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Article

Continuous-Flow Analysis for Determination of Nitrate Using Hydrazine-Copper Method in Plan

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This study is to describe continuous-flow analysis (CFA) for the determination of nitrate using hydrazine-copper in plant material and to test precision of this method compared with that of methods,which are RQflex method and salicylic acid method. Samples were leaves of watermelon, cucumber, melon and tomato. Nitrate values measured by the RQflex method were greater than those measured by CFA or salicylic acid method. The correlation of nitrate values between those measured by CFA and salicylic acid method was R^2=0.9671, and those measured by CFA between those measured by RQflex method was R^2=0.9739.

Recovery rate of nitrate added to tissue extract by CFA method was 99.7 ±0.25%.

Key words : Nitrate, Continuous-flow analysis (CFA), Salicylic acid method, and RQflex

Introduction

Nitrate or nitrite is always investigated for protecting environment at water, agriculture, and food. The concentration of nitrate in plants is generally 0.02mM-162mM (Brinkered, E. F and O. E. Kolari, 1975). This concentration is dependent on plant variety, species, maturity, region, and cultivated condition such as temperature, light intensity, and deficiency of some nutrients and use of fertilizers. Nitrate taken at human body is reduced to nitrite, and it changes N-nitrosoamine compounds which are potent carcinogens to combine with amine, and it can react with hemoglobin influencing the oxygen transport system(MAFF, 1999). Many of nitrate detection methods have been developed until now at varies materials like foods, water, biological materials, soils and plants. They include, kinetic analysis (Liang B et al., 1994), Flow injection analysis, HPLC, gas chromatogratphy (Butt, S. B. et al., 2001), capillary electrophoresis (Öztekin, N. et al., 2002), amperometry (Bertotti, M. and D. Pletcher, 1997), potentiometry (Li, J. W. et al., 1994), voltammetry and chemiluminescence methods (Behzad, H. and T. Abdolah, 2001). Howevermost commonly used method is spectrophosmety which is reduced nitrate to nitrite. This method is AOAC official method that is reacted nitrite with N-(1-naphthyl)ethylenediamine and sulfanilamide (AOAC method ,1995). Also a variety of reducing agents have been investigated to facilitate this conversion and include zinc, amalgamated cadmium, hydrazine-copper and copperised cadmium (Mattew, J. et al., 2001).

This study is to describe CFA for the determination of nitrate in plant material using hydrazine-copperand to test precision of this method compared with those of other methods which are RQflex method and salicylic acid method.

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Materials and Methods

Plant samples were leaf of watermelon, melon, cucumber and tomatoes. They were obtained from a lot of farm households except leaves of tomato. Leaves of tomato were obtained from experimental field of National Horticultural Research Institute.

Plant samples were homogenized under liquid nitrogen. And they were preserved until analyze at -80℃. Homogenized samples (0.5g) were weighted and extracted in 5mL of deionized H_2O for 1h and shaken (140rpm). Then they were filtered with NO.2 filter paper.

Nitrate was measured by CFA, salicylic acid method and RQflex method. Table 1 lists the methods used and the nitrate standard range used for each method. (Table 1)

A diagram of the CFA instrument (Auto Analyzer 3: BRAN+LUEBBE) manifold employed is shown in Fig 1.

Nitrate is reduced to nitrite by hydrazine sulfate under

Table 1. Nitrate measurement ranges used to assay nitrate in tissue extracts.

Method	Nitrate detection principle	NO_3^--N standard range mg N L^{-1}
CFA	Hydrazine-copper conversion of nitate to nitrite followed by color reaction and absorbance measurement	5-15
Salicylic acid	The complex formed by nitration of salicylic acid under highly acidic conditions	5-50
RQflex	Test strip chemical conversion of nitrate to nitrite with color reaction and reflectometry measurement	1.1-50.8

