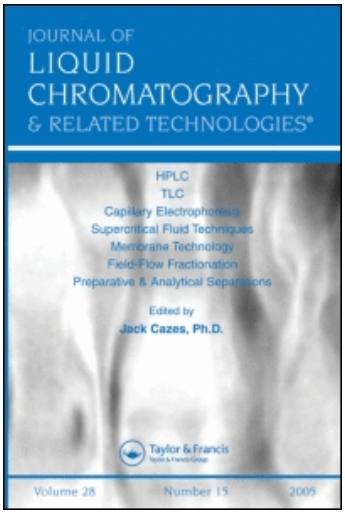

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Quantitative Determination and Stress Degradation Studies on a Biomarker Trigonelline by a Validated Stability-Indicating HPTLC Method

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Abstract: The present study describes stress degradation studies on a biomarker, trigonelline following the International Conference on Harmonization (ICH) guidelines under different stress conditions and establishment of a stability-indicating HPTLC assay method. Analysis of trigonelline was performed on TLC aluminium plates precoated with silica gel 60F-254 and mobile phase consisting of n-propanol-methanol-water (4:1:4 v/v/v). Spectrodensitometric scanning was carried out in absorbance mode at 269 nm for trigonelline ($R_f = 0.46 \pm 0.02$). Trigonelline was subjected to acid and alkali hydrolysis, oxidation, dry heat, wet heat treatment, and photodegradation. Statistical analysis proves that the developed HPTLC method is reproducible and selective. As the method could effectively separate the drug from its degradation products, it can be employed as a stability-indicating one. Moreover, the method was utilized to investigate the kinetics of the acidic and alkaline degradation processes at different temperatures and to study degradation in constant ionic strength buffer solutions within the pH range 1–11.

Keywords: Trigonelline, High performance thin layer chromatography, Stability-indicating, Stress degradation, Kinetics of degradation, pH-rate profile, Chemoprofiling

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INTRODUCTION

In recent times, chromatographic techniques such as high performance thin-layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) have been used to generate profiles of various chemical constituents of herbal drugs, which is known as chemoprofiling. Chemical constituents are isolated in an increasing order of polarity with specific organic solvents and a compound specific to that particular species (e.g., alkaloid, terpene, etc.) is characterized as a chemical marker. Current editions of herbal pharmacopoeia place a strong emphasis on the need for chemoprofiling of crude drugs and their subsequent standardization with respect to their composition. When these chemical marker compounds possess an intrinsic biological activity they are known as biomarkers. Some examples of this are ginsenosides from ginseng and hypericin from St. John's wort.

Biomarkers have gained increased attention primarily in European nations and in countries like China and India, which have a long history of using herbal medicines. The European Scientific Cooperative on Phytotherapy (ESCOP) has clearly specified the requirement of the standardization of phytopharmaceuticals on the basis of biomarkers that are unique to that species.[1]

Trigonella foenum-graecum (TFG) is an aromatic herbaceous plant widely known for its various pharmacological activities, including antiseptic, antimigraine, antitumor, and mutagenic properties.[2–10] One of the active principles reported to be responsible for various actions is trigonelline. Chemically trigonelline is 1-methylnicotinic acid (Figure 1) and is an important alkaloid constituent, which can be used as a biomarker for TFG.

Literature survey reveals the spectrophotometric[11] and high performance liquid chromatography (HPLC) method for the determination of trigonelline in TFG and in biological fluids.[12] Other analytical methods such as over pressured liquid chromatography (OPLC),[13,14] thin layer chromatography (TLC),[15] and high performance thin layer chromatography (HPTLC) also find application in trigonelline analysis.

The parent drug stability test guidelines (Q1AR) issued by International Conference on Harmonization (ICH) requires that stress testing of drug substance should include the effects of temperature, humidity, light, and susceptibility across a range of pH values.[16] Stress testing is suggested as a development strategy under ICH requirements, and is carried out under more severe conditions

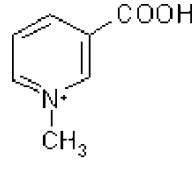

Figure 1. Chemical structure of trigonelline.

than accelerated studies. Further, stress testing should be carried out to establish the inherent or intrinsic stability characteristics of the molecule by establishing the degradation pathways to help in the validation of analytical methods to be used in stability studies of the analyte. Accordingly, the present study puts the ICH recommendations into practice by subjecting trigonelline to the variety of suggested stress test conditions to establish inherent stability of the drug, and to develop the validated stability indicating HPTLC assay.

