

Equivalency of Spectroquant®

Nitrite Method Azo Dye Test Kits

Nitrite Method Iron Redox Test Kit

Reactions and Photometry

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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 free of nitrite that is used as a zero standard and to calibrate the spectrophotometer
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 Nitrite 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, storm runoff, industrial or domestic wastewater.

Introduction

The method flexibility allowed in the EPA rules 40 CFR part 136.6 [1] lay out the requirements a modified analytical method must meet to be considered equivalent to a promulgated analytical method. These requirements are explained in detail in a memo authored by Richard Redding [2]:

The Spectroquant® Test Kits 1.14547, 1.14776 and 1.00609 is covered under the method flexibility allowed in the EPA rules 40 CFR part 136.6 [1] The rule lays out the requirements a modified analytical method must meet to be considered equivalent to a promulgated analytical method. These requirements are explained in detail in a memo authored by Richard Redding [2]:

The March 12th Methods Update Rule promulgated 136.6 which allows the regulated community more flexibility that includes:

- 1. Changes in equipment operating parameters such as minor changes in the monitoring wavelength of a colorimeter*
- 2. Adjusting sample sizes or changing extraction solvents to optimize method performance in meeting regulatory requirements*
- 3. Minor changes in reagents used where the underlying reaction and principles remain virtually the same:*
 - a. Changes in complexing reagent provided that the change does not produce interferences.*
 - b. Changes in reactants provided that the change does not produce interference.*

The method equivalency report for Spectroquant® Test Kits 1.14547, 1.14776 and 1.00609 will directly compare this kit with the allowed method modifications listed in Richard Reading's memo. The MDL results along with the IDC, LRB and LFB and % RSD all show good agreement with the Quality Control parameters that is expected in 40 CFR part 136.7 [1].

This equivalency checklist will directly compare the Spectroquant® nitrite test kits with the simple method modifications listed in Richard Reading's memo and allow a laboratory to establish method equivalency for their analyses and reporting to both users of the results and regulators.

Nitrite Reaction

Chemical Reactions and Spectroscopy

Nitrite Chemistry

The Spectroquant® test kits and all of the Spectroquant® Quality Control (QC) samples identified the active ingredient used in their analyses as Nitrite-N ($\text{NO}_2^- - \text{N}$). The Nitrite (NO_2^-) can be calculates from this value by multiplying by a factor of 46.0055

$$\text{NO}_2^- - \text{N} \times 3.2845 = \text{NO}_2^-$$

Figure 1: Nitrite-Nitrogen to Nitrite Conversion Formula

The EPA approved methods that utilize spectroscopic determination identify the active ingredient used in their analyses as Nitrite (NO_2^-) or Nitrite-N ($\text{NO}_2^- - \text{N}$). The conversion formula or its reciprocal in Figure 1 is utilized in the EPA results.

Classical Azo Dye (Griess) Reaction

The azo dye (Griess) reaction has been utilized for the detection of nitrite in aqueous samples with in-depth studies published. [3-8] The overall reaction proposed is a 3-step process in which an azo dye is formed (Figure 2).

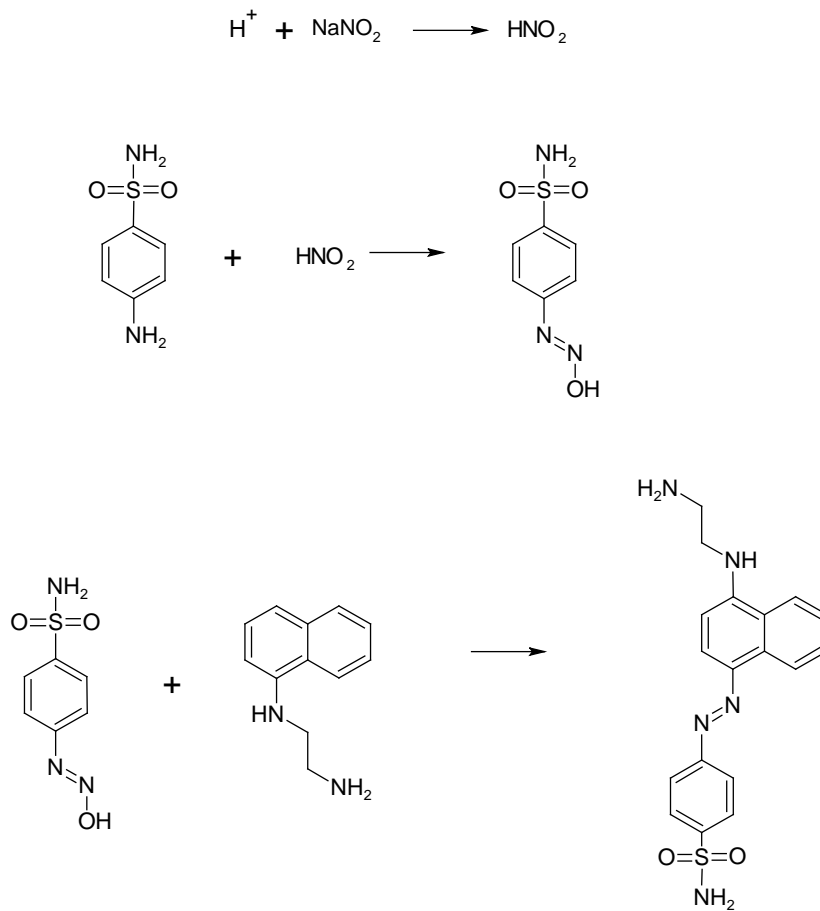


Figure 2: Classical Nitrite Azo Dye Reaction (Griess reaction)

