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GREEN ANALYTICAL CHEMISTRY APPROACH IN RP-HPLC FOR QUANTITATIVE ESTIMATIONS OF TYROSINE KINASE INHIBITOR

Manish Kumar*[1], Manish Sharma[2], Prof. (Dr.) Jitendra Banweer[3]

[1]Research Scholar, Sage Institute of Research and Technology Pharmacy, Sanjeev Agrawal Global Educational University, Bhopal.

[2]Assistant Professor, Sage Institute of Research and Technology Pharmacy, Sanjeev Agrawal Global Educational University, Bhopal.

[3]Dean, Sage Institute of Research and Technology Pharmacy, Sanjeev Agrawal Global Educational University, Bhopal.

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***Corresponding Author: Manish Kumar**
Research Scholar, Sage Institute of Research and Technology Pharmacy, Sanjeev Agrawal Global Educational University, Bhopal.
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ABSTRACT

Tyrosine kinase inhibitors (TKIs) have revolutionized the management of several malignancies by selectively targeting aberrant signaling pathways that drive tumor growth and survival. As their clinical use expands, there is a growing need for robust, sensitive, and environmentally sustainable analytical methods to support quality control, pharmacokinetic monitoring, and regulatory compliance. Reversed-phase high-performance liquid chromatography (RP-HPLC) remains the workhorse technique for quantifying TKIs in pharmaceutical formulations and biological matrices; however, conventional RP-HPLC methods often rely on toxic organic solvents, high flow rates, and energy-intensive operations, raising concerns about environmental and occupational safety. In response, the concept of “green RP-HPLC” has emerged, integrating analytical quality-by-design (AQbD), solvent substitution, miniaturization, and multi-metric greenness assessment into method development workflows. This paper presents a comprehensive account of green RP-HPLC method development for selected TKIs, illustrated with recent data tables, mechanistic diagrams, and quantitative greenness evaluation using tools such as Analytical Eco-Scale, AGREE, GAPI, and NEMI.

KEYWORDS: Green, Tyrosine, Kinase, HPLC, Sustainability.

1. BACKGROUND AND RATIONALE

1.1 Role of Tyrosine Kinase Inhibitors in Oncology

Tyrosine kinase inhibitors are one of the most important developments in precision oncology, wherein the drug selectively affect abnormal signalling pathways that lead to malignant transformation, tumor progression and resistance to therapy. Tyrosine kinases control important processes in the cell such as cell proliferation, differentiation, apoptosis, and blood vessel formation through the mechanism of phosphorylation mediated signal transduction. Dysregulation of these enzymes either due to gene mutation, gene amplification or chromosomal rearrangement is a hallmark sign of a number of cancers in humans. TKIs exert their anticancer effects mainly due to their ability to inhibit ATP binding sites or substrate recognition domains of receptor and non-receptor tyrosine kinases and in turn inhibit downstream oncogenic signalling pathways, such as PI3K AKT and MAPK pathways.[1,2]

Clinically, TKIs have been shown to be remarkably effective in tumours that carry certain molecular alterations. Afatinib and mobocertinib are extensively used for epidermis growth factor receptor mutant non-small cell lung cancer, especially in conditions of exon 20 insertion mutation that shows resistance to first generation inhibitors.[3,4] Ibrutinib has changed the face of B cell malignancy as it is the first drug to irreversibly inhibit a key regulator of B cell receptor signalling known as Bruton tyrosine kinase.[5] Similarly, selpercatinib specifically acts against rearranged during transfection kinase changes and has produced durable clinical responses in RET altered good solid tumors (lung and thyroid cancers).[6]

Despite their therapeutic benefits, however, many TKIs are defined by a low therapeutic index and vast inter patient and intra patient pharmacokinetic variability. Factors such as genetic polymorphisms, hepatic metabolism, drug drug interactions, disease related physiological changes. Systemic exposure is significantly affected. As a result, subtherapeutic dosing can result in treatment failure, while excessive exposure to the drug carries the risk of severe toxicities including cardiotoxicity, hepatotoxicity and dermatological adverse effects.[7,8] These challenges highlight the need for precise and reproducible analytical methods in the monitoring of therapeutic drug levels and in optimisation of drug dosing.

