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Determination of the Content of α-Hydroxy Acids in Cosmetics by HPLC

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Abstract: A HPLC method was established for the determination of 10 α - hydroxy acids, glucuronic acid, tartaric acid, glycolic acid, malic acid, l actic acid, citric acid, 2-hydroxybutyric acid, mandelic acid, diphenylethanoic acid and hydroxyoctanoic acid in cosmetics. The sample was separated on the CAPCELL PAK C18 MG column(4.6×250,5μm),with 0.1 mol / L diammonium hydrogen phosphate solution as mobile phase. The flow rate was 1 mL·min-1, and the column temperature was 25 ℃. Linearity of glucuronic acid, tartaric acid, glycolic acid, malic acid, lactic acid, citric acid,2-hydroxybutyric acid, mandelic acid, diphenylethanoic acid and hydroxyoctanoic acid standards were established within the range of 103.5~2070 μg·mL-1,65~1300 μg·mL-1, 101.5~2025 μg·mL-1, 113.35~2025 μg·mL-1, 113.35~2267 μg·mL-1, 250~5000 μg·mL-1, 105~2100 μg·mL-1, 126.35~2527 μg·mL-1, 2.96~59.2 μg·mL-1,3.1~62 μg·mL-1, and 105.75~2115 μg·mL-1 with the correlation coefficient above 0.997 respectively. The average recoveries were 90.46%-96.48%,90.13%- 100.16%, 92.81%-98.73%,92.84%-97.74%,94.11%-100.17%,86.73-115.12,90.13-98.99,87.04-96.73, 90.23-95.41,92.54-96.55and91.76-100.44 ,respectively. Tartaric acid and malic acid were not detected in four samples. The contents of hydroxyacetic acid were 3.31%-10.02%. The content of lactic acid was 1.51% in one sample. The content of citric acid was 0.03% in one sample. In 10 batches of samples, α - hydroxy acid, glucuronic acid, tartaric acid, glycolic acid, malic acid, mandelic acid and diphenyl alcohol acid were not detected. The content of lactic acid S11 was 1.57%, citric acid S1 and S2 were 0.22% and 0.17%, 2-hydroxybutyric acid S1 and S8 were 2.71% and 1.47%, and hydroxyoctanoic acid S11 was 0.12%. All 12 kinds of cosmetics were qualified and did not exceed the national content requirements. The method is simple, sensitive and reproducible, and can be used for the determination of α - hydroxy acid in cosmetics.

1. Introduction

Hydroxy acids refer to acids that contain a hydroxyl group, which includes not only acids with a hydroxyl group at the α-position but also those that have hydroxyl groups at other positions in addition to the α-position [1]. These compounds are mainly used in cosmetics as exfoliants, moisturizers, antioxidants, etc. Once the stratum corneum forms, α-hydroxy acids accelerate the shedding of epidermal cells by reducing the adhesion between them, thereby improving the appearance of the skin [2]. However, excessive use of α-hydroxy acids can lead to significant irritation, such as redness, changes in skin color, and swelling [3]. According to China's Cosmetic Hygiene Standards, the total amount of α-hydroxy acids in cosmetic formulations must not exceed 6%, and the product's pH in use must not be lower than 3.5. Therefore, it is necessary to establish a high-efficiency, sensitive, and accurate method for detecting the amount of α-hydroxy acids in cosmetics [4]. According to the Cosmetic Hygiene Supervision Regulations, cosmetics are defined as daily chemical industrial products that are applied by rubbing, spraying, or other similar methods to any part of the human body (such as skin, hair, nails, lips, etc.) to achieve the purposes of cleaning, eliminating unpleasant odors, skincare, beauty, and decoration [5]. Although some articles have used liquid chromatography methods to detect α-hydroxy acids in cosmetics, they either involve fewer cosmetic products or fewer types of α-hydroxy acids [6,7]. This study selected various products, including facial cleansers, shampoos, lotions, masks, serums, makeup removers, and shower gels, and applied HPLC to determine the content of 10 α-hydroxy acids: glucuronic acid, tartaric acid, glycolic acid, malic acid, lactic acid, citric acid, 2-hydroxybutyric acid, mandelic acid, diphenylethanoic acid, and hydroxyoctanoic acid. The study verified that this method can effectively analyze different types of cosmetics.

