Original Article

Development and validation of a stability indicating HPTLC-densitometric method for lafutidine

Abstract

Background: A simple, selective, precise, and stability indicating high-performance thin layer chromatographic method has been established and validated for analysis of lafutidine in bulk drug and formulations. **Materials and Methods:** The compounds were analyzed on aluminum backed silica gel 60 F_{254} plates with chloroform:ethanol:acetic Acid (8:1:1) as mobile phase. Densitometric analysis of lafutidine was performed at 230 nm. **Result**: Regression analysis data for the calibration plots were indicative of good linear relationship between response and concentration over the range 100-500 ng per spot. The correlation coefficient (r^2) was 0.998±0.002. **Conclusion:** Lafutidine was subjected to acid, base, peroxide, and sunlight degradation. In stability tests, the drug was susceptible to acid and basic hydrolysis, oxidation, and photodegradation.

Key words:
High-performance thin layer chromatography, lafutidine, stability indicating assay, stress testing, Ultra violet spectroscopy

Introduction

The parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH)[1] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability indicating and should be fully validated. Lafutidine is -(furan-2-ylmethylsulphinyl)-N-[(Z)-4-[4-(piperidinyl-methyl)-pyridin-2-yl) oxybut-2-enyl] acetamide [Figure 1].[2] It is freely soluble in methanol, whereas it is practically insoluble in water. It is a second generation histamine H_2-receptor antagonist and acts as anti-ulcerative.[2]

The literature survey reveals that lafutidine was analyzed by GC-MS or LC-MS,[2] HPLC.[3] However, HPTLC method had not been reported till date in literature for analysis of lafutidine as bulk drug or in formulations.

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HPTLC is a widely used analytical technique due to its advantages of low operating cost, high sample throughput,

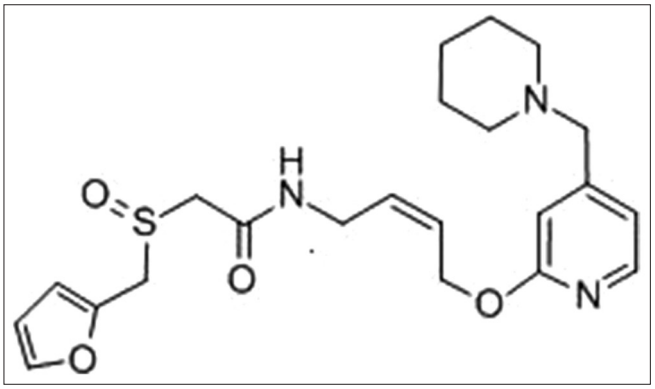

Figure 1: Structure of lafutidine

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and need of minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus reducing the analysis time and cost per analysis.[4] Hence, the objective of the present study was to develop a stability-indicating HPTLC[5] method for estimation of lafutidine as bulk drug and in formulations and to perform stress studies under a variety of ICH recommended test conditions.[1,6] The proposed method was validated for linearity, accuracy (recovery studies), specificity, precision, robustness, ruggedness, limit of detection (LOD), limit of quantitation (LOQ), and repeatability according to the ICH guidelines[7,8] and its updated international convention.[9]

Experimental

Materials

Lafutidine pure drug sample was obtained from Alkem Research Center, Panvel, Mumbai, as a gift sample with 99.9% w/w assay value. It was used without any purification. All other chemicals and reagents used were of analytical grade.

Instrumentation

The samples were spotted in the form of bands of width 10 mm with a DESSAGA 10 microliter sample syringe on silica gel pre-coated aluminum plate 60 F–254 plates, (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany) using a CAMAG IV sample applicator. A constant application rate of 0.1 µl/s was used, and the space between two bands was 10 mm. The scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times, and baseline correction was done. The mobile phase consisted of chloroform:ethanol:acetic acid (8:1:1 v/v), and 25 ml of mobile phase was used per chromatograph run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 25 min at room temperature (25±2˚C) at relative humidity of 60% ±5%. The length of each chromatogram run was 8 cm. Following the development, these TLC plates were dried in a current of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow rate in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a CAMAG TLC scanner in the reflectance absorbance mode at 231 nm and operated by CAMAG. The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatograph were determined from the intensity of the diffused light. Evaluation was done by peak areas with linear regression.

