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Development and validation of a Stability-indicating Reversed-Phase High Performance Liquid Chromatography Method for assay of Prothionamide in Pure and Pharmaceutical Dosage form

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Abstract: A novel stability-indicating gradient reverse phase-high performance liquid chromatographic (RP-HPLC) method was developed for the determination of Prothionamide in pure and pharmaceutical dosage form was developed and validated. The chromatographic conditions comprised of a reversed-phase C_{18} column (250 x 4.6 mm), 5 µ with a mobile phase consisting of a mixture of Methanol: Buffer (0.02M KH_2PO_4) solution in the ratio (85:15) and pH adjusted to 4.5. Flow rate was 1 mL / min. Detection was carried out at 290 nm. The retention time of Prothionamide was 4.8 min. Prothionamide was subjected to acid and alkali hydrolysis, oxidation, photochemical degradation and thermal degradation. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 200-1200µg/ml. The value of correlation coefficient, slope and intercept were, 0.9998, 175.73and 672.36, respectively. The method was validated for precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, photochemical and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.
Key words: Prothionamide, chromatography, stability indicating, degradation.

INTRODUCTION:

Prothionamide, chemically, 2-propylpyridine-4-carbothioamide[1] is a drug used in the treatment of tuberculosis. It has also been tested for use in the treatment of leprosy[2,3] (**Figure: 1**). Literature survey reveals that UV, HPLC for determination of content uniformity and simultaneous estimation of Prothionamide is reported[4, 5, 6], but there is no stability indicating high-performance liquid chromatography

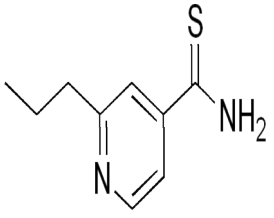

Figure:1 Chemical structure of Prothionamide

(HPLC) method for the determination of Prothionamide from its tablets, as its Pharmaceutical dosage form.

The International Conference on Harmonization (ICH) guideline entitled 'Stability Testing of New Drug Substances and Products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests[7, 8] (ICH, 1993, 1996). The hydrolytic and the photolytic stability are also required. An ideal stability-indicating method is one that quantifies the drug per se and also resolves its degradation products. A very viable alternative for stability-indicating analysis of Prothionamide is HPLC.

The aim of the present work was to develop an accurate, specific, reproducible, and stability indicating method for the determination of Prothionamide in the presence of its degradation products and related impurities as per ICH guideline.

MATERIALS AND METHODS

Materials

Prothionamide was supplied by Lupin Pharma, Mumbai, India and tablets (Label Claim: 250 mg per tablet, Product Name: PETHIDE and Manufacturer: Lupin Pharma was procured from the market. 0.02M Sodium dihydrogen Orthophosophate monohydrate LR Grade, Methanol and Orthophosphoric acid LR Grade were purchased from RFCL Ltd., New Delhi, India. High purity water was prepared by using Millipore Milli-Q plus water purification system.

Instrument used

The HPLC used was a shimazdu HPLC LC-20AT series with SPD-20A UV photodiode array detector and LCsolution software, Japan was used for all the experiments. The column used was XTerra® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Thermal Stability studies were performed in a dry air oven (Thermo labs, India). Micrositer syringer- 50 µ L (Hamilton Company, USA).

Methods

i. Chromatographic conditions

Chromatographic separation was achieved at ambient temperature on a reversed phase column using a mobile-phase consisting of a mixture of 0.02M Sodium dihydrogen Orthophosophate monohydrate and Methanol in water. Adjust pH of solution to 4.5 ± 0.05 with orthophosphoric acid) in the ratio (85:15). The mobile phase so prepared was filtered through 0.22 µm nylon membrane filter and degassed by sonication. Flow rate of 1 mL / min was maintained. Detection was carried out at 290 nm. The injection volume was 20 µL for assay and degradation level.

