ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development of Analytical Method for Oxybutynin Hydrochloride by Spectrofluorimetry.

Arthy L[1], Subha S[1], Arun R[2], and Anton Smith A[1]*.

[1]Department of Pharmacy, Annamalai University, Annamalai Nagar -608002, India.
[2]Karpagam University, Pollachi Main Road, Eachanari Post, Coimbatore – 641021, India.

ABSTRACT

A simple, rapid, precise and accurate spectrofluorimetric method has been developed and validated for the assay of oxybutynin hydrochloride using acetonitrile as solvent. The fluorescence was measured at the excitation of 230nm and the emission was determined at 290nm. Beer's law was obeyed in the range of 10-60µg/ml. The LOD and LOQ were found to be 1.3µg/ml and 4.3µg/ml respectively. The percentage recovery was found to be 100.8±0.69% indicating no interference of the tablet excipients. The results demonstrate that proposed method is accurate, precise and reproducible while being simple and rapid too for the determination of oxybutynin hydrochloride in tablet dosage form.

Keywords: oxybutynin hydrochloride, spectrofluorimetry, formulation, determination.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Oxybutynin hydrochloride is a white, crystalline solid. It is readily soluble in water and acids, but relatively insoluble in alkalis. Its chemical name is 4-(diethylamino) but-2-yn-1-yl-2-cyclohexyl-2-hydroxy-2-phenylacetate; hydrochloride [1]. The structure of the compound oxybutynin hydrochloride is presented in figure 1. It exerts a direct antispasmodic effect on smooth muscles and inhibits the muscarinic action of acetylcholine on smooth muscle. It is indicated for the relief of symptoms of reflex neurogenic bladder [2]. Literature survey revealed that few methods have been performed for the analysis of oxybutynin hydrochloride such as spectrofluorimetry [3] spectrophotometry [4]-[9], charge transfer complexation reaction [10], volatammetry [2], HPTLC [11]-[12], HPLC [13]-[16], colorimetry [17]. Even though spectrofluorimetric estimataion has been developed, that method has complex reaction process. Hence the aim of present work was to develop a simple, accurate and precise spectrofluorimetric method using acetonitrile as solvent for the determination of oxybutynin hydrochloride in pure drug and pharmaceutical formulations.

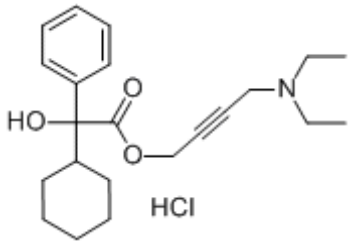

Fig.1: Structure of Oxybutynin hydrochloride

MATERIALS AND METHODS

Experimental

Instrumentation

The instruments used for the development process are UV-Visible Spectrophotometry (Systronics 2202), Spectrofluorimetry (Perkin Elmer LS 55), Sonicator (Branson 2510), Electronic balance (ADAIR-Precisa 92 SM-202A).

Chemicals and Reagents

Oxybutynin hydrochloride working standard was received as a gift sample. Formulations were purchased from the local pharmacies and used for analysis. Acetonitrile solution HPLC grade, Water: Double distilled water and all other chemicals used in the analysis were AR grade.

Procedure

Preparation of stock solution

10mg of pure drug Oxybutynin hydrochloride was weighed and transferred to a 10ml volumetric flask, about 5ml of acetonitrile was added to the above flask, ensured the complete solubility and the volume was made up with the acetonitrile.

Preparation of sample solution

The average weight of the tablets was determined by weighing 20 tablets and these were powdered. Tablet powder equivalent to 5mg of Oxybutynin hydrochloride was weighed and transferred to a 10ml volumetric flask. About 5 ml of acetonitrile was added and sonicated for 5mins for the complete dissolution of drug. The volume was made up with acetonitrile and filtered through Whattman filter paper no 1. Further dilutions were made with acetonitrile to attain a concentration of 10μg/ml. Six replicate analyses were carried out with sample weighed individually. The average weight of tablet was found to be 0.190g.

