Nitrate Spectroquant® Analytical Test Kit Method

Spectroquant® Test Kits of Merck KGaA, Darmstadt, Germany: 1.00614, 1.01842, 1.09713, 1.14542, 1.14556, 1.14563, 1.14764, 1.14773, 1.14942

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Scope and Application

1. This method determines the level of nitrate-nitrogen (NO_3-N) in waters and wastewater matrices.
2. This method is for use in the United States Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.
3. This method is intended for the analysis of NO_3-N in drinking and surface waters, ground water, industrial wastes, and other wastewater matrices.
4. The method detection limit has been determined by a single laboratory validation study summarized in the Method Performance section [1]
5. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedures in Quality Control section.

Summary Method

1. This method consists of convenient, ready to use cell test kits for determination of nitrate (NO_3-N).
2. This method is based on "Standard Methods for the Examination of Water and Wastewater", 22nd edition, SM 4500 NO_3^- E (1997/2011) [2], and International Standard Organization ISO Standard No.7890-1 [3].
3. The test kit is suitable for both on-site testing and typical laboratory testing.
4. The photometric determination can be conducted on either a Spectroquant® system photometer or any other spectrophotometric device.
5. Quality is assured through the use of quality control samples (QCS), calibration of the instrumentation by using calibration test solutions and operation of a formal quality assurance program.

Definitions

1. Nitrite Stock Standard Solution: A concentrated solution containing method analyte prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.
2. Calibration Blank: A volume of reagent water with the same matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the nitrate analyzer
3. Calibration Standard: A solution prepared from the dilution of stock standard solutions. These solutions are used to calibrate the instrument response with respect to analyte concentration.

4. Detection Limit (DL), also called Method Detection Limit (MDL) -: The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.
5. Dynamic Range (DR): The concentration range over which the instrument response to an analyte is first order linear or second order quadratic.
6. Instrument Performance Check (IPC) Solution: A solution of method analyte, used to evaluate the performance of the instrument system with respect to a defined set of method criteria.
7. Laboratory Fortified Blank (LFB) - An aliquot of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation compounds are added. The LFB is processed and analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.
8. Laboratory Fortified Sample Matrix/Duplicate (LFM/LFMD) also called Matrix Spike/Matrix Spike Duplicate (MS/MSD): An aliquot of an environmental sample to which known quantities of nitrate is added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.
9. Laboratory Reagent Blank (LRB) - A volume of reagent water or other blank matrix that is processed exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, surrogates and internal standards that are used in the extraction and analysis batches. The LRB is used to determine if the method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
10. Minimum Reporting Level (MRL) - The minimum concentration that can be reported by a laboratory as a quantitated value for a method analyte in a sample following analysis. This concentration must not be any lower than the concentration of the lowest calibration standard for that instrument.
11. Water Sample: For the purpose of this method, a sample taken from one of the following sources: drinking water, surface water, ground water , and storm runoff, industrial or domestic wastewater.

Interferences

1. Suspended matter, color and turbidity may interfere with the photometric measurement. To counter this potential positive interference, filter the sample through a membrane filter with 0.45 micrometer pore diameter SM 4500 NO_3^- (E) (1997/2011) [2]
2. A detailed overview of interfering ions up to concentrations of 1-1000 mg/l is given in each

test kit product brochure. .

Safety

1. This method does not address all safety issues associated with its use. The toxicity or carcinogenicity of reagents used in this method has not been fully established. Each chemical and environmental sample should be regarded as a potential health hazard and exposure should be minimized. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (SDSs) should be available to all personnel involved in these analyses.
2. The toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level. It is suggested that the laboratory perform personal hygiene monitoring of each analyst using this method and that the results of this monitoring shall be made available to the analyst.
3. Samples of unknown origin may possess potentially hazardous compounds. Samples should be handled with care (e.g., under a hood), so as to minimize exposure.
4. As samples of unknown origin may contain compounds which could react violently with the reagents, pipette the sample into the test tube under a hood, and direct the opening of the test tube away from anyone in the area.
5. This method employs the use of Spectroquant® Nitrite Tests containing pre-measured reagents, which limits the handling of hazardous chemicals.

Equipment and Supplies

1. Membrane filters, 0.45 µm pre-wash by soaking in deionized water.
2. Class A volumetric flasks-various sizes, acid washed.
3. Class A volumetric pipettes-various sizes, acid washed.
4. Rectangular cuvettes 1-5 cm, or round cuvettes 13-16 mm or equivalent cuvettes, as needed
5. Pipetter- Fixed or adjustable volume.
6. Pipetter tips, Nitrite free.
7. Visible spectrophotometer capable of measuring absorbance within a wavelength range listed in Table 3-4, and with cell compartment for rectangular cuvettes 1-5 cm, or round cuvettes 13-16 mm or equivalent.
8. Laboratory timer.
9. Rack for cells.
10. Dry cloth or wipe for cleaning cells.

Reagents and Standards

1. Nitrite free deionized (DI) water
2. Spectroquant® Nitrite Test kit.
3. $NaNO_3$ (Sigma S5506, Assay 99.5 %) which was dried overnight at 104 ºC and stored in a desiccator until cool.
4. Purchased NO_3-N Standard Stock Solution (1,000 mg/L) or prepared NO_3-N stock solution (1000 mg/L).

