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REVIEW ARTICLE

Analytical Strategies for Assaying Newly Approved Anticancer Agents: A Comprehensive Review

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ABSTRACT
The rapid evolution in anticancer therapeutics, with several targeted therapies, biologics, and antibody–drug conjugates (ADCs) approved recently, demands robust and precise analytical methods to support quality control, pharmacokinetics, and regulatory approval. The following review is a state-of-the-art review of contemporary assay technologies being used for the analysis of recently approved anticancer drugs. Focuses on the choice and use of advanced chromatographic methods like high-performance liquid chromatography (HPLC), ultra-high-performance liquid chromatography (UHPLC), and liquid chromatography–mass spectrometry (LC-MS/MS), as well as spectroscopic and bioanalytical platforms like ELISA, UV-visible spectroscopy, fluorescence, and FTIR. The article considers future technologies like biosensors and nanotechnology-based assays and provides insights into the prospects for their use in point-of-care diagnostics and high-sensitivity detection. Particular emphasis is placed on method validation parameters—accuracy, precision, linearity, specificity, and sensitivity—set by ICH, FDA, and EMA guidelines. Additionally, the review provides a summary of sample preparation methods required for assay performance and highlights pitfall issues introduced by challenging biological matrices. Targeted therapy and immunotherapy case studies provide real-world analytical perspective, and a comparative assay method comparison demonstrates their strength and limitation. By harmonizing regulatory views with technological advances and understanding, the present paper offers a strategic approach to the decision and validation of assays suitable for the particular analytical needs of contemporary anticancer drug development.
Keywords*: ADCs, HPLC, FTIR, FDA, anticancer therapeutics*

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INTRODUCTION

Background and Rationale

Cancer still continues to be one of the major causes of morbidity and mortality globally and a call for expedited development and approval of newer anticancer drugs with better efficacy and safety(1). With the ongoing development of new targeted agents, Immunotherapeutics, and small molecule inhibitors by the drug industry, there has been an increased call for strong, sensitive, and reliable analytical tools for their assay(2). Sensitive assay procedures are problematic not only for precise dosage determination in pharmaceutical products but also for drug stability, quality control, regulatory issues, and therapeutic effectiveness(3). Furthermore, the intricate chemical structures and multiple mechanisms of new anticancer drugs make it difficult for conventional analytical methods to be sensitive, selective, and reproducible(4). Thus, the use of sophisticated analytical techniques—HPLC, UHPLC, and LC-MS/MS—

has been critical for the quantitative and qualitative determination of these drugs(5). This review attempts to point out the novel developments and methodological strategies to the assay of newly approved anticancer medications, i.e., their applications in drug development and clinical use. HPLC LC-MS/MS UHPLC(6).

Scope of the Review

This review is specifically on the analytical approaches applied to the assessment of new anticancer drugs approved in recent times, specifically those licensed in the past ten years by prominent regulatory bodies like the U.S. FDA and European Medicines Agency (EMA). The review narrows to small-molecule chemotherapeutics, targeted agents, and biologics such as kinase inhibitors, monoclonal antibodies, and immune checkpoint inhibitors that have shown considerable clinical promise(7). It points out the different analytical methods employed for quantitative estimation of these drugs in bulk as well as formulation samples. Through a critical overview of existing assay methods like HPLC, LC-MS/MS, UV-spectrophotometry, and other novel techniques, the review intends to give a comparative insight into their appropriateness, sensitivity, and regulatory acceptability(8). The scope of the thesis does not include detailed pharmacodynamic and pharmacokinetic description but rather the method development, validation, and issues encountered in actual assaying of these new therapeutic drugs(9).

Objectives

The aim of this review is to provide a critical summary of the analytical techniques used for the assay of newly approved anticancer pharmaceuticals(10). As new oncology drugs keep surfacing, there is an increasing need to understand and assess the approaches being undertaken to guarantee the quality, safety, and potency of such medicines(11). This review aims to explore the most accessible and validated techniques, e.g., high-performance liquid chromatography (HPLC), ultra-high-performance liquid chromatography (UHPLC), liquid chromatography-mass spectrometry (LC-MS/MS), and other spectrophotometric methods(12). The primary issue is to assess the reliability, sensitivity, and regulatory acceptability of these analysis techniques in bulk drug substances and pharmaceutical formulations. Moreover, the review attempts to find the most critical issues related to method development and validation, especially for complex molecules with distinctive chemical characteristics(13). By thorough examination of these facets, this review aims to provide useful information for researchers, pharmaceutical scientists, and regulatory experts working in anticancer drug analysis and quality control(14).

