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METHOD DEVELOPMENT AND VALIDATION OF ANTICANCER DRUGS (NILOTINIB AND GEMCITABINE) BY RP-HPLC METHOD

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ABSTRACT

A simple, fast, accurate and precise UV-spectroscopic method and RP-HPLC method were developed and validated for the estimation of Nilotinib/Gemcitabine per ICH guidelines. Acetonitrile and water (50:50) was used as the solvent. The λ max of Nilotinib/Gemcitabine was found to be 242 nm and it was proved linear in the concentration range of Nilotinib 0.5-3µg/ml and for Gemcitabine 1-6µg/ml with a correlation coefficient value of 0.999. Accuracy studies of UV-spectroscopy method was performed at three different levels, i.e., 50%, 100%, and 150% and recovery was found to be in the range of 99.6 to 100.8% for Nilotinib and the range of 98.3 to 101.2% for Gemcitabine respectively. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 0.217 and 0.658 µg/ml for Nilotinib and 0.103 and 0.312 f or Gemcitabine RP-HPLC method was developed by using Acetonitrile: water, 0.1% ortho phosphoric acid (50:50). The method was developed in Eclipse C18 column (100 mm × 4.6 mm, 3.5µm particle size). In RP-HPLC method was found to be linear in the range of Nilotinib/Gemcitabine is 0.25-1.5µg/ml with a correlation coefficient value of 0.999. The accuracy studies of RP-HPL C method was performed at three different levels, i.e., 50%, 100%, and 150% and recovery was found to be in the range of 98.24 to 100.3% for Nilotinib and the range of 98.18 to 99.98% and for Gemcitabine respectively. The limit of detection (LOD) and Li mit of Quantification (LOQ) were found to be 0.0421 and 0.1276 µg/ml for Nilotinib and 0.047.and 0.1424 µg/ml for Capecit abine for RP-HPLC method. The % RSD is <2% which indicates the accuracy and precision of the method. The abo ve method was a rapid tool for routine analysis of Nilotinib/Gemcitabine in the bulk and in the pharmaceutical dosage form.

KEYWORDS: Anticancer, Nilotinib, Gemcitabine, Acetonitrile, UV, RP-HPLC and ICH.

INTRODUCTON

Nilotinib is a cancer medication prescribed to treat leukemia and gastrointestinal tumors. It operates by inhibiting proteins associated with cancer cell growth in order to relieve symptoms, prevent the spread of cancer cells, and aid other treatments. Nilotinib is one of the newest anticancer drugs in the market and was one of the first drugs to be pushed through Food and Drug Administration's (FDA) fast track designation for approval. The drug is designed to inhibit tyrosine kinases such as Bcr-Abland is used in the treatment of Chronic Myeloid Leukemia (CML) and gastrointestinal stroma tumors. Chemically Gemcitabine is designated as pentyl[1-(3,4-dihydroxy-5-methyl tetrahydrofuran-2-yl)-5-fluoro-2-oxo-1H-pyrimidin-4-yl]aminomethanoate1, it is used as antineoplastic agent for the treatment of breast and colorectal cancer. Literature survey reveals a few LC-MS methods reported for the determination of Gemcitabine and its metabolites in biological fluids. and a single HPLC method in tablet formulation7-8. Keeping this point into consideration, an attempt was made to develop a simple, accurate and validated stability indicating RP-HPLC method for the estimation of Nilotinib and Gemcitabine in pure and tablet form. The proposed Method was validated as per ICH guidelines Q2A.

MATERIALS AND METHODS
Chemicals and Reagents

Analytically pure sample of Nilotinib (TASIGNA) with purity greater than 99% manufactured by Cipla Limited was used. Acetonitrile (HPLC grade) was obtained from Merck water (HPLC grade), potassium dihydrogen (AR grade), acetic acid (AR Grade) were obtained from SD Fine chemicals (Hyderabad, India).

Instrumentation

Double beam UV-Visible Spectrophotometer (Shimadzu-1800) connected to a computer loaded with Shimadzu UV Probe 2.33 software was used for all the spectrophotometric measurements in all proposed spectrophotometric methods.

