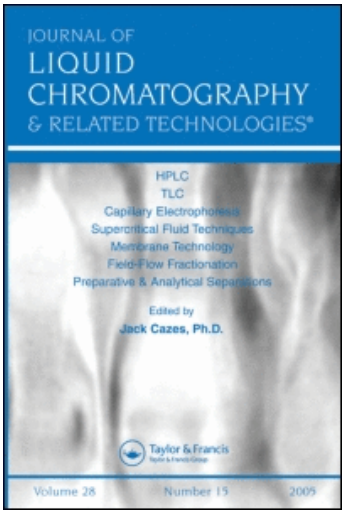

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

HPLC Determination of Lactic Acid in Milrinone Injection and Oral Solution Using Ion-Exchange Sample Preparation Methods

Terry D. Wilson[a]
[a] Analytical Chemistry Department, Sterling-Winthrop Research Institute, Rensselaer, New York

To cite this Article Wilson, Terry D.(1985) 'HPLC Determination of Lactic Acid in Milrinone Injection and Oral Solution Using Ion-Exchange Sample Preparation Methods', Journal of Liquid Chromatography & Related Technologies, 8: 9, 1629 — 1650
To link to this Article: DOI: 10.1080/01483918508074083
URL: http://dx.doi.org/10.1080/01483918508074083

JOURNAL OF LIQUID CHROMATOGRAPHY, 8(9), 1629-1650 (1985)

HPLC DETERMINATION OF LACTIC ACID IN MILRINONE INJECTION AND ORAL SOLUTION USING ION-EXCHANGE SAMPLE PREPARATION METHODS

Terry D. Wilson
Analytical Chemistry Department
Sterling-Winthrop Research Institute
Rensselaer, New York 12144

ABSTRACT

Lactic acid has been measured in Milrinone Injection and Oral Solution by HPLC using an ion-exclusion polymeric column coupled with a reversed-phase guard column. This dual-column chromatography was preceded by classical ion-exchange sample preparation to eliminate interfering excipient components. The lactic acid lactate content of USP lactic acid was converted to lactic acid in processing. It was also further characterized using a TLC separation.

INTRODUCTION

Lactic acid, $C_3H_6O_3$ (2-hydroxypropanoic acid), is a product of bacterial fermentation of milk and certain plant derivatives, a product of energy producing carbohydrate metabolism and other natural

 0148-3919/85/0809-1629$3.50/0

sources. Its pharmaceutical applications include acidification and salt formation with organic bases, use in physiological buffers and in biodegradable drug delivery systems. Milrinone (1,6-dihydro-2-methyl-6-oxo(3,4'-bipyridine)-5-carbonitrile), a potent new cardiotonic agent with inotropic activity, contains lactic acid as acidulant in both injection and oral solution (Wincardin^{TM}) dosage forms.

Previous analytical methods for lactic acid include acid-base titration (1), colorimetry (2), infra-red spectroscopy (3), iodimetric titration (4) and enzymatic **analysis**(5). Selective and sensitive chromatographic methods have been applied to lactic acid including gas chromatography (6-9), chiral gas chromatography (10), GC-MS (11-13) and thin-layer chromatography(14-16). A variety of column liquid chromatographic procedures are known for organic acids including lactic acid. Reversed-phase HPLC using UV detection at 190 nm (17) or 210 nm (18-20) and post-column reaction detection of lactic acid enantiomers (**21**) **have** been reported. Other column chromatographic methods have included ion chromatography with suppression (22-23) and without suppression (24-28).

The advent of microparticulate polymeric strong cation-exchange HPLC columns has given a new dimen-

sion to organic acid analysis. The separation mechanism described as ion-exclusion (IE) or ion-moderated partition (IMP) was discussed early by Harlow and Morman (29) and reviewed by Wood, Cummings and Jupille (30). General descriptions of the capabilities of these columns have followed (31-33). Their application to the separation of organic acids among bacterial metabolites as a means of monitoring fermentation (34), in urine as a metabolic disorder screen (35-36) and in certain foods (37) have been described. The present study concerns the application of such a polymeric organic acid column in conjunction with a reversed-phase guard column to the quantitation of lactic acid in Milrinone Oral Solution and Injection dosage forms. This method was used following a classical ion-exchange sample cleanup to eliminate interfering components from these formulations.

