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Novel Spectrophotometric Method with Enhanced Sensitivity for the Determination of Nitrite in Vegetables

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Abstract

The aim of the present work is to develop a novel, sensitive, fast, and cost-effective spectrophotometric method for the detection and quantitation of nitrite ions in vegetables. The method is based on the diazotization-coupling reaction of nitrite with solid sulfanilic acid and N-(1-naphthyl) ethylenediamine dihydrochloride in acidic medium. The final product is an azo dye with a maximum absorption at 542 nm. Variables such as amounts of solid diazo-coupling reagents, pH, and mass of charcoal, are optimized. The method detection and quantitation limits are respectively 0.010 mg L^{-1} and 0.030 mg L^{-1}. The precession of the method reported as %RSD is below 5%, and the linear range is extended between 0.05 and 1 mg L^{-1} with R^2 reaching 0.9999. The performance criteria of the proposed method under the most optimal conditions are validated and found to be superior compared to the performance of two selected reference methods and also to the results of the same method when diazo-coupling reagents are used as solutions. The proposed and validated method is used to quantitate nitrite in four leafy and root vegetables marketed in Yemeni. The method's recovery in the four tested vegetables is between 98.00% and 102.00% which is an indication of the high extraction efficiency and sensitivity of the developed method.

Keywords: Nitrite, Sulfanilic acid, N-(1- naphthyl) ethylenediamine dihydro chloride, Vegetables, Spectrophotometry.

1. Introduction

Nitrite is one of the reactive species of nitrogen that has received increased attention in the recent years due to possible adverse and beneficial effects associated with it. Nitrite has been linked to the formation of N- nitrosamines in acidic media including the stomach via its reactions with secondary amines and amides (Santamaria, 2006); (Sindelar and Milkowski, 2012). The lower molecular weight N-nitrosamines have been unequivocally proven to be carcinogenic to humans and animals (Straif et al., 2000; Preussmann, 1983); Bryan et al., 2012); (Bogovski and Bogovski, 1981). Thus, nitrite is considered as a precursor of N-nitrosamine formation.

Despite the above established knowledge, the direct link between nitrite in the diet and gastric cancer, however, has not been proven conclusively as concluded by three recent review and meta-analysis articles (Bryan et al., 2012; Parvizishad et al., 2017; Song et al., 2015). The association of nitrite with methaemoglobinaemia is also questionable (Merino, 2009a). Studies on the curative effect of nitrite to humans have been on the rise. Recent review articles concluded that the dietary intake of nitrite proved to be an alternative pathway for the formation of nitric oxide (NO) which is a potent cardiovascular relaxant and vasodilator (Machha and Schechter, 2011); Jackson et al., 2018; Lundberg and Weitzberg, 2009). Nitrite may also serve as a plasma biomarker for NO bioavailability (Lundberg and Weitzberg, 2009; Bryan, 2006).

Despite the positive shift in the appreciation of the role of nitrite, the daily consumption of nitrite is still regulated in many countries. The proposed acceptable daily intake (ADI) of nitrite is estimated to be 0.06 mg kg^{-1} bodyweight which is translated to be 3.6 mg day^{-1} for 60 kg person (SCF, 1995).

The ubiquitous nature of nitrite and the complexity of the matrices present a challenge for scientists to develop an adequate method for the detection and quantitation of nitrite. A variety of analytical methods have been developed and applied for the determination of nitrite in food, water, vegetables, biological fluids, and other matrices. These methods include spectrophotometry (Moorcroft et al., 2001; Ozcan and Akbulut, 2007), chromatography (Gennaro and Bertolo, 1989; Cheng and Tasng, 1998; Bosch et al., 1995; Sui and Henshall, 1998), polarographic method (Ximenes et al., 2000), flow injection analysis (Ensafi and Kazemzadeh, 1999; Kazemzadeh and Ensafi, 2001), and capillary electrophoresis (Jimidar et al., 1995; Gaspar et al., 2005). Two review articles (Moorcroft et al., 2001; Wang et al., 2017), have summarized scientific endeavors to assess the level of nitrite ions in

