GLC Assay for Lidocaine in Human Plasma

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Abstract □ A simple, rapid, and highly sensitive method for the GLC analysis of plasma lidocaine is described. Mepivacaine hydrochloride is added as the internal standard; the plasma is deproteinated and centrifuged, and the supernate is alkalinized and extracted into a small volume of carbon disulfide. The column is connected to a flame-ionization detector, and the column oven temperature is programmed from 130 to 260°. Plasma lidocaine concentrations between 0.04 and 8.0 μg/ml can be measured accurately, and there is no interference from the monoethylglycinexylidide or glycinexylidide metabolites of lidocaine or from many commonly used drugs and diagnostic agents. The sensitivity, simplicity, and speed of this assay are important in pharmacokinetic studies of lidocaine.

Keyphrases □ Lidocaine—GLC assay in human plasma □ GLC—analysis, lidocaine in human plasma □ Anesthetics, local—lidocaine, GLC assay in human plasma

Several methods for measuring plasma lidocaine have been described, but many have limited usefulness in pharmacokinetic studies. Some require lengthy extractions (1–5), which reduce the number of samples that can be analyzed in a day. Other methods lack the sensitivity needed for measuring low concentrations of lidocaine (6–8), and one method requires a benzene extraction step with the attendant hazards of benzene exposure (9). Kline and Martin (10) reported a GLC lidocaine assay that avoided complex extractions and provided reasonable sensitivity. However, the method had not been evaluated systematically for interference from other commonly used drugs, and the sensitivity was not adequate for the present study.

This paper reports efforts to improve the assay sensitivity of the Kline and Martin method and to evaluate the method for interference from commonly used medications.

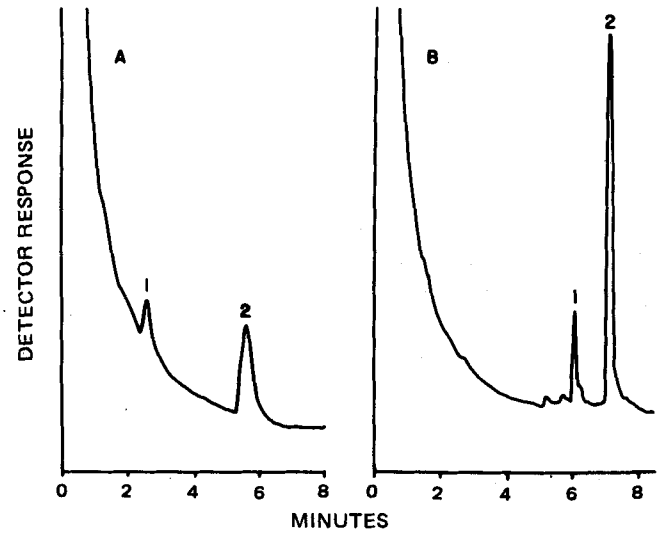

Figure 1—*GLC plasma lidocaine determination using 4 μl of carbon disulfide-extracted plasma containing 325 ng of lidocaine/ml (1) and mepivacaine (2) as the internal standard. Determinations were from the same sample. Key: A, chromatogram using the isothermal method described by Kline and Martin (10); and B, chromatogram using a programmed oven temperature operation from 130 to 260°.*

EXPERIMENTAL

Reagents—Lidocaine hydrochloride monohydrate[1], mepivacaine hydrochloride[2], analytical reagent grade trichloroacetic acid[3], carbon disulfide[3], and sodium hydroxide[3] were used as provided. Lidocaine concentrations are expressed as the free base.

Apparatus—A gas chromatograph[4] equipped with oven temperature programming capability and a flame-ionization detector was fitted with a 1.8-m × 2-mm i.d. glass column, packed with 3% OV-17 on 100–120-mesh Gas Chrom Q. The operating temperatures were 200 and 280° for the injector and detector, respectively. The column oven temperature was programmed for an initial 1-min hold at 130°, and then the oven temperature was raised 24°/min to 260° and held for 4 min. The carrier gas (nitrogen) and hydrogen flow rates were 45 and 30 ml/min, respectively. A 2-mv recorder[5] was used.