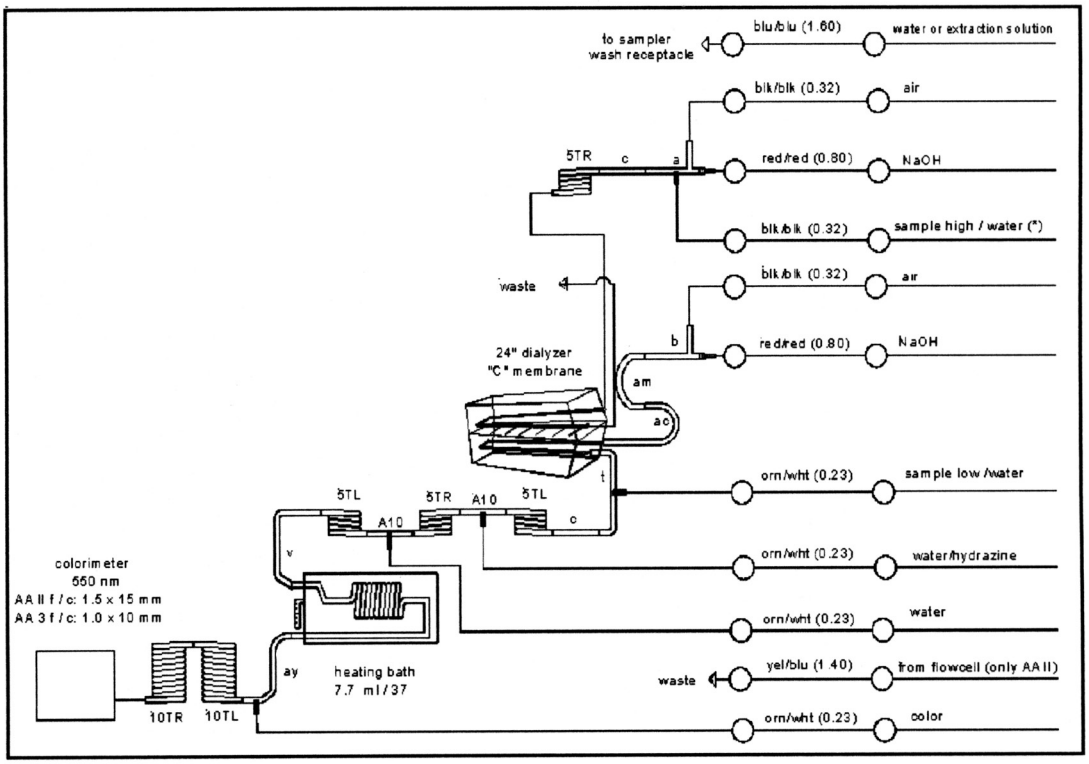

Fig 1. A diagram of the CFA instrument. (http://www.bran-luebbe.com)

alkaline solution, witha copper catalyst and reacted with sulphanilamide and N-(1-naphthyl-ethylenediamine dihydrochloride(NEDC) to form red-purple compound measured at 540nm. The addition of zinc to the reducing agent suppresses the complexing of copper by organic material. A dialyzer for the high range on MT7 eliminated interference from colored samples and suspended solids. Regents were same as below.

Buffer solution

- NaOH 10 g
- Ortho-phosphoric acid 3mL
- DI water to 1000mL
- 50% Tritonx (in ethanol) 2mL

Working solution (reduce nitrate to nitrite)

- 0.1% cupric sulphate 10mL
- 1% zinc sulphate 10mL
- Hydrazine sulphate 2g
- DI water to 1000mL

Color regent

- 2% sulfanilamide in 3N HCl: 0.1% NEDC = 1:1 (store at dark bottle)

The Merck RQflex 2 handheld instrument #16954 and Reflectoquant nitrate test strips, #11696-1 was used according to directions included with the instrument and test strips. Calibration was achieved with a preprogrammed bar code.

Salicylic acid method was based on D. A. Cataldo (1975). 0.1mL of extracted plant sample and 0.4mL of 5% (W/V) salicylic acid in concentrated H_2SO_4 were mixed thoroughly. After 20minutes at room temperature, 9.5mL of 2N NaOH were added slowly to raise the pH above 12. Samples were cooled to room temperature and absorbance at 410nm was determined in a spectrophotometer(Hitachi U-2000).

