



***Biomass Analysis Technology Team
Laboratory Analytical Procedure***

**DRAFT
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**Procedure Title: Determination of Sugars,
Byproducts, and Degradation Products in Liquid
Fraction Process Samples**

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1. Introduction

- 1.1 Carbohydrates make up a major portion of biomass samples. These carbohydrates are polysaccharides constructed primarily of glucose, xylose, arabinose, galactose, and mannose monomeric subunits. During certain pretreatments of biomass, a portion of these polysaccharides are hydrolyzed and soluble sugars are released into the liquid stream. This method is used to quantify the total amount of soluble carbohydrates released into solution as well as the amount of monomeric sugars released into solution. The soluble sugars in the liquid fraction of process samples can be quantified by HPLC with refractive index detection. If the sugars are present in oligomeric form further processing into their monomeric units is required prior to HPLC analysis.
- 1.2 The liquid portion may also contain carbohydrate degradation products, such as HMF and furfural, as well as other components of interest, such as organic acids and sugar alcohols. This method is used to measure the level of these degradation products and byproducts. These components are analyzed by HPLC with refractive index detection to determine optimal production process parameters or to monitor ongoing processes.
- 1.3 The concentrations of monomeric sugars (soluble monosaccharides) and cellobiose, total sugars (monosaccharides and oligosaccharides), as well as carbohydrate degradation products and sugar alcohols can be determined using this procedure. Monomeric sugars are quantified by HPLC with refractive index detection. Oligomeric sugars are converted into the monomeric form using acid hydrolysis and quantified by HPLC with refractive index detection. Byproducts and degradation products are quantified by HPLC with refractive index detection.

2. Scope

- 2.1 This procedure is used to characterize liquid process samples, including pretreatment liquors, liquid fermentation samples, and liquid fractions of process solids.
- 2.2 This procedure is appropriate for biomass containing the components listed throughout the procedure. Any biomass containing other interfering components (such as co-eluting constituents) must be further investigated.
- 2.3 All analyses should be performed in accordance with an appropriate laboratory specific Quality Assurance Plan (QAP).

3. Terminology

3.1 None

4. Significance and Use

4.1 This procedure is used to determine the composition of liquid fraction process samples. Other optional procedures can be used in conjunction with this procedure, including a measure of acid soluble lignin in LAP “Determination of Structural Carbohydrates and Lignin in Biomass”.

4.2 This procedure is used, in conjunction with other procedures to determine the chemical composition of biomass samples, see LAP “Summative Mass Closure for Biomass Samples”.

5. Interferences

5.1 When analyzing for carbohydrate degradation products and sugar alcohols, the following interferences should be noted:

5.1.1 Arabitol coelutes with xylitol. If the sample is thought to contain arabitol, the experimentally determined xylitol concentration should be flagged as potentially being biased high due to the suspected arabitol component.

5.1.2 Some samples may contain sorbitol, which elutes about a minute earlier than xylitol on the Aminex HPX-87H column, and will appear as a peak in between the xylose and arabinose peaks.

5.1.3 Some samples may contain glycerol, which elutes at the same time as formic acid on the Aminex HPX-87H column.

5.2 Certain guard columns for carbohydrate quantification may cause artifact peaks. Individual carbohydrates should be run on new columns and guard columns to verify the absence of artifact peaks.

6. Apparatus

6.1 Analytical balance, accurate to 0.1mg

6.2 pH meter, accurate to 0.01pH unit

6.3 Autoclave, suitable for autoclaving liquids, set to $121^{\circ} \pm 3^{\circ}\text{C}$

6.4 HPLC system equipped with refractive index detector and the following columns:

6.4.1 Biorad Aminex HPX-87P column (or equivalent) with ionic form $\text{H}^{+}/\text{CO}_3^{-}$ deashing guard column

6.4.2 Biorad Aminex HPX-87H column (or equivalent) with corresponding guard column

7. Reagents and materials

7.1 Reagents

7.1.1 High purity standards

7.1.1.1 D-cellobiose, D-(+)glucose, D-(+)xylose, D-(+)galactose, L-(+)arabinose, and D-(+)mannose

7.1.1.2 Xylitol, succinic acid, L-lactic acid, glycerol, acetic acid, ethanol, 5-hydroxy-2-furaldehyde (HMF), and furfural

7.1.2 Second set of high purity standards, as listed above, from a different source (manufacturer or lot), to be used to prepare calibration verification standards (CVS)

7.1.3 Sulfuric acid, concentrated, ACS reagent grade

7.1.4 Sulfuric acid, 72% w/w (specific gravity 1.6338 at 20°C)- (also commercially available as a reagent for the determination of fluorine, from Fluka #00647)

7.1.5 Calcium carbonate, ACS reagent grade

7.1.6 Water, HPLC grade, 0.2 μm filtered

7.2 Materials

7.2.1 Erlenmeyer flasks, 20 mL

7.2.2 Glass bottles, autoclave safe, crimp top, with rubber stoppers and aluminum seals to fit

7.2.3 pH paper (range 2-9)

7.2.4 Disposable syringes, 3 mL, fitted with 0.2 μm syringe filters

7.2.5 Autosampler vials with crimp top seals to fit

7.2.6 Volumetric pipets, class A, of appropriate sizes or corresponding pipettors

7.2.7 Volumetric flasks, class A, of appropriate sizes for standard and CVS dilution

7.2.8 Adjustable pipettors, covering ranges of 10 μl to 10 ml

8. ES&H Considerations and Hazards

8.1 Sulfuric acid is corrosive and should be handled with care

8.2 Follow all applicable NREL chemical handling procedures

9. Sampling, Test Specimens and Test Units

9.1 Vigorously shake or vortex the sample to suspend any entrained solids. Samples may be filtered prior to analysis if entrained solids are not of interest.