Forced degradation studies

A stock solution containing 10 mg lafutidine in 10 ml methanol was prepared. This solution was used for forced degradation to provide an indication of the stability indicating property and specificity of the proposed method. In all degradation studies, the average peak area of lafutidine after application (100 ng) of six replicates was obtained. In order to study the degradation products of lafutidine using the HPTLC method, most of the study was carried out by single development of the TLC plate to prevent the movement of the non-polar degradation products to the extreme end of the plate.

Acid- and base-induced degradation studies

Acid decomposition studies were performed by using 0.1, 0.2, 0.5 and 1N HCl, and basic decomposition studies were performed by using 0.1 and 0.2N NaOH. The resulting solutions were applied to TLC plate in such a way that applied concentration was 100 ng for both acid and base degradation products, and the chromatograms were run as described above.

Hydrogen peroxide-induced degradation

To study hydrogen peroxide-induced degradation, initial studies were performed in 3% hydrogen peroxide at room temperature for 4 hr. Subsequently, the drug was exposed to 30% hydrogen peroxide at room temperature for a period of 4 hrs. For the HPTLC study, the resultant solutions were applied to TLC plate in such a way that applied concentration was 100 ng, and the chromatograms were run as described above.

Photolytic degradation

Photo-degradation studies were performed by directly exposing lafutidine to sunlight during the daytime (60,000-70,000 lux) for 2 days. Then, 1 µl of the solution (100 ng) was applied to TLC plates, and chromatograms were run as described above.

Neutral hydrolysis

To study the degradation behavior of drug under neutral conditions, it was dissolved in methanol and the solution was kept for 4 hrs.

Optimization of the stability-indicating HPTLC method

The HPTLC procedure was optimized with a view to develop a stability-indicating assay method. Both the pure and degraded drug solution was spotted on to TLC plates and run in different solvent systems. Initially, petroleum ether, chloroform, ethyl acetate, and methanol were tried in different ratios. Finally, the mobile phase consisting of chloroform:ethanol:acetic acid in the ratio of 8:1:1 v/v was found to be optimum. In order to reduce the neckless effect, TLC chamber was saturated for 25 min using saturation pads. The mobile phase was run up to a distance of 8 cm, which takes approximately 15 min for complete development of the TLC plate. During optimization of the HPTLC method, we had achieved good separation of the drug as well as the degradation products on normal phase TLC plates, which are more economical compared with reverse phase TLC plates.

Validation of the method

Validation of the optimized HPTLC method was carried out with respect to the following parameters.

Linearity and range

Standard solutions of lafutidine were prepared in the concentration range of 100-500 ng/µl. Then, 1 µl of each standard solution was spotted on the TLC plate to apply concentrations of 100-500 ng/spot. Each concentration was applied three times to the TLC plate. The plate was then developed using the previously mentioned mobile phase, and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves [Figure 2].

Precision

The precision of the method was determined by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (100, 200, 300 ng/spot) of the drug at three times on the same day. The intermediate precision of the method was checked by repeating studies on two different days. Additionally, the developed HPTLC method was checked by separation studies on the mixture of reaction solutions on different chromatographic plates on a different day.

Limit of detection and limit of quantitation

To determine the limits of detection and quantification, concentrations in the lower part of the linear range of the calibration plot were used. Stock solution of lafutidine (1000 µg mL^{-1}) was prepared, and dilutions of different concentrations as 100, 200, 300, 400, and 500 ng/µl were prepared. 0.1 µl of each of these dilutions were applied on the plate in triplicate. Amounts of lafutidine per band were plotted against average response (peak area), and the regression equation was determined. The standard deviations (SD) of responses and the average standard deviations (ASD) were calculated. Detection limit was calculated as (3.3 × ASD)/b, and quantification limit was calculated as (10 × ASD)/b, where 'b' denotes the slope obtained in the linearity study.

