Original Article

Reconstituted powder for suspension of antitubercular drugs formulated as microspheres for pediatric use

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ABSTRACT: The aim of the present investigation was to develop a novel dosage form of rifampicin and isoniazid to minimize degradation of rifampicin in acidic medium, to modulate the release of rifampicin in the stomach and isoniazid in the intestine, and to provide pediatric compliance. Rifampicin slowly diffuses out through this hydrogel matrix, thereby sustaining its release (50.08%). The release of isoniazid was thus very low in an acidic environment, *i.e.* simulated gastric fluid (SGF) pH 1.2 (18.98%), while in simulated intestinal fluid (SIF) pH 7.4 the release was sustained and prolonged (76.98%). Good results were obtained for a period of 36 h in SIF pH 7.4 with isoniazid-alginate microspheres. The drug content was calculated on the basis of the drug entrapment efficiency of the individual microsphere formulation (gelatin, 82.32% and sodium alginate blends, 89.31%). Results revealed that an optimized formulation had a sedimentation volume of 0.4. This optimized formulation was found to be stable. Degradation of isoniazid was faster than that of rifampicin. The degradation rate constant at 25°C was found to be 1.9286 × 10^{-4} (day^{-1}), so the formulation was predicted to have a shelf life of 1.518 years.

Keywords: Reconstituted powder, Suspension, Antitubercular drugs, Degradation, Pediatric use

Introduction

Tuberculosis is a major cause of childhood morbidity and mortality. Estimates indicate that about 6-8%, *i.e.* 1.3 million, of all new Tuberculosis (TB) cases and 4.5 lac deaths are in the pediatric age group (*1*). Although children can present with TB at any stage, the majority of the cases is seen at ages 1-4 due to children's low resistance to progression of disease infection. Despite this, a surprising fact is that, globally, national TB control programs have continued to accord a low priority to childhood TB (*2*).

Sodium alginate is a purified carbohydrate product extracted from brown seaweed by the use of dilute alkali. It consists chiefly of the sodium salt of alginic acid; a polyuronic acid composed of β-(1→4)-D-mannosyluronic acid and α-(1→4)-L-gulosyluronic acid residues linked so that the carboxyl group of each unit is free while the aldehyde group is shielded by a glycosidic linkage (*3,4*).

Chitin, the polysaccharide polymer from which chitosan is derived, is a cellulose-like polymer consisting mainly of unbranched chains of *N*-acetyl-D-glucosamine. Deacetylated chitin, or chitosan, is comprised of chains of D-glucosamine. When ingested, chitosan can be considered a dietary fiber (*5,6*).

Gelatin contains a large number of glycine (almost 1 in 3 residues, arranged every third residue), proline and 4-hydroxyproline residues. A typical structure is [Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro] (*7*).

Isoniazid (INH) is an antimycobacterial agent widely used in first-line therapy for tuberculosis. The drug is characterized by a short half-life ranging from 1-4 h, depending on the rate of metabolism. INH has a pronounced absorption from all the three sections of the small intestine (*8*) and from intramuscular (IM) injection sites. INH is inactivated in the liver, mainly by acetylation and dehydrazination; the rate of acetylation is genetically determined and subject to individual variation. Long-term continuous therapy with INH leads to hepatotoxicity and peripheral neuritis. Thus, a drug formulation must have controlled release of INH, especially in the small intestine. Rifampicin was selected as a model drug for this research. Rifampicin with a naphthaquinone group is an antitubercular drug used for the treatment of tuberculosis. Rifampicin is

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completely eliminated from the gut after oral intake. On account of the high dose, attempts were made to develop a controlled-release formulation of rifampicin for sustained action (*9*).

Studies are being carried out in the authors' laboratory to determine the reason for the drop in bioavailability of rifampicin from certain fixed-dose combination (FDC) antituberculosis products. Data were provided earlier to show that rapid decomposition of rifampicin in the presence of isoniazid in FDC antituberculosis products in an acidic stomach environment was the likely reason behind the appearance of the problem (*10-13*). In one recent investigation, the focus was primarily on understanding why the bioavailability problem of rifampicin in FDC products appeared more prominent when bioequivalence studies were done on multiple marketed formulations (*14,15*) than when studies involved single test formulations (*16,17*).

