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QUALITY BY DESIGN APPROACH TO ANALYTICAL RP-HPLC METHOD DEVELOPMENT AND ITS VALIDATION

Khemeshwari L. Sarve[1]*, Atul T. Hemke[1] ,Tanvi M. Anandpara[2], Krishna R. Gupta[3] Milind J. Umekar[4]

Smt. Kishoritai Bhoyar College of Pharmacy, New Kamptee, Nagpur, Maharashtra-441002(India)

Corresponding author E-mail: atulhemke321@gmail.com*

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ABSTRACT

Mangiferin is an active constituent obtained from the dried parts such as leaves and barks of the mango tree (Mangifera indica L.) which belongs to the family Anacardiaceae. The present work is dealing with analytical RP-HPLC method development and validation for the determination of Mangiferin in Mangifera Indica. Quality by design (QbD) refers to the achievement of certain predictable quality with desired and predetermined specifications. A very useful component of the QbD is the understanding of factors and their interaction effects by a desired set of experiments. The proposed study describes the development of RP-HPLC method for the estimation of mangiferin using QbD approach and validation of proposed method as per ICH guidelines. An efficient experimental design based on systematic scouting of two key components of the RP-HPLC method (mobile phase and flow rate) is presented. The solution of mangiferin was made in methanol and absorption maximum was found to be 302nm. The chromatographic conditions were optimized with design expert software 11.0 version, Agilent C_{18} column (250 × 4.6 mm, 5µm)used as stationary phase, mobile phase comprisesof methanol and 0.1% OPA (53:47) and flow rate was 0.7 mL/min. The developed method was found linear (R^2-0.998) within the range of 10-50 µg/mL concentrations. The precision, ruggedness and robustness values were also within the prescribed limits. The proposed method can be used for routine analysis of mangiferin in quality control laboratories.

INTRODUCTION

Mangiferin is (1,3,6,7-tetrahydroxy2-[(2S,3R,4R,0-3,4,5 trihydroxy (hydroxymethyl) oxane-2-yl]-xanthen-9-one **(Figure 1)**, widely distributed in higher plants such as Mangifera indica L. and Anemarrhena Asphodeloides, showing antidiabetic, antitumor, antiviral, antioxidant, immunomodulatory and anti-inflammatory activities.[1] Quality by Design (QbD) is an important process in pharmaceutical industry which is introduced by USFDA. It is modern, scientific methods that formalize product design, automates manual testing and [2]According to the International Council for Harmonization (ICH),"Quality by Design is a systematic approach to drug development, Which begins with predefined objectives, and uses science and risk management approaches to gain product and process understanding and ultimately process control."[3] streamline troubleshooting.

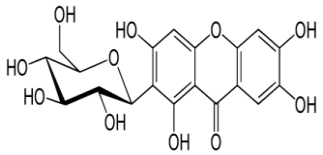


Figure 1Structure of Mangiferin (MGN)

A Design of experiment (DOE) approach will used to identify the optimum conditions for analysis during method development. The iterative procedure used in the studies included

performing experiments in the region of the best-known solution, fitting a response model to the experimental data and then optimizing the estimated response model. The conventional practice of modification of a single factor at a time may result in poor optimization as other factors are maintained at constant levels that do not depict the combined effect of all the factors involved in a separation. This approach is also time consuming and requires a vast number of experiments to establish optimum levels. These limitations can be eliminated by collectively optimizing all parameters using DOE.So the proposed work related to method development and its validation using QBD approach.[4] The literature survey revealed that very few analytical methods were reported for estimation of Mangiferin in bulk and from plant extract including RP-HPLC[5-7] and HPTLC[8]. The assay method based on QbD approach was not located in literature. Hence proposed work reprsents QbD based development and validation of RP-HPLC menthod for estimation of Mangiferin.

MATERIAL AND METHOD

Reagents and chemicals

Mangiferin API was purchased from Yucca Enterprises pvt. Ltd. Mumbai, methanol and acetonitrile were procured from LOBA Chemie and were HPLC grade. Ortho-Phosphoric acid, ethanol, Potassium dihydrgen orthophosphate and dimethyl formamide were used of GR grade.