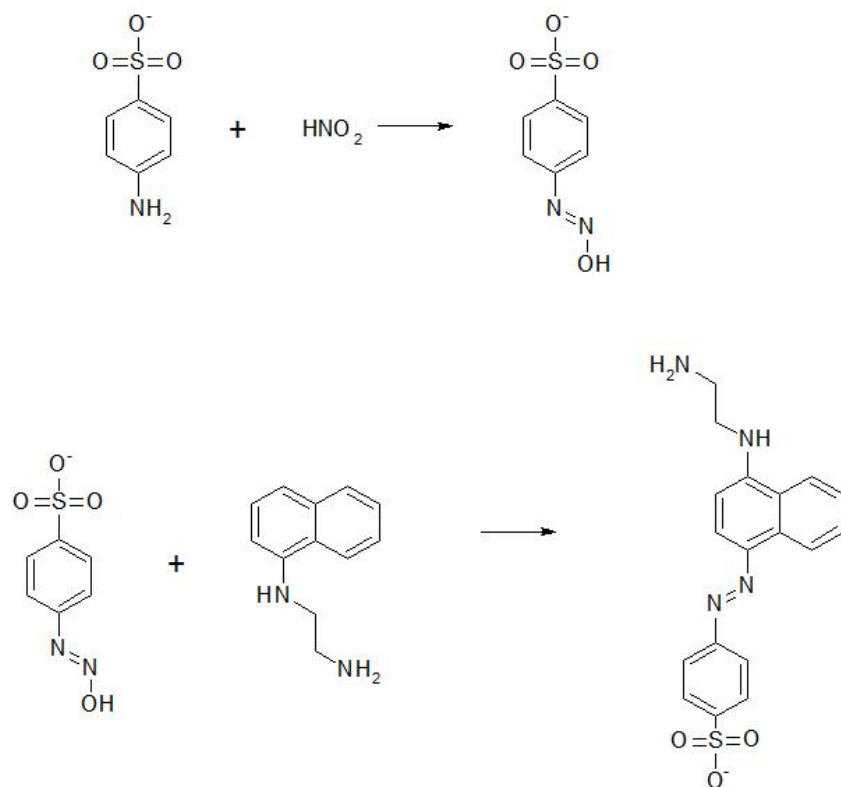
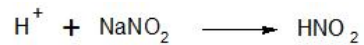


Figure 3: Spectroquant® Azo (Griess) Reaction

The azo dye product is stable for one to two hours. Maximum absorbance is measured over a broad wavelength range. Slight variations in pH can cause either hypsochromic (Blue) or bathochromic (Red) shifts in the absorbance maxima wavelengths. This broadness of the peak maxima allows the test method to utilize various wavelengths for nitrite quantitation.

Nitrite-Iron (II) Redox Reaction

The reaction of nitrite in an acidic solution of Iron (II) proceeds through a redox oxidation of Iron (II) to Iron (III) and the production of nitrous oxide (NO). The nitrous oxide then reacts through an inner hydration sphere for ligand substitution to Iron (II) Sulfate producing a compound that absorbs light in the 430-460 nm range [9, 10]. (Figure 3)

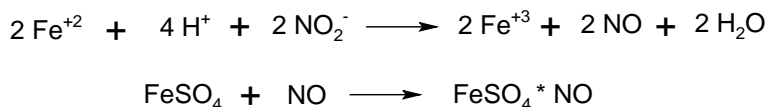


Figure 4: Nitrite Iron (II) Redox Reaction

Nitrite Reaction-Product Spectra

The nitrite azo dye and Iron (II) reactions produce absorbance maximum (maxima) in the ranges listed in Table 1 below. The visible spectra for each analytical test are illustrated below (Figures 4-6). The minimum and maximum absorbance wavelengths listed in Table 1 are identified for each analytical test spectra.

