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ANALYTICAL QUALITY BY DESIGN (AQbD): APPLICATION TO DEVELOPMENT AND VALIDATION OF THREE SPECTROPHTOMETRIC METHODS FOR DETERMINATION OF OXYBUTININ HYDROCHLORIDE IN PHARMACEUTICALS

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ABSTRACT

Three spectrophotometric methods *viz.* zero order, area under curve (AUC) and second derivative were developed for determining oxybutnin hydrochloride (OXN) in bulk and, pharmaceutical formulation. A novel approach of quality by design (QbD) was followed to develop robust spectrophotometric methods. Key method variables such as scanning speed and sampling interval were optimized using DoE and their effect on absorbane was studied. The method linearity was observed over a range of 10-80µg/mL of OXN. The developed method was also subjected to validation studies such as specificity, accuracy (>98%), precision (< 2%) etc. Utilizing QbD approach ensured development of analytical methods devoid of any quality risks. The developed methods were found suitable for determining analyte in both bulk as well as in drug formulation. Overall the methods were reliable, robust and possess the potential of application in routine quality control testing purposes.

KEYWORDS: Oxybutinin, AQbD, spectrophotometry, validation, robustness.

INTRODUCTION

Analytical quality by design (QbD) provides a clear knowledge on method variables, which may affect the analytical attributes.[1] It has several systematic steps for developing and

optimizing a quality analytical method. It helps ensuring method robustness and establishing optimum method conditions.

Oxybutinin hydrochloride (OXN), i.e. 4-(diethylamino)but-2-ynyl (2S)-2-cyclohexyl-2-hydroxy-2-phenylacetate; hydrochloride is a drug used in urinary incontinence.[2] Literature reveals few spectrophotometric methods were reported for quantifying OXN in pharmaceuticals.[3-5] Methods reported in literature possessed drawbacks like lack of information on sensitivity studies, molar extinction coefficients, and not addressing the additional peaks obtained in the spectrum, etc. moreover; these reported methods fail to establish the robustness of the method. Thus, efforts were made to develop an analytical quality-by-design driven superior ultra-violet spectrophotometric method to determine OXN in tablets.

In the present study, the analytical target profile (ATP) and critical analytical attribute (CAA) for developing robust UV spectrophotometric methods were defined rationally. Suitable risk assessment followed by risk management using experimental design based investigation of critical method variables (CMVs) for their effect on CAA was carried out. Further, method control strategies were framed for achieving continuous improvement of method performance. Therefore, based on the above intent successful attempts were made to accentuate the AQbD-based development and validation of three robust UV spectrophotometric methods for reliable determination of OXN in pharmaceutical formulations.[6]

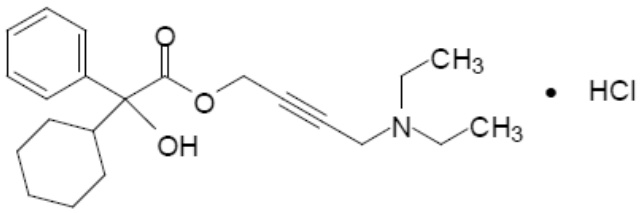

Figure 1: Chemical structure of oxybutinin hydrochloride.

MATERIALS AND METHODS

Materials

OXN (purity > 98%) was obtained from RA Chem Pharma Ltd., India. Methanol from Merck Ltd., Mumbai, India was used. The in-house tablet formulation containing 5mg of OXN was analyzed by the developed methods.

Instrumentation

A SHIMADZU 1800 UV spectrophotometer (Kyoto, Japan) along with a high-precision analytical balance and an ultrasonicatior (Enertech, India) was used for the purpose.

Methods

Establishment of ATP

Investigation of literature and analyte profile helped towards the establishment of method objective. This primarily contained developing a robust and cost-effective analysis of OXN in in-house tablet formulation. In order to meet the ATP, three UV spectrophotometric methods were selected considering absorbance of OXN as CAA.

Risk Assessment

To understand the causal-effect relation between the potential method variables and CAA, a typical fish-bone diagram can be used. Hence, a fish- bone diagram addressing various method variables affecting CAA was prepared (not shown in figures). A Control-Noise-Experimental(C-N-X) approach along with a suitable assessment matrix was employed for discovering the highest risk variables controlling the CAA. Scores were assigned to each variable and total score were evaluated to identify the CMVs. Variables *viz.* types of solvent, detection, scanning speed, sampling interval, sample intactness, etc. was evaluated for this purpose. Design of experiment approach was utilized for investigation and subsequent optimization of the sorted CMVs.

