IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2020 Vol.:20, Issue:1

Polymorphic Stress Studies of Nilotinib Hydrochloride Hydrates and Its Characterization

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Cheddi Srinivasa Rao, R. Sreenivas, *Vangala Ranga Reddy, Durga Prasad, Gopal Vaidyanathan

Natco Pharma Ltd, Natco Research Centre, B-13, Industrial Estate, Sanath Nagar, Hyderabad-500 018, India

Submitted: 01 November 2020
Revised: 20 November 2020
Accepted: 10 December 2020

Keywords: Nilotinib HCl, Hydrate, Thermal, humidity, Pressure, Non-stoichiometry, Dynamic Vapor Sorption

ABSTRACT

Nilotinib HCl (Figure-1) is a class of pharmacologic drugs known as kinase inhibitors. The crystalline hydrate forms have been selected for the formulation. The polymorphic stress studies of hydrates were performed for additional controls of the successful development of the formulation process by taking into consideration of temperature, humidity and compression/pressure studies. The Nilotinib monohydrate contains one mole of water is an integral part of the crystal lattice structure and the remaining corresponds to surface water. The dihydrate exhibits variable hydration states with the same PXRD patterns with typical crystal lattice channels that can accommodate additional loosely bound water results in the non-stoichiometric ratio. The overall water content was determined by Karl Fischer titration and thermogravimetric analysis (TGA) combined with the information obtained from the Dynamic Vapor Sorption, Differential Scanning Calorimetry, Powder X-ray diffraction, Thermogravimetry and Mass Spectroscopy (TG-MS), and Microscopy.

INTRODUCTION:

The study to control and understand the polymorphism of APIs during the manufacturing and formulation process is an integral part of pharmaceutical development. Many drug molecules exhibiting different polymorphic or pseudo polymorphic forms often show differences in physicochemical properties like melting point, hygroscopicity, solubility, stability, and processability [1,2]. In some of the cases, hydrates are the most reasonable choice for the formulation process development. As per the literature, most of the drug substances of about 75% are capable of forming hydrates [3] which is usual among larger drug molecules [4,5]. The hydrate forms of the API drugs that exist in the market are nedocromil sodium [6], Cephalexin hydrate [7], etc., For many pharmaceutical materials, phase transitions can be influenced by the amount of water vapour surrounding the sample which can adsorb only on the surface, absorb deep into the bulk structure, chemisorb to the surface, act as a plasticizing agent forcing a glass transition and potentially inducing spontaneous recrystallization, chemically react with the solid [8].

Several API's can also exhibit multiple hydrated states and the most hydrated form is the stable form at the lowest temperature and ambient pressure. The hydration state of crystalline substances is of particular concern in the pharmaceutical industry. For instance, some hydrated materials can affect solubility, dissolution rate, flowability, and compressibility. These factors affect the entire chain of the drug development process from pre-formulation to solid form development to packaging and storage without which pharmaceutical scientists cannot develop and understand the material properties, to anticipate and circumvent potential problems [9]. For these reasons, there has been increased regulatory pressure to fully characterize and control the physical form of active drugs [1] in the initial and the formulation stages.

Understanding and controlling the polymorphic form during the stress condition leads to obtain a stable API in the market. First and foremost, predicting any possible polymorphic contamination during stress studies may reduce the time to obtain a stable API. Failure to catch polymorphic contamination results in life-threatening consequences in some cases. Understanding these factors early in the drug development stages of the API indicates the stability of a more rugged process. The present work involves the study of Nilotinib HCl hydrates and their stress studies with respective polymorphic forms.

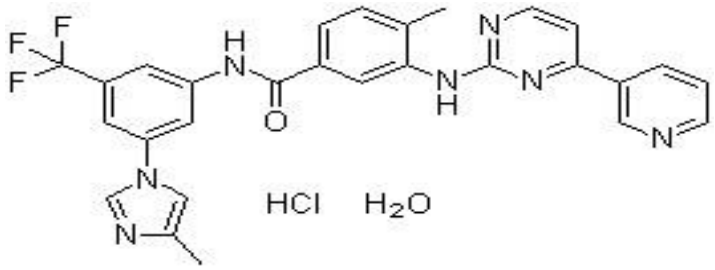


Figure No. 1: Chemical Structure of Nilotinib Hydrate

Nilotinib sold under the brand name Tasigna, is a medication used to treat chronic myelogenous leukemia which has the Philadelphia chromosome. It may be used both in initial cases of chronic phase CML as well as in accelerated and chronic phase CML. Chemically it is 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl)phenyl]-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-benzamide, monohydrochloride. The solubility of Nilotinib hydrochloride hydrate in aqueous solutions at 25°C strongly decreases with increasing pH, and it is practically insoluble in buffer solutions of pH 4.5 and higher pH values [8]. Nilotinib hydrochloride is characterized as a BCS Class IV compound with low/moderate aqueous solubility and low permeability. The Class IV drugs adding a challenge of designing an optimal formulation.

Nilotinib HCl dihydrate shows a unique tendency to form nonstoichiometric hydrates which are variable, but a specific composition, with water content varying continuously from 3.0 to 5.0 %w/w i.e., from Monohydrate to Dihydrate. This paper describes the unusual hygroscopic behavior of Nilotinib hydrates and understanding the polymorphic form during the stress conditions that have been investigated using solid state characterization techniques like Powder X-ray diffraction, Differential Scanning Calorimetry, Dynamic Vapor Sorption, and Microscopic studies.

MATERIALS AND METHODS:

Crystallization procedure for Monohydrate:

Nilotinib base 75 g and 1820 mL of methanol were taken into RB flask. Add 14 g of conc. HCl dissolved in methanol into the reaction mixture. Stir the reaction mass for 30 minutes at 50-55 ° C. Cool the reaction mass to 0-5 °C and filter the product and wash with chilled methanol and get the desired compound 70 g with more than 99.9% purity.

Nilotinib HCl (0.25 g) was taken into the reaction mixture and maintain at 0-5 °C for 3 hrs. Filter under vacuum and wash the cake with 25 mL chilled isopropyl alcohol. Dried the product at 30-35 °C under high vacuum for 1 hour to obtain the Nilotinib HCl Monohydrate.

Crystallization procedure for Dihydrate:

Nilotinib base 25 g, 400 mL of methanol, and 43.7 mL of water into round bottom flask. Add 4 milliliters of conc. HCl dissolved in methanol into the reaction mixture. Stir the reaction mass for 30 minutes at 60-65 °C. Add seed material and cool to 0-5 °C. Stir the suspension at 0-5 °C for 180 minutes. Filter the product and wash it with chilled isopropanol and get the desired compound 22 g with more than 99.9% purity [11].

Nilotinib HCl (0.25 g) was taken into a reaction mixture of Methanol and water in the ratio (1: 15:1.75) and maintain at 0-5 °C for 3 hrs. Filter under vacuum and wash the cake with 0.25 mL chilled isopropyl alcohol. Dried the product at 30-35 °C under high vacuum for 1 hour to obtain the Nilotinib HCl Dihydrate.

