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Review Article

Exploring the analytical method development for ibrutinib: A review

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Abstract

Ibrutinib is a first-in-class, orally active Bruton's tyrosine kinase (BTK) inhibitor that has transformed the treatment of various B-cell malignancies, including chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), Waldenström's macroglobulinemia (WM), and marginal zone lymphoma (MZL). It works by irreversibly binding to BTK, thereby blocking B-cell receptor (BCR) signaling, which is essential for the proliferation and survival of malignant B-cells. Clinical studies have shown that ibrutinib significantly improves progression-free survival (PFS) and overall response rates (ORR), even in patients with high-risk genetic mutations such as del(17p). Ibrutinib is generally well tolerated, with manageable adverse effects including diarrhea, fatigue, bleeding, atrial fibrillation, and hypertension. However, resistance can develop over time due to mutations in BTK (e.g., C481S) or in downstream signaling proteins, posing challenges to long-term efficacy. To address this, ongoing research is exploring combination therapies and second-generation BTK inhibitors. Ibrutinib marks a significant advancement in targeted cancer therapy by offering an effective and less toxic alternative to conventional chemotherapy, thus improving quality of life and survival in patients with B-cell malignancies. In present Paper diverse approaches for the analysis of Ibrutinib in bulk drug along with its formulations are considered the abnormal proteins that instruct cancer cells to proliferate are stopped. This prevents cancer cells from proliferating. Using a range of analytical methods, such as UV spectroscopy, high performance thin liquid chromatography (HPTLC), high pressure liquid chromatography (HPLC), reversed phase high performance liquid chromatography (RP-HPLC), ultra performance liquid chromatography (UPLC), liquid chromatography-tandem mass spectroscopy (LC-MS/MS), and capillary electrophoresis (CE), it has been possible to identify ibrutinib.

Keywords: Ibrutinib, HPLC, LC-MS/MS, HPTLC, UPLC, CE

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1. Introduction

A little drug called ibrutinib functions as a strong, irreversible inhibitor of Burton's tyrosine kinase. Clinical investigations indicate that it is a promising treatment for B-cell malignancies and is classified as a targeted covalent medication.[1] Developed by Pharmacyclics Inc., ibrutinib was initially approved by the FDA in November 2013 under accelerated approval for the treatment of mantle cell lymphoma (MCL). However, the medication manufacturer revoked the accelerated approvals for ibrutinib in the United States in April 2023.[2]

Health Canada and the EMA both approved it ibrutinib in November and October of 2014, respectively. It was authorized in August 2017 to treat a number of illnesses, including Waldenström's Macroglobulinemia, chronic lymphocytic leukemia (CLL), and chronic graft versus host disease (cGVHD). The FDA authorized ibrutinib as the first treatment for cGVHD in children in August 2017.[3]

Chemically, ibrutinib (IBR) is 1-[(3R)-3-[4-amino-3-(4-phenoxyphenyl) pyrazolo[3,4-d] 1-ylpyrimidin Piperidin-1-yl prop-en-1-one. By establishing a covalent link with a cysteine residue in the active site of a BTK (Cys481), the medication ibrutinib inhibits Bruton's tyrosine kinase (BTK), a protein situated at the center of a B-cell receptor signaling pathway. It stops substrates like Phospholipase C Gamma from becoming phosphorylated. Therefore, an anticancer response results from the reduction of cell growth, transcription regulation, and inflammation.[4]

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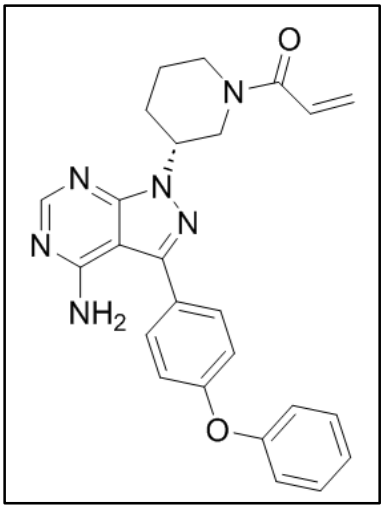

