



## Improvement of extraction efficiency for spectrophotometric determination of $\text{NO}_3^-$ and $\text{NO}_2^-$ in Gunshot residues; An application for a shooting distance study

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### Abstract

This research studied parameters affecting the extraction efficiency of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  from gunshot residue samples (GSRs). The spectrophotometric determination of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  was carried out using the principle of a Griess reaction. For this work, both sampling and extraction parameters were investigated before selecting the optimal condition for a study of shooting distance. The results showed that a mixed solution of 1% Sulfanilamide and 1% N-(1-Naphthyl) ethylene diamine (NED) in 0.5 molL<sup>-1</sup> HCl (Griess reagent) and 30 min reaction time provided good sensitivity for the determination of  $\text{NO}_2^-$  at 540 nm. In the case of  $\text{NO}_3^-$ , 1% VCl<sub>3</sub> in Griess reagent was used to reduce  $\text{NO}_3^-$  to  $\text{NO}_2^-$  before analysis with the same principle. The optimal condition for a sampling procedure used 150  $\mu\text{L}$  of room temperature deionized water per cotton brush swab for two replicated GSR collections. Samples were stored for 1 week before analysis could be carried out at 4 °C in a refrigerator or at room temperature without significantly different results. On the other hand, 4 mL of room temperature deionized water with and without ultrasonic extraction times of 5 or 30 min respectively were the selected parameters for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  extraction from GSRs.

The proposed method could be applied completely for spectrophotometric determination of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in GSRs. The limits of detection (LOD) of  $\text{NO}_2^-$  and  $\text{NO}_2^- + \text{NO}_3^-$  were  $0.27 \times 10^{-6}$  and  $3.58 \times 10^{-6}$  molL<sup>-1</sup> respectively with a relative standard deviation  $\leq 11.6\%$  of the given slope. A wide linearity range between  $2.17 \times 10^{-6}$  to  $217 \times 10^{-6}$  molL<sup>-1</sup> was obtained and a percentage of recovery in the range of 96.24 – 102.32 and 98.04 – 105.96 for  $\text{NO}_3^-$  and  $\text{NO}_2^-$  was achieved, respectively. This method can be performed for determination of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  content at a maximum 304.8 centimeter shooting distance. From these findings, the proposed sampling, extracting and determining methods for  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in GSRs are highly sensitive, precise, robust, simple and inexpensive, with a short analysis time and provide a useful alternative procedure for analytical chemists or forensic scientists in their work.

**Keywords:** Extraction,  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , Gunshot residues

### Introduction

Nowadays, firearms are extensively used by criminals for crime in a large number of areas. Thus, investigators have to prove and clarify existing evidence for use as credible evidence in prosecution litigation processes in court (Bolton–King, 2016). This leads to the identification of criminals or innocent parties accurately and reliably. Moreover, justices can utilize proven evidence to consider a case judgment in a fair manner with good governance. Chemical compositions evaluated in Gunshot Residue Samples (GSRs) are crucial evidence for forensic science because they add a great deal of weight to what justices accept for a trial. In general, the critical chemical compositions of GSRs are nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ), which are widely accepted in forensic science because they are the main components of gunpowder. The amount of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  compounds at the target area and/or the area of the hand or the clothing worn by the shooter are correlated to the firing distance and post-firing time. One report stated the amount of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in GSRs decreased with increasing firing distance and post-firing time (Puttinil & Suppaluknaree, 2012). Therefore, the information obtained from

analysis of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in GSRs was very important to accompany the development of a case for prosecution by investigators.

Quantification of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in GSRs has been carried out by several methods. A colorimetric method has been performed by reacting the  $\text{NO}_2^-$  compound with Griess solution, sulfonic acid solution (sulfanilamide) and N-(1-Naphthyl) ethylene diamine (NED) solution under acidic conditions (Patton & Kryskalla, 2011). A purple color was produced and the amount of  $\text{NO}_2^-$  was related to the color intensity, as demonstrated in Fig 1. Moreover,  $\text{NO}_3^-$  compound can be analyzed with a similar method by adding a reducing agent to reduce the  $\text{NO}_3^-$  to  $\text{NO}_2^-$  before measurement of absorbance by a spectrophotometric method. Alternatively, a Griess reaction could be performed by sequential injection analysis (SIA) and flow injection analysis, (FIA) (Chen, Yuan, Huang, Zhang & Bergman, 2008, p. 82 and Pasquali, Gallego-Pico, Hernando, Velasco & Alegria, 2010, p.79) and paper-based analytical devices (PADs) (Bhakta, Borba, Taba, Garcia & Carrilho, 2014, p. 117). However, there was also a report that the  $\text{NO}_2^-$  compound could react with 1-Butyl-3-methylimidazolium (1-Butyl-3-methylimidazolium-modified methyl red) as a color reagent. Afterwards the quantity of apparent color intensity was measured with the same methods mentioned previously (Zhang, Qi, Dong, Chen, Xu, Ma & Chen, 2014, p. 429). In addition, separation techniques such as capillary electrophoresis (CE) (Betta, Vitali, Fett & Costa, 2014, p. 23), high performance liquid chromatography (HPLC) (Ferreira & Silva, 2008, p. 1598) and gas chromatography (GC) (Pagliano, Meija & Mester, 2014, p. 36) have been effective methods for analysis of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in GSRs.

