CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Artilide in Human Plasma

Purpose

Determination of artilide fumarate in human plasma.

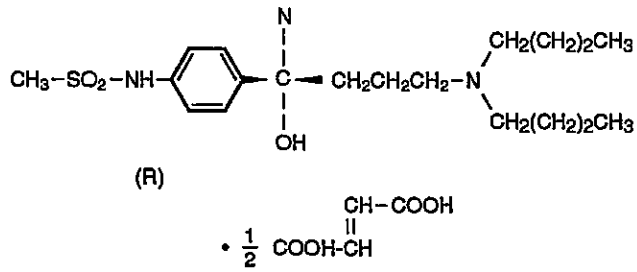

Figure 1. Structure of artilide fumarate.

Artilide fumarate [F1] is a class III antiarrhythmic agent being tested for the treatment of atrial flutter and fibrillation [1]. Due to its low therapeutic range, quantitation was achieved using LC coupled with fluorescence detection and column switching [2]. The procedure involves a complex column-switching technique which cannot be described in detail here.

Conditions

System: BAS-201 Liquid Chromatograph, coupled with three BAS PM-80 pumps to provide flow to the various columns.
Analytical Column: C_8, 150 x 4.6 mm, 5 µm particle size.
Guard Columns: Two cyano, 15 x 3.2 mm, 7 µm particle size.
Detector: BAS FL-45 Fluorescence Detector
Excitation Wavelength: 224 nm
Emission Wavelength: 346 nm cutoff filter
Mobile Phase A: 50:50:0.1:0.1 (v:v) acetonitrile:water:triethylamine (TEA):trifluoroacetic acid (TFA)
Mobile Phase B: 30:70 (v:v) methanol:water
Mobile Phase C: 40:40:20:0.2:0.2 (v:v) acetonitrile:methanol:water:TEA:TFA
Mobile Phase D: 30:70:0.15:0.15 (v:v) acetonitrile:water:TEA:TFA
Flow Rate: 2 mL/min
Linear Range: 0.2 to 100 ng/mL

Sample Preparation

Samples of plasma containing the internal standard (U-70,226E) were passed through solid-phase extraction columns, washed with several solvents, and eluted with an acetonitrile:acetone:TEA mixture. The eluents were dried, then derivatized for 15 minutes with a 1-napthylisocyanate reagent. The reaction was quenched with TFA and the samples transferred to autosampler vials. Aliquots of 600 µL were injected onto the system.

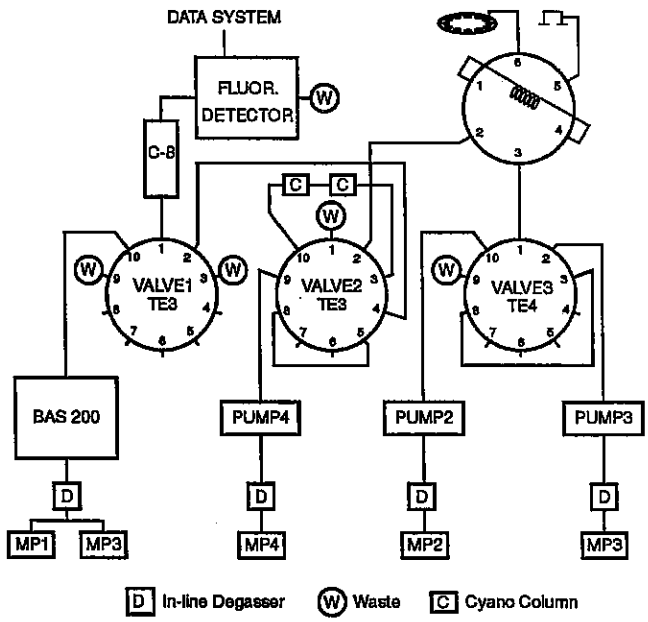

Figure 2. Diagram of column-switching system

System Description

Refer to F2. The analytical column was flushed with mobile phase C in a clean-up step while the autosampler injected a new sample. The sample was immediately washed by mobile phase B onto two cyano columns in series, for approximately nine minutes. Four minutes after sample injection, the flow on the analytical column was switched to mobile phase A. At 8.7 minutes the 10-port valves switched, allowing sample to wash from the cyano columns onto the analytical column, using mobile phase D. At 9.7 min-

utes the valves switched back to their starting positions. When the IS eluted, the analytical column was switched to mobile phase C for cleaning. The entire cycle took about 27 minutes.

