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Method Development and Validation for the estimation of Ketorolac Tromithamine by RP-HPLC and UV-Spectroscopy

Adhiraj Chauhan[1]*, Dr. Mukesh Bansal[2], Dr. Dilip Agrawal[2]

[1]Research Scholar, Mahatma Gandhi College of Pharmaceutical Sciences, Sitapura, Jaipur.
[2]Mahatma Gandhi College of Pharmaceutical Sciences, Sitapura, Jaipur.
*Mail ID: adhirajchauhan011@gmail.com

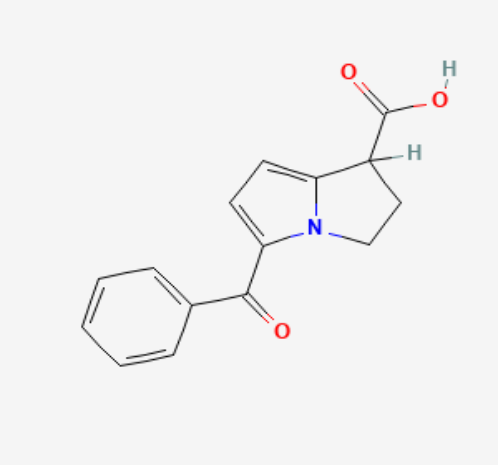
Abstract

Ketorolac tromethamine, a potent nonsteroidal anti-inflammatory drug, is widely used for the management of moderate to severe pain. This study aimed to develop and validate a reliable method for the simultaneous estimation of ketorolac tromethamine by reversed-phase high-performance liquid chromatography (RP-HPLC) and UV spectroscopy. The RP-HPLC method was optimized using a C4 column with a mobile phase consisting of Buffer: Acetonitrile: IPA in a (78:12:10) ratio, adjusted to pH 3.0 with orthophosphoric acid. The flow rate was set at 1.5 mL/min, and detection was performed at 322 nm. The method was validated for linearity, precision, accuracy, specificity, and robustness. The UV spectroscopic method involved the measurement of absorbance at 322 nm in 0.1 N HCl. Both methods demonstrated good linearity over a concentration range of 5-30 µg/mL for ketorolac tromethamine. The methods were found to be precise, with %RSD values less than 2%, accurate, with recovery values within 98-102%, and specific, showing no interference from excipients. The developed methods are simple, rapid, and can be successfully applied for the routine analysis of ketorolac tromethamine in pharmaceutical formulations.

Keywords: Ketorolac tromethamine, NSAIDS, UV spectroscopy, RP-HPLC

Introduction

Ketorolac tromethamine is a non steroidal anti-inflammatory drug which exhibits pronounced analgesic and moderate anti-inflammatory activity. It is a synthetic pyrrolizine carboxylic acid derivative. Ketorolac tromethamine is used to treat moderately severe pain and inflammation, usually after surgery .Ketorolac tromethamine works by blocking the production of prostaglandins, compounds that cause pain ,fever and inflammation Ketorolac tromethamine($C_{19}H_{24}N_2O_6$)has a molecular weight 376.4 g/mol and it was estimated by various analytical methods such as spectrophotometry HPTLC, voltammetry, fluorophotometry ,HPLC and in biological fluids such as human plasma, human serum human eye samples, serum and synovial fluids and post mortem blood samples in the literature. In the present study the authors have proposed five UV spectrophotometric methods for the determination of Ketorolac tromethamine in pharmaceutical dosage forms and the methods were validated as per ICH guidelines.



