ISSN : 0974-7419 Volume 16 Issue 3

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 16(3) 2016 [119-127]

Stability-indicating high performance liquid chromatographic determination of rivastigmine with kinetic study of rivastigmine alkaline degradation

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ABSTRACT

Stability-indicating high performance liquid chromatographic determination of rivastigmine in its binary mixture with kinetic study of rivastigmine alkaline degradation

Stability-indicative determination of rivastigmine hydrogen tartarate (RIV) in the presence of its degradate was investigated. The degradation product was isolated, *via* alkaline-degradation, characterized and confirmed. Selective quantification of RIV in bulk form, pharmaceutical formulations and/or in the presence of RIV degradate was demonstrated. The analytical technique adopted for quantification was high performance liquid chromatography (HPLC). Separation was performed using a Xbridge ODS column with a mobile phase consisting of phosphate buffer pH (3.4): acetonitrile 70: 30 (v/v) with UV detection at 262 nm. The method showed high sensitivity with good linearity over the concentration range of 20 to 160 μg mL^{-1} for RIV. The HPLC method was used to study the kinetics of RIV alkaline degradation that was found to follow a first-order reaction. The activation energy could be estimated from the Arrhenius plot and it was found to be 9.854 Kcal $mole^{-1}$.

KEYWORDS

Rivastigmine;
High performance liquid chromatography;
Kinetic study.

INTRODUCTION

Rivastigmine [123441-03-2] (Figure 1) is ethyl methyl carbamic acid 3-[(1S)-1-(dimethyl amino) ethyl phenyl ester, (S)-N-ethyl-3-[(1-dimethylamino) ethyl]-N-methyl phenyl carbamate]. $C_{14}H_{22}N_2O_2$, mol.wt 250.34[1]

Rivastigmine is classified as a reversible acetylcholine esterase inhibitor of the carbamate type. It is central nervous system selective used for the symptomatic treatment of Alzheimer's disease.[2] (Put Figure 1 here)

Several methods have been reported for the determination of RIV, these include, spectrophotometric[3-5], thin layer chromatographic[6], high performance liquid chromatographic[7-13], capillary zone

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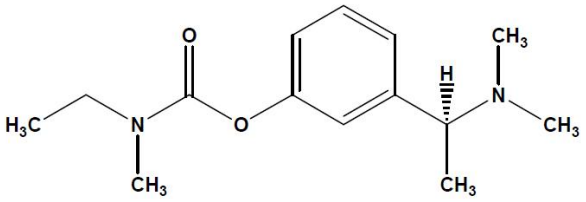

Figure 1 : Chemical structure of rivastigmine

electrophoresis[14, 15] and electrochemical[16, 17].

In modern analytical laboratory, there is a need for significant stability-indicating methods of analysis. From these procedures, only the spectrophotometric[5] HPLC[9], TLC[6] and electrochemical[16, 17] techniques were recommended as stability indicating assays. Rivastigmine retention time in reference[9] was 8 min, which make the suggested method more rapid.

The focus of the present study was to develop and validate simple stability-indicating method for the quantification of RIV in bulk form and/or in the presence of RIV alkaline-degradate. Moreover, using the suggested method to monitor the kinetics of RIV degradation to solve problems encountered in quality control and to predict the expiry dates of pharmaceutical products.

EXPERIMENTAL

Instruments

- Precoated HPTLC plates, (Macheray-Nagel (Germany)) silica gel 60 F_{245} 20 x 20 cm, 0.2 nm thickness.

