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DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF KETOROLAC TROMETHAMINE IN DRUG SUBSTANCE AND ITS PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A rapid and sensitive reverse phase High Performance Liquid Chromatographic [RP-HPLC] method was developed for the estimation of Ketorolac tromethamine [KTT] in pure and its capsule dosage forms. The method was validated as per International Conference on Harmonization [ICH] guidelines. The mobile phase used in this study is a mixture of tetrahydrofuran and monobasic ammonium phosphate buffer (pH-3.0) in the ratio of 30:70% v/v. Stationary phase was waters spherisorb C_8 reverse phase column (250×4.6mm, 5µm) dimensions at 40°c temperature. The analysis was performed with run time of 10.0 minutes at a flow rate of 1.50ml/min. The KTT was monitored at 313nm with UV detection and KTT was eluted at 7.20min. The method was linear (r2 =0.999) at concentration ranging from 100 to 600µg/ml, precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (mean recovery = 99.5%), specific and robust. Detection and quantification limits were 27.0µg/ml and 92.0µg/ml, estimated from linearity by regression method. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of KTT in bulk, its capsule dosage forms.

***Keywords**: Ketorolac tromethamine, RP-HPLC, Validation, Dosage form.*

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General Introduction:

Ketorolac tromethamine [(+/-)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxymethyl)-1,3-propanediol] is a highly potent member of non steroidal anti-inflammatory drugs. The compound shows potent prostaglandin cyclooxygenase inhibitory activity [1]. Ketorolac, when administered intramuscularly or orally, is a safe and effective analgesic agent for the short-term management of acute postoperative pain and can be used as an alternative to opioid therapy[2].

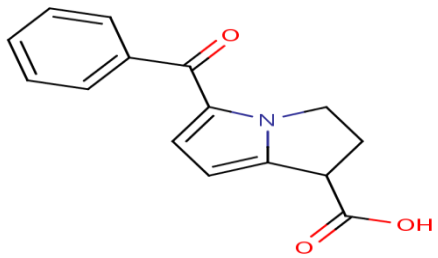

Fig.1. Structure of Ketorolac tromethamine.

A few analytical methods were published in literature that describe the quantification of Ketorolac tromethamine in plasma by liquid chromatography, fluorescence detection, and UV detection but lacking of stability indication observed. In the present investigation the authors propose a simple, sensitive, reproducible and stability indicating method for the determination of Ketorolac tromethamine.

Comprehensive literature survey reveals that several analytical methods have been reported for the estimation of KTT which includes Reverse Phase HPLC [7,8], Spectrophotometry[4,5], HPTLC[10], Capillary Zone Electrophoresis,[11] fluorimetric assay,[6] micellar electrokinetic chromatography[12] and differential pulse polarography[13].

The proposed method was validated with respect to Stability indication besides selectivity, linearity, precision, and accuracy, limit of quantitation (LOQ) and limit of detection (LOD) according to ICH requirements [14-18] to show it could be used for determination of KTT in pharmaceutical formulations.

Experimental Chemicals and Reagents:

HPLC grade tetrahydrofuran and water as well as monobasic ammonium phosphate, Orthophosphoric acid, A.R. grade were purchased from Fisher scientific, Mumbai, India. All other chemicals used were of HPLC grade or A.R. grade.

Milli-Q Water, Tetrahydrofuran (HPLC Grade), Orthophosphoric acid (GR Grade), monobasic ammonium phosphate (GR Grade) were obtained from Fisher scientific, Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

Instrumentation and Chromatographic Conditions

Instrumentation:

The analysis of the drug was carried out on a waters LC system equipped with 2695pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Sperisorb C-8 ((Make: Waters); 250 mmx4.6 mm I.D; particle size 5 µm)) was used. The output of signal was monitored and integrated Agilent EZ Chrome Elite software.

Buffer preparation:

Dissolve 2.72g of monobasic ammonium Phosphate in 1000mL of Milli-Q Water, adjust pH to 3.0 with dilute ortho phosphoric acid and Filter the solution through 0.45µm membrane filter.

