Asian Journal of Pharmaceutical Technology & Innovation
ISSN: 2347-8810

Research Article

Analytical Quality by Design (QbD) Approach to RP-HPLC Method Development and Validation of Meloxicam

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Received on: 14-08-2016
Accepted on: 20-08-2016
Published on: 15-10-2016

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ABSTRACT

Quality by Design (QbD) refers to a holistic approach towards drug development. Meloxicam is a Non steroidal anti-inflammatory drug (NSAID) which acts by inhibition of prostaglandin synthetase (cyclooxygenase 1 and 2) leading to the inhibition of prostaglandin synthesis, thereby exerts anti-inflammatory, antipyretic and analgesic effects. Though, various analytical methods have been reported, but they are tedious, difficult to reproduce and time consuming. So there is need to develop a novel RP-HPLC method to overcome this problem. A simple, fast and robust HPLC analytical method was developed by employing experimental design. In experimental design, Analysis of variance (ANOVA) was performed to study the significance of the method critical factors such as % composition of mobile phase, flow rate and wavelength on the response (asymmetric factor) was tested. The Reverse Phase (RP) HPLC method was successfully developed and validated according to International Conference of Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, robustness, LOD, LOQ. The polynomial regression data for the calibration plots exhibited linear relationship (r^2 = 0.999) over a concentration range of 25–125µg/ml and the linear regression equation was y = 41905x – 53177. The % coefficient of variation for both intra-day and inter-day precision was less than 2%. The good recoveries of MLX were obtained as more than 99%. The proposed method has proven that method is accurate, precise, reproducible and suitable for regulatory flexibility.

Key-words: Quality by design, Meloxicam, RP-HPLC, ANOVA

Cite this article as:
Anup A. Puranpole, Vijayalaxmi A. Chavan, Priyanka Kodrikar, Ganesh Mali, Analytical Quality by Design (QbD) Approach to RP-HPLC Method Development and Validation of Meloxicam, Asian Journal of Pharmaceutical Technology & Innovation, 04 (20); , 2016. www.asianpharmtech.com

INTRODUCTION:-

Quality by design (QbD encompasses designing and developing formulations and manufacturing processes which ensures predefined product specifications. QbD is a holistic approach where product specifications, manufacturing process and critical parameters are included in order to ease the final approval and ongoing quality control of new drug.[1-7] ICH guidance Q8 (R2) describes QbD as, "a systematic approach to pharmaceutical development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management".[8] Meloxicam is a 4-hydroxy-2-methyl-N-(5-methyl-1,3-thiazol-2-yl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide having molecular formula $C_{14}H_{13}N_3O_4S_2$, molecular weight 351.4, melting point 254ºc. The chemical structure of Meloxicam is given in figure-1.[9]

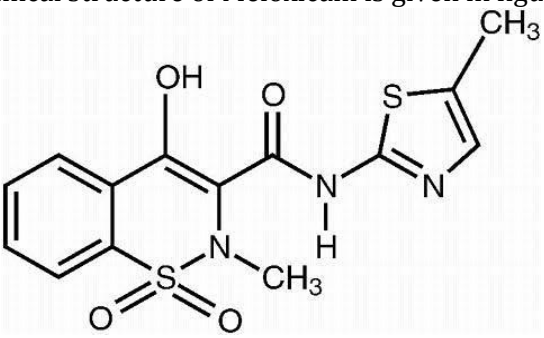


Fig. 1: Structure of Meloxicam

There are number of methods reported in the literature to determine meloxicam. Literature survey reveals that many researchers have adopted QbD principles to the development of analytical methods and they are termed analytical QbD (AQbD).[10-14] Also, there are some publications on HPLC method development strategy for Meloxicam[15-21] but the method development approach for RP-HPLC specifically focused on pharmaceutical development in QbD environment have not been discussed. Therefore there is unmet need to develop a systematic HPLC method development using QbD principles to ensure the quality of the method throughout the product lifecycle.