- A Liquid chromatograph consisted of a Waters HPLC system serial no. 01757-3696 USA with Waters 2998 PDA detector and Water e2695 separation modules pump with auto injector used along with Waters XBrigde™ C18 column (4.6 x 250 mm, 5µm). Empower 2 software; Waters Corporation was utilized for data acquisition. Waters Corporation 34 Maple Street, Milford, Massachusetts

The chromatographic conditions were: Stationary phase: Waters XBrigde™ C18 column (4.6 x 250 mm, 5µm). Mobile phase: solution pH 3.4 (Dissolve 1.74 gm of dipotassium hydrogen phosphate anhydrous and dilute to a volume of 900 mL with deionised water, add 1 mL triethylamine and 0.1 % sodium heptane sulphonate and adjust to a pH of 3.4 with 85% phosphoric acid): acetonitrile 70: 30 (v/v). The mobile phase was filtered through 0.45 µm Millipore membrane filter and was degassed for 30 min in an ultrasonic bath prior to use. UV detection was done at 262 nm. The system was operated at ambient temperature. The flow rate was isocratic at 1.0 mL min^{-1}. The samples were filtered also through a 0.45 µm membrane filter, and were injected by the aid of a 25µL Hamilton® analytical syringe.

Materials and reagents

Rivastigmine hydrogen tartrate (mol.wt 400.4) (ID 3102936) reference standard was kindly supplied by Novartis Pharm Co, its purity was certified to be 99.69%. Its purity was also checked in our laboratory according to the reported spectrophotometric method[5] (2nd derivative at 262 nm) and it was found to be 99.70 ± 0.578.

Pharmaceutical formulations: Exelon® capsules batch number B 8173 and B 3003 (exp 3/2010), were purchased from the Egyptian market. Each capsule is claimed to contain 3 mg or 6 mg of rivastigmine hydrogen tartrate. Exelon® capsules are manufactured by Novartis Company (Basle, Switzerland).

All chemicals used were of analytical grade and de-ionized water was HPLC grade. Sodium hydroxide, hydrochloric acid, methanol, chloroform, ethyl acetate, acetonitrile for HPLC, dipotassium hydrogen phosphate anhydrous, sodium heptane sulphonate, phosphoric acid and triethylamine were obtained from Merck (Germany).

Standard solutions

RIV stock solution (0.5 mg mL^{-1}) in acetonitrile. RIV degradation product stock solution (0.5 mg mL^{-1}) in acetonitrile.

Procedures

Degradation of rivastigmine [5]

Five hundred milligrams of RIV were dissolved in 50 mL of 0.5 M sodium hydroxide and then refluxed at 100^{o}_{C} for 20 min. 1 mL was cooled to room temperature and then diluted with methanol. The degraded solution and standard solution were spotted on HPTLC plates. The plates were placed in chromatographic tanks previously saturated for 1 h with

the mobile phase of methanol: butanol: H_2O: NH_4OH (5:4:1:0.01 v/v/v/v) and then air-dried. The spots were visualized under UV light at 254 nm. The medium was rendered acidic using concentrated hydrochloric acid to precipitate the degradation product. The degradation product was filtered and then recrystallized from isopropyl alcohol.

Linearity

Portions 1.0- 8.0 mL from RIV stock solution (0.5 mg mL^{-1}) were transferred separately into a series of 25 mL measuring flask then completed to volume with acetonitrile. 25 µL of the previous solutions were injected into the liquid chromatograph using the chromatographic conditions described under *2.1. Instruments*. The corresponding peak areas were measured and calibration curve representing the ratio of the relative peak area of RIV to that of the external standard (30 µg mL^{-1} of RIV) was constructed, versus the corresponding concentrations of RIV in µg mL^{-1}.

Kinetic studies

For studying the kinetic order of the reaction

Into a 50-mL measuring flask, 0.25 g of RIV in 0.5 M NaOH was dissolved and completed to the mark with the same solvent. This solution was transferred into another clean dry conical flask and refluxed in a thermostatically controlled water bath at 80 $^{o}_{C}$ for 20 min. 1.0 mL sample solutions were taken at 4.0 min intervals, i.e after 4, 8, 12, 16 and 20 min placed into 25-mL measuring flasks, flasks half filled with cold acetonitrile, neutralized with 1.0 mL of cold 0.5 M hydrochloric acid (neutralize to stop the hydrolysis reaction) and the volume was completed with cold acetonitrile. The solutions (initial concentration C_o = 200 µg mL^{-1}) were injected in the liquid chromatograph using the chromatographic conditions described above. The concentration of RIV was calculated from the regression equation. The log % of remaining concentration against time was plotted.

