IJPSR (2020), Volume 11, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 September 2019; received in revised form, 05 March 2020; accepted, 20 March 2020; published 01 September 2020

DEVELOPMENT OF RP-HPLC METHOD FOR THE ESTIMATION OF ACOTIAMIDE HYDROCHLORIDE HYDRATE USING AQbD APPROACH

Samruddhi S. Gawande, Atul T. Hemke *, Krishna R. Gupta and Milind J. Umekar

Department of Pharmaceutical Chemistry, Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur - 441002, Maharashtra, India.

Keywords:

Quality by Design (QbD), RP-HPLC, Box-Behnken Design, ANOVA

Correspondence to Author:
Dr. Atul T. Hemke

Professor,
Department of Pharmaceutical Chemistry, Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur - 441002, Maharashtra, India.

E-mail: atulhemke321@gmail.com

ABSTRACT: The current studies details QbD enable the development of a simple, rapid, and cost-effective reverse phase high performance liquid chromatographic method for estimation of Acotiamide hydrochloride hydrate. The simple analytical RP-HPLC method was developed using Box-Behnken Design (BBD). In the present work, three independent factors were used, such as org phase (A), aqueous phase (B), and flow rate (C). Totally 17 experimental runs were suggested by the software for analyzing the interaction, and the tailing factor (R1), retention time (R2), and area (R3) were considered as response factors (dependent factors). The developed experimental design was statistically analyzed using ANOVA, counterplots, and surface response curves. The optimal chromatographic separation was achieved using Hyperchrom ODS C_{18} (4.6×250 mm, 5µ) analytical column with a mobile phase containing potassium dihydrogen phosphate buffer (pH 6.8): Acetonitrile (60:40 v/v) with the flow rate of 1 ml/min using PDA detector. The retention time of the drug was found to be 5.2646 min at a detection wavelength of 282 nm. The method was found to be linear in the concentration range of 2-10 µg/ml. The method was validated as per the ICH guidelines for precision, accuracy, linearity, ruggedness, robustness, the limit of detection, and limit of quantitation. The proposed method was found simple, economical, and robust, which can be used for routine analysis of the Acotiamide hydrochloride hydrate.

INTRODUCTION: Acotiamide is N-[2-[bis (1-methylethyl) amino] ethyl]-2-[(2-hydroxy-4, 5 dimethoxybenzoyl) amino] thiazole-4-carboxamide monohydrochloride trihydrate **Fig. 1**. Acotiamide is a prokinetic agent with gastrointestinal (GI) motility-enhancing activity [1]. It is used for the treatment of functional dyspepsia.

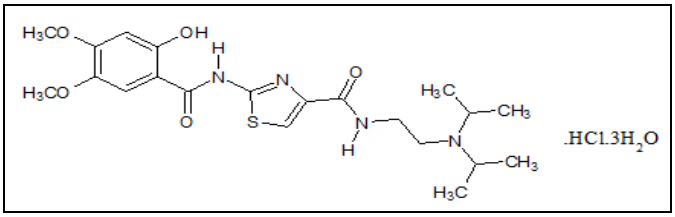

FIG. 1: CHEMICAL STRUCTURE OF ACOTIAMIDE HYDROCHLORIDE HYDRATE

It inhibits acetylcholinesterase (AChE) an enzyme responsible for the breakdown of acetylcholine (ACh). Increased ACh concentrations lead to an improvement of gastric emptying and GI motility and eventually to a reduction of dyspepsia symptoms [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(9).4530-39

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(9).4530-39

Quality by Design (QbD) is an important process in the pharmaceutical industry, which is introduced by USFDA. It is modern, scientific methods that formalize product design automates manual testing, and streamline troubleshooting [3]. 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 [4].

A Design of the experiment (DOE) approach will be 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 [5].

The literature survey revealed that very few analytical methods were reported for the estimation of acotiamide hydrochloride hydrate in tablet formulation using HPLC [6-9], Spectrophotometer [10,] and Spectrofluorimeter [11]. It was found that QbD based analytical method not located in literature for the estimation of said drug. Hence, the aim of the present work was to develop and validate the RP-HPLC method for the determination of Acotiamide hydrochloride hydrate using a QbD approach.

MATERIALS AND METHODS:

Chemicals and Reagents: Acotiamide hydrochloride hydrate was procured as a gift sample from Lupin Pvt. Ltd., Pune. HPLC grade acetonitrile and methanol were purchased from Merck Life Sciences Pvt. Ltd. Potassium dihydrogen phosphate was used for GR grade. The commercially available formulation of ACT was purchased from local market and used for assay.

Instruments and Software: HPLC analysis was carried out using **a** Shimadzu 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 200-400nm exercised using UV probe software. For applying quality by design, Design Expert® – Full version 11.0 Software was used.

Preparation of Standard Solution: An accurately weighed about 10 mg of Acotiamide hydrochloride hydrate and transferred into a 10 ml volumetric flask, add about 2 ml of diluent and sonicated to dissolve and make volume up to the mark with diluent (stock solution). 1 ml of this stock solution further diluted to 10 ml with diluent. Again 1 ml of the above working stock solution was transferred in 10 ml of volumetric flask and volume was made up to the mark with diluent (10 µg/ml).