2. Instruments and Reagents

2.1 Instruments

Dionex U3000 high-performance liquid chromatograph (Agilent Technologies, USA);PH meter ST3100; XSE205DU electronic balance;WVC-D22H ultrasonic cleaner with digital display; TGL16M tabletop high-speed refrigerated centrifuge;HH-6 thermostatic water bath with digital display; CAPCELL PAK C18 MG (4.6 × 250 mm, 5 μm) chromatographic column.

2.2 Reagents

Standards: Glucuronic acid (batch no. 140648-201804, purity 99.80%), tartaric acid (batch no. 190072-201501), glycolic acid (batch no. 190058-201501), malic acid (batch no. 190014-201302), lactic acid (batch no. 4073088, purity 90%), citric acid (batch no. 111679-201602, purity 97.00%), 2-hydroxybutyric acid (batch no. AL19630-10, purity 99.80%), mandelic acid (batch no. 100980-200701), diphenylethanoic acid (batch no. XK190123-04, purity 99.80%), and hydroxyoctanoic acid (batch no. MK190825-07, purity 99.40%) were all purchased from Dr. Ehrenstorfer GmbH.Malic acid (batch no. 190013-201001, purity 100.0%) and citric acid (batch no. 111679-200401, purity 100.0%) were purchased from the National Institutes for Food and Drug Control, China.Methanol (chromatographic grade, Merck, Germany), formic acid (analytical grade, China National Pharmaceutical Group), and ultrapure water were used.

Samples: S1 (Pond's facial cleanser), S2 (Opera Coix seed water), S2 (Correction niacinamide essence), S3 (Garnier makeup remover), S4 (Osmunda facial cleanser), S5 (Huimei beauty mask), S6 (Wen Biquan mask), S7 (Franlinka mask), S8 (Mediheal mask), S9 (Pearl makeup remover), and S10 (Encounter fragrance shower gel) were sourced from sample collections at the Dali Institute for

Food and Drug Quality Control.

3. Methods and Results

3.1 Chromatographic Conditions

The CAPCELL PAK C18 MG (4.6 × 250 mm, 5 μm) column was used with a column temperature of 25 ℃. The mobile phase consisted of 0.1 mol/L diammonium hydrogen phosphate solution, adjusted to a pH of 3.0 with phosphoric acid. Methanol was used, with a flow rate of 0.4 mL/min, and the injection volume was 5 μL.

3.2 Preparation of Standard Solutions

Accurately weigh 16.56 mg of glucuronic acid, 10.40 mg of tartaric acid, 16.20 mg of glycolic acid, 18.14 mg of malic acid, 40.00 mg of lactic acid, 16.80 mg of citric acid, 20.22 mg of 2-hydroxybutyric acid, 5.92 mg of mandelic acid, 6.20 mg of diphenylethanoic acid, and 16.92 mg of hydroxyoctanoic acid. Dissolve each in water using ultrasonication to prepare 1 mg/mL stock solutions of each standard. Store the solutions at 4 ℃. When needed, dilute the stock solutions with ultrapure water to prepare a mixed standard solution, containing approximately 1 mg/mL of each standard (glucuronic acid, tartaric acid, glycolic acid, malic acid, lactic acid, citric acid, 2-hydroxybutyric acid, mandelic acid, diphenylethanoic acid, and hydroxyoctanoic acid), as shown in Table 1.

Table 1: Standard Series Concentrations of 10 Types of α-Hydroxy Acids

α-Hydroxy Acid Components	Glucuronic Acid	Tartaric Acid	Glycolic Acid	Malic Acid	Lactic Acid	Citric Acid	2-Hydroxybutyric Acid	Mandelic Acid	Diphenylethanoic Acid	Hydroxyoctanoic Acid
Mixed Standard Solution, mg/L	103.5	65.0	101.3	113.35	250.0	105.0	126.4	3.0	3.1	105.8
	207.0	130.0	202.5	226.7	500.0	210.0	252.7	5.9	6.2	211.5
	414.0	260.0	405.0	453.4	1000.0	420.0	505.4	11.8	12.4	423.0
	828.0	520.0	810.0	906.8	2000.0	840.0	1010.8	23.7	24.8	846.0
	1035.0	650.0	1012.5	1133.5	2500.0	1050.0	1263.5	29.6	31.0	1057.5
	2070.0	1300.0	2025.0	2267.0	5000.0	2100.0	2527.0	59.2	62.0	2115.0

