Improved GLC Determination of Ethambutol

Keyphrases □ Ethambutol—GLC analysis in biological fluids □ GLC—analysis, ethambutol in biological fluids □ Tuberculostatic antibacterials—ethambutol, GLC analysis in biological fluids

To the Editor:

Several GLC procedures have been reported for the determination of ethambutol in biological fluids (1–4). Only the methods (3, 4) using electron-capture detection are sensitive enough to measure ethambutol concentrations in patient samples of plasma and dialysate. We modified the previously published method (3, 4) to provide a more sensitive and precise procedure that is particularly useful for the determination of low ethambutol levels in biological samples.

Appropriate plasma aliquots (10–100 μl) were mixed with 50 μl of the internal standard [(+)-2,2′-(ethylenediimino)di-1-propanol] solution (10 μg/ml) and deionized water to give a final volume of 0.5 ml. The mixtures were extracted with 5 ml of chloroform for 10 min under alkaline conditions. Portions of the chloroform extract were transferred to another tube and evaporated to dryness under nitrogen. Residues were dissolved in 0.5 ml of spectroquality ethyl acetate.

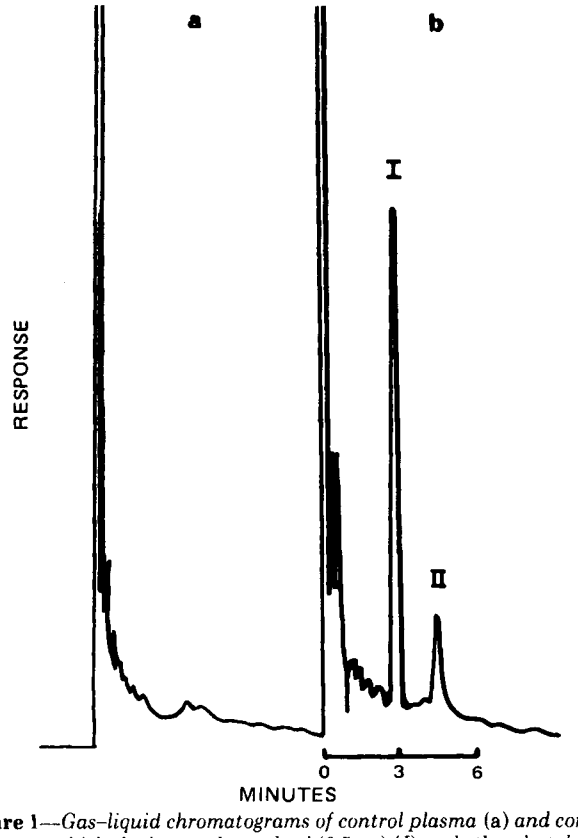

Figure 1—*Gas–liquid chromatograms of control plasma* (a) *and control plasma to which the internal standard (0.5 μg) (I) and ethambutol (0.01 μg) (II) were added* (b).

Table I—Recovery of Ethambutol from Plasma using Electron-Capture Detection (n = 5)

Ethambutol Added, ng	Ethambutol Found, ng	Recovery[a], %
10	9.37 ± 0.84	93.7 ± 8.4
20	18.79 ± 1.13	93.4 ± 5.7
30	30.15 ± 2.72	103.5 ± 9.1
50	51.19 ± 1.13	102.4 ± 2.3
70	68.49 ± 5.02	97.8 ± 7.2
100	98.70 ± 4.92	98.7 ± 4.9
10–100[b]	—	98.3 ± 4.2

[a] Expressed as mean ± *SD*. [b] n = 30.

Derivatization was initiated by adding 20 μl of trifluoroacetic anhydride and was complete in 1 hr at 50°. The contents of the tube were dried with a nitrogen stream to remove excess trifluoroacetic anhydride. Residues of the trifluoroacyl derivatives were reconstituted in 0.5 ml of anhydrous ethyl acetate, and 1–2 μl was injected into the gas–liquid chromatograph equipped with a ^{63}Ni-electron-capture detector. GLC conditions were the same as those described previously (3, 4).

Figure 1 presents gas–liquid chromatograms of control and blank plasma to which 0.01 μg of ethambutol and 0.5 μg of the internal standard were added. Retention times of 3 and 5 min were observed for the internal standard and the drug, respectively. No interfering peaks were found in the control plasma specimens (Fig. 1*a*). Table I summarizes the results obtained following electron-capture analysis of various amounts of ethambutol (10–100 ng) added to plasma. The mean recovery of ethambutol from control plasma in the 10–100-ng range was 98.3 ± 4.2% (n = 30).

The modified procedure offers the following advantages:

1. No catalyst is needed for derivatization. In the old procedure, pyridine is required.
2. The excess trifluoroacetic anhydride can be eliminated by direct evaporation. In the old procedure, the excess derivatizing agent was decomposed by the addition of 0.01 *M* HCl. The acid hydrolysis may adversely affect the yield of the trifluoroacyl derivative.
3. The detection sensitivity of the modified method, 10 ng/ml of plasma, is a significant improvement over that of the previous procedure, 100 ng/ml of plasma. Therefore, only a very small biological sample volume is required for ethambutol detection.

The minimum detectable quantity for ethambutol using this procedure is 10 ng. The optimal therapeutic plasma level in humans was reported as 3–5 μg/ml for ethambutol (5). In this concentration range, only 1–2 μl of plasma would be adequate for an accurate determination of the ethambutol content. This procedure would be helpful in pharmacokinetic studies where low ethambutol concentrations are encountered (6). It should be particularly useful for the microdetermination of ethambutol in bio-

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logical fluids such as saliva, cerebrospinal fluid, and dialysate.