9.2 Care must be taken to ensure a representative sample is taken for analysis at each step.

When measuring volumes for analysis, the sample should be at room temperature.

9.3 Store samples in sealed containers so the volatile component concentration remains consistent. Samples should be stored in a refrigerator until ready to use.

10. Procedure

10.1 Measure and record the pH of each sample to the nearest 0.01 pH unit

10.2 Analyze the sample for byproducts and degradation products as follows

10.2.1 Prepare 0.01 N sulfuric acid for use as a HPLC mobile phase. In a 1L volumetric flask, add 278 μl concentrated sulfuric acid and bring to volume with HPLC grade water.

Filter through a 0.2 μm filter and degas before use. If concentrated sulfuric acid is not available, standardized 10 N sulfuric acid may also be used. 2.00 mL of 10 N sulfuric acid in a 2 L volumetric flask, brought to volume with HPLC grade water will also produce 0.01 N sulfuric acid.

10.2.2 Prepare a series of calibration standards containing the compounds that are to be quantified, referring to Table 1 for suggested concentration ranges. Use a four point calibration. If standards are prepared outside of the suggested ranges, the new range for these calibration curves must be validated. The linear range of HMF and furfural is limited by their solubility. Add these two components to the standards after the ethanol has been added to increase the HMF and furfural solubility. Filter the standard solutions through 0.2 μm filters into autosampler vials. Seal and label the vials.

10.2.2.1 The retention times of xylitol and succinic acid are close. Test the column to verify adequate peak separation and quantification. If adequate separation is not achieved, regenerate or replace the column and confirm improved separation.

10.2.2.2 A fresh set of standards is not required for every analysis. A large batch of standards may be produced, filtered through 0.2 μm filters into autosampler vials, sealed and labeled. The standards and CVS samples may be stored in a freezer and removed when needed. Thaw and vortex frozen standards prior to use. During every use, standards and CVS samples should be observed for unusual concentration behavior. Unusual concentrations may mean that the samples are compromised or volatile components have been lost. Assuming sufficient volume, standards and CVS samples

should not have more than 12 injections drawn from a single vial. In a chilled autosampler chamber, the lifetime of standards and CVS samples is approximately seven days.

10.2.2.3 Table 1- Suggested concentration ranges for 10.2.2 calibration standards

| Component | Approximate Retention time (min) | Suggested concentration range (mg/ml) |
|---------------|----------------------------------|---------------------------------------|
| Xylitol | 11.6 | 0.2 – 6.0 |
| Succinic acid | 12.0 | 0.2 – 10.0 |
| L-Lactic acid | 13.2 | 0.2 – 12.0 |
| Glycerol | 14.2 | 0.2 – 8.0 |
| Acetic acid | 15.5 | 0.2 – 12.0 |
| Ethanol | 22.7 | 1.0 - 15.0 |
| HMF | 29.4 | 0.02 – 5.0 |
| Furfural | 42.8 | 0.02 - 5.0 |
| CVS | - | Middle of linear range |

10.2.3 Prepare an independent calibration verification standard (CVS) for each set of calibration standards. Use reagents from a source or lot other than that used in preparing the calibration standards. Prepare the CVS at a concentration that falls in the middle of the validated range of the calibration curve. The CVS should be analyzed on the HPLC after each calibration set and at regular intervals throughout the sequence, bracketing groups of samples. The CVS is used to verify the quality and stability of the calibration curve(s) throughout the run.

10.2.4 Prepare the sample(s) for HPLC analysis by passing it through a 0.2 µm filter into an autosampler vial. Seal and label the vial. Prepare each sample in duplicate if desired. If an analyzed sample falls outside of the validated calibration range, dilute as needed and analyze the sample again. The concentrations should be corrected for dilution after running. See sections 11.1 and 11.2 for calculations.

10.2.5 Analyze the calibration standards, CVS, and samples by HPLC using a Biorad Aminex HPX-87H column.

HPLC conditions:

Sample volume: 10 - 25 µL, dependent on sample concentration and detector limits

Mobile phase: 0.01 N sulfuric acid, 0.2 µm filtered and degassed

Flow rate: 0.6 mL / minute

Column temperature: 55 – 65 °C

Detector temperature: as close to column temperature as possible

Detector: refractive index

Run time: 50 minutes

10.3 Analyze the sample for monomeric sugars and cellobiose as follows

10.3.1 Prepare a series of calibration standards containing the compounds that are to be quantified, referring to Table 2 for suggested concentration range. Use a four point calibration. If standards are prepared outside of the suggested ranges, the new range for these calibration curves must be validated.

