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Evaluation of physicochemical stability and degradation kinetics of bedaquiline in hydrolytic solutions of different pH

S. J. Rajput* and P. J. Vanavi

Abstract

Background: Tuberculosis is an infection that has high mortality rate in human as well as in animals if it remains unattained for long time. Scientists are always desirous to discover new molecules against *Mycobacterium tuberculosis*; one of them is bedaquiline which was recently approved to treat multidrug resistance TB. During the clinical study of new molecule stability and impurity are the key aspects to develop formulation. Stability issues in bulk drug are dangerous to drug safety and needs careful attention in formulation development. Bedaquiline stability study was completed with reversed-phase high-performance liquid chromatography (HPLC) and utilized in degradation kinetic study of bedaquiline in aqueous condition under different pH, temperature, and concentrations of degradant.

Results: Linearity was obtained in 50.0-250.0μg/ml, correlation coefficient, and regression line equation were 0.998 and Y=18528x + 7E+06 respectively. Intraday, inter day precision, and repeatability RSD were less than 2.0%. Average recovery in accuracy study was more than 98.0% showed that good recovery was obtained. Degradation kinetics parameters like activation energy (Ea), half-life (t_{50}), rate constant (k), and shelf life (t_{90}) were calculated under different condition for bedaquiline. Entropy and enthalpy of reaction was studied to gather knowledge about energy of system.

Conclusion: The result explained that bedaquiline degradation was pH-dependant, as increase in concentration of degradant and temperature, there was increase in degradation rate of bedaquiline. Bedaquiline was stable in neutral aqueous condition and at lower temperatures, shows that drug is hydrophobic in nature. Kinetic data showed that bedaquiline followed first order kinetics in acidic and alkaline pH.

Keywords: Bedaquiline, RP-HPLC, Degradation kinetics, HPLC-PDA, Degradation products, Anti-TB, Stability study, Impurity

Background

Bedaquiline ((1R, 2S)-1-(6-bromo-2 methoxy-3-quinolinyl)-4-(dimethylamino)-2-(1-naphthalenyl)-1-phenyl-2-butanol), which was approved in 2012, first new molecule approved after 40 years to treat TB. It is diarylquinoline derivative, currently used to treat MDR (multi-drug resistance) TB (Fig. 1) [1].

MDR-TB is most common in recent years and difficult to treat; it is associated with HIV, diabetes, or some other degenerative disease. Unlike other anti TB drugs, bedaquiline acts directly on ATP (adenosine triphosphate) synthase enzyme to stop energy chain of *Mycobacterium tuberculosis*; thus, bedaquiline acts as a bactericidal antibiotic with very common side effects [2, 3]. RP-HPLC analytical method, LC/MS method and HPLC method in plasma is reported till date, no stability or degradation data is reported [4–6].

* Correspondence: sjrajput@gmail.com
Pharmaceutical Quality Assurance Laboratory, Centre for Relevance and Excellence in NDDS, Pharmacy Department, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat 390002, India

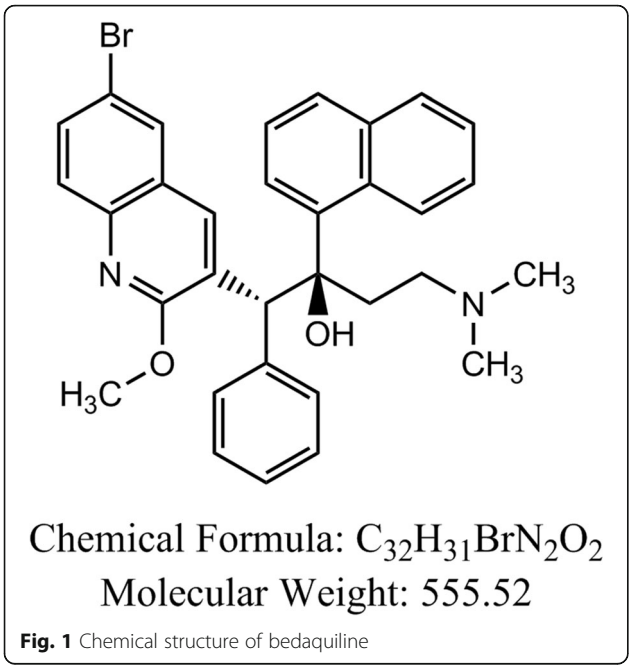

Fig. 1 Chemical structure of bedaquiline

The bedaquiline study of changes occurs in aqueous condition under different pH, and temperature can be advantageous, these studies would assist in formulation development, shelf life, and storage-related studies and toxicity studies. The degradation product formed can play role in altering therapeutic effect, can itself have toxic effect/s or have profound effect on adverse effect/s.

