decyltrichlorosilane chemically bonded to a small diameter (<10 μm) silica particle. The smaller particle size results in faster analysis time with improved resolution.

The retention times of penicillin G potassium and several degradation products formed during acid hydrolysis of penicillin G potassium at pH 2.83 are given in Table I. Identification of penicillin G potassium and related compounds was accomplished by comparing the retention time of the unknown with that of known standards. An unidentified compound with a retention time of 4.9 min was detected and designated as Compound F. A representative chromatogram for pH 2.83 is shown in Fig. 1. No penicillamine and penamaldic acid were detected at this pH.

The stability of penicillin G potassium and its degradation products in the HPLC mobile phase also was determined. Samples of penicillin G potassium and its degradation products were aged in the acetonitrile–phosphate mobile phase for a period at least equal to the retention for the particular compound. Samples were injected onto the HPLC column to observe any change in peak height or any increase in the number of peaks. Additionally, solutions containing mixtures of penicillin G potassium and its degradation products were aged in the mobile phase and subjected to the same test. No change in the number of peaks or peak height was noted.

Figure 2 illustrates the results obtained for penicillin G potassium degradation at pH 2.83 using the described HPLC process. The studies are continuing, and the complete kinetic scheme will be reported in a subsequent article.

REFERENCES

(1) R. R. Goodall and A. A. Levi, *Analyst*, **72**, 277 (1947).

(2) I. J. McGilveray and R. D. Strickland, *J. Pharm. Sci.*, **56**, 77 (1967).

(3) J. R. Fooks and G. L. Mattock, *ibid.*, **58**, 1357 (1969).

(4) E. Evrard, M. Claesen, and H. Vanderhaeghe, *Nature*, **201**, 124 (1964).

(5) C. Hishta, D. L. Mays, and M. Garofalo, *Anal. Chem.*, **43**, 1530 (1971).

(6) J. M. Blaha, A. M. Knevel, and S. L. Hem, *J. Pharm. Sci.*, **64**, 1384 (1975).

(7) P. Adriens, B. Meesschaert, H. Eyssen, E. Roets, and H. Vanderhaeghe, *J. Chromatogr.*, **144**, 93 (1977).

(8) M. Schwartz, *J. Pharm. Sci.*, **58**, 643 (1969).

(9) M. Cole, M. D. Kenig, and V. A. Hewitt, *Antimicrob. Agents Chemother.*, **1973**, 463.

(10) J. V. Vasani, Ph.D. thesis, Philadelphia College of Pharmacy and Science, Philadelphia, Pa., 1977.

(11) J. L. Longridge and D. Timms, *J. Chem. Soc., B*, **1971**, 852.

(12) L. R. Snyder and J. J. Kirkland, "Introduction to Modern Liquid Chromatography," Wiley, New York, N.Y., 1974, p. 89.

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Sensitive High-Pressure Liquid Chromatographic Determination of Disopyramide and Mono-*N*-dealkyldisopyramide

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Received March 19, 1979, from the *College of Pharmacy, North Dakota State University, Fargo, ND 58105.* Accepted for publication April 5, 1979.

Abstract □ A high-pressure liquid chromatographic procedure for the accurate determination of disopyramide and its chief metabolite in plasma is presented. The method is suitable for monitoring patients receiving disopyramide therapy. A reversed-phase cyanopropylsilane column is utilized with a mobile phase of 50% acetonitrile and 50% 0.01 *M* sodium acetate buffer at pH 4.0. Absorption was monitored at 254 nm with a detection limit of 0.2 μg/ml of plasma. The reproducibility and precision of the procedure were demonstrated on samples containing 0.50–12 μg/ml of plasma.

