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Forced Degradation Study for Assay Method of Rifampicin, Isoniazid and Pyrazinamide in Combined Pharmaceutical Dosage Form

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ABSTRACT

As HPLC method is time consuming, very accurate and precise method was developed for simultaneous estimation of Rifampicin, Isoniazid and Pyrazinamide from Rifampicin, Isoniazid, Pyrazinamide and Ethambutol dosage form by UPLC. Forced degradation study was performed on this method to establish the method is stability indicating.

The liquid forced degradation and solid degradation study was carried out on Acquity UPLC @BEH C18, (100 mm x 2.1 mm), 1.7 µm column using the gradient composition of mixture of phosphate buffer pH 6.8 and acetonitrile in ratio of 96:4 v/v as mobile phase A and mixture of phosphate buffer pH 6.8 and acetonitrile in ratio of 45:55 v/v used as mobile phase B at flow rate 0.25 mL/min and detection wavelength 238 nm.

Key-words: Development and validation of UPLC method for Rifampicin, Isoniazid and Pyrazinamide.

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1 Introduction:

The new developed assay method on UPLC is simple, accurate, precise, linear and rugged. Method was validated as per ICH guidelines[1,2,3] for simultaneous estimation of Rifampicin, Isoniazid, and Pyrazinamide in tablet dosage form. To demonstrate that the developed method is stability indicating the forced degradation study preformed[4].

Rifampicin (7*S*,9*E*,11*S*,12*R*,13*S*,14*R*,15*R*,16*R*,17*S*,18*S*,19*E*,21*Z*)-2,15,17,27,29-pentahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-26-{(E)-[(4-methylpiperazin-1-yl)imino]methyl}-6,23-dioxo-8,30-dioxa-24-azatetracyclo triaconta-1(28),2,4,9,19,21,25(29),26-octaen-13-yl acetate is a semisynthetic derivative of rifamycin, an antibiotic produced by Streptomyces mediterranei. It is active in vitro against gram positive and gram negative cocci, some enteric bacteria, mycobacteria and chlamydia.

Isoniazide is pyridine-4-carbohydrazide is a chemo not antibiotic. Isoniazid is the most active drug for the treatment of tuberculosis caused by susceptible strains.

Pyrazinamide is pyrazine-2-carboxamide is a relative of nicotinamide, stable and slightly soluble in water.

Purpose of this study is demonstrate the assay method is stability indicating method for combination drug dosage form of Rifampicin, Isoniazid, Pyrazinamide and Ethambutol tablet.

2 MATERIALS AND METHODS

2.1 Chemical and Reagents

Rifampicin, Isoniazide and Pyrazinamide API, used having purity 99.8%, 99.2% and 99.7%, respectively. The combination tablet dosage form of Rifampicin, Isoniazid, Pyrazinamide and Ethambutol contain 150, 75, 400 and 275mg strength tablets of Macleods Pharmaceutical Ltd. Mumbai, India were used. Water (Milli-Q), Anhydrous dibasic sodium phosphate, Methanol, Acetonitrile, Orthophosphoric acid, were purchased from Ranchem. All the chemical used were of HPLC grade.

2.2 Instruments / Equipments

Waters Acquity UPLC with UV detector having Acquity UPLC @BEH C18, (100 mm x 2.1 mm) 1.7 µm , Analytical Balance (Sartorius), pH meter(Lab India), used for the study.

2.3 Method:

The separation of drug was achieved with gradient method on a reverse phase Acquity UPLC @BEH C18, (100 mm x 2.1 mm) 1.7 µm column at wavelength 238nm, injection volume 1.7µl and column oven temperature was 35°C and sample temperature was 10°C. The gradient program is of 6 minutes and is as follow.

Tabel 1: Gradient program

Time (mins)	Mobile phase Ratio A:B (%)	Comment
0	95:05	Linear gradient
1.5	0:100	Linear gradient
3.5	0:100	Linear gradient
3.51	95:05	Linear gradient
6.00	95:05	Re-equilibration

Buffer solution:

Buffer was prepared by dissolving about 1.4 g of anhydrous dibasic sodium phosphate in 1000 mL of water, mixed. Adjusted the pH of the solution to 6.8 ± 0.05 with orthophosphoric acid, mixed. Filtered.

Diluent: Prepared a mixture of methanol and buffer solution (4 : 96 v/v), degas.

Mobile phase A: Prepared a mixture of buffer solution and acetonitrile (96 : 4 v/v), degassed

Mobile phase B: Prepare a mixture of buffer solution and acetonitrile (45 : 55 v/v), degassed.

