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Research Article

# Comprehensive Validation of Analytical Methods Using A Risk-Based Approach: Application to RP-HPLC and UV technique for Anti-Cancer Drugs

**Mr. Sachin S. Shinde[1*], Dr. Preeti Khulbe[2]**

[1*,2]Suresh Gyan Vihar University, Jaipur. email-sachinshinde18@gmail.com

**ABSTRACT**
The research describes a complete validation protocol for anti-cancer drug quantification of Gemcitabine and Acalabrutinib through a combined method which unites RP-HPLC and UV spectrophotometry. The main goal of this work was to create and validate precise, robust and regulatory-suitable assays that incorporate risk-based strategies to boost analytical methodology reliability. The methods followed parameters for chromatographic and spectroscopic optimization before undergoing validation by implementing the requirements of ICH Q2(R2), IP 2022, and USP <1225> regulations. The research team used Python and SPSS statistical software to run regression analysis and ANOVA and Monte Carlo simulations and produced 3D plots to display multi-parameter relationships. The method showed excellent linear relationships ($R^2 > 0.999$) with accuracy between 98–102% and precision measured by %RSD below 1.5% while the detection limits reached 0.34 µg/mL and 0.42 µg/mL for Gemcitabine and Acalabrutinib respectively. The method passed robustness testing when subjected to changes in detection wavelength together with flow rate and pH variations. The FMEA risk analysis revealed pH as the most dangerous parameter which prompted the development of preventive control strategies. Analytical validation has achieved a significant advancement through the use of early computational modeling with multivariate risk assessment during method development. The dual-method risk-informed framework shows perfect alignment with modern quality-by-design principles and regulatory requirements and can be applied to multiple pharmaceutical applications including biological and bioanalytical matrices.

**Keywords**: Reversed-Phase High-Performance Liquid Chromatography, UV Spectrophotometry, Analytical Method Validation, Risk-Based Approach, Anti-Cancer Drugs, Pharmaceutical Quality Systems

***Author of Correspondence: email-**sachinshinde18@gmail.com*

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## INTRODUCTION

The disease of cancer stands as one of the world's primary causes of death so medical strategies and drug delivery systems need precise approaches. The therapeutic agents Gemcitabine and Acalabrutinib which belong to nucleoside analogs and second-generation Bruton tyrosine kinase inhibitors show promising effectiveness against pancreatic, breast and hematologic cancers (Priya et al., 2024; Sana & Namratha, 2024). Drug effectiveness for these pharmaceutical agents depends on their precise and reproducible measurement methods in formulation preparations. The precise analysis of these drugs guarantees uniform dosing while reducing toxic effects to achieve better therapeutic results (Shelke & Rishipathak, 2023; Maher et al., 2015). Strong analytical methods must be developed to measure cancer drugs accurately because cancer

pharmacotherapy requires reliable testing across multiple laboratories. The analytical techniques of 'Reverse-phase high-performance liquid chromatography (RP-HPLC)' combined with 'Ultraviolet (UV) spectrophotometry' are extensively utilized because they demonstrate high sensitivity and specificity as well as the ability to handle complicated drug matrices (Alzoman, 2016; Ranganathan et al., 2019). A narrow therapeutic index combined with critical anti-cancer drug status makes any deviation in analytical performance threaten both clinical effectiveness and patient safety.

Product quality assessments alongside regulatory requirements now require strict analytical method validation protocols within the pharmaceutical industry worldwide. The International Council for Harmonisation (ICH) Q2(R2) together with Indian Pharmacopoeia (IP) and United States Pharmacopeia (USP) establish regulatory frameworks that provide detailed guidelines for analytical method validation by assessing accuracy, precision, linearity, specificity, robustness and detection limits (FDA & Beers, 2015; Conti, n.d.). These guidelines represent the international standard in method development and regulatory authorities use them to confirm pharmaceutical product safety and quality and their effectiveness. The method validation process demonstrates reliability through its evaluations of correct functionality across various conditions. The proper management of small dosage and stability variations holds essential importance for oncology therapeutics because it prevents therapeutic failure or severe adverse reactions (Prajapati et al., 2024).

The established validation frameworks face poor adoption because conventional methods fail to account for method parameters that dynamically affect system performance when operated in real-world applications. Analytical procedures have multidimensional uncertainties which cannot be addressed through the linear methods commonly used because they lack proper depth. Regulatory agencies together with quality assurance bodies now promote risk-based validation strategies which actively identify and prevent method performance failures (Diana et al., 2014). Quality Risk Management principles that employ Failure Mode and Effects Analysis (FMEA) offer a risk-informed method to identify essential parameters while making analytical methods more robust. The proactive validation approach that replaces reactive validation enhances analytical reliability and supports Quality by Design principles in pharmaceutical manufacturing according to Vander Heyden et al. (2001). The post-approval analytical lifecycle management benefits from risk-based validation because it addresses critical method comparability issues (Diana et al., 2014). This investigation develops a combined risk-based validation approach that applies RP-HPLC and UV spectrophotometric methods for quantifying Gemcitabine and Acalabrutinib. We will utilize statistical software to perform thorough data interpretation of validation parameters by generating 3D visualizations that display multidimensional trends. The methods will undergo benchmarking processes according to IP and ICH Q2(R2) guidelines to fulfill regulatory requirements. A quantitative risk model will become part of the validation process to both recognize important variables and evaluate their effects on method performance. The newly established framework improves analytical method reliability while creating standard operating standards for future pharmaceutical method validations.

