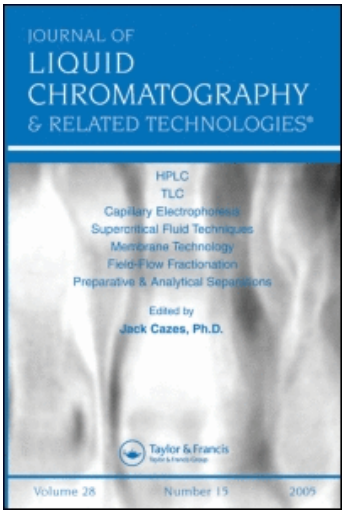

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Tsina, Irene , Chu, Frances , Kaloostian, Martin , Pettibone, Marian and Wu, Anne(1996) 'HPLC Method for the Determination of Ketorolac in Human Plasma', Journal of Liquid Chromatography & Related Technologies, 19: 6, 957 — 967
To link to this Article: DOI: 10.1080/10826079608001926
URL: http://dx.doi.org/10.1080/10826079608001926

J. LIQ. CHROM. & REL. TECHNOL., 19(6), 957–967 (1996)

HPLC METHOD FOR THE DETERMINATION OF KETOROLAC IN HUMAN PLASMA

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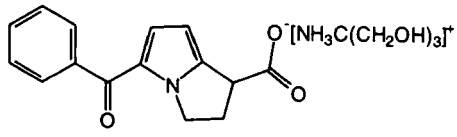
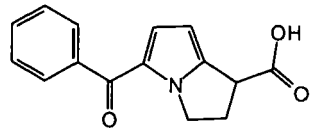
ABSTRACT

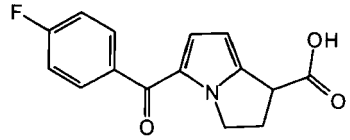
A precise and accurate HPLC method for the quantification of ketorolac in human plasma is described. Ketorolac and an added internal standard (a fluoro analog of ketorolac) are extracted from acidified plasma samples and the extract is further purified. The reconstituted sample is injected onto a reverse phase HPLC column. The method has a linear concentration range of 0.010-3.00 μg of ketorolac per aliquot of plasma, using 0.1 to 1.0 mL of plasma for analysis. The quantification limit of the method is 0.010 μg/mL. The intra- and inter-assay % CVs were less than 5.5%, and the mean recoveries ranged from 96% to 109%. The absolute recovery was 92%.



Ketorolac Tromethamine
Structure I



Ketorolac
Structure II



Internal Standard (IS)
Structure III

Figure 1. Molecular structures of ketorolac tromethamine (I), ketorolac (II), and internal standard (III).

INTRODUCTION

Ketorolac tromethamine (**I**, Figure 1) is a potent non-narcotic analgesic. The activities of the drug arise from its ability to inhibit prostaglandin synthesis.[1-4] Ketorolac tromethamine is marketed as a racemic mixture, although its biological activity is associated with the (-)*S* enantiomer.[5] It is available for intravenous (IV), intramuscular (IM), and oral administration.

To support the evaluation of the pharmacokinetics of ketorolac tromethamine in humans, we have developed a sensitive, specific, and accurate HPLC method for the determination of ketorolac in plasma. Previous unoptimized versions of this method have been summaried briefly,[6,7] but the fully optimized method with complete validation data is presented here. Two other methods for the determination of ketorolac in plasma or serum using HPLC have been reported.[8,9] These methods have quantification limits two to five times greater than those of the method reported here and are not always adequately sensitive for the study of ketorolac pharmacokinetics in humans.

EXPERIMENTAL

Chemicals and Supplies

Ketorolac tromethamine, (±)-5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2a] pyrrole-1-carboxylic acid, tris(hydroxymethyl)aminomethane salt (**I**; Figure 1); ketorolac, (±)-5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2a]pyrrole-1-carboxylic acid (**II**; Figure 1); and internal standard, RS-37414-000, (±)-5-p-fluorobenzoyl-1,2-dihydro-3H-pyrrolo[1,2a]-pyrrole-1-carboxylic acid (IS, **III**; Figure 1) were obtained from Syntex Research (Palo Alto, CA, USA). HPLC-grade methanol, acetonitrile, hexane, and ethyl acetate were purchased from Burdick and Jackson Laboratories (Muskegon, MI, USA), and water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Reagent grade phosphoric acid, 85%, analytical grade sodium acetate, and glacial acetic acid were purchased from Mallinckrodt (St. Louis, MO, USA). An aqueous solution of 0.5 M sodium acetate buffer, pH 3, was prepared in house. Heparinized human control plasma (blank plasma) was obtained from normal, healthy volunteers from the Clinical Studies Unit, Syntex Research (Palo Alto, CA, USA).

