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Determination of α-Hydroxyacids in Cosmetics

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ABSTRACT

A high performance liquid chromatographic method was developed for the simultaneous determination of four α-hydroxyacids (AHAs, Glycolic acid, dl-Malic acid, Lactic acid and Citric acid) in cosmetics.

Samples were analyzed using a reverse-phase C_{18} column (Capcell PAK C18 UG120 S-5 μm) with 2% phosphoric acid (pH 2.0) as mobile phase at 210 nm. Calibration curves of four α-hydroxyacids were constructed in the range of 50-500 μg/mL and their correlation coefficients were in the range of 0.9992-0.9995. The relative standard deviations of four α-hydroxyacids for intraday and interday analyses were 0.05~1.49% and 0.72~3.24%, respectively. The average recoveries of four α-hydroxyacids ranged from 96.3% to 99.2%.

Key words: high performance liquid chromatography, α-hydroxyacids, AHAs

INTRODUCTION

Environmental pollution, ultraviolet radiation and long-term disadvantagious factors generate skin wrinkles and early aging. As a result, a new, global trend has occurred in the development of anti-aging ingredients for skin care [(1)]. Organic acid with hydroxyl group (-OH) in its α carbon is called AHAs, with a general name “fruit acid”. Fruit acid improves the metabolism of epithelium cells, skin luster, melioration of surface wrinkles, moisturization and inteneration of keratin. The most frequently used in cosmetics are Glycolic acid, dl-Malic acid, Lactic acid and Citric acid, among which, Glycolic acid and Lactic acid are proven to have the best effects on reduction of wrinkles and stimulating skin cell renewal. This has been provn by scientific evidence [(2-3)].

The quality of AHAs products in the US and Japan is not officially regulated. The distribution of these products is independently managed by cosmetic dealers. To protect consumers, the ROC Department of Health (Executive Yuan) announced that cosmetics which contain fruit acid and related compounds (Glycolic acid and Lactic acid) should not have a pH value lower than 3.5 and should label uses and warnings (Nov. 4, 1998)[(4)].

The long-term safety of AHAs products hasn’t been completely established[(5,6)], and market-available AHAs cosmetics rarely label concentration levels. It has been reported that the change of concentration of AHAs and pH value of final formulation are likely to affect the skin and cause such side effects as: rash, irritation, burning, bleeding and a change in sun sensitivity[(7)]. Therefore, it is a primary job to assure the safety of consumers by monitoring the pH value and AHAs concentration in cosmetics. The most widely used quantification method for organic acids is chromatography, which is also widely applied in analyses of food, medicine and plants such as physiological fluids, silage, tobacco, fruits, drinks and injections[(8-24)]. Only quantification analysis of Glycolic acid can be found in current cosmetic-related literature[(12)]. This study employed a simple and accurate reversed-phase HPLC method to rapidly identify and quantify the four AHAs ingredients in cosmetics.

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MATERIALS AND METHODS

I. *Materials*

Glycolic acid (99.5%), dl-Malic acid (99.2%) and Citric acid (99.5%) were purchased from Chem Service (U.S.A.). Lactic acid (90.0%) was purchased from Fluka (Japan). Maleic acid was purchased from Aldrich (U.S.A.) and served as the internal standard. Phosphoric acid (85%) was purchased from Merck (Germany). Ammonia water (25%) was purchased from R.D.H. (Germany). Formic acid was purchased from Merck (Germany).

II. *Instruments*

HPLC, Waters Model 510 Pump, Waters In-Line Degasser, Waters 600 Controller with which Waters 717 plus Autosampler connected and Waters 996 Photodiode Array Detector was used in this study. Water purification equipment here is Milli-Q Waters Purification System (Milli-pore Corp.).

III. *Methods*

(I) *Analysis Condition*

The chromatography column was Capcell PAK C18 UG120 S-5 μm (4.6 × 250 mm); mobile phase was 2% phos-

phoric acid (the pH was adjusted ammonia water to 2.0). The flow rate was 0.5 mL/min. The detective wavelength was 210 nm. Injection value for each time was 25 µL.

