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Research Article

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

K.Kranthi Kiran, A.Sravya, B.Ramadhevi, B.Aruna, Ch.Ramadhevi

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

Corresponding Author: K.Kranthi Kiran

Email Id: kothapallikranthikiran@gmail.com

ABSTRACT

A simple and selective LC method is described for the determination of Albuterol and Ipratropium Bromide in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 80 volumes of methanol and 20 volumes of water with detection of 239 nm. Linearity was observed in the range 36-84 µg /ml for Albuterol (r^2 =0.996) and 6-14 µg /ml for Ipratropium Bromide (r^2 =0.997) 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: Albuterol and Ipratropium**,** 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].

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds [2]. There are two main types of analysis – Qualitative and Quantitative analysis. Qualitative analysis is performed to establish composition of a

substance [3]. It is done to determine the presence of a compound or substance in a given sample or not [4-6]. 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 Albuterol and ipratropium bromide pharmaceutical dosage form.

Plan of work

- Solubility determination of Albuterol and ipratropium bromide 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

Mobile phase

A mixture of 80 volumes of Methanol and 20 volumes of Water. The mobile phase was sonicated for 10min to remove gases.

Determination of working wavelength (λmax)

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

Preparation of standard stock solution of albuterol

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

Preparation of standard stock solution of ipratropium

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

RESULTS AND DISCUSSIONS

Solubility studies

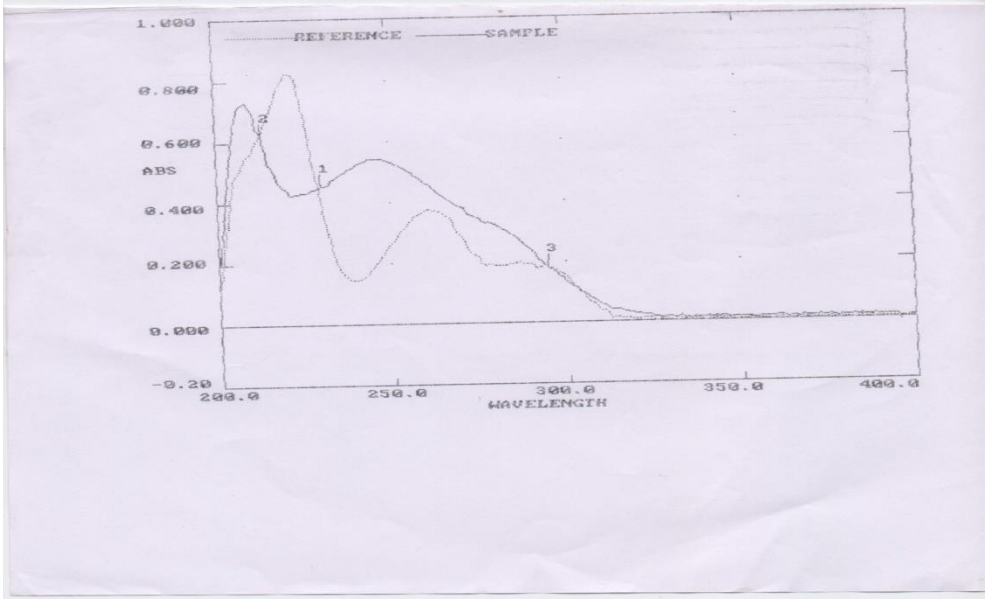
These studies are carried out at 25 ^{0}C

Albuterol

Freely soluble in methanol,water and mixed phosphate buffer.

Ipratropium

Freely soluble in ethanol and methanol, and slightly soluble in acetone and very slightly soluble in 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 and the absorption curve shows the isobestic point was found to be 239 nm for the combination.

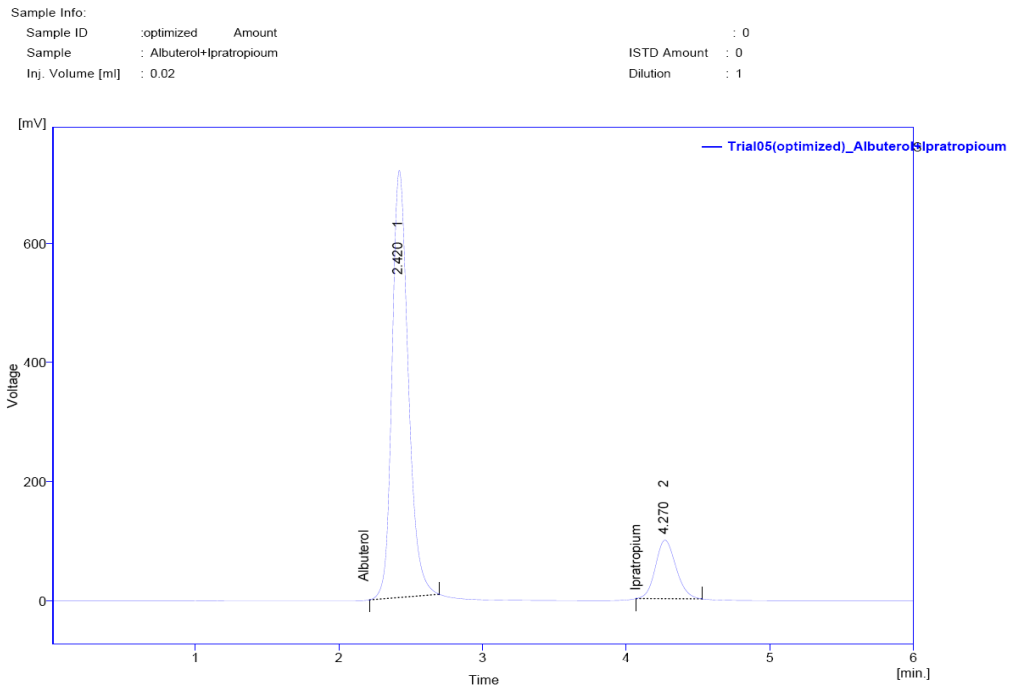
Isobestic point of Albuterol and Ipratropium

METHOD DEVELOPMENT OF ALBUTEROL AND IPRATROPIUM BROMIDE

Trial- 1

Preparation of standard solution

Weigh accurately 60 mg of ALBUTEROL and 40 mg of IPRATROPIUM BROMIDE 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 60 µg/ml of ALBUTEROL and 40 µg/ml of IPRATROPIUM BROMIDE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.



Result Table (Uncal - Trial05_Albuterol+Ipratropioum)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.420	5640.876	717.248	85.23	87.9	0.12
2	4.270	977.839	98.345	14.77	12.1	0.16
	Total	6618.715	815.593	100.00	100.0	

Column Performance Table (From 50% - Trial05_Albuterol+Ipratropioum)

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.420	0.120	1.414	0.00	2253	45062	-
2	4.270	0.157	1.256	0.00	4115	82308	7.869

Fig. 3: Chromatogram of IPRATROPIUM AND ALBUTEROL

Observation

- Peak Asymmetry factor for Ipratropium and Albuterol meet the system suitability requirements. The run time is very correct. Theoretical plates were more than 2000.Hence it is taken for optimization.

Table 1: Optimized chromatographic conditions

Mobile phase	**METHANOL:WATER(80:20)**
Ph	-
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	239
Injection volume	20 µl
Run time	6 min
Retention time	About2.420 min for ALBUTEROL and 4.270 min for IPRATROPIUM BROMIDE.

ASSAY

Preparation of samples for assay

Preparation of standard solution

Preparation of mixed standard solution

Weigh accurately 60 mg of ALBUTEROL and 40 mg of IPRATROPIUM BROMIDE 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 60 µg/ml of ALBUTEROL and 40 µg/ml of IPRATROPIUM BROMIDE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains IPRATROPIUM BROMIDE- 100 mg ALBUTEROL-600 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of IPRATROPIUM BROMIDE and ALBUTEROL (µg/ml) were prepared by dissolving weight equivalent to 10 mg of IPRATROPIUM BROMIDE and 60 mg of ALBUTEROL 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 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of IPRATROPIUM BROMIDE and 60µg/ml of ALBUTEROL was made by adding 1 ml of stock solution to 10 ml of mobile phase. Calculation: The amount of ALBUTEROL and IPRATROPIUM present in the formulation by using the formula given below, and results shown in above table:

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

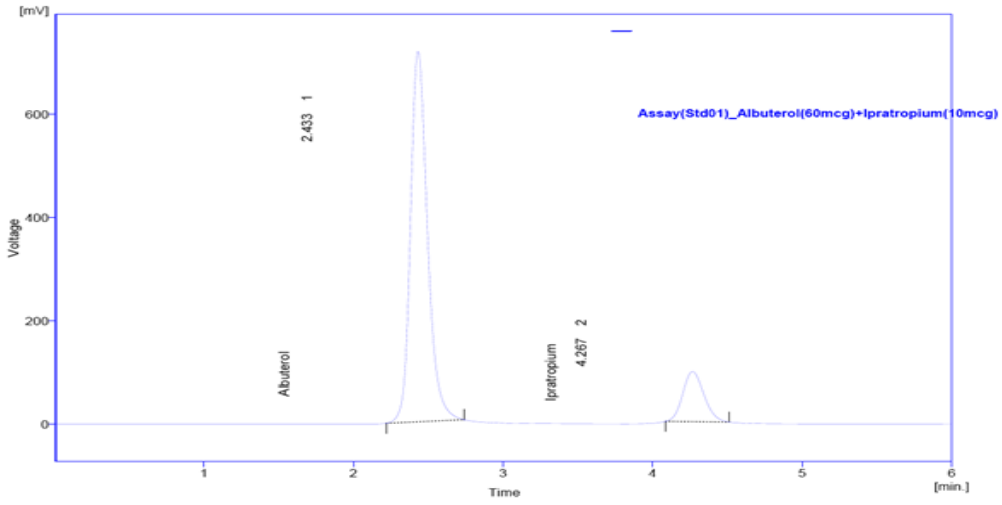


Fig: Chromatogram of Assay standard preparation-1

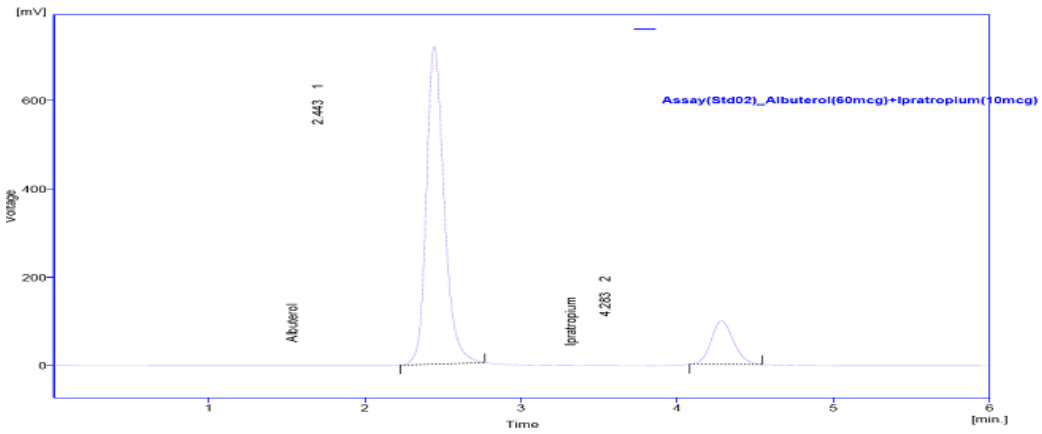


Fig: Chromatogram of Assay standard preparation-2

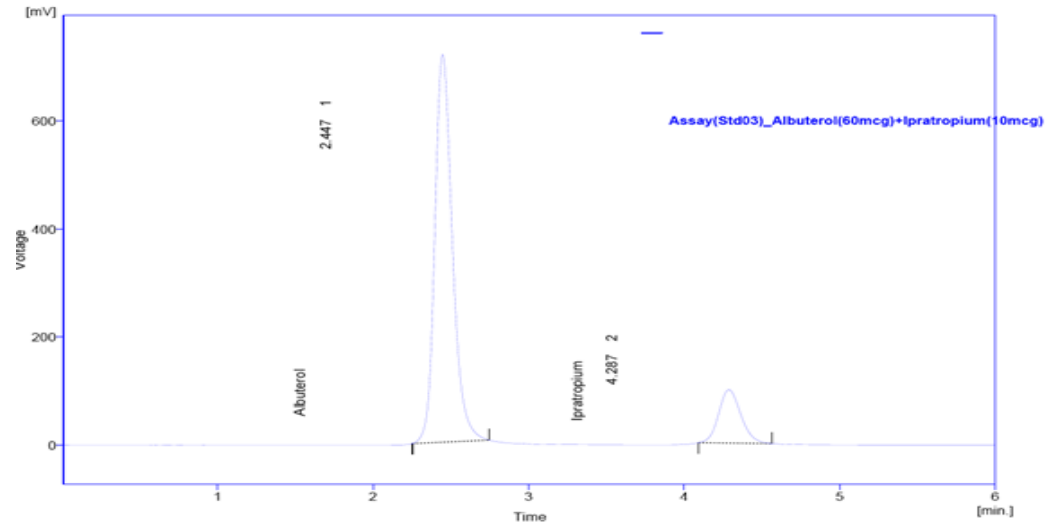


Fig: Chromatogram of Assay standard preparation-3

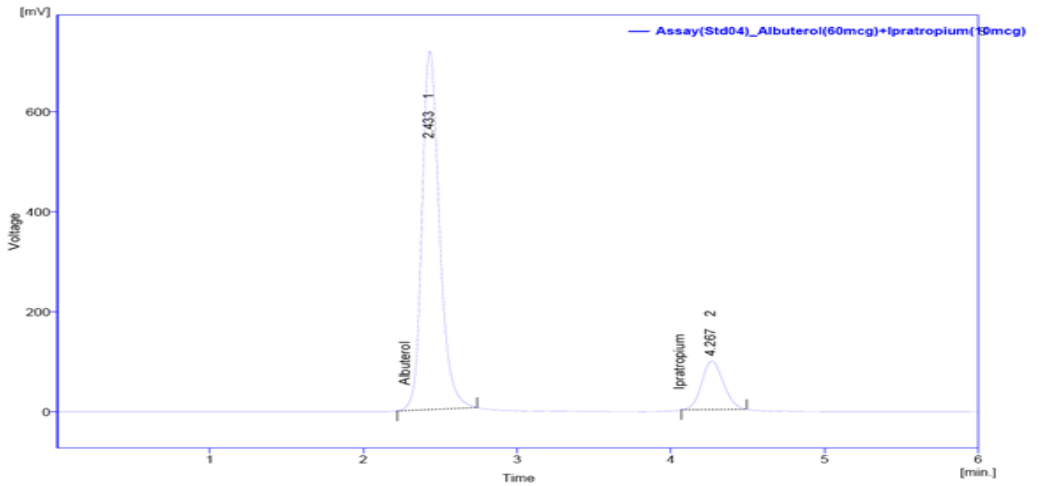


Fig: Chromatogram of Assay standard preparation-4

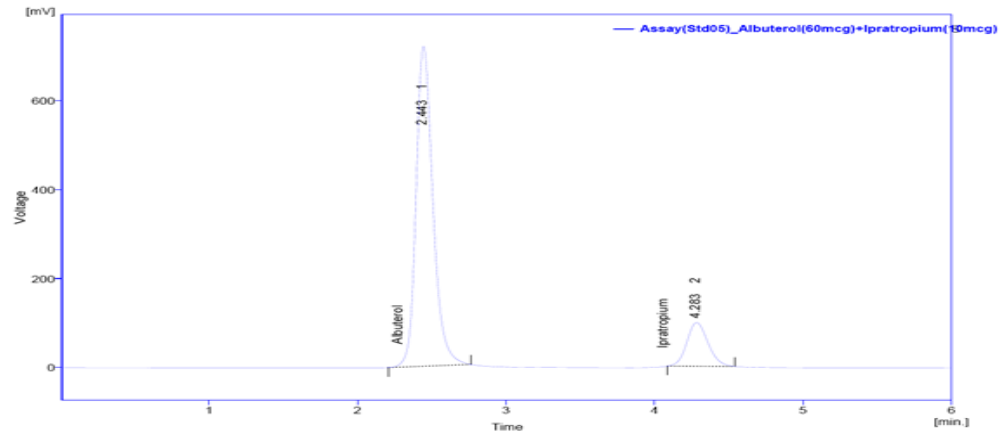


Fig: Chromatogram of Assay standard preparation-5

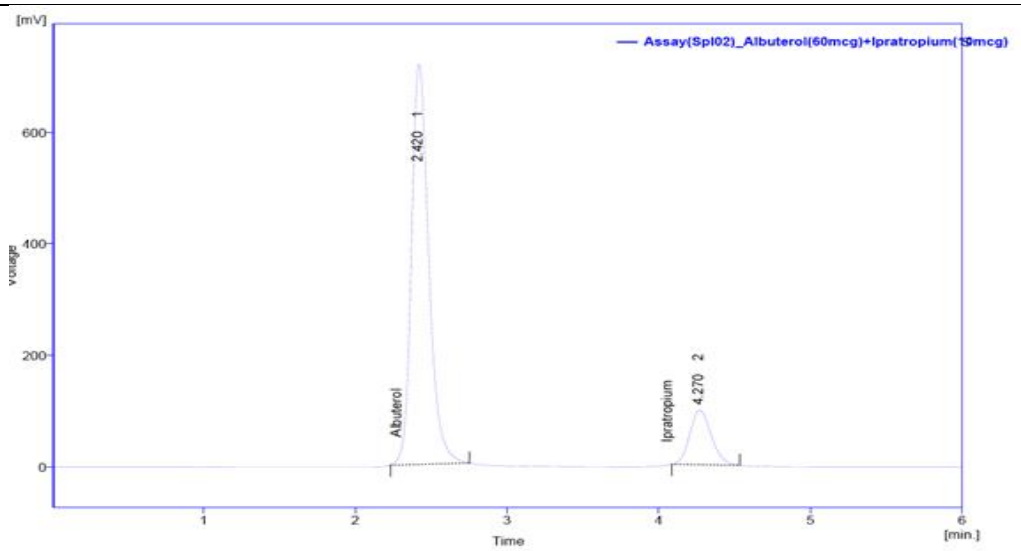


Fig: Chromatogram of Assay sample preparation-2

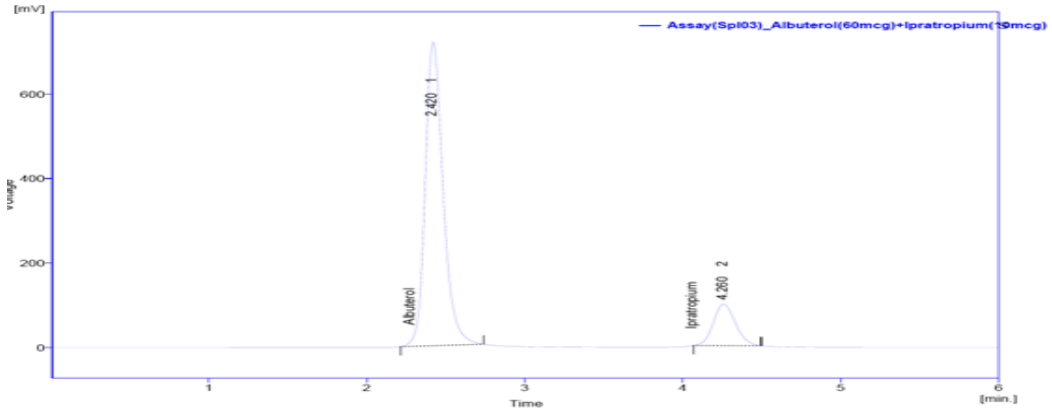


Fig: Chromatogram of Assay sample preparation-3

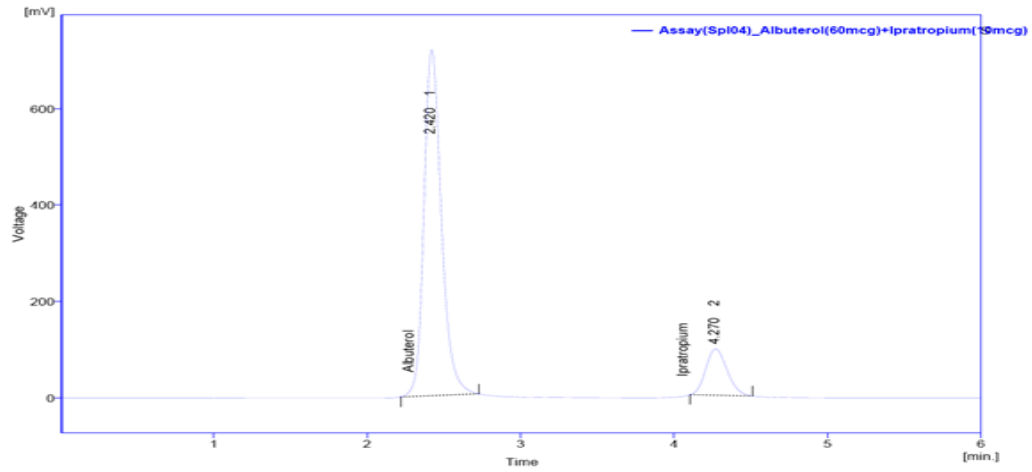


Fig: Chromatogram of Assay sample preparation-4

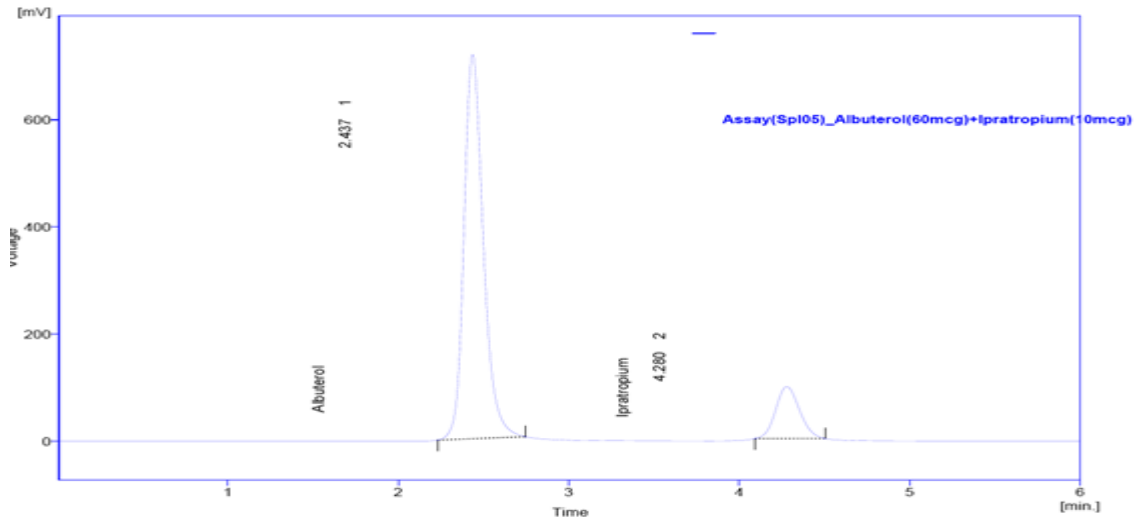


Fig: Chromatogram of Assay sample preparation-5

Table: Assay Results

ALBUTEROL			**IPRATROPIUM BROMIDE**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	5673.243	5701.197	953.186	991.133
Injection-2	5693.944	5688.381	980.027	963.992
Injection-3	5645.959	5673.079	979.597	956.883
Injection-4	5673.243	5677.094	954.493	928.917
Injection-5	5690.410	5673.471	971.682	943.128
Average Area	5675.36	5682.644	967.797	956.8106
Standard Deviation	19.00822		13.17558	
%RSD	0.002126		0.024482	
Assay(%purity)	**100.1284**		**98.8648**	

Observation

The amount of Ipratropium and Albuterol present in the taken dosage form was found to be 100.12% and 98.86 % 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 analyses in their dosage form.

Preparation of mixed standard solution