10.3.2 Table 2- Suggested concentration ranges for 10.3.1 calibration standards

| Component | Suggested concentration range (mg/ml) |
|---------------|---|
| D-cellobiose | 1.2 – 24.0 |
| D(+)glucose | 1.2 – 24.0 |
| D(+)xylose | 1.2 – 24.0 |
| D(+)galactose | 1.2 – 24.0 |
| L(+)arabinose | 1.2 – 24.0 |
| D(+)mannose | 1.2 – 24.0 |
| CVS | Middle of linear range, concentration not equal to a calibration point (12.0 suggested) |

- 10.3.3 A fresh set of standards is not required for every analysis. A large batch of standards may be produced, filtered through 0.2 µm filters into autosampler vials, sealed and labeled. The standards and CVS samples may be stored in a freezer and removed when needed. Thaw and vortex frozen standards prior to use. During every use, standards and CVS samples should be observed for unusual concentration behavior. Unusual concentrations may mean that the samples are compromised or volatile components have been lost. Assuming sufficient volume, standards and CVS samples should not have more than 12 injections drawn from a single vial. In a chilled autosampler chamber, the lifetime of standards and CVS samples is approximately three to four days.
- 10.3.4 Prepare an independent calibration verification standard (CVS) for each set of calibration standards. Use reagents from a source or lot other than that used in preparing the calibration standards. Prepare the CVS at a concentration that falls in the middle of the validated range of the calibration curve.. The CVS should be analyzed on the HPLC after each calibration set and at regular intervals throughout the sequence, bracketing groups of samples. The CVS is used to verify the quality and stability of the calibration curve(s) throughout the run.
- 10.3.5 Measure and record pH or refer to step 10.1 for pH measurement. If the pH is less than 5, use calcium carbonate to neutralize an aliquot (10 mL is recommended) of each sample in an Erlenmeyer flask. Neutralize to pH 5 – 6. Avoid neutralizing to a pH greater than 6 by monitoring with pH paper. Add the calcium carbonate slowly upon reaching a pH of 4. Swirl the sample frequently. After reaching pH 5 – 6, allow the sample to settle and decant off the clear liquid. The pH of the liquid after settling will be approximately 7. Samples with a pH greater than 9 cannot be analyzed using the HPX-87P column.
- 10.3.6 Prepare the sample for HPLC analysis by passing the decanted liquid through a 0.2 µm filter into an autosampler vial. Seal and label the vial.. Prepare each sample in duplicate if desired. If it is suspected that the sample concentrations may exceed the calibration range, dilute the samples as needed, recording the dilution. The concentrations should be corrected for dilution after running. If necessary, neutralized samples may be stored in the refrigerator for three or four days. After this time, the samples should be considered compromised.

10.3.7 Analyze the calibration standards, CVS, and samples by HPLC using a Biorad Aminex HPX-87P column equipped with the appropriate guard column.

HPLC conditions:

Injection volume: 10 – 50 μ L, dependent on concentration and detector limits

Mobile phase: HPLC grade water, 0.2 μ m filtered and degassed

Flow rate: 0.6 mL / minute

Column temperature: 80 - 85°C

Detector temperature: as close to column temperature as possible

Detector: refractive index

Run time: 20 minute data collection plus 15 minute post run (with possible adjustment for later eluting compounds)

See sections 11.1 and 11.2 for calculations.

10.4 Analyze the sample for total sugar content (monosaccharides and oligosaccharides)

10.4.1 Refer to steps 10.3.1 through 10.3.4 for preparation of calibration standards and CVS samples. It is often useful to combine the analyses from 10.3 and 10.4 into one HPLC sequence.

10.4.2 Pipette duplicate representative aliquots of sample into autoclave safe, crimp top, glass bottles. Aliquots of 5.0, 10.0, or 20.0 mL may be used, depending on available sample volume.

10.4.3 Measure and record the pH of the sample of refer to step 10.1 for pH measurement. Based on sample pH, calculate the amount of 72% w/w sulfuric acid required to bring the acid concentration of each aliquot to 4% (refer to section 11.3 for example calculations and section 15.1 for a quick reference sheet). Add the required amount of acid while swirling the sample. Stopper the bottles and crimp aluminum seals into place. Using a permanent marker, label the aluminum seals with sample identification.

10.4.4 Prepare a set of sugar recovery standards (SRS) that will be taken through the analysis and used to correct for losses due to decomposition of sugars during dilute acid hydrolysis. Refer to Table 3 for SRS concentration suggestions. SRS concentrations should be chosen to most closely resemble the concentrations of sugars in the sample. Weigh out the required amounts of each sugar, to the nearest 0.1 mg, and transfer to a crimp top bottle. Add 10.0 mL HPLC grade water.

10.4.5 Table 3- Suggested concentrations for 10.4.4 sugar recovery standards

| SRS type | Sugar concentrations (mg / mL) | | | | |
|----------|--------------------------------|--------|-----------|-----------|---------|
| | glucose | xylose | galactose | arabinose | mannose |
| High | 40 | 100 | 20 | 20 | 10 |
| Meduim | 20 | 50 | 10 | 10 | 5 |
| Low | 4 | 10 | 2 | 2 | 1 |

10.4.6 Add the appropriate amount of 72% sulfuric acid to each sugar recovery standard (refer to section 11.3 for example calculations). For a starting pH of 7, the amount of 72% sulfuric acid needed will be 348.5 μ L. Stopper the bottles and crimp aluminum seals into place. Using a permanent marker, clearly label the aluminum seals with sample identification.

