Advances in Bioresearch

Adv. Biores., Special Issue (3) 2025: 212-223 ©2024 Society of Education, India Print ISSN 0976-4585; Online ISSN 2277-1573 Journal's URL:http://www.soeagra.com/abr.html CODEN: ABRDC3 DOI: 10.15515/abr.0976-4585. SPL3.25.212223



A Review on Modern Methods for The Detection and Quantification of Anticancer Drugs in Pharmaceutical Products

Yogita Yadnik, Chandrashekhar Patil, Shweta Gunjal, Shounak Mande, Pritam Dube*
Sandip Foundation's Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik-422213,
Maharashtra, India
*Corresponding Author Email: pritamdube@gmail.com

ABSTRACT

This paper reviews various advanced analytical methods developed to study and ensure the quality of anticancer drugs like Brigatinib and Busulfan. These methods, including advanced techniques like RP-HPLC (Reversed-Phase High-Performance Liquid Chromatography) and UPLC-MS/MS (Ultra-Performance Liquid Chromatography-Mass Spectrometry), were designed to test drug purity, detect impurities, and study how the drugs behave in the body. For example, an RP-HPLC method for Brigatinib was highly accurate, with the drug detected at 5.6 minutes, making it ideal for routine quality control. Another method, UPLC-MS/MS, measured Brigatinib in blood plasma in under a minute, helping researchers study how the drug is processed in the body. Similarly, methods for Busulfan showed excellent precision and recovery rates, proving their reliability for pharmaceutical use. These techniques are robust, sensitive, and adhere to international standards, ensuring their application in both drug manufacturing and clinical studies. **Keywords**: Drug analysis, Brigatinib, Busulfan, RP-HPLC, UPLC-MS/MS, Quality control, Pharmacokinetics.

Received 14.05.2025 Revised 19.06.2025 Accepted 23.07.2025

How to cite this article:

Yogita Y, Chandrashekhar P, Shweta G, Shounak M, Pritam D. A Review on Modern Methods for The Detection and Quantification of Anticancer Drugs in Pharmaceutical Products. Adv. Biores. Special Issue [3] 2025. 212-223

INTRODUCTION

Cancer remains one of the most significant global health challenges, necessitating the development of highly effective and precise pharmaceutical treatments [1-3]. The detection and quantification of anticancer drugs in pharmaceutical products are critical for ensuring their efficacy, safety, and regulatory compliance. Advanced analytical techniques play a vital role in monitoring drug composition, stability, and potency, enabling accurate dosage administration and minimizing adverse effects [4,5]. This review aims to provide a comprehensive overview of modern analytical techniques used for the detection and quantification of anticancer drugs, highlighting their principles, applications, and advancements. By examining the strengths and limitations of these methodologies, this study seeks to contribute to the continuous improvement of pharmaceutical analysis, ensuring the availability of high-quality anticancer medications for effective patient treatment [6].

METHODOLOGY

This review systematically assesses modern analytical approaches for the detection and quantification of anticancer drugs in pharmaceutical products. The methodology encompasses an extensive literature survey, followed by structured data extraction, classification, and synthesis from multiple research papers, emphasizing drug type, analytical techniques, method specifications, mobile phase compositions, and retention times. The data gathered from selected research papers were systematically extracted and classified according to drug type, analytical technique, mobile phase composition, retention time, and key validation parameters such as accuracy, precision, linearity, and robustness. To enable effective comparative analysis, the extracted information was organized into structured tables. Furthermore, the study conducted a critical evaluation of various analytical methods employed for detecting antihypertensive drugs, focusing on their efficiency, sensitivity, and reproducibility.

ANALYTICAL METHODS

Analytical methods are scientifically validated procedures used to detect, identify, quantify, and study the chemical composition and structure of substances. In pharmaceutical analysis, particularly for anticancer drugs, these methods are essential to ensure the quality, safety, efficacy, and stability of drug formulations. Analytical methods can be broadly categorized into qualitative methods (which determine the presence or identity of a substance) and quantitative methods (which measure the exact amount of a substance present).

Chromatographic Methods

High-Performance Liquid Chromatography (HPLC)

HPLC is one of the most commonly used techniques in medicine testing. It is simple, reliable, and gives very accurate results. It helps scientists check how much of a drug is present and if it is pure. In this method, a liquid is pushed at high pressure through a tube filled with special particles. Different parts of the sample move through the tube at different speeds, which helps separate them clearly [7-10]. Table 1 show the different Modes of HPLC Method

Table 1: Different Modes of HPLC Method

Mode	Principle	Stationary	Mobile Phase	Used For	Example
	•	Phase			(Anticancer Drugs)
Reverse Phase (RP-HPLC)	Separation based on polarity (non- polar column retains non-polar compounds longer)	Non-polar (e.g., C18, C8)	Polar (water, methanol, acetonitrile)	Widely used for most drugs	Doxorubicin, Paclitaxel, Imatinib
Normal Phase (NP-HPLC)	Separation based on polarity (polar column retains polar compounds)	Polar (silica, alumina)	Non-polar (hexane, chloroform)	Non-polar compounds or isomer separation	Plant alkaloids, lipophilic drugs
Ion-Exchange Chromatography (IEC)	Separation based on ionic charge (positive or negative)	Charged resins (cation or anion exchangers)	Buffered aqueous solutions (pH controlled)	Charged molecules like amino acids, peptides, proteins	Methotrexate, Monoclonal antibodies
Size-Exclusion Chromatography (SEC)	Separation based on molecule size (larger molecules elute faster)	Porous polymer or silica beads	Aqueous or organic (non-reactive solvents)	Large biomolecules, polymers	Antibody-drug conjugates (ADCs), Enzymes
Affinity Chromatography	Separation based on specific binding between molecule and ligand	Ligand- immobilized resin (e.g., antibody, enzyme)	Buffer solutions	Highly specific purification of proteins or targeted drugs	Protein-based drugs, Cancer biomarkers

HPLC Applications for Specific Drug Classes

Table 2 shows the analytical method validation of various anticancer drugs by HPLC method.

Alkylating Agents

Purpose - Stability testing, degradation studies, quantification in formulations

Example - Cyclophosphamide, Ifosfamide

Mode of HPLC -Reverse Phase (RP-HPLC) [11].