Weigh accurately 60 mg of ALBUTEROL and 40 mg of IPRATROPIUM BROMIDE 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 60 µg/ml of ALBUTEROL and 40 µg/ml of IPRATROPIUM BROMIDE is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains IPRATROPIUM BROMIDE- 400 mg, ALBUTEROL-600 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of IPRATROPIUM BROMIDE and ALBUTEROL (µg/ml) were prepared by dissolving weight equivalent to 400 mg of IPRATROPIUM BROMIDE and 600 mg of ALBUTEROL 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 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 40 µg/ml of IPRATROPIUM BROMIDE and 60µg/ml of ALBUTEROL was made by adding 1 ml of stock solution to 10 ml of mobile phase.

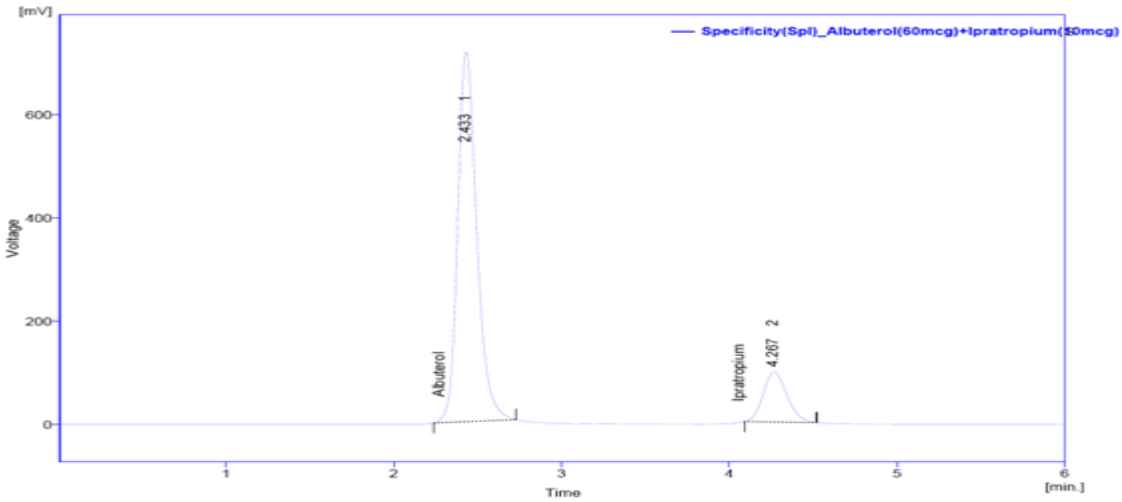


Fig: Chromatogram for specificity ofIpratropium and ALBUTEROL sample

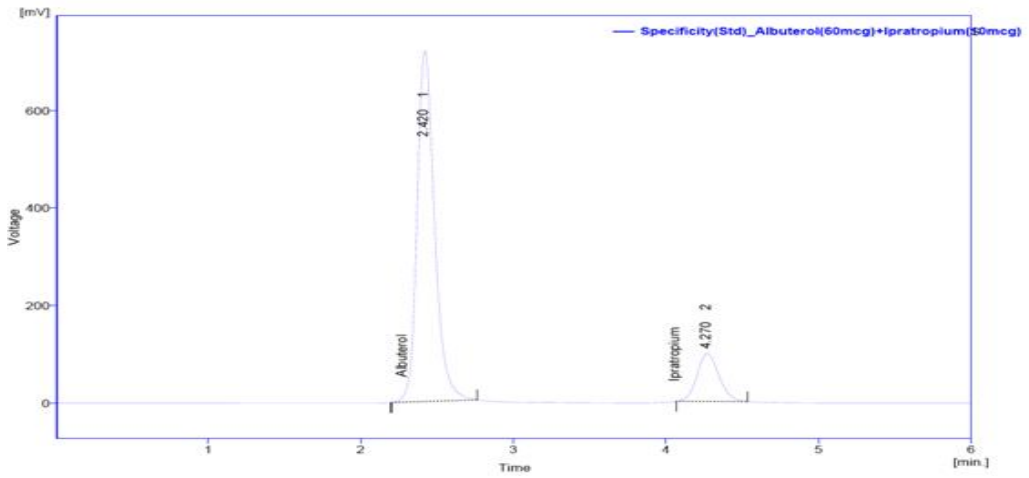


Fig: Chromatogram for Specificity of Ipratropium and ALBUTEROL standard

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Observation

It is observed from the above data; diluents or excipients peaks are not interfering with the Ipratropium and ALBUTEROL peaks.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of ALBUTEROL and IPRATROPIUM BROMIDE (microgram/ml) were prepared by dissolving 60 mg of ALBUTEROL and 40 mg of IPRATROPIUM BROMIDE dissolved in sufficient mobile phase and dilute to 100 ml with mobile phase. Further dilutions were given in the table

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)	
			ALBUTEROL	IPRATROPIUM BROMIDE
Preparation 1	0.6	10	36	6
Preparation 2	0.8	10	48	8
Preparation 3	1.0	10	60	10
Preparation 4	1.2	10	72	12
Preparation 5	1.4	10	84	14

