ION Nutritional Labs	Standard Operating Procedure **Pterostilbene Determination by HPLC with UV/Vis Spectroscopy**	**SOP Number** **D-798**	**Revision** **1**
		Effective Date 04/24/24	**Page** **Page 1 of 9**

Written by/ Date	**Reviewed by/ Date**	**Approved by/ Date**
SAS 04/08/24	CJP 04-09-24	AJS 04/21/24
Title: Analytical Development Scientist	**Title: Analytical Development Scientist**	**Title: QC Laboratory Manager**

1.0 Purpose

This document describes the analytical procedure for the determination of Pterostilbene (PSB) in raw materials and finished products.

2.0 Scope

This procedure applies to the identification and quantification of PSB in raw materials and finished products.

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 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 **AD** – Analytical Development

4.3 **PSB** – Pterostilbene

4.4 **ACN** – Acetonitrile

4.5 **MeOH** – Methanol

4.6 **H3PO4** – Phosphoric Acid

4.7 **ACS** – American Chemical Society

4.8 **HPLC** – High Performance Liquid Chromatography

4.9 **UV/Vis** – Ultraviolet & Visible Electromagnetic Spectra

5.0 References

5.1 PRTCL-20-0043, Protocol, Pterostilbene Determination by HPLC Using UV/Vis Spectroscopy

5.2 RPT-20-0036, Report, Pterostilbene Determination by HPLC Using UV/Vis Spectroscopy

5.3 D-793, SOP, Cryogenic Grinding of Chewable Gels

6.0 Supplies

6.1 Chemicals – All reagents are ACS grade or better.

6.1.1 Milli-Q Water

6.1.2 ACN

6.1.3 H3PO4

6.1.4 MeOH

6.1.5 PSB Reference Standard

6.2 Supplies and Glassware

6.2.1 HPLC vials, 12mm X 32mm with screw cap enclosures w/ septa

6.2.2 Volumetric glassware and/or adjustable pipettes and tips

6.2.3 Weigh paper and/or funnels

6.2.4 Syringes with 0.45µ Nylon Syringe Filters

6.3 Equipment

6.3.1 Suitable gradient HPLC system consisting of a pump, autosampler, column oven and UV detector with a chromatographic data handling system

6.3.2 Analytical Balance

6.3.3 Wrist Action Shaker

6.3.4 Sonicator Bath

7.0 Procedure

7.1 Mobile Phase & Diluent Preparation

7.1.1 Mobile Phase

7.1.1.1 Mobile Phase A: Add 1000 µL of H3PO4 to 1000 mL of water and mix well.

7.1.1.2 Mobile Phase B: ACN

7.1.2 Extraction Solvent = Diluent = MeOH

7.1.3 Preparations may be scaled as necessary

7.2 Standard Prep

7.2.1 Accurately weigh and transfer about 25 mg of PSB reference standard into a 100 mL volumetric flask. Add ~50 mL of Diluent and sonicate for five minutes.

7.2.2 Cool to ambient then dilute to volume with Diluent and mix well – this is the PSB Stock. Dilute the PSB Stock 2:100 with Diluent – this is the PSB Working Standard.

7.3 Sample Preparation

7.3.1 Specific sample testing details are provided in each products profile. If a specific testing details section is not available, then follow preparation procedure as described below, maintaining concentration within the linear range listed below.

7.3.2 The validated range for the analytical method is 0.00246 – 0.0123 mg/mL.

1.1.1 For raw materials: weigh no less than 25 mg into a suitably sized volumetric flask of no less than 100 mL volume. Fill the flask to about 50% of the flask volume with Diluent and shake mechanically for 15 minutes. Sonicate for 5 minutes, cool to ambient then dilute to volume with Diluent.

1.1.2 For solid and liquid dose finished products: Combine and homogenize no less than ten dosage units. Based on the label claim and fill weight (capsules), serving size (powders and liquids) or tablet weight per dose, weigh no less than 100 mg of the pooled dosages into a suitably sized volumetric flask of no less than 100 mL. Fill the flask to about 50% of the flask volume with Diluent and shake mechanically for 15 minutes. Sonicate for 5 minutes, cool to ambient then dilute to volume with Diluent.

1.1.3 For chewable gels (gummies), homogenize at least 10 dosage units according to the procedure outlined in D-793 Cryogenic Grinding of Chewable Gels. Quickly weigh no less than 200 mg of the pooled and homogenized dosages into a

suitably sized beaker. Add a volume of Diluent equivalent to 50% of the desired flask volume, add a stir bar, and stir until dissolved. Transfer the solution to a volumetric flask of the desired size. Use several small portions of Diluent to rinse any remaining residue from the beaker into the volumetric flask ensuring complete transfer, and dilute to volume using Diluent.

