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Original Article

Implementation of QbD Strategies for HPLC Cleaning Method Development and Validation of Cinacalcet in API and Dosage Form

Lalitha Repudi[1], Baby Nalanda Revu[2], Buggana Siva Jyothi[3], Raghavarapu Prasanthi[4], Vasanth P M[5], Swetha Sri Remidicherla[6,*]

[1]Department of Pharmacy, Chaitanya (Deemed to be University), Gandipet, Himayath Nagar, Hyderabad, Telangana, INDIA.
[2]Department of Pharmaceutical Analysis, GITAM School of Pharmacy, GITAM (Deemed to be) University, Visakhapatnam, Andhra Pradesh, INDIA.
[3]Department of Pharmaceutical Quality Assurance, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya (Co-Ed), Secunderabad, Telangana, INDIA.
[4]Department of Pharmaceutics, Sree Dattha Institute of Pharmacy, Sheriguda, Hyderabad, Telangana, INDIA.
[5]Department of Pharmaceutical Analysis, SVU College of Pharmaceutical Sciences, Sri Venkateswara University, Tirupati, Andhra Pradesh, INDIA.
[6]Department of Pharmaceutical Analysis, TRR College of Pharmacy, Meerpet, Telangana, INDIA.

ABSTRACT

Background: Cinacalcet HCl is used to treat hyperthyroidism and hypercalcisim. QbD approach used to assess risk and develop cleaning method for Cinacalcet HCl. The Method Operable Design Region (MODR) was obtained by CCD concluded from surface and contour plot Analytical Target Profile defined with 15 variables verified for quality. **Materials and Methods:** The contour diagram displays the anticipated and optimized data, which includes the composition of the eluent phase consisting of potassium dihydrogen ortho Phosphate buffer with a pH of 3.5 and acetonitrile (CH_3CN) in a ratio of 20:80 v/v. The flow rate was set at 1 mL/min for a duration of 10 min. The analysis was performed using a Kinetex C_{18} column at a temperature of 25°C, and the wavelength chosen for detection was 279 nm. **Results:** After injecting swabbed samples from SS plate(10*10 cm), sharp and resolved peak of CIN at 2.8 min of retention was observed by a typical recuperation rate of 92.4% (±2.1%, *n*=3).The set method had a LOQ of 0.740 µg mL^{-1} and a LOD of 0.244 µg mL^{-1}.The measured linearity of the calibration curve ranges from 0.25 to 12.5 gmL^{-1}, with a r^2 of 0.999. All validation parameter findings fell within the permitted range. % RSD of system suitability 0.03%, inter-day and intra-day precision % RSD value 1.07 and 0.95%, respectively, and accuracy (0.059-0.189%). **Conclusion:** QbD approach is applied effectively to optimize HPLC method for Cinacalcet estimation in formulation and API manufacturing.

Keywords: Cinacalcet, Central Composite Design, Modr, Cleaning Method, Analytical Target Profile, Method Validation , Qbd.

Correspondence:
Mrs. Swetha Sri Remidicherla
Department of Pharmaceutical Analysis, TRR College of Pharmacy, Meerpet, Telangana, INDIA.
Email: sweety.rss@gmail.com

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INTRODUCTION

Cinacalcet plays a role as a calcimimetic and a P450 inhibitor[1] and improves the sensitivity of calcium receptors on parathyroid cells to reduce parathyroid hormone levels and hence decrease serum calcium levels. It also has a role in Hypercalcemia and Kidney Disease (CKD) in persons with parathyroid cancer.[2] Cinacalcet HCl has the chemical formula $C_{22}H_{22}F_3N.HCl$ and a molecular weight of 357.41 g/mol as a freebase and 393.87 g/mol as a salt of HCl.[3] It is a white to off-white, crystalline solid that is somewhat soluble in water, methanol, and 59% ethanol. Cinacalcet HCl has a Pka value of 8.72 (Strongest Base: 10.3) and a melting point between 175 and 177 °C.[4] Validating cleaning method is a process used to show that a cleaning method successfully removes impurities or residues from surfaces or equipment.[5] It guarantees that the cleaning technique can reliably and consistently achieve the specified level of cleanliness.[6] Quality by Design (QbD) in analytical method development focuses on ensuring the quality of pharmaceutical products through systematic planning and risk management. A key step in this approach is the identification of Critical Method Parameters (CMPs), which directly influence the quality of data generated. Once identified, CMPs are optimized using Design of Experiments (DOE) to study their impact on method performance and to establish method robustness. Alongside optimization, a control strategy is formulated, which defines acceptance criteria such as accuracy, precision, linearity, and range, ensuring the method consistently meets desired

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specifications. The Analytical QbD (AQbD) concept extends these principles to analytical techniques, providing risk-based, life-cycle oriented, and regulatory-flexible development. Literature supports AQbD as a valuable tool for robust Analytics, with applications in separation, impurity profiling, and chiral analysis of Cinacalcet. For instance, Reddy *et al.* developed a stability-indicating RP-UPLC method for Cinacalcet impurities using an Acquity BEH Shield RP18 column, while Yang *et al.* demonstrated chiral separation of Cinacalcet and its intermediates via HPLC. Overall, applying QbD principles helps identify and manage variability, delivering precise, reliable, and regulatory-compliant analytical methods. Figure 1 illustrates the key steps required to fulfill AQbD requirements and establish an Analytical Target Profile (ATP) for cleaning method development. A literature survey revealed that only a limited number of analytical techniques have been reported for Cinacalcet estimation, primarily UV spectroscopy,[7-13] HPLC,[14-28] and LC-MS.[29-49]

MATERIALS AND METHODS

Dr Reddy's supplied the reference standard for Cinacalcet HCl. The tablet dosage form of Cinacalcet HCl is PTH 30 tablet by Intas pharmaceuticals. Potassium dihydrogen ortho Phosphate, ortho phosphoric acid were acquired from SD Fine Chemical Tech. Methanol of HPLC grade from Rankem chemicals. Acetonitrile HPLC grade from Finar chemicals. A Waters HPLC system (Waters 1525, For HPLC analysis, a binary pump, and UV-visible detector 2487 were utilized. Separation was accomplished in an analytical C18 Kinetex column with internal diameter measurements of (100×4.6×5) µm (internal diameter). Double Beam UV-visible spectrophotometer (Lab India UV-3200), cyclomixer (CM 101) made by Remi Equipment's, swabs (polypropylene sticks) by Hi-media Lab, stainless steel plate (10× 10 ×2 mm) thickness, Analytical balances Make by Shimadzu, sonicator by Soltec (2200MH).

Using QbD for Optimization

Experiments with different factor interactions on dependent variables were designed using QbD. Two variables-the composition of the mobile phase and the pH of the buffer-were considered independent in the design of the CCD investigation as in Table 1, while retention time, tailing factor, and resolution were considered dependent response factors. In order to optimize the independent factors and assess the dependent variables, eleven runs were conducted.

