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DEVELOPMENT & VALIDATION OF A RP-HPLC METHOD FOR THE DETERMINATION OF KETOROLAC TROMETAMOL AND ITS IMPURITIES IN PHARMACEUTICAL DOSAGE FORM 30 MG/ML SOLUTION FOR INJECTION

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ABSTRACT

The analysis of established HPLC technique for the separation and quantification of Ketorolac trometamol and its impurities are described. Samples are analysed by reverse phase (RP-HPLC) using stationary phase Inert sustain (250 x 4.6mm, 5µm) column and the movable segment consisted of water, methanol and glacial acetic acid in the proportion of (49:50:1 %volume/volume/volume). The run velocity is 1.0 mL/min, the column oven was preserved at 40°C and sampler cooler oven was preserved 5°C, infused 10µL and wavelength fixed at 313nm UV-detection. The established HPLC technique was authenticated with admiration to specificity, precision, linearity, accuracy, LOD, LOQ and solution stability. Corroboration study compared as stated by ICH instruction.

Key words: Ketorolac trometamol, assessment of related substances, liquid chromatography.

1.0 Introduction

Ketorolac trometamol, chemically it is, 2-Amino-2-(hydroxymethyl)propane-1,3-diol-(1-RS)-5-benzoyl-2, 3-dihydro-1 H-pyrrolizine-1-carboxylate is a selective COX-I inhibitor, highly potent class of nonsteroidal anti-inflammatory drugs (NSAIDs) are used for short term treatment of post operative pain and local inflammation associated with musculoskeletal, joint disorders and some operative procedures. [1-5] When administered as eye drops it demonstrated analgesic, anti-histaminic, anti-inflammatory and anti-pyretic activity. The mechanism of action is to inhibit prostaglandin biosynthesis and given systemically does not cause pupil constriction.

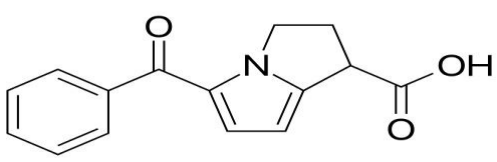

Fig No. 01- Chemical structure of Ketorolac trometamol

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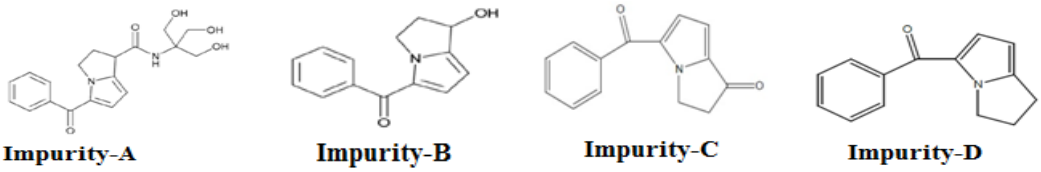

Fig No. 02- Chemical structures Ketorolac impurities

An all-embracing literature assessment was approved and established a few high performance liquid chromatographic (HPLC) techniques for the fortitude of Ketorolac trometamol in dissimilar biological fluids [6-12] and formulations [13-16] were reported for the impurity profile study of Ketorolac trometamol. Stability indicating and simultaneous determination methods [17-18] in human plasma and formulations were also reported. One HPTLC method [19] and an automatic fluorescence method [20] were also developed for the determination of Ketorolac trometamol.

The main objective of the proposed method is to develop a steadiness representative HPLC technique and authenticated by means of ICH and USP corroboration instructions for the inference of Ketorolac trometamol and its impurities in pharmaceutical dosage forms (Parenteral).

2.0 Experimental

2.1 Reagents and chemicals

Methanol, Glacial acetic acid was procured from Merck. Water (Milli-Q). All chemicals were of an analytical grade and used as received. Impurities are procured from SynZeal Research Private Limited, Ahmedabad, Gujarat. Ketorolac injection 30mg/mL (ALMAJECT INC) was procured from local market.

2.2 Instrumentation

Chromatographic partition was achieved by using an waters alliance e2695, Empower^3 software using an Inert sustain (250 x 4.6mm, 5µm) and the movable segment consisted of water, methanol and glacial acetic acid in the proportion of (49:50:1 %volume/volume/volume). The run velocity is 1.0 ml/min. The column oven was preserved at 40°C and sampler cooler oven was 5°C, infused 10µL and wavelength 313nm. The sprint instance was 40 minutes.