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various matrices. Generally speaking, each method has its limitations and advantages. Spectrophotometric based-methods that are classified into three categories: Griess Assay methods, nitrosation procedures and catalytic methods are by far the most popular approaches for the determination of nitrite due to their simplicity and feasibility (Wang et al., 2017). Even though Griess Assay has been used extensively for the detection of nitrite with dozens of color producing reagents (diazo-coupling agents) available for the reaction with nitrite, this approach suffers from interferences and low sensitivity. The performance of spectrophotometric methods in general is compromised by the addition of access reagents that causes unnecessary dilution. Thus, the present work aims at developing and validating a spectrophotometric method based on the use of a new combination of diazotizing and coupling agents; sulfanilic acid and N-(1-naphthyl) ethylenediaminedihydrochloride (NEDD) in solid forms to determine nitrite ions in vegetables. In this study, this method is applied for nitrite quantitation in four leafy and root vegetables (mint, coriander, white radish, and carrots). This approach and to the best of the authors' knowledge has not been reported previously.

2. Methodology

2.1. Chemicals and Reagents

All chemicals and reagents used in the present work were of analytical grade. The charcoal, hydrochloric acid 37%, sodium nitrite, sodium hydroxide (99.9%), sulfanilamide (99%) and sulfanilic acid (99%) were from BDH. The N-(1-naphthyl) ethylenediamine. 2HCl (99%) and Methyl anthranilate (99%) were purchased from Merck and Himedia respectively. De-ionized water (with specific conductance of 0.05 µS cm^{-1}) was produced in-house, and was used for the preparation of all solutions.

2.2. Instruments

Spectrophotometer (UV/Visible) model Spectroscan 60DV, Biotech Engineering, was used for all absorbance measurements. Deionized water was prepared in-house using DirectQ3 (Millipore-USA). Electronic balance, model no: E51120-4, Biotech Engineering and pH meter, Biotech Engineering were also used.

2.3. Preparation of Nitrite Standards and Reagents Solutions

2.3.1. Nitrite Stock Standard Solution

The stock standard solution of nitrite (400 mg L^{-1}) was prepared by placing 0.15 g of sodium nitrite in a 250 mL volumetric flask and de-ionized water was then added to the mark. The stock solutions were kept in a refrigerator at 4 °C. All other working standard solutions were then made fresh from the 400 mg L^{-1} stock solution by making the appropriate dilution using de-ionized water.

2.3.2. Hydrochloric Acid and Sodium Hydroxide

HCl solution was prepared by diluting an appropriate volume of the concentrated HCl reagent in de-ionized water to make 0.1 M. The 2N NaOH solution was prepared by weighing an appropriate amount of the solid pellets in de-ionized water to make a final concentration of 2N.

2.3.3. Preparation of the Color Producing Reagents (diazo-coupling reagents)

Sulfanilic acid (SA), sulfanilamide, and NEDD were prepared by weighing 0.6 g, 1 g and 0.6 g respectively into three separate volumetric flasks. De-ionized water was added to the flasks. Methyl anthranilate was prepared by diluting 0.5 mL in 100 mL of alcohol.

2.4. Vegetable Samples

Two leafy plants (mint, and coriander) and two root vegetables (carrots and white radish) were used for this study. These vegetables are amongst the most common cultivated crops in Yemen. A total of twenty samples (five samples for each vegetable) were collected between February and December, 2013 which represnt the dry and rainy seasons. The five samples of each kind of vegetable represented five local markets (Ali Muhssan, Bab al Qa'a, Bab Alyemen, Shumailah and Daris markets) where all the market located in the capital Sana'a. The markets and samples were chosen randomly. The vegetables are brought fresh from suburb areas and other governorates on a dialy basis.

2.5. Development and Validations Procedures

The performance criteria of the developed method included linearity, accuracy, precision (repeatability), detection limit, quantitation limit and stability (robustness) were validated. The validation of these parameters was done as follows:

2.5.1. Comparison and Selection of Color Reagents Used in Nitrite Determination Methods

For the sake of comparison, two published combinations of diazo-coupling reagents used for spectrophotometric determination of nitrite ions were selected based on their stability and sensitivity. The first combination involved sulfanilic acid with methyl anthranilate (Narayana and Sunil, 2009). In this paper, it is named as "Ref. method 1". The second diazo-coupling reagents were sulfanilinamide with NEDD as color reagents (Merino, 2009b), and it was given the name of "Ref. method 2". These two names were used throughout this paper. The results of the two reference methods were compared with each other and also with the results of our developed method in which sulfanilic acid with NEDD were used as diazo-coupling reagents.

