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Note

Separation and analysis of azosemide in urine and in serum by high-performance liquid chromatography

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Azosemide, 5-(4-chloro-5-sulphamyl-2-thenylamino-phenyl)tetrazole, is a new diuretic in the initial stages of testing which resembles the potent diuretic furosemide in chemical structure (Fig. 1), and in its site of action [1—3]. We have developed an assay for azosemide by high-performance liquid chromatography (HPLC) which allows facile measurement in serum and in urine. A previous assay utilized fluorometric detection and involved derivatization and extraction of the samples [3]. This paper describes a new assay of azosemide in serum and in urine using reversed-phase HPLC with ultraviolet detection and requiring no extraction or derivatization of the sample. An internal standardization technique employed phenobarbital as the standard. The method is simple,

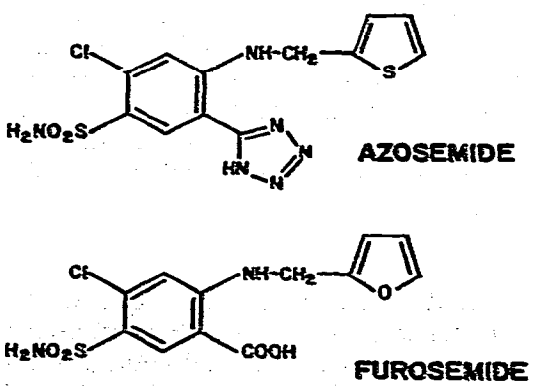

Fig. 1. Chemical structures of azosemide and furosemide.

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reliable and has been automated for urine samples to allow ease of drug measurement, a prerequisite for large-scale clinical evaluation and characterization of the drug.

EXPERIMENTAL

Materials

Azosemide was obtained from Merrell-National Labs. (Cincinnati, Ohio, U.S.A.) and the phenobarbital from Sigma (St. Louis, Mo., U.S.A.). Distilled-in-glass acetonitrile was obtained from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.) and glacial acetic acid (A.C.S. grade) from Mallinckrodt (St. Louis, Mo., U.S.A.). Water used in the assay was triple distilled and filtered (0.45 μm pore size). All solvents were degassed before use.

Chromatography

All analyses were performed using a Perkin-Elmer (Norwalk, Conn., U.S.A.) Series 3 high-performance liquid chromatograph with a Perkin Elmer Model LC 65-T variable-wavelength ultraviolet detector. A Dupont (Wilmington, Del., U.S.A.) Zorbax ODS column (25 cm × 4.6 mm C_{18} reversed-phase, 5 μm particle size) preceded by a 7 cm × 2.1 mm precolumn packed with CO:PELL ODS (Whatman, Clifton, N.J., U.S.A.) was used to perform the separations. Azosemide and phenobarbital were measured at 239 nm and a gradient elution using acetonitrile and acetate buffered water, pH 4.05 (0.6 ml glacial acetic acid added to 1 l of water and buffered to pH 4.05 with 4 *N* sodium hydroxide) was employed. A linear gradient from 10 to 40% acetonitrile over a 10-min period was delivered at a flow-rate of 2 ml/min. The system was then purged with 40% acetonitrile for 2 min and allowed to equilibrate for 5 min before the next sample was injected.

Sample preparations

To each 0.3-ml volume of filtered urine (0.45-μm pore size) were added 0.3 ml distilled water and 50 μl of the internal standard (0.412 mg/ml phenobarbital in ethanol). The samples were prepared in 1-ml serum vials, capped, vortexed and a volume of 10 μl was injected automatically using a Perkin-Elmer Model 420 autosampler. To serum samples were added 20 μl of phenobarbital followed by the addition of 0.4 ml acetonitrile with mixing to precipitate serum proteins. Each sample was then centrifuged and the supernate was decanted and evaporated to dryness. The residue was reconstituted in 50—100 μl of buffer and 5—20 μl was injected manually.