EXPERIMENTAL

Chemicals and Reagents

Lactic acid (USP), acetic acid and sodium chloride reagent were from J.T. Baker (Phillipsburg, NJ). Pyruvic acid (Fisher Scientific, Fairlawn, NJ), sulfuric acid (Corco Chemical, Fairless Hills, PA) and sodium

hydroxide (MCB Reagents, Cincinnati, OH) were reagent grade. Water was Nanopure (Sybron/Barnstead, Boston, MA). The anion-exchange resin used was AG 1-X2, 50-100 mesh, 3.5 meq/dry g, and the cation-exchange resin used was AG 50W-X2, 50-100 mesh, 5.2 meq/dry g, both from Bio-Rad (Richmond, CA).

Apparatus

A modular HPLC system was used at ambient temperature consisting of a Waters 6000A solvent delivery pump (Waters Associates, Milford, MA) operated at 1.0 mL/min, a Kratos SF770R variable wavelength UV-visible detector set at 210 nm (Kratos Inc., Westwood, NJ), a Rheodyne 7125 manual injection valve equipped with a 20μL loop (Rheodyne, Cotati, CA) and a Series 5000 Fisher Recordall (Fisher Scientific, Springfield, NJ). The analytical column used was a 9μ 300x7.8 mm Aminex HPX-87H organic acid ion-exclusion column (Bio-Rad Labs, Richmond, CA). The guard column used was a 5μ 30x4.6 mm RP-18 Spheri-5 cartridge in a Brownlee MPLC 3 cm guard column holder (Brownlee Labs, Santa Clara, CA). The lab automation system used was a HP 3354 from Hewlett-Packard (Avondale, PA).
The mobile phase used in the separation was 0.0045 N sulfuric acid with a pH of 2.3.

Preparation of Ion-Exchange Resin

Anion-Exchange Resin for Solution Dosage Form

A 70 g portion of resin in the Cl^- form was slurried with two 400 mL portions of 1 N NaOH and rinsed with water until the rinse was neutral to wide-range pH indicator paper. Glass columns 120 mm long by 9 mm (I.D.) equipped with a 15 mL reservoir and a stopcock were then filled with the resin.

Cation-Exchange Resin for Injection Dosage Form

A 70 g portion of resin in the H^+ form was slurried with two 400 mL portions of 1 N HCl and rinsed with water until the rinse was neutral to wide-range pH indicator paper. Glass columns were then filled with resin as above.

Solution Dosage Form

Standard Preparation

A standard lactic acid solution of about 1 mg/mL ($C_3H_6O_3$) as determined by USP titration (1) was prepared. The pH of 10.0 mL of this solution was adjusted to 4.5 with 0.1 N NaOH and it was transferred quantitatively to an anion-exchange column prepared above. This solution was drained through the column at 1-2 mL/min and the eluate was discarded. The beaker containing the original solution was washed with two

10 mL portions of water which were passed through the column and discarded. Following this the beaker was washed with two 5 mL portions of 2.5 N NaOH which were passed through the column and collected in a 50 mL volumetric flask. The beaker was further washed with two 10 mL portions of 1 N NaCl which were passed through the column and collected in the same flask. The solution in the flask was acidified with 7 N H_2SO_4, cooled to room temperature and diluted to volume with water giving about 0.21 mg/mL $C_3H_6O_3$.

Sample Preparation

The pH of 10.0 mL sample in a 50 mL beaker was adjusted to 4.5 with 0.1 N NaOH. The method for standard preparation was then followed beginning with the quantitative transfer to a prepared anion-exchange column.

Injection Dosage Form

Standard Preparation

A standard lactic acid solution of about 2.3 mg/mL $C_3H_6O_3$ was prepared, an aliquot was transferred to a 50 mL beaker and acidified with 1 N H_2SO_4. This solution was quantitatively transferred to a prepared cation-exchange column, was allowed to pass through at a rate of 1-2 mL/min and was collected in a 50

mL volumetric flask. The beaker was then rinsed with four 5 mL portions of water transferring each to the column when the last had drained to the top of the resin. The eluate from all rinses were collected in the same volumetric flask. The solution in the flask was then hydrolyzed with 1 N NaOH for 10 min at room temperature, neutralized with 1 N H_2SO_4 and diluted to the mark with water to give about 0.23 mg/mL $C_3H_6O_3$.

Sample Preparation

Ten mL of sample was pipetted into a 50 mL beaker, acidified with 1 N H_2SO_4 and transferred quantitatively to a prepared cation-exchange column. Subsequently the method for the standard preparation above was followed.