ii. Standard preparation

100 mg of Prothionamide working standard was accurately weighed and transferred to a 100 mL volumetric flask. Solution was sonicated and diluted up to the mark with mobile phase. A series of standard solutions in the concentration range of 200, 400, 600, 800, 1000 and 1200µg/ml were prepared followed by a suitable dilution of stock solution with the mobile phase.

iii. Sample preparation:

20 tablets were weighed and finely powdered. Blend equivalent to 50 mg of Prothionamide was transferred to a 100 mL volumetric flask. About 60 mL of mobile phase was added and the solution was sonicated for 15 min and make up to the mark with mobile phase. The resulting solution was filtered through 0.22µm nylon membrane filter. The solution was mixed well and centrifuged at 2500 RPM for 10 min.

iv. Preparation of calibration graph

The linearity of response for Prothionamide assay method was determined by preparing and injecting solutions with concentrations of about 200, 400, 600, 800, 1000 and 1200µg/ml of Prothionamide.

Method validation

i. Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application was carried out using six replicates of the same standard concentration (500 µg / mL for standard application). Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of marketed sample (500 µg / mL for sample application). It showed very low % relative standard deviation (% RSD) of peak area of Prothionamide.

ii. Accuracy

Accuracy (Recovery) study was performed by spiking 30, 50 and 70% of Prothionamide working standard to a preanalysed sample. The preanalysed sample was weighed in such a way that final concentration is half or 50% of the sample preparation before spiking. The percentage sum level of preanalysed sample and spiked amount of drug should be 80, 100 and 120% of simulated dosages nominal or target concentration of sample preparation. The accuracy of the analytical method was established in duplicate across its range according to the assay procedure.

$$\textbf{\% Recovery} = \frac{\%Amount\,\mathrm{Recov}ered}{\%SumLevel} \times 100$$

iii. Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The robustness and ruggedness of the method was established as the % deviation from mean assay value obtain from precision study is less than ±2.0%.

iv. Analysis of marketed formulation

20 tablets were weighed and finely powdered. Transfer blend equivalent to 100 mg of Prothionamide to a 100 mL volumetric flask. Add about 60 mL of mobile phase and sonicate for 15 min and make up volume with mobile phase. Mix well and centrifuge the solution at 2500 RPM for 10 min. Dilute the solution up to the desired concentration and inject it into the HPLC system.

Forced degradation studies

i. Preparation of acid and based- induced degradation product

Tablet powder equivalent to 100 mg of Prothionamide was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 1 N HCl was added and 5 mL of 1 N NaOH were added separately. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. The acidic forced degradation and the alkaline forced degradation was performed in dark in order to exclude the possible degradative effect of light. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

ii. Preparation of hydrogen peroxide - induced degradation product

Tablet powder equivalent to 100 mg of Prothionamide was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. To it 5 mL of 3.0% H_2O_2 was added. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

iii. Photodegradation product

Tablet powder equivalent to 100 mg of Prothionamide (previously kept in UV light for 24 hr) was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking and diluted up to the mark with mobile phase. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC system.

iv. Thermal degradation product

Tablet powder equivalent to 100 mg of Prothionamide was transferred to 100 mL volumetric flask. To it, 10 mL of mobile phase was added and sonicated for 15 min with intermittent shaking. The sample was heated on a boiling water bath for 30 min, cooled to room temperature and diluted to volume with mobile phase, mixed well. This solution was centrifuged at 2500 rpm for 10 min and 5 mL of supernatant liquid was transferred to 25 mL volumetric flask, diluted to volume with mobile phase, mixed well and injected into the HPLC. The specificity degradation study data for the determination of Prothionamide and its degradants in pharmaceutical dosage form is given in **Table: 4 & 6.** The no stress treatment sample (as control) has been evaluated relative to the standard concentration where as rest of the stressed condition samples **(Figure: 2 to 6)** is evaluated relative to the control sample with respect to the % assay and % degradation. The percentage degradation results are calculated by area normalization method.

