Review Article

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Overall review on annalytical method development and validation of Nilotinib Hydrochloride

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Abstract

In this review, the article determines the different analytical methods for the quantitative establishment of Nilotinib by using HPLC, HPLC-MS, HPLC-UV, and LC-MS/MS. Pharmaceutical analytical method development of Nilotinib requires valid analytical procedures for quantitative and qualitative analysis in Pharmaceuticals dosage formulations and human serum. This assessment explains that the superiority of the HPLC/LC-MS methods reviewed is based on the quantitative analysis of drugs in formulations, (API), and biological fluids such as serum and plasma.

Keywords: Method development, High performance Liquid Chromatography (HPLC/LCMS Nilotinib

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Introduction

Nilotinib, sold under the brand name Tasigna marketed worldwide by Novartis, is a medication used to treat chronic myelogenous leukemia (CML) which has the Philadelphia chromosome [1]. It may be used both in initial cases of chronic phase CML as well as in accelerated and chronic phase CML that has not responded to Imatinib [2][3]. It is taken by mouth.

Common side effects may include low platelets, low white blood cells, anemia, rashes, vomiting, diarrhea, and joint pains. Other serious side effects may include QT prolongation, sudden death, pancreatitis, and liver problems. It is not safe for use during pregnancy. Nilotinib is a Bcr-Abl tyrosine kinase inhibitor and works by interfering with signaling within the cancer cell. Nilotinib was approved for medical use in the United States in 2007 [3]. It is on the World Health Organization's List of Essential Medicines [4].

Medical uses

Nilotinib is used to treat Philadelphia chromosome (Ph+)-positive chronic myelogenous leukemia.

Adverse effects

Nilotinib has a number of adverse effects including headache, fatigue, gastrointestinal problems such as nausea, vomiting, diarrhea and constipation, muscle and joint pain, rash and other skin conditions, flu-like symptoms, and reduced blood cell count. Less typical side effects are those of the cardiovascular system, such as high blood pressure, various types of arrhythmia, and prolonged QT interval. Nilotinib can also affect the body's electrolyte and glucose balance [5]. Though lung-related adverse effects are rare when compared with Imatinib and Dasatinib, there is a case report of acute respiratory failure from diffuse alveolar hemorrhage in a people taking nilotinib [6].

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Nilotinib carries a black box warning in the United States for possible heart complications [8]. Contraindications include long QT syndrome, hypokalemia, hypomagnesaemia, pregnancy, planned pregnancy, lactation and galactose/lactose intolerance [9].

Pharmacology

Nilotinib inhibits the kinases BCR-ABL, [15] KIT, LCK, EPHA3, EPHA8, DDR1, DDR2, PDGFRB, MAPK11 and ZAK.Structurally related to Imatinib, [17] It is 10–30 fold more potent than Imatinib in inhibiting Bcr-Abl tyrosine kinase activity and proliferation of Bcr-Abl expressing cells.

Review on Nilotinib Hydrochloride

Ivaturi et al. Reported that to develop a rapid, accurate, linear, sensitive and stability indicating RP-HPLC method for the determination of nilotinib in bulk and pharmaceutical dosage forms in the presence of its four related substances. The RP-HPLC method was developed for the chromatographic separation of nilotinib and its impurities by using waters Xterra RP-18 (150*4.6 mm, 3.5 µm) column with a mobile phase combination of 10 mM ammonium formate with pH-3.5 and acetonitrile in gradient mode. An injection volume of 20 µl. Flow rate was 1.0 ml/min and detection was carried a wavelength of 250 nm. The retention time for nilotinib and its four impurities were found to be 4.37, 7.40, 8.96, 10.21 and 10.87 min respectively. The linear regression analysis data for the calibration plots showed the good linear relationship in the concentration range of 0.04-3.0 ppm for the nilotinib impurities. The % recovery of nilotinib impurities was found to be 96.8-99.4% in the linearity range. The detection limit (LOD) values were about 0.014, 0.016, 0.005 and 0.03 ppm respectively and the quantification limit (LOQ) values were 0.042, 0.048, 0.014 and 0.09 ppm respectively. The % degradation at various stress conditions like acid, alkaline, oxidative, thermal and photolytic stress was found to be 8.92, 18.35,5.63, 0.88 and 3.89 respectively.

