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Research article

Optimized and Validated Box–Behnken Design Combined Response Surface Methodology to Quantify Acetic Acid Content in Potassium Clavulanate Using High–Performance Liquid Chromatography

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A simple and robust high-performance liquid chromatography method with UV detection was developed to quantify acetic acid in potassium clavulanate. The chromatographic separation was performed on the Hypersil BDS C_{18} column (250×4.6 mm, 5 µm) with optimized response surface methodology combined Box–Behnken experimental design. The mobile phase comprises of a buffer prepared by ammonium dihydrogen orthophosphate (20 mM) containing 0.01% triethylamine, maintained pH between 2.8–3.2 using phosphoric acid and % acetonitrile as per Box–Behnken factorial design. The detection was performed at 235 nm with a mobile phase flow rate of 1 ml/min. The method was validated as per the ICH guidelines. The linearity of the proposed method was rectilinear within the range of 2.5–24.8 µg/ml. The value of the limit of detection (LOD) & limit of quantitation (LOQ) by the validated method was found to be 1.9 and 5.8 µg/ml, respectively. The robustness and ruggedness studies were conducted by small changes with parameters in the proposed method. The % RSD values were within the accepted limit of criteria. This developed method can quantify routine acetic acid content analysis in potassium clavulanate pharmaceutical formulations.

1. INTRODUCTION

Potassium Clavulanate (PC) is chemically known as Potassium (Z)–(2R, 5R)–3–(2–hydroxyethylidene)–7–oxo–4–oxa–1–azabicyclo[3.2.0]–heptene–2–carboxylate [$C_8H_8KNO_5$]. It is a semi-synthetic β–lactamase inhibitor, possibly isolated from streptomyces. It is able to inactivate a wide range of β–lactamase enzymes commonly found in microorganisms resistant to penicillins and cephalosporins. It contains a β–lactam ring and attaches firmly to β–lactamase at or near its active site. In addition, it protects other β–lactam antibiotics from beta-lactamase catalysis.[1,2]

Pure acetic acid (CH_3COOH) is trivially known as glacial acetic acid in an anhydrous state. It is a colorless, hygroscopic, and mild chelating agent with 99.5–100% available concentrations. It is a harmful chemical that has been correlated with numerous adverse drug actions concerning patients recently. But proper dilutions of it have a variety of medicinal uses. It acts as an irritant when its concentration is between 10–25% (1.67–4.16 mol/L); however, it is corrosive when its concentration is greater than 25% (>4.16 mol/L) and should be handled in the fume hood.[3] Typically, acetic acid is prepared using acetaldehyde and oxygen through a catalytical oxidation process. The reaction includes manganese acetate as a catalyst. The reaction is controlled so that almost 4% of acetaldehyde will be unreacted and recycled to the process again after separating from the crude acetic acid. As a result, several by-products are formed, such as ethylidene diacetate, acetoxyglycolic acid ester, succinic acid derivatives, and acetoacetic acid derivatives.[4] Acetic acid chemical exposure can cause irritations to humans after contact with the nose, cough and throat. The other symptoms are headaches, eye pain, and nausea.[5–8] It was determined in vinegar,[9] wine,[10,11] fermentation broth,[12] gefitinib,[13] birch wood[14] and heritage collections.[15]

During the synthesis of pharmaceuticals were required residual solvent in the process. However, unwantedly minimal amounts remained in the final product as impurity like acetic acid content in the pharmaceutical products becomes risky to administer; thus, they should be identified and quantitated.[16] An acid-base titrimetric method was developed and validated in amlodipine besylate to determine

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acetic acid content, where sodium hydroxide was used as a strong base against acetic acid as a weak acid.[17] An automated enzymatic spectrophotometric method was employed to quantify acetic acid in vinegar. The process encountered several validation parameters such as linearity, sensitivity and precision but could not verify the accuracy.[9] So, it is not only essential to develop an easy method but also to validate the method's parameters based on the regulatory guidelines. Therefore, the proposed method is developed and validated per the international conference on harmonization (ICH) guidelines[18–21] to quantify its amount in the final formulation products of potassium clavulanate.

Response surface methodology (RSM) combined Box–Behnken design (BBD) is now favoured to optimize and develop the analytical method in pharmaceuticals.[22–24] Due to its fewer experimental trials and fewer chemicals and solvent utilization, the analysis cost is reduced. It was favoured and applied to achieve the optimum analytical conditions for quantifying acetic acid content. It has numerous advantages in increasing the processes in which independent parameters are involved with distinct combinations to correlate relations.

