Rapid and Micro High-Pressure Liquid Chromatographic Method for Simultaneous Determination of Procainamide and *N*-Acetylprocainamide in Plasma

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Abstract □ A rapid and simple high-pressure liquid chromatographic method was developed for the simultaneous determination of plasma levels of procainamide and its major metabolite, *N*-acetylprocainamide. Plasma samples were deproteinized with acetonitrile, and the supernate was chromatographed on a cation-exchange column. The assay can be carried out on as little as 20 μl of plasma and requires only about 7 min for each sample. No interference was found in plasma samples from cardiac patients receiving procainamide. This method is simple, fast, and useful for routine therapeutic monitoring and for pharmacokinetic studies of procainamide and its metabolite.

Keyphrases □ Procainamide—high-pressure liquid chromatographic analysis simultaneously with *N*-acetylprocainamide in plasma □ *N*-Acetylprocainamide—high-pressure liquid chromatographic analysis simultaneously with procainamide in plasma □ High-pressure liquid chromatography—analyses, procainamide and *N*-acetylprocainamide simultaneously in plasma □ Cardiac depressants—procainamide, high-pressure liquid chromatographic analysis simultaneously with *N*-acetylprocainamide in plasma

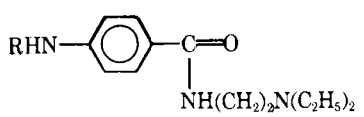
The importance of monitoring plasma procainamide concentrations has been realized for more than 2 decades (1). As much as a sevenfold difference in plasma procainamide concentrations could result from the same daily dose regimen (1, 2). Large variations were found in the daily dose of procainamide necessary to suppress ventricular arrhythmias in different patients (3).

BACKGROUND

The individual disparity in drug absorption, metabolism, and excretion was postulated as a plausible explanation for observed variations (4). These observations indicate that the plasma procainamide concentration correlates more closely than the dose regimen to the therapeutic and toxic responses in patients. The optimal antiarrhythmic activity of procainamide occurs in the 4–8-μg/ml plasma concentration range. Concentrations between 8 and 16 μg/ml are considered potentially toxic. Toxicity, such as arrhythmia and hypertension, often is associated with concentrations greater than 16 μg/ml (1).

N-Acetylprocainamide, the major metabolite of procainamide, also has antiarrhythmic activity comparable to that of the parent drug (5); its concentrations in plasma could range from 60 to 180% of procainamide (6). Therefore, it is highly desirable to monitor simultaneously plasma concentrations of procainamide and *N*-acetylprocainamide. Methods to measure one or both of these compounds in biological samples include spectrophotometry (1, 6), fluorometry (1), GLC (4, 7), mass fragmentography (8), TLC densitometry (6), and high-pressure liquid chromatography (HPLC) (9, 10).

The spectrophotometric (1, 6) and fluorometric (1) methods are less selective and cannot measure procainamide and *N*-acetylprocainamide simultaneously. The mass fragmentographic method (8) is probably not convenient for routine monitoring. The GLC (7) and TLC (6) methods can measure the two compounds simultaneously in biological fluids. The HPLC method has been used for procainamide and lidocaine in serum (9). More recently, an HPLC determination of procainamide and *N*-acetylprocainamide was described (10). All these reported methods require 1 or 2 ml of sample and use up to 6 ml of solvent for extraction.



I: R = H
II: R = CH_3CO

This paper reports a simple and fast HPLC method for the simultaneous determination of procainamide and *N*-acetylprocainamide with as little as 20 μl of plasma.

EXPERIMENTAL

Reagents—Procainamide hydrochloride[1], *N*-acetylprocainamide hydrochloride[1], ammonium phosphate[2], phosphoric acid[2], and acetonitrile[3] were used.

Apparatus—The HPLC system consisted of solvent delivery pumps[4], a septumless sample injector[5], an ion-exchange column[6], and a spectrophotometric detector[7].

Procedure—Aliquots of 0.1 ml of plasma in 13 × 100-mm culture tubes were vortexed with 0.25 ml of acetonitrile for 10 sec and centrifuged for 1 min. The clear supernate was poured directly into another set of culture tubes (although this step is not necessary). Aliquots of 100 μl were injected into the column and chromatographed.

The mobile phase consisted of 80% (v/v) 0.1 *M* ammonium phosphate acidified with phosphoric acid (0.05%) to pH 2.5 ± 0.05 and 20% acetonitrile; the flow rate was 2 ml/min. The separated sample components from the column effluent were monitored by UV absorption at 274 nm, and the chromatograms were recorded on a potentiometric recorder at 0.02 absorbance unit full scale and a chart speed of 4 mm/min.

