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Sequential flow injection analysis of ammonium and nitrate using gas phase molecular absorption spectrometry

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Abstract

A flow injection method on the basis of gas phase molecular absorption is described for the sequential determination of ammonium and nitrate. Two hundred microliters of sample solution is injected into the flow line. For ammonium determination, the sample zone is directed to a line in which reacts with NaOH (13 M) and produces ammonia. But for nitrate determination, the sample zone is passed through the on-line copperized zinc (Zn/Cu) reduction column and produces ammonium ion and in the follows ammonia. The produced ammonia in both cases is purged into the stream of N_2 carrier gas. The gaseous phase is separated from the liquid phase using a gas–liquid separator and then is swept into a flow through cell, which has been positioned in the cell compartment of an UV-Vis spectrophotometer. The absorbance of the gaseous phase is measured at 194 nm. Under selected conditions for sequential analysis of ammonium and nitrate, linear relations were found between the peak heights of absorption signals and concentrations of ammonium (10–650 $\mu g\,ml^{-1}$) and nitrate (20–800 $\mu g\,ml^{-1}$). The limit of detections for ammonium and nitrate analysis were 8 and 10 $\mu g\,ml^{-1}$, respectively. The relative standard deviations of repeated measurements of 50 $\mu g\,ml^{-1}$ of ammonium and nitrate were 2.0, 2.9%, respectively. Maximum sampling rate was about 40 samples/h. The method was applied to the determination of ammonium in pharmaceutical products and the sequential determination of ammonium and nitrate in spiked water samples.

Keywords: FIA; GPMAS; Ammonium; Nitrate

1. Introduction

Ammonia is a significant alkaline pollutant in the atmosphere. Ammonia is released to the atmosphere from two main sources: soil and water. Ammonia reaches soil and water usually from industrial processes, natural or synthetic fertilizer application, animal excreta, decaying organic matter, or natural fixation from the atmosphere. Ammonia in soil and water can volatilize to the atmosphere or undergo microbial transformation to nitrate or nitrite anions, or be taken up by plants. Ammonia emitted into the troposphere is readily trapped by acidic cloud droplets and neutralizes the acidity of the droplets to form ammonium salt or reacts with acidic gases to form aerosol [1]. Therefore, the determination of ammonium ion in wet deposition is important in atmospheric chemistry.

Nitrate is naturally present in plants and its concentration varies enormously. The nitrate content in vegetable may be influenced by factors related to the plant and the environment. Furthermore, nitrate, as well as nitrite, have been added intentionally at the curing process of certain meat products, due to their ability to inhibit the outgrowth spores of *Clostridium botulinum* and to impart characteristics color and flavor to this kind of foodstuffs. The significance of nitrate to human health is related to the fact that nitrate, after being metabolized or reduced to nitrite, can react with secondary or tertiary amines to form N-nitroso compounds, which are potent carcinogens. Nitrite can also interact with hemoglobin influencing the oxygen transport mechanism, giving rise to methemoglobinemia [2].

The frequent presence of ammonium (or ammonia), nitrate and nitrite in a wide variety of environmental, clinical

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and industrial samples has stimulated the development of a large number of methods for their determination.

The most widely used methods for determination of ammonium (or ammonia) and nitrate (after reduction to nitrite) are spectrophotometric and based on the Berthelot [3] (or to a lesser extent the Nessler) and Griess reactions [3,4], respectively. A variety of analytical methods such as chromatographic [5–7], potentiometric [8], conductimetric [9], and chemiluminescence [10] for ammonium determination and chromatographic [5], fluorimetric [11], amperometric [12], voltammetric [13], chemiluminescence [14,15], and capillary electrophoresis [16] for the determination of nitrate have been developed. Two papers critically review the performances, merits and shortcomings of some papers on ammonium (or ammonia) [17] and nitrate [18] determination deal with flow procedures like flow injection analysis.

Most of the reported methods use expensive or unstable reagents that must be prepared fresh daily or assemblies including membranes that must be periodically cleaned and calibrated as their performances change with time. Many of them entail prior separation of the analyte or masking of potential interferences, this makes batch processes time-consuming, especially when distillation is required.

