

Introduction of a spectrophotometric method for simultaneous determination of nitrite and nitrate in water samples using partial least squares

M. Shariati-Rad¹ · M. Irandoust¹ · M. Haghighi¹

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Abstract A simple and sensitive spectrophotometric method for simultaneous determination of nitrite and nitrate was introduced. The method is based on the reaction of nitrite and nitrate with 2,4-xylenol as reagent in the concentrated sulfuric acid. Calibration curves were linear in the range of 0.2–20.0 and 0.5–7.0 mg L⁻¹ for nitrite and nitrate, respectively. Due to the severe spectral overlapping of the reaction products of two anions with 2,4-xylenol, partial least squares (PLS) was used for simultaneous determination of nitrite and nitrate in the mixtures. For calibration and validation, 25 binary mixtures of nitrite and nitrate (17 calibration and eight external test samples) were designed by simplex lattice design with lattice degree of one. PLS models with Q^2 values of 0.988 and 0.976 were constructed for nitrite and nitrate, respectively. The validated models were used to analyze real water samples. With the recommended method, simultaneous determination of nitrite and nitrate in water samples is possible with satisfactory results.

Keywords Nitrite · Nitrate · 2,4-xylenol · Partial least squares · Water samples

Introduction

Excess concentration of nitrite and nitrate in drinking water is hazardous to health, especially for pregnant women (Kazemzadeh and Ensafi 2001). Main threats to health that

arise from the ingestion of these ions are reported as “blue baby” syndrome and gastric cancer (Swann 1975; Bruning-Fann and Kaneene 1993; Eaton et al. 1978; Cox and Frank 1982). These anions react with secondary amines in the acidic conditions of the stomach. The result is the production of carcinogenic nitrosamines which implicate in the pathology of gastric cancer (Swann 1975; Kuiper et al. 1994). In addition, they cause methemoglobinemia (oxygen deficiency) in infants (Bruning-Fann and Kaneene 1993). Passage of nitrite into the bloodstream results in the irreversible conversion of hemoglobin to methemoglobin which results in uptake in transportation of oxygen (Bruning-Fann and Kaneene 1993).

The current standards established by the US Environmental Protection Agency (EPA) for drinking water are 1 mg L⁻¹ for nitrite–nitrogen (NO₂⁻-N), 10 mg L⁻¹ for nitrate–nitrogen (NO₃⁻-N) and 10 mg L⁻¹ for the sum of NO₂⁻-N and NO₃⁻-N (Environmental Protection Agency 1991). The National Research Council (NRC) subcommittee on nitrate and nitrite in drinking water agreed that current drinking water standards of EPA for these anions are adequate to protect human health (National Research Council 1995).

Few techniques possess sufficient generic applicability to enable detection of nitrite and nitrate among the huge number of potential interferences that can be encountered in environmental, food, industrial and physiological samples (Moorcroft et al. 2001). Simultaneous determination of nitrite and nitrate is important in environmental chemistry, and numerous analytical methods have been presented for this purpose. Techniques used for simultaneous determination of nitrite and nitrate include electrochemical (Gross et al. 2015; Betta et al. 2014; Madasamy et al. 2014; Doherty et al. 1996; Davis et al. 2000), molecular absorption spectrometry (Brandao et al. 2014), flow injection analysis (Feng et al. 2013) and capillary

✉ M. Shariati-Rad
mohmasrad@gmail.com

¹ Department of Analytical Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran

electrophoresis (Fukushi et al. 2000; Jimidar et al. 1995; Alonso and Prego 2000; Bories et al. 1999) methods, whereby the analytes are detected independently in a single measurement.

Still, the most frequently employed methodology for the determination of nitrite and nitrate is the Griess reaction (Greiss 1879; Abbas and Mostafa 2000; Kiso et al. 2006; Pasquali et al. 2007, 2010; Nagaraja et al. 2010). However, for determination of nitrate, a reduction step is needed to reduce nitrate to nitrite. This conversion is accomplished by using a reduction step which is especially preferred when using injection methodologies (FIA and SIA), because it provides quantitative reduction within a short reaction time (Garcia-Robledo et al. 2014; Pasquali et al. 2010; Legnerova et al. 2002; Cerda et al. 1998; Karlicek et al. 1988). The total nitrite content (initial nitrite plus nitrite produced by reduction of nitrate) is then determined spectrophotometrically by Griess method at a single wavelength.