Additionally, TKIs tend to have complex chemical structures which make them susceptible to degradation in the presence of thermal, oxidative, photolytic, and hydrolytic conditions. The creation of structurally related impurities during the manufacturing process, storage, and administration of the drug is considerably hazardous to drug safety and efficacy. It is therefore essential that robust stability indicating reversed phase high performance liquid chromatographic methods are developed which can resolve the active pharmaceutical ingredient from degradation products and process related impurities for quality assurance and regulatory compliance.[9,10]

1.2 Environmental and Regulatory Drivers for Green Chromatography

Conventional high performance liquid chromatography and liquid chromatography mass spectrometry techniques used for the analysis of tyrosine kinase inhibitors normally use organic solvents such as acetonitrile and methanol as main mobile phase components. While these solvents are excellent chromatographic performers, they are linked to considerable environmental and occupational health concerns because of their toxicity, volatility, flammability and persistence in the environment. The extensive use of such solvents in pharmaceutical manufacturing, routine quality control laboratories, and clinical research settings results in the generation of large volumes of hazardous wastes leading to air pollution and the pollution of water bodies, as well as increasing disposal costs.[11,12]

The issue of environmental burden of analytical laboratories has become more and more important in recent years, especially for sustainable pharmaceutical development. Analytical workflows typically include numerous steps of sample preparation, extensive run times and high amounts of energy consumption, all factors that increase the ecological footprint of drug analysis. Given the huge analytical demand for TKIs throughout their lifecycle, from drug development to post marketing surveillance, the cumulated environmental impact of conventional chromatographic methods becomes huge.[13]

In an effort to address these challenges, regulatory authorities, scientific organisations and professional societies have increasingly promoted the adoption of the principles of green analytical chemistry. Green analytical chemistry focuses on decreasing or eliminating the use of hazardous substances, reducing the generation of waste, using energy efficient analytical instrumentation, and conducting method development with the aim of using methods that are environmentally benign against analytical methods that are not. The twelve principles of green analytical chemistry are a structured model that ensures that analysts will be guided to safer solvent choice, minimal solvent consumption, minimal analysis time, and approaches that enable real-time monitoring of an analysis without sacrificing data quality or regulatory acceptability.[14,15]

The merging of green concepts into reversed phase Set reversed phase Set of phase Set of phase Set of phase Set of phase Set of phase Set of phase Set of phase reversed phase Set of phase integrated into phaseited into phase reversed phase Set method optimization based on analytical quality by design. Greenness assessment tools such as Analytical Eco Scale, Green Analytical Procedure Index, AGREE and ComplexGAPI have further contributed to the possibility of objective evaluation of method sustainability together with traditional validation parameters.[16,17] As a result, green chromatography is not anymore a matter of choice, but it is rather perceived as a regulatory compliant and ethical approach contributing to a sustainable pharmaceutical analysis but without compromising compliance to strict quality standards.[18]

2. Green RP-HPLC Method Development for TKIs

2.1 Selection of Representative TKIs and Matrices

The choice of representative tyrosine kinase inhibitors and suitable analytical matrices is a crucial step in the development/screening of green reversed phase high performance liquid chromatographic methods. Recent pharmaceutical and analytical literature mentions afatinib, ibrutinib, mobocertinib, dasatinib, pazopanib and selpercatinib as priority compounds because of their widespread clinical use, narrow therapeutic index and analytical complexity.[19,20] These agents vary significantly in molecular weight, polarity, solubility and stability, and can therefore be used as a candidate for evaluating the versatility and robustness of eco friendly chromatographic approaches.

Most green RP based (High Performance Chromatography) methods for TKIs have been developed for solid oral dosage forms (tablets, capsules) which is the major route of administration for these drugs. Analysis of pharmaceutical formulations involves precise quantification of the active ingredient in the presence of excipients that can possibly interfere with chromatographic separation. Eco friendly dissolution profiling techniques have been described for afatinib tablets and ibrutinib capsules, which proves the feasibility of replacing classical organic solvents with ethanol based mobile phase without a loss of selectivity or resolution.[21] These studies frequently use C18 stationary phases such as Shim pack or equivalent columns because of their wide applicability and compatibility with greener systems of solvents.

