Selenium

DOC316.53.01129

Diaminobenzidine Method[1]

Method 8194

0.01 to 1.00 mg/L

Scope and Application: For water and wastewater

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater.*

Test preparation

How to use instrument-specific information

The *Instrument-specific information* table displays requirements that may vary between instruments. To use this table, select an instrument then read across to find the corresponding information required to perform this test.

Table 375 Instrument-specific information

Instrument	Sample cell	Cell orientation
DR 6000	2612602	Fill line faces right
DR 5000	2612602	Fill line faces user
DR 3900	2612602	Fill line faces user
DR 3800, DR 2800, DR 2700	2612602	Fill line faces right

Before starting the test:

Distillation is required for determining total selenium. See *Distillation* at the end of the procedure. Use the distillate as the sample in step 2.
Acetone[1] is a suitable solvent for removing toluene from glassware after results are measured.
Toluene (F005) solutions are regulated as hazardous waste by the Federal RCRA. Do not pour these materials down the drain. Water saturated with toluene, toluene solutions and the cotton plug used in the delivery tube of the separatory funnel should be collected for disposal with laboratory solvent wastes. Refer to the current MSDS for safe disposal and handling information.
If there are visible water bubbles on the bottom of the cell, decant the top portion into a clean, dry cell prior to reading the sample.
In bright light conditions (e.g. direct sunlight) it may be necessary to close the cell compartment with the protective cover during measurements.

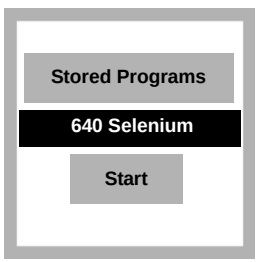
[1] See *Optional reagents and apparatus*.

Collect the following items:

Description	Quantity
Buffer Solution, sulfate type, pH 2.0	10 mL
Cotton Ball	1
Cylinder, Graduated: 50- and 100-mL	1 of each
Diaminobenzidine, tetrahydrochloride	0.1 g
Distillation reagents and apparatus	—
Dropper, 0.5 and 1.0 mL marks, one glass and one plastic	1 of each
Flask, Erlenmeyer, 500-mL	2
Funnel, separatory, 250 mL	2
Hot Plate, 7-inch	1
Pipet, volumetric, 5-mL, plus safety bulb filler	1
Potassium Hydroxide Standard Solution, 12 N	4 mL
Ring support (3-inch) and stand	2
Sample Cells (see *Instrument-specific information*)	2
Spoons, measuring, 0.2 and 0.05 g	1 of each
TitraVer® Hardness Reagent	0.4 g
Toluene, ACS	60 mL
Water, Deionized	100 mL

See *Consumables and replacement items* for reorder information.

Diaminobenzidine method



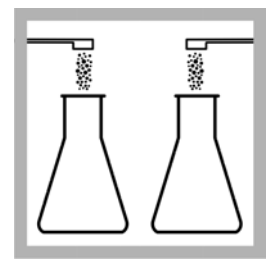
1. Select the test.

Insert an adapter if required (see *Instrument-specific information*).

2. Measure 100 mL of deionized water into a 500-mL Erlenmeyer flask. Label the flask “blank”.

Measure 100 mL of sample into a 500-mL Erlenmeyer flask. Label the flask “sample”.

3. Add a 0.2-g spoonful of TitraVer® Hardness Reagent to each flask. Swirl to mix.



4. Add a 0.05-g spoonful of diaminobenzidene tetrahydrochloride to each flask. Swirl to mix.

Diaminobenzidine method (continued)

5. If you have not distilled the sample, add 5.0 mL of Buffer Solution, sulfate type, pH 2.0 to each flask. Swirl to mix.

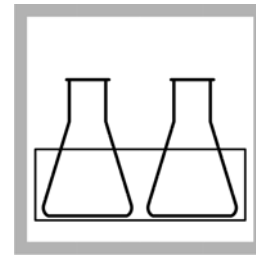
If the sample has been distilled, adjust the pH of the distillate to pH 2.7 ± 0.2 using 5 N Sodium Hydroxide Standard Solution. Adjust the blank to pH 2.7 ± 0.2 using 5.25 N Sulfuric Acid Standard Solution.

6. Heat each flask on a hot plate. Bring the contents to a gentle boil.

7. Start the instrument timer.

A five-minute reaction period will begin. Continue to boil the contents gently during this time period.

A yellow color will develop if selenium is present.

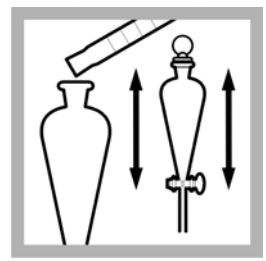


8. When the timer expires, remove both flasks. Cool to room temperature using a water bath.

Do not boil more than one minute after the timer expires.

