Analysis of Mepivacaine, Bupivacaine, Etidocaine, Lidocaine, and Tetracaine

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Received August 20, 1979, from the *Department of Drug Metabolism and Disposition, Sterling-Winthrop Research Institute, Rensselaer, NY 12144.* Accepted for publication December 4, 1979.

Abstract □ A GLC method, employing a nitrogen–phosphorus-sensitive detector, is described for the analysis of mepivacaine, bupivacaine, etidocaine, lidocaine, and tetracaine in biological fluids. The method is simple, reliable, and sensitive, with a practical limit of sensitivity of ~2.5 ng/ml, well below therapeutic plasma levels. Extensive start-up procedures and sample preparation are not required.

Keyphrases □ GLC—analysis, mepivacaine, bupivacaine, etidocaine, lidocaine, and tetracaine in biological fluids □ Anesthetics, local—simultaneous GLC analysis of mepivacaine, bupivacaine, etidocaine, lidocaine, and tetracaine in biological fluids □ Mepivacaine—simultaneous GLC analysis with bupivacaine, etidocaine, lidocaine, and tetracaine in biological fluids □ Bupivacaine—simultaneous GLC analysis with mepivacaine, etidocaine, lidocaine, and tetracaine in biological fluids □ Etidocaine—simultaneous GLC analysis with mepivacaine, bupivacaine, lidocaine, and tetracaine in biological fluids □ Lidocaine—simultaneous GLC analysis with mepivacaine, bupivacaine, etidocaine, and tetracaine in biological fluids □ Tetracaine—simultaneous GLC analysis with mepivacaine, bupivacaine, etidocaine, and lidocaine in biological fluids

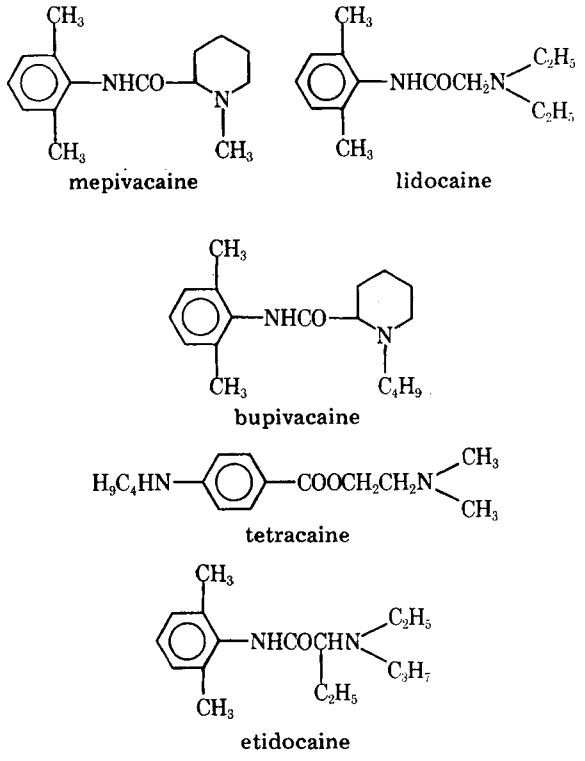
GLC has been the method of choice for the quantitative analysis of basic drugs in biological media for many years. Goehl and Davison (1) reported a method, developed in these laboratories, for the analysis of meperidine by GLC with flame-ionization detection; an improved, general flame-ionization GLC method for basic drugs was described by Mather and Tucker (2). Other methods for basic drugs, based on GLC with various detectors, have been published (3–10). The most sensitive methods utilize GLC–mass spectrometry (11, 12).

This paper describes a GLC method, employing a nitrogen–phosphorus-sensitive detector, that was used to determine mepivacaine, bupivacaine, etidocaine, lidocaine, and tetracaine in various biological fluids including plasma, cerebrospinal fluid, placental blood, and umbilical cord blood. It has proven to be facile, dependable, and equally applicable to a single sample or a large number of samples. Start-up time is minimal, and the sensitivity of the assay is several times greater than that of other reported GLC methods and is comparable to reported GLC–mass spectrometry methods.

EXPERIMENTAL

Reagents—Bupivacaine hydrochloride[1], mepivacaine hydrochloride[2], etidocaine hydrochloride[3], reagent grade ether, methanol, hydrochloric acid, and sodium hydroxide were used as received.

