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INNOVATIVE APPROACHES IN THE QUANTITATIVE ANALYSIS OF IBRUTINIB

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ABSTRACT

Ibrutinib is an irreversible Bruton's tyrosine kinase (BTK) inhibitor widely used in the treatment of various B-cell malignancies, including chronic lymphocytic leukemia, mantle cell lymphoma, and Waldenström's macroglobulinemia. Accurate and reliable analytical methods are essential for its quality control, stability evaluation, and bioanalysis. This review presents a comprehensive overview of analytical techniques reported for the determination of ibrutinib in bulk drug, pharmaceutical formulations, and biological matrices. Various spectrophotometric, RP-HPLC, UPLC, and LC-MS/MS methods have been discussed with respect to their sensitivity, linearity, accuracy, precision, and validation according to ICH guidelines. Spectrophotometric methods offer simplicity and cost effectiveness but exhibit lower sensitivity, whereas RP-HPLC methods demonstrate high accuracy, robustness, and reproducibility, making them suitable for routine pharmaceutical analysis. Advanced UPLC and LC-MS/MS techniques provide enhanced sensitivity at nanogram levels and are particularly valuable for impurity profiling, stability studies, and pharmacokinetic investigations. Stability studies consistently indicate that ibrutinib is susceptible to degradation under acidic, basic, and oxidative conditions while remaining stable under neutral, photolytic, and thermal stress. Overall, this review highlights RP-HPLC as the preferred method for quality control applications, with UPLC and LC-MS/MS serving as indispensable tools for advanced bioanalytical and stability assessments.

KEYWORDS: Ibrutinib; Bruton's tyrosine kinase; RP-HPLC; UPLC; LC-MS/MS; Stability studies.

INTRODUCTION

Ibrutinib belongs to a family of drugs known as kinase inhibitors[1]. Ibrutinib, a United States Food and Drug Administration approved drug, chemically known as 1-[(3R)-3 -[4- Amino-3-(4-phenoxyphenyl)-1H-pyrazolo [3, 4-d] pyrimidin-1-yl] piperidin-1-yl] prop- 2-en-1-one is a white to off-white solid powder soluble in polar solvents like acetonitrile, methanol and water. It is irreversible and elective small molecule which is used in the management of patients with chronic lymphocytic leukemia by binding perpetually to a protein, Bruton's tyrosine kinase (BTK), that inhibits B cell antigen receptor (BCR) signaling in human B cells via specific active-site occupancy.[2] t works by stopping the function of the abnormal protein that sends a proliferation signal to cancer cells.[1] It is useful in the treatment of Waldenstrom's macro globulinemia, lymphocytic leukemia and second-line treatment for marginal zone lymphoma, chronic graft versus host disease and mantle cell lymphoma.[3]

ibrutinib

REVIEW OF THE LITERATURE

1. **Sultana et al. (2022)** 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 2-14 µg/ml, it obeys beer's law with a correlation coefficient (R^2) 0f 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 5.226 µg/ml and 2.760µg/ml. The proposed analytical methods were validated according to the guideline of ICH and gave good result in terms of range, linearity, precision, accuracy, robustness and insensitivity.[4]