For studying the effect of NaOH concentration on the reaction rate

Into a series of 50 mL measuring flasks, 0.25 g of RIV were dissolved in 0.5, 0.375, and 0.25 mole L^{-1} NaOH and completed to the mark with the same solvent. These solutions were transferred into other clean dry conical flasks, and then refluxed in a thermostatically controlled water bath at 80 $^{o}_{C}$ for 20 min. 1.0 mL sample solutions were taken at 4.0 min intervals and then complete as described under (*For studying the kinetic order of the reaction)*. The log % of remaining concentration against time was constructed for different molarities of NaOH and the rates constant and t1/2 were calculated.

For studying the effect of the temperature on the reaction rate

Three portions each of 0.25 g of RIV were dissolved in 50 mL measuring flasks and completed to volume with 0.5, 0.375, and 0.25 mole L^{-1} NaOH respectively. These solutions were transferred into other clean dry conical flasks and then refluxed in a thermostatically controlled water bath at 60 $^{o}_{C}$, 70 $^{o}_{C}$, 80 $^{o}_{C}$ and 90 $^{o}_{C}$ for 20 min. 1.0 mL sample solutions were taken at 4.0 min intervals and then complete as described under (*For studying the kinetic order of the reaction)*. The log % of remaining concentration against time at different temperatures was plotted. Also the Arrhenius plot for the effect of temperature on the rate of hydrolysis was constructed.

RESULTS AND DISCUSSION

High performance liquid chromatographic analysis

A simple isocratic high-performance liquid chromatographic method was developed for the determination of RIV in the presence of RIV degradation product without prior separation. To optimize the HPLC assay parameters, type of column and its dimensions, mobile phase conditions, and choice of detection wavelength were investigated. Different types of stationary phase C_8 and a ZORBAX ODS column with different dimensions and particle size were used. It was found that the ZORBAX ODS column (250 mm x 4.6 mm I.D) with a particle size of 5 µm gave the most suitable resolution. The mobile phase was chosen after several trials to reach the optimum stationary/mobile-phase matching. The peak shape improved dramatically by increasing the

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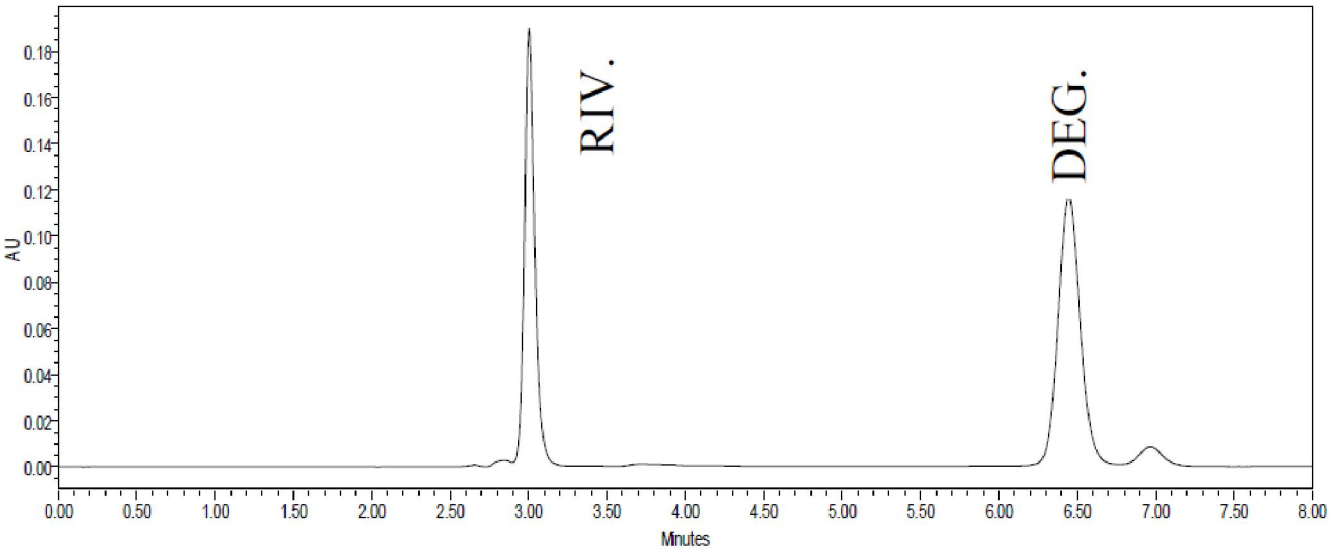