Apparatus—The gas–liquid chromatograph[4] was fitted with a nitrogen–phosphorus detector. Columns (122 and 183 cm) packed with OV-1 or OV-17 on 100–120-mesh Ultrabond[5] or on 100–120-mesh Gas Chrom Q[6] were used, depending on the resolution required. Typical operating parameters were: column temperature, 200 or 250°; injector temperature, 300°; detector temperature, 350°; helium carrier gas flow, 35 ml/min; hydrogen detector gas flow, 5 ml/min; air detector gas flow, 50 ml/min; and detector bead voltage, 20 v.



Procedure—To a 12-ml, round-bottom centrifuge tube fitted with a fluoroplastic-lined screw cap were added 1.0 ml of plasma, 25 μl of the internal standard solution (10 μg/ml in methanol), and 5.0 ml of ether. The tube was shaken on a horizontal shaker for 10 min and centrifuged. After the aqueous layer was frozen in a dry ice–acetone bath, the ether layer was transferred to a clean 12-ml centrifuge tube and treated with 0.2 ml of 1.0 *N* HCl. The tube was shaken, centrifuged, and frozen as before, and the ether phase was discarded.

Following the addition of 0.2 ml of 2.0 *N* NaOH, the aqueous phase was extracted with 5.0 ml of ether and frozen. The ether phase was decanted into a 6-ml, glass-stoppered conical tube and dried at 50° with a gentle stream of dry air. The residue was dissolved in 50 μl of methanol, and 1.0 μl of the solution was analyzed by GLC.

For the analysis of related esters, such as tetracaine, hydrolysis of the ester function must be prevented (13) by: (*a*) utilizing sodium arsenite (50 mg/ml of blood) as an esterase inhibitor, and (*b*) eliminating the back-extraction step.

RESULTS AND DISCUSSION

Figure 1 is a chromatogram, obtained with 3% OV-17 on Ultrabond, of a sample containing equal amounts of etidocaine, bupivacaine, and mepivacaine. Figure 2 shows chromatograms, obtained with 10% OV-1 on Gas Chrom Q, of a blank plasma standard, a 0.2-μg/ml plasma standard, and a clinical plasma sample using bupivacaine as an internal

[1] Marcaine Hydrochloride, Sterling-Winthrop Research Institute, Rensselaer, N.Y.
[2] Carbocaine Hydrochloride, A. B. Bofors, Nobelkrut, Sweden.
[3] Duranest Hydrochloride, Astra Pharmaceutical Products, Worchester, Mass.
[4] Model 5710A, Hewlett-Packard, Palo Alto, Calif.
[5] RFR Corp., Hope, R.I.
[6] Applied Science Laboratories, State College, Pa.

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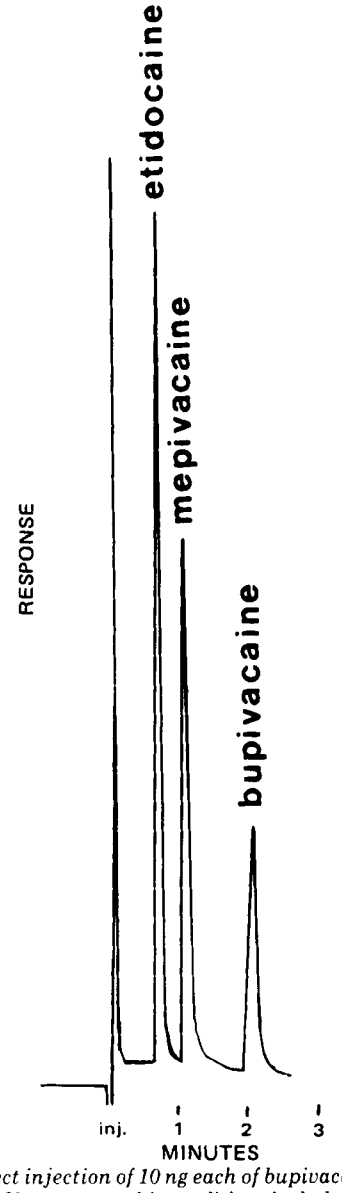

Figure 1—*Direct injection of 10 ng each of bupivacaine, mepivacaine, and etidocaine. Chromatographic conditions included a 122-cm × 2-mm (i.d.) column packed with 3% OV-17 on 100–120-mesh Ultrabond, an injector temperature of 300°, a column temperature of 200°, a detector temperature of 350°, a carrier gas flow rate of 35 ml/min, and detector gases of hydrogen and air at flow rates of 5 and 50 ml/min, respectively.*

