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Forced Degradation Study of Pyrazinamide In Bulk and Formulation by UHPLC Method

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ABSTRACT

The present study was undertaken to determine the forced degradation of Pyrazinamide, performed by various conditions such as acid, alkali, oxidation, thermal and photolytic. The study includes both Pyrazinamide in bulk and tablet formulation. The study based on available guidelines and main reference .Pyrazinamide has a Pyrazine nucleus. It is easily hydrolyzed by acid and alkali. The assay value of degraded products measured by intraday (30mins, 60mins, 90mins) and interday (1st, 3rd, 5th day) by UHPLC. Extensive degradation was observed in alkali hydrolysis method, and the degraded products were analysed by using UHPLC. At 90mins of intraday study using 0.1M NaOH, the degradation assay value of bulk and formulation were found to be 84.50% and 83.40% respectively. Intraday study, the degradation of bulk and formulation was observed on 1st day with the assay value of 23.62% and 25.42% respectively. However complete degradation of Pyrazinamide was observed on 3rd day and 5thday. It was determined that Pyrazinamide was found to be extremely unstable under alkali condition.

Keywords: Pyrazinamide, Kromasil C_{18} Column, Buffer, Acetonitrile UHPLC determination

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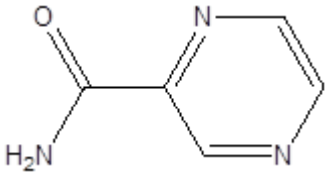
INTRODUCTION

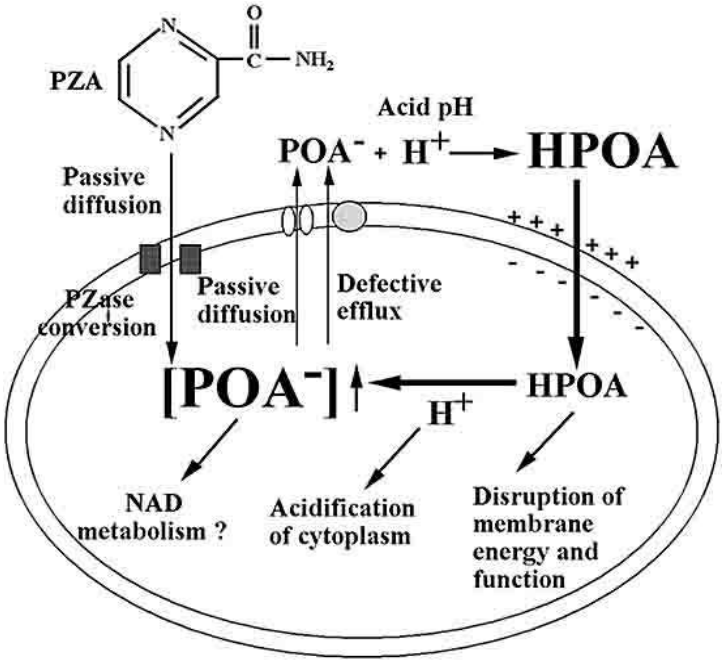
Pyrazinamide is chemically Pyrazine 2 carboxamide Molecular formula $C_5H_5N_3O$ Molecular weight 123.1g/mol .Pyrazinamide –Pyrazine analoge of Nicotinamide

Chemical Structure:



It is an Antitubercular agent, its administered orally first line drugs. The present developed method was simple precise, specific and accurate.

Pyrazinamide acts as bacteriostatic. It stops the growth of Mycobacterium tuberculosis by acting as a pro drug. Inside the granuloma of Mycobacterium tuberculosis under acidic conditions, the enzyme pyrazinamidase converts pyrazinamide in to its active form pyrazinoic acid. This pyrazinoic acid which slowly comes out converts to protonated conjugate acid might diffuse easily back in to the bacilli and accumulates inside the bacillus at acid pH.[1]



Pyrazinoic acid was thought to inhibit the enzyme fatty acid synthase (FAS) in, which is required by the bacterium to synthesize fatty acids although this has been discounted.

Hence the present study is aimed to developing stability indicating method for the determination of Pyrazinamide. Forced degradation studies have been reported individually for its determination. Hence, the present study in aimed for the determination of forced degradation of Pyrazinamide in various condition like acid /alkali/oxidation etc,

MATERIALS AND METHOD

Chemical and Reagents

All the experiment was performed with pharmaceutical Pyrazinamide. Pyrazinamide reference standard obtained from Micro labs, HPLC Grade acetonitrile was purchased from E. Merck co, Mumbai, India and potassium dihydrogen phosphate and sodium hydroxide AR Grade was purchased from Mumbai India. Solvents were filtered through 0.45µ membrane filter. All dilutions were performed in standard volumetric flasks.

The determination of Pyrazinamide was carried out on water UHPLC Model Infinity 1220 LC equipped with UV detector using data handling system –water alliance empower two software.wensar,PGB-200 electronic balance was used for weighing the samples. Ultrasonic bath was used for degassing and mixing the mobile phase.