The proposed investigation was suggested with the aims: (i) Optimization and design of a quadratic model based on the HPLC system, which included independent variables like pH, ACN and flow rate as per Box–Behnken design. (ii) Reduces the error with the number of experimental trials (iii) Selections of the column and chromatographic conditions that enhance separation efficiency (iv) Validated the procedures as per ICH regulatory guidelines. Hence, RSM-BBD combinations optimized the developed procedures involved a simple, sensitive and accurate high–performance liquid chromatographic (HPLC) due to its enhanced and precise instrumentation to determine acetic acid content in potassium clavulanate. The procedure can also quantify acetic acid contents with other pharmaceuticals in academic and quality control laboratories.

2. EXPERIMENTAL

2.1. CHEMICALS AND REAGENTS

The glacial acetic acid was purchased from Sigma Aldrich through a local vendor. The pharmaceutical formulations of potassium clavulanate such as Augmentin 625, Megamox 625 and Klavox 625 were bought from the local pharmacy. Ammonium dihydrogen orthophosphate, triethylamine, acetonitrile and phosphoric acid (H_3PO_4) were purchased from Sigma Aldrich. All the chemicals were of analytical grade and utilized without any additional purification.

2.2. INSTRUMENTATION

The chromatography analysis was carried out with the Shimadzu LC–2010 CHT HPLC, including a quaternary pump, autosampler, degassing unit and photodiode array (PDA) detector. All the chromatograms are integrated by LC solution software. The sample's best separation was achieved with a C_{18} (250 mm×4.6 mm, 5 µm) ODS hypersil column at wavelength 235 nm.

2.3. BUFFER AND STANDARD SOLUTION

The ammonium dihydrogen orthophosphate (20 mM) comprising 0.01% triethylamine adjusted pH (2.8–3.2) with a diluted phosphoric acid. The specific amount of glacial acetic acid (99.9%) was transferred into a 50 ml volumetric flask & diluted with water up to the mark as a stock solution (5000 µg/ml) and then diluted as per the requirements with the HPLC water.

2.4. OPTIMIZATION OF BOX–BEHNKEN DESIGN

Three-factor and three-level factorial design (3^3) optimised the HPLC chromatographic conditions. A. pH, B. ACN (%), and C. flow rate (ml/min) as the most preferred independent factors, which were at low, medium and high three levels. Several runs were performed according to the trial plan of 3^3 factorial designs to investigate its impact on dependent variables. The peak area and the theoretical plate's number (N) are responses to its constraints. Optimization performed at, A: 2.8 (-1), 3 (0), 3.2 (+1); B: 10 (-1), 15 (0), 20 (+1); C: 0.8 (-1), 1 (0), 1.2 (+1) were low (-1), centre (0) and high (+1) levels (Table 1). The Design-Expert software (trail version 8.0.4.1) was used to create and estimate the statistical experimental design, including 17 experiments to optimize the chromatographic conditions and correlate the relationship between independent and dependent variables. The best probable conditions obtained by employing graphical and statistical analysis are established on the response surface and contour plots with desirability function.

2.5. PROCEDURE TO DETERMINE ACETIC ACID IN PHARMACEUTICAL FORMULATIONS

One tablet (claiming 625mg) was accurately weighed and finely powdered. The specific amount of powder was transferred into a 100 ml separating funnel and added 25 ml of distilled for extractions. The same procedure was followed twice with a similar volume of distilled water. Then, it was filtered through a 0.45 µm Millipore filter paper and obtained a concentration of 50 µg/ml with suitable dilution with the diluent as the mobile phase. Dilution followed the proposed procedure to quantify its nominal content using the established quadratic equation.

2.6. METHOD VALIDATION

Validation of analytical procedures is critical for regulatory purposes and their practical and reliable lengthy-term application. The ICH guidelines attained a remarkable agreement in harmonizing the explanations of mandatory validation parameters, calculations and analysis. The analyst's responsibility is to identify factors relevant to performing a given analytical process, design proper validation protocols, including acceptance criteria, and execute an adequate evaluation.[16–19] The validation characteristics necessary for the different test procedures and their usual requirements are well recognized. The specificity and selectivity of the current method are assessed with the retention time of acetic acid. It is established with common excip-

Table 1. Optimized Box-Behnken experimental design with independent with dependent variables.

Independent	Unit	Codes	Levels			Dependent
			Low	Medium	High	
pH		A	2.8	3.0	3.2	Peak Area
ACN	%	B	10	15	20	
Flow rate	ml/min	C	0.8	1.0	1.2	Theoretical plate

ients such as magnesium stearate, microcrystalline cellulose, polydextrose and titanium dioxide. The proposed method's linearity was constructed and calculated using the linear regression analysis and processed by the least square regression method. The parameters, limit of detection (LOD) and limit of quantitation (LOQ), were agreed upon based on the response and slope of the linear regression equation. The accuracy and precision of the technique were estimated within the linear dynamic range. Five independent analyses were presented at every concentration level within one day (intraday precision) and seven consecutive days (interday precision). Recovery studies ascertained the accuracy. The robustness and ruggedness parameters are evaluated by changing slight variations with the proposed procedure regarding flow rate, detection wavelength, and column models and locations. The amount of acetic acid was quantified in pharmaceutical formulations using the regression equation.