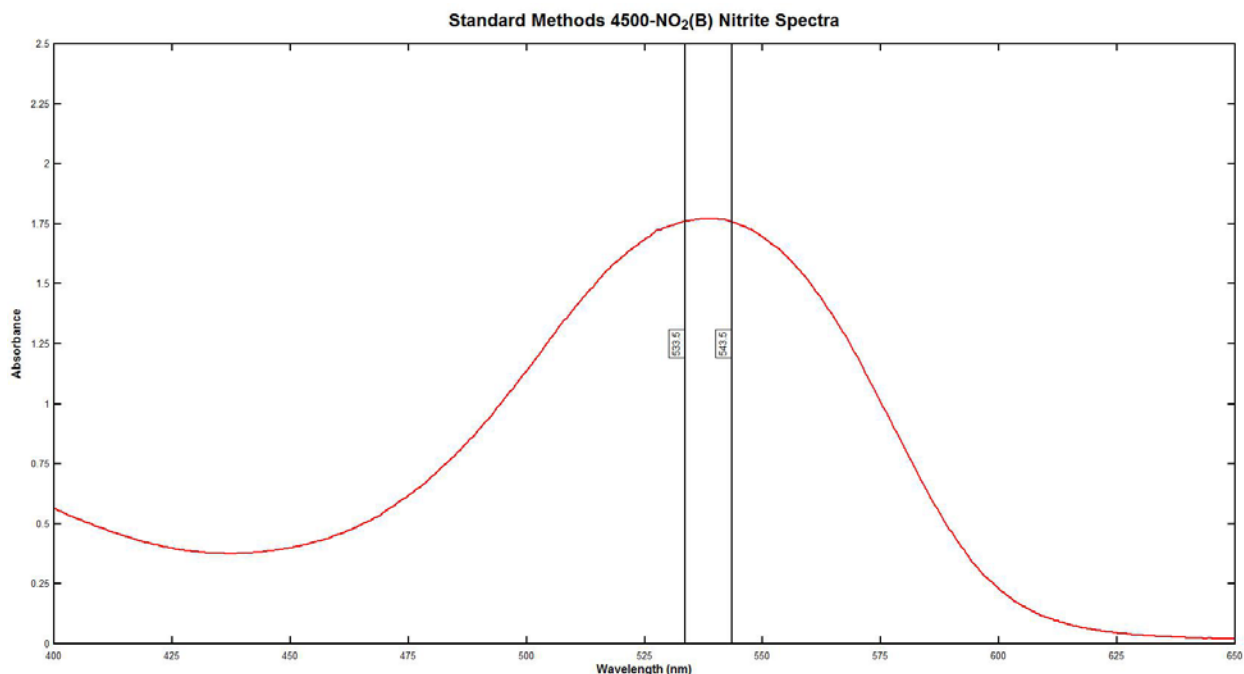


Figure 5: Classical Standard Methods 4500 NO₂ (B) Spectra

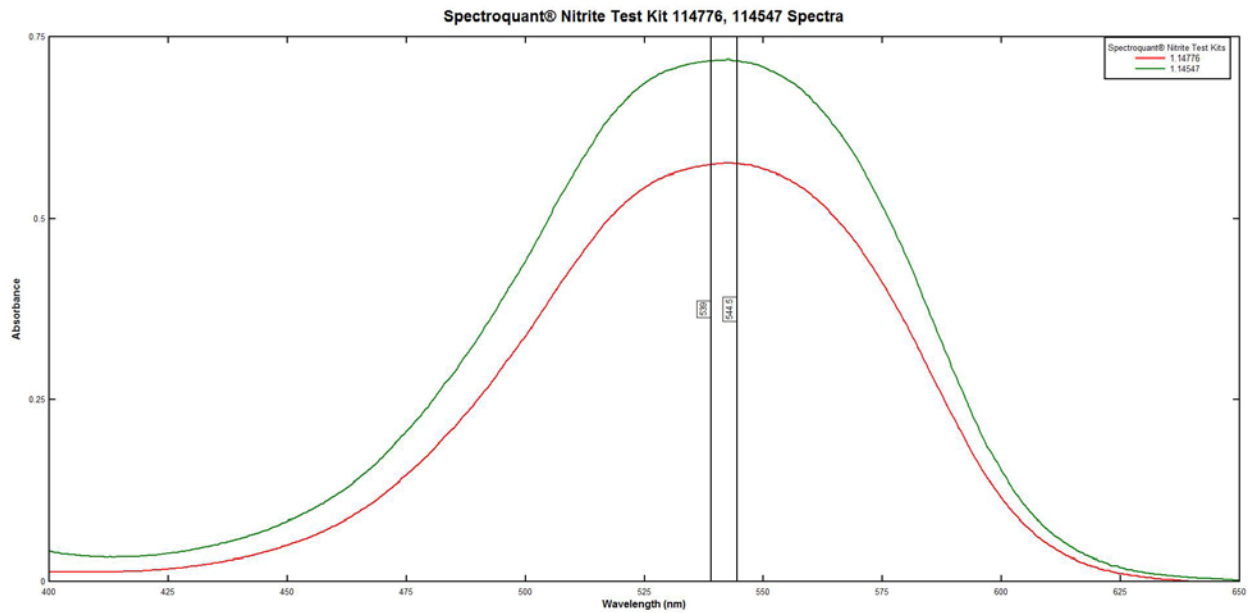


Figure 6: Spectroquant® Test Kits (114776, 114547) Absorbance Spectra

In all cases, a maxima band can be identified and accurate quantitation of the azo dye or the Iron (II)-NO compound can be determined.

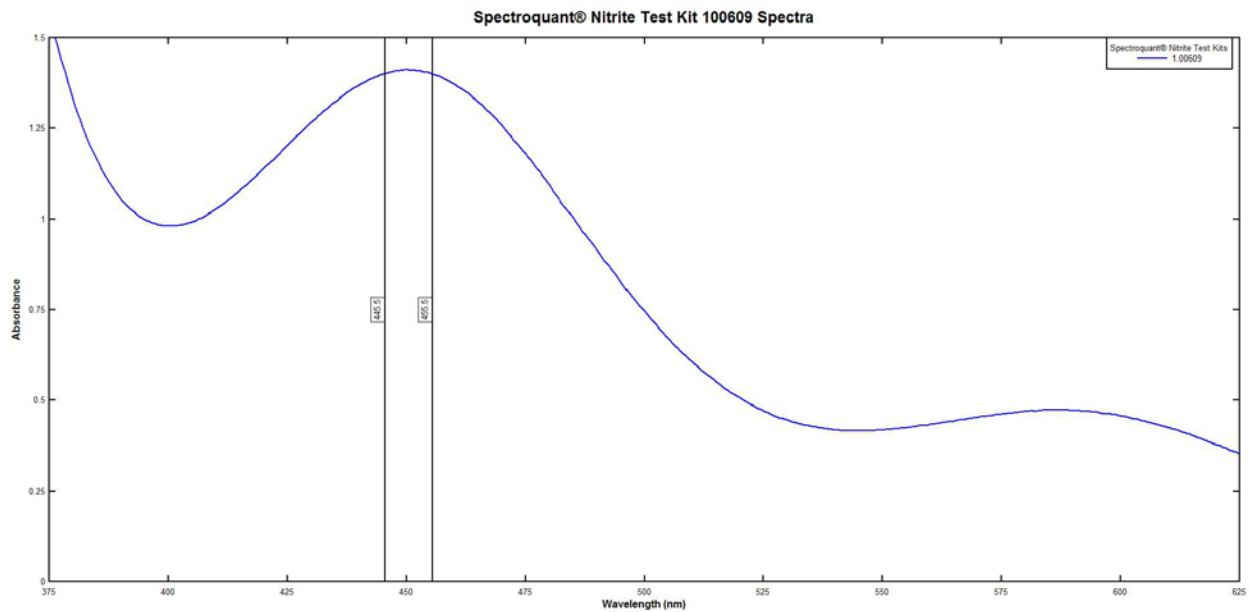


Figure 7: Spectroquant® Test Kit (100609) Absorbance Spectra

Maxima Wavelength Range

The broad band azo dye maxima seen for each Spectroquant® test kit fell within the same range wavelength maxima seen for the Methods 4500-NO₂ (B). To determine the maximum wavelength for each test kit, a standard for nitrite whose concentration was at or near the maximum concentration listed in the test kit product sheet was analyzed. These maximum wavelengths are summarized in Table 1 below. The wavelengths used for both the Spectroquant® Test Kits (114776, 114547) and Standard Methods (4500-NO₂ (B)) are highlighted in yellow. It should be noted that Standard Methods lists a quantitation wavelength of 543 nm, but that method analyses was performed at the maximum wavelength recorded with the Thermo Genesys 10S spectrophotometer. This small shift in maximum wavelength is expected with the azo dye and is due to slight variations in the final pH of the reaction products [3, 11].

The same procedure was utilized to determine absorbance maxima for the Iron (II) test kit. Absorbance maxima seen was not effected with minor pH variations, but instead can be changed at acid concentration of 10% to 90% [9]

Wavelength (nm)	Azo Dye Absorbance			Wavelength (nm)	Fe(II)-NO Absorbance
	114776	114547	Standard Methods 4500-NO ₂ (B)		100609
533.5			1.7610	445.5	1.4017
534			1.7627	446	1.4035
534.5			1.7639	446.5	1.4055
535			1.7651	447	1.4070
535.5			1.7666	447.5	1.4084
536			1.7684	448	1.4091
536.5			1.7696	448.5	1.4103
537			1.7708	449	1.4109
537.5			1.7705	449.5	1.4113
538			1.7702	450	1.4112
538.5			1.7699	450.5	1.4114
539		0.7170	1.7698	451	1.4107
539.5		0.7174	1.7699	451.5	1.4105
540		0.7178	1.7704	452	1.4100
540.5	0.5752	0.7178	1.7704	452.5	1.4093
541	0.5755	0.7182	1.7691	453	1.4085
541.5	0.5759	0.7181	1.7690	453.5	1.4069
542	0.5759	0.7184	1.7673	454	1.4053
542.5	0.5762	0.7183	1.7652	454.5	1.4045
543	0.5757	0.7185	1.7621	455	1.4024
543.5	0.5756	0.7174	1.7583	455.5	1.4004
544	0.5752	0.7171			
544.5	0.5756	0.7171			

Analytical Standards

For this study, all analytical standards were prepared as a NO_2^- -N. The nitrite standard was prepared from NaNO_2 (Sigma S2252, Assay 99.8 %) which was dried overnight at 104 °C and stored in a desiccator until cool. This sodium nitrite was then used within 8 hours of cooling to prepare the standards listed in Tables 2-3 below.