10.4.7 A fresh SRS is not required for every analysis. A large batch of sugar recovery standards may be produced, filtered through 0.2 μ m filters, dispensed in 10.0 mL

aliquots into sealed containers, and labeled. They may be stored in a freezer and removed when needed. Thaw and vortex the frozen SRS prior to use. If frozen SRS are used, the appropriate amount of acid must be added to the thawed sample and vortexed prior to transferring to a glass crimp top bottle.

- 10.4.8 Autoclave the sealed samples and sugar recovery standards for one hour at 121°C, usually the liquids setting. After completion of the autoclave cycle, allow the hydrolyzates to slowly cool to near room temperature before removing the seals and stoppers.
- 10.4.9 Use calcium carbonate to neutralize each sample to pH 5 – 6. Avoid neutralizing to a pH greater than 6 by monitoring with pH paper. Add the calcium carbonate slowly upon reaching a pH of 4. Swirl the sample frequently. After reaching pH 5 – 6, allow the sample to settle and decant off the clear liquid. The pH of the liquid after settling will be approximately 7.
- 10.4.10 Repeat steps 10.3.6 and 10.3.7, analyzing calibration standards, CVS, SRS, and samples. Refer to sections 11.1, 11.2, 11.4, and 11.5 for calculations.

10.5 Analyze the sample for acid soluble lignin content

- 10.5.1 See section 10.3 in LAP “Determination of Structural Carbohydrates and Lignin in Biomass” for a method for determining acid soluble lignin. Filter the liquor prior to this analysis if necessary.

11. Calculations

- 11.1 Create a calibration curve for each analyte to be quantified using linear regression. From these curves, determine the concentration in mg/mL of each component present in the samples analyzed by HPLC, correcting for dilution if required.
- 11.2 Calculate and record the amount of each calibration verification standard (CVS) recovered following HPLC analysis.

$$\% \text{ CVS recovery} = \frac{\text{conc. detected by HPLC, mg/mL}}{\text{known conc. of standard, mg/mL}} \times 100$$

- 11.3 For each sample and standard, calculate the volume of 72% sulfuric acid required to bring the acid concentration to a 4% final acid concentration. The molar concentration of hydrogen ions, [H⁺], in a sample, can be calculated from the pH as follows,
pH = -log[H⁺], therefore, [H⁺] = antilog(-pH).

The volume of 72% sulfuric acid needed is then calculated from the following equation:

$$V_{72\%} = \frac{[(C_{4\%} \times V_s) - (V_s \times [H^+] \times 98.08 \text{g } H_2SO_4 / 2 \text{ moles } H^+)]}{C_{72\%}}$$

Where:

$V_{72\%}$ is the volume of 72% acid to be added, in mL
 V_s is the initial volume of sample or standard, in mL,
 $C_{4\%}$ is the concentration of 4% w/w H₂SO₄, 41.0 g/L
 $C_{72\%}$ is the concentration of 72% w/w H₂SO₄, 1176.3 g/L
 $[H^+]$ is the concentration of hydrogen ions, in moles/L

Example #1: Calculate the amount of 72% H₂SO₄ needed to prepare a sample with a pH of 2.41 for 4% acid hydrolysis. If the pH is 2.41, then [H⁺]=0.00389 M. Therefore:

$$\frac{[(41.0 \text{ g/L}) \times (20 \text{ mL}) - (20 \text{ mL}) \times (0.00389 \text{ moles/L}) \times (98.08 \text{ g/2 moles})]}{1176.3 \text{ g/L}} = 0.694 \text{ mL}$$

Example #2: Calculate the amount of 72% H₂SO₄ needed to prepare a sugar recovery standard for 4% acid hydrolysis, assuming that the pH of the water is 7. If the pH is 7, [H⁺]=0.0000001 M, a value small enough to be ignored in the following calculation.

$$(41.0 \text{ g/L} \times 20 \text{ mL}) / 1176.3 \text{ g/L} = 0.697 \text{ mL}$$

Also reference Appendix 1 for a quick reference sheet.

- 11.4 For the sugar recovery standards (SRS), calculate the amount of each component sugar recovered after dilute acid hydrolysis, accounting for any dilution made prior to HPLC analysis. Average any replicate (%R_{sugar}) values obtained for each individual sugar and report %R_{avg, sugar}.

$$\% R_{sugar} = \frac{\text{conc. detected by HPLC, mg/mL}}{\text{known conc. of sugar before hydrolysis, mg/mL}} \times 100$$

- 11.5 Use the percent hydrolyzed sugar recovery values calculated in step 11.3 to correct the corresponding sugar concentration values obtained by HPLC for each of the hydrolyzed samples (C_{cor. sample}), accounting for any dilution made prior to HPLC analysis.

$$C_x = \frac{C_{HPLC} \times \text{dilution factor}}{\% R_{ave. sugar} / 100}$$

Where:
 C_{HPLC} = conc. of a sugar as determined by HPLC, mg/mL.
 % R_{ave. sugar} = average recovery of a specific SRS component.
 C_x = C_{cor. sample}, concentration in mg/mL of a sugar in the hydrolyzed sample after correction for loss on 4% hydrolysis.