Analytical Standards

1. Standard Stock Solution 1 (1000 mg/L NO_3^- - N):
   - 1.1. 6.0983 g (± 0.0001 g) of the dried sodium nitrate was dissolved in 800 mL of nitrate free DI water in a Class A 1000 mL volumetric flask.
   - 1.2. Nitrate free water was added to the mark (1000 mL).
   - 1.3. Stock Solution 1 was mixed completely by flask inversion (Minimum 20 times).
2. Standard Stock Solution 2 (500 mg/L NO_3^- - N):
   - 2.1. 500 mL of Standard Stock Solution 1 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.
   - 2.2. Nitrate free DI water was added to the mark (1000 mL).
   - 2.3. Stock Solution 2 was mixed completely by flask inversion. (Minimum 20 times)
3. Standard Stock Solution 3 (100 mg/L NO_3^- - N):
   - 3.1. 100 mL of Standard Stock Solution 1 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.
   - 3.2. Nitrate free DI water was added to the mark (1000 mL).
   - 3.3. Stock Solution 3 was mixed completely by flask inversion (Minimum 20 times).
4. Standard Stock Solution 4 (10 mg/L NO_3^- - N):
   - 4.1. 100 mL of Standard Stock Solution 3 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.
   - 4.2. Nitrate free DI water was added to the mark (1000 mL).
   - 4.3. Stock Solution 4 was mixed completely by flask inversion (Minimum 20 times).
5. Standard Stock Solution 5 (1.0 mg/L NO_3^- - N):
   - 5.1. 100 mL of Standard Stock Solution 4 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.

5.2. Nitrate free DI water was added to the mark (1000 mL).

5.3. Stock Solution 5 was mixed completely by flask inversion (Minimum 20 times).

6. Standard Stock Solution 6 (0.1 mg/L NO_3^- - N):

6.1. 100 mL of Standard Stock Solution 5 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.

6.2. Nitrate free DI water was added to the mark (1000 mL).

6.3. Stock Solution 6 was mixed completely by flask inversion (Minimum 20 times).

7. All other standards are prepared from Stock Standards following the dilution volumes listed in Table 3.

7.1. The Stock Standard volume listed in Table 1 was transferred with a Class A volumetric pipet to a Class A 100 mL volumetric flask.

7.2. Nitrate free DI water was added to the mark (100 mL).

7.3. Standard was mixed completely by flask inversion (Minimum 20 times).

8. Fresh standards are prepared every 14 days.

Table 1: Nitrate Standard Preparation		
Stock Standard	**Volume of Stock Standard Diluted to 100 mL**	**Final Standard Concentration**
1		1000
2	45	225
2	40	200
2	30	150
2	25	125
3	100	100
3	75	75
3	50	50
3	30	30
3	25	25
3	20	20
3	23	23
3	18	18
3	17	17
3	15	15
3	12	12
4	100	10
4	75	7.5
4	50	5
4	30	3
4	20	2
5	50	0.5
5	30	0.3
5	20	0.2
5	10	0.1

Sample Collection, Preservation, and Storage

1. Collect approximately 1-L, or a minimum of 100 ml, of a representative sample in a new plastic or glass bottle, following conventional sampling techniques.
   1.1. For dissolved NO_3-N filter samples through a 0.45 µm Whatman PES syringe filter for immediately upon sampling.
2. Analyze the samples for NO_3-N as soon as possible**.**
3. Refrigerate samples at ≤ 6°C from the time of collection. Maximum holding time is 48 hrs. 40 CFR 136, Table II [4].
4. Collect an additional two aliquots of a sample for each batch (of at least 20 samples) for the matrix spike and matrix spike duplicate.

Quality Control

1. Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an Initial Demonstration of Capability, a LRB, LFB, LFM, LFMD, and CCV for each Sample Batch and the use of control charts to determine Ongoing Demonstration of Capability. The laboratory is required to maintain performance records that define the quality of the data that are generated.
2. A LRB, LFB, LFM, LFMD, and CCV will be run with each sample batch. Failure of any QC sample in sample batch will require a corrective action and may require the sample batch to be reanalyzed.

Initial Demonstration of Capability (IDC)

1. Before new analysts run any samples, verify their capability with the method. Run a LFB at least four times.
2. Calculate the standard deviation of the four samples. The data is summarized in Table 11.
3. All 4 LFB values must fall within the LFB recovery limits for the analyst to pass the IDC
4. The LFB's recovery limits are:

   LFB's initial recovery limits = Mean ± (5.84 × Standard Deviation)

5. Where:

   5.84 = the two-sided Student's t factor for three degrees of freedom.

Method Detection Limit (MDL)

1. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using Type 1 Nitrate Free Deionized (DI) Reagent Water fortified at a concentration of one to five times the estimated instrument detection limit.
2. To determine MDL values, take seven replicate aliquots of the fortified reagent water and

process through the entire analytical method.

3. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = t \times S$$
$$where,\ t\ =\ Student's\ t\ value\ for\ a\ 99\%\ confidence\ level$$
$$and\ a\ standard\ deviation\ estimate\ with\ n-1\ degrees\ of\ freedom$$
$$[t\ =\ 3.14\ for\ seven\ replicates]$$
$$S\ =\ standard\ deviation\ of\ the\ replicate\ analyses$$

4. MDLs should be determined every six months, when a new operator begins work or whenever there is a significant change in the background or instrument response.