Antimetabolites

Purpose - Assay in biological fluids, impurity profiling, dissolution studies

Example -5-Fluorouracil, Methotrexate, Cytarabine

Mode of HPLC - HPLC, HILIC [12].

Anthracyclines (Antibiotics)

Purpose - Detection in plasma and tissues, stability in light/heat, drug release studies

Example - Doxorubicin, Daunorubicin, Epirubicin

Mode of HPLC - RP-HPLC, Fluorescence Detection

Tyrosine Kinase Inhibitors (TKIs)

Purpose -Monitoring drug levels in blood, bioequivalence studies

Example - Imatinib, Erlotinib, Gefitinib

Mode of HPLC - RP-HPLC, LC-MS/MS [13].

Advantages of HPLC for Anticancer Drug Analysis

High Sensitivity and Accuracy -HPLC can detect very small amounts of anticancer drugs even in complex samples like blood or plasma.

Excellent Separation Efficiency -Anticancer drugs often exist with impurities, degradation products, or similar compounds (like isomers). HPLC can separate and identify each compound clearly, which helps in checking drug purity [14].

Ultra-Performance Liquid Chromatography (UPLC)

Ultra-Performance Liquid Chromatography (UPLC) is an advanced form of traditional HPLC, designed to provide higher resolution, faster analysis, and greater sensitivity. It uses smaller particle size columns (< $2~\mu m$) and operates at higher pressures (up to 15,000 psi) compared to HPLC (typically up to 6,000 psi). UPLC has become increasingly popular in pharmaceutical and clinical laboratories, especially for the analysis of potent drugs like anticancer agents, where speed, precision, and sensitivity are crucial [15-17].

Applications for Specific Drug Classes

UPLC techniques find diverse applications across various drug classes in oncology. For alkylating agents like cyclophosphamide and ifosfamide, UPLC is used to quantify drug levels in formulations and plasma, as well as analyze degradation products. Antimetabolites such as methotrexate, 5-fluorouracil, and gemcitabine benefit from UPLC for plasma and urine level monitoring, stability testing, and degradation analysis [18]. Anthracycline antibiotics like doxorubicin and daunorubicin utilize fluorescence-based UPLC for sensitive detection in biological samples. Tyrosine kinase inhibitors (TKIs) including imatinib, erlotinib, and gefitinib employ UPLC-MS/MS for therapeutic drug monitoring and pharmacokinetic profiling [19]. These applications demonstrate the versatility and importance of UPLC in oncology drug analysis and monitoring.

Advantages

UHPLC offers several key advantages over traditional HPLC methods. It enables faster analysis with significantly reduced run times, often under 5 minutes, making it particularly suitable for high-throughput screening applications. The technique provides higher resolution, allowing for improved separation of closely related compounds, which is essential for accurate impurity profiling. Additionally, UHPLC demonstrates greater sensitivity, facilitating the detection of trace-level drugs or impurities in biological fluids and pharmaceutical formulations. These combined benefits make UHPLC a powerful analytical tool for various applications in pharmaceutical research and quality control.

UV-Visible Spectroscopy:

Principle of UV-Visible Spectroscopy

UV-Visible spectroscopy is based on the absorption of ultraviolet (200–400 nm) and visible light (400–800 nm) by molecules. When a compound absorbs light in this region, electrons in the molecule are promoted from a lower energy level (ground state) to a higher energy level (excited state) [20-22].

Applications for Specific Drug Classes

UV-visible spectroscopy finds diverse applications in analyzing various classes of anticancer drugs. For antimetabolites like 5-Fluorouracil and Methotrexate, it is used for assay in tablets/injections and degradation studies. Anthracyclines such as Doxorubicin and Daunorubicin utilize visible region absorbance for content and stability assessment. The technique is employed for API assay in bulk and formulations of alkylating agents like Cyclophosphamide and Busulfan [23]. For Vinca Alkaloids such as Vincristine and Vinblastine, it aids in estimation within IV formulations. UV-visible spectroscopy offers several advantages: it is simple and fast, providing quick results ideal for routine quality control; cost-effective compared to HPLC or MS, making it suitable for basic labs; and non-destructive, allowing samples to be reused or further analyzed.

Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid Chromatography–Mass Spectrometry (LC-MS) is a hybrid analytical technique that combines the separation power of liquid chromatography (LC) with the detection and identification abilities of mass spectrometry (MS).LC separates compounds based on their chemical properties (like polarity, size, or charge).MS detects and identifies compounds based on their mass-to-charge ratio (m/z) by ionizing them and analyzing the resulting ions [24].

Applications for Specific Drug Classes

Mass spectrometry applications in oncology drug analysis span various drug classes, including alkylating agents for detecting active drugs and metabolites in plasma, antimetabolites for pharmacokinetic studies in biological fluids, platinum compounds for identifying drug-DNA adducts, monoclonal antibodies for peptide mapping and glycan analysis, and hormonal therapies for pharmacokinetic and metabolite tracking [25, 26]. However, several challenges exist in this field, such as matrix interference requiring

careful sample preparation, ion suppression/enhancement necessitating the use of internal standards or isotope-labeled drugs, high cost and maintenance limiting routine use in smaller facilities, and complex metabolite profiling demanding high-resolution MS techniques like QTOF or Orbitrap [24]. These applications and challenges highlight the diverse and complex nature of mass spectrometry in oncology drug analysis, emphasizing the need for advanced techniques and careful consideration of potential limitations.

RESULTS AND DISCUSSION

The comprehensive review of modern analytical methods for the detection and quantification of anticancer drugs in pharmaceutical products has provided valuable insights into the effectiveness, reliability, and advancements of various analytical techniques. The extracted information was systematically categorized and tabulated, facilitating comparative analysis, as presented in Table 2. The data from multiple research studies highlight key parameters such as drug type, analytical method, mobile phase composition, detection limits, and retention times.

The findings demonstrate that High-Performance Liquid Chromatography (HPLC) remains widely used for quantitative analysis, providing precision and reproducibility essential for pharmaceutical quality control. Additionally, Gas Chromatography (GC) has shown efficiency in separating volatile anticancer compounds, particularly in specialized formulations.

