125°; NMR [$(D_3C)_2SO$]: ppm (δ) 2.92 (s, 3), 3.0 (broad s, 4), 3.4 (broad s, 4), 3.63 (s, 2), 6.02 (s, 1), 6.30 (s, 2), 7.1 (d, 1), and 7.3–7.9 (m, 6).

Anal.—Calc. for $C_{22}H_{22}Cl_2N_4O_3 \cdot C_4H_4O_4 \cdot \frac{1}{2}H_2O$: C, 53.25; H, 4.64; Cl, 12.09; N, 9.55. Found: C, 52.96; H, 4.53; Cl, 11.96; N, 9.46.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one *N*-(2-Dimethylaminoethyl)-*N*-methylglycinate Dihydrochloride (VII)—The title compound was prepared by the same procedure as that described in the preparation of V*a* but from XII and *N,N,N'*-trimethylethylenediamine. The product was obtained from ethanol–ether in 20% yield, mp 221–223° dec.; NMR [$(D_3C)_2SO$]: ppm (δ) 2.95 (s, 6), 3.00 (s, 3), 3.65 (broad s, 4), 4.52 (broad s, 2), 6.10 (s, 1), 7.02 (d, 1), and 7.4–7.8 (m, 6).

Anal.—Calc. for $C_{22}H_{24}Cl_2N_4O_3 \cdot 2HCl \cdot 2H_2O$: C, 46.17; H, 5.28; N, 9.79. Found: C, 45.96; H, 5.36; N, 9.85.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one 4-(Hydroxyethyl)-1-piperazineacetate Dihydrochloride (VIII)—The title compound was prepared as described for VII but from XII and *N*-(2-hydroxyethyl)piperazine. The product, obtained in 46% yield from ethanol–ether, became dark above 195° and decomposed above 202°; NMR [$(D_3C)_2SO$]: ppm (δ) 3.1–4.2 (broad m, 16), 4.55 (broad s, 2), 6.03 (s, 1), 7.01 (d, 1), and 7.3–7.8 (m, 6).

Anal.—Calc. for $C_{23}H_{26}Cl_4N_4O_4 \cdot 2HCl \cdot 2\frac{1}{2}H_2O$: C, 45.33; H, 5.13; H_2O, 7.39; N, 9.29. Found: C, 45.34; H, 4.70; H_2O, 7.39; N, 9.33.

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GLC Determination of Propranolol, Other β-Blocking Drugs, and Metabolites in Biological Fluids and Tissues

THOMAS WALLE

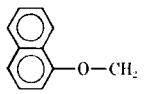
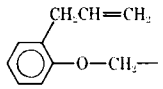
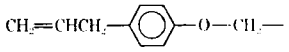
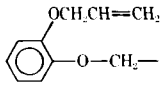
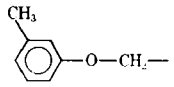
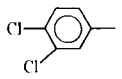
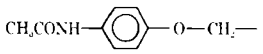
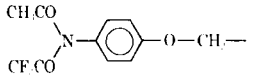
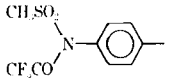
Abstract □ A highly sensitive and specific electron-capture GLC method was developed for β-blocking drugs, including propranolol, oxprenolol, alprenolol, pronethalol, dichloroisoproterenol, practolol, and sotalol, and several of their metabolites. The drugs are separated and detected as their trifluoroacetyl derivatives. Minimum detectable amounts ranged from 0.1 to 1.1 pg. The chemical structures of the derivatives were confirmed by GLC–mass spectrometry. Propranolol was extracted from plasma by either a single- or a back-extraction procedure, with oxprenolol as the internal standard. The minimum detectable concentration was 0.1 ng/ml plasma. The standard curve was linear from 0.5 to 500 ng/ml. Applications of this method demonstrated quantitatively detectable plasma propranolol levels 24 hr after a small (0.05 mg/kg) intravenous dose. Accurate plasma determinations could be made in patients receiving numerous other drugs. The method was shown to be applicable to propranolol and two metabolites, 1-(α-naphthoxy)-2,3-propylene glycol and *N*-desisopropylpropranolol, in tissue. Determinations of picogram amounts of oxprenolol and its four major urinary metabolites demonstrate the general applicability of the method to all β-blocking drugs and most of their metabolites.

Keyphrases □ Propranolol and two metabolites—GLC determination in biological fluids and tissues □ β-Blocking drugs—electron-capture GLC determination of propranolol, oxprenoiol, alprenolol, pronethalol, dichloroisoproterenol, practolol, sotalol, and several metabolites in biological fluids and tissues □ GLC—determination of propranolol, other β-blocking drugs, and metabolites in biological fluids and tissues

Large variations in response to β-blocking drug therapy have been demonstrated in numerous clinical studies, making the task of evaluating the clinical efficacy of this chemical and pharmacological class of compounds extremely difficult. These variations have been attributed in part to individual differences in the metabolic disposition of these drugs.

A better understanding of variations in the metabolic disposition of β-blocking drugs during treatment requires reliable measurements of these drugs in plasma, other biological fluids, and tissues. Most β-blocking drugs are extensively metabolized (1, 2),

Table I—Electron-Capture Response and Relative Retention Times for Trifluoroacetyl Derivatives of β-Blocking Drugs

$R-CH(OCOCF_3)CH_2N(COCF_3)CH(CH_3)_2$		MDQ		
		Moles per Second $\times 10^{-16}$	Picograms[a]	Relative Retention[b]
	Propranolol	2.0	0.5	8.90
	Alprenolol	2.8	0.7	2.20
	H 64/52	3.2	0.8	3.00
	Oxprenolol	4.3	1.1	3.30
	Toliprolol	3.8	0.9	1.48
	Pronethalol	2.6	0.6	3.87
	Dichloroisoproterenol	0.5	0.1	1.59
	Practolol	2.9	0.7	26.3
	Practolol	2.8	0.7	7.2
	Sotalol	2.4	0.7	7.0

[a] MDQ (minimum detectable quantity) expressed in picograms is valid at t_R = 3 min for a column with 3800 theoretical plates. [b] Using 1-bromonaphthalene (t_R = 1.00 min) as the reference compound.

and some of the resulting metabolites have important pharmacological properties (3, 4). Therefore, a method for drug determination should include such metabolites if possible.

