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*Amy E. Kinkennon,*[1] *Ph.D.; David L. Black,*[1] *Ph.D.; Timothy A. Robert,*[1] *Ph.D.; and Peter R. Stout,*[1] *Ph.D.*

# Analysis of Nitrite in Adulterated Urine Samples by Capillary Electrophoresis

**ABSTRACT:** A simple method for analyzing nitrite in urine has been developed to confirm and quantify the amount of nitrite in potentially adulterated urine samples. The method involved separation of nitrite by capillary electrophoresis and direct UV detection at 214 nm. Separation was performed using a bare fused silica capillary and a 25 mM phosphate run buffer at a pH of 7.5. Sample preparation consisted of diluting the urine samples 1:20 with run buffer and internal standard, and centrifuging for 5 min at 2500 rpm. The sample was hydrodynamically injected, then separated using −25 kV with the column maintained at 35°C. The method had upper and lower limits of linearity of 1500 and 80 μg/mL nitrite, respectively, and a limit of detection of 20 μg/mL. The method was evaluated using the National Committee for Clinical Laboratory Standards (NCCLS) protocol (Document EP10-A2), and validated using controls, standards, and authentic urine samples. Ten anions, $ClO^-$, $CrO_4^{-2}$, $NO_3^-$, $HCO_3^-$, $I^-$, $CH_3COO^-$, $F^-$, $SO_4^-$, $S_2O_8^{-2}$, and $Cl^-$, were tested for potential interference with the assay. Interferences with quantitation were noted for only $CrO_4^{-2}$ and $S_2O_8^{-2}$. High concentrations of $Cl^-$ interfered with the chromatography. The method had acceptable accuracy, precision, and specificity.

**KEYWORDS:** forensic sciences, urine adulteration, nitrites in urine, capillary ion electrophoresis

Continual issues arise in urine drug testing with adulteration of samples. Nitrite ($NO_2^-$) compounds are sometimes used as adulterants to destroy traces of drugs in urine samples. Many laboratories use either a general or specific oxidant colorimetric test to screen for the presence of nitrite or other oxidants. However, for forensic acceptability, it is necessary to have a second confirmatory test of samples that screened positive, preferably using a distinctly different chemical basis.

The goal of this study was to develop a capillary electrophoresis (CE) method to confirm and quantitate $NO_2^-$ in urine samples that had previously screened positive. The manufacturer of the CE used in these experiments supplied a method for the separation of nitrite and nitrate anions. This indirect detection method, which employed $CrO_4^{-2}$ and cetyltrimethylammonium bromide (CTAB) in the run buffer, separated nitrite and nitrate with good peak shapes and resolution. However, it was determined that anions endogenous in the urine severely interfered with the nitrite peak. Therefore, it was necessary to investigate other methods.

Ferslew et al. published a CE method that accomplished the desired analytical goals (1). They analyzed $NO_2^-$ in urine samples and obtained good analytical results with minimal or no interference from endogenous anions. However, the buffer employed, 4.0 mM tetradecyltrimethylammonium hydroxide (OFM-OH) with various modifiers (pH = 9.1), was relatively expensive (Waters Corporation Product # WAT049385), and the article did not present limits of linearity, a limit of detection, or extensive validation studies.

Other published methods for analysis of nitrite were also investigated. Methods used various buffer systems such as pyridinedicarboxylic acid with cetyltrimethylammonium hydroxide at a pH of 12.1 (2), chromate with trimethyltetradecylammonium bromide at pH of 8–11 (3) or chromate with didodecyldimethylammonium bromide at pH of 2–4 (4), boric acid with lithium sulfate and a polymer modifier at pH of 2.3–8.5 (5), artificial seawater (6), imidazole–sulfate with various modifiers (7), sodium sulfate with NICE-Pack OFM Anion-BT at a pH of 4.8 (8), and sodium acetate at a pH of 4.0 (9). Among the nine authors cited, two analyzed urine samples (1,8), and two included internal standards (1,9).

The goal of this research was to develop a fast, reproducible CE method to analyze $NO_2^-$ in urine with no interferences from endogenous anions. A simple buffer system, an appropriate pH, and a stable internal standard were investigated. The method also needed to be inexpensive, require simple preparation steps for buffers and samples, and have a broad dynamic range.

## Materials and Methods

Monosodium dihydrogen phosphate was obtained from Fisher (Pittsburgh, PA). Reagent grade sodium thiosulfate ($Na_2S_2O_3$), tetrabutylammonium hydrogen sulfate (TBAS), and potassium nitrite were obtained from Sigma (St. Louis, MO). Negative urine used was obtained from a laboratory volunteer. Samples and run buffers were made with deionized water with a resistance of 16–17.5 MΩ.

A Beckman Coulter (Fullerton, CA) P/ACE MDQ Capillary Electrophoresis System was used for these experiments. The capillary column was uncoated fused silica (Agilent Part 160-2644-5, Palo Alto, CA) with an inner diameter of 0.75 μm and an effective length of 40 cm (total length of 50 cm). A window was burned in the polyimide coating with a lighter for direct UV detection at 214 nm. The method employed a hydrodynamic injection, and the anions were separated using −25 kV. The column temperature was maintained at 35°C by a liquid cooling system. Each buffer reservoir consisted of a 2 mL vial containing 1.3 mL of run buffer. Each reservoir was used for no more than three injections.