X-ray Powder Diffraction (PXRD):

The Powder X-ray Diffractograms were obtained by PANalytical, X'Pert pro diffractometer. A silicon standard was used to check the instrument peak position. The sample was exposed to CuKα1 (1.5406 Å) radiation with a voltage and current of 45 (kV) and 40 (mA) respectively. The X-ray powder diffraction was collected with a scan range from 3.0 to 50.0° 2θ

Differential Scanning Calorimetry (DSC):

The thermal profiles were produced using a Mettler Toledo DSC. Approximately 3.9 mg of the sample was weighed into 40 µL alumina pans. Samples were heated from 30 to 300 °C, with a rate of 10°C/min. An Indium standard was used to check the instrument's performance.

Dynamic Vapor Sorption Analyser (DVS):

The isotherms were produced using SMS DVS analyzer. Typically a sample size of about 40 mg was loaded into the sample pan and the sample was analyzed on DVS automated sorption analyzer at an isothermal temperature of 25°C. The absorption of relative humidity was increased from 0% to 90% RH with an increment of 10% RH. The description of relative humidity was then decreased in the same manner to accomplish a full cycle.

Polarized Light Microscopy (PLM):

The microscopic images were collected using Carl Zeiss/Axio Lab-A1 Polarized Light Microscope at a magnification of 40x.

Karl Fischer titration:

Karl Fisher titrations for water content determination were performed using a Metrohm 835 Titrando titrator (Metrohm Ltd., Herisau, Switzerland). HYDRANAL®-Methanol dry (Sigma-Aldrich Laborchemikalien GmbH) was used as working medium, and HYDRANAL®-Composite (Sigma-Aldrich Laborchemikalien GmbH) was used as a titration agent.

Thermogravimetry and Mass Spectroscopy (TG-MS):

The TG-MS analysis was performed on Q600 TGA mass spec. of TA instruments. The analysis was performed by selecting the water and CO_2 as degrading products formed during heating.

RESULTS AND DISCUSSION:

CHARACTERIZATION OF HYDRATES:

PXRD of Nilotinib Monohydrate and Dihydrate:

Figure-2 shows the overlaid powder X-ray diffraction pattern of Nilotinib HCl Monohydrate, Dihydrate and both the diffraction patterns show characteristic X-ray diffraction and are different from each other. The monohydrate diffractogram shows good crystallinity with sharp well defined and resolved peaks compared to Dihydrate.

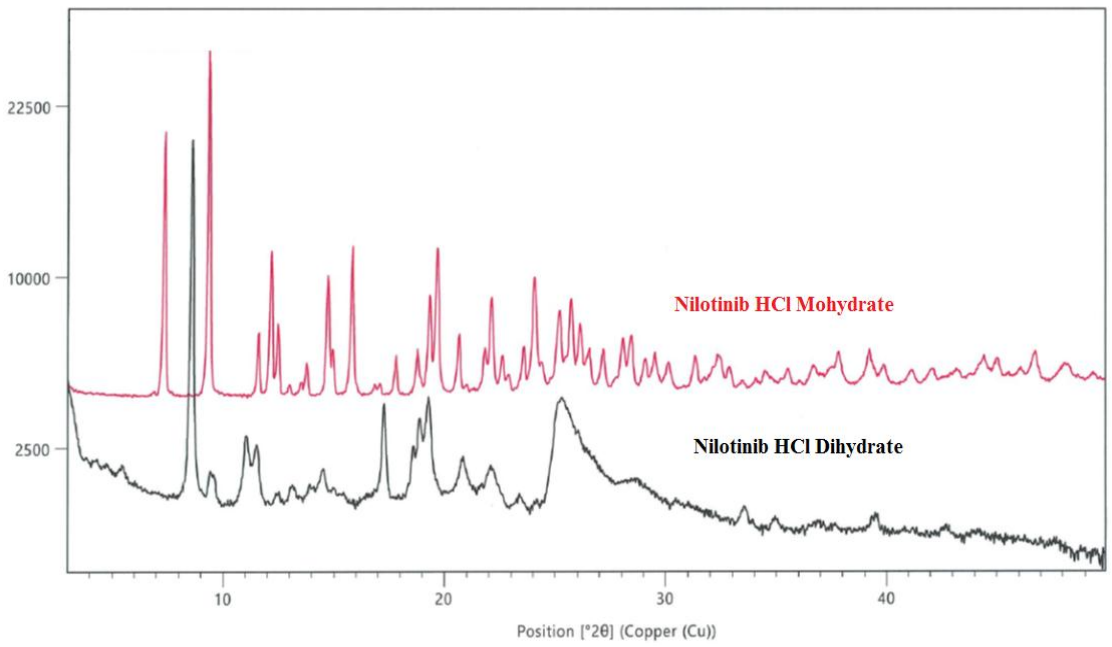


Figure No. 2: PXRD pattern of Nilotinib HCl Monohydrate and dihydrate

DSC and TGA of Nilotinib Monohydrate and Dihydrate:

The Overlaid DSC and TGA thermogram of Nilotinib HCl Monohydrate is depicted in Figure-3. The first small endotherm at about 50 °C corresponds to the loss of surface water about 1 %w/w. The second weight loss at about 140 °C corresponds to dehydration of lattice bound water at about 3.2 %w/w equivalent to 1 mole followed by melt with decomposition endotherm at about 190 °C. The water by KF shows a 3.2 %w/w which is equivalent to the theoretical monohydrate content.