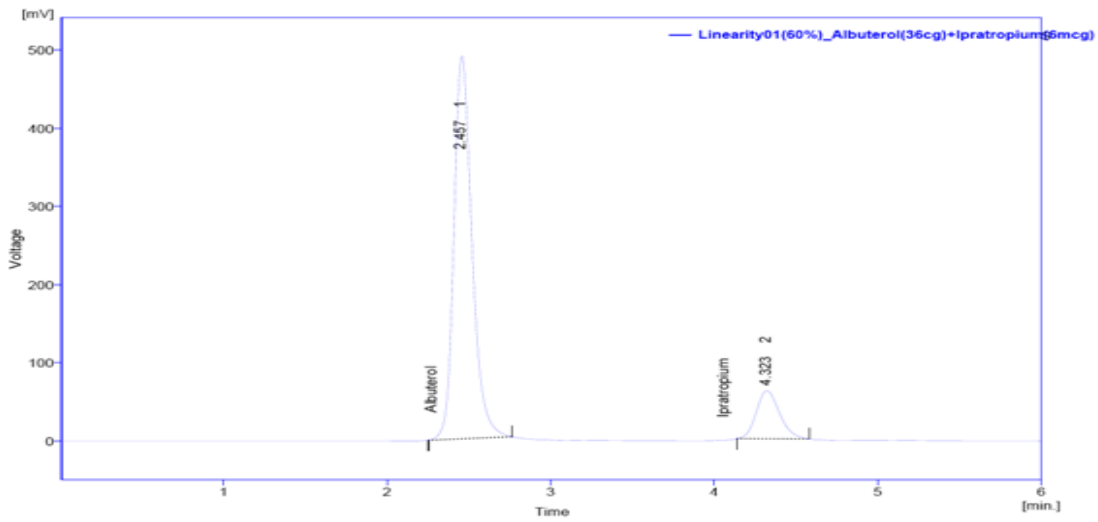


Fig: Chromatogram of Ipratropium and ALBUTEROL preparation-1

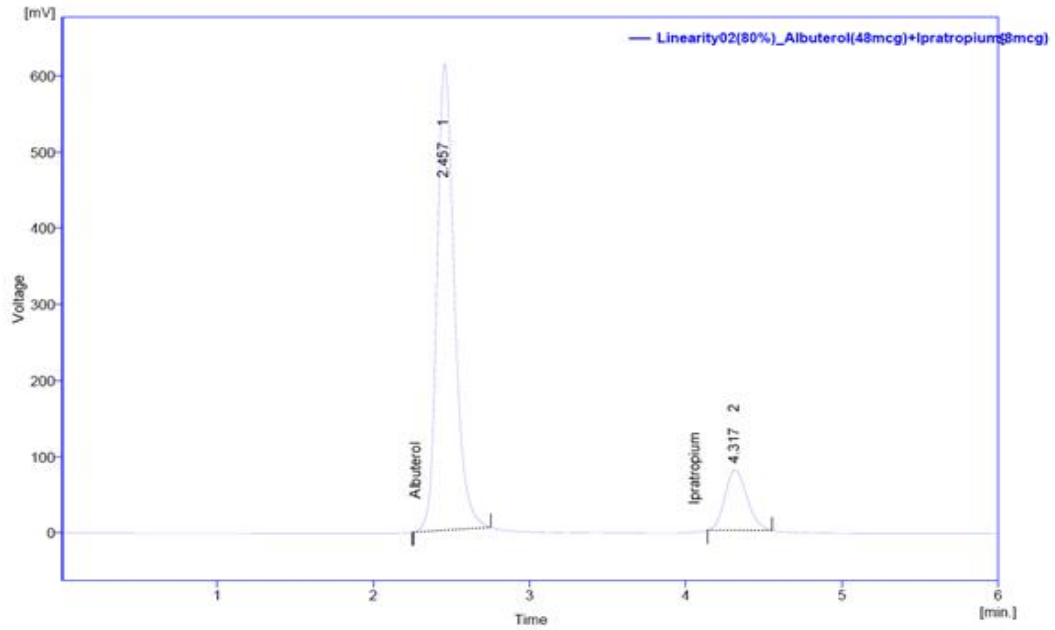


Fig: Chromatogram of Ipratropium and ALBUTEROL preparation-2

Fig: Chromatogram of Ipratropium and ALBUTEROL preparation-3

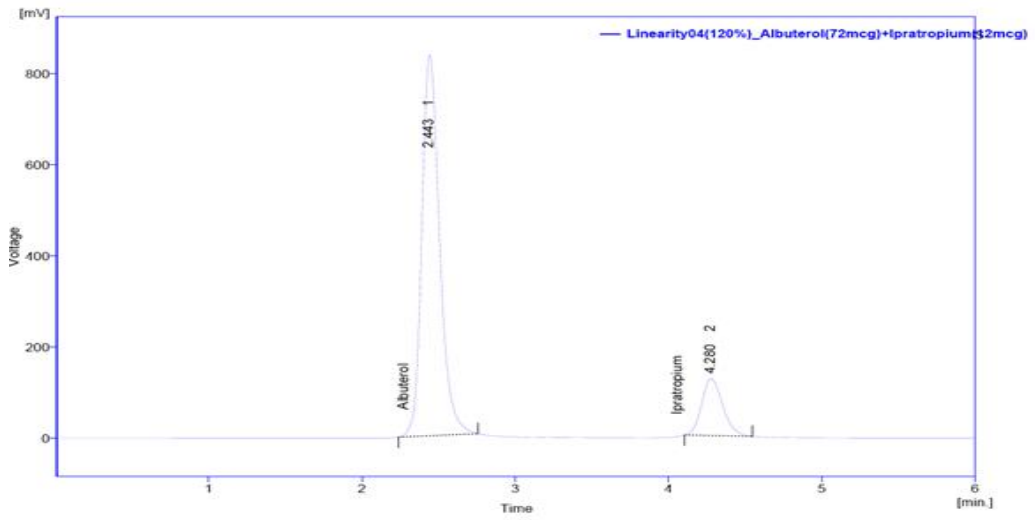


Fig: Chromatogram of Ipratropium and ALBUTEROL preparation-4

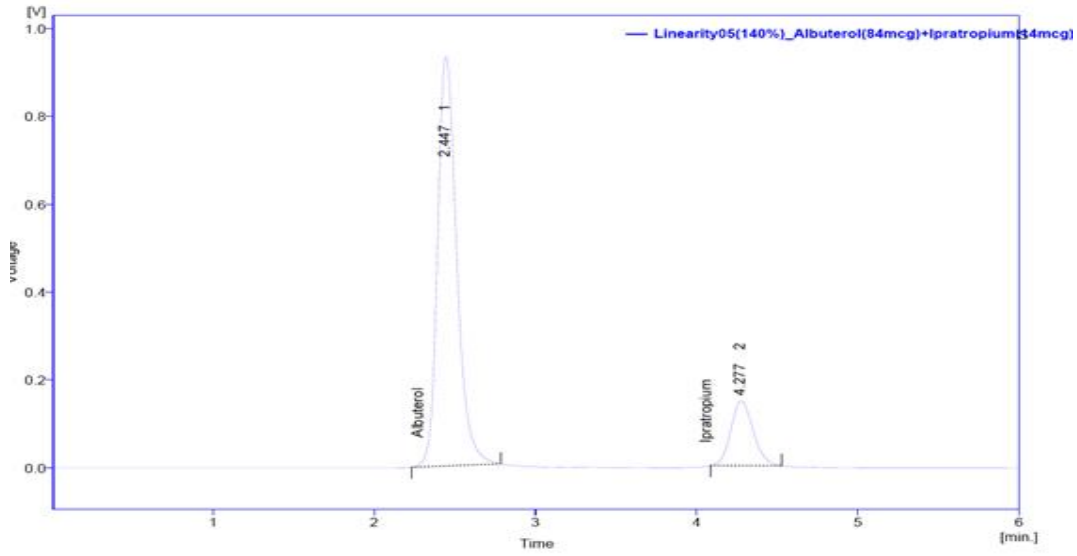


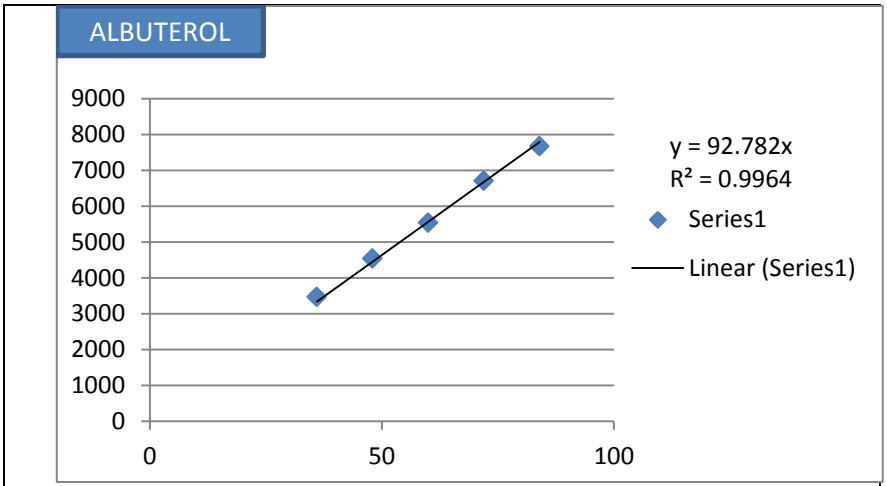
Fig: Chromatogram of Ipratropium and ALBUTEROL for preparation-5

