1.0 Purpose

This document describes the analytical procedure for the determination of Phenylcapsaicin in raw materials.

2.0 Scope

This procedure applies to the identification and quantification of Phenylcapsaicin in raw materials. This method was validated under protocol PRTCL-22-0054.

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 **MeOH** – Methanol

4.4 **H3PO4** – Phosphoric Acid

4.5 **ACS** – American Chemical Society

4.6 **HPLC** – High Performance Liquid Chromatography

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

5.0 References

5.1 PRTCL-22-0054, Protocol, Validation of an Analytical Method for the Determination of Phenylcapsaicin by HPLC-UV

5.2 Analytical Method for Quantification of Phenylcapsaicin in aXivite® (aXichem MOA-1102-01 Version 02)

6.0 Supplies

6.1 Chemicals – All reagents are ACS grade or better

6.1.1 Milli-Q Water

6.1.2 MeOH

6.1.3 ACN

6.1.4 H3PO4

6.1.5 Capsaicin Reference Standard

6.1.5.1 Apply Relative Response Factor (RRF) in Section 7.7.1

6.1.5.2 Alternatively, Phenylcapsaicin may be used without correction.

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

7.0 Procedure

7.1 Mobile Phase & Diluent Preparation

7.1.1 Mobile Phase

7.1.1.1 Combine 500ml ACN + 500ml water + 1ml H3PO4 and mix well.

7.1.2 Extraction Solvent / Diluent

7.1.2.1 Combine 500ml MeOH + 250ml water and mix well.

7.2 Standard Prep

7.2.1 Prepare capsaicin stock at 1 mg/ml in MeOH. Sonicate for 5 minutes to ensure dissolution. 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 Alternatively, prepare the standard as above using Phenylcapsaicin.

7.2.3 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 The validated linear ranges for the analytical method are 60.3 – 140.8 µg/mL Phenylcapsaicin and 64.5 – 150.5 µg/mL Capsaicin.

7.3.2 Extract sufficient sample (based on the raw material manufacturer assay value) with Extraction Solvent in order to generate a concentration that is within the validated linear range. Sonicate for 60 minutes. Shake vigorously then filter a 5ml aliquot for analysis, discarding the first 3-4ml of filtrate.

7.4 HPLC Parameters

7.4.1 Column: Agilent 5 HC-C_{18}(2), 4.6 x 250mm, 5µm (Or Equivalent)

7.4.2 Column Temperature: 35°C

7.4.3 Flow rate: 1.0 mL/min

7.4.4 Mobile Phase: Isocratic

7.4.5 Wavelength: 282 nm

7.4.6 Injection Volume: 10 µL

7.4.7 Run Time: 25 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 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 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 $$\% = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{V_{spl}}{SA} \times 100 \text{ x RRF}$$

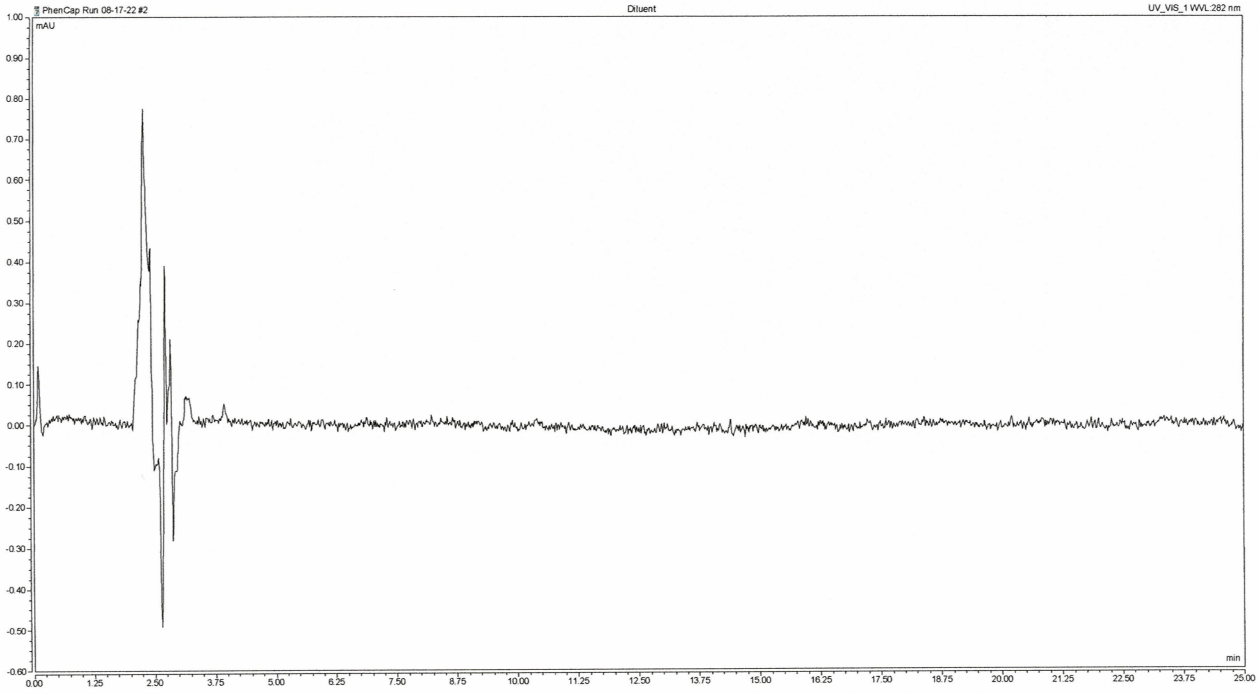
R_u	Sample peak area
R_s	Mean (n=5) standard peak area
Wt_{std}	Weight of the reference standard, mg
V_{std}	Volume of the standard preparation accounting for dilutions, mL
P	Purity of the reference standard in decimal format
SA	Sample amount, mg
V_{spl}	Volume of the sample preparation accounting for dilutions, mL
RRF	Multiply result by 1.07 when using Capsaicin as Reference Standard.