G. Chaitanya and A. K. M. **Pawar**Reported that simple, accurate, precise and sensitive UV spectrophotometric method was developed for the determination of Nilotinib hydrochloride in bulk and pharmaceutical dosage form. The solvent used is Methanol: Water (1:1) and the wavelength corresponding to the maximum absorbance of the drug was found at 263 nm. Beers law was observed in the concentration range of 7- 12µg/mL with correlation coefficient R^2 = 0.9984. The linear regression equation obtained by least square regression method were y = 0.1094x - 0.3008, where y is the absorbance and x is the concentration of the pure drug solution. The method was validated for several parameters like accuracy, precision as per ICH guidelines. The values of the relative standard deviation and % recovery were found to be satisfactory, indicating that the proposed method is precise and accurate and hence can be used for the routine analysis of Nilotinib hydrochloride in bulk and pharmaceutical formulation

Barla and Buralla reported that the main objective was to identify the robust chromatographic conditions where an adequate separation of the components with quality peaks, within acceptable run time can be achieved. Nilotinib in bulk and formulations were analyzed and quantification. Nilotinib in bulk and Pharmaceutical dosage form were analyzed on Phenomenex enable C18 column (15x4.6mm, 5µm particle size) as stationary phase. Mobile phase was composed of acetonitrile and phosphate buffer (pH 5) in the ratio of 60:40 %v/v at a flow rate of 1ml/min. The elution was analyzed using PDA detector at a detection wavelength of 260nm. The proposed method was validated by International Council for Harmonization (ICH) guidelines. In this study, the chromatographic peaks of Nilotinib showed good resolution with retention time of 5.401min. Nilotinib showed an excellent linearity with 0.999 of correlation coefficient. The LOD was about 10.43 ng/ml and LOQ were about 31.63 ng/ml. Other validation parameters including precision, specificity, accuracy and robustness demonstrated good reliability in the quantification of Nilotinib.

Ramana Reddy Gopireddy reported that selective RP-HPLC method for separation and determination of potential related impurities (process related, and degradants) of Nilotinib (NTB) drug substance has been developed and validated. The separation was accomplished on YMC Triart C-18 (150 × 4.6 mm, 3.0 µm) column connected to a photodiode array (PDA) detector using 10 mM $NH_4H_2PO_4$ (pH: 3.5 ± 0.05 adjusted with diluted Ortho phosphoric acid) as a buffer and acetonitrile in a ratio of 85:15 (%v/v) respectively as mobile phase-A, and 10 mM $NH_4H_2PO_4$ buffer, acetonitrile and methanol in a ratio of 20:27.5:52.5 (%v/v/v) respectively as mobile phase B, under gradient elution. The flow rate and column oven temperature were 1.0 mL/min and 50 °C respectively. Detection was carried out at 230 nm. NTB was stressed to degrade under various stress conditions of photolysis, thermal, hydrolysis and oxidation stress as per ICH Q1A (R2). The drug was highly susceptible to acidic and alkaline hydrolytic stress conditions. The drug degraded to two

degradation products (DPs), while the remains stable under thermal, photolytic, neutral hydrolytic and oxidative stress conditions. Characterization of DPs was performed by 1D and 2D NMR, FT-IR and LC/MS/MS. Optimized method was validated in agreement with ICH guidelines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, robustness and ruggedness. This optimized method can be used for quality control of both drug substance and drug product of NTB.

Sudhakar Babu Kondral reported thatA novel stability-indicating ultra-performance liquid chromatographic (UPLC) method has been developed for quantitative determination of nilotinib hydrochloride in active pharmaceutical ingredients along with four impurities (imp-1, imp-2, imp-3 and imp-4). The method is applicable to the quantification of related compounds and assay of nilotinib hydrochloride drug. Efficient chromatographic separation was achieved on a Shim-pack XR-ODS II, 75 × 3.0 mm, 1.8-μm column with a gradient mobile phase combination. Quantification was carried at 260 nm at a flow rate of 0.6 mL min−1. Stress degradation conditions were established for nilotinib hydrochloride by subjecting it to acid, base, oxidation, humidity, thermal and photolysis. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 97.0%. The developed UPLC method was validated according to the present International Conference on Harmonization guidelines for specificity, detection limit, quantitation limit, linearity, accuracy, precision, intermediate precision and robustness. The resolution between nilotinib hydrochloride and four potential impurities is found to be >2.0. Regression analysis shows as r value (correlation coefficient) of >0.999 for nilotinib hydrochloride and four potential impurities.