Fig.1 Ibrutinib Structure

1.1. Mechanism of action

BTK (Bruton's tyrosine kinase) is inhibited by ibrutinib. It inhibits BTK by creating a covalent connection with a cysteine residue in the active site (Cys481). Since BTK inhibition affects B-cell receptor signaling, ibrutinib stops downstream substrates like PLC-γ from being phosphorylated.[5]

1.2. Ibrutinib Pharmacodynamics

Studies conducted in vitro have demonstrated that pro-survival factors can still induce CLL cell death. Additionally, it has been documented to hinder cell migration, limit CLL cell survival and proliferation, and decrease the release of chemokines including CCL3 and CCL4. Regression in xenograft mice models has been demonstrated to result from the latter impact. [5]

In phase I and II clinical trials, the total response rate for relapsed/refractory CLL was about 71%. About 70% of individuals with relapsed or refractory mantle cell lymphoma who were evaluated showed some degree of response.[6] A partial response was recorded in 15-20% of patients in clinical trials for relapsed/refractory diffuse large B-cell lymphoma, but nearly 75% of patients evaluated for relapsed/refractory Waldenstrom's macroglobulinemia had a partial response. Lastly, a partial to full response was achieved in roughly 54% of patients with relapsed/refractory follicular lymphoma.[7]

2. Ibrutinib Pharmacokinetics

2.1. Absorption

Oral Bioavailability: Ibrutinib has a moderate bioavailability when taken orally, while precise statistics about its absolute bioavailability are not easily found in the literature. Peak plasma concentrations within one to two hours of treatment are the result of relatively quick oral absorption.[8]

Effect of Food on Absorption: Ibrutinib absorption is somewhat impacted by food consumption. AUC (Area Under the Curve) and Cmax (highest plasma concentration) may rise by about 30% when ibrutinib is taken with food, according to studies. These modifications, however, are not clinically significant enough to call for dosage modifications. You can take it with or without food.[9]

2.2. Distribution

Volume of Distribution (Vd): Ibrutinib has a large volume of distribution, which suggests that it has significantly penetrated tissue. Usually, the Vd falls between 43 and 57 L. This implies that the medication spreads broadly throughout the body, including into bone marrow and lymphoid tissues, which are areas where B-cells are active).[10]

Binding to Plasma Proteins: Ibrutinib binds to plasma proteins (~97%), primarily albumin. Only the unbound (free) fraction of the medication is pharmacologically active, and the bound fraction may serve as a reservoir for prolonged release, therefore this high binding affinity is crucial.[11]

Blood-Brain Barrier Crossing: It is unknown how much ibrutinib passes through the blood-brain barrier (BBB), but its high plasma protein binding probably limits its supply to the central nervous system.[10]

2.3. Metabolism

Hepatic Metabolism (CYP3A4): The cytochrome P450 enzyme CYP3A4 is mostly responsible for the liver's metabolism of ibrutinib and is essential to its removal. Understanding possible drug-drug interactions requires an understanding of this metabolism.[12]

2.3.1. Metabolite profile

The main metabolite of ibrutinib is PCI-45227, which has pharmacological activity but is much weaker than the original medication. This metabolite likewise inhibits BTK, although less potently, according to studies, which adds to its therapeutic impact. Other trace metabolites have been found, although their contribution to the medication's therapeutic effectiveness is negligible.[13]

2.4. Enzyme involvement

The main enzyme in charge of ibrutinib metabolism is CYP3A4, hence patient medication levels can be greatly impacted by this enzyme's activity. Patients should have their ibrutinib dosage modified if they are using powerful CYP3A4 inducers (like rifampin) or inhibitors (like ketoconazole). Higher ibrutinib concentrations could result from CYP3A4 inhibition, raising the possibility of side effects, whereas induction could lessen the effectiveness of treatment.[14]

2.5. Excretion

Half-Life (T1/2): Ibrutinib's elimination half-life for the parent medication is between four and six hours. The prolonged half-life of PCI-45227, the drug's active metabolite, probably prolongs the pharmacological activity of the medication.[15]

Excretion Pathways: About 80% of the administered dose of ibrutinib and its metabolites are eliminated through feces, with the remaining 10% being eliminated through urine. Research indicates that the liver's considerable first-pass metabolism of ibrutinib accounts for both its high volume of distribution and low oral bioavailability.[16]

2.6. Chromatographic overview

The amount of Ibrutinib in pharmaceutical preparations and human plasma can be determined using a variety of techniques. There have been descriptions of analysis techniques reported by UV, HPLC, HPTLC, TLC, UPLC, LC/MS, and capillary electrophoresis. The most used technique among them for analyzing ibrutinib is HPLC. A few key analytical techniques that have been applied recently for the analysis of Ibrutinib have been compiled in this review.

