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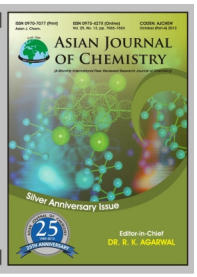

Ion Chromatographic Determination of Nitrite and Nitrate in Tobacco Leaf

LI WU[1], ZE-MIN SONG[2], YONG-AN CHEN[2], HONG-YING ZHU[3], ZHANG-HAI LI[3,*], YUN GAO[3], JUN YANG[3] and YI-DE HUNG[1,*]

[1]Department of Agriculture, Anhui Agricultural University, Hefei 230036, Anhui Province, P.R. China
[2]Qiannan Tobacco Branch Company, Guizhou Tobacco Company, Duyun 558000, Guizhou Province, P.R. China
[3]Research Center of Tobacco and Health, University of Science and Technology of China, Hefei 230052, Anhui Province, P.R. China

*Corresponding authors: Tel/Fax: +86 551 63492072; E-mail: lzhai@ustc.edu.cn; yidehuang@126.com

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A method for simultaneous determining NO_2^- and NO_3^- in tobacco leaf with ion chromatography was established by using internal standard quantitation based on Br^-. Tobacco samples were pretreated by ultrasonic extraction and solid phase extract clean up and then the resulting supernatants were injected into a high capacity anion exchange column with suppressed conductivity detection. The detection limits of NO_2^- and NO_3^- were 4.7 and 2.4 μg/L, respectively. Recovery were both above 95 % and RSD for either ion was less than 5 %. The method is simple, rapid, accurate, reliable and suited for the analysis of NO_2^- and NO_3^- in tobacco leaf samples.

Key Words: Ion chromatography, NO_2^-, NO_3^-, Tobacco leaf.

INTRODUCTION

The concentration levels of NO_2^- and NO_3^- in tobacco leaf are important indicators of tobacco quality and are also associated with carcinogenic of tobacco, because they are the major precursors of tobacco-specific nitrosamines[1,2]. However, as the levels of NO_2^- and NO_3^- in tobacco leaf are generally low, sensitive method is necessary for quantification.

The commonly used methods for determination of NO_2^- and NO_3^- were spectrophotometry[3,4] and fluorometry[5]. Whereas, NO_3^- was reduced to NO_2^- through a reductive column filled with zinc granules[3] or copperized-cadmium[4,5] and was finally determined as the summation[3-5] of NO_2^- and NO_3^-. Those determinations were performed by flow injection analysis[3-6] with higher sample throughput. However, the methods are not necessarily simple in the measurement system and problems with the stability and handling of the reduction column have been reported. Thus, the development of simple, fast and sensitive methods is desirable for the simultaneous determination of NO_2^- and NO_3^- in tobacco leaf.

Ion chromatography with ultraviolet and electrochemical detection have been used to determine ions[7-9], including NO_2^- and NO_3^{-}[10,11]. The columns of the chromatography are packed with ion exchanging resins instead of silica derived adsorbents. The detection is usually based on changes in conductivity, a nonspecific but rather sensitive technique. Conductivity has the advantage that it covers an enormous linear dynamic signal range of five orders of magnitude, which is far better than spectroscopic techniques, these are generally limited to two orders of magnitude[10]. NO_2^- and NO_3^- are quantified based upon standard curves obtained with authentic NO_2^- and NO_3^- solutions of different concentrations. It is expected that the introduction of internal standard in this method would improve the stability and accuracy of the method. It is important to remove the interferences due to large amounts of matrix anions such as Cl^-, SO_4^{2-} before ion chromatography[12,13]. The common purification methods were column-switching ion chromatography[13] and cationic surfactant coated ODS column[12]. In these methods, the analytes were measured with longer retention times (15-17 min) to remove the interferences by matrix ions[12,13]. Solid phase extraction is a simple and less time consuming purification method[14,15]. Thus solid phase extraction was chosen to purify the extract before determined by ion chromatography in this study.

In this paper, a new method for simultaneous determination of NO_2^- and NO_3^- in tobacco leaf samples by using the combination of solid phase extractions (C_{18}, Ag^+ and Na^+ solid phase cartridges) and ion chromatography with suppressed conductivity detection was described.

EXPERIMENTAL

A Dionex ICS-3000 (Sunnyvale, CA, USA) ion chromatograph consisted of a suppressed conductivity detection ASRS was used. The Ion Pac AS23- HC (250 mm × 4 mm) anion analysis column and Ion Pac AG23- HC (50 mm × 4 mm) protect column were obtained from Dionex. The eluent used

was composed of 4.5 mM Na_2CO_3-0.8 mM $NaHCO_3$. The injection volume was 25 μL and the eluent flow-rate was 1 mL/min.

