Determination of Tolmetin in Human Plasma by GLC and Spectrophotometric Procedures

WILLIAM A. CRESSMAN ˣ, BARBARA LOPEZ, and DARRELL SUMNER *

Abstract □ GLC and spectrophotometric methods for the determination of tolmetin in plasma were developed. The methods can detect tolmetin in concentrations above 0.5 μg/ml. The plasma levels obtained with a 600-mg dose of tolmetin to a human subject are reported.

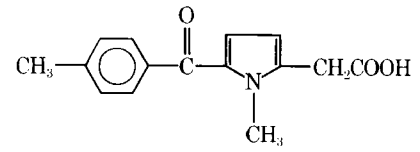
Keyphrases □ Tolmetin—GLC and spectrophotometric analyses, plasma □ GLC—analysis, tolmetin in plasma □ Spectrophotometry—analysis, tolmetin in plasma

The pyrrole derivative 1-methyl-5-*p*-toluoylpyrrole-2-acetic acid, tolmetin[1] (I), is a new anti-inflammatory agent currently undergoing clinical trials in rheumatoid arthritis (1, 2). GLC and spectrophotometric assays were developed to evaluate the absorption, distribution, and excretion of tolmetin in human subjects. These procedures were used to evaluate dose–response relationships, bioavailability, and the potential drug interaction between tolmetin and aspirin. The spectrophotometric assay has primary utility in bioavailability and other studies conducted under controlled conditions. The GLC assay has broader utility for monitoring blood levels of tolmetin in clinical therapy.

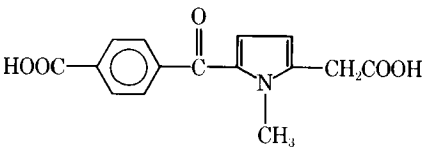
The major tolmetin metabolite in humans (3) is 5-(*p*-carboxybenzoyl)-1-methylpyrrole-2-acetic acid (II).

EXPERIMENTAL

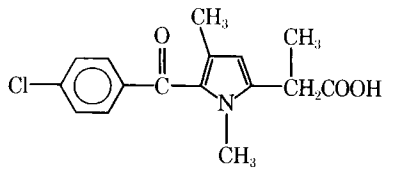
Reagents—Citric acid buffer was prepared by dissolving 210 g of citric acid in 1000 ml of water and adjusting the pH to 2.5 with 5 *N* NaOH. Phosphate buffer was prepared by dissolving 69 g of monobasic sodium phosphate in 1000 ml of water and adjusting to pH 7.0 with 5 *N* NaOH.



I



II



III

[1] Tolectin, McNeil Laboratories.

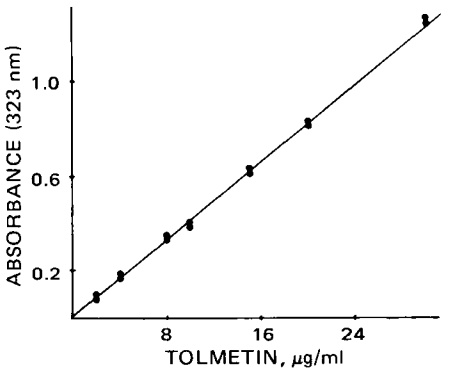


Figure 1—*Standard curve for the spectrophotometric determination of tolmetin in plasma (blank absorbance = 0.03 absorbance unit).*

The GLC internal standard (III) was dissolved in ether to give a concentration of 0.5 μg/ml. Diazomethane was prepared by adding 50 mg of *N*-nitrosomethylurea to 5 ml of ether and 3 ml of 40% KOH. The resulting diazomethane–ether solution was dried over potassium hydroxide pellets prior to use.

Spectrophotometric Assay—*Step 1*—To a 3-ml plasma sample in a 30-ml (1-oz) disposable glass screw-capped bottle with a polyethylene-lined cap were added 1 ml of water, 3 ml of pH 2.5 citric acid buffer, and 20 ml of 1% isopentyl alcohol in methylcyclohexane. The bottle was shaken for 20 min on a wrist-action shaker.

Step 2—Eighteen milliliters of the 1% isopentyl alcohol–methylcyclohexane layer was transferred to a clean 30-ml (1-oz) bottle, 2.5 ml of pH 7.0 phosphate buffer was added, and the mixture was shaken for 20 min.

