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Short communication

# Simple and sensitive method for the analysis of ketorolac in human plasma using high-performance liquid chromatography

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ABSTRACT

*Objective:* To develop a simple and sensitive method of ketorolac in drug free human plasma using high-performance liquid chromatographic (HPLC).
*Method:* Ketorolac from blank plasma was extracted using protein precipitation method. Trichloro acetic acid (10%) was used as a protein precipitating agent and the percentage of recovery was calculated by adding known volume of dexibuprofen as internal standard. The HPLC separation was achieved on reversed-phase $C_{18}$ column and the separations were detection by Photodiode Array (PDA) detector at 306 nm. Acetonitrile and 5 mM ammonium acetate (pH 3.5), in the ratio of 60:40% v/v was used as mobile phase at the isocratic flow rate of 1.0 ml/min. The method was validated according to FDA Guidance for Industry.
*Result:* The percentage of recovery of ketorolac was 93.7 ± 0.12 (mean ± SD; $n = 5$). The linearity was achieved in this method range of 10.0–125.0 ng/ml with regression coefficient range of 0.999.
*Conclusion:* Ketorolac bioanalytical method was developed and validated according to FDA Guidance for Industry.


## 1. Introduction

Ketorolac [(6)-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, USA) to treat moderate pain with reduced opioid doses. Only limited methods have been reported for estimation of ketorolac in plasma such as spectrophotometric, fluorimetric assay, HPLC, HPTLC, and capillary electrochromatography.[2–6] To the best of our knowledge no reports were found for the validation of ketorolac in drug free human plasma using HPLC according to FDA Guidance for Industry. Hence the present study was planned to develop a method for estimation of ketorolac in human plasma using HPLC and validated as per FDA guidance 2001.

## 2. Material and methods

The chromatographic separation was achieved on a Shimadzu high-pressure liquid chromatographic system equipped with a binary LC-20AD solvent delivery system, SPD-20A Photodiode Array (PDA) detector and SIL-20ACHT injector with 50 μL loop volume. The LC solution version 1.25 data acquisition system was used for data collecting and processing (Shimadzu Corporation, Japan). Thermo $C_{18}$ (250 mm × 4.6 mm i.d., 5.0μ) column was used for present analysis (Thermo scientific, USA).

The HPLC grade of acetonitrile and ammonium acetate was obtained from Merck, Darmstadt, West Germany. A molecular biology grade chemical of ammonium acetate was obtained from Systerm Laboratory Chemicals and Reagents, Malaysia. Analytical grade of potassium dihydrogen phosphate was obtained from HmBG Chemicals, Malaysia. The gift sample of ketorolac was obtained form Zontron Pharmaceuticals, Malaysia. Dexibuprofen was purchased form Noven Lifesciences Private Limited, India.

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*2.1. Preparation of the calibration standards and quality control (QC) samples*

The 1.0 mg/ml stock solutions of ketorolac and dexibuprofen were prepared using mixture of water and acetonitrile (1:1) solution. The working standards of ketorolac was prepared from stock solution at the concentrations of 10, 20, 40, 60, 80, 100 and 125 ng/ml. Ketorolac working solution was used to prepare the spiking stock solutions for construction of seven-point calibration curve. The QC samples at three different levels *viz.*, 20, 80, 125 ng/mL were prepared and stored at 2–8 °C until analysed.

*2.2. Sample preparation for analysis*

In 2 ml centrifugation tube 0.5 ml of plasma was taken. In that 0.5 ml of calibration standard or validation QC, 0.5 ml (15 μg/ml) of internal standard (dexibuprofen) and 0.5 ml of precipitating agent (10% v/v trichloro acetic acid) were added and vortexed for 2 min. The resulting solution was centrifuged at 3000 rpm for 10 min and the supernatant layer was separated and used for chromatographic analysis.

*2.3. Chromatographic conditions*

Reverse phase higher pressure liquid chromatographic (RP-HPLC) method was used for standardization of ketorolac. The mobile phase used was acetonitrile and 5 mM ammonium acetate (pH 3.5), in the ratio of 60:40% v/v. The mobile phase flow rate was maintained at 1 ml/min and the sample injection volume was 50 μl. The analytes was detected using PDA at 306 nm.