2.5.1.1. Nitrite Determination Using "Ref. Method 1" (Narayana and Sunil, 2009):

A volume of 10 mL of working nitrite standards 8 to 80 mg L^{-1} were transferred into a series of 100 mL calibrated flask. To each flask, 10 mL of 0.1 M HCl and 10 mL of 0.6% sulfanilic acid were added, then the solution was mixed properly. Thereafter, 10 mL of 1% methyl anthranilate and 10 mL of NaOH (2N) were added, and the contents were diluted to 100 mL using de-ionized water. After the formation of the yellow colored dye, the λ_{max} was determined by scanning the absorbance in the range of 380-800 nm. Finally, the absorbance of the solutions was measured at λ_{max} (410 nm) against the corresponding reagent blank.

2.5.1.3. Nitrite Determination Using Sulfanilic Acid with NEDD as Diazo-coupling Reagents (the proposed method)

The volumes of 10 ml of working nitrite standards of 0.5 to 10 mg L^{-1} were transferred into a series of 100 mL calibrated flasks. To each flask, 10 mL of 0.1 M HCl, 10 mL of 0.6% sulfanilic and 10 mL of 0.6% NEDD were added and diluted to 100 mL using de-ionized water. After the formation of the pink colored dye, the λ_{max} was determined by scanning the absorbance in the range 380-800 nm. Finally, the absorbance of the solutions at λ_{max} (542 nm) was measured against the corresponding reagent blank.

2.6. Optimization and Development of the Procedures

For the purpose of enhancing the sensitivity and suitability of the method, few parameters were optimized to establish the best conditions. These variables included charcoal mass, pH, physical state and quantity of the diazo-coupling reagents, and pH. .

2.7. Application of the Developed Method for Nitrite Determination in Vegetables

The application of the developed method and the validation for the quantitation of nitrite in four kinds of leafy and root vegetables were done as follows:

2.7.1. Preparation of Test Sample (nitrite extraction)

A weight of 250 g of root vegetables and 100 g of leafy vegetables were rinsed with tap water and then with de-ionized water. After cutting the vegetable samples into small parts, they were ground by a blender, and were filtered, and washed using de-ionized water. After shaking, a volume of 50 mL of the samples was then centrifuged using Compact Laboratory Centrifuges Digital LC 8 (Chemglass Life Science) and the supernatant was collected into a glass bottle. The pigments were removed from the sample by adding an approprate amount of charcoal, and were shaken properly and centrifuged. The supernatant was collected from which a volume 10 mL was transferred into separate flasks. To each flask, 1 mL of 0.1 M HCl, 6 mg of sulfanilic acid and 6 mg of NEDD were added, and the content was shaken by hand until all reagents were dissolved. The absorbance of the pink-colored dye was measured at 542 nm against the corresponding reagent blank. Each sample was analyzed in triplicates, and the result was expressed as mg kg^{-1} fresh weight of vegetable.

2.7.2. Spiking and Recovery Calculations

For calculating the method recovery and studying the matrix effects, 0.05 to 1 mg L^{-1} of nitrite spiked samples were prepared and thereafter the nitrite concentrations were measured. The differences between the pairs of results obtained from the spiked and unspiked samples were used to calculate the recovery

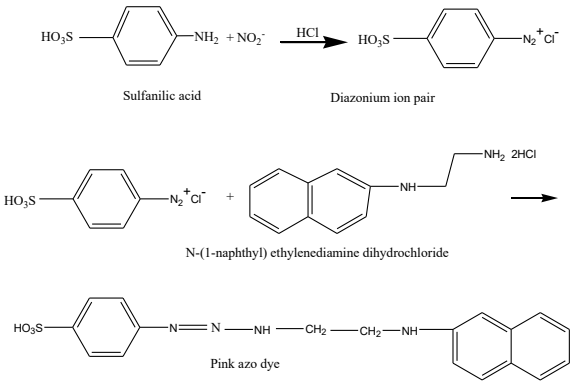
2.7.3. Preparation of Spiked Samples of Nitrite

Spiked samples with the concentrations of 0.05 to 1mgl^{-1} were prepared by adding various volumes (5.5 to 110 µL) of 100 mg L^{-1} nitrite standard solutions to 10 mL of the filtrated vegetable sample. Each spiked sample was treated with 1 mL of 0.1 M HCl, 6 mg of sulfanilic acid and 6 mg of NEDD to develop the color, and then the solution was shaken. Absorbance of the pink- colored dye was measured at 542 nm against the corresponding reagent blank.

