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# Development and validation of stability indicating HPLC method for the estimation of etodolac in pharmaceutical dosage form

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**Abstract**
A rapid, simple, precise, accurate, and isocratic high performance liquid chromatography (HPLC) method has been developed for routine quality control of etodolac in pharmaceutical formulations. Separation was carried out by C18 column. The mobile phase was a mixture of solvent A (80:20%v/v – buffer and acetonitrile) and solvent B (75:25%v/v acetonitrile and buffer) in gradient mode at a flow rate of 1 mLmin$^{-1}$. The ultraviolet (UV) detection and column temperature were 227 nm and ambient in nature. The run time was 15 min under these chromatographic conditions. Excellent linear relationship between peak area and etodolac concentration in the range of 0.04-1 µg mL$^{-1}$ has been observed ($r^2$, 0.998). Developed method has been found to be sensitive, precise (the interday and intraday relative standard deviation (RSD) values for peak area was less than 0.4, accurate (recovery, 97.3-97.7%), specific and robust (% RSD were less than 1.00, for system suitability parameters). Proposed method has been successfully applied for quantification of etodolac in pharmaceutical formulations.

**Keywords:** etodolac, RP-HPLC, inflammation, NSAID, indole

## Introduction

Etodolac is a monocarboxylic acid [1] that is acetic acid in which one of the methyl hydrogens is substituted by a 1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl moiety. A preferential inhibitor of cyclo-oxygenase 2 and non-steroidal anti-inflammatory, it is used for the treatment of rheumatoid arthritis and osteoarthritis, and for the alleviation of postoperative pain. Administered as the racemate, only the (S)-enantiomer is active. It is a non-steroidal anti-inflammatory drug [2] (NSAID) with anti-inflammatory, analgesic and antipyretic properties. Its therapeutic effects are due to its ability to inhibit prostaglandin synthesis. It inhibits the activity of cyclooxygenase I and II, thereby preventing the formation of prostaglandin which is involved in the induction of pain, fever, and inflammation. It also inhibits platelet aggregation by blocking platelet cyclooxygenase and the subsequent formation of thromboxane A2. Chemically (±) 1,8-diethyl-1,3,4,9-tetrahydropyrano-[3,4-b]indole-1-acetic acid and extensively metabolized in the liver. The hydroxylated-etodolac metabolites undergo further glucuronidation followed by renal excretion and partial elimination in the feces (16% of dose). The elimination half-life [3] of etodolac is between 6 and 8 hours in plasma and white crystalline compound, insoluble in water but soluble in alcohols, chloroform, dimethyl sulfoxide, and aqueous polyethylene glycol (Fig 1).

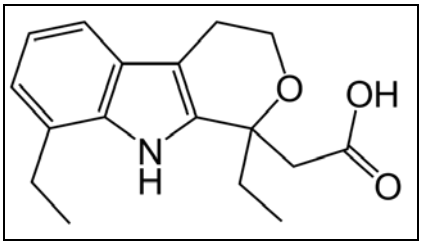


**Fig 1:** Chemical structure of etodolac

According to the literature search, there are few published high performance liquid chromatography (HPLC) methods for estimation of etodolac in dosage forms [4, 7].

## Experimental

### Chemicals

Analytical grade chemicals were used without further purification in this study. Sodiumdihydrogen phosphate, ortho-phosphoric acid, HPLC-grade acetonitrile and methanolwere purchased from fisher scientific. Ultra-pure water was obtained from water purification unit (Millipore Elix$^R$).Etodolacwas obtained as a gift sample from adept pharma and bio science excellence pvt. Ltd., Hyderabad, india.

### Stock Standard Solution

One hundred milligram pure drug was accurately weighed, dissolved in about 30 mL of deionized water and transferred to a 100 mL volumetric flask. Then the volume was completed to 100 mL with deionized water to obtain 1 mg mL1 of stock solution. The resulting stock solution was sonicated and filtered through a 0.45 mm filter. The stock solution was further diluted with deionized water to obtain the required concentration of standard solutions (10–100 mg mL1) before being injected into the system for analysis.

