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Short communication

Ion chromatographic method for simultaneous determination of nitrate and nitrite in human saliva

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Abstract

A simple, rapid, accurate and sensitive method is proposed for the simultaneous determination of nitrite and nitrate in human saliva. Nitrite and nitrate present in the human saliva were determined after 10- to 100-fold dilution with ion chromatography (IC) using suppressed conductivity detection. Recoveries of nitrite and nitrate were found to be ranged between 95% and 101%. The method was linear (r^2=0.9991) over the concentration working range. The detection limits were found to be 15.0 μg/l and 33.5 μg/l, for nitrite and nitrate, respectively. Ions that are present in human saliva and several other ions that are suspected to affect nitrite and nitrate determination were checked. It was found that most of the ions did not cause any interference in the determination. The method allows simultaneous determination of nitrite and nitrate in human saliva.

Keywords: Nitrate; Nitrite

1. Introduction

Extensive interest has been attained in determining nitrite and nitrate in biological materials. Nitrite and nitrate are generally regarded as hazardous compounds, because they are highly toxic to humans and especially to infants, which usually results in methemoglobinemia and possibly death [1,2]. Nitrite has the possibility to react with secondary amines and amides and form various carcinogenic *N*-nitroso compounds [3].

Salivary nitrite is produced due to microbial reduction of nitrate in the oral cavity. Therefore, ingested nitrite through foods and salivary nitrite can react in vivo with various amines and amides in the food in the acidic environment of human stomach or other parts of the body to form *N*-nitroso compounds [3]. This reason suggests the need for accurate control and monitoring of nitrite and nitrate level in human saliva, Many researchers have studied the effect of various nitrate-rich diets on salivary nitrite levels and ultimately on vivo *N*-nitrosation [3].

Several methods have been reported for the determination of nitrite and nitrate in foods and biological materials [4–8], these methods include high-performance liquid chromatography (HPLC) or flow injection with chemiluminescence detection [4], gas chromatography (GC) [5,6], HPLC using a micellar mobile phase [7], HPLC by reversed-phase ion pair liquid chromatography [8] and spectrophotometric methods [9–11]. Where a spectrophotometric method is widely used, it is based on a diazotization of various aromatic amines with nitrite in acidic medium and on a subsequent coupling of the

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diazonium ions with *N*-(1-naphthyl)ethylenediamine [9], 8 quinoline [10], or resorcinol [11]. In general, spectrophotometric methods are subject to various interferences and lack of specificity. GC methods in general need a derivatization reaction and then partitioning to the organic layer prior to analysis, which is a complicated process. In liquid chromatography, nitrite is liable to be oxidized in the acidic medium during the process [12].

In the present communication we describe a selective and sensitive analytical method in which nitrite and nitrate are determined directly from human saliva using ion chromatography (IC) using an Ion Pac AS12A column, with suppressed conductivity detection. The method allows simultaneous determination of nitrite and nitrate in human saliva.

2. Experimental

2.1. Instrumentation

A Dionex (Sunnyvale, CA, USA) a suppressed conductivity detection ASR-II, Auto suppression-II, recycle mode and LC25 enclosure system, was used. Chromatographic data were analyzed using a C-R6A chromatopac chart. The Ion Pac AS12A (200×4 mm) column was obtained from Dionex.

2.2. Materials

Human saliva samples were collected from different volunteers. Sodium nitrite and sodium nitrate were obtained from (Wako, Japan). Sodium carbonate and sodium hydrogencarbonate were also obtained from (Wako).

2.3. Ion chromatography

Nitrite and nitrate are separated using the isocratic conditions with an Ion Pac AS12A analytical column (200×4 mm). The eluent used was composed of 2.7 m*M* Na_2CO_3–0.3 m*M* $NaHCO_3$. The injection volume was 25 μl and the eluent flow-rate was 1.5 ml/min. Anions were detected with suppressed conductivity detection.

2.4. Calibration curves

A calibration graph was constructed by plotting the peak areas against the concentrations of the standard injection for nitrite and nitrate. Nitrate was injected in duplicate at 12 levels ranging between 0.05 μg/l to 400 μg/ml and nitrite was injected in duplicate at 12 levels ranging from 0.03 μg/l to 300 μg/ml. Peak areas (three points) were collected against nitrite and nitrate concentration and used for construction of the calibration graph.

2.5. Method for determining nitrite and nitrate in human saliva

Human saliva was collected from individual volunteers, the solid matter was dissolved in deionized water and the insoluble matter removed by centrifugation and then analyzed by IC. The concentrations of nitrite and nitrate were calculated from the calibration graph for each anion separately.

3. Results and discussion

Fig. 1 shows the chromatogram of the standards nitrate and nitrite. Chromatograms of nitrite and nitrate for saliva samples obtained from different volunteers are shown in Fig. 2a and b. The chromatograms of of the spiked saliva with nitrite and nitrate are shown in Fig. 2c and d. The simultaneous determination of nitrite and nitrate in saliva by the Ion Pac AS12A column was performed with suppressed conductivity detection. The dissolved solution of solid saliva is centrifuged and was directly used for nitrite and nitrate determination. The chromatograms of saliva are similar to the ones obtained for standard nitrite and nitrate. Nitrate and nitrite in human saliva was determined by the recommended method and HPLC method [7]. The results are shown in Table 1 and agreed well.

