Journal of Chromatography, 232 (1982) 435—439
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1385

Note

Determination of propafenone in serum or plasma by electron-capture gas chromatography

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(First received March 26th, 1982; revised manuscript received June 7th, 1982)

Propafenone, 2'-[3-(propylamino)-2-(hydroxy)-propoxy] -3-phenyl-propiophenone, is a new antiarrhythmic agent recently introduced in clinical practice. Its efficacy has been demonstrated in suppressing supraventricular and ventricular arrhythmias [1, 2]. It has been shown that propafenone serum levels are significantly correlated with changes in atrioventricular conduction times [3]. In the literature a high-performance liquid chromatographic (HPLC) method for the determination of propafenone plasma concentrations is described [4] but it requires large blood samples and injection volumes. To overcome these problems a rapid and sensitive gas—liquid chromatographic (GLC) method with electron-capture detection (ECD) which allows the detection of 10 ng/ml of the drug is described in this paper.

MATERIAL AND METHODS

Gas—liquid chromatography

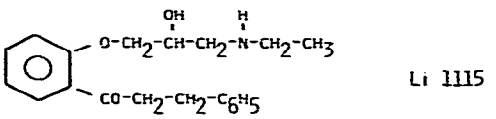
A Perkin-Elmer Model Sigma 4 gas chromatograph equipped with a ^{63}Ni electron-capture detector was used. A glass column (190 cm × 2 mm I.D.) was packed with 3% OV-101 on GCPS 100—120 mesh (Carlo Erba Strumentazione, Milan, Italy). The carrier and purge gas was 10% methane—argon (1:9) at flow-rates of 15 ml/min and 50 ml/min, respectively. The column temperature was 245°C and the temperatures of injection port and detector were 300°C.

0378-4347/82/0000—0000/$02.75

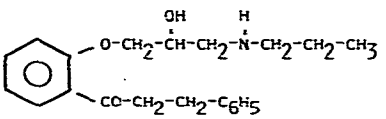
Reagents and materials

Propafenone · HCl and its internal standard Li 1115 (Fig. 1) were supplied by Knoll (Ludwigshafen, Rhein, G.F.R.). Trifluoroacetic anhydride (TFAA) and heptafluorobutyric anhydride (HFBA) were obtained from Pierce Chemicals (Rockford, IL, U.S.A.) and stored at −20°C. All organic solvents were RS grade (Carlo Erba).

Glassware was washed with hot nitric acid, rinsed with distilled water, dried at 105°C and silanized overnight with 5% dimethylchlorosilane in toluene. It was then washed with methanol and dried.



Li 1115



PROPAFENONE

Fig. 1. Structural formulae of propafenone and Li 1115 (internal standard).

Extraction procedure

Plasma samples (100—500 μl) were pipetted into glass stoppered tubes and internal standard (150 ng) was added. The samples were made alkaline (pH 11) with 1 *N* sodium hydroxide and extracted twice by shaking for 10 min with 4 ml of benzene each time. After centrifugation the combined organic extracts were evaporated to dryness under a stream of dry nitrogen at 40°C.

Derivatization

To the dried extract 300 μl of toluene and 50 μl of TFAA were added. The tubes, after being tightly stoppered, were heated for 45 min in a water bath at 40°C. The reaction mixture was then evaporated to dryness, the residue reconstituted with 200 μl of cyclohexane and 0.5—1.0 μl was injected.

The completeness of the reaction was checked by reacting fixed amounts of propafenone and TFAA at the constant temperature of 40°C. The results, shown in Fig. 2, indicate that quantitative derivatization was achieved in the conditions described above. An alternative procedure was also developed using HFBA as derivatizing agent. The HFBA derivatives of propafenone and its internal standard were stable and gave a greater response than TFAA derivatives in the electron-capture detector. However, it was not possible to achieve a satisfactory separation of these compounds on the common stationary phases.