### Sample Solution

Ten etodolac tablets were accurately weighed and transferred to a dry and clean mortar, then ground into a fine powder. Next, tablet powder equal to 250 mg etodolac was transferred to a volumetric flask of 250 mL. About 100 mL deionized water was added and this flask was attached to a rotary shaker for 10 min. to completely disperse the ingredients. The mixture was sonicated for 30 min, diluted to volume with deionized water to give a solution containing 1,000 mg mL1 and then filtered through a 0.45 mm filter.

**Chromatographic Conditions**

Chromatographic analysis was performed on a column of Kromasil RP C18 (4.6 mm X 250 mm X 5.0 mm). The mobile phase consisted of ACN:Sodium phosphate buffer (pH-4.5) in ratio of 80:20 (solvent A) and 75:25 v/v (solvent B). The mobile phase was filtered and degassed through a 0.45 mm membrane filter before use and then pumped at a flow rate of 1 mL $min^{-1}$. The column temperature has been ambient in nature. The run time was 15 min under these conditions.

**Method Validation**

The analytical method validation has been performed as per ICH guidelines of Validation of Analytical Procedure: Q2 (R1) [8, 9]. The validation parameters such as system suitability, linearity, the limit of detection (LOD), the limit of quantification (LOQ), accuracy, specificity, precision, and robustness were addressed.

**Linearity**

Standard calibration has been prepared using six standard solutions within the concentration range of 0.04-1 µg $mL^{-1}$ has been observed ($r^2$, 0.998). In optimized chromatographic conditions, each standard solution was chromatographed for 15 min three times. Least squares linear regression analysis of the average peak area versus concentration data were used to evaluate the linearity of the method.

**Specificity/Selectivity**

Selectivity is the ability of the analytical method to produce a response for the analyte in the presence of other interference. The selectivity of the method was tested by comparing the chromatograms obtained for etodolac standard, tablet, and blank solutions. The parameters retention time and tailing factor were calculated in order to prove that the method chosen was specific

**Limit of Detection and Limit of Quantification**

These values were determined using the standard error (s) and slope of the regression line (m) as shown in following equations:

LOD = 3.3*s/m

LOQ = 10*s/m

**Precision**

Precision was analyzed by calculating variations of the method in intraday (repeatability performed by analyzing standard solution on the same day) and inter-day (repeatability carried out by analyzing standard solution on three different days). Precision study was performed by injecting six times of standard solution at three different concentrations, on the same day and three consecutive days.

**Accuracy**

Recovery studies were conducted by the standard addition technique to confirm the accuracy of the proposed method. In this method, 50, 100, 150% of three different levels of pure drug were added to the previously analysed sample solutions, and etodolac recovery was calculated for each concentration.

**Robustness**

A robustness analysis was performed to determine the impact of minor yet systematic differences in chromatographic conditions. The modifications include different flow rate, pH of the buffer, Buffer concentration and wavelength range. After each change, System suitability parameters were checked by injecting the sample solution into the chromatographic system and the results were compared with those under the original chromatographic conditions.

**Solution Stability**

The stability of sample and standard solutions were monitored over a 24 h period. For this, standard and sample solutions were injected into the system at 8 h periods, and the peak area and retention time were evaluated. During the stability study, standard solutions have been stored at ambient temperature (25ºC) and protected from light.

**Result and Discussion**

**Determination of λmax**

The wavelength corresponding to maximum absorbance (λmax) was determined as 227 nm from the UV spectrum of standard solution.

**Method Development**

Several preliminary studies were conducted to optimize the chromatographic conditions for the quantification of etodolac. Mobile phases consisting of several buffer systems were tried at the beginning of the study; they could not meet the required system parameters. Then only phosphate buffer system was tested without using organic modifiers, long analysis times were obtained. Different acetonitrile solution ratios were investigated to obtain optimum conditions. The acetonitrile ratio was determined as 10% against 50 mM sodiumdihydrogen phosphate solution (pH 4.5) due to the etodolac peak being well shaped and symmetrical using this system. Eventually, it was found that the mobile phase consisting of a mixture of solvent A (80:20%v/v – buffer and acetonitrile) and solvent B (75:25%v/v acetonitrile and buffer) in gradient mode provided stronger theoretical plates (>2,000) and peak tailing factor (<1.0).

