Sustainable Stability Indicating HPTLC Method For Ponatinib HCl

Dr. Mrinalini Damle[1]*, Sunita Sanap[2], Divya mode[3], Gauri Shirekar[4]

[1]Head of Department, Pharmaceutical Quality Assurance, All India Shri Shivaji Memorial Society's College of Pharmacy, Pune, Maharashtra, India, 411001.

[2]Master of Pharmacy, Department of Quality Assurance, All India Shri Shivaji Memorial Society's College of Pharmacy, Pune, Maharashtra, India, 411001.

[3]Master of Pharmacy, Department of Quality Assurance, All India Shri Shivaji Memorial Society's College of Pharmacy, Pune, Maharashtra, India, 411001.

[4]Master of Pharmacy, Department of Quality Assurance, All India Shri Shivaji Memorial Society's College of Pharmacy, Pune, Maharashtra, India, 411001

ABSTRACT

Ponatinib HCl is Tyrosine kinase inhibitor (TKI) used primarily to treat certain types of leukemia. A sustainable stability-indicating High-Performance Thin Layer Chromatography (HPTLC) method was developed and validated for the estimation of Ponatinib HCl. The method was optimized to using a mobile phase of Chloroform: Methanol: Formic acid in the ratio of 9:1:0.3 (v/v/v), providing sharp and well-resolved peak with an Rf value of 0.49 ± 0.03. Detection was performed at 266 nm. Linearity was established in range 100–500 ng/band with correlation coefficient (R^2) of 0.9996. Stability studies performed under acid, alkaline, oxidative, neutral, thermal, and photolytic conditions as per ICH Q1A(R2) and Q1B guidelines, indicate method's stability-indicating capability. The validation of the analytical method for Ponatinib HCl by HPTLC was conducted in accordance with the ICH Q2 (R1) guideline. The method showed good accuracy with recovery, and precision with %RSD <2%. The LOD and LOQ found to be 1.74 and 6.90 ng/band (method 1), and 5.27 and 20.93 ng/band (method 2), respectively. The method also proved robust and the solution was stable for 24 hours. Furthermore, the environmental sustainability of the developed method was assessed under Green analytical chemistry (GAC) and white analytical chemistry (WAC) using MoGAPI (Modified green analytical procedure index), BAGI (Blue applicability grade index), confirming its eco-friendly nature. In conclusion, the developed stability indicating HPTLC method is valid and environmentally friendly.

Keywords Ponatinib HCl, Method validation, stability study, HPTLC method, Sustainability Analysis

INTRODUCTION

Ponatinib hydrochloride is a tyrosine kinase inhibitor used primarily in leukemia [1,2] USFDA approved Ponatinib HCl in December 14, 2012 for CML which create resistant for Ph+ALL then it is approved in March 19, 2024 for combination Chemotherapy [3] Structure of Ponatinib HCl is given in Fig. 1

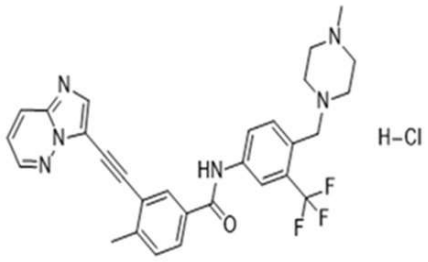

Fig. 1: Structure of Ponatinib HCl

The stability testing related guideline Q1A (R2), issued by the ICH, recommends conducting of degradation checking on a drug to determine stability properties [4,5] Additionally, it specifies that the analytical methods utilized for stability samples and must be subjected to thorough validation [6] This study focuses on the method for Ponatinib determination by SI-HPTLC. GAC [7] and WAC [8] emphasizes reducing environmental impact of analytical methods. To evaluate sustainability, tools like MoGAPI, [9] BAGI, [10] are used, each assessing different aspects such as environmental impact, safety, and sample preparation efficiency. This study applies these greenness assessment softwares for checking greenness of an analytical method, offering a comprehensive view of its sustainability.

