	Title	Revision n°	Date	Document N°
	DETERMINATION OF GLYCOLIC ACID AND LACTIC ACID (ALPHA HYDROXY ACID) BY HIGH PERFORMANCE LIQUID CHROMATOGRAPH(HPLC) USING REVERSED PHASE SYSTEM		19/06/19	ACM 010

1. SCOPE AND FIELD OF APPLICATION

The method describes the determination of alpha hydroxyl acid (glycolic acid and lactic acid) in cosmetic products.

2. PRINCIPLE

The alpha hydroxyl acid (AHAs) such as glycolic acid and lactic acid are used in skin care products for skin whitening and anti-aging through gentle peeling. These substances are water soluble which can be separated by HPLC. The concentrations of glycolic acid and lactic acid in cosmetic products can be determined from the peak area of glycolic acid and lactic acid in sample solution with the calibration curve of glycolic acid and lactic acid standards.

3. REAGENTS

General: all reagents used shall be of analytical purity.
Water shall be distilled water, or water of at least equal purity.

3.1 Potassium dihydrogen phosphate (KH_2PO_4), AR grade
3.2 2 M Phosphoric acid (H_3PO_4)
3.3 Distilled or deionized water
3.4 1 M Sodium hydroxide (NaOH)
3.5 Mixed Standard solution
Prepare 0.1 % (w/v) solution of glycolic acid and lactic acid in H_2O
3.6 Standard calibration solutions
Using the mixed standard stock solutions, prepare standard calibration solutions concentration of 10, 20, 30, 40 and 50 μg/mL in mobile phase. Pipette the mix standard solution 100, 200, 300,400 and 500 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.
3.7 Mobile phase: 0.2 M phosphate buffer pH 3.2
Preparation of 0.2 M phosphate buffer pH 3.2
Dissolve 27.22 g (± 0.1 mg) of potassium dihydrogen phosphate (KH_2PO_4) in 800 mL distilled water into 1000 mL of volumetric flask with stopper. Add about 1.0 mL of phosphoric acid. Measure the pH of the buffer with pH meter. The pH value should be 3.2, otherwise adjust to pH 3.2 by the addition of 1 M NaOH or 2 M H_3PO_4 and adjust to volume with distilled water.

4. APPARATUS

Normal laboratory equipment and:

4.1 High Performance Liquid Chromatograph (HPLC) with a photodiode array detector and autosampler
4.2 Analytical column: Prevail Organic Acid 5 μm, 4.6 mm (id) x 250 mm; Alltech (Silica based column coated with sulfonated polystyrene divinylbenzene or equivalent)
4.3 Pipette, 100 – 1000 μL
4.4 Volumetric flask 10, 25, and 1000 mL
4.5 Disposable syringe filter 0.45 μm (PVDF or equivalent)
4.6 Ultrasonic bath
4.7 Electronic balance, 0.1 mg
4.8 pH meter

5. PROCEDURE

5.1 Preparation of sample solution:

5.1.1 Weigh Accurately 0.5 g of sample (duplicate A and B) into 25 mL volumetric flask. Add about 10 mL of methyl alcohol to the volumetric flask and mixed well by vortexing 3-5 minutes. Add about 10 mL of mobile phase to dissolve by vortexing 5 minutes +/- sonicate 10-15 minutes as necessary until cream was dispersed. Make up to volume with mobile phase when the sample was at room temperature. Dilute quantitatively and stepwise if necessary, with mobile phase. Filter sample solution through disposable syringe filter (4.5) into vial with cap.

Note 1. Add small volume of the anti-foaming agent such as ethanol or methanol to decreasing the foam before make up volume.

2. The amount of the final concentration of sample solution shall be within calibration curve as following guide.

Concentration of GA or LA in sample (%w/w)	Weight of sample (g) in 25 mL	Dilution		Final conc. of sample (µg/mL)
		Pipetted volume (mL)	Make up volume (mL)	
0.1 - 0.15	0.50	-	-	20-30
0.2 - 0.5	0.50	3	10	12 – 30
0.6 – 1.0	0.50	1	10	12 – 20
1.1 – 2.0	0.50	0.50	10	11 - 20
3.0 -4.0	0.50	0.25	10	15 – 20

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 Pipette mixed standard stock solution or weigh accurately of standard and transfer into the spiked sample volumetic flask.