Optimised chromatographic conditions were achieved using an gradient mobile phase comprising a mixture of solvent A (80:20%v/v – buffer and acetonitrile) and solvent B (75:25%v/v acetonitrile and buffer) in gradient mode at a flow rate of 1.0 mL $min^{-1}$on an Kromasil RP C18 (4.6 mm X 250 mm X 5.0 mm) that was kept at 30˚C. The analysis was conducted which offers a lot of advantages such as good chromatographic peak shape, enhanced column efficiency, and low-column pressure, in addition to being economic. The eluate was monitored using a UV detector set at 227 nm. Under the chromatographic conditions etodolac was eluted at retention time5.30 min.

**Method Validation**

**Linearity**

The stock standard solution of etodolac was diluted appropriately with deionized water to obtain standard solutions within the concentration range of µg $mL^{-1}$ has been observed ($r^2$, 0.998). Each standard solution was injected three times into the HPLC system under the above-mentioned chromatographic working conditions. Linearity of the proposed method has been estimated at 8 concentration levels in the range of µg $mL^{-1}$by regression analysis. The calibration curve was developed by plotting average peak area versus standard concentration (Fig. 2).

The correlation coefficient, slope, and intercept of the regression line were determined using the least squares method. The relation between mean peak area Y (n = 3) and concentration, X expressed by equation Y = a+bX, was linear. Values of slope, intercept, and correlation coefficient (r) were 538815, 8279.9 and 0.998, respectively as shown in Table 1.

**Precision**

Precision study was performed by injecting six times of standard solution at three different concentrations, on the same day and three consecutive days on the same day and three consecutive days. All RSD values for retention time and peak area for selected etodolac concentrations were less than 0.5 and 2.0%, respectively. In this case, the method is precise and can be used for our intended purpose.

**Accuracy study**

A known quantity of standard solution has been added to the sample solutions previously analyzed at three different levels (50%, 100% and 150%). The amount recovered for etodolac has been calculated for three concentrations. The recovery data were summarized in Table 2. Percent RSD values for all analyses were less than 2% indicating that excipients found in pharmaceutical formulations do not interfereand analytical method is very accurate.

**Robustness**

The results showed that the change in flow rate and mobile phase concentration had little effect on the chromatographic behaviour of etodolac. The small change in theflow rate, pH of the buffer, Buffer concentration and wavelength range. The change in the column temperature did not have a significant effect on the method.

**Degradation Behaviour of Etodolac**

**Degradation under Acidic Conditions**

When an etodolac was treated with 1N HCl kept at 70˚C for 15hrs. Sufficient degradation was achieved. Degradation was observed as shown in figure3. Impurity was formed.

**Degradation under Basic Conditions**

When Etodolac was treated with 5N NaOH, kept at $70^0$C for 50hrs, no degradation was observed as shown in figure 4.

**Degradation under Neutral Conditions**

When Etodolac was treated with water kept at $70^0$C for 50hrs, no degradation wasobserved as shown in figure 5.

**Degradation under Oxidative Conditions**

When Etodolac was treated with 3%H2O2 at normal room temperature in a dark for 6hrs, it was degradedto11.88% as shown in figure 6. Two types of impurities were formed.

**Degradation under Photolytic Condition**

When Etodolac was exposed to light providing an overall illumination of 3.6 million hrs and an integrated near ultraviolet energy of 600 Watt hours/squaremeter, no degradation was observed as shown in figure 7.

**Thermal degradation**

When Etodolac powder was spread in petridish and placed in hot air oven at70˚C for 10days, no degradation was observed.