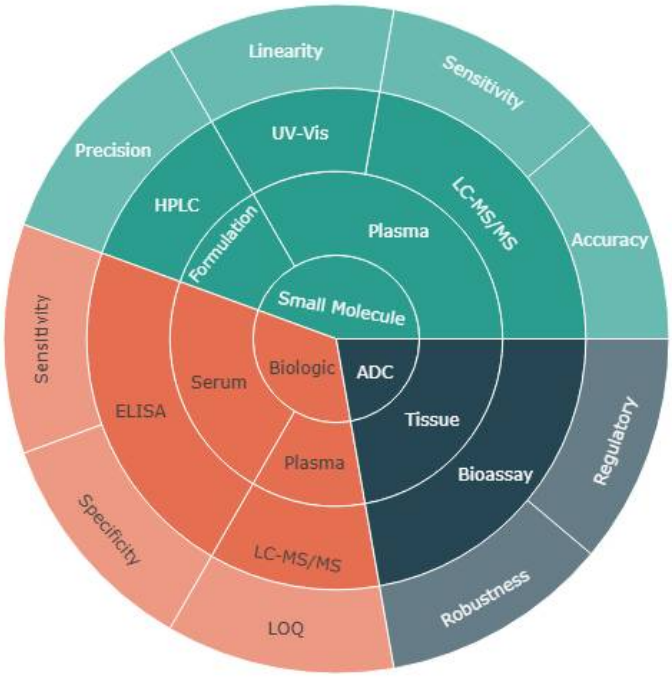

Figure 1. *Flowchart of holistic analytical approach to development and validation of recently launched anticancer drugs. The flowchart illustrates a hierarchical approach starting from the drug class classification—i.e., small molecules, biologics, and antibody–drug conjugates (ADCs). The drug class is linked to unique biological or pharmaceutical sample matrices like plasma, serum, formulation, or tissue that determine the choice of analytical method. Analytical techniques are LC-MS/MS, HPLC, ELISA, UV-Visible*

spectrophotometry, and cell-based bioassays, selected based on drug type compatibility as well as matrix characteristics. The outer ring indicates the most crucial validation parameters based on regulatory needs like sensitivity, specificity, accuracy, precision, robustness, and compliance with regulations (ICH/FDA/EMA). This figure illustrates that assay strategies are tailored based on the physicochemical character of the drug, biological complexity of the matrix, and the requirement to achieve tough quality and performance requirements in pharmaceutical analysis.

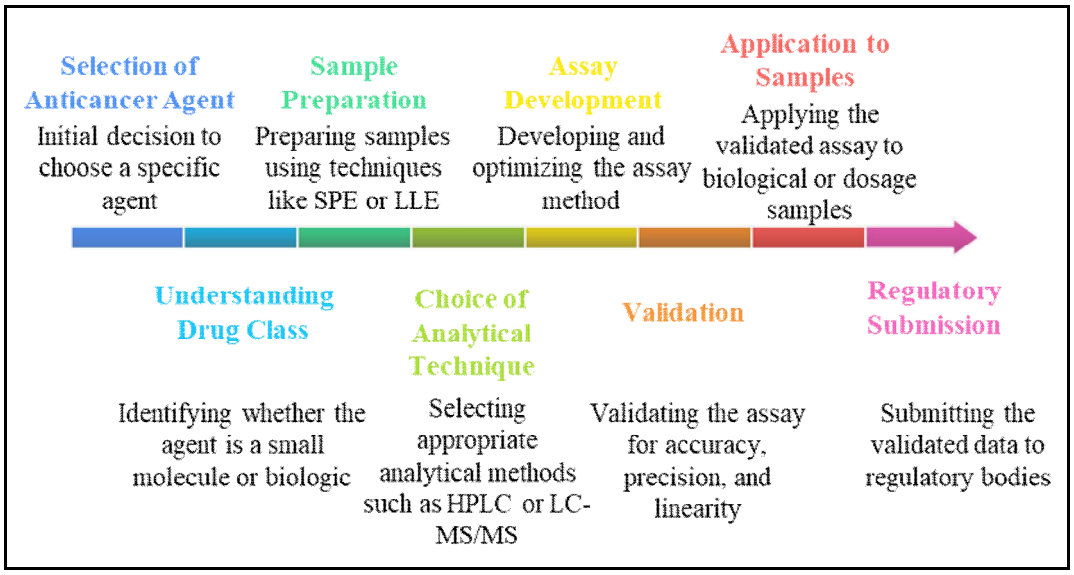

Fig no. 2: Anticancer Agent Selection and Validation Process

Overview of Newly Approved Anticancer Drugs

Over the past decade, cancer treatment has been revolutionized with the approval of many anticancer drugs that are more efficacious, selective, and less systemically toxic than conventional chemotherapeutic agents(15). The recently approved new drugs are of a few major categories, which include targeted therapy, immunotherapy, antibody-drug conjugates (ADCs), and small-molecule inhibitors(7). Targeted agents like tyrosine kinase inhibitors (e.g., osimertinib, lorlatinib) aim to specifically inhibit the growth of tumor cells and proliferation and survival signal pathways selectively, usually against mutant proteins or overexpressed receptors(16). Immunotherapies like immune checkpoint inhibitors pembrolizumab and nivolumab aim at the augmentation of the body's immune effect to identify and kill cancer cells by inhibiting the PD-1/PD-L1 pathway(17). ADCs, like trastuzumab deruxtecan, are a hybrid strategy that takes cytotoxic drugs directly to cancer cells in the form of a monoclonal antibody carrier with the aim of achieving maximum efficacy while reducing off-target exposure(18). Their action is representative of a trend toward non-selective cytotoxicity to precision therapy against tumor biology in a specific patient(19). A trend in the rate of anticancer agent development is seen in the sequence of approvals in the past decade(20). Regulatory agencies like the United States Food and Drug Administration (FDA), European Medicines Agency (EMA), and India's Central Drugs Standard Control Organization (CDSCO) have introduced fast-track review mechanisms to allow quicker access to life-saving medicines. Medicines such as larotrectinib, for tumors with NTRK gene fusion regardless of tumor type, are indicative of the new tissue-agnostic paradigm of cancer treatment(21). In addition, increases in orphan drug and breakthrough therapy designations indicate increasing focus on the treatment of rare aggressive cancers of unmet medical requirements(22).

Table 1 Summary of analytical methods used for recently approved anticancer drugs, including assay purpose and sample matrix.