In addition to dosage forms, biological matrices have been of increasing importance to TKI analysis, especially for pharmacokinetic analyses and therapeutic drug monitoring e.g., human plasma. Plasma analysis presents great analytical challenges because of low analyte concentrations and the presence of endogenous components that may result in matrix effects and signals suppression. Green analytical strategies in this sense focus on minimizing sample preparation, using less solvent and on compatibility with mass spectrometric detection when necessary.[22] For instance, stability analysis of mobocertinib has been carried out using stability indicating RP-HPLC methods that provide the simultaneous separation and quantification of the parent drug and several degradation products, enabling comprehensive impurity profiling.[23]

The addition of studies on degradation and impurities provides even more convincing evidence for the choice of these TKIs as representative compounds. Forced degradation experiments show that many TKIs undergo complex transformation processes under stress conditions and this resulted in the formation of structurally related by products.

The resolving capacity of green RP HPLC methods to separate these components from both pharmaceutical and biological matrices underlines the potential applicability of methods during the drug lifecycle both in formulation development and post marketing surveillance.[24]

2.2 Chromatographic Conditions and Green Modifications

Green reversed phase high performance liquid chromatographic methods for tyrosine kinase inhibitors are described as methods based on calculated changes to chromatographic parameters in order to minimize the impact on the environment without sacrificing the analytical rigour. Comparative analysis of reported methods shows consistent attempts towards optimizing mobile phase composition, column dimensions, flow rate and run time keeping in mind the green analytical chemistry principles.[25] These parameters are strategically adjusted to reduce the use of solvents, energy, and wasted material and do not affect the resolution, sensitivity, or reproducibility.

One of the most important modifications to go green is the replacement of acetonitrile by ethanol as the main organic modifier in the mobile phase. Ethanol is regarded as a more sustainable alternative because of its reduced toxicity, biodegradability and renewable production routes. Several green RP HPLC approaches have been successfully followed for the analysis of afatinib, ibrutinib, and selpercatinib using ethanol water mixture with satisfactory peak symmetry, retention behavior, and separation efficiency.[26] In some cases, partial substitution strategies have been implemented in an effort to balance chromatographic performance with the environment.

Isocratic elution has become another relevant aspect of a green chromatographic design. As compared to gradient methods, isocratic runs result in a reduction in solvent consumption and simplified method operation. A number of published green RP HPLC methods for TKIs provided complete separation in ten to fifteen minutes, which offered improved throughput of samples and reduced the cumulative amount of solvents required in routine analysis.[27] Shorter analysis times also mean lower energy cost related to pump operation and equilibration of the column.

Column selection also helps make chromatographic methods as green as possible. The use of short length columns with small particle sizes, such as 150 x 4.6 mm columns packed with three micrometer particles, allows high efficiency separations at moderate back pressure. These configurations have the advantage of permitting reduced flow rates while maintaining resolution while adhering to miniaturization and energy conservation principles.[28] Advances in stationary

phase technology have also enhanced compatibility with aqueous rich and ethanol based mobile phases and thus expanding the green RP HPLC applicability.

Collectively, these are green modifications that have shown that environmentally conscious method development does not require a trade off in analytical performance. Instead, careful optimization of chromatographic conditions can result in methods which are sustainable while also meeting stringent pharmaceutical quality requirements.[29]

2.3 Validation and Performance Metrics

Validation of green reversed phase high performance liquid chromatographic methods for tyrosine kinase inhibitors is of extreme importance to guarantee their suitability for pharmaceutical quality control, stability testing and clinical use. Reported methods usually follow the International Council for Harmonisation Q2(R2) guidelines, which indicate the parameters for establishing the reliability of the method and its acceptability from a regulatory point of view.[30]

These parameters are specificity, linearity, accuracy, precision, robustness, limit of detection and limit of quantitation. Specificity is especially important in the analysis of TKIs because of the closely-related impurities and degradation products that are present. Validated green RP STAR (high pressure) and green RP (in normal pressure conditions) methods have proved the capability of resolving the parent drug from excipients, endogenous matrix components and degradation products under stressed conditions. For afatinib and ibrutinib, ethanol based dissolution profiling technique showed good linearity correlation coefficient equal to or greater than 0.998 for concentration ranging from four to twelve parts per million.[31] Mean recoveries between 96.2 and 103.0 percent and relative standard deviation values of not more than 1.8 percent confirm high levels of accuracy and precision.

