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Development of A Spectrophotometric Method with Enhanced Sensitivity for the Determination of Nitrate Contamination in Vegetables

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Abstract:- Sensitive and environmental friendly sequential method for the determination of nitrate in vegetables was proposed. The method was based on the use of a new combination of Griess reagents (sulfanilic acid and N(1-napthyl) ethylenediamine dihydrochloride) in solid form added directly to nitrate solution in acid medium. The method was optimized for the amounts of charcoal, zinc, diazo-coupling reagents and pH. The validated method had detection and quantitation limits of 0.022 and 0.066 ppm respectively. The dynamic linear range extended between 0.088 and 1.2 ppm (R2 = 0.9999). The method recovery in aqueous solutions was between 96.25% ∓4.40% and 99.70% ∓ 4.20% for seven measurements with an average of 98.52% ∓4.11% indicating high method accuracy and optimum conversion of nitrate to nitrite. The method was successfully applied for the determination of nitrate in two leafy crops (mint and coriander) and two root vegetables (white radish and carrot) collected from five different local markets in the capital of Yemen, Sana'a. The average recovery values of nitrate in the four vegetables matrices ranged between 95.63% and 107.50%. The assessment of nitrate in the vegetables samples revealed that carrots contained the least amount of nitrate (77.90 mg/kg) among the tested vegetables flowed by white radish (641.84 mg/kg), mint (786.86 mg/kg) and coriander (1740.00 mg/kg). The data of the present study confirmed that the proposed method has the required sensitivity to determine nitrate bellow the regulated level in various kinds of leafy and root vegetables.

Keywords:- *Gries Reagents, Nitrate, Nitrite, N-(1- Naphthyl) Ethylenediamine Dihydro Chloride, UV-Vis Spectrophotometry, Sulfanilic Acid, , Vegetables.*

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I. INTRODUCTION

Nitrate (NO3-) is one of the nitrogen compounds that is widely spread in nature. It could be found as a contaminant in food specially vegetables, water, soil and the environment [1], [2]. Human exposure to nitrate mainly comes from the consumption of vegetables which constitutes more than 75% of the daily intake of nitrate [3], [4]. Nitrate itself is relatively safe but a small portion of nitrate may be reduced in the oral cavity to nitrite that could undergo nitrosation reactions with secondary amines and amides in the stomach to form nitrosamines. The formation of notorious carcinogenic nitros compounds has put nitrate in disgrace [5][6]. To the contrary, nitrate may act as a precursor in the generation of nitric oxide (NO) which is a biological messenger that is involved in many potential functions in human physiology [7]. Recent upsurge in scientific research on the role of NO generated from nitrate-nitrite pathway indicates that NO could serve –among other functions as a blood pressure reducer [8], back up pathway for NO synthesis in patients suffering from dysfunction of L-arginine NO synthesis path-way [7], and reducing platelets aggregation [9]. Even though, these new evidences suggest that the role of nitrate-nitrite as cancer causing agents may be overestimated, regulations on the use of nitrate and daily dietary intake are still enforced. Joint Expert Committee of the Food and Agriculture (JECFA) and the European Commission's Scientific Committee on Food (SCF) have set maximum limits for acceptable daily intake of nitrate (ADI) to be 0 - 3.7 mg/kg bodyweight per day while the USA Environmental Protection agency Reference Dose (RFD) has its limits for nitrate (7 mg/kg body-weight per day NO3) [10], [11].