The calibration curves were prepared by analyzing 0.1-ml plasma samples spiked with procainamide and *N*-acetylprocainamide (0.88 mg/ml–21.22 μg/ml) from concentrated stock solutions in methanol and plotting the peak heights *versus* the concentrations. Peak height measurements were used for all quantitations. All separations were carried out at ambient temperature.

RESULTS AND DISCUSSION

The UV spectra of procainamide (I) and *N*-acetylprocainamide (II) are shown in Fig. 1. With the mobile phase as a solvent, procainamide showed absorption maxima at 282 and 204 nm while *N*-acetylprocainamide showed absorption maxima at 268 and 205 nm. These data suggest that the most sensitive detections can be obtained by monitoring absorbance at 205 nm. Although UV absorbance measurements at wavelengths as low as 199 nm have been used in liquid chromatography (9) to obtain very sensitive detection, attempts to monitor these compounds at 205 nm encountered some difficulty because of the interference of some endogenous plasma compounds. Therefore, the absorbance at 274 nm appears to be the optimal compromise between sensitivity and selectivity.

Since procainamide is a weak base [pKa 9.4 (11)] and is ionized at a low pH, a cation-exchange column was chosen. Although a reversed-phase column had been used successfully for the quantitation of procainamide (9), the ion-exchange column was preferred in that it separated both the parent drug and its metabolite. A good linear relationship between peak height measurements and concentrations was observed even when a large volume (up to 100 μl) of sample was injected. When a large volume of

[1] E. R. Squibb & Sons, Princeton, N.J.
[2] Fisher Scientific, Fair Lawn, N.J.
[3] Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Model A-6000, Waters Associates, Milford, Mass.
[5] U6K, Waters Associates, Milford, Mass.
[6] Partisil PXS 10/25 SCX, Whatman, Clifton, N.J.
[7] Model LC 55, Perkin-Elmer Corp., Norwalk, Conn.

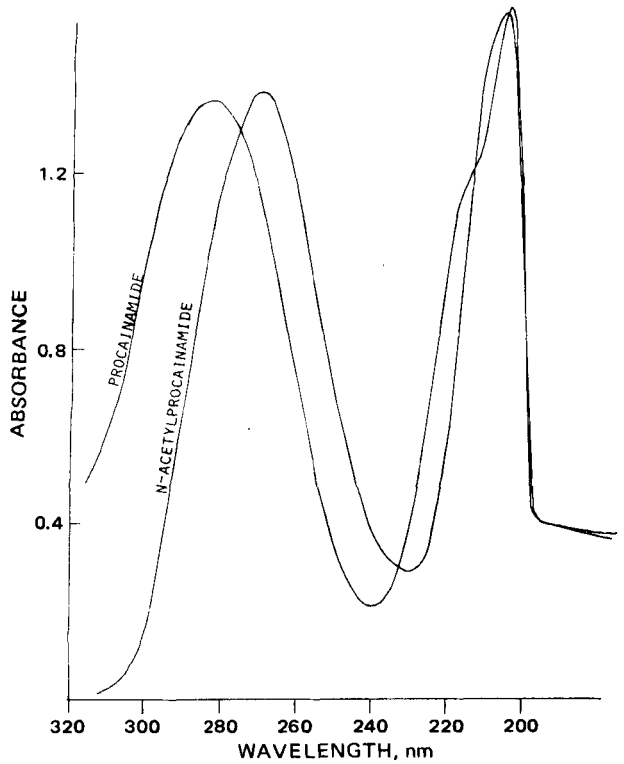


Figure 1—*UV spectra of procainamide hydrochloride (20 μg/ml) and N-acetylprocainamide hydrochloride (20 μg/ml) in the HPLC mobile phase.*

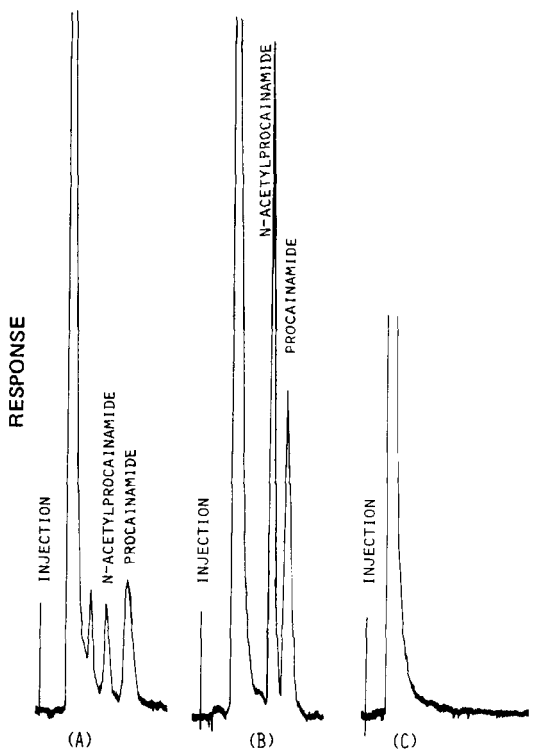


Figure 2—*Chromatograms of procainamide (2.8 μg/ml) and N-acetylprocainamide (1.1 μg/ml) from plasma of a patient on procainamide (A), from spiked (7.5 μg/ml) plasma (B), and plasma blank (C).*