Standard preparation:

16 mg Rifampicin reference/ working standard, 8 mg of Isoniazid reference/ working standard and 43 mg of Pyrazinamide reference/ working standard was taken in to a 100 mL volumetric flask. Added 4 mL methanol; sonicated for 2-3 min. Added about 50 mL of buffer solution and sonicated to dissolve. Allowed to equilibrate to room temperature and make up the volume with buffer and mix.

Sample solution preparation:
Weighed 20 tablets selected at random and crushed them to a fine powder. Transferred an accurately weighed quantity of crushed tablet powder, equivalent to 8 mg of Isoniazid to a 100 mL volumetric flask. Added 4 mL of methanol and sonicated for 2-3 min. Added 50 mL of buffer. Sonicated for 5 min. Allowed to equilibration to room temperature, diluted with buffer to volume. Filtered through PVDF syringe filter (Axiva or equivalent) by discarding the first few mL of the filtrate. The final concentration of solution was 160, 80 and 430 µg/mL of Rifampicin, Isoniazide and Pyrazinamide, respectively.

3 EXPERIMENTALS

3.1 Forced degradation study:

Stress testing[5] (forced degradation study) help to identify the degradation products, stability of the molecules and also validate the stability[6] and specificity of the analytical procedure. Study was performed for following factor.

- Thermal degradation with Heated the powder at 80°C for 14 hours.
- Photolytic degradation[7], Powder covered with aluminum foil exposed. Also powder was directly exposed (without aluminum foil) in the photo stability chamber, as per ICH guidelines.
- Thermal and Humidity degradation with at 40°C/75%RH for 24 hours exposed.
- Acid degradation with acid media, powder was kept in 0.01M HCl for 1 hrs at 40°C on water bath.
- Base degradation with base media, powder was kept in 0.01M NaOH for 2 hrs. at room temperature.
- Oxidative degradation with Oxidative degradation media, powder was kept in 5 ml of 0.03% H_2O_2 for 2 hrs. at 40°C on water bath.
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3.1.1 Summary of forced degradation results:

The summary of degradation given with Tabel 2. The maximum degradation was observed in thermal degradation (Solid degradation) and acid degradation (Liquid degradation) chromatograms of same were shown in Figure 1 & 6.

4 Results

4.1 Forced degradation study:

Table 2: Forced degradation summary result for Assay method of Rifampicin, Isoniazide and Pyrazinamide

Force Degradation condition	% Degradation			Peak Purity		
	Rifa	Iso	Pyra	Rifa	Iso	Pyra
Initial	----	----	----	Peak Pure	Peak Pure	Peak Pure
Heat at 80°C in oven for 14 hours (Thermal Degradation)	X	13.6	0.8	Peak Pure	Peak Pure	Peak Pure
Photolytic degradation, as per ICH guidelines (Control)	----	----	----	Peak Pure	Peak Pure	Peak Pure
Photolytic degradation, as per ICH guidelines (Exposed)	3.8	X	X	Peak Pure	Peak Pure	Peak Pure
Thermal and Humidity at 40°C/75% RH for 24 hours	3.4	4.9	X	Peak Pure	Peak Pure	Peak Pure
5 mL of 0.01 M HCl heated at 40°C for 1 hr on water bath.	0.8	8.7	4.5	Peak Pure	Peak Pure	Peak Pure
5 mL of 0.01 M NaOH kept at room temperature.	5.7	X	2.6	Peak Pure	Peak Pure	Peak Pure
5 mL of 0.03 % H_2O_2 heated at 40°C for 2 hours on water bath	X	3.5	0.5	Peak Pure	Peak Pure	Peak Pure

Note: X indicates No degradation, Rifa indicates Rifampicin; Iso indicates Isoniazide and Pyra indicates Pyrazinamide

The peaks due to Rifampicin, Isoniazide and Pyrazinamide were found to the spectrally pure in all the degradation conditions, indicating that there was no co-elution with main peaks. Based on the above results it was concluded that the method for assay of Rifampicin, Isoniazide and Pyrazinamide estimation in Rifampicin, Isoniazide, Pyrazinamide and Ethambutol Hydrochloride tablets (150 mg / 75 mg / 400 mg/ 275mg) tablets was specific and stability indicating.

