Development and Validation of Stability Indicating HPTLC Method for Estimation of Dasatinib and Characterization of Degradation Products by Using Mass Spectroscopy

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ABSTRACT

Dasatinib which is available in market as a brand name of Sprycel tablets. Method was validated according to the ICH guidelines. The HPTLC method was developed using Camag HPTLC system. Silica G60 F_{254} precoated TLC plates were used as stationary phase. Mobile phase was used toluene: methanol (6:4). Dasatinib analysis was carried out in the absorbance mode at 240 nm. The drugs were satisfactorily show peak with RF 0.65 ± 0.03 for Dasatinib. Development method was validated as per ICH guideline using validation parameter like specification, linearity, accuracy, LOD, LOQ, precision, and robustness. The method was found to be linear in the range of 200ng-1.2μg and correlation coefficient value 0.997.dasatinib. In stability testing, Dasatinib were found susceptible to alkaline degradation. Because the method could effectively separate the drugs from their degradation products, it can be used as a stability indicating method. Degradation product of Dasatinib in alkaline condition was carried out and its degradation product is successfully separated and isolated by HPTLC method. Degradation product was identified by using MS-MS technique.

Keywords: Dasatinib, HPTLC, stability indicating assay, isolation of degradation product, MS-MS identification

INTRODUCTION

Dasatinib (anticancer), belongs to tyrosine kinase inhibitors (TKIs). Dasatinib is thiazole – based ATP competitive dual src/Abl kinase inhibitor, approval for the treatment of imatinib- resistant cancer patient [1-3]. Dasatinib is chemically N-(2-chloro-6-methylphenyl)-2-((6-(4-(2-hydroxyethyl)piperazin-1-yl)2-methylpyrimidin-4-yl)amino) thiazole-5-carboxamide, which melt in the range of 273.28º C. Some HPLC and UV analytical methods were reported in the literature. The detailed literature review revels that, there is NO HPTLC method was reported [4].

For accurate quantitative determination of interest in combination, it is necessary to isolate drugs from impurities, degradent, formulation excipients and analyze separately.

As there is no HPTLC-MS method was reported till date, it is thought worthwhile, to develop and validate the stability indicating HPTLC method and to perform forces degradation studies for estimation of Dasatinib in pharmaceutical dosage form [5,6].

Moreover, HPTLC-MS/MS studies were also planned for isolation and identification of degradant products. Based on HPTLC-MS/MS studies the degradation pathway is also studied and reported [7-9].

EXPERIMENTAL WORK

Pure Dasatinib drug was provide as gift sample from Intas Pharmaceutical Ltd. Ahamdabad, Gujrat. The analytical grades chemicals toluene, methanol, NaOH, HCL and H_2O_2 was used.

Instruments

The validation of Dasatinib was performed with the help of CAMAG LINOMAT-5 automatic applicator, for the saturation twin glass chamber used and the degradation study of Dasatinib was carried out with the help of water bath, UV chamber BIT make (model no.49).

Preparation of Standard Stock Solution

Accurately weighted quantity of 20.0 mg Dasatinib was transfer to 10.0ml volumetric flask, add 5.0ml of methanol and sonicate for 10.0min then make up volume with methanol (Concentration obtained 2000µl).

Chromatographic Condition

The mixture of mobile phase mixture in the ratio of 6:4 v/v was optimized for high performance thin layer chromatography plate development. The chamber was saturated with the mobile phase at room temperature for 10 min and then developed plate were dried at room temperature for 5 min and scanned under 200-700 nm UV rang the maximum absorption was found to be at 240 nm using deuterium lamp in absorption-reflectance mode [9-11].

METHOD VALIDATION

The method was validated as per Q2 (Analytical validation) ICH guideline [12,13].

Linearity

Different concentration of Dasatinib [200-12000 ng per band] were applied on TLC plate with the help of micro syringe using LINOMAT-V automatic sample applicator. The peak area was recorded for each drug concentration vs peak area was constructed for Dasatinib.

Precision

The same concentration of drug solution application on TLC plate [0.4 µl] in interday and intraday precision evaluated by determining three similar responses. Three time response on same day and on three times response on different day for Dasatinib.

The result was found to be in % relative standard deviation (%RSD) and repeatability was carried out by applying Dasatinib solution (0.4 µl/ml) 6 times on TLC plate and result found in % relative standard deviation.

Accuracy

The accuracy studies were conducted at three levels i.e. 80%, 100% and 120%. It was done by weighing tablet powder equivalent to 20.0 mg of Dasatinib and transferring them to 9 different 10.0 ml volumetric flasks and adding 8.0 mg, 10 mg and 12 mg of pure drug in 9 flasks in triplicate form. Solutions were done as per procedure described in sample solution preparation and amount of drug recovered was calculated [14-16].

Limit of Detection and Limit of Quantification

The limit of detection and limit of qutification were calculated by using given formula

LOD – 3.3 SD/ S and LOQ- 10 SD / S

where SD is the standard deviation and S is the slope of calibration curve.