7.8 System Wash, Column Wash and Column Storage

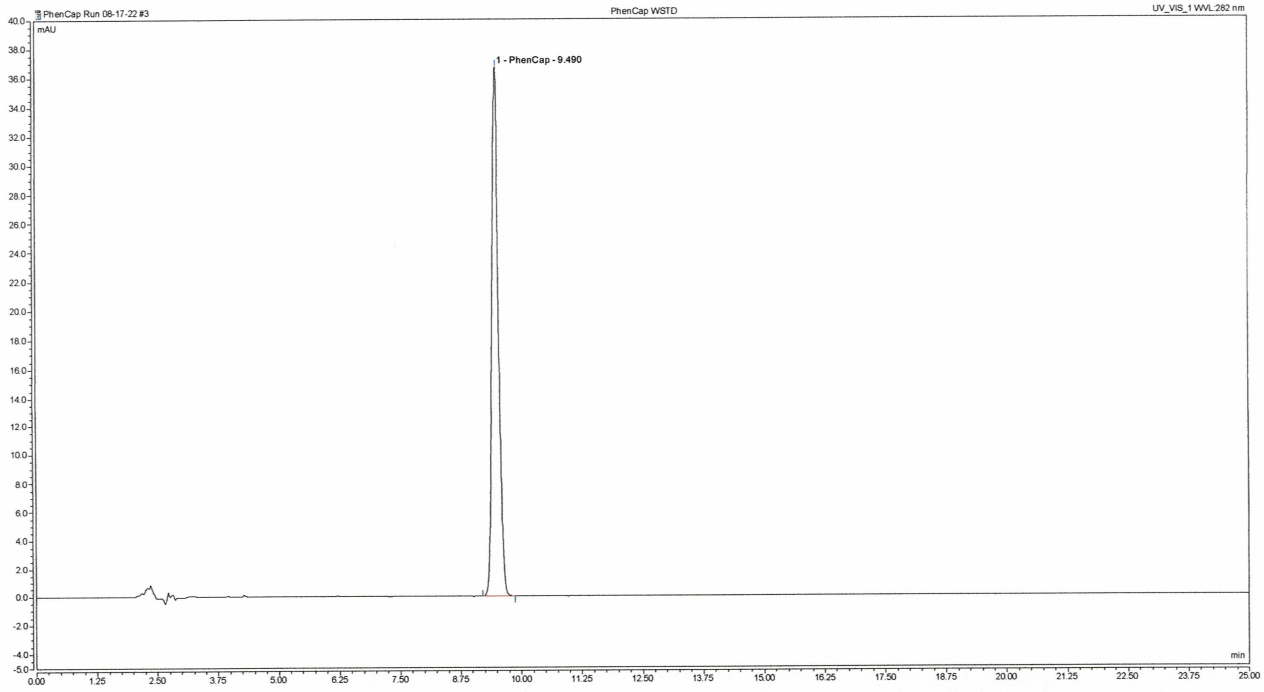
7.8.1 Wash and store the column in 75:25 ACN / Water

