1.0 Purpose

This document describes the analytical procedure for the determination of Paraxanthine in raw materials and finished products.

2.0 Scope

This procedure applies to the identification and quantification of Paraxanthine in raw materials and finished products. This method was validated under protocol PRTCL-22-0052.

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 **ACN** – Acetonitrile

4.3 **ACS** – American Chemical Society

4.4 **HPLC** – High Performance Liquid Chromatography

4.5 **UV-Vis** – Ultraviolet-Visible (Detection)

5.0 References

5.1 PRTCL-22-0052, Protocol, Validation of an Analytical Method for the Determination of Paraxanthine by HPLC-UV

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 Paraxanthine 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

6.2.3 Weigh paper

6.2.4 Syringes with 0.45µ nylon filters

6.3 Equipment

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

6.3.2 Analytical Balance

6.3.3 Sonicator Bath

6.3.4 Wrist Action Shaker

7.0 Procedure

7.1 Mobile Phase & Diluent Preparation

7.1.1 Mobile Phase

7.1.1.1 Mobile Phase A = Water

7.1.1.2 Mobile Phase B = ACN

7.1.2 Extraction Solvent / Diluent

7.1.2.1 Water

7.2 Standard Prep

7.2.1 Prepare standard stock solution at 0.2 mg/ml in water. Sonicate for 10 minutes to ensure dissolution, then shake vigorously. Cool to room temperature, then dilute 1:10 with diluent. Shake vigorously then filter a 5ml aliquot for analysis, discarding the first 3-4ml of filtrate.

7.2.2 Alternative standard preparations are acceptable as long as the preparations are within the linear range of this method.

7.3 Sample Preparation

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

7.3.2 The validated linear range for the analytical method is 0.01284 – 0.02996 mg/ml. Prepare raw material samples like standards.

7.3.3 Prepare finished product sample stock solution at 0.2 mg/ml in water (based on the finished product profile). Shake mechanically at ~ half volume for 15 minutes then QS to volume. Sonicate for 10 minutes then shake vigorously. Dilute 1:10 with diluent and shake vigorously. Filter a 5ml aliquot for analysis, discarding the first 3-4ml of filtrate.

7.4 HPLC Parameters

7.4.1 Column: Phenomenex Kinetex XB-C_{18}, 4.6 x 150mm, 2.6µm SPP (Or Equivalent)

7.4.2 Column Temperature: 40°C

7.4.3 Flow rate: 1.0 mL/min

7.4.4 Mobile Phase Gradient:

Time, min	% A	%B
0	97	3
2	97	3
10	84	16
12	50	50
12.1	97	3
15	97	3

7.4.5 Wavelength: 271 nm

7.4.6 Injection Volume: 5 µL

7.4.7 Run Time: 15 minutes

7.4.8 Recommended 3-D Spectral Range (for Identification): 210nm - 350nm

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 the Working Standard.

7.5.3 Make a single injection of each Sample Preparation.

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

7.6 System Suitability Requirements

7.6.1 The %RSD of five (5) consecutive standard injections is NMT 2%.

7.6.2 The %RSD of all standard injections is NMT 3%.

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 % assay:

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

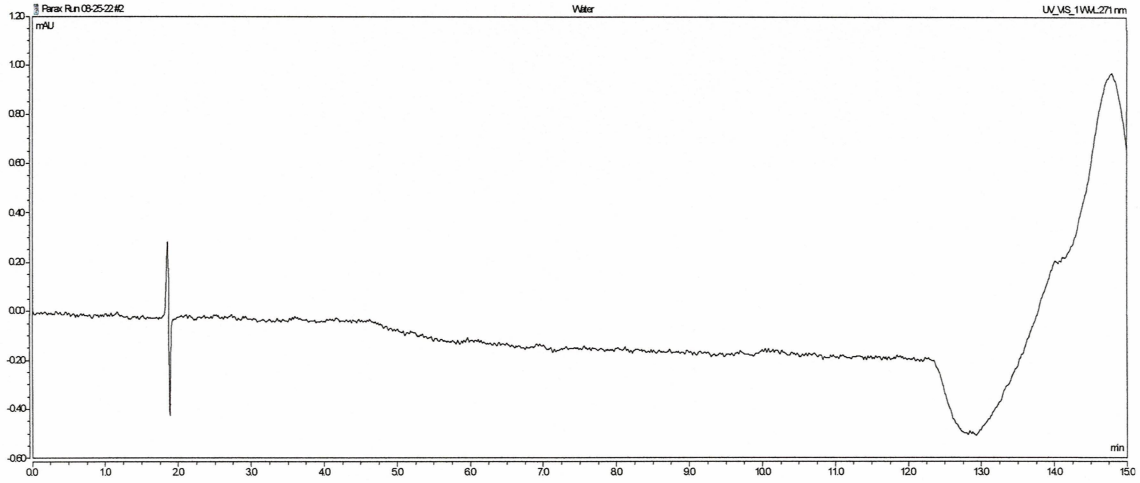
R_u	Sample peak area
R_s	Mean (n=5) standard peak area
Wt_{std}	Weight of the reference standard, mg
V_{std}	Volume of the standard preparation accounting for dilutions, mL
P	Purity of the reference standard in decimal format
SA	Sample amount, mg
V_{spl}	Volume of the sample preparation accounting for dilutions, mL
SS	Use average dosage weight for solids or use serving size specified in Product Profile for powders and liquids. Use "1" for raw materials
LA	Label Amount (Use 1 for Raw Materials)

7.8 System Wash, Column Wash and Column Storage

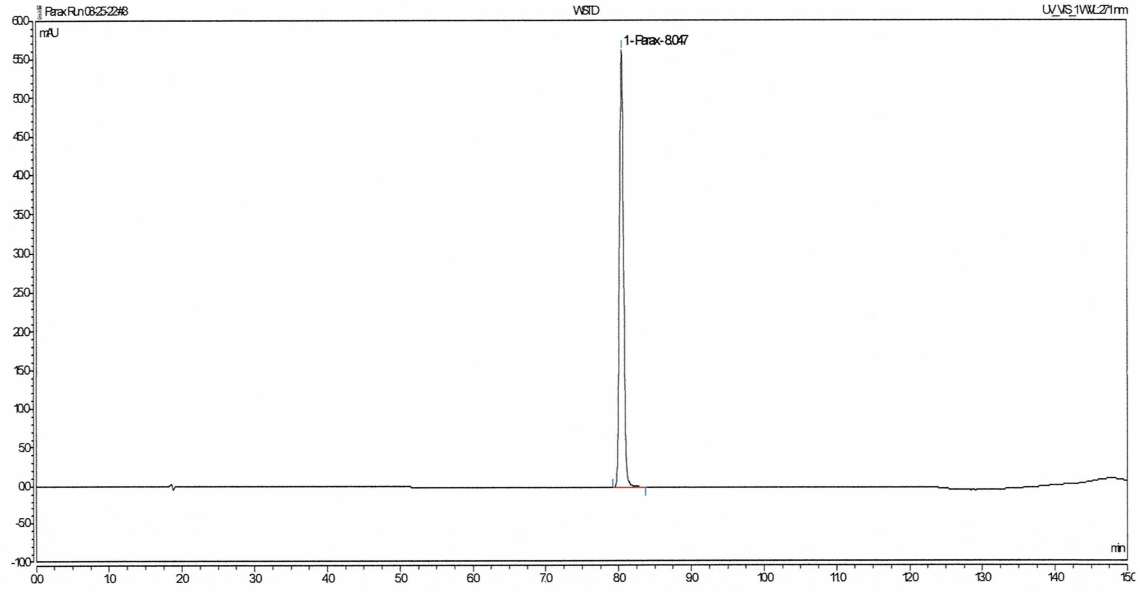
7.8.1 Wash and store the column in 75:25 ACN / Water