INSTRUMENTATION & CHROMATOGRAPHIC CONDITIONS

Preparation of standard stock solution

Accurately weighed Pyrazinamide (80mg) was transferred into a 200mL clean dry volumetric flask. The drug was dissolved in water with sonication and final volume adjusted water up to mark to prepare a 40µg/mL stock solution. The solution was filtered through 0.45µ membrane filter paper. The solution was preserved and was used as standard stock solution for the preparation of calibration curve.

Preparation of sample solution

10 tablets were accurately weighed and calculated the average weight of each tablet then the weight equivalent to 0.5mg (80mg) tablets was transferred into 200mL volumetric flask, and dissolved in distilled water to achieve concentration of 40µg/ml. Before the sample bulk and standard solution were filtered through a 0.45µ membrane filter. The chromatographic separation of Pyrazinamide was carried out on a Kromasil C18 analytical column25mmx4.6mm.1.7µparticles) at a temperature of 30˚C under reversed phase chromatographic conditions. Separation was carried out using mobile phase of buffer (pH=3 potassium dihydrogen phosphate buffer) and acetonitrile taken in the ratio 900:100v/v.The mobile phase was delivered at a flow rate of 1.0mL/min in isocratic conditions .PDA detector was used to detecting the separated components. The data was analysed on empower 2 software version. Before analysis, the mobile phase was degassed by use of sonicated and filtered through a 0.45µm filter. The system was equilibrated before the injection.

Mobile Phase

Accurately 6.8045gm of potassium di hydrogen phosphate and add 1.844gm of sodium hydroxide in 900mL of water and pH adjusted to3.0 with ortho phosphoric acid and diluted to 1000ml with water. The solution was filtered through 0.45µ membrane filter and was degassed. A freshly prepared buffer and acetonitrile in a ratio of 900:100v/v was used as mobile phase. The mobile phase was used as diluents for preparing the working solution of the drug.[2]

Forced Degradation Studies

Forced degradation studies were carried out for standard drug at different stress conditions like oxidation, acidic, alkaline, heat, photo stability etc. The following procedure was adopted for forced degradation studies. [3,4]

INTRADAY/INTER DAY STUDY BULK AND FORMULATION

Alkali degradation studies

To 4ml of stock solution of Pyrazinamide,1ml of 0.1M sodium hydroxide was added separately The solution were kept for 90mins .For UHPLC study ,resultant solution was diluted to obtain 40µg/ml and 10µl were injected into the system and the chromatogram were recorded to assess the stability of sample. The effect of alkali degradation illustrated in (Figure 1-2)

Acid degradation studies

To 4ml of stock solution of Pyrazinamide, 1ml of 0.1M Hydrochloric acid was added separately. The solution were kept for 90mins .For UHPLC study, resultant solution was diluted to obtain 40µg/ml and 10µl were injected into the system and the chromatogram were recorded to assess the stability of sample. The effect of acid degradation illustrated in (Figure 3-4)

Oxidation studies

To 4ml of stock solution of Pyrazinamide, 1ml of 0.1M Hydrochloric acid was added separately. The solution were kept for 90mins.For UHPLC study, resultant solution was diluted to obtain 40µg/ml and 10µl were injected into the system and the chromatogram were recorded to assess the stability of sample. The effect of oxidative degradation illustrated in (Figure 4-5)

Thermal degradation studies

1gm of Pyrazinamide tablets were weighed and transferred to a petri dish. This petri was placed in a hot air oven at the temperature of 50˚c.The third day 80mg of Pyrazinamide tablets were weighed from a petric dish and transferred to 200ml volumetric flask. An aliquot solution was diluted with mobile phase to get final concentration. [5 -7]

Photo stability studies

1gm of Pyrazinamide tablets were weighed and transferred to a petri dish. This petri was placed in a hot air oven at the temperature of 70˚c.The third day 80mg of Pyrazinamide tablets were weighed

from a petric dish and transferred to 200ml volumetric flask. An aliquot solution was diluted with mobile phase to get final concentration.[8]

INTER DAY STUDY

Standard Preparation

The standard preparation was prepared in a similar manner as interday.

Standard Preparation (Stress)

In the stress study, the standard was prepared in the same way as above. The final solution was scanned and absorption was measured at the time intervals 1st, 3rd, 5th day.

Sample Preparation (Stress)

Sample preparation in stress conditions same methods was followed, the final solution was scanned and absorption was measures at the following time duration 1st, 3rd, 5th day

Blank Preparation (Both Inter day and Intraday)

100ml of 0.1M HCl/0.1M NaOH/5% H_2O_2 was taken in a 100ml of volumetric flask. The solution kept at room temperature. The next day an aliquot solution was diluted water to get final concentration. This procedure is repeated for 3rd and 5th day.

RESULTS AND DISCUSSION

The results of forced degradation shows that, sample undergoes greater degradation compared with that of standard in all methods used. An important feature in this study was that sample undergoes hydrolytic degradation (both acid &alkali) than other degradation methods. This is because the amide group in pyrazinamide undergoes hydrolysis to form acid. There was only mild degradation in oxidation.Table-1 shows results of intraday degradation and the assay values of standard and sample were found to be 84.50 % and 83.46 % respectively at end of 90mins degradation. In interday study, both standard and sample have undergone maximum degradation on 1st day. Table-1 shows the assay values of standard and sample were found to be 23.62 % and 25.42 % respectively. Complete degradation has been observed 3rd day onwards. The above point states that pyrazinamide was unstable in alkali condition.

