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Note

Rapid high-performance liquid chromatographic analysis of oxaprozin, a non-steroidal anti-inflammatory agent

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Oxaprozin (Fig. 1) is a non-steroidal anti-inflammatory agent of the proprionic acid class which is under investigation for the treatment of pain and inflammation [1, 2]. Similar to other drugs in this category [3], high-performance liquid chromatography (HPLC) has been successfully used for quantitation of oxaprozin in biological fluids [4]. The present report describes a rapid and sensitive HPLC technique for analysis of oxaprozin in plasma. Analytic cost is greatly reduced with the use of the radial compression separation system [3, 5]. With the use of an automatic sampler, large numbers of samples can be quantitated with minimal technical time.

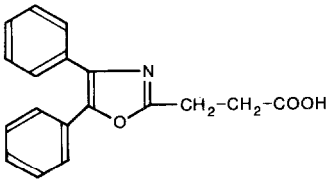

Fig. 1. Structural formula of oxaprozin.

METHODS

Reagents and standards

Pure samples of oxaprozin (Wyeth Labs., Radnor, PA, U.S.A.) and ketoprofen (Ives Labs., New York, NY, U.S.A.) were kindly provided by their pharmaceutical manufacturers. All other reagents, analytical grade or better, were purchased from commercial sources and used without further purification.

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Standard solutions were prepared by dissolving 100 mg of oxaprozin or ketoprofen each in 100 ml of methanol. Further dilutions with methanol were made as necessary.

Instrumentation

Analyses were performed using a Waters Assoc. (Milford, MA, U.S.A.) HPLC system, consisting of a solvent delivery system (M6000), ultraviolet absorbence detector (M440 or M480), data processor (M730), and automatic sampling system (WISP 710B). The column was a reversed-phase C_{18} radial compression cartridge (spherical 10-μm particles, non-postsilanized), 10 cm × 5 mm I.D., which was housed in an RCM-100 radial compression module. Column effluent was quantitated with the absorbance detector set at 280 nm. The mobile phase consisted of acetonitrile—water (45:55), to which were added 2.5 ml of acetic acid per liter. The flow-rate was 1 ml/min, and analyses were performed at room temperature.

Preparation of samples

Ketoprofen (50 μg) was added to a series of 13-ml round-bottom glass culture tubes with PTFE-lined screw top caps. Calibration standards were prepared by adding variable amounts of oxaprozin, ranging from 5 through 100 μg, to a series of these tubes. All other tubes contained only the internal standard. To each of the calibration tubes is added 1.0 ml of drug-free control plasma; 1 ml of "unknown" plasma is added to all other tubes. The samples are acidified with 0.2 ml of 1.0 *M* hydrochloric acid, and 4—5 ml of ethyl acetate are added. The samples are agitated in the upright position on a vortex-type mixer for 90—120 sec. After centrifugation for 10 min at 400 *g*, an aliquot of the upper organic layer is transferred to a tapered centrifuge tube and evaporated to dryness under mildly reduced pressure at 40—50°C. The extracts are redissolved in 0.2 ml of methanol and transferred to an automatic sampling vial equipped with a limited volume insert. The automatic sampler is programmed to deliver 20 μl per sample.

Clinical pharmacokinetic study

A healthy male volunteer participated after giving written informed consent. After an overnight fast, he received a single 1200-mg dose (two 600-mg tablets) of oxaprozin with 100—200 ml of tap water. He remained fasting until 3 h after dosage. Venous blood samples were drawn into heparinized tubes at multiple points in time over the next fourteen days. Blood samples were centrifuged, and the plasma separated and frozen until the time of assay. Concentrations of oxaprozin in all samples were determined by the method described above.

RESULTS

Evaluation of the method

Oxaprozin and the internal standard ketoprofen gave two well resolved chromatographic peaks (Fig. 2). Extracts of drug-free plasma samples were consistently free of endogenous contaminants at the retention times

corresponding to these two compounds. Oxaprozin plasma concentration was linearly related to the peak height ratio of oxaprozin versus ketoprofen. The equation of a typical calibration line is: $Y = 0.0273X + 0.023$ ($r = 0.999$), where X is plasma oxaproxin concentration and Y is peak height ratio. Coefficients of variation for identical samples ($n = 6$ at each concentration) were: at 1 μg/ml, 6.8%; at 10 μg/ml, 7.4%; at 50 μg/ml, 2.4%; and at 100 μg/ml, 4.4%. The sensitivity limits are approximately 0.5 μg/ml of plasma. This can be improved by minor modifications such as injection of larger aliquots of the final reconstituted extract.