(II) *Preparation of Standard Solutions*

1. Maleic acid 100 µg/mL was prepared as the internal standard stock solution.

2. 2,000 µg/mL of Glycolic acid, dl-Malic acid, Lactic acid and Citric acid was prepared as the standard stock solution.

3. Standard solutions were prepared from stock solutions. Concentration of the standard solution was 200 µg/mL, and concentration of the internal standard was 2 µg/mL.

(III) *Standard Curve*

25, 50, 100, 200, 400 and 500 µg/mL standard solutions were analyzed respectively with 2 µg/mL internal standard. Linear regression equations and correlation coefficients were obtained from plots of concentration versus peak area ratio of standard to internal standard.

(IV) *Validation*

1. Precision

Within the standard calibration range, the standard stock solution and the internal standard stock solution were quantified precisely and diluted with water to 120, 240 and 360 µg/mL for each of the Glycolic acid, dl-Malic acid, Lactic acid and Citric acid with 2 µg/mL Maleic acid internal standard in each standard fluid. They were injected into HPLC for analysis three times on the same day and the successive five days. The standard deviation (S.D.) and relative standard deviation (R.S.D.) were then calculated.

2. Accuracy

Ingredients with known concentrations were added in the placebo sample solutions and injected into HPLC for analysis after filtration. The recovery rate and accuracy were calculated. 0.5g AHAs free cream substrate was weighed and put in 10 mL flasks respectively. Glycolic acid, dl-Malic acid, Lactic acid and Citric acid standard stock solutions and internal standard stock solution were added to the flask to 125, 250 and 500 µg/mL for standard solutions and 2 µg/mL for internal standard solution, centrifuged for 10 minutes at 6000 rpm. The supernate was filtered through 0.45 µm filter and the filtrate was collected and analyzed in HPLC for three replicates. The recovery rate was calculated from average peak area ratio of sample to internal standard by the obtained linear equation.

3. Limit of Detection

Four standard solutions were respectively diluted by water into solutions in a concentration gradient and analyzed by HPLC. The limit of detection was obtained from the concentration when the signal peak area was three times the noise peak area.

(V) *Identification and Quantification*

Six commercially available samples were weighed precisely, mixed with an appropriate amount of the internal standard stock solution, dissolved with water, and sonificated for 30 minutes. Sonicated samples were diluted with water to the final concentration of the internal standard 2 µg/mL and centrifuged for 10 minutes at 6000 rpm. The supernate was filtered with 0.45 µm filter and the filtrate was taken for HPLC analysis. By comparing the ratio of the peak area of the sample to the internal standard and calibration curve, we obtained the concentration of each sample.

