

**Report of the Hach Method 10242 Revision 1.2  
TKN Equivalency Validation Study**

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## ***Acknowledgments***

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## *Executive Summary*

A single-laboratory equivalency validation study following the requirements at 40 CFR 136.6 was successfully completed using nine different matrices with Hach Method 10242 Revision 1.2 and EPA Reference Method 351.2, for the measurement of Total Kjeldahl Nitrogen.

Based on the comparative side-by-side results of all matrices, Hach Method 10242 Revision 1.2 for the measurement of Total Kjeldahl Nitrogen, was determined to be equal or better in performance to that of EPA Reference Method 351.2 and EPA approved Hach Method 10242 Revision 1.1 for regulatory compliance monitoring and reporting.

## ***Section 1***

### ***Introduction***

Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4$  and subsequently analyzed colorimetrically using salicylate or phenate chemistry with nitroprusside. Organic Kjeldahl nitrogen is the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value. Total nitrogen (TN) is the sum of TKN (free-ammonia and organic nitrogen) plus nitrate and nitrite.

$$[\text{TKN}] = [(\text{NH}_3 + \text{NH}_4^+)] + [\text{Nitrogen}_{\text{org}}]$$

$$[\text{Nitrogen}_{\text{org}}] = [\text{TKN}] - [(\text{NH}_3 + \text{NH}_4^+)]$$

$$[\text{TN}] = [\text{TKN}] + [\text{NO}_3] + [\text{NO}_2]$$

EPA Methods 351.1, EPA Method 351.2, and Standard Methods 4500-Norg and 4500-NH<sub>3</sub> are promulgated procedures for the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes.

Hach Method 10242 Revision 1.2, also known as TNTplus 880 is a simplified green chemistry alternative to that of EPA Reference Methods 351.1, 351.2 and the approved alternatives methods of Hach Method 10242 Revision 1.1, SM 4500-Norg and 4500-NH<sub>3</sub>. In the simplified total Kjeldahl (s-TKN) method, inorganic ( $\text{NH}_3$ ,  $\text{NH}_4^+$ ,  $\text{NO}_2$ ) and organic nitrogen are oxidized to nitrate by digestion with peroxodisulfate. The nitrate ions react with 2,6-dimethylphenol in a solution of sulfuric and phosphoric acid to form a nitrophenol. Oxidized forms of nitrogen in the original sample (nitrite + nitrate) are determined in a second test vial and then subtracted, resulting in TKN.

$$[\text{TKN}] = [\text{TN}] - [\text{NO}_3 + \text{NO}_2]$$

Hach Method 10242 Revision 1.2 is being validated as an equivalent method to Hach Method 10242 Revision 1.1, following the requirements of 40 *CFR* part 136.6 for the determination of total Kjeldahl nitrogen. The differences between Hach Method 10242 Revision 1.1 and 1.2 is the later revision digests the sample at 120° C. for 30 minutes and does not require the addition of sodium sulfite and sodium azide to remove any residual persulfate oxidant after digestion.

The quality control (QC) requirements incorporated into Hach Method 10242 Revision 1.2 exceed and improve upon that of the currently approved 40 *CFR* part 136 total Kjeldahl nitrogen methods and are consistent with 40 *CFR* 136, Appendix A methods for determination of organic analytes. The QC acceptance criteria in this method were derived from the results of the single-laboratory study. This report gives the details of the validation study.

## ***Section 2***

### ***Single-laboratory Validation Study***

This section of the report provides the details of the single-laboratory equivalency validation study conducted by Hach for the determination of comparability of measurement of total Kjeldahl nitrogen with the Hach Method 10242 Revision 1.2 to EPA Reference Method 351.2.

#### **2.1 Study Objectives**

The purposes of the single-laboratory study were to validate Hach Method 10242 Revision 1.2 according to the EPA requirements at 40 CFR 136.6.