- 11.6 To report or calculate the relative percent difference (RPD) between two samples, use the following calculation

$$RPD = \left(\frac{(X_1 - X_2)}{X_{mean}} \right) \times 100$$

Where:
 X₁ and X₂ = measured values
 X_{mean} = the mean of X₁ and X₂

- 11.7 To report or calculate the root mean square deviation (RMS deviation) or the standard deviation (st dev) of the samples, use the following calculations.
 First find the root mean square (RMS), of the sample using

$$RMS = x_m = mean = \sqrt{\left(\frac{\sum_{i=1}^n x_i}{n}\right)^2}$$

Then find the root mean square deviation, or standard deviation, using

$$RMSdeviation = \sigma = stdev = \sqrt{\frac{\sum_{i=1}^n (x_i - x_m)^2}{n}}$$

Where:

x_m =the root mean square of all x values in the set

n =number of samples in set

x_i =a measured value from the set

12. Report Format

12.1 Report concentrations, in mg / mL, of analytes of interest. RPD and RMS deviation may also be reported.

13. Precision and Bias

13.1 Determined by data quality objectives and laboratory specific Quality Assurance Plan.

14. Quality Control

14.1 Reported Significant Figures or Decimal Places: Determined by data quality objectives and laboratory specific Quality Assurance Plan.

14.2 Replicates: Analyze for monomeric sugars and cellobiose in duplicate. All other analyses may be run in duplicate if desired.

14.3 Blank: An optional instrument blank may be run, using HPLC grade water analyzed by HPLC in the same manner as a sample.

14.4 Relative percent difference criteria: Determined by data quality objectives and laboratory specific Quality Assurance Plan.

14.5 Calibration verification standard: Calibration verification standards should be independently prepared and analyzed as described in the procedure section.

14.6 Sample size: 15 – 80 mL (50 mL minimum recommended)

14.7 Sample storage: Store samples in sealed containers so the volatile component concentration remains consistent. Samples should be stored in a refrigerator. If necessary, neutralized samples may be stored in a refrigerator for three to four days. After this time the samples should be considered compromised. Neutralized samples may need to be refiltered after storage in a refrigerator if precipitate is visible.

14.8 Standard storage: The standards should be stored in a freezer and removed when needed. Thaw and vortex frozen standards prior to use.

14.9 Standard preparation: Standards should be prepared as described in the procedure.

14.10 Definition of a batch: Any number of samples which are analyzed and recorded together. Samples within a batch should be of the same matrix. The maximum size of a batch will

be limited by equipment constraints.

14.11 Control charts: All CVS and SRS recoveries should be control charted.

15. Appendices

15.1 See Appendix 1 at the end of the document for a quick reference sheet for calculation 11.3.

16. References

- 16.1 Ehrman, C.I., and M.E. Himmel. 1994. "Simultaneous Saccharification and Fermentation of Pretreated Biomass: Improving Mass Balance Closure." *Biotechnology Techniques*, 8(2):99-104.
- 16.2 NREL Ethanol Project CAT Task Laboratory Analytical Procedure #013, "HPLC Analysis of Liquid Fractions of Process Samples for Monomeric Sugars and Cellobiose".
- 16.3 NREL Ethanol Project CAT Task Laboratory Analytical Procedure #014, "Dilute Acid Hydrolysis Procedure for Determination of Total Sugars in the Liquid Fraction of Process Samples".
- 16.4 NREL Ethanol Project CAT Task Laboratory Analytical Procedure #015, "HPLC Analysis of Liquid Fractions of Process Samples for Byproducts and Degradation Products".

