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Research Article

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Development and validation of a headspace gas chromatographic method for the determination of ethyl alcohol in ketorolac tromethamine injection

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ABSTRACT

A simple, sensitive, accurate, precise and repeatable HS-GC method was developed for the determination of ethyl alcohol content in Ketorolac Tromethamine injection USP.Head space Gas chromatography was performed on Agilent GC with FID detector using Helium as carrier gas at 5.0 ml/min flow with DB-Wax column (30 m x 0.53 mm ID, film thickness 1µ). The developed method was validated and parameters were found to be within the limits of USP. The retention time for ethyl alcohol and 1-propanol individually and in spiked standard solution was determined.The linearity was observed in the concentration range of 25% to 150%.Correlation coefficient was 0.9999 and Y-Intercept was 0.000734. The relative standard deviation of six replicates for intraday was 1.2 % and Inter day precision was 3.1 % were found to be within the limit.The % recovery for ethanol ranged from 98.1% to 103.6% was found to be within the limit. Hence the proposed method was simple, specific, precise and accurate for the determination of ethyl alcohol content in Ketorolac Tromethamine injection.

Key words: Ketorolac Tromethamine, HS-GC method, Intrnal standard

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INTRODUCTION

Ketorolac Tromethamine Injection, USP is a member of the pyrrolo-pyrrole group of Non-Steroidal Anti-inflammatory Drugs (NSAIDs). The chemical name for Ketorolac Tromethamine is (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, compound with 2-amino-2-(hydroxymethyl)-1,3-propanediol (1:1). **Fig 1** shows the structure of Ketorolac Tromethamine.

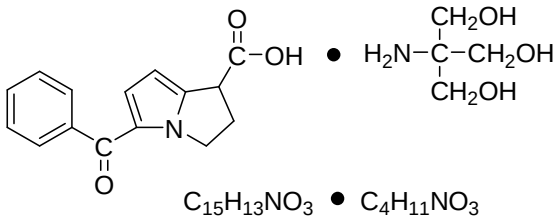

Fig 1: Structure of Ketorolac Tromethamine

Ketorolac Tromethamine is a racemic mixture of [-]S and [+]R Ketorolac Tromethamine.It is used for the short-term (≤5 days) management of moderately severe acute pain that requires analgesia at the opioid level, usually in a postoperative setting [1-4].

Ethyl alcohol is organic volatile solvent it is used in the manufacture of drug substances or excipients or in the preparation of drug products. An addition of ethyl alcohol also may be applied to increase solubility of some ingredients which are less soluble in water [5], Since there is no therapeutic benefit from it, it should be removed completely or should be within the limit [6]. As ethyl alcohol is class 3 solvent, as per USP Should not cross 50 mg per day [7] (corresponding to 5000 ppm or 0.5% when the daily dose does not exceed 10 gms).

EXPERIMENTAL SECTION

Chemicals and Materials: Ethyl alcohol and 1-Propanol obtained from Sigma-Aldrich. Ketorolac Tromethamine injection USP available commercially with brand name Torolac injection from Lupin Laboratories Limited with a label claim of 30 mg/ml.All chemicals were GC grade and used as received. HPLC grade water (Milli-Q water) was used as diluent.

Instrumentation: The analysis was performed on Agilent gas chromatograph equipped with head space sampler and a flame ionization detector (FID).

Chromatographic conditions: Column used was DB-Wax column (30 m Length x 0.53mm ID), film thickness 1µ, column temperature is 40°C for 4.5 minutes, Ramp 60°C/minute to 220°C,Hold time 2 minutes. Carrier gas is helium with flow rate 5.0 ml/min. Hydrogen flow set as 45.0 ml/min and air flow rate is 450.0 ml/min; make up flow (Helium) 25.0 ml/min. Split ratio 1:5, Injector temperature is 150°C and detector temperature is 260°C, total run time 9.5 min.

Head space parameters: Vial temperature, needle temperature and transfer line temperatures are 90°C, 110°C, 130°C respectively. Vial equilibration time, pressurization time and loop filling time are 12.0 mins, 3.0 mins, 0.5 mins respectively. Loop equilibration time,injection time and GC cycle times are 0.2 mins, 0.08 mins, 15.0 mins respectively.