RESULTS AND DISSCUSSION

I. *Analysis Method*

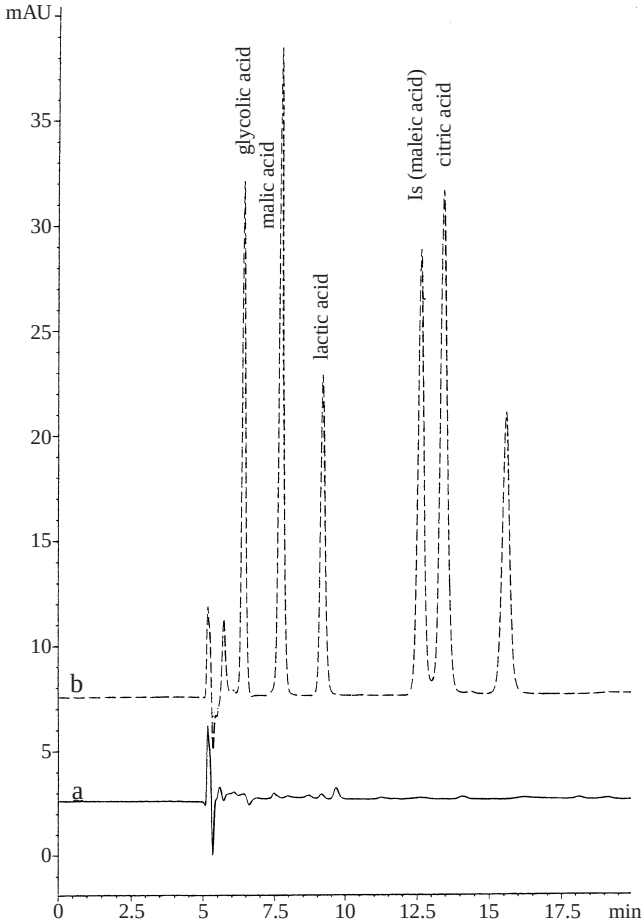

Figure 1. HPLC Chromatograms of a cream blank extract (a) and calibrators (b).
Conditions:column, Capcell PAK C18 UG120; mobile phase, 2% phosphoric acid (pH 2.0); flow-rate, 0.5 mL/min.

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The chromatography analysis was carried out through a reverse phase C_{18} column, Capcell PAK C18 UG120 S-5 μm, with 2% phosphoric acid (pH 2.0) as the mobile phase, Maleic acid as the internal standard and detected under 210 nm. The chromatograms are shown in Figure 1. The retention time of Glycolic acid, dl-Malic acid, Lactic acid and Citric acid was 6.4, 7.7, 9.1 and 13.4 minutes respectively. The retention time for the internal standard, Maleic acid, was 12.6 minutes. Through the analysis of LC-MS, the peak shown at 15.5 minutes was proved to be an impurity of dl-Malic acid, whose detailed composition and structure needs further verification.

Four AHAs, Glycolic acid, dl-Malic acid, Lactic acid and Citric acid, which were analyzed in this study, have a chemical structure shown in Figure 2. Four AHAs are all acidic compounds with short retention time when using polar solvent as mobile phase and therefore, could not be separated completely. We tested the concentration and pH impact of liquid solution on capacity factor of four AHAs for the reference of selection of mobile phase. There was tailing in the citric acid peak when Formic acid was used. It could not be meliorated by changing the concentration and pH value of formic acid. The concentration and pH value of diluted phosphoric acid were shown to have influence on AHAs retention time and thus, we started to discuss the capacity factors. The pKa value of Glycolic acid, dl-Malic acid, Lactic acid and Citric acid was 3.82, 3.40, 3.86 and 3.13 respectively, and all were larger than 3. If we controlled the pH value under pKa, the compound molecules remained uncharged. Therefore, we studied the influence of mobile phase pH value on their capacity factors when 2% phosphoric acid with pH value 2.0, 2.25 and 2.5 was employed. As shown in Figure 3, the pH value of diluted phosphoric acid affected the retention time of target compounds. The 2% phosphoric acid (pH 2.0) gave the best separation effect. Maleic acid and Citric acid were sensitive to pH variation in the mobile phase. In an investigation of different concentrations of diluted phosphoric acid solution (i.e., 1.0%, 1.5% and 3.0%) when mobile phase was pH 2.5, the phosphoric acid concentration had little effect on the retention time and capacity factor.

When the Cosmosil 5C18-MS column was employed in this research, it showed low separation efficiency of Maleic acid and drag in the peak tail. This effect was improved when Capcell PAK C18 UG120 S-5 μm (4.6 × 250 mm) was employed. The silica surface was coated silicon polymer in this column, which gave the advantage for eliminating the possible cause of peak tailing, silanols. It can be operated in wild pH range, pH 2-10 and has excellent separation effect on polar compounds.

The linear regression equation, correlation coefficient (r) and the limit of detection in the analysis protocol for Glycolic acid, dl-Malic acid, Lactic acid and Citric acid standard are listed in Table 1. Within the range of concentration of 50~500 μg/mL, all the calibration curves of Glycolic acid, dl-Malic acid, Lactic acid and Citric acid were in good linear correlation with correlation coefficient of 0.9992-0.9995.