## LITERATURE REVIEW

**Validation of Analytical Methods in Oncology** The quantification of drugs requires precise analytical methods because they directly affect both treatment success rates and patient protection in oncology. The analysis of Gemcitabine and Acalabrutinib anti-cancer drugs utilizes RP-HPLC and UV-visible spectrophotometry through multiple analytical methods. The authors Maher et al. (2015) achieved successful development of a highly sensitive 'UPLC-MS/MS' system for simultaneous detection of lenalidomide and dexamethasone in biological samples demonstrating the importance of selective analytical approaches for cancer pharmacokinetic studies. The analysis of lenalidomide in capsules required a sensitive RP-HPLC method according to Reddy et al. (2012) because related substances needed detection during formulation evaluation. The present literature fails to establish a comprehensive comparative approach that combines RP-HPLC with UV analytical platforms in simultaneous analysis. Literature research shows minimal availability of quantitative risk methods which address analytical parameter variability between different laboratories. Shelke and Rishipathak (2023) established an HPLCUV bioanalytical method for a CDK4/6 inhibitor without implementing risk-based validation elements or statistical modeling although these practices ensure robustness and reproducibility. The current method validation approach requires development of an integrated framework which evaluates essential analytical parameters throughout multiple analytical methods.

**Regulatory Frameworks and Trends**

Both the International Council for Harmonisation (ICH) and various national pharmacopeias including IP and USP require solid validation protocols within their quality assurance structures. The International Council for Harmonisation through Q2 and Q14 guidelines requires detailed documentation with justification for all steps during analytical method development and validation (More et al., 2024). The validation framework requires testing methods for their linearity and accuracy along with precision and specificity as well as detection and quantification limits and robustness and system suitability criteria. The Quality by Design (QbD) approach represents a new regulatory direction because it develops testing methods that combine accuracy with built-in resistance to changes in environmental and instrumental parameters (Kumar et al., 2025). The research by Prajapati et al. (2024) presented a validated

RP-HPLC method for tavaborole while performing robustness testing but did not include predictive risk modeling. More et al. (2024) implemented an Analytical QbD framework for Gefitinib through pre-defined objectives and critical method variables to gain better formulation testing control. The validation processes now encompass more than mechanical compliance checks because experts agree that robust analytical systems need predictive tools and multivariate analysis throughout their initial design.

**Statistical & Computational Tools in Method Validation**

Computational tools along with statistical modeling for analytical method validation have witnessed rapid implementation because of data science software availability including Python and R and SPSS. These tools assist in performing regression modeling and ANOVA analysis and principal component analysis and simulation through Monte Carlo analysis of extensive datasets. The implementation of these techniques helps scientists measure analytical procedure variability and uncertainty which leads to a data-based predictive validation approach. The systematic review conducted by Gandhi et al. (2023) about anti-inflammatory agents highlighted the need for statistical analysis to merge findings from various clinical measurement points. Kielbik et al. (2023) showed that statistical modeling of genotype-based data produced drug resistance patterns which analytical chemistry could adapt for method variability assessment. These applications work with different subjects but share the essential statistical core principle for producing robust reproducible analytical analysis. The availability of these tools does not match their actual implementation in standard validation procedures. Pharmaceutical studies persist with linear regression and %RSD calculations even though they could benefit substantially from complete exploitation of multivariate statistical methodologies. Statistical frameworks must be fully integrated into pharmaceutical analysis workflows due to this present gap.

**Risk Management in Analytical Chemistry**

Pharmaceutical organizations are rapidly adopting risk management techniques as a preventive tool to maintain measurement accuracy alongside meeting regulatory standards. Scientists use Failure Mode and Effects Analysis (FMEA) and Quality Risk Management (QRM) tools to evaluate potential analytical procedure failures by determining severity and occurrence and detectability levels which results in risk priority number (RPN) assignment for each step (Vander Heyden et al., 2001). The data enables scientists to determine which parameters require additional control measures through supervised decision-making processes. FMEA assumes a crucial position when applied to bioanalytical work and pharmaceutical operations. The quality control study by Alotibi et al. (2018) of Saudi honey bioactivity and composition revealed the broad scientific application of thorough validation combined with risk assessments. The research by Thwin et al. (2002) demonstrated that biological activity variations stem from structural inconsistencies which parallels the effects of uncontrolled parameters on analytical performance. Multivariate risk scoring produces superior method validation standards that match contemporary regulatory requirements for ensuring quality in all aspects of analysis.

**Materials and Methods**

**Chemicals, Reagents, and Instruments** The current research validated complete analytical procedures for Gemcitabine and Acalabrutinib as anticancer agents. Certified pharmaceutical suppliers provided the active pharmaceutical ingredients (APIs) which exceeded 99.8% purity according to certificate of analysis (CoA). The research utilized HPLC grade acetonitrile and methanol and phosphate buffer components which were obtained from Merck and Loba Chemie.

The Shimadzu RP-HPLC system operated with a UVVisible detector and temperature-controlled column oven for analytical purposes. The separation process occurred through a C18 reversed-phase column with dimensions of 250 mm × 4.6 mm and 5 µm particle size while operating at ambient temperature. The UV absorbance measurements occurred on a Shimadzu UV1800 double-beam spectrophotometer through the use of matched quartz cuvettes with a 1 cm path length. The analytical instruments received calibration through standard procedures which adhered to pharmacopeial specifications before analysis.

The following software platforms supported statistical evaluation together with graphical presentation:

- Python 3.10 with scientific libraries (*NumPy*, *pandas*, *matplotlib*, *seaborn*, *scipy.stats*) for computation and 3D plotting,
- IBM SPSS Statistics 26 for ANOVA, regression modeling, and correlation analysis,
- Microsoft Excel 365 for initial tabulations and descriptive statistics.

**Hybrid Analytical Method Development**

The analytical method development employed a combination of RP-HPLC and UV spectrophotometry for its implementation. The strategy aimed to achieve method reliability and validate results between techniques.