3. RESULTS AND DISCUSSION

3.1. BOX–BEHNKEN FITTING MODEL AND RESPONSE SURFACE PLOTS

The three-level factorial summary with the outcomes of 17 experimental runs was exhibited (Table 2), where peak area and theoretical plate were selected as a response. While pH, ACN (%) and flow rate (ml/min) are independent variables for this analysis. The BBD optimization was fitted with quadratic models with lower standard deviation and higher F values. The adjusted and predicted correlation coefficient (R^2) was close to 1 (Table 3 & 4), indicating the quadratic model's competency. The multivariate analysis of the assessed results correlated a quadratic equation between peak area, theoretical plate numbers, and their independent variables. The expected value could be estimated employing the below equations in terms of coded factors:

$$\text{Peak Area} = 48543 - 87.37\ \text{A} + 6\ \text{B} + 141.63\ \text{C} + 43.75\ \text{AB} - 228.5\ \text{AC} + 230.25\ \text{BC} - 879.25\ \text{A}^2 - 776.5\ \text{B}^2 - 965.75\ \text{C}^2$$

$$\text{Theoretical Plate (N)} = 7742 + 63\ \text{A} - 53.5\ \text{B} + 34\ \text{C} + 126.5\ \text{AB} + 38\ \text{AC} + 65\ \text{BC} - 96.75\ \text{A}^2 - 117.75\ \text{B}^2 - 16.75\ \text{C}^2$$

Equations in terms of actual factors:

$$\text{Peak Area} = -191551 + 136507\text{pH} + 571.5\ \text{ACN} + 62679.4\ \text{flow rate} + 43.75\ \text{pH*ACN} - 5712.5\ \text{pH*flow rate} + 230.25\ \text{ACN*flow rate} - 21981.25\ \text{pH}^2 - 31.06\ \text{ACN}^2 - 24143.75\ \text{flow rate}^2$$

$$\text{Theoretical Plate (N)} = -6942.25 + 11980\ \text{pH} - 313.9\ \text{ACN} - 2817.5\ \text{flow rate} + 126.5\ \text{pH*ACN} + 950\ \text{pH*flow rate} + 65\ \text{ACN*flow rate} - 2418.75\ \text{pH}^2 - 4.71\ \text{ACN}^2 - 418.75\ \text{flow rate}^2$$

Analysis of variance (ANOVA) studied the model equation's statistical importance in calculating the peak area and theoretical plate's number. The independent variables were statistically meaningful if the p-values were <0.05 and computed each variable's percent contribution (PC) (Table 5 & 6). It has been recommended that the model terms be considered significant when $p < 0.05$. The p-value for the quadratic models is significantly less than 0.05, indicating competency and an effective quadratic model to quantify acetic acid in the potassium clavulanate dosage forms.

The normal probability graph of the residuals is almost linear, maintaining that the errors are typically scattered. The current investigation (Figures 1a and 1d) represents a lesser error linear graph. The residuals against the predictor plot are spread residuals. The plot bounces haphazardly and forms an almost straight group with zero residual line. A crucial plot (Figure 1b & 1e) rises arbitrarily to form a horizontal line close to the zero residual with no value points, keeping the additional residuals' basic random pattern. The perturbation plot describes a relation between the independent and dependent variables, significantly impacting the responses. The outcomes of the models highly depend on the independent factors (Figures 1c & 1f). The flow rate (ml/min) is more influential than pH and ACN (%) enhanced and improved the model to quantify acetic acid content. Response surface plots were produced for the quadratic model to investigate the relationship between independent factors against peak area and theoretical plate number. The dependent response's influence on the 2D contour (Figure 2) and 3D plots (Figure 3) explained the variable's influence on the dependent response. The plot showed that the peak area and theoretical plate response boosted with the ACN (%) increase and pH. Consequently, the acetic acid separation altered with A, B and C. The best optimized chromatographic conditions were 3.14 pH and 11.1 % ACN with a 1.1 ml/min flow rate to validate the HPLC method as per ICH guidelines with unity desirability.