We compared interference between samples to change sampling rate and sample vs wash ratio. This aimed at setting up maximum sampling rate. Samples were 5, 10,

15mg L^{-1} (NO_3^--N) and inserted two DI water between different NO_3^--N levels. Sampling rate were 90, 70, 50 h^{-1} and sample vs wash ratio were 4:1, 3:1, 2:1.

Nitrate assay interference associated with tissue extract was evaluated by adding known nitrate to an extract from leaf samples. Leaf samples were mixed leaf of watermelon, leaf of melon, leaf of cucumber. The extracted leaf samples were added nitrate (0, 2.5, 5, 7.5, 15 mg L^{-1}: CFA method)

Results and Disscution

Analysis rate of CFA Recovered NO_3^--N of 15, 10, and 5 mg L^{-1} were each of 14.80±0.24, 9.98±0.06, and 5.05±0.01 mg L^{-1}, respetively when sample wash rate was 4, and it was no difference sample wash rate 3 and 2 under sampling rate 90 h^{-1}. But DI water was interfered with 15, 10, 5 mg L^{-1} samples. Interference rates of 1st DI-water after 15, 10, 5 mg L^{-1} samples were 0.35±0.06, 0.24±0.01, and 0.15±0.00 mg L^{-1}. Interference rates of 2nd DI-water after 15, 10, 5 mg L^{-1} samples were 0.10±0.01, 0.09±0.00 and 0.07±0.01 mg L^{-1} when sample wash rate was 4. Interference rates of sample wash rate 3 and 2 were lower than sample wash rate 4 under 90 h^{-1} sampling rates. (Table 2)

Recovered NO_3^--N of 15, 10, 5 mg L^{-1} were each of 14.65±0.01, 9.75±0.01, 4.95 ±0.04 mg L^{-1} when sample wash rate was 4, and it was no difference sample wash rate 3 and 2 under sampling rate 70 h^{-1}. There was no interference with DI water after sample except 15mg L^{-1} sample under 70 h^{-1} sampling rates. (Table 3)

Recovered NO_3^--N of 15,10,5 mg L^{-1} were each of 14.85±0.13, 10.01±0.02, 4.99 ±0.00 mg L^{-1} when sample wash rate was 4, and it was no difference sample wash rate 3 and 2 under sampling rate 50 h^{-1}. There was no interference with DI water after samples under 50 h^{-1} sampling rates. (Table 4)

Nitrate standard addition Standards ranging from 0 to 15 mg NO_3^--N L^{-1} in plant extracted were utilized (Table 5). NO_3^--N concentrate of mixed plant extracted sample was 1.52 mg L^{-1}. Recovered NO_3^--N concentrates were 4.01±0.01, 6.49±0.02, 8.99±0.01 and 16.55±0.01 at added NO_3^--N 2.5, 5, 7.5 and 15 mg L^{-1} in plant extracted sample. Recovery rates were 99.6±0.266, 99.4±0.515, 99.6±0.222 and 100.2±0.233

Table 2. Recovery of levels of NO_3^--N in standard solutions and interference with DI water under 90 h^{-1} sampling rate

Sample: Wash	Initial levels of NO_3^--N mg L^{-1}	Recovered NO_3^--N	1st DI-Water mg L^{-1}	2nd DI-Water
4:1	15	14.80±0.24	0.35±0.06	0.10±0.01
	10	9.98±0.06	0.24±0.01	0.09±0.00
	5	5.05±0.01	0.15±0.00	0.07±0.01
3:1	15	14.77±0.14	0.15±0.01	0.00±0.00
	10	9.91±0.04	0.04±0.02	0.00±0.00
	5	4.92±0.02	0.00±0.00	0.00±0.00
2:1	15	14.78±0.07	0.14±0.02	0.00±0.00
	10	9.98±0.03	0.05±0.01	0.00±0.00
	5	4.92±0.01	0.00±0.00	0.00±0.00

