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Determination of Procainamide and *N*-Acetylprocainamide in Biological Fluids by High-Pressure Liquid Chromatography

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Abstract □ A modification of a high-pressure liquid chromatographic method for the simultaneous determination of procainamide and *N*-acetylprocainamide in plasma is described. The deficiencies in the specificity of the existing method were overcome by replacing the cation-exchange column and the mobile phase. The recovery and reproducibility of both procainamide and *N*-acetylprocainamide from human, dog, and rat plasma and urine spiked with either compound were excellent in the concentration range of 0.05–10 μg/ml for plasma and 0.5–20 μg/ml for urine. The comparison of this method with a specific extraction method for sets of plasma samples from human subjects and rats receiving *N*-acetylprocainamide and procainamide, respectively, showed no statistically significant difference.

Keyphrases □ Procainamide—simultaneous high-pressure liquid chromatographic determination with *N*-acetylprocainamide in biological fluids □ *N*-Acetylprocainamide—simultaneous high-pressure liquid chromatographic determination with procainamide in biological fluids □ High-pressure liquid chromatography—analysis, procainamide and *N*-acetylprocainamide in biological fluids □ Antiarrhythmic agents—procainamide, simultaneous high-pressure liquid chromatographic determination with *N*-acetylprocainamide in biological fluids

High-pressure liquid chromatographic (HPLC) procedures for the determination of procainamide and its major metabolite, *N*-acetylprocainamide, in plasma have been described (1–6). One approach requires selective extraction of the drugs from plasma with an organic solvent, followed by reextraction or evaporation of the organic solvent prior to assay (1–4). The other approach involves deproteinization of plasma with acetonitrile and direct injection of the supernate following centrifugation (5). The former procedure usually is tedious. The latter procedure, while simple and rapid, may exhibit specificity and recovery deficiencies under certain conditions.

Nation *et al.* (7) suggested that the extraction method, although tedious and time consuming, may be a prudent approach for the determination of procainamide and *N*-acetylprocainamide. The present investigators experienced similar difficulties in using the deproteinization method. The purpose of this report is to describe a modified deproteinization method which produced consistent results and eliminated the lack of specificity and recovery. This result was achieved by replacing the cation-exchange column and the mobile phase utilized previously (5, 7).

EXPERIMENTAL

Materials—*N*-Acetylprocainamide hydrochloride[1] (an investigational new drug), procainamide hydrochloride USP[2], dioxane[3], toluene[3], acetonitrile[3], TLC plates[4], heparin USP[5], and a carbonated beverage[6] were obtained from commercial sources. Dioxane, toluene, and acetonitrile were glass distilled and purchased commercially. [^{14}C]Procainamide hydrochloride (specific activity 32 μCi/mg) and [^{14}C]-*N*-acetylprocainamide hydrochloride (specific activity 23.1 μCi/mg) were obtained commercially[7]. The internal standard, *p*-amino-*N*-(2-dipropylaminoethyl)benzamide, was synthesized in these laboratories.

Apparatus and Conditions—The high-pressure liquid chromatograph[8] was equipped with a 30-cm long × 4-mm i.d. alkylphenyl column[9] and a UV detector[10] at 280 nm. The column was eluted with a mobile phase that was a 60:40 mixture of acetonitrile and phosphate buffer (0.01 *M* NaH_2PO_4 and 0.005 *M* Na_2HPO_4, pH 6.6). The final pH of the mobile phase was 7.7. The flow rate was 2.0 ml/min. The solutions containing the drugs and the internal standard were injected into the chromatograph *via* an automatic processor[11].

The TLC plates were developed in an ascending fashion in a saturated chamber containing dioxane–toluene–ammonium hydroxide (80:30:1). The radioactivity on the developed TLC plates was detected by a radiochromatographic scanner[12], and the activity was determined on a liquid scintillation counter[13].

Preparation of Standards—The stock solution of the internal standard was prepared in methanol at a concentration of 2 mg/ml. This solution was diluted with acetonitrile to give a working standard solution of 1.5 μg/ml.

