Anal.—Calc. for $C_{27}H_{54}B_{10}N_2O_4S_2$: C, 50.45; H, 8.47. Found: C, 50.58; H, 8.33.

DL -S-(10 - Dimethylsulfidooctahydrodecaborane)methionine (I)—The oily free acid, VIII (1.4 g, 3 mmoles), was treated with 20 ml of 15% HBr–acetic acid for 1 hr. Ether was added, and the mixture was cooled. A white solid was obtained, which was recrystallized from methanol–ether, yielding 1 g, mp 152° dec. The hydrobromide salt (0.21 g) was suspended in water, and the pH was adjusted to 6 with dilute sodium hydroxide solution. The resulting white solid was insoluble in cold water but soluble in hot water and in methanol. It was ninhydrin positive and palladium chloride positive, and it had an R_f of 0.41 (methanol) and a melting point of 185°; IR (potassium bromide): 3550, 3350, broad (NH_2), 2980, 2900 (CH_2), 2500 (BH), 1600 (CO_2^-), 1500 (CH_2), 1400, 1320, 1040, and 1000 cm^{-1}.

A satisfactory microanalysis could not be obtained due to incomplete combustion and the formation of boron carbide.

***N*-Carbobenzoxy-DL-*S*-(10-dimethylsulfidooctahydrodecaborane)methionylglycine Ethyl Ester (IX)**—The dicyclohexylammonium salt of VIII (330 mg, 0.51 mmole) was converted to the acid by treatment with 2 *N* H_2SO_4 and extraction into ethyl acetate. After drying over magnesium sulfate and evaporation, the residue was dissolved in 10 ml of methylene chloride plus 1 ml of dimethylformamide and cooled to 0°. Glycine ethyl ester hydrochloride (140 mg, 1 mmole) was added along with 0.14 ml (1 mmole) of triethylamine. Dicyclohexylcarbodiimide (123 mg, 0.6 mmole) was then introduced. After the mixture was stirred overnight, it was filtered, evaporated, and partitioned between ethyl acetate and water.

The organic layer was washed sequentially with 2 *N* H_2SO_4, sodium bicarbonate solution, and saline. This layer was then dried over magnesium sulfate and evaporated. The residue was purified by preparatory TLC to give 50.2 mg of recovered acid VIII (R_f 0.57, methanol) and 89.9 mg of the dipeptide (R_f 0.25, 20% ethyl acetate–chloroform) as a noncrystalline foam, mp 81–83° (begins to change structure at 65°); IR (chloroform): 3400 (NH), 3030, 2930 (CH), 2500 (BH), 1720 (CH_2OC=ONH), 1705 (ester), 1670 (amide), and 1200 (ester) cm^{-1}; NMR (deuterochloroform): δ 7.3 (5H, s, C_6H_5), 5.5–6.0 (2H, m, 2NH), 3.9–4.3 (7H, m, $NHCH(CH_2CH_2$—$CONHCH_2COCH_2CH_3)$, and 3.0 (9H, d, 3SCH), and 1.0–1.5 (5H, m, CH_2CH_3 and $CHCH_2CH_2$).

Anal.—Calc. for $C_{19}H_{38}B_{10}N_2O_5S_2$: C, 41.75; H, 7.01; S, 11.71. Found: C, 42.03; H, 7.24; S, 11.91.

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[x] To whom inquiries should be directed.

High-Speed Liquid Chromatographic Determination of Procaine in Pharmaceuticals

S. K. WAHBA KHALIL [x] and W. H. SHELVER

Abstract □ The operating conditions for a quantitative method of determining procaine in pharmaceutical preparations by high-speed liquid chromatography are described. The presence of decomposition products and the possible interference of other ingredients usually present in pharmaceutical preparations were found to have no effect. The method, because of its simplicity, is highly suited for routine analysis of pharmaceutical preparations containing procaine.

Keyphrases □ Procaine—high-speed liquid chromatographic analysis, pharmaceutical preparations □ High-speed liquid chromatography—analysis, procaine, pharmaceutical preparations

Several methods for the quantitative determination of procaine and its hydrochloride in pharmaceutical preparations are available. The USP XVIII (1) monographs for procaine hydrochloride and sterile procaine hydrochloride incorporate a method based on titration with standard sodium nitrite solution. This analytical scheme is satisfactory for the drug substance or its solution, but other substances containing a primary amine would interfere with the analysis. The assay for procaine hydrochloride in procaine hydrochloride injection requires an extraction procedure, followed by spectrophotometric determination (2). The USP does not recommend any method of assay for the procaine portion in penicillin G procaine, although a method is recommended for the penicillin moiety (3).

Several colorimetric (4–6), spectrophotometric (7), and acidimetric (8) methods have been developed for the determination of procaine. These methods show high sensitivity but do not have the specificity and simplicity that would be anticipated with a chromatographic procedure. In addition, since these procedures often require separation of the procaine from the interfering substances, they are quite time consuming.

Table I—Calibration Data for HSLC Procaine Analysis[a]

Concentration Added, μg/ml	Peak Area of Procaine / Peak Area of Standard	SD	CV
25[b]	0.318	0.0085	2.7
50[c]	0.672	0.0164	2.4
125[c]	1.608	0.0783	4.9

[a] Regression analysis gave the following equation: concentration = 0.927 + 78.05 (area of procaine/area of standard). [b] Average of nine replicate injections. [c] Average of five replicate injections.

