1. SCOPE AND FIELD OF APPLICATION
   This test method describes the determination of salicylic acid in cosmetic products (cream).

2. PRINCIPLE
   Salicylic Acid is a Beta Hydroxy Acid (BHA). Salicylic acid is used in skin-care products for skin exfoliate and acne care. It is soluble in ethyl alcohol and methyl alcohol which can be separated by HPLC. The concentration of salicylic acid in cosmetic products can be determined from its peak area in sample with the calibration curve of salicylic acid standard.

3. REAGENTS
   3.1 General: all reagents used shall be of analytical purity.
   3.1.1 salicylic acid, Reference standard
   3.1.2 glacial acetic acid, AR grade
   3.1.3 methyl alcohol, HPLC grade (MeOH)
   3.1.4 Water shall be distilled water (H2O)
   3.2 Standard stock solution
      Prepare 0.1 % (w/v) solution of salicylic acid in methyl alcohol.
   3.3 Standard calibration solutions
      Prepare standard calibration solutions concentration of 2, 6, 10, 20 and 30 µg/mL in mobile phase. Pipette the mix standard solution 20, 60, 100, 200 and 300 µL, respectively with automatic pipette to each 10 mL volumetric flask. Make up to volume with mobile phase and mix well. Label as S1, S2, S3, S4 and S5, respectively.
   3.4 Mobile phase: MeOH : 1.5% v/v glacial acetic acid (55 : 45 by volume)

4. APPARATUS
   Normal laboratory equipment and:
   4.1 High Performance Liquid Chromatograph (HPLC) with a photodiode array detector and autosampler
   4.2 Analytical column: Hypersil GOLD C18, 5 µm, 4.6 mm x 150 mm (Thermo SCIENTIFIC™) or equivalent.
   4.3 Disposable syringe filter 0.45 µm (PVDF or equivalent)
   4.4 Ultrasonic bath
   4.5 Electronic balance, 0.1 mg (readability)

5. PROCEDURE
   5.1 Sample solution preparation:
      5.1.1 Weigh accurately 0.25 g of sample (duplicate A and B) into 25 mL volumetric flask.
      5.1.2 Dissolve with 10 mL of methyl alcohol by vortexing or sonicate or warm at 60 °C for 2-5 mins as necessary until cream is dispersed.
      5.1.3 Let the sample cool down to room temperature, then make up to volume with mobile phase and mix well. Dilute quantitatively and stepwise if necessary, with mobile phase.
      5.1.4 Filter sample solution through disposable syringe filter (4.3) into vial with cap.

Note: The amount of the final concentration of sample solution shall be within calibration curve. If necessary, appropriate dilutions may be done as following guide.
5.2 Preparation of spiked sample solution for determination of percent recovery

5.2.1 Accurately weigh sample as in 5.1.1 (duplicate C and D).

5.2.2 Add known amount of standard to the sample, then follow procedure 5.2.1. Calculate the concentration of spiked standard at level 100% or 50% of concentration of SA in sample.

5.2.3 Dissolve spiked sample as in 5.1.1 and dilute quantitatively, and stepwise if necessary, with mobile phase. Filter spiked sample solution through disposable syringe filter (4.3) into vial with cap.

Note: The amount of standard addition to be calculated at level 100% or 50% of concentration of SA in sample and the final concentration shall be within calibration curve as following guide.

5.3 High performance liquid chromatography (HPLC)

5.3.1 Chromatographic conditions

5.3.1.1 Mobile phase: MeOH : 1.5% glacial acetic acid (55 : 45) by volume
5.3.1.2 Flow rate: 1.0 mL/minute
5.3.1.3 Photodiode array detection wavelength: 200 – 350 nm and λmax at 236 or 302 nm
5.3.1.4 Column: Hypersil Gold C18, 5 µm, 150 mm. x 4.6 mm. Id
5.3.1.5 Run time: 5 mins (for Standard calibration solutions) 8-15 mins (for sample solutions)

5.4 Sequence of injection to the HPLC system.
Sequently inject the prepared solution to HPLC and record peak area as follows:

5.4.1 System suitability: Inject standard solution, S1 (3.3) to examine the retention time and triplicate injections to determine standard deviation of peak area, tailing factor, resolution and K prime. The acceptance criteria are as follows

<table>
<thead>
<tr>
<th>Name</th>
<th>%RSD of peak area (n=3)</th>
<th>Tailing factor</th>
<th>K prime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic acid</td>
<td>≤ 3</td>
<td>≤ 2.0</td>
<td>≥ 2</td>
</tr>
</tbody>
</table>

5.4.2 Inject S1, S2, S3, S4 and S5 (3.3), respectively for construction of the calibration curve.

5.4.3 Inject S3 (to compare the peak area with the peak area from calibration curve, % RPD should be < 3 %)

5.4.4 Inject mobile phase

5.4.5 Inject sample solution 1A, 1B, 1C, 1D

5.4.6 Inject S3 (to compare the peak area with the peak area from calibration curve, % RPD should be < 3 %)

5.4.7 Inject mobile phase

5.4.8 If there are more samples, inject S3 and mobile phase for each 4-10 injections. % RPD should be < 3 %

5.4.9 Last vial is S3 to compare the peak area with the peak area from calibration curve, % RPD should be < 3 %
6. CALCULATION

6.1 Construction of calibration curve between concentration and peak area of standard salicylic acid solutions. From linear regression equation:

\[ A_0 = b_1 C_0 + b_0 \]

Where

- \( b_1 \) = slope
- \( b_0 \) = intercept
- \( C_0 \) = concentration of salicylic acid \( \mu \text{g/mL} \)
- \( A_0 \) = peak area

6.2 Calculation salicycic acid; SA in percentage by mass, using the formula:

\[ \text{salicylic acid (\% w/w)} = \frac{\text{conc. of SA in sample soln (\mu g/mL) \times dilution factor}}{\text{sample weight (g) \times 1,000 \times 1,000}} \times 100 \]

\[ \text{dilution factor} = \frac{\text{initial volume of sample soln (mL) \times dilution volume (mL)}}{\text{volume from stock sample solution (mL)}} \]

6.3 % Recovery

\[ \% \text{recovery} = \frac{S - U}{C_{SA}} \times 100 \]

Where,

- \( S \) = concentration of salicylic acid in spiked sample, % w/w
- \( U \) = concentration of salicylic acid in un-spiked sample, % w/w
- \( C_{SA} \) = concentration of salicylic acid added, % w/w

6.4 % Relative Percent Different

\[ \% \text{RPD} = \frac{A_2 - A_1}{A_1 + A_2} \times 100 \]

Where,

- \( A_1 \) = peak area of S3 in calibration curve
- \( A_2 \) = peak area of S3 when inject interval in sequence of injection
7. REMARKS

7.1 Method validation information

7.1.1 Precision

7.1.1.1 Within day

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cream product (%)</th>
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<tr>
<td>Within day (7 replicates) : %RSD</td>
<td>0.04</td>
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7.1.1.2 Different days (Intermediate precision)

<table>
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<tr>
<th>Parameter</th>
<th>Cream product</th>
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<tbody>
<tr>
<td>Between-day (5 days/7 replicates each) : p-value</td>
<td>0.47</td>
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7.1.2 Limits

<table>
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<th>Parameter</th>
<th>Cream product (%w/w)</th>
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<tbody>
<tr>
<td>Limit of Detection (LOD)</td>
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<tr>
<td>Limit of Quantitation (LOQ)</td>
<td>0.01</td>
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7.1.3 Expanded uncertainty

<table>
<thead>
<tr>
<th>Salicylic acid in Cream product</th>
<th>Concentration (%w/w)</th>
<th>Expanded uncertainty at 95% confidence level % w/w</th>
<th>Relative</th>
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<tr>
<td></td>
<td>5.00</td>
<td>0.45</td>
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7.2 Chromatogram and spectrum of BHA (salicylic acid)
<table>
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<th>Title</th>
<th>Revision n°</th>
<th>date</th>
<th>Document No</th>
</tr>
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<td>DETERMINATION OF SALICYLIC ACID (BETA HYDROXY ACID) IN COSMETIC PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)</td>
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