Thus aim of this work is to improve the oral bioavailability of isoniazid in the presence of rifampicin by minimizing the chemical interaction between the metabolites of rifampicin with isoniazid under acidic conditions. Isoniazid and rifampicin are both first-line antitubercular drugs with a half-life ranging from 3-4 h and are available on the market in solid dosage form, leading to low compliance in pediatrics. Hence, formulation of a reconstituted powder for suspension using micro-particulate technology can resolve this problem.

Materials and Methods

Isoniazid was generously donated by Lupin Research Park (Pune, India), sodium alginate was purchased from Central Drug House (CDH, India), and all other materials and solvents were from Sigma (St. Louis, MO, USA). Rifampicin was generously donated by Lupin Research Park (Pune, India). Gelatin type B (250 blooms, pH of 1% aqueous solution of 4-5) was purchased from Sigma. Sucrose was purchased from CDH (India) and all other materials and solvents were from Sigma.

Preparation of microspheres

Sodium alginate solutions (3-5%) were prepared by dissolving sodium alginate in 10 mL of warm water. To this solution was added 1% of chitosan dissolved in acetic acid. The whole solution was stirred for 2 h at a temperature of 40ºC and then 0.8 mL of plasticizer, dibutyl phthalate, and isoniazid (5%) were added. The resulting polymer solution was emulsified in light liquid paraffin containing 2% Span 80. The emulsion was stirred for 1 h to ensure complete emulsification. To this was added calcium chloride solution, and the dispersion was stirred for another 10 min. Microspheres were collected by filtration, washed with isopropanol three times, and finally dried at room temperature.

To the 15% gelatin solution were added 3-5% of sucrose and 2% rifampicin. The resulting solution was warmed to 40°C and the warm solution was mixed with 40 mL of liquid paraffin which was kept at 40°C. The mixture was stirred for 5 min with a three-blade paddle (diameter: 35 mm) at 2,000 rpm to form a water-in-oil emulsion. Next, the emulsion was rapidly cooled to 15°C and then 150 mL of acetone were added in order to dehydrate and flocculate the coacervated droplets. The residual solvent was removed with *n*-hexane. After preparation, the microspheres were maintained at room temperature in a desiccator until use.

Preparation of reconstituted powder for suspension

The oral drug delivery system was prepared by using an equivalent amount of isoniazid-alginate blends and rifampicin-gelatin microspheres as in marketed FDC products. Sugar and vanilla were added to the equivalent amount of microspheres as a sweetening agent and flavoring agent, respectively, to increase the palatability of the formulation. Xanthum gum and sodium benzoate were added to the formulation as a viscosity enhancer and preservative, respectively.

Evaluation of the reconstituted powder for suspension

Sedimentation volume determination

Sedimentation volume (F) was determined by measuring the ultimate volume of sediment with respect to the total volume of suspension. The optimized formulation of powder was dissolved in 10 mL of distilled water. The suspension was kept undisturbed for 10 min in a 10-mL measuring cylinder. The volume of sediment was measured after 10 min.

In vitro dissolution studies

In vitro dissolution studies of the formulation were carried out in simulated gastric fluid (SGF) pH 1.2 and simulated intestinal fluid (SIF) pH 7.4 to study drug interaction and to assess the efficacy of the combination product. Samples were taken at regular intervals and replaced with fresh dissolution medium. The absorbance was measured and the concentration was determined by the UV method at 262 nm and 473 nm for isoniazid and rifampicin, respectively.

In vivo study

Gamma scintigraphy studies were carried out to determine the location and distribution of microspheres upon oral administration and the extent of transit through the gastro-intestinal tract (GIT). For this study, the microspheres were labeled with ^{99m}Tc-pertechnetate,

eluted from a ^{99}Mo-^{99m}Tc generator supplied by Amersham Biosciences (USA), and the experimental animals were New Zealand white rabbits (3-3.5 kg) and Adult Wistar rats of either sex (300-350 g, 15-20 weeks old).

^{99m}Tc-pertechnetate-isoniazid-alginate blend microspheres were prepared by dissolving 3.5 mg of microspheres in 1 mL of distilled water in a sterile glass vial followed by different concentrations of stannous chloride dehydrates (25-200 µg); pH varied from 5-7.5. The contents were filtered through a 0.22-mm membrane filter into a sterile vial. Approximately 18.5 MBq ^{99m}Tc-pertechnetate were added to this, and the result was mixed and incubated for 5-10 min. Then, the complex was subjected to quality control.

Radiolabelling efficiency was evaluated with Instant Thin Layer Chromatography-Silica gel (ITLC-SG) strips as the stationary phase and acetone 100% as the mobile phase.

$$\% \text{ Radiolabelling} = \frac{\text{Radioactivity (counts) retained in the lower half of the strip}}{\text{Initial radioactivity associated (total count present) with the strip}} \times 100$$

Radiochemical impurity that is likely to exist in the form of unconjugated technetium was determined by ascending ITLC-SG.

$$\% \text{ R/H technetium} = \frac{\text{Counts present in the lower part of strip}}{\text{Total count present in the strip}} \times 100$$

For whole-body imaging, 37 MBq of activity in 1 mL of preparation was administered orally to four rabbits weighing between 3-3.5 kg. The microspheres were administered by gavage in a dose of 2 mg/mL after overnight fasting for 8-10 h. Animals were given free access to water, but food was restored 1-2 h after dosing. The animals were anaesthetized with diazepam and serial scintigraphic examination was done at 4 and 24 h to assess the mobilization of the microspheres in the GIT using a large field view gamma camera (Siemens) equipped with a high-resolution, parallel-hole collimator and linked to a dedicated computer. Images were recorded for a preset time of 5 min/view with a 15% window centered to include the 140 KeV photo peak of ^{99m}Tc.

Stability study of the formulation

A stability study of optimized formulation was carried out as per WHO Guidelines. For the estimation of drug content in the microspheres, the stability-indicating HPTLC method was used. Microspheres were packed in laminated aluminium foil and kept ina stability chamber maintained at temperatures of 40 ± 0.5°C, 50 ± 0.5°C, and 60 ± 0.5°C for 90 days. Samples were taken at intervals of 0, 30, 60, and 90 days. The samples were analyzed for their drug content by HPTLC analysis using a standard curve (For isoniazid, AUC = 1.7426 × concentration; for rifampicin, AUC = 4.4781 × concentration).

The slope of each line was obtained and degradation rate constant (K) was calculated by the formula

$$\text{Slope} = -K/2.303$$

where K is the degradation rate constant. The value of K at 25°C (K_{25}) was obtained by extrapolation of the plot and shelf life was then calculated by substituting K_{25} in the following equation

$$T_{0.9} = 0.1054/K_{25}$$

where $T_{0.9}$ is the time required for 10% drug degradation and is referred to as shelf life.

Result and Discussion

Sugar was added as a sweetening agent and vanilla was used as a flavoring agent in an equivalent amount of microspheres, increasing the palatability of the formulation. Xanthum gum and sodium benzoate were added to the formulation as a viscosity enhancer and preservative, respectively (Table 1).

The present study was an attempt to develop a suitable oral drug delivery system for isoniazid and rifampicin in order to prevent their interaction in gastric medium. Microparticulate technology has been substantially explored for the targeted delivery of drugs by the parenteral route. It has also been utilized for oral delivery of drugs and bioactives primarily to bypass first-pass hepatic metabolism and sustain drug levels. An extensive survey of the literature survey indicated the potential for such a delivery system to achieve the desired purpose.

Reported data on drug permeability studies suggested that rifampicin was absorbed well from the stomach and isoniazid from the intestine (*18*). A wide range of both enteric as well as non-enteric biodegradable polymers was thus screened for the study. Sodium alginate and gelatin were selected due to the ease of preparation of microspheres from these polymers.

Chemically, sodium alginate is a carbohydrate and is

Table 1. Formula of optimized formulation of the reconstituted powder for suspension

Ingredients	Formulae
Microspheres	266.20 mg
Sugar	133.10 mg
Xanthum Gum	3%
Vanilla flavor	1%
Sodium benzoate	0.5%

made up of homopolymeric blocks of mannuronic and guluronic acids. Calcium ions bind to the polyuronic acid groups present in the polymer chains to form a 3D-network, which swells when water is consumed. In situ drug loading was done by dissolving or dispersing a known quantity of each drug in an aqueous polymer solution before emulsification in the oil phase. Water-soluble drugs are present in the internal phase of the w/o emulsion and thus are efficiently entrapped in the hydrogel matrix.

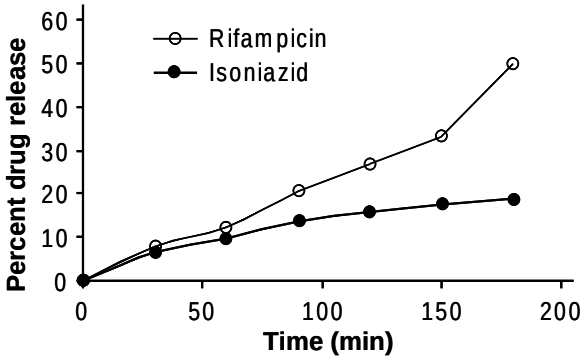
Chemically, gelatin is a protein and is made up of large numbers of glycin, proline, and 4-hydroxyproline amino acids. Sugar binds to the free amino groups present in the polymer chains to form a 3D-network, which swells when water is consumed. In situ drug loading was done by dissolving or dispersing a known quantity of each drug in an aqueous polymer solution before emulsification in the oil phase.

Gelatin swells in an acidic environment (SGF pH 1.2), forming minute hydrogel microparticles. Rifampicin slowly diffuses out through this hydrogel matrix, thereby sustaining its release (50.08%). However, burst release was observed in SIF pH 7.4 due to erosion of the matrix. Sodium alginate-blend microspheres also release the drug by diffusion in SGF pH 1.2 while in SIF pH 7.4 the drug release mechanism involves both diffusion of the drug through the capillaries formed as well as slow erosion of the matrix. The release of isoniazid was thus very low in an acidic environment (18.98%), while in SIF pH 7.4 the release was sustained and prolonged (76.98%). Good results were obtained for a period of 36 h in SIF pH 7.4 with isoniazid-alginate microspheres.

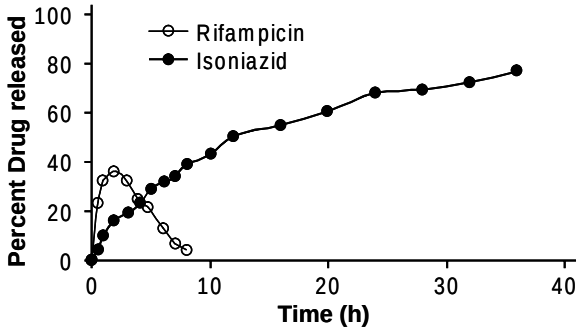
A reconstituted powder for suspension was formulated to consist of an accurately weighed amount of drug-loaded microspheres, sweetening agent, viscosity enhancer, and flavoring agent for greater compliance in pediatrics. The drug content was calculated on the basis of the drug entrapment efficiency of the individual microsphere formulation (gelatin, 82.32% and sodium alginate blends, 89.31%) and drug-loaded microspheres containing an amount of drug (182.22 mg of rifampicin-gelatin microspheres and 83.98 mg of isoniazid-alginate blends microspheres) equivalent to that of the standard FDC product approved by the WHO (150 mg rifampicin + 75 mg isoniazid) were used.

The sedimentation volume normally ranges from less than one to one and it may exceed one. The sedimentation volume decreases with an increase in xanthum gum, *i.e.* from 0.1% to 0.9%. The optimized formulation was concluded to have a sedimentation volume of 0.4.

In vitro dissolution studies of marketed formulations consisting of single drugs and a combination of isoniazid and rifampicin suggested the existence of an interaction between the two drugs in SGF pH 1.2 (Figure 1). Only minor differences were seen in the extent of drug release of both drugs in SIF pH 7.4 (Figure 2).



Figue 1. Dissolution profile of oral formulation of isoniazid and rifampicin (combination) in SGF pH 1.2.



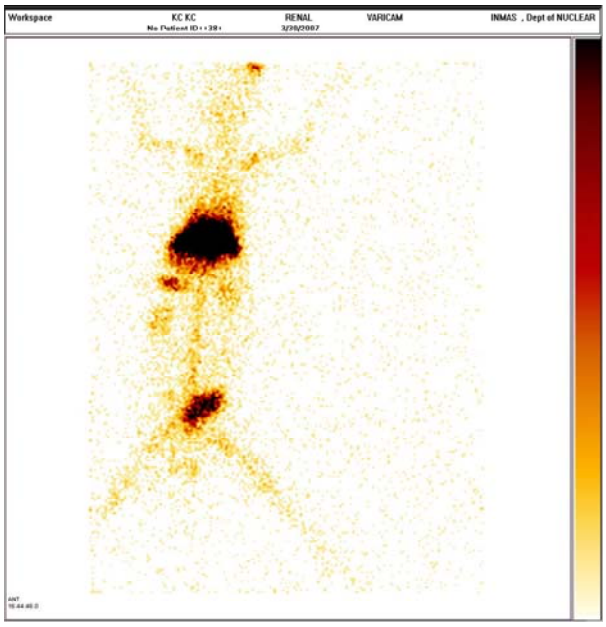
Figue 2. Dissolution profile of oral formulation of isoniazid and rifampicin (combination) in SIF pH 7.4.

However, this drug delivery system was largely successful in preventing the drug interaction due to reduced release of isoniazid from alginate microspheres into the gastric medium. The maximum extent of rifampicin was released into the gastric medium while gelatin microspheres had a burst release in SIF.

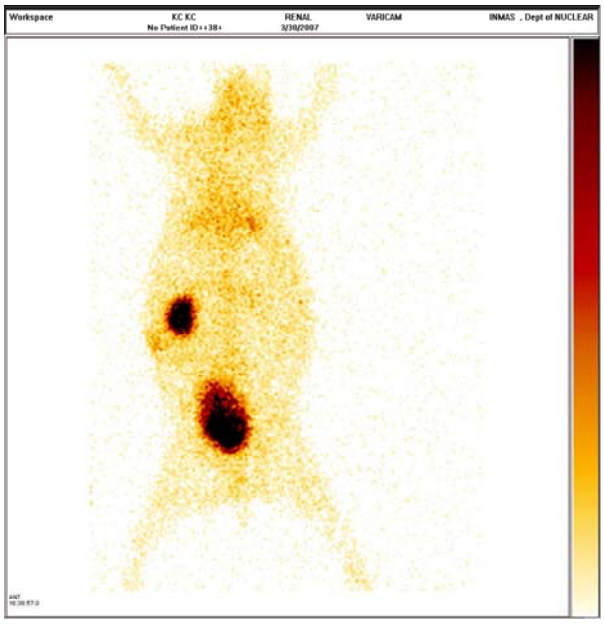
In gamma scintigraphy analysis, the section of GIT was analyzed in detail, revealing substantial differentiation. The presence of microspheres was marked in the intestinal lumen after 4 h (Figure 3). Microspheres were also be detected in different organs like the kidney, liver, lungs and lower part of the intestine after 24 h (Figure 4). Such prolonged retention of the formulation could be due to the small particle size of the formulation and the bio-adhesive nature of the preparation.

HPTLC analysis of the drug combination was based on the reported method (*19*). The following are the instrument specifications and chromatographic conditions: Solvent system: *n*-hexane/2-propanolol/acetone/ammonia/formic acid (3:3.8:2.8:0.3:0.1, v/v); wavelength at 254 nm (Figure 5).

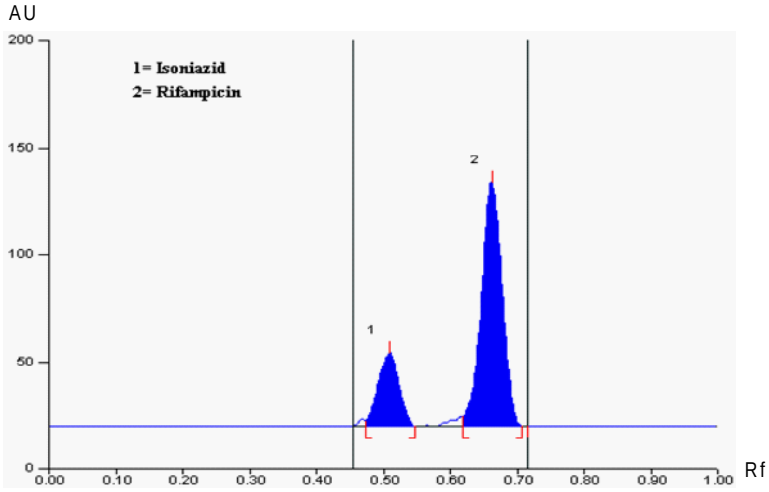
Results of observations are shown in Tables 2 and 3. The log % of the remaining drug was plotted against time (Figures 6 and 7). The effect of temperature on the degradation was studied by plotting Log *K* versus 1/*T*



Figue 3. Whole body γ-scintigraphy of rabbit after 4 h of oral administration of ^{99m}Tc-pertechnetate-reconstited powder of microspheres.



Figue 4. Whole body γ-scintigraphy of rabbit after 24 h of oral administration of ^{99m}Tc-pertechnetate-reconstited powder of microspheres.



Figue 5. HPTLC graph of isoniazid and rifampicin by the reported method.

(Figures 8 and 9).

The optimized formulation was found to be stable. Degradation of isoniazid was faster than that of rifampicin. Thus, the shelf life can be predicted using the isoniazid shelf life. Higher temperatures ($K = 2.303 \times 10^{-4}$ day^{-1} at 60°C) led to more than 2% degradation of isoniazid at the end of 90 days. The degradation rate constant at 25°C was found to be 1.9286×10^{-4} (day^{-1}), so the formulation was predicted to have a shelf life of 1.518 years.

Conclusions

The present work was an attempt to develop a reconstituted powder for suspension of antitubercular drug microspheres for pediatric use in order to reduce the interaction between two drugs. The following conclusions were drawn from the results obtained:

(i) *In vitro* drug release was obtained for 36 h for isoniazid-alginate microspheres and for 4 h for rifampicin-gelatin microspheres.

(ii) The developed formulation provided more prolonged release of the drug than did the marketed preparation.

(iii) Gamma-scintigraphic analysis revealed the presence of alginate microspheres in the intestine for a period of more than 24 h.

(iv) Stability studies indicated that the developed formulation was stable for three months.

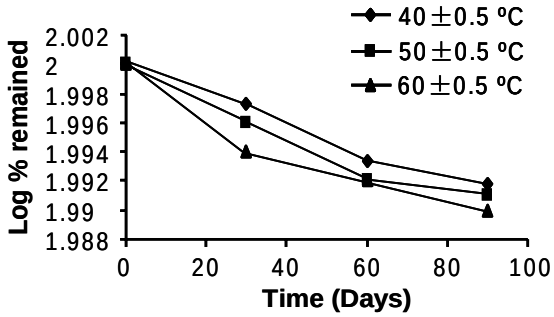
(v) The formulation, a reconstituted powder for

Table 2. Degradation of isoniazid in the optimized formulation according to WHO guidelines

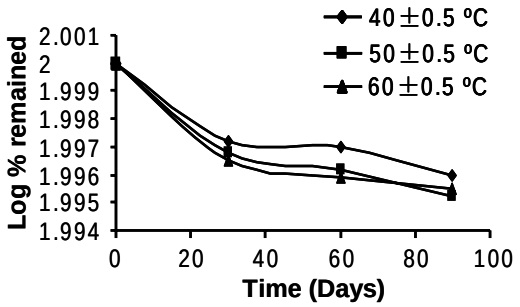
Temp (°C)	Time (Days)	Mean area (±SD)	Conc. (ng/µL)	Contents (mg)	% Remained	% Log remained
40 ± 0.5	0	1306.95	750.0	75.00	100.00	2.000
	30	1298.93	745.4	74.54	99.39	1.997
	60	1286.74	738.4	73.84	98.45	1.993
	90	1282.38	735.9	73.59	98.12	1.991
50 ± 0.5	0	1306.95	750.0	75.00	100.00	2.000
	30	1297.01	744.3	74.43	99.24	1.996
	60	1285.69	737.9	73.78	98.37	1.992
	90	1280.46	734.8	73.48	97.97	1.991
60 ± 0.5	0	1306.95	750.0	75.00	100.00	2.000
	30	1296.49	744.0	74.00	98.67	1.994
	60	1282.72	736.1	73.61	98.15	1.992
	90	1279.59	734.3	73.43	97.91	1.990

Table 3. Degradation of rifampicin in the optimized formulation according to WHO guidelines

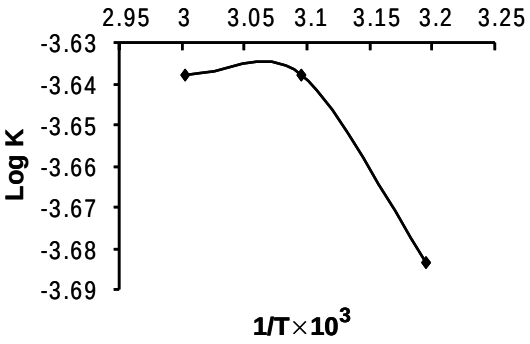
Temp (°C)	Time (Days)	Mean area (±SD)	Conc. (ng/µL)	Contents (mg)	% Remained	% Log remained
40 ± 0.5	0	6717.25	1500.0	150.00	100.00	2.000
	30	6674.16	1490.4	149.04	99.36	1.997
	60	6671.47	1489.8	148.98	99.32	1.997
	90	6656.25	1486.4	148.64	99.09	1.996
50 ± 0.5	0	6717.25	1500.0	150.00	100.00	2.000
	30	6668.34	1489.1	148.91	99.27	1.996
	60	6659.83	1487.2	148.72	99.15	1.996
	90	6651.77	1485.4	148.54	99.03	1.995
60 ± 0.5	0	6717.25	1500.0	150.00	100.00	2.000
	30	6663.41	1488.0	148.80	99.20	1.996
	60	6655.35	1486.2	148.62	99.08	1.995
	90	6649.53	1484.9	148.49	98.99	1.995



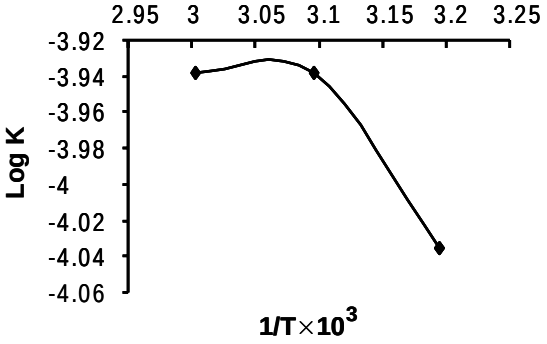
Figue 6. Degradation kinetics of isoniazid in optimized formulation according to WHO guidelines.



Figue 7. Degradation kinetics of rifampicin in optimized formulation according to WHO guidelines.



Figue 8. Graph for calculation of shelf life of isoniazid (Log degradation rate constant, K versus $1/T \times 10^3$).



Figue 9. Graph for calculation of shelf life of rifampicin (Log degradation rate constant, K versus $1/T \times 10^3$).

suspension, thus has potential clinical usefulness and may contribute to patient compliance through better delivery of two first-line antitubercular agents.

Extensive chemotherapeutic studies are however, required to adjust the dosage regimen for this formulation.

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