3. Results and Discussion

3.1. Method Development for NO_2^- Determination

The development of the proposed spectrophotometric method for nitrite determination in vegetables was based on a modifed Geris reaction in which a diazotization reaction took place between nitrite ion and sulfanilic acid in an acidic medium. The product was coupled with NEDD to produce an azo dye as proposed in the scheme bellow.



Scheme showing a proposed chemical structure of the pink azo dye produced by the reaction of sulfanilic acid and NEDD with NO_2^-

Both reactions were done at room temperature. Figure 1 below depicts the visible spectra obtained from the proposed method and the two referencemethods. Noticeably, in the Ref. method 1, the amount of nitrite standard was 80 mg L-1 since it was difficult to get equivalent absorbance to that generated by Ref. method 2, and the proposed method in which only 4 mg L^{-1} of nitrite standard have to be used. The azo dyes produced by the proposed method and Ref. method 2 showed maximum abornbances at longer wavelengths (542 and 543 nm respecttively) compared to that obtained from Ref. method 1 where the maximum absorbance was observed at 410 nm. The molar absorptivities of the products from the the proposed method, Ref. method 2 and Ref. methods 1 were 7.50 $x10^3$, 7.07 $x10^3$ and 6.47 $x10^2$ L mol^{-1} cm^{-1} respectively. The molar absorptivity data revealed that the method proposed in the present work showed a higher value compared to both reference methods. Further comparision was also made in Table 1 showing some initial analytical merits of the proposed method against the two refrence methods.

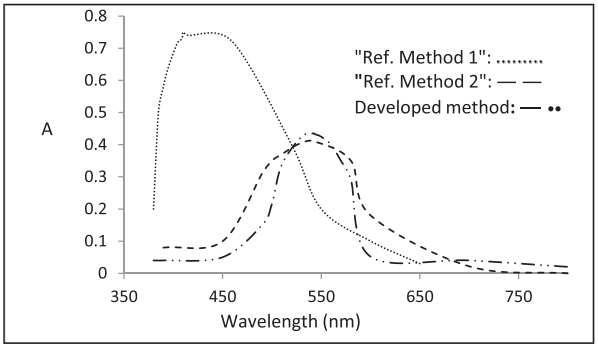


Figure 1. Visible spectra of azo dyes that were produced by the two reference methods and the developed method.

Table 1. Comparison Table between some initial performance criteria of the developed method with the two reference methods.

NO.	Method	λ_{max} (nm)	Equation	R^2	LOD mg L^{-1}
1	"Ref. method 1"	410	y = 0.009x + 0.006	0.9997	1.10
2	"Ref. method 2"	543	y = 0.103x - 0.003	0.9994	0.092
3	Proposed method	542	y = 0.108x + 0.007	0.9998	0.090

LOD = 3.3 × sd/s, where sd is the standard deviation of the blank and s is the slope of the calibration curve. R^2 was calculated from the respected calibration curves of the three methods.

Even though, the performances of the proposed method and Ref. method 2 look similar under initial experimental conditions, the data uneqivocally proved that the proposed method has LOD that is 12 times less than that of the Ref. method 1. With possible further fine tunning, the analytical merits of the proposed method could be even improved to make the proposed method a better alternative for nitrite determination. Therefore, further development and validation steps as discussed in the following sections were considered to improve the performance of the proposed method.

