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

Table I—Recovery and Reproducibility of Valproic Acid and Valpromide in Human Plasma

Conc., μg/mL	Valproic Acid			Valpromide		
	Recovery[a], %	*SD*	*CV*, %	Recovery[a], %	*SD*	*CV*, %
5	104.25	7.18	6.89	104.14	5.70	5.42
10	100.32	5.58	5.57	102.09	5.37	5.26
20	104.49	6.46	6.19	100.54	7.48	7.44
30	102.73	7.29	8.49	99.43	8.56	8.61
40	103.45	7.89	7.63	98.85	7.98	8.07
50	104.19	6.69	6.42	99.76	8.59	8.62
60	104.21	6.85	6.57	99.81	7.64	7.66

[a] Mean of 10 determinations.

μL of chloroform, 20 μL of internal standard solution, and 0.5 mL of 1 M HCl. The sample was vortexed for 15 s, shaken for 15 min, and centrifuged at 4000 rpm for 15 min. Three microliters of the organic phase was injected into the gas chromatograph.

To determine the precision of the assay, 12 mL of human plasma was spiked with appropriate aliquots of the aqueous valproic acid and valpromide stock solutions and was stored at −20°C during the 3-month study. On different days 1-mL aliquots were taken from the various stored samples and analyzed against a fresh calibration curve made according to the extraction procedure on the same day.

RESULTS AND DISCUSSION

Typical chromatograms of extracts from plasma with reference to drug-free plasma samples is presented in Fig. 1. Under the assay conditions the following retention times were obtained: valproic acid, 0.96 min; internal standard, 1.8 min; and valpromide, 2.23 min. There was no interference from endogeneous plasma components.

Calibration curves from plasma extracts showed a linear correlation between peak height ratio (y) of valpromide or valproic acid against the internal standard and plasma concentration of the drugs (x). The linear calibration equation was $y = 0.0968x - 0.044$ ($r = 0.997$) for valproic acid and $y = 0.0713x + 0.0018$ ($r = 0.997$) for valpromide. A least-squares linear regression method was used to calculate these curves. The minimal detectable concentration was ~1-2 μg/mL in plasma.

Analytical recoveries of substances were established as follows. Various amounts (5, 10, 20, 30, 40, 50, and 60 μg taken from the aqueous stock solutions) of valproic acid and valpromide were dissolved in 1 mL of drug-free plasma. After acidification, plasma samples were extracted into 0.5 mL of chloroform which contained 20 μL of the internal standard solution. A series of external standards were prepared by adding 20 μL of the internal standard solution to 0.48 mL of chloroform which contained the various amounts taken from the organic stock solution (5, 10, 20, 30, 40, 50, and 60 μg) of valproic acid and valpromide. Recoveries were calculated by comparing peak height ratios of the extracted standard to the ratios of the external standards (Table I). The standard deviation of the analytical recoveries can serve as a good estimate for reproducibility.

Precision or accuracy of the assay was determined by performing 10 replicate analyses of five control samples containing 20, 30, 40, 50, and 60 μg/mL of both drugs on different days over a 3-month period (Table II). The recovery percentage of valpromide and valproic acid, as presented in Table I, does not have a statistically significant difference from 100% ($p > 0.05$). The observed values of the various valpromide and valproic acid concentrations (Table II) were not different statistically from the added concentrations ($p > 0.05$) (12).

In a preliminary pharmacokinetic study, valpromide (400 mg) was administered intravenously to a mongrel dog (20 kg). This 20-mg/kg dose was of the same order of magnitude as the therapeutic dose in humans, which is 900-1800 mg (13-26 mg/kg) in divided doses (6). The plasma levels of valpromide and valproic acid obtained in this study are presented in Fig. 2 (mean ± *SD* of three replicates). The curves presented in Fig. 2 were obtained after a computer fitting of the observed data using the NONLIN computer program (13). Valpromide was partly biotransformed to valproic acid, and the plasma levels of these two compounds were assayed by the proposed method throughout the duration of the 12-h study. The *CV* among the three replicates of each data point of valproic acid and valpromide was <10%. As in previous studies of valpromide, plasma levels of valpromide and valproic acid were