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Pharmacokinetic Studies of Propoxyphene IV: Effect of Renal Failure on Systemic Clearance in Rats

Keyphrases □ Propoxyphene—pharmacokinetics, effect of renal failure on systemic clearance, rats □ Pharmacokinetics—propoxyphene, effect of renal failure on systemic clearance, rats □ Analgesics—propoxyphene, pharmacokinetics, effect of renal failure on systemic clearance, rats

To the Editor:

There is considerable concern about the safety of the widely used analgesic agent propoxyphene (dextropropoxyphene) (1). Under certain circumstances, death from propoxyphene may occur following ingestion of quantities only slightly larger than the upper limit of the recommended therapeutic dosage (1).

Propoxyphene is subject to pronounced presystemic ("first-pass") biotransformation; only a small fraction of the absorbed dose enters the general circulation in unmetabolized form (2–4). This effect may be due to both hepatic and prehepatic biotransformation of the drug during absorption (5). Plasma concentrations of propoxyphene in patients without functioning kidneys are considerably higher than in normal subjects after oral administration of the drug (6). Indirect evidence suggests that this result is due to decreased presystemic biotransformation in the patients (6). However, since propoxyphene could not be administered by intravenous injection, a decreased systemic clearance or apparent volume of distribution of the drug in anephric patients could not be excluded definitively. For this reason, the systemic clearance and apparent volume of distribution of propoxyphene after intravenous injection have now been determined in normal rats and in rats with renal failure.

Male Sprague–Dawley rats, 260–350 g, received a single 5-mg/kg iv dose of either uranyl nitrate (7) or an equal volume of saline solution. Five days later, when serum urea nitrogen concentrations had increased to 150 ± 37 mg/100 ml (mean ± *SD*) in the uranyl nitrate-treated animals (as compared to 15 ± 8 mg/100 ml in normal rats), all animals received a single dose of tritium-labeled propoxyphene, 8.3 ± 1.9 μg/kg (mean ± *SD*), through an indwelling cannula in the jugular vein (8).

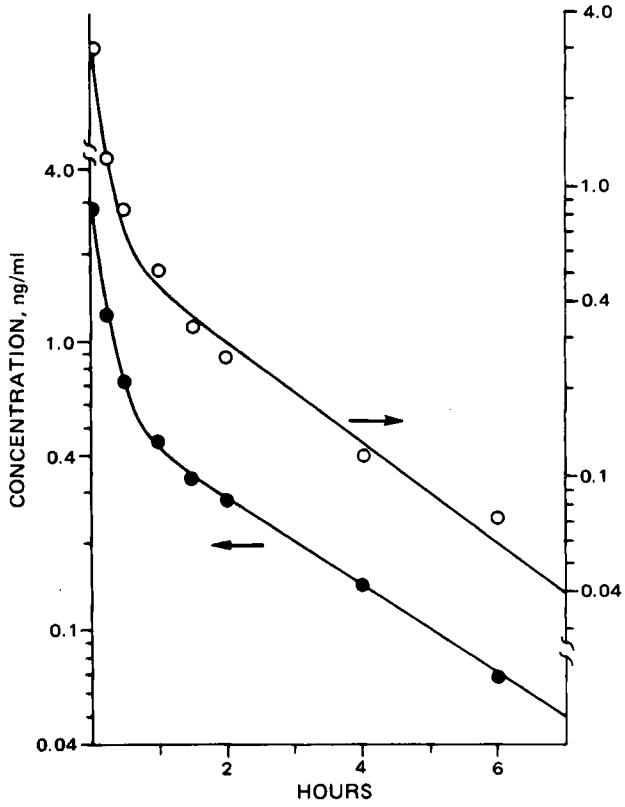

Figure 1—*Serum concentrations of propoxyphene as a function of time after intravenous injection of ^{3}H-propoxyphene at ~8 μg/kg. Key: ●, normal rat; and ○, rat with experimental renal failure. The curves were fitted to the data by nonlinear least-squares regression analysis. (The two vertical axes are displaced relative to one another.)*

Blood samples (0.25–1 ml) were obtained serially for 6–8 hr after injection. Serum was separated, adjusted to pH 9.8, and extracted with butyl chloride; the unmetabolized drug was isolated by TLC (9) and quantified by scintillation spectrometry. An aliquot of the injected solution was assayed similarly. The concentration–time curve was fitted to a biexponential equation by a digital computer (10), with the concentrations weighted as their reciprocals (Fig. 1). The systemic clearance was calculated from the injected dose and the area under the concentration–time curve. The apparent volume of distribution was calculated by dividing the systemic clearance by β.

Compared to surgical methods, injection of uranyl nitrate produces a more reproducible (as determined by serum creatinine and urea nitrogen concentrations) renal failure model. The animals appear to be in better health than after surgery. There was no evidence of hepatocellular damage after a single injection of uranyl nitrate in that glutamic–pyruvic transaminase concentrations in serum were normal[1]. Concomitant injection of tritium-labeled and unlabeled propoxyphene and assay of serum samples by scintillation spectrometry (after TLC) and by GLC

[1] K. M. Giacomini, S. M. Roberts, and G. Levy, unpublished data.

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