Application Note

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Rapid Determination of 10 α-Hydroxy Acids

Quantifying cosmetic ingredients using the Agilent InfinityLab Poroshell 120 Aq-C18 column and the Agilent 1260 Infinity II Prime LC System

Authors

Baogang Yang
Shanghai Institute of Quality Supervision and Inspection Technology

Hui Xu, Bo Chen, and Xinlei Yang
Agilent Technologies (China) Co., Ltd

Abstract

This application note describes a rapid HPLC method using the Agilent 1260 Infinity II Prime LC system and the Agilent InfinityLab Poroshell 120 Aq-C18 (4.6 × 100 mm, 2.7 µm) column for 10 α-hydroxy acids (glucuronic acid, tartaric acid, glycolic acid, malic acid, lactic acid, citric acid, 2-hydroxybutyric acid, mandelic acid, diphenylglycolic acid, and hydroxyoctanoic acid) quantification in cosmetic products. The 10 α-hydroxy acids were determined in 12 minutes by a gradient method using methanol and 0.1 mol/L diammonium hydrogen phosphate aqueous solution as mobile phases. Compared with the *Detection Methods for 10 α-Hydroxy Acids in Cosmetics* in Circular No. 12 in 2019 by the China National Medical Products Administration (NMPA), the method developed in this note provides higher resolution and better reproducibility in a shorter analysis time, and it is suitable for rapid analysis of α-hydroxy acids in cosmetics.

Introduction

α-Hydroxy acids are a class of organic acids with hydroxyl (or ketone) groups at the α position of the molecular structure, and are widely used for cleansing, skin care, and other cosmetic purposes. These compounds have the effects of slowing cell aging, enhancing skin cell regeneration, promoting stratum corneum peeling, and removing wrinkles. However, the use of such ingredients can irritate the skin and enhance the skin's sensitivity to ultraviolet rays, and so increasing attention has been paid to their safety. A study on α-hydroxy acids released by the US FDA pointed out that long-term use of cosmetics containing α-hydroxy acids may weaken the skin's resistance to ultraviolet rays, age the skin, accelerate the destruction or death of cells, cause skin redness, blistering, and burns, and lead to permanent damage to the skin. Therefore, α-hydroxy acids are an important indicator of the hygienic management of cosmetics.[1,2,3]

China's Cosmetic Safety and Technical Specifications 2015 edition (referred to as the Specifications from this point onwards) stipulates that the total amount of α-hydroxy acids in cosmetic formulas shall not be greater than 6%, and describes detection methods for five α-hydroxy acids, including tartaric acid. However, these methods only cover a few components and are not enough to achieve comprehensive monitoring.[4] For this reason, Circular No. 12 issued by the China NMPA in 2019 provides detection methods for 10 α-hydroxy acids in cosmetics (referred to as the Notification methods from this point onwards)[5], which is used to replace the 2015 version of the Specification method and expand the testing scope of α-hydroxy acids detection. The assay uses a conventional C18 column (4.6 × 250 mm, 5 µm) and takes 28 minutes for one analysis. Also, tartaric acid interacts with the matrix, resulting in poor separation.

In this application note, an HPLC method for rapid analysis of 10 α-hydroxy acids in cosmetics was developed with a 1260 Infinity II Prime HPLC system and an InfinityLab Poroshell 120 Aq-C18 (4.6 × 100 mm, 2.7 µm) column. Compared with the Notification methods, this method provides higher resolution, faster analysis, and higher throughput.

Experimental

Reagents and samples

Mixed standard solutions of 10 α-hydroxy acids were purchased from First Standard (concentrations: 8,002.7 mg/L glucuronic acid, 4,002.0 mg/L tartaric acid, 7,994.3 mg/L glycolic acid, 8,008.0 mg/L malic acid, 10,001.6 mg/L lactic acid, 8,000.3 mg/L citric acid, 10,002.6 mg/L 2-hydroxybutyric acid, 201.4 mg/L mandelic acid, 200.8 mg/L diphenylglycolic acid, and 4,002.8 mg/L hydroxyoctanoic acid); diammonium hydrogen phosphate was purchased from Anpel (Shanghai); methanol and isopropanol were purchased from Merck; and phosphoric acid was purchased from DIKMA, all of which were HPLC grade. High-purity water was obtained from the Milli-Q water purifier system (USA); cosmetic samples were provided by customers.

