

Rapid and Sensitive Reverse-phase High-performance Liquid Chromatography Method for Estimation of Ketorolac in Pharmaceuticals Using Weighted Regression

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Dubey, *et al.*: New RP-HPLC Method for Estimation of Ketorolac

A reliable, rapid and sensitive isocratic reverse phase high-performance liquid chromatography method has been developed and validated for assay of ketorolac tromethamine in tablets and ophthalmic dosage forms using diclofenac sodium as an internal standard. An isocratic separation of ketorolac tromethamine was achieved on Oyster BDS (150×4.6 mm i.d., 5 µm particle size) column using mobile phase of methanol:acetonitrile:sodium dihydrogen phosphate (20 mM; pH 5.5) (50:10:40, %v/v) at a flow rate of 1.0 ml/min. The eluents were monitored at 322 nm for ketorolac and at 282 nm for diclofenac sodium with a photodiode array detector. The retention times of ketorolac and diclofenac sodium were found to be 1.9 min and 4.6 min, respectively. Response was a linear function of drug concentration in the range of 0.01-15 µg/ml ($R^2=0.994$; linear regression model using weighing factor $1/x^2$) with a limit of detection and quantification of 0.002 µg/ml and 0.007 µg/ml, respectively. The % recovery and % relative standard deviation values indicated the method was accurate and precise.

Key words: Diclofenac sodium, ketorolac tromethamine, method validation, reverse phase high-performance liquid chromatography-PDA, weighted regression

Ketorolac tromethamine, chemically, 5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-(hydroxymethyl)-1,3-propanediol (fig. 1a), is a nonsteroidal antiinflammatory drug which exhibits pronounced analgesic and moderate antiinflammatory activity^[1-4]. It is indicated for the short-term management of moderate to severe pain. Literature survey showed that very few analytical methods have been reported for the estimation of ketorolac in single or in combination such as spectrophotometric^[5], flow injection analysis^[6], high-performance liquid chromatography (HPLC)^[7-15], high-performance thin layer chromatography (HPTLC)^[16] and gas chromatography-mass spectrometry^[17], which are either less economical or less sensitive. For routine analysis, a simple, rapid and most sensitive analytical method is preferred. The objective of the present study is to develop a simple, reliable, rapid and sensitive analytical method with better detection range, for the estimation of ketorolac tromethamine in bulk and in dosage forms using diclofenac sodium as an

internal standard (fig. 1b). The developed method was validated as per International Conference on Harmonization (ICH) guidelines and suitable statistical tests were performed on validation data.

MATERIALS AND METHODS

Ketorolac tromethamine reference standard was provided by Torrent Pharmaceuticals, Ahmedabad, India. Diclofenac sodium was procured from Aarti Drugs Ltd., Mumbai, India, tablets of ketorolac tromethamine, Ketorol DT, with a 10 mg label claim, manufactured by Dr. Reddys Laboratories Ltd., and eye drops, Acular LS with a labelled claim of 4 mg/ml, manufactured by Allergan were procured from local market. HPLC grade acetonitrile, methanol

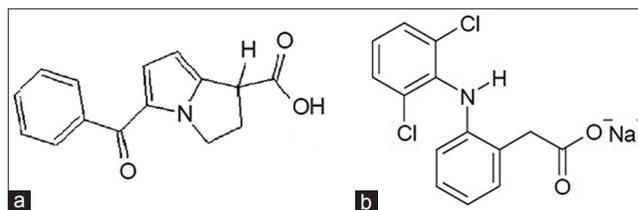


Fig. 1: Chemical structure of analytes. (a) Ketorolac and (b) Diclofenac sodium

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and analytical grade hydrochloric acid, sodium hydroxide pellets and sodium dihydrogen phosphate were obtained from Merck India Limited, Mumbai, India. High purity deionized water was prepared by TKA smart2pure, Niederelbert, Germany purification system. In addition, an electronic balance (AG-135, Mettler-Toledo, Germany), pH meter (pH tutor, Eutech Instruments, Singapore), a sonicator (Toshiba, New Delhi) were used.

Chromatography:

The chromatographic system used to perform development and validation of this method consisted of an LC-20AD binary pump, an SPD-M20A photodiode array detector, SIL 20 AC auto sampler, connected to a communication and Bus module CBM 20A (Shimadzu, Kyoto, Japan). Chromatographic analysis was performed on Oyster BDS (150×4.6 mm i.d., 5 µm particle size) column using mobile phase at a flow rate of 1.0 ml/min in isocratic mode. The mobile phase consists of methanol:acetonitrile:sodium dihydrogen phosphate (20 mM, pH 5.5) (50:10:40, v/v). The eluent was monitored using photo diode array (PDA) detector at wavelengths 322 nm and 282 nm for ketorolac and diclofenac sodium, respectively. The column was maintained at ambient temperature and injection volume of 50 µl was used.