Preparation of standard and sample vials:
Internal standard: Pipetted 2 ml of 1-Propanol into a 200 ml volumetric flask, made up to volume with diluent and mixed well.

Standard solution: Weighed about 745 mg of ethyl alcohol into a 50 ml volumetric flask, made up to volume with diluent and mixed well. Pipetted 10 ml each of above solution and the internal standard solution into a 50 ml volumetric flask, made up to volume with diluent and mixed well.

Sample solution and placebo solution: Pipetted 1.6 ml of the sample into a 50 ml volumetric flask added 10 ml of internal standard and made up to 50 ml with diluent and mixed well. Placebo solution prepared by taking 1.6 ml of placebo and 10 ml of internal standard and made up to 50 ml with diluent.

Procedure: Taken all the prepared solutions each 5 ml in to each 20 ml head space vial and sealed immediately with crimp cap.

METHOD VALIDATION

The Proposed method was validated for different parameters like specificity, precision, linearity, accuracy and robustness as per ICH guidelines and these are key parameters according to US-FDA [8, 9].

RESULTS AND DISCUSSION

Specificity: The specificity of the method was evaluated by injecting blank, Ethyl alcohol(3000 ppm) and 1-propanol individually at specification level into the GC system. The specified solvents have separated from one another and also from blank (**Table.1**). and (**Fig. 2 to 6**).