The standard solutions in plasma (human, dog, and rat) were prepared by spiking known amounts of procainamide and *N*-acetylprocainamide from a stock solution containing 1 mg/ml of each drug as the

[1] Napp Chemicals, Lodi, N.J.
[2] Ganes Chemicals, New York, N.Y.
[3] Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Aluminum oxide 60F-254, E. Merck, Rahway, N.J.
[5] Eli Lilly and Co., Indianapolis, Ind.
[6] Coca Cola.
[7] Custom synthesized, New England Nuclear, Boston, Mass.
[8] Model ALC/GPC 204, Waters Associates, Milford, Mass.
[9] μBondapak phenyl column, Waters Associates, Milford, Mass.
[10] Model 440, Waters Associates, Milford, Mass.
[11] Model 710 WISP, Waters Associates, Milford, Mass.
[12] Model 7201, Packard Instrument Co., Downers Grove, Ill.
[13] Model 2425, Packard Instrument Co., Downers Grove, Ill.

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Table I—Recovery and Reproducibility of Procainamide in Plasma and Urine

Concentration, μg/ml	Recovery[a], %				Reproducibility[b]			
	Plasma			Human Urine	Plasma			Human Urine
	Human	Dog	Rat		Human	Dog	Rat	
0.05	96.3 ± 4.98	100.5 ± 3.91	93.6 ± 9.53	—	94.8	96.1	89.8	—
0.1	94.3 ± 5.01	101.9 ± 4.37	96.2 ± 1.91	—	94.7	95.7	98.0	—
0.25	103.5 ± 1.96	94.7 ± 4.32	84.2 ± 3.64	—	98.1	95.4	95.7	—
0.5	99.1 ± 0.960	99.9 ± 0.808	99.0 ± 0.591	94.7 ± 1.01	99.0	99.2	99.4	98.9
0.75	101.0 ± 1.58	98.1 ± 0.693	96.4 ± 0.0	—	98.4	99.3	100.0	—
1.0	96.9 ± 1.15	100.1 ± 0.700	99.3 ± 0.730	102.6 ± 1.27	98.8	99.3	99.3	98.8
2.5	97.6 ± 2.10	98.6 ± 0.650	96.4 ± 0.0	100.9 ± 0.766	97.8	99.3	100.0	99.2
5.0	100.7 ± 2.01	97.9 ± 2.00	94.7 ± 1.23	99.0 ± 1.07	98.0	98.0	98.7	98.9
7.5	99.1 ± 1.40	—[c]	—[c]	99.1 ± 3.39	98.6	98.6	98.5	96.6
10.0	100.0 ± 1.21	100.0 ± 0.231	100.8 ± 0.500	98.3 ± 2.54	98.8	99.8	99.5	97.4
15.0	—	—	—	101.6 ± 2.46	—	—	—	97.6
20.0	—	—	—	102.9 ± 0.877	—	—	—	99.1
Mean	98.8	99.1	95.6	99.9	97.7	98.1	97.9	98.3
SD	3.45	2.99	5.56	3.08	1.60	1.69	3.11	0.972

[a] Mean ± *SD*, $n = 4$. [b] Values equal 100 minus percent coefficient of variation. [c] Contaminated aqueous standards.

Table II—Recovery and Reproducibility of *N*-Acetylprocainamide in Plasma and Urine

Concentration, μg/ml	Recovery[a], %				Reproducibility[b]			
	Plasma			Human Urine	Plasma			Human Urine
	Human	Dog	Rat		Human	Dog	Rat	
0.05	88.9 ± 3.62	95.9 ± 6.99	89.8 ± 0.299	—	95.9	92.7	99.7	—
0.1	93.8 ± 2.88	94.2 ± 3.89	92.9 ± 2.05	—	96.9	95.9	97.8	—
0.25	98.8 ± 2.19	91.2 ± 1.98	81.5 ± 0.750	—	97.8	97.8	99.1	—
0.5	98.3 ± 0.681	93.2 ± 0.751	92.5 ± 0.493	92.2 ± 0.503	99.3	99.2	99.5	99.5
0.75	97.3 ± 1.26	93.7 ± 0.693	92.7 ± 1.10	—	98.7	99.3	98.8	—
1.0	93.4 ± 1.10	93.6 ± 0.800	93.7 ± 0.939	101.7 ± 2.19	98.8	99.4	99.0	97.8
2.5	95.6 ± 1.15	92.9 ± 0.650	91.4 ± 0.450	100.6 ± 1.11	98.8	99.3	99.5	98.9
5.0	96.8 ± 2.08	91.8 ± 1.73	89.4 ± 1.14	97.7 ± 1.45	97.9	98.1	98.7	98.5
7.5	95.8 ± 1.54	—[c]	—[c]	98.6 ± 3.62	98.4	98.1	98.4	96.3
10.0	97.3 ± 1.65	95.5 ± 2.38	95.4 ± 0.245	96.9 ± 2.68	99.8	97.5	99.7	97.2
15.0	—	—	—	101.2 ± 2.46	—	—	—	97.6
20.0	—	—	—	102.0 ± 1.27	—	—	—	98.8
Mean	95.6	93.6	91.0	98.9	98.1	97.7	99.0	98.1
SD	3.33	2.98	3.94	3.65	1.02	2.08	0.616	1.04

[a] Mean ± *SD*, $n = 4$. [b] Values equal 100 minus percent coefficient of variation. [c] Contaminated aqueous standards.

free base in water. The plasma standards were 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0, 7.5, and 10 μg/ml of each drug as the free base.

