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Short communication

Ion chromatographic method for the simultaneous determination of nitrite and nitrate by post-column indirect fluorescence detection ☆

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Abstract

This short paper highlights the suitability of ion chromatography with post-column indirect fluorescence detection to determine simultaneously nitrite and nitrate based on the quenching of tryptophan native fluorescence. The method uses an enhanced fluorescence mobile phase containing tryptophan and detects the suppression of fluorescence of the mobile phase due to the elution of the target ions. The phenomenon of fluorescence quenching of tryptophan is highly induced by the presence of phosphate ions. The quenched fluorescence intensity exhibits concentration dependence in the range 1–25 mg/l and 3–65 mg/l for nitrite and nitrate, respectively. The relative standard deviation for five replicates of a standard solution containing a mixture of 5 mg/l of nitrite and 10 mg/l of nitrate lies around 2.8%. This simple coupling technique results in a relatively sensitive, fast, and accurate method, allowing for both qualitative and quantitative analysis of nitrite and nitrate. The method can easily be implemented to real samples such as foodstuffs, fertilizers and soils and is proven to be precise and accurate when compared with reference methods.

Keywords: Indirect detection; Detection, LC; Food analysis; Soil; Fertilizers; Environmental analysis; Nitrite; Nitrate; Inorganic anions

1. Introduction

Nitrate and nitrite are commonly monitored for environmental protection purposes in water bodies owing to their high solubility and mobility within the soil and the gradually increasing eutrophication of natural waters [1–3]. A contentious health concern related to these agents is the possible formation of carcinogenic nitrosamines as a result of the reaction of nitrite with secondary or tertiary amines present in the human body [4,5]. The presence of nitrate in foods is mainly due to plants, taking nitrogen from the soil in this ionic form. The use of nitrogen-containing fertilizers increases the nitrate concentration in the soil and therefore, the nitrate content in plants grown therein is above the normal level. Finally, a small amount of nitrite is deliberately added to meat products to protect them against *Clostridium botulinum* and to enhance flavor [6]. The growing need to monitor these ions in the context of food control programs has increased [7,8]. Several

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methods have been proposed for the simultaneous determination of these species, based on flow-injection analysis [9–11] and ion chromatography (IC) [12,13]. In some cases, nitrate is reduced to nitrite, which subsequently reacts to form a highly colored azo-dye. Representative surveys of the scientific literature covering the detection of these important analytes have been compiled over recent years [14–16].

Quenching of tryptophan fluorescence due to the presence of some ions such as nitrite, iodide and caesium is well known [17]. The aim of this short communication is to develop an ion chromatographic method for the determination of nitrite and nitrate with a simple modular IC–post-column reaction indirect fluorescence detection system, exploiting the property of both these ions to quench tryptophan fluorescence. The method uses an enhanced fluorescence mobile phase containing tryptophan and detects the suppression of fluorescence of the mobile phase due to the elution of the target ions. The phenomenon of fluorescence quenching of tryptophan is highly induced by the presence of phosphate ions, which are utilized as buffer solution components in the flow stream for the post-column reaction.

2. Experimental

2.1. Chemicals and apparatus

Individual stock solutions of nitrite and nitrate at concentrations of 100 mg/l with respect to the anions were prepared by respective sodium salts in double-distilled water (DDW). Tryptophan was of 99% purity and its solutions were prepared at a concentration of 5 mg/l in 30 m*M* sodium phosphate with pH 12. All the chemicals were obtained form Sigma (Sigma–Aldrich, Hellas). Light salami was obtained from a local supermarket and the two employed fertilizers were from Laboratoire Algochimie (France), under the trade name ALGOFLASH. Filter papers were obtained from Whatman (Whatman Ltd, UK).

2.2. Equipment

The chromatographic separation employed an anionic column (Shim-pack IC-A1, Shimadzu) connected in series with a precolumn (Shim-pack IC-GA1, Shimadzu), both being thermostatted at 40 °C. Detection was carried out with an RF-551 fluorescence detector (Shimadzu) set to the high sensitivity mode. An Ismatec peristaltic pump at 30 r.p.m. (Ismatec Glattburg-Zurich, Switzerland) delivered a continuous and pulse-free flow necessary for the sensitive detection. Signal acquisition and processing for the IC–post-column–fluoresence system were carried out with the Shimadzu CLASS VP, Version 4.3 Chromatography Software.

2.3. Chromatographic conditions

The mobile phase consisted of a buffer solution of citric acid–NaOH 0.08 *M* at a flow-rate of 1.5 ml/min. Subsequent to the ion separation, the mobile phase merged with a phosphate solution of tryptophan, flowing at 1 ml/min. A glass-bead PTFE tube 50 cm long, was connected after merging with a view to enhance mixing of the two streams [18]. Upon mixing, the pH of the flowing liquid raises to 9.5. Under these experimental conditions the separation and quantitation was possible within 9.5 min. The volume injected onto the chromatograph was 50 μl.

