



**RESEARCH ARTICLE**

**An Assay Method for the Simultaneous Estimation of Albuterol and Ipratropium Bromide Using RP-HPLC Technology**

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**ABSTRACT**

A basic and specific RP-HPLC technique is depicted for the determination of Albuterol and Ipratropium Bromide in dosage forms. Chromatographic separation was accomplished on a C18 column using mobile phase consisting of a mixture of mixed Phosphate buffer pH: 3.4 Methanol (30:70 v/v), with detection of 239 nm and flow rate at 1.mL/min. Linearity was observed in the range 36-84 µg /ml for Albuterol ( $r^2 = 0.996$ ) & 6-14µg /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 strategies were validated. The exactness of the method was evaluated by recovery studies at three distinct levels. Recovery experiments showed the nonattendance of interference from regularly experienced pharmaceutical added substances or additives. The technique was observed to be exact as demonstrated by the repeatability analysis, indicating %RSD less than 2. All statistical data demonstrates legitimacy of the techniques, sensitivity, accuracy or precision and reproducibility. It can be utilized for routine investigation of pharmaceutical dose form.

**KEYWORDS**

Assay Studies, Albuterol and Ipratropium Bromide by RP-HPLC

**INTRODUCTION**

**ALBUTEROL**

Albuterol is also known as salbutamol. Albuterol is a 4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol. Salbutamol is a short-acting, selective beta2-adrenergic receptor agonist used in the treatment of asthma and COPD. It is 29 times more selective for beta2 receptors than beta1 receptors giving it higher specificity for pulmonary beta receptors versus beta1-adrenergic receptors located in the heart<sup>1-5</sup>.

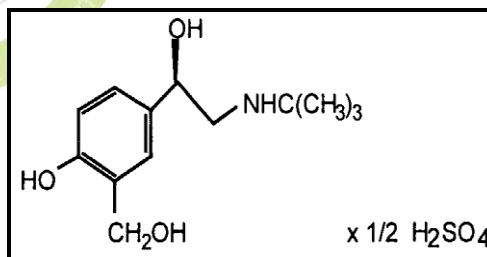


Figure 1: Molecular Structure of ALBUTEROL

**IPRATROPIUM**

Ipratropium is a 1R, 3R, 5S, 8R)-3-[(3-hydroxy-2-phenylpropanoyl) oxy]-8-methyl-8-(propan-2-yl)-8-azabicyclo [3.2.1] octan-8-ium bromide. A muscarinic antagonist structurally related to atropine but often considered safer and more effective for inhalation use. It is used for various bronchial disorders, in rhinitis, and as an antiarrhythmic<sup>4-7</sup>.

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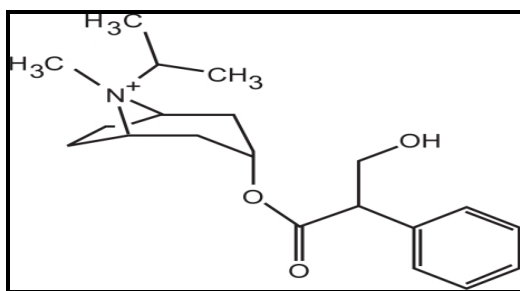


Figure 2: Molecular Structure of Ipratropium Bromide

The existing literature review shows that HPLC and derivative spectroscopic methods have been carried out. However there is a need to develop an easier and cost effective RP HPLC method development which can be carried out on small scale laboratory basis also. Hence by observing literature, this work is carried out to obtain a low retention time, and an easier and cost effective method.

## MATERIAL AND METHODS

### Materials

Albuterol and Ipratropium Bromide were gifted samples obtained from Chandra labs; Hyd. Methanol (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45  $\mu\text{m}$  filtered water used for HPLC analysis and preparation of buffer. Buffers and all other chemicals were analytical grade.

### Instrumentation

An Agilent -1220 HPLC system consisting of an Agilent pump - 2690, an inbuilt auto sampler, a column oven and Agilent 2998 wavelength absorbance detector (PDA) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was Inertsil ODS C18 (250 x 4.6 mm, 5  $\mu\text{m}$ ).

A Band line sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment. The mobile phase is a mixture of 30 volumes of mixed Phosphate Buffer pH 4.5:70 volumes of methanol were prepared with isocratic flow programming was used as mobile phase at 1 mL/min. The column was maintained at ambient temperature.

