



Research Article

REVERSE PHASE ISOCRATIC HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULPHATE AND BECLOMETHASONE DIPROPIONATE IN ROTACAPS FORMULATION DOSAGE FORMS

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ABSTRACT

A new, rapid and sensitive RP isocratic HPLC method for simultaneous estimation of Salbutamol Sulphate and Beclomethasone Dipropionate in Rotacaps formulation has been developed. The determination was performed using HPLC system with an octadecylsilane column and a solvent system comprising of water and Acetonitrile in the volume ratio of (40:60 v/v). The detection was carried out using a UV detector set at 230 nm wavelength. The method was validated with respect to the linearity, accuracy, specificity and robustness. The method has been successfully applied for analysis of drugs in Rotacaps pharmaceutical formulation and is suitable for the routine quality control analysis.

Keywords: RP Isocratic HPLC, Rotacaps, Salbutamol Sulphate, Beclomethasone Dipropionate.

INTRODUCTION

Rotacaps, is a Dry-Powder-Inhaler system. The drug is inhaled as cloud of the fine particles, filled into a hard gelatin capsule which is loaded into a device prior to the use. Dry-Powder-Inhalers are propellant free, and do not contain any excipients other than a carrier (invariably lactose). They are breath actuated, avoiding the problems of inhalation/actuation coordination encountered with Metered Dose Inhalers and consequently they are particularly useful for young children¹.

OBJECTIVE

Salbutamol Sulphate a β_2 Agonist, Antiasthmatic, and Beclomethasone Dipropionate is an Anti-inflammatory agent used in the mentioned Rotacap formulation. Salbutamol Sulphate in pharmaceuticals has been assayed using visible spectrophotometric methods based on reactions such as redox, reducing and then chelating, oxidative coupling, diazotization and coupling, nitrosation, nitration, nitration followed by Meisenheimer complex formation and charge-transfer complex formation²⁻¹². However, any of these procedures suffer from some disadvantage, such as poor sensitivity, heating or extraction step, critical working conditions or the use of organic solvents, and are hence unsatisfactory for routine analysis¹³. The official methods for determination of Betamethasone Dipropionate in different pharmaceutical dosage forms are prescribed in the USP^{14, 15}. There is no known method available for the estimation of these two drugs in the commercially available formulation. Hence the present work depicts the simultaneous Reverse Phase (RP) isocratic High Performance Liquid Chromatographic method, which is fast, simple, and robust for routine quality control determination of Salbutamol Sulphate and Beclomethasone Dipropionate in the Rotacap formulation. The choice of octadecylsilane column and UV detector was considered so the main objective of developing a method applicable for routine quality control monitoring is achieved.

MATERIALS AND METHODS**Solubility studies**

Solubility of both the drugs Salbutamol Sulphate and Beclomethasone Dipropionate was carried out in water, acetonitrile and methanol in order to optimize the mobile phase composition in the RP-HPLC system. Beclomethasone Dipropionate is insoluble in water¹⁶, and Salbutamol Sulphate is freely soluble in water¹⁶. Both the drugs are soluble in Acetonitrile and methanol solvent.

Evaluation of wavelength

The UV scans of Salbutamol Sulphate and Beclomethasone Dipropionate in methanol was performed using a Shimadzu spectrophotometer. The wavelength maxima of Salbutamol Sulphate were found to be 227 nm and 278 nm (figure 1B) and for Beclomethasone Dipropionate were found to be 239 nm (figure 1A). Hence, 230 nm was considered to be the wavelength of choice for the simultaneous estimation.

Evaluation of column

Various RP columns such as octyl silyl, octadecylsilane of different brands like Agilent, Waters were screened so as to obtain a good separation between Salbutamol Sulphate and Beclomethasone Dipropionate peaks in the short period of time. The octadecylsilane column of Waters was found to be the most suitable. The retention time for Salbutamol Sulphate and Beclomethasone Dipropionate was found to be 2-5 minutes and 7 minutes respectively (figure 2) (table 1). The run time for complete elution was found to be about 10 minutes.

Evaluation of mobile phase composition

The different ratios of water in methanol and water in acetonitrile were mixed and the samples of Salbutamol Sulphate and Beclomethasone Dipropionate were injected onto the octadecylsilane column. It was observed that water and acetonitrile mixture in the volume ratio 40:60 v/v yielded sharp peaks in optimum time for Salbutamol Sulphate and Beclomethasone Dipropionate.

Preparation of standard solution

Solution A: 500ppm of Beclomethasone Dipropionate was prepared accurately by dissolving 25mg of drug in the mobile phase and diluted upto 50ml with the same solvent mixture¹⁷.

