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METHOD DEVELOPMENT AND VALIDATION OF NILOTINIB HYDROCHLORIDE MONOHYDRATE BY UV-VISIBLE SPECTROSCOPY

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ABSTRACT
This study aimed to develop and validate a simple, cost-effective, and precise UV-visible spectrophotometric method for quantifying nilotinib hydrochloride monohydrate (NHM) in pharmaceutical formulations. The procedure's solvent was acetonitrile (ACN):water (1:1 v/v), and NHM's maximum absorbance was recorded at 303 nm. The method demonstrated excellent linearity in the concentration range of 0–50 µg/mL (R2 = 0.9998), as per the International Council for Harmonization (ICH) guidelines. High reproducibility was shown by precision studies with intra-day and inter-day precision <2% and assay analysis %RSD values of 0.58%. Accuracy was confirmed by recovery studies at 100%, 150%, and 200% levels; recoveries ranging from 99 to 101% (%RSD = 0.612) were obtained. The limits of quantification (LOQ) and detection (LOD) were determined to be 4.719 µg/mL and 1.557 µg/mL, respectively, indicating high sensitivity. The assay of capsule formulations showed a percentage purity of 100.03% w/v, which is in line with the acceptance criteria (90–110%). The created technique demonstrated robustness, specificity, and excipient interference-free operation. This UV-visible spectroscopy method is ideal for routine quality control of NHM in pharmaceutical industries because it offers benefits like simplicity, speed, and cost-effectiveness. It also ensures regulatory compliance and improves analytical efficiency.

KEYWORDS: UV spectrophotometric method, analysis, method validation, nilotinib hydrochloride monohydrate, nilotinib, CML.

INTRODUCTION
Nilotinib hydrochloride monohydrate salt is a synthetic amino pyrimidine, a second-generation tyrosine kinase inhibitor, that is used to treat chronic myelogenous leukemia that is resistant to imatinib. Nilotinib exhibits in vitro activity against numerous imatinib-resistant mutants and has an affinity that is about 20 times greater than that of imatinib.[1, 2] The NLH is chemically designated as 4-methyl-N-[3-(4- methyl-1H-imidazol-1-yl)-5-(trifluoromethyl) phenyl]-3- [(4-pyridin-3-yl pyrimidin-2-yl) amino] benzamide salt) with an empirical formula and molecular weight of C28H22F3N7O.HCl and 565.98 g/mol, respectively. Tasigna is its trade name, and it is a small molecule that is a salt of hydrochloride monohydrate. It is a tyrosine kinase inhibitor (TKI) that is approved for the treatment of chronic granulocytic leukemia (CGL), another name for chronic myelogenous leukemia (CML).[3-6]

It is a second-generation TKI[2] that has a 20-fold greater affinity for the adenosine triphosphate (ATP) binding sites in vitro than imatinib, one of the current TKIs. CML is a member of the family of clonal disorders of pluripotent hematopoietic stem cells in the bone marrow known as chronic myeloproliferative diseases.[7-8]

Based on the crystal structure of the imatinib-ABL complex, researchers rationally designed novel inhibitors that proved effective against imatinib-resistant mutants of the BCR-ABL protein. Nilotinib is a novel, selective BCR-ABL inhibitor that fits into the ATP-binding site of the BCR-ABL protein with a higher affinity than imatinib.[2]

Slightly soluble in methanol and dimethyl sulfoxide, it is a powder that ranges from slightly yellow to slightly greenish yellow.[9] Because of NTB's novel therapeutic action, long-term efficacy, and safety, its use has grown over the last ten years.[10]

People who have tested positive for the Philadelphia chromosome, a common genetic abnormality associated with chronic myeloid leukemia (CML), are treated with it. Another name for CML is chronic granulocytic leukemia (CGL). It is a white blood cell cancer brought on by the unchecked and accelerated growth of myeloid cells in bone marrow and their subsequent accumulation in the blood. The proliferation of mature granulocytes and their precursors is a hallmark of CML, a clonal bone marrow stem cell disease. Since the first tyrosine kinase inhibitor was introduced, CML has been primarily treated and targeted with medications such as imatinib, nilotinib, and dasatinib, which have improved long-term survival rates. A drug substance's and a drug product's percentage level of impurities must be controlled because their presence can significantly affect the product's quality, safety, and efficacy.[11–17] Nilotinib's Risk Evaluation and Mitigation Strategy (REMS) has been authorized by the FDA.[18]

According to the literature review, there is only one UV method and a small number of HPLC methods for Nilotinib Hydrochloride Monohydrate.[19–22] To determine the amount of nilotinib hydrochloride in bulk and its commercial formulations, the current study aims to develop a UV spectrophotometric method. This will be followed by analytical method validation in accordance with ICH recommended conditions.