In early stage bedaquiline stability studies, it was observed that bulk drug of bedaquiline appears as white and amorphous powder and stable under thermal condition (at 80 °C) and under UV light for more than 28 days without any physical change; addition of degradant, change in ionic strength of degradant, or change in pH other than neutral in above described conditions made chemical changes in bedaquiline reaction system. To establish safety of drug, stability of molecule, the ICH (International Conference on Harmonization) suggests that stability studies should be performed extensively [7]; moreover, degradation products will also be identified and characterized, so stability and degradation kinetic of bedaquiline was thought of interest.

The aim of the study was to carry out stability study and degradation kinetic of bedaquiline in aqueous medium using different pH and temperature to identify degradation products formed. This study would be informative in case of bedaquiline stability and can help in formulation development and to find out appropriate storage condition for bedaquiline.

Method

Materials and reagents

The bulk drug of bedaquiline was obtained from Dishman Pharmaceuticals (India). Hydrochloric acid (HCl), sodium hydroxide (NaOH) pellets, O-phosphoric acid (OPA), and sodium dihydrogen ortho phosphate (NaH_2PO_4) buffer were purchased from S.D fine Chem Limited, India. Methanol (MeOH) was purchased from Avantor performance materials India Ltd. India. Deionization process was used to prepare distilled water in laboratory. Since, bedaquiline formulation is not available in the Indian market, synthetic mixture (as per tablet formulation Sirturo^{TM}) [8] was prepared in laboratory for analysis. All the chemicals and reagents used were analytical and HPLC grade and commercially available.

Chromatographic conditions

The HPLC system (Waters 515) consisting of PDA detector, binary pump, 20 μL manual injector with pump module controller was used for chromatographic analysis on Thermo hypersil C_8 (250×4.6mm, 5 μm) column. Nylon 0.22 μm Millipore filter and Hamilton syringe (25μL capacity) were used for filtering and injecting sample in HPLC system respectively. Shimadzu AUX 220 weigh balance was used for weighting purpose. Mobile phase consisting of 10 mm sodium dihydrogen ortho phosphate buffer adjusted to pH 2.5 with O-phosphoric acid (%A) and methanol (%B) in gradient mode (Table 1). Detection was carried out at 227 nm. System suitability data (tailing factor, resolution, theoretical plates, and capacity factor) were obtained using the software Empower3.

Degradation kinetic study sample preparation

Acidic pH degradation

Accurately weighed 10 mg bedaquiline was dissolved in 1 ml methanol in three separate volumetric flasks; remaining volume was made up with 0.1 N, 1 N HCl, and 2 N HCl separately. Solutions were kept at preset temperature of 25 °C, 50 °C, and 80 °C. In total, 0.2 ml aliquot was withdrawn at 30 min time interval and freeze immediately to douse the reaction. Sample was neutralized with respective concentration of NaOH (0.1 N, 1 N, and 2 N) before injecting into chromatographic system.

Alkaline pH degradation

Accurately weighed 10 mg bedaquiline API was dissolved in 1 ml methanol in three separate volumetric

Table 1 Gradient scheme for bedaquiline stability indicating method

Time	%A	%B
0	45	55
40	20	80
50	45	55

flasks, remaining volume was made up with 0.1 N, 1 N, and 2 N NaOH separately. Solutions were kept at preset temperature of 25 °C, 50 °C, and 80 °C. In total, 0.2 ml aliquot was withdrawn at 30 min time interval and freeze immediately to douse the reaction. Sample was neutralized with respective concentration of HCl (0.1 N, 1 N, and 2 N) before injecting into chromatographic system.