Table 2: Nitrite Standard Stock 1 as NO_2^- - N	
1000 mg/L Stock Preparation NO_2^- -N	
Molecular Weight Sodium Nitrite Standard (g)	69.0000
Molecular Weight Nitrogen (g)	14.0067
Mole Fraction Nitrogen in Sodium Nitrite	0.2030
Grams NaNO_2 to Provide 1000 mg/ NaNO_2	4.9262
Purity NaNO_2 Sigma Aldrich Lot# MKBX1577V	0.998
Corrected Grams NaNO_2 to Provide 1000 mg/ NaNO_2	4.9361

1. Standard Stock Solution 1 (1000 mg/L NO_2^- - N):
 - a. 4.9361 g (± 0.0001 g) of the dried sodium nitrite was dissolved in 800 mL of nitrite free DI water in a Class A 1000 mL volumetric flask.
 - b. Nitrite free water was added to the mark (1000 mL).
 - c. Stock Solution 1 was mixed completely by flask inversion (Minimum 20 times).
2. Standard Stock Solution 2 (100 mg/L NO_2^- - N):
 - a. 100 mL of Standard Stock Solution 1 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.
 - b. Nitrite free DI water was added to the mark (1000 mL).
 - c. Stock Solution 2 was mixed completely by flask inversion. (Minimum 20 times)
3. Standard Stock Solution 3 (10 mg/L NO_2^- - N):
 - a. 100 mL of Standard Stock Solution 2 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.
 - b. Nitrite free DI water was added to the mark (1000 mL).
 - c. Stock Solution 3 was mixed completely by flask inversion (Minimum 20 times).
4. Standard Stock Solution 4 (1 mg/L NO_2^- - N):

- a. 100 mL of Standard Stock Solution 3 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.
 - b. Nitrite free DI water was added to the mark (1000 mL).
 - c. Stock Solution 4 was mixed completely by flask inversion (Minimum 20 times).
5. Standard Stock Solution 5 (0.1 mg/L NO₂⁻ - N):
- a. 100 mL of Standard Stock Solution 4 was transferred with a Class A 50 mL pipet to a Class A 1000 mL volumetric flask.
 - b. Nitrite free DI water was added to the mark (1000 mL).
 - c. Stock Solution 5 was mixed completely by flask inversion (Minimum 20 times).

Fresh standards were prepared every 14 days.

Table 3: Nitrite Standard Preparation		
Stock Standard Used	mL of Stock into a 100 mL Volumetric Flask	Final Concentration (mg/L NO₂-N)
5	2	0.002
5	10	0.01
5	20	0.02
5	30	0.03
4	5	0.05
4	10	0.1
3	5	0.5
3	7	0.7
3	10	1
3	15	1.5
3	20	2
3	23	2.3
3	50	5
2	10	10
2	20	20
2	30	30
2	40	40
1	5	50
1	6	60
1	7	70
1	8	80
1	9	90

Other Sample Preparation

The samples used in this method equivalency study were either standards prepared in nitrite free

DI water or Mississippi River water that was filtered through a 0.45 µm Whatman PES syringe filter to remove turbidity.

The Standard Methods for the Examination of Water and Wastewater [12] methods approved by EPA [1] allows the analyses of these nitrite samples with filtration if needed. Filtration is identified in the Spectroquant® product flyers and is acceptable under the EPA approved method 4500-NO₂⁻ B-2000 (2011).

Table 4: Spectroquant® Test Kit Wavelengths	
Test Kit or Method	Quantitation Wavelength (nm)
1.14776	542
1.14547	542
1.00609	450
Standard Methods 4500-NO ₂ (B)	537

Calibration and Linear Correlation Coefficient

Utilizing both the absorbance maximum determined experimentally for the Spectroquant® test kit and Standard Methods 4500-NO₂ (B), the Thermo Scientific Genesys 10S was 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.

The correlation coefficient was >0.999 in all cases indicating that the Spectroquant® Test Kits can produce a linear or quadratic calibration. The calibration standards using mg/L (NO₂-N) for each test kit along with the linear or quadratic Correlation Coefficients are summarized in Table 5-6 below.

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*	Standard 9*	Correlation Coefficient
											Linear Intercept Through Zero
114547	542	0.000	0.010	0.030	0.050	0.100	0.500	0.700			0.99983
											Quadratic Intercept Through Zero
100609	450	0.000	1.000	2.000	5.000	10.000	20.000	40.000	60.000	90.000	0.99919
											Quadratic, Intercept Calculated
1.14776	542	0.000	0.020	0.030	0.050	0.100	0.500	0.700	1.000		0.99997

* Standard Concentration NO₂-N mg/L

Table 6: Standard Methods 4500-NO₂ (B) Calibration Standards and Correlation Coefficient

Test Kit	Wavelength (nm)	Standard 1*	Standard 2*	Standard 3*	Standard 4*	Standard 5*	Standard 6*	Correlation Coefficient
								Quadratic Intercept Through Zero
Standard Methods 4500-NO ₂ (B)	537	0.000	0.030	0.050	0.100	0.700	1.000	0.99977

* Standard Concentration NO₂⁻N mg/L

Accuracy, Percent Recovery and Precision

Accuracy (Spectroquant® QC Sample-LFB)

Quality control check samples were obtained from Spectroquant® (Table 7) and used in the equivalency evaluation of the Spectroquant® Test Kits ability to recover nitrite. The control check concentration (accuracy) and precision are summarized in Tables 8-9 below. The percent recoveries for each Spectroquant® test kit and Standard Methods 4500-NO₂ (B) [12] for the quality control check samples used are summarized in Table 9.

