| ION LABS | Standard Operating Procedure<br>**Quantification of Procyanidins (Acid Butanol Method) by Visible Light Spectroscopy** | **SOP Number**<br>**D-790** | **Revision**<br>**1** |
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| | | **Effective Date**<br>03/31/23 | **Page**<br>**Page 1 of 6** |

| **Written by/ Date** | **Reviewed by/ Date** | **Approved by/ Date** |
|---|---|---|
| 03/14/23 | SAS 03/16/23 | 03/29/23 |
| **Title: Quality Assurance Director** | **Title: Analytical Development Scientist** | **Title: Quality Control Director** |

## 1.0 Purpose

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

## 2.0 Scope

This procedure applies to the quantification of procyanidins in raw materials and finished products. The Acid Butanol method involves the HCl catalyzed depolymerization of condensed tannin in butanol to yield a red anthocyanin product that can be detected spectrophotometrically. The method is by nature non-specific, and expresses procyanidin content as equivalents USP Maritime Pine Extract. This method was validated under Protocol MV-LAB-19-183.

## 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 **USP** – United States Pharmacopeia

4.3 **MPE** – Maritime Pine Extract

4.4 **FAS** – Ferric Ammonium Sulfate Dodecahydrate

## 5.0 References

5.1 MV-LAB-19-183, Protocol, Quantification of Procyanidins (Acid Butanol Method) by Visible Light Spectroscopy

5.2 USP Monograph: Maritime Pine Extract, Current Edition.

## 6.0 Supplies

6.1 Chemicals: (Use reagent grade or better.)

6.1.1 Milli-Q Water

6.1.2 HCl

6.1.3 Butanol

6.1.4 Methanol

6.1.5 FAS

6.1.6 USP MPE

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

6.2.4 Weigh Paper & Boats

6.2.5 Graduated Cylinders

6.2.6 Beakers

6.2.7 15 mL Polypropylene Centrifuge Tubes

6.2.8 40 mL Glass Vials w/ Bonded Septum Caps

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

6.3.9 Centrifuge

## 7.0 Procedure

7.1 Instrument, Water Baths & Reaction Solution Preparations

7.1.1 Turn on the spectrophotometer and allow it 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 551 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 40mL vials required to contain the blank, standards and samples. Fill ~1/3 full with DI water. Cover one with aluminum foil and place in a freezer. Retrieve the beaker in the freezer occasionally and break up the surface in order to obtain an ice bath. Place the other in a 95°C water bath.

7.1.3 Reagent Solution A – Add 5 mL HCl to 95 mL Butanol and mix well. (Preparations may be scaled as necessary. Make fresh daily.)

7.1.4 Reagent Solution B – Dissolve 2g FAS in a mixture of 100 mL water + 17.5 mL HCL and mix well. (Preparations may be scaled as necessary. Solution is good for 15 days.)

7.2 Standard Prep

7.2.1 Transfer ~67 mg USP MPE into a 50 mL volumetric flask. Add ~30 mL of methanol and swirl to dissolve. Dilute to volume with methanol and mix well. This is the USP MPE Stock Solution.

7.2.2 Transfer 600, 800, 1000, 1200 & 1400 uL aliquots of the USP MPE Stock Solution into individual 10 mL volumetric flasks. Dilute to volume with methanol and mix well.

7.3 Sample Extraction

7.3.1 The validated range for the analytical method is 0.054 – 0.126 mg/mL.

7.3.2 Raw Materials: Given the (vendor declared) raw material assay value, dissolve the sample in methanol at a concentration at or below 2.5 mg/mL, then dilute to a convenient concentration within the validated range.

7.3.3 Finished Products: Given the finished product formulation, transfer a calculated mass of sample to a 100 mL volumetric flask such that the concentration is at or below 0.0825 mg/mL. Add 50 mL of methanol and shake mechanically for 15 minutes, then dilute to volume with methanol and mix thoroughly. Sonicate for 20 minutes, mix thoroughly and allow to cool to ambient temperature. Transfer ~10 mL to a 15 mL centrifuge tube and spin down at 4000 rpm for 3 minutes. Be careful not to disturb the clear supernatant for use in the chromogen formation reaction.

7.4 Chromogen Formation Reactions

7.4.1 Assemble and run the reactions in the 40 mL glass vials as indicated in the table below:

| | Blank | Standard | Sample |
|---|---|---|---|
| Reagent Solution A, mL | 6.0 | 6.0 | 6.0 |
| Reagent Solution B, µL | 250 | 250 | 250 |
| Standard Prep, uL | --- | 1000 | --- |
| Sample Prep, uL | --- | --- | 1000 |
| Methanol, uL | 1000 | | |
| Cap tightly, vortex mix then submerge in the 95°C bath for 40 minutes. | | | |
| Transfer the reaction vials to the ice bath for ~1 minute then vortex mix and allow to stabilize at ambient temperature. | | | |

7.5 Absorbance Readings

7.5.1 Transfer the blank reaction solution 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 Calculations for Raw Material % Assay

7.7.1 Sample Concentration, mg/mL = (Sample Mass, mg (Corr. for Water) / Stock Volume, mL) * Dilution Factor, mL/mL

7.7.2 USP MPE Eq's in Sample, mg/mL = (Absorbance – Intercept)/Slope

7.7.3 Content of Procyanidins, % = (USP MPE Eq's in Sample, mg/mL / Sample Concentration, mg/mL) * 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: updated logo and format. | CC-23-0140 | K. Burris |