Volume of 72% H2SO4 (in uL) required to bring liquor acid concentration to 4%

| pH | Vol. of liquor (mL) | | | pH | Vol. of liquor (mL) | | | pH | Vol. of liquor (mL) | | | pH | Vol. of liquor (mL) | | | pH | Vol. of liquor (mL) | | | pH | Vol. of liquor (mL) | | | pH | Vol. of liquor (mL) | | | | | | | | | | | | | | |
|------|---------------------|-------|-------|------|---------------------|-------|-------|------|---------------------|-------|-------|------|---------------------|-------|-------|------|---------------------|-------|-------|------|---------------------|-------|-------|------|---------------------|-------|-------|------|-----|-----|-----|------|-----|-----|-----|------|-----|-----|-----|
| | 5.00 | 10.00 | 20.00 | | 5.00 | 10.00 | 20.00 | | 5.00 | 10.00 | 20.00 | | 5.00 | 10.00 | 20.00 | | 5.00 | 10.00 | 20.00 | | 5.00 | 10.00 | 20.00 | | 5.00 | 10.00 | 20.00 | | | | | | | | | | | | |
| 0.01 | * | * | * | 0.50 | 108 | 217 | 433 | 1.0 | 153 | 307 | 614 | 1.50 | 168 | 335 | 671 | 2.0 | 172 | 344 | 689 | 2.50 | 174 | 347 | 694 | 3.0 | 174 | 348 | 696 | 3.50 | 174 | 348 | 697 | 4.0 | 174 | 349 | 697 | 4.50 | 174 | 349 | 697 |
| 0.02 | * | * | * | 0.51 | 110 | 220 | 439 | 1.01 | 154 | 308 | 616 | 1.51 | 168 | 336 | 671 | 2.01 | 172 | 344 | 689 | 2.51 | 174 | 347 | 695 | 3.01 | 174 | 348 | 696 | 3.51 | 174 | 348 | 697 | 4.01 | 174 | 349 | 697 | 4.51 | 174 | 349 | 697 |
| 0.03 | * | * | * | 0.52 | 111 | 223 | 445 | 1.02 | 154 | 309 | 617 | 1.52 | 168 | 336 | 672 | 2.02 | 172 | 345 | 689 | 2.52 | 174 | 347 | 695 | 3.02 | 174 | 348 | 696 | 3.52 | 174 | 348 | 697 | 4.02 | 174 | 349 | 697 | 4.52 | 174 | 349 | 697 |
| 0.04 | * | * | * | 0.53 | 113 | 226 | 451 | 1.03 | 155 | 310 | 619 | 1.53 | 168 | 336 | 672 | 2.03 | 172 | 345 | 689 | 2.53 | 174 | 347 | 695 | 3.03 | 174 | 348 | 696 | 3.53 | 174 | 348 | 697 | 4.03 | 174 | 349 | 697 | 4.53 | 174 | 349 | 697 |
| 0.05 | * | * | * | 0.54 | 114 | 228 | 457 | 1.04 | 155 | 311 | 621 | 1.54 | 168 | 337 | 673 | 2.04 | 172 | 345 | 689 | 2.54 | 174 | 347 | 695 | 3.04 | 174 | 348 | 696 | 3.54 | 174 | 348 | 697 | 4.04 | 174 | 349 | 697 | 4.54 | 174 | 349 | 697 |
| 0.06 | * | * | * | 0.55 | 116 | 231 | 462 | 1.05 | 156 | 311 | 623 | 1.55 | 168 | 337 | 674 | 2.05 | 172 | 345 | 690 | 2.55 | 174 | 347 | 695 | 3.05 | 174 | 348 | 696 | 3.55 | 174 | 348 | 697 | 4.05 | 174 | 349 | 697 | 4.55 | 174 | 349 | 697 |
| 0.07 | * | * | * | 0.56 | 117 | 234 | 467 | 1.06 | 156 | 312 | 624 | 1.56 | 169 | 337 | 674 | 2.06 | 172 | 345 | 690 | 2.56 | 174 | 347 | 695 | 3.06 | 174 | 348 | 696 | 3.56 | 174 | 348 | 697 | 4.06 | 174 | 349 | 697 | 4.56 | 174 | 349 | 697 |
| 0.08 | 1 | 2 | 4 | 0.57 | 118 | 236 | 473 | 1.07 | 157 | 313 | 626 | 1.57 | 169 | 337 | 675 | 2.07 | 173 | 345 | 690 | 2.57 | 174 | 347 | 695 | 3.07 | 174 | 348 | 696 | 3.57 | 174 | 348 | 697 | 4.07 | 174 | 349 | 697 | 4.57 | 174 | 349 | 697 |
| 0.09 | 5 | 10 | 19 | 0.58 | 119 | 239 | 478 | 1.08 | 157 | 314 | 628 | 1.58 | 169 | 338 | 675 | 2.08 | 173 | 345 | 690 | 2.58 | 174 | 347 | 695 | 3.08 | 174 | 348 | 696 | 3.58 | 174 | 348 | 697 | 4.08 | 174 | 349 | 697 | 4.58 | 174 | 349 | 697 |
| 0.10 | 9 | 17 | 35 | 0.59 | 121 | 241 | 483 | 1.09 | 157 | 315 | 629 | 1.59 | 169 | 338 | 676 | 2.09 | 173 | 345 | 690 | 2.59 | 174 | 347 | 695 | 3.09 | 174 | 348 | 696 | 3.59 | 174 | 348 | 697 | 4.09 | 174 | 349 | 697 | 4.59 | 174 | 349 | 697 |
