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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETERMINATION OF TULOBUTEROL IN TRANSDERMAL DRUG DELIVERY SYSTEM

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ABSTRACT

A sensitive, accurate and reproducible RP-HPLC method has been developed and validated for the determination of Tulobuterol in transdermal drug delivery system. Chromatographic separation was achieved isocratically on a Prontosil C_{18} column (250 x 4.6 mm, 5 µm) with a mobile phase consisting of Acetonitrile:0.02M Potassium dihydrogen phosphate buffer (60:40), adjusted to pH 3.0 with Orthophosphoric acid at the flow rate of 1.0 mL/min. UV detection was performed at 215 nm. The retention time for tulobuterol was found to be 2.880 minute. The proposed method was validated by parameters such as selectivity and specificity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness and assay according to the ICH guidelines. The calibration curve of tulobuterol was linear in the range of 25 – 75 µg/mL with correlation coefficient (r^2) of 0.9998. Both intra and inter-day precision and accuracy were within acceptable limits. The limit of detection and limit of quantification for tulobuterol were found out to be 2.90 and 0.95 respectively. The robustness was also estimated on the small fluctuations in the mobile phase compositions, wavelength and the flow rate. The developed method can be applicable for routine quantitative analysis.

KEYWORDS: Tulobuterol, Transdermal drug delivery, RP-HPLC, Method Validation.

INTRODUCTION

The Tulobuterol patch is the first bronchodilator transdermal delivery system developed for a long acting β_2 receptor agonists (LABA) and has been widely used in the management of asthma and chronic obstructive pulmonary disease (COPD). Tulobuterol is official in

Japanese Pharmacopoeia. The molecular formula of tulobuterol is C_{12} H_{18}ClNO and chemical name is (RS) -2-tert-Butylamino-1- (2-chlorophenyl) ethanol.[1-4]

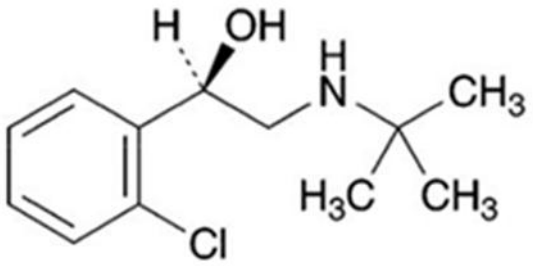

Figure 1: Structure of Tulobuterol

Nocturnal asthma is defined as variable exacerbation of the underlying asthma condition, which is associated with increased symptoms of airway hyper-responsiveness and/or worsening of lung function. Nocturnal asthma exaggerates and decrease in lung function in the early morning which known as the "morning dip". Hence tulobuterol patch was developed to prevent the morning dip and to sustain drug efficacy over 24 hours.[5] The tulobuterol patch is a unique transdermal drug delivery system prepared by using crystal reservoir technology. It's a directly acting sympathomimetic and has selective β_2 receptor stimulating activity. It's easy to use and requires only once-daily application. It should not be used in the patients with a history of hypersensitivity. The patch are available in three different doses: Tuloplast™ 0.5 (Each patch of 2.5 cm^2 contains 0.5 mg Tulobuterol), Tuloplast™ 1 (Each patch of 5 cm^2 contains 1.0 mg Tulobuterol), Tuloplast™ 2 (Each patch of 10 cm^2 contains 2.0 mg Tulobuterol).[6-7]

The literature survey showed a very few chromatographic methods[8-12] were developed for the determination of tulobuterol in human plasma. Hence, there's no specific method for the determination of tulobuterol by RP-HPLC by using this particular mobile phase in transdermal drug delivery system. The proposed method is optimized and validated as per the international conference on harmonization (ICH) guidelines.[13]

EXPERIMENTAL

Materials and reagents

Tulobuterol was obtained as a gift sample from Vamsi Labs Ltd., Solapur, India. The available pharmaceutical formulation (Tuloplast™ 2 Transdermal Patch) was obtained commercially (Manufactured by Teikoku Seiyaku Co. Ltd., Kagawa, Japan). Each patch of

10 cm^2 contains 2.0 mg of Tulobuterol were used in analysis. HPLC grade acetonitrile (Thomas Baker) and water, Potassium dihydrogen phosphate (LOBA CHEM), Orthophosphoric Acid.