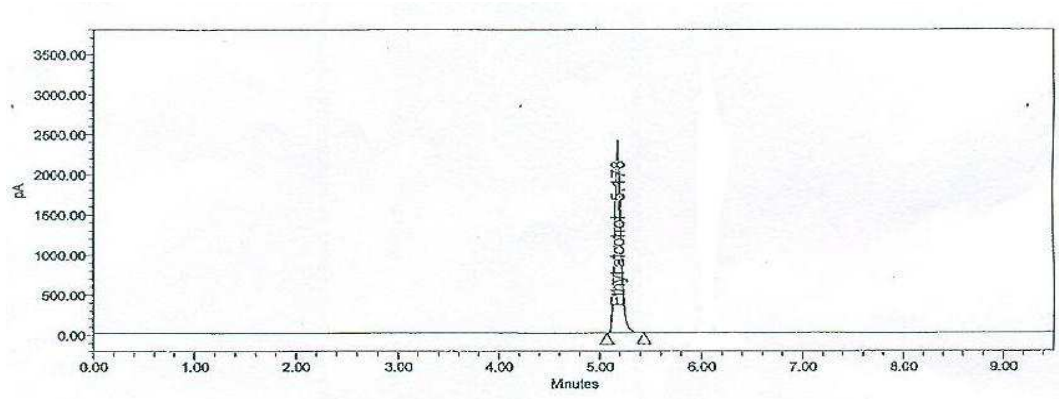

Fig 2: Chromatogram of Blank Solution

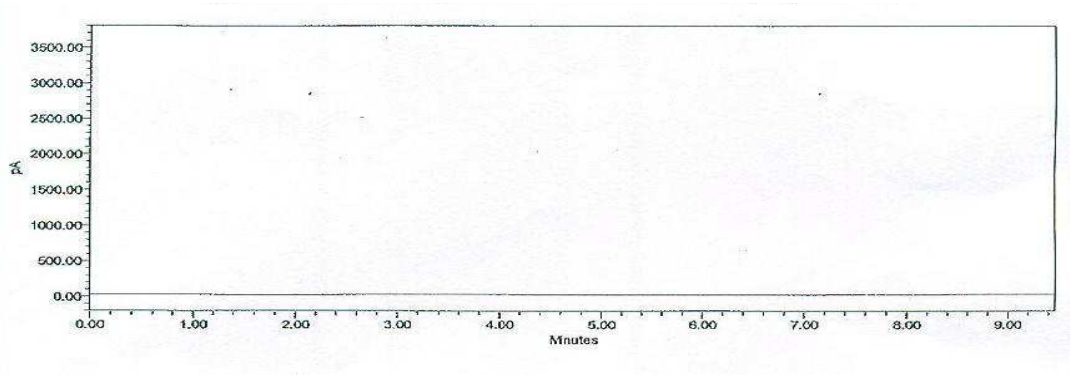

Fig 3: Chromatogram of Ethyl alcohol Individual Solution

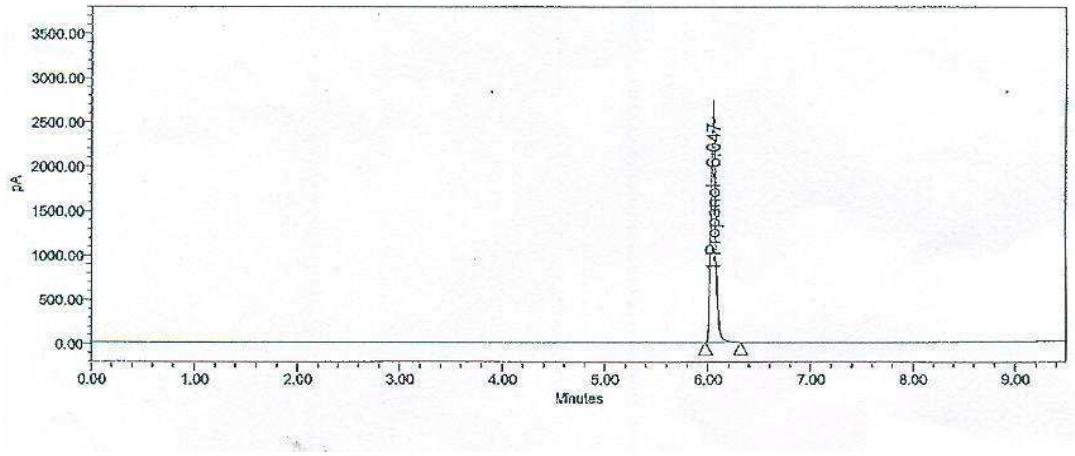

Fig 4: Chromatogram of Placebo Solution (with Internal Standard)