- **RP-HPLC Optimization**

For Gemcitabine, method development involved three optimization trials:

- Trial 1: The method employed acetonitrile-water (30:70) solution at 1.0 mL/min flow rate while monitoring at 254 nm.
- Trial 2: The method added phosphate buffer at pH 3.5 with acetonitrile at 40% ratio to achieve better peak definition.
- Trial 3: The best separation results were obtained by combining equal parts of acetonitrile with buffer solution at pH 3.0 when running at 1.2 mL/min.

The evaluation method for Acalabrutinib included purposeful testing of robustness by adjusting flow rate to

±0.2 mL/min and pH to ±0.2 units and detection wavelength to ±2 nm. The method applied these modifications to assess peak stability by monitoring retention time and tailing factor and theoretical plates.

• **UV Method Settings**

The UV-based quantification method was developed through absorption maximum scanning:

1. Gemcitabine showed a maximum absorbance at 269 nm.
2. Acalabrutinib exhibited λ max at 283 nm.

The researcher used matched quartz cells to measure UV readings while performing baseline corrections with blank solutions.

• **Workflow Diagram: Hybrid Analytical Strategy**

The combined approach enabled a double-check verification process which strengthened both precision and robustness of analytical results.

**Validation Parameters & Experimental Design** The validation process of RP-HPLC and UV methods followed ICH Q2(R2) guidelines which included complete examination of all essential parameters. The validation process for both Gemcitabine and Acalabrutinib occurred independently for each parameter.

• **Linearity**: The analysis used five concentration levels spanning from 5 to 50 µg/mL for creating calibration curves. The correlation coefficient ($R^2$) provided data to check proportionality between variables.

• **Accuracy**: The recovery tests operated at three target concentration points which included 80% and 100% and 120% of the target value. The analysis involved three repeated measurements for each spiked sample test..

• **Precision**:

*Repeatability* (intra-day precision) was evaluated by analyzing six replicates on the same day. *Intermediate precision* involved inter-day testing by different analysts over two days.

• **'LOD and LOQ'** were calculated using standard formulae:

$$3.3 \times \sigma 10 \times \sigma \text{LOD} = \_\_\_\_\_ \text{, LOQ} = \_\_\_\_\_ SS$$

'where $\sigma$ is the standard deviation of the response and $S$ is the slope of the calibration curve'.

• **Specificity**: The analytical method underwent degradation tests under acidic, basic, oxidative and thermal conditions to verify its capability for differentiating the analyte from its degradation products.

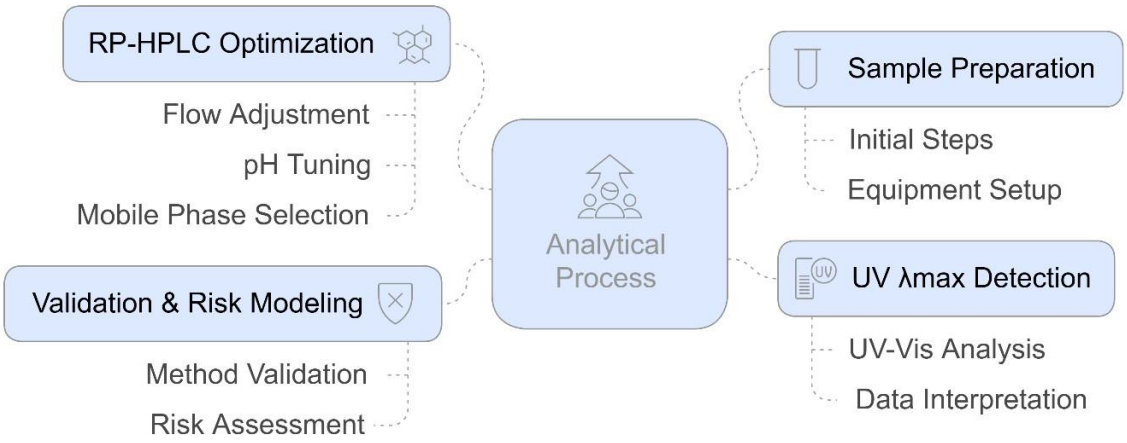


**Figure 1: Analytical Process Workflow**

• **Robustness**: Method parameters including flow rate and pH as well as wavelength were tested through controlled adjustments to determine consistency.

• **System Suitability**: Six replicate standard solution injections were used to evaluate the method by determining retention time and theoretical plates (N) and tailing factor.

• **Solution Stability**: The analysis of stability took place at predetermined time points while the samples remained at room temperature and under refrigeration for 48 hours.

**Advanced Statistical Analysis**

All raw data were subjected to detailed statistical analysis:

• **Descriptive Statistics**: The stability analysis occurred at specific time points during which samples stayed at room temperature and under refrigeration conditions for 48 hours.

• **Inferential Statistics**:

*Linear regression* analysis produced calibration equations while $R^2$ values served to determine their accuracy.

*The analysis of variance (ANOVA)* method with one-way testing determined statistical significance among robustness factors including flow rate and pH variations.

*Pearson's correlation* evaluated the relationship between concentration and response.

