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STABILITY-INDICATING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY FOR LACTIC ACID IN LOTIONS

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SUMMARY

A stability-indicating high-performance liquid chromatographic method using an amine column and a UV detector at 214 nm is developed for lactic acid in dermatological products. The detector response for lactic acid is linear in the range 8.8–39.9 μg. Repeatability of the chromatographic peak area is 0.9% relative standard deviation (R.S.D.) (n = 6). Precision of the method is 1.3% R.S.D. (n = 9). Recovery of lactic acid from lactate lotion is 100.4% with 0.4% R.S.D. (n = 9). The method is particularly suitable for products containing parabens.

INTRODUCTION

Lactic acid has been widely used in the food industry as an additive, acidulant and preservative[1]. The hygroscopic properties of sodium lactate and lactic acid–sodium lactate mixtures were found to amplify the emollient properties of hand cream[2], and have also been utilized in cosmetic formulations and in the treatment of skin diseases. In 1977, Van Scott *et al.*[3] found applications of lactic acid in the treatment of dry skin and acne. They also found that keratoses of the skin may be successfully prevented or treated with lactic acid[4].

Several methods have been described for the determination of lactic acid. The colorimetric method[5] using complexation with ferric ion is not very rugged and accurate results could be obtained only with strict control of experimental conditions. Gas chromatographic methods[1,6,7] require cumbersome derivatization of the acid before analysis. The liquid chromatographic methods are relatively simple, and they were developed mainly for lactic acid in either food products or plasma[8–12]. However, these high-performance liquid chromatographic (HPLC) methods are not applicable to dermatological products due to excipient interference or deterioration of column performance by excipients. Also, some of these methods require expensive columns and operations at elevated temperatures. This paper describes the development of a specific, accurate and precise HPLC method for lactic acid in lotions.

EXPERIMENTAL

Apparatus

Chromatography was performed using a Waters Assoc. (Milford, MA, U.S.A.) Model 204 chromatograph with a M-6000A pump, a U6K injector and a UV detector (Model 441) set at 214 nm. A 30 cm × 3.9 mm I.D. μBondapak amine column (Waters Assoc.) was connected to the HPLC system. Output was monitored on an OmniScribe recorder (Houston Instruments, Houston, TX, U.S.A.) and a Model 3352B laboratory data system (Hewlett-Packard, Avondale, PA, U.S.A.). A Hewlett-Packard Model 1040A diode array spectrophotometric detector was used in HPLC peak purity evaluation.

Reagents

Lactic acid (88% solution) was purchased from McKesson Chemical Co. (Buffalo, NY, U.S.A.). HPLC-grade acetonitrile and ACS-grade methanol and sodium hydroxide were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). HPLC-grade phosphoric acid (85%) was purchased from Fisher Scientiific (Fairlawn, NJ, U.S.A.). A lactate lotion, Lac-Hydrin® from Westwood Pharmaceuticals (Buffalo, NY, U.S.A.), and its placebo were used in validating the method. The excipients in Lac-Hydrin include light mineral oil, glyceryl stearate, PEG-100 stearate, propylene glycol, polyoxy 40 stearate, glycerin, magnesium aluminum silicate, laureth-4, cetyl alcohol, methyl cellulose, fragrance, quaternium-15, and methyl and propyl parabens.

HPLC operating conditions

The eluent was water–acetonitrile (1:1) containing 3.2 m*M* ammonium dihydrogen phosphate. The flow-rate was 1.0 ml/min. Injection volume was 20 μl. Peak areas were used to quantitate lactic acid.

Sample and standard preparation

Lactic acid reagent, which contains *ca.* 88% lactic acid, was standardized by titration as described in ref. 13. The sample and standard solutions for HPLC analysis were prepared as follows.

A sample equivalent to 120 mg of lactic acid was weighed into a 150-ml beaker, 20 ml of 0.1 *N* sodium hydroxide was added, and the beaker was covered with a watch glass and boiled gently on a hot plate for 20 min. After cooling to room temperature, it was diluted to *ca.* 40 ml with water, adjusted to pH 6.0 with dilute phosphoric acid and then quantitatively transferred and diluted to 100 ml with water. The solution was then clarified by filtration.