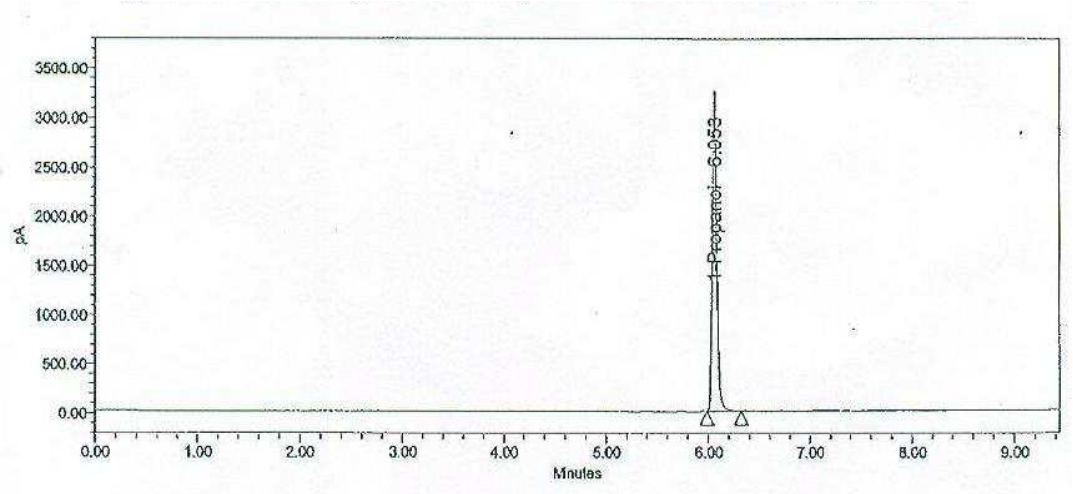

Fig 5: Chromatogram of Internal Standard Solution

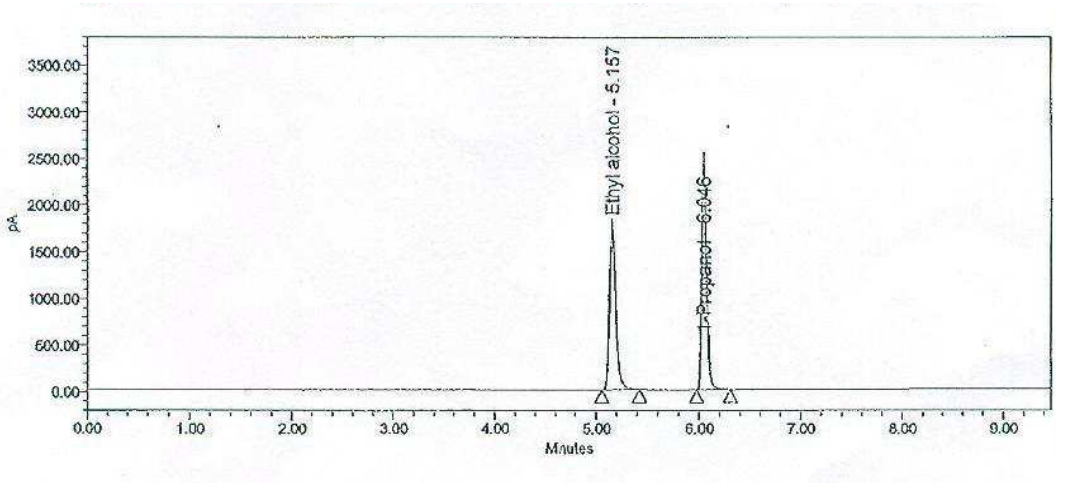

Fig 6: Chromatogram of Ethyl alcohol Spiked Sample Ssolution

TABLE 1: Specificity

S.No	Sample name	Name of the solvent	RT
1.	Individual Ethyl alcohol solution	Ethyl alcohol	5.178
2.	Individual 1-propanol solution (Internal standard)	1-propanol	6.053
3.	Spiked sample	Ethyl alcohol	5.157
4.	Spiked sample	1-propanol	6.046
5.	Placebo solution	No (no peak at the RT of Ethyl alcohol)	No (no Interference)

Precision: The precision of test method was evaluated by preparing and injecting six samples of same batch of Ketorolac Tromethamine injection as per test method. Chromatogram of ethyl alcohol standard solution shown in the **Fig 7.** Peak areas ratio of ethyl alcohol and 1-propanol was used to calculate the % of ethyl alcohol in all the calculations. The % RSD of ethyl alcohol (%) present in sample was 0.5 intermediate precision (ruggedness) of the method was also evaluated on different day with different column. The % RSD for Intermediate precision was 2.0. The results of method precision and intermediate preceision were given in **Table 2.**

