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DETERMINATION OF PRODUCT SHELF LIFE AND EVALUATION OF KINETIC PARAMETERS OF THERMAL DECOMPOSITION OF ISONIAZID IN SOLID STATE BY HPLC.

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ABSTRACT:
A new high-performance liquid chromatography (HPLC) method for the quantitation of Isoniazid has been developed and validated. Isoniazid was subjected to thermal decomposition at 120, 130, 140, 150, 160,170°C. Samples were withdrawn at intervals of 7, 14, 21 and 28 days and analyzed for remaining drug content by HPLC assay method. The samples were eluted on a Cosmosil C18 column (250 mm x 4.6 mm, 5.0 µm) at 30°C, with a mobile phase of Methanol : Water :: 80 : 20, (v/v) . The flow rate was 1.0 mL/min, Injection volume: 20µL and detection by PDA detector with detection wavelength at 266 nm. Isoniazid shows thermal-degradation after exposure to high temperature. The shelf life of Isoniazid was determined by accelerated stability studies on the basis of first order degradation kinetics. The reaction rate constants, activation energy and half life were also calculated using Arrhenius equation and plots. The shelf life of Isoniazid was found to be approximately (t_{90}) 5.75 years. Amounts of Isoniazid in all samples were determined by HPLC and the results were compared with those from Infra red spectroscopy and X-ray diffraction technology for qualitative comparison.

Key words: Isoniazid, Arrhenius equation, accelerated stability testing, shelf-life, HPLC.

I .INTRODUCTION:

Isoniazid (Laniazid, Nydrazid) **(pyridine-4-carbohydrazide)** (Figure 1) also known as isonicotinyl hydrazine (INH) or isonicotinic acid hydrazide is an organic compound that is the first-line medication in prevention and treatment of tuberculosis. (35) Isoniazid is a bactericidal agent active against organisms of the genus Mycobacterium, specifically *M. tuberculosis*, *M. bovisand*, *M. kansasii*. It is a highly specific agent, ineffective against other microorganisms. Isoniazid is bactericidal to rapidly dividing mycobacteria, but is bacteriostatic if the mycobacterium is slow growing.

Isoniazid is odourless, and occurs as a colourless or white crystalline powder or as white crystals. It is freely soluble in water, sparingly soluble in alcohol, and slightly soluble in chloroform and in ether. Isoniazid is slowly affected by exposure to air and light. Isoniazid is an antibacterial, available as 100 mg or 300 mg tablets for oral administration. It is also available as syrup. Isoniazid is also available as part of fixed dose combinations with other TB drugs such as Pyrazinamide and Rifampicin (Rifater is an example).
Many HPLC , LC-MS methods have been cited in the research papers for the qualitataive and quantitative analysis of Isoniazid as bulk drugs, various formulations and its degradation products (1,8,9,11,18,22,23,25,27,30,31,34).

This paper describes a stability indicating High Performance Liquid Chromatographic (HPLC) method for analysis of Isoniazid and Isoniazid high temperature degradation samples were developed, followed by validation of the method in accordance with ICH guidelines. With the help of Arrhenius equation the reaction rate constants, activation energy were calculated. Shelf life and half life of Isoniazid were also predicted using Arrhenius plot. Qualitative comparison of HPLC results with those obtained by IR and XRD analysis was also carried out.

To ensure the quality, efficacy and safety of pharmaceutical products, stability studies form an essential part of the pharmaceutical pre- and post-formulation studies (Loyd et al., 2003; Watterman et al., 2007; Yunqi and Reza, 2005). Instabilities in modern formulations are often detectable only after considerable storage under normal conditions. To assess the stability of the formulation, it

is usual to expose it to high stress conditions to enhance its deterioration and therefore reduce considerably the time required for testing (Olaniyi, 2000; Tson et al., 2003; Djira et al., 2008). Accelerated stability studies have proved an effective alternative to the time consuming and uneconomical practice of storing the product at room temperature for the time the products would be likely to remain in stock and use. A comprehensive stability plan is an essential and pertinent extension of the quality assurance programme. The effect of temperature on the rate of decomposition as described by the Arrhenius equation (Alfonso et al.,1985) states that:

$$k=Ae^{-Ea/RT}$$

Where k = specific rate constant, T= absolute temperature, Ea = Energy of activation, R =Gas Constant (8.314J/K.Mol), A = Frequency factor. The constants A and Ea may be evaluated by determining k at several temperatures and plotting log k against 1/T. The slope of the graph is –Ea/2.303R and the intercept on the y-axis is log A from which the values of Ea and A may be calculated. Stability of a pharmaceutical product may be defined as the capability of the particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications. It is the time from the manufacture and packaging of the formulation until its chemical and biological activity is not less than a predetermined level of labeled potency while its physical characteristics remain unchanged (Wolfgang, 1987; Repeto, 2000; Sprandel et al., 2005).

The physical stability of a suspension is normally assessed by the measurement of its rate of sedimentation, the final volume or height of the sediment and the ease of re-dispersion of the product. An assessment of these three parameters at elevated temperatures would give speedier indication of a rank of order of instability but it is essential to correlate these results with those taken from suspensions stored at ambient temperatures (Sethi, 1997; Jiben, 2002).There have been several reports on accelerated Stability studies, evaluation of kinetic parameters of decomposition.

Alibrandi Giuseppe et al. in 2003 carried out Fast drug stability by LC variable-parameter kinetic experiments. Al Omari Mahmoud M. et al. in 2007 observed effect of light and heat on the stability of montelukast in solution and in its solid state. Anderson Geoffrey et al. in 1991 determined Product Shelf Life and Activation Energy for Five Drugs of Abuse. Chena Gang et al. in 2002 determined the rate constants and activation energy of acetaminophen hydrolysis by capillary electrophoresis. Sungthongjee Srisagul in 2004 used "Application of Arrhenius Equation and Plackett-Burman Design to Ascorbic Acid Syrup Development. Syed Akheel A. et al. in 2001 developed LC method for finasteride and applied it to storage stability studies.

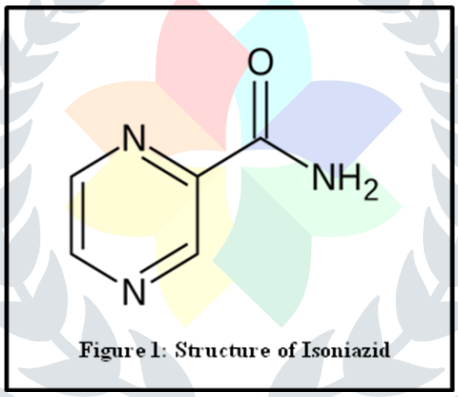

Figure 1: Structure of Isoniazid

I .MATERIALS AND METHODS:

Isoniazid API, received as a gift sample from Calyx chemicals and pharmaceuticals LTD., Mumbai.
Methanol HPLC grade, water HPLC grade.
Isoniazid Sample Solonex 300mg, Macleods pharmaceuticals LTD. (obtained from market)

Instrumentation and chromatographic conditions employed:

The Agilent HPLC (model 1100) system consisted of quaternary pump (model G1311 A), with an auto sampler (model G1313A) having injection capacity of 100 µl. PDA detector was used (model G1515B), data was integrated using Agilent Chemstation software (Ver.10.02) and Cosmosil C18 column (250 mm x 4.6 mm, 5.0µm) and Phenomenex HPLC guard column cartridge (4.0 x 3.0mm, 5.0µm) was also used during analysis.

Chromatographic parameters selected:

Column : Cosmosil C18 column (250 mm x 4.6 mm, 5.0 µm),HPLC guard column cartridge (4.0 x 3.0mm, 5.0µm), Mobile phase:- Methanol : Water :: 80 : 20, (v/v), Flow rate: 1.0 ml/min. Detection wavelength: 266 nm, Injection volume: 20.0 µl, Column compartment temperature: 30oC, Diluent:- Methanol : Water :: 78 : 22 , (v/v)

Preparation of Isoniazid standard stock solution:

50.0 mg of Isoniazid standard was accurately weighed and transferred to a 100.0 ml volumetric flask, 70.0 ml of diluent was added and sonicate till dissolved. Volume make up to 100.0 ml with diluent. Further diluted 5.0 ml from stock solution to 50.0 ml with diluent.