The run buffer consisted of 25 mM phosphate with 3.5 mM TBAS as a modifier to slow the electro-osmotic flow. The pH was

[1] Aegis Sciences Corporation, 345 Hill Ave., Nashville, Tennessee 37210.
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TABLE 1—*Regeneration and injection parameters on the CE.*

| Cycle | Time | Pressure | Solution |
|---|---|---|---|
| Initial | 8 min | 20 psi | 0.5 M NaOH |
| Regeneration/Equilibration | 2 min | 20 psi | Water |
| | 10 min | 20 psi | Run buffer |
| Within-Run | 2 min | 20 psi | 0.5 M NaOH |
| Regeneration/Equilibration | 2 min | 20 psi | Water |
| | 3 min | 20 psi | 5 M phosphate with 7 mM TBAS |
| | 6 min | 20 psi | Run buffer |
| Injection | 5 s | 0.5 psi | Sample |
| Separation | 9 min | −25 kV | |

adjusted to 7.5 with NaOH. The run buffer was filtered through Gelman (Pall Corporation, Ann Arbor, MI) IC Acrodisk filters with Supor® membranes (0.45 μm pore size), and stored at 8°C.

At the start of each batch of samples, the column was conditioned with an initial regeneration/equilibration cycle described in Table 1. Before each sample was injected, the column was flushed for 1 min with run buffer. After every three samples, the column was regenerated with NaOH and buffer. All of the regeneration, injection, and separation parameters are given in Table 1.

Authentic urine samples were prepared by diluting 100 μL of urine with 1850 μL of run buffer and 50 μL of $S_2O_3^{-2}$ (stock internal standard at a concentration of 2000 μg/mL $S_2O_3^{-2}$ in water). This resulted in a 1:20 dilution of the original urine and a final internal standard concentration of 50 μg/mL $S_2O_3^{-2}$. Samples were centrifuged at 2500 rpm for 5 min before being transferred to a vial for injection. If the nitrite in a sample exceeded the upper limit of linearity of the method, the sample was diluted with run buffer.

## Results and Discussion

### *Linearity Study*

To prepare standards for the generation of a calibration curve, 100 μL of urine and 50 μL of 2000 μg/mL $S_2O_3^{-2}$ (the internal standard) were mixed with an appropriate volume of either 10,000 or 100 μg/mL $NO_2^-$. These standards were then diluted with the appropriate amount of run buffer to a total volume of 2 mL. Therefore, the dilution factor for the urine was 1:20, and all of the standards contained 50 μg/mL of internal standard. The amount of nitrite ranged from 2–400 μg/mL in the standards, which correlated to 40–8000 μg/mL $NO_2^-$ in original urine samples.

Each standard (from 40 to 8000 μg/mL $NO_2^-$) was injected three times, and the averaged data plotted. Following linear regression analysis, the standards were reanalyzed as unknown samples. Using this procedure, it was determined that the error of the 80 μg/mL standard exceeded 5%. However, if the highest datum at 8000 μg/mL was removed from the graph, the concentration of the 80 μg/mL standard was calculated within 5% of the actual value.

The linearity study was repeated three times over the course of two weeks using new standards and buffers each time. The migration times of the $NO_2^-$ relative to the internal standard were averaged for the three sets of data. Each set consisted of 14 standards (excluding the 8000 μg/mL standard) injected three times for a total of 126 measurements of the migration time. The overall average relative migration time and standard deviation for $NO_2^-$ were $1.152 \pm 0.026$ (2.2% RSD). However, it was noted the relative migration time became progressively longer with increasing $NO_2^-$ concentration. Imposing a maximum allowable shift of ±3% around the migration time of the 500 μg/mL standard resulted in an upper limit of linearity of 1500 μg/mL. At 6000 μg/mL, the relative migration time had shifted by approximately 7.5%.

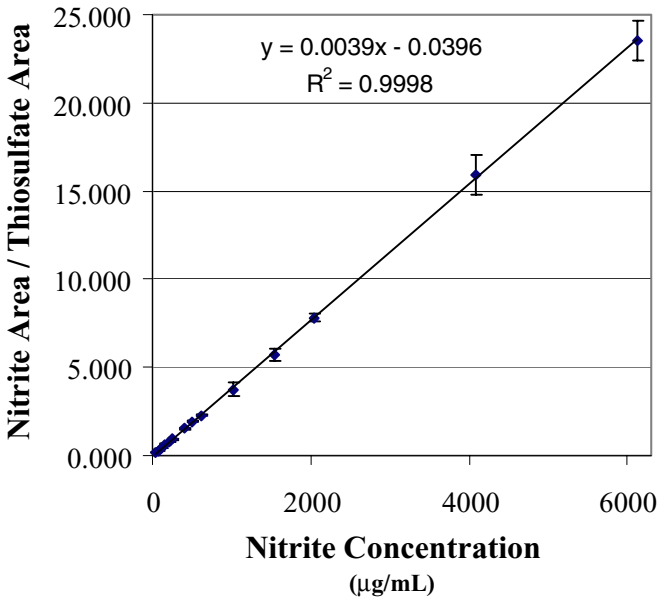


FIG. 1—*Averaged linearity data from three separate sets of standards analyzed on three separate days. (Thiosulfate concentration was 51.4 μg/mL in all standards.)*

