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VALIDATED RP - HPLC METHOD FOR THE ESTIMATION OF OXYBUTYNIN IN FORMULATION

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Oxybutynin in tablet dosage form. Isocratic elution at a flow rate of 1.0ml/min was employed on a symmetry C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of 1% orthophosphoric acid: acetonitrile: methanol 40:45:15 (V/V/V). The UV detection wavelength was 205nm and 20µl sample was injected. The retention time for Oxybutynin was 2.435 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Oxybutynin in tablet dosage form.

Keywords: Oxybutynin, RP-HPLC, UV detection, Recovery, Precise.

INTRODUCTION

Oxybutynin molecular formula $C_{22}H_{31}NO_3$ and weight 357.486 g/mol IUPAC name 4-(diethylamino)but-2-yn-1-yl2-cyclohexyl-2-hydroxy -2-phenylacetate. Oxybutynin (Ditropan, Lyrinel XL) is an anticholinergic medication used to relieve urinary and bladder difficulties, including frequent urination and inability to control urination (urge incontinence), by decreasing muscle spasms of the bladder. It competitively antagonizes the M_1, M_2, and M_3 subtypes of the muscarinic acetylcholine receptor.[1-4] It also has direct spasmolytic effects on bladder smooth muscle as a calcium antagonist and local anesthetic, but at concentrations far above those used clinically. It is available orally in generic formulation and as the brand-names Ditropan and Lyrinel XL, as a transdermal patch under the brand name Oxytrol, and as a topical gel under the brand name Gelnique. Also Ditrospam by Avenzor Syria.Oxybutynin is also a possible treatment of hyperhidrosis, or hyper-active sweating.[5-12]

MATERIALS AND METHODS

Chemicals and Reagents

HPLC grade acetonitrile, orthophosphoric acid and methanol were purchased from Merck Specialties Pvt. Ltd.

Instrumentation and Analytical Conditions

The analysis of drug was carried out on a PEAK HPLC system equipped with a reverse phase C18 column (250x4.6mm, 5µm in particle size), a LC-P7000 isocratic pump, a 20µl injection loop and a LC-UV7000 absorbance detector and running on PEAK Chromatographic Software version 1.06. Isocratic elution with 1% orthophosphoric acid: acetonitrile: methanol 40:45:15 (V/V) (P^H-6.5) was used at a flow rate of 1.0ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

Stock and Working Standard Solutions

Accurately weigh and transfer 10mg of Oxybutynin working standard into a 10ml volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm nylon filter paper and finally12ppm were prepared. The calibration curve was plotted with the five concentrations of the 2ppm – 12ppm working standard solutions. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of Zolmitriptan Tablets

Weigh 20 Oxybutynin (Oxyspas - 5mg) tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of Oxybutynin in to a 10ml volumetric flask. Add diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45um filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark with diluents and finally 12ppm were prepared. Mix well and filter through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Oxybutynin was measured for the determination. The results are furnished in Table 3.

Validation Procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 2ppm to 12ppm prepared in triplicates to test linearity. The peak area of Oxybutynin was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Oxybutynin test solution in the same equipment at a concentration value of 100% (12ppm) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of the Oxybutynin was determined and precision was reported as %RSD.

Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of Oxybutynin at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Oxybutynin recovered in the samples. Sample solution short term stability was tested at ambient temperature (20 ± 10^0C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48 hours at ambient temperature and compared with freshly prepared solutions.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug Oxybutynin is non-polar. Non-

polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of orthophosphosphoric acid, acetonitrile and methanol was selected as mobile phase and the effect of composition of mobile phase on the retention time of Oxybutynin was thoroughly investigated. The concentration of the orthophosphoric acid, acetonitrile and methanol were optimized to give symmetric peak with short run time (Fig.3).

Validation of Method

Linearity

Five points graphs was constructed covering a concentration range 2-12 ppm (Three independent determinations were performed at each concentration). Linear relationships between the peak area signals of Oxybutynin the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table1.

Precision

The validated method was applied for the assay of commercial tablets containing Oxybutynin. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on two consecutive days indicated a RSD of 0.6355. This indicates good method precision.

Stability

The stability of Oxybutynin in standard and sample solutions containing determined by storing the solutions at ambient temperature (20 ± 10^0C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that Oxybutynin is stable and standard and sample solutions for at least 2 days at ambient temperature.

System suitability

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits (Table.3). The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Oxybutynin in tablet formulation. The results are furnished in Table 3.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Oxybutynin in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Oxybutynin in pharmaceutical dosage form.