3.3 Preparation of Reference Solution

Accurately weigh 5.92 mg of mandelic acid and 6.20 mg of diphenylethanoic acid into a 100 mL volumetric flask, and dilute to volume with 20% methanol solution. Shake well. Then, accurately weigh 16.56 mg of glucuronic acid, 10.40 mg of tartaric acid, 16.20 mg of glycolic acid, 18.14 mg of malic acid, 40.00 mg of lactic acid, 16.80 mg of citric acid, 20.22 mg of 2-hydroxybutyric acid, and 16.92 mg of octanoic acid. Dissolve these in 8 mL of the mixed standard solution of mandelic acid and diphenylethanoic acid, shake well, and set aside for use.

3.4 Preparation of Test Solution

Weigh 1 g of the sample (accurate to 0.0001 g) and place it in a 10 mL stoppered colorimetric tube. Heat it in a 90 ℃ water bath for 30 minutes to remove volatile organic solvents. Add water to the 10 mL mark, and vortex thoroughly for 30 seconds. Ultrasonically extract at 60 ℃ for 30 minutes. Take an appropriate amount of the sample and centrifuge it at high speed (10,000 rpm) for 15 minutes. Filter the supernatant through a 0.45 μm membrane to obtain the test solution.

3.5 Linearity, Detection Limit, and Quantification Limit

Accurately pipette an appropriate amount of the mixed reference solution into a 10 mL volumetric flask, and dilute with water to obtain a series of concentrations of the mixed reference solutions. Accurately inject 5 μL of each concentration of the mixed reference solution for analysis. Use concentration ($\mu g \cdot mL^{-1}$) as the x-axis and peak area as the y-axis to perform linear regression. The detection limit for each component is calculated with a signal-to-noise ratio (S/N) of 3, and the quantification limit is calculated with an S/N of 10. The results are shown in Table 2, indicating good linearity within the respective linear ranges of each component, as presented in Table 2.

Table 2: Detection Limits and Quantification Limits

No.	Component Name	Detection Limit (μg)	Quantification Limit (μg)	Regression Equation	R^2
1	Glucuronic acid	0.08	0.24	Y = 0.0031X + 0.21119	0.9977
2	Tartaric acid	0.03	0.09	Y = 0.0084X + 0.3472	0.9972
3	Glycolic acid	0.02	0.06	Y = 0.003X + 0.1964	0.997
4	Malic acid	0.02	0.06	Y = 0.0049X + 0.3551	0.9973
5	Lactic acid	0.05	0.15	Y = 0.0004X + 0.0629	0.9977
6	Citric acid	0.02	0.06	Y = 0.0046X + 0.3289	0.9971
	Tartaric acid	0.08	0.24	Y = 0.0336X + 1.4232	0.9974
7	2-Hydroxybutyric acid	0.04	0.12	Y = 0.0028X + 0.1819	0.9985
8	Mandelic acid	0.001	0.003	Y = 0.1507X + 0.2719	0.9977
9	Diphenylethanoic acid	0.001	0.003	Y = 0.249X + 0.4526	0.9988
10	Octanoic acid	0.02	0.06	Y = 0.002X + 0.0882	0.9992

3.6 Precision Test

Accurately measure 400 μL of each of the 10 α-hydroxy acid standard solutions, dilute with 20% methanol to 1 mL, and shake well for use. Under the same system conditions as the test solution, inject six consecutive times. The relative standard deviations (RSDs) of the chromatographic peak areas for glucuronic acid, tartaric acid, glycolic acid, malic acid, lactic acid, citric acid, tartaric acid, 2-hydroxybutyric acid, mandelic acid, diphenylethanoic acid, and octanoic acid were 2.0%, 1.7%, 2.1%, 2.1%, 2.4%, 2.2%, 2.1%, 2.2%, 1.8%, 2.1%, and 1.7%, respectively, indicating good precision.