Hydrolytic degradation indicated that Pyrazinamide was unstable in acid condition. Intraday degradation both standard and sample exhibits meager amount of degradation. However sample undergoes greater degradation than standard at 90 mins.Table-1 shows the assay value of standard and sample was found to be 82.17 %and 79.16% respectively. Table-1 shows inter day study, the assay value of standard and sample were found to be 19.57%and 18.57 % respectively. Complete degradation was observed on both standard and sample from 3rd onwards

Oxidative degradation table -1 shows that pyrazinamide was found to be mild degradation in oxidative condition. In intraday analyses both sample and standard was found to be 88.93%and 83.63 % at end of 90mins degradation.Table-1 shows interday degradation study on 1st day standard and sample undergone maximum degradation. The assay value of standard and sample were found to be 15.53 % and 18.43% respectively. Complete degradation was observed on both standard and sample from 3rd on wards.

Table: 1 Forced degradation study of Pyrazinamide (Intraday /Inter day) Assay of acid/alkali/oxidation/thermal/photo light BY UHPLC

Stress Condition	Time	Standard	Sample	Remarks
Hydrochloric Acid 0.1M	90 mins	82.17 %	79.6 %	Degradation observed
Sodium Hydroxide 0.1M	90 mins	84.50 %	83.40 %	Degradation observed
Oxidation	90 mins	88.93 %	83.63 %	Degradation observed
Hydrochloric Acid 0.1M	1st day	19.57 %	18.57 %	Degradation observed
Sodium Hydroxide 0.1M	1st day	23.62 %	25.42 %	Degradation observed
Oxidation	1st day	15.53 %	18.43 %	Degradation observed
Thermal 50˚c	3rd day	82.42 %	83.18 %	Degradation observed
Photo light (sunlight)	3rd day	83.15 %	79.58 %	Degradation observed

In thermal and photolytic degradation studies shows Table-1 small amount degradation observed to 3rd day .In thermal the assay value of bulk and sample were found to be 82.42%and 83.18 %.where as in photolytic the assay value of bulk and sample were found to be 83.15 % and 79.58 at end of 3rd day degradation.

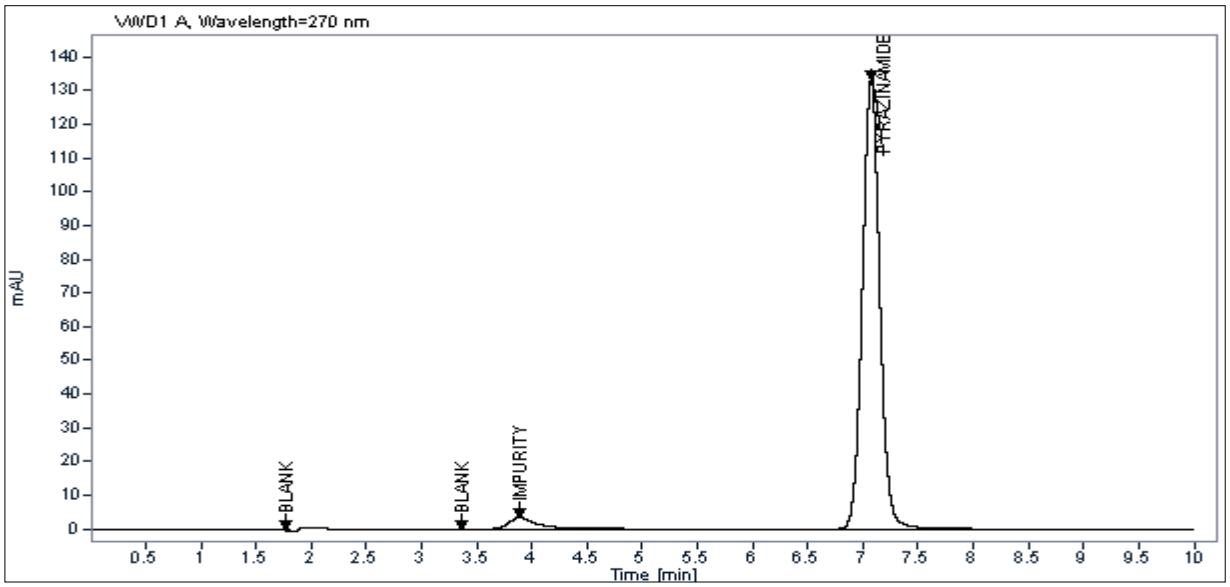


Figure 1: Chromatogram of Pyrazinamide Bulk in 0.1M NaOH (90mins)