The standard solutions in human urine were prepared from a stock solution containing 1 mg/ml each of procainamide and *N*-acetylprocainamide as the hydrochloride salts. The concentrations of the standards were 0.5, 1.0, 2.5, 5.0, 7.5, 10, 15, and 20 μg/ml of procainamide hydrochloride and *N*-acetylprocainamide hydrochloride.

Preparation of Samples—To compare the deproteinization method with the extraction method (7), plasma samples were obtained from a clinical study in which a normal subject was given a 500-mg *N*-acetylprocainamide hydrochloride tablet every 6 hr for 54 hr and from a metabolism study in which rats received procainamide hydrochloride (1280 mg/kg/day) mixed in the diet for 6 months. The human plasma samples were collected at 30, 36, 48, 49, 50, 54.5, 55.0, 56, 58, and 60 hr following the first dose.

Procedure—To a 0.1- or 0.5-ml plasma or urine sample containing 0.05–20 μg each of *N*-acetylprocainamide and procainamide/ml was added an equal or double volume, respectively, of acetonitrile containing 1.5 μg of the internal standard [*p*-amino-*N*-(2-dipropylaminoethyl)-benzamide]/ml. The mixture was vortexed for 10 sec and then centrifuged for 5 min at 4500 rpm. The supernatant solution was decanted, and 50 μl was applied for HPLC analysis.

The solvent extraction method consisted of the following sequences. A mixture of 0.1 ml of plasma, 0.5 ml of water, and 0.1 ml of 1 *M* NaOH was vortexed for 1 min with 3 ml of ethyl acetate. After centrifugation, most of the organic layer was transferred to a test tube and vortexed for 1 min with 0.1 ml of 0.5% phosphoric acid. The tube was centrifuged, and the aqueous phase was separated, evaporated to dryness, and reconstituted in water–acetonitrile (1:2). An aliquot of this solution was injected.

The specificity of the HPLC method was examined using [^{14}C]-*N*-acetylprocainamide and [^{14}C]procainamide and TLC. Briefly, plasma samples containing both drugs were deproteinized with two volumes of acetonitrile, vortexed, and centrifuged. Fifty microliters of the supernate was spotted on TLC plates, and the chromatograms then were developed. The developed plates were examined by a radiochromatographic scanner, and the activity corresponding to *N*-acetylprocainamide and procainamide spots was determined by liquid scintillation. Additionally, 20-sec fractions of the HPLC eluate were collected for radioactivity determination to establish the entire high-pressure liquid chromatogram.

RESULTS AND DISCUSSION

The HPLC retention times of *N*-acetylprocainamide, procainamide, and the internal standard were 3.5, 4.4, and 5.6 min, respectively. Tables I and II summarize the recovery and reproducibility of the assay for human, dog, and rat plasma as well as for human urine for a wide range of procainamide and *N*-acetylprocainamide concentrations. The relationships between the peak height ratios and drug concentrations were linear over the entire range studied for both plasma and urine. The assay recovery of procainamide for human, dog, and rat plasma was 98.8, 99.1, and 95.6%, respectively; the recovery of *N*-acetylprocainamide was 95.6, 93.6, and 91.0%, respectively. The recovery of procainamide and *N*-acetylprocainamide for human urine was 99.9 and 98.9%, respectively. Both plasma and urine recoveries were concentration independent throughout the range studied.

The average reproducibility of the assay for each set of samples (n = 32–40) was ~98%. The use of an internal standard with similar physicochemical characteristics to the drugs contributed to the accuracy and high reproducibility. The TLC–HPLC comparison produced essentially the same results with respect to the specificity and recovery of the assay. Additionally, 48-hr incubation of plasma samples containing *N*-acetylprocainamide and procainamide at room temperature showed 93.5 ± 8.31 and 95.6 ± 3.76% (mean ± *SD*, n = 10) of the spiked values, respectively, indicating no significant degradation of either drug. The resolution of the column as tested by radioactivity measurement was consistent at all times, regardless of aging of the alkylphenyl column, and symmetrical peaks were produced with no indication of interfering compounds. Similarly, the radiochromatographic scanning of TLC plates showed no interfering peaks.