Validation of Method and Minimum Detectable Limit

Linearity for the lactic acid assay procedures was determined by adding lactic acid to placebos at 0, 80, 100 and 120% of the theoretical value in duplicate for each dosage form. These were then processed by the respective procedures outlined above.

The processed standard for the injection dosage form was diluted serially with water giving the following concentrations: 0.23, 0.115, 0.023, 0.0115,

0.0023, 0.00115 and 0.00023 mg/mL $C_3H_6O_3$ which were then chromatographed.

Separation of Structural Analogs-Resolution Factor

A mixed standard solution was prepared by hydrolyzing 10 mg of USP lactic acid in a 50 mL volumetric flask with 1 N NaOH and neutralizing with 1 N H_2SO_4. To this was added pyruvic acid and acetic acid giving final concentraitons of 0.1 mg/mL and 0.16 mg/mL respectively for the latter two when diluted to volume with water. The resolution factor was determined between lactic acid and acetic acid using the relationship:

$$R = \frac{2 (t_2 - t_1)}{w_2 + w_1}$$

where t_2 and t_1 are the respective retention times and w_2 and w_1 the respective peak widths measured at the bases by extrapolating the straight sides of the peaks to the baseline.

Separation of Components in USP Lactic Acid

A 50 mL sample of USP lactic acid was distilled under vacuum (aspirator) and fractions were collected with the boiling ranges: 60-90° and 90-120°. These along with the residue were examined after appropriate dilution with water by HPLC and TLC. The residue

fraction was dissolved initially in CH_3CN:H_2O (1:1) because of low solubility in water. The TLC system consisted of cellulose F precoated 20x20 cm plates (0.1 mm thickness) obtained from E. Merck with a mobile phase composed of the organic layer from toluene-propionic acid-water (45:45:5) modified from Munier, Drapier and Faivre (15). Visualization was by 0.1% bromcresol green.

RESULTS AND DISCUSSION

USP lactic acid is described as 'a mixture of lactic acid ($C_3H_6O_3$) and lactic acid lactate ($C_6H_{10}O_5$) equivalent to a total of not less than 85.0 percent and not more than 90.0 percent, by weight, of $C_3H_6O_3$'(1). This mixed character of lactic acid is clearly evident in Figure 1A which shows a 0.15 mg/mL standard solution diluted directly from a USP sample with water and run under the conditions of the assay. When lactic acid at the same level was hydrolyzed with 1 N NaOH for 10 min at room temperature and then neutralized with 1 N H_2SO_4 the chromatogram shown in Figure 1B was obtained. The separation found between the main peak for lactic acid with a retention time of 8 min and the second peak depended on both

