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Note

High-performance liquid chromatographic determination of pimeprofen and its metabolite ibuprofen in sheep plasma and lymph

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Pimeprofen (I) is a pyridyl ester of ibuprofen (II) (Fig. 1) which has shown anti-inflammatory activity when applied topically [1–3]. Metabolic studies using radioactive techniques [4–6] and gas chromatography–mass spectroscopy [7] indicate that I is initially hydrolyzed in the bloodstream to form II and 2-pyridylmethanol.

A number of gas chromatographic and high-performance liquid chromatographic (HPLC) methods for the analysis of II in biological fluids have been described [8–24]; however, no chromatographic methods for the routine determination of I in biological fluids have been published.

This paper describes the development of such a method using solid-phase extraction of the analytes and reversed-phase HPLC. The method has been applied to the analyses of I and II in sheep plasma and lymph.

EXPERIMENTAL

Materials and reagents

Acetonitrile and methanol of HPLC grade were purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Pimeprofen (I) was obtained from Hisamitsu Pharmaceutical (Tosu, Saga, Japan). Deionized water, progesterone (the internal standard), ibuprofen (II) and blank sheep plasma were obtained from The Upjohn Company (Kalamazoo, MI, U.S.A.). Glacial acetic acid of reagent grade was purchased from Mallinckrodt (Paris, KY, U.S.A.). Sodium hydroxide and sulfuric acid solutions were prepared with Accululates purchased from Anachemia Chemicals (Champlain, NY, U.S.A.). Disposable C_{18} solid-phase extrac-

Pimeprofen (I) Ibuprofen (II)

Fig. 1. Chemical structures of pimeprofen (I) and ibuprofen (II).

tion cartridges (Sep-Pak) were purchased from Waters Assoc. (Milford, MA, U.S.A.).

Chromatographic system

The analyses were performed with a 250×4.6 mm I.D. Zorbax C_8 (5 μm) column (DuPont, Wilmington, DE, U.S.A.) at ambient temperature. The column was preceded by a 0.5-μm column inlet filter (Rheodyne, Cotati, CA, U.S.A.). The need to flush the analytical column after each injection necessitated using two mobile phases: one for the analytical separation and one for flushing the column. The pumping and switching of the mobile phases were accomplished with two Model 110A pumps (Altex Scientific, Berkeley, CA, U.S.A.) connected to a Model 7010 air-driven valve (Rheodyne). The valve switching was controlled by a digital valve sequence programmer (Valco Instrument, Houston, TX, U.S.A.). A WISP 710B (Waters Assoc.) was utilized as the injector. Detection was accomplished with a Spectromonitor D variable-wavelength UV–VIS detector set at 265 nm (LDC/Milton Roy, Riviera Beach, FL, U.S.A.). Chromatograpic data were collected and processed with laboratory data system developed by The Upjohn Company [25].

The analytical mobile phase was acetonitrile–deionized water–0.5 *M* sodium hydroxide–glacial acetic acid (550:410:45:5) at an apparent pH of 5.7, and was degassed and filtered through a 0.45-μm Nylon 66 membrane (Pall Pneumatic Product, Ocala, FL, U.S.A.). The mobile phase utilized for flushing the column was 100% acetonitrile. The flow-rate of the mobile phases was 1.2 ml/min. The injection volume was 100 μl and the detector attenuation was 0.01 a.u.f.s. The run time of 43 min included an initial 31 min of the analytical mobile phase to

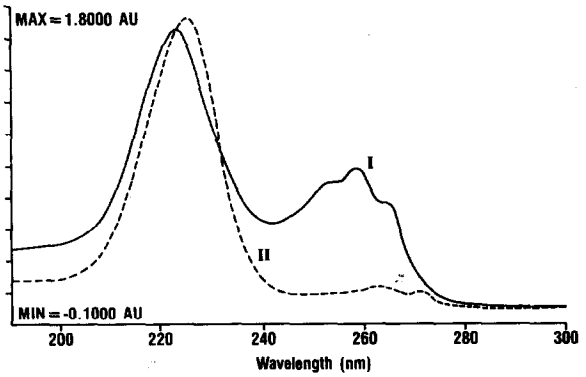

Fig. 2. UV spectra of pimeprofen (I) and ibuprofen (II) in analytical mobile phase.

