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Research Article

A new analytical method development and validation for the simultaneus estimation of ibuprofen and tramadol using RP-HPLC

K.Kranthi Kiran, K.Saritha, K.Maryrani, L.Thomas, Santhikumari

Assoc. Professor & Jogaiah institute of Technology & Sciences College of Pharmacy, Kalagampudi. A.P India

Corresponding Author: K.Kranthi Kiran

Email: kothapallikranthikiran@gmail.com

ABSTRACT

A simple and selective LC method is described for the determination of Ibuprofen and Tramadol in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 60 volumes of Triethylamine buffer, 40 volumes of acetonitrile with detection of 227 nm. Linearity was observed in the range 50-150 µg/ml for Ibuprofen (r^2 =0.983) and 50-150 µg /ml for Tramadol (r^2 =0.985) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Keywords: Liquid chromatography (LC), RSD Relative standard deviation, R^2 correlation coefficient, Ibuprofen and tramadol**,** Reverse phase HPLC.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books [1-3].

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination [4-6], quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis. Qualitative analysis is performed to establish composition of a substance.

It is done to determine the presence of a compound or substance in a given sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half-life etc. [7-10].

AIM AND PLAN OF WORK

Aim

To develop new RP HPLC method for the simultaneous estimation of Ibuprofen and Tramadol pharmaceutical dosage form.

PLAN OF WORK

Solubility determination of Ibuprofen and Tramadol various solvents and buffers.

- Determine the absorption maxima of both the drugs in UV–Visible region in different solvents/buffers and selecting the solvents for HPLC method development.
- Optimize the mobile phase and flow rates for proper resolution and retention times.
- Validate the developed method as per ICH guidelines.

METHODOLOGY

A mixture of Triethylamine buffer (pH): ACN were prepared. The mobile phase was sonicated for 10min to remove gases and filtered through 0.45µ membrane filter for degassing of mobile phase.

Determination of Working Wavelength (λmax)

In estimation of drug wavelength maxima is used.. So this wavelength is used in estimation to estimate drug accurately.

Determination Of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of IBUPROFEN

100 mg of IBUPROFEN was weighed and transferred in to 100ml volumetric flask and dissolved in water and then make up to the mark with water and prepare 100 µg /ml of solution by diluting 1ml to 10ml with water.

Preparation of standard stock solution of TRAMADOL

100 mg of TRAMADOL was weighed in to 100ml volumetric flask and dissolved in water and then dilute up to the mark with water and prepare 100 µg /ml of solution by diluting 1ml to 10ml with water.

RESULTS AND DISCUSSIONS

Solubility Studies

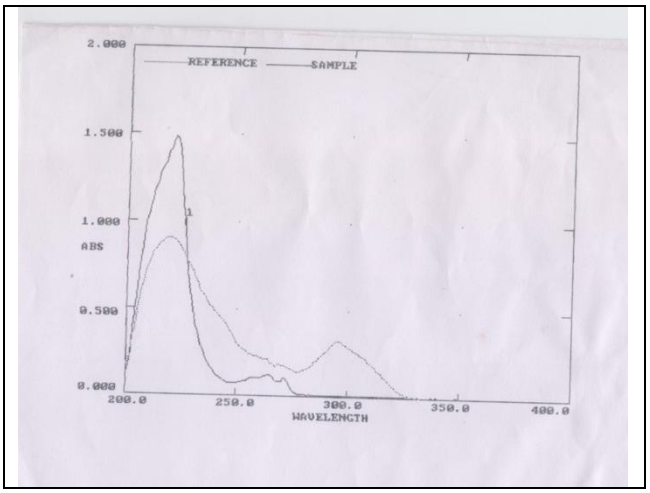
These studies are carried out at 25 ^{0}C

Ibuprofen

Freely soluble in ethanol and methanol, and slightly soluble in acetone and isopropanol and very slightly soluble in water.

Tramadol

Freely soluble in methanol and water.



RESULTS

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig. no. 8.1, and the isobestic point was found to be 227 nm for the combination.

ISOBESTIC POINT OF IBUPROFEN AND TRAMADOL METHOD DEVELOPMENT OF IBUPROFEN AND TRAMADOL

Trial- 1

Chromatographic conditions

Mobile phase : Triethylamine buffer (pH): ACN

pH : 4.0
Ratio : 60:40
Column : Inertsil ODS, (250×4.6× 5µ)
Wavelength : 227 nm
Flow rate : 1ml/min

Preparation of mixed standard solution

Weigh accurately 10 mg of IBUPROFEN and TRAMADOL in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of IBUPROFEN and TRAMADOL is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

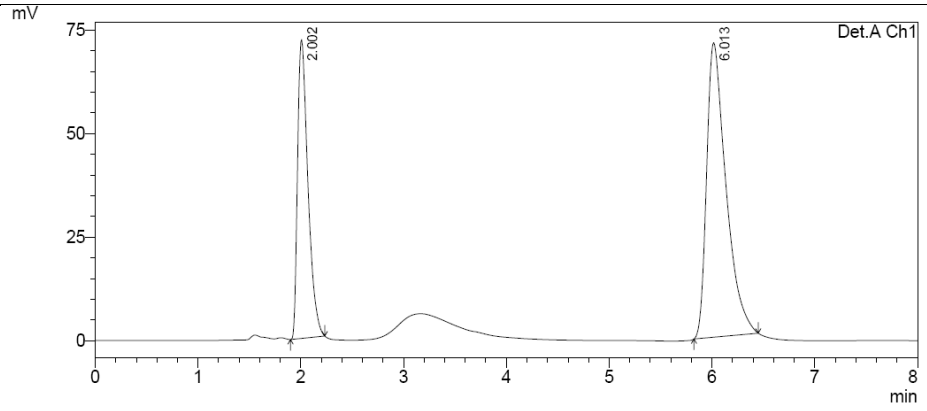


Fig. 8.3.5: Chromatogram of TRAMADOL and IBUPROFEN by using mobile phase

Observation

- All the system suitability requirements were met.
- The peak Asymmetry factor was less than 2 for both TRAMADOL and IBUPROFEN.
- The efficiency was more than 2000 TRAMADOL and IBUPROFEN.
- Resolution between two peaks >1.5.
- The details are given in the figure 8.3.8; hence this method was for optimized.

Table 8.3.8: Optimized chromatographic conditions

Mobile phase	Triethylamine buffer (pH):ACN (60:40)
Ph	4.0
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	227
Injection volume	20 µl
Run time	6 min
Retention time	About 2.323 min for IBUPROFEN and 3.967 min for TRAMADOL.

Assay

Preparation of samples for Assay

Preparation of standard solution

Weigh accurately 10mg of IBUPROFEN and 10 mg of TRAMADOL in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10 µg/ml of IBUPROFEN and TRAMADOL is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains TRAMADOL-05 mg IBUPROFEN-50 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of TRAMADOL and IBUPROFEN (µg/ml) were prepared by dissolving weight equivalent to 10 mg of TRAMADOL and IBUPROFEN and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of TRAMADOL and IBUPROFENwas made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Ibuprofen and Tramadol present in the formulation by using the formula given below, and results shown in above table:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