MDLs must be determined as per the current promulgated 40 CFR part 136 Appendix B.[5]

Laboratory Reagent Blank (LRB)

1. DI water that is analyzed as a sample. Analyzed at a frequency of no less than once per sample batch. The blank result will be below the MDL.
2. If LRB fails, immediately cease analyzing samples and initiate corrective action.

Laboratory Fortified Blank (LFB)

1. A sample of known concentration of nitrate in DI water. Analyzed at a frequency of no less than once per sample batch.
2. The concentration of the LFB should be between 10% and 50% of the calibration range set by the calibration standards.
3. The minimum acceptable recovery will be 90% to 110%.
4. If LFB fails, immediately cease analyzing samples and initiate corrective action.
5. The LFB QC (Ongoing Demonstration of Capability) charts will set the acceptable recovery limits for the continuing LFB recoveries.
6. Percent Recovery LFB calculation:

$$\left(\frac{Experimental\,Value}{Expected\,Value}\right) * 100 = \text{ Percent Recovery LFB}$$

$Experimental\,Value$ = LFB Concentration determined experimentally
$Expected\,Value$ = Known LFB concentration

Laboratory Fortified Matrix Spikes (LFM and LFMD) or Matrix Spikes (MS/MSD)

1. A duplicate set of water samples spiked with a known amount of nitrate so that the overall concentration of the spiked sample is within the dynamic calibration range of the instrument.

2. Analyzed at a frequency of no less than once per sample batch.
3. A Relative Percent Difference will be calculated for each LFM/LFMD or MS/MSD set.
4. The LFM/LFMD or MS/MSD recovery and RPD should be between a minimum acceptable level of

   4.1. . LFM/LFMD or MS/MSD recovery of 90% to 110%

   4.2. RPD $\leq$ 20%

5. The LFM QC (Ongoing Demonstration of Capability) charts will set the acceptable recovery limits for the continuing LFM recoveries.
6. If LFM/LFMD fails, initiate corrective action.
7. Percent Recovery LFM Calculation

$$\left(\frac{Spiked\,Value - (s\;x\,Unspiked\,Value)}{Concentration\,of\;Spike}\right) * 100 = \text{ Percent Recovery LFM}$$

$$Spiked\,Value = \text{LFM concentration determined experimentally}$$
$$Unspiked\,Value = \text{Concentration of sample before spiking}$$
$$s = Dilution\,Correction$$

8. The RPD QC (Ongoing Demonstration of Capability) charts will set the acceptable recovery limits for the continuing RPDs.
9. Relative Percent Difference (RPD) Calculation

$$\left(\frac{(LFM - LFMD)}{\left(\frac{LFM + LFMD}{2}\right)}\right) * 100 = \text{RPD}$$

LFM = Concentration determined for LFM

$LFMD$ = Concentration determined for LFM duplicate

Continuing Calibration Verification (CCV)

1. Check standards will be prepared so as to have a concentration between the ML and upper calibration standard. They will be run at least once per batch.
2. For the calibration verification to be valid, check standard results must not exceed ± 10% of its true value,
3. If calibration verification fails, immediately cease analyzing samples and initiate corrective action.

Dynamic Range (DR)

1. The DR must be determined initially and with each day of use. This is performed by calibrating the instrument for either a linear or quadratic range as per the manufacturer's directions.

Instrument Performance Check (IPC) Solution

1. Solutions consisting of the LRB, LFB, LFM/LFMD and CCV ran per each batch of samples. These results will be charted on control charts to determine ODC for the laboratory.

Sample Batch

1. A group of samples which behave similarly with respect to the sampling or the testing procedures being employed and which are processed as a unit.
2. For QC purposes, if the number of samples in a group is greater than 20, then each group of 20 samples or less will all be handled as a separate batch.
3. A batch cannot span between laboratory work days (24 hrs.).
4. New batches must be started each laboratory work day.
5. Sample Batch: Typical sample analysis sequence .
   - 5.1. Calibration zero
   - 5.2. Calibration Standards, Linear of Quadratic
   - 5.3. LRB
   - 5.4. LFB
   - 5.5. Sample used for LFM/LFMD
   - 5.6. LFM
   - 5.7. LFMD
   - 5.8. Samples (First half of batch)
   - 5.9. CCV
   - 5.10. Samples (Second half of batch)
   - 5.11. Repeat CCV (Optional)

Control Charts (ODC)

1. Control charts will be kept for LFB and LFM percent recovery per sample batch, LFM and LFMD RPD per sample batch, LRB concentration and CCV concentration per sample batch.

2. Trends will be graphed and warning limits calculated to show whether the values determined go outside the set by the laboratory. (*Refer to minimum acceptable values above for each QC parameter)*
3. If these trends exceeds the maximum limits of this method, then corrective action (Root Cause Analysis) must be performed.

