WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 7.632

Volume 11, Issue 7, 553-566 Review Article ISSN 2278 – 4357

A REVIEW: NOVEL ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF SELECTED ANTICANCER AND ANTIVIRAL DRUGS

Santhosh Illendula[1]* and Naveen Kumar Singhal[2]

[1]Research Scholar, Department of Pharmacy, Shyam University, Dausa-303511, Rajasthan, India.

[2]Professor, Department of Pharmacy, Shyam University, Dausa-303511, Rajasthan, India.

Article Received on 23 April 2022,

Revised on 13 May 2022,
Accepted on 03 June 2022

DOI: 10.20959/wjpps20227-22475

***Corresponding Author**

Santhosh Illendula

Research scholar,

Department of pharmacy,

Shyam university, Dausa-303511, Rajasthan, India.

ABSTRACT

This review article determines the different analytical methods for the quantitative establishment of selected acalabrutinib, lorlatinib and olaparib and selected antiviral drug molnupiravir by using HPLC and LC-MS/MS. Pharmaceutical analytical method development of acalabrutinib, lorlatinib and olaparib and selected antiviral drug molnupiravir requires valid analytical procedures for quantitative and qualitative analysis in Pharmaceuticals dosage formulations. An Huge survey for determination of acalabrutinib, lorlatinib and olaparib anticancer and selected antiviral drug molnupiravir follow from the research articles published in various pharmaceutical and analytical chemistry Journals. This assessment explain that the superiority of the HPLC/LC-MS methods reviewed is based on the quantitative analysis of drugs in formulations.

KEYWORDS: Method Validation, High performance Liquid Chromatography (HPLC/LCMS), acalabrutinib, lorlatinib, olaparib and molnupiravir.

INTRODUCTION

Cancer is defined as a ‘group of diseases characterized by the uncontrolled growth and spread of abnormal cells’ and is one the deadliest diseases globally. Cancer represents the second most common cause of death in Europe and USA after cardiovascular diseases according to cancer facts and figure of 2016, a publication distributed by the American cancer society.[1] Acalabrutinib is a highly selective Bruton’s tyrosine kinase inhibitor, is associated with high

overall response rates and used for treated chronic lymphocytic leukemia.[2] Acalabrutinib binds covalently to Cys481 in the ATP binding pocket of BTK.[3] Acalabrutinib is chemically, (4-[8- amino3-[(2S)-1-but-2-ynoylpyrrolidin-2-yl]imidazo[1,5-a]pyrazin1-yl]-N-(2-pyridyl) benzamide)].[4] The chemical structure of acalbrutinib shown in figure 1.

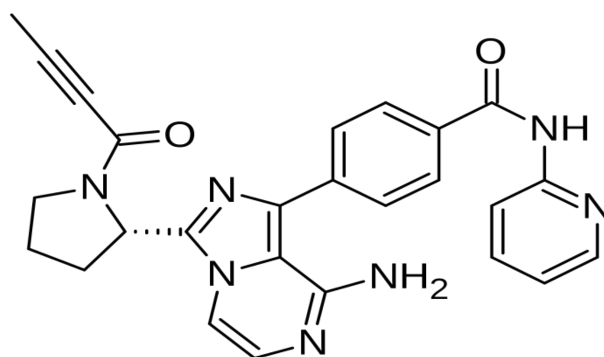

Figure 1: Chemical structure of acalbrutinib.

Lorlatinib (fig.2) is chemically7-amino-12-fluoro2, 10,16-trimethyl-15-oxo-10, 15,16,17-tetrahydro-2H-4,8-
methenopyrazolomethenopyrazolo[4,3h][2,5,11]enzoxadiazacyclotetradecine-3-carbonitrile. Is a kinase inhibitor indicated for the treatment of patients with anaplastic lymphoma kinase (ALK)-positive metastatic non-small cell lung cancer (NSCLC).[5] Lorlatinib is available in markets as conventional tablets with a trade name of LORBRENA. It has shown survival benefits in the treatment of lung cancer in phase III trials. Some high-performance liquid-chromatographic (HPLC) methods with ultraviolet (UV) have been developed. Some methods with tandem mass spectrometry (MS=MS) each with its own advantages and limitations has been reported for the assay of Lorlatinib or other ALK drugs in human plasma.[6-10]

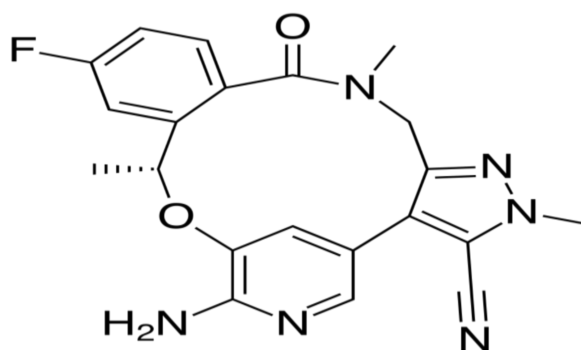

Figure 2: Chemical structure of lorlatinib.