Sample preparation

Preparation of standard stock solution: 5 mg of Cinacalcet HCl was weighed and placed in a 5 mL volumetric flask. It was then diluted with acetonitrile up to the mark, (1000 µg/mL) (stock solution 1). 1 mL of this solution was made to the 10 mL graduation with acetonitrile (100 µg/mL) (stock solution 2). Further serial dilutions were made from stock solution 2 to obtain concentrations of 1, 1.25, 2, 2.5, 5, 10, 15, 20, 25, 30, 50, 75, and 100 µg/mL.

Buffer preparation

After precisely measuring 3.4 g of potassium dihydrogen phosphate, the substance was carefully transferred to a 500 mL volumetric flask. Then, using ortho phosphoric acid, the pH was adjusted to 3.5. Similarly, the solution was changed to the requisite pH levels of 3, 3.25, and 3.5. After passing the solution through a 0.45 membrane filter, it was sonicated for 10 min to remove any remaining particles.

Mobile Phase Preparation

Acetonitrile of HPLC grade is taken in a chromatographic bottle and sonicated for 10 min, The prepared Buffer and Acetonitrile was taken in a required ratio and together used as a mobile phase. After experimenting with different ratios, the mixture of buffer and acetonitrile (20:80 v/v, pH 3.5) was selected as the mobile phase. A sharper peak with less tailing was produced by this composition, which held better than higher buffer fractions.

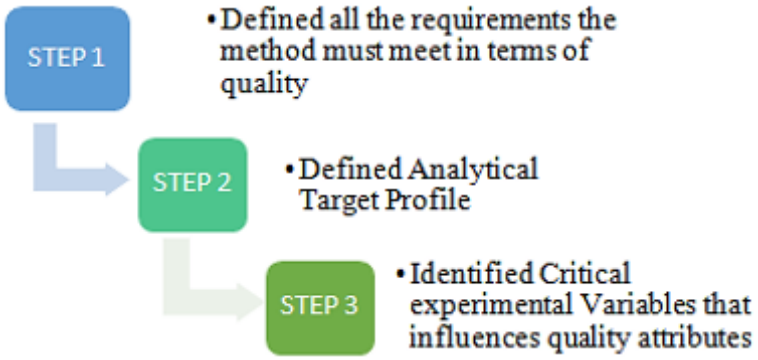


Figure 1: Steps in Analytical QbD.

Table 1: Matrix Design according to Central Composite Design (CCD) for Cinacalcet HCl.

Run Order	S	Coded Factor Level	
		FA	FB
1	8	65	3.5
2	6	80	3.25
3	7	65	3
4	2	80	3
5	4	80	3.5
6	10	65	3.25
7	9	65	3.25
8	1	50	3
9	3	50	3.5
10	5	50	3.25
11	11	65	3.25
	Effects of component levels		
Value set	Lower (−1)	Middle (0)	Higher (+1)
FA: Acetontrile	50	65	80
FB: Buffer PH	3	3.25	3.5

S- standard; FA- factor A; FB-factor B.

Cleaning method

Direct surface sampling (swab method)

The most common sampling method involves swabbing a surface methodically with a probe, also known as a "swab," SS plate(stainless steel plate) was taken and gently wiped with chosen solvent (Acetonitrile), then pipetted out 1 mL of solvent and spread drop wise on SS plate, dried the SS plate by using hair dryer, then with the help of rinsed swab stick (swab stick was placed in a solvent and sonicated for 5 min) collect the sample left on SS plate by horizontal, vertical and diagonal strikes then enclose the swab in test tube with solvent selected and mix in cyclomixer for 4 min and made up the solution up to 10 mL with Acetonitrile and measure its RT and absorbance, consider this as blank, now repeat the same with different concentrations (2, 4, 5, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100) ppm, the same is applied on the manufacturing equipment to detect the traces left from the previous batch production.

RESULTS

Optimization

Experimental design optimization can develop a mathematical model to identify ideal chromatographic parameters is exemplified in Table 2.

Retention time

Utilizing three independent factors and a significant analytical model (p-values <0.0500), CCD was able to optimize the retention duration effectively in Figures 2a and 2b. Due to the fact that the signal-to-noise ratio of 28.846 suggests that there is adequate signal, the model can be utilized to navigate the design space.

Tailing factor

Utilizing CCD surface response and ANOVA for the linear model, the tailing factor was optimized. The model was significant (p-values <0.0500) and the signal-to-noise ratio of 39.082 indicates an adequate signal. This model can be used to navigate the design space as in Figures 2c and 2d.

Resolution

Optimization of resolution for the simplified quadratic model in Figures 2e and 2f was accomplished by the utilization of CCD surface response and ANOVA. With p-values less than 0.0500 and a signal-to-noise ratio of 25.246, the model was determined to be statistically significant, indicating that the signal is enough.

System suitability

The study's findings regarding the optimal operating variables for Cinacalcet swab sample separation are displayed in Figure 2g. Numerical values displayed in Table 3 details on the compatibility of HPLC system with the method developed, considering standard deviation as 348.600 for 6 injections of CIN, its calculated relative % obtained as 0.30.

Linearity

Beginning with a lower concentration as depicted in Table 3, swabbed sample of Cinacalcet was proved for the operating parameters that are selective, by CCD which is projected in

Figure 3a, where its corresponding overlain is Figured in Figure 3b. Tested five concentrations has showed good results.

Precision

Having considering lower concentration, Cinacalcet counterpart was eluted at 2.871 min and 2.865 min respectively for Intra-day as well as Inter-day. Mean response for the same was down through the limit, seen as 1.07% and 0.95%.

Accuracy

Specified concentration was swabbed, Cinacalcet attained mean % recovery getting through the limits. Its % relative standard deviation was acquired as 0.090, 0.189, 0.059 respectively at each spike level.

Robustness

Our HPLC approach for Cinacalcet at 2.5 µg/mL was tested for reliability under normal analytical fluctuations by intentionally varying critical chromatographic parameters. By carefully measuring the reference standard and then diluting it with the mobile phase to attain the target concentration, a Cinacalcet standard solution was produced. For the chromatographic settings, a YMC Triart C18 column (250 × 4.6 mm, 5 µm), a mobile phase of 10 mM ammonium bicarbonate and acetonitrile in a 65:35 v/v ratio, 1.0 mL/min flow rate, detection at 279 nm, and 30°C column temperature was used. The robustness of the method was tested under various conditions, including as flow rate (±0.1 mL/min), mobile phase composition (60:40 and 70:30), wavelength shifts (278 nm and 280 nm), and column temperature (±5°C). For each condition, 2.5 µg/mL solution was injected six times.

