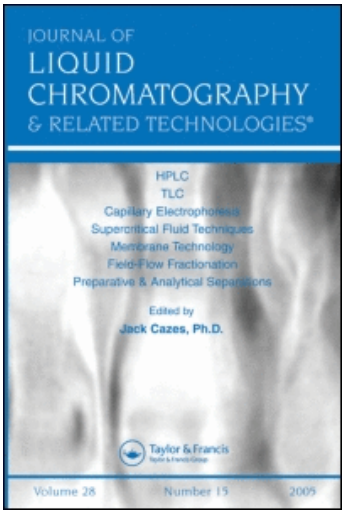

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High Pressure Liquid Chromatography of (Bay o 9867) Ciprofloxacin in Serum Samples

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HIGH PRESSURE LIQUID CHROMATOGRAPHY OF (BAY o 9867) CIPROFLOXACIN IN SERUM SAMPLES

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ABSTRACT

Quantitative analysis of ciprofloxacin using liquid chromatography and a traditional microbiological assay were compared and had a correlation coefficient of 0.9701. Liquid chromatography (LC) was a modification of that of Wingender et al. (1) and used a mobile phase consisting of water, acetonitrile, phosphoric acid, and tetrabutylammonium hydroxide. Separation on a reverse phase C18 column gave a retention time of 3.6 minutes.

INTRODUCTION

Ciprofloxacin, a new oral antibacterial agent belonging to a group of compounds termed quinoline carboxylic acids, has undergone extensive in vitro

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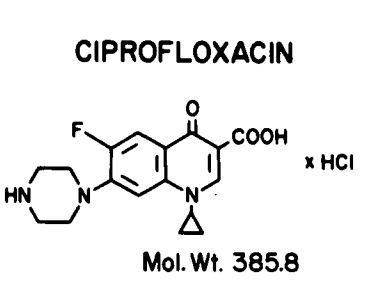

FIGURE 1. Structure of ciprofloxacin.

testing (2-8). Reports of animal and human studies (9-11) are now increasing and valid quantitative antibiotic concentration data from blood and body fluids in these studies is important for correlation of therapy outcome with the in vitro data.

The compound's structure is shown in Figure 1, having two ionizable groups it displays amphoteric behavior.

EXPERIMENTAL

LC Assay

The chromatographic system used was a Varian (Walnut Creek, CA.) liquid chromatograph model 5020 with a Waters (Waters Associates, Milford, MA.) 420E fluorescence detector and Varian strip chart recorder. Deproteinated samples (100 mcl were injected by a fixed loop injector and chromatographed on a micro-BondaPak-C18 column (Waters Associates) using a mobile phase mixture consisting of 88% 25 mM H_3PO_4 (Fisher

Scientific, Pittsburg, PA.) and 30 mM tetrabutylammonium hydroxide, (Sigma Chemical, St. Louis, MO.) at pH 3.0 with 12% acetonitrile (Fisher Scientific, HPLC grade). The flow rate was 2.0 ml/min. Fluorescence detection was achieved using a mercury lamp with 254 nm wavelength excitation filter and a 425 nm long pass emission filter and full detector sensitivity. Calculations were based on an external standard method using peak height ratios. Each sample was injected twice and the value averaged to obtain the final result.

Microbiological Assay

A standard agar well diffusion technique employing E. coli ATCC (American Type Culture Collection, Rockville, MD.) 10536 as the indicator organism was used (12). The assay plates (100 mm X 15 mm, Falcon Plastics, Cockeysville, MD.) consisted of 10 ml of a 0.20% suspension of an overnight growth of indicator organism in antibiotic media #1 (Difco, Detroit, MI.). Twenty-five microliter volumes of sample or standards were placed into 6.0 mm diameter wells previously cut in the agar, and the plates were then incubated at 37°C for 24 hours. Resultant zone diameters were read to the nearest 0.1 mm and sample concentrations were determined by averaging diameters from three replicate plates, and comparing the results to a standard curve.

Samples

Serum samples for assay development and evaluation were prepared by adding ciprofloxacin to pooled rabbit or human serum. Thirty serum samples obtained from rabbits receiving ciprofloxacin (10 mg/kg IM) were used for comparing the two assay techniques. Deproteination of serum samples in preparation for LC was achieved using methanol precipitation (2 volumes methanol; 1 volume sample) followed by centrifugation at 12,000 X g using an Eppendorf Centrifuge, model 5414 (Brinkman Instruments, Inc., Westburg, NY).