Several methods have been reported for quantitative determinations of β-blocking drugs. Propranolol and sotalol have been determined fluorometrically (5, 6), practolol has been determined colorimetrically (7), and alprenolol has been determined by GLC (8). A recently published method for propranolol also utilizes GLC (9). The specificity of the fluorometric method for propranolol has been demonstrated to be inadequate[1]. The fluorometric method for sotalol and the colorimetric method for practolol are limited with respect to both specificity and sensitivity. None of the methods has been demonstrated to be applicable to the determination of metabolites.

This investigation describes the improvement and extension of the electron-capture GLC method originally reported for alprenolol (8) to include propranolol and eight other β-blocking drugs.

Applications demonstrate the method to be useful for both plasma, tissue, and urine determinations and to include drug metabolites.

EXPERIMENTAL

Reagents—Trifluoroacetic anhydride[2] in 1-ml ampuls was used as the derivatization reagent. To protect the anhydride from hydrolysis by moist air after opening the ampuls, it was stored in airtight 3-ml vials[3]. Trimethylamine (1 *M*) in benzene was prepared from gaseous trimethylamine[4]. Nanograde benzene[5] was used without prior distillation. Sulfuric acid (0.2 *N*), sodium hydroxide (5 *N*), and pH 6.0 phosphate buffer (0.5 *M*) were prepared with glass-distilled water and stored in glass bottles.

Propranolol[6], alprenolol[7], 1-(*p*-allylphenoxy)-3-isopropylamino-2-propanol[8], oxprenolol[9], 1-(*m*-toloxy)-3-isopropylamino-2-propanol[10], pronethalol[6], dichloroisoproterenol[6], and sotalol[9] were obtained as their hydrochlorides and practolol[6] was obtained as the free base from commercial sources. Their purity was checked by GLC as the trifluoroacetyl derivatives and by TLC. The chemical structures of their trifluoroacetyl derivatives are shown in Table I.

[1] K. Walle and T. Walle, to be published.

[2] Pierce Chemical Co., Rockford, Ill.
[3] Reacti-Vials, Pierce Chemical Co.
[4] Eastman-Kodak Co., Rochester, N.Y.
[5] Mallinckrodt Chemical Works, St. Louis, Mo.
[6] Imperial Chemical Industries, Ltd., Wilmslow, Great Britain.
[7] AB Hässle, Mölndal, Sweden
[8] H 64/52, AB Hässle.
[9] William S. Merrell Co., Cincinnati, Ohio.
[10] Toliprolol, ICI 45763, Imperial Chemical Industries, Ltd.

Glassware—All glassware was cleaned in chromic acid. Glass-stoppered 10-ml centrifuge tubes were then silanized with dimethyldichlorosilane[2] and rinsed with methanol and toluene. Glassware treated this way could be used repeatedly for several months. Between such treatments, all glassware was cleaned in a 2% cleaning solution[11].

Preparation of Derivatives—Propranolol as its hydrochloride or as its free base after extraction was taken up in 50–200 μl of benzene in glass-stoppered 10-ml centrifuge tubes. Twenty-five microliters of trimethylamine (1 *M*) in benzene and 50 μl of trifluoroacetic anhydride were added. The tubes, after being tightly stoppered, were heated for 5 min in a 50° water bath. After cooling, the reaction mixtures were shaken vigorously for 30 sec with 1 ml of pH 6.0 phosphate buffer (0.5 *M*) and centrifuged. Part of the benzene phase (1–5 μl) was taken for electron-capture GLC analysis.

The completeness of the reaction under various conditions was studied with 1-bromonaphthalene[12] as the internal standard. Extended reaction times from 5 to 60 min with varying amounts of reagents [10–75 μl of trimethylamine (1 *M*) in benzene and 10–75 μl of trifluoroacetic anhydride] gave identical recoveries, indicating quantitative derivatization conditions for propranolol. The same conditions also gave quantitative reactions with alprenolol, 1-(*p*-allylphenoxy)-3-isopropylamino-2-propanol, oxprenolol, 1-(*m*-tolyoxy)-3-isopropylamino-2-propanol, pronethalol, and dichloroisoproterenol.

Quantitative derivatization of practolol and sotalol required 75 μl of trimethylamine (1 *M*) in benzene and 75 μl of trifluoroacetic anhydride. The final wash was made with 2 ml of pH 6.0 phosphate buffer (0.5 *M*).

GLC—A gas chromatograph[13] equipped with a ^{63}Ni electron-capture detector was used. A 150-cm glass column with an internal diameter of 2 mm was packed with 2% OV-17 on resilanized (10) Chromosorb W,AW-DMCS (80–100 mesh). The nitrogen flow rate was 30 ml/min. (The column was conditioned at 250° for 72 hr.) The injector temperature was 230°, the column temperature was 170°, and the detector temperature was 270°.

The ^{63}Ni detector gave a linear response for quantities ranging from 15 to 800 pg of trifluoroacetylated propranolol.