Gas-phase molecular absorption spectrometry (GPMAS) was introduced by Syty [19] and developed in Cresser's laboratory [20]. The GPMAS technique has been applied to the determination of anions and cations in solution by conversion of the determinant into a volatile molecular species followed by their molecular absorbance measurements in the gas phase. A stream of carrier gas carries the gaseous product into a flow-through absorption cell, which is positioned in the light path of the spectrometer in the space normally occupied by the flame of the atomic absorption spectrometer. The absorbance signal of the compound is measured at the selected wavelength, which is corresponding to the absorption maximum of the evolved compound. Ammonium [21] and nitrate [22,23] have been previously determined using GPMAS method and AAS as the detection system. The introduction of diode-array detection systems in UV-Vis spectrophotometers allows new development in GPMAS by Sanz and co-workers [24]. Using conventional UV-Vis spectrophotometer instead of an AAS as the detection system in conjugation with FIA [25–28] allows the GPMAS became simpler, faster and reliable than before.

This paper describes the development and application of a FIA–GPMAS method for the determination of ammonium and nitrate in different matrices, which allows to solve many limitations of the previous works. In the proposed method, ammonium which is originally present in sample solution and produced ammonium from reduction of nitrate in sample solution using on-line copperized zinc (Zn/Cu) reduction column react with the stream sodium hydroxide solution. The evolved ammonia gas is purged into the stream of N_2 carrier gas. The gaseous phase is separated from the liquid stream in a home-made gas–liquid separator [25] and then is swept by the carrier N_2 gas into a home-made flow-through cell [25], which has been positioned in the space of the cell compartment of an UV-Vis spectrophotometer. The transient absorbance of the gaseous phase was measured at 194 nm.

2. Experimental

2.1. Apparatus

The flow system used in this work is shown in Fig. 1. A Watson Marlow 505Du peristaltic pump propelled carrier and sodium hydroxide solutions using silicon manifold tubing (1.02 and 0.76 mm i.d.), respectively. The sample solution was injected via a Rheodyne six-way Teflon rotary valve type 50 into the carrier stream. The flow lines were made from Supelco Teflon tubing (0.5 mm i.d.). The gaseous phase was separated form liquid phase using a home-made gas–liquid separator. The absorbance was measured with a Pharmacia Ultrospec 4300 *pro* UV-Vis spectrophotometer with a home-made flow through cell (a cylindrical block of polyethylene with 50 mm length, 30 mm o.d., 5 mm i.d. and quartz windows). Pharmacia Biotech MultiTemp III thermostatic water circulator was used to study of temperature effect. The copperized zinc (Zn/Cu) reduction column was made from a glass column (3 mm i.d. × 8 cm) filled with the copperized zinc (particle size: 0.3–1.5 mm). The transient absorption data was collected at 1 s intervals using SWIFT II software.

2.2. Reagents

All reagents were of analytical reagent grade from Fluka and were used without further purification. Working solutions were freshly prepared by accurate dilution of the stock solutions. Deionized, triply distilled water was used for preparing all solutions and diluting them.

Ammonium standard solution: A stock solution of ammonium (2000 μg ml^{-1}) was prepared by dissolving 0.5946 g of NH_4Cl to 100 ml with water.

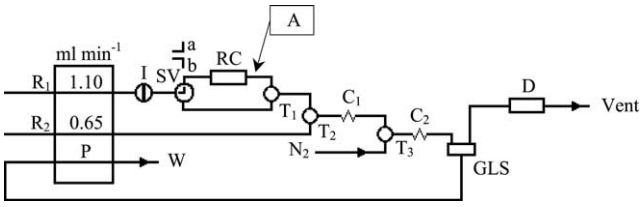

Fig. 1. Schematic FIA diagram for sequential determination of ammonium and nitrate. P, pump; I, injection valve (200 μl); SV, selection valve; T_1, T_2, T_3, three way connectors; D, spectrophotometric detector with a home-made flow cell (194 nm); GLS, gas–liquid separator; W, waste. Conditions: R_1, NaOH (3 M); R_2, NaOH (13 M); RC, Zn/Cu reduction column (3 mm i.d. × 8 cm); C_1, reaction coil (0.5 mm i.d. × 40 cm); purging coil (0.5 mm i.d. × 40 cm); N_2, carrier gas (79.5 ml min^{-1}); A, disconnection point for column regeneration.

