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Short communication

Determination of ketorolac in human plasma by reversed-phase high-performance liquid chromatography using solid-phase extraction and ultraviolet detection

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Abstract

An improved high-performance liquid chromatographic method has been developed to measure human plasma concentrations of the analgesic nonsteroidal anti-inflammatory drug ketorolac for use in pharmacokinetic studies. Samples were prepared for analysis by solid-phase extraction using Bond-Elut PH columns, with nearly complete recovery of both ketorolac and the internal standard tolmetin. The two compounds were separated on a Radial-Pak C_{18} column using a mobile phase consisting of water–acetonitrile–1.0 mol/l dibutylamine phosphate (pH 2.5) (30:20:1) and detected at a UV wavelength of 313 nm. Using only 250 μl of plasma, the standard curve was linear from 0.05 to 10.0 μg/ml.

Keywords: Ketorolac

1. Introduction

Ketorolac [(±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid] is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic efficacy similar to that of the opioids [1]. The drug is administered intravenously, intramuscularly, or orally as the water soluble tromethamine salt (Toradol; Hoffmann-La Roche, Nutley, NJ, USA) to treat moderate pain or, together with reduced opioid doses, for severe pain.

Previously described methods of ketorolac analysis include both high-performance liquid chromatography (HPLC) [2–5] and gas chromatography–mass spectrometry (GC–MS) [6]. Wu and Massey [3] developed an HPLC assay for ketorolac that consumes 1 ml of plasma for sample preparation by a tedious three-stage liquid–liquid extraction technique that uses a total of 13 ml of diethyl ether and 3 ml of hexane; the sensitivity of this assay is 0.05 μg/ml. The other reported ketorolac HPLC assays are either a slight modification of the assay of Wu and Massey [5] or brief descriptions of sample preparation and chromatographic conditions without assay validation data [2,4]. Kelm et al. [6] described a GC–MS assay developed to measure ketorolac concentrations in plasma and gingival crevicular fluid after oral, topical administration. In this assay, solid-phase

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extraction was used for sample preparation, the dried effluent was derivatized with ethereal diazomethane, and residual baseline interference was filtered out by mass spectrometry; the reported limit of quantitation of the assay was 0.5 ng ketorolac/ml of plasma, with a relative standard deviation of less than 20%, which is well below that needed for the quantitation of ketorolac in normal clinical conditions in which the EC_{50} (median effective concentration) is 0.37 μg/ml [7]. The purpose of the present study was to develop a simple and sensitive HPLC assay for the measurement of human plasma ketorolac concentrations for use in pharmacokinetic studies of systemically administered ketorolac.

2. Experimental

2.1. Chemicals

Ketorolac tromethamine was donated by Hoffmann-La Roche. Sodium tolmetin was obtained from Sigma (St. Louis, MO, USA). HPLC-grade acetonitrile, methanol, and acetone were obtained from Burdick and Jackson (Muskegon, MI, USA) while organic-reagent grade dibutylamine, reagent-grade hydrochloric acid, and analytical-reagent grade phosphoric acid were obtained from Mallinckrodt (Paris, KY, USA).

2.2. Preparation of stock and plasma standard solutions

Because ketorolac tromethamine dissociates into the anion form of ketorolac at physiological pH [8], the measured concentration is referred to ketorolac; 14.7 mg of ketorolac tromethamine was dissolved in 10 ml of methanol to provide a free acid concentration of 1.0 mg/ml [9]. Appropriate dilutions were made in methanol to produce final concentrations of 500, 250, 100, 50, 25, 10, and 5 μg/ml in stock solutions, 10 μl of which was added to 1.0 ml of human plasma to give final standard plasma concentrations of 10.00, 5.00, 2.50, 1.00, 0.50, 0.25, 0.10, 0.05 μg/ml, respectively. A 10-mg amount of the internal standard sodium tolmetin [5] was dissolved in 10 ml of methanol and was further diluted to 20 μg/ml in methanol to prepare the working internal standard solution.

2.3. Sample collection

Venous blood samples (2 ml) were obtained by syringe through an intravenous catheter and transferred to Vacutainer (Becton-Dickinson, Rutherford, NJ, USA) blood collection tubes containing sodium heparin. The plasma samples were removed after centrifugation of the blood for 10 min at 1800 *g*, transferred to polypropylene tubes, and stored at −25°C until prepared for analysis.