Instruments and software

HPLC analysis was carried out usingShimadzu HPLC series 1100. The wavelength of maximum absorbance was detected by UV-Visible spectrometer(double beam), Shimadzu UV-1700 model and wavelength scanning range was 200-400nmwas exercised using UV probe software. For applying quality by design Design Expert® – Full Version 11.0software was used.

Preparation of solutions

Preparation of Diluent: It consists of mixture of Methanol: 0.1% OPA in ratio 50:50.

Preparation of 0.1% Ortho-phosphoric acid: A 1.0 mL of ortho-phosphoric acid (OPA) was transferred in 1000.0 mL volumetric flask, and volume was made upto the mark with double distill water, sonicated and filter through 0.45µm membrane filter paper.

Preparation of Mobile Phase:

50 mL of Methanol and 50 mL of 0.1% Ortho-phosphoric acid (pH 3.0) were mixed and sonicated for 15 min. to remove the air bubbles. The prepared mobile phase was sonicated and filtered through 0.45µm membrane filter.

Preparation of Standard Stock Solution:

Weighed and transferred accurately about 10 mg of Mangiferin standard in a 10 mL volumetric flask, 5mL of diluent was added, sonicated to dissolve and diluted up to the mark with diluent. 0.1 mL portion of this solution was further diluted to 10 mL with diluent. (10 µL)

Selection of Wavelength:

Accurately weighed 10 mg of standard Mangiferin, dissolved in the 10 mL of diluent and mixed well. Transferred 0.2 mL of this solution into 10 mL volumetric flask and volume was made up to the mark. The final solution of Mangiferin standard was scanned in the range of 400-200 nm in 1.0 cm cell against blank and spectrum was recorded. The study of spectra shows that the peak maxima for Mangiferin was found to be at 302 nm and was selected for further studies. The Spectrum was recorded is shown in **Figure 2.**

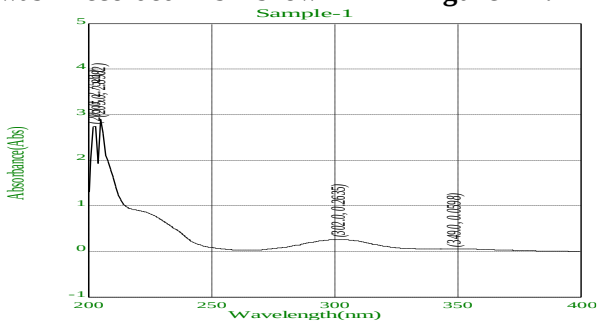


Figure 2 UV-Spectra of standard Mangiferin

Box-Behnken Design (BBD):[9-12]

BBD was chosen as a DOE tool for optimizing the method developed here, since it provides second-order equations to correlate the studied factors with the obtained responses. BBD is considered to be an alternative to the central composite design (CCD) that provides suitable mathematical models with a reduced number of experimental runs. BBD avoids the extreme experimental conditions that are usually employed in CCD, which could lead to unacceptable results. In this work, BBD was used to optimize the HPLC method and to find

the effect of various dependent and Independent Factors.

Analytical Target Profile (ATP):

ATP defines the analytical variables to be measured (i.e. level of a specified impurity), as well as performance characteristic to be obtained by this measurement (i.e. Accuracy, Precision and range). The ATP provides the link between the eventual analytical method and the chemical and formulation process.

Design of Experiment (DoE):

Optimization was done by response surface methodology, applying a three level Box Behnken design with three centre points.

Selection of Independent and Dependent Factors: Preliminary experiments were performed to identify the critical factors and to set their levels (maximum and minimum) for the experimental design. In this step the following parameters were investigated: The independent variables are and their Low, Medium and High levels are described in **Table 1, 2 and 3.** The evaluated responses (Dependent Variables) are Response Y1 (Theoretical plates), Response Y2 (Tailing Factor) and Response Y3 (Retention Time).