In our previous study, we have developed and validated an HPTLC assay method for trigonelline in herbal extract and pharmaceutical dosage form.[17] The proposed HPTLC densitometric method is simple, accurate, specific, and repeatable. It reduces the duration of analysis and, hence, is suitable for routine determination of trigonelline and its stability in bulk and pharmaceutical dosage forms.[18] In the present study, we investigated the different stress conditions on the stability of the drug with the help of a previously described validated HPTLC method. Acid- and base-induced degradation kinetics was investigated by quantitation of drugs by a developed and validated HPTLC method. The apparent pseudo first-order rate constant, half-life, and activation energy were calculated from the Arrhenius plot. Additionally, the pH rate profile for degradation of trigonelline in constant ionic strength buffer solutions within the pH range 1–11 was studied.[16,19] The endeavor was to quantify the amount of degradation of trigonelline under various stress conditions. There is no literature survey yet on these aspects for this drug. An ideal stability-indicating method shall quantify the drug per se and also resolves its degradation products. Therefore, it was necessary to study the stability of trigonelline towards acidic, alkaline, oxidative, UV, and photodegradation processes.[16,17]

EXPERIMENTAL

Materials

Standard trigonelline hydrochloride was purchased from Sigma Aldrich Chemicals Pvt. Ltd. (New Delhi, India). *Trigonella foenum-graecum* was procured from the local suppliers, and was assessed biologically by the Department of Botany. All chemicals and reagents used were of analytical grade and were purchased from Merck Ltd. (Mumbai, India).

Instrumentation and Chromatographic Conditions

The samples were spotted in the form of bands of width 6 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm × 10 cm, 200 μm thickness, E. Merck, Darmstadt, Germany), using a Linomat V (Camag, Muttenz, Switzerland) sample applicator. A constant application rate of 150 nl s^{-1} were employed and space between two bands were 10 mm. The slit dimension was kept at 5 mm × 0.45 mm and 20 mm s^{-1} scanning speed was employed. The mobile phase consisted of

n-propanol-methanol-water (4:1:4 v/v/v). Linear ascending development was carried out in a twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25°C ± 2°C) at relative humidity of 55% ± 5%. The length of the chromatogram run was 80 mm. Subsequent to development, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner III in the absorbance mode at 269 nm. The source of radiation utilized was a deuterium lamp.

Forced Degradation Studies on Trigonelline

Forced degradation studies are useful in providing an indication of the stability-indicating property and specificity of the proposed analytical method and were carried out after subjecting trigonelline to acid and alkali hydrolysis, oxidation, dry heat and wet heat treatment, photochemical, and UV-degradation. The procedure used to study these parameters was the same as we previously reported for other HPTLC analytical methods.[20]

A stock solution containing 100 mg of trigonelline in 100 mL methanol was prepared. This stock solution (1000 mg mL^{-1}) was used for forced degradation study under various stress conditions.

Preparation of Acid- and Base-Induced Degradation Product

To 10 mL of methanolic stock solution of trigonelline, 10 mL of 5 M HCl and 10 mL of 5 M NaOH were added separately. These mixtures were refluxed separately for 3 h at 80°C. The forced degradation in acidic and basic media was performed in the dark, in order to exclude the possible degradative effect of light. Two microlitres (1000 ng $spot^{-1}$) of the resultant solutions were applied on TLC plates and the chromatograms run as described in Instrumentation and Chromatographic section.

Preparation of Hydrogen Peroxide-Induced Degradation Product

To 10 mL of methanolic stock solution of trigonelline, 10 mL of hydrogen peroxide (H_2O_2) (30% v/v) was added. This solution was heated in a boiling water bath for 10 min to remove completely the excess of hydrogen peroxide and then refluxed for 3 h at 80°C. Two microlitres (1000 ng $spot^{-1}$) of the resultant solution was applied on TLC plates and the chromatograms run as described in Instrumentation and Chromatographic section.

Dry Heat-Induced Degradation Product

The powdered trigonelline was stored at 100°C for 8 h under dry heat conditions to study the inherent stability of trigonelline. The methanolic stock solution of this dry heat exposed drug was prepared as described in Forced

Degredation Studies, and 10 mL of the prepared stock solution was diluted to 20 mL with methanol (500 μg mL^{-1}). Two microlitres (1000 ng spot^{-1}) of the resultant solution was applied on TLC plates and the chromatograms run as described in Instrumentation and Chromatographic section.

Wet Heat-Induced Degradation Product

The methanolic stock solution of 10 mL of trigonelline was diluted to 20 mL with methanol (500 μg mL^{-1}) and refluxed for 3 h in a boiling water bath to study the wet heat degradation. Two microlitres (1000 ng spot^{-1}) of the resultant solution was applied on TLC plates and the chromatograms run as described in Instrumentation and Chromatographic section.

Photochemical and UV Degradation Product

The methanolic stock solution of 10 mL of trigonelline was diluted to 20 mL with methanol (500 μg mL^{-1}) and the photochemical stability of the drug was studied by exposing the solution to direct sunlight for three days (from 09:00 to 17:00 h at 30°C, total 24 h) on a wooden plank and kept on a terrace. The drug solution was also exposed to UV irradiation at 254 nm for 8 h in a UV-chamber. Two microlitres (1000 ng spot^{-1}) of the resultant solutions were applied on TLC plates and the chromatograms run as described in Instrumentation and Chromatographic section.

Phosphate Buffer (pH 4.5) Degradation Product

The methanolic stock solution of 10 mL of trigonelline was diluted to 20 mL with pH 4.5 phosphate buffer (500 μg mL^{-1}) and refluxed for 3 h in a boiling water bath to study the degradation of trigonelline, if any. Two microlitres (1000 ng spot^{-1}) of the resultant solution was applied on TLC plates and the chromatograms run as described in Instrumentation and Chromatographic section.

In all degradation studies, the average peak area of trigonelline after application (1000 ng spot^{-1}) of six replicates was obtained.