II. *Validation*

The testing results of the interday and intraday run of four AHAs are listed in Table 2. The relative standard deviation of the interday and intraday run was between 0.05~1.49% and 0.72~3.24% which showed that the analysis

Figure 2. Structure of four α-hydroxyacid and internal standard.

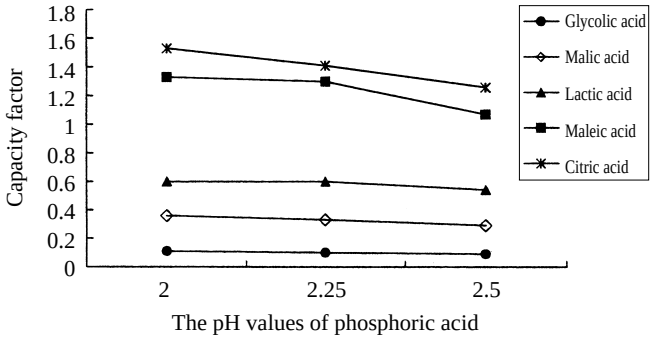

Figure 3. The effect of phosphoric acid pH values (2.0%) on capacity factor.

Table 1. Calibration curve and detection limits of AHAs

AHAs	Concentration (μg/mL)	Regression equation	r^2	Limit of detection
Glycolic acid	25, 50, 100, 200, 400, 500	Y=0.002149X+0.014272	0.9994	5 μg/mL
dl-Malic acid	25, 50, 100, 200, 400, 500	Y=0.002864X+0.029034	0.9994	5 μg/mL
Lactic acid	25, 50, 100, 200, 400, 500	Y=0.001597X+0.009457	0.9992	5 μg/mL
Citric acid	25, 50, 100, 200, 400, 500	Y=0.003842X+0.021829	0.9995	1 μg/mL

Table 2. The relative standard deviations of intraday and interday run of AHAs

AHAs	Concentration (μg/mL)	Mean ± S.D.(R.S.D.%) Intraday[a]	Interday[b]
Glycolic acid	120	118.12 ± 0.39 (0.34)	117.02 ± 1.66 (1.42)
	240	239.81 ± 0.28 (0.12)	239.71 ± 1.72 (0.72)
	360	362.37 ± 0.16 (0.05)	352.20 ± 11.06 (3.14)
dl-Malic acid	120	112.45 ± 0.09 (0.09)	116.17 ± 3.76 (3.24)
	240	238.00 ± 0.22 (0.09)	236.24 ± 2.18 (0.92)
	360	336.15 ± 0.40 (0.12)	343.71 ± 8.19 (2.39)
Lactic acid	120	117.18 ± 1.74 (1.49)	118.00 ± 1.29 (1.10)
	240	235.61 ± 1.38 (0.59)	239.03 ± 3.14 (1.31)
	360	354.29 ± 3.33 (0.94)	356.50 ± 3.56 (1.00)
Citric acid	120	120.05 ± 0.66 (0.55)	121.00 ± 2.41 (2.00)
	240	235.52 ± 2.95 (1.25)	236.50 ± 2.30 (0.98)
	360	356.92 ± 1.25 (0.35)	351.16 ± 6.67 (1.90)

[a] n=3, Repeat injection three times on the same day.
[b] n=15, Repeat injection three times each day and a successive five-day.

Table 3. Recoveries of AHAs in synthetic samples

AHAs	Theoretical conc. (μg/mL)	Estimated conc. (μg/mL)	Recovery (%)	mean(%)± S.D.[a]	R.S.D. (%)
Glycolic acid	125	122.7	98.2	99.2 ± 0.0094	0.95
	250	250.0	100.0		
	500	497.1	99.4		
dl-Malic acid	125	118.0	94.4	96.3 ± 0.018	1.84
	250	241.9	96.8		
	500	489.4	97.9		
Lactic acid	125	123.2	98.6	99.0 ± 0.010	1.06
	250	250.5	100.2		
	500	491.3	98.3		
Citric acid	125	122.5	98.0	97.6 ± 0.0151	1.54
	250	247.1	98.9		
	500	479.6	95.9		

[a] n=3.

