NCASI METHOD POLY W109.01

POLYPHENOLICS IN PULP MILL WASTEWATERS BY SPECTROPHOTOMETRY

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This method is included in NCASI's *Methods Manual* and is a modified Folin reagent method for tannin-lignin (polyphenols) determination in wastewater. It is included in the *Methods Manual* as a proposed method. Those electing to apply the method are strongly encouraged to conduct rigorous QA/QC or validation so that the quality of data generated can be evaluated. Please note that this proposed method is not suitable for use as a regulatory monitoring or compliance method.

NCASI METHOD POLY W109.01

POLYPHENOLICS IN PULP MILL WASTEWATERS BY SPECTROPHOTOMETRY

1.0 Scope and Application

1.1 Method Poly W109.01 procedures utilize a spectrophotometer to measure the absorbance of light as it passes through a sample that has been prepared by reaction with TanniVer 3® Folin phenol reagent. Concentrations of all hydroxylated compounds (polyphenols) that give a proportional blue color are determined by comparison of the absorbance of the sample to the absorbance of tannic acid solutions of known concentrations.

1.2 This method has been validated at the single laboratory level in wastewater treatment plant influents, biologically treated effluents from pulp and paper mills, and receiving waters. Demonstration of method performance for specific matrix types is recommended.

1.3 The estimated minimum detection limit (MDL) achievable is instrument and light path length dependent and was found to be 0.02 mg/L in reagent water using a 10 mm light path length. This value is provided as guidance. Due to variables in instrumentation and matrix effects, each laboratory should establish its own MDL. The lower instrument calibration limit (LCL) for this method is approximately 0.08 mg/L. Lower calibration levels can be obtained by using an increased light path length. The concentration range used during a single method validation was from 0.08 to 8.0 mg/L. Sample values above 8.0 mg/L may be determined by quantitative dilution (Section 12.3).

2.0 Summary of Method

2.1 Sample Preparation

A sample volume of preserved or unpreserved wastewater is prepared to a volume of 25 mL in a 40 mL vial or a 50 mL round bottom tube using reagent grade water for dilution. The amount of sample utilized depends on previous experience with the sample to obtain an absorbance within the range of the calibration curve (Section 7.4). Folin phenol reagent (0.5 mL) is added and mixed with each sample or each prepared calibration point. Immediately, 5.0 mL (5 g) of sodium carbonate-tartrate buffer solution is added and mixed with each sample or prepared calibration point. Samples are allowed to react for a minimum of 30 minutes at room temperature to produce a blue- to green-hued solution.

2.2 Quantitative Analysis

The absorbance of the solution is measured with a spectrophotometer set at a wavelength of 700 nanometers (ηm). The corrected concentration of the sample is calculated using the linear regression equation (Section 12.1) determined for the six point calibration curve prepared using tannic acid and the dilution factor for the sample.

2.3 Quality Assurance

Quality is assured through reproducible calibration and testing of sample preparation and the spectrophotometer system. Calibration is verified on each day of analysis by freshly preparing and analyzing a point from the calibration curve. A method blank is analyzed with each sample set (samples started through the process on a given day, to a maximum of 20), along with sample duplicates to ensure reproducibility in the matrix. A fortified sample is assessed to determine recovery in the matrix. A complete description of quality control procedures, calculations, and method performance criteria are listed in Sections 9, 12, and 17, respectively.

3.0 Definitions

3.1 These definitions are specific to this method, but conform to common usage as much as possible.

3.1.1 May – this action, activity, or procedural step is neither required nor prohibited

3.1.2 Method Detection Limit (MDL) – As defined by EPA (1), the MDL is the lowest concentration that can be identified at the 99% confidence level as being greater than background.

3.1.3 Must not – this action, activity, or procedural step is prohibited

3.1.4 Must – this action, activity, or procedural step is required

3.1.5 Should – this action, activity, or procedural step is suggested, but not required

4.0 Interferences

4.1 Reagents, glassware, and other sample processing equipment may contribute analytical interferences, resulting in inaccurate absorbance readings. Run method blanks initially and with each subsequent sample set to demonstrate that the reagents, glassware, and other sample processing hardware are free from interferents under the conditions of the method.