This report describes a high-speed liquid chromatographic (HSLC) method. The method permits measurement of procaine in the presence of its degradation products and other chemicals commonly included with procaine in pharmaceutical products. This procedure was applied to several commercial preparations containing procaine[1].

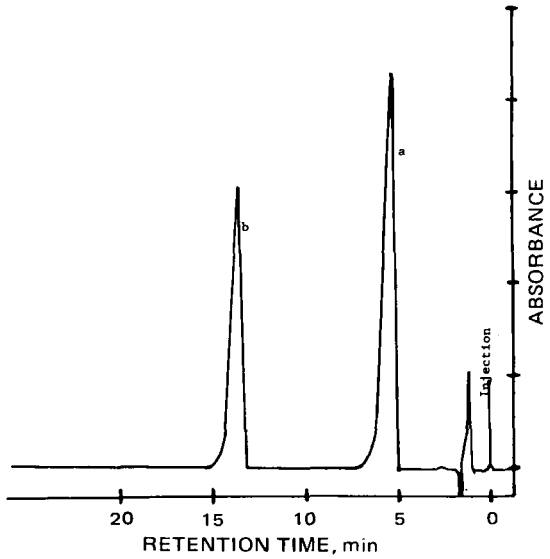

Figure 1—*Representative chromatogram. Key: a, pyrrocaine; and b, procaine.*

EXPERIMENTAL[2]

Reagents and Chemicals—Analytical grade procaine hydrochloride[3] and pyrrocaine hydrochloride[4] were used.

Mobile Phase—The mobile phase, consisting of 60% acetonitrile, 40% water, and 0.01% concentrated ammonium hydroxide solution (28%) was prepared daily. The mobile phase was degassed by applying a water aspirator vacuum to the solution for 10 min.

Internal Standard Solution—An aqueous solution of pyrrocaine hydrochloride, 1 mg/ml (calculated on the free base weight), was used.

Standard Procaine Hydrochloride Solution—A 5-mg/100 ml (calculated on the free base weight) procaine hydrochloride aqueous solution was used.

Conditions for Chromatographic Analysis—The degassed mobile phase was pumped through the column at a flow rate of 3 ml/min (1000–1500 psi) at room temperature until a stable baseline was obtained. Replicate 20-μl injections of a 1:1 mixture of the procaine hydrochloride and the pyrrocaine hydrochloride solutions were made using a 25-μl syringe[5]. The chromatogram was recorded[6] with a chart speed of 0.51 cm (0.2 in.)/min with the output from the UV monitor at 0.04 absorbance unit fed into the electronic integrator. The peak areas were measured using an electronic integrator.

Interferences—The possible interferences from *p*-aminobenzoic acid, diethylaminoethanol, and epinephrine were studied by adding different amounts to a standard procaine hydrochloride solution and performing the analysis.

Sample Preparation and Assay—For liquid preparations, measure an aliquot of the procaine preparation containing 10 mg of procaine, as the free base, into a 100-ml volumetric flask and dilute to volume with water. In case of penicillin procaine, weigh accurately about 10 mg of the powder, dissolve in water, and dilute to 100 ml with water. Mix an equal volume of the test solution with standard pyrrocaine solution, and inject 10 μl of the mixture. Calculate the amount of procaine by comparing with a standard.

Table II—Comparison between HSLC Method and Other Methods

Preparation	Labeled Amount, mg/ml	Recovery, % HSLC Mean	HSLC n[a]	HSLC CV	Other Methods Mean	Other Methods n	Other Methods CV
Procaine hydrochloride[b], 2-ml ampul	100	99.6	6	1.8	101.4[c]	3	0.8
Procaine hydrochloride[b], 2-ml ampul	1000	100.0	4	0.9	100.2[c]	2	0.2
Procaine hydrochloride[b] and adrenalin	100	100.0	4	0.4	100.6[d]	2	0.1
Penicillin G procaine	40	99.7	5	2.3	100.3[d]	2	0.1

[a] *n* = number of replicate determinations. [b] Novocain. [c] Volumetric determination using sodium nitrite. [d] Colorimetric determination using sodium nitrite and α-naphthylethylamine.

RESULTS AND DISCUSSION

The separation of amines by partitioning on-column requires suppression of the ionization of the amino group to obtain sharp peaks. The addition of a very small amount of ammonium hydroxide solution to the mobile phase gave excellent results and avoided the addition of any solid, such as ammonium carbonate, which could interfere with the operation of the chromatographic system by blocking the column, injection system, or detector. Utilization of a mobile phase consisting of 60% acetonitrile and 40% water modified with 0.01% ammonium hydroxide gave well-resolved sharp peaks for pyrrocaine and procaine with retention times of 5.6 and 14.3 min, respectively (Fig. 1).

Under these conditions, *p*-aminobenzoic acid, diethylaminoethanol, and epinephrine were eluted with the solvent front and thus did not interfere with the analysis. The output of the UV detector

[1] Obtained from the College of Pharmacy preparations laboratory.

[2] A Waters Associates liquid chromatograph (model 202) equipped with an M-6000 pump, a single-wavelength UV monitor (254 nm), a U6K Universal liquid chromatograph injector, an integrator (Hewlett Packard model 3370B) with digital printout, and a column containing octadecylsilane bonded to μCorasil (*μBondapak C 18*), 300 mm long × 4 mm i.d., obtained from Waters Associates, were used.

[3] Merck.

[4] Lot 64 C 025, Endo Laboratories.

[5] Precision Sampling Corp., Baton Rouge, La.

[6] Beckman 25.4-cm (10-in.) laboratory recorder model 1005.

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.