The primary objective behind the study was to implement QbD approach to develop and validate RP-HPLC[22-31] method for Meloxicam and by establishing thorough understanding of the method and build in quality during the method development; it will ensure optimum method performance over the lifetime of the product. Through the systematic method development by applying the statistical parameters [32] to optimization and evaluation of method, it will be supporting the design space concept of QbD.

MATERIALS AND METHODS:-

Preparation of stock solution:

Accurately weighed 10 mg of Meloxicam was transferred to 10 ml volumetric flask containing little amount of methanol and water in the ratio of 60:40. The volume was made up to the mark using same mixture of mobile phase to get 1000 ppm concentration.

Preparation of working solution:

From the stock solution, withdraw 0.5 ml and transfer it to volumetric flask and dilute it with the mobile phase upto 10 ml (50 ppm). The resulting solution is sonicated for 10 min.

Equipment:

Chromatographic separation was carried on a HPLC Binary Gradient System with integrated 4-liquid gradient system, high-speed and cooled auto sampler, temperature controlled column compartment and UV-3000-M detector.

Chromatographic condition:

Chromatographic separations were achieved on C_{18} Hexon (250 mm x 4.6 ID, Particle size: 5 micron). The output signal was monitored and processed using HPLC Workstation software. Temperature of column temperature was at 25˚C. Mobile phase consisting of 60% of methanol and 40% water, at a flow rate of 0.8 ml/min. Sample injection volume was 20 µl. Run time was 6 min. The standard and sample preparation was made with mobile phase methanol: water (60:40). Prior to the injection of drug solution, column was equilibrated with the mobile phase flowing through the system. Detection was done at 363nm.

Method design:

Method Optimization of the analytical method was tested applying multifactor response surface methodology. Experiment was performed by running 17 runs in triplicate and final optimized condition was analyzed by Design Expert-10 software. Injection volume was 20 µl and oven temperature was 25°C. The factors selected for optimization were flow rate, wavelength and concentration of the mobile phase. Box Behnken design as given in Table-1, was applied to these factors for optimization. Application of multivariate regression analysis resulted in a fitted full quadrate model for the average responses for peak USP tailing given by the equation

$$Y= \beta_0+\beta_1X_1+\beta_2X_2+ \beta3X_3+ \beta_{11}X_1^2+ \beta_{22}X_2^2+ \beta_{33} X_3^2 + \beta_{12} X_1 X_2+ \beta_{13} X_1X_3+ \beta_{23}X_2X_3$$

Where Y is the response,

β_0 is the arithmetic mean response,

β_1, $\beta_2$and β_3 are regression coefficients of the factor X_1, X_2 and X_3 respectively,

β_{11}, β_{22}, β_{33} are squared coefficients,

β_{12}, β_{13} and β_{23} are interaction coefficients

TABLE 1: BOX BEHNKEN EXPERIMENTAL DESIGN

Chromatographic Condition	Levels Used		
	Low (-)	Center (0)	High (+)
% Composition (X_1)	40	50	60
Flow Rate (X_2)	0.8	1	1.2
Wavelength (X_3)	361	363	365

Critical quality attributes:

In accordance with QbD principles, the optimal conditions should be surrounded with satisfactory design space in order to provide adequate robustness of the method. Therefore the optimal conditions were searched as experimental point where maximal selectivity factor of peak in minimal analysis duration and with sufficient surrounding design space could be obtained. The factors such as flow rate, mobile phase concentration, and wavelength found to be critical. For this they were determined by using the Design Expert 10 software. The Box Behnken optimization for Meloxicam is given in Table-2.