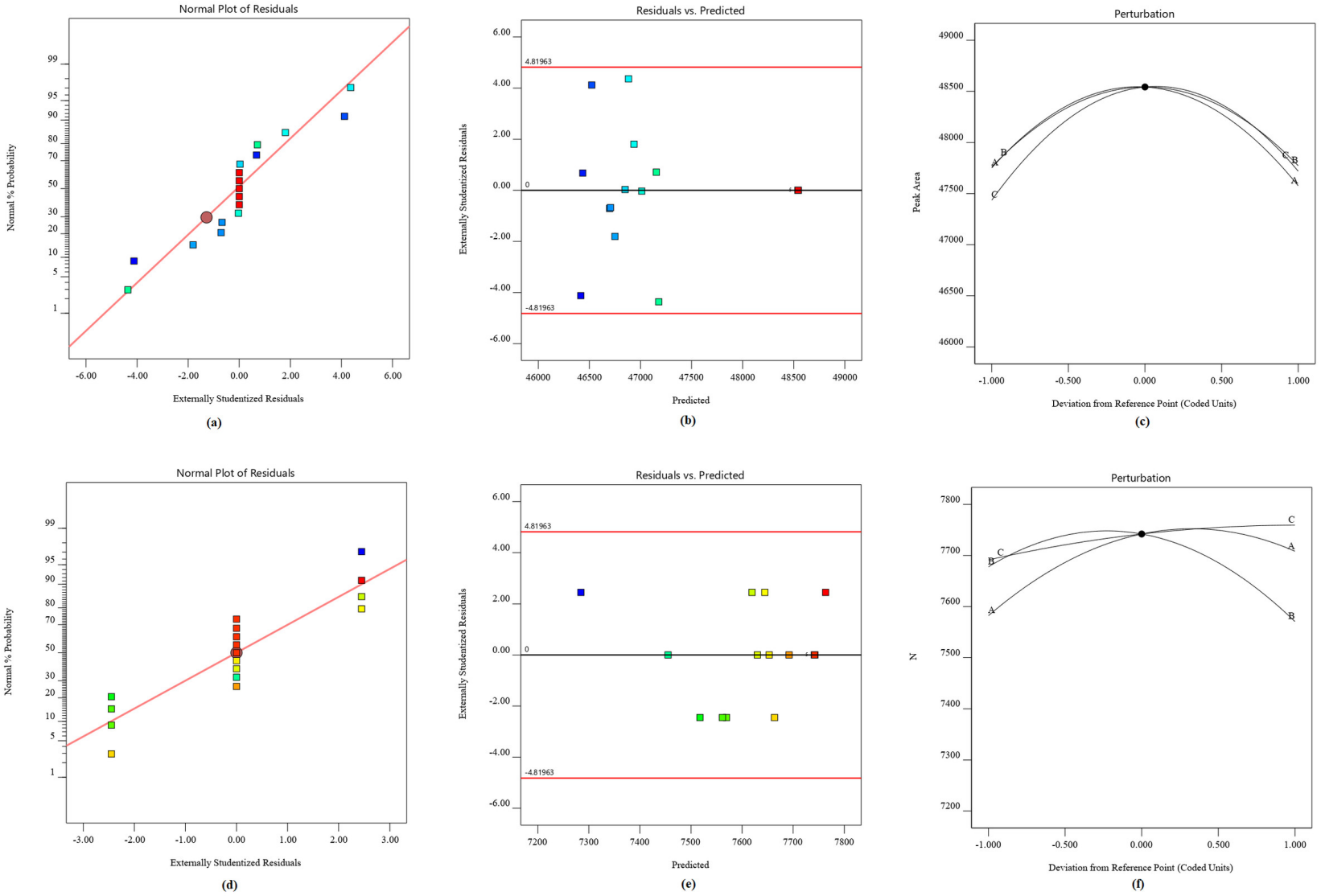

Figure 1. Box-Behnken design with peak area (a) Normal plot of residuals (b) Predicted vs Actual (c) Perturbation; with theoretical plate (d) Normal plot of residuals (e) Predicted vs Actual (f) Perturbation; with the theoretical plate to determine acetic acid content in potassium Clavulanate.