Preparation of Sample Solution: Weigh and powder twenty tablets of Acotiamide hydrochloride hydrate. The tablet powder equivalent to 10 mg of Acotiamide hydrochloride hydrate was taken and transferred into a 10 ml of the volumetric flask, add about 2 ml of diluent and sonicated for 25 minutes to dissolve it completely and volume was made up to the mark with diluent. The solution gets filtered through Whatman filter paper, and 1 ml of this solution diluted to 10 ml with diluent. Further 1 ml of the above solution transferred in 10 ml of volumetric flask and volume was made up to the mark with diluent (10 µg/ml).

Design of Experiment (DOE): Optimization was done by response surface methodology and applying a three-level Box-Behnken design with three center points.

Box-Behnken Design (BBD): BBD was chosen as a DOE tool for optimizing the developed method 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 proposed 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 characteristics to be obtained by this measurement. The ATP provides the link between the eventual analytical method and the chemical formulation process.

Model Design Optimization: The significance of the model so obtained was evaluated in two ways, ANOVA method and Good fit evaluation.

ANOVA: ANOVA is a statistical method based on the F-test to estimate the significance of the model. It involves subdividing total variation into variation due to residual error, main effects, and interactions.

Main Effect (Lack of Fit): The lack of Fit is one of the components of the partition of the sum of squares in an ANOVA, which can tell that the proposed model is fit or not.

Method Development: After solubility determination, various mobile phases were tried containing the different composition of the organic phase and aqueous phase at varying pH. Out of several tried combinations as suggested by BBD, the optimized mobile phase comprises of potassium dihydrogen phosphate buffer (pH 6.8): Acetonitrile (60:40 v/v) at flow rate of 1.0 ml/min gave efficient chromatographic separation on Hyperchrome ODS C_{18} column (Log P value-1.62) of ACT (10 µg/ml).

Method Validation:

Accuracy: Accuracy of the method was evaluated from the recovery study of Acotiamide hydrochloride hydrate through 20 µg/ml solution spiked with 50, 100, and 150% extra quantity of standard Acotiamide hydrochloride hydrate. The standard deviation (SD) and % RSD was calculated.

Precision: Acotiamide hydrochloride hydrate tablet solution was prepared as described under preparation of the sample, and such six replicates were injected into the HPLC system and area under curve determined.

System Suitability Test: The system suitability was assessed by using six replicates of Acotiamide hydrochloride hydrate sample solution, and the retention time, theoretical plate, and peak asymmetry was noted.

Linearity: The Linearity of the method was determined by diluting the standard stock solution (2-10 µg/ml) of Acotiamide hydrochloride hydrate. The linearity plot was constructed between concentration and area under the curve.

Ruggedness: The ruggedness of the proposed method has been verified by analyzing the tablet sample used for method precision by two different analysts using the same instrument and also by interday and intraday variation study. The overall mean, standard deviation (SD) and % RSD was calculated.

Robustness: The robustness of the method was evaluated by injecting the sample at deliberately varying chromatographic conditions which includes a change in composition of organic phase in mobile phase, pH of the buffer, flow rate, and wavelength.

LOD and LOQ: The Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated from slope (S) of linearity plot and standard deviation of the response to the blank sample.

RESULTS AND DISCUSSION:

Selection of Wavelength: The standard solution of Acotiamide hydrochloride hydrate (10 µg/ml) was scanned in the range of 400-200 nm in 1.0 cm cell against blank and spectrum was recorded. From the spectra, Acotiamide hydrochloride hydrate has peak maxima at 282 nm and was selected for further studies. The Spectrum was recorded is shown in **Fig. 2.**

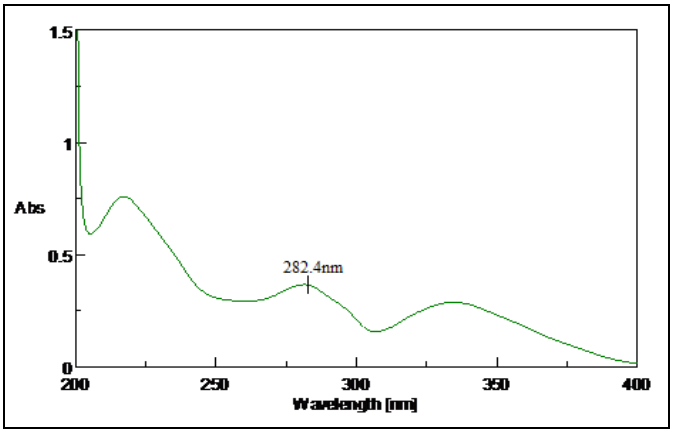

FIG. 2: UV SPECTRA OF ACOTIAMIDE HYDROCHLORIDE HYDRATE

Experiment Design: A 3^2 factorial design using BBD was applied for observing the effect of three independent factors such as an organic phase (A), aqueous phase (B), flow rate (C) on three responses such as tailing factor (R1), retention time (R2), area (R3) as parameters for calculation of proposed method. The chromatographic conditions and ranges fixed for selected factors are given in **Table 1**.