Corrective Action (Root Cause Analysis)

1. The laboratory analyst(s) and laboratory management will perform a root cause analysis for any QC failures. The analysis will have at a minimum the following areas described in detail:
   - 1.1. Identify the problem: Identify the QC failure. Include instrument, reagent, sampling, personnel and any other problems.
   - 1.2. Investigate to identify the root cause: Determine how each problem identified interacted with each other to create the QC problem.
   - 1.3. Come up with the solution: Develop an encompassing solution to address all problems that created the QC failure.
   - 1.4. Implement the solution: Develop an implementation plan that includes all components of the developed solution and have laboratory management implement it.
   - 1.5. Document the solution: Document all corrective action steps taken under laboratory management implementation of the corrective action.
   - 1.6. Communicate the solution: Develop training and management programs to communicate and evaluate all personnel included in the corrective action solution.
   - 1.7. Evaluate the effectiveness of the solution: Document QC results in trend charts and laboratory staff performance to validate corrective action solution.

Calibration and Standardization

1. Visible Spectrophotometer
   - 1.1. The Spectroquant® system photometer
     - 1.1.1. Has factory calibrations included. These factory calibrations can be used for *non-regulatory* nitrate analyses. The calibration curve can be verified, and the data from this verification can be stored, modified or re-entered at anytime. However, the factory program settings cannot be changed by the user. When appropriate, the manufacturer supplies a new microchip (transponder) containing new calibration data.
     - 1.1.2. Can be calibrated by the user to meet the requirements of this method.
   - 1.2. For visible spectrophotometers, other than the Spectroquant® system photometer, plot a calibration curve with a minimum of five (5) data points, from standards prepared from a

NO_3-N standards solution appropriate to the range to be tested. The calibration curve should also include a blank DI water.

2. Prepare standard curves from the NO_3-N solution. The curve should include the lowest and highest concentrations for the range tested as set by the Spectroquant® test kit product insert.
3. Apply linear or polynomial curve-fitting statistics, as appropriate, to analyze the concentration–instrument response relationship. The appropriate linear or nonlinear correlation coefficient for standard concentration-to-instrument response should be ≥ 0.995.
4. Back calculate the concentration of each calibration point. The back-calculated and true concentrations should agree within ± 10. At the lower limit of the operational range, acceptance criteria are usually wider.
5. Repeat initial calibration daily or when starting a new batch of samples, unless the method permits calibration verification between batches.
6. Run CCVs as per the QC requirements.

Procedure

1. Choose a Spectroquant® Nitrate Test concentration range appropriate for the sample matrix to be tested, using prior knowledge of the particular waste stream.
2. Table 2 contains the Dynamic Range for each test kit.

Table 2: Test Kit Dynamic Range

Spectroquant® Test Kit	**Measuring range (mg/L NO_3-N)**
1.14764	1.0-50.0
1.14563	0.5-25.0
1.09713	0.10-25.0
1.00614	23-225
1.14542	0.5-18.0
1.14942	0.2-17.0
1.14773	0.2-20.0
1.14556	0.10-3.00
1.01842	0.3-30.0

Note: Refer to the specific Spectroquant® product insert for additional detailed information.

3. Pipette sufficient sample depending on the concentration range of pre-treated sample into a Spectroquant® test kit tube or other reagent tube.
4. Check pH range as per test kit insert.
5. Mix well by inversion.
6. Proceed with the additional reagent addition and reaction time as per the specific Spectroquant® Test Kit.
7. Warm up the instrument as per manufacturer's suggestion for operation.
   7.1. Set the instrument to a wavelength as outlined in Tables 3-4 below.

Table 3: Test Kit Maxima Wavelengths Used with Thermo Genesys 10S

Spectroquant® Test Kit or Method	Quantitation Wavelength (nm)
1.14764	337
1.14563	334
1.09713	357
1.00614	345
1.14542	520
1.14942	499
1.14773	516
1.14556	504
1.01842	500

Table 4: Spectroquant® Test Kits Absorbance Maxima Range

nm	1.14764	1.14563	1.09713	1.00614	nm	1.14542	1.14942	1.14773	1.14556	nm	1.01842
332	1.4592	1.0257	0.49	1.4193	491	1.7019	2.4017	1.5957	1.6773	495	0.2374
332.5	1.4611	1.0265	0.4886	1.4201	491.5	1.7078	2.4111	1.6007	1.6864	495.5	0.237
333	1.4627	1.0267	0.4881	1.4205	492	1.714	2.4195	1.6057	1.6945	496	0.2361