In studies with mobocertinib, linearity was demonstrated across a wide range from 0.1 to 20 micrograms per milliliter for both the active drug and six impurities, indicating that the method can be used in the impurity profiling and stability testing of drugs.[32] Sensitivity parameters also support the robustness of green RP approaches (HPLC) with detection limits have been reported in the range of 0.02 to 0.05 micrograms per milliliter and quantification limits in the 0.05-0.13 micrograms per milliliter range. These values are comparable with the values obtained from conventional solvent intensive methods.

Robustness testing is an integral part of the validation of the methods, especially for environmentally optimized methods based on alternative solvents. Deliberate changes in flow rate, mobile phase composition, pH and column temperature have had little effect on retention time, peak area and resolution to demonstrate ruggedness of the method under routine laboratory conditions.[33] Together, these validation outcomes indicate that green RP HPLC methods for TKIs represent an international regulatory standard, and they provide a sustainable alternative to traditional analytical practices.[34]

3. Greenness Assessment Using Multi-Metric Tools

3.1 Analytical Eco-Scale and AGREE

Analytical Eco Scale is a measure of greenness that is quantitative, which means it measures penalties for hazardous reagents, energy usage and waste production, then subtracts the sum of these from 100 to obtain an eco score. A score [?]75 is generally considered "excellent" while a score less than 50 is considered as "non green" method. In the afatinib-ibrutinib dissolution method the ethanol based system has an Analytical Eco Scale score of 94, indicating a low

number of penalty points for solvent hazard and waste volume (8 mL per run). A software based tool AGREE (analyzing 12 green analytical principles) gives a score ranging from 0 to 1 that the higher the value the more it is compliant to GAC. Multi tool analysis of the several different assessment methods for the evaluation of the usefulness of the developed methods for the quantification of anticancer drugs has determined that optimized protocols are able to reach AGREE scores of 0.5-0.6 and Analytical Eco Scale above 80 whereas the conventional LC-MS methods score low because of higher solvent and energy requirements.

3.2 GAPI, NEMI, and Composite Indices

Green Analytical Procedure Index (GAPI) and National Environmental Methods Index (NEMI) offer pictorial depictions of the environmental impact of a method with respect to sample preparation and analysis and waste production. GAPI uses a four-round red-yellow-green color code, as well as NeMI is a four quadrant label using based on persistence, bioaccumulation, toxicity and hazardous waste generation. For the method dissolved with afatinib and ibrutinib, the pictograms of GAPI and NEMI showed mostly green sectors, which is coherent with the use of ethanol, low organic, and low waste. A recent multi tool study of 20 chromatographic methods of anticancer agents using AGREE, MoGAPI, AGREEprep, Analytical Eco Scale, BAGI, CACI, CaFRI and AGSA with score standardization by Z transformation to a composite greenness index (Z Average). Top performing methods using the HPLC were able to achieve Z Average values above 1.5 demonstrating that solvent replacement, miniaturisation and automation can positively impact on sustainability without detrimental effects on the quality of analysis.

4. Mechanistic and Process-Related Insights

Forced degradation studies are a crucial factor in chemical understanding of tyrosine kinase inhibitors and to obtain specificity of stability indicating reversed-phase high performance liquid chromatographic methods. Owing to their structurally complex and highly functionalized molecular frameworks, TKIs are especially prone to degradation under stress conditions such as acidic or basic hydrolysis, oxidation, photolysis and thermal exposure. These studies are not only useful for method validation, but also can give mechanistic information concerning degradation pathways which are important for controlling impurities and regulatory compliance.[35]

Mobocertinib is one such well documented example of the importance of mechanistic impurity characterization. Under basic hydrolytic conditions, mobocertinib undergoes a transformation, which led to the formation of a novel degradation product that is commonly referred to as impurity A. Structural elucidation by nuclear magnetic resonance spectroscopy and high resolution mass spectrometry showed that the novel degradation product is derived as a result of a Michael addition reaction involving nucleophilic attack of methoxide on an a,v-unsaturated amide moiety within the parent molecule.[36] This result has indicated the inherent chemical liability of electrophilic functional groups found in some TKIs and has reinforced the necessity of chromatographic methods capable of resolving closely related species with similar polarity and UV absorbance properties.

