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DEVELOPMENT AND VALIDATION OF A REVERSED-PHASE HPLC METHOD FOR ANALYSIS OF TIOTROPIUM BROMIDE AND BUDESONIDE IN METERED DOSE INHALATION FORM

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ABSTRACT
A simple, sensitive, rapid and reproducible reversed- phase HPLC method has been developed and validated for estimation of Tiotropium bromide and Budesonide. The assay involved an isocratic elution of these two components on Kromosil C_{18} column (150 X 4.6 mm, 5µm) using a mobile phase composition of Buffer: Acetonitrile (65:35, v/v). The flow rate was 2.0 mL/min; Column oven temperature 30°C and the analytes monitored at 235nm.Calibration curves were linear with coefficient correlation between 0.999 to 1.000 over a concentration range of 0.037 to 0.561 µg/mL of Tiotropium bromide and 0.405 to 6.075 µg/mL for Budesonide respectively. All the validation parameters were within the acceptance range according to ICH norms. The Method has been successfully applied for analysis of drugs in pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

***Key words*:** Tiotropium bromide; Budesonide; Reversed-phase HPLC; Antiasthma tic. *etc.*

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INTRODUCTION

Tiotropium Bromide is an anticholinergic bronchodilator used in the management of chronic obstructive pulmonary disease (COPD) [1].It is a muscarinic receptor antagonist, often referred to as an antimuscarinic or anticholinergic agent. Although it does not display selectivity for specific muscarinic receptors, on topical application it acts mainly on M3 muscarinic receptors located in the airways to produce smooth muscle relaxation, thus producing a bronchodilatory effect [2].

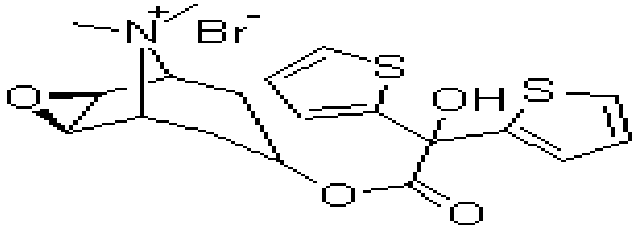
Budesonide, 16a(R), 17-(Butylidenebis(oxy))-11b,21-dihydroxypregna-1,4-diene-3,20-dione, is a glucocorticoid used by inhalation in the management of asthma and allergic rhinitis[1].

Budesonide is official only in European pharmacopoeia (EP) [1], which suggests a liquid chromatography method for the estimation of budesonide in bulk. The different analytical methods that are reported for its determination include ELISA [3-4] and HPLC [5-6].

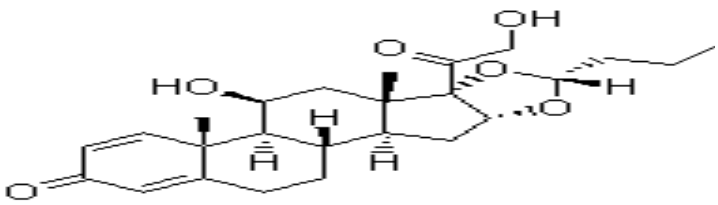
The aim of this study Tiotropium bromide and Budesonide is latest combination of anti asthmatic drugs. It is available in dry powder inhalation and metered dose inhalation form. Both the drugs were individually official in Indian pharmacopoeia [7], United States pharmacopoeia [8] and British pharmacopoeia [9].

Literature survey revealed spectrophotometric and chromatographic methods reported for estimation of Tiotropium bromide and Budesonide individually or in combination with other drugs. However, there is no analytical method reported for the simultaneous determination of these drugs in a pharmaceutical formulation. Present work describes simple, rapid, accurate and precise method for simultaneous determination of Tiotropium bromide and Budesonide in metered dose inhalation. The proposed methods were validated as per ICH guidelines [10].

Tiotropium Bromide



Budesonide



MATERIALS AND METHODS

Chemicals and reagents:

Working standards of pharmaceutical grade Tiotropium bromide and Budesonide were obtained as generous gifts from Hetero Labs Ltd (Hyderabad, India) and was used as such without further purification. Acetonitrile and Methanol (HPLC Grade), Sodium dihydrogen orthophosphate monohydrate (AR Grade), decane sulphonic acid sodium salt (AR Grade), Orthophosphoric acid (AR Grade) purchased from Merck specialties Pvt.Ltd, (Mumbai, India) and double distilled water were used in analysis.

Instrumentation and Chromatographic conditions:

Shimadzu HPLC system LC-2010 CHT consisting of UV/VIS detector and LC solutions software was used for analysis. Separation was carried out on Kromosil C_{18} (150 x 4.6 mm i.d.) column using Buffer pH 3.0: Acetonitrile in ration of (65:35 v/v) as mobile phase at flow rate of 2.0 ml/min and Column oven temperature 30°C. Samples were injected using auto injector with 100µL loop and detection was carried out at 235 nm. All weighing were done on Shimadzu balance (Model AY-120).