4.2 Any substance that will produce a reaction with the Folin phenol reagent has the potential to cause interference. Organic compounds such as hydroxylated aromatics, proteins, humics, and amines can produce false positives (2). Ferrous iron, sodium sulfite, manganese, nitrite, cyanide, bisulfite, sulfide, and hydroxylamine hydrochloride also give a positive response when reacted with the Folin phenol reagent (2,3).

4.2.1 Ferrous iron interference at 2 mg/L will produce an increase in absorbance equivalent to 1 mg/L as tannic acid. Interference from ferrous iron up to 20 mg/L can be eliminated by adding 200 mg sodium pyrophosphate before reagents are added to the prepared sample (3).

4.2.2 Sodium sulfite interference at 125 mg/L will produce an increase in absorbance equivalent to 1 mg/L as tannic acid. Interference from sodium sulfite can be eliminated by adding 1 mL formaldehyde solution just before reagents are added to the prepared sample (3).

4.3 All glassware must be clean and free of scratches. Cells used for spectrophotometric measurement of samples must be free of all oil or residue that may cause interference in the absorbance measurement. It is recommended that the same cell be utilized for measurements of the calibration curve, daily calibration checks, blanks, and samples.

5.0 Safety

5.1 Each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to a level protective of human health. This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of data handling sheets (MSDS) should also be made available to all personnel involved in these analyses.

5.2 The Folin phenol reagent solution is corrosive. It contains phosphoric (H_3PO_4) and hydrochloric acid (HCl) pH <2. Take appropriate measures to avoid contact with chemicals by wearing chemical-resistant gloves, eye protection, and other protective clothing.

5.3 The carbonate-tartrate buffer reagent solution is corrosive. It contains sodium carbonate and sodium tartrate. The pH of this solution is >13, and it should be prepared and used with caution. Take appropriate measures to avoid contact with the chemicals by wearing chemical-resistant gloves, eye protection, and other protective clothing.

5.4 All chemicals should be treated as potential health hazards. It is recommended that prudent practices for handling chemicals in the laboratory be employed (4). As with all samples, precautions should be taken to avoid exposure to potentially toxic, caustic, or nuisance odor compounds.

6.0 Equipment and Supplies

Note: *Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 A spectrophotometer with a tungsten lamp source and a 10 ηm spectral slit width is required. The instrument must be capable of emitting light at a selectable wavelength of 700 ηm with an operating range of 400 to 700 ηm. Verify that the spectrophotometer is calibrated correctly by following the directions from the manufacturer for your specific instrument.

6.2 Do not use glassware with any star fractures, chips, cracks, or severe scratches. All glassware should be washed with detergent, rinsed with tap water, and rinsed with deionized water prior to use.

6.3 Equipment for Sample Preparation

6.3.1 40 mL amber glass VOA vials or 50 mL centrifuge tubes with Teflon™-lined caps

6.3.2 Adjustable automatic pipette, 1 to 1000 µL

6.3.3 Adjustable automatic pipette, 2 to 5 mL

6.4 Equipment and Supplies for Standard and Reagent Preparation

6.4.1 200 mL volumetric flask for tannic acid standard preparation

6.4.2 250 mL amber glass bottle for storage of standard

6.4.3 10, 25, 50, and 100 µL glass syringes for preparation of calibration curve

6.4.4 1 or 2 L glass beakers for sodium carbonate-tartrate preparation

6.4.5 Hot plate with magnetic stirrer

6.4.6 2 or 3 inch stir bar

6.4.7 Thermometer, 0 to 100°C

6.4.8 1 L volumetric flask for sodium carbonate-tartrate preparation

6.4.9 1 L amber glass bottle for storage of sodium carbonate-tartrate reagent

6.5 Other Apparatus

6.5.1 Sample cuvettes (absorption cell) for measurement of absorbance; cell path length is determined by required MDL, path length of 10 mm was utilized for the single laboratory evaluation