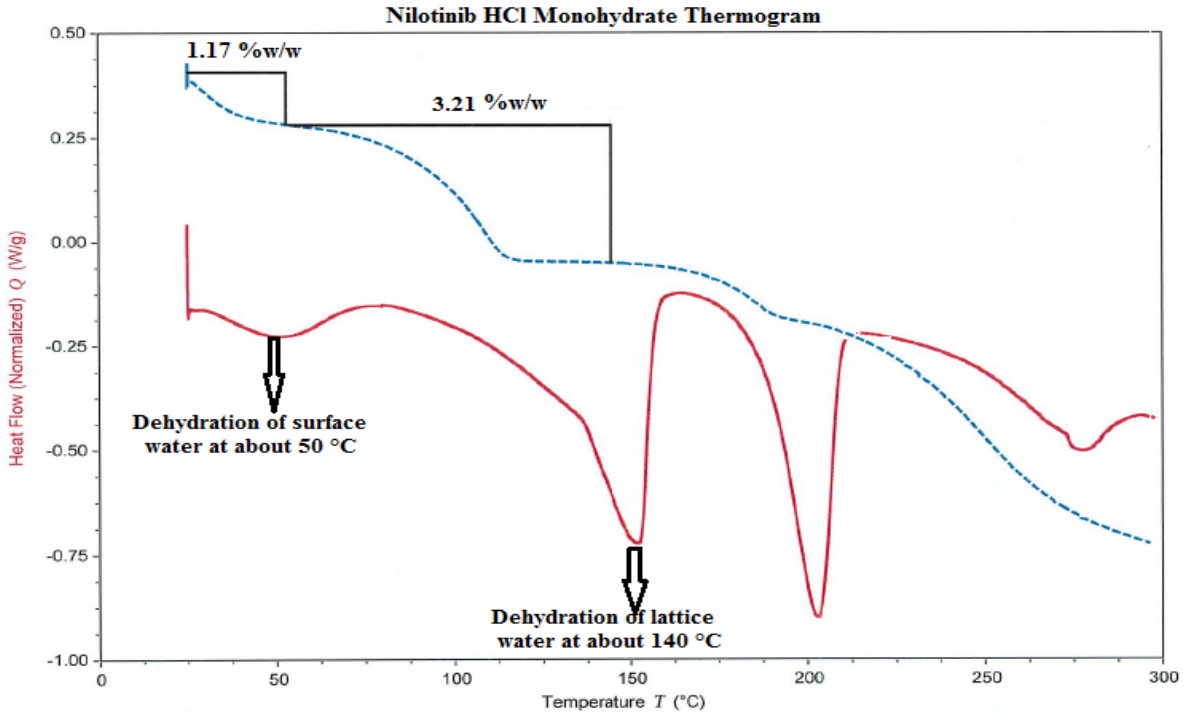


Figure No. 3: Overlaid DSC and TGA thermogram of Nilotinib HCl Monohydrate

The DSC and TGA thermogram of Dihydrate is depicted in Figure-4. The first broad endotherm shows a gradual decrease in the water of about 1.7 %w/w. The second and third endotherm corresponds to dehydration of about 3.3 %w/w followed by melt with decomposition. As per theoretical, the dihydrate shows a weight loss of 5.9 %w/w but the overall weight loss by TGA was 4.96% w/w which corresponds to a variable hydrate. However, dihydrate content will vary from 4 to 6% w/w depending on the environmental conditions like temperature and humidity and the same phenomenon was observed in our studies.

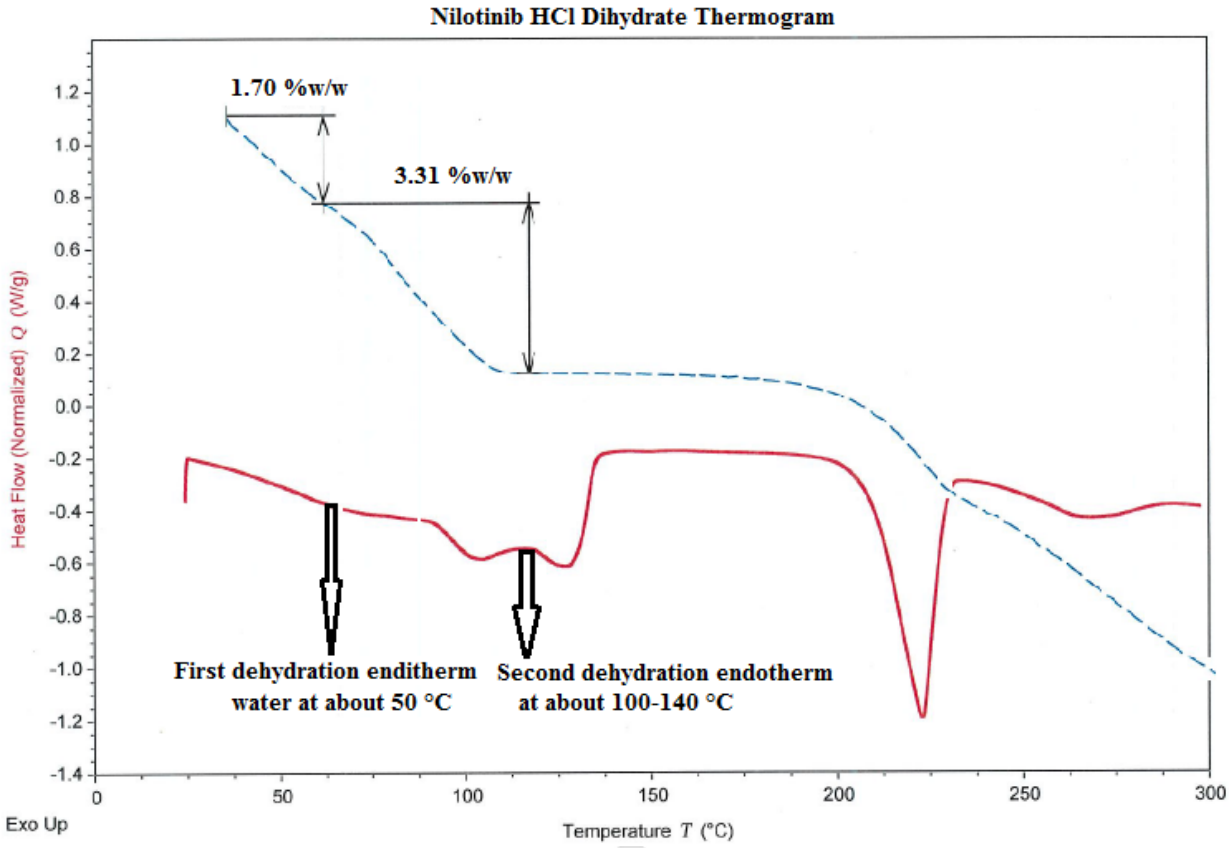


Figure No. 4: Overlaid DSC and TGA thermogram of Nilotinib HCl Dihydrate

Morphology of Nilotinib Monohydrate and Dihydrate:

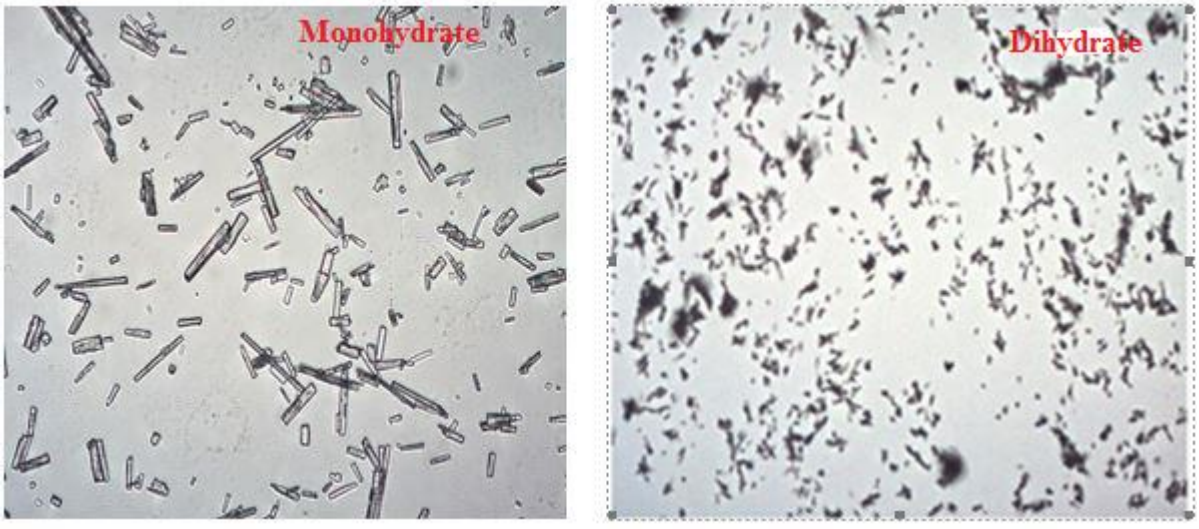


Figure No. 5: Microscopy images of Nilotinib HCl monohydrate and Dihydrate at a magnification of 40x