Keyphrases □ Disopyramide—analysis, high-pressure liquid chromatography, plasma, metabolites, humans □ Disopyramide—metabolites, analysis, high-pressure liquid chromatography, humans □ Mono-*N*-dealkyldisopyramide—analysis, high-pressure liquid chromatography, plasma, humans □ Antiarrhythmic agents—disopyramide, high-pressure liquid chromatographic analysis, plasma, humans

Disopyramide phosphate[1] is a relatively new antiarrhythmic drug with electrophysiological properties similar to quinidine, but it is reported to be better tolerated (1). The wide range of plasma half-lives (2) of disopyramide phosphate in humans (4–18 hr) indicates that patients may require individualized dosage regimens. The side effects of the drug (3, 4) and the antiarrhythmic effects of disopyramide phosphate have been related to concentration (5, 6). Consequently, measurement of the concentration may provide a method to maximize therapeutic effect while minimizing potential side effects.

Direct spectrophotometric (7) and spectrofluorometric (8) determinations have been reported. Both these techniques are nonspecific because the major metabolite (mono-*N*-dealkyldisopyramide) has similar spectral characteristics to the parent compound (7, 8). GLC procedures also have been reported, but they either require derivatization of the compound (9) or a selective (nitrogen) detector (10). Quantitation of disopyramide and its metabolite has also been performed by high-pressure liquid chromatography (HPLC). The reported methods require the ion-pair modification of octadecylsilane reversed-phase chromatography (11) or the use of a multiwavelength detector (12, 13).

This paper describes a rapid, specific method for the determination of disopyramide and its major metabolite by HPLC using a reversed-phase cyanopropylsilane column and a 254-nm detector. The method is applicable to the direct determination of plasma levels, even in the

[1] Norpace.

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Table I—Precision and Percent Recovery of Disopyramide Phosphate and Mono-*N*-dealkyldisopyramide

Level, μg/ml	*n*	Recovery, %	*CV*
	Disopyramide Phosphate		
0.5	4	90.8	12.0
1.5	5	101.5	6.4
4.0	5	103.3	2.9
6.0	4	104.7	1.7
9.0	4	101.7	1.4
12.0	5	99.5	2.0
	Mono-*N*-dealkyldisopyramide		
0.5	4	111.1	12.5
1.5	5	96.0	12.8
4.0	5	91.5	4.1
6.0	5	99.1	5.2
9.0	5	96.8	3.5
12.0	4	100.9	6.9

presence of other antiarrhythmic drugs. The applicability was demonstrated by the analysis of plasma from patients taking disopyramide.

EXPERIMENTAL

Instrumentation—A high-pressure liquid chromatograph[2] was equipped with a cyanopropylsilane[3] column, 300 mm long × 4 mm i.d. The degassed mobile phase was pumped through the column at 1.2 ml/min (1800–2000 psi) at ambient temperature until a stable baseline was obtained.

Chemicals and Reagents—Sodium acetate, sodium hydrogen phosphate, acetic acid, and phosphoric acid were ACS certified grade. HPLC grade methanol, methylene chloride, and acetonitrile were used. Disopyramide phosphate[4], mono-*N*-dealkyldisopyramide[4], and cyclomethycaine sulfate[5] were supplied by the manufacturers.

Mobile Phase—Sodium acetate solution, 0.01 *M*, was prepared in distilled water, and the pH was adjusted to 4 by the gradual addition of acetic acid. An equal volume of acetonitrile was added to the buffer, and the mixture was degassed under vacuum.

Stock Solutions—*Disopyramide Phosphate and Mono-*N*-dealkyldisopyramide*—A solution containing 4 mg of the drug and 4 mg of its metabolite in 100 ml of methanol was prepared. The solution was stored in a refrigerator and made fresh each month.

Cyclomethycaine Sulfate (Internal Standard)—A solution was prepared by dissolving 8 mg in 5 ml of methanol and diluting to 100 ml with methylene chloride. This solution was prepared fresh daily.

Working Internal Standard Solution—With a volumetric pipet, 1.0 ml of the stock cyclomethycaine solution was transferred to a 500-ml volumetric flask and diluted with methylene chloride to volume (0.16 μg/ml).

Sample Preparation—To 0.5 ml of heparinized plasma in a 15-ml screw-capped centrifuge tube, an aliquot of the methanolic solution of disopyramide phosphate and dealkyldisopyramide containing 0.25–6.0 μg of each drug, 0.5 ml of phosphate buffer (pH 7.4, 0.1 *M*), and 5.0 ml of methylene chloride containing 0.16 μg of cyclomethycaine sulfate/ml were added. The tubes were vortexed for 10 sec and centrifuged for 5 min at 900×*g*. A 4.0-ml volume of the organic phase was transferred to special concentration tubes[6] and evaporated to dryness at ambient temperature under a gentle nitrogen stream.