Instrumentation

The HPLC system consisted of a Hewlett-Packard Model 1090 L ternary solvent delivery system and autosampler (Santa Clara, CA, USA), a Kratos Spectroflow 785A variable wavelength UV detector (Kratos Analytical Instruments, Ramsey, NJ, USA), and a Nelson 6000 Laboratory Data System (PE-Nelson, Cupertino, CA, USA).

Chromatographic Conditions

For the determination of ketorolac, aliquots of sample extract (20 µl) were injected onto a Nova-Pak C_{18}, 4-µm, 4.6 mm x 150 mm column (Waters Associates, Milford, MA, USA) using a mobile phase of acetonitrile-0.05% aqueous phosphoric acid (34:66, v/v) and a flow rate of 1 mL/min. The UV detector was set at 317 nm. A 0.5-µm precolumn filter (Upchurch Scientific Inc., Oak Harbor, WA, USA) was connected to the analytical column and replaced every 1,000 injections.

Sample Preparation

1. Spiking procedure

A stock solution was prepared by dissolving ketorolac tromethamine in methanol. The stock solution was further diluted with methanol-water (9:1, v/v) to prepare spiking solutions at equivalent ketorolac free acid concentrations of 0.1, 0.2, 0.4, 1, 2, 10, 20, and 30 µg/mL. An internal standard spiking solution (IS) of 2 µg/mL was prepared by dissolving the internal standard in methanol and then further diluting the solution in methanol-water (9:1, v/v). For preparation of the calibration standards used for the construction of the calibration curve and for validation of the method, 0.1-mL aliquots of the ketorolac spiking solutions were added to 1.0-mL aliquots of blank human plasma to prepare a set of calibration standards at ketorolac concentrations of 0.01, 0.02, 0.04, 0.1, 0.2, 1.0, 2.0, and 3.0 µg/mL.

2. Extraction of calibration standards

To each calibration standard was added 0.1 mL of the internal standard spiking solution and 0.1 mL of the 0.5 M sodium acetate buffer. The combination was vortexed briefly, and 6 mL of ethyl acetate-hexane (3:7, v/v) was added to each tube. Tubes were vortexed vigorously for 5 minutes, then

centrifuged for 2 to 5 minutes at 2000 rpm, and then placed in a dry ice/isopropanol or dry ice/methanol bath to freeze the aqueous layer. The organic layer was then decanted, and the organic extract was evaporated to dryness at 38°C under a stream of nitrogen. Each tube then received 0.5 mL of methanol-water (9:1, v/v) and 3 mL of hexane. Tubes were sonicated for 15 seconds and then vortexed vigorously for 3 minutes. The contents were allowed to settle for at least 5 minutes before the upper hexane layer was aspirated and the remaining methanol-water layer was evaporated to dryness at 38°C under a stream of nitrogen. Then 0.1 mL of methanol was added, followed by 0.1 mL of the mobile phase, acetonitrile-aqueous 0.05% phosphoric acid (34:66, v/v). Tubes were sonicated for 30 seconds, then vortexed for 30 seconds. Tube contents were then transferred to HPLC vials.

3. Extraction of clinical samples

Samples of heparinized plasma obtained from healthy volunteers or patients treated with ketorolac were stored at -20°C before analysis. Samples were thawed at room temperature, vortexed briefly, and centrifuged for 2 minutes at approximately 2500 rpm; an aliquot of 0.1-1.0 mL was dispensed for analysis. When less than 1.0 mL was used for analysis, water was added to bring the total volume to 1.0 mL. The samples were extracted using the same procedure described for the calibration standards. The concentration of ketorolac in samples was calculated by reference to calibration curves generated from calibration standards analyzed along with each batch of clinical samples. Concentrations were reported in terms of μg of ketorolac free acid per mL of plasma.

Data Handling and Calculations

Linear least-squares regression was performed on the peak height ratio (analyte peak height:internal standard peak height) versus concentration data generated by the calibration standards to construct a linear standard curve of the form *peak height ratio = m (concentration) + b*. Calibration standards with ketorolac concentrations from 0.01-0.20 μg/mL ketorolac were used in the unweighted linear regression to construct a calibration curve, and calibration standards of higher concentrations were used to verify extrapolations of the curve up to 3.0 μg/mL. Concentrations in unknowns were then determined from their peak height ratios by the standard curve equation, with appropriate corrections for sample aliquot volumes that were less than 1.0 mL.