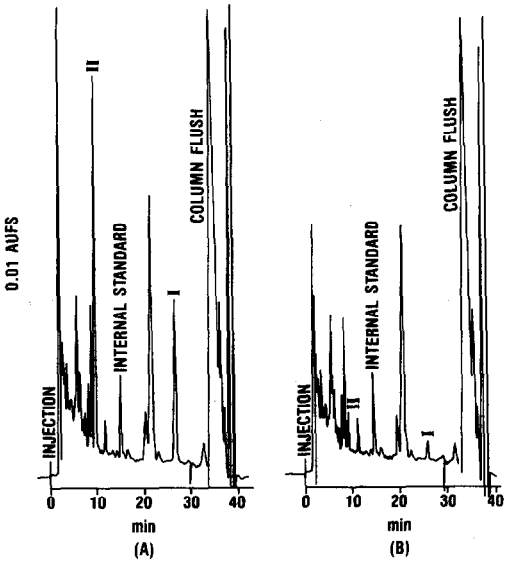

Fig. 3. Chromatograms of sheep plasma standards containing (A) 0.8 μg/ml I and 4.8 μg/ml II and (B) 0.08 μg/ml I and 0.5 μg/ml II.

perform the separation, 6 min of the flush mobile phase to remove non-polar contaminants, and 6 min of the analytical mobile phase to prepare the column for the next injection. The retention times of I, II, and progesterone (the internal standard) were approximately 27, 9, and 17 min, respectively.

Preparation of standards

A stock solution was prepared by dissolving an accurately weighed amount of approximately 17 mg of I and 120 mg of II in 50 ml of acetonitrile. Standard

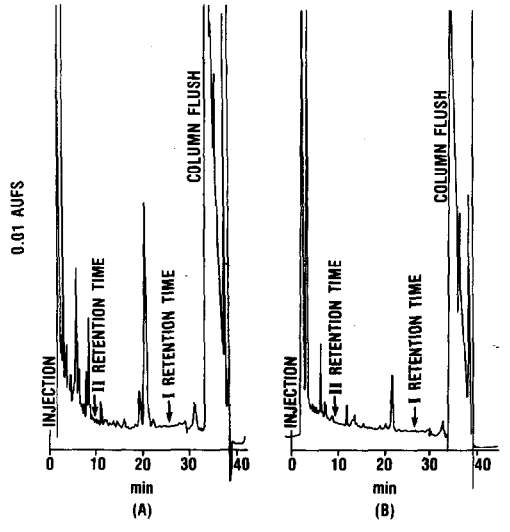

Fig. 4. Chromatograms of blank sheep plasma (A) and blank sheep lymph (B).

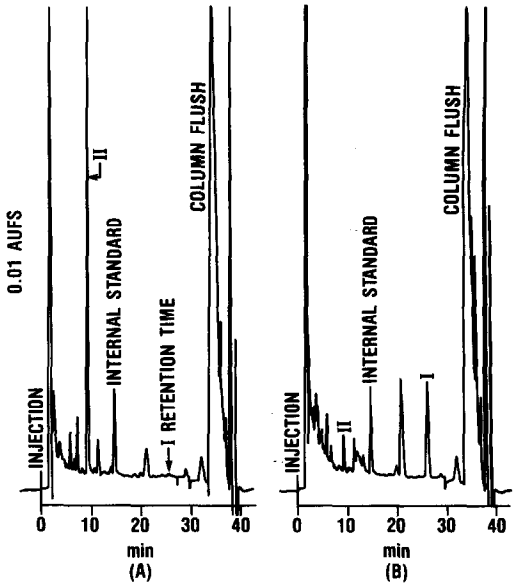

Fig. 5. Chromatograms of plasma (A) and lymph (B) from sheep dosed with pimeprofen (I).

solutions utilized for spiking blank plasma were 1:2, 1:10, 1:50, and 1:100 dilutions of the stock solution with acetonitrile–deionized water (50:50). The internal standard solution was a 0.4 μg/ml solution of progesterone in acetonitrile–deionized water (50:50). Plasma standards employed in the construction of standard curves were prepared by spiking blank sheep plasma with 25- or 50-μl aliquots of the standard solutions prepared above. Lymph samples were quantitated with the plasma standard curves since sufficient quantities of blank sheep lymph were not available.

TABLE I

RECOVERY OF PIMEPROFEN AND IBUPROFEN FROM PLASMA ($n=7$)

Concentration (μg/ml)	Mean recovery (%)	Coefficient of variation (%)
Pimeprofen		
7.5	67	9
0.8	69	7
0.3	75	10
0.2	67	10
0.08	72	8
Ibuprofen		
47.8	51	11
4.8	59	10
1.9	65	15
1.0	58	14
0.5	66	16

TABLE II

TYPICAL STANDARD CURVES FOR I AND II IN PLASMA

In the linear regression equations, y = response, x = concentration and r^2 = correlation coefficient.