Instrumentation

RP-HPLC was performed using Shimadzu HPLC system consisting of a pump LC-20AD, rheodyne sample injection port with 20 microlitre loop, SPD-20A UV-Detector, Spinchrom software, column used was Prontosil C18 (250 x 4.6 mm, 5 µm), Weighing was done on Contech CA-123 balance and pH was adjusted using PCI analytics Digital pH meter 111.

Principle

Reversed phase liquid chromatography isocratic elution with SPD-20A UV detection.

Chromatographic Conditions

Column : Prontosil C18 (250 x 4.6 mm, 5 µm particle size)

Mobile Phase : Acetonitrile: 0.02 M Potassium dihydrogen phosphate buffer (60:40), adjusted to pH 3.0 with Orthophosphoric acid

Flow Rate : 1.0 mL/min

Wavelength : 215 nm

Retention Time : 2.880 minute

Injection Volume : 20 µL

Runtime : 7 minutes

Elution : Isocratic

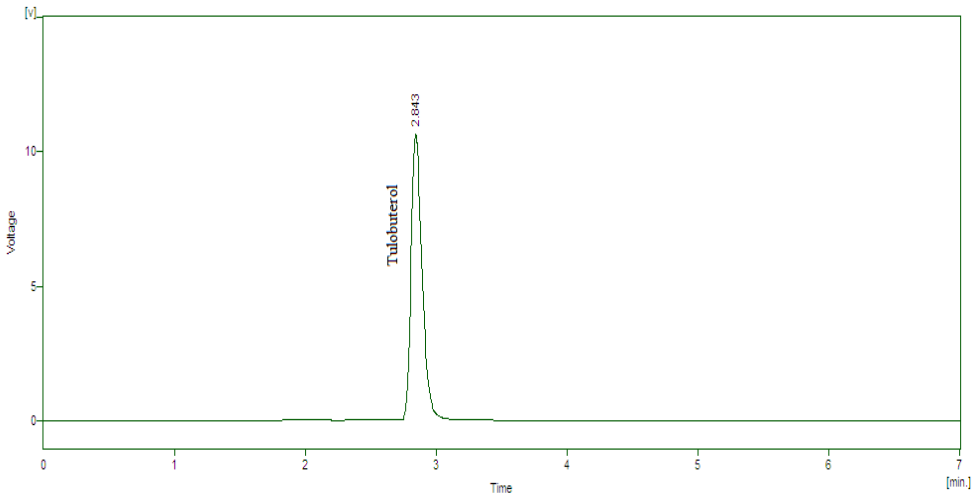

Figure 2: Chromatogram of Tulobuterol Standard

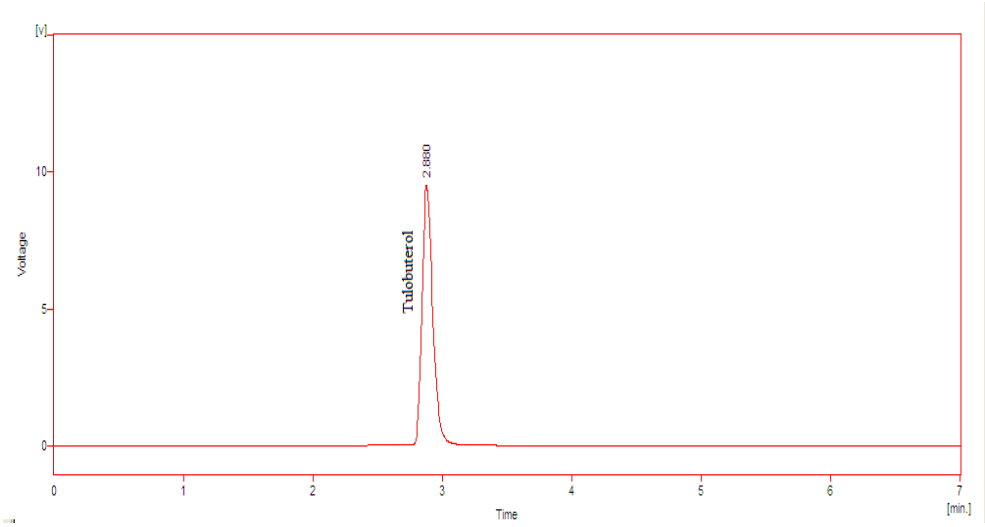

Figure 3: Chromatogram of Tulobuterol Patch

Preparation of 0.02 M Potassium dihydrogen orthophosphate (pH 3.0)

