

Risk Based Identification and Experimental Optimisation of Critical Parameters in a Stability Indicating RP HPLC Method Using Design of Experiments

Jaipal Singh Patel¹, Prof (Dr.) Sushila Kalra²,
Prof (Dr.) Neetesh Kumar Jain³

¹Research Scholar, Department of Pharmacy, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India

²Supervisor, Department of Pharmacology, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India

³Co-Supervisor, Department of Pharmacology and Toxicology Modern Institute of Pharmaceutical Sciences, Indore ((NBA Accredited), Visiting Professor Centro Escolar University, Manila Philippines

Abstract:

Robust chromatographic performance depends on understanding how method parameters, their interactions, and normal operational variability influence analytical outputs. This paper presents a risk-based Design of Experiments strategy for identifying and optimising critical parameters in a stability-indicating RP-HPLC procedure for simultaneous analysis of metformin hydrochloride and dapagliflozin in combined tablets. An Ishikawa analysis organised potential variability across materials, method, instrument, measurement, analyst, and environment. Failure Mode and Effects Analysis prioritised buffer pH, initial acetonitrile proportion, gradient time, column chemistry, and flow rate. A screening design was used to distinguish influential factors from low-risk variables, followed by a three-factor central composite response-surface design examining buffer pH, initial acetonitrile, and gradient time. Responses included critical-pair resolution, peak tailing, and runtime. Illustrative quadratic modelling indicated significant linear, interaction, and curvature effects, with initial organic proportion exerting the strongest negative influence on critical resolution and gradient time exerting a positive effect within the studied region. Model diagnostics supported predictive use within the experimental domain. Multiple-response optimisation identified pH 3.20, initial acetonitrile 20.0%, and gradient time 6.05 min as a normal operating point, with predicted critical resolution of 2.31, tailing of 1.13, and cycle time of 10.5 min. Confirmation runs showed close agreement with model predictions. The study demonstrates how risk tools and DoE can convert empirical method adjustment into documented knowledge suitable for an analytical control strategy. All numerical findings are illustrative and must be replaced with authenticated experimental data before publication.

Keywords: Analytical Quality by Design; Design of Experiments; FMEA; Ishikawa analysis; response-surface methodology; RP-HPLC; critical method parameters; stability-indicating method

1 Introduction:

A chromatographic procedure is reliable only when it continues to meet its intended analytical purpose despite unavoidable small changes in preparation, equipment, environment, and materials. Traditional one-factor-at-a-time experiments can identify a locally acceptable condition, but they do not estimate interactions efficiently and may obscure curvature. For a stability-indicating method, this limitation is serious because selectivity can deteriorate even when retention time and peak appearance seem acceptable.

Analytical Quality by Design places method development within a science- and risk-based lifecycle. ICH Q14 describes an enhanced approach in which prior knowledge and risk assessment can guide multivariate experimentation, parameter ranges, robustness evaluation, and the analytical procedure control strategy. ICH Q2(R2) links validation to intended purpose, while ICH Q9(R1) emphasises systematic risk identification, analysis, control, communication, and review. The purpose of DoE is not to decorate a final method with statistical plots; it is to quantify relationships that improve decisions and reduce the probability of reporting an incorrect result.

2 Objectives:

1. Map potential sources of analytical variability through an Ishikawa analysis.
2. Prioritise failure modes using severity, occurrence, and detectability scoring.
3. Screen candidate parameters and identify those with material effects on method performance.
4. Develop quadratic response-surface models and evaluate their adequacy using ANOVA and diagnostic statistics.
5. Interpret significant interactions, identify a robust operating region, and confirm the selected normal operating point.

