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ACKNOWLEDGMENTS AND ADDRESSES

Received April 15, 1974, from the **Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow 226003, India,* and the *‡Department of Physiology and Pharmacology, University of North Dakota School of Medicine, Grand Forks, ND 58201*

Accepted for publication June 11, 1974.

Supported in part by the Council of Scientific and Industrial Research, New Delhi, India, the Indian National Science Academy, New Delhi, India, and the U.S. Public Health Service National Institutes of Health [Grant 1 T01 HL 05939-01A1 and 5 S01 RR0 5407 (School of Medicine University of North Dakota General Research Support Grant)].

The authors thank Professor K. P. Bhargava and Professor Stanley J. Brumleve for their advice and encouragement. Grateful acknowledgment is made to the Council of Scientific and Industrial Research, New Delhi, India, and the Indian National Science Academy, New Delhi, India, for providing Senior Research Fellowships to A. K. Chaturvedi and A. Chaudhari, respectively.

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Comparison of Procainamide Analyses in Plasma by Spectrophotofluorometry, Colorimetry, and GLC

JOHN STERLING, STEPHEN COX, and W. G. HANEY ˣ

Abstract □ A procedure for analysis of procainamide in plasma based on the reaction of procainamide with fluorescamine was developed. The accuracy and precision of results obtained using this method were compared to those of results obtained using colorimetric and GLC methods. The utility of the three procedures in the routine determination of procainamide in plasma is discussed.

Keyphrases □ Procainamide—comparison of spectrophotofluorometric, colorimetric, and GLC analyses, plasma samples □ Methodology—procainamide in plasma, comparison of spectrophotofluorometric, colorimetric, and GLC analyses

Procainamide appears to be one of the numerous drugs (1) for which the monitoring of drug plasma concentration is often necessary to ensure effective therapy. Large variations in procainamide plasma concentration resulting from a given daily dose have been noted (2, 3), and plasma concentration appears to relate predictably to the patient's response to the drug (4). Thus, rapid and accurate procedures for determination of procainamide in plasma are necessary.

Procainamide (as well as other drugs containing a primary amine on an aromatic ring) has most often been determined by diazotization, coupling with *N*-(1-naphthyl)ethylenediamine, and subsequent colorimetric analysis (5). While this procedure has been used for a long time (6), it requires 1–2 hr for a single assay, sodium nitrite and ammonium sulfamate solutions must be prepared at the time of analysis, and the color produced is unstable (7). The utility of direct spectrophotofluorometry for this determination has been investigated (3). However, a metabolite of procainamide (*N*-acetylprocainamide) interfered in the analysis, and accuracy and precision were not determined. Finally, GLC has been used for the analysis of procainamide in plasma (8) and undoubtedly has utility, although nonlinear calibration curves and relatively unstable column packing material limit its usefulness.

Spectrophotofluorometric procedures have a high daily capacity for sample analysis, are more amenable to application by less sophisticated personnel than are GLC techniques, and generally are more precise. Therefore, the development of such an assay for procainamide as a possible substitute for the colorimetric assay is of interest.

A general procedure for the microanalysis of drugs containing the primary aromatic amino substituent was presented previously (9). This procedure is based on selective reaction of fluorescamine with the primary aromatic amino substituent and subsequent de-

termination of the derivative by spectrophotofluorometry. This report presents results of the application of this procedure to the analysis of procainamide in plasma. To allow an investigation of the specificity of the analytical procedure, a modified GLC assay was developed, and results of the two procedures were compared. Finally, results and utility of the well-accepted colorimetric procedure were compared and contrasted with those of the GLC and spectrophotofluorometric procedures.

EXPERIMENTAL

Apparatus—A spectrophotofluorometer[1] was equipped as described previously (9). Operating conditions were also the same, except that the band passes were maintained at 2.0 and 1.0 mm for excitation and emission wavelengths, respectively.