Mobile phase preparation:

Prepare a filtered and degassed mixture of Buffer and Tetrahydrofuran in the ratio of 700:300 v/v respectively.

Diluent preparation:

Mobile Phase is used as diluent.

Standard preparation:

Accurately weigh and transfer about 20mg of KTT into a 50 mL volumetric flask, add 30 mL of diluent and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with diluent.

Sample preparation:

Weigh and mix powder not fewer than 20 tablets. Accurately weigh and transfer equivalent to 20mg of KTT into a 50 mL volumetric flask, add 30 mL of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter.

Chromatographic conditions:

A Spherisorb C-8 ((Make: Waters; 250 mmx4.6 mm I.D; particle size 5µm)) Column was used for analysis at 40°C column temperature. The mobile phase was pumped through the column at a flow rate of 1.5mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 313nm for the detection and Chromatographic runtime was 10 minutes.

RESULTS AND DISCUSSION

Method development[11-15]:

Spectroscopic analysis of compound showed that KTT has maximum UV absorbance (λmax) at 313 nm. Therefore, the chromatographic detection was performed at 313nm using a Photo diode array detector. To develop a suitable and robust LC method for the determination of KTT, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Spherisorb C-8 (Make: Waters; 250 mmx4.6 mm I.D; particle size 5 µm) with the following mobile phase. Filter the solution through 0.45µm membrane filter. Prepare a filtered and degassed mixture of Buffer and Methanol in the ratio of 500:500 v/v respectively. It was observed that a non symmetrical peak was observed.

For next trial the mobile phase composition was changed slightly. The mobile phase composition was Buffer and acetonitrile in the ratio of 600:400 v/v. above trail the peak shape was little broad but the peaks are separated. Again the mobile phase composition changed slightly to Buffer and tetrahydrofuran in the ratio of 300:700 v/v respectively as eluent at flow rate 1.5 mL/min. UV detection was performed at 313nm. The retention time of KTT is 7.2 minutes (**Fig-2**.) and the peak shape was good.

Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in the mobile phase. Different experiments were performed to optimize the mobile phase and adequate separation of drugs achieved. The optimized mobile phase was determined as a mixture of Buffer and tetrahydrofuran (700:300) at a flow rate of 1.5 mL.min-1. Under these conditions KTT was eluted at 7.2 min, with a run time of 10 min.

The chromatogram of KTT standard using the proposed method is shown in **(Fig-2.)** System suitability results of the method are presented in **Table-1.**

A typical chromatogram for estimation of KTT obtained by using the aforementioned mobile phase from 10 µL of the assay preparation is illustrated in **Fig. 2.**

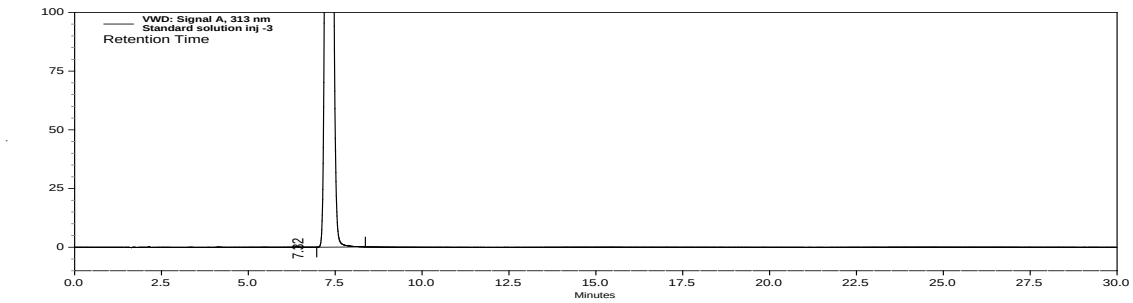

Figure 2: A typical HPLC Chromatogram showing the peak of KTT

Method validation:

The developed RP-LC method extensively validated for assay of KTT using the following Parameters.

Specificity:

Blank and Placebo interference:

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution **(Fig.-3)** showed no peaks at the retention time of KTT peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of KTT in KTT tablets. Similarly Chromatogram of Placebo solution **(Fig.-4)** showed no peaks at the retention time of KTT peak. This indicates that the Placebo used in sample preparation do not interfere in estimation of KTT in KTT tablets.