Selection of wavelength of Nilotinib/Gemcitabine 10µg/ml of Nilotinib/Capcitabine were prepared in acetonitrile:water (50:50v/v) solvent. The resulting solutions were scanned from 190 to 400 nm in UV-Visible spectrophotometer. The optimal response for two of them was obtained at 242 nm. Hence the complete method was processed at the wavelength of 242 nm.

Preparation of mobile phase of Nilotinib/Gemcitabine
Take HPLC water 500 ml (50%) and 500ml Acetonitrile (50%) HPLC into a 1000 ml volumetric flask to this add (0.1%v/v) of ortho phosphoric acid was mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Selection of flow rate of Nilotinib/Gemcitabine As increase in the flow rate results in decrease in the retention time. Hence sufficient flow rate of 0.8 mLmin-1 was chosen to avoid overlap between the peaks and the loss of its acceptable resolution values. Preparation of Standard Stock Solution of **Nilotinib/Gemcitabine.**

Standard stock solution of Nilotinib/Gemcitabine (1 mg/ml) was prepared by transferring into a 10 ml volumetric flask containing 4mL of acetonitrile:water (50:50v/v)diluent. It was then sonicated for 15 minutes and solution was diluted up to the volume by acetonitrile: water (50:50v/v). From these, further dilutions were made using acetonitrile: water.

Preparation of sample solution of Nilotinib/Gemcitabine
20 Tablets of contents were weighed and the average weight was determined. They were crushed in to fine powder with glass mortar. The quantity of powder equivalent to 10 mg of active ingredient present in Nilotinib/ powder equivalent to 50mg of Gemcitabine was transferred into a 10 ml clean dry volumetric flask, 7 ml of diluents acetonitrile:water (50:50v/v) was added to it and was shaken by mechanical stirrer and sonicated for about 10 minutes by shaking at intervals of five minutes and diluted up to the mark with diluent to give a concentration of 1000 µg/ml for Nilotinib and 5000µg/ml for Gemcitabine The solution was filtered through the whatman filter paper. The filtrate contains 10µg/ml of Nilotinib/Gemcitabine to give the respective concentrations as par with standard solution.

System Suitability studies of Nilotinib/Gemcitabine According to the USP, system suitability tests are an integral part of chromatographic methods. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute anintegral system that can be evaluated as a whole. The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, columns, analysts) is suitable for the intended application.

System Suitability Test Parameters are
Plate number or number of theoretical plates (n)
Capacity factor (capacity ratio) k
The selectivity or Separation Factor (relative retention) α.
Peak Resolution rS
Peak asymmetry factor (As).
These are measured on a peak or peaks of known retention time and peak width.

Linearity and range of Nilotinib/Gemcitabine
The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in the sample within the range. The range of the analytical method is the interval between the upper and lower levels that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Procedure for Nilotinib/Gemcitabine
Each level solution was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted and the correlation coefficient was calculated. The linearity of the method was demonstrated over the concentration range of Nilotinib/Gemcitabine 0.25-1.5µg / ml. Aliquots of six levels were prepared from sample solution. The solutions were injected in to HPLC system as per test procedure.

Preparation of sample stock solution of Nilotinib/Gemcitabine About 10mg of Nilotinib/Gemcitabine samples was weighed in to volumetric flask, it was dissolved with diluents acetonitrile:water (50:50v/v) and the volume was made up to the mark with same diluent (10µg/ml of Nilotinib/Gemcitabine).

Precision studies of Nilotinib/Gemcitabine
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 homogeneous samples. It provides an indication of random error results and is expressed as relative standard deviation (% RSD).
Precision of the method was demonstrated by
• Intraday precision
• Inter day precision
• Repeatability

Repeatability (Method precision) of Nilotinib/Gemcitabine
Repeatability expresses the analytical variability under the same operating conditions over a short interval of time. The repeatability studies were carried out by taking 2µg/ml for Nilotinib and 4µg/ml for Gemcitabine as 100% test concentration and repeating it for six times.

Intermediate precision
Intraday precision and inter day precision of Nilotinib/Gemcitabine
Variation of results within the same day (intra-day) and variation of results between days (inter-day) were analysed. Intra-day precision was determined by analyzing single concentration of Nilotinib/Gemcitabine for 6 times on the same day at 242nm. Inter-day precision was determined by analyzing single concentrations of Nilotinib/Gemcitabine on the preceding day at 242nm and % RSD was calculated.