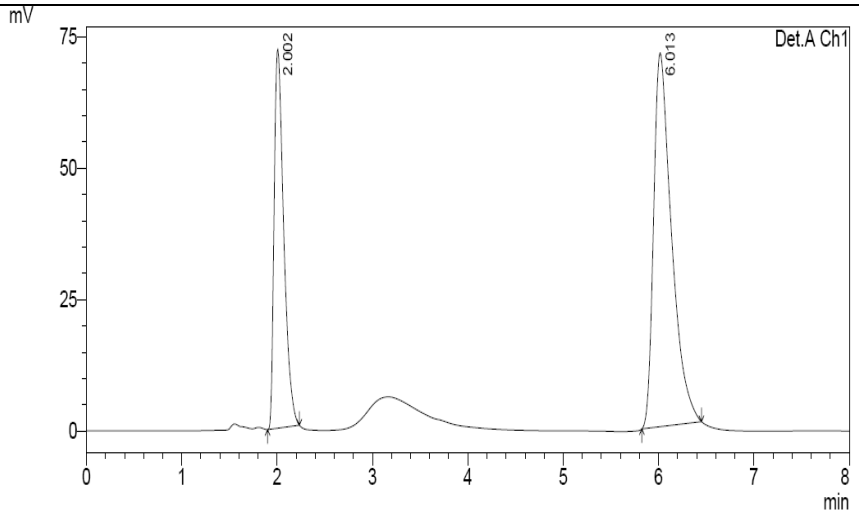


Fig: Chromatogram of Assay standard preparation-1

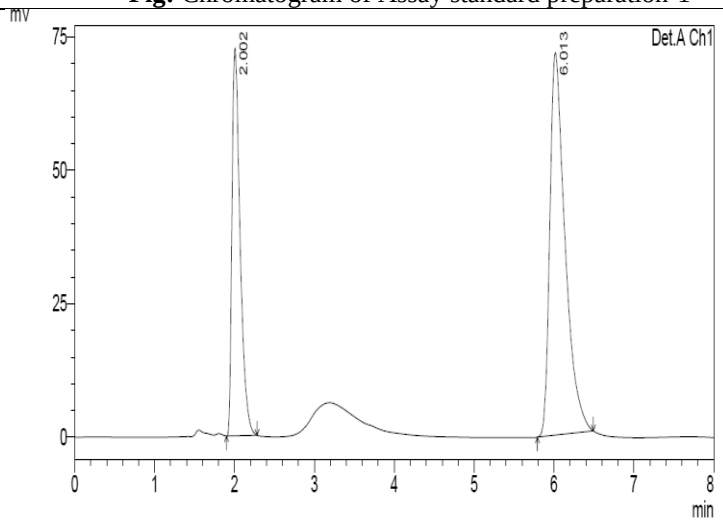


Fig: Chromatogram of Assay standard preparation-2

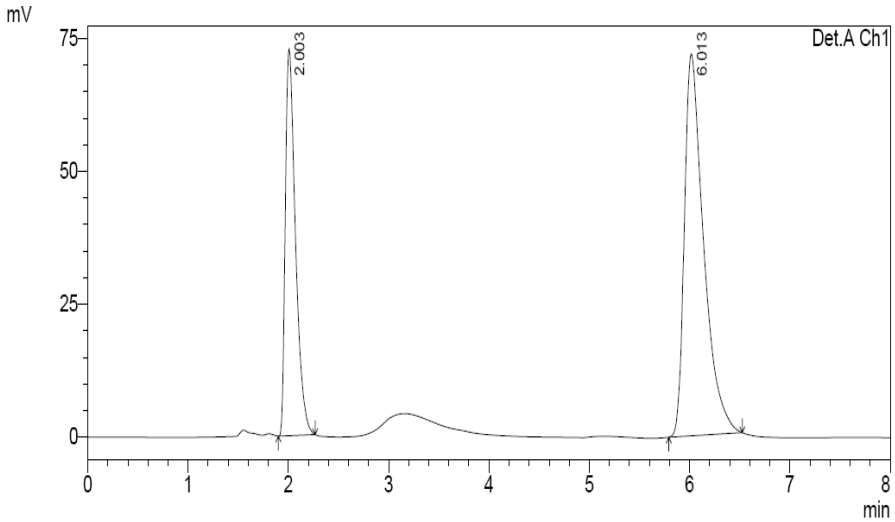


Fig: Chromatogram of Assay standard preparation-3

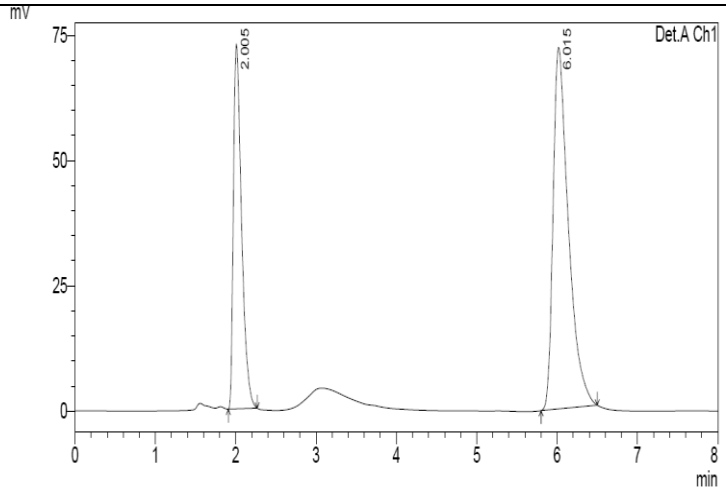


Fig: Chromatogram of Assay standard preparation-4

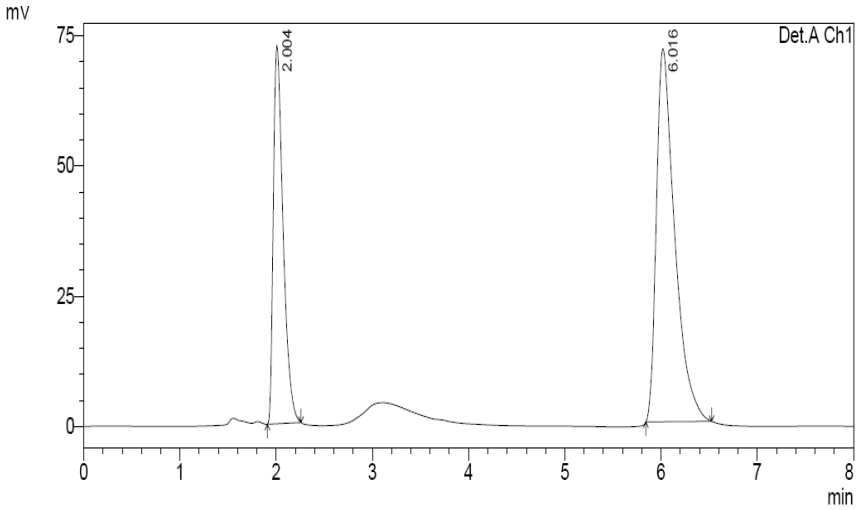


Fig: Chromatogram of Assay standard preparation-5

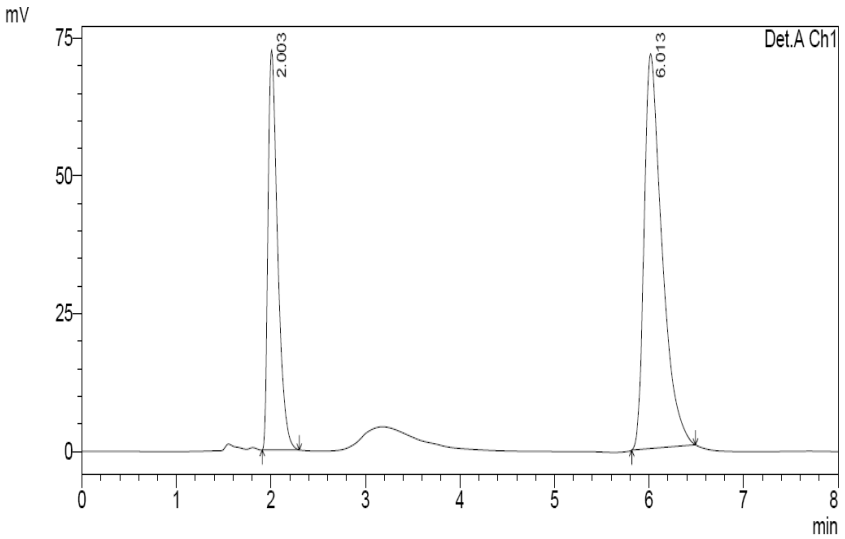


Fig: Chromatogram of Assay sample preparation-1

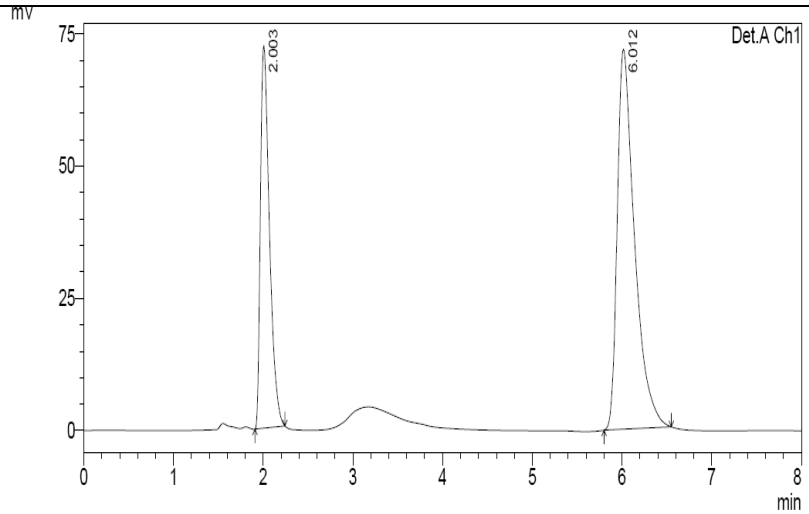


Fig: Chromatogram of Assay sample preparation-2

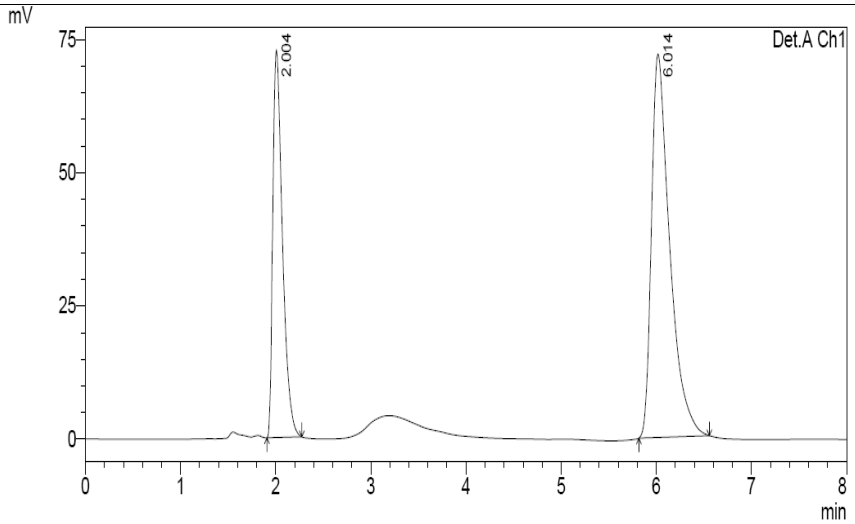


Fig: Chromatogram of Assay sample preparation-3

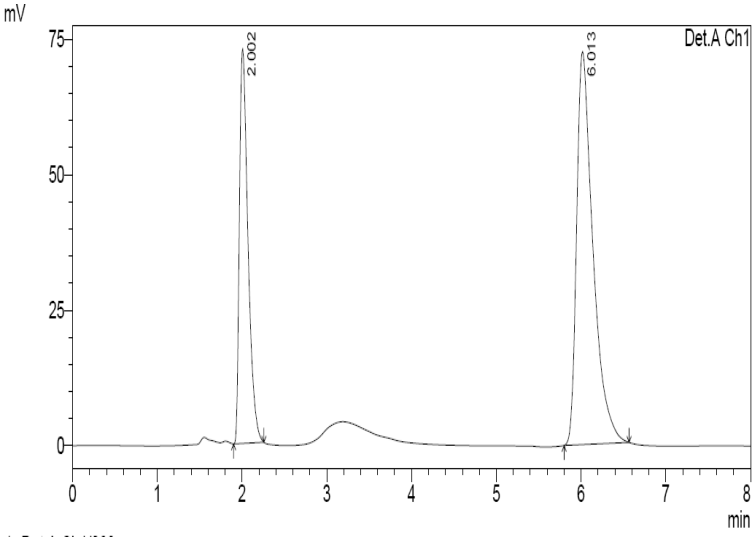


Fig: Chromatogram of Assay sample preparation-4

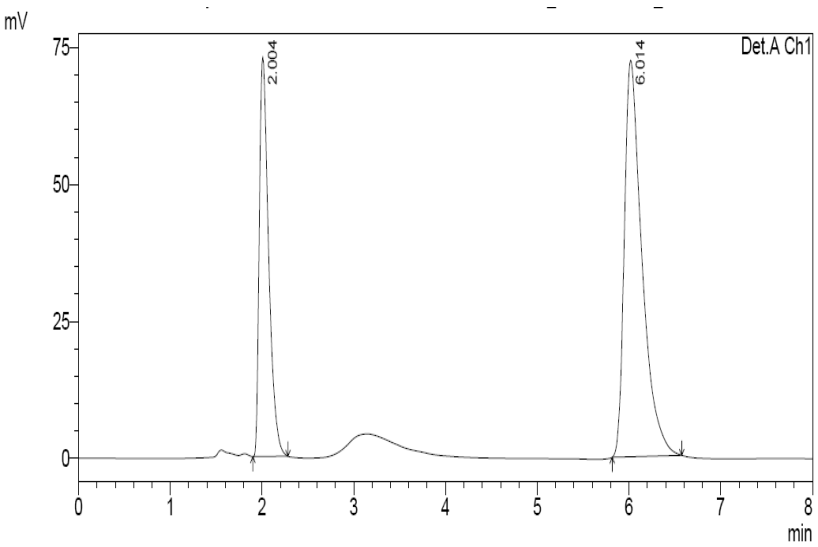


Fig: Chromatogram of Assay sample preparation-5

Table: Assay Results

	IBUPROFEN		IBUPROFEN BROMIDE	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	506899	515556.000	931844	947876.000
Injection-2	514450.000	508489	950651.000	962290
Injection-3	512714	513449.000	958312	960335
Injection-4	513898	513770	958137	972115
Injection-5	513154.000	516509	948997.000	969359.000
Average Area	512223.000	513554.600	949588.2	962395
Assay(%purity)	100.259965		101.348669	

Observation

The amount of IBUPROFEN and TRAMADOL present in the taken dosage form was found to be 100.25 % and 101.34 % respectively.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of mixed standard solution