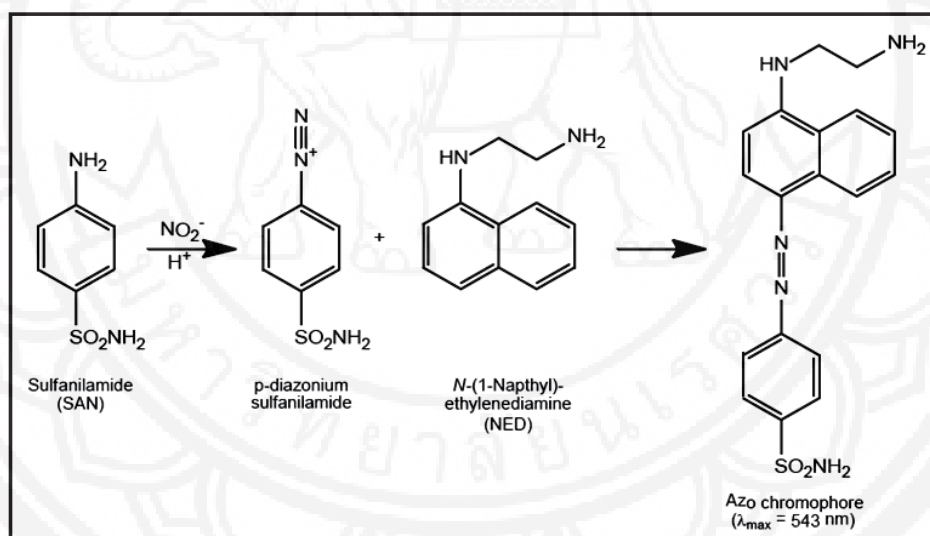


Figure 1 The principle of a Griess reaction used for colorimetric detection of  $\text{NO}_2^-$  (Patton & Kryskalla, 2011)

The Royal Thai police forensics science center normally applies a Griess reaction for qualitative analysis of  $\text{NO}_2^-$  in GSRs. However, limitations of this method are low sensitivity and being unable to analyze  $\text{NO}_3^-$  at the same time. Therefore, this project aimed for development of spectrometric detection of  $\text{NO}_2^-$  using a Griess reaction while applying  $\text{VCl}_3$  as a reducing agent to reduce  $\text{NO}_3^-$  to  $\text{NO}_2^-$  before spectrometric detection of generated  $\text{NO}_2^-$  using the existing principle. In this way, the inconvenient Cd column reducing method (Ansanan, Poosittisak & Thanoorad, 2013, p. 46) could be eliminated. Furthermore, an optimal condition for sampling efficiency including sampling times (replication of cotton swab number), solvent temperature, solvent volume and stored temperature were investigated. Various parameters used for extraction of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  from GSRs



were also studied in terms of extraction temperature, extraction time, extraction solvent volume and sonication time, respectively. Finally, an appropriate condition was examined for sampling, extraction and spectrometric determination of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in collected GSRs around a firing target at various shooting distances.

## Experiment, Instruments and Chemicals

### 1. Instruments and Chemicals

The instruments and apparatus used in this study included a 9 mm semiautomatic type of automatic pistol (CZ) with a 3.5 inch caliber length (model 75D Compact, Czech Republic), jacket hollow point bullets (THAIARMS, Thailand), a cotton brush (Ambulance brand, Thailand), a white cotton target, an ultrasonic cleaning bath (Transsonic Digital, Elma, Germany), a dry block heater (JSR, JSBL-01T, Korea) and a spectrophotometer (UH5300, Hitachi, Japan).