The data from the three linearity studies were averaged, and a regression line fitted to the data (Fig. 1). Each data point represents an average of the three measurements with the standard deviation. As the 40 μg/mL standard was not calculated within acceptable error limits, the lower limit of linearity (LLOL) for this method was determined to be 80 μg/mL $NO_2^-$ in urine (4 μg/mL on-column concentration.) Although the quantitative values were acceptable up to 6000 μg/mL, the relative migration time restricted the upper limit of linearity (ULOL) to 1500 μg/mL.

### *Detection Limits*

A minimum signal-to-noise ratio (S:N) of 10:1 was implemented as the determinant for the limit of detection (LOD). A set of standards were prepared at 10, 20, 60, and 80 μg/mL in urine (0.5, 1, 2, 3, and 4 μg/mL on-column concentrations, respectively). These standards were analyzed, and the Beckman software used to calculate the S:N. The LOD for this method was determined to be 20 μg/mL $NO_2^-$ in urine with a S:N of approximately 11.

## NCCLS Evaluation

The National Committee for Clinical Laboratory Standards (NCCLS) Preliminary Evaluation of Quantitative Clinical Laboratory Methods (10) was performed to determine if bias, precision, carryover, nonlinearity, and/or drift were acceptable within the analytical range of 80–6000 μg/mL of $NO_2^-$ in urine. The NCCLS procedure required that three standards be prepared at 80, 3040, and 6000 μg/mL. These three standards were analyzed in a specific order five times. The NCCLS procedure then detailed all the calculations to be performed using these data. Following the analytical procedure the calculations revealed that there was a positive bias (12%) at 80 μg/mL, and negative biases at 3036 μg/mL (−1.4%) and 5991 μg/mL (−4.0%). There was excellent precision in the

TABLE 2—*Results of the NCCLS preliminary evaluation.*

| | Intercept | Slope | % Carryover | Nonlinearity | Drift |
|---|---|---|---|---|---|
| Value | 40.47 | 0.958 | 0.225 | −0.000008 | 3.30 |
| Significant (Yes/No) | Y | Y | N | Y | N |

analysis with the middle concentration (3036 μg/mL) having the highest coefficient of variation of 1.89% throughout the five days.

The NCCLS evaluation protocol culminated with a final data table summarizing the findings (Table 2). These results indicated that neither drift nor carryover were significant in this procedure. There was some nonlinearity, but the value was extremely small. The slope was approximately 1, and the non-zero intercept may have been caused by endogenous nitrite in the urine. The NCCLS preliminary evaluation indicated the analytical procedure was acceptable.

### *Precision and Accuracy Test with Axiom Trilevel Controls*

Axiom Test True™ Truetrol™ Adulteration Controls (Tampa, FL) were obtained as nitrite method controls. Level 1 contained 0 μg/mL $NO_2^-$, Level 2 contained 600 μg/mL, and Level 3 contained 375 μg/mL. Levels 2 and 3 were diluted to make a series of controls to check the accuracy at the test thresholds of 500 and 200 μg/mL, and at 40% of the test thresholds. The Truetrol™ controls were not available in a urine matrix, and no urine was added before the analysis. Dilutions of the Level 2 and 3 controls were made with DI water, then 100 μL of each control, 50 μL of 2000 μg/mL $S_2O_3^{-2}$, and 1850 μL of run buffer were mixed together. The prepared controls were then injected on the CE.

The controls were prepared and analyzed on three separate days. The averaged data are shown in Table 3. The precision of the data was acceptable with the RSDs of the measured concentrations consistently below 2%. The accuracy of these analyses was acceptable, as the concentrations obtained for all of the samples (except 64 μg/mL) were within the acceptable range of ±20%.

### *Interference Study*

Several anions were studied to determine if they would interfere with the analysis of the $NO_2^-$. The anions were supplemented in urine at concentrations of 100 or 1000 μg/mL. Interference with the $NO_2^-$ quantitation was checked at the lower limit of linearity (80 μg/mL) and at the threshold concentration (500 μg/mL). The results are shown in Tables 4 through 7.

These data indicated no serious interferences at the threshold of 500 μg/mL. However, $CrO_4^{-2}$, $S_2O_8^{-2}$, and $Cl^-$ caused erroneously high results at the lower limit of linearity (80 μg/mL $NO_2^-$). None of the other anions evaluated interfered with the quantitative analysis of the $NO_2^-$.