Table 4: Spectroquant® Test Kits Absorbance Maxima Range

nm	1.14764	1.14563	1.09713	1.00614	nm	1.14542	1.14942	1.14773	1.14556	nm	1.01842
333.5	1.4642	1.027	0.4877	1.4214	492.5	1.7221	2.4381	1.6135	1.7049	496.5	0.2352
334	1.4655	1.0272	0.4878	1.4222	493	1.7286	2.443	1.619	1.7161	497	0.2345
334.5	1.4663	1.0273	0.4884	1.4238	493.5	1.7358	2.4486	1.625	1.7255	497.5	0.2338
335	1.467	1.0271	0.489	1.4242	494	1.7431	2.4547	1.631	1.7351	498	0.2335
335.5	1.4679	1.0271	0.4902	1.4248	494.5	1.7503	2.4619	1.637	1.7438	498.5	0.2327
336	1.4682	1.0269	0.4914	1.4259	495	1.7579	2.4663	1.6425	1.7549	499	0.2322
336.5	1.4688	1.0265	0.4935	1.4274	495.5	1.7642	2.4728	1.6474	1.7615	499.5	0.2316
337	1.4694	1.0263	0.4956	1.4287	496	1.7704	2.4759	1.6517	1.7688	500	0.2311
337.5	1.4696	1.0259	0.4986	1.4297	496.5	1.7753	2.4817	1.6566	1.775	500.5	0.2307
338	1.4693	1.0253	0.5012	1.4311	497	1.7816	2.4832	1.6608	1.7817	501	0.2298
338.5	1.4693	1.0249	0.504	1.4327	497.5	1.7875	2.4829	1.6656	1.7875	501.5	0.2297
339	1.4691	1.0242	0.5077	1.4332	498	1.7931	2.4844	1.6702	1.7942	502	0.2289
339.5	1.4686	1.0237	0.5117	1.4343	498.5	1.7995	2.4844	1.6754	1.7992	502.5	0.2283
340	1.4678	1.0226	0.5155	1.4356	499	1.8054	2.4849	1.6795	1.8035	503	0.2282
340.5	1.4667	1.0213	0.5199	1.4364	499.5	1.8104	2.4838	1.6834	1.8076	503.5	0.2279
341	1.4655	1.0202	0.5248	1.4377	500	1.816	2.4813	1.6871	1.8118	504	0.2272
341.5	1.4645	1.0194	0.5297	1.4392	500.5	1.8216	2.4777	1.6915	1.8163	504.5	0.227
342	1.4627	1.0175	0.5355	1.4398	501	1.8267	2.4751	1.6951	1.8184	505	0.2265
342.5	1.461	1.0157	0.5413	1.4407	501.5	1.8311	2.4754	1.6987	1.8214		
343	1.4586	1.0139	0.5471	1.4417	502	1.8367	2.4722	1.7024	1.8245		
343.5	1.4564	1.0118	0.5524	1.442	502.5	1.8418	2.4702	1.7064	1.8262		
344	1.4545	1.0102	0.558	1.443	503	1.8473	2.4644	1.7095	1.8283		
344.5	1.452	1.0082	0.563	1.4432	503.5	1.8523	2.4626	1.7131	1.8292		
345	1.4491	1.0058	0.5682	1.4431	504	1.8577	2.457	1.7172	1.8302		
345.5	1.4461	1.0036	0.5736	1.4428	504.5	1.8615	2.4546	1.7202	1.8298		
346	1.4431	1.0012	0.5788	1.4423	505	1.8657	2.4468	1.7229	1.8291		
346.5	1.4392	0.998	0.5841	1.4416	505.5	1.8702	2.4415	1.7256	1.8281		
347	1.4351	0.9958	0.5888	1.4403	506	1.875	2.4361	1.7292	1.8259		
347.5	1.4307	0.9923	0.5946	1.4402	506.5	1.8795	2.4284	1.7315	1.8242		
348	1.4263	0.9889	0.6004	1.439	507	1.8824	2.4248	1.7337	1.8216		
354	1.3601	0.9398	0.6476	1.4072	507.5	1.8857	2.4195	1.7363	1.8183		
354.5	1.3525	0.9342	0.6494	1.402	508	1.889	2.4139	1.7388	1.8151		
355	1.3448	0.9285	0.6512	1.3961	508.5	1.8923	2.4091	1.7405	1.8106		
355.5	1.3397	0.9226	0.6524	1.3906	509	1.8971	2.4031	1.7425	1.8068		

Table 4: Spectroquant® Test Kits Absorbance Maxima Range

nm	1.14764	1.14563	1.09713	1.00614	nm	1.14542	1.14942	1.14773	1.14556	nm	1.01842
356	1.3282	0.9161	0.6529	1.3857	509.5	1.8995	2.3983	1.7442	1.801		
356.5	1.3203	0.9103	0.6546	1.378	510	1.903	2.3925	1.7447	1.7957		
357	1.3126	0.9046	0.6524	1.3717	510.5	1.9055	2.3854	1.7476	1.7889		
357.5	1.3054	0.8991	0.6527	1.366	511	1.9085	2.3783	1.7493	1.781		
358	1.2973	0.8932	0.6518	1.3589	511.5	1.9104	2.3714	1.7503	1.7741		
358.5	1.288	0.8867	0.6509	1.3513	512	1.914	2.3653	1.7514	1.7672		
359	1.2776	0.879	0.6489	1.3421	512.5	1.9163	2.3582	1.7522	1.759		
					513	1.9179	2.3529	1.753	1.7492		
					513.5	1.9198	2.3428	1.7543	1.7395		
					514	1.9208	2.3377	1.7547	1.7301		
					514.5	1.9234	2.3305	1.7557	1.718		
					515	1.9256	2.3196	1.7548	1.7059		
					515.5	1.9263	2.3148	1.756	1.6942		
					516	1.9288	2.3061	1.7556	1.6814		
					516.5	1.9295	2.2988	1.7557	1.6667		
					517	1.9302	2.2934	1.7555	1.6569		
					517.5	1.9308	2.2873	1.7549	1.6444		
					518	1.9321	2.2815	1.7542	1.6318		
					518.5	1.9319	2.2731	1.7534	1.6196		
					519	1.9323	2.2666	1.7527	1.6068		
					519.5	1.9325	2.2555	1.751	1.5892		
					520	1.9322	2.2456	1.7501	1.5767		
					520.5	1.9311	2.236	1.7488	1.562		
					521	1.9311	2.2249	1.7469	1.5451		
					521.5	1.9295	2.2151	1.7447	1.5284		
					522	1.9289	2.2052	1.7427	1.5139		
					522.5	1.927	2.1947	1.7401	1.4969		
					523	1.9267	2.1825	1.7377	1.4806		
					523.5	1.9247	2.1733	1.7347	1.4655		
					524	1.923	2.1603	1.7319	1.4477		
					524.5	1.921	2.1507	1.7285	1.4313		
					525	1.9193	2.139	1.7249	1.4147		
					525.5	1.9162	2.1258	1.7213	1.3971		
					526	1.9139	2.1118	1.7178	1.3799		