3.7 Stability Test

The test solution for recovery was stored at room temperature and injected for analysis at 0, 4, 8, 12, and 24 hours. The relative standard deviations (RSDs) of the peak areas for each component were 2.0%, 1.3%, 0.9%, 0.5%, and 2.6%, respectively, indicating that the test solution remained basically stable within 24 hours.

3.8 Reproducibility Test

Sample S1 was prepared according to the method in section 2.4 to obtain the test solution. The content of citric acid was measured with the following results: 52.5147, 52.5093, 51.7757, 51.0273, 53.9937, and 54.6524. The relative standard deviation (RSD) was 2.57%, indicating good

reproducibility of the method.

3.9 Spike Recovery Test

Nine portions of sample S1, each weighing approximately 0.2 g, were accurately weighed. A suitable amount of mixed reference solution at three different concentration levels was added to each portion. The test solution was prepared according to the method in section 2.4, and samples were injected for analysis. The recovery rates were calculated, and the results are shown in Table 3. The table indicates that the recovery rate for this method is satisfactory.

Table 3: Spike Recovery Test Results

Component	Measured Result	Amount in Sample	Amount in Sample	Average Recovery Rate (%)	Average Recovery Rate (%)
Glucuronic acid	139.7999	0	144.9	96.48	1.23
	378.3531	0	414	91.39	1.88
	987.4478	0	1035	90.46%	7.10
Tartaric acid	91.1501	0	91	100.16	2.05
	234.3335	0	260	90.13	2.05
	627.4181	0	650	96.52	7.96%
Glycolic acid	140.4624	0	141.75	98.73	1.76
	374.7021	0	405	94.18	4.56
	960.5856	0	1012.5	92.81	7.41
Malic acid	155.0993	0	158.69	97.74	2.24
	421.0485	0	453.5	92.84	2.86
	1079.111	0	1133.5	95.20	6.29
Lactic acid	350.5968	0	350	100.17	3.68
	943.1617	0	1000	94.38	7.92
	2352.853	0	2500	94.11	5.25%
Citric acid	278.0558	168.7709	147	86.73	4.21
	420	168.7709	652.26	115.12	6.09
	1151.322	168.7709	1050	93.57	6.74
Tartaric acid	90.0797	0	91	98.99	5.41
	234.3335	0	260	90.13	2.05
	622.9029	0	650	95.83	7.27
2-Hydroxybutyric acid	171.1062	0	176.89	96.73	1.82
	440.0039	0	505.4	87.04	3.46
	1142.9220	0	1263.5	90.46	6.60
Mandelic acid	3.8216	0	4.144	92.22	1.82
	10.68278	0	11.84	90.23	2.66
	28.2424	0	29.6	95.41	6.85
Diphenylethanoic acid	4.1901	0	4.34	96.55	3.12
	11.47548	0	12.40	92.54	5.28
	29.7729	0	31	96.04	6.65
Octanoic acid	148.7026	0	148.05	100.44	2.22
	423	0	389.4166	92.06	5.89
	970.4323	0	1057.5	91.76	8.55

3.10 Determination of Sample Content

Each sample was tested according to the prescribed method, and the results are shown in Table 4.

Table 4: Sample Content Determination Results (n=2, Units: μg/mL)

Sample No.	Glucuronic Acid	Tartaric Acid	Glycolic Acid	Malic Acid	Lactic Acid	Citric Acid	2-Hydroxybutyric Acid	Mandelic Acid	Diphenylethanoic Acid	Octanoic Acid
S1	ND	ND	ND	ND	ND	216.05	2710.795	ND	ND	ND
S2	ND	ND	ND	ND	ND	171.39	ND	ND	ND	ND
S3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S8	ND	ND	ND	ND	ND	ND	1473.59	ND	ND	ND
S9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S11	ND	ND	ND	ND	1574.75	ND	ND	ND	ND	119.65
S12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: "ND" means not detected

4. Discussion

This study aims to establish a highly efficient and sensitive high-performance liquid chromatography (HPLC) method to determine the content of 10 types of α-hydroxy acids in cosmetics, including glucuronic acid, tartaric acid, glycolic acid, malic acid, lactic acid, citric acid, 2-hydroxybutyric acid, mandelic acid, diphenylacetic acid, and octanoic acid. Through the analysis of various cosmetic samples, the suitability and accuracy of this method have been demonstrated.