RESULTS AND DISCUSSION

Choice of detector

Lactic acid is a poor UV absorber, and it can only be detected at wavelengths below 220 nm. Derivatization as a means of improving detection appears possible but would unnecessarily lengthen the procedure. Detection by refractive index detector is less sensitive than UV detection at 214 nm. Therefore, the method was developed using the latter.

Chromatography

Polystyrene-based ion-exchange columns have found wide application to the analysis of organic acids such as lactic acid but they require column operation at elevated temperatures[8–10]. The reported separations on other types of columns (amine and octadecylsilane) for lactic acid[11,12] are not adequate for analyzing lotion-type products due to excipient interferences. Using an ion-pairing approach we were able to improve this separation.

Excellent separation of lactic acid from excipients was obtained using a Waters μBondapak C_{18} column and 0.005 *M* tetrabutylammonium hydroxide (pH adjusted to 7 with phosphoric acid) as eluent. However, this system is not suitable for analyzing products containing parabens, a common preservative in topical products. Heating the sample with 0.1 *N* sodium hydroxide during sample preparation, to hydrolyze intermolecular esters of lactic acid, also hydrolyzes parabens to *p*-hydroxybenzoic acid. The latter elutes very late, thus extending the chromatographic run time to over 40 min (Fig. 1).

Efforts to reduce the analysis time by changing the ion-pairing agent or by adding methanol to the eluent were not successful due to interference from other positive or negative peaks. Also, it was noted that some product excipients were held up on th column unless a stronger eluent with 20% or more methanol is used. Circumventing the problem of late-eluting components by the use of two columns and the column switching technique led to a temporary digression of the baseline in

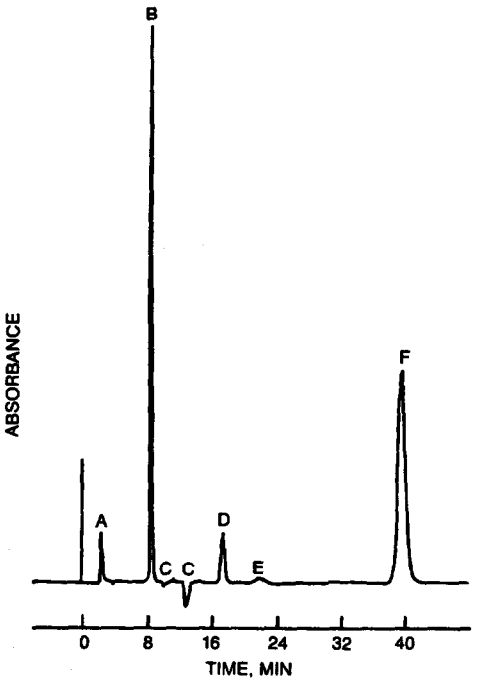
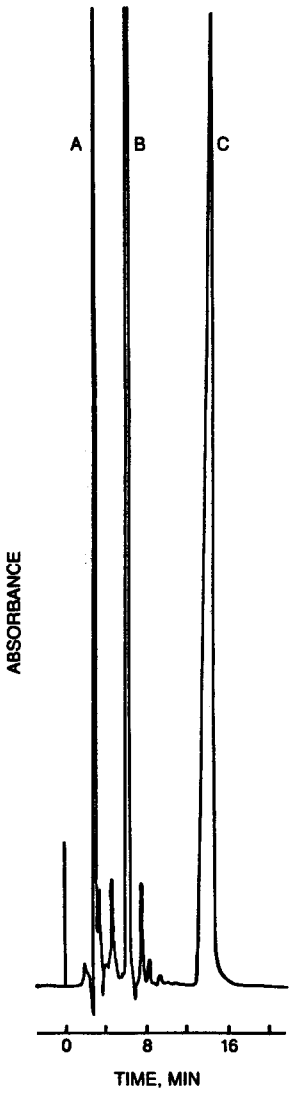