GLC–Mass Spectrometry—The combination instrument[14] was operated at an accelerating voltage of 3.5 kv, an ionization voltage of 20 ev, and a trap current of 65 μamp. The column, 90 cm long and 2 mm i.d., was packed with the same liquid phase and support as already described. The instrument was equipped with an accelerator voltage alternator for multiple-ion detection (11).

Propranolol Determinations in Plasma—*Single Extraction*—To samples of 1.00 ml of plasma were added 50–100 μl of an aqueous solution of the internal standard (oxprenolol hydrochloride) and 0.1 ml of 5 *N* NaOH (pH 13) in a glass-stoppered 10-ml centrifuge tube. The sample was shaken with 3 ml of benzene for 5 min and centrifuged at 3000 rpm for 5 min.

The benzene phase was transferred to another tube and evaporated to a small volume (50 μl) with a gentle stream of nitrogen at 70°. Derivatization and buffer wash were done according to procedures described under *Preparation of Derivatives*. For electron-capture analysis, 1–5-μl volumes of the final benzene phase were injected.

Back Extraction—The initial benzene phase from the single-extraction procedure (3 ml) was transferred to a tube containing 1 ml of 0.2 *N* H_2SO_4. The tube was shaken for 1 min and centrifuged. The acidic aqueous phase was transferred to another tube, made alkaline (pH 13) with 5 *N* NaOH, and shaken with 3 ml of benzene. The benzene phase was then treated according to the single-extraction procedure.

Standard Curve—One-milliliter control plasma samples were spiked with propranolol hydrochloride and extracted following either the single-extraction procedure (5–15 ng/ml) or the back-extraction procedure (0.5–4 ng/ml), using 25 ng/ml oxprenolol hydrochloride as internal standard. Peak area ratios of propranolol–oxprenolol were measured and plotted as a function of propranolol concentration.

[11] Micro, International Products Corp., Trenton, N.J.
[12] Pfalz and Bauer, Inc., Flushing, N.Y.
[13] Varian model 1440, Varian-Aerograph, Walnut Creek, Calif.
[14] LKB 9000 S, LKB Instruments, Inc., Rockville, Md.

Table II—Plasma Propranolol Levels after Chronic Oral Administration in Humans

Daily Oral Dose, mg	Propranolol[a], ng/ml
120	40 ± 4
120	77 ± 3
160	26 ± 2
160	72 ± 2
240	38 ± 3
320	67 ± 6

[a] Mean of four determinations.

Plasma Determinations—A dose of 3.3 mg of propranolol hydrochloride was given intravenously to a 72-year-old patient. Five-milliliter heparinized blood samples were drawn 0.5, 1, 1.5, 2, 3, 6, 12, 18, and 24 hr after the dose. After separating the plasma by centrifugation (15 min at 6000×*g*), 1-ml samples were taken for analysis following the back-extraction procedure.

Blood samples from six patients on chronic oral propranolol therapy, 120–320 mg daily in four divided doses, were drawn 2 hr after the morning dose (Table II). These samples were handled identically. The patients were treated with numerous other drugs concomitantly including methyldopa, digoxin, furosemide, aldactone, guanethidine, allopurinol, propoxyphene, chlordiazepoxide, and dicyclomine.

Propranolol and Metabolites in Heart Tissue—Two hundred milligrams of heart tissue (wet weight) from a propranolol-treated blood-perfused dog heart–lung preparation (12) was homogenized[15] in 4 ml of ice-cold 0.4 *N* $HClO_4$. After centrifugation, the supernate was taken for analysis following the single-extraction procedure, with 6 ml of benzene as the extractant.

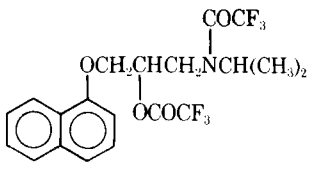
Electron-Capture Response—Trifluoroacetyl derivatives of propranolol and eight other β-blocking drugs, prepared according to procedures described under *Preparation of Derivatives*, were analyzed in triplicate, either alone with 1-bromonaphthalene as the reference compound or in mixtures. The electron affinity is expressed as the minimum detectable quantity in moles per second × 10^{-16} (10). The minimum detectable quantity, defined as the quantity that gives a signal three times the background noise level, was determined for 1-bromonaphthalene (2.6×10^{-16} mole/sec).

The response of the trifluoroacetylated drugs was determined in relation to this compound. Detector cell temperatures ranging from 200 to 270° did not significantly affect the sensitivity. The effect of the detector cell temperature on the electron-capture detector response was reviewed by Wentworth and Chen (13). The higher detector temperature, 270°, reduced contamination of the detector.

Oxprenolol and Oxprenolol Metabolites in Rat Urine—One-milliliter urine samples from rats treated with 10 mg of oxprenolol hydrochloride intraperitoneally were hydrolyzed with β-glucuronidase and sulfatase[16] (2). Ten microliters of these hydrolysates was taken for analysis following the single-extraction procedure but extraction was with ethyl acetate at pH 10.

RESULTS AND DISCUSSION

Preparation of Derivatives—Trifluoroacetylation of propranolol with trifluoroacetic anhydride, as described in the *Experimental* section, yields a derivative containing two trifluoroacetyl groups in the side chain:



This structure has been confirmed by GLC–mass spectrometry (14). The derivative has excellent GLC properties and gives a very high response on an electron-capture detector. The minimum de-

[15] Polytron, Brinkmann Instruments, Inc., Westbury, N.Y.
[16] Glusulase, Endo Laboratories, Garden City, N.Y.