Olaparib is an orally active inhibitor of poly (ADP-ribose) polymerases (PARP), of which PARP-1 is the most common. This enzyme assists with base-excision repair, fixing single-strand DNA breaks[11] that, if not repaired, become double-strand breaks when encountered by the replication fork. Double-strand breaks are often repaired via homologous recombination (HR), where BRCA1 and BRCA2 play an important role.[12] Deleterious mutations in BRCA1 or BRCA2 predispose carriers to breast and other cancers. When PARP is inhibited, BRCA1/2-deficient cells can no longer efficiently repair double-strand DNA breaks, leading to accumulation of DNA lesions, chromatid breaks, and eventual apoptosis. Thus, tumor cells with BRCA1/2 mutations are potentially sensitized to PARP inhibitors. Murai et al. reported that, in addition to inhibiting base-excision repair, PARP inhibitors exhibit further cytotoxic activity by trapping PARP at single-strand DNA breaks.[13] The mechanism of PARP inhibitors offer the possibility of selectively killing cancer cells while avoiding the toxicities associated with most chemotherapy. Olaparib chemical structure was shown in figure 3.

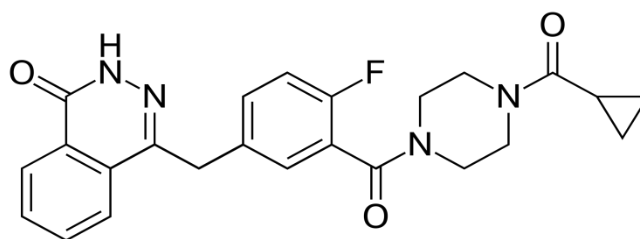

Figure 3: Chemical structure of olaparib.

Molnupiravir is an antiviral medication that inhibits the replication of certain RNA viruses.[14] It is used to treat COVID-19 in those infected by SARS-CoV-2.Molnupiravir is a prodrug of the synthetic nucleoside derivative N^4-hydroxycytidine and exerts its antiviral action through introduction of copying errors during viral RNA replication[15,16] Molnupiravir was originally developed to treat influenza at Emory University by the university's drug innovation company, Drug Innovation Ventures at Emory (DRIVE), but was reportedly abandoned for mutagenicity concerns.[17] It was then acquired by Miami-based company Ridgeback Biotherapeutics, which later partnered with Merck & Co. to develop the drug further.[18] Based on positive results in placebo-controlled double-blind randomized clinical trials.[19 & 20] Molnupiravir was approved for medical use in the United Kingdom in November 2021.[21&22] In December 2021, the U.S. Food and Drug Administration (FDA) granted an emergency use authorization (EUA) to molnupiravir for use in certain populations

where other treatments are not feasible. The emergency use authorization was only narrowly approved because of questions regarding efficacy and concerns that molnupiravir's mutagenic effects could create new variants that evade immunity and prolong the COVID-19 pandemic.[23,24and25] Molnupiravir chemical structure was shown in figure4.

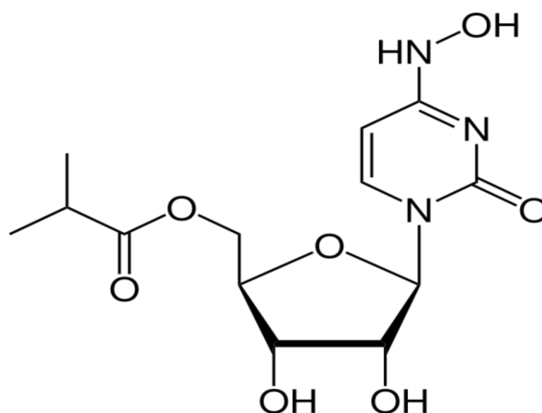

Figure 4: Chemical structure of molnupiravir.

