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Rapid Determination of Procainamide and Its *N*-Acetyl Derivative in Human Plasma by High-Pressure Liquid Chromatography

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Abstract □ A rapid, specific, high-pressure liquid chromatographic method for the determination of procainamide and its *N*-acetyl derivative in plasma was developed. The procedure is fast enough (15 min from receipt of blood to reporting value) to be used for emergency determinations. The sensitivity, precision, and accuracy are sufficient for routine monitoring of therapeutic levels in patients. The assay is specific enough to be valid in the presence of a number of drugs and dietary substances present in clinical samples.

Keyphrases □ Procainamide and *N*-acetyl derivative—high-pressure liquid chromatographic analyses, human plasma □ High-pressure liquid chromatography—analyses, procainamide and *N*-acetyl derivative, human plasma □ Cardiac depressants—procainamide and *N*-acetyl derivative, high-pressure liquid chromatographic analyses, human plasma

Procainamide (I) is an antiarrhythmic compound which demonstrates marked intersubject variability in absorption and elimination (1). Its *N*-acetyl metabolite (II) in humans (2) has antiarrhythmic activity comparable to that of I (3) but demonstrates an even greater variability in plasma concentration with a given I dosage schedule. Methods of monitoring plasma I concentrations that do not also determine II are not adequate.

Recently, several methods for the simultaneous determination of I and II were reported. A fluorometric method required more than 4 ml of blood for a duplicate determination and involved reextraction into acid, addition of alkali, and two fluorescence measurements (4). No mention was made of the possible interference of the fluorescent base quinidine, which would be extracted under these conditions and might be present in patients receiving procainamide.

A GLC method was reported for I and II determinations in urine samples (5), but no mention was made of its application to blood samples. Another GLC method for the determination of both I and II in blood, urine, and saliva samples was reported (6). However, each duplicate analysis probably required 1 hr or more because of a three-stage acid–base extraction, evaporation, and the 23.5-min retention time for II.

A quantitative TLC approach to the problem was developed (2). The reported standard deviations of 9% for I and 11% for II reflect a well-known difficulty of quantitative TLC.

None of the reported methods claimed the combination of small sample size, rapidity, specificity, and precision desirable for routine patient monitoring. This paper describes a method for performing a duplicate analysis in 16 min on as little as 300 μl of whole blood with sufficient accuracy, precision, and specificity for clinical samples.

EXPERIMENTAL

Materials—UV grade hexane, 2-propanol, methanol, 1-butanol, and chloroform[1] and reagent grade concentrated ammonium hydroxide[2] were used as obtained. High purity samples of I and II[3] were used in the preparation of standards.

Apparatus—A high-pressure liquid chromatograph[4] was equipped with a septumless injection port[5] and a fixed wavelength (280 nm) UV absorption detector[6]. The detector was operated at 0.005 and 0.01 absorbance unit full scale.

Chromatographic Parameters—The mobile phase was prepared by diluting concentrated ammonium hydroxide 10:1 with distilled water. One milliliter of this solution was added to 1 liter of 2-propanol and 1 liter of hexane, and the solution was diluted to 5 liters with methanol. The mobile phase was pumped at 5.0 ml/min (3500 psi) through a stainless steel column (4 mm i.d. × 30 cm) packed with a high efficiency bonded-phase absorption packing[7].

Analytical Procedure—Two 50-μl aliquots of each plasma sample were placed in 1.5-ml disposable centrifuge tubes[8]. To each tube was added 10 μl of 5 *N* NaOH solution and 600 μl of an organic extraction solution containing 20% 1-butanol, 20% chloroform, and 60% hexane by volume. The two tubes, together with any other samples being run at the same time, were shaken for 30 sec at 1350 oscillations/sec[9] and then centrifuged for 30 sec at 15,000 rpm[10].

[1] Burdick and Jackson Laboratories, Muskegon, Mich.
[2] Mallinckrodt Chemical Works, St. Louis, Mo.
[3] E. R. Squibb & Sons.
[4] Model 6000A, Waters Associates, Milford, Mass.
[5] Model U6K, Waters Associates, Milford, Mass.
[6] Model 440, Waters Associates, Milford, Mass.
[7] μBondapak CN, Waters Associates, Milford, Mass.
[8] Brinkmann Instruments, Westbury, N.Y.
[9] Eppendorf Micro Shaker, Brinkmann Instruments, Westbury, N.Y.
[10] Eppendorf Micro Centrifuge, Model 3200, Brinkmann Instruments, Westbury, N.Y.

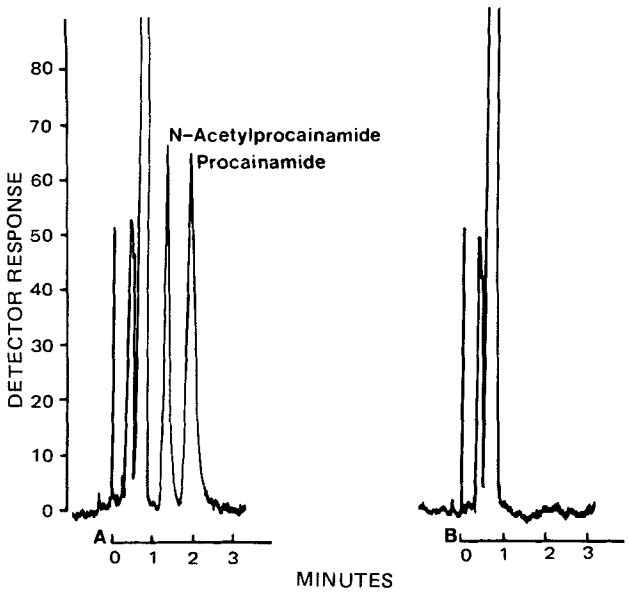

Figure 1—*High-pressure liquid chromatograms of human plasma extracts. Key: A, plasma containing 5 μg of I/ml and 5 μg of II/ml; amd B, control plasma.*

After centrifugation, exactly 100 μl of the supernate from the first tube was measured accurately with a syringe[11] and injected into the chromatograph. Three minutes after the first injection, 100 μl from the second tube was injected. Three minutes after the second injection, the chromatogram was removed and the peak heights were measured in millimeters. The peak heights were converted to plasma I and II concentrations by comparison to a standard curve.