Based on the results obtained during the preliminary studies, the method chosen for assay method development with the shortest analysis time was threefactors, three-level BBD with three replicates at the centre point (middle level).

Optimization ofthe method: The method optimization was done by studying parameters such as system suitability test linearity of response and % estimation of drug.

Method validation:[13]

Precision: The intermediate precision of an analytical procedure expresses the closeness of agreement (Degree of Scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. For Intraday and Interday variation, the sample was prepared as per the procedure described earlier, analysed at specified intervals and % Label Claim was calculated as shown in **Table 4.**

Recovery Study (Accuracy):

The Accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or as an accepted reference value and the value found.

Table 1 Selection of independent factors and their levels

Factor	Name	Units	Type	Low	High	Actual	Actual	Low Coded	High Coded
A	Org. Phase	%	Numeric	30	70	-1.000	1.000	50.00	14.14
B	Aq. Phase	%	Numeric	30	70	-1.000	1.000	50.00	14.14
C	Flow Rate	mL/min	Numeric	0.6	0.8	-1.000	1.000	0.7	0.07

Table 2 Chromatographic factors and response variables for Box Behenken experimental design

Factor	Name	Units	Low	Level used Centre	High
A	Org. Phase	%	30	50	70
B	Aq. Phase	%	30	50	70
C	Flow Rate	mL/min	0.6	0.7	0.8

Table 3 Box-Behnen design used in HPLC method optimization

Std. Runs	Sr. No.	Organic phase (%)	Aqueous phase (%)	Flow Rate (mL/min)
6	1	70	50	0.6
10	2	50	70	0.6
12	3	50	70	0.8
3	4	30	70	0.7
13	5	50	50	0.7
5	6	30	50	0.6
9	7	50	30	0.6
2	8	70	30	0.7
17	9	50	50	0.7
7	10	30	50	0.8
16	11	50	50	0.7
11	12	50	30	0.8
8	13	70	50	0.8
15	14	50	50	0.7
1	15	30	30	0.7
4	16	70	70	0.7
14	17	50	50	0.7

Table 4 Observations and results of intermediate precision

Sr. No.	Time (Hrs)	AUC (mAU)	% Label Claim	Day	AUC (mAU)	% Label Claim
1.	0^{th}	581.192	101.00	1^{st}	596.011	100.80
2.	3^{rd}	581.278	100.48	2^{nd}	598.069	101.17
3.	5^{th}	582.309	101.44	3^{rd}	597.892	98.28
Mean			100.97	Mean		100.08
±SD			0.480	±SD		1.572
%RSD			0.475	%RSD		1.57

Preparation of Sample: An accurately weighed quantity of preanalysed Mangiferin sample was transferred in a series of 10 mL volumetric flasks and Mangiferin standard drug was added at three different levels (80%, 100% and 120%), 5 mL of diluent was added and sonicated for 20 min. The volume was made up to the mark and filtered through 0.45µm membrane filter. A 0.1 mL portion was diluted to 10 mL with diluent. A 20µl volume of each final dilution were injected separately and chromatographed. The result was tabulated in **Table 5.**

Robustness: Deliberate change was made in the optimized chromatographic parameter and robustness of the method was studied by evaluating system suitability parameter data after varying the mobile phase composition, detection wavelength and flow rate. The observations recorded in**Table 6.**

Limit of Detection and Limit of Quantitation:

Limit of Detection (LOD): The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantified as an exact value.

Limit of Quantitation (LOQ): The quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ are calculated based on standard deviation of response and slope. For the present study, the LOD and LOQ were calculated by following formula and the calculated values are shown in **Table 7.**

$$\text{Limit of Detection (LOD)} = \frac{3.3\,\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

$$\text{Limit of Quantitation (LOQ)} = \frac{10\,\sigma}{S}$$

RESULTS

Design Model Evaluation

The significance of model so obtained can be evaluated by ANOVA method.ANOVA is a statistical method based on F-test to estimate the significance of model. It involves sub-dividing total variation into variation due to Main effects and Interactions.

