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Note

Simultaneous determination of ketorolac and its hydroxylated metabolite in plasma by high-performance liquid chromatography

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Ketorolac tromethamine (KT, Toradol®) is an orally and parenterally active non-steroidal and non-narcotic analgesic with cyclo-oxygenase inhibitory activity [1–4].

Following oral or intramuscular administration of KT, ketorolac (K) and the *p*-hydroxy metabolite (KM) are the major drug-related components found in human plasma [5]. KM is an essentially inactive metabolite, having less than one fifth the anti-inflammatory activity and less than one hundredth the analgesic activity of KT in animals [5] and therefore it does not contribute significantly to the overall clinical effect of KT However, to investigate any major differences in the metabolism of KT as assessed by the metabolic profile in plasma following different routes of administration, it was necessary to develop a method suitable for the simultaneous quantification of K and KM in plasma. The simple and specific high-performance liquid chromatographic (HPLC) method that we have developed for the simultaneous determination of K and KM is described in this report.

EXPERIMENTAL

Chemicals and reagents

Ketorolac, (±)-5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid (I), *p*-hydroxyketorolac (II) and the internal standard, *m*-hydroxyketorolac (III), were obtained from Syntex Research (Palo Alto, CA, U.S.A.) (Fig. 1).

Acetonitrile and methanol were HPLC grade (J. T. Baker, Phillipsburg, NJ, U.S.A.), diethyl ether and hexane were distilled in glass (Burdick & Jackson Labs., Muskegon, MI, U.S.A.) and all other chemicals were of reagent grade. Phosphate buffer (0.02 *M*, pH 6) was prepared by mixing 88.9 parts of 0.02 *M* KH_2PO_4 with 11.1 parts of 0.02 *M* Na_2HPO_4.

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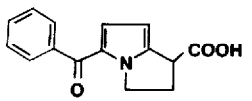

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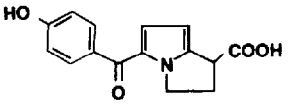

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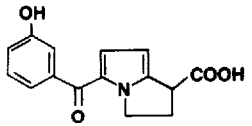

III

Fig 1. Structures of ketorolac (I). *p*-hydroxyketorolac (II) and *m*-hydroxyketorolac (III).

High-performance liquid chromatography

A Waters Model 204 series HPLC system equipped with a Model 6000A solvent delivery system, a Model 710A WISP autosampler (Waters Assoc., Milford, MA, U.S.A.) and a fixed-wavelength UV absorption detector (Model 160, Beckman Instruments, Palo Alto, CA, U.S.A.) was used. Separations were performed on a Regis Spherisorb ODS column (25 cm × 4.6 mm I.D; particle size 5 μm) (Regis, Morton Grove, IL, U.S.A) with a Whatman HC Pellosil C_{18} guard column (7 cm × 2.1 mm I.D ; particle size 30–38 μm) (Whatman, Clifton, NJ, U S.A.) using acetonitrile–methanol–0.02 *M* phosphate buffer/0.01 *M* tetrabutylammonium phosphate (pH 6) (15·20:65) as the mobile phase. The chromatography was performed at ambient temperature using a flow-rate of 0.8 ml/min. The chromatographic peaks of interest were monitored by UV absorption at 313 nm (0.005 a.u.f.s) and the chromatographic data were collected and processed by a Nelson 6000 data acquisition and processing system (Nelson Analytical, Cupertino, CA, U.S.A.).

Standard solutions

Stock solutions of K (I) at a concentration of 1 mg/ml and KM (II) at 200 μg/ml were prepared in methanol. The working solution of internal standard (III) contained 150 ng of III per 500 μl of aqueous methanol (water–methanol, 9:1). Spiking solutions containing 50, 100, 300 and 500 ng of K or 10, 25, 50 and 100 ng

of KM per 500 μl of aqueous methanol (water–methanol, 9:1) were prepared by serial dilution of the stock solutions.