Drug Name	Approval Year	Class	Analytical Method Used	Matrix	Purpose of Assay	Reference
Tucatinib	2020	HER2 Tyrosine Kinase Inhibitor	LC-MS/MS	Human Plasma	Pharmacokinetics	(23)
Belantamab Mafodotin	2021	Antibody-Drug Conjugate	ELISA	Serum	Therapeutic monitoring	(24)
Mobocertinib	2021	EGFR Inhibitor	UHPLC	Tablet Dosage Form	Content uniformity, assay	(25)
Tislelizumab	2022	PD-1 Inhibitor	ELISA + Bioassay	Serum	Immunogenicity testing	(26)
Trastuzumab Deruxtecan	2023	ADC (Anti-HER2)	LC-MS/MS + Immunoassay	Plasma + Injection	Potency + PK profiling	(27)

The therapeutic impact of these innovative treatments is significant, frequently establishing prolonged survival, enhanced response rates, and quality of life in patients with advanced or refractory cancers(28). Nevertheless, the characteristics of such drugs necessitate similar highly sophisticated and dependable analysis tools for their assay. Such quantitation not only proves to be essential to preserve therapeutic uniformity and safety, but also to provide regulatory compliance at the time of manufacturing, quality control, and post-marketing surveillance(14). Their increasing therapeutic efficacy is thus deserving of the utilization of precise, validated, and sensitive assay techniques for enabling their clinical safe and effective use(29).

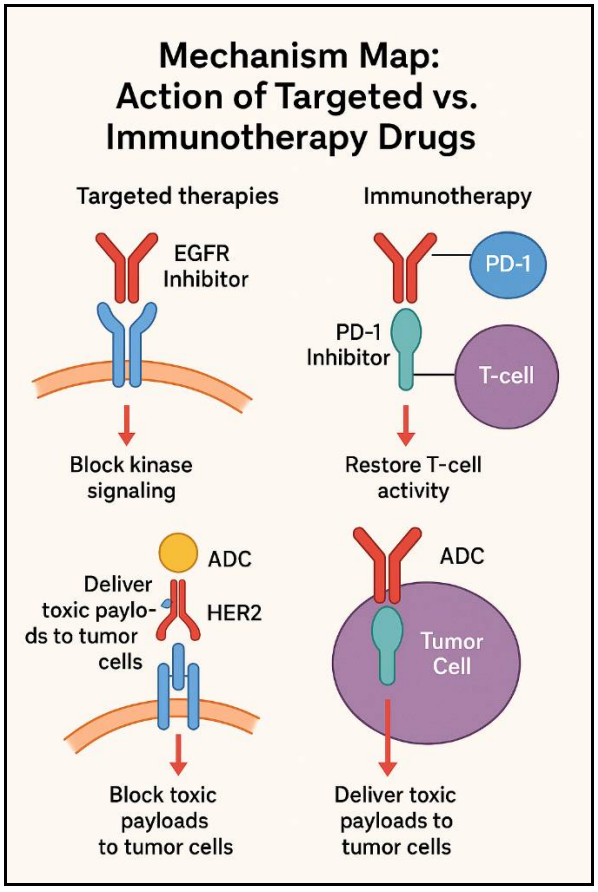

Figure no. 3 Mechanism-based anticancer drug classification of new drugs with focus on major drug classes and targets. The figure shows representative therapeutic classes like small molecules, monoclonal antibodies, ADCs, and checkpoint inhibitors, with their respective mechanisms of action and clinical relevance.

ANALYTICAL TECHNIQUES EMPLOYED IN ASSAY DEVELOPMENT

Table 2. Comparative analysis of the analytical methods utilized in anticancer drug assays with emphasis on detection systems, appropriateness of sample, advantages, and disadvantages.

Analytical Technique	Detection System	Sample Type	Suitable for Drug Type	Strengths	Limitations	Reference
HPLC	UV / PDA Detector	Bulk Drug, Tablet, Capsule	Small Molecules	Cost-effective, widely used	Lower sensitivity in complex matrices	(30)
LC-MS/MS	Triple Quadrupole MS	Plasma, Serum, Tissue	Kinase Inhibitors, ADCs	High sensitivity and specificity	Expensive, needs skilled personnel	(31)
ELISA	Colorimetric	Biological Fluids	Monoclonal Antibodies	Biologically relevant, validated method	Cross-reactivity, antibody dependent	(32)
Biosensor Technology	Electrochemical / Optical	Plasma, Serum	ADCs, Small Molecules	Rapid, portable, point-of-care use	Not widely standardized or validated	(33)
UV-Visible Spectro photometry	UV Detector	Bulk Drug	Simple APIs	Quick, economical, no derivatization	Poor selectivity in mixtures	(34)

Chromatographic Methods

Chromatographic techniques are the cornerstone of contemporary pharmaceutical chemistry and are widely employed to assay anticancer medication because they are sensitive, reproducible, and capable of separating intricate mixtures(35). They do this by distributing the constituents of a sample between a stationary phase and a mobile phase in a way that individual detection and measurement are made feasible. Of all the chromatographic methods, High-Performance Liquid Chromatography (HPLC), Ultra-High-Performance Liquid Chromatography (UHPLC), and Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) are extensively employed in the determination of newly approved anticancer drugs(36). These provide the selectivity and sensitivity required to handle complex chemical structures and low therapeutic levels of novel oncological agents(37). Their use for a variety of sample matrices such as bulk drug, dosage forms, and body fluids renders them inevitable in research and regulatory environments(38).

High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is an old, tried analytical technique for the separation and quantitation of active pharmaceutical ingredients (APIs), e.g., anticancer drugs(39). HPLC is based on partitioning between a mobile phase (liquid solvent) and stationary phase (typically silica-based columns), where parts are discriminated according to their interaction with the phases(40). HPLC is an adaptable method of analysis, which can separate polar as well as non-polar compounds(41).