Accuracy studies of Nilotinib/Gemcitabine Accuracy is the closeness of the results obtained by the method to the true value. Recovery studies were carried out at 50%, 100% and 150% by adding known amount of standard drug solution of Nilotinib/Gemcitabine i.e. (0.75, 1, 1.25 µg/ml) to the sample solution whose concentration is maintained constant for Nilotinib/Gemcitabine i.e. 0.5µg/ml. The %recovery was calculated and reported.

Robustness of Nilotinib/Gemcitabine
Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is carried out by changing the flow rate of mobile phase from 0.8 to 1.2mL min-changing the detection wavelength from 240 to 242nm.

Specificity of Nilotinib/Gemcitabine
The term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. The analyte was assessed in the presence of components and it was found that there was no interaction with the analyte.

System suitability testing (SST) of Nilotinib/Gemcitabine
System suitability tests are an integral part of chromatographic method. They were used to verify that the reproducibility of the chromatographic system is adequate for the analysis. It is defined as tests to measure that the method can generate result of acceptable accuracy and precision.

Limits of detection and limits of quantification of Nilotinib/Gemcitabine
The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation.

Procedure
The Limit of Quantification (LOQ) and Limit of Detection (LOD) were based on the residual standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1).

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where,
σ = the residual standard deviation of the response and S = slope of the calibration curve

The flow rate was varied for Nilotinib/Gemcitabine at 0.9 mLmin-1 to 0.7 mLmin-1.
Standard solution of Nilotinib/Gemcitabine was analysed at 0.9 and 0.7mL min-1 i.e. at ± 0.8unit of optimized flow rate (1mL/min).The detection wavelength was varied at 240 to 244nm. Standard solution of Nilotinib/Gemcitabine was analysed at 240 and 244nm i.e. at ± 1unit of optimized wavelength (242nm).

Selectivity studies of Piloting/Gemcitabine
The solutions were prepared and analysed with change in the analytical conditions like different instrument and different analyst. Standard concentrations of Nilotinib/Gemcitabine 1µg/ml were carried used to carry out the ruggedness studies.

Assay studies of Nilotinib/Gemcitabine
Assay studies were carried out by weighing twenty tablets of Nilotinib/Gemcitabine formulation and powdered. The powder equivalent to 10mg was taken and the solution equivalent to 1000µg/ml was prepared and was used for further dilutions.

RESULTS AND DISCUSSION
Selection of mobile phase and flow rate of Nilotinib/Gemcitabine Initially various mobile phase compositions were tried, to separate title ingredients. Mobile phase composition and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor) and run time. The system with acetonitrile:water 0.1% ortho phosphoric acid(50:50v/v) of 0.8ml/min flow rate was found to be quite robust. The optimum wavelength for detection was 242nm at which better detector response for Nilotinib/Gemcitabine was obtained. The retention time was found to be 1.0min for Nilotinib and 1.6min for Gemcitabine and the total runtime for this method along with the elution of and indicates that the developed method is quite fast and economical. Calibration curve for Nilotinib/Gemcitabine Appropriate aliquots from standard Nilotinib/Gemcitabine stock solutions were transferred into different volumetric flasks of 10ml capacity and the volume is adjusted to the mark to obtain concentrations of 0.25, 0.5, 0.75, 1, 1.25, 1.5 µg/ml of Nilotinib/Gemcitabine

Linearity of Nilotinib/Gemcitabine
The linearity was found in the concentration range of 0.25-1.5 µg/ml for the developed HPLC method and the results are shown in Table No.1 and 2.

Precision studies of Nilotinib/Gemcitabine Repeatability studies were carried out by taking 100 % test concentration and repeating it three times. Interday and intraday precision were done by taking 100% testconcentration and repeating it three times and values

for both system precision and method precision in terms of % RSD were found to be <2.0% .

The results show that the % RSD value for repeatability, intraday and intraday is 0.43, 0.56, 0.55 for Nilotinib the results shown in Table No.3 and 0.49,0.40,0.21 for Gemcitabine the results shown in Table No.4 which indicate that they meet the acceptance criteria and hence the method is said to be precise.

System suitability studies of Nilotinib/Gemcitabine System suitability tests are an integral part of chromatographic method. They were used to verify that the reproducibility of the chromatographic system is adequate for the analysis. It is defined as tests to measure that the method can generate result of acceptable accuracy and precision. For Nilotinib the results have shown in Table No.9 and for Gemcitabine the result shown in Table No.10.