Procedure—To a 12-ml glass centrifuge tube were added 1 ml of plasma, 2 ml of mepivacaine hydrochloride (1 μg/ml in water) as the internal standard, and 1 ml of 1.8 *N* trichloroacetic acid; the solution then was mixed gently on a vortex mixer for 10 sec. After the tube was centrifuged at 2200 rpm for 10 min, the supernate was removed and centrifuged for 10 min.

The final supernate was transferred to a clear 13-ml glass-stoppered centrifuge tube, and 1 ml of 5 *N* NaOH and 100 μl of carbon disulfide were added. After vigorous vortexing for 45 sec, the tube was centrifuged for 10 min; then 4 μl of the carbon disulfide layer was injected onto the GLC column.

Interference Study—To test for interference from lidocaine metabolites or commonly used drugs, plasma samples were spiked with one of the following: monoethylglycinexylidide[1] (1 or 5 μg/ml), glycinexylidide[1] (1 or 5 μg/ml), acetaminophen (20 μg/ml), aminophylline (100 μg/ml), aspirin (100 or 200 μg/ml), atropine (0.2 μg/ml), bretylium tosylate (70 μg/ml), chlordiazepoxide (3 μg/ml), cimetidine (5 μg/ml), diazepam (2.5 μg/ml), diazoxide (60 μg/ml), digoxin (2 ng/ml), dobutamine (0.2 or 0.5 μg/ml), dopamine (0.7 μg/ml), ethacrynic acid (20 μg/ml), furosemide (20 μg/ml), heparin (2 units/ml), hydralazine (1 μg/ml), hydrocortisone (200 μg/ml), indocyanine green (10 or 20 μg/ml), isoproterenol (10 ng/ml), meperidine (1 μg/ml), methadone (1 μg/ml), methyldopa (50 μg/ml), methylprednisolone (10 μg/ml), morphine (0.1 μg/ml), nitroglycerin (0.1 μg/ml), nitroprusside (0.2 μg/ml), norepinephrine (10 ng/ml), ouabain (1 ng/ml), pentazocine (0.2 μg/ml), pentobarbital (20 μg/ml), phenytoin (20 μg/ml), physostigmine (0.4 μg/ml), procainamide (6 μg/ml), prochlorperazine (1 μg/ml), propranolol (0.2 or 1.0 μg/ml), quinidine (3.75 or 8.0 μg/ml), trimethaphan (1 μg/ml), or warfarin (10 μg/ml).

RESULTS AND DISCUSSION

Comparison with Published Method—Kline and Martin (10) described a simple, rapid GLC assay for plasma lidocaine that was quite satisfactory for measuring usual therapeutic lidocaine levels. One problem, however, is that the lidocaine peak comes off the column during the rapid decline phase of the solvent front (Fig. 1A). Sensitivity with the Kline–Martin technique is limited to ~250 ng of lidocaine/ml of plasma. To improve sensitivity while retaining the simplicity of the extraction, oven temperature programming was employed. This modification clearly separates the lidocaine and internal standard peaks from the solvent front and improves peak resolution and peak height (Fig. 1). With the oven

[1] Gift from Astra Research Laboratory, Framingham, Mass.
[2] Gift from Sterling-Winthrop Research Institute, Rensselaer, N.Y.
[3] Scientific Products, Rochester, N.Y.
[4] Model 900, Perkin-Elmer Corp., Norwalk, Conn.
[5] Speedomax X/L 680, Leeds & Northrup Co., North Wales, Pa.

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Table I—Lidocaine GLC Assay Precision with Column Oven Temperature Programming

Actual Plasma Lidocaine Concentration, μg/ml	Measured Lidocaine Concentration, μg/ml
4.00	4.01 ± 0.13 (21)[a]
1.62	1.61 ± 0.04 (15)
0.16	0.17 ± 0.01 (15)
0.08	0.08 ± 0.01 (18)
0.04	0.04 ± 0.002 (14)

[a] Mean ± *SE* (*n*).

temperature programming modification, plasma lidocaine concentrations as low as 30 ng/ml can be measured. The retention times for lidocaine and mepivacaine (internal standard) are 6.0 and 7.1 min, respectively.