Preparation of Quality Control Samples

Quality control samples (QCs) prepared by spiking ketorolac into control human plasma were stored at -20°C in a manner similar to that used for the clinical samples. QCs were prepared at the following three ketorolac concentrations: QC #1 (0.020 μg/mL), QC #2 (0.415 μg/mL), and QC #3 (13.3 μg/mL). To prepare the bulk QCs, ketorolac was dissolved in methanol-water (9:1, v/v) and added to the appropriate amount of blank human plasma, which was then swirled briefly and stirred on a magnetic stirrer for 10 minutes before being apportioned into polypropylene tubes for storage at - 20°C. Two QCs of each of the three different concentrations were analyzed with each batch of clinical samples to monitor the performance of the method during routine use.

RESULTS AND DISCUSSION

Quantification Limit

The quantification limit of the method is 0.010 μg/mL using 1.0 mL of plasma for analysis. Concentrations below 0.010 μg/mL are reported as below the quantification limit of the method (BQL). At the quantification limit, the signal-to-noise ratio of the HPLC peak was approximately 8:1.

Precision and Accuracy

The precision of the assay was assessed by the intra-assay (within-day) and inter-assay (between-day) coefficients of variation (%CVs) of the method. The accuracy of the method was evaluated by the recovery, defined as the ratio of the concentration of ketorolac found in the sample to that added to the sample (found/added). Data for the intra- and inter-assay %CVs and the recoveries obtained using calibration standards are presented in Table 1. Corresponding data for the QC samples are also presented in Table 1. All intra-assay %CVs were less than 4% and all inter-assay %CVs were less than 6%. All recoveries were between 96% and 109%.

Specificity

The analysis of blank human plasma from six different sources showed no interfering peaks at the retention times of ketorolac and the internal standard.

Representative chromatograms from blank plasma, from a spiked calibration standard, and from a patient sample are shown in Figure 2.

Linearity

The linear range of the method, using 0.1 to 1.0 mL of plasma for analysis, was 0.010-3.0 μg per aliquot of plasma.

Table 1

Precision and Accuracy of Ketorolac HPLC in Plasma

	Nominal Concentration (μg/m L)	Replicates Intra-assay	Replicates Inter-assay	Mean Concentration Found (μg/mL) Intra-assay	Mean Concentration Found (μg/mL) Inter-assay	Precision (%CV) Intra-assay	Precision (%CV) Inter-assay	Accuracy (% Recovery) Intra-assay	Accuracy (% Recovery) Inter-assay
Calibration Standards	0.0100	4	4	0.0109	0.0103	3.85	3.84	109	103
	0.0200	4	4	0.0207	0.0201	1.86	2.45	103	101
	0.0400	4	4	0.0402	0.0402	0.610	3.81	101	101
	0.100	4	4	0.0983	0.0983	0.985	1.34	98.3	98.3
	0.200	4	4	0.200	0.201	0.289	0.642	100	101
	1.00	4	4	1.00	1.00	1.44	1.92	100	100
	2.00	4	4	2.07	1.99	0.623	1.71	103	99.5
	3.00	4	4	3.14	3.00	1.16	2.78	105	100
QC Samples									
QC #1	0.0200	4	48	0.0199	0.0192	3.99	5.31	99.5	96.0
QC#2	0.415	4	48	0.401	0.400	1.23	2.83	96.6	96.4
QC#3	13.3	4	48	14.1	13.5	0.816	2.40	106	102

Absolute Recovery

The absolute recovery of ketorolac from plasma, determined by the analysis of plasma spiked with [14C]-ketorolac, was 92%.

Effect of Volume

The effect of varying the volume of plasma used in the range of 0.1 to 1.0 mL was examined using QC samples. The results with aliquots of 0.1, 0.2, and 0.5 mL were equivalent to results with 1.0-mL aliquots. These data indicate that volumes of plasma from 0.1 to 1.0 mL may be used for the analysis.