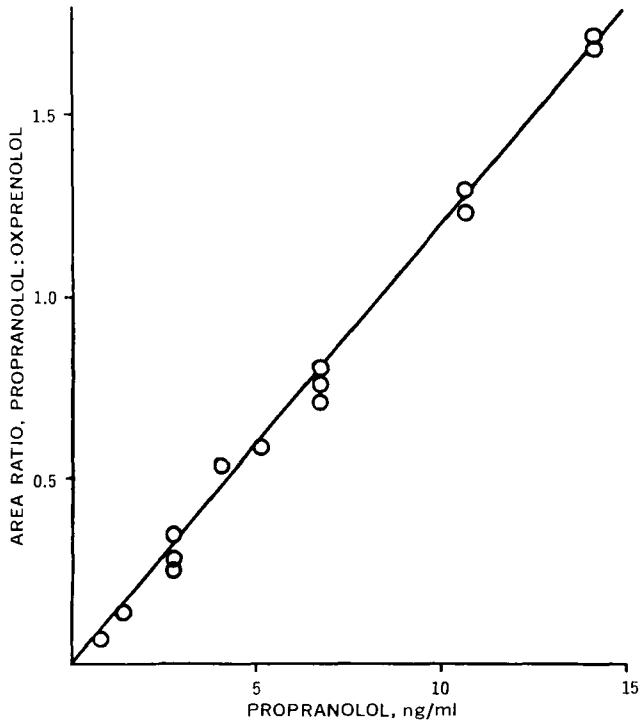


Figure 1—*Standard curve for the determination of propranolol in plasma. Single-extraction procedure for concentrations of 5–15 ng/ml, and back-extraction procedure for concentrations of 0.5–4 ng/ml, with oxprenolol as the internal standard. Each point represents the mean of two to five independent determinations. The curve is obtained by the method of least squares from a total number of 38 determinations.*

tectable quantity (10) is 2.0×10^{-16} mole/sec, corresponding to 0.5 pg under the GLC conditions used.

Heptafluorobutyric anhydride[2] as a reagent yielded the corresponding derivative with two heptafluorobutyryl groups in the side chain. This derivative was only slightly more sensitive, 0.9×10^{-16} mole/sec. This finding agrees with sensitivities reported for other similar trifluoroacetyl and heptafluorobutyryl derivatives of bifunctional molecules (10, 15).

Heptafluorobutyric anhydride was selected as the reagent for propranolol by Di Salle *et al.* (9). Trifluoroacetylation in this study, however, provided much higher selectivity and thereby

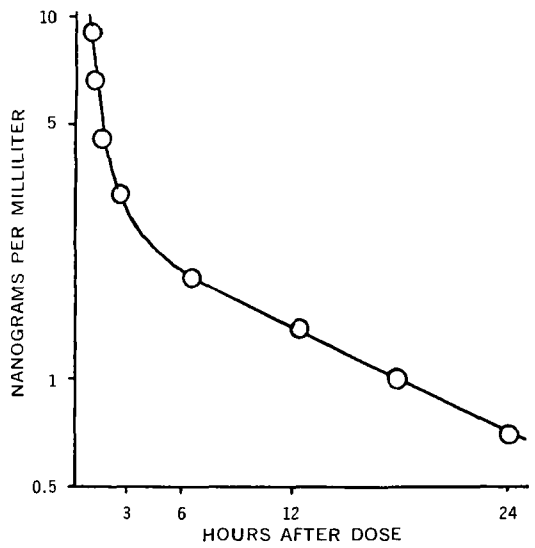


Figure 2—*Plasma propranolol levels after a 3.3-mg iv dose, 0.05 mg/kg, in a 72-year-old man. Each point represents the mean of three determinations.*

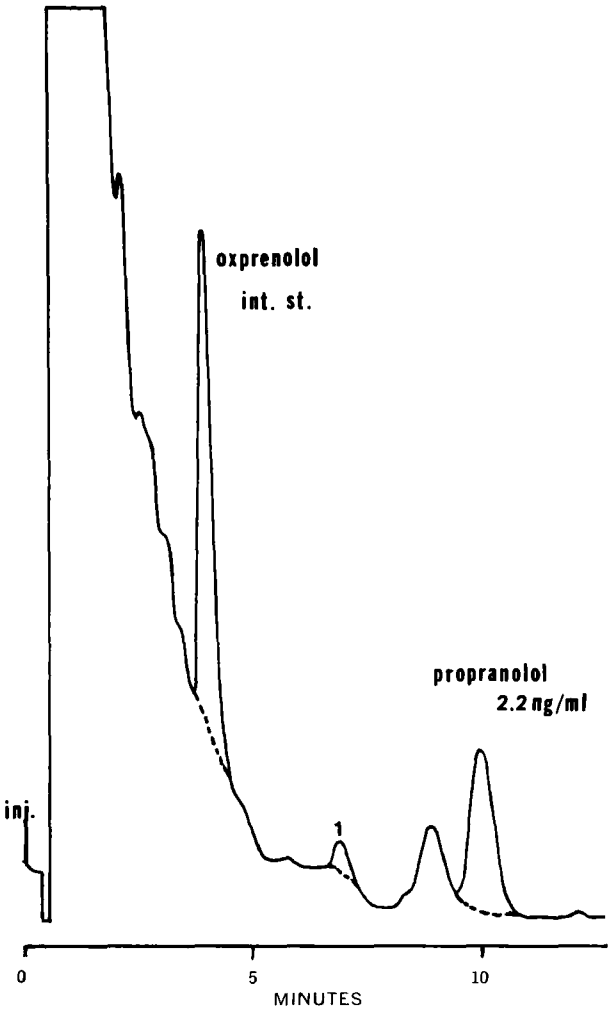


Figure 3—*Gas chromatogram of a plasma extract (back extraction) obtained 6 hr after the dose from the patient in Fig. 2. 1 = N-desisopropylpropranolol. Dashed lines represent the background noise from control plasma.*

higher sensitivity than heptafluorobutyrylation for determinations in complex biological extracts. Coextracted endogenous monofunctional amino, alcoholic, or phenolic compounds with heptafluorobutyric anhydride yield derivatives that have a high response on an electron-capture detector (10, 16) while the response of the corresponding derivatives with trifluoroacetic anhydride is only 1/100 to 1/1000, making the trifluoroacetyl derivatives less likely to interfere (15, 16).

