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Shaker Bath Adaptation to the Environmental Protection Agency's Cadmium Column Reduction Method (Method 353.3) for the Determination of Nitrate in Water Samples

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Nitrate is found as a drinking water contaminant in nearly every state in the United States. Fertilizer use and the microbial degradation of biotic and abiotic nitrogen -containing compounds also contribute to nitrate contamination (Spaulding & Exner 1991). Nitrate is a growth- limiting nutrient for marine phytoplankton (Morris & Riley 1963) and many photosynthetic autotrophs (American Public Health Association, 1975). The nitrate ion is highly mobile in water and therefore a potential source of ground and surface water contamination.

Accumulation of nitrate in ground and surface waters possess a health risk to animals (Crespi & Ramazzotti 1991) and elevated levels of nitrate in well water has been associated with human infant methemoglobinemia (Comly, 1945). Elevated nitrate levels in drinking water can lead to the formation of nitrosoamines which have also been suspected to participate in the development of human cancers (Correa, 1988).

The following adaptation of the USEPA Method 353.3 (USEPA, 1979) was made to allow for the determination of nitrate in water samples without the need to purchase expensive probes, analyzers or specialized glassware. The procedure is useful for the laboratory which has a transient interest in aqueous nitrate concentrations, and it allows for the quick, sequential analysis of up to ten samples in a twenty minute period.

This study was undertaken to answer two questions. First, is the proposed modification comparable to Method 353.3? Second, does the efficiency of the cadmium metal to reduce nitrate decline over sequential samples?

MATERIALS AND METHODS

All chemicals were ACS grade and purchased from Fisher Scientific. The 0.45µm, 47mm diameter membrane filters were purchased from Gelman Sciences.

Ten grams of granulated cadmium were placed into a 250 -mL Erlenmeyer flask and washed with 80 mL of 6N HCl, which gives the cadmium a bright silver color. The acidified cadmium was then washed with distilled water to remove the excess HCl and then washed twice with 80 mL of 2% copper sulfate (20g $CuSO_4 \cdot 5H_2O$ per liter of distilled water). This produces a black colloidal precipitate of Copper which is removed with distilled water (at least 5 washes).

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The oxidized metal can not be allowed to dry and is preserved between uses with dilute ammonium chloride-EDTA (300 mL of ammonium chloride-EDTA and 200 mL of distilled water) which is decanted before sample addition. The ammonium chloride-EDTA was prepared by mixing 13g ammonium chloride and 1.7g disodium ethlyene tetracetate in 900 mL of distilled water and adjusting the pH to 8.5 with concentrated ammonium hydroxide and diluting to one liter.

To determine the concentration of nitrate, 25 mL of sample (pH adjusted between 5.0 and 9.0) was filtered (0.45µM glass fiber filter) into a flask containing 10 g of the prepared cadmium. Within 15 minutes, 75 mL of the ammonium chloride-EDTA solution were added, the flask stoppered and shaken for 20 min. at room temperature at a rate of approximately 125 agitations/min. Fifty mL of solution were decanted into a separate 100 mL beaker to which 2.0 mL of color reagent 10 g of sulfanilamide + 1.0 g of N-(1-napthyl)-ethylenediamine dihydrochloride + 100 mL of phosphoric acid (conc.)/L] were added. Samples were incubated for 30 min. but no longer than two hours and absorbency was measured at 540 nm.

Between each set of samples it was necessary to wash the cadmium at least twice with distilled water and then follow the above steps for the next analysis. According to previous work (Morris and Riley 1963; American Public Health Association, 1975) the cadmium is stable and does not need to be recharged for several analyses providing that it is not allowed to dry and is stored in dilute NH_4Cl-EDTA. A separate experiment of 25 sequential samples of a 0.5 ppm standard showed no loss of efficiency and verified that the cadmium was indeed stable for several analysis.

Validation of the modified method was accomplished by running purchased nitrate standards (Hach) and unknowns by both methods. The standard curves were generated with 7.218g of potassium nitrate dissolved in 500 mL of distilled water and diluted to one liter (Stock nitrate solution 1.0 mL = 1.0 mg NO_3-N). Solution preservation is discussed in Method 3533. Table 1 and graphs 1A &1B show the comparison of 14 samples that were split and analyzed using both methods.

RESULTS AND DISCUSSION

In order to determine if this method was comparable to USEPA Method 653.3 and to see if there was a decreased efficiency of the cadmium over prolonged use, samples and spikes were split and analyzed according to the USEPA Method 353.3 and with the proposed adaptation. The loss of cadmium efficiency over time was tested by subjecting three grades of cadmium metal- new, single use preserved, multi-use air dried- to 25 sequential trails of 0.5 µg/I. Nitrate-Nitrogen.