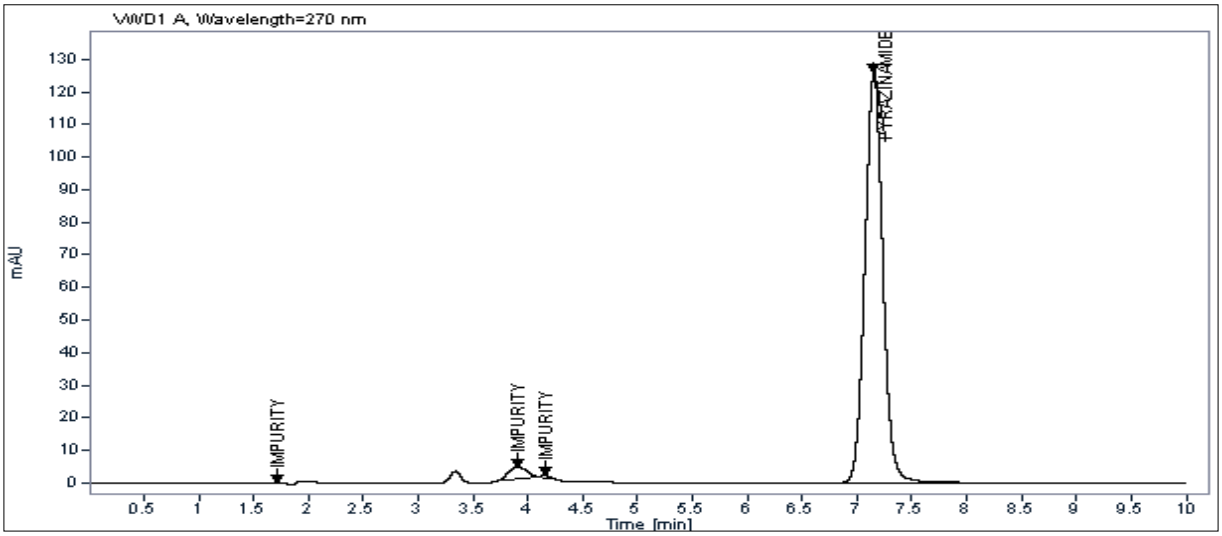


Figure 2: Chromatogram of Pyrazinamide Sample in 0.1M NaOH (90mins)

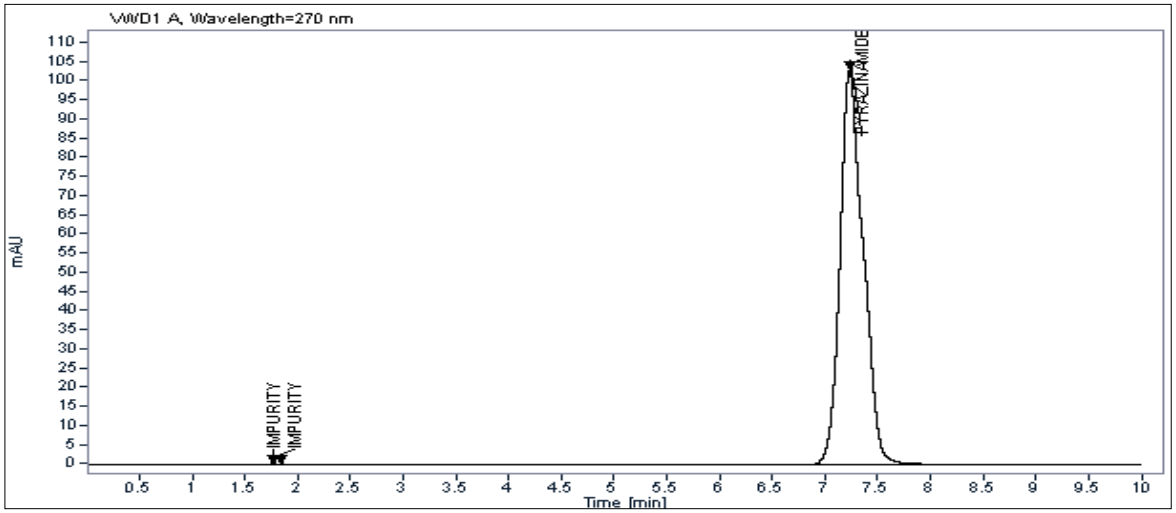


Figure : 3 Chromatogram for Pyrazinamide Bulk in 0.1 M HCL (90mins)