Kinetic Investigation

Accurately weighed 100 mg of trigonelline was dissolved in 100 mL methanol to prepare the standard solution (1000 μg mL^{-1}). Five millilitre aliquots were transferred into separate 100 mL double neck round bottom flasks and mixed, respectively, with 5 mL of 5 M HCl and 5 M NaOH to get a final concentration of 500 μg mL^{-1}. These flasks were then refluxed at different temperatures (40, 50, 60, 70, 80, and 90°C) to study the acidic and alkaline degradation for different time intervals. At the specified time, the contents of the flask were neutralized to pH 7.0 using predetermined volumes of 1 M HCl and 1 M NaOH.

The contents of the flask were quantitatively transferred to 25 mL volumetric flasks with the help of a microsyringe and appropriately diluted to volume with methanol, and estimated by the HPTLC method. Each experiment was repeated three times at each temperature and time interval. Four microlitres (800 ng $spot^{-1}$) of the resultant solutions were applied on TLC plates, the chromatograms were run as described in Instrumentation and Chromatographic section, and the concentration of the remaining trigonelline was calculated. Data was further processed and degradation kinetics constants were calculated.

pH-Rate Profile

Accurately weighed 100 mg of trigonelline was transferred into separate 100 mL volumetric flasks and diluted to volume with constant ionic strength buffer solutions prepared as per Indian Pharmacopoeia.[21] The pH values of the buffer solutions used for measurement of the pH rate profile of the degradation of trigonelline was as follows, pH 1.2, 1.8, 2.8, 3.8, 4.5, 5.7, 6.8, 8.0, 9.2, 9.7, and 10.8. The pH values of these buffer solutions were checked before and after the reaction and were unchanged. Separate 8 mL aliquots of the buffer solution containing trigonelline (1000 $\mu g\ mL^{-1}$) were transferred into separate stoppered round bottom flasks, which were refluxed at 40°C at several time intervals. At the specified time interval, the contents of the flasks were neutralized to pH 7.0 using 1M NaOH or 1M HCl solution. The contents of the flasks were transferred into 10 mL volumetric flasks and diluted to volume with the buffer solutions. One microlitre (800 ng $spot^{-1}$) of each solution was applied on TLC plates, the chromatograms were run as described in Instrument and Chromatographic Section and the concentration of the remaining trigonelline was calculated at each pH value and time interval.

RESULTS AND DISCUSSION

Stability of Trigonelline in Sample Solution

Solutions of three different concentrations of trigonelline (200, 400, and 800 ng $spot^{-1}$) were prepared from the sample solution and stored at room temperature for 3, 6, 9, 12, 18, and 24 h, respectively. The solutions were stored in tightly capped volumetric flasks protected from light on a laboratory bench. They were then applied on the same chromatoplates and after development the chromatograms were evaluated for additional spots, if any. The S.D., % R.S.D. and S.E. for the samples analyzed at different elapsed assay times were found to be <2 (Table 1). Thus, the drug was stable in the solution state. There was no indication of compound instability in sample solution.

Table 1. Stability of trigonelline in sample solutions (n = 6)

Actual amount (ng spot^{-1})	Area mean	Area range	S.D.	R.S.D. (%)	S.E.
200	1038.57	1013.66–1068.29	1.32	0.127	0.54
400	1851.88	1829.16–1886.31	1.92	0.104	0.78
800	3508.83	3479.97–3547.22	1.83	0.052	0.75

Spot Stability of Trigonelline

The time the sample is left to stand on the solvent prior to chromatographic development can influence the stability of separated spots and, hence, is required to be investigated for validation.[22] Two-dimensional chromatography using same solvent system was used to determine any decomposition occurring during spotting and development. If decomposition occurs during development, peak(s) of the decomposition product(s) shall be obtained for the analyte, both in the first and second direction of the run. No decomposition was observed for trigonelline during spotting and development (Figure 2).

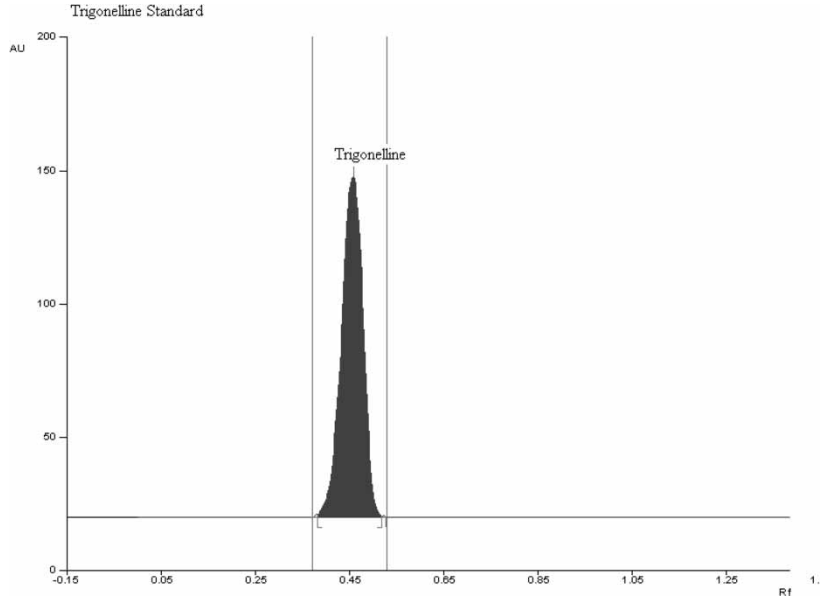

Figure 2. Chromatogram of standard trigonelline (1000 ng spot^{-1}): peak 1 ($R_f = 0.46$), mobile phase: n-propanol-methanol-water (4:1:4 v/v/v).