All chemicals used in this work were analytical reagent grade. A  $100 \text{ mg NO}_2^- \text{ L}^{-1}$  solution was prepared by weighing  $0.1500 \text{ g}$  of  $\text{NaNO}_2$  (Sodium nitrite, RCI-Labscan, Thailand) and dissolving it in  $1,000 \text{ mL}$  of deionized water ( $R=10^8 \Omega$ , DI water) in a volumetric flask. Using the same procedure,  $0.1371 \text{ g}$  of  $\text{NaNO}_3$  (Sodium nitrate, RCI-Labscan, Thailand) was weighed and dissolved in  $1,000 \text{ mL}$  of deionized water to prepare a  $100 \text{ mg NO}_3^- \text{ L}^{-1}$  solution. Also,  $41.7 \text{ mL}$  of concentrated HCl solution (Conc. Hydrochloric acid, HCl, RCI-Labscan, Thailand) was pipetted and diluted to  $1,000 \text{ mL}$  DI water to obtain a  $0.5 \text{ molL}^{-1}$  HCl solution. Griess reagent was prepared by weighing  $1.00\text{xx} \text{ g}$  of N-(1-Naphyl)-ethylenediamine (NED, Sigma-Aldrich, United States of America) and  $1.00\text{xx} \text{ g}$  of Sulfanilamide (Sigma-Aldrich, China) and then dissolving these in a  $0.5 \text{ molL}^{-1}$  HCl solution. To prepare Mixed Reducing and Griess reagent (MRG),  $1.00\text{xx} \text{ g}$  of NED, Sulfanilamide and  $\text{VCl}_3$  (Vanadium III chloride, Merck, France) were weighed and dissolved in a  $0.5 \text{ molL}^{-1}$  HCl solution.

### 2. Experiment

#### 2.1 The study of an optimal condition for construction of a calibration graph for $\text{NO}_3^-$ and $\text{NO}_2^-$

Firstly, the reaction of  $\text{NO}_2^-$  and Griess reagent was studied. The calibration graph of  $\text{NO}_2^-$  in range of  $0.22 \times 10^{-6}$  to  $1.09 \times 10^{-6} \text{ molL}^{-1}$  ( $10 - 50 \mu\text{g NO}_2^- \text{ L}^{-1}$ ) was constructed. Mixed Griess reagent with various concentrations of NED and Sulfanilamide were investigated for selecting an optimal condition. In the part of  $\text{NO}_3^-$  analysis, effect of reaction time for reducing  $\text{NO}_3^-$  to  $\text{NO}_2^-$  in range of  $0 - 60 \text{ min}$  with controlled  $0.25\% \text{ VCl}_3$  was studied. Mixed Reducing and Griess reagents (MRG) with  $0.25\%$ ,  $1\%$  and  $2\%$  (w/v)  $\text{VCl}_3$  were used for  $\text{NO}_3^-$  calibration graph construction, and an optimal  $\text{VCl}_3$  amount was then selected by consideration on the reducing efficiency in term of the slope. As mentioned above, the selected optimum condition was used to construct a calibration curve of mixed  $\text{NO}_2^- + \text{NO}_3^-$  standard solutions in ranges of  $80 \times 10^{-6}$  to  $400 \times 10^{-6} \text{ molL}^{-1}$  ( $5$  to  $25 \text{ mg NO}_3^- \text{ L}^{-1}$ ) and  $32.2 \times 10^{-6}$  to  $160 \times 10^{-6} \text{ molL}^{-1}$  ( $2$  to  $10 \text{ mg NO}_3^- \text{ L}^{-1}$ ), respectively.

Preliminary test of sample solution found that the amount of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were in the range of  $4.0 \times 10^{-6}$  to  $6.0 \times 10^{-6} \text{ molL}^{-1}$ . Thus, the working range used in this work was between  $2.2 \times 10^{-6}$  to  $10.8 \times 10^{-6} \text{ molL}^{-1}$  of the mixed standard solutions of  $\text{NO}_2^- + \text{NO}_3^-$  using Griess and MRG reagents for quantification of  $\text{NO}_2^-$  and  $\text{NO}_3^-$ , respectively.

#### 2.2 An investigation of an optimal condition for sampling and extraction of $\text{NO}_3^-$ and $\text{NO}_2^-$ in GSRs



This primary experiment started with a sampling procedure by collecting GSRs from inside a gun caliber at 3 cm depth. Various parameters such as sampling cotton brush replicates, sampling solvent temperature, sampling solvent volume and stored sample temperature were investigated by determination of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . The amount of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in GSRs collected using those parameters were evaluated to allow selection of an optimal condition for that sampling step before applying it to the next extraction procedure.

The extraction efficiency was then studied with the control parameters of pistol and bullet type, constant shooting distance, 150  $\mu\text{L}$  sampling solvent, duplicate cotton brush swab sampling ( $n=2$ ) and GSRs collected from 3 cm inside the gun caliber. Finally, extraction parameters such as extraction time, extraction volume, extraction temperature and ultrasonic extraction time were then studied to choose an appropriate condition for determination of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ .

### 2.3 Validation study of the proposed method

The experiment for calibration graph construction was conducted more than 30 times to investigate precision in terms of standard deviation (SD) and relative standard deviation (%RSD) of the slope and intercept (absorbance of blank solution, C). Inter-day and intra-day comparisons, limits of detection ( $\text{LOD}=3\text{SD of Blank/Slope}$ ) and limits of quantification ( $\text{LOQ} = 10\text{SD of Blank/Slope}$ ) were computed and reported. For the next step, the linearity range was studied. 5 sample solutions and added 10.48  $\mu\text{M}$  of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in the same set of 5 sample solutions were determined the amount of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  by using the proposed method. Percentage of recovery (%R) was then calculated using Equation 1.

$$\%R = \left[ \frac{(\text{Added standard sample})-(\text{sample})}{(\text{Added standard})} \right] \times 100 \quad \dots\dots\dots 1$$

Finally, collection of GSRs within a 3 cm radius of the target with various shooting distances was performed before applying the proposed sampling, extracting and determining methods for quantification of the amounts of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  at those shooting distances.

## 3. Results and discussion

### 3.1 The optimal condition for calibration curve construction of $\text{NO}_3^-$ and $\text{NO}_2^-$

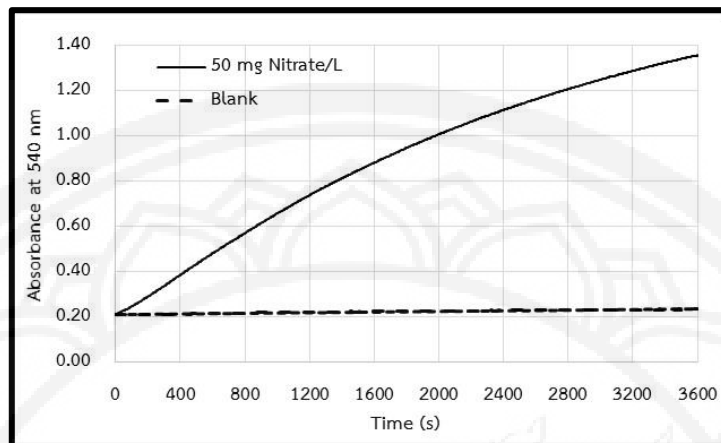
The results found that 1.00% (w/v) of both sulfanilamide and NED in 0.5  $\text{molL}^{-1}$  HCl (Griess reagent) was an optimal concentration for calibration curve construction of  $\text{NO}_2^-$ . In the case of mixed standard  $\text{NO}_3^- + \text{NO}_2^-$  solutions, an equal concentration of each solution in the range of 2.2 to 10.9  $\mu\text{M}$  were blended. 1.00 mL of each concentration was separately added to 3.00 mL of Griess reagent and mixed well before measurement of absorbance at 540 nm and construction of a calibration graph (Fig. 3B). The linear equation 2 was obtained, where the Y axis is absorbance at 540 nm, the  $X_1$  axis is  $\text{NO}_2^-$  concentration in  $\text{molL}^{-1}$ , m is the slope of the graph ( $\text{Abs.mol}^{-1}\text{L}$ ) and C is an intercept as follows.

$$Y = mX_1 + C \quad \dots\dots\dots 2$$

For  $\text{NO}_3^-$  analysis, the kinetics of the reducing reaction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  by  $\text{VCl}_3$  was first studied. The result was that the reaction rate increased with increasing reaction time as shown in Fig. 2. A 30 minute reaction time was selected with optimized sensitivity and reasonable analysis time. The effect of the amount of  $\text{VCl}_3$  on reduction efficiency was also investigated by reduction of  $\text{NO}_3^-$  concentration in the range of



$1.60 \times 10^{-4}$  to  $8.00 \times 10^{-4}$  molL<sup>-1</sup>. The obtained slopes were  $320.68 \pm 3.35$ ,  $705.35 \pm 11.80$  and  $716.13 \pm 8.48$  (Abs. mol<sup>-1</sup>L) when using 0.25%, 1% and 2% of VCl<sub>3</sub> in MRG reagent, respectively. However, to save chemicals with appropriate sensitivity, the MRG reagent was prepared from 1.00% (w/v) of sulfanilamide, NED and VCl<sub>3</sub> in 0.5 molL<sup>-1</sup> HCl, respectively.