Preparation of stock solutions and calibration curve:

Stock solutions were prepared by weighing 5 mg and 25 mg of ketorolac and diclofenac sodium individually and transferred into two separate 50 ml volumetric flasks. Volumes were made up to the mark with methanol to obtain a solution containing 100 µg/ml of ketorolac and 500 µg/ml of diclofenac sodium. Appropriate aliquots of ketorolac stock solution were taken in different 10 ml volumetric flasks and to each volumetric flask 100 µl of diclofenac stock solution were added then diluted up to the mark with mobile phase to obtain final concentrations of 0.01-15 µg/ml of ketorolac each containing 5 µg/ml of internal standard. The solutions were analysed and chromatograms were recorded. Calibration curves were constructed by plotting average peak areas ratios of ketorolac and diclofenac sodium versus concentration of analyte and regression equation was computed.

Analysis of marketed formulations:

Two tablets (Ketorol DT, 10 mg) were transferred into a 100 ml volumetric flask; 10 ml of water was added

and sonicated for 10 min. After sonication 40 ml of methanol was added and again sonicated for 10 min and finally volume was made up to the mark by adding methanol. This solution was centrifuged for 10 min. To 5 ml of above supernatant, 5 ml of working solution of internal standard (100 µg/ml) was added and the volume was made up to 50 ml with mobile phase. From this solution 0.5 ml was taken and made up to 1 ml with mobile phase and this solution was analysed.

Five millilitres of ophthalmic solution (Acular LS, 4 mg/ml, 5 ml) was transferred into 100 ml volumetric flask and the volume was made by using methanol. Then 5 ml of above solution was taken, to that 5 ml of working solution of internal standard (100 µg/ml) was added and the volume was made up to 50 ml with mobile phase. From this solution 0.5 ml was taken and made up to 1 ml with mobile phase and it was analysed.

Method validation:

The method of analysis was validated as per the recommendations of ICH^[18-20] and United States Pharmacopoeia (USP)^[21] for the parameters such as linearity, accuracy, precision, detection limit, quantification limit and robustness.

Linearity:

For preparing the calibration curve, seven solutions containing 0.01, 0.1, 0.5, 1, 3, 5, 10 and 15 µg/ml ketorolac tromethamine were used. Each solution was injected in triplicate and linearity was evaluated by weighted linear-regression analysis.

Accuracy:

Accuracy was assessed by determining % of nominal concentration for three levels of quality control standards (lower quality control (LQC), medium quality control (MQC), high quality control (HQC) corresponds to 50 ng/ml, 4 µg/ml, 12 µg/ml, respectively). At each level, three replicates ($n=3$) were prepared and analysed.

Precision:

Precision study was carried out for intraday and interday, by estimating the relative standard deviation for the three levels of quality control standards (LQC, MQC, HQC corresponds to 50 ng/ml, 4 µg/ml, 12 µg/ml, respectively). At each level, three replicates ($n=3$) were prepared and analysed.

Limit of detection and quantification:

The LOD and LOQ were calculated using following formulae: $LOD=3.3\sigma/S$ and $LOQ=10\sigma/S$, where σ =standard deviation of intercept of calibration curve and S =average of the slope of the calibration curves.

System suitability:

System suitability tests are an integral part of chromatographic method which is used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level of 10 $\mu\text{g/ml}$.

Robustness:

For robustness evaluation of HPLC method a few parameters like flow rate, different lots of solvent and pH of mobile phase were deliberately changed. One factor was changed at one time to estimate the effect. Each factor selected was changed at three levels (-1, 0, +1) with respect to optimized parameters. Robustness of the method was done at the concentration level 10 $\mu\text{g/ml}$.

Stock solution stability:

Stability in solution was evaluated for the stock solution. The solutions were stored at ambient temperature without protection from light and tested after 12, 24, 36 and 48 h. The responses for the aged solution were evaluated by comparison with freshly prepared solutions.

Specificity:

The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. Effect of excipients of formulation was studied, whether they interfere with the analyte.

RESULTS AND DISCUSSION

The mobile phase of methanol:acetonitrile: sodium dihydrogen phosphate (20 mM, pH 5.5) (50:10:40, v/v), at flow rate of 1 ml/min gave two sharp, well-resolved peaks with minimum tailing factor for analyte and internal standard. The eluent

was monitored at 322 nm for ketorolac and at 282 nm for diclofenac sodium. As it is an isocratic reverse phase (RP)-HPLC method, retention time of solutes depends on their physicochemical properties. Diclofenac sodium, used as an internal standard is acidic in nature like analyte and its pKa is equivalent to analyte's pKa and its response was proportional to ketorolac response which is most essential. The retention times for ketorolac and diclofenac were 1.9 min and 4.6 min, respectively (fig. 2). Data analysis was conducted on the pooled data using prism software. Variance test (F-test) used indicated the presence of heteroscedasticity in the response data.