RESULTS AND DISCUSSION

Under the chromatographic conditions used the peaks corresponding to propafenone and internal standard derivatives had retention times of 7.51 and

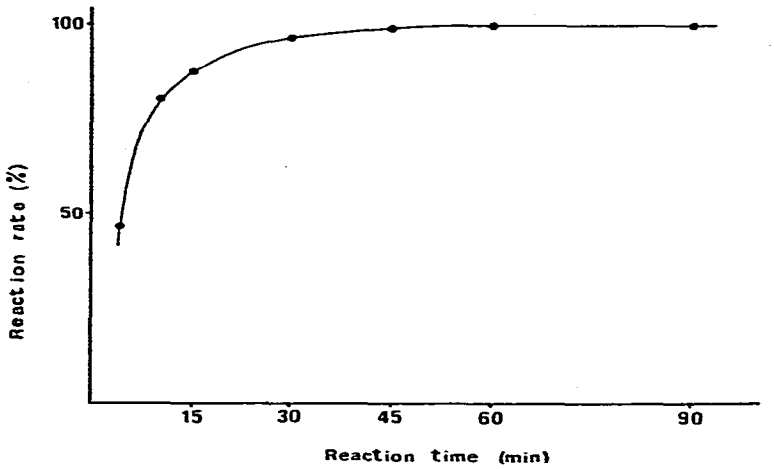


Fig. 2. Time course of TFAA-derivative formation from propafenone and TFAA.

6.54 min, respectively. A typical chromatogram of a plasma sample spiked with 200 ng/ml propafenone is shown in Fig. 3a, while a chromatogram of blank plasma is shown in Fig. 3b. In general no peaks that interfered with propafenone or internal standard peaks were encountered in the analysis of plasma from different patients.

The quantitative determinations of propafenone in plasma were performed by adding known amounts of drug in the range 50—200 ng/ml. The calibration

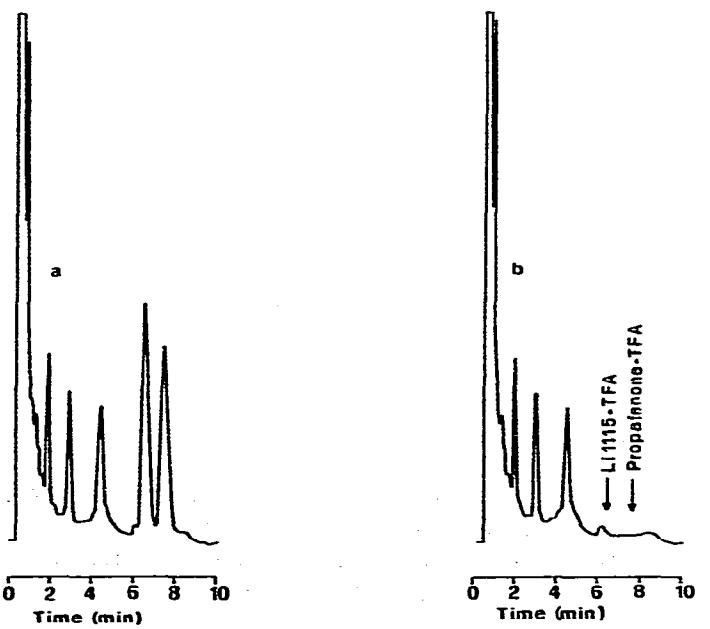


Fig. 3. Chromatograms obtained analyzing (a) an extract from plasma sample spiked with 200 ng/ml propafenone and 150 ng/ml Li 1115, and (b) plasma blank.

curves were linear within the range used and the minimum detectable amount was estimated as 100 pg injected.

To demonstrate the accuracy and precision of this method replicate determinations were carried out; the results are shown in Table I. Restandardization was carried out each day in order to maintain precision, although day-to-day variation in detector response was small.

TABLE I

REPRODUCIBILITY AT A GIVEN PLASMA CONCENTRATION FOR PROPAFENONE

Propafenone added (ng/ml)	*n*	Propafenone found ± S.D. (ng/ml)	C.V. (%)
50	6	52.5 ± 1.0	1.9
100	6	103.3 ± 3.5	3.4
150	6	151.0 ± 3.7	2.4
200	6	204.8 ± 7.6	3.7
Mean			2.8

TABLE II

CONCENTRATION OF PROPAFENONE (ng/ml) IN PATIENT PLASMA SAMPLES AS DETERMINED BY GLC—ECD AND HPLC

Plasma sample	GLC—ECD	HPLC
1	96	65
2	138	122
3	612	571
4	101	110
5	624	569
6	222	200
7	280	265

Moreover, we performed a parallel analysis of patient plasma samples taken after chronic propafenone administration using HPLC [4] and the new GLC—ECD method (Table II). Linear regression analysis of HPLC and GLC values showed good agreement and gave a regression coefficient of 0.998.

The high sensitivity of this method will permit an accurate determination, particularly after the administration of low doses of propafenone, using small blood sample volumes. This feature is most important when the pharmacokinetic study requires many and frequent plasma determinations.

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