7.0 Reagents and Standards

7.1 Reagents

7.1.1 Tannic acid, $C_{76}H_{52}O_{46}$, CAS Number 1401-55-4; ACS reagent, Sigma Cat #T-8406

7.1.2 TanniVer 3® Folin phenol reagent; commercially obtained, HACH Cat #256049

7.1.3 Sodium carbonate, Na_2CO_3, CAS Number 497-19-8; Sigma-Aldrich, ACS reagent, Cat #223530

7.1.4 Sodium L tartrate, $C_4H_4Na_2O_6 \cdot 2H_2O$, CAS Number 6106-24-7; Sigma-Aldrich, ACS reagent, Cat #228729

7.1.5 Reagent water free of polyphenolic as determined by this method

7.1.6 Sulfuric acid, H_2SO_4, CAS Number 7664-93-9; ACS reagent grade

7.2 Standard Preparation

7.2.1 Prepare a 200 mg/L tannic acid stock standard. Dry approximately 100 mg tannic acid in a drying oven at 105°C for 2 hours. Let it cool in a dessicator. Weigh 40.0 mg tannic acid and transfer to a 200 mL volumetric flask. Dissolve the solids in approximately 90 mL reagent water and bring it to a final volume of 200 mL. Transfer the standard to a 250 mL amber glass bottle. Refrigerate and protect from light when not in use. Prepare a fresh stock every four to five months or as calibration checks indicate that a new standard and calibration are warranted.

7.3 Reagent Preparation

7.3.1 Prepare the sodium carbonate buffer reagent by slowly dissolving 200 g Na_2CO_3 in approximately 700 to 800 mL preheated (~50°C) reagent water in a 1 L beaker using a hot plate stirrer with a magnetic stir bar. Slowly increase solution temperature to 75 to 80°C while adding the Na_2CO_3. After the Na_2CO_3 solids are completely dissolved gradually add 12 g sodium L tartrate. Cover and cool the solution before transferring to a 1 L volumetric flask. Allow the flask to cool to room temperature and adjust final volume to 1 L. Store the reagent in a 1 L amber glass bottle or two 500 mL amber glass bottles (2).

7.3.2 Use H_2SO_4 to preserve samples by adjusting sample pH to <2. Prepare a 1:3 vol/vol solution by gently adding ~34 mL concentrated H_2SO_4 to approximately 60 mL reagent grade water using a stir bar and stir plate. Bring the volume to 100 mL once heat has dissipated.

7.4 Calibration Curve

7.4.1 A six to seven point calibration curve (minimum six points) should be prepared every five months or as results indicate. If a calibration curve is not prepared on the day of analysis, a continuing calibration verification (CCV) must be performed before sample analysis (Section 7.5).

7.4.2 Prepare the calibration curve. Remove the tannic acid standard (Section 7.2.1) from the refrigerator, thoroughly shake it, pour approximately 35 mL into an amber glass vial, and bring to room temperature. Mark seven vials or tubes with numbers for identification and place them in a rack (Section 17, Table 1).

7.4.3 Add 25.0 mL reagent water to the method blank vial using a volumetric pipette (or a variable volume pipette and balance for gravimetric verification of 25 g).

7.4.4 Calibration curve concentrations are ~0.08, ~0.2, ~0.40, ~0.80, ~2.0, ~4.0, and ~8.0 mg/L. Make the dilutions in the vials or tubes as outlined in Section 17, Table 1 by adding appropriate amounts of reagent water and appropriate amounts of tannic acid stock standard solution (Section 7.2.1).

7.5 Continuing Calibration Verification

7.5.1 Analyze a CCV with each set of samples to verify the stability of the original calibration. Prepare the CCV by selecting one point of the calibration curve and preparing fresh on the day of analysis (Section 7.4.4).

8.0 Sample Collection, Preservation, and Storage

8.1 Sample Collection

Collect grab or composite samples using clean glass or plastic sampling containers that are free from contaminants that may interfere with analyses. Composite samples should be refrigerated at 4°C during the sampling period. Samples should be refrigerated as soon as possible after collection if analysis will not be completed within 24 hours.

8.2 Sample Preservation and Storage

Samples can be stored for up to 30 days before analysis when preserved at pH 2 using a 1:3 H_2SO_4 solution after sampling (Section 7.3.2). Preserved samples must be refrigerated (4°C) prior to analyses.

