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Method Development and Validation for Simultaneous Estimation of Levosalbutamol Sulphate and Budesonide in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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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 Budesonide in bulk and suspension for inhalation 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 40:60 (%v/v). The flow rate was 0.8 ml/min and effluent was monitored at 266 nm. Retention time was found to be 3.16 min., 17.94 min. and 20.90 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 be 0.43, 0.72, 0.97 and 1.24 µg/ml respectively. No chromatographic interference from placebo and degradants were found. The proposed method was successfully used for estimation of Levosalbutamol sulphate and Budesonide in bulk and suspension for inhalation dosage forms.

Keywords: Levosalbutamol sulphate, Budesonide, 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- adrenoreceptor 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 bronchodilation can be achieved with doses that substantially decrease β-mediated side effects.[1]

Budesonide, 16a(R), 17-(Butylidenebis(oxy)-11b,21-dihydroxypregna-1,4-diene-3,20-dione (Figure 1b), is a glucocorticoid used by inhalation in the management of asthma and allergic rhinitis1 Budesonide is official only in European pharmacopoeia (EP)1, which suggests a liquid chromatography method for the estimation of budesonide in bulk.[2]

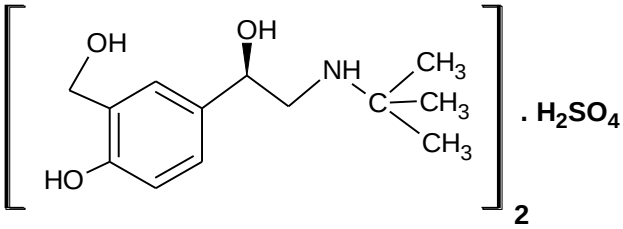

Figure 1(a): Structure of Levosalbutamol sulphate.

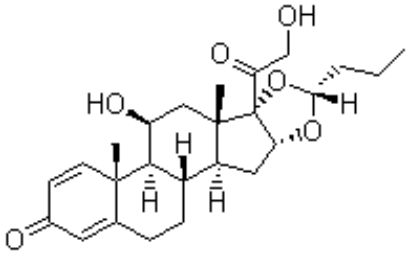

Figure 1(b): Structure of budesonide.

The aim of this study was to develop a RP HPLC method for the quantitative simultaneous determination of Levosalbutamol sulphate and Budesonide. 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 Specialities. The working standards of Levosalbutamol sulphate and Budesonide were procured from Aarti Drugs. 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 'A' class volumetric glassware. All other reagents were of analytical grade.

Instrument and Chromatographic Conditions

Shimadzu LC 2010 C_{HT} HPLC was used for the chromatographic separation equipped with autosampler 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 (2.6 g Potassium dihydrogen Phosphate, 2.77 g Heptane 1-Sulfonic acid sodium salt in 950 ml of water. Adjust pH 3.00 with orthophosphoric acid and dilute to 1000 ml with water.) In the ratio 40: 60. 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 20 µL. Oven temp was set at 30^{o}C. The mobile phase was pumped at 0.8 ml/min at room temperature. Detection was carried by using wavelength 266.

Preparation of standard and test solution

Take 50 mg Levosalbutamol Sulphate working standard and 20 mg Budesonide in 100 mL volumetric flask and make up volume upto the mark by Diluent. Further dilute 5 mL of this solution upto 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 Budesonide and Levosalbutamol sulphate were scanned on photo diode array detector for selecting the optimum wavelength. A typical HPLC chromatogram for simultaneous determination of Budesonide and Levosalbutamol sulphate from pharmaceutical formulation is shown in figure 2 and 3. Results of the developed method are shown in table 1.

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.

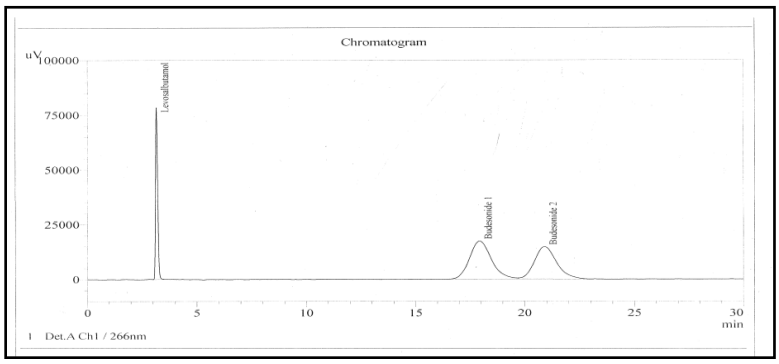

Figure 2: Chromatogram of Budesonide and Levosalbutamol sulphate in standard preparation

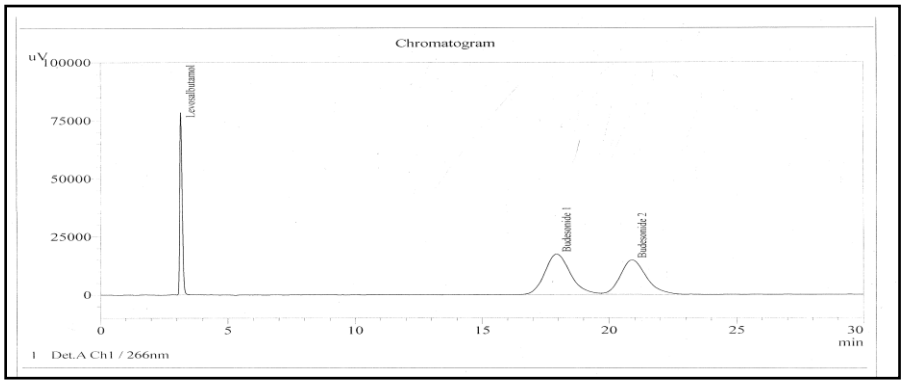

Figure 3: Chromatogram of Budesonide and Levosalbutamol sulphate in sample preparation