Robustness

It was calculated by change the ratio of mobile phase and saturation time of mobile phase. The result was shown in **Table 3**.
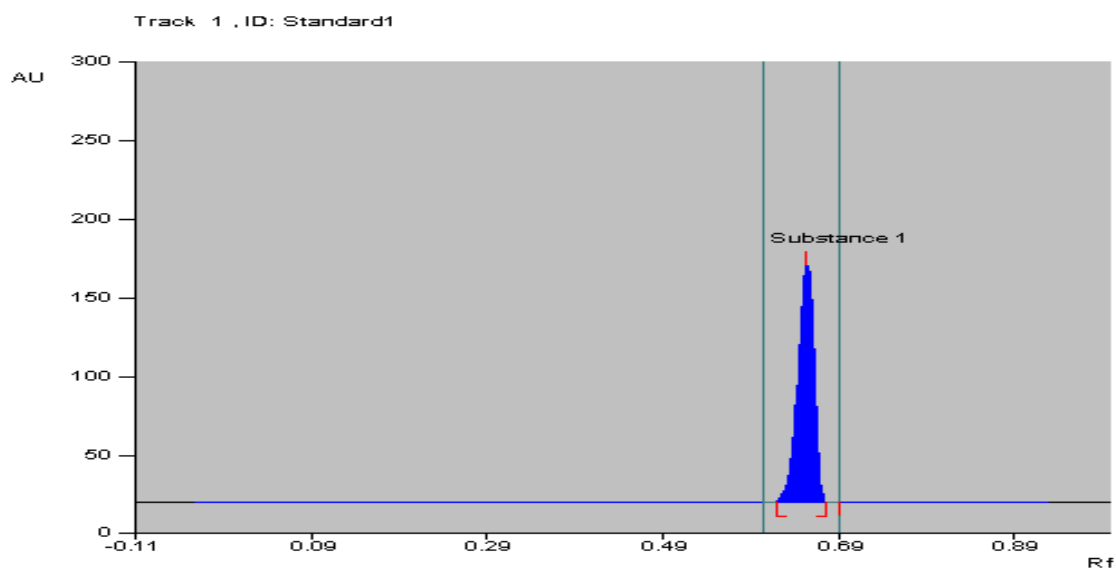
Figure 1. Standard Densitogram of Dasatinib

Forced Degradation Study of Dasatinib

In forced degradation study the six samples was prepared by transferring 20 mg of Dasatinib in drug in 10ml volumetric flask and add 3 ml of 0.1 HCL, 0.1 NaOH, 30% $H_2O_2$and distilled water first 4 flask respectively. Heated in water bath for acidic and basic 2hrs, for oxidative 1hrs and in distilled water 3hrs at 80^0 C respectively. For photolytic degradation of pure drug exposed in UV light at 257nm for 48 hrs and thermal degradation at 100^0 C for 1hrs. Then transfer to remaining two flask respectively [17-18].

ISOLATION AND IDENTIFICATION OF DEGRADATION PRODUCTS BY HPTLC-MS/MS

The degradation analysis was performed on six stress condition. Alkaline and neutral stress condition was used in HPTLC-MS/MS studies, because the percentage of degradation is found more compared with other stress condition. The degraded products isolate and identify done by applying degraded sample solution on TLC plates (9µl-band) and plate was developed under optimized chromatographic conditions. After drying the plate, it was observed under UV cabinet and degraded band was identified and marked. The identify degraded band was scrapped out and soaked overnight in methanol. On next day, the sample was filtered through Whatmann filter paper and subjected to MS/MS. Two types of spectra was obtained by MS-MS studies. Q1 (used for identification of parent compound) and Q3 (used identification of fragmentation patterns). The MS-MS spectra was shown in **Figure 9** to **Figure 12**.

RESULT AND DISCUSSION

Optimization of Mobile Phase

The same analysed by HPTLC using mobile phase Toluene: Methanol (7:3) the peak was slightly broad then toluene: methanol: glacial acid (6:4:0.4) the retention factor value of peak was very less and then using toluene: methanol: ammonia (7:3:0.2) the retention factor of peak area was very broad so by using toluene: methanol (6:4) ratio was used. They give best result and the retention factor was 0.65 ±0.03 (**Figure 1**).