Representative electropherograms are shown in Figs. 2 through 6 for the 80 μg/mL standard. (Data in Figs. 2–4 were obtained in one experiment, and data in Figs. 5 and 6 were obtained approximately one month later.) As documented in Figs. 3 and 4, chromate and

TABLE 3—*Nitrite ($NO_2^-$) precision and accuracy data obtained using Axiom Test True™ Truetrol™ Axiom Adulteration Controls (μg/mL).*

| Day 1 | Day 2 | Day 3 | Average | Standard Deviation | % RSD | Actual | % Error |
|---|---|---|---|---|---|---|---|
| 49.5 | 49.5 | 49.3 | 49.4 | 0.1 | 0.24 | 64* | −22.76 |
| 86.8 | 86.2 | 86.4 | 86.4 | 0.3 | 0.33 | 80 | 8.06 |
| 99.4 | 101.7 | 103.1 | 101.4 | 1.9 | 1.83 | 96 | 5.64 |
| 162.7 | 163.6 | 160.4 | 162.2 | 1.6 | 1.01 | 160 | 1.39 |
| 198.7 | 199.5 | 195.5 | 197.9 | 2.1 | 1.08 | 200 | −1.06 |
| 224.2 | 230.1 | 227.6 | 227.3 | 3.0 | 1.32 | 240 | −5.29 |
| 352.6 | 350.2 | 355.5 | 352.8 | 2.6 | 0.75 | 375† | −5.93 |
| 362.4 | 356.3 | 361.8 | 360.2 | 3.4 | 0.93 | 400 | −9.95 |
| 432.9 | 438.0 | 446.8 | 439.2 | 7.0 | 1.60 | 500 | −12.16 |
| 512.2 | 515.4 | 517.8 | 515.1 | 2.9 | 0.55 | 600† | −14.14 |

* 64 μg/mL is below the LLOL.
† Concentrations of undiluted Axiom Adulteration Controls. All other samples are dilutions of one of these controls.

TABLE 4—*Nitrite interferants study—Part 1.*

| Anion* | Measured $[NO_2^-]$† (μg/mL) | % Error |
|---|---|---|
| $ClO^-$ | 91.3 | 14.1 |
| $CrO_4^{-2}$ | 105.7 | 32.2 |
| $NO_3^-$ | 94.2 | 17.8 |
| $HCO_3^-$ | 93.5 | 16.9 |
| $I^-$ | 93.8 | 17.3 |
| $CH_3COO^-$ | 94.0 | 17.5 |
| $F^-$ | 95.3 | 19.1 |
| $SO_4^{-2}$ | 90.6 | 13.3 |
| $S_2O_8^{-2}$ | 94.9 | 18.6 |
| $Cl^-$ (1000 μg/mL) | 90.9 | 13.6 |

* Anion concentration: 100 μg/mL.
† Actual $NO_2^-$ concentration: 80 μg/mL.

TABLE 5—*Nitrite interferants study—Part 2.*

| Anion* | Measured $[NO_2^-]$† (μg/mL) | % Error |
|---|---|---|
| $ClO^-$ | 93.8 | 17.2 |
| $CrO_4^{-2}$ | 99.7 | 24.6 |
| $NO_3^-$ | 91.0 | 13.8 |
| $HCO_3^-$ | 92.9 | 16.2 |
| $I^-$ | 92.4 | 15.5 |
| $CH_3COO^-$ | 93.6 | 16.9 |
| $F^-$ | 91.0 | 13.8 |
| $SO_4^{-2}$ | 91.5 | 14.4 |
| $S_2O_8^{-2}$ | 106.8 | 33.5 |
| $Cl^-$ (10,000 μg/mL) | 104.5 | 30.6 |

* Anion concentration: 1000 μg/mL.
† Actual $NO_2^-$ concentration: 80 μg/mL.

TABLE 6—*Nitrite interferants study—Part 3.*

| Anion* | Measured $[NO_2^-]$† (μg/mL) | % Error |
|---|---|---|
| $ClO^-$ | 532.2 | 6.4 |
| $CrO_4^{-2}$ | 545.1 | 9.0 |
| $NO_3^-$ | 520.5 | 4.1 |
| $HCO_3^-$ | 531.9 | 6.4 |
| $I^-$ | 496.7 | −0.7 |
| $CH_3COO^-$ | 530.2 | 6.0 |
| $F^-$ | 518.5 | 3.7 |
| $SO_4^{-2}$ | 533.5 | 6.7 |
| $S_2O_8^{-2}$ | 512.6 | 2.5 |
| $Cl^-$ (1000 μg/mL) | 502.2 | 0.4 |

* Anion concentration: 100 μg/mL.
† Actual $NO_2^-$ concentration: 500 μg/mL.

TABLE 7—*Nitrite interferants study—Part 4.*

| Anion* | Measured $[NO_2^-]^\dagger$ (μg/mL) | % Error |
|---|---|---|
| $ClO^-$ | 522.9 | 4.6 |
| $CrO_4^{-2}$ | 501.8 | 0.4 |
| $NO_3^-$ | 517.1 | 3.4 |
| $HCO_3^-$ | 511.1 | 2.2 |
| $I^-$ | 491.2 | −1.8 |
| $CH_3COO^-$ | 492.7 | −1.5 |
| $F^-$ | 493.9 | −1.2 |
| $SO_4^{-2}$ | 520.6 | 4.1 |
| $S_2O_8^{-2}$ | 519.0 | 3.8 |
| $Cl^-$ (10,000 μg/mL) | 519.7 | 3.9 |

* Anion concentration: 1000 μg/mL.
† Actual $NO_2^-$ concentration: 500 μg/mL.