Spectroscopic methods, including UV-VIS Spectrophotometry and Fourier Transform Infrared (FTIR) Spectroscopy, have been explored for rapid detection and structural characterization of anticancer agents. These techniques offer cost-effective alternatives for routine quality control, though they often require complementary chromatographic methods for enhanced accuracy.

This review establishes a strong foundation for researchers and pharmaceutical professionals seeking effective analytical techniques for anticancer drug evaluation, emphasizing the need for continued innovation in method development. Future advancements should focus on enhancing sensitivity, minimizing environmental impact, and integrating automation and artificial intelligence to optimize pharmaceutical analysis for improved cancer treatment outcomes.

Table 2. Analytical method validation of various anticancer drugs by HPLC method.

Drug	Method	Specification	Mobile phase	Retention	Reference
				times	
Brigatinib	RP-HPLC	C18 column (ID: 5-micron particle size and a 100 Å pore size) Flow rate was at 1 ml/min Injection volume 10 µl	Methanol and distilled water (75%:25%) w	5.6 min	27
UP - HPLC UPLC-MS/MS	UP – HPLC	Luna C ₁₈ (100 x 2.6 mm) 1.6 µm column Flow rate of 1 ml/min Injection Volume 10µl	Acetonitrile, and 0.1 % Tri ethyl amine (TEA) (80:20 v/v)	-	28
	UPLC-MS/MS	BEH C18 column (2.1 mm × 50 mm, 1.7 μm).	Acetonitrile and 0.1% formic acid in water	0.56 min)	29
	HPLC	Lichrospher® C18 (250 mm × 4.6 mm Column WL: 261 nm Flow rate 1.0 ml/min	Methanol, acetonitrile, phosphate buffer Ph4.5 in 55:25:20 (v/v)	4.60 min, 12.28 min, 3.37 min, 7.34 min and 8.39 min	30
Busulfan HPLC HPLC RP-HPLC Metho	HPLC	Column: YMC Pack ODS-A (150 x 4.6) mm, 3µm Flow rate: 1.5 ml / min Injection volume: 20 µl	Water, acetonitrile and tetrahydrofuran at 30:65:5 (V/V) ratio	4.4 min	31
	HPLC	Novapakoctadecylsilyl (ODS) (150×3.9 mm I.D.) analytical column	Methanol-water (80:20, v/v) a	0.8 min	32
	RP-HPLC Method	C18 column (Length: 150mm, Diameter: 4.6mm) Flow rate was	Acetonitrile, Water and Tetrahydrofuran in the ratio of 66:32:2	14 min	33

		1.0ml/min	(v/v/v)		
	RP-HPLC	Zorbax SB C8; 250mmx4.6mm, 5µm or Equivalent Flow rate of 0.5 ml/min	Acetonitirle: Formic acid 50:50:1.00v/v/v	8 min	34
	LC-MS/MS	Acquity UPLC BEH C18 LC column (2.1 mm × 100 mm, i.e. 1.7 µm The flow rate was set at 400 µl/min	Ammonium acetate 5 mm and formic acid 0.1% v/v in water (phase A) and formic acid 0.1% v/v in ACN (phase B)	2.71 min	35
	GC-FID	Silica capillary column (0.53 mm × 30 m, 1.0 µm, USP Phase G42) Oven temperature programming from 60 to 220 ° C Inlet temperature of 250 ° C, A split ratio of 1:1 An injection volume of 2 µl.	(35%-phenyl)- methylpolysiloxane		36
5-Fluorouracil	HPLC	C8 Phenomenex (100 mm x 4.6 mm, 5 μm, 110A) Injection volume was 30 μl	Methanol: Water (10:90) v/v, acidified with 0.05% (v/v) orthophosphoric acid (ph4.5)		37
	HPLC	C18 column A flow rate of 1 ml/min Injection volume was 100 µl	Acetonitrile and water (10:90, v/v		38
	RP-HPLC	BDS hypersil C18 4.6 mm x 250 mm Flow rate was 0.8 ml/min. Injection volume 20 µl	Methanol: water (10:90 v/v)	6.2±0.1 min	39
	RP-HPLC	ODS hypersil C18 column having 4.6 mm x 250 mm Flow rate was 1 ml/min Injection volume 10 µ	Acetonitrile: water (10:90) at ph6	4.5 min	40
	HPLC	R-HS,C-18, 5micrometer Flow rate of 1 ml/min	5mmol/L potassium dihydrogen phosphate (ph6.0) and Methanol of (96:4 v/v)	3.457 min	41
	RP-HPLC	C-18 reversed phase column (Phenomenex; Prodigy ODS3V, 250×4.6 mm, 5 μ) Flow rate of 1.2 ml/min	50mm KH2 PO4 (ph, 5.0)		42
	UPLC-MS/MS	UPLC BEH C18 column (2.1 × 100 mm; 1.7 μm) Flow rate of 0.15 ml/min	Acetonitrile- ammonium acetate 1 mm (95:5)		43
Gemcitabine	HPLC	Agilent TC C18 (250*4.6mm) Flow rate 1.0ml/ min	Acetonitrile and water 50:50 v/v	4.340 min	44
	RP-HPLC	C18 (250 X 4.6) mm Flow Rate : 0.8 ml/minute Injection Volume : 10 µl	0.1% Orthophosphoric acid in water and 100% Methanol	40 min	45
L	RP-HPLC	AninertsilODS-3V	Water: acetonitrile	4.8 min	46