Although the lack of specificity of the assay may result from drug metabolites, it often may be due to the presence of endogenous and/or

Table III—Comparison of Direct Deproteinization Method to Extraction Method for the HPLC Determination of Procainamide and *N*-Acetylprocainamide in Plasma

Clinical Study[a]			Metabolic Study of Procainamide in Rats[b]					
Steady-State Plasma Concentrations of *N*-Acetylprocainamide, μg/ml			Procainamide Concentration, μg/ml			*N*-Acetylprocainamide Concentration, μg/ml		
Deproteinization Method (I)	Extraction Method (II)	I/II	Deproteinization Method (I)	Extraction Method (II)	I/II	Deproteinization Method (I)	Extraction Method (II)	I/II
3.27	3.33	0.982	0.720	0.762	0.945	0.672	0.776	0.866
4.10	4.20	0.976	0.833	0.897	0.929	0.610	0.617	0.987
4.08	4.09	0.998	2.23	2.06	1.08	0.904	0.970	0.932
4.39	4.33	1.01	1.67	1.68	0.994	0.877	1.06	0.827
5.65	5.82	0.971	0.698	0.706	0.948	1.09	1.15	0.948
4.32	4.52	0.956	0.281	0.351	0.801	2.27	2.22	1.02
4.49	4.58	0.980	0.338	0.310	1.09	0.397	0.411	0.966
6.95	6.89	1.01	0.737	0.744	0.991	0.820	0.823	0.996
5.54	5.50	1.01	1.34	1.33	1.01	1.17	1.13	1.04
4.34	4.40	0.986	1.02	1.02	1.00	0.726	0.767	0.947
Mean		0.988			0.979			0.953
SD		0.0186			0.082			0.066

[a] A normal subject received 500 mg of *N*-acetylprocainamide hydrochloride every 6 hr for 54 hr. [b] Rats received procainamide hydrochloride mixed in the diet of 1280 mg/kg/day for 6 months.

exogenous compounds. Plasma samples often are heparinized, and traces of heparin can be a source of interference with the drug peaks, particularly in the deproteinization techniques. This problem was observed with at least two HPLC columns differing in their polarity and under various elution systems. Unless a suitable chromatographic system is selected for a given compound, heparin probably will be a source of error in the assay of drugs. In this method, the retention time of heparin was 2.2 min and that of components of coffee or a carbonated beverage was <2 min. Previous investigators (8, 9) noticed that chemicals contained in vacutainer stoppers may interfere with extraction procedures for lidocaine and procainamide for a GLC measurement.

The application of both methods to human and rat plasma samples produced results with no statistically significant differences (paired *t* test) between the method described in this study and the specific extraction method (7) with respect to *N*-acetylprocainamide in humans and procainamide and *N*-acetylprocainamide in rats (Table III). The concentration ratio of the deproteinization method to the extraction method averaged 95.3–97.9%.

The proposed assay offers the same simplicity of the deproteinization procedure published previously (5, 7). However, the use of the alkylphenyl column with an appropriate mobile phase produces more consistent and reliable results. This procedure was tested with numerous samples obtained from subjects or animals given *N*-acetylprocainamide, procainamide, or both without any difficulties or specificity problems.

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Synthesis and Biological Evaluation of *p*-Bromospiperone as Potential Neuroleptic Drug

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Received February 19, 1980, from the **Chemistry Division, Argonne National Laboratory, Argonne, IL 60439,* and the ‡*Department of Radiology and the* §*Department of Psychiatry, University of Chicago, Chicago, IL 60637.* Accepted for publication March 18, 1980.

Abstract □ *p*-Bromospiperone was prepared from the reaction of spiperone with bromine. It was tested for dopamine receptor binding affinity *in vitro* and its ability to stimulate prolactin secretion *in vivo*. The results indicate no significant change of biological activities due to the bromination of spiperone.

Keyphrases □ Spiperone—*p*-bromo analog, synthesis and biological evaluation as a potential neuroleptic □ Neuroleptics, potential—*p*-bromospiperone, synthesis and biological evaluation □ Prolactin secretion—stimulation by *p*-bromospiperone

Spiperone (I) is one of the most potent neuroleptic drugs in clinical use. This drugs binds extensively to the dopamine receptors in the brains of rats and mice (1, 2). Attempts have been made to study the receptor–drug inter-

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