Ruggedness

Various settings were used to test the HPLC method for Cinacalcet at a concentration of 2.5 µg/mL. These settings included different analysts, columns, and days. The standard solution of Cinacalcet, with a concentration of 2.5 µg/mL, was prepared using the previously published method and mobile phase. Under the same chromatographic conditions, Analyst 2 utilized Luna C18 on Day 2 instead of the primary column (e.g., Kinetex C18) employed on Day 1. For each condition, three injections of the prepared standard solution were administered. Theoretical plates, peak area, tailing factor, retention time, and peak time were all recorded for comparison. Consistency and reliability in regular lab settings were proven by confirming ruggedness when retention time and area %RSD stayed below acceptable values (≤2%) across analysts, columns, and days.

LOD and LOQ

Applying the formula, detectable and quantifiable amount of Cinacalcet gained is 0.244 µg/mL and 0.740 µg/mL respectively.

DISCUSSION

Optimization

The findings from an investigation into the best way to separate Cinacalcet swabs utilized operational variables. The independent variables of moving phase component are flow rate and acetonitrile concentration, and the dependent variables are the tailing factor and retention time. The F-test for linear model table shows that the model is statistically significant (p<0.0001), indicating that the independent variables have a significant effect on the dependent variables. F-value for acetonitrile is much higher than the F-value for buffer, indicating that acetonitrile has a greater effect on the dependent variables. Relationships between independent and

Table 2: Analyzing experimental findings and choosing the final method conditions.

		F- 1	F- 2	R-1	R-2	R- 3
Std	Run	A: Acetonitrile (%)	B: Buffer pH	Retention time (Min)	Tailing Factor	Resolution
8	1	65	3.5	4.6	1	2
6	2	80	3.25	2.4	0.7	2.8
7	3	65	3	3.1	1.3	1.7
2	4	80	3	2	0.9	2.2
4	5	80	3.5	2.8	0.4	3.4
10	6	65	3.25	3.5	1.1	1.9
9	7	65	3.25	3.5	1.1	1.9
1	8	50	3	5.1	2	1.4
3	9	50	3.5	5.7	1.5	1.7
5	10	50	3.25	5.4	1.8	1.5
11	11	65	3.25	3.5	1.1	1.9

F- factor; R-response.

Table 3: Thorough data table combining the outcomes for the validation parameters of the Cinacalcet HCl HPLC technique.

Validation Parameter	Condition/Value
System Suitability"n"	Retention Time (RT): 2.884 min; Area: 117232.34; Theoretical Plates: 6005-6907; Tailing Factor: 1.179-1.187
Precision (Intra-day)"n"	RT: 2.871 min; Apex Mean: 56391.30; %RSD (RT): 0.30; %RSD (Apex): 1.07
Precision (Inter-day)"n"	RT: 2.865 min; Apex Mean: 63634.65; %RSD (RT): 0.95; %RSD (Apex): 0.95
Linearity	Concentration Range: 2.5-12.5 µg/mL; RT: constant at 2.876 min; R^2 (implied): close to 1.000
Linearity (Area)	2.5 µg/mL: 48157; 5 µg/mL: 117504; 7.5 µg/mL: 189850; 10 µg/mL: 262807; 12.5 µg/mL: 328756
Accuracy (% Recovery)"n"	50%: 99.54-99.67% (Mean: 99.57; %RSD: 0.090); 100%: Mean: 99.05 (%RSD: 0.189); 150%: Mean: 99.69 (%RSD: 0.059)
Robustness - Flow Rate"n"	0.9 mL/min: RT=3.319, Apex=162729, TF=1.19; 1.1 mL/min: RT=2.741, Apex=148631, TF=1.05
Robustness - MP Comp."n"	+5% MP: RT=2.850, Apex=108486, TF=1.1; -5% MP: RT=2.880, Area=120027, TF=1.08
Ruggedness - Day/ Column"n"	RT Mean (Day-1): 2.871; Apex Mean: 56391.30; RT Mean (Day-2): 2.871; Apex Mean: 56826.67
Ruggedness - %RSD"n"	RT: 0.0008-0.007; Area: SD=602.1-768.8; %RSD: RT=0.031%, Area=1.07-1.35

TF: Tailing factor; SD: Standard deviation; RSD: % Relative standard Deviation; "n"=6; (*n*)=3.

dependent variables are shown by the response layer plots. Tailing factor decreases as the acetonitrile concentration increases. The retention time increases as the acetonitrile concentration increases and as the flow rate decreases. The optimal chromatographic conditions are acetonitrile concentration app 79.65% and buffer pH 3.5. These conditions result in a tailing factor of 0.418838 and a retention time of 2.8 min.

System Suitability and Precision

Table 3 showcases the results for system accuracy and appropriateness. With a retention period of 2.871 min intra-day and 2.865 min inter-day, the approach demonstrated remarkable repeatability. With mean areas of 56,391.30 and 63,634.65, respectively, there was very little change in peak area from one day to the next. With an average of 602.1 theoretical plate counts, the column efficiency was good, and the tailing factor was below 1.2, proving that the peaks were symmetrical. Both the retention time and area %RSDs were well within acceptable precision standards, at 0.30% and 0.95%, respectively.

Linearity

The method's linearity can be seen in Table 3 from 2.5 to 12.5 µg/mL. With a linear increase in peak areas from 48,157 to 328,756, the method's robust linear response and dependable quantification over the tested range were confirmed by a constant retention time of 2.876 min for all concentrations. This method has apparent advantages over HPLC methods, which take 5.7 min but have less sensitive linearity ranges, and LC-MS methods, which require more complicated equipment. Shorter retention time and wider linearity range improve sample throughput and analysis.

Accuracy

At three different spike intensities, the accuracy data was offered in Table 3. Mean recoveries were 99.57%, 99.05%, and 99.69% at 50%, 100%, and 150%, respectively, with recovery rates ranging from 98.85% to 99.76%. Cinacalcet was consistently and truthfully recovered from the matrix with %RSD values that were noticeably low (≤0.189%).