2. **Naresh et al. (2020)** developed and validated a novel stability-indicating method for separation and identification of degradation products using RP-HPLC/PDA and QDa mass detectors. Separation of impurities could be achieved using stationary phase column (X- select CSH, C18, 150 mm x 4.6 mm x 3.5µm) with a flow rate of 1ml/minute and total run time was 85 minutes. Ibrutinib is sensitive to acid, base and peroxide conditions as evident from its degradation studies. Ibrutinib drug was found to be stable under heat, UV, humidity and water conditions. The proposed method was validated as per ICH Q2 (R1) guidelines. According to validation and degradation results, the method was found to be a good stability indicating process.[5]
3. **Hima Bindu et al. (2018)** developed a new HPLC method for the quantification of Ibrutinib. Shimadzu Model HPLC system with Kromosil column (250mm x 4.6 mm, 5µm particle size) was used for this method development. Mixture of phosphate buffer and acetonitrile (45: 55, v/v) with 1.0 ml/min flow rate are the optimized chromatographic conditions and the system was monitored at 295 nm. Ibrutinib shows linearity range of 3.5- 2100 µg/ml with regression correlation coefficient (R2 = 0.9999). The LOD and LOQ were found to be 0.6927 µg/ml and 2.1578 µg/ml respectively. The studies were performed and method was validated. Assay of marketed formulations was conducted and the system suitability parameters were within the limits.[6]
4. **Siva Prasad et al. (2019)** developed a new reverse-phase HPLC method for the determination of Ibrutinib capsules and its excipients with stability-indicative quality. A column (X-Bridge C18 150 x 4.6 mm, 3µm) is used for efficient separation of Ibrutinib and its impurities with a stationary phase with a stable bond and two different mobile phases A and B. All compounds are monitored using an array of photodiodes detector at 220nm. The developed technology was proved to be robust in a particular design mode and the flow gradient was optimized. Ibrutinib degrades under various stress test conditions according to International Council of Harmonization and parameters; linearity, stability, specificity, precision, accuracy, limit of detection and limit of quantification were evaluated. Injected volumes and test concentrations were optimized to achieve the limit of quantification less than the reporting threshold. The developed method is successful, reliable and supporting as per the requirements of the regulatory bodies offering HPLC analysis techniques.[7]
5. **Vykuntam et al. (2016)** developed a simple, accurate and precise method for the estinfation of Ibrutinib using RP-HPLC technique. The chromatographic conditions used are the stationary phase Kromos 150 mm x 4.6 mm x 5 µm, the mobile phase 0.1% orthophosphoric acid: acetonitrile in the ratio 40:60 and the flow rate at 1 ml/min, detection wavelength at 296nm and the column temperature at 30°. Conditions were completed using the optimize method. The suitability parameters of the system were investigated by injecting the standard six times and the results were clearly within the acceptance criteria. A linear test was performed a the 25% to 150% level, the R^2 value was found to be 0.999. The repeatability accuracy wa found to be 1.01 and 1.50 for medium accuracy. The LOD and LOQ reported were 0.394 µg/m and 1.194 µg/ml, respectively. Using the above method, 100.55% was achieved for the marketed formulation. Degradation studies of Ibrutinib were performed, and under a onditions the purity threshold was greater than the purity angle and within the acceptab ange.[8]
6. **Sureshbabu et al. (2016)** developed a stability-indicating RP-HPLC method and validated it according to International Guidelines for Chemical Harmonization and is approved for the determination of Ibrutinib in bulk and tablet form. Chromatographic separation was performed using a Waters (Alliance) HPLC 2695 series system equipped with a UV-visible spectrophotometer as a detector. A mobile phase consisting of 0.1% orthophosphoric acid buffer and acetonitrile in a ratio of 70:30 v/v was allowed to flow through a 100 mm x 4.6 mm, 5 µm Inertsil Octadecyl silica column at a flow rate of 0.8 ml/min, keeping the column temperature at 30°. About 20 µl of the standard or sample solution was injected into the column and the components were detected at 320 nm. The accuracy of the developed method as a percentage of the average yield at three different concentrations of 50%, 100% and 150% of the target concentration was determined. Peak area was found to be proportional to Ibrutinib concentration. The developed method was found to be robust and applied in quality control that analyzes pharmaceutical products. The stability of the drug under different degradation conditions was investigated and found to be stable. The proposed method was found to be simple and recommended as an alternative method in quality control laboratories.[9]
7. **Raman et al. et al. (2021)** developed and validated a selective RP-HPLC method for separating and determining of potential-related impurities (process related and degradants) of Ibrutinib drug. Separation was performed on an X-Bridge C18 column, (150 V

4.6 mm x 3.5 µm) connected to a photodiode array detector by using 10 mM potassium dihydrogen phosphate with 0.025% trifluoroacetic acid (pH~5.5 adjusted with KOH) acetonitrile solution in a ratio of 85:15 as mobile phase A and 10 mM potassium dihydrogen phosphate with 0.07% trifluoroacetic acid (pH 5.5 adjusted with KOH solution) and acetonitrile ratio of 30:70 as mobile phase B respectively, under gradient elution. The optimized chromatographic conditions such as flow rate and detection wavelength were 1.0 ml/min and 220 nm, respectively. Preparative HPLC was used to enrich and separate two unknown impurities that were detected in HPLC analyses above 0.1% under Ibrutinib thermal stability conditions. 1H NMR, 13C NMR, mass spectroscopy, and FT-IR spectroscopy were used to confirm the structures of impurities. Specificity, limit of detection, limit of quantification, linearity, accuracy, precision, robustness, and robustness of method performance were validated in accordance with International Conference on Harmonization guidelines.[10]