Acalabrutinib Reported Methods

Priyanka P and Shyamala et al., (2019) were developed on novel isocratic reverse phase liquid chromatography method for determination of Acalabrutinib was developed and validated after optimization of various chromatographic conditions. Chromatographic conditions used are stationary phase kromosil C18 column (250×4.6 mm5µ) with mobile phase 0.1% O-Phosphoric acid and methanol in ratio of 50:50 and flow rate was maintained at 1.0 ml/min, detection wavelength was 236nm.[26] Pooja Singh et al., (2021) reported on quantification and stability indicating UPLC method development and validation of acalabrutinib in bulk and pharmaceutical dosage form. This method was developed by using BEH C 18 (50 mm x 2.1 mm, 1.7µm) column was run through with Mobile phase 0.01N KH2PO4: Methanol in the ratio of 50:50 at a flow rate of 0.3 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected for the separation was 234nm. Retention time was achieved 1.148minute in optimized chromatogram.[27] Atchutarama krishna et al., (2020) reported on development and validation of novel HPLC bioanalytical analysis method for acalabrutinib: an anticancer drug in human plasma. This method accomplished optimized chromatography conditions consist of methanol, acetonitrile and 0.1%orthophosphoric acid in the ratio of 45:35:20 (v/v) as a mobile phase with knauer Euros her II C18 Column (250 × 4.6 mm, 5µ) as stationary phase. Isocratic elution with 0.9 mL flow separates acalabrutinib at 4.6 min.[28] Anusha et al., (2019) developed Stability indicating RP-HPLC method

development and validation for the determination of Acalabrutinib in bulk drug and capsule dosage form. In this method separation was done using Zodiasil C18 150 x 4.6 mm, 5 µ column. The mobile phase (Water and methanol 60:40%v/v) was pumped at 0.8ml/min and effluent was detected at 230nm using a PDA detector. The retention time was 2.76 ± 0.1 min.[29] Valluri, V. R et al., (2022) developed on A novel LC-MS/MS method for simultaneous estimation of acalabrutinib and its active metabolite acalabrutinib M_{27} in human plasma and application to a human pharmacokinetic study. This method accomplished separation of the analytes was carried out on Zorbax Eclipse XDB-C_{18} (150 × 4.6 mm, 5 µm) column with a mixture of acetonitrile and 10 mM ammonium formate in 0.1% formic acid buffer (65 : 35, v/v) as mobile phase at a flow rate of 1 mL/ min.[30] The acalabrutinib summary reported methods shown in table 1.

Table 1: Acalabrutinib reported methods.

Sample	Instrument	Chromatographic conditions	References
API	RP-HPLC	C18(250 X 4.6 mm, 5µ) 0.1% O-Phosphoric acid and methanol in ratio of 50:50, 236 nm	[26]
API and formulation	UPLC	BEH C 18 (50 mm x 2.1 mm, 1.7µm) Mobile phase 0.01N KH2PO4 : Methanol in the ratio of 50:50, 234 nm	[27]
Human Plasma	HPLC	Euros her II C18 Column (250 × 4.6 mm, 5µ) methanol, acetonitrile and 0.1%orthophosphoric acid in the ratio of 45:35:20 (v/v) as a mobile phase, 242 nm	[28]
API and formulation	RP-HPLC	Zodiasil C18 150 x 4.6 mm, 5 µ column Water and methanol 60:40%v/v, 230 nm	[29]
Human plasma	LC-MS/MS	Zorbax Eclipse XDB-C_{18} (150 × 4.6 mm, 5 µm), acetonitrile and 10 mM ammonium formate in 0.1% formic acid buffer (65 : 35, v/v), m/e 466.1–372.1	[30]

Lorlatinib Reported Methods

Kalyan Chakravarthi et al., (2021) developed on RP-HPLC Method Development and Validation for the Determination of lorlatinib in Bulk and Its Pharmaceutical Formulation. This method accomplished C18 column (250mm X 4.6mm, 3µm) with mobile phase consisting of potassium dihydrogen orthophosphate, acetonitrile and methanol (45:30:25%V/V) having pH 5.8 was adjusted by using orthophosphoric acid. The flow rate was 1.0mL and effluents were monitored at 310nm. The Retention time of Lorlatinib was 7.871/min.[31] Veerman et al., (2021) reported on quantify the small-molecule kinase inhibitors ceritinib, dacomitinib, lorlatinib, and nintedanib in human plasma by liquid

chromatography/triple-quadrupole mass spectrometry. In this method Acquity UPLC® BEH C18 column 1.7 µm, 50 mm x 2.1 mm was used with a binary gradient of pure water/formic acid/ammonium formate (100:0.1:0.02, v/v/v) and methanol/formic acid (100:0.1, v/v). Calibration curves for all small-molecule kinase inhibitors were 5.00-500 ng/mL.[32] Anuradha et al., (2019) reported on RP-HPLC method development and validation for the determination of lorlatinib in bulk and its pharmaceutical formulation. In this method C18 column (250mmX4.6mm, 3µm) with mobile phase consisting of potassium dihydrogen orthophosphate, acetonitrile and methanol (50:30:20%V/V) having pH 4.2 was adjusted by using orthophosphoric acid. The flow rate was 1.0mL and effluents were monitored at 310nm. The Retention time of lorlatinib was 7.871/min.[33] Sparidansa et al., (2018) reported on bioanalytical assay for the quantification of the ALK inhibitor lorlatinib in mouse plasma using liquid chromatography-tandem mass spectrometry. This method is explained about compounds were eluted at 0.5 mL/min and separated on a 3-µm particle-size, polar embedded octadecyl silica column by gradient elution using 0.1% of formic acid (in water) and methanol. Compounds were monitored with positive electrospray ionization using a triple quadruple mass spectrometer in selected reaction monitoring mode.[34] The lorlatinib summary reported methods shown in table 2.