Weigh accurately 10mg of IBUPROFEN and 10 mg of TRAMADOL in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of IBUPROFEN and TRAMADOL is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains TRAMADOL–0.5 mg IBUPROFEN -5 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of TRAMADOL and IBUPROFEN (µg/ml) were prepared by dissolving weight equivalent to 10 mg of TRAMADOLand 20 mg of IBUPROFEN and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of TRAMADOLand IBUPROFEN was made by adding 1 ml of stock solution to 10 ml of mobile phase.

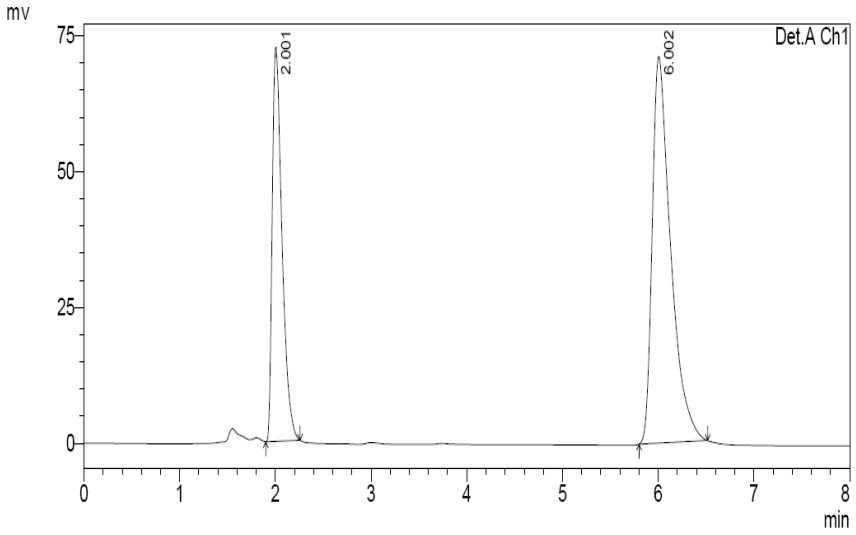


Fig: Chromatogram for specificity of Ibuprofen and IBUPROFEN sample

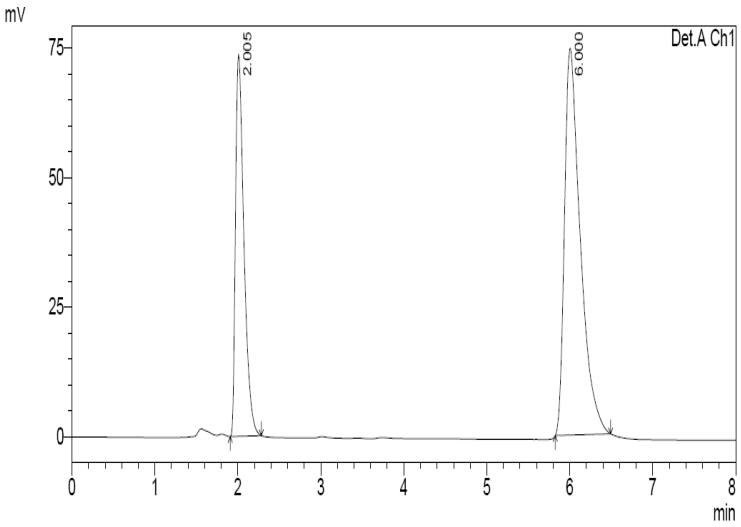


Fig: Chromatogram for Specificity of Ibuprofen and IBUPROFEN standard

Observation

It is observed from the above data, diluent or excipient peaks are not interfering with the IBUPROFEN and TRAMADOL peaks.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of IBUPROFEN and TRAMADOL (microgram/ml) were prepared by dissolving 10 mg of IBUPROFEN and TRAMADOL dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase.

Table 9.3 .1: Linearity Preparations

Preparations	Volume from standard stock transferred in ml		Volume made up in ml (with mobile phase)	Concentration of solution(µg /ml)	
				IBUPROFEN	TRAMADOL
Preparation 1	0.50	0.50	10	50	50
Preparation 2	0.75	0.75	10	75	75
Preparation 3	1	1	10	100	100
Preparation 4	1.25	1.25	10	125	125
Preparation 5	1.50	1.50	10	150	150