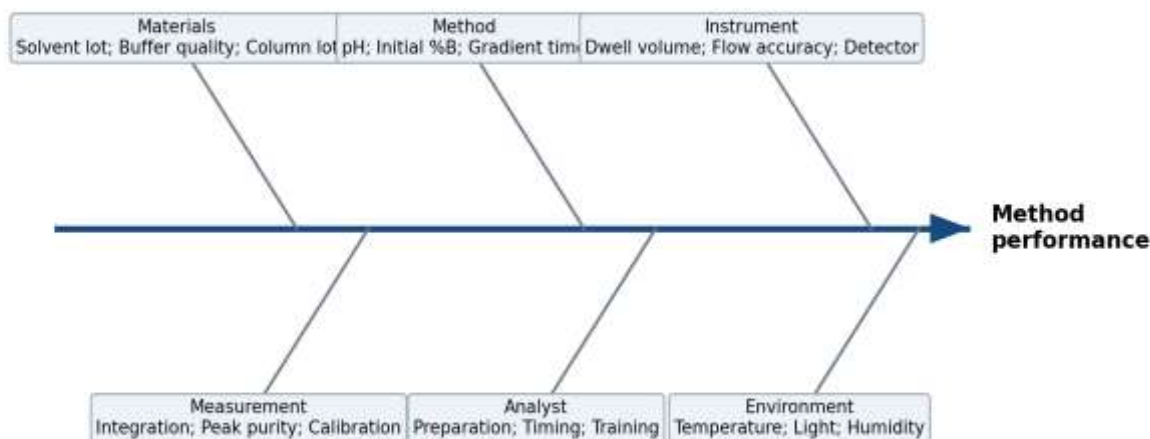
3 Materials and Methods:

The model procedure was intended for simultaneous assay and stability-indicating evaluation of metformin hydrochloride 500 mg and dapagliflozin 10 mg immediate-release tablets. A C18 column, phosphate-buffer-acetonitrile gradient, UV detection near 225 nm, and a target cycle below 12 min formed the preliminary platform. The key performance requirements were critical-pair resolution not less than 1.80, tailing factor not more than 1.50, adequate efficiency, and reproducible retention within a validated reportable range.

A cross-functional assessment considered sources that could influence sample preparation, chromatographic separation, detection, integration, and the reportable result. Factors were recorded without prematurely labelling all of them critical. Each causal pathway was linked to a potential performance failure, such as co-elution, excessive tailing, low response, extraction bias, or unstable retention.

Figure 1:

Ishikawa Map of Potential Sources of RP HPLC Variability



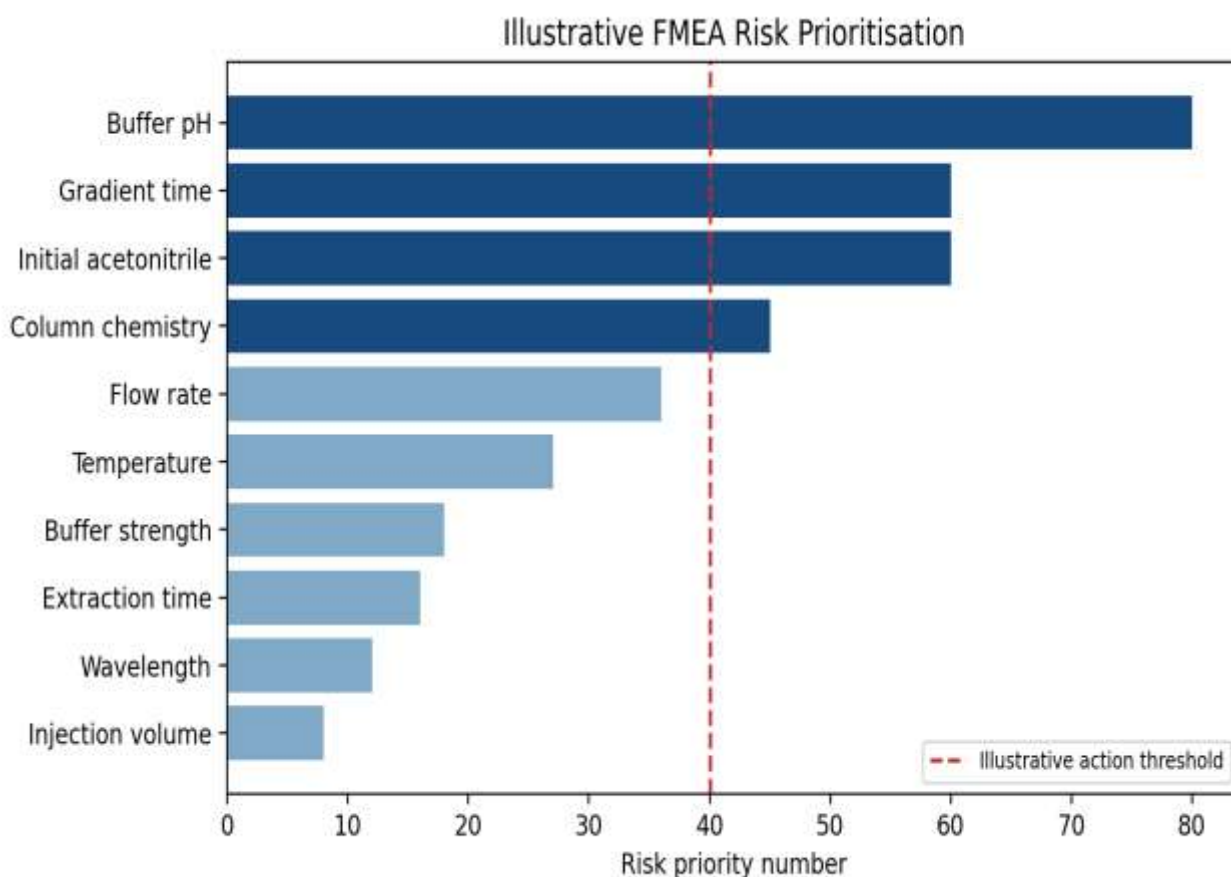
Failure Mode and Effects Analysis:

Each candidate factor was assigned an illustrative score from 1 to 5 for severity, occurrence, and detectability. Risk priority number was calculated as $RPN = S \times O \times D$. High RPN did not by itself prove criticality; it identified where additional knowledge or control was needed. Scores were documented with rationale and were intended to be revised after experimental evidence became available.

Factor	Severity	Occurrence	Detectability	RPN	Initial action
Buffer pH	5	4	4	80	Experimental study
Initial acetonitrile	5	4	3	60	Experimental study
Gradient time	5	3	4	60	Experimental study
Column chemistry	5	3	3	45	Experimental study
Flow rate	4	3	3	36	Control or verify
Temperature	3	3	3	27	Control or verify
Buffer strength	3	2	3	18	Control or verify
Injection volume	2	2	2	8	Control or verify
Wavelength	3	2	2	12	Control or verify

Factor	Severity	Occurrence	Detectability	RPN	Initial action
Extraction time	4	2	2	16	Control or verify

Figure 2 Illustrative FMEA risk prioritisation:



Screening Design:

A two-level fractional factorial screening design with centre points was proposed for buffer pH, initial acetonitrile percentage, gradient time, flow rate, temperature, and buffer strength. Runs were randomised to reduce time-related bias. Centre points assessed short-term repeatability and provided an initial indication of curvature. Column chemistry was evaluated as a prior categorical scouting factor because changing the stationary-phase family could alter the separation mechanism more fundamentally than a small quantitative adjustment.

Factor	Low level	Centre	High level	Screening conclusion
A Buffer pH	3.05	3.20	3.35	Retained
B Initial acetonitrile	18.5%	20.0%	21.5%	Retained
C Gradient time	5.70 min	6.05 min	6.40 min	Retained

Factor	Low level	Centre	High level	Screening conclusion
D Flow rate	0.90 mL/min	1.00 mL/min	1.10 mL/min	Controlled; lower effect
E Temperature	27 degrees C	30 degrees C	33 degrees C	Controlled; lower effect
F Buffer strength	15 mM	20 mM	25 mM	Fixed after low effect

Illustrative screening effects showed that pH, initial acetonitrile, and gradient time materially influenced critical resolution. Flow altered runtime and pressure but did not meaningfully improve selectivity within the studied interval. Temperature and buffer strength showed smaller effects and were fixed at practical centre conditions for response-surface optimisation.

