ION LABS QUALITY FIRST	Standard Operating Procedure Cordycepic Acid (Mannitol) Determination by HPLC with RI Detection	SOP Number D-752	Revision 0
		Effective Date 04/03/21	Page Page 1 of 4

Written by/ Date	Reviewed by/ Date	Approved by/ Date
04-16-21	04/16/21	04/16/21
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1.0 Purpose

This document describes the analytical procedure for the determination of Cordycepic Acid (Mannitol) in raw materials.

2.0 Scope

This procedure applies to the identification and quantification of Cordycepic Acid (Mannitol) in raw materials. This method was verified under protocol PRTCL-21-0008.

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 the QC Laboratory Management to implement this procedure and to ensure that the procedure is being followed.

3.3 It is the responsibility of the QC Laboratory Management and AD 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 ACS – American Chemical Society

4.4 HPLC – High Performance Liquid Chromatography

4.5 RI – Refractive Index

5.0 References

5.1 PRTCL-21-0008: Verification of an Analytical Method for the Determination of Cordycepic Acid (Mannitol) by HPLC-RI

6.0 Supplies

6.1 Chemicals – All reagents are ACS grade or better.

6.1.1 Milli-Q Water

6.1.2 Mannitol 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 HPLC system consisting of a pump, autosampler and RI detector with a chromatographic data handling system

6.3.2 External Column Heater

6.3.3 Calibrated Thermocouple

6.3.4 Analytical Balance

6.3.5 Wrist Action Shaker

7.0 Procedure

(WARNING – SEE CAUTIONS RELATED TO USE OF COLUMN BELOW PRIOR TO BEGINNING WORK!)

7.1 Mobile Phase & Diluent

7.1.1 Mobile Phase

7.1.1.1 Use Milli-Q water.

7.1.1.2 **Caution: The only mobile phase suitable for use in this column is pure water! Accidental contact with organic solvents or buffer salts will ruin the column!**

7.1.1.3 **Caution: Do not allow the pump pressure to exceed 800 psi when using this column! Heat the column in the oven to at least 65°C prior to introducing a low flow (≤ 0.1 ml/min)! Do not increase the flow again until the column is temperature equilibrated at 90°C!**

7.1.1.4 **Caution: At each installation, flush the column into a beaker for 30 minutes before attaching it to the inlet of the detector!**

7.1.2 Extraction Solvent / Diluent – Use Milli-Q water.

7.2 Standard Preparation

7.2.1 Accurately weigh and transfer about 100 mg of Mannitol reference standard into a 50 mL volumetric flask. Add ~30 mL of water and swirl to dissolve. QS to volume and mix well. This is the working standard.

7.3 Sample Preparation

7.3.1 The validated range for the analytical method is 1.01 – 5.05 mg/mL.

7.3.2 Extract sufficient sample (based on the manufacturer assay value) with Diluent in order to generate a concentration that is within the validated linear range.

7.3.3 Samples can be dissolved in Diluent at any volume starting from 25 mL. The volume chosen must be in the solubility range of Mannitol (validated at ~2 mg/ml). To manage large volumes, the sample can be initially dissolved in a smaller volume that is within the solubility range and a portion further diluted to bring the Mannitol concentration into the linear range.

7.3.4 Fill the flask to about 50% of the chosen volume with Diluent and shake mechanically for 10 minutes. QS to volume, mix well, then filter a 5ml aliquot for analysis, discarding the first 3-4ml of filtrate.

7.3.5 For raw materials being analyzed for the first time using this method, an in process validation is required to demonstrate baseline separation of peaks, and extraction efficiency as a part of system suitability before data can be reported using this method.

7.4 HPLC Parameters

7.4.1 Column: Agilent MetaCarb Ca Plus, 300 x 7.8 mm (Or USP L-19 Equivalent)

7.4.2 Mobile Phase: Water (Isocratic)

7.4.3 Flow rate: 0.45 mL/min

7.4.4 Optical Unit Temperature: 37°C

7.4.4.1 (Note: The RI detector is extremely sensitive to changes in temperature and may take several hours to equilibrate. Once equilibrated, purge the reference cell for 10 minutes.)

7.4.5 Column Temperature: 90°C

7.4.5.1 (Note: The external column heater temperature display is for reference only. Adjust, if necessary, using the calibrated thermocouple.)

7.4.6 Injection Volume: 20 µL

7.4.7 Run Time: 35 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 Mannitol Working Standard.

7.5.3 Make a single injection of each Sample Preparation.

7.6 System Suitability Requirements

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

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

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

7.7.1 $$\% = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{1}{SA} \times \frac{V_{spl}}{1} \times 100$$

R_u Sample peak area

R_s Mean (n=5) standard peak area

Wt_{std} Weight of the reference standard

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

P Purity of the reference standard in decimal format

SA Sample amount

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

7.8 System Wash, Column Wash and Column Storage

7.8.1 Wash and store the column in water.

8.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	04/14/21	New	N/A	C. Perry