EXPERIMENTAL

Materials

Chemical and reagents

Ponatinib HCl was obtained from Sun Pharmaceutical industries, Gurugram, Haryana. Methanol, Chloroform, Formic acid, NaOH, HCl and 30% w/v H_2O_2 from Loba ChemiePvt. and HPLC grade water.

Instrumentation

HPTLC

A chromatographic analysis was conducted utilizing the HPTLC system manufactured by CAMAG. The stationary phase consisted of TLC plates measurements are 20 cm x 20 cm, pre-coated with Silica gel 60 F_{254} and a layer thickness 0.2 mm (Merck). An exact volume was applied using the Linomat-5 Applicator in conjunction with a HAMILTON syringe (100 µl). The winCATS software facilitated the application process, while the CAMAG TLC Scanner 3 was utilized for peak detection. The development of the TLC plate was performed in a twin-trough glass chamber. The sustainability analysis was performed using MoGAPI, BAGI.

Sample preparation

Selection of solvent

Ponatinib HCl is soluble in methanol. This led to the selection of Methanol as the solvent for method development.

Preparation of solution (1000 µg/mL and 50 µg/mL)

Ponatinib HCl working standard was weighed precisely, and dissolved in methanol**.** It was diluted appropriately to obtain standard solutions of required strengths.

Selection of detection wavelength

The wavelength selected for further research was 266 nm, based on the spectrum obtained using a UV-visible Spectrophotometer as shown in Fig. 2

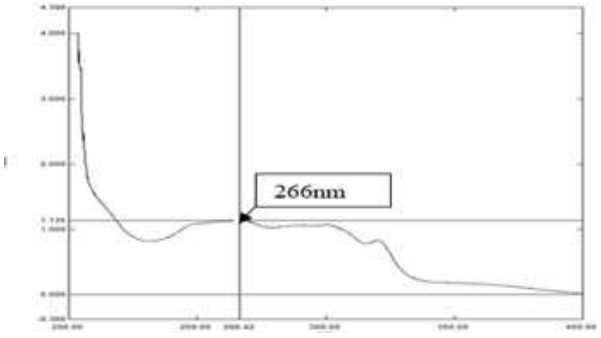

Fig. 2: UV Spectrum of Ponatinib HCl (20 µg/ml)

Chromatographic Conditions

For HPTLC, after several experiments, the ideal mobile phase selected was Chloroform: Methanol: Formic acid (9:1: 0.3v/v/v). The wavelength used for densitometric analysis was 266 nm. A visual representation of the densitogram can be seen in Fig. 3.

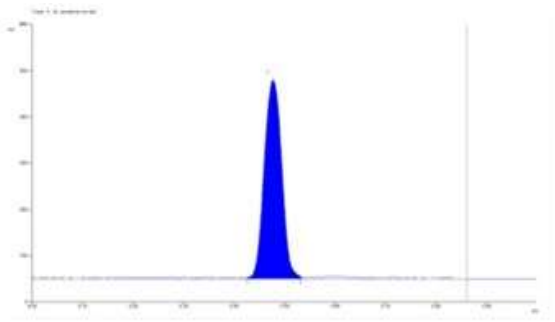

Fig. 3: Representative Densitogram of Ponatinib HCl (500 ng/band, Rf =0.49±0.03)

Forced Degradation studies

The stress testing, conducted as per ICH Q1A (R2) and Q1B guidelines guidelines, involved subjecting drug to various stress conditions for varying time intervals. Adjustments were made to the reagent strength and exposure time to achieve a degradation level ranging from 10% to 30% (with a corresponding recovery range of 90–70%). The optimized stress conditions were used for HPTLC method.

Multiwavelength scanning

After optimizing the forced degradation studies, we observed that there are no any peaks of degradation products. In order to look for the degradation product peaks; higher concentrations of samples were exposed to stress degradation study and applied on TLC plate. Concentration used for multiwavelength scanning is also 10 times greater than concentration used in forced degradation studies. The obtained densitogram is shown in Fig. 4.