A gas chromatograph[2] with isothermal control and flame-ionization detector was used. Columns (1.82 m, spiral-shaped, borosilicate glass) were packed with 100–120-mesh, acid-washed, flux-calcined diatomite coated with 0.5% polyamide[3] and 6% (3,3,3-trifluoropropyl)methylsilicone. Prior to use, the column was conditioned at 250° for 24 hr. Injector port (fitted with glass injector sleeve), column, and detector temperatures were 270, 235, and 280°, respectively. Gas flow rates were: hydrogen, 40 ml/min; air, 225 ml/min; and nitrogen as carrier gas, 40 ml/min. Peak areas were determined using an automatic electronic digital integrator[4] with printout.

A spectrophotometer[5] was used for colorimetric determinations.

Reagents and Solutions—Dibucaine hydrochloride was obtained as a USP reference standard. Standard solutions of dibucaine (20.0 mg/100 ml) and procainamide were prepared in distilled water, and the pH was adjusted to 5.0 with 0.1 *N* HCl and/or 0.1 *N* NaOH. These solutions were diluted quantitatively prior to use. For recovery studies, a solution of dibucaine (2.125 μg/ml) was prepared by extracting a solution of dibucaine in pH 9.5 borate buffer (10.0 ml) with chloroform (80 ml) and adding chloroform to a volume of 100.00 ml.

Other reagents and solutions were obtained or prepared as previously indicated (13).

Preparation of Calibration Curves—Calibration curves for the GLC assay were prepared by mixing solutions (1.0–20.0 μg/ml in 1.0 ml) of procainamide with internal standard solution (1.0 ml of 8.5 μg/ml), pH 9.5 borate buffer (0.5 ml), and methylene dichloride (8 ml). This mixture was shaken for 2 min and centrifuged for 3 min at 2000 rpm. The methylene dichloride extract was filtered through phase-separating filter paper, and the filtrate was evaporated to dryness in a water bath under a stream of dry nitrogen. The residue was reconstituted in carbon disulfide (50 μl), and a volume (3–5 μl) of this solution was injected into the chromatograph. The ratio of the area of the peak representing procainamide to that representing dibucaine was plotted against the concentration of procainamide in the solution. Calibration curves were prepared on 10 consecutive days.

Calibration curves for spectrophotofluorometric studies were prepared by mixing solutions (1.0–20.0 μg/ml in 1.0 ml) of procainamide with pH 5.5 phosphate buffer (0.5 ml). Fluorescamine solution (0.5 ml of a solution of 25 mg/100 ml in acetone) was added with shaking. After 7 min, fluorescence of the solution was determined at 400 and 498 nm for excitation and emission wavelengths, respectively, against a reagent blank. Calibration curves were prepared on 10 consecutive days.

Calibration curves for colorimetric determinations were prepared by adding various quantities of procainamide solution to pooled plasma and treating the spiked samples as previously noted (3).

Extraction—Procainamide solution was added to plasma (20.0 ml) to give concentrations of 2.0–20.0 μg/ml. An aliquot (2.0 ml) of this plasma was mixed with pH 9.5 borate buffer (1.0 ml), extracted with methylene dichloride (16.0 ml), and centrifuged at 2000 rpm for 3 min. The extract was passed through phase-separating filter paper. An aliquot (6.0 ml) was removed, evaporated to dryness, and reconstituted with pH 5.5 phosphate buffer. Fluorescence of the solution was determined against a reagent blank.

An aliquot (6.0 ml) of the remaining methylene dichloride extract was combined with the solution of dibucaine in chloroform (3.0 ml), evaporated to dryness, reconstituted with carbon disulfide (50 μl), and injected into the chromatograph. Recoveries were calculated by correcting for the volume of extract taken and by reference to previously derived calibration curves.

Accuracy and Precision—Procainamide solution was added to plasma (90.0 ml) to give concentrations of 2.0–20.0 μg/ml, together with sufficient dibucaine solution to give a final concentration of 8.5 μg/ml and pH 5.5 phosphate buffer (6.0 ml). Aliquots (1.0 ml) of these solutions were added to vials, and the vials were capped and maintained at −6°. On 10 consecutive days, the contents of nine vials were thawed, and three each were analyzed for procaina-

[1] American Instruments Co.
[2] Varian model 575.
[3] Versamid 900, Analabs, North Haven, Conn.
[4] Varian model 475.
[5] Cary model 118.