Method optimization and data analysis

A central composite design (CCD) was applied for identifying optimum method conditions and to ensure robustness. A robust analytical control space was established after selecting a CCD domain, with the intent of obtaining fewer experiments (thirteen experiments with five center points). Absorbance of standard drug 20 µg/mL at 204 nm was measured as the response variable and assessed as per CCD.

Different effects among the CMVs were unearthed using a selected mathematical model. The analysis of variance (ANOVA), lack of fit, the coefficient of correlation (R^2), predicted residual sum of squares (PRESS) etc. were the various parameters which were evaluated for data analysis purpose. Also polynomial equations, 2-D and 3-D plots were evaluated to assess model suitability. Further, the optimized chromatographic conditions were established by verifying numerical desirability function within the ACS region.

Analytical Control strategy

Control strategies were defined based on the results obtained for CAA. Control strategy was established for both the CMVs as per results obtained from numerical and graphical optimization.

Preparation of stock and working solution

Standard stock solution of concentration 1000 µg/mL of OXN was prepared by dissolving 25 mg of OXN in 5mL of methanol kept in a 25 mL volumetric flask. Finally, ultrasonication was carried out for 5 min, and volume was made up with methanol. Further, this solution was used to prepare the required dilutions of concentration ranging from 10-80 µg/mL of OXN.

Assay of in-house tablets

Finely ground and powdered tablet equivalent to 25 mg of OXN was ultrsonicated for 20 min in a 25 mL volumetric flask, having methanol. Finally, volume was made up followed by subsequent filtration using Whatmann filter paper. Drug content in the above solution was determined using the calibration curves.

Method Validation

Specificity

Specificity of the UV spectrophotometric method was evaluated by spectral verification UV spectra for any interference at the absorption maximum of drug because of excipients.

Linearity

Suitable aliquots from stock solution of standard OXN in separate 10 ml volumetric flasks were diluted to obtain concentration of 10-80 µg/mL. Dilution up to 10 ml was carried out with methanol. Spectral measurements were done at 204 nm (zero order), 215-225 nm (area under curve) and 237 nm (second derivative), respectively. Calibration curves were generated by taking the response *vs.* concentration and were later utilized for quantification purpose.

Accuracy and precision

Accuracy of the method using standard addition method was conducted at 80,100 and 120% (n=3) of the test concentration (20 µg/mL). The amount of drug standard added to the recovery solutions were calculated. The precision (intraday and interday) was assessed by hexaplicate analysis of OXN (20 µg/mL) and relative standard deviation (RSD) values were determined.

RESULTS AND DISCUSSION

The present paper describes development of three AQbD based UV spectrophotometric methods for analysis of OXN in tablets. OXN was insoluble in solvents other than methanol. Further, the studies were conducted using methanol as solvent. Standard OXN solution shows absorption maximums (λ_{max}) at 204 nm in methanol and was selected as the detection wavelength (Figure 2). CMVs were identified using fish-bone diagram and a C-N-X based risk estimation matrix (Table 1). The various method variables were assigned with total score and selected accordingly for DoE investigation.

Table 1: C-N-X based risk assessment matrix.

Method Variables	Risk Level on CAA	Score	C,N,X	Strategy
Scanning speed	10	100	X	DoE
Sampling interval	10	100	X	DoE
Wavelength	6	60	C	204nm
Sample integrity	5	50	N	Quality Assesed
Solvent grade	5	50	C	Controlled
Sample preparation	4	40	C	Controlled
Detector Equillibration	2	20	C	Controlled

C, N, X-Controlled, Noise, Experimental; Risk Level: 1-Negligible, 5-Low, 10-High; Final Score=(Risk Level of CMV×10).

Thirteen randomized experiments as per a CCD, were performed on a UV spectrophotometer to investigate the effect of CMVs on the CAA. Details of the experimentation strategy along with obtained results are listed in Table 2.

Table 2: Experimental Design Matrix as per CCD.