The linear equation considered was: $y=ax+b$; where 'y' is ratio of response of ketorolac and diclofenac and 'x' is ketorolac concentration; a , b are slope and intercept of regression line. Weighing factors considered were 1, $1/x$, $1/x^2$, $1/y$ and $1/y^2$. The best regression model and weighing factor was chosen according to the sum of absolute percentage relative error (% RE) values. The best model will be that which gives the least sum of the % RE across the whole concentration range. Here we applied weighted regression analysis taking $1/x^2$ and the weighted regression equation was $y=0.3022x+0.00046$. The selection of weighing factor is given in Table 1.

The accuracy study shown that, this method was accurate (Table 2) and the precision for

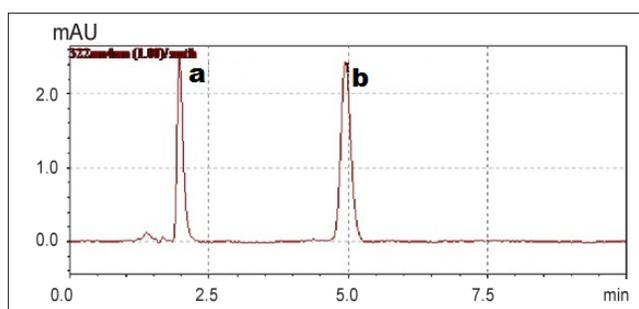


Fig. 2: Chromatogram of the standard resolution solution. Chromatogram showing completely resolved peaks of ketorolac (a, 100 ng/ml) at retention time of 1.9 min and diclofenac sodium (b, IS, 5 $\mu\text{g/ml}$) at retention time of 4.6 min.

TABLE 1: WEIGHTED LEAST SQUARES REGRESSION ANALYSIS

Weighting factor	1	1/x	1/x ²	1/y	1/y ²
a \pm SD	0.284 \pm 0.020	0.290 \pm 0.003	0.30 \pm 0.007	0.290 \pm 0.001	0.300 \pm 0.006
b \pm SD	0.025 \pm 0.003	0.001 \pm 0.001	0.0004 \pm 0.0002	0.0011 \pm 0.001	0.0004 \pm 0.0001
R ²	0.9991	0.9986	0.9940	0.9986	0.9948
$\Sigma\%$ RE	4134.19	185.38	108.82	197.31	110.08

a and b are slope and intercept of regression line, respectively, SD=Standard deviation for $n=3$ observations, RE=Relative error

TABLE 2: VALIDATION PARAMETER-ACCURACY OF METHOD

Nominal conc. ($\mu\text{g/ml}$)	Calculated conc. ($\mu\text{g/ml}$)	% Bias	% of nominal conc.
0.05 (LQC)	0.050	0.985	99.015
4.0 (MQC)	3.897	2.580	97.420
12.0 (HQC)	12.004	0.029	100.029

$n=3$ observations, LQC=Lower quality control, MQC=Medium quality control, HQC=High quality control

TABLE 3: PRECISION OF METHOD

Time	Nominal conc. ($\mu\text{g/ml}$)	Calculated conc. ($\mu\text{g/ml}$)	% RSD
Intraday precision			
8.30 AM	0.05 (LQC)	0.050	1.096
	4.0 (MQC)	3.897	1.806
	12.0 (HQC)	12.004	1.480
12.00 PM	0.05 (LQC)	0.051	0.863
	4.0 (MQC)	3.934	0.050
	12.0 (HQC)	11.693	0.127
5.00 PM	0.05 (LQC)	0.049	0.999
	4.0 (MQC)	3.894	0.961
	12.0 (HQC)	11.915	0.661
Interday precision			
Day 1	0.05 (LQC)	0.050	1.096
	4.0 (MQC)	3.897	1.806
	12.0 (HQC)	12.004	1.480
Day 2	0.05 (LQC)	0.050	1.566
	4.0 (MQC)	3.979	0.414
	12.0 (HQC)	11.964	0.630
Day 3	0.05 (LQC)	0.048	1.284
	4.0 (MQC)	3.893	0.931
	12.0 (HQC)	11.935	1.139

$n=3$ observations. Intraday precision carried out at three different times in a day, Interday precision carried out on three different days, LQC=Lower quality control, MQC=Medium quality control, HQC=High quality control, RSD=Relative standard deviation

TABLE 4: SUMMARY OF VALIDATION AND SYSTEM SUITABILITY TEST PARAMETERS

Parameter (units)	
Linearity range ($\mu\text{g/ml}$)	0.05-15
Correlation coefficient	0.994 \pm 0.00038
LOD ($\mu\text{g/ml}$)	0.0024
LOQ ($\mu\text{g/ml}$)	0.0074
Recovery (%)	97.42-100.02
Intraday precision (% RSD, $n=3$)	0.050-1.80
Interday precision (% RSD, $n=3$)	0.41-1.81
Robustness	Robust
Retention time (min)	1.9 \pm 0.2
Resolution	2.335
Theoretical plates	4500
Tailing factor (asymmetry factor)	1.02