		column (250mm x 4.6mm; 5µm) Flow rate of 1ml/min, Injection Volume 10µl	(90:10; ph3.5)		
	RP-HPLC	Zorbax RXC8, (250mm× 4.6mm, 5µ) Flow rate 1.2 ml/min Injected volume 20 µl	Phosphate buffer (ph3.0) and methanol (85:15 v/v)	40 min	47
	RP-HPLC	ACE C18 (250x4.6 mm, 5 µm particle size) Flow rate 1 ml/min Injection volume was 20 µl	Phosphate buffer, ph3/acetonitrile at a 98:2 ratio,	13 min	48
	RP-HPLC	Hypersil BDS C18 column (250 x 4.6mm x 5 µ) Flow rate was 1.0ml/min Injected Volume 10 µl	Buffer and acetonitrile 93:7v/v.	3.927min	49
	UV spectrophotomet ric	Double beam systronics Model UV2201 (India) Spectral bandwidth of 1 nm	Phosphate buffer (ph7.4) Phosphate buffer (ph-6.8) 0.2 M potassium dihydrogen orthophosphate 0.2 M sodium hydroxide		50
	HPTLC	Concentration range 500-3000 ng/band Wavelength 268 nm Correlation coefficient of 0.997	Toluene-methanol- chloroform in the ratio of 3.6:3.6:3 (v/v/v)		51
	UV	Concentration range of 50-250µg/m Absorption maxima at 554nm			52
Imatinib	RP-HPLC	Symmetry C18 (150 mm ' 4.6 mm) 5m Flow rate 1.0 ml/minute Injection volume 20µl	Ortho-phosphoric acid as buffer solution and methanol ratio of 50:50 (V/V)	17.602 min	53
	RP-HPLC	HiqSil C18 (250 mm × 4.6 mm, 5 µm) Flow rate 1.0 ml/min Injection volume was 20 µl.	Methanol and acetate buffer ph3.5 in the ratio of 80:20 v/v	8.060 and 11.398 min	54
	RP-HPLC	C18 G column (250 x 4.6 mm, 5 µm) Flow rate of 1.0 ml/min Injection Volume 20 µl	O-Phosphoric acid (0.1% v/v): Acetonitrile 70:30 (v/v)	3.25 min	55
	RP-HPLC	HiqSil C18 (250 x 4.6 mm, 5µm) Flow rate was 1.0 ml/min Injection volume was 20 µl	Methanol and Acetate Buffer ph3.5 in the ratio of 80: 20 v/v	6.208 Min	56
	RP-HPLC	Eclipse XDB-C18 (150 mm X 4.6 mm) 5µ Flow rate of 1.0 ml/minute. Injection volume 20 µl	Mixture of methanol and acetonitrile in the ratio of (300:200)	5.360 Min	57
Letrozole	RP-HPLC	KromasilODS C18 column (250mmx4.6mm, 5µ) Flow rate of 1.0ml/min Injection volume is 20µl	Potassium dihydrogen phosphate and methanol (85:15) V/V	3.42 Min	58

	RP-HPLC	FinepakC8 column (4.6 mm § 250 mm, Jasco, Japan) Flow rate of 1 ml/min Injection volume was 20 ul	Water, acetonitrile And methanol (50:30:20 v/v/v)	10 min	59
	HPLC	RP8 Macherge Nagel (L7 reversed- phase column (150mm × 4.6mm), 5 microns, Flow rate of 1.0 ml.min-1 Injection volume 20µl	Water: Acetonitrile (530: 470 v/v)	5.7 Min	60
	RP-HPLC	Zorbax C18 column (250 x 4.6 mm ID; 5.0 μm particle size) Flow rate 0.7 ml/min	Methanol: 0.1% orthophosphoric acid (60:40)	5.94 Min	61
Methotrexate	HPLC	Zorbax Eclipse XDB-C8 150 x 4.6 mm Flow rate 1.0 ml/min Volume of injection: 20 ul	Acetonitrile in the ratio of 93:7		62
1	HPLC	Inertsil ods-3V C-18, 4.6x250mm Injection Volume 10 or 20µl Flow rate of 1ml/min	Buffer (0.1 M dihydrogen phosphate and 0.01 M TRIS; ph5.7): methanol: acetonitrile(70:20:1 0)	4.6 and 9.5 min	63
	UV Spectrophotomet er (Jasco V630)	Concentration range of 2-10 µg/Ml Amaxat 303nm Correlation Coefficient 0.9987	Phosphate buffer solution		64
	Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotomet er	Amax at 307 nm Concentrations ranged from 80 to 120% Correlation coefficient (r = 0.999)	Poly(ε- caprolactone), 0.1 ml/l hydrochloric acid		65
	UV Spectrophotomet er	Concentration ranges 5- 30 µg ml-1 Correlation coefficient (r2) 0.9999 Absorption maxima) 610 nm	Dimethylesulfoxide, (0.5n) hydrochloric acid		66
	HPLC	RP-C8 (250 mm 4.6 mm, 5 mm) Flowrate1 ml/min	ACN and H20 containing 0.1% trifluoroacetic acid with a ratio of 1:1	6 min	67
Nilotinib	HPLC	Phenomenex C18 column (15x4.6mm, 5µm) Flow rate 1ml/min Volume of injection 20µl	Acetonitrile and phosphate buffer (ph5) 60:40 %v/v	5.401min	68
	RP-HPLC	Thermo Scientific C18 column (250mm x 4.6mm i.d.5µ) 20µl injection volume Flow rate was 1.0ml/min	0.1% trifluoroacetic buffer: acetonitrile in the ratio of 65:35 v/v	5.888min	69
	RP-HPLC	Xterra RP-18 (150*4.6 mm, 3.5 μm) Injection volume of 20 μl	10 mm ammonium formate with ph-3.5 and acetonitrile 50:50 V/V	7.41 Min	70