TABLE 2: BOX BEHNKEN OPTIMIZATION FOR MELOXICAM

Run	Coding (X1,X2,X3)	% Composition (Methanol: Water)	Flow Rate (ml/min)	Wavelength (nm)
1	-0-	40-60	1	361
2	0-+	50-50	0.8	365
3	0--	50-50	0.8	361
4	+-0	60-40	0.8	363
5	000	50-50	1	363
6	+0+	60-40	1	365
7	0+-	50-50	1.2	361
8	000	50-50	1	363
9	-+0	40-60	1.2	363
10	000	50-50	1	363
11	-0+	40-60	1	365
12	+0-	60-40	1	361
13	++0	60-40	1.2	363
14	000	50-50	1	363
15	--0	40-60	0.8	363
16	000	50-50	1	363
17	0++	50-50	1.2	365

Method validation:

To confirm the suitability of the method for its intended purpose, the method was validated in accordance with the ICH guidelines ICH guideline Q2 (R1) for system suitability, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), percentage recovery and robustness.

Linearity:

The linearity of an analytical method is its ability to elicit test results that are proportional to the concentrations of analyte in samples within a given range. 0.25, 0.5, 0.75, 1.0, 1.25 ml stock solution was pipette out in 10 ml of flask containing small amount of mobile phase and volume was made up to the mark. The resultant solutions were sonicated for 10 min. Finally all of these solutions were injected in triplicate to given chromatographic conditions and area equivalent to each concentration was determined. Calibration curve was constructed between concentrations versus peak area.

Precision:

The precision of an analytical method expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. Precision was evaluated by injecting concentration of 75µg/ml. of Meloxicam and the peak area was recorded for intraday and interday precision in three replicates as per ICH guidelines Q2 (R1). The results for the same are usually expressed as the variance, standard deviation or confidence level of a series of measurements.

Accuracy:

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy should be established across the specified range of the analytical procedure. The ICH document on validation methodology recommends accuracy to be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g., three concentrations/three replicates each). From the calibration range three QC standards were defined as 25, 75 and 125 µg/ml. Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value.

Percentage Recovery:

Percent recovery is determination of percent purity of given analyte in finished product. The accuracy of the methods was determined by calculating recoveries of Meloxicam by the standard addition method. Known amount of standard solutions of Meloxicam (50 µg/ml) was added to its sample solution of (25, 50, 75 µg/ml) representing 50, 100, 150% recovery levels. Each of this solution was injected in triplicate and the mean area in each case was determined. The equivalent mean measured concentrations for each level were determined. Finally percentage recovery was calculated.

LOD and LOQ:

The detection limit of an individual analytical procedure is the lowest amount of analyte in sample, which can be detected but not necessarily quantified as an exact value. LOD is expressed as a concentration at a specified signal to noise ratio. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in sample, which can be quantitatively determined with suitable precision and accuracy.

Robustness:

The robustness of analytical method is the 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. Experiment was performed by changing condition such as wavelength (+2 nm). 75 µg/ml working solution of Meloxicam was selected for robustness study.

RESULTS AND DISCUSSION:-

HPLC method optimization by QbD approach:

QbD is a systematic approach to development that begins with predefined objectives and emphasizes product, process understanding and process control, based on sound science and quality risk management. As analytical techniques and method are used for quality control of pharmaceutical compounds and there by assure patient safety and efficacy, they have become an essential part of pharmaceutical QbD. The initial chromatographic conditions and the data obtained from seventeen QbD trails were given in Table-3 and Table-4 respectively.

TABLE 3: INITIAL CHROMATOGRAPHIC CONDITION

Column	Hexon C18 (250mm x 4.6ID, Particle size: 5 micron)
Flow Rate	0.8ml/min
Wavelength	363nm
Injection Volume	20µl
Run Time	6min
Mobile Phase Ratio	Methanol: Water (60:40)
Temperature	30°C

