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Note

High-performance liquid chromatographic assay for zomepirac and its main metabolite in urine

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Zomepirac (Z) [5-(4-chlorobenzoyl)-1,4-dimethyl-1H-pyrrole-2-acetic acid] is an orally active, non-narcotic analgesic agent [1, 2].

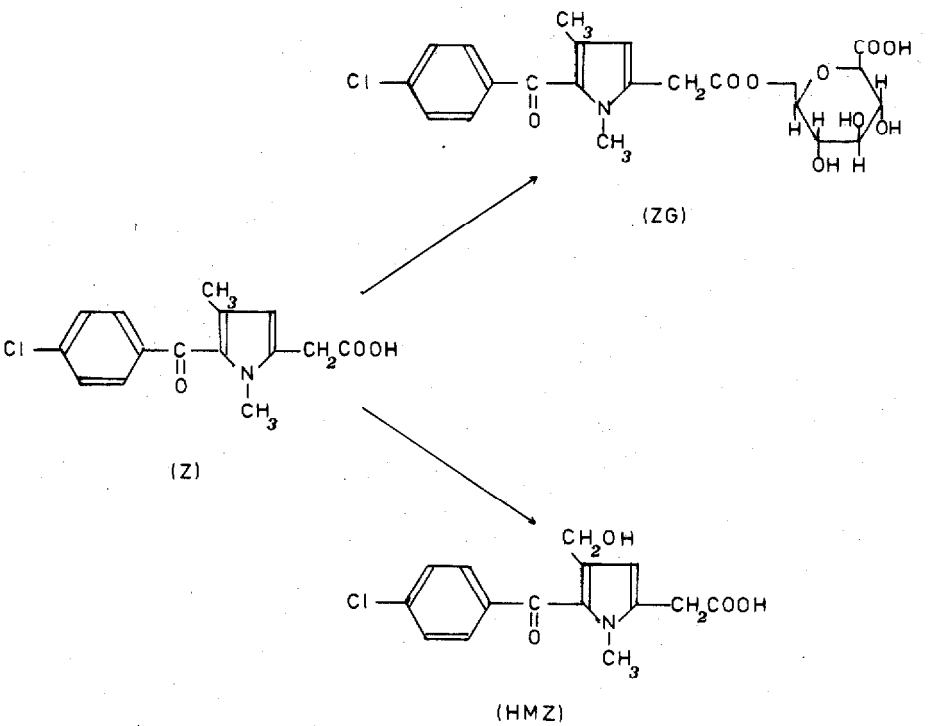

Fig. 1. Metabolic pathway for zomepirac in man.

The metabolism of zomepirac was studied using [^{14}C]zomepirac and the major urinary metabolites in man were identified by chromatographic and spectroscopic data [3]. Zomepirac is primarily metabolized by conjugation with glucuronic acid to give zomepirac glucuronide (ZG), while hydroxylation yielding hydroxymethylzomepirac (HMZ) is a minor pathway (Fig. 1).

Zomepirac was determined in plasma by normal- [4] and reversed-phase [5] high-performance liquid chromatography (HPLC). Recently gas chromatography—mass spectrometry for determining zomepirac in urine was reported [6]. However, this method required hydrolysis of the conjugated drug followed by extraction and derivatization, and allows the detection only of free zomepirac. In continuing our work on the application of HPLC in biomedical analysis [7, 8], we developed a simple and sensitive method for the quantitative determination of zomepirac and zomepirac glucuronide in human urine. The procedure described involves direct reversed-phase HPLC, using propyl 4-hydroxybenzoate or dantrolene sodium as internal standard.

The peaks were detected at 254 or 317 nm and quantities of each assay component as low as 100 ng/ml could be measured.

EXPERIMENTAL

Materials

Zomepirac sodium was provided by SIT (Mede, Italy).

Zomepirac glucuronide was extracted from urine samples and separated by chromatography on a Sephadex LH-20 column [3]; its structure was confirmed by mass spectra.

Propyl 4-hydroxybenzoate (Fluka, Buchs, Switzerland) and dantrolene sodium (SIT) have been used as internal standards for the assay at 254 and 317 nm, respectively.

All the solvents were of HPLC grade (LiChrosolv; Merck, Darmstadt, G.F.R.). Water was deionized, distilled from alkaline permanganate and filtered through a 0.45-μm membrane filter (Type HA, Millipore, Bedford, MA, U.S.A.).

Chromatography

Chromatographic analyses were performed on a component system consisting of a Model 6000A pump, equipped with a Model U6K universal injector, a Lambda-Max Model 480 ultraviolet (UV) detector, and a Model 730 data module (Waters Assoc., Milford, MA, U.S.A.).