Linearity and Calibration Curve

The calibration curve indicate that increase area was directly proportional to concentration. The linearity curve was found to be linear and regression coefficient was found 0.995 with equation y= 1168.7x+1219.3. The linearity curve has been shown in **Figure 2**. The results for precision, accuracy and robustness were mentioned in **Table 1** to **Table 3**.
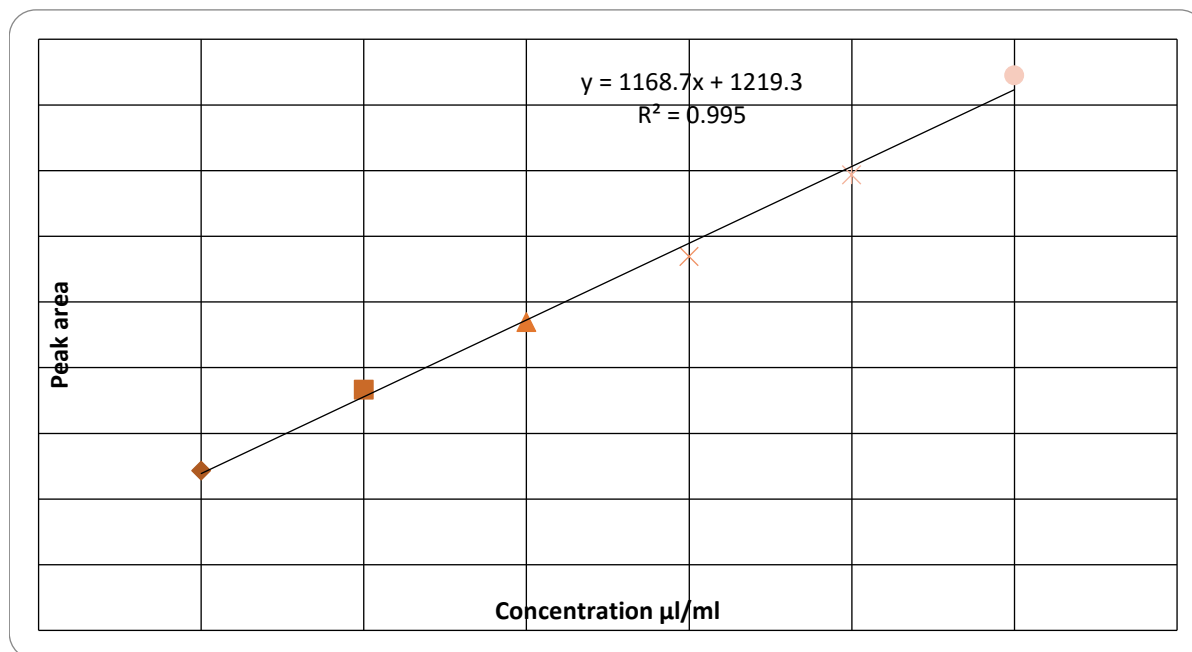
$$y = 1168.7x + 1219.3$$
$$R^2 = 0.995$$

Figure 2. Calibration Curve

Table 1. Result of Precision study

Amount (ng band^{-1})	Intraday (n=3) ± %RSD	Interday (n=3) ± %RSD
800ng/ml	4602.50 ± 1.12	4691.92±1.10
800ng/ml	4490.35 ± 1.09	4990.35± 1.09
800ng/ml	4581.45 ± 1.07	4750.10 ± 1.11

Table 2. Result of Recovery study

Level	Mean % recovery	R.S.D. (R≤2)
80	99.8	0.59
100	99.98	0.59
120	100.8	0.59

Table 3. Result of Robustness study

Sr no.	Factor	(±) S.D. for Peak Area
1	Saturation time (± 5 mins)	1.5
2	Total mobile phase (± 1ml)	1.6
3	Mobile phase composition (± 0.1 ml)	1.1
4	Time from development to scanning (±mins.)	1.6

## Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of the method was performed by standared reported procedure and found to be 0.92 μg/ml. and 2.817 μg/ml respectively.

Forced Degradation Study

Forced degradation studies of Dasatinib were carried out under various stress condition like acidic, alkaline, oxidative, neutral, photolytic and thermal. The degradation found maximum in alkaline condition and minimum in acidic condition. The result of forced degradation studies was shown in below (**Table 4** and **Figure 3** to **Figure 8**).

