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Development of New Spectrophotometric Methods for the Simultaneous Estimation of Levosalbutamol Sulphate and Beclomethasone Dipropionate in Bulk Drug and Pharmaceutical Formulations(ROTACAP)

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Abstract: Two simple, precise and accurate UV methods have been developed for the simultaneous estimation of Levosalbutamol sulphate (LS) and Beclomethasone dipropionate (BD) in bulk and pharmaceutical formulation. Method A applied Area Under Curve (AUC) for the analysis of LS in the wavelength range of 272-282nm and for BD in the wavelength range of 234-244nm.Method B is Q-absorbance methodbased on the measurement of absorptivity at 269nm(as an iso-absorptive point) and 277 nm.LS and BD shows a maximum absorption at 277nm and 239nm respectively with a linearity range of 10-50 µg/ml and 5-25 µg/ml respectively for both the methods. Results of analysis were statistically validated with recovery studies and were found to be satisfactory.
Keywords: Levosalbutamolsulphate,Beclomethasonedipropionate,Area under curve, Q-value analysis.

INTRODUCTION

Levosalbutamol Sulphate is an orally inhaled β_2-adrenoreceptor agonist, indicated for the relief of bronchospasm in conditions such as asthma and chronic obstructive pulmonary disease (COPD).[1]It is chemically (R)-1-(4-hydroxy-3-hydroxy-methyl phenyl)-2-(ter-butylamino)ethanolsulphate[2] Figure:1). Levosalbutamol is a single isomer β_2- adrenoreceptor agonist that differs from racemic Salbutamol by elimination of (S)-Salbutamol. Clinical and mechanistic studies have demonstrated that (R)-Salbutamol alone provides the β_2-agonist activity that is needed for the relief of broncho constriction.

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. [3]

Beclomethasone dipropionate is an orally inhaled adrenocortico-steroid which suppresses bronchial inflammation, increases peak expiratory flow rate, reduces need for rescue β_2-agonist inhalations and prevents episodes of acute asthma.It is chemically9α-chloro-11β-hydroxy-16β-methyl-3, 20-dioxo-1, 4- pregnadiene-17, 21-diyldipropionate (Figure:2).When administered via inhalation, there is an extensive conversion of Beclomethasone dipropionate to active metabolite Beclomethsone-17-monopropionate within the lungs prior to systemic

absorption. [4]It is an official drug Indian pharmacopoeia 1996, [5] United states pharmacopoeia 2004[6] and British Pharmacopoeia 2003. [7]

The combination of Levosalbutamol sulphate and Beclomethasone Dipropionate is very useful in the treatment of asthmatic bronchoconstriction.

Literature survey reveals that, only few spectrophotometric[8-11] and bio-analytical methods by LC-MS and HPLC were found using human plasma[12-14] , urine[15] , blood [16] and biological fluids[17]for the quantitative estimation of Levosalbutamol sulphate and Beclomethasone Dipropionate in bulk and pharmaceutical formulations have been developed. Hence an attempt has been made to develop new UV methods for its estimation in bulk and pharmaceutical formulation with good accuracy, simplicity and precision.

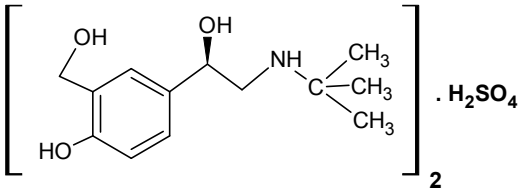

Figure 1: Chemical Structre of Levosalbutamolsulphate

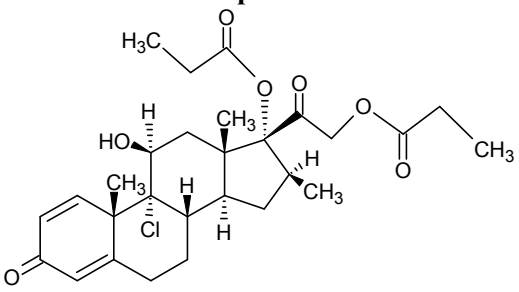

Figure 2: Chemical Structure of Beclomethasonedipropionate

EXPERIMENTAL

Materials and Method

Pure sample of both the drugs were obtained from Cipla, Sikkim, India as gift samples. A Shimadzu UV-1700 UV/VIS Spectrophotometer was used with 1cm match quartz cell. Rotacaps of 400 µg of Levosalbutamol sulphate and 200 µg of Beclomethasonedipropionate were procured from local pharmacy.