Step 3—The 1% isopentyl alcohol–methylcyclohexane layer was removed and discarded, 2 ml of ether was added to the phosphate buffer layer, and the mixture was shaken for 15 min.

Step 4—The ether layer was then removed and discarded. An additional 2 ml of ether was added, the mixture was shaken for 15 min, and the new ether layer was removed and discarded.

Step 5—The absorbance of the pH 7.0 phosphate buffer layer was read at 323 nm against a pH 7.0 phosphate buffer blank. A plasma blank was also run, and each sample absorbance reading

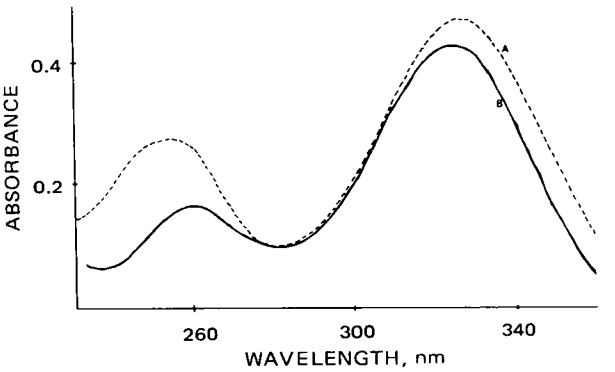


Figure 2—*Spectrum of 10-μg/ml solutions of tolmetin (B) and the tolmetin metabolite (A) in pH 7.0 buffer.*

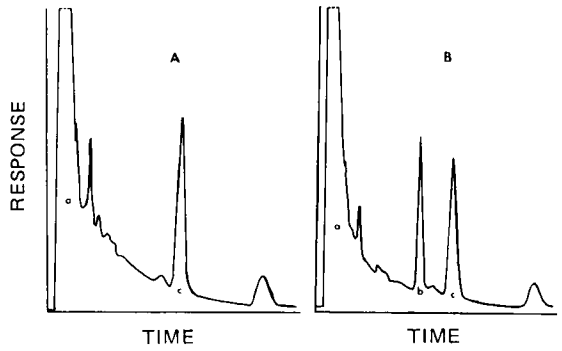


Figure 3—*Chromatograms of a blank human plasma sample (A) and a plasma sample containing 16 μg/ml of tolmetin (B). Key: a, solvent front; b, tolmetin methyl esther peak; and c, internal standard (III) methyl ester peak.*

was corrected for the blank (absorbance observed was usually 0.01–0.02 absorbance unit).

GLC Assay—*Instrumental Conditions*—A gas chromatograph[2] equipped with a flame-ionization detector was used. The column was a 1.8-m (6-ft) × 7-mm o.d. (4-mm i.d.) glass coil packed with 3% OV-1 on 60 – 80 - mesh Gas Chrom Q[3]. The injection port was maintained at 265°, the column was maintained at 200°, and the detector was maintained at 310°. Nitrogen carrier gas was used at a flow rate of 60 ml/min. The column was silylated[4] regularly each morning prior to use.

Under these conditions, the following retention times were observed: tolmetin (I) (methyl ester), 6 min; internal standard (III) (methyl ester), 8.5 min; and tolmetin metabolite (II) (dimethyl ester), > 13 min.

Sample Preparation—The first four steps of sample preparation are similar to those noted for the spectrophotometric assay with the following minor changes. In Step 1, only 1 ml of plasma was required; in Step 2, 5 ml of buffer was used; and in Steps 3 and 4, 3 ml of ether was used. The assay was then continued as follows.

The ether layer was removed and discarded; 4.5 ml of the aqueous layer (pH 7 phosphate buffer) was transferred to a clean 30-ml (1-oz) bottle, and the pH was adjusted to ~1 with 3.0 ml of 1 *N* HCl. Then 20 ml of the ethereal internal standard solution was added and shaken for 20 min.

Eighteen milliliters of the ether layer was removed and evaporated to about 2 ml. The sample was placed in a hood, and 2–3 ml of the anhydrous, ethereal diazomethane solution was added (sample should retain a yellow color). Additional reagent was added if the yellow color was dissipated, and the mixture was allowed to stand for 10 min or until the bubbling stopped. The excess diazomethane was evaporated with nitrogen in a hood until the sample turned colorless. The sample was evaporated to dryness using nitrogen and a warm water bath (30–50°). At this point, the sample was stored in a refrigerator.