Sample preparation

To 1 ml of plasma in a 15-ml culture tube were added 1 ml of aqueous methanol (water–methanol, 9:1), 500 μl of the internal standard solution (*m*-hydroxyketorolac, 150 ng), 100 μl of 0.5 *M* sodium acetate (pH 3) and 5 ml of diethyl ether. The mixture was placed in a mechanical shaker for 5 min and then centrifuged at 3500 *g* for 5 min. The organic layer was transferred to another 15-ml culture tube and 3 ml of hexane and 2 ml of 0.1 *M* sodium hydroxide were added. The mixture was shaken for 5 min and then centrifuged for 5 min. After centrifugation, the organic layer was removed and discarded by using a vacuum aspirator. The aqueous alkaline layer was made acidic by the addition of 500 μl of 2 *M* hydrochloric acid. The mixture was extracted with 8 ml of diethyl ether as described previously. The resulting organic layer was transferred to a clean tube and was evaporated to dryness under a steady stream of nitrogen at 45°C. The residue was reconstituted in 100 μl of the mobile phase by vortex-mixing for 15 s. The solution was transferred to an autosampler vial, and 30 μl were injected into the HPLC system for analysis.

Quantification

Concentrations of K and KM in samples of plasma were calculated by reference to their corresponding calibration curves. For generation of calibration curves 1-ml aliquots of blank human plasma were spiked with standard solutions of K and KM to yield samples containing K and KM at concentrations of 50 and 10, 100 and 25, 300 and 50, and 500 and 100 ng/ml. These samples were processed in the manner described above under *Sample preparation*.

Calibration curves were obtained by plotting the ratio of the peak height for the analyte to that of the internal standard (y) against the quantity of the analyte added (x) and fitting the data to a straight line by linear least-squares regression analysis.

RESULTS AND DISCUSSION

Using the chromatographic conditions described, KM, the internal standard and K yielded sharp and well resolved peaks with retention times of 7.2, 8.3 and 14.3 min, respectively (Fig. 2B). In the method described here, K and KM are extracted from acidified plasma into diethyl ether. The crude extract obtained is not suitable for HPLC analysis owing to the presence of numerous endogenous components that interfere with the quantification of K and KM. To further purify the extract prior to HPLC analysis, acid–base partitioning is employed. However, the analytes cannot be extracted efficiently from diethyl ether into aqueous base. Efficient extraction of the analytes into aqueous base requires that

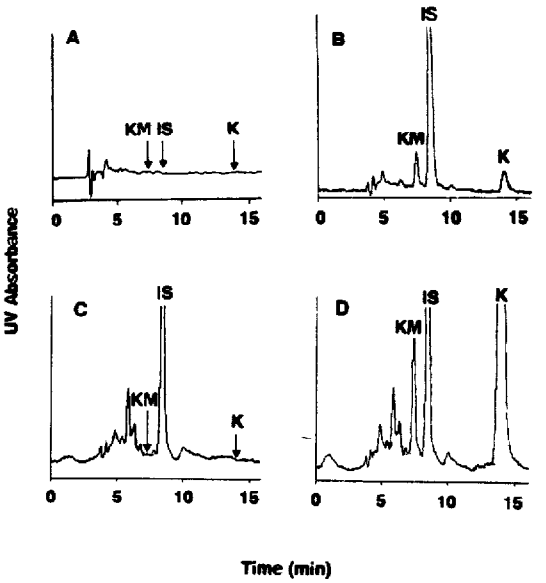

Fig 2 HPLC profiles of 1 ml of (A) blank human plasma, (B) human plasma spiked with 150 ng of internal standard (IS), 50 ng of ketorolac (K) and 10 ng of metabolite (KM), (C) plasma collected from a subject pre-dose and (D) plasma from a subject 2 h following intramuscular injection of 60 mg of ketorolac tromethamine

the polarity of the initial ether extract be reduced by addition of hexane. The aqueous extract obtained is acidified, and the analytes are back-extracted into diethyl ether.

To achieve chromatographic separation of KM and the internal standard from endogenous components it is necessary to add tetrabutylammonium phosphate (TBA) to the mobile phase to serve as a counter-ion. In the absence of TBA, some samples of plasma, especially those that were more than two years old, contain endogenous components that cannot be adequately resolved chromatographically from KM and the internal standard. A typical example of a chromatogram obtained for blank human plasma processed by this method is shown in Fig. 2A. A representative chromatogram for blank plasma spiked with 10 ng of KM, 50 ng of K and 150 ng of internal standard and processed by this method is shown in Fig. 2B.