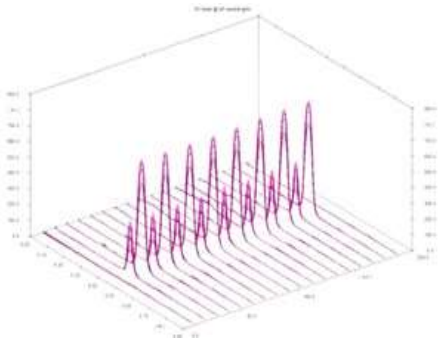

Fig. 4. Densitogram of Multiple Wavelength Scanning

(Track 1- Blank methanol, 2 and 3- Standard Ponatinib HCl 500 & 5000 ng/band, Track 4 & 5- Acid degradation 500 & 5000 ng/band, Track 6 and 7-Alkaline degradation 500 & 5000 ng/band, Track 8 and 9- Oxidative degradation 500 & 5000 ng/band, Track 10 & 11- Neutral degradation 500 & 5000 ng/band, Track 12 and 13- Thermal degradation 500 & 5000 ng/band, Track 14 and 15- UV light degradation 500 & 5000 ng/band, Track 16 & 17- Fluorescent light degradation 500 & 5000 ng/band, Track 18- Blank methanol)

Method Validation

The developed HPTLC method was validated as per ICH Q2 (R1).

Specificity

The method's specificity was assessed through peak purity profile studies. This process included analysing the correlation coefficients between the spectra obtained at the initial slope and the peak maximum ($r_{s,m}$), in addition to the correlation coefficient between the spectra at the peak maximum and the final slope ($r_{m,e}$).

Linearity and range

Standard solution of Ponatinib HCl (50 µg/ml) was utilized to spot volumes of 2,4,6,8 and 10 µL onto the TLC plate, resulting in spotted quantities ranging from 100 to 500 ng/band. The calibration curve was plotted using the peak area against amount spotted. The regression equation was calculated, and the correlation coefficient was determined

Assay

Assay of Ponatinib HCl was done on spiked blend due to unavailability of its marketed preparation in local market. For the preparation of blank blend, 105 mg starch and 105 mg lactose were mixed in the mortar pestle to made a 210 mg of blend. For preparing a spiked blend, 90 mg Ponatinib HCl drug was mixed with 210 mg of blank blend to get the 300 mg spiked blend amount of spiked blend equivalent to 10 mg Ponatinib HCl was dispersed in methanol. The solution was sonicated and filtered using Whatman filter paper. It was then diluted to get 50 µg/ml working solution. Solution was applied to a TLC plate in a 5 µl volume and plate was developed and scanned at 266 nm, with the peak area recorded followed by calculating the percent recovery.

Accuracy

A method of recovery study was used at 80, 100, and 120% level by adding different amounts of standard to a spiked blend that had been assayed.

Precision

Precision was assessed by evaluating repeatability and intermediate precision studies. In repeatability study, six replicates of a standard (100 ng/band) were spotted to a TLC plate on the same day at different time intervals. For intermediate precision, six replicates of the standard (100 ng/band) were spotted to a TLC plate over three consecutive days and then % RSD was calculated.

Detection Limit and Quantification Limit

The LOD and LOQ for the developed HPTLC method was determined in accordance with ICH guidelines, utilizing the slope of the calibration line and standard deviation of the peak area at lowest concentration (method 1) and slope of the calibration line and standard deviation of the y-intercept (method 2)

Robustness

The robustness was assessed by making small modifications in the mobile phase composition, saturation time, time from application to development, time from development to scanning and analyst variation.

Solution stability

Solution stability was evaluated using a 50µg/ml sample solution, which was kept at room temperature for 24 hours. The peak area was calculated by spotting initially and after 24 hours.

RESULT AND DISCUSSION

Forced degradation studies

The developed Stability indicating HPTLC method was evaluated through forced degradation studies, which were optimized to reach a degradation level of 10-30%, A summary of the results is presented in Table 1

