reagents. Easy automation and a shorter analysis time are advantages of the kinetic assay over the chromogenic procedure. By incorporation of standards containing the pancreatic or salivary isoamylase, results can be calculated with a simple calculator, avoiding the need to use a nonlinear calibration curve (13, 14).

We thank Mrs. J. M. A. van den Berg-Coppens for her skillful technical assistance, Mrs. C. L. M. Curta for her secretarial help, and Mrs. T. Vahlkamp (St. Liduina Hospital, Purmerend) for her cooperation in part of this study. Pharmacia Nederland BV supplied us with Phadebas® reagents.

References

CLIN. CHEM. 29/6, 1104—1108 (1983)

Liquid-Chromatographic Determination of Aminoglutethimide in Plasma

B. A. Robinson and F. N. Cornell

A simple, rapid “high-performance” liquid-chromatographic procedure is presented for the determination of aminoglutethimide in plasma. After precipitation of the protein with acetonitrile, an aliquot of the supernate is injected directly onto a radially compressed, reversed-phase column. The aminoglutethimide is isocratically eluted with a mobile phase of acetonitrile/water/tert-butyl ammonium phosphate. The method is both accurate and precise and has been in routine use in our laboratory for more than 12 months.

Additional Keyphrases: anticonvulsant drugs · drug assay

Aminoglutethimide was first developed as an anticonvulsant 21 years ago, but was considered unsuitable because of its suppressive effect on adrenal function (1). A powerful inhibitor of the desmolase enzyme system, the drug blocks the conversion of cholesterol to Δ5-pregnenolone; it also inhibits the aromatase system, preventing the conversion of androgens to estrogens in nonadrenal tissues (2).

Clinically, aminoglutethimide has been used to induce medical adrenalectomy and treat steroid-dependent breast cancer. However, side effects of drowsiness, ataxia, and rash necessitate in some cases cessation of treatment (1, 2).

Prior methods for the measurement of plasma aminoglutethimide have been based on organic extraction, followed by color development with a relatively nonspecific reagent (3). We have developed a simple method involving protein precipitation by acetonitrile, liquid-chromatographic separation, and detection of ultraviolet absorbance. The method is accurate, precise, rapid, and simple to perform.

Materials and Methods

Apparatus. The chromatographic system consisted of a M600A pump, a U6K universal injector, a Model 440 absorbance detector, a Model 450 variable wavelength detector, a RCM-100 radial compression module (all from Waters Associates Inc., Milford, MA 01757), and an Omniscrype Model EBS217-I recorder (Houston Instruments Inc., Austin, TX 78753). The chromatographic column was an uncapped octadecyl (C18) silica reversed-phase column, 0.8 cm (i.d.) × 10 cm, with a 10-μm particle size (Radial Pak, part no. 83720; Waters Associates).

Reagents. Acetonitrile and tert-butyl ammonium phosphate ("Pic A" Low UV), both HPLC grade, were obtained from Waters Associates. Water was distilled, then purified with a Milli Q Reagent Grade water system (Millipore Corp., Bedford, MA 01730).
The mobile phase consisted of 3 mL of Pic A added to a mixture of 100 mL of acetonitrile and 100 mL of distilled water. The solution was adjusted to pH 6.3 ± 0.2 with orthophosphoric acid. Immediately before use the mobile phase was filtered through a 0.5-μm pore-size filter (Milli-pore Corp.; FHUP 04700) and degassed. A stock aminoglutethimide standard was prepared by dissolving 23.2 mg of aminoglutethimide ("Elipten"; obtained from Ciba-Geigy Australia Ltd., and used without further purification) in 100 mL of distilled water (1 mmol/L). The working standard was prepared by diluting 10 mL of stock solution to 100 mL of mobile phase (0.1 mmol/L). Both standard solutions were stable for at least three months when stored at 4 °C.

Procedure. To a 0.1-mL aliquot of plasma in a 1.5-mL centrifuge tube (EV929; Filtrona Plastics, Thomastown, Victoria, Australia 3074) add 0.2 mL of acetonitrile. Vortex-mix for 15 s and then centrifuge at 12 500 × g for 1 min (Eppendorf Centrifuge 5412, Hamburg, F.R.G. D/2000). Decant the supernate carefully and recentrifuge to remove any remaining debris. Inject 20 μL of the supernate or 5 μL of working standard solution into the chromatograph.

The flow rate of the mobile phase is 2.0 mL/min and the column is kept at ambient temperature (22 °C). The detection wavelengths are set at 240/254 nm with a span of 0.02 absorbance units full-scale deflection.

Measure peak heights of samples, controls, and standard at 240 and 254 nm and calculate the plasma aminoglutethimide concentrations at each wavelength according to the following equations.

\[
\text{Plasma aminoglutethimide} = \frac{\text{sample peak height}}{\text{standard peak height} \times \text{conc of standard}}
\]

If the results calculated at the two wavelengths differ by more than 5%, then a co-eluting compound is assumed to be present. Accurate quantification in this case necessitates a change in chromatographic conditions to separate the interfering compound from the aminoglutethimide.

Results

Figure 1 shows a chromatogram of an aminoglutethimide standard and two patients' samples. The aminoglutethimide has a retention time of 2.5 min, corresponding to a retention volume of 5 mL. Although aminoglutethimide is not resolved completely from the preceding peak, other experiments (not shown) on patients’ sera before medication, or after cessation of treatment, indicate that the preceding peak has returned to baseline before 2.5 min.

Results using this method are linear to a plasma concentration of at least 1.5 mmol/L (10 mmol injected onto the column), 30-fold the upper limit of the therapeutic range. As estimated from the peak-to-noise ratio, the limit of detectability of aminoglutethimide is less than 1 μmol/L in plasma.

We performed recovery and imprecision studies on pooled plasma supplemented with aminoglutethimide at two concentrations. Recovery at 20 μmol/L ranged from 100% to 110%, mean 107% (n = 20); within-batch imprecision (CV) was 2.3% (n = 20) and between-batch was 3.6% (n = 10). Recovery at 100 μmol/L ranged from 96 to 105% mean 102% (n = 20); within-batch imprecision (CV) was 2.0% (n = 20) and between-batch was 2.2% (n = 10).

Also with pooled plasma, we assessed the stability of drug-containing specimens stored at 4 and −20 °C. At 4 °C the plasma aminoglutethimide concentration decreased by 10–20% over a seven-day period whereas at −20 °C it was stable for at least three months.

At the time of publication we have analyzed more than 800 samples. In approximately 2% of these samples, we could not quantify the aminoglutethimide because of the presence of interfering peaks as yet unidentified. Interfering compounds that co-eluted with aminoglutethimide were detected by a change in the peak height ratio at the two detection wavelengths.

Discussion

With this procedure aminoglutethimide can be accurately and precisely determined in plasma. Reagent costs are low and the method is both rapid and robust, making it an attractive alternative to current colorimetric techniques.

Extra peaks other than aminoglutethimide are observed on chromatograms of patients receiving medication. These are presumably metabolites of aminoglutethimide and further work is in progress to confirm this assumption. Despite the fact that little is known about the pharmacological effects of aminoglutethimide metabolites, the measurement of plasma aminoglutethimide has proved useful clinically and has been in routine use in our institute for approximately 12 months. Our results indicate toxic concentrations are reached at approximately 70 μmol/L in plasma, the provisional therapeutic range being 20–50 μmol/L.

References