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

Experimental Value = LFB Concentration determined experimentally

Expected Value = Known LFB concentration

Figure 8: LFB Percent Recovery

Precision (Spectroquant® QC Sample-LFB)

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).[13-15]

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

Figure 9: Percent Relative Standard Deviation

Table 7: Spectroquant® Quality Control Check Samples (LFB-IPC)			
Spectroquant® Check Sample	Label Concentration (NO₂⁻-N mg/L)	Check Sample Actual Concentration (NO₂⁻-N mg/L)	Check Sample Precision (± mg/L)
1.25041.0100	0.200	0.196	0.009
1.25042.0100	40.0	40.9	1.3

The % RSD was calculated for the Spectroquant® and Standard Methods 4500-NO₂ (B) [12] check samples that were used for the accuracy evaluation. The number of replicate concentration determinations at the maximum wavelength (nm) determined for each Spectroquant® test kit and 4500-NO₂ (B) was five (5) replicates. The mean and standard deviation was then calculated and these values were used to determine % RSD. The % RSD for each quality controls sample is summarized in Table B below.

The accuracy or recovery of the Spectroquant® check samples by either the Spectroquant® test kits or 4500-NO₂ (B) was no lower than 100.00 % and no higher than 107.58 %. The % RSD of

the Spectroquant® check samples by either the Spectroquant® test kits or the classical phenate method was no greater than 1.160 %.

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-NO₂ (B) [12].

Table A: LFB Recovery Comparison					
Spectroquant® Check Sample	Label Concentration (NO₂-N mg/L)	Standard Methods 4500-NO₂ (B)	1.14776	1.14547	1.00609
		537 nm	542 nm	542 nm	450 nm
1.25041	0.196	100.00%	102.55%	107.58%	
1.25042	40.9				102.22%

Table B: % RSD Comparison					
Spectroquant® Check Sample	Label Concentration (NH₄-N mg/L)	Standard Methods 4500-NO₂ (B)	1.14776	1.14547	1.00609
		537 nm	542 nm	542 nm	450 nm
1.25041	0.196	0.881 %	1.086 %	1.160 %	
1.25042	40.9				1.136 %

Laboratory Reagent Blank (LRB)

A volume of DI reagent water 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 8 below. In all cases, nitrite was not seen at a level above 0.012 mg/L NO₂-N

Table 8: Laboratory Reagent Blank (mg/L NO₂-N)			
Standard Methods 4500-NO₂ (B)	1.14776	1.14547	1.00609
0.012	0.011	0.008	< 0.000

Initial Demonstration of Capability (IDC)

Control samples of nitrite were prepared and analyzed with each Spectroquant® Test Kit. The IDC was calculated from the average of 4 control samples run 5 times each and the standard deviation based on the formula in Standard Methods 4020 [12].

$$\text{IDC Limits} = \text{Average} \pm (\text{Standard Deviation} \times 5.84)$$

Figure 10: IDC Calculation for 4 Control Samples

The results are summarized in Figures 10-12 below. Each IDC analysis value for each test kit was between upper and lower limits calculated for a passing IDC. All IDC samples passed for each Spectroquant® Test Kit.