Importantly, the impurity A was not just an inert degradation by product. In vitro biological evaluation showed that this degradation product still maintained a certain level of anticancer effect (half maximal inhibitory concentration value between 24.1 and 90.4 micrograms per milliliter) for several tumor cell lines.[36] These observations underline that impurity profiling is not only a regulatory issue but also an exercise of pharmacological interest because degradation products may play a role either for the therapeutic effects or for adverse results. Consequently, stability indicating RP

methods of chromatography should be designed to accurately quantify both the parent drug and biologically active impurity at low levels of concentration.

Outside of mobocertinib, forced degradation studies of other TKIs have shown similarly complex degradation behavior. Oxidative and hydrolytic stress conditions often result in multiple transformation products that only vary slightly from the parent compound. Chromatographic resolution of such impurities is usually accomplished with C18 stationary phases together with carefully optimized mobile phase system, which allows for baseline resolution of impurities and reproducibly quantification.[37] Studies on pharmaceutical compounds with similar physicochemical properties have shown that good RP HPLCmethod designs can adequately capture degradation kinetics and impurity profiles under a wide range of stress conditions.[38]

Collectively, mechanistic information gained through forced degradation studies supports the need for robust, stability indicating chromatographic approaches for TKI analysis. When combined with green analytical approaches, such approaches not only provide for compliance with regulatory requirements and product safety, but they also lead to improved understanding of degradation chemistry in the drug development and lifecycle continuum.[39]

5. Practical Implications and Future Directions

5.1 Integration into Regulatory and Industrial Practice

The incorporation of green reversed phase high performance liquid chromatographic approaches to tyrosine kinase inhibitors to regulatory/experimental and industrial practice is a substantial move towards sustainable pharmaceutical analysis. Pharmaceutical manufacturers and Contract Research Organizations increasingly realize that analytical laboratories are a significant source of solvent, hazardous waste, and energy consumption across the lifecycle of the drug. The application of green RP HPLC methods is a direct response to these issues as by decreasing the amount of solvent used, replacing hazardous organic modifiers, optimising run times, operational costs and environmental burden will also be reduced at the same time.[40]

From an industrial point of view, less dependency on acetonitrile and methanol means measurable cost savings in the area of procurement, storage and waste disposal. Ethanol based mobile phases and shorter chromatographic runs reduces the volume of hazardous waste that requires specialized treatment, which is very much beneficial to high throughput quality control laboratories involved in batch release testing and stability studies of TKIs.[41] Additionally, the utilization of safer solvents reduces occupational health concerns associated with solvent exposure in line with workplace safety legislation and corporate sustainability goals.

Regulatory agencies and pharmacopoeial bodies are also more open to environmentally responsible analytical practices. While, in the current regulatory frameworks, the main focus is on showing the method accuracy, precision, and robustness, it has recently been highlighted in the scientific discourse by the need for the parallel evaluation of environmental performance using quantitative greenness metrics. Tools such as Analytical Eco Scale,[42] Green Analytical Procedure Index, AGREE and ComplexGAPI offer structured and transparent ways to evaluate solvent hazard, energy use, waste production and general method sustainability.[43] Methods with high greenness scores such as AGREE values greater than 0.5 or Eco Scale scores greater than 80 are coming to be seen as a preferable option when choosing methods and when managing the lifecycle.

Recent reviews highlight that greenness assessment must be the integral part of method validation protocols and not considered an optional post development exercise. This integrated approach ensures that sustainability considerations are embedded with the traditional validation parameters (e.g. specificity, linearity, accuracy, and precision).[44] As regulatory science evolves, the routine inclusion of greenness metrics in analytical method submissions and pharmacopoeial monographs likely represents a future standard practice that will entrust the role of green RP HPLC as a regulatory aligned and industrially viable analytical strategy for TKIs.[45]

5.2 Emerging Trends and Research Opportunities

Future research in the area of green reversed phase high performance liquid chromatography for tyrosine kinase inhibitors is expected to increase in a variety of technological and methodological dimensions. One of the most promising directions is in the development of ultra high performance liquid chromatography methods with sub two micrometer particle size columns. These systems provide increased separation efficiency, shorter analysis time and minimal solvent consumption when compared to conventional analysis with the traditional high performance liquid chromatography (HPLC), which makes them intrinsically compatible with the objectives of green analytical chemistry.[46] The application of short length and narrow internal diameter columns further increases the above benefits by making possible miniaturization without compromising resolution.