Preparation of Standard solution

Both the pure drugs of about 50mg were weighed accurately and dissolved in 50ml of redistilled ethanol to give the standard stock solution of 1000µg/ml (Stock A). Aliquots of standard stock solution were pipetted out and suitably diluted with ethanol to get a final concentration of the standard solution.

Method A: Area Under Curve method (AUC)

The AUC method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 272 nm and 282nm for LS (Figure:3) and 234 nm and 244 nm for BD (Figure:4). Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. Suitable dilutions of Stock A of the both the drugs were prepared and scanned in the spectrum mode in the wavelength range 400-200nm and the calibration curve was plotted (Figure: 6,7).

As the results obtained were satisfactory, the methods were applied for the pharmaceutical formulations.

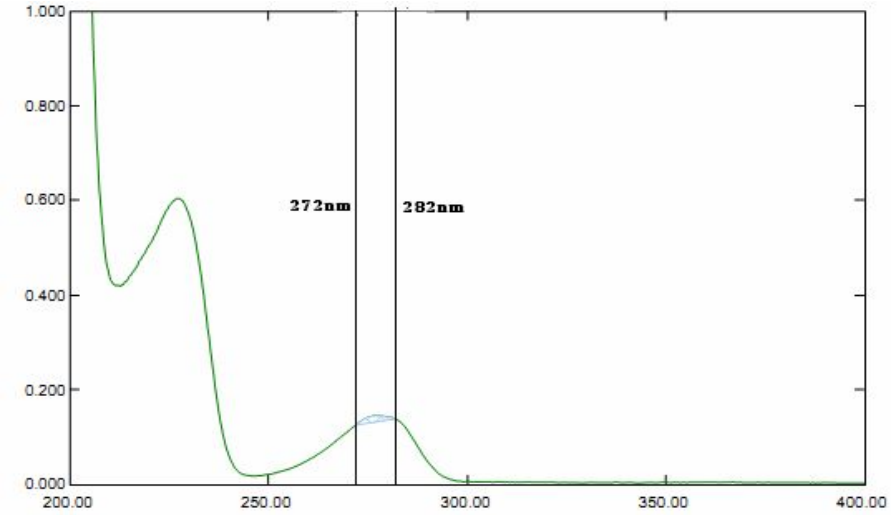

Figure 3: Spectra for LS at 272-282 nm in ethanol by AUC

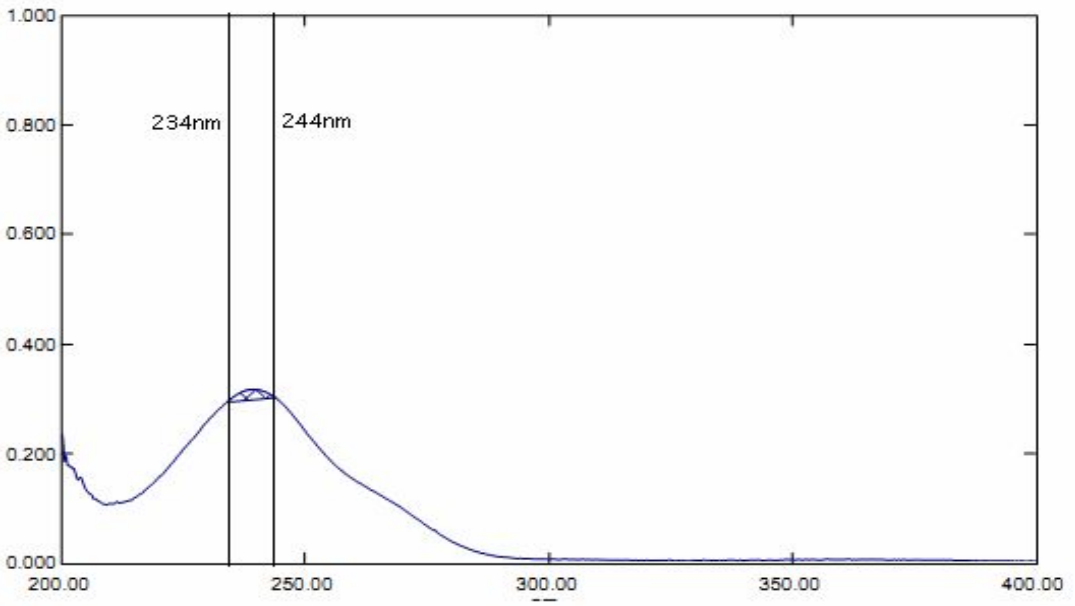

Figure 4: Spectra for BD at 234-244nm in ethanol by AUC

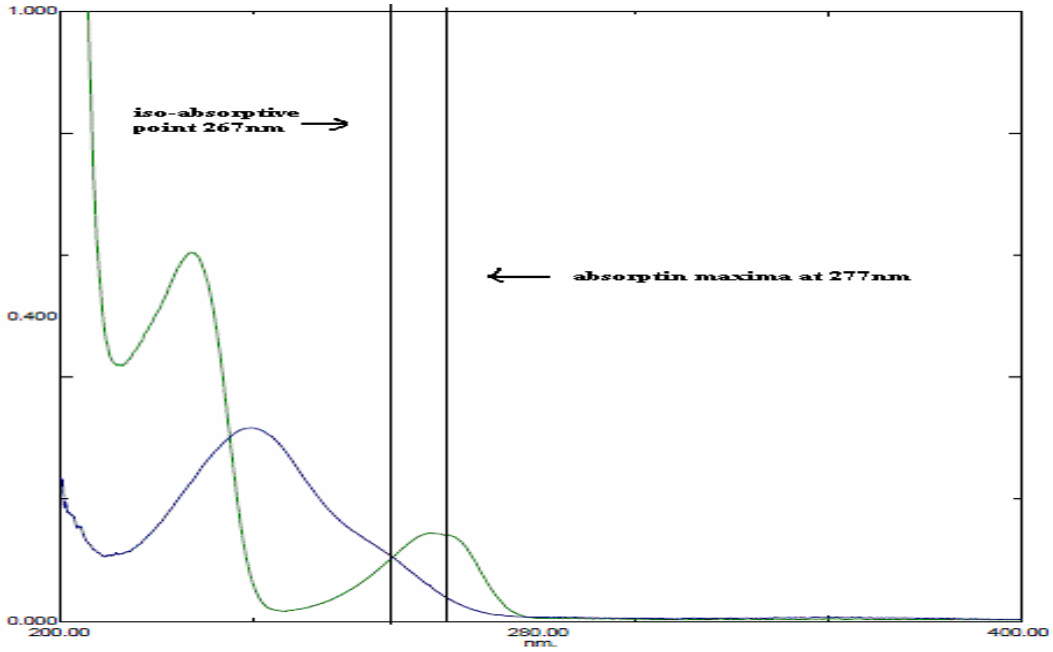

Figure 5: Isoabsorptive point of Levosalbutamol sulphate and Beclomethasone dipropionate at 269 nm.