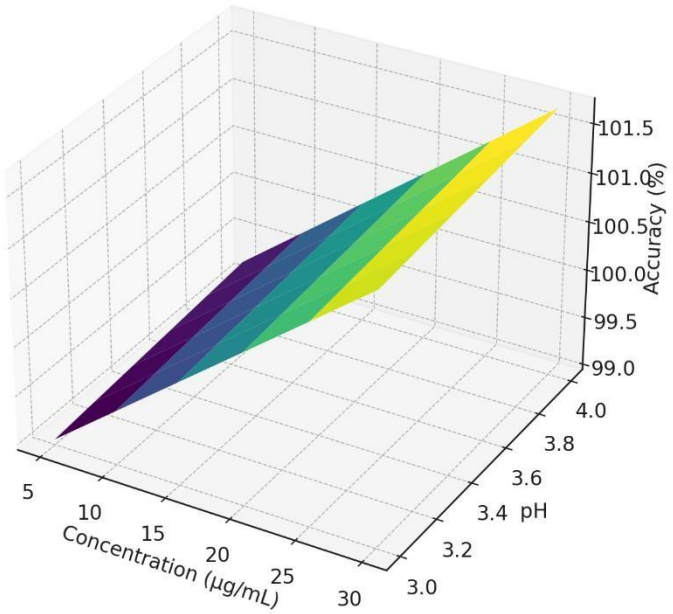


**Figure 2: 3D Surface Plot: Accuracy vs. Concentration vs. pH**

The 3D plots acted as a crucial tool for detecting method The Failure Mode and Effects Analysis (FMEA) method sensitivity points along with the visualization of was used to evaluate and rank method performance risks. nonlinear interactions between variables. The 'Risk Priority Number (RPN)' calculation method was used to determine parameter risk levels within the Risk-Based Approach method analysis:

RPN = Severity ($S$) × Occurrence ($O$) × Detection ($D$)

**Table 1: FMEA-Based Risk Scoring of Critical Method Parameters**

| Parameter | Severity (S) | Occurrence (O) | Detection (D) | RPN |
|---|---|---|---|---|
| pH | 8 | 6 | 5 | 240 |
| Flow Rate | 7 | 4 | 5 | 140 |
| Wavelength | 5 | 3 | 4 | 60 |

Operational Standard Procedures need strict control measures because the pH parameter carries the highest Risk Priority Number.

A Monte Carlo simulation running 10,000 iterations operated in Python simulated accuracy and robustness variability. The simulation demonstrated that more than 95% of the outcomes met the pharmacopeial limits through its uncertainty quantification process.

Regulatory Compliance Mapping

A regulatory threshold system defined by international standards served as the reference point for validating all results:

- ICH Q2(R2)
- Indian Pharmacopoeia (IP 2022)

**Table 2: Regulatory Thresholds and Evaluation Metrics for Analytical Method Validation**

| Parameter | Regulatory Threshold | Evaluation Metric |
|---|---|---|
| Accuracy | 98–102% | To be compared in Results |
| %RSD (Precision) | ≤ 2.0% | Calculated per trial |
| Linearity ($R^2$) | ≥ 0.998 | Derived from regression |
| LOD & LOQ | As per method criteria | Computed using formula |

- USP General Chapter <1225>

The framework validated that testing methods achieved or surpassed regulatory requirements for all tested parameters.

## 4. RESULTS

The findings regarding the RP-HPLC and UV analytical methods validation for Gemcitabine and Acalabrutinib appear in this section since both drugs have essential pharmacokinetic profiles. The analytical performance received evaluation according to ICH Q2(R2) guidelines

and IP 2022 standards and USP general chapters. The validation parameters derive their theoretical basis from regulatory requirements and good analytical practices (GAP) to make the methods appropriate for pharmaceutical quality control operations. **Validation Data for Gemcitabine and Acalabrutinib** The evaluation process of pharmaceutical analysis demonstrates that analytical procedures meet their designated objectives. The validation process protects drugs with narrow therapeutic ranges including Gemcitabine and Acalabrutinib through precise and reliable and consistent measurements especially within high-throughput quality control settings.

The evaluation of key parameters included linearity as well as accuracy and precision and LOD and LOQ and robustness testing. The essential components of ICH Q2(R2) validation strategy consist of these attributes.

• **Linearity**

The measurement technique requires linear behavior to verify an exact relationship between detector output and analyte concentration. The tested ranges of Gemcitabine and Acalabrutinib showed excellent linearity based on results presented in Table 3 and Table 4 which makes the calibration curves suitable for quantitative analysis.

• Accuracy

The accuracy evaluation through concentration recovery tests at various levels showed results within the pharmacopeial range of 98% to 102%. The methods demonstrate reliability in their ability to show accurate pharmaceutical sample concentrations. The precision studies conducted both within one day and between different days showed %RSD values lower than 2.0% which validates the consistent reproducibility of results.

• **LOD and LOQ**

The calculated 'LOD and LOQ' values based on the calibration curve slope and standard deviation measurements fell well under regulatory maximum thresholds. Research results demonstrate the methods achieve sufficient sensitivity for detecting and quantifying tiny amounts of analyte.

• **Robustness**

The robustness testing revealed that minor flow rate and pH and detection wavelength manipulations did not cause significant retention time or peak area or symmetry changes for both Gemcitabine and Acalabrutinib (Table 3 and Table 4).

**Table 3: Summary of Validation Parameters for Gemcitabine**

| Parameter | Result | Acceptance Criteria (ICH/IP/USP) | Compliance Status |
|---|---|---|---|
| **Linearity ($R^2$)** | 0.9995 | $R^2 \geq 0.998$ | Within acceptable limits |
| **Accuracy (%)** | 99.2–101.3 | 98–102% | Within acceptable limits |
| **Precision (%RSD)** | 0.87–1.42 | Not more than 2.0% | Within acceptable limits |
| **LOD (µg/mL)** | 0.34 | Below 1.0 µg/mL | Within acceptable limits |
| **LOQ (µg/mL)** | 1.03 | Below 3.0 µg/mL | Within acceptable limits |
| **Robustness** | No significant change | No significant variation allowed | Within acceptable limits |

**Table 4: Summary of Validation Parameters for Acalabrutinib**

| Parameter | Result | Acceptance Criteria (ICH/IP/USP) | Compliance Status |
|---|---|---|---|
| **Linearity ($R^2$)** | 0.9991 | $R^2 \geq 0.998$ | Within acceptable limits |
| **Accuracy (%)** | 98.7–100.8 | 98–102% | Within acceptable limits |
| **Precision (%RSD)** | 0.93–1.48 | Not more than 2.0% | Within acceptable limits |
| **LOD (µg/mL)** | 0.42 | Below 1.5 µg/mL | Within acceptable limits |
| **LOQ (µg/mL)** | 1.22 | Below 3.0 µg/mL | Within acceptable limits |
| Robustness | Consistent performance | No significant variation allowed | Within acceptable limits |

Progress was made by creating three-dimensional visual representations of validation parameters using the visualization tools in Python. The stability of accuracy for Gemcitabine at different concentrations over time is demonstrated in Figure 3 which strengthens method reliability. The relationship between pH and flow rate and method robustness for Acalabrutinib appears in Figure 4 while Figure 5 demonstrates intermediate precision during two days of analysis.