Robustness

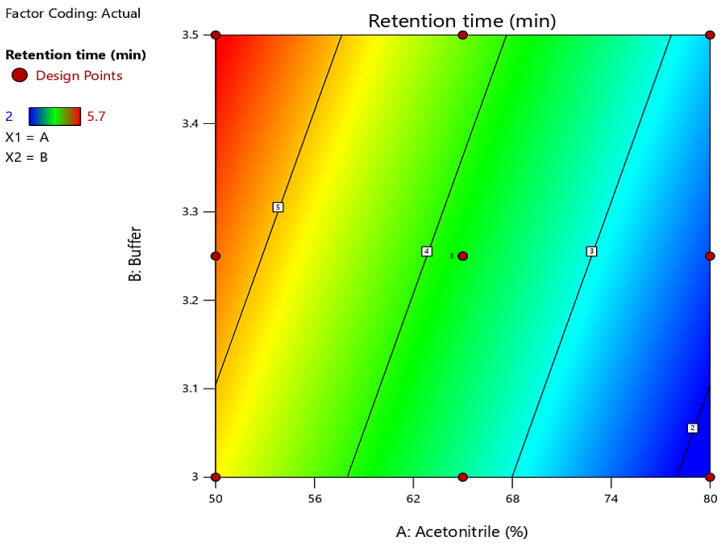
Sturdiness was the main topic of Table 3. Retention time (3.319 min) and tailing factor (1.19 and 1.05, respectively) were slightly affected by switching fluid flow from 0.9 raising to 1.1 mL/min. Likewise, the method's resilience was confirmed when small wavelength adjustments (278 and 280 nm) and ±5% changes in the mobile phase composition resulted in acceptable fluctuations in retention time and area, without affecting the peak form.

Ruggedness

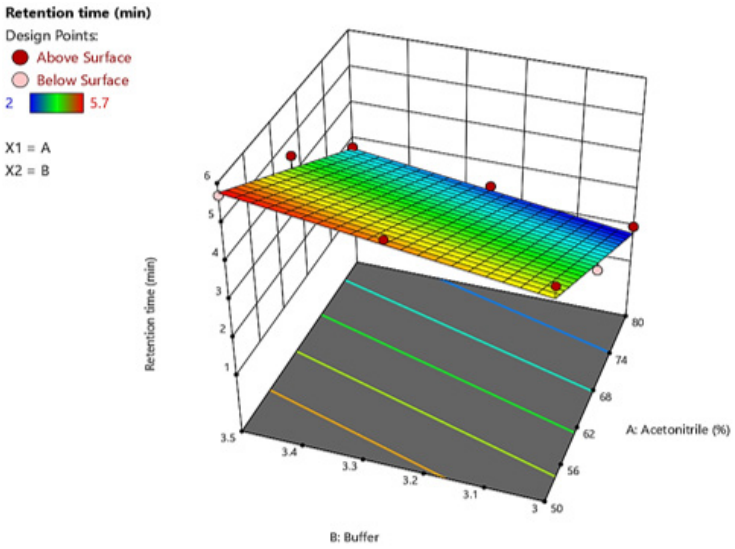
In Table 3, we see how two separate analysts and columns (Kinetex and Luna) performed over the course of two days when it came to toughness. There was little change in the mean peak areas (56,391.30 vs. 56,826.67), while the average retention time was steady at 2.871 min. Underscoring the method's repeatability under diverse settings, the %RSD for retention time was 0.031% and for peak area, it was 1.35%.

Assay of Cinacalcet by HPLC

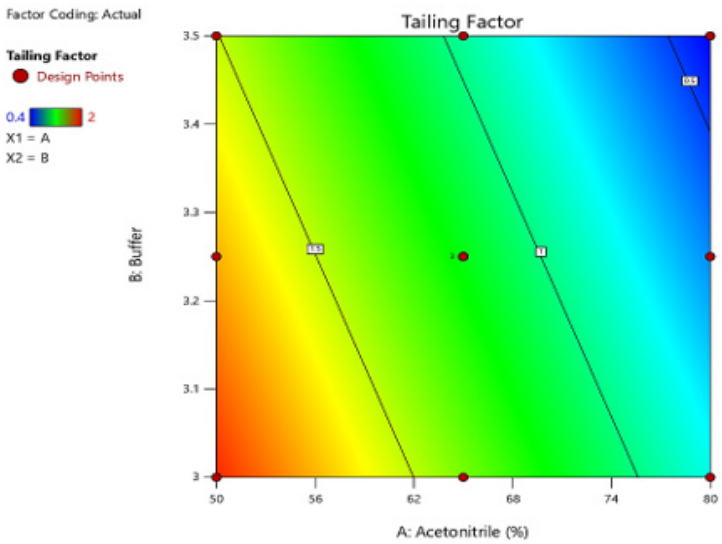
The assay of Cinacalcet in the tablet formulation was determined using a validated HPLC method, yielding a result of 101.17% of the labeled claim. This value was derived by comparing the chromatographic area response of the sample (217311) to that of the standard (217470), and applying correction factors for standard weight (10.1 mg), sample weight (10.2 mg), API purity (99.9%), and tablet weight corresponding to label strength (152 mg vs. 150 mg). The calculated percentage falls well within the acceptable Pharmacopoeial range of 98.0%-102.0%. The low variation between sample and standard responses, along with consistent dilution and weighing accuracy, confirms the integrity of the analytical procedure and the quality of the tested formulation.


Factor Coding: Actual
Retention time (min)
Design Points
2
5.7
X1 = A
X2 = B
Retention time (min)
B: Buffer
A: Acetonitrile (%)

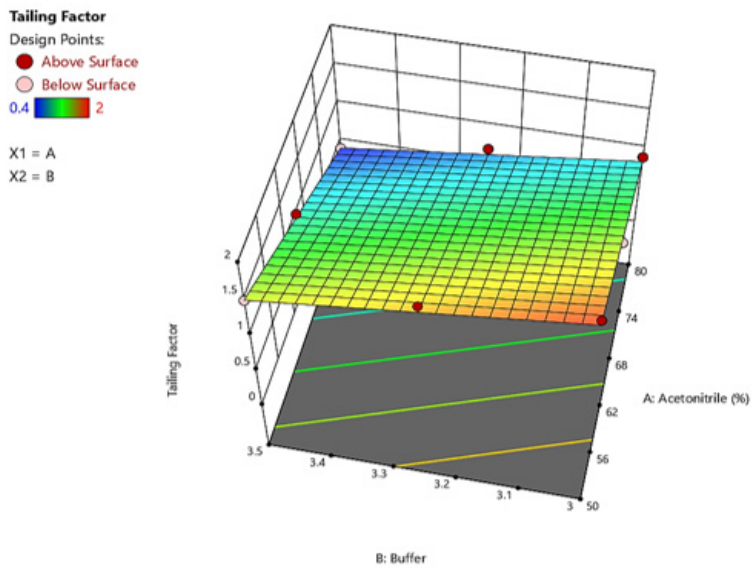
(a)


Retention time (min)
Design Points:
Above Surface
Below Surface
2
5.7
X1 = A
X2 = B
Retention time (min)
A: Acetonitrile (%)
B: Buffer