ANOVA Technique:

The ANOVA (One-way Analysis of Variance) is used to determine whether there are any significant differences between the means of three or more independent groups.

Theoretical Plates: The Model F-value of 3.19 implies the model is significant. There is only a 5.13% chance that a Model F-Value this large may be occurring due to noise.P-Values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicates the model terms are not significant.In this case, B and AB are significant model terms.

Tailing Factor: The Model F-value of 5.04 implies the model is significant. There is only a 2.23% chance that a Model F-Value this large may be occurring due to noise. P-Values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicates the model terms are not significant. In this case A, B and C^2 are significant model terms.

Retention Time: The Model F-value of 16.54 implies the model is significant. There is only a 0.06% chance that a Model F-Value this large may be occur due to noise.P-Values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicates the model terms are not significant.In this case A, C, AC, B^2, C^2 are significant model terms.

Table 5 : Observation and results of recovery study

Accuracy Level (%)	Amount of Std. drug added (mg)	AUC of Sample (mV)	Amount of drug recovered (mg)	% Recovery*
80	8.05	257.66	7.92	98.38
100	9.98	284.49	9.86	98.95
120	12.05	313.26	11.98	99.25
Mean				98.86
±SD				1.61
%RSD				1.63

***Each observation is mean of three observations.**

Table 6 Observation of robustness study

Sr. No.	Deliberate condition	Retention time (min)	AUC (mAU)	Asymmetry	Theoretical plate
1.	Standard condition	3.256	591.106	0.60	10921
2.	Mobile Phase (Methanol: 0.1% OPA) (49:51)	3.258	591.107	0.61	10935
3.	Mobile phase (Methanol: 0.1%) (51:49)	3.267	590.946	0.64	11686
4.	Wavelength (303 nm)	3.264	591.563	0.62	10970
5.	Wavelength (301 nm)	3.263	591.907	0.63	11309
8.	Flow rate (0.6mL/min)	3.264	591.115	0.60	9756

9.	Flow rate (0.8mL/min)	2.866	580.126	0.64	9677
Mean		3.2054	589.695	0.62	10750.57
±SD		0.0719	5.2330	0.017	511.45
%RSD		1.67	1.28	1.97	1.84

Table7 Results for LOD and LOQ

Mangiferin (mg/mL)	
LOD	1.123
LOQ	3.404

Table 8(a) Model summary statistics for response Y1 (Theoretical plate)

Sr. No.	Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
1	Linear	2997.72	0.3285	0.1736	-0.3783	2.398E+08
2	2FI	2443.98	0.6567	0.4507	-0.6504	2.871E+08
3	Quadratic	2684.93	0.7100	0.3370	-3.6407	8.074E+08
4	Cubic	0.0000	1.0000	1.0000		

Table 8(b)Model Summary Statistics for response Y2 (Tailing Factor)

Sr. No.	Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
1	Linear	0.3359	0.5577	0.4557	0.1825	2.71
2	2FI	0.3739	0.5784	0.3254	-0.7159	5.69
3	Quadratic	0.2518	0.8662	0.6942	-1.1408	7.10
4	Cubic	0.0000	1.0000	1.0000		

Table 8(c)Model Summary Statistics for response Y3 (Retention Time)

Sr. No.	Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
1	Linear	0.3142	0.5193	0.4084	0.1667	2.22
2	2FI	0.3279	0.5974	0.3558	-0.3497	3.60
3	Quadratic	0.1309	0.9551	0.8974	0.2815	1.92
4	Cubic	0.0000	1.0000	1.0000		

Main Effects (Lack of Fit)

The Lack of Fit is one of the components of partition of the sum of squares in an ANOVA which can tell that proposed model is fit or not. Results are shown in **Table 8(a) to 8(c).**

Interactions

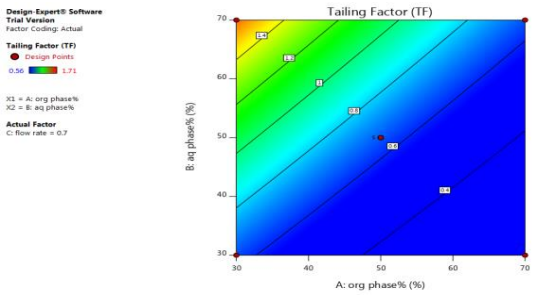
It helps to determine the effects between various factors, also used to determine the quadratic equation.