Spiked recovery experiments were performed to determine the % recoveries of known amounts of nitrite and nitrate standard solutions added to saliva solution. The spiked saliva samples were allowed to

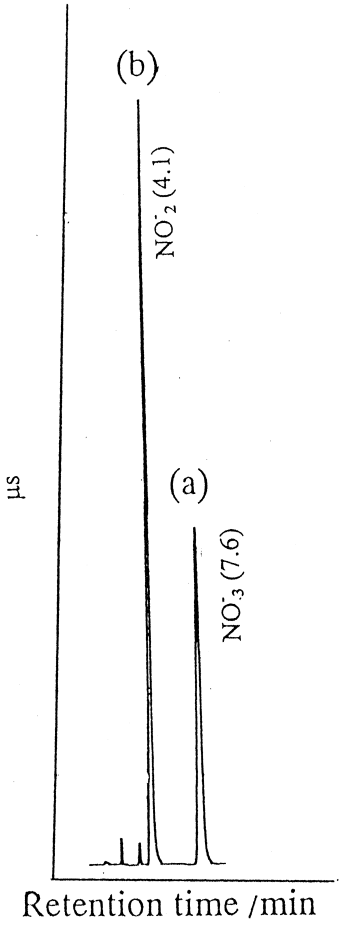

Fig. 1. Chromatogram of nitrate and nitrite standards. Peaks: (a) nitrate, 5 mg/l; (b) nitrite, 5 mg/l. Column: AS12A. Eluent: 2.7 m*M* Na_2CO_3–0.3 m*M* $NaHCO_3$, flow-rate, 1.5 ml/min. Detection: suppressed conductivity detection. Injection volume: 25 μl.

stand for few minutes with shaking. Then the concentrations of the spiked nitrate and nitrite were determined by the recommended procedure. The statistical analysis results of the spiked saliva with a known standard concentration of nitrite and nitrate are as follows: when 2 μg/ml of nitrate and nitrite is spiked with saliva sample, the relative standard deviations (RSDs) (n=10) are 2.56 and 2.95% for nitrite and nitrate, respectively. When 50 μg/ml of nitrate and nitrite is spiked with saliva sample, the RSDs (n=10) are 1.98 and 1.83% for nitrite and nitrate, respectively. When 100 μg/ml of nitrate and nitrite is spiked with saliva sample, the RSDs (n=10) are 1.21 and 1.56% for nitrite and nitrate, respectively Recoveries obtained ranged between 95% and 101% for both nitrite and nitrate, respectively.

The validity of the proposed method to determine the degree of agreement among separate tests and the results checked for human saliva were expressed as RSDs. The results obtained are summarized in Table 2. RSDs for peak areas and retention times of nitrite and nitrate for human saliva were found to be less than 3.0% for peak areas and less than 0.5% for retention time. The method shows the absence of detectable change in retention time for human saliva.

Ions that are present in human saliva and several other ions that are suspected to affect nitrite and nitrate determination were checked. It was found that most of the ions did not cause any interference in the determination.

The linearity data and coefficient constant for simultaneous determination of nitrite and nitrate was calculated. It was observed that a linearity of peak area and nitrite concentration in the range 30 μg/l–300 μg/ml is obtained with coefficient constant, r^2=0.0001. A linearity between peak areas and nitrate concentration is obtained in the range 50 μg/l–400 μg/ml, with r^2=0.999. The detection limits for nitrite and nitrate were determined at three times the noise and found to be equal to 15 μg/l and 33.5 μg/l, respectively.

4. Conclusion

The proposed analytical method for determining nitrate and nitrite in human saliva is simple, rapid and accurate. The anion-exchange column in conjunction with suppressed conductivity detection was adopted to provide to a selective determination of nitrate and nitrite from human saliva without interference from the matrix components present in the saliva. The saliva sample needs only centrifugation to remove the insoluble matter and no sample preparation or clean-up process is necessary. The method works in a narrow range for nitrite and nitrate determination.

Fig. 2. Chromatogram of nitrate and nitrite of human saliva, with suppressed conductivity detection. (a) Unspiked saliva; (b) unspiked saliva; (c) spiked with nitrate (2 μg/ml); (d) spiked with nitrite (2 μg/ml). Nitrite retention time=4.1 min and nitrate retention time=7.5 min. Other conditions as in Fig. 1.

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Table 1
Results of the analysis of human saliva samples

Saliva sample No.	Proposed method (μg/ml)[a]		Reference method (μg/ml)[a,b]	
	Nitrite	Nitrate	Nitrite	Nitrate
1	4.8	15.9	4.5	17.8
2	3.7	17.5	3.3	18.0
3	6.8	10.5	6.2	12.4
4	4.1	18.0	4.0	19.2

[a] Three determinations for each sample.
[b] Ref. [7].

Table 2
Linearity data for human saliva for nitrite and nitrate and relative standard deviations

Nitrite peak area RSD (%)	Nitrate peak area RSD (%)	Nitrite retention time RSD (%)	Nitrate retention time RSD (%)	Nitrite concentration range and r^2	Nitrate concentration range and r^2
2.93	2.31	0.28	0.45	30 μg/l–300 μg/ml r^2=0.9992	50 μg/l–400 μg/ml r^2=0.999

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