Nitrate Reductor Helpful Hints

As shown in the video, the Nitrate Reductor is very simple to use. However, when analyzing samples with complex matrixes, pre-analytical sample preparation is very important. A thorough discussion of this subject is found in the previously cited reference. This reference may be accessed at no charge by using the Digital Object Identifier (DOI).
http://dx.doi.org/10.5740/jaoacint.10-454

1. The Reductor was designed to use with Nunc 96-well microplates, available from Fisher Scientific. These microplates are of the proper depth and have built-in positioning holes that enable the Reductor to be attached securely to the microplate with locking pins. If another brand of microplate is used, the Reductor may be fitted into place and securely taped to the microplate. However, some brands of microplates may not be suitable if the wells are of insufficient depth (i.e. less than 10.5 mm). The Reductor pins must not be allowed to touch the bottom of the plate. Any small scratch in the optical surface will scatter light when the microplate is read and will cause significant (and usually undetectable) error in the analysis.

2. If available, a digital dilutor (e.g., Hamilton Microlab 500) may be used to prepare samples for analysis and to make calibration standards. This instrument will make more accurate serial dilutions than regular air displacement pipettes and will produce extremely precise standard curves.

3. A one hour reduction time was found to be optimal for most applications. A shorter time may be satisfactory for samples with high nitrate concentrations. However, if the nitrate concentrations are very low, an extended reduction time may be necessary. The rate of reduction of nitrate to nitrite is dependent on the probability of nitrate ions encountering active reducing sites on the surface of cadmium pins. If the nitrate concentrations are low, it will take longer to achieve near complete reduction.

4. When a full plate of samples is not required, it is good technique to fill the entire plate with the buffer solution plus matrix blank to equalize wear on the cadmium pins. After reduction, Griess reagent may also be added to these extra wells. In the event that some samples are over-range, a good approximation of their concentration may be made by merely estimating the necessary dilution factor and withdrawing an appropriate amount of liquid from the extra blanks and then adding the same amount from each of the over-range samples. For example, if the total volume of liquid, after addition of to the Griess reagent, is 280 µL, to make a 10X dilution, withdraw 28 µL from a well containing the matrix blank and discard. Then add 28 µL of the over-range sample to the blank well. The microplate may then be shaken briefly and reread on the plate reader. This technique will not work if the over-range samples are so high that a precipitate is observed shortly after the plate is read.

5. If mathematical curve fitting options (i.e., quadratic) are available with the microplate-reader software, the calibration standard range may be somewhat extended with little effect on the accuracy of the analysis. However, multiple, evenly-spaced standards should be used to better define the calibration curve.

6. When using the pH 10 buffer system, a slight increase in the speed of the titer-plate shaker (e.g., 6.5 to 7.5) may be necessary to achieve proper mixing after the Griess reagent is added.

7. Although urine samples may require dilutions of 1/50 or more, the use of the Somogyi reagent cleanup procedure is still highly recommended.

8. It is unnecessary to change the cleaning solution frequently. A few µL (~50) of the 2% copper sulfate solution should be added after 10 uses to maintain the reductive capacity.

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