Table 4. The contents of AHAs in commercial products

products	Labeled amount		Found(% of labeled amount)	
	Glycolic acid	Lactic acid	Glycolic acid	Lactic acid
Cream 1	2.0%	6.0%	1.9% (95.0%)	5.5% (91.7%)
Cream 2	1.0%	3.0%	0.9% (90.0%)	2.8% (93.3%)
Cream 3	4.0%	3.0%	3.7% (92.5%)	2.8% (93.3%)
Essential solution	8.0%	–	7.7% (96.3%)	–
Lotion	1.0%	3.0%	0.9% (90.0%)	2.8% (93.3%)
Moisture lotion (unknow)	–	–	0.4%	1.0%

result was good when this HPLC methodology was applied on the assay of Glycolic acid, dl-Malic acid, Lactic acid and Citric acid. Recoveries of AHAs in synthetic samples are shown in Table 3. The recovery rates of these four AHAs in cosmetics were 94.4-100.2%. The recovery rate of Glycolic acid was 99.2 ± 0.94%, 96.3 ± 1.8% for dl-Malic acid, 99.0 ± 1.0% for Lactic acid and 97.6 ± 1.51% for Citric acid. The R.S.D. of recovery rate in these four compounds was 0.95~1.84%.

III. *The Contents of AHAs in Commercial Products*

Contents of Glycolic acid, dl-Malic acid, Lactic acid and Citric acid in samples were analyzed by HPLC after filtration. The methodology was applied to analyze target compounds in six different commercial products. The contents of the commercial samples all agreed with 90-110% of the labeled content. The results are shown in Table 4. Chromatograms of sample 'cream1' and 'essential solution' are shown in Figure 4 and Figure 5.

This study established a feasible HPLC reverse phase analysis method. As a whole, this analysis contributes a good, simple, precise and fast way to identify and quantify four AHAs ingredients in cosmetics.

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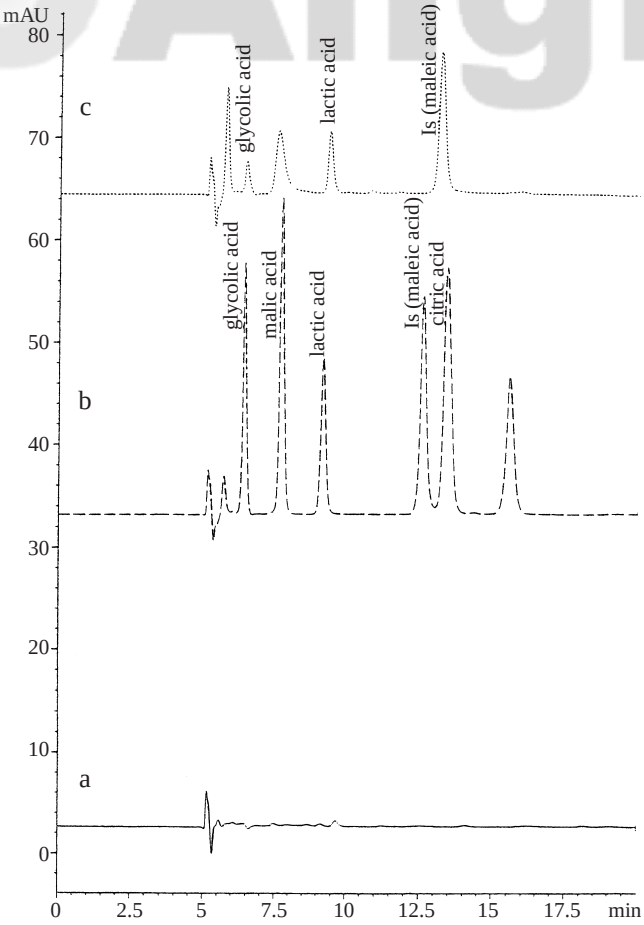

Figure 4. HPLC Chromatograms of blank(a), four α-hydroxyacids reference and internal standard(b), commercial product-creaml (c).

