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Original Article

Simultaneous Determination of Salbutamol Sulphate and Ambroxol Hydrochloride in Solid Dosage Form by RP-HPLC

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ABSTRACT

The A new reverse phase high performance liquid chromatographic method for the simultaneous determination of salbutamol and ambroxol in tablet form has been developed and validated. Salbutamol and ambroxol are frequently associated with bronchospasm. The separation was carried out by using a mobile phase which consists of methanol and 0.1 percent triethylamine at pH 3.0 (50:50). The study was carried out on Younglin isocratic HPLC System having UV detector at the wavelength of 224 nm. The stationary phase used was Cosmosil c-18 column having 4.6 mm inner diameter, 250 mm length and particle size of 5 µm, the column temperature was maintained at 25° C and injection volume of 20 µl. The retention time were found to be 3.5 minutes for ambroxol and 6 minutes for salbutamol. The peaks were well resolved and the percent recovery of salbutamol and ambroxol were within limit of 98.0 % to 102.0 %. The developed method was accurate, reproducible and therefore suitable for routine analysis.

Keywords: Ambroxol, HPLC, Salbutamol.

1. INTRODUCTION

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. Salbutamol is formulated as a racemic mixture of the R- and S-isomers. The R-

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isomer has 150 times greater affinity for the beta2-receptor than the S-isomer and the S-isomer has been associated with toxicity. This lead to the development of levalbuterol, the single R-isomer of salbutamol. However, the high cost of levalbuterol compared to salbutamol has deterred wide-spread use of this enantiomerically pure version of the drug. Salbutamol is generally used for acute episodes of bronchospasm caused by bronchial asthma, chronic bronchitis and other chronic bronchopulmonary disorders such as chronic obstructive pulmonary disorder (COPD). It is also used prophylactically for exercise-induced asthma. Ambroxol is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. The substance is a mucoactive drug with several properties including secretolytic and secretomotoric actions that restore the physiological clearance mechanisms of the respiratory tract which play an important role in the body's natural defense mechanisms. It stimulates synthesis and release of surfactant by type II pneumocytes. Surfactants act as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents.

Several methods for analysis of salbutamol and ambroxol are available in the literature [1-4]. One of these methods requires dual wavelengths in a single run [2]. Some methods use HPTLC method for simultaneous determination [5]. The use of gradient mobile phase for determination is also reported in literature [6].

The aim of this study is to develop a simple a reliable isocratic reverse phase HPLC method to quantify the analytes under study.

2. MATERIALS AND METHODS

The reference standards of salbutamol and ambroxol were obtained from paragon organics formulations private limited. Triethylamine, and orthophosphoric acid acid were used of merck hplc grade. Sal Mucolite was purchased, manufactured by Dr. Reddys. The HPLC analysis was carried out on younglin the pH measurements of mobile phase were carried out on a mettler toledo pH meter.

Chromatographic conditions

The newly optimized method used an isocratic mobile phase. The separation was carried out by using a mobile phase which consists of methanol and 0.1 percent triethylamine at pH 3.0. The study was carried out on Younglin isocratic HPLC System having UV detector at the wavelength of 224 nm. The stationary phase used was Cosmosil c-18 column having 4.6 mm inner diameter, 250 mm length and particle size of 5 µm, the column temperature was maintained at 25° C and injection volume of 20 µl. The retention time were found to be 3.5 minutes for ambroxol and 6 minutes for salbutamol.

Standard preparation: weighed 4 mg salbutamol and 60 mg ambroxol in 50 mL volumetric flask, and dissolved in 40 mL of methanol and made upto volume with the same, further transferred 5.0 mL of the above solution to 50 ml and make up to the mark with methanol.

Sample preparation: weighed 10 tablets determined the average weight of tablets, powdered and weighed powder equivalent to 4 mg salbutamol and 60 mg ambroxol in a 100 mL volumetric flask, added 70 mL of diluent, sonicate for 5 minutes with intermittent shaking ensure complete dissolution and make up to the mark with methanol. Filter 20 ml of aliquot through 0.45 µm nylon filter and transferred 10.0 mL of this solution to 50 mL volumetric flask make up to the mark with methanol.

3. RESULTS AND DISCUSSION

Initially method development was made on an isocratic system using 0.05 percent orthophosphoric acid and methanol in a proportion of 70:30 using cosmosil C-18 column 250 mm length, 4.5 mm inner diameter and 5

µm particle size using a wavelength of 224 nm and 20 µl injection volume and column temperature at 25°C, using these chromatographic conditions separations were obtained, but the peak shape of salbutamol was not proper. The salbutamol peak did not meet the acceptance criteria for asymmetry factor.

Proceeding further with few more mobile phase combinations triethylamine was used to reduce the peak tailing; also the pH 3.0 enhanced the resolution between the two peaks. The method thus developed was found to be successful for proper peak separation. The method was further subjected to validation.

Method validation

Specificity refers to the extent to which a method can determine particular analyte in mixtures or matrices without interferences from other components. In this assay, each individual excipient solution was analyzed as well as the mixture of placebo was prepared and analyzed there is no peak in the retention times corresponding to the analytes. The mixture of standard was injected and the peak of two analytes was well resolved.