3.2. Optimization of the Proposed Method

The kinetices study of the various diazo-coupling reagents used in Gries reactios for nitrite detrmination reported previously (Fox, 1979) indicated that optimum conditions for each diazo-coupling reagents varried and should be determined experimentally. Thus, For enhancing the sensitivity and suitability of the proposed method for the determination of nitrite in vegetables, different variables such as the quantity of charcoal, pH, the physical state (liquid or solid) and amount of diazo-coupling reagents were optimized to establish the most optimal experimental conditions. The optimization processes are discribed as follows:

3.2.1 Effect of Quantity of Charcoal on the Method's Performance

Pigments from vegetables' extract may cause interferences in nitrite determination and thus compromise the method performance (Wang et al., 2017). Some earlier works (Prasad and Chetty, 2008; Mao et al., 2009) used activated charcoal for pegment removal prior to the spectrophotometric assessment of nitrite in vegetables. Although, activated charcoal is effective in pigment removal, its cost is higher than charcoal. Thus, the latter was selected as an alternative in the proposed method. To test the effectiveness of charcoal and find out its optimum mass for the removal of pigments from cooriander, carrots, mint and white radesh, various amounts of charcoals (0.1-1 g)were used. As might be expected, the extracts of colored and leafy vegetables needed higher amounts of charcoal than the juicy or colorless vegetables. An amount of 0.300 g charcoal was enough to remove the pigment from the 10 mL white radish extract, while the same volume of the extracts of mint, coriander and carrots required 0.600 g of charcoal. For further investigations, nitrite recovery calculations in the presence of three different amounts of charcoal are shown in Table 2.

Table 1. Recoveries results of nitrite ions standards after applying different amounts of charcoal.

NO.	Nitrite actual Conc. mg l^{-1} (n =3)	%Recovery after using 0.1 g charcoal	%RSD	%Recovery after using 0.5 g charcoal	%RSD	% Recovery after using 1 g charcoal	%RSD
1	0.05	99.54	0.40	97.70	0.41	97.76	0.42
2	0.20	100.92	0.40	101.84	0.40	102.30	0.42
3	0.40	100.46	0. 30	99.31	0.32	102.76	0.40
4	0.80	100.92	0.35	100.92	0.30	102.07	0.35
5	1.00	98.25	0.30	100.09	0.30	98.25	0.35

Recovery = [(calculated Conc.(mg L^{-1})/actual Conc.(mg L^{-1})] × 100, n = number of replications.

3.2.2. pH Effect on Nitrite Ions Determination

In general, the determination of nitrite in foodstuffes based on Griess reaction requires a careful control of the pH for both diazotization and coupling reactions to prevent the converion of nitrite to nitrous acid or nitrous oxide (Hsu et al., 2009). Even though the pH optimum value varries according to the type of diazo-coupling reagents and needs to be determined experimentally, the sulfonic acid diazotization reaction has to be done in acidic media (Fox, 1979; Narayana and Sunil, 2009; Rider and Mellon, 1946). In the proposed method, the optimum pH for the azo dye formation as a result of the diazo-coupling reactions was determined as shown in Figure 2.

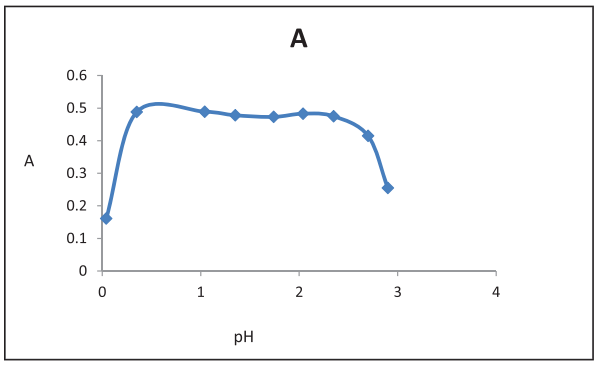


Figure 2. pH Effect on nitrite ions determination using the developed method.

