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Note

Determination of pindolol in biological fluids by an electron-capture gas—liquid chromatographic method on a wall-coated open tubular column

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Pindolol, 4-(2-hydroxy-3-isopropylaminopropoxy)indole, a beta-blocking drug, has until now been determined in plasma by the fluorimetric method described by Pacha [1]. Recently, an electron-capture gas—liquid chromatographic (GLC) procedure using a trifluoroacetyl derivative of pindolol was proposed [2]. On some occasions, these two methods demonstrate, however, a lack of specificity. In the fluorimetric assay, interfering fluorescent material can be coextracted from biological fluids. For the GLC determinations, impurities present in some batches of the reagent may lead to insufficient specificity. A rapid and highly specific electron-capture GLC method using a wall-coated open tubular column is described in this paper.

EXPERIMENTAL

Instrumentation

A Hewlett-Packard gas chromatograph Model 5713A was equipped with a ^{63}Ni electron-capture detector and a capillary injection system 18740B, and was connected to a Sefram P.E. recorder with a scale range of 1 mV. A Hewlett-Packard integrator system 3352C was used for integration and quantitation.

The OV-1 coated open-tubular glass capillary column (25 m × 0.3 mm I.D.) was pretreated and tested before use.

The splitless injection mode was used, a 2-μl sample being injected. The injection period was 30 sec.

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The column temperature was isothermal at 80°C for 2 min, and then programmed from 80°C to 170°C at a rate of 10°C/min. Detector and injector temperatures were 300°C and 250°C, respectively.

The make-up gas (argon with 10% methane) had a flow-rate of 35 ml/min. The flow-rate of the carrier gas (helium) was 3 ml/min. Under these conditions the retention times of propranolol and pindolol were 13 min 5 sec, and 13 min 30 sec, respectively.

Standard solutions

The standard stock solutions (0.1 mg/ml) of pindolol and propranolol were prepared by dissolving these compounds in methanol. The solutions were stored at 4°C and dilutions were made just before use.

Analytical procedure

Pindolol with propranolol as internal standard (20 ng/ml) were extracted from plasma as described previously [2]. The dry extract was dissolved in 100 µl of a 4 *M* trimethylamine solution in iso-octane and derivatized with 10 µl of trifluoroacetylimidazole for 30 min at room temperature. The reaction mixture was shaken vigorously for 5 sec with 1 ml of 0.5 *M* phosphate buffer (pH 5.4) and centrifuged. A 2-µl aliquot of the iso-octane phase was taken for electron-capture GLC analysis.

Standard curves

Under these conditions the calibration curve of pindolol, with propranolol as the internal standard, was linear over the range of 2.5—30 ng/ml (Fig. 1).

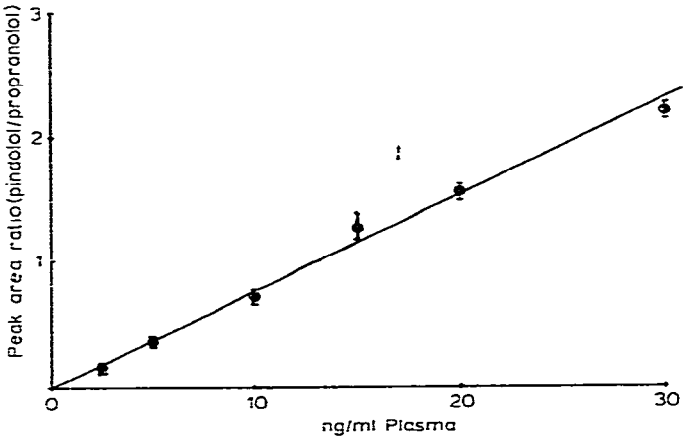

Fig. 1. Calibration curve for the described method.

