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Development and validation of a stability indicating RP-HPLC method for the estimation of triamcinolone in bulk and in tablet formulation

Abstract

Introduction: A simple, rapid, precise, sensitive and reproducible reverse phase High Performance Liquid Chromatographic (RP-HPLC) method has been developed for quantitative analysis of Triamcinolone in bulk and pharmaceutical tablet dosage form. Chromatographic separation was achieved on BDS C18, (250mm x 4.6mm, 5µm particle size). The flow rate was 1.0mL/min and detection was carried out by absorption at 238nm using photodiode array detector. The mobile phase methanol:water (70:30) with pH adjusted to 10.5 using triethylamine was selected as it achieves symmetrical peak and sensitivity.

Result: The method is validated within the range of 3-18µg/mL and correlation coefficient was found to be (r^2=0.998). Excellent mean recovery studies for precision, repeatability, ruggedness, robustness and sensitivity were obtained when method was validated as per ICH guidelines. For stress studies the drug was subjected to acid, alkali, oxidation and photolytic degradation. The degradation studies indicated the drug to be susceptible to alkalines degradations.

Conclusion: Thus the method found to be accurate, specific and reliable, and can be successfully used for the quality control on bulk product and pharmaceutical tablet dosage form.

Keywords: triamcinolone, RP-HPLC, validation, degradation

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Introduction

Triamcinolone (Figure 1) is chemically (9α-fluoro-11β,16α,17,21 tetrahydroxypregna-1, 4-diene-3, 20-Dione.[1,2] It is a long–acting synthetic corticosteroid given orally, by injection, inhalation, or as a topical ointment. Early anti-inflammatory effects include the inhibition of macrophage and leukocyte movement and activity in the inflamed area by reversing vascular dilation and permeability.[3] Later, inflammatory processes, collagen deposition, keloid (scar) formation also are inhibited by corticosteroids. Clinically, these actions correspond to decreased edema, erythema, pruritus, plaque formation and scaling of the affected skin.[4] Recently, this anti-inflammatory compound has been administered intravitreally route for the treatment of chronic inflammation of the posterior segment of the eye.[5]

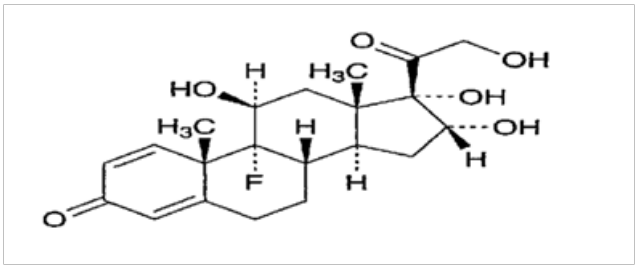

Figure 1 Chemical structure of Triamcinolone.

Literature survey reveals that a few analytical methods have been reported for its quantitative estimation in pharmaceutical formulation, which includes UV and HPLC method.[3–7] The objective of present work is to develop a simple, sensitive, rapid and economic RP-HPLC method for the quantitative estimation of Triamcinolone in bulk and pharmaceutical tablet formulation.

Materials and methods

Chemicals and reagents

Triamcinolone was supplied as gift sample by Glenmark Pharmaceuticals Ltd, Mumbai (India). Commercial tablet formulations (Kenacort-4mg; Abbott healthcare Ltd.) were purchased from local market. All chemicals and reagents used were of analytical and HPLC grade, obtained from Merck and Loba chemie.

Instruments and chromatographic conditions

A High Performance liquid Chromatographic system Agilent Technologies with Analytical Column Qualisil BDS C18 (250mm x 4.6mm, 5µm), equipped with Photo Diode Array Detector and Quaternary pump was used for the analysis. All weights were taken on electronic balance (Shimadzu AUX 120). It was equilibrated with the mobile phase methanol:water:(70:30 *v/v*) and pH adjusted to 10.5 with triethylamine. The flow rate was maintained at 1.0mL/min and eluents were monitored at 238nm. The sample was injected using a 20µL fixed loop. The total sample run time was 10min. Retention time of triamcinolone is 5.46min (Table 1).