The major merits of HPLC are satisfactory resolution, satisfactory sensitivity, and compatibility for routine quality control and stability testing. It facilitates quantitation of drugs despite the presence of impurities and degradation products(42). Its drawbacks, however, include longer analysis times, higher solvent consumption, and lack of sensitivity to determine ultra-trace levels except when used in combination with other detectors. In spite of these, HPLC is employed most frequently in the pharma lab because of its predictability and regulatory acceptability(43).

Ultra-High-Performance Liquid Chromatography (UHPLC)

Ultra-High-Performance Liquid Chromatography (UHPLC) is a next-generation HPLC that employs columns with sub-2-micron particles and runs at much higher pressures(44). This configuration increases separation efficiency so that more speed run times and greater resolution are possible. UHPLC is particularly suited where the priority is analysis speed, for example, high-throughput drug screening or stability testing(45). Relative to conventional HPLC, UHPLC allows for shorter retention times, increased peak capacity, and reduced solvent usage, thus being a cost- and environment-saving choice. Its sensitivity is high and makes it ideal for the analysis of analytes at low concentrations, for example, cytotoxic drugs or trace impurities in anticancer therapies(46). The only limitation is the requirement of specialized equipment and maintenance, which may not be cost-effective for all laboratories.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) is considered one of the most powerful techniques for the assay of anticancer agents, especially in complex biological matrices like

plasma, urine, or tissue homogenates(47). After chromatographic separation, the analytes are ionized and identified based on their mass-to-charge ratio (m/z), and further fragmented to provide structural information, enhancing specificity(48). LC-MS/MS offers unmatched sensitivity, selectivity, and the ability to quantify analytes at picogram or nanogram levels, which is crucial for drugs with narrow therapeutic windows. It also supports multi-analyte detection in a single run, making it ideal for pharmacokinetic and therapeutic drug monitoring studies(49). Despite its significant advantages, LC-MS/MS requires expensive instrumentation, skilled personnel, and careful method optimization, limiting its routine use in some settings. However, in oncology drug development and bioanalysis, it has become an indispensable tool(50).

Spectroscopic Methods

Spectroscopic methods are of primary importance in qualitative and quantitative analysis of drugs, e.g., anticancer drugs(10). Spectroscopic methods are founded on interaction of electromagnetic radiation with matter in which changes in absorption, emission, or vibration provide vast information regarding the structure and concentration of analytes. Spectroscopic methods are simpler, faster, and more cost-effective compared to chromatographic methods. These are applied to a significant extent in regular quality control, determination of functional groups, and preliminary assay methods(39). Out of all the spectroscopic techniques, UV-Visible Spectrophotometry, Fluorescence Spectroscopy, and Fourier Transform Infrared (FTIR) Spectroscopy are generally used to investigate anticancer drugs because they are legitimate and available(51).

UV-Visible Spectrophotometry

UV-Visible Spectrophotometry is one of the most fundamental and widely used techniques in pharmaceutical analysis. It is based on the principle that molecules absorb ultraviolet or visible light at specific wavelengths, leading to electronic transitions between molecular orbitals. The absorbance is directly proportional to the concentration of the analyte, following Beer–Lambert's Law(52).

This technique is commonly used for the quantitative estimation of anticancer drugs in both bulk and formulation forms. It is particularly useful in early-stage drug development for determining solubility, pKa, and preliminary assay studies(53). Case studies include the assay of drugs like methotrexate, imatinib, and tamoxifen using simple UV methods(10). The advantages of UV spectrophotometry are its simplicity, low cost, and minimal sample preparation. However, its limitations include lower specificity and sensitivity compared to chromatographic methods, making it unsuitable for complex mixtures or biological matrices without prior extraction or purification(54).

Fluorescence Spectroscopy

Fluorescence spectroscopy is an ultrasensitive method of measuring the light emission by a material upon illumination or exposure to electromagnetic radiation. When light of a definite wavelength is absorbed by a molecule, the molecule emits light as it comes back to ground state after being excited to a higher energy state. Fluorescence intensity varies directly with analyte concentration(55). This approach is mainly useful in the quantification of low concentrations of anticancer drugs, for example, doxorubicin, paclitaxel, and some kinase inhibitors, in plasma or serum(47). Fluorescence spectroscopy has widespread use in bioanalytical work, drug-drug interaction studies, and microanalytical work. It is very sensitive and selective in nature but can only work if the analyte itself or its derivative is fluorescent in nature. Quenching effects, photobleaching, and matrix effects can also be troublesome(56).

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) Spectroscopy is a vibrational spectroscopic method that gives structural information by analyzing the absorption of infrared radiation by chemical bonds within a molecule(57). IR radiation is absorbed by molecular functional groups at characteristic frequencies, giving a molecular fingerprint of the molecule(58). FTIR use in anticancer drug analysis is fundamentally for structural identification, functional group identification, and API-excipient compatibility studies. FTIR is further used in the determination of polymorphs of anticancer drugs such as anastrozole and 5-fluorouracil. FTIR is a non-destructive technique, and it also has negligible sample preparation requirements, with frequent employment in solid-state identification. But its drawback is lesser quantitative potency and a likelihood of overlapping of the bands of absorption, hence is not as suitable for complex mixtures or trace analysis(59).