TABLE 8—*Interference study with commercial products.*

| | | 80 μg/mL $NO_2^-$ | | 500 μg/mL $NO_2^-$ | |
|---|---|---|---|---|---|
| | % V/V | Measured $[NO_2^-]$ (μg/mL) | % Error | Measured $[NO_2^-]$ (μg/mL) | % Error |
| Chlorox Bleach | 50 | 0 | ... | 0 | ... |
| SoftCide Soap | 50 | 97.2 | 21.5 | 603.8 | 20.8 |
| Tide Detergent | 50 | 97.1 | 21.4 | 518.8 | 3.8 |
| Visine | 25 | 94.6 | 18.3 | 524.0 | 4.8 |

persulfate co-eluted with the nitrite, which caused the errors in the quantitative analysis. A high concentration of chloride caused the internal standard peak to become very narrow and develop a front (Fig. 5). This front was excluded in the peak integration which increased the area ratio of the analyte. The high chloride content also shifted the two peaks closer together. Iodide eluted just before the internal standard (Fig. 6), but did not seriously affect the quantitation of the nitrite.

Four commercially-available products were also tested as potential interferants. Urine was adulterated with either soap, bleach, or Visine well beyond what a person might normally do if attempting to adulterate a urine sample. Bleach and two soaps were mixed with urine at 50% v/v, and the Visine was mixed at 25% v/v at nitrite concentrations of 80 and 500 μg/mL.

The results of these analyses are shown in Table 8. The bleach completely destroyed the nitrite, whereas the soap products caused the concentrations to be reported slightly elevated. Visine did not significantly affect the nitrite quantitation. Although complete studies were not pursued, it was concluded that bleach would seriously affect the nitrite analysis. However, small amounts of soap or Visine (<10% v/v) should not affect the nitrite quantitation.

### *Analysis of 20 Positive and 20 Negative Urine Samples*

The laboratory had frozen urine samples that had previously screened positive for nitrite using sulfanilic acid and N,N-dimethyl-1-napthylamine (Sigma Chemical, St. Louis, MO) on a Syva 30R (San Jose, CA). Twenty samples were randomly selected to be analyzed using the CE. Twenty urine samples that had screened negative were analyzed concurrently. All 40 samples were rescreened on a Syva 30R in addition to undergoing the confirmation testing on the CE. The results for all 40 samples are given in Table 9. The samples were analyzed by alternating negative and positive urine samples. (All odd-numbered samples should have been negative, and all even-numbered samples should have been positive.) It became immediately obvious that many of the samples that had previously screened positive had degraded during storage at −32°C. Nitrite was still detectable in many of those samples, but was below the 500 μg/mL threshold. However, there was very good agreement between the Syva 30R screening results and the CE confirmation results. The only exception was sample #36 which screened at 311 μg/mL $NO_2^-$, but no nitrite was detected in the CE confirmation. Sample numbers 6, 34, and 38 were diluted with run buffer and reanalyzed approximately one month after these data were obtained. The nitrite concentrations were lower, but within 7% of the concentrations reported in the table. The data from sample 32 are shown in Fig. 7 as a representative electropherogram.

### *Analysis of 100 Random Urine Samples*

Ninety-nine urine samples were analyzed using the CE method. Samples were processed in batches of 20 following initial screening. (One sample was discarded before it could be analyzed, so one batch contained only 19 samples.) Of the 99 samples analyzed, none were positive for nitrite. Due to problems with the relative migration times exceeding the 3% window, it was determined the relative migration time window should be widened to ±4% for routine analysis.