3. HPLC Method

Developed a simple and reliable HPLC method for determining plasma ibrutinib concentration. Separation was performed on a Canpcell Pack C18 MG II column (250 × 4.6 mm) using a mobile phase of acetonitrile and 0.5% KH_2PO_4 buffer (pH 3.0) in a 52:48 (v/v) ratio at a flow rate of 1.0 mL/min. Detection was carried out using UV absorbance. The method showed a lower limit of quantification (LOQ) of 10 µg/mL, with a calibration curve of $y = 0.01x + 0.113$ (r^2 = 0.9999). Recovery ranged from 84% within a concentration range of 10–500 ng/mL. Intra- and interday variation coefficients ranged from 2.6–7.7% and 4.0–6.6%, respectively, confirming the method's accuracy and reproducibility for plasma ibrutinib analysis.[17]

Developed a sensitive, stability-indicating RP-HPLC method for quantifying ibrutinib. Analysis was performed using a Shimadzu HPLC system with a Kromosil C18 column (250 × 4.6 mm, 5 µm) and a mobile phase of phosphate buffer and acetonitrile (45:55, v/v) at 1.0 mL/min. Detection was carried out at 295 nm. The method showed excellent linearity over a range of 3.5–2100 µg/mL with a regression equation $y = 15528x + 251.16$ and correlation coefficient $r^2 = 0.9999$. Sensitivity was confirmed with an LOD of 0.6927 µg/mL and an LOQ of 2.1578 µg/mL, demonstrating high precision and accuracy.[17]

Developed and validated a UHPLC-MS/MS method for simultaneous quantification of ibrutinib and its active metabolite PCI-45227 in human cerebrospinal fluid (CSF). Separation was achieved using a Waters BEH C18 column (50 × 2.1 mm, 1.7 µm) with gradient elution. The mobile phase consisted of acetonitrile with 0.1% formic acid and 5 mM ammonium formate buffer (pH 3.2), at a flow rate of 400 µL/min. The method demonstrated high sensitivity, with LODs of 0.01 and 0.03 ng/mL, and a lower limit of quantification (LLOQ) of 0.17 ng/mL[18]

Developed and validated a stability-indicating RP-HPLC method for ibrutinib, focusing on the identification and separation of degradation products, known impurities, and genotoxic contaminants. The method employed an X-Select CSH C18 column (150 × 4.6 mm, 3.5 µm) with a mobile phase of phosphate buffer and methanol at a flow rate of 1.0 mL/min and a total run time of 85 minutes. Detection was performed using PDA and QDa mass detectors, ensuring robust impurity profiling and method suitability for stability studies.[19]

Optimized a liquid chromatographic method for anti-leukemic drugs using a Box-Behnken design. Separation was achieved on an Onyx monolithic C18 column (100 × 4.6 mm) with a mobile phase of 0.01M potassium dihydrogen orthophosphate, methanol, and acetonitrile (30:34.29:35.71, v/v) at pH 3.5 and a flow rate of 0.723 mL/min. Detection was performed at 260 nm using a UV detector. The method showed good linearity ($R^2 = 0.997$), with LOD and LOQ of 26.169 ng/mL and 79.3 ng/mL, respectively, and a retention time of 3.81 minutes, making it suitable for routine analysis.[20]

Developed and validated an HPLC method for enantioseparation of ibrutinib under normal-phase conditions. Separation was achieved using a Chiral-Pack-IC column (cellulose tris(3,5-dichlorophenylcarbamate) on silica gel) with a mobile phase of 0.1% diethylamine, 0.3% trifluoroacetic acid, n-hexane, and ethanol (55:45, v/v). Optimal conditions included a flow rate of 0.9 mL/min, column temperature of 30°C, detection at 260 nm, and a 10 µL injection volume. The method enabled precise and reliable resolution of ibrutinib enantiomers.[21]