Figure 1 : Chrmatograph of Acid Degradation

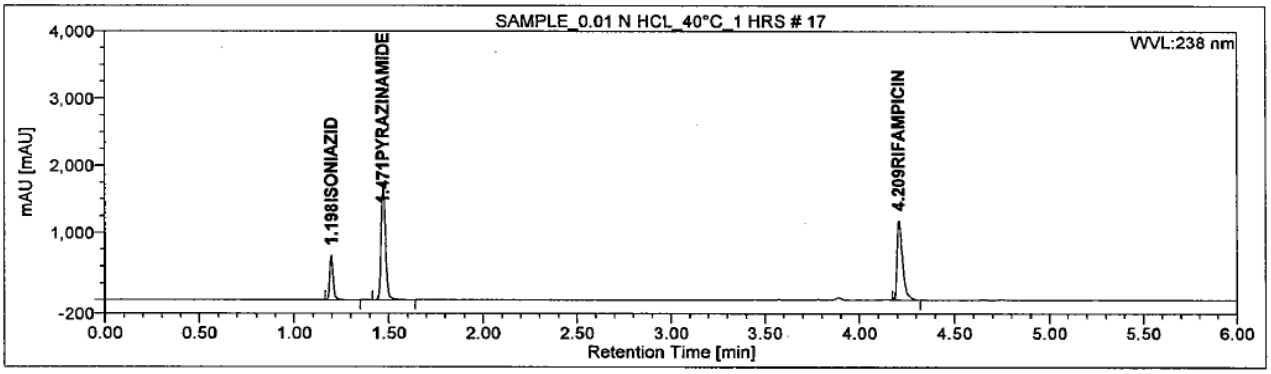


Figure 2 : Chrmatograph of Base Degradation

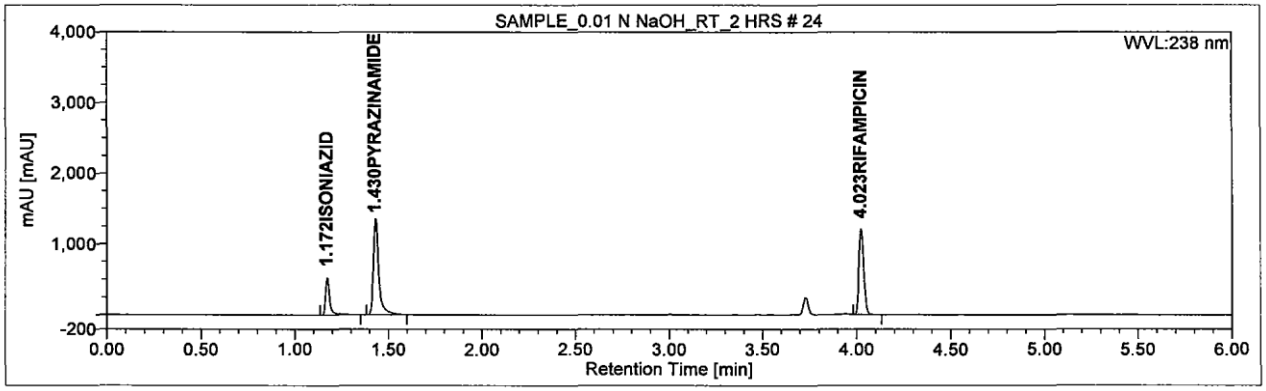


Figure 3 : Chrmatograph of Peroxide Degradation

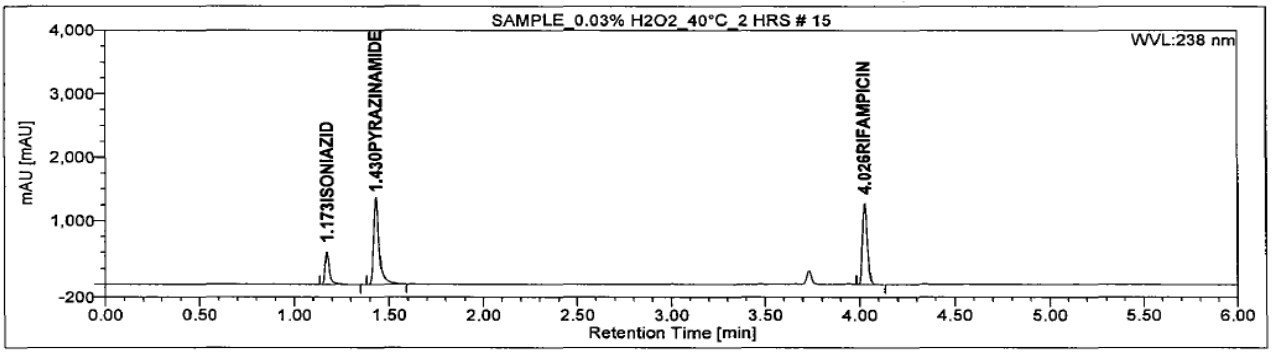


Figure 4 : Chrmatograph of Photocontrol Degradation

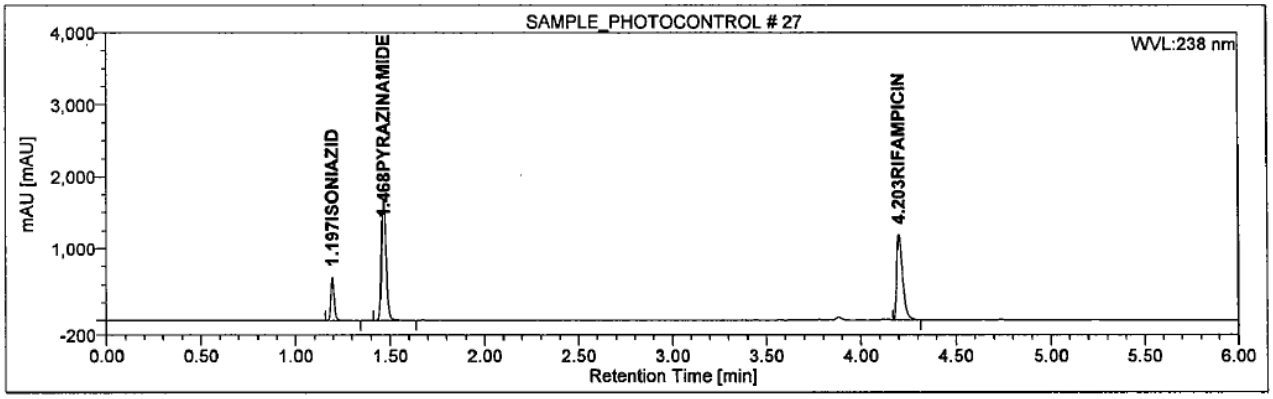


Figure 5 : Chrmatograph of Photo Degradation

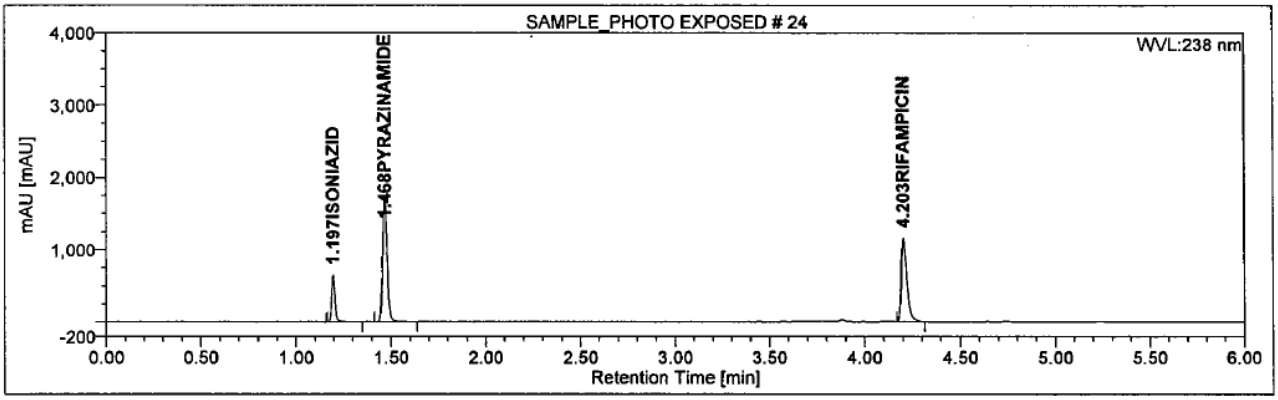


Figure 6 : Chrmatograph of Thermal Degradation

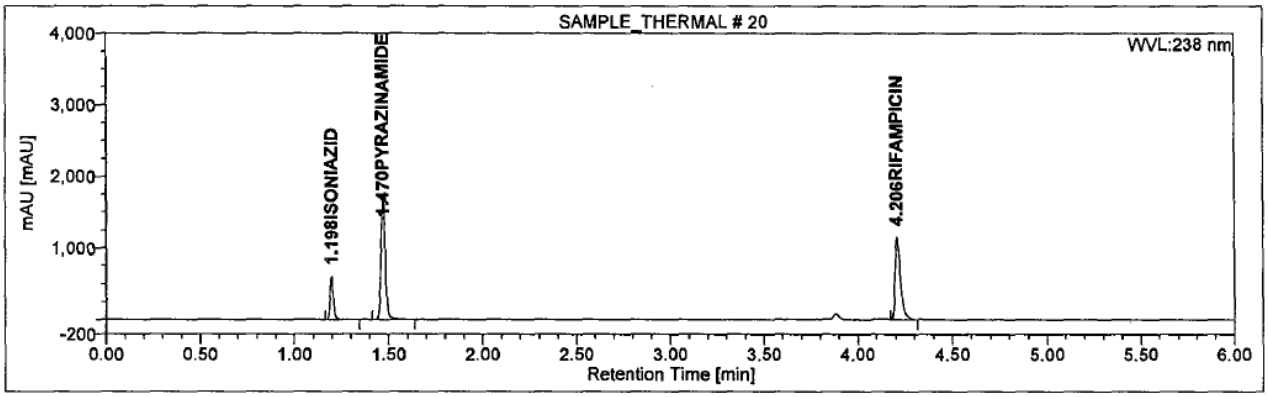
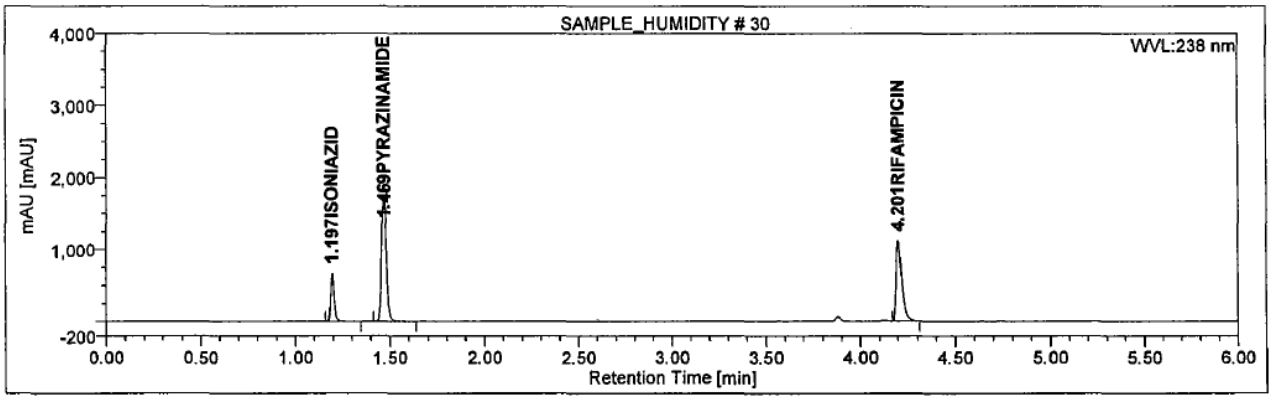


Figure 7 : Chrmatograph of Thermal & Humidity Degradation



5 Conclusion

The forced degradation study was performed on validated method for the determination of assay of Rifampicin, Isoniazide and Pyrazinamide in Rifampicin, Isoniazide, Pyrazinamide