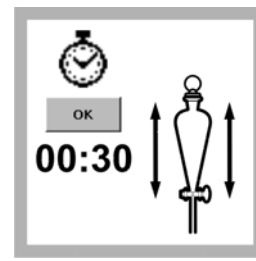
9. Transfer the contents of each flask to separate 250-mL separatory funnels. Label the funnels “blank” and “sample”. Place each flask in a support ring on a stand.

10. Add 2.0 mL of 12 N Potassium Hydroxide Standard Solution to each funnel using a calibrated 1.0-mL plastic dropper. Stopper. Shake each funnel to mix.



11. Add 30-mL of toluene to each funnel. Stopper. Swirl and invert each funnel, then open the stopcock to vent the funnel. Close the stopcock. Repeat twice with each funnel.

Use toluene only with adequate ventilation.



12. Start the instrument timer.

A 30-second reaction period will begin. During this time, vigorously shake the funnel that contains the blank.

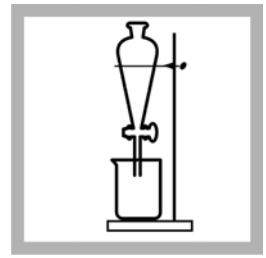
Diaminobenzidine method (continued)

13. Start the instrument timer.

A 30-second reaction period will begin. During this time, vigorously shake the funnel that contains the sample.

14. Start the instrument timer.

A four-minute reaction period will begin.



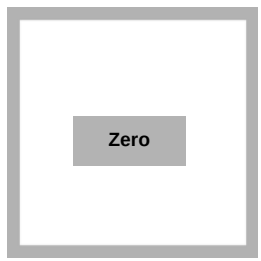
15. When the timer expires, remove the stopper and drain the lower water layer from each funnel and discard.

Complete steps 16–19 within five minutes after the timer expires. The developed color is stable, but should be measured as soon as possible.

16. Insert a cotton plug into the delivery tube of each separatory funnel. Slowly drain the toluene into respective sample cells labeled “blank” and “sample”. Stopper the sample cells.

Filtering the toluene through dry, absorbent cotton will remove water or suspended particles.

17. Wipe the blank and insert it into the cell holder.

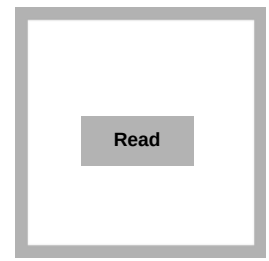


18. **ZERO** the instrument.

The display will show:

0.00 mg/L Se

19. Wipe the prepared sample and insert it into the cell holder.



20. **READ** the results in mg/L Se.

Interferences

There are no positive inorganic interferences with this method. Any other interferences can be removed by distilling the sample.

Table 376 Interfering substances

Interfering substance	Interference level
Ferric iron	Up to 2.5 mg/L. Distill sample to eliminate interference.
Manganese	Will not interfere.
Strong oxidizing agents (i.e., iodine, bromine or chlorine)	Can react with the indicator to give low results. Distill sample to eliminate interference.

Sample collection, preservation and storage

- Collect samples in clean glass or plastic containers.
- Adjust the pH to 2 or less with Nitric Acid* (about 1.5 mL per liter).
- Preserved samples can be stored for up to six months at room temperature.
- Correct the test result for volume additions.

Distillation

CAUTION
Always perform this procedure under a fume hood.

This distillation involves the use of a strong acid and oxidizer at high temperatures. To avoid personal injury, observe all laboratory safety precautions when operating the distilling apparatus.

1. Measure 500 mL of sample into a 1000-mL beaker.

2. Add 1 mL of Methyl Orange Indicator Solution. Stir with a glass rod.

3. Use a dropper to add 0.1 N Hydrochloric Acid Standard Solution dropwise until the solution becomes pink. Then add an additional 2 mL.

4. Use a pipet to add 5.0 mL Calcium Chloride Solution. Mix well.

5. Use a dropper to add 1-g/L Potassium Permanganate Standard Solution drop-wise until the solution is purple.

6. Place the beaker on a hot plate. Evaporate the solution to approximately 250 mL. Periodically add 1-g/L Potassium Permanganate Solution to keep the solution purple.

7. Any precipitate formed at this step is manganese dioxide and may be ignored.

8. Cool the solution. While cooling, set up the distillation apparatus for the general purpose distillation as shown in the distillation manual.

9. Pour the treated sample solution into the distillation flask. Add a stirring bar to the flask.

10. Pipet 5.0 mL of 0.1 N Sodium Hydroxide Standard Solution into the flask. Turn the stirrer power switch to ON. Set the stir control to 5.