Stability Indicating Property

The chromatograms of the samples treated with acid, base, wet heat, photochemical, and UV light (254 nm), showed well separated spots of pure trigonelline, as well as some additional peaks at different R_f values. The degradants identification was based on the comparison of the UV spectra of "stressed samples" with that of the "standard solution". The spots of the degraded products were well resolved from the drug spot (Figure 3–8) and the number of degradation products with their R_f values, content of trigonelline remained, and percentage recovery are listed in Table 2. The ANOVA for % recovery of trigonelline at 95% confidence level showed no significant difference ($p < 0.05$) for H_2O_2, dry heat, and phosphate buffer (pH 4.5) stressed samples.

Acid- and Base-Induced Degradation Product

The chromatogram of the acid degraded sample for trigonelline showed 4 additional peaks at R_f values of 0.05, 0.60, 0.84, and 0.91, respectively (Figure 3). The chromatogram of the base degraded sample also showed 4

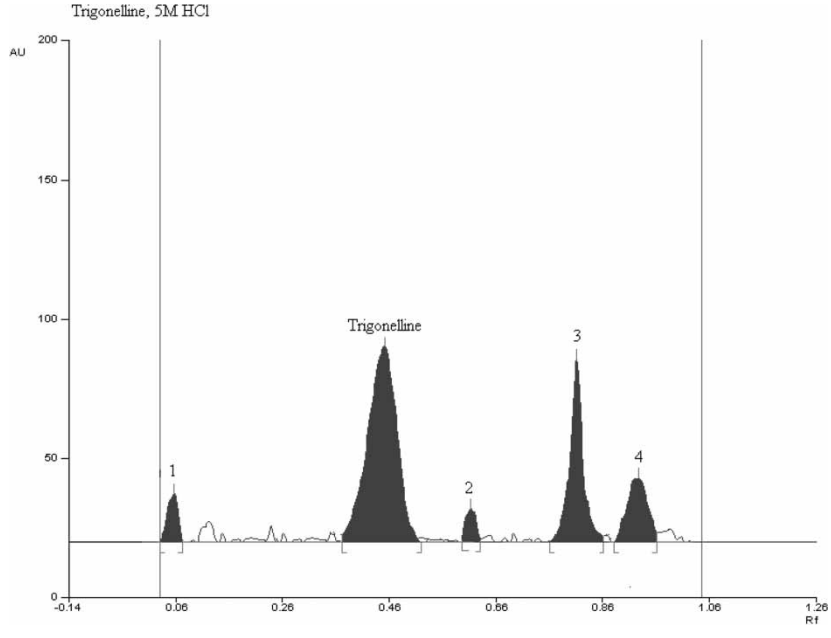

Figure 3. Chromatogram of acid (5 M HCl, reflux for 3 h at 80°C) treated trigonelline: peak 1, degradant ($R_f = 0.05$); peak 2, degradant ($R_f = 0.60$); peak 3, degradant ($R_f = 0.84$); peak 4, degradant ($R_f = 0.91$).

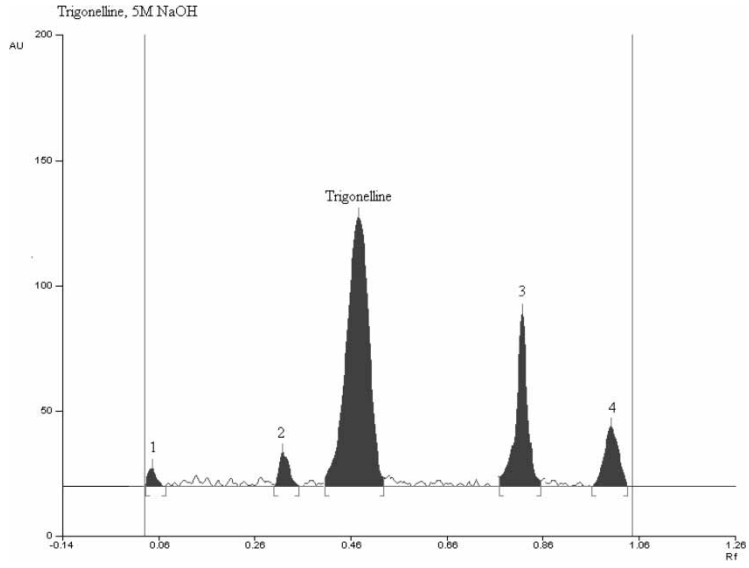

Figure 4. Chromatogram of base (5 M NaOH, reflux for 3 h at 80°C) treated trigonelline: peak 1, degradant ($R_f = 0.03$); peak 2, degradant ($R_f = 0.35$); peak 3, degradant ($R_f = 0.79$); peak 4, degradant ($R_f = 0.93$).