Structure of Ketorolac Tromithamine

List of chemicals:

Table 1: List of chemicals

S. No	Chemicals	Supplied by
1.	Ketorolac Tromithmine	GNH India
2.	Acetonitrile	Vizag Chemicals, Vizag
3.	Iso- Propyl Alcohol	Hemadari Chemicals, Mumbai
4.	Hydrogen Peroxide (30%)	Reagent Grade, Merck, India
5.	Ortho- Phospharic Acid	Global industial Distributors
6.	Hydrochloric acid	Reagent Grade, Merck, India
7.	Milli-Q water	It was purified by Millipore Corporation's system

List of equipments:

Table 2: List of equipments

S. No	Equipment	Company/ Model
1.	HPLC	CYBERLAB (LC-100)
2.	UV-visible spectrophotometer	UV- 2450, Shimadzu, Japan
3.	Analytical Balance	AD 265S, Mettler Toledo, Switzerland.
4.	pH Meter	Labindia, India
5.	Sonicator	5510, Branson Ultrasonic Corporation, Danbury, CT, USA.
6.	Hot air oven	Labline, India
7.	Photo stability chamber	SVI equipments, Germany

Method

Standard stock preparation:

Drug-: Ketorolac Tromethamine

Weigh and transfer about 100 mg of Ketorolac Tromethamine reference standard to a 100 ml volumetric flask. Add about 50 ml of 0.2M HCL, sonicate to dissolve, and then add 35ml acetonitrile and mix make up the volume with acetonitrile and mix.

Standard solution preparation:

Drug-: Ketorolac Tromethamine

Weigh and transfer about 100 mg of **Ketorolac Tromethamine** reference standard to a 100 ml volumetric flask. Add about 50 ml of 0.2M HCl, sonicate to dissolve, then Add 35ml acetonitrile and mix, make up the volume with acetonitrile and mix. Pipette out 4 ml of solution to a 25 ml volumetric flask and dilute up to the mark with diluent and mix. (160ppm)

Sample preparation

Weigh 250mg Drug and transfer into 250ml volumetric flask. Add 125mL of 0.2M HCl; shake for 15mins in a mechanical shaker. Sonicate for about 30 minutes with occasional shaking and cool to room temperature. Add 75mL of Acetonitrile and sonicate again for another 30minutes with occasional shaking. Make the volume up to the mark with Acetonitrile and mix. Allow to settle for 10mins. Filter the sample through 0.45µm Nylon filter. Discarding first few mL of filtrate. Pipette out 4ml sample stock solution to a 100ml volumetric flask and dilute up to the mark with diluents and mix.

Buffer Preparation

25 mg of Ketorolac tromethamine was accurately weighed and transferred in to a 25 ml volumetric flask and dissolved in methanol (1000 µg/ml) and a series of dilutions were prepared with respective buffers as per the requirement. The reagents were prepared as given below.

Preparation of phosphate buffer (pH 5.0)

6.8 grams of potassium dihydrogen phosphate was dissolved in water and the pH was adjusted to 5.0 with 10M potassium hydroxide in a 1000 ml volumetric flask.

Preparation of phosphate buffer (pH 8.0)

50 ml of 0.2M potassium dihydrogen phosphate was placed in a 200 ml volumetric flask and 46.1 ml of 0.2M sodium hydroxide was added and the volume was made up to 200 ml.

Preparation of acetate buffer (pH 4.7)

8.4 grams of sodium acetate and 3.35 ml of acetic acid was dissolved in sufficient water to make 1000 ml in a 1000 ml volumetric flask.

Preparation of 0.1N sodium hydroxide

4 grams of sodium hydroxide was dissolved in sufficient water and made up to 1000 ml in 1000 ml volumetric flask.

Preparation of borate buffer (pH 9.0)

6.20 grams of boric acid was dissolved in 500 ml of water and the pH was adjusted with 1M sodium hydroxide (about 41.5 ml) and diluted with water to produce 1000 ml in a 1000 ml volumetric flask.

Mobile phase

Use Buffer, Acetonitrile and isopropyl alcohol in the ratio of 78:12:10. Sonicate to degas.

Diluent

Take 8.5ml HCl dissolve up to 1000ml with Water: ACN (50:50).

0.2M HCl

Dilute 17mL of Conc. Hydrochloric acid to 1 Liter with water.

Blank Solution: Use diluent as blank.