**Conclusion**

A very quick, cost-effective, precise and accurate HPLC method for the determination of FVP has been developed and validated in compliance with ICH guidance Q2. Besides the short run time (15min), retention time (5.3) and flow rate of mobile phase (1 mL $min^{-1}$) made the method attractive because these features save analysis time and cost. In short, this method is sensitive, selective, reproducible and rapid for etodolac in bulk and tablet. The accuracy and precision are within reasonable limits finally analytical method is reliable and robust.

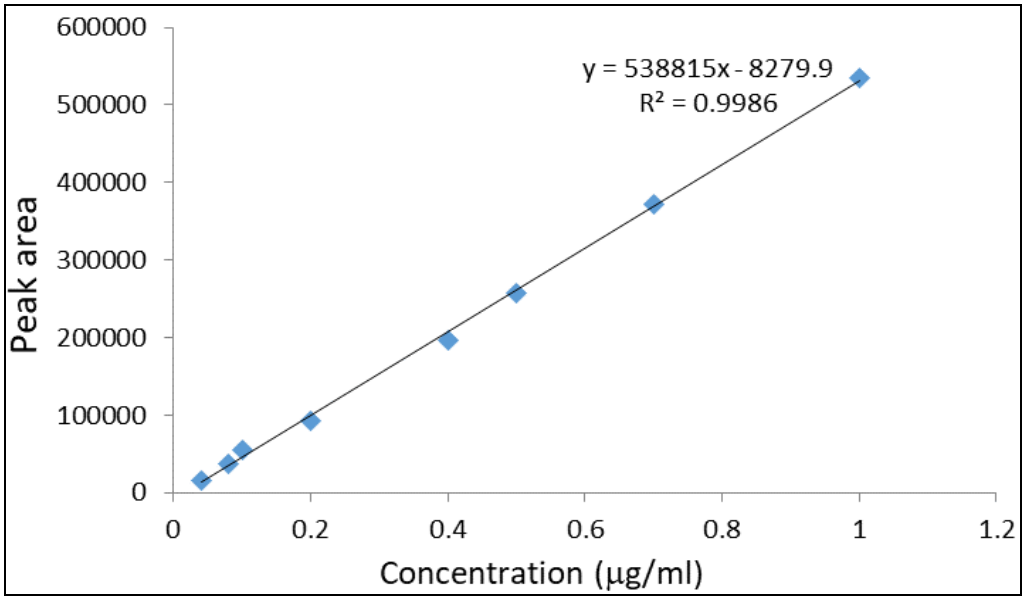