8.0 Chromatograms

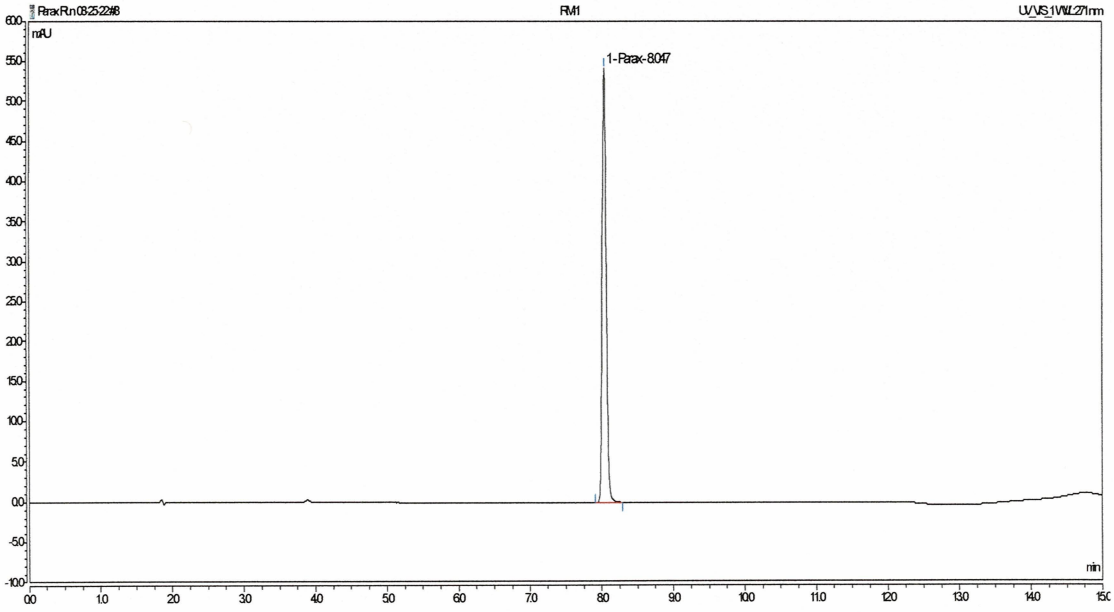
8.1 Typical Diluent Chromatogram



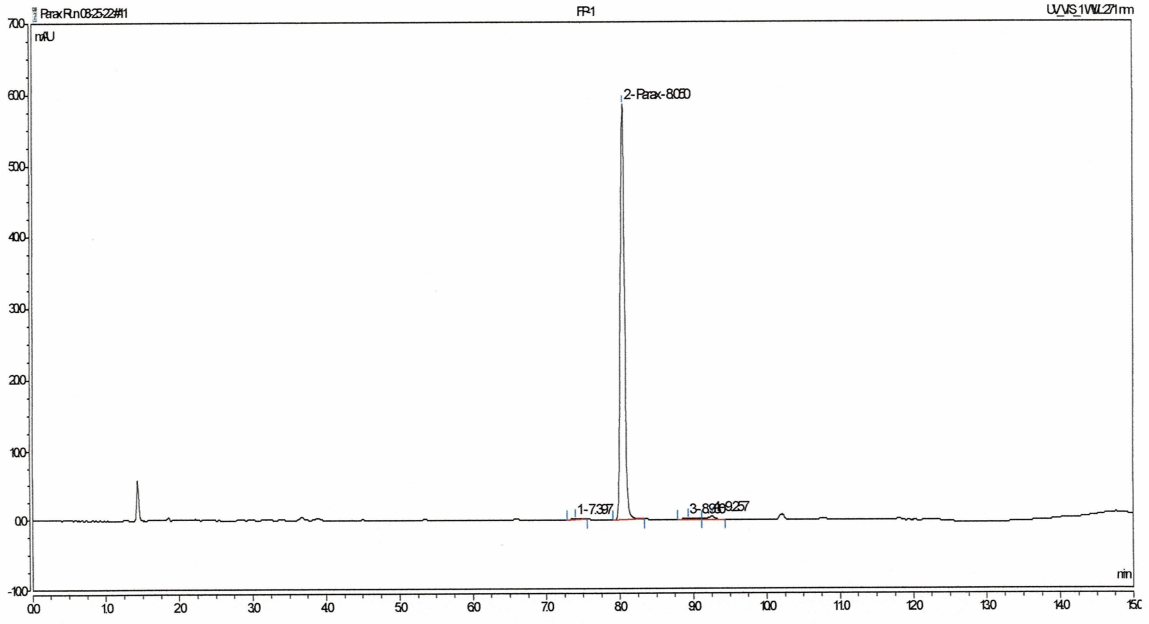
8.2 Typical Standard Chromatogram



8.3 Typical Raw Material Chromatogram



8.4 Typical Finished Product Chromatogram



9.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	11/10/22	New procedure.	N/A	C. Perry