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Research Paper**VALIDATION AND ANALYTICAL METHOD FOR THE SIMULTANEOUS DETERMINATION OF ALBUTEROL SULPHATE AND IPRATROPIUM BROMIDE****Mohit Singhal*, Dilip Agrawal, Rakesh Goyal**

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A simple, sensitive and specific RP-HPLC method was developed for the quantification of related impurities of albuterol sulfate (AS) and ipratropium bromide (IB) in liquid pharmaceutical dosage form. The chromatographic separation employs gradient elution using anPeerless basic C8 (150 X 4.6 mm, 5 μ m) columns. Mobile phase consisting of mobile phase A (Mixture of anhydrous potassiumdihydrogen orthophosphate and 1-pentane sulphonicacid sodium salt monohydrate, pH 4.0 $\pm$ 0.05 (Sol. A),acetonitrile (95:5 v/v)) and mobile phase B (Mixture of anhydrous potassiumdihydrogen orthophosphate and 1-pentane sulphonicacid sodium salt monohydrate, pH 4.0 $\pm$ 0.05 (Sol. B),acetonitrile (70:30 v/v)) delivered at a flow rate of 1.0 ml min⁻¹. The analytes were detected and quantified at 276 nm and 220 nm using photodiode array (PDA) detector. The method was validated as per ICH guidelines, demonstrating to be accurate and precise (repeatability and intermediate precision level) within the corresponding linear range of known impurities of AS and IB. All these results provide the stability indicating capability of the method.

Keywords: albuterol sulfate, ipratropium bromide High Performance Liquid Chromatography, Validation**INTRODUCTION**

Racemic (R, S)—albuterol is 1:1 mixture of R-enantiomer which has bronchodilatory and anti-inflammatory effects, and S-enantiomer which is associated with increased airway hyperreactivityand pro-inflammatory effects. Ipratropium bromide (IB) (also known as Sch1000) is a new atropine-like bronchodilator drug, whose mechanism of action is via an anticholinergic pathway and may decrease cyclic guanosine monophosphate.¹ Ipratropium nasal spray is safe, effective way to treat chronic rhinorrhea in laryngectomized patients,

improving their quality of life. The potential of anticholinergics to provide bronchodilatory benefits over short-acting 2-agonists (SABA) alone in patients with moderate-to-severe persistent asthma has not been well defined AS + IB resulted in significantly greater improvement in forced expiratory volume in 1 s (FEV1) and longer duration of response compared to AS alone in patients with moderate-to-severe persistent asthma.²⁻³ The literature survey reveals that, AS is reported in USP while it is reported as salbutamol in EP. Few HPLC methods are reported for the



determination of salbutamol or albuterol, related substances and enantiomeric separation.⁴The other methods available in the literature are based on CE, LC–MS and GC–MS.⁵ Articles on the determination of IB and related substances by HPLC and spectrophotometric methods are very few. However the exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals includes these drugs in combination for the simultaneous determination of related substances of AS and IB and the information regarding the stability of the drugs is not available. So it is felt essential to develop a liquid chromatographic procedure which will serve a reliable, accurate, sensitive and stability indicating HPLC method for the simultaneous determination of related substances of AS and IB in AS + IB nasal solution.⁵⁻⁶

Regulatory agencies recommend the use of stability indicating methods (SIMs) for the analysis of stability samples.⁷This requires stress studies in order to generate the potential related impurities under stressed conditions, method development and validation. With the evident of the International Conference on Harmonization (ICH) guidelines, requirements for the establishment of SIMs have become more clearly mandated. Environmental conditions including light, heat and the susceptibility of the drug product towards

hydrolysis or oxidation can play an important role in the production of potential impurities.⁸ Stress testing can help identifying degradation products and provide important information about intrinsic stability of the drug product.

EXPERIMENTAL

Chemicals and Reagents:

Double distilled water was used. AR grade solvents were used during the whole study. HPLC grade Methanol (Merck) was used for the mobile phase. Disodium hydrogen phosphate and sodium dihydrogen phosphate were of also AR grade (Merck).

Instrument:

HPLC system (Waters Milford, USA) equipped with inbuilt autosampler and quaternary gradient pump with an on-line degasser was used. The column compartment having temperature control and photodiode array (PDA) detector was employed throughout the analysis. Chromatographic data was acquired using empower software.

Drug Standard Stock Solution:

Solutions were prepared by dissolving about 100.0 mg of albuterol sulphate and 45.0 mg of ipratropium bromide with small amount of diluent in separate 25 mL and 50 mL volumetric flasks and the volume was made up with the diluent to obtain concentrations corresponding to 4.0 mg/mL and 0.9 mg/mL respectively. (primary stock). Further 5.0 mL of each of the above solutions were transferred to a 50 mL

volumetric flask and diluted to volume with the diluent to get a 0.4 mg/mL solution of albuterol sulphate and 0.09 mg/mL of ipratropium bromide (working standard). Further dilutions were made from the working standard solution in the required concentration range.

Preparation of sample Solution:

Sample solutions were prepared by suitably diluting the albuterol sulphate and ipratropium bromide respires. Accurately pipetted about 30.0 mg equivalent of albuterol sulphate and 5.0 mg equivalent of ipratropium bromide sample was transferred into a 50 mL volumetric flask, 20 mL of diluent was added and the flask was shaken for 10 min. so as to completely extract all the drugs. The volume was then made up to the mark with diluent to get a solution containing 0.6 mg/mL of albuterol sulphate and 0.1 mg/mL of ipratropium bromide. Further dilutions were made from the above solution in the required concentration range.