HPLC Separation and Quantitation—The residue was dissolved in 200 μl of acetonitrile, and 50–100-μl injections were made using a 50-μl syringe[7]. A standard curve was constructed utilizing a minimum of four replicate plasma extractions simulating concentrations of disopyramide phosphate and mono-*N*-dealkyldisopyramide from 0.5 to 12 μg/ml. The chromatograms were recorded[8] at a chart speed of 5 mm/min.

Disopyramide and its metabolite were identified on the basis of retention time. The peak heights were measured, and the disopyramide phosphate/cyclomethycaine and mono-*N*-dealkyldisopyramide/cyclomethycaine ratios were calculated and plotted *versus* concentration, expressed as micrograms per milliliter of plasma.

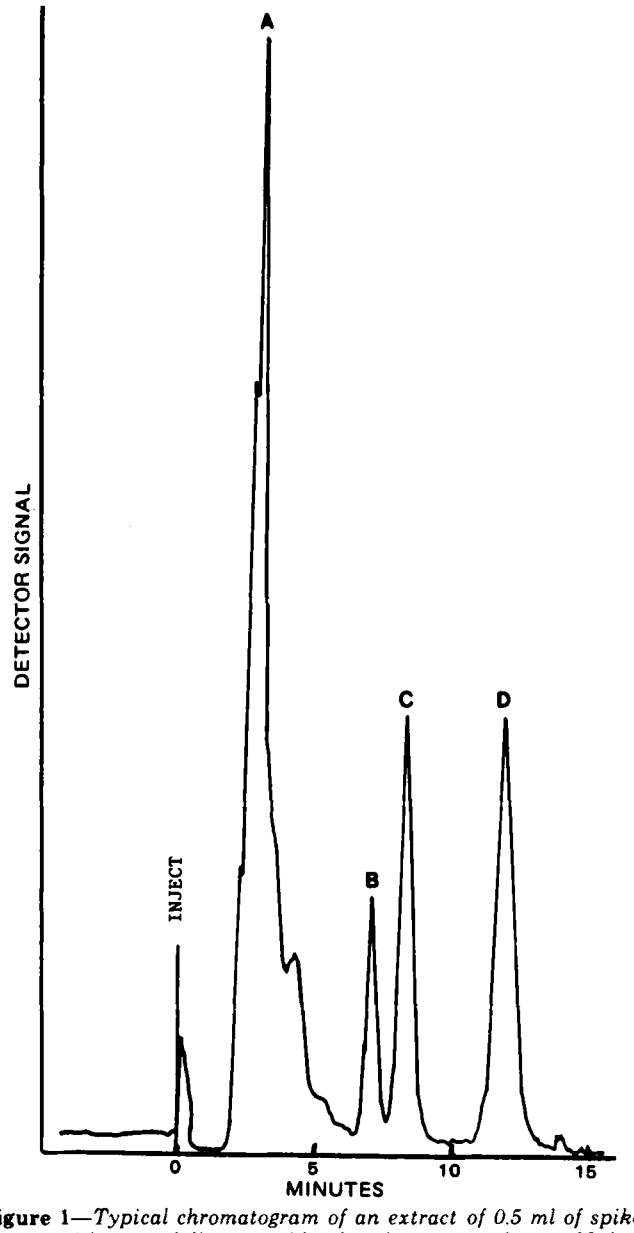

Figure 1—*Typical chromatogram of an extract of 0.5 ml of spiked plasma with 2 μg of disopyramide phosphate, 2 μg of mono-*N*-dealkyldisopyramide, and 8 μg of the internal standard. Key: A, plasma extractable; B, mono-*N*-dealkyldisopyramide; C, disopyramide; and D, internal standard.*

Interferences—Possible interferences from normal plasma constituents and other antiarrhythmic drugs were studied by adding therapeutic amounts to plasma containing disopyramide phosphate, its metabolite, and the internal standard and performing the analysis.