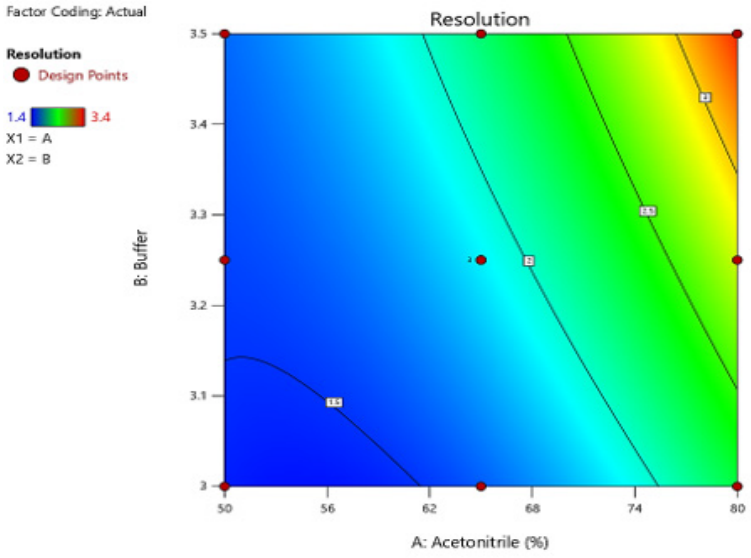
(b)


Factor Coding: Actual
Tailing Factor
Design Points
0.4
2
X1 = A
X2 = B
Tailing Factor
B: Buffer
A: Acetonitrile (%)

(c)


Tailing Factor
Design Points:
Above Surface
Below Surface
0.4
2
X1 = A
X2 = B
Tailing Factor
A: Acetonitrile (%)
B: Buffer

(d)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Resolution
Design Points
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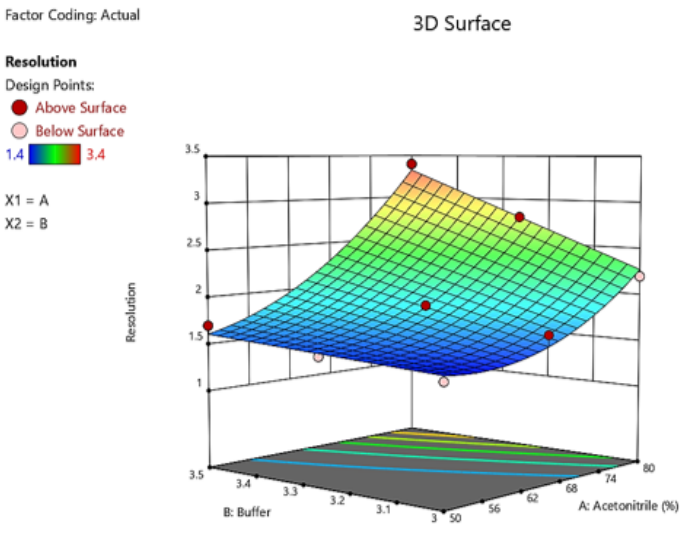
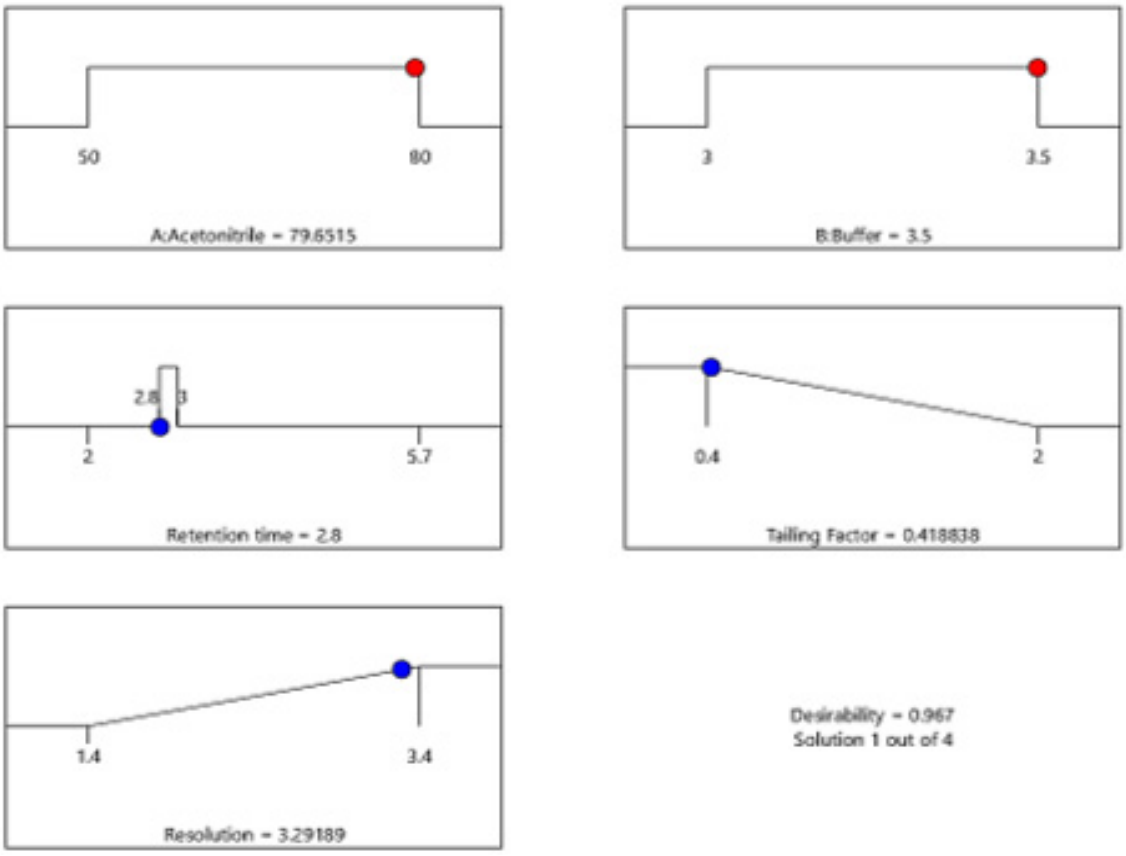
3.4
X1 = A
X2 = B
Resolution
B: Buffer
A: Acetonitrile (%)

(e)