Run No.	Scanning Speed	Sampling Interval
1	1.0	-1.0
2	0	0
3	0	0
4	-1.0	1.0
5	0	1.0
6	1.0	0
7	-1.0	0
8	1.0	1.0
9	0	0
10	0	-1.0
11	0	0
12	-1.0	-1.0
13	0	0
Coded Level		
1(Low)	Slow	0.5nm
0(Nominal)	Medium	1.0nm
+1(High)	Fast	2.0nm

The optimization data was subjected to appropriate mathematical models for analysis. Polynomial equation (Eq.1) consisting of model terms for both main effects and interaction effects were generated for the CAAs. It helped to unearth the connection among the CMVs and CAAs, for sound understanding of the various effects.

$$Absorbance\ at\ 204nm = 0.28 + 0.0006A + 0.0005B + 0.0015AB + 0.010A^2 + 0.0027B^2 \ldots Eq.1$$

Where A= Scanning speed, B= Sampling interval.

Assessment of ANOVA ($P<0.05$) along with satisfactory values of r^2 ($r^2>0.9$) advocated for the adequacy of the selected mathematical model for obtaining optimum values of CAAs. Minimal interaction among both the CMVs was noticed for CAA, as the factor lines were not intersecting each other. Response surface evaluation was performed employing 3-D plot (Figure 3-(A). Figure 3(a) depicts a declining trend in absorbance at mid levels of scanning speed. A stationary "minima" was noticed for the same. Sampling interval was found to have no significant effect on CAA. Analogous interpretation was noticed from the 2D contour plot (Figure 3(B)) supporting the 3D response surface.

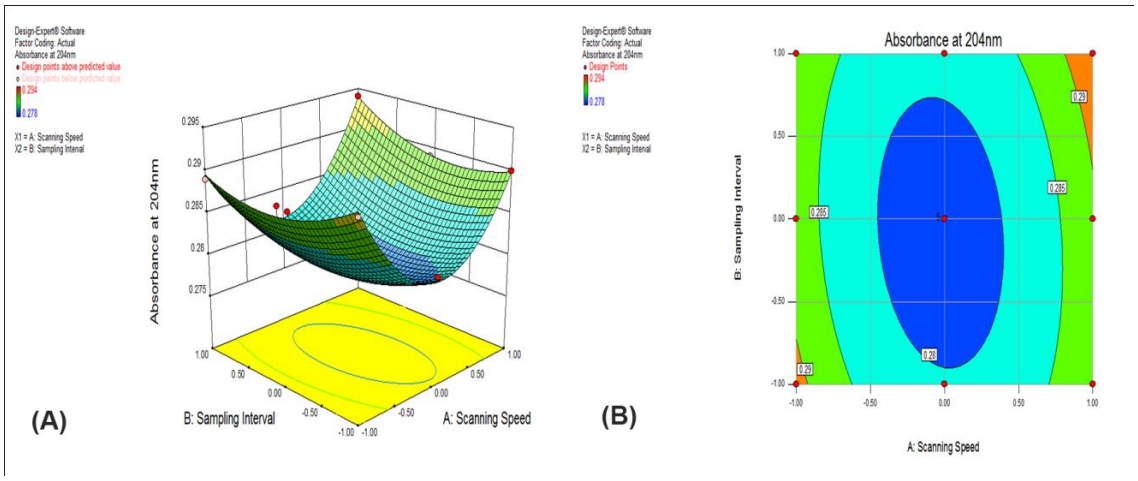

Figure 3: 3-D response surface obtained for absorbance at 204nm.

The desirability as well as overlay plot (Figure 4) represented spectrophotometric conditions for obtaining optimum values of all the three CAAs. Based on the above obtained conditions the method was performed for validation studies.