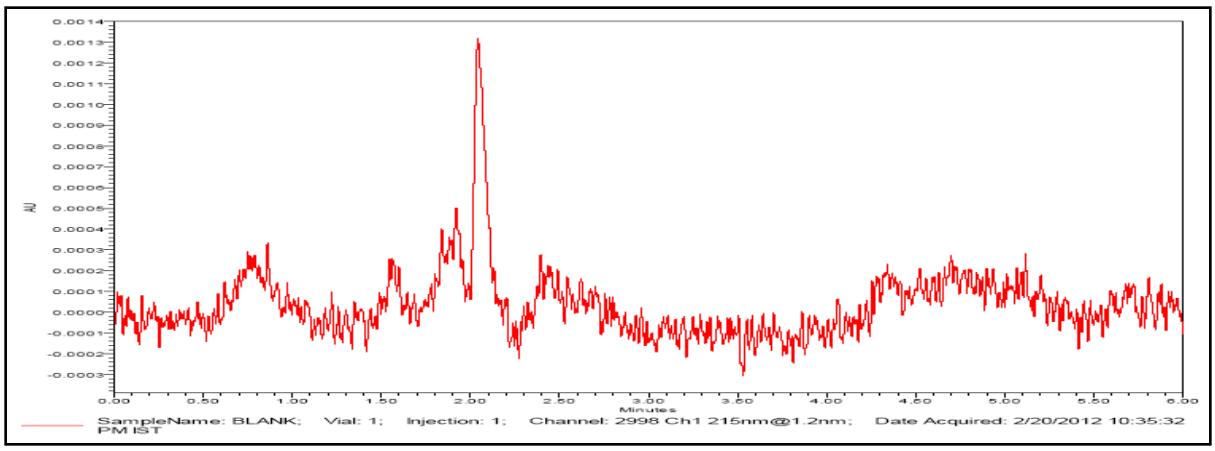

Figure 3: A typical HPLC Chromatogram showing the no interference of diluent for KTT

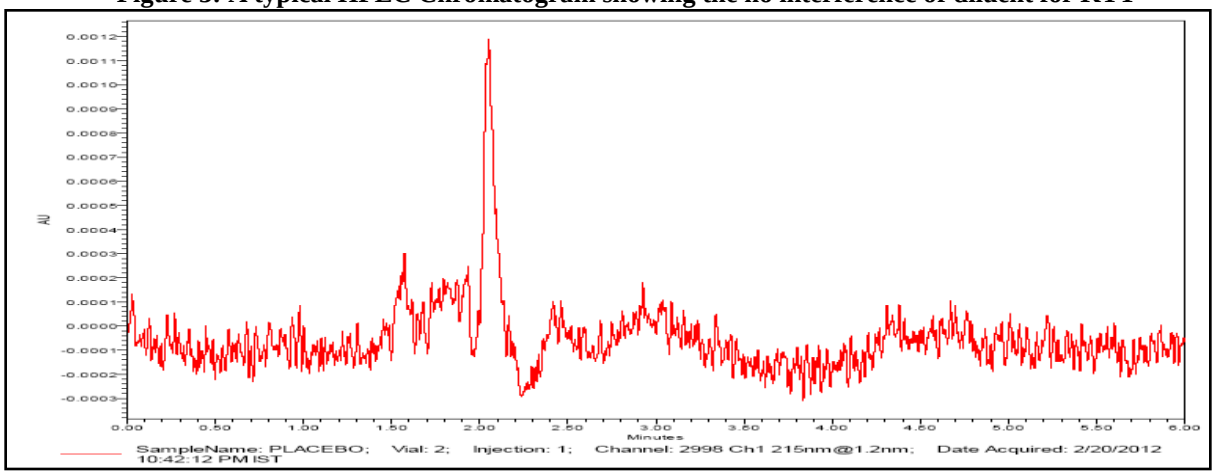

Figure 4: A typical HPLC Chromatogram showing the no interference of placebo for KTT

Table 1: System suitability parameters for KTT by proposed method

Name of the Compound	Retention Time	Theoretical plate	Tailing factor
Ketorolac tromethamine	7.2	8536	1.29

The HPLC chromatograms recorded for the placebo showed almost no peaks at the retention time of KTT. The peak for KTT is clearly separated from other excipients of the formulations. As there is no blank interference is observed at the retention time of KTT, the HPLC method presented in this study is specific for KTT. Prepare the standard solution and test solution as per the proposed analytical method.