Immunoassays and Bioassays

Immunoassays and bioassays represent an important set of analytical methods of special value in the quantitative and qualitative determination of biologics and therapeutic proteins increasingly prevalent among newly approved anticancer drugs(60). Of these, enzyme-linked immunosorbent assay (ELISA) is among the most prevalent methods due to its high specificity, sensitivity, and applicability for high-throughput quantitation. ELISA is an antigen–antibody principle technique wherein the analyte to be

found (most often a protein or a monoclonal antibody) is bound by a known conjugated antibody with an enzyme(61). Upon addition of substrate, the enzyme catalyzes a detectable signal that may be a color change in proportion to the concentration of analyte. ELISA has been extensively applied in the analysis of biologics like bevacizumab, nivolumab, and trastuzumab in formulation analysis and pharmacokinetic and immunogenicity analysis(62). ELISA is especially useful in therapeutic drug monitoring and drug-antibody interaction assessment. In addition to ELISA, other bioanalytical assays like radioimmunoassay (RIA), western blot, flow cytometry, and electrochemiluminescence immunoassays (ECLIA) are also utilized in oncology drug development and clinical studies(63). These approaches provide enhanced selectivity and sensitivity, which are ideal for the detection of low-abundance biomolecules in complicated biological matrices such as blood, serum, or tissue. Furthermore, cell-based bioassays, where the biological activity of a compound is measured within intact cells, are especially valuable in determining the functional potency of anticancer drugs, particularly those that target specific cellular pathways or immune checkpoints. These assays are pivotal in the qualification and design of immune-modulating therapy, monoclonal antibodies, and ADCs(64). Nonetheless, immunoassays and bioassays are not free from problems. They involve very specific reagents (e.g., monoclonal antibodies), rigorous validation procedures, and are most frequently plagued by matrix interferences and cross-reactivity(65). Optimization and development are also expensive and time-consuming. Notwithstanding these limitations, immunoassays and bioassays are still priceless in assay and characterization of anticancer therapy of today, providing biologically significant insights not available by pure physicochemical procedures(65).

Emerging and Novel Methods

As anticancer drugs are becoming more sophisticated in terms of newly developed complexity particularly biologics and targeted therapy conventional analysis methods occasionally lag in sensitivity, pace, and in-capability of real-time detection. Hence, scientists have engineered newer and advanced analytical methods in the form of biosensor technologies and nanotechnology-based assays(66). These next-generation tools are supporting more and more qualitative and quantitative analysis of anticancer drugs, particularly in clinical and point-of-care applications(67).

Biosensor Technologies

Biosensor technologies include analytical devices which integrate a biological recognition component, e.g., an enzyme, antibody, aptamer, or nucleic acid, and a physical transducer for converting the biological interaction to a quantifiable signal(68). Biosensors are renowned for delivering real-time, label-free, and extremely sensitive analyte detection, including anticancer agents. The ensuing signal is generally optical, electrochemical, piezoelectric, or thermal in nature depending on biosensor construction. In cancer, biosensors have demonstrated immense potential in the quantitation of trace amounts of chemotherapy agents such as doxorubicin, methotrexate, and cisplatin in serum or plasma(69). Electrochemical biosensors, for instance, have been extensively used because of their sensitivity, rapid response, and suitability for miniaturization(69). Optical biosensors such as surface plasmon resonance (SPR)-based biosensors have facilitated online monitoring of drug-protein interactions, allowing for pharmacodynamic and pharmacokinetic studies. Additionally, coupling biosensor platforms with wearables and microfluidics for point-of-care testing and real-time monitoring is promising to reveal cancer therapy(70). As much as it presents many advantages, biosensors must be calibrated with best-in-class and with sensitive optimization, and their extensive clinical applications remain undermined by regulatory and standardization problems.

Nanotechnology-Based Assays

Nanotechnology assays employ nanomaterials like gold nanoparticles (AuNPs), quantum dots (QDs), carbon nanotubes, and magnetic nanoparticles to improve the analytical performance of standard assay systems(71). These nanomaterials possess exclusive properties, e.g., high surface area, improved optical and electric properties, and good drug-binding, and are thus superb candidates to improve sensitivity and selectivity in drug detection. For anticancer drug analysis, colorimetric assays based on gold nanoparticles have been applied for quick detection that is visible color change upon drug interaction(72). Quantum dots have also been utilized in fluorescent immunoassays that allow for multiplexed detection of a panel of drugs or biomarkers within a single assay. Carbon nanotubes and magnetic nanoparticles have also been utilized after functionalization with antibodies or aptamers to selectively interact and detect anticancer agents at nanomolar or even picomolar levels(73). These nanotechnology-based assays are of great promise for the drug analysis in biological matrices and in real-time cellular imaging applications. But problems such as batch-to-batch reproducibility, toxicity issues, as well as regulation need to be addressed before these systems can be largely applied in clinical and industrial settings(74).

SAMPLE PREPARATION AND EXTRACTION METHODS

Sample preparation is a key component of the analytical process, particularly in biological and pharmaceutical matrices that are complex. In effective assay of anticancer drugs newly synthesized and approved, suitable sample preparation is responsible for analyte stability, sensitivity, and minimizing matrix interferences(75). It also ensures reproducibility and regulatory acceptability. The involved techniques are solid-phase extraction (SPE), liquid-liquid extraction (LLE), protein precipitation, and filtration(76). These analyses are necessary for the separation of the compound of interest from the impurities and for their concentration to be quantified at detectable levels, especially in biological matrices such as plasma, serum, or tissue homogenates. Evolving requirements for high-throughput, precise, and sensitive analyses have also driven the design of automated and miniaturized sample handling systems for rapid and efficient analysis of advanced anticancer treatments(77).