Table 1: System Suitability Parameters

S. No.	System suitability parameters	Limit	Observation Levosalbutamol sulphate	Budesonide 1	Budesonide 2
1	Theoretical plate	NLT 2000	4645.17	2754.24	2688.31
2	Tailing Factors	NMT 2.3	1.24	1.39	1.41
3	Resolution	NLT 1.5	-----	2.202	2.189
4	% RSD of area response for six replicate standard	NMT 2.0%	0.166%	0.803	0.872
5	% RSD of retention time response for six replicate standard	NMT 1.0%	0.756%	0.642	0.672

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 Budesonide and Levosalbutamol sulphate obtained from the graph obtained by plotting area count on Y axis and concentration on X axis. Correlation coefficient of Budesonide and Levosalbutamol sulphate are shown in table 2.

Table 3: Correlation Coefficient

Levosalbutamol sulphate	Budesonide 1	Budesonide 2
0.9996	0.9998	0.9995

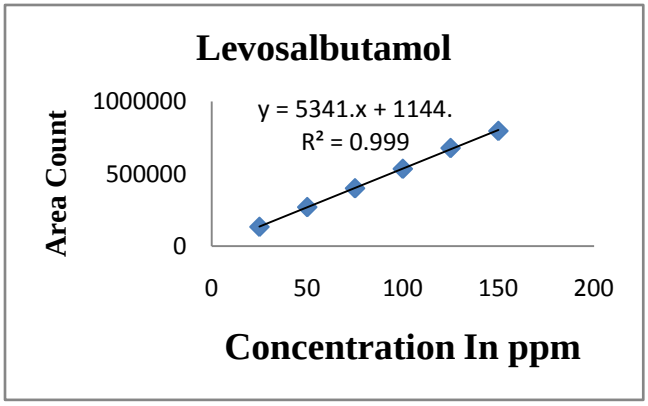

Figure 4: Linearity of Levosalbutamol sulphate.

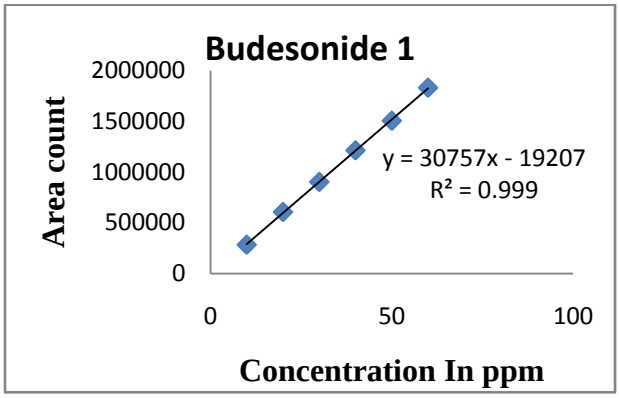

Figure 5: Linearity of Budesonide 1.

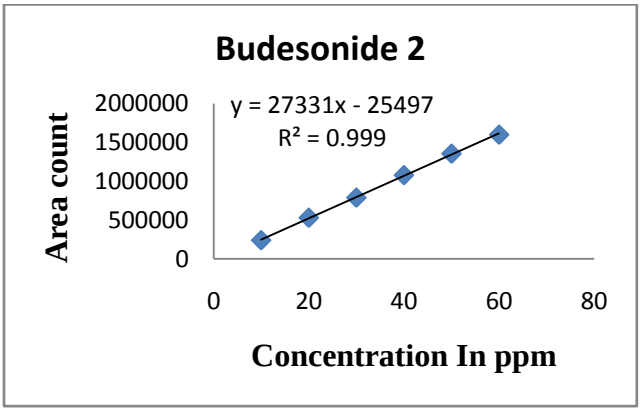

Figure 6: Linearity of Budesonide 2.

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 Budesonide and Levosalbutamol sulphate peak was determined and shown in table 3.

Method Precision

Six sample of a single batch of Budesonide and Levosalbutamol sulphate peak were analyzed by proposed method and their assay was calculated and results are shown in table 3.

Table 3: System Precision and Method Precision

System Precision		
% RSD	**Levosalbutamol sulphate**	**Budesonide**
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 Budesonide 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 Budesonide and Levosalbutamol sulphate and results are shown in table 4.

Table 4: Accuracy (Recovery)

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

Limit of Detection and Quantification

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

$$LOD = \frac{3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

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

Table 5: LOD and LOQ

	Budesonide	**Levosalbutamol sulphate**
LOD (ppm)	0.43	0.72
LOQ (ppm)	0.97	1.24

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		
	Budesonide	**Levosalbutamol sulphate**
% 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 25°C		
	Budesonide	**Levosalbutamol sulphate**
% RSD	0.16	0.20
Tailing factor	1.34	1.67
Theoretical plates	2865.29	2812.16
at Temp 35°C		
% RSD	0.21	0.39
Tailing factor	1.42	1.39
Theoretical plates	2953.63	4245.71

CONCLUSION

The present study shows that the method developed for the determination of Budesonide and Levosalbutamol sulphate were specific, linear, accurate, precise and robust. Wavelength 266nm was used in order to optimize the response of Levosalbutamol Sulphate as its concentration was higher than Budesonide 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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