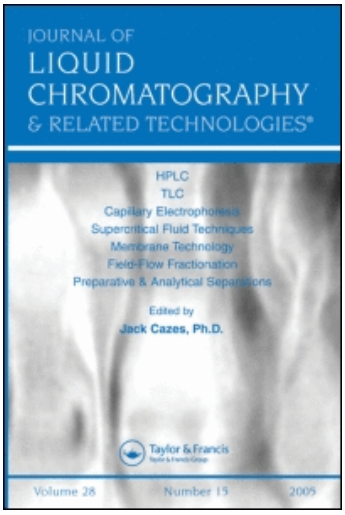

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF LACTIC ACID AND LACTIC ACID LACTATE IN AMRINONE LACTATE FORMULATIONS

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ABSTRACT

A high performance liquid chromatographic method for the quantitation of lactic acid in Amrinone Lactate formulations is described. Samples were separated using a Dionex HPICE-AS1 ion chromatography exclusion column and a mobile phase of 1 mM sodium octanesulfonate. A conductivity detector equipped with an anion micromembrane suppressor was used and regenerated with 5 mM tetrabutylammonium hydroxide. The method demonstrated a recovery of 100.3% with a linear correlation coefficient of the standards of 0.9999. The selectivity of the method was demonstrated by showing the absence of chromatographic interference by other formulation components. The method also separates lactic acid lactate from lactic acid. Studies conducted on samples of Amrinone Lactate Injection and placebo demonstrated a temperature related equilibrium between lactic acid and lactic acid lactate.

INTRODUCTION

Amrinone Lactate ([3,4'-Bipyridin]-6(1H)-one, 5-amino-, 3-hydroxypropanoate) is a cardiotonic agent, distinct from digitalis glycosides or catecholamines, which has been shown to have inotropic activity in humans(1). Lactic acid is added to the formulation to enhance Amrinone's solubility. A

High Performance Ion Chromatography (HPIC) method has been developed to evaluate the stability and concentration of lactic acid in Amrinone Lactate Injection.

Several analytical methods for lactic acid have been described in the literature. These methods include acid-base titration(2); colorimetry; infra-red spectrophotometry; iodimetric titration; enzymatic analysis; gas chromatography; gas chromatography/mass spectrophotometry; and thin layer chromatography. Several liquid chromatographic methods have also been described. Reverse-phase high performance liquid chromatographic methods using UV detection have been reported as well as ion chromatography methods(3). Methods for the quantitation of lactic acid in Amrinone Lactate and Milrinone Lactate Injection, a compound similar to Amrinone, have been described (3, 4). However, they involve complex or lengthy sample preparation to remove interfering compounds and quantitation by titration. None of these methods are suitable for our purposes, they are time consuming and do not facilitate analysis of numerous samples. The reported method involves a simple dilution of the sample followed by chromatography with conductimetric detection. This facilitates fast, simple and accurate analysis of samples and eliminates extensive sample preparation.

EXPERIMENTAL

Reagents

Amrinone Lactate (Inocor® Lactate; Winthrop Pharmaceuticals) and sodium octanesulfonate (HPLC grade; Regis Chemical Company) were used as obtained from the vendors. Lithium lactate (Spectrum Chemical Corp.) was reagent grade, lactic acid (Sigma Chemical Company) was USP grade and water was prepared using a Milli-Q™ water system.

Equipment

All experiments were carried out on a chromatographic system consisting of an autosampler (Waters 712 WISP®), a gradient pump (Dionex GPM-2) and a conductivity detector (Dionex CDM-2). All data were collected and analyzed on a Fisons Multichrom® data acquisition system.

Standard Preparation

Approximately 460 mg of lithium lactate was dissolved in 50 mL of water. Three standards were prepared by pipetting 3.00, 5.00 and 7.00 mL of this solution into separate 25-mL volumetric flasks and diluting to volume with water. Lithium lactate was chosen as a standard in lieu of lactic acid, since lactic acid polymerizes in solution forming cyclic dimers and linear polymers(6); the oligomer concentration varies as a function of concentration and temperature, a titration is necessary to determine the concentration of free lactic acid in solution. Lithium lactate solutions do not polymerize and are stable indefinitely at 2-4° C.

Sample Preparation

Amrinone Lactate samples were prepared by pipetting 3.0 mL of the injection into a 10.0 mL volumetric flask and diluting to volume with water. These samples were thoroughly mixed and injected on the HPIC.

Oligomer Preparation

Lactic acid oligomers were produced by refluxing USP lactic acid for 12 hours at 120 °C(7).

Chromatography

All experiments were conducted at ambient temperatures and on Dionex HPICE-AS1 ion chromatography exclusion columns. The mobile phase consisted of 1.0 mM sodium octanesulfonate. It was filtered and degassed by passing it through a 0.2 µm filter (Nylon 66 Alltech Associates) under vacuum. The column was periodically washed with 0.1 N hydrochloric acid to remove any compounds that do not elute under these conditions. To decrease background conductivity, a Dionex Anion MicroMembrane-ICE (AMMS-ICE) suppressor was used.