1.1.4 To manage large volumes, the sample can be initially dissolved in a smaller volume and a portion further diluted using Diluent to bring the analyte concentration into the linear range. Dilutions can be made using volumetric glassware and/or adjustable pipettes. Dilutions can be prepared in HPLC vials.

7.3.3 Filter through a 0.45 µm membrane discarding the first 3 – 4 mL before collecting a portion for analysis. Alternatively, centrifuge an aliquot of the final sample at 10,000 rpm for 5 min to remove particulates.

7.4 HPLC Parameters

7.4.1 Column: Restek Raptor ARC-18, 4.6 x 150mm, 2.7µm (Or Equivalent)

7.4.2 Column Temperature: 35°C

7.4.3 Flow rate: 1.0 mL/min

7.4.4 Mobile Phase: Isocratic 50% A / 50% B

7.4.5 Wavelength: 330 nm

7.4.6 Injection Volume: 5 µL

7.4.7 Run Time: 10 minutes

7.5 Recommended Sequence

7.5.1 Make at least 2 injections of the Diluent.

7.5.2 Make at least five (5) injections of PSB Working Standard.

7.5.3 Make a single injection of each Sample Preparation.

7.5.4 Make a single injection of the Standard Solution after every ten (10) sample injections and/or at the end of a run.

7.6 System Suitability Requirements

7.6.1 The %RSD of five (5) consecutive standard injections is NMT 2.0%

7.6.2 The %RSD of all standard injections is NMT 3.0%.

7.6.3 If present, any interference in the diluent should be subtracted out of the sample and standard peak areas.

7.7 Example calculations for determining finished product % LC or raw material % assay:

7.7.1 $$\% = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{SS}{SA} \times \frac{V_{spl}}{LA} \times 100$$

R_u Sample peak area

R_s Mean (n=5) standard peak area

Wt_{std} Weight of the reference standard in mg

V_{std} Volume of the standard preparation accounting for dilutions in mL

P Purity of the reference standard in decimal format

SA Sample amount in g

SS Serving size in g: Average weight of ten dosage units for tablets, fill weight for capsules, mass of a single serving for powders, volume of a single serving from the theoretical formula for liquids, or 1 for raw materials.

V_{spl} Volume of the sample preparation accounting for dilutions in mL

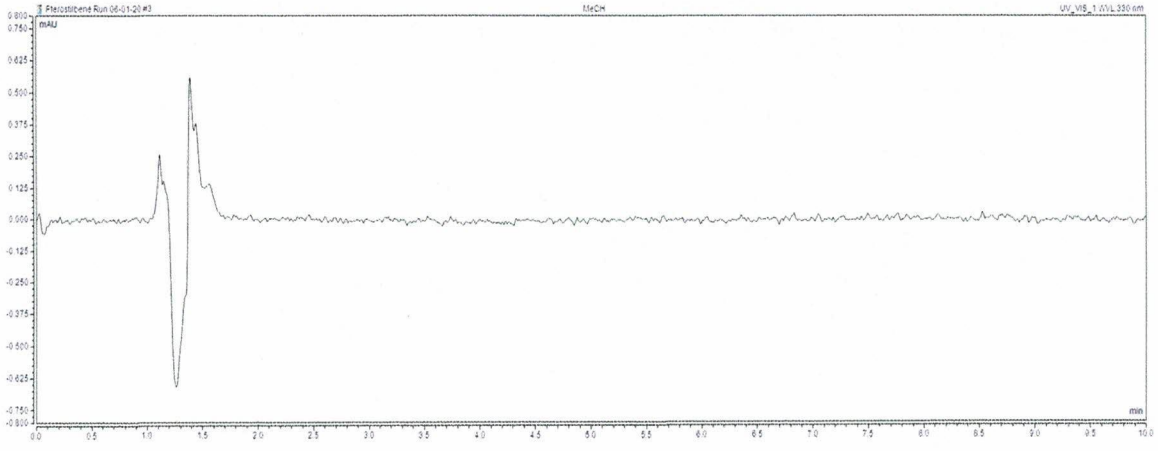
LA Label amount of analyte in mg. (Use 1 for raw materials.)