TABLE 4: DATA OBTAINED FROM QBD RUNS STUDY

Run	Coding (X_1,X_2,X_3)	% Composition (Methanol: Water)	Flow Rate (ml/min)	Wavelength (nm)	Tailing Factor
1	-0-	40-60	1	361	2.15
2	0-+	50-50	0.8	365	1.39
3	0--	50-50	0.8	361	1.36
4	+-0	60-40	0.8	363	0.8
5	000	50-50	1	363	1.47
6	+0+	60-40	1	365	0.85
7	0+-	50-50	1.2	361	1.37
8	000	50-50	1	363	1.47
9	-+0	40-60	1.2	363	2.29
10	000	50-50	1	363	1.47
11	-0+	40-60	1	365	2.38
12	+0-	60-40	1	361	0.78
13	++0	60-40	1.2	363	0.82
14	000	50-50	1	363	1.47
15	--0	40-60	0.8	363	2.22
16	000	50-50	1	363	1.47
17	0++	50-50	1.2	365	1.45

Method design:

Box Behnken Multivariate regression analysis was applied and fitted full quadratic model was obtained for the USP tailing factor of peak. Factor considered here are flow rate, mobile phase composition and wavelength. Regression coefficient and p-values obtained from software generated report are given in Table 5. Analysis of variance (ANOVA) was performed to study the significance of the factors and interaction terms on the response i.e. USP tailing of the peak. The p-value simply provided the cut-off beyond which we emphasized that the findings were statistically significant by convent ion ($p < 0.05$).

TABLE 5: REGRESSION COEFFICIENTS AND ASSOCIATED PROBABILITY VALUES (P-VALUES) FOR USP TAILING OF MELOXICAM

Term	Coefficient	p value
Intercept	1.47	
%Composition	-0.72375	< 0.0001
Wavelength	0.05125	0.0008
Flow rate	0.02	0.0625
%Composition x Wavelength	-0.04	0.0166
Wavelength x Flow rate	0.0125	
% Composition x Flow rate	-0.0125	
%Composition x %Composition	0.105	< 0.0001
Wavelength x Wavelength	-0.035	0.0262
Flow rate x Flow rate	-0.0425	0.0113

The p-value was found to be less than 0.05, hence model was found to be significant for prediction of response s. Entire model was fitted well for optimization. Significant factors found were interaction of flow rate x flow rate (p= 0.0113), %composition x %composition (p-value < 0.0001) and wavelength x wavelength (p-value=0.0262). Three of the factors were found to affect the peak response from their respective coefficients namely flow rate, wavelength, flow rate x wavelength. All of these three factors were shown positive relationship with tailing.

Response surface and contour plot were studied to visualize effect of factor and their interaction so as to develop design space for robust method. The contour plot is a two-dimensional (2D) representation of the response (tailing factor) plotted against combinations of numeric factors and/or mixture components. It can show the relationship between the responses, mixture components and/or numeric factors.

From Figure-2, Figure-3, Figure-4 it was concluded that for reliability of the method for routine analysis of the Meloxicam, flow rate, % Composition and wavelength should have been 0.8 ml, 60% and 363 nm respectively.