| 0.11 | 12 | 25 | 50 | 0.60 | 122 | 244 | 488 | 1.10 | 158 | 315 | 631 | 1.60 | 169 | 338 | 676 | 2.10 | 173 | 345 | 690 | 2.60 | 174 | 348 | 695 | 3.10 | 174 | 348 | 696 | 3.60 | 174 | 348 | 697 | 4.10 | 174 | 349 | 697 | 4.60 | 174 | 349 | 697 |
| 0.12 | 16 | 32 | 65 | 0.61 | 123 | 246 | 492 | 1.11 | 158 | 316 | 632 | 1.61 | 169 | 338 | 677 | 2.11 | 173 | 345 | 691 | 2.61 | 174 | 348 | 695 | 3.11 | 174 | 348 | 696 | 3.61 | 174 | 348 | 697 | 4.11 | 174 | 349 | 697 | 4.61 | 174 | 349 | 697 |
| 0.13 | 20 | 39 | 79 | 0.62 | 124 | 249 | 497 | 1.12 | 158 | 317 | 634 | 1.62 | 169 | 339 | 677 | 2.12 | 173 | 345 | 691 | 2.62 | 174 | 348 | 695 | 3.12 | 174 | 348 | 696 | 3.62 | 174 | 348 | 697 | 4.12 | 174 | 349 | 697 | 4.62 | 174 | 349 | 697 |
| 0.14 | 23 | 47 | 93 | 0.63 | 125 | 251 | 502 | 1.13 | 159 | 318 | 635 | 1.63 | 169 | 339 | 678 | 2.13 | 173 | 345 | 691 | 2.63 | 174 | 348 | 695 | 3.13 | 174 | 348 | 696 | 3.63 | 174 | 348 | 697 | 4.13 | 174 | 349 | 697 | 4.63 | 174 | 349 | 697 |
| 0.15 | 27 | 53 | 107 | 0.64 | 127 | 253 | 506 | 1.14 | 159 | 318 | 637 | 1.64 | 169 | 339 | 678 | 2.14 | 173 | 346 | 691 | 2.64 | 174 | 348 | 695 | 3.14 | 174 | 348 | 696 | 3.64 | 174 | 348 | 697 | 4.14 | 174 | 349 | 697 | 4.64 | 174 | 349 | 697 |
| 0.16 | 30 | 60 | 120 | 0.65 | 128 | 255 | 510 | 1.15 | 160 | 319 | 638 | 1.65 | 170 | 339 | 678 | 2.15 | 173 | 346 | 691 | 2.65 | 174 | 348 | 695 | 3.15 | 174 | 348 | 697 | 3.65 | 174 | 348 | 697 | 4.15 | 174 | 349 | 697 | 4.65 | 174 | 349 | 697 |
| 0.17 | 33 | 67 | 133 | 0.66 | 129 | 257 | 515 | 1.16 | 160 | 320 | 639 | 1.66 | 170 | 339 | 679 | 2.16 | 173 | 346 | 691 | 2.66 | 174 | 348 | 695 | 3.16 | 174 | 348 | 697 | 3.66 | 174 | 348 | 697 | 4.16 | 174 | 349 | 697 | 4.66 | 174 | 349 | 697 |
| 0.18 | 37 | 73 | 146 | 0.67 | 130 | 259 | 519 | 1.17 | 160 | 320 | 641 | 1.67 | 170 | 340 | 679 | 2.17 | 173 | 346 | 691 | 2.67 | 174 | 348 | 695 | 3.17 | 174 | 348 | 697 | 3.67 | 174 | 348 | 697 | 4.17 | 174 | 349 | 697 | 4.67 | 174 | 349 | 697 |
| 0.19 | 40 | 79 | 159 | 0.68 | 131 | 261 | 523 | 1.18 | 161 | 321 | 642 | 1.68 | 170 | 340 | 680 | 2.18 | 173 | 346 | 692 | 2.68 | 174 | 348 | 695 | 3.18 | 174 | 348 | 697 | 3.68 | 174 | 348 | 697 | 4.18 | 174 | 349 | 697 | 4.68 | 174 | 349 | 697 |
| 0.20 | 43 | 86 | 171 | 0.69 | 132 | 263 | 527 | 1.19 | 161 | 322 | 643 | 1.69 | 170 | 340 | 680 | 2.19 | 173 | 346 | 692 | 2.69 | 174 | 348 | 695 | 3.19 | 174 | 348 | 697 | 3.69 | 174 | 348 | 697 | 4.19 | 174 | 349 | 697 | 4.69 | 174 | 349 | 697 |
| 0.21 | 46 | 91 | 183 | 0.71 | 134 | 267 | 535 | 1.21 | 161 | 323 | 646 | 1.71 | 170 | 340 | 681 | 2.21 | 173 | 346 | 692 | 2.71 | 174 | 348 | 695 | 3.21 | 174 | 348 | 697 | 3.71 | 174 | 348 | 697 | 4.21 | 174 | 349 | 697 | 4.71 | 174 | 349 | 697 |
| 0.22 | 49 | 97 | 195 | 0.72 | 135 | 269 | 538 | 1.22 | 162 | 323 | 647 | 1.72 | 170 | 341 | 681 | 2.22 | 173 | 346 | 692 | 2.72 | 174 | 348 | 695 | 3.22 | 174 | 348 | 697 | 3.72 | 174 | 348 | 697 | 4.22 | 174 | 349 | 697 | 4.72 | 174 | 349 | 697 |
| 0.23 | 52 | 103 | 206 | 0.73 | 135 | 271 | 542 | 1.23 | 162 | 324 | 648 | 1.73 | 170 | 341 | 682 | 2.23 | 173 | 346 | 692 | 2.73 | 174 | 348 | 695 | 3.23 | 174 | 348 | 697 | 3.73 | 174 | 348 | 697 | 4.23 | 174 | 349 | 697 | 4.73 | 174 | 349 | 697 |