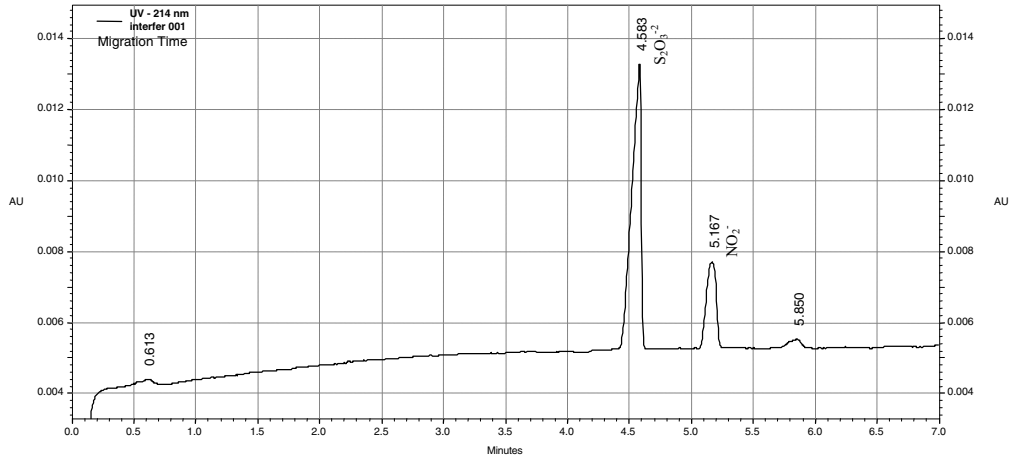


FIG. 2—*Example electropherogram of 80 μg/mL nitrite in urine.*

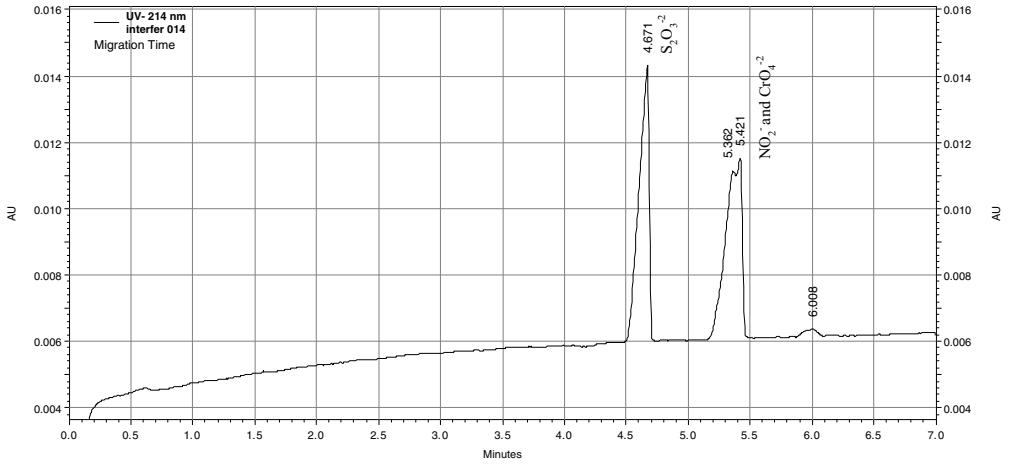


FIG. 3—*Example electropherogram of 80 μg/mL nitrite in urine with 1000 μg/mL* $CrO_4^{-2}$.

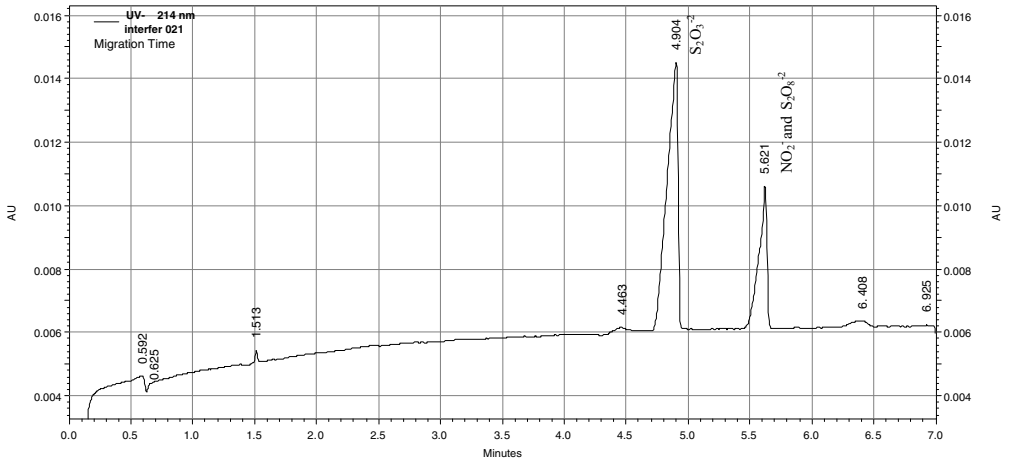


FIG. 4—*Example electropherogram of 80 μg/mL nitrite in urine with 1000 μg/mL* $S_2O_8^{-2}$.

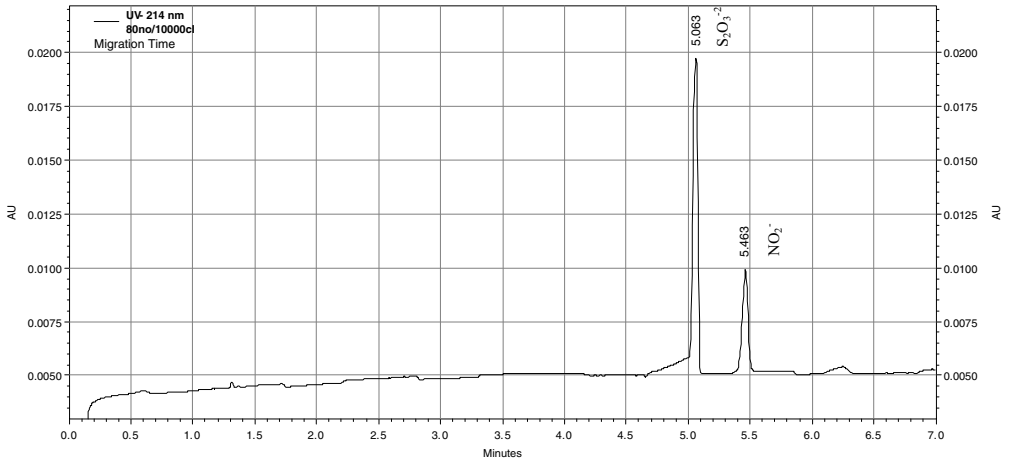


FIG. 5—*Example electropherogram of 80 μg/mL nitrite in urine with 10,000 μg/mL* $Cl^-$.

