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Simultaneous Determination of Nitrate and Nitrite in Toothpastes by High-Performance Liquid Chromatography

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Abstract □ A stability-indicating analytical method is described for the simultaneous determination of nitrate, and if present, its reductive degradation product, nitrite, in toothpastes. Nitrate and nitrite were extracted from the toothpaste using distilled water and separated from other water-soluble excipients by two RP-8 columns (250 mm × 4 mm i.d.) using a mobile phase containing 0.2% (w/v) sodium acetate and 2.5% (v/v) glacial acetic acid in distilled water. A UV detector set at 313 nm was used for quantitation. The method was applied to commercial toothpastes containing 5% potassium nitrate and yielded an average recovery of 100.1% with a relative standard deviation of 1.43%. Average recovery of nitrate and nitrite from spiked samples were 100.6% and 96.4%, respectively. The minimum detectable concentration for nitrite was 50 μg/g of toothpaste.

Keyphrases □ Nitrate—simultaneous determination with nitrite, toothpastes, HPLC □ Nitrite—simultaneous determination with nitrate, toothpastes, HPLC □ Dentifrices—simultaneous determination of nitrate and nitrite, HPLC

Potassium nitrate, reportedly a tooth desensitizing agent (1-3), is the active ingredient in commercial dentrifrices for the treatment of dental hypersensitivity. As nitrate ion can be converted to nitrite by reducing agents or bacteria, a simple, specific, and sensitive method for simultaneous determination of intact nitrate and its reductive degradation product is desirable for routine quality control and stability evaluation.

The widely used colorimetric procedures for nitrate (4, 5) and nitrite (6-12) are subject to interference by other ions and generally require tedious sample treatment. In addition, those methods involving the cadmium reduction of nitrate to nitrite (6, 7) prior to color development often suffer from nonstoichiometric conversion.

Methods involving derivatization followed by HPLC (13) or GC (14, 15) are lengthy. The potentiometric method with a nitrate ion-selective electrode (16-18), differential pulse polarography (19), and enthalpimetric analysis for nitrate (20) are specific, but they measure only one ion (nitrate or nitrite). The newly developed ion chromatography (21) and its variations (22-27) are selective, sensitive, and suitable for one-step differentiation and determination of nitrate and nitrite. However, the need for additional expensive instrumentation (an ion chromatography system with conductivity or electrochemical detector and ion-exchange columns) hinders the use of this technique.

Recently, Skelly (28) reported the use of an eluant containing the octylamine salt of a mineral acid to separate inorganic anions on a conventional reverse-phase column, with detection at 205 nm. Leuenberger *et al.* (29) and Cortes (30), using phosphate buffer as an eluant, have described the resolution of nitrate from nitrite on an amino normal-phase column. The separation mechanism of the former is dynamic ion-exchange, while the second is weak base ion-exchange. Both methods, which directly determined nitrate and nitrite by conventional HPLC with low-wavelength detection, are simple and sensitive, but they have not been applied to the toothpaste matrix.

The purpose of this paper is to report the separation and simultaneous determination of nitrate and nitrite by high-performance liquid chromatography (HPLC) using two RP-8 columns with acetate buffer as mobile phase. The separated nitrate and nitrite were monitored by a UV detector set at 313 nm. No sample treatment or cleanup was required, and the method was found to be simple, rapid, precise, and selective for the measurement of nitrate and nitrite in a complex toothpaste matrix.

EXPERIMENTAL SECTION

Chemicals and Reagents—Potassium nitrate[1] and sodium acetate[2] were used without further purification. Glacial acetic acid[3] and 0.1 M sodium nitrite solution[4] were used as received.

Apparatus—The liquid chromatograph[5] was fitted with a manual septumless injector[6], a fixed-wavelength UV detector (313 nm)[7], and a strip-chart recorder[8]. The recorder was connected to a laboratory data system[9] through an A/D converter[10]. Two 250 mm × 4.0-mm i.d. columns containing 10-μm Lichrosorb RP-8 packing[11] and one precolumn containing 37-40-μm octadecylsilane packing[12] were used.

