1.0 Purpose

The purpose of this procedure is to define the method for the quantification and identification of Salicylic Acid and its Organic Impurities in raw materials and finished products by HPLC/UV.

2.0 Scope

This procedure applies to the quantification and identification of Salicylic Acid in raw materials and finished products by HPLC/UV. This procedure applies to the quantification and identification of the Organic Impurities of Salicylic Acid in raw materials by HPLC/UV. This procedure is intended for use in the QC laboratory.

3.0 Responsibility

3.1 It is the responsibility of QC Chemists to follow this procedure.

3.2 It is the responsibility of QC Laboratory Management to ensure that this procedure is being followed.

3.3 It is the responsibility of QC Laboratory Management and/or Analytical Development to keep this procedure aligned with current practices.

4.0 Definitions

4.1 **HPLC/UV** – High Performance Liquid Chromatography with Ultraviolet Detection

4.2 **QC** – Quality control

4.3 **AD** – Analytical development

4.4 **SAL** – Salicylic acid

4.5 **RCA** – Salicylic acid related compound A (4-hydroxybenzoic acid)

4.6 **RCB** – Salicylic acid related compound B (4-hydroxyisophthalic acid)

4.7 **H_2O** – Deionized water

5.0 References

5.1 PRTCL-20-0075, Protocol, Verification of an Analytical Method for the Determination of Salicylic Acid and its Organic Impurities by HPLC

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

6.0 Supplies

6.1 Chemicals: All reagents are HPLC grade or better

6.1.1 SAL reference standard

6.1.2 RCA reference standard

6.1.3 RCB reference standard

6.1.4 Phenol reference standard

6.1.5 MeOH

6.1.6 Glacial acetic acid

6.2 Glassware

6.2.1 Volumetric glassware as required for standard and sample preparations

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 Centrifuge

6.3.5 0.45µm luer-lock syringe filter and syringe barrel

6.3.6 Adjustable pipette and tips

7.0 Assay of Salicylic Acid

7.1 Mobile Phase Preparation

7.1.1 Mobile Phase

7.1.1.1 Transfer 400 mL of methanol to a 1 L media bottle.

7.1.1.2 Add 10.0 mL of glacial acetic acid.

7.1.1.3 Add 600 mL of H_2O.

7.1.1.4 Mix well.

7.1.2 Diluent A

7.1.2.1 Use Mobile Phase.

7.2 Standard Preparation

7.2.1 Accurately weigh and transfer about 25 mg of SAL reference standard to a 50-mL volumetric flask.

7.2.2 Dissolve in and dilute to volume with Diluent.

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 linear range for SAL is 0.35 mg/mL – 0.65 mg/mL.

7.3.3 For raw materials: weigh no less than 25 mg into a suitably sized volumetric flask of no less than 25 mL volume to generate an analyte concentration that is within the validated linearity range. Fill the flask to about 65% of the calculated volume with Diluent A and shake mechanically for 20 minutes. Sonication for up to 10 minutes can also be used to assist dissolution. If sonication is used, allow the sample to equilibrate to room temperature before bringing to final volume. Bring to final volume using Diluent A.

7.3.4 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 25 mL to generate an analyte concentration that is within the validated linear range. Fill the flask to about 65% of the calculated volume with Diluent A and shake mechanically for 20 minutes. Sonication for up to 10 minutes can also be used to assist dissolution. If sonication is used, allow the sample to equilibrate to room temperature before bringing to final volume. Bring to final volume using Diluent A.

7.3.5 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 A equivalent to 50% of the desired flask volume, add a stir bar, and stir until dissolved. Transfer the solution to a volumetric flask of size suitable to generate an analyte concentration that is within the validated linear range. Use several small portions of Diluent A to rinse any remaining residue from the beaker into the volumetric flask ensuring complete transfer, and dilute to volume using Diluent A.

1.1.1 To manage large volumes, the sample can be initially dissolved in a smaller volume and a portion further diluted using Diluent A 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.