Table: linearity of ALBUTEROL

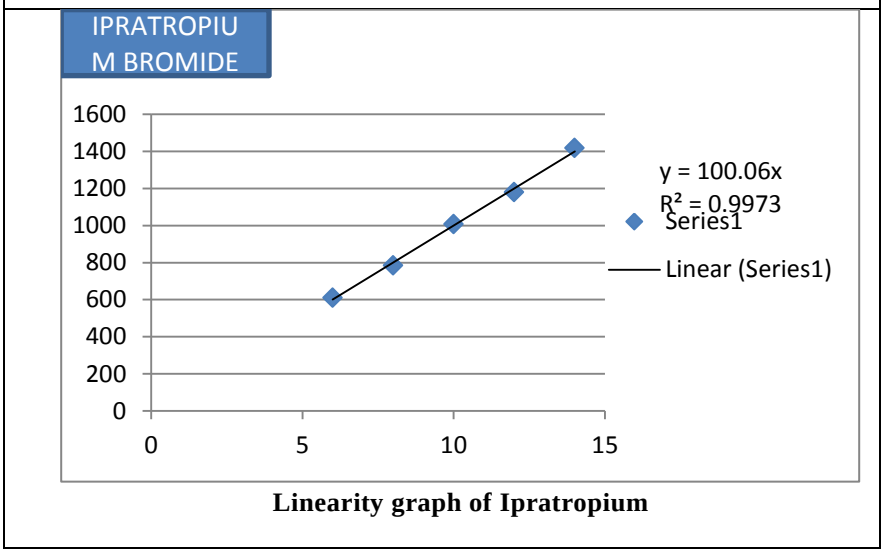
S. No.	Conc.(µg/ml)	Area
1	36	3769.742
2	48	4743.960
3	60	5538.159
4	72	6714.107
5	84	7678.012

Table: linearity of IPRATROPIUM BROMIDE

S. No.	Conc.(µg/ml)	Area
1	6	609.077
2	8	774.576
3	10	1007.518
4	12	1180.863
5	14	1417.216



Linearity graph of ALBUTEROL



Linearity graph of Ipratropium

The relationship between the concentration of ALBUTEROL and IPRATROPIUM BROMIDE and area of ALBUTEROL and IPRATROPIUM BROMIDE 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 ALBUTEROL and IPRATROPIUM BROMIDE is 0.996 and 0.997. The relationship between the concentration of ALBUTEROL and IPRATROPIUM BROMIDE and area of ALBUTEROL and IPRATROPIUM BROMIDE 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%.

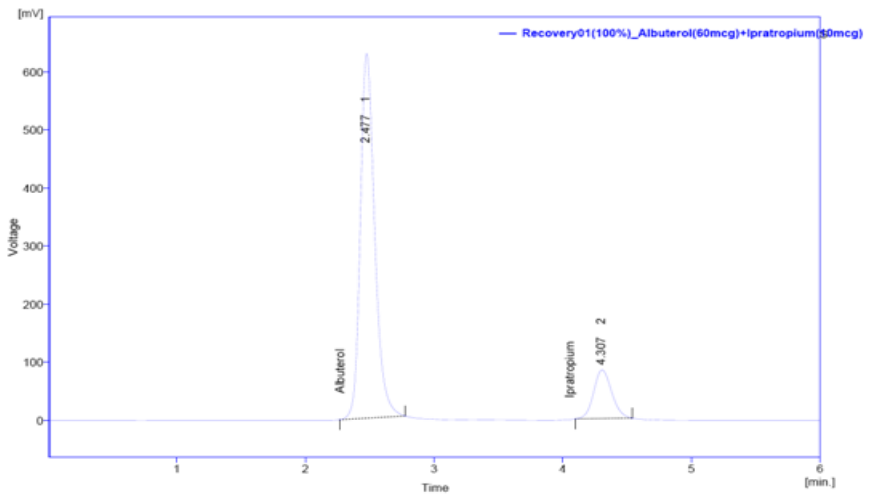


Fig: Chromatogram of 50% recovery (injection 1)

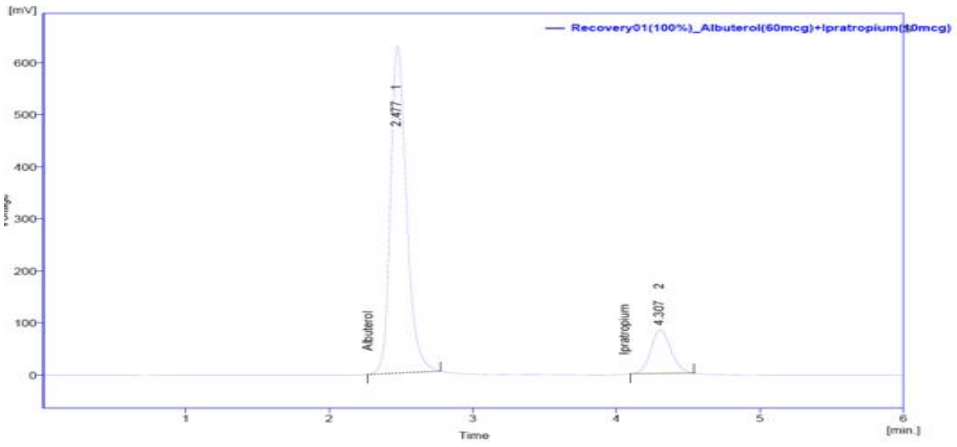


Fig: Chromatogram of 100% recovery (injection 2)

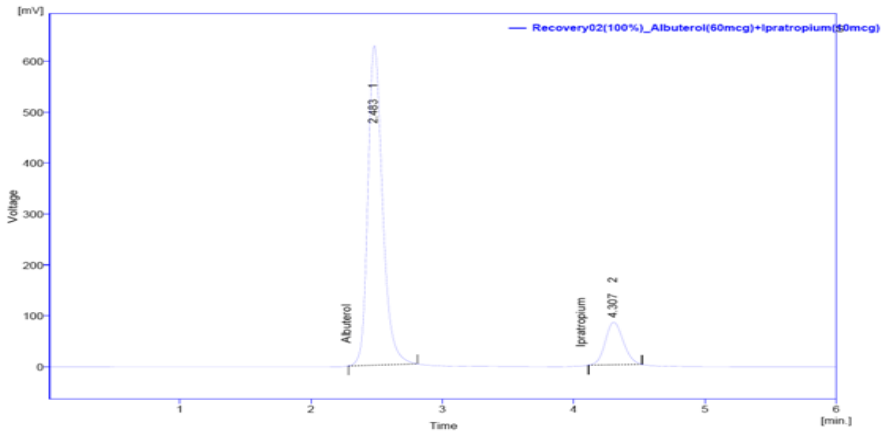


Fig: Chromatogram of 150% recovery (injection 3)

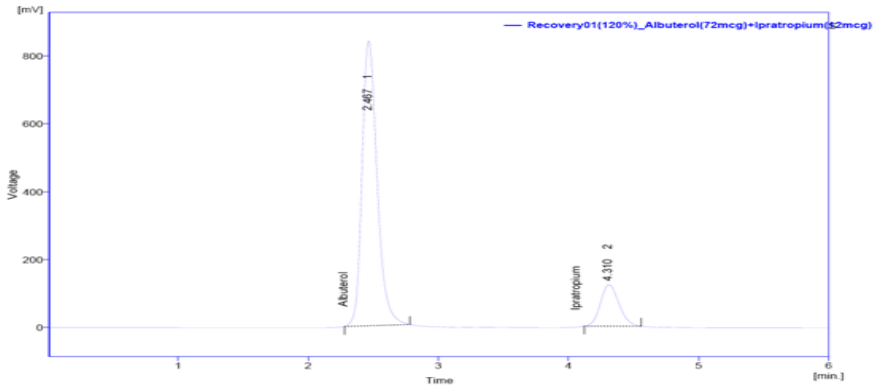


Fig: Chromatogram of 50% recovery (injection 1)

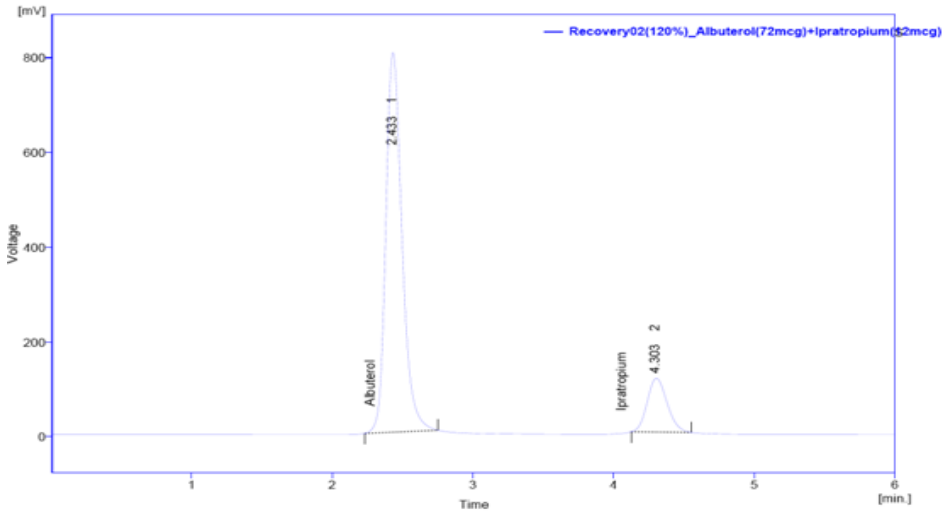


Fig: Chromatogram of 100% recovery (injection 2)

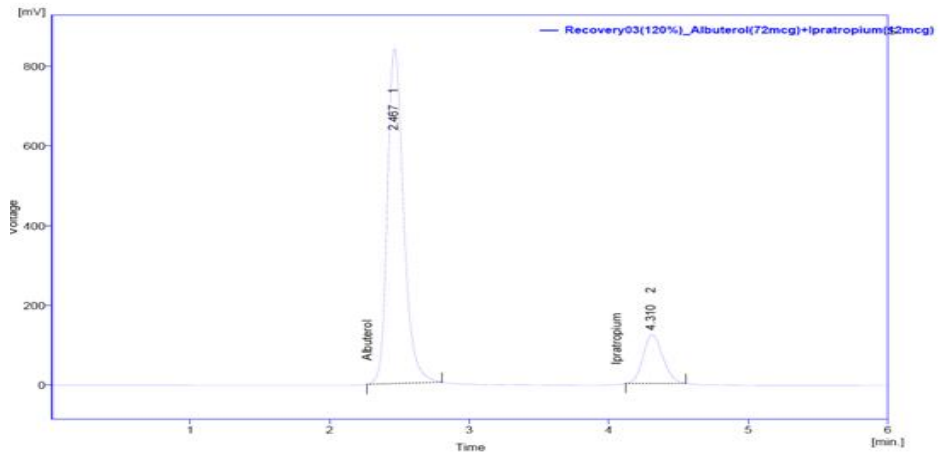


Fig: Chromatogram of 150% recovery (injection 3)

