ION LABS	Standard Operating Procedure **Quantification of Total Polyphenols (Folin-Ciocalteu Method) by Visible Light Spectroscopy**	**SOP Number** **D-791**	**Revision** **1**
		Effective Date 03/31/23	**Page** **Page 1 of 5**

Written by/ Date	**Reviewed by/ Date**	**Approved by/ Date**
03/14/23	SAS 03/16/23	03/29/23
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1.0 Purpose

This document describes the analytical procedure for the quantification of total polyphenols in raw materials and finished products.

2.0 Scope

This procedure applies to the quantification of total polyphenols in raw materials and finished products. Folin-Ciocalteu reagent reacts with phenols (as well as nonphenolic reducing substances) to form chromogens that can be detected spectrophotometrically. The method is by nature non-specific, and expresses total polyphenol content as equivalents gallic acid. This method was validated under Protocol MV-LAB-19-182.

3.0 Responsibility

3.1 It is the responsibility of QC and Analytical chemists who have verified their ability to execute this procedure to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development personnel to keep this procedure current with the associated monographs and laboratory practices.

4.0 Definitions

4.1 **QC** – Quality Control

4.2 **FCR** – Folin-Ciocalteu Reagent

5.0 References

5.1 MV-LAB-19-182, Protocol, Quantification of Total Polyphenols (Folin-Ciocalteu Method) by Visible Light Spectroscopy

5.2 Naturex, Inc. Test Method CQ-MO-232 Quantification of Total Polyphenols (Folin-Ciocalteu Method) by Spectrophotometry, Version H, 11-07-19

6.0 Supplies

6.1 Chemicals: (Use reagent grade or better.)

6.1.1 Milli-Q Water

6.1.2 Sodium Carbonate, Anhy. (Sigma-Aldrich S2127 or Equiv.)

6.1.3 Folin-Ciocalteu Reagent (Sigma-Aldrich 47641 or Equiv.)

6.1.4 1000 ppm Gallic Acid Standard (Ricca Chemical R3224100 or Equiv.)

6.2 Supplies and Glassware

6.2.1 Polystyrene (PS) Cuvettes (Micro or Semi-Micro)

6.2.2 Volumetric Glassware

6.2.3 Adjustable Pipettes & Tips

6.2.3 Weigh Paper & Boats

6.2.4 10 ml Polypropylene Syringes

6.2.5 17mm x 0.45u Nylon Syringe Filters

6.2.6 15 mL Polypropylene Centrifuge Tubes

6.2.7 22 mL Glass Scintillation Vials

6.3 Equipment

6.3.1 UV/Vis Spectrophotometer

6.3.2 Analytical Balance

6.3.3 Sonicator Bath

6.3.4 Wrist Action Shaker

6.3.5 Vortex Mixer

6.3.6 Heated Water Bath

6.3.7 Thermometer

6.3.8 Timer

7.0 Procedure

7.1 Instrument, Water Baths & Sodium Carbonate Solution Preparations

7.1.1 Turn on the spectrophotometer and allow to initialize. Open the PerkinElmer WinLab software and log in. Click on Instruments in the Folder List, then click Manual Control in the uppermost navigation ribbon. In Settings, enter 755 nm in the Wavelength dialog box. Leave the remaining settings unchanged at the default values. Click the Apply button and let the instrument warm up for ~30min.

7.1.2 Select two beakers, each being large enough to accommodate the number of centrifuge tubes required to contain the blank, standards and samples. Fill ~2/3 full with DI water. Cover one with aluminum foil and refrigerate. Place the other in a 55°C water bath.

7.1.3 Dilute 12.5 g of sodium carbonate to volume in a 100 mL volumetric flask. Shake and/or sonicate until fully dissolved. (Preparations may be scaled as necessary.)

7.2 Standard Prep

7.2.1 Transfer 400, 600, 800, 1000 & 1200 uL aliquots of the 1000 mg/L gallic acid reference standard into individual 10 mL volumetric flasks. Dilute to volume with water and mix well.

7.3 Sample Extraction

7.3.1 The validated range for the analytical method is 40.0 – 120.0 mg/L.

7.3.2 Given the raw material assay value / finished product label claim, extract the sample at a concentration of 50 mg/L (expected total polyphenols) in a 100 mL volumetric flask.

7.3.3 Prepare the sample as follows: to the calculated mass of sample transferred to a 100 mL volumetric flask, add 50 mL of water and thoroughly wet the material with the aid of a vortex mixer for several minutes. Next, shake mechanically for 15 minutes, then dilute to volume with water and mix thoroughly. Finally, sonicate for 20 minutes, mix thoroughly and allow to cool to ambient temperature.

7.4 Chromogen Formation Reaction

7.4.1 Assemble and run the reactions in the 15 mL centrifuge tubes as indicated in the table below:

	Blank	Standard	Sample
Water, mL	5.333	5	5
Standard Prep, uL	---	333	---
Sample Prep, uL	---	---	333
FCR, uL	333	333	333
Vortex mix then let stand at ambient temperature for 5 minutes.			
12.5 % Sodium Carbonate, mL	1	1	1
Vortex mix then incubate at 55°C for 15 minutes. Immediately transfer to the cold water bath for 3-5 minutes. Vortex mix then filter via syringe, sending the first 2 mL to waste before collecting the balance of the reaction solution into a scintillation vial.			

7.5 Absorbance Readings

7.5.1 Transfer the blank reaction filtrate to a cuvette. Orient at the 1 cm pathlength in position 1 of the sample holder.

7.5.2 Click the Actions tab then select Autozero. Click OK in the UV WinLab dialog box when prompted in order to zero the instrument on the blank.

7.5.3 Record a single absorbance reading for each of the standard and sample preparations.

7.6 System Suitability Requirements

7.6.1 Graph the standard concentration versus the absorbance and obtain the regression line equation and correlation coefficient (r^2).

7.6.2 The assay is considered valid if $r^2 \geq 0.99$.

7.7 Example Calculations for Raw Material % Assay

7.7.1 Sample Concentration, mg/L = Sample Mass, mg (Corr. for Water) / 0.100 L

7.7.2 Gallic Acid Eq's in Sample, mg/L = (Absorbance – Intercept)/Slope

7.7.3 Total Polyphenols, % = (Gallic Acid Eq's in Sample, mg/L / Sample Concentration, mg/L) * 100

7.8 Example Calculation for Finished Product % Label Claim

7.8.1 Label Claim, % = (% Assay/100 * Serving Size, mg)/ Label Claim, mg) * 100

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	11/27/19	New	N/A	C. Perry
1	03/16/23	Scheduled review: update logo and format.	CC-23-0143	K. Burris