Method validation for nitrate analysis in water using spectrophotometer visible with cadmium reduction

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Abstract. The modified method for nitrate analysis in water sample by means of spectrophotometer visible using cadmium reduction has been validated. The method was validated according with analytical parameters. The result of this study were linear range 0.069-5 mg.L⁻¹, limit detection and quantitation were 0.025 mg.L⁻¹ and 0.069 mg.L⁻¹, respectively. repeatability of the method 2.19 % rsd and accuracy with 102.7 % recovery. Statistically, the results were in good agreement. The accuracy of this modified method has improved considerably in total. The method showed a good performance and can be applied for nitrate monitoring in water sample.

1. Introduction
Nitrate is a part of nitrogen cycle in the earth’s crust and naturally occurring in ionic species. It commonly exists in the environment in fairly water-soluble forms, in association with different ionic species such as sodium and potassium [1]. Nitrate is stable in the environment, however it can be reduced into nitrite through biological processes. Inorganic fertilizer, oxidizing agent and making glass are kinds of man-made activities which can produce of nitrate [2]. Nitrate can enter into surface and groundwater from agriculture activities and wastewater treatment [3]. It can percolate in large quantities under aerobic condition and can be denitrified and degraded to nitrogen under anaerobic condition [4].

Human exposure to nitrate through air, water or soil depends on local nitrate concentration [5] and the total amount of NO produced in the blood [6]. Nitrate can be reducing into nitrite in stomach and incorporate into carcinogenic N-nitrosamine compounds when it enters the body and nitric can react with hemoglobin in the blood and reduce the ability of hemoglobin to carry oxygen. Methemoglobinemia is a kinds of baby disease caused by nitrate where a condition in which more than one percent of the hemoglobin in red blood cells take the form of the methemoglobin [7]. The maximum contamination level (MCL) for nitrate in drinking water is 10 mg/L nitrate-nitrogen (Nitrate-N) by The US Environmental Protection Agency (US EPA) [8].

Various method reported for determination of nitrate including spectrometric (Griess reaction) [9], chemiluminescent [10], electrochemical [11], chromatography [12], and spectrofluorimetric [13]. Spectrometric method (Griess reaction) is the most favorite method for routine analysis since low cost and easy feasibility. According with Proficiency Testing (PT) for nitrate analysis in drinking water conducted by National Accreditation Committee (KAN), 40 laboratories testing participated in this scheme where 11 laboratories use UV Screening method, 9 laboratories use cadmium reduction method, 12 laboratories use Brucine method, and 8 laboratories use other method. The result of this scheme that
only 19 laboratories testing showed satisfactory result and 21 laboratories gave unsatisfactory result. There were 4 laboratory used cadmium reduction method showed unsatisfactory result. It can be seen that spectrometric method based on Griess reaction is the most popular method for nitrate analysis in water since it good sensitivity, on the other hand, it is very difficult because of interferences.

The principle of spectrometric method based on Griess reaction is nitrate react with sulphanilic acid in acidic medium to form diazonium salt that reacts further alphanaphthylamine to produce pink-colored azo dye in wavelength 543 nm. Nitrate is reduced to nitrite, usually by means of cadmium copperized cadmium column. The concentration of nitrate is calculated by subtracting the nitrite concentration with the total concentration. However, alphanapthylamine used in this method is carcinogenic so that it is replaced by N-1-napthyl ethylendiamine (NED) as a coupling component for sulfanilamide in Griess reaction [14].

Since nitrate is an important analyse for environmental, food and human health monitoring, thus the method performance for determination of nitrate in water sample by means of spectrometric method (Griess reaction) based on cadmium reduction should be validated according with analytical parameters to proof that the method fit for purpose. This method can be applied for nitrate monitoring in water samples.

2. Methodology
The nitrate analysis method adopted from Standard Water and Wastewater treatment 4500-NO₃⁻ Nitrogen (Nitrate) was modified in pH, flow rate, CuSO₄ concentration.

All of reagents used in this experiment were analytical grade and water used for analysis is ultrapure water (18.3 MΩ cm⁻¹ resistivity). Cadmium (Cd) granule was purchase from Merck, Germany with i.d 3-6 mm. CuSO₄ pro analysis from Merck, Germany was used as a coupling agent with Cd. 1000 mg.L⁻¹ made from NaNO₃ and NaNO₂ pro analysis (Merck, Germany).

2.1. Optimization method
Parameters examined to get a reliable result were sample pH, sample flowrate and CuSO₄ concentration.

2.2. Spectrophotometer verification
The performance of analytical instrument, Spectrophotometer UV Vis U-1800 (Hitachi, Japan), was evaluated by checking the accuracy of absorbance and wavelength by means of K₂Cr₂O₇ standard solution and Holmium oxide glass filter.