### Chromatographic Conditions

The chromatographic elution was carried out in isocratic mode using a mobile phase which is a mixture of 30 volumes of mixed Phosphate Buffer pH 4.5: 70 volumes of Methanol. The analysis was performed at ambient temperature using a flow rate of 1.2 mL/min with a run time of 6 mins. The eluent was monitored using PDA detector. The mobile phase was filtered through 0.45  $\mu\text{m}$  micron filter prior to use.

### Preparation of Standard Stock solution of Albuterol

60 mg of Albuterol and was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10  $\mu\text{g}$  /ml of solution by diluting 1 ml to 10ml with methanol.

### Preparation of Standard Stock Solution of Ipratropium Bromide

40 mg 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  $\mu\text{g}$  /ml of solution by diluting 1ml to 10ml with methanol.

### Preparation of Mixed Standard Solution

Weigh accurately 60 mg of Albuterol and 40mg 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 100 $\mu\text{g}$ /ml of Albuterol and 40 $\mu\text{g}$ /ml of Ipratropium bromide is prepared by diluting 4ml to 10ml with mobile phase. This solution is used for recording chromatogram.

### Preparation of Sample Solution

20 tablets (each tablet contains 60mg of Albuterol and 10mg of Ipratropium bromide) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Albuterol (100 $\mu\text{g}$ /ml) and Ipratropium bromide (10 $\mu\text{g}$ /ml) were prepared by dissolving weight equivalent to 60mg of Albuterol and 10mg of Ipratropium bromide 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 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 100µg/ml of Albuterol and 10µg/ml of Ipratropium bromide was made by adding 1ml of stock solution to 10 ml of mobile phase.

### **Method Validation**

The developed method was validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

### **Specificity Study**

The specificity of the RP-HPLC method was checked by comparison of chromatograms obtained from standard, sample and the corresponding placebo.

### **Linearity and Range**

The linearity of the method was determined at five concentration levels ranging from 36-84 µg/mL for Albuterol and 6 -14 µg/mL for Ipratropium bromide. The calibration curves were constructed by plotting peak areas versus concentration of Albuterol and Ipratropium bromide. The slope, Y-intercept and correlation coefficient were calculated.

### **Accuracy (% Recovery)**

The accuracy of the method was evaluated in triplicate at three concentration levels, 80, 100 and 120 % of the target test concentration (60 µg/mL of Albuterol and 10 µg/mL of Ipratropium bromide). The percentages of recoveries were calculated.

### **Precision**

Precision was investigated using the sample preparation procedure for six pure samples of Albuterol and Ipratropium bromide.

### **Method Precision (Intra-day)**

The precision of the method was evaluated by carrying out six independent assays of 60 µg/mL of Albuterol and 10 µg/mL of Ipratropium bromide test samples against qualified reference standard. Six test samples were assayed against reference standard.

### **Limit of Detection and Limit of Quantification**

The limit of detection (LOD) and limit of quantification (LOQ) were estimated using signal - to - noise ratio of 3:1 and 10:1 as per ICH guidelines.

### **Robustness**

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions: Flow rate ( $\pm 0.2$ ), column temperature ( $\pm 5^\circ\text{C}$ ) and wavelength of detection ( $\pm 2\text{nm}$ ).

### **Ruggedness**

The ruggedness of the method was evaluated by two different analyst with different instruments and lab.

### **System-Suitability Test**

The system suitability tests represent an integral part of the method and are used to ensure adequate performance of the chromatographic system. The parameters, retention time (RT), theoretical plates (N), tailing factor (T), peak asymmetry (As) and repeatability were evaluated using five replicate injections of the drugs at a concentration of 60 µg/mL of Albuterol and 10 µg/mL of Ipratropium bromide.

## **RESULTS AND DISCUSSION**

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for the simultaneous estimation of Albuterol and Ipratropium bromide in tablet dosage form, different chromatographic conditions for its validation were applied & the results observed are presented (Shown in Table 1 and 2). The results of Correlation coefficient (r) LOD, LOQ, Accuracy, Precision, Robustness and Ruggedness are shown in Table 1. The results of System Suitability Parameters consisting of Retention time, Theoretical plates, Asymmetry are shown in Table 2. The standard chromatogram of simultaneous estimation of Albuterol and Ipratropium bromide in tablet dosage form is shown in figure 5. The calibration curve of Tramadol and Acetaminophen is shown in Figure 3 and 4 respectively.