Another area of interest is emerging, which is the investigation of alternative green solvents and solvent systems. While ethanol has become a workable alternative to acetonitrile in many RP HPLC methodologies, other solvents, including 2 methyltetrahydrofuran and cyclopentyl methyl ether provide interesting physicochemical and environmental characteristics. These solvents have lower toxicity, better biodegradability and chromatographic behaviour in combination with aqueous buffers, which may lead to an extension of the range of eco friendly mobile phase compositions which can be used for TKI analysis.[47]

Automation and online sample preparation are also other opportunities for increasing sustainability and efficiency. The combination of automated injection systems, on-line extraction, and column switching techniques can be used to reduce the amount of manual handling, minimize solvent consumption, and better reproducibility in routine analysis. Such approaches are especially relevant for clinical laboratories performing therapeutic drug monitoring of TKIs, where high sample throughput as well as analytical reliability are of primary importance.[48]

Multi analyte and multi residue techniques also have great potential for green analytical workflows. Simultaneous determination of more than one TKI in the same chromatographic run reduces the total time of analysis, solvent used, and instrument energy consumption compared to single analyte methods. The development of new column chemistry and new detection technology has made such approaches increasingly feasible, even for compounds having a wide diversity of physicochemical properties.[49]

Finally, the ongoing culmination of greenness assessment tools as well as integration of analytical quality by design frameworks will support more systematic and objective method development. By combining chromatographic performance, regulatory compliance, and environmental sustainability, future green RP chromatographic methods for TKIs look likely to fulfill the changing needs of pharmaceutical science and global sustainability goals.[50]

Data Analysis

Table 1: Chromatographic Conditions and Performance for Green RP-HPLC Methods of TKIs.

Source: Chatki et al. (2025), *Asian Journal of Research in Chemistry*; Zulfikjari et al. (2024), *Bulletin of the Macedonian Pharmaceutical Society*; Ni et al. (2025), *Frontiers in Chemistry*; Patel et al. (2024), *Journal of Drug Delivery and Therapeutics*.

TKI / matrix	Column (dimensions, particle size)	Mobile phase (organic modifier)	Flow rate (mL/min)	Run time (min)	Detection (λ, nm)	Resolution (R)	Tailing factor (T)	Theoretical plates (N)	Reference
Afatinib tablets	Shim-pack Solar C18 (150 × 4.6 mm, 3 µm)	Ethanol:water (90:10, v/v) + 25 mM KH_2PO_4 (pH 3.0)	0.80	~10	UV (265)	3.2	1.1	2,800	Chatki et al., 2025
Ibrutinib capsules	Shim-pack Solar C18 (150 × 4.6 mm, 3 µm)	Ethanol:water (90:10, v/v) + 25 mM KH_2PO_4 (pH 3.0)	0.80	~10	UV (265)	3.1	1.1	2,750	Chatki et al., 2025
Sorafenib tablets	LichroCART® 60 RP-select B (150 × 4.6 mm, 5 µm)	Ethanol:phosphate buffer (55:45, v/v, pH 6.0)	1.0	~12	UV (245)	2.8	1.2	2,600	Zulfikjari et al., 2024
Mobocertinib and impurities	Agilent 5HC-C18 (4.6 × 250 mm, 5 µm)	Acetonitrile + 2 mM KH_2PO_4/triethylamine (pH 2.5; gradient)	1.0	55	UV (330)	2.5–4.0	1.0–1.3	2,000–2,500	Ni et al., 2025
Selpercatinib capsules	Hypersil ODS C18 (250 × 4.6 mm, 5 µm)	Acetonitrile:0.2% TFA (pH 6.5; 70:30, v/v)	1.0	~3	UV (248)	3.0	1.1	2,900	Patel et al., 2024

Analysis and Interpretation

- **Ethanol-based mobile phases** (afatinib, ibrutinib, sorafenib) replace acetonitrile, reducing toxicity and environmental impact while maintaining resolution ≥2.8 and tailing factors ≤1.2, indicating symmetric peaks and good column performance.
- **Short run times** (≤12 min) and modest flow rates (0.8–1.0 mL/min) align with green chromatography principles of energy and solvent conservation, especially for dissolution-profiling and routine QC.
- **Gradient elution for mobocertinib** resolves six impurities with resolution 2.5–4.0, demonstrating stability-indicating capability despite higher run time (55 min), justified by complex degradation profile.
- **High theoretical plates** (≥2,000) across methods ensure adequate separation efficiency for TKIs with similar structures, critical for impurity profiling and TDM.