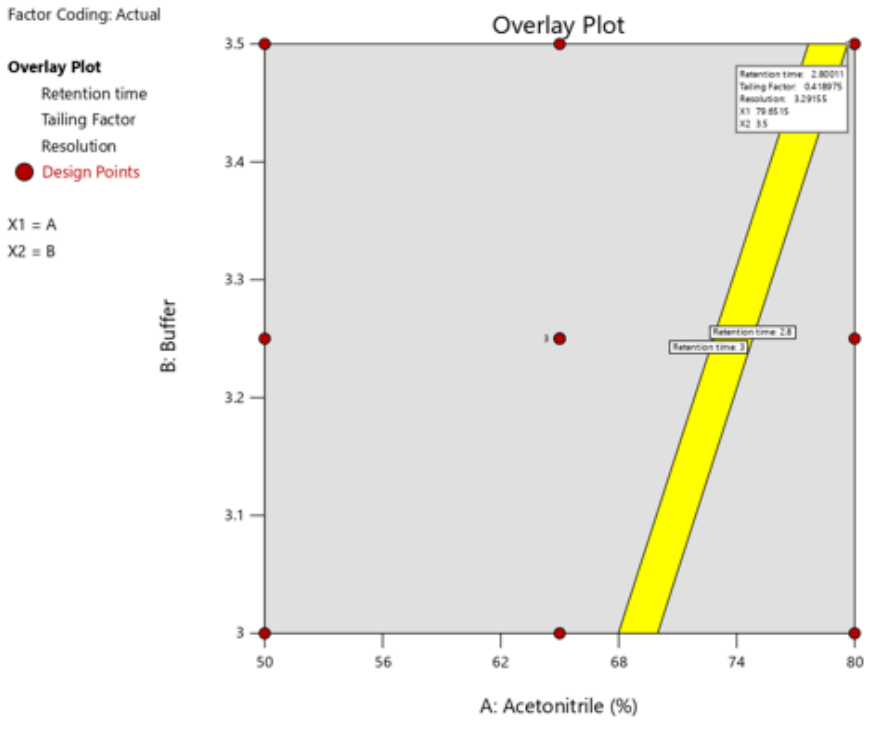

Factor Coding: Actual
3D Surface
Resolution
Design Points:
Above Surface
Below Surface
1.4
3.4
X1 = A
X2 = B
Resolution
B: Buffer
A: Acetonitrile (%)

(f)

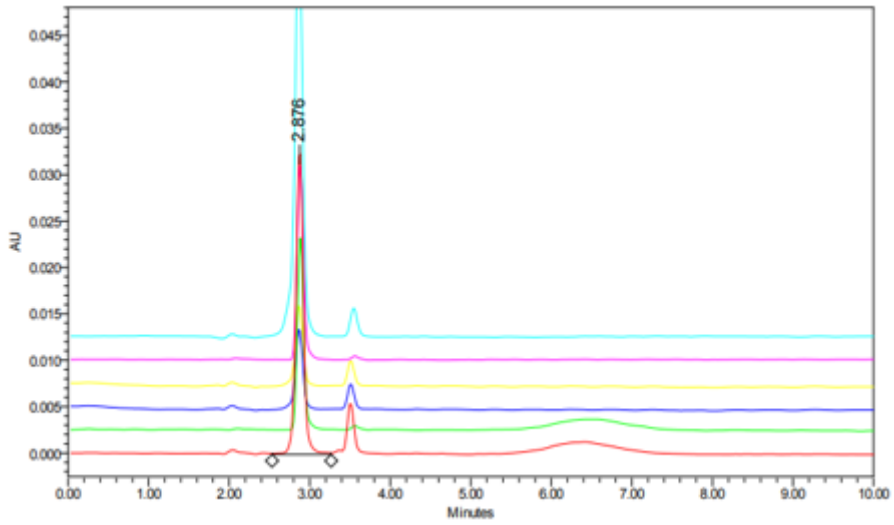


(g)

Figure 2: (a) A contour chart illustrating the effect of the independent variable on retention duration. (b) The impact of a standalone component on retention time as shown in three dimensions: the response region. (c) The influence that an independent component has on the contour plot of the tailing factor. (d) The influence of an independent component on the tailing factor in the three-dimensional response surface. (e)An independent component impact towards the Resolution -contour plot. (f) An independent element's influence on the tailing value in a three-dimensional response surface. (g) Optimized QbD parameters.



(a)



(b)

Figure 3: (a) Operating parameters that are selective, as shown by CCD$ (b) Overlay chromatogram obtained for swabbed CIN$ Central Composite Design.

CONCLUSION

An AQbD approach was used to develop, optimize, and validate a method for estimating Cinacalcet in bulk and formulation. The method meets ICH requirements and is robust, linear, accurate, specific, sensitive, and precise. The criticality of each targeted parameter was determined, and risk-bearing parameters were carefully evaluated. The method was optimized, and the design space was established using a central composite design. To show how the mobile phase composition and the retention factor are related, design expert software was used to create contour plots and 3-D response surface graphs. For examining bulk and formulation quality control samples of Cinacalcet in the pharmaceutical industry, the developed method is suitable. On Analyzing CCD design obtained from QbD it is observed that the effect of buffer Ph is inversely proportional to the tailing factor and effect of buffer Ph and acetonitrile is directly proportional to the retention time and Resolution. The method's verified performance, speed, and robustness make it ideal for industrial Cinacalcet quality control, cleaning validation, and regulatory-compliant batch release.

ACKNOWLEDGEMENT

The Cinacalcet HCl API was provided by Dr. Reddy's, and the authors would like to express their gratitude to them for their assistance in carrying out the project.

ABBREVIATIONS

mL: Millilitre; **g:** Gram; **mg:** Milligram; **µg:** Microgram; **NMT:** Not More Than; **RSD:** Relative Standard Deviation; **VF:** Volumetric Flask; **mcg:** Microgram.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

A Cinacalcet HCl cleaning technique was created, optimized, and validated using Quality by Design (QbD). Method Operable Design Region (MODR) creation and risk assessment were part of Central Composite Design. The best High-Performance Liquid Chromatography (HPLC) method used a 20:80 v/v mobile phase of acetonitrile and potassium dihydrogen orthophosphate buffer (pH 3.5), a flow rate of 1 mL/min for 10 min, a Kinetex C18 column at 25°C, and detection at 279 nm. QbD parameters were modified for 11 runs: retention time, tailing factor, and resolution were dependent, while buffer pH and mobile phase composition were independent. CCD models showed statistical significance for optimizing retention time (p-values <0.0500, signal-to-noise ratio 28.846), tailing factor (p-values <0.0500, 39.082), and resolution (p-values <0.0500, 25.246). Try a buffer pH of 3.5 and acetonitrile concentration of 79.65% for optimum results. This yields 0.418838 tailing factor and 2.8 min retention. This method passed all validation tests. System suitability was indicated by a tailing factor <1.2, high column efficiency (602.1 theoretical plates), and a relative %RSD of 0.30% for 6 Cinacalcet injections. Using a constant retention period of 2.876 min, linearity ranged from 0.25 to 12.5 µg/mL with a r^2 of 0.999 Peak areas increased linearly from 48,157 to 328,756. Precision's %RSD was 0.95% between days and 1.07% within. Mean recoveries were 99.57%, 99.05%, and 99.69% for 50%, 100%, and 150% spike intensities, while accuracy ranged from 0.059% to 0.189% %RSD. Robustness tests (57 and 77) showed acceptable changes in retention duration and area when varying flow rate (±0.1 mL/min), mobile phase composition (60:40 and 70:30), wavelength (278 and 280 nm), and column temperature (±5°C). Retention length and peak area

had %RSDs of 0.031% and 1.35%, respectively, demonstrating ruggedness across analysts, columns, and days. These limits of detection were 0.244 and 0.740 μg mL^{-1}, respectively. Cinacalcet tablets tested 101.17% of the advertised amount. Finding that buffer pH is directly proportional to retention time and resolution and inversely proportional to tailing factor, the study optimized the HPLC Cinacalcet quantification method utilizing QbD.