RESULTS

The retention time for ciprofloxacin was 3.6 min. The chromatogram from serum is shown in Figure 2. The LC had a linear detector response to 2.0 mcg/ml and had a lower detection limit of 0.05 mcg/ml. Drug recovery during the deproteintation process calculated using peak heights of spiked serum sample at 5.0 mcg/ml compared to an identically prepared nonprotein containing sample was 63% from human and 80% from rabbit sera. The coefficient of variation of LC assay using a sample at 1.5 mcg/ml measured with ten replicates was 5.9%.

The microbiological assay gave a standard curve which was linear when log concentration was plotted against zone diameter from 0.125 mcg to 2.0 mcg/ml shown in Figure 3.

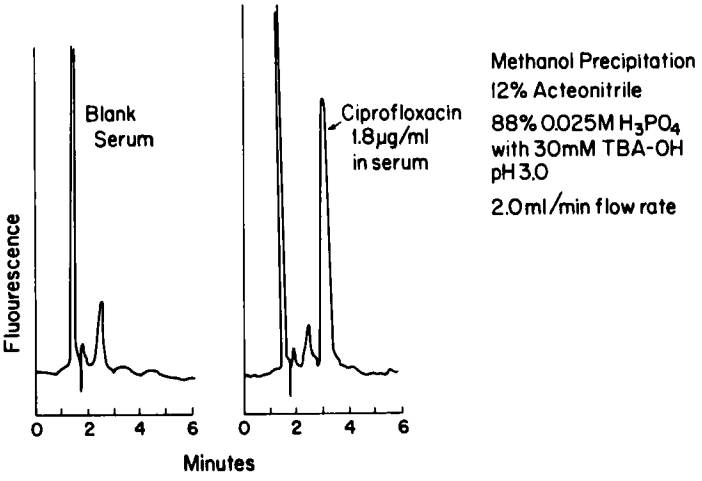

FIGURE 2. Chromatograms from serum samples.

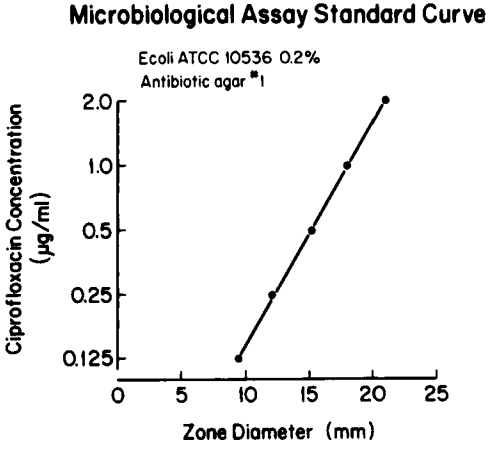

FIGURE 3. Standard curve for microbiological assay.

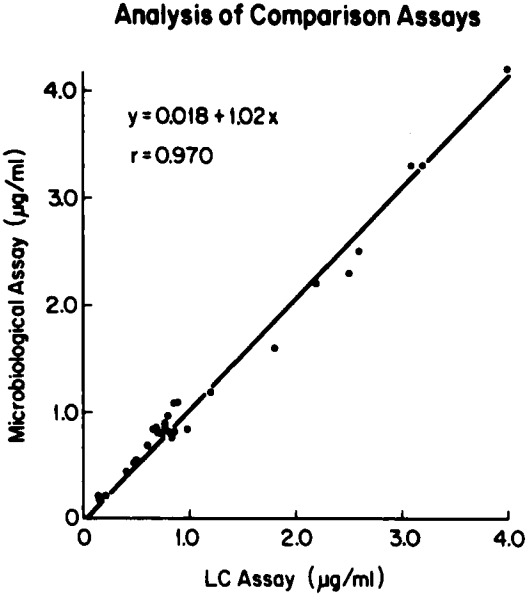

FIGURE 4. Statistical comparison of LC and Microbiological methods.

Comparison of the two assay methods using in vivo serum samples gave the regression analysis shown in Figure 4. The correlation coefficient was 0.9701. Comparison using 14 rabbit serum samples spiked, in vitro, with varying ciprofloxacin concentrations gave a linear regression of Y = - 0.08 + 1.18 X and a correlation coefficient of 0.9933.

DISCUSSION

Results indicate that a near perfect direct relationship was obtained. This favorable comparison shows the LC method to be accurate. The correlation

coefficient and coefficient of variation represent that the LC results were precise and reliable. The comparison analysis on *in vivo* samples and *in vitro* samples demonstrate that there are no major *in vivo* metabolite interferences in the serum assay. Indications for use of LC over microbiological methods are the increased rapidity of quantitation and removal of interference from other antimicrobial agents which is encountered with a microbiological system.

ACKNOWLEDGMENTS

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