# GLC Method for Quantitative Determination of Amitriptyline in Human Plasma

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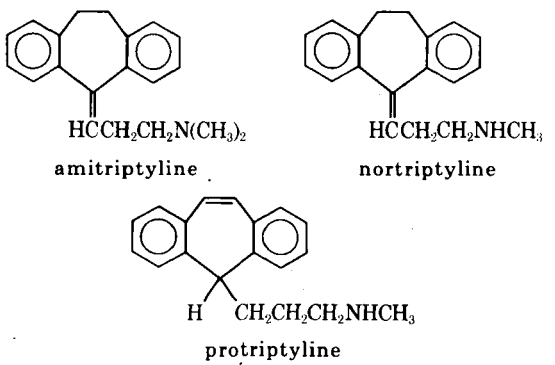
**Abstract** □ A GLC method was developed for the measurement of steady-state levels of amitriptyline and nortriptyline in human plasma after therapeutic doses of amitriptyline. The drugs were extracted from alkalinized samples with heptane–3% isoamyl alcohol and back-extracted into 0.1 *N* HCl. After subsequent extraction into ether, the drugs were analyzed by GLC. The method is accurate, specific, and sensitive, being capable of measuring concentrations as low as 25 ng/ml of these drugs. After 6 days of amitriptyline administration (75 mg/day), levels of amitriptyline and nortriptyline in three subjects were approximately 40–90 and 40 ng/ml, respectively, 12 hr after the last dose.

**Keyphrases** □ Amitriptyline—GLC determination in plasma □ Nortriptyline—GLC determination in plasma □ GLC—determination of steady-state levels of amitriptyline and nortriptyline in human plasma

Published methods for the determination of amitriptyline (1–5) lack either the sensitivity or specificity for determination of the drug in human plasma after therapeutic doses in the presence of its metabolite, nortriptyline. Recently, a GLC method for amitriptyline was published (6) with sensitivity sufficient to measure "steady state" plasma levels of the drug and its metabolite in man. However, difficulties were encountered with this method because plasma extracts produced multiple interfering peaks on the chromatograms and an unstable baseline. Accordingly, the GLC method described in the present paper was developed.

## EXPERIMENTAL

**Reagents**—Pesticide quality *n*-heptane[1] was used without further purification. Reagent grade isoamyl alcohol[2] was washed successively with 1 *N* NaOH, 1 *N* HCl, and water before use. Ether[3] and ethyl acetate[4] were redistilled immediately before use. Double-distilled water was used to prepare 0.5 *N* NaOH and 0.1 *N* HCl. Amitriptyline, nortriptyline, and protriptyline were used as the hydrochloride salts, with all concentrations expressed in terms of the free base. Drugs were dissolved in double-distilled water.




**Apparatus**—Analyses were performed on a gas chromatograph[5] equipped with a flame-ionization detector. The column, 1.82 m (6 ft) × 4 mm (i.d.), was packed with 1.5% OV-17 on Gas Chrom Q[6], 80–100 mesh. The packed column was conditioned before use by heating at 250° overnight under helium flow. Chromatographic conditions were as follows: column oven temperature, 212°; detector temperature, 280°; injection port temperature, 260°; hydrogen, 50 ml/min; air, 700 ml/min; and helium carrier gas, 100 ml/min. The recorder[7] was used on range 1 ($4 \times 10^{-12}$ amp) and attenuation 32, 16, or 8 as required. Disposable glass tubes with constricted tips[8] were used for evaporation of the final ether extract and were cleaned by washing with detergent, rinsing thoroughly with double-distilled water, draining, and drying in a vacuum oven at 100°.