Preparation of Isoniazid sample (Solonex 300mg) solution for assay:
As per label claim, each tablet of Solonex 300mg (manufactured by Macleods pharmaceuticals LTD, Mumbai) contains 300.0 mg of Isoniazid. Hence, 5 tablets (weighing about 2.76 grams) were crushed in mortar and pestle. From that powder 0.1841 grams of powder equivalent to 100 mg of Isoniazid was weighed and transferred to a 100.0 ml of volumetric flask. 70.0 ml of diluent was added sonicated for 10 minutes. Volume make up to 100.0 ml with diluent. Further diluted 5.0 ml from stock solution to 100.0 ml with diluent. The sample solution thus prepared was filtered through a 0.22 µm membrane filter paper and finally chromatographed.

Preparation of Isoniazid degradation sample solution for kinetic and accelerated stability study:
50.0 mg of Isoniazid sample was accurately weighed and transferred to a 100.0 ml volumetric flask, 70.0 ml of diluent was added and sonicate till dissolved. Volume make up to 100.0 ml with diluent. Further diluted 5.0 ml from stock solution to 50.0 ml with diluent.

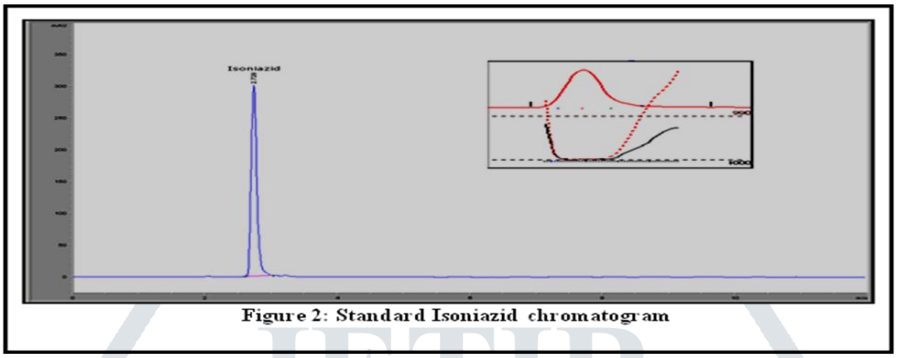

Figure 2: Standard Isoniazid chromatogram

II .METHOD VALIDATION:
The objective of the method is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The above method was validated according to ICH guidelines to establish the performance characteristic of a method (expressed in terms of analytical parameters) to meet the requirements for the intended application of the method. They were tested using the optimized chromatographic conditions and instruments.

Specificity:
The specificity of the method was determined by checking the interference due to excipients. The proposed method was established by checking the peak purity of Isoniazid. To check specificity; diluent, mobile phase, Solonex 300mg sample and Isoniazid standard were chromatographed to see co – elution/ interference of excipients of sample at the retention time of Isoniazid standard. The peak purity of the drug was also checked during analysis of samples after high temperature forced degradation conditions to assess the interference due to degradation products.

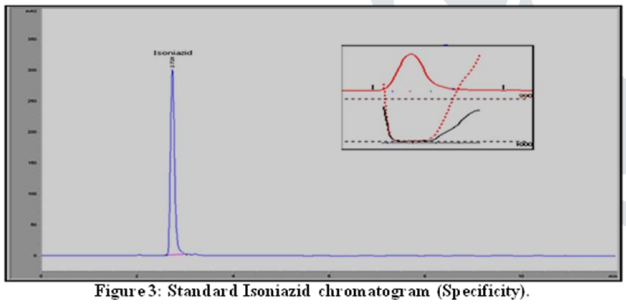
Figure 3: Standard Isoniazid chromatogram (Specificity).

