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Report

Determination of a Novel Antiarrhythmic Agent ACC-9358 in Human Plasma by High-Performance Liquid Chromatography

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A liquid chromatographic method for the determination of ACC-9358, an antiarrhythmic agent, in human plasma is described. ACC-9358 was extracted from plasma into chloroform and back extracted into 0.01 *N* hydrochloric acid. ACC-9358 in the acidic extract was eluted from a Novapak C18 column using a 87:13:0.1 (by volume) mixture of phosphate buffer (pH 3.0), acetonitrile, and triethylamine as the mobile phase and measured at 280 nm. The average extraction recoveries were 78.4 and 85.4% at the 0.5- and 5-μg/ml level, respectively. Standard curves were linear and reproducible over the concentration range of 0.01–10 μg/ml, with coefficients of determination better than 0.999. Coefficients of variation for both within-day and day-to-day analysis were less than 11%. The assay method is sensitive, reproducible, and suitable for disposition studies of ACC-9358 in humans.

KEY WORDS: antiarrhythmic agent; ACC-9358; high-performance liquid chromatography; human plasma.

INTRODUCTION

ACC-9358 [4-hydroxy-*N*-phenyl-3,5-bis(l-pyrrolidinylmethyl)benzamide] is a new class Ic antiarrhythmic agent (Fig. 1). Preclinical studies have demonstrated that it is effective in controlling ventricular arrhythmia and atrial flutter in dogs (1). Elimination half-life of ACC-9358 averaged 4.5 and 1.2 hr in dogs (2) and rats (3), respectively.

ACC-9358 is currently undergoing clinical trials. To study the pharmacokinetics of this agent in humans, we have developed a selective, sensitive, and reproducible liquid chromatographic method for its measurement in plasma. This paper describes the assay methodology and its application to the determination of ACC-9358 in human plasma.

MATERIALS AND METHODS

Chemicals. ACC-9358 was synthesized at Du Pont Critical Care (Waukegan, Ill.). Internal standard, timolol maleate, was obtained from Merck, Sharp and Dohme (West Point, Pa.). Acetonitrile and chloroform were of liquid chromatography grade (American Burdick & Jackson, Muskegon, Mich.). Triethylamine (100%) was purchased from J. T. Baker Chemical Co. (Phillipsburg, N.J.). Hydrochloric acid (0.01 *N*), phosphoric acid (87%), and monobasic potassium phosphate were of analytical reagent grade and were purchased from commercial sources.

High-Performance Liquid Chromatography (HPLC). A Waters HPLC system (Waters Associates, Milford, Mass.) equipped with a Model 590 solvent delivery system, a Model 710B automatic sample injector, a Model 440 UV detector set at 280 nm, and a Novapak C18 column (150 mm × 3.9-mm i.d.) was used. The mobile phase consisted of a 87:13:0.1 (by volume) mixture of 0.05 *M* monobasic potassium phosphate (pH 3.0), acetonitrile, and triethylamine. The mobile phase was filtered through a 0.45-μm Nylon-66 membrane (Rainin Instrument, Woburn, Mass.) and further degassed using a vacuum pump. The mobile-phase flow rate was 1.0 ml/min at a column inlet pressure of 1000 psi. Chromatograms were recorded on a strip chart recorder (Linear Instruments Corp., Reno, Nev.) and peak area ratios and all concentrations were generated by a Hewlett-Packard Model 3357 laboratory automation system (Hewlett-Packard, Palo Alto, Calif.)

Standard Solutions. A stock solution of ACC-9358 was prepared in 0.01 *N* hydrochloric acid at a concentration of 0.1 mg/ml (free base). Working standards in the concentration range of 0.1–100 μg/ml were prepared by appropriate

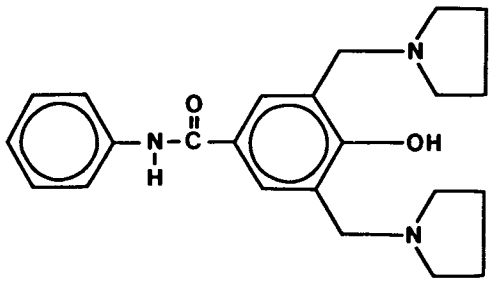

Fig. 1. Chemical structure of ACC-9358.

