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REVIEW ARTICLE

A Review on Bioanalysis of Recently-Approved Kinase Inhibitors

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ABSTRACT

Several steps in the development of cancer are linked to protein kinase dysregulation. There has been a dramatic change in the way cancer is treated since the introduction of protein kinase inhibitors (KIs). The Food and Drug Administration (FDA) has licensed a number of protein kinase inhibitors throughout the past few decades. Recent years (2020-2023) have seen an increase in the number of kinase inhibitors receiving authorization from the FDA. Consequently, there is a growing need for bioanalytical techniques to qualitatively and quantitatively analyze these drugs, and many articles have reported the development, validation, and adoption of such techniques in the context of KIs. The analytical procedures that may quantify KIs in plasma, CSF, urine, tissue, and liver microsomes are described in detail in the majority of published works. Most papers discuss the technological framework that has enabled the assessment of drug concentrations in a range of samples. Plasma, dried blood spots, and tissue analyses all fall under this category. This article provides a comprehensive overview of the several bioanalytical methods now available for determining the concentration of newly licensed kinase inhibitors in biological samples.

Keywords: *Kinase inhibitors, Bioanalysis, Serum, plasma, liquid chromatography-tandem mass spectroscopy (LC-MS/MS), Ultra-high performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS), pharmacokinetics.*

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INTRODUCTION

A critical function for kinases in the tumorigenesis and metastasis of numerous cancers has been disclosed by the latest advances in our knowledge of the basic molecular pathways underpinning tumor cell signaling. The majority of protein kinases are linked to cancer development due to their role in promoting cell proliferation, survival, and migration upon persistent overexpression or activation. Evidence from investigations of kinase mutations throughout the genome suggests that some hereditary variations of certain kinases are directly linked to cancer development, promotion, progression, and recurrence. Mutations and chromosomal rearrangements have been found in various types of cancer

during the last three decades, and these changes have been linked to the regulation and malfunction of protein and lipid kinases and inactive phosphatases. In addition to their roles in metabolic and cell cycle control, protein kinases also play important roles in preserving cells and specialization. Intracellular enzymes called protein kinases govern cell division and proliferation, in addition to the initiation and modulation of immunological responses. Protein kinases are phosphotransferases that attach phosphate group to the serine, threonine, or tyrosine side chain residues in cells. To begin immune cell signaling inside the cell, kinases are required. For example, kinases attach to the internal element found in T and B cell membrane receptors, and when these cells are stimulated by their extrinsic ligands, they initiate intracellular signaling pathways. Protein kinases catalyze the transfer of phosphate (P) from ATP to side chains of serine, threonine, or tyrosine residue in a protein (Figure 1). This phosphorylation serves as a "molecular shift" that may activate or inactivate proteins. In contrast, protein phosphatases are enzymes that remove phosphate groups from proteins, so blocking the activity of kinases and reversing the effects of phosphorylation.