Simultaneous determination of two or more compounds in the same mixture without preliminary separation is the main problem of spectrophotometric multicomponent analysis. Frequently, the multivariate calibration methods like partial least squares (PLS) (Martens and Naes 1989; Geladi and Kowalski 1986) have been applied to mixtures without resorting to time-consuming chemical separation like chromatography. PLS has been successfully used for the simultaneous determination of pollutants in water and environmental samples (Gholivand et al. 2012; An et al. 2010; Gao et al. 2011; Sarnacchiaro et al. 2012).

In this paper, a UV–Vis spectrophotometric method is proposed for simultaneous determination of nitrite and nitrate in water samples using PLS multivariate calibration. The method is based on the reaction of nitrite and nitrate with 2,4-xylenol as reagent in acidic conditions.

Theory

Partial least squares (PLS)

In multivariate calibration, a linear model is built between the measured instrumental signals included in matrix \mathbf{X} and the property we are seeking it included in vector \mathbf{y} (Martens and Naes 1989; Geladi and Kowalski 1986). In PLS, instead of the original variables, new latent variables are used which are the result of the compression and abstracting of them. In matrix notation, the linear model can be written as $\mathbf{y} = \mathbf{X}\mathbf{b}$ where \mathbf{b} contains the regression coefficients which are determined during the calibration step.

Performances of the PLS models can be expressed in terms of root mean square error of cross-validation

(RMSECV), root mean square error of prediction (RMSEP) and cross-validated correlation coefficient (Q^2) (Vandeginste et al. 1998):

$$\text{RMSECV} = \sqrt{\frac{\sum_{i=1}^m (c_i - \hat{c}_i)^2}{m}} \quad (1)$$

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^n (c_i - \hat{c}_i)^2}{n}} \quad (2)$$

$$Q^2 = 1 - \frac{\sum_{i=1}^n (c_i - \hat{c}_i)^2}{\sum_{i=1}^n (c_i - c_{\text{mean}})^2} \quad (3)$$

where c_i , \hat{c}_i and c_{mean} are the real and predicted concentration of the component, and mean of the real concentrations, respectively. In Eq. (1), c_i and \hat{c}_i are concentrations in calibration set, and c_i , \hat{c}_i and c_{mean} in Eqs. (2) and (3) are concentrations in validation set. m and n are the number of samples in the calibration and validation set, respectively.

Materials and methods

Apparatus and software

An Agilent spectrophotometer model 8453 with diode array detector was used for recording spectra in a 1-cm path-length quartz cell. All spectra were recorded against the reagent blank. PLS multivariate calibrations were performed in MATLAB 6.5 environment (MATLAB 6.5, The Mathworks Inc., Natick).

Reagents and solutions

All chemicals were of analytical reagent grade. Sodium nitrite, sodium nitrate, 2,4-xylenol and sulfuric acid (98 % (w/v)) were supplied by Merck (Darmstadt, Germany).

Standard solutions

Stock 1000.0 mg L⁻¹ solutions of sodium nitrite and sodium nitrate were prepared in doubly distilled water. Stock 8.20 × 10⁻³ mol L⁻¹ solution of 2,4-xylenol was prepared and used as reagent.

Recommended procedure

An aliquot of the stock solution of 2,4-xylenol (30.54 μL, 8.20 × 10⁻³ mol L⁻¹) was added to the quartz cell (1-cm

path-length) containing 2.0 mL concentrated sulfuric acid (98 %w/v). Then, a given volume of nitrite or nitrate (1000.0 mg L^{-1}) was added, and the resulting mixture was diluted to 2.5 mL by doubly distilled water. The mixture was gently mixed, and the absorbances of products were measured at 245 and 255 nm for nitrite and nitrate, respectively against the reagent blank.

Synthetic binary mixtures of nitrite and nitrate for PLS multivariate calibration

In order to perform PLS modeling, 25 synthetic binary mixtures of nitrite and nitrate were designed and prepared. The mixtures were designed based on the simplex lattice design with lattice degree of one. Dividing the samples into 17 calibration and eight validation samples was carried out by Kennard–Stone algorithm (Kennard and Stone 1969). The compositions of the 25 binary mixtures are included in Table 1.