Notes

A chromatogram of a blank plasma sample is shown in F3. A quality-control sample appears in F4, and a pharmacokinetic profile is shown in F5.

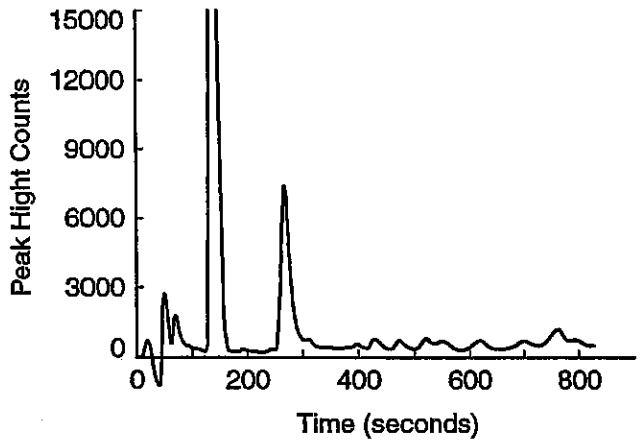

Figure 3. Blank plasma sample.

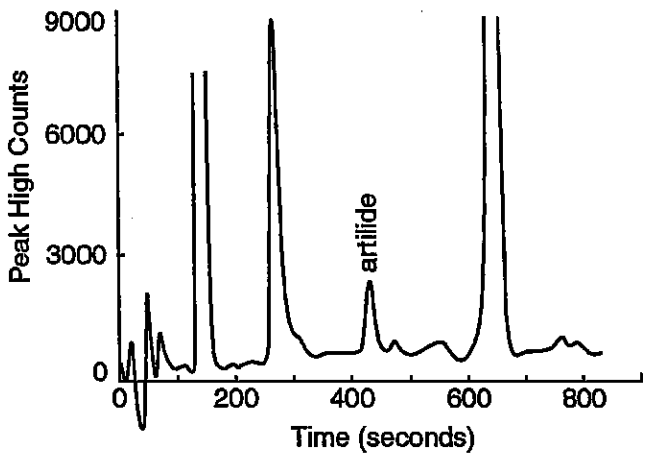

Figure 4. 0.405 ng/mL quality-control sample.

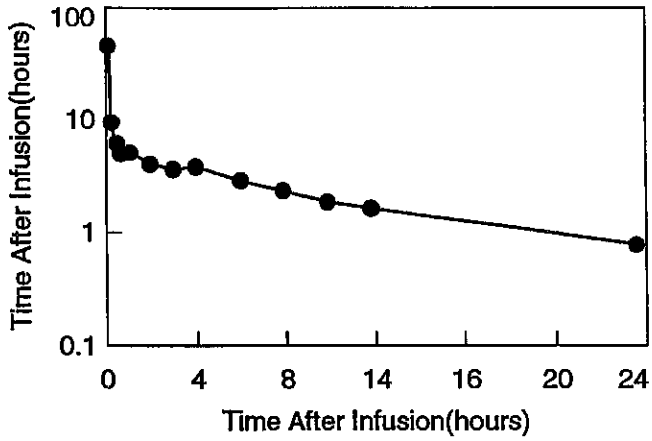

Figure 5. Pharmacokinetic profile.

References

1. *C.-Y. L. Hsu and R.R. Walters, J. Chromatogr. 667 (1995): 115-128.*
2. *K.L. Steele, R.E. Shoup and N.K. Hopkins, poster session presented to The American Association of Pharmaceutical Scientists 8th Annual Meeting, Orlando FL, 1993.*