RESULTS AND DISCUSSION

In the previous paper [2] the method described involved the use of a glass

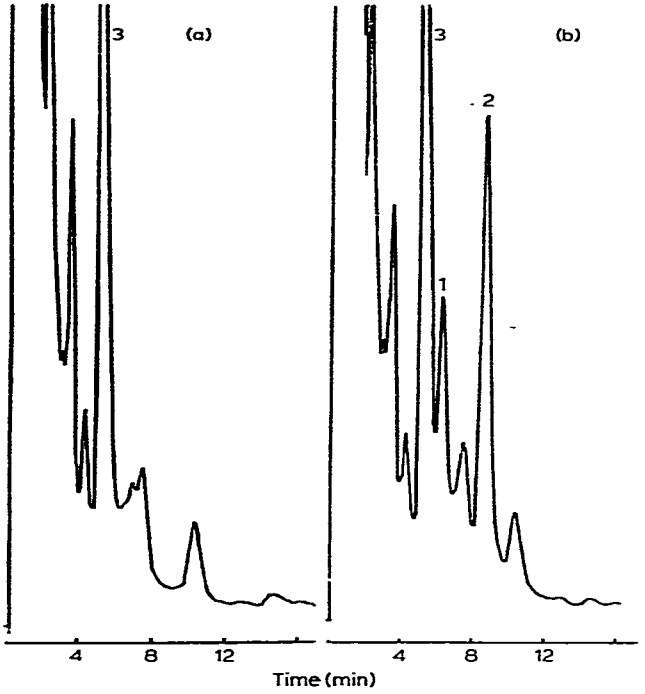

Fig. 2. Gas chromatograms obtained with a 2% OV-17 packed column from (a) a blank human plasma sample and (b) a human plasma spiked with 10 ng/ml pindolol (peak 1) and 50 ng/ml propranolol (peak 2) as internal standard (peak 3 results from the reagent).

column packed with 2% OV-17 on Gas-Chrom Q (100—120 mesh). Pindolol and propranolol were determined with good sensitivity and specificity. Unfortunately, the acetylating reagent trifluoroacetylimidazole had not always the same batch-to-batch purity. This resulted in an interfering peak appearing sometimes on the chromatogram with a retention time slightly shorter than that of pindolol. Fig. 2 demonstrates the poor separation of the two peaks of the "interfering substance" and pindolol. Moreover, if no precautions were taken in the washing procedure for the glassware, interfering peaks appeared on the chromatogram with one of them having nearly the same retention time as pindolol. To avoid this potential source of error in the accuracy and reproducibility of the method, elaborate and time-consuming washing of the glassware was necessary.

The use of a wall-coated OV-1 capillary column with a high efficiency overcame these problems. In a test assay during which no precautions were taken during either the washing of glassware or in the quality of the reagent, the method was validated. This has since been confirmed by numerous analyses. As demonstrated in Fig. 3, under these conditions, interfering substances were completely absent from drug peaks after extraction of human plasma spiked with 10 ng of pindolol and 20 ng of propranolol per ml. The two peaks are well-separated.

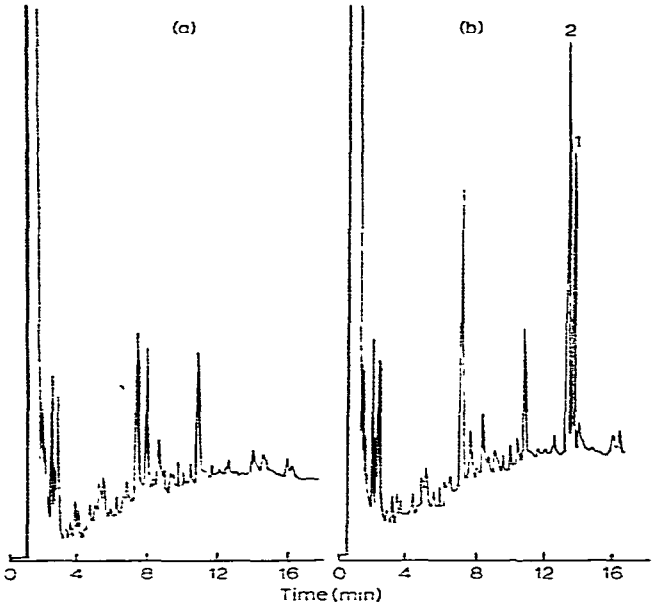

Fig. 3. Gas chromatograms obtained with an OV-1 wall-coated open tubular column from (a) a blank human plasma sample, and (b) a human plasma spiked with 10 ng/ml pindolol (peak 1) and 20 ng/ml propranolol (peak 2) as internal standard.

