is high accuracy, a high precision, low cost, fast, has an easy procedure, and minimizes to use of hazardous reagents [8,10]. The methods can be developed as a routine test methods for water quality parameters, but not all laboratories have HPLC. This instrumentation is relatively expensive and requires relatively expensive maintenance costs.

In addition, nitrite can be analyzed by an ion-selective electrode based on diazotization between nitrite ion and a p-phenylenediamine [1]. This method has a high selectivity for detecting nitrite at low concentration levels, but this equipment is not available in all laboratories.

Determination of nitrite in water according to the standard method was carried out using UV-Vis spectrophotometry [12]. Nitrite in acidic condition at pH 2.0 – 2.5 will react with sulfanilamide and N-(1-naphthyl) ethylene dihydrochloride (NEDA) forms dye azo compound which is purplish-red [12-13]. The color formed is measured by its absorbance using the UV-Vis spectrophotometer at the maximum wavelength of 543 nm [12]. Modification of the spectrophotometric method was developed by replacing reagents such as sulfanilic acid, NEDA, and methyl anthranilate [14], Griess reagent (sulfanilic acid, alpha naphthylamine, acetic acid) [14–16], p-aminobenzoic acid (PABA), and N-(1-naphthyl) ethylene dihydrochloride [19].

The method also requires a large number of the sample so it requires the use of large amounts of reagents. The N-(1-naphthyl) ethylene dihydrochloride solution is unstable. The solution should be refrigerated in a dark bottle and cannot be used for more than 1 month. These reagent are relatively expensive and are carcinogenic. The use of a lat of chemicals and the disposal of expired reagents add new problem to handling of laboratory wastewater.

Therefore, modification of the method was carried out to minimize the number of samples, minimize the reagents, reduce the cost, and efficient. Direct UV-Vis spectrophotometer using commercially available colorimetric kits for nitrite can be used as a routine test method [20]. The nitrite test kits are available in the form of reagents such as 1) phosphoric acid, acid sulfanilamide, and N-(-naphthyl)-ethylenediamine dihydrochloride, 2) sulfanilic acid and N-(-naphthyl)-ethylenediamine dihydrochloride and 3) ethylene diammonium sulfate.

Modifications to the method to be developed as green analysis procedure must be validated. This study is focused on the validation of nitrite determination using test kit [21] nitrite from phosphoric acid, acid sulfanilamide, and N-(-naphthyl)-ethylenediamine dihydrochloride. Method validation was carried out using a smaller sample size [14,22]. The study refers to the principle of green analysis for determination of samples with UV-Vis spectrophotometry. The green analysis methods is carried out a simple, efficient, low-cost, and effective procedure [22]. The validation parameters studied include linearity, the limit of detection, the limit of quantification, precision, accuracy, and uncertainty. The validation results are compared with the acceptance requirements in the Indonesian National Standard for nitrite test [8].

Actually, the study on green analytical methods for nitrite has been carried out. Green method for determination of nitrite can be carried out based on the a novel diazo coupling reaction[23]. This method is carried out through the reduction stage. The diazo coupling reaction use pyrogallol reagent[23]. This reagent is safe, but the diazo coupling reaction requires aromatic amine precursor and heating at 70°C for 15 minutes. This method also uses a large number of reagents. The method has the limit of detection above 1 mg/L. This method is not suitable for routine testing of nitrite with low concentration levels.

Therefore, the method proposed in this study is expected to be simpler, using a small sample volume, less reagent, and lower analysis costs. The validation method on green analysis of nitrite can be recommended in the routine testing of water quality. The lower sample size minimizes the number of reagents thereby minimizing the production of laboratory waste.

Materials and Methods

Materials

Materials used in this study are certified reference material (CRM) nitrite standard solution 0.200 mg/L, free nitrite distilled water, and nitrite test kit which contain sulfanilamide, N-(-naphthyl)-ethylenediamine dihydrochloride, and phosphoric acid.