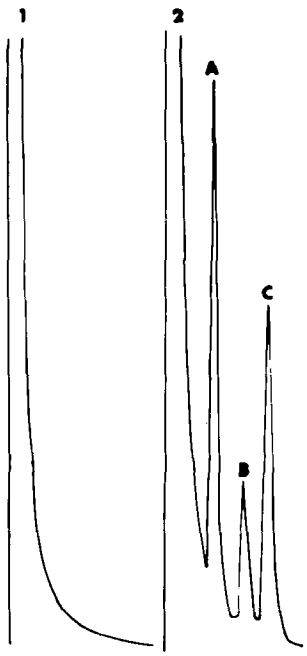

Figure 1—*Typical chromatograms of a human plasma blank (1) and valproic acid and valpromide in human plasma (2). Key: (A) valproic acid, 30 μg/mL; (B) IS, 10 μg/mL; (C) valpromide, 30 μg/mL.*

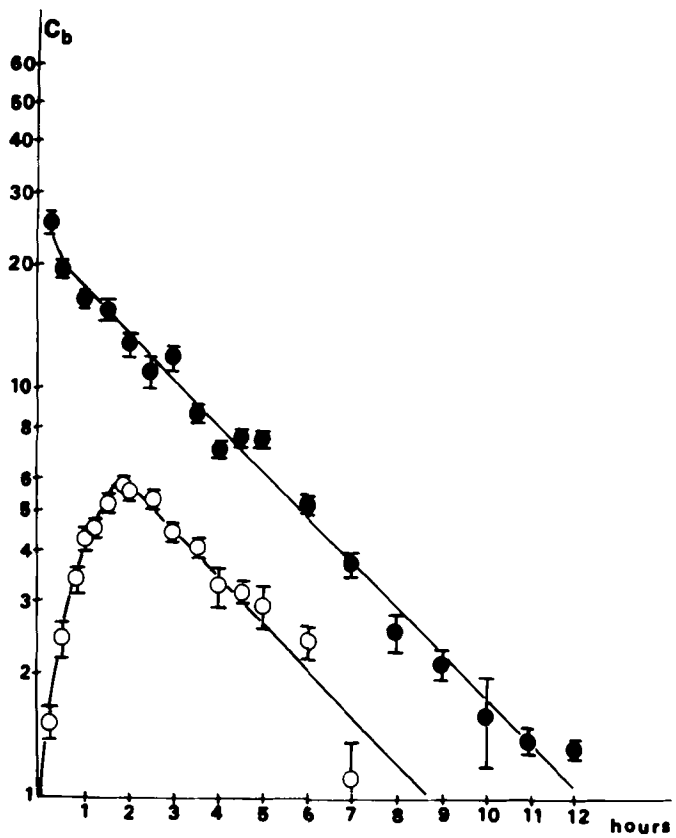

Figure 2—*Plasma levels of valpromide (●) and valproic acid (○) obtained after intravenous administration of 400 mg of valpromide to a dog.* C_b *is expressed in μg/mL.*

Table II—Precision of the Assay for Valproic Acid and Valpromide in Human Plasma

Conc., μg/mL	Valproic Acid: Conc. Found[a], μg/mL	Valproic Acid: SD	Valproic Acid: CV, %	Valpromide: Conc. Found[a], μg/mL	Valpromide: SD	Valpromide: CV, %
20	20.30	1.86	9.18	19.53	0.91	4.66
30	30.52	1.71	4.21	31.76	3.00	9.45
40	41.88	3.24	7.74	41.50	2.80	6.75
50	49.84	3.97	7.96	49.08	3.95	8.00
60	57.84	4.75	8.21	62.5	5.12	8.19

[a] Mean of 10 determinations.

assayed separately by two different systems (2, 4). The proposed method is very useful and advantageous in any pharmacokinetic or metabolic study of valpromide.

REFERENCES

(1) F. Pisani, A. Fazio, G. Oteri, and R. Di Perri, *Ther. Drug. Monit.*, **3**, 297 (1981).

(2) F. Pisani, A. Fazio, G. Oteri, and R. Di Perri, *J. Pharm. Pharmacol.*, **34**, 45 (1982).

(3) P. Favel, J. Cartier, J. P. Gratadou, and G. Gratadou, *Epilepsia*, **14**, 329 (1973).

(4) F. Pisani and R. Di Perri, *Ital. J. Neurol. Sci.*, **4**, 245 (1980).