sample was chromatographed on a reversed-phase column, peak broadening was generally observed, and accurate measurement of the peak height became more difficult.

Figure 2 shows a chromatogram of procainamide and *N*-acetylprocainamide from a plasma sample obtained immediately after administration of 750 mg of procainamide by intravenous infusion over 20 min to an adult patient. Chromatograms of a plasma blank with and without addition of procainamide and *N*-acetylprocainamide are also included. Plasma samples from both healthy subjects and renal patients showed no peak that would interfere with the assay. Under the conditions employed, procainamide and *N*-acetylprocainamide were eluted from the ion-exchange column at 5 and 4 min, respectively (Fig. 2).

The linearity of standard curves of procainamide and *N*-acetylprocainamide is shown in Table I. The peak heights were proportional to plasma concentrations for both compounds in all ranges studied (up to 21 μg/ml), as indicated by the fairly constant response factors (peak height/concentration). The coefficients of variation for the intraassay ($n = 4$) and interassay ($n = 6$) of procainamide (1.73 μg/ml) were 6.36 and 8.67%, respectively. The coefficients of variation for *N*-acetylprocainamide (1.77 μg/ml) were 1.72 and 2.25% for the intraassay ($n = 4$) and interassay ($n = 6$), respectively. By this method, the low limit of detection was 0.2 μg/ml for both compounds in plasma, which is better than the reported GLC assay (7).

Table I—Linearity of Standard Curves for Procainamide and *N*-Acetylprocainamide

Concentration[a] Spiked in Plasma, μg/ml	Peak Height, cm		Response Factor[b]	
	Procainamide	*N*-Acetylprocainamide	Procainamide	*N*-Acetylprocainamide
0.88	1.0	2.3	1.14	2.61
1.77	2.0	4.5	1.13	2.54
2.65	3.0	6.85	1.13	2.58
3.54	4.1	9.0	1.16	2.54
7.07	8.1	17.9	1.15	2.53
10.61	11.9	26.8	1.12	2.53
14.14	16.0	36.1	1.13	2.55
21.22	23.8	54.0	1.12	2.54
		Average	1.135	2.553
		SD	0.013	0.028

[a] As free base of drugs. [b] Peak height/concentration.

This procedure was employed successfully to monitor the plasma procainamide and *N*-acetylprocainamide levels of patients receiving procainamide and other drugs such as phenobarbital. In all patient samples analyzed, no interference was encountered. In samples showing excessive hemolysis of red blood cells, successful assays also could be performed. Analysis of plasma supplemented with phenytoin, salicylic acid, sulfisoxazole, chloramphenicol, and acetaminophen showed that these drugs did not interfere with the assay.

By using the simple deproteinization method for sample preparation, no solvent extraction, transfer, or evaporation steps were needed. The simplicity of the procedure can help in reducing experimental errors.

Acetonitrile precipitated plasma proteins as a solid mass, adhering to walls of the culture tube after mild centrifugation (2000 rpm), to give a clear supernate which was easy to pour out, almost quantitatively. Other water-miscible organic solvents, such as methanol, ethanol, and acetone, were less satisfactory in that a clear supernate could not be obtained with mild centrifugation. Although procainamide and its metabolite are partially bound to proteins in plasma (6), the recovery from plasma by acetonitrile deproteinization was essentially 100%, based on the peak area analysis, as compared to the addition of acetonitrile to the aqueous solutions of both compounds in the ratio of 2.5:1 (v/v). However, the peak heights from the procainamide-spiked plasma were 20% shorter from equal concentrations spiked in water. This result may be due to the peak widening effect. However, this effect was not found with *N*-acetylprocainamide.

Since, at higher concentrations (>1 μg/ml), chromatograms from the injection of 50 μl of supernates of the acetonitrile–plasma mixture gave peak heights sufficient for accurate quantitation, the assay can be carried out on as little as 20 μl of plasma deproteinized with 50 μl of acetonitrile. This method should be useful when only a small amount of sample is available. The results can be obtained in about 7 min for a single sample. In terms of speed, sensitivity, and simplicity, this method is superior to most methods reported for monitoring plasma levels of procainamide and *N*-acetylprocainamide.