Fig. 1. Chromatogram of lactate lotion. Column: μBondapak C_{18}; eluent: 0.005 *M* tetrabutylammonium hydroxide in water (pH adjusted to 7.0 with phosphoric acid); flow-rate: 1.0 ml/min. Peaks: A = solvent front; B = lactic acid; C = negative peak; D = impurity of lactic acid; E = excipient; F = late-eluting excipient peak.

chromatograms, which interfered with the quantitation of the lactic acid peak. The digression is apparently due to pressure transients during column switching by the HPSC Model 410 system (Autochrom, Milford, MA, U.S.A.), which became more prominent at 214 nm, the wavelength of detection.

The problem of late-eluting peaks and non-eluting components was successfully resolved using a μBondapak amine column and 3.2 mM phosphate buffer in



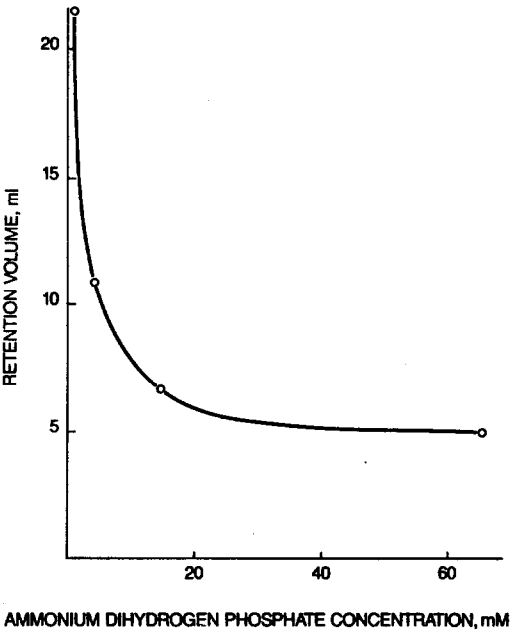


Fig. 2. Typical chromatogram of lactate lotion sample. Eluent: water–acetonitrile (1:1) containing 3.2 mM ammonium dihydrogen phosphate; flow-rate: 1.0 ml/min. Peaks: A = solvent front; B = excipient peak; C = lactic acid.

Fig. 3. Effect of ionic strength on retention of lactic acid. Mobile phase: 50% acetonitrile–water (pH = 5.3) containing various amount of ammonium dihydrogen phosphate.

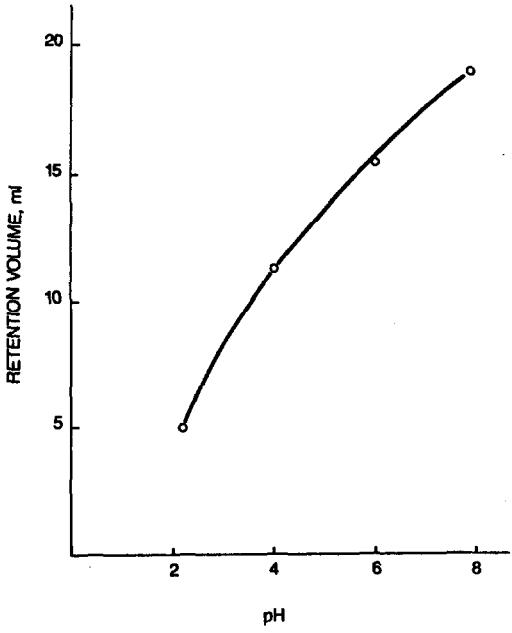


Fig. 4. Effect of pH on retention of lactic acid.

acetonitrile–water (1:1) as eluent (Fig. 2). The lactic acid peak is well resolved from the excipient peaks. As shown in the method validation section, it is well suited for quantitation. The amine column, although normally operating as a reversed-phase column, acts as an ion-exchange column in this system. Change in the cation by replacing the ammonium ion in the eluent with potassium ion or tetramethylammonium ion has no effect on the retention time. An increase in ionic strength with ammonium dihydrogen phosphate, while keeping the pH constant, decreased the retention volume (Fig. 3). An increase in the pH of the eluent led to an increase in retention volume (Fig. 4). These observations support the ion-exchange separation mechanism of an acid. A log plot of the capacity factor k' *vs.* concentration of acetonitrile in the eluent (Fig. 5) showed a linear relationship, typical of reversed-phase chromatography and well-known in ion-exchange chromatography[14].