**Procedure**—From 3 to 5 ml of human plasma, 0.1 ml of protriptyline internal standard (1 or 2 μg/ml), 1 ml of 0.5 *N* NaOH, and 25 ml of heptane containing 3% isoamyl alcohol were combined in a 45-ml glass-stoppered centrifuge tube. The mixture was shaken for 15 min and centrifuged. Most of the organic phase was transferred to a similar tube containing 5 ml of 0.1 *N* HCl, and the mixture was shaken for 10 min. After centrifugation, the organic phase was discarded by aspiration and the aqueous phase was shaken three times with 25 ml each of *n*-heptane (to remove

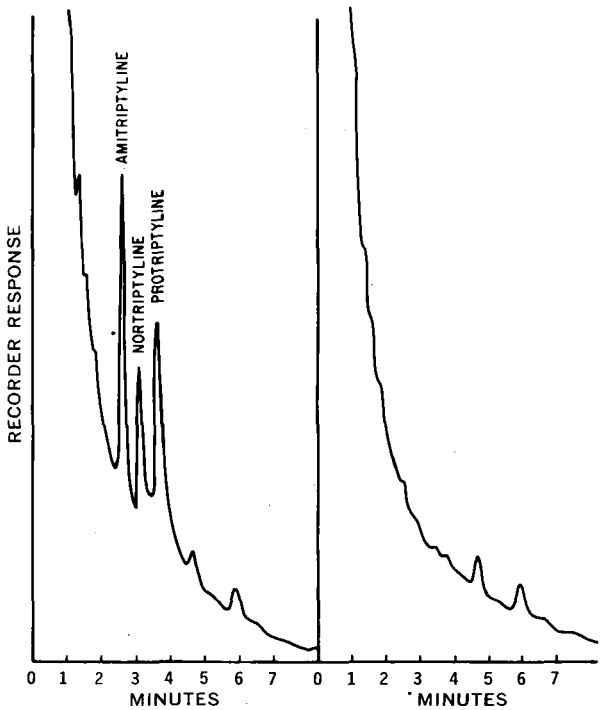



**Figure 1**—*Gas chromatogram (left) of an extract of 3 ml of human plasma containing 100 ng/ml of amitriptyline, 100 ng/ml of nortriptyline, and 200 ng/ml of protriptyline. The right-hand curve depicts an extract of 3 ml of control human plasma containing no drugs.*

[1] Matheson, Coleman and Bell.
[2] J. T. Baker.
[3] Anhydrous, ACS, Fisher.
[4] Fisher Certified, ACS.
[5] Hewlett-Packard (F&M) model 810.
[6] Applied Science Labs., Inc.
[7] Hewlett-Packard, model 7123A.
[8] Laboratory Research Co., Los Angeles, Calif.

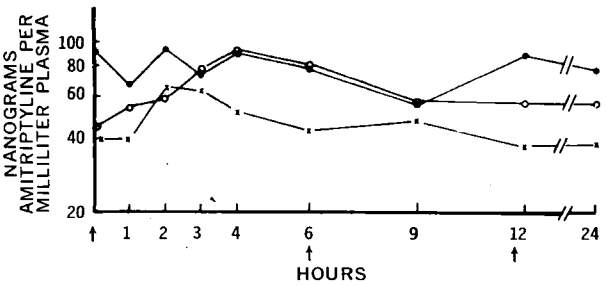



**Figure 2**—*Plasma levels of amitriptyline in three human subjects after 6 days of daily administration of 3 × 25-mg tablets of amitriptyline. Arrows indicate the time of administration of 1 × 25-mg tablet of amitriptyline on the day of analysis (Day 7).*

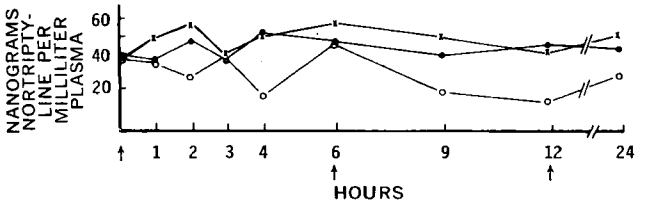



**Figure 3**—*Plasma levels of nortriptyline in the three human subjects described in Fig. 2.*

isoamyl alcohol). After aspirating the final heptane wash, most of the aqueous phase was transferred to a 13-ml glass-stoppered centrifuge tube. Five milliliters of freshly distilled ether and 1.5 ml of 0.5 *N* NaOH were added and the tube was shaken for 15 min. After centrifugation, as much as possible of the ether layer was transferred with a pipet (Pasteur) to a glass tube with constricted tip. The ether was evaporated in a water bath at 40°, material on the sides of the tube being washed down by periodically chilling the tube in ice water (7). The residue was dissolved in 25 μl of ethyl acetate, and 5 μl was injected into the gas chromatograph. All samples were assayed in duplicate.