Preparation of Nitrite Standard Series

The nitrite standard series was prepared with concentration of 0; 0.12; 0.14; 0.16; 0.18; and 0.2 ppm of nitrite standard solution CRM. A total of 4 mL of the standard was put into the test tube. The formation of dye azo is carried out by adding 1 spatula of nitrite test kit which contain sulfanilamide, N-(-naphthyl)-ethylenediamine dihydrochloride, and phosphoric acid, homogenized, and allowed to stand for 10 minutes. Standard series absorbance was measured using UV-Vis spectrometric at the wavelength of 543 nm.

Sampling and Sample Preparation on Green Analysis of Nitrite

This study was conducted by using domestic wastewater taken from the inlet and outlet of the domestic wastewater treatment plant. Sampling was carried out with water sampler equipment. The domestic wastewater samples used are a grab sample type. The collection is carried out at the inlet and outlet tubs. The domestic wastewater samples are homogenized [21].

The homogeneous domestic wastewater samples were filtered using nitrite-free filter paper with a pore of 0.45 μm. The domestic wastewater samples were put into dark colored bottle free from nitrite contamination. A total of 4 mL of the domestic wastewater samples were put into the test tube. The formation of dye azo is carried out by adding 1 spatula of nitrite test kit which contain sulfanilamide, N-(-naphthyl)-ethylenediamine dihydrochloride, and phosphoric acid, homogenized, and allowed to stand for 10 minutes. The absorbance was read at the wavelength of 543 nm. The test was carried out with 7 replications.

Determination of Linearity

Linearity is determined based on the correlation coefficient and determination coefficient of the calibration curve. The linearity of calibration curve is acceptable if the correlation coefficient $\geq$ 0.995. Determination of linearity refers to the Indonesian National Standard procedure for nitrite[12].

Determination of The Limit of Detection (LOD) and The Limit of Quantification (LOQ)

The limit of detection and the limit of quantification on the determination of nitrite were calculated from residual standard deviation values and slope on the linear regression equation. The residual standard deviation is determined from standard series absorbance measurement which has been corrected by linear regression equation. Determination of the limit of detection and the limit of quantification refers to the Indonesian National Standard procedure for nitrite[12].

Determination of Precision

Determination of precision is done by using replication 7 samples. Precision is determined to form the percentage of relative standard deviation (%RSD) value. The test method has good precision if the %RSD value is less than 2%. The % RSD was determined from the standard deviation of domestic wastewater measurement. Determination of precision refers to the Indonesian National Standard procedure for nitrite[12].

Determination of Accuracy

Determination of accuracy using certified reference material (CRM). A total of 4 mL of the domestic wastewater samples and CRM solution were put into the test tube added 1 spatula of nitrite test kit, homogenized, and allowed to stand for 10 minutes. The absorbance was read at the wavelength of 543 nm. The accuracy is determined based on the percentage of recovery (%R). The accuracy value can be accepted if the %R value is in the range of 90 - 110%. Determination of accuracy refers to the Indonesian National Standard procedure for nitrite[12].

Determination of Uncertainty

Determination of measurement uncertainty includes 4 stages, 1) specification of measurement on green analysis of nitrite, 2) identifying and analyzing of uncertainty sources on green analysis of nitrite, 3) quantifying the uncertainty components on green analysis of nitrite, and 4) calculating the combined standard and expanded uncertainties on green analysis of nitrite. Green analysis of nitrite presented in Figure 1. Based on Figure 1, the uncertainty sources can be identified. The identification of the uncertainty sources is presented in the fishbone diagram in Figure 2.

The components quantification is based on Figure 2 by calculating the uncertainty of volume of domestic wastewater samples, the concentration of nitrite, and precision. The standard uncertainty of sample volume of domestic wastewater samples, $\mu(V_c)$ obtains from the pipette calibration and the expansion factor of temperature. The standard uncertainty of nitrite concentration, $\mu(C_x)$ is obtained from the calibration curve and the precision uncertainty, μ_p is determined from the standard deviation of the sample measurement. The combined standard uncertainty of nitrite (μ_c) is calculated by relative

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uncertainty and the expanded uncertainty (μ) is calculated using the capacity factor with interval confidence of 95%.