Table 4. Results of forced degradation study

Sr. no.	Stress condition	Temperature and time	% assay of active substance
1	Acid (0.1 M HCL)	80° C for 2 hrs.	95.88%
2	Alkali (0.1 M NaOH)	80° C for 2 hrs.	89.79%
3	Oxidative (3% H_2O_2)	80° C for 1 hrs.	94.06%
4	Neutral	80° C for 3 hrs.	84.45%
5	UV degradation	48 hrs.	97.99%
6	Thermal	100° for 1 hrs	98.66%
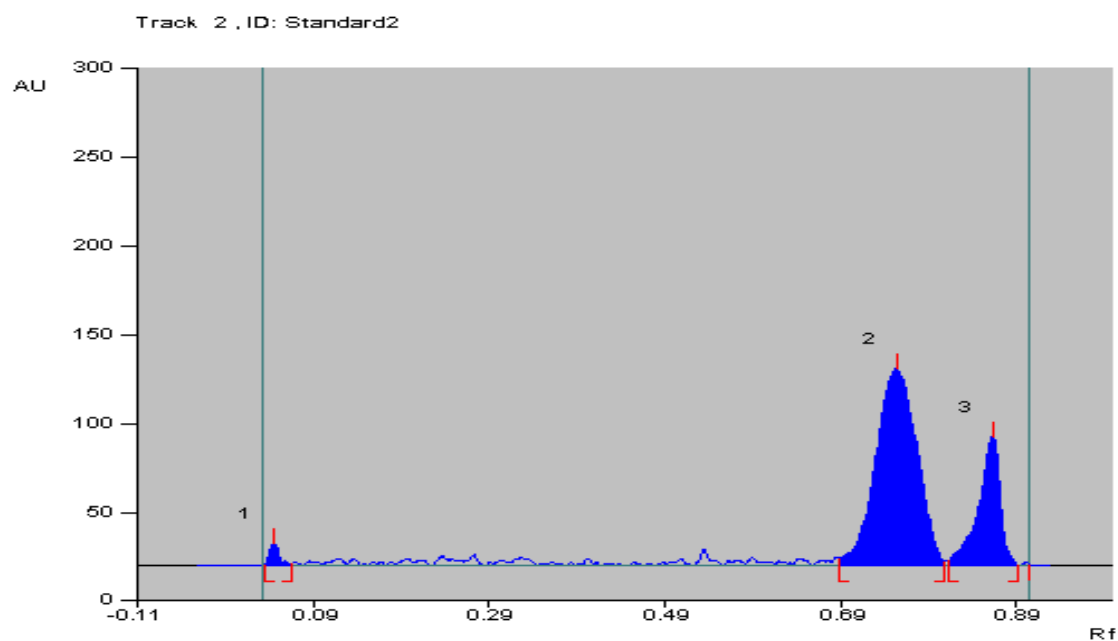
Figure 3. Densitogram of sample treated with 0.1 M HCL
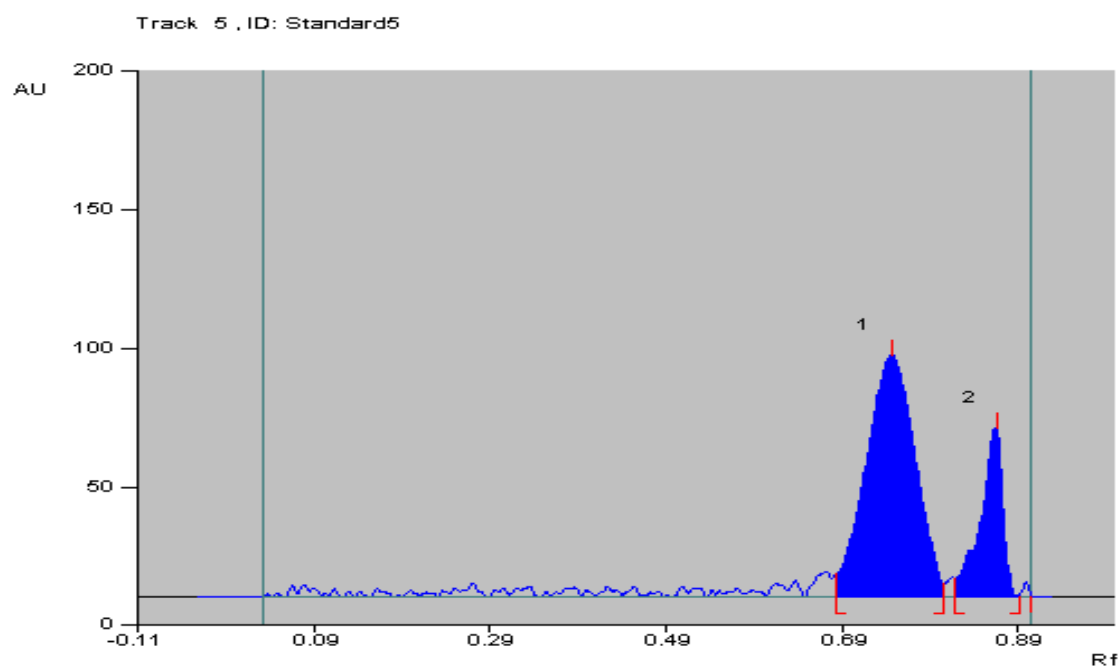
Figure 4. Densitogram of sample treated with 0.1 M NaOH
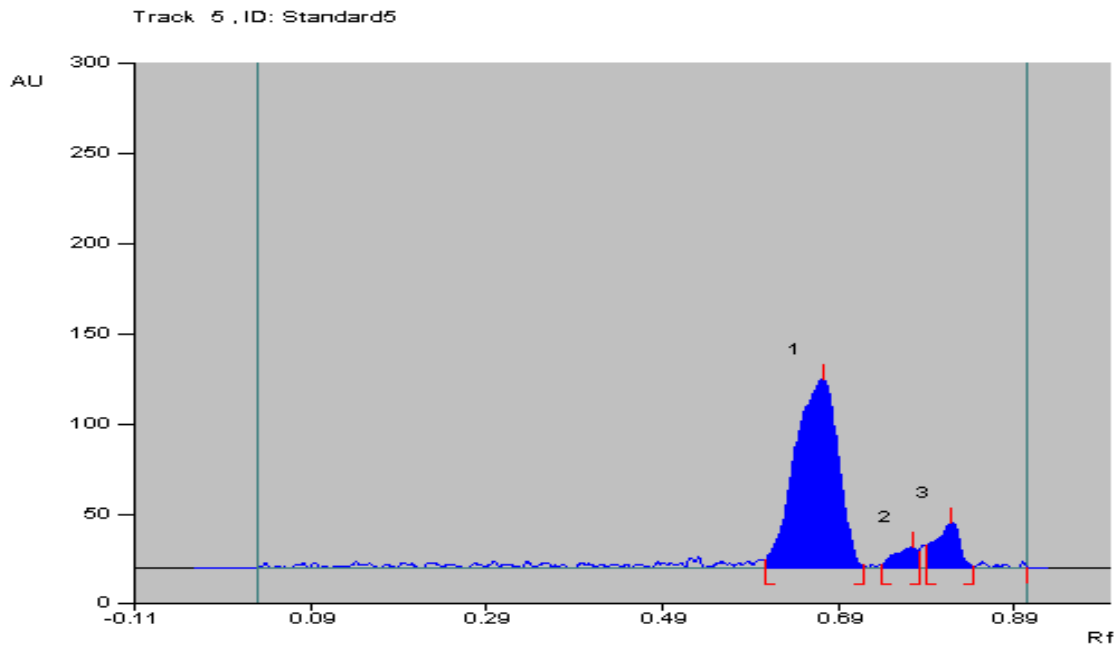
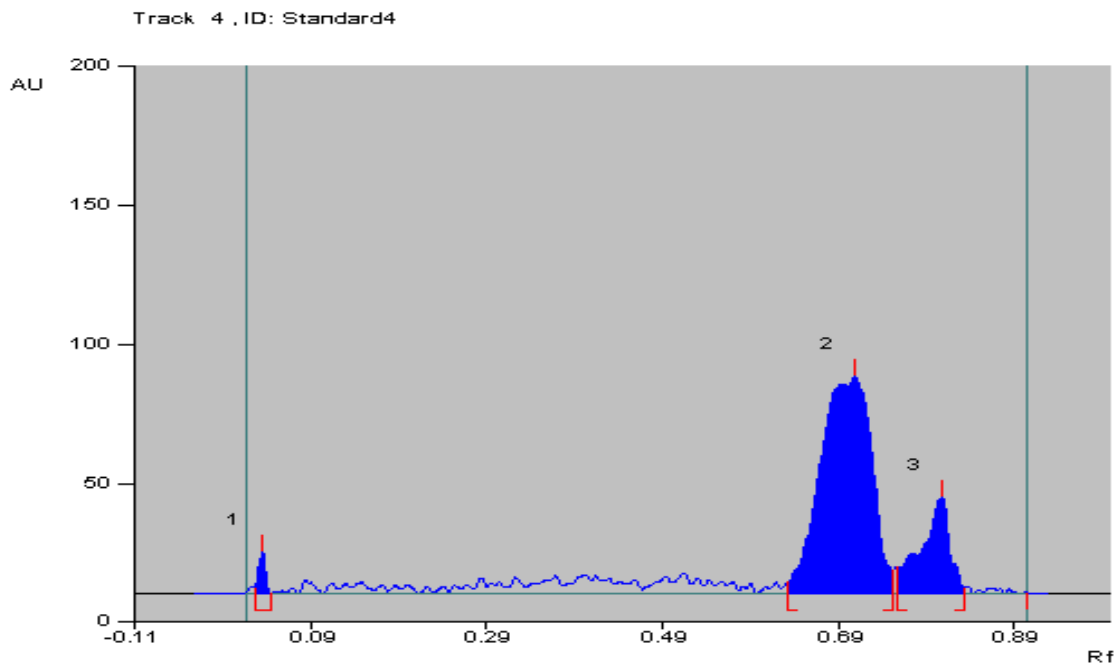**Figure 5.** Densitogram of sample treated with 3% H_2O_2