#### **2.2 Sample Source**

The single-laboratory validation study required collection of a variety of industrial, municipal, and treated drinking water samples in order to assess the effect of sample type on the digestion chemistry of the Hach revised TKN method. When selecting these sites, various factors were considered and included the following criteria:

- The expected range of TKN-N concentrations, with a range of 0.1 – 16 mg/L
- Industrial categories with existing TKN effluent limitations
- Waste streams that have measurable quantities of nitrate and total nitrogen
- Accessibility of waste streams

#### **2.3 Sample Collection and Handling Activities**

At each site, sample material was collected in clean polyethylene containers. Industrial and municipal waste samples were collected, preserved, and shipped to the study sponsor facility (Hach) according to the specifications in 40 CFR part 136, Table II. Aliquots were preserved on-site with H<sub>2</sub>SO<sub>4</sub> to a pH of less than 2, shipped overnight to the sponsor facility at a temperature not greater than 6° C., but not less than 1° C. Drinking Water sample matrices were collected at various service taps associated with the drinking water treatment facility, then immediately preserved.

Upon receipt of collected industrial and municipal wastes samples to the sponsor facility, each matrix was screened by the Hach 10242 revised TKN procedure to determine the background concentration of total nitrogen, total nitrate, and TKN-N.

## 2.4 Analytical Study Design

The single-laboratory validation study consisted of four parts tests, the method detection limit (MDL) and minimum limit (ML) tests, an initial precision and recovery (IPR) test, a matrix and matrix spike duplicate test, and linearity. The participating validation laboratories are to perform these tests for the proposed Hach Method 10242 Revision 1.2 and EPA Reference Method 351.2.

### 2.4.1 MDL and ML Tests

The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero. To determine the MDL, the laboratories were required to follow the procedure in Appendix B to 40 *CFR* part 136. This procedure consists of the analysis of seven aliquots of reagent water that are spiked with the analyte(s) of interest. For the MDL study, ammonium chloride ( $\text{NH}_4\text{Cl}$ ) specified in EPA Reference Method 351.2 was used. The MDL is calculated by multiplying the standard deviation of the seven replicate analyses by the student's t value for (n-1) degrees of freedom, where n equals the number of replicates.

The ML is defined as the level at which the entire analytical system produces a recognizable signal and an acceptable calibration point and is determined by multiplying the MDL by 3.18 and rounding the result to the nearest factor of 10 multiple of 1, 2, or 5. The value 3.18 represents the ration between the student t multiplier used to determine the MDL and the 10 times multiplier used in the American Chemical Society (ACS) limit of quantity (LOQ) calculations (i.e.  $10 \div 3.143 = 3.18$ ). For example, if the calculated MDL is 5.8, the resulting ML will be equal to 5.8 times 3.18, or 18.4. Rounding to the nearest factor of 10 multiple of 1, 2, or 5 then establishes the ML at 20.

### 2.4.2 IPR Test

The IPR test is used for initial demonstration of a laboratory's capability to produce results that are at least as precise and accurate as results from practice of the method by other laboratories. The IPR test also is used to demonstrate that a new method or method modification will produce results that are as precise and accurate as the reference method. The IPR test consists of analyzing at least four replicate aliquots of a reference matrix spiked with the analytes of interest and with either surrogate compounds or, for isotope dilution analysis, labeled compounds. The concentration of the target analytes in the spike solution may vary between one and five times the concentration used to establish the lowest calibration point (e.g., one to five times the ML). The spiked aliquots are carried through the entire analytical process. Specifications are developed for the permissible range of recovery for each analyte and for an upper limit on the standard deviation or RSD of recovery.

### 2.4.3 Matrix, Matrix Spike and Matrix Spike Duplicate Test

The matrix and matrix spike/matrix spike duplicate (MS/MSD) test is used to assess method performance in the sample matrix. Analytes of interest are added to a field sample aliquot that is then thoroughly homogenized and split into two spiked replicate aliquots. One of these replicates is identified as the matrix spike sample and the other is identified as the matrix spike duplicate sample. The recoveries of the analytes, relative to the spike, are determined in each sample. The precision of the determinations also is assessed by measuring the relative percent difference (RPD) between the analyte concentrations measured in the MS and MSD.

## 2.5 Data Validation

Data generated from this study were reviewed and validated according to the USEPA validation protocol at 40 CFR 136.6. Final results, including all calculations, were verified from the raw data and bench sheets. All quality control requirements in the study plan and draft version of Hach Method 10242 Revision 1.2 and USEPA Reference Method 351.2 were met, and all data reviewed were determined to be of acceptable quality.

