	Title	Revision n°	date	Document No
	DETERMINATION OF SALICYLIC ACID (BETA HYDROXY ACID) IN COSMETIC PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)	0	17/11/2017	009

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 (H_2O)

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)

Preparation of mobile phase

Transfer 550 mL of methyl alcohol, 450 mL of 1.5% v/v glacial acetic acid into glass bottle 1000 ml, mix thoroughly upon ultrasonic bath for 5-10 min. (or degas process by unchanged ratio of mobile phase)

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 λ_{max} at 236 or 302 nm

5.3.1.4 Column: Hypersil Gold C18, 5 µm, 150 mm. x 4.6 mm. Id

5.3.1.4 Injection volume: 20 µL

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.

Sequentially 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

Name	%RSD of peak area (n=3)	Tailing factor	K prime
Salicylic acid	≤ 3	≤ 2.0	≥ 2

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:

$$A_0 = b_1C_0 + b_0$$

Where

b_1 = slope
b_0 = intercept
C_0 = concentration of salicylic acid µg/mL
A_0 = peak area

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

$$salicylic\ acid\ (\%\,w/w) = \frac{conc.of\ SA\ in\ sample\ soln\ \left(\frac{\mu g}{ml}\right) \times\ dilution\ factor}{sample\ weight\ (g) \times\ 1{,}000\ \times\ 1{,}000} \times\ 100$$

$$dilution\ factor = \frac{initial\ volume\ of\ sample\ soln\ (ml)\ \times\ dilution\ volume\ (mL)}{volume\ from\ stock\ sample\ solution\ (mL)}$$

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

$$\%\,\text{RPD} = \frac{A_2 - A_1}{\frac{A_1 + A_2}{2}} \times 100$$

Where,
A1 = peak area of S3 in calibration curve
A2 = 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

Parameter	Cream product (%)
Within day (7 replicates): %RSD	0.04

7.1.1.2 Different days (Intermediate precision)

Parameter	Cream product
Between-day (5 days/7 replicates each) : p-value	0.47

7.1.2 Limits

Parameter	Cream product (%w/w)
Limit of Detection (LOD)	0.005
Limit of Quantitation (LOQ)	0.01

7.1.3 Expanded uncertainty

Salicylic acid in	Concentration (%w/w)	Expanded uncertainty at 95% confidence level	
		% w/w	Relative
Cream product	5.00	0.45	0.04

7.2 Chromatogram and spectrum of BHA (salicylic acid)