Chromatographic Conditions—The chromatographic solvent was 0.2% (w/v) sodium acetate in distilled water containing 2.5% (v/v) glacial acetic acid, adjusted to pH 3.0 ± 0.1 with glacial acetic acid or sodium hydroxide. This solvent was vacuum-filtered[13] through a 0.45-μm filter[13] and deaerated

[1] Baker Analyzed Reagent, J. T. Baker Chemical Co., Phillipsburg, N.J.
[2] MCB Manufacturing Chemical Inc., Cincinnati, Ohio.
[3] VWR Scientific Inc., South Plainfield, N.J.
[4] Orion Research Inc., Cambridge, Mass.
[5] Model 6000 A Pump; Waters Associates, Milford, Mass.
[6] Valco Model CV6-UHpa-N6o, 7000 psi sample injection valve equipped with a 100-μL sampling loop.
[7] Model 440; Waters Associates, Milford, Mass.
[8] Houston Instrument, Austin, Tex.
[9] Model 21MX Computer with model 2645A terminal; Hewlett-Packard, Avondale, Pa., and model DP 8000 printer; Anadex, Chatsworth, Calif.
[10] Model 18652A; Hewlett-Packard, Avondale, Pa.
[11] Cat. #9318; Merck, Elmsford, N.Y.
[12] Waters Associates, Milford, Mass.
[13] Millipore Corp., Bedford, Mass.

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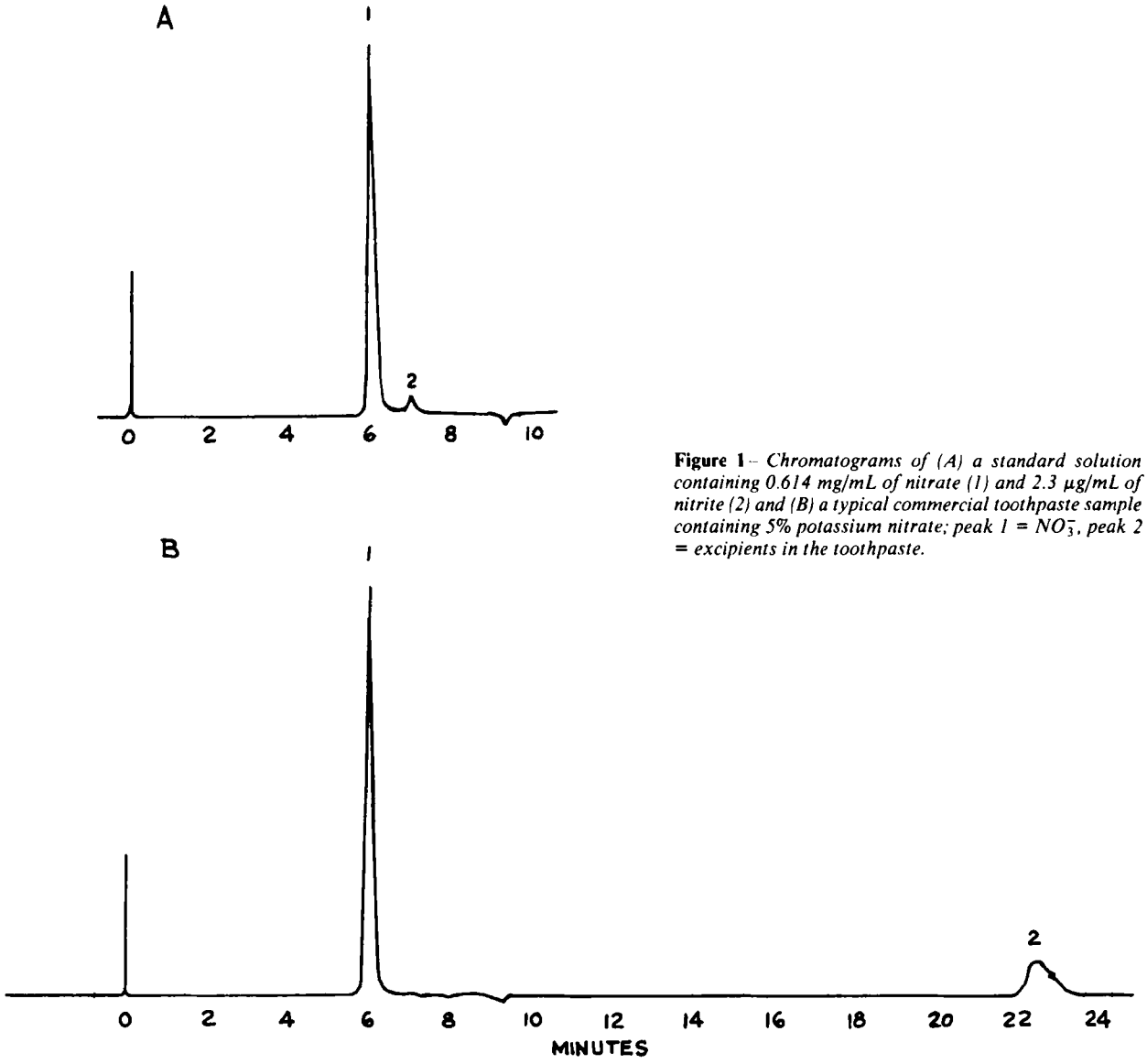