The microscopy images were collected by wet mount sample preparation at a magnification of 40x. The needle shape morphology of Nilotinib HCl monohydrate results a good crystalline X-ray diffraction pattern. The Dihydrate shows an irregular of fine particles results a relatively poor crystalline X-ray diffraction pattern as shown in Figure-5.

POLYMORPHIC STRESS STUDIES OF APIs:

The stress studies of Nilotinib HCl Monohydrate and Dihydrate were carried to understand the polymorphic phase change due to the impact of temperature, humidity and pressure which helps in deciding the stability and suitability of API in the formulation process.

Pressure Stress Studies of Nilotinib HCl Monohydrate and Dihydrate:

The PXRD data of pressure stress studies of API's at initial and the sample after Hydraulic pressure stress at about 10 tons for 1 hour is shown in Figure-6 and Figure-7. The diffraction pattern after hydraulic pressure stress is matching with initial with a decrease in the peak intensities as well as peak broadening with the halo. The degree of crystallinity of initial and the sample after 10 tons of pressure were calculated from the PXRD data by using the relative constant background method and the results are tabulated below in Table-1. The Nilotinib HCl Monohydrate shows a strong decrease in crystallinity after compression at 10 tons for 1 hour compared to the Dihydrate [12].

Table No. 1: % crystallinity of Monohydrate and Dihydrate by PXRD

Condition	% Crystallinity by PXRD		Remarks
	Monohydrate	Dihydrate	
API Initial	78.70	70.49	% Crystallinity was calculated by using the relative constant background method
API after compression at 10 tons for 1 hour	46.36	50.24	

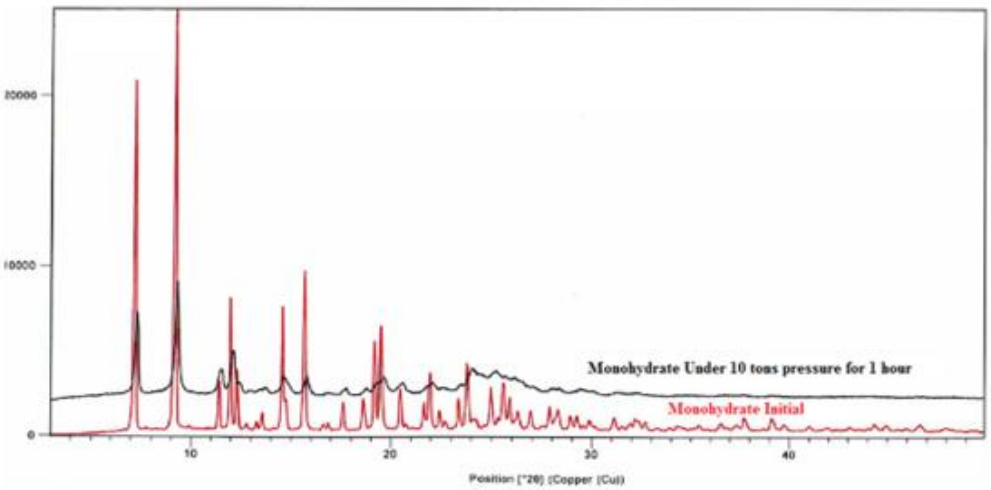


Figure No. 6: Overlaid PXRD pattern of Nilotinib HCl Monohydrate Initial and exposure at 10 tons pressure for 1 hour.

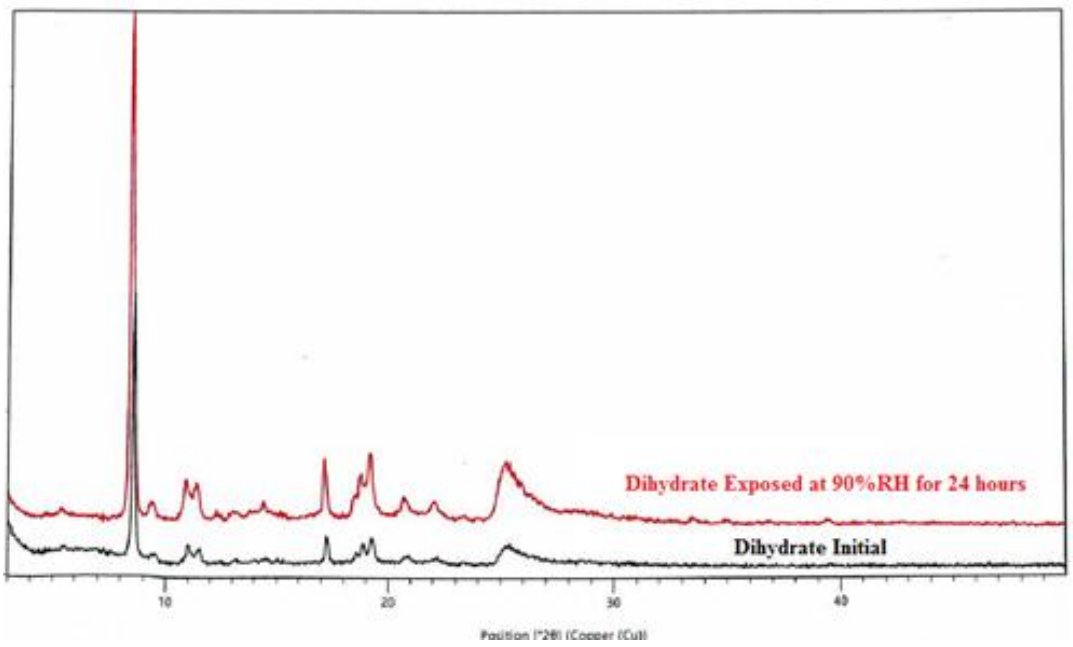
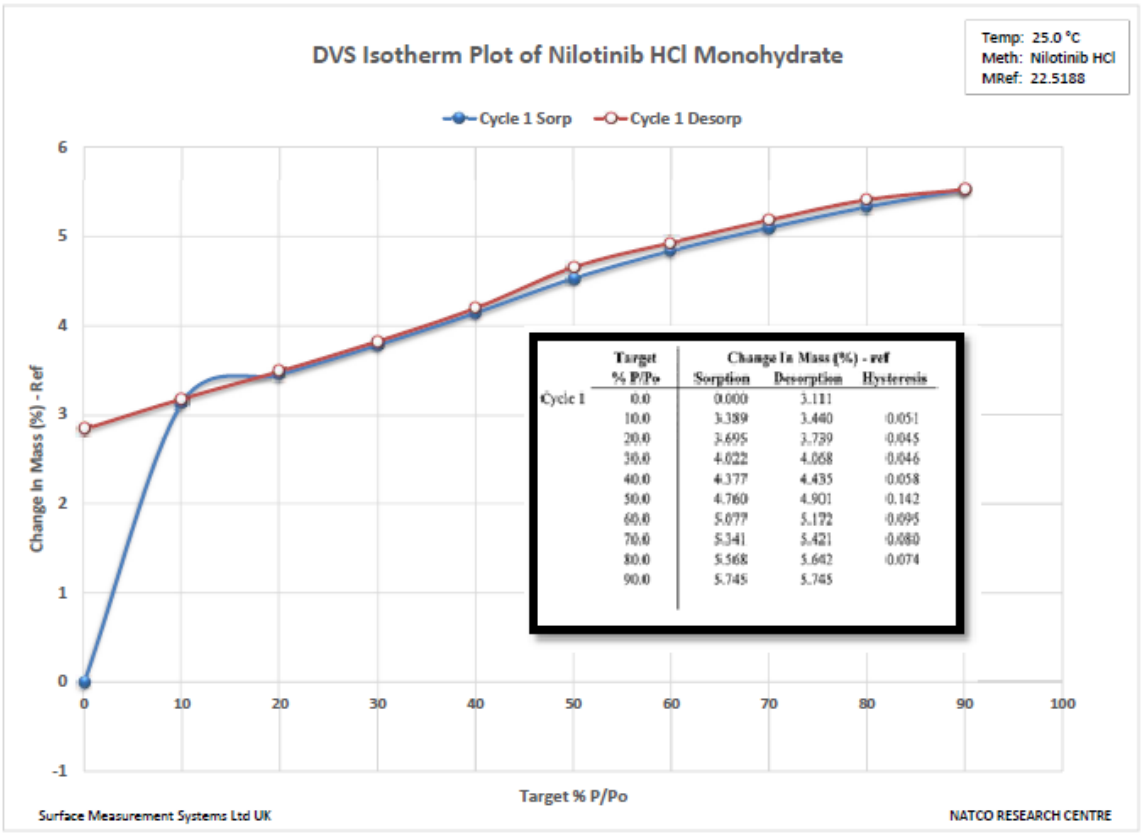