Figure 2 : Liquid chromatographic separation of RIV (40 µg mL^{-1}) and RIV degradation product (40 µg ml^{-1}) using the chromatographic conditions described in the text

TABLE 1 : Parameters of system suitability test of HPLC method

Parameter	Obtained value		Reference value
	Rivastigmine	Deg.product	
Resolution (R)	9.12		R > 2
T (tailing factor)	1.15	1.0	T = 1 for a typical symmetric peak
α (relative retention)	2.14		> 1
k' (column capacity)	5.42	2	1- 10 acceptable
N (column efficiency)	206.05	100	Increases with efficiency of the separation
HETP	0.12	0.25	The smaller the value, the higher the column efficiency

percentage of buffer to acetonitrile in the mobile phase[18]. Tailing of the drug peak was reduced by the addition of triethyl amine. Optimum separation was obtained with a mobile phase consisting of solution pH 3.4 added to it 1 mL triethylamine and 0.1 % sodium heptane sulphonate: acetonitrile 70: 30 (v/v) with a retention time of 6.42 ± 0.03 min. for RIV and 3.00 ± 0.02 min. for RIV degradation product. (Figure 2). System suitability parameters were tested by calculating the capacity factor, tailing factor, the sensitivity factor, column efficiency, and selectivity factor (resolution)[19-22] [TABLE 1]. The chromatographic system described in this work allowed complete base line separation of RIV from its degradation product.

Linear relationships were obtained between the relative peak area at the selected wavelength 262 nm and the corresponding concentrations in the range of 20- 160 µg mL^{-1} for RIV by adopting the external standard method for calibration.

The regression equations were computed and found to be:

A = 0.4637 C + 0.0303 r = 0.9996

Where A is the relative peak areas to that of the external standard, C is the concentration in µg mL^{-1} for RIV and r is the correlation coefficient.

HPLC validation

The selectivity and specificity of the proposed method was proved by the analysis of laboratory prepared mixtures containing different ratios of the selected analytes. (TABLE 2)

To ascertain the accuracy of the proposed procedure, it was successfully applied for the determination of RIV in Exelon capsules (TABLE 3). It is clear from the table that the company method failed to quantify RIV in the expired Exelon capsules. The validity was assessed by applying the standard ad-

TABLE 2 : Determination of rivastigmine in laboratory prepared mixtures by the proposed HPLC method

Concentration (μg mL^{-1})		Ratio	HPLC method
RIV	Deg.product	RIV: Deg. product	Recovery%
			RIV
140	20	7: 1	98.09
120	40	3: 1	98.77
100	60	5: 3	99.67
80	80	1: 1	101.12
60	100	3: 5	100.21
40	120	1: 3	99.45
20	140	1: 7	100.32
	Mean ± S.D.		99.66 ± 1.014

TABLE 3 : Determination of rivastigmine in exelon capsules by the proposed methods