Sample Preparation and Assay—Heparinized plasma samples from patients on oral disopyramide phosphate therapy were processed in duplicate as described. The amounts of disopyramide and its metabolite were calculated by comparison with the standard curve prepared daily.

RESULTS AND DISCUSSION

Methylene chloride was used as the organic solvent in the extraction procedure because it exhibits good extractibility for disopyramide, propranolol, and other antiarrhythmic amines (14). Moreover, it extracts a minimum of interfering constituents from plasma and evaporates easily. The extraction at pH 7.4 gave excellent recoveries of disopyramide and the internal standard (90–95%). The recovery of the metabolite was only 32%.

[2] Model 202 chromatograph, M 600 pump, and U6K universal injector, Waters Associates, Milford, Mass.
[3] μBondapak CN, Waters Associates, Milford, Mass.
[4] Searle Laboratories, Chicago, Ill.
[5] Eli Lilly, Indianapolis, Ind.
[6] Concentratubes, Laboratory Research Co., Los Angeles, Calif.
[7] Hamilton Co., Reno, Nev.
[8] Model 56, Perkin-Elmer, Norwalk, Conn.

The ratios of the peak heights of disopyramide and its metabolite to the peak height of the internal standard were calculated. Statistical analysis of the data (Table I) by linear regression indicated excellent linearity and reproducibility with correlation coefficients of 0.9991 and 0.9955, slopes of 0.3655 and 0.3100, and intercepts of 0.0030 and −0.0038 in the range of 0.5–12.0 μg/ml of plasma for disopyramide and its metabolite, respectively. This range includes the therapeutic range of the drug.

The use of reversed-phase chromatography on a cyanopropylsilane column and a mobile phase of 50% acetonitrile and 50% acetate buffer (pH 4, 0.01 *M*) afforded excellent separation with sharp peaks for disopyramide, mono-*N*-dealkyldisopyramide, and cyclomethycaine (Fig. 1).

The method was applied for the determination of disopyramide and its metabolite in patient plasma before and after administration of disopyramide phosphate. No interference was noted from commonly utilized cardiac drugs. Quinidine, lidocaine, procainamide, and the more recent β-blockers metoprolol and timolol were eluted at different retention times. Although propranolol and its 4-hydroxy metabolite have the same retention times as disopyramide and its mono-*N*-dealkylated metabolite, respectively, the accurate determination of the two drugs is still possible since disopyramide and its metabolite do not fluoresce while propranolol and its metabolite fluoresce but have no appreciable absorption at 254 nm. Cyclomethycaine is an ideal internal standard since it absorbs at 254 nm and fluoresces at 276-nm excitation with a 340-nm cutoff filter (14).

Major advantages of the proposed method are its simplicity and rapidity. Both disopyramide and its metabolite are determined in a single procedure using a standard single wavelength UV detector. Moreover, propranolol can be determined using the same extraction and chromatographic conditions by the incorporation of a spectrofluorometric detector. Other chromatographic methods require separate conditions for the determination of each drug or even for the determination of metabolites. In addition, the method gave excellent separation without the ion-pair technique, which results in limited use of the column and a decrease in the capacity factor (15).

REFERENCES

(1) M. E. Josephson, A. R. Caracta, and S. H. Lau, *Am. Heart J.*, **86**, 771 (1973).

(2) "Facts and Comparisons," E. K. Kastrup and J. R. Boyd, Eds., Facts and Comparisons, Inc., St. Louis, Mo., 1977, p. 146c.

(3) F. A. Peters and R. A. Winkle, *Hosp. Form.*, **13**, 989 (1978).

(4) P. P. Mathur, *Am. Heart J.*, **84**, 764 (1972).

(5) L. A. Vismara, D. T. Mason, and E. A. Amsterdam, *Clin. Pharmacol. Ther.*, **16**, 330 (1974).

(6) L. A. Vismara, Z. Vera, and R. R. Miller, *Am. J. Cardiol.*, **39**, 1027 (1977).