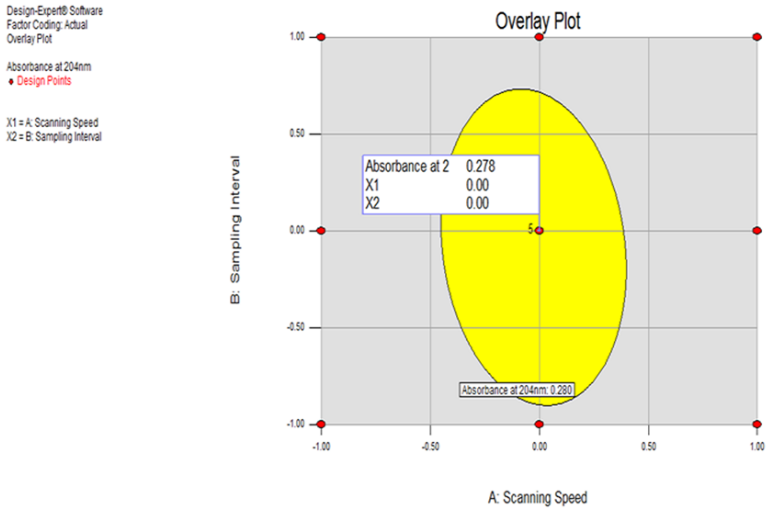

Figure 4: Analytical control space obtained for the optimized method.

A typical spectrum (Figure 5) of OXN in tablets revealed optimum peak shape in the predicted experimental conditions. Further, two varied approaches were derived from the base spectrum viz. area under curve (AUC) at 215-225nm (Figure 6) and second derivative at 237nm (Figure 7) were followed for further spectrophotometric measurements.

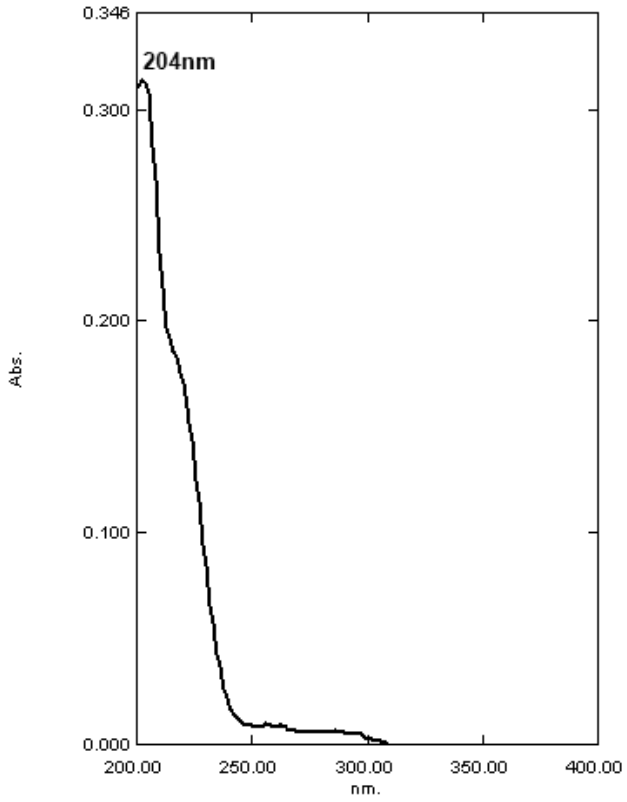

Figure 5: UV absorbance spectrum of OXN in methanol (zero order, Method-I at 204nm).

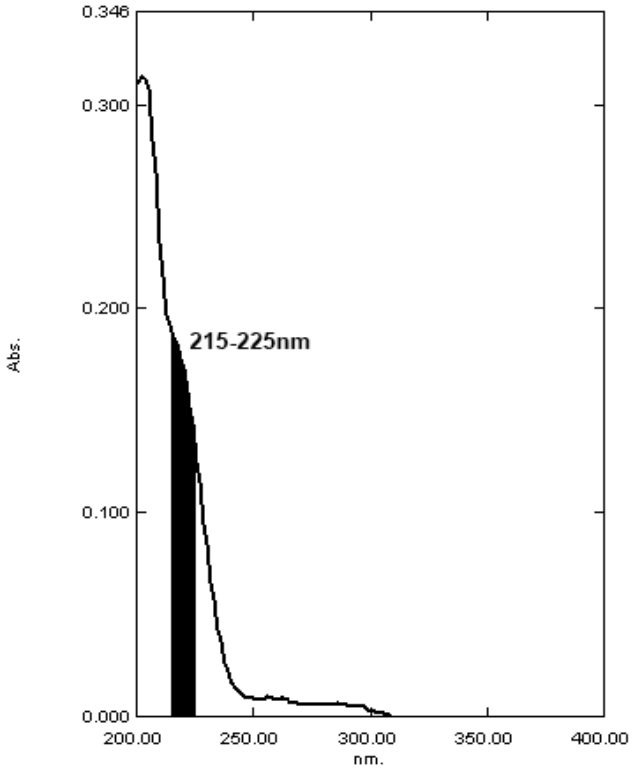

Figure 6: UV absorbance spectrum of OXN in methanol (AUC, Method-II at 215-225nm).