Figure No. 7: Overlaid PXRD pattern of Nilotinib HCl Dihydrate Initial and exposure at 10 tons of pressure for 1 hour.

Hygroscopic Study of Nilotinib HCl Monohydrate and Dihydrate (DVS):

The hygroscopic study by DVS was carried to understand the kinetics of water sorption and desorption behavior of Nilotinib HCl Monohydrate and Dihydrate as shown in Figure-8 and Figure-10. The study was performed by generating the relative humidity of sorption from 0 to 90% RH and desorption from 90 to 0% RH with an increment of 10% RH to accomplish a full

cycle. The sorption, desorption and hysteresis values of Nilotinib HCl Monohydrate and Dihydrate are summarized in Table is shown in the Inset of Figure-8 and Figure-10.



	Target % P/Po	Change In Mass (%) - ref Sorption	Desorption	Hysteresis
Cycle 1	0.0	0.000	3.111	
	10.0	3.389	3.440	0.051
	20.0	3.695	3.739	0.045
	30.0	4.022	4.068	0.046
	40.0	4.377	4.435	0.058
	50.0	4.760	4.901	0.142
	60.0	5.077	5.172	0.095
	70.0	5.341	5.421	0.080
	80.0	5.568	5.642	0.074
	90.0	5.745	5.745	

Figure No. 8: DVS Isotherm plot of Nilotinib Hydrochloride Monohydrate API

The Nilotinib Hydrochloride Monohydrate API shows a step increase in the relative humidity from 0 to 3.38% w/w at 10% RH shows the strong bonding interaction between adsorbate and adsorbent. The relative humidity was gradually increased from 10 to 90% RH with a total weight gain of 5.74% w/w. The desorption isotherm shows negligible hysteresis with retention of 3.11% w/w of water at 0% RH in the crystal lattice. The PXRD pattern of the sample after sorption and desorption shows no significant change in the crystal lattice as shown in Figure-9 [13].