**Fig 2:** Calibration curve of etodolac

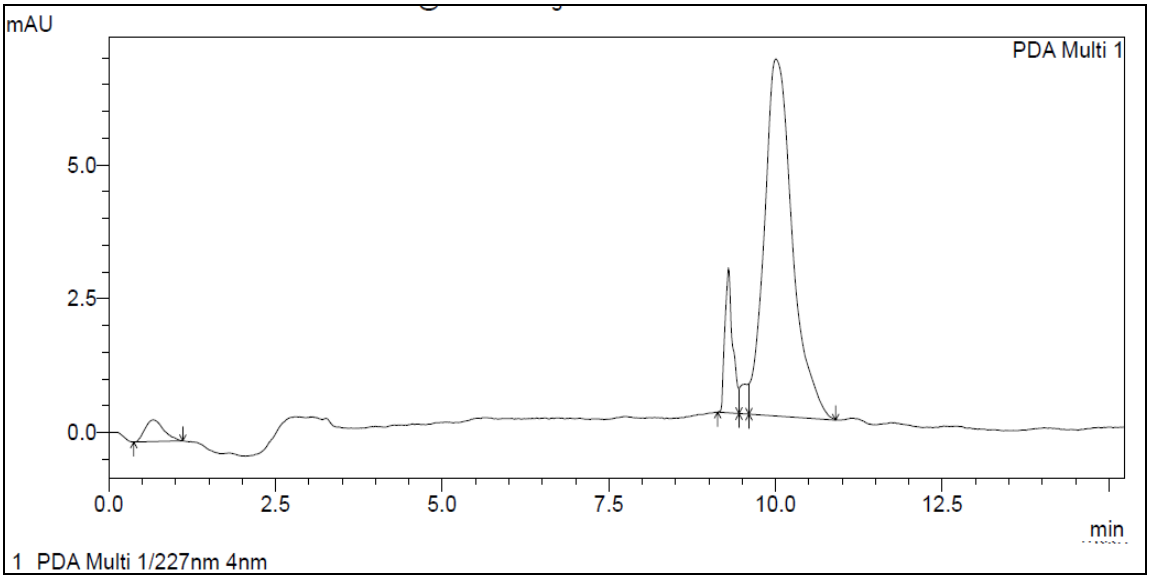


**Fig 3:** Acid degradation of Etodolac

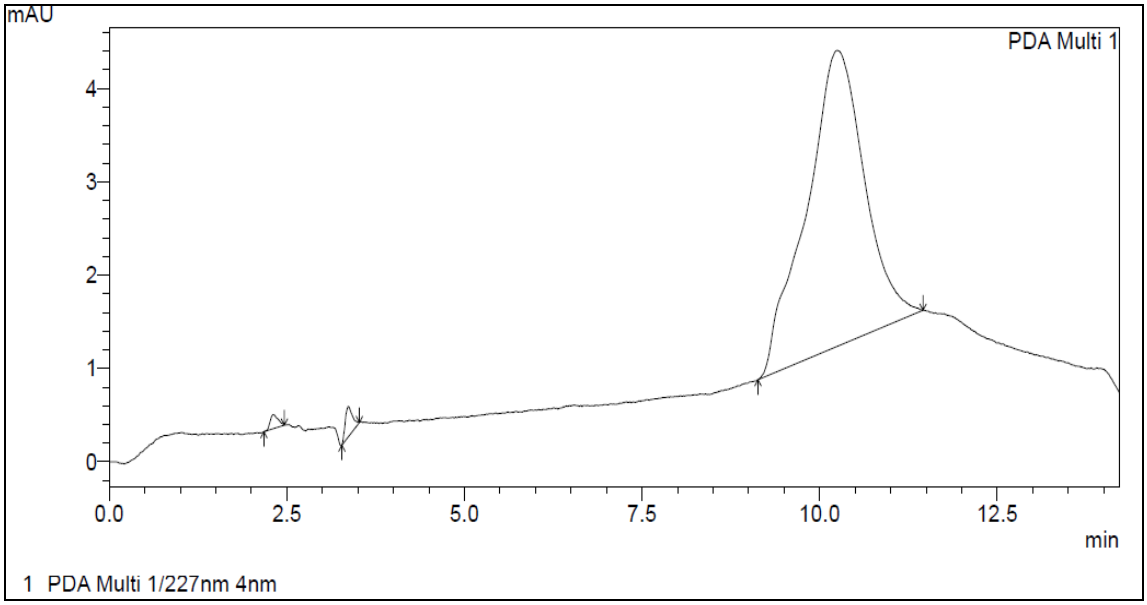


**Fig 4:** Base degradation of Etodolac

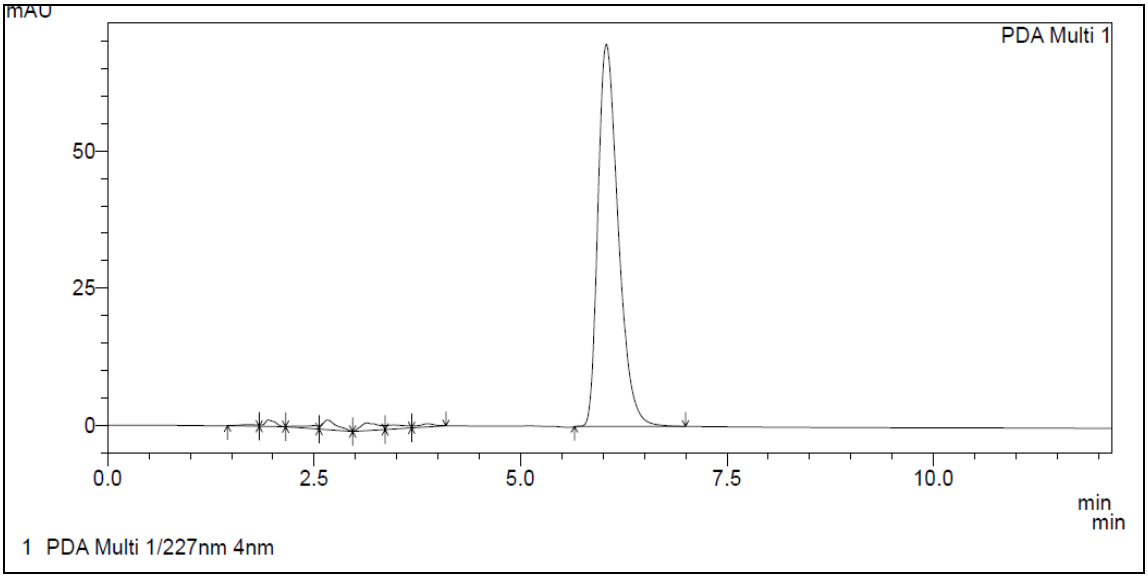


**Fig 5:** Neutral degradation of Etodolac

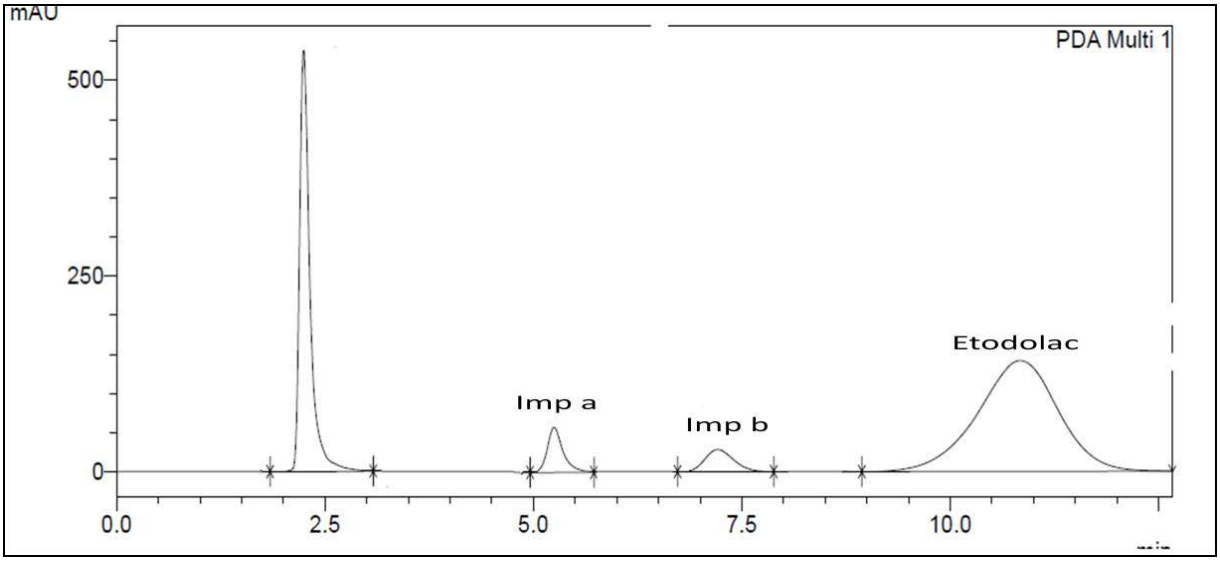


**Fig 6:** oxidative degradation of Etodolac

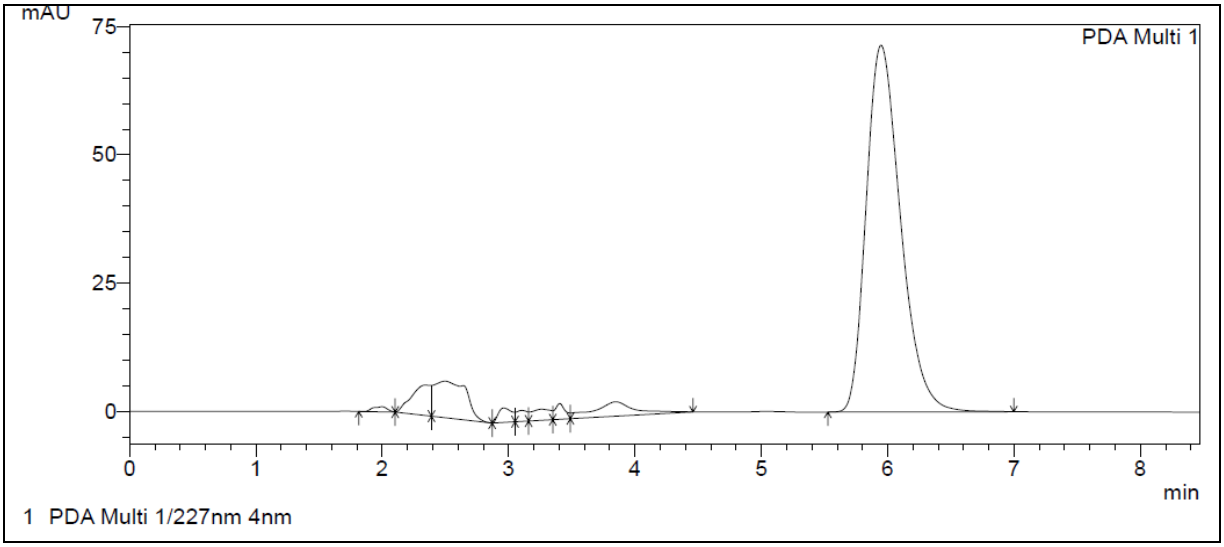


**Fig 7:** Photolytic degradation of Etodolac

**Table 1:** Statistical data of etodolac

| Parameters | Values |
|---|---|
| Slope | 538815 |
| Intercept | 8279.9 |