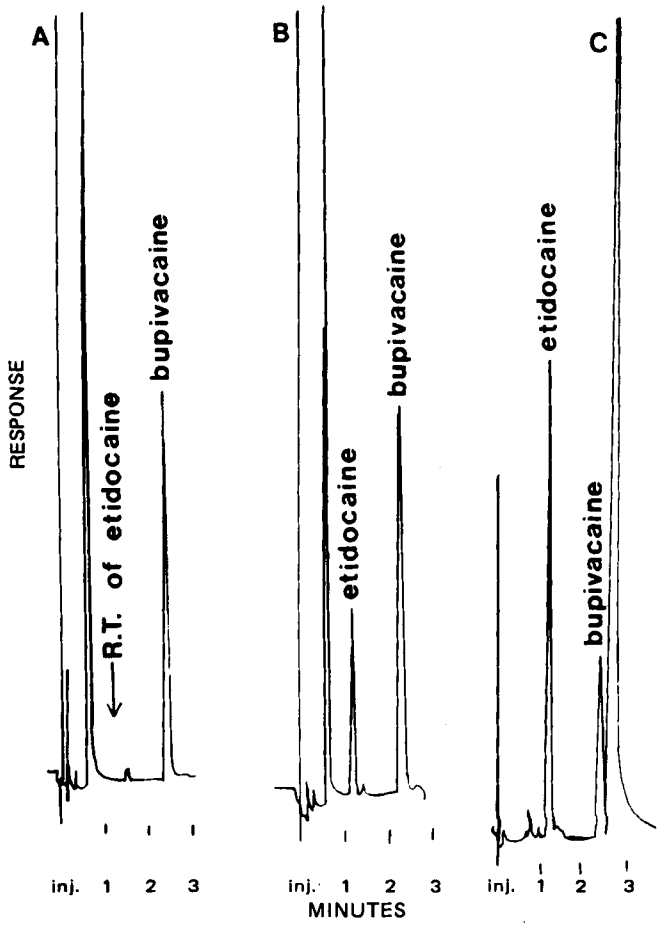

Figure 2—*Analysis of etidocaine extracted from plasma with bupivacaine (250 ng/ml) as the internal standard. Key: A, plasma blank (attenuation 128); B, 200 ng/ml of plasma standard (attenuation 128); and C, clinical sample (attenuation 256). Chromatographic conditions included a 183-cm × 2-mm (i.d.) column packed with 10% OV-1 on 100–120-mesh Gas Chrom Q, an injector temperature of 300°, a column temperature of 250°, a detector temperature of 350°, a carrier gas (helium) flow rate of 35 ml/min, and detector gases of hydrogen and air at flow rates of 5 and 50 ml/min, respectively.*

standard for etidocaine. The resolution obtained from the different columns is more of a function of the efficiency of the column, the carrier gas flow rate, and the temperature than of the stationary phase used. This effect is an added advantage since a particular column is not required, depending on the resolution requirements.

The plasma sample represented in Fig. 2C was one of 72 samples in a study involving anephric patients. The large peak eluting just after bupivacaine in this chromatogram was seen in the plasma of all of the patients studied but was not observed in the standards. Similar peaks, observed in samples collected in tubes with rubber stoppers, were identified by mass spectral analysis as the plasticizer tributoxyethyl phosphate. This phenomenon was reported by previous investigators (14), and it did not interfere with the analysis. By avoiding this plasticizer, the analysis time can be shortened; if high sensitivity is not required, the back-extraction step in sample preparation can be eliminated.

The analytical method works equally well for mepivacaine, bupivacaine, etidocaine, lidocaine, and tetracaine. However, tetracaine is hydrolyzed rapidly by plasma pseudocholinesterase, making its detection after normal doses extremely difficult (13). The minimum quantifiable level, defined as the concentration whose lower 80% confidence limit just encompasses zero[7], was ~20 ng/ml for each of the five drugs when duplicate standards of 0 and 50–500 ng/ml of the drug were used to produce the calibration curve. The minimum quantifiable level is dependent on the concentrations of the standards used, and lower values can be generated by using lower concentration standards. For example, a minimum quantifiable level of 0.8 ng/ml was calculated using duplicate mepivacaine standards of 0 and 2.5–20 ng/ml in plasma with bupivacaine as an internal standard. The practical limit of sensitivity is 2.5 ng/ml, with the size of the solvent front as the limiting factor. The standard error of the assay, calculated from the pure error term of the least-squares fit of the standards for three assays, using standards of 0 and 50–500 ng/ml was ±2 ng/ml.