Robustness

Robustness of the method was determined by introducing small changes in the mobile phase composition (±0.1 ml for each component), the effects on the result was examined. Mobile phases having different compositions, e.g., chloroform:ethanol:acetic acid (7.9:1:1, v/v), (7.7:1:1, v/v), (7.6:1:1 v/v), and (7.5:1:1 v/v) were tried, and chromatograms were run. The amount of mobile phase was varied over the range of ±5%. The time from spotting to chromatography and from chromatography to scanning was varied from ±10 min. The robustness of the method was determined at three different concentration levels at 100, 300, and 500 ng per spot.

Accuracy

The accuracy of the developed method was determined by recovery studies as per ICH guidelines. Pre-analyzed samples of lafutidine were spiked with known concentrations of pure drug corresponding to 80%, 100%, and 120% and then analyzed in triplicate to check recovery.

Analysis of a marketed formulation

Ten tablets (Brand name: Lafukem label claim: 10 mg lafutidine per tablet) were accurately weighed and finely powdered. The weight of the tablet triturate equivalent to 10 mg of lafutidine was transferred into a 100 ml volumetric flask containing 20 ml methanol, sonicated for 30 min, and diluted to 100 ml with methanol. Then, 1 ml of the above filtered solution was diluted to produce a concentration of 10 µg/ml and 1 µl of this solution (100 ng/spot). These samples were then applied to a TLC plate, which was developed and scanned as described above. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

Results and Discussion

Stability-indicating property HPTLC studies of the samples obtained during the stress testing of lafutidine under different conditions using chloroform:ethanol:acetic acid (8:1:1 v/v) as the mobile phase suggested the following degradation behavior. Figure 3 shows the chromatogram of pure lafutidine (Rf value 0.85).

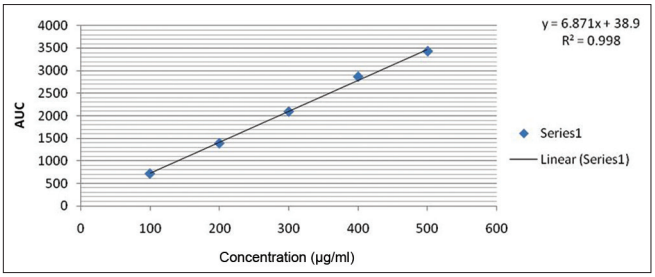

Figure 2: Linearity range of lafutidine

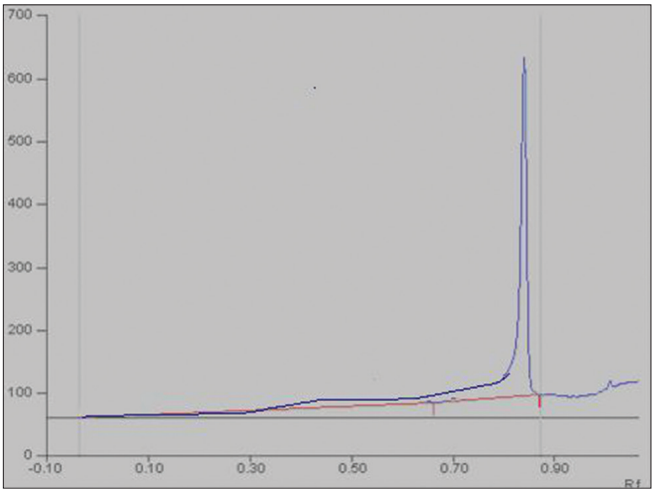

Figure 3: HPTLC chromatogram of lafutidine

Acid-induced degradation

Acid degradation studies showed the presence of one extra peak at Rf value 0.73 other than lafutidine, which shows its peak at Rf value 0.85 [Figure 4]. This extra peak represents the formation of degraded product. However, acid degradation is lesser when compared to alkali degradation.

Base-induced degradation

The drug was found to undergo alkaline degradation very readily. The reaction in 0.1 M sodium hydroxide was so fast that around 60% of the drug was degraded in 10 min. Complete degradation of drug was observed after 4 h. Base-induced degradation showed presence of two peaks at different Rf values 0.38 and 0.71 other than lafutidine at Rf 0.85 as shown in Figure 5. These extra peaks confirm the formation of two degradation products.