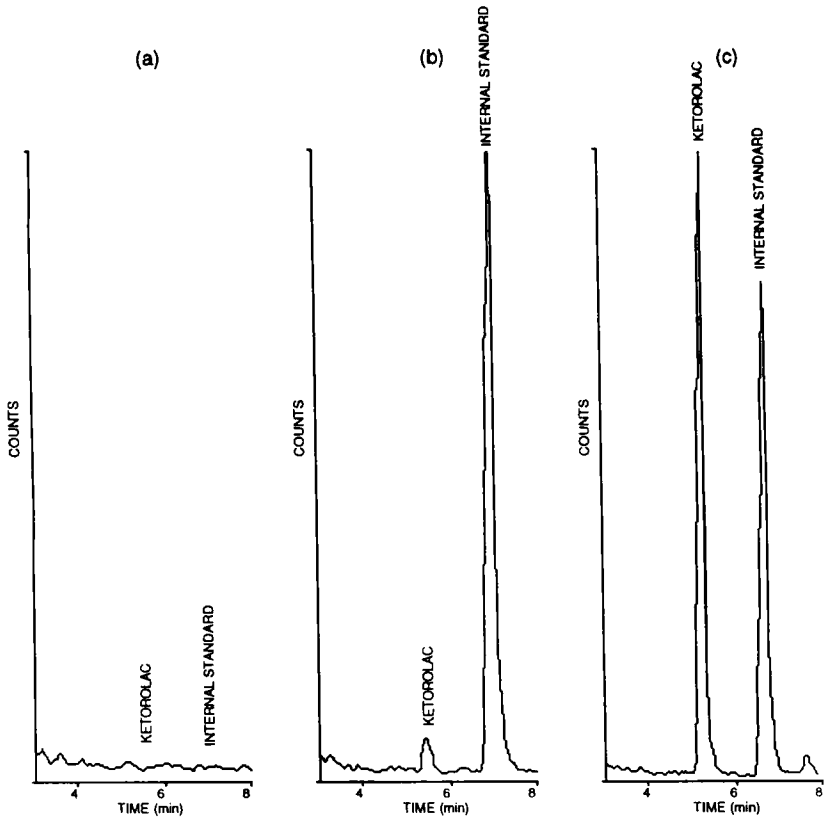


Figure 2. Chromatograms of (a) a blank human plasma from untreated subject; (b) a blank human plasma spiked with 0.010 μg/mL of ketorolac tromethamine and 0.2 μg/mL of internal standard; and (c) a patient plasma sample 40 minutes following oral administration of 10 mg of ketorolac tromethamine.

Stability

Ketorolac concentrations in QC plasma samples were found to be stable when stored in a -20°C freezer for at least 6 months. Ketorolac in QC plasma samples was found to be stable for at least 24 hours when stored on the laboratory bench at room temperature under normal laboratory conditions.

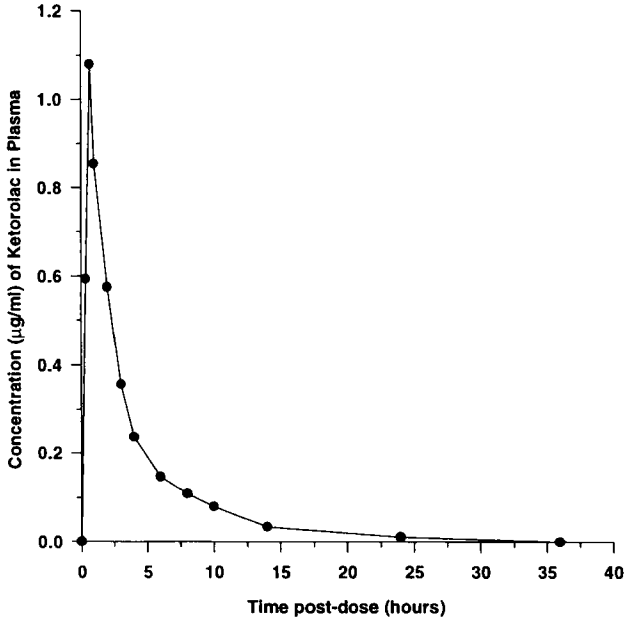


Figure 3. A representative plasma concentration vs time profile of a healthy subject following oral administration of 10 mg of ketorolac tromethamine. (Values reported as BQL are plotted as 0 µg/mL.)

Using refreezing in a -20°C freezer after thawing samples, ketorolac in both QC and clinical plasma samples was found to be stable for at least three freeze/thaw cycles.

Extracts prepared for HPLC analysis from calibration standards, from QC samples, and from clinical samples were analyzed by HPLC on the day samples were processed and several times after storage. They were stored refrigerated at 1-4°C or at room temperature (20-23°C) under normal laboratory conditions. Resulting data indicated that ketorolac in the extract is stable for at least 24 hours at room temperature and for at least 2 weeks when stored at 1-4°C.

Application

This method has been applied to the analysis of plasma from healthy subjects and patients treated with oral, IM, and IV ketorolac tromethamine. A representative profile for a healthy subject who received 10 mg of oral ketorolac is shown in Figure 3.

CONCLUSIONS

The method described here for the determination of ketorolac tromethamine in plasma is precise and accurate and can be used in samples obtained from subjects receiving ketorolac tromethamine by intramuscular, intravenous, or oral administration.

ACKNOWLEDGEMENTS

The authors wish to thank Bee-Lian Huang for technical contributions and Jennifer Porro for assistance in writing the manuscript.

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Received October 5, 1995
Accepted October 30, 1995
Manuscript 4006