Arthanareeswari Maruthapillai et al. (2021) developed and validated a stability-indicating RP-HPLC method using a QbD approach for separating process-related and unknown impurities of ibrutinib. Separation was performed on an X-Bridge C18 column (150 × 4.6 mm, 3.5 µm) with gradient elution using a mobile phase of 10 mM potassium dihydrogen phosphate, 0.07% trifluoroacetic acid (pH ~5.5, adjusted with KOH), and acetonitrile (30:70). Detection was carried out at 220 nm with a flow rate of 1.0 mL/min. Impurity characterization was further supported by NMR spectroscopy and ESI-MS, ensuring robust method validation.[22]

Developed and validated a QbD-based LC method for determining ibrutinib in finished dosage forms. Separation was performed on a Luna C18 column (150 × 4.6 mm, 3.0 µm) using gradient elution. The mobile phase consisted of 10 mM phosphate buffer with 1 mL triethylamine (pH 5.6, adjusted with orthophosphoric acid) and acetonitrile (95:5,

v/v) as phase A, and 85% acetonitrile as phase B. Chromatographic conditions included a flow rate of 1.0 mL/min, 10 µL injection volume, 40°C column temperature, and detection at 258 nm. The method demonstrated excellent linearity ($R^2 = 0.9999$) over the 20–200% range and high recovery (98–102%), confirming its accuracy and reliability for pharmaceutical analysis.[23]

Developed and validated a stability-indicating UHPLC method for estimating ibrutinib and trace-level impurities using a QbD approach. The method utilized an AQUITY UPLC BEH C18 column with a mobile phase of 0.02M formic acid in acetonitrile and water (1:1, v/v), at pH 2.5. Optimal conditions included a flow rate of 0.55 mL/min and a column temperature of 28°C. The method demonstrated excellent linearity ($R^2 > 0.9995$) with LODs of 0.01/0.025 µg/mL and LOQs of 0.015/0.0187 µg/mL, confirming its reliability for ibrutinib stability and impurity profiling.[24]

Developed and validated an RP-HPLC method for quantifying ibrutinib in human and porcine skin. Separation was achieved using a Discovery® C18 column (150 × 4.6 mm, 5 µm) with a mobile phase of acetonitrile and 0.01 M phosphoric acid (pH 3.5) in ratios of 40:60 or 35:65 (v/v), at a flow rate of 1.0 mL/min and oven temperatures of 30°C or 35°C. Detection was performed at 259 nm using Shimadzu LC software. The method showed excellent linearity (0.2–15.0 µg/mL), with LOD and LOQ of 0.01 µg/mL and 0.02 µg/mL, respectively, and high recovery (>89.5% for pig skin and >92.0% for human skin), confirming its precision, accuracy, and robustness.[25]

3.1. Overview of LC-MS method

Simultaneous quantification of lenalidomide, ibrutinib and its active metabolite PCI-45227 in rat plasma by LC-MS/MS: application to a pharmacokinetic study. A reverse-phase C18 column (YMC Pack ODS AM, 150 mm × 4.6 mm, 5 µm) kept at 40°C was used in the analytical procedure. After injecting a 5 µL sample into the column, the analytes were separated using a gradient elution technique with a mobile phase made up of 0.1% formic acid buffer (B) and acetonitrile (A). After post-column elution, the flow split was 70%, and the flow rate was set at 1.0 mL/min. This approach's robustness and dependability were demonstrated when it was successfully used in pharmacokinetic research in rats.[26]

Development and Validation of a Simultaneous Quantification Method of 14 Tyrosine Kinase Inhibitors in Human Plasma Using LC-MS/MS. An Acquity UPLC BEH C18 column (1.7 µm, 2.1 × 50 mm, pore size 1.9 µm) installed in a thermostated column oven kept at 40°C was utilized in the analytical procedure. Phase A of the mobile phase was a 10 mmol/L ammonium formate buffer containing 0.1% formic acid, while Phase B was acetonitrile containing 0.1% formic acid. In order to maintain accuracy and reduce carryover, the autosampler was also flushed in between injections using a third solution (Phase C) that contained isopropanol. This approach has been shown to work well for tyrosine kinase inhibitor pharmacokinetic research and therapeutic applications.[27]

