Simplified GLC Assay for Lidocaine in Plasma

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Abstract □ A simple, rapid GLC method for the determination of lidocaine in plasma is described. After addition of mepivacaine as an internal standard, the plasma sample is deproteinated and centrifuged. The supernate is alkalinized, and the lidocaine and internal standard are extracted into a microvolume of carbon disulfide. By using GLC with a flame-ionization detector, linear concentration-response curves were obtained in the 1-6-μg/ml range. The method can easily determine plasma concentrations at the 250-ng/ml level when a 1-ml plasma sample is analyzed.

Keyphrases □ Lidocaine—GLC analysis in plasma □ GLC—analysis, lidocaine in plasma □ Anesthetics, topical—lidocaine, GLC analysis in plasma

Interest in the analysis of lidocaine in biological media has persisted for over 25 years. The acid-dye methods were subject to interference by metabolites and other endogenous alkaline substances (1, 2) and, therefore, did not give reliable estimates of lidocaine concentrations. Other than for metabolism, disposition, and excretion studies, there has not been a critical need for plasma lidocaine level determinations until its recent application in the control of ventricular arrhythmias by intravenous infusion (3).

GLC has been the analytical method of choice (4–13), but none of the literature procedures met all of this laboratory's requirements. A simple, rapid GLC method using a flame-ionization detector is presented here.

EXPERIMENTAL

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

Apparatus—A gas chromatograph[4] fitted with a 1.8-m (6-ft) × 2-mm (i.d.) glass column, packed with 3% OV-17 on 100–120-mesh Gas Chrom Q, and a flame-ionization detector was used. The column oven was operated at 210° with an optional (see *Results and Discussion*) program involving a 5.0-min initial hold, a 40°/min program to 270°, and a final temperature hold of 2.5 min. The injector temperature was 240°, and the flame-ionization detector temperature was 280°. The carrier gas was nitrogen at a flow rate of 30 ml/min. A 10-mv recorder was used, and the electrometer range was set at 10^{-11} ampere full scale.

Procedure—To a 12-ml glass-stoppered centrifuge tube were added 1 ml of plasma, 2 ml of mepivacaine hydrochloride solution (2 μg/ml in water) as the internal standard, and 1 ml of 1.8 *N* trichloroacetic acid aqueous solution, in that order. The tube was mixed gently for 5–10 sec on a vortex mixer and centrifuged at 2200 rpm. The supernate was transferred to a clean 12-ml centrifuge tube, and 1 ml of 5 *N* NaOH and 100 μl of carbon disulfide were added.

Then the tube was vigorously mixed on a vortex mixer for 45 sec and centrifuged at 2000 rpm, and 3 μl of the carbon disulfide layer was injected into the GLC column. The lidocaine concentration in the plasma sample was determined from a calibration curve of lidocaine/mepivacaine peak height ratio *versus* lidocaine concentration from drug-free plasma or aqueous standard solutions.

[1] Gift from Astra Pharmaceutical Products, Worcester, Mass.
[2] Gift from Sterling-Winthrop Research Institute, Rensselaer, N.Y.
[3] Fisher Scientific Co., Pittsburgh, Pa.
[4] Model 90885, Packard Instrument Co., Downers Grove, Ill.

RESULTS AND DISCUSSION

Evaluation of Published Procedures—Most published GLC procedures initially extracted lidocaine into an organic solvent from alkalinized plasma. At that point, some investigators evaporated the organic solvent and dissolved the residue in a microvolume of injection solvent (5–7). Others performed multiple extractions between organic and aqueous media, presumably to eliminate interferences (8–12). Several methods utilized an alkaline flame-ionization detector to achieve selectivity and sensitivity (7, 11–13).

Initially, the method of Benowitz and Rowland (6) was investigated because of its claimed sensitivity (0.01 μg/ml) but was abandoned because the extraction of lidocaine into ether from an alkaline aqueous medium appeared to be incomplete and, during extraction, ether dissolved enough water to interfere in the GLC determination. Freezing the aqueous layer (9) or drying the ether extract with a desiccant did not eliminate the latter problem. Because of the need for a rapid assay, a procedure involving multiple extractions or an evaporation step should be avoided. After the development of this method, a high-pressure liquid chromatographic (HPLC) procedure for lidocaine (and procainamide) in serum was published (14). It also involved extraction and evaporation and, because of the low UV absorptivity of lidocaine, required a 205-nm UV detector.

Selection of Extraction and Injection Solvent—The use of benzene for direct extraction and injection (13) was abandoned because of its large solvent peak with the flame-ionization detector. Methylene chloride similarly gave a large solvent peak and yielded intractable emulsions during the extraction step. Carbon disulfide is an injection solvent that gives minimal response with the flame-ionization detector and extracts lidocaine from alkaline solution (6). However, initial attempts at extracting alkalinized plasma with carbon disulfide produced emulsions that could not be broken by centrifuging.