Generalised 2FI equation for Response Y1 (Theoretical plates)

7609.41+A-B+C+AB-AC+BC

Generalised Quadratic equation for Response Y2(Tailing Factor)

$0.6600-A+B-C-AB+AC-BC+A^2+B^2+C^2$



Generalised Quadratic equation for Response Y3 (Retention Time)

$3.29\text{-}A+B\text{-}C+AB+AC\text{-}BC+ A^2+B^2+C^2$

Table 9(a) to 9(c) represents the final equation in terms of Actual factors.

The observations of counter plots are shown in **Figure 3(a)-3(c).**

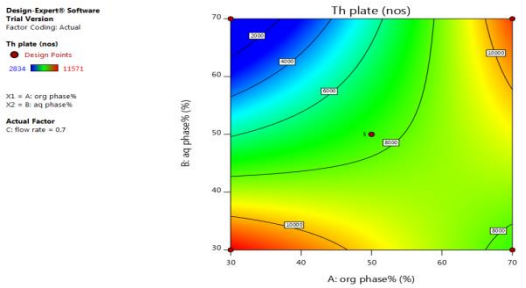


Figure 3(a)Counter plot for Y1 response for Theoretical Plates

Figure 3(b) Counter plot for Y2 response for Tailing Factor

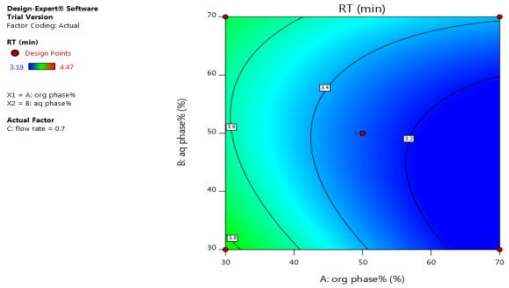


Figure 3(c) Counter plot for Y3 response for Retention Time

Final predicted responses for Dependent Factors:

After performing the study software has predicted the values for our selected factors (**Table 10)** based on above predicted chromatographic conditions and expected response

System Suitability Test (SST):

The standard solution prepared above was used to study the system suitability test. After equilibrium of column with mobile phase, five replicate injections of 20µg/mL solution were injected through the manual injector and the recorded chromatogram is shown in the **Figure 5** andthe peak area were measured. The observations of SST are shown in **Table 11.**From the obtained chromatogram, Mangiferin have retention time 3.264min. The asymmetry of the peak was 0.57 and %RSD was found to be 0.44 indicating the system is suitable for analysis.

Linearity of Test Method:

The linearity of an analytical procedure is its ability (within a given range) to obtain a test results which are directly proportional to the concentration of analyte present in the sample.

Procedure:

Accurately weighed quantity of about 10 mg Mangiferin API was transferred to a 10 mL volumetric flask, dissolved in sufficient quantity of diluent and diluted up to mark with diluent. From the standard stock solution, accurately pipette out 0.1 mL to 0.5 mL were transferred to a series of 10 mL volumetric flasks and diluted upto the mark with diluent to get the solution having the concentration of 10 µg/mL to50 µg/mL respectively. All the solutions prepared above were injected to the HPLC system under optimized chromatographic parameters.