The trifluoroacetylation reaction is quantitative in less than 5 min using trimethylamine as the catalyst. The effect of trimethylamine on the acylation of primary and secondary amines, alcohols, and phenols was described earlier (10, 16). Excess reagent is removed by shaking the reaction mixture with a pH 6.0 phosphate buffer (16). Under these conditions, the derivative is stable for at least 24 hr.

Interference in the derivatization reaction by free acid in the trifluoroacetic anhydride reagent, as earlier reported for heptafluorobutyric anhydride (10), was observed. By careful handling of the trifluoroacetic anhydride reagent according to the *Experimental* section, this problem can be avoided.

Plasma Propranolol Determinations—Propranolol is quantitatively (>95%) extracted from plasma at high pH with benzene. Oxprenolol, used as internal standard, was added to the plasma before extraction. This procedure (single extraction) including the GLC separation takes 45 min, permitting analysis of 12–15 samples/day on a single-column instrument. For very low concentrations (<5 ng/ml), a back-extraction clean-up procedure may be necessary. The back-extraction clean-up procedure removes neutral compounds from the initial benzene extract that may interfere

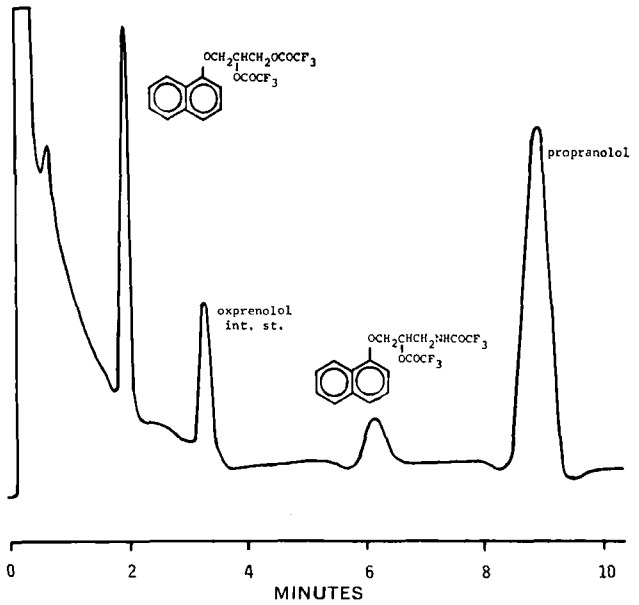


Figure 4—*Gas chromatogram of a heart tissue extract (single extraction) obtained from a propranolol-treated blood-perfused dog heart–lung preparation. Two metabolites, 1-(α-naphthoxy)-2,3-propylene glycol and* N*-desisopropylpropranolol, are shown.*

in the analysis and is particularly valuable for the analysis of plasma samples from patients taking numerous drugs. The back-extraction procedure including GLC takes about 70 min.

Figure 1 shows the standard curve obtained for propranolol determinations in plasma with oxprenolol as the internal standard, following both the single-extraction procedure (5–15 ng/ml) and the back-extraction procedure (0.5–4 ng/ml). The results obtained from the two procedures fit the same standard curve. The curve is linear within a 30-fold range and goes through the origin. The minimum detectable concentration is about 0.1 ng/ml of plasma.

The precision of the method after repeated analysis is about ±15% at the 3-ng/ml level (back extraction, $n = 8$) and about ±8% at the 25-ng/ml level (single extraction, $n = 8$). The standard curve can be extended to 500 ng of propranolol/ml of plasma by limiting the amount of trifluoroacetyl derivative injected to 800 pg (see *Experimental*).

By using this method, the plasma levels of propranolol were followed for 24 hr in a 72-year-old patient receiving 3.3 mg of propranolol (0.05 mg/kg) intravenously (Fig. 2). Propranolol was still detectable (0.7 ng/ml) 24 hr after the administration. The calculated plasma half-life of 12 hr greatly exceeds that reported for normal volunteers (5). Whether this unusually long half-life is related to the physical condition of the patient or to the use of the more sensitive method, permitting a more accurate determination of the second phase of the biphasic elimination curve, is not known.

A GLC tracing of a 6-hr plasma sample (back extraction) from this patient is shown in Fig. 3. In addition to propranolol and the internal standard, a major metabolite of propranolol in blood, *N*-desisopropylpropranolol (2), is recognized. Criteria of identity of propranolol include retention time and characteristic molecular ion and base peak by multiple-ion detection GLC–mass spectrometry. Other peaks in the chromatogram are normal constituents of human plasma under the conditions used. The absence of interfering compounds was tested by repetitive scanning analysis.

Table II demonstrates plasma concentrations in six patients treated chronically with 120–320 mg of propranolol hydrochloride orally per day[17]. The patients were treated with numerous other drugs as well. Concentrations ranging from 26 to 77 ng/ml were obtained with the back-extraction method, with a precision ranging from 2.8 to 10%. No interferences from other drugs were observed.