Optimized HPLC Parameters:

Instrument: High Performance Liquid Chromatography with UV detection
Column: Inertsil ODS 3, 100mm X 2.1mm, 5µm
Flow Rate: 1.5 mL/min
Injection Volume: 10 µL
Column Temperature: 40°C
Sample Cooler Temperature: 5°C
Detection: By UV at 322 nm
Mobile Phase: Buffer: Acetonitrile: IPA (78:12:10)
Diluent: 0.1M HCl in Water: ACN (60:40)
Blank Preparation: 0.1M HCl in Water: ACN (60:40)

Result and Discussion

Specificity:

Interference from blank, placebo and impurities:

Procedure

Prepared blank, standard, and sample for 200+50+200mg Drug as per method. Prepared placebo preparation, impurity preparations, sample spiked with impurity as follows.

Placebo preparation

Weigh 2.4g placebo powder and transfer into 250ml volumetric flask. Add 125mL of 0.2M HCl; Shake for 15minute in a mechanical shaker. Sonicate for about 30 minutes with occasional shaking and cool to room temperature. Add 75mL of Acetonitrile and sonicate again for another 30 mins with occasional shaking. Make the volume up to the mark with Acetonitrile and mix. Allow to settle for 10mins. Filter the sample through 0.45µm Nylon filter. Discarding first few mL of filtrate, Pipette out 4 ml of solution to a 100 ml volumetric flask and dilute up to the mark with +diluent and mix.

Individual impurity solution preparations at 5% level with Respect to Sample concentration:

Impurity Preparation

Weighed and transferred accurately about 1 mg of each Impurity into a 10 ml volumetric flask. Added about 5ml of diluent and sonicate to dissolve and diluted to volume with diluent and mixed (100 ppm),

Preparation of Sample spiked with impurity

Weigh 4.8 g sample powder and transfer into 250ml volumetric flask Add 125mL of 0.2M HCl; Shake for 15mins.in a mechanical shaker. Sonicate for about 30 minutes with occasional shaking and cool to room temperature. Add 75mL of Acetonitrile and sonicate again for another 30mins with occasional shaking. Make the volume up to the mark with Acetonitrile and mix. Allow to settle for 10mins. Filter the sample through 0.45µm Nylon filter. Discarding first few mL of filtrate, Pipette out 4 ml of solution to a 100 ml volumetric flask and dilute up to the mark with diluent and mix. Add Individual Impurity in the volumetric flask.

Prepare sample spiked with impurity in triplicate and inject in duplicate

Inject diluent as blank once, standard preparation five times, placebo preparation once, standard solutions once, unspiked sample preparation and spiked sample preparations in duplicate. Check the peak purity for the main peak in standard preparation and sample preparation.