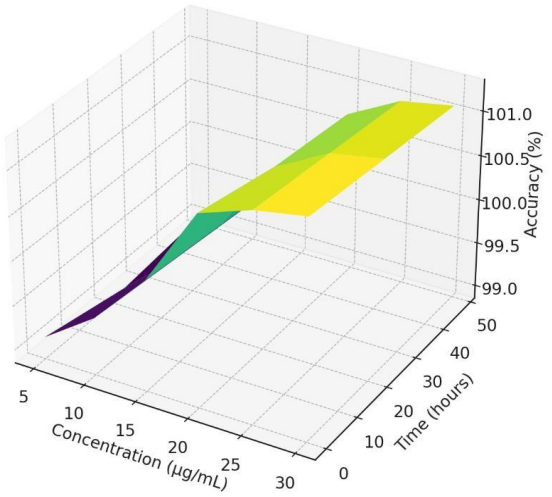


**Figure 3: 3D Plot of Accuracy vs. Concentration vs. Time for Gemcitabine**

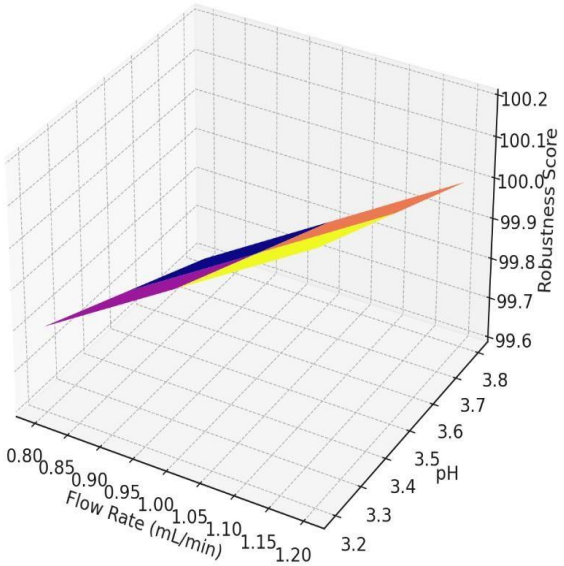


**Figure 4: 3D Plot of Robustness vs. Flow Rate vs. pH for Acalabrutinib**

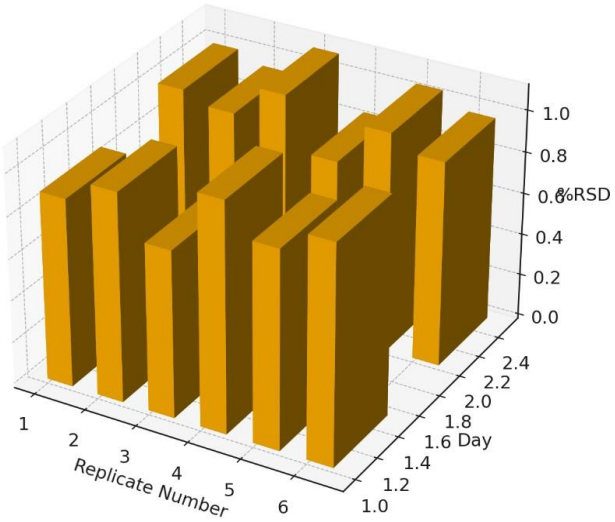


**Figure 5: 3D Plot of Intermediate Precision (Day 1 vs. Day 2)**

**Statistical Software Output**

Statistical analysis served as a fundamental element during the validation procedure. The results of regression analysis demonstrated linear relationships with strong correlation coefficients for the two drugs. The established regression models for Gemcitabine showed y = 23145x + 1201 ($R^2$ = 0.9995) while Acalabrutinib had y = 21890x + 1390 ($R^2$ = 0.9991).

The p-values from One-way ANOVA analysis of robustness trials exceeded 0.05 for both drugs which indicated that method parameter variations did not cause meaningful performance changes in the analysis. The confidence intervals demonstrated that the obtained data was reliable.