TABLE I

REPRODUCIBILITY AND ACCURACY OF THE METHOD

Added to plasma (ng/ml)	Recovered from plasma (ng/ml; mean ± S.E.M. $n = 3$)	Coefficient of variation (%)	Accuracy (mean ± S.E.M.)
1	1.05 ± 0.05	8.2	8.0 ± 2.7
2.5	2.66 ± 0.14	9.3	9.7 ± 2.6
5	4.74 ± 0.12	4.3	5.2 ± 2.4
10	10.14 ± 0.20	3.4	2.9 ± 0.9
15	14.94 ± 0.19	2.3	1.8 ± 0.2
20	20.90 ± 0.75	3.0	2.2 ± 0.6
30	29.82 ± 0.60	3.5	2.7 ± 0.7

The experimental procedure, described above, guarantees a better quality of data.

The results of the reproducibility study to validate the method are shown in Table I. The coefficient of variation ranged from 2.3 to 9.3%. The average reproducibility of an assay over the concentration range 2.5—30 ng/ml is ± 4.9%. The accuracy of the method is given by the mean percentage deviation of all concentrations from the theoretical value (see Table I). This value ranged from 1.8 to 9.7 with a mean value of 4.6.

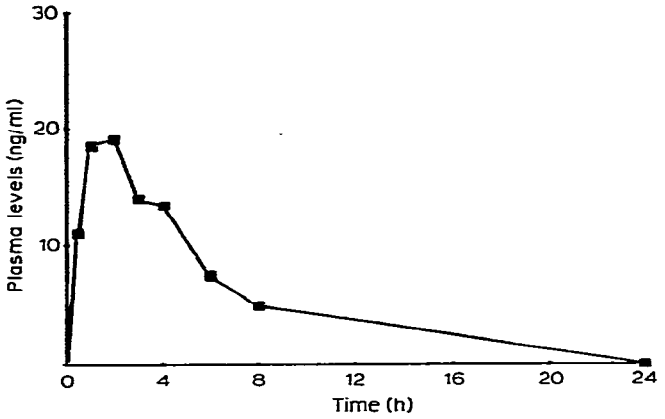

Fig. 4. Plasma levels of pindolol in one subject after a single oral dose of 5 mg of drug administered orally in tablet form.

Fig. 3a demonstrates that in a plasma blank there is no interference with the pindolol peak. The sensitivity of this chromatographic determination is about 5 pg injected on to the column. It is therefore possible to detect with good precision 500 pg of pindolol per ml of plasma using a 2-ml sample. If only 1 ml of plasma is available, the iso-octane phase can be concentrated before injection on to the column to maintain the same limits of sensitivity, but this additional step is time-consuming.

A plasma concentration curve obtained using this method in one volunteer who took 5 mg of pindolol orally is displayed in Fig. 4. The procedure can also be applied to the determination of propranolol with pindolol as internal standard.

CONCLUSIONS

The high resolution and excellent sensitivity obtained by the use of a wall-coated open tubular column compared to the conventional packed column led to the development of an accurate and convenient electron-capture GLC method for pindolol in plasma. This method allows the determination of pindolol in plasma samples from clinical investigations of patients to whom other medications are administered, by minimizing the potential analytical interference.

In addition, the time for sample preparation is reduced, due to simplification of the washing procedure for the glassware. Moreover, the automatization of data processing is reliable and easier, and facilitates the collection of data for population characteristics of pharmacokinetic parameters in different disease states and under various therapeutic regimens.

Because of these advantages, the described analytical method offers a worthwhile alternative to the conventional and currently used procedure.

ACKNOWLEDGEMENTS

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