Preparation of mobile phase and standard stock solutions

Mobile phase was prepared by mixing of methanol and water to get the proportion of (70:30*v/v*) and finally the pH was adjusted to 10.5 with triethylamine. The mobile phase was sonicated for 15min and filter through 0.45µ membrane filter. The standard stock solution of triamcinolone was prepared by dissolving 10mg drug in 100mL of mobile phase to get 100µg/mL volume was made up to the mark with mobile phase.

Table 1 Finalized chromatographic conditions

Chromatographic mode	Chromatographic condition
Standard solution	100µg/mL of Triamcinolone in mobile phase
Mobile phase	Methanol: water (70:30*v/v*) adjusted pH to 10.5
Detection wavelength	238nm
Flow rate	1.0mL/min
Rt	5.46

Analysis of tablet formulation

Commercially available tablets of triamcinolone were selected for estimation of total drug content by proposed method. Twenty tablets were accurately weighed, powdered and the quantity equivalent to 10mg of drug was transferred to a 100mL volumetric flask and volume made up to 50mL with methanol and sonicated for 20min. The solution was filtered through 0.45µ membrane filter and pipette out 1.2mL the resultant solution was diluted with mobile phase to get final concentration 12µg/mL. The amount of drug present in sample solution was determined using the calibration curve of standard drug shown in Table 2 and Figure 2.

Table 2 Analysis of tablet formulation

Amount Taken (µg/Ml)	Amount found (µg/Ml) Mean±S.D.	%Amount Found*Mean ±S.D.	%R.S.D.
12	11.96±0.15	99.73±1.30	1.31

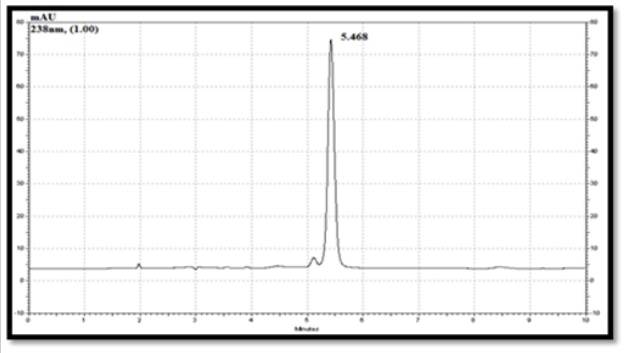

Figure 2 Chromatogram of TRI marketed formulation (12µg/ml) in methanol: water: triethylamine (70:30:0.1 v/v) (showing RF=5.46).

Validation of proposed method

The proposed method provides accurate and precise quality control tool for routine analysis of Triamcinolone according to the ICH guidelines for various parameters like accuracy, precision, repeatability, sensitivity, ruggedness.[8,9]

Linearity

Appropriate known volumes of aliquots form standard triamcinolone stock solution were transferred to series of six 10mL volumetric flasks. The volume was adjusted to the mark with methanol to get concentrations of 3-18µg/mL. Separately calibration curve was plotted for this method as absorbance *vs* concentration (Figure 3) and Results are shown in Table 3.

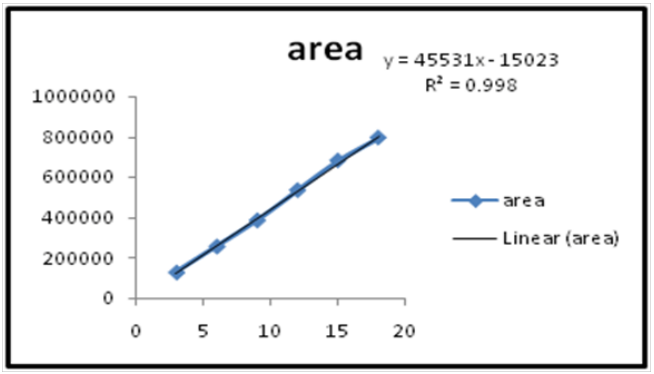

Figure 3 Calibration curve of Triamcinolone.