5.2.3 Prepare the spiked sample solutions according to steps 5.1.2 to 5.1.4.

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

Concentration of GA or LA in sample (%w/w)	Weight of sample (g) in 25 mL	50 % standard addition (µg)	Dilution		Final conc. of spiked sample (µg/mL)
			Pipetted volume (mL)	Make up volume (mL)	
0.1 - 0.15	0.50	250-375	-	-	30 - 45
0.2 - 0.5	0.50	500-1250	3	10	18 - 45
0.6 – 1.0	0.50	1500-2500	1	10	18 - 30
1.1 – 2.0	0.50	2750-5000	0.50	10	16.5 - 30
3.0 -4.0	0.50	7500-10000	0.25	10	22.5 – 30

5.3 High performance liquid chromatography (HPLC)

5.3.1 Chromatographic conditions

5.3.1.1 Mobile phase: 0.2 M phosphate buffer pH 3.2

5.3.1.2 Flow rate: 1.2 mL/min.

5.3.1.3 Photodiode array detection wavelength : 200 – 350 nm and λmax = 210 nm

5.3.1.4 Runtime : 6 mins.

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 20 µL of mixed standard solution, S1 (3.6) 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	Retention time (min)	%RSD of peak area (n=3)	Tailing factor	Resolution	K prime
Glycolic acid	3.0 ± 0.5	≤ 3	≤ 1.3	≥ 1.5	≥ 1.0
Lactic acid	4.2 ± 0.5	≤ 3	≤ 1.3	≥ 5	≥ 1.8

5.4.2 S1, S2, S3, S4, S5 (3.6) for plotting the calibration curve.

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

5.4.4 Mobile phase

5.4.5 Sample solution 1A, 1B, 1C and 1D

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

5.4.7 Mobile phase

5.4.8 If there are more samples, inject S3 and mobile phase for each 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 Plotting calibration curve between concentration and peak area of each standard glycolic acid or lactic acid solutions. From linear regression equation:

$$A_0 = b_1C_0 + b_0$$

When

b_1 = slope

b_0 = intercept

C_0 = concentration of glycolic acid or lactic acid µg/mL

A_0 = peak area

6.2 Calculation glycolic acid; GA or lactic acid; LA in percentage by mass, using the equation

$$\text{GA or LA (\%w/w)} = \frac{\text{conc. of GA or LA in sample solution (µg/mL) x dilution factor}}{\text{sample weight (g)} \times 1{,}000 \times 1{,}000} \times 100$$

$$\text{dilution factor} = \frac{\text{initial volume of sample sol}^{n}\text{ (mL) x dilution volume (mL)}}{\text{volume from stock sample solution (mL)}}$$

6.3 % Recovery

$$\%\,\text{recovery} = \frac{S - U}{C_{SA}} \times 100$$

When

S = concentration of glycolic acid or lactic acid in spiked sample, % w/w
U = concentration of glycolic acid or lactic acid in sample, % w/w
C_{SA} = concentration of standard glycolic acid or lactic acid added, % w/w

6.4 % Relative Percent Different

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

When

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 of glycolic acid and lactic acid in body lotion

7.1.1 Precision

7.1.1.1 Within day

Parameter	ga	la
- Precision (system) : standard (expressed as % RSD)	2.56	2.75
- Precision (method) : Repeatability : expressed as % RSD (within day)	0.61	0.53

7.1.1.2 Different days

Parameter	ga	la
- Intermediate precision : expressed as %RSD (between day)	p = 0.20	p = 0.28

7.1.2 Accuracy

Parameter	ga	la
: expressed as recovery (%w/w)	93 -106	96 -104

7.1.3 Linearity and Range

7.1.3.1 System linearity

Parameter	ga	la
Linearity and Range	10-50µg/mL	10-50µg/mL
- System linearity : expressed as correlation coefficient (r)	0.9998	0.9993

7.1.3.2 Method linearity (50% - 150%)

Parameter	ga	la
- Method linearity 50% - 150%) : expressed as correlation coefficient (r)	0.999	0.999