Following chromatography, a baseline was drawn and peak heights of amitriptyline, nortriptyline, and protriptyline were measured. The ratio of amitriptyline and nortriptyline peak heights to that of the internal standard corrected for attenuation was calculated, and the concentration of each was obtained by reference to the standard curve.

A standard curve was constructed by analysis of samples to which known amounts of amitriptyline, nortriptyline, and internal standard had been added. When the ratio of peak heights of each compound to that of protriptyline internal standard was plotted *versus* concentration of amitriptyline and nortriptyline in nanograms per milliliter of plasma, the best-fit straight line was drawn that passed through the experimental points and origin.

## RESULTS AND DISCUSSION

As shown in Fig. 1, amitriptyline, nortriptyline, and the internal standard were adequately separated under these conditions, with retention times of 2.9, 3.4, and 4.0 min, respectively. Blank plasma samples assayed in the same manner gave no significant peaks on the chromatogram. Several known metabolites of amitriptyline and nortriptyline, *i.e.*, the 10-hydroxy and 10,11-dihydroxy analogs of both drugs, were found not to interfere, having retention times of 5.3–12.6 min under the assay conditions (Table I).

The precision and accuracy of the method were demonstrated by analysis (in quadruplicate) of plasma samples containing 25, 50, 75, and 100 ng/ml each of amitriptyline and nortriptyline.

**Table I**—Relative Retention Times of Amitriptyline, Nortriptyline, and Their Metabolites

| Compound | Relative Retention Time |
|---|---|
| Amitriptyline | 1.0 |
| Nortriptyline | 1.18 |
| 10-Hydroxyamitriptyline | 1.96 |
| 10-Hydroxynortriptyline | 2.38 |
| 10,11-Dihydroxyamitriptyline | 3.89 |
| 10,11-Dihydroxynortriptyline | 4.67 |

The concentrations found were equal to 97 ± 8% (mean ± *SD*) of the actual values for amitriptyline and 96 ± 9% of the actual values for nortriptyline over this range of values. The lower limit of sensitivity was approximately 20 ng/ml, and the range of linearity extended to approximately 100 ng/ml for nortriptyline and was somewhat higher for amitriptyline. The absolute recoveries were approximately 100% for amitriptyline, 85% for nortriptyline, and 90% for protriptyline.

The results of analysis for the drug and its metabolite, nortriptyline, in plasma from three human subjects receiving amitriptyline are depicted in Figs. 2 and 3. Levels of amitriptyline varied between 38 and 94 ng/ml, while those of nortriptyline were somewhat lower, ranging from 26 to 57 ng/ml. These values are similar to those reported previously (6). However, the spread of plasma levels was less than the ranges of 20–303 and 20–178 ng/ml, respectively, reported by previous authors (6) for six subjects on the same amitriptyline dosage regimen (25 mg tid). Thus, it seems possible that the smaller variation between subjects with the present method is a consequence of analyzing samples with less endogenous contaminants and of the use of a mass internal standard (8) which reflects losses during extraction.

The present method appears to have a number of advantages over the procedure of Braithwaite and Widdop (6). Reproducibility is improved by the use of an internal standard which is carried through the entire procedure. There is no interference from endogenous constituents in plasma extracts, but in the previous method (6) care must be taken to avoid interference during repetitive analyses from large, slowly eluting impurity peaks.

No need was found to use silanized glassware. Preparation of the gas chromatographic column used in the present method was much simpler, and the column packing itself was of known stability, requiring no precautions for use as did the mixed phase column used by the previous authors.

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