(7) D. Martin, L. Burke, W. Nordin, and S. Ehen, *Clin. Chem.*, **24**, 991 (1978).

(8) R. E. Ranney, R. R. Dean, A. Karim, and F. M. Radzialowski, *Arch. Int. Pharmacodyn. Ther.*, **191**, 162 (1971).

(9) T. C. Hutsell and S. J. Stachelski, *J. Chromatogr.*, **106**, 151 (1975).

(10) A. M. J. A. Duchateau, F. W. H. M. Merkus, and F. Schobben, *ibid.*, **109**, 432 (1975).

(11) P. J. Meffin, S. R. Harapat, and D. C. Harrison, *ibid.*, **132**, 503 (1977).

(12) P.-O. Lagerstrom and B.-A. Persson, *ibid.*, **149**, 331 (1978).

(13) L. A. Broussard and C. S. Frings, *Clin. Chem.*, **24**, 1007 (1978).

(14) G. Nygard, W. H. Shelver, and S. K. W. Khalil, *J. Pharm. Sci.*, **68**, 379 (1979).

(15) G. Schill, K. O. Borg, R. Modin, and B.-A. Persson, in "Progress in Drug Metabolism," vol. 2, L. F. Chasseaud and J. Bridges, Eds., Wiley, New York, N.Y., 1977, chap. 5.

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Plasma Binding of Benzodiazepines in Humans

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Received December 29, 1978, from the *Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine and Veterans Administration Hospital, Nashville, TN 37203.* Accepted for publication April 5, 1979.

Abstract □ Plasma binding of chlordiazepoxide, diazepam, lorazepam, and oxazepam was determined by equilibrium dialysis in 20 male, healthy volunteers, 25–86 years old. A wide range of binding was observed, with the free fraction varying twofold for lorazepam, fourfold for chlordiazepoxide and diazepam, and over 20-fold for oxazepam. Statistically significant linear relationships were not observed between the degree of binding and age, serum albumin, or total protein for any of the drugs. There was, however, a correlation between the extent of binding for the four drugs. Because of the importance of unbound benzodiazepine levels in eliciting any pharmacological response and also in disposition, consideration of the wide interindividual variability in plasma binding must be made in interpreting pharmacodynamic and pharmacokinetic data.

Keyphrases □ Benzodiazepines—plasma binding, equilibrium dialysis, humans, individual variation, age □ Sedatives—benzodiazepines, plasma binding, equilibrium dialysis, humans, individual variation, age □ Plasma binding—benzodiazepines, humans, individual variation, age

During a study of the effects of age on lorazepam disposition in humans, the results obtained from 11 apparently healthy subjects, 15–73 years old, suggested that the extent of plasma binding of this drug decreased with age (1). This result was in contrast to previous findings with the related 1,4-benzodiazepines, diazepam (2), chlordiazepoxide (3), and oxazepam (4). Accordingly, studies of lorazepam binding were performed with plasma from a larger group of subjects to determine whether the observed trend was real or had occurred by chance. The investigation also provided an opportunity to compare directly the relative plasma binding behavior of the various benzodiazepines.

EXPERIMENTAL

^{14}C-Chlordiazepoxide[1] (59.95 mCi/mmole), ^{14}C-diazepam[1] (13.01 mCi/mmole), ^{14}C-lorazepam[2] (10.79 mCi/mmole), and ^{14}C-oxazepam[2] (5.91 mCi/mmole) were examined for radiopurity by TLC. All except lorazepam were >98% pure. Purification of the latter to this level was achieved by preparative TLC using silica GF plates[3] and a mobile phase of ethyl acetate[4]–ethanol[5]–ammonium hydroxide[4] (5:5:1 v/v). Separate drug standard solutions were prepared in pH 7.4 buffer as follows:

[1] Hoffmann-La Roche, Nutley, NJ 07110.
[2] Wyeth Laboratories, Radnor, PA 19087.
[3] I.C.N. Pharmaceuticals, Cleveland, OH 44128.
[4] ACS grade, Fisher Scientific Co., Pittsburgh, PA 15219.
[5] USI absolute ethyl alcohol, USP-NF reagent.

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