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METHOD DEVELOPMENT AND VALIDATION OF TULOBUTEROL IN API AND ITS PHARMACEUTICAL DOSAGE FORMS BY UV SPECTROPHOTOMETRY

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ABSTRACT: A simple, accurate, sensitive and reproducible UV spectrophotometric method has been developed for the determination of Tulobuterol (TLB) in bulk and also in its pharmaceutical dosage formulations. The proposed method showed absorbance maxima at 212 nm. Beer's law is obeyed over a concentration range of 25-125µg/mL. The respective linear regression equation being Y =0.009x +0.014 for TLB. Results of analysis for the method established, was validated statistically and also by recovery studies. The apparent molar absorptivity and Sandell's Sensitivity values are $0.43x10^4$ $L^{mol-1}cm^{-1}$ and 0.0371 μgcm^{-2} respectively. The assay and recovery studies were found to be 99.16% and coefficient correlation(r) was found to be 0.999. The different experimental parameters effecting the development and stability were studied carefully and optimized. No interference was observed in the presence of common pharmaceutical excipients. The validity of the methods was tested by analyzing the drug in its pharmaceutical preparations. Good recoveries were also obtained. The developed method employed was successful for the determination of TLB in various pharmaceutical preparations.

INTRODUCTION: Tulobuterol (TLB) (Empirical Formula: $C_{12}H_{16}ClNO$, Mol.Weight: 227.730 g/mol) chemically is, (*RS*)-2-(*tert*-butylamino)-1-(2-chlorophenyl) Ethanol **(Figure 1)**. Chronic Obstructive Pulmonary Disease (COPD) is an inflammatory lung disease that occurs as a result of inhalation of harmful particles, such as those in cigarette smoke. There is some concern that the number of COPD patients will increase with the aging of the population.

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The global initiative for Chronic Obstructive Lung Disease (COLD) recommends the use of long-acting bronchodilators [1-5], such as anti cholinergics, β_2 adrenergic receptor agonists and methyl xanthenes for the management of stable COPD patients. TLB is a direct-acting sympathomimetic with selective action on β_2-receptors. Thus, TLB is a selective β_2 adrenergic agonist with minimal nonselective inhibitory effect [6-9] on airway and vascular smooth muscle.

It also facilitates adrenergic neurotransmission, which may help to explain its bronchodilator effect in the intact organism. TLB does not activate β_1 adrenoceptors and has no direct positive chronotropic effect [10-13]. As highlighted earlier, the

use of the above drug has become, very wide spread.

The survey of literature showed a very few chromatographic methods and biological analytical methods [14-18] irrespective of any single spectrophotometric method for the analysis of selected drug at the time of commencement of these investigations.

So in order to bridge this gap, the authors are fascinated in choosing this drug. A detailed account of all analytical methods existing for the drug is made to avoid duplication of the method developed.

The authors has made some humble attempts, hoping to fulfill and bridge this gap, in succeeding the developed extractive analytical method by using spectrophotometry, verifying the efficacy and safety of TLB. The results of this labor of love are set forth in this article.

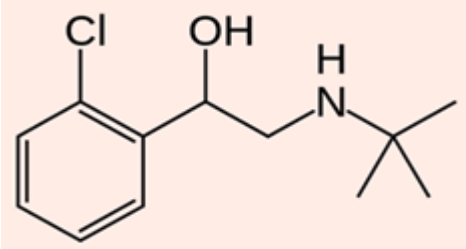

FIGURE 1: TULOBUTEROL

MATERIALS & METHODS:

Instrument: Shimadzu double beam Ultra Violet – Visible Spectrophotometer UV-1800 with 1 cm matched quartz cells were used for all spectral measurements.

Chemicals & Reagents: All the chemicals used were of analytical & extra pure reagent grade, procured from SD Fine Chemicals (SDFC), Mumbai, India. All the solutions were freshly prepared.

1. Acid Phthalate Buffer pH 2.4
2. Distilled Water
3. Hydrochloric Acid
4. Methanol AR grade
5. Potassium Hydrogen Phthalate EP

Preparative Analytical Methodology:

Preparation of Phthalate buffer pH 2.4: Add 250 ml of 0.2 M potassium Hydrogen Phthalate to 10 ml of 0.2 M HCL make up the final volume with water to produce 1000 ml.