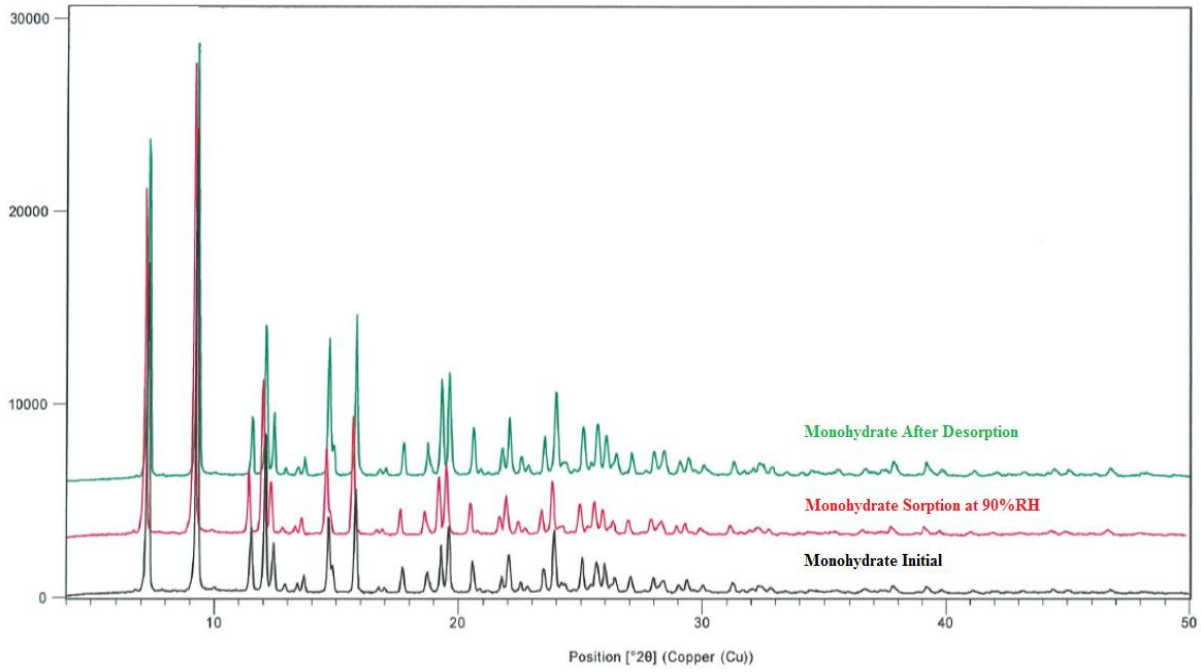
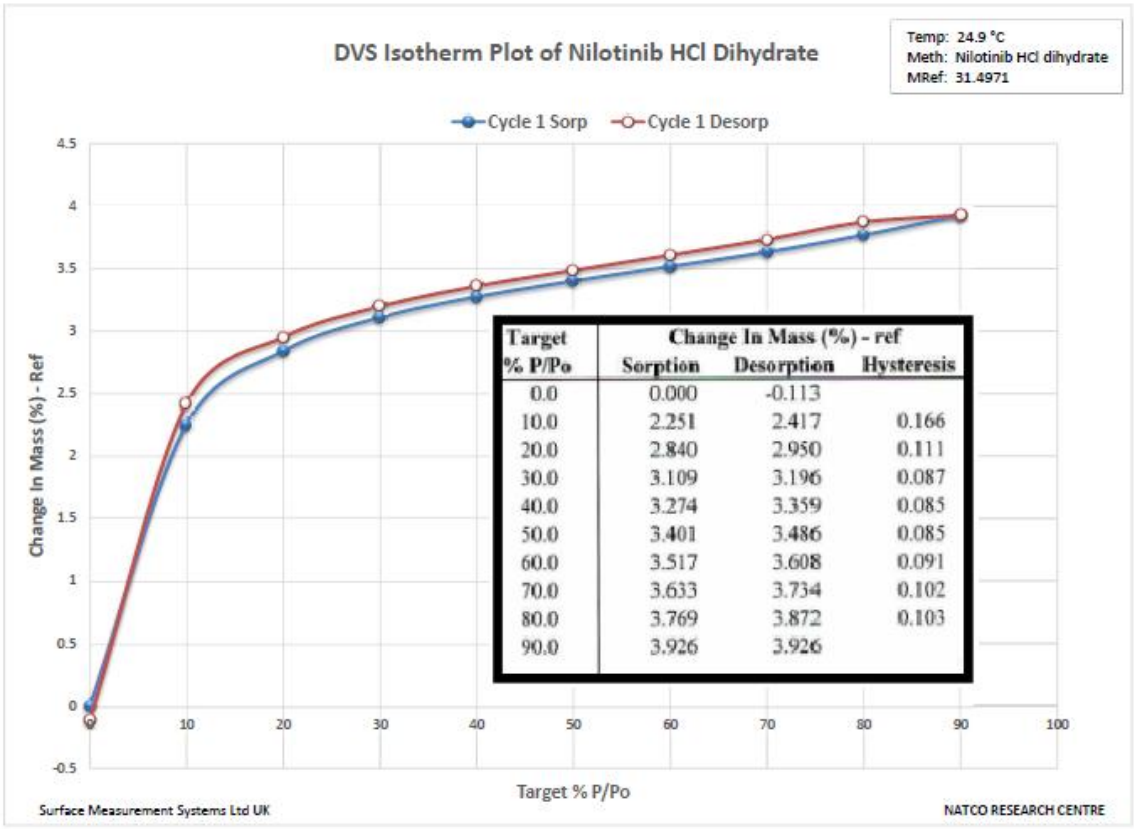


Figure No. 9: Overlaid PXRD pattern of Nilotinib HCl Monohydrate Initial, Sorption at 90% RH and after desorption at 0% RH

The sorption isotherm of dihydrate shows a significant increase in moisture uptake at low partial pressure i.e., at 20% RH indicating the strong water-solid interactions. The substantial mass gain is followed by progressive sorption at intermediate and high relative humidity is 3.926% w/w compared to the theoretical calculation for dihydrate (6.3% w/w). This shows that the water present in the crystal lattice is non-stoichiometric. The sorption and desorption isotherm is reversible with no significant difference in the hysteresis, confirming that the hydrate does not undergo phase change during sorption and desorption analysis which is further confirmed by the PXRD data as shown in Figure-11. The PXRD pattern of the desorption sample is matching with the Initial, indicates the isomorphic dehydrate. The presence of an iso-structural dehydrate is a typical feature of the non-stoichiometric hydrate [12]. The fact that there is no change in water sorption indicates, water is loosely bounded along the lattice channel.



Target % P/Po	Change In Mass (%) - ref Sorption	Desorption	Hysteresis
0.0	0.000	-0.113	
10.0	2.251	2.417	0.166
20.0	2.840	2.950	0.111
30.0	3.109	3.196	0.087
40.0	3.274	3.359	0.085
50.0	3.401	3.486	0.085
60.0	3.517	3.608	0.091
70.0	3.633	3.734	0.102
80.0	3.769	3.872	0.103
90.0	3.926	3.926	

Figure No. 10: DVS Isotherm plot of Nilotinib Hydrochloride Dihydrate API

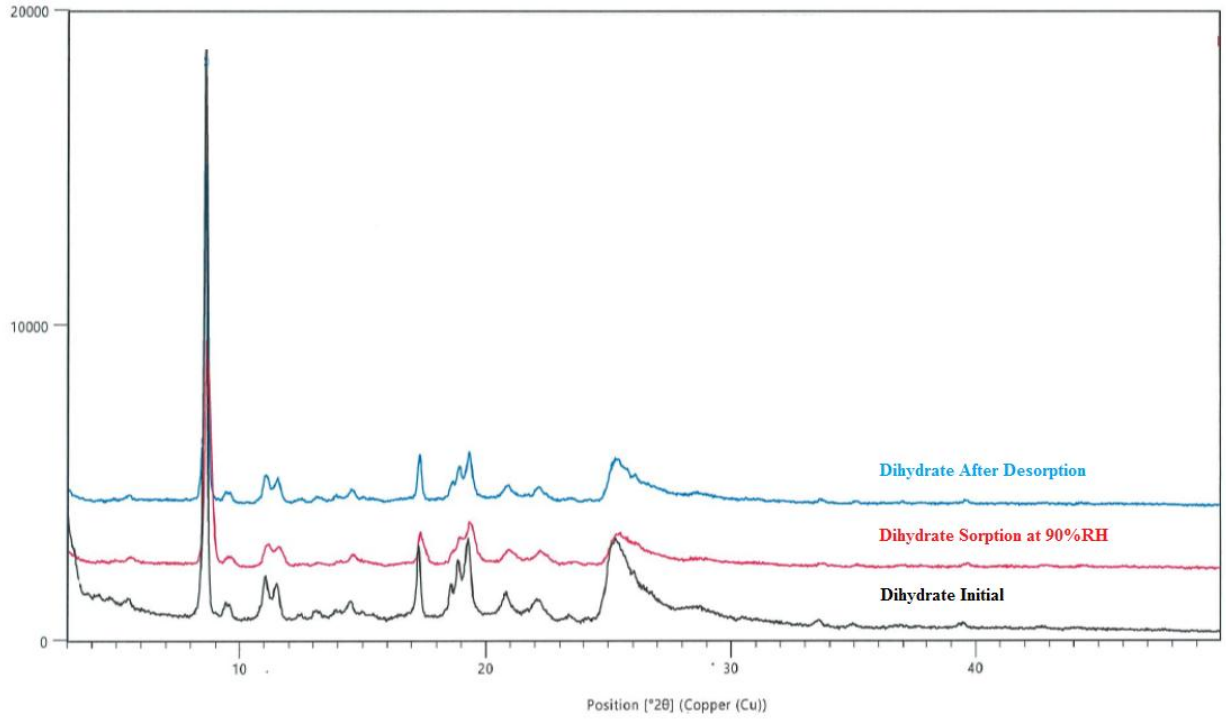


Figure No. 11: Overlaid PXRD pattern of Nilotinib HCl Dihydrate Initial, Sorption at 90% RH and after desorption at 0% RH