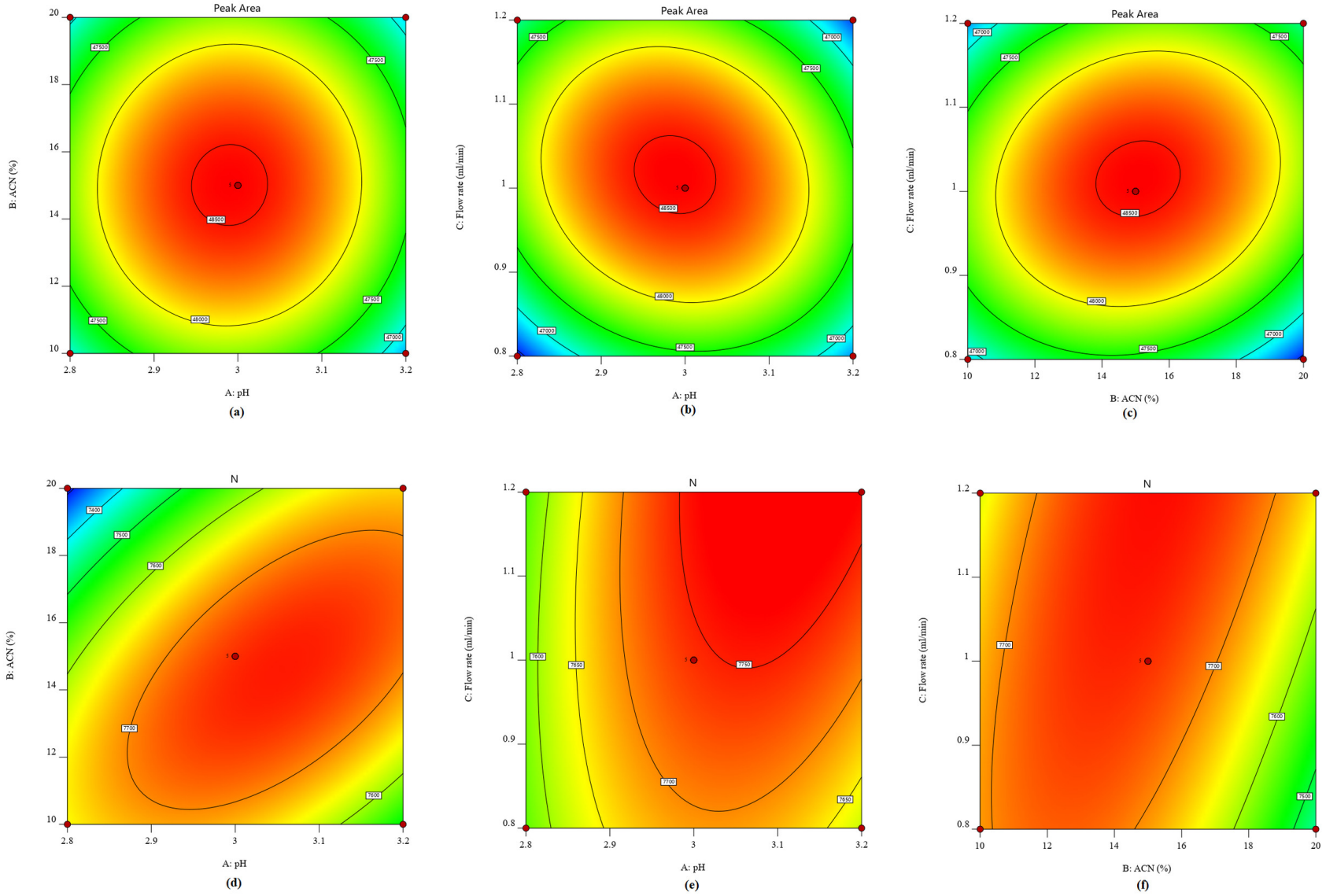

Figure 2. 2D contour plot with peak area signifying the relations between (a) pH and ACN (b) pH and flow rate (c) ACN and flow rate; with theoretical plate (d) pH and ACN (e) pH and flow rate (f) ACN and flow rate to quantify acetic acid content in pharmaceutical formulations.