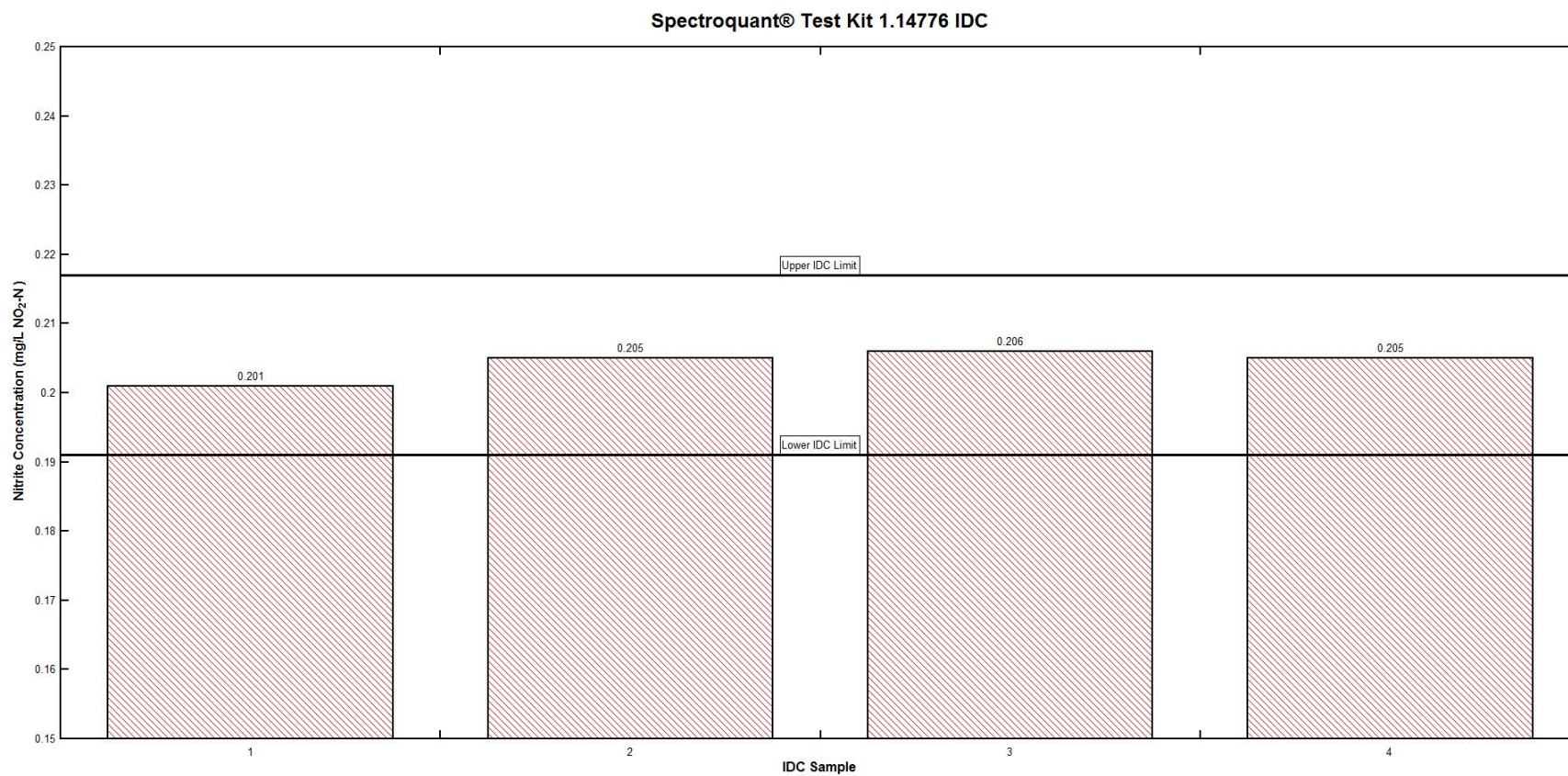


Figure 11: Spectroquant® Test Kit 1.14776 IDC

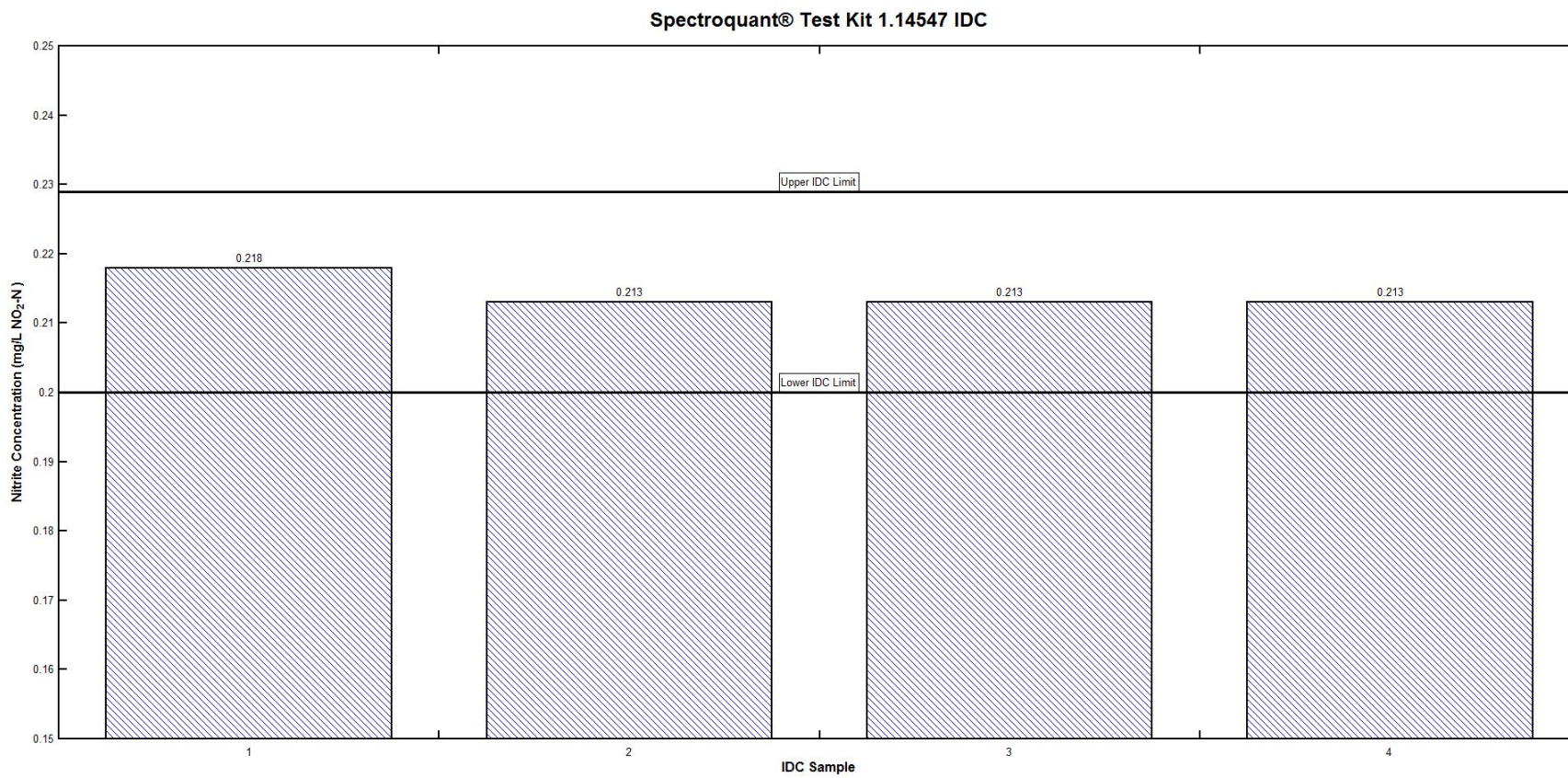


Figure 12: Spectroquant® Test Kit 1.14547 IDC

Spectroquant® Test Kit 1.00609 IDC

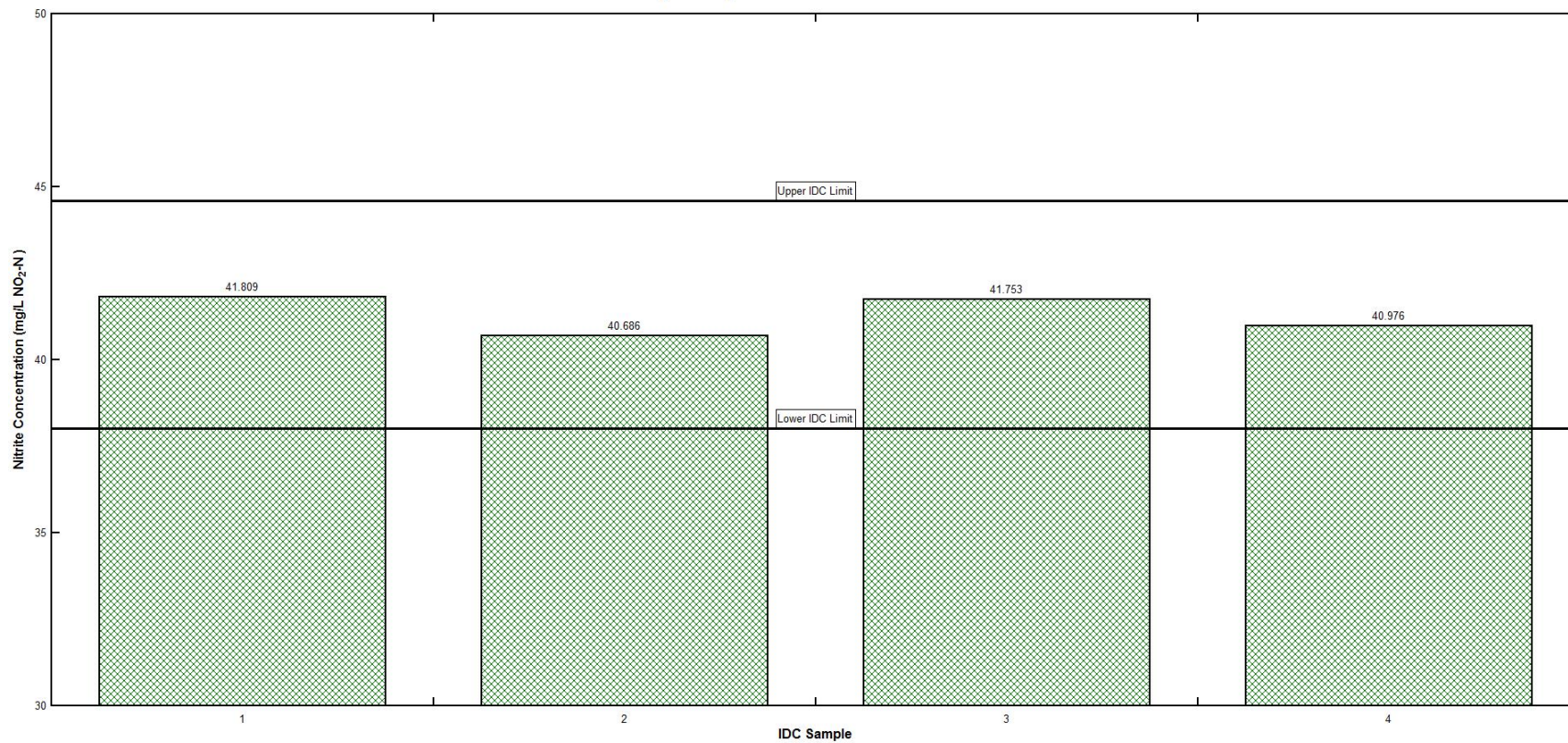


Figure 13: Spectroquant® Test Kit 1.00609 IDC

Matrix Spikes

A Mississippi River 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).

A sample of the Mississippi River was obtained from boat dock in Muscatine, Iowa. This sample was then filtered through a 0.45 µm syringe filter prior to matrix analyses.

The USGS has indicated that nitrite concentrations in the Mississippi River are in the range of 0.05 to 0.3 mg/L [16]. The Spectroquant® test kit 1.14776 and 1.14547 were selected for the test as they have the lowest reporting limit. The matrix spike concentration was selected to be at the midpoint of the Spectroquant® test kit range (0.1 mg/L NO₂-N). Each spectroscopic analysis was measured five (5) times and the average is reports and the standard deviation was used in the % RSD calculation.

Figures 9-10 provide the calculations used. The percent recovery, RPD and % RSD for the samples are summarized in Table 9-10 below.

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

Spiked Value = LFM concentration determined experimentally

Unspiked Value = Concentration of sample before spiking

s = *Dilution Correction*

Figure 14: Percent Recovery LFM

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

LFM = Concentration determined for LFM

LFMD = Concentration determined for LFM duplicate

Figure 15: Relative Percent Difference (RPD)

The results show that the Spectroquant® test kit can determine nitrite in a matrix regulated under the Clean Water Act (CWA)

Table 9: Matrix Spike Percent Recovery and RPD		
	1.14776	1.14547
Mississippi River (NO₂-N mg/L)	0.030	0.023
Matrix Spike (NO₂-N mg/L)	0.142	0.135
Matrix Spike Percent Recovery	112 %	112%
Matrix Spike Duplicate (NO₂-N mg/L))	0.138	0.135
Matrix Spike Duplicate Percent Recovery	108 %	112 %
Relative Percent Difference	3.00 %	0.00 %

Table 10: Matrix Spike and Spike Duplicate % RSD		
	1.14776	1.14547
Mississippi River	0.00%	0.00%
Matrix Spike	0.00%	0.00%
Matrix Spike Duplicate	0.00%	0.00%

Method Detection Limits

The Method Detection Limit (MDL) was calculated for each Spectroquant® test kit following the requirements in Appendix B, 40 CFR part 136 [17]. Each Spectroquant® test kit product flyer and test kit contained a nitrite range for which the test kit reagents were at a concentration to produce complete reaction of nitrite. 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.

The results for the Spectroquant® test kit MDL are at or below the MRL listed on the Spectroquant® product flyer (Table 11). These MRL vs. MDL results for the Spectroquant® test kits concludes that the Spectroquant® test kits can provide either an azo dye or Fe(II)-NO chromophore that responds like the Standard Methods 4500-NO₂ (B) method and that the dynamic range listed on each Spectroquant® product flyer is accurate.