Procedure
System suitability standard solution which contained 10μg/ml of Nilotinib/Gemcitabine was prepared by appropriately diluting and mixing the corresponding stock standard solutions. System suitability was determined from six replicate injections of the system suitability standard before sample analysis. Parameters such as a number of theoretical plates (N), tailing factor and retention time, resolution were calculated. Specificity studies of Nilotinib/Gemcitabine The term specific generally refers to a method that produces a response for a single analyte only while the term selectivity refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. The analyte was assessed in the presence of components and it was found that there was no interaction with the analyte.

Robustness studies of Nilotinib/Gemcitabine Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is carried out by changing the flow rate of mobile phase from 0.8 to 1.2 mL min-1 changing the detection wavelength from 240 to 242nm.for Nilotinib. The results shown in Table Nos.11, 12, 13 and14. Accuracy studies of Nilotinib/Gemcitabine Accuracy is the closeness of the results obtained by the method to the true value. Recovery studies were carried out at 50%, 100% and 150% by adding known amount of standard drug solution of Nilotinib / Gemcitabine i.e. (0.75, 1, 1.25Âμg/ml), to the sample solution whose concentration is maintained constant for Nilotinib/Gemcitabine i.e. 0.5Âμg/ml. The %recovery was calculated. The results for accuracy indicate that the % recovery values are in the range of 98.24-100.3 for Nilotinib. The results shown in table no.15 and 98.18- 99.98 for Gemcitabine The results shown in table no.16: which indicate that the method is accurate as it meets the necessary criteria. Application to pharmaceutical dosage form of Nilotinib/Gemcitabine.

Assay studies were carried out by taking twenty tablets of Nilotinib/Gemcitabine formulation which were weighed and powdered. The powder equivalent to 10mg was taken and the solution equivalent to 1000Âμg/ml and was used for further estimation and the results are shown in Table No.19. The results show that the % purity was found to be 99.55% for Nilotinib. The results shown in table and 99.33%for Gemcitabine The results shown in Table No.20 hence meets the necessary criteria. Limit of detection (LOD) and Limit of quantification (LOQ) of Nilotinib/Gemcitabine The LOD and LOQ were calculated by using the slope and SD of response (intercept). The mean slope value and the SD of response were obtained from the calibration curve and LOD and LOQ were shown in Table No.20

Table No. 1: Linearity results for Nilotinib at 242nm.

S.No	Linearity Level	Concentration(μg/ml)	Peak Area
1	I	0.25	1711603
2	II	0.5	3243280
3	III	0.75	4773302
4	IV	1.00	6144426
5	V	1.25	7552279
6	VI	1.5	8916832
Correlation Coefficient			0.999

Table No. 2: Linearity results for Gemcitabine.

S.No	Linearity Level	Concentration(μg/ml)	Area
1	I	0.25	1367088
2	II	0.50	2641638
3	III	0.75	3829585
4	IV	1.0	5148424
5	V	1.25	6387895
6	VI	1.5	7830709
Correlation Coefficient			0.999

Table No. 3: Repeatability Results for of Nilotinib.

S.No	Conc.(µg/ml)	Absorbance (242nm)	Area mean± S.D(n=6)	% RSD
1	1	6249423		
2	1	6249423		
3	1	6249423	61984942± 6810.4	0.43
4	1	6249423		
5	1	6249423		

Table No.4: Repeatability Results for Gemcitabine.

S.No	Concentration(µg/ml)	Absorbance (242nm)	Area mean± S.D (n=6)	% RSD
1	1	5147832		
2	1	5147832		
3	1	5147832	5169153±25816.7	0.49
4	1	5147832		
5	1	5147832		
6	1	5147832		

Table No.5: Intermediate Precision Intraday Results for Nilotinib.

Concentration (µg/ml)	Injection No	Peak Area	R_t
	1	6243294	1.077
	2	6243294	1.065
	3	6243294	1.074
1	Mean	6209408	
	SD	35056.4	
	% RSD	0.56	

Table No.6: Intermediate Precision Intraday Results for Gemcitabine.