Propranolol and Metabolites in Tissues—The method is applicable to tissue as well as to plasma samples. Figure 4 shows a gas chromatogram of a heart tissue extract. The tissue was obtained from a propranolol-treated (10 mg of propranolol hydrochloride) blood-perfused dog heart–lung preparation (12). In addition to propranolol, two pharmacologically active propranolol metabolites, 1-(α-naphthoxy)-2,3-propylene glycol (4) and *N*-desisopropylpropranolol[18], were detected. Criteria of identity for these two metabolites include retention times and the presence of characteristic molecular ions and base peaks by multiple-ion detection GLC–mass spectrometry. The presence of the two metabolites reflects extrahepatic metabolism (12).

Two trifluoroacetyl groups are incorporated in these metabolites, giving them an electron-capture detector response the same as or higher than propranolol. This should be true for any metabolite of propranolol containing either an alcohol and an amino or two alcohol groups in the side chain (2, 17). Noticeable too is the fairly clean GLC tracing of heart tissue, indicating very little interference from endogenous material. Propranolol and the two metabolites have also been measured in a rat liver microsomal preparation using the same method (18).

Preliminary investigations demonstrated that two other metabolites of propranolol, the active 4-hydroxypropranolol (3) and the ring hydroxylated 1-(α-naphthoxy)-2,3-propylene glycol, can also be determined using a modification of the same method.

Applications to Other β-Blocking Drugs and Their Metabolites—Trifluoroacetylation of several other β-blocking drugs, using the same derivatization conditions as for propranolol, yielded derivatives with high electron-capture detector response. Retention times and minimum detectable quantity of these derivatives are given in Table I. The peak symmetry was excellent even

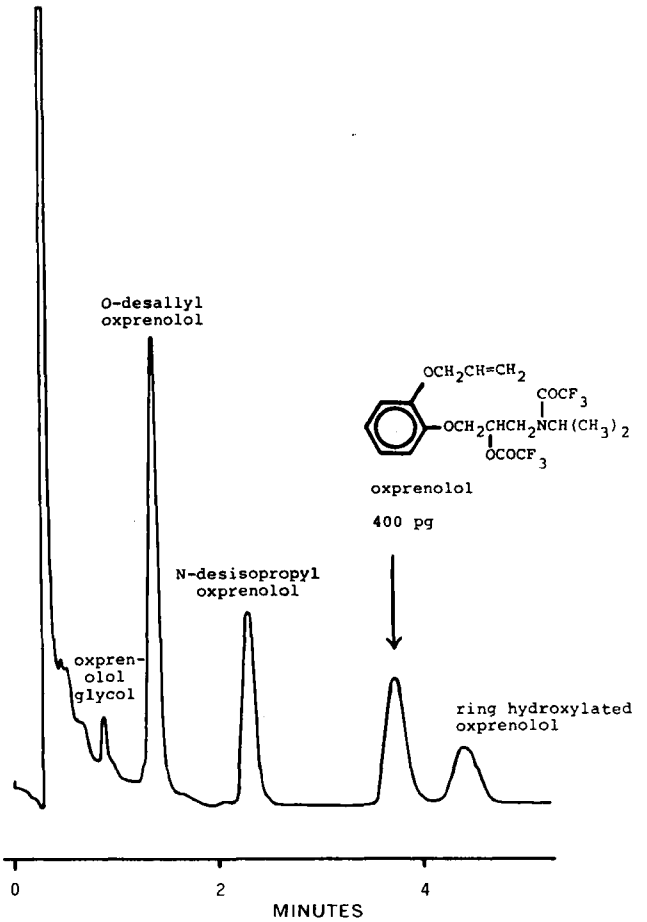


Figure 5—*Gas chromatogram of an extract (single extraction, pH 9) of hydrolyzed urine (10 μl) obtained from a rat administered 10 mg of oxprenolol hydrochloride. For identification of metabolites, see Ref. 1.*

[17] T. Walle, E. Conradi, K. Walle, and T. Gaffney, to be published.

[18] Pharmacological activity. J. Pruett, unpublished data from this laboratory.