Calibration Curve—Calibration curves were constructed with the ratio of lidocaine–mepivacaine peak heights plotted along the ordinate and the lidocaine concentration plotted along the abscissa. Initially, curves were developed by spiking blank plasma with 0.03, 0.08, 0.16, 0.40, 0.80, 1.60, 4.0, and 8.0 μg of lidocaine/ml and analyzing the samples as described. Calibration data were fit to a single straight line using a least-squares regression[6] with $r = 0.994$. Despite the apparent fit, the calculated regression line did not coincide adequately with the observed peak height ratios for low lidocaine concentrations. Instead, the calibration curves were described best by two straight lines: one for lidocaine concentrations above 0.8 μg/ml and another for concentrations below 0.8 μg/ml.

The calculated correlation coefficients and equations were $r = 0.994$, ratio = 2.121 [lidocaine concentration (μg/ml)] − 1.04 for the upper range, and $r = 0.999$, ratio = 1.149 [lidocaine concentration (μg/ml)] + 0.05 for the lower range. Curves prepared from aqueous standards (same procedure but without the first two centrifugations) were the same as those using plasma standards; therefore, aqueous standards were used for daily calibration.

Precision—Assay precision was evaluated by analyzing multiple plasma samples spiked with 0.04, 0.08, 0.16, 1.62, or 4.00 μg of lidocaine/ml (Table I). Over this concentration range, assay precision was quite satisfactory.

Interference—Neither the monoethylglycinexylidide or glycinexylidide lidocaine metabolites nor any of the tested drugs produced interfering peaks or alterations in the lidocaine–mepivacaine (internal standard) peak height ratios with this method. Meperidine (1 μg/ml) generated a sharp peak with a peak height almost 75% as large as the internal standard, but the meperidine peak (retention time of 5.1 min) eluted before the lidocaine and mepivacaine peaks and did not interfere with the lidocaine assay.

Conclusions—The GLC lidocaine assay reported by Kline and Martin (10) is a simple and rapid method; however, the sensitivity is not adequate for many pharmacokinetic studies. Incorporating column oven temperature programming improved the assay sensitivity so that plasma lidocaine concentrations as low as 40 ng/ml could be measured readily. The modification retains the simplicity, speed, and sample capacity of the original method.

[6] TI Program ST 1-08, Texas Instruments Inc., Dallas, Tex.

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COMMUNICATIONS

Specific Radioimmunoassay for Flunitrazepam

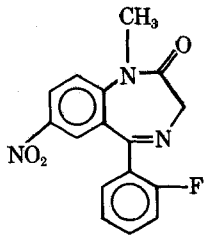
Keyphrases □ Flunitrazepam—quantitation by radioimmunoassay, compared with electron-capture GLC, human plasma □ Radioimmunoassay—quantitation of flunitrazepam, compared with electron-capture GLC, human plasma □ Hypnotic agents—flunitrazepam, quantitation by radioimmunoassay, human plasma

To the Editor:

Flunitrazepam[1] [5-(2-fluorophenyl)-1,3-dihydro-1-methyl-7-nitro-2*H*-1,4-benzodiazepin-2-one, I], a benzodiazepine derivative, is clinically effective as a hypnotic when administered orally in the 0.5–2-mg dose range and may be administered parenterally, usually in combination with other drugs, for the induction of anesthesia (1, 2). Flunitrazepam has been marketed for several years in Europe and is presently under clinical evaluation in the United States.

In humans, I is completely metabolized; apart from intact I, the major metabolites found in plasma are the *N*-desmethyl and 7-amino derivatives (3). However, intact I is primarily responsible for the hypnotic activity of the drug (3) and has a half-life of 20–36 hr on chronic administration (4, 5).

Electron-capture GLC has been the method of choice



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[1] Rohypnol, Hoffmann–La Roche.

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