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SIMULTANEOUS QUANTIFICATION OF PROCAINAMIDE AND N-ACETYLPROCAINAMIDE WITH HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A rapid, precise and specific method for the simultaneous determination of procainamide and N-acetylprocainamide (NAPA) in plasma using high-performance liquid chromatography is described. N-Formylprocainamide was utilized as internal standard. The coefficients of variation of the method for both procainamide and NAPA were 3.6% in the range of plasma levels to be expected clinically. The method is especially useful for rapid determination of acetylator phenotype in patients requiring procainamide for control of arrhythmias.

INTRODUCTION

The therapeutic index of procainamide (PA)* is not large, and the interpatient variation in steady-state plasma concentrations of the drug is considerable[1]. Thus, assessment of levels of active drug in plasma should allow for better individualization of dosage regimens. This objective is complicated however, by recent evidence suggesting that PA's major metabolite, N-acetylprocainamide (NAPA), also has antiarrhythmic activity[2]. Having a lower clearance than the parent drug, this metabolite will accumulate to a greater degree. Steady-state levels of NAPA will be considerably higher in rapid acetylators than in those individuals whose drug acetylation is slow. Accordingly, it would be helpful to have a rapid, precise and specific method for measuring, simultaneously, the levels of parent drug and active metabolite in plasma. In addition to potential value in therapy and in research on this drug, such a method should allow classification of patients receiving the drug according to acetylator phenotype without having to discontinue PA therapy to administer a second acetylation substrate such as dapsone or sulfamethazine. Knowledge of any possible relationship of acetylation phenotype to the lupus erythematosus-like syndrome produced by PA would be of considerable value.

Reidenberg *et al.* have developed a thin-layer densitometric technique for

* *p*-Amino-N-(2-diethylaminoethyl)benzamide.

determining acetylator phenotype based on a ratio of PA to NAPA in plasma[3]. Although such ratios can be determined by this method, it is inconvenient for routine laboratory usage and yields calibration curves which have standard deviations of 9 and 11% for PA and NAPA, respectively. Karlsson *et al.*[4] have described a method for the simultaneous measurement of PA and NAPA in urine by gas–liquid chromatography (GLC) but were unable to use this technique for plasma because of an interfering peak which prevents quantification of PA. Atkinson *et al.*[5] have measured PA in plasma utilizing GLC with a standard deviation of the method averaging as much as 8%. Although NAPA does not interfere with this assay, it was not measured simultaneously. Elson *et al.*[6] have simultaneously measured PA and NAPA in plasma using GLC with a precision of 4 and 5%, respectively. Other methods are less specific. Colorimetric analysis is susceptible to interference by commonly used drugs such as chlordiazepoxide; an example of the non-specificity that renders the method unreliable for general usage[7].

The spectrophotofluorometric method of Klein and Koch-Weser[8] is probably the most commonly used technique for routine determination of concentrations of PA in plasma. However, interference by NAPA limits the specificity of the test. Accordingly, we have investigated the utility, sensitivity and specificity of high performance liquid chromatography (HPLC) for the simultaneous analysis of PA and NAPA in human plasma.

EXPERIMENTAL

Apparatus

All analyses were performed utilizing a Waters Assoc. (Milford, Mass., U.S.A.) high-performance liquid chromatograph. A M6000 pump and a U6K injector were coupled to a μBondapak C_{18} column. Procainamide and N-acetylprocainamide were quantified by measuring absorbency with a Waters Assoc. UV detector at 254 nm and recorded on a 10 mV and 100 mV full-scale dual-pen Linear Instruments recorder. Plasma concentrations of PA and NAPA were determined by comparison of peak height ratios with a known amount of N-formylprocainamide (NFPA) added as an internal standard.

Reagents

Procainamide hydrochloride was obtained from Pfaltz & Bauer (Stamford, Conn., U.S.A.) and NAPA was either prepared from PA in our laboratory by reaction with acetic anhydride or obtained from Arnar-Stone Labs. (Mt. Prospect, Ill., U.S.A.). N-formylprocainamide (NFPA) was synthesized by reaction of PA with formic acid. Because of incompleteness of the reaction, NFPA required purification through separation with the HPLC method described below. Glass-distilled chloroform and acetonitrile were purchased from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.) and all other chemicals were ACS reagent grade.

Analysis of samples

PA and NAPA were extracted from human plasma by mixing 500 μl of plasma, 100 μl of NFPA (1.0 μg) in water, 400 μl of 0.5 *N* NaOH and 10.0 ml of 10% *n*-propanol in chloroform in a 15-ml polypropylene tube with a Caplug®. The mixture was

shaken for 5 min on an Eberbach electronic shaker and centrifuged for 2 min. The aqueous phase was aspirated and discarded; 7 ml of the organic phase were transferred with a silanized Mohr pipet to a silanized Schwarz vial. The solvent was evaporated to dryness with a gentle stream of dry nitrogen at approximately 40°. The residue was redissolved in 400 μl of distilled water and 100-μl samples were injected for analysis.