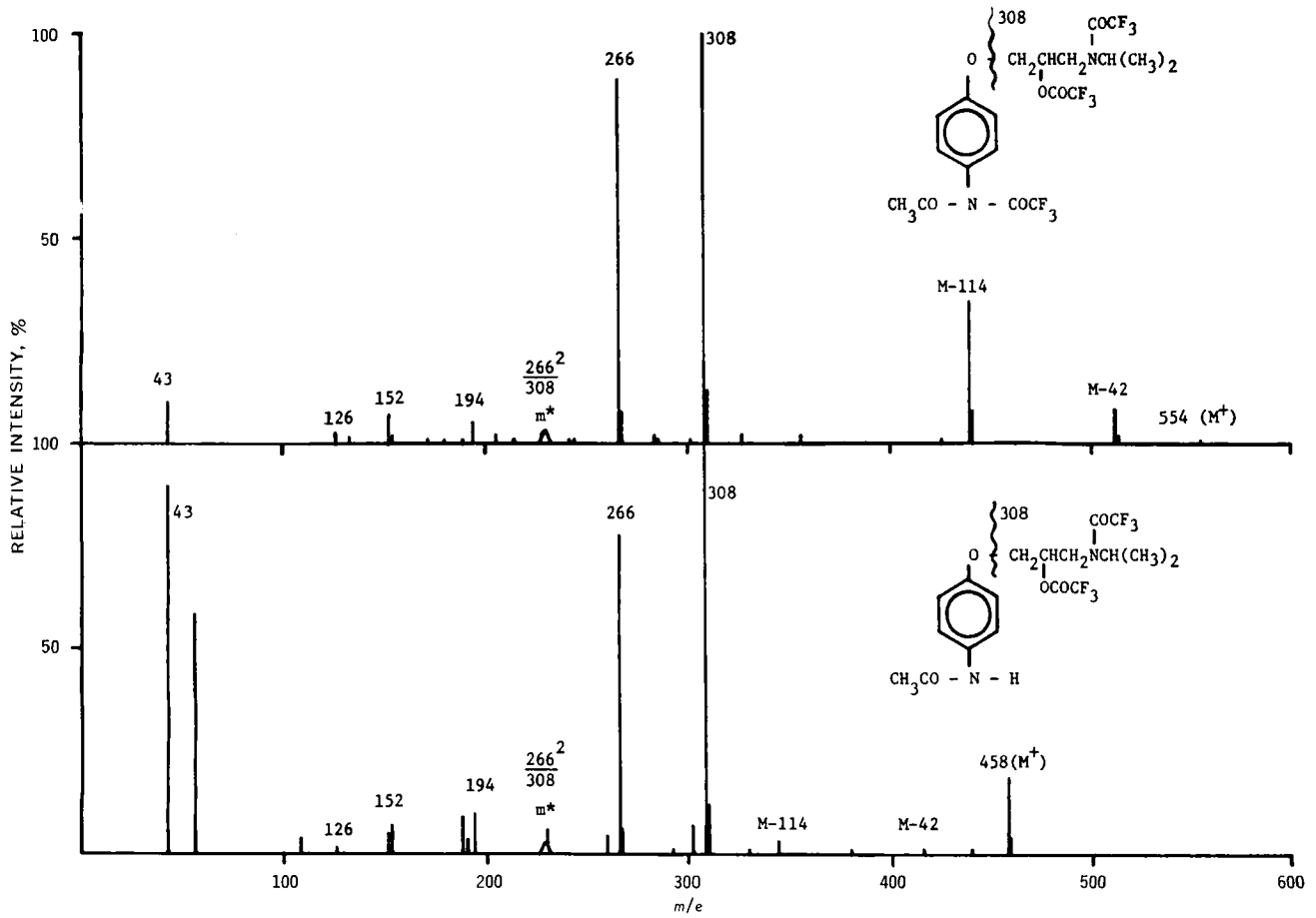


Figure 6—*Normalized mass spectra of the di- (bottom) and tri- (top) trifluoroacetyl derivatives of practolol.*

in low picogram quantities. Dichloroisoproterenol, with the contribution of its two chlorine atoms, could be detected down to 0.1 pg. Minimum detectable amounts for the other β-blocking drugs had a narrow range of 0.5–1.1 pg, demonstrating the trifluoroacetylated β-blocking side chain to be the single most important contributor to the high electron-capture response. The chemical structures of most of these derivatives were confirmed earlier by GLC–mass spectrometry (14).

Isothermal separation of all nine β-blocking drugs in Table I could be accomplished in about 10 min, with the shortest retention time (1.48 min) for 1-(*m*-toloxy)-3-isopropylamino-2-propanol. Separation factors of more than 1.10 were obtained for all of these drugs except dichloroisoproterenol–1-(*m*-toloxy)-3-isopropylamino-2-propanol (1.07) and practolol–sotalol (1.03).

The extraction conditions described for propranolol from plasma and tissues can also be applied to alprenolol, 1-(*p*-allylphenoxy)-3-isopropylamino-2-propanol, oxprenolol, 1-(*m*-toloxy)-3-isopropylamino-2-propanol, pronethalol, and dichloroisoproterenol. It can also be applied to metabolites of these drugs, if optimum pH for their extraction is considered. Thus, picogram quantities of oxprenolol and its four major urinary metabolites in rats (ring hydroxylated oxprenolol, *N*-desisopropyloxprenolol, *O*-desallyloxprenolol, and a glycolic oxprenolol metabolite) have been simultaneously determined after extraction with ethyl acetate at pH 10 (Fig. 5). The chemical structures of these metabolites were described in other studies (1)[19].

Fast (<5 min) and quantitative derivatization of practolol and sotalol required larger quantities of both trimethylamine [75 μl of trimethylamine (1 *M*) in benzene] and trifluoroacetic anhydride (75 μl). Under these conditions, practolol formed a tri-trifluoroacetyl derivative with the third trifluoroacetyl group incorporated in the acetamide moiety (Table I). The mass spectrum of this derivative is shown in Fig. 6 (top half). The molecular ion at *m/e* 554 is very weak. Distinct fragment ions are seen at *m/e* 512, M − 42 (CH_2CO), and at *m/e* 440, M − 114 (CF_3COOH). The base peak at *m/e* 308 and most of the remaining fragment ions are characteristic of all aryloxy β-blocking drugs (14).

This derivative is stable for at least 8 hr under the conditions used. Prolonged storage (>24 hr) of the derivative in contact with the pH 6.0 buffer quantitatively yields the corresponding di-trifluoroacetyl derivative (Table I). Figure 6 (bottom half) shows the mass spectrum of this derivative. The molecular ion at *m/e* 458 is prominent. Characteristic ions at *m/e* 416, M − 42 (CH_2CO), and at *m/e* 344, M − 114 (CF_3COOH), are obtained (*cf.*, spectrum on top) in addition to ions characteristic of the β-blocking side chain (14). Note the large difference in retention times between the di- and tri-trifluoroacetyl derivatives (Table I) and also the similarity in electron-capture response.