		Flow rate was 1.0 ml/min			
		Column temperature of 40 °C			
	RP-HPLC	C-18 column (250mm x 4.6mm i.d.,5µm) Flow rate of 1ml/min Injection volume of 20 µl Column temperature of 25°C	Water and acetonitrile in the ratio of 50:50 %v/v	6 Min	71
	UV spectrophotomet ric	Maximum absorbance 263 nm Concentration range of 7- $12\mu g/ml$ Correlation coefficent $r^2 = 0.9984$.	Methanol:water (1:1)		72
Prednisolone	RP-HPLC	C18 column (15cmx 0.46 cm. 5µm) Flow rate 1ml/min Column temperature 45°C Injection volume: 20µl	Tetrahydrofuran: acetonitrile in ratio (75: 15: 10)	7.5, 9.98 and 10.7 min	73
	RP-HPLC	HypersilODS C18 (250 × 4.6 mm, packed with 5 micron) Flow rate 1.0 ml/min Injection volume: 20μl	Methanol:water(58: 42)	7.029 and 1.681 min	74
	RP-HPLC	Bondapak C ₁₈ column	Aetonitrile- citrophosphate buffer (ph5; 45:55 v/v		75
Fulvestrant	HPLC	ACE C_{18} analytical column (250 mm × 4.6 mm I.D., 5 μ m) Flow rate 1.0 ml min ⁻¹ Injection volume 10 μ l.	1% orthophosphoric acid -methanol (80:20, v/v)	3.1 min	76
	RP – HPLC	Zorbax XDB C18; 150 × 4.6 mm, 3.5 Flow rate 2 ml/min	Acetonitrile, Methanol and Water (490:410:100)	21 min	77
	HPLC	Cyano column (4.6 mm x 25 cm, 5 µm) The flow rate 1.5 ml/min Injection volume 10 µl	N-hexane and isopropyl alcohol (70 : 30 v/v).	30 Min	78
Capmatinib	HPLC	Column: Zorbax Eclipse XDB-C18 (4.6 mm *150 mm, 5 µm) Flowrate: 1 ml/min Wavelength: 255 nm. Injection volume was 20 µl	Phosphate buffer: methanol (50:50 v/v)	6.5 min	79
	HPLC	Column: C18 (250 x 4.6mm,5µ) Flowrate: 1 ml/min. Wavelength: 231 nm	D buffer (PH 6.5): meoh(60:40) v/v	4.327 min	80
	HPLC	Column: C18 Column (150 mm x 4.6 mm i.d., 5µ) Flowrate: 0.6 ml/min. Wavelength: 218 nm Injection volume was 20 µl	Methanol: Phosphate buffer ph3 (65:35 % v/v)	4.3 min	81
Lorlatinib	RP-HPLC	Column: Eclipse plus C18(250 mm×4.6 mm,3	Potassium Dihydrogenorthoph	7.87 min	82

Erdafitinib	HPLC	µm). Flowrate: 1 ml/min. Wavelength: 310 nm Injection volume was 10 µl Column persil™ ODS C18 Column (150 mm × 4.6 mm, 5 µ). Wavelength: 310 nm Flow rate: 1 ml/min	osphate, Acetonitrile, and Methanol (50:30:20 v/v). 20mm Sodium acetate buffer (ph4. ±0.02), methanol and acetonitrile (60:10:30 v/v/v)	3.38 min	83
Selpercatinib	RP-HPLC	Injection volume 10 μl Column: Zorbax C18 column (150×.6 mm, 5μ). Flowrate: 1 ml/min Wavelength: 220 nm Injection Volume 10 μl	0.1% orthophosphoric acid and acetonitrile (60:40 v/v)	2.653 min	84
Zanubrutinib	HPLC	Column: Luna C18 (250x 4.6 Mm, 5 µ) Wavelength: 220 nm Flowrate:1 ml/min Injection Volume 10 µl	0.1% ortho phosphoric acid and acetonitrile 50:50 v/v.	4.358 min	85
Rucaparib	RP-HPLC	Column: C-18 ODS (25 cm,0.46 cm diameter, 5 µm). Wavelength: 286 nm Flowrate:1ml/min.	Phosphate (0.02 M) methanol (65:35 % v/v)	5.484 min	86
Talozoparib	RP-HPLC	Inertsil ODS C18 (4.6mm x 250mm, 5µm). Wavelength: 225 nm Flowrate: 1 ml/min.	Methanol: Acetate Buffer (ph-4.2) (40:60% v/v))	3.388 min	87
TalozoparibTo sylate	HPLC	Column: Spherisorb ODS2 C18 column (250mm × 4.5 mm; 5µm). Wavelength: 269 nm Flowrate: 1 ml/min	Methanol: acetonitrile: 0.1 % sodium perchlorate in the ratio of 25:75:05 (v/v)	2.74 min	88
Dacomitinib	RP-HPLC	Column: Zorbax Eclipse (250x4.6 mm,5 µm). Wavelength: 253 nm Flowrate: 1 ml/min	0.1M sodium perchlorate, acetonitrile 20:80 (v/v)	5.8 min	89

CONCLUSION

Based on the review, it can be concluded that RP-HPLC, UPLC-MS/MS, and GC-FID are key analytical techniques widely used for the quality control and analysis of anticancer drugs. RP-HPLC remains the most commonly used method for testing the purity, stability, and impurity profiles of drugs like Brigatinib, Busulfan, and 5-Fluorouracil. The use of columns such as C18 and Zorbax Eclipse, combined with buffers like phosphate and solvents like acetonitrile, are critical in ensuring the method's efficiency and accuracy. UPLC-MS/MS has proven highly effective for pharmacokinetic studies and therapeutic drug monitoring due to its superior sensitivity and faster analysis times, though it requires specialized equipment and expertise. GC-FID is particularly useful for impurity profiling, especially for volatile compounds, but its application is limited to drugs that are thermally stable. The methods discussed are generally in compliance with international regulatory guidelines such as those from the International Council for Harmonization (ICH), ensuring their reliability and acceptance in global pharmaceutical quality control. However, there is a need for continued innovation to develop multi-analyte methods and more adaptable techniques, especially to accommodate new drug delivery systems and complex drug formulations like biologics and nanoparticles. Future research should focus on improving the speed, accessibility, and sensitivity of these methods, with particular attention to enhancing their application in clinical and pharmaceutical settings.