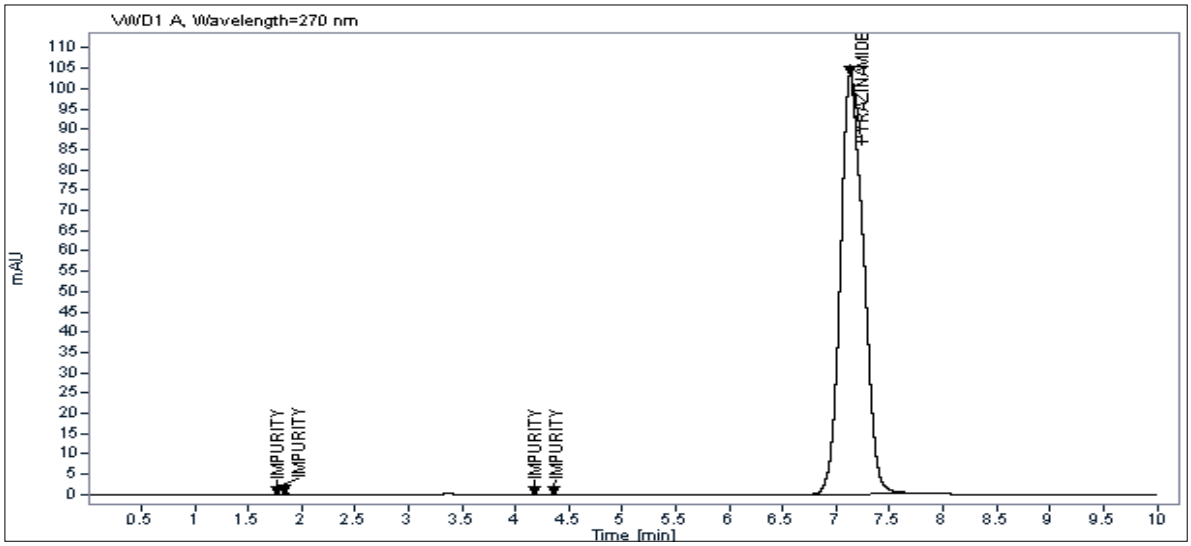


Figure 4: Chromatogram for Pyrazinamide Sample in 0.1 M HCL (90mins)

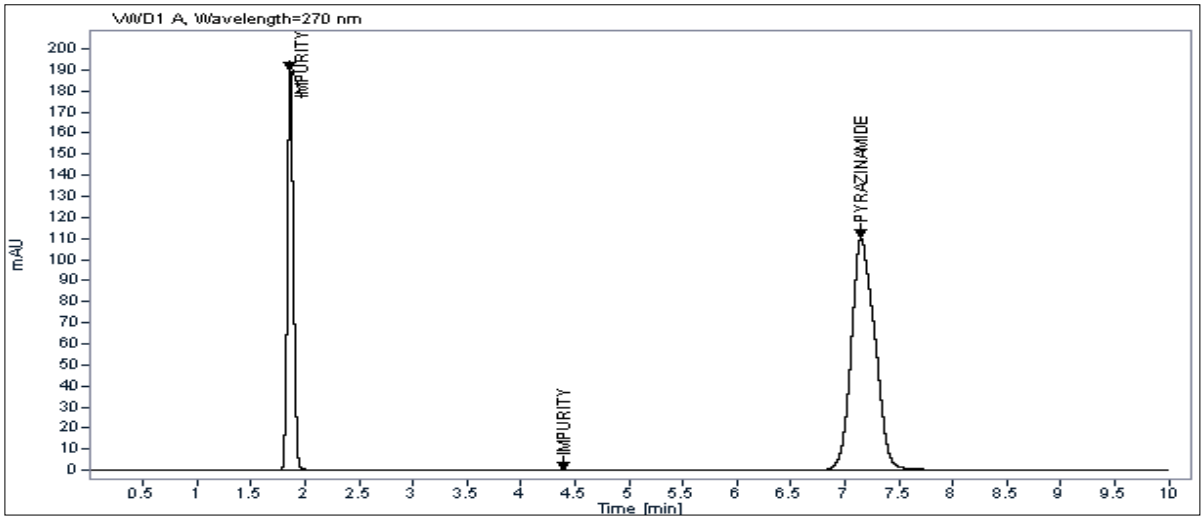


Figure 5: Chromatogram for Pyrazinamide Bulk in 5% H_2O_2 (90mins)

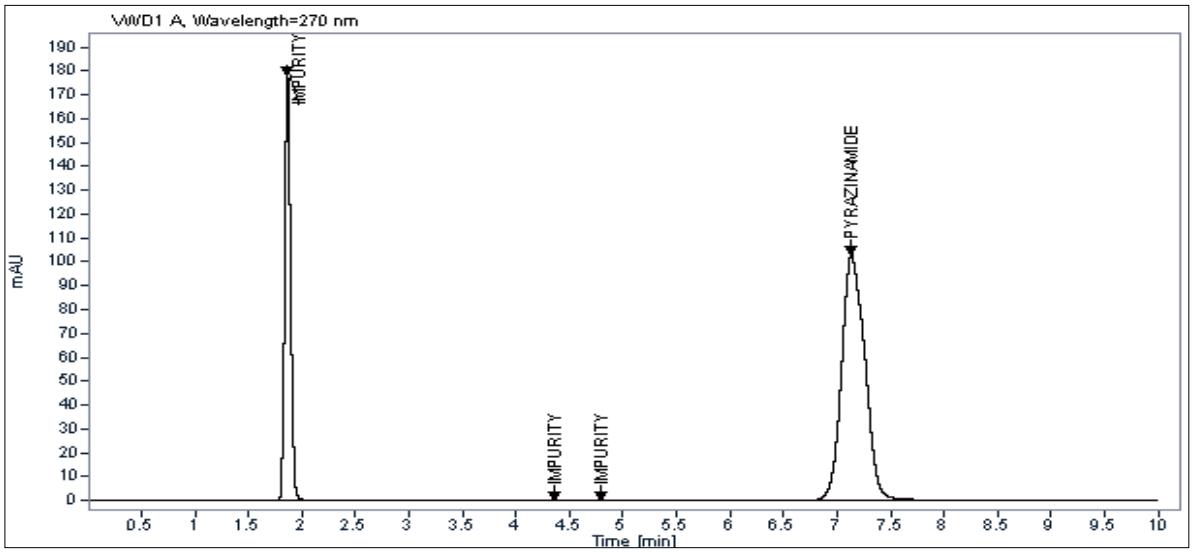


Figure :6 Chromatogram for Pyrazinamide sample in 5% H_2O_2 (90mins)