Figure 6. Densitogram of sample treated with distilled water
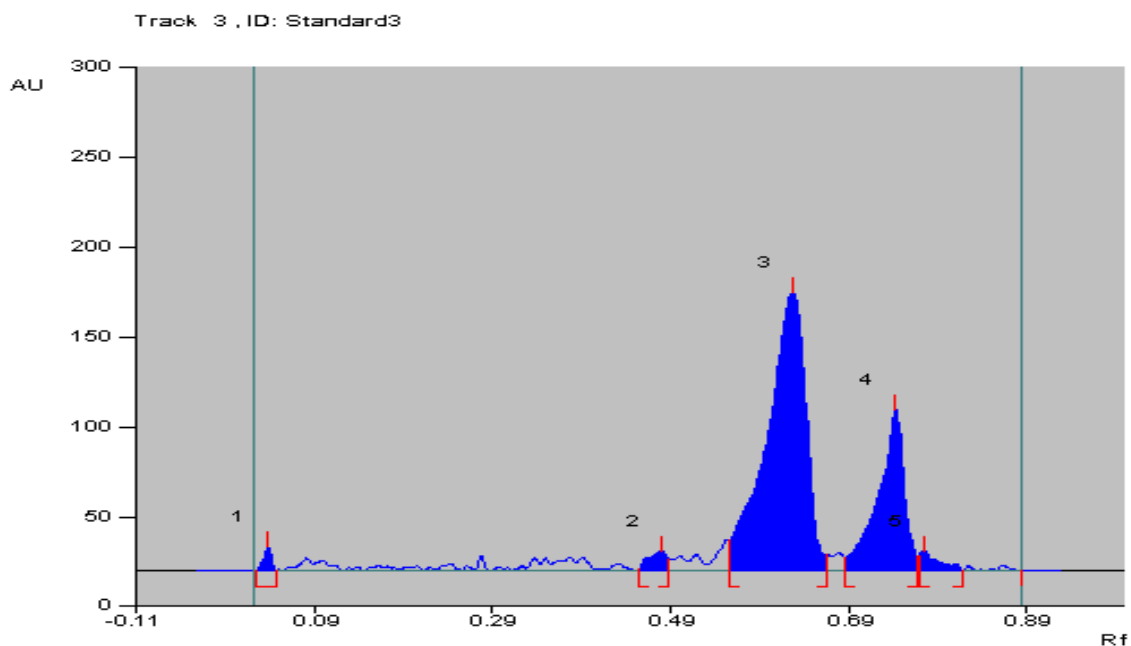
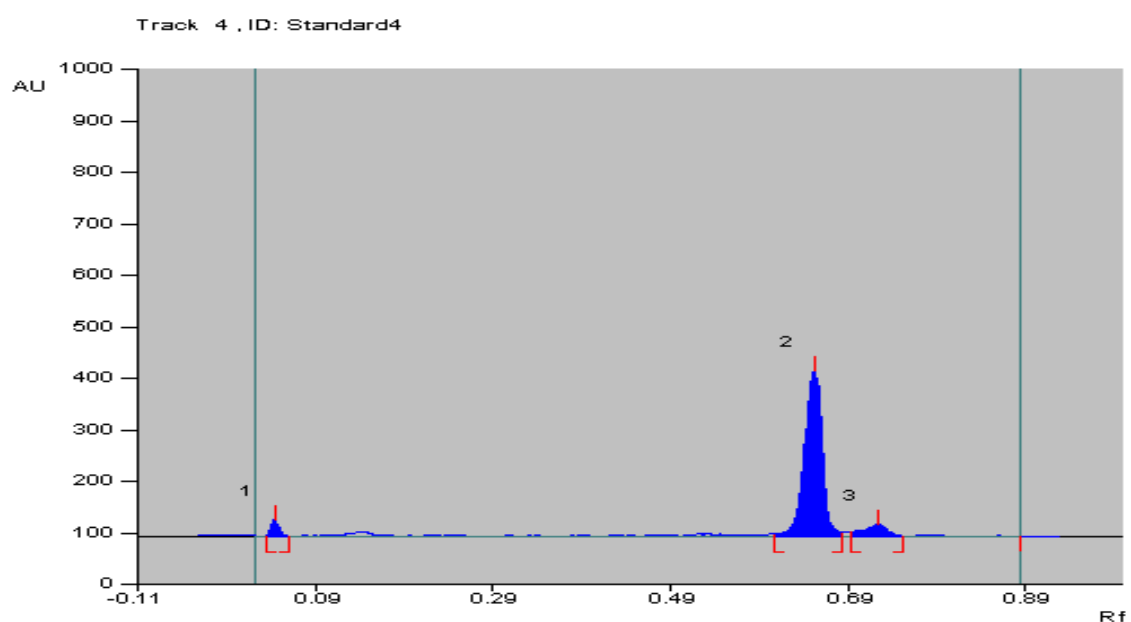**Figure 7.** Densitogram of sample treated with UV