Specificity

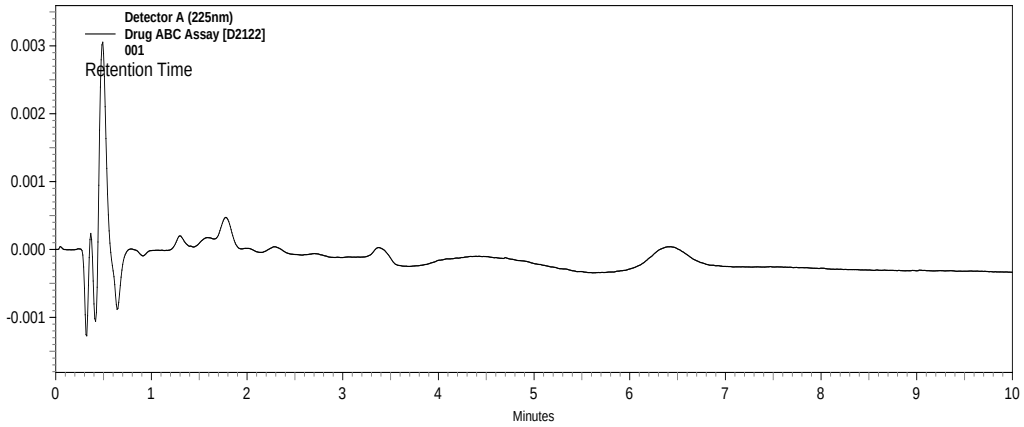


Figure 1: Peak Purity for the Blank

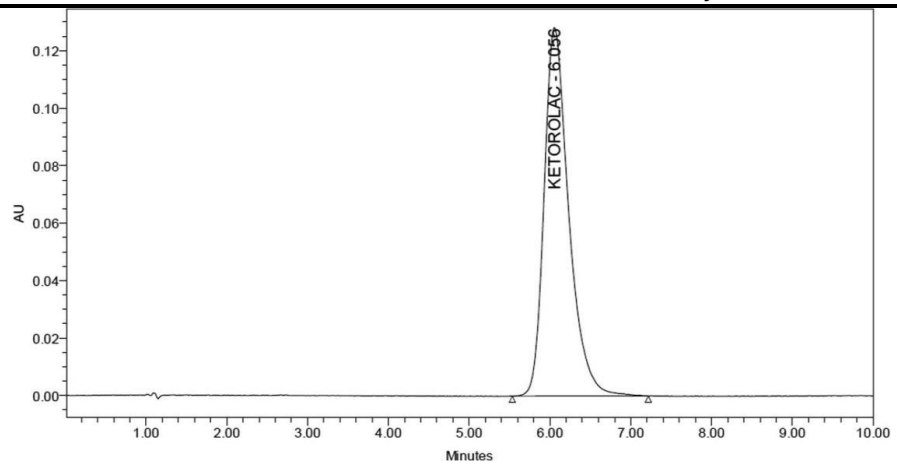


Figure 2: Peak Purity for the Standard

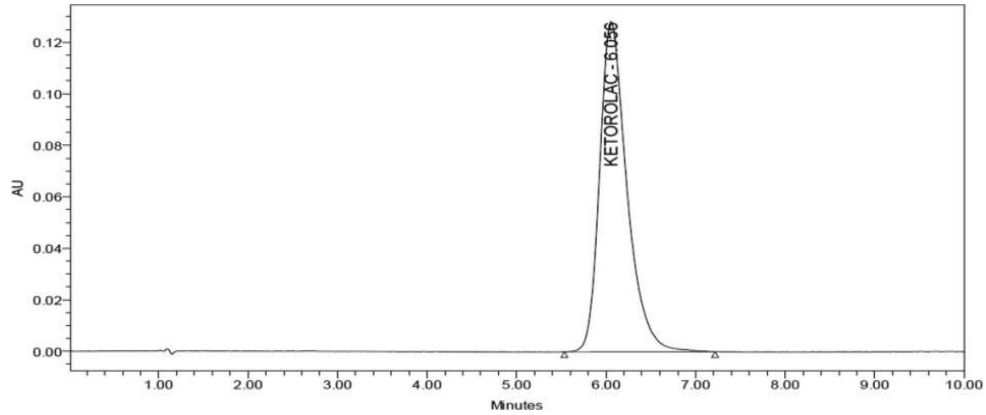


Figure 3: Sample Spike in HPLC

The peak purity index for the main peak in standard preparation and sample preparation was determined and recorded in Table 3.

Table 3: Data indicating peak purity index of Ketorolac Tromithamine

Sample	Peak Purity Index
	Ketorolac Tromithamine
Standard preparation	0.9999
Sample preparation	0.9999

Acid degradation: Heated for 3 hour in 1N hydrochloric acid at 80°C.

Base degradation: Heated for 1 hour in 0.1N sodium hydroxide at 80°C.

Peroxide degradation: Heated for 30Minutes in 3% hydrogen peroxide at 80°C.

Thermal degradation: Heated for 24 hour at 105°C in oven.

All the solutions were passed through 0.45 µ filter before making injections.

Note: Minimum 10 to 30% degradation should be achieved in minimum 2 conditions. Final concentration of all the placebo and sample preparation should be approximately equal to the test concentration specified in diluent.