2.3 Preparation of solutions

Preparation of mobile phase

Prepared a mixture of 500 mL of methanol, 490 mL of water and 1 mL of glacial acetic acid in the ratio 50:49:1 (%v/v/v). Filtered through 0.45 µm casing sift and sonicated to degas.

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Preparation of diluent

Prepared a combination of 500 mL of irrigate and 500 mL of Carbinol in the proportion of 50:50 (%v/v), filtered through 0.45 µm membrane sift and sonicated to degas.

Preparation of standard stock solution

Weighed accurately 60.0 mg of Ketorolac trometamol working standard into a 50 mL volumetric thermos, added 35 mL of diluent sonicated for 2 minutes to dissolved, diluted to quantity with diluent and mixed well. Pipette out 2.0 mL of this solution into 50 mL volumetric thermos, made up to quantity with diluent and mixed well.

Preparation of standard solution

Further diluted 1.0 mL of standard stock solution keen on 20 mL volumetric thermos, made up to quantity with diluent and mixed well.

Preparation of sensitivity solution

Transferred 3.0 mL of standard solution into 20 mL volumetric thermos and diluted to quantity with diluent and mixed well.

Preparation of placebo solution

Transferred 2.0 mL of placebo solution into 50 mL volumetric thermos, added 35 mL diluent and shaked for 10 minutes to dissolve and diluted to quantity with diluent and mixed well

Preparation of test solution

Transferred 2.0 mL (30mg/mL) of sample solution into a 50 mL volumetric thermos, added 35 mL diluent and shaked for 10 minutes to dissolve and diluted to quantity with diluent and mixed well.

3.0 Method development

Method optimization parameters

An sympathetic of the character of API (functionality, acidity, or basicity), the synthetic procedure, related impurities, the possible degradation pathways and their degradation products are needed for successful method development in reverse-phase HPLC. In addition, successful method development should result a robust, simple and time efficient method that is capable of being utilized in manufacturing setting.

Selection of wavelength

The sensitivity of the HPLC method depends upon the selection of detection wavelength. An ideal wavelength is one that gives good response for related substances and the drugs to be detected. The wavelength for measurement was selected as 313 nm from the absorption spectrum.

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Selection of stationary phase

Proper selection of the stationary phase depends up on the nature of the sample and chemical profile. The drug selected for the present study was polar compound and could be separated either by normal phase chromatography or reverse phase chromatography. From literature survey, it was found that different C18 columns could be appropriately used for the separation of related substances for Ketorolac trometamol.

Selection of mobile phase

Different mobile phases are employed to develop a suitable LC method for the quantitative determination of impurities in Ketorolac trometamol, different mobile phase composition were tried to get good peak shapes and selectivity for the impurities present in Ketorolac trometamol.

4.0 Method validation

4.1 Specificity

Specificity was demonstrated by infused blank solution, placebo solution, standard solution, sample solution, spiked sample and creature impurities as well as scrutinized as stated by the test technique. It was scrutinized that identified impurities are not co eluting with apiece additional and foremost analyte crest.

Table no. 01- Impurity interference data (Specificity results)

S.No	Sample	Retention time (min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo	ND	NA	NA
3	Standard solution	21.652	No	No
4	Sample solution	21.63	No	No
5	Spiked sample solution	21.637	No	No
6	Impurity-A	13.883	No	No
7	Impurity-B	18.797	No	No
8	Impurity-C	8.366	No	No
9	Impurity-D	24.214	No	No