Table 2: Lorlatinib reported methods.

Sample	Instrument	Chromatographic conditions	References
API and formulation	RP-HPLC	C18 column (250mm X 4.6mm, 3µm) with mobile phase consisting of potassium dihydrogen orthophosphate, acetonitrile and methanol (45:30:25%V/V) having pH 5.8 was adjusted by using orthophosphoric acid, 310 nm.	[31]
Human plasma	LC-MS/MS	Acquity UPLC® BEH C18 column 1.7 µm, 50 mm x 2.1 mm was used with a binary gradient of pure water/formic acid/ammonium formate (100:0.1:0.02, v/v/v) and methanol/formic acid (100:0.1, v/v).	[32]
API and formulation	RP-HPLC	C18 column (250mmX4.6mm, 3µm) with mobile phase consisting of potassium dihydrogen orthophosphate, acetonitrile and methanol (50:30:20%V/V) having pH 4.2 was adjusted by using orthophosphoric acid.310 nm.	[33]
Mouse plasma	LC-MS/MS	3-µm particle-size, polar embedded octadecyl silica column by gradient elution using 0.1% of formic acid (in water) and methanol. Compounds were monitored with positive electrospray ionization using a triple quadrupole mass spectrometer in selected reaction monitoring mode	[34]

Olaparib Reported Methods

Dasameswara Rao et al.,(2019) developed on new rapid stability indicating rp-uplc method for the determination of olaparib, its related substances and degradation products in bulk drug and dosage form. In this method UPLC® BEH C18 (2.1 mm × 100 mm, 1.7 micron) column, using buffer (1.0ml Orthophosphoric acid in 1000ml water) and acetonitrile in gradient elution mode. The flow rate was kept at 0.4mL/min.[35] Daumar et al., (2018) reported on development and validation of a high-performance liquid chromatography method for the quantitation of intracellular parp inhibitor olaparib in cancer cells. This method accomplished C_{18} column (150 × 3.9 mm, 4 μm) using a mobile phase consisting of acetonitrile and ultra-pure water in gradient mode, at a constant 1.2 mL/min flow rate, at 35 °C. Detection was carried out at 254 nm.[36] Ressiat et al., (2018) reported on Development and Validation of a Simultaneous Quantification Method of Ruxolitinib, Vismodegib, Olaparib, and Pazopanib in Human Plasma Using Liquid Chromatography Coupled with Tandem Mass Spectrometry. This method accomplished he mobile phase consisted of a gradient elution of 10-mmol/L formate ammonium buffer containing 0.1% (vol/vol) formic acid (phase A) and acetonitrile with 0.1% (vol/vol) formic acid (phase B) at a flow rate at 300 μL/min.[37] William D. Figg et al., (2014) reported on A Sensitive and Robust Ultra HPLC Assay with Tandem Mass Spectrometric Detection for the Quantitation of the PARP Inhibitor Olaparib AZD2281) in Human Plasma for Pharmacokinetic Application. This method explains about Waters UPLC® BEH C18 column (2.1 × 50 mm, 1.7 μm) and mass spectrometric detection. The mass transitions m/z 435.4→281.1 and m/z 443.2→281.1.[38] Priyanka Waje et al., (2021). Method development and validation for quantitative estimation of olaparib in tablet dosage form by rp-hplc method. The chromatographic separation was achieved on Thermo Scientific C18 column (250mm x 4.6mm i.d.5μ). The mobile phase selected was 0.1% Trifluoroacetic buffer: Acetonitrile in the ratio of 60: 40 v/v at flow rate 1.0ml/min with column temperature maintained at 35°C and 10μl injection volume. The detection was carried out at 276nm[39]. Pagidi rajagopaludu et al., (2022) were developed on method development and validation of erythromycin and olaparib in human plasma by liquid chromatography–tandem mass spectrometry. The chromatography was carried out at 40°C on a phenomenax Luna column (C18, 5 m, 100×4.6 mm). It is set up as 75% A and 25% B for the first 0.5 min, then 10% A and 90% B for the next 3.5 min. Then, it steadily increased to 75% A and 255% B by 3.75 min and stayed there at 5.0 min at a flow rate of 0.5 mL/min. Olaparib mass-to-charge ratios of 435.08–102.04. In In the turbo electrospray interface, a positive ionization mode was

adopted. T the turbo electrospray interface, a positive ionization mode was adopted[40]. · The olaparib summary reported methods shown in table 3.