Internal standard

No suitable internal standard was found, although we tested several compounds: 2-bromoacetic acid, 2-hydroxybutyric acid, ammonium acetate, maleic acid, hexanoic acid, *p*-hydroxybenzoic acid, ethyl glycolate, glycolic acid, ammonium oxalate and glyceric acid. Only glycolic acid resolved well from lactic acid, but the peak showed tailing. Because of the lack of a good internal standard, we developed the method using external standard only.

Chromatographic precision

Chromatographic precision of the method was determined by six replicate injections of a lactic acid standard (1.1 mg/ml). The lactic acid peak area was reproduced with a relative standard deviation (R.S.D.) of 0.9%.

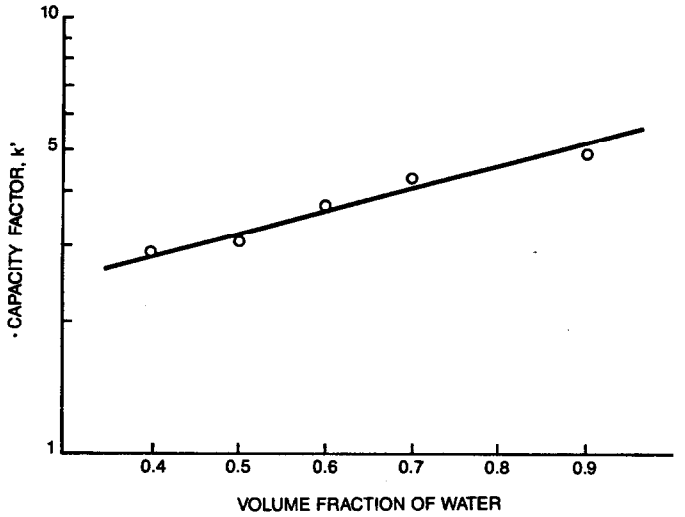


Fig. 5. Effect of solvent strength on retention of lactic acid at constant pH and ionic strength. Eluent: acetonitrile–water with 3.2 m*M* ammonium dihydrogen phosphate (pH 5.3).

Linearity of detector response

Seven lactic acid standard solutions in the concentration range 0.44–2.0 mg/ml were prepared and analyzed. Linear regression analysis of the peak area data gave a correlation coefficient (r) of 0.9996 and a y-intercept equivalent to −0.2% of the normal response (12% lactic acid lotion).

Method validation

Placebo of lactic acid lotion was analyzed by this method. No interference

TABLE I

ACCURACY DATA

Spiked sample		*Lactic acid*	
Placebo (g)	*Lactic acid (mg)**	*Assay (mg)*	*% Recovery*
0.5	119.9	120.6	100.6
0.5	119.9	119.6	99.8
0.5	119.9	121.1	101.0
1.0	119.9	120.7	100.7
1.0	119.9	119.6	99.8
1.0	119.9	119.8	99.9
1.5	119.9	120.6	100.6
1.5	119.9	120.5	100.5
1.5	119.9	120.6	100.5
		Mean	100.4
		R.S.D.	0.4%

* Spiked as aqueous lactic acid solution.