TABLE 1: SELECTION OF INDEPENDENT FACTORS AND THEIR LEVELS

Factor	Name	Units	Type	Min.	Max.	Coded	Values	Mean	Std. Dev.
A	Org. phase	%	Numeric	30	50	-1.00	1.000=50	40	7.07107
B	Aq. phase	%	Numeric	50	70	-1.00	1.000=70	60	7.07107
C	Flow rate	ml/min	Numeric	0.8	1.2	-1.00	1.000=1.2	1	0.141421

The sum of total 17 runs was obtained for fixed variables by selecting three center repetitions. Each combination of mobile phase composition, flow rate, and pH suggested by BBD were finally run on the system and observed for the responses such as peak area, tailing factor, and retention time is represented in **Table 2**. After performing the trials, the values predicted by the software are given in **Table 3,** and some of the recorded chromato-grams are shown in **Fig. 3(a)** to **3(c).**

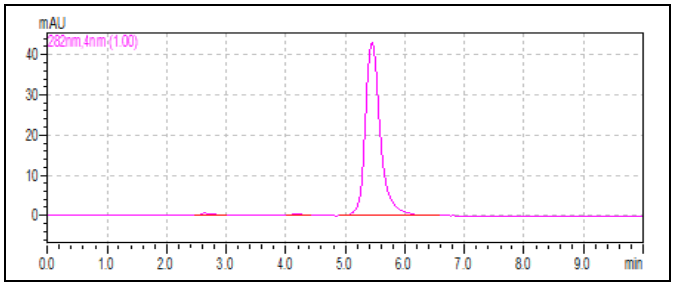

FIG. 3A: CHROMATOGRAM OF ACOTIAMIDE HYDROCHLORIDE HYDRATE (STD. RUN 3)

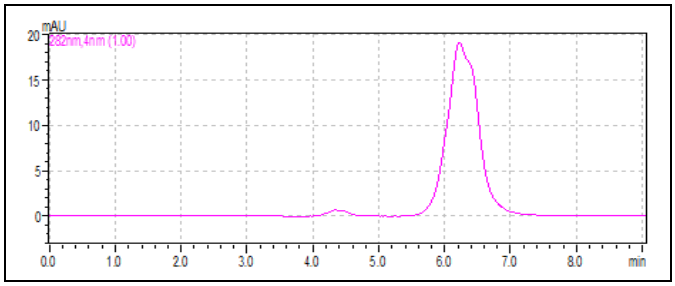

FIG. 3B: CHROMATOGRAM OF ACOTIAMIDE HYDROCHLORIDE HYDRATE (STD. RUN 8)

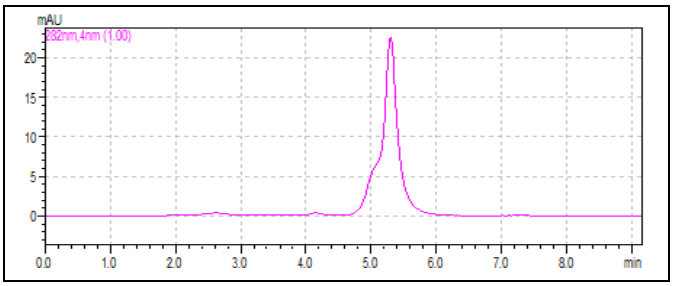

FIG. 3C: CHROMATOGRAM OF ACOTIAMIDE HYDROCHLORIDE HYDRATE (STD. RUN 17)

TABLE 2: BOX-BEHNKEN EXPERIMENTAL DESIGN USING FACTORS AND RESPONSES

Std Runs	A: Org phase (%)	B: Aq. phase (%)	C: Flow rate (ml/min)	Tailing factor	RT	Area
4	50	70	1	1.282	4.784	367692
12	40	70	1.2	1.255	4.888	422958
3	30	70	1	1.243	5.452	426914
17	40	60	1	1.339	5.278	295130
9	40	50	0.8	0.921	6.294	650282
2	50	50	1	1.12	4.191	493476
7	30	60	1.2	1.29	5.415	497654
1	30	50	1	1.17	6.216	623311
15	40	60	1	1.339	5.278	295130
16	40	60	1	1.339	5.278	295130
5	30	60	0.8	1.245	7.999	388616
8	50	60	1.2	1.249	3.31	379254
6	50	60	0.8	0.917	5.095	771322
10	40	70	0.8	1.103	5.298	699332
14	40	60	1	1.339	5.278	295130
11	40	50	1.2	1.251	3.381	411104
13	40	60	1	1.339	5.278	295130

TABLE 3: SUMMARY OF DEPENDENT FACTORS

Response	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
R1	Polynomial	0.917	1.339	1.22006	0.134891	1.4602	None	Quadratic
R2	Polynomial	3.31	7.999	5.21841	1.07203	2.41662	None	2FI
R3	Polynomial	295130	771322	447504	153677	2.6135	None	Quadratic

Design Model Evaluation:

ANOVA Technique: The model was validated by the application of analysis of variance (ANOVA) to both the responses and variables to examine the significance of the model, which showed that both the responses achieved significant differences in their values. The model F-value 12.02 (Tailing Factor), 20.60 (Retention Time), 5.01 (Area) implies that the model is significant. Results are shown in **Table 4(a)-4(c).**

TABLE 4A: ANOVA FOR RESPONSE SURFACE QUADRATIC MODEL (TAILING FACTOR R1)