Table 3. Recovery of levels of NO_3^--N in standard solutions and interference with DI water under 70 h^{-1} sampling rate

Sample: Wash	Initial levels of NO_3^--N mg L^{-1}	Recovered NO_3^--N	1st DI-Water mg L^{-1}	2nd DI-Water
4:1	15	14.65±0.01	0.01±0100	0.00±0.00
	10	9.75±0.01	0.00±0.00	0.00±0.00
	5	4.90±0.04	0.00±0.00	0.00±0.00
3:1	15	14.54±0.24	0.10±0.01	0.02±0.03
	10	9.98±0.05	0.00±0.00	0.00±0.00
	5	5.01±0.05	0.00±0.00	0.00±0.00
2:1	15	15.01±0.06	0.02±0.02	0.00±0.00
	10	9.92±0.06	0.00±0.00	0.00±0.00
	5	5.17±0.00	0.00±0.00	0.00±0.00

Table 4. Recovery of levels of NO_3^--N in standard solutions and interference with DI water under 50 h^{-1} sampling rate

Sample: Wash	Initial levels of NO_3^--N mg L^{-1}	Recovered NO_3^--N	1st DI-Water mg L^{-1}	2nd DI-Water
4:1	15	14.85±0.13	0.00±0.00	0.00±0.00
	10	10.01±0.02	0.00±0.00	0.00±0.00
	5	4.99±0.00	0.00±0.00	0.00±0.00
3:1	15	14.90±0.10	0.00±0.00	0.00±0.00
	10	10.06±0.04	0.00±0.00	0.00±0.00
	5	5.02±0.03	0.00±0.00	0.00±0.00
2:1	15	15.01±0.02	0.02±0.02	0.00±0.00
	10	10.02±0.03	0.00±0.00	0.00±0.00
	5	5.11±0.15	0.00±0.00	0.00±0.00

Method Comparisons There was positive correlation between NO_3^--N by CFA method between NO_3^--N by alternate methods (salicylic acid method and RQflex method) (Fig 2). The correlation NO_3^--N values obtained by CFA and NO_3^--N values obtained by salicylic acid method was R^2=0.9671 and the correlation NO_3^--N values obtained by CFA and NO_3^--N values obtained by RQflex method was R^2=0.9739.

We could obtain high quality analyzed results at plant by CFA method to compare the other nitrate determination methods. Usually using method of nitrate determination is Cu-Cd method because of its high sensitive of nitrate reduction to nitrite. But this method is the efficiency of nitrate reduction by Cu-Cd reduction can be adversely affected by interferences in plant extracts (B. J. Alves et al., 2000). This CFA method to using hydrazine-copperhas high sensitive nitrate reduction in plant extracts (table 5). Also it has simplicity at determination process to compare the other methods. But CFA method has disadvantage which be interfered with previous sample (Table 2-4). But disadvantage can overcome to reduce sampling rate at 50 h^{-1}.

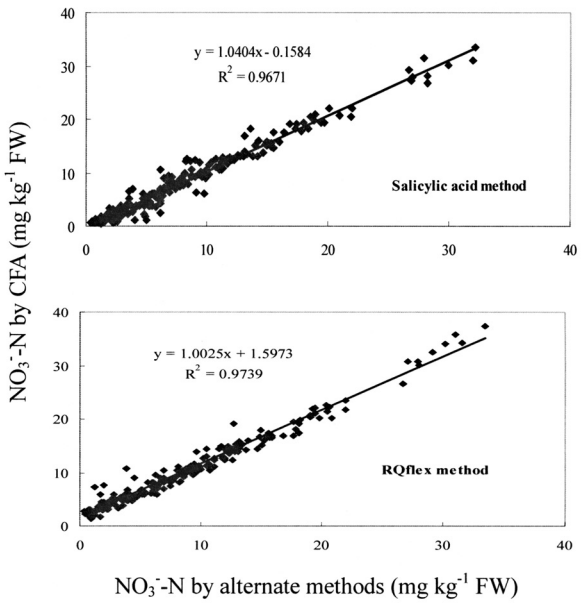

Fig 2. The relationship between NO_3^--N by CFA method and alternate methods (salicylic acid method and RQflex method).