Table 3 Linearity study of triamcinolone

Concentration(µg/mL)	Mean peak area# ± S.D.	% R.S.D.
3	120606 ± 2400.62	1.99
6	250488.3 ± 5186.33	2.07
9	372399.3 ± 6211.71	1.66
12	524392.7 ± 4741.77	0.9
15	682221.3 ± 8219.71	1.2
18	795690.7 ± 14884.94	1.87

#n=3

Precision

Precision of the method was studied as intraday and inter-day variations. Intra-day precision was determined by analyzing 6,12 and 18µg/mL of Triamcinolone solutions for three times on the same day. Inter-day precision was determined by analyzing daily for three consecutive days over a period of week using same concentration. Results are shown in Table 4.

Table 4 Results of precision studies (Intra-day and Inter-day)

Conc. (µg/Ml)	Intra-Day Amount Found# (%)		Inter-Day Amount Found# (%)	
	Mean ± S.D.	% R.S.D.	Mean ± S.D.	% R.S.D.
6	97.55 ± 0.07	1.24	97.43 ± 0.05	0.91
12	99.59 ± 0.10	0.87	99.59 ± 0.15	1.27
18	99.84 ± 0.33	1.83	98.74 ± 0.11	0.64

#n=3

Accuracy

To the pre-analyzed sample solution, a known amount of standard stock solution was added at different levels i.e. 80, 100 and 120%. The absorbance of solution was recorded, result of recovery studies are reported to assess the accuracy of the proposed method confirmed that proposed method is accurate for determination of Triamcinolone in pharmaceutical formulation**.** Results are shown in Table 5.

Table 5 Results of recovery studies

Initial amount of drug (µg/Ml)	Excess drug added to the analyzed (%)	Amount added (µg/Ml)	% Recovery# Mean±S.D.	R.S.D.
9	80	7.2	101.56±0.30	1.87
9	100	9	101.97±0.14	0.76
9	120	10.8	102.46±0.20	1

#n=3

Repeatability

Repeatability was determined by analyzing 9µg/mL concentration of Triamcinolone solution for six times was recorded and spectra were area measured for each sample. Results are shown in Table 6.

Table 6 Results of repeatability study

Concentration (µg/Ml)	Amount Found*Mean±S.D.	% R.S.D.
9	100.38 ± 0.11	1.23

*n=6

Sensitivity

Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of Quantitation (LOQ). The LOD and LOQ were calculated by the use of equation, LOD=SD/Slope x 3.3 and LOQ=SD/Slope x 10, where SD is the residual standard deviation of the peak areas of the drug (n=3) and Sensitivity was performed between 3-5µg/mL range. Results are shown in Table 7.

Table 7 LOD and LOQ

LOD (µg/mL)	LOQ (µg/mL)
3.8	11.53

#n=3

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two different analysts using same operational and environmental conditions. Results are shown in Table 8.

Table 8 Results of ruggedness study

Analyst	Concentration (µg/Ml)	% Amount Found* (Mean ± S.D.)	% R.S.D.
I	9	100.70±0.08	0.94
II	9	99.37±0.07	0.86

*n=6

Robustness

Robustness of the method was studied by deliberate variations of the analytical parameters such as flow rate (±0.2mL/min), concentration of mobile phase (±5mL). The results are shown in Table 9.

Table 9 Results of robustness study

Change mobile phase ± 5.0mL in same flow rate 1.0mL/min

Runs	Mobile Phase Composition (V/V)	Flow Rate (Ml/ Min)	Area	Retention Time (Min)	Tailing Factor
1	65:35:00	1	454512	5.42	1.03
2	70:30:00	1	459592	5.46	1.09
3	75:25:00	1	462920	5.56	1.13

Change flow rate ± 0.2mL in same mobile phase 65:35 (v/v)

Runs	Mobile Phase Composition (V/V)	Flow Rate (Ml/ Min)	Area	Retention Time (Min)	Tailing Factor
1	65:35:00	0.8	520990	5.78	1.08
2	65:35:00	1	454512	5.42	1.03
3	65:35:00	1.2	438119	4.55	1.11

Forced degradation studies

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative and photolytic degradations. The sample was exposed to under following conditions.[10]

Degradation in acidic condition

About 10mg of pure drug was accurately weighed and taken in 100mL round bottom flask and dissolved in 10mL of 0.1N methanolic HCl and kept in at 40°C for 6h and cooled, neutralized with 0.1N NaOH solution and the concentrations of 10µg/mL same sample solution was prepared and 20µL of the sample solution was injected in to the HPLC system. Result is shown in Table 10 and chromatogram of acidic condition is showed in Figure 4.