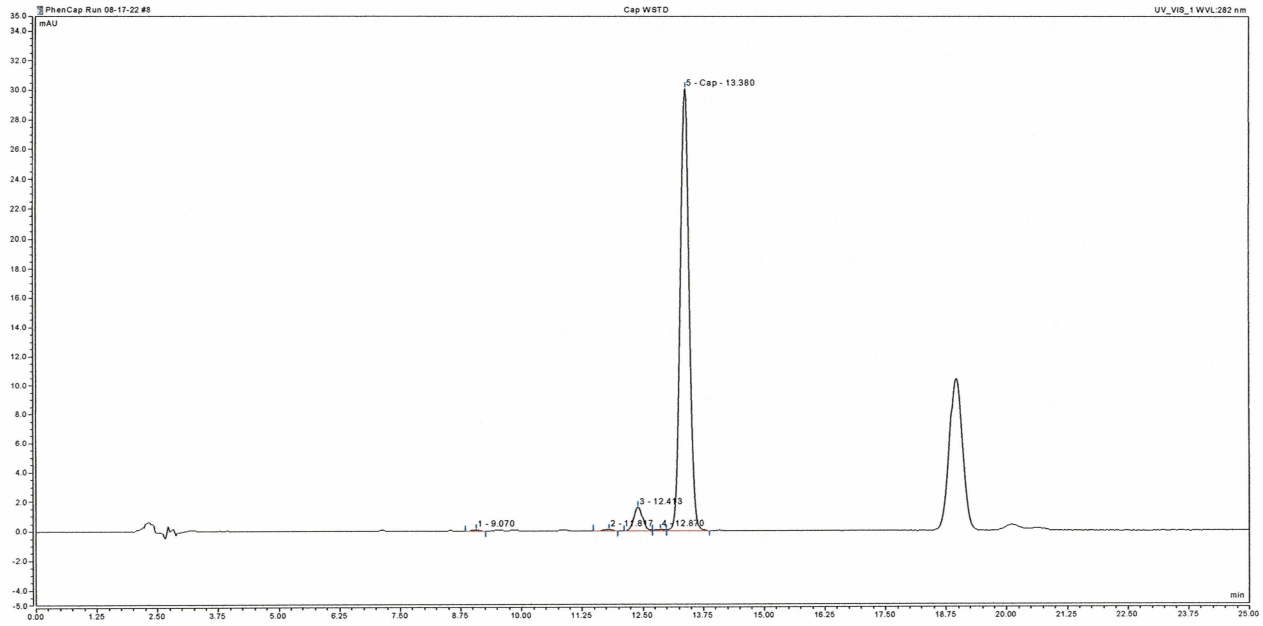
8.0 Chromatograms

8.1 Typical Diluent Chromatogram

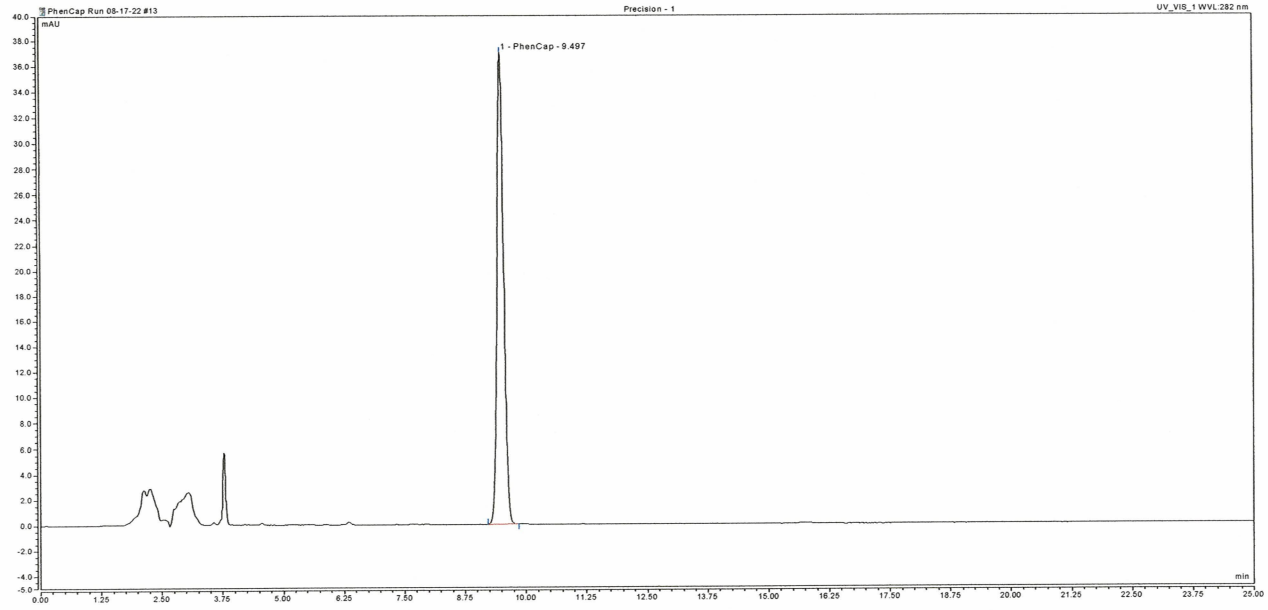


8.2 Typical Standard Chromatograms





8.3 Typical Raw Material Chromatogram



9.0 Revision History

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