TABLE II

PRECISION DATA

Lactate lotion (mg)	*Lactic acid assay (%)*
494.6	13.06
493.3	12.69
505.4	12.64
1002.6	12.83
1016.3	12.63
992.2	13.08
1503.4	12.71
1517.2	12.81
1500.0	12.83
Mean	12.81
R.S.D.	1.3%

peaks at the retention time of lactic acid were observed. Nine synthetic samples were made by spiking the placebo with solutions containing known amounts of standard lactic acid at levels corresponding to 67–200% of the normal concentration. As shown in Table I, the average recovery obtained by the method is 100.4% (0.4% R.S.D.). The lack of any trend in recovery values with increase in amount of placebo suggests that the lactic acid/placebo ratio has no effect on the assay. Nine samples of lactate lotion with a label content of 12% lactic acid were analyzed by the HPLC

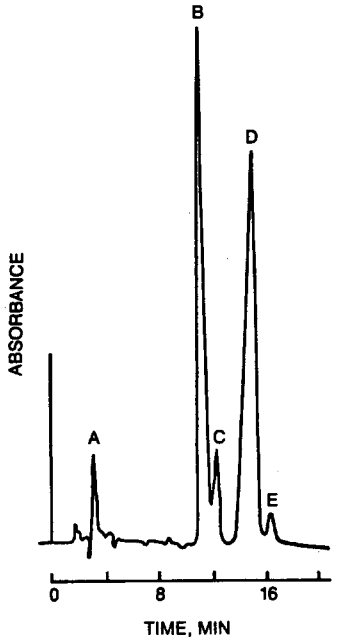


Fig. 6. Chromatogram of lactic acid and its possible degradation products. Peaks: A = solvent front; B = pyruvic acid; C = acetic acid; D = lactic acid; E = impurity from pyruvic acid.

procedure. The average of nine assays is 12.81%, with a relative standard deviation of 1.3% (Table II). The lack of any trend in assay results with change in sample size used for analysis indicates that larger or smaller samples can be used for analysis. The specificity of the method was checked by chromatographing a mixture of lactic acid with its possible degradation products. Fig. 6 shows that pyruvic acid and acetic acid peaks are resolved from the lactic acid peak. Oxalic acid and acetaldehyde were retained on the column. The purity of the separated lactic acid was further checked by recording the UV spectra (200–400 nm) at different points on the lactic acid peak using the diode array spectrophotometric detector. When normalized, all the spectra were found to be superimposable. Similar spectra were observed for the lactic acid peak of the lactate lotion sample stored at 30°C for 55 months. Hence, any degradation of placebo or lactic acid does not lead to interferences in the assay. Therefore, the method is stability-indicating for lactic acid.

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REFERENCES

1 C. H. Holten, *Lactic Acid*, Verlag Chemie, Weinheim/Bergster., 1971, pp. 21–23 and 192–216.
2 F. K. Bagatell and W. Smott, *Cutis*, 18 (1976) 591.
3 E. J. Van Scott, S. Lane and R. J. Yu, *U.S. Patent*, 4021572, May 3, 1977.
4 E. J. Van Scott and R. J. Yu, *U.S. Patent*, 4234599, November 18, 1980.
5 *Official Methods of Analysis*, Assoc. Off. Anal. Chem., Arlington, VA, 13th ed., 1980.
6 D. B. Drucker, *J. Chromatogr.*, 208 (1981) 279.
7 A. T. G. Steverink, *Analyst (London)*, 109 (1984) 174.
8 D. J. Woo and J. R. Benson, *LC, Liq. Chromatogr. HPLC Mag.*, 1 (1983) 238.
9 R. Anderson and B. Hedlund, *Z. Lebensm. Unters.-Forsch.*, 176 (1983) 440.
10 S. H. Ashoor and J. Welty, *J. Sci. Food Agric.*, 287 (1984) 452.
11 P. E. Shaw and C. J. Wilson, *J. Sci. Food Agric.*, 34 (1983) 1255.
12 J. A. Owens and J. S. Robinson, *J. Chromatogr.*, 307 (1984) 380.
13 *The United States Pharmacopeia*, 20th rev., U.S. Pharmacopeial Convention, Rockville, MD, 1980, p. 438.
14 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, John Wiley, New York, 2nd ed., 1979, p. 426.