the factors that influence the disposition and response to acetazolamide are in progress.

REFERENCES

(1) B. Lehmann, E. Linner, and P. J. Wistrand, in "Schering Workshop in Pharmacokinetics," G. Raspe, Ed., Pergamon, Oxford, 1969, p. 197.

(2) B. R. Friedland, J. Mallonee, and D. R. Anderson, *Arch. Ophthalmol.*, **95**, 1809 (1977).

(3) F. G. Berson, D. L. Epstein, W. M. Grant, T. Hutchinson, and P. C. Dobbs, *Arch Ophthalmol.*, **98**, 1051 (1980).

(4) M. Inui, H. Azuma, T. Nishimura, and N. Hatada, in "Advances in Epileptology: XIIIth Epilepsy International Symposium," H. Akimoto, H. Kazamatsuri, M. Seino, and A. Ward, Eds., Raven, New York, N.Y., 1982, p. 307.

(5) D. M. Woodbury and J. W. Kemp, in "Antiepileptic Drugs," D. M. Woodbury, J. K Penry, and C. E. Pippenger, Eds., Raven Press, New York, N.Y., 1982, p. 771.

(6) J. B. West, "Respiratory Physiology," Williams and Wilkins Co., Baltimore, Md., 1979, p. 74.

(7) W. F. Bayne, G. Rogers, and N. Crisologo, *J. Pharm. Sci.*, **64**, 402 (1975).

(8) R. D. Hossie, N. Mouseau, S. Sved, and R. Brien, *J. Pharm. Sci.*, **69**, 348 (1980).

(9) D. M. Chambers, M. H. White, and H. B. Kostenbauder, *J. Chromatogr.*, **225**, 231 (1981).

(10) T. H. Maren, *J. Pharmacol. Exp. Ther.*, **130**, 26 (1960).

(11) G. J. Yakatan, C. A. Martin, and R. V. Smith, *Anal. Chim. Acta*, **84**, 173 (1976).

(12) S. M. Wallace, V. P. Shah, and S. Riegelman, *J. Pharm. Sci.*, **66**, 527 (1977).

(13) T. H. Maren, J. R. Haywood, S. K. Chapman, and T. J. Zimmerman, *Invest. Ophthalmol. Vis. Sci.*, **16**, 730 (1977).

Amperometric Determination of Hydralazine Hydrochloride in a Flowing Stream at the Glassy Carbon Electrode

MUMTAZ H. SHAH and JAMES T. STEWART ˣ

Received March 11, 1983, from the *Department of Medicinal Chemistry, College of Pharmacy, The University of Georgia, Athens, GA 30602.* Accepted for publication May 25, 1983.

Abstract □ A flow-injection method for the determination of hydralazine hydrochloride based on electrochemical oxidation at the glassy carbon electrode is presented. The amperometric method is highly specific and may be used to determine hydralazine hydrochloride in the presence of other drugs commonly found in its pharmaceutical dosage forms or administered concurrently in therapeutic situations. By using an electrode potential of +650 mV *versus* an Ag/AgCl reference electrode, a calibration curve was found to be linear in the 1–50-μg/mL concentration range, with minimum detectability at 10 ng (signal-to-noise ratio, 2). When the method was applied to the analysis of hydralazine hydrochloride in selected pharmaceutical dosage forms, it showed good accuracy and precision. Although automation was not used in this study, the method could readily be incorporated in automated systems because it employs the technique of continuous analysis in a flowing stream.

Keyphrases □ Hydralazine hydrochloride—amperometric determination in a flowing stream, glassy carbon electrode □ Flow-injection method—amperometric determination, hydralazine hydrochloride, glassy carbon electrode □ Electrochemical oxidation—glassy carbon electrode, flow-injection method, determination of hydralazine hydrochloride

Hydralazine hydrochloride, a widely prescribed antihypertensive agent, has been analyzed by diverse methodologies, including titrimetry (1, 2), spectrophotometry (3, 4), fluorometry (5), GC, and HPLC (6–10). Interest in this laboratory in the development of new continuous assay methods for drugs in flowing streams led us to investigate the oxidation of hydralazine at the glassy carbon electrode. There is almost no information in the literature concerning the electrochemistry of hydralazine. One report has indicated that the drug can be reduced at the dropping mercury electrode to give two half-wave potentials of −700 and −950 mV *versus* the standard calomel electrode (11). The reduction occurs in a solution containing 1 M HCl as a supporting electrolyte and gelatin as a surfactant. The report did not comment on the sensitivity, accuracy, precision, and specificity of the method. There appears to be no data in the literature on the electrochemical oxidation of the drug.