REFERENCES

- 1. Swartz ME, (2005). UPLC: An introduction and review. J Liq Chromatogr Relat Technol.;28(7-8):1253–1263.
- Mohsen Safaei, Mostafa Bahrami, Ling Shing Wong, Douglas Law S, Afroz RR. (2025). Zinc Oxide Nanoparticles in Oral Cancer Therapy: A Systematic Review of Their Potential and Efficacy. J Appl Organomet Chem. 25;1000-1009.
- 3. Bello Umar A, Abdullahi SH, Uzairu A, Shallangwa GA, Uba S. (2023). Molecular Docking Studies of some Coumarin Derivatives as Anti-Breast Cancer agents: Computer-Aided Design and Pharmacokinetics Studies. Prog Chem Biochem Res [Internet]. 6(3):229–43. Available from: www.pcbiochemres.com
- 4. Zhang J, Zhang Y, Zhang Q, (2021). Development and validation of a rapid and sensitive HPLC-UV method for the determination of Brigatinib in rat plasma. J Chromatogr Sci.;59(2):161–167.
- 5. Pawar SD, Shanmugasundaram P. (2024). Synthesis, Characterization and Evaluation of Anticancer Activity of Some 6-(Chloro / Methoxy/ Methyl) -N- (4-Substitutedphenyl)-4-Thiomorpholinoquinazolin-3(4H)-Amine Derivatives. Adv J Chem Sect A.7(5):643–58.
- 6. Bouligand J, et al. (2019). Busulfan monitoring using LC-MS/MS: Inter-laboratory proficiency testing and long-term performance. Clin Chim Acta.495:66–71.
- 7. Kazakevich Y, Lobrutto R. (2007). HPLC for Pharmaceutical Scientists. Hoboken (NJ): John Wiley & Sons.
- 8. Shinde SA, Deshmukh AD, More UK. (2023). Neonatal Sickle Cell Disease Assessment in Rural Indian Community: Demand for Point of Care Testing (POCT). J Med Chem Sci.7(1):1–8.
- 9. Chandramore KR, Sonawane SS, Ahire RS, Reddy H, Ahire SB, Jadhav PB, et al. (2025). Development and Validation of Stability Indicating LC Method for Selexipag: In-Silico Toxicity Study and Characterization of its Degradation Products. Chem Methodol.9:427–47.
- 10. Preeti N. Yadav, Chhalotiya Usmangani K, Patel Kesha M, Tandel Jinal N. (2020). Quantification of A β Adrenergic Receptor Drug Mirabegron by Stability Indicating LC Method and Uv–visible Spectroscopic Method in Bulk and Pharmaceutical Dosage Form. Chem Methodol;4(53):340–58. Available from: http://chemmethod.com
- 11. Liu D, Zhang L, Yang T, Zhao H.(2003). Determination of cyclophosphamide in plasma by RP-HPLC. J Pharm Biomed Anal. 32(1):169–174.
- 12. Zamek-Gliszczynski MJ, et al. (2005). Quantitative HPLC method for 5-fluorouracil and metabolites. Biomed Chromatogr. 19(5):394–402.
- 13. Filippone S, et al. (2014).Monitoring Imatinib by LC-MS/MS in plasma for therapeutic drug management. J Chromatogr B.;960:160-165.
- 14. Swartz ME. (2005). HPLC: Essential concepts and applications. Anal Chem. 77(12):404A-410A.
- 15. Kumar P, Mahadik KR, Paradkar AR. (2011). Development and validation of UPLC method for quantification of cytotoxic drugs in bulk and dosage forms. J Chromatogr Sci. 49(2):98–103.
- 16. Prasath H, Azhakesan A, Manikandan G. (2025). Analytical Methods for Quantification of Gemcitabine in Pharmaceutical and Biological Samples: An Overview of Developments in the Last Decade. Asian J Green Chem. ;9:457–75.
- 17. Elumalai S, Sharma M, Dantinapalli VLS, Palanisamy M. (2025). Novel Stability Indicating UPLC Method Development and Validation for Simultaneous Quantification of Perindopril Arginine and Bisoprolol Fumarate in Pure and Pharmaceutical Dosage Form. Adv J Chem Sect A. 8(9):1488–507.
- 18. Karu K, Hornshaw M, Woffendin G, Bodin K, Hamberg M, Gaskell SJ. (2007). Liquid chromatography-mass spectrometry using UPLC for rapid separation and analysis of urinary drug metabolites. Anal Chem. 79(19):7583–7590.
- 19. Xu X, Qian X, Zhao Y. (2022). A validated UPLC–MS/MS method for rapid determination of Imatinib in plasma: application to pharmacokinetics. Biomed Chromatogr. 36(1):e5257.
- 20. Al-Azzam KM, Alzoubi KH, AbuRuz SM. (2005). Development and validation of a spectrophotometric method for the determination of 5-fluorouracil in pharmaceutical formulations. Il Farmaco. 60(6-7):555–558.
- 21. Morah EJ, Eboagu NC, Nwakife NC, Chinelo Ezeonu C. (2024). Characterization and Phytochemical Evaluation of the Seed, Seed Oil and Leaves of Moringa oleifera. Adv J Chem Sect B Nat Prod Med Chem [Internet].;6(2):147–65. Available from: http://www.aichem-b.com/https://doi.org/10.48309/aicb. 2023.394689. 1166
- 22. Baraga WM, Shtewia FA, Ulsalam Tarrousha AA, Al-Adiwisha WM, Altounsib MK. (2025). Green Synthesis of Silver Nanowires Using Aqueous *Brassica tournefortii* Leaves Extract and Evaluation of Their Antibacterial and Antioxidant Activities. J Appl Organomet Chem. 2025;5(1):13–27.
- 23. Mohamed ME, Refat MS. (2014). Spectrophotometric determination of busulfan in pure form and in pharmaceutical formulations. Spectrochim Acta A Mol Biomol Spectrosc.120:448–454.
- 24. Panuccio G, Barco S, et al. (2018). LC-MS/MS-based quantification of anticancer agents: Current strategies and challenges in clinical pharmacology. J Pharm Biomed Anal.152:48–61.
- 25. Relling MV, et al. (2004). Cyclophosphamide and metabolites by LC-MS/MS in patient samples: Pharmacokinetics and metabolism. Clin Pharmacol Ther. 75(5):442–449.
- 26. Wu J, Madero M, et al. Characterization of monoclonal antibody Trastuzumab and related variants using LC–MS peptide mapping. Anal Bioanal Chem. 2017;409(5):1205–1216.
- 27. Akter, M. R., Hossain, M. S., Alam, K. M. K., & Rafiquzzaman, M. (2023). Development and validation of RP-HPLC method for the determination of anticancer drug brigatinib. GSC Biol. Pharm. Sci., 23(03):030–041.