Neutral pH degradation

Bedaquiline was found stable in neutral pH media and did not degraded sufficiently to identify degradation products.

The concentration of all samples was 200.0 μg/ml. Time interval for samples withdrawn was 0, 30, 60, 90, 120, 150, and 180 min to understand effect of pH and concentration of acid/alkali. Samples were filtered through 0.22 μm membrane before injecting in chromatographic system. The blank samples were prepared by without adding API.

Validation sample preparation

For linearity study, 50.0-250.0 μg/ml sample was prepared from stock solution of 1000 μg/ml (accurately weighed 50 mg sample dissolved in 50 ml methanol), aliquot of 0.5, 1.0, 1.5, 2.0, and 2.5 ml was withdrawn in volumetric flasks to get 50.0, 100.0, 150.0, 200.0, and 250.0 μg/ml respectively.

For precision study, intraday and inter day samples were prepared from same stock solution for 50.0, 100.0, and 150.0 μg/ml. Repeatability study was carried out for 100.0 μg/ml. Accuracy study was completed by adding 100.0 μg/ml in synthetic mixture sample containing 80, 100, and 120% of sample separately.

The withdrawn aliquots were transferred in separate 10 ml volumetric flask and diluted with methanol to get desired concentration. All samples were filtered through 0.22 μm syringe filter before injecting in chromatographic condition.

Result

HPLC condition optimization

Several trials were taken to get paramount system suitability and separation between DPs and bedaquiline peak in chromatographic system, 10 mm sodium dihydrogen orthophosphate (pH 2.5 using OPA) in water and methanol was good to get ideal system suitability for peaks. To achieve appropriate resolution between bedaquiline and DP peak gradient run was chosen (Table 1).

Method validation

A linear calibration curve was obtained for the series 50.0-250.0 μg/ml and characteristic calibration curve equation was $y = 5.8 \times 10^5 x - 5 \times 10^5$ ($r = 0.998$). In the equation, y denotes peak area of bedaquiline and x is concentration of bedaquiline. Table 2 showed precision and trueness, analysis of table showed that bedaquiline was precise and recovered in prescribed criteria in above described chromatographic condition.

Stability study in different pH

Stability of bedaquiline in different pH was studied and method was developed to separate degradation product peaks and bedaquiline peak. The characteristic chromatogram of bedaquiline and its degraded sample is shown in Fig. 2.

Kinetic parameters and calculation

To calculate order of reaction, a chart of %C, *log* (C_0/C_t) and *1/ln C* were plotted versus time for hydrolytic (acidic and alkaline) degradation conditions. Correlation coefficients obtained for each graph, among them strongest correlation coefficients were identified and degradation mechanism illustrated by that order of reaction. Bedaquiline showed strong correlation coefficients in the plot of *log* (C_0/C_t) versus time was a sign that it followed first order of reaction. The equations for calculation of kinetic parameters are as follows:

Rate constant (k) was calculated from first order chart based on Eq. 1

$$\log\left(\frac{Ct}{Co}\right) = -kt \tag{1}$$

Here, C is concentration, C_0 is initial concentration, and C_t is concentration at time t. The correlation coefficient for first degradation plots were more than 0.9.

Half-life ($t_{1/2}$) for bedaquiline degradation kinetic was studied by Eq. 2

$$t1/2 = \ln 2/k \tag{2}$$

Arrhenius plot was created by taking log of rate constant (k) versus inverse of temperature in Kelvin to calculate activation energy (E_a) by Eq. 3 [9]

$$\text{slope} = -\frac{Ea}{R} \tag{3}$$

Table 2 Trueness, repeatability, and precision result in method validation

Parameter	Concentration (μg/ml)	Recovery (%)	RSD (%)
Accuracy	150.0	100.63±0.679	1.0
	200.0	102.72 ± 0.882	
	250.0	101.26 ± 0.907	
Precision	100.0		0.704
Repeatability	100.0		0.750