Table 3: Olaparib reported methods.

Sample	Instrument	Chromatographic conditions	References
API and formulation	RP-HPLC	UPLC® BEH C18 (2.1 mm × 100 mm, 1.7 micron) column, using buffer (1.0ml Orthophosphoric acid in 1000ml water) and acetonitrile in gradient elution mode,	[35]
API	RP-HPLC	C_{18} column (150 × 3.9 mm, 4 μm) using a mobile phase consisting of acetonitrile and ultra-pure water in gradient mode, at a constant 1.2 mL/min flow rate, at 35 °C. Detection was carried out at 254 nm	[36]
Human plasma	LC-MS/MS	10-mmol/L formate ammonium buffer containing 0.1% (v/v) formic acid (phase A) and acetonitrile with 0.1% (v/v) formic acid (phase B) at a flow rate at 300 μL/min	[37]
Human plasma	UHPLC-MS	Waters UPLC® BEH C18 column (2.1 × 50 mm, 1.7 μm) and mass spectrometric detection. The mass transitions m/z 435.4→281.1 and m/z 443.2→281.1	[38]
Formulation	RP-HPLC	Thermo Scientific C18 column (250mm x 4.6mm i.d.5μ). The mobile phase selected was 0.1% Trifluoroacetic buffer: Acetonitrile in the ratio of 60: 40 v/v,276 nm.	[39]
Human plasma	LC-MS/MS	Phenomenax Luna column (C18, 5 m, 100×4.6 mm), Olaparib mass-to-charge ratios of 435.08–102.04.	[40]

Molnupiravir Reported Methods

Tuba Recber et al., (2022) reported on A stability indicating RP-HPLC method for determination of the COVID-19 drug molnupiravir applied using nano formulations in permeability studies C18 Column (75 X 4.6 mm, 3 μm) was used at 30 °C. Isocratic elution was performed with ACN: water (20:80 v/v) mixture. The flow rate was 0.5 mL/min and UV detection was at 240 nm. Molnupiravir eluted within 5 min.[41] Amara A eta al., (2021) reported on the development and validation of a novel LC-MS/MS method for the simultaneous quantification of Molnupiravir and its metabolite ß-d-N4-hydroxycytidine in human plasma and saliva. Chromatographic separation was achieved using a polar Atlantis C_{18} column with gradient elution of 1 mM Ammonium acetate in water (pH4.3) and 1 mM Ammonium acetate in acetonitrile. Analyte detection was conducted in negative ionisation mode using SRM. Analysis was performed using stable isotopically labelled (SIL) internal

standards (IS). The m/z transitions were: MPV (328.1→126.0). Validation was over a linear range of 2.5-5000 ng/ml for both plasma and saliva.[42] Bhumika Parmar et al., (2022) were developed on uv visible spectroscopy method development and validation for estimation of molnupiravir in solid dosage form. Standard and sample solutions for molnupiravir were prepared in distilled water. Absorbance correction method for molnupiravir 280 nm was measured. The method followed linearity in range of 0.2-1 µg/ml with correlation value of 0.9998 for molnupiravir. This method was validated for various parameters according to ich guidelines.[43] Pritam Jain et al., (2022) were reported on Quantitative estimation of molnupiravir by UV- Spectrophotometric method. UV-spectrophotometric method has been developed for estimation of Molnupiravir from bulk and pharmaceutical formulation. The λmax of Molnupiravir in distilled water was found to be 235 nm. The drug follows linearity in the concentration range 5-30 µg/ml with correlation coefficient value 0.999. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 99.99 % was found in good agreement with the label claim.[44] Molnupiravir chromatographic reported methods were shown in table 4 and spectrophotometric condition were shown in table 5.

Table 4: Molnupiravir chromatographic reported methods.

Sample	Instrument	Chromatographic conditions	References
API	RP-HPLC	C18 Column (75 X 4.6 mm, 3 µm) was used at 30 °C. Isocratic elution was performed with ACN: water (20:80 v/v) mixture, 240 nm.	[41]
Human plasma and saliva	LC-MS/MS	C_{18} column with gradient elution of 1 mM Ammonium acetate in water (pH4.3) and 1 mM Ammonium acetate in acetonitrile. The m/z transitions were: MPV (328.1→126.0).	[42]

Table 5: Molnupiravir spectro photometric reported methods.