INTER DAY STUDY

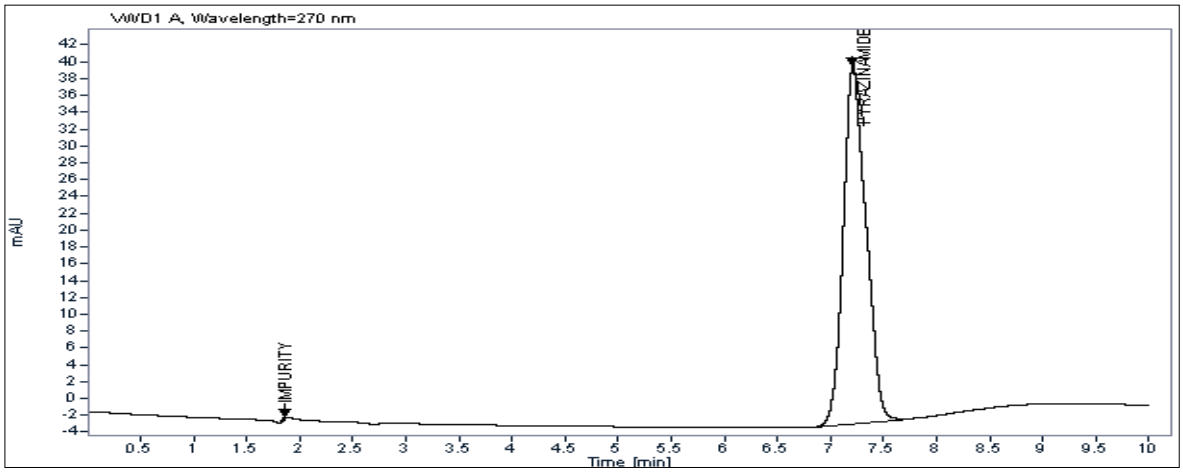


Figure 7 : Chromatogram for Pyrazinamide Bulk in 0.1M NaOH (3rd day)

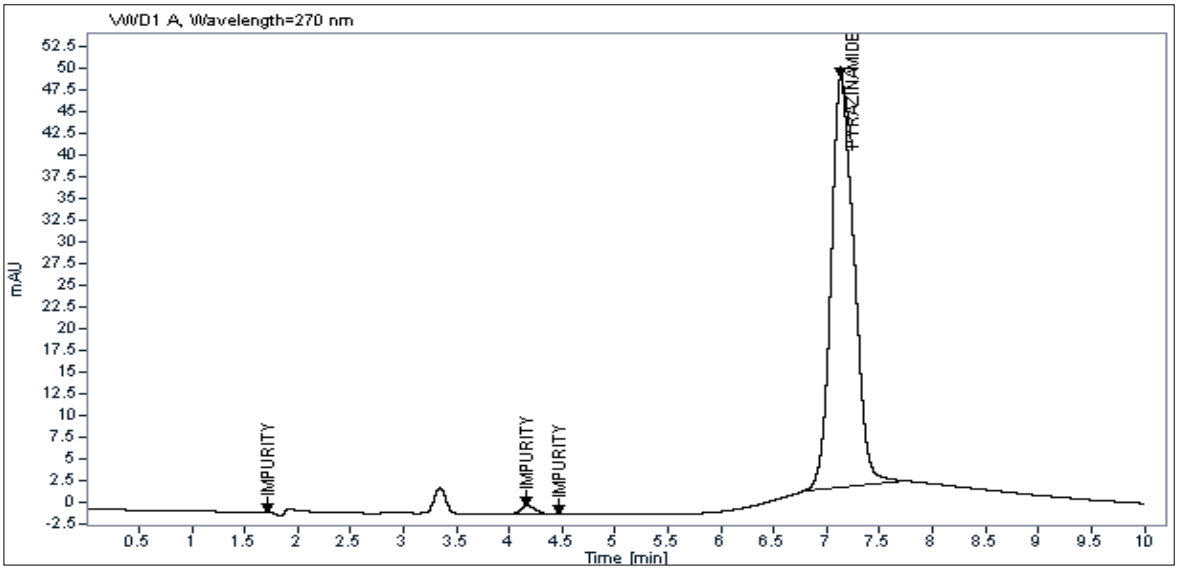


Figure 8: Chromatogram for Pyrazinamide Sample in 0.1M NaOH (3rd day)