Instruments

The 1260 Infinity II Prime HPLC system was equipped with the following modules:

- Agilent 1260 Infinity II Prime flexible pump (G7104C)
- Agilent 1260 Infinity II vialsampler (G7129C)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A)
- Agilent 1260 Infinity II DAD (G7117C)

LC column

Agilent InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm (part number 695975-742).

Software

Agilent OpenLab CDS 2.6 software for instrument control and data analysis.

Preparation of mixed standard working solutions

Pipette the mixed standard stock solutions 12.5, 25, 50, 100, 250, and 500 µL, respectively. Dilute with water up to a volume of 1 mL. Mix well and prepare a series of mixed standard working solutions. The concentrations are shown in Table 1.

Table 1. Concentration of mixed standard working solution.

Compound	Concentration (mg/L)					
Glucuronic Acid	100	200	400	800	2,001	4,001
Tartaric Acid	50	100	200	400	1,001	2,001
Glycolic Acid	100	200	400	799	1,999	3,997
Malic Acid	100	200	400	801	2,002	4,004
Lactic Acid	125	250	500	1000	2,500	5,001
Citric Acid	100	200	400	800	2,000	4,000
2-Hydroxybutyric Acid	125	250	500	1,000	2,501	5,001
Mandelic Acid	2.5	5	10	20	50	101
Diphenylglycolic Acid	2.5	5	10	20	50	100
Hydroxyoctanoic Acid	50	100	200	400	1,001	2,001

Sample preparation

Sample preparation was carried out according to the Notification method[5]; the specific procedure was as follows:

Weigh 1 g of sample (accurate to 0.0001 g) into a 10 mL stopper tube.

↓

Place in a 90 °C water bath for 30 min to remove volatile organic solvents.

↓

Add water to a volume of 10 mL and mix well by vortexing for 30 s (if the sample is in a dosage form that is difficult to disperse in water, such as "water-in-oil", 1 mL of isopropanol can be added first, the sample vortexed, and mixed for 30 s to make the dispersion uniform. Then, add water to a volume of 10 mL).

↓

Perform ultrasonic extraction at 60 °C for 30 min; take an appropriate amount of sample and centrifuge it at 10,000 rpm for 15 min.

↓

Filter the supernatant through a 0.45 µm membrane as the solution to be measured.

Chromatographic conditions

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm
Column Temperature	25 °C
Injection Volume	5 µL
DAD	UV wavelength: 214 nm Reference wavelength: 360 nm, 10 Hz
Mobile Phase A	100 mmol/L diammonium hydrogen phosphate solution (pH 2.8)
Mobile Phase B	Methanol
Gradient	Time (min) B (%) Flow Rate (mL/min) 0 0 0.7 2.3 0 0.7 2.4 0 1.0 4.0 50 1.0 4.5 65 1.0 7.5 65 1.0 7.6 0 0.7 10 0 0.7 Postrun 2 min

Results and discussion

Selection of column

A conventional C18 column (250 mm × 4.6 mm, 5 µm) was used in the Notification method. To improve analysis efficiency and shorten analysis time, various column chemistries that can be used with 100% aqueous phase. Agilent columns SB-AQ, ZORBAX Bonus-RP, Polaris C18-A, Aq-C18, PFP, SB-Phenyl, and Phenyl-Hexyl were compared for the analysis of 10 α-hydroxy acids. By optimizing the buffer concentration, pH value, and gradient setting for the mobile phase, all the 10 α-hydroxy acids were well retained and separated on the InfinityLab Poroshell 120 Aq-C18 and Polaris C18-A columns (Figure 1). Considering its resolution and analytical efficiency, the InfinityLab Poroshell 120 Aq-C18 Column (4.6 × 100 mm, 2.7 µm) was selected for this method. Each of the target compounds achieved a minimum resolution of more than 2.5 on this column, and analysis time was reduced to 12 minutes. This method is faster and more efficient than the Notification method.