Preparation of Buffer (pH 3.0)

Weighed and transferred 1.38 gm of Sodium dihydrogen orthophosphate monohydrate into a beaker containing 1000 ml of water and 1.22 gm of decane sulphonic acid sodium salt and mix to dissolve. Adjusted the pH of the solution to 3.0 with orthophosphoric acid.

Preparation of standard stock solutions:

Tiotropium bromide standard stock solution:

Accurately weighed and transferred 18.7 mg of Tiotropium bromide working standard into a 100 ml volumetric flask. Added 50 ml of mobile phase

and sonicate to dissolve. Dilute to volume with mobile phase and mix. Transferred 10 ml of the above solution in to a 100 ml volumetric flask and dilute to volume with mobile phase.

Budesonide standard stock solution:

Accurately weighed and transferred 40.5 mg of Budesonide working standard into a 100 ml volumetric flask. Added 50 ml of methanol and sonicate to dissolve. Dilute to volume with mobile phase and mix.

Preparation of standard solutions:

Transferred 2.0 ml of Tiotropium bromide standard stock solution and 1.0ml of Budesonide standard stock solution into a 100 ml volumetric flask dilute to volume with mobile phase.

Preparation of sample solution A:

Took a container, fit in an actuator & prime the valve by wasting first two actuations. Shacked the container for at least five seconds in-between each actuation. Remove the container from its actuator & wash with methanol. Clean the valve and valve stem internally and externally with an airline fitted with a narrow jet. Place a disc in clean and dry 100 ml beaker; add about 50 ml of acetonitrile. Hold the container in inverted position, shake for 5 seconds and deliver one actuation in beaker through the hole provided in the center of the disc. Similarly deliver further nine actuations in the same beaker with constant shaking for at least five seconds in between each delivery.

Transferred the solution from beaker into a 100-ml volumetric flask. Wash the beaker and disc with acetonitrile and transferred into the same volumetric flask. Make up the volume up to the mark with acetonitrile. Transferred 10 ml of the above solution in to 25ml volumetric flask, dilute to volume with mobile phase and mix.

Sample Preparation for Actuator Retention B:

Washed the actuator with mobile phase and dry gently. Fit the container in actuator & deliver 10 actuations with constant shaking for at least 5 seconds in between each delivery. Remove the container from actuator, wash the actuator 3-4 times with 10 ml acetotrile, collect in 25 ml volumetric flask & then make up the volume up to the mark with mobile phase.

After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the metered dose sample solution A and actuator retention B solution was injected, chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per inhalation was estimated from the respective calibration curves.

Content of Active Ingredient Delivered per actuation = µg of Sample A - µg of Sample B
% Labeled Amount of Active Ingredient Delivered per actuation

$$= \frac{[\mu g \text{ of Sample A} - \mu g \text{ of Sample B}] \times 100}{\text{Label Claim}}$$

System suitability:

The system suitability was assessed by five replicate injections of the Tiotropium bromide containing 0.374 µg/ml and Budesonide containing 4.050 µg/ml of both the drugs. The peak asymmetry, number of theoretical plates, the percentage relative standard deviation of standard solution five injections were calculated as represented in **Table 1**. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination.

Table 1: System Suitability parameters for RP-HPLC method

Parameters	Values		
	Tiotropium bromide	Budesonide-Epimer-B	Budesonide-Epimer-A
Theoretical plates	4650	7150	7263
Asymmetry Factor	1.15	0.80	0.75
%RSD	0.65	0.71	0.89

METHOD VALIDATION

The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.

Linearity:

To evaluate linearity of the method, six calibration standards of Tiotropium bromide containing 0.037, 0.094, 0.187, 0.374, 0.449, and 0.561 mg/mL and Budesonide containing 0.405, 1.013, 2.025, 4.050, 4.860, and 6.075 mg/mL were analyzed. A plot of peak areas versus Tiotropium bromide and Budesonide concentrations was linear in the range from 0.037 to 0.561 mg/mL of Tiotropium bromide and 0.405 to 6.075 mg/mL of Budesonide with a correlation coefficient of 0.9991 and 0.9995. This result demonstrates linearity of this method over the specified range.

One set of three different concentrations of mixed standard solutions of Tiotropium bromide and Budesonide were prepared. All the solutions were analyzed thrice, in order to record any intraday variations in the results. For inter day variations study three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days. The peak areas were recorded and relative standard deviation (RSD) was calculated.

Accuracy:

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% and 150%.The percentage of recoveries were calculated, results of which are represented in **Table 2**.

Precision:

Table 2: Recovery studies of Tiotropium bromide (TB) and Budesonide (BD)

Drug	% Level	Amount added(µg/ml)	Amount found(µg/ml)	% Recovery*	% RSD
TB	50	0.189	0.190	100.5	0.51
	100	0.375	0.372	99.2	0.85
	150	0.566	0.561	99.1	0.12
BD	50	2.058	2.049	99.6	0.52
	100	4.085	4.052	99.2	0.34
	150	6.078	6.054	99.6	0.84

*Average of three determinations.