Standard Curve—Stock solutions of 0.10 mg of I/ml and 0.10 mg of II/ml were prepared by dissolving weighed drug samples in methanol. Appropriate aliquots of these standards were added to conical centrifuge tubes and evaporated under nitrogen. Sufficient pooled human plasma was added to each tube to produce final concentrations of 2, 4, 6, 8, 10, 15, and 20 μg/ml. The tubes were vortexed to dissolve the drugs, and then 100-μl aliquots were removed for analysis by the described procedure.

Eight analyses were done at each concentration. The heights of the chromatographic peaks were measured in millimeters. A straight-line fit of the data was made by linear regression analysis, and the correlation coefficient was determined.

Accuracy and Precision—The accuracy and precision of the method were evaluated by performing 48 duplicate analyses on spiked samples of human plasma. Twelve duplicate analyses were performed at each of the following concentrations: 2, 4, 6, and 8 μg/ml. The spiked samples were prepared by adding appropriate aliquots of the 0.10-mg/ml stock solution to centrifuge tubes, evaporating the methanol in a nitrogen stream, and then dissolving I and II in 100-μl aliquots from selected plasma samples.

The 48 plasma samples used were left over from blood samples originally drawn for analyses of other drugs from patients known not to be receiving I or II. After preparation, the spiked samples were analyzed as described; the mean and standard deviation of the measured values were determined.

Evaluation of Interferences—Samples of caffeine, theobromine, theophylline, 3-methylxanthine, quinidine, and propranolol were dissolved in the extraction solution. Aliquots (100 μl) of these solutions were injected into the chromatograph, and the retention times of the drugs were determined. The possibility of interferences also was tested by performing the analysis on a series of plasma samples from patients receiving drugs other than procainamide. Any interferences from quinidine, primidone, phenobarbital, phenytoin, and their metabolites were detected in this way.

RESULTS AND DISCUSSION

Figure 1A shows the chromatogram obtained by injecting 100 μl of the extract of the plasma sample spiked with 5 μg of I/ml and 5 μg of II/ml. The method of analysis described under *Analytical Procedure* was used.

[11] Precision Sampling Corp.

Table I—Accuracy and Precision of HPLC Assay for I and II

I Added, μg/ml	II Added, μg/ml	I Found, μg/ml	*SD* of I Found, %	II Found, μg/ml	*SD* of II Found, %
4	4	3.95	3.0	3.93	2.4
6	6	5.94	2.2	5.96	2.2
8	8	8.04	2.1	7.92	1.4
10	10	10.13	1.2	10.06	1.4

Figure 1B shows the chromatogram of a 100-μl injection of a plasma sample extracted in exactly the same way but with no added I or II. Figure 1B shows that all significant artifact peaks and baseline variations due to the injection of 100 μl of extraction solvent plus components extracted from plasma were complete within about 1 min after injection. Figure 1A shows that the I and II peaks were well separated from each other and from the artifact peaks.

The I and II peak heights (in millimeters) measured on the chromatograms were plotted against the concentrations (in micrograms per milliliter) of I and II in the plasma standard solutions to prepare a standard curve. Twelve plasma samples were assayed at each concentration: 2, 4, 6, 8, 10, 15, and 20 μg/ml of both I and II. The curves obtained by linear regression analyses of these data were $y = 22.8x - 4.76$ for I and $y = 22.5x - 4.6$ for II. The regression coefficients were 0.9999 for I and 0.9996 for II.

The method is sufficiently accurate and precise for routine analysis of clinical samples (Table I). The time required for a duplicate analysis, from centrifugation of the blood to final calculations, is only 16 min. In this laboratory, all routine I and II analyses are run once a day along with external standards. The stat samples are run as soon as received and are checked by external standards, which are run after the values are reported. It has never proven necessary to recall a stat value.

Several drugs that might be taken by patients also taking procainamide were tested for interference with the procainamide assay. Plasma samples from patients having therapeutic levels of quinidine, primidone, phenobarbital, and phenytoin but no procainamide were analyzed. The samples were extracted and chromatographed in exactly the same way as for a I and II analysis. In none of these cases was there a peak giving a false I or II level. In some cases, this result was probably due to the fact that the drug or its metabolites did not extract under the assay conditions. Injection of solutions of caffeine, theobromine, theophylline, 3-methylxanthine, quinidine, and propranolol made up in the extraction solvent failed to reveal any interferences. Only quinidine gave a peak, with a retention time of about 1 min, and it was well resolved from II.

A total of 37 clinical samples have been run by this method. In all of these samples as well as in all controls, no significant peaks with retention times longer than the procainamide have been observed. This result is perhaps due partly to the fact that substances more polar than procainamide tend to be left behind in the aqueous phase during extraction. An important conclusion is that the extractions into less polar solvents followed by normal phase liquid chromatography can lead to short chromatographic runs and short analysis times.

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