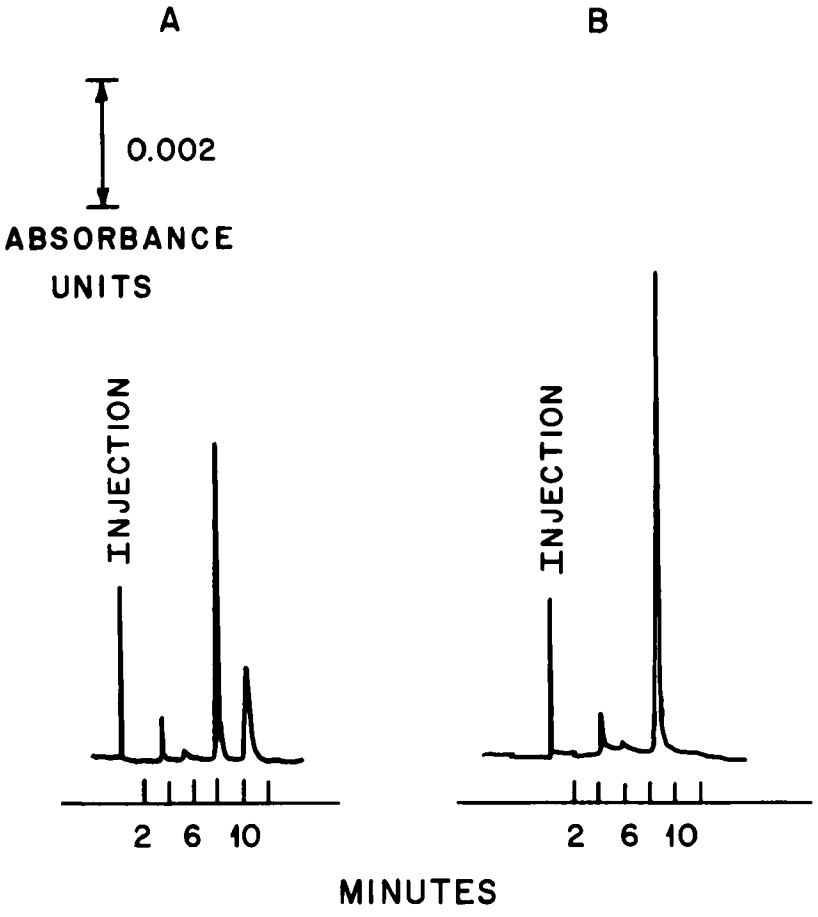

Figure 1. Chromatograms of USP lactic acid (A) and hydrolyzed USP lactic acid (B). Chromatographic conditions: column- Aminex HPX-87H 9μ 30 cm, guard column- Spheri-5 RP-18, mobile phase- 0.0045 N H_2SO_4, flow rate- 1.0 mL/min, detection- UV-210 nm, sensitivity- 0.02 AUFS.

the mobile phase composition and the guard column used. While the mobile phase for resin-based microparticulate ion-exclusion columns can contain acetonitrile, it was found unnecessary to use mixed solvents here. The mobile phase pH, however, was adjusted in an early attempt to eliminate the second lactic acid peak. Sulfuric acid normality was varied from 0.0126 to 0.001. This resulted in little change in retention time for the main peak although k' of the second peak did change dramatically as seen in Figure 2. The retention time for the second peak also depended on the particular C_{18} guard cartridge used with little relationship to age or back-pressure found. The decreased polarity of the component at the second peak, the ester, was indicated by placing two C_{18} guard columns upstream to the organic acid column which gave an increased retention time for this component only. The usual configuration utilized with organic acid columns is an ion-exclusion guard column with the analytical column of the same packing material. An additional element of selectivity was used here by addition of the reversed-phase guard column. Such dual-column HPLC has been described earlier by Buchanan and Thoene where an Aminex HPX-87H column was used upstream or downstream to a C_{18}

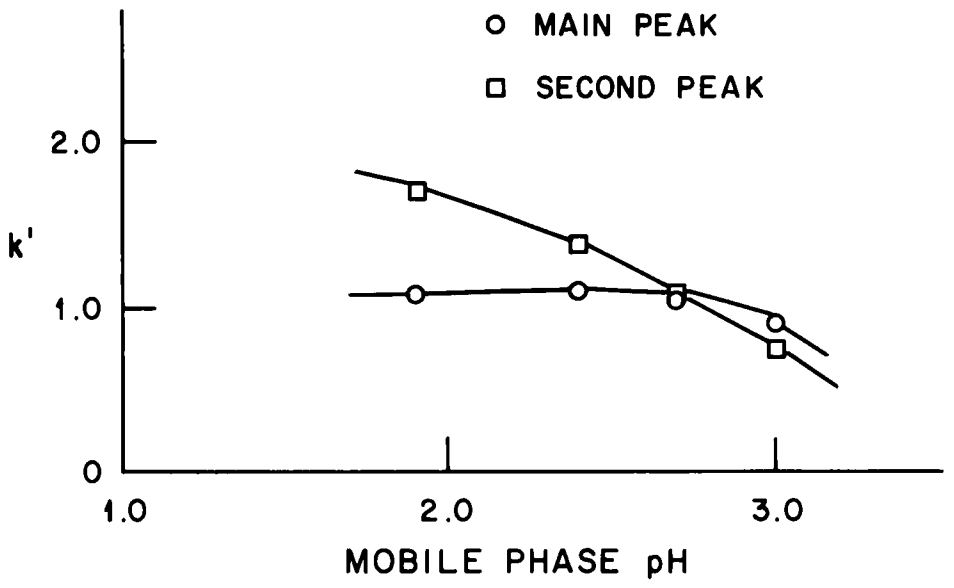

Figure 2. Effect of mobile phase pH on capacity factors of two components of USP lactic acid.

analytical column for the separation of urinary organic acids (35).

In order to circumvent the problem of the second peak of lactic acid means were found to eliminate it. Hydrolysis of the ester with NaOH on a steam bath was more severe than necessary since this is easily accomplished at the concentration of the assay in 10 min at room temperature. This means was used in the injection dosage form whereas in the oral solution, hydrolysis occurs in processing on the anion-exchange column.