S. no.	Source	Sum of squares	DF	Mean square	F value	p-value Prob > F
1	Model (Significant)	0.27	9	0.030	12.02	0.0017
2	A-Org phase	0.018	1	0.018	7.14	0.0319
3	B-Aq phase	0.022	1	0.022	8.76	0.0211
4	C-Flow rate	0.092	1	0.092	36.48	0.0005
5	AB	0.00198	1	0.00198	0.78	0.4055
6	AC	0.021	1	0.021	8.14	0.0246
7	BC	0.007921	1	0.007921	3.13	0.1200
8	A^2	0.009007	1	0.009007	3.56	0.1011
9	B^2	0.033	1	0.033	13.19	0.0084
10	C^2	0.058	1	0.058	22.99	0.0020
11	Residual	0.018	7	0.002528		
12	Lack of Fit	0.018	3	0.0059		
13	Pure Error	0.000	4	0.000		
14	Cor Total	0.29	16			

TABLE 4B: ANOVA FOR RESPONSE SURFACE 2FI MODEL (RETENTION TIME R2)

S. no.	Source	Sum of squares	DF	Mean square	F value	p-value Prob > F
1	Model	17.01	6	2.84	20.60	< 0.0001
2	A-Org phase	7.42	1	7.42	53.88	< 0.0001
3	B-Aq phase	0.014	1	0.014	0.10	0.7526
4	C-flow rate	7.40	1	7.40	53.74	< 0.0001
5	AB	0.46	1	0.46	3.35	0.0973
6	AC	0.16	1	0.16	1.16	0.3068
7	BC	1.57	1	1.57	11.38	0.0071
8	Residual	1.38	10	0.14		
9	Lack of Fit	1.38	6	0.23		
10	Pure Error	0.000	4	0.000		
11	Cor Total	18.39	16			

TABLE 4C: ANOVA FOR RESPONSE SURFACE QUADRATIC MODEL (AREA R3)

S. no.	Source	Sum of squares	DF	Mean square	F value	p-value Prob > F
1	Model	327072486573.72	9	36341387397	5.01	0.0226
2	A-Org phase	707801500.1	1	707801500.1	0.098	0.7639
3	B-Aq phase	8533208841	1	8533208841	1.18	0.3141
4	C-Flow rate	79716651341	1	79716651341	10.99	0.0129
5	AB	1246548492	1	1246548942	0.17	0.6909
6	AC	62776805809	1	62776805809	8.65	0.0217
7	BC	345885604	1	345885604	0.048	0.8334
8	A^2	22441199069	1	22441199069	3.09	0.1221
9	B^2	50681747119	1	506817447119	6.98	0.0333
10	C^2	83799886505	1	83799886505	11.55	0.0115
11	Residual	50794825935	7	7256403705		
12	Lack of Fit	50794825935	3	16931608645		
13	Pure Error	0.000	4	0.000		
14	Cor Total	377867312508.47	16			

Main Effects (Lack of Fit): The lack of fit is one of the components of the partition of the sum of squares in an ANOVA, which can tell that that purpose model is fit or not. The model summary statistics are shown in **Table 5(a)-5(c).**

TABLE 5A: MODEL SUMMARY STATISTICS (TAILING FACTOR R1)

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	0.11	0.4549	0.3291	0.0933	0.26
2FI	0.11	0.5597	0.2955	-0.2883	0.38
Quadratic	0.050	0.9392	0.8610	0.0273	0.28
Cubic	0.000	1.0000	1.0000		+

TABLE 5B: MODEL SUMMARY STATISTICS (RETENTION TIME R2)

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	0.52	0.8063	0.7616	0.5954	7.44
2FI	0.37	0.9252	0.8802	0.6193	7.00
Quadratic	0.35	0.9529	0.8924	0.2470	13.85
Cubic	0.000	1.0000	1.0000		+

TABLE 5C: MODEL SUMMARY STATISTICS (AREA R3)

Source	Std. Dev.	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS
Linear	149100	0.2354	0.0590	-0.3042	492800000000
2FI	149800	0.4058	0.0492	-0.8214	688300000000
Quadratic	85184.53	0.8656	0.6927	-1.1508	812700000000
Cubic	0.000	1.0000	1.0000		+

Interactions: The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. The following **Table (6a-6c)** shows the final equation in terms of actual factors.

TABLE 6A: FINAL EQUATION IN TERMS OF ACTUAL FACTORS (TAILING FACTOR R1)

S. no.	Factors	Tailing Factor
		-5.57112
1	Org phase	-0.016975
2	Aq. phase	+0.12541
3	Flow rate	+6.31188
4	Org phase × Aq. phase	0.0002225
5	Org phase × Flow rate	+0.035875
6	Aq. phase × Flow rate	-0.022250
7	Org phase^2	-0.0004625
8	Aq. phase^2	-0.00089
9	Flow rate^2	-2.93750

TABLE 6B: FINAL EQUATION IN TERMS OF ACTUAL FACTORS (RETENTION TIME R2)

S. no.	Factors	Tailing Factor
		+44.53141
1	Org phase	-0.39970
2	Aq. phase	-0.44432
3	Flow rate	-27.57500
4	Org phase × Aq. phase	0.00339249999
5	Org phase × Flow rate	+0.099875
6	Aq. phase × Flow rate	+0.31287

Fig. 4(a) to **4(b)** indicates that an increase % concentration of the organic phase in the mobile phase resulted in a decrease in tailing factor and retention time respectively while **Fig. 4(c)** indicates increase in area. **Fig. 5(a)** to **5(c)** shows responses as quadratic surface model for tailing factor, 2 FI model for retention time, and quadratic model for an area that measures signal to noise ratio.