Response Surface Design:

A rotatable central composite design was selected for three quantitative factors. Coded levels included factorial points at -1 and +1, axial points at plus or minus 1.682, and six replicated centre points, giving 20 runs. The factors were A, buffer pH; B, initial acetonitrile percentage; and C, gradient time. Critical-pair resolution, tailing factor, and runtime were modelled. Factor transformations were $\text{pH} = 3.20 + 0.15A$, $\text{initial acetonitrile} = 20.0 + 1.5B$, and $\text{gradient time} = 6.05 + 0.35C$ min.

Run	A	B	C	pH	Initial B %	Gradient min	Resolution	Tailing	Runtime min
1	-1	-1	-1	3.050	18.50	5.70	1.90	1.17	10.33
2	1	-1	-1	3.350	18.50	5.70	1.59	1.28	10.39
3	-1	1	-1	3.050	21.50	5.70	1.91	1.18	10.05
4	1	1	-1	3.350	21.50	5.70	1.20	1.45	10.11
5	-1	-1	1	3.050	18.50	6.40	2.44	1.10	10.89
6	1	-1	1	3.350	18.50	6.40	2.49	1.22	10.95
7	-1	1	1	3.050	21.50	6.40	1.97	1.12	10.61
8	1	1	1	3.350	21.50	6.40	1.64	1.41	10.67
9	-1.68	0	0	2.948	20.00	6.05	2.16	1.15	10.45
10	1.68	0	0	3.452	20.00	6.05	1.65	1.50	10.55
11	0	-1.68	0	3.200	17.48	6.05	2.19	1.16	10.74
12	0	1.68	0	3.200	22.52	6.05	1.47	1.32	10.26
13	0	0	-1.68	3.200	20.00	5.46	1.59	1.18	10.03
14	0	0	1.68	3.200	20.00	6.64	2.42	1.09	10.97

Run	A	B	C	pH	Initial B %	Gradient min	Resolution	Tailing	Runtime min
15	0	0	0	3.200	20.00	6.05	2.31	1.13	10.50
16	0	0	0	3.200	20.00	6.05	2.32	1.13	10.50
17	0	0	0	3.200	20.00	6.05	2.30	1.13	10.50
18	0	0	0	3.200	20.00	6.05	2.31	1.13	10.50
19	0	0	0	3.200	20.00	6.05	2.32	1.13	10.50
20	0	0	0	3.200	20.00	6.05	2.30	1.13	10.50

Statistical Analysis:

A second-order polynomial was fitted to each response: $Y = \text{beta0} + \text{betaA} A + \text{betaB} B + \text{betaC} C + \text{betaAB} AB + \text{betaAC} AC + \text{betaBC} BC + \text{betaAA} A^2 + \text{betaBB} B^2 + \text{betaCC} C^2$. Terms were evaluated using ANOVA at an illustrative alpha level of 0.05. Model hierarchy was preserved when interactions or quadratic terms were retained. Adequacy was assessed using model significance, lack of fit, adjusted and predicted R squared, adequate precision, residual behaviour, influence diagnostics, and agreement between predicted and confirmation results.

4 Results:

4.1 FMEA Outcome and Risk Reduction:

The highest initial RPN was assigned to buffer pH (80), followed by initial acetonitrile (60), gradient time (60), and column chemistry (45). These factors had plausible direct effects on ionisation, retention, selectivity, and critical-pair separation. Flow rate and temperature were moderate risks because they could change efficiency and runtime, while injection volume and wavelength were lower under the defined conditions. After experimental evaluation, risk was controlled through fixed settings, justified ranges, system suitability, column qualification, and preparation instructions rather than by reducing scores without evidence.

4.2 Screening Results:

The screening model ranked initial organic proportion, gradient time, and pH as the dominant contributors to critical-pair resolution. Increasing initial acetonitrile reduced retention and compressed the early separation. Increasing gradient time improved resolution within the studied range, although excessively long gradients increased cycle time. Higher pH increased tailing and altered selectivity. The interaction pattern suggested that the effect of pH depended partly on organic composition, supporting response-surface investigation rather than isolated one-factor adjustment.