Various spectrophotometric methods have been developed and used for the determination of nitrate in environmental samples including vegetables [12][13][14]. Comprehensive literature survey of the spectrophotometric methods for nitrate assessment could be found in three re-cent review articles [15][16][17] that overview their principles, strengths and weaknesses. In spectrophotometric approach, nitrate is indirectly determined as nitrite via its di-azo-coupling reaction (Griess assay), nitrosation reaction or catalytic reaction. These methods are preferred for routine assessment of nitrate for simplicity, low cost and availability of equipment in lab with limited recourses. However, these advantages are counterbalanced by strict controls of reaction conditions, interferences from ions, and organics. In addition, the presences of pigments and other matrix components in vegetables could result may cause low methods' sensitivity which is generally in the range of 0.02 – 2 M [17]. As a result, the aim of the present work focused on the development and validation of simple, cost effective and ecofriendly spectrophotometric method with enhanced sensitivity to detect and quantitate nitrate in vegetables. The proposed approach was based on the combined use of sulfanilic acid (SA) and N(1-naphthyl) etheylendiamine dihydrochloride (NEDD) as diazo-coupling agents. In addition, the use of SA and NEDD in solid forms that were directly added to the reaction vessels containing nitrate solutions was a new approach. To the best of our knowledge, this approach is unique and has not been reported previous-ly for nitrate detection and quantitation in leafy and root vegetables.

II. METHODOLOGY

A. Chemicals and Instrumentations

All chemicals were of analytical grades and used without further purification. They were purchased from either BDH, Himedia or Merck. Deionized water with specific onductance of 0.05 µS cm-1 (DirectQ3, Millipore-USA) was prepared in-house and used for the preparation of all solutions when needed. UV/Visible spectrophotometer, model Spectroscan 60DV, Biotech Engineering, was used for absorbance measurements. pH meter (HI-5321) and electronic balance (ESJ120-4) both from Biotech Engineering were also used.

B. Preparation of Reagents, Collection of Vegetables and Nitrate Extraction

Stock solutions of nitrate and nitrite (400 ppm each) were prepared from their sodium salts in deionized water and further dilutions with deionized water were carried out to make the desired standard working solutions. The preparation 0.1 M HCl, 2 N NaOH, Sulfanilamide Sulfanilic acid (from BDH), Methyl anthranilate (from Himedia) and N-(1-naphthyl) ethylenediamine.2HCl, vegetables' collection and nitrate extraction were done similarly to that reported for nitrite assessment and described elsewhere by our group[18]. In brief, a stock solution of 400 ppm nitrite was prepared from its sodium salt in deionized water and used for the preparation of fresh working standard solutions. Dilute solutions of 0.1 M HCl, 2 M NaOH, 0.6 % w/v SA, (0.6 % w/v), 0.6% NEDD, and 1 % w/v sulfaniamide were prepared in deionize water. Methyl anthranilate of 0.5% v/v strength was prepared in ethyl alcohol. In the case where solid NEED and SA were used, the specified amounts were directly added to the reaction vessels. Four crops (mint, coriander, white radish and carrots) were collected from five local markets in Sana'a, Yemen. Nitrate extraction was done as follows; a weight of 250 g of each carrots and white radish and 100 g of each mint and coriander were rinsed with tap and de-ionized water respectively. Each crop was blended using an electrical blender and then filtered to remove solid materials. A volume of 50 mL of the filtrate of each crop was further centrifuged using Compact Laboratory Centrifuges Digital LC 8 (Chemglass Life Science) and the supernatant was transferred to a glass bottle. Appropriate amount of charcoal was added to the vegetables extract to remove pigment. The content was centrifuged again and 10 mL volume of the supernatant of each vegetable was transferred into separate volumetric flasks. Further treatment to optimise the reduction of nitrate to nitrite and the formation of the azo dye was then carried out as described in section 2.3.

C. Development and Validation Procedures Comparison and Selection of Diazotization and Coupling Agents

Three different combinations of Griess diazo-coupling reagents were chosen to form azo dyes with nitrate as nitrite. The first and second combinations consisted of sulfanilic acid (SA)/methyl anthranilate (MA) and sulphanilamide (SAD)/N(1-napthyl) ethylenediamine dihydrochloride

