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ACKNOWLEDGMENTS

The authors acknowledge the clinical observations of neurological deficiencies in the newborn by L. J. Graziani, Thomas Jefferson University School of Medicine, and Barbara H. Korberly, Philadelphia College of Pharmacy and Science, which prompted this investigation.

GLC Microdetermination of Indomethacin in Plasma

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Received May 18, 1979, from the *Department of Pharmacology, University of Illinois at the Medical Center, Chicago, IL 60680.* Accepted for publication August 27, 1979.

Abstract □ A GLC method utilizing an electron-capture detector is described for analysis of indomethacin in blood plasma. Indomethacin is extracted with ethyl acetate from plasma buffered to pH 5.0. The ethyl acetate is evaporated to dryness, and indomethacin is derivatized to a pentafluoropropionyl ester prior to chromatography. A GLC standard is used for peak height quantitation of indomethacin. The extraction efficiency from plasma is 92 ± 3%, and as little as 1 ng of indomethacin can be quantitatively determined.

Keyphrases □ Indomethacin—GLC analysis, human plasma □ GLC—analysis, indomethacin in human plasma □ Anti-inflammatory agents—indomethacin, GLC analysis in human plasma

Indomethacin[1] is an extensively used anti-inflammatory drug with potent inhibitory action on prostaglandin synthesis. Recent studies demonstrated that the drug is also successful in the management of patent ductus arteriosus in the premature infant (1). Since complications of indomethacin therapy in the premature infant are associated with high plasma levels, a rapid and sensitive assay was needed for monitoring therapeutic plasma drug levels utilizing small samples. Several investigators recently reported sensitive analytical procedures for indomethacin in biological fluid; however, these procedures require several extractions (2, 3), lack an internal standard (4), and require an initial plasma sample of 0.5 ml (5). Therefore, an electron-capture GLC method was developed for monitoring therapeutic indomethacin levels in limited plasma samples (0.1 ml) utilizing a GLC standard for quantitation.

EXPERIMENTAL

Apparatus—A gas chromatograph[2] equipped with a ^{63}Ni-electron-capture detector was maintained with a high purity nitrogen column gas flow of 70 ml/min. The column oven temperature was 295°; the injection port and detector temperatures were maintained at 310°.

Column—A glass column, 2 m × 2 mm, was packed with 10% SP 2250 on 100–120-mesh Supelcoport[3]. The column was rinsed before packing with methanol and acetone; it was then dried and conditioned for 2 hr with a 20% solution of dimethyldichlorosilane[4] in toluene. Following silylation, the column was rinsed with acetone and dried under nitrogen before packing.

Procedures—Plasma (0.1 ml) was transferred to 1.5-ml polypropylene disposable micro test tubes[5], buffered to pH 5.0 with 0.1 ml of 0.2 *M* acetate buffer, and extracted with 1.0 ml of ethyl acetate. The ethyl acetate (0.9 ml) was transferred by disposable pipet to a dry, clean 1.5-ml micro test tube and evaporated to dryness using a vacuum centrifuge[6]. The residue was mixed with 10 μl of a mixture of 2,2,3,3,3-pentafluoro-1-propanol in pentafluoropropionic anhydride[7] (1:4, v/v) and heated to 75° for 20 min. Following derivatization, the sample was evaporated to dryness using the vacuum centrifuge; the residue was redissolved in 10 μl of ethyl acetate containing 2.5 μg of *p*-bromobenzaldehyde isonicotinoylhydrazone/ml as the internal standard.

One-microliter samples were injected into the gas chromatograph for analysis. The internal standard was prepared according to Timbrell *et al.* (6) by mixing equimolar amounts of isoniazid and *p*-bromobenzaldehyde in methanol and refluxing for 1 hr at 60°. After cooling for 24 hr at −20°, the crystalline product was filtered, washed twice with warm methanol, and recrystallized. The final product, *p*-bromobenzaldehyde isonicotinoylhydrazone, was found to be better than 98% pure by GLC and TLC.

A standard calibration curve was prepared by adding 0.1 ml of plasma to known amounts of indomethacin to give final indomethacin concentrations ranging from 0.01 to 1 μg/ml. Thirty samples, five per concentration, were analyzed to prepare the standard curve. Each sample was injected in duplicate, and the determination of the entire standard curve was replicated at once. 2-^{14}C-Indomethacin[8] was used to estimate the extraction efficiency of indomethacin from plasma by measurement of the total radioactivity in the extract.

RESULTS AND DISCUSSION

The electron-capture GLC retention times for derivatized indomethacin and the internal standard were 5.2 and 6.8 min, respectively. Chromatograms from a control human plasma and a plasma sample containing 31 ng of indomethacin, both with the internal standard, are shown in Fig. 1. Since peaks for both agents were symmetrical, quantitation was made by comparison of peak heights. Detector response and the calibration curve were linear over 0.01–1.0-μg/ml range. Blanks were prepared from plasma of drug-free subjects, and no peaks were observed that would interfere with the measurement of indomethacin or the internal standard. Plasma samples containing 5 μg/ml of salicylate or furosemide also did not interfere with indomethacin quantitation.