Both methods are useful for the determination of nitrate concentrations between 0.1 ppm and 1.0 ppm. However, the proposed adaptation requires less specialized equipment to achieve this range of detection. Samples containing concentrations of 1.0 ppm or greater, will have to be diluted with distilled water prior to analysis. Separate nitrate and nitrite measurements can be made by analyzing samples with and without the reduction step.

Method 353.3 requires the manufacture or purchase of a cadmium reduction column containing no less than 18.5 cm of the prepared cadmium metal. All reagents and sample quantities are identical in both methods. In Method 353.3,25 mL of the sample and 75 mL of the ammonium chloride-EDTA solution are placed into the reservoir of the column and allowed to percolate through the column at a drip rate of 7 to 10 mL per min. The initial 30 mL of the reduced sample are discarded and the remaining solution is collected for analysis. All remaining steps are the same as mentioned above.

The comparison of the two methods by the splitting of 14 samples is summarized in the following table:

Table 1. (Comparison of standard recoveries (ppm)).

Method 353.3	Shaker Method	Actual Concentration
0.405	.764	0.25
0.50	0.65	0.50
0.72	0.42	0.75
0.48	1.35	1.00
0.45	1.14	1.00
0.48	1.17	1.00
0.943	1.09	1.00
0.82	0.934	1.00
1.70	2.40	2.00
1.90	2.76	2.00
4.40	4.98	5.00
4.55	5.10	5.00
4.99	5.10	5.00
5.56	5.53	5.00

Figure 1 shows the fitted regressions obtained by plotting measured concentrations against actual concentrations for each method. The R^2 values for both regressions indicate a high degree of correlation (0.98 & 0.97 for the shaker and column methods, respectively). The slopes of the regression lines for the two methods did not differ significantly, though the intercepts were different indicating a difference in the population sampled.

The question of the loss of efficiency of the cadmium to reduce the nitrate after several sequential samples was addressed in order to determine at what point should the cadmium be cleaned and recharged. This experiment was designed using varying degrees of used cadmium between the two methods (Table 2). Although previous work has stated that the metal is stable for several weeks (Morris and Riley 1963; American Public Health Association, 1975), these data suggest that recharging after 25 samples ensures that the cadmium is most efficient in reducing the nitrate.

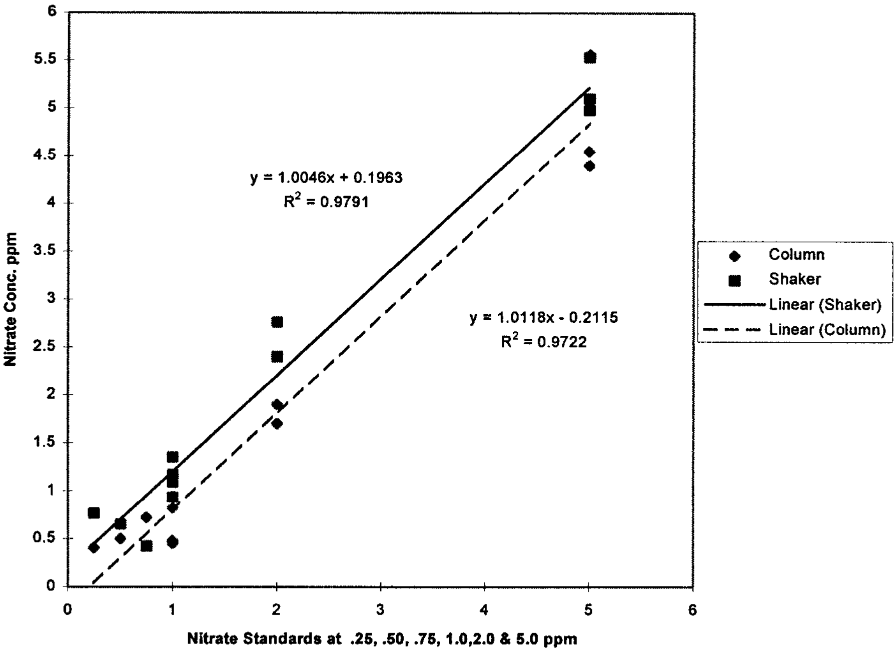
Table 2. (Comparison of methods for loss of efficiency by comparison of the mean of the absorbency values.)