9.0 Laboratory Quality Control/Quality Assurance (QA/QC)

9.1 Each laboratory using this method is required to operate a formal quality assurance program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and ongoing analyses of standards and blanks as a test of continued performance. Compare laboratory performance to established performance criteria to determine if results of analyses meet performance characteristics of the method (Section 17). Minimum requirements for this program include an initial demonstration of laboratory capability and analyses of CCV standards (Section 7.5), method blanks (Section 9.4), replicate samples to assess method reproducibility (Section 9.5), and matrix spikes (MS/MSD pairs, Section 9.6) to characterize precision and accuracy in wastewater matrices.

9.2 Spectrophotometer Performance and Calibration Verification

9.2.1 Fill the sample cuvette with reagent grade water and place it in the spectrophotometer. Adjust the absorbance reading to zero. Verify zero after every calibration and after every six to eight sample readings.

9.2.2 Determine that the spectrophotometer system is operating within acceptable parameters by conducting CCVs before each set of samples are analyzed (Section 7.5). Calibration verification involves preparing an independent standard from the initial calibration curve using current tannic acid standard and reagents used to prepare the calibration curve (Sections 7). Percent recovery determined for the calibration check should be within calculated warning limits (Section 17, Table 2). Prepare a new check standard if calibration verification fails to meet the acceptance criterion. If this fails to correct the calibration verification difficulties, prepare a new tannic acid standard and calibration curve for analysis (Section 7).

9.2.3 Verify that the spectrophotometer is operating properly and is calibrated electronically in accordance with the instrument manual.

9.3 Frequency

One sample per analytical batch of no more than twenty samples of similar matrix type should be allocated for quality control (i.e., duplicate, matrix spike analyses). A representative sample from each new or untested source or sample matrix should be treated as a quality control sample.

9.4 Method Blanks

9.4.1 Demonstrate that reagents and dilution water used for sample preparation are free of polyphenolics by analyzing a blank with each sample set (20 samples or less). Prepare a method blank using the same procedure outlined in Section 11.1 utilizing reagent grade water for the sample. A summary of method blank statistics observed during the single laboratory evaluation of this method are provided in Section 17, Table 3.

9.5 Sample and Duplicate Precision

Analyze a sample and duplicate for each matrix type with each set of samples to assess method reproducibility. Calculate the relative percent difference (RPD) in concentration for each replicate pair using Equation 1.

Equation 1

$$\textit{Relative Percent Difference} = \frac{\textit{Sample Conc. (mg/L)- Sample Duplicate Conc. (mg/L) x 100}}{\textit{Average Concentration of the Sample and Sample Duplicate}}$$

A summary of the precision determined in the single laboratory validation is provided in Section 17, Table 4 for pulp mill wastewaters and receiving waters.

9.6 Matrix Spikes and Matrix Spike Duplicates (MS/MSD)

A matrix spike analysis should be performed with each set of samples (batch of samples no greater than 20). A known amount of tannic acid solution (Section 7.2.1) should be added to a sample so that the native plus the spike level of tannic acid is at least one times

the native level. The percent recovery of the matrix spike should be determined and the results charted to document recovery. Section 17, Table 5 lists recoveries found during single laboratory validation studies.

9.7 Field Replicates and Field Spikes

Depending on specific program requirements, field replicates may be required to assess precision and accuracy of sampling, preservation, and transport techniques.

10.0 Calibration and Standardization

10.1 Select a wavelength of 700 ηm on the spectrophotometer, verify that it is operating correctly in accordance with the instrument manual, zero it (Section 9.2.1), and utilize the same conditions to measure the absorbance of all samples, method blanks, calibration curves, CCVs, and quality control samples (Section 9).

10.2 Quantitation

10.2.1 Analyze calibration standards (Section 7.4) at a wavelength of 700 ηm using the procedure described in Section 11.2.4. Process a blank with the curve to confirm that glassware, sample cuvettes, reagents, and other components are free from contamination. Prepare the blank with reagent water using the procedure for preparation of the samples (Section 11). Subtract the absorbance of the method blank from calibration curve points to correct for background absorbance associated with dilution water and reagents prior to plotting the calibration curve. Construct a calibration curve by plotting the absorbance versus concentration as tannic acid (mg/L) for the calibration curve points. Generate a linear regression equation ($y = mx + b$) for the calibration curve. An example of a calibration curve plot is shown in Section 17, Figure 1.