Concentration (µg/ml)	Injection No	Peak Area	R_t
	1	5144782	1.775
	2	5144782	1.610
	3	5144782	1.810
4	Mean	5151041	
	SD	20905.4	
	% RSD	0.40	

Table No. 7: Interday Results for Nilotinib.

Concentration (µg/ml)	Injection No	Peak Area	R_t
	1	6177323	1.077
	2	6177323	1.007
	3	6177323	1.074
1	Mean	6206826	
	SD	34475.2	
	% RSD	0.55	

Table No. 8: Interday Results for Gemcitabine.

Concentration (µg/ml)	Injection No	Peak Area	R_t
	1	5133783	1.775
	2	5133783	1.677
	3	5133783	1.810
1	Mean	5146409	
	SD	11313.1	
	% RSD	0.21	

Table No.9: System Suitability Values for Nilotinib.

S.No	R_t	Peak Area	USP Plate count	Asymmetric factor
1	1.007	6241234	2645	1.1
2	1.007	3210865	2754	1.5
3	1.007	6198632	2663	1.6

Tailing factor Obtained from the standard injection is 1.7 Theoretical Plates Obtained from the standard injection is 2496

Table No.10: System Suitability Values for Gemcitabine.

S.No	R$_t$	Peak Area	USP Plate count	Asymmetric factor
1	1.677	5134732	4012	1.5
2	1.677	5118491	4025	1.2
3	1.677	5089132	4137	1.5

Tailing factor Obtained from the standard injection is 1.51 Theoretical Plates Obtained from the standard injection is 4137

Table No.11: Robustness Results for Nilotinib (flow rate).

S.No	Flow Rate	R$_t$	Area	Height	Plate count	Tailing
1	Less Flow	1.401	421480	45332	2741.1	1.71
2	More Flow	1.007	343858	43270	2543.2	1.58

Table No.12: Robustness Results for Nilotinib (Wavelength).

S.No	Wavelength	R$_t$	Area	Height	Plate count	Tailing
1	244 nm	1.007	6597945	39645	2980.4	1.60
2	240 nm	1.010	6597790	48101	2423.5	1.64

Table No.13: Robustness Results for Gemcitabine. (flow rate)

Flow Rate	R$_t$	Area	Height	Plate count	Tailing	Resolution
Less Flow	2.253	2558248	234950	4162	1.57	4.53
More flow	1.680	2084296	225397	3921.4	1.48	4.43

Table No.15: Values for Accuracy of Nilotinib.

S.No	Spiked Level (%)	Formulation Conc. (µg/ml)	Pure Drug Conc. (µg/ml)	Amount found	% Recovery	% Mean recovery ±SD	% RSD
1	50	0.5	0.75	0.74876	99.83	99.08±0.797	0.8043
		0.5	0.75	0.74386	99.18		
		0.5	0.75	0.73686	98.24		
2	100	0.5	1	0.99473	99.47	99.470±0.567	0.5701
		0.5	1	1.0003	100.03		
		0.5	1	0.98014	98.90		
3	150	0.5	1.25	1.24677	99.74	99.597±0.267	0.2684
		0.5	1.25	1.24701	99.76		
		0.5	1.25	1.24110	99.28		

Table No.14: Robustness Results for Capcitabine (Wavelength).

S.No	Wavelength	R$_t$	Area	Height	Plate count	Tailing
1	244 nm	1.693	4026730	211957	4457.1	1.44
2	240 nm	1.693	4270708	245935	3712.3	1.56

Table No.16: Chromatogram Values for Accuracy of Capcitabine.

S.No	Spiked level (%)	Formulation Conc. (µg/ml)	Pure Drug Conc (µg/ml)	Amount found	% Recovery	% Mean recovery ± SD	% RSD
1	50	0.5	0.75	0.7465	99.53	99.48±0.2759	0.2773
		0.5	0.75	0.7480	99.73		
		0.5	0.75	0.7439	98.18		
2	100	0.5	1	0.9950	99.50	99.653±0.134	0.135
		0.5	1	0.9976	99.76		
		0.5	1	0.9969	99.69		
3	150	0.5	1.25	1.2372	98.98	98.897±0.244	0.247
		0.5	1.25	1.2386	99.98		
		0.5	1.25	1.232	98.62		

Table No.17: Assay Results for Nilotinib.