Table 1
Selected chemical and FIA parameters for determination of ammonium while the SV is in "b" position

Parameters	Studied range	Selected value	Experimental observation on signal intensity	Mathematical model of the effect of parameter (X) on signal intensity (Y)[a]
Reagent flow rate				
R_1 (ml min^{-1})	1.10–3.90	1.10	Decreased exponentially	$Y = 0.2819\exp(-0.322X)$
R_2 (ml min^{-1})	0.65–2.29	0.65		
Gas flow rate (ml min^{-1})	31.2–71.5	55.4	Increased linearly	$Y = -0.103 + 0.0048X$
Length of reaction coil				
C_1 (cm)	10–60	40	No significant effect	$Y = 0.1531 \pm 0.01$
C_2 (cm)	10–60	40	No significant effect	$Y = 0.1704 \pm 0.01$
Size of sample loop (μl)	50–300	200	Increased logarithmic	$Y = 0.1255\ln(X) - 0.410$
Temperature				
C_1, C_2, GLS (°C)	25–50	25	No significant effect up to 40 °C and then increased linearly	$Y = 0.240 \pm 0.01$
				$Y = -0.1765 + 0.0105X$
Concentration of reagents				
$R_1 = H_2O$				
R_2 = NaOH (M)	4–14	13	Increased exponentially	$Y = 0.004\exp(0.2647X)$

[a] $[NH_4^+] = 500\ \mu g\ ml^{-1}$.

Nitrate standard solution: A stock solution (2000 μg ml^{-1}) was prepared by dissolving 0.3260 g of potassium nitrate in water to give a 100 ml solution.

Sodium hydroxide solution: Working solution of sodium hydroxide (13 M) was prepared by dissolving 52.0 g of NaOH to 100 ml with water.

Copper sulfate solution: A stock solution of copper sulfate (5%) was prepared by dissolving 2.5 g of $CuSO_4{\cdot}5H_2O$ to 50 ml with water.

2.3. Procedures

2.3.1. Preparation of Zn/Cu reduction column

The copperized zinc reduction column was prepared and conditioned using on-line procedure. For this purpose, the glass column (3 mm i.d. × 8 cm) was filled carefully with new or used Zn granules (particle size: 0.3–1.5 mm). The filled Zn column was positioned in the proposed manifold (Fig. 1). The selection valve was set to "a" position and the flow line was disconnected at "A". The waste of manifold in the on-line reductor column generation was "A". The Zn column was washed (1 min) and rinsed (2 min) with HCl solution (5 M) and distilled water as the carrier solutions, respectively. The carrier solution was replaced by a $CuSO_4$ solution (5%), which flowed into the column for 1 min or until black colloidal precipitates was developed. Finally, the Zn/Cu column was washed with distilled water (as the carrier for 2 min) to remove all colloidal precipitated Cu. The flow line was reconnected again at "A" for nitrate reduction. Flow rate of carrier solution in all steps was 2 ml min^{-1}. The

Table 2
Selected chemical and FIA parameters for determination of nitrate while the SV is in "a" position

Parameters	Studied range	Selected value	Experimental observation on signal intensity
Length of Zn/Cu column (cm)	2–10	8	Reached to the plateau
Reagent flow rate			
R_1 (ml min^{-1})	0.60–2.80	1.10	Passed over maximum (slightly)
R_2 (ml min^{-1})	0.35–1.65	0.65	
Gas flow rate (ml min^{-1})	31.2–87.6	79.5	Passed over maximum
Length of reaction coil			
C_1 (cm)	10–60	40	No significant effect
C_2 (cm)	10–60	40	No significant effect
Size of sample loop (μl)	50–300	200	Increased logarithmic
Temperature			
C_1, C_2, GLS (°C)	25–50	25	No significant effect up to 40 °C and the increased linearly
Concentration of reagents			
R_1 = NaOH (M)	1–5	3	Reached to the plateau
R_2 = NaOH (M)	4–14	13	Increased exponentially

Table 3
Analytical features for ammonium determination using proposed method under conditions given in Table 1

Calibration equation	r	Linearity ($\mu g\,ml^{-1}$)	RSD (%, $n = 6$)	LOD ($\mu g\,ml^{-1}$)	S (samples h^{-1})
$A = -0.0018 + 0.00054C$	0.9984	10–1200	2.6 ($x = 250\,\mu g\,ml^{-1}$)	8	40 (maximum)
$A = 0.2364 + 0.00033C$	0.9984	1200–4600	1.5 ($x = 500\,\mu g\,ml^{-1}$)		
			1.2 ($x = 1000\,\mu g\,ml^{-1}$)		

A, absorbance; C, amount of ammonium ($\mu g\,ml^{-1}$); r, correlation coefficient; RSD, relative standard deviation; LOD, limit of detection; S, sample throughput.