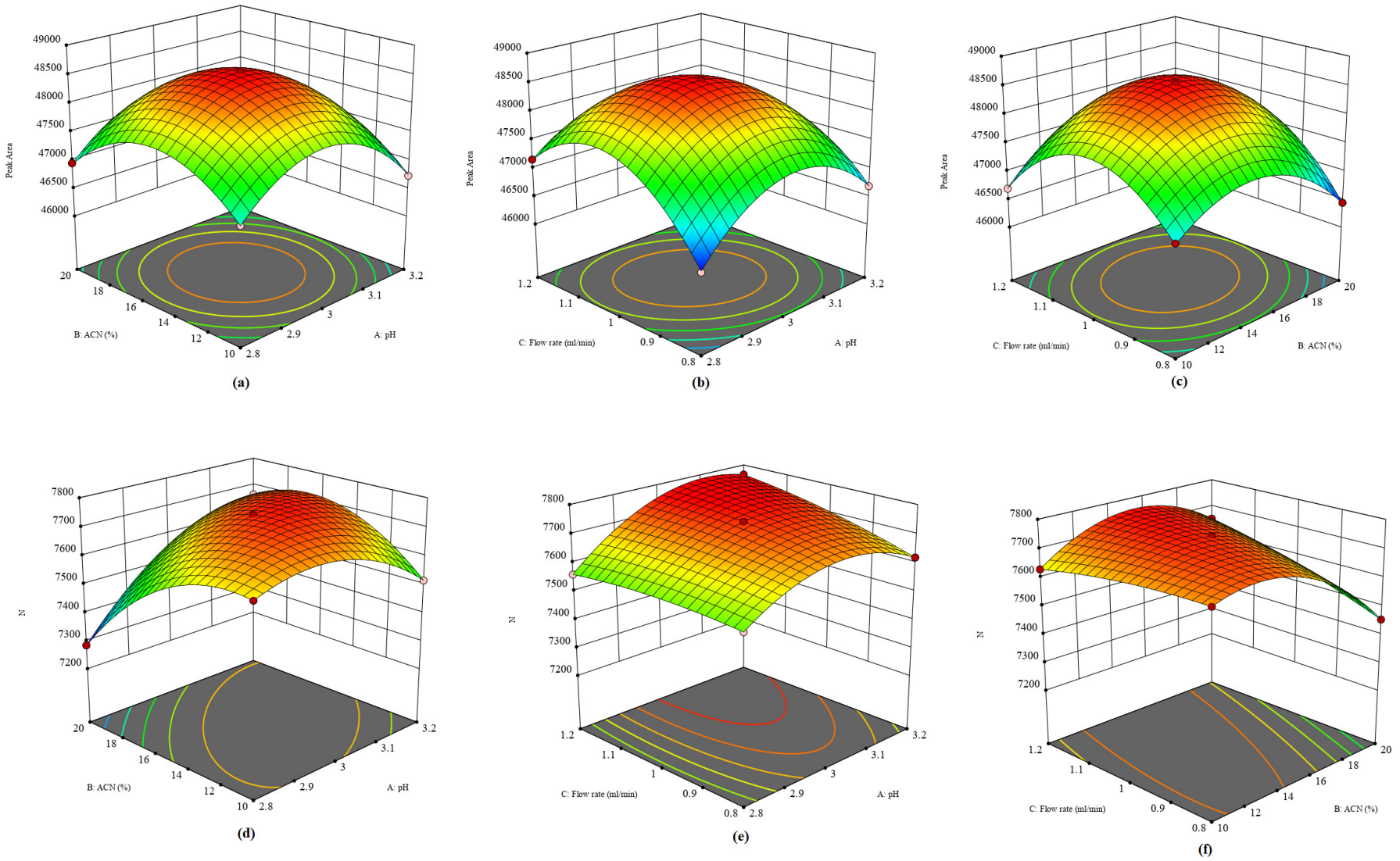

Figure 3. 3D surface plot with peak area signifying the relations between (a) pH and ACN (b) pH and flow rate (c) ACN and flow rate; with theoretical plate (d) pH and ACN (e) pH and flow rate (f) ACN and flow rate and their influences on the responses to determine acetic acid content.

Table 2. The three-level factorial summary includes independent variables and their responses.

Runs	Independent factors			Dependent factors	
	A: pH	B: ACN (%)	C: Flow rate (ml/min)	Peak Area	N
1	3 (0)	10 (-1)	0.8 (-1)	46910	7692
2	3 (0)	10 (-1)	1.2 (+1)	46698	7630
3	3.2 (+1)	20 (+1)	1 (0)	46850	7663
4	3.2 (+1)	15 (0)	1.2 (+1)	46550	7764
5 (c)	3 (0)	15 (0)	1 (0)	48543	7742
6	2.8 (-1)	10 (-1)	1 (0)	47012	7645
7 (c)	3 (0)	15 (0)	1 (0)	48543	7742
8 (c)	3 (0)	15 (0)	1 (0)	48543	7742
9	2.8 (-1)	15 (0)	0.8 (-1)	46389	7569
10	3.2 (+1)	15 (0)	0.8 (-1)	46689	7620
11	3 (0)	20 (+1)	1.2 (+1)	47152	7653
12	3.2 (+1)	10 (-1)	1 (0)	46732	7517
13 (c)	3 (0)	15 (0)	1 (0)	48543	7742
14	2.8 (-1)	15 (0)	1.2 (+1)	47164	7561
15 (c)	3 (0)	15 (0)	1 (0)	48543	7742
16	3 (0)	20 (+1)	0.8 (-1)	46443	7455
17	2.8 (-1)	20 (+1)	1 (0)	46955	7285

Table 3. The sequential fitting model with peak area as a response to quantify acetic acid content.

Sequential sum of squares					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Total vs Mean	3.805E+10	1	3.805E+10		
Linear vs Mean	2.218E+05	3	73941.42	0.0852	0.9669
Linear vs 2FI	4.286E+05	3	1.429E+05	0.1316	0.9391
Quadratic vs 2FI	1.085E+07	3	3.617E+06	6788.91	< 0.0001
Cubic vs Quadratic	3729.75	3	1243.25		
Residual	0.0000	4	0.0000		
Total	3.806E+10	17	2.239E+09		
Summary statistics					
Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**Press**
Linear	931.67	0.0193	-0.2070	-0.4151	1.628E+07
2FI	1041.90	0.0565	-0.5096	-1.1961	2.527E+07
Quadratic	23.08	0.9997	0.9993	0.9948	59676.00
Cubic	0.0000	1.0000	1.0000		

3.2. METHOD VALIDATION

3.2.1. SYSTEM SUITABILITY

The system suitability test was one of the main parameters for validation. The method was evaluated by injecting six replicates of the acetic acid solution (20 µg/ml) from the same vial. The percentage relative standard deviation (% RSD) of peak areas and retention time were calculated with an acceptable limit of 2%.