To achieve post-column indirect fluorescence detection (IFD), the detector electronic offset was used to zero the detector signal as the post column fluorescent solution passed through the detector. Fluorescence detection was performed by monitoring the tryptophan quenching, appearing as a negative peak, at an emission wavelength of 355 nm after excitation at 270 nm.

2.4. Extraction of nitrite and nitrate

2.4.1. Food sample

Ten grams of salami were removed from its commercial package and 70 ml of warm DDW were added. The mixture was then homogenized in a blender for 1 min and the homogenized sample was heated at a temperature maintained around 70 °C, for 15 min. After cooling to room temperature, the sample was centrifuged at 1370 g for 10 min. Following centrifugation, the supernatant was removed and filtered successively through a Whatman filter paper No. 40 and a GF/A filter. The filtrate was

then collected in a volumetric flask and diluted up to 100 ml, before being used for analysis.

2.4.2. Soil sample

The soil sample analyzed was collected from a leek plantation in Epirus, Greece. About 10 g of soil was mixed with 70 ml of water, shaken for 30 min at 70 °C, decanted and filtered. The retentate was rinsed with 20 ml of warm DDW and the filtrate along with the washing solution was transferred to a 100 ml volumetric flask, filled up to the final volume and filtered as in food samples through GF/A filter, before analysis.

2.4.3. Fertilizers

A portion of 100 μl of the product was diluted with DDW up to 100 ml and the solution was filtered as in food samples, before analysis.

3. Results and discussion

The optimum chromatographic and reaction conditions for IC–IFD were established after performing pertinent experiments. The emission spectra of tryptophan at different pH ambiences, as well as in the presence of phosphate ions are given in Fig. 1. The influence of the pH and concentration of phosphate ions on the fluorescence quenching are illustrated in Fig. 2. Highest fluorescence of tryptophan as well as quenching due to the action of the nitrite and nitrate is seen at pH 9.5, where tryptophan exists in the anionic state. It seems that at this pH value, the hydrophobic indole ring of tryptophan is much exposed to the attack of the quenchers, a fact being induced by the presence of phosphate ions. Table 1 collects the optimum experimental conditions.

The quenched fluorescence intensity exhibits concentration dependence in the range 1–25 mg/l and 3–65 mg/l for nitrite and nitrate, respectively. Up to 80 μl of sample can be injected into the system without causing significant peak broadening or deteriorating of resolution, thus increasing the sensitivity of the system. The relative standard deviation for five replicates of a standard solution containing a mixture of 5 mg/l of nitrite and 10 mg/l of nitrate lies around 2.8%.

The method can easily be implemented to real samples such as foodstuffs, fertilizers and soils and is proven to be precise and accurate when compared with reference methods [19,20]. Given the capacity of IC for ion separation and the limited number of anions, which quench the fluorescence of tryptophan, it is hardly anticipated that any interferences may be encountered. Native contents of nitrite and nitrate as determined by the two applied methods, are given in Table 2. Recoveries from fortified samples were quantitative in the range 94–99% for the three different matrices. A typical chromatogram of the analytical method for a soil sample spiked with nitrite is illustrated in Fig. 3 and reveals satisfactory peak separation and absence of interferences.

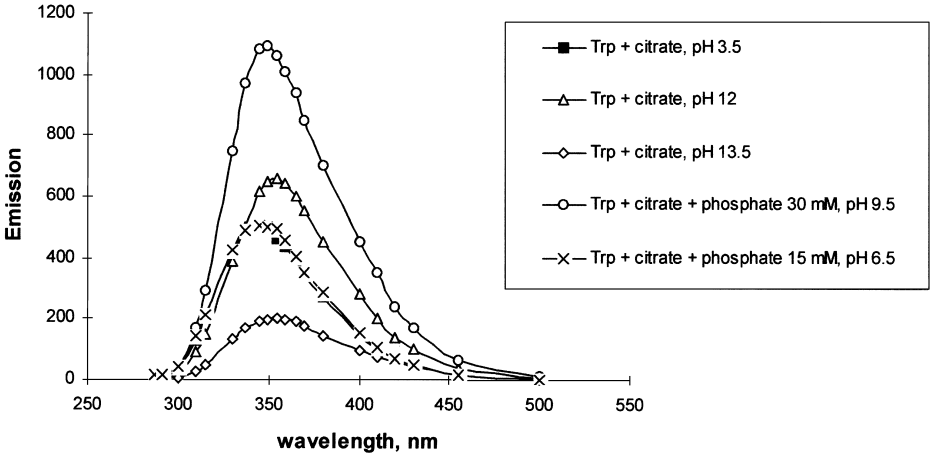

Fig. 1. Effect of the composition of reaction medium and pH on the fluorescence of tryptophan.

