
	<p style="text-align: center;">Standard Operating Procedure</p>  <p style="text-align: center;">PRG pollution research group</p>	Effective Date: 20 June 2013	Version: 002
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SOP_Chem_005 Chemical Analysis_ Spectroquant Nitrate Test			Page #: 1 of 4

Standard Operation Procedure – Nitrates Test, Spectroquant Cell Test (Cat. No. 1.09713)

1. Scope and Application

- Test measures the nitrate concentration, in the range of 0.10 – 25.0 mg/l NO₃-N, of solutions with a maximum of 0.2% sodium chloride and 50 mg/l NO₂⁻.

2. Summary

- In sulphuric and phosphoric solution, nitrate ions react with 2,6-dimethylphenol (DMP) to form 4-nitro-2,6-dimethylphenol that is determined photometrically.

3. Apparatus and Glassware

- Pipettes.
- Rectangular cells.
- Spectrophotometer.

4. Interferences

Concentrations of foreign substances in mg/l or %					
Al ³⁺	1000	Hg ²⁺	100	Surfactants	1000
Ca ²⁺	500	Mg ²⁺	1000	COD (K-Hydrogen phthalate)	500
Cd ²⁺	250	Mn ²⁺	1000		
Cl ⁻	1000	NH ₄ ⁺	1000	Organic substances (glucose)	500
CN ⁻	100	Ni ²⁺	500		
Cr ³⁺	500	NO ₂ ⁻	5	Na-acetate	25%
Cr ₂ O ₇ ²⁻	50	Pb ²⁺	100	NaCl	0.2%
Cu ²⁺	500	PO ₄ ³⁻	1000	Na ₂ SO ₄	25%
F ⁻	1000	SiO ₃ ²⁻	500		
Fe ³⁺	100	Zn ²⁺	1000		

5. Collection, Preservation and Storage

- Collect faecal samples in 1L plastic buckets.
- Preferably, analyse samples immediately after sampling.
- Store samples at 4 °C or freeze dry samples.
- Preserve wastewater samples by acidifying with concentrated sulphuric acid to pH 2 and faecal samples by freeze drying or freezing.
- Determine NO₂-N on well- homogenised samples.
- Check the chloride content, with Merckoquant® Chloride Test, if concentration range is unknown.
- Samples containing more than 1000mg/l Cl- must be diluted with distilled water.
- Check the nitrite content, if necessary, eliminate interfering nitrite ions (stated amounts apply for nitrate contents of up to 50 mg/l).
- To 10 ml of sample add approximately 50mg of amidosulphuric acid and dissolve.
- The pH of this solution must be within the range of 1-3.
- Adjust, if necessary with sulphuric acid.
- Check the nitrate content with the Merckoquant® Nitrate Test.
- Samples containing more than 25.0 mg/l NO₃-N (110.7 mg/l NO₃-) must be diluted with distilled water.
- Filter turbid samples.

6. Safety Precautions

- Handle concentrated acid with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- After analysis, clean bottles and beakers with distilled water keep.
- Dispose used gloves after completion of analysis.
- Clean hands using antiseptic soap and disinfect with ethanol solution.
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

7. Sample Preparation –Faecal Sludge

1. Weigh out 2.0000g of well-mixed faecal sludge sample.
2. Blend the weighed sample with 500ml of distilled water in a 1L blender for 30 seconds on the highest speed.
3. Add 250ml distilled water and blend on highest speed until the sample is homogenised (this could range from 30 to 60 seconds).
4. Transfer the blended mixture into a 1L volumetric flask.
5. Add 200ml of blender washings into the flask and top up to 1L with distilled water.
6. Transfer the 1L solution to a plastic bottle and store at 4 °C.

8. Reagents

- Reagent NO₃-1.
- Reagent NO₃-2.
- Amidosulphuric acid.
- Sulphuric acid.
- Universal indicator strips pH 0 – 14.

9. Calibration

- To check the photometric measurement system (test reagents, measurement device, and handling) and the mode of working, the nitrate standard solutions CRM, 0.500 mg/l NO₃-N, CAT No. 125036, 2.50 mg/l NO₃-N Cat No 125037, and 15.0 mg/l NO₃-N Cat No 125038 can be used.
- Alternatively make up equivalent solutions.
- Prepare a series of at least three standards, covering the desired range, and a blank by diluting suitable volumes of standard solutions. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor. Report only those values that fall between the lowest and the highest calibration standards. Dilute and reanalyse samples exceeding the highest standard.. Report results in mg/L.

10. Procedure

- Pipette 4.0 ml of reagent NO₃-1 into a dry test tube.
- Add 0.50 ml of pre-treated sample (5°C- 25°C), do not mix.
- Add with pipette 0.5 ml of reagent NO₃-2 (Wear eye protection as the mixture becomes hot) and mix, holding only the upper part of the tube.
- Leave the hot reaction to stand for 10 min (reaction time).
- Do not cool with water.
- Fill the sample into the rectangular cell and measure in the photometer.

Notes on the measurement:

- Analyse immediately after sampling.
- Reclose the reagent bottles immediately after use.
- For photometric measurement, the cells must be clean. Wipe, if necessary, with a dry paper towel.
- Measurement of turbid solutions yields false-high readings.
- The colour of the measurement solution remains stable for 30 min after the end of the reaction time stated above. (After 60 min the measurement value would have increased by 5%).

11. Chemical Waste Disposal

- Dilute 10 ml into 1000 ml.

- Slowly add NaCO_3 until pH 6-8 is reached.
- Flush down the sink with excess water.

12. Data Quality

Measurement	0.1 – 5.0 mg/l $\text{NO}_3\text{-N}$
Standard Deviation (mg/l $\text{NO}_3\text{-N}$)	± 0.11
Confidence Interval (mg/l $\text{NO}_3\text{-N}$)	± 0.3
Sensitivity (mg/l $\text{NO}_3\text{-N}$)	0.04
Accuracy (mg/l $\text{NO}_3\text{-N}$)	± 0.10

13. References

<http://www.merckmillipore.com/ZA/en/products/analytcs-sample-prep/test-kits-and-photometric-methods/instrumental-test-systems-for-quantitative-analyses/photometric-measurements-spectroquant-system/spectroquant-tests/>

APPROVAL OF STANDARD OPERATING PROCEDURE

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