Table I—Efficiency of Extraction of Procainamide from Plasma as Determined by GLC and Spectrophotofluorometry

Method	Procainamide Added, μg	Procainamide Recovered, μg	Recovery, %	*SD*, %[a]
GLC	2.00	1.97	98.50	5.43
Spectrophotofluorometry	2.00	2.02	101.00	2.73
GLC	4.00	4.13	103.75	4.51
Spectrophotofluorometry	4.00	3.96	98.75	2.30
GLC	8.00	7.85	98.12	1.84
Spectrophotofluorometry	8.00	7.93	99.12	1.94
GLC	12.00	12.31	102.58	2.47
Spectrophotofluorometry	12.00	12.14	101.16	2.11
GLC	16.00	15.53	97.06	4.14
Spectrophotofluorometry	16.00	15.74	98.30	1.76
GLC	20.00	21.06	105.33	5.91
Spectrophotofluorometry	20.00	20.40	102.00	2.14

[a] $n = 10$.

Table II—Accuracy and Precision of Three Methods of Procainamide Determination

Method	Procainamide Quantity, μg	Procainamide Found, μg	Recovery, %	*SD*, %[a]
GLC	2.00	1.94	97.00	7.57
Spectrophotofluorometry	2.00	2.02	101.00	3.41
Colorimetry	2.00	2.09	104.50	9.84
GLC	4.00	4.23	105.75	6.90
Spectrophotofluorometry	4.00	4.13	103.25	4.11
Colorimetry	4.00	3.81	95.25	8.72
GLC	8.00	8.31	103.87	5.74
Spectrophotofluorometry	8.00	8.07	100.87	3.02
Colorimetry	8.00	8.39	104.87	6.59
GLC	12.00	12.60	105.00	6.94
Spectrophotofluorometry	12.00	11.71	97.59	3.17
Colorimetry	12.00	11.55	96.25	6.87
GLC	16.00	16.03	100.19	8.42
Spectrophotofluorometry	16.00	15.87	99.19	2.54
Colorimetry	16.00	15.34	95.88	7.22

[a] $n = 30$.

Table III—Procainamide Concentration in Plasma as Determined by Three Methods

Sample	Other Drugs	Apparent Procainamide Concentration[a]		
		GLC	Spectrophotofluorometry	Colorimetry
1	Nitrofurantoin, methaqualone	5.40	5.12	5.59
2	Digoxin, glutethimide	7.21	6.84	7.36
3	Chlordiazepoxide, codeine	2.74	2.92	4.16
4	Tolbutamide, diazepam	6.14	6.23	5.71
5	Aspirin, phenacetin, caffeine, propoxyphene	2.10	2.34	2.61
6	Dicumarol, erythromycin	6.41	6.29	6.53
7	Aspirin, codeine, chlordiazepoxide	5.37	5.54	8.97
8	Methaqualone, chlorothiazide	10.74	9.96	10.92

[a] Expressed in micrograms per milliliter.

mide content by spectrophotofluorometry, colorimetry, and GLC.

Patient Plasma Procainamide—Blood from patients taking procainamide was mixed with pH 5.5 phosphate buffer (1.0 ml) and centrifuged, and the plasma was stored at −6°. At the time of analysis, samples were thawed and split into aliquots (1.0 ml). Dibucaine solution (1.0 ml of a 8.5 μg/ml solution) was added to the aliquot to be analyzed by GLC, and analysis of each aliquot by spectrophotofluorometry, colorimetry, and GLC proceeded as described.