This laboratory has reported previously on continuous analysis in flowing streams by oxidation of drugs such as ascorbic acid and methyldopa at the tubular carbon electrode (12, 13). The glassy carbon electrode has supplanted the tubular carbon electrode and has shown general usefulness as a sensitive tool for the determination of oxidizable drugs in flowing stream systems such as HPLC (14–16). These kinds of electrodes can be easily incorporated into automated or semiautomated systems such as would be used in dosage form analysis.

In this report, amperometric determination of hydralazine hydrochloride in a flowing stream utilizing oxidation at the glassy carbon electrode is reported. The flow-injection method

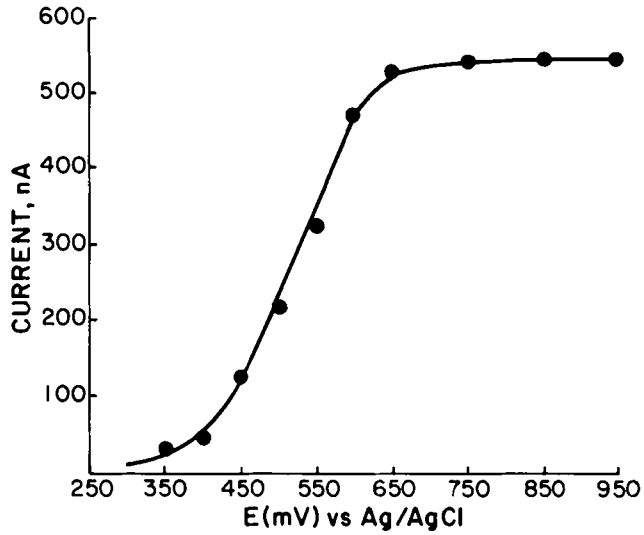

Figure 1—*Hydrodynamic voltammogram of hydralazine hydrochloride (50 μg/mL) in a 40:60 mixture of Walpole acetate buffer (pH 4.2)-absolute methanol at a flow rate of 1 mL/min.*

0022-3549/84/0700-0989$01.00/0

Table I—Percent Recovery of Hydralazine Hydrochloride in Synthetic Drug Mixtures

Drug	Concentration, μg/mL[a]			
	8	16	32	100
Hydrochlorothiazide	99.79 ± 1.39	99.19 ± 1.25	99.19 ± 0.92	85.91 ± 1.52
Chlorothiazide	99.59 ± 0.60	99.39 ± 0.92	99.19 ± 0.92	90.52 ± 1.19
Reserpine	99.39 ± 0.92	99.38 ± 0.61	99.79 ± 1.39	78.96 ± 1.21
Propranolol hydrochloride	99.39 ± 0.92	99.19 ± 1.25	100.19 ± 0.61	99.99 ± 0.92
Triamterene	99.39 ± 0.92	99.99 ± 0.92	99.59 ± 1.21	99.19 ± 0.35
Methyldopa	99.59 ± 0.60	100.19 ± 0.61	99.99 ± 0.92	99.59 ± 1.21
Guanethidine monosulfate	99.39 ± 0.92	99.79 ± 1.39	99.19 ± 0.92	99.19 ± 0.35
Chlorthalidone	100.40 ± 0.35	100.60 ± 0.60	100.20 ± 0.35	100.20 ± 0.35

[a] Concentration of each drug in mixtures that also contained 16 μg/mL of hydralazine hydrochloride. Values represent mean percent recovery ± *SD* of hydralazine hydrochloride in the drug mixture. The data were based on quadruplicate determinations of each mixture.

detects the drug in the 1-50-μg/mL range with good accuracy, precision, and specificity. The procedure was shown to be applicable to the analysis of hydralazine hydrochloride in pharmaceutical dosage forms.