LQC=Lower quality control, LOD=Limit of detection, RSD=Relative standard deviation

both intraday and interday evaluated given percentage relative standard deviation (% RSD) below 2 (Table 3). The LOD and LOQ were 0.0024 and 0.0074 $\mu\text{g/ml}$, respectively. The

TABLE 5: ROBUSTNESS PARAMETERS

Factor	Level	Retention time	Asymmetry
Ketorolac			
Flow rate (ml/min)			
0.9	-1	2.04	1.19
1.0	0	1.96	1.15
1.1	+1	1.88	1.11
Mean \pm SD ($n=3$)		1.96 \pm 0.88	1.15 \pm 0.04
pH of mobile phase			
5.4	-1	1.87	1.18
5.5	0	1.96	1.15
5.6	+1	1.99	1.12
Mean \pm SD ($n=3$)		1.96 \pm 0.05	1.15 \pm 0.03
Solvents of different lots			
First lot		1.96	1.15
Second lot		1.95	1.17
Mean \pm SD ($n=3$)		1.96 \pm 0.01	1.16 \pm 0.01

SD=Standard deviation

TABLE 6: STOCK SOLUTION STABILITY DATA

Time (h)	Found concentration ($\mu\text{g/ml}$)	% Recovery
0	100	100
12	99.78	99.78
24	99.34	99.34
36	99.30	99.30
48	99.20	99.20

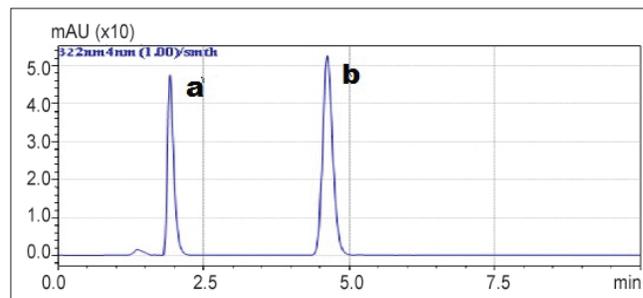


Fig. 3: Chromatogram of marketed formulation 1. Chromatogram of ketorolac (a, Ketorol DT, 10 mg) and diclofenac sodium (b, IS, 5 $\mu\text{g/ml}$).

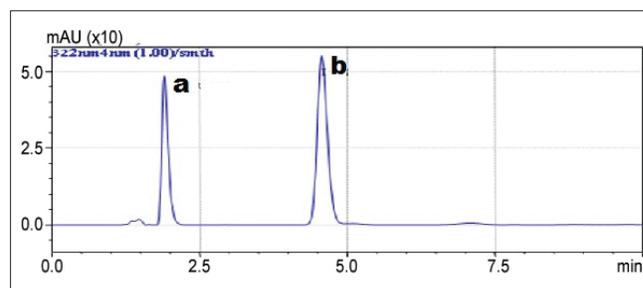


Fig. 4: Chromatogram of marketed formulation 2. Chromatogram of ketorolac (allergen 5 ml) and diclofenac sodium (IS, 5 $\mu\text{g/ml}$).

results for validation and system suitability test parameters are summarized in Table 4. Results for robustness evaluation are given in Table 5. Insignificant differences in peak areas and less

TABLE 7: ASSAY DATA OF KETOROLAC TROMETHAMINE DOSAGE FORMS

Dosage form	Labelled claim	Found amount (mg)	Mean amount (mg)	% Recovery
Tablet (Ketorol DT, 10 mg)	10 mg	10.04	9.93	99.93
		9.94		
		9.78		
Eye drops (Acular LS, 4 mg/ml, 5 ml)	4 mg/ml (20 mg)	19.92	19.93	99.65
		19.89		
		19.94		

variability in retention times observed indicates robustness of method. Stock solution stability study indicates this stock solution is stable up to 48 h which is greater than total analysis process (Table 6). Assay results for pharmaceutical dosage forms using proposed method were comparable with the corresponding labelled amounts (Table 7; figs. 3 and 4).

It can be concluded that the developed method was found to be simple, sensitive and selective for analysis of ketorolac in bulk and formulations. Statistical analysis proved that method was accurate, precise, and reproducible. The method can be used for routine analysis, without batch to batch variation as internal standard was used. The method is also very robust and enables quality-control analysis of ketorolac with large sample throughput.

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Accepted 05 February 2013

Revised 24 January 2013

Received 22 April 2012

Indian J Pharm Sci 2013;75(1):89-93