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STABILITY-INDICATING REVERSED-PHASE HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY/DENSITOMETRY ESTIMATION OF LAFUTIDINE IN BULK AND TABLETS

Lafutidine (LTD) is a histamine H_2 receptor antagonist, with anti-ulcer and mucosal protective activity. A sensitive and selective stability-indicating reversed-phase high-performance thin-layer chromatography (RP-HPTLC)/densitometry method is suggested for the determination of LTD in the presence of its acid, alkaline, oxidative and dry heat degradation products. Stress testing of LTD was done according to the International Conference on Harmonization (ICH) guidelines in order to validate the stability-indicating power of the analytical procedures. Stress testing demonstrated that LTD underwent acid, alkaline, oxidative and dry heat degradation; on the other hand, it showed stability towards neutral and photo degradation. Chromatography was performed on aluminum-backed silica gel 60 RP-18 F_{254} S plates with methanol:water:triethylamine 8:2:0.5 (v/v) as the mobile phase. Densitometric scanning was performed at 276 nm. The system gave compact bands for LTD (Rf 0.67±0.02). Linear regression analysis data for the calibration plots showed a good linear relationship with r^2 > 0.99 in the working concentration range 500 to 3000 ng per band. The limits of detection and quantification were 23.51 ng and 71.26 ng, respectively. The developed method can routinely be used for analysis of LTD in bulk and pharmaceutical formulations.

Keywords: lafutidine, RP-HPTLC, validation; stability- indicating.

Lafutidine (LTD), 2-[(2-furanylmethyl)-sulfinyl]--*N*-[(2*Z*)-4-[[4-(1-piperidinylmethyl)-2 -pyridinyl] oxy]--2-butenyl] acetamide [1] (Figure 1) is a histamine H_2 receptor antagonist, with anti-ulcer and mucosal protective activity [2].

In addition to being a potent H_2 receptors antagonist, LTD also activates capsaicin-sensitive afferent neurons and stimulates the release of calcitonin generelated peptide (CGRP), which inhibits acid secretion and stimulates mucosal blood flow. The gastroprotective action of LTD includes increase in mucin biosynthesis *via* stimulation of nitric oxide production, increasing the thickness of the surface mucus gel layer and maintaining gastric mucosal blood flow and bicarbonate response [3].

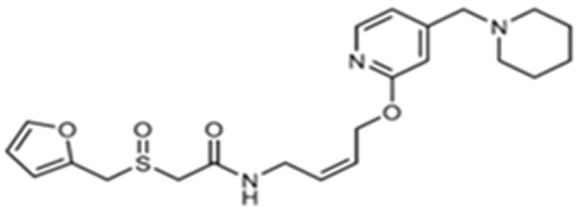

Figure 1. The chemical structure of LTD.

In the literature, several analytical methods including LC-ESI-MS [4], high-performance liquid- chromatography-electrospray ionization mass spectrometry [5] and LC tandem mass spectrometry [6] have been studied for analysis of LTD in biological samples. Also, RP-HPLC [7] and UV-spectrophotometric [8] methods have been established for the determination of LTD in pharmaceutical formulations.

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Analysis of pharmaceuticals is an imperative and chief aspect of the complete drug development process. Therefore, simple and rapid methods for quality control of commercial formulations are needed. High-performance thin-layer chromatography (HPTLC) is an important separation technique, since many samples can be chromatographed simultaneously with small amounts of solvent [9].

Forced degradation experiments are used to relieve the development of analytical methodology, to achieve better insight of the stability of the active pharmaceutical ingredient (API) and the drug product, and to provide information about degradation pathways and degradation products [10].

However, no literature is available for dealing with the stress degradation profile of LTD in accordance with ICH guidelines using any of the above-mentioned analytical techniques.

The objective of this work was to develop a stability-indicating reversed-phase high-performance thin-layer chromatography (RP-HPTLC)/densitometry method for analysis of LTD. Since there are no reports of analysis of LTD alone in formulations, the method has also been used for analysis of LTD in tablets. This paper describes an accurate, specific, reproducible, and stability-indicating method for analysis of LTD in the presence of its degradation products. The method was validated in accordance with the guidelines of the US Pharmacopoeia [11] and the International Conference on Harmonization (ICH) [12].

MATERIALS, INSTRUMENTATION AND METHODS

Chemicals

Lafutidine (purity 99.5%) was supplied as a gift sample by Ajanta Pharma Ltd., Mumbai, India. Pharmaceutical formulation containing Lafutidine 10 mg per tablet were purchased from local market. All chemicals and reagents used were of analytical grade and were purchased from Merck Ltd., Mumbai, India.

Instrumentation

Balance: Shimadzu AUX -120; ultrasonicator: Enertech Electronics Pvt. Ltd.; syringe: Hamilton - Bonaduz, Schweiz (100 µl); stationary phase: 20 cm× ×10 cm aluminum-backed RP-HPTLC plates coated layers of silica gel 60 RP-18 F254 S (prewashed by methanol and activated at 105 °C for15 min prior to chromatography).

HPTLC System: Camag (Muttenz, Switzerland); applicator: Linomat 5; scanner: Camag TLC Scanner 3; data processor: winCATS (version 1.3.0)

Chromatographic conditions

Mobile phase: methanol:water:triethylamine, 8:2:0.5 (v/v/v); mobile phase volume: 10 mL; TLC chamber saturation time: 20 min at ambient temperature (30±1 °C); sample application rate: 150 nL/s; slit dimension: 6 mm×0.45 mm; development distance: 8 cm; detection: densitometrically, using a Camag TLC scanner 3 at 276 nm in the reflectance–absorbance mode.

Preparation of stock standard solution and study of calibration curve

A stock standard solution was prepared by dissolving 25 mg of LTD in 25 mL methanol to obtain concentration 1 mg/mL. Appropriate volumes in the range of 1–6 mL were transferred into series of 10 mL volumetric flasks and volumes were made up to mark with methanol. From each volumetric flask, 5 µL of solution was applied on RP-HPTLC plate to get concentration in the range of 500–3000 ng per band. After evaporation of solvents at room temperature for 5 min, chromatography was performed as described above. The calibration curve was established by plotting peak area against drug quantity per band. Calibration equations were determined by use of linear regression analysis and correlation coefficients (r^2) were calculated. All measurements were repeated six times.

Development and validation of RP-HPTLC method

The proposed method was validated according to ICH guidelines [12].

Precision and recovery

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

The repeatability of sample application and measurement of peak area were measured using six replicates of the same band (1000 ng per band of LTD).

Intra-day variation was determined by analyzing three different concentrations for three times within a day and inter-day precision was assessed by three