1.0 Purpose

This document describes the analytical procedure for the determination of Beta Carotene (when present as beadlets encapsulated in alginate or gelatin) in raw materials and finished products.

2.0 Scope

This procedure applies to the identification and quantification of Beta Carotene (when present as beadlets encapsulated in alginate or gelatin) in raw materials and finished products. This method was validated under protocol PRTCL-24-0024.

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 **H_2O** – Deionized Water (>18MΩ·cm)

4.3 **NH_4OH** – Ammonium Hydroxide

4.4 **THF** – Tetrahydrofuran

4.5 **BHT** – Butylated Hydroxytoluene

4.6 **H_3PO_4** – Phosphoric Acid

4.7 **ACS** – American Chemical Society

5.0 References

5.1 PRTCL-24-0024, Validation of an Analytical Method for the Determination of Beta Carotene by Visible Spectroscopy

6.0 Supplies

6.1 Chemicals – (All reagents are ACS grade or better.)

6.1.1 H_2O

6.1.2 NH_4OH

6.1.3 H_3PO_4

6.1.4 THF

6.1.5 BHT

6.1.6 Alkaline Protease (Use BioCat Alkaline Protease R, or equivalent.)

6.1.7 Cyclohexane

6.2 Supplies and Glassware

6.2.1 1cm Cuvettes

6.2.2 **Low Actinic** Volumetric Glassware

6.2.3 Weigh Paper

6.2.4 Syringes with 0.45µ Teflon or Glass Fiber Syringe Filters

6.3 Equipment

6.3.1 Spectrophotometer

6.3.2 Analytical Balance

6.3.3 pH Meter

6.3.4 Heated Ultrasonic Bath

6.3.5 Digital Timer

6.3.6 Digital Thermometer

7.0 Procedure

7.1 Extraction Solvent & Diluent Preparation (Preparations may be scaled.)

7.1.1 Extraction Solvent – 4% Ammonium Hydroxide Buffer

7.1.1.1 Add 36ml Ammonium Hydroxide to 214ml H_2O and mix well. Adjust pH to 9.5 with H_3PO_4. *The accuracy of the pH adjustment is of critical importance!*

7.1.2 Diluent – Stabilized THF Solution (0.1% BHT)

7.1.2.1 Add 500mg BHT to 500ml THF and mix well.

7.2 Sample Preparation

(Use low actinic glassware! Minimize sample exposure to light and oxygen!)

7.2.1 Specific sample testing details are provided in each product profile. If a specific testing details section is not available, follow preparation procedure as described below, maintaining concentration within the linear range of this method.

7.2.2 The validated linear range for the analytical method is 0.26 – 4.23 µg/mL.

(Use low actinic glassware! Minimize sample exposure to light and oxygen!)

7.2.3 Pool at least 20 dosage units and homogenize as appropriate (e.g. grind tablets / capsule fill / powders / stick pack contents by mortar and pestle, cryogenically powder and dissolve gummies, etc.) To prepare the stock sample, weigh sufficient sample (based on the raw material manufacturer assay value / finished product profile) to deliver ~10mg active into a 100ml volumetric flask.

7.2.4 Add 20ml Extraction Solvent and two drops of alkaline protease and swirl vigorously to thoroughly wet the sample. Place in a heated ultrasonic bath preheated to 55°C. *Submerge and immobilize the flask carefully, making sure that the level of the water in the water bath is below the level of the sample in the flask.* Sonicate for 20 minutes, swirling vigorously every 5 minutes.

7.2.5 Remove the sample from the sonicator and allow to equilibrate to room temperature. QS to volume with diluent then add a stir bar and mix on a stir plate for 20 minutes. *If undissolved beadlets remain, add two drops of NH_4OH and continue to stir for 10 minutes more.*

7.2.6 Allow the mixture to settle then dilute 3:100 with cyclohexane. Shake vigorously then filter a 5ml aliquot for analysis, discarding the first 1-2ml before directing the filtrate into a cuvette.

7.3 Spectrophotometer Parameters

7.3.1 Assay Wavelength: 456nm

7.3.2 Identification Wavelength (Absorbance Ratio): A_{455nm} / A_{483nm}

7.3.3 Cell Path: 1cm

7.3.4 Blank: Cyclohexane

7.4 Calculation for determining percentage:

7.4.1 $T = A/(F*C)$

Where:

T Percentage of Total Carotenoids as Beta Carotene ($C_{40}H_{56}$)

A Absorbance of the sample solution

F 2505, Coefficient of Extinction ($E^{1\%}$) of pure all-trans-beta carotene in Cyclohexane ($100ml * g^{-1} * cm^{-1}$)

C Concentration of the sample solution (g/ml)

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	03/12/24	New procedure.	N/A	C. Perry