| Correlation coefficient | 0.998 |
| LOD | 0.027, 0.018, 0.064 |
| LOQ | 0.09, 0.06, 0.21 |

**Table 2:** Recovery data of etodolac

| Compound | % level | Spiked impurity quantity | % recovery | Mean % recovery |
|---|---|---|---|---|
| Imp-a | 0.05 | 0.151 | 96.7 | 97.73 |
| | 0.10 | 0.302 | 100.3 | |
| | 0.15 | 0.453 | 96.2 | |
| Imp-b | 0.05 | 0.148 | 96.6 | 98.66 |
| | 0.10 | 0.296 | 98.3 | |
| | 0.15 | 0.444 | 101.1 | |
| Imp-c | 0.05 | 0.152 | 94.8 | 97.36 |
| | 0.10 | 0.304 | 98.4 | |
| | 0.15 | 0.456 | 98.9 | |
| Etodolac | 0.1 | 149 | 100.6 | 99.8 |
| | 0.5 | 298 | 99.7 | |
| | 1 | 447 | 99.1 | |

**Table 3:** Degradation studies of etodolac

| Conditions | | Time | % of degradation |
|---|---|---|---|
| Acidic hydrolysis | Reflux at 70˚C in 5N HCl | 48hrs | 18% was observed |
| Base hydrolysis | Reflux at 70˚C in 5N NaOH | 50hrs | No |
| Neutral hydrolysis | Reflux at 70˚C in water | 70hrs | No |
| Oxidative degradation | 3% H2O2 in dark at room temperature | 12hrs | 15.3% was observed |
| Photolytic | Visible exposer-3.6 million hrs<br>UV exposer – 600 watt hours/square meter | —— | No |
| Thermal | 70˚C in Hot air oven | 10days | No |

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