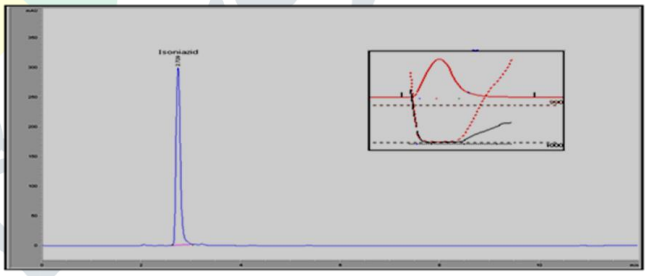
Figure 4: Isoniazid sample (Solonex 300mg) chromatogram (Specificity).

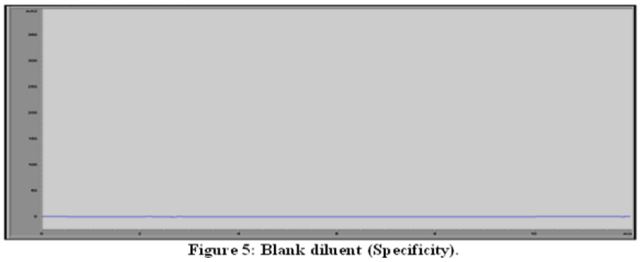
Figure 5: Blank diluent (Specificity).

Linearity:
For the establishment of linearity standard solution of different concentration (ranging from ± 50% and ±3 levels of concentrations of the working range) were prepared from a single stock solution. These solutions were chromatographed and peak areas were obtained subsequently. Peak areas were plotted against corresponding concentrations to obtain the calibration graph.

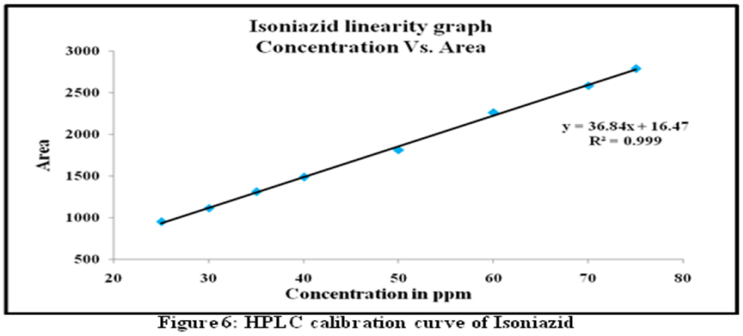

Figure 6: HPLC calibration curve of Isoniazid

Solution stability study:
Stability of drug in solution was assessed for the standard preparation of 50 ppm of Isoniazid. The solution was kept at room temperature without protection of light and tested after 2, 4, 6, 8, 12, 24 and 48 hrs. The responses for the aged solution were evaluated by comparison with freshly prepared solution.

Precision:
Precision was established by evaluating system precision, method precision and intermediate precision. For system precision six replicate injections of standards and samples were chromatographed. For method precision, six injections of six different standards and samples were chromatographed. Intermediate precision is merely the repetition of method precision but on another day.

Limit of Detection (LOD):
LOD was calculated from the formula: LOD = 3.3 σ / S
Where; σ = Standard deviation of the responses of calibration curve
S = Slope of the calibration curve

Limit of Quantitation (LOQ):
LOQ was calculated from the formula:
LOQ = 10 σ / S
Where; σ = Standard deviation of the responses of calibration curve
S = Slope of the calibration curve

Robustness of the Method:
Robustness of the method was studied at different parameters viz.
Change in the mobile flow rate ± 0.1ml of 1.0 ml
Change in the detection wavelengths ± 2 of 266nm
Change in the column oven compartment temperature ± 2°C of 30°C

Accuracy:
To check the accuracy of the method, recovery studies were carried out by addition of standard drug to sample at three different levels 80 %, 100 % and 120 %. For this 25 ppm of sample solution was prepared. Addition of standard drug was done by spiking from 1000 ppm solution of standard Isoniazid to the sample in their respective volumetric flasks. In 100 % recovery study for Isoniazid, amount of standard drug added was 35 ppm. In 80 % and 120 % recovery study for Isoniazid, the amount of standard drug added was 28 ppm and 42 ppm respectively. Samples were then chromatographed and peak areas were obtained. At each level of the amount, six determinations were performed. Amount of drug recovered was calculated.