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Synthesis of Potential Antineoplastic Agents XXVI: 1,3,4,6,7,11b-Hexahydro-9,10-dimethoxy-2*H*-benzo[*a*]2-quinolizinone Derivatives

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Received May 23, 1977, from the **Department of Chemistry, University of Missouri–Kansas City, Kansas City, MO 64110,* and the ‡*Department of Chemistry, Clarkson College of Technology, Potsdam, NY 13676.* Accepted for publication September 22, 1977.

Abstract □ A number of 3-alkyl-1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-2*H*-benzo[*a*]2-quinolizinones and 2-substituted 3-ethyl-1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-2*H*-benzo[*a*]quinolizines were prepared and submitted for antineoplastic and anticonvulsant screening.

Keyphrases □ Benzo[*a*]quinolizines, various substituted—synthesized, evaluated for antineoplastic and anticonvulsant activity □ Antineoplastic activity—evaluated in various substituted benzo[*a*]quinolizines □ Anticonvulsant activity—evaluated in various substituted benzo[*a*]quinolizines □ Structure–activity relationships—various substituted benzo[*a*]quinolizines evaluated for antineoplastic and anticonvulsant activity

During work directed toward the synthesis of analogs of emetine (I), 1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-3-ethyl-2*H*-benzo[*a*]2-quinolizinone (II) was synthesized (1). The hydrochloride salt of II was active in the L-1210 lymphoid leukemia system.

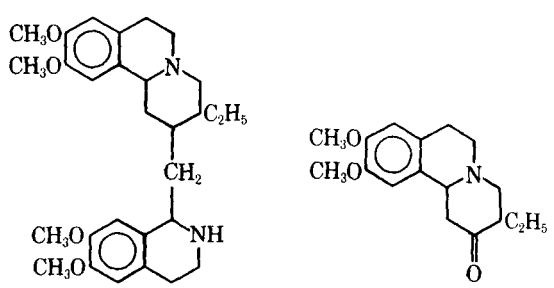
DISCUSSION

To determine the effect of structure on the antineoplastic activity of II hydrochloride, a series of related compounds was prepared. The compounds investigated fall into two categories: analogs in which the ethyl group of II has been replaced by a series of other alkyl groups (III*a*–III*i* and V, Table I) and analogs in which the carbonyl group of II has been replaced by other groups (IV*a*–IV*i*, VI, and VII, Table II).

The compounds in Table I were prepared by reaction of 3,4-dihydro-6,7-dimethoxyisoquinoline and the appropriate Mannich bases (1). New compounds, including the dimer V, are discussed under *Experimental*. All known compounds had melting points in agreement with reported values (2–4).

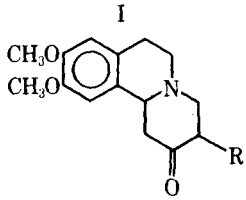
Compounds IV*a*–IV*c* were prepared by the method of Openshaw and Whittaker (5), as was the propyl analog VI. The oxime (IV*d*) (6) and IV*e* and IV*f* (7) were prepared by previously reported methods. The preparation of the remaining compounds in Table II is reported under *Experimental*.

The compounds in Tables I and II were screened through the Drug Evaluation Branch of the National Cancer Institute in the L-1210 lymphoid leukemia or P-388 lymphocytic leukemia systems. None of the compounds with groups in place of the 2-carbonyl possessed any significant antineoplastic activity (Table II). A number of the analogs in Table

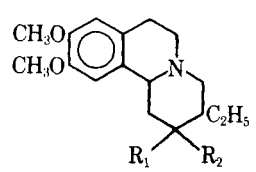


I

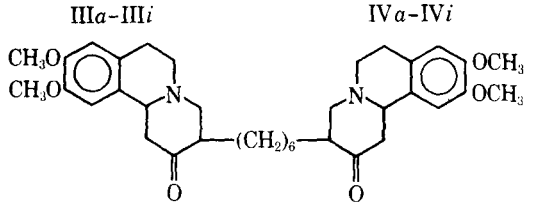
II



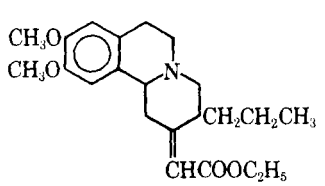
III*a*–III*i*



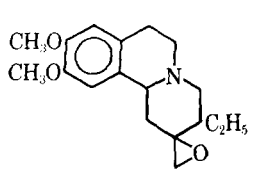
IV*a*–IV*i*



V



VI



VII