Table 4
Analytical features for sequential analysis of ammonium and nitrate using proposed method under conditions given in Table 2

Species	Calibration equation	r	Linearity ($\mu g\,ml^{-1}$)	RSD (%, $n = 6$)	LOD ($\mu g\,ml^{-1}$)	S (samples h^{-1})
NH_4^+	$A = -0.0064 + 0.00080C$	0.9993	10–650	2.0 ($x = 50\,\mu g\,ml^{-1}$)	8	40 (maximum)
				1.4 ($x = 100\,\mu g\,ml^{-1}$)		
NO_3^-	$A = -0.00085 + 0.00010C$	0.9984	20–800	2.9 ($x = 50\,\mu g\,ml^{-1}$)	10	
				1.8 ($x = 200\,\mu g\,ml^{-1}$)		

A, absorbance; C, amount of species ($\mu g\,ml^{-1}$); r, correlation coefficient; RSD, relative standard deviation; LOD, limit of detection; S, sample throughput.

Zn/Cu reduction column was regenerated by the procedure mentioned every day.

2.3.2. Sequential determination of ammonium and nitrate

Sample solution containing ammonium and nitrate was injected at I into the carrier stream containing sodium hydroxide solution (3 M). The switch in the "b" position resulted in a bypass of the Zd/Cu column by the analyte zone and mixing with sodium hydroxide solution (13 M), directly at T_2. The reaction of ammonium with sodium hydroxide was completed during the flowing in C_1. N_2 gas was introduced into the flow line at T_3. The evolved gaseous product (ammonia) diffused into the N_2 segments, during the flow in C_2. The gaseous phase was separated form the liquid phase using a gas–liquid separator and then was swept into the flow-through cell, which had been positioned, in the optical path of the UV-Vis spectrophotometer. The molecular absorbance of the gaseous phase was recorded at 194 nm and at 1 s intervals. At this mode of operation only ammonium of analyte zone reacted with the sodium hydroxide solution (13 M) and gave an absorption signal. But, the switch in the "a" position passed the analyte zone through the Zn/Cu column, where nitrate was reduced to ammonium, and the absorption signal arising from the sum of both ammonium and nitrate was measured. Nitrate was determined from the difference between intensities of the absorption signals.

3. Results and discussion

3.1. Optimization of the flow injection manifold

The background and product (ammonia) absorption spectra of the gaseous phase (Fig. 2) were obtained by continuous propelling of distilled water and ammonium solution (500 $\mu g\,ml^{-1}$), respectively, as the carrier stream (R_1) in the proposed manifold while the selection valve (SV) was in "b" position. Using the SV, while it was in "a" or "b" position, the proposed flow injection manifold was optimized for determination of nitrate and ammonium, separately. Separate standard solutions (1000, 500, and 200 $\mu g\,ml^{-1}$) of ammonium and nitrate were used. A wavelength of 194 nm was employed and using univariate optimization method the influences of the hydrodynamic and chemical parameters on the magnitude of the peak height (peak intensity) were studied. Tables 1 and 2 show the result of the optimization of working conditions for ammonium and nitrate determination, separately. During the course of optimization the reproducibility of the results ($n = 3$) was better than 4%.

Obtained calibration curve in each step of optimization procedures for both nitrate and ammonium determination showed linear behavior. The behaviors of changes C_1 and C_2, temperature, sample loop, and concentration of NaOH

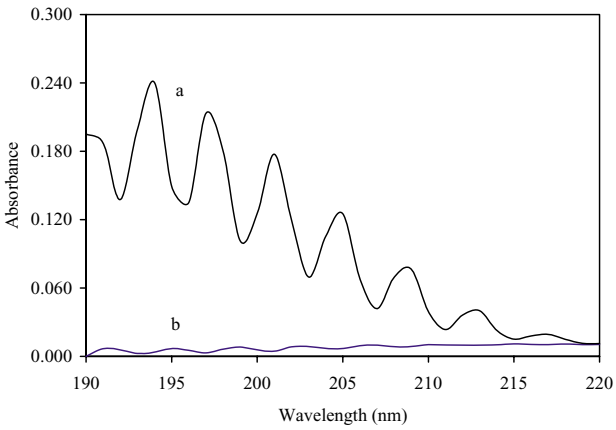

Fig. 2. The background (b) and product (a) absorption spectra of the gaseous phase when distilled water and ammonium solution (500 $\mu g\,ml^{-1}$) were propelled continuously, as the carrier stream (R_1) while SV was in "b" position.