Thermal Study of Nilotinib HCl Monohydrate and Dihydrate:

The thermal studies of Nilotinib Hydrochloride hydrates were performed by heating the sample in the TGA instrument at their respective first and second thermal transition temperature of the initial thermogram. The sample was further characterized by PXRD to understand the polymorphic phase changes during thermal analysis.

Monohydrate API heated up to 120 °C and 160 °C:

Figure 12 shows the overlaid diffractogram of the initial, sample heated in a TGA instrument up to 50 °C and 120 °C. The PXRD pattern of the sample heated at 50 °C is matching with the initial represents the loss of surface water. Whereas the sample heated at 120 °C shows a slight shift in the 2θ peak positions towards the right of the initial due to loss of weak lattice bond water.

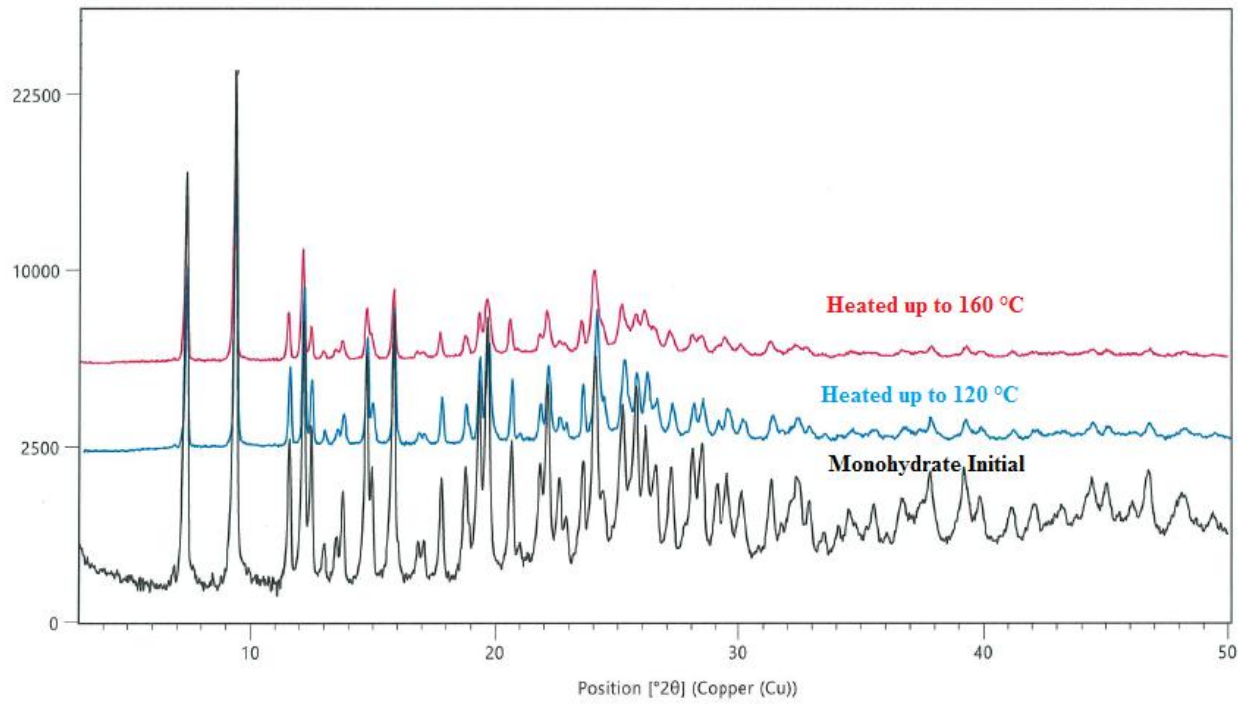


Figure No. 12: Overlaid diffractogram of Nilotinib HCl monohydrate API at initial and sample heated at about 50 °C and 120 °C

Dihydrate API heated up to 50 °C and 120 °C:

Figure-13 shows the overlaid diffractogram of the initial, sample heated in a TGA instrument up to 50 °C and 120 °C and both the diffraction patterns are matching with the initial dihydrate.

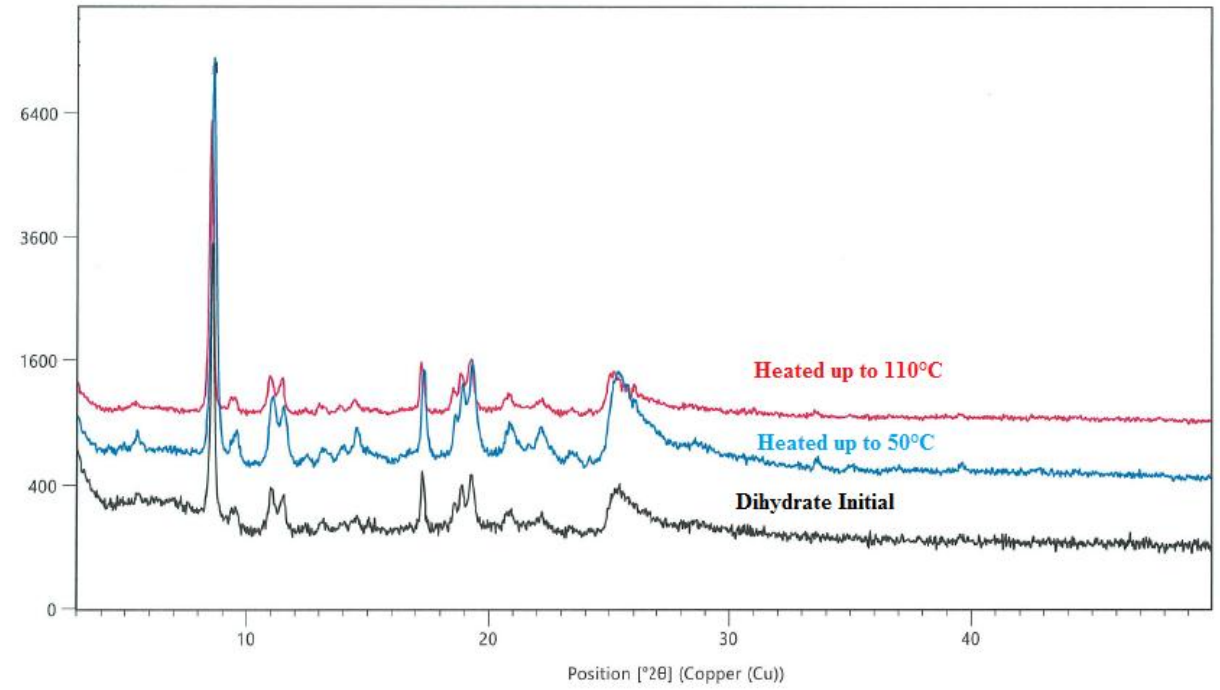


Figure No. 13: Overlaid diffractogram of Nilotinib HCl Dihydrate API at initial and sample heated at about 50 °C and 120 °C

To understand further, the evolved gases with respective weight change was observed in Nilotinib HCl Monohydrate using the TGA-MS analysis as shown in Figure-14 [14].