RESULTS AND DISCUSSION

The selectivity of the spectrophotofluorometric procedure was examined by comparing results of the method with those obtained from a GLC procedure. Nonlinear calibration curves and incomplete extraction necessitated modifications in the previously proposed GLC procedure (8). Since it appeared that nonlinearity was due to adsorption of procainamide onto the solid support, a more polar stationary phase and a higher liquid load were used. In addition, a small amount of a polyamide was used to coat the solid support prior to coating with (3,3,3-trifluoropropyl)methylsilicone. The polyamide in this capacity acts to deactivate potential adsorptive sites on the solid support (10). The packing material was stable over 3 months.

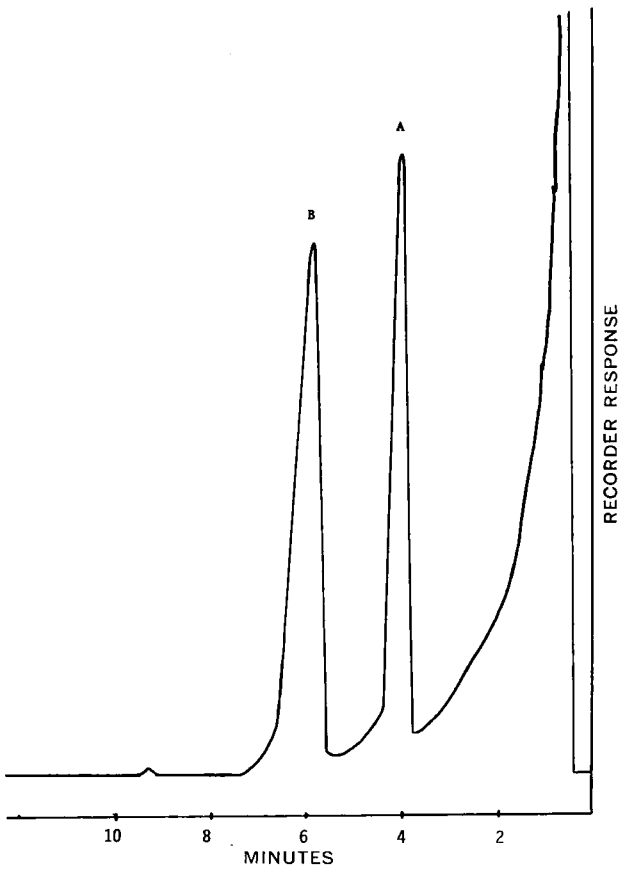

Figure 1—*Chromatographic trace of an extract of plasma containing procainamide (6.5 μg/ml, A) and internal standard (8.5 μg/ml, B). (See text for chromatographic conditions.)*

Calibration curves for the GLC assay were prepared on 10 consecutive days, and the standard deviation of the curve slope was 1.6%. The average correlation coefficient was 1.022, and the average y-intercept was +0.007.

Dibucaine was used as an extracted internal standard in the GLC assay. Because of the use of dibucaine in drug therapy, it may not be the internal standard of choice in routine GLC determinations of procainamide in plasma. However, it is well suited for this comparative study, since it does not interfere with results of the spectrophotofluorometric or colorimetric assay. Therefore, it may be added to plasma before the sample is split into three aliquots for analysis, ensuring that differences in results obtained *via* the three procedures are due to differences in analytical procedures rather than to differences in sampling.

Calibration curves for the spectrophotofluorometric assay were prepared on 10 consecutive days. The standard deviation of the slope of the curve was 0.72%, the average correlation coefficient was 1.009, and the average y-intercept was −0.005.

Results of the investigation of extraction efficiency as determined by GLC and by spectrophotofluorometry are presented in Table I. The therapeutic concentration of procainamide in plasma has been estimated to be 4–10 μg/ml (3). Extraction efficiency in this range is satisfactory as determined by either method. However, results of the GLC assay are much less precise at 2.0, 16.0, and 20.0 μg/ml than results at other concentrations and results of the spectrophotofluorometric assay. This finding appears to reflect a diminution in analytical precision as the difference between the area of procainamide and the internal standard increases rather than erratic recovery.