Validation

Various methods for analysis of Oxybutynin hydrochloride in bulk and formulation were carried out as per ICH-Q2R1 guidelines[18].

Linearity and range

The method was validated according to ICH Q2A guidelines for the validation of analytical procedures in order to determine the linearity, precision and accuracy of the Oxybutynin hydrochloride. Six point calibration curves were generated with the appropriate volumes of the working standard solution by spectrofluorimetry method. The intensity of fluorescence was measured by keeping 220 nm as excitation wavelength and 290nm as emission wavelength. The linearity was evaluated by the least-square regression method.

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicate at different concentration levels covering the entire linearity range. The precision of the assay was determined by repeatability (intra-day) and reported as RSD % for a statistically significant number of replicate measurements.

Recovery study (accuracy)

Accuracy is the percent of analyte recovered by assay from a known added amount of drug. Data from nine determinations over three concentration levels covering the specified range were obtained. The accuracy was determined with standard quality control samples prepared in triplicate at three different concentration levels (50%, 100% and 150%) covering the entire linearity range with the pre-estimated formulation by standard addition method.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations

$$LOD = 3\,\sigma/s;\ LOQ = 10\,\sigma/s$$

Where σ is the standard deviation of the absorbance (response) of the sample and s is the slope of the related calibration graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same sample under a variety of normal test conditions, such as different analysts, different instruments, lots of reagents, different elapsed assay times, different days etc. Ruggedness is normally expressed as the lack of influence on the test results, of operational and environmental variables, of the analytical method. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst.

The degree of reproducibility of the test results is then determined as function of the assay variables. This reproducibility may be compared to the precision of the assay under normal conditions to obtain a measure of the ruggedness of the analytical method.

ISSN: 0975-8585

Robustness

The robustness of the analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Stability

The stability of Oxybutynin hydrochloride in acetonitrile solution (30µg/ml) was studied by the spectrofluorimetry method. Sample solution was prepared and fluorescence intensity was noted down at 0hr, 6hrs and 24hrs.

RESULTS AND DISCUSSION

An absorption maximum was found out for the sample solution of 30 µg/ml concentration as described in Beer's law. In that we observed (λmax) being 220nm giving incremental absorbance, while the concentration has been increased. Hence 220 nm was used as excitation wavelength and 290nm as emission wavelength. The UV- Visible and fluorescence spectra are presented in Fig 2 and Fig 3 respectively.

