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Letter to the Editor

Determination of bumetanide in plasma by high-performance liquid chromatography

Sir,

Bumetanide (3-*n*-butylamino-4-phenoxy-5-sulphamoyl benzoic acid) is a very potent diuretic with a pharmacological action similar to furosemide [1]. After a single oral administration of the usual therapeutic dose (1–3 mg), plasma levels reach only the lower nanogram range and after 6–8 h sub-nanogram levels, especially if retarded galenic formulations are administered [1].

Only a few assays for the different galenic forms of oral bumetanide have been published [1–7], and most of those did not have sufficient sensitivity or were laborious to establish.

This paper describes a sensitive high-performance liquid chromatographic (HPLC) method for the determination of bumetanide in plasma using fluorescence detection and an internal standard (3-*n*-butylamino-4-phenoxy-5-methylsulphamoyl benzoic acid).

EXPERIMENTAL

Chemicals and standards

Bumetanide was obtained from Krämer and Martin (Siegburg, F.R.G.). The internal standard was synthesized in our laboratories (Schwarz Pharma, Monheim, F.R.G.) by methyl iodide alkylation of bumetanide. The crude product was purified by HPLC on a semi-preparative C_{18} column, and identified by mass spectrometry. Diethyl ether (nanograde) was obtained from Promochem (Wesel, F.R.G.). All other chemicals were of analytical grade and purchased from Merck (Darmstadt, F.R.G.).

Instruments

A Waters HPLC pump M590 and Waters WISP 710B autosampler (Waters Assoc., Milford, MA, U.S.A.) were used. The RF 530 spectrofluorimeter (Shimadzu, Kyoto, Japan) was set at high range (excitation 340 nm, emission 440 nm) (flow-cell 12 μl, time constant 1.5 s). The chromatographic system consisted

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of a Waters stainless-steel precolumn (2.2 cm×0.39 cm I.D.), which was manually filled with Corasil-C_{18}, 37–50 μm irregular particles (Waters), and an analytical C_8 bonded silica column packed with 3-μm spherical particles, LC-8-DB (7.5 cm×0.46 cm I.D.) (Supelco, Houston, TX, U.S.A.). Retention and integration data were collected by a Model 6000 laboratory data system (Nelson, Cupertino, CA, U.S.A.) on an HP 1000 computer (Hewlett Packard, Cupertino, CA, U.S.A.).

Chromatographic conditions

Chromatography was performed at ambient temperature. The eluent was a mixture of 0.03 *M* sodium phosphate buffer (pH 3.0) and acetonitrile (200:125, v/v), degassed in an ultrasonic bath prior to use. The flow-rate was 1 ml/min.

Solutions

Stock solutions (10 μg/ml) of bumetanide and internal standard were prepared in methanol. Bumetanide working solutions of 0.05 and 0.5 μg/ml were prepared freshly every day by diluting the stock solution with water. An internal standard solution of 0.5 μg/ml was prepared by the same procedure.

Calibration curves

Calibration curves were obtained by adding 0.5–25 ng/ml bumetanide and 15 ng/ml internal standard to pooled blank plasma. Validation data were also evaluated using spiked samples.

Extraction

To 1 ml of plasma were added 15 ng of internal standard, 0.2 ml of 1 *M* sulphuric acid and 6 ml of diethyl ether. The sample was extracted for 20 min in a shaker, and phase separation was achieved by a 5-min centrifugation (20°C, 1000 *g*). A 4-ml volume of extract was evaporated in a nitrogen stream at 30°C. The residue was dissolved in 150 μl of eluent, and 100 μl of the resulting solution were injected.

RESULTS

The retention times for bumetanide and internal standard were 4.5 and 6.5 min, respectively. Based on a signal-to-noise ratio of 3, the detection limit was ca. 0.1 ng/ml (Fig. 1). Bumetanide recoveries were measured at 2.5, 5 and 10 ng/ml, and ranged between 85% and 95% when measured on different days. Calibration curves were evaluated by $1/c$ weighted linear regression; regression coefficients were better than 0.998. Intra- and inter-assay validation data were determined in the concentration range of the calibration curves. Intra-assay accuracies and precisions were better than 8% ($n=6$) and inter-assay data were in the same range except at the lowest calibration point (0.5 ng/ml), where 14 and 15% were the values, respectively ($n=6$).

Owing to the lack of efficient methods for the determination of bumetanide in biological fluids, the method presented here offers the possibility of rapid and accurate measurements for drug investigation purposes.

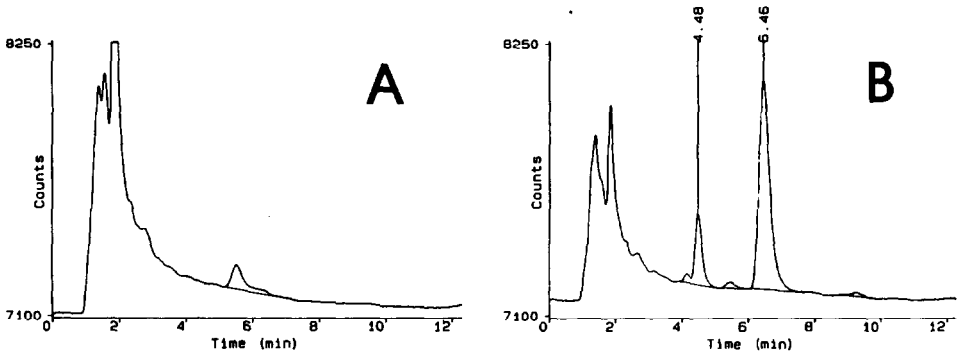

Fig. 1. Chromatograms of (A) blank plasma and (B) plasma from a volunteer 1 h after oral administration of 0.5 mg of retarded bumetanide. Retention times: bumetanide, 4.48 min (3.9 ng/ml); internal standard, 6.46 min (15.0 ng/ml).

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