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(NEED). The third combination consisted of SA/ NEED. The preparation of the azo dyes from each group of reagents was done as follows: In the first case where SA and MA were used as diazotization and coupling agents respectively, 10 mL of working standards (16 to 80 ppm) were transferred into a series of 100 mL calibrated flasks. To each flask, 10 mL of 0.1 M HCl, 5 mg of zinc powder with 100 mg of sodium chloride and 10 mL of 0.6% SA were added consecutively, and then the solution was mixed properly. The mixed solution was filtered into a series of 100 mL calibrated flasks using Whatman No 41 filter paper. The filter paper was washed with 10 mL of 0.1 M HCl. After that, 10 mL of 1% MA and 10 mL of NaOH (2M) were added and the contents were diluted to 100 mL using de-ionized water. After the formation of the yellow-colored dye, λmax was determined by scanning the absorbance in the range 380-800 nm. Finally, absorbance measurements were acquired at λmax (410 nm) against the corresponding reagent blank. For the case of using the other two combinations SAD /NEED and SA/NEED, similar steps were carried out as described above for SA/MA with the exception that 0.6% of SAD, NEED and SA were used and the NaOH was not added. Absorption measurements of the formed azo dye between the reduced nitrate and SAD/NEED were done at λmax = 543 nm while spectra were acquired at 542 nm for the case when SA/NEED diazo-coupling agents were used.

➢ *Variables' Optimization Procedure*

Variables that include charcoal mass, pH effect, quantities of diazo-coupling reagents, and zinc were optimized one at a time. The optimization of the mass of charcoal was done by using various masses ranging from 0.1-1 g. Zinc quantity was varied between 2 and 20 mg. The pH effect on the stability of the developed azo dye was evaluated using various concentrations of HCl in which 1 mL volumes of 0.05 to 10 M were used. The concentrations of the proposed diazo-coupling reagents (SA and NEDD) were optimized as solutions as well as solid where reagents were added directly to reaction vessels. Using the optimized conditions, sample preparation for absorption measurement could be summarized as follows: A volume of 10 mL of nitrate solution (0.088, to 1.2 ppm) was pipetted into a series of 25 mL flasks. To each flask, 1 mL of 0.1 M HCl, 5 mg of zinc powder, 100 mg of NaCl, 6 mg of SA and 6 mg of NEDD were consecutively added and the solution was shaken by hand for couple Min. Finally, the absorbance of the pink colored dye was measured at 542 nm against the corresponding reagent blank.

➢ *Validation Study*

Validation parameters; precision, linearity, limit of detection (LOD), limit of quantification (LOQ), recovery, and accuracy of the developed method were determined. The precision results were reported as %RSD. The linear range for nitrate solutions and regression coefficient was calculated from the linear regression equation (y = mx + b). The lower part of the linear range of the calibration plot was used to determine the limit of detection (LOD) and limit of quantitation (LOQ).

D. Preparation of Test Samples and Spiking

Extraction procedure for nitrate from vegetables was carried out as described in section 2.2 above. Upon obtaining the colorless supernatants of vegetables, 10 mL of the supernatant was transferred to a glass tube and then 5 mg Zn, 100 mg NaCl, and 1 mL (0.1 M) HCl were added consecutively to the tube followed by shacking the tube. Finally, optimum amounts of SA (6 mg) and NEDD (6 mg) were weighed and directly added to the tube. The constituents were shaken properly until the pink colour was developed. Each sample was analyzed in duplicate and the results were expressed as mg/kg fresh weight.

For calculating the method recovery and studying the matrix effects, filtrated vegetable samples were fortified with various nitrate standards (0.088 to 0.440 ppm). The fortified samples were treated as described for vegetable's extract above. The differences between the pairs of results obtained from the fortified and unfortified samples were used to calculate the recovery. The method was applied for nitrate quantification in vegetables collected from five different markets in the capital Sana'a. Each vegetable was analysed five times and the average concentration of NO3- was calculated.

III. RESULTS AND DISCUSSIONS

In the present work, we reported the use of a novel combination of solid diazo-coupling agents SA/NEDD which was evaluated for its sensitivity and efficiency to determine nitrate concentration in vegetables. To do so, three different combi-nations of Griess diazo-coupling agents were examined for their efficiency to react with nitrate as nitrite and subsequently determine the latter spectrophotometrically. Initial data from the performance of the three diazo-coupling combinations were shown in Table 1. Noticeably, these data along with UV-Vis spectra [18] generated from the three different diazo-coupling combinations revealed that the pro-posed combination (SA/NEDD) had higher analytical merits (LOD, calibration sensitivity and molar absorptivity) compared to the results obtained by the use of the other two combinations (SA/MA) and (SAD /NEED). Therefore, the proposed combination was selected for further development and optimization studies.