EXPERIMENTAL SECTION

Apparatus—Voltammograms and cyclic voltammetry measurements were made with a conventional three-electrode system[1] consisting of a glassy carbon working electrode, a platinum auxiliary electrode, and a silver-silver chloride reference electrode. A wall-jet-type methyl methacrylate electrochemical cell[2] and associated electronics[2] were used for the flowing stream analysis. The cell contained a glassy carbon working electrode, a silver-silver chloride reference electrode, and a second glassy carbon auxiliary electrode. The mobile phase was pumped through the cell at a fixed flow rate with a reciprocating pump[3]. Samples were manually injected into the sample injection system[4] with a microsyringe[5]. The pump, injector, and electrochemical cell were connected with standard HPLC stainless steel tubing (0.02 cm) and fittings. Cell currents were recorded at ambient temperature with a strip-chart recorder[6].

Chemicals—Hydralazine hydrochloride[7] powder was obtained for use in the analytical study. All other chemicals were commercially available and were used as received. A stock solution containing 0.1 mg/mL of hydralazine hydrochloride was prepared in Walpole acetate buffer, pH 4.2 (17). Further dilutions of the solution were made to provide working standards in the 1-50-μg/mL range.

Procedures—A 40:60 mixture of Walpole acetate buffer (pH 4.2)-absolute methanol was pumped through the electrochemical cell[2] at a flow rate of 1.0 mL/min. Aliquots (50 μL) of hydralazine hydrochloride solutions in acetate buffer were injected into the flowing stream, and the current was measured with an applied potential of +650 mV *versus* an Ag/AgCl reference electrode.

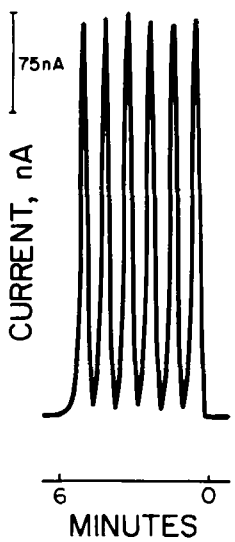

Figure 2—*Replicate injections of hydralazine hydrochloride (16 μg/mL) into the flowing stream system at a rate of 60 samples/h.*

To determine whether other antihypertensive drugs or compounds commonly found in dosage forms with hydralazine hydrochloride interfered with the assay by altering the current flow of the drug or were oxidized at the glassy carbon electrode, the following studies were performed. Individual solutions (0.1 mg/mL) of guanethidine monosulfate, reserpine, hydrochlorothiazide, chlorothiazide, triamterene, methyldopa, propranolol hydrochloride, and chlorthalidone were prepared in acetate buffer (pH 4.2)-methanol (40:60). Accurately pipetted aliquots of these solutions were then used to prepare various mixtures containing the individual drugs at the 8-100-μg/mL range, with the hydralazine concentration maintained at 16 μg/mL. Each mixture was then assayed for hydralazine content. The data obtained from each mixture were then compared with those of a pure solution of hydralazine hydrochloride to calculate the degree of interference, if any, at the various concentration levels of the added drugs.

Analysis of solid dosage form—Tablets and/or capsules containing hydralazine hydrochloride were sonicated in Walpole acetate buffer (pH 4.2). The resulting solution was then filtered, if necessary, and diluted to the 1-50-μg/mL range. An aliquot of the diluted solution was then assayed for hydralazine hydrochloride as described above.

RESULTS AND DISCUSSION

Figure 1 shows a hydrodynamic voltammogram of hydralazine hydrochloride (50 μg/mL) when subjected to electrochemical oxidation at the glassy carbon electrode in acetate buffer (pH 4.2)-methanol (40:60). A half-wave potential of +510 mV was observed. A potential of +650 mV was selected for the amperometric determination since it represented the point on the wave at which maximum drug sensitivity could be obtained.

By using the optimum electrode potential of +650 mV, a hydralazine hydrochloride calibration curve was obtained in the 1-50-μg/mL range. Linear regression analysis of the calibration data gave typical slope, intercept, and r values of 19.82, 8.79, and 0.9990 (n = 18), respectively.