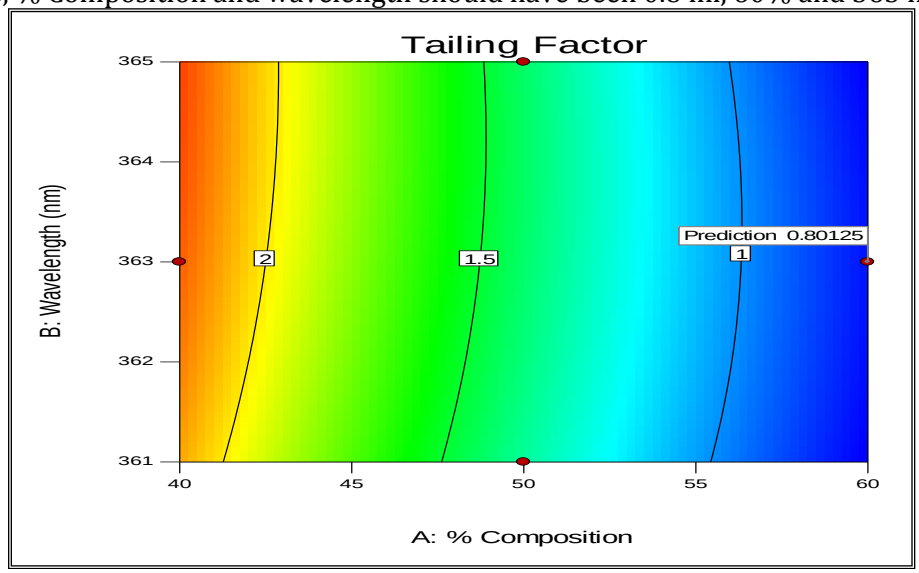


Fig. 2: Effect of % composition and wavelength on USP tailing factor of Meloxicam