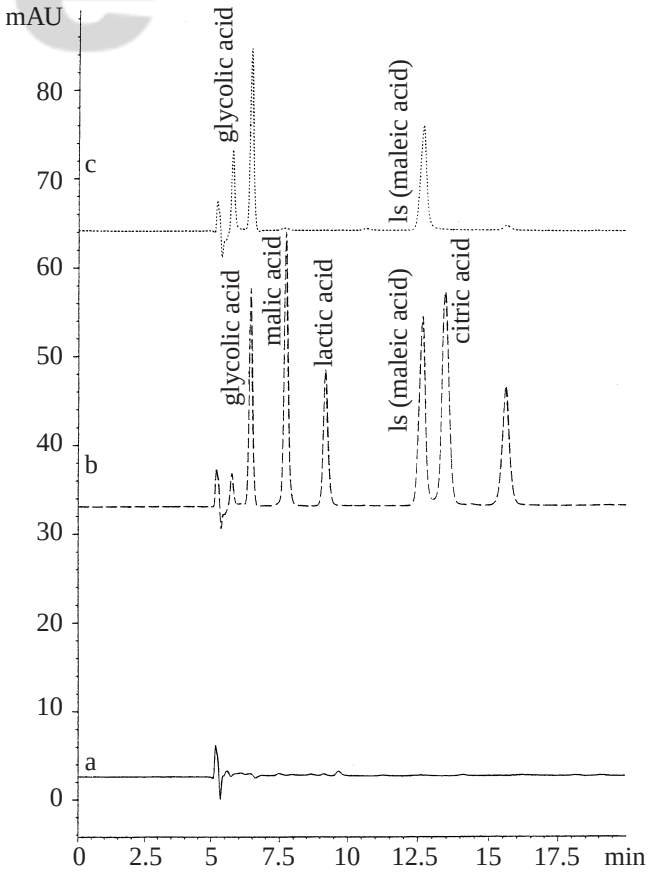

Figure 5. HPLC Chromatograms of blank(a), four α-hydroxyacids reference and internal standard(b), commercial product-essential solution(c).

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REFERENCES

1. Nacht, S. 1995. 50 Years of advances in skin care. Cosm. & Toil. 110: 69-82.
2. Idson, B. 1985. "Natural" moisturizers for cosmetics. Drug & Cosmetic Industry May: 24-26.
3. Smith, W. P. 1996. Comparative effectiveness of α-hydroxy acids on skin properties. International Journal of Cosmetic Science 18: 75-83.
4. Department of Health, Executive Yuan. 1998. The regulations of the pH value, purpose and warning of the α-hydroxyacids and the related compound.(Glycolic acid, Lactic acid)Announcement NO. 87058604. Taipei.
5. Kurtzweil, P. 1998. Alpha hydroxy acids for skin care smooth sailing or rough seas. FDA Comsumer Magazine Mar-April: 1-6.
6. Walter, P. S. 1994. Hydroxy acids and skin aging. Cosm. & Toil. 109: 41-48.
7. Garrett, A. W. 1997. AHAs and more. Drug & Cosmetic Industry Jan: 8-10.
8. Buchanan, D. N., Bonasso, F. and Thoene, J. G. 1983. Volatile carboxylic acid profiling in physiological fluids. J. Chromatogr. 278: 133-138.
9. Fussell, R. J. and McCalley, D. V. 1987. Determination of volatile fatty acids(C2-C5) and lactic acid in silage by gas chromatography. Analyst 112: 1213-1216.
10. Reyes, F. G. R., Wrolstad, R. E. and Cornwell, C. J. 1982. Comparison of enzymic, gas-liquid chromatographic, and high performance liquid chromatographic methods for determining sugars and organic acid in strawberries at three stages of maturity. J. Assoc. Off. Anal. Chem. 65: 126-131.
11. Clark, T. J. and Bunch, J. E. 1997. Derivatization solid-phase microextraction gas chromatographic-mass spectrometric determination of organic acids in tobacco. J. Chromatogr. Science 35: 209-212.
12. Scalia, S., Callegari, R. and Villani, S. 1998. Determination of glycolic acid in cosmetic products by solid-phase extraction and reversed-phase ion-pair high-performance liquid chromatography. J. Chromatogr. A. 795: 219-225.
13. Skelly, N. E. 1982. Separation of inorganic and organic anions on reversed-phase liquid chromatography column. Anal. Chem. 54: 712-715.
14. Peldszus, S., Huck, P. M. and Andrews, S. A. 1996.