Table 10 Results of degradation of triamcinolone under various stress conditions.

Chemicals	Condition	No. of Deg. Peaks	Rt	% Degradation
0.1N HCl	6h (400°C)	---	---	13.06%
0.1N NaOH	90min (400°C)	1	2.25	97.69%
6 % H_2O_2	6h (400°C)	1	3.02	13.90%
Photolytic	12h	---	5.46	33.87%

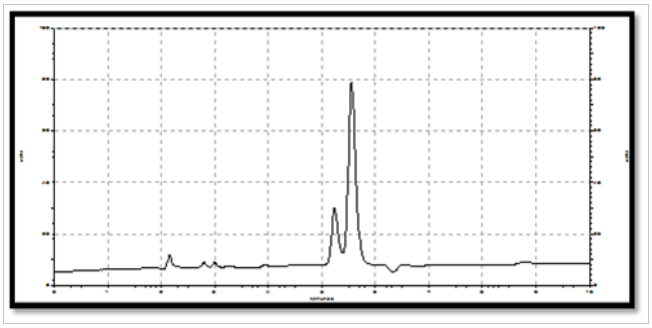

Figure 4 Acidic degradation 0.1N HCl 6hr at 40°C.

Degradation in basic condition

About 10mg of pure drug was accurately weighed and taken in 100mL round bottom flask and dissolved in 10mL of 0.1N methanolic NaOH and kept in 90min heated at 40°C and cooled, neutralized with 0.1 N HCl and the concentration of 10µg/mL sample solution was prepared and 20µL of the sample solution was injected in to the HPLC system. Result is shown in Table 10 and chromatogram of acidic condition is showed in Figure 5.

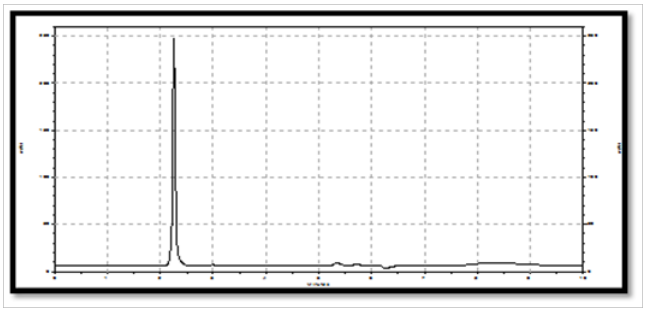

Figure 5 Basic degradation 0.1N NaOH 90min at 40°C.

Degradation in oxidative condition

About 10mg of pure drug was accurately weighed and taken in 10mL volumetric flask and dissolved in 10mL of 6% methanolic H_2O_2 and kept in 40°C at 6h and the concentration of 10µg/mL sample solution was prepared and 20µL of the sample solution was injected in to the HPLC system. Result is shown in Table 10 and chromatogram of acidic condition is showed in Figure 6.

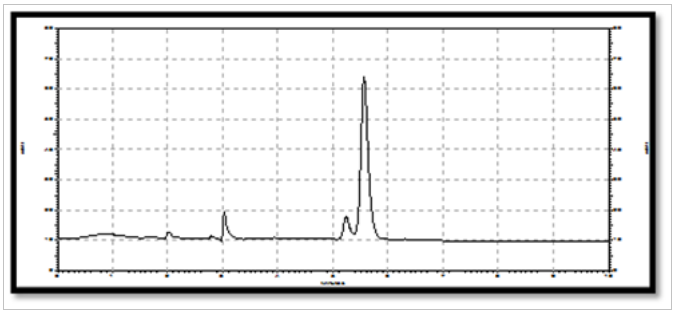

Figure 6 Oxidative degradation 6% H_2O_2 6hr at 40°C.

Degradation in photolytic condition

About 100mg of pure drug was taken in a clean petridish and exposed to sunlight for 12h. From this sample was prepared in 10mg of Triamcinolone was taken and dissolved in 100 mL mobile phase and the pipette out 1.0mL to get final concentration of 10µg/mL sample solution was prepared and 20µL of the sample solution was injected in to the HPLC system. Result is shown in Table 10 and chromatogram of acidic condition is showed in Figure 7.