Table 4: Results of forced degradation study of samples using the proposed method, indicating specificity of the developed method

Stress condition/ duration/ state	% Degradation Drug-A	Peak Purity Index Drug-A
Acidic/1NHCl/80°C/ 3hr/ solution	1.24	0.9999
Alkaline/ 0.1N NaOH / 80°C /1hr/solution	14.04	0.9999
Oxidative/3% H_2O_2 / 80°C/ 1hr /solution	10.29	0.9999
Thermal/105°C/24hr/ solid	1.06	0.9998

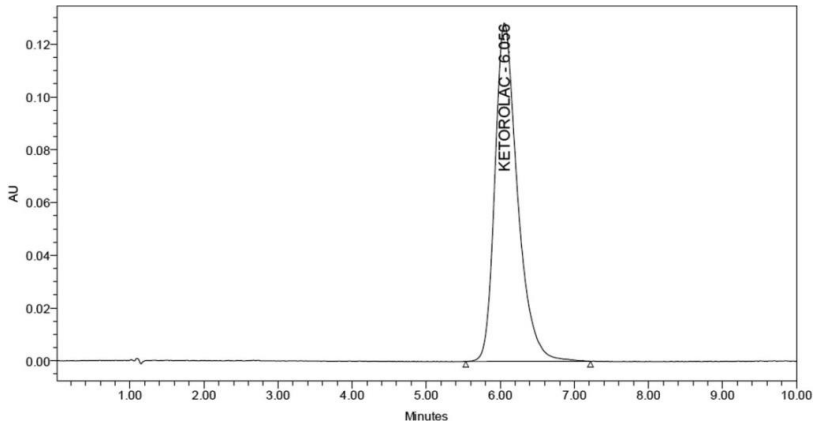


Figure 4: Linearity Chromatogram for Level

Table 5: Linearity of Ketorolac Tromethamine by HPLC method

Linearity level	Conc. (µg/ml)	Area	Mean Area
Level – 1 (25%)	5	14367.8 14413.4	14321
Level – 2 (37.5%)	10	30633.7 28855.9	29413
Level – 3 (50%)	25	82716.1 82796.6	82237.9
Level – 4 (75%)	50	153708.2 152393.2	151221.2
Level – 5 (100%)	75	224027.2 225609.2	224312.1
Level – 6 (112.5%)	100	319468.7 318363.7	316923.1
Level – 7 (150%)	150	469270.3 465679.3	466276
Correlation coefficient (r)		**0.9991**	
Slope of regression line		**3116.9x**	
Y-intercept		**1259.8**	

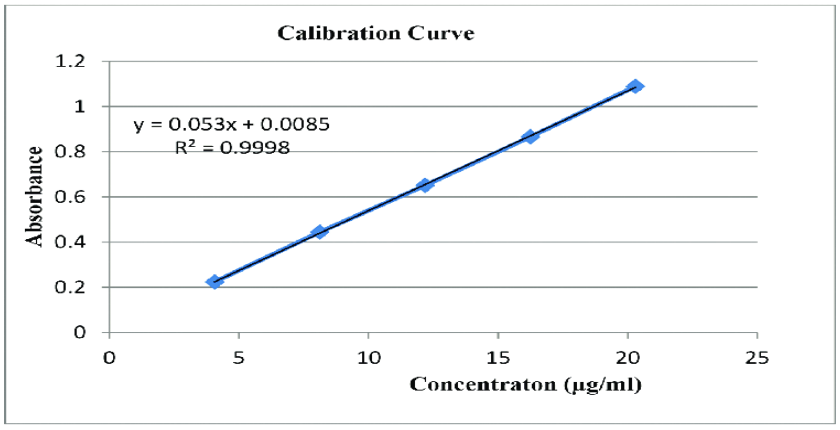


Figure 5: Linearity Graph of Ketorolac Tromethamine

Table 6: Data indicating recovery study of Ketorolac Tromethamine

Drug ($\mu g\ mL^{-1}$)	Amount spiked (%)	Amount spiked (μgmL^{-1})	Concentration found (μgmL^{-1})	%Mean Recovery ±S.D.	% RSD
KTT (50)	80	40	39.94 39.98 40.21	100.11± 0.35	0.35
	100	50	49.67 49.67 49.82	99.44± 0.16	0.16
	120	60	60.01 59.74 59.76	99.73± 0.25	0.25

Method Precision (Repeatability)

Procedure

Method precision should be established by analyzing six sample preparations under same conditions as per test procedure for Assay using same lot of sample. Individual assay value, mean assay value, 95% Confidence Interval and % RSD shall be calculated for the results obtained and recorded.