Figure 1—*Chromatograms of (A) a standard solution containing 0.614 mg/mL of nitrate (1) and 2.3 μg/mL of nitrite (2) and (B) a typical commercial toothpaste sample containing 5% potassium nitrate; peak 1 = NO_3^-, peak 2 = excipients in the toothpaste.*

for 5 min with stirring before use. The temperature was ambient, the solvent flow rate was 1.0 mL/min, and the inlet pressure was 1800 psi. The detector sensitivity was 0.1 AUFS, and the chart speed was 1.0 cm/min.

Standard Solutions—Three standard solutions containing 30.7, 61.4, and 92.1 mg of nitrate and, respectively, 115, 230, and 460 μg of nitrite per 100 mL were prepared in distilled water and filtered as described above prior to injection.

Assay for Commercial Products—An accurately weighed 2.0-g portion of the toothpaste was transferred to a 100-mL beaker, 20 mL of distilled water was added, and the contents were mixed with a spatula to complete dispersion. The dispersion was then quantitatively transfered to a 100-mL volumetric flask, diluted to volume with distilled water, and mixed well. A portion of this solution was centrifuged at 3000 rpm for 5 min and filtered through a 0.45-μm filter before injection.

Spiked Samples—Accurately measured quantities of nitrate and nitrite were admixed with a placebo portion of toothpaste. These mixtures were formulated to contain 30.7, 61.4, and 92.1 mg of NO_3 and 115, 230, and 460 μg of NO_2, respectively, per 2.0-g sample. These samples were assayed as described above for commercial products.

Quantitation—Since peak areas of nitrate and nitrite were found to be directly proportional to their concentrations, all results were calculated by interpolation from standard curves of peak areas *versus* concentrations.

Table I—Recovery Data for Nitrate from Spiked Samples

Amount Added, mg	Amount Found, mg	Recovery, %
0.5062	0.5167 (*RSD* = 0.69%, *n* = 3)	102.07
1.0124	1.0199 (*RSD* = 1.11%, *n* = 4)	100.75
1.5186	1.5045 (*RSD* = 1.72%, *n* = 3)	99.07
Mean *N* = 10		100.6
SD		1.63
RSD		1.62

RESULTS AND DISCUSSION

Linearity responses for nitrate and nitrite were observed, respectively, over the range of 0.307–0.921 mg/mL with a correlation coefficient of 0.999 and 1.15–9.2 μg/mL with a correlation coefficient of 0.999. The relationship of peak area to concentration can be presented by the linear regression equations

Table II—Assay Results for Toothpaste Containing 5% Potassium Nitrate

Sample	Potassium Nitrate Found, %	Percent of Label Claim
1	5.09	101.8
2	4.99	99.8
3	5.07	101.4
4	5.00	100.0
5	4.90	98.0
6	5.02	100.4
7	5.02	100.4
8	4.89	97.8
9	5.07	101.4
Mean		100.1
SD		1.43
RSD		1.43