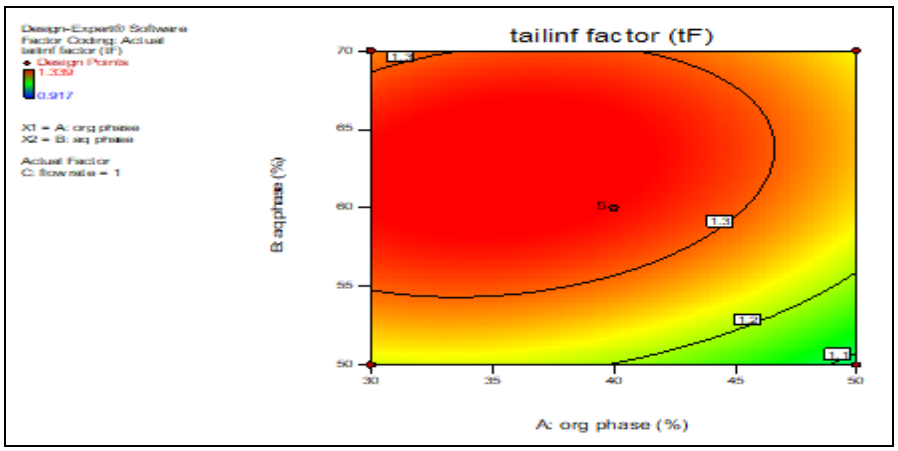

FIG. 4A: COUNTER PLOT FOR RESPONSE R1 (TAILING FACTOR)

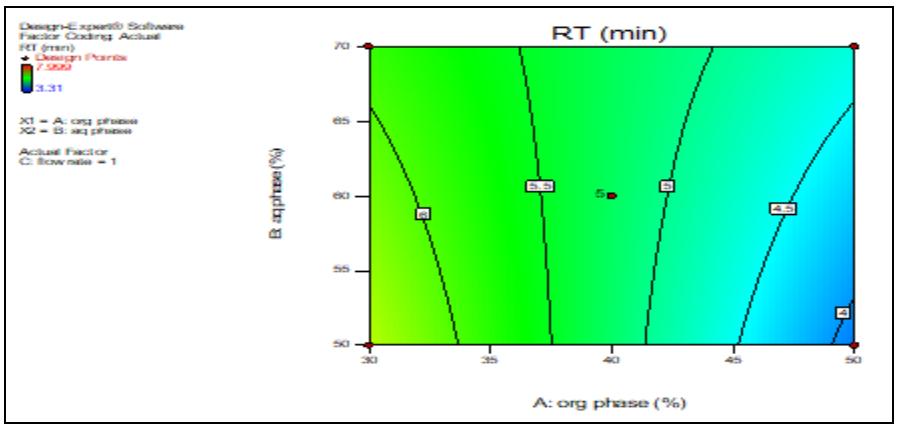

FIG. 4B: COUNTER PLOT FOR RESPONSE R2 (RETENTION TIME)

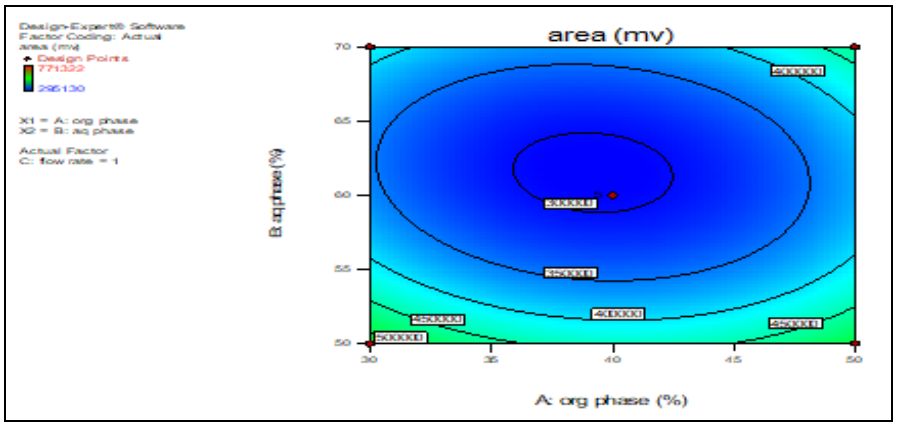

FIG. 4C: COUNTER PLOT FOR RESPONSE R3 (AREA)

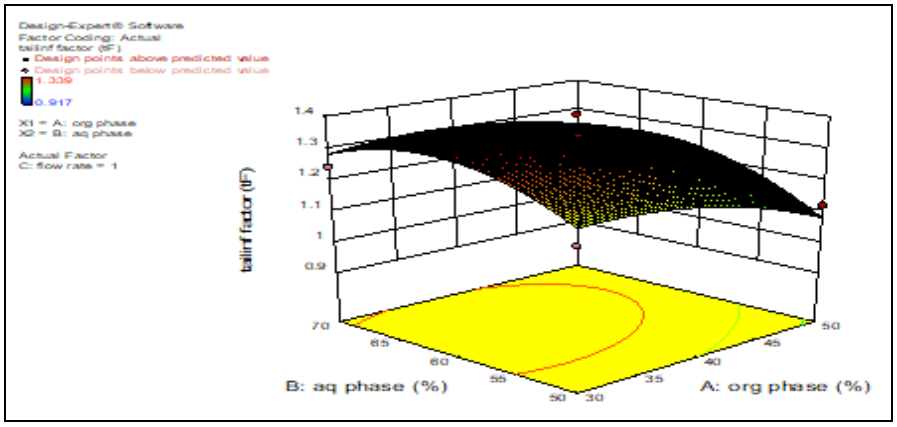

FIG. 5A: SURFACE RESPONSE CURVE FOR R1 (TAILING FACTOR)