Table 1. Summary of degradation parameters for Ponatinib HCl

Sr	Stress Condition	Temperature and Time	% Recovery	Peak Purity	
				$r_{(s,m)}$	$r_{(m,e)}$
1	Acid hydrolysis (1 N HCl)	Immediate at room temperature	86.82	0.998	0.999
2	Alkaline hydrolysis (1N NaOH)	Immediate at room temperature	89.56	0.998	0.999
3	Oxidation Degradation (30% v/vH_2O_2)	2hr at room temperature	84.81	0.998	0.997
4	Neutral degradation (D.W)	1 hr at room temperature	82.54	0.999	0.998
5	Thermal degradation	80 ºC for 4 h	85.23	0.997	0.999
6	UV degradation	200-watt hr./m^2	87.81	0.999	0.998
7	Fluorescence Degradation	1.2 million LUX hours	84.52	0.997	0.998

The degradation study demonstrated that the drug Ponatinib HCl was found to be relatively stable under acidic, alkaline, oxidative, thermal, neutral hydrolysis and photolytic stress. The recovery was obtained in the range of 70-90%

Method Validation

Specificity

Evaluation of the drug peak's, peak purity under stress conditions using winCATS software revealed that it falls within the specified limits for method. This indicates that there are no interferences within drug peak.

Linearity and range

The method was determined to be linear within a range of 100 to 500 ng/band for Ponatinib HCl. The R^2 was found to be 0.9996 with equation y = 21.048x + 1372.8. The 3D Densitogram obtained in HPTLC is shown in Fig. 5. Calibration Curve for Ponatinib HCl is given in Fig. 5.

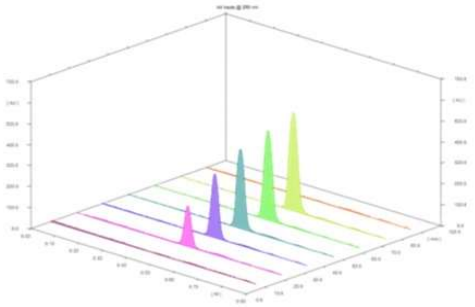

Fig. 5: 3D Densitogram of Ponatinib HCl Linearity (100-500 ng/band)

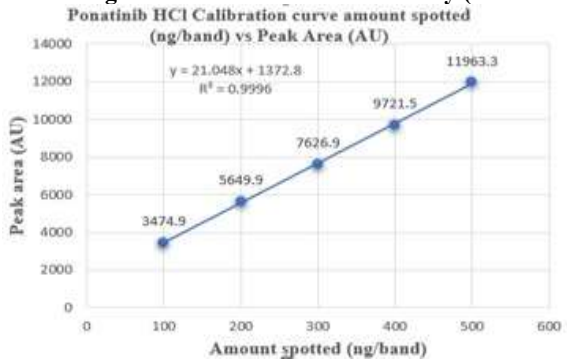

Fig. 6: Calibration Curve for Ponatinib HCl (100-500 ng/band)

Assay

Assay of Ponatinib HCl from spiked blend was found to be 100.34%.

Accuracy

Recovery studies conducted at 80%, 100%, and 120% of concentration in accordance with ICH guidelines for HPTLC resulted in findings that fell within acceptable limits. The representative densitogram for accuracy and assay are shown in Fig. 7

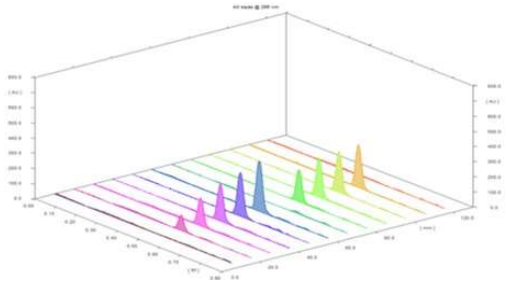

Fig. 7: Accuracy for Ponatinib HCl (Track 2-6 standard linearity, track 8 @ assay, track 9 Std addition @ 80%, Track 10 Std addition @ 100%, Track 11 std addition @ 120%.

Precision

The % RSD for both Interday and Intraday precision was found less than 2, which indicates that the method is precise. The 3D densitogram is shown in Fig. 8

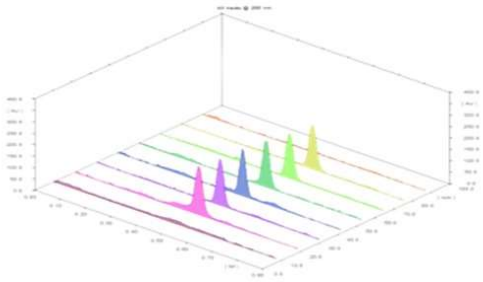

Fig. 8: Precision for Ponatinib HCl (100 ng/band)

LOD and LOQ

LOD and LOQ for Ponatinib HCl was found (by method 1) to be 1.74 and 6.90 ng/band and by method 2 5.27 and 20.93 ng/band.