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dilution of the stock solution in 0.01 *N* hydrochloric acid. A solution of the internal standard, timolol maleate, was prepared in water at a concentration of 20 μg/ml.

Extraction of Plasma. One milliliter of plasma (containing ACC-9358), 0.1 ml (20 μg/ml) of internal standard, and 0.1 ml of water were transferred into a 20 × 125-mm glass culture tube with a Teflon-lined screw cap and vortexed. After the addition of 10 ml of chloroform, the tube was capped tightly, shaken mechanically for 10 min, and centrifuged at 1900 *g* (3000 rpm) for 10 min. The aqueous layer was discarded and the chloroform layer was transferred into a 16 × 125-mm glass culture tube containing 0.3 ml of 0.01 *N* hydrochloric acid. The tube was shaken mechanically for 10 min, then centrifuged for 10 min to clarify the phases, 0.2–0.25 ml of the acid phase was transferred into a microinsert contained in an autosampler vial, and a 100-μl aliquot was analyzed by HPLC.

Standard Curves. Duplicate standards were prepared by transferring 0.1 ml of the working standards (instead of 0.1 ml of water above) and 1.0 ml of drug free plasma into glass culture tubes to encompass the standard curve range of 0.01–10 μg/ml. After the addition of 0.1 ml of internal standard, the tubes were treated as described under Extraction of Plasma.

Extraction Recovery and Assay Reproducibility. The recovery of ACC-9358 from plasma was determined at two different concentrations. Known amounts of ACC-9358 were added to drug-free plasma, and the area responses of ACC-9358 in the extracted spiked samples were compared with those obtained by direct injection of standard solutions containing equivalent amounts of ACC-9358.

Assay reproducibility was assessed at low, medium, and high concentrations of ACC-9358. Within-day reproducibility was calculated from six replicate analysis. Day-to-day reproducibility was determined from duplicate analysis on 3 different days.

Stability of ACC-9358 in Plasma. Plasma samples spiked with ACC-9358 (0.5 and 5 μg/ml) were stored at −20°C and analyzed at different times over a 12-month period. Area ratios of ACC-9358 to internal standard in these samples were compared with those from freshly prepared controls.

Application of the Method. Blood samples were collected from volunteers after the intravenous administration of ACC-9358. Plasma was harvested after centrifugation and analyzed for ACC-9358 concentration using the method described in this report.

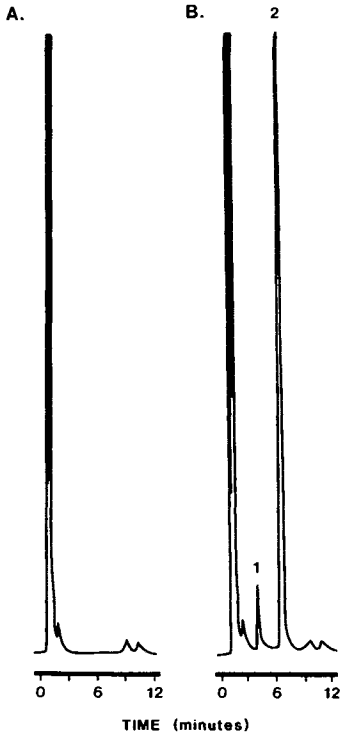

Fig. 2. Chromatograms of plasma (1-ml) extracts: A, blank plasma; B, plasma spiked with 0.01 μg of ACC-9358 (1) and 2 μg of internal standard (2). 0.01 A full scale.

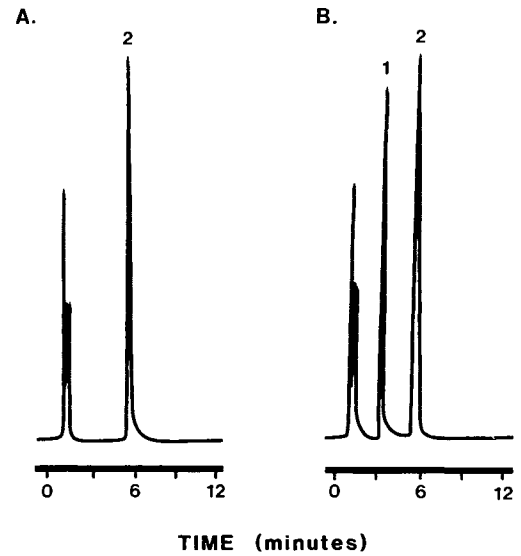

Fig. 3. Chromatograms of plasma extract before (A) and 5 min after (B) intravenous administration of ACC-9358 to a volunteer. 1, ACC-9358; 2, internal standard. 0.1 A full scale.