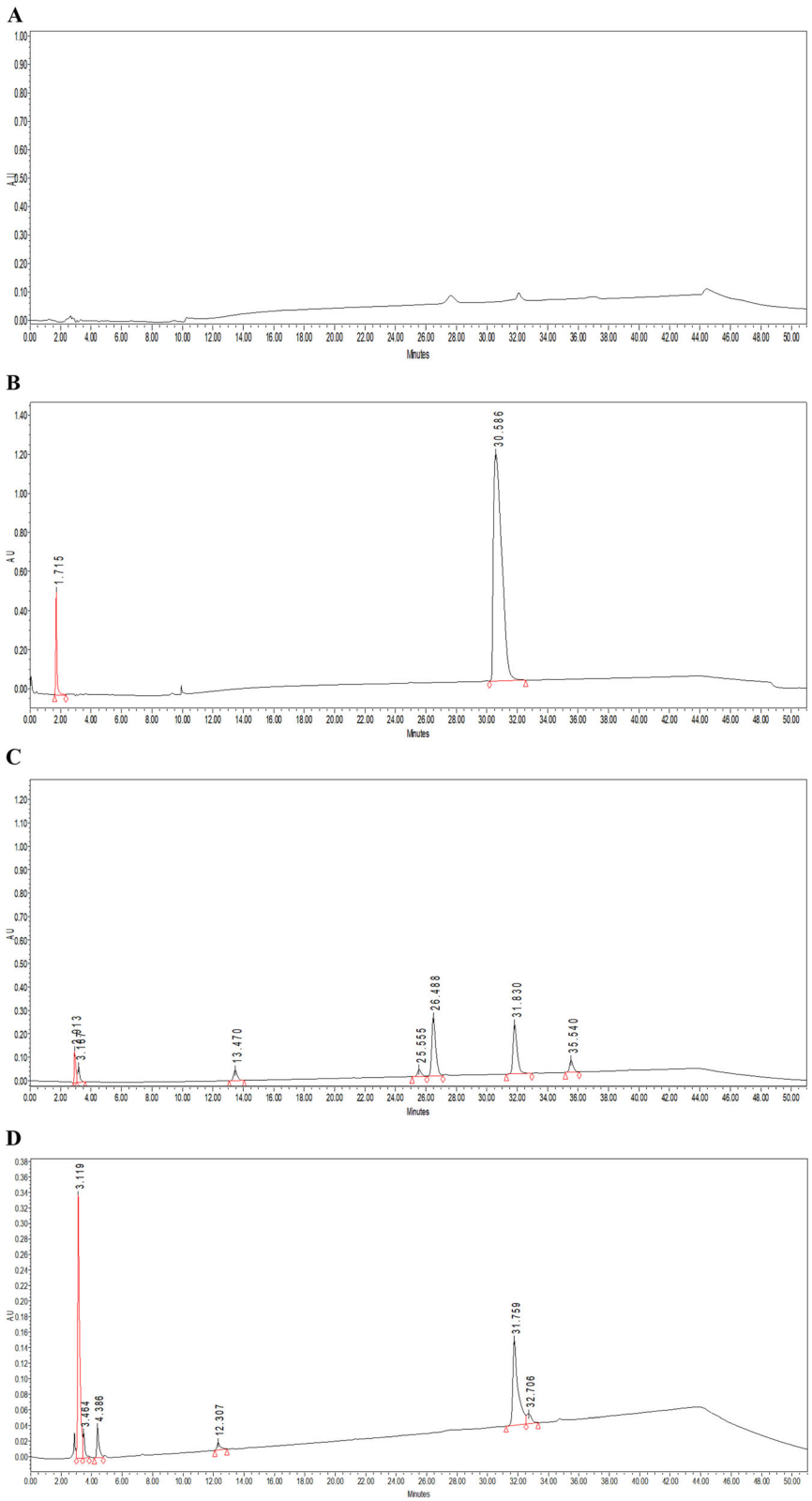

Fig. 2 **a** Blank chromatogram. **b** Blank added with bedaquiline API. **c** Acid-treated bedaquiline. **d** Alkali-treated bedaquiline

Here, R denotes universal gas constant (value is 8.314 $\times 10^{-3}$ kJ/mol*K).