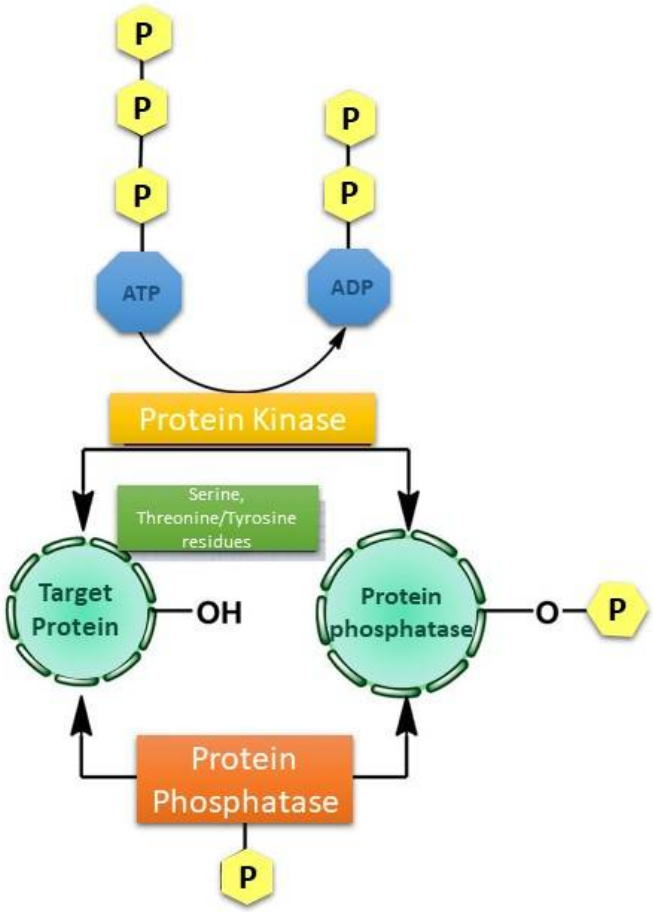

Figure 1: Protein kinase phosphorylation

Several steps in the development of cancer are linked to protein kinase dysregulation. There has been a dramatic change in the way cancer is treated since the introduction of protein kinase inhibitors. The Food and Drug Administration (FDA) has licensed a number of protein kinase inhibitors throughout the past few decades. Recent years (2020-2023) have seen an increase in the number of kinase inhibitors receiving authorization from the FDA, and this study will concentrate on the bioanalytical methods utilized to quantify these compounds.

Avapritinib

Avapritinib (Ayvakit™, Blueprint Medicines Corp.) has been approved by the FDA for the treatment of adults with advanced systemic mastocytosis (AdvSM), which includes patients with aggressive systemic mastocytosis (ASM), systemic mastocytosis with a comorbid hematological neoplasm (SM-AHN), and mast cell leukemia (MCL). Xu et al. looked into a rapid and accurate UPLC-MS/MS technique to confirm and quantify avapritinib content in rat plasma. Avapritinib and IS produced believable improvements in terms of recovery, stability, and matrix impact. A single oral dosage of avapritinib (30 mg/kg) was given

to the rats. Avapritinib concentrations in pharmacokinetic studies were determined using the proposed technique [1]. To develop an LC-MS/MS-based approach that is both straightforward and reliable for measuring avapritinib in plasma from rats. Both the intraday and interday %CV readings were confirmed to be acceptable. Freeze-thaw, Autosampler, benchtop, and long-term stability testing all showed the medication to be stable [2].

Brigatinib

Brigatinib (ALUNBRIG, ARIAD Pharmaceuticals Inc.) was authorized by the FDA for the treatment of adults with anaplastic lymphoma kinase (ALK)-positive, metastatic non-small cell lung cancer (NSCLC). For the purpose of quantifying brigatinib, alectinib, and lorlatinib in human plasma samples, an LC-MS/MS assay was designed and validated. Brain, liver, kidney, and spleen homogenates were also used to partly validate the procedure, along with diluted mouse plasma. Most of the conditions tested did not affect the compounds' stability. The effects of brigatinib on the body and how it's distributed in the body's tissues have been documented in a preliminary investigation [3]. The concentration of brigatinib in human plasma was determined using HPLC-ESI-MS/MS and a brigatinib-D6 internal standard (IS) prepared in accordance with a standardized protein precipitation procedure. The linear regression model yields a standard curve spanning a range of 15.00-120.00 pg/ml and a correlation coefficient (r2) exceeding 0.999 [4].

Cabozantinib

The FDA authorized Cabozantinib (Cabometyx, Exelixis, Inc.) on September 17, 2021, for the treatment of adults and children over the age of 12 with metastatic differentiated thyroid cancer (DTC) that continues to advance even with VEGFR-targeted therapy and in individuals who are unsuitable for or resistant to radioactive iodine. For the determination of Cabozantinib in human plasma, Srikanth Inturi et al. developed a straightforward, sensitive, and specific LC-MS/MS approach. Freeze-thaw, benchtop, and postoperative stability investigations all showed that Cabozantinib was stable [5]. A pharmacokinetic investigation of Cabozantinib in rats using this technique yielded positive results [6]. High throughput is ensured by an efficient and reliable assay, which has been effectively utilized in the monitoring of KI levels in patients [7]. Another analytical technique has been established for the routine measurement of Cabozantinib levels in human plasma. The half-life of the medication in plasma is 48 hours at room temperature or 4 °C [8], while in whole blood it is at least 6 hours at room temperature (after sample).