S.No	Concentration (µg/ml)	Formulation	Label claim	Amount found	% Purity	% RSD
1	1µ g/ml	Tasigna (100mg)	100mg	0.0016	99.55%	0.3522

Table No.18: Assay Results for Gemcitabine.

S.No	Concentration (µg/ml)	Formulation	Label claim	mount found	% Purity	an peak ± S.D	% RSD
1	1(µ g/ml)	Gemzar 500mg	500mg	0.00018	99.33	51411823112.34	0.4519

Table No.19: LOQ, LOD Results for Nilotinib/Capecitbine at 242nm.

S.No	Parameter	Nilotinib	Gemcitabine
1	LOD	0.0421	0.047
2	LOQ	0.1276	0.1424

Table No.20: Summary of HPLC Validation Parameters for Nilotinib/Gemcitabine

S.No	Parameter	Nilotinib	Gemcitabine
1	λ max (nm)	242nm	242nm
2	Linearity range µ g/ml	0.25 to 1.5µ g/ml	0.25-1.5µg/ml
3	Correlation coefficient (r^2)	0.999	0.999
4	Regression equation (y= mx +c)	y=6E+06x+3576	y=5E+06x+46652
5	Slope (m)	6E+06x	5E+06x
6	Intercept(c)	3576	46652
7	Accuracy	98.24 – 100.3	98.18-99.98
8	Precision (% RSD)	0.43	0.49
9	LOD	0.0421	0.047
10	LOQ	0.1276	0.1424
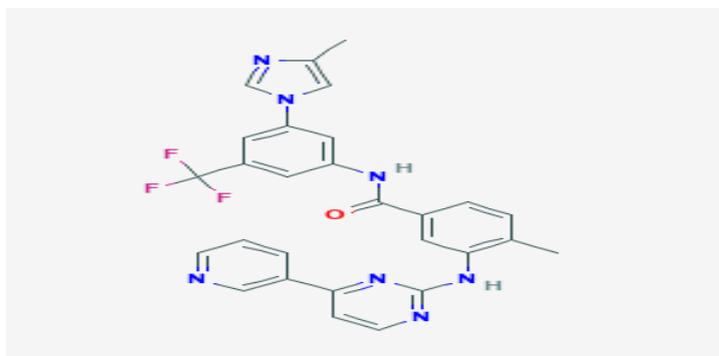
Fig.1: Chemical structure of Nilotinib.
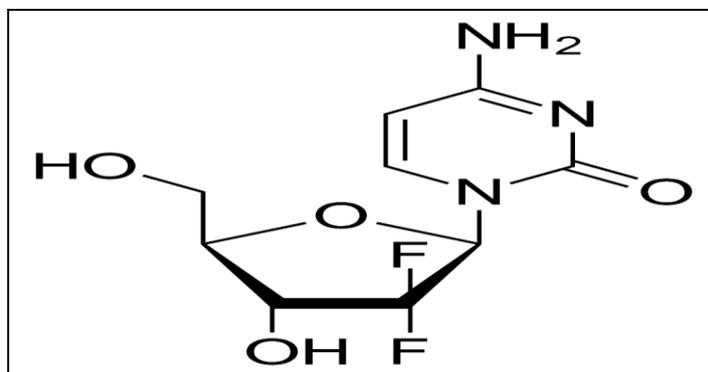
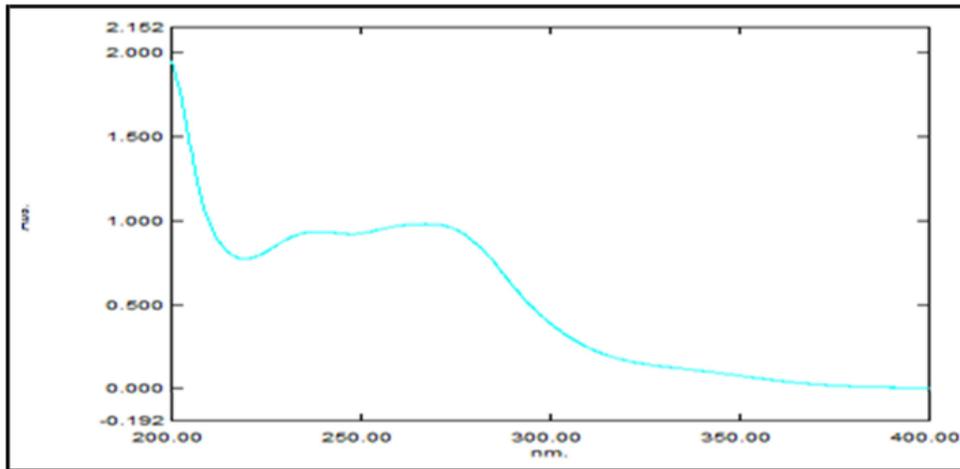**Fig.2: Chemical structure of Gemcitabine.**
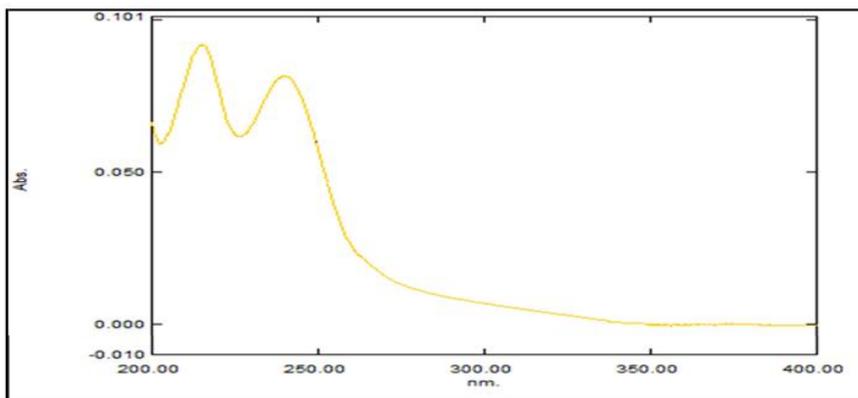
Fig.3: Absorption spectrum of Nilotinib.

