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Review Article

AQbD-Based RP-HPLC Method Development for Simultaneous Estimation of Antineoplastic Agents in Combined Dosage Forms: A Critical Review

Dr. Rahul Solunke*, Dhage Pratiksha

Godavari institute of Pharmacy Kolpa

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ABSTRACT

Analytical Quality by Design (AQbD) has emerged as a risk-based, regulatory-aligned framework for building robustness into reversed-phase high-performance liquid chromatography (RP-HPLC) methods from the earliest stages of development, rather than relying on validation as an afterthought. Antineoplastic therapy is increasingly delivered as fixed-dose or co-administered combination regimens, which places growing demand on analytical methods capable of resolving structurally dissimilar actives — small molecules, prodrugs, and, in some regimens, biologics — within a single stability-indicating run. This review consolidates recent (2021–2025) AQbD- and Design of Experiments (DoE)-driven RP-HPLC methods reported for antineoplastic agents, both singly and in simultaneous-estimation combinations, and maps them against ICH Q8(R2), Q9(R1), Q2(R1), and the newly finalized ICH Q14 analytical procedure development guideline. Recurring gaps are identified in design-space documentation, use of green/white analytical chemistry metrics, and stability-indicating validation for multi-drug oncology combinations approved in 2023–2025. The review argues for a structured AQbD workflow — Analytical Target Profile, risk assessment, DoE-based design-space mapping, and control-strategy definition — as the appropriate response to this gap.

INTRODUCTION

Combination antineoplastic regimens — whether co-formulated or co-administered — are now central to oncology practice because they allow simultaneous engagement of multiple resistance

pathways while improving dosing convenience. This trend has direct analytical consequences: quality control, stability testing, and regulatory submission all require RP-HPLC methods capable of resolving two or more actives of differing

***Corresponding Author:** Dr. Rahul Solunke

Address: Godavari institute of Pharmacy Kolpa.

Email ✉: solunkerahul1986@gmail.com

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polarity, pKa, and UV response within a single run, while remaining stability-indicating under ICH-mandated forced-degradation conditions.

Analytical Quality by Design (AQbD) reframes method development as a risk-based design exercise rather than a trial-and-error optimization. Building on the QbD principles of ICH Q8(R2) and the risk-management framework of ICH Q9(R1), and now formalized for analytical procedures in ICH Q14 (Analytical Procedure Development), AQbD begins with an Analytical Target Profile (ATP), proceeds through risk assessment of critical method parameters (CMPs), uses Design of Experiments (DoE) — commonly Box-Behnken or central composite designs — to map a design space against critical analytical attributes (CAAs), and concludes with a defined control strategy. This structured approach is progressively displacing empirical, one-factor-at-a-time method development in the pharmaceutical analytical literature.

2. Regulatory and Clinical Relevance

The urgency of this review is underscored by regulatory activity in the antineoplastic combination space. On 20 December 2024, the US FDA granted accelerated approval to the three-drug combination of encorafenib (Braftovi), cetuximab (Erbix), and mFOLFOX6 chemotherapy for adult patients with BRAF V600E-mutant metastatic colorectal cancer — one of several combination-regimen oncology approvals granted in that approval cycle. Encorafenib alone, and in its combined form with cetuximab, has already been the subject of a dedicated stability-indicating RP-HPLC simultaneous-estimation method (Sriramula & Shivaraj, 2025), illustrating how quickly analytical method development follows clinical approval in this space. Rising global incidence of colorectal, prostate, and BRAF-mutant cancers — alongside the accompanying growth in fixed-dose and co-

administered antineoplastic regimens — creates sustained downstream demand for validated, AQbD-compliant RP-HPLC methods capable of supporting batch release, stability studies, and regulatory dossiers for these products.

3. AQbD Workflow for Antineoplastic Combinations

Across the methods surveyed in Section 4, a broadly consistent AQbD workflow recurs: (i) definition of the Analytical Target Profile specifying the analytes, matrix, and required specificity/sensitivity; (ii) an Ishikawa or risk-ranking exercise to shortlist critical method parameters — typically mobile-phase organic ratio, buffer pH, flow rate, and column temperature; (iii) a Plackett-Burman or similar screening design to reduce the parameter list, followed by a Box-Behnken or central composite design to model the design space against retention time, resolution, tailing factor, and peak area as critical analytical attributes; (iv) generation of a design space and normal operating region using response-surface modelling (commonly in Design-Expert software); and (v) definition of a control strategy and full ICH Q2(R1) validation — specificity, linearity, accuracy, precision, robustness, and forced-degradation stability-indication.

4. Literature Review

A representative body of recent AQbD- and DoE-driven RP-HPLC work on antineoplastic agents is summarised below.

Sriramula and Shivaraj (2025) developed and validated a stability-indicating RP-HPLC method for the simultaneous quantification of encorafenib and cetuximab in bulk and pharmaceutical dosage forms, using a Waters X-Terra RP-18 column with an acetonitrile/0.1% TEA buffer mobile phase and UV detection at 240 nm, reporting linearity,



accuracy, and precision within ICH-acceptable limits — directly relevant to the December 2024 FDA-approved encorafenib-cetuximab-chemotherapy regimen.

