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Method Development and Validation of Levosalbutamol by RP-HPLC In Bulk And Nebulizer Dosage Form

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ABSTRACT

A simple, precise, accurate and stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method is developed for estimation of Levosalbutamol sulphate and Ipratropium Bromide in bulk and nebulizer dosage form. The method employed, with reverse phase Inertsil® 5µ C18 (250 × 4.0 mm) column in an isocratic mode, with mobile phase of acetonitrile: buffer in the ratio 77:23 (%v/v). The flow rate was 1.3 ml/min and effluent was monitored at 210 nm. Retention time was found to be 3.05 min., and 10.59 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 25 – 150% of the working concentration (r2 > 0.999) respectively. The LOD and LOQ values for were found to be0.72,0.43, 1.24and 0.97 and µg/ml respectively. No chromatographic interference from placebo and degradants were found. The proposed method was successfully used for estimation of Levosalbutamol sulphate and Ipratropium bromide in bulk and nebulizer dosage forms.

Keywords: Levosalbutamol sulphate, Ipratropium bromide, RP-HPLC, Validation, Stability-indicating method.

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INTRODUCTION

Levosalbutamol sulphate is β2-adrenoreceptor agonist, indicated for the relief of bronchospasm in conditions such as asthma and chronic obstructive pulmonary disease. It is chemically (R)-1-(4-hydroxy-3-hydroxy-methyl phenyl)-2-(ter-butylamino) ethanol sulphate (Figure 1a) Levosalbutamol is a single isomer β2- adrenoceptor agonist that differs from racemic Salbutamol by elimination of (S)-Salbutamol. Clinical and mechanistic studies have demonstrated that (R)-Salbutamol alone provides the β2-agonist activity that is needed for the relief of bronchoconstriction. Evidence from clinical studies shows delayed recovery from exacerbation of asthma by patients who are exposed to high concentrations of (S)-Salbutamol.Thus, when compared with racemic salbutamol, clinically comparable Broncho-dilation can be achieved with doses that substantially decrease β-mediated side effects.1

Ipratropium bromide, (1R, 3r, 5S, 8r)-3-[[(2RS)-3-hydroxy-2-phenylpropanoyl] oxy]-8-methyl-8-(1-methylethyl)-8-azoniabicyclo [3.2.1] octane bromide monohydrate (Figure 1b), is a Short-acting Bronchodilators, Anticholinergic used by inhalation in the management of asthma and allergic rhinitis.

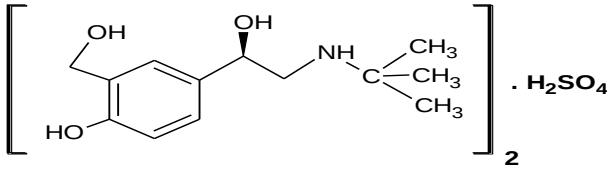

Figure 1(a): Structure of Levosalbutamol sulphate.

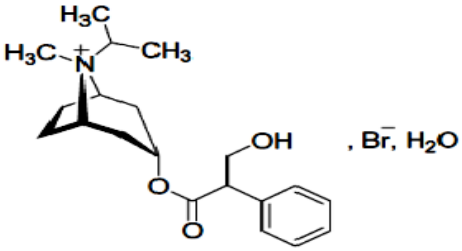

Figure 1(b): Structure of Ipratropium bromide.

The aim of this study was to develop a RP HPLC method for the quantitative simultaneous determination of Levosalbutamol sulphate and Ipratropium bromide. The method developed was validated as per ICH Q2 (R1).3-4

MATERIALS AND METHODS

Chemicals and Reagents

HPLC grade acetonitrile, Potassium dihydrogen, Heptane-1-Sulphonic acid sodium salt and orthophosphoric acid were used to prepare the mobile phase and were purchased from Merck Specialties. Deionized and purified water using a Milli-Q system (Millipore) was used for the

mobile phase and the standard solutions preparation. All experiment was performed using class volumetric glassware. All other reagents were of analytical grade.