Chromatographic separation was carried out on an X-Bridge phenyl column (150 × 4.6 mm, 3.5 µm) and an isocratic mobile phase made up of 50:50 acetonitrile and 0.1% ortho-phosphoric acid (OPA). A constant flow rate of 1.0 mL/min at room temperature was maintained. With a linear calibration curve spanning a concentration range of 5–100 ng/mL and a correlation coefficient (r2) of 0.99924, the approach proved to be accurate and dependable for pharmacokinetic investigations.[28]

Bioanalysis of ibrutinib, and its dihydrodiol- and glutathione cycle metabolites by liquid chromatography-tandem mass spectrometry. A ReproSil-pur 120 C18 column (250 × 10 mm, 10 µm particle size) supported the chromatographic separation. Mobile Phase A contained 0.1% (v/v) formic acid in water, and Mobile Phase B contained 0.1% (v/v) formic acid in acetonitrile. These two components made up the mobile phase. A flow rate of 6 mL/min was used to transport the mobile phases, guaranteeing effective elution of ibrutinib and its metabolites.[29]

Quantification of eight hematological tyrosine kinase inhibitors in both plasma and whole blood by a validated LC-MS/MS method. Acquity UPLC® BEH C18 analytical column (100 mm × 2.1 mm, 1.7 µm particle size) and corresponding BEH C18 guard column were used for the chromatographic analysis. To preserve sample stability, the autosampler was held at 4°C and the column temperature was fixed at 60°C.Phase A of the mobile phase included 10 mM ammonium formate and 0.1% formic acid in water, while Phase B contained 0.1% formic acid in acetonitrile. A flow rate of 0.5 mL/min was used to provide the mobile phase.[30]

LC–MS/MS method for the quantification of potential genotoxic impurity 4‑phenoxyphenyl‑boronic acid in ibrutinib. The Atlantis T3 column (150 × 4.6 mm, 5.0 µm particle size) was used for the analysis. With a flow rate of 1.0 mL/min, a gradient elution mode was used. The mobile phases were acetonitrile (Mobile Phase B) and 10 mM ammonium formate buffer (Mobile Phase A). Using the regression equation y=1242.6x+21.052 and a correlation coefficient (r) of 0.999, the approach showed excellent linearity. 0.45 to 5.0 µg/mL was determined to be the linearity range. The results showed that the quantitation limit (LOQ) was 0.45 µg/mL and the detection limit (LOD) was 0.134 µg/mL [30]

Table 1: HPLC Method for analysis of Ibrutinib.