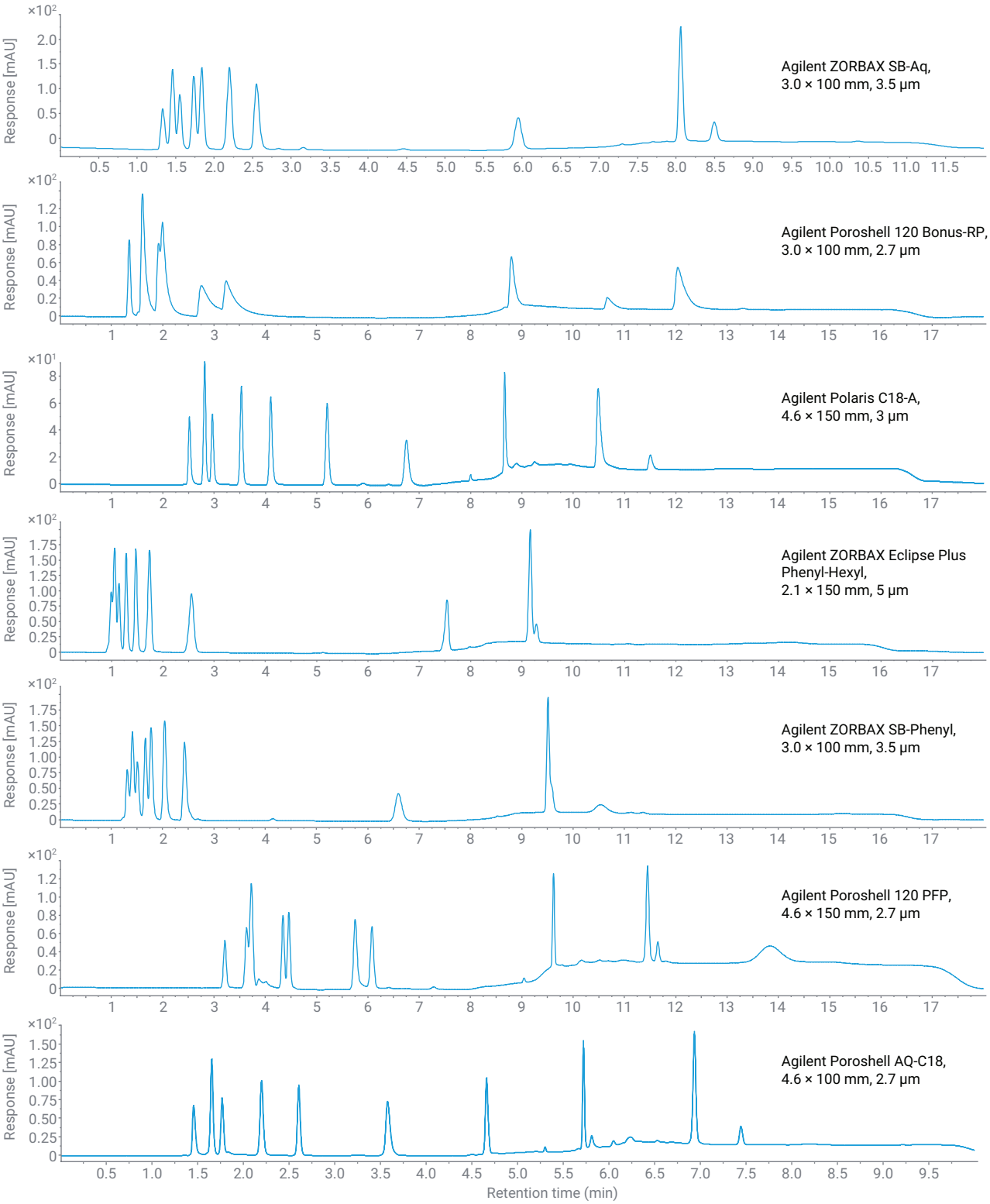

Figure 1. Chromatograms of separation of 10 mixed standard solutions of α-hydroxy acids on various columns.

Retention time and peak area precision

Under the chromatographic conditions described in the Experimental section, six injections were repeated using the midpoint concentration of the mixed standard working solution to obtain the average retention time and RSD values for the peak area. The results are shown in Table 2. As shown in Table 2, the retention time and the RSD for the peak area were less than 0.05% and 0.5%, which indicate good method reproducibility and precision.