Table 11: Method Reporting Limit - Method Detection Limits

Spectroquant® Test Kit	<i>MRL</i> <i>Lowest Concentration Listed for</i> <i>Spectroquant® Test Kit</i> <i>(NO₂-N)</i>	MDL (NO₂-N)
1.00609	<i>1.0</i>	0.327
1.14547	<i>0.010</i>	0.006
1.14776	<i>0.020</i>	0.005

Spectroquant®-EPA Promulgated Methods (Test Kits 1.14776, 1.14547)

An overall review of the nitrite test kits 1.14547 and 1.14776 spectrophotometric analytical reagents and their equivalency to EPA promulgated method are summarized in Table 12. In the Introduction section in this report, the conditions that are approved under the currently promulgated 40 CFR part 136.6 were expanded on in detail in the Richard Reding memo [2]:

1. *Changes in equipment operating parameters such as minor changes in the monitoring wavelength of a colorimeter*
2. *Adjusting sample sizes or changing extraction solvents to optimize method performance in meeting regulatory requirements*
3. *Minor changes in reagents used where the underlying reaction and principles remain virtually the same:*
 - a. *Changes in complexing reagent provided that the change does not produce interferences.*
 - b. *Changes in reactants provided that the change does not produce interference.*

The chemistry and analytical procedure for Spectroquant® Test Kits 1.14776 and 1.14547 has only been changed by the replacement the phosphoric acid-sulfanilamide with sulfanilic acid. The sulfanilic acid provides the hydrogen proton to produce the low pH that was provided by the phosphoric acid and the sulfanilic acid moiety produces the same adduct group for the Griess reaction. The MDL results along with the IDC, LRB, LFB, MS/MSD, RPD and % RSD all show good agreement with the Quality Control parameters that is expected in 40 CFR part 136.7 [1].

Spectroquant® Equivalent Method (Test Kit 1.00609)

An overall review of the nitrite test kit 1.00609 spectrophotometric analytical reagents and their equivalency to EPA promulgated method are summarized in Table 12. In the Introduction section in this report, the conditions that are approved under the currently promulgated 40 CFR part 136.6 were expanded on in detail in the Richard Reding memo [2]:

4. *Changes in equipment operating parameters such as minor changes in the monitoring wavelength of a colorimeter*
5. *Adjusting sample sizes or changing extraction solvents to optimize method performance in meeting regulatory requirements*
6. *Minor changes in reagents used where the underlying reaction and principles remain virtually the same:*
 - a. *Changes in complexing reagent provided that the change does not produce interferences.*
 - b. *Changes in reactants provided that the change does not produce interference.*

The reagent changes when compared to Standard Methods 4500-NO₂ (B) were:

1. Iron (II)-NO redox reaction replaced the azo dye reaction.

2. A different pH range and acid concentration was used.
3. A different wavelength range was used to quantitate the Iron (II)-NO product.