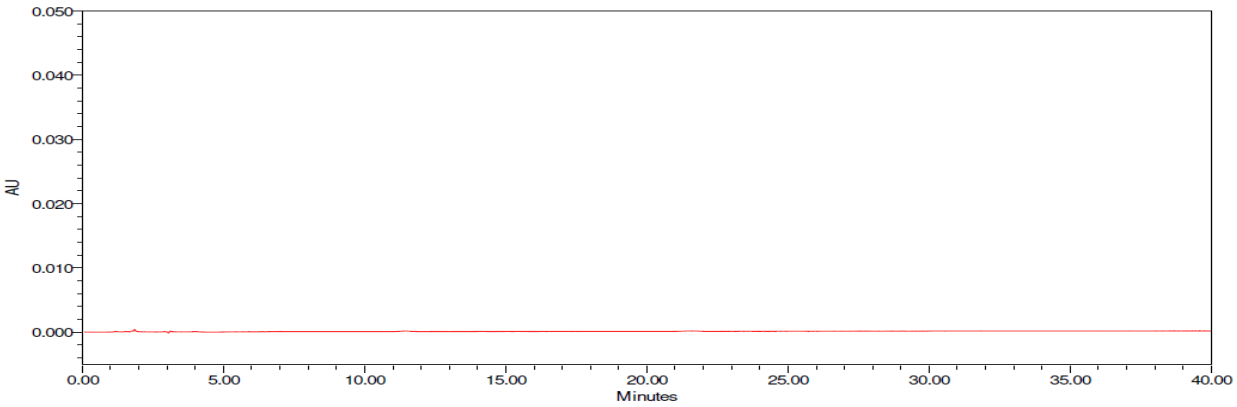

Fig No. 03- typical chromatogram of blank

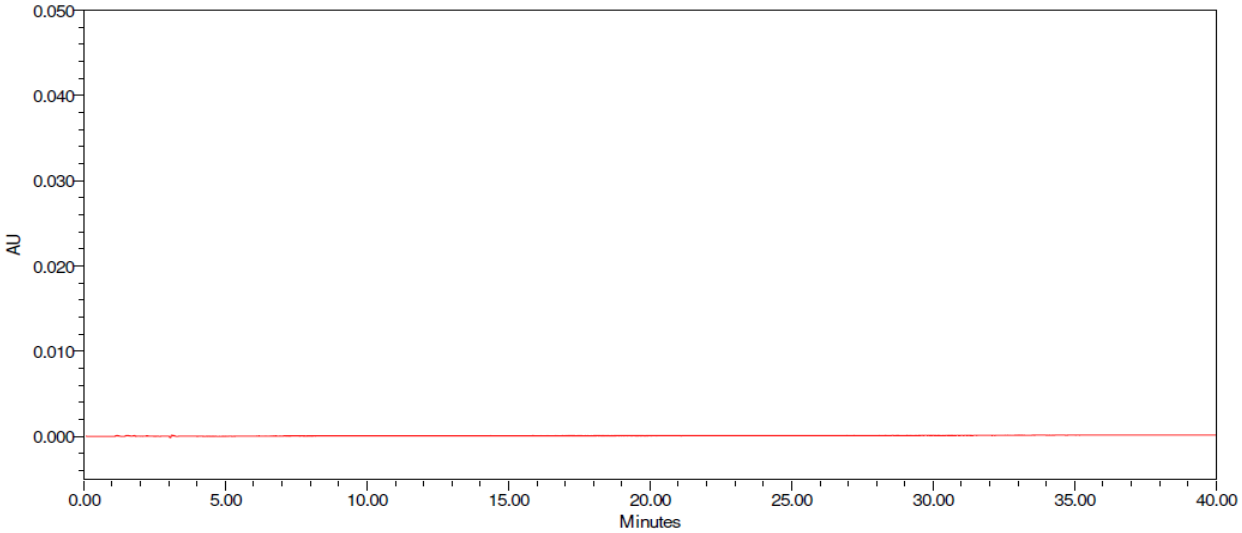

Fig No. 04- typical chromatogram of placebo

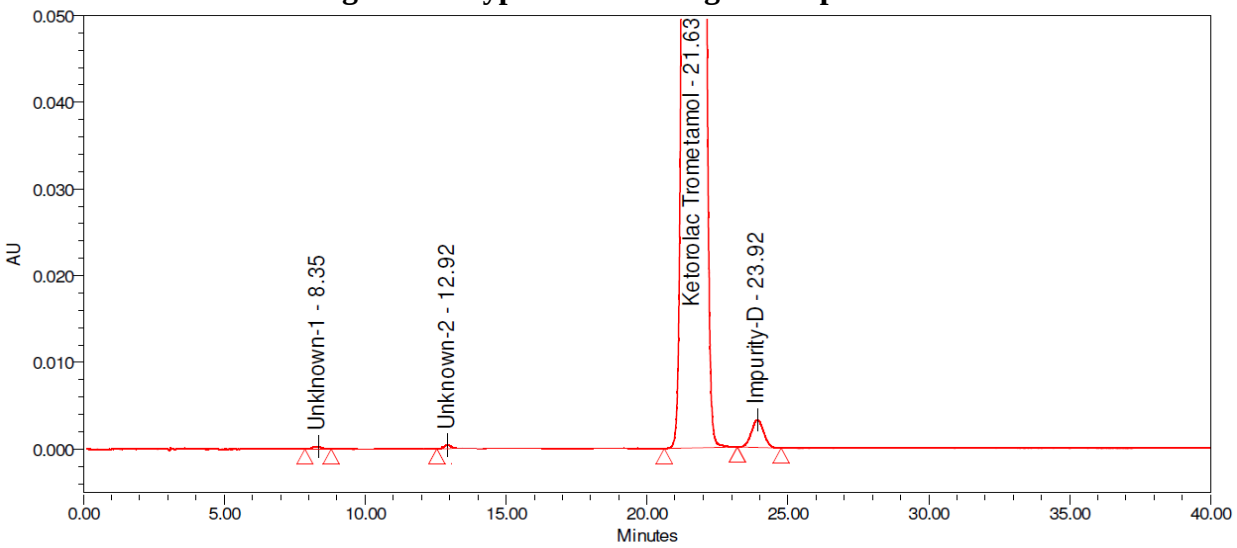

Fig No. 05- typical chromatogram sample

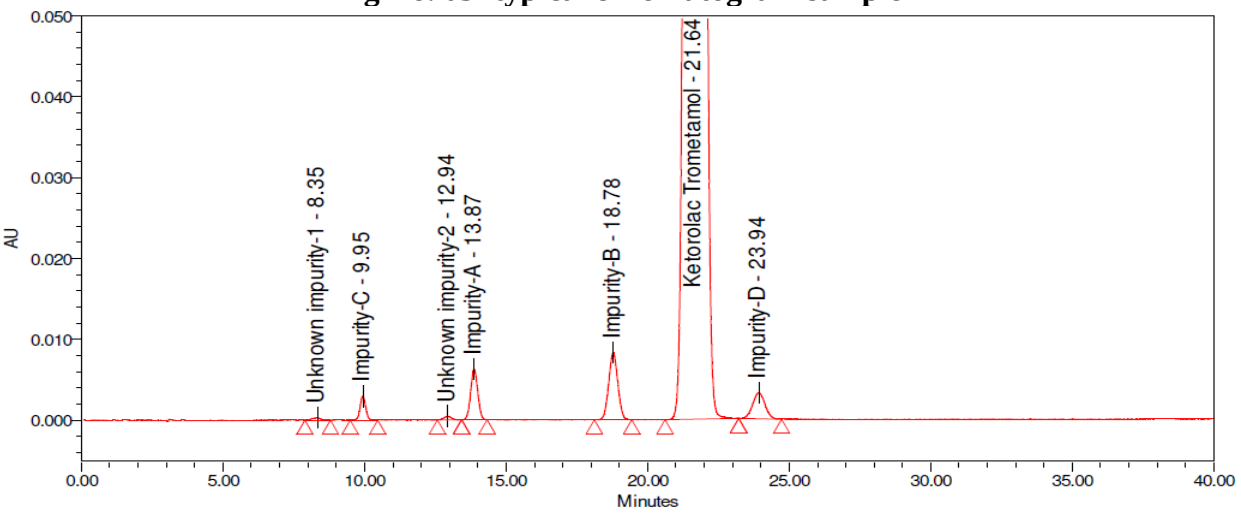

Fig No. 06- typical chromatogram spiked Sample

Forced degradation study

Sample solutions and placebo solutions were exposed to the following stress conditions to degradation. Stressed and unstressed samples were injected into the HPLC system with photo

diode array detector. All degrading peaks were resolved from Ketorolac trometamol peak in the chromatograms of all samples and placebo did not show any interference at the retention time of Ketorolac trometamol and impurities.