PROCEDURE:

Preparation of standard stock and working sample solution: Weigh 0.5 gm of bulk drug (TLB) and dissolve in 50 ml of methanol, shake well till it dissolves and make up to 100 ml with the same. From the above stock solution, working sample solution was prepared from 0.25-1.25 ml (25-125µg/mL) respectively.

Assay: Aliquots of standard drug solution of TLB containing 0.25-1.25 ml (25-125 µg/mL) were taken and transferred into test tubes. 2 ml of Phthalate buffer pH 2.4 and 5 ml of methanol were added. The contents are shaken thoroughly for 5 min and allowed to stand for 15 minutes.

The absorbance was measured at 212 nm against reagent blank and a calibration curve was constructed. The absorbance of the sample solution was measured, and the amount of TLB was determined by referring to the calibration curve or computed from the regression equation as illustrated in **Figure 2, 3 & 4.**

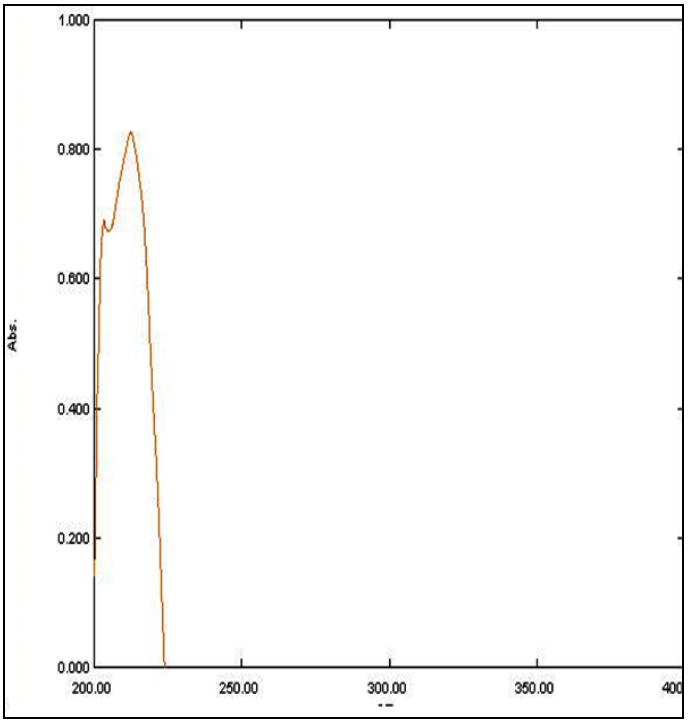

FIGURE: 2: ABSORPTION SPECTRUM OF TLB

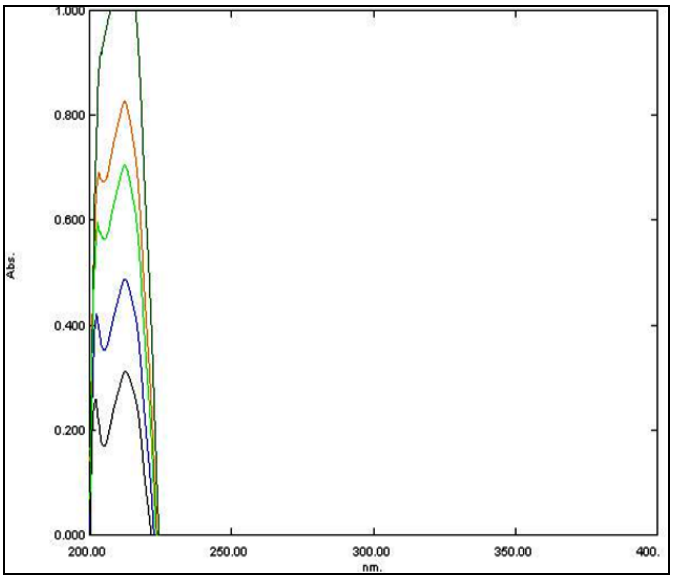

FIGURE 3: OVERLAY ABSORPTION SPECTRA OF ALL THE CONCENTRATIONS

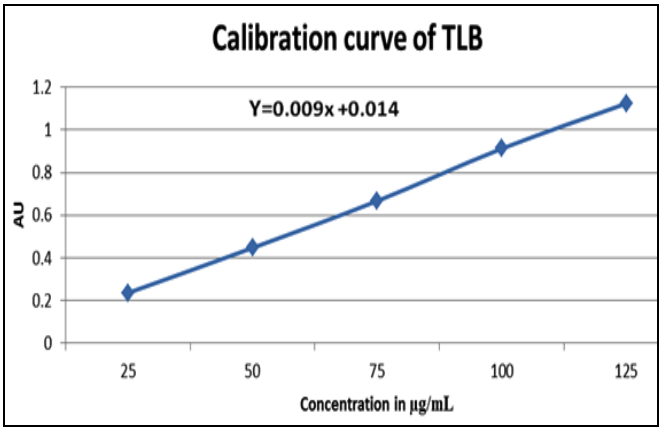

FIG: 4: STANDARD CALIBRATION CURVE OF TLB

Preparation of the sample solution: Ten tablets of TLB were accurately weighed and powdered. Tablet powder equivalent to 100 mg of TLB was dissolved in 50 ml of methanol, sonicated for 15 mins and filtered. The filtrate is combined and the final volume was made to 100 ml with methanol for the above method. The solution was suitably diluted and analyzed as given under the assay procedure for bulk sample and the linearity absorbance range observed for TLB was shown in **Table 1**. The analysis procedure was repeated three times with tablet formulations and the result of analysis was determined as depicted and shown in **Table 2.**

TABLE 1: LINEARITY ABSORBANCE RANGE OF TLB

Concentration(µg/mL)	Absorbance
25	0.236
50	0.447
75	0.667
100	0.912
125	1.124

Recovery Studies: To ensure the accuracy and reproducibility of the results obtained, known amounts of the pure drug was added to the previously analyzed formulates samples and these samples were reanalyzed by the proposed method and also by performing recovery studies. The percentage recoveries, thus obtained for this method were given in Table 2.

TABLE 2: ASSAY & RECOVERY STUDIES OF TLB IN TABLET FORMULATION

Tablet Formulation	Amount Claim (mg/tablet)	*Amount obtained (mg) by the proposed method	**% Recovery by the proposed method
1	5	4.93	102.28
2	5	4.82	101.19
3	5	4.87	100.83

*Average of three determinations, **After spiking the sample