4.3 Quadratic Model for Critical Resolution:

In coded units, the illustrative fitted relationship was: $\text{Resolution} = 2.31 - 0.16A - 0.21B + 0.24C - 0.10AB + 0.08AC - 0.12BC - 0.14A^2 - 0.17B^2 - 0.11C^2$. The negative B coefficient confirmed that a higher initial organic proportion reduced critical resolution. The positive C coefficient showed that a longer gradient improved separation near the centre. Negative quadratic terms indicated curvature and a local optimum rather than indefinite improvement at an extreme setting.

Source	df	Sum of squares	Mean square	F value	p value
Model	9	2.184	0.2427	92.6	<0.0001
A pH	1	0.349	0.3490	133.2	<0.0001
B Initial organic	1	0.602	0.6020	229.8	<0.0001
C Gradient time	1	0.786	0.7860	300.0	<0.0001
AB	1	0.080	0.0800	30.5	0.0003
AC	1	0.051	0.0510	19.5	0.0013
BC	1	0.115	0.1150	43.9	<0.0001
Quadratic terms	3	0.201	0.0670	25.6	<0.0001
Residual	10	0.026	0.00262		
Lack of fit	5	0.018	0.00360	2.25	0.196
Pure error	5	0.008	0.00160		
Total	19	2.210			

The illustrative model R squared was 0.988, adjusted R squared 0.977, and predicted R squared 0.944. The difference between adjusted and predicted values was small, and lack of fit was not significant relative to pure error. Adequate precision exceeded the usual signal-to-noise benchmark. These statistics supported interpolation inside the studied domain, not extrapolation beyond it.

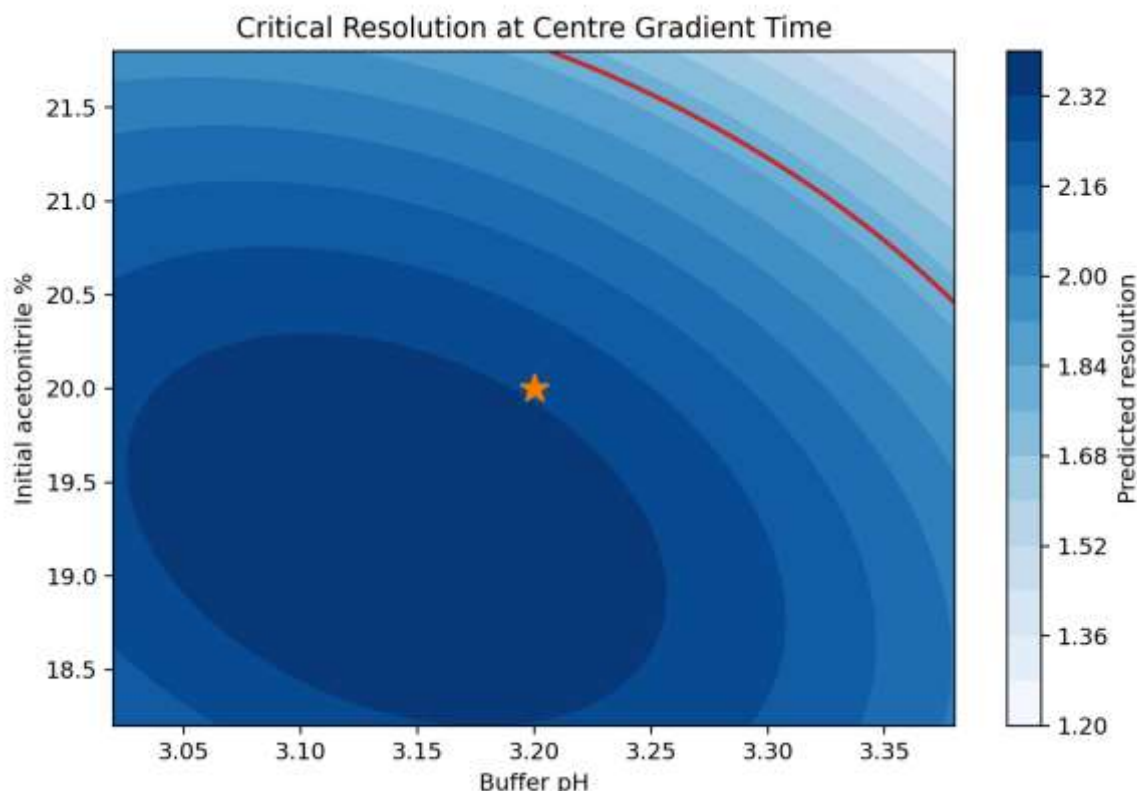
4.4 Models for Tailing and Runtime:

Tailing was described by: $\text{Tailing} = 1.13 + 0.10A + 0.05B - 0.03C + 0.04AB + 0.07A^2 + 0.04B^2$. pH had the largest effect, consistent with changes in ionisation and residual silanol interaction. Runtime was adequately represented by a linear model: $\text{Runtime} = 10.50 + 0.03A - 0.14B + 0.28C$. A longer gradient increased runtime, while higher initial organic reduced it. The optimisation therefore required a compromise between resolution and throughput.

4.5 Factor Interactions

The AB interaction showed that the adverse effect of increasing initial organic was greater at higher pH. The BC interaction indicated that extending gradient time partially compensated for resolution lost at higher initial organic, but the benefit diminished toward the edge of the region. The AC interaction was smaller but still meaningful: gradient time influenced separation differently across the pH range. These findings explain why independent factor ranges cannot always be combined safely without considering their joint effects.

Figure 3 Predicted critical resolution across pH and initial organic composition



Note. The red contour represents predicted resolution of 1.80 at the centre gradient time. The star marks the illustrative normal operating point.

4.6 Model Diagnostics:

Studentised residuals were distributed without a systematic relationship to predicted response or run order in the illustrative dataset. The normal probability pattern was acceptably linear, and no point exceeded the usual influence threshold. Replicated centre points produced a small pure-error estimate. Diagnostic interpretation remained response specific: strong R squared alone was not considered evidence of adequacy, and terms were not removed solely to increase adjusted R squared.

4.7 Multiple Response Optimisation:

Desirability functions were assigned to maximise critical resolution, keep tailing below 1.50, and minimise runtime while retaining practical conditions. Resolution received the greatest importance because loss of stability-indicating selectivity posed the highest analytical risk. The composite desirability reached an illustrative value of 0.91 near pH 3.20, initial acetonitrile 20.0%, and gradient time 6.05 min. This point was intentionally placed away from model boundaries to improve tolerance to routine variation.

4.8 Method Operable Region and Normal Operating Point:

The provisional predicted region satisfying resolution at least 1.80, tailing not more than 1.50, and cycle time not more than 12 min extended approximately across pH 3.05-3.35, initial acetonitrile 18.5-21.5%, and gradient time 5.70-6.40 min, subject to interactions. A narrower routine working region of pH 3.15-3.25, initial acetonitrile 19.5-20.5%, and gradient time 5.90-6.20 min was proposed. This distinction preserves operational discipline: a statistical feasible region is not automatically an approved MODR, and authentic validation and regulatory assessment are required.

4.9 Confirmation Experiments:

Response	Predicted	Illustrative observed mean	Relative prediction error	Criterion	Assessment
Critical resolution	2.31	2.29	-0.87%	Within 5% and ≥ 1.80	Meets
Tailing factor	1.13	1.15	1.77%	Within 5% and ≤ 1.50	Meets
Cycle time	10.50 min	10.54 min	0.38%	Within 5% and ≤ 12 min	Meets

Three illustrative confirmation preparations at the normal operating point produced results close to predictions. Confirmation should also include selected edge or worst-case combinations and stressed samples. Agreement at the centre establishes local predictive adequacy but does not validate every point in a proposed operating region.