The described method has proven to be simple, reliable, and sensitive, giving a linear response in the analysis of mepivacaine, bupivacaine, etidocaine, lidocaine, and tetracaine. Any of these compounds can be used as an internal standard for one or all of the others. Except for tetracaine, the sensitivity of the assay is well below that required to determine therapeutic levels and, for specific applications, can be extended to somewhat lower levels. Previously, this sensitivity was possible only through GLC–mass spectrometry. Since extensive start-up procedures and sample pretreatment are not required, the method is equally applicable to a single sample or numerous samples. It has been used to analyze plasma, cerebrospinal fluid, placental blood, and umbilical cord blood for drug concentrations.

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Dependence of Area under the Curve on Proquazone Particle Size and *In Vitro* Dissolution Rate

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Received November 14, 1979, from the *Pharmaceutical Department, Sandoz Ltd., Basel, Switzerland.* Accepted for publication January 11, 1980.

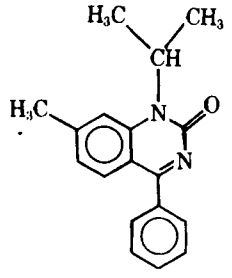
Abstract □ The *in vitro* dissolution and GI absorption of various sieve fractions of proquazone were studied (particle-size ranges of 45–74, 160–300, and 500–1000 μm). The dissolution rates of preparations F45, F160, and F500 were determined *in vitro* in a flow-through assembly in artificial gastric juice at 37°. The time required for 63% of the maximum amount of soluble drug to pass into solution was characterized by the dissolution variable τ_D. The *in vitro* dissolution rates for the preparations differed significantly in the order $\tau_{D,F45} < \tau_{D,F160} < \tau_{D,F500}$. After oral administration of 300 mg of the fractions to each of eight rhesus monkeys, the area under the plasma level–time curve (AUC) differed significantly in the order $AUC_{F45} > AUC_{F160} > AUC_{F500}$. The dissolution rate increased with decreasing particle size. The AUC increased with decreasing particle size and with increasing dissolution rate. These results indicate that the dissolution rate probably determines the extent of absorption when dissolution is rate limiting.

Keyphrases □ Proquazone—effect of particle size on area under the curve □ Dissolution rate, *in vitro*—proquazone, dependence on particle size □ Particle size—proquazone, effect on *in vitro* dissolution rate

After oral administration of solid dosage forms, absorption from the GI tract can be described as a sum of two consecutive transport processes: (*a*) dissolution of the drug from the dosage form (which produces a solution, micelles, or a solubilized entity), characterized by the dissolution rate constant k_1 for dissolution *in vivo;* and (*b*) transport of the drug to and through the intestinal membranes and its penetration into the general circulation, characterized by the total absorption rate constant k_2.

It is possible to distinguish between two fundamentally different cases (1): either the dissolution proceeds more slowly than the absorption ($k_1 < k_2$), or the absorption proceeds more slowly than the dissolution ($k_1 > k_2$). When $k_1 < k_2$, it should be possible to increase the absorption rate by increasing k_1 through a reduction in the particle size (2). When $k_1 > k_2$, a reduction in the particle size cannot affect the absorption rate.

The effect of particle size on relative absorbability has been demonstrated for several drugs, *e.g.*, griseofulvin (3), tetracycline (4), tolbutamide (5), and benoxaprofen (6). There also have been reviews on this subject (7–9). The relationship between particle surface area and GI absorption holds when absorption is dissolution rate limited, *i.e.*, when $k_1 < k_2$. For example, for proquazone, with a saturation solubility of 0.1% in artificial gastric juice, the area under the curve as a function of particle size and as a function of the *in vitro* dissolution rate was investigated. Proquazone[1] (I) is a quinazolidine anti-inflammatory drug.



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EXPERIMENTAL

The experimental preparation was crystallized from ethyl acetate. A sonic sifter[2] followed by an air-jet sieve[3] was used to fractionate the product into the following ranges: 45–74 μm (F45), 160–300 μm (F160), and 500–1000 μm (F500). Care was taken to ensure that the particle-size ranges did not overlap. The experimental fractions were packed by hand in hard gelatin capsules for oral administration. The packing appeared to be very loose when the content was inspected.

The measurement of the dissolution rate was carried out in a flow-through assembly at a rate of 33 ml/min and at 37° (10). The solvent consisted of 0.082 *N* HCl and 0.034 *M* NaCl at pH 1.2. Samples were drawn after 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, and 240 min. The concentration of free drug was determined spectrophotometrically at 232 nm.

Male rhesus monkeys (*Macaca mulatta*), ~3 years old and 8–10 kg, received no food for a period extending from 20 hr before administration to 4 hr after it, but they had free access to water. A 300-mg proquazone

[1] Biarison, Sandoz Ltd.
[2] Allen Bradley, Fisher Scientific Co.
[3] Alpine, Augsburg, West Germany.

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