Table: 1.11 Specificity parameters for KTT Standard by proposed method

KTT standard	Inj-1	Inj-2	Inj-3	Avg.	%RSD
RT	7.22	7.18	7.16	7.19	0.34
Area	133261425	133899914	134047140	133736160	0.31

Table: 1.12 Specificity parameters for KTT Sample by proposed method

KTT sample	Inj-1	Inj-2	Inj-3	Avg.	%RSD
RT	7.16	7.14	7.15	7.15	0.12
Area	130414935	130554929	130026295	130332053	0.21

Forced Degradation:

Control Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 20 mg of Ketorolac tromethamine into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent.(Figure 1.21)

Acid Degradation Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 20 mg of Ketorolac tromethamine into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature. Then add 10ml of 5N HCl, refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 5N NaOH and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 1.21)

Base Degradation Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 20 mg of Ketorolac tromethamine into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature. Then add 10ml of 5N Base (NaOH), refluxed for 30min at 60°C, then cooled to room temperature, neutralize with 5N Acid (HCl) and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 1.21)

Peroxide Degradation Sample:

Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 20 mg of Ketorolac tromethamine into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature. Then add 2ml of 30% Peroxide, refluxed for 30min at 60°C, then cooled to room temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 1.21)

Thermal Degradation Sample:

Powder collected from 20 tablets are exposed to heat at 105°C for about 5days. Then Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 20 mg of Ketorolac tromethamine into a 100 ml volumetric flask add about 70 ml of diluent, and sonicate for 30minutes with intermittent shaking at controlled temperature and dilute to volume with diluent and mix. Filter the solution through 0.45 µm membrane Filter.

Transfer 5.0 ml of the above solution into a 100 ml volumetric flask and dilute to volume with diluent. (Figure 1.21)

Similarly Humidity, UV-Light exposure, Sunlight exposure and Water hydrolysis stress samples are prepared and checked for their purity by proposed method.