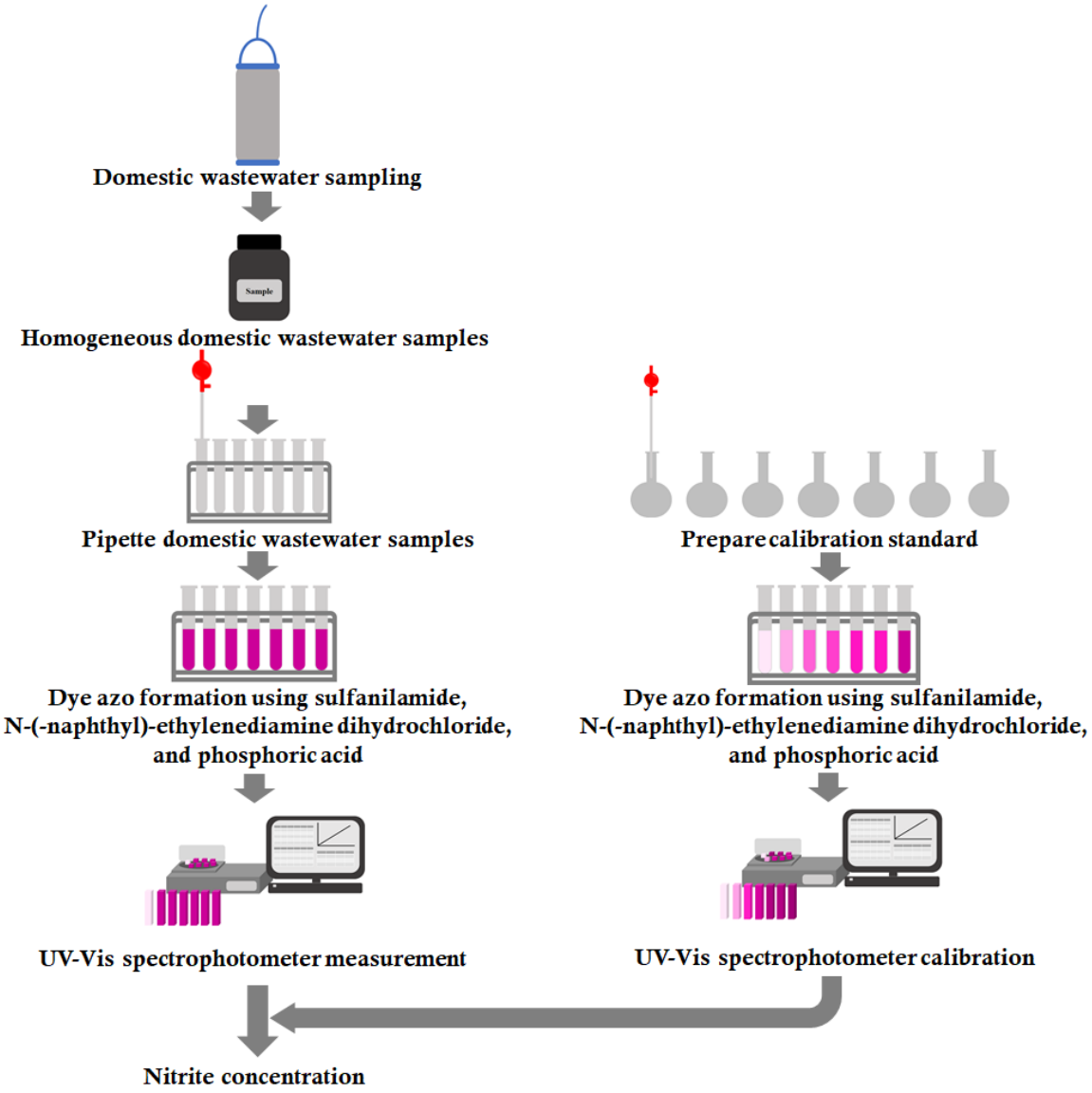

Figure 1. Green analysis procedure of nitrite

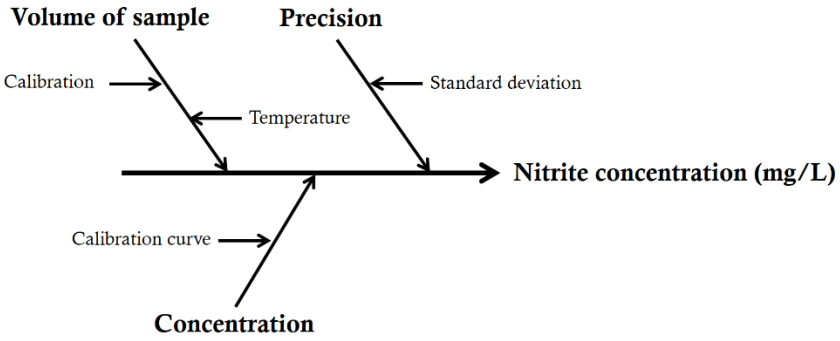

Figure 2. Fishbone diagram on green analysis of nitrite

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Result and Discussion

Determination of Linearity

Linearity is used to determine the ability of the nitrite method to obtain test results that are suitable for nitrite in the domestic wastewater samples at a certain concentration range. Linearity of calibration curve on green analysis of nitrite prepared form CRM is shown in Figure 3. (a). Figure 3. (a) shows the calibration curve of the standard series measurements. Based on Figure 3. (a) shows that the calibration curve on the nitrite determination has the linear regression equation of y = 2.8405x + 0.0043. The correlation coefficient (R) for the calibration curve is 0.9992 and the coefficient determination (R^2) is 0.9985. Linearity of calibration curve for nitrite standard series is following the predicted absorption calculation results. The