Table 4: Spectroquant® Test Kits Absorbance Maxima Range

nm	1.14764	1.14563	1.09713	1.00614	nm	1.14542	1.14942	1.14773	1.14556	nm	1.01842
					526.5	1.9107	2.0969	1.7135	1.3616		
					527	1.9068	2.0785	1.7077	1.3404		
					527.5	1.9055	2.0771	1.7124	1.3237		
					528	1.9005	2.0462	1.6971	1.3059		

7.2. Zero the instrument with reagent water / blank which has been prepared in the same manner as the standards and samples.

7.3. Place the cell into the cell compartment/cell holder.

7.4. Record the absorbance reading from the instrument.

7.5. Plot the absorbance reading on the calibration curve, to obtain the concentration NO_3-N in mg/L.

Data Analysis and Calculations

1. If no pre-dilution was performed upon the sample, no calculation is necessary.
2. If pre-dilution was required, calculate the NO_3-N (mg/L) by multiplying the concentration by the dilution factor.
3. Report results to the significant digits for concentrations found in the product insert.

Method Performance

1. The method performance was measured by a single analyst/laboratory and is detailed in Tables 6-16 in the "*Method Equivalency Spectroquant® Nitrate Test Kit: Reactions and Photometry*" report [1] and the Tables, Diagrams, Flowcharts, and Validation Data section below.

Calibration and Linear Correlation Coefficient

1. Utilizing the absorbance maximum determined experimentally for the Spectroquant® test kit, the spectrophotometer is calibrated with a minimum of six (6) standards and a linear or quadratic correlation coefficient was determined. The standards bracketed the Spectroquant® test kit concentrations listed in the product flyer.
2. The correlation coefficients are >0.995 in all cases. The Correlation Coefficients are summarized in Table 5-6.

Accuracy (Spectroquant® QC Sample-LFB)

1. Quality control check samples were obtained from Spectroquant® (Table 7) and used in the equivalency evaluation of the Spectroquant® test kits ability to recover free nitrate.
2. The control check concentration (accuracy) is summarized as the percent recoveries for each Spectroquant® test kit for the quality control check samples used and are summarized in Tables 8-9.

Precision (Spectroquant® QC Sample-LFB)

1. Precision is defined in Standard Methods for the Examination of Water and Wastewater, Part 1000 and in EPA guidance documents as either the confidence interval about the mean or the Percent Relative Standard Deviation (%RSD).[6-8]

$$\text{Percent Relative Standard Deviation} = \left(\frac{s}{\bar{X}}\right) \times 100$$

2. The %RSD was calculated for the Spectroquant®, Hach TNT and Standard Methods 4500-NO_3 (E) [2] check samples that were used for the accuracy evaluation. The number of replicate concentration determinations at the maximum wavelength determined for each Spectroquant® test kit was five (5) replicates. The mean and standard deviation was then calculated and these values were used to determine %RSD. The %RSD for reach quality controls sample is summarized in Table 9.
3. The accuracy or recovery of the Spectroquant® check samples by either the Spectroquant® test kits, the classical Cadmium Column (SM 4500 NO_3^- (E)) or Hach TNT method was no lower than 86.29% and no higher than 102.62%. The %RSD of the Spectroquant® check samples by the Spectroquant® test kits, the classical Cadmium Column (SM 4500 NO_3^-- (E)) or Hach TNT method was no greater than 6.962 %..
4. These efficient recoveries of 3rd party check samples and the low %RSD shows that the Spectroquant® will produce equivalent accurate and precise results when compared to Standard Methods (4500 NO_3^-- (E)) [2].

Laboratory Reagent Blank (LRB)

1. A volume of reagent water or other blank matrix was processed exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, that are used in the analysis. The LRB was run five times for each Spectroquant® test and the average is reported in Table 10 below. In all cases, nitrate was not seen at a level above 0.106 mg/L NO_3-N.

Matrix Spikes

1. A wastewater matrix was utilized in determining the matrix effects on the spike percent recovery, precision and the relative percent difference between the matrix spike (MS) and the

duplicate spike (MSD).

2. A sample of Final Effluent was obtained from a boat dock in Muscatine, Iowa. This sample was then filtered through a 0.45 µm syringe filter prior to matrix analyses.
3. The Spectroquant® Test Kits 1.14556, 1.14563 and 1.01842 were selected for the test. Matrix spike concentration was selected to be at the midpoint of the Spectroquant® test kit range (2.00 mg/L NO_3-N). Each spectroscopic analysis was measured five (5) times and the average is reports and the standard deviation was used in the % RSD calculation.
4. The percent recovery, RPD and %RSD for the samples are summarized in Tables 12-13.

