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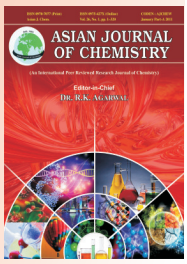

Simultaneous Determination of Nitrate and Nitrite in Wrapper by High-Performance Liquid Chromatography with Cloud-Point Extraction

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A simple, fast, sensitive and accurate reversed-phase HPLC methodology for simultaneous monitoring of nitrate and nitrite in wrapper is described. Nitrite was determined directly using the Griess diazo-coupling reaction and the formed azo dye was measured at 510 nm by HPLC with cloud point extraction. Nitrate based on the ion pair chromatography to be quantified by HPLC when UV light absorption responses at 210 nm. Ultrasonic-assisted extraction was utilized to reduce extraction time and improve extraction efficiency when nitrate and nitrite were extracted from the paper. The effective parameters of the extraction process such as ultrasonic auxiliary extraction time, the volume of nonionic surfactant (Triton X-114), sample pH, ionic strength, equilibration temperature and time were optimized. Under the optimum conditions, this method was characterized by an acceptable linear range of 5 to 800 ng/mL for nitrite, 0.1 to 10 μg/mL for nitrate and correlation coefficients of the calibration curves were both higher than 0.996. The limits of detection are 5 ng/mL and 100 ng/mL for nitrite and nitrate respectively. The proposed method was successfully applied to the extraction and determination of nitrite and nitrate in wrapper and the satisfactory relative recoveries (86.1-96.6 %) were obtained.

Keywords: Nitrite, Nitrate, Cloud point extraction, High-performance liquid chromatography, Wrapper.

INTRODUCTION

Nitrite and nitrate are ubiquitous within environmental, food manufactures, other industrial processes and biological fluids[1]. Packaging materials, such as cigarette paper also contains nitrite and nitrate, and characteristics of cigarette paper directly affect the combustibility, aroma components and flavor of cigarette[2]. The simultaneous determination and speciation of nitrite and nitrate have increasing attention in recent years because of their potential harmful impact on human health. Many analytical methods for the determination of nitrite and nitrate have been developed, including spectrophotometry[3-6], spectrofluorimetry[7-11], gas chromatography[12,13], high performance liquid chromatography[14,15], capillary electrophoresis[16], flow injection analysis[17] and ion chromatography[18]. However, they are limited for cross-reactivity and poor interlaboratory reproducibility. Furthermore, some methods needed complicated procedures of extraction or expensive instruments. The commonly used methods for nitrite determination include the spectrophotometric detection based on the Griess reaction[19], in which nitrite is diazotized with sulfanilamide and then reacted with N-(1-naphthyl)ethylenediamine to form a colored product. For the measurement of concentration of nitrate, it is necessary to reduce the nitrate to nitrite with copperised cadmium and the process of reduction is very complicated and time-consuming[20]. In addition, nitrite is usually present in paper in relatively low amounts compared to nitrate anion and its concentration falls under the detection limit. Because of this, a cloud point extraction was used to quantify the trace nitrite.

The goal of this paper was to obtain a simple, sensitive, fast and cost-effective method for the detection of nitrite and nitrate in wrapper. In the present study, a commonly available and easy to operate method was to develop for analyzing the nitrite and nitrate in wrapper by cloud point extraction using Triton X-114 as the extraction solvent.

EXPERIMENTAL

Experiments were performed using a HPLC system consisting of a vacuum degasser, an auto sampler, a quaternary pump and a diode-array detector (Agilent 1200 Series, Agilent Technologies, Calif, USA). An ultrasonic cleaner with temperature control (Shanghai, China) was used for ultrasonic extraction. A centrifuge with calibrated centrifugal tubes (Shanghai, China) was used for the phase separation process.

Standards of sodium nitrite and sodium nitrate were purchased from Aladdin Chemical Co. (Shanghai, China).

Acetonitrile and methanol were chromatographic grade (Tedia Company, USA). Except where noted, all reagents were of analytical grade and all solution preparations were made using doubly distilled-deionized water. Several different kinds of wrappers were purchased from local supermarket.