Batch number	HPLC method	Reported method *
	Found % ± S.D.** of RIV	Found % ± S.D.** of RIV
B 8173	99.28 ± 1.124	99.54 ± 0.954
B 3003 (exp.date 3/2010)	80.46 ± 1.110	80.55 ± 1.107

*** Stability indicating spectrophotometric method (2nd derivative at 262 nm)[5]; ** Average of four determinations.**

TABLE 4 : Statistical comparison for the results obtained by the proposed methods and the reported method

Item	HPLC method	Reported method*
Mean	100.09	99.58
S.D.	0.761	1.011
Variance	0.579	1.022
N	8	6
F test	1.76 (4.362)	
Student's t test	1.08 (2.179)	

The figures in parenthesis are the corresponding tabulated values at P=0.05[23]; * Stability indicating spectrophotometric method (2nd derivative at 262 nm) (5).

dition technique. The small relative standard deviations indicate that the method is accurate.

The results obtained for the analysis of RIV in the pure powdered form were statistically compared with those from a previously reported method[5]. A significant difference was not observed[23]. (TABLE 4)

The precision of the suggested method was also expressed in terms of relative standard deviation of the inter-day and intra-day analysis. The robustness of the HPLC method was investigated by analysis of samples under a variety of experimental conditions such as small changes in the pH (3.0–3.5), and changing the column using a 250 mm × 4.6 mm i.d. C_{18} Lichrosorb 10 μm analytical column. The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and the mobile phase pH were varied. During these investigations, the retention times were modified, however the areas and peaks symmetry were conserved. Changes in instruments or personnel did not alter the results, which indicate the ruggedness of the proposed method. The obtained assay parameters and a validation sheet[19] are presented in TABLE 5.

Kinetics of the degradation

The linear relationship (Figure 3) between the log % of remaining concentration against time indicated first-order degradation. Since the hydrolysis was performed in a large excess of NaOH (0.5 mole L^{-1}), therefore it follows a pseudo-first order reac-

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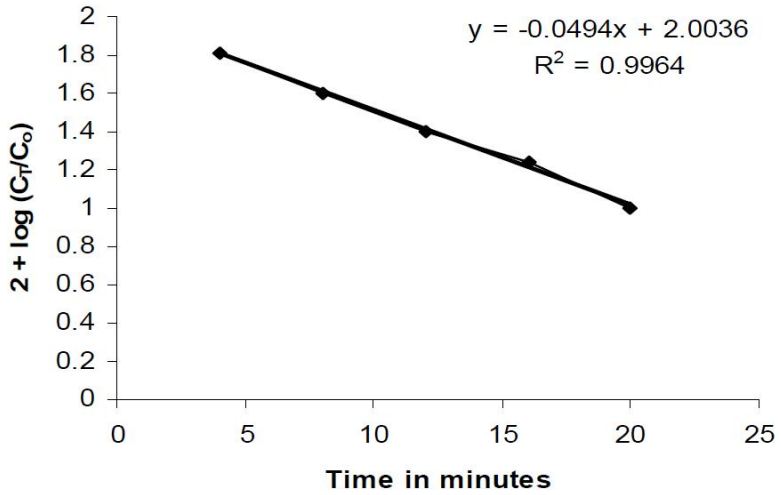

Figure 3 : First order plot of the hydrolysis of RIV (1000 mg %) with 0.5 M NaOH at 80 °C

TABLE 5 : Assay parameters and method validation for rivastigmine[19]

Parameter	HPLC method
Range (μgmL^{-1})	20-160
Slope	0.4637
Intercept	0.0303
Mean	100.09
S.D.	0.761
Variance	0.579
Correlation coefficient (r)	0.760
Coefficient of variation	0.9996
* RSD%[a]	0.893,0.868
*RSD%[b]	1.265,1.147

***RSD%[a], RSD%[b]: the intra-day, inter-day respectively (n=5) relative standard deviation of concentrations (80 and 120 μg mL^{-1})**

tion rate[24] which is the term used when two reactants are involved in the reaction but one of them is in such a large excess (NaOH) that any change in its concentration is negligible compared with the change in concentration of the other reactant (drug).