RESULTS AND DISCUSSION

A method for the simultaneous quantification of PA and the active metabolite, NAPA, from human plasma utilizing HPLC is described. Levels of PA and NAPA in the range of 1–15 μg/ml were measured by UV absorption in less than 15 min after a simple extraction process.

Various solvent systems were evaluated for their extraction efficiencies and ability to separate the compounds of interest from impurities. During preliminary studies it was found that PA and NAPA could not be recovered quantitatively when added to plasma in glass tubes in amounts to yield concentrations less than 2 μg/ml. This problem was overcome by using polypropylene tubes during the extraction process and silanized glassware thereafter. This greatly improved the recovery at levels above 1 μg/ml. The exact pH for optimal extraction was not found to be crucial except that recovery decreased at very high pH or at pH below 8. Optimal recovery was obtained at 0.2 *N* NaOH. Finally, a mixture of 10% *n*-propanol in chloroform was chosen because it exhibited a high extraction efficiency and did not extract any interfering components. Fig. 1A is a chromatogram of blank plasma and demonstrates the lack of interfering compounds with the extraction procedure. The HPLC parameters are given in the legend.

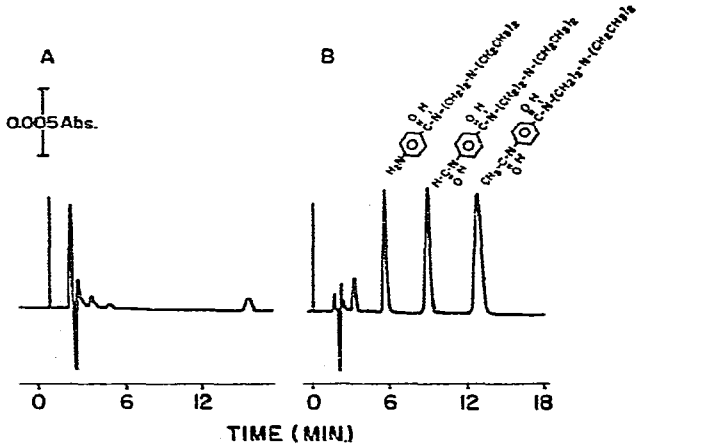

Fig. 1. Chromatograms of (A) blank plasma and (B) plasma with 2 μg/ml each of procainamide, N-formylprocainamide and N-acetylprocainamide added. Column, Waters Assoc. μBondapak C_{18} (10 μm), 30 cm × 4 mm I.D.; pressure, 2000 p.s.i.; temperature, ambient; solvents, isocratic 4 g sodium acetate; 1000 ml distilled water; 40 ml acetic acid; 50 ml acetonitrile; flow-rate, 2.0 ml/min; sample size, 100 μl; detector, Waters Assoc. UV (254 nm) Model 440. Compounds separated, procainamide, N-acetylprocainamide and N-formylprocainamide.

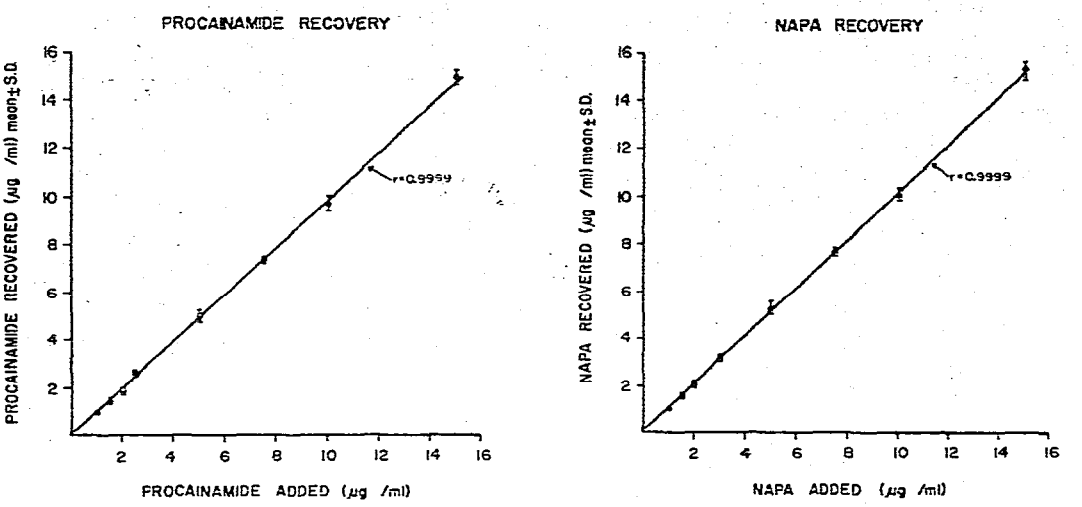

Fig. 2. Standard curves for procainamide and N-acetylprocainamide in plasma: each point represents the mean ± standard deviation for 6 separate determinations.