Sample	Spectro photometric conditions	References
Formulation	Absorbance correction method for molnupiravir 280 nm, linearity in range of 0.2-1 µg/ml with correlation value of 0.9998.	[43]
API and formulation	The λmax of Molnupiravir in distilled water was found to be 235 nm. The drug follows linearity in the concentration range 5-30 µg/ml with correlation coefficient value 0.999.	[44]

CONCLUSION

A sensitive and accurate HPLC and LC-MS/MS methods were developed for the estimation of the above selected anti-cancer and anti viral drugs acalabrutinib, lorlatinib, olaparib and

molnupiravir in pharmaceutical dosage forms. The above methods were evaluated for Specificity, Linearity, Accuracy, Precision, Ruggedness and Robustness as per ICH&FDA guidelines. From this study it is clear that it is possible to develop a new sensitive and accurate HPLC, UPLC and LC-MS/MS methods for anti cancer and anti viral drugs.

REFERENCES

1. Punna Venkateshwarlu and Mehul M. Patel. A Review: Method Development Validation and Degradation Studies of some Anticancer Drugs. Research J. Pharm. and Tech., 2021; 14(10): 5443-5448.
2. Viral Kumar Patel, Betty Lamothe, Mary L Ayres. Pharmacodynamics and Proteomic Analysis of Acalabrutinib Therapy: Similarity of On-Target Effects to Ibrutinib and Rationale for Combination Therapy. Leukemia, 2018; 32(4): 920–930.
3. Sarah E.M. Herman, Arnau Montraveta, Carsten U Niemann. The Bruton Tyrosine Kinase (BTK) Inhibitor Acalabrutinib Demonstrates Potent OnTarget Effects and Efficacy in Two Mouse Models of Chronic Lymphocytic Leukemia. Cancer Therapy: Preclinical, 2016; DOI: 10.1158/1078- 0432.CCR-16-0463.
4. Tjeerd Barf, Todd Covey, Raquel Izumi, Bas van de Kar, et al., Acalabrutinib (ACP-196): A Covalent Bruton Tyrosine Kinase Inhibitor with a Differentiated Selectivity and In Vivo Potency Profiles. J Pharmacol Exp Ther., 2017; 363: 240–252.
5. B. Golding, A. Luu, R. Jones, and A. M. Viloria-Petit. The function and therapeutic targeting of anaplastic lymphoma kinase (ALK) in non-small cell lung cancer (NSCLC)," Molecular Cancer, 2018; 17(1): 1-15.
6. 2 Zhang W, Siu LL, Moore MJ, and Chen EX. Simultaneous determination of OSI-774 and its major metabolite OSI-420 in human plasma by using HPLC with UV detection. J. Chromatogr. B., 2005; 814(1): 143–147.
7. Zhao M, He P, Rudek MA, Hidalgo M, and Baker SD. Specific method for determination of OSI-774 and its metabolite OSI-420 in human plasma by using liquid chromatography–tandem mass spectrometry. J. Chromatogr. B., 2003; 793(2): 413–420.
8. Dowell J, Minna JD, and Kirkpatrick P. Erlotinib: Small-molecule targeted therapy in the treatment of non-small-cell lung cancer. Nat. Rev., 2005; 4: 13–14.
9. Masters AR, Sweeney CJ, and Jones DR. The quantification of erlotinib (OSI-774) and OSI-420 in human plasma by liquid chromatography–tandem mass spectrometry. J. Chromatogr. B., 2007; 848(2): 379-383.