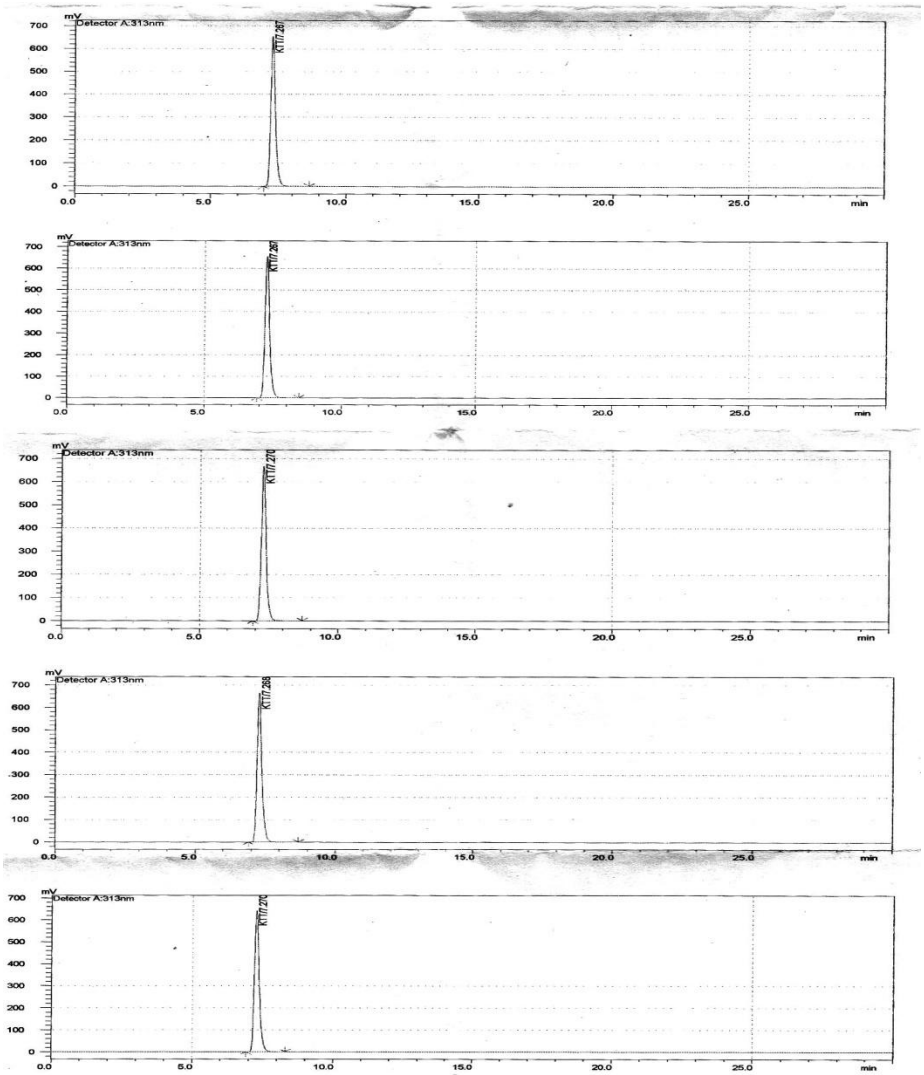

Figure 1.21: A typical HPLC Chromatogram showing the degradation profile of Ketorolac tromethamine by proposed method.

Precision:

In the study of the instrumental system precision study for six standard preparations showed a %RSD of retention time 0.13% was obtained, % RSD 0.37% for the area obtained respectively. In the study of the instrumental system precision study for six sample preparations showed a %RSD of retention time 0.19% was obtained, % RSD 0.10% for the area obtained respectively.

In the study of the instrumental system precision where, a RSD of 0.37% was obtained for the standard area obtained corresponding to the first day, being 0.96% for the second day, respectively. The method precision study for six sample preparations in marketed samples showed a RSD of 1.25 % and the 95% confidence interval of 0.2 with the assay range of 99.9-100.4

For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as inter-day RSD corresponded to 0.3 %(For Standard). The same study was carried out for different analysts (n = 6 number of samples per analyst) obtaining a RSD of 0.5 %(Intermediate Precision) and 95% confidence interval of 0.5 with the assay range of 99.8-100.6 The Overall %RSD for n=12 is 0.3. Both results together with the individual results are showing that the proposed analytical technique has a good intermediate precision.

Table 2: Method Precision (Inter and Intraday) studies for KTT by proposed method

Summary showing Method Precision by Proposed Method	
Method Precision (Inter &Intra Day)	
100.1	100.20
100.7	100.4
99.4	99.6
99.3	99.7
99.2	99.5
100.5	99.6
Overall Avg.	99.9
Overage Std Dev.	0.51
Over all %RSD	0.50

Accuracy:

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of KTT, analyzed as per the proposed method. The percentage recoveries with found in the range of 99.3 to 100.9 with an overall %RSD of 0.5 for KTT and From the data obtained which given in **Table-1.17** to **Table-1.22** the method was found to be accurate.

Table: 1.17: Authentic level KTT Working Standard Areas

KTT	AREA
Inj-1	133922965
Inj-2	134086659
Inj-3	134112650
Inj-4	134341797
Inj-5	134175567
Inj-6	134737464
Mean Area	134229517

Table: 1.18: Authentic level KTT Sample Areas

KTT	AREA
Inj-1	131035764
Inj-2	130933403
Inj-3	131813023
Inj-4	131055990
Inj-5	131218276
Inj-6	131522187
Mean Area	131263107