Hydrogen peroxide-induced degradation

The drug was highly degraded in 30% hydrogen peroxide at room temperature. Hydrogen peroxide-induced degradation showed one extra peak at Rf 0.71 other than lafutidine [Figure 6].

Photolytic degradation

The samples were degraded in sunlight for two days. The corresponding rate of degradation in dark was much slower. Photolytic degradation showed only one extra peak at Rf 0.71 [Figure 7].

Neutral hydrolysis

There was no degradation products observed in neutral hydrolysis.

Validation of the stability-indicating method

The results of validation studies [Table 1] on the stability indicating method developed for lafutidine in the current study involving chloroform:ethanol:acetic acid (8:1:1) as the mobile phase for HPTLC are given below.

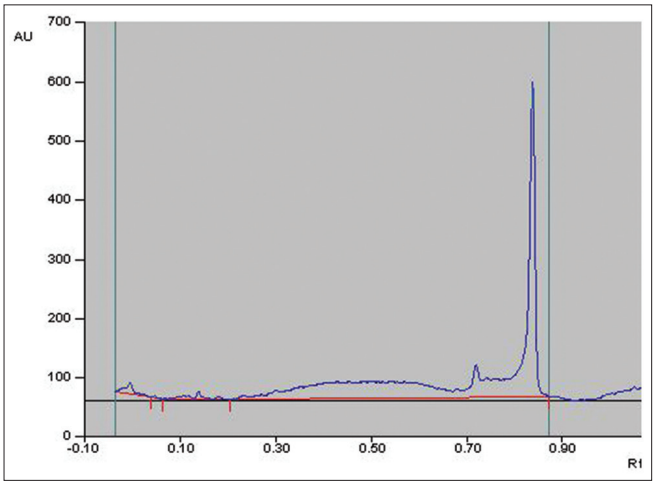

Figure 4: HPTLC chromatogram of acid degraded (0. 2 M HCl) lafutidine

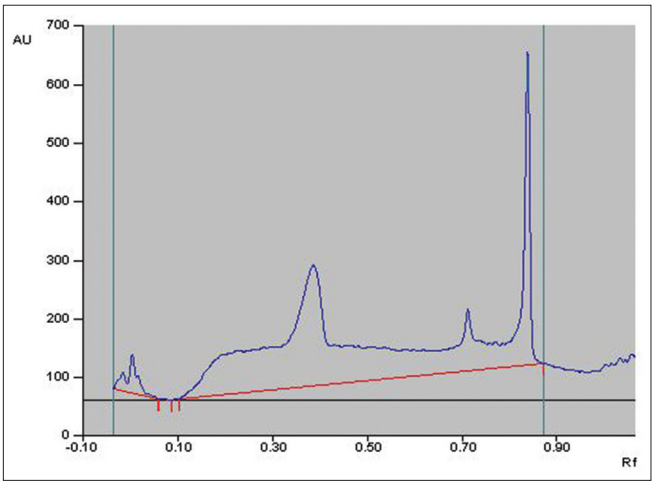

Figure 5: HPTLC chromatogram of base degraded (0.1 M NaOH) lafutidine

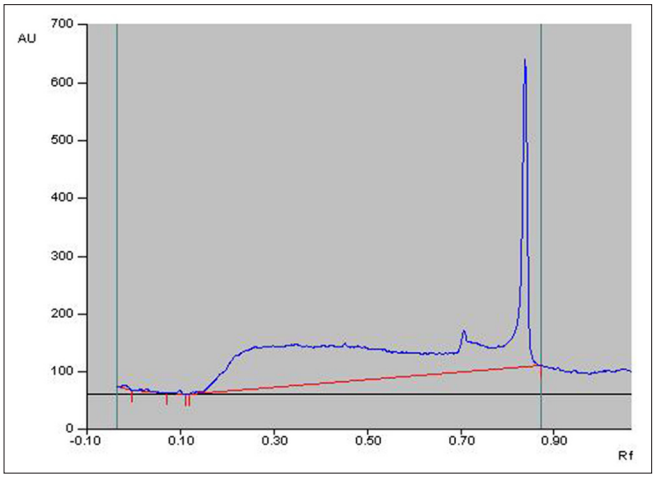

Figure 6: HPTLC chromatogram of H_2O_2 degraded (30% hydrogen peroxide) lafutidine