2.3. Method validation
The method for nitrate analysis was validated according with analytical parameters. For instance, linearity, precision, limit of detection, limit of quantitation and accuracy and uncertainty [15].

2.4. Method application
The method was applied for determination of nitrate in water sample.

3. Results and discussion

3.1. Optimization method
In order to optimize the method for nitrate analysis in water. The effects of flowrate sample, sample pH and CuSO₄ concentration were studied.

The flow rate of sample in this method means the contact time between nitrate and Cd-Cu granule in the column to achieve a good reduction process ranging from 2.5-10 mL.min⁻¹. The results are shown in figure 1.

The highest absorbance was achieved at 6 mL.min⁻¹ by means of 0.1 mg.L⁻¹ of nitrate standard solution.
pH condition is critical point for nitrate reduction by spectrometric with Cd-Cu reduction, since the nitrate can reduce to nitrite in specific pH. The pH condition was optimized in of pH 6-10, in figure 2. In figure 2, the absorbance maximum of 0.1 mg. L\(^{-1}\) standard nitrate solution was achieved at 8.5.

Cadmium granules can be directly used to reduce nitrate to nitrite. However, to accelerate the reduction process CuSO\(_4\) solution is used so that the Cd-Cu complex is formed. Since, the concentration of CuSO\(_4\) solution has an effect on the nitrate reduction process to nitrite, so that it is necessary to be optimized. The results of the optimization of CuSO\(_4\) solution can be seen in figure 3.

In figure 3, the result showed that the concentration of CuSO\(_4\) at concentration 1-2% of CuSO\(_4\), the absorbance of nitrate was increased then decreased and went to stable. At concentration 2% of CuSO\(_4\), the absorbance of 0.1 mg. L\(^{-1}\) nitrate standard solution gave the maximum absorbance.

Effect matrix was examined by comparing of slope from two calibration curves. One calibration made by spiked sample and one is made without spike sample. The results are shown in figure 4. We can see that the slope from both of calibration curve were similar. It means the effect of matrix is not influent for nitrate analysis in water sample, the external calibration curve can be used for quantifying of nitrate concentration in water sample.

**Figure 1.** flowrate optimization by using 0.1 mg. L\(^{-1}\) of nitrate standard.

**Figure 2.** pH optimization by using 0.1 mg. L\(^{-1}\) of nitrate standard.

**Figure 3.** Optimization of CuSO\(_4\) concentration.
By using the optimized experimental condition described above. The method was validated according with analytical parameter. The results are shown in Table 1.

| Figure of merit          | Result | Unit     | Acceptable criteria         |
|--------------------------|--------|----------|----------------------------|
| Linearity                | Until 5| mg L⁻¹   |                            |
| Repeatability, (n=7)     | 1.61   | %rsd     | ≤ 2/3 CV Horwitz           |
| Recovery, (n=7)          | 102.7  | %        | 80-115%                    |
| Limit of detection (LOD), (n=10)* | 0.025  | mg L⁻¹   |                            |
| Limit of Quantitation, LoQ, (n=10)* | 0.069  | mg L⁻¹   |                            |

In Table 1, the calibration curve was linear until 5 mg.L⁻¹ with coefficient correlation $R^2=0.999$ and the precision was studied as repeatability and expressed relative standard deviation for n=7 were 1.61 %rsd, the method showed a good precision since the precision of result is below than 2/3 CV Horwitz. The accuracy of method examined by spike sample was 102.7%. the method showed a good agreement 80-115% (AOAC, 2005). The limit of detection (3sd) and quantitation (10sd) were 0.025 and 0.069 mg. L⁻¹, respectively.

The uncertainty of method was evaluated by means bottom up approach [16]

$$u_x = \sqrt{\left(\frac{u_{V_0}}{c_0}\right)^2 + \left(\frac{c_{\text{rec}}}{c_{\text{nom}}}ight)^2 + \left(\frac{c_{\text{nom}}}{V_{\text{vol nitrate}}}ight)^2 + \left(\frac{u_{\text{vol nitrate}}}{V_{\text{vol nitrate}}}ight)^2 + \left(\frac{u_{\text{repe}}}{\text{repeat}}\right)^2}$$

By this equation, the uncertainty value of sample quantified was 1.21 mg. L⁻¹.

The method was applied for surface water, and the nitrate concentration was 18.938±1.2 mg.L⁻¹. This result showed the concentration of nitrate in surface water was higher than the maximum contamination level in drinking water. It can be caused by man-mad activities.

4. Conclusion
The modified method for nitrate analysis with cadmium reduction by means of spectrophotometer Visible showed a good performance with 102.7 % of recovery and 2.19 %rsd of precision, the concentration minimum which can be detected was 0.025 mg. L⁻¹. Thus, this method can be applied for nitrate monitoring in low concentration in water samples.
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