The standard solution of 1000 mg/L $NaNO_3$ and 1000 mg/L $NaNO_2$ were purchased from MERCK Corp. (Germany). Potassium bromide was purchased from Sinopharm Chemical Reagent Co., Ltd. (Analytical grade, China). Deionized water was prepared by a Milli-Q Plus system at 18.2 M (MilliPore, Bedford, MA). C_{18} solid phase cartridges (1 mg), Ag^+ solid phase cartridges (1 mg) and Na^+ solid phase cartridges (1 mg) were purchased from Shanghai ANPEL Scientific Instrument Co., Ltd. (China).

Preparation of solid phase extraction columns: Prior to use, C_{18} cartridge (1 mg) was conditioned with 10 mL of methanol followed by 15 mL of deionized water under gravity. Ag^+ and Na^+ solid phase cartridge (1 mg) was actived with 10 mL of water under gravity.

Method for determining NO_2^- and NO_3^- in tobacco leaf samples: About 5 g of tobacco leaf samples and 100 mL of deionized water were added to 200 mL Erlenmeyer flask. The mixtures were homogenized and sonicated for 0.5 h at room temperature. Then, the supernatants were filtered using a type 1 filter paper (Whatman Co., Maidstone, UK). 20 mL of the extract was purified by through C_{18}, Ag^+ and Na^+ solid phase cartridges in turn. Only 10 mL of the extract was reserved and 0.2 mL of KBr (500 mg/L) was added as an internal standard. The extract was filtered with a microfilter 0.45 μm pore size (Teknokroma, Barcelona, Spain) and then analyzed by ion chromatography. The concentrations of NO_2^- and NO_3^- were calculated from the internal standard method for each anion separately.

RESULTS AND DISCUSSION

Solid phase extraction purification: Tobacco leaf sample is a well known complex matrix. Its extract contains some low weight organic matters such as pigments which can danger the column of ion chromatography. And some anions such as Cl^-, SO_4^{2-}, which can interfere with the target analysis[12,13] of NO_2^- and NO_3^-. So it is necessary to remove them before determined by ion chromatography. C_{18} solid cartridge was always used to remove organic matters from tobacco leaf extract[14]. And it was found that the colour of the extract became lighter after it passed the C_{18} cartridge, so it was chosen in our study. Ag^+ solid phase cartridge was chosen to remove Cl^-, SO_4^{2-}, because Cl^-, SO_4^{2-} can react with Ag^+ and be retained on the cartridge. And the colour of the cartridge became dark from light yellow after the extract passed it. However, some precipitations were also found in the extract, it might be due to the fact that some Ag^+ were washed out during the purification, so Na^+ cartridge was chosen to remove Ag^+.

Choice of the internal standard: The relative low concentration of NO_2^- and NO_3^- and the instability of instrument make its hard to quantitatively analyze NO_2^- and NO_3^- yields for tobacco samples, so internal standard method was chosen to quantity NO_2^- and NO_3^- in tobacco leaf sample. During the experiment, Br^- in tobacco leaf sample was not found, it might be due to its concentration very low. And Br^- does not interfere with NO_2^- and NO_3^-, as shown in Fig. 1. They can separate sufficiently from each other. Therefore, KBr was chosen as an internal standard in this study.