Linearity and Range were carried out over a range of 30 to 150 percent of working level concentration. The linearity regression correlation coefficient, % Y-intercept and % RSD for peak area response and retention time for lower and higher range were calculated. The linearity regression correlation coefficient for the component was found within limit (Not less than 0.999). The % Y-intercept for the component was found within the limit (Not more than +2.0).

Accuracy was determined by spiking the placebo preparation with 50, 80, 100, 120 and 150 percent of working level concentration of analyte mixture, prepared in triplicate for each level in six replicates for 100 % level and the percentage recovery were calculated for each level separately. The percentage recoveries observed for the levels were found well within the limit set for the accuracy study (Not less than 98.0% and not more than 102.0%).

For precision six injections of standard solution and six sample preparations were injected into the chromatographic system and the assay were calculated. For intermediate precision same sequence of precision was injected using new standard and sample preparation on the next day by another analyst. The difference in assay results of precision and intermediate precision was between ± 2.0 %. [7, 8]

The robustness of method was carried out by changing the different chromatographic conditions (one at a time) such as:

1. Change in flow rate from 1.0 to 1.1 ml/min
2. Change in flow rate from 1.0 to 0.9 ml/min
3. Change in wavelength 224 nm to 228 nm
4. Change in wavelength 224 nm to 220 nm

The % RSD of standards and the assay values was found to be within limit for each change in parameter.

Table 1: Statistical evaluation of linearity data

Component	correlation coefficient	Slope	Intercept
Salbutamol	0.999	0.0344	0.02
Ambroxol	0.9996	1.20532	1.14

Table 2: Statistical evaluation of accuracy data

Recovery level	Salbutamol	Ambroxol
50	98.2	98
80	99.2	99.2
100	98.5	98.7
120	99.6	98.6
150	98.4	99.3

Table 3: Statistical evaluation of precision data

Component	Analyst I	Analyst II	Difference
Salbutamol	100.8	99.9	0.9
Ambroxol	99.8	98.6	1.2

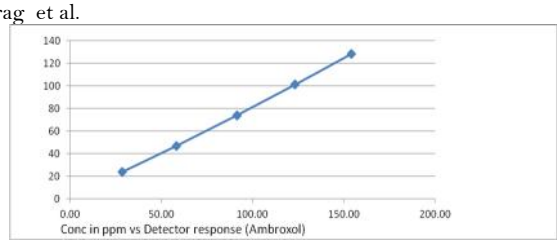

Fig 1: Concentration in ppm versus detector response for Ambroxol

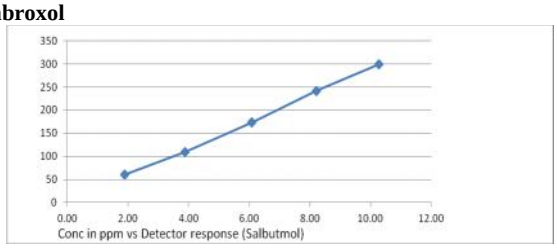

Fig 2: Concentration in ppm versus detector response for Salbutamol

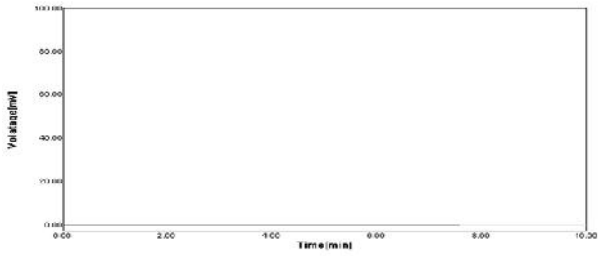

Fig 4: Chromatogram of diluent

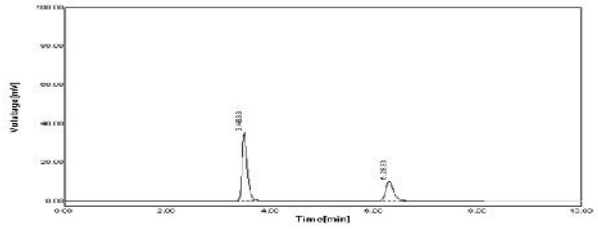

Fig 5: Chromatogram of standard solution

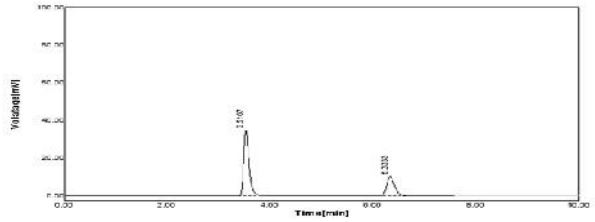

Fig 6: Chromatogram of sample solution

4. CONCLUSION

The method developed in this work is simple, sensitive, precise and accurate and hence can be used for the routine analysis of salbutamol and ambroxol in tablet dosage form.

5. ACKNOWLEDGEMENTS

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