Table: 1.19 Assay Preparation (50%) for KTT Sample by proposed method

KTT	**RT**	**AREA**
Inj-1	7.20	66282960
Inj-2	7.20	66505399
Inj-3	7.24	66339299
AVG	7.22	66375886
%RSD	0.22	0.17

Table: 1.20 Assay Preparation (100%) for KTT Sample by proposed method

KTT	RT	AREA
Inj-1	7.26	132279622
Inj-2	7.27	132559113
Inj-3	7.21	132781766
AVG	7.25	132540167
%RSD	0.31	0.19

Table: 1.21 Assay Preparation (150%) for KTT Sample by proposed method

KTT	RT	AREA
Inj-1	7.19	189121033
Inj-2	7.18	189439078
Inj-3	7.18	189931659
AVG	7.19	189497257
%RSD	0.08	0.22

Table: 1.22 % Recovery of Ketorolac tromethamine

Name	Level – 1 (50%)	Level –2 (100%)	Level –3 (150%)
Ketorolac	99.9	99.98	99.88

Linearity of detector response:

The standard curve was obtained in the concentration range of 100-600µg/ml. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in **Fig: 1.30** and **Table: 1.23** to demonstrate the linearity of the method.

Table: 1.23 Linearity of Response for KTT by proposed method

Linearity of Response for Ketorolac		
% Level (Approx.)	**Concentration (µg/ml)**	**Average Area**
25	100	33529388
50	200	65190691
75	300	98857127
100	400	129736371
125	500	165224031
150	600	191902593
% Y-intercept		1.38
CC		0.9991

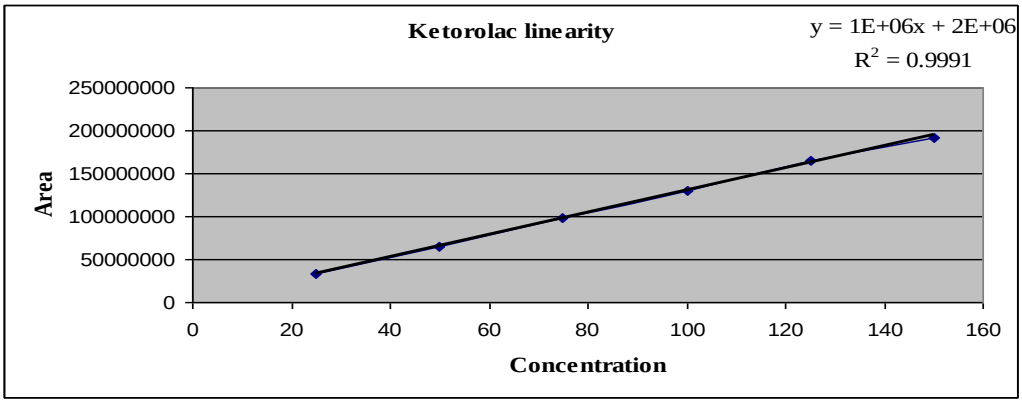

Fig: 1.30 - Linearity curve for Ketorolac

LOD and LOQ:

Limit of detection was found to be 27µg/ml and Limit of quantification was found to be 92µg/ml.

Table: 1.24 Limit of Detection for KTT by proposed method

	LOD	Ketorolac		
	RT	Area	Height	S/N
	7.26	8788	813	3.25
	7.25	8442	909	3.42
	7.27	8964	839	3.30
AVG	10.26	8731	854	3.32
%RSD	0.09	3.04	5.82	2.63

Table: 1.25 Limit of Quantification for KTT by proposed method

	LOQ	Ketorolac		
	RT	Area	Height	S/N
	7.27	32807	2550	10.38
	7.29	33310	2504	9.78
	7.26	31371	2501	9.97
	7.25	35666	2595	10.07
	7.25	33300	2495	9.98
	7.26	33761	2564	10.27
AVG	7.26	33369	2529	10.04
%RSD	0.18	4.18	1.7	2.19

CONCLUSION:

A fast, simple and reliable analytical method for determination of KTT in pharmaceutical preparation using HPLC with UV detection. An analytical run takes about 7.25min. Separation of compounds is very fast, with good reproducibility and peak asymmetry. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision.

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