Azra Takhvar reported that in this research, a reversed phase high performance liquid chromatographic method was developed for the simultaneous determination of two tyrosine kinase inhibitors, nilotinib and sorafenib. Separation was performed on an Agilent C18 column (4.6×250 mm, 5μm) with mobile phase composition of potassium dihydrogen phosphate buffer (25 mM, pH 4.2) and acetonitrile (35:65 v/v) at 1.2 mL/min with UV detection at 265 nm. Specificity, linearity, precision, accuracy, and robustness of the proposed method were all assessed. Nilotinib and sorafenib had estimated retention times of 5.1 and 5.9 minutes, respectively. Linear concentration ranges for nilotinib and sorafenib, were determined as 0.05-1 μg/mL and 10-45 μg/mL with comparable coefficient correlations (0.999). For nilotinib and sorafenib, the limits of detection (LOD) were determined as 0.030 and 0.020 μg/mL, while the limits of quantification (LOQ) were 0.101 and 0.069 μg/mL respectively.

Harika Reported that A RP - HPLC method in Isocratic mode was developed for the estimation of Nilotinib in bulk and pharmaceutical dosage forms. The method was employed on C - 18 column using Water and Acetonitrile in the ratio 50:50 v/v as mobile phase at a flow rate of 1mL/min. The UV detection wavelength selected was 254nm.The retention time for Nilotinib was found to be 3.874 min. The linearity for the method was observed in a concentration range of 5 - 250 μg/mL with the correlation coefficient of 0. 999.The developed method was validated as per ICH guidelines. The method was found to be simple, accurate and precise.

M.A. Fouad and E.F. Elkady reported that simple, selective, and precise stability-indicating reversed-phase liquid chromatographic method was developed and validated for the determination of nilotinib. Nilotinib was subjected to acid and alkali hydrolysis, oxidation, thermal, and photo-degradation. The degradation products were well separated from the pure drug. The method was based on isocratic elution of nilotinib and its degradation products on reversed phase C18 column (100 mm × 4.6 mm, 3.5 μm) — Zorbax Eclipse Plus using a mobile phase consisting of 10 mM KH2PO4: acetonitrile (54.5:45.5%, v/v) at a flow rate of 1 mL min−1. Quantitation was achieved with UV detection at 265 nm. Linearity, accuracy and precision were found to be acceptable over the concentration range of 0.1–80 μg mL−1. The drug was found to be susceptible to acid and base hydrolysis but resistant to oxidation, dry heat degradation, and photo degradation. The proposed method was successfully applied to the determination of nilotinib in bulk and in its pharmaceutical preparation.

M Rajavardhan Reddy et al. reported that The present study describes a newly developed, optimized and validated isocratic RP-HPLC method for the separation of two tyrosine kinase inhibitors (Dasatinib-DST and Nilotinib-NLT) with Methyl paraben-MPB as internal standard (IS), in bulk and pharmaceutical formulations with the aid of Chemo metrics, multi criteria decision making (MCDM) approach. The separation was achieved by using Phenomenex Enable C18 column (15×4.6 mm id, 5μm particle size) and PDA-UV-detection at 277nm.The range of independent variables used for the optimization were MeOH: 60-70%, pH: 2-2.5 and flow rate:0.3-0.8ml/min. The influence of these

independent variables on the output responses: capacity factor of the first peak (k1), resolution (Rs) and separation (α) of the second peak and retention time (tR3) were evaluated. Using this strategy, mathematical model was defined, and response surface were derived for the separation. The coefficients of determination R2 were more than 0.9258 for all the models. The four responses were simultaneously optimized by using Derringer's desirability functions and MCDM approach. Optimum conditions chosen for assay were MeOH, 0.01mM KH2PO4 (pH 2.5±0.5) adjusted with diluted orthophosphoric acid solution (68.03:31.97v/v) and flow rate of 0.8 mL/min. Peak area ratio of the analyte and internal standard was used for the quantification of pharmaceutical formulation samples. Total chromatographic analysis time per sample was approximately 9.0 min with DST, MPB (IS) and NLT eluting with retention times of 2.7, 3.2, and 6.0 minutes respectively. The optimized assay condition was validated as per ICH guidelines and applied for the quantitative analysis Sprycel-DST tablet and Tasigna - NLT capsule.