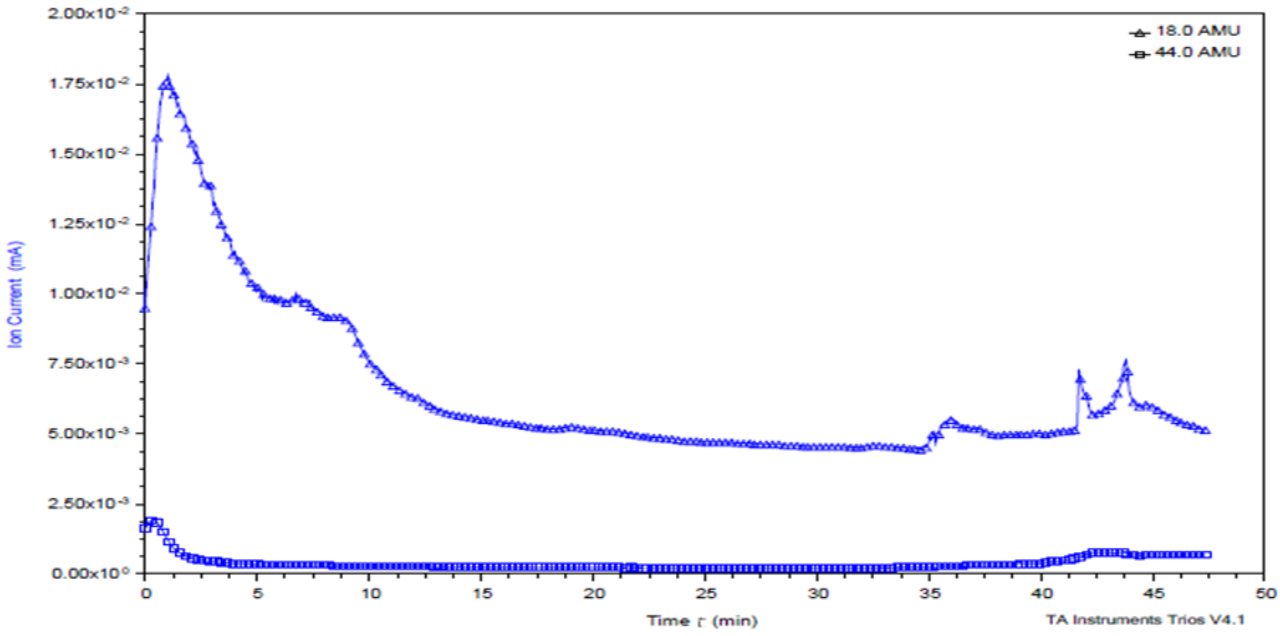


Figure No. 14: TG-MS spectrograph of Nilotinib HCl Monohydrate

The TGA-MS spectrograph of the drug substance shows two curves. The first curve is due to the elimination of evolved gas captured at 18 AMU (Atomic Mass Unit) which attributes to water loss observed at lower temperatures i.e., below 100 °C indicating that water is not firmly bound in the crystal lattice. The second curve shows a weight loss corresponds to 44 AMU (Atomic Mass Unit) due to the liberation of CO_2 followed by the decomposition process of the parent molecule. The results are in good agreement with TGA and DVS data.

CONCLUSION:

The Nilotinib HCl hydrates show different types of water bonding interactions like the surface, Lattice Channel and Lattice bound water. The DVS studies confirmed the different types of water interactions of two hydrates respectively. The Monohydrate has surface water and weak lattice bound water corresponds to 1 mole whereas the dihydrate has loosely bond lattice channel water which shows a variation in water content due to change in temperature, humidity and pressure with retention of the crystal lattice. The polymorphic stress studies show that both the hydrates are stable and are promising and suitable API's for the formulation studies.

REFERENCES:

1) S. R. Byrn, R.R. Pfeiffer, J.G. Stowell, Solid-state chemistry of Drugs, SSCI, West Lafayette 1999
2) H.P. Stahl, D.D. Braimar (Ed.), Towards better safety of Drugs and Pharmaceutical Products, Biomedical Press, Elsevier North Holland, 1980; 265-280
3) T.L. Threlfall, Analysis of Organic polymorphs A Review, Analyst, October 1995; 120: 2435
4) Bernstein, Polymorphism in molecular crystals, 2002
5) D. Giron, Application of thermal analysis and coupled techniques in pharmaceutical industry, J. of Thermal analysis and Calorimetry 2002; 68: 335-357.
6) Rajendra Khankari, Linna Chen, David J.W. Grant, J. Pham. Sci, 1998; 9(87): 1052-1061,
7) A. R. Kennedy, M. O. Okoth, D. B. Sheen, J. N. Sherwood, S. J. Teat and R. M. Vrcelj, Acta Cryst. 2003; C**59**, o650-o652
8) Pienaar, E.W, Caira M. R and lotter A.P, J. Cryst. Spectr Res 1993; 23(9): 739-744
9) K. R. Morris, Structural aspects of hydrates and solvates, Marcel Dekkar, Inc, 95: 126-179
10) EMA Scientific discussion of Nilotinib
11) Amala Kompella, et al., US 2015/0183762A1
12) Characterization of crystalline and partially crystalline solids by PXRD, USP general chapters, <941>
13) Tieger Eszter et al., CrystEngComm. 2016; 18: 3819
14) M. Schubnell, Thermogravimetry and gas analysis, Part 1: Basic principles and overview, UserCom 45, 1-9.

	***Author Name – Corresponding Author*** ***Dr. Vangala Ranga Reddy*** *ARD-DGM* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*
	***Cheedi Srinivas Rao*** *ARD- eputy Manager* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*
	***R. Srinivas*** *CRD-Senior Manager* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*
	***Dr. K. Durga Prasad*** *Sr. Vice President-CRD* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*
	***Dr. Gopal Vaidyanathan*** *Sr. Vice President-ARD & DQA* *NATCO Research Centre, B-11, 13 &14* *Industrial Estate, Sanathnagar* *Hyderabad – 500 018, Telangana* *INDIA*