About 2.7218 g of Potassium dihydrogen orthophosphate was accurately weighed and transferred to 1000 ml volumetric flask and dissolved in 900 ml of water. The pH was adjusted to 3.0 ± 0.05 with Orthophosphoric acid, volume was made up to 1000 ml with water. The solution was then filtered using 0.45 µ membrane filter.

Preparation of Mobile Phase

Mixed Acetonitrile and 0.02 M Potassium dihydrogen orthophosphate buffer adjusted to pH 3.0 with Orthophosphoric acid in the ratio 60:40 and was sonicated.

Preparation of Standard Solution

About 100 mg of Tulobuterol was accurately weighed and transferred into 100 ml volumetric flask and dissolved in mobile phase and final volume was made up to the mark with mobile phase. Final concentration of tulobuterol of 50 µg/ml are made by suitable dilutions.

Preparation of Sample solution

Take the patch equivalent to 2 mg, then remove the release liner. After removal of release liner, their rims were carefully and completely cut into pieces and then patch was transferred into 25 ml conical flask containing 10 ml of mobile phase, sonication for 15 minutes; after sonication volume make up to 20 ml with mobile phase and again sonicate for 15 minutes. It was cooled down at room temperature for 15 minutes. Solution was filtered using Whatman filter paper. Final concentration of tulobuterol of 50 µg/ml are made by suitable dilutions.

RESULTS AND DISCUSSION

The proposed RP-HPLC method was validated as per ICH guidelines. The parameters studied for validation were selectivity and specificity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness and assay.

Selectivity and Specificity

To assess the selectivity of the developed method solution of drug were injected into the system then observe sharp peak of Tulobuterol were obtained at retention time of 2.880 min in reference to standard solution. Specificity was determined by comparison of the chromatogram of standard and sample solution. As the retention time of standard drug and the retention time of the drug in sample solution were same, so the method was specific. The parameters like resolution (R_s) and asymmetric factor were calculated. Good correlation was found between the results of standard and sample solution. Result are shown in the Table 1.

Linearity

The linearity of an analytical method is its ability to obtain results, which are directly proportional to the concentration of analyte in the sample. It was carried out by preparing the sample solution containing 25-75 µg/ml. A calibration curve was drawn by plotting concentration on X-axis Vs area on Y-axis and regression equation, correlation coefficient, y-intercept, slope of the equation was calculated. Result are shown in the Table 2 and Figure 4.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 75%, 100% and 125%. The recovery studies were carried out by adding known amounts of standard Tulobuterol were added to pre-analyzed samples and they were subjected to proposed HPLC method. The recoveries result of Tulobuterol in transdermal patch are shown in the Table 3.

Precision

Precision study was performed to find out intraday and interday variations. The intraday and interday precision study of Tulobuterol was carried out by estimating the correspondence response 3 times on the same day and on 3 different days for 3 different concentrations of tulobuterol and the results are reported in terms of % relative standard deviation (% RSD) however, all result fall within acceptance limits (RSD < 2), as shown in Table 4.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD is ability of analytical method able to detect the lowest concentration of the analyte. LOQ is lowest concentration of the analyte which can be quantitatively analyzed with acceptable precision and accuracy. It was calculated based on the slope and blank response from the calibration curve as per ICH guidelines. LOD and LOQ were calculated based on the standard deviation of the response and slope. Result are shown in Table 4.

Robustness

The robustness study was done by making small changes in the optimized method parameters like ± 2% change in mobile phase ratio, ± 2% change in flow rate and ± 2% change in wavelength. There was no significant impact on the retention time and tailing factor.