The system was allowed to equilibrate by passing the mobile phase through chromatographic column. After equilibration, standard solutions having a volume of 20µL was injected in ascending order of different concentration levels for Mangiferin. Linearity of test response was established by plotting a graph between peak areas versus concentration of drug in µg/mL. The correlation coefficient for Mangiferin was found to be 0.998 indicates proposed method is linear as shown in **Figure 6.**

Table 9(a) Final equations in terms of actual factor (Theoretical plates)

Sr. No.	Factors	Theoretical plates
		+33189.41176
1	Org phase	-319.92500
2	Aq. Phase	-672.08125
3	Flow rate	-1812.50000
4	Org phase × aq phase	+9.40687
5	Org phase × flow rate	-91.75000
6	Aq phase × flow rate	+142.37500

Table 9(b) Final equations in terms of actual factor (Tailing Factor)

Sr. No.	Factors	Tailing Factor
		+22.88000
1	Aq phase	-0.016875
2	Flow rate	+0.024875
3	Org phase × aq phase	-64.05000
4	Org phase × flow rate	-0.000325
5	Aq phase × flow rate	-2.40437E-16
6	Org phase²	-0.007500
7	Aq phase²	+0.000163
8	Flow rate²	+0.000138
9	Org phase	+46.00000

Table 9(c) Final equations in terms of actual factor (Retention Time)

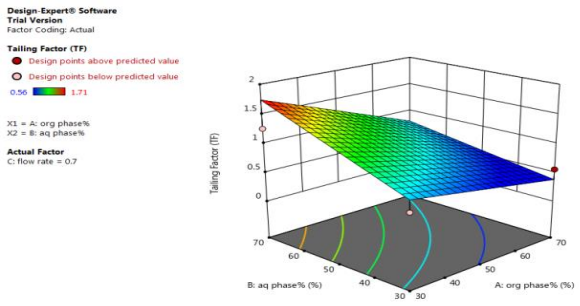
Sr. No.	Factors	Retention Time
		+32.29250
1.	Aq phase	-0.111625
2.	Flow rate	-0.053750
3.	Org phase × aq phase	-67.15000
4.	Org phase × flow rate	+0.000275
5.	Aq phase × flow rate	+0.100000
6.	Org phase²	-0.002500
7.	Aq phase²	+0.000144
8.	Flow rate²	+0.000444
9.	Org phase	+42.25000

Table 10 Predicted and actual values of dependent factors

Sr. No.	Dependent Factors	Values Predicted	Dependent Factors	Mean	Model
1.	Organic phase (%)	53	Th.Pl.	7609.41	Quadratic
2.	Aqueous phase (%)	47	Tailing Factor	0.9329	Quadratic
3.	Flow Rate(ML/min.)	0.7	Rt	3.60	Quadratic

Table 11 Observations of system suitability test

Sr. No.	Weight of Standard Drug taken (mg)	Area Under Curve (AUC) in mV
1	10.0	580.279
2		579.777
3		580.223
4		579.917
5		580.351
Mean		580.1094
± SD		0.2487
% RSD		0.44%
Theoretical Plate/Column		9658.6
Retention time		3.264
Asymmetry		0.57



4(a)Surface response curve for Y1 response (Theoretical plates)

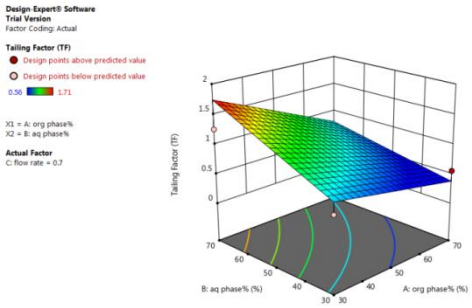


Figure 4(b)Surface response curve for Y2 response (Tailing factor)

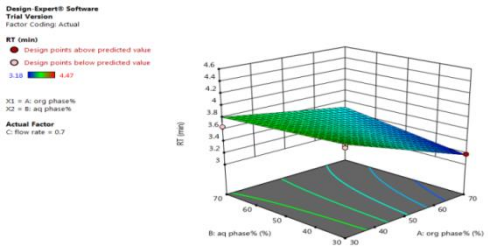


Figure 4(c)Surface response curve for Y3 response (Retention Time)