A stability-indicating RP-HPLC method for the antineoplastic combination of niraparib and abiraterone in synthetic mixtures and combined tablet formulations was reported in Discover Applied Sciences (2025), using a Zodiac C18 column with an acetonitrile/sodium phosphate buffer mobile phase, achieving baseline resolution with retention times of 2.2 and 2.7 minutes for the two actives.

Venkatachari and Ramasamy (2025) applied a Box-Behnken design to RP-HPLC method development for irinotecan hydrochloride trihydrate, using QbD risk-assessment tools to shorten retention time while maintaining robustness — illustrating the single-analyte AQbD workflow that underpins the simultaneous-estimation methods discussed above.

An AQbD-driven RP-HPLC method for abiraterone acetate, quantifying the drug in the presence of its degradants, used a Box-Behnken design with three critical method parameters (mobile-phase composition, flow rate, and buffer pH) across seventeen experimental runs, with retention time and peak area modelled as critical analytical attributes using Design-Expert software (PMC, 2022).

A QbD-based, Central Composite Design-optimized stability-indicating RP-HPLC method was reported for larotrectinib, combining Plackett-Burman screening with CCD optimization and an integrated white/green/blue analytical-sustainability assessment — an increasingly common addition to AQbD antineoplastic method papers (Journal of Applied Pharmaceutical Research, 2025).

Outside strict oncology indications, Box-Behnken- and CCD-based AQbD RP-HPLC methods for other simultaneous-estimation

combinations — for example rutin and ciprofloxacin in a bilosomal nanoformulation (PMC, 2023) and linagliptin and empagliflozin (Research Square, 2025) — provide useful methodological precedent for design-of-experiments strategy applied to structurally dissimilar co-formulated actives, even though the drugs themselves are not antineoplastic.

5. Identified Gaps and Novelty Statement

Three gaps recur across the literature surveyed. First, published AQbD-RP-HPLC methods for antineoplastic combinations are almost entirely single-method reports; no review to date has consolidated AQbD-specific (ATP-to-control-strategy) workflows across multiple antineoplastic combination methods to identify shared design-space and robustness patterns. Second, integration of green/white/blue analytical-chemistry sustainability metrics remains inconsistent, appearing in only a minority of the antineoplastic-focused papers reviewed. Third, several antineoplastic combinations receiving FDA approval in 2023–2025 (including the December 2024 encorafenib-cetuximab-chemotherapy approval) do not yet have a corresponding AQbD-optimized, stability-indicating RP-HPLC method in the peer-reviewed literature, representing an active method-development opportunity rather than a reviewable gap at present.

6. CONCLUSION

AQbD-driven RP-HPLC method development offers a regulatory-aligned, risk-based route to robust, stability-indicating methods for antineoplastic combination therapies at a time when clinical approvals of such combinations are accelerating. The literature reviewed here demonstrates consistent uptake of Box-Behnken and central composite designs for individual and combined antineoplastic actives, but reveals an



emerging lag between newly approved combination regimens and the publication of corresponding AQbD-optimized analytical methods. Closing this lag, and standardizing incorporation of green-chemistry metrics into the AQbD control strategy, represent the principal opportunities for future analytical method development in this therapeutic area.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. Sriramula JR, Shivaraj S. Analytical Method Development and Validation for the Estimation of Combined Antineoplastic Agents by RP-HPLC. *Int J Pharm Drug Anal.* 2025;13(3):1-5. doi:10.47957/ijpda.v13i3.632
2. A novel RP-HPLC stability-indicating method for simultaneous estimation of Niraparib and Abiraterone in synthetic mixtures and combined tablet formulations. *Discov Appl Sci.* 2025. doi:10.1007/s42452-025-06930-5
3. Venkatachari KM, Ramasamy V. Box-Behnken Design Approach to Method Development for Irinotecan Hydrochloride Trihydrate by Using RP-HPLC Method. *Biomed Pharmacol J.* 2025;18(1).
4. AQbD Driven Development of an RP-HPLC Method for the Quantitation of Abiraterone Acetate for its Pharmaceutical Formulations in the Presence of Degradants. PMC8744445.
5. A QbD-based stability-indicating RP-HPLC method for larotrectinib: degradation kinetics and integrated white, green, and blue analytical assessment. *J Appl Pharm Res.* 2025.
6. QbD-Engineered Development and Validation of a RP-HPLC Method for Simultaneous Estimation of Rutin and Ciprofloxacin HCl in Bilosomal Nanoformulation. PMC10286274. 2023.
7. Center Composite Design Assisted Optimization of RP-HPLC Method for Simultaneous Estimation of Linagliptin and Empagliflozin Using AQbD Perspective. Research Square. 2025.
8. FDA's December 2024 Oncology Approvals and Designations: Accelerated approval of encorafenib, cetuximab and mFOLFOX6 for BRAF V600E-mutant metastatic colorectal cancer (20 December 2024). Targeted Oncology.
9. ICH Harmonised Guideline. Q8(R2): Pharmaceutical Development. International Council for Harmonisation.
10. ICH Harmonised Guideline. Q9(R1): Quality Risk Management. International Council for Harmonisation.
11. ICH Harmonised Guideline. Q2(R1): Validation of Analytical Procedures: Text and Methodology. International Council for Harmonisation.
12. ICH Harmonised Guideline. Q14: Analytical Procedure Development. International Council for Harmonisation.

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