	Shaker Method		Method 353.3	
Cd Metal Usage	Mean	Variance	Mean	Variance
Air Dried	0.190	1.2e-5	0.210	7.1e-5
Single Use	0.194	3.9e-5	0.242	8.5e-5
New (unused)	0.184	3.1e-5	0.224	3.2e-5

The mean values are the absorbencies of each sample. The shaker method is represented by ten values in the mean (one sample equally divided between two flasks and then sub divided into five separate samples for analysis), and Method 353.3 is represented by five samples per mean.

Statistical analysis of the data utilized the SAS software to perform linear regression and analysis of variance.

Figure 1 Comparison of Methods to Nitrate Standards



Statistical analysis was performed to determine differences in the cadmium efficiency between methods and cadmium types. F-tests produced values below the critical values and the analysis showed a highly significant difference between both cadmium efficiency and cadmium types between the two methods.

Table 3 represents the use of this modification for the determination of nitrate and selected pesticides in a recent study on three North Carolina golf courses (*Ryals ET AL 1998, Environmental Toxicology and Chemistry).*

Table 3. (Nitrate Concentrations (ppm) on three Eastern North Carolina golf courses)

Sample Date	Nitrate-Nitrogen C I	Nitrate-Nitrogen C II	Nitrate Nitrogen C III
5-Jan	0.00	0.00	0.00
19-Jan	0.00	0.00	0.00
2-Feb	0.00	0.00	0.00
2-Mar	0.00	0.04	0.00
16-Mar	0.01	0.05	0.00
30-Mar	0.11	0.03	0.01
13-Apr	0.54	1.98	0.09
27-Apr	0.27	0.05	0.00
25-May	0.27	0.70	0.00
8-Jun	0.28	0.67	0.00
22-Jun	0.00	0.73	0.00
6-Jul	0.30	0.19	0.01
20-Jul	0.24	0.41	0.11
3-Aug	0.01	0.07	0.01
17-Aug	0.03	0.09	0.01
31-Aug	0.03	0.02	0.02
14-Sep	0.09	0.03	0.02
28-Sep	0.06	0.03	0.08
12-Oct	0.07	0.22	0.01
26-Oct	0.06	0.08	0.05
9-Nov	0.04	0.07	0.05
23-Nov	0.02	0.13	0.01
7-Dec	0.02	0.10	0.03

*All samples were collected in 1994 and values of 0.00 indicate lost data.

The results suggest that both methods are comparable in the detection of nitrate in water. The shaker modification offers a less expensive analytical alternative with the cost of the reagents as the only initial expenditure, as opposed to the initial expenditure of hundreds of dollars for screening techniques or thousands for automated analyzers with associated data handling systems and the subsequent expenditures for reagents and maintenance. It allows for more samples to be analyzed at a time with less direct operator observation as compared to Method 353.3. The shaker method requires less direct maintenance since the researcher does not have to be present to observe that the column reservoir does not run dry and allow the cadmium to dry or introduce air bubbles into the column matrix which can cause loss of flow rate. Both methods are susceptible to drifting values due to loss of column efficiency. In addition, the shaker modification is a time and monetary saving compared to Method 353.3 resulting in the equivalent detection levels.

REFERENCES

American Public Health Association (1975), Standard methods for the examination of water and waste water, 14th edition, p. 423, Method 419C American Public Health Association, Washington, DC.

Comly H (1945) Cyanosis in infants caused by nitrates in well water. In: Bogardi and Kuzelka (ed), Nitrate contamination (exposure, consequence and control). NATO ASI Series G Vol. 30. Springer-Verlag, New York, p. 14.

Correa P (1988) Nitrate exposure and human cancer. In: Bogardi and Kuzelka (ed), Nitrate contamination (exposure, consequence and control). NATO ASI Series G Vol. 30. Springer-Verlag, New York, p.281.

Crespi and Ramazzotti (1991) Evidence that the N-Nitroso compounds contribute to the causation of certain human cancers. In: Bogardi and Kuzelka (ed), Nitrate contamination (exposure, consequence and control). NATO ASI Series G Vol. 30. Springer-Verlag, New York, p.233.

Morris AW and Riley JP (1963) The determination of nitrate in seawater. Anal Chim Acta 29: 272-279.

Spalding R and Exner M (1991) Nitrate contamination in the contiguous United States. In: Bogardi and Kuzelka (ed), Nitrate contamination (exposure, consequence and control). Nato ASI Series G Vol. 30. Springer-Verlag, New York, p. 13.

United States Environmental Protection Agency (1979) Methods for chemical analysis of water and wastes. EPA Monitoring and Support, Doc. EPA-600/4-79-020, Cincinnati.