Solution B: 1200ppm of Salbutamol Sulphate was prepared accurately by dissolving 60mg of drug in the mobile phase and diluted upto 50 ml with the same solvent mixture¹⁷.

From solution A and solution B, 2ml each was mixed and volume was made upto 100ml with the mobile phase and the so obtained solution was filtered through 0.45 micron membrane filter.

Preparation of sample solution

Ten 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 in a 200ml volumetric flask.

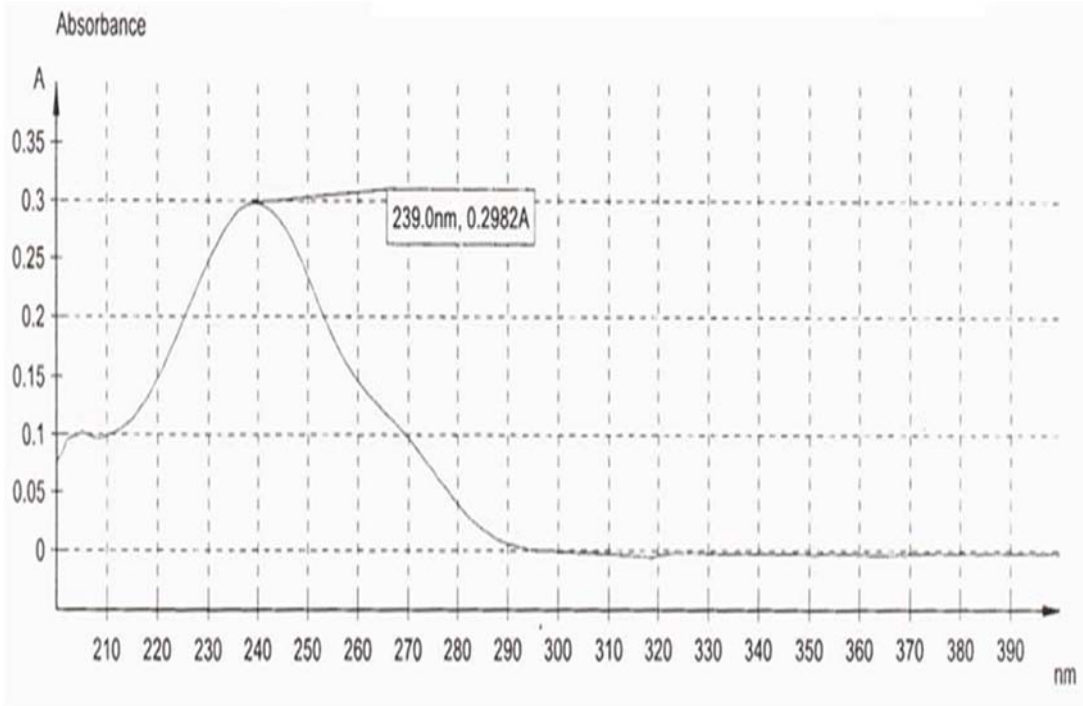


Fig. 1A: UV scan of Beclomethasone Dipropionate

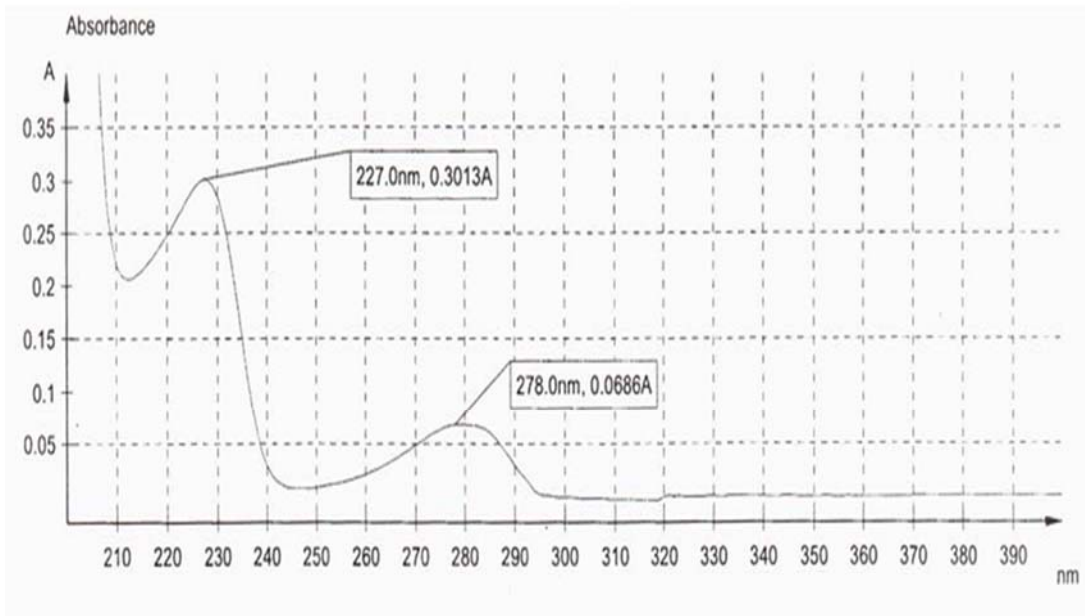


Fig. 1B: UV scan of Salbutamol sulphate

Table1: Data obtained from chromatogram of Salbutamol and Beclomethasone Dipropionate

