Nitrate

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SUMMARY

This procedure is used for measuring nitrate in water samples using Hach TNT 835 low range nitrate kits.

EQUIPMENT AND SUPPLIES

Hach DR2800 Spectrophotometer
1000µl pipette
kimwipes
sample racks
pipette tips
Eye Droppers
Acid washed squeeze bottle
Analytical balance with at least 2 decimal places
200µl pipette
Hach TNTplus 835 kits
alcohol swabs
timer
100mL Volumetric Flasks
Ultrapure water (Type 1)
Gloves

NOTES

- All glassware (with the exception of the Hach kit vials) used for samples, standards, or blanks needs to be acid-washed prior to use.
- Samples need to be analyzed as soon as possible (within 6 hours of receipt at the lab).
- Never pour leftover chemicals down the drain or in the trash. There are appropriate receptacles located in the hood in Room 105.
- Check the date balances were last calibrated before using. They should be calibrated by a professional on an annual basis and checked using calibrated weights monthly.

- Pipettes used in this procedure need to be calibrated annually and checked using a balance on a monthly basis or as problems arise.
- Wear gloves at all times during procedure!
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REAGENTS

1. All chemicals used in this procedure are included in the Hach TNT 835 kit. Use care when pipetting these reagents and dispose of appropriately.

STANDARDS

1. 100mg/L NO3-N (NIST) stock solution for making standards is purchased from the Hach company (product # 194749). This solution is stable in the refrigerator for about six months after opening or until expiration date on bottle (whichever comes first).
2. For all standards, mix stock in ultrapure (type 1) water. These should be prepared no more than 24 hours before each sampling event. Place a 100mL volumetric flask on the analytical balance, and tare. Add amount of 100mg/L stock standard given below using a new eye dropper. Use the squeeze bottle to dilute to mark with type 1 ultrapure water. Store in acid washed, glass, amber bottle. 0ppm is simply ultrapure water.

0.5ppm: 0.5g stock solution	2.500ppm: 2.5g stock solution
7.500ppm: 7.5g stock solution	10.00ppm: 10g stock solution .

   These standards are run by lab personnel at the beginning of each sampling day to test the efficiency of laboratory equipment. If the standards are off by more than 10%, recalibrate spec and rerun standards. If they are still off, record the values and make sure that linearity is retained in the event that sample values need adjusted at a later date (ex. purchased standards are also off by same amount).
3. In addition to prepared standards, a wastewater standard (Hach # 2833249), drinking water standard (Hach # 2833049), and 1mg/L No3-N standard (Hach #

204649) are run through as samples to test the accuracy of the volunteer running the samples. These standards need to be within 10% of their expected values in order for accuracy to be considered achieved.

SAMPLE PREPARATION AND STORAGE

Sample are collected in coolers containing ice packs to keep them as cool as possible in the field and should be stored at 4˚ C immediately upon arrival in the lab. Samples are to be analyzed within 6 hours of arrival at the lab. If it is not possible to analyze samples within this time range, samples must be frozen. Freezing samples should only happen in cases of extreme emergency or complete equipment failure.

SAMPLE ANALYSIS

1. Make sure vial is marked with correct sample number.
2. Check to make sure spectrophotometer is on and reads “please insert barcode cuvette”. If this is not the case, find a lab manager and inform them of the issue. If you are unfamiliar with using a pipette, please see a lab manager. **Please note:** Check the lot numbers on the sample box prior to starting a new box. Whenever the lot number on the sample vials changes, please rerun a blank and a standard before running any more samples. If the values are off or you are unsure how to do this, please see a lab manager.
3. Pipette 1000µL (1mL) of sample into the reagent vial using a new pipette tip and the 1000 µL (blue top) pipette. Place in test tube rack. You can set up about 5 samples before continuing to step 4.
4. Pipette 200µL of solution A into each of the reagent vials using a new pipette tip and the 200 µL (yellow top) pipette. Caution: Vial will get very warm upon addition of this chemical. Cap quickly and handle vial by the lid.
5. After capping, invert vial 2-3 times until no more streaks are present in the solution. Set timer for 15 minutes.

6. While reaction is occurring, wipe down vials with an alcohol swab to remove and dust, fingerprints, etc.
7. **Sample one is used to blank the machine. Please see a lab manager when this sample is ready.** When timer beeps, remove cover from spec. Place the vial into the cell holder, making sure to align barcode with arrow, and quickly replace cover. This test is time sensitive! Read results as quickly after reaction time as possible.
8. Record value on the data sheet. Note: If the machine reads **Under Range**, make sure to record that on the data sheet. If the machine reads **Over Range**, put sample aside to be diluted and rerun. For dilution: Pipette 500µL of sample and 500µL of ultrapure water into a new vial and repeat steps 3-6. Make sure to mark the sample on the sheet as diluted. Retain both undiluted and diluted values.

CLEAN UP

Throw used pipette tips, kimwipes, and alcohol swabs in the trash. Place capped reaction vials in designated disposal bin. Straighten bench space.