NOTES

Improved Electron-Capture GLC Determination of Alprenolol and Oxprenolol in Serum Using a Wall-Coated Open Tubular Column

D. DeBRUYNE, H. KINSUN ˣ, M. A. MOULIN, and M. C. BIGOT

Received March 31, 1978, from the *Laboratory of Pharmacology, Faculty of Medicine, Functional Explorations B Department, University Hospital Center of Caen, 14032 Caen Cedex, France.* Accepted for publication September 5, 1978.

Abstract □ An accurate, rapid, and sensitive GLC determination of alprenolol and oxprenolol in serum is described. This method combines an electron-capture detector with a wall-coated open tubular column. The lowest detectable amount of the halogenated (heptafluoroacyl) derivatives of alprenolol and oxprenolol is 2 pg. The high resolving power and the rapid elution time of the wall-coated open tubular column dramatically improve the performance (accuracy and sensitivity) of the conventional electron-capture detector and packed column system in these determinations. This method allows the use of small volumes of sample (100–200 µl), does not require redistilled reagents, and has a simplified extraction procedure.

Keyphrases □ Alprenolol—electron-capture GLC determination of serum levels, wall-coated open tubular column □ Oxprenolol—electron-capture GLC determination of serum levels, wall-coated open tubular column □ GLC—determination of alprenolol and oxprenolol in serum using wall-coated open tubular column and electron-capture detection

Because of their lower therapeutic serum levels [10–100 ng/ml (1, 2)], the β-blocking agents alprenolol and oxprenolol have been determined by electron-capture GLC (3–7) as their halogenated derivatives. Unfortunately, these methods require a large blood sample (1–4 ml), involve a time-consuming extraction step, and sometimes have poor reproducibility (3).

The purpose of this work was to develop a fast and more convenient GLC determination for alprenolol and oxprenolol in blood by the simultaneous use of an electron-capture detector and a wall-coated open tubular column.

EXPERIMENTAL

Instrumentation—The gas chromatograph[1], equipped with a ^{63}Ni electron-capture detector[2] and a splitter injector[3], was connected to a recorder[4] with a scale range of 1 mv.

The 0.3-µm OV-101 phase thickness glass capillary column[5] (25 m × 0.25 mm) was pretreated and tested by the manufacturer. According to the spot test, it had an effective number of theoretical plates (N) of 38,000 (endrin[6]) at 200° for a carrier gas (nitrogen) with a velocity of 16 cm/sec and a capacity factor of 6.08.

The test conditions were: sample injected, 1 µl; injector temperature, 270°; column temperature, 205° (assay of alprenolol) or 185° (assay of oxprenolol); split ratio, 1:12; linear velocity of carrier gas, 34 (assay of alprenolol) or 58 (assay of oxprenolol) cm/sec; makeup gas (nitrogen) flow rate, 30 ml/min; detector temperature, 280°; duration and interval of pulsed mode, 0.8–4 and 100–200 µsec, respectively; sensitivity and attenuation, 0.1 × 64 and 1 × 32; and chart speed, 10 mm/min.

Standard Solutions—The standard stock solutions (0.2 µg/ml) of pure alprenolol[7] and oxprenolol[8] were prepared by dissolving them directly in 0.2 N HCl; these solutions were stored at 4° in a cold chamber. Chemical purity of standards was checked by high-resolution (wall-coated open tubular column) GLC.

Extraction and Preparation of Derivatives—To 200 µl of normal human serum[9] in glass test tubes (polytef-lined screw cap) were added, successively, 5–30 ng of alprenolol, 40 ng of oxprenolol from stock solutions as the internal standard, 25 µl of 1 N NaOH, and 5 ml of ether. The mixture was shaken for 5 min. After washing with 0.5 ml of distilled water and centrifugation (5 min at 15° and 3500 rpm), the ethereal phase was evaporated to dryness on a water bath at 50°. Then 100 µl of heptafluorobutyryl anhydride[10] solution in ethyl acetate (1:3 v/v) was added as the acylating reagent (8), and the temperature was raised to 60° for 15 min. Excess heptafluorobutyryl anhydride was eliminated by evaporation in a rapid current of air. After it was completely discharged, 100 µl of hexane was added and the mixture was evaporated once again.