Table no. 02- Forced Degradation results

S.No	Name of the Solution	A%	B%	C%	D%	Total impurities (%)	Net Degradation (%)	Mass balance (%)
1	Unstressed Sample	ND	0.01	ND	0.01	0.04	NA	NA
2	Acid stress sample (2N HCl/5mL/60°C/2hrs)	ND	0.03	ND	0.01	3.10	3.06	99.86
3	Base stress sample (2N NaOH/5mL/60°C/2hrs)	ND	0.01	ND	0.01	0.11	0.07	100.87
4	Peroxide stress sample (30%H2O2/5mL//4.0Hrs @ RT)	ND	0.03	0.02	0.01	0.18	0.14	99.94
5	Water Stress sample (5mL /60°C/4hrs)	ND	0.01	ND	0.01	0.09	0.05	101.71
6	Thermal Stress sample (60°C/4hrs)	ND	0.01	ND	0.01	0.07	0.03	101.65

Ketorolac trometamol was sensitive to stress condition like acidic. The results proved that the developed method has good selectivity and specificity. Hence it is suitable for determination of impurities in Ketorolac trometamol liquid dosage form.

4.2 Precision

4.2.1 System exactitude

System exactitude was exhibited by organized standard solution as stated by the test technique and infused for six times keen on HPLC system. The preservation instance and vicinity rejoinder of analyte crest were recorded.

Table no. 03- System precision data for standard

S.No.	Area response
1	73578
2	74245
3	74479
4	73827
5	74509
6	74290
Average	74155
STDV	373.3977
% RSD	0.5

The %RSD of crest vicinity for Ketorolac trometamol standard was established 0.5% which is underneath 5.0% designates that the system gives precise result.

4.2.2 Method exactitude

Method exactitude was revealed by organized six samples by spiking of impurities at designed level and analyzed as stated by the test technique.

Table no. 04- Results of method precision

S.No.	Sample Details	Impurity (% Recovery)			
		Imp-A	Imp-B	Imp-C	Imp-D
1	Method Precision Spiked Prep-1	99.0	105.8	96.5	102.0
2	Method Precision Spiked Prep-2	98.1	104.9	96.1	101.0
3	Method Precision Spiked Prep-3	100.5	106.8	98.8	103.9
4	Method Precision Spiked Prep-4	99.5	104.7	98.7	101.9
5	Method Precision Spiked Prep-5	98.9	105.2	99.1	102.1
6	Method Precision Spiked Prep-6	100.1	103.9	98.9	100.9
Avg.		99.4	105.2	98.0	102.0
SD		0.8712	0.9948	1.3423	1.0801
%RSD		0.88	0.95	1.37	1.06

The consequences were well inside the limits. From the above consequences, it is concluded that technique is precise.

4.3 Limit of Quantitation (LOQ)

A solution containing 0.2448 µg/mL of Ketorolac trometamol, 0.1536 µg/mL of Impurity-A, 0.1548 µg/mL of impurity-B, 0.2328 µg/mL impurity-C, 0.2292 µg/mL impurity-D was injected six times. The %RSD areas of each impurity and standard were calculated.

Table no. 05- LOQ for Ketorolac trometamol and impurities

S.No.	Imp-A	Imp-B	Imp-C	Imp-D	Ketorolac trometamol
1	6883	10153	3189	8157	7083
2	6895	10039	2892	7915	7183
3	6738	10136	3127	8541	6519
4	6596	9815	3010	7737	6291
5	6799	9544	3065	8724	6730
6	7180	10171	2903	9007	6918
Avg.	6849	9976	3031	8347	6787
Std.Dev.	195.7864	249.5866	119.5676	491.9733	341.3741
%RSD	2.9	2.5	3.9	5.9	5.0

The limit of quantitation values obtained for each impurity and Ketorolac trometamol are within the acceptance criteria.

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4.4 Linearity

The linearity of detector rejoinder for Ketorolac trometamol and its impurities was demonstrated by preparing solutions over the range of 0.1% level to 1.0 % level of target concentration level. A plot of concentration vs. area response of peak was done. The correlation co-efficient between concentration and area response was evaluated.