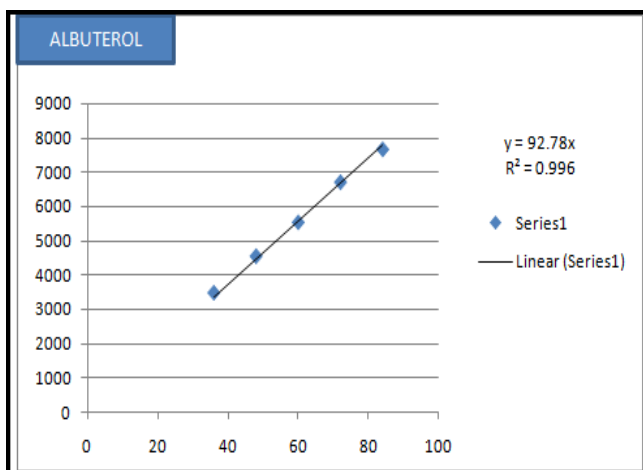


Figure 3: Calibration curve for Albuterol

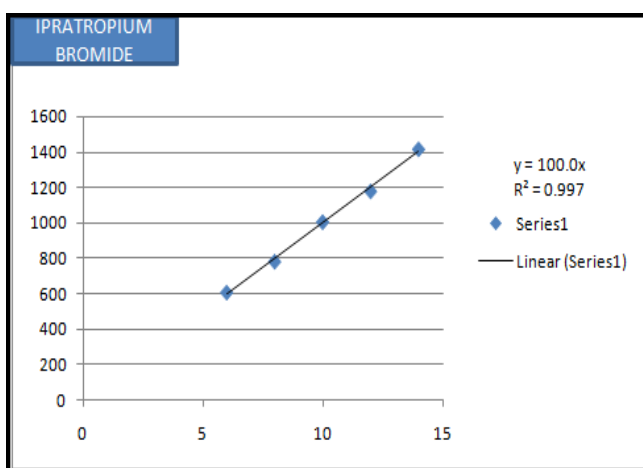


Figure 4: Calibration Curve for Ipratropium Bromide

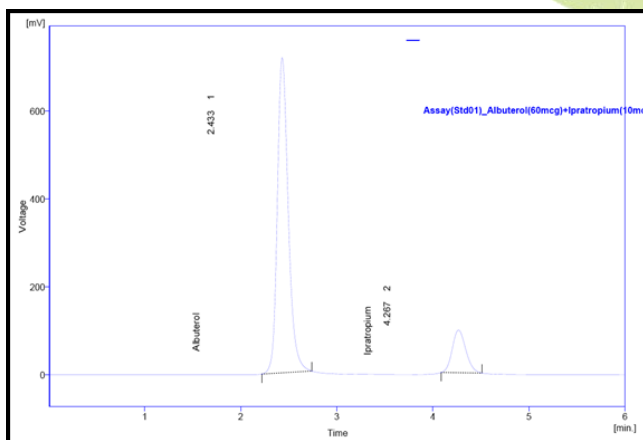


Figure 5: Standard chromatogram of simultaneous estimation of Albuterol and Ipratropium Bromide. (The amount of Albuterol and Ipratropium present in the taken dosage form was found to be 100.128% and 98.864 % respectively.)

Table 1: Results Analysis and Calibration Curves

Parameters	Albuterol	Ipratropium bromide
Correlation coefficient(r)	0.996	0.997
LOD ( $\mu\text{g/mL}$ )	0.81	0.53
LOQ ( $\mu\text{g/mL}$ )	2.46	1.633
Accuracy	98.33%	102.45%
Precision	0.76	1.33
Robustness	0.54	0.62
Ruggedness	0.13	0.42

Table 2: Results of System Suitability Parameters

Parameters	Albuterol	Ipratropium bromide
Retention time	2.503	5.177
Theoretical plates	2054	3591
Asymmetry	1.679	1.354

## CONCLUSION

A simple RP-HPLC method has been developed & validated for the simultaneous estimation of Albuterol and Ipratropium Bromide in tablet dosage form. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is suitable for assay studies which can help in the analysis of Albuterol and Ipratropium bromide in combination for tablet dosage form with the developed method, it is cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical



pharmacokinetic studies in near future. More development for the quality control studies can be performed with different techniques is highly recommended for future research work.

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