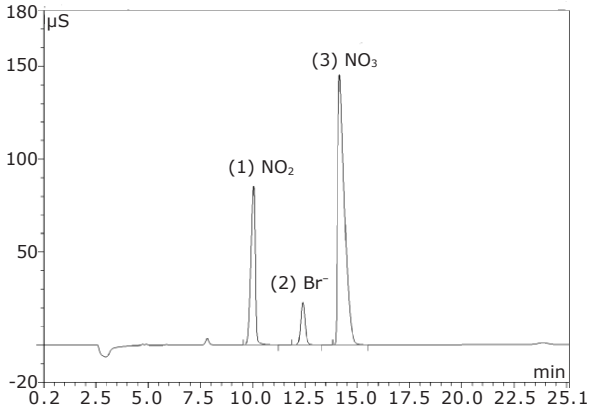

Fig. 1. Chromatogram of standard solution: (1) NO_2^-; (2) Br^-; (3) NO_3^-

Linearity, detection limits and reproducibility: Under the optimum experiment conditions, the analytes showed good linear relationship, sensitivity and reproducibility. The linearity data and coefficient constant for simultaneous determination of NO_2^- and NO_3^- was calculated. The linear ranges of the calibration plots for NO_2^- and NO_3^- were 5.0-500 μg/L (r^2 = 0.9993) and 5.0-100 mg/L (r^2 = 0.9997), respectively. The detection limits for NO_2^- and NO_3^- were determined at three times the noise and found to be equal to 1.7 and 2.4 μg/L, respectively. To assess the repeatability of this method, experiments were performed using spiked tobacco leaf sample. The analytical method recovery (%) and RSD (%) obtained for the NO_2^- and NO_3^- are listed in Table-1. Recovery of NO_2^- and NO_3^- from tobacco leaf samples spiked at low and high spiked levels were 94.4-95.8 and 98.0-100.5 %, respectively. RSDs (n = 4) for these two levels were in the ranges 3.2-4.6 and 2.6-3.8 %.

TABLE-1
RECOVERIES AND RSDs OF NO_2^- AND NO_3^-

Anion	Spiked (mg/L)	Determined (mg/L)	Recovery (%)	RSD (%)
NO_2^-	0.0500	0.0472	94.4	4.6
	0.1000	0.0958	95.8	3.2
NO_3^-	50.00	50.26	100.5	3.8
	100.0	98.04	98.0	3.6

Analysis of real tobacco leaf samples: To assess the applicability of the method to real tobacco leaf samples, eight tobacco leaf samples were analyzed according to the experimental conditions. Fig. 2 shows the chromatogram of tobacco leaf sample, from which it can be seen the analytes are separated sufficiently. The results was shown in Table-2. Therefore, it could be concluded that the present method was successfully applied to the fast and sensitive determination of NO_2^- and NO_3^- in real tobacco leaf samples.

Conclusion

The proposed analytical method using Br^- as internal standard for determining NO_2^- and NO_3^- in tobacco leaf is rapid, precise and accurate. The protocol of high purification efficiency of solid phase extractions combined with good separation of

TABLE-2
CONTENTS OF NO_2^- AND NO_3^- IN EIGHT TOBACCO LEAF SAMPLES

Sample	Anion							
	XL01	XL02	XL03	XL04	XL05	XL06	XL07	XL08
NO_2^- (μg/g)	8.05	0.11	6.67	0.76	1.02	1.50	16.1	24.6
NO_3^- (mg/g)	2.24	0.09	2.54	0.12	0.11	0.12	1.02	1.02

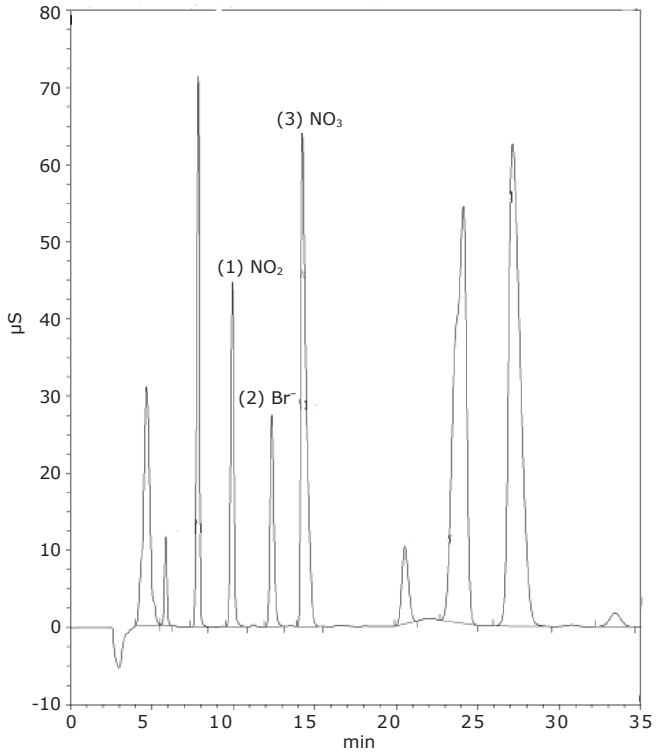

Fig. 2. Chromatogram of tobacco sample solution: (1) NO_2^-; (2) Br^-; (3) NO_3^-

anion exchange column was adopted to provide to a selective determination of target NO_2^- and NO_3^- in tobacco leaf sample without interference from the matrix components present in the tobacco leaf.

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