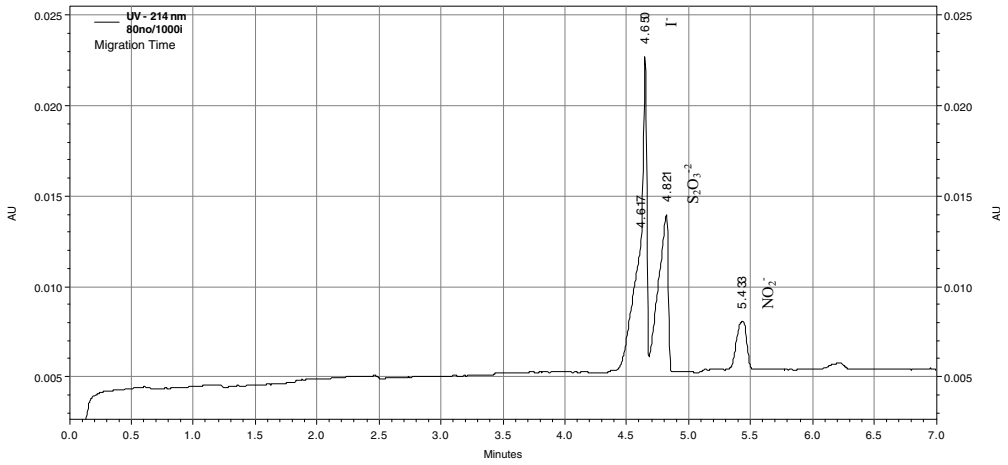


FIG. 6—*Example electropherogram of 80 μg/mL nitrite in urine with 1000 μg/mL $I^-$.*

TABLE 9—*Nitrite concentrations in 20 positive and 20 negative urine samples (μg/mL).*

| | 30R Screening Results | CE Confirmation Results | % Difference | | 30R Screening Results | CE Confirmation Results | % Difference | |
|---|---|---|---|---|---|---|---|---|
| 1 | 3 | ND* | | | 21 | ND | ND | |
| 2 | 49 | <80† | | | 22 | 4594 | 4097 | 12.1 |
| 3 | 6 | ND | | | 23 | 5 | ND | |
| 4 | 77 | <80 | | | 24 | 5258 | 4893 | 7.5 |
| 5 | ND | ND | | | 25 | ND | ND | |
| 6 | 12300 | 11552 | 6.5 | | 26 | 2129 | 1954 | 8.9 |
| 7 | 52 | <80 | | | 27 | ND | ND | |
| 8 | 65 | <80 | | | 28 | ND | ND | |
| 9 | 3 | ND | | | 29 | 1 | ND | |
| 10 | 3631 | 3865 | −6.0 | | 30 | 103 | 97 | 6.0 |
| 11 | ND | ND | | | 31 | 20 | ND | |
| 12 | 308 | 288 | 7.1 | | 32 | 1053 | 1001 | 5.2 |
| 13 | ND | ND | | | 33 | 5 | ND | |
| 14 | 4086 | 4131 | −1.1 | | 34 | 10404 | 10169 | 2.3 |
| 15 | ND | ND | | | 35 | 5 | ND | |
| 16 | 2243 | 2318 | −3.2 | | 36 | 311 | ND | |
| 17 | 21 | ND | | | 37 | ND | ND | |
| 18 | 4682 | 4987 | −6.1 | | 38 | 11620 | 10286 | 13.0 |
| 19 | ND | ND | | | 39 | ND | ND | |
| 20 | 9 | ND | | | 40 | 138 | 136 | 1.6 |

* ND = Not detected.
† Nitrite was detected, but was below the LLOL of 80 μg/mL.