Matrices and Challenges Thereunto

Anticancer drug analysis covers a large variety of sample matrices ranging from bulk drug substance and formulation to biological matrices like blood, plasma, serum, urine, and tissue samples(10). Each of these matrices presents unique challenges in terms of sample complexity, interferences, and analyte stability. Biological fluids, for example, are known to contain proteins, lipids, and other endogenous constituents that can interfere with the assay performance, decrease sensitivity, or lead to ion suppression with mass spectrometry(78). Solid dose products can include excipients that influence extraction efficiency or chromatographic behavior. Tissue specimens should be homogenized and handled gently to avoid degradation of labile analytes. Moreover, the extensive lipophilicity, solubility problems, or instability at certain pH of most recently launched anticancer agents are additional sample preparation issues(79). Thus, appropriate extraction procedure selection and optimization specific to the matrix is critical for obtaining accurate, reproducible results.

Extraction and Cleanup Procedures

Effective extraction and clean-up methods are crucial to separate the drug from its matrix and eliminate interfering material. Solid-Phase Extraction (SPE) is among the most commonly used techniques used as a result of its selectivity, recovery yield, and suitability in most systems of detection(76). SPE entails the flow of the sample through an adsorbent material packed in a cartridge, where the analyte is adsorbed and later eluted with a suitable solvent(80). Liquid-Liquid Extraction (LLE) is another popular technique that involves separation of compounds according to solubility in two immiscible phases—the most typical being an aqueous phase and a solvent organic phase. LLE is low-cost and straightforward but can be non-selective and laborious. Other techniques such as protein precipitation, applied mainly for plasma or serum samples, provide a convenient and rapid means of eliminating proteins prior to LC or LC-MS/MS analysis(81). Technique selection depends on the matrix, drug characteristics, required sensitivity, and downstream analysis method. Clean-up properly not only enhances accuracy and precision but also the life of chromatography columns and detectors(82).

Automation and High-Throughput Approaches

With the growing need for high-throughput and rapid anticancer drug screening, automation of sample preparation has been a major trend of recent pharmaceutical analysis(83). Automated liquid-handling stations, online sample preparation modules, and robotic SPE systems are used today in an effort to enhance throughput, reproducibility, and safety. These systems minimize labor, human mistakes, and ensure uniform sample treatment for hundreds of samples in a matter of hours. High-throughput platforms integrated with LC-MS/MS systems enable direct injection of clean-up samples, simplifying workflows without compromising analytical quality(84). Microextraction techniques like solid-phase microextraction (SPME) and dispersive liquid-liquid microextraction (DLLME) are being made smaller and automated for use on small-volume samples(85). These developments have specific applications in clinical pharmacokinetic studies, where drug monitoring with timely rapidity is essential. Generally, automation not only boosts efficiency but also supports compliance with regulations through improved traceability and consistency of the data on analysis runs(86).

METHOD QUALITY ASSURANCE AND VALIDATION

Analytical method validation is an important step towards the quality control assurance that the procedures of the assay applied to anticancer agents are precise, accurate, and reproducible under specified conditions. It ensures that the method will be appropriate for its purpose, whether for general quality control, stability testing, or bioanalytical research(87). With growing structural complexity of recently launched anticancer drugs and its high regulatory interest, method validation became an indispensable requirement for both small molecules and biologics. It includes diverse parameters like

accuracy, precision, specificity, linearity, detection limits, and robustness(88). In addition, compliance with international regulatory standards, including those from the FDA (U.S. Food and Drug Administration), EMA (European Medicines Agency), and ICH (International Council for Harmonisation), is mandatory in global pharmaceutical compliance. Comparative case studies also reveal that proven methods are seamlessly performed in practice scenarios to illustrate their efficiency within drug development and quality control processes(89).

Validation Parameters

The most important aspect of validation of analytical method is an assessment of some performance characteristics that dictate the quality of the method. Accuracy is used to describe the closeness of the experimental value to the true value, such that the method will quantify the right amount of the drug. Precision measures the extent to which the results can be reproduced under the same conditions in the repeated tests and is depicted as intra-day and inter-day variation(90). Specificity is the measure of the capacity of the method to quantify the intended analyte in the absence of interference from excipients, degradation products, or endogenous impurities of significance in drug matrices of anticancer complexity(91). For purposes of this discussion, linearity is the capacity of a method to give results in proportion to the analyte concentration within a specified range, typically presented by the correlation coefficient (r^2). Limit of Detection (LOD) and Limit of Quantitation (LOQ) are the lowest reliable concentration that can be measured or detected, respectively. These are crucial to ensure the method is sensitive enough to track drug concentrations even at traces since it involves very potent or low-dose anticancer drugs(92).

Table 3 Summary of main validation parameters applied to the development of analytical procedures for anticancer drug assays, such as definitions, acceptance criteria, and utility in oncology.

Validation Parameter	Definition	Acceptance Criteria	Importance in Oncology	Reference
Accuracy	Closeness to the true value	98–102% recovery	Ensures drug is accurately dosed	(93)
Precision	Repeatability under same conditions	RSD ≤ 2%	Ensures reliability for clinical batches	(94)
Specificity	Ability to distinguish analyte from other components	No interference observed	Necessary for complex formulations	(95)
Linearity	Direct response between analyte concentration and signal	$r^2 \geq 0.999$	Important for quantitation across dose range	(96)
LOD	Minimum amount detected, not necessarily quantifiable	As per method sensitivity	Crucial for impurity detection	(97)
LOQ	Lowest quantifiable concentration with acceptable precision	Signal-to-noise ≥ 10:1	Important for low-dose drug monitoring	(98)

Regulatory Guidelines and Standard Protocols

Regulatory agencies like the FDA, EMA, and ICH offer legislative guidelines for method validation, to which any analytical process in drug development, approval, or quality control must adhere strictly(99). The ICH Q2(R1) guideline "Validation of Analytical Procedures: Text and Methodology" is the most commonly cited protocol for the validation of an analytical method(100). It provides requirements for method properties like specificity, linearity, accuracy, precision, detection limits, quantitation limits, and robustness. The FDA Bioanalytical Method Validation Guidance also defines the requirements for bioanalytical methods applied to pharmacokinetics and pharmacodynamics studies, including stability parameters, carryover, matrix effects, and recovery(101). The EMA also publishes guidelines for validating bioanalytical methods for human and veterinary medicines. Compliance with these principles guarantees that the analytical techniques employed are scientifically sound, repeatedly reproducible, and acceptable for regulatory acceptance at a global and regional level(102).