Robustness

The robustness of method performed by making alterations in parameters of developed HPTLC method and the % RSD was < 2 confirming the developed method is robust.

Solution stability

Solution stability was evaluated using a 50µg/ml drug solution, which was kept for 24 hrs. at room temperature. The peak area was calculated upon spotting initially and after 24 hours it showed marginal reduction in peak area.

A summary of the validation parameters given in Table 2

Table 2. Summary of validation parameters

Sr. No.	Validation Parameter	HPTLC
1	Linearity Range	
2	Intraday Precision	1.43
	Interday Precision	1.20
3	Assay	100.34%
4	Accuracy	
	Level 80%	101.15
	Level 100%	100.99
	Level 120%	100.28
5	LOD	
	Method 1	1.74 ng/band
	Method 2	5.27 ng/band
6	LOQ	
	Method 1	6.90 ng/band
	Method 2	20.93 ng/band
7	Robustness	Robust
8	Specificity	Specific
9	Solution stability	98.92%

SUSTAINABILITY ANALYSIS

A comprehensive sustainability analysis of analytical method was carried out utilizing four well-established sustainability assessment models MoGAPI**,** BAGI. Each tool provides a different perspective on the environmental and procedural impact of the method, offering a multifaceted evaluation of its greenness. MoGAPI focuses on visualizing the ecological burden of each step in the analytical process, while BAGI integrates health, safety, and environmental factors. The greenness scores obtained from these tools confirming that the developed stability indicating HPTLC method is environmentally sustainable and aligns well with the principles of GAC and WAC

MoGAPI

Modified GAPI is an enhanced version of the original GAPI tool, designed to more accurately and comprehensively assess the environmental sustainability of analytical procedures.

MoGAPI was applied using the following key parameters:

A. Sample Preparation

1. **Sample collection method-** on the basis of sample collection HPTLC is typically an off line sample collection method.
2. **Preservation** –No preservatives are added in samples.
3. **Transport** –No transport required
4. **Storage** –Samples are stored under special conditions refrigeration 2°C to 8°C.
5. **Type of Method** – Direct, extraction-based, or derivatization. The sample is analysed with direct method.
6. **Scale of Extraction** –on the basis of extraction required or not options are given. Extraction was not required.
7. **Solvents/Reagents Used** –on the basis of solvents greenness Chloroform methanol and formic acid hydrogen peroxide, sodium hydroxide and hydrochloric acid falls under non-green solvents.
8. **Additional Treatment** – (clean up, solvent removal, derivatization, mineralization etc.) simple methods like clean up and solvent removals are used.

B. Reagents And Solvents

9. **Amount** – (ranges from <10, 10-100, >100mL/g.) <10 drug was used for method.
10. **Health Hazard** – Toxicity (based on National Fire Protection Association NFPA health hazard score 0-4). Toxicity was between 0-2
11. **Safety Hazard** – (Flammability, instability score 0-4). Score for the method was between 0-1

C. Instrumentation

12. **Energy** – (Based on Power usage or instrument time per run <0.1, 0.1-1.5, >1.5 kWh per sample) 0.25kWh per hour energy was required for HPTLC
13. **Occupational Hazard** – (on the basis of vapours release options are given) Hermetic sealing method was applied for HPTLC.
14. **Waste** – (Quantity of waste generated <1, 1-10, >10mL) Total estimated waste generated per hour for HPTLC is 1-10 mL score is greater than 10.
15. **Waste Treatment** – (On the basis of waste treatment used options are given) No waste treatment procedure was applied for developed method.
16. **Quantification** – (yes or no) HPTLC method includes calibration, peak area measurement, and uses a standard curve so it is yes.

Greenness of method by AGREE prep is shown in Fig. 9.

