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Note

High-performance liquid chromatographic method for quantitative determination of Yutac®, a new antiarrhythmic agent, in dog plasma

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Among the wide variety of chemical compounds used in the therapy of cardial arrhythmia, bispidine derivatives [1—3] belong to the first group of the classification of Vaughan Williams [4]. As a bispidine derivative, 3-methyl-7-ethyl-9-α-ol-(4-chlorobenzoyloxy)-3,7-diazabicyclo (3.3.1)-nonane monohydrochloride (I) (Fig. 1) acts mainly via membrane stabilization, but possesses calcium-antagonistic activity as well [5]. The compound is soluble in methanol and water, with a molecular weight of 322.8. The pK_a values of the functional N atoms are 1.67 and 12.3, respectively. The maximum UV absorbance occurs at 243 nm and does not show any pH dependence.

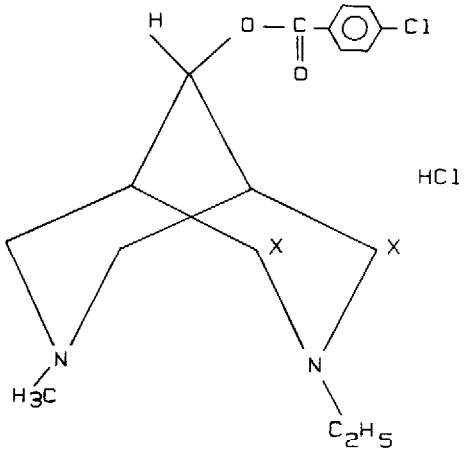

Fig. 1. Structural form of I. The position of the ^{14}C label is marked by X.

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In the present paper a method for the extraction of I from plasma and a high-performance liquid chromatographic (HPLC) procedure suitable for pharmacokinetic studies in dogs are described.

EXPERIMENTAL

Chemicals

I and internal standard [II, 3,7-dimethyl-9-(2′-naphthoyloxy)-3,7-diazabicyclo (3.3.1)-nonane dimethane sulphonate] were produced by the Chemical Works of Gedeon Richter (Budapest, Hungary). ^{14}C-Labelled I (specific radioactivity: 334.42 MBq/mmol) was prepared by the Central Research Institute for Chemistry, Hungarian Academy of Sciences (Budapest, Hungary). Methanol, acetonitrile, diethyl ether and Extrelut were purchased from E. Merck (Darmstadt, F.R.G.). All other chemicals used were the products of Reanal (Budapest, Hungary) and were of analytical purity.

Sample treatment and preparation of calibration curves

To 0.8 ml of dog plasma, an aqueous solution of 50 ng of II (internal standard) and 0—500 ng of ^{14}C-labelled I were added. The pH of plasma samples was adjusted to 9 using 20 μl of conc. NH_4OH. The samples were applied to the top of the chromatographic columns (15 × 5 mm) filled with 500 mg of Extrelut, allowed to soak for 5 min and eluted with 7 ml of diethyl ether. The recovery of I was checked by liquid scintillation counting. Diethyl ether was evaporated under a stream of nitrogen; the dry residue was dissolved in 0.1 ml of methanol by shaking and 30-μl aliquots were injected onto the analytical column.

Radioactivity of samples was counted in a Packard Tri-Carb 2660 liquid scintillation spectrometer. The ^{14}C radioactivity in 100-μl plasma aliquots was measured in Insta-Gel® (Packard, Downers Grove, IL, U.S.A.) and that of the diethyl ether samples (100-μl aliquots) in a toluene-based liquid scintillation solution (5 g of 2,5-diphenyloxazole, 0.1 g of dimethyl 1,4-bis(5-phenyloxazolyl-2)benzene, 1000 ml of toluene).

Chromatographic conditions

HPLC assay was performed on a Hewlett-Packard 1081 B chromatograph equipped with a variable-wavelength Cecil 2112 UV detector (Cecil Instruments, Cambridge, U.K.) and a 3380 S integrator. A Chromsil Si 60 prepacked column (25 × 4.6 mm I.D.), 10 μm particle size (Labor-MIM, Budapest, Hungary), was used for the separation of the compounds. The flow-rate was 1.3 ml/min at a pressure of 0.7 kPa; the temperature was 30°C. The absorbance of the effluent was monitored at 243 nm.

In order to study the influence of pH and water content of eluent on column efficiency, the pH of eluents, consisting of methanol—acetonitrile—0.01 *M* sodium perchlorate (100:100:2) were changed from pH 3.7 to pH 2.7 (pH 3.7, 3.4, 3.0, 2.7) using perchloric acid, and water content of the eluent was changed from 0.1% to 2.0% (0.1, 0.5, 1.0, 2.0%) of the total volume.