RESULTS AND DISCUSSIONS: The UV spectrophotometric methods are more popular due to their sensitivity in assay of the drug and hence UV spectrophotometric methods have gained considerable attention for quantitative determination of many pharmaceutical preparations. This proposed method is UV spectrophotometric methods for the determination of TLB by using methanol as a solvent form in its formulation i.e. tablets.

The working conditions of this method were established by varying one parameter at a time and keeping the other parameters fixed by observing the effect produced on the absorbance. Various parameters involved in this method were optimized. The proposed method was validated statistically and also by recovery studies. The molar absorptivity and Sandell's sensitivity values show the sensitivity of method while the precision was confirmed by % RSD (Relative Standard Deviation). The optical characteristics such as absorption maxima (nm), molar absorptivity (lit. mol $^{-1}$ cm^{-1}), correlation coefficient (r) and Sandell's sensitivity (mg/cm^2/0.001) were

calculated and are summarized in **Table 3**. Assay results of recovery studies are given in **Table 2**.

Results are in good agreement with labeled values. The percent recovery obtained indicates non-interference from the common excipients used in the formulation. The reproducibility, repeatability and accuracy of these methods were found to be good, which is evidenced by low standard deviation.

The proposed method is simple, sensitive, accurate, precise and reproducible. Hence, they can be successfully applied for the routine estimation of TLB in bulk and pharmaceutical dosage form even at very low concentration and determination of stability of drug in formulation such as tablets.

TABLE 3: OPTICAL CHARACTERISTICS AND REGRESSION ANALYSIS OF THE PROPOSED METHOD

Parameters	Proposed method
Measured λ_{max} (nm)	212
Beers law limit (µg/mL)	25-125
Molar absorptivity (L/mole/cm)	$1.028x10^4$
Sandell's sensitivity (mcg/cm^2/0.001 Absorbance unit)	0.0371
Regression Equation (Y = mx + c)	Y (0.0039) =0.009x +0.014
Slope (m)	0.009
Intercept (c)	0.0039
Standard Error of Estimate	0.014
Correlation coefficient (r)	0.999
Precision (% Relative standard deviation)	0.3068
Confidence intervals (upper limit =1)	0.963-0.985
LOD (µg/mL)	0.13
LOQ (µg/mL)	0.39

CONCLUSION: TLB was estimated successfully by UV spectrophotometric method, both as a pure compound and also the constituent of a tablet formulation. The method is simple, rapid, accurate, or cost-effective with high accuracy & precision and does not involve any critical reaction conditions, or tedious sample preparation. It is unaffected by slight variations in experimental conditions such as pH, shaking time and temperature. The applicability of the new procedure for routine quality control of TLB in pharmaceutical formulations was established.