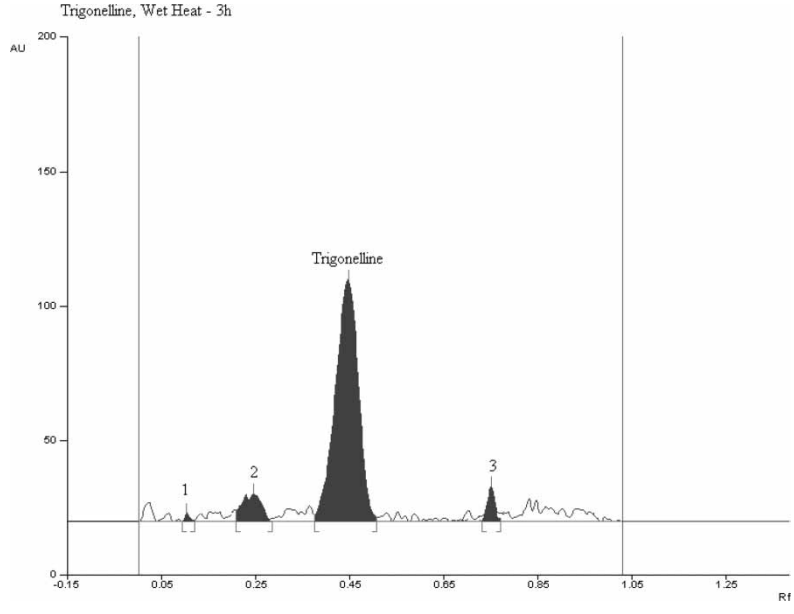

Figure 5. Chromatogram of trigonelline exposed to wet heat (100°C for 3 h): peak 1, degradant ($R_f = 0.11$); peak 2, degradant ($R_f = 0.24$), peak 3, degradant ($R_f = 0.75$).

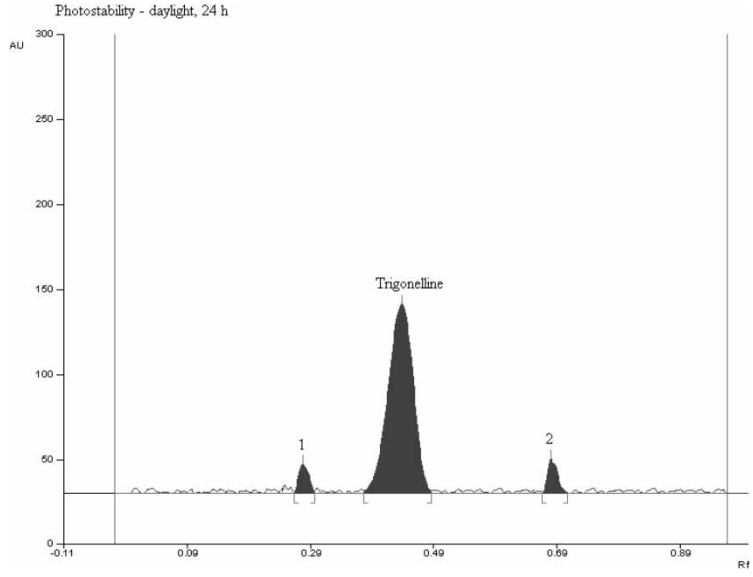

Figure 6. Chromatogram of trigonelline exposed to direct sunlight (photostability, 24 h): peak 1, degradant ($R_f = 0.28$); peak 2, degradant ($R_f = 0.68$).

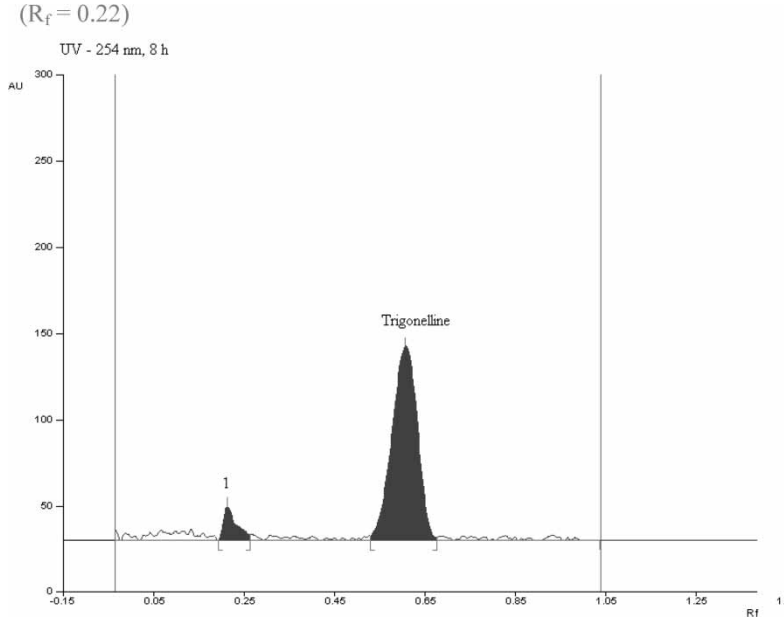

Figure 7. Chromatogram of trigonelline exposed to UV irradiation (254 nm, 8 h): peak 1, degradant ($R_f = 0.22$).