These modification in the Spectroquant® test kit 1.00609 falls under the detailed guidelines in Reding memo and as such the Spectroquant® test kit 1.00609 evaluated is equivalent to promulgated EPA azo dye nitrite promulgated method.

The MDL results along with the IDC, LRB, LFB and % RSD all show good agreement with the Quality Control parameters that is expected in 40 CFR part 136.7 [1].

Table 12: Test Kit Method Equivalency Summary

Spectroquant® Test Kit	Spectroquant® Change	136.6 Requirement
<p>1.14547 1.14776</p>	<p>Phosphoric acid-Sulfanilamide with Sulfanilic acid</p>	<p>Changes Allowed Under Method Equivalency.</p> <ul style="list-style-type: none"> • Minor changes in reagents used where the underlying reaction and principles remain virtually the same: • Changes in complexing reagent provided that the change does not produce interferences. • Changes in reactants provided that the change does not produce interference. • Accuracy, Precision, MRL and MDL from the experimental data were acceptable.
<p>1.14547 1.14776</p>	<p>Absorbance Maxima Changed from (533.5-543.5) to (539-544.5)</p>	<p>Changes Allowed Under Method Equivalency.</p> <ul style="list-style-type: none"> • Changes in equipment operating parameters such as minor changes in the monitoring wavelength of a colorimeter • Accuracy, Precision, MRL and MDL from the experimental data were acceptable.

Table 12: Test Kit Method Equivalency Summary

Spectroquant® Test Kit	Spectroquant® Change	136.6 Requirement
1.00609	Iron (II)-NO Redox Reaction Replaced Azo Dye Reaction	<p>Changes Allowed Under Method Equivalency.</p> <ul style="list-style-type: none"> • Minor changes in reagents used where the underlying reaction and principles remain virtually the same: • Changes in complexing reagent provided that the change does not produce interferences. • Changes in reactants provided that the change does not produce interference. • Accuracy, Precision, MRL and MDL from the experimental data were acceptable.
	Absorbance Maxima Changed from (533.5-543.5) to (445.5-455.5)	<p>Changes Allowed Under Method Equivalency.</p> <ul style="list-style-type: none"> • Changes in equipment operating parameters such as minor changes in the monitoring wavelength of a colorimeter • Accuracy, Precision, MRL and MDL from the experimental data were acceptable.

Summary and Conclusion

Summary

This study has provided the literature review information, EPA 40 CFR part 136.6 requirements and experimental data to support that Spectroquant® Nitrite Tests Kits 1.14776 and 1.14547 and Spectroquant® Nitrite Test Kit 1.00609 is equivalent to the EPA promulgated method Standard Methods 4500-NO₂ (B)

Conclusion

In conclusion, these changes in the Spectroquant® test kits (or future test kits with the same chemistry) produce an identical or equivalent result when compared to the colorimetric nitrite methods listed in 40 CFR part 136 .

References

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Appendix

EPA Method Equivalency Check-Off Table from Richard Reding Memo, *Flexibility to Modify CWA Methods, 2007*

Equivalency Requirement	Section in Report
Concentrations of calibration standards. Document the range of the concentrations of material used to establish the relationship between response of the measurement system and analyte concentration.	Yes, Tables 5 and 6
% RSD or correlation coefficient of calibration regression.	Yes, Tables 5, 6 and B
Performance range tested with units.	Yes, Tables 5 and 6
Sample(s) used in initial demonstration have the recommended preservative, where applicable.	Yes
Sample(s) used in initial demonstration met recommended holding times, where applicable.	Yes
Interferences.	None, MS/MDS Tables 9 and 10
Document the qualitative identification criteria used.	LFB percent recovery from 3 rd party QC samples. Table A. % RSD from 3 rd party QC samples. Table B. MDL determination and comparison to MRL of Spectroquant® test kits. Table B. MS/MSD . Tables 9 and 10
Performance evaluation studies performed for analytes of interest, where available.	See Tables 7, 8, 9, 10, 11, A and B Figures 11-13
Latest study sponsor or title	NA
Latest study number.	NA
Analysis of external reference material	See Table A.
Results of analyses on reference material from a source different from that used to prepare the calibration standards, if applicable.	See Table A.
Sources of external reference material, if applicable.	See Table 7
Surrogates used, if applicable.	Not Required
Concentrations of surrogates, if applicable.	Not Required

EPA Method Equivalency Check-Off Table from Richard Reding Memo, *Flexibility to Modify CWA Methods, 2007*

Equivalency Requirement	Section in Report
Recoveries of surrogates appropriate to the proposed use, if applicable.	Not Required
Sample preparation.	As per Spectroquant® test kit product flyer or Standard Methods 4500-NO ₂ (B) or report text.
Clean-up procedures.	As per Spectroquant® test kit product flyer or Standard Methods 4500-NO ₂ (B) or report text..
Method blank result.	Table 8
Matrix (reagent water, drinking water, effluent)	Mississippi River
Matrix spikes.	Matrix Spike section in the report. See Table 9, and 10
Spiking system, appropriate to the method and application.	Matrix Spike section in the report. See Table 9, and 10
Spike concentrations (with units corresponding to the final sample concentration) and recoveries.	Matrix Spike section in the report. See Table 9, and 10
Source of spiking material.	Muscatine Boat Dock, Mississippi River
Number of replicate spikes	MS and MSD analyzed and Spectroquant® LFBs. Each sample analyzed spectroscopically and an average and standard deviation determined for the % RSD. See Table A, B, 9, and 10
Initial demonstration of capability.	See Figures 10-12
Precision (analyte by analyte) Duplicates.	See Table A, B, 9, and 10
Bias (analyte by analyte).	See Table A, B, 9, and 10
Detection limit (with units; analyte by analyte).	See Table 11
Confirmation of detection limit, if applicable.	See Table 11
Quantitation limit (with units; analyte by analyte) Minimum level (ML), practical quantitation level (PQL) or limit of quantitation (LOQ).	Quantitation limit, Minimum Level, PQL and LOQ set by Spectroquant® product flyer. Table 11 confirms that the Spectroquant test limits in the product flyer are met.

EPA Method Equivalency Check-Off Table from Richard Reding Memo, *Flexibility to Modify CWA Methods*, 2007

Equivalency Requirement	Section in Report
Qualitative confirmation.	Not Required