Capmatinib

Capmatinib (Tabrecta, Novartis Pharmaceuticals Corp.) received full FDA approval on August 10, 2022, for the treatment of adult patients with metastatic NSCLC whose tumors possess a genetic mutation that results in mesenchymal-epithelial transition (MET) exon 14 deletions. Following oral therapy of 5, 10, and 20 mg/kg of Capmatinib in rats, pharmacokinetic research was conducted, and UPLC-MS/MS indicated exceptional results in linearity, precision, reliability, and stability [9]. Capmatinib in plasma was quantified for the first time using two novel HPLC techniques that use Fluorescence detection (FLD) and Photodiode Array Detection (DAD). These techniques may be used to augment pharmacokinetic research, especially in bioanalytical laboratories without LC-MS/MS instruments [10]. Capmatinib concentration in human plasma was determined using an LC-MS/MS approach that was subsequently validated for use in rabbit pharmacokinetic studies. Pharmacokinetics were studied following oral therapy of Capmatinib to healthy rabbits, validating the established approach [11]. Capmatinib (INC280) in rat plasma was quantified using an LC-MS/MS technique. This validated technique is currently being employed for the measurement of Capmatinib in pre-clinical investigations after having been successfully deployed to assess the pharmacokinetics of the drug using plasma samples from rats [12].

Crizotinib

Crizotinib (Xalkori, Pfizer Inc.) was authorized by the FDA on July 14, 2022, for the treatment of adults and children older than 1 year who have unresectable, relapsed, or refractory inflammatory ALK-positive myofibroblastic tumors (IMT). In order to determine alectinib (ALC), ceritinib (CER), and crizotinib (CRZ) in rat plasma simultaneously, a novel UPLC-MS/MS technique was designed and validated. To further investigate the potential PK interaction between bromelain and the selected drugs in Wistar rats, the suggested approach was used. The results showed that the ingestion of bromelain with CER or CRZ induced a considerable drop in plasma levels of these drugs [13]. To assist current clinical and preclinical pharmacokinetic research, an LC-ESI-MS/MS technique for measuring crizotinib in human and mouse plasma was established. Crizotinib levels in plasma samples were effectively analyzed using this approach in phase I pediatric trials [14], suggesting the usage of crizotinib for the therapy of pediatric brain tumors. Rat plasma [15] and human plasma [16] crizotinib concentrations were determined using an LC-MS/MS technique. Crizotinib was effectively quantified and its pharmacokinetics were studied in rats

using this LC-MS/MS test after both intravenous and oral administration of the drug. Oral administration of crizotinib resulted in a 68.6 ± 9.63% absolute bioavailability in rats [17]. Researchers have also used LC-MS/MS to analyze the pharmacokinetics of CRZ and CRZ-lactam in human plasma following a single oral dosage of 250 mg. According to the findings, CRZ was promptly converted into its metabolite, crizotinib-lactam, which had in vivo exposure that was 38.50 percent lower than that of crizotinib [18]. The toxicity caused by crizotinib is a major clinical concern. Investigating which bodily systems this chemical affects would need tissue distribution research. A simple LC-MS/MS technique for the detection of crizotinib in different mouse tissues was devised. The research indicated that the highest concentrations of crizotinib were in the digestive system, with the lungs, liver, and spleen serving as secondary targets. Crizotinib toxicity might be better understood because of this study since it gives a dependable approach to measuring the drug [19]. Therapeutic drug monitoring (TDM) of TKIs has been proven to increase treatment success and decrease adverse events in many trials. This led to the development and validation of an LC-MS/MS technique for the TDM of 12 TKIs, including crizotinib, in individuals with NSCLC, which is now used for regular TDM of these TKIs. NSCLC patients may benefit from individualized dosage modification and better management of side effects by monitoring the plasma levels of TKIs [20].