Sample preparation for determination of nitrite and nitrate in water and wastewater samples

Drinking water samples were collected without adding any preservative in polyethylene bottles and analyzed within 6 h. Water samples were filtered through a Whatman No. 41 filter paper. An appropriate volume of the filtrate

Table 1 Mixtures designed for calibration and prediction of nitrite and nitrate by PLS

Calibration set samples		Prediction set samples	
NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})	NO_2^- (mg L^{-1})	NO_3^- (mg L^{-1})
S1	6.00	0.00	S1 3.00 3.00
S2	1.00	3.00	S2 2.00 2.00
S3	0.00	4.00	S3 0.75 2.25
S4	0.00	3.00	S4 5.00 0.00
S5	1.75	5.25	S5 1.50 4.50
S6	1.25	3.75	S6 4.50 1.50
S7	7.00	0.00	S7 3.00 0.00
S8	3.50	3.50	S8 0.00 5.00
S9	2.50	2.50	
S10	2.25	0.75	
S11	5.25	1.75	
S12	0.00	6.00	
S13	3.00	1.00	
S14	4.00	0.00	
S15	0.00	7.00	
S16	3.75	1.25	
S17	1.50	1.50	

without dilution was transferred to the 1-cm path-length quartz cell containing 2.0 mL concentrated sulfuric acid and 30.54 μL of 2,4-xyleneol stock solution.

Results and discussion

Optimization

It was observed that in the reaction of nitrite and nitrate with 2,4-xyleneol, the absorbance of the reaction product increases with the volume of the concentrated sulfuric acid, especially for nitrite. Only in the presence of large volumes of the concentrated sulfuric acid, nitrate produced detectable absorbances. Variation of the absorbance of the products against volume of the concentrated sulfuric acid and concentration of 2,4-xyleneol is shown in Fig. 1a. Based on the figure, volume of 2 mL was selected as the optimal volume of the concentrated sulfuric acid for simultaneous determination of nitrite and nitrate.

Effect of the concentration of 2,4-xyleneol on the absorbance of the reaction product was also investigated. For both analytes, in the presence of 2,4-xyleneol with

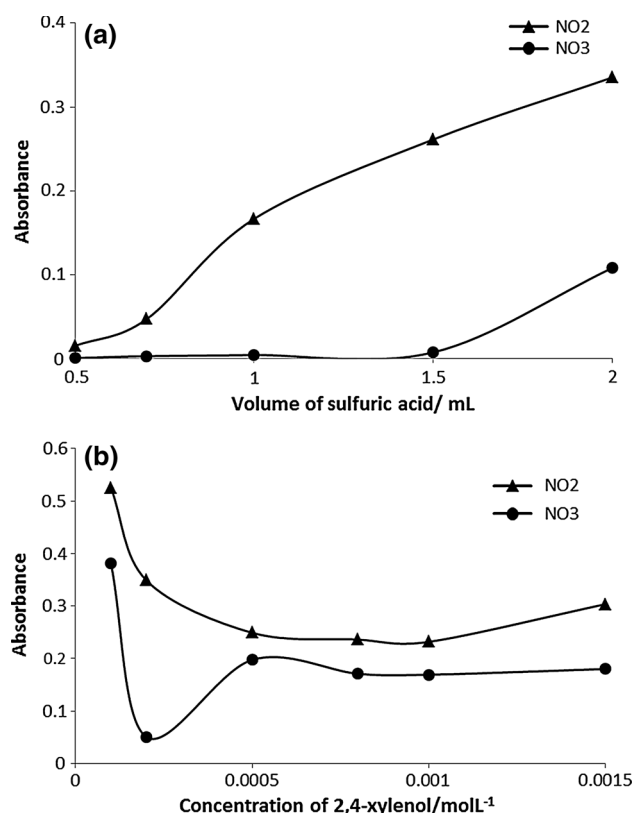


Fig. 1 Variation of the response of the reaction between 2,4-xyleneol and nitrate and nitrite (2 mg L^{-1}) with **a** volume of the concentrated sulfuric acid and **b** concentration of 2,4-xyleneol

concentration of 1.0×10^{-4} mol L⁻¹ (Fig. 1b), the maximum intensity of absorbance was observed.

