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Letter to the Editor

Improved method for the determination of furosemide in plasma by high-performance liquid chromatography

Sir,

Furosemide (F, 4-chloro-N-furfuryl-5-sulphamoylanthranilic acid) is a potent loop diuretic agent, which is widely used for the therapy of oedema. Measurements of F plasma levels for pharmacokinetic and pharmacodynamic studies are commonly performed by high-performance liquid chromatography (HPLC). Several such methods have been published, but they are not sensitive enough to measure levels below 100 ng/ml [1–8] or are too laborious in sample pretreatment [9,10]. We therefore developed a new method, which simplifies the handling without loss of sensitivity. F is extracted from plasma by the use of solid-phase extraction columns and analysed on a reversed-phase column within 16 min. Under the conditions of sample preparation and chromatography, F is stable enough for automated HPLC separation.

EXPERIMENTAL

Materials

F for standard solutions was obtained from Sigma (Deisenhofen, F.R.G.). Solid-phase extraction columns (C_{18}, for 1-ml samples) were purchased from Baker (Gross-Gerau, F.R.G.). HPLC-grade solvents were from Merck (Darmstadt, F.R.G.).

Plasma samples

A plasma pool (Deutsches Rotes Kreuz) was used for the development of the method. Plasma samples from two patients receiving F in combination with triamterene (Diutrix®) orally were analysed.

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Chromatographic system

The HPLC system consisted of an L-6200 liquid chromatograph equipped with an F-1050 fluorescence spectrophotometer and a D-2500 chromato-integrator (Merck-Hitachi, Darmstadt, F.R.G.). A PRP-1 column, 250 mm×4.1 mm I.D., 10 μm particle size (Hamilton, Darmstadt, F.R.G.), protected by a 4 mm ×4 mm I.D. guard column containing LiChrosorb RP-18, 5 μm particle size (Merck) was used for separations. Sample injection was performed using a Rheodyne valve with 50-μl loop. F was eluted isocratically with 3% phosphoric acid–acetonitrile (67:33, v/v) at a flow-rate of 1.4 ml/min at room temperature. It was detected at excitation and emission wavelengths of 268 and 410 nm, respectively.

Sample preparation

A 1.0-ml plasma sample was acidified with 1.0 ml of 3% phosphoric acid. After shaking, it was transferred to an extraction column that had been conditioned with 2 ml of methanol, 1 ml of water and 1 ml of 1.5% phosphoric acid. Afterwards the dry column was eluted with 0.5 ml of methanol containing 0.1 ml phosphoric acid (85% in water) per 100 ml. The sample analysis volume was 50 μl.

RESULTS AND DISCUSSION

To evaluate potential interferences with the determination of F, some common pure standard drugs were tested under the chromatographic conditions described above. None of the following drugs that give a fluorescence signal at the wavelengths of furosemide detection interfered with the analysis of F: dipyridamole, sulphametoxazole, verapamil, salicylic acid/acetylsalicylic acid, indomethacin, propranolol and triamterene. A typical chromatogram of the analysis of F in plasma is shown in Fig. 1.

Extraction recoveries were greater than 90% and were reproducible, so the use of an internal standard was not necessary. Blank plasma samples were spiked with F for calibration. A linear correlation was found between the peak area and the concentration of F in the range 10–500 ng/ml ($r=0.998$). The limit of detection was ca. 1 ng/ml, with a signal-to-noise ratio of ca. 1:4.

Precision and accuracy were checked by analysing two blank plasma samples spiked with 100 and with 50 ng F per ml, respectively. Plasma concentrations were determined according to the described method and ranged from 96.1 to 103.3 and 47.0 to 52.4 ng/ml with coefficients of variation of 3.7 and 5.0%, respectively ($n=5$ each).

The method was applied to determine F in plasma of two patients who were treated with Diutrix (Medice, Iserlohn, F.R.G.; 50 mg of F combined with 60 mg of triamterene, one dose daily in the morning). Samples were taken on days 2–5 at 2 p.m. Plasma F levels were 141, 113, 113 and 194 ng/ml, respectively,

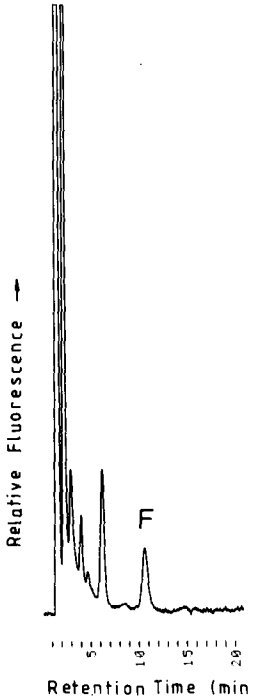

Fig. 1. Determination of furosemide (F) in human plasma. The peak of F represents a concentration of 58 ng/ml. Analytical conditions are described in Experimental.

for patient 1, and 54.4, 56.1, 106 and 128 ng/ml, respectively, for patient 2. No interference with the analysis of F was observed from plasma components. Results of a full pharmacokinetic study will be published elsewhere.

Kerremans et al. [7] observed a low stability of F when hydrochloric acid was used for acidification. We tested the stability of F in methanol acidified with phosphoric acid as described above, and did not observe any decomposition of F when the solution was kept at room temperature in the dark for five days. Since F is stable under these conditions, extracted samples can be analysed using an autosampler.

In conclusion, the presented method is useful for the determination of F in plasma because of very simple sample preparation and high sensitivity.

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