Degradation kinetics analysis

Persuade of pH on the steadiness of bedaquiline

Bedaquiline forced degradation study was carried out in acidic (≤2) and alkaline (≥ 8) pH, Table 3 is the study of rate constant and half-life in different pH and temperature (25 °C to 80 °C). The stability of bedaquiline at 80 °C in different pH is shown in Fig. 3.

Rate constant increased with increase in temperature and concentration of acid/alkaline pH, it can be seen in Table 3, and this leads to draw a conclusion that degradation of bedaquiline was driven by pH and temperature together.

The persuade of temperature on the steadiness of bedaquiline

As discussed, above temperature alone do not have any role in degradation of bedaquiline, so addition of pH along with temperature can provoke degradation of bedaquiline. Another related equation of enthalpy of activation ($\Delta H^{\updownarrow}$) and entropy of activation ($\Delta S^{\updownarrow}$) are shown in Eqs. 4 and 5 respectively [9–11]; every reaction system has particular energy for carrying out change in system, this change of energy is enthalpy of activation.

Table 3 Half-life ($t_{1/2}$) and rate constant (k) at different pH and temperature

Parameters	Temperature (°C)		
	25	50	80
Acidic pH (HCl)			
Rate constant (k)			
0.1 N	0.00184	0.00230	0.00322
1 N	0.00392	0.00576	0.00643
2 N	0.00507	0.00668	0.00748
Half-life ($t_{1/2}$) min			
0.1 N	376.14	300.91	214.94
1 N	177.01	120.36	107.85
2 N	136.78	103.76	92.59
Alkaline pH (NaOH)			
Rate constant (k)			
0.1 N	0.00138	0.00184	0.00230
1 N	0.00184	0.00230	0.00276
2 N	0.00368	0.00484	0.00553
Half-life (t1/2) min			
0.1 N	501.52	376.14	300.91
1 N	376.14	300.91	250.76
2 N	188.07	143.29	125.38

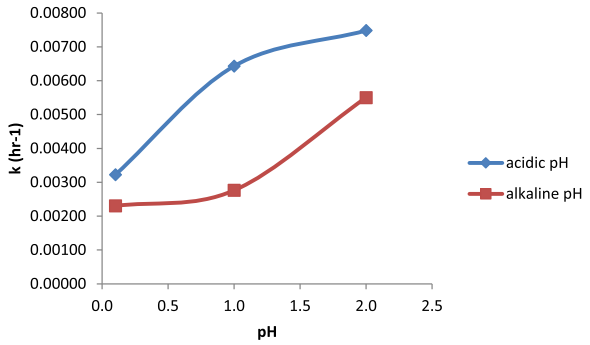

Fig. 3 Relationship between rate constant (k) and pH in various pH solution at 80 °C

$$\Delta H\updownarrow = Ea - RT \tag{4}$$

Where Ea is activation energy in KJ/mol, R is universal gas constant (8.314 J/K/mol), and T is absolute temperature (K).

The reaction system brings out rearrangement of molecules and leads disarrangements in system, this disarrangement of system at start and end of reaction can be measured by entropy, it can be seen from Table 4.

$$(\Delta S\updownarrow) = \frac{\Delta H\updownarrow}{T} - R\ln\frac{T}{k} - R\ln\frac{k}{h} \tag{5}$$

Where R is the universal gas constant (8.314 K/J/mol), T is absolute temperature (K), k is Boltzmann constant (1.3807 $\times 10^{-23}$ J s) and h is Plank's constant (6.626 $\times 10^{-34}$ J s).

Arrhenius plot of Log k versus 1/T at various pH values and calculated kinetic parameters are listed in Table 4.

Table 4 shows linear equations of Arrhenius plot (r>0.90) for the range of temperature 25-80 °C. Slope of the equations Arrhenius equation (Ea/R) were large. Bedaquiline molecule was stable at high pH (acid/alkaline) and this statement is reliable in concern of effect of pH on stability of bedaquiline.