In vivo experiments

Male and female Beagle dogs were used as experimental animals. I in physiological saline was administered intravenously to dogs into the cephalic vein or orally in capsules at a dose of 2 mg/kg of body weight in randomized "cross-over". Blood samples were taken from the cephalic vein at times indicated in Fig. 6 and collected into heparinized test-tubes. To the plasma obtained from blood after centrifugation at 1500 *g* for 10 min, 50 ng of II in 1 μl of water were added and the samples were processed as above.

RESULTS AND DISCUSSION

Fig. 2 shows the values of capacity and selectivity factors for I and internal standard at different pH values. At pH 3.7 the retention time was too long (k' = 7.0) and the width of the peaks was fairly large. The theoretical number of plates was also very low (N = 1550). By increasing the acid concentration of the eluent, the capacity factor decreased (k' = 2.0) and the theoretical number of plates increased (N = 2700). Alteration of the pH did not essentially change the values of the selectivity factors. Decreasing the pH resulted in an increase of N, but it was smaller than required.

The influence of water on k' and N is shown in Figs. 3 and 4. The k' value for both derivatives increased significantly with increasing water content in

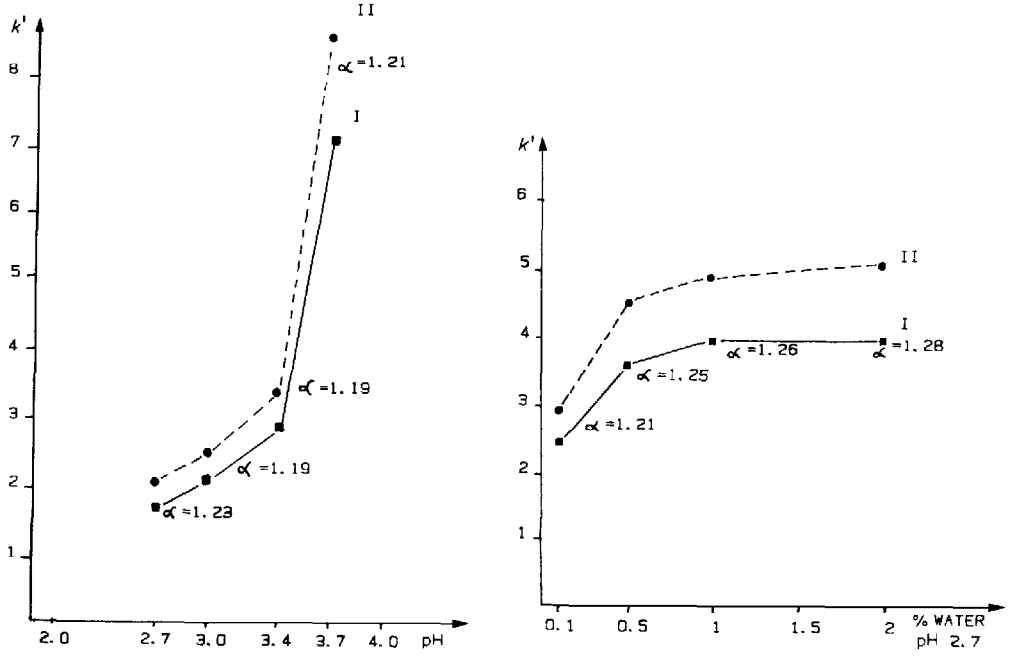

Fig. 2. Effect of pH on values of capacity (k') and selectivity (α) factors of I and II. Mobile phase: acetonitrile—methanol—0.01 *M* sodium perchlorate pH 2.7—3.7 (100:100:2).

Fig. 3. Effect of water on values of capacity (k') and selectivity (α) factors of I and II. Mobile phase: acetonitrile—methanol—0.01 *M* sodium perchlorate (100:100:0.2) in water (0.1—2%) at pH 2.7.

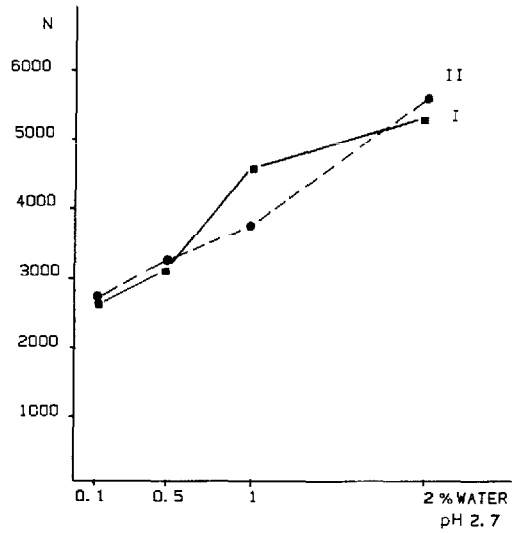

Fig. 4. Effect of amount of water (as a moderator) on theoretical number of plates (N) of the column.