The scheme developed for sample and standard preparation was necessitated by interference from excipients and decreased recoveries found when more straightforward procedures were followed. Interference from alcohols present in the oral solution was eliminated by use of the water wash in the anion-exchange treatment. Lactic acid at this time is quantitatively attached to the resin due to the prior pH adjustment. The 2.5 N NaOH converts any remaining lactic acid ester to the acid salt and exchanges for it on the resin. One N NaCl completes this latter process with a higher selectivity for the strongly basic ammonium sites on the anion-exchanger. A final acidification was used to obtain uniform hydrogen ion concentration and ionic strength. Figure 3 shows chromatograms of a processed standard (A), a 100% spiked placebo (B) and a placebo (C) for the oral solution dosage form with lactic acid eluting at 8 min. The large solvent front peak at 4 min is due to the added H_2SO_4.

Lactic acid in Milrinone Injection presented a somewhat less complex picture than the oral solution. The anion-exchange procedure could have been used here as well, however a simpler approach was followed because of the absence of alcohols from this formula.

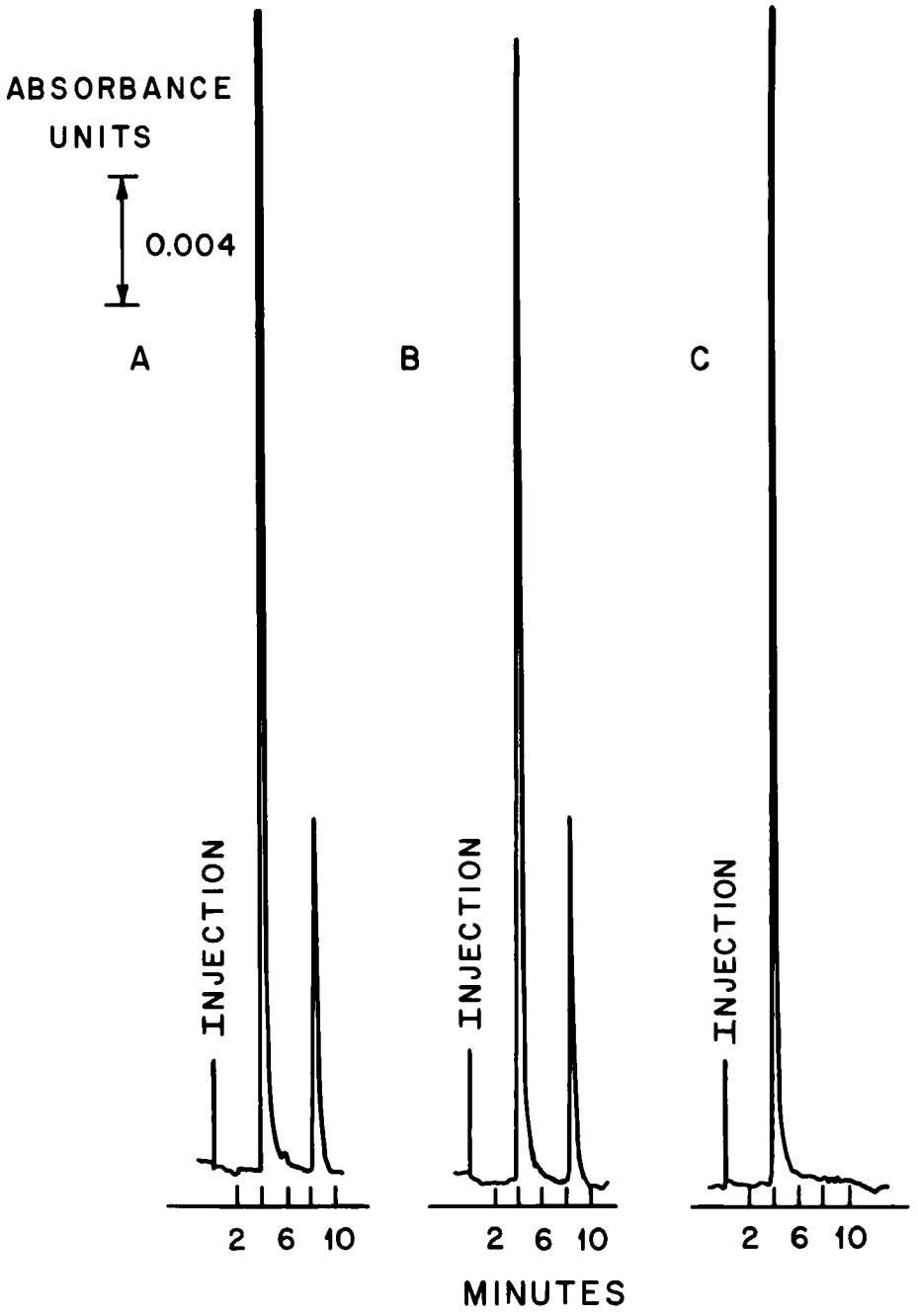