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

7.4 HPLC Parameters

7.4.1 Column: Restek Roc C18, 5µm, 4.6 mm x 100 mm

7.4.2 Column Temperature: 30 °C

7.4.3 Flow rate: 1.0 mL/min

7.4.4 Wavelength: 270 nm

7.4.5 Injection Volume: 10 µL

7.4.6 Run Time: 10 minutes

7.4.7 Recommended Spectral Range (for Identification)- 220 nm to 400 nm

7.5 Retention Time

7.5.1 The retention time of SAL is about 7.2 minutes.

7.6 Recommended Sequence

7.6.1 Make at least 2 injections of the diluent.

7.6.2 Make five injections of Standard Solution.

7.6.3 Make a single injection of each Sample Preparation.

7.7 System Suitability Requirements

7.7.1 The %RSD of five consecutive standard injections is NMT 0.73%.

7.7.2 The USP tailing factor is NMT 2.5

7.7.3 No significant (>0.5%) interference are present in the diluent injection.

7.8 Column Wash and Storage

7.8.1 Store the column in Methanol / H_2O (50/50)

8.0 Organic Impurities of Salicylic Acid

8.1 Mobile Phase Preparation

8.1.1 Prepare as directed in Section 6.1.

8.2 Diluent B

8.2.1 Transfer 350 mL of methanol to a media bottle.

8.2.2 Add 20 mL of glacial acetic acid.

8.2.3 Add 150 mL of H_2O.

8.2.4 Mix well.

8.3 Phenol Solution Preparation

8.3.1 Accurately weigh and transfer about 25 mg of phenol reference standard to a 25-mL volumetric flask.

8.3.2 Dissolve in and dilute to volume with Diluent B.

8.4 Peak ID Solution

8.4.1 Transfer 0.1 mL of Phenol Solution to a 10-mL volumetric flask.

8.4.2 Dilute to volume with Diluent B.

8.5 Stock Standard Preparation

8.5.1 Accurately weigh and transfer about 50 mg of RCA to a 100-mL volumetric flask.

8.5.2 Accurately weigh and transfer about 25 mg of RCB to the same 100-mL volumetric flask.

8.5.3 Transfer 10.0 mL of Phenol Solution to the same 100-mL volumetric flask.

8.5.4 Dissolve in and dilute to volume with Diluent B.

8.6 Working Standard Preparation

8.6.1 Use the actual purity from the reference standard CofA in your calculations.

8.6.2 Accurately weigh and transfer about 25 mg of SAL reference standard to a 50-mL volumetric flask.

8.6.3 Transfer 5.0 mL of Stock Standard to the same 50-mL volumetric flask.

8.6.4 Dissolve in and dilute to volume with Diluent B.

8.7 Sample Preparation

8.7.1 Accurately weigh and transfer about 2.5 g of raw material sample to a 50-mL volumetric flask.

8.7.2 Add about 45 mL of Diluent B.

8.7.3 Sonicate until completely dissolved.

8.7.4 Equilibrate to room temperature.

8.7.5 Dilute to volume with Diluent B.

8.8 HPLC Parameters

8.8.1 Column: Kinetex XB-C18, 5µm, 4.6 mm x 150 mm

8.8.2 Column Temperature: 30 °C

8.8.3 Flow rate: 0.75 mL/min

8.8.4 Wavelength: 270 nm

8.8.5 Injection volume: 2 µL

8.8.6 Run Time: 12 minutes

8.8.7 Recommended Spectral Range (for Identification)- 220 nm to 400 nm

8.9 Retention Times

Note: The elution order of RCB and Phenol may switch. Use the *Peak ID Solution* to evaluate the retention time of these compounds.