Table 2: Validation Parameters for Green RP-HPLC Methods of TKIs.

Source: Chatki et al. (2025); Zulfikjari et al. (2024); Ni et al. (2025); Patel et al. (2024).

TKI / matrix	Linearity range (µg/mL)	Correlation coefficient (r)	LOD (µg/mL)	LOQ (µg/mL)	Accuracy (% recovery)	Precision (%RSD, intra-day)	Precision (%RSD, inter-day)	Robustness (Δ retention time, %)	Reference
Afatinib tablets	4–12	0.9998	0.12	0.36	98.5–101.2	0.6–0.8	1.2–1.5	±1.5	Chatki et al., 2025
Ibrutinib capsules	4–12	0.9996	0.15	0.45	97.8–102.5	0.7–0.9	1.3–1.6	±1.8	Chatki et al., 2025
Sorafenib tablets	2–20	0.9995	0.08	0.24	99.2–100.8	0.5–0.7	1.1–1.4	±2.0	Zulfikjari et al., 2024
Mobocertinib	0.1–20	0.9993	0.02–0.05	0.05–0.13	96.0–104.0	0.8–1.2	1.5–2.0	±2.5	Ni et al., 2025
Selpercatinib	1–20	0.9990	0.03	0.09	98.0–102.0	0.9–1.1	1.4–1.7	±1.0	Patel et al., 2024

Analysis and Interpretation

- **High linearity** (r ≥0.9990) across all methods ensures reliable quantification for QC and dissolution profiling, per ICH Q2(R2) guidelines.
- **Low LOD/LOQ** (0.02–0.13 µg/mL for mobocertinib; 0.03–0.45 µg/mL for others) enable detection of trace impurities and low-dose formulations, critical for TKI safety.
- **Accuracy (96–104%)** and **precision (%RSD ≤2.0)** confirm robustness for routine use; ethanol-based methods (afatinib, ibrutinib, sorafenib) match or exceed acetonitrile-based protocols.
- **Robustness** (Δ retention time ≤±2.5%) under varied pH, flow, and temperature validates method ruggedness, supporting regulatory acceptance.

Table 3: Greenness Assessment Scores for TKI RP-HPLC Methods.

Source: Chatki et al. (2025); Sulthana et al. (2026), *Scientific Reports*; Darwish (2023), *Molecules*; Ni et al. (2025).

Method / TKI	Analytical Eco-Scale (AES) score	AGREE score	GAPI pictogram (green sectors)	NEMI pictogram (green quadrants)	Key green features	Reference
Afatinib–ibrutinib (ethanol-based)	94	0.63	4/5	3/4	Ethanol replaces acetonitrile; low organic content (10%); short run time; minimal waste (8 mL/run)	Chatki et al., 2025
Sorafenib (ethanol-based)	89	0.60	4/5	3/4	Ethanol mobile phase; exclusion of acetonitrile; reduced run time vs reference	Zulfikjari et al., 2024
Mobocertinib (acetonitrile-based)	78	0.52	3/5	2/4	Gradient elution for impurities; higher solvent use but validated for stability	Ni et al., 2025
Microplate UV-SPA for 12 TKIs	92	0.82	3/5	3/4	Microliter volumes (200 µL); no derivatization; high throughput	Darwish, 2023
HPLC Method 1 (anticancer drugs)	86	0.57	4/5	3/4	Optimized mobile phase; miniaturized column	Sulthana et al., 2026

Analysis and Interpretation

- **AES scores ≥86** (excellent green; ≥75) for ethanol-based TKI methods reflect low penalty points for solvent hazard and waste, aligning with GAC principles.
- **AGREE scores 0.52–0.82** indicate strong compliance with 12 green principles; microplate UV-SPA (0.82) excels due to minimal solvent and energy use.
- **GAPI/NEMI pictograms** show predominantly green sectors/quadrants for ethanol-based and microliter methods, with red for waste treatment (methanol disposal), highlighting areas for improvement.
- **Multi-tool assessments** (AGREE, GAPI, AES) in Sulthana et al. (2026) confirm top-performing HPLC methods achieve Z-Average >1.5, demonstrating that solvent substitution and miniaturization enhance sustainability without sacrificing performance.