Drug quantitation

Azosemide in a patient sample was quantified by comparing the peak height ratio of azosemide to phenobarbital (A/P) in the sample to the A/P of standard samples of urine or serum containing known amounts of azosemide and phenobarbital. A linear plot of the peak height ratio versus known concentration of standard samples was constructed. For a given peak height ratio of a patient sample a corresponding amount of standard drug was obtained from the curve and then converted to a specific concentration of drug per sample.

RESULTS AND DISCUSSION

Fig. 2 depicts a typical chromatogram of the analysis of a human urine sample. The retention time of azosemide was 10 min and the total analysis time was 17 min. No other substances with a retention time comparable to that of azosemide were seen during the sample analyses. Commonly used drugs which were tested for and showed no interference included acetaminophen, aspirin, chlorothiazide, chlorpromazine, hydrochlorothiazide, procainamide, quinidine, sulfamethazole, theophyllin, and tolbutamide.

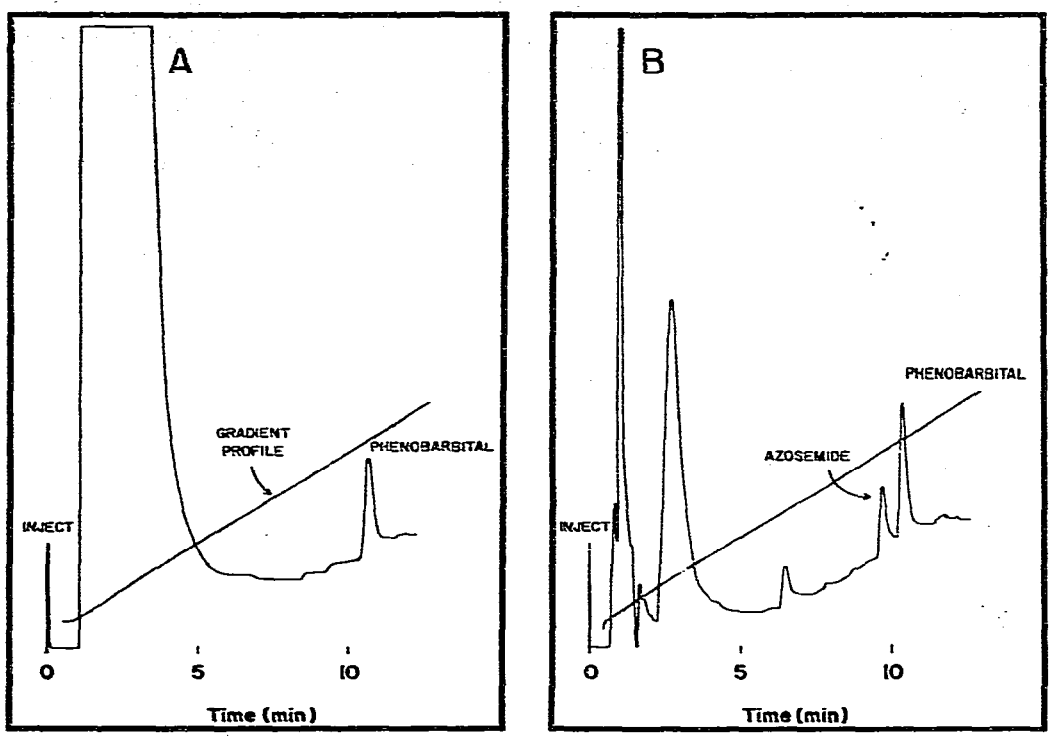

Fig. 2. Typical chromatogram of an aliquot of urine from a subject before (A) and 2 h after (B) receiving 40 mg of azosemide by mouth.

Recovery of drug was assessed by comparison of serum samples processed in the normal manner (i.e., deproteinated, centrifuged, filtered and evaporated) with a standard curve obtained from non-processed samples. Recovery of azosemide was 97.4% at each concentration tested.

The relationship between the peak height ratio (peak height of azosemide/peak height of phenobarbital) and the concentration of standard drug used to calculate the amount of azosemide in urine and serum samples was linear over the concentration range studied, verified by the consistently high correlation coefficient ($r > 0.99$) obtained from a linear regression analysis of the data.