Table 1 Comparison of the Initial Data Obtained by Using Three Different Combinations of Diazocoupling Agents

NO	Diazo-coupling Agents	λ_{max} (nm)	Molecular Absorptivity (cm-1mol-1L)	Equation	R2	LOD (ppm)
1	SA and MA	410	596	y = 0.0047x + 0.0012	0.9996	4.95
2	SAD and NEDD	543	6150	y = 0.0299x + 0.0057	0.9990	0. 36
3	SA and NEDD	542	6600	y = 0.0367x + 0.0014	0.9996	0.29

SA: sulfanilic acid, MA: methyl anthranilate, SAD: sulfanilinamide, NEDD: N-(1- naphthyl) ethylene diaminedihydrochloride.
LOD is 3 times sthe noise level.

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E. Optimization The Conditions of The Proposed Method for Nitrate Reduction

Methods based on Griess reactions for the determination of nitrate as nitrite requires experimental optimization of reactions condition. This is due to the variation in the optimum values of the various parameters involved in the formation of the azo dye such as pH, amount of diazo-coupling reagents, type and amount of reducing agents, and reactions' temperature [20]. Thus, parameters optimization in the present work was carried out one at a time where one parameter was changed and the rest were kept constant

➢ *Charcoal Optimization*

Vegetables under investigation contained colours and pigments which may negatively affect the accuracy of developed method. Previous researchers have recommended the use of active charcoal (activated carbon) [21] for pigment removal from vegetables. In our work, ordinary charcoal was selected due to its availability and cheapness compared to activated charcoal. The effect of charcoal on nitrate recovery is depicted in Fig. 1.

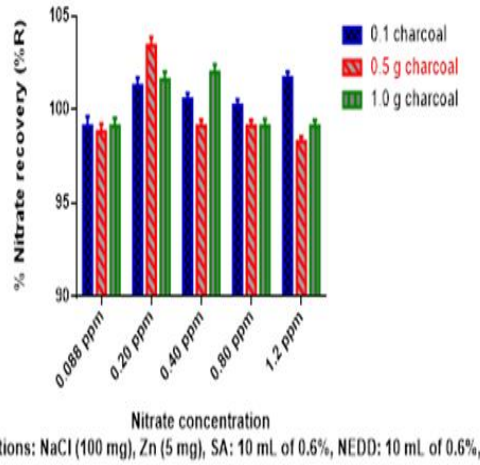

Fig 1 Optimization of the Mass of Charcoal at Different Concentration of Nitrate

According to this data, recovery of nitrate ranged from 98.28% to 102.01% with SD ≤0.5 which indicated the effectiveness of charcoal in removing pigments. The optimum mass of charcoal depends on the type of vegetables. Colored and leafy vegetables (coriander, mint and carrot) require higher amount of charcoal (0.6 mg/10 mL extract) while only 0.3 mg/10 mL extract is needed in the case of white radish. It should be mentioned that charcoal treatment of extract was made after blending the vegetable with deionized water at room temperature which was in line with the recommendation for maximum nitrate recovery from vegetables [22].

➢ *Effect of Zinc Quantity on The Reduction of Nitrate*

The effect of zinc on the reduction of nitrate to nitrite was investigated and the results were presented in Fig. 2.