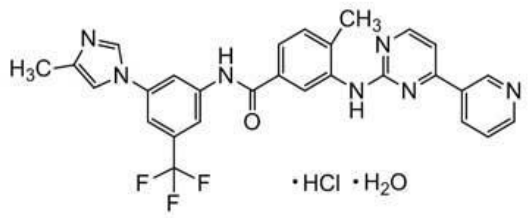

Figure 1: Structure of Nilotinib Hydrochloride Monohydrate.

MATERIALS AND METHOD

INSTRUMENTATION AND SOFTWARE

The study made use of top-notch lab glassware and sophisticated analytical tools. Using a double-beam UV/ visible spectrophotometer (LABINDIA UV-3000) with UV Win software support, accurate absorbance measurements were made at a few chosen wavelengths. Using a WENSAR electronic balance, precise weighing of the samples and reagents was accomplished. Borosilicate glass, which is prized for its longevity and resistance to heat and chemicals, was used to make all of the glassware used in the procedure. This included instruments like volumetric flasks for creating precise solution concentrations, dropping pipettes for cautious liquid handling, and beakers for routine lab work. The study's consistent outcomes were guaranteed by the careful selection of tools and supplies.

SOLVENT

The solvent for nilotinib hydrochloride monohydrate is a mixture of acetonitrile and water [1:1]. The reagents used in the study are of analytical grade.

METHOD DEVELOPMENT

Selection of solvent

- Based on Nilotinib Hydrochloride Monohydrate's solubility, the solvent was chosen.
- ACN, Dimethyl Sulfoxide, Ethanol, Water, ACN: Water, and Ethanol: Water Mixtures were among the solvents in which Nilotinib Hydrochloride Monohydrate was tested for solubility.
- The drug demonstrated good solubility in water and an ACN (e.g., 50:50 v/v), which was chosen as the study's solvent.

Preparation of Acetonitrile-Water Mixture

- ACN and water were mixed in equal volumes [1:1] to create a 50:50 (v/v) ACN and water ratio.
  However, when the solvent ratio was 1:1 acetonitrile to water, the maximum sensitivity and sufficient solubility were noted.

Preparation of Standard Stock Solution:

A concentration of 1 mg/ml (1000µg/ml) was achieved by dissolving 100 mg of Nilotinib Hydrochloride Monohydrate in 100 ml of ACN and water (1:1). This resulted in a standard stock solution (primary). From the primary solution, 0.1 ml is taken and makeup with 10 ml to get concentration of 0.01 mg/ml(10µg/ml).

Determination of Absorption Maxima

Nilotinib Hydrochloride Monohydrate standard stock solutions were scanned in the UV/Visible spectrophotometer between 200 and 400 nm in wavelength. The spectra were used to determine the absorbance maxima (λ max) for nilotinib.

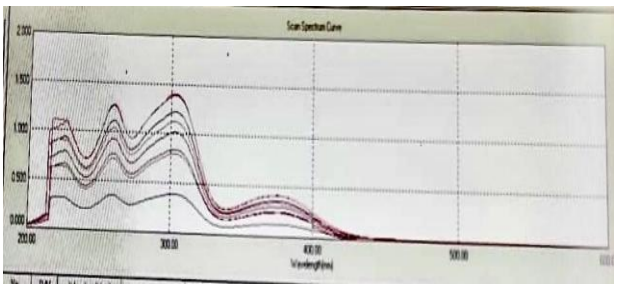

Figure 2: Absorption maxima of Nilotinib Hydrochloride Monohydrate.

Determination of drug content capsule dosage form: (Assay)

To determine the actual weight of the Nilotinib Hydrochloride Monohydrate powder in capsules, weigh 10 capsules initially and record the total weight (W1). Open the capsules carefully, remove the powder and

weigh the capsules shells (W2). To determine the total weight of the Nilotinib Hydrochloride Monohydrate powder, subtract the weight of the empty capsules from the weight of the filled capsules (W1-W2). After dissolving 100 mg of the medication (nilotinib hydrochloride monohydrate) in 100 milliliters of ACN and water, the mixture was vigorously shaken for fifteen minutes. A concentration of 10 µg/ml was obtained by pipetting 0.1 ml of this solution into a 10 ml standard flask and diluting it with water and ACN to the appropriate level. Nilotinib Hydrochloride Monohydrate's absorbance was measured at its corresponding λ max values in comparison to a blank.