10.2.2 If the R-squared value determined for the curve is ≥ 0.991, the calibration curve is assumed to be linear and acceptable. The linear equation determined from the curve can then be used to calculate sample concentration (Section 12). If the curve is not linear, evaluate the problem, undertake appropriate remedial action, and reanalyze the calibration curve solutions. If remedial actions and reanalysis fail to produce an R-squared value of ≥ 0.991, prepare a new tannic acid standard and calibration curve and reanalyze. Statistics for calibration curve linearity determined during a single laboratory validation of this method are included in Section 17, Table 6.

10.3 Verify calibration prior to analysis of samples (Sections 9.2) by analyzing a CCV (Section 7.5) prior to analysis of each set of samples. It is recommended that the selected CCV vary over time in order to verify calibration of the instrument over the full range of the method. Recalibrate if percent recovery for the CCV is outside of the warning criteria (Section 17, Table 2).

11.0 Procedure

This section includes procedures used in preparation of calibration standards, CCVs, samples, quality control samples, and method blanks for colorimetric analysis.

11.1 Sample Preparation

11.1.1 Remove sample bottles from the refrigerator, warm to room temperature, and shake to ensure homogeneity.

11.1.2 Final volume must be 25.0 mL before reagent addition. The amount of sample utilized will depend on previous experience with the sample to obtain an absorbance within the range of the calibration curve. Prepare the sample by adding reagent water to each vial or tube and the selected volume (or mass in grams) of sample to bring the total volume to 25.0 mL (25 g). Record the amount of sample utilized and the amount of reagent water added. For example, a common sample dilution is prepared by adding 15 mL (15 g) reagent water to 10 mL (10 g) sample for a total final volume of 25.0 mL (25 g). Remember to correct the final result by the dilution factor as indicated in Section 12.

11.1.3 Add 0.5 mL (0.5 g) of Folin phenol reagent (TanniVer 3®, Section 7.1.2) to each sample using an automatic pipette. Cap the tube or vial with a Teflon-lined screw cap and invert at least two times. A vortex mixer can be used with round bottom tubes for approximately 5 seconds.

11.1.4 Within 1 minute of adding the Folin phenol reagent, add 5.0 mL sodium carbonate tartrate buffer solution (Section 7.3.1) to each sample or each prepared calibration point, using an automatic pipette. Cap and invert at least two times. A vortex mixer can be used with round bottom tubes for approximately 5 seconds.

11.1.5 Allow samples to react for a minimum of 30 to 40 minutes at room temperature.

11.1.6 Measure absorbance within 20 minutes using the procedures outlined in Section 9.

11.2 Spectrophotometer Analysis

11.2.1 **S**pectrophotometer conditions should be set according to the criteria described in Section 9.2.3.

11.2.2 Perform an initial calibration by analyzing the calibration curve (Section 9.4) or a CCV as outlined in Section 9.5.

11.2.3 Verify that the spectrophotometer is zeroed (Section 9.2.1).

11.2.4 Rinse the cell with a small amount of sample and discard the rinse. Transfer enough sample to the sample cuvette, filling above the reference line. Measure and record absorbance at 700 ηm and discard the sample. Repeat with each sample, periodically verifying that the spectrophotometer is zeroed.

12.0 Data Analysis and Calculations

12.1 Quantitation

12.1.1 Use the linear regression equation from the calibration curve (Section 10.2) to calculate corresponding color values of the samples. Calculate color values using Equation 2.

Equation 2

$y=mx+b$

where:
y is the absorbance measured in the sample
m is the slope
b is the y-intercept
x is the calculated concentration as mg/L tannic acid

Calculate the concentration for each sample utilizing the measured absorbance value and solving the resulting linear equation for x (Section 10.2).