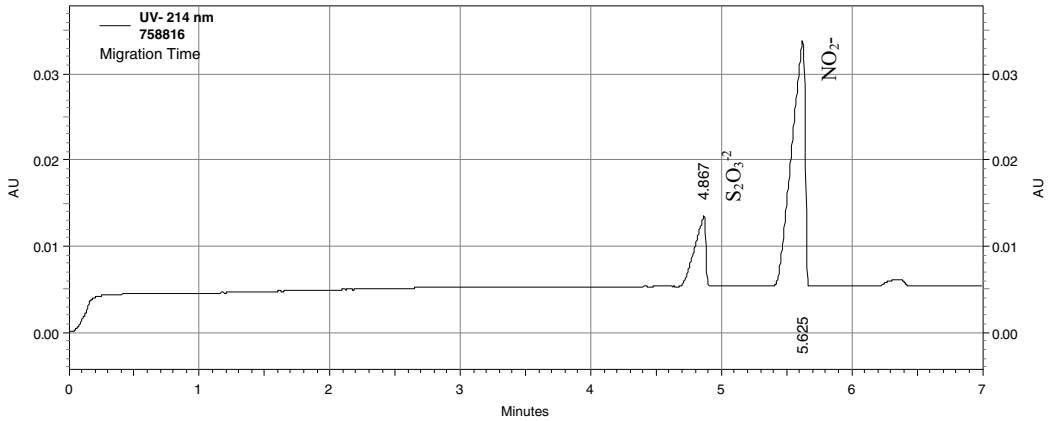


FIG. 7—*Example electropherogram of 6000 μg/mL nitrite in urine.*

## Conclusions

This method for analyzing $NO_2^-$ in urine has good accuracy and precision. Of the ten anions studied as potential interferants, only $CrO_4^{-2}$ and $S_2O_8^{-2}$ interfered with the nitrite quantitation. Data also indicated that a salt concentration exceeding 10,000 μg/mL may affect the peak shapes and change the integration results. Of the four commercial products studied as potential adulterants, only bleach seriously interfered with the nitrite quantitation.

There were two main issues noted with this method. The first was that it was difficult to attain reproducible migration times. The migration times became progressively longer with each injection, and no amount of buffer rinsing helped. It was decided to use a 1 min buffer rinse between each injection to flush the column and replenish the electrolyte, then perform a 13 min regeneration cycle after each third injection. This method resulted in the 2.2% RSD for the relative migration time of nitrite reported. (The absolute migration times had approximately 7% RSD.) The error in the migration time could possibly be reduced by regenerating after each injection. However, it was concluded the reduced error in the migration time was not justified by the time required to regenerate after each injection.

The second issue was the peak shape of both the nitrite and the internal standard. Due to differences in the mobilities of the analytes relative to the phosphate run buffer, both peaks fronted. Alternate buffer systems were evaluated, but none were successful. Given the ease of preparation and low cost, it was decided that the phosphate buffer provided adequate results.

Despite these two issues, the method had a suitable range of linearity, with acceptable quantitative precision and accuracy. The relative migration times were contained within a precision window of ±4%. The method had few interferences, and the buffers and samples were simple and inexpensive to prepare. The validated method was successfully used to test more than 100 authentic urine samples. The method had an initial 33-min period in which the capillary column was conditioned, then it had a throughput of four samples per hour. The threshold evaluated made the method readily applicable to SAMSHA testing guidelines, and the extended linear range makes the method acceptable for potentially lower thresholds as well.

In addition to nitrite, the CE may also be applied to the quantitation of chromate, another common adulterant. The interferance data indicated that chromate was detected using UV detection at 214 nm. However, this may not be the optimal method, and efforts should be expended to optimize and validate the separation and detection parameters for chromate. Other oxidants that are commonly used include pyridinium chlorochromate, bleach, and peroxidase/peroxide and iodine ions. Although chlorochromate could probably be detected by UV, the other analytes may not be amenable to CE analysis with UV detection. In addition, finding internal standards stable enough to withstand such strong oxidizing reagents may be difficult.

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## References

1. Ferslew KE, Hagardorn AN, Robert TA. Capillary ion electrophoresis of endogenous anions and anionic adulterants in human urine. J Forensic Sci 2001;46(3):615–26. [PubMed]
2. Soga T, Tajima I, Heiger DN. Capillary electrophoresis for the determination of forensic anions in adulterated foods and beverages. Amer Laboratory 2000;32(3):124–7.
3. Barciela Alonso MC, Prego R. Determination of silicate, simultaneously with other nutrients (nitrite, nitrate, and phosphate), in river waters by capillary electrophoresis. Anal Chim Acta 2000;416:21–7.
4. Melanson JE, Lucy CA. Ultra-rapid analysis of nitrate and nitrite by capillary electrophoresis. J Chrom A 2000;884:311–6.
5. Li J, Ding W, Fritz JS. Separation of anions by ion chromatography-capillary electrophoresis. J Chrom A 2000;879:245–57.
6. Fukushi K, Ishio N, Urayama H, Takeda S, Wakida S, Hiiro K. Simultaneous determination of bromide, nitrite, and nitrate ions in seawater by capillary zone electrophoresis using artificial seawater as the carrier solution. Electrophoresis 2000;21:388–95. [PubMed]
7. Padarauskas A, Olsauskaite V, Paliulionyte V, Pranaityte B. Simultaneous separation of nitrate, nitrite and ammonium by capillary electrophoresis. Chromatographia 2000;52(3/4):133–6.
8. Morcos E, Wiklund NP. Nitrite and nitrate measurement in human urine by capillary electrophoresis. Electrophoresis 2001;22:2763–8. [PubMed]
9. Hage DS, Chattopadhyay A, Wolfe CAC, Grundman J, Kelter PB. Determination of nitrate and nitrite in water by capillary electrophoresis. J Chem Educ 1998;75(12):1588–90.
10. National Committee for Clinical Laboratory Standards Document EP10-A2 (ISBN 1-56238-482-1). Preliminary evaluation of quantitative clinical laboratory methods; approved guideline. 2nd ed. Wayne, PA: NCCLS.

Additional information and reprint requests:
Peter R. Stout, Ph.D.
Aegis Sciences Corporation
345 Hill Ave.
Nashville, TN 37210
E-mail: pstout@aegislabs.com