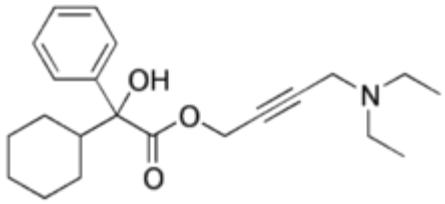

Figure1: Chemical Structure of Oxybutynin

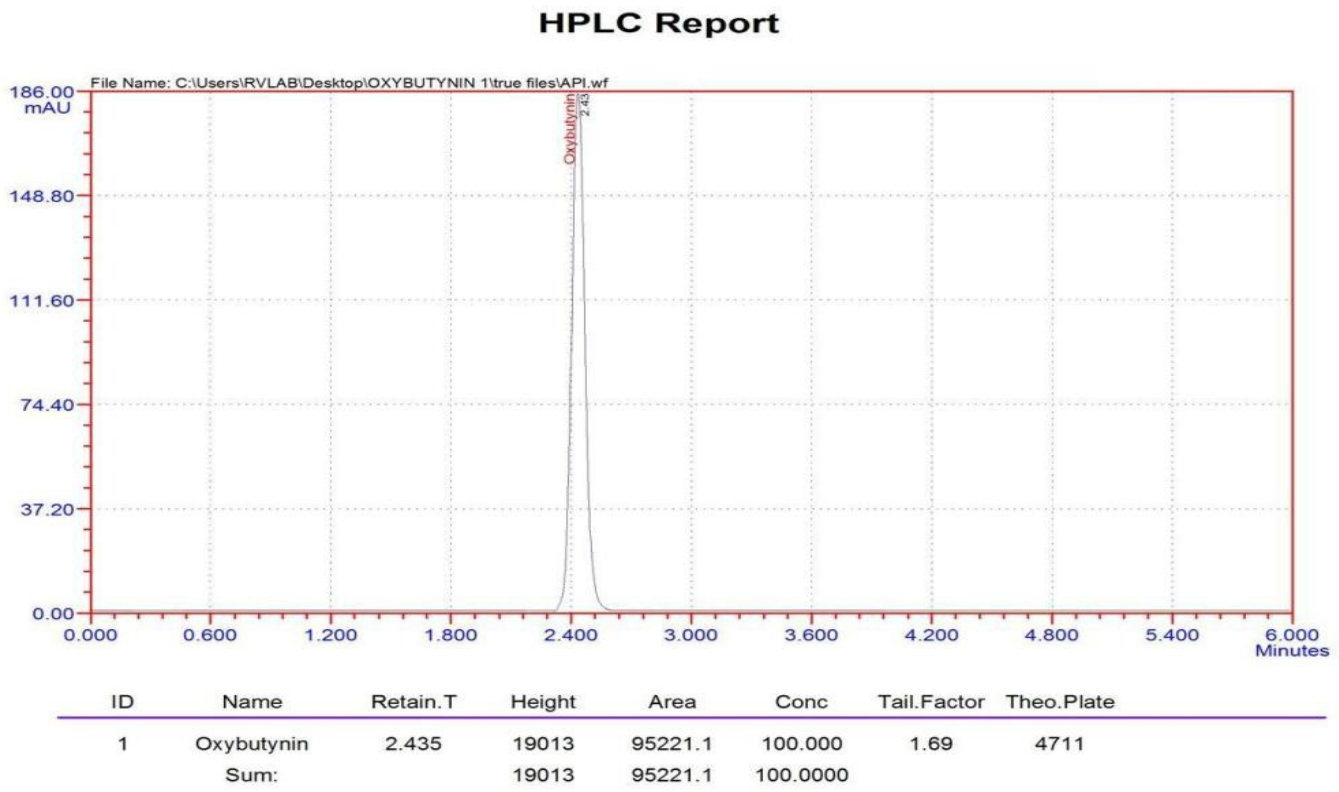

Figure2: Typical chromatogram of Oxybutynin formulation

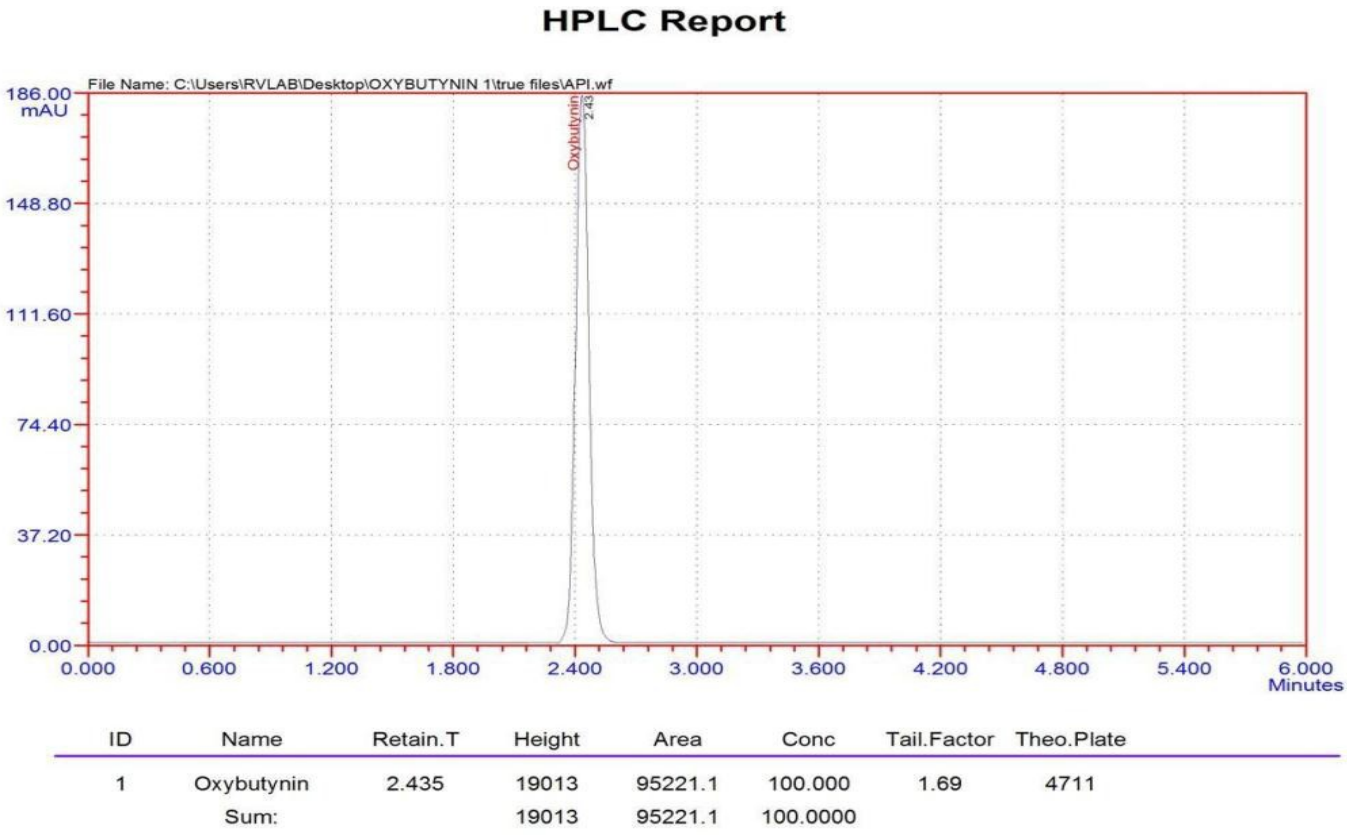

Figure3: Typical chromatogram of Oxybutynin

Table 1: Linearity of Oxybutynin

S.No.	Linearity level	Concentration	Area
1	I	2ppm	62161.7
2	II	4ppm	130828.1
3	III	6ppm	198704.9
4	IV	8ppm	274458.3
5	V	10ppm	343516.3
6	VI	12ppm	402085.0

Table 2: Recovery studies of Oxybutynin

% Concentration	% Recovery	Mean Recovery
50%	98.24%	98.95%
100%	99.23%	
150%	99.38%	

Table 3: System stability parameters

Parameters	Values
λ max (nm)	205
Beer's law limit (ppm)	2-12
Correlation coefficient	0.999
Retention time	2.435
Theoretical plates	4711.09
Tailing factor	1.69
Limit of detection (ppm)	0.24
Limit of quantification (ppm)	0.8
Slope	34445.33
Intercept	-6051.066667
accuracy	99.32%
R.S.D.	0.6355
% of Oxybutynin in formulation	3.95%

Table 4: Assay

Formulation	Label claim (mg)	% Amount found
Oxyspas	5mg	3.95%

Table 5: Chromatographic condition

Mobile phase	**1% Orthophosphoric acid:Methanol :Acetonitrile (40:15:45)**
PH	6.5
UV detection	205nm
Analytical column	C18
Flow rate	1.0ml/min
Temperature	ambient
Injection volume	20µl
Runtime	6min
Retention time	2.435 min

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