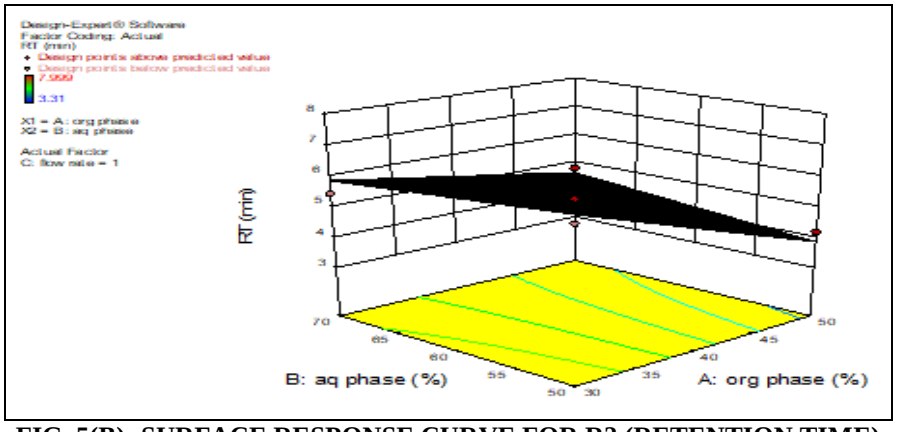

FIG. 5(B): SURFACE RESPONSE CURVE FOR R2 (RETENTION TIME)

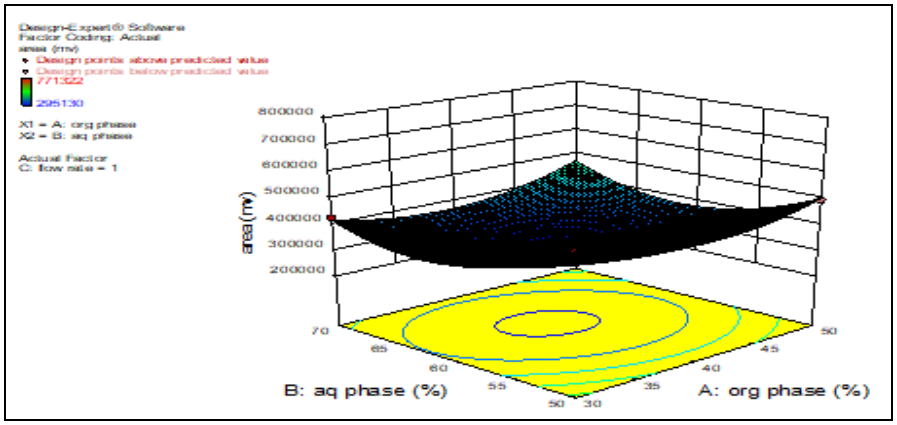

FIG. 5C: SURFACE RESPONSE CURVE FOR R3 (AREA)

TABLE 6C: FINAL EQUATION IN TERMS OF ACTUAL FACTORS (AREA R3)

S. no.	Factors	Tailing Factor
		7236410
1	Org phase	-5417.38750
2	Aq. phase	-137333
3	Flow rate	-4768420
4	Org phase × Aq. phase	+176.53250
5	Org phase × Flow rate	-62638.25000
6	Org phase^2	+730.05375
7	Aq. phase^2	+1097.12875
8	Flow rate^2	+3.52690E+006

Final Predicted Response for Dependent Factors: After performing the trials as per design, the values predicted by the software for selected factors are shown in **Table 7.**

System Suitability Study: The standard solution prepared by the mentioned procedure was used to study the system suitability test. After the equilibrium of the column with the mobile phase, six replicate injections of 20 µg/ml solution were injected through the manual injector separately, and the chromatograms were recorded. The observations of SST are shown in **Table 8** indicates the system is suitable for analysis of the said drug.

TABLE 7: PREDICTED AND ACTUAL VALUES OF DEPENDENT FACTORS

S. no.	Dependent factors	Values predicted	Dependent factors	Actual values	Model
1	Tailing Factor	1.29439	Tailing Factor	1.344	Quadratic
2	Retention Time	5.65388	Retention Time	5.2646	2FI
3	Area	339373	Area	292544.33	Quadratic

TABLE 8: OBSERVATIONS FOR SYSTEM SUITABILITY STUDY

Acotiamide hydrochloride hydrate (10 µg/ml)	
Mean Peak Area (µV)	292544.33
% RSD	0.72
Retention Time (min)	5.24
HETP	53.45
Tailing factor	1.33

Estimation of Acotiamide in Pharmaceutical Dosage Form: The developed RP-HPLC method was applied for the estimation of ACT in pharmaceutical formulations. The sample solutions were prepared as mentioned earlier procedure and injected in the system after equilibration of the column with the mobile phase. The content of Acotiamide hydrochloride hydrate in each sample was calculated by comparing the peak area of the sample with that of standard. The replicate estimation of the Acotiamide hydrochloride hydrate sample yields quite concurrent results indicating the method is precise. The representative chromatogram of standard and sample are shown in **Fig. 6(a)** and **6(b)**. The observations and results are tabulated in **Table 9.**