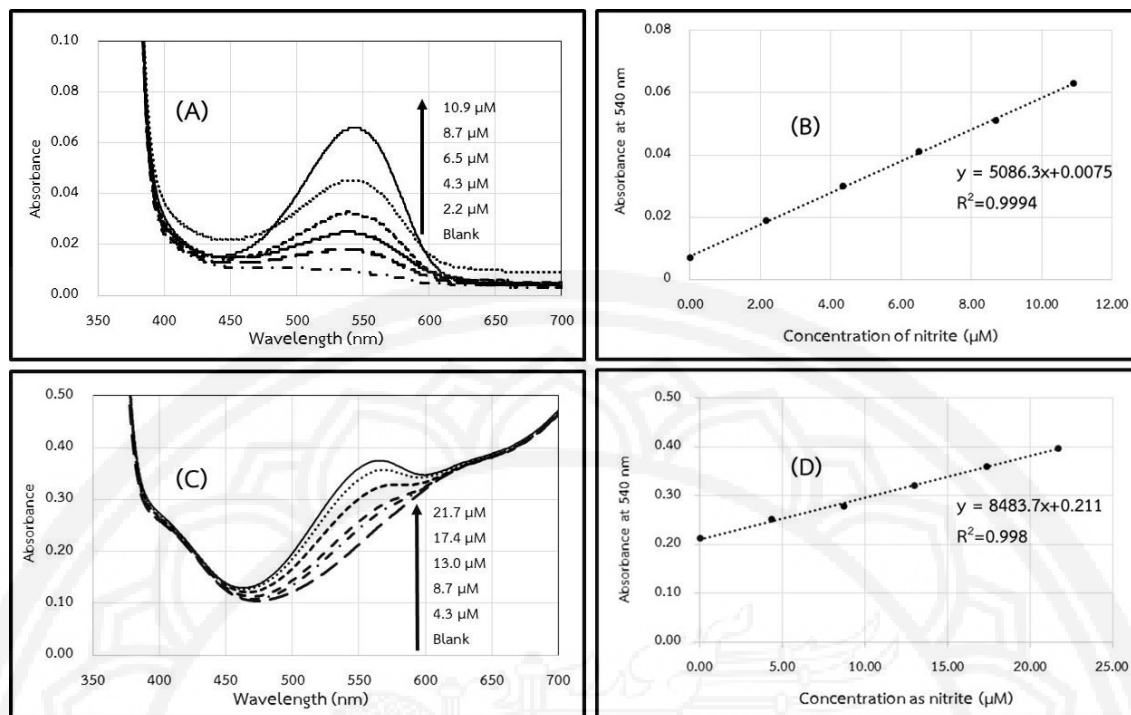
**Figure 2** Kinetic study of a reducing reaction of 50 mg NO<sub>3</sub><sup>-</sup> L<sup>-1</sup> and blank solution using 0.25% VCl<sub>3</sub> in MRG reagent

A mixed standard solution of NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> at the same concentration range of between  $2.2 \times 10^{-6}$  to  $10.9 \times 10^{-6}$  molL<sup>-1</sup> (NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> =  $4.3 \times 10^{-6}$  –  $21.7 \times 10^{-6}$  molL<sup>-1</sup>) was prepared and 1.00 mL of these solutions were separately added to 3.00 mL of MRG and mixed well with a 30 minute reaction time before measurement of absorbance at 540 nm. The obtained calibration curve and linear equation (Eq. 3) is shown in Fig. 3D. For the linear equation, X<sub>2</sub> and the Y axis represent NO<sub>2</sub><sup>-</sup> concentration in molL<sup>-1</sup> and absorbance at 540 nm, respectively while the slope (m) is the ratio of absorbance at 540 nm per concentration of NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> (molL<sup>-1</sup>) with an intercept C.

$$Y = mX_2 + C \quad \dots\dots 3$$

Determination of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> in GSRs was handled by measurement of absorbance at 540 nm of the reacted sample solution with Griess reagent and calculation the concentration of NO<sub>2</sub><sup>-</sup> in molL<sup>-1</sup> using Eq. 2, given as A molL<sup>-1</sup>. The same set of sample solutions was reacted with MRG reagent and also measured for absorbance at 540 nm before calculation of the concentration of NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> (as molNO<sub>2</sub><sup>-</sup> L<sup>-1</sup>) using Eq. 3, given as B molL<sup>-1</sup>. Therefore, the concentration of NO<sub>3</sub><sup>-</sup> in molL<sup>-1</sup> is equal to B-A. The spectrum and obtained calibration graph are shown in Figure 3.