The same procedure was used for the standardization of oxprenolol with 5 ng of alprenolol as the internal standard.

The residue was dissolved in 50 µl of hexane, and 1 µl of the mixture was injected into the chromatograph.

RESULTS AND DISCUSSION

Figure 1 illustrates the chromatographic responses of the alprenolol (α) and oxprenolol (β) heptafluorobutyryl derivatives. The chromatogram (Fig. 1*a*) obtained from 3% OV-1 on a 100–120-mesh Gas Chrom Q packed stainless steel column[3] (2.17 mm × 2 m) with the back-extraction procedure of Di Salle *et al.* (8) for propranolol clearly shows fewer peaks than that obtained from the wall-coated open tubular column (Fig. 1*b*). Figure 1*b* shows retention times relative to the solvent of 6 min for alprenolol and 7.5 min for oxprenolol. The lowest detectable amount was 2 pg.

Comparison of the two chromatograms obtained with equivalent nonpolar phases depicts the poor separation of interfering substances from alprenolol and oxprenolol owing to the insufficient resolving power of the packed column (9). This result could constitute a source of error in the accuracy and reproducibility of any method.

The high efficiency (10–12) of the wall-coated open tubular column is substantiated by Fig. 1c. It was obtained after using the described simple extraction procedure with reagents that had not been redistilled (ether and heptafluorobutyryl anhydride). Interfering substances were completely absent from drug peaks. Under these conditions, the calibration curve of alprenolol (with oxprenolol as the internal standard) (13) was linear over a range of 0–150 ng/ml. The slope of the regression line was 0.014 with a correlation coefficient of 0.999. The standard deviation varied from 3% at 25 ng/ml to 1.5% at 150 ng/ml over 2–3 months. The calibration curve of oxprenolol (with alprenolol as the internal standard) was linear over a range of 0–150 ng/ml. The slope of the regression line was 0.012 with a correlation coefficient of 0.999. The standard deviation varied from 3% at 25 ng/ml to 2% at 150 ng/ml over 2–3 months. The recovery was 97 ± 4.5% for both drugs.

[1] Girdel model 3000 FC 1 ERPT, Suresnes, France.
[2] Girdel model 3000, Suresnes, France.
[3] Girdel, Suresnes, France.
[4] Servotrace PU Sefram, Paris, France.
[5] Catalog No. 2101-201, LKB, Bromma, Sweden.
[6] Applied Sciences, Inglewood, CA 90304.
[7] Aptine, Lematte Boinot, Paris, France.
[8] Trasicor, Ciba-Geigy, Rueil Malmaison, France.
[9] Biotrol 00A, Biotrol, Paris, France.
[10] Pierce Chemical Co., Rockford, IL 61105.