Figure 3. Chromatograms of lactic acid in Milrinone Oral Solution, processed standard (A), 100% spiked placebo (B) and placebo (C). Chromatographic conditions as in Figure 1 with sensitivity- 0.04 AUFS.

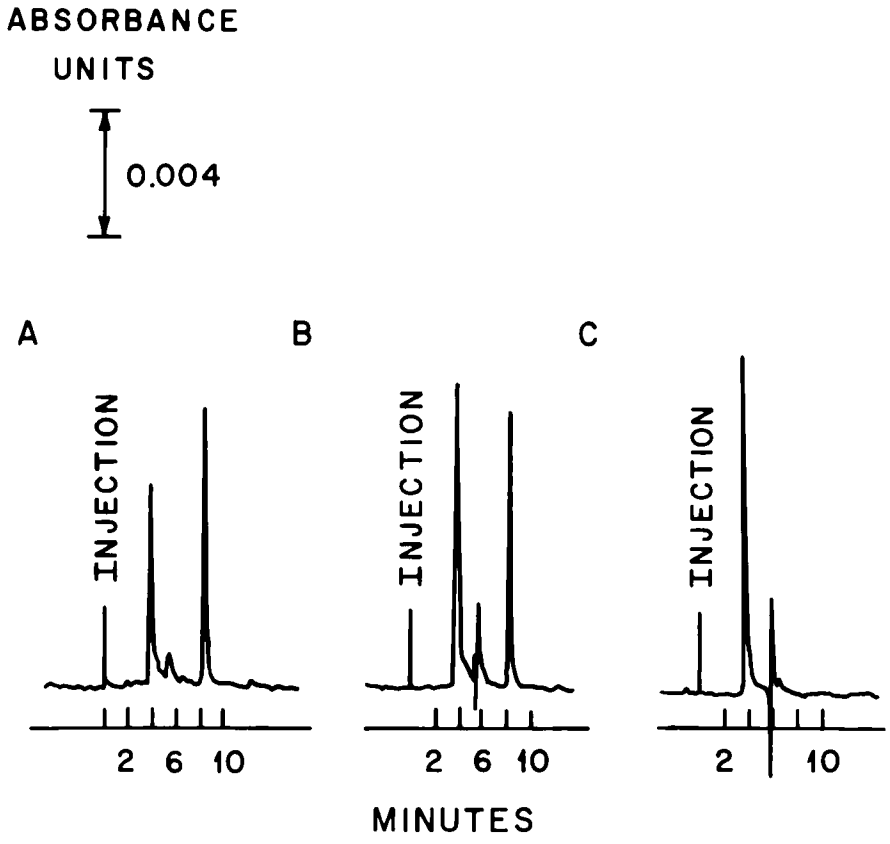

Figure 4. Chromatograms of lactic acid in Milrinone Injection, processed standard (A), 100% spiked placebo (B) and placebo (C). Chromatographic conditions as in Figure 3.

Here it was found necessary only to remove Milrinone from lactic acid which occurs in passing through the cation-exchange column at acidic pH. The acid was naturally not retained by this resin and was quantitatively eluted with water. Lactic acid at this

point still contained the ester material so a hydrolysis step was needed using NaOH. Figure 4A is a chromatogram of standard lactic acid processed by this procedure with a retention time of 8 min, while 4B is a 100% spiked placebo. A blank is seen in Figure 4C with the negative/positive peak at 6 min due to dextrose which follows lactic acid through the processing.

A chromatogram of a mixed standard containing pyruvic, lactic and acetic acids is shown in Figure 5. These compounds are well separated from each other as is an unknown component ③ originating in the pyruvic acid. The minimum acceptable resolution factor between lactic and acetic acids was set at 1.5.