Instrument and Chromatographic Conditions

Shimadzu LC 2010 CHT HPLC was used for the chromatographic separation equipped with auto sampler and Photo diode array (PDA) detector. The software used was LC Solution. The chromatographic separation of Levosalbutamol sulphate and Budesonide were carried out using Inertsil C18 250 x 4.0 mm, 5µ reverse phase analytical column. Mobile phase consisted of Acetonitrile: Buffer (2.5 g Potassium dihydrogen Phosphate, 2.77 g Heptane 1-Sulfonic acid sodium salt in 950 ml of water. Adjust pH 3.85 with orthophosphoric acid and dilute to 1000 ml with water.) In the ratio 77: 23.The mobile phase was filtered by passing it through 0.45 µm nylon membrane filter and the filtrate is degassed by using bath sonicator. Mobile phase is used as diluent. Injection volume was 50 µL. Oven temp was set at 35o C. The mobile phase was pumped at 1.3 ml/min at room temperature. Detection was carried by using wavelength 210 nm.

Preparation of standard and test solution

Take 30 mg Levosalbutamol Sulphate working standard and 25 mg Ipratropium bromide in 100 mL volumetric flask and make up volume up to the mark by Diluent. Further dilute 5 mL of this solution up to 50 mL with Diluent. (100 and 40 ppm)

RESULTS AND DISCUSSION

Method Development 5-10

Different columns containing L1 and L7 stationary phase were tried for separation and resolution. Inertsil-3V column was found satisfactory over the other columns. The UV spectrum of Levosalbutamol sulphate and Ipratropium bromide were scanned on photo diode array detector for selecting the optimum wavelength. A typical HPLC chromatogram for simultaneous determination of Levosalbutamol sulphate from Ipratropium bromide pharmaceutical formulation is shown in figure 2 and 3. Results of the developed method are shown in table 1.

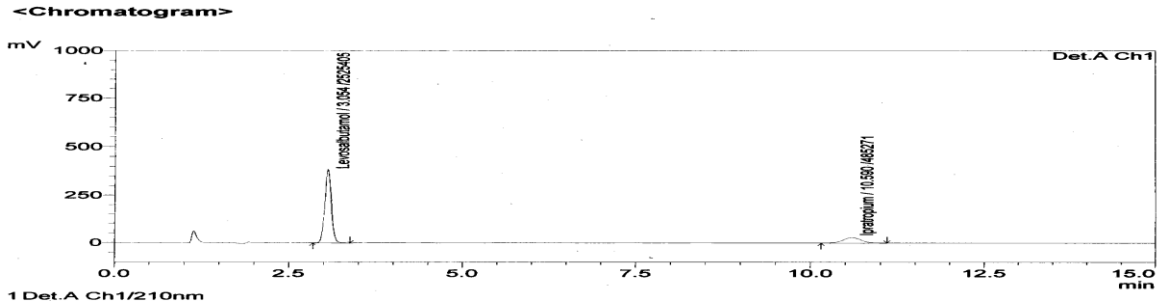

Figure 2: Chromatogram Levosalbutamol sulphate and Ipratropium bromide in standard preparation

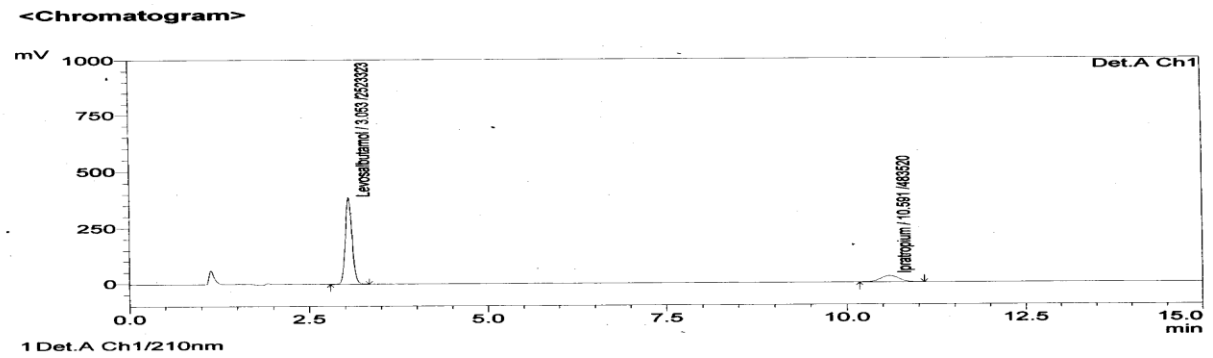

Figure 3: Chromatogram Levosalbutamol sulphate and Ipratropium bromide in sample preparation

Method Validation

Specificity

The test was carried out by injecting 20 µl standard solutions of Levosalbutamol sulphate (1.25 mg/2.5ml), and Budesonide (0.5 mg/2.5ml) in five replicates. The RSD values for areas of Budesonide and Levosalbutamol sulphate standard were found 0.12%, 0.15 % respectively. Resolution, Theoretical plates and Tailing factor were determined. Results are shown in table 1.