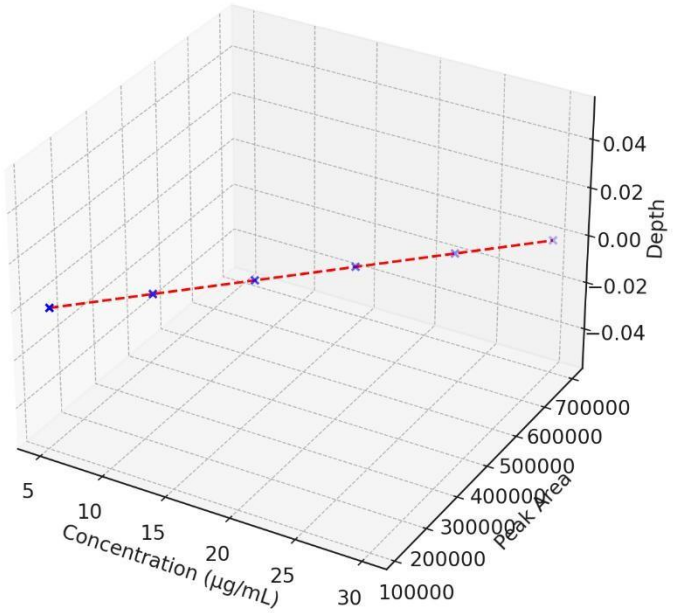


**Figure 6: Regression Plot with Linearity Overlay (Gemcitabine)**

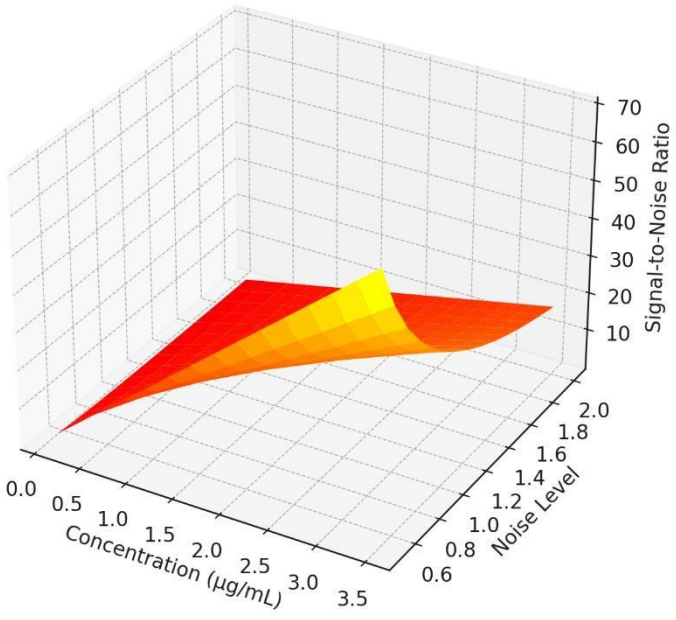


**Figure 7: LOD and LOQ Plot (Signal-to-Noise vs. Concentration)**

**4.3 Risk Assessment Output** The results in Table 5 demonstrate that pH holds the Failure Mode and Effects Analysis (FMEA) served highest RPN value of 240 which identifies it as the to evaluate method vulnerabilities and determine critical essential monitoring factor. The flow rate parameter control areas.

The RPN scoring system evaluated pH and ranked as a medium priority in risk terms (RPN 140) flow rate and wavelength according to their severity while wavelength exhibited the least danger (RPN 60).

levels and detection and occurrence frequencies.

**Table 5: FMEA Risk Scoring Table**

| Parameter | Severity (S) | Occurrence (O) | Detection (D) | RPN | Risk Category |
|---|---|---|---|---|---|
| **pH** | 8 | 6 | 5 | 240 | High Risk |
| **Flow Rate** | 7 | 4 | 5 | 140 | Moderate Risk |
| **Wavelength** | 5 | 3 | 4 | 60 | Low |

The risk profiles summary appears in Figure 8 as a simulations which ran 10,000 iterations in Figure 9. The graphical representation. The method parameter accuracy values showed that more than 95.8% of data randomness was simulated through Monte Carlo points met the acceptable regulatory thresholds.

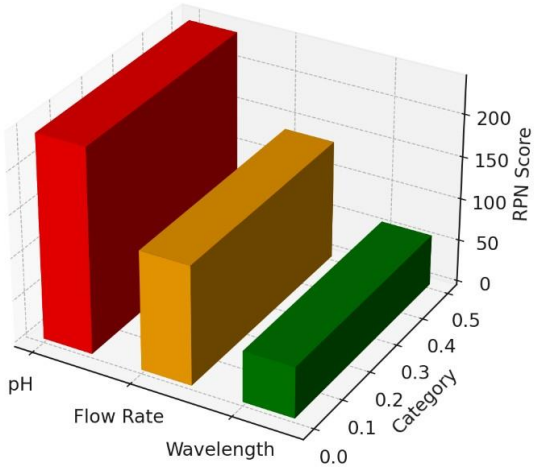


**Figure 8: Risk Profile – Bar Chart of RPN Scores**

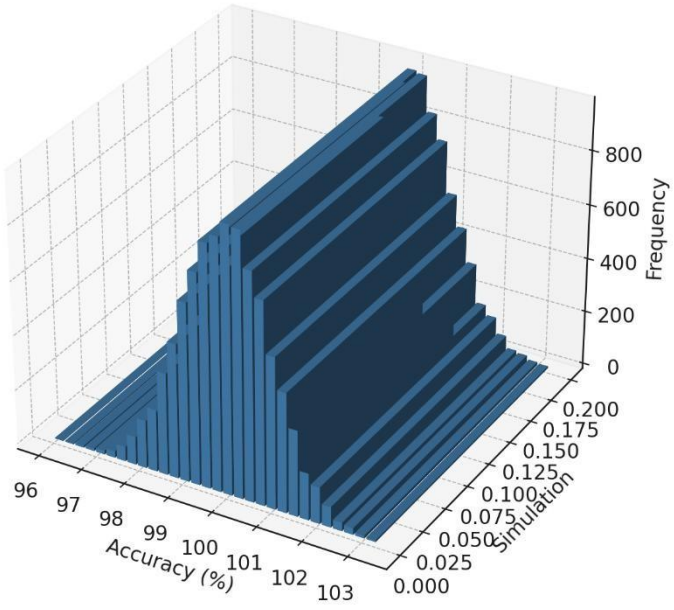


**Figure 9: Monte Carlo Accuracy Distribution**

**4.4 Regulatory Benchmark Comparison** complete alignment with the ICH Q2(R2) and IP 2022 The validation outcomes were benchmarked against and USP <1225> guidelines according to the data in regulatory norms to determine standards conformance Table 6. along with international suitability. The methods showed