RESULTS AND DISCUSSION

Method recovery was determined by spiking known concentrations of lactic acid into solutions containing amrinone and the other formulation

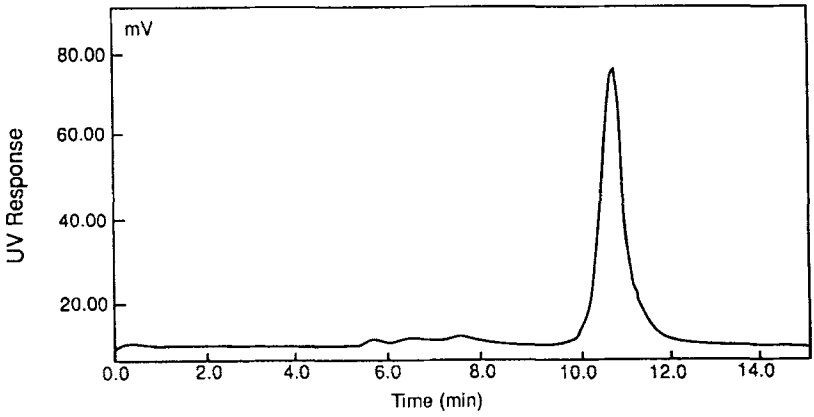

Figure 1. A chromatogram of a 6.25 mg/mL solution of USP lactic acid

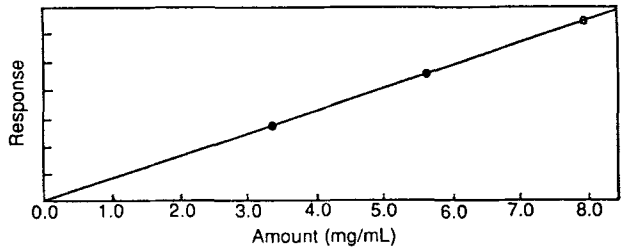

Figure 2. A standard curve for lithium lactate standards prepared as described in the text. A linear correlation coefficient of 0.9999 was acheived.

ingredients. Lactic acid was titrated by the USP assay procedure to determine potency(2). Samples were prepared at concentrations of approximately 5.0, 6.3 and 7.5 mg/mL lactic acid. The samples were chromatographed by the described procedure yielding a mean recovery of 100.3% for nine spiked samples. Figure 1 is an example of the chromatography for one of these samples. Linearity of standard response was determined for a range of 3.5 mg/mL to 8.0 mg/mL, and a linear correlation coefficient of 0.9999 was achieved (Figure 2).

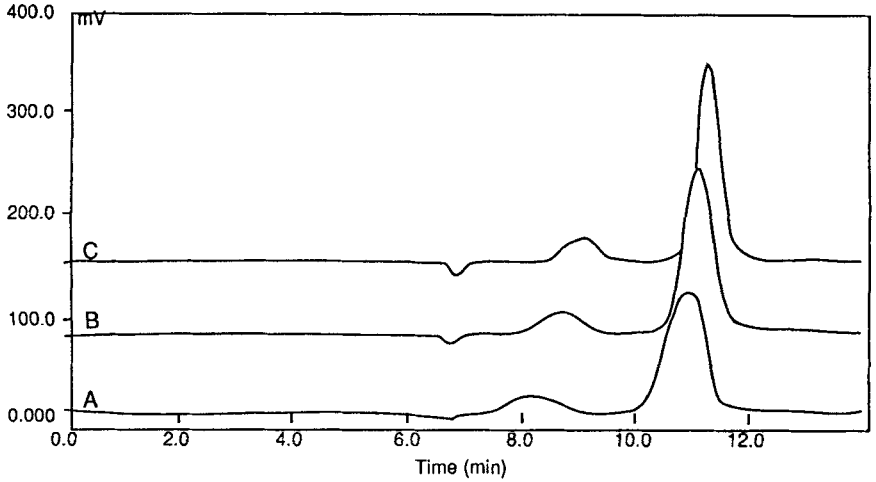

Figure 3. An isometric plot of chromatograms of USP lactic acid separated by mobile phases with varing concentrations of sodium octanesulfonate. A) 0.5 mM; B) 1.0 mM; C) 1.5mM

The selectivity of the method was shown by the absence of chromatographic interferences from the other formulation ingredients and the lactic acid oligomers. Amrinone, sodium metabisulfite and lactic acid were chromatographed individually and in combinations to demonstrate that there was no co-elution or other chromatographic interferences. Lactic acid oligomers were also produced as described and chromatographed. No chromatographic interferences were detected in either set of experiments.