Firstly, this study expands upon previous research by covering more types of α-hydroxy acids and cosmetic products. This is particularly important for quality control in the cosmetics industry. α-Hydroxy acids are widely used in skincare and personal care products for their exfoliating, moisturizing, and antioxidant properties. By reducing the adhesion between corneocytes, α-hydroxy acids accelerate the shedding of these cells, improving skin texture and appearance. However, excessive use can cause skin irritation and other side effects. Therefore, accurate determination of the α-hydroxy acid content is crucial for ensuring the safety of cosmetics.

In this study, methodological evaluations were conducted, including tests for linearity, detection limits, quantification limits, precision, stability, repeatability, and spiked recovery. The results showed that the linearity of each α-hydroxy acid within the specified concentration range was excellent ($r > 0.997$), and both the detection and quantification limits were within reasonable ranges, indicating that the method has sufficient sensitivity. In precision tests, the relative standard deviation (RSD) of the α-hydroxy acids was less than 2.5%, demonstrating the method's accuracy and consistency. Additionally, the spiked recovery test showed that recovery rates ranged from 86.73% to 115.12%, meeting testing standards and further validating the method's reliability.

The study found that while most samples did not detect α-hydroxy acids, a small number of samples, such as citric acid and 2-hydroxybutyric acid in S1, and lactic acid and octanoic acid in S11, showed low detection levels, all within the national safety limits. These results suggest that the common cosmetics on the market generally comply with national health regulations concerning α-hydroxy acid usage, ensuring consumer safety.

However, the study also highlights some limitations. Although the methodological evaluations were comprehensive, covering various cosmetic types and α-hydroxy acids, differences in content between different batches of samples may still be influenced by factors such as cosmetic formulations, manufacturing processes, and storage conditions. Future research could expand the sample size to analyze the α-hydroxy acid content in products from different brands and batches, enhancing the method's applicability in practical use. Moreover, although this study examined numerous α-hydroxy acids, the continuous development of cosmetic formulations and the emergence of new α-hydroxy acid derivatives may place higher demands on detection methods. Therefore, future research should focus on developing more efficient and diverse detection techniques.

5. Conclusion

This study successfully established an HPLC method for the determination of 10 types of α-hydroxy acids in cosmetics. The method has undergone rigorous validation for linearity, detection limits, quantification limits, precision, stability, repeatability, and spiked recovery, proving to be sensitive, accurate, and reproducible, making it suitable for analyzing various types of cosmetics.

First, the results demonstrate that the detection rate of α-hydroxy acids in different types of cosmetics (such as facial cleansers, serums, masks, makeup removers, etc.) was low, with all results falling within the safety limits set by national regulations. This provides an effective tool for quality control in the cosmetics industry, ensuring product safety. Specifically, widely used α-hydroxy acids in skincare products, such as lactic acid, citric acid, and 2-hydroxybutyric acid, were detected at levels below the regulated limits, further proving that this method can be used to ensure consumer safety.

Secondly, the method’s broad applicability is evident in its cosmetic detection, and its high sensitivity and accuracy lay a foundation for future research. As the cosmetics industry evolves and new α-hydroxy acids are introduced, this method can be continually optimized and expanded to address the detection needs of a wider range of active ingredients in cosmetics.

However, the study also identifies areas for improvement. Although the current detection results show that cosmetics on the market comply with national standards, it is necessary to further expand the sample size to include more brands and different product batches, improving the generalizability of the detection results. Additionally, with the diversification of cosmetic formulations, more α-hydroxy acid derivatives may be introduced, necessitating the development of more sensitive and faster detection technologies to meet future regulatory requirements.

In summary, the HPLC method established in this study has broad application prospects and is of significant importance for quality control in the cosmetics industry. Through the widespread application of this method, cosmetic manufacturers and regulatory agencies can more effectively monitor the content of active ingredients in products, ensuring product safety and quality. At the same time, future research should further refine and optimize this detection technology to adapt to the ever-changing market demands and technological advancements, contributing to the healthy development of the cosmetics industry.

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