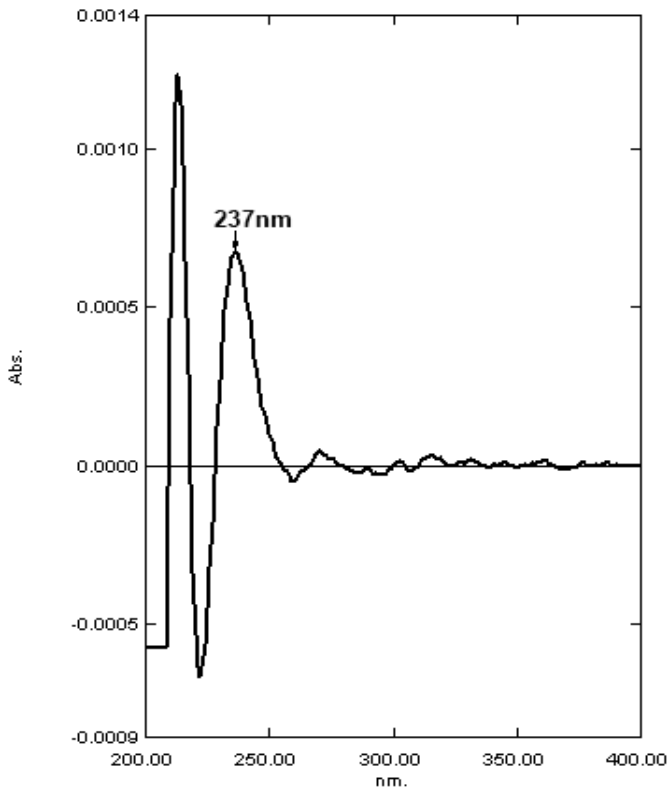

Figure 7: UV absorbance spectrum of OXN in methanol (second derivative, Method-III at 237nm).

The optical features of the developed analytical method were found satisfactory (Table 4). Specificity as well as selectivity of the method was achieved due to non-interference of the commonly used excipients present in the tablets. Linearity over concentration range of 10-80 µg/mL of OXN was established. The recovery of standard OXN from tablets was found to be within 99.13-99.8% (n=3) at all the λ_{max}. The results of accuracy (98.91-100.21%) and precision (%RSD < 2) were acceptable. The results from the validation study indicate the method is interference free and complies with the ICH requirements.

Table 4: Summary of obtained results for the three analytical methods.

Parameters	**Observed Values**		
Method Type	**Method-I, Zero order**	**Method-II, AUC**	**Method-III, Second Derivative**
Wavelength(nm)	204	215-225	237
Linearity range (µg/mL)	10-80	10-80	10-80
Sandell's sensitivity (µg/cm2/0.001AU)	0.056	…	…
Molar Extinction Co-efficient(L/mol.cm)	7.012×10^4	…	…
Regression Equation	Y=0.016x+0.017	Y=0.093x+0.074	Y=0.00003+0.00004
Correlation coefficient (R2)	0.999	0.999	0.999
Precision(%R.S.D) Intraday Interday	 0.13 0.19	 0.02 0.04	 0.08 0.09
Accuracy (%Recovery ± S.D.) 80% 100% 120%	 98.95±0.56 98.91±0.41 99.95±0.045	 99.87±0.06 100.18±0.35 99.98±0.81	 99.99±0.065 100.21±0.7 100.12±0.29
% Range of Error 0.05 confidence limits 0.01 confidence limits	 ±0.0399 ±0.0526	 ±0.0399 ±0.0526	 ±0.0040 ±0.0053

CONCLUSION

Three robust UV spectrophotometric methods were developed for determination of OXN in tablets, using the systematic AQbD approach. Two CMVs *viz.* scanning speed and sampling interval required systematic investigation and optimization to obtain a quality analytical method. The results of the study suggested method novelty, simplicity, accuracy and preciseness. The obtained values of method validation as per statistical studies indicated fitness of the method for routine analysis purpose. Lack of interference in analysis due to commonly used excipients established method specificity. Therefore, this AQbD based analytical method can be employed for determination of OXN in pharmaceuticals.

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