10. Lepper ER, Swain SM, Tan AR, Figg WD, and Sparreboom A. Liquid-chromatographic determination of erlotinib (OSI-774), an epidermal growth factor receptor tyrosine kinase inhibitor. J. Chromatogr. B., 2003; 796(1): 181-188.
11. Dantzer F., de La Rubia G., Menissier-de Murcia J., Hostomsky Z., de Murcia G., Schreiber V. Base excision repair is impaired in mammalian cells lacking Poly(ADP-ribose) polymerase-1. Biochemistry, 2000; 39: 7559–7569.
12. Farmer H., McCabe N., Lord C.J., Tutt A.N., Johnson D.A., Richardson T.B., Santarosa M., Dillon K.J., Hickson I., Knights C. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature, 2005; 434: 917–921.
13. Murai, J., Huang S.Y., Das B.B., Renaud A., Zhang Y., Doroshow J.H., Ji J.,Takeda, S., Pommier, Y. Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. Cancer Res., 2012; 75: 5588–5599.
14. Toots M, Yoon JJ, Cox RM, Hart M, Sticher ZM, Makhsous N, et al. "Characterization of orally efficacious influenza drug with high resistance barrier in ferrets and human airway epithelia". Science Translational Medicine, October 2019; 11 (515): eaax5866. doi:10.1126/scitranslmed.aax5866. PMC 6848974. PMID 31645453.
15. Toots M, Yoon JJ, Hart M, Natchus MG, Painter GR, Plemper RK. "Quantitative efficacy paradigms of the influenza clinical drug candidate EIDD-2801 in the ferret model". Translational Research, April 2020; 218: 16–28. doi: 10.1016/j.trsl.2019.12.002. PMC 7568909. PMID 31945316.
16. Cohen B, Piller C (13 May 2020). "Emails offer look into whistleblower charges of cronyism behind potential COVID-19 drug".
17. Cully M). "A tale of two antiviral targets - and the COVID-19 drugs that bind them". Nature Reviews. Drug Discovery, January 2022; 21(1): 3–5. doi:10.1038/d41573-021-00202-8.
18. Jump up to: Aleccia J (29 September 2021). "Daily pill to treat COVID could be just months away". ABC News. Kaiser Health News. Archived from the original on 29 September 2021. Retrieved 29 September 2021.
19. Jayk Bernal A, Gomes da Silva MM, Musungaie DB, Kovalchuk E, Gonzalez A, Delos Reyes V, et al. "Molnupiravir for oral treatment of COVID-19 in nonhospitalized patients". The New England Journal of Medicine, December 2021; 386(6): 509–520. doi:10.1056/NEJMoa2116044. PMC 8693688. PMID 34914868.

20. Singh AK, Singh A, Singh R, Misra A. "Molnupiravir in COVID-19: A systematic review of literature". Diabetes & Metabolic Syndrome, November, 2021; 15(6): 102329. doi:10.1016/j.dsx.2021.102329. PMC 8556684. PMID 34742052.
21. Merck and Ridgeback's Molnupiravir, an Oral COVID-19 Antiviral Medicine, Receives First Authorization in the World". Merck (Press release). 4 November 2021. Retrieved 4 November 2021.
22. Robbins R, Khan AJ, Specia M (4 November 2021). "Britain Becomes First to Authorize an Antiviral Pill for Covid-19". The New York Times. Retrieved 27 November 2021.
23. Kimball S (30 November 2021). "FDA advisory panel narrowly endorses Merck's oral Covid treatment pill, despite reduced efficacy and safety questions". CNBC. Retrieved 1 January 2022.
24. Jump up toLin MZ (24 December 2021). "A new drug to treat covid could create a breeding ground for mutant viruses". The Washington Post.
25. Jump up to:[a b] Service RF (7 November 2021). "A prominent virologist warns COVID-19 pill could unleash dangerous mutants. Others see little cause for alarm". www.science.org. Retrieved 24 December 2021.
26. Priyanka P., Shyamala, Mounika. D, Nadeemuddin and Abdul Moyeez. Development and validation of RP-HPLC method for determination of new anticancer agent acalabrutinib in bulk and its pharmaceutical formulation. Ejbps, 2019; 6(4): 465-470.
27. Mohan Goud V, Pooja Singh N.quantification and stability indicating UPLC method development and validation of acalabrutinib in bulk and pharmaceutical dosage form. International Journal of Life science and Pharma Research, 2021; 11(5): 41-50.
28. Atchutarama Krishna, G., Srinivasarao, P., Benarji Patrudu, T., Chidanandaswamy, R. Development and Validation of Novel HPLC Bioanalytical Analysis Method for Acalabrutinib: An Anticancer Drug in Human Plasma. Asian Journal of Chemistry, 2020; 32(10): 2606-2610.
29. Anusha A., Pushpa Latha E., Uttam Prasad Panigrahy., Rama Mohan Reddy T and Abbulu K. Stability indicating RP-HPLC method development and validation for the determination of Acalabrutinib in bulk drug and capsule dosage form. International Journal of Bio-Pharma Research, 2019; 8(8): 2758-2762.
30. Valluri VR, Katari NK, Khatri C, Kasar P, Polagani SR, Jonnalagadda SB. A novel LC-MS/MS method for simultaneous estimation of acalabrutinib and its active metabolite acalabrutinib M_{27} in human plasma and application to a human pharmacokinetic study. RSC Adv, 2022; 12(11): 6631-6639.