Linearity of recovery data is shown in Tables 1 and 2 for the oral solution and injection respectively. In each dosage form peak height measurement appeared moderately more precise based on standard deviations. The minimum quantifiable limit for lactic acid was 0.0023 mg/mL as determined by the serial dilution study.

High-performance liquid chromatograms of the distillation fractions revealed little lactic acid and no ester in the 60-90° range. The 90-120°

TABLE 1

Lactic Acid Recovery from Oral Solution

	Measurement by Peak Height		Peak Area	
mg/mL added	mg/mL found	% recovery	mg/mL found	% recovery
0.859	0.848	98.7	0.845	98.4
0.859	0.864	100.6	0.893	104.0
1.07	1.08	100.9	1.09	101.9
1.07	1.07	100.0	1.08	100.9
1.29	1.32	102.3	1.28	99.2
1.29	1.32	102.3	1.32	102.3
Average Percent Recovery		100.8		101.1
Standard Deviation of y		.00816		.0226
Correlation Coefficient		.99938		.99457

TABLE 2

Lactic Acid Recovery from Injection

	Measurement by Peak Height		Peak Area	
mg/mL added	mg/mL found	% recovery	mg/mL found	% recovery
0.904	0.900	99.6	0.905	100.1
0.904	0.902	99.8	0.896	99.1
1.13	1.13	100.0	1.16	102.6
1.13	1.12	99.1	1.13	100.0
1.36	1.34	98.5	1.37	100.7
1.36	1.34	98.5	1.36	100.0
Average Percent Recovery		99.2		100.4
Standard Deviation of y		.00517		.0142
Correlation Coefficient		.99973		.99812

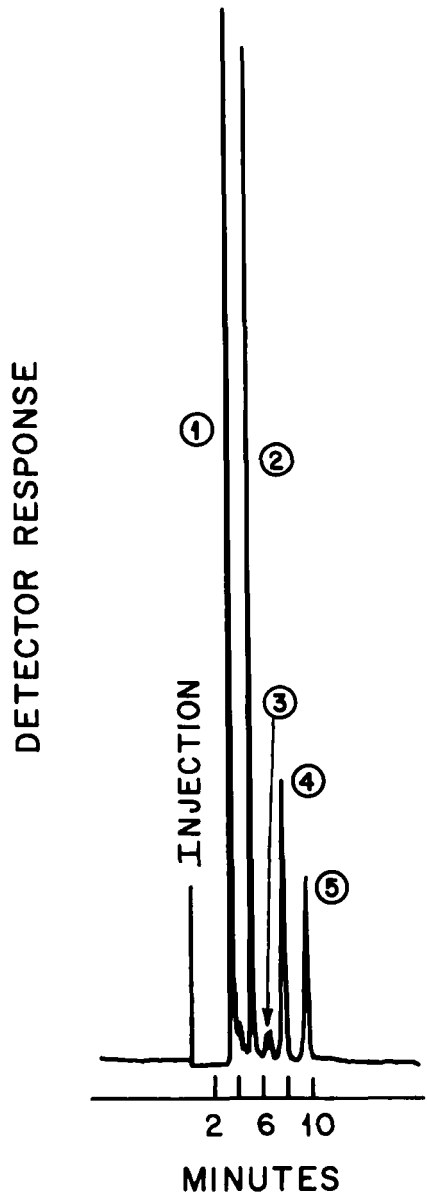

Figure 5. Chromatogram of mixed standard preparation including: solvent front (1), pyruvic acid (2), unknown (3), lactic acid (4) and acetic acid (5). Chromatographic conditions as in Figure 3.

fraction contained about 13 times more lactic acid than ester while the residue had 1.4 times more ester content than acid as determined by peak height measurement. An unhydrolyzed lactic acid standard by comparison gives a peak height ratio of between 3 and 4 to 1 for lactic acid to ester depending on relative retention times. This enrichment of ester in the residue seen here by removal of lactic acid appears to proceed at a somewhat faster rate (2 hours) than observed by Alén and Sjöström who obtained a ratio of 2.2/1 for lactic acid to ester in 6 hours at 100° and 1 atmosphere(13). Results by TLC on cellulose F supported the HPLC findings on separation of distillation fractions with an R_f of 0.35 for lactic acid and 0.48 for the ester. This agrees with the work of Munier, Drapier and Faivre (15) who correctly suggested that the higher R_f spot was an ester.