To estimate the reproducibility of the electrode response by the amperometric method, eight replicate injections at hydralazine hydrochloride concentrations of 4-, 16-, and 40-μg/mL were run. Mean peak currents of 51 ± 1.15, 165.63 ± 1.25, and 367.50 ± 1.25 nA, respectively, were obtained. The

Table II—Analysis of Hydralazine Hydrochloride in Pharmaceutical Dosage Forms

Product Components	Hydralazine Declared/Sample, mg	Hydralazine Found/Sample, mg[a]	Recovery, %
Hydralazine hydrochloride	25	25.55 ± 0.25	102.10
Hydralazine hydrochloride Hydrochlorothiazide	25	24.98 ± 0.10	99.90
Hydralazine hydrochloride Reserpine	50	50.85 ± 0.52	101.70
Hydralazine hydrochloride Hydrochlorothiazide Reserpine	25	24.90 ± 0.42	99.35

[a] Values represent mean ± *SD* based on quadruplicate determinations of each sample.

[1] Model 174 A polarograph; Princeton Applied Research.
[2] Model EA-1096 plexiglas cell with Model E-611 detector; Metrohm.
[3] Model M-6000A Pump; Waters Associates, Milford, Mass.
[4] Model U6K Injector; Waters Associates, Milford, Mass.
[5] 50-μL syringe; Hamilton Co., Reno, Nev.
[6] Houston Omniscribe Recorder.
[7] CIBA-GEIGY Corp., Summit, N.J.

precision of these measurements is expressed by relative standard deviations of 2.30, 0.75, and 0.34% for the 4-, 16-, and 40-μg/mL levels, respectively.

Interference studies were performed to establish the specificity of the method for hydralazine hydrochloride in the presence of other drugs that might be found in its combination dosage forms. There was little or no interference from these miscellaneous compounds, except for hydrochlorothiazide, chlorothiazide, and reserpine, in which appreciable interference was noted when their concentrations were six- to sevenfold greater than the hydralazine level (Table I).

Applications of the method to the assay of hydralazine hydrochloride in commercial dosage forms was then studied. After sample preparation and dilution to the 1–50-μg/mL calibration range, the drug solutions were analyzed, and the concentration in each dosage form was calculated using the slope and intercept values generated from linear regression analysis of the hydralazine calibration data. The results of the assay shown in Table II indicate that hydralazine content in dosage forms can be conveniently determined by the amperometric method described herein with good accuracy and precision.

By using the parameters established for the assay, hydralazine samples can be injected into the flowing stream system at the rate of 60 samples/h (Fig. 2). The sensitivity of the assay, based on a signal-to-noise ratio of two, is 10 ng of drug.

REFERENCES

(1) "U.S. Pharmacopeia," 20th Rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 377.

(2) R. Soliman and S. A. Belal, *J. Drug Res.*, **6**, 7 (1974).

(3) J. T. Stewart and Y. Chang, *J. Assoc. Off. Anal. Chem.*, **62**, 1107 (1979).

(4) S. B. Zak, M. F. Bartlett, W. E. Wagner, J. T. Gilleran, and G. Lukas, *J. Pharm. Sci.*, **63**, 225 (1974).

(5) D. V. Naik, B. R. Davis, K. M. Minnet, and S. G. Schulman, *J. Pharm. Sci.*, **65**, 274 (1976).

(6) K. D. Haegele, H. B. Skrdlant, N. W. Robie, D. Lalka, and J. L. McNay, *J. Chromatogr.*, **126**, 517 (1976).

(7) K. M. Smith, R. N. Johnson, and B. T. Kho, *J. Chromatogr.*, **137**, 431 (1977).

(8) D. B. Jack, S. Brechbuhler, P. H. Degen, Z. Zbinden, and W. Riess, *J. Chromatogr.*, **115**, 87 (1975).

(9) W. J. Proveaux, J. P. O'Donnell, and J. K. H. Ma, *J. Chromatogr.*, **176**, 480 (1979).

(10) T. M. Ludden, L. K. Goggin, J. L. McNay, R. D. Haegele, and A. M. M. Shepherd, *J. Pharm. Sci.*, **68**, 1423 (1979).

(11) Z. Modras, *Chem. Anal.*, **17**, 1349 (1973); through *Chem. Abstr.*, **78**, 128457g (1973).

(12) W. D. Mason, T. D. Gardner, and J. T. Stewart, *J. Pharm. Sci.*, **61**, 1301 (1972).

(13) J. T. Stewart, H. C. Loo, and W. D. Mason, *J. Pharm. Sci.*, **63**, 954 (1974).