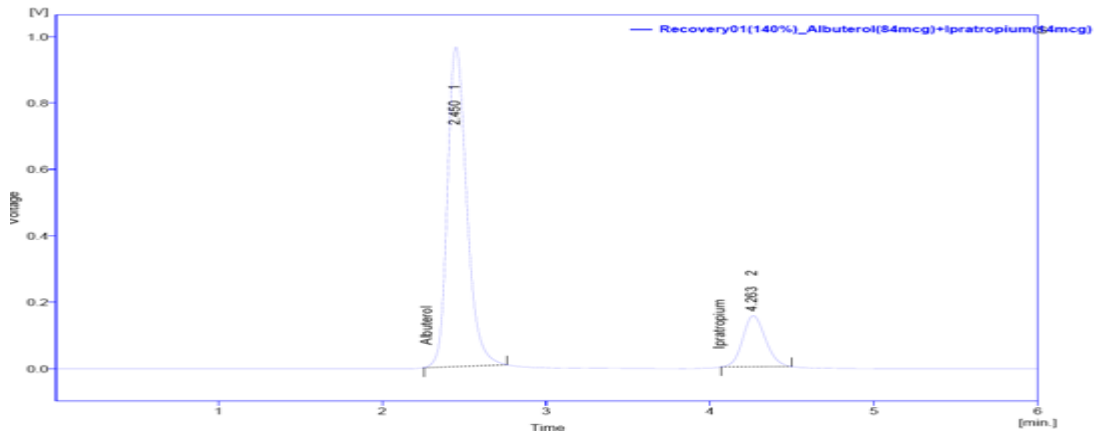


Fig: Chromatogram of 50% recovery (injection 1)

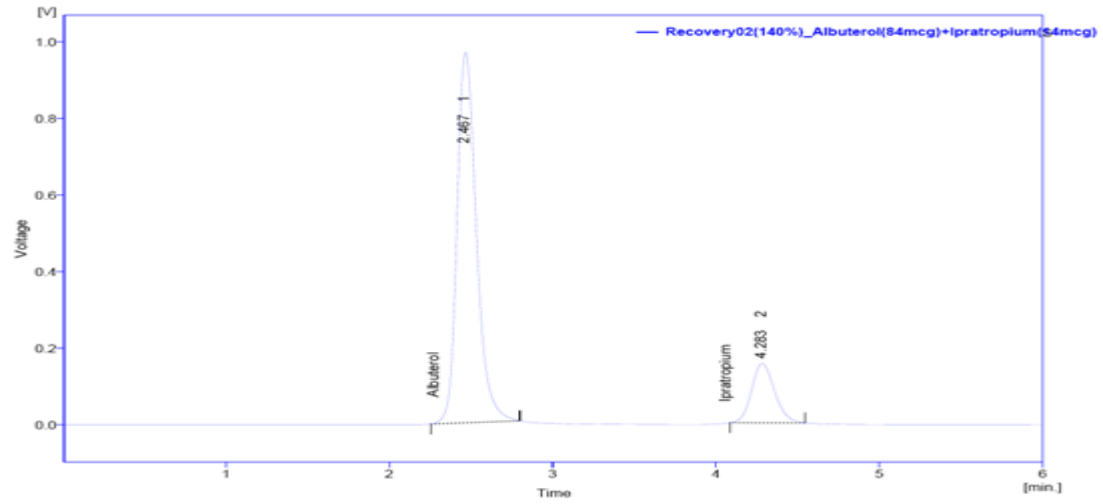


Fig: Chromatogram of 100% recovery (injection 2)

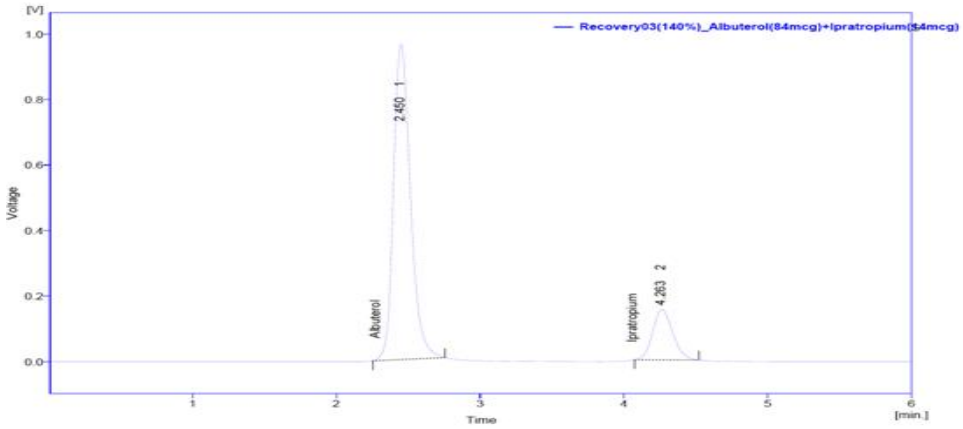


Fig: Chromatogram of 150% recovery (injection 3)

Acceptance criteria

The % recovery of Ipratropium and ALBUTEROL should lie between 98% and 110%.

Table: Recovery results for IPRATROPIUM

Recovery level	Accuracy ALBUTEROL					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
100%	60	4879.059	4878.164	31.78	85.95	98.33
	60	4874.809				
	60	4880.624				
120%	72	6715.130	6642.086	40.04	117.03	
	72	6416.984				
	72	6794.146				
140%	84	7889.449	7914.480	79.76	92.03	
	84	7976.993				
	84	7876.999				

Table: Recovery results for ALBUTEROL

Recovery level	Accuracy IPRATROPIUM BROMIDE					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
100%	10	815.841	808.663	8.7	83.55	102.45
	10	793.602				
	10	816.547				
120%	12	1211.449	1177.796	10.02	121.69	
	12	1115.460				
	12	1206.480				
140%	14	1483.271	1497.44	13.03	102.11	
	14	1515.624				
	14	1493.453				

Observation

The percentage mean recovery of Ipratropium and Albuterol is 104.74% and 109.08 % respectively.

Precision

Method precision

Method precision

Prepared sample preparations of IPRATROPIUM BROMIDE and ALBUTEROL as per test method and injected 6 times in to the column.

Acceptance criteria

The % Relative standard deviation of Assay preparations of IPRATROPIUM BROMIDE and ALBUTEROL should be not more than 2.0%.