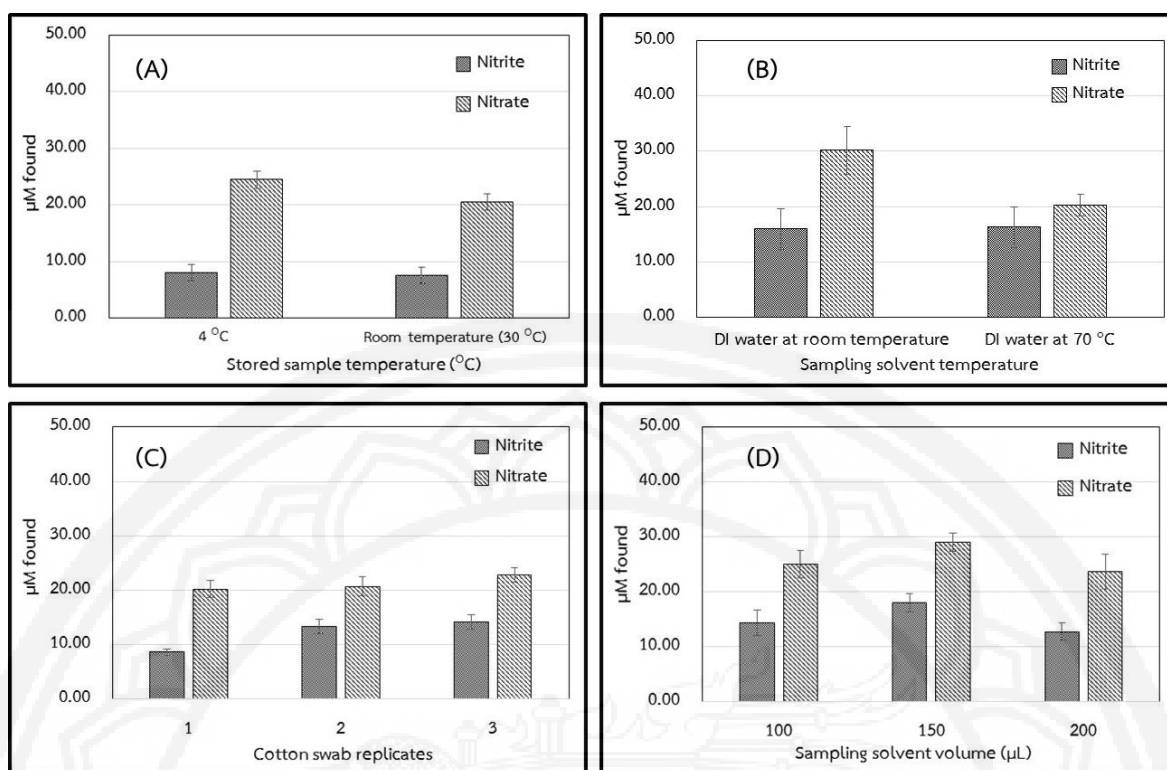
The result of the calibration curve construction 7 times (n=7) provided good performance in term of good linearity with  $R^2 = 0.99xx$  and high precision with  $\%RSD \leq 11.6\%$ . For inter-day experiments, the LOD and LOQ for determination of NO<sub>2</sub><sup>-</sup> were  $0.27 \times 10^{-6}$  and  $0.90 \times 10^{-6}$  molL<sup>-1</sup>, while LOD and LOQ for determination of NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> were  $3.58 \times 10^{-6}$  and  $11.93 \times 10^{-6}$  molL<sup>-1</sup>, respectively. With this performance, the developed method can be applied appropriately for determination of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> in GSRs. The linear equation obtained in the range of 2.17 to 217 μM (0.1 – 10 mgNO<sub>2</sub><sup>-</sup> L<sup>-1</sup>) were  $Y = 5724.1X_1 + 0.0043$ ,  $R^2 = 0.9998$  for NO<sub>2</sub><sup>-</sup> and  $Y = 7614.4X_2 + 0.2134$ ,  $R^2 = 0.9995$  for NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup>, respectively. The above result gave a very wide linearity range. Therefore, this may be possible to apply for determination of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> in other samples such as food and drinks.



**Figure 3** Spectrum and calibration curves obtained from mixed standard  $\text{NO}_2^- + \text{NO}_3^-$  by using Griess reagent (A and B) and MRG reagent (C and D)

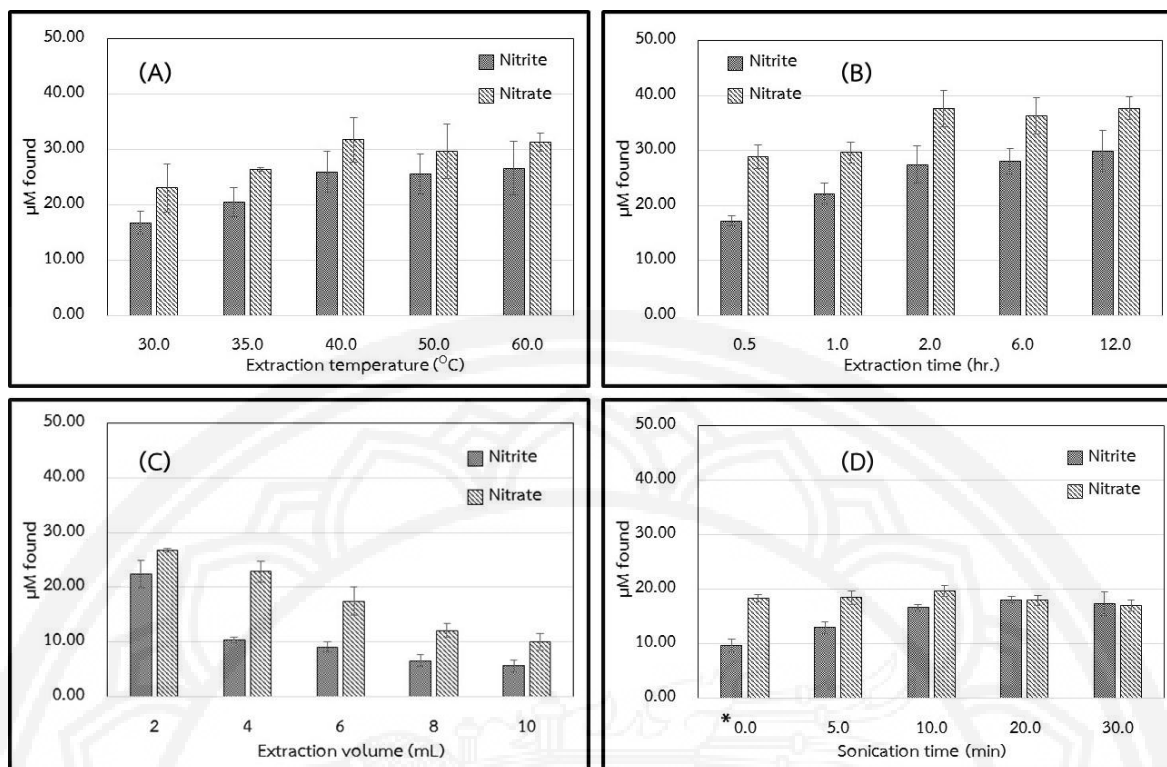
### 3.2 An optimal condition for sampling and extraction of $\text{NO}_3^-$ and $\text{NO}_2^-$ from GSRs