## 2.6 Results

A total of 10 matrices (reagent water 3 municipal wastewater effluents, 3 industrial wastewater effluents, and 3 treated drinking water effluents) for TKN determination for each of the three performance tests (MDL/ML, IPR, Matrix and MS/MSD), by the proposed Hach Method 10242 Revision 1.2 and USEPA Reference Method 351.2. For each reference matrix (reagent water) and field sample, the analytical results were subjected to statistical evaluation as described in section 3.3 above. The calculations of analytical results are presented by performance test in tables 5 through 10.

## 2.7 Discussions

### 2.7.1 MDL and ML Test

The MDL with Hach Method 10242 Revision 1.2 was determined to be 0.08 mg/L and 0.12 mg/L for the USEPA Reference Method 351.2. Likewise, the ML and rounded ML for each method was determined to be 0.26/1.0 mg/L and 0.39/1.00 mg/L, respectively.

This similarity in MDL and ML results indicates Hach Method 10242 Revision 1.2 is equally effective and comparable to Hach Method 10242 Revision 1.1, and USEPA Method 351.2 with respect to method detection in the standard reference matrix of reagent water.

### 2.7.2 IPR Test

The mean percent recovery and standard deviation of mean percent recoveries with Hach Method 10242 Revision 1.2 was determined to be 96.9% and 2.2, respectively. The mean

percent recovery and standard deviation of mean percent recoveries with USEPA Reference Method 351.2 was determined to be 109% and 0.85, respectively.

The Hach Method 10242 Revision 1.2 results of mean percent recovery, standard deviation of mean percent recoveries, further indicates equal effectiveness and comparability to Hach Method 10242 Revision 1.1 and USEPA Reference Method 351.2 in the standard reference matrix of reagent water.

### 2.7.3 Matrix Spike Matrix Spike Duplicate Test

The mean percent recovery and standard deviation of mean percent recoveries across all matrices with Hach Method 10242 Revision 1.2 was determined to be 95.6 and 2.13, respectively. The mean percent recovery and standard deviation of mean percent recoveries across laboratories with USEPA Reference Method 351.2 was determined to be 104 and 7.29, respectively.

Hach Method 10242 Revision 1.2 results of mean percent recovery, standard deviation of mean percent recoveries indicates equal effectiveness and comparability to USEPA Reference Method 351.2 in various matrices of drinking water, treated wastewater from POTW's, and industrial treated wastewater effluents.

## 2.8 Conclusions

A single-laboratory equivalency validation study was successfully completed using nine different matrices with Hach Method 10242 Revision 1.2 and EPA Reference Method 351.2, for the measurement of Total Kjeldahl Nitrogen.

Based on the comparative side-by-side results of all matrices, Hach Method 10242 Revision 1.2 for the measurement of Total Kjeldahl Nitrogen, is equal or better in performance to that of EPA Reference Method 351.2 and EPA approved Hach Method 10242 Revision 1.1 for regulatory compliance monitoring and reporting.



**Table 1**  
**Single-laboratory Validation Results**  
**Method Detection and Method Limit**  
**Hach Method 10242 Revision 1.2**  
**TKN (mg/L)**

<i>MDL Replicate</i>	<b>Hach Method 10242 Rev. 1.2</b>	<b>EPA Reference Method 351.2</b>
<b>Rep #1</b>	1.19	1.26
<b>Rep #2</b>	1.22	1.23
<b>Rep #3</b>	1.26	1.23
<b>Rep #4</b>	1.25	1.30
<b>Rep #5</b>	1.20	1.33
<b>Rep #6</b>	1.20	1.23
<b>Rep #7</b>	1.22	1.26
<b>Standard Deviation</b>	0.026	0.039
<b>MDL</b>	0.083	0.12
<b>ML</b>	0.264	0.39
<b>ML (Rounded)</b>	1.0	1.0

**Table 2**  
**Single-laboratory Validation Results**  
**Initial Precision and Recovery**  
**Hach Method 10242 Revision 1.2**  
**TKN Percent Recovery**

