was fed into the electronic integrator, and the ratio of the area of the procaine peak to the area of the internal standard (pyrrocaine) was calculated. Regression analysis of these data indicated excellent linearity and reproducibility (Table I). The amount of procaine that could be measured was as low as 0.25 μg of total free base injected.

Application of this method to four pharmaceutical dosage forms containing procaine was carried out to determine the suitability of the procedure for this type of analysis. The results of the HSLC method (Table II) were compared with the sodium nitrite titration or the colorimetric determination, which required an extraction. Although the coefficient of variation of the HSLC method is somewhat higher than the other method, it is sufficiently low to be acceptable. The agreement between the HSLC method and the other methods was excellent. The convenience of the HSLC method, together with its increased specificity and simplicity, makes this method a desirable alternative.

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[x] To whom inquiries should be directed.

GLC Analysis of Lidocaine in Blood Using an Alkaline Flame-Ionization Detector

T. R. IRGENS, W. M. HENDERSON, and W. H. SHELVER [x]

Abstract □ A method was developed for the quantitative GLC determination of lidocaine and its major metabolite monoethylglycinexylidide. By using the specificity of the alkaline flame-ionization detector, this simple analytical procedure is extremely sensitive, rapid, and easily performed.

Keyphrases □ Lidocaine—GLC analysis in blood □ Monoethylglycinexylidide—major metabolite of lidocaine, GLC analysis, blood □ GLC—analysis, lidocaine and monoethylglycinexylidide (major metabolite), blood □ Antiarrhythmics—lidocaine, GLC analysis in blood

The therapeutic and toxic effects of many drugs are directly related to the concentration of the drug and metabolites in the plasma of the patient. To regulate the dose properly, a rapid, precise, and specific procedure for measuring plasma concentrations of the drug and significant drug metabolites is required.

Lidocaine is used routinely in the acute management of ventricular arrhythmias, particularly those that occur during acute myocardial infarction. Lidocaine is metabolically dealkylated at the basic nitrogen (1, 2), and methods of measuring the monodealkylated product (3) monoethylglycinexylidide and the didealkylated product (4) glycinexylidide or both (5) have been developed. Lidocaine occasionally shows toxic side effects (6), and both monoethylglycinexylidide (7) and glycinexylidide (8) have been reported to have significant pharmacological actions that could contribute to the antiarrhythmic activity and to the side effects of lidocaine. Animal experiments suggest that monoethylglycinexylidide is the most potent (8).

Element-specific detectors such as the alkaline flame-ionization detector are useful for the analysis of compounds containing nitrogen (9). The alkaline flame-ionization detector has proven to be a suitable detector for monitoring drugs containing nitrogen (10–13).

Several investigators reported the quantitative determination of lidocaine by GLC, usually utilizing the flame-ionization detector (14–17); other workers utilized GLC–mass spectrometry (3, 4, 8). After this study was undertaken, a report appeared on the use of the alkaline flame-ionization detector (18) in the determination of lidocaine and its metabolites, but precision was reported only for a range of samples.

EXPERIMENTAL

Apparatus—The gas chromatograph[1] was equipped with an alkaline flame-ionization detector and a flame-ionization detector. An electronic integrator[2] was used for measuring peak areas. A glass U-shaped column, 1.8 m × 0.3 cm (6 ft × 0.125 in.), was packed with 3% KOH plus 2% polyethylene glycol[3] coated onto 80–100-mesh Chromosorb W DMSC/AW.

The column was conditioned at 240° for 24 hr, and the operating conditions were: oven temperature, 210°; detector temperature, 220°; and injection port temperature, 200°. Nitrogen was used as the carrier gas at a flow rate of 15 ml/min. Hydrogen and air were adjusted to give optimum recorder response. For this analysis, the hydrogen was set at 45 ml/min and air was at 290 ml/min.

Chemicals and Reagents—Lidocaine[4] and pyrrocaine[5] were

[1] Model 420, Packard Instrument Co., Downers Grove, Ill.
[2] Model 3370B, Hewlett-Packard Co., Palo Alto, Calif.
[3] Carbowax 20 M.
[4] Astra Pharmaceutical Products, Worcester, Mass.
[5] Endo Laboratories, Richmond Hill, N.Y.

Table I—Statistical Analysis of the Calibration Curve for Lidocaine and Monoethylglycinexylidide

Compound	Slope	*SE*	*r*	*n*
Monoethylglycinexylidide	0.511 (0.489–0.533)	0.010	0.998	12
Lidocaine	0.320 (0.311–0.330)	0.005	0.998	19

obtained from commercial sources. Monoethylglycinexylidide was synthesized using a modified procedure of Moore and Dalrymple (19). The melting point for monoethylglycinexylidide was 51° [lit. (4) mp 51°], and its NMR spectra were compatible with the proposed structure.