Absorption spectra

The electronic absorption spectra of the products of the reaction of nitrite and nitrate with 2,4-xyleneol are shown in Fig. 2. As can be seen in Fig. 2, both spectra are broad and highly overlapped. The main absorption band of the two products is located at about 250 nm. This band is more intense for reaction product of nitrite. The other bands which are less intense have been located at 525 and 360 nm for nitrite and nitrate, respectively.

The strong overlapping of the spectra of the reaction products needs separation or analysis by multivariate calibration methods like PLS.

Univariate calibration

In order to find the dynamic linear range of calibration for each compound, the absorbance of a series of solutions containing varying amounts of nitrite or nitrate in optimal conditions was recorded at 245 and 255 nm for nitrite and nitrate, respectively. The univariate calibration curves were linear in the range of 0.2–20.0 mg L⁻¹ for nitrite and 0.5–7.0 mg L⁻¹ for nitrate.

In the previous studies performed on the reaction of nitrite with xyleneol derivatives (2,6-xyleneol), a dynamic linear range of 0–10 mg L⁻¹ was reported (Hartley and Bly 1963; Hartley and Asai 1963). Spectrophotometric determination of nitrite based on the Griess reaction has characteristics which may be superior over the xyleneol method. However, because of the simultaneous reaction of nitrite and nitrate with xyleneol derivatives (in the present work, 2,4-xyleneol), this reaction is preferred for simultaneous spectrophotometric determination of these species using multivariate calibration.

Interference study

The effect of potential interferences in the determination of 2.0 mg L⁻¹ of nitrite and nitrate by the developed method was studied. The results are given in Table 2. As can be seen, in most cases interference occurs in high concentration of the examined interferent. The most serious interferent is Fe³⁺. The interference of cations specially Fe³⁺ can be attributed to the complexation of the cations with the reagent (2,4-xyleneol).

Determination of nitrite and nitrate in synthetic binary mixtures using PLS multivariate calibration

The spectra of the standard mixtures collected in Table 1 were recorded in the wavelength range of 220–600 nm

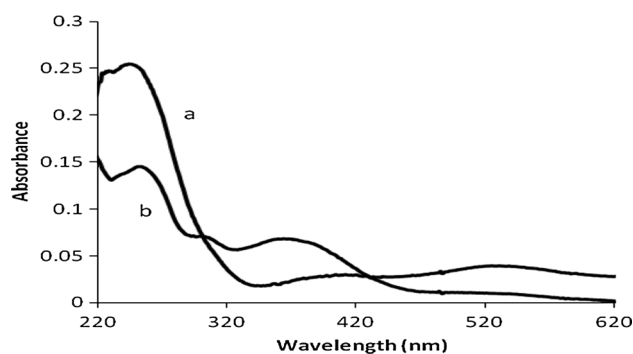


Fig. 2 Spectrum of the reaction product of **a** nitrite (2.0 mg L⁻¹) and **b** nitrate (2.0 mg L⁻¹) with 2,4-xyleneol (1.0×10^{-4} mol L⁻¹) in the presence of 2.0 mL of the concentrated sulfuric acid

Table 2 Tolerance limit of different ions in determination of nitrite and nitrate with concentrations of 2.0 mg L⁻¹

Interferent	Added as	Tolerance limit (mg L ⁻¹)	
		Nitrite	Nitrate
Fe ³⁺	FeCl ₃	5.0	7.0
Ca ²⁺	CaCl ₂	60.0	7.0
Mg ²⁺	MgCl ₂	8.0	70.0
NH ₄ ⁺	NH ₄ Cl	7.0	20.0
F ⁻	NaF	20.0	25.0
SO ₄ ²⁻	Na ₂ SO ₄	15.0	20.0
Cl ⁻	NaCl	17.0	26.0

Table 3 Statistical parameters of the PLS models

Parameters	PLS		PCR	
	Nitrite	Nitrate	Nitrite	Nitrate
Number of latent variables	3	4	2	7
RMSEP	0.086	0.080	0.147	0.143
RMSECV	0.134	0.124	0.346	0.315
Q ²	0.988	0.976	0.888	0.768
SEL ^a	0.007	0.006		
SEN ^a	0.638	0.168		
LOD ^a	0.136	0.273		

^a Parameters calculated based on the net analyte signal (NAS) concept

with 1-nm intervals. In multivariate analysis, the wavelength ranges of 220–300 and 220–600 nm were used for determination of nitrite and nitrate, respectively.

