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Note

High-performance liquid chromatographic assay for monitoring indapamide and its major metabolite in urine

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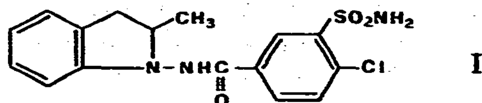
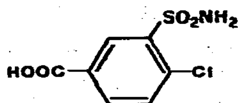
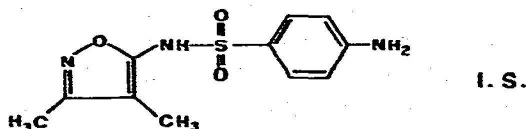
Indapamide (I) [3-(aminosulfonyl)-4-chloro-N-(2,3-dihydro-2-methyl-1H-indol-1-yl)-benzamide] is still widely used in the treatment of mild to moderate hypertension [1]. The comparative pharmacokinetics and metabolism of I were studied using [^{14}C]indapamide [2]. Although the drug is extensively metabolized to several metabolites, 3-aminosulfonyl-4-chlorobenzoic acid (II) was identified by mass spectrometry as the major urinary metabolite [3].



I



II



I. S.

Drug levels in biological fluids were measured by thin-layer chromatography using both unlabeled and labeled I [4, 5], and recently a fluorometric assay for urinary I was also reported [6]. By this method reaction of I with sodium

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hydroxide at 100°C for 45 min is necessary to cleave the amide bond and produce fluorescence from the indoline moiety. This procedure was adapted to the analysis of intact I in urine and is unable to assay the major urinary metabolite. Very recently, an enzymatic assay was developed [7] for I in a drug—rodent—food mixture that is based on the inhibition of carbonic anhydrase by I. However, this method cannot be applied to the analysis of I in biological fluids.

We report here a simple and rapid high-performance liquid chromatographic (HPLC) method for the quantitative determination of I and II in human urine. The procedure described involves direct reversed-phase HPLC, using sulfisoxazole as internal standard (I.S.), and is sensitive to 10 ng of I and II. Further, this method has been applied to the assay of I as bulk material or as a component of a pharmaceutical preparation, even in the presence of II as an impurity.

EXPERIMENTAL

Materials

I and II were obtained from Ricerchimica (Milan, Italy). Sulfisoxazole was supplied from Serva Feinbiochemica (Heidelberg, G.F.R.).

All the solvents were of HPLC grade (Lichrosolv; Merck, Darmstadt, G.F.R.). Water was distilled from an all-glass still; other reagents were of analytical grade.

Chromatographic procedure

The chromatographic unit consisted of a Model 6000A solvent delivery system, a model U6K universal injector, a Model 440 ultraviolet detector and a Model 730 data module (Waters Assoc., Milford, MA, U.S.A.). Ultraviolet absorption was measured at 254 nm. Chromatography was performed at room temperature on a 30 cm × 4 mm I.D., 10 μm μBondapak C_{18} column with acetonitrile—water (35:65. v/v) as mobile phase. Before mixing, water was brought to pH 2.8 with 10% phosphoric acid and degassed. The flow-rate was 2 ml/min (140 bars); a precolumn (2 cm × 4 mm I.D.) of Bondapak C_{18} Corasil was used.

Solutions

Stock solutions: stock solutions of I and II were prepared with a final concentration of 0.1 mg/ml in methanol. Sulfisoxazole, the internal standard (I.S.), was dissolved in methanol to give a concentration of 0.15 mg/ml.

Standard solutions: solutions of I and II (5—25 μg/ml) containing 15 μg/ml I.S. were prepared from the stock solutions. Fresh solutions if I in amber glass were prepared each day of analysis because of the reported [2] instability of I in solutions.

Sample preparation

Bulk drug substance and tablets: to an accurately weighed amount of bulk drug (10 mg) or of tablets equivalent to 10 mg of I, were added 10 ml of I.S. solution and the volume was made to 100 ml with methanol.

Calibration curve for urine

Into individual 15-ml tubes were placed 1.0 ml of each standard solution of I and II. Then 1.0 ml of blank urine was added and the resulting mixture was extracted with ethyl acetate (10 ml) by vortex mixing, followed by centrifugation. The organic phases were pipetted into a conical tube and evaporated to dryness under a nitrogen stream. The mobile phase (1.0 ml) was added and a 10-μl aliquot was injected into the chromatograph.

Recovery

The recovery from urine was assessed by comparing peak area ratios for urine standards subjected to the described procedure to those using standard solutions of the same concentration.