In order to reduce the chance of pipetting and extraction errors, the N-formyl derivative of PA was synthesized (structure confirmed by gas chromatography–mass spectroscopic analysis) for use as an internal standard. Standard solutions of NFPA were stable when kept at —4° for as long as 8 weeks. Solutions of PA, NAPA or NFPA were not stable unless kept frozen between assays. Fig. 1B is a chromatogram which demonstrates the position of the peak of the internal standard, NFPA, relative to PA and NAPA. Fig. 2 shows the average recovery and standard deviation for six standard curves derived from known amounts of PA and NAPA added to plasma containing none of the drug. In the range for PA (4–8 μg/ml) and NAPA to be expected clinically, the coefficient of variation was 3.6% for each compound.

Fig. 3 demonstrates chromatograms from the plasma of two patients receiving 250 mg PA orally every 6 h. Both had been receiving this dosage for 3 or more days and the plasma was collected 3 h after a dose as recommended by Reidenberg *et al.* for determination of acetylator phenotype[3]. Patient A has a ratio of NAPA–PA equal to 0.33, indicative of slow acetylation. In contrast, the ratio for patient B was 1.12, indicating rapid acetylation. The last peak (t_R = 15.6 min) is frequently seen in random plasma samples and has a t_R value which corresponds to caffeine. A small peak at t_R = 7.1 is also frequently seen in patients receiving PA and is possibly a metabolite of PA which has been noted by previous investigators[9].

The identities of the peaks for PA and NAPA were confirmed by several steps. First, both compounds were isolated in fractions of the HPLC effluent and identified by their mass spectra. Also, plasma known to contain PA and NAPA was extracted and then treated with acetic anhydride. The acetylated plasma extract produced a chromatogram which was devoid of a peak corresponding to PA and the NAPA peak had increased in height. A portion of the same plasma extract treated with concentrated hydrochloric acid produced a chromatogram in which no NAPA peak was seen and the PA peak was larger than before.

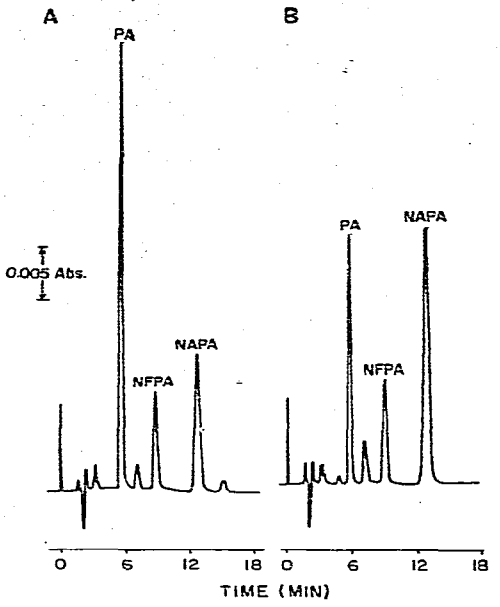

Fig. 3. A, Chromatogram of plasma from a patient who is a "slow acetylator" of procainamide. Concentrations: [PA] = 8.22 μg/ml and [NAPA] = 2.74 μg/ml; [NAPA]/[PA] = 0.33. B, Chromatogram of plasma from a patient who is a "rapid acetylator" of procainamide. [PA] = 4.75 μg/ml, [NAPA] = 5.30 μg/ml, [NAPA]/[PA] = 1.12. Chromatographic parameters same as in Fig. 1.

Because of the previously reported interference by chlordiazepoxide with the colorimetric method[7] and the frequent presence of the drug in plasma samples, the assay was tested for interference by chlordiazepoxide and diazepam. Neither drug altered the chromatogram when added to plasma containing NAPA and PA.

CONCLUSIONS

The rapid and simultaneous determination of plasma levels of NAPA and PA is possible by the above described technique. The precision of the method is equal to or better than other described methods using GLC or thin-layer densitometry. The method is more specific than colorimetry or spectrophotofluorometry and the utility of the method allows assay of large numbers of samples in a short period of time. It is particularly useful for determination of acetylator phenotype as the ratio of NAPA to PA can be determined directly from the peak heights.

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