Thee results showed that the suitable pH values were in the range of (0.35-2.35) which corresponded to a maximum absorption. Unlike the case when sulfonic acid and methyle anthranilate were used as dizo-coupling reagents (Narayana and Sunil, 2009), where a higher molar concentraion (up to 2 M) HCl was used, the diazo-coupling reactions in the proposed method did not work at pH values lower than 0.35. At these extreem pH, the formed azo dye was degraded and the appearance of a dark color in the solution was observed. Beyond pH 2.35, the absorbance of the formed azo dye was less, indicating unfaverable conditions for the dye formation. It should be mentioned that all pH measurments were done in HCl solution rather than in buffer system to simplify the diazonium ion formation and the subsequent coupling of the formed ion with NEDD

3.2.3. Minimizing the Dilution Factor Through Using Solid Diazo-coupling Reagents:

Enhancing the method sensitivity relies heavily on the concentration of color-producing reagents. Consequently, we tried to minimize the effect of dilution by adding volumes of higher concentrations of the color-producing reagents; sulfanilic acid and NEDD. This attempt, however, failed due to the solubility problem. For this reason, a different approach has been used in which the solid reagents were added directly to the reaction vessel. Interestingly, this approach overcame the solubility problem and resulted also in minimizing the volume of the needed hydrochloric acid (HCl) where only a volume of 1 mL of 0.1M HCl was enough to adjust the pH. The advantages of this approach is not limited to minimizing the dilution-factor effect, but also, the preparation steps are less, and the method is economic, simpler and environmental friendly. This attempt has not been reported previously in the determination of nitrite ions, as far as the researchers know.

3.2.4. Quantity of Coupling Reagents

The amounts of sulfanilic acid and NEDD were also optimized. The investigation was carried out using concentrations of nitrite (0.05 to 1 mg L^{-1}) which covered the method range. The results in Table 3 confirmed that the amount of 6 mg of each sulfanilic acid and NEDD was sufficient to generate repeatable linear results over the method range.

Table 3. Effect of the quantity of coupling reagents.

Nitrite actual Conc. mg L^{-1}	Quantity of sulfanilic acid (mg)	Quantity of NEDD (mg)	A
0.05	6	6	0.056
0.05	12	12	0.057
0.05	18	18	0.057
0.40	6	6	0.440
0.40	12	12	0.440
0.40	18	18	0.440
1.00	6	6	1.080
1.00	12	12	1.080
1.00	18	18	1.090

Experiments were done at pH = 2.

The investigation was done at pH 2. Higher amounts of reagents have no effect on the results. The lack of repeatability of nitrite determination using Griess reaction is attributed to the presence of excessive nitrite ions compared to the concentration of the diazo-coupling reagents (Nicoholas and Fox, 1973).

With all optimum conditions in hand, confirmation experiments were carried out to check if these optimized conditions would have improved the calibration sensitivity and LOD of the developed method. Table 4 compared the results of the developed method under optimum and initial conditions.

Table 4. Data comparison under initial and optimum conditions.

NO.	Method	Condition	*Reagent's phase	Calibration curve equation	R^2	LOD mg L^{-1}
1	Proposed method in the present work	Initial (no optimization)	As as solution	y = 0.108x + 0.005	0.9998	0.090
2	Developed method in the present work	Optimal conditions	Added as solid	y = 1.1x + 0.004	0.9999	0.010

**Reagents: sulfanilic acid and NEDD*

The calibration sensitivity and LOD under optimum conditions were substantially improved more than nine times. It is also evident from the data (when compared to Table 1), that the developed method under optimum conditions is 120 times more sensitive than Ref. method 1, and its LOD is 110 times lower. Furthermore, the developed method has a calibration sensitivity nine times better than that of Ref. method 2, and the detection limit is 111 times lower.

3.3. Validation Study

Method validation is an essential step to ensure the accuracy and validity of the data obtained by the developed method. Various performance criteria were validated as outlined below:

3.3.1. Precision (repeatability)

The repeatability (intraday precision) of the developed method was calculated at three different concentrations covering the method range (0.05 1 mg L^{-1}), in triplicate. Results of the method precision, as % RSD, were shown in Table 5.

Table 5. Results of repeatability studies.