2.4. Solid-phase extraction procedure

Ketorolac was extracted from plasma using Bond-Elut 100-mg PH solid-phase extraction columns on a Vac-Elut 10 place vacuum manifold (Varian, Harbor City, CA, USA). The columns were conditioned by sequential flushes (under vacuum) of two column volumes of methanol, two volumes of acetone, two volumes of water, and two volumes of 0.05 *M* hydrochloric acid (HCl). Then 250 μl 0.05 *M* HCl, a 250 μl plasma sample, 10 μl of the working internal standard solution, and 250 μl 0.05 *M* HCl were loaded onto the column and the vacuum was reapplied. The column was next washed with 500 μl 0.01 *M* HCl under vacuum. Subsequent application of 500 μl acetone, under vacuum, eluted the ketorolac and tolmetin into 1.5-ml microcentrifuge tubes. Samples were then dried under vacuum using the Alltech vacuum manifold and drying attachment (Alltech, Deerfield, IL, USA). After the sample was dried (approximately 30 min), it was reconstituted with 250 μl of the HPLC mobile phase (described below), mixed on a vortex mixer, and centrifuged at 12,800 *g* for 5 min. The supernatant was transferred to an autosampler injection vial.

2.5. Chromatographic conditions

The HPLC system consisted of a Waters Associates (Milford, MA, USA) Model 510 solvent delivery system, a 717 WISP autosampler, a guard column packed with 35 to 50 μm C_{18} Corasil, a Guard-Pak 10-μm C_{18} pre-column insert, an RCM-100 radial compression module with a Radial-Pak

10-μm C_{18} (10 cm×8 mm) cartridge, and an ABI Spectroflow 757 variable-wavelength UV detector (Perkin-Elmer, Foster City, CA, USA) set at 313 nm [2]. Ketorolac and tolmetin were eluted isocratically at ambient temperature and at 1.8 ml/min with a water–acetonitrile–1.0 mol/l dibutylamine phosphate (DBAP, pH 2.5) (30:20:1) mobile phase that had been filtered through a 0.22-μm Durapore Filter (Waters). The chromatograms were recorded, the peaks were identified and integrated, and concentrations were reported on the basis of the internal standard area ratio method by a Hewlett-Packard 3395 integrator (Wilmington, DE, USA).

2.6. Evaluation of the method

The linearity, accuracy, and precision of the assay was assessed by the measurement of ketorolac concentration of replicate plasma standards containing 0.05 to 10.00 μg/ml ketorolac prepared as described above over a period of several weeks. Recovery was evaluated by comparing the area units of the peaks from the extracted plasma standards with the known area units of the stock solutions.

2.7. Clinical study

The usefulness of this assay for clinical pharmacokinetic studies was evaluated in a 13-year-old, 45 kg male patient scheduled for an elective operative procedure after obtaining institutionally approved written parental informed consent. Blood samples (2 ml) were obtained before and 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480 and 600 min after ketorolac tromethamine (Toradol), 0.5 mg/kg, administration intravenously for postoperative analgesia.

3. Results and discussion

This method provided us with a simple HPLC method of measuring plasma ketorolac concentrations which is sufficiently sensitive for use in pharmacokinetic studies of systemically administered ketorolac, which has an EC_{50} of 0.37 μg/ml [7]. Representative chromatograms are shown in Fig. 1. Ketorolac and the internal standard tolmetin were