TABLE 9: OBSERVATIONS AND RESULTS OF ASSAY

S. no.	Weight of tablet powder (mg)	Area of standard (µv)	Area of the sample (µv)	Amount of drug estimated (mg)	% Label Claim
1	40.5	297921	294051	9.90	99.73
2	40.3		295422	9.91	100.32
3	40.9		293680	9.89	98.65
4	40.2		297703	9.99	99.65
5	40.2		296309	9.94	100.88
		Mean			99.68
		±SD			0.8488
		%RSD			0.85

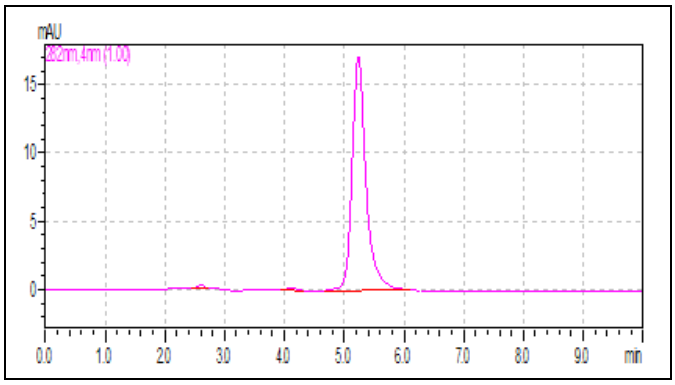

FIG. 6A: CHROMATOGRAM OF STANDARD ACOTIAMIDE HYDROCHLORIDE HYDRATE

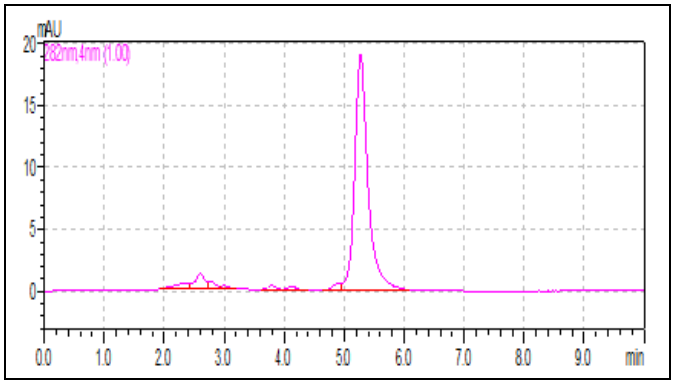

FIG. 6B: CHROMATOGRAM OF SAMPLE OF ACOTIAMIDE HYDROCHLORIDE HYDRATE

Validation of Proposed Method:

Accuracy: Accuracy of the proposed method was ascertained on the basis of recovery studies performed by the standard addition method. The result of the recovery study was found very close to 100%, representing the accuracy of the method and also shows that excipients have no interference in the estimation. The results and statistical data are summarized in **Table 10.**

TABLE 10: RESULTS OF RECOVERY STUDY

S. no.	Weight of tablet powder taken (mg)	Amount of pure drug added (mg)	AUC (µv)	Amount recovered (mg)	% Recovery*
1	40.5	5.2	448937	5.21	100.19
2	40.9	10.4	608324	10.6	101.92
3	40.8	15.7	758936	15.71	100.06
		Mean			100.723
		±SD			1.038
		%RSD			1.03

*Each value is mean of three observations

Linearity: Linearity study was performed on Acotiamide hydrochloride hydrate API by preparing the solutions having a concentration from 2 to10 µg/ml. A plot of concentration (µg/ml) and area under the curve (AUC) was constructed in **Fig. 7**. The correlation coefficient of Acotiamide hydrochloride hydrate API was found to 0.998, which indicates that the proposed method is linear.

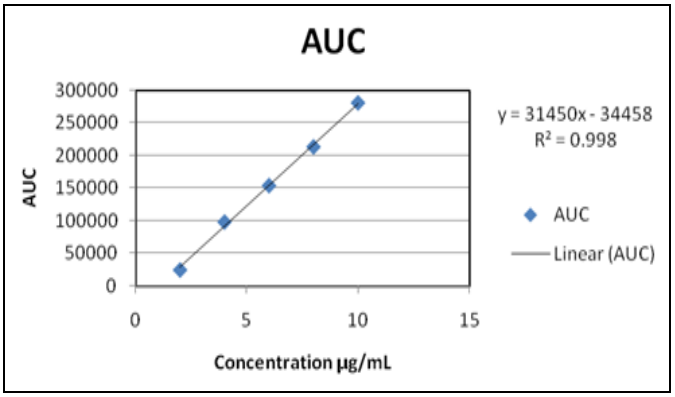

FIG. 7: PLOT OF LINEARITY CURVE FOR ACT

Ruggedness: The studies were carried out for two different parameters *i.e.* Different analysts and Days (Intraday and Interday). The results of the estimation of Acotiamide hydrochloride hydrate **Table 11** was found very much reproducible indicating the ruggedness of the method in the hands of different analysts

TABLE 11: RESULTS OF RUGGEDNESS STUDY

S. no.	Parameter	*Mean % label claim	±SD	%RSD
1	Analyst to analyst variation	99.87	1.575	1.58
2	Intraday	100.26	0.554	0.55
3	Interday	99.42	1.06	1.05