Case Studies and Comparative Analysis

A few case studies have proved the utility of certified analytical procedures for newly approved anticancer drugs. To illustrate, an HPLC method validated for osimertinib, a third-generation EGFR inhibitor, was proven for specificity, linearity ($r^2 > 0.999$), and LOQ 0.1 µg/mL and thus qualified to be formulated as well as in pharmacokinetic studies. Concurrently, an LC-MS/MS approach for pembrolizumab, a monoclonal antibody employed in immunotherapy, was validated for selectivity and sensitivity in plasma samples for effective therapeutic drug monitoring for clinical applications(103). In another application, a UHPLC approach for lorlatinib—an ALK small-molecule inhibitor—was validated

for high accuracy and low detection levels for application in bulk drug testing as well as stability studies. Such examples show how validated methods can be adapted to particular drugs and matrices and provide excellent reproducibility under various analytical conditions(104). Comparative evaluation of different validated methods also assists in determining the most appropriate method based on such considerations as sensitivity, speed, cost, and compatibility in the matrix.

APPLICATION OF ASSAY METHODS TO SPECIFIC ANTICANCER AGENTS

Application of analytical assay technologies in particular anticancer drugs is essential to achieve drug efficacy, safety, and regulatory approval during the product lifecycle. As more new therapeutic agents are approved, for instance, targeted therapies and immunotherapies, their respective assay technologies need to be accurate and flexible. Targeted therapy agents like tyrosine kinase inhibitors (TKIs) target single molecular targets that are responsible for cancer formation(105). These include drugs such as osimertinib, lorlatinib, alpelisib, and entrectinib that call for subtle and discriminating analysis owing to their low doses, intricate pharmacokinetic profiles, and structural similarity with naturally occurring compounds(106). High-Performance Liquid Chromatography (HPLC) and Ultra-High-Performance Liquid Chromatography (UHPLC) are utilized extensively for these compounds owing to their superior separating properties with a wide range of accompanying detection systems. But problem arises when confronted by highly similar metabolites, decomposition products, or matrix interferences in biological matrices that require high-level approaches like LC-MS/MS for higher sensitivity and specificity. For example, while doing human plasma osimertinib assay, LC-MS/MS techniques were seen to do a better work of tracking trace amounts with less interferences(31). In addition, targeted therapies usually require demanding stability-indicating approaches to follow degradation under stress, and HPLC with DAD or MS is useful to impurity profile and degradation pathways. Prior to their use, for immunotherapies, mostly monoclonal antibodies (mAbs), immune checkpoint inhibitors (nivolumab, pembrolizumab, atezolizumab) and cytokine modulators, assay development presents a different challenge set(107). These large biomolecules are temperature, pH, and enzymatic labile, and the assay platforms used for them must account for structural heterogeneity, post-translational modifications, and the potential for immunogenicity. Common chromatographic assays are usually not appropriate for immunotherapeutics; rather, immunoassay-based methods like ELISA, electrochemiluminescence immunoassays (ECLIA), and surface plasmon resonance (SPR) are utilized because they can measure intact proteins in complex biological matrices(108). ELISA continues to be the most universally applied procedure for quantitation of therapeutic antibody in plasma with high specificity, good reproducibility, and cost-effectiveness for regular analysis. It nevertheless needs to be validated for the employed antibody reagents and is prone to cross-reactivity. Ligand-binding assays were successful in accurate measurement of serum drug concentrations of nivolumab and pembrolizumab in extended treatment durations in clinical pharmacokinetic studies(109). Furthermore, techniques like CE and SEC are generally employed to track aggregation, fragmentation, and heterogeneity of products in mAb products. Hybrid LC-MS/MS techniques are also applied for peptide mapping and quantitation of therapeutic proteins in certain situations, although these require sophisticated apparatus and technical know-how(110). A comparative analysis of analytical techniques used on anticancer drugs shows that there is no one technique to fit all; instead, the method should be optimized according to the drug's physicochemical characteristics, formulation form, and target matrix. In the case of small-molecule targeted therapies, chromatographic techniques are still the gold standard owing to their capacity to deliver accurate quantitation, ruggedness, and flexibility for bulk and formulated products(111). But these approaches are not sensitive enough for trace-level measurement in complicated biological matrices, where LC-MS/MS is the method of choice because it is ultra-sensitive and highly selective. Immunoassays, on the other hand, are a must for large-molecule pharmaceuticals such as monoclonal antibodies since they provide biologically meaningful information and are serum and plasma friendly(112). But they cannot be applied to small molecules or degradation profiling. Methods such as UV-visible spectrophotometry and fluorescence spectroscopy, while effective for initial assays or high-dose formulations, are not usually specific enough for contemporary oncology applications. Recent methodologies based on biosensor technologies and nanotechnology-based assays provide better prospects, particularly for fast, real-time, and point-of-care analysis. These techniques hold promise for drug monitoring and diagnostic aid but are yet in the developmental phases and are confronted with issues regarding standardization and regulatory approval(113). Finally, combining several analytical methods is usually the most effective strategy in anticancer drug assay. As an example, the use of UHPLC for formulation analysis supplemented by LC-MS/MS for bioanalysis and ELISA for the monitoring of immunogenicity guarantees complete information about the performance of the drug in various

environments. The dynamic phase of oncology drug development dictates relentless progress in assay technologies, coupled with stringent validation and regulatory compliance. With anticancer treatments becoming more personalized and molecularly heterogenous, the analytical approaches to characterize and quantify these drugs need to keep pace accordingly so that clinical responses are backed by strong, reliable, and scientifically valid methodologies(114).