Differences in chromatographic conditions were evaluated to determine their significance. Mobile phases consisting of concentrations of 50%, 100% and 150% of the suggested component concentrations were evaluated to determine their impact on chromatographic retention and resolution. Whereas, retention times increased slightly at the higher concentrations of sodium octanesulfonate, the chromatography was not adversely affected until the sodium octanesulfonate concentration was greater than 2 mM. At higher concentrations, the background from the acid increases and sensitivity is lost. This is explicable when the role of the the sodium octanesulfonate is

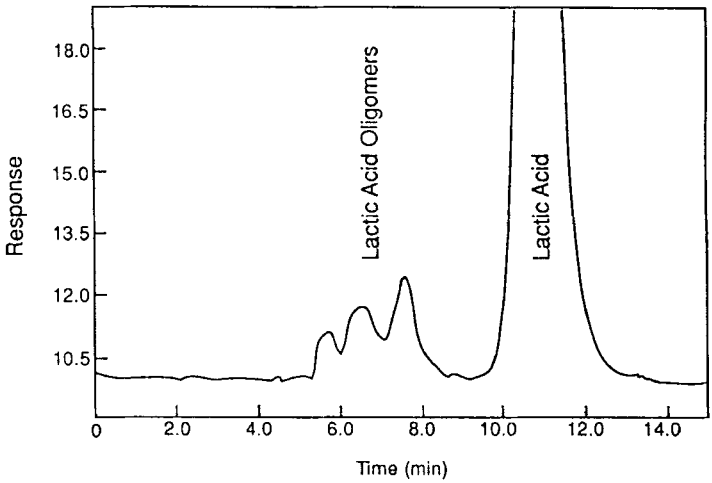

Figure 4. A chromatogram of lactic acid and the separation of lactic acid oligomers from the unpolimerized acid.

considered. Sodium octanesulfonate is added to the mobile phase to lower the pH of the mobile phase and to reprotonate the dissociated weak acid. The undissociated acid is not affected by Donnan exclusion and can permeate the Donnan membrane to interact with the stationary phase. Reducing mobile phase pH such that virtually all of the lactic acid is protonated and neutrally charged improves peak shape and increases retention. (Figure 3) However, once the acid concentration is high enough such that all the lactic acid is neutral, no chromatographic advantage is gained and background conductivity increases.(7)

In addition to the quantitation of lactic acid in Amrinone Lactate formulations, this method also resolves oligomers of lactic acid. Lactic acid oligomers were produced as described above This solution was analyzed by the above method. Figure 4 shows the resulting chromatogram.

Samples containing 6.8 mg/mL lactic acid were prepared and stored at 4 °C, 30 °C, 40 °C and 60 °C for 4 weeks. Three peaks with retention times corresponding to the lactic acid oligomers appear. To confirm the presence of

TABLE 1

Analysis of Lactic Acid and Lactic Acid Lactates
Temperature Dependent Polymerization

Temp (°C)	Assay Result	Post Hydrolysis
4	6.2	6.7
30	6.4	6.7
40	6.5	6.8
60	6.6	6.7

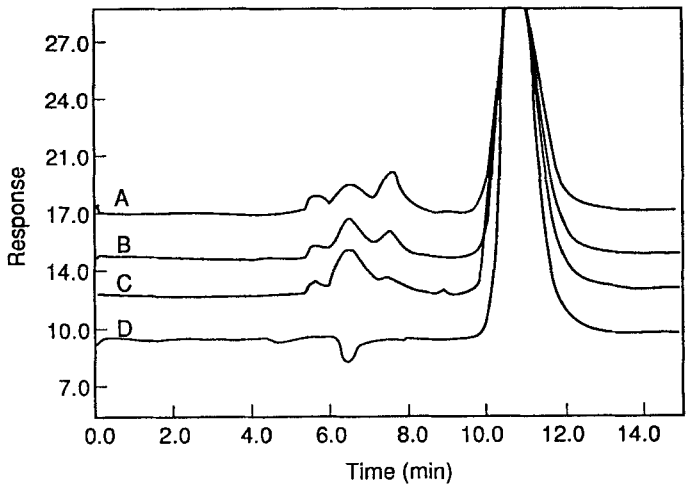

Figure 5. Chromatograms of lactic acid solutions stored under controlled temperatures for 4 weeks. A) 4 °C; B) 30 °C; C) 40 °C; D) 60 °C

lactic acid lactates being formed, samples from each temperature were hydrolyzed to convert any lactic acid lactate present to lactic acid. The results are summarized in Table 1. A temperature dependent equilibrium was demonstrated and could be monitored by the above method. Figure 5 is an overlay of chromatograms for samples stored at these conditions.

CONCLUSIONS

The HPIC method described is a fast, simple, and reliable method for the determination of lactic acid in amrinone lactate injection. The accuracy and precision of the method is suitable for use in a research or quality control laboratory. This method can be modified to analyze other pharmaceutical and non-pharmaceutical products for lactic acid and lactic acid lactate content.

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