11. Turn on the water and adjust so a constant flow is maintained through the condenser. Set the heat control to 10.

12. When only a few milliliters are left in the distillation flask, turn the power switch off. The distillate in the Erlenmeyer flask may be discarded.

CAUTION
Perform step 13 under a fume hood.

13. When the flask has cooled, add 50 mL of 19.2 N Sulfuric Acid Standard Solution to the flask. Add 10 grams of Potassium Bromide† to the flask.

14. Fill a 250-mL beaker to the 75-mL mark with deionized water. Place it under the drip tube. Elevate the beaker with a laboratory jack so the tube extends below the level of the water.

* See *Optional reagents and apparatus*.

† Purchase Potassium Bromide from a local chemical supplier.

15. Add 1.0 mL of 30% hydrogen peroxide solution to the flask. Turn the stir control to 5 and the heat control to 10. Cap the distillation flask.
16. Heat the distillation flask until the yellow color is gone from the complete distillation apparatus, including the J-tube and condenser. Remove the beaker from under the drip tube.
17. Turn off the heater switch. When the J-tube and condenser have cooled, rinse them with deionized water. Add the washings to the 250-mL beaker. Total volume in the beaker should be approximately 100 mL.
18. Add the Phenol Solution drop-wise to the distilled sample to discharge the bromine color (a white precipitate of tribromophenol will form).
19. Allow the precipitate to settle. Using a dropper, collect about 5 mL of the clear, colorless distillate and transfer to a test tube.
20. Test the solution for completeness of precipitation by adding 2 drops of Phenol Solution. If the solution becomes cloudy or white precipitate forms, residual bromine is still present (proceed to next step). If no cloudiness occurs, the sample is ready for analysis.
21. Transfer the 5-mL aliquot back to the beaker and continue to add Phenol Solution until no turbidity is formed in subsequent 5-mL aliquots.
22. Transfer the entire sample into a 500-mL volumetric flask. Rinse the beaker with deionized water and add to the flask.
23. Dilute to volume with deionized water, stopper and mix well. The distillate is now ready for analysis.

Accuracy check

Standard additions method (sample spike)
Required for accuracy check:

- Selenium Standard Solution, 1000 mg/L
- 100-mL volumetric flask
- 10 mL volumetric pipet and pipet bulb
- Deionized water
- TenSette Pipet and tips
- Mixing cylinders (3)

1. After reading test results, leave the sample cell (unspiked sample) in the instrument.
2. Select standard additions from the instrument menu: **OPTIONS>MORE>STANDARD ADDITIONS**.
3. Accept the default values for standard concentration, sample volume and spike volumes. After the values are accepted, the unspiked sample reading will appear in the top row. See the user manual for more information.
4. Prepare a 100-mg/L selenium standard solution:
   a. Pipet 10 mL of 1000-mg/L selenium standard solution into a 100-mL volumetric flask.
   b. Dilute to volume with demineralized water.
5. Use the TenSette Pipet to prepare spiked samples: add 0.1 mL, 0.2 mL and 0.3 mL of the prepared standard to three 100-mL portions of fresh sample.

6. Follow the *Diaminobenzidine method* test procedure for each of the spiked samples, starting with the 0.1 mL sample spike. Measure each of the spiked samples in the instrument.
7. Select **GRAPH** to view the results. Select **IDEAL LINE** (or best-fit) to compare the standard addition results to the theoretical 100% recovery.

Standard solution method
Note: *Refer to the instrument user manual for specific software navigation instructions.*

Required for accuracy check:

- 1000 mg/L Selenium Standard Solution
- Deionized water
- 100-mL volumetric flask
- 200-mL volumetric flask
- 500-mL Erlenmeyer flask
- Volumetric pipet
- TenSette Pipet

1. Prepare a 0.5 mg/L Selenium standard solution:
   a. Prepare a 100-mg/L selenium standard solution: Pipet 10 mL of 1000-mg/L selenium standard solution into a 100-mL volumetric flask. Dilute to volume with demineralized water.
   b. Pipet 1.00 mL of the 100-mg/L solution into a 200-mL volumetric flask. Dilute to volume with deionized water.
   c. Transfer 100 mL of the standard into a 500-mL Erlenmeyer flask
2. Use this solution in place of the sample. Follow the *Diaminobenzidine method* test procedure.
3. To adjust the calibration curve using the reading obtained with the standard solution, navigate to Standard Adjust in the software: **OPTIONS>MORE>STANDARD ADJUST**.
4. Turn on the Standard Adjust feature and accept the displayed concentration. If an alternate concentration is used, enter the concentration and adjust the curve to that value.