N. Venkateswara Rao reported thatA new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay method has been developed for estimation of Nilotinib in capsules dosage forms. The separation was achieved by using column YMC-Pack-ODS-AQ, (150 x 4.6 mm, 5µ) mobile phase consisted of 20 mmol/L ammonium acetate buffer and methanol in the ratio of (20:80 volume/volume). The flow rate was 1.0mL/min. Nilotinib was detected using UV detector at the wavelength of 250 nm. Column temperature 40°C and sample temperature ambient and injection volume 10µL, run time 10 minutes. The retention time of Nilotinib was noted to be 4.50 min respectively. The proposed HPLC method was linear over the range of 10.05-30.016 µg/mL, the correlation coefficient was found to be 0.999. Relative standard deviation for method precision was found to be 0.59. Recovery study results are found to be good in the range of 99.8-100.6 respectively. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Jing Zeng reported that sensitive, rapid, simple and economical ultra-performance liquid chromatography–tandem mass spectrometric method (UPLC–MS/MS) was developed and validated for simultaneous determination of Imatinib, Dasatinib and nilotinib in human plasma using gliquidone as internal standard (IS). Liquid-liquid extraction method with ethyl acetate was used for sample pre-treatment. The separation was performed on an Xtimate Phenyl column using isocratic mobile phase consisting of A (aqueous phase: 0.15% formic acid and 0.05% ammonium acetate) and B (organic phase: acetonitrile) (A:B=40:60, v/v). The flow rate was 0.25 mL/min and the total run time was 6 min. The multiple reaction monitoring (MRM) transitions, *m/z* 494.5→394.5 for imatinib, 488.7→401.5 for dasatinib, 530.7→289.5 for nilotinib and 528.5→403.4 for IS, were chosen to achieve high selectivity in the simultaneous analyses. The method exhibited great improvement in sensitivity and good linearity over the concentration range of 2.6–5250.0 ng/mL for imatinib, 2.0–490.0 ng/mL for dasatinib, and 2.4–4700.0 ng/mL for nilotinib. The method showed acceptable results on sensitivity, specificity, recovery, precision, accuracy and stability tests. This UPLC–MS/MS assay was successfully used for human plasma samples analysis and no significant differences were found in imatinib steady-state trough concentrations among the *SLC22A5* −1889T>C or *SLCO1B3* 699G>A genotypes (*P*>0.05). This validated method can provide support for clinical therapeutic drug monitoring and pharmacokinetic investigations of these three tyrosine kinase inhibitors (TKIs).

CONCLUSION

A sensitive and accurate RP-HPLC methods, stability-indicating HPLC, HPLC-PDA, HPLC-UV, stability indicating HPTLC and HPLC-MS, with solid phase extraction methods was developed for the estimation of Nilotinib, in pharmaceutical dosage forms, human plasma, the above methods was evaluated for Specificity, Linearity, Accuracy, Precision, Ruggedness and Robustness as per ICH&FDA guidelines.

Reference

1. Konatham Teja Kumar Reddy, & M. Akiful Haque. (2022). Develop and validate a highly sensitive method for the estimation of Molnupiravir in rat plasma by high-performance liquid chromatography-tandem mass spectroscopy and its application to pharmacokinetic studies. *Journal of Pharmaceutical Negative Results*, 28–34. https://doi.org/10.47750/pnr.2022.13.S01.0
2. Konatham Teja Kumar Reddy, Penke Vijaya Babu, Rajinikanth Sagapola, & Peta Sudhakar. (2022). A REVIEW OF ARTIFICIAL INTELLIGENCE IN

TREATMENT OF COVID-19. *Journal of Pharmaceutical Negative Results*, 254–264. https://doi.org/10.47750/pnr.2022.13.S01.31