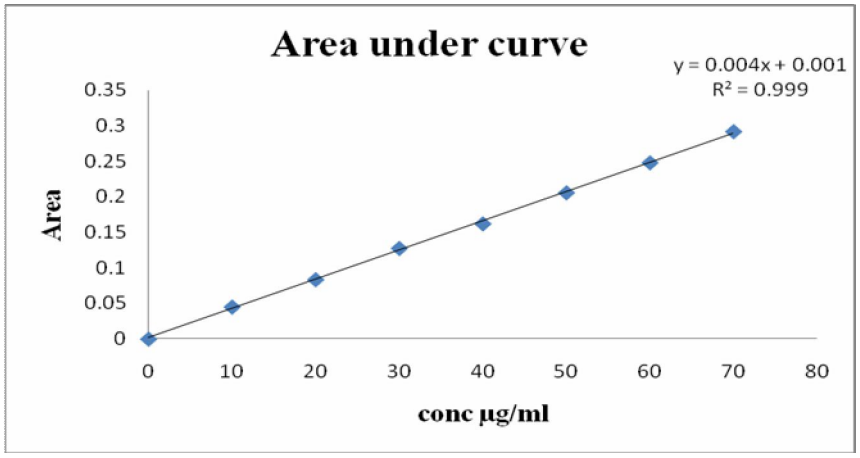

Figure 6: Calibration curve of for Levosalbutamol sulphate at 272– 282 nm in ethanolby Area Under Curve method.

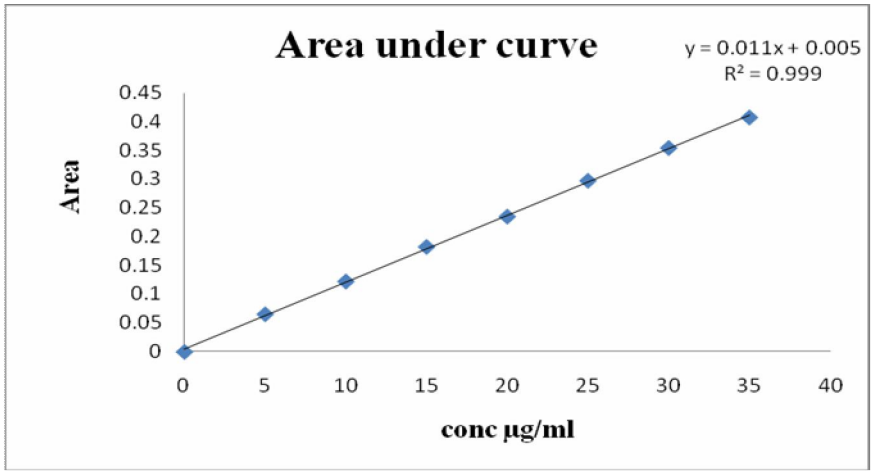

Figure 7: Calibration curve of Beclomethasone dipropionate at 234-244 nm in ethanol by Area Under Curve method.

Determination of Maximum Wavelength and Iso-absorptive point

By appropriate dilution of two standard drug solutions with ethanol,solutions containing 50µ g/ml of Levosalbutamol sulphate and 25µg/ml of Beclomethasonedipropionate were scanned separately in the range of 200-400nm to determine the wavelength of maximum absorption for both the drugs. Levosalbutamol sulphate showed absorbance maxima at 277 nm(λ_1) and 229 nm and Beclomethasonedipropionateshowed maxima absobance at 239 nm(λ_2).The overlain spectra showed λ_{max} ofboth drugs and alsoiso-absorptive points at 269 nm(Figure 5).

Method B: Q-Analysis Method or Absorption Ratio Method

From the overlain spectrum of Levosalbutamol sulphate and Beclomethasone dipropionate (Figure 5),two wavelengths were selected one at 269 nm which isisoabsorptive point for both the drugs and the other is277 nm $\lambda_{max}$of Levosalbutamol sulphate. Suitable dilutions of Stock A of the drug were prepared and scanned in the spectrum mode in the wavelength range 400-200nm and the absorbance ratio values for both the drugs at selected wavelengths were also calculated.