Method Development and Validation:

After a series of trial and error experiments, the following HPLC conditions mentioned in Table 5.3 were optimized for the assay of albuterol sulphate and ipratropium bromide in bulk samples and nasal solutions

Parameter	Value
Column	Peerless basic C8 (150 X 4.6 mm, 5 µm) column
Mobile phase	Mobile phase A: Mixture of anhydrous potassium dihydrogen orthophosphate

	and 1-pentane sulphonic acid sodium salt monohydrate, pH 4.0 ± 0.05 (Sol. A), acetonitrile (95:5 v/v) Mobile phase B: Mixture of anhydrous potassium dihydrogen orthophosphate and 1-pentane sulphonic acid sodium salt monohydrate, pH 4.0 ± 0.05 (Sol. B), acetonitrile (70:30 v/v)
Elution mode	Gradient
Flow rate	1.0 mL/min.
Detection wave length	Albuterol sulphate 276 nm Ipratropium bromide 220 nm
Column temperature	30 °C
Volume of injection	20 µL
Run time	22.0 min.
Retention time obtained	Albuterol sulphate 2.927 min. Ipratropium bromide 10.479 min.

RESULTS AND DISCUSSION

System suitability:

System suitability testing is an integral part of many analytical procedures. System suitability studies were carried out at the beginning of each validation study by injecting five replicates of the mixed standard solution of albuterol sulphate and ipratropium bromide and the % RSD (NMT 2.0) for the peak areas obtained was calculated (system precision).

Specificity:

The specificity of the method was established by studying the albuterol sulphate and ipratropium bromide peaks in the presence of excipients. The commonly present excipients

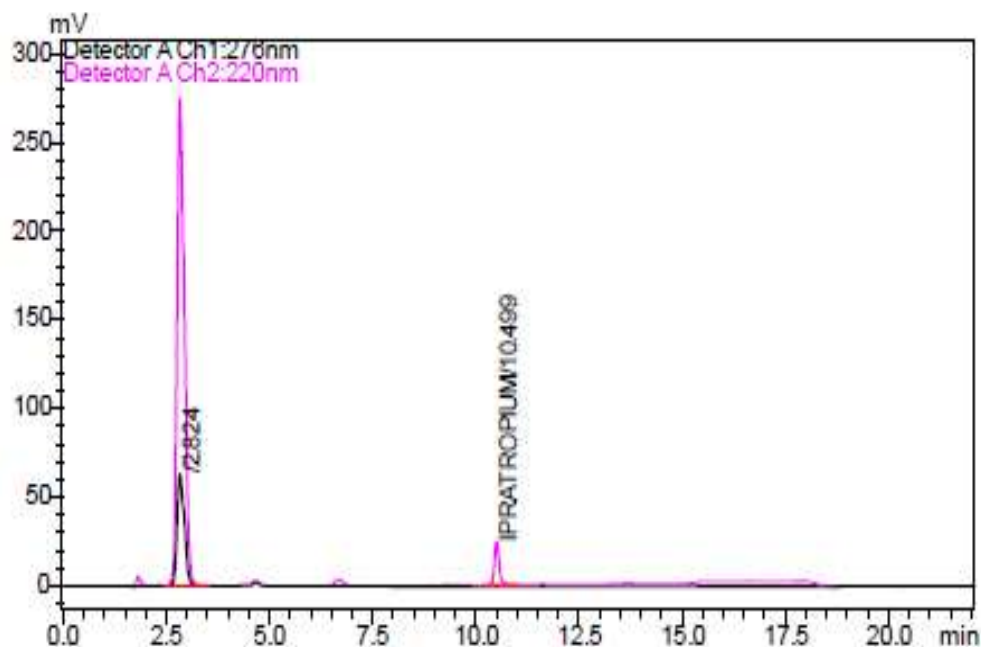


Figure 1: Chromatogram showing the separation of drugs

did not pose any interference at the retention time of the drug as they were not identified.

Linearity:

Linearity can be measured by using external standard regression method. The method obeyed linearity in the range of 0.0100 – 0.2080 mg/mL and 0.0023– 0.0468 mg/mL for albuterol sulphate and ipratropium bromide respectively as observed from the linearity curves. The regression equations for albuterol sulphate and ipratropium bromide were found to be $y = 71,64,037.851x - 2618.92$ ($R^2 = 1.0000$) and $y = 97,33,941.999x - 1469.59$ ($R^2 = 1.0000$) respectively

Precision:

Precision is the degree of repeatability. The

repeatability and intermediate precision were studied by analysing the sample solutions of albuterol sulphate and ipratropium bromide. The low coefficients of variation obtained in the intraday (0.8 % – 1.0 %) and inter day precision (1.1 % – 1.4 %) study are indicative of the precision of the method.

Accuracy:

Accuracy is a measure of degree of closeness of the experimental value with the actual value, and it can be expressed in terms of percentage of recovery. High recovery values for albuterol sulphate (98.0 – 102.0 %) and ipratropium bromide (98.1 – 101.9 %) obtained from the analysis of combined dosage forms by the proposed method indicates the accuracy of the



method.

Robustness:

Robustness is a measure of reproducibility of the results obtained by varying the test conditions. The deliberate changes in the method (flow rate and temperature) have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust (% RSD < 1.00).

CONCLUSION:

The retention times of the drugs obtained in this method were far away giving sufficient resolution for the analytes where in some of the reported methods the retention of both the drugs was very close which may be a limiting factor under deliberate variations in the method. The optimized run time was short within in which the method was proved to be stability indicating and most of the reported methods were not stability indicating.

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Conflict of Interest

The authors declare that they have no conflict of interest