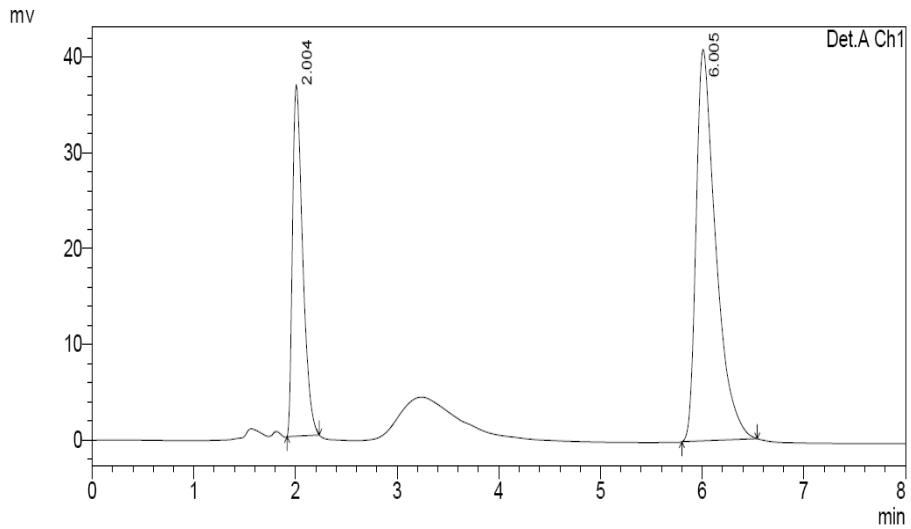


Fig: Chromatogram of Ibuprofen and IBUPROFEN preparation-1

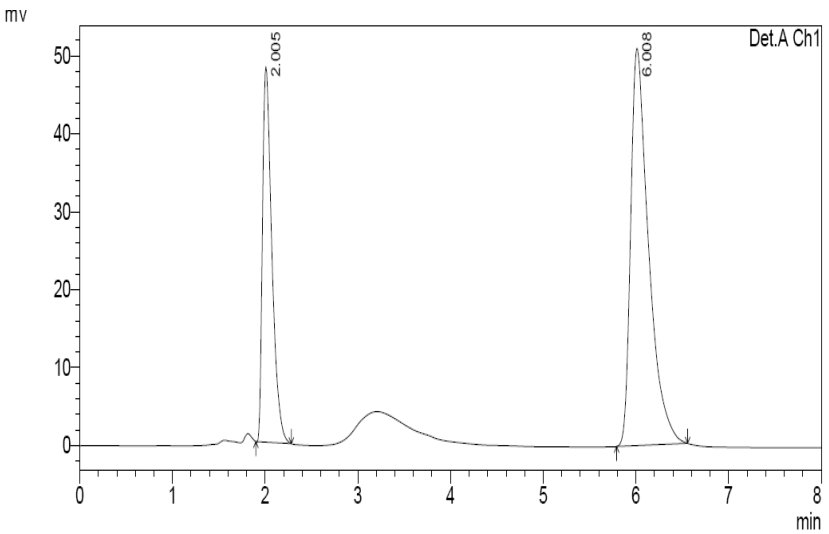


Fig: Chromatogram of Ibuprofen and IBUPROFEN preparation-2

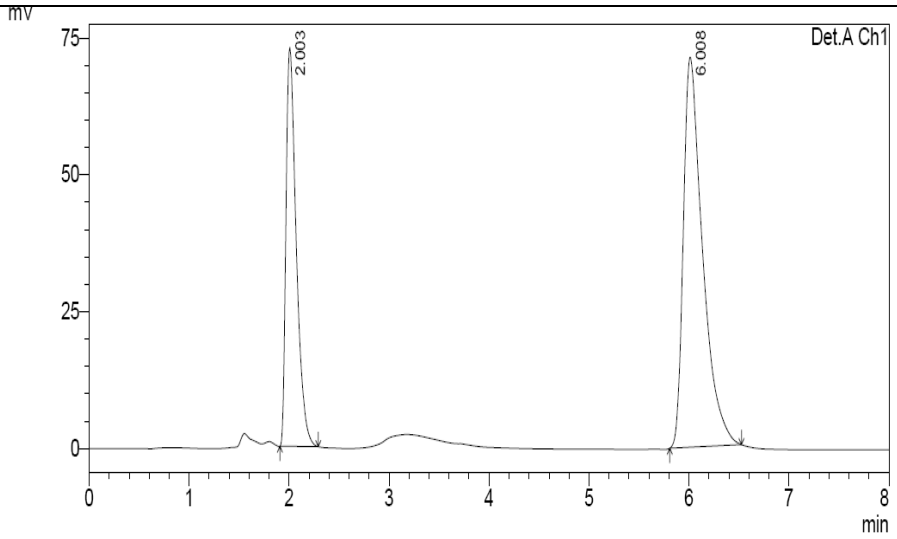


Fig: Chromatogram of Ibuprofen and IBUPROFEN preparation-3

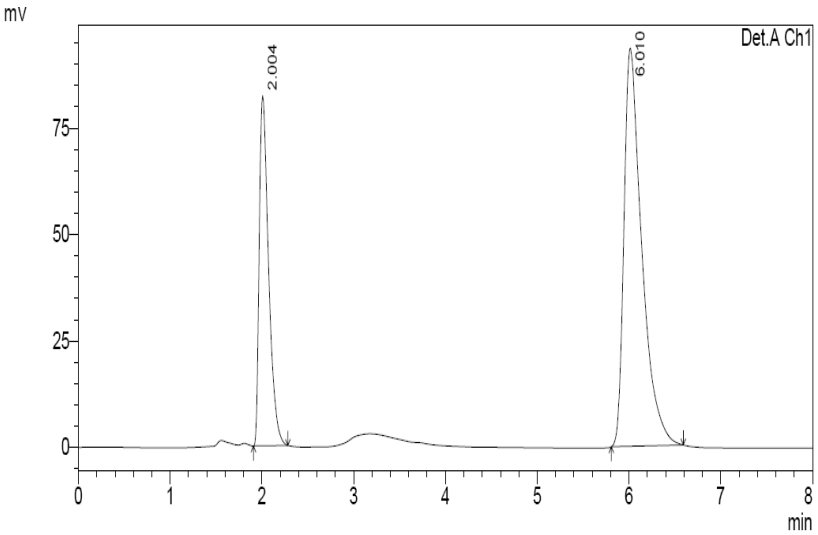


Fig: Chromatogram of Ibuprofen and IBUPROFEN preparation-4

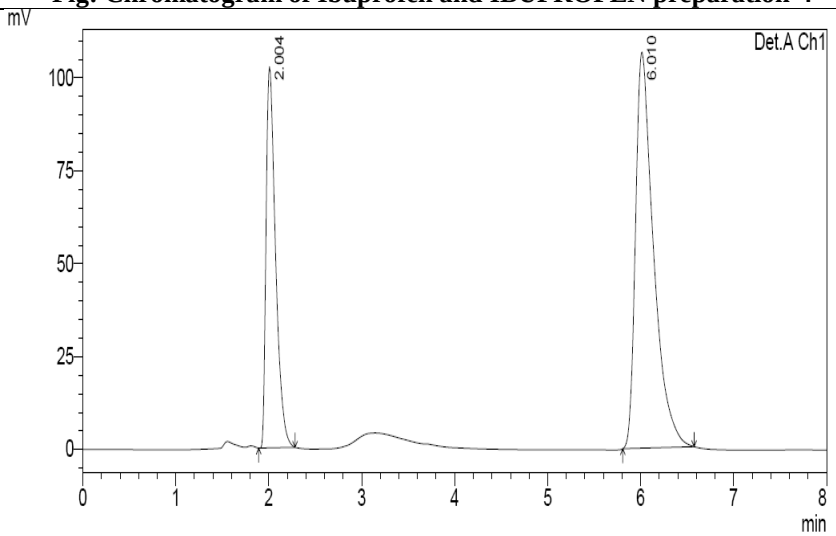


Fig: Chromatogram of Ibuprofen and IBUPROFEN for preparation-5

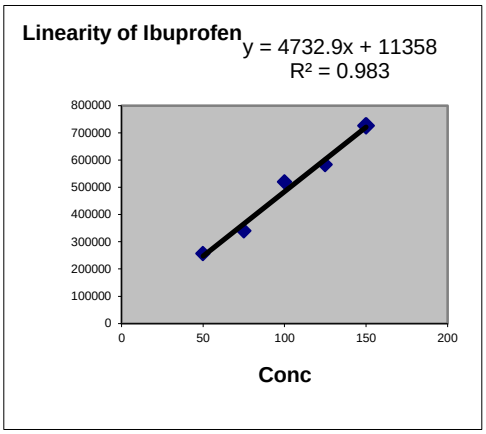
Table: linearity of IBUPROFEN

S. No.	Conc.(µg/ml)	Area
1	50	256242
2	75	339099
3	100	519076
4	125	582857
5	150	725978

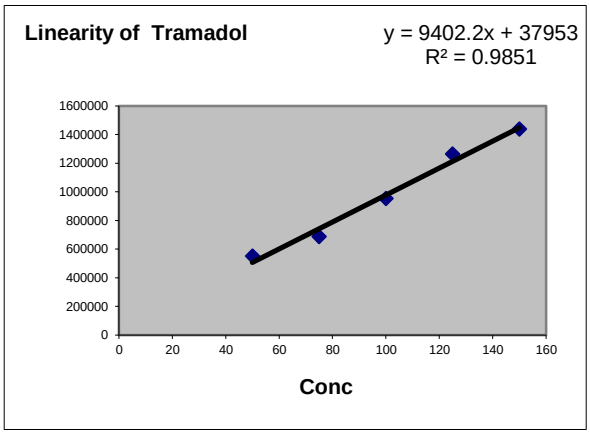
Table: linearity of IBUPROFEN BROMIDE

S. No.	Conc.(µg/ml)	Area
1	50	550613
2	75	686138
3	100	953262
4	125	1263825
5	150	1437050

Linearity graph of IBUPROFEN



Linearity graph ofIbuprofen



Linearity graph of Tramadol

Acceptance criteria

The relationship between the concentration of IBUPROFEN and TRAMADOL and area of IBUPROFEN and TRAMADOL should be linear in the specified range and the correlation should not be less than 0.99.