S. No.	Matrix/Dosage form	Stationary phase	Mobile phase	Detection (nm)	Flow Rate (ml/min)	Retention time(ml/min)	Detector	Ref no.
1	Human plasma	Column: Canpcell Pack C18 MG II (250 nm × 4.6 mm)	Acetonitrile and 0.5% monopotassium phosphate (KH_2PO_4) buffer (pH 3.0) in a 52:48 ratio (v/v)	260 nm.	Flow rate: 1.0 mL/min	-	UV-Detector	17
2	Tablet	Kromosil (250 mm × 4.6 mm, 5 µm particle size).	Phosphate buffer and acetonitrile in a 45:55 (v/v) ratio	295 nm	1.0 mL/min	-	Ultraviolet (UV) detector	17
3	Human cerebrospinal fluid.	Column**:**BEH C18 (50.0 × 2.1 mm; 1.7 µm particle size).	Gradient elution with: Ammonium formate buffer (5 mM, pH 3.2). -Acetonitrile with 0.1% formic acid	210 nm or 254 nm	400 µL/min	-	Ultraviolet (UV) detector	18
4		X-Select CSH C18 column (150 mm × 4.6 mm × 3.5 µm).	Phosphate buffer with acetonitrile.		1 mL/min.	85 minutes.	PDA (Photodiode Array) and QDa (Quadrupole Detector Array)	19
5	Bulk dosage form	Onyx monolithic-C18, 100 × 4.6 mm.	Potassium dihydrogen orthophosphate (0.01M): 30% Methanol: 34.29% Acetonitrile: 35.71%	260 nm.	0.723 mL/min.	3.81 minutes	UV detector	20
6		Chiral-Pack-IC (Cellulose tris (3,5-dichlorophenylcarbamate) immobilized on silica gel).	-n-Hexane and ethanol in a ratio of 55:45% v/v. -Added modifiers: Diethyl amine and 0.3% Trifluoroacetic acid.	260 nm.	0.9 mL/min.	-	photodiode array (PDA) detector	21
7	degradation	X-Bridge C18 (150 mm × 4.6 mm, 3.5 µm particle size).	A mixture of 10 mM potassium dihydrogen phosphate with 0.07% trifluoroacetic acid (pH ~ 5.5 adjusted with KOH solution) and acetonitrile in a 30:70 (v/v) ratio	220nm	1.0 mL/min.	-	photodiode array (PDA) detector	22
8	Solid dosage form	Luna C18 column (150 × 4.6 mm,	Phase A- 10 mM phosphate buffer with 1 mL triethylamine (pH	258 nm.	1.0 mL/min.	-	Photodiode array detector	23

		3.0 µm particle size).	adjusted to 5.6 using diluted orthophosphoric acid) and acetonitrile in a 95:5 v/v ratio. Phase B: 85% acetonitrile.					
9	Tablet	AQUITY UPLC BEH C18	Equal parts of 0.02M formic acid in water and 0.02M formic acid in acetonitrile.		0.55 mL/min.	-	Photodiode Array (PDA**)** detector	24
10	Topical formulation	Discovery® Supelco C18 column, dimensions: 150 × 4.5 mm, particle size: 5 µm	Acetonitrile and 0.01 mol/L Phosphoric acid (pH 3.5) in varying ratios: • 40:60 v/v or 35:65 v/v.	259 nm	1.0 mL/min.	-	UV-Detector	25

Table 2: LC-MS Method for analysis of Ibrutinib.

S. No.	Dosage form	Stationary phase	Mobile phase	Detection (nm)	Flow rate	Retention time	Ref no
1	Plasma	reverse phase C18 Column YMC Pack ODS AM (150mm×4.6mm,5µm)	A: Acetonitrile B: 0.1% Formic acid buffer	Electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) in positive mode	1.0 mL/min	-	26
2	Human Plasma (Claire)	Acquity UPLC BEH C18. Particle Size: 1.7 µm	Phase A: 10 mmol/L ammonium formate buffer with 0.1% (vol/vol) formic acid. Phase B: Acetonitrile with 0.1% (vol/vol) formic acid. Phase C: Isopropanol (used for flushing the autosampler between injections).	Tandem mass spectrometer (MS/MS).	0.2 mL/min to 0.5 mL/min,		27
3		X-Bridge phenyl column (150 mm × 4.6 mm, 3.5 µm particle size).	An isocratic mixture of 0.1% ortho-phosphoric acid (OPA) in water and acetonitrile in a 50:50 ratio.	Electrospray ionization (ESI) source operating in positive ion mode.	1.0 mL/min		28
4	Metabolite in human plasma	Column used**:** ReproSil-Pur 120 C18 Column (250 × 10 mm, 10 µm particle size).	A: 0.1% (v/v) formic acid in water. B:0.1% (v/v) formic acid in acetonitrile.	tandem mass spectrometry (MS/MS 5.5 minutes	6 mL/min	5.5 minutes	29
5	Plasma (EDTA and heparin) and whole	Acquity UPLC® BEH C18 Column (100 mm length × 2.1 mm i.d., 1.7 µm particle size)	Phase A: 10 mM ammonium formate with 0.1% formic acid in water	Tandem mass spectrometry (LC-MS/MS) for detection.	0.5 mL/min	4 minutes	30

	blood samples.		Phase B: 0.1% formic acid in acetonitrile				
6	Drug substance	Atlantis T3, dimensions 150 × 4.6 mm, particle size 5.0 µm	- Phase A: Ammonium formate buffer (10 mM). - Phase B: Acetonitrile.	Mass spectrometry in negative ion mode using selected ion monitoring (SIM) at m/z 231.02 for 4-phenoxyphenyl-boronic acid.	flow rate of 1.0 mL/min		30

Table 3: HPTLC Method for analysis of Ibrutinib.