REFERENCES

1. Padhi D, Harris R. Clinical pharmacokinetic and pharmacodynamic profile of cinacalcet hydrochloride. ClinPharmacokinetic. 2009;48(5):303-11. doi:10.2165/00003088-200948050-00002
2. National Center for Biotechnology Information. PubChem Compound Summary for CID 156418, cinacalcet hydrochloride.
3. Mostafa GA, Al-Badr AA. Cinacalcet hydrochloride. In: Profiles of Drug Substances, Excipients and Related Methodology. Academic Press; 2007;42:1-90.
4. Soni P, Patel D, Patel H, Patel M. Development and validation of RP-HPLC method for simultaneous estimation of Cinacalcet hydrochloride and Calcium carbonate in pharmaceutical dosage form. J Anal Sci Technol. 2015;6:20. doi:10.1186/s40543-015-0067-1.
5. Raj Abhishek. Cleaning validation in pharmaceutical industries. J Atoms Molecules. 2014;4(4):779-83.
6. Lakshmana S, Prabhu. Cleaning validation and its importance in pharmaceutical industry. Pharma Times. 2010;42(7):21-5.
7. Panda SS, Rath J, Bera VVRK. QbD-driven development and validation of UV spectrophotometric method for estimation of paliperidone in extended release tablet dosage form. Anal Chem Lett. 2018;8(4):510-18. doi:10.1080/22297928.2018.1446845
8. Manjula A, Chandana-Deepthi G, Vijayaraj S. Spectrophotometric determination of cinacalcet hydrochloride in bulk. Int J Pharm Rev Res. 2012;2(2):111-18.
9. Maruthi R, Chandan RS, Barath M, Datta GN, D'Silva M, Kumari FK, Ahmad F, Geetha R. Analytical method development and validation of teneligliptin by UV spectroscopy. Res J Pharm Technol. 2021;14(1):75-8. doi:10.5958/0974-360X.2021.00014.7
10. Kumar D, Nallagatla VR, Vagolu S, *et al.* Analytical method development and validation for the estimation of cinacalcet hydrochloride in bulk and tablet dosage form by UV spectroscopy. Int J Pharm Sci Res. 2012;3(7):2304-8.
11. Sreejith KR, Rajagopal PL, Premaletha K. Analytical method development and validation of anastrozole in pure and tablet dosage form by UV spectroscopy. Res J Pharm Technol. 2017;10(4):1015-19. doi:10.5958/0974-360X.2017.00183.4
12. Sumithra M, Yuvanesh P, Mistry A. Analytical method development and validation of ambroxol hydrochloride by UV spectroscopy and forced degradation study and detection of stability. Res J Pharm Technol. 2016;9(7):794-800. doi:10.5958/0974-360X.2016.00152.9
13. Panda SS, Rath J, Bera VVRK. QbD-driven development and validation of UV spectrophotometric method for estimation of paliperidone in extended release tablet dosage form. Anal Chem Lett. 2018;8(4):510-18. doi:10.1080/22297928.2018.1446845
14. Soni P, Patel D, Patel H, Patel M. Development and validation of RP-HPLC method for simultaneous estimation of cinacalcet hydrochloride and calcium carbonate in pharmaceutical dosage form. J Anal Sci Technol. 2015;6:20. doi:10.1186/s40543-015-0067-1
15. Chandrasekhar KB, Rao JR, Kumar BG, Rao JV. Development and validation of a stability-indicating HPLC method for cinacalcet hydrochloride and its related impurities in bulk drug and dosage forms. J Liq Chromatogr Relat Technol. 2015;38(16):1608-15. doi:10.1080/10826076.2015.1061885
16. Gawai AA, Charhate K, Shaikh F, Khan A, Chaubey A, Biyani KR. RP-HPLC method development and validation for hyperlipidemic agent atorvastatin in pharmaceutical dosage form. Res J Pharm Technol. 2017;10(6):1780-7.
17. Chundawat RS, Sarangdevot YS, Rathore RPS, Sisodiya DS, Rathore US. Method development and validation for simultaneous estimation of montelukast and fexofenadine in pharmaceutical dosage form by HPLC. Res J Pharm Technol. 2013;6(10):1102-6.
18. Naik A, Nazareth C, Gaonkar SN. Cleaning method development and validation for simultaneous estimation of olmesartan and atorvastatin by HPLC. Res J Pharm Technol. 2020;13(5):2125-8. doi:10.5958/0974-360X.2020.00382.0
19. Parida AK, Rao KS, Patnaik AK. A novel validated RP-HPLC method for the estimation of Ticagrelor in bulk and pharmaceutical dosage forms. Res J Pharm Technol. 2018;11(3):867-72. doi:10.5958/0974-360X.2018.00161.0
20. Murugan S, Chandrasekhar R. Method development and validation of Fingolimod in bulk and tablet dosage form by RP-HPLC. Res J Pharm Technol. 2017;10(8):2573-6. doi:10.5958/0974-360X.2017.00456.5
21. Pai NR, Sawant SS. Development and validation of RP-HPLC method for estimation of Olmesartan medoxomil in tablet dosage form. Res J Pharm Technol. 2013;6(11):1279-84.
22. Tanuja A, Ganapaty S, Murthy VSN. Stability-indicating RP-HPLC method development and validation for solifenacin succinate in bulk and pharmaceutical formulation. Res J Pharm Technol. 2021;14(7):3509-14. doi:10.52711/0974-360X.2021.00608
23. Waghmare SA, Sumithra M. QbD based development and validation of RP-HPLC method for Nintedanib esylate: application to bioanalytical and stability study in plasma. Anal Chem Lett. 2021;11(3):392-408. doi:10.1080/22297928.2021.1930581
24. Yahya BA, Bawazir AS, Sangshetti JN, Baig SS, Shaikh SS. QbD-based development and validation of an efficient RP-HPLC method for estimation of abiraterone acetate in bulk, tablet, and nano-formulation. Anal Chem Lett. 2021;11(1):112-30. doi:10.1080/22297928.2021.1888794
25. Jagan Mohan TSS, Jogia HA, Mukkanti K. A stability-indicating UHPLC method for simultaneous estimation of perindopril and indapamide in presence of impurities: application of QbD for robustness study. Anal Chem Lett. 2020;10(4):477-97. doi:10.1080/22297928.2020.1817776
26. Sanap SN, Bisen AC, Choudhury AD, Verma SK, Kumar M, Bhatta RS. A QbD driven approach for development and validation of an RP-UHPLC method for simultaneous estimation of ofloxacin and fluconazole: application to pharmaceutical analysis. Anal Chem Lett. 2022;12(3):310-21. doi:10.1080/22297928.2022.2063069
27. Vyas AJ, Gol D, Patel AI, Patel AB, Patel NK, Chavda JR, Lumbhani A, Chudasama A. Implementing analytical quality by design (AQbD) approach for simultaneous estimation of tadalafil and macitentan by RP-HPLC method. Anal Chem Lett. 2021;11(4):539-52. doi:10.1080/22297928.2021.1938215
28. Ramalingam P, Bhadraya K, Reddy YP. Analytical quality by design: a tool for regulatory flexibility and robust analytics. Int J Anal Chem. 2015; 2015:868727. doi:10.1155/2015/868727
29. Thota N, Chowdary KP, Kanumula NV, Naveen Kumar M, Kantevari S. Development and validation of an LC-MS/MS method for quantification of cinacalcet in human plasma and its application to pharmacokinetic study. Biomed Chromatogr. 2015;29(5):795-802. doi:10.1002/bmc.3342
30. Sahu RK, Kumar S, Kumar A, *et al.* Development and validation of an LC-MS/MS method for quantification of cinacalcet in rat plasma: application to pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci. 2015;1000:71-77. doi:10.1016/j.jchromb.2015.07.038
31. Farnoudian-Habibi A, Jaymand M. Development and validation of a quantitative assay for the determination of cinacalcet and its main metabolites in human plasma using RP-HPLC method. Microchem J. 2017;130:377-83. doi:10.1016/j.microc.2016.10.017
32. Patel K, Shah UA, Joshi HV, Patel JK, Patel CN. QbD-stressed development and validation of a stability-indicating RP-HPLC method for simultaneous estimation of linagliptin and metformin HCl in pharmaceutical dosage form. Res J Pharm Technol. 2022;15(5):1917-23. doi:10.52711/0974-360X.2022.00319
33. Reddy NB, Reddy KG, Reddy KJ, Srinivas NR. Rapid and sensitive determination of cinacalcet in human plasma by LC-MS/MS: application to a bioequivalence study. J Chromatogr B Analyt Technol Biomed Life Sci. 2012;897:128-33. doi:10.1016/j.jchromb.2012.03.033
34. Tegegne HA, Raju KVSN, Reddy PVGK, Kumar MK, Annapurna MM. Development and validation of a rapid and sensitive LC-MS/MS method for quantification of cinacalcet in human plasma. Biomed Chromatogr. 2018; 32(4)\:e4134. doi:10.1002/bmc.4134
35. Tella SR, Mohan GM, Ravi TK, Reddy PV, Reddy PV. Development and validation of LC-MS/MS method for determination of cinacalcet in rat plasma and its application to pharmacokinetic studies. Lat Am J Pharm. 2013;32(10):1548-55.
36. Kancherla P, Srinivas K, Alegate P, Albaseer SS. LC-MS/MS characterization of forced degradation products of repaglinide quality by design approach for stability-indicating LC method development and validation for degradation products. Anal Chem Lett. 2020;10(6):740-57. doi:10.1080/22297928.2020.1861975
37. Kumar PMV, Rao AS. Synergistic spectrophotometric strategies for precise sartans quantification in pharmaceuticals: unifying UV derivative and AUC approaches with solvent consistency. SEEJPH. 2025;26:3349-63. doi:10.70135/seejph.vi.5377
38. Remidicherla SS, Chakravarthi G, Malothu N. A validated LC-MS/MS method for determination at trace level of nitrosamine impurities in doxofylline API. Indian J Pharm Educ Res. 2024;58(3):1-9.
39. Richard P, Vothani S, Theerthala S, Boddu K, Vasanth PM. Strengthening Finerenone assessment: a central composite design viewpoint for liquid chromatographic method development. Indian J Pharm Educ Res. 2024;59(4):1-10.
40. Remidicherla SS, Chakravarthi G, Reddy A, Rao GK. Method development and validation for quantification of imatinib mesylate spiked in *in vitro* saliva by LC-MS/MS. Indian Drugs. 2024;61(4):46-56.
41. Swetha Sri R, Bhavya Sri K, Mounika C. A review on comparative study of HPLC and UPLC. Res J Pharm Technol. 2020;13(3):1570-4. doi:10.5958/0974-360X.2020.00284.X
42. Nithisha M, Ashwini P, Swetha Sri R. Implementation of hydrotropic solvents for UV spectrophotometric assessment of esomeprazole-loaded microspheres: greenness evaluation. Asian J Pharm Technol. 2024;14(2):123-7. doi:10.52711/2231-5713.2024.00022
43. Swetha Sri R, Pravallika S, Chakravarthy G, Bhavya Sri K, Sumakanth M. Cleaning method development and validation by UV method for quantitative assessment of favipiravir residue in manufacturing area. Ann Phytomed. 2022;S1-S6. doi:10.54085/ap.trips.2022.11.1.0