(5) F. Pisani, A. A. D'Agostino, A. Fazio, G. Oteri, G. Primerano, and R. Di Perri, *Epilepsia*, **23**, 115 (1982).

(6) "Martindale, The Extra Pharmacopoeia," 27th ed. Pharmaceutical Press, London, 1977, p. 1752.

(7) R. M. Pinder, R. N. Brogden, T. M. Speight, and G. S. Avery, *Drugs*, **13**, 81 (1977).

(8) R. Gugler and E. von-Unruh, *Clin. Pharmacokinet.*, **5**, 67 (1980).

(9) A. Soufi, D. Colussi, and F. Marfil, *J. Chromatog.*, **182**, 241 (1980); and references cited therein.

(10) A. E. Hershey, J. R. Patton, and K. H. Dudley, *Ther. Drug. Monit.*, **1**, 217 (1979); and references cited therein.

(11) F. Pisani, R. Di Perri, and G. Nistico, *J. Chromatogr.*, **174**, 231 (1979).

(12) R. V. Smith and J. T. Stewart, "Textbook of Biopharmaceutic Analysis," Lea and Febiger, 1981, p. 79.

(13) C. M. Metzler, O. L. Elfind, and A. J. McEwen, "A User's Manual for NONLIN and Associated Program Research Biostatistics," The Upjohn Co., Kalamazoo, Mich., 1974.

ACKNOWLEDGMENTS

This work was supported by Grant Number 2127 from the Israel National Council for Research and Development, and is included in the dissertation project of Abraham Rubinstein, as a partial fulfilment of the Doctor of Philosophy degree requirements of the Hebrew University of Jerusalem.

The authors thank Miss Dana Lev for her skillful technical assistance.

Pharmacokinetics of Iohexol, a New Nonionic Radiocontrast Agent, in Humans

JEROME EDELSON ˣ, DAVID SHAW, and GERARD PALACE

Received Feburary 17, 1983, from *Sterling-Winthrop Research Institute, Rensselaer, NY 12144.* Accepted for publication May 20, 1983.

Abstract □ Sixteen healthy men received iohexol intravenously at a concentration of 346 mg of iodine/mL. Doses of 500, 750, 1000, and 1500 mg of iodine/kg of body weight were administered to four volunteers each. Neither clearance nor percent of dose excreted in the urine showed any significant correlation with size of the dose. The overall mean (±*SD*) renal and total body clearances were 120 ± 18.6 and 131 ± 18.6 mL/min, respectively. The overall mean apparent volume of distribution was 165 (±30.7) mL/kg. Urine contained 92.3 ± 4.4% of the dose. Most of the drug (89.9%) was excreted within the first 12 h. An open three-compartment body model gave the best fit to the experimental data. The mean apparent first-order terminal elimination (γ-phase) half-life was 12.6 h.

Keyphrases □ Iohexol—pharmacokinetics, intravenous administration, humans □ Pharmacokinetics—iohexol intravenous administration, humans □ Radiocontrast agents—iohexol, plasma and urine levels, intravenous administration, pharmacokinetics, humans

Ionic contrast media that are approved for human use are hyperosmolar to plasma. Administration of the large volumes necessary for visualization can result in large detrimental fluid shifts within the body. Prior to conducting intensive clinical trials with a new contrast medium, the route and rate of excretion must be adequately assessed. Previous studies with ionic contrast media have suggested that excretion occurs almost exclusively *via* the kidney at a rate that is consistent with passive handling by glomerular filtration (1).

Iohexol[1], 5-[acetyl(2,3-dihydroxypropyl)-amino]-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide, is a new nonionic radiographic contrast agent which is intended for vascular and intrathecal use in humans. The biological properties of iohexol (2) are similar to those of metrizamide, the first nonionic contrast medium approved for clinical use (3). A significant advantage of iohexol is its stability in solution to terminal heat sterilization, and its preparation as a ready-to-use solution.

This report describes the results of our investigations into the excretion of iohexol, and includes a nonlinear least-squares estimate of the pharmacokinetic parameters of iohexol following intravenous administration.

EXPERIMENTAL SECTION

Study in Human Volunteers—Four groups of four healthy male volunteers, between the ages of 18 and 50 years, received iohexol intravenously at doses

[1] Omnipaque; Sterling Drug, New York, N.Y.