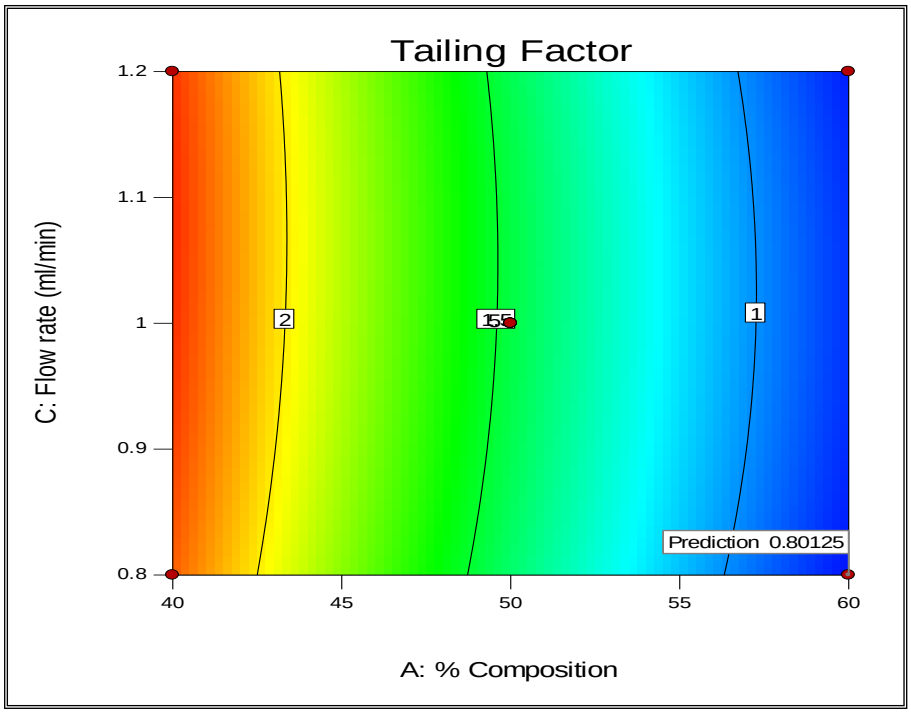


Fig. 3: Effect of % composition and flow rate on USP tailing factor of Meloxicam

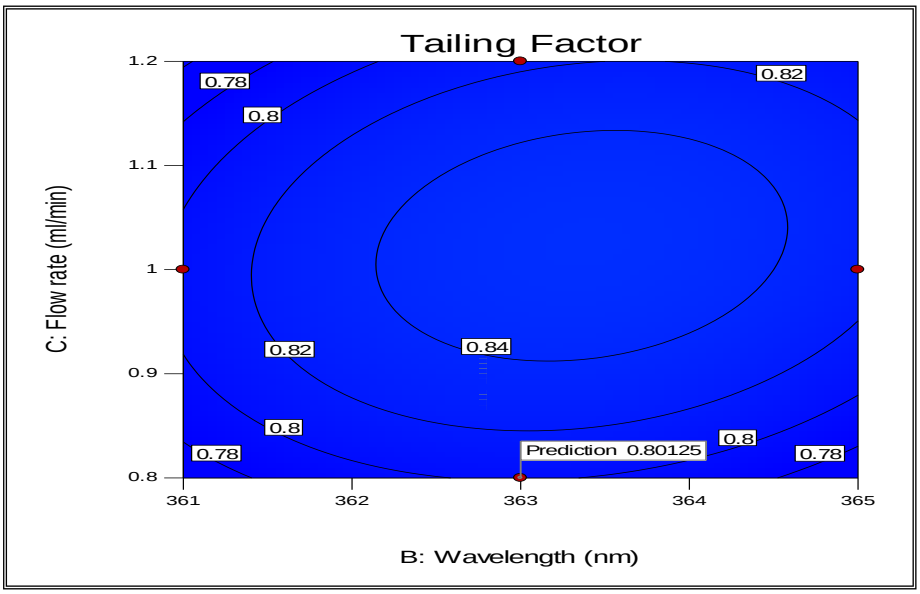
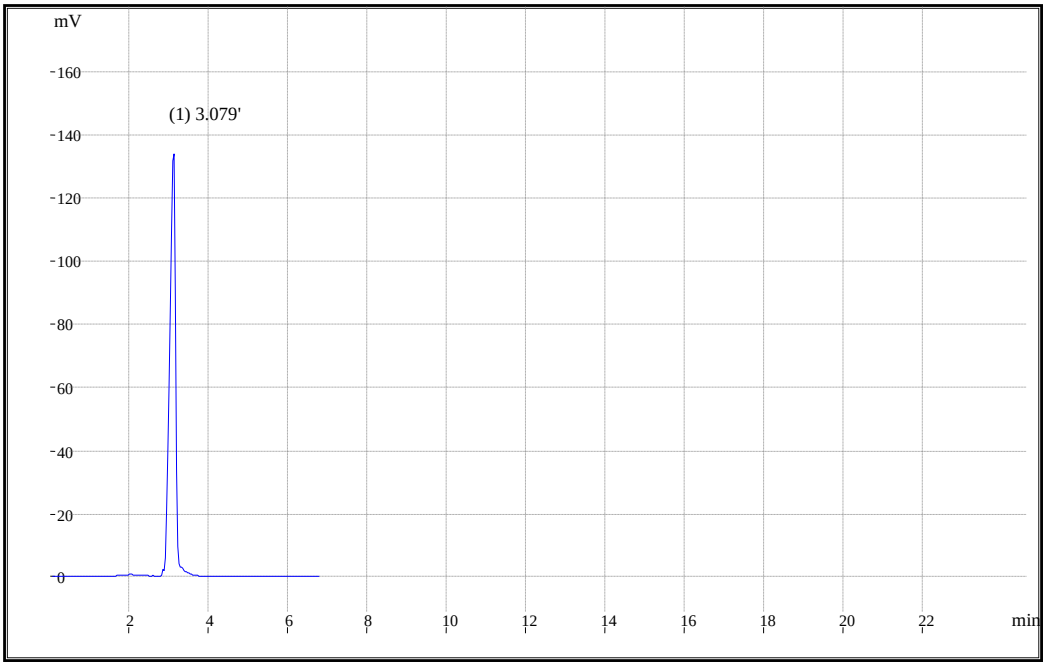


Fig. 4: Effect of wavelength and flow rate on USP tailing factor of Meloxicam

Therefore suggested optimum conditions were selected as given in Table-6 and the chromatogram of the optimized condition was given in Figure-5.

TABLE 6: SUGGESTED OPTIMIZED CONDITION FOR QBD APPROACH

Flow Rate (ml/min)	% Composition (Methanol: Water)	Wavelength (nm)
0.8	60:40	363



Validation parameters:

Linearity:

From the observations given in Table-7, calibration curve was constructed between concentrations versus area as shown in figure-6. It was found to be linear with corresponding correlation coefficient (r^2) of 0.999. The equation of line, slope and intercept were determined and expressed in an equation as below,

TABLE 7: DATA OBTAINED FROM LINEARITY STUDY BY HPLC

Sr. No.	Concentration (µg/ml)	Area
1	25	559151
2	50	1510933
3	75	2602861
4	100	3659410
5	125	4722991

y = 41905x – 53177

From correlation coefficient (r^2) obtained, it was found that there was a linear correlation between the concentration and observed area. The linearity was found within the range of 25-125 µg/ml. Hence this method could be used to determine the concentration of Meloxicam quantitatively within given range.