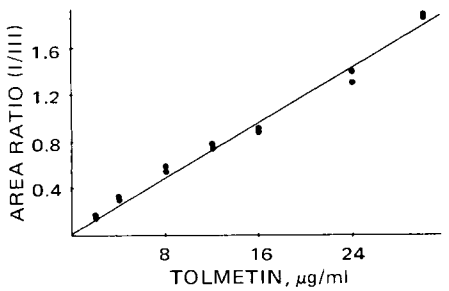


Figure 4—*Standard curve for the GLC determination of tolmetin in plasma.*

[2] Perkin-Elmer model 900.
[3] Applied Science Labs., State College, Pa.
[4] Silyl-8, Pierce Chemical Co., Rockford, Ill.

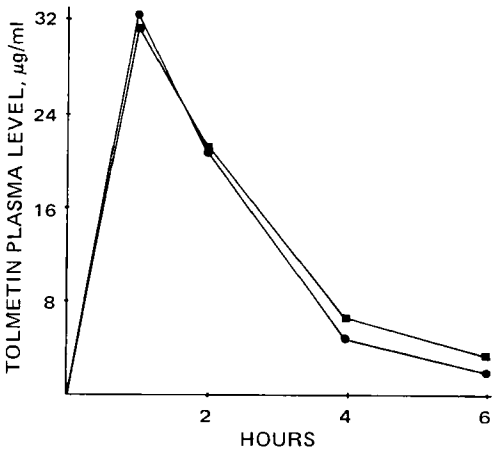


Figure 5—*Plasma and urine levels of tolmetin in a human subject administered 600 mg of tolmetin as an oral solution. Key: ●, GLC assay; and ■, spectrophotometric assay. One hundred percent of the radioactivity from an oral dose of ^{14}C-tolmetin is excreted in the urine within 24 hr.*

The dried residue was dissolved in 30 μl of methanol, and the sides of the tube were washed down with methanol. One-tenth of the sample (3 μl) was injected into the gas chromatograph using a 1-μl methanol plug in the syringe behind the sample. The appropriate attenuation setting for a 30-μg/ml plasma sample is 1×10^{-10} amp full-scale deflection.

In these studies, with this and other GLC plasma assays, the results were always more reproducible when the internal standard was added in the last step prior to evaporation or derivatization, *i.e.*, to monitor derivative formation and/or injection variables. In most cases, addition of the internal standard in earlier steps resulted in increased variability, which appears to increase with the number of extraction steps involved in the assay.

Standard Curves—Standard samples were prepared by dissolving tolmetin sodium dihydrate in water at appropriate concentrations so that 1 ml of the seeded solution could be used in place of the 1 ml of water that is added to each sample in Step 1 of the assay. The standard samples were prepared using heparinized human plasma from an untreated subject.

RESULTS AND DISCUSSION

Spectrophotometric Assay—A typical standard curve for the spectrophotometric assay, using duplicate seeded samples, is shown in Fig. 1. The lower limit of detection of the assay is 0.5 μg/ml. Samples of urine carried through the procedure gave low blank absorbance values (0.02 absorbance unit at 323 nm). Since II (the major metabolite of tolmetin in human subjects) also shows an absorption maximum at 323 nm with approximately the same absorptivity (Fig. 2), the potential interference of this compound was evaluated.

Less than 2% of the initial amount of II was recovered in the final assay layer. Studies with ^{14}C-tolmetin have shown that plasma levels of II are always less than 50% of the levels of tolmetin and that the plasma half-life of II is similar to that of tolmetin[5]. Therefore, no significant interference from II is expected.

GLC Assay—Using the chromatographic conditions described, tolmetin has a retention time of 6 min. A typical gas chromatogram of plasma extracted with and without the addition of tolmetin is shown in Fig. 3. The use of larger volumes of plasma to increase the sensitivity of the procedure was explored, but this procedure was accompanied by an equivalent increase in background "noise" and/or minor interfering peaks and was not pursued further. The dimethyl ester of II has a retention time greater than 13 min and, therefore, should not interfere in the assay. In addition, only 2% of the II seeded into plasma was carried through the assay.

A typical standard curve for the GLC assay, using duplicate seeded samples, is shown in Fig. 4. The lower limit of detection

[5] Data on file at McNeil Laboratories, Inc.