The results of this labor of love are set forth by developing a simple, precise and accurate method for the estimation of TLB in bulk drug sample and also in its pharmaceutical dosage forms, used for routine analysis of TLB.

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REFERENCES:

1. Global initiative for COPD. Global strategy for diagnosis, management and prevention of COPD. Update 2008.online. Available http:||www.gold copd.com|Guidelineitem.asp?L1=2&L2=1&intld=2003.
2. Littner MR, ilowite JS, Tashkin DP, *et al.* Long-acting bronchodilation with once daily dosing of Tiotropium (spiriva) in stable COPD .Thorax 2000:55:289-94.
3. Niewoehner DE, Rice K, Cote C, et al .prevention of exacerbations of COPD with tiotropium, a once-daily inhaled anticholinergic bronchodilators: A randomized trail .Ann intern Med 2005:143:317-26.
4. Barr RG, Bourbeau J, Camargo CA, Ram FS. Tiotropium for stable COPD: A meta-analysis. Thorax 2006; 61; 854-62.
5. Uemastu T, Nakano M, Kosuge K, Kanamaru M, Nakashima M. The pharmacokinetics of the β2-adrenoreceptors agonist, tulobuterol, given transdermally and by inhalation. Eur J Clin pharmacol 1993:44:361-4.
6. Fukuchi Y, Nagai A, Seyama K *et al.* Pharmacokinetics and pharmacodynamics of Tulobuterol in the treatment of stable COPD..An open label comparison with inhaled Salmeterol .treat respire Med-2005-4.447-55.
7. Taruma G, Ohta K. Adherence to treatment by patients with asthma or COPD: comparison between inhaled drugs and transdermal patch Respir Med 2007:101:1895-902.
8. Tashkin DP, Copper CB. The role of long-acting bronchodilators in the management of stable COPD. Chest 2004:125:249-59.
9. Hogg JC. Pathophysiology of air flow limitation in COPD .Lancet 2004:364:709-21.
10. Barnes PJ. Neural control of human airways in health and disease. Am Rev Respir Dis 1986:134:1289-314.
11. Fan VS, Curtis JR, TU SP, McDonell MB, Fihn SD.Ambulatory care quality improvement project investigators. Using quality of life to predict hospitalization and mortality in patients with obstructive lung diseases. Chest 2002:122:429-36.
12. Jones PW .Quality of life measurement for patients with diseases of the airway. Thorax 1991; 46:676-82.
13. de Torres JP, Casanova C, Hernandez C, et al. Gender associated differences in determinants of

quality of life in patients with COPD: A case series study .Health Qual life outcomes 2006; 4:72.
14. Xu F, Zhang Z, Tian Y, Jiao H, Liang J, Gong G, High-performance liquid chromatography electrospray ionization mass spectrometry determination of Tulobuterol in rabbit's plasma, J Pharm Biomed Anal. 2005 Feb 7; 37(1):187-93.
15. Thienpont LM, Verhaeghe PG, De Leenheer AP, Measurement of Tulobuterol in human plasma by capillary gas chromatography and selected ion monitoring detection, Biomed Environ Mass Spectrom. 1987 Nov; 14(11):613-6.
16. Matsumura K, Kubo O, Sakashita T, Adachi Y, Kato H, Watanabe K, Hirobe M, Quantitative determination of Tulobuterol and its metabolites in human urine by mass fragmentography, Journal of chromatography 1981; 222(1) 53-60
17. K Matsumura, O Kubo, T Tsukada, K Nishide, H Kato, K Watanabe, M Hirobe, Determination of Tulobuterol in human serum by electron-capture gas-liquid chromatography, Journal of Chromatography, 07/1982; 230(1):148-53.
18. Dudley RE, Patterson SE, Machotka SV, Kesterson JW, One-month inhalation toxicity study of Tulobuterol hydrochloride in rats and dogs, Fundam Appl Toxicol. 1989 Nov; 13(4):694-701.

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