Different parameters that affect the rate of the reaction were studied. The temperature dependence of rivastigmine degradation was studied by conducting the reaction at different temperatures using different concentrations of the base solution (Figure 4). At each temperature the rate constant and $t_{1/2}$ were calculated then the log of the rate constant was plotted against the reciprocal of the temperature in Kelvin units (Arrhenius plot, Figure 5) to demonstrate the effect of temperature on the rate constant. It was concluded that as the temperature increased the rate of hydrolysis increased with a decrease in the $t_{1/2}$ (TABLE 7). Also, the energy of activation was determined by calculating the rate constant from the following equation[25].

$$\log \frac{k2}{k1} = \frac{Ea}{2.303R}\left(\frac{T2-T1}{T1T2}\right)$$

TABLE 6 : Kinetic data of rivastigmine alkaline degradation

Molarity of NaOH	Temperature	k (min^{-1})	$t_{1/2}$ (min)
0.5 M	90°C	0.160	4.33
	80°C	0.105	6.60
	70°C	0.070	9.90
	60°C	0.047	14.73
0.375 M	90°C	0.120	5.77
	80°C	0.077	9.02
	70°C	0.051	13.59
	60°C	0.035	19.82
0.25 M	90°C	0.079	8.77
	80 °C	0.057	12.15
	70 °C	0.037	18.72
	60 °C	0.022	31.51