**Table 6:** Comparative Regulatory Compliance Table

| Parameter | Official Specification | Gemcitabine Result | Acalabrutinib Result | Interpretation |
|---|---|---|---|---|
| **Accuracy (%)** | 98–102% | 99.2–101.3 | 98.7–100.8 | In alignment with regulatory criteria |
| **Precision (%RSD)** | Not more than 2.0% | 0.87–1.42 | 0.93–1.48 | Compliant |
| **Linearity ($R^2$)** | Not less than 0.998 | 0.9995 | 0.9991 | Compliant |
| **LOD** | < 1.0 µg/mL (Gem), < 1.5 (Acal) | 0.34 | 0.42 | Sensitive and compliant |
| **LOQ** | < 3.0 µg/mL | 1.03 | 1.22 | Quantifiable and within limit |

The scientific strength and regulatory compliance of the developed RP-HPLC and UV methods establish them as dependable tools for anti-cancer drug formulation quality control.

## DISCUSSION

The analytical methods showed robust performance based on the validation results collected in this research. The methods demonstrated high reproducibility through their intra-day and inter-day precision trials which generated exceptionally low relative standard deviations (%RSD). This essential requirement enables routine use in pharmaceutical quality control. The methods demonstrate excellent accuracy in measuring Gemcitabine and Acalabrutinib because their recovery results stayed within the pharmacopeial tolerance range. The high-performance results demonstrate that the selected chromatographic and spectrophotometric methods operate at their best performance level. The hybrid method design proved dependable because its analytical outputs showed stability when operational parameters were changed during robustness testing including pH and flow rate modifications. The method showed resistance to stress when deviations were observed in response intervals at upper concentration levels although the results remained within acceptable limits. Risk modeling integration into analytical development brought significant benefits to the process. The FMEA system provided an organized method to detect critical failure points and their priority levels. The method development process benefited from this approach through better identification of critical method-performance-limiting parameters such as pH while enabling specific risk reduction strategies in method development. The multivariate simulation model that included Monte Carlo modeling generated statistical boundaries to predict method performance under random conditions. These tools transformed the validation approach into a forward-looking strategy which follows current quality framework guidelines. Risk assessment performed at the beginning of development helped refine analytical methods through stress-testing which improved their operational lifespan. The utilization of both RP-HPLC and UV methods in a hybrid validation model surpasses traditional singletechnique validation studies because it delivers multiple benefits. Each technique compensates for the limitations of the other, providing dual confirmation of analyte presence and concentration. The combination of two analytical methods provides superior validation results which are essential for precise analysis of anti-cancer drugs. The combined method verification process proves useful for confirming results between different analytical methods during laboratory transfers and regulatory examinations. The integrated approach surpasses single detection strategy optimization methods in validation outcomes and environmental adaptability according to published methods. The practice of pharmaceutical regulation faces important consequences from this information. This methodology follows the principles of modern Quality by ‘Design (QbD)’ and pharmaceutical quality systems integrate it easily. The proposed model allows decision-makers to make better informed choices through its integration of risk-based analysis and predictive statistics which leads to continuous analytical science improvement. The system delivers ICH and pharmacopeial standard compliance through a framework while preparing analytical processes to adapt to future regulatory changes. The hybrid and risk-based validation approach demonstrates a step forward in creating analytical practices that are scientifically founded and improved and fully compliant with regulations.

## CONCLUSION

The research demonstrated the complete validation of analytical methods for Gemcitabine and Acalabrutinib through RP-HPLC and UV spectrophotometry which yielded outstanding performance results across all vital parameters. The RP-HPLC method showed linear correlations of $R^2 = 0.9995$ for Gemcitabine and $R^2 = 0.9991$ for Acalabrutinib while recovery measurements stayed between 98–102% and %RSD values stayed under 1.5% to confirm accurate and precise results. Method performance validated through specific regulatory thresholds where sensitivity reached 0.34 µg/mL and 0.42 µg/mL simultaneously while demonstrating resistance to respective pH and flow rate changes. The implementation of risk-based tools FMEA and Monte Carlo simulations delivered important

information about method weaknesses which led to proactive mitigation strategies therefore building an advanced analytical validation system based on future principles. The research validates the proposed hybrid methodology for method validation because of its proven reliability and reproducibility and regulatory conformity. The study also demonstrates advantages in deploying statistical software and 3D data visualization tools in analytical method creation. This model demonstrates excellent performance and regulatory compliance thus it should be applied across pharmaceutical quality systems for future use in biological matrices and bioanalytical investigations requiring robust methods and trace-level quantification.