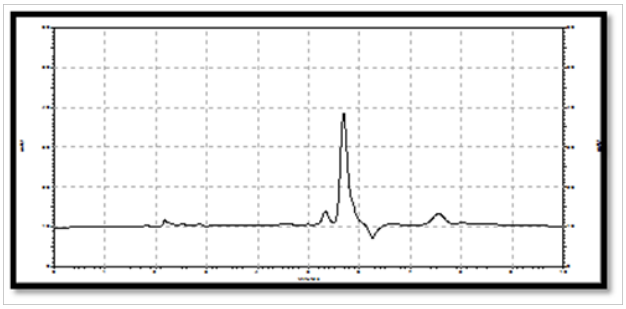

Figure 7 Photolytic degradation of direct sunlight.

Results and discussion

Method development

A new RP-HPLC method was adopted with a view to develop an accurate and reproducible process for estimation of triamcinolone. Optimization of the method was done by altering mobile phase composition, pH, column packing, flow rate, temperature, detection wavelength, and the effects on retention and peak shape were monitored for the selected drug candidate. The final chromatographic conditions have been set for stationary phase giving satisfactory resolution and run time with reversed phase Qualisil BDS C18 (250mm x 4.6mm, 5µm particle diameters) column.

A series of mobile phases varying the pH and volume fractions of methanol and water have been also tested with changing the pH and the best results obtained by use of mobile phase consisting of methanol: water (70:30*v/v*) with pH adjusted to 10.5 by means of triethylamine. This gives a well resolved, sharp peak for triamcinolone with a retention time of 5.46. The flow rate of 1.0mL/min and detection wavelength was chosen as 238nm. System suitability parameters were studied by injecting five replicate injections of working standard solution (10µg/mL). The results obtained for method development indicates that the proposed method followed linearity over specified range of drug concentration. The range of concentration corresponds to the upper and lower limits for quantitation of analytical determination showing precision, accuracy and adequate linearity.

Method validation

The proposed method was validated according to the ICH guidelines for several parameters.[8,9] The HPLC methodology used showed adequate linearity in the concentration range (3-18µg/mL) recorded at 238nm for quantitative analysis of triamcinolone in experimental condition described. The correlation coefficient value was obtained as r^2=0.998. The calibration curve was plotted as shown in Figure 3 and results of linearity are mention in Table 3. Precision is an important analytical parameter representing the variability in the result in repeated serial analysis of the sample under identical experimental conditions. In the present work intra and interday precision were evaluated. The results are shown in Table 4 and % R.S.D for precision was found to be less than 2. This indicates that the proposed method is precise.

Accuracy is one of the most important analytical parameters of a methodology and it can be expressed as the percent recovery of the

known amount of drug added to a sample. Accuracy describes the degree of veracity of the quantitative analysis. For repeated analysis of a reference drug, the deviation of the mean from the certified value shall not outside the limits ±10%. For pharmaceutical products, the established reasonable percent recovery limits are 80-110%. Accuracy (recovery) of the method was determined by spiking 80, 100 and 120% of working standard. The percentage recoveries found to be in the range of 101.46-102.56% as shown in Table 5 and are in agreement with established limits. Repeatability was measured in terms of application and measurement. Study was carried out for six replicated of the standard at a concentration of 9µg/mL. The R.S.D calculated was less than 2.0% as shown in Table 6. To standardize the methodology, a standard drug sample was run and the limit of detection and quantitation of the equipment were determined. Thus, the smallest amount of drug that can be reliable detected in the sample is the limit of detection (LOD) under the experimental conditions established. The limit of quantitation (LOQ), the lowest content of the drug, which can be measured with reasonable statistical certainty, was used. If both accuracy and precision are constant over a concentration range around the limit of detection, then the limit of quantitation is numerically equal to six times the standard deviation of the mean of blank determination. The LOD for proposed method was found to be 3.80 and LOQ was found to be 11.52 as shown in Table 7.