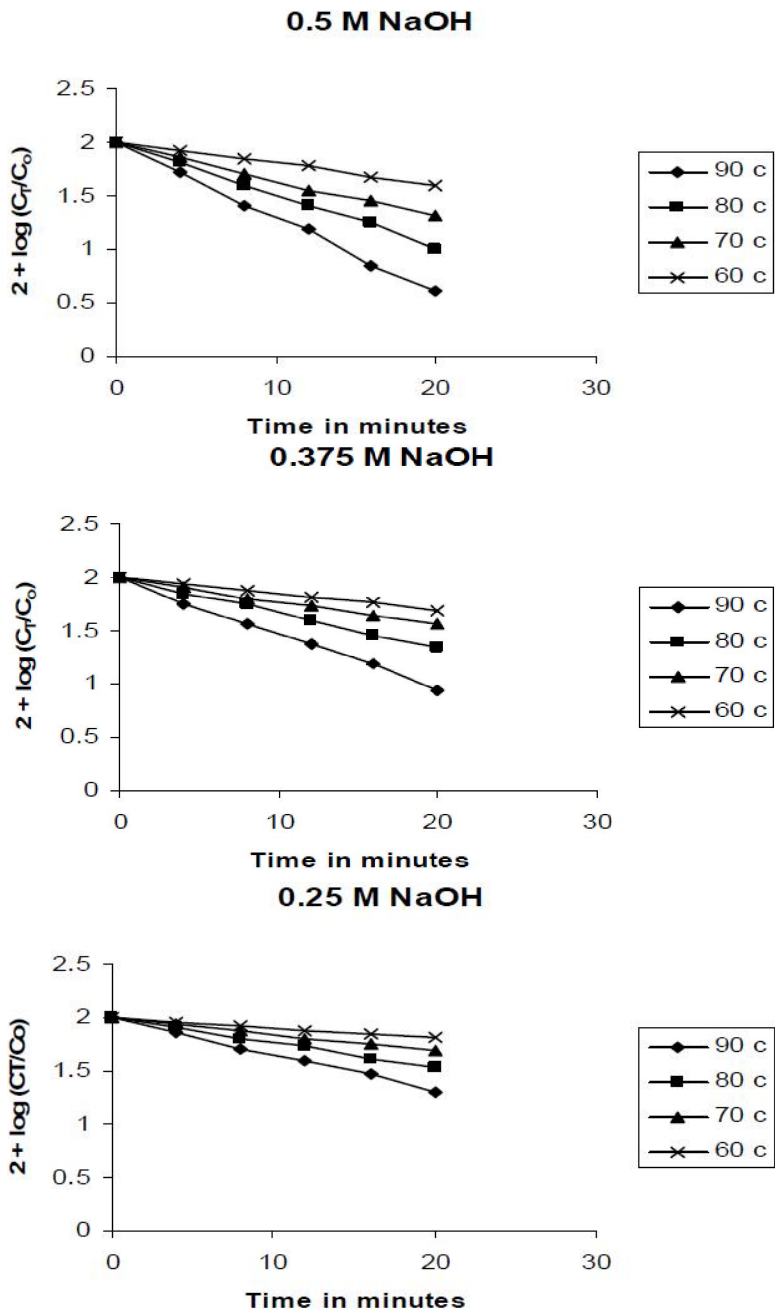

Figure 4 : First order plot of the hydrolysis of RIV (1000 mg %) with 0.5M, 0.375 and 0.25 NaOH at different temperatures

Where "E_a" is the activation energy, "T_1" and "T_2" are the two temperatures degrees in Kelvin, "R" is the gas constant, and "k_1" and "k_2" are the rate constants at the two temperatures used.

The calculated "E_a" was found to be 9.854 Kcal mole^{-1} which was a comparatively low value for amides, suggesting the instability in alkaline medium[26].

Another factor that affects the rate of the reaction is the base strength of NaOH, thus different normalities were used to study the hydrolysis reaction. The rate of hydrolysis increased with an increasing NaOH concentration, although the effect was minor compared to the effect of temperature (Figure 4) and (TABLE 6).

In conclusion, the alkaline hydrolysis of rivastigmine was found to follow a pseudo first order reaction rate. Also the reaction rate increases with increase in the temperature and the strength of the alkaline solution.

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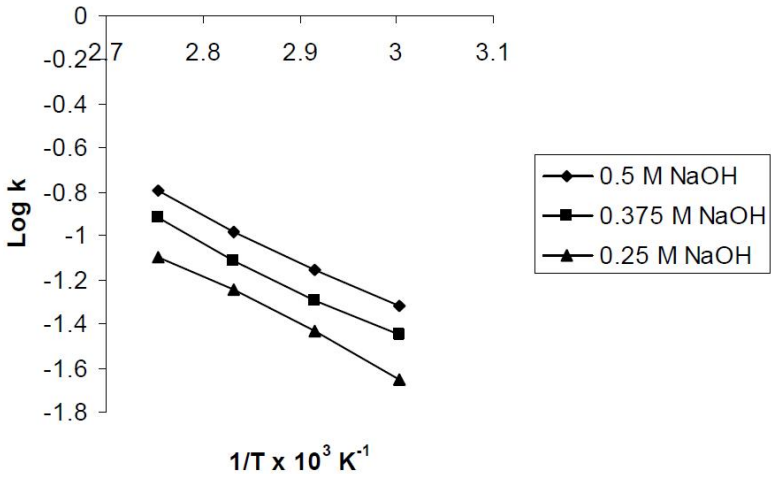

Figure 5 : Arrhenius plot for the hydrolysis of RIV (1000 mg %) with 0.5, 0.375 and 0.25 M NaOH

CONCLUSION

The proposed HPLC method provides a simple, sensitive, and selective method suitable for the quality control analysis of RIV either in the pure powdered form or available pharmaceutical dosage forms with no interference from excipients or the degradation product.

ACKNOWLEDGEMEN

The authors would like to thank Institute of Scientific Research and Revival of Islamic Heritage at Umm Al-Qura University (Project ID 43410009) for the financial support.

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