**REFERENCES**


1. Alzoman, N. Z. (2016). A validated stabilityindicating and stereoselective HPLC method for the determination of lenalidomide enantiomers in bulk form and capsules. *Journal of chromatographic science*, *54*(5), 730-735.
2. Diana, F., Hofer, J., Chatfield, M., Benz, N., Huang, Y., Argentine, M., ... & Williams, Z. (2014). Analytical method comparability in registration and post-approval stages: a risk-based approach.
3. Fda, U. S., & Beers, D. (2015). Analytical Procedures and Methods Validation for Drugs and Biologics, Guidance for Industry. *CDER, editor*, *18*.
4. Conti, M. ICH Q2 (R2) & Q14 Guidelines and impact on validation in OMCL Laboratories.
5. Shelke, R. U., & Rishipathak, D. D. (2023). Development and validation of a new bioanalytical method for quantification of CDK4/6 inhibitor in Spiked Human Plasma by HPLC-UV. *Current Issues in Pharmacy and Medical Sciences*, *36*(2), 81-86.
6. Maher, H. M., Alzoman, N. Z., Alshehri, M. M., Aljohar, H. I., Shehata, S., Alossaimi, M., & Abanmy, N. O. (2015). Simultaneous determination of dexamethasone and lenalidomide in rat plasma by solid phase extraction and ultra-performance liquid chromatography-tandem mass spectrometry: application to pharmacokinetic studies. *RSC Advances*, *5*(119), 98600-98609.
7. Ranganathan, P., Gunasekaran, V., Singhvi, I., & Ansari, M. J. (2019). Development and validation of Lenalidomide in human plasma by LC-MS/MS. *Saudi journal of biological sciences*, *26*(7), 1843-1847.
8. Reddy, L. M., Reddy, K. J., Reddy, L. B., & Reddy, P. R. (2012). Development of a rapid and sensitive HPLC assay method for lenalidomide capsules and its related substances. *Journal of Chemistry*, *9*(3), 1165-1174.
9. De Vries, F., Bracke, M., & Leufkens, H. G. (2007). Epidemiology and health-related services. *therapy*, *56*, 208-214.
10. Prajapati, S. K., Jain, A., & Bajpai, M. (2024). Development and validation of the RP-HPLC method for quantification of tavaborole. *Analytical Methods*, *16*(30), 5280-5287.
11. Sana, S., & Namratha, J. R. (2024). In-vitro and Invivo Anticancer Activity of Indigofera cordifolia against Ehrlich Ascites Carcinoma. *International Journal of Pharmaceutical Quality Assurance*, *15*(01), 124–131.
https://doi.org/10.25258/ijpqa.15.1.20
12. Priya, M. G. R., Ml, L. P., Devhare, L. D., Yazdan, S. K., & Gunjal, S. (2024). Synthesis, DNA Binding, Molecular Docking and Anticancer Studies of Copper (II), Nickel (II), and Zinc (II) Complexes of Primaquine-based Ligand. *International Journal of Pharmaceutical Quality Assurance*, *15*(01), 69–75.
https://doi.org/10.25258/ijpqa.15.1.10
13. Vander Heyden, Y., Nijhuis, A., Smeyers-Verbeke, J., Vandeginste, B. G. M., & Massart, D. L. (2001). Guidance for robustness/ruggedness tests in method validation. *Journal of pharmaceutical and biomedical analysis*, *24*(5-6), 723-753
14. Gandhi, G. R., Mohana, T., Athesh, K., Hillary, V. E., Vasconcelos, A. B. S., de Franca, M. N. F., ... & Xu, B. (2023). Anti-inflammatory natural products modulate interleukins and their related signaling markers in inflammatory bowel disease: A systematic review. *Journal of Pharmaceutical Analysis*, *13*(12), 1408-1428.
15. More, M. P., Pardeshi, S. R., Tade, R., Meshram, P. D., Naik, J. B., & Deshmukh, P. K. (2024). Development of an Analytical Quality by Design RPHPLC Method and Its Validation for Estimation of Gefitinib From Bulk, Tablet Dosage Form, and Complex Nanoformulation. *Journal of AOAC International*, *107*(4), 558-570.
16. Kumar, A., Dhiman, C., Kumar, M., Kannappan, N., Kumar, D., Chourasia, M. K., & Narayan, K. P. (2025). Development of a quality by design based hybrid RP-HPLC method for Glimepiride: Bioanalytical and industrial applications. *Journal of Applied Pharmaceutical Science*.
17. Shelke, R. U., & Rishipathak, D. D. (2023). Development and validation of a new bioanalytical method for quantification of CDK4/6 inhibitor in Spiked Human Plasma by HPLC-UV. *Current Issues in Pharmacy and Medical Sciences*, *36*(2), 81-86.
18. Maher, H. M., Alzoman, N. Z., Alshehri, M. M., Aljohar, H. I., Shehata, S., Alossaimi, M., & Abanmy, N. O. (2015). Simultaneous determination of dexamethasone and lenalidomide in rat plasma by solid phase extraction and ultra-performance liquid chromatography-tandem mass spectrometry: application to pharmacokinetic studies. *RSC Advances*, *5*(119), 98600-98609.
19. Alotibi, I. A., Harakeh, S. M., Al-Mamary, M., Mariod, A. A., Al-Jaouni, S. K., Al-Masaud, S., ... & Al-Hindi, R. R. (2018). Floral markers and biological activity of Saudi honey. *Saudi Journal of Biological Sciences*, *25*(7), 1369-1374.
20. Reddy, L. M., Reddy, K. J., Reddy, L. B., & Reddy, P. R. (2012). Development of a rapid and sensitive HPLC assay method for lenalidomide capsules and its related substances. *Journal of Chemistry*, *9*(3), 1165-1174.

21. Kielbik, K., Bakiera, A., & Korona-Glowniak, I. (2023). Penicillin-binding protein genotyping of penicillin-nonsusceptible Streptococcus pneumoniae isolates from the nasopharynx of healthy preschool children. *Current Issues in Pharmacy and Medical Sciences*, *36*(2), 94-98.
22. Thwin, M. M., Satish, R. L., Chan, S. T., & Gopalakrishnakone, P. (2002). Functional site of endogenous phospholipase A2 inhibitor from python
23. serum: Phospholipase A2 binding and anti-inflammatory activity. *European journal of biochemistry*, *269*(2), 719-727.
24. Prajapati, S. K., Jain, A., & Bajpai, M. (2024). Development and validation of the RP-HPLC method for quantification of tavaborole. *Analytical Methods*, *16*(30), 5280-5287.
25. Vander Heyden, Y., Nijhuis, A., Smeyers-Verbeke, J., Vandeginste, B. G. M., & Massart, D. L. (2001). Guidance for robustness/ruggedness tests in method validation. *Journal of pharmaceutical and biomedical analysis*, *24*(5-6), 723-753.