As expected, the plot reliably passed very near the origin. The precision of the method was also tested over the concentration range studied. The coefficient of variation of ten measurements at each of several concentrations varied

from 1.6% at 0.86 μg/ml to 13.3% at 0.055 μg/ml. We considered the lower limit of reliable detection of azosemide to be 0.05 μg/ml. Concentrations as low as 0.007 μg/ml were detected but this was at or below the limits of the standard curve in some cases and thus was not considered reliable.

The chromatographic determination described above resulted in good separation and quantitation of azosemide and consequently allowed a preliminary pharmacokinetic study of azosemide. Fig. 2 shows a chromatogram of human urine prior to administration of azosemide and a trace 2 h after an oral dose of 40 mg of the drug. The concentration of diuretic in urine samples ranged from 0.007 to 1.651 μg/ml and in serum samples from 0.052 to 1.073 μg/ml.

The profiles of drug excretion rate (urine) and drug concentration (serum) with time for one subject are shown in Fig. 3. A lag time of approximately 60 min was observed in the appearance of azosemide in the serum. From the profile of concentration or excretion rate with time, the half-life of drug absorption was estimated to be 49 min in both urine and serum and the half-life of drug elimination was estimated at 89 min.

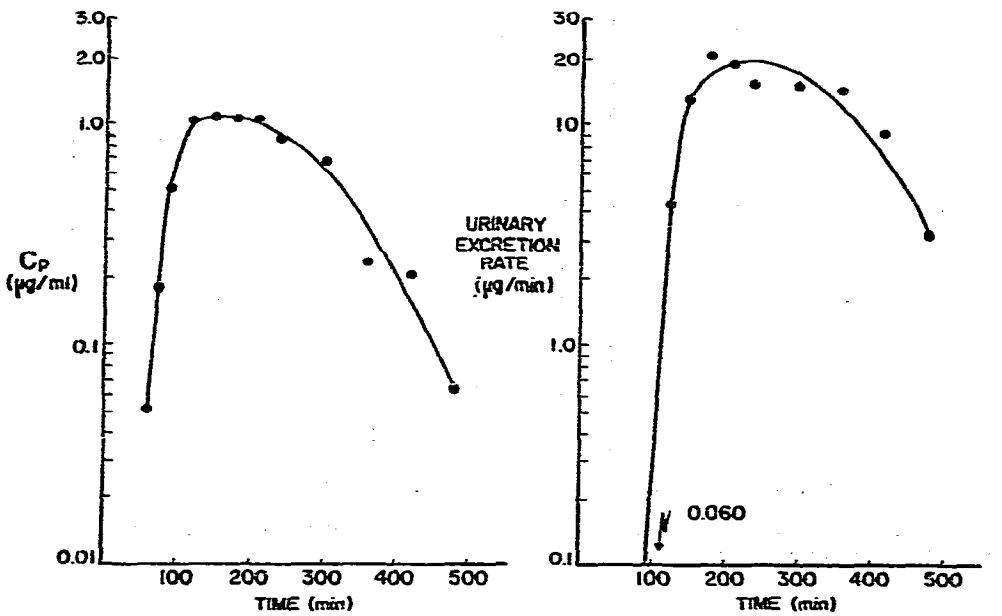

Fig. 3. Time course of azosemide urinary excretion rate (right) and concentration in serum (left) for one subject.

This assay provides an efficient and accurate method for analysis of azosemide in large numbers of biologic samples. A similar approach should be feasible with other structurally similar compounds allowing accurate determination of pharmacokinetics and of pharmacodynamic relationships.

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REFERENCES

1 D.C. Brater, Clin. Pharmacol. Ther., 25 (1979) 428.
2 D.C. Brater, S.A. Anderson and S. Strowig, Clin. Pharmacol. Ther., 25 (1979) 435.
3 F. Kruck, W. Bablok, E. Besenfelder, G. Botzien and B. Kaufmann, Eur. J. Clin. Pharmacol., 14 (1978) 153.