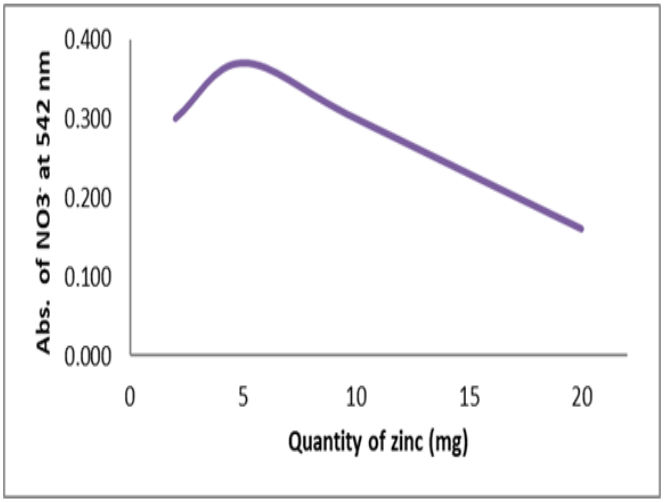

Fig 2 Effect of Quantity of Zinc on The Reduction of Nitrat

Conditions: NaCl: 100 mg, SA: 10 mL of 0.6%, NEDD: 10 mL of 0.6%, pH 2.00. The plotted data were the means of three measurements for each plotted point.

The results revealed that the optimum amount of zinc metal was 5 mg. The smaller quantities of zinc metal appeared to be less effective in reducing nitrate to nitrite while the higher quantities than 5 mg caused solubility problem that negatively affected the absorbance measurement in general.

➢ *PH Effect on Nitrate Ions Determination*

The successful diazotization reaction of SA with nitrite that is formed from the reduction of nitrate requires an acidic medium[21].

Thus, investigating the effect of the pH is critical to ensure successful detection and determination of nitrate ions. Consequently, we have tested different pH mediums using HCl to find out the optimum value of the pH. The results presented in Fig. 3 revealed that the suitable pH value was in the range of 1.74 - 2.35 which corresponded to maximum absorption of the developed azo dye. Lower pH values than 1.74 (higher concentrations of hydrogen ion) caused a degradation.

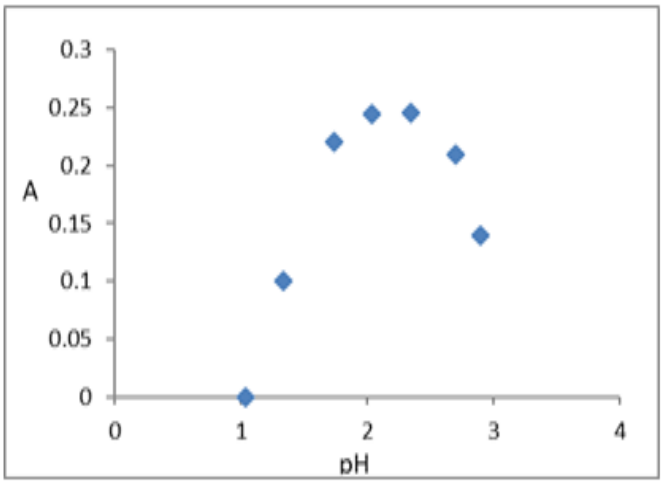

Fig 3 Effect of pH on Nitrate Determination

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Conditions were as follows; [NO_3-]: 0.88 ppm, zinc: 5mg, NaCl: 100 mg, SA: 10 mL of 0.6%, NEDD: 10 mL of 0.6%of the dye and a formation of a darker colour. Higher pH seems to be less effective in the dye formation where lower absorbance is observed.

➢ *Effect of The Concentration of The Diazo-Coupling Reagents*

The method sensitivity is affected by various factors including sample dilution due to the addition of large volumes of acid, and diazo-coupling reagents (SA) and (NEDD). In this work, we have tried to minimize the effect of dilution by adding smaller volumes of SA and NEDD in higher concentrations. This attempt failed due to a solubility problem. For this reason, we have used a different approach in which the solid reagents were added directly to the reaction vessel as we have reported in our previous work [18]. As a result of this approach, the volume of the HCl was also reduced from 10 mL to 1 mL which was sufficient enough to adjust the solution's pH Furthermore, the working procedure became simpler and the method was faster, cheaper and more environmental friendly. The method LOD (3 times the noise level) in the case of using solid SA and NEDD reagents was calculated to be 0.022 ppm while its value (shown in Table 1) in the case of using the same reagents as solutions was 0.290 ppm. This indicated an improvement by more 13 times in the method sensitivity in favor of using solid reagents which made the validated method capable of detection trace amounts of nitrate ions well below the regulated level. The linear calibration curves equations using SA and NEDD as solids and solutions were y = 0.287x + 0.001 and y = 0.0367x + 0.001 respectively which is translated to approximately 8 times enhancement in the calibration sensitivity.