31. K.Kalyan Chakravarthi and K. Himabindu. RP-HPLC Method Development and Validation for the Determination of lorlatinib in Bulk and Its Pharmaceutical Formulation. International Journal of Research Publication and Reviews, 2021; 2(9): 958-963.
32. Veerman GDM, de Bruijn P, Dingemans AC, Mathijssen RHJ, Koolen SLW. To quantify the small-molecule kinase inhibitors ceritinib, dacomitinib, lorlatinib, and nintedanib in human plasma by liquid chromatography/triple-quadrupole mass spectrometry. J Pharm Biomed Anal, 2021; 193: 113733.
33. Anuradha N. D. and Syed Azhar Nizami. RP-HPLC method development and validation for the determination of lorlatinib in bulk and its pharmaceutical formulation. world journal of pharmacy and pharmaceutical sciences, 8(6): 1142-1150.
34. Claudia Spataria, b, Wenlong Lic, Alfred H. Schinkelc, Gaetano Ragnob, Jan H.M. Schellensa, d, Jos H. Beijnena, d,e, Rolf W. Sparidansa. Bioanalytical assay for the quantification of the ALK inhibitor lorlatinib in mouse plasma using liquid chromatography-tandem mass spectrometry.J Chromatogr B Analyt Technol Biomed Life Sci., 2018; 1083: 204-208.
35. DasameswaraRao Kavitapu, Arthanareeswari Maruthapillai, S. Devikala, J. Arockia Selvi, M. Tamilselvi, Sudarshan Mahapatra, G. Pradeep Kumar and Pradeep Kumar Tyagi.New Rapid Stability indicating RP-UPLC Method for the Determination of Olaparib, its Related Substances and Degradation Products in Bulk drug and Dosage Form. Materials Today Proceedings, 2019; 14(2): 492-503.
36. Daumar P, Dufour R, Dubois C, Penault-Llorca F, Bamdad M and Mounetou E. Development and validation of a high-performance liquid chromatography method for the quantitation of intracellular PARP inhibitor Olaparib in cancer cells. J Pharm Biomed Anal, 2018; 152: 74-80.
37. Ressiat C, Huynh HH, Plé A, Sauvageon H, Madelaine I, Chougnet C, Le Maignan C, Mourah S and Goldwirt L. Development and Validation of a Simultaneous Quantification Method of Ruxolitinib, Vismodegib, Olaparib, and Pazopanib in Human Plasma Using Liquid Chromatography Coupled With Tandem Mass Spectrometry. Ther Drug Monit, 2018; 40(3): 337-343.
38. Jeffrey Roth , Cody J. Peer , Baskar Mannargudi , Helen Swaisland , Jung-Min Lee , Elise C. Kohn and William D. A Sensitive and Robust Ultra HPLC Assay with Tandem Mass Spectrometric Detection for the Quantitation of the PARP Inhibitor Olaparib

(AZD2281) in Human Plasma for Pharmacokinetic Application. Figg Chromatography, 2014; 1: 82-95.

39. Priyanka Waje, Preeti Kulkarni, Smita George, Vijaya Kumar Munipalli, Raman Mohan Singh and Bhaskar Vaidhun. Method development and validation for quantitative estimation of olaparib in tablet dosage form by rp-hplc method. World Journal of Pharmaceutical Research, 2021; 10(12): 2162-2174.
40. Pagidi rajagopaludu1, nimmakayala saritha, devanna , srinivas maddi. Method development and validation of erythromycin and olaparib in human plasma by liquid chromatography–tandem mass spectrometry. Asian J Pharm Clin Res., 2022; 15(4): 28-33.
41. Tuba Reçber, Selin Seda Timur, Sevilay Erdoğan Kablan, Fatma Yalçın, Tutku Ceren Karabulut, R. Neslihan Gürsoy, Hakan Eroğlu, Sedef Kır, Emirhan Nemutlu, A stability indicating RP-HPLC method for determination of the COVID-19 drug molnupiravir applied using nanoformulations in permeability studies, Journal of Pharmaceutical and Biomedical Analysis, Nov 30, 2022; 214: 114693.
42. Amara A, Penchala SD, Else L, Hale C, FitzGerald R, Walker L, Lyons R, Fletcher T, Khoo S. The development and validation of a novel LC-MS/MS method for the simultaneous quantification of Molnupiravir and its metabolite ß-d-N4-hydroxycytidine in human plasma and saliva. J Pharm Biomed Anal, Nov 30, 2021; 206: 114356.
43. Bhumika Parmar, Aadil Raj, Divya Chauhan, Aakash Vasava, Vishal Chauhan, Mitali Dalwadi and Umesh Upadhyay. UV Visible spectroscopy method devlopment and validation for estimation of molnupiravir in solid dosage form. IJCRT., 2022; 10(4): 2320-2882.
44. Pritam Jain, Manali Bhamare and Sanjay Surana (2022). Quantitative estimation of molnupiravir by UV- Spectrophotometric method. International Journal of Pharmaceutical Chemistry and Analysis, 2022; 9(1): 35–39.