Figures 4 and 5 show the effect of various parameters on sampling and extraction efficiency for determination of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in GSRs. In Figure 4, it was found that storage of sample for 7 days in a refrigerator (at 4  $^{\circ}\text{C}$ ) and room temperature (RT) provided no significantly different result. It also found that indifferent results was also obtained when room temperature DI water and 70  $^{\circ}\text{C}$  was used for sampling. These results gave a flexible condition for analytical chemists. Due to the reason of sensitivity and analysis time, 150  $\mu\text{L}$  of sampling solvent volume and cotton swab duplicates were selected as an appropriate sampling condition.



**Figure 4** The amount of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  found in GSRs with various sampling parameters ( $n=5$ ), A = stored sample temperature, B = sampling solvent temperature, C = cotton swab replicates and D = sampling solvent volume

Consideration of Figure 5 A, B and D, found that increasing temperature, time and sonication time provided higher amounts of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  according to the theory because increasing temperature increases the solubility of both  $\text{NO}_2^-$  and  $\text{NO}_3^-$  into DI water. Similarly, increasing extraction time or sonication time also gave higher mass transfer rate of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  from GSRs to DI water solvent. However, in some laboratories, a 30 min extraction time without an ultrasonic cleaning bath might be performed as an alternative choice (Figure 5 D). As expected, increasing extraction solvent volume (Figure 5 C) decreased the amount found of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  according to the increased dilution factor at a higher solvent volume. For both sensitivity and sample consumption reasons, 4 mL of solvent volume was selected as an optimum condition which was enough for spectrophotometric determination of both  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in GSRs. However, 2 mL solvent volume might be chosen for application to other lower sensitivity methods. A summary of investigated parameters and selected condition are shown in Table 1.



**Figure 5** Extraction efficiency study of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in GSRs at various extraction parameters (n=3) A = Extraction temperature, B = Extraction time, C = Extraction volume and D = Ultrasonic extraction time (\*extraction by DI water for 30 min without ultrasonic sonication)

**Table 1** The results of sampling and extraction efficiency studies for determination of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  from GSRs

| Sampling parameters          | Studied range  | Selected condition                                    |
|------------------------------|--|---|
| Cotton swab replicates       | 1 – 3 cotton swabs replicates                          | duplicates  |
| Sampling solvent temperature | DI water at room temperature and 70 $^{\circ}\text{C}$ | DI water at room temperature (30 $^{\circ}\text{C}$ ) |
| Sampling solvent volume      | 100 – 200 $\mu\text{L}$                                | 150 $\mu\text{L}$                                     |
| Stored sample temperature    | Room temperature and 4 $^{\circ}\text{C}$              | Room temperature and 4 $^{\circ}\text{C}$             |
| Extraction parameters        | Studied range  | Selected condition                                    |
| Extraction time              | 0.5 – 12.0 hr.   | 0.5 hr.   |
| Extraction volume            | 2.0 – 10.0 mL  | 4.0 mL  |
| Extraction temperature       | 30.0 – 60.0 $^{\circ}\text{C}$                         | 30.0 $^{\circ}\text{C}$                               |
| Ultrasonic extraction time   | 5 – 30 min   | 5 min   |