Precision:

The mean area of six determinations of 100% of the test concentration (75µg/ml) was statistically analyzed to determine SD and % RSD. Results given in Table-8 showed that method is precise for selected calibration range as % RSD was less than 2%.

TABLE 8: DATA OBTAINED FROM PRECISION STUDY

	Intraday		Interday	
Concentration (µg/ml)	Mean area ± SD	RSD	Mean area ± SD	RSD
75	2617315 ± 21650.47	0.83	2645310 ± 17050.45	0.65

Hence this method might be use precisely for the analysis of bulk Meloxicam. Consequently the method was set to be precise for selected calibration range**.**

Accuracy:

The results for the accuracy study are given in Table-9. As the % RSD values are less than 2, the proposed method was found to be accurate.

TABLE 9: DATA OBTAINED FROM ACCURACY STUDY

Sr. No.	Concentration (µg/ml)	Area	Mean	SD	%RSD
1	25	569151	576050	7135.16	1.23
	25	583400			
	25	575600			
2	75	2602861	2626819	20905.6	0.79
	75	2636238			
	75	2641358			
3	125	4722991	4712854	8885.38	0.18
	125	4706414			
	125	4709157			

% Recovery:

The results for the accuracy study are given in Table-10. The recovered amount of Meloxicam was found to be within the limits as per compendial standards.

TABLE 10: DATA OBTAINED FROM %RECOVERY STUDY

Recovery Level	Concentration Taken (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery
50% Recovery	50	25	75.42	100.56
100% Recovery	50	50	100.11	100.11
150% Recovery	50	75	124.61	99.688

Robustness:

From the results given in Table-11, it was observed that even after deliberate variations in the experimental condition, the method has no significant impact.

TABLE 11: DATA OBTAINED FROM ROBUSTNESS STUDY

Concentration (µg/ml)	Area	Mean	SD	%RSD
75	2640707			
75	2630977	2637881	6012.06	0.23
75	2641960			

LOD and LOQ:

The values of LOD and LOQ are given in Table-12.

TABLE 12: DATA OBTAINED FROM LOD AND LOQ STUDY

Conc.(µg/ml)	LOD	LOQ
25	0.554	1.68
75	1.624	4.923
125	0.69	2.092

The QbD approach had been successfully used to develop HPLC method for Meloxicam (API). All key aspect of QbD were tried to be implemented in said study. Systematic approach was utilized to develop an efficient and robust method which included beginning with determination of target profile characteristics, risk assessment, design of experiment and validation. It is then of core importance to demonstrate that the level of quality required for the CQAs can be met with high probability. Three factors (mobile phase, flow rate and wavelength) that were significantly affectingly peaks were then analysed to determine their interactions and quadratic effects with the least possible runs by using Box-Behnken model in conjunction with response surface methodology. Response surface diagrams and contour plots were studied and it was noticed that mobile phase concentration, wavelength, and flow rate remarkably affected response **(**USP tailing factor**).**

Desirability function was applied to get best possible outcome. Optimum run condition was obtained; the one with the higher desirability was chosen. Replicates of run having optimized condition were taken to confirm the predicted response with the actual response.

Moreover, this approach provides an in depth knowledge and enables the creation of chromatographic database that can be utilize to provide alternative method conditions at a future time.

ACKNOWLEDGEMENT:-

The authors would like to acknowledge the contributions of "RAP Analytical Research and Training Center, Nashik" for providing the facilities and support for the research work.

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