Table 1: System Suitability Parameters

S. No	System suitability parameters	Limit	Observation	
			Levosalbutamol sulphate	Ipratropium bromide
01	Theoretical plate	NLT 3000	4360.21	8387.79
02	Tailing Factors	NMT 2.0	1.11	1.01
03	Similarity Factor	0.980-1.020	0.99	0.99
04	% RSD of area response for six replicate standard	NMT 2.0 %	0.083%	0.510%
05	% RSD of retention time response for six replicate standard	NMT 1.0 %	0.0267%	0.0162%

Linearity

The linearity of an analytical procedure within a given range is its ability to obtain test results, which are directly proportional to the concentration of analyte in the standard. The range is derived from the linearity studies. A linearity standard solution was prepared at about 25%, 50%, 75%, 100%, 125% and 150% of the standard solution concentration and then linearity correlation coefficient of Levosalbutamol sulphate and Ipratropium bromide obtained from the graph obtained by plotting area count on Y axis and concentration on X axis. Correlation coefficient of Levosalbutamol sulphate and Ipratropium bromide are shown in table 2.

Table 2: Correlation Coefficient

Levosalbutamol sulphate	Ipratropium bromide
0.9985	0.9937

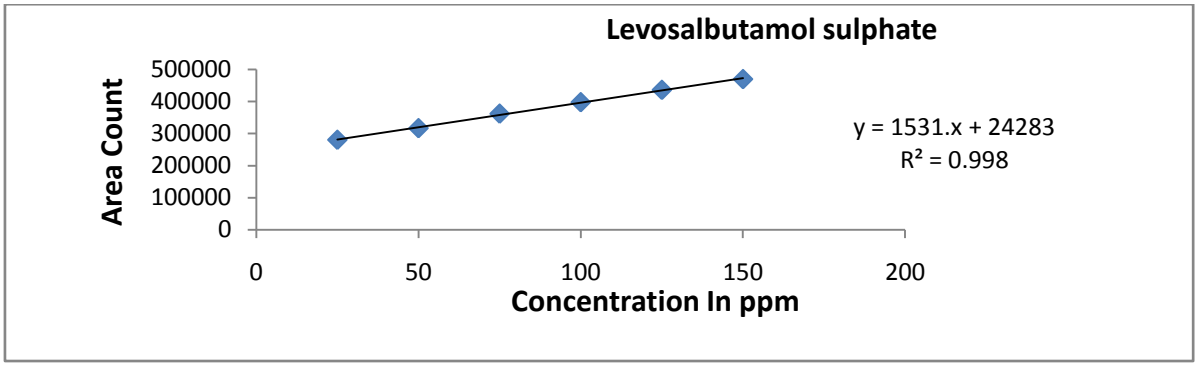

Figure 4: Linearity of Levosalbutamol Sulphate.

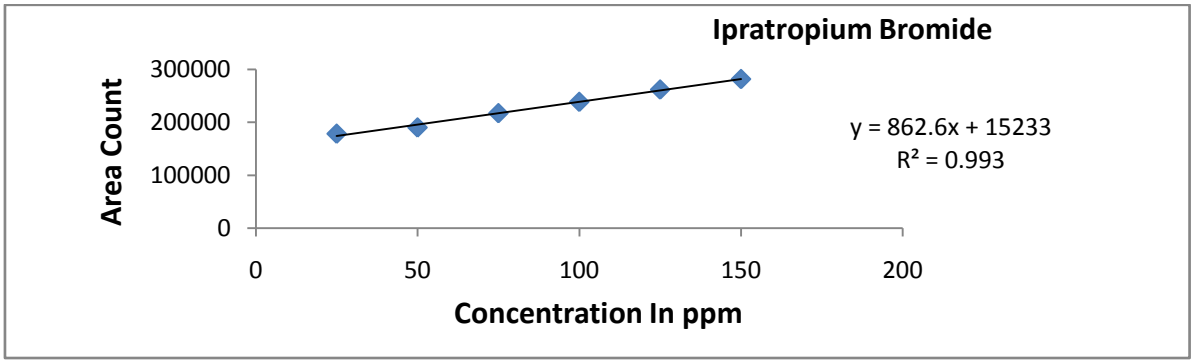

Figure 4: Linearity of Ipratropium bromide.