Samples were chromatographed at room temperature on a μBondapak C_{18} column (30 cm × 4 mm I.D., 10 μm) (Waters Assoc.). A pre-column (2.5 cm × 4 mm I.D.) packed with μBondapak C_{18} Corasil was used to prevent deterioration of the main column.

The separations were obtained using acetonitrile—water brought to pH 2.8 with phosphoric acid (50:75) as a mobile phase with the flow-rate of 2 ml/min at 140 bars.

Peaks were detected by 254 or 317 nm absorbance measurements (a.u.f.s. 0.02) with the linearity verified for assay components quantities up to 125 μg/ml.

Solutions

Stock solutions of zomepirac sodium and zomepirac glucuronide were prepared with a concentration of 0.5 mg/ml in the mobile phase. Propyl-4-hydroxybenzoate and dantrolene sodium were dissolved in the mobile phase to give a concentration of 0.1 mg/ml.

Calibration curves

Into individual 10 ml volumetric flasks were placed 1 ml of urine, 1 ml of internal standard (I.S.) solution and accurately pipetted volumes (in the range of 0.005—0.25 ml) of zomepirac sodium and zomepirac glucuronide standard solutions. After dilution to 10 ml using the mobile phase, replicate injections of 10 μl were made for each sample.

Assay method

To 1.0 ml of urine was added 1.0 ml of internal standard solution and diluted to 10 ml with the mobile phase.

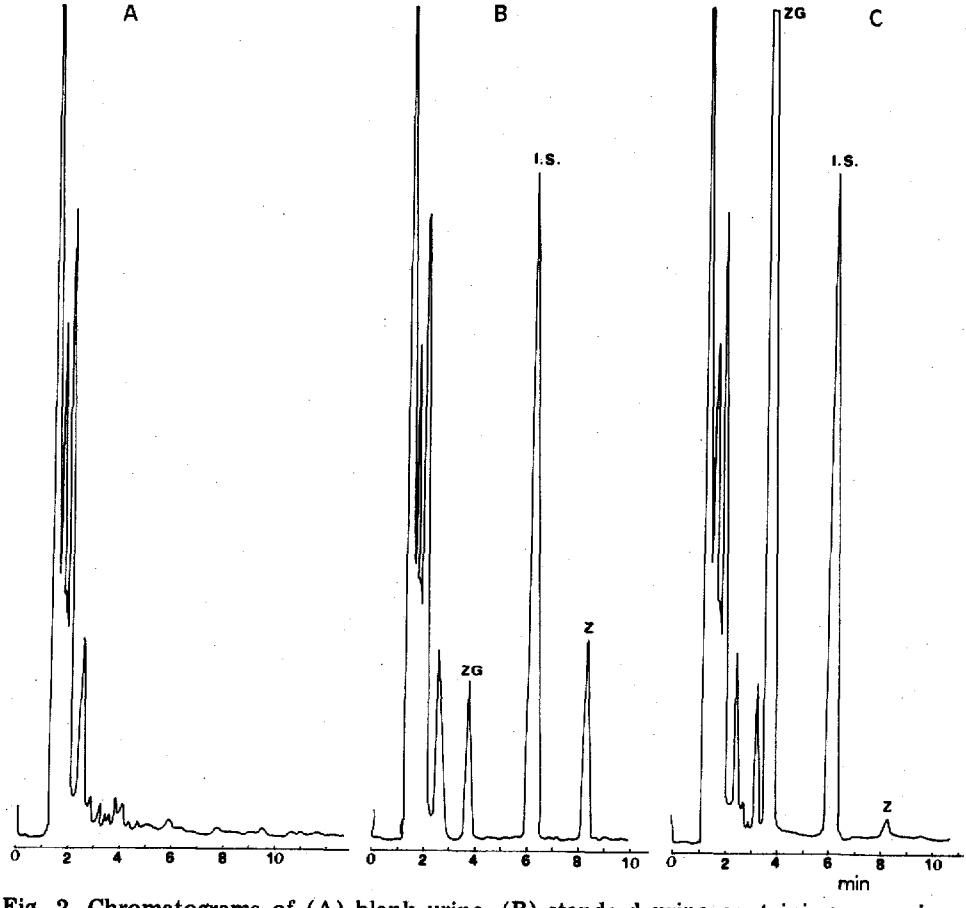

Fig. 2. Chromatograms of (A) blank urine, (B) standard urine containing zomepirac (Z), zomepirac glucuronide (ZG), and propyl-4-hydroxybenzoate (I.S.), 100 ng of each, and (C) urine sample from a volunteer 2 h after the ingestion of a 100-mg tablet of sodium zomepirac. UV detection: 254 nm.