Table: 1 Regression characteristics of the proposed HPLC method

S. No	Drug	Prothionamide
1.	Range (µg/ml)	200-1200µg/ml
2.	Mean 'R^{2}' value	0.9998
3.	Slope (m)	175.73
4.	Intercept (c)	672.36
5.	Retention Time (min)	4.8
6.	Theoretical Plates (N)	9746
7.	Tailing Factor	1.03

Table: 2 Method precision of Prothionamide

Sample Preparation	% Assay Prothionamide	% Deviation From Mean Assay value Prothionamide
1	99.87	0.03
2	100.35	0.51
3	99.45	-0.39
4	98.77	-1.07
5	99.83	-0.01
6	100.76	0.92
Mean	99.84	
±SD	0.633	
%RSD	0.634	

Table: 3 Ruggedness and robustness of Prothionamide

Parameter	Normal (Original)	Changed conditions
Column make	X Terra® RP18 column; 250 x 4.6 mm; 5 µ	Luna C_8 (Octylsilane), 250 x 4.6 mm; 5 µ
Flow rate	1 mL/min	1.5 mL/min
Mobile phase Composition	Buffer: Methanol Adjust pH4.5, (85:15).	Buffer: Methanol Adjust pH4.5, (75:25).
Pump	Jasco PU-2080 plus series	Shimazdu LC-20AT
Detector	Jasco UV-2075 plus series	Shimazdu UV-VIS detector
Analyst	Kalyan.B	Narasimha.V
% assay of Prothionamide	99.84%	99.81%
% deviation from mean assay value obtained in method precision studies for Prothionamide : 0.38%		

Table: 4 Recovery of Prothionamide

Sample preparation	% simulated dosage normal	% sum level	% amount recovered	% recovery	Mean % recovery
Preanalysed sample				99.84	
1	80	80.53	81.24	100.88	100.95
2	80	79.93	81.54	101.02	
1	100	99.75	101.81	102.07	101.51
2	100	100.43	101.42	100.95	
1	120	119.45	121.25	101.51	101.46
2	120	119.82	121.52	101.42	

Table: 5 Stressed study data of Prothionamide

S. No	Condition	% assay Prothionamide	Retention time of drug (min)	% Degradation
1	No stress treatment	99.84	4.8	Nil
2	Acid	99.58	2.15, 3.49	0.07
3	Alkali	99.49	2.62, 3.31	0.14
4	H_20_2	-	-	-
5	UV	98.67	3.12	0.05
6	Thermal	-	-	-

Table: 6 Summary of forced degradation results

S. No	Stress condition	Time	%Assay of active substance	Mass balance (%assay+ degradation products)	Remarks
1	Acid degradation (1 N HCl)	1/2 hr	99.58	99.65	No degradation products formed
2	Alkali degradation (1N NaOH)	1/2 hr	99.49	99.63	No degradation products formed
3	H_2O_2 degradation (3%)	1/2 hr	-	-	-
4	UV degradation	24 hr	98.67	98.72	Mild degradation formed
5	Thermal degradation 60^0C)	1/2 hr	-	-	-

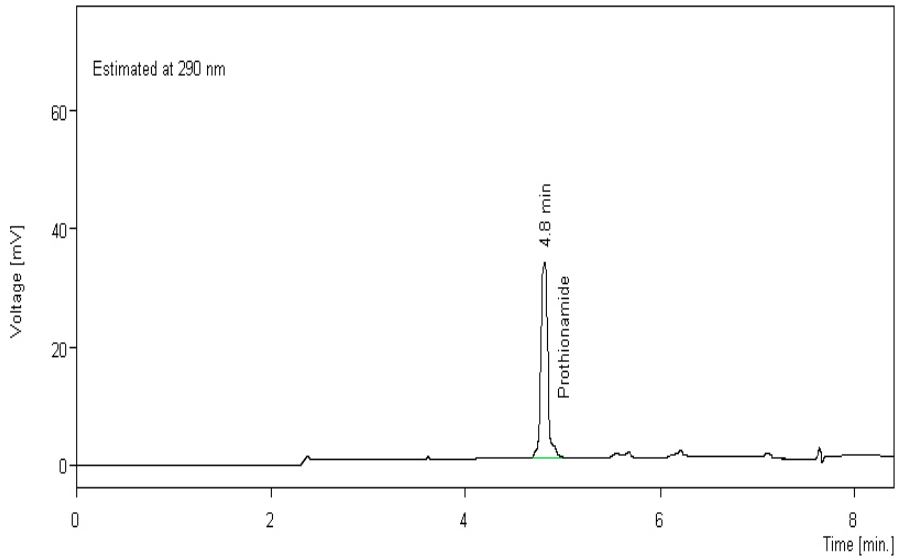

Figure: 2 The simple chromatogram of standard Prothionamide

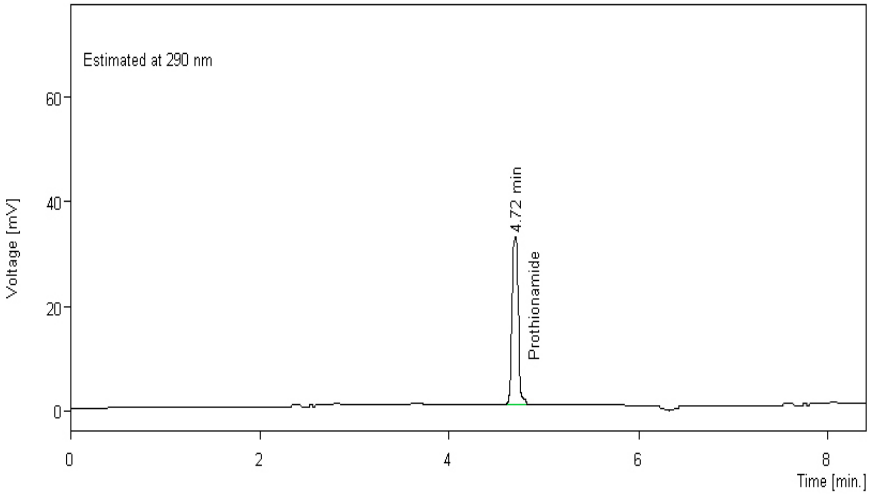

Figure: 3 The simple chromatogram of test Prothionamide

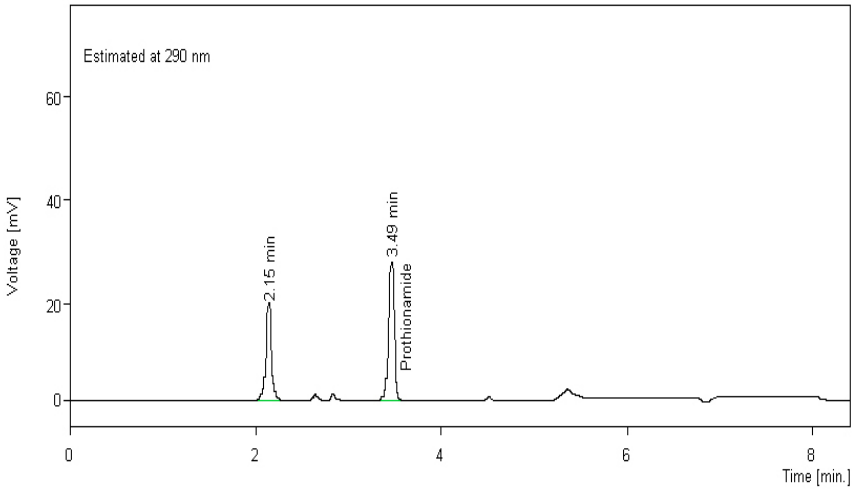

Figure: 4 The simple chromatogram of acid degraded sample.

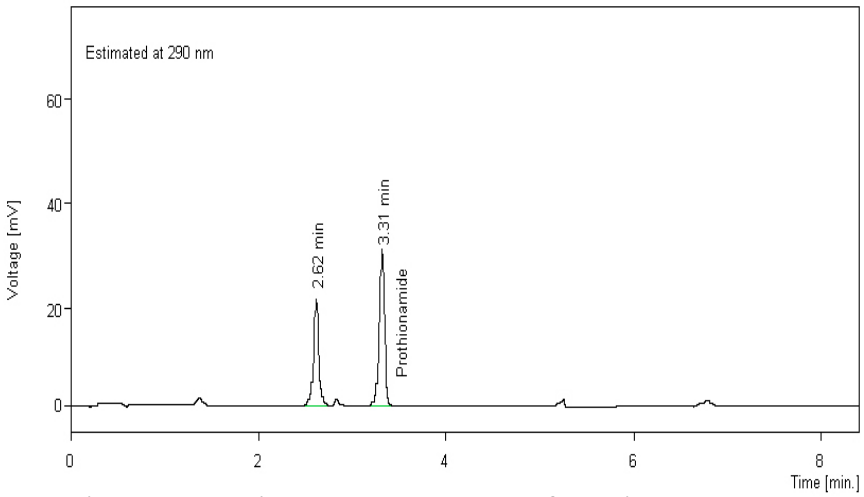

Figure: 5 The simple chromatogram of alkali degraded sample.

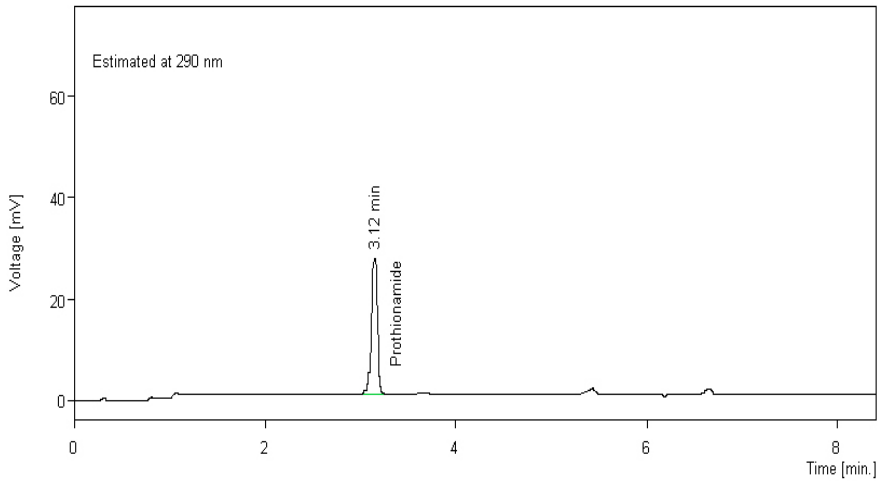

Figure: 6 The simple chromatogram of UV degraded sample.

RESULTS AND DISCUSSION

Method of development

The chromatographic conditions were optimized with a view to develop a stability- indicating assay method. Two different columns were tried as under chromatographic conditions namely, XTerra® RP18, 250 x 4.6 mm, 5 µ (water, Ireland) and Luna C_8 (Octylsilane), 250 x 4.6 mm, 5 µ (Phenomenax, USA). Luna C8 gave good peak shape but a lower retention. XTerra® RP18 column had given a good peak shape with response at affordable retention time. The chromatographic conditions finally comprised of a mixture of 0.02M Sodium dihydrogen Orthophosophate monohydrate and Methanol in water in the ratio (85:15) at a flow rate of 1 mL / min using XTerra® RP18 column; 250 x 4.6 mm; 5 µ (G. L. Sciences, Japan) at 290 nm.

Calibration curve

These results indicate that the response is linear over the range of 200, 400, 600, 800, 1000 and 1200µg/ml of Prothionamide with coefficient of regression, R^2, value as 0.9998. The value of correlation coefficient, slope and intercept were, 0.9998, 175.73 and 672.36, respectively as shown in **Table: 1**.

Validation of the method

i. Precision

The %RSD for repeatability of sample preparation is 0.634%. This shows that precision of the method is satisfactory as % relative standard deviation is not more than ± 2.0%. Table: 8.2 represent the precision of method.

ii. Ruggedness and robustness of the method

Method robustness and ruggedness was determined by analysing same sample at normal operating conditions and also by changing some operating analytical conditions such as column make, mobile phase composition, flow rate, instrument and analyst. The deliberate aforementioned changes in parameters alters the result of Prothionamide 0.38% to method precision study, which is not a significant change. The robustness and ruggedness of the method is established as the % deviation from mean assay value obtain from precision study is less than ±2.0%. **Table: 3** represent the ruggedness and robustness of the method.

iii. Accuracy

The accuracy of the method was established by recovery studies. Results indicate that the individual recovery of Prothionamide ranges from 100.88% to 102.07% with mean recovery of 101.31% and % relative standard deviation of 0.414%. The recovery of Prothionamide by proposed method is satisfactory as % relative standard deviation is not more than ± 2.0% and mean recovery between 98.0 - 102.0%.

iv. Analysis of the marketed formulation

The drug content was found to be 99.81% with a % RSD of 0.634%. It was noted that no degradation of Prothionamide had occurred in the marketed formulation that were analysed by this method. The low RSD value indicated the suitability of this method for routine analysis of Prothionamide in pharmaceutical dosage form.

v. Stability- indicating property

The chromatogram of no stress treatment sample (as control) showed no additional peak **(Figure: 2&3).** The retention time (RT) of standard and sample were 4.8 min and 4.77 min respectively. The chromatogram of acid degraded sample showed additional peaks at retention time (RT) of 2.15 and 3.49 min respectively **(Figure: 4).** The chromatogram of alkali degraded sample showed additional peaks at RT of 2.62 and 3.31 min respectively (**Figure: 5).** The chromatogram of hydrogen peroxide degraded sample showed no additional peaks. The chromatogram of UV degraded sample showed additional peaks at RT of 3.12 min **(Figure: 6).** The chromatogram of thermal degraded sample showed no additional peak the values were shown in **Table: 5**. Rest of the peaks, if any, were from its blank or placebo in each of these specified conditions. In each forced degradation samples were additional peaks were observed, the response of the drug was changing from the initial control sample. This indicates that the drug is susceptible to acid-base hydrolysis degradation and UV degradation.

CONCLUSION

The developed HPLC technique is precise, specific, accurate and stability indicating. Statistical analysis proves that the method is reproducible and selective for the analysis of Prothionamide in pharmaceutical dosage form. The method can be used to determine the purity of the drug available from various sources. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

REFERENCES:

1. Wang F, Langley R, Gulten G. Mechanism of thioamide drug action against tuberculosis and leprosy. The Journal of Experimental Medicine, 2007, 204 (1), 73–8.
2. Fajardo TT, Guinto RS, Cellona RV, Abalos RM, Dela Cruz EC, Gelber RH. A clinical trial of ethionamide and prothionamide for treatment of lepromatous leprosy. American Journal of Tropical Medicine and Hygiene, 2006, 74 (3): 457–61.
3. K. Venkatesan. Clinical Pharmacokinetic considerations in the treatment of Patients with Leprosy. Clinical Pharmacokinetics, 1989, 16, 365-386.
4. The International pharmacopoeia By World Health Organization, 4th edition, 2006, 804.
5. Elvira Richter, Sabine Rüsch-Gerdes and Doris Hillemann. Drug-susceptibility testing in TB: current status and future prospects. Expert Review of Respiratory Medicine, 2009, 3(5), 497-510.
6. P. J. Jenner, G. A. Ellard, P. J. K. Gruer and V. R. Aber. A comparison of the blood levels and urinary excretion of ethionamide and prothionamide in man. Journal of Antimicrobial Chemotherapy, 1984, 13, 267-277.
7. ICH, Q2B (1993). Validation of Analytical Procedure: Methodology,International Conference on Harmonization, Geneva, March 1996.
8. ICH, Q1A Stability Testing of New Drug Substances and Products, International Conference on Harmonization, Geneva.

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