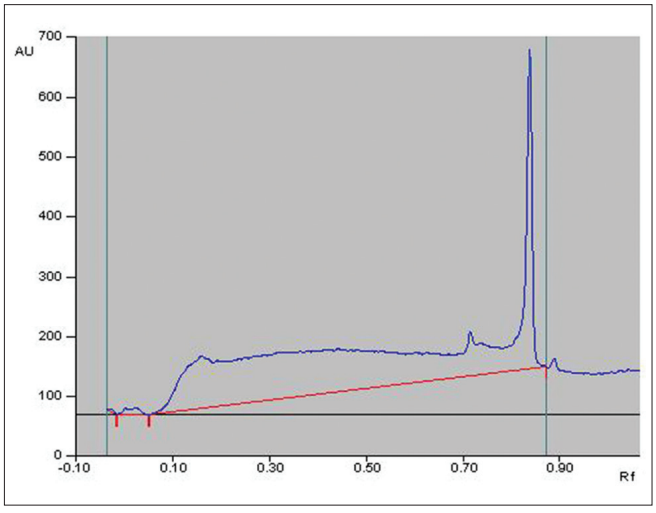

Figure 7: HPTLC chromatogram of photo-degraded lafutidine

Linearity

Five different concentrations of lafutidine have been used. Method used in determination of linearity was as specified above. The peak area increases as the concentration of spot increases. It indicates that the linear relationship between the concentration of spot and peak area is maintained.

The drug response was linear (r^2=0.991) over the concentration range between 100-500 ng/spot. The mean (±RSD) values of the slope, intercept, and correlation coefficient were 6.871±0.24, 577.733±0.033 and 0.998±0.002, respectively. Results are shown in Table 2.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 3. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were <2% as recommended by ICH guideline. Separation of the drug and different degradation products in stressed samples were found to be similar when analyzes were performed using different chromatographic system on different days. The low values of % RSD are indicativ e of the high repeatability of the method.

Robustness of the method

When the standard deviation of peak area was calculated for each change of conditions, RSD was found to be less than 2%. These low RSD values [Table 4] indicated the method is robust.

LOD and LOQ

The limits of detection and quantification calculated as described above were 11.4 ng and 33.83 ng, respectively. This indicates that the sensitivity of the method is adequate. Results are shown in Table 1.

Recovery studies

As observed from results in Table 5, good recoveries of the drug in the range from 98.12% to 100.54% were obtained at various added concentrations, which indicated the accuracy of the method was adequate.

Analysis of marketed formulation

Experimental results of the amount of lafutidine in tablets, expressed as a percentage of label claims, were in good agreement with the label claims, thereby suggesting that there was no interference from any of the excipients, which are normally present in tablets. The drug content was found to be 98.122%. Results are shown in Table 4 and Figure 8. The low value of % RSD indicated the method was suitable for routine analysis of lafutidine in pharmaceutical dosage forms.

Conclusion

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. The developed HPTLC technique is precise, specific, accurate, and stability-indicating. Statistical analysis proves that the method is suitable for the analysis of lafutidine as bulk drug

Table 1: Summary of validation data

Method characteristic	Value
Linear range (ng band^{-1})	100-500 ng
Correlation coefficient	0.998
Limit of detection (ng band^{-1})	11.4 ng
Limit of quantitation (ng band^{-1})	34.2 ng
Recovery (%)	99.161
Ruggedness (% RSD)	
Analyst I (*n*=3)	0.67
Analyst II (*n*=3)	0.85
Precision (% RSD)	
Inter-day (*n*=3)	1.04
Intra-day (*n*=3)	0.67
Robustness	1.24

RSD – Relative standard deviation

Table 2: Linear regression data

Linear range (ng per spot)	100-500 ng
r^2 ± SD	0.998 ± 0.0015
Slope ± SD	6.871 ± 0.247
Intercept ± SD	577.73 ± 0.033