3. Konatham Teja Kumar Reddy, Kumaraswamy Gandla, Penke Vijaya Babu, M Vinay Kumar Chakravarthy, Pavuluri Chandrasekhar, & Rajinikanth Sagapola. (2022). A CRITICAL REVIEW ON BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FEW ONCOLOGY DRUGS BY USING LC-MS-MS. *Journal of Pharmaceutical Negative Results*, 16–27. https://doi.org/10.47750/pnr.2022.13.S01.03
4. Reddy, K. T. K., & Haque, M. A. (2022). Bioanalytical method development and validation of atrasentan in human plasma using verapamil as internal standard by liquid chromatography coupled with tandem mass spectrometry. International Journal of Health Sciences, 6(S8), 625–638. https://doi.org/10.53730/ijhs.v6nS8.10470
5. Konatham Teja Kumar Reddy et.al High Performance Liquid Chromatography for The Simultaneous Estimation of Anti-Ulcer Drugs in Pharmaceutical Dosage Form, journal of Positive School Psychology, Vol. 6, No. 9, 4524-452
6. Reddy KTK, Haque MA. Development and Validation of a High Throughput Lc-Ms/MS Method for Quantitation of Ipilimumab in Human Plasma. International Journal of Pharmaceutical Quality Assurance. 2022;13(3):303-307
7. Teja Kumar Reddy Konatham, M. Anuradha (2020), a stability indicating method development and validation of Telmisartan and Nifedipine in pure form using RP-HPLC. International Journal of Pharmaceutical, Biological and Chemical Sciences, 9(3): 36-44
8. Teja Kumar Reddy Konatham, Satyanarayana Reddy K., Anuradha Manipogo, a Review on viruses that originated from china; Sars, mers and covid-19 World Journal of Pharmaceutical Research, Vol 9, Issue 5, 2020, 2010-2015.
9. Teja Kumar Reddy Konatham et al, A Systematic Review on Method Development and Validation of Few Antiviral Drugs by Using RP-HPLC. Ijppr.Human, 2021; Vol. 21 (3): 651-661.
10. Konatham Teja Kumar Reddy and Kumaraswamy Gandla. Novel Vesicular Drug Delivery Systems Proniosomes. Pharm Res 2022, 6(3): 000272.
11. Konatham et al. synthesis and evaluation of some novel oxadiazole derivatives, World Journal of Pharmaceutical Research, Vol 9, Issue 4, 2020.
12. BaigShahed Mirza, Haque Akiful Mohammad, Konatham Reddy Teja Kumar, Mohammad DuzaBadrud, YahyaAateka Barrawaz, Saffiruddin Sana Shaikh, Siddiqui A. Falak and Khan L. Sharuk*, Recent Advancements in Hyperthermia-Driven Controlled Drug Delivery from Nanotherapeutics, Recent Advances in Drug Delivery and Formulation 2022; 16(4) . https://dx.doi.org/10.2174/2667387816666220902091043
13. Yelampalli, Suresh Reddy, Kumaraswamy Gandla, Konatham Teja Kumar Reddy, Adel Ehab Ibrahim, and Sami El Deeb. 2023. "Determination of Sodium, Potassium, and Magnesium as Sulfate Salts in Oral Preparations Using Ion Chromatography and Conductivity Detection" *Separations* 10, no. 2: 99. https://doi.org/10.3390/separations10020099
14. Teja, M.; Konatham, T.; Muralidharan, V.; Murugesan, A.; Vasantha, N.; Hyandavi, M. A Review on Biosensors for COVID-19. *IJAPSR* 2022, *7*, 9-14.
15. Boini, K.M., singh, A. and Koka, S.S., 2021. Gut Microbial Metabolite Trimethylamine N-oxide Enhances Endoplasmic Reticular Stress and Promotes Endothelial Dysfunction. Circulation, 144(Suppl_1), pp.A14071-A14071.
16. K. Sudheer Kumar / "Formulate and evaluate the herbal bath soap" using extracts of three plants having ethnic and dermatological importance in Ayurveda, namely Azadirecta indica, curcuma longa, ocimum tenuiaflorum "NEUROQUANTOLOGY | OCTOBER 2022 | VOLUME 20 | ISSUE 12 | PAGE 1048-1054| DOI: 10.14704/NQ.2022.20.12. NQ77087
17. Shiv Chandra Singh, A., Yu, A., Chang, B., Li, H., Rosenzweig, A. and Roh, J.D., 2021. Exercise Training Attenuates Activin Type II Receptor Signaling in the Aged Heart. Circulation, 144(Suppl_1), pp.A14259-A14259.