Table no. 06- Linearity solution preparation for impurity-A

S.No.	Linearity Level	Concentration in ppm	Area response
1	LOQ	0.15	6849
2	25	0.62	26478
3	50	1.23	52956
4	100	2.41	103829
5	125	3.08	133605
6	150	3.72	158299
Correlation coefficient (r^2)			0.9998
Slope			42,796.5330
Intercept			377.8108
%Y-Intercept			0.36

Fig No. 07- Linearity graph of impurity-A

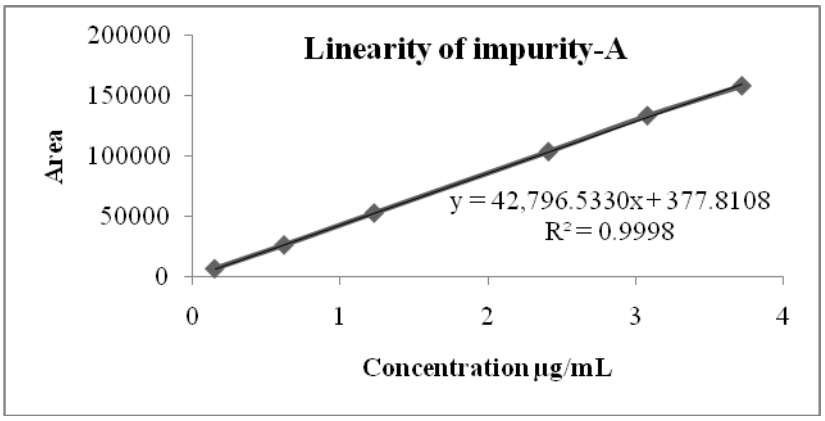

Table no. 07- Linearity solution preparation for impurity-B

S.No.	Linearity Level	Concentration in ppm	Area response
1	LOQ	0.15	9976
2	25	0.62	42690
3	50	1.23	85380
4	100	2.41	170760
5	125	3.08	213450
6	150	3.72	256140
Correlation coefficient (r^2)			0.9996
Slope			69,337.6862
Intercept			71.1934
%Y-Intercept			0.04

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Fig No. 08- Linearity graph of impurity-B

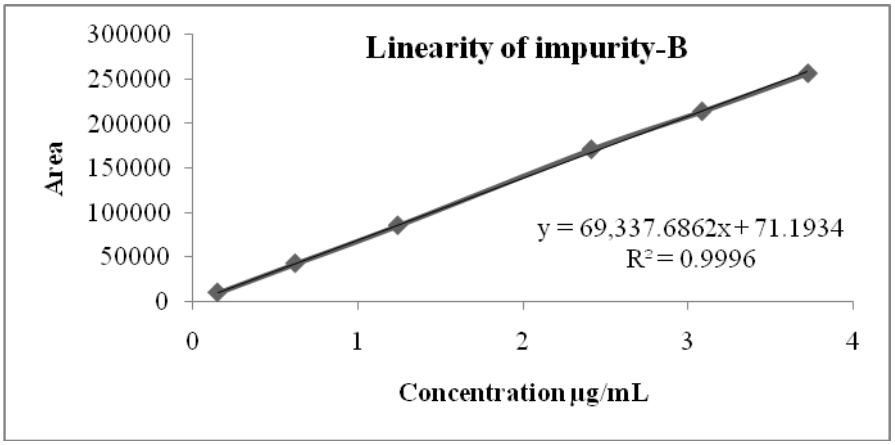

Table no. 08- Linearity solution preparation for impurity-C

S.No.	Linearity Level	Concentration in ppm	Area response
1	LOQ	0.15	3031
2	25	0.62	9682
3	50	1.23	19363
4	100	2.41	38726
5	125	3.08	48408
6	150	3.72	58089
Correlation coefficient (r^2)			0.9996
Slope			15593.8847
Intercept			389.2689
%Y-Intercept			1.01