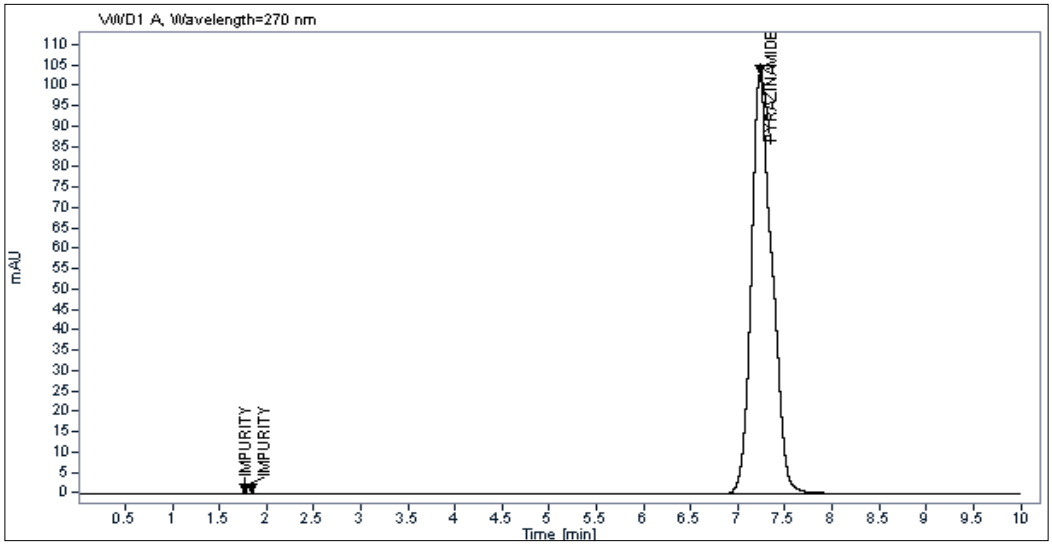


Figure 9: Chromatogram for Pyrazinamide Bulk in 0.1M HCl (3rd day)

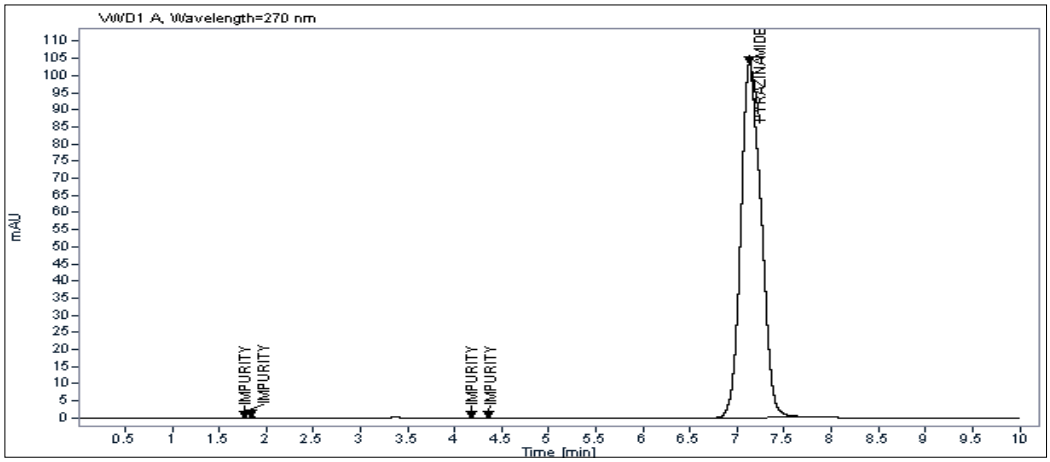


Figure 10: Chromatogram for Pyrazinamide sample in 0.1M HCl (3rd day)

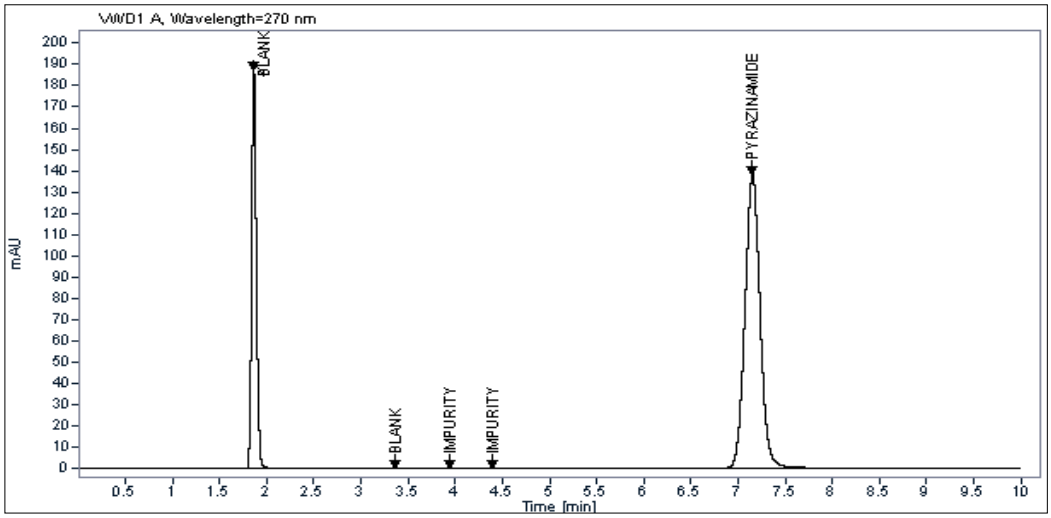


Figure 11: Chromatogram for Pyrazinamide blank in 5% H_2O_2 (3rd day)