*2.4. Validation*

The method was validated for selectivity, sensitivity, recovery, linearity, precision, accuracy and stability according to FDA 2001 guidelines.[7]

*2.4.1. Selectivity*

The selectivity of the method was evaluated by comparing the chromatograms obtained from the samples containing ketorolac and the internal standard with those obtained from blank samples.

*2.4.2. Sensitivity*

Sensitivity was achieved in terms of Lower Limit of Quantification (LLOQ) where the response of LLOQ was at least five times greater than the response of interference in blank matrix at the retention time or mass transitions of the analyte.

*2.4.3. Linearity*

Different concentrations of ketorolac standard solutions were prepared in the range of 10.0–125.0 ng/ml in plasma. In 2 ml centrifugation tube 0.5 ml of plasma, 0.5 ml of ketorolac (calibration standard), 0.5 ml of (15 μg/ml) of internal standard and 0.5 ml of precipitating agent (10% v/v trichloro acetic acid) were added and vortexed for 2 min. The resulting solution was centrifuged at 3000 rpm for 10 min and the supernatant layer was separated and used for chromatographic analysis. These solutions were analysed, the peak areas and response factors were calculated. The calibration curve was plotted using response factor *Vs* concentration of the standard solutions.

*2.4.4. Precision and accuracy*

The precision of the method was determined by intraday precision and interday precision. The intra-assay precision and accuracy was calculated for five replicates at each LLOQ, Low Quality Control (LQC), Middle Quality Control (MQC) and High Quality Control (HQC) levels, each on the same analytical run, and inter-assay precision and accuracy was calculated after repeated analysis in three different analytical runs.

*2.4.5. Stability studies*

Stability study was carried out at room temperature stock solution stability, refrigerated stock solution stability, freeze thaw stability, short term stability and long term stability testing procedures. Room temperature stock solution stability was carried out at 0, 3 and 8 h by injecting four replicates of prepared stock dilutions of ketorolac equivalent to middle quality control sample concentration and the stock dilution of internal standard equivalent to the working concentration. Comparison of the mean area response of ketorolac and internal standard at 3 and 8 h was carried out against the zero hour value. Refrigerated stock solution stability was determined at 7, 14 and 27 days by injecting four replicates of prepared stock dilutions of the analyte equivalent to the middle