Assay

The amount of Tulobuterol per patch was calculated by comparing the peak area of the standard solution and sample. Result are shown in Table 5.

Table 1: System suitability parameters

System Suitability Parameters	Tulobuterol
Retention time (min)	2.880
Resolution	2.86
Theoretical Plates	4681
Asymmetric Factor	1.62

Table 2: Linearity of Tulobuterol

Sr No.	Concentration (µg/ml)	Peak Area
1.	25	593.926
2.	37.5	897.493
3.	50	1189.737
4.	62.5	1490.556
5.	75	1771.21
Regression equation		y = 23.581x + 9.532
Correlation coefficient (R^2)		0.9998
Slope		23.581
Intercept		9.532

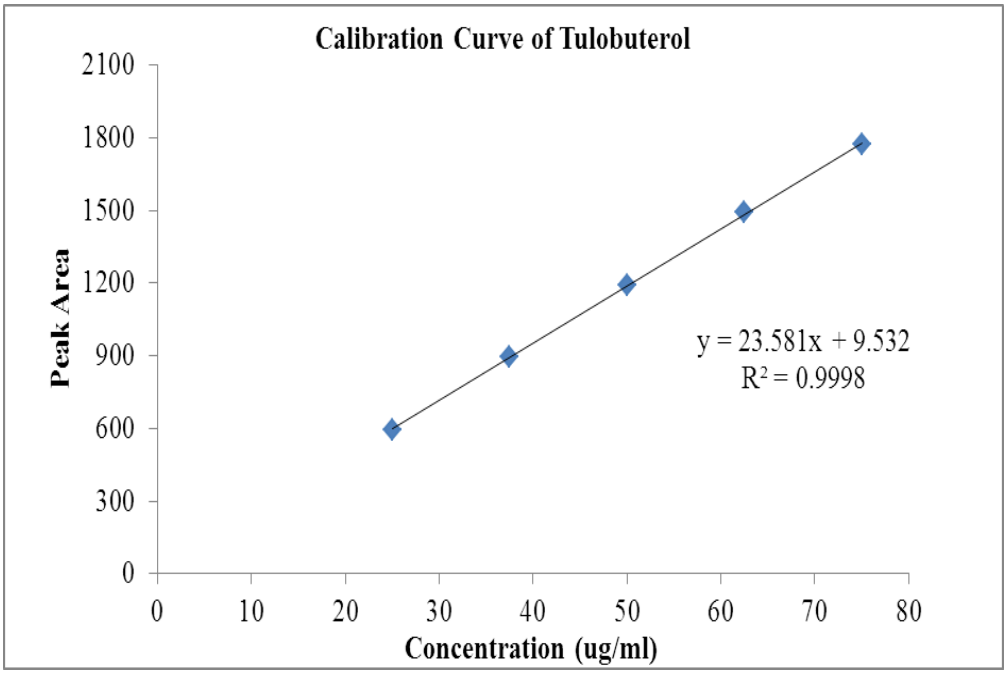

Figure 4: Calibration curve of Tulobuterol

Table 3: Accuracy Studies of Tulobuterol

Pre-analyzed sample solution (µg/ml)	Sample concentration (µg/ml)	Excess drug added (µg/ml)	Amount recovered (µg/ml)	% Recovery
Tulobuterol	25	12.5	37.13	99.00%
	25	25	49.59	99.19%
	25	37.5	62.10	99.36%

Table 4: Precision Studies, LOD and LOQ of Tulobuterol

Parameters	Precision(%RSD)		Limit of detection	Limit of Quantitation
	Intra-day (n=3)	Inter–day (n=3)		
Tulobuterol	0.42	0.35	2.90	0.95

Table 5: Assay Determination of Tulobuterol

Brand	% Amount found
Tuloplast™ 2 (Each patch of 10 cm^2 contains 2.0 mg Tulobuterol).	99.235%

CONCLUSION

The proposed HPLC method for the determination of tulobuterol in transdermal drug delivery system was found to be sensitive, accurate and reproducible. This conventional method has not been reported before. All the validated parameters were found to be within the range.

Thus the method developed can be used as alternative method for the routine determination of the drug in quality control laboratories as it has taken very short run time of around 3 min.

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