(R_2) on signal intensities were very similar in both separate determination of ammonium and nitrate using proposed manifold (Tables 1 and 2). Additionally, in the determination of ammonium by replacing H_2O (R_1) with NaOH 3 M (i.e. R_1 with its optimum value in nitrate determination), no significant changes in results of ammonium determination were observed. So, it seems the dominant species in nitrate determination at T_2 is ammonium ion, which in the follows transforms to ammonia. In other word, in the Zn/Cu column nitrate is reduced to the ammonium and because of lack of time and/or reagent no extensive conversion to ammonia is done. So, on the basis of the mentioned results the optimum conditions, which have been shown in Table 2, were selected for determination of ammonium and nitrate in a sample, sequentially.

3.2. Analytical performance of the proposed methods

The calibration graphs for ammonium determination under conditions given in Table 1 and for sequential ammonium and nitrate determination under conditions given in Table 2, obtained using proposed method with the injection of a series of standard ammonium and nitrate solutions. The limit of detections were determined experimentally on the basis of three times of the standard deviation of the measurements of blank signals [29]. The reproducibility of the proposed method was determined by repetitive injection of standard ammonium and nitrate solutions. Typical calibration trace for nitrate determination is shown in Fig. 3. The analytical features such as calibration equation, correlation coefficient, linear dynamic range, limit of detection, and sample throughput have been summarized in Tables 3 and 4.

3.3. Interferences and removal of interfering ions

The influence of co-existing ions was examined by testing a series of solutions containing 200 $\mu g\,ml^{-1}$ ammonium (or nitrate) plus potential interfering ions (20 000 $\mu g\,ml^{-1}$).

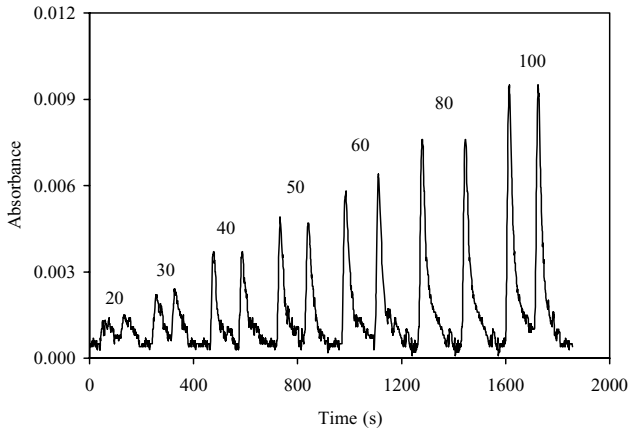

Fig. 3. Typical calibration trace for a series of standard nitrate solutions. Number above peaks denotes concentration of nitrate in $\mu g\,ml^{-1}$.