Observation

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of IBUPROFEN and TRAMADOL is 0.999 and 0.996. The relationship between the concentration of IBUPROFEN and TRAMADOL and area of IBUPROFEN and TRAMADOL is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%

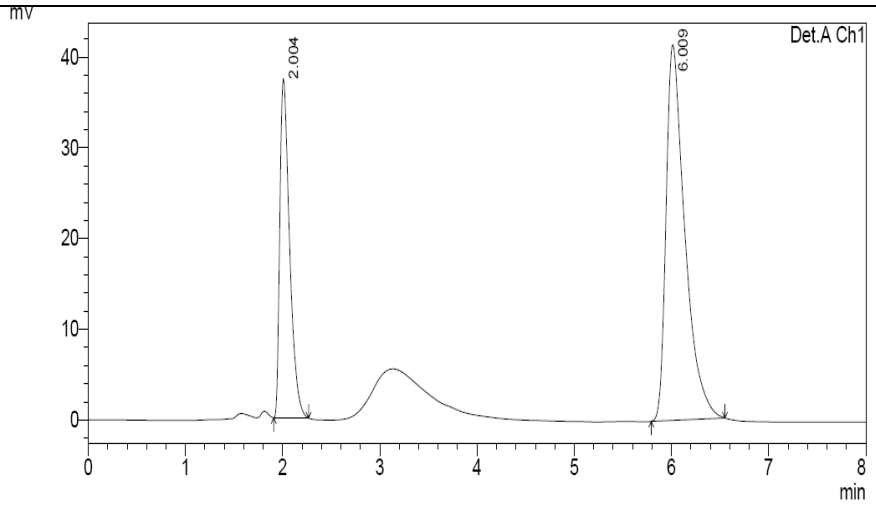


Fig: Chromatogram of 50% recovery (injection 1)

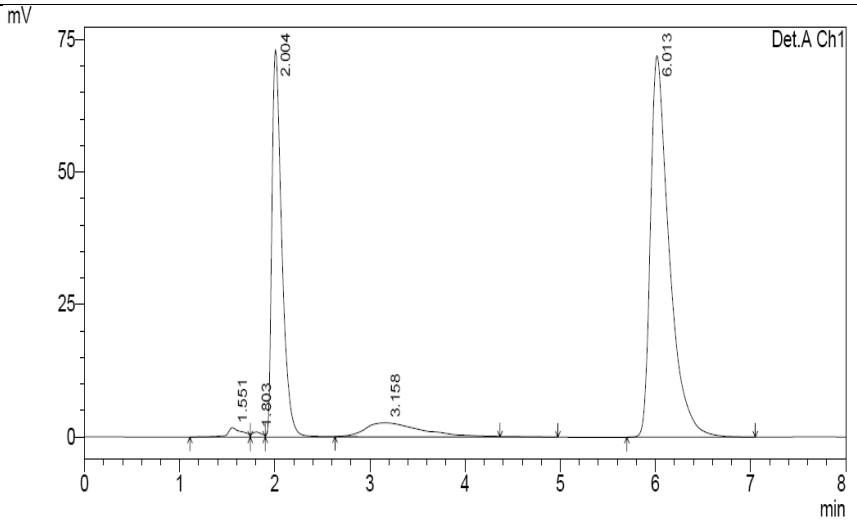


Fig: Chromatogram of 100% recovery (injection 2)

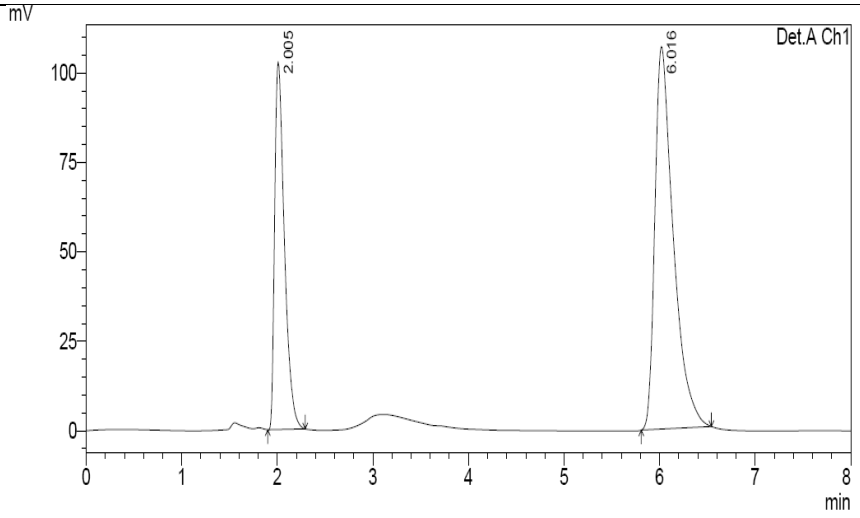


Fig: Chromatogram of 150% recovery (injection 3)

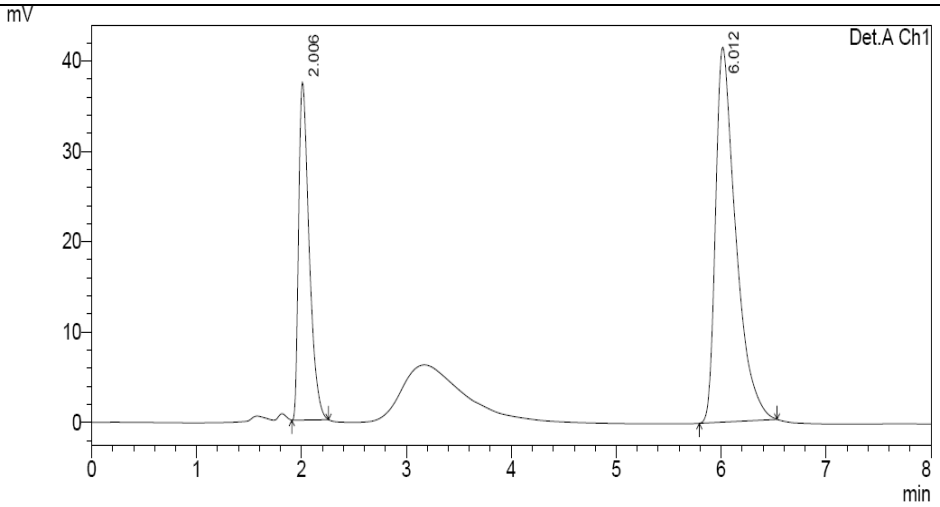


Fig: Chromatogram of 50% recovery (injection 1)

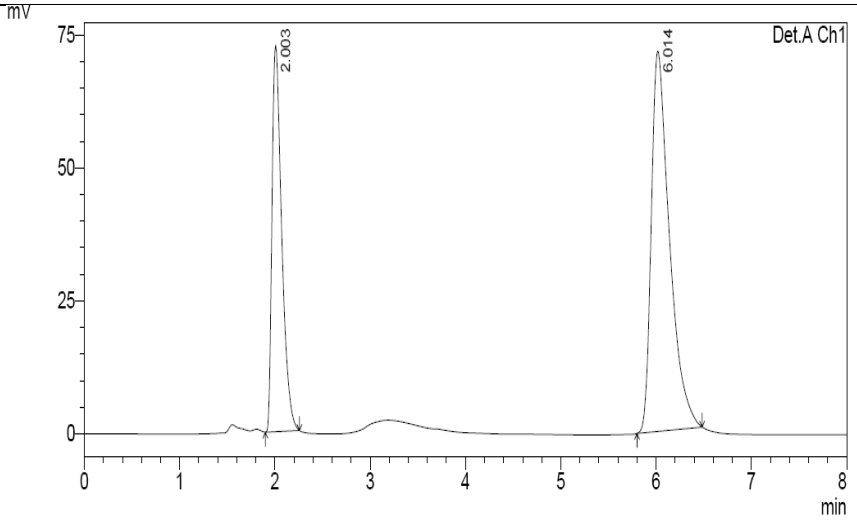


Fig: Chromatogram of 100% recovery (injection 2)

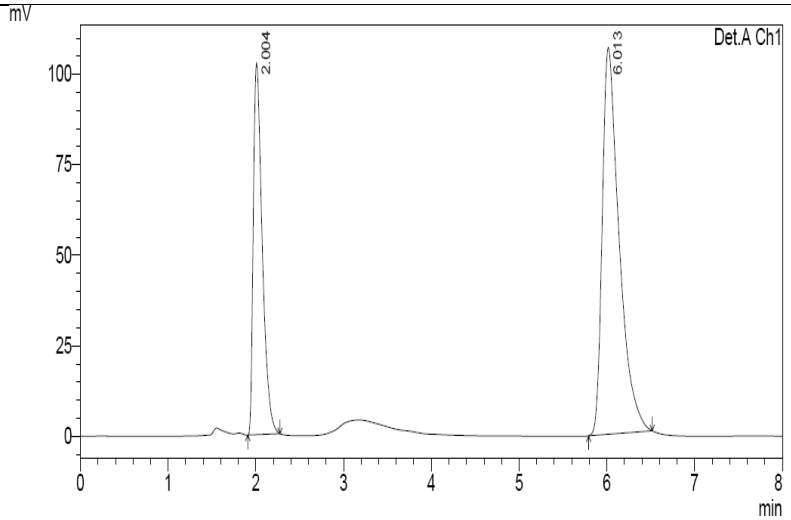


Fig: Chromatogram of 150% recovery (injection 3)