8.9.1 RCA ≈ 3.1 minutes

8.9.2 Phenol ≈ 4.7 minutes

8.9.3 RCB ≈ 5.7 minutes

8.9.4 SAL ≈ 9.0 minutes

8.10 Recommended Sequence

8.10.1 Make at least 2 injections of Diluent B.

8.10.2 Make five injections of the Working Standard.

8.10.3 Make a single injection of the Peak ID Solution.

8.10.4 Make a single injection of each Sample Solution.

8.11 System Suitability Requirements

8.11.1 The %RSD of six consecutive standard injections of the Working Standard is NMT 5.0%.

8.11.2 The USP resolution between phenol and RC-B is NLT 2.0.

8.11.3 No significant (>0.5%) interference are present in the injection of Diluent B.

8.12 Column Wash and Storage

8.12.1 Store the column in Methanol / H_2O (50/50)

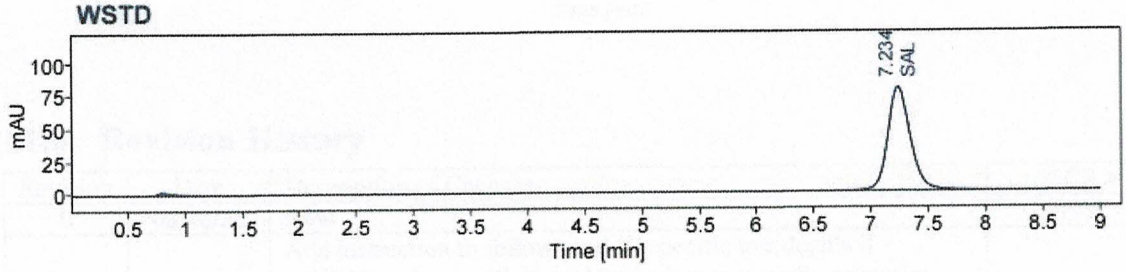
9.0 Example calculation

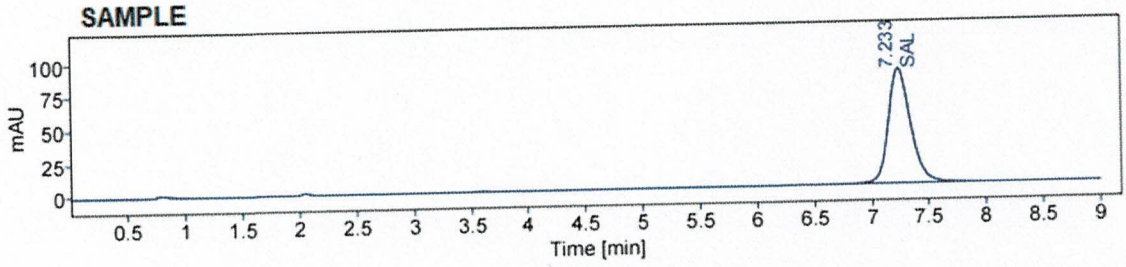
9.1.1 $$\% \text{ assay} = \frac{R_u}{R_s} \times \frac{Wt_{std} \times P}{V_{std}} \times \frac{SS}{Spl_{wt}} \times \frac{V_{spl}}{LA} \times 100$$

R_u	Sample peak area
R_s	Mean standard peak area
Wt_{std}	Weight of 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
SS	Serving size: Average weight of ten dosage units in mg for tablets and capsules, weight of a single serving from the theoretical formula in mg for liquids, or 1 for raw materials.
Spl_{wt}	Sample weight in mg
V_{spl}	Volume of the sample preparation accounting for dilutions in mL
LA	Label amount in mg (use 1 for raw materials)

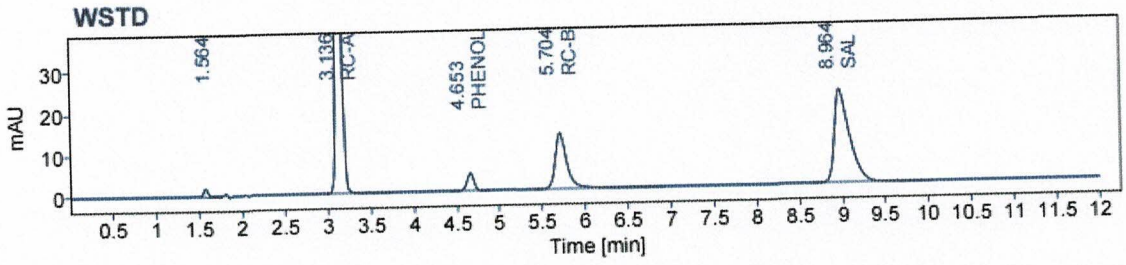
10.0 Representative Chromatograms

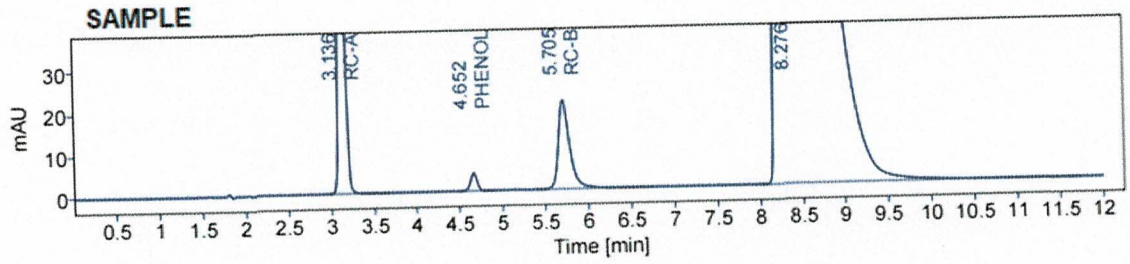
10.1 Assay of Salicylic Acid





10.2 Organic Impurities





11.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	08/18/20	New	N/A	S. Sassman
1	04/03/24	Add instruction to follow product specific test details if available, add specific sample prep instructions for gummies, correct calculation error, edit for consistency with current methods.	CC-24-0132	S. Sassman