➢ *Effect of The Quantities of Solid Diazo-Coupling Reagents*

The optimum amounts of SA and NEDD were also determined to ensure reliable results. Data in Figure 4 (a and b) confirmed that an amount of 6 mg solid of each SA and NEDD were sufficient to generate repeatable linear results. The use of excess amounts of reagents produced no further increase in the absorbance.

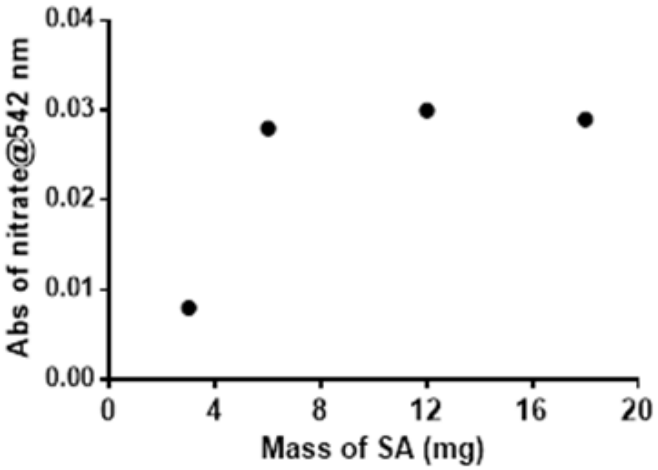

Fig 4 (a) Effect of the Quantity of SA Reagent on Nitrate Ion Determinaton

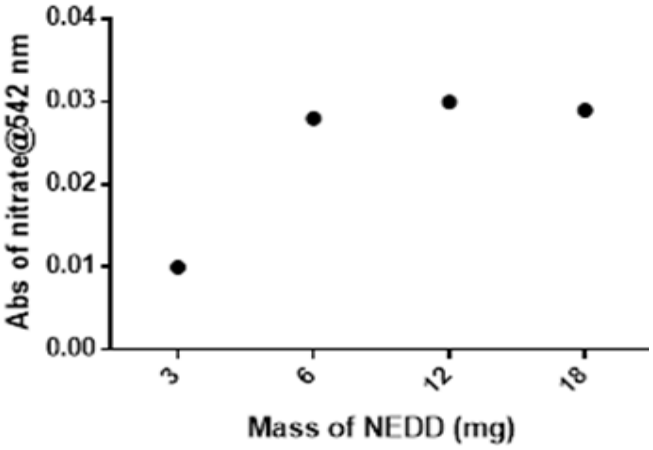

Fig 4 (b) Effect of the Mass of NEED Reagent on the Determination of Nitrate

B. Validation Results

➢ *Linearity and Accuracy*

Fig. 5 and Table 2 showed the corresponding data of linearity and accuracy of our optimized developed method.

Table 2 Method's Repeatability

Sample #	%Recovery (n = 3)	%RSD
1	96.25	4.4
2	98.50	4.1
3	99.25	3.7
4	99.40	4.0
5	99.63	4.3
6	99.70	4.2
7	96.92	4.1
Average	98.52	4.1

The linear range using optimum conditions was found to extend between 0.088 and 1.2 ppm. The calibration sensitivity of the validated method as shown in the insert of Figure 5 was found to be 64 and 10 times higher than results obtained by the of use of the two other combinations (SA/MA) and (SAD/NEDD) shown in Table 1 respectively. Interestingly, the optimization procedures of the developed method have led also to an increase in the developed method sensitivity by 8 times. In addition, the linear regression data (R^2 = 0.9999) for the calibration plot was an indication of a good linear relationship between absorbance and concentration of nitrate over the method's dynamic range. The low value of intercept of the ordinate indicated insignificant deviation from linearity. Data in Table 2 provided that the method's % recovery ranged between 96.25% ∓ 4.40% and 99.70% ∓4.20% and the average value was 98.52% ∓ 4.11% for seven measurements. This indicated a high accuracy of the method.