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Reference

Alves, B. J., R, L., Zotarelli, A.S Resende, J.C. Polidoro, S. Urquigara, and R. M. Boddey. 2000. Rapid and sensitive determination of nitrate in plant tissue using flow injection analysis. Commumnicaion of Soil Sci. Plant Anal. 31 : 2739-2750.

AOAC method .1995. Official methods of analysis 16th edn. AOAC,

Table 5. Recovery of levels of NO_3^--N added to those of plant determined by CFA under the 50 h^{-1} sampling rate

Initial NO_3^--N mg L^{-1}		Added NO_3^--N mg L^{-1}	Recovered NO_3^--N mg L^{-1}	Recovery rate %
1.52	+	2.5	4.01±0.01	99.6±0.266
1.52	+	5	6.49±0.02	99.4±0.514
1.52	+	7.5	8.99±0.01	99.6±0.222
1.52	+	15	16.55±0.01	100.2±0.233

pp8-9.

Bertotti, M., and D. Pletcher .1997. Amperometric determination of nitrite via reaction with iodide using microelectrodes. Anal. Chin. Acta. 337 : 49-55.

Brinkered, E. F., and O. E. Kolari. 1975. The history and use of nitrite and nitrate in the curing of meet. Food Cosm. Toxi. 13: 655-661.

Buttv S. B., M. Riaz, and M. Z. Iqbal .2001. Simulaneous determination of nitrate in the curing of meet. Food and Cosmetics Toxicology. 13 : 655-661.

Cataldo, D.A., M. Haroon, L.E. Schrader, and V.L. Youngs. 1975. Rapid colorimetric determination of nitrate in plant tissue. Comm. Soil Sci. Plant Anayl. 6 : 71-80.

Haghighi, B., and A. Tavassoli. 2001. Flow-injection analysis of nitrate by reduction to nitrite and gas-phase molecular absorption spectrometry. Fresenius J. Anal. Chem. 371: 1113-1118.

Liang, B., M. Iwatsuki, and T. Fukasawa. 1994. Catalylic spectrophotometric determination of nitrite using the chlorpromazine hydrogen-peroxide dedox reaction in acetic-acid medium. Analyst. 119 : 2114-2117.

Li, J. Z., X. C. Wu, R.Yuan, H. G..Lin and R. Q.Yu .1994. Cobalt phthalocyanine derivatives as neutral carriers for nitrite-sensitive poly (vinyl chloride) membrane electrodes. Analyst. 119 : 1363-1366.

MAFF. Ministry of Agriculture, Fisheries and Food .1992. Nitrate and nitrite and N-niroso compounds in food: second report, Food surveillance paper 32.

Mattew J. M., D. James, G. C. Richard. 2001. Detection and determination of nitrate and nitrite : a review. Talanta. 54 : 785-803.

Öztekin, N., M.S. Nuku and F. B. Erim .2002. Simultaneous determination of nitrite and nitrate in meat products and vegetables by capillary electrophoresis. Food Chem. 76 : 103-106.

Hydrazine-copper 방법을 이용한 연속흐름제어장치를 통한 식물체의 nitrate 분석

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이연구의 목적은 연속흐름제어장치를 통한 식물체의 nitrate 분석방법 및 RQflex 방법 및 salicylic acid 방법과 비교를 통해 그 정확성을 확인하고자 함에 있다. 분석은 메론, 수박, 오이, 그리고 토마토 잎을 현장에서 채취 후, 분석시까지 초저온 냉동고에 보관후 분석하였다. RQflex로 측정한 결과가 CFA방법이나 salicylic acd 방법보다 약간 높게 나왔으며, CFA방법과 salicylic acid 방법과의 상관관계는 R^2=0.9671을 보였으며, RQflex와의 상관관계는 R^2=0.9739를 보였다.