Like practolol, sotalol forms a tri-trifluoroacetyl derivative with the third trifluoroacetyl group incorporated in the sulfonamide moiety (Table I). Its mass spectrum is shown in Fig. 7. The molecular ion at *m/e* 560 is weak. Distinct fragment ions are obtained at *m/e* 481, M − 79 (CH_3SO_2), and at *m/e* 446, M − 114 (CF_3COOH). The base peak at *m/e* 168 is a result of α-cleavage of the major side chain. The tri-trifluoroacetyl derivative of sotalol is stable in contact with pH 6.0 buffer for at least 8 hr. Extended storage (>24 hr) results in partial hydrolysis, loss of the trifluoroacetyl group from the sulfonamide moiety, and loss of trifluoroacetic acid from the β-blocking side chain.

Since practolol and sotalol are considerably less lipophilic than the other β-blocking drugs, neither compound can be quantitatively extracted with benzene. Both can, however, be extracted with ethyl acetate.

SUMMARY

A highly sensitive and specific electron-capture GLC method for propranolol was described. The drug is extracted from plasma fol-

[19] D. Garteiz, Division of Clinical Pharmacology, University of Cincinnati College of Medicine, Cincinnati, OH 45219, personal communication.

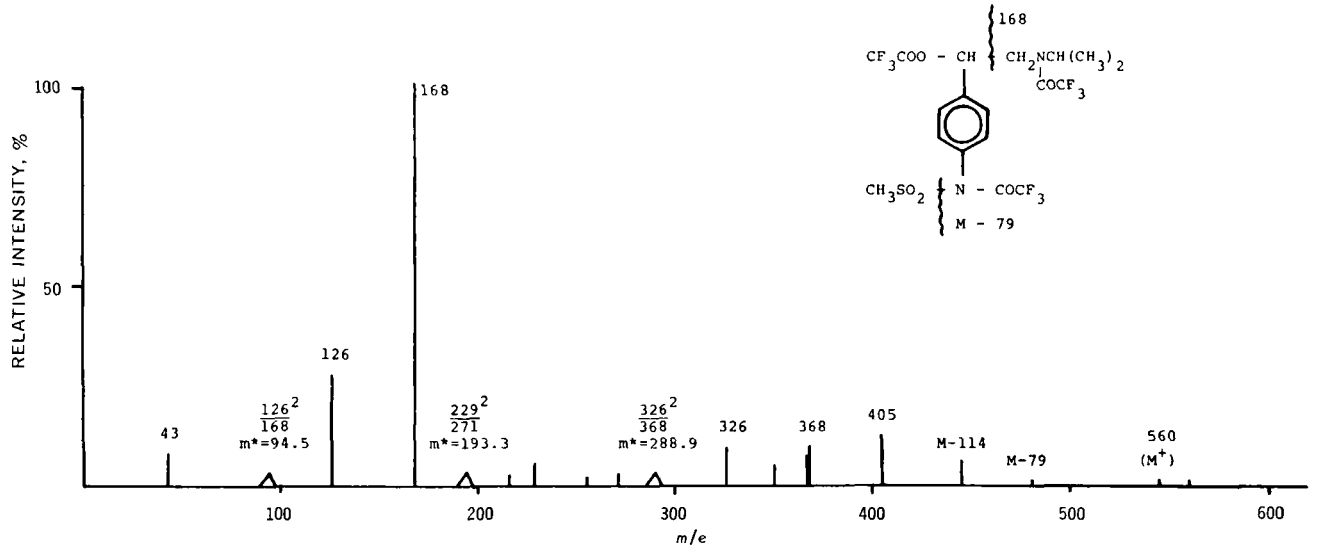


Figure 7—*Normalized mass spectrum of the tri-trifluoroacetyl derivative of sotalol.*

lowing either a single- or a back-extraction procedure, with oxprenolol as the internal standard. Extracted propranolol and internal standard are separated and detected as their di-trifluoroacetyl derivatives. The minimum detectable amount of propranolol is 0.5 pg. The minimum detectable concentration of propranolol in plasma is 0.1 ng/ml. The standard curve is linear from 0.5 to 500 ng/ml.

Applications of the method demonstrated quantitatively detectable plasma propranolol levels 24 hr after a small (0.05 mg/kg) intravenous dose. Accurate plasma determinations could be made in patients receiving numerous other drugs. The method is applicable to propranolol and two metabolites, 1-(α-naphthoxy)-2,3-propylene glycol and *N*-desisopropylpropranolol, in heart tissue.

The electron-capture response and retention times were also reported for eight other β-blocking drugs. Alprenolol, 1-(*p*-allylphenoxy)-3-isopropylamino-2-propanol, oxprenolol, 1-(*m*-toloxy)-3-isopropylamino-2-propanol, pronethalol, and dichloroisoproterenol form di-trifluoroacetyl derivatives. Practolol and sotalol form tri-trifluoroacetyl derivatives with incorporation of the third trifluoroacetyl group in the acetamide and sulfonamide moieties, respectively. The chemical structures of these derivatives were confirmed by GLC–mass spectrometry. Minimum detectable amounts of these derivatives by electron-capture detection ranged from 0.1 to 1.1 pg. The method for propranolol was shown to be applicable to the separation of picogram quantities of oxprenolol and four of its major urinary metabolites.

These results strongly indicate that the method described is generally applicable to quantitative determinations of all β-blocking drugs and most of their metabolites in body fluids and tissues. Trifluoroacetylation of biological extracts has already proved most valuable in the identification of numerous propranolol and oxprenolol metabolites by GLC–mass spectrometry (1, 2, 4, 18).

The high sensitivity of the method will permit more accurate half-life determinations, particularly after low doses, and will permit analysis of very small sample sizes, *e.g.*, serial blood samples from infants or biopsy samples. It should be possible to do complete pharmacokinetic studies of this entire chemical and pharmacological class of compounds, including their metabolites. Application of the method could lead to a better understanding of the complex metabolic disposition and pharmacology of this class of compounds and may be a useful aid in clinical drug therapy.

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