Sample	Nitrite actual Conc. mg L^{-1}	Absorbance (n =3)	Calculated Conc. mg L^{-1}	% Recovery	Average % RSD	Acceptable % RSD (AOAC 1998)
SMP _1	0.05	0.059	0.051	102.00	4.78	15 (for 100 ppb)
	0.05	0.057	0.049	98.00		
	0.05	0.054	0.046	92.00		
SMP _2	0.40	0.460	0.420	105.00	4.74	11 (for 1 mg L^{-1})
	0.40	0.450	0.411	102.75		
	0.40	0.420	0.383	95.75		
SMP _3	1.00	1.030	0.946	94.60	4.26	11 (for 1 mg L^{-1})
	1.00	1.090	1.001	100.10		
	1.00	1.120	1.029	102.90		
Overall Average % RSD					4.59	

% Recovery = (Calculated conc. in mg L^{-1}/actual Conc. mg L^{-1}) × 100

The low values of % RSD were indicative of the high repeatability of the method. The average % RSD values of the developed method (%4.59) are within the accepted limit suggested by the AOAC Manual for the Peer-Verified Methods program (AOAC 1998) and very close to the reported value (%4.51) using capillary electrohoresis (Oztekin et al., 2002)

3.3.2. Accuracy

Accuracy of the developed method was also calculated and shown in Table 6.

Table 6. Accuracy of the developed method.

Name of sample	Nitrite Actual Conc. mg L^{-1}	Absorbance (n = 3)	Calculated Conc. mg L^{-1}	% Recovery	% RSD	% Bias
Blank	Negligible	Negligible				
STD_1	0.05	0.062	0.0487	97.40	4.2	-2.60
STD_2	0.10	0.119	0.0992	99.20	3.7	-0.80
STD_3	0.20	0.230	0.1975	98.75	3.5	-1.25
STD_4	0.40	0.450	0.3924	98.10	3.8	-1.90
STD_5	0.60	0.680	0.5961	99.35	3.8	-0.65
STD_6	0.80	0.900	0.7910	98.87	3.5	-1.13
STD_7	1.00	1.120	0.9858	98.58	4.0	-1.42

% Recovery = (Calculated concentration in mg L^{-1} /actual Conc. mg L^{-1}) × 100
% Bias = % Recovery - % 100 , calculated concentration in mg L^{-1} was calculated from the linear regression equation (y = 1.1x + 0.004), n = number of replications.

The method showed a small bias which indicated that errors affecting the accuracy of the developed method were under control.

3.3.3. Method Linearity

The linearity of the developed method was tested by constructing a calibration curve of the data in Table 6. The obtained results shown in Figure 3 indicated that the developed method possessed high linearity with R^2 = 0.9999 within the method linear range (0.05 – 1 mg L^{-1}). The linearity of the developed method (0.9999) was close to the linearity of HPLC method (Chou et al., 2003) reported previously (1.000) and similar to the value reported by Kmel and co-workers (Kmel et al., 2019). It should be indicated that the average recovery of the standards used to construct the calibration curve was 98.61% (%RSD 3.80) which indicated a high accuracy method.

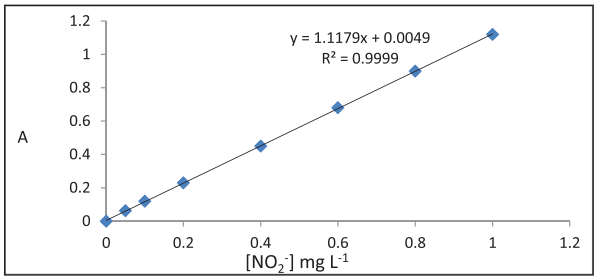


Figure 3. Calibration curve of nitrite standard solutions using the developed method.

3.3.4. Limits of Detection (LOD) and Quantification (LOQ)

Nitrite concentrations in the lower part of the linear range of the calibration plot were used to determine the limit of detection (LOD) and limit of quantification (LOQ). They were determined from the slope of the calibration plot(s) and standard deviation (sd) of the blank using the following two equations (Jimidar et al., 1995):

LOD = 3.3 × sd/s

LOQ = 10 × sd/s

The LOD and LOQ were 0.010 and 0.030 mg L^{-1}, respectively, for nitrite, which indicated that the sensitivity of the method was adequate enough to detect nitrite level well below the safe limit (European Commission, 1995). The above LOD is much lower than that reported for Capillary electrophoresis methods for nitrite in vegetables (Jimidar et al., 1995) and the multichannel contineous flow analyzer accredited by the Solvenion Accreditation Board (Susin et al., 2006).

3.3.5 Recovery Calculation of Nitrite in Vegetables:

Table 7 (a-d) sumarizes the recovery calculations of nitrite in the matricies of the four selected vegetables (carrots, white radish, mint and coriander). The overall recovery of proposed method ranged between 98.00% and 102.00%. This is an indication of the high sensitivity and suitability of the method to determine nitrite in root and leafy vegetables.