Peak no.	Retention time (min)	Peak name	Peak type	Area μ AU ² (SEC)	Height μ AU	Area %
1	2.521	Salbutamol	BMB	883615	104425	64.915
2	7.726	Beclomethasone Dipropionate	BMB	477579	34121	35.085
TOTAL				1361194	138546	100.000

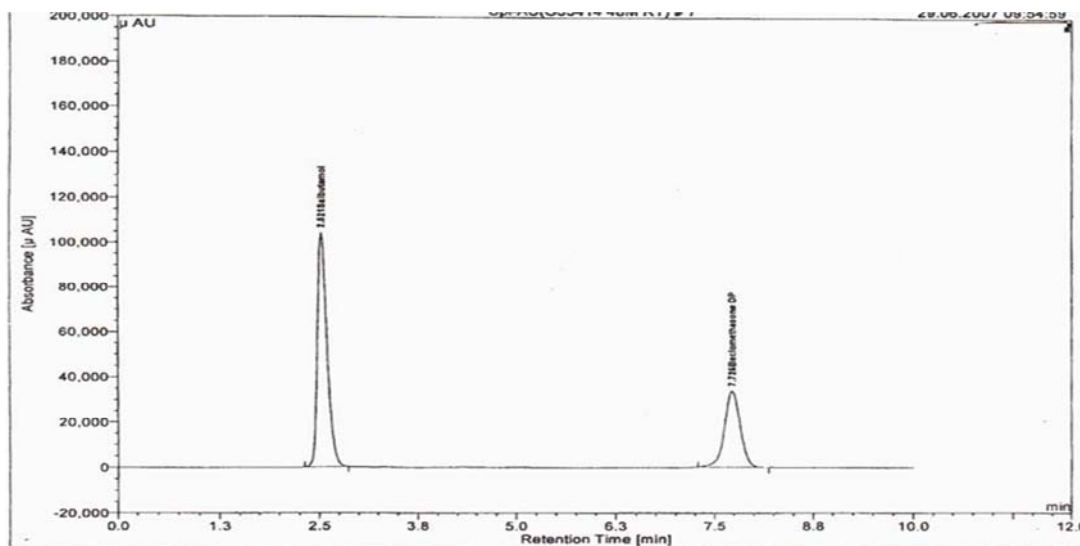


Fig. 2: Chromatogram of Salbutamol and Beclomethasone Dipropionate

The inner sides of the capsule were washed twice with the mobile phase and the washings were transferred into the same volumetric flask. The inner sides of the capsule cap were washed with the mobile phase and similarly the washings were transferred into the same volumetric flask.

All the remaining capsules were treated in the same manner, adding the contents and washing to the same volumetric flask. About 70ml of mobile phase was added and sonicated to dissolve. The volume was made up with the mobile phase and then filtered through 0.45 micron membrane filter

Procedure

About 20µl of the standard solution and sample solution were separately injected onto the HPLC column and the contents of each drug in the sample was measured.

Validation

The method was validated for specificity, robustness, linearity, accuracy.

Specificity: Different ratios of mobile phase containing acetonitrile and water were examined and the phase mixture comprising of 40:60 v/v (water: acetonitrile) was selected as optimal for detection and quantification was 230 nm at which the best detector response for the two aforementioned drugs was obtained.

Robustness: Robustness of the method was checked after deliberate alteration of the wavelength and flow rate of mobile phase showed that the retention time of the peak of interest remained unaffected by the small changes of the operational parameters.

Linearity: The calibration curve obtained by plotting peak areas against concentration showed linearity in the range of 12 to 36 ppm for Salbutamol Sulphate and 5 to 15 ppm for Beclomethasone Dipropionate with the correlation coefficient better than 0.999.

Accuracy: The accuracy of the method established using recovery was found to be in the range of 98.0% to 102.0%.

CONCLUSION

Based on the validation data it could be concluded that the proposed method is accurate, precise, simple and selective for the analysis of Salbutamol Sulphate and Beclomethasone Dipropionate, Using this method it is possible to quantify the two drugs on the same

“ISOCRATIC HPLC” method which makes it suitable for the routine quality-control monitoring.

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