12.1.2 The calibration curve slope and y-intercept will vary depending on the light path length of the sample cuvette used in each different spectrophotometer. Therefore, it is important to use the same light path length for all measurements. The following is an example calculation:

Absorbance for the sample (y)	0.419
Linear regression equation	$0.419 = 0.0845x + 0.0025$
Solve for x	$x = \frac{0.419 - 0.0025}{0.0845}$
Calculated concentration (x)	$x = 4.93$ mg/L

12.2 Data Review Requirements

12.2.1 Review data to assess the accuracy and precision of calculated and corrected concentrations. Assess any spectrophotometer problems, interferences, and bias using the guidance provided in Sections 9 and 10. Correct any problems prior to reporting analytical results.

12.2.2 Assess the need for sample dilutions. The procedure for conducting sample dilutions and reanalysis is described in Section 12.3.

12.2.3 Resolve inconsistencies between duplicates as necessary.

12.2.4 If review of the data shows any problems that could affect subsequent analyses, discontinue analyses until problems are resolved.

12.3 Results Outside Calibration Range

If the measured absorbance or calculated concentration exceeds the highest calibration point, dilute an aliquot of the sample 1:1 with the reacted blank from the sample set to bring absorbance within the calibration range of the method. Continue sample analysis as described in Section 11.2.

13.0 Method Performance

Single laboratory performance for this method is detailed in Section 17, Tables 2 through 6.

14.0 Pollution Prevention

Pollution prevention approaches have not been evaluated for this method.

15.0 Waste Management

15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 Dispose of all samples as required by federal, state, and local regulations.

15.3 For further information on waste management, the Environmental Protection Agency suggests you consult *The Waste Management Manual for Laboratory Personnel*, and *Less is Better: Laboratory Chemical Management for Waste Reduction*. Both are available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC, 20036.

16.0 References

1. *Federal Register* 49 (209). Appendix B to Part 136 – Definition and Procedure for the Determination of the Method Detection Limit–revision 1.11. October 26, 1984.

2. *Standard Methods for the Examination of Water and Wastewater*, 20th Edition. American Public Health Association, Washington, DC. pp 5-51 to 5-52. 1998.

3. *HACH Water Analysis Handbook*. Tannin and Lignin Method 8193. pp 1009 to 1012. 2003.

4. *Prudent Practices in the Laboratory*. National Research Council (NRC). National Academy Press, Washington, DC. 1995.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Calibration Curve Preparation Volumes

Vial Identification Number	Calibration Point (mg/L)	Reagent Water (mL)	Tannic Acid Standard (μL)	Tanni-Ver Reagent (mL)	CarbonateBuffer Solution (mL)
1	blank	25.0	----	0.5	5.0
2	~0.08	~25.0	10	0.5	5.0
3	~0.2	~25.0	25	0.5	5.0
4	~0.8	24.9	100	0.5	5.0
5	~2.0	24.8	250	0.5	5.0
6	~4.0	24.5	500	0.5	5.0
7	~6.0	24.3	750	0.5	5.0
8	~8.0	24.0	1000	0.5	5.0

Table 2. Continuing Calibration Verification (CCV) Criteria

Average Recovery[a] (%)	Standard Deviation[b]	Warning Limits[c] (%)	Action Limits[d] (%)	Concentration Range (mg/L)
100	3.0	89.7 – 107	91 – 109	0.4 – 8.0

[a] average recovery for 140 CCVs
[b] standard deviation of recoveries for 140 CCVs
[c] average recovery plus or minus two times standard deviation
[d] average recovery plus or minus three times standard deviation

Table 3. Method Blank Summary Statistics

Sample Type	Average Absorbance	Range of Absorbances	N =
Method blanks	0.007	0.003 – 0.018	90

Table 4. Single Laboratory Precision

Range RPD	Average RPD	N =
<0.01 – 3.63	1.18	90

Table 5. Single Laboratory Accuracy

Average Recovery (%)	Standard Deviation	Relative Standard Deviation (%)	Range of Recoveries (%)	N =
94.3	5.8	6.2	78.5 – 105	90

Table 6. Calibration Curve Linearity

R-Squared Average	R-squared Range	RSD (%)	N =
0.9987	0.971 – 1.000	0.5	34

Figure 1. Typical Calibration Curve