Result:

Individual % assay, mean % assay, % RSD and 95% confidence interval were calculated and recorded in Table 7.

Table 7: Method precision data for analysis of Ketorolac Tromethamine Working Standard

Sample No.	Area	Mean Area	% Assay
1	152712.7		
2	151528.9		
3	152002.5	152105.48	98.42
4	152134.6		
5	152145.7		
Mean Assay			**98.42**
SD			**895**
% RSD			**0.38**
95% C.I			**NA**

Intermediate Precision (Ruggedness)

Procedure

The procedure followed for method precision shall be repeated on a different day by different analyst, using a different HPLC system and different column of same make using same lot of sample. Individual assay value, mean assay value, 95% Confidence Interval and %RSD shall be calculated. The mean assay value shall be compared with the average assay value obtained in method precision study. The difference in the mean assay values shall be calculated and recorded.

Table 10: Intermediate precision data for analysis of Ketorolac Tromethamine Working Standard

Sample No.	Area	Mean Area	% Assay
1	178867.9		
2	179874.9		
3	178209.2	179452.56	**99.24**
4	179732.2		
5	180578.6		
Mean Assay			**99.24**
SD			**819.75**
% RSD			**0.02**
95% C.I			**NA**

Table 11: Comparison for Ketorolac Tromethamine

Comparison	Method Precision	Intermediate Precision
Results	**% Assay**	**% Assay**
Test Sample-1	98.82	98.02
Test Sample-2	98.06	98.91
Test Sample-3	98.37	101.90
Test Sample-4	98.5	98.41
Test Sample-5	98.9	98.99
Test Sample-6	98.2	98.04
Mean Assay	**98.42**	**99.24**
Std. Dev	895	857
% RSD	0.38	0.02
95% C.I	NA	NA
Absolute Difference	-0.82	

Solution Stability

Procedure

Standard and sample preparation was prepared as per test procedure and assay of standard and sample was determined as per method. Standard and sample solution was stored for 48 hours at room temperature. Assay of standard and sample solution after 2 and 4 hours was determined using freshly prepared standard. The assay obtained was compared with the initial assay value and recorded.

Table 12: Solution stability data for Ketorolac Tromethamine

Time	Standard			Sample		
	Area	% Assay	Absolute Difference	Area	% Assay	Absolute Difference
Initial	154812.5	100.0	-	154803.1	99.9	-
After 2 hrs	154623.9	99.8	0.2	154812.8	100.8	0.9
After 4 hrs	153978.8	99.9	0.1	154822.4	101.0	1.1

Filter media interference

Procedure

Filter media interference was determined by comparing results of sample filtered through 0.45µ nylon filter and unfiltered sample. Three-three replicates of filtered and unfiltered samples were prepared and injected in duplicate.

Table 13: Data indicating %assay of filtered and unfiltered sample for Ketorolac Tromethamine

Sample Preparation No.	Filtered sample		Unfiltered sample	
	Average Area	Assay (%)	Average Area	Assay (%)
1.	154812.3	100.8	154811.8	100.1
Difference of % Assay		0.7		

Robustness:

NOTE: For Robustness study different batch of Ketorolac Tromethamine is used.

Procedure

Change following parameters one by one and observe their effect on system suitability.

- Change in flow rate of mobile phase by ± 10% [use flow rate 1.40 ml/min and 1.60 ml/min].
- Change in column oven temperature by ± 5°C. (Use column oven temperature 35°C and 45°C).
- Change in pH by ± 0.10 [use pH 2.2 and 1.8].