0022-3549/ 79/ 0400-0511$01.00/ 0
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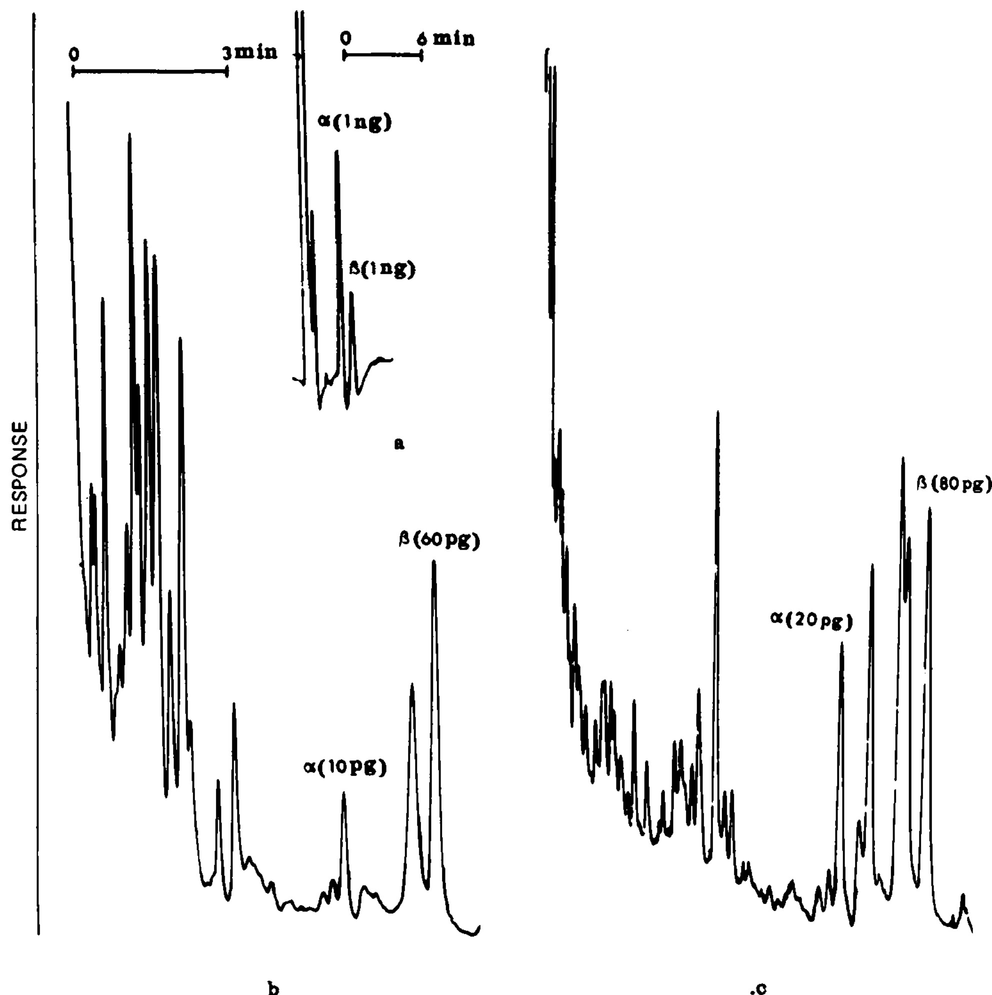

Figure 1—(a) Chromatogram according to the extraction procedure of Di Salle et al. *(8) but with a 3% OV-1 packed column. The test conditions were: injector temperature, 260°; detector temperature; 280°; column temperature, 185°; nitrogen pressure, 2 bar; duration and pulse interval, 0.8 and 100 μsec; sensitivity and attenuation, 1 × 32; and chart speed, 2.5 mm/min.* (b) *Chromatogram according to the extraction procedure described in Fig. 1*a *but with the wall-coated open tubular column and chromatographic conditions described under* Experimental. (c) *Chromatogram according to the single extraction procedure with chromatographic conditions similar to those used for Fig. 1*b. *The α and β are peaks of alprenolol and oxprenolol, respectively.*

The most troublesome interference from endogenous substances coextracted with the drugs, such as amines and alcohols that react with heptafluorobutyryl anhydride or aromatic and oxygen derivatives, was not found under the test conditions because of the high efficiency of the wall-coated open tubular column. Exogenous substances such as clonazepam[11], flunitrazepam[11], diazepam[11], endrin[6], dicophane[6], and propranolol[12] do not coelute with alprenolol and oxprenolol.

The method has been used in clinical pharmacology and pharmacokinetic studies. In monitoring blood oxprenolol levels, patients receiving an oral oxprenolol dose of 0.66–1.5 mg/kg/8 hr from tablets[8] showed blood concentrations of 100–385 ng/ml. In dogs, blood alprenolol levels reached 448 ± 13.5 ng/ml 1.5 hr after a single oral dose of 15 mg of alprenolol/kg from tablets[7] and decreased rapidly to 148 ± 4.5 ng/ml 3 hr after administration. The chromatograms obtained in both cases, *i.e.*, alprenolol in dogs and oxprenolol in humans, were of the same form as in Figs. 1*b* and 1*c*.

The high resolving power and sensitivity (14) of the wall-coated open tubular column relative to the conventional packed column (in which the number of theoretical plates is limited by the length of the column) led to the development of a fast, accurate, and convenient electron-capture GLC method for alprenolol and oxprenolol determinations in blood. The small volume of working sample (100–200 μl instead of 1–4 ml) and the simplified extraction procedure are desirable in the analysis of β-blockers (15–18).

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ACKNOWLEDGMENTS

The authors thank A. Berra, E. Come, V. Liard, and M. Perault for their help in preparing this manuscript.