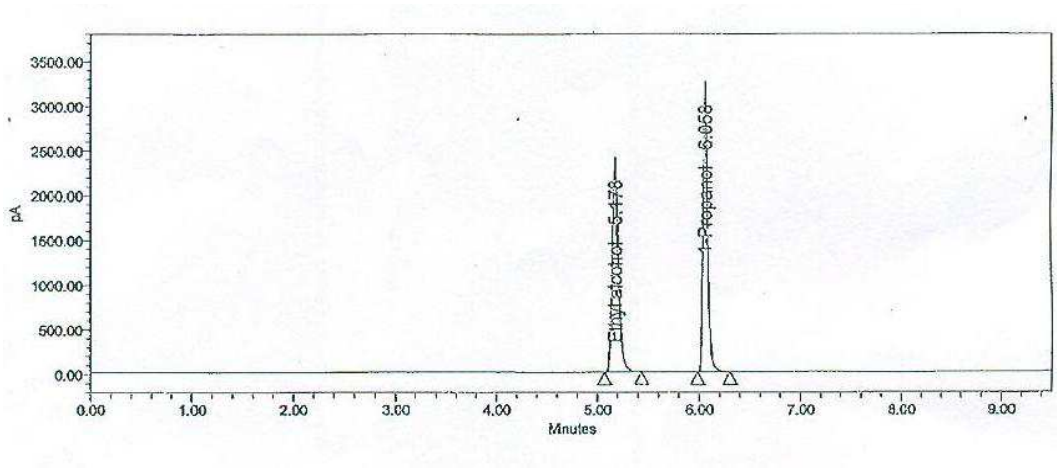

Fig 7: Chromatogram of Ethyl alcohol Standard Solution

TABLE 2: Results of Method Precision and Intermediate Precision

S.No	Ethyl alcohol(%)	
	Method precision	Intermediate precision
1	97.7	98.6
2	98.4	98.8
3	97.7	96.0
4	97.8	95.4
5	98.0	99.7
6	98.8	95.5
Average	98.1	97.3
% RSD	0.5	2.0

Linearity: The linearity of detector response was evaluated by preparing a series of solutions from 25% to 150% (i.e 25%, 50%, 75%, 100%, 125% & 150%) of the target concentration and injected into GC system. Plotted the graph with concentration in mg/ml on X-axis and area ratios on Y-axis. The correlation coefficient for ethyl alcohol was 0.99999965. Results of linearity of detector response were given in **Table 3** and **Fig 8.**

TABLE 3: Results of Linearity of Detector Response

S.No.	Ethyl alcohol	
	Concentration(µg/ml)	Peak area ratio
25%	739.2520	0.2656
50%	1478.5040	0.5306
75%	2217.7560	0.7949
100%	2957.0080	1.0602
125%	3696.2600	1.3258
150%	4583.3624	1.6425
Slope	0.000358	
Y-Intercept	0.000734	
Correlation coefficient	0.99999965	
% Y intercept (relative to 100% std)	0.0692%	

Linearity graph.

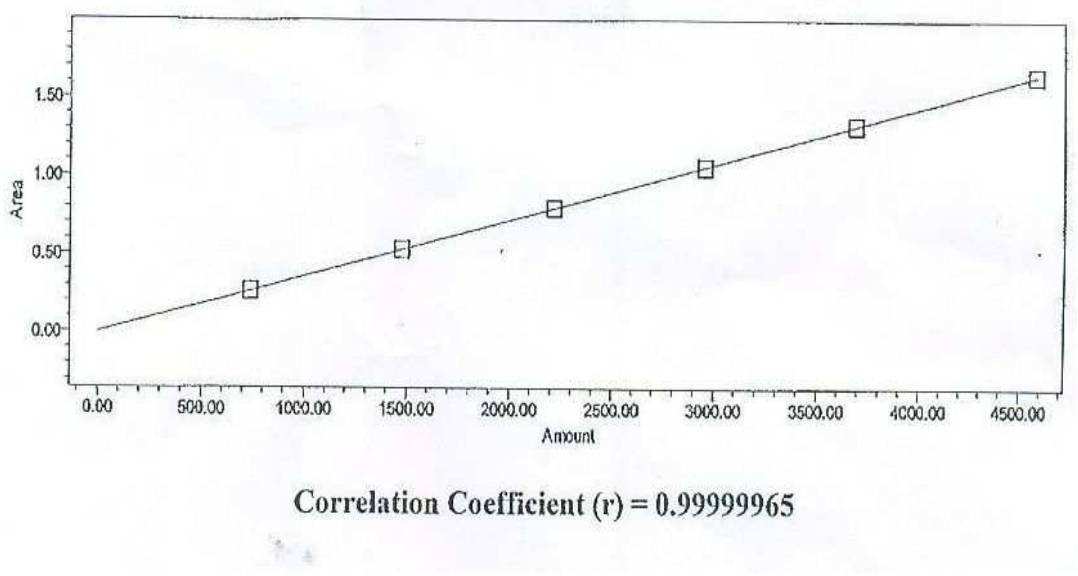

Fig 8: Chromatogram of Ethyl alcohol Standard Solution

Accuracy and Linearity of Test Method: The accuracy and linearityof test method for ethyl alcohol was established by preparing the test solution in triplicate by spiking the ethyl alcohol into the placebo of Ketorolac Tromethamine injection as per the test method at 25% (six times), 50%, 75%, 100%, 125% and six times at 150% of 3000 ppm and injected into the GC system. Plotted the graph with concentration (µg/ml) addedon X-axis and concentration (µg/ml) fond on Y-axis. The % recovery, correlation coefficient and % RSD of ethyl alcohol, recovery for six preparations of 25% and 150% accuracy level was found to be within the limits. Results for accuracy and linearity of test method results were shown in **Table 4, 5, 6 and Fig 9.**