High performance liquid chromatography-DAD detection: Chromatographic separations were carried out using a reversed phase C18 analytical column of 150 × 4.6 mm (Agilent TC-C18). The mobile phase consisted of acetonitrile, methanol and doubly distilled-deionized water contained 1 % (v/v) tetrabutylammonium hydroxide (Aladdin, China) at pH 4. The gradient elution program was shown in Table-1. To get the optimum results, mobile phase with a flow rate of 1 mL/min and column temperature at 25 °C were used. The injection volume was 20 µL and the DAD detector was set at 220 and 510 nm which are the proper absorption wavelengths of nitrate and nitrite, respectively.

TABLE-1
GRADIENT ELUTION PROGRAM OF HPLC

Time (min)	Acetonitrile (%)	Methanol (%)	1 % Tetrabutylammonium hydroxide (%)
0	10	0	90
5.0	10	0	90
7.0	10	46	44
11.0	10	46	44
12.0	10	0	90

Preparation of solutions: Standard stock solutions of nitrate and nitrite were prepared by dissolving dried sodium nitrate and sodium nitrite (at 110 °C for 2 h) in doubly distilled-deionized water at a concentration of 500 µg/mL. Working solutions were prepared by an appropriate dilution of the stock solutions on the day of use. Griess A reagent, 100 mg *p*-amino-benzene sulfonic acid was dissolved in a mixture consisting of 5 mL acetic acid and 5 mL purified water by water bath heating (45 °C). Griess B reagent was prepared by dissolving 10 mg 1-naphtylamine in 10 mL acetic acid. 10 mL of Triton X-114 (Sigma, USA) was dissolved in 100 mL double distilled-deionized water for a Triton X-114 (10 %).

Preparation of sample: 0.2 g wrapper was weighed accurately and placed in a screw-cap glass centrifuge tube with a conical bottom with 10 mL doubly distilled-deionized water. The centrifuge tube was placed in ultrasonic cleaner at 25 kHz of ultrasonic frequency and 25 °C for 10 min. The extraction solution was filtered using qualitative filter paper into a new tube. Afterwards, pH of sample was adjusted to pH 5 by the addition of dipotassium hydrogen phosphate-potassium dihydrogen phosphate buffer solution. The 5 mL extraction solution was subjected to the cloud-point extraction procedure.

Cloud-point extraction procedure: A 5 mL prepared extraction solution of paper containing nitrite was placed in a screw-cap glass centrifuge tube with a conical bottom. Then 100 µL of Triton X-114 and 0.3 g $(NH_4)_2SO_4$ were added into the sample solution and mixing uniformity by vortex. A cloudy solution was formed after the solution was placed in a thermostat bath at 45 °C for 10 min. After centrifugation at 4000 rpm for 10 min, the emulsion was detached. The aqueous phase was removed and the surfactant-rich phase was deposited at the bottom of the tube. Then the surfactant-rich phase was diluted to 0.4 mL with methanol and sample was filtered through 0.45 µm nylon syringe filter (troody, China) in order to remove particles. 20 µL of solution was injected into the HPLC system for analysis. A blank solution was also run using water instead of nitrite.

RESULTS AND DISCUSSION

The variables investigated were extraction way and the type of ion pair reagent, volume of Triton X-114, pH, the addition of $(NH_4)_2SO_4$, the equilibration temperature. Quantitation was carried out by the peak area method. In this experiment, 5 mL of prepared sample spiked with 5 µg/mL of nitrate and 100 ng/mL of nitrite was used for the study. All the experiments were performed 5 times and the averages of the results were used for optimization.

Effect of sonication time: Ultrasound has been used to induce physical or chemical effects in a medium by using low-frequency ultrasound for a period time. This extraction can be accelerated by ultrasonic waves and experiments show the enhancement of the absorbance in the presence of ultrasonic waves. The ultrasonic time was also investigated. It was found that an exposure time of 10 min is enough for the best sensitivity.

Effect of sample pH: An acid medium is necessary for NO_2^- reacts with Griess reagent to form diazo compound, but too excessive amount of H^+ is disadvantageous to extraction efficiency. The effect of pH on the absorbance at a constant concentration of nitrite in surfactant-rich phase was investigated in the range 3-7. As can be Fig. 1, the highest signal intensity of nitrite was obtained at pH 5. Therefore the optimum pH was used as 5 in the experiment.