*Each value is mean of three observations

TABLE 12: RESULTS OF ROBUSTNESS STUDY

Parameters	Retention Time (min)	HETP	Tailing factor
Standard Condition	5.25	57.294	1.338
λmax 277 nm	5.209	57.821	1.341
λmax 287 nm	5.213	58.025	1.325
Pot. dihydrogen phosphate	4.941	57.056	1.312
Buffer : ACN (55:45)			
pH -7	4.927	58.920	1.336
pH -6.6	5.032	56.829	1.290
Flow rate 0.8 ml/min	6.294	57.786	1.320
Flow rate 1.2 ml/min	4.881	57.951	1.345
Mean		57.71	1.3258
%RSD		1.14	1.38
Mean RSD		1.26	

Robustness: The robustness study carried out at varying chromatographic conditions. The results and observations are given in **Table 12**. The proposed method was found to be robust as mean RSD 1.26.

Limit of Detection and Limit of Quantitation: DL and QL were calculated based on the standard deviation of response and slop (from linearity) of Acotiamide hydrochloride hydrate. The result of DL and QL value was found to 0.5292 and 1.6037, respectively.

CONCLUSION: A novel, simple, fast, and robust RP-HPLC analytical method of Acotiamide hydrochloride hydrate was successfully developed by employing AQbD approach (BBD Design) and further validated according to ICH guidelines. AQbD approach in method development provided a better performing and robust method in less time as compared to the manual method development.

ACKNOWLEDGEMENT: Authors are thankful to Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee Nagpur, Maharashtra, India, for providing all the research requirements and also thankful to Lupin Private Ltd. Pune, Maharashtra, India, for providing the API of Acotiamide hydrochloride hydrate as a gift sample.

CONFLICTS OF INTEREST: None

REFERENCES:

1. https://pubchem.ncbi.nlm.nih.gov/compound/Acotiamide (Accessed on 4 July 2018)
2. https://www.drugbank.ca/drugs/DB12482 (Accessed on 4 July 2018)
3. Vogt F and Kord A: Development of quality by design analytical method. Journal of Pharmaceutical Science 2011; 100(3): 797-800.
4. Peraman R, Bhadrya K and Reddy Y: Analytical quality by design: A tool for regulatory flexibility and robust analytics. International Journal of Analytical Chemistry 2015; 6(2): 101-16.
5. Vinayak A and Lateef S: Automated QbD based method development and validation of oxidative degraded Atorvastatin. Agilent Technologies 2014; 5(20): 1-12.
6. Dineshchandra SO, Darji CV, Patel J and Patel B: Development and validation of stability indicating RP-HPLC method for estimation of Acotiamide hydrochloride in tablet dosage form. Indo American Journal of Pharmaceutical Sciences 2018; 05(04): 2563-71.
7. Vani R and Sunitha M: Analytical method development and validation for the determination of Acotiamide hydrochloride using reverse phase HPLC method in bulk and tablet dosage form. World Journal of pharmacy and Pharmaceutical Sciences 2017; 6(10): 768-75.
8. Ladumore V, Chaudhari G, Shah P and Khoja S: Development and validation of stability indicating RP-HPLC method for Acotiamide hydrochloride hydrate in pharmaceutical dosage form. European Journal of Biomedical and Pharmaceutical Sciences 2018; 5(5): 621-27.
9. Damle MC and Harne UV: Development and validation of stability indicating HPLC method for determination of Acotiamide hyrochloride. International Journal of Pharmaceutical Sciences and Research 2018; 9(10): 4410-15.
10. Pasha IS and Varanasi MB: Chromogenic visible spectrophotometric quantification of Acotiamide in bulk drug and its formulation. World Journal of Pharmaceutical Research 2017; 6(17): 1261-67.
11. Attia AM, Nassar WI and Ramzy S: Spectrofluorimetric determination of Acotiamide hydrochloride trihydrate in the presence of its oxidative degradation product by different ratio spectra manipulating methods. Innoriginal International Journal of Sciences 2018; 5(3): 46-50.
12. Deepa M, Reddy KR and Satyanarayana SV: A review on quality by design approach for analytical method development. Journal of Pharmacy Research 2017; 11(4): 272-77.
13. Bajaj M and Nanda S: Analytical quality by design (AQbd): New paradigm for analytical method development. International Journal of Development Research 2015; 5(02): 3589-99.
14. Ranga S, Jaimini M, Sharma SK, Chauhan B and Kumar A: A review on design of experiments (DOE). International Journal of Pharmaceutical and Chemical Sciences 2014; 3(1): 216-44.
15. Ferreira S, Bruns R, Ferreira H, Matos G, David J, Brandao G, Silva E, Portugal L, Reis P, Souza and Santos W: Box- Behnken design: An alternative for the optimization of analytical method. Analytica Chemica Acta 2007; 597(2): 179-86.
16. ICH Harmonised Tripartite Guideline. Stability Testing of New Drug Substances and Products Q1A (R2). International Conference on Harmonisation. Geneva: Switzerland 2003: 1-24.

How to cite this article:
Gawande SS, Hemke AT, Gupta KR and Umekar MJ: Development of RP-HPLC method for the estimation of acotiamide hydrochloride hydrate using AQbD approach. Int J Pharm Sci & Res 2020; 11(9): 4530-39. doi: 10.13040/IJPSR.0975-8232.11(9).4530-39.