Parameter	HPLC Validation report of Isoniazid
Linearity range	**25 – 75 ppm**
Limit of detection (LOD)	**1.94 ppm**
Limit of quantitation (LOQ)	**5.88 ppm**
Accuracy (%Recovery)	**Between 99 – 101%**
Solution stability	**Stable up to 48 Hours**
% Assay	**Solonex 300mg : 100.07%**
Precision (% RSD) **System precision** **Method precision (Inter – day)** **Intermediate precision (Intra – day)**	 **Less than 2.0 %** **Less than 2.0 %** **Less than 2.0 %**
Robustness	**Robust**
Specificity	**Specific**

Table 1: Statistical data for the validation of Isoniazid by HPLC method

III .EXPERIMENTAL PROCEDURES:

In accordance with ICH stability testing of drug substances and products, Isoniazid standard were stored at 120, 130, 140, 150, 160,170°C. Stress testing of a drug, in this case Isoniazid, which can in turn help establish the intrinsic stability of the molecules, and validate the stability indicating power of the analytical procedures used (1,8,9,11,18,22,23,25,27,29,30,31,33). The nature of the stress testing depends on the chemical and physical characteristic of drug and the type of formulation. Stress testing according to ICH is likely to be carried out on a single batch of the drug substance. It should include the effect of temperature (in 10°C increments; for accelerated testing). In this work, thermostability was determined under dry and dark conditions. At the accelerated storage condition, a minimum of three time points, including the initial and final time points. Samples were taken at 7, 14, 21 and 28 days after being stored at 120, 130, 140, 150, 160,170°C. and analyzed by HPLC and IR and XRD technique for qualitative purpose. All samples were analyzed simultaneously and comparison was made with time zero Isoniazid standard at room temperature on HPLC, to establish the percentage of drug remaining in the high temperature forced degradation samples. The data thus generated from this accelerated high temperature degradation study was used to calculate reaction rate constants, activation energy, shelf life t_{90} and half life $t_{1/2}$.

DATA ANALYSIS:

The temperature dependence of the rate of chemical degradation of Isoniazid in bulk was investigated by determining the concentration of active principal (Isoniazid) by HPLC as a function of time. A linear correlation was obtained at each temperature, and the observed rate constant (k) was calculated as the slope of these curves. The activation energy (Ea) was derived from the slope of the natural logarithm of the observed rate constants (ln k) plotted against the inverse absolute temperature (1/T) using the Arrhenius equation ln k = ln A – Ea/RT Eq. 1 where the constant (A) is the frequency factor; Ea is the energy of activation; R is the universal gas constant; and T is the absolute temperature. Shelf life t90 and half life t1/2 were also calculated from the apparent rate constant value from the Arrhenius plot.

ASSAY OF ISONIAZID:

The content of authentic Isoniazid was determined in samples as a function of time and temperature (Table II). The changes occurring in the assay values with respect to temperature are listed in the following table. Data of temperature sets from 120 to 160°C was considered for stability calculations since the higher temperature did not have enough data points for calculations.

	Time 0	1week	2week	3week	4week
26°C (RT)	100.00	100.06	99.94	100.00	100.06
120°C	100.17	99.00	97.51	96.79	94.03
130°C	100.06	96.95	96.07	93.24	90.20
140°C	99.78	96.00	92.78	88.84	85.95
150°C	99.78	95.39	89.39	85.51	81.18
160°C	99.61	93.44	88.38	81.98	72.86
170°C	99.50	6.40	1.73	0.00	0.00

Table 2: Amount of drug remaining in %

	Time 0	1week	2week	3week	4week
26°C (RT)	4.6052	4.6057	4.6046	4.6052	4.6057
120°C	4.6068	4.5952	4.5800	4.5726	4.5436
130°C	4.6057	4.5742	4.5651	4.5352	4.5020
140°C	4.6030	4.5644	4.5303	4.4868	4.4538
150°C	4.6030	4.5580	4.4931	4.4486	4.3966
160°C	4.6013	4.5373	4.4816	4.4065	4.2885
170°C	4.6002	1.8568	0.5458	NA	NA