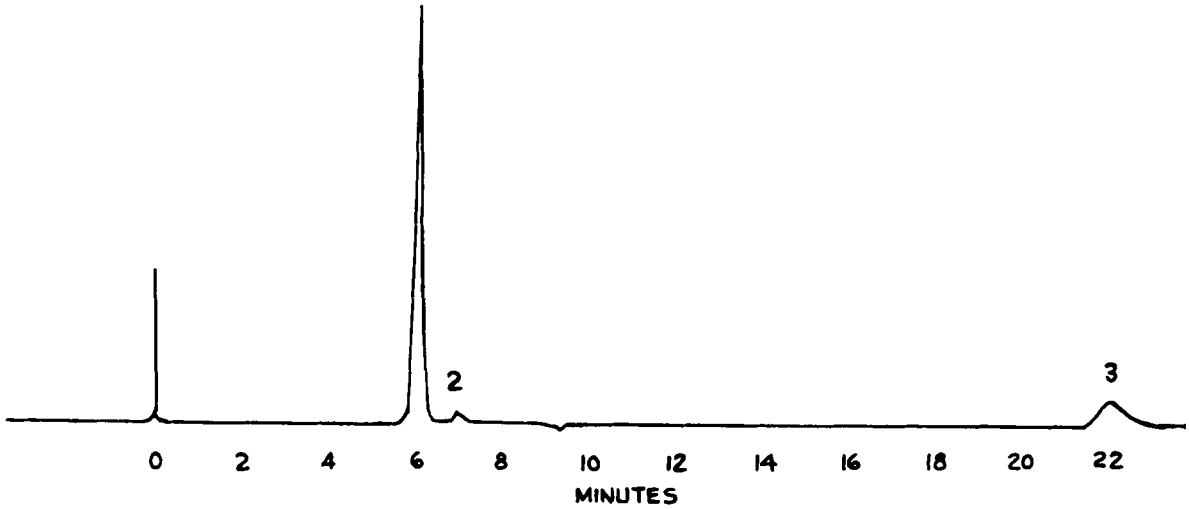

Figure 2—*Chromatogram of a toothpaste placebo spiked with both nitrate (1) and nitrite (2). The spiked placebo was prepared by adding 100 mg of potassium nitrate and 2 mL of 5.0 mM sodium nitrite solution into 2 g of toothpaste placebo; peak 3 = excipients in the toothpaste.*

$y = 35789.8x + 1127.5$ for nitrate and $y = 668322x + 312$ for nitrite, where y is the peak area obtained by the laboratory data system and x is the concentration in mg/mL.

Chromatograms obtained for a standard solution of nitrate and nitrite and for a typical commercial toothpaste containing 5% potassium nitrate are presented in Fig. 1A and B, respectively. The nitrate ion was well resolved from the other excipients, and the recovery data for nitrate from spiked samples of toothpaste (Table I) illustrate the validity of the method for these formulations. Average recovery for placebo dentifrices spiked with three concentration levels (50, 100, and 150% of label concentration) of nitrate was 100.6% with a standard deviation of 1.63%. This method has been used routinely in a product stability program and found to be accurate and precise with a relative standard deviation of 1.43% ($n = 9$). Representative data are summarized in Table II.

Although nitrite was not detected in any commercial toothpaste samples, it was necessary to demonstrate the selectivity of the method for nitrate in the presence of nitrite; therefore, spiked toothpaste samples containing both nitrate and nitrite were prepared and assayed. A representative chromatogram of a spiked sample containing 61.4 mg of nitrate and 115 μg of nitrite per 100 mL is reproduced in Fig. 2. Excellent resolution of nitrite from nitrate, an essential requirement for a stability-indicating assay, was obtained. Average recovery at the 2.3–9.2-μg/mL nitrite level was 96.4%, with a relative standard deviation of 6.7% ($n = 8$). The minimum detectable concentration of nitrite was 50 μg/g of toothpaste sample.

The retention of nitrate and nitrite on a reverse-phase column may be attributed to the interaction of these anions with the protonated silanol backbone of stationary phase at the acidic condition (pH 3.0 ± 0.1) employed in this experiment. In addition to this electrostatic attractive force, solute anions may also experience the steric exclusion provided by the hydrophobic long-chain octyl substituents of the stationary phase. The balance between these two opposing forces could determine the retention of anions on the column and the separation of one anion from the others. The unsatisfactory results obtained with an RP-18 column may be due to the overwhelming steric exclusion provided by the more hydrophobic octadecyl substituents of the stationary phase.

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