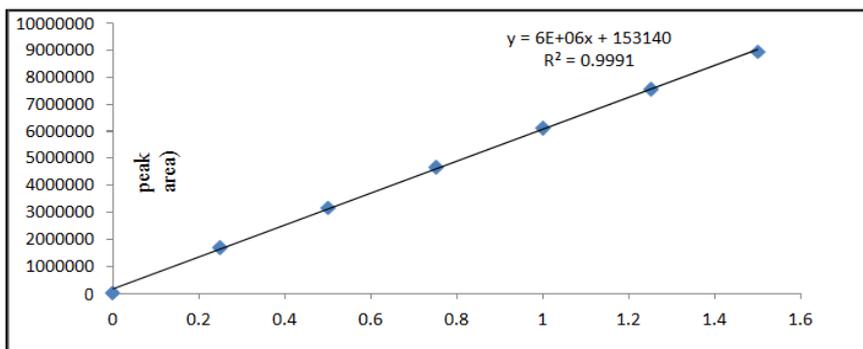**Fig.4: Absorption spectrum of Gemcitabine.**

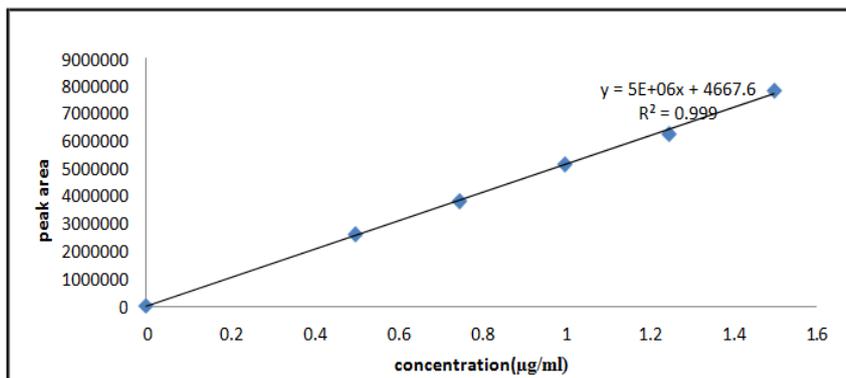**Figure No.5: Calibration Curve for Nilotinib at 242nm**