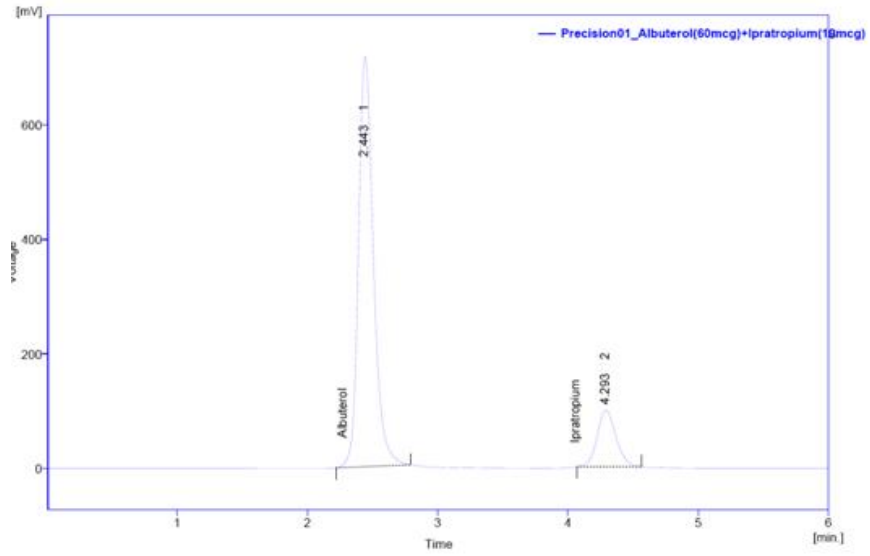


Fig: Chromatogram of precision injection 1

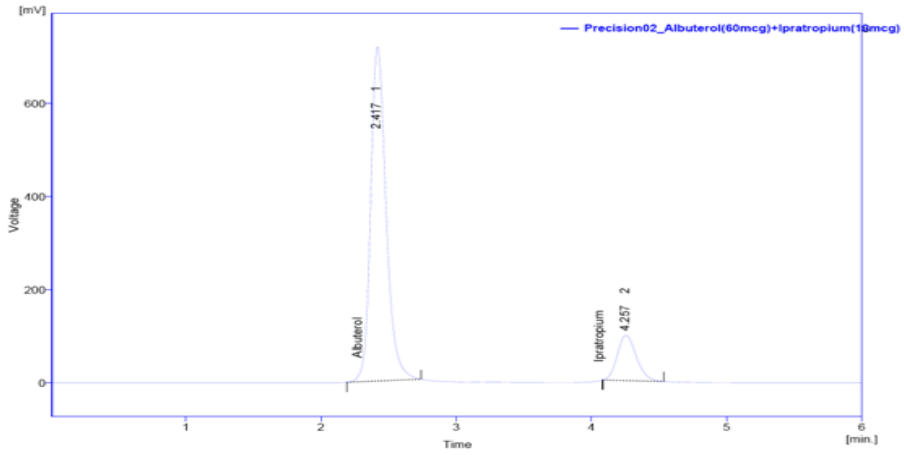


Fig: Chromatogram of precision injection 2

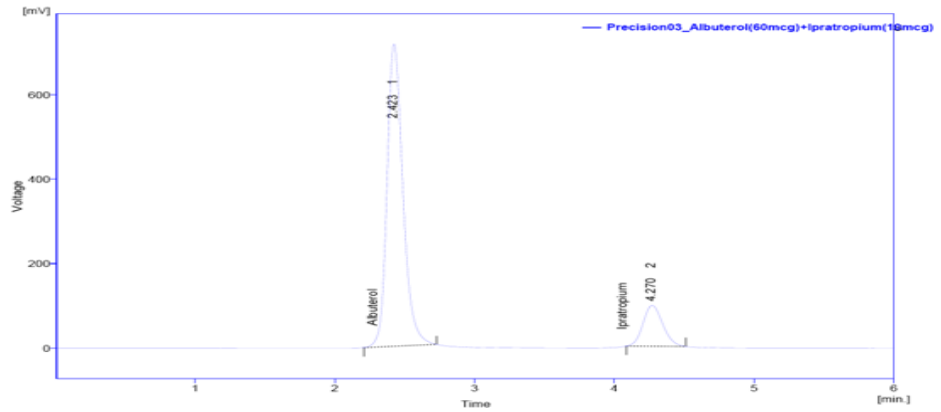


Fig: Chromatogram of precision injection 3

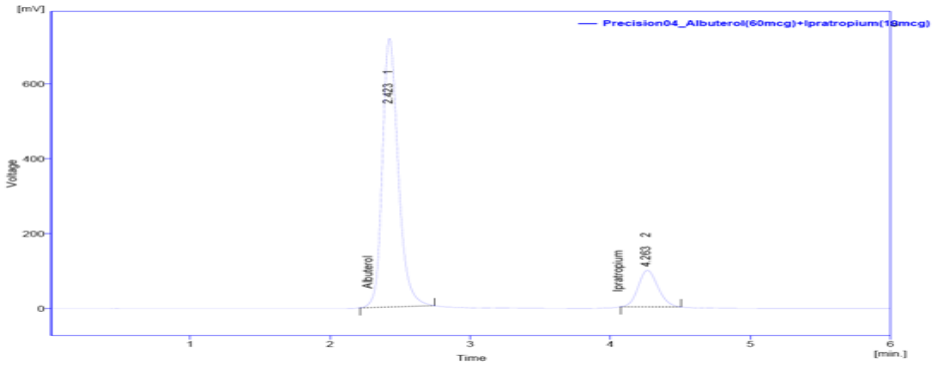


Fig: Chromatogram of precision injection 4

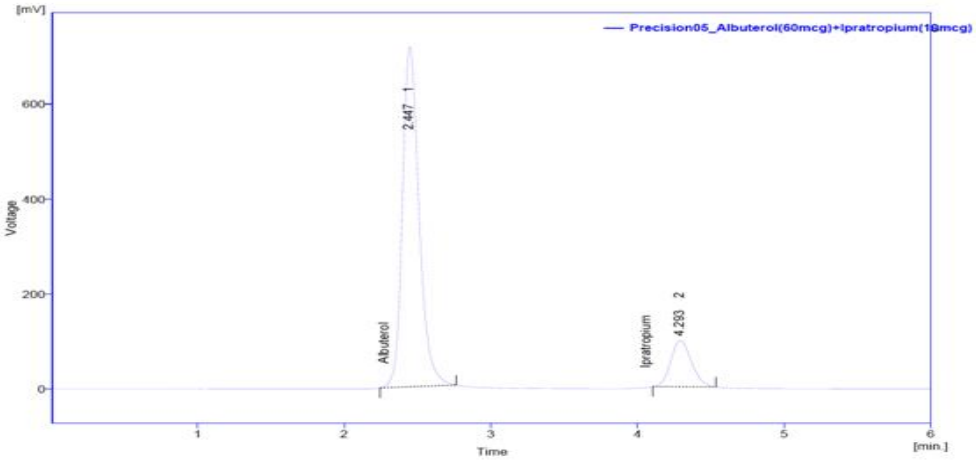


Fig: Chromatogram of precision injection 5

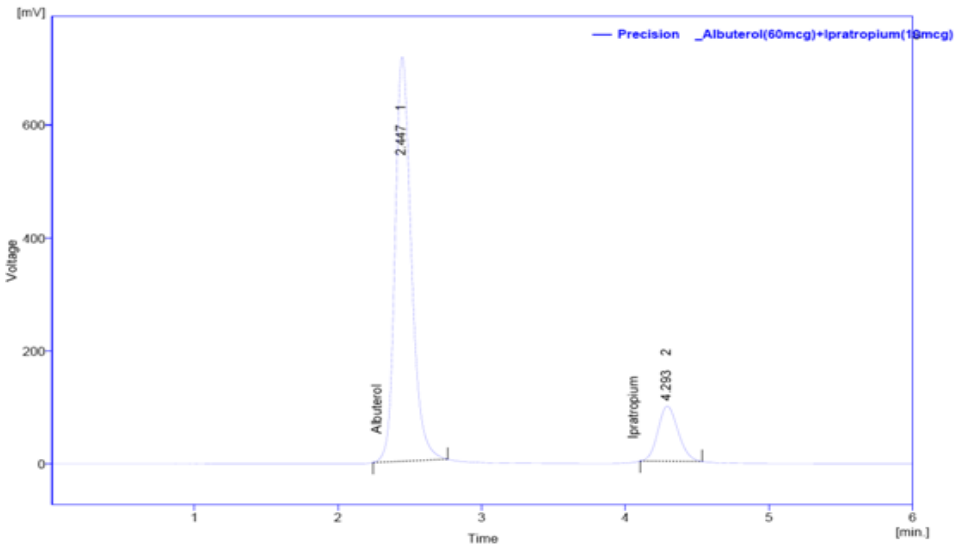


Fig: Chromatogram of precision injection 6

Table: Results for precision of Ipratropium and ALBUTEROL

ALBUTEROL			IPRATROPIUM BROMIDE	
Rt	Area	S.No.	Rt	Area
2.443	5710.568	1	4.293	991.742
2.417	5683.849	2	4.257	954.143
2.423	5662.646	3	4.270	948.278
2.423	5679.338	4	4.263	955.360
2.447	5659.977	5	4.293	951.175
2.423	5645.244	6	4.267	968.288
2.429333	5673.604	Avg	4.273833	961.4977
0.01242	22.86586	Stdev	0.015471	16.33345
0.005113	0.00403	**%RSD**	0.00362	0.016988

Observation

Test results for Albuterol and Ipratropium are showing that the % RSD of Assay results are within limits.

Robustness

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.

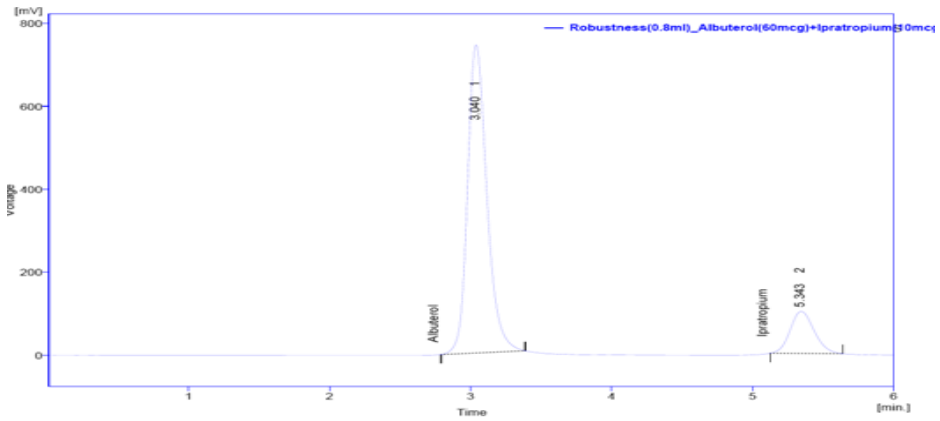


Fig: Chromatogram of Ipratropium and ALBUTEROL Robustness (0.8 ml/min)

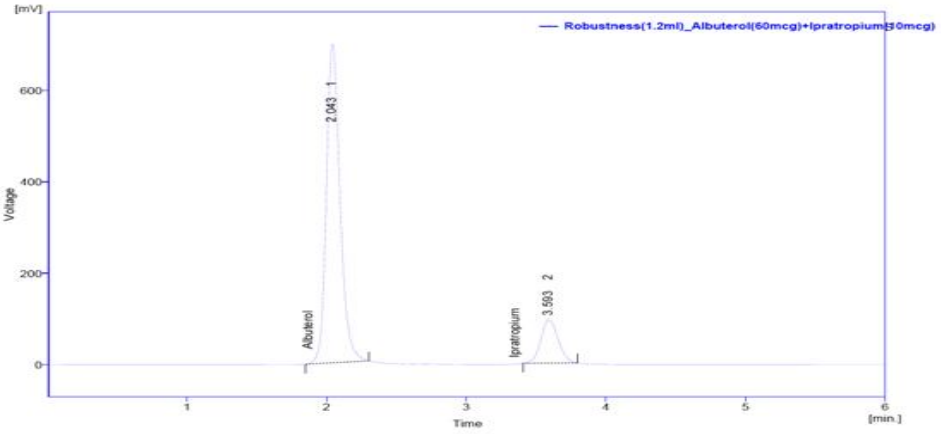


Fig: Chromatogram of Ipratropium and ALBUTEROL for Robustness (1.2 ml/min)