validation curve shows the regression equation y = 2.8405x + 0.0043 with linearity (R) of 1.000 and the coefficient determination (R^2) of 1.000. Base on Figure 3. (a) the linearity of the calibration curve obtained form standard series has good linearity which correlation coefficient $\geq$ 0.995 [12].

Based on the study showed that the reduction in the volume of standard series from 50 mL to 4 mL and the use of test kit which contain sulfanilamide, N-(-naphthyl)-ethylenediamine dihydrochloride, and phosphoric acid provides linearity according to the requirements in the standard. Based on Table 2. green analysis of nitrite have good linearity as well as some previous methods which include HPLC [8–10], ion chromatography [9–11], and UV-Vis spectrophotometry with sulfanilic acid-NEDA-methyl anthranilate [14], Griess reagent [16,17], PABA-NEDA [19], and colorimetric kit [15].

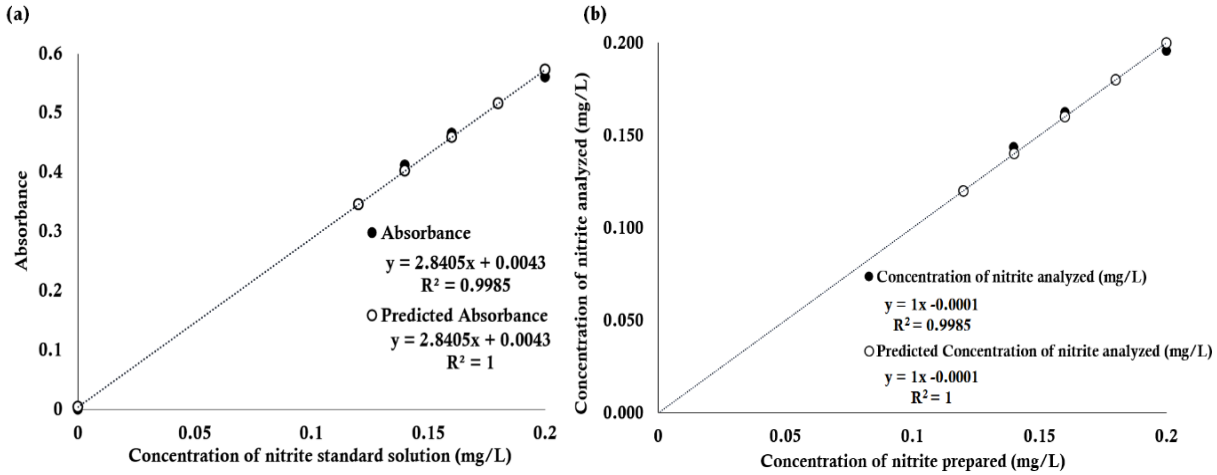

Figure 3. (a) Calibration curve of nitrite and (b) Model of linear regression for concentration of nitrite