Determination of I and II in urine

For I and II levels higher than 100 ng/ml, 1.0 ml of urine and 1.0 ml of I.S. solution were diluted to 10 ml with the mobile phase. After vortex mixing, a 10-μl aliquot was injected.

For lower I and II levels (up to 100 ng/ml), 1.0 ml of urine and 1.0 ml of I.S. solution were placed into 15-ml tubes. Then, each sample was treated as described under Calibration curve for urine. Control blanks were also performed.

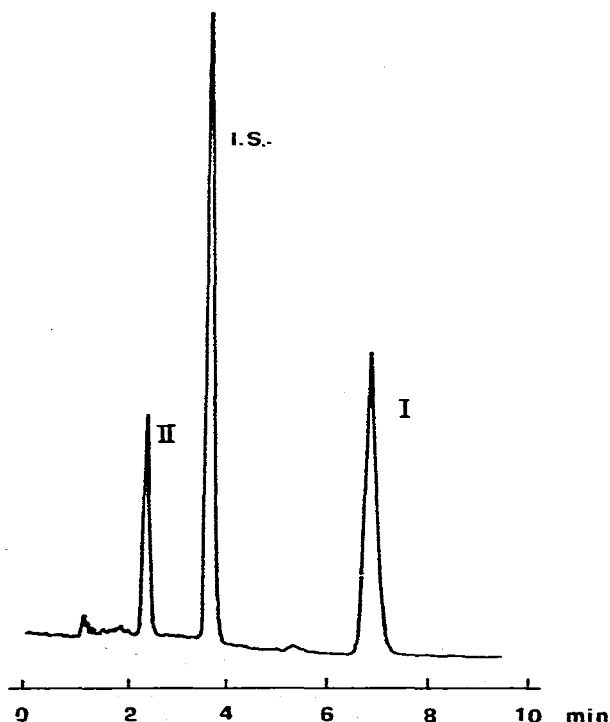

Fig. 1. High-performance liquid chromatogram of indapamide (I) and 3-aminosulfonyl-4-chlorobenzoic acid (II). I, II: 30 ng injected. I.S.: 50 ng injected.

RESULTS AND DISCUSSION

Although several mobile phases were evaluated, the system acetonitrile–water (pH 2.8) was optimal. The I.S., I and II gave capacity factors of 2.2, 5.0 and 1.1, respectively (Fig. 1). This eluent allowed the separation of the components from endogenous urine substances (Fig. 2A and B) as well as from the pharmaceutical preparations.

A linear relationship between the peak height ratio (peak height of I or II/I.S. peak height) and the amount was found for both compounds over the range investigated: 0.025—0.125 μg. Typical calibration curves are shown in Fig. 3.