Dabrafenib

On March 16, 2023, the FDA authorized the combination of dabrafenib (Tafinlar, Novartis) and trametinib (Mekinist, Novartis) for the systemic treatment of children and young adults aged 1 and older who have been diagnosed with low-grade glioma (LGG) with a BRAF V600E mutation. Both medications have had new oral formulations authorized by the FDA that make them easier for individuals with swallowing difficulties to use. Using EMA recommendations, a micellar liquid chromatographic technique was designed and validated for the determination of dabrafenib and other kinase inhibitors in plasma. With little investment of money, time, energy, and potentially less harmful substances, the process was successfully completed. The technique proved helpful for clinical analysis [21] since it allowed for the determination of the medications' target concentrations. Human plasma (22-24] and mouse plasma [25] dabrafenib concentrations were determined using an LC-MS/MS method that was developed and validated. The proposed approach has been shown to be useful for pharmacokinetics and bioequivalence research due to its high sensitivity, higher accuracy, precision, and excellent recovery for the plasma samples [26]. The LC-MS/MS generic test has shown to be a valuable resource in the quest to better characterize the pharmacology of dabrafenib, and it might serve as a foundation for the development of drug-specific analyses with an even greater characterization of their performance [27]. The clinical viability of volumetric absorptive micro sampling (VAMS) was confirmed by its effective implementation in real-world situations. Samples of capillary blood were analyzed for dabrafenib concentration using VAMS by certified medical personnel or by patients themselves at home [28].

Encorafenib

Encorafenib (BRAFTOVI, Array BioPharma Inc.) in combination with cetuximab has been authorized by the FDA for the treatment of adults with BRAF V600E mutation–positive metastatic colorectal cancer (CRC) after previous therapy. The concentration of Encorafenib in rat plasma was determined using a recently designed and completely validated LC-MS/MS bioanalytical technique. When administered orally, Encorafenib (20 mg/kg), pharmacokinetic parameters may be efficiently quantified attributable to the proposed assay's established broad range of calibration curves. The current method stands out due to its efficient extraction recovery and its resilience to matrix influence. Having a total run duration of 2 minutes and a verified sensitivity of 0.2 ng/mL, this assay is suitable for efficient routine tests in pharmacokinetic investigations [29].

Futibatinib

Futibatinib (Lytgobi, Taiho Oncology, Inc.) was given expedited authorization by the FDA on September 30, 2022, for the treatment of adults with recurrent, inoperable, or metastatic intrahepatic cholangiocarcinoma that contains a fusion or reorganization of the fibroblast growth factor receptor (FGFR)-2 gene. For use in the metabolic stability test, a UPLC-MS/MS analytical technique for quantifying futibatinib was developed and validated. This technique provides a precise, sensitive, and time-efficient way of assessing the microsomal stability of futibatinib in HLMs [30] The approach has been effectively used to study the pharmacokinetics of futibatinib in beagle dogs, and it was also an excellent tool for determining its levels in plasma. Drug-drug interaction (DDI) research might also benefit from this methodology [31].

Ibrutinib

Ibrutinib (Imbruvica, Pharmacyclics LLC) was authorized by the FDA on August 24, 2022, for the treatment of children patients above 1 year of age, who have been suffering from cGVHD after having

previously failed on 1 or more lines of systemic medication. It was stated that the drug remained stable for longer periods under different stability settings, and the established LC-MS/MS technology was effectively adaptable to the routine investigation of ibrutinib in biological matrices [32]. Ibrutinib (IBR) and its metabolite dihydrodiol-ibrutinib (DIBR) in human plasma were quantified using LC-MS/MS and shown to be reliable throughout a concentration range of 0.5 to 100 ng/ml. Bile acids were shown to interact with DIBR during the evaluation of plasma samples from a clinical investigation in people with hepatic impairment. Samples from people with hepatic impairment showed considerable interference, but this had no effect on the outcomes of any of the other clinical investigations reviewed [33]. Whether kept in the fridge or the freezer, both IBR and DIBR were shown to be stable [34]. Human CSF and plasma samples [35], beagle dog plasma [36], and rat plasma [37] were used to effectively quantify ibrutinib and PCI-45227 using UHPLC-MS/MS. Lenalidomide, ibrutinib, and the active metabolite PCI-45227 were all simultaneously estimated using an LC-MS/MS to assist pharmacokinetic research in Wistar rats. In a re-analysis study, Veeraraghavan et al. (2015) showed that the assay could be replicated with reliability using data from 18 replicate samples.

Infigratinib

Infigratinib (Truseltiq, QED Therapeutics, Inc.) recently received expedited authorization by the FDA for the treatment of patients with already treated, inoperative locally progressed or metastatic cholangiocarcinoma who also have an FGFR2 fusion or other rearrangements. Using an LC-MS/MS analytical technique, the metabolic stability of INF was evaluated, and it was shown to have a modest extraction ratio, suggesting relatively excellent anticipated oral bioavailability in HLMs in vitro tests [38]. By administering 10 mg/kg INF through gavage to SD rats, the pharmacokinetics of this compound were studied, and the major pharmacokinetic characteristics were derived via the use of an analytical technique devised using UPLC-MS/MS to measure the content in plasma. Xu et al. found that patients using numerous oral medicines (e.g., CYP3A inducers or inhibitors) or individuals with liver or renal impairment may need TDM to obtain customized doses of INF [39].