Figure 3. (b) shows the model linear regression for concentration for nitrite standard solution. The regression model of y = 1x – 0.0001. The CRM of nitrite gives the high linearity to the calibration curve and has measured proximity to the true value. Figure 3. (b) shows that the reduction of the standard series volume and the addition of nitrite test kit has conformity with the CRM concentration value with the average of trueness value 100.35%.

Limit of Detection and Limit of Quantification.

The data in Table 1. is used to calculate the limit of detection and the limit of quantification from residual standard deviation. The limit of detection shows the lowest concentration of nitrite in the domestic wastewater samples detected in the analysis using UV-Vis spectrometric with nitrite test kits which contain sulfanilamide, N-(-naphthyl)-ethylenediamine dihydrochloride, and phosphoric acid. Based on the calibration curve, the LOD value of 0.01 mg/L. The limit of quantification shows the lowest concentration of nitrite in the domestic wastewater sample quantified in the analysis. Based on the data LOQ of 0.03 mg/L.

Table 1. The absorbance of nitrite for determination of LOD and LOQ

Concentration (mg/L)	Absorbance (Y)	Absorbance calculated (Y_i)	$(Y-Yi)^2$
0	0.000	0.0043	1.85×10^{-5}
0.12	0.345	0.345	2.56×10^{-8}
0.14	0.412	0.402	1.01×10^{-4}
0.16	0.465	0.459	3.87×10^{-5}

0.18	0.516	0.516	1.68×10^{-7}
0.2	0.560	0.572	1.54×10^{-4}
$\Sigma (Y-Yi)^2$			0.0003
Residual standard deviation			0.0088
Limit of detection (mg/L)			0.01
Limit of quantification (mg/L)			0.03

Based on Table 2., the green analysis is capable of detecting and quantifying nitrite at low concentration levels. The limit of detection and the limit of quantification this method lower than limit of detection in HPLC [8–10] and ion chromatography [9–11]. The lower standard series volume of with commercial test kit able to detect nitrite at low concentration levels, as the standard method with sulfanilic acid-NEDA [12] and sulfanilic acid-NEDA-methyl anthranilate [14]. This method can be used for measuring nitrite at low concentration levels with good precision and accuracy.

Table 2. The comparison of linearity, LOD, and LOQ for determination of nitrite

Methods	Linear regression equation	Coefficient correlation	LOD (mg/L)	LOQ (mg/L)	References
HPLC	y = 0.0608x + 0.0050	0.9995	0.45	1.50	[10]
	Y=1.6294X+0.73367	0.9994	0.25	0.8	[8]
	-	0.999	1.5	4.9	[9]
Ion chromatography	-	-	0.14	0.49	[10]
	y = 0.0017x + 0.009	0.9993	0.13	0.44	[11]
	-	0.999	1.2	0.7	[9]
UV-Vis spectrophotometry					
SA-NEDA-MA	y = 0.108x + 0.007	0.9998	0.01	0.03	[14]
PABA-NEDA	-	$\geq$0.9990	-	-	[19]
Griess reagent	-	0.9982	-	-	[16]
Griess reagent	y = 0.0506x - 0.0261	0.964	-	-	[17]
Colorimetric kits	y = 0.71x + 0.02	0.99842	0.018	0.062	[15]
Green method based on diazo coupling reaction	-	0.9994	1.8	-	[21]

SA-NEDA-MA : sulfanilic acid, N-(1-naphthyl) ethylene dihydrochloride, and methyl anthranilate
PABA-NEDA : p-aminobenzoic acid and N-(1-naphthyl) ethylene dihydrochloride
Griess reagent : sulfanilic acid, alpha naphthylamine, acetic acid

Precision

Table 3 shows the replication data on the determination of nitrite on inlet and outlet domestic wastewater samples. Based on Table 3, the results show that this method has good precision with the percentage relative standard deviation of 1.55 and 1.67%. The percentage relative standard deviation is just below 5%. The method provides good precision for routine testing of domestic wastewater samples. This method can provide uniformity of nitrite concentration on the precision.