| 0.24 | 54 | 109 | 217 | 0.74 | 136 | 273 | 545 | 1.24 | 162 | 325 | 649 | 1.74 | 170 | 341 | 682 | 2.24 | 173 | 346 | 692 | 2.74 | 174 | 348 | 695 | 3.24 | 174 | 348 | 697 | 3.74 | 174 | 348 | 697 | 4.24 | 174 | 349 | 697 | 4.74 | 174 | 349 | 697 |
| 0.25 | 57 | 114 | 228 | 0.75 | 137 | 274 | 549 | 1.25 | 163 | 325 | 650 | 1.75 | 171 | 341 | 682 | 2.25 | 173 | 346 | 692 | 2.75 | 174 | 348 | 695 | 3.25 | 174 | 348 | 697 | 3.75 | 174 | 348 | 697 | 4.25 | 174 | 349 | 697 | 4.75 | 174 | 349 | 697 |
| 0.26 | 60 | 119 | 239 | 0.76 | 138 | 276 | 552 | 1.26 | 163 | 326 | 651 | 1.76 | 171 | 341 | 683 | 2.26 | 173 | 346 | 693 | 2.76 | 174 | 348 | 695 | 3.26 | 174 | 348 | 697 | 3.76 | 174 | 348 | 697 | 4.26 | 174 | 349 | 697 | 4.76 | 174 | 349 | 697 |
| 0.27 | 62 | 125 | 249 | 0.77 | 139 | 278 | 556 | 1.27 | 163 | 326 | 652 | 1.77 | 171 | 341 | 683 | 2.27 | 173 | 346 | 693 | 2.77 | 174 | 348 | 695 | 3.27 | 174 | 348 | 697 | 3.77 | 174 | 348 | 697 | 4.27 | 174 | 349 | 697 | 4.77 | 174 | 349 | 697 |
| 0.28 | 65 | 130 | 260 | 0.78 | 140 | 279 | 559 | 1.28 | 163 | 327 | 653 | 1.78 | 171 | 342 | 683 | 2.28 | 173 | 346 | 693 | 2.78 | 174 | 348 | 695 | 3.28 | 174 | 348 | 697 | 3.78 | 174 | 348 | 697 | 4.28 | 174 | 349 | 697 | 4.78 | 174 | 349 | 697 |
| 0.29 | 67 | 135 | 269 | 0.79 | 140 | 281 | 562 | 1.29 | 164 | 327 | 654 | 1.79 | 171 | 342 | 684 | 2.29 | 173 | 346 | 693 | 2.79 | 174 | 348 | 695 | 3.29 | 174 | 348 | 697 | 3.79 | 174 | 348 | 697 | 4.29 | 174 | 349 | 697 | 4.79 | 174 | 349 | 697 |
| 0.30 | 70 | 140 | 279 | 0.80 | 141 | 282 | 565 | 1.30 | 164 | 328 | 655 | 1.80 | 171 | 342 | 684 | 2.30 | 173 | 346 | 693 | 2.80 | 174 | 348 | 695 | 3.30 | 174 | 348 | 697 | 3.80 | 174 | 348 | 697 | 4.30 | 174 | 349 | 697 | 4.80 | 174 | 349 | 697 |
| 0.31 | 72 | 144 | 289 | 0.81 | 142 | 284 | 568 | 1.31 | 164 | 328 | 656 | 1.81 | 171 | 342 | 684 | 2.31 | 173 | 347 | 693 | 2.81 | 174 | 348 | 695 | 3.31 | 174 | 348 | 697 | 3.81 | 174 | 348 | 697 | 4.31 | 174 | 349 | 697 | 4.81 | 174 | 349 | 697 |
| 0.32 | 75 | 149 | 298 | 0.82 | 143 | 285 | 571 | 1.32 | 164 | 329 | 657 | 1.82 | 171 | 342 | 684 | 2.32 | 173 | 347 | 693 | 2.82 | 174 | 348 | 695 | 3.32 | 174 | 348 | 697 | 3.82 | 174 | 348 | 697 | 4.32 | 174 | 349 | 697 | 4.82 | 174 | 349 | 697 |
| 0.33 | 77 | 154 | 307 | 0.83 | 143 | 287 | 574 | 1.33 | 165 | 329 | 658 | 1.83 | 171 | 342 | 685 | 2.33 | 173 | 347 | 693 | 2.83 | 174 | 348 | 695 | 3.33 | 174 | 348 | 697 | 3.83 | 174 | 348 | 697 | 4.33 | 174 | 349 | 697 | 4.83 | 174 | 349 | 697 |
| 0.34 | 79 | 158 | 316 | 0.84 | 144 | 288 | 577 | 1.34 | 165 | 329 | 659 | 1.84 | 171 | 343 | 685 | 2.34 | 173 | 347 | 693 | 2.84 | 174 | 348 | 695 | 3.34 | 174 | 348 | 697 | 3.84 | 174 | 348 | 697 | 4.34 | 174 | 349 | 697 | 4.84 | 174 | 349 | 697 |
| 0.35 | 81 | 162 | 325 | 0.85 | 145 | 290 | 579 | 1.35 | 165 | 330 | 660 | 1.85 | 171 | 343 | 685 | 2.35 | 173 | 347 | 693 | 2.85 | 174 | 348 | 695 | 3.35 | 174 | 348 | 697 | 3.85 | 174 | 348 | 697 | 4.35 | 174 | 349 | 697 | 4.85 | 174 | 349 | 697 |
| 0.36 | 83 | 167 | 333 | 0.86 | 146 | 291 | 582 | 1.36 | 165 | 330 | 661 | 1.86 | 171 | 343 | 686 | 2.36 | 173 | 347 | 693 | 2.86 | 174 | 348 | 695 | 3.36 | 174 | 348 | 697 | 3.86 | 174 | 348 | 697 | | | | | | | | |