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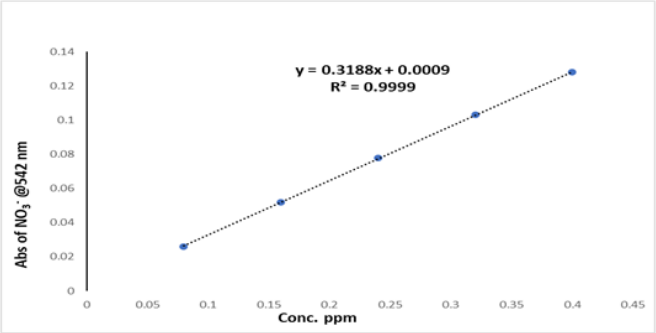

Fig 5 Standard curve of known concentrations of NO3- in vegetables matrix under optimum conditions:

Conditions: pH =2, zinc = 5 mg, NaCl = 100 mg, charcoal 6 mg, SA and NEDD 6 mg each. Coriander extracts were spiked with various known nitrate standards. The reported nitrate concentration was the difference between spiked and non-spiked sample.

➢ *Precision (Repeatability)*

The repeatability (intraday precision) of the method was determined as intraday variation for determination of nitrate at concentrations covering the method range (0.080 ppm to 1.2 ppm) in triplicate. The results showed that the mean recovery of NO_3^- ranged between 99.65% and 104.00% with an average of 101.43%. The average %RSD for all measurements was 3.86%. The %RSD is a good agreement with the limits set by the AOAC manual for the Peer-Verified Methods Program which indicates that the accepted limit of %RSD is 7.3 – 15 for 10 ppm to 100 ppb concentration [23].

➢ *LOD and LOQ*

The LOD (3 times the noise level) and LOQ (10 times the noise level) of the present work were calculated and found to be 0.022 and 0.066 ppm respectively for nitrate. When the value of LOD was compared to those shown in Table 1, a significant improvement of the method sensitivity under the optimized conditions was noticed.

➢ *Method's Recovery*

The recovery of the method was evaluated using four types of vegetables; two leafy (coriander and mint) and two root and juicy (carrots and white radish) as shown in Table 3. The average recovery ranged between 95.63% and 107.50%. These recovery values are compared well with those obtained with FIA (85.4%-107.4%) [26], capillary electrophoresis (90.40%-112.80%)[27], and capillary liquid chromatography (87.28%-107.54% [28].

Table 3 Recovery

Sample	Taken Conc (ppm)	Absorbance (n=2)	Calculated Conc. (ppm)	Recovery
Coriander				
Un-spiked sample	0	0.149	--	--
Spiked sample 1	0.080	0.175	0.083	103.75
Spiked sample 2	0.16	0.201	0.170	106.25
Spiked sample 3	0.24	0.227	0.257	107.08
Spiked sample 4	0.32	0.252	0.340	106.25
Spiked sample 5	0.40	0.278	0.427	106.75
Average				106.02
Mint				
Un-spiked sample	0	0.144	--	
Spiked sample 1	0.080	0.168	0.077	96.25
Spiked sample 2	0.16	0.192	0.157	98.13
Spiked sample 3	0.24	0.216	0.237	98.75
Spiked sample 4	0.32	0.240	0.317	99.06
Spiked sample 5	0.40	0.264	0.397	99.25
Average				98.29
White radish				
Un-spiked sample	0	0.04	--	--
Spiked sample 1	0.080	0.066	0.083	103.75
Spiked sample 2	0.16	0.091	0.167	104.38
Spiked sample 3	0.24	0.116	0.250	104.17
Spiked sample 4	0.32	0.142	0.337	105.31
Spiked sample 5	0.40	0.170	0.430	107.50
Average				105.02
Carrots				
Un-spiked sample	0	0.041	--	--
Spiked sample 1	0.080	0.065	0.077	96.25
Spiked sample 2	0.16	0.088	0.153	95.63
Spiked sample 3	0.24	0.112	0.233	97.08
Spiked sample 4	0.32	0.135	0.310	96.88
Spiked sample 5	0.40	0.160	0.393	98.25
Average				96.82