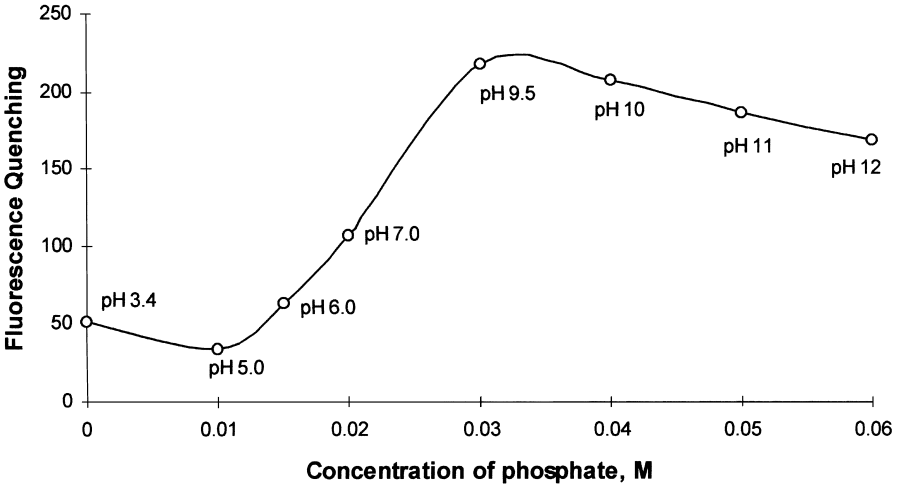

Fig. 2. The impact of phosphate concentration on the quenching of tryptophan.

Table 1
Optimum chromatographic and post-column reaction conditions

Parameter	Optimized value
Mobile phase	8 m*M* citric acid, pH 3.4
Mobile phase flow-rate	1.5 ml/min
Column temperature	40 °C
Post-column reagent	0.5 mg tryptophan/100 ml of 30 m*M* sodium phosphate
Post-column reagent flow-rate	1 ml/min

4. Conclusions

This short paper summarizes the results of studies on the suitability of the IC with post-column IFD to determine simultaneously nitrite and nitrate. The intrinsic fluorescence of tryptophan, which is dramatically affected by the pH, vanishes due to the quenching action of these ions. The proposed simple coupling technique method results in a relatively sensitive, fast, and accurate analysis of nitrite and nitrate in a variety of matrices. Furthermore, the use of fluorescence detection renders the method free of

Table 2
Performance assessment of the method

Sample	Concentration of nitrate (μg/g)[a]			Concentration of nitrite (μg/g)[a]		
	Method			Method		
	IC–IFD	AOAC	Error (%)	IC–IFD	AOAC	Error (%)
Salami	84±2	88±3	4.5	54±1	56±2	3.6
Soil sample	81±2	83±2	2.4	n.d.	0.9±0.1	–
Fertilizer 1	111±3	105±3	5.7	n.d.	n.d.	–
Fertilizer 2	139±3	132±3	5.3	n.d.	n.d.	–

n.d., non-detectable.
[a] Average of three runs ±SD.

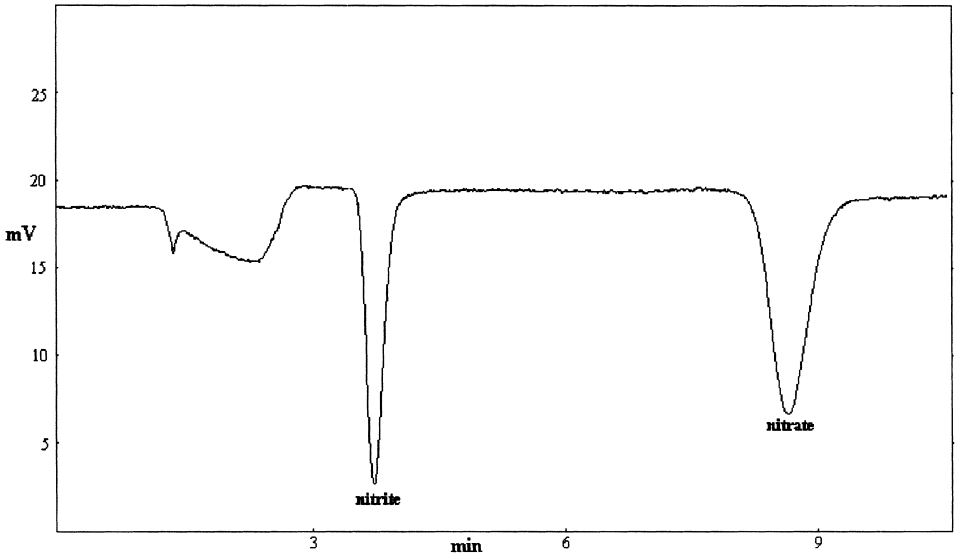

Fig. 3. Typical chromatogram for nitrate and nitrite detection in a soil sample fortified with nitrite.

interferences because components, which may decrease the background fluorescence of tryptophan, are barely found in the real samples.

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