- 28. Gunturu, R., & Kantipudi, R. (2022). A study of stability indicating development and validation of a method for simultaneous estimation of brigatinib and alectinib using reverse phase ultra performance liquid chromatography. J. Pharm. Res. Int., 34(9A):46-55.
- 29. Wen, J., Bao, S., Cai, Y., Zhang, B., Wang, R., Wang, C., & Hu, G. (2017). A reliable and stable method for determination of brigatinib in rat plasma by UPLC-MS/MS: Application to a pharmacokinetic study. J. Chromatogr. B, 1068–1069:84–89.
- 30. Attada, T., Potnuru, J., Kumar, B. S., Prasad, K. T., & Rao Anna, V. (2023). Qualitative and quantitative assessment of process-related impurities in brigatinib raw material and formulations using HPLC. Anal. Sci. Technol., 36(4):180–190.
- 31. SwamySekhar, K., Kishore, C. V., Rao, T. V., & Babu, K. R. (2021). HPLC method for the development and validation of busulfan in pharmaceutical formulation. GSC Adv. Res. Rev., 6(3):136–142.
- 32. Heggie, J. R., Wu, M., Burns, R. B., Ng, C. S., Fung, H. C., Knight, G., et al. (1997). Validation of a high-performance liquid chromatographic assay method for pharmacokinetic evaluation of busulfan. J. Chromatogr. B, 692(1-2):437-444.
- 33. Rewaria, S., & Swamy, V. B. M. (2013). Analytical method development and validation for assay method of busulfan injection by RP-HPLC method. Res. J. Pharm. Biol. Chem. Sci., 4(2):1781-1791.
- 34. Sridhar, J. V. S., Andrews, B. S. A., Uppala, L. K., & Abbaraju, V. D. N. K. (2019). New RP-HPLC method for development and validation of busulfan assay in liquid formulation. Pramana Res. J., 9(4).
- 35. Cafaro, A., Pigliasco, F., Baiardi, G., Barco, S., Stella, M., Bandettini, R., et al. (2023). Development and validation of a novel LC-MS/MS method for TDM-guided personalization of HSCT conditioning with high-dose busulfan in children. Biomedicines, 11(2):530.
- 36. Khan, A., Winter, G., & Tan, S. (2021). Stability-indicating method development and validation for busulfan drug substance by GC-FID. J. Chromatogr. Sci., 59(2):112–119.
- 37. Viegas, J. S. R., Praça, F. S. G., & Bentley, M. V. L. B. (2018). Development and validation of a HPLC method for 5-Fu quantification following in vitro skin permeation assay. J. Pharm. Sci., 2(1):113.
- 38. De Mattos, A. C., Khalil, N. M., & Mainardes, R. M. (2013). Development and validation of an HPLC method for the determination of fluorouracil in polymeric nanoparticles. Braz. J. Pharm. Sci., 49(1):27-36.
- 39. Minhas, M. U., Ahmad, M., Sohail, M., & Siddique, F. (2015). Fast and new reversed-phase HPLC method for analysis of 5-fluorouracil in human and rabbit plasma. Pak. Vet. J., 35(1):71-75.
- 40. Sethi, A., Ahmad, M., Ahmad, I., & Sultana, S. (2022). RP-HPLC method for 5-FU in human and rabbit plasma samples: Identification and quantification. Am. J. Adv. Drug Deliv., 10:31.
- 41. Hanif, M., Akhtar, M. F., Naeem, S., Wahid, M., Shehzad, M. A., Saadullah, M., et al. (2017). Development and validation of a new HPLC method for detection of 5-fluorouracil in mobile phase and plasma. Curr. Pharm. Anal., 13(0).
- 42. Sinha, V. R., Kumar, R. V., & Bhinge, J. R. (2009). Stability-indicating RP-HPLC assay method for 5-fluorouracil. Indian J. Pharm. Sci., 71(6):704-708.
- 43. Harahap, Y., Amarta, V., & Saputri, F. A. (2024). Development and validation of UPLC-MS/MS method for 5-fluorouracil quantification in dried blood spot. Heliyon, 10, Article e29990.
- 44. Syed, A., Prachet, P., Munwar, S., & Nadendla, R. R. (2020). Analytical method development and validation of gemcitabine in tablets by HPLC by different analytical techniques. IOSR J. Pharm. Biol. Sci. (IOSR-JPBS), 15(1), 24-29.
- 45. Shaik, A. H., Kotanka, R. R., Singh, H., Naidu, B. G., Rao, S. G., & Nalla, K. (2024). Development and validation of a novel method for analysis of impurities in gemcitabine hydrochloride using RP-HPLC. J. Chem. Pharm. Res. (ICHR), 14(1), 522-530.
- 46. Mishra, S., Narenderan, S. T., Babu, B., Mukherjee, K., & Meyyanathan, S. N. (2019). Validated method for gemcitabine estimation in formulations by RP-HPLC. Res. J. Pharm. Technol., 12(11).
- 47. Nataraj, K. S., BadrudDuza, M., Kalyani, N. V. V. S., & Kiran Kumar, D. (2012). RP-HPLC method for gemcitabine in bulk and dosage forms. Res. J. Pharm. Biol. Chem. Sci. (RJPBCS), 3(4), 410-416.
- 48. Vidal, H., Goncalinho, H., Monteiro, J., Das Neves, J., Sarmento, B., Diniz, C., & Fresco, P. (2014). Simple RP-HPLC method for gemcitabine in plasma. Int. J. Pharm. Pharm. Sci., 6(9).
- 49. Siddartha, B., Sudheer Babu, I., Ravichandra Gupta, C., & Parthiban, C. (2014). RP-HPLC method for gemcitabine in bulk and dosage form. World J. Pharm. Res., 3(2).
- 50. Kaur, T., Kaur, S., & Kaur, P. (2017). UV-spectrophotometric methods for gemcitabine in bulk and nanoparticles. Int. J. Appl. Pharm., 9(5).
- 51. Borisagar, S., Patel, H., & Patel, C. (2000). HPTLC method for gemcitabine estimation in dosage form. J. Planar Chromatogr. Mod. TLC.
- 52. Sarsambi, P. S., Kalyan K., Raju, S. A., & Danki, L. S. (2011). Spectrophotometric methods for gemcitabine in bulk and formulations. Res. J. Pharm. Technol., 4(6), 951–954.
- 53. Shah, P., & Shah, R. (2015). RP-HPLC method for related substances of Imatinib. Int. J. Pharm Tech Res., 8(6), 128-146.
- 54. Kuna, A. K., Seru, G., & Venkata Radha, G. (2018). RP-HPLC for imatinibmesylate and its impurity. Asian J. Pharm. Clin. Res., 11(3), 136-139.
- 55. Sindhu, S. N., Rao, Y. S., Kumar, T. H., & Rao, K. V. P. (2015). RP-HPLC for imatinibmesylate in pure and dosage form. Der Pharmacia Lettre, 7(3), 33-38.