Lenvatinib

The FDA has authorized the first-line therapy of adults with advanced renal cell carcinoma (RCC) with a combination of lenvatinib (Lenvima, Eisai) and pembrolizumab (Keytruda, Merck). Using LC-MS/MS, Talari et al. (2022) validated the bioanalytical technique and analyzed the pharmacokinetics of Lenvatinib and its metabolites in rat plasma. In a different investigation, an LC-MS/MS technique was established for the determination of lenvatinib concentrations in human plasma [40]. Lenvatinib was shown to be stable in both human serum and phosphate-buffered saline (PBS) throughout a range of stability tests. Results from clinical trials demonstrating lenvatinib's strong protein binding in serum confirmed its effective application for in vivo protein binding investigations [41]. Five different labs developed seven different bioanalytical procedures using LC-MS/MS, and the results imply that lenvatinib levels in human plasma may be analyzed across labs and clinical trials [42]. Qualitative investigation [43] confirmed the viability of this approach for assessing Lenvatinib's kinetic distribution. Lenvatinib in human plasma was quantified by RP-HPLC for therapeutic and pharmacokinetic research [44]. Lenvatinib was shown to reduce the systemic intake of telmisartan, as determined by simultaneous estimate using UPLC-MS/MS. Cui et al. (2022) found evidence of a possible pharmacological link between lenvatinib and telmisartan.

Lorlatinib

Lorlatinib (Lorbrena, Pfizer Inc.) was given authorization by the FDA on March 3, 2021, for the treatment of patients with metastatic NSCLC with ALK-positive tumors, as identified by an FDA-approved test. Preliminary pharmacokinetic investigations in male and female wild-type mice were successful because of the development and validation of a bio-analytical LC-MS/MS assay for lorlatinib in mouse plasma [45] and tissue homogenate [46]. The maximum blood levels of lorlatinib (2,705.683 ± 539.779 μg/L) were reached at 0.625 ± 0.231 h after oral treatment. The kidneys had the lowest concentration (548.83 ng/100 mg), while the liver had the highest (3,153.93 ng/100 mg) and the stomach had the third-highest (2,159.92 ng/100 mg) [47]. The results of a second, completely separate experiment (cross-validation) on lorlatinib homogenate samples confirmed the original results. The therapeutic use of UPLC-MS/MS for quantifying lorlatinib in human plasma was proven by quantifying numerous samples from a pharmacokinetic trial for individuals with lung cancer [48].

Mobocertinib

Mobocertinib (Exkivity, Takeda Pharmaceuticals, Inc.) received an expedited authorization by the FDA for the treatment of adults with NSCLC who experience progression of disease despite receiving platinum-based chemotherapy and whose tumors have been shown to have epidermal growth factor receptor (EGFR) exon 20 insertion modifications, as per an FDA-approved test. Rat plasma Mobocertinib concentrations were determined using an LC-MS/MS technology that was designed and optimized

specifically for this purpose. Mobocertinib pharmacokinetics were investigated in rats given 2, 6, and 18 mg/kg by oral gavage using this methodology. The results showed that mobocertinib was stable in the investigated settings. After being given orally to rats at doses ranging from 2.0 to 18.0 mg/kg, mobocertinib exhibited linear pharmacokinetic properties [49].

Neratinib

On February 25, 2020, neratinib plus capecitabine received authorization from FDA for use in adult patients with advanced or metastatic HER2-positive breast cancer who were given more than 2 previous anti-HER2-based therapies in the metastatic setting. Determination of neratinib was performed using a bioanalytical UPLC-MS/MS approach in rat plasma and tissue homogenates [50], in human plasma [51-52] and in rat plasma [53]. In biological metrics, the approach was successfully used for the pharmacokinetic study of pure formulations. Oral simultaneous treatment with neratinib and apigenin was investigated for potential DDIs by Maher et al. (54]. TDM of cancer sufferers on such regimens will benefit greatly from the findings of this investigation. Results from estimating neratinib and naringenin in rat plasma by UPLC-MS/MS [55] and neratinib and curcumin in human plasma supported the method's sustainability. Quantitation of neratinib in human plasma was also performed using an LC-MS/MS and a stable internal standard [56].