Method Detection Limits

1. The Method Detection Limit (MDL) was calculated for each Spectroquant® test kit following the requirements in Appendix B, 40 CFR part 136 [9].
2. Each Spectroquant® test kit product flyer and test kit contained a nitrate range for which the test kit reagents were at a concentration to produce complete reaction of the nitrate.
3. The concentration of sample utilized for each Spectroquant® test kit MDL was determined to be 2-5 times the concentration of the lowest concentration listed on the Spectroquant® test kit or product flyer.
4. The results for the Spectroquant® test kit MDL are at or below the MRL listed on the Spectroquant® product flyer (Table 14).
5. These MRL vs. MDL results for the Spectroquant® test kits concludes that the Spectroquant® test kits are produce MDL results that are less than the lower limits of dynamic range listed on each Spectroquant® product flyer.

Pollution Prevention

1. The reagents used in this method pose little threat to the environment, when managed properly.
2. Reagents should be ordered consistent with laboratory use, to minimize the amount of expired materials to be disposed.

Waste Management

1. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restriction. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations.
2. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" and "Less is Better: Laboratory Chemical Management for Waste

Reduction," both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

References

1. Askew, E.F., *Method Equivalency Spectroquant® Nitrate Test Kit: Reactions and Photometry*. 2017.
2. Eaton, E., Baird, R., Rice, E., , ed. *Standard Methods for the Examination of Water and Wastewater, 22nd Edition*. 22 ed. 2012, APHA, AWWA, WEF.
3. ISO, *Water quality - Determination of nitrate -Part 1 : 2,6-Dimethylphenol spectrometric method*, in *7890-1*. 1986: Switzerland. p. 1-5.
4. EPA, *Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; Analysis and Sampling Procedures*. 2012. p. 29758-29846.
5. EPA, *Part 136-Guidelines Establishing Test Procedures for the Analysis of Pollutants Appendix B*. 1973, EPA.
6. EPA, *Chapter 5: Calculation Of Precision, Rias, And Method Detection Limit For Chemical And Physical Measurements*. 1984.
7. EPA, *Protocol for the Evaluation of Alternate Test Procedures for Organic and Inorganic Analytes in Drinking Water*. 2015.
8. EPA, *Protocol for Review and Validation of New Methods for Regulated Organic and Inorganic Analytes in Wastewater Under EPA's Alternate Test Procedure Program*. 2016.
9. EPA, *Subchapter D 40 CFR part 136 Guidelines Establishing Test Preoceedures for the Analysis of Pollutants*. 2001.

Tables, Diagrams, Flowcharts, and Validation Data

Table 5: Spectroquant® Test Kit Calibration Standards and Correlation Coefficient										
Test Kit	**Wavelength (nm)**	**Standard 1***	**Standard 2***	**Standard 3***	**Standard 4***	**Standard 5***	**Standard 6***	**Standard 7***	**Standard 8***	**Correlation Coefficient**
1.14764	337	0.00	1.00	5.00	10.00	15.00	25.00	30.00	50.00	Linear Intercept Through Zero 0.99997
1.14563	334	0.00	0.50	1.00	3.00	5.00	10.00	15.00	25.00	Linear Intercept Through Zero 0.999954
1.09713	357	0.00	1.00	3.00	5.00	10.00	15.00	20.00	25.00	Linear Intercept Through Zero 0.99977
1.00614	345	0.00	23.00	30.00	50.00	100.00	150.00	200.00	225.00	Linear Intercept Through Zero 0.99999
1.14542	520	0.00	0.50	1.00	3.00	5.00	10.00	15.00	18.00	Linear Intercept Through Zero 0.99794
1.14942	499	0.00	0.20	0.50	1.00	5.0	10.00	15.00	17.00	Quadratic, Intercept Calculated 0.99952
1.14773	516	0.00	0.20	0.50	2.00	5.00	10.00	15.00	20.00	Linear Intercept Through Zero 0.99933
1.14556	504	0.00	0.10	0.30	0.50	1.00	2.00	3.00		Quadratic Intercept Calculated 0.99914

Table 5: Spectroquant® Test Kit Calibration Standards and Correlation Coefficient										
Test Kit	**Wavelength (nm)**	**Standard 1***	**Standard 2***	**Standard 3***	**Standard 4***	**Standard 5***	**Standard 6***	**Standard 7***	**Standard 8***	**Correlation Coefficient**
										Quadratic Intercept Through Zero 0.99868
1.01842	500	0.00	0.30	0.50	1.00	5.00	10.00	25.00	30.00	

* Standard Concentration NO_3-N

Table 6: Standard Methods 4500-NO_3 (E) and Hach TNT Calibration Standards and Correlation Coefficient									
Test Kit	**Wavelength (nm)**	**Standard 1***	**Standard 2***	**Standard 3***	**Standard 4***	**Standard 5***	**Standard 6***	**Standard 7***	**Correlation Coefficient**
									Quadratic, Intercept Calculated 0.99930
Standard Methods 4500-NO_3 (E)	**537**	0.000	0.200	0.300	0.500	1.000			
									Quadratic, Intercept Calculated 0.99823
Hach TNT 835	**335**	0.000	0.300	0.500	1.000	5.000	10.000	12.000	
									Quadratic, Intercept Calculated 0.99984
Hach TNT 836	**335**	0.000	5.000	10.000	15.000	20.000	25.000	30.000	