Concentration of I (μg/ml)	Peak-height ratio I/I.S.	Concentration of II (μg/ml)	Peak-height ratio II/I.S.
7.5	14.230	47.8	23.830
0.8	1.374	4.8	2.557
0.3	0.603	1.9	1.065
0.2	0.267	1.0	0.507
0.08	0.136	0.5	0.273
$y=1.8854x-0.0063$ ($r^2=0.9999$)		$y=0.4972x+0.0943$ ($r^2=0.9999$)	

Standard and sample processing

A 1-ml volume of sample or spiked plasma standard was pipetted into separate 13×100 mm glass culture tubes. A 200-μl volume of 1 *N* sulfuric acid was added to each tube. After vortexing, 1 ml was withdrawn from each tube and placed on a C_{18} cartridge which had been prewashed with 2 ml of methanol and 5 ml of deionized water. The loaded cartridges were washed with 10 ml of methanol–deionized water (50:50) and adjusted to pH 4.0 with glacial acetic acid. The analytes were then eluted with 6 ml of acetonitrile. The eluent was collected in 16×125 mm glass culture tubes that were then placed in a 55°C water-bath. The acetonitrile was reduced to dryness under a stream of nitrogen. The standards and samples were ready for injection after reconstitution with 250 μl of the internal standard preparation.

Standard curves were constructed daily by plotting the peak-height ratios (analyte height/internal standard height) of the plasma standards against the concentration of the standard.

RESULTS AND DISCUSSION

Chromatograpy

Reversed-phase HPLC was chosen because of its ability to separate compounds which differ in the functional groups present and its capability to analyze carboxylic acids without derivatization. The analytical mobile phase selected was a sodium acetate-buffered acetonitrile–deionized water system at an apparent pH of 5.7. A pH between 4 and 7 was required to eliminate possible hydrolytic degradation of I under strong acid or alkaline conditions [26]. Good peak shape was observed for II even though the pH of the mobile phase was far enough above the pK_a of ibuprofen to convert it to the ionized form. The flush mobile phase was chosen because it quickly removed non-polar endogenous interferences from the column with minimal equilibration time between injections. Several reversed-phase columns were evaluated, including a Waters μBondapak C_{18}, Brownlee RP-8 Spheri-5, and a DuPont Zorbax C_8. The Zorbax column produced the best chromatography based on peak shape and separation efficiency. Three Zorbax columns tested had similar chromatographic properties.

Fig. 2 shows the UV spectra of I and II. Both analytes have absorption maxima near 225 and 260 nm. A wavelength of 265 nm was selected for quantitation because it elicited good analyte response while reducing endogenous component response. The 225-nm region was unsuitable because of interferences from endogenous components.

Fig. 3 presents chromatograms of typical sheep plasma standards. The retention times of I, II, and internal standard were 27, 9, and 17 min, respectively. The drug, metabolite, and internal standard were well resolved from each other and from endogenous components in the plasma. Fig. 4 shows chromatograms of blank sheep plasma and lymph. No significant interfering peaks were observed in either blanks. Fig. 5 presents chromatograms of plasma and lymph from sheep dosed with I. The chromatograms demonstrate that the sensitivity of the method is adequate and that additional metabolites of I do not interfere with the compounds of interest.

Recovery from plasma

The recoveries of I and II from blank sheep plasma were determined by comparing the peak-height ratios from seven standard curves prepared on different days to the peak-height ratio obtained for an acetonitrile–deionized water (50:50) solution of the analytes analyzed concurrently with each standard curve (Table I). The results of the recovery studies indicate no significant dependence of recovery on concentration. The lack of quantitative recovery is primarily a result of incomplete extraction of the analytes from the plasma. A thorough study of the recoveries of the analytes from sheep lymph was not conducted due to insufficient quantities of blank lymph. In order to assure the validity of using plasma standard curves for lymph sample quantitation, a lymph sample which gave no response for the analytes was spiked and analyzed versus an acetonitrile–deionized water (50:50) solution of the analytes. The recoveries of I and II from the lymph were similar to those from the plasma.

Calibration curves

Standard curves were prepared over the concentration range 0.08–7.5 μg/ml I and 0.5–48 μg/ml II. Table II lists experimental data from typical standard curves. The calibration data from seven standard curves for each analyte were well described by linear equations with correlation coefficients ranging from 0.9998 to 0.9999 for I and 0.9985 to 0.9999 for II. The coefficient of variation of the slopes of the seven calibration lines ranged from 0.26 to 0.65% for I and 0.36 to 1.9% for II. The mean intercepts were 0.02 and 0.1 μg/ml for I and II, respectively. Based on a signal-to-noise ratio of 3:1, the limits of detection were 0.04 and 0.5 μg/ml for I and II, respectively.

Precision and accuracy

The analytes were added to blank sheep plasma to give six replicate samples with theoretical concentrations of 0.33 μg/ml I and 2.16 μg/ml II. The precision of the method, determined by processing the samples in groups of three on two consecutive days, was 7.2 and 5.8% for I and II, respectively. The accuracy of the

method, calculated by comparing the results from the precision study to the theoretical values expected, was 97% for I and 94% for II.

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