Limit of Detection and Limit of Quantification:

LOD and LOQ were calculated as 3.3 ∞/S and 10 ∞/S respectively; where ∞ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness:

In the robustness study, the influence of small, deliberate variations of the analytical parameters on retention time of the drugs was examined. The following three factors were selected for change: flow rate of the mobile phase (2.0± 0.04 ml/min), a wavelength at which the drugs were recorded (235±1 nm).One factor at the time was changed to estimate the effect. A number of replicate analyses (n=3) were conducted at 3 levels of the factor (-, 0, +).It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

For RP-HPLC method different mobile phases were tried and the mobile phase containing Buffer: Acetonitrile (65:35, v/v) was found to be optimal for obtaining well defined and resolved peaks with mean retention times 4.93 ± 0.01 and 10.350 ± 0.02 min & 11.268 ± 0.02 min (Mean ± S.D.) for Tiotropium bromide and Budesonide epimer B and Budesonide epimer A respectively. The representative chromatogram of the standard solution of mixture is shown in **Figure 1**.

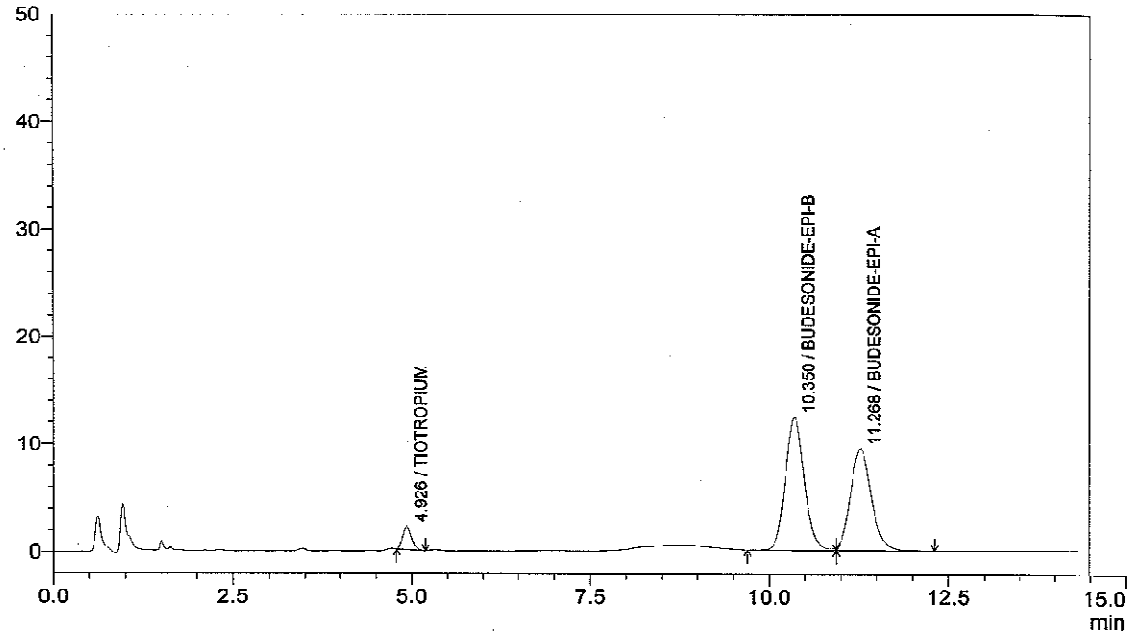


Figure 1: Representative chromatogram obtained for standard mixture of Tiotropium bromide (0.374 µg/ml, 4.93 ± 0.01 min), Budesonide (4.050 µg/ml, 10.350 ± 0.02 min, Epimer B & 11.268 ± 0.02 min, Epimer A)

Results were found to be linear in the concentration range of 0.374 to 0.561 µg/mL of Tiotropium bromide and 0.405 to 6.075 µg/mL for Budesonide respectively .The correlation coefficients for the plots were 0.9991 for Tiotropium bromide and 0.9995 for Budesonide. The proposed method was also evaluated by the assay of commercially available Inhalations

containing Tiotropium bromide and Budesonide. The % assay was found to be 99.3 ± 1.041 for Tiotropium bromide and 99.4 ± 0.012 for Budesonide (mean ± S.D., n = 6).The method was found to be accurate and precise, as indicated by recovery studies and % RSD not more than 2.Robustness of the method (data not shown), checked after deliberate alterations of the analytical parameters shown no marked changes in the chromatogram (RSD < 2), which demonstrated that the RP-HPLC method developed is robust. The summary of validation parameters of proposed HPLC method is given in **Table 3**.

Table 3: Summary of validation parameters of proposed RP-HPLC method

Parameters	Tiotropium bromide	Budesonide
Linearity range (µg/ml)	0.037-0.561	0.405-6.075
Correlation co-efficient	0.9991	0.9995
Slope(m)	182574.36	115241.25
y-Intercept(c)	1022.51	1074.63
Accuracy (% recovery)	100.5-99.1	99.2-99.6
Precision(%RSD)		
Intraday (n =3)	0.211	0.851
Inter day (n =3)	0.571	1.021

CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Tiotropium bromide and Budesonide in combined metered dosage inhalation form.

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