Method performance

Program	Standard	Precision—95% Confidence Limits of Distribution	Sensitivity—ΔConcentration per 0.010 ΔAbs
640	0.50 mg/L Se	0.47–0.53 mg/L Se	0.01 mg/L Se

Summary of method

An EDTA masking agent is added to the sample to remove interferences such as iron prior to the test. The addition of a sulfate buffer adjusts the sample to the optimum pH of 1 to 2. Under these conditions, diaminobenzidine reacts with all selenium present as selenite (Se^{4+}) to give a yellow-colored piazselenol complex which is extracted and the color intensity measured colorimetrically. Selenium present as Se^{2+} and Se^{6+} is not detected unless the sample is distilled. Test results are measured at 420 nm.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
Selenium Reagent Set (100 tests[1]), includes:			2244200
(1) Buffer Solution, sulfate type, pH 2.0	10 mL	500 mL	45249
(1) Diaminobenzidine, tetrahydrochloride	0.1 g	5 g	706222
(2) Potassium Hydroxide Standard Solution, 12 N	4 mL	100 mL	23032
(1) TitraVer® Hardness Reagent, ACS	0.4 g	100 g	20426
(1) Toluene, ACS	60 mL	4 L	1447017
Water, deionized	100 mL	4 L	27256

[1] This test requires a reagent blank. The number of tests shown refers to any combination of samples and blanks.

Required apparatus

Description	Quantity	Unit	Catalog number
Cotton Balls, absorbent	1	100/pkg	257201
Cylinder, graduated, 50-mL	1	each	50841
Cylinder, graduated, 100-mL	1	each	50842
Dropper, 0.5 & 1.0 mL marks, glass	1	5/pkg	1419705
Dropper, 0.5 & 1.0 mL marks, plastic	1	20/pkg	2124720
Flask, Erlenmeyer, 500-mL	2	each	50549
Funnel, separatory, 250-mL	2	each	52046
Hot Plate, 7x7-inch, 120 VAC	1	each	2881500
Hot plate stirrer 7x7-inch, 220-240 VAC	1	each	2881602
Pipet, volumetric, 5-mL	1	each	1451537
Pipet filler, safety bulb	1	each	1465100
Ring, support, (3-inch) 83-mm	2	each	58000
Sample Cells, 1-inch square, 25 mL with stopper, matched pair	2	2/pkg	2612602
Spoon, measuring, 0.05-g	1	each	49200
Spoon, measuring, 0.2-g	1	each	63800
Support, ring stand, (5 x 8 inch) 127 x 203 mm	2	each	56300

Distillation reagents and apparatus

Description	Unit	Catalog number
Calcium Chloride Solution	1000 mL	42853
Hydrochloric Acid Standard Solution, 0.1 N	1000 mL	1481253
Hydrogen Peroxide, 30%, ACS	473 mL	14411
Methyl Orange Indicator Solution, (0.50-g/L)	500 mL	14849
Phenol Solution, 30-g/L	29 mL	211220
Potassium Permanganate Solution, 1-g/L	100 mL	1416442
Potassium Bromide, ACS grade[1]	—	—
Sodium Hydroxide Standard Solution, 0.100 N	1000 mL	19153

Distillation reagents and apparatus

Description	Unit	Catalog number
Sulfuric Acid Standard Solution, 19.2 N	500 mL	203849
Distillation Apparatus Set, general purpose	each	2265300
Distillation Apparatus Heater, 115 VAC	each	2274400
Distillation Apparatus Heater, 230 VAC	each	2274402
Beaker, 1000 mL	each	50053
Glass stirring rod	3/pkg	177001
Stir bar, magnetic	each	2095351
Beaker, 250 mL	each	50046H
Test tubes, 24 mL, 16 mm diameter	10/pkg	56524

[1] Purchase Potassium Bromide from a local chemical supplier

Recommended standards

Description	Unit	Catalog number
Selenium Standard Solution, 1000-mg/L	100 mL	2240742

Optional reagents and apparatus

Description	Unit	Catalog number
Acetone, ACS	500 mL	1442949
Nitric Acid, ACS	500 mL	15249
Sodium Hydroxide, 5.0 N	100 mL	245032
Sulfuric Acid, 5.25 N	100 mL	244932
Pipet, TenSette®, Pipet, 0.1–1.0 mL	each	1970001
Pipet Tips, for TenSette Pipet 1970001[1]	50/pkg	2185696
Pipet Tips, for TenSette Pipet 1970001[1]	1000/pkg	2185628
Sampling Bottle with cap, low density polyethylene, 250 mL	12/pkg	2087076
pH Paper, 0–14 pH range	100/pkg	2601300
Flask, Class A volumetric, 100 mL	each	1457442
Flask, Class A volumetric, 200 mL	each	1457445
Pipet, Class A volumetric, 10 mL	each	1451538
Pipet, Class A volumetric, 1 mL	each	1451535

[1] Other sizes are available

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