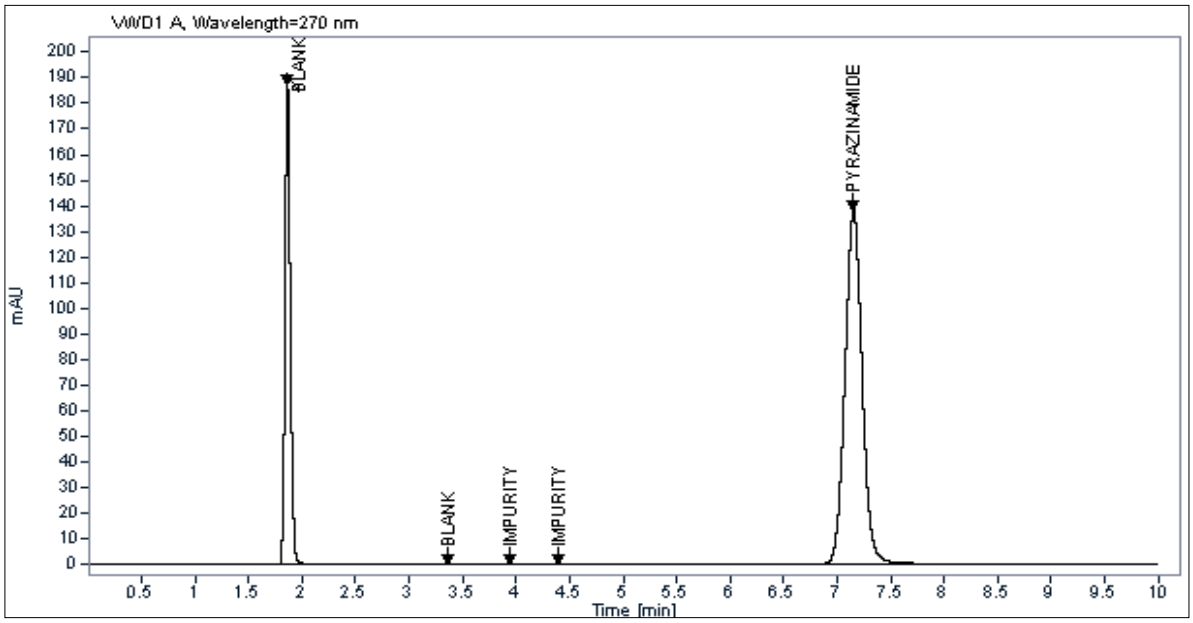


Figure 12: Chromatogram for Pyrazinamide Bulk in 5% H_2O_2 (3rd day)

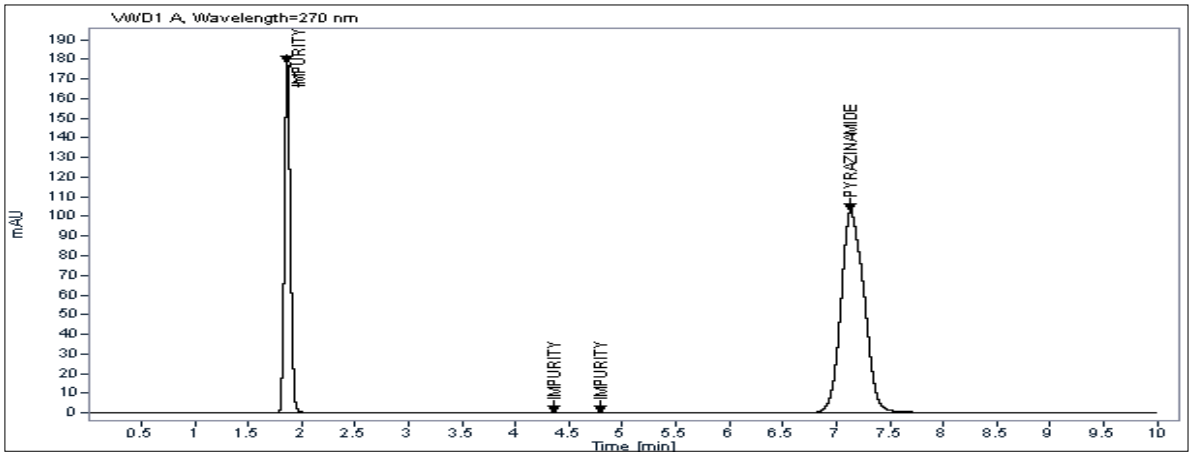


Figure 13: Chromatogram for Pyrazinamide sample in 5% H_2O_2 (3rd day)

CONCLUSION

The present study involves the stress induced stability studies such as alkali and acid hydrolytic degradation, oxidative degradation, thermal and photolytic degradation. An important feature in this study was that sample undergoes greater hydrolytic degradation (both acid and alkali) than other degradation methods used. This is because the amide group in pyrazinamide undergoes hydrolysis to form acid. There was only mild degradation in oxidation. Whereas thermal and photolytic degradation shows only little amount of degradation.

ACKNOWLEDGEMENTS

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