Determination of short-chain aliphatic, oxo- and hydroxy-acids in drinking water at low microgram per liter concentrations. J. Chromatogr. A. 723: 27-34.
15. Wilson, T. D. 1985. HPLC determination of lactic acid in milrinone injection and oral solution using ion-exchange sample preparation methods. J. Liq. Chromatogr. 8: 1629-1650.
16. Pecina, R., Bonn, G., Burtscher, E. and Bobleter, O. 1984. High-performance liquid chromatographic elution behaviour of alcohols, aldehydes, ketones, organic acids and carbohydrates on a strong cation-exchange stationary phase. J. Chromatogr. 287: 245-258.
17. Cherchi, A., Spanedda, L., Tuberoso, C. and Cabras, P. 1994. Solid-phase extraction and high-performance liquid chromatographic determination of organic acid in honey. J. Chromatogr. A. 669: 59-64.
18. Bevilacqua, A. E. and Califano, A. N. 1989. Determination of organic acid in dairy products by high performance liquid chromatography. J. Food Sci. 54: 1076-1079.
19. Zyren, J. and Elkins, E. R. 1985. Interlaboratory variability of methods used for detection of economic adulteration in apple. J. Assoc. Off. Anal. Chem. 68: 672-676.
20. Chen, P., Nie, L. and Yao, S. 1995. Determination of lactic acid and pyruvic acid in serum and cerebrospinal fluid by ion-exclusion chromatography with a bulk acoustic wave detector. J. Chromatogr. B. 673: 153-158.
21. Fritz, J. 1991. Principles and applications of ion-exclusion chromatography. J. Chromatogr. 546: 111-118.
22. Fischer, K., Bipp, H., Bieniek, D. and Kettrup, A. 1995. Determination of monomeric sugar carboxylic acids by ion-exclusion chromatography. J. Chromatogr. A. 706: 361-373.
23. Okada, T. 1988. Redox suppressor for ion-exclusion chromatography of carboxylic acids with conductometric detection. Anal. Chem. 60: 1666-1669.
24. Widiastuti, R., Haddad, P. R. and Jackson, P. E. 1992. Approaches to gradient elution in ion-exclusion chromatography of carboxylic acids. J. Chromatogr. 602: 43-50.

化粧品中α-Hydroxyacids之分析

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摘　要

本研究利用高效液相層析法，分析化粧品中四種α-hydroxyacids（AHAs）（Glycolic acid、dl-Malic acid、Lactic acid、Citric acid）之含量，本實驗採用C_{18}逆相層析管柱Capcell PAK C18 UG120 S-5 μm（4.6 × 250 mm），移動相為2% 磷酸溶液（pH 2.0），內部標準品為Maleic acid，檢測波長為210 nm，得到良好分析結果。四種AHAs於50-500 μg/mL均呈現良好線性關係，其相關係數(r)為介於0.9992~0.9995之間，四種AHAs之同日內及異日間試驗之相對標準偏差，分別為0.05~1.49% 及0.72~3.24%，四種AHAs之添加回收率為介於96.3% 至99.2% 之間。

關鍵詞：α-hydroxyacids，高效液相層析法，AHAs