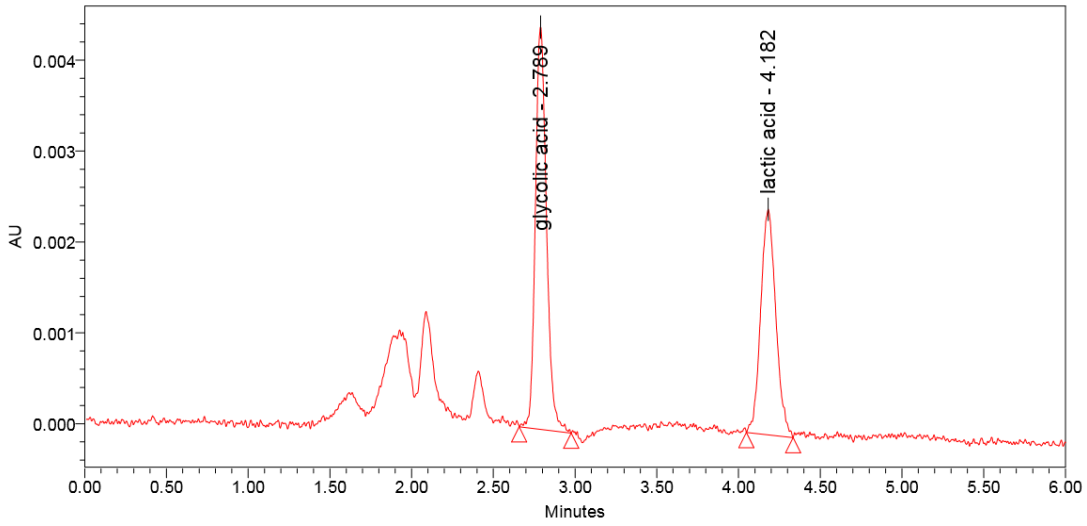
7.1.3.3 Determination limits

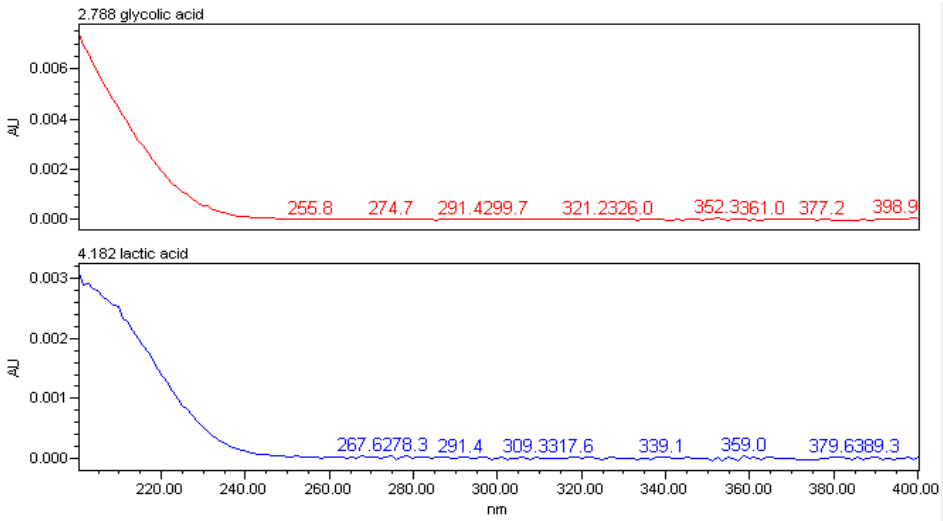
Parameter	ga	La
Limit of Quantitation (LOQ)	0.05 %w/w	0.05 %w/w
Limit of Detection (LOD)	0.025 % w/w	0.02 %w/w

7.2 Expanded uncertainty

Alpha-hydroxy acid	Concentration of alpha-hydroxy acid, % w/w	Expanded uncertainty	
		Relative	% w/w
Glycolic acid	0.78	0.02	0.04
Lactic acid	0.56	0.03	0.09

7.3 Chromatogram and spectrum of glycolic acid and lactic acid reference standard solution.




2.788 glycolic acid
0.006
0.004
0.002
0.000
AU
255.8
274.7
291.4299.7
321.2326.0
352.3361.0
377.2
398.9
4.182 lactic acid
0.003
0.002
0.001
0.000
AU
267.6278.3
291.4
309.3317.6
339.1
359.0
379.6389.3
220.00
240.00
260.00
280.00
300.00
320.00
340.00
360.00
380.00
400.00
nm