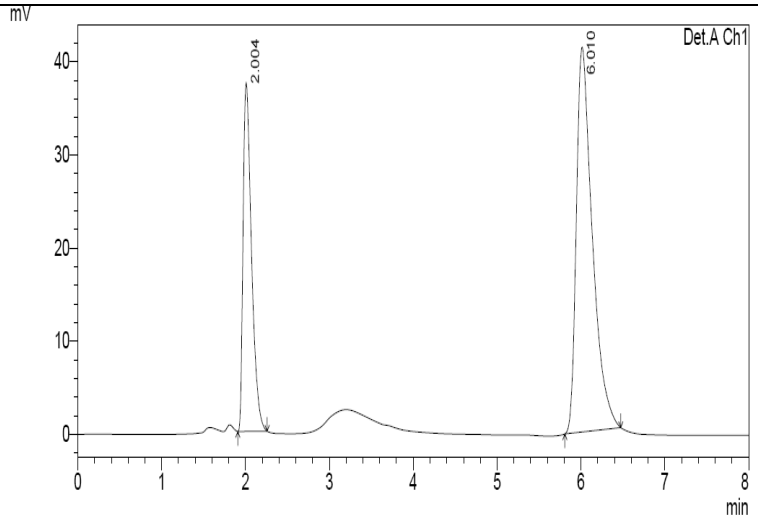


Fig: Chromatogram of 50% recovery (injection 1)

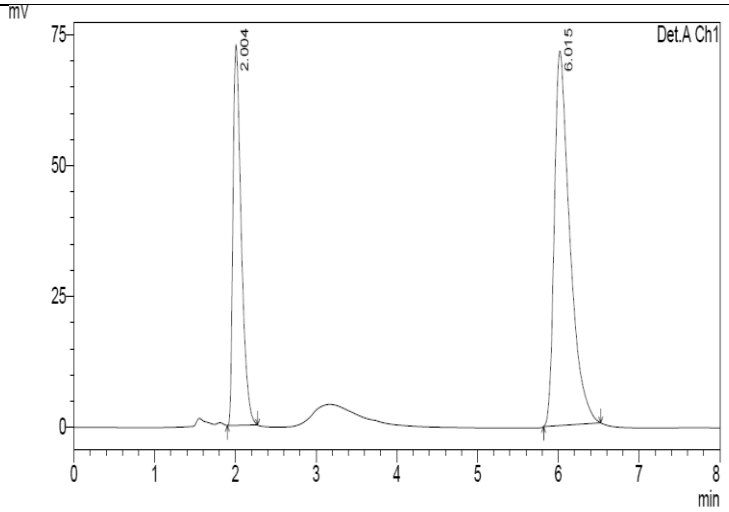


Fig: Chromatogram of 100% recovery (injection 2)

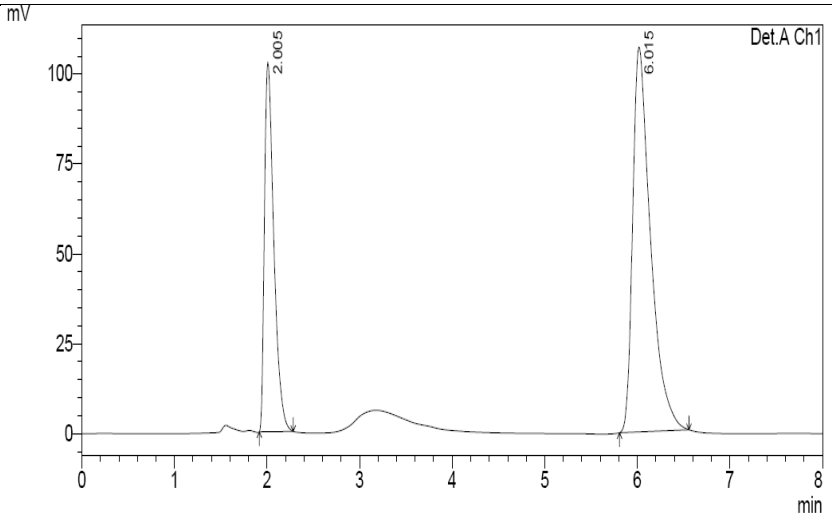


Fig: Chromatogram of 150% recovery (injection 3)

Acceptance criteria

The % recovery of Ibuprofen and IBUPROFEN should lie between 98% and 110%.

Recovery level	Accuracy IBUPROFEN			Average % Recovery
	Amount taken(mcg/ml)	Area	%Recovery	
100	**50**	**261419**		
	50	**260850**	**100.7138839**	**100.6450511**
	50	**261452**	**100.4946719**	
			100.7265975	
120	**100**	**540729**		**101.0050751**
	100	**514176**	**104.1602136**	
	100	**518144**	**99.04532951**	
			99.80968232	**99.1287765**
140	**150**	**869667**		
	150	**857028**	**100.5139733**	
	150	**846351**		
			99.05318878	
			97.81916738	

Recovery results for TRAMADOL

Recovery level	**Accuracy TRAMADOL**			Average % Recovery
	Amount taken(mcg/ml)	Area	%Recovery	
100	50	**557310**		**102.83**
	50	**556033**	**111.1521079**	
	50	**547688**	**110.897418**	
			109.2330582	**99.36**
120	100	**988685**		
	100	**949923**	**98.59362098**	**99.103**
	100	**955604**	**94.72819778**	
140	150	**1435140**	**95.29471833**	
	150	**1430786**	**85.86899724**	
	150	**1437821**	**85.60848355**	
			86.02941	

Observation

The percentage mean recovery of IBUPROFEN and TRAMADOL is 99.19 % and 99.89 % respectively.

Precision

Method precision

Prepared sample preparations of TRAMADOL and IBUPROFEN as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of TRAMADOL and IBUPROFEN should be not more than 2.0%.