Precision

System precision

The six injections of standard solutions were injected to the chromatographic system. The relative standard deviation for area and retention time of Levosalbutamol sulphate and Ipratropium bromide peak was determined and shown in table 3.

Method Precision Six sample of a single batch of Levosalbutamol sulphate and Ipratropium bromide peak were analyzed by proposed method and their assay was calculated and results are shown in table3.

Table 3: System Precision and Method Precision

System Precision		
% RSD	**Levosalbutamol sulphate**	**Ipratropium bromide**
AREA	0.12%	0.15 %
RT	0.08%	0.05%
	Method Precision	
% RSD of assay	0.43	0.59

Accuracy (Recovery)

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the found value. Recovery samples were prepared in triplicate and injected each sample in duplicate

to the chromatography system. Levosalbutamol sulphate and Ipratropium bromide peak working standard was added with placebo and recovery solutions were prepared so that, the final concentration contains 50%, 100% and 150 % of the recovery levels of Levosalbutamol sulphate and Ipratropium bromide and results are shown in table 4.

Table 4: Accuracy (Recovery)

Analyte	Conc. Added (ppm)	RSD (%)	Mean (%) Recovery
Levosalbutamol sulphate	50	0.209	100.06
	100	0.598	100.28
	150	0.334	100.13
Ipratropium Bromide	20	0.502	100.33
	40	0.706	100.43
	60	0.436	100.31

Limit of Detection and Quantification

The limit of detection and Quantification were calculated as per formulas given below

$$LOD = 3\,\sigma / S$$

$$LOQ = 10\,\sigma / S$$

Where σ is standard deviation and S is the slope of the calibration curve. The LOD and LOQ values of Levosalbutamol sulphate and Ipratropium bromide are shown in table 5.

Table 5: LOD and LOQ

	Levosalbutamol sulphate	Ipratropium bromide
LOD (ppm)	0.72	0.43
LOQ (ppm)	1.24	0.97

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The analysis was carried out used the method outlined in the method of analysis and by carried out the following alterations and results are shown in table 6 and 7. a) By changing the flow rate of the HPLC System by (0.1 mL/min. b) By changing the column oven temperature by + 5°.

Table 6: Robustness by changing flow rate

At flow rate 1.0 ml/ min		
	Levosalbutamol Sulphate	**Ipratropium Bromide**
% RSD	0.14	0.16
Tailing factor	1.21	1.36
Theoretical plates	2818.36	2857.13
at flow rate 0.6 ml/ min		
% RSD	0.15	0.17
Tailing factor	1.31	1.64
Theoretical plates	2829.31	4351.01

Table 7: Robustness by changing temperature at Temp 25oC

	Levosalbutamol sulphate	**Ipratropium Bromide**
% RSD	0.21	0.16
Tailing factor	1.62	1.34
Theoretical plates	3812.16	2865.29
at Temp 35oC		
% RSD	0.39	0.21
Tailing factor	1.39	1.42
Theoretical plates	4245.71	2953.63

CONCLUSION

The present study shows that the method developed for the determination of Levosalbutamol sulphate and Ipratropium bromide were specific, linear, accurate, precise and robust. Wavelength 210nm was used in order to optimize the response of Levosalbutamol Sulphate as its concentration was higher than Ipratropium bromide in the sample. The method clearly shows that all the peaks had tailing factor less than 2. The RSD for areas and theoretical plates (> 2500) was also found to be satisfactory. Validation parameters were performed according to ICH Q2 (R1) guidelines. The recoveries achieved were highly significant in the developed method. Hence it can be concluded that the method developed can be effectively used in the industries as well as research purposes.

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