Preparation and analysis of the rotacap formulation

Twenty capsules were chosen and tapped so as to accumulate all the contents in the capsule body. Capsules were opened carefully and their contents were emptied into a 50 ml volumetric flask and dissolved in 25 ml ethanol and the content was kept in ultrasonicator for 20 min. Finally the volume was made up to the mark with ethanol. The solution was filtered through Whatmann filter paper No.41 and this solution was used as stock A solution.

From the above stock A solution, 3.5 ml of the aliquot

was pipetted out and was transferred to a 10 ml volumetric flask. The volume was made up to 10 ml with ethanol to obtain a solution with final concentration of LS and BD, 56 µg/ml and 28 µg/ml respectively. Six replicates were prepared and analysed at the selected analytical wavelengths, were statistically validated

Validation of the methods[18]

Allthe methods were validated according to ICH guidelines by carrying out analysis of six replicate samples of tablet. Recovery studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug to previously analyzed tablet powder sample. From the amount of drug found, percentage recovery was calculated **(**Table 1).

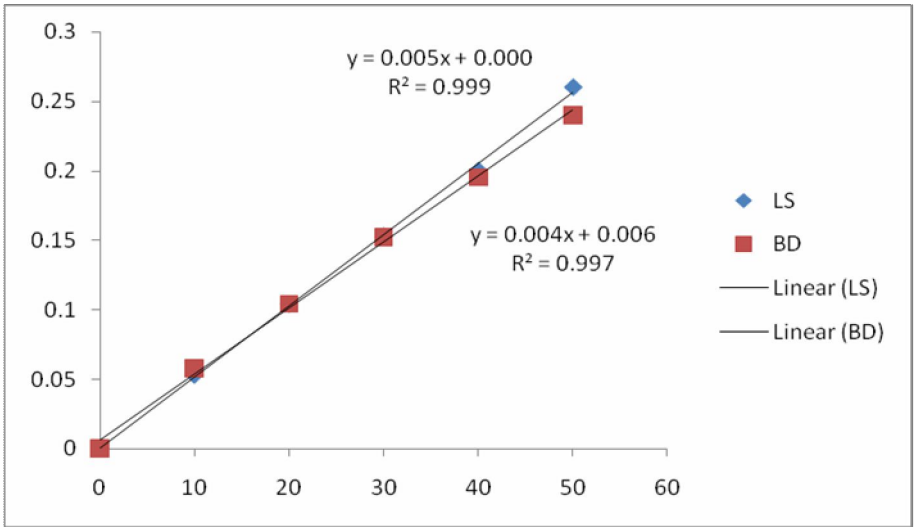

Figure 8: Calibration curve of Levosalbutamolsulphate and Beclomethasonedipropionate at 269 nm in ethanolby Q-Absorption ratio method.