5 Discussion:

The combined Ishikawa-FMEA-DoE sequence created a defensible chain from potential cause to evidence and control. Ishikawa analysis encouraged broad identification of variability. FMEA then prioritised investigation, while screening reduced the factor set efficiently. Response-surface modelling quantified curvature and interactions that would have been difficult to recognise through one-factor experiments. The result was not merely an optimised chromatogram; it was a structured explanation of how operating conditions affect method performance.

Risk scores remain subjective unless supported by defined scales, multidisciplinary reasoning, and periodic review. RPN also has mathematical limitations: different combinations of severity, occurrence, and detectability can produce the same product. High-severity risks should therefore not be ignored because their RPN is numerically moderate. In this study, critical resolution and selectivity were given priority because failure could compromise the stability-indicating claim and the correctness of the reportable result.

ANOVA was interpreted alongside diagnostic evidence and scientific plausibility. A significant term does not necessarily have a practically important effect, and a non-significant effect may still require control when prior knowledge or severity warrants caution. Similarly, an operating region should not be defined solely by a coloured contour plot. Prediction uncertainty, model hierarchy, experimental coverage, confirmation, measurement capability, and transfer conditions must be considered.

5.1 Proposed Control Strategy:

Element	Proposed control	Evidence source
Buffer pH	Target 3.20; tightly defined preparation and measurement procedure	Strong A and AB effects
Initial acetonitrile	Target 20.0%; controlled solvent preparation and pump proportioning	Strong B, AB, and BC effects

Element	Proposed control	Evidence source
Gradient time	Target 6.05 min; programmed range and dwell-volume awareness	Strong C and interaction effects
Flow rate	1.0 mL/min; qualified pump and periodic verification	Screening and runtime model
Temperature	30 degrees C; qualified oven	Screening and robustness
Column	Approved C18 product; lot and use history	High FMEA severity and scouting knowledge
System suitability	Critical resolution ≥ 1.80 , tailing ≤ 1.50 , efficiency criterion	ATP and development knowledge

6 Practical and Regulatory Significance:

The approach aligns development decisions with the analytical procedure lifecycle described in ICH Q14. Development data can inform robustness, parameter ranges, system suitability, and change management when studies are scientifically sound and properly documented. The proposed ranges do not themselves confer regulatory flexibility. Established conditions, reporting categories, and movement within a PAR or MODR depend on the approved dossier and regional requirements.

For a quality-control laboratory, the models provide practical troubleshooting knowledge. A fall in resolution accompanied by a change in initial organic delivery suggests a different corrective pathway from tailing driven by pH preparation or stationary-phase ageing. Training can therefore focus on cause-and-effect relationships rather than mechanical repetition of the procedure.

7 Limitations:

The numerical dataset, ANOVA, FMEA scores, equations, and confirmation results are illustrative. They do not demonstrate actual method performance or validation. The final paper requires authenticated randomised run order, raw chromatograms, calculation files, software version, residual and influence plots, model-selection rationale, failed trials, deviations, column details, forced-degradation selectivity, and independently reviewed confirmation results. The proposed risk thresholds and acceptance criteria must be approved before experimentation and justified for the intended product and laboratory.

8 Conclusion:

A risk-based experimental strategy was developed for a stability-indicating RP-HPLC method. Ishikawa analysis identified a broad set of possible sources of variability, and FMEA prioritised pH, initial acetonitrile, gradient time, column chemistry, and flow. Screening reduced the experimental domain to three principal quantitative factors. Central composite modelling quantified linear, interaction, and quadratic effects and supported a practical normal operating point at pH 3.20, 20.0% initial acetonitrile, and 6.05 min gradient time. The illustrative models predicted acceptable resolution, tailing, and runtime, and confirmation results were close to those predictions. The study shows that DoE is most valuable when it connects risk, scientific understanding, operating ranges, confirmation, and lifecycle control.

Authentic experimental evidence is essential before any performance, MODR, or regulatory claim is made.