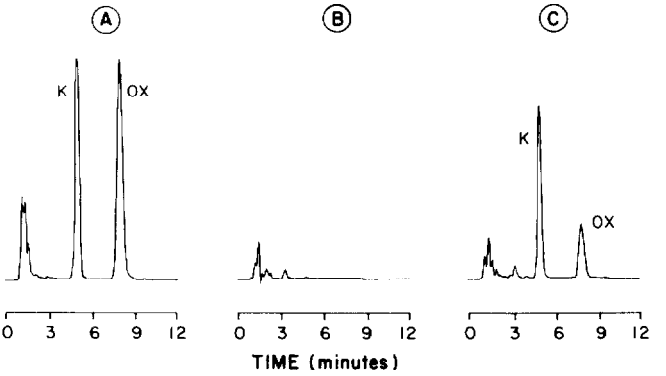

Fig. 2. Chromatograms of plasma extracts. (A) Calibration standard containing 50 μg/ml ketoprofen (K) and 50 μg/ml oxaprozin (OX). (B) Plasma sample from a subject prior to ingestion of oxaprozin. (C) Plasma sample (to which was also added the internal standard) from a subject taken 96 h after a single 1200-mg dose of oxaprozin.

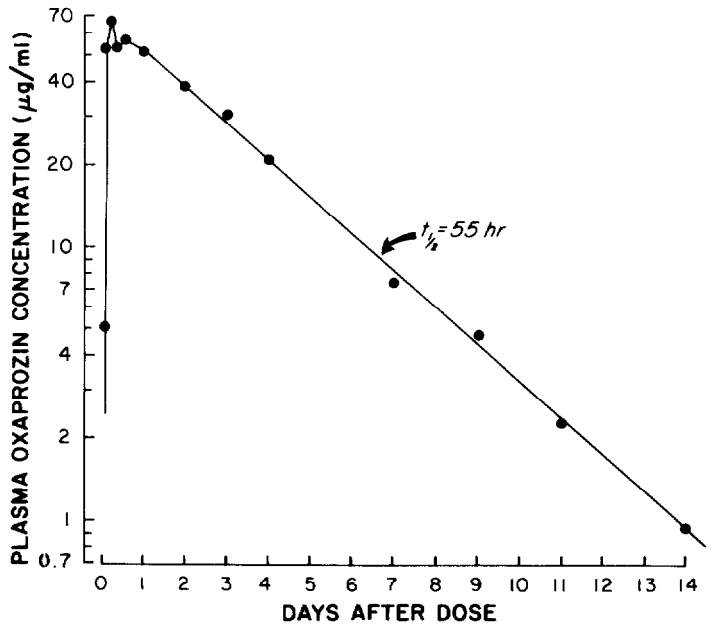

Fig. 3. Plasma concentrations of oxaprozin in the volunteer subject who participated in the pharmacokinetic study.

Potential assay interferences by other analgesic agents were evaluated for those compounds of which pure reference standards were available. Salicylic acid and acetaminophen had considerably shorter retention times than the internal standard ketoprofen, while indomethacin and phenybutazone had longer retention times than oxaprozin. Ibuprofen and piroxicam did not yield detectable chromatographic peaks under the described conditions. Therefore the above compounds do not interfere with the assay. Flurbiprofen and fenoprofen had retention times similar to oxaprozin and therefore are potential assay interferences.

Pharmacokinetic results

A peak plasma oxaprozin concentration of 67 μg/ml was reached at 6 h after dosage. Thereafter, plasma concentrations declined exponentially with an apparent half-life of 55 h (Fig. 3). Under the assumption that the entire 1200-mg dose of oxaprozin was available to the systemic circulation, apparent volume of distribution and total metabolic clearance were calculated as described previously [6]. The calculated values were: volume of distribution, 18 l; total clearance, 3.8 ml/min.

DISCUSSION

The present report describes a rapid and sensitive HPLC method for quantitation of oxaprozin in plasma. After addition of a structurally related internal standard, acidified plasma samples are directly extracted into ethyl acetate. The extract is separated, evaporated to dryness, and chromatographed on a reversed-phase C_{18} radial compression separation system. The overall approach is analogous to that previously developed by McHugh et al. [4]. However, the radial compression system has advantages in that the solvent consumption is low, and the cartridges themselves are considerably less expensive and have a longer average life than stainless-steel columns [3, 5]. Furthermore the automatic injection system allows quantitation of large numbers of plasma samples. One technical person can easily prepare up to 100 samples for analysis in a single working day, while the actual chromatography can proceed overnight using the automatic sampling system.

The applicability of the method to pharmacokinetic studies of oxaprozin in humans is demonstrated. Due in part to its extensive binding to plasma protein, the extent of oxaprozin distribution is very limited [7—9]. This, together with its low metabolic clearance, leads oxaprozin to have a very long elimination half-life [7—9]. Thus, oxaprozin has the potential to be effective as a non-steroidal anti-inflammatory agent with a once daily or once every other day dosing schedule.

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