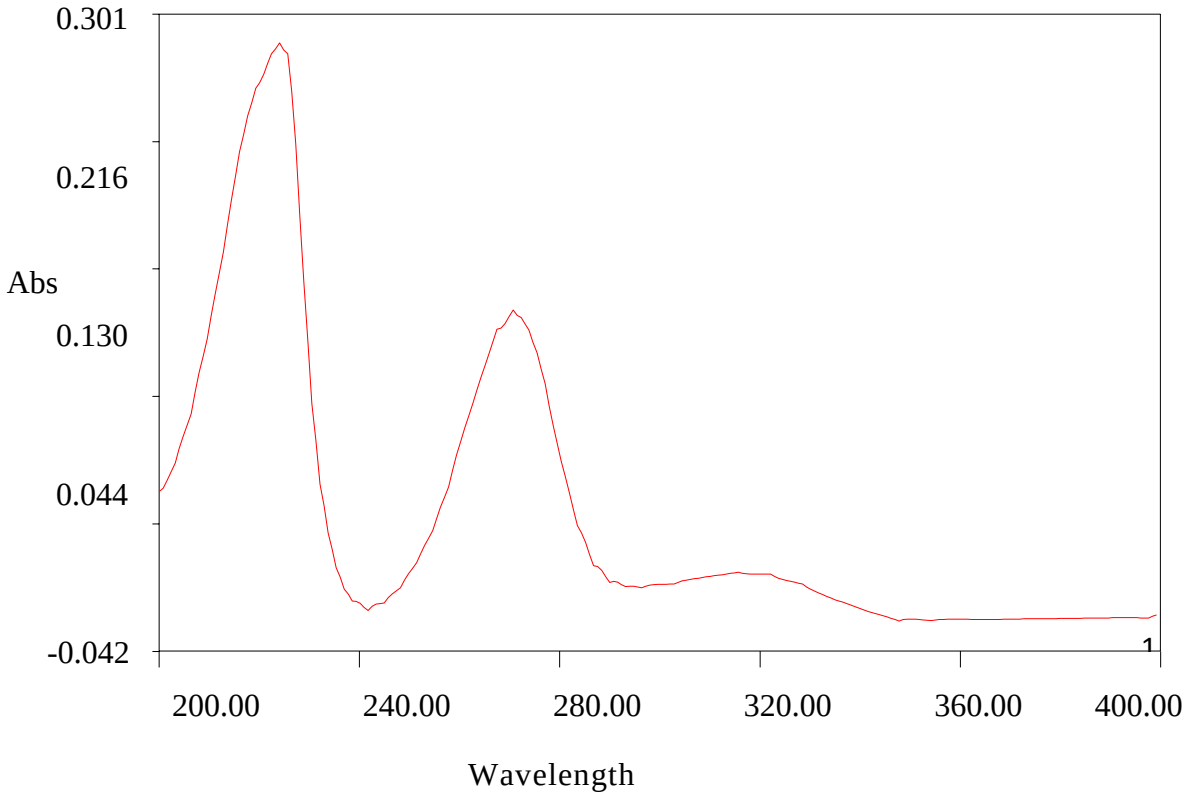

Fig 2: UV spectrum of Oxybutynin hydrochloride

ISSN: 0975-8585

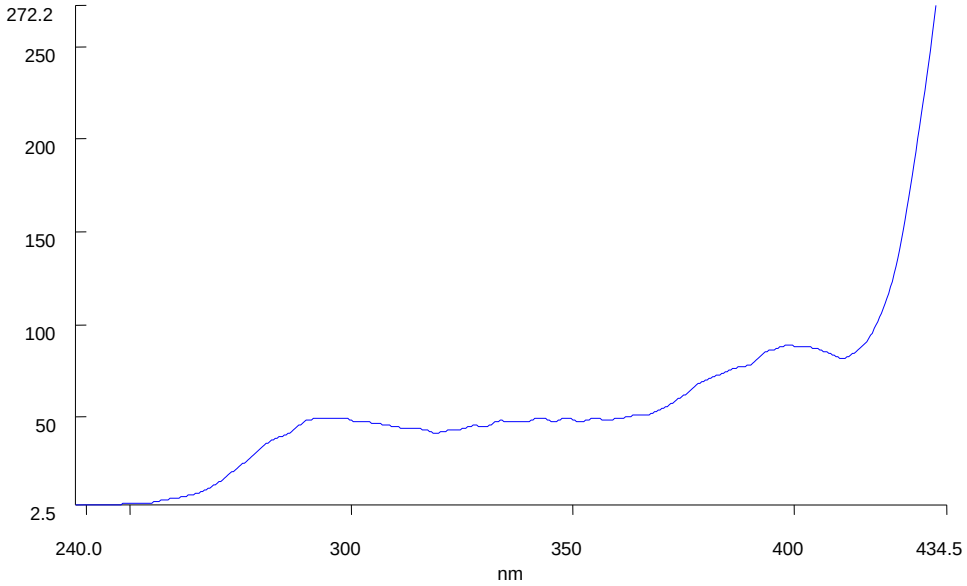

Fig 3: Fluorescence spectrum of Oxybutynin hydrochloride

Validation

Calibration curve data was constructed in the range of the expected concentrations of 10µg/ml to 60µg/ml was obeyed over this concentration range. The regression equation was found to be y = 8.273 x. The correlation coefficient (r^2) of the standard curve was found to be greater than 0.999. The stock solutions and working standards were made in acetonitrile. The λ_{max} of the drug for analysis was determined by taking scans of the drug sample solutions in the selected intensity and emission wavelength. Hence the assay can be carried out with in this concentration, which may produce reproducibility. The linearity profile in the different concentrations was presented in table 1 and the graph was presented in Fig.4 respectively.