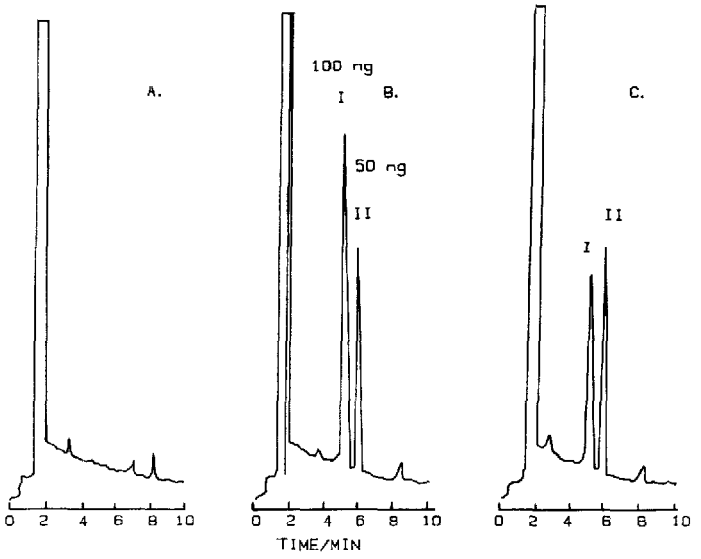

Fig. 5. Chromatograms of dog plasma extracts: (A) blank plasma extract; (B) plasma spiked with 100 ng of I and 50 ng of II; (C) plasma extract after intravenous administration of I at a dose of 2 mg/kg. Separation of two bispidine derivatives on a Chromsil Si 60 (10 μm particles size) column. Eluent: methanol—acetonitrile—0.01 M sodium perchlorate/perchloric acid (100:100:2) at pH 2.7.

the range of 0.1—2% of water (Fig. 3). The theoretical number of plates also increased (Fig. 4). The best separation was obtained when the eluent consisted of 100 ml of methanol, 100 ml of acetonitrile and 2 ml of 0.01 M sodium perchlorate/perchloric acid (pH 2.7).

The calibration curves were linear in the concentration range of interest (15—500 ng/ml). The linear regression curve for the data used for calibration

could be described by the equation $y = 0.006x + 0.018$ ($r = 0.994$) where y is the ratio of peak heights of I to II and x is the concentration of I.

The day-to-day coefficient of variation of the slope of the calibration curves was 3.6% ($n = 6$). Coefficients of variation for identical samples were 11.2% at 25 ng/ml, 7.6% at 100 ng/ml, 6.3% at 200 ng/ml ($n = 5$ at each concentration). The detection limit was found to be 15 ng of I per ml of plasma. Recovery of I from plasma was checked using radiolabelled drug and was found to be 73.1 ± 3.08% (S.D.) ($n = 6$).

Fig. 5 shows chromatograms of dog plasma extracts. Under the HPLC conditions used no interference by endogenous compounds has been observed.

The pharmacokinetic curve in Fig. 6 illustrates the successful application of the method. The results presented demonstrate that the method is appropriate for routine analysis and pharmacokinetic studies.

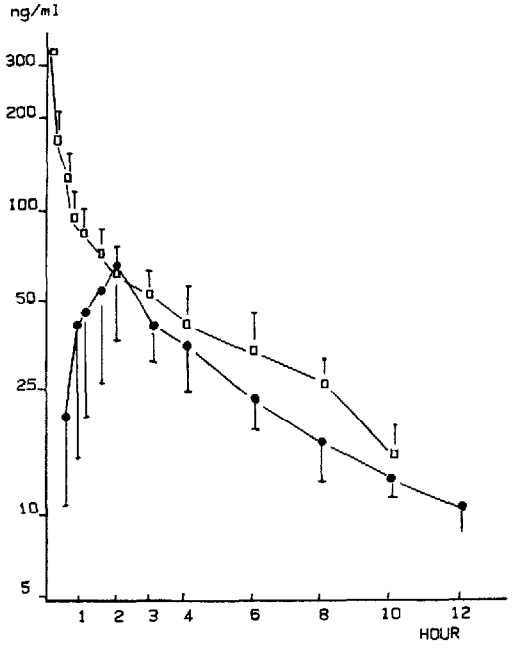

Fig. 6. Pharmacokinetic curve of I after intravenous (□) and oral (●) administration of the drug at a dose of 2 mg/kg of body weight.

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