S.No.	Matrix/ Dosage form	Stationary phase	Mobile phase	Detection	Rf	Linearity range	Ref
1	Degradants	High-Performance Thin-Layer Chromatography (HPTLC) aluminum plates (10 × 10 cm) precoated with silica gel 60 F254 (E. Merck, Darmstadt, Germany).	A mixture of methanol, ethyl acetate, and ammonia in the ratio of 2:8:0.2 (v/v/v)	286 nm using a deuterium lamp	0.60	range of 100–500 ng per	31

Table 4: UPLC Method for analysis of Ibrutinib.

S.No.	Matrix/ Dosage form	Stationary phase	Mobile phase	Detection	Flow rate	Retention time	Linearity range	Ref
1	Plasma	Acquity BEH C18 column (50 × 2.1 mm, 1.7 µm) at 40°C	f 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in 10 mM ammonium acetate (B)	tandem mass spectrometer (MS/MS) equipped with an electrospray ionization (ESI) source	0.250 mL/min.	0.62 min and 0.47 min	0.35 to 400 ng/mL	32
2	Nanocrystalline	Agilent Zorbax SB-C18 Rapid Resolution HD column (2.1 × 50 mm, 1.8 µm)	10 mM ammonium acetate and 0.1% formic acid (A) and pure acetonitrile (B)	positive electrospray ionization	flow rate of 0.4 ml/min	3.0 minutes.	0.5 to 500 ng/mL	33
3	Degradant Product	Acquity BEH C-18 (100 mm × 2.1 mm, 1.7 µm)	Eluent A: Phosphate buffer containing 0.1% triethylamine, pH adjusted to 6.0 Eluent B: Acetonitrile	Photodiode array (PDA) detector at 215 nm	0.3 mL/min	15 minutes	25 to 250 ng/mL	34

Table 5: Capillary electrophoresis Method for analysis of Ibrutinib.

S.No.	Matrix/ Dosage form	Detection	Capillaries (Fused Silica capillary)	Separation voltage	Temperature	Ref
1	-	diode array detector (DAD) at 200 nm	Uncoated fused-silica capillaries (50 µm I.D. ×58.5 cm total length (50 cm effective length)) from Polymicro Technologies (Phoenix, AZ, USA)	30 kV.	25°C	36

3.2. Overview of HPTLC method

Thin layer chromatographic method for separation and estimation of anticancer drug Ibrutinib in presence of its degradants. HPTLC aluminum plates (10 × 10 cm) precoated with silica gel 60 F254 (E. Merck, Darmstadt, Germany) were used for the chromatographic analysis. Methanol, ethyl acetate, and ammonia (2:8:0.2, v/v/v) made up the mobile phase utilized for plate development. The generated chromatograms were scanned densitometrically at 286 nm with a deuterium lamp serving as the radiation source. At a scanning rate of 20 mm/s, scans were conducted using slit dimensions of 5 mm in length and 0.45 mm in breadth. With a retention factor (Rf) of 0.60, the technique demonstrated good linearity over the 100–500 ng/band range. The limit of quantitation (LOQ) was 64 ng/band, and the limit of detection (LOD) was 21 ng/band.[31]

3.3. Overview of UPLC method

Simple and Sensitive UPLC-MS/MS Method for High-Throughput Analysis of Ibrutinib in Rat Plasma: Optimization by Box-Behnken Experimental Design. A 50 × 2.1 mm, 1.7 µm Acquity BEH C18 column kept at 40°C was used for the chromatographic separation. At a flow rate of 0.250 mL/min, the mobile phase was composed of 0.1% formic acid in acetonitrile (Phase A) and 0.1% formic acid in 10 mM ammonium acetate (Phase B) in an 80:20 ratio. Ibrutinib and the internal standard (IS) were effectively separated under these circumstances, with retention durations of 0.62 and 0.47 minutes, respectively. The calibration curves showed good linearity using weighted ($1/x^2$) least squares linear regression over the concentration range of 0.35–400 ng/mL.[32]