Osimertinib

For use in individuals with NSCLC with tumors that have EGFR exon 19 omissions or exon 21 L858R modifications, as identified by an FDA-approved test, FDA granted authorization to osimertinib (TAGRISSO, AstraZeneca Pharmaceuticals LP) as adjuvant therapy following tumor surgery. A precise bioanalytical UHPLC-MS/MS technique for osimertinib and its metabolites was developed to investigate their metabolic pathway. Osimertinib showed only moderate stability. Samples from patients receiving 80 mg of osimertinib once a day showed that the test was clinically applicable since they contained measurable and quantifiable quantities of all studied chemicals [57]. Osimertinib is among the several kinase inhibitors that have been studied and analyzed using an LC-MS/MS that has been validated for use in normal clinical practice [58-61]. High throughput was achieved using this quick and sensitive technique to track kinase inhibitor levels in patients [62]. An HPLC-UV/DAD was validated for quantifying osimertinib along with other KIs in order to offer a more practical substitute. After comparing results to LC-MS/MS, researchers concluded that the newly developed HPLC-UV/DAD technology is "fit-for-TDM" in clinical practice and provides a viable alternative to LC-MS/MS.

Pemigatinib

Pemigatinib (Pemazyre, Incyte Corporation) was authorized by the FDA on August 26, 2022, to treat individuals with recurrent or refractory myeloid/lymphoid neoplasms (MLNs) that have FGFR1 translocation. In order to quantitatively evaluate the metabolic stability of pemigatinib in human liver microsomes (HLM), an LC-MS/MS analytical approach was devised. The extraction ratio for PMB was modest, indicating high bioavailability [63]. A UPLC-MS/MS technique has been established to quantify pemigatinib concentration in rat plasma. In a pharmacokinetic investigation, the test was also acceptable for detecting the blood levels of pemigatinib following a single oral administration of 1.35 mg/kg to rats [64].

Pralsetinib

Pralsetinib (GAVRETO, Blueprint Medicines Corporation) was granted FDA approval for the treatment of adults and children over the age of 12 with RET-mutant medullary thyroid cancer (MTC) who need chemotherapy, as well as those with RET fusion-positive thyroid cancer who need chemotherapy and are radioactive iodine-refractory. K2-EDTA plasma [65] and mouse plasma [66] were analyzed for Pralsetinib concentrations using LC-MS/MS. Both plasma samples showed stability for a minimum of 7 days when stored at 2-8 degrees Celsius, and for at least 24 hours when stored at 15-25 degrees Celsius.

Ripretinib

In adults with advanced gastrointestinal stromal tumors (GIST), ripretinib (QINLOCK, Deciphera Pharmaceuticals, LLC.) has been authorized by the FDA for use in combination with three or more KIs. Ripretinib was measured in rat plasma using HPLC-FLD, and this approach may be useful for pharmacokinetics and bioequivalence investigations of ripretinib in plasma samples [67]. To determine the ripretinib levels in the plasma of beagle dogs, a novel and robust UPLC-MS/MS method was devised and improved to its utmost potential. Itraconazole and voriconazole were shown in a pharmacokinetic investigation to enhance the plasma clearance of ripretinib in beagle dogs by inhibiting its metabolism [68].

Ruxolitinib

The FDA granted approval to ruxolitinib (Jakafi, Incyte Corp.) on September 22, 2021, for the treatment of chronic graft-versus-host disease (cGVHD) in patients above 12 years who had previously failed on one or

two lines of systemic therapy. Human plasma and serum were analyzed using LC-MS/MS to determine the concentrations of ruxolitinib and other kinase inhibitors [69,70]. Charlier et al. (2019) verified RP-HPLC with FLD in plasma samples, proposing that it should be taken into account alongside other methods. For the purpose of tracking the drug levels of kinase inhibitors, including ruxolitinib, in dried capillary blood, a volumetric absorptive micro sampling (VAMS) approach was developed and validated [71].