Fig No. 09- Linearity graph of impurity-C

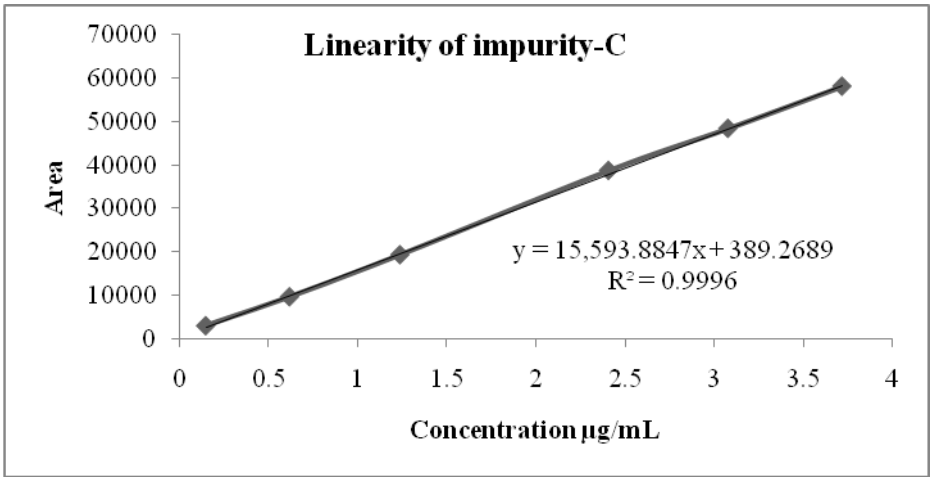

Table no. 09- Linearity solution preparation for impurity-D

S.No.	Linearity Level	Concentration in ppm	Area response
1	LOQ	0.15	8347
2	25	0.62	22790
3	50	1.23	45580

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4	100	2.41	91160
5	125	3.08	113950
6	150	3.72	136740
Correlation coefficient (r^2)			0.9994
Slope			36501.1054
Intercept			1504.0996
%Y-Intercept			1.65

Fig No. 10- Linearity graph of impurity-D

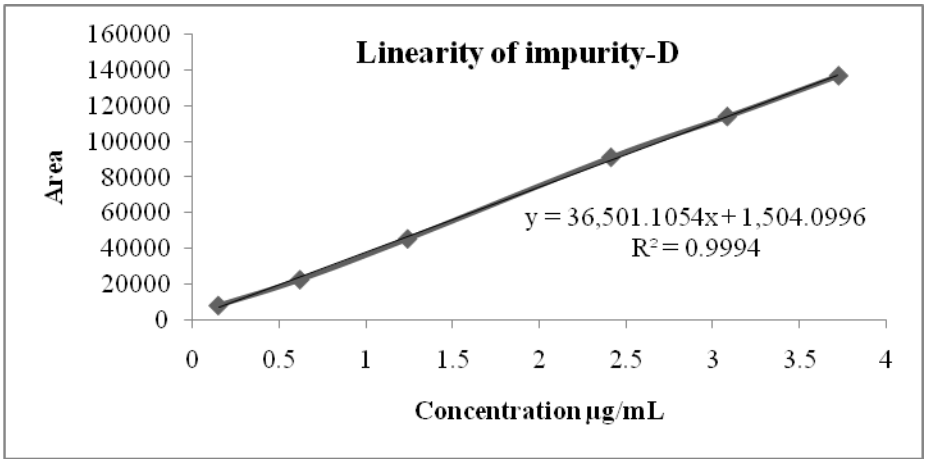
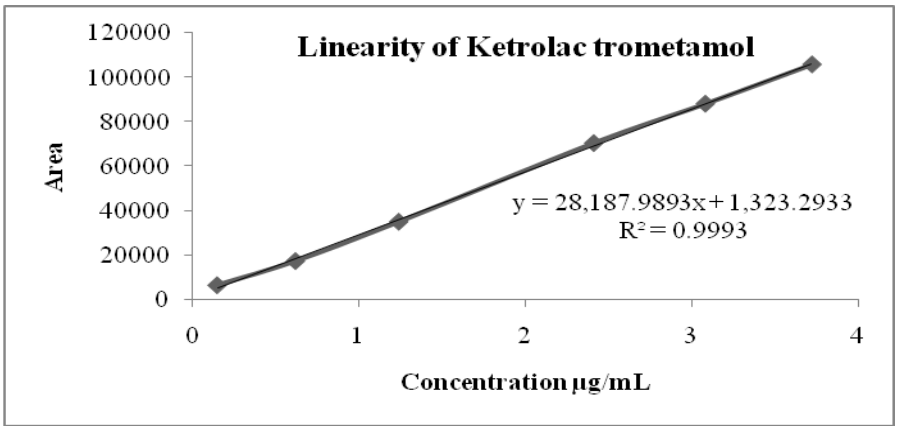