44. Anusha T, Prasanthi R, Tirunagari M, Buggana SJ, Mitta C, Remidicherla SS. QbD-driven approach to cleaning method development and validation for darunavir analysis in oral films. Int J Pharm Qual Assur. 2024;15(3):1575-80.
45. Remidicherla SS, Chakravarthi G, Malothu N, Reddy AR. A comprehensive LC-MS/MS method for detecting genotoxic nitrosamine impurities in favipiravir API. Indian J Pharm Educ Res. 2025; 59(1s)\:s256-63.
46. Remidicherla SS, Chakravarthi G, Madhavi A, Malothu N. Development and validation of an LC-MS/MS method for nitrosamine impurity detection in tamsulosin hydrochloride. Int J App Pharm. 2025;17(2):432-40.
47. Asha BR, Goudanavar P, Rao GSN, Gandla K, Naveen R, Majeed S, Muthukumarasamy R. QbD-driven targeted pulmonary delivery of dexamethasone-loaded chitosan microspheres: biodistribution and pharmacokinetic study. Saudi Pharm J. 2023;31(9):101711. doi:10.1016/j.jsps.2023.101711
48. Mayasa V, Patil AS, Gandla K, Nelson VK, Repudi L. Phytochemical evaluation and screening of anti-depressant and anxiolytic activities of *Bacopa monnierain* rats. Res J Pharm Technol. 2024;17(6):2587-91. doi:10.52711/0974-360X.2024.00404
49. Shareef I, Gandla K. Stability-indicating UPLC method development, validation, and forced degradation studies of sulfamethoxazole and clindamycin in bulk and formulated dosage forms. Saudi J Med Pharm Sci. 2025;11(5):437-43. doi:10.36348/sjmps.2025.v11i05.012.

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