Selpercatinib

For adults with locally advanced or metastatic NSCLC with a rearranged during transfection (RET) gene fusion, selpercatinib (Retevmo, Eli Lilly, and Company) was licensed by the FDA on September 21, 2022. LC-MS/MS was used to design and validate a bioanalytical test for selpercatinib and Pralsetinib in mouse plasma [72], in mouse tissue homogenates [73] and human K2-EDTA plasma [74] samples. Drug stability was not affected by matrix effects or extraction inefficiencies under any of the scenarios tested. Additionally, this approach was used in a selpercatinib mouse pilot trial, which was followed by a cost-effective reanalysis of the original sample.

Selumetinib

Selumetinib (KOSELUGO, AstraZeneca) was granted FDA approval for the treatment of incurable plexiform neurofibromas (PN) in children and young adults with neurofibromatosis type 1 (NF1). The clinical study is being supported by the application of robust and sensitive LC-MS/MS techniques for the detection of selumetinib along with its metabolites, which were validated using human biological samples [75,76].

Tivozanib

Tivizanib (Fotivda, AVEO Pharmaceuticals, Inc.), a kinase inhibitor, has been authorized by the FDA for the treatment of adults with relapsed or refractory advanced renal cell carcinoma (RCC) after two or more previous systemic therapies. A preliminary bioanalytical assay for tivozanib has been developed and validated across a linear range of 0.5–5000 ng/mL in human plasma, mouse plasma, and tissue homogenates. In this study, they showed that the linear range could be increased from 2-4 decades by detecting two MRM transitions for tivozanib. Pharmacokinetic experiments in mice and a transport test both used the LC-MS/MS assay with acceptable results [77]. To determine the concentration of tivozanib in rat plasma and liver microsomes, researchers created and employed two novel HPLC techniques combined with FLD or DAD The presented techniques are appropriate for facilitating in vivo and in vitro tivozanib research, notably DDI studies, especially in bioanalytical laboratories without LC-MS/MS capabilities [78] due to their accessibility, speed, and cost-effectiveness.

Tucatinib

Tucatinib (Tukysa, Seagen Inc.) alongside trastuzumab received accelerated approval from the FDA on January 19, 2023, for the treatment of RAS wild-type, HER2-positive colorectal cancer that has advanced after receiving fluoropyrimidine, oxaliplatin, and irinotecan-based chemotherapy and is incurable or has metastasized. To investigate the impact of quercetin on tucatinib metabolism in rats, a UPLC-MS/MS was built and effectively used to measure tucatinib levels in rat plasma. There may be therapeutic relevance to the interaction between quercetin and tucatinib at high doses. Three labs employing five different approaches analyzed tucatinib plasma levels for pharmacokinetic analysis utilizing MS/MS approach. Using a 'base' bioanalysis done by one laboratory and technique, a five-way cross-validation approach was designed to verify the accuracy of the other four procedures. For a more comprehensive population pharmacokinetic study, researchers were able to pool data from various clinical trials of tucatinib using this strategy [79].

Zanubrutinib

On Jan 19, 2023, FDA approval was granted to zanubrutinib (Brukinsa, BeiGene USA, Inc.) for chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL). Clinical patient plasma levels of zanubrutinib were quantified using a validated LC-MS/MS technique. Mouse plasma zanubrutinib levels were also quantified using the same technique. After orally administering zanubrutinib to beagle dogs, a UPLC-MS/MS technique was established for its quantification in the dogs' plasma [80].

Table 1: A Summary of the bioanalytical techniques reported in this article

Drug	Analytical technique	Biological matrix
Avapritinib	UPLC-MS/MS	Rat plasma
	LC-MS/MS	Rat plasma
Brigatinib	LC-MS/MS	Human plasma
	HPLC-ESI-MS/MS	Human plasma
Cabozantinib		Human plasma
		Rat plasma