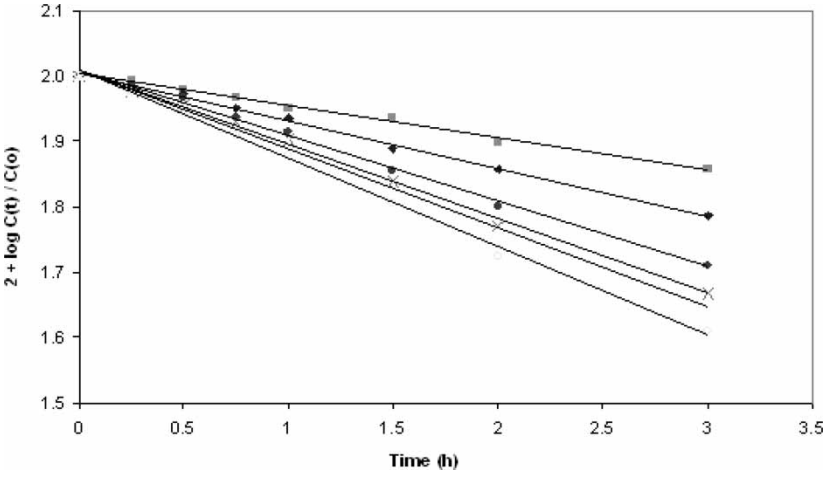

Figure 8. Pseudo first-order plots for the degradation of trigonelline with 5M HCl at various temperatures. *Key:* 90°C (*o*), 80°C (▲), 70°C (×), 60°C (●), 50°C (◆), 40°C (■); C_t, concentration at time t; C_0, concentration at time zero.

additional peaks at R_f value of 0.03, 0.35, 0.79, and 0.93, respectively (Figure 4). The areas of the base-degraded product peaks were found to be lower than the areas of the acid-degraded product peaks and standard drug concentration (1000 ng spot^{-1}). Drug recovery at the level of 42.73% and

Table 2. Forced degradation study of trigonelline (n = 3)

Sr. no	Exposure conditions	Time (h)	R_f value of degradation products	Figure	Drug Remained (ng/1000 ng) (±S.D., n = 3)	Recovery (%)
1	Acid, 5M HCl, refluxed	3	0.05, 0.60, 0.84, 0.91	Figure 3	427.3 ± 7.44	42.73
2	Base, 5M NaOH, refluxed	3	0.03, 0.35, 0.79, 0.93	Figure 4	746.2 ± 6.38	74.62
3	H_2O_2 (30% v/v), refluxed	3	None detected	—	990.6 ± 2.81	99.06
4	Dry heat (100°C)	8	None detected	—	993.8 ± 5.73	99.38
5	Wet heat (100°C)	3	0.11, 0.24, 0.75	Figure 5	926.7 ± 2.68	92.67
6	Photostability - daylight	24	0.28, 0.68	Figure 6	952.1 ± 4.79	95.21
7	UV (254 nm)	8	0.22	Figure 7	979.3 ± 2.18	97.93
8	Phosphate buffer, pH 4.5	3	None detected	—	1006.4 ± 3.22	99.14

74.62% from acid- and base-stressed samples, respectively, suggests significant degradation of trigonelline in acidic and basic conditions. The spots of the degraded products were well resolved from the drug spot.

Hydrogen Peroxide-Induced Degradation Product

The chromatogram of the sample of trigonelline treated with 30% v/v H_2O_2 showed no additional peaks other than the standard trigonelline peak at $R_f = 0.46 \pm 0.02$, suggesting the stability of trigonelline towards the oxidation induced degradation.

Dry Heat and Wet Heat Degradation Product

The chromatogram of the wet heat stressed sample of trigonelline showed 3 additional peaks of degradants at R_f values of 0.11, 0.24, and 0.75 (Figure 5). The dry heat stressed sample, on the other hand, did not show appearance of any additional peaks of degradants.

Photochemical and UV Degradation Product

The chromatogram of the sample exposed to photochemical degradation showed 2 additional peaks other than the trigonelline peak at R_f values of 0.28 and 0.68 (Figure 6). The chromatogram of the sample exposed to UV light at 254 nm showed an additional peak at R_f values of 0.22 (Figure 7). Percent recovery values suggest that the drug is more unstable towards the photochemical degradation than UV irradiations.

Phosphate Buffer (pH 4.5) Degradation Product

The chromatogram of the sample of trigonelline treated with phosphate buffer (pH 4.5) showed no additional peaks other than the standard trigonelline peak at $R_f = 0.46 \pm 0.02$, suggesting the stability of trigonelline at acidic pH of 4.5.