Table 2. Retention time and peak area precision (n = 6).

Compound	Area RSD (%)	Retention Time RSD (%)
Glucuronic Acid	0.135	0.007
Tartaric Acid	0.213	0.013
Glycolic Acid	0.232	0.009
Malic Acid	0.228	0.034
Lactic Acid	0.181	0.017
Citric Acid	0.416	0.094
2-Hydroxybutyric Acid	0.184	0.020
Mandelic Acid	0.141	0.005
Diphenylglycolic Acid	0.088	0.018
Hydroxyoctanoic Acid	0.108	0.026

Standard curve

The mixed standard working solutions were injected according to the chromatographic conditions described in the Experimental section. The concentration of each compound was used as the X-axis and the corresponding peak area was used as the Y-axis to establish a standard curve for each compound. The linear equations and associated coefficients are shown in Table 3. As shown, 10 α-hydroxy acids produced good linearity over the concentration range investigated.

Table 3. Linear equations and correlation coefficients (n = 6) for standard curves.

Compound	Solution Concentration Range for Standard Curve	Curve Equation	R^2
Glucuronic Acid	100 to 4,001 mg/L	y = 0.1931x + 1.6989	0.99971
Tartaric Acid	50 to 2,001 mg/L	y = 0.7149x – 2.9819	0.99859
Glycolic Acid	100 to 3,997 mg/L	y = 0.2085x + 3.1055	0.99942
Malic Acid	100 to 4,004 mg/L	y = 0.3390x – 6.5032	0.99924
Lactic Acid	125 to 5,001 mg/L	y = 0.2130x – 2.2917	0.99967
Citric Acid	100 to 4,000 mg/L	y = 0.3373x – 5.2263	0.99936
2-Hydroxybutyric Acid	125 to 5,001 mg/L	y = 0.2218x – 0.2268	0.99976
Mandelic Acid	2.5 to 101 mg/L	y = 10.4509x – 2.5589	0.99968
Diphenylglycolic Acid	2.5 to 100 mg/L	y = 17.6563x – 2.8104	0.99989
Hydroxyoctanoic Acid	50 to 2,001 mg/L	y = 0.0776x – 0.3621	0.99944

Real sample and spiked test

The real cosmetic samples and spiked samples were analyzed using the described method. The results are shown in Figure 2 and Table 4. It can be seen that α-hydroxy acids were not detected in either sample (moisturizing water and moisturizing cream, respectively); recovery for 10 target compounds in the two spiked samples ranged from 85% to 110%, and the interference peak at 1.58 minutes in the sample can be separated from tartaric acid.