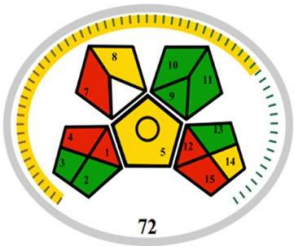

Fig. 9: Greenness assessment of Developed HPTLC Method by Modified Green Analytical Procedure Index (MoGAPI)

Indicate values of 72 for the HPTLC method, it implies that the HPTLC method that has been developed is environmentally friendly.

BAGI

The BAGI used to check the practicality and applicability of method, complementing traditional green metrics like AGREE and GAPI. BAGI focuses on the "blue" principles of White Analytical Chemistry, which emphasize the practical aspects of analytical procedures

BAGI was applied using the following key parameters:

1. **Type of Analysis**
   Determines whether the method is qualitative, quantitative, screening, Quantitative and confIrmatory. HPTLC analysis falls under quantitative and confirmatory analytical method
2. **Multi- or Single-Element Analysis**
   Indicates whether the method analyses one specific analyte (single-element) or multiple analytes simultaneously (multi-element) 2-5, 6-15, >15 compounds. Single element analysis was performed.

3. **Analytical Technique**
   On the basis of level of advanced to simple instruments scores are given. HPTLC falls under the simple instrument
4. **Simultaneous Sample Preparation**
   (Measures how many samples can be prepared at once. 1, 1-12, 13-95, >95) 1 sample was prepared at a time.
5. **Sample Preparation**
   (Depend upon number of steps and complexity of sample preparation scores are given) for developed HPTLC method simple sample preparation required
6. **Samples per Hour**
   (Indicates method throughput—how many samples can be analysed in one hour. <1, 2-4, 5-10, >10) 5-10 samples were analysed in one hour.
7. **Reagents and Materials**
   (On the basis of preparation process of reagents and its cost scores are given) for HPTLC common commercially available reagents was widely used in methods — both for sample preparation and mobile phases.
8. **Preconcentration**
   (Whether preconcentration required or not on the basis of that scores are given) No preconcentration was required in developed HPTLC method.
9. **Degree of Automation**
   On the basis of degree of automation HPTLC falls under semi-automated with common devices
10. **Amount of sample**
    Refers to how much sample volume is required for analysis. For developed HPTLC method <100 µL (or mg) was required

    Each attribute is scored on a scale of 25 to 100 points, corresponding to shades from white (lowest) to dark blue (highest). The blunnes and practicability scores by BAGI given in Fig. 10

Fig.10: Blueness assessment by Blue Applicability Grade Index (BAGI)

Indicate values 70 for the HPTLC, it also implies that the HPTLC method that have been developed is environmentally friendly.

DISCUSSION

Based on the literature search conducted for Ponatinib HCl, previous studies determination of ponatinib HCl in plasma using HPLC,[11] LC-HRMS and NMR studies,[12] Quality by design approach for analytical method for quantifying ponatinib in rat plasma,[13] simultaneous quantification of ponatinib using HPLC-PDA,[14] LC-MS/MS,[15] liquid chromatography-tandem mass spectrometric assay[16] and its impurities and degradation products[17] have reported it was observed that there were no reported Stability indicating HPLC and HPTLC methods available for Ponatinib HCl. In light of this, our proposed stability indicating HPTLC method aims to comply with ICH guidelines by optimizing stress-induced degradations ranging from 10% to 30%. Furthermore, we have developed and optimized sustainable stability indicating HPTLC method that underwent validation in accordance with ICH guidelines. A sustainability analysis of analytical method done using four well-established greenness assessment models MoGAPI**,** BAGI**.**

CONCLUSION

The newly developed Sustainable stability indicating HPTLC method can be used to determine Ponatinib HCl. The validation process followed the guidelines set by ICH [Q2(R1)]. It was observed that Ponatinib HCl exhibited relative stability in all stress conditions. Overall, the method can be

considered green and sustainable**.** This multi-tool approach strengthens the reliability of the sustainability assessment and supports the method's alignment with the principles of Green and white analytical chemistry.