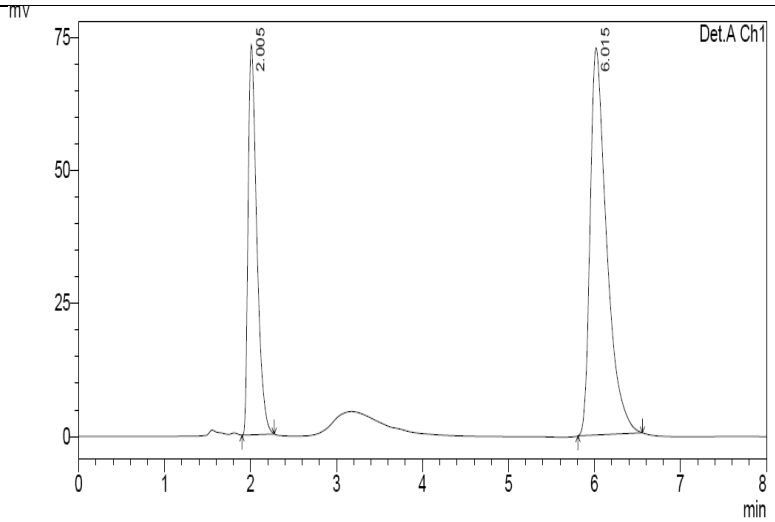


Fig: Chromatogram of precision injection 1

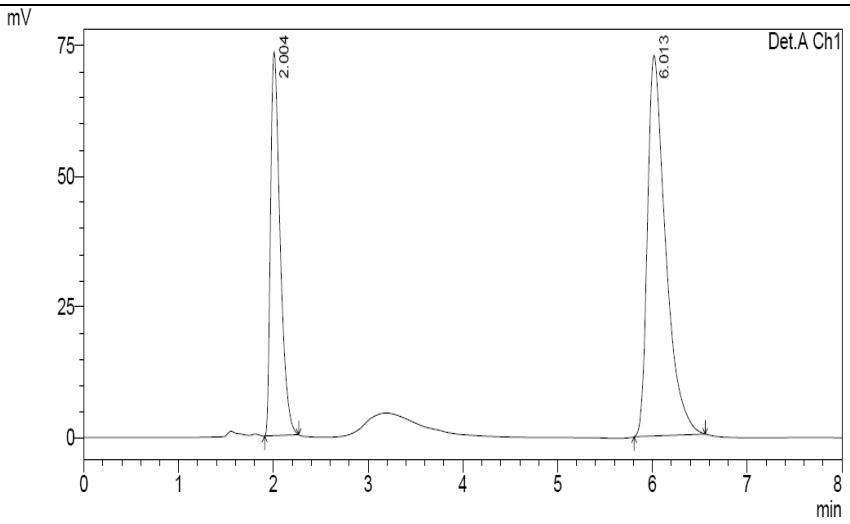


Fig: Chromatogram of precision injection 2

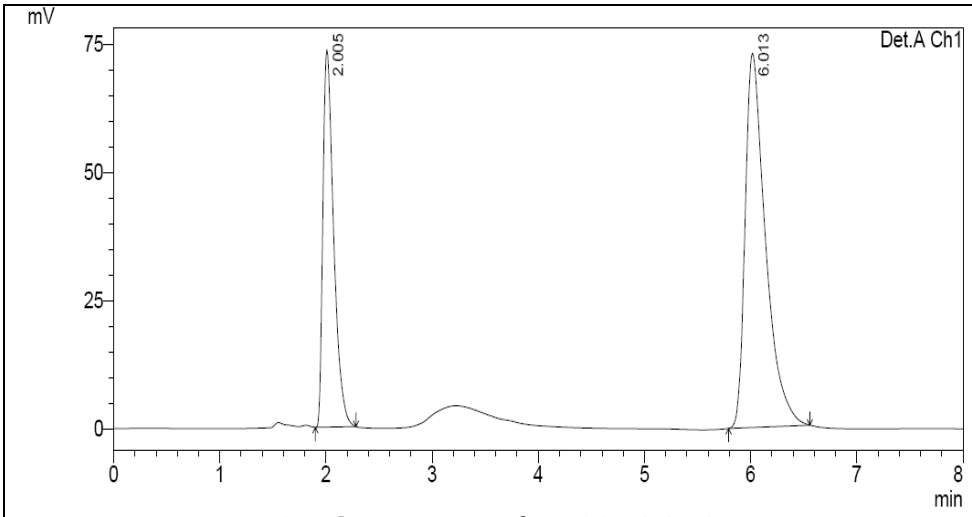


Fig: Chromatogram of precision injection 3

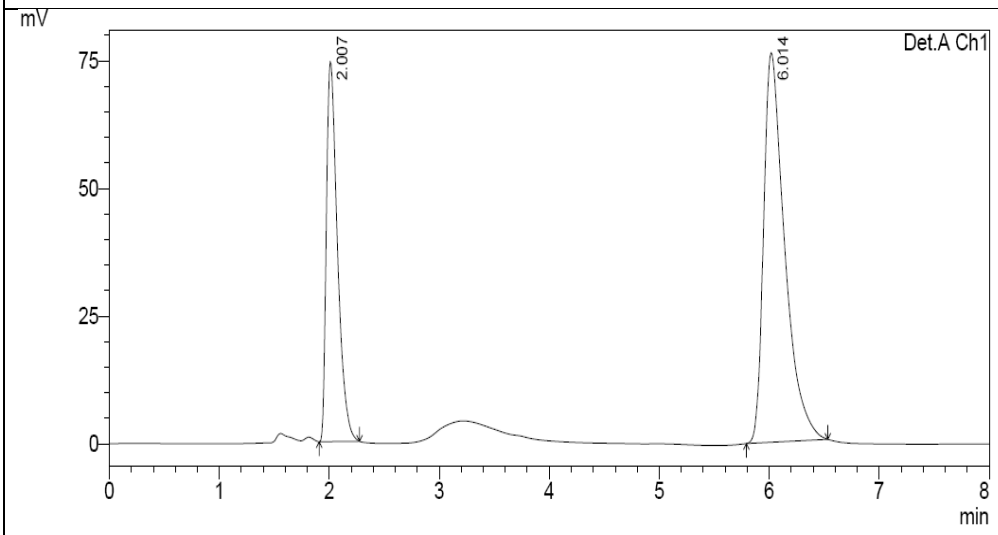


Fig: Chromatogram of precision injection 4

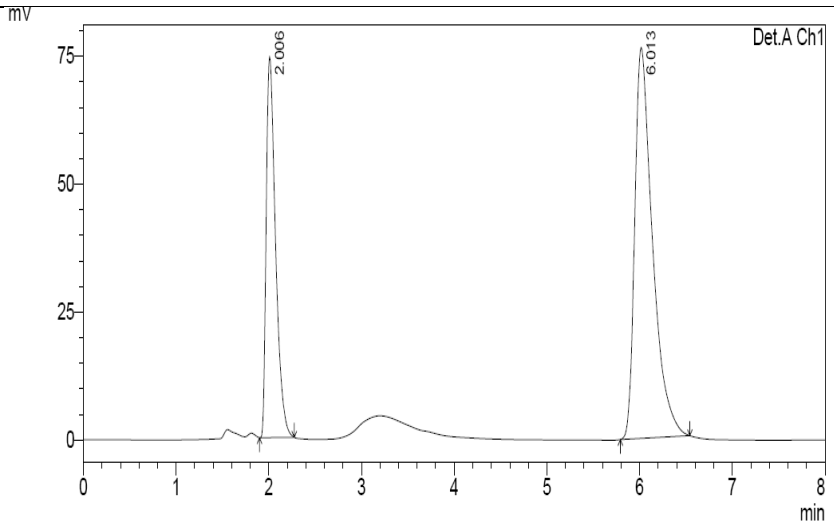


Fig: Chromatogram of precision injection 5

IBUPROFEN			TRAMADOL		
S. No.	Rt	Area	S. No.	Rt	Area
1	2.005	517461.000	1	2.005	517461.000
2	2.004	517192.000	2	2.004	517192.000
3	2.005	518753.000	3	2.005	518753.000
4	2.007	521539.000	4	2.007	521539.000
5	2.006	521945.000	5	2.006	521945.000
6	2.006	521320.000	6	2.006	521320.000
avg	2.0055	519701.667	**avg**	2.0055	519701.667
stdev	0.0010	2156.215	**stdev**	0.0010	2156.215
%RSD	**0.05**	**0.41**	**%RSD**	**0.05**	**0.41**

Observation

Test results for TRAMADOL and IBUPROFEN are showing that the %RSD of Assay results are within limits. The results were shown in table

Robustness

Chromatographic conditions variation

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like flow rate and wavelength. System suitability parameters were compared with that of method precision.

Acceptance criteria

The system suitability should pass as per the test method at variable conditions.

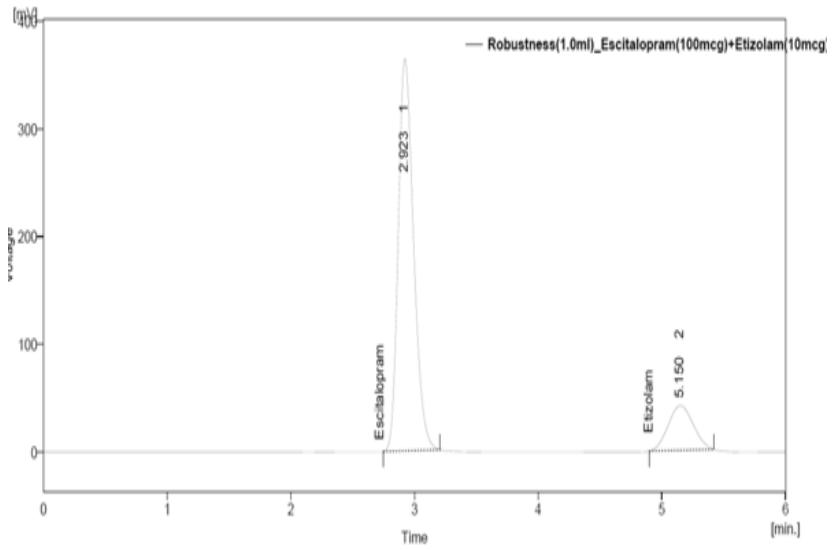


Fig: Chromatogram of Ibuprofen and IBUPROFEN Robustness (0.8 ml/min)

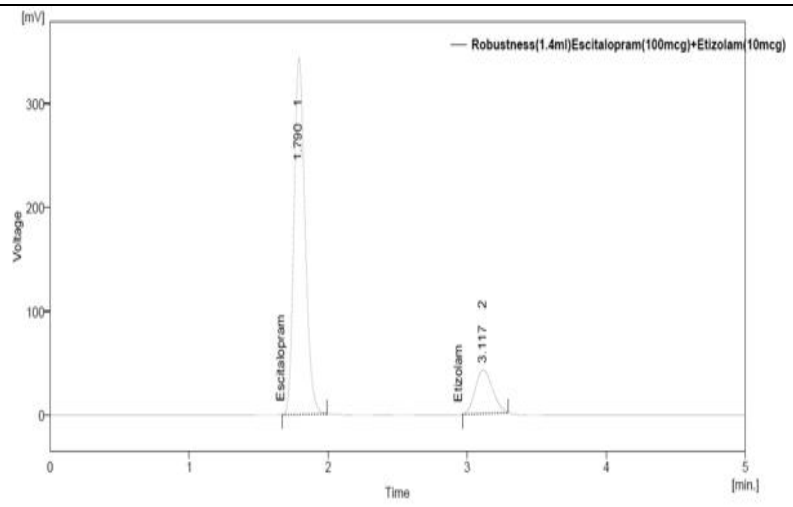


Fig: Chromatogram of Ibuprofen and IBUPROFEN for Robustness (1.2 ml/min)

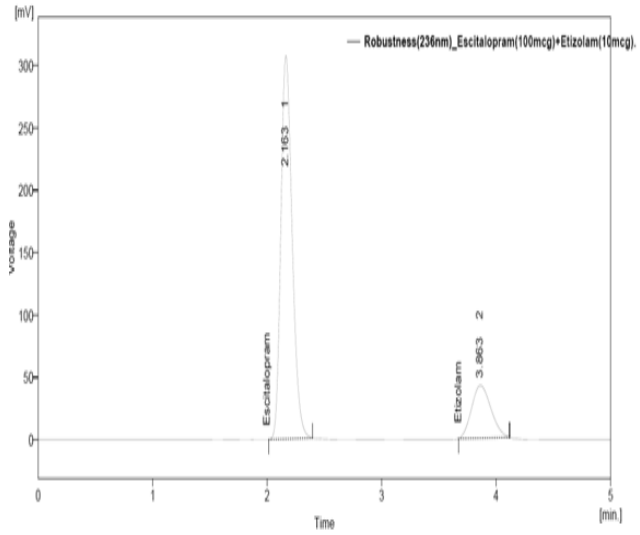


Fig: Chromatogram of Ibuprofen and IBUPROFEN for Robustness (236)