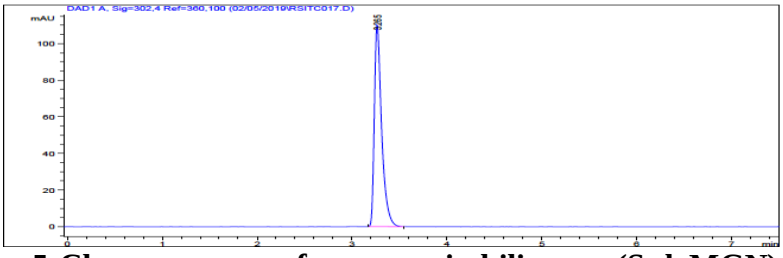


Figure 5:Chromatogram of system suitability test (Std. MGN)

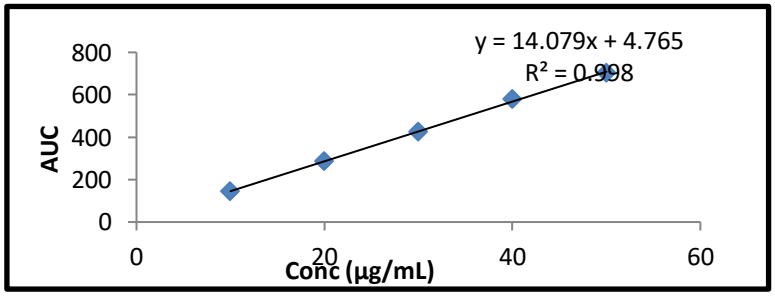


Figure 6: Plot of linearity curve for MGN

Percent estimation of MGN

An accurately weighed quantity of previously prepared solid dispersion (Powder form) equivalent to 10 mg of MGN was transferred to 10 mL of volumetric flask, sonicated for 15 min with sufficient quantity of diluent (mobile phase) and volume was made up to mark with diluent. The content of flask was filtered through 0.45µm filter paper. A 1 mL portion of the filtered was further diluted to 10 mL with diluent. Further 1 mL of solution was transferred in 10 mL volumetric flaskand volume was made up to the mark with mobile phase. After equilibration of stationary phase, such five sample solutions were prepared from same working stock solution, injected separately and chromatogram were recorded. A chromatogram so obtained depicted in **Figure 7and 8.** The content of MGN in each sample was calculated by comparing the peak area of sample with that standard using formula. The results are shown in **Table 12**represents proposed method was found precise.

DISCUSSION: There were few works reported on implementation of quality by design in analytical method development. But the sequence of implementation has to be considered as per FDA. Some articles have reported a method based on stability assay by considering resolution, as a method response to support specificity in robustness. However, method verification in design space, method performance has to be added. The knowledge based QTPP for the product of Mangiferin was constructed with the assessment of criticality for its critical attribute. Analytical target profile (ATP) was derived based on QTPP profile and then objective of this analytical QbD work was considered as assay component of QTPP of product specifications. To initiate the QbD work, organic phase (X1), aqueous phase (X2) and flow rate (X3)considered as independent variables whereas theoretical plates (Y1), tailing factor (Y2) and retention time (Y3) was selected as dependent variables. BBD is considered to an alternative to CCD. BBD avoid an extreme experimental condition that are usually employed in CCD and was used to optimise the HPLC method. Agilent C_{18}(250X4.6mm, 5µm) column was chosen as stationary phase due to wide acceptability of pharmaceuticals and high reproducibility. The mobile phase composed of methanol and 0.1% ortho-phosphoric acid (53: 47) and wavelength selected for analysis was 302nm. The obtained experimental results was subjected to various statistical parameter for better understanding and was found nonlinear relationship between input variable and response. The prediction form MODR has been verified by actual experimental results indicating its robustness. Thus the method developed based on AQbD is more precise, accurate, and robust during method transfer and also cost effective. This method satisfy the design space concept for analytical method (MODR) and suitable for regulatory submission under regulatory flexibility.

