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Note

Determination of furosemide concentrations in plasma and urine using high-speed liquid chromatography

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A simple method for the determination of furosemide, without derivatization, in biological fluids has been described by Hajdú and Häussler[1,2]. They utilized a spectrofluorimetric technique applied directly on a plasma sample or plasma extract. Interferences by metabolites and other substances may cause problems using their method. The method described in this paper utilizes high-speed liquid chromatography of an extract of either plasma or urine. This technique is more specific and is simple to perform.

EXPERIMENTAL

The liquid chromatograph used was a Waters Ass. (Framingham, Mass., U.S.A.) Model ALC 202, equipped with a UV detector, operated at 280 nm and attenuated to 0.02 absorbance unit full scale. The column (1.8 m × 2 mm I.D., stainless steel) was dry-packed with Bondapak C_{18}/Corasil (Waters Ass.). The mobile phase consisted of 0.02 *M* buffer of pH 2 (potassium chloride–hydrochloric acid) mixed with 26% acetonitrile when plasma samples were analyzed and 24% when urine samples were analyzed. The system was operated at a flow-rate of 0.9 ml/min at 25°.

One millilitre of plasma or urine was transferred into a 10-ml screw-capped tube, then 2 ml of water and 150 μl of 4 *M* hydrochloric acid were added. The sample was then extracted twice with diethyl ether (6 ml and 3 ml). The combined ether extract was evaporated with a gentle stream of nitrogen and allowed to dry under vacuum in a desiccator for 2 h. The residue was dissolved in 0.5 ml of the eluent mixture (see above). After filtration through a pasteur pipette stoppered with glass-wool, an aliquot of the solution (40 μl) was injected on to the liquid chromatograph.

RESULTS AND DISCUSSION

Figs. 1a–1d illustrate chromatograms obtained from the analyses of plasma and urine samples. Fig. 1a represents the on-column injection of 2.4 μg extracted from a plasma sample and Fig. 1c the injection of 1.6 μg extracted from a urine sample (the amounts injected are calculated from the original plasma and urine concentra-

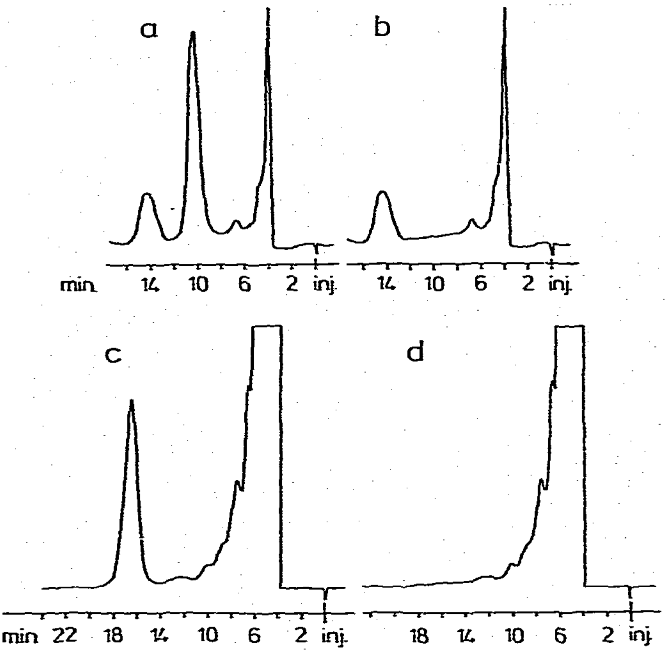

Fig. 1. Chromatograms of plasma and urine samples: (a) and (c) show chromatograms of a plasma and a urine sample, respectively, containing furosemide; (b) and (d) are blank controls of a plasma and a urine sample, respectively.

tions). No interfering compounds were extracted from either plasma or urine with diethyl ether (Figs. 1b and 1d).

Reversed-phase liquid chromatography was chosen because it was found to be suitable for the samples and the polar furosemide molecule. The mobile phase was maintained at a low pH because of the acidity of furosemide. Decreasing the amount of acetonitrile in the eluent prolonged the retention time of the furosemide peak. The residue obtained after evaporation of the diethyl ether extract contained residual water, which was preferably removed by drying under vacuum in a desiccator (in the dark) rather than by adding desiccant to the ether solution before evaporation. The residue was dissolved in 0.5 ml of the eluent with ultrasonification just before the analysis on the chromatograph. Dissolution of the sample in 250 µg of the eluent was also tested, but it resulted in a greater spreading of the test results when the analysis was carried out under routine conditions. Filtration of the dissolved sample was necessary in order to avoid plugging the column.

The output of the UV detector is linear in absorbance units, and the area under the peak is proportional to the concentration. The standard curve (Fig. 2) was constructed by analyzing samples to which had been added different amounts of furosemide (1–24 µg) and then plotting the resulting peak areas against the concentration. The peak height can also be utilized, but it depends a great deal on how the injection is performed. The standard deviation was determined by analyzing five plasma samples containing 4 µg/ml of furosemide according to the method described. The recovery (± standard deviation) was 3.9 ± 0.23 µg/ml. The method can be used for the deter-

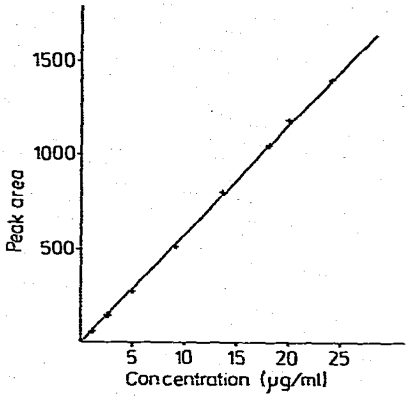

Fig. 2. Detector response of furosemide.

mination of plasma levels just below 1 µg/ml. Determinations of lower concentrations, using the same original sample volume, can be carried out by increasing the aliquot of the injected sample.

ACKNOWLEDGEMENT

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