A rapid and sensitive method for quantification of ibrutinib in rat plasma by UPLC–ESI–MS/MS: validation and application to pharmacokinetic studies of a novel ibrutinib nanocrystalline. The Agilent ZORBAX SB-C18 Rapid Resolution HD column (2.1 × 50 mm, 1.8 µm) was used in the isocratic analysis. The mobile phase was composed of pure acetonitrile in a 10:90 (v/v) ratio, deionized water with 10 mM ammonium acetate and 0.1% formic acid, and a flow rate of 0.4 mL/min. Because of its exceptional sensitivity, accuracy, and precision, the approach can be used for pharmacokinetic studies of a new nanocrystalline formulation of ibrutinib. This methodology guarantees dependable and effective quantification, enabling more investigation into the pharmacokinetics of ibrutinib.[33]

Development and Validation of Novel and Highly Sensitive Stability-Indicating Reverse Phase Ultra Performance Liquid Chromatography Method for Quantification of Ibrutinib and its ten Degradation Products. Gradient method of elution on an AQUITY CSH C-18 column (100 mm × 2.1 mm, 1.7 µm) was used to produce chromatographic separation. At a flow rate of 0.3 mL/min, the mobile phase was composed of Eluent A (phosphate buffer with triethylamine, pH 6) and Eluent B (acetonitrile). The column temperature was kept at 45°C, and the run period was 15 minutes. The newly made mobile phase was filtered through a 0.22 µm filter after being sonicated. At 215 nm, a PDA detector was used for detection, and the injection volume was 5 µL. Excellent linearity was demonstrated by the approach using the equation y=100.1880x−130.8966 over a concentration range of 25–250 ng/mL (R2 = 0.9999). Analyzing the stability and degradation of ibrutinib in pharmaceutical formulations is reliable thanks to this approved approach.[34]

3.4. Overview of UV method

Developed a simple, sensitive, accurate, rapid, and economical spectrophotometric and colorimetric method for the determination of Ibrutinib in pure drug and tablet form. Absorbance was measured at 248nm and 552nm using ethanol as solvent system. In the concentration range of 2-14µg/ml and 1-5µg/ml, it obeys Beer's law with a correlation coefficient (R2) of 0.998 and 0.996, respectively. The limit of detection (LOD) was found to be 1.226 µg/ml and 1.000 µg/ml. The limit of quantification (LOQ) was determined to be 5.226µg/ml and 2.760µg/ml. The proposed analytical methods were validated according to the guidelines of ICH and gave good results in terms of range, linearity, precision, accuracy, robustness and in sensitivity.[35]

3.5. Overview of capillary electrophoresis

Enantioseparation and ecotoxicity evaluation of ibrutinib by Electrokinetic Chromatography using single and dual systems. Ibrutinib enantioseparation was made sensitive and effective with electrokinetic chromatography employing single and dual chiral selector devices. Using formate buffer and uncoated fused-silica capillaries (50 µm I.D., 58.5 cm total length, and 50 cm effective length), a 30 kV applied voltage was used. A UV detector at 260 nm for enantiomeric analysis and a diode array detector (DAD) at 200 nm were used for detection. Sulfated-γ-cyclodextrin (S-γ-CD) was used as the chiral selector in the single system, which

separated the molecules in 4.2 minutes with an enantioresolution of 1.5. Combining S-γ-CD with a chiral ionic liquid ([TMA][L-Lys]), the dual system separated the two in 8.1 minutes with an enantioresolution of 3.3. S-ibrutinib, an enantiomeric impurity, could be detected using both techniques at concentrations as low.[36]

4. Conclusion

This review highlights various analytical techniques used to determine Ibrutinib and its combinations in pharmaceutical and biological samples. HPLC was the most commonly employed method, along with UV, HPTLC, UPLC, and LC/MS. The findings may aid researchers in formulation development and quality control of Ibrutinib.

5. Source of Funding

None.

6. Conflict of Interest

None.

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