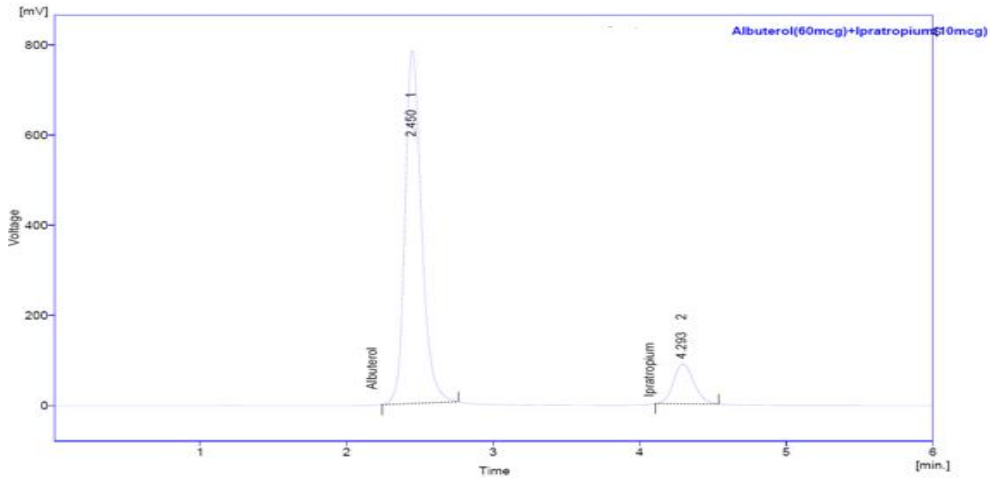


Fig: Chromatogram of Ipratropium and ALBUTEROL for Robustness (236)

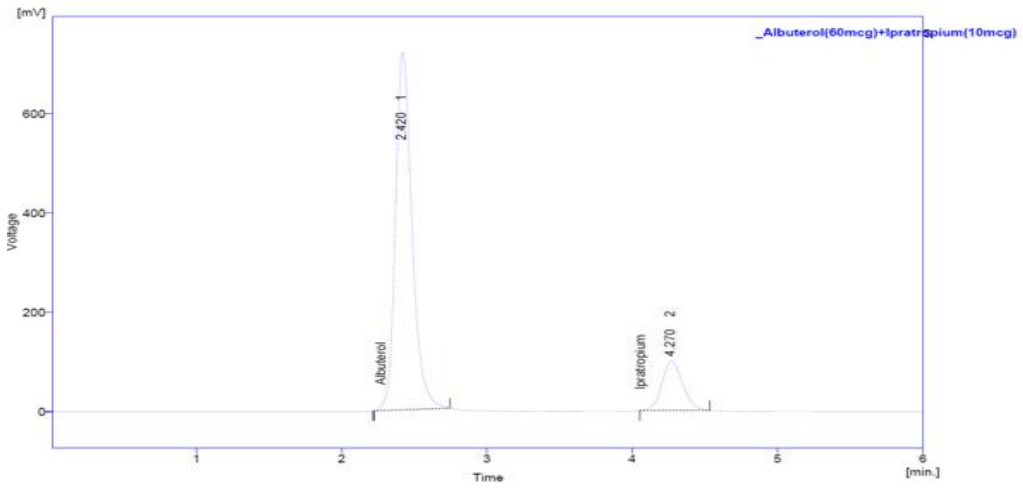


Fig: Chromatogram of Ipratropium and ALBUTEROL for Robustness (241)

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%.

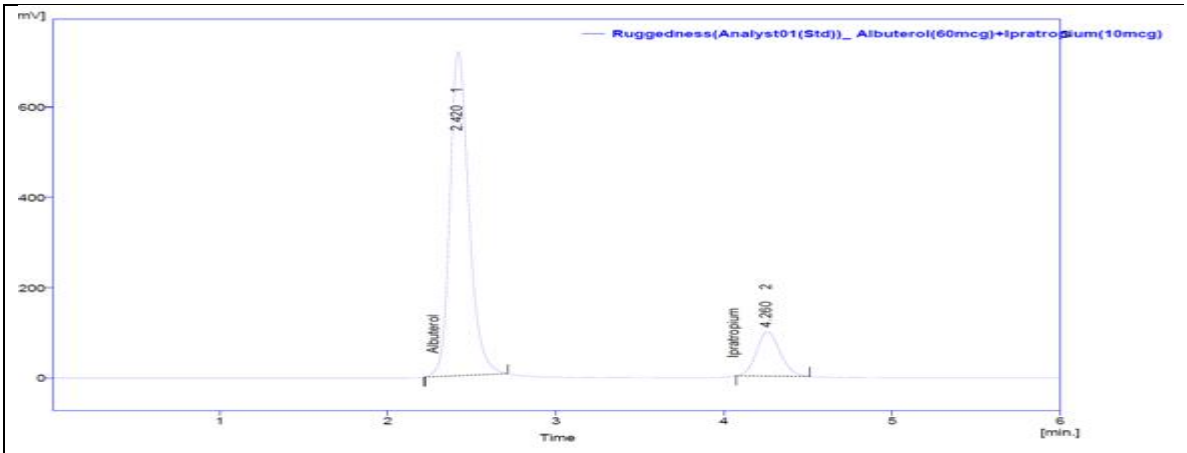


Fig: Chromatogram of Analyst 01 standard preparation

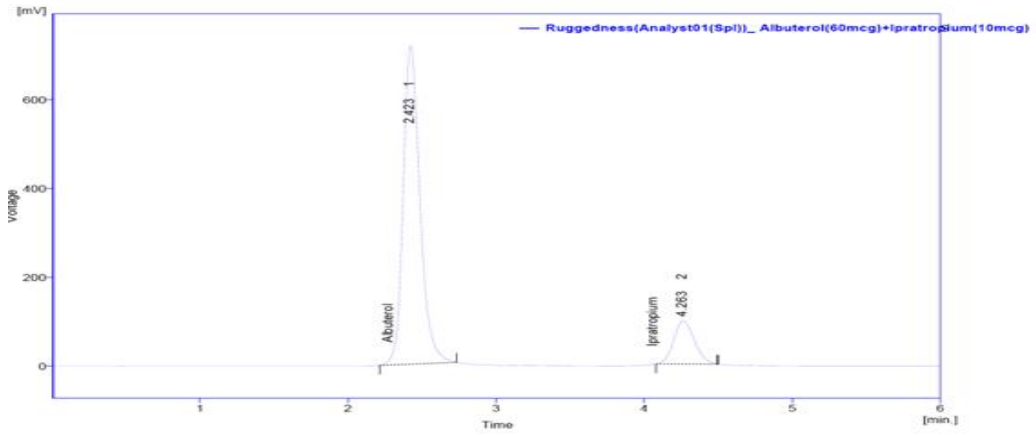


Fig: Chromatogram of Analyst 01 sample preparation

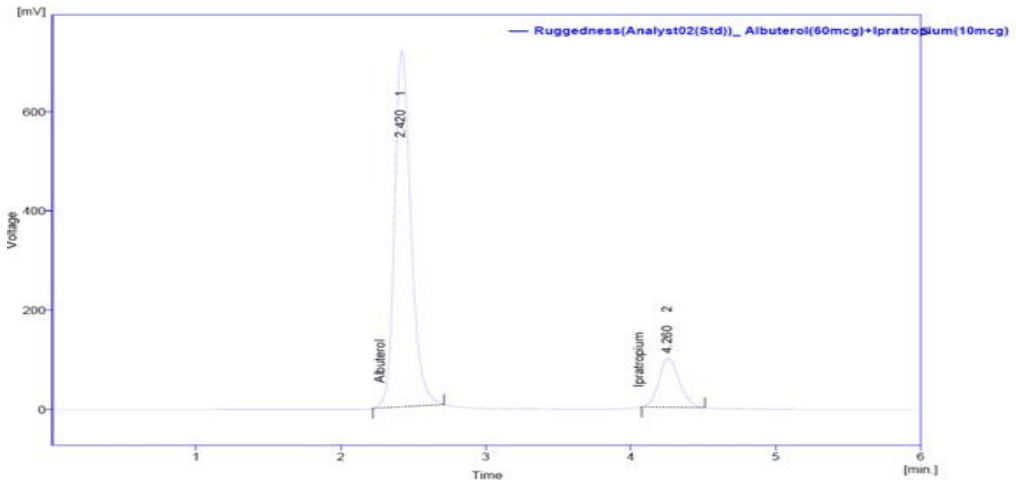


Fig: Chromatogram of Analyst 02 standard preparation

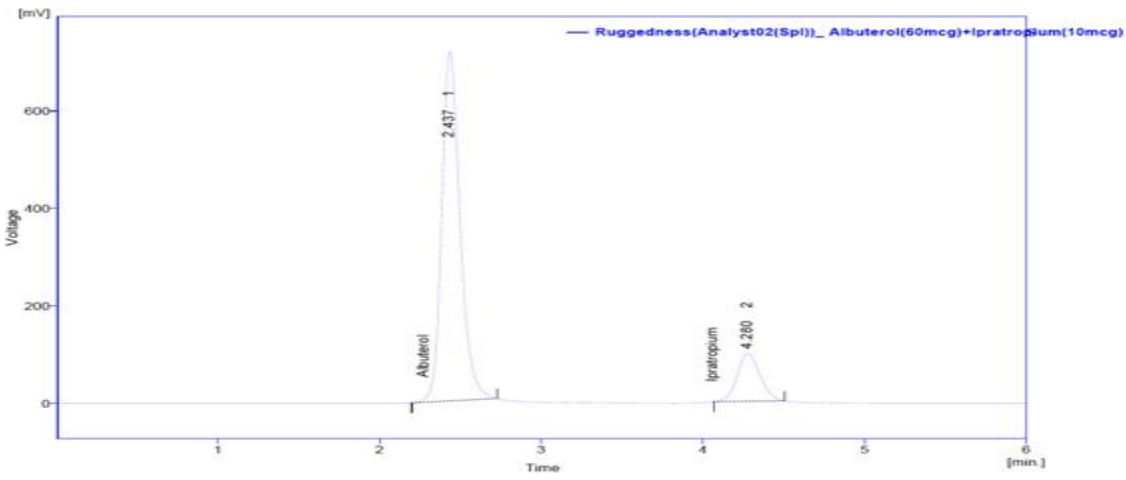


Fig: Chromatogram of Analyst 02 sample preparation

Table 9.9.5: Results for Ruggedness

ALBUTEROL	%Assay	IPRATROPIUM 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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