Models were internally validated employing the well-known leave-one-out cross-validation, and the number of latent variables for prediction was chosen in agreement with the Haaland–Thomas criterion (Haaland and Thomas 1988). For comparison, the multivariate data were also analyzed by principal component regression (PCR). The statistical parameters including the number of latent

Table 4 Results of the application of the recommended method for simultaneous determination of nitrite and nitrate in water samples

	NO ₂ ⁻				Griess method	NO ₃ ⁻			
	Added (mg L ⁻¹)	Found (mg L ⁻¹)	Recovery	RSD %		Added (mg L ⁻¹)	Found ^a (mg L ⁻¹)	Recovery	RSD %
Water 1	0.00	N. D. ^b	–	–	N. D.	0.00	18.10	–	4.51
	2.00	1.96	98.0	4.45	–	2.00	20.08	99.2	0.94
Water 2	0.00	N. D.	–	–	N. D.	0.00	18.30	–	7.47
	2.00	1.96	98.1	7.81	–	2.00	20.26	98.5	1.50

^a Obtained concentrations have been corrected for dilution

^b Not detected

variables, RMSECV, RMSEP and Q^2 are collected in Table 3. As can be seen, all of the statistical parameters resulted by PLS modeling are superior over the counterparts of PCR. High Q^2 values and low RMSECV and RMSEP values obtained by PLS modeling indicate that this multivariate calibration and the analytical method are reliable. The constructed PLS models have high predictivities (high Q^2 values).

In Table 3, some of the parameters were calculated based on the net analyte signal (NAS) concept (Booksh and Kowalski 1994; Al-Degs et al. 2012). These are multivariate figure of merits. The multivariate limit of detection (LOD) can be calculated by

$$LOD = \Delta(\alpha, \beta)\delta r \quad (4)$$

where $\Delta(\alpha, \beta)$ is the non-centrality parameter which can be extracted from the statistical tables and δr is the standard deviation of the measurements. Selectivity (SEL) for k th component can be calculated as

$$SEN = \frac{S_k^*}{\|S_k\|} \quad (5)$$

And its sensitivity (SEN) is

$$SEN = \frac{1}{\|b\|} \quad (6)$$

In the above equations, S_k , S_k^* and b are pure spectrum of the k th component, its NAS and the PLS regression coefficients, respectively.

Application of the method

In order to examine the suitability of the recommended method for simultaneous determination of nitrite and nitrate in the real samples, it was used to analyze drinking water samples. The results are collected in Table 4. Data in Table 4 show that the method is accurate since percent recoveries are between 98.0 and 100.0 %. For assessment of the proposed method, we used Griess method (Greiss 1879, Irandoust et al. 2013) as a standard method for determination

of nitrite. The results of the recommended method conform well to the Griess standard method. Precision of the method is satisfactory. In most cases, RSD % values are below 5 %.

Conclusion

A simple spectrophotometric method was introduced for simultaneous determination of nitrite and nitrate. Partial least squares multivariate calibration was used for resolution of mixtures of the analytes. The method can successfully be used for determination of nitrite and nitrate in water samples. The recommended method can be considered as an alternative to the expensive methods like ion chromatography for simultaneous determination of nitrite and nitrate. Moreover, in the analyzed drinking water samples, the level of the more hazardous nitrite located below the thresholds established by the relating standard agencies.

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References

- Abbas MN, Mostafa GA (2000) Determination of traces of nitrite and nitrate in water by solid phase spectrophotometry. *Anal Chim Acta* 410:185–192
- Al-Degs YS, El-Sheikh AH, Issa AA, Al-Ghouti MA, Sunjuk M (2012) A simple and accurate analytical method for determination of three commercial dyes in different water systems using partial least squares regression. *Water Sci Technol* 66:1647–1655
- Alonso MCB, Prego R (2000) Determination of silicate, simultaneously with other nutrients (nitrite, nitrate and phosphate), in river waters by capillary electrophoresis. *Anal Chim Acta* 416:21–27
- An L, Deng J, Zhou L, Li H, Chen F, Wang H, Liu Y (2010) Simultaneous spectrophotometric determination of trace amount of malachite green and crystal violet in water after cloud point extraction using partial least squares regression. *J Hazard Mater* 175:883–888
- Betta FD, Vitali L, Fett R, Oliveira Costa AC (2014) Development and validation of a sub-minute capillary zone electrophoresis