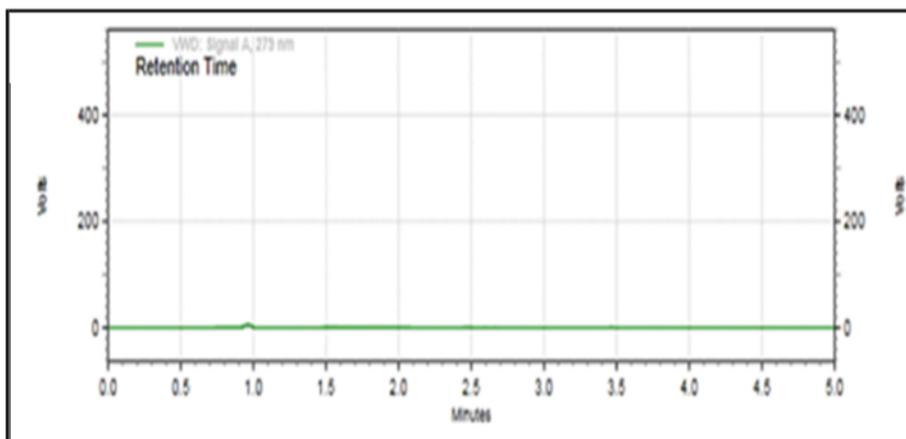**Figure No. 6: Calibration Curve for Gemcitabine**
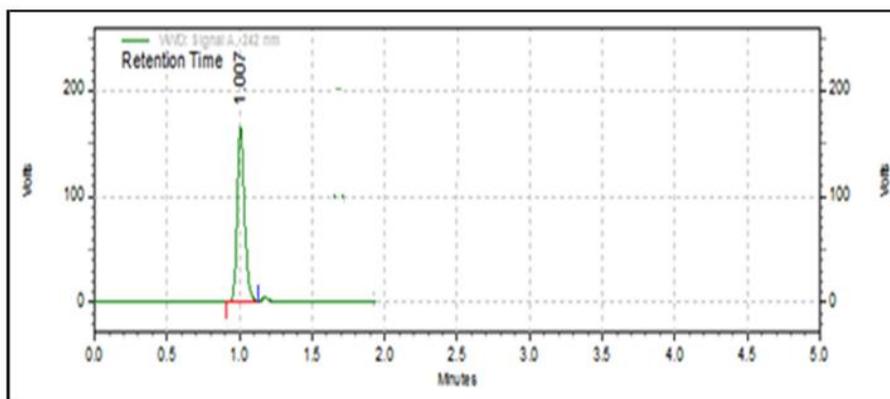
Figure No.7: Chromatogram of Specificity.
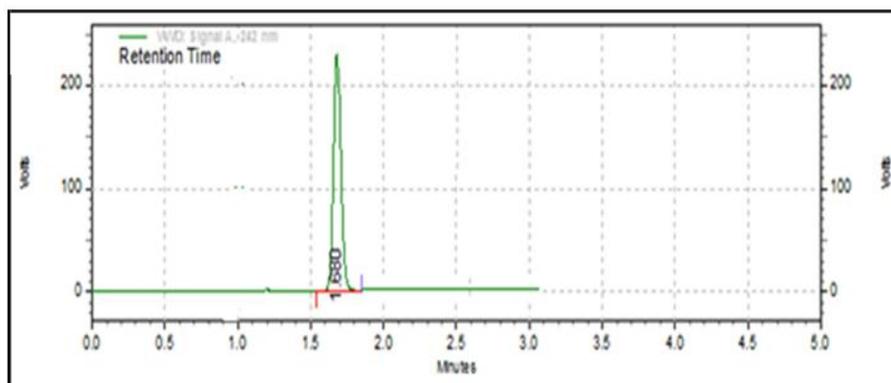

Figure No.8: Chromatogram of Nilotinib.

Figure No.9: Chromatogram of Gemcitabine.

CONCLUSION
High performance liquid chromatography at present one of the most sophisticated tool of the analysis. The estimation of Nilotinib myselate and Capcitabine was done by RP-HPLC Method. The mobile phase was optimized with consists of Acetonitrile. Water mixed in the ratio of 50:50 % v/ v. A C18 column C18 (4.6 x 150mm, 5µm, Make: XTerra) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of Nilotinib myselate and Capcitabine were found to be from 25-125 µg/ml of Nilotinib myselate and Capcitabine. Linear regression coefficient was not more than 0.999.The maximum absorbance is found to be at 242 nm. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 97-102% of Nilotinib myselate and Capcitabine. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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