8. **Santhoshillendula et al. (2019)** developed a simple, rapid, accurate, precise and sensitive reverse phase liquid chromatography method for determining ibrutinib drug in bulk and pharmaceutical dosage forms. The chromatographic method was standardized by using develosil octadecyl silica HG-5 RO C18, 5 µm, 15cm × 4.6mm i.d column with uv detection at 287 nm and a mobile phase of 0.1% orthophosphoric acid:methanol with a ratio of 35:65, flow rate was 1.0 ml/min. the method was successfully applied for the determination of ibrutinib in bulk and dosage forms. The method was linear between 0-14 µg/ml. The yield varied 98% to 102% and the limit of detection was 0.09µg/ml and the limit of quantification was 0.29µg/ml. various analytical performance parameter such as accuracy, precesion, limit of detection, limit of quantification and reliability was determined according to International Conference of Harmonization (ICH) guidelines.[11]
9. **Mehta et al. (2020)** developed and validated a new, simple, robust, stability-indicating, highly sensitive and specific reversed-phase, ultra performance liquid chromatography method and validated for the determination of Ibrutinib using water-loaded surface hybrid C-18 (100 mm x 2.1 mm, 1.7 µm) column where the mobile phase consisting of a mixture of Eluent-A phosphate buffer, 0.1% triethylamine (pH 6.0) and Eluent-B acetonitrile with a flow rate of 0.3 ml/min. Expression was monitored at 215 nm using a photodiode array detector. Linearity was observed between 25 and 250 ng/ml. According to the guidelines of the Council for International Harmonization Q1, Ibrutinib was subjected to stressful conditions. It is considered to be the first highly sensitive and stable high-performance liquid chromatography method capable of separating Ibrutinib and its ten degradation products at the nanogram (ng) level. The method was validated according to the guidelines of the International Council for Harmonization.[12]
10. **Shuangshuang et al. (2020)** described a new nanocrystal of Ibrutinib to improve bioavailability in their study and a rapid and reliable UPLC-MS/MS method was developed for accurate quantification of Ibrutinib in rat plasma. Chromatographic separation was performed on an Agilent Zorbax SB-C18 fast Solution HD column (2.1 x 50 mm, 1.8 µm). The mobile phase comprised of deionized water (containing 10 mM ammonium acetate and 0.1% formic acid) and pure acetonitrile. An isocratic elution (water-acetonitrile 10:90 v/v) was used and the flow rate of 0.4 ml/min was maintained. The column temperature was set at 40º. Vilazodone was used as an internal standard in this assay method. Several reaction monitoring methods using positive electrospray ionization were selected for the detection of Ibrutinib and Vilasodone. For extraction of plasma samples, acetonitrile was used to precipitate proteins. There was no endogenous interference with Ibrutinib and Vilazodone, and the linear range of this method was 1-2000 ng/ml. The yields were 98.4, 97.4, and 102.7% at low, medium, and high concentrations respectively. The matrix effect was 96.6, 111.1 and 99.6%, respectively. Using this validated method, the pharmacokinetic differences between crude Ibrutinib and the new nanocrystalline Ibrutinib were successfully investigated in rats.[13]
11. **Hepsebah et al. (2012)** developed a rapid and highly sensitive bioanalytical LC-MS/MS technique for the quantification of Ibrutinib in human plasma. Chromatography was performed on a reversed-phase symmetrical C18 column (75 mm × 4.6 mm, 3.5 µm), gradient elution with acetonitrile, methanol and 0.1% v/v formic acid as mobile phase. The chromatographic peaks were separated with a flow rate of 0.7 ml/min. The extraction of drug was done with ethyl acetate solvent by liquid-liquid extraction method. The calibration curve for Ibrutinib was linear over the concentration range of 1-600 ng/ml with a regression coefficient (R2) value > 0.99. The %RSD values were less than 8.5% for inter- day precision and accuracy. The method produced the percentage values of lower quality control (LQC), medium quality control (MQC) and higher quality control (HQC) samples were 101.86%, 102.8% and 99.28%, respectively. The drug was stable for a long time. Time under different stability conditions and the method was successfully applied to the routine analysis of Ibrutinib in biological matrices.[14]

RESULT AND DISCUSSION

Various analytical methods have been developed and validated for the determination of ibrutinib in bulk drug, formulations, and biological samples. Spectrophotometric techniques are simple and cost-effective but less sensitive, while RP-HPLC

methods consistently show high accuracy, precision, and linearity, making them reliable for routine pharmaceutical analysis. Advanced UPLC and LC-MS/MS methods provide superior sensitivity, detecting ibrutinib at nanogram levels, and are particularly useful for stability and pharmacokinetic studies. Stability investigations reveal that ibrutinib degrades under acidic, basic, and oxidative conditions but remains stable under neutral, photolytic, and thermal environments. Overall, spectrophotometry is suitable for preliminary screening, RP-HPLC serves as the gold standard for quality control, and UPLC/LC-MS/MS are indispensable for bioanalytical and advanced stability assessments.

CONCLUSION

The review of literature concludes that diverse analytical methods have been successfully developed and validated for the determination of ibrutinib in bulk drug, formulations, and biological matrices. Spectrophotometric techniques offer simplicity and cost-effectiveness, while RP-HPLC methods provide high accuracy, precision, and robustness, making them suitable for routine quality control. Advanced UPLC and LC-MS/MS methods deliver superior sensitivity at nanogram levels, enabling detailed stability and pharmacokinetic studies. Stability investigations consistently show that ibrutinib is vulnerable to acidic, basic, and oxidative conditions but remains stable under neutral, photolytic, and thermal environments. Overall, the findings establish a comprehensive toolkit of validated methods, with RP-HPLC as the gold standard for pharmaceutical analysis and UPLC/LC-MS/MS indispensable for bioanalytical and advanced stability assessments.

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