CHALLENGES, LIMITATIONS, AND FUTURE PERSPECTIVES

Current Technical and Methodological Limitations

Notwithstanding recent developments in analytical technology, there are some constraints to influence the reliability and efficiency of anticancer agent assay methods. The best constraint is the matrix effect, especially in biological matrices like tissues, serum, and plasma(75). The effects may lead to ion suppression or enhancement, especially with LC-MS/MS techniques, to provide incorrect quantitation of the analyte. Biologic matrices also contain endogenous compounds that can interfere with assay detection and validate assay specificity. For immunoassay-based techniques such as ELISA, non-specific binding, cross-reactivity, and non-homogeneous quality of antibodies are some among the issues that further constrain reproducibility. In the case of biologic anticancer drugs such as monoclonal antibodies, absence of standardized procedures and certified reference materials is a hurdle to laboratory comparison and method validation(115). Furthermore, most conventional methods are not only labor intensive but also require elaborate sample preparation including several extraction, centrifugation, or derivatization steps, thus higher likelihood of human error and variability. Even advanced technology like LC-MS/MS, although extremely sensitive, is expensive and requires professional staff, thus ruling out their heavy usage in routine quality control. These restrictions highlight the importance of robust, reproducible, and uncomplicated analysis platforms that have universal application for numerous classes of drugs and matrices(116).

Future Prospects and Technological Advancements

In order to address today's analysis requirements, new technologies and new methods are being explored that have the potential to revolutionize anticancer drug assays in the future(117). Among those, the combination of artificial intelligence (AI) and machine learning (ML) technologies in analyses holds the most hope. AI has the potential to facilitate method optimization by predicting chromatographic conditions, automate peak identification, reduce data processing time, and eliminate human bias. Machine learning codes are being developed more and more to analyze complex sets of data from LC-MS/MS, NMR, and biosensor systems to perform rapid and precise drug quantitation even in noisy biological samples(118). Also, biosensor methods such as electrochemical and optical biosensors are demonstrating tremendous potential for real-time point-of-care detection of anticancer agents with high sensitivity and specificity. These instruments are especially useful for therapeutic drug monitoring and intraoperative analysis. The evolution of lab-on-a-chip and microfluidic platforms also holds out the potential for miniaturized, fast, and cost-effective assay design, enabling analysis of several samples or biomarkers in parallel. In addition, the incorporation of nanomaterials, such as gold nanoparticles, quantum dots, and magnetic nanobeads, into assay design has offered new opportunities for signal amplification and ultra-sensitive detection(119). These advances are not only intended to reduce the time and expense of analysis but to enable the evolution toward customized medicine, in which quick and accurate monitoring of drugs is essential to tailor therapy to individual patients' specific needs.

Effect on Clinical and Regulatory Environments

The evolution of analytical assay technologies will have a significant influence on the clinical as well as on the regulatory aspect of cancer therapy(20). Clinically, enhanced analytical accuracy and lower turnaround time will facilitate quicker decision-making in personalized therapy, drug monitoring, and the early diagnosis of drug resistance or toxicity. Real-time monitoring through biosensors or wearable devices may become the norm in oncology practice, augmenting both patient compliance and treatment response(20). For regulators, the FDA, EMA, and ICH are increasingly promoting the use of new analytical strategies provided that they are properly validated and in accordance with internationally accepted quality standards. The shift towards risk-based validation strategies, embracing real-world evidence, and data integrity and automation is highly consistent with the use of AI-based platforms and next-generation analytical tools. These new trends will, however, necessitate new validation strategies, strong regulatory systems, and international harmonization to ensure consistent quality and safety(120). In addition, as biologics, biosimilars, and ADCs gain wider acceptance, regulatory authorities can anticipate soon demanding even advanced analytical characterization to determine potency, stability, and immunogenicity(121). Therefore, the changing analytical landscape not only holds promise for scientific

and operational advantages but also necessitates foresight adaptation by regulatory infrastructure and clinic stakeholders.

CONCLUSION

The unprecedented rate of progress in the area of oncology drug development has resulted in approval of novel therapeutic agents including small molecules, biologics, and antibody–drug conjugates. To determine their clinical efficacy and safety, integration of precise, valid, and accurate analytical techniques becomes unavoidable. This review highlighted the pivotal role played by methods like LC-MS/MS, HPLC, ELISA, UHPLC, and advanced biosensor technology in qualitative and quantitative analysis of drug agents. Each approach has definite benefits based on the drug class, matrix sample, and purpose of assay, i.e., pharmacokinetics, immunogenicity, and potency. Compliance with proper sample preparation, consideration of the matrix, and rigid compliance with guidelines also preserves assay integrity. In advancing in areas of discovery and development, integration of high-throughput platforms and emerging detection platforms will increasingly improve analytical performance. Thus, the strategic selection and validation of assay methods not only facilitate compliance with demands but also lead to best-achievable therapeutic results in the treatment of cancer.

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