The ruggedness is degree of reproducible of the results obtained under a variety of conditions, these includes different laboratory conditions and analysts. To evaluate ruggedness of the developed method, deliberated variations were made in the method parameters such as analysts. The results are found to be % R.S.D of 0.86-0.94 as shown in Table 8. The robustness method was determined by making slight changes in the chromatographic conditions such as flow rate and mobile phase. In the present method, deliberated variations were made in flow rate of mobile phase (±0.2mL/min) and ratio of mobile phase (±5.0mL). The results are shown in Table 9.

Forced degradation study

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using acid, alkaline, oxidative and photolytic conditions. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

Acid degradation of triamcinolone was done using 0.1N HCl for 6h at 40°C. The reaction was initiated by exposing the drug with HCl and slowly the exposure time is increased. The % degradation was found to be 13.06%. As no peak for degradant was observed; indicated that the drug is quite stable under acidic environment. The obtained chromatogram for degradation is shown in Figure 4A. Basic degradation of triamcinolone was performed by using 0.1N NaOH kept in 90min at 40°C. At very initial stage only high amount of drug (97.69%) found to be degrading which is attributed to presence of peak at Rt 2.25. This is clearly distinct form peak of original drug as shown from obtained chromatogram in Figure 4B. As far as the oxidative degradation is concern, triamcinolone is found to be less degraded under oxidative stress condition. The % degradation in 6% H_20_2 was found to be 13.9% at 40°C with a separate peak at Rt 3.02 as shown in Figure 4C. This supports to the fact that triamcinolone is stable against oxidative condition. The report analyzed for the degradation in direct sun light and the obtained chromatogram was shown in Figure 4D. The results explored that the drug is unstable in light and degraded around 34%. All the results of degradation studies under several stress conditions are tabulated in Table 10.

Conclusion

In this study, a selective and validated stability indicating RP-HPLC method for triamcinolone was developed according to ICH guidelines. The proposed method proved to be simple, accurate, precise, specific and selective across all the studied parameters. Moreover the proposed method proved to be applied successfully for its pharmaceutical tablet formulation. This also indicated as there is no interference of excipients. Forced degradation studies indicate that the drug is highly unstable under alkaline condition as it degraded rapidly and in higher fraction. Thus, it can be conclude d that the proposed method may be supportive for routine analysis of triamcinolone in bulk and in tablet formulation as well.

Acknowledgments

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Conflicts of interest

The author declares that there is no conflict of interest.

References

1. Indian Pharmacopoeia. Ministry of health & family welfare, India. 2014;3:2906.
2. The European Pharmacopoeia. Convention on the Elaboration of European Pharmacopoeia, 5th Ed. 2005;2:2613.
3. Gupta SK, Kumar B, Sharma PK. Development and validation of Spectrophotometric method for estimation of Triamcinolone in solid dosage form. *Asian J Pharm Ana*. 2013;3(2):42–43.
4. Dave BR, Jiladia MA, Vidysagar G. Simultaneous estimation of Chlorhexidine and Tramcinolone by HPLC. *Int Jou of Pharm Sci*. 2012;3(4):2850–2860.
5. Emirhan N, Filiz S, Umut A, et al. Determination of Tramcinolone in subretinal fluid by avalidated HPLC method. *Hacettepe University, Journal of Faculty of Pharmacy*. 2005;25(2):71–78.
6. Aquino GDA, Stopilha RT, Pedrosa KSCR, et al. Validation of quantitative analysis method for Triamcinolone in ternary complexes by UV -Vis spectrophotometry. *Rev Cienc Farm Basica Apl*. 2011;32(1):35–40.
7. Silva AA, Formariz TP, Scarpa MV, et al. Development and validation of HPLC method for quantitative analysis of Triamcinolone in biodegradable microparticales. *Rev Cienc Farm Basica Apl*. 2006;27(2):119–126.
8. ICH-International Conference on Harmonization of Technical Requirements of Pharmaceuticals for human use. *Validation of Analytical Procedures: Methodology*. 1996.
9. ICH Guidelines Q2 (R1). Validation of Analytical Procedures: Text and Methodology International Conference on Harmonization. 2005:1–13.
10. Ranjit S, Rehman Z. Current trends in forced degradation study for pharmaceutical product development. *J Pharm Educ Res*. 2012;3(1):54–63.