Degradation Kinetics

The kinetic of degradation of trigonelline was investigated in 5 M NaOH and 5 M HCl, since the decomposition rate of trigonelline at the lower strength of 1 M HCl and 1 M NaOH was too slow to obtain reliable kinetic data. Each experiment was repeated three times at each temperature and time interval. The mean concentration of trigonelline was calculated for each experiment. A regular decrease in the concentration of trigonelline with increasing time intervals was observed for higher temperatures. At the selected temperatures (40, 50, 60, 70, 80, and 90°C for acidic and alkaline degradation) the degradation process followed pseudo first order kinetics (Figures 8–9). From the slopes of the straight lines, it was possible to calculate apparent first order

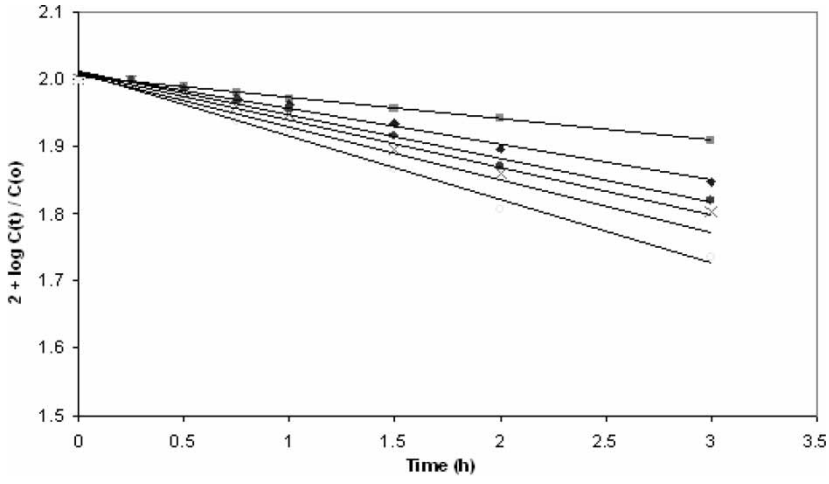

Figure 9. Pseudo first-order plots for the degradation of trigonelline with 5 M NaOH at various temperatures. *Key:* 90°C (○), 80°C (▲), 70°C (×), 60°C (●), 50°C (◆), 40°C (■); C_t, concentration at time t; C_0, concentration at time zero.

degradation rate constant (K_{obs}), half-life ($t_{1/2}$), and t_{90} (i.e., time where 90% of original concentration of the drug is left), at each temperature for acidic and alkaline degradation processes using the HPTLC method (Table 3). Data obtained from first order kinetics treatment was further subjected to fitting in Arrhenius equation:

$$\log \mathrm{K} = \log \mathrm{A} - \frac{\mathrm{Ea}}{2.303\,\mathrm{RT}}$$

where K is rate constant, A the frequency factor, Ea the energy of activation (cal mol^{-1}), R the gas constant (1.987 cal K^{-1} mol^{-1}), and T is absolute temperature (K).

The Arrhenius plot shows the temperature dependence of the drug in the form of a natural logarithm of the rate coefficient versus the reciprocal temperature. An Arrhenius plot is extremely useful if the data is determined experimentally. It also shows, at a glance, if the scatter of the data points is small or large; if there exists an Arrhenius relation at all (i.e. a straight line); and if there are enough data points to get unambiguous values for A and Ea. The plot of $(2 + \log\ K_{\mathrm{obs}})$ versus $(1/\mathrm{T} \times 10^3)$ gave the Arrhenius plot (Figure 10), which was found to be linear in the temperature range 40°–90°C. The correlation coefficient for the line fittings was found to be 0.9928 and 0.9912, respectively, for the acidic and basic degradation process.

The activation energy and the Arrhenius frequency factor were calculated for acidic and alkaline degradation processes. The method of accelerated testing of pharmaceutical products based on principles of chemical kinetics

Table 3. Degradation rate constant (K_{obs}), half-life ($t_{1/2}$) and t_{90} for trigonelline

Temperature (°C)	K_{obs} (h^{-1})	$t_{1/2}$ (h)	t_{90} (h)	R^2 for Pseudo First-order plots
In 5 M HCl				
40	0.0723	9.59	1.45	0.9926
50	0.0782	8.86	1.34	0.9967
60	0.0881	7.87	1.19	0.9958
70	0.1087	6.38	0.97	0.9969
80	0.1208	5.74	0.87	0.9981
90	0.1291	5.37	0.81	0.9973
In 5 M NaOH				
40	0.0315	22.00	3.33	0.9965
50	0.0427	16.23	2.46	0.9993
60	0.0554	12.51	1.90	0.9959
70	0.0705	9.83	1.49	0.9987
80	0.0800	8.66	1.31	0.9913
90	0.0979	7.08	1.07	0.9934

was used to obtain a measure of the stability of the drug under said conditions.[23,24] The degradation rate constant at room temperature (K_{25}) is obtained by extrapolating to 25°C (where $1000/T = 3.356$) and by inserting this into Eq. (1) and $t_{1/2}$ and t_{90} are calculated (Table 4).