Procedure—Into a 20-ml screw-topped test tube was placed 2.5 ml of plasma containing lidocaine. To this tube was added 5 ml of a benzene solution containing pyrrocaine as the internal standard in a concentration of 5 μg/ml. Then 0.2 ml of 5 *N* sodium hydroxide was added to make the mixture alkaline.

The mixture was mixed for 15 min and then centrifuged for 5 min. The organic phase was transferred to a pear-shaped tube and evaporated to dryness on a water bath, equipped with a vacuum, at 50°. Fifty microliters of benzene was used to dissolve the residue. A 5-μl aliquot was injected directly on the column with a 10-μl microsyringe.

Calibration Curve—Samples for the lidocaine calibration ranged from 0.1 to 3 μg/ml. Five extractions were made at each concentration, and the calibration curve was plotted utilizing a linear regression line. Regression statistics are shown in Table I.

Samples for the monoethylglycinexylidide calibration curve ranged from 0.25 to 4 μg/ml, and the calibration curve was plotted utilizing a linear regression line. Regression statistics are shown in Table I.

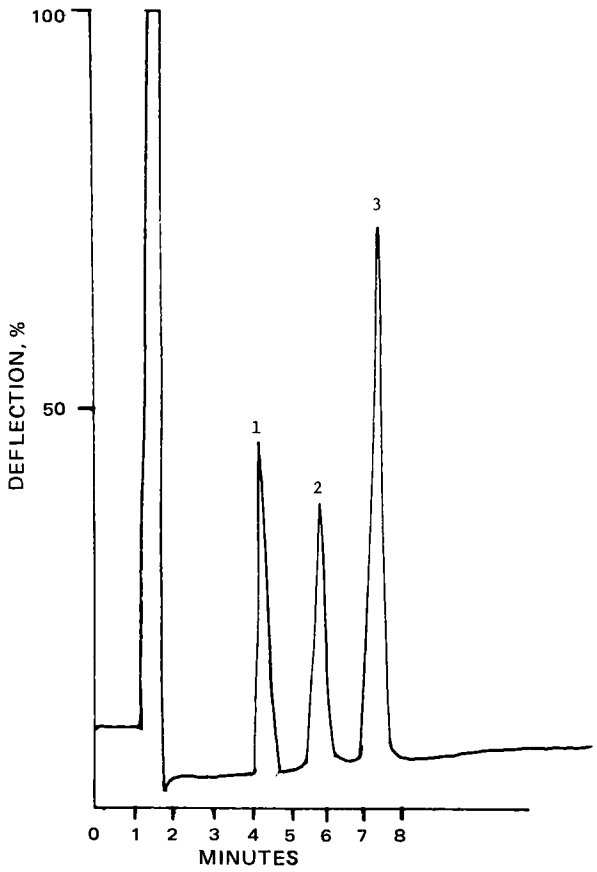

Figure 1—*Gas chromatographic separation of lidocaine (peak 1), monoethylglycinexylidide (peak 2), and pyrrocaine (peak 3). The sample contained 100 ng of lidocaine, 100 ng of monoethylglycinexylidide, and 500 ng of pyrrocaine.*

Table II—Precision of the Lidocaine Analysis at Different Plasma Levels

Plasma Concentration, μg/ml[a]	Coefficient of Variation, %
0.1	18.0
0.4	9.1
1.0	6.5
3.0	3.6

[a] Mean of five determinations.

Table III—Precision of the Monoethylglycinexylidide Analysis at Different Plasma Levels

Plasma Concentration, μg/ml[a]	Coefficient of Variation, %
0.25	5.53
0.50	3.16
2.0	2.27
4.0	1.91

[a] Mean of three determinations.

Relative Recovery—A standard solution in benzene of lidocaine-free base and pyrrocaine was prepared in a concentration that would be precisely the concentration attained after extraction of a 1-μg/ml lidocaine sample. The same procedure was followed for extraction of lidocaine, and a 2.5-ml aliquot was used and evaporated to dryness. The peak area ratios were recorded for the extraction and the standard solution. The mean relative recovery was 91.9% (SD = 5.0, n = 4). The mean relative recovery for monoethylglycinexylidide was 88.7% (SD = 3.65, n = 3)

RESULTS

Figure 1 shows a typical chromatogram of lidocaine, monoethylglycinexylidide, and pyrrocaine extracted from plasma. The retention times were 4 min for lidocaine, 6 min for monoethylglycinexylidide, and 7.5 min for pyrrocaine.