- method for determination of nitrate and nitrite in baby foods. *Talanta* 122:23–29
- Booksh KS, Kowalski BR (1994) Theory of analytical chemistry. *Anal Chem* 66:782A–791A
- Bories PN, Scherman E, Dziedzic L (1999) Analysis of nitrite and nitrate in biological fluids by capillary electrophoresis. *Clin Biochem* 32:9–14
- Brandao GC, Matos GD, Pereira RN, Ferreira SLC (2014) Development of a simple method for the determination of nitrite and nitrate in groundwater by high-resolution continuum source electrothermal molecular absorption spectrometry. *Anal Chim Acta* 806:101–106
- Bruning-Fann CS, Kaneene JB (1993) The effects of nitrate, nitrite and N-nitroso compounds on human health: a review. *Vet Human Toxicol* 35:521–538
- Cerda A, Oms MT, Forteza R, Cerda V (1998) Sequential injection sandwich technique for the simultaneous determination of nitrate and nitrite. *Anal Chim Acta* 371:63–71
- Cox RD, Frank CW (1982) Determination of nitrate and nitrite in blood and urine by chemiluminescence. *J Anal Toxicol* 6:148–152
- Davis J, Moorcroft MJ, Wilkins SJ, Compton RG, Cardoso MF (2000) Electrochemical detection of nitrate and nitrite at a copper modified electrode. *Analyst* 125:737–742
- Doherty AP, Stanley MA, Leech D, Vos JG (1996) Oxidative detection of nitrite at an electrocatalytic [Ru(bipy)₂poly-(4-vinylpyridine)₁₀Cl]Cl electrochemical sensor applied for flow injection determination of nitrate using a Cu/Cd redox column. *Anal Chim Acta* 319:111–120
- Eaton AD, Clescheri LS, Greenberg AE (1978) Standard methods for the examination of water and wastewater, 16th edn. American Public Health Association, New York
- EPA (Environmental Protection Agency) (1991) National primary drinking water regulations, final rule. *Fed Reg* 56:3526–3597
- Feng S, Zhang M, Huang YM, Yuan DX, Zhu Y (2013) Simultaneous determination of nanomolar nitrite and nitrate in sea water using reverse flow injection analysis coupled with along path length liquid waveguide capillary cell. *Talanta* 117:456–462
- Fukushi K, Ishio N, Sumida M, Takeda S, Wakida S, Hiroyuki K (2000) Improvement of capillary zone electrophoresis sensitivity with artificial seawater as the background electrolyte utilizing transient isotachopheresis for the determination of nitrite and nitrate ions in seawater. *Electrophoresis* 21:2866–2871
- Gao J-F, Wang J-H, Yang C, Wang S-Y, Peng Y-Z (2011) Binary biosorption of Acid Red 14 and Reactive Red 15 onto acid treated okara: simultaneous spectrophotometric determination of two dyes using partial least squares regression. *Chem Eng J* 171:967–975
- Garcia-Robledo E, Corzo A, Pappaspyrou S (2014) A fast and direct spectrophotometric method for sequential determination of nitrate and nitrite at low concentrations in small volumes. *Marine Chem* 162:30–36
- Geladi P, Kowalski BR (1986) Partial least-squares regression: a tutorial. *Anal Chim Acta* 185:1–17
- Gholivand MB, Shariati-Rad M, Karimian N, Torkashvand M (2012) A chemometrics approach for simultaneous determination of cyanazine and propazine based on a carbon paste electrode modified by a molecularly imprinted polymer. *Analyst* 137:1190–1198
- Greiss JP (1879) Ueber einige Azoverbindungen. *Ber Dtsch Chem Ges* 12:426–428
- Gross AJ, Holmes S, Dale SEC, Smallwood MJ, Green SJ, Winlove CP, Benjamin N, Winyard PG, Marken F (2015) Nitrite/nitrate detection in serum based on dual-plate generator-collector currents in a microtrench. *Talanta* 131:228–235
- Haaland DM, Thomas EV (1988) Partial least-squares methods for spectral analyses. I. Relation to other quantitative calibration methods and the extraction of qualitative information. *Anal Chem* 60:1193–1202