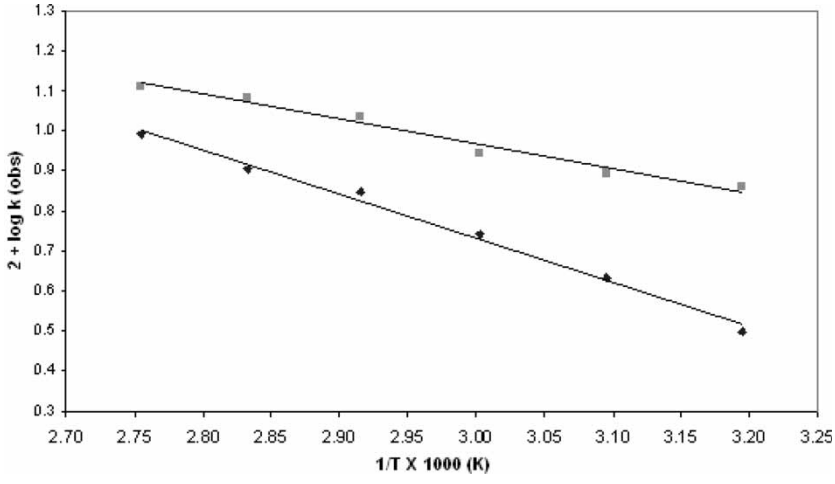

Figure 10. Arrhenius plot for the degradation of trigonelline in presence of 1M HCl (■) and 1M NaOH (◆).

Table 4. Summary of degradation kinetic data for trigonelline at 25° ± 2°C

Parameters	5 M HCl	5 M NaOH
E_a (Kcal mol^{-1})[a]	6.80×10^{-3}	5.05×10^{-3}
K_{25} (h^{-1})[b]	5.56×10^{-2}	2.18×10^{-2}
$t_{1/2}$ (h)[c]	12.46	31.75
t_{90} (h)[d]	1.89	4.81
A[e]	2.85	110.33

[a]Activation energy.
[b]Degradation rate constant.
[c]Half-life.
[d]Time for 90% potency left.
[e]Arrhenius frequency factor.

The pH rate profile of degradation of trigonelline in constant ionic strength buffer solutions was studied at 40°C using the HPTLC method (Figure 11). The apparent first order degradation rate constant, $t_{1/2}$ and t_{90} were calculated for each pH value (Table 5). From the degradation kinetics data, it can be concluded that the drug is highly susceptible to acidic and alkaline degradation. The pH rate profile study shows that the trigonelline is most stable at pH of 4.5 to 6.8, which further suggests that the developed HPTLC method can be extended for quantitative estimation of the drug in plasma and other biological fluids.

The method, however, is not suggested to establish material balance between the extent of drug decomposed and formation of degradation

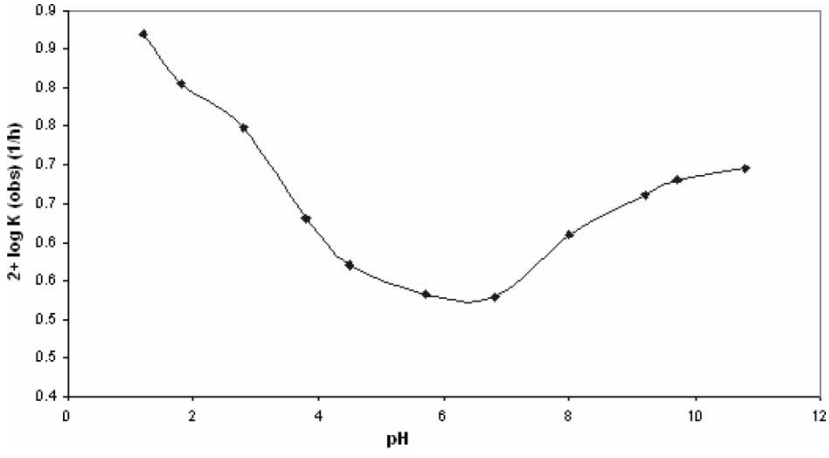

Figure 11. pH-rate profile for the decomposition of trigonelline at constant ionic strength buffer solutions at 40° ± 2°C.

Table 5. Degradation rate constant (K_{obs}), half-life ($t_{1/2}$) and t_{90} for trigonelline at constant ionic strength buffer at different pH values and at a temperature of $40^{\circ} \pm 2^{\circ}C$

pH Value	K_{obs} (h^{-1})	$t_{1/2}$ (h)	t_{90} (h)
1.2	0.0741	9.35	1.42
1.8	0.0638	10.86	1.65
2.8	0.0561	12.35	1.87
3.8	0.0427	16.23	2.46
4.5	0.0372	18.63	2.82
5.7	0.0341	20.32	3.08
6.8	0.0338	20.50	3.11
8.0	0.0407	17.03	2.58
9.2	0.0458	15.13	2.29
9.7	0.0479	14.47	2.19
10.8	0.0497	13.94	2.11

products. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

CONCLUSION

This study is a typical example of development of a stability indicating assay, established following the recommendations of ICH guidelines. It is one of the rare studies where forced decomposition studies were done under all different suggested conditions and the degradation products were well resolved for a biomarker, trigonelline. This study may further be extended to study the degradation kinetics of drug and to predict degradation pathways. This method can be proposed for the analysis of trigonelline and its degradation products in stability samples in industry. The degradation rate constant, half-life, and t_{90} of trigonelline can be predicted for acid and alkaline degradation processes. It can also be extended for quantitative estimation of trigonelline in plasma and other biological fluids. A new finding of this study is that the trigonelline is unstable in acid, base, wet heat, photochemical, and UV-stressed samples. As the method separates the drug from its degradation products, it can be employed as a stability indicating one.

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