Table 3: Intra-day and inter-day precision of the HPTLC method

Amount (ng band^{-1})	Mean area	SD	RSD (%)
Intra-day precision			
100	724	6.580274	1.22
300	2093.5	7.766917	0.374
500	3425	14.6731	0.42
Inter-day precision			
100	715	9.731393	1.35
300	1989	17.07923	0.877
500	3384	29.22841	0.88

n=3; SD – Standard deviations; RSD – Relative standard deviation

Table 4: Robustness of method

Conditions	SD of peak area	% RSD
Mobile phase composition		
Chloroform:Ethanol:Acetic acid (8:1:1)	3.002941	1.45
	12.91962	1.45
	15.88238	0.5
	17.49286	1.65
Mobile phase volume		
26.25	4.203173	0.771
23.75	3.86221	0.69
Development distance		
7	9.591663	1.7
7.5	7.141428	1.31
8	9.146948	1.7

SD – Standard deviations; RSD – Relative standard deviation

Table 5: Result from recovery study

Initial amount (ng)	Amount added		Amount recovered (ng)	% Recovery	Mean±% RSD
	%	ng			
100	0	0	390.489	98.122	99.160±1.019
100	80	320	712.386	98.942	
100	100	400	804.372	100.546	
100	120	480	871.497	99.034	

RSD – Relative standard deviation

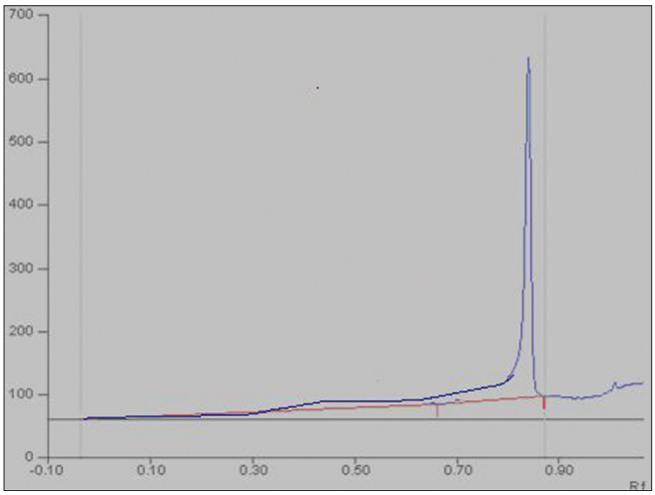

Figure 8: HPTLC chromatogram of marketed formulation of lafutidine

and in pharmaceutical formulation without any interference from the excipients. This stability-indicating assay method of lafutidine was established using ICH guidelines. The method can be used to determine the purity of drug available from various sources by detecting any related impurities. Because the method separates the drug from its degradation products, it can be used as a stability-indicating method. It is proposed for the analysis of drug in degraded products of stability samples obtained during industrial production.

References

1. ICH, Stability testing of new drug substances and products Q1A (R2), International Conference on Harmonization, IFPMA, Geneva, 2003.
2. Chen WD, Liang Y, Li H, Xiong Y, Liu XD, Wang GJ, *et al*. Simple, sensitive and rapid LC–ESI–MS method for the quantitation of lafutidine in human plasma: Application to pharmacokinetic studies. J Pharm Biomed Anal 2006;41:256-60.
3. HPLC Methods for Recently Approved Pharmaceuticals, 333 Lafutidine Published Online, 2005.
4. Sethi PD. High performance thin layer chromatography, quantitative analysis of pharmaceutical formulations. 2nd ed. New Delhi, India: CBS Publishers and Distributors; 1996. p. 56-63.
5. Bakshi MS, Singh SS. Development of validated stability- indicating assay methods critical review. J Pharm Biomed Anal 2002;28:1011-40.
6. Singh SS, Bakshi MS. Guidance on conduct of stress tests to determine inherent stability of drugs. Pharm Tech Online 2000;24:1-14.
7. ICH Guidance on analytical method validation, in: Proceedings of the international convention on quality for the pharmaceutical industry, Toronto, Canada, 2002.
8. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, 1994.
9. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, 1996.

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