Table 3. The nitrite concentration of wastewater samples

Replication	The nitrite concentration, x (mg/L)	
	Inlet	Outlet
1	0.0460	0.0369
2	0.0455	0.0379
3	0.0452	0.0383
4	0.0465	0.0374
5	0.0471	0.0387
6	0.0452	0.0384
7	0.0464	0.0382
Standard deviation	0.0007	0.0006
The percentage of relative standard deviation (%RSD)	1.55	1.67

Based on Table 3. and Table 5. green analysis of nitrite have good precision as well as some previous methods which include HPLC [8], ion chromatography [10,11], and UV-Vis spectrophotometry with sulfanilic acid-NEDA-methyl anthranilate [14], Griess reagent [16], PABA-NEDA [19], and green method based on diazo coupling reaction [23].

Accuracy

Determination of accuracy was done with calculating the percentage of recovery from spiked sample. Table 4 shows the data of concentration of spiked samples. Based on Table 4, the average of percentage of recovery for determination of nitrite of 109.61%. The data for determination of nitrite using developed method with UV-Vis spectrometry method are in the range 85 - 110%. The green analysis of nitrite using UV-Vis spectrophotometry with test kit nitrite which contain phosphoric acid, acid sulfanilamide, and N-(-naphtyl)-ethylenediamine dihydrochloride show good accuracy.

Table 4. The nitrite concentration for determination of accuracy

Replication	Concentration of nitrite		%Recovery
	Sample	Spike sample	
1	0.0549	0.0662	111.55
2	0.0549	0.0658	109.61
3	0.0549	0.0655	107.68
Average			109.61

Table 5. The comparison of precision and accuracy for determination of nitrite

Methods	Precision (%RSD)	Accuracy (%R)	References
HPLC	1.4 – 5.2	-	[10]
	4.85	99 – 104	[8]
Ion chromatography	0.44 – 3.6	98 – 102	[10]
	1.2	95 – 114	[11]
UV-Vis spectrophotometry			
SA-NEDA-MA	4.59	98 - 102	[14]
PABA-NEDA	0.31 – 1.83	83 – 96	[19]
Griess reagent	3.02 – 24.82	-	[16]
Griess reagent	-	99.23	[17]
Green method based on diazo coupling reaction	0.033	96.44	[23]

SA-NEDA-MA : sulfanilic acid, N-(1-naphthyl) ethylene dihydrochloride, and methyl anthranilate
PABA-NEDA : p-aminobenzoic acid and N-(1-naphthyl) ethylene dihydrochloride
Griess reagent : sulfanilic acid, alpha naphthylamine, acetic acid

Based on Table 4. and Table 5. green analysis of nitrite have good precision as well as some previous methods which include HPLC [8,10], ion chromatography [10,11], and UV-Vis spectrophotometry with sulfanilic acid-NEDA-methyl anthranilate [14], Griess reagent [17], and PABA-NEDA [19].

Uncertainty

Table 6. shows the uncertainty values on determination of nitrite using UV-Vis spectrometric with nitrite test kit. Based on Table 6., the standard uncertainty value of domestic wastewater samples volume, concentration of nitrite, and precision should be determined. The combined uncertainty is determined from all sources to calculate the expanded uncertainty value. The expanded uncertainty is presented as the uncertainty value the should be included in the nitrite test results.

Table 6. shows the expanded uncertainty for determination of nitrite in domestic wastewater using green analysis procedure have expanded uncertainty of 0.01 mg/L for inlet and outlet sample. This uncertainty value is used to determine the acceptability limit of data from traceable source. Therefore, based on Table 3. and Table 6. it can be reported that the concentration of nitrite in domestic wastewater is 0.05 $\pm$ 0.01 mg/L for inlet sample and 0.04 $\pm$ 0.01 mg/L for outlet sample.