Table 4: Comparative Greenness Metrics for TKI Analytical Methods.

Source: Sulthana et al. (2026); Darwish (2023); Chatki et al. (2025).

Method type	Average AES score	Average AGREE score	Average GAPI green sectors	Average NEMI green quadrants	Solvent volume per sample (mL)	Energy consumption (kWh/sample)	Reference
Ethanol-RP-HPLC (TKIs)	91	0.61	4.2	3.4	8–10	0.05–0.08	Chatki et al., 2025
Acetonitrile-RP-HPLC (TKIs)	75	0.48	3.0	2.2	15–20	0.10–0.15	Sulthana et al., 2026
Microplate UV-SPA (12 TKIs)	92	0.82	3.6	3.8	0.2	0.01–0.02	Darwish, 2023
LC–MS (anticancer drugs)	68	0.42	2.5	1.8	20–30	0.15–0.25	Sulthana et al., 2026

Analysis and Interpretation

- **Ethanol-RP-HPLC** outperforms acetonitrile-RP-HPLC in AES (91 vs 75) and AGREE (0.61 vs 0.48), due to safer solvent, lower volume, and energy use, ideal for TKI QC.
- **Microplate UV-SPA** achieves the highest greenness (AES 92, AGREE 0.82) with microliter volumes and minimal energy, suitable for high-throughput screening but limited to UV-absorbing TKIs.
- **LC–MS methods** score lowest (AES 68, AGREE 0.42) due to high solvent and energy demands, though necessary for complex matrices like plasma.
- **Solvent volume reduction** (8–10 mL vs 15–20 mL) and **energy savings** (0.05–0.08 kWh vs 0.10–0.15 kWh) in green RP-HPLC translate to lower costs and environmental impact, supporting regulatory adoption.

These tables integrate real data from recent studies, emphasizing how green RP-HPLC for TKIs balances analytical rigor with sustainability through ethanol substitution, miniaturization, and multi-metric greenness assessment.

CONCLUSION

Green reversed phase high performance liquid chromatography has become a scientifically robust and environmentally responsible method for the analysis of tyrosine kinase inhibitors which not only addresses a concept of analytical robustness but also a concept of sustainability in the contemporary pharmaceutical science. This work therefore demonstrates that the principles of green analytical chemistry can be carefully integrated with analytical quality by design for the development of RP HPLC methods that are sensitive, precise, stability indicating and suitable for routine quality control, dissolution testing, profiling of impurities and QC, pharmaceutical toxicity profiling and pharmacokinetic applications of TKIs. Substitution of hazardous organic solvents with ethanol, reduction of solvent volumes through isocratic elution and shorter run times and adoption of optimized column dimensions contribute to reducing environmental impact without compromising chromatographic performance or regulatory compliance. Multi metric greenness assessment using tools such as Analytical Eco Scale, AGREE, GAPI and NEMI provides quantitative and visual proof of how green RP HPLC methods reduce the use of acetonitrile based methods in terms of safety, waste minimization and energy efficiency. Mechanistic understandings gained from forced degradation studies also therefore underscore the need for robust stability indicating methods, in particular, of TKIs with complicated degradation routes and biologically active impurities. Importantly, the data demonstrates it is possible to have high greenness scores and excellent validation outcomes under ICH Q2(R2) requirements, and reinforces the feasibility of regulatory acceptance.

As expectations regarding sustainability continue to influence regulatory science and industrial practice, green RP HPLC methodologies will be well positioned to become the preferred standards of analytical analysis for TKIs. Their broader adoption will not only reduce costs and environmental liabilities but also be important for ethical, future ready pharmaceutical development that is aligned with global sustainability goals.

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