ACKNOWLEDGMENT

Authors are thankful to the Principal and the management of the AISSMS College of Pharmacy, Pune, Maharashtra, India for providing required facilities for research work. The authors would like to thank Sun Pharmaceuticals for the generous gift of Ponatinib HCl.

REFERENCES

1. Drugbank for Ponatinib Hydrochloride, https://go.drugbank.com/salts/DBSALT000142, accessed on 27th Jan 2025
2. https://pubchem.ncbi.nlm.nih.gov/compound/ Ponatinib hydrochloride, accesses on 27th Jan 2025
3. Attwa MW, Alkahtani HM, El-Azab AS, Abdel-Aziz AA, Abdelhameed AS, Kadi AA, Hassan SB, Zeidan DW, Bakheit AH. Ponatinib: A comprehensive drug profile. Profiles of Drug Substances, Excipients and Related Methodology. 2024 Jan 1; 49:81-114.
4. ICH Guideline Q1A (R2), Stability testing of new drug substances and products, 2003. Revision 2. Accessed December 16, 2024.
5. ICH Guideline Q1B, Stability Testing: Photostability testing of new drug substances and products, 1996. Accessed December16, 2024.
6. ICH Guideline Q2(R1), ICH Q2(R1) Guideline on Validation of Analytical Procedures, 2005. Accessed December 16, 2024.
7. Armenta S, Garrigues S, de la Guardia M. Green analytical chemistry. TrAC Trends in Analytical Chemistry. 2008 Jun 1;27(6):497-511.
8. Nowak PM, Wietecha-Posłuszny R, Pawliszyn J. White analytical chemistry: an approach to reconcile the principles of green analytical chemistry and functionality. TrAC Trends in Analytical Chemistry. 2021 May 1; 138:116-123.
9. Mansour FR, Płotka-Wasylka J, Locatelli M. Modified GAPI (MoGAPI) tool and software for the assessment of method greenness: case studies and applications. Analytica. 2024 Sep 6;5(3):455-59.
10. Manousi N, Wojnowski W, Płotka-Wasylka J, Samanidou V. Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality. Green chemistry. 2023;25(19):7598-604.
11. Yasu T, Momo K, Kobayashi S, Kuroda S, Tojo A. Simple determination of plasma ponatinib concentration using HPLC. Biological and Pharmaceutical Bulletin. 2018 Feb 1;41(2):254-58.
12. Golla VM, Kushwah BS, Dhiman V, Velip L, Samanthula G. LC-HRMS and NMR studies for characterization of forced degradation impurities of ponatinib, a tyrosine kinase inhibitor, insights into in-silico degradation and toxicity profiles. Journal of Pharmaceutical and Biomedical Analysis. 2023 Apr 1; 227:152-161.
13. Koo N, Lee EJ, Kim MJ, Park M, Lee KR, Chae YJ. Quality by design-based approach for the development of an analytical method for quantifying ponatinib in rat plasma. Heliyon. 2024 Oct 15;10(19):367-76.
14. Yokoyama Y, Nozawa E, Morita M, Ishikawa E, Mori T, Sakurai M, Kikuchi T, Matsuki E, Yamazaki R, Kataoka K, Jibiki A. Simultaneous quantification of dasatinib, nilotinib, bosutinib, and ponatinib using high-performance liquid chromatography–Photodiode array detection. Journal of Clinical Laboratory Analysis. 2022 Aug;36(8):245-53.
15. Kadi AA, Darwish HW, Attwa MW, Amer SM. Validated LC-MS/MS method for the quantification of ponatinib in plasma: application to metabolic stability. PLoS One. 2016 Oct 20;11(10):64-67.
16. Sparidans RW, Kort A, Schinkel AH, Schellens JH, Beijnen JH. Liquid chromatography–tandem mass spectrometric assay for ponatinib and N-desmethyl ponatinib in mouse plasma. Journal of Chromatography B. 2016 Jun 15;1023: 24-29.
17. Wang J, Zhu Y, Qin J, Wu W, Huang R, Cai L. Chromatographic analysis of ponatinib and its impurities: method development, validation, and identification of new degradation product. Frontiers in Chemistry. 2024 Nov 12;12: 48-57.