Table 3: Natural log of % drug remaining

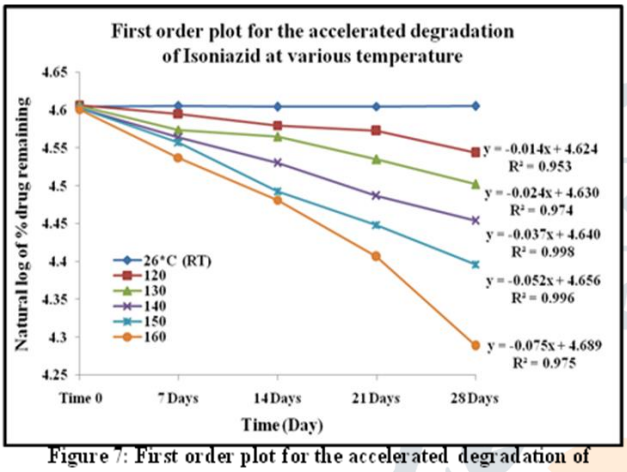

Figure 7: First order plot for the accelerated degradation of Isoniazid at various temperatures.

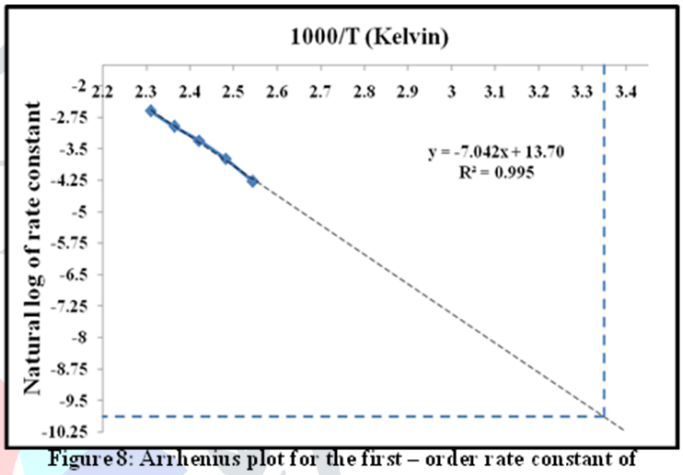

Figure 8: Arrhenius plot for the first – order rate constant of Isoniazid degradation over the temperature range of 120 – 160°C

From the first order plot for the accelerated degradation of Isoniazid (Fiure 8) at various temperatures, the rate constants were obtained, which were equal to the slope of the lines for the particular degradation temperature.

Temperature (T)	T (Kelvin)	1000/T	Rate constant	ln of Rate constant
120°C	393	2.5445	0.014	-4.2687
130°C	403	2.4813	0.024	-3.7297
140°C	413	2.4213	0.037	-3.2968
150°C	423	2.3640	0.052	-2.9565
160°C	433	2.3094	0.075	-2.5903

Table 4: Results obtained by first order degradation study for Isoniazid

IV . SHELF LIFE AND HALF LIFE CALCULATIONS:

0.1054/ kapp = t_{90}

Where; kapp = Reaction rate constant at particular temperature

t_{90} = Shelf life of the compound

A perpendicular is dropped on X axis from a value 3.35 (Inverse of Room temperature 26°C) (Figure 8)which coincides at a particular point on the extrapolated slope line. From this point again a perpendicular is dropped on Y axis which corresponds to a particular point which suggests the natural log value of rate constant at that temperature. The antilog of this value gives the direct value of rate constant at room temperature.

From the above graph (Figure 8) the rate of reaction at 26°C is found out to be approximately 0.0000502 day $^{-1}$.

Substituting this value of rate constant at 26°C in the above equation t_{90} can be calculated.

Therefore,
t_{90} = 0.1054/0.0000502
t_{90} = 2099.60 days
t_{90} = 5.75 years.