Linear calibration curves are obtained for K ($r = 1.0$) at concentrations of 50–500 ng/ml and for KM ($r = 1.0$) at concentrations of 10–100 ng/ml. Data for the recovery of K and KM from samples of plasma fortified with these compounds in the range 50–500 ng for K or 10–100 ng for KM are shown in Table I. The mean recovery for K spiked in plasma ranged from 98.8 to 101.2% and for KM spiked in plasma ranged from 98.4 to 105%. The low coefficients of variation for the recovery of K ($\leq$ 6.3%, $n = 4$) and KM ($\leq$ 3 9%, $n = 4$) provide evidence

TABLE I

RECOVERY OF KETOROLAC AND ITS *p*-HYDROXY METABOLITE FROM 1 ml PLASMA DURING HPLC ASSAY

Amount spiked (ng)	Amount recovered (ng)					C V (%)	Mean recovery (%)
	1	2	3	4	Mean ± SD		
Ketorolac							
50	49 1	47 3	54 7	51.3	50 6±3 2	6 3	101 2
100	103 1	102 7	101 7	97 3	101 2±2 7	2 6	101 2
300	295 7	300.7	286.1	302 6	296 3±7 4	2 5	98 8
500	502 0	499.3	507.5	498 9	501 9±4.0	0 8	100.4
Metabolite							
10	10.2	11 0	10.1	10 6	10 5±0 4	3 9	105 0
25	24 9	25 7	23 6	24 0	24.6±0 9	3.8	98 4
50	49 7	47 0	51 9	50 4	49.8±2 1	4.1	99 6
100	100 1	101 2	99 4	100.0	100.2±0 8	0 7	100 2

of the reproducibility of the method. The quantification limit of this method is adequate to allow measurement of KM and K in samples of plasma collected up to 8 and 24 h, respectively, after oral or intramuscular administration of a single 30-mg dose of KT to human volunteers.

This assay has been used for the quantification of K and KM in plasma collected from twelve subjects during a study to compare the bioavailability and metabolic fate of KT administered orally (30 mg) and intramuscularly (30, 60 and 90 mg). Representative chromatograms obtained for samples of plasma collected from one subject during the study are shown in Fig. 2C and D. The average

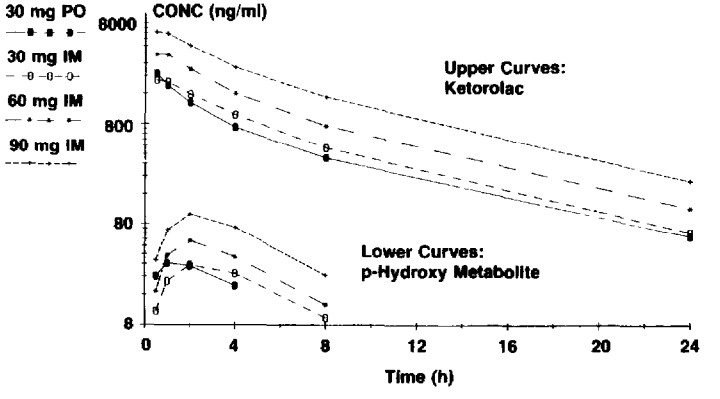

Fig 3. Average concentrations of ketorolac and its *p*-hydroxy metabolite in plasma from five subjects following administration of 30 mg orally (PO) and 30, 60 and 90 mg intramuscularly (IM) of ketorolac tromethamine

concentration–time profiles for K and the metabolite KM obtained using data generated by this method are shown in Fig. 3. At all collection times following oral or intramuscular administration of KT, the concentration of the metabolite in the plasma was less than 5% that of K.

In conclusion, the HPLC method described here is simple and specific and can be partially automated for the simultaneous analysis of ketorolac and its *p*-hydroxy metabolite present in plasma at concentrations of ≥ 50 and ≥ 10 ng/ml, respectively.

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