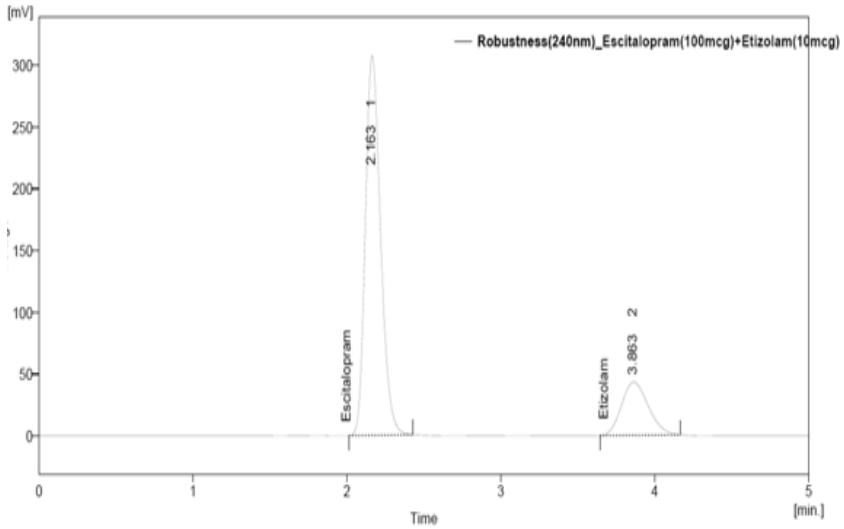


Fig: Chromatogram of Ibuprofen and IBUPROFEN for Robustness (241)

Observation

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

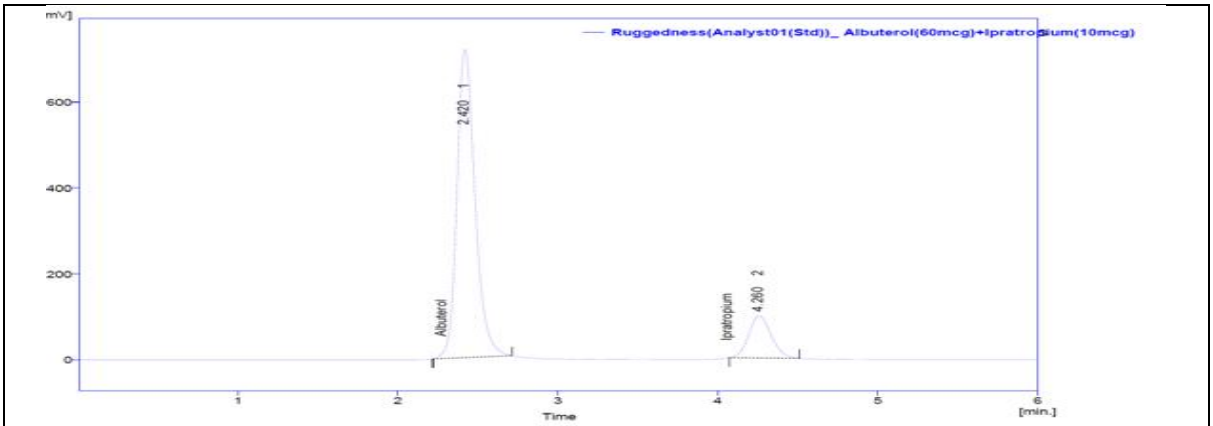


Fig: Chromatogram of Analyst 01 standard preparation

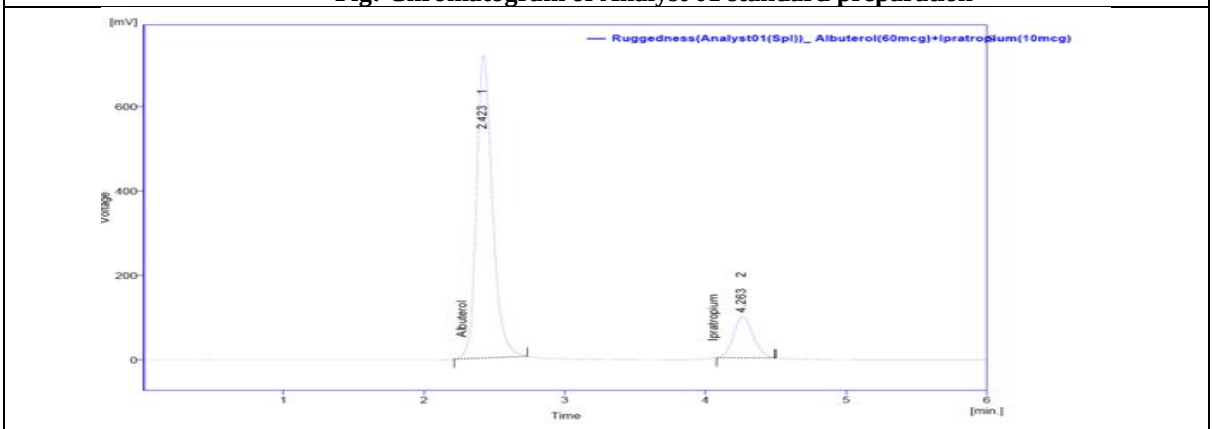


Fig: Chromatogram of Analyst 01 sample preparation

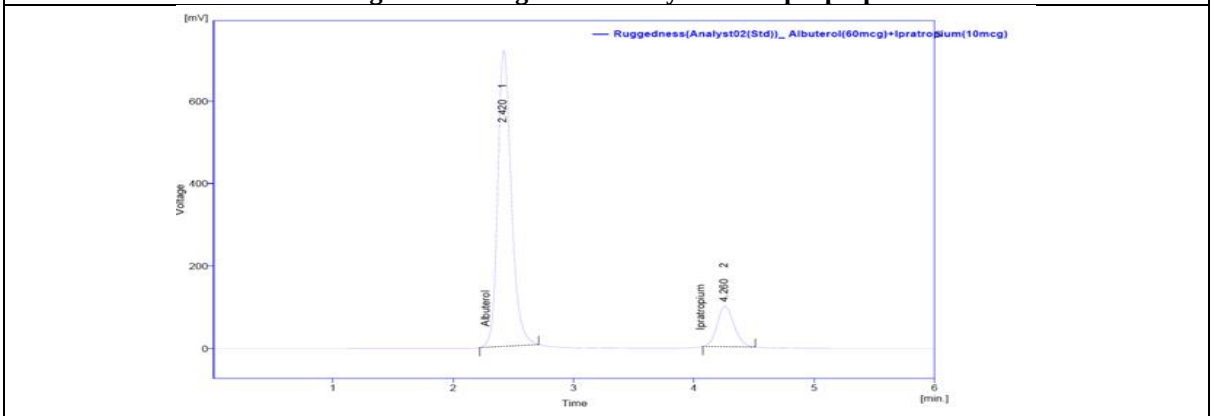


Fig: Chromatogram of Analyst 02 standard preparation

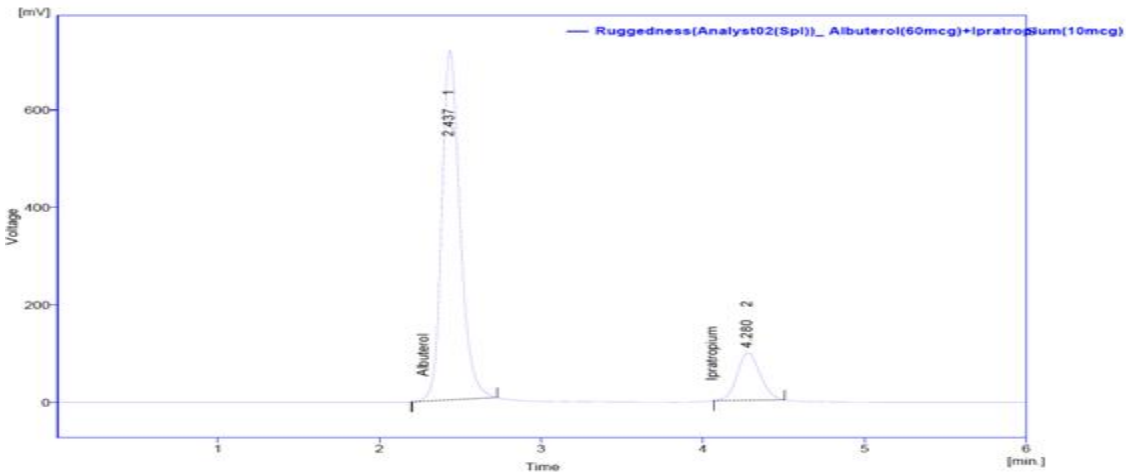


Fig: Chromatogram of Analyst 02 sample preparation

Table 9.9.5: Results for Ruggedness

IBUPROFEN	%Assay	IBUPROFEN BROMIDE	%Assay
Analyst 01	100.53	**Analyst 01**	98.65
Anaylst 02	100.40	**Anaylst 02**	100.41

Observation

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

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