After vortex mixing, replicate injections of 10 μl were made. To assess specificity, a control urine sample (1 ml) was fortified at 100 μg/ml with zomepirac sodium and zomepirac glucuronide and processed through the assay as specified above.

RESULTS AND DISCUSSION

Fig. 2A and B show chromatograms obtained from blank urine (A) and from standard urine (B) containing zomepirac, zomepirac glucuronide, and propyl-4-hydroxybenzoate (internal standard), 100 ng of each. Fig. 2C is a chromatogram of a representative sample from a volunteer following the oral ingestion of a 100-mg zomepirac sodium tablet (Zomax: Cilag-Chemie). These chromatograms demonstrate the lack of interference and the specificity of the assay procedure for the measurement of zomepirac and its main metab-

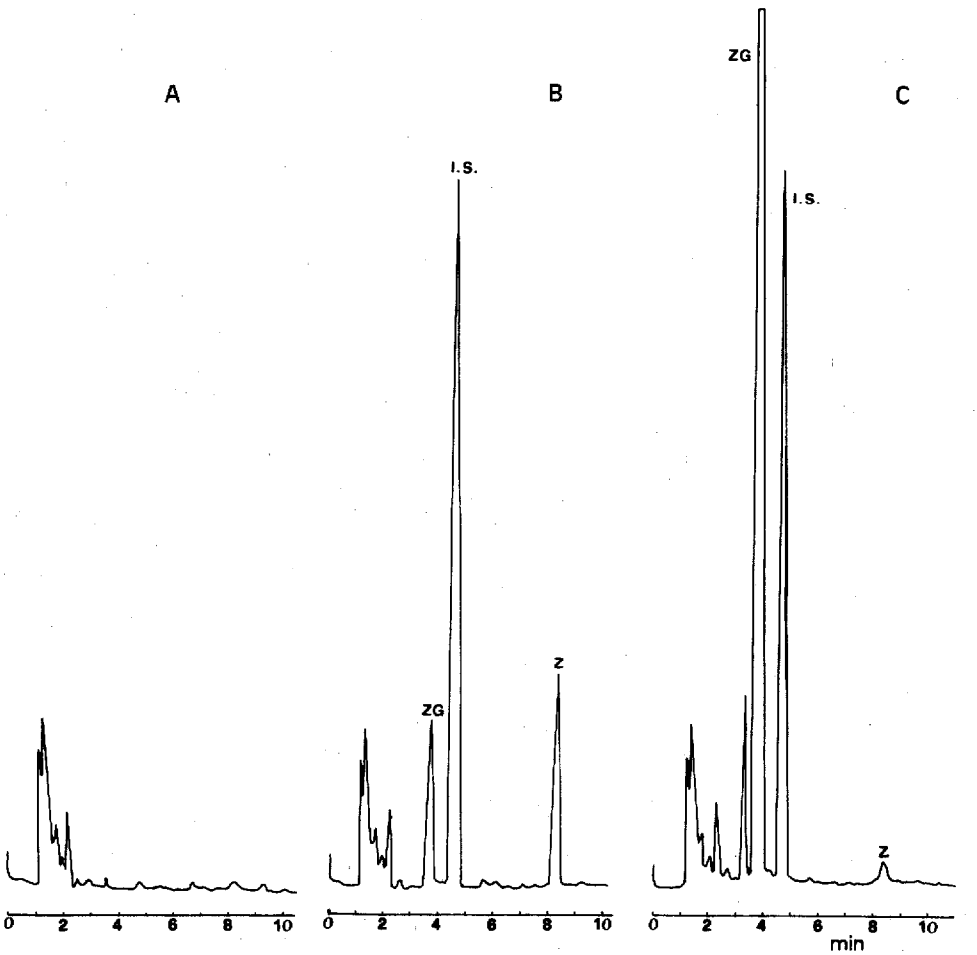

Fig. 3. Chromatograms of (A) blank urine, (B) standard urine containing zomepirac (Z), zomepirac glucuronide (ZG) and sodium dantrolene (I.S.), 100 ng of each, and (C) urine sample from a volunteer 2 h after the ingestion of a 100-mg tablet of sodium zomepirac. UV detection: 317 nm.

olite in urine. The peak eluted with a retention time of 3.2 min and detected at both 254 and 317 nm is probably due to hydroxymethylzomepirac. However, owing to the difficulty in isolating this minor metabolite, no direct evidence can be produced. Interfering peaks from endogenous substances were diminished by monitoring the eluate at 317 nm, where zomepirac and zomepirac glucuronide absorb strongly. In these conditions, sodium dantrolene has been used successfully as the internal standard (Fig. 3A—C).