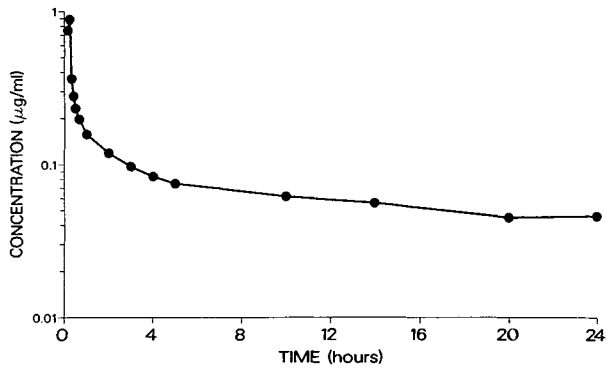

Fig. 4. Plasma ACC-9358 concentration–time profile following intravenous administration (0.5 mg/kg) to a volunteer.

RESULTS AND DISCUSSION

Absolute recoveries (mean ± SD) of ACC-9358 from human plasma using the described extraction procedures were 78.4 ± 1.9 and 85.4 ± 1.6% at the 0.5- and 5-μg/ml level, respectively. The recovery of the internal standard was 81.2 ± 0.8%.

Figure 2 illustrates the chromatograms obtained from extracts of blank plasma and plasma spiked with ACC-9358 and internal standard. Both compounds were well resolved from each other and there was no chromatographic interference from the endogenous plasma components in the extracts. Initially we used a Waters μBondapak phenyl column. In this column, ACC-9358 eluted as a broad and tailing peak after the internal standard peak. The Novapak C18 column used in the present work provided favorable retention times for both compounds. The higher efficiency of the Novapak column also enabled a greater assay sensitivity to be achieved. The elution order was ACC-9358 at 3.5 min and internal standard at 5.9 min, and as we did not observe any late peaks in the chromatogram, the chromatographic run could be completed within 10 min. We have analyzed 300 samples on a single column without any chromatographic problem.

Absorbance of ACC-9358 was measured at 280 nm and not at the wavelength of maximum absorbance of 300 nm because the latter wavelength was not available in the UV detector used for this work.

Reproducibility was excellent for both within-day and day-to-day analysis. Coefficients of variation for within-day analysis ($N = 6$) were 10.7% at 0.01 μg/ml, 1.5% at 1 μg/ml, and 2.0% at 10 μg/ml. The corresponding values for day-to-day analysis ($N = 6$) were 10.6, 2.0, and 1.8%.

The limit of assay sensitivity was 0.01 μg/ml, with a linear range of 0.01–10 μg/ml. Standard curves prepared on different days showed a good reproducibility. Linear regression analysis, using SAS (4), of 10 standard curves provided a slope (standard error) of 1.2815 (±0.0029), an intercept of 0.0216 (±0.011), and a coefficient of determination of 0.9995.

At the 0.01-μg/ml level, the signal-to-noise ratio was 15 and the coefficient of variation was less than 11%. Furthermore, ACC-9358 eluted as a sharp peak without any interference from the extractive plasma components. Therefore, a lower quantitation limit of 0.005 μg/ml with an acceptable precision is still attainable.

The stability of ACC-9358 in plasma stored at −20°C was assessed over a 12-month period. We did not observe a decrease in ACC-9358 levels with time. The average recovery from 1-, 3-, 6-, 9-, and 12-month spiked plasma samples was 100.7 ± 5.0% at the 0.5-μg/ml level and 98.9 ± 4.1% at the 5-μg/ml level.

The assay method was applied to determine plasma levels of ACC-9358 in humans. Figure 3 illustrates the representative chromatograms of plasma extracts before and after the intravenous administration (0.5 mg/kg) of ACC-9358 to a volunteer. The plasma ACC-9358 concentration versus time profile in the same volunteer is shown in Fig. 4. Plasma ACC-9358 concentrations were measured for 30 hr following administration of the drug and the decline in plasma concentration appears to be biphasic. Figure 4 also demonstrates that the sensitivity of this assay is adequate to define the elimination profile of ACC-9358 in humans.

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