- 56. Kuna, A. K., Seru, G., & Radha, G. V. (2018). RP-HPLC method for imatinibmesylate and acid impurity. The Pharma Innov. J., 7(12), 418-422.
- 57. Shah, P., Shah, N., & Shah, R. (2015). Stability-indicating RP-HPLC method for Imatinib in tablets. J. Pharm. Sci. Res., 6(10), 4453-4468.
- 58. S, P., PM, V., T, R., & Malothu, R. (2012). RP-HPLC method for letrozole in bulk and dosage form. Int. Res. J. Pharm., 3(11).
- 59. Mondal, N., Pal, T. K., & Ghosal, S. K. (2009). Development and validation of RP-HPLC method to determine letrozole in different pharmaceutical formulations and its application to studies of drug release from nanoparticles. Acta Poloniae Pharmaceutica Drug Research, 66(1), 11–17.
- 60. Ahmed, N. R., Lottfie, S. N., & Hassan, Y. S. (n.d.). (2021). HPLC method for estimation of letrozole in pharmaceutical formulation: Application to content uniformity testing. World Journal of Pharmacy and Pharmaceutical Sciences, 11(1), 10–18.
- 61. Priyadarsini, S., & Lahoti, S. R. (2022). RP-HPLC method development and validation for quantification of letrozole solid lipid nanoparticle. Journal of Research in Pharmacy, 26(2), 421–430.
- 62. De Abreu, C. C., et al. (2015). Development and validation of HPLC method to determine methotrexate in children oncologic patients. Eur. Rev. Med. Pharmacol. Sci., 19, 1373–1380.
- 63. Nagulu, M., et al. (n.d.). Development and validation of rapid and sensitive HPLC method for the determination of methotrexate in human serum. Stamford Journal of Pharmaceutical Sciences, 2(1), 8–13.
- 64. Rajnani, N., & Kurup, N. S. (2018). Method development of methotrexate in phosphate buffer solution by UV-visible spectroscopy. Int. J. Trend Sci. Res. Dev. 2(4).
- 65. Mara da Costa, V., et al. (2012). UV spectrophotometric method for methotrexate in implants: Application to quality control. Int. J. Pharm. Pharm. Sci., 4(Suppl 4), 1–8.
- 66. Ramachandra, B., et al. (2013). UV-visible spectrophotometric method for methotrexate analysis in formulations. Der Pharma Chemica, 5(4), 71–79.
- 67. Karami, F., et al. (2019). Analytical methodologies for methotrexate and metabolites in pharma, bio, and environment. J. Pharm. Anal., 9, 373–391.
- 68. Barla, A., & Buralla, K. K. (2020). RP-HPLC method for nilotinib in bulk and dosage form. Int. J. Pharm. Investig., 10(3), 364–367.
- 69. Jain, S., et al. (2023). RP-HPLC method for nilotinib in bulk and formulation. J. Chem. Health Res., 13(2), 410-421.
- 70. Ivaturi, R., et al. (2016). Stability indicating HPLC method for nilotinib hydrochloride. Int. J. Pharm. Pharm. Sci., 8(9), 92–97.
- 71. Harika, M., & Kumar, G. S. (n.d.). (2020). RP-HPLC method for nilotinib in bulk and formulations. Int. J. Pharm. Investigation, 10(3):364-367
- 72. Chaitanya, G., & Pawar, A. K. M. (2015). UV spectrophotometric method for Nilotinib hydrochloride (orphan drug). J. Chem. Pharm. Res., 7(12), 1087–1093.
- 73. Gad Kariem, E. A., et al. (2016). RP-HPLC for prednisolone and its degradation products in tablets. EC Pharm. Sci., 2(3), 312–324.
- 74. Ghosh, S., et al. (2011). RP-HPLC validation for prednisolone in tablets. Asian J. Chem., 23(11), 5092-5094.
- 75. Singh, D. K., & Verma, R. (2007). Second derivative-spectrophotometric and RP-HPLC for prednisolone. J. Anal. Sci., 23, 1241–1243.
- 76. Atila, A., et al. (2016). HPLC vs voltammetric method for fulvestrant. Iran. J. Pharm. Res., 15(3), 369-378.
- 77. Ghosh, S., et al. (2021). RP-HPLC method validation of fulvestrant injection. Egypt. J. Chem., 64(4), 2047-2055.
- 78. Varanasi, M. B., et al. (2010). RP-HPLC for fulvestrant in pharmaceutical dosage forms. Int. J. Chem. Sci., 8(2), 1215–1225.
- 79. Dalvi, S., et al. (2021). RP-HPLC method for Capmatinib quantification in tablets. IJPPR, 21(3), 314-329.
- 80. Darshnakiritbhai, S., et al. (2022). Stability indicating RP-HPLC for Capmatinib. IJPRA, 7(3), 712-719.
- 81. Sivaganesh, M. M., et al. (2022). RP-HPLC for Capmatinib in bulk and tablets. YMER, 21(1), 638-662.
- 82. Chakravarthi, K. K., & Himabindu, K. (2021). RP-HPLC method for Lorlatinib. IJRPR, 2(9), 958-963.
- 83. Maithani, M., et al. (2020). RP-HPLC method for Erdafitinib. J. Biomed. Pharm. Res., 9(3), 29–32.
- 84. Singamsetty, N., & Sundararajan, R. (2021). RP-HPLC method for Selpercatinib. IJRPS, 12(1), 931-939.
- 85. Kumari, M. V., & Reddy, C. H. B. (2020). RP-HPLC for Zanubrutinib in bulk and formulations. AJPCR, 159-162.
- 86. Suchitra, D., & Battu, S. (2021). Stability indicating RP-HPLC for Rucaparib. Am. J. Anal. Chem., 12(4), 96-107.
- 87. Sudhakar, N., et al. (2023). RP-HPLC method for Talazoparib in bulk and dosage forms. YMER, 22(1), 63-76.
- 88. Satyanaraya, V., et al. (2023). Stability indicating method for Talazoparib Tosylate via HPLC. YMER, 22(1), 421–434.
- 89. Kamani, V., et al. (2021). RP-HPLC for Dacomitinib and related impurities in bulk and solid forms. J. Pharm. Res. Int., 33(40B), 187–199.

Copyright: © **2025 Author**. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.