3.2.2. SYSTEM SUITABILITY

It is established with the presence of common excipients such as magnesium stearate, microcrystalline cellulose, polydextrose and titanium dioxide. The retention time was similar, even though the peak areas differed and found %RSD within the limit of ±2%.

3.2.3. LINEARITY AND RANGE

The Beer Lambert's law was compliant with the regression plots in the concentration range of 2.5–24.8 µg/ml with a

Table 4. The sequential fitting model with theoretical plate number as a response to quantify acetic acid content.

Sequential sum of squares					
Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**
Total vs Mean	9.905E+08	1	9.905E+08		
Linear vs Mean	63898.00	3	21299.33	1.43	0.2784
Linear vs 2FI	86685.00	3	28895.00	2.71	0.1013
Quadratic vs 2FI	1.066E+05	3	35535.82	1.244E+05	< 0.0001
Cubic vs Quadratic	2.00	3	0.6667		
Residual	0.0000	4	0.0000		
Total	9.908E+08	17	5.828E+07		
Summary statistics					
Source	**Std. Dev.**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**Press**
Linear	121.94	0.2484	0.0750	-0.3512	3.475E+05
2FI	103.25	0.5855	0.3368	-0.2067	3.104E+05
Quadratic	0.5345	1.0000	1.0000	0.9999	32.00
Cubic	0.0000	1.0000	1.0000		

Table 5. ANOVA with peak area response in the contribution of parameters in the Quadratic model to determine acetic acid content.

Source	Sum of squares	df	Mean square	F-value	p-value
Model	1.150E+07	9	1.278E+06	2398.60	< 0.0001
A–pH	61075.12	1	61075.12	114.63	< 0.0001
B–ACN	288.00	1	288.00	0.5405	0.4861
C–Flow rate	1.605E+05	1	1.605E+05	301.15	< 0.0001
AB	7656.25	1	7656.25	14.37	0.0068
AC	2.088E+05	1	2.088E+05	391.97	< 0.0001
BC	2.121E+05	1	2.121E+05	397.99	< 0.0001
A^2	3.255E+06	1	3.255E+06	6109.13	< 0.0001
B^2	2.539E+06	1	2.539E+06	4764.72	< 0.0001
C^2	3.927E+06	1	3.927E+06	7370.28	< 0.0001
Residual	3729.75	7	532.82		
Lack of Fit	3729.75	3	1243.25		
Cor Total	1.151E+07	16			
Std. Dev.	23.08		R^2	0.9997	
Mean	47309.35		Adjusted R^2	0.9993	
C.V. %	0.0488		Predicted R^2	0.9948	
			Adeq Precision	120.1861	

correlation coefficient (r^2) of 0.9965 with a linear graph between concentration and peak areas with excellent linearity over the working concentration ranges.

3.2.4. ACCURACY AND PRECISION

The instrument precision was determined by performing a system suitability test. The standard acetic acid solution was prepared by adding varying amounts of acetic acid. The intraday precision attempts were conducted over duplicate analysis (n=5) of acetic acid corresponding to 7, 14 and 21 µg/ml. The within-day precision was estimated with replicate analysis for seven consecutive days with a similar concentration as intraday precision. The results showed %recovery and %RSD values for intraday and interday precision were 95.72–96.97%, 92.34–95.43 % and 0.44–0.77%, 0.52–0.98%, respectively. Thus, the precision results are satisfactory. The % recovery is 93.32–94.84%, within the 80–120% limit. All the results were within the range of (80 – 120 %) as per the regulatory requirements.

Table 6. ANOVA with theoretical plate response in the contribution of parameters in the Quadratic model to quantify acetic acid content.