(14) R. E. Shoup (Ed.), "Recent Reports on Liquid Chromatography with Electrochemical Detection," BAS Press, West Lafayette, Ind., 1981.

(15) D. D. Koch and P. T. Kissinger, *J. Chromatogr. Biomed. Appl.*, **164**, 441 (1979).

(16) L. A. Pachla and P. T. Kissinger, *Anal. Chem.*, **48**, 364 (1976).

(17) M. Brezina and P. Zuman, "Polarography in Medicine, Biochemistry and Pharmacy," Interscience, New York, N.Y., 1958, p. 731.

Rapid Gas Chromatographic Assay for Monitoring Valproic Acid and Valpromide in Plasma

MEIR BIALER ˣ, MICHAEL FRIEDMAN, and ABRAHAM RUBINSTEIN

Received July 1, 1982, from the *Department of Pharmacy, School of Pharmacy, Hebrew University, P.O. Box 12065, Jerusalem 91120, Israel.* Accepted for publication May 23, 1983.

Abstract □ A gas chromatographic (GC) method for monitoring valproic acid and valpromide in plasma was developed. The procedure involved a single solvent extraction of drugs from acidified plasma samples, followed by a GC injection of the organic phase. This rapid, sensitive, specific, and reproducible method is a key factor in pharmacokinetic and stability studies of valpromide. Pharmacokinetic application of the new GC method is presented by a simultaneous plasma monitoring of valpromide and valproic acid levels obtained after intravenous administration of valpromide to a dog.

Keyphrases □ Valproic acid—GC, plasma, valpromide □ Valpromide—GC, plasma, valproic acid

Valpromide (2-propylvaleramide), commonly used in clinical practice as an antiepileptic and antipsychotic drug (1–6), is a primary amide of the more widely known valproic acid, which is used to treat different types of epilepsy, particularly petit mal epilepsy (7, 8). Recent reports claimed that valpromide is biotransformed to valproic acid before reaching the systemic circulation. Only traces of valproic acid have been observed in patients who were on chronic oral treatment of valpromide (1, 4, 5); after treatment with valpromide (600 mg t.i.d.) to 42 epileptic patients, the therapeutic plasma concentrations of valproic acid ranged from 50 to 60 μg/mL with minimal fluctuations in plasma level (4). This is within the therapeutic range of valproic acid (8). After single oral administration of valpromide (600 mg) to humans, the plasma levels of valproic acid ranged from 7 to 20 μg/mL for >30 h after the valpromide administration (4, 5).

During the last decade various methods for analyzing valproic acid in biological fluids have been published (9, 10). Recently a gas chromatographic (GC) method for monitoring valpromide in plasma was reported (11). The aim of this work was to develop a rapid GC method for a routine valproic acid and valpromide assay in plasma. This assay is fundamental in pharmacokinetic and stability studies of valpromide, since valpromide may serve as a prodrug for valproic acid (1, 2).

EXPERIMENTAL SECTION

Reagents—Organic stock solutions of valproic acid[1] and valpromide[2] were prepared by dissolving the drugs separately in chloroform. Aqueous stock solutions were prepared by dissolving sodium valproate[1] and valpromide separately in water. The concentration of all stock solutions was 1 mg/mL. Caprylic acid[2] was used as an internal standard and was dissolved in chloroform at a concentration of 1 mg/mL. Stock solutions were stored at 4°C.

Apparatus—The gas chromatograph[3] was equipped with a flame-ionization detector and a recorder[4]. The glass column, 180 cm × 2 mm i.d., was packed with 5% free fatty acid phase[5] on 80–100 mesh Chrom Q. Flow rates were as follows: hydrogen, 40 mL/min; air, 400 mL/min; carrier gas (nitrogen), 40 mL/min. The system temperatures were: column, 175°C; injector, 180°C; detector, 220°C.

Extraction Procedure—To 1.0 mL of plasma spiked with the appropriate aliquots of valproic acid and valpromide aqueous solutions were added 480

[1] Labaz, Paris, France.
[2] BDH, Poole, England.
[3] Model 7421; Packard, Downers Grove, Ill.
[4] Unicorder 225; Panto, Kyoto, Japan.
[5] Applied Science Labs., State College, Pa.

0022-3549/84/0700-0991$01.00/0