TABLE I

COMPARISON BETWEEN PEAK AREA RATIOS USING URINE SAMPLES AND STANDARD SOLUTIONS

	I	II
Slope	1.038	0.995
Correlation coefficient ($n = 8$)	0.9981	0.9975

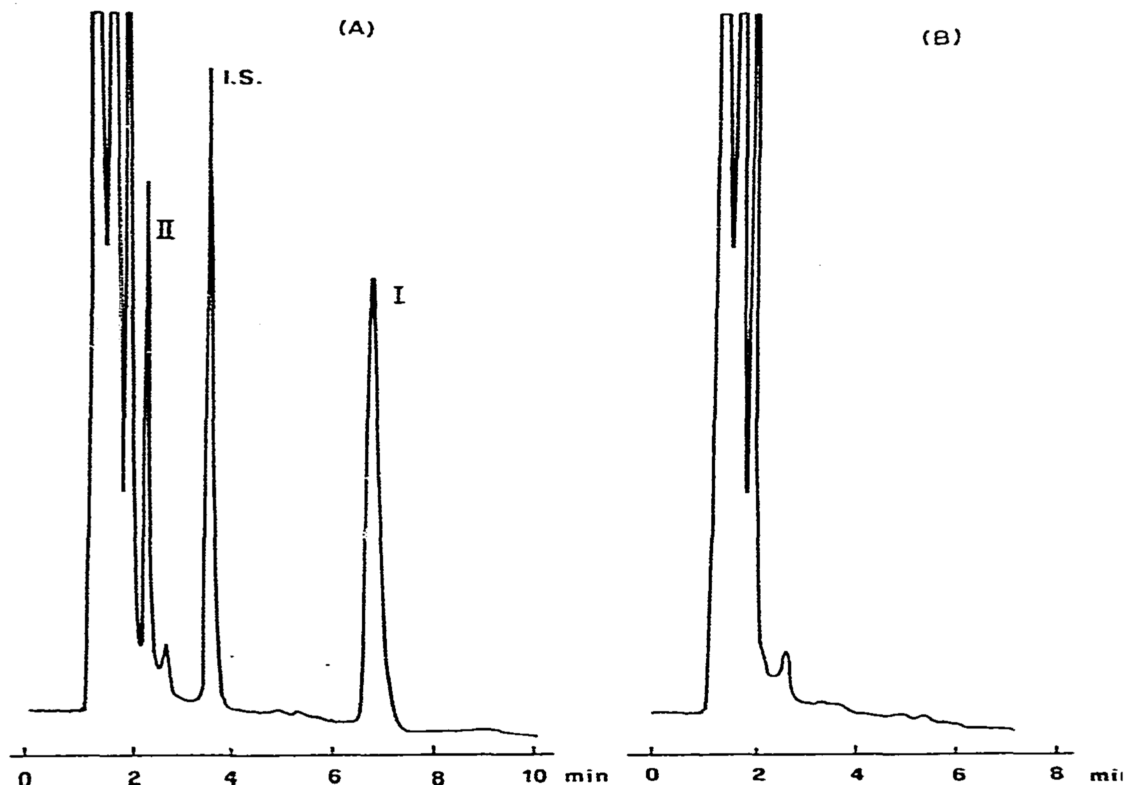

Fig. 2. High-performance liquid chromatogram of (A) urine and (B) blank urine. I, II: 60 ng injected. I.S.: 50 ng injected.

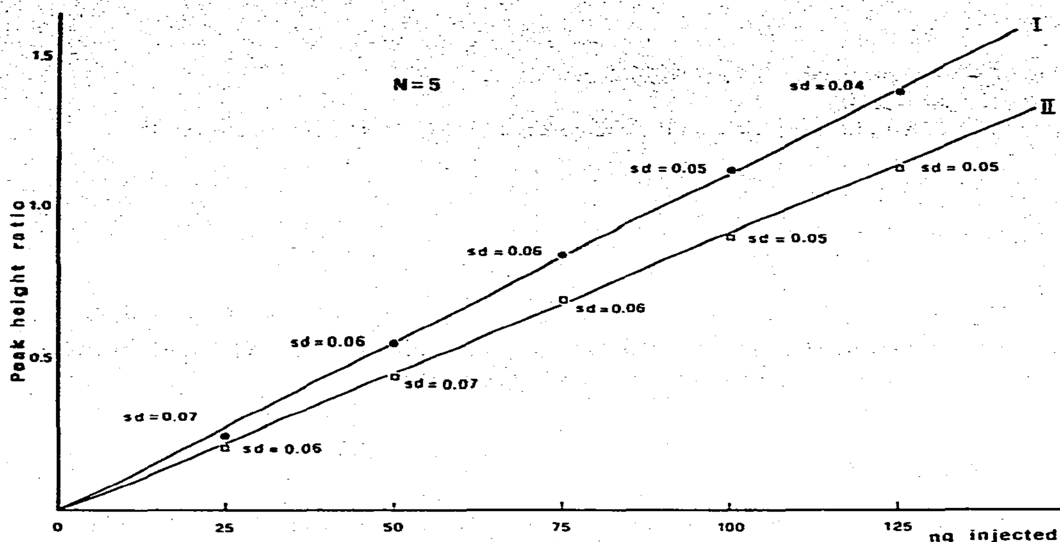

Fig. 3. Calibration graph of the peak height ratio (peak height of I or II/I.S. peak height) versus the amount of I and II injected. sd = standard deviation.

The long-term reproducibility of the assay was determined by assaying freshly prepared sample solutions once a week for a period of six weeks. The coefficients of variation (%) were 2.95 and 3.50 for I and II, respectively.

The correlation between results obtained using spiked urine samples and standard solutions was very good. The results, summarized in Table I, indicate that the recovery from urine was virtually complete.

The limits of sensitivity were 25 ng/ml, and are adequate for monitoring I and II at the usual dosage regimen (2.5–5 mg daily) [2]. Urine can be injected directly after dilution with the mobile phase, so that assay of I and II levels higher than 100 ng/ml is very simple and rapid.

In conclusion, the procedure described above can be successfully used either for monitoring I and II in urine, or for assaying I as bulk material or as a component of pharmaceutical preparations.

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