The responses of mentioned test solutions were compared with those obtained from the uncontaminated ammonium (or nitrate) solutions. In these studies a 5% error criterion was adopted. Nitrate, nitrite, carbonate, phosphate, sulfide, sulfite, sulfate, chloride, bromide, iodide, cyanide, and thiocyanide did not interfere in ammonium determination. Sulfide and nitrite interfered in nitrate determination. Sulfide decreased the reduction efficiency of the Zn/Cu column and regeneration of the Zn/Cu column was essential for further uses. The observed overall reduction and conversion efficiency of nitrate to ammonia at the optimum conditions of Table 2 was about 90%, but after injection of a sulfide solution (200 $\mu g\,ml^{-1}$) the observed overall efficiency decreased to 20%. Nitrite reduced to ammonium as the same overall reduction and conversion efficiency for nitrate (i.e. 90%). Acidification and heating eliminated the interference from S^{2-} and NO_2^-. Cations including Ca^{2+}, Ba^{2+}, Mg^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, and Fe^{3+} were examined. They produced insoluble metal hydroxides and blocked the flow line in the studied range of 1000–20 000 $\mu g\,ml^{-1}$. Investigation on sample solutions containing 200 $\mu g\,ml^{-1}$ of nitrate plus 200 $\mu g\,ml^{-1}$ of cations showed the flow line is not blocked by the colloidal precipitate and the error due to their presence are less than 5%. Cations (2000 $\mu g\,ml^{-1}$) were removed from sample solution using OH^- (2 M) prior to ammonium and nitrate determination. Drop by drop adding of NaOH (2 M) to the sample solution until is no further precipitate seen, and also adding of NaOH to the sample solution to make a 2 M sample solution with respect to NaOH were performed. Both procedures caused precipitation of bulk amounts of metal ions with no evolution of ammonia. The results showed, cations (2000 $\mu g\,ml^{-1}$) are removed from sample solution by precipitate removal and the error due to the presence of remaining cations (soluble or even complex forms) and adsorption of analyte by precipitate are less than 5%.

Study on Acetaminophen, Clobutinol, Chlorpheniramine showed that the amine groups in the mentioned organic compounds did not cleavage under experimental conditions and did not interfere in ammonium determination.

4. Application

The presented procedures were successfully applied to the determination of ammonium in some pharmaceutical products and sequential determination of ammonium and nitrate in spiked water samples. Water samples were examined directly using the procedure proposed without any pretreatment. Pharmaceutical products samples were diluted two times before FIA measurement. To examine the reliability of the method certain amounts of standard ammonium and nitrate solutions were added to the sample solutions and analyzed according to the proposed method. Recovery of each measurement was calculated by comparing the results obtained before and after adding the ammonium and nitrate

Table 5
Determination of ammonium and nitrate in some spiked samples ($n = 3$) using proposed procedures

Sample	Amount of ammonium ($\mu g\,ml^{-1}$)					Amount of nitrate ($\mu g\,ml^{-1}$)				
	Determined	Added	Found	RSD (%)	Recovery (%)	Determined	Added	Found	RSD (%)	Recovery (%)
Diphenhydramine (s)	82	–	–	2.0	98[a]	n.d.				
	200	–	–	1.5	100[b]					
Expecturant (s)	80	–	–	2.3	100[a]	n.d.				
	206	–	–	1.2	103[b]					
Well water[c]	None									
		80	88	2.0	110	30	30	29	2.8	97
		120	118	1.7	98		75	77	2.5	103
		160	166	1.5	104		120	123	2.1	102
Tap water[d]	None					None				
		20	19	2.7	95		50	54	3.1	92
		50	42	2.3	84		70	62	2.8	89
		200	184	1.1	92		500	569	1.1	87
Well water[e]	None									
		40	40	2.5	100	220	40	40	1.9	100
		80	80	2.1	100		80	75	1.6	94
		120	130	1.3	108		120	115	1.2	96
		160	160	1.4	100		160	155	1.3	97

RSD, relative standard deviation; s, syrup; n.d., not determined.
[a] Certified value = 80 $\mu g\,ml^{-1}$.
[b] Certified value = 200 $\mu g\,ml^{-1}$.
[c] IASBS well water.
[d] Zanjan tap water.
[e] Mahabad well water.

standard solutions. The amounts of ammonium and nitrate in the samples taken and their recoveries have been shown in Table 5.

5. Conclusions

The method described in this paper is safe, simple and low cost and can be applied to the various types of samples containing more than 10 $\mu g\,ml^{-1}$ of ammonium and nitrate. Reliability, reproducibility, time saving, low reagent consumption, need of small sample volume, large dynamic range and high sample throughput (up to 40 samples h^{-1}) are important features of the proposed FIA system. The method is practically attractive because of introducing of UV-Vis spectrophotometer instead of AAS as the detection system [21]. Determination of nitrate using copperized zinc (Zn/Cu) reduction column with respect to copperized cadmium (Cd/Cu) reduction method [26] offered shorter linear dynamic range. Interferences in the proposed method are few and are readily overcome. Amine groups in Acetaminophen, Clobutinol, Chlorpheniramine did not interfere in ammonium determination. The application of the proposed method to the determination of ammonium and nitrate in some pharmaceutical products and spiked water samples has demonstrated the usefulness of the method.

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