* Standard Concentration NO_3^-N mg/L

Table 7: Spectroquant® Quality Control Check Samples (LFB-IPC)			
Spectroquant® Check Sample	**Label Concentration (NO_3^--N mg/L)**	**Check Sample Actual Concentration (NO_3^--N mg/L)**	**Check Sample Precision (± mg/L)**
1.25036.0100	0.50	0.49	0.05
1.25037.0100	2.50	2.48	0.06
1.25038.0100	15.0	14.9	0.4
1.25039.0100	40.0	40.2	1.0
1.25040.0100	200	200	5

Table 8: LFB Recovery Comparison													
Spectroquant® Check Sample	**Label Concentration (NO_3-N mg/L)**	**Standard Methods 4500-NO_3 (E)**	**Hach TNT 836**	**1.14764**	**1.14563**	**1.09713**	**1.00614**	**1.14542**	**1.14942**	**1.14773**	**1.14556**	**1.01842**
1.25036.0100	0.50	99.49									91.84	
1.25037.0100	2.50			98.79	100.81	99.19		97.98	86.29	100.00	100.00	
1.25038.0100	15.0		99.47	99.13	99.87	99.13		97.99	100.81	100.94		102.62
1.25039.0100	40.0						98.91					
1.25040.0100	200						97.99					

Table 9: % RSD Comparison												
Spectroquant® Check Sample	Label Concentration (NO_3-N mg/L)	Standard Methods 4500-NO_3 (E)	Hach TNT 836	1.14764	1.14563	1.09713	1.00614	1.14542	1.14942	1.14773	1.14556	1.01842
1.25036.0100	0.50	0.931									5.917	
1.25037.0100	2.50			1.633	0.596	4.633		6.962	1.966	3.077	2.683	
1.25038.0100	15.0		2.037	0.897	1.848	0.415		0.965	1.028	1.269		0.065
1.25039.0100	40.0						0.315					
1.25040.0100	200						2.988					

Table 10: Laboratory Reagent Blank (mg/L NO_3-N)								
1.14764	1.14563	1.09713	1.00614	1.14542	1.14942	1.14773	1.14556	1.01842
0.034	0.006	0.003	0.106	-0.030	-0.009	0.000	0.071	-0.010

Table 11: Initial Demonstration of Capability (IDC)															
IDC Values (mg/L NO3-N)	**Average**	**IDC Low**	**IDC High**	**Average**	**IDC Low**	**IDC High**	**Average**	**IDC Low**	**IDC High**	**Average**	**IDC Low**	**IDC High**	**Average**	**IDC Low**	**IDC High**
Standard Methods 4500-NO_3 (E)	0.491	0.465	0.518												
Hach TNT 836							15.015	13.299	16.801						
1.14764				2.500	2.260	2.740	14.913	14.131	15.694						
1.14563				2.520	2.430	2.610	14.790	13.193	16.387						
1.09713				2.550	1.860	3.230	14.860	14.500	15.220						
1.00614										39.833	39.099	40.566	196.563	162.265	230.860
1.14542				2.390	1.420	3.350	14.530	13.711	15.349						
1.14942				2.090	1.850	2.330	15.018	14.116	15.919						
1.14773				2.440	2.000	2.880	14.973	13.863	16.082						

Table 11: Initial Demonstration of Capability (IDC)															
IDC Values (mg/L NO3-N)	**Average**	**IDC Low**	**IDC High**	**Average**	**IDC Low**	**IDC High**	**Average**	**IDC Low**	**IDC High**	**Average**	**IDC Low**	**IDC High**	**Average**	**IDC Low**	**IDC High**
1.14556	0.423	0.277	0.569	2.420	2.040	2.800									
1.01842							15.295	15.237	15.353						

Table 12: Matrix Spike Percent Recovery and RPD (2.00 mNO_3-N mg/L)			
	1.14556	**1.14563**	**1.01842**
Final Effluent (NO_3-N mg/L)	0.58	0.82	1.46
Matrix Spike (NO_3-N mg/L)	2.35	2.87	3.53
Matrix Spike Percent Recovery	89%	103%	104%
Matrix Spike Duplicate (NO_3-N mg/L)	2.39	2.86	3.59
Matrix Spike Duplicate Percent Recovery	91%	102%	107%
Relative Percent Difference	1.69%	0.35%	1.69%

Table 13: Final Effluent, Matrix Spike and Spike Duplicate % RSD			
	1.14556	**1.14563**	**1.01842**
Final Effluent	0.00%	0.00%	0.68%
Matrix Spike	0.00%	0.00%	0.56%
Matrix Spike Duplicate	0.00%	0.00%	1.11%

Table 14: Method Reporting Limit - Method Detection Limits			
Spectroquant® Test Kit	**Spectrometer Cell Width (mm)**	***MRL*** ***Lowest Concentration Listed for Spectroquant® Test Kit*** ***(NO_3-N)***	**MDL** **(NO_3-N)**
1.14764	**10**	***1.00***	**0.456**
1.14563	**10**	***0.50***	**0.114**
1.09713	**10**	***1.00***	**0.120**
1.00614	**10**	***23.00***	**2.101**
1.14542	**10**	***0.50***	**0.001**
1.14942	**10**	***0.20***	**0.136**
1.14773	**10**	***0.50***	**0.040**
1.14556	**10**	***0.10***	**0.073**
1.01842	**50**	***0.30***	**0.159**