Results of the study designed to examine day-to-day accuracy and precision of each of the three analytical procedures are presented in Table II. While the precision of the results obtained both by the GLC and spectrophotofluorometric procedures is less than that obtained in the extraction studies, this difference is larger with the GLC method. The precision of the colorimetric method is much less than was previously reported (3). While other factors may contribute to this discrepancy, one major consideration is that precision, in the latter study, was determined by analyzing many samples in 1 day. Results gathered on numerous days apparently would yield more meaningful information concerning precision, since this approach more closely approximates conditions under which the assay will be performed. The precision of results obtained by GLC analysis is comparable to that found previously (8).

Since the study was performed on plasma for 10 consecutive days, it is noteworthy that plasma concentration of procainamide

on Day 1 was not significantly different from that on Day 10, as determined by each of the three procedures. Procainamide in plasma stored under the conditions in this study appears to be stable for at least 6 weeks. However, in initial studies, a large quantity of plasma was spiked with procainamide, frozen, and thawed daily before samples were taken. When using this procedure, 21% of procainamide degraded by the 7th day. This result necessitated freezing procainamide in separate aliquots for analysis.

The specificity of the colorimetric and spectrophotofluorometric assays was investigated by determining apparant procainamide concentration in plasma of patients taking various drugs. Results were compared with results of the GLC assay (Table III).

Interferences in the colorimetric assay would be limited to those substances that possess a primary aromatic amine substituent or that have significant absorption at 550 nm and are extracted from the plasma. While fluorescamine will react with any primary amine under the proper conditions, its reactivity is limited in this procedure by adjusting the pH of the reaction medium to 5.5. Under these conditions, primary aliphatic amines do not react with fluorescamine (9). Thus, the selectivity of the two spectral procedures should be comparable.

Results of the three procedures differ significantly in only two cases, both of which were characterized by apparently higher levels of procainamide as determined by colorimetry. Since both samples were obtained from patients taking chlordiazepoxide, the potential interference of chlordiazepoxide in the colorimetric determination was investigated by using colorimetry to determine apparent procainamide levels in the plasma of patients taking only chlordiazepoxide. For three such patients, apparent procainamide levels were 2.12, 3.47, and 1.87 μg/ml. The explanation of this interference is not apparent, since known metabolites of the drug should not participate in the colorimetric reaction.

The utility of the procedures was also investigated by using each of the three methods to determine the procainamide concentration in the plasma of 17 patients taking only procainamide. Concentrations ranged from 1.23 to 15.31 μg/ml, and none of the values obtained by the three procedures differed by more than 17%.

The proposed spectrophotofluorometric procedure has several important advantages over the colorimetric procedure. Since extraction is quantitative, it is not necessary to extract spiked plasma samples to prepare calibration curves. Solutions (pH 5.5 phosphate buffer, pH 9.5 borate buffer, and fluorescamine) used in the assay are stable for at least 6 weeks, as opposed to the instability of the sodium nitrite and ammonium sulfamate solutions used for the colorimetric procedure. The proposed procedure appears to be somewhat more specific than the colorimetric procedure, as evidenced by results obtained from plasma of patients taking chlordiazepoxide. Precision of results obtained by the spectrophotofluorometric procedure is greater than that obtained by colorimetry. Finally, time is much less critical in this procedure, since the fluorescence has been shown to be stable for up to 24 hr (9), in contrast to the instability of the chromophore in the colorimetric procedure (7).

With respect to the GLC assay, a major advantage is the increased capacity for sample analysis by spectrophotofluorometry. Fifteen samples can easily be processed in 2 hr by a single technician. It is a simple, easily mastered procedure. Additionally, precision is greater than precision of the GLC assay. The spectrophotofluorometric procedure should not, however, be applied to analysis of procainamide in plasma of patients also taking other drugs (such as sulfonamides) that contain the primary aromatic amino substituent.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 4, 1974, from the *School of Pharmacy, University of Missouri–Kansas City, Kansas City, MO 64110*

Accepted for publication June 11, 1974.

Supported by Marion Laboratories, Inc.

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