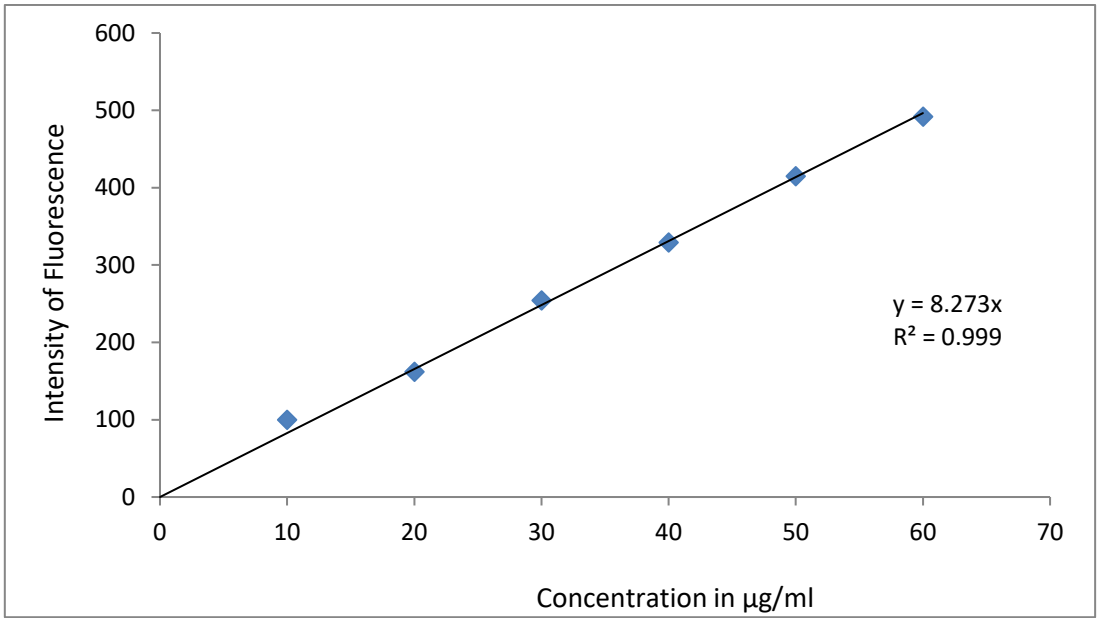

Fig 4: Linearity profile of Oxybutynin hydrochloride

ISSN: 0975-8585

Table 1: Linearity Data for Oxybutynin hydrochloride

Oxybutynin hydrochloride concentration (µg/ml)	Intensity of Fluorescence
10	100
20	162
30	254
40	329
50	415
60	492

The precision was carried out and the results were presented in table 2. The values obtained in the repeatability (precision) shows that there is no significant difference in the precision value. Hence the developed method can be used to analyte the Oxybutynin hydrochloride in tablet formulation. There is no evidence of interference of excitation with Oxybutynin hydrochloride. It was found to be that the mean precision value was found to be is 100.36 ±0.83 %. The precision results were observed from 99 to 101.20%.

Table 2: Precision study of oxybutynin hydrochloride

S.no	Weight of the sample (mg)	Intensity of fluorescence	Drug content (mg)	Percentage found (%)
1	189	443.81	4.94	99.00
2	189.2	447.05	5.00	100.50
3	190	444.04	5.02	99.80
4	190	448.81	5.01	101.00
5	189	450.37	4.99	101.20
6	189	449.01	5.00	100.70
Mean				100.36
SD				0.83
RSD				0.82

The recovery study was carried out as described and that was presented in table 3, where drug - drug interactions, drug-excipients interactions and drug-solvent interactions has not been found. Hence there is no interference of any component with the drug has been proved. The percentage recovery was found to be 100.08± 0.69.