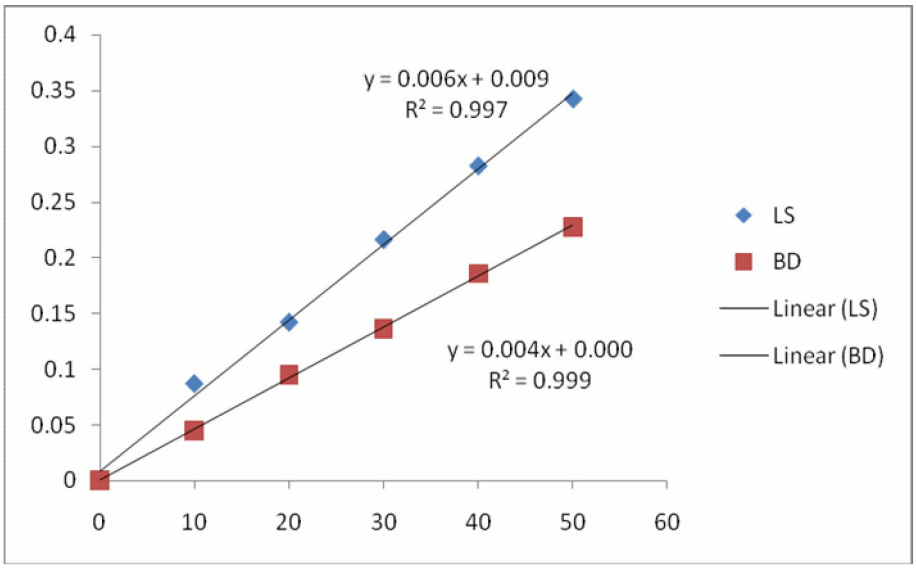

Figure 9: Calibration curve of Levosalbutamolsulphate and Beclomethasonedipropionate at 277 nm in ethanol by Q-Absorption ratio method.

Table 1: Optical characteristics and other parameters for Method A

Parameters	**Levosabutamol sulphate**	**Beclomethasone dipropionate**
λ_{max} / wavelength range (nm)	272 - 282	234 - 244
Linearity range (µg/ml)	10-50	5-25
Molar absorbtivity (litre, $mole^{-1}$ cm^{-1})	237	51.16
Coefficient of Correlation	0.9995	0.9995
Slope* (m)	0.0041	-0.0006
Intercept* (c)	0.0014	-0.0003
Accuracy (%RSD) 80% 100% 120% 120%	 0.5163 0.6278 0.2804	 0.3593 0.2516 0.2804 0.5123
Precision (%RSD) Intra-day Inter-day	 0.8788 0.9827	 0.3867 0.6262
LOD	0.3082	0.4054
LOQ	0.9340	4.0540

*y = mx + c; when x is the concentration in µg/ml and y is absorbance unit.

Table 1: Optical characteristics and other parameters for Method B

Parameters	**Levosalbutamol sulphate**	**Beclomethasone dipropionate**
Wavelength range (nm)	277	277
Iso-absorptive point (nm)	269	269
Linearity range (µg/ml)	10-50	5-25
Molar absorbtivity (litre, $mole^{-1}$ cm^{-1})	12.85	26.83
Coefficient of Correlation	0.9995	0.9992
Slope* (m)	0.0051	0.0095
Intercept* (c)	0.005	0.0049
Accuracy (%RSD) 80% 100% 120%	 0.275 0.201 0.284	 0.297 0.201 0.290
Precision (%RSD) Intra-day Inter-day	 0.5736 1.106	 0.3867 0.7694
LOD	0.5039	0.0168
LOQ	1.5271	0.0511

Table 2: Results of formulation

METHOD	Brand Name	Label claim of LS (µg)	Label claim of BD (µg)	Amount found for LS (µg)	Amount found for BD (µg)	%Recovery ± SD**for LS	%Recovery ± SD**for BD
A	Aerocort	400	200	398.8	198.5	99.71±0.8199	99.2±0.3881
B		400	200	369.9	199.0	99.41±0.8333	99.5±0.1910

**Average of six determinations

RESULTS AND DISCUSSION

The estimation of Levosalbutamolsulphate and Beclomethasonedipropionate in rotacapsformulation was found to be accurate and reproducible with a linearity of 10-50 µg/ml and 5-25 µg/ml respectively for both the methods and the correlation coefficient 0.999 and 0.998 for the methods A and B respectively.The optical characteristics such as linearity range, molar absorptivity, percentage relative standard deviation of recovery studies and precision in each method were calculated and the results were reported in Table 1. Also the regression characteristics like slope (m), intercept (c) and correlation coefficient (r) were calculated and are presented in Table 1. The accuracy of the methods was a different levels i.e. 80%, 100% and 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The %RSD value was less than 2, an indicative of the accuracy of the methods.

CONCLUSION

Thus, it can be concluded that the methods developed in the present investigation were simple, sensitive, accurate, rapid and precise. Hence, the above said methods can be successfully applied for the estimation of Levosalbutamol sulphate and Beclomethasone dipropionate in pharmaceutical formulation.

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