	LC-MS/MS	Human serum and plasma
		Human plasma
Capmatinib	UPLC-MS/MS	Rat plasma
	HPLC	Rat plasma, human liver microsomes
	LC-MS/MS	Human and rabbit plasma
		Rat plasma
Crizotinib	UPLC–MS/MS	Rat plasma
	LC-ESI–MS/MS	Human and mouse plasma
	LC-MS/MS	Human plasma
		Rat plasma
		Human plasma
		Mouse tissues
		Plasma samples from patients with NSCLC
Dabrafenib	Micellar liquid chromatography	Plasma
	LC-MS/MS	Human plasma
		Mouse plasma
	Volumetric absorptive microsampling (VAMS)	Plasma samples
Encorafenib	LC-MS/MS	Rat plasma
Futibatinib	UPLC-MS/MS	Human liver microsomes
		Beagle dog plasma
Ibrutinib	LC-MS/MS	Human plasma
		Wistar rat plasma
	UHPLC-MS/MS	Human CSF and plasma
		Beagle dog plasma
		Rat plasma
Infigratinib	LC-MS/MS	Human liver microsomes
	UPLC-MS/MS	SD rats plasma
Lenvatinib	LC-MS/MS	Rat plasma
		Human plasma
		Human serum
		Human plasma
	RP-HPLC	Human palsma
	UPLC-MS/MS	Rat plasma
Lorlatinib	LC-MS/MS	Mouse plasma and tissue homogenate
		Mouse plasma
		Mouse serum and tissue sample
	UPLC-MS/MS	Human plasma
Mobocertinib	LC-MS/MS	Rat plasma
Neratinib	UPLC–MS/MS	Rat plasma and tissue homogenates
		Human plasma
		Rat plasma
	LC-MS/MS	Human plasma
Osimertinib	UHPLC–MS/MS	Human plasma
	LC-MS/MS	Human serum
		Human plasma
		Serum and plasma
		Plasma and CSF
	HPLC-UV/DAD	Human serum
Pemigatinib	LC-MS/MS	Human liver microsomes
	UPLC-MS/MS	Rat plasma

Pralsetinib	LC-MS/MS	K2-EDTA plasma
		Mouse plasma
Ripretinib	HPLC-FLD	Rat plasma
	UPLC-MS/MS	Beagle dog plasma
Ruxolitinib	LC-MS/MS	Human plasma
		Human serum and plasma
	RP-HPLC	Plasma samples
	LC-MS/MS with volumetric absorptive microsampling (VAMS)	Dried capillary blood
Selpercatinib	LC-MS/MS	Human K2-EDTA plasma
		Mouse plasma and tissue homogenates
		Mouse plasma
Selumetinib	LC-MS/MS	Human biological matrices
Tivozanib	LC-MS/MS	Mouse plasma
	HPLC	Rat plasma and liver microsomes
Tucatinib	UPLC–MS/MS	Rat plasma
	MS/MS	Human plasma
Zanubrutinib	LC-MS/MS	Human plasma
		Mouse plasma
	UPLC–MS/MS	Beagle dog plasma

FUTURE PROSPECTS

Simple, high-throughput procedures that need few or no human interactions are ideal for bioanalysis. The use of chromatographic separation continues to be vital, and the decrease in analysis time shown over the last several decades is a clear indication of this. We expect that MS detection will continue to be the gold standard for bioanalysis, particularly for quantitative purposes. As MS detectors have become more reasonably priced, they may be found at a wider variety of research and academic institutions. Some scientists choose to use Q-TOF or Q-Orbitrap equipment for their high-resolution mass spectrometry. Not mentioned previously in this paper is the ion-mobility interface, which has the potential to improve chromatography-free bioanalysis and might potentially distinguish isobaric chemicals or even chiral molecules.

Using rat/HLMs, various research looked into the drug metabolites and biotransformation routes of different kinase inhibitors. The results highlight the need to include liver microsomes in subsequent bioanalyses of KI candidates to strengthen metabolic studies.

KIs bioanalysis is crucial for the study of cancer and therapeutic development. In recent years, numerous assays have been developed to shed light on topics such as drug metabolism These techniques, in conjunction with research into the desired therapeutic drug level and the clinical dose-effect connection, may be used for TDM to increase clinical effectiveness and decrease the toxicological effects of KIs.

CONCLUSION

The bioanalysis of KIs is a crucial technique for gaining a deeper understanding of the complete efficacy-toxicity ratio of KIs, which can then be utilized to enhance their therapeutic efficacy. While multianalyte and metabolite analysis are on the rise, chromatographic techniques remain the most employed separation method for KI bioanalytical tests. The majority of KI bioanalytical procedures use the US FDA and EMA criteria as their validation framework. To sum up, LC-MS/MS is the gold standard separation method. Future qualitative investigations may make more use of UPLC-MS/MS.

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