Table 3: Accuracy of oxybutynin chloride

S.no	Level added (%)	Pure drug added (mg)	Mean as Recovery
1	50	2.5	99.63±0.47
2	100	5	100.09± 0.66
3	150	7.5	100.53± 0.81

n=3

The stability of Oxybutynin hydrochloride in acetonitrile solution was evaluated to verify whether any spontaneous degradation occurs, when the samples were prepared. The results were expressed as a percentage of the drug remaining. The obtained data (table 4) showed that the sample solutions were stable up to 24 hours.

ISSN: 0975-8585

Table 4: Stability Study Data

Time	Fluorescence intensity
0 0 hours	14 324.3
30 6 hours	10 325.1
60 12 hours	10 324.7
90 24 hours	10 324.9

The LOD was found to be 1.3 µg/ml and LOQ concentration was found to be 4.3µg/ml. The ruggedness studies were carried out as described as above. The concentration used for the above study was 30µg/ml. It was found to be that there no deliberate difference was found which was presented in table 5. The robustness study was carried out as described as above. From the below data (Table 6) it has been proved that there is no significant change when the drug analysed in different wavelength (±2 nm).

Table 5: Ruggedness

S. No.	Analyst – 1	Analyst – 2
	Intensity of fluorescence	
1.	324	325
2.	325	324
3.	326	325

Table 6: Robustness

S.No.	Solvent	Intensity of fluorescence
		290 nm
1 1.	Qualigen's	324
2 2.	Fischer's	327

CONCLUSION

A spectrofluorimetric method for quantifying Oxybutynin hydrochloride in formulation has been developed and validated. The assay is selective, precise, accurate and linear over the concentration range from 10-60 µg/ml. LOD & LOQ were found to be 1.3µg/ml and 4.3µg/ml respectively. The method is simple and suitable for the determination of Oxybutynin hydrochloride in pure form and pharmaceutical preparations.

REFERENCES

[1] The United States Pharmacopoeia (USP 34). National Formulary (NF 29); 2011.
[2] Jain R, Radhapyari K and Jadon N, J Colloid Interface Sci 2007; 314: 572–577.
[3] Walash MI, Belal F, El-Enany N and Elmansi H, J Fluoresc 2011;21:715–722.
[4] Srikanth K, Emmanuel KA and Raju KR, Rasayan J Chem 2010;3(1): 179-187.
[5] Patel R, Subrahmanyam EVS and Sharbaraya AR, J Chem Pharm Res 2012;4(9):4342-4351.
[6] Ramadan NK, Mohamed HM and El Laithy MM, Bull Faculty of Pharmacy Cairo University 2007;45:31-40.
[7] Varma MVS, Kaushal AM and Garg S, J Pharm Biomed Anal 2004;36:669–674.
[8] Wagieh NE, Hegazy MA, Abdelkawy M and Abdelaleem EA, Talanta 2010; 80:2007–2015
[9] Manthera VSV, Aditya MK, Sanjay Garg, J Pharm Biomed Anal 2004; 36: 669 – 74.
[10] Sonia TH, Awatef EF, Marianne AM and Eman AM, , Int J Pharm Chem Sci 2013; 2(4): 2024-2033.
[11] Nour EW, Mahq AH, Talanta 2010; 80(5): 2007 –15.
[12] Sitadevi P and Rao MKLP, Anal Chem 2010; 9: 378-383.
[13] Mamatha J, Devanna N, Sandya Rani J, Int J Adv Sci Engg Tech 2017; 5(1): 125-130.
[14] El-Gindy A, Il Farmaco 2005; 60: 689-699.

[15] Avula S, Babu NK and Ramana VM, Pharmacophore 2011;2:156-162.
[16] Hassan A, Jordan J Pharm Sci 2011; 4: 114-123.
[17] Rohit Patel, Subrahmanyam EVS and Sharbaraya A R, J Chem Pharm Res 2012; 4(9):4342-4351.
[18] International Conference on Harmonization (ICH) Guidelines Q2 (R1), validation of analytical procedures: Text and methodology.2005.