- Hartley AM, Asai RI (1963) Spectrophotometric determination of nitrite as 4-nitroso-2,6-xyleneol. *Anal Chem* 35:1215–1218
- Hartley AM, Bly RM (1963) Polarographic determination of nitrite as 4-nitroso-2,6-xyleneol. *Anal Chem* 35:2094–2100
- Iranidoust M, Shariati-Rad M, Haghghi M (2013) Nitrite determination in water samples based on a modified Griess reaction and central composite design. *Anal. Methods* 5:5977–5982
- Jimidar M, Hartmann C, Cousement N, Massart DL (1995) Determination of nitrate and nitrite in vegetables by capillary electrophoresis with indirect detection. *J Chromatogr A* 706:479–492
- Karlicek R, Dolejsova J, Polasek M (1988) Simultaneous determination of nitrites and nitrates using the flow injection analysis. *Agrochimica* 28:119–122
- Kazemzadeh A, Ensafi AA (2001) Sequential flow injection spectrophotometric determination of nitrite and nitrate in various samples. *Anal Chim Acta* 442:319–326
- Kennard RW, Stone LA (1969) Computer aided design of experiments. *Technometrics* 11:137–148
- Kiso Y, Jung Y, Kuzawa K, Seko Y, Saito Y, Yamada T, Nagai M (2006) Visual determination of nitrite and nitrate in waters by color band formation method. *Chemosphere* 64:1949–1954
- Kuiper MA, Visser JJ, Bergmans PL, Scheltens P, Wolters EC (1994) Decreased cerebrospinal fluid nitrate level in Parkinson's disease, Alzheimer's disease and multiple system atrophy. *J Neurol Sci* 121:46–49
- Legnerova Z, Solich P, Sklenarova H, Satinsky D, Karlicek R (2002) Automated simultaneous monitoring of nitrate and nitrite in surface water by sequential injection analysis. *Water Res* 36:2777–2783
- Madasamy T, Pandiaraj M, Balamurugan M, Bhargava K, Kumar Sathy N, Karunakaran C (2014) Copper, zinc superoxide dismutase and nitrate reductase coimmobilized bienzymatic biosensor for the simultaneous determination of nitrite and nitrate. *Biosens Bioelectron* 52:209–215
- Martens H, Naes T (1989) *Multivariate calibration*. Wiley, Chichester
- Moorcroft MJ, Davis J, Compton RG (2001) Detection and determination of nitrate and nitrite: a review. *Talanta* 54:785–803
- Nagaraja P, Al-Tayar N, Shivakumar A, Shrestha A, Gowda A (2010) A simple and sensitive spectrophotometric method for the determination of trace amounts of nitrite in environmental and biological samples using 4-amino-5-hydroxynaphthalene-2,7-disulphonic acid monosodium salt. *Spectrochim. Acta A* 75:1411–1416
- NRC (National Research Council), (1995) *Nitrate and nitrite in drinking water*. National Academy Press, Washington, DC, Subcommittee on Nitrate and Nitrite in Drinking Water
- Pasquali CEL, Hernando PF, Alegria JSD (2007) Spectrophotometric simultaneous determination of nitrite, nitrate and ammonium in soils by flow injection analysis. *Anal Chim Acta* 600:177–182
- Pasquali CEL, Gallego-Pico A, Hernando PF, Velasco M, Alegria JSD (2010) Two rapid and sensitive automated methods for the determination of nitrite and nitrate in soil samples. *Microchem J* 94:79–82
- Sarnacchiaro P, Diez S, Montouri P (2012) Polycyclic aromatic hydrocarbons pollution in a coastal environment: the statistical analysis of dependence to estimate the source of pollution. *Current Anal Chem* 8:300–309
- Swann PF (1975) The toxicology of nitrate, nitrite and *n*-nitroso compounds. *J Sci Food Agric* 26:1761–1770
- Vandeginste BGM, Massart DL, Buydens LMC, de Jong S, Lewi PJ, Smeyers-Verbeke J (1998) *Handbook of chemometrics and quality metrics: part B*. Elsevier, Amsterdam