Table no. 10- Linearity solution preparation for Ketorolac trometamol

S.No.	Linearity Level	Concentration in ppm	Area response
1	LOQ	0.15	6787
2	25	0.62	17635
3	50	1.23	35269
4	100	2.41	70538
5	125	3.08	88173
6	150	3.72	105807
Correlation coefficient (r^2)			0.9993
Slope			28187.9893
Intercept			1323.2933
%Y-Intercept			1.88

Fig No. 11- Linearity graph of Ketorolac trometamol



The linearity results for Ketorolac trometamol and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.

4.5 Accuracy

Recovery of Ketorolac trometamol impurities in Ketorolac trometamol liquid dosage formulation was performed. The sample was taken and varying amounts of Ketorolac trometamol impurities spiking at LOQ level to 150 % of specification level were added to the flasks.

Table no. 11- Accuracy study of Ketorolac trometamol

S.No.	Theoretical (%)	% Mean Recovery			
		Impurity-A	Impurity-B	Impurity-C	Impurity-D
1	LOQ	100.0	92.3	110.5	94.7
2	50	94.2	106.8	90.7	99.0
3	100	94.2	101.0	92.6	97.0
4	150	97.4	102.6	95.6	98.7

Accuracy at LOQ level to 150% level for impurity-A, impurity-B, impurity-C and impurity-D is meeting the acceptance criteria. From the above results, it is concluded that method is accurate.

4.6 Solution stability

Stability of solutions such as standard solution and sample solutions was established at various conditions such as bench top and in refrigerator (2-8°C). The response of these was compared with respect initial standard solution and spiked sample solution.

Solution stability parameter was established, standard solution were stable upto 48 hrs on bench top in refrigerator and sample solutions were stable upto 24 Hours on bench top and 36 hrs in refrigerator condition.

5.0 Results & Discussion

A uncomplicated, fiscal, accurate and precise HPLC technique was productively urbanized. In this technique it was carried out by using stationary phase Inert sustain (250 x 4.6mm, 5µm) column and the movable segment consisted of irrigate, Carbinol and glacial acetic acid in the proportion of (49:50:1 %volume/volume/volume). The run velocity is 1.0 ml/min, the column oven was preserved at 40°C and sampler cooler oven was preserved 5°C, infused 10µL and wavelength 313nm. The consequences obtained were accurate and reproducible. The technique urbanized was statistically authenticated in terms of selectivity, accuracy, linearity, precision and stability of solution.

For selectivity, the chromatograms were recorded for standard, sample and spiked sample solutions of Ketorolac trometamol and its related substances. Selectivity studies reveal that the peaks are well separated from each other. Therefore the method is selective for the determination

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of related substances in Ketorolac trometamol. There is no interference of blank and placebo at Ketorolac trometamol and impurities peaks. The elution order and the retention times of impurities and Ketorolac trometamol obtained from individual standard preparations and mixed standard preparations are comparable.

The limit of quantitation (LOQ) for 0.2448 µg/mL of Ketorolac trometamol, 0.1536 µg/mL of impurity-A, 0.1548 µg/mL of impurity-B, 0.2328 µg/mL impurity-C, 0.2292 µg/mL impurity-D respectively.

The linearity results for Ketorolac trometamol and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.

The accuracy studies were shown as % recovery for Ketorolac trometamol and its impurities at specification level. The limit of % recovered shown is in the range of LOQ and 150% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

For precision studies six replicate injections were performed. %RSD was determined from the peak areas of Ketorolac trometamol and its impurities. The acceptance limit should be not more than 5.0, and the results were found to be within the acceptance limits.

Solution stability parameter was established, standard solution were stable upto 48 hrs on bench top in refrigerator and sample solutions were stable upto 24 Hours on bench top and 36 hrs in refrigerator condition.

6.0 Conclusion

The new-fangled HPLC method developed and validated for determination of Ketorolac trometamol pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of drug in its liquid dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control.

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Conflict of interests

The authors claim that there is no conflict of interest.

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