VALIDATION

Linearity

100 mg of Nilotinib Hydrochloride Monohydrate is weighed and transferred into a standard flask and dissolved with 100 ml of ACN: water (1:1). The content was shaken well to give a concentration of 1mg/ml. From the above solution, pipette out 0.1,0.2,0.3,0.4 and 0.5ml into an individual 10 ml standard flask and make up volume with ACN and water. Every solution has 10, 20, 30, 40, and 50 µg/ml. The solution's absorbance at 303 nm is measured in comparison to a blank.

Table 1: Linearity Data of Nilotinib Hydrochloride Monohydrate.

Concentration (µg/ml)	Absorbance (303 nm)
0	0
10	0.328
20	0.628
30	0.928
40	1.228
50	1.528
R^2value	**0.9998**
Slope value	**0.03000**
Intercept value	**0.028**

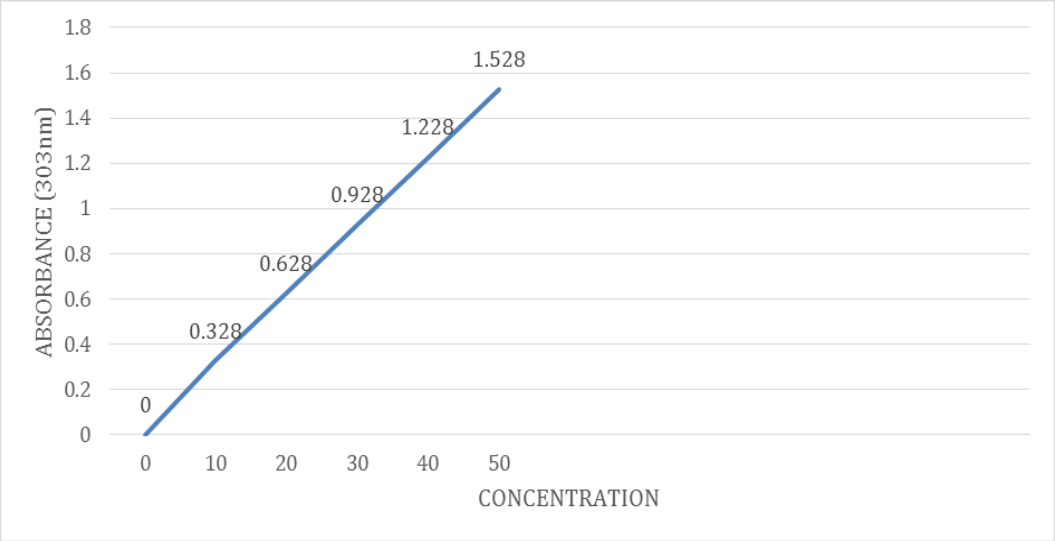

Figure 3: Calibration curve of Nilotinib Hydrochloride Monohydrate.

Determination of drug content in tablet dosage form (Assay)

The percentage purity of the capsule dosage form was found to be 100.03% w/v. The % RSD for analysis of formulation was found to be within the limit (<2%).

Table 2: Assay value of Nilotinib Hydrochloride Monohydrate.

Number of sample preparation	Amount of drug Present mg	%purity
1	10.05	100.50%
2	10.12	101.20%
3	9.98	99.80%
4	10.08	100.80%
5	9.97	99.70%
6	10.02	100.20%
	Mean	**100.03%**
	Standard deviation	**0.58**
	%RSD(NMT:2.0%)	**0.58**
	Acceptance criteria	**90-110%**

Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of a homogenous sample. Measured quantity values obtained by repeating measurements on the same or similar objects under specific conditions are said to be closely agreed upon. The method's repeatability was verified by analyzing the pure drug (Standard) and formulation (Sample) six times at the same concentration. The amount of each drug present in the pure drug (Standard) and formulation (Sample) were calculated. The percentage RSD was determined.

Table 3: Repeatability study for Nilotinib Hydrochloride Monohydrate (Pure drug).

S.No	Concentration (µg/ml)	Absorbance	Amount found (µg)	Assay (%)
1	10	0.512	9.85	98.50%
2	10	0.534	9.92	99.20%
3	10	0.521	10.05	100.50%
4	10	0.529	9.88	98.80%
5	10	0.537	10.1	101.00%
6	10	0.53	9.95	99.50%
			Mean	**99.58%**
			S.D	**0.9325**
			%RSD	**0.9365**

Precision(sample)

A percentage of 100.07% ± 0.89 of Nilotinib Hydrochloride Monohydrate was found to be present in the formulation (sample).

Table 4: Repeatability study for Nilotinib Hydrochloride Monohydrate.