### 3.3 Comparison of results between the proposed and other methods

The results of %R for determination of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in GSRs was studied by spiking both  $\text{NO}_2^-$  and  $\text{NO}_3^-$  at the same amount of 10.48  $\mu\text{M}$  into 5 GSR samples. %R as calculated by Eq. 1 were in the ranges of 96.24 to 102.32 and 98.04 to 105.96 for  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , respectively, which were acceptable results. A comparison of figure of merit between this proposed method and other methods can be considered using Table 2. In conclusion, the proposed method is simple and has a low detection limit. It also saves in terms of cost, time and energy for suitable determination of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in GSRs. The amount of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  found in GSRs collected from 3 cm around targets at various shooting distances is presented in figure 6. This demonstrates



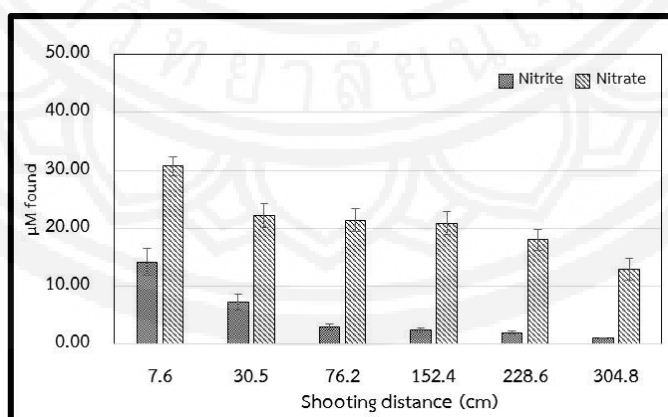


that the proposed method can determine  $\text{NO}_2^-$  and  $\text{NO}_3^-$  at maximum 304.8 cm shooting distance. From these findings, this provides a new alternative choice with high sensitivity and potential for determination of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in GSRs that is acceptable for application in the forensic field.

**Table 2** A comparison of merits of the proposed and other methods for determination of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in samples

| Determination method | Sample           | Extraction  | Reduction condition   | Limit of detection (LOD, $\mu\text{M}$ ) |                 | References  |
|----------------------|------------------|---|---|--|-----------------|---|
|                      |                  |   |   | $\text{NO}_2^-$                          | $\text{NO}_3^-$ |   |
| FIA spectrometry     | soil             | Shaking in 50 mL of 2 molL <sup>-1</sup> KCl for 90 min | Cd column   | 0.48                                     | 1.60            | Pasquali, Gallego-Pico, Hernando, Velasco & Alegria, 2010, p. 79                    |
| Micro plate reader   | Sea water        | -   | 0.8% $\text{VCl}_3$ in 1 molL <sup>-1</sup> HCl at 45 °C for 60 min                       | $6 \times 10^{-4}$                       | *0.002          | Schnetger & Lehnert, 2014, p. 91  |
| Spectrometry         | Environment      | -   | 2% $\text{VCl}_3$ in 6 molL <sup>-1</sup> HCl at 60 °C for 25 min                         |  | 0.05            | Garca-Robledo, Corzo & Papaspyrou, 2014, p. 30                                      |
| CE spectrometry      | Biological fluid | -   | -   | 41                                       | 26              | Miyado, Tanaka, Nagai, Takeda, Saito, Fukushi, Yoshida, Wakida & Niki, 2004, p. 185 |
| IEC                  | GSRs             | Ultrasonic extraction in DI water for 30 min            | -   | 0.46                                     | 0.16            | Gilchrist, Jongekrijg & Harvey, 2012, p. 50   |
| IEC                  | GSRs             | Organic solvent/water                                   | -   | 4.35                                     | 1.61            | Fernandez de la Ossa & Lopez-Lopez 2011, p. 1740                                    |
| IEC                  | GSRs             | Ultrasonic extraction in 2 mL DI water for 60 min       | -   | 4.8                                      | 8.46            | Srestheesombat & Shoosakulkriang., 2014, p. 64                                      |
| Spectrometry         | GSRs             | Ultrasonic extraction in 4 mL DI water for 5 min        | 1% $\text{VCl}_3$ in 0.5 molL <sup>-1</sup> HCl at RT and Ultrasonic extraction for 5 min | 0.27                                     | *3.58           | This work   |

**Note:** FIA = Flow injection analysis, CE = Capillary electrophoresis and IEC = Ion exchange chromatography, RT = room temperature and \* = LOD presented for quantification of  $\text{NO}_2^- + \text{NO}_3^-$



**Figure 6** Found amount of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in GSRs at various shooting distances (n=5)



### Summary and conclusion

The proposed sampling, extracting and determining method of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in GSRs provided a good sensitivity and a wide linearity range. This method is simple, convenient, cheap and requires a short analysis time. It was able to determine  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in GSRs collected around the target at maximum 304.8 cm shooting distance. Finally, it can be concluded that this method has great potential for application of GSR analysis in forensics field.

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