

Determination of nitrate by spectrophotometer

Reference: Cataldo et al. (1975) Rapid colorimetric determination of nitrate in plant tissues by nitration of salicylic acid. Commun. Soil Science and Plant Analysis 6(1) 71-80

An analysis is described for the rapid determination of nitrate-N in plant and soil extracts. The complex formed by nitration of salicylic acid under highly acidic conditions absorbs maximally at 410 nm in basic (pH>12) solutions. Absorbance of the chromophore is directly proportional to the amount of nitrate-N present. Ammonium, nitrite and chloride ions do not interfere.

Standards

Stock solution 0.25 g/L NO₃-N (=250mg/L, 250 ug/mL)

- In a 1.0L Standard Flask containing approximately 600mL Type-I-water, dissolve 1.805 g potassium nitrate.
- Ensure all KNO₃ is dissolved, make up to the mark with Type-I-water, mix and store in a suitably labelled plastic container.

Prepare standards containing ~0 to 60 ug NO₃-N in a 0.25 mL aliquot:

Amount of NO ₃ -N	Vol stock (mL)	Vol extractant (or H ₂ O)
62.5	0.25	0.00
50	0.20	0.05
37.5	0.15	0.10
25	0.10	0.15
12.5	0.05	0.20
0	0.00	0.25

Blanks

A blank of 0.25 mL extractant (or H₂O) with the normal reagents is normally sufficient.

For pigmented samples a separate blank may be required for each sample. This blank shall consist of the extract, 0.8 mL of conc H₂SO₄ (minus salicylic acid) and 19 mL of 2 N NaOH

Reagents

Salicylic acid-H₂SO₄

Dissolve 5g of salicylic acid in 100 mL of conc H₂SO₄. The salicylic acid-H₂SO₄ reagent should be made fresh every week and stored in a brown bottle. Nitrate standards should be stored at 4C.

2 N NaOH

In a 250 mL beaker dissolve 40.0 g of NaOH pellets in ~100 mL of water. Transfer to a 500 mL volumetric flask and make up to 500.0 mL with DI water

Procedure

1. Turn on spectrophotometer
2. pipette an aliquot (e.g. 0.25 mL) of extract or standard into a 50-mL Erlenmyer flask
3. mix thoroughly with 0.8 mL of 5% (w/v) salicylic acid in conc. H₂SO₄
4. After 20 minutes at room temperature, add 19 mL of 2 N NaOH to raise the pH above 12
5. Cool samples to room temperature
6. Measure absorbance at 410 nm