Figure 8. Densitogram of sample treated with thermal

ISOLATION AND IDENTIFICATION OF DEGRADE PRODUCT BY HPTLC-MS/MS (TANDUM MASS SPECTROSCOPY) METHOD

The degradation product was isolated by using HPTLC method and the structures of degrandants product are determined by using MS-MS study. The fragmentation pattern of the drug was established by carrying out MS-MS studies in positive electrosprayionization (ESI) mode in the mass range of 50–1500 daltons (Da) (**Figure 9**). The drug (concentration of 5µg/ml) was directly infused using a syringe pump into MS/MS in methanol: water (50:50v/v). To optimized the mass parameters which clearly inform about the molecular ion peak of the drug. These were further modified to get complete fragmentation of the drug. High purity nitrogen was used as the nebulizer as well as the auxiliary gas. Fragmentation of various precursor ions formed in MS/MS studies was achieved at different collision energies.
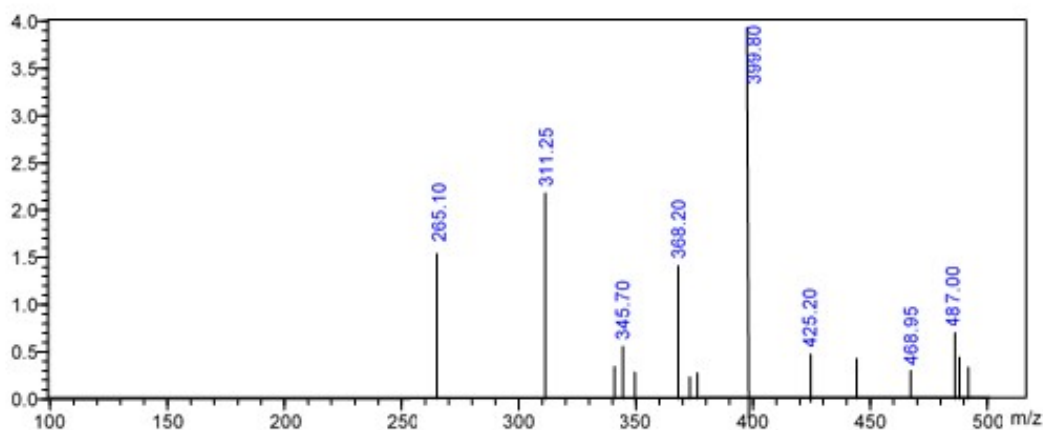
Figure 9. MS/MS spectrum of the m/z= 487.3 Dasatinib fragment in the positive mode.
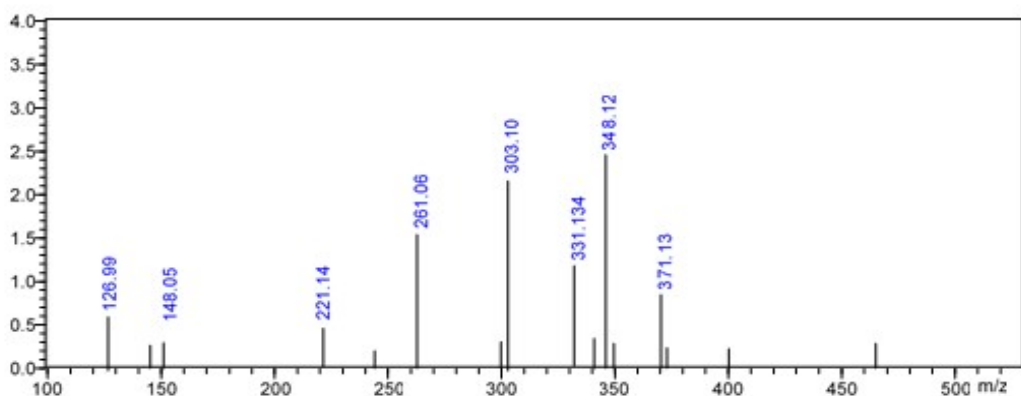

Figure 10. MS/MS spectrum of the DP-1

As evident in **Figures 3-8**, the drug degraded primarily into four DPs (denoted as DPs I–IV in accordance with the sequence in which the peak appeared from left to right in the chromatogram). DP-I was formed in both alkaline and neutral stress solutions, while DP-II and DP-III were formed in oxidative stress conditions.

After the degradation formation of four fragmented product which was identify based on IR spectra. The four degrading product were DP1, DP2, DP3 and DP4 respectively. The result of the HPTLC-MS/MS was given below,

DP-1(m/z 348.12) as shown in **Figure 10**. The parent ion of m/z 348.12 is observed in mass spectra. The DP-I was formed by hydrolysis of amide bond. The hydrolytic product is seen all three degradation condition, acid, base, and neutral condition.