Table 6. Determination of uncertainty on domestic sample

The sources of uncertainty	Unit	Inlet sample			Outlet sample		
		Value (x)	$(\mu_{(x)})$	$(\mu_{(x)})/x$	Value (x)	$(\mu_{(x)})$	$(\mu_{(x)})/x$
Volume of sample, $\mu(V_c)$	mL	5	0.0206	1.69×10^{-5}	5	0.0206	1.69×10^{-5}
Concentration of nitrite, $\mu(C_x)$	mg/L	0.0459	0.0027	0.0036	0.0459	0.0027	0.0036
Precision, μ_p	mg/L	1.55	0.0003	3.01×10^{-8}	1.67	0.0002	2.05×10^{-8}
Combined uncertainty, μ_C	mg/L			0.0036			0.0029
Expanded uncertainty, μ	mg/L			0.01			0.01

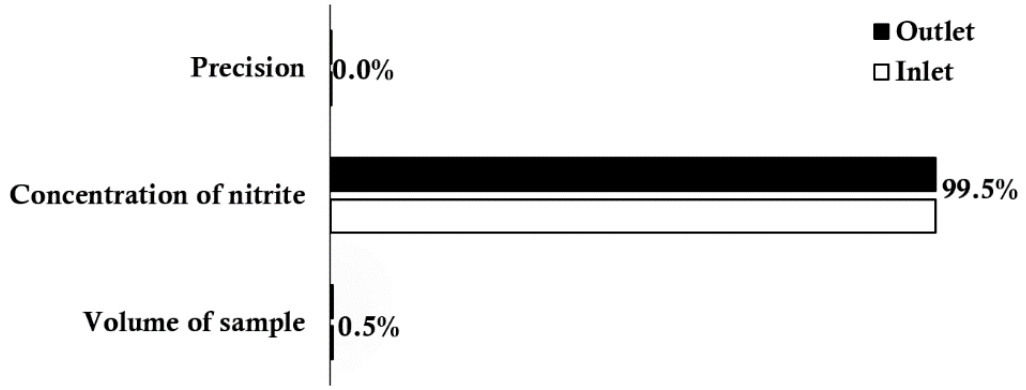

Figure 4. The percentage of uncertainty contribution on determination of nitrite

Figure 4 shows the percentage contributors to the measurement uncertainty. The nitrite measurement result provide good precision and good linearity. Green analysis with low volume of domestic wastewater samples contribute to very small measurement uncertainty. Figure 4 shows that the largest contributor for uncertainty value comes from the concentration of nitrite. The standard uncertainty of nitrite concentration from calibration curve. Based on Figure 3. (a) and Figure 3. (b) the linearity of the calibration curve obtained form standard series has good linearity which correlation coefficient.

Figure 4. also shows that the reduction in the volume of domestic wastewater samples and the reagents does not contribute significantly to the measurement uncertainty value. Therefore, the concept of green analysis in this study can be recommended as a routine test method. Based on the results, it can be concludes that the green analysis of nitrite on domestic wastewater can be accepted by following quality assurance and quality control [22]. The results have been accompanied by validation of the development of green analytical methods and measurement uncertainty evaluation [22].

Conclusion

Green analysis for determination of nitrite using small sample size with test kit nitrite from phosphoric acid, acid sulfanilamide, and N-(-naphtyl)-ethylenediamine dihydrochloride have good linearity with linear regression equation of y = 2.8405x + 0.0043 with correlation coefficient of 0.9992. Green analysis can be used detection and quantification nitrite in domestic wastewater at low concentration levels with LOD of 0.01 mg/L and LOQ of 0.03 mg/L. This method has good precision and accuracy with % RSD of 1.61% and % recovery of 109.61%. Based on the study, determination of nitrite with green analysis method in domestic wastewater can be used at low concentration levels. The concentration of nitrite is 0.05 $\pm$ 0.01 mg/L for inlet sample and 0.04 $\pm$ 0.01 mg/L for outlet sample. The method is simple, efficient, low-cost, and effective for green analysis of nitrite.

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