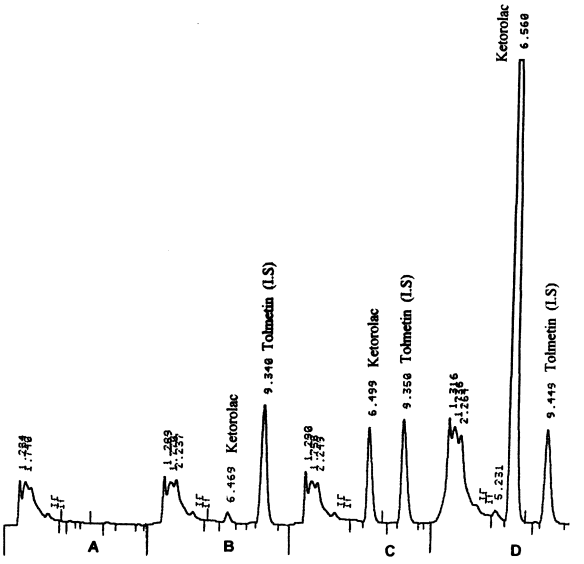

Fig. 1. Chromatograms of ketorolac extracted with the internal standard sodium tolmetin, 0.8 μg/ml. The ordinate is the UV detector's response and the abscissa is time in minutes. The retention times for the peaks are indicated on the chromatograms. (A) Blank plasma; (B) plasma standard with 0.05 μg/ml ketorolac; (C) plasma standard with 0.50 μg/ml ketorolac; (D) plasma standard with 5.00 μg/ml ketorolac.

well resolved, with retention times of 6.5 min and 9.4 min, respectively, and there was no interference from endogenous plasma constituents.

It is often necessary to buffer the aqueous mobile phase or add various salts to it to reduce band tailing in a reversed-phase system [10]. In the present method, band tailing and resolution of ketorolac and tolmetin were optimized by the addition of DBAP, pH 2.5 [11] to the mobile phase.

The accuracy and precision of the HPLC technique for the measurement of ketorolac are summarized in Table 1. The lowest standard ketorolac concentration studied for which we were able to obtain satisfactory accuracy and precision was 0.05 μg/ml. Linear regression analysis of the standard ketorolac concentration from 0.05 to 10.00 μg/ml versus ketorolac:tolmetin (internal standard) area ratios verified the linearity of the standard curve (r^2=0.998, y=0.709x+0.014). Relative errors of 5% or less were observed throughout the entire concentration range studied. The relative standard deviations for six replicate measurements of each standard

Table 1
Accuracy and precision for the plasma ketorolac assay (n=6)

Ketorolac added (μg/ml)	Ketorolac measured (μg/ml) (mean±SD)	Mean error (μg/ml)	Relative error (%)	RSD (%)
0.05	0.050±0.004	0.000	0.0	8.5
0.10	0.095±0.007	0.005	5.0	7.6
0.25	0.257±0.022	0.007	2.8	8.5
0.50	0.502±0.036	0.002	0.4	7.2
1.00	0.995±0.031	0.005	0.5	3.1
2.50	2.487±0.048	0.013	0.5	1.9
5.00	4.888±0.371	0.112	2.2	7.6
10.00	10.011±0.306	0.011	0.1	3.1

concentration over a period of several weeks were 8.5% or less. The average recoveries for ketorolac and tolmetin from the 48 replicate plasma standard samples were 103.9±7.9 and 96.6±4.6%, respectively.

The plasma ketorolac concentration versus time relationship in the patient is illustrated in Fig. 2. The present HPLC technique for the measurement of ketorolac is able to measure accurately and easily its plasma concentration for at least 10 h after administration of a standard clinical dose, hence is suitable for use in pharmacokinetic studies in humans.

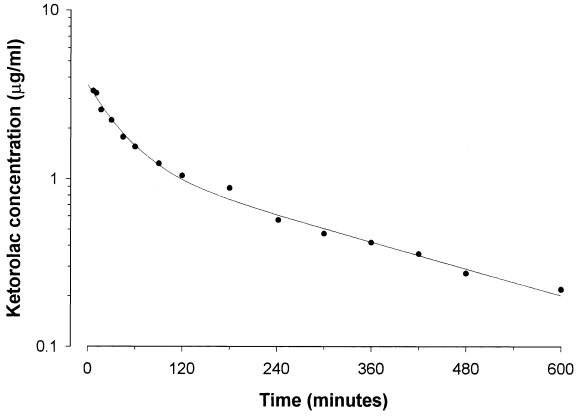

Fig. 2. Plasma ketorolac concentration versus time relationship after the administration of 0.5 mg/kg ketorolac tromethamine (Toradol) to a 13-year-old, 45 kg male patient for postoperative analgesia. The solid line is a computer-derived non-linear least-squares regression line through the actual plasma concentrations measured in the patient's plasma over 10 h.

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