The method was evaluated over a concentration range of 2.5—125 μg of zomepirac sodium and zomepirac glucuronide per ml of urine. The spiked samples were taken through the analytical procedure. Linear relationships between the ratios of peak areas (zomepirac sodium and zomepirac glucuronide to internal standards) and concentrations (μg/ml) of zomepirac sodium and

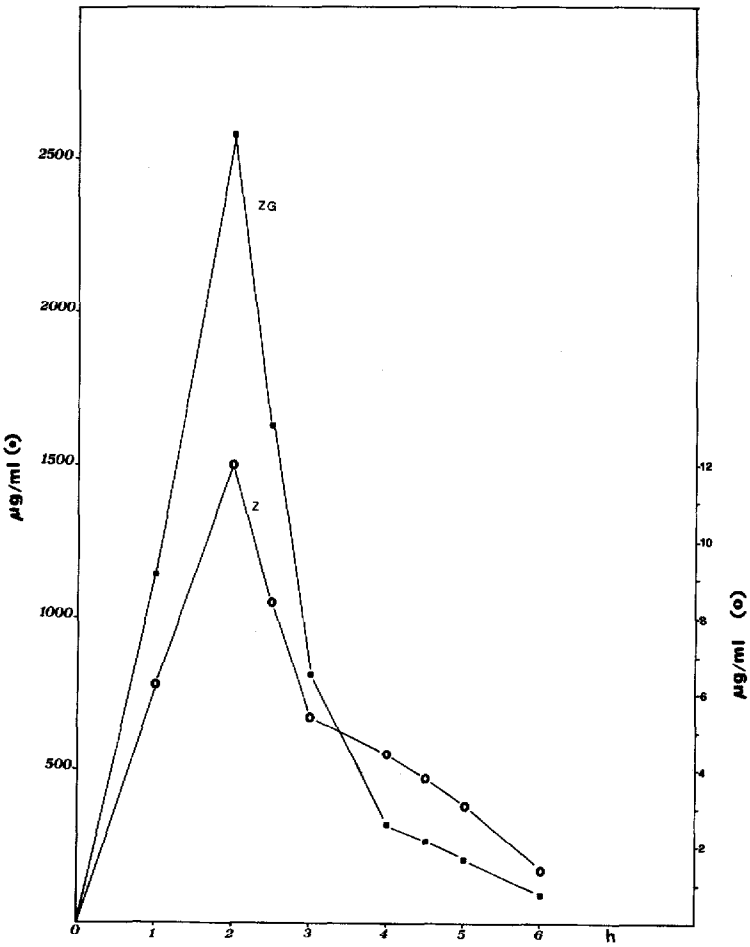

Fig. 4. Urine zomepirac glucuronide (■) and zomepirac (○) concentration—time curves obtained in a volunteer following oral administration of a 100-mg tablet of zomepirac sodium.

zomepirac glucuronide were found and can be expressed by the following equations

zomepirac sodium

$Y = 0.0043X - 0.0162$ $r = 0.996$ (I.S. = propyl-4-hydroxybenzoate)
$Y = 0.0049X - 0.0165$ $r = 0.982$ (I.S. = sodium dantrolene)

zomepirac glucuronide

$Y = 0.0028X - 0.0181$ $r = 0.986$ (I.S. = propyl-4-hydroxybenzoate)
$Y = 0.0032X - 0.0149$ $r = 0.989$ (I.S. = sodium dantrolene)

where Y represents the peak area ratio and X the concentration (μg/ml).

Intra-day assay variations (C.V. %) were determined by assaying the spiked samples four times on the same day and were 4.2% and 3.2% for zomepirac sodium and zomepirac glucuronide, respectively. Inter-day assay variations were obtained by analyzing the samples daily for two weeks and resulted in 3.8% and 4.5% for zomepirac sodium and zomepirac glucuronide, respectively.

The lowest limit of detection has been found to be approximately 100 ng/ml. This is adequate since the peak urine concentrations of zomepirac and zomepirac glucuronide following a therapeutic dose (100 mg) are much higher.

A representative plot of urine zomepirac and zomepirac glucuronide concentrations vs. time for a volunteer subject following a 100-mg oral dose is shown in Fig. 4. The results indicate that in man the drug is cleared almost entirely by conjugation.

In conclusion, this HPLC method is a simple, reproducible and sensitive procedure.

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