Result:

The effect of changes was observed on system suitability values and recorded in the Table

Flow rate:

Table 14: Change the flow rate of Mobile Phase for Ketorolac Tromethamine

Standard repetitions	1.50 ml/min (As per Method)	1.40 ml/min (-0.10 ml/min)	1.60 ml/min (+0.10 ml/min)
	Drug- Area		
1	154812	164512	154543
2	154834	154856	154876
3	154765	154567	154231
4	154976	154987	154543
5	154819	154812	154453
Mean Area	154816	155032	154876
% RSD	**0.12**	**0.17**	**0.07**

Sample preparation	1.5 ml/min (As per Method)	1.40 ml/min (-0.10 ml/min)	1.60 ml/min (+0.10 ml/min)
	Drug-Area		
1	154765	155476	155435
2	154865	154787	154654
Mean Area	155543	154345	154893
% Assay	**97.6**	**98.2**	**98.1**
Absolute Difference	**-**	**0.6**	**0.5**

Standard repetitions	1.5 ml/min (As per Method)	1.40 ml/min (-0.10 ml/min)	1.60 ml/min (+0.10 ml/min)
		Drug-Area	
1	154345	154236	154789
2	154762	154768	154794
3	154789	154765	154885
4	154985	154879	154798
5	154334	154788	154725
Mean Area	896651	953846	848013
% RSD	**0.13**	**0.11**	**0.13**

Column oven Temperature:

Table 15: Change the Column Oven Temperature for Ketorolac Tromethamine

Standard repetitions	40°C (As per Method)	35°C (-5°C)	45°C (+5°C)
	Drug- Area		
1	154211	154443	154883
2	154765	154985	154647
3	154984	154932	154856
4	154433	154854	154799
5	154884	154879	149765
Mean Area	154324	154356	154567
% RSD	**0.12**	**0.12**	**0.15**

Sample preparation	40°C (As per Method)	35°C (-5°C)	45°C (+5°C)
		Drug- Area	
1	153765	154745	154864
2	154565	154762	154653
Mean Area	154733	154543	154982
% Assay	**97.6**	**98.5**	**99.3**
Absolute Difference	**-**	**0.9**	**1.7**

Summary

Literature survey reveals that there is only no method of assay development of ketorolac tromethamine drugs that is RP-HPLC. Hence, an attempt has been made to develop simple, rapid and accurate methods for estimation of ketorolac tromethamine drugs by High Performance Liquid Chromatography.

- A Reverse Phase HPLC Method was developed for simultaneous estimation of drug. The separation was achieved by Inersil ODS C_4 Column and Buffer: ACN: IPA (78:12:10 v/v) as mobile phase, at a flow rate of 1.5 ml/min. The detection was carried out at 322nm. The retention time of ketorolac tromethamine was found to be at 1.8min, 2.8min and 6.6min.
- **Specificity:** There was no any interference from peaks due to blank, placebo and impurities with the main peak. And peak purity was found to be 1.000. These result indicate specificity of method.
- **Linearity:** was assessed by a plot of concentration versus area. The graphs were found to be linear in range of 40-240µg/ml for ketorolac tromethamine with correlation coefficient values 0.99832.
- **Accuracy:** Three replicate injections, each of three different test concentrations in the range of 50 to 125 % for Assay and has yielded the results within 99.5-101.7% for ketorolac tromethamine.
- **Precision**: Precision studies were carried out using parameters like different days, repeatability. Results showed that the % RSD was found 0.16 for ketorolac tromethamine, i.e. less than 2. This study signifies the precision of the method.
- **Ruggedness**: Ruggedness studies were carried out using different analyst parameter. Results showed that the % RSD was 0.13 for ketorolac tromethamine, i.e. less than 2 for different analyst studies. This study signifies the ruggedness of the method under varying conditions of its performance.
- **Robustness**: Robustness studies were carried out by different condition like change in mobile phase composition, change in pH, change in flow rate, and change in temperature. The system suitability criteria and the difference in the % assay value obtained as per method and by varied condition were found less than 2.0% absolute.

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