Table 4 Arrhenius plot and activation parameters of bedaquiline in different pH solutions

Acid	Activation	r	Ea (kJ/mol)	$\Delta H^{\updownarrow}$	$\Delta S^{\updownarrow}$
0.1N	*ln* k= −500.4*x*−1.181	0.98	4.16	6.43	−54.93
1.0N	*ln k*= −457.3*x*−1.007	0.90	3.80	6.07	−53.62
2.0N	*ln k*= −400.1*x*−1.196	0.94	3.33	5.60	−51.89
Alkali	**Activation**	**r**	**Ea (kJ/mol)**	**$\Delta H^{\updownarrow}$**	**$\Delta S^{\updownarrow}$**
0.1N	*ln k* = −424.5*x*−1.430	0.99	3.52	2.274	−39.71
1.0N	*ln k* = −337.0*x*−1.601	0.99	2.80	2.273	−39.71
2.0N	*ln k* = −336.8*x*−1.293	0.96	2.80	2.273	−39.71

Furthermore, at lower pH for instance in 0.1 N, 1.0 N, and 2.0 N at 80 °C, t_{90} was 32.57, 16.34, and 14.03 min and at higher pH for instance in 0.1 N, 1.0 N, and 2.0 N at 80 °C, t_{90} was 45.59, 37.99, and 19.00 min.

Persuade of concentration on steadiness of bedaquiline

The influence of concentration of acid/alkali on rate constant is shown at 80 °C in Fig. 3. Bedaquiline degradation rate increased with increase in temperature and acid/alkaline concentration. These illustrate that bedaquiline degradation reaction is acid/alkali catalyzed and after1.0 N of alkali; increase in concentration of alkali adds no change in degradation parameters. Moreover, temperature also plays synergistic role in degradation of bedaquiline, degradation rate constant increased with increase in temperature, obtained degradation rate constant for 80 °C was more than 25 °C.

Persuade of oxidation on steadiness of bedaquiline

Bedaquiline stability was studied in three H_2O_2 concentrations (1%, 6%, and 12%) to explain effect of oxidative agent on bedaquiline stability. The linear plot of $ln\ C_t/C_0$ versus time in different % of H_2O_2 is shown in Fig. 4, the degradation rate constant were 0.0008, 0.0014, and 0.0028 in 1%, 6%, and 12% H_2O_2, respectively. This result indicates that bedaquiline was extremely unstable under H_2O_2 condition. As seen in Fig. 4, bedaquiline reaction is slow and steady in 1% and 6% H_2O_2 but reaction rate noticeably increased in 12% H_2O_2. Precaution should be taken in storage of bedaquiline; it should be stored away from oxidative environment.

Persuade of irradiation on steadiness of bedaquiline

Bedaquiline powder was stored under indirect sun light at room temperature for 28 days; sample was withdrawn at alternate day interval to check if any irradiation reactions proceeded. Bedaquiline did not decomposed, no change in physical appearance, and in chromatogram observed. This result demonstrates that bedaquiline is highly stable under indirect UV irradiation; for precautionary step, it should be stored away from direct sun light exposure for long time.

Discussion

Bedaquiline has very poor solubility in water showed that it is semi polar in nature; although it was soluble in acetonitrile and very soluble in methanol directed use of methanol in mobile phase rather than acetonitrile and water alone. Due to solubility of bedaquiline in water and acetonitrile, they were not a good choice of solvent; moreover, pH near to neutral was also subtracted from choice. Acidic pH buffer (pH 2.5 ±0.5) was tried in different ionic strength such as sodium dihydrogen phosphate buffer showed good system suitability for DPs and API peaks. Ionic strength (up to 50 mm) did not show adequate effect on peak symmetry and other chromatographic parameters, so possible minimum ionic strength for buffer was chosen.

Method was validated as per ICH Q2 (R1) guidelines with concern of linearity, repeatability, precision, selectivity, and exactness. An excellent peak shape was obtained by above mentioned chromatographic condition without hindrance of any DPs [12, 13].

Bedaquiline degradation reaction was acid/alkali catalyzed in presence of temperature (80 °C) but maximum stability in presence of pH was obtained at lower temperature and lower ionic strength of degradants. Analysis of result showed that bedaquiline degradation was pH dependent. Acid stability of bedaquiline in comparison to alkaline pH showed that bedaquiline is more stable in alkaline pH. Freeze/thaw and long-term stability of bedaquiline samples were established by LC-MS/MS method and bedaquiline was reported stable for long term and freeze/thaw stability in plasma [14].