References:

1. Armstrong, N. A. (2006). *Pharmaceutical experimental design and interpretation* (2nd ed.). CRC Press.
2. Borman, P., Nethercote, P., Chatfield, M., Thompson, D., & Truman, K. (2010). The application of quality by design to analytical methods. *Pharmaceutical Technology*, 34(12), 142-152.
3. Box, G. E. P., & Draper, N. R. (2007). *Response surfaces, mixtures, and ridge analyses* (2nd ed.). Wiley.
4. Box, G. E. P., Hunter, J. S., & Hunter, W. G. (2005). *Statistics for experimenters: Design, innovation, and discovery* (2nd ed.). Wiley.
5. Dejaegher, B., & Vander Heyden, Y. (2011). Experimental designs and their recent advances in set-up, data interpretation, and analytical applications. *Journal of Pharmaceutical and Biomedical Analysis*, 56(2), 141-158.
6. Ferreira, S. L. C., Bruns, R. E., Ferreira, H. S., Matos, G. D., David, J. M., Brandao, G. C., da Silva, E. G. P., Portugal, L. A., dos Reis, P. S., Souza, A. S., & dos Santos, W. N. L. (2007). Box-Behnken design: An alternative for the optimization of analytical methods. *Analytica Chimica Acta*, 597(2), 179-186.
7. International Council for Harmonisation. (2023a). Q14: Analytical procedure development. https://database.ich.org/sites/default/files/ICH_Q14_Guideline_2023_1116.pdf
8. International Council for Harmonisation. (2023b). Q2(R2): Validation of analytical procedures. [https://database.ich.org/sites/default/files/ICH_Q2\(R2\)_Guideline_2023_1130.pdf](https://database.ich.org/sites/default/files/ICH_Q2(R2)_Guideline_2023_1130.pdf)
9. International Council for Harmonisation. (2023c). Q9(R1): Quality risk management.
10. Molnar, I., Rieger, H.-J., & Monks, K. E. (2010). Aspects of the design space in high-pressure liquid chromatography method development. *Journal of Chromatography A*, 1217(19), 3193-3200. <https://doi.org/10.1016/j.chroma.2010.02.001>
11. Montgomery, D. C. (2019). *Design and analysis of experiments* (10th ed.). Wiley.
12. Myers, R. H., Montgomery, D. C., & Anderson-Cook, C. M. (2016). *Response surface methodology: Process and product optimization using designed experiments* (4th ed.). Wiley.
13. Orlandini, S., Pinzauti, S., & Furlanetto, S. (2013). Application of quality by design to the development of analytical separation methods. *Analytical and Bioanalytical Chemistry*, 405, 443-450. <https://doi.org/10.1007/s00216-012-6302-2>
14. Peraman, R., Bhadraya, K., & Reddy, Y. P. (2015). Analytical quality by design: A tool for regulatory flexibility and robust analytics. *International Journal of Analytical Chemistry*, 2015, Article 868727. <https://doi.org/10.1155/2015/868727>
15. Reid, G. L., Cheng, G., Fortin, D. T., Harwood, J. W., Morgado, J. E., Wang, J., & Xue, G. (2013). Reversed-phase liquid chromatographic method development in an analytical quality by design framework. *Journal of Liquid Chromatography & Related Technologies*, 36(18), 2612-2638.
16. Rozet, E., Lebrun, P., Debrus, B., Boulanger, B., & Hubert, P. (2013). Design spaces for analytical methods. *TrAC Trends in Analytical Chemistry*, 42, 157-167. <https://doi.org/10.1016/j.trac.2012.09.007>

17. Sahu, P. K., Ramiseti, N. R., Cecchi, T., Swain, S., Patro, C. S., & Panda, J. (2018). An overview of experimental designs in HPLC method development and validation. *Journal of Pharmaceutical and Biomedical Analysis*, 147, 590-611.
18. Vander Heyden, Y., Nijhuis, A., Smeyers-Verbeke, J., Vandeginste, B. G. M., & Massart, D. L. (2001). Guidance for robustness and ruggedness tests in method validation. *Journal of Pharmaceutical and Biomedical Analysis*, 24(5-6), 723-753.
19. Vogt, F. G., & Kord, A. S. (2011). Development of quality-by-design analytical methods. *Journal of Pharmaceutical Sciences*, 100(3), 797-812. <https://doi.org/10.1002/jps.22325>