DP-2 (m/z 503.15) as shown in **Figure 11**. The parent ion m/z 503.12 is observed in mass spectrum. The DP-II was formed by oxidation of nitrogen on piperazine ring. The two degradation product was observed by N-oxide formation on the either of the nitrogen of piperazine ring.
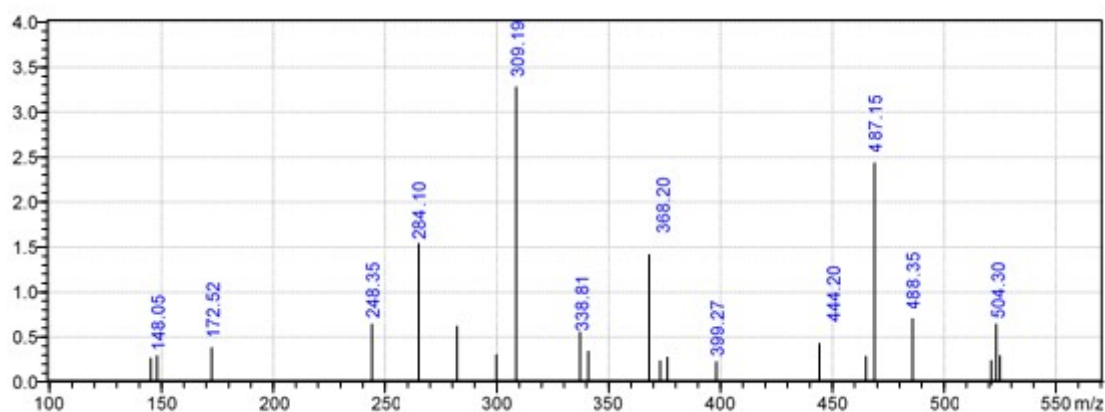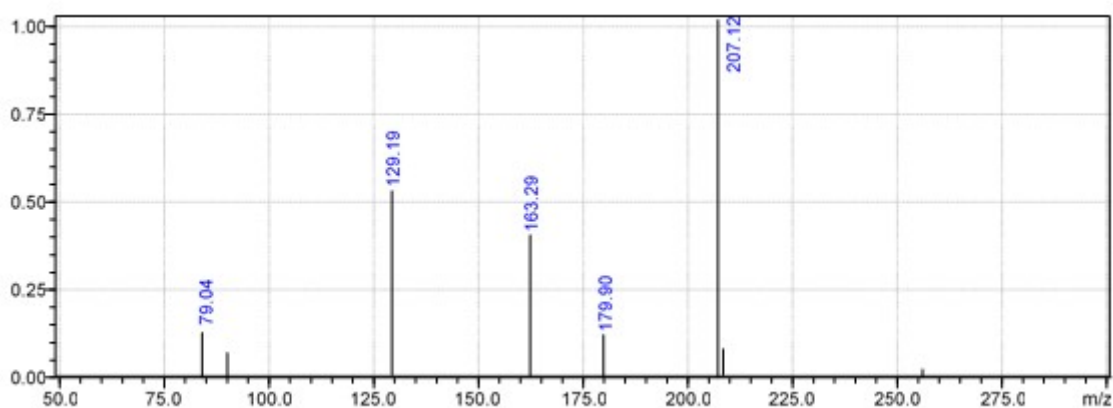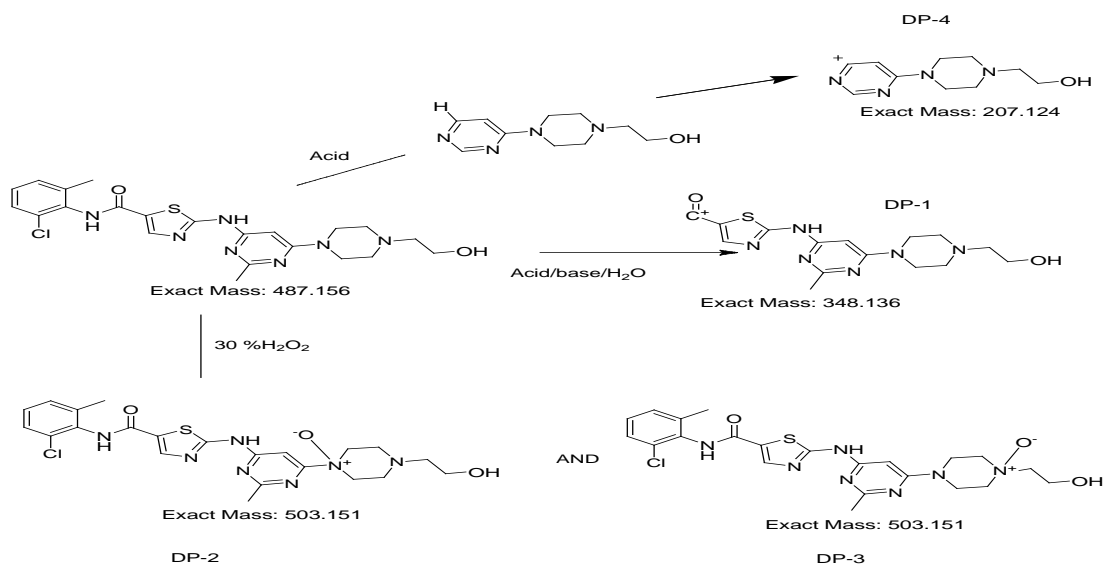
Figure 11. MS/MS spectrum of DP-2

Figure 12. MS/MS spectrum of DP-4

DP-4 (m/z 207.12) as shown in **Figure 12**. The parent ion of m/z 207.12 is observed in mass spectra. The DP-IV formation is due to breakdown of the bond between –NH and pyrimidine ring.

Fragmentation Pattern of the Dasatinib Drug

Formation of DP-1, DP-2, DP-3 and DP-4 after stress degradation study.

CONCLUSION

The proposed HPTLC method for estimation of Dasatinib in pharmaceutical dosage form was found to be accurate, precise, specific and less time consuming. The developed HPTLC method was able to quantitative Dasatinib in presence of its degradation products. Thus, it can represent good method for analysis of Dasatinib as there are no reported methods for the same. Hence, the developed HPTLC methods can be used for routine quality control of pharmaceutical formulations containing Dasatinib.

By performing HPTLC-MS/MS of degraded products, fragmentation patterns were predicted by comparing IR spectrum of pure drug and by observing m/z ratios of different peaks in MS spectra. By observing the spectra, it was concluded that degradation in alkali and neutral media was more than the degradation in UV light and both of products follow same degradation pathway.

ACKNOWLEDGEMENT

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