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C. Application of The Developed Method for The Assessment of Nitrate in Vegetables

The method's applicability for the determination of nitrate in real samples were shown in Table 4. Five samples from each market were analyzed and the average concentrations were tabulated. A general observation on these data can be stated that leafy vegetables (mint and coriander) contained higher nitrate levels compared to root and juicy vegetables (carrots and white radish) which was consistent with previous reports [21].

If we assume that an average adult weighing 60 kg consumes an amount of 400 g of the above four fresh vegetables (i.e. 100 g of each) per day from any of the five markets listed above without peeling or cooking, this person will exceed the acceptable daily intake (ADI) level (220 mg/day) [29]. The average value of nitrate in carrots (77.90 mg/kg) reported in our study was comparable to nitrate levels in crops grown in Greece (87 mg/kg), and much less in carrots grown in Slovenia (264 mg/kg), Korea (316 mg/kg), Belgium (287 mg/g), UK (170-210 mg/kg) and France (113-394 mg/kg) [30]. The low value of nitrate in carrot is desirable since carrot is usually used for babies' food and babies may be vulnerable to methemoglobinemia as some researchers reported[31].

Radish which belongs to the family Brassicaceae was classified by Santamaria[10] as a very high nitrate accumulator (> 2500 mg kg-1). Nitrate levels in radish grown in Switzerland (3500 mg kg-1)[32], and Korea (1878 mg kg-1)[33] were also high. Our assessment of nitrate in the roots of white radish was (641.84 mg kg-1). This variation in nitrate level of the same vegetable grown in different regions was not unusual since various factors including light intensity, used fertilizers, harvesting time, storage conditions, air temperature, and soil composition all have an influence on nitrate level to various degrees[34].

Mint and coriander are green leafy vegetables that are expected to show high nitrate content due to the presence of laminae in their leaves [34]. Our assessments of nitrate in mint (786.86 mg kg-1) and coriander (1740.00 mg kg-1) were moderately high compared to root vegetables (carrot and white radish) discussed above. Literature surveys of nitrate levels in mint grown in different parts of the world show wide variation (154.4-349.6 mg kg-1)[35] and (5450 mg kg-1)[36]. In the case of coriander, the reported levels of nitrate are also varied according to the season (2523 mg kg-1) in Summer and (1747 in Winter) [37].

Table 4 Nitrate Concentration in Real Samples

Sample Source	**C Ppm (N = 5)**			
	Carrots	White Radish	Mint	Coriander
Ali Muhssan Market	106.67	457.60	667.33	1130.00
Bab Al Qa'a Market	64.00	873.60	939.33	2595.00
Bab Alyemen Market	61.33	860.80	928.67	1640.00
Shumailah Market	61.33	700.80	746.67	2445.00
Daris Market	37.33	694.40	886.67	2145.00
Average Values	66.13	717.44	833.74	1663.00

IV. CONCLUSION

The present work detailed the development and validation of sensitive spectrophotometric method for nitrate determination in vegetables. The method relied on the reduction of nitrate to nitrite and subsequent diazotization and coupling of nitrite with new combination of modified Griess reagents (SA and NEDD) in solid form. The method had LOD and LOQ (0.022 ppm and 0.066 ppm respectively. The nitrate recovery in vegetables extended between 95.63% and 107.50%. The method was successfully applied for the determination of nitrate contamination in root and leafy vegetables. The developed method shows high sensitivity for the determination of nitrate in vegetables well bellow the regulated limits.

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