Peak area ratios were used to prepare calibration curves. The standard curve was linear over the range of 0.1–3 μg/ml of plasma for lidocaine and of 0.25–4 μg/ml of plasma for monoethylglycinexylidide. The precision of the extraction procedure for lidocaine was checked at the 0.1-, 0.4-, 1-, and 3-μg/ml levels. Five extractions were made at each concentration (Table II). For monoethylglycinexylidide, the precision was tested at 0.25, 0.5, 2.0, and 4 μg/ml of plasma (Table III).

As shown, subtherapeutic quantities of licocaine can be measured with a high degree of precision by this method. The column used gave excellent separation of lidocaine from the metabolite monoethylglycinexylidide, and no interference was noted from compounds normally present in blood.

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[x] To whom inquiries should be directed.

Onset of Enzyme Induction with Pregnenolone-16α-carbonitrile in Male and Female Rats

S. J. STOHS [x] and H. ROSENBERG

Abstract □ Male and female rats treated with a single 20-mg/kg ip dose of pregnenolone-16α-carbonitrile (PCN) produced parallel increases in hepatic cytochrome P-450 and aniline hydroxylase activity. However, the onset of increase in aniline hydroxylase activity and cytochrome P-450 content was slower in male than female animals. The maximal levels achieved in male rats were 89% of the values observed in the female animals. Furthermore, the hepatic aniline hydroxylase activity and P-450 content declined more rapidly in male than in female rats.

Keyphrases □ Pregnenolone-16α-carbonitrile—effect on hepatic cytochrome P-450 and aniline hydroxylase activity, male and female rats compared □ Cytochrome P-450 activity—effect of pregnenolone-16α-carbonitrile, male and female rats compared □ Aniline hydroxylase activity—effect of pregnenolone-16α-carbonitrile, male and female rats compared

3β-Hydroxypregn-5-ene-20-one-16α-carbonitrile, commonly known as pregnenolone-16α-carbonitrile or PCN (I), is a catatoxic steroid with no known hormonal activity. However, it is rapidly becoming well known as a potent inducer of hepatic enzymes (1–11). As is characteristic of enzyme inducers such as phenobarbital, I increases liver weight, cytochrome P-450 content, aniline hydroxylase, ethylmorphine *N*-demethylase, and cytochrome c reductase but does not increase the microsomal protein per gram of liver content (5).

Ultrastructural changes in rat hepatocytes following I treatment have been extensively examined (12–17). Compound I produced marked proliferation of smooth-surfaced endoplasmic reticulum, with disorganization of the rough endoplasmic reticulum occurring. Most cytoplasmic changes returned to normal within 10 days after the last I administration (15).

The potential for using I to promote detoxification of many xenobiotics exists; and when I is given to female rats several days before a toxicant, it can protect against a variety of substances (3). If I is to be useful in promoting xenobiotic detoxification, the rate of onset of enhanced hepatic drug-metabolizing activity must be determined since the applicability of I lies in its administration after a toxicant has been ingested by a patient.

All cited studies were conducted with female rats. Therefore, it was of interest to learn how quickly I can enhance the activity of the hepatic mixed function oxidase system in male rats and to compare these results with the onset, maximal effects, and duration of induction in female rats.

EXPERIMENTAL

Animal Pretreatment—Male and female rats[1], 120–140 g, were maintained on food[2] and tap water *ad libitum*. To determine the onset of action of I upon hepatic mixed function oxidase activity, both male and female rats were given a single dose of I (20 mg/kg ip) in 0.2% polysorbate 80 in isotonic saline or only the polysorbate 80–saline vehicle and sacrificed at various times after injection.

Hepatic aniline hydroxylase activity (nanomoles of *p*-aminophenol formed per minute per milligram of microsomal protein) and hepatic microsomal cytochrome P-450 content (nanomoles of P-450 per milligram of microsomal protein) were determined in control and experimental animals injected and sacrificed at corresponding times. Three control and three experimental rats were treated and sacrificed at each time point for each sex.

Enzyme, Protein, and Cytochrome P-450 Assays—The 10,000×*g* supernatant fraction was used to determine aniline hydroxylase activity, using an NADPH-generating system (18) and the assay technique of Kato and Gillette (19). Microsomal P-450 was determined by the method of Omura and Sato (20). Microsomal protein was measured by the Folin phenol method (21). These determinations were performed on three pairs of rats sacrificed at 10, 15, 20, 25, 30, 48, 60, and 72 hr after treatment. Averages in corresponding control and experimental groups were compared (Table I).

RESULTS AND DISCUSSION

The effect of a single injection of pregnenolone-16α-carbonitrile (I) at 20 mg/kg on the onset and time course of hepatic aniline hy-

[1] Sasco, Inc., Omaha, Neb.
[2] Purina Lab Chow.