TABLE:4 Results for Accuracy of Ethyl Alcohol

Recovery Level	Preparation	µg/ml Added	µg/ml recovered	(%) recovery	Average (%) Recovery
25%	1	750.972	732.537	97.5	98.1
	2		738.468	98.3	
	3		738.738	98.4	
50%	1	1501.945	1506.594	101.0	101.0
	2		1517.109	101.6	
	3		1525.736	102.5	
75%	1	2252.917	2308.151	102.5	101.4
	2		2276.337	101.0	
	3		2270.136	100.8	
100%	1	3003.889	3079.512	102.5	101.6
	2		3021.546	100.6	
	3		3051.203	101.6	
125%	1	3754.861	3889.158	103.6	103.6
	2		3847.907	102.5	
	3		3932.026	104.7	
150%	1	4505.834	4523.826	100.4	102.4
	2		4713.363	104.6	
	3		4609.023	102.3	

TABLE: 5 Results for Precision at Lower And Heigher Level

Solvent name	Ethyl alcohol	Ethyl alcohol
Concentration(%)	Accuracy at (25%)	Accuracy at (150%)
S.No	% Recovery	% Recovery
1	97.5	100.4
2	98.3	104.6
3	98.4	102.3
4	100.3	106.6
5	100.2	105.2
6	100.9	104.8
Average	99.3	104.0
%RSD	1.4	2.2

TABLE: 6 Results For Precision at Lower and Heigher Level

Recovery level	Ethyl alcohol	
	Concn (µg/ml)	Concn (µg/ml)
25%	750.972	745.523
50%	1501.945	1516.480
75%	2252.917	2284.875
100%	3003.889	3050.754
125%	3754.861	3889.697
150%	4505.834	4685.189
Slope	1.049453	
Y-Intercept	62.966333	
Correlation Coefficient	0.999875	