Table 7 (a). Recovery results of nitrite in carrots sample.

Sample	Spiked Conc. ppm	Abs.	A. Spiked sample-A. unspiked	Calculated Conc. (ppm)	% Recovery	% Bias
Unspiked sample	0.00	0.056				
Spiked sample_1	0.05	0.114	0.058	0.050	100.00	0.00
Spiked sample_2	0.20	0.281	0.225	0.204	102.00	2.00
Spiked sample_3	0.40	0.491	0.435	0.397	99.25	-0.75
Spiked sample_4	0.80	0.921	0.865	0.794	99.25	-0.75
Spiked sample_5	1.00	1.131	1.075	0.987	98.70	-1.30

Table 7 (b). Recovery results of nitrite in white radish sample

Sample	Spiked Conc. ppm	Abs.	A. Spiked sample-A. unspiked	Calculated Conc. (ppm)	% Recovery	% Bias
Unspiked sample	0.00	0.110				
Spiked sample_1	0.05	0.167	0.057	0.049	98.00	-2.00
Spiked sample_2	0.20	0.330	0.220	0.199	99.50	-0.50
Spiked sample_3	0.40	0.545	0.435	0.397	99.25	-0.75
Spiked sample_4	0.80	0.975	0.865	0.794	99.25	-0.75
Spiked sample_5	1.00	1.195	1.085	0.996	99.60	-0.40

Table 7 (c). Recovery results of nitrite in mint sample.

Sample	Spiked Conc. ppm	Abs.	A. Spiked sample-A. unspiked	Calculated Conc. (ppm)	% Recovery	% Bias
Unspiked sample	0.00	0.090				
Spiked sample_1	0.05	0.147	0.057	0.049	98.00	-2.00
Spiked sample_2	0.20	0.310	0.220	0.199	99.50	-0.50
Spiked sample_3	0.40	0.530	0.440	0.402	100.50	0.50
Spiked sample_4	0.80	0.960	0.870	0.798	99.75	-0.25
Spiked sample_5	1.00	1.190	1.100	1.010	101.00	1.00

Table 7 (d). Recovery results of nitrite in coriander sample.

Sample	Spiked Conc. ppm	Abs.	A. Spiked sample-A. unspiked	Calculated Conc. (ppm)	% Recovery	% Bias
Unspiked sample	0.00	0.130				
Spiked sample_1	0.05	0.187	0.057	0.049	0.049	-2.00
Spiked sample_2	0.20	0.355	0.225	0.204	0.204	2.00
Spiked sample_3	0.40	0.570	0.440	0.402	0.402	0.50
Spiked sample_4	0.80	0.995	0.865	0.794	0.794	-0.75
Spiked sample_5	1.00	1.200	1.070	0.982	0.982	-1.80

3.4. Real sample analysis

Table 8. summarizes the results of nitrite assessment in vegetables.

Table 8. Nitrite concentration in some vegetable samples from different markets.

Sample Source	mg.kg^{-1}			
	Carrots	White radish	Mint	Coriander
ALI MUHSSAN MARKET sample1_1	0.13	4.94	1.19	3.29
ALI MUHSSAN MARKET sample1_2	0.13	4.95	1.24	3.24
ALI MUHSSAN MARKET (Spiked sample)	0.15	4.92	1.20	3.24
BAB AL QA'A MARKET	0.02	4.19	0.71	2.14
BAB ALYEMEN MARKET	0.08	3.95	0.98	1.93
SHUMAILAH MARKET	0.38	2.18	1.27	1.47
DARIS MARKET	0.10	4.31	1.04	3.35
Average	0.14±0.11	4.20 ± 0.98	1.09 ± 0.20	2.80 ± 0.72

4. Conclusion

This paper presented the development and validation of a spectrophotometric method for the determination of nitrite in vegetables. The method was based on the use of sulfanilic acid with NEDD in solid forms added directly to reaction vessels containing HCl and nitrite solutions. The method showed higher performance criteria compared to those of two methods used as references. The developed method was successfully applied for the determination of nitrite in green leafy and root vegetables.

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