Table I—Urine Levels (Percent of Dose)

Method	0–12 hr	12–25 hr
GLC	2.9	0.31
UV	2.4	0.25

used in this procedure is 0.5 μg/ml. A similar GLC assay designed to measure both I and II was reported recently (4).

Interference of Aspirin and Salicylic Acid—Since tolmetin often could be used with large doses of aspirin, the interference of aspirin or salicylic acid in the spectrophotometric and GLC assay for tolmetin was evaluated. Plasma was seeded with 500 μg/ml[6] of aspirin or 500 μg/ml of salicylic acid and assayed by both procedures.

No interference was observed from either aspirin or salicylic acid with the GLC assay, but significant interference was observed in the spectrophotometric assay. For 500 μg/ml of aspirin, the spectrophotometric assay gave an absorption equivalent to ~10 μg/ml of tolmetin. For 500 μg/ml of salicylic acid, the assay gave absorption equivalent to 50 μg/ml of tolmetin. Since the levels of tolmetin at therapeutic doses (400–600 mg) are between 10 and 30 μg/ml, the spectrophotometric assay would not be suitable for the determination of plasma levels of tolmetin in patients who are simultaneously receiving aspirin products.

With the spectrophotometric assay, one can easily assay 36 samples/day. With the GLC assay, using a dual-column gas chromatograph and manual injection, analytical productivity is reduced by approximately half. Therefore, the spectrophotometric assay offers significant economic advantages over the GLC assay in controlled situations, *e.g.*, bioavailability studies (6). Because of the greater specificity of the GLC procedure, it offers greater utility in the assay of plasma levels of tolmetin in clinical practice (6).

[6] The 500-μg/ml level of aspirin or salicylic acid is at least two to three times the level expected from a 4.5-g dose of aspirin per day (5).

Comparison of the Two Analytical Procedures—To evaluate the applicability of the assay procedures, plasma and urine samples from a fasted subject dosed with 600 mg of tolmetin as an oral solution were assayed using both methods. The results are shown in Fig. 5. Studies with ^{14}C-tolmetin (3) showed that 100% of the administered dose of radioactivity is excreted in the urine within 24 hr. These radioactive studies also demonstrated that only 2–3% of the administered dose is excreted in the urine as intact drug. Therefore, the urine assay (Table I) provides a severe test of both assay procedures. The agreement of the urine and plasma assays by both methods, as well as the agreement of the results with data from other studies using radioactive and nonradioactive tolmetin (3, 4), validates the utility of both assay procedures.

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GLC Determination of Procainamide in Biological Fluids

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Abstract □ A GLC method for the determination of procainamide in biological fluids is presented. By using a dipropyl analog of procainamide as an internal standard, both compounds can be chromatographed directly, yielding linear calibration curves and a sensitivity that allows quantitative determination of concentrations as low as 0.1 μg/ml. The extraction procedure was carefully modified to avoid hydrolysis of *N*-acetylprocainamide, a major metabolite of procainamide. The usefulness of the procedure is demonstrated by following the disappearance of procainamide from the plasma and urine of human subjects treated with the drug.

Keyphrases □ Procainamide—GLC analysis, biological fluids, therapeutic range □ GLC—analysis, procainamide in biological fluids

The effectiveness of procainamide[1] (I) in the prevention or treatment of cardiac arrhythmias has been firmly established (1, 2). Careful clinical studies demonstrated that plasma levels of procainamide may be correlated with pharmacological responses. The range of therapeutic plasma concentrations is 4–8 μg/ml (2–4).

Due to individual differences in absorption and elimination of procainamide, dosages necessary for therapeutic effects may vary widely (2). Several methods are presently available for the determination of procainamide plasma concentrations within the therapeutic range.

The most commonly used method of determining procainamide in biological fluids is a colorimetric method (1) where, following complexation with *N*-(1-naphthyl)ethylenediamine, absorbance is read at 550 nm. This method has been used by many investigators (2, 4–11) and has been shown to have satisfactory precision above 2–3 μg. However, its specificity recently has been questioned. Gibson *et al.* (12) reported that *N*-acetylprocainamide, the major metabolite in humans (5), is hydrolyzed to procainamide under the conditions of the procedure of Mark *et al.* (1).

Spectrophotofluorometric detection is also possible

[1] Pronestyl, E. R. Squibb & Sons. The chemical name is *p*-amino-*N*-[2-(diethylamino)ethyl]benzamide hydrochloride.