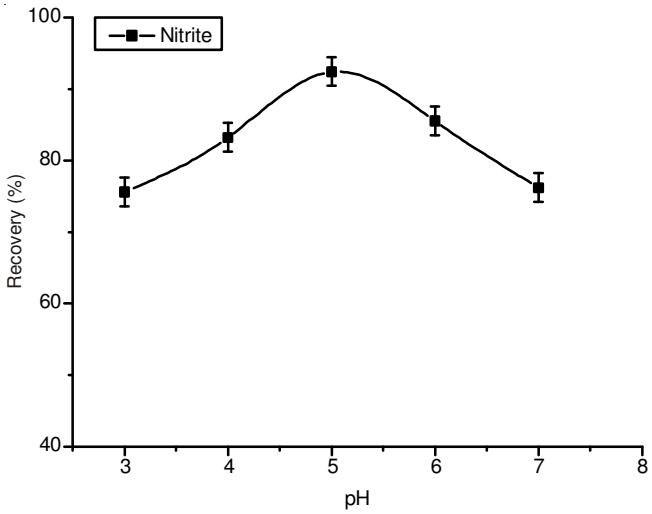

Fig.1. Effect of sample pH

Effect of Triton X-114 concentration: The surfactant concentration is a critical factor for effective extraction and should be sufficient for quantitative extraction of the trace nitrite. The extraction solvent should have similar hydrophobicity to the target analytes and form a stable cloudy system with the presence of Triton X-114 and $(NH_4)_2SO_4$. The effect of the concentration of Triton X-114 on the extraction efficiency was investigated in the range 0.2-1.8 % (v/v). As Fig. 2 shows, 1 % Triton X-114 was selected in the subsequent experiments.

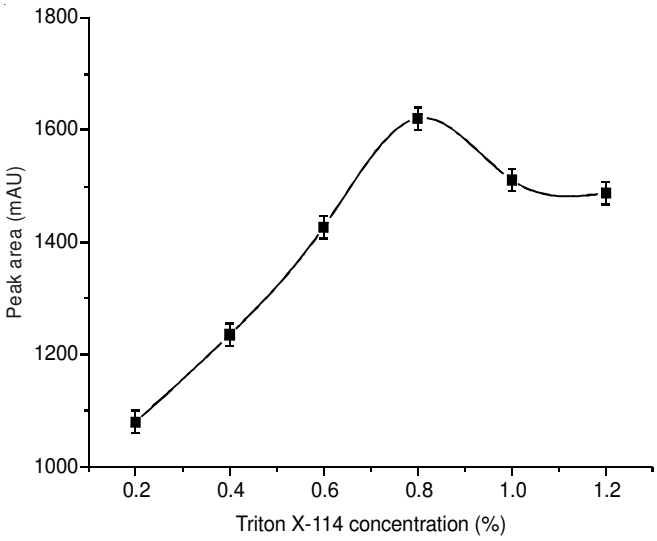

Fig. 2. Effect of Triton X 114 concentration

Effect of salt amount: The addition of salt can increase the incompatibility between the water structures in hydration shells of ions and surfactant macromolecules. Addition of salt can decrease the cloud-point temperature and accelerate the phase separation by enhancing the micellar concentration in the surfactant-rich phase. The addition of $(NH_4)_2SO_4$ was investigated in the concentration range of 0.1-0 5g. AS shown in Fig. 3. The adequate addition of $(NH_4)_2SO_4$ is 0.3 g to achieve quantitative extraction.