S.No	Concentration (µg/ml)	Absorbance	Amount found (µg)	Assay (%)
1	10	0.512	9.98	99.80%
2	10	0.518	10.15	10150%
3	10	0.51	9.95	99.50%
4	10	0.515	10.08	100.80%
5	10	0.509	9.93	99.30%
6	10	0.508	9.9	99.00%
			Mean	**100.07%**
			S. D	**0.89**
			RSD	**0.89**

Recovery study: (Accuracy)

The percentage recovery study was conducted using the following ranges: 100%, 150%, and 200 percent.

Each sample was examined six times at concentrations of 10, 15, and 20 µg/ml.

100%

After the sample weighed at around 0.11g, it was placed in a 10-ml standard flask and diluted with water and ACN until the concentration was 1 mg/ml. Pipette out 0.1 ml of the solution mentioned above. into a separate standard flask and make up 10ml with ACN and water (10µg/ml). Then measure the absorbance at 303nm.

150%

Weigh out approximately 0.165g of the sample, then transfer it to a 10ml standard flask and make it up to the mark with water and ACN to get 1.5mg/ml. From the above solution pipette out 0.1ml in a separate standard flask and makeup10ml with ACN and water (15µg/ml). Then measure the absorbance at 303nm.

200%

Weigh out approximately 0.22g of the sample, then transfer it to a 10ml standard flask and make it up to the mark with water and ACN to get 2 mg/ml. Pipette 0.1 ml of the above solution into a different standard flask, then make 10 ml with water and ACN (20µg/ml). Then measure the absorbance at 303nm.

Table 5: Recovery study of Nilotinib Hydrochloride Monohydrate.

S.No	Concentration (%)	Amount present (µg/ml)	Amount Recovered (µg/ml)	Percentage recovery (%)
1	100%	10	10.05	100.5
			9.98	99.8

			10.02	100.2
2	150%	15	14.95	99.66
			15.1	100.66
			14.98	99.86
3	200%	20	19.92	99.6
			19.98	99.9
			20.04	100.2
			Mean	99.94%
			Standard deviation	**0.612**
			%RSD	**0.612**

Limit of detection (LOD)

An individual analytical procedure's detection limit is the lowest concentration of analyte in a sample that can be identified but may not be precisely quantified. LOD=3.3 σ/s, Where σ is standard deviation of y intercept of calibration curve and s is slope of regression equation. The LOD value 1.557 µg/mL.

Limit of quantification (LOQ)

The lowest concentration of an analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the quantification limit of a particular analytical procedure. LOQ =10 σ/s Where σ is standard deviation of y intercept of calibration curve and s is slope of regression equation. The LOQ value is 4.719 µg/mL.

RESULT AND DISCUSSION

Using UV spectrophotometry, the suggested method for analyzing nilotinib hydrochloride monohydrate is straightforward, precise, cost-effective, and practical. The Maximum wavelength was found to be 303nm. Excellent linearity was demonstrated by the developed analytical method for nilotinib hydrochloride monohydrate, which followed Beer-Lambert's law and had a correlation coefficient (r2) of 0.9998. The calibration curve showed that absorbance at 303 nm and concentration (0–50 µg/ml) were strongly correlated. In order to ensure compliance with the acceptance criteria (90-110%), the dosage form's assay confirmed a percentage purity of 100.03% w/v with a % RSD of 0.58, well within the acceptable limit (<2%). High reproducibility was demonstrated by precision studies; the assay for the formulation was 100.07% ± 0.89 and for the pure drug it was 99.58% ± 0.9325, both of which had percentage RSD values within allowable bounds. A recovery study at three concentration levels (100%, 150%, and 200%) was used to validate accuracy. The results showed that there was no excipient interference, with percentage recovery ranging from 99 to 101% and an RSD of 0.612. With a quantification limit (LOQ) of 4.719 µg/ml and a detection limit (LOD) of 1.557 µg/ml, the technique showed excellent sensitivity.

Table 6: Validation summary of the UV spectrophometric method.

S.NO	Parameters	Result
1	Absorption Maxima (nm)	303
2	Linearity Range (µg/ml)	0-50
3	Assay (sample)	100.03%
4	Precision (standard))	99.58% ± 0.9325
5	Precision (sample)	100.07% ± 0.89
5	Correlation Coefficient (R^2)	0.9998
6	Slope	0.03
7	Intercept	0.28
8	Accuracy (% ± SD)	99-101% ±0.612
9	LOD µg/ml	1.557
10	LOQ µg/ml	4.719

CONCLUSION

A simple and reliable UV spectrophotometric method is now available for estimating. Nilotinib hydrochloride monohydrate in pharmaceutical forms. We adhere to ICH guidelines and combine strong accuracy, precision, and linearity. The method's low percentage RSD and high recovery rates demonstrated its efficacy. It is suitable for routine quality control and drug analysis.

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Conflict of interests

We declare that there is no conflict of interest.

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