Linear regression analysis of the individual data points (n = 60) from the standards plotted as the ratio of the peak height of derivatized in-

[1] Indocin, Merck & Co.
[2] Varian model 3700.
[3] Supelco, Inc., Bellefonte, Pa.
[4] Applied Science Laboratories, State College, Pa.
[5] Bio-Rad Laboratories, Richmond, Calif.
[6] Savant Instruments, Hicksville, N.Y.
[7] Regis Chemical, Morton Grove, Ill.
[8] New England Nuclear, Boston, Mass.

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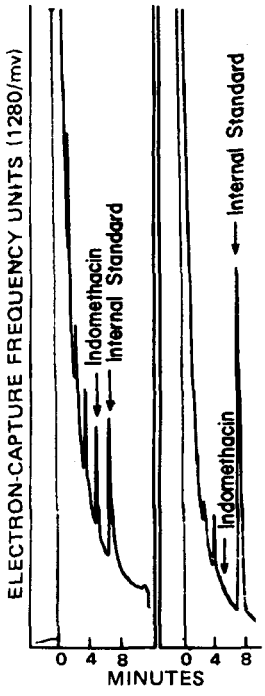

Figure 1—*Gas chromatograms of human plasma. Key: left: plasma (0.1 ml) containing 31 ng of indomethacin and internal standard; and right: control plasma with internal standard.*

domethacin divided by the peak height of the internal standard *versus* the plasma indomethacin concentration gave a computed slope of 42.8/ng of derivatized indomethacin and a coefficient of determination (r^2), a measure of accuracy, of 0.994. Extraction efficiency based on results from 10 samples using ^{14}C-indomethacin was estimated at 92 ± 3%.

Results of indomethacin analysis in a premature infant with patent ductus arteriosus following therapeutic intravenous administration of a 0.2-mg/kg dose of indomethacin sodium trihydrate are shown in Fig. 2. The pharmacokinetic parameters for the intravenous study were evaluated using a modified IGPHARM program (7). The calculated half-life was 15.5 hr, but further study is needed before any definitive statements can be made concerning the indomethacin half-life in the premature infant. Alvan *et al.* (8) demonstrated that the half-life of the β-phase of indomethacin in adults ranged from 2.6 to 11.2 hr. This rapid and sensitive method for indomethacin analysis has been useful in the

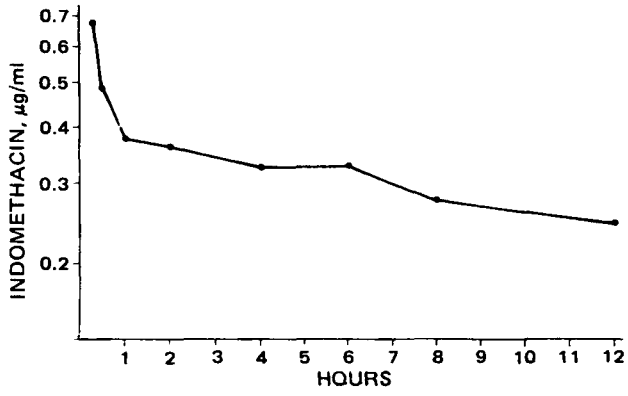

Figure 2—*Plasma concentration–time profile for indomethacin in a premature infant receiving intravenous indomethacin sodium trihydrate ($t_{1/2}$ = 15.5 hr, V_D = 0.37 liter/kg, and Cl_p = 15.3 ml/kg/hr). Sample size ranged from 0.05 to 0.15 ml of plasma.*

therapeutic monitoring of plasma drug levels when the sample volume is necessarily limited.

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ACKNOWLEDGMENTS

Supported in part by U.S. Public Health Service Grant DA 01877 and University of Illinois Research Grant 7658.

Peristaltic Dissolution Apparatus: Prediction of Relative *In Vivo* Performance of Prednisone Tablets in Humans

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Received March 23, 1979, from the *R & D Laboratories, Frank W. Horner Limited, Montreal H3C 2W6, Canada.* Accepted for publication August 29, 1979.

Abstract □ By utilizing a previously established correlation concept, the relative *in vivo* performance in humans of seven unknown prednisone tablets was predicted accurately from peristaltic dissolution rate data. A general discussion is presented on the role of the peristaltic apparatus in selecting a suitable dosage form at the developmental stage.

Keyphrases □ Prednisone—tablets, prediction of relative *in vivo* performance in humans, peristaltic dissolution apparatus □ Dissolution apparatus, peristaltic—prednisone tablets, prediction of relative *in vivo* performance in humans □ *In vitro–in vivo* correlation—prednisone tablets, prediction of relative *in vivo* performance in humans, peristaltic dissolution apparatus

In 1975, a peristaltic dissolution rate apparatus was developed that was capable of predicting the *in vivo* performance of tolbutamide tablets in beagle dogs (1). This work was extended later to include meprobamate tablets (2) and tolbutamide tablets marketed in Canada (3). Despite the excellent *in vitro–in vivo* correlations obtained, a prime concern was the reliance on beagles as an *in vivo* model and the absence of human data in the correlations.

The performance of the apparatus on tablets that had been evaluated in a human bioavailability study was tested in this study. Seven prednisone tablets commercially available in the United States, each containing 5 mg of prednisone, were submitted in coded form by the Food and Drug Administration (FDA). Based on the peristaltic dissolution data, the relative *in vivo* performance of the tablets was predicted accurately.

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