Similarly $\mathbf{t_{1/2}}$ can also be calculated using following formula,
$\mathbf{0.6931/ k_{app} = t_{1/2}}$
$t_{1/2}$ = 0.6931/0.0000502
$t_{1/2}$ = 13806.77 days
$t_{1/2}$ = 37.83 years

Activation energy:
The - slope of above graph = -Ea/R
Therefore
-7.042 = -Ea/R
Ea = 7.042 × 8.314
Ea = 58.547 kJ/Mol
Or
Ea = 58547 J/mol

Finally from the graph in (figure 8), looking at the straight line nature of the graph it can be stated that Isoniazid degradation follows a first order reaction mechanism.

Temperature	Rate constant (day^{-1})	t_{90} (days) at 26°C	t_{90} (years) at 26°C	$t_{1/2}$ (days) at 26°C	$t_{1/2}$ (years) at 26°C	Activation energy (Ea) kJ/mol
120°C	0.0140	2099.60	5.75	13806.77	37.83	58.55
130°C	0.0240					
140°C	0.0370					
150°C	0.0520					
160°C	0.0750					

Table 5: Summary of Results obtained by first order degradation study for Isoniazid

V .XRD ANALYSIS WAS CONDUCTED AS FOLLOWS:
Samples were filled in a glass holder and exposed to Cu Kα radiation (40 kV x 30 mA) in a wide angle X-ray diffractometer at ambient temperature. The instrument was operated in a continuous scan mode, over the angular range of 5 to 80° 2θ. X -ray powder diffraction patterns of the Isoniazid time 0 standard and the degradation samples (140, 150, 160°C) are given below. Based on the results, generated by HPLC technique, it was decided to analyze only two, three and four week samples for XRD.

VI . (DRS) IR SPECTROSCOPIC ANALYSIS WAS CONDUCTED AS FOLLOWS:
FTIR spectra of Isoniazid degradation samples were obtained using Shimadzu FTIR spectrophotometer using diffuse reflectance technique (KBr disc technique) as a part of qualitative analysis by comparing it with the spectra of Isoniazid standard. Samples of Isoniazid degradation sample powder and Isoniazid standard were previously ground and mixed with KBr, an infrared transparent matrix. The KBr discs were prepared by compressing the powder and the scans were obtained in the mid-infrared regions of the spectrum from 4000 – 400 cm-1. Infrared spectroscopic results (spectrum/pattern) of the Isoniazid time 0 standard and the degradation samples (140, 150, 160°C) are given below. Based on the results, generated by HPLC technique, it was decided to analyze only two, three and four week samples for IR spectroscopy.

VII .SUMMARY AND CONCLUSIONS:
A stability indicating High Performance Liquid Chromatographic (HPLC) method for routine analysis in a Quality Control (QC) lab was developed for the assay of Isoniazid. The developed HPLC method is a stability-indicating. Stability indicating nature of the method was established after subjecting the drug to accelerated stress temperature parameter. The method was properly validated and showed satisfactory data for all the method validation parameters tested. The HPLC method developed and validated for Isoniazid is rapid, precise and selective. The developed method was found "specific" to the drug as the peaks of the degradation products did not interfere with the drug peak. Thus the proposed method can be employed for assessing the stability of Isoniazid as a bulk drug.

Stability testing is usually performed to determine the product shelf life during the early stages of product development. The results obtained in the study demonstrate that Arrhenius equation could be applied for calculating Isoniazid shelf life, half life, rate constant and activation energy.

A secondary objective was to qualitatively compare the high temperature degradation assay results of HPLC with those of Infrared spectroscopy and X – Ray diffraction methods. Infrared spectroscopic and X – Ray diffraction results of degraded samples also showed significant changes in the graphical patterns when compared with fresh samples. Thus the data generated from these two techniques can be used as supporting data with data of HPLC analysis. HPLC analysis shows no changes in retention time of peak of interest with respect to temperature and time. However, differences are seen in x-ray diffraction and diffuse reflectance infrared spectroscopic studies. It may be due to internal morphology changes e.g. particle size, aggregation, loss of moisture, crystallanity etc. In summary, accelerated stability testing and Arrhenius equation available for interpreting the data can provide valuable information for evaluating reagents and control products. By optimizing the analytical precision and other aspects of test protocol design, one can expect both real time and accelerated stability studies to provide more valid information.

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