Deproteination of the plasma with trichloroacetic acid provided a medium from which the lidocaine could be extracted after alkalinization. It was determined that the lidocaine and mepivacaine were stable in the carbon disulfide-alkalinized plasma mixture at least overnight under refrigeration. Benowitz and Rowland (6) stated that similar samples were stable for 7 days in the carbon disulfide layer.

Development of GLC Conditions—OV-17 was selected as the liquid phase because it already had been used in the GLC analysis of lidocaine (6). After screening numerous compounds, mepivacaine was selected as the internal standard because of its structural similarity to lidocaine (both anilides) and its desirable extraction and chromatographic properties in this procedure.

Figure 1A is a chromatogram of an actual plasma sample containing 500 ng of lidocaine/ml, and Fig. 1B is a chromatogram of drug-free plasma analyzed by this procedure. Under the described conditions, the retention time of lidocaine was 1–2 min and that of mepivacaine was 3–4 min. No interference was observed in the blank plasma scan at these retention times.

The optional column oven temperature program was required in the analysis of some patient samples to prevent a large peak that eluted after the mepivacaine from interfering with subsequent injections. Since this interference was not consistently observed, it was presumed to be another drug and not the plasticizer from the blood collection tube stopper. This substance has been reported to interfere in other plasma GLC analyses (15, 16). No attempt was made to identify this interference since it was readily eliminated by the brief temperature program.

Calibration Curve—Calibration curves were initially constructed by spiking blank plasma with 1, 2, 4, and 6 μg of lidocaine/ml and analyzing these standards by the described procedure. A calibration curve of peak height ratio *versus* lidocaine concentration was linear with a correlation coefficient of 1.000, a slope of 0.778, and a *y*-intercept of 0.004. Calibration curves prepared with water (but eliminating the initial centrifugation since no protein was present) were essentially identical to those prepared with blank plasma. Further confirmation of this result comes from the fact that the mean value for a 2-μg/ml spiked plasma control repetitively analyzed by three different technicians using aqueous

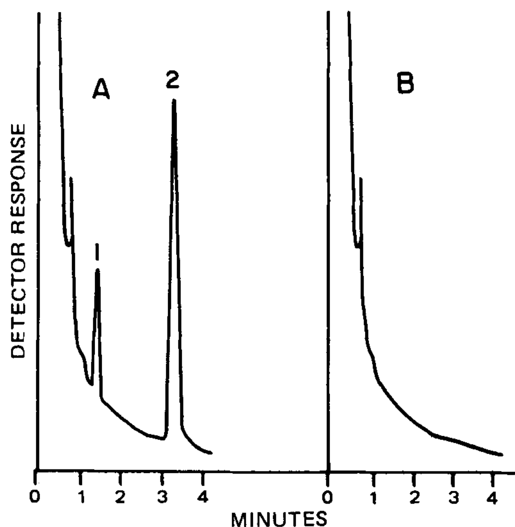

Figure 1—*Gas–liquid chromatograms. Key: A, human plasma sample containing 500 ng of lidocaine/ml (1) and mepivacaine (internal standard) (2); and B, blank plasma.*

calibration curves was 2.0 μg/ml. Since the method is linear, calibration curves were replaced by single-point calibrations. Linearity was confirmed down to 500 ng/ml.

Precision—A synthetic sample was prepared by spiking blank plasma with lidocaine at the 2-μg/ml level. This sample was then analyzed 10 times. The coefficient of variation for the peak height ratio was 5.7%.

Sensitivity—As can be seen from Fig. 1A, 500 ng/ml was easily measurable while 250 ng/ml was readily detectable. By increasing instrumental sensitivity or sample volume, it may be possible to detect even lower levels, but such levels are considerably subtherapeutic (17).

Interferences—Although authentic metabolites of lidocaine were never tested in this procedure, the lidocaine GLC peaks from several patient samples were examined on a gas chromatograph–mass spectrometer. The mass spectra corresponded to that of lidocaine, indicating that no interfering compounds (metabolites or other drugs) were eluting with the lidocaine. During pharmacokinetic profiles of 18 patients, the peak height of mepivacaine remained essentially constant within each run, suggesting that other medications or lidocaine metabolites were not eluting with the internal standard peak. Therefore, it is concluded that the method is specific for lidocaine in the presence of its metabolites.

Extraction Recovery—An aqueous solution of lidocaine hydrochloride containing the lidocaine base equivalent of 2 μg/ml was analyzed in this procedure. The chromatographic peak was compared to that obtained by direct injection of a carbon disulfide solution containing the equivalent concentration of lidocaine base. With the assumption of equal injection volumes, the peak height comparison indicated that at least 94% of the lidocaine was extracted from the alkaline aqueous solution by the microvolume of carbon disulfide.

Conclusions—A method has been described for the GLC analysis of plasma lidocaine levels. It is simple and rapid because it avoids multiple extractions and solvent evaporations and utilizes a flame-ionization detector. The method is linear, precise, accurate, and sensitive. It has been in use for over 1 year in this pharmacokinetics laboratory where it has been applied to the generation of pharmacokinetic profiles on patients who have been administered lidocaine as an endotracheal spray.

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