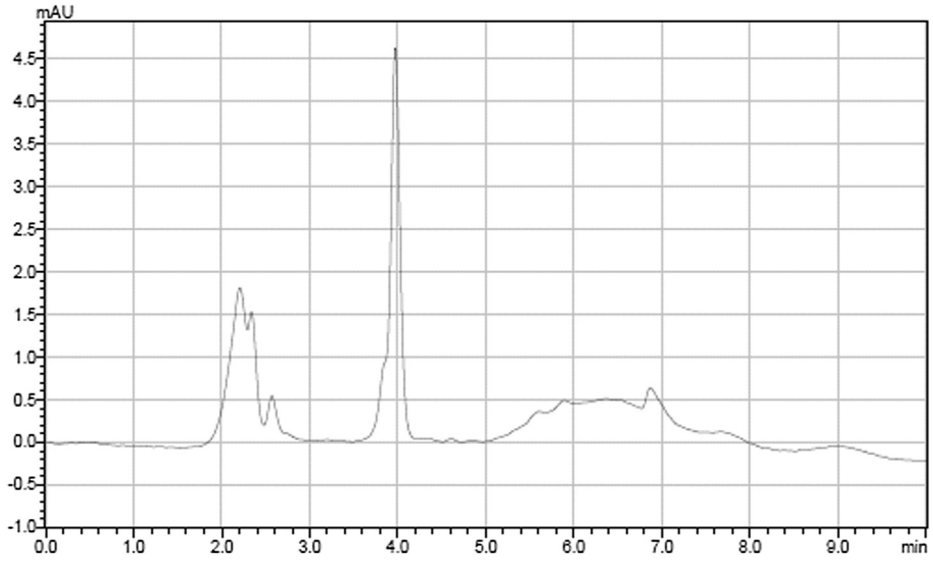


**Fig. 1.** Typical chromatogram of blank plasma chromatogram obtained with blank plasma showed plasma interference.

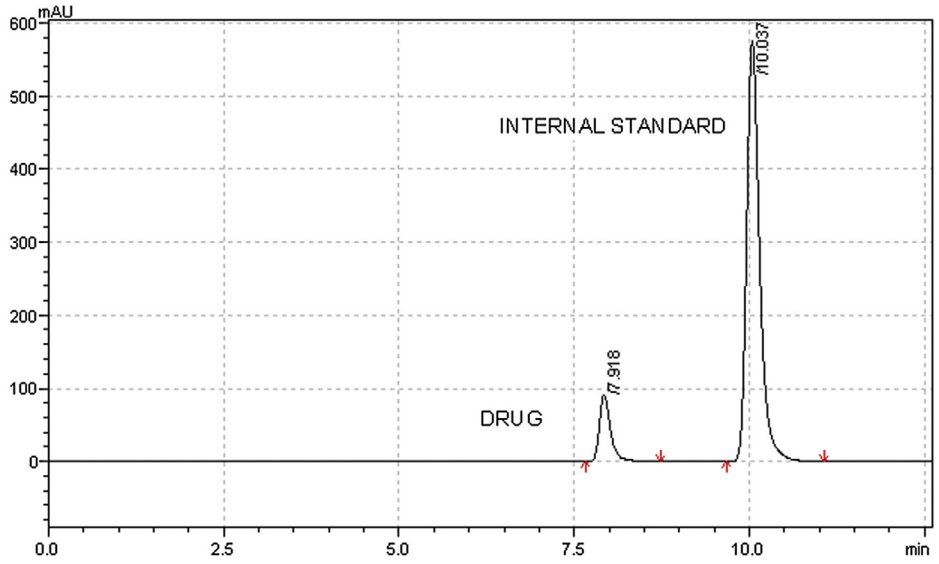


**Fig. 2.** Typical Chromatogram of ketorolac sample. Chromatogram obtained with plasma extracts of 1 μg/ml, 100 μg/ml of ketorolac (RT = 7.9) and Dexibuprofen (RT = 10.0) respectively.

quality control sample concentration and the stock dilution of internal standard equivalent to the working concentration. The stability studies of plasma samples spiked with ketorolac were subjected to three freeze–thaw cycles, short term stability at room temperature for 3 h and long term stability at − 70 °C over four weeks. In addition, stability of standard solutions was performed at room temperature over 6 h and after freezing for four weeks. The stability of triplicate spiked human plasma samples following three freeze thaw cycles was analysed. The mean concentrations of the stability samples were compared to the theoretical concentrations. The stability of triplicate short term samples spiked with ketorolac was investigated at room temperature for 1–3 h before extraction. The plasma samples for long term stability were stored in the freezer at −70 °C until the time of analysis.

## 3. Results and discussion

### 3.1. Selectivity

No interfering endogenous compound peak was observed at the retention time of drug and internal standard (Fig. 1). Under chromatographic conditions, the retention times of ketorolac and dexibuprofen were 7.9 min and 10.0 min respectively. Representative chromatogram of LLOQ and one study sample containing ketorolac is shown in Fig. 2.

**Table 1**
Inter-run accuracy and precision of plasma calibration standards for ketorolac.

| Standard concentration (ng/mL) | Average calculated concentration (ng/mL) Mean ± SD |
|---|---|
| 10 | 9.78 ± 0.69 |
| 20 | 19.89 ± 1.08 |
| 40 | 38.99 ± 1.25 |
| 60 | 59.98 ± 0.37 |
| 80 | 79.85 ± 0.68 |
| 100 | 99.89 ± 0.87 |
| 125 | 124.98 ± 0.54 |

SD = Standard deviation

### 3.2. Sensitivity (LLOQ)

The sensitivity of the experiment was carried out according to the LLOQ level. The LOD and LOQ were 4 ng/ml and 9 ng/ml respectively. The present study may be more sensitivity than study published by elsewhere.