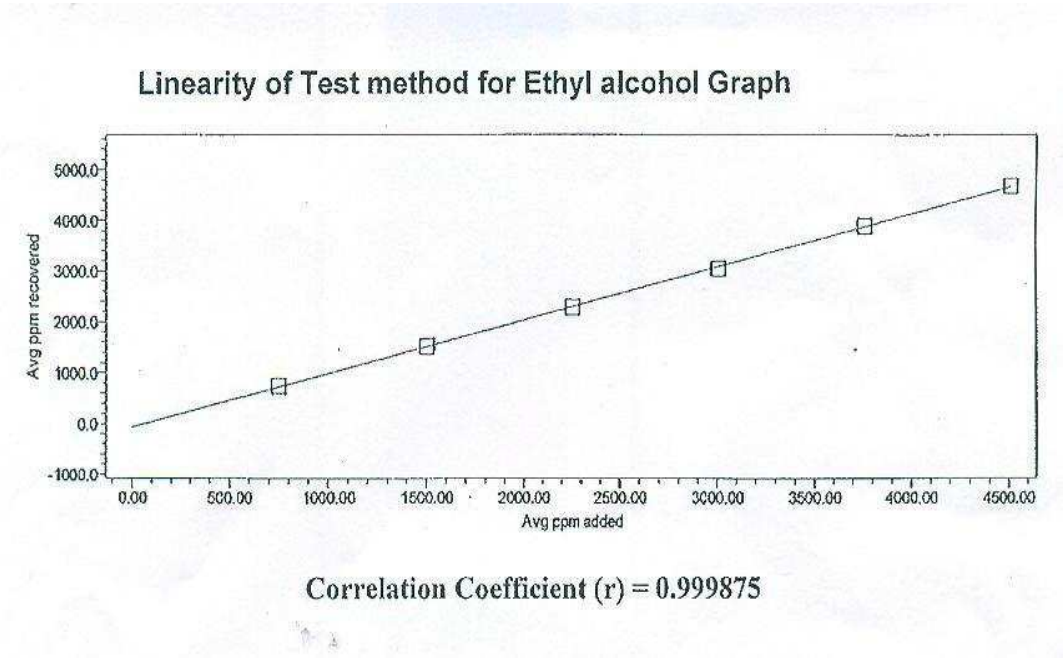

Fig 9: Linearity of Test method for Ethyl alcohol

Robustness: Robustness of a method was defined as a measure of its capacity to remain unaffected by small but deliberate changes in method parameters and provides an indication of its reliability during normal usage [10]. To determine the robustness parameters were varied column oven temperature, injector temperature, thermostating time. Prepared and injected standard solution and test solutions with varied conditions. The system suitability and RRT of ethyl alcohol were evaluated.

TABLE:7 Results For Robustness

Variation in condition		Resolution between Ethyl alcohol and 1- Propanol	%RSD from six replicates of standard solution	RRT for Ethyl alcohol
Coumn oven temperature	50°C(+ change)	10.4	1.9	0.74
	40°C(control)	9.5	2.7	0.85
	30°C(-change)	8.7	2.2	0.89
Injector temperature	155°C(+ change)	9.4	1.7	0.85
	150°C(control)	9.5	2.7	0.85
	145°C(-change)	9.5	1.3	0.85
Thermostating time	13.2min(+ change)	6.6	1.7	0.85
	12 min(control)	6.9	3.1	0.85
	10.8min(-change)	6.6	2.3	0.85

Acceptance limit for resolution is not less than 4.0,for%RSD from six replicates of standard solution is not more than 5.0 (fixed as per USP and ICH guidelines) [11].

Range of test method: The specified range is normally derived from linearity studies and depends on the intended application of the procedure [12]. Based on linearity, precision and accuracy data it was concluded that the range of the test method is from 25% to 150% level.

CONCLUSION

The developed GC–Head space method was found to be precise, linear, specific, robust and accurate for the determination of ethyl alcohol content in Ketorolac Tromethamine injection. Hence the validated method can be used for the routine determination and quantification of ethyl alcohol in Ketorolac Tromethamine for injection formulation in quality control laboratories in pharmaceutical industry.

REFERENCES

[1] Ketorolac Tromethamine injection http://www.drugs.com/monograph/keterolac-tromethamine.html
[2] JEF Reynolds; Martindale, The extra pharmacopoeia., Pharmaceutical press, London, **2009,** 36, 74.
[3] SN Razzq; I Mariam; IU Khan; I Ashaq, *J.Liq.chrom.rel.Tech.*, **2012**, 35, 651-661.
[4] J Squella; I Lemus; J Sturm; L Nunezvergara, *Analytical letters.*, **1997**, 30, 553-564.
[5] Elzbieta Huzar; Alicja Wodnicka, *Acta Poloniae Pharmaceutica- research.*, **2013**, 70, 41-49.
[6] Praveen Kumar Baliyan; RP Singh; Surabh Arora, *Asian journal of chemistry.*, **2009**, 21, 1739-1746.
[7] ICH, International conference on harmonization, February **2011**, Guideline for residual solvents Q3C(R5).
[8] FDA, Food and drug administration, Centre of drug evaluation and research, **1994**, Reviewer guidance validation of chromatographic methods.
[9] ICH, International conference on harmonization, October **1994**, Text on validation of analytical procedures Q2A.
[10]ICH, International conference on harmonization, October **1994**, Harmonised tripartite guideline, Text and methodology on validation of analytical procedures Q2(R1).
[11]United states pharmacopoeia (USP), **2006**, 1517-1578.
[12]ClecioS Ramos, *International journal of pharmaceutical Science Iinvention* , **2013**, 2, 2319.