and Ethambutol Hydrochloride tablets (150 mg / 75 mg / 400 mg/ 275mg) and found that method is stabilitry indicating hence concluded that the method has been suitable for its intended use, i.e. to determine the assay of Rifampicin, Isoniazide and Pyrazinamide in Rifampicin, Isoniazide, Pyrazinamide and Ethambutol Hydrochloride tablets (150 mg / 75 mg / 400 mg/ 275mg).

References:

1 International Conference on the Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Validation of Analytical Procedures:Text and Methodology Q2 (R1). (2005). Available from http://www.ich.org

2 International Conference on Harmonization (ICH) Q8(R2): Pharmaceutical Development (August 2009).

3 International Conference on Harmonization (ICH) Q11: Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities) (May 2011)

4 United States Pharmacopeia/National Formulary
General Chapter <621> Chromatography
General Chapter <1010> Analytical Data-Interpretation and Treatment
General Chapter <1224> Transfer of Analytical Procedures
General Chapter <1225> Validation of Compendial Procedures
General Chapter <1226> Verification of Compendial Procedures

5 Sudha T. et al : Stability indicating RP-HPLC method for the simultaneous estimation of the anti-retroviral drugs and in tablet dosage forms IJBPAS, October, 2012, 1(9): 1322-1335.

6 Chirlange S., et al.: Stability Indicating HPTLC method for the Simultaneous estimation of Rilpivirin, Isoniazide and Pyrazinamide in Bulk and Combined Pharmaceutical Dosage Form. Journal of Advanced Drug Delivery 2014; 1(4); 157-172

7 ICH Harmonised Guidleine Stability Testing: Photostability Testing of New Drugs Substances and Products Q1B.