### 3.3. Linearity

The calibration curves correlation coefficient was >0.999. The calibration curve data of ketorolac presented in Table 1 and Fig. 3.

### 3.4. Precision and accuracy

Intraday and interday accuracy and precision of the method were determined by analysis of the control plasma spiked with ketorolac at LLOQ, LQC, MQC and HQC. All QCs concentration was calculated using the calibration curve. The accuracy and precision of the method were described as a percentage bias and the percentage relative standard deviation; the results are given in Table 2.

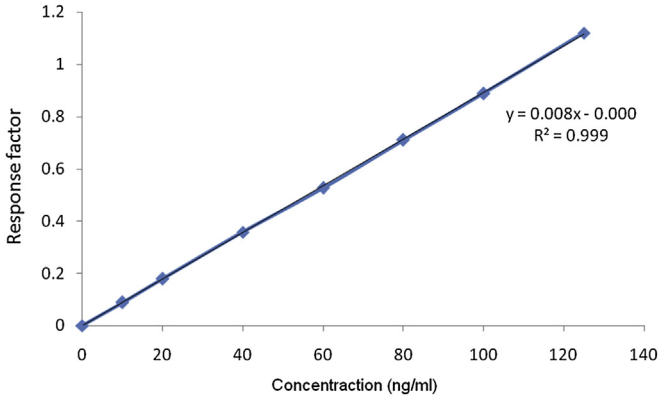


**Fig. 3.** Calibration curve of ketorolac. Calibration curve of ketrolac at the concentration range of 0–120 ng/ml.

**Table 2**
Intraday and interday accuracy and precision of ketorolac in plasma.

| Standard concentration (ng/mL) | Average calculated concentration (ng/mL) Mean ± SD |
|---|---|
| Interday ($n = 3$) | |
| 10 | 9.87 ± 0.07 |
| 80 | 79.47 ± 0.46 |
| 125 | 124.54 ± 0.52 |
| Intraday ($n = 3$) | |
| 10 | 9.79 ± 0.08 |
| 80 | 79.87 ± 0.71 |
| 125 | 124.49 ± 0.60 |
| SD = Standard deviation | |

**Table 3**
Stability study of ketorolac.

| Standard concentration (ng/mL) | Average calculated concentration (ng/mL) Mean ± SD |
|---|---|
| Bench top ($n = 5$) | |
| 80 | 79.35 ± 0.47 |
| 125 | 124.54 ± 0.52 |
| Freeze thaw stability ($n = 5$) | |
| 80 | 79.14 ± 0.57 |
| 125 | 123.99 ± 0.98 |
| Long term stability ($n = 5$) | |
| 80 | 89.57 ± 1.02 |
| 125 | 124.58 ± 1.05 |
| SD = Standard deviation | |

### 3.5. Stability

Stock solution analysis was performed at 100.0 ng/mL. Proper storage after for 15 days at 2–8 °C and at room temperature for 6 h, more than 98% of ketorolac remained unchanged, based on peak areas in comparison with freshly prepared solution of Ketorolac. This suggests that the ketorolac in standard solution is stable for at least 15 days when stored at 2–8 °C and for 6 h at room temperature. Bench top stability of ketorolac in plasma was investigated at LQC and HQC levels. This revealed that the ketorolac in plasma was stable for at least 6 h at room temperature. It was confirmed that repeated freezing and thawing (three cycles) of plasma samples spiked with ketorolac at LQC and HQC level did not affect the stability of ketorolac long term stability of the Ketorolac in plasma at −70 °C was also performed after 30 days of storage at LQC, HQC levels. The results of the stability studies are shown in Table 3. The average long term stability was 96.82%. The above results indicated that the ketorolac was stable in the studied conditions.

## 4. Conclusion

A simple and sensitive method for the determination of ketorolac in plasma by HPLC was developed and validated. Adequate specificity, precision and accuracy of the proposed method were demonstrated over the concentration range of 10.0–125.0 ng/ml. The method was accurate, reproducible, specific and applicable to the evaluation of pharmacokinetic study of ketorolac.

## Conflicts of interest

All authors have none to declare.

## Acknowledgement

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