<b>IPR Replicate</b>	<b>Hach Method 10242 Rev. 1.2 (%)</b>	<b>EPA Reference Method 351.2 (%)</b>
<b>Rep #1</b>	96.0	108
<b>Rep #2</b>	97.2	109
<b>Rep #3</b>	94.6	110
<b>Rep #4</b>	99.8	110
<b>Mean % Recovery</b>	96.9	109
<b>Standard Deviation</b>	2.2	0.85

**Table 3**  
**Single-laboratory Validation Results**  
**Matrix Spike and Spike Duplicate Recovery**  
**Hach Method 10242 Revision 1.2**  
**TKN Percent Recovery**

Matrix	Hach Method 10242 Revision 1.2					EPA Reference Method 351.2				
	Matrix mg/L	Matrix Spike mg/L	Matrix Spike Dup. mg/L	Mean % Rec.	Std. Dev.	Matrix mg/L	Matrix Spike mg/L	Matrix Spike Dup. mg/L	Mean % Rec.	Std. Dev.
<b>Muni. WW #1</b>	0.42	4.97	5.08	92.1	1.6	0.80	6.82	6.59	118	3.3
<b>Muni. WW #2</b>	1.90	6.55	6.72	94.7	2.4	1.06	6.30	6.33	105	0.42
<b>Muni. WW #3</b>	0.82	5.52	5.87	97.5	5.0	1.20	5.87	5.98	94.5	1.6
<b>Ind. WW #1</b>	0.69	5.50	5.60	97.2	1.4	0.41	5.56	5.55	103	0.14
<b>Ind. WW #2</b>	1.70	6.42	6.44	94.6	0.28	2.00	7.15	7.22	104	0.99
<b>Ind. WW #3</b>	1.01	5.88	5.99	98.5	1.6	1.16	6.20	5.98	98.6	3.1
<b>DW #1</b>	0.00	4.73	4.77	95.0	0.57	0.15	5.60	5.90	112	4.2
<b>DW #2</b>	0.12	4.78	4.77	93.1	0.14	0.33	5.28	5.46	101	2.6
<b>DW #3</b>	0.15	4.95	5.08	97.3	1.84	0.00	4.92	4.92	98.4	0.14

**Table 4**  
**Single-laboratory Linearity Validation Results**  
**Hach Method 10242 Revision 1.2**  
**Coefficient of Variation**

Standard Concentration (mg/L TKN as NH <sub>3</sub> -N)	Measured Concentration (mg/L TKN as NH <sub>3</sub> -N)
1.00	1.08
2.00	1.97
5.00	4.79
8.00	7.53
16.0	15.7
Coefficient of Variation (R <sup>2</sup> ) = 0.9993	

**Table 5**  
**Single-laboratory Quality Control Performance Results**

Quality Control Parameter	Hach Method 10242 Revision 1.2	EPA Method 351.2
<b>MDL</b>	0.08 mg/L TKN as NH <sub>3</sub> -N	0.12 mg/L TKN as NH <sub>3</sub> -N
<b>ML</b>	0.26 mg/L TKN as NH <sub>3</sub> -N	0.39 mg/L TKN as NH <sub>3</sub> -N
<b>Rounded ML</b>	1.00 mg/L TKN as NH <sub>3</sub> -N	1.00 mg/L TKN as NH <sub>3</sub> -N
<b>IPR Mean % Recovery</b>	96.9	109
<b>IPR SD</b>	2.2	0.85
<b>IPR % Limit of Recovery Range</b>	90% - 110%	90% - 110%
<b>Matrix Spike/Duplicate Mean % Recovery</b>	95.6	104
<b>Matrix Spike SD</b>	2.2	7.3
<b>Matrix Spike % Limit of Recovery Range</b>	90 - 110%	90% - 110%

*Appendix A*  
*Wastewater and Drinking Water Sources*

**Matrix Category – Municipal Wastewater (Muni WW)**  
Colorado, Utah, Ohio

**Matrix Category – Industrial (Ind WW)**  
Dairy processing, Meat Processing, Brewery

**Matrix Category – Treated Drinking Water (DW)**  
Colorado, Florida, New Hampshire