K28 Analysis of Nitrite in Adulterated Urine Specimens by Capillary Electrophoresis

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The purpose of this study was to develop and validate a simple, inexpensive, and robust method for the detection and quantitation of nitrite in urine for the confirmation of screening results. A method was developed that was specific, accurate, and precise with a wide dynamic range.

This research presents methods that improve the detection of nitrites in urine, which have been used to adulterate samples submitted for forensic urine drug testing. Thus, this presentation provides a method to make this testing more reliable.

Continual issues arise in urine drug testing with adulteration of samples. Nitrite (NO2-) compounds are sometimes used as adulterants to destroy traces of drugs in urine samples. Many laboratories use either specific or general oxidant colorimetric tests to screen for the presence of nitrite or other oxidants. However, for forensic acceptability, it is necessary to have a second test of the samples, preferably using a distinctly different chemical basis to confirm the initial findings.

A Beckman Coulter P/ACE MDQ Capillary Electrophoresis System was used for these experiments. The capillary was uncoated fused silica with an inner diameter of 0.75 mm 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 analytes were separated using -25 kV. The column temperature was maintained at 35ºC 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 adjusted to 7.5 with NaOH.

At the start of each batch of samples, the column went through an initial regeneration/equilibration cycle that included washes with NaOH and buffer. Before each sample was injected, the column was flushed for 1min with run buffer. After every three samples, the column was regenerated with NaOH and buffer.

The lower limit of linearity for this method was determined to be
80 mg/mL NO2in urine (4mg / mL on-column concentration.) Although the quantitative values were acceptable up to 6000 mg/mL, the relative migration time restricted the upper limit of linearity to 1500 mg/mL. The LOD for this method was determined to be 20 mg/mL NO2in urine with a S:N of approximately 11.

The precision and accuracy of the method were determined by analyzing Axiom Test True™ Truetrol™ Adulteration Controls. Controls were analyzed as received, and were also diluted to span more of the linear range. The precision of the data was good with the relative standard deviations of the calculated concentrations consistently below 2%. The accuracy of these analyses was acceptable, as the concentrations obtained for all of the samples within the linear range were within
+ 20% of the actual values.

Several anions were studied to determine if they would interfere with the analysis of the NO2-. The anions were fortified in urine at concentrations of 100 or 1000 mg/mL, except chloride, which was fortified at concentrations of 1000, or 10,000 mg/mL. Interference with the NO2quantitation was checked at the lower limit of linearity (80 mg/mL) and at the threshold cutoff concentration (500 mg/mL). These data indicated that, among these anions, there were no serious interferences noted at the threshold level of 500 mg/mL. However, CrO4-2, S2O8-2, and Clcaused erroneously high results at the lower limit of linearity (80 mg/mL NO2-). None of the other anions that were tested interfered with the quantitative analysis of the nitrite.

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-minute buffer rinse between each injection to flush the column and replenish the electrolyte, then perform a 13-minute regeneration cycle after each third injection. This method resulted in the 2.2% RSD for the relative migration time of nitrite that was reported earlier in this paper. (The absolute migration times had approximately 7% RSD.) Regenerating after each injection could readily reduce the error in the migration time. However, it was decided that the increased precision in the migration time was not worth the large amount of time that would be required to regenerate after each injection.

The second main 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. The only means to correct this problem would be to change the buffer system. Various buffer systems were tested, but none performed well. Given the simplicity of the phosphate buffer system, the ease of making it, and the low cost of the chemicals, it was decided that the phosphate buffer provided adequate results.

The method had an acceptable range of linearity, with good quantitative precision and accuracy. The precision in the relative migration times was not as impressive, as it is recommended that a 4% window be allowed rather than the standard 2% window applied to most chromatographic methods. The method had few interferences, and the buffers and samples were simple and inexpensive to prepare. The method passed a

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rigorous validation protocol, and was successfully used to test more than 100 real urine samples.

Adulteration Testing, Nitrite in Urine, Capillary Ion Electrophoresis

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