7.8 System Wash, Column Wash and Column Storage

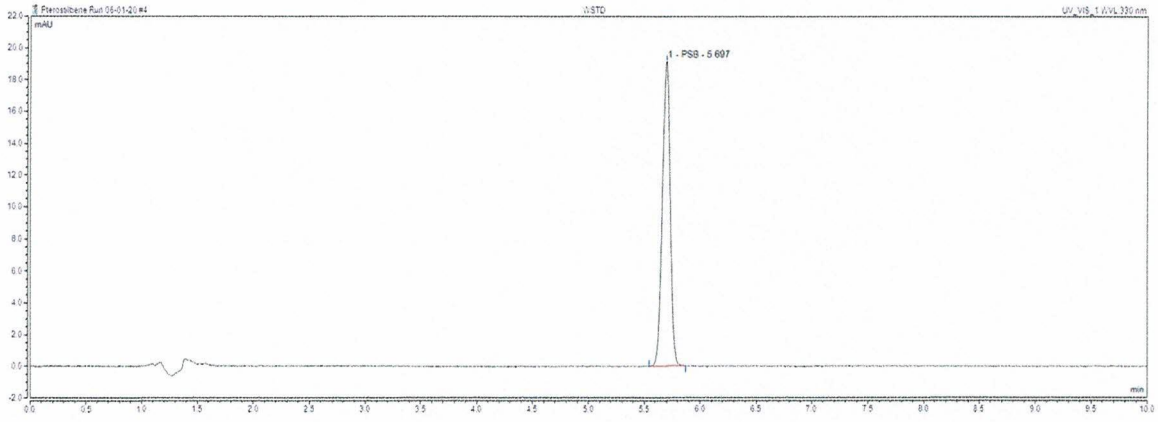
7.8.1 Wash and store the column in 50:50 ACN / Water.

8.0 Example Chromatograms

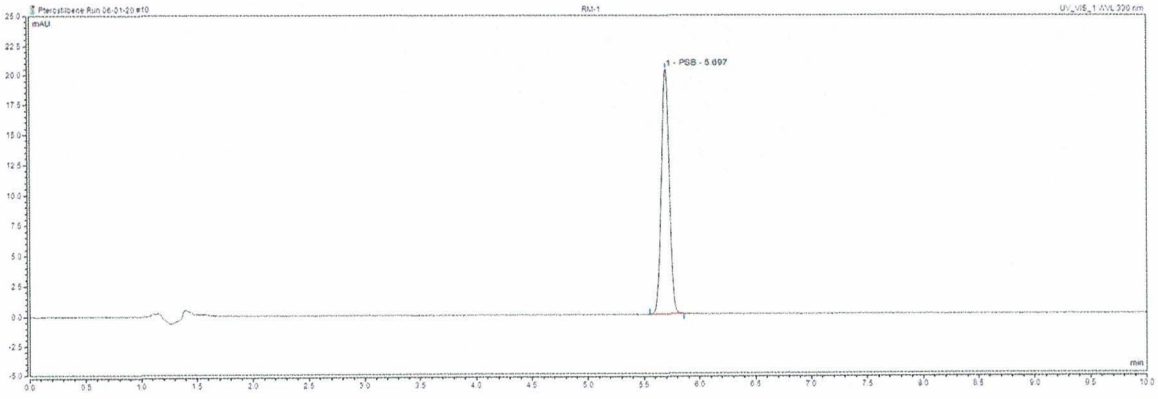
8.1 Typical Diluent Chromatogram



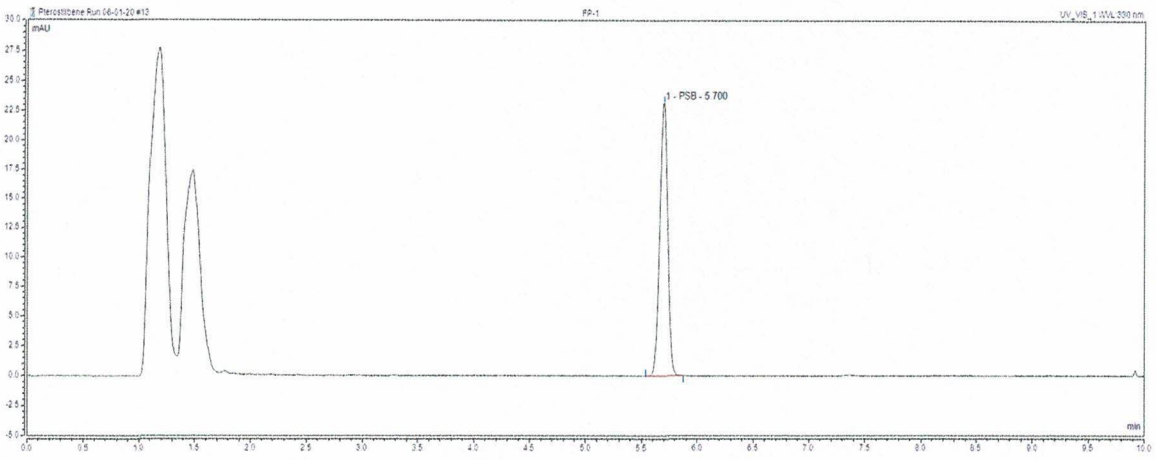
8.2 Typical Working Standard Chromatogram



8.3 Typical Raw Material Chromatogram



8.4 Typical Finished Product Chromatogram



9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	09/01/20	New	N/A	C. Perry
1	04/03/24	Add instruction to follow product specific test details if available, add specific sample prep instructions for gummies, edit for consistency with current methods.	CC-24-0129	S. Sassman