Source	Sum of squares	df	Mean square	F-value	p-value	PC (%)
Model	31752.00	1	31752.00	1.111E+05	< 0.0001	
A–pH	22898.00	1	22898.00	80143.00	< 0.0001	10.51
B–ACN	9248.00	1	9248.00	32368.00	< 0.0001	4.25
C–Flow rate	64009.00	1	64009.00	2.240E+05	< 0.0001	29.388
AB	5776.00	1	5776.00	20216.00	< 0.0001	2.651
AC	16900.00	1	16900.00	59150.00	< 0.0001	7.76
BC	39412.89	1	39412.89	1.379E+05	< 0.0001	18.1
A^2	58379.21	1	58379.21	2.043E+05	< 0.0001	26.8
B^2	1181.32	1	1181.32	4134.61	< 0.0001	0.54
C^2	2.00	7	0.2857			0.001
Residual	2.00	3	0.6667			
Lack of Fit	31752.00	1	31752.00	1.111E+05	< 0.0001	
Cor Total	2.572E+05	16				
Std. Dev.	0.5345		R^2	1.0000		
Mean	7633.18		Adjusted R^2	1.0000		
C.V. %	0.0070		Predicted R^2	0.9999		
			Adeq Precision	1168.4066		

3.2.5. LOD AND LOQ

The standard deviation (S_o) of concentration vs respective peak area was calculated using the linearity results. The LOD and LOQ values were evaluated after determining the slope of the straight line. The values were 1.9 and 5.8 ppm, respectively. The precision of the LOD value was investigated. Since 2.48 ppm gave a peak area of 18937, therefore, carried out precision with 0.5 ppm, giving a peak area of approx. 4000, the lowest area where a detectable peak appears in normal conditions. The % RSD of 0.505 ppm solution was 15.19% of six replicate injections and met the acceptance criteria of NMT 20%. The derivatized samples (5.86 and 5.05 ppm) were injected six times separately. The % RSD was 6.65 and 16.06 %, respectively, so 5.86 ppm was very low compared to 5.05 ppm. The % RSD value for 5.05 ppm was very close to the acceptance limit of 20%. Therefore, the method's LOD and LOQ arrived at 0.5 and 5.05 ppm, respectively.

3.2.6. ROBUSTNESS AND RUGGEDNESS

The robustness of the proposed method was evaluated by slight change with mobile phase combination and wavelength within the limit ± 10%. As a result, the % RSD values are within the range of ±2% per the ICH guidelines. The ruggedness test was confirmed by utilizing the various model of the HPLC column and performed in two different laboratories. The % RSD was 0.47–1.02% with the acceptable limit and indicated the robust method.

3.3. ACETIC ACID CONTENT IN PHARMACEUTICAL FORMULATIONS

The optimized Box–Behnken chromatographic conditions were applied in pharmaceutical dosage forms to quantify acetic acid. It is remarkably effective and anticipated to generate toxicity that exhibits unforeseen pharmacological acts toxic to human health. It is necessary to control it as impurity is critical for the patients. It's also a paramount regulatory concern in the generic drug office and considerably influences its approvals. The content was undetected in all pharmaceutical formulations. As well, the higher pharmaceutical doses do not have any impact on the content of acetic acid. Therefore, the acetic acid content is absent in potassium clavulanate's dosage products.

3.4. ANALYTICAL TECHNIQUES TO QUANTIFY ACETIC ACID CONTENT

A convenient microanalytical flow system was applied to quantify acetic acid during wine ageing. The diminished system is established using a gas-diffusion membrane and a pH–ion-sensitive field-effect transistor.[10] Due to the hindering consequences of acetic acid in various fermentation practices, a gas-diffusion online technique was developed for determining acetic acid based on a flow diffusion analysis. It included the UV-absorbance, which was detected at 205 nm. The amount was attained by parting an acceptor and a carrier stream operating a gas permeable polytetrafluoroethylene membrane.[12] The titrimetric method involved in quantifying it in amlodipine besylate utilizing the conventional acid-base concept. The technique was developed per the ICH guidelines, but drawbacks during end point detection.[17] The automated enzymatic spectropho-

tometric method determines in vinegar. It followed many validation obligations except accuracy. Transferring knowledge with effective procedures will avoid the recurrence of extended and laborious validation practices requiring higher costs and wasting time.[9] Therefore, needed a method to develop without any clean-up procedure with lesser trials and validated as per regulatory guidelines. The proposed approach is based on the HPLC technique, which is highly sensitive and optimized with BBD-RSM. It reduces error, the experiment trials and the cost to determine acetic acid content with a wider linear dynamic range.

4. CONCLUSION

The analytical method for estimating acetic acid in potassium clavulanate in pharmaceutical formulations has been successfully validated. The proposed procedures involved optimizing the chromatographic conditions using response surface methodology via Box–Behnken design, which decreases the number of experiments. The pH, ACN and flow rate interactions with peak area and theoretical plate number were evaluated with 2D contour and 3D surface plots. The proposed method ensures no additional procedure like any cleanup procedure for its quantification. It has a broader linear dynamic range and excellent accuracy and precision. It is a simple, accurate and time-consuming method. Nevertheless, the %RSD value was obtained accurate and within the standard limit of ICH guidelines. Thus, the present method is excellent for determining acetic acid content in pharmaceutical formulations of potassium clavulanate.

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