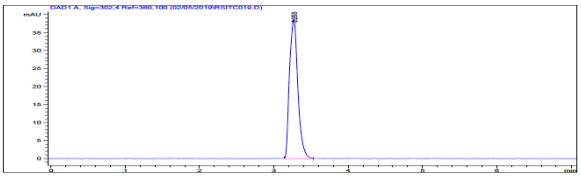


Figure 7- Chromatogram of Standard MGN

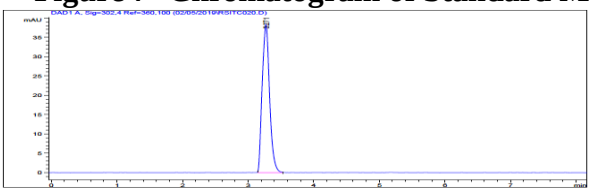


Figure 8- Chromatogram of sample

Table 12 Observations for estimation of MGN

Sr. No.	Wt. of Std. taken (mg)	Wt. of sample taken (mg)	AUC Sample (mAU)	Amt. of drug estimated (mg)	% Label Claim
1.	10.0	20.28	581.125	10.11	99.79
2.			585.225	10.12	99.88
3.			588.477	10.15	99.95
4.			592.785	10.21	100.72
5.			598.211	10.18	100.08
				Mean	100.09
				±S.D.	0.383
				%RSD	0.38

CONCLUSION

A novel simple, fast and robust RP-HPLC analytical method of MGN was successfully developed by employing QbD approach and further validated according to ICH guidelines. By the use of DOE approach where box-behnken designs was used to analyse the various analytical target profile. The automated QbD method development approach provide a better performing and more robust method in less time as compared to the manual method of development.

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REFERENCES

1. Chemistry of Mangiferin: https://en.m.wikipedia.org/wiki/Mangiferin. (Accessed on 12 July, 2018).
2. Vogt F, Kord A. Development of Quality by Deign analytical method. Journal of Pharmaceutical Science. 2011; 100(3):797-800.
3. Peraman R, Bhadrya K, Reddy Y. Analytical Quality by design: A tool for regulatory flexibility and Robust Analytics. International Journal of Analytical Chemistry. 2015; 6(2):101-116.
4. Vinayak A, Lateef S. Automated QbD based Method Development and Validation of Oxidative Degraded Atorvastatin. Agilent Technologies. 2014; 5(20):1.12.
5. Bossunia M, Urmi K, Shaha C. Quality-by-Design approach to stability indicating RP-HPLC analytical method development for estimation of Canagliflozin API and its validation. Pharma Method. 2017; 8(2):92-101.
6. Kammalla A, Ramasamy M, Agarwal A, Dubey G. Development and Validation of a RP-HPLC method for the simultaneous determination of Mangiferin, Ellagic acid and Hydroxycitric acid in polyherbal formulation. Pharmacognosy Journal. 2014; 6(3):23-28.
7. Kumar P, Srivastava V, Kumar L. Development of new reversed-phase HPLC method for the determination of Mangiferin in Mangifera indica Linn.Biosciences, Biotechnology Research Asia. 2008; 5(1):383-388.
8. Padh H, Parmar S, Patel B. Stability indicating HPTLC method for estimation of Mangiferin in bulk and dosage form. International Journal of Pharmacy and Biological Sciences. 2017; 7(3):71-77.
9. Hemant B, Mulchand K, Saranjeet S, Sarwar B, and Bhupinder S. QbD in analytical sciences: An overview. Pharma Times. 2014; 46 (8):71-75.
10. Loyd D, Bergum J. Application of Quality by Design (QbD) to the development and validation of

analytical methods. Journal of Pharmaceutical Sciences. 2011; 100(3):39-52.

11. Karmarkar S, Garber R, Genchanok Y, George S, Yang X, Hammond R. Quality by design (QbD) based development of stability indicating HPLC method for drug and impurities. Journal of Chromatographic Science. 2011; 4(2):49-57.
12. Ferreira S, Bruns R, Ferreira H, Matos G, and David J. Box-Behnken design: An alternative for the optimization of analytical methods. Analytica Chimica Acta. 2007;597:179-186.
13. ICH, Validation of Analytical Procedure: Text and methodology Q2 (R1) current step 4 version. Patent guideline.1994:630-632.