The newly developed method described above gives precise and accurate measurement of lactic acid in two liquid dosage forms. This or slightly modified methods could be applied to other organic acids or acidic counterions which are not retained well by reversed-phase columns even with ionic suppression. It also provides an approach to studying certain esterification equilibria.

REFERENCES

1. The U.S. Pharmacopeia. 21st ed, USP Convention, Inc., Rockville, Md, 1985, p. 581.

2. Tolbert, C.E. and Kenner, C.T., J. Pharmaceut. Sci., 60, 596 (1971).

3. Riva, A. and Bisognani, M., Anal. Chem., 44, 2101 (1972).

4. Nema, S.N. and Verma, R.M., Analyst, 104, 691, (1979).

5. Fine, A., Brosnan, J.T. and Herzberg, G.R., Metabolism Clin. and Experimental, 33, 393 (1984).

6. Buchanan, D.N., Bonasso, F. and Thoene, J.G., J. Chromatogr., 278, 133 (1983).

7. Borenstein, D.G., Gibbs, C.A. and Jacobs, R.P., Arthritis and Rheumatism, 25, 947 (1982).

8. Lambert, M.A. and Moss, C.W., J. Chromatogr., 74, 335 (1972).

9. Hyppänen, T., Sjöström, E. and Vuorinen, T., J. Chromatogr., 261, 320 (1983).

10. Munegumi, T. and Harada, K., J. Chromatogr., 291, 354 (1984).

11. Ng, K.J., Andresen, B.D., Hilty, M.D. and Bianchine, J.R., J. Chromatogr., 276, 1 (1983).

12. Rizzo, A.F., Cronache di Chimica, 68, 19 (1982).

13. Alén, R. and Sjöström, E., Acta Chem. Scand B, 34, 633 (1980).

14. Vonderheid, C. Funk, W. and Hille, J., Proc. Int. Symp. Instrum. HPTLC, 2nd, 209 (1982).

15. Munier, R.L., Drapier, A.M. and Faivre, B., Chromatographia, 6, 468 (1973).

16. Stamer, J.R., Weirs, L.D. and Mattick, L.R., Food Chem., 10, 235 (1983).

17. Owens, J. and Robinson, J.S., J. Chromatogr., 307, 380 (1984).

18. Distler, W., J. Chromatogr., 152, 250 (1978).

19. Gonnet, C., Marichy, M. and Philippe, N., Analusis, 7, 370 (1979).

20. Nakamura, K., Morikawa, Y. and Matsumoto, I., Bunseki Kagaku, 29, 314 (1980).

21. Benecke, I., J. Chromatogr., 291, 155 (1984).

22. Rich, W., Johnson, E., Lois, L., Kabra, P., Stafford, B. and Marton, L., Clin. Chem., 26, 1492 (1980).

23. Rokushika, S., Sun, Z.L. and Hatano, H., J. Chromatogr., 253, 87 (1982).

24. Busch, H., Hurlbert, R.B. and Potter, V.R., J. Biol. Chem., 196, 717 (1952).

25. Lunder, T.L. and Messori, F., Chromatographia, 12, 716 (1979).

26. Saitoh, H. and Oikawa, K., Bunseki Kagaku, 32, 273 (1983).

27. Symonds, P., Ann. Nutr. Alim., 32, 957 (1978).

28. Rapp, V.A. and Ziegler, A., Deutsche Lebensmittel-Rund. 12, 396 (1979).

29. Harlow, G.A. and Morman, D.H., Anal. Chem., 36, 2438 (1964).

30. Wood, R., Cummings, L. and Jupille, T., J. Chromatogr., Sci., 18, 551 (1980).

31. Woo, D.J. and Benson, J.R., Liquid Chromatogr., 1, 238 (1983).

32. Woo, D.J. and Benson, J.R., American Laboratory, 16, 50 (1984).

33. Pecina, R., Bonn, G., Burtscher, E. and Bobleter, O., J. Chromatogr., 287, 245 (1984).

34. Marsili, R.T., J. Chromatogr. Sci., 19, 451 (1981).

35. Buchanan, D.N. and Thoene, J.G., Anal. Biochem., 124, 108 (1982).

36. Bennett, M.J. and Bradey, C.E., Clin. Chem., 30, 542 (1984).

37. Ashoor, S.H. and Welty, J., J. Chromatogr., 287, 452 (1984).