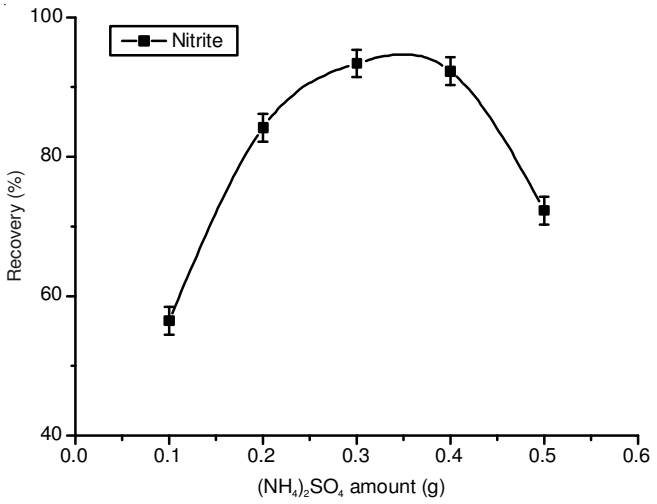

Fig. 3. Effect of salt amount

Effect of the equilibration temperature and time: Equilibration time and equilibration temperature are two important parameters in cloud point extraction. It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature as a compromise between completion of extraction and efficient separation of phases. The dependence of extraction efficiency upon equilibrium temperature and time was studied over ranges of 30-70 °C and 10-50 min, respectively. The results showed that an equilibrium temperature of 45 °C is appropriate and 10 min as equilibration time is enough for nitrite derivatives in order to achieve quantitative extraction.

Characteristics of analytical method: Table-2 shows some analytical characteristics of the optimized method, including regression equation, linear range, limits of detection and enrichment factors. The linearity of nitrite was in the range 5-800 ng/mL and of nitrate in the range 0.1-10 µg/mL. The detection limits were 5 ng/mL for nitrite and 100 ng/mL for nitrate, respectively. The relative standard deviation (RSD) of 100 and 5 µg/mL for nitrite and nitrate was less than 4.5 %.

TABLE-2
PERFORMANCE CHARACTERISTICS OF THE PROPOSED METHOD

Analyte	Regression equation	Linear range (ng/mL)	RSD (%) n=6	r^2	Limit of detection (ng/mL)
Nitrate	Y=1.34X+89.114	10-5000	4.33	0.9980	100.0
Nitrite	Y=14.76X+14.571	5-800	3.85	0.9961	5.0

Application: The accuracy and validity of the proposed method was checked by applying the determination of nitrite and nitrate concentration in various wrapper samples. Recovery studies were also carried out after it was spiked to samples known concentrations of nitrite at levels of 100 and 300 ng/g and of nitrate at levels of 5 and 10 µg/g. The accuracy, precision and reproducibility were verified by recovery studies. As presented in Tables 3 and 4, the recoveries for the addition of different concentrations of nitrite and nitrate to samples are in the range of 86.1-96.6 %. The results show that the proposed method is effective for the determination of trace amounts of nitrate and nitrite in the real cigarette paper samples.

TABLE-3
NITRATE AND NITRITE FOUND IN WRAPPER SAMPLES AND RECOVERIES OBTAINED AFTER SPIKING THE SAMPLES WITH NITRATE AND NITRITE AT TWO CONCENTRATION LEVELS

Sample	Spiked		Found		Recovery (%)	
	Nitrite (µg/mL)	Nitrate (ng/mL)	Nitrite (ng/mL)	Nitrate (µg/mL)	Nitrite (ng/mL)	Nitrate (µg/mL)
1	0.0	0.0	10.63 ± 0.1	4.82±0.04		
	100.0	5.0	108.22±0.1	9.12±0.1	88.6	86.1
	300.0	10.0	300.51±0.1	13.53±0.1	96.6	87.1
2	0.0	0.0	18.7±0.2	3.56±0.1		
	100.0	5.0	112.57±0.1	8.12±0.2	92.8	91.2
	300.0	10.0	298.35±0.1	12.35±0.1	93.2	87.9

TABLE-4
NITRATE AND NITRITE FOUND IN BLANK WRAPPER SAMPLES, AND RECOVERIES OBTAINED

	Samples		
	1	2	3
Nitrite (ng/mL)	0.014	0.024	0.022
Nitrate (µg/mL)	4.67	3.48	5.22

Conclusion

The new proposed procedure gives a sensitive and selective for the determination of nitrate and nitrite ions in wrapper samples. In this study, ion pair chromatography was developed for direct determination for nitrate. The cloud point extraction preconcentration was used to analysis the trace nitrite. The results indicate the method has high recovery and good repeatability,

short extraction time and good linearity over the investigated concentration range. The use of easily available equipments, low cost, easy sample preparation and better performance than the other published methods is the superiority of this method. The results showed that the nitrite and nitrate could be effectively monitored by this method and it was also a good reference for wrapper.

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