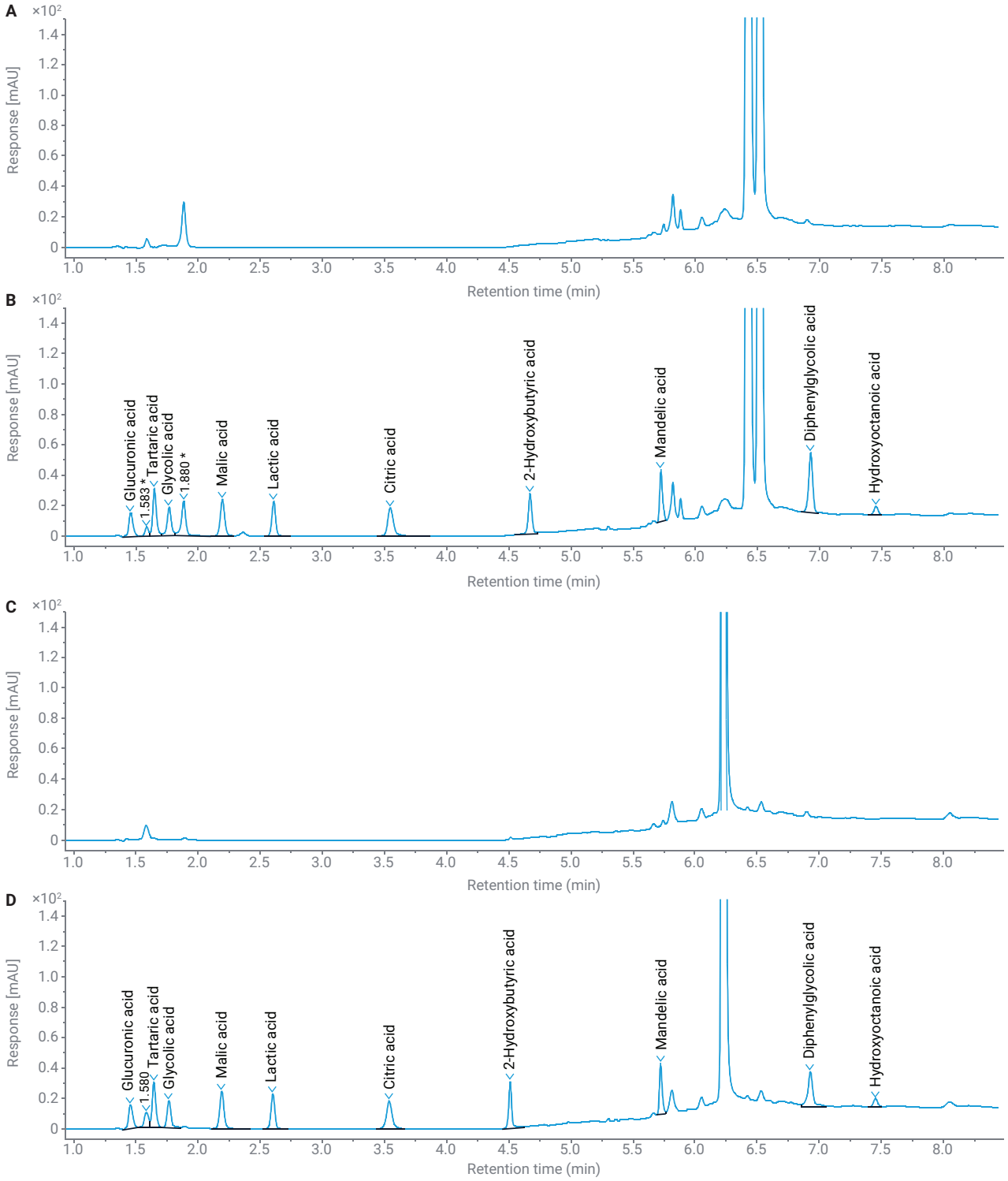

Figure 2. Chromatograms of real samples and spiked samples: (A) Moisturizing water sample; (B) spiked moisturizing water sample; (C) moisturizing cream sample; (D) spiked moisturizing cream sample.

Table 4. Recovery results of spike samples.

No.	Compound	Spiked Concentration (mg/L)	Moisturizing Water		Moisturizing Cream	
			Measured Concentration (mg/L)	Recovery (%)	Measured Concentration (mg/L)	Recovery (%)
1	Glucuronic acid	200	194.838	97.42	190.384	95.19
2	Tartaric acid	100	96.019	96.02	89.995	90.00
3	Glycolic acid	200	204.166	102.1	178.737	89.37
4	Malic acid	200	200.047	100.0	197.768	98.88
5	Lactic acid	250	243.496	97.40	241.390	96.56
6	Citric acid	200	203.240	101.6	194.982	97.49
7	2-Hydroxybutyric acid	250	243.496	97.40	245.834	98.33
8	Mandelic acid	5	5.416	108.3	5.129	102.6
9	Diphenylglycolic acid	5	5.149	103.0	4.314	86.28
10	Hydroxyoctanoic acid	200	185.194	92.60	178.658	89.33

Conclusion

A method was developed with the Agilent 1260 Infinity II Prime HPLC system and the Agilent InfinityLab Poroshell 120 Aq-C18 column (4.6 × 100 mm, 2.7 µm) for the rapid analysis of 10 α-hydroxy acids in cosmetics. This method separated all 10 α-hydroxy acids in 12 minutes with a minimum resolution of 2.5 for each compound and complete separation of interfering substances in the samples. The precision and accuracy of the method were good, and the RSD of the peak area and the retention time of six injections were less than 0.05% and 0.5%, respectively. Recovery results for spiked sample were in the range of 85% to 110%. This method is suitable for rapid quantitative analysis of α-hydroxy acids in cosmetics.

References

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DE96289756

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Printed in the USA, June 28, 2023
5994-6084EN

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