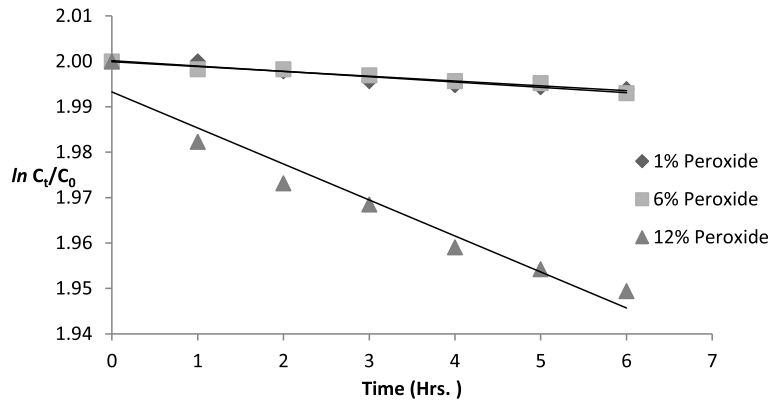

Fig. 4 *ln* C_t/C_0 versus time in different % of H_2O_2

Few studies reported methods to improve absorption of bedaquiline and pharmacokinetics of bedaquiline in rat plasma, the results showed that bedaquiline is safe without interaction or degradation in plasma [15, 16]; therefore, it was thought of interest to study the effect of pH and temperature on degradation of bedaquiline in hydrolytic solutions by degradation kinetics study. Degradation kinetic study of bedaquiline showed that the pH and ionic strength of acid/alkali had profound effect on rate constant. Shelf life and half-life of bedaquiline degradation decreased with increase in acid concentration and temperature. Table 4 showed enthalpy and entropy values indicate that at highest acid concentration of study had lowest enthalpy; this clearly means that acid and temperature catalyze the reaction and limits enthalpy value. All degradation reactions were endothermic as positive enthalpy values were obtained. Highest entropy value was obtained in highest acid concentration of study indicates that increase in ionic strength led the reaction to occur spontaneously; here, temperature played as catalyst. While in alkaline pH medium, entropy values remained constant indicate that bedaquiline is stable in alkaline medium. Entropy and enthalpy value do not affect by reaction path and reaction mechanism. Bedaquiline degradation followed first order of reaction in acidic and alkaline medium. Kinetic parameters were calculated and can be used to understand the reaction mechanism, practically this data would help in how to make reaction faster and synthesize degradation product. From the kinetic study of bedaquiline in each pH and in oxidized medium, storage condition of bedaquiline can be stored well in dry, away from sun light and oxidizing agent.

Conclusion

RP-HPLC method was inducted and validated in present study; this method was utilized for stability study and degradation kinetic study of bedaquiline under different pH solution of acid and alkali. As discussed in above section, degradation of bedaquiline is influenced by pH, temperature, and concentration of degradant; however, bedaquiline was stable in neutral pH media, lower alkaline pH, irradiation, and lower temperature. Bedaquiline was highly unstable under higher temperature with acid, alkaline, and oxidative media. Bedaquiline followed first order kinetics in degradation reaction. Avoiding the pH, temperature, concentration, oxidation, and long-term irradiation exposure should be the suggested storage condition for bedaquiline

The conditions for stress degradation of bedaquiline were as per ICH recommended guidelines to analyze degradation products. A total of four degradation products were obtained and will be identified in further study. The established stability—indicating method can be typical example for developing stability indicating method. The knowledge gained from this study will be helping hand in understanding the stability of bedaquiline and further study of bedaquiline in clinical studies.

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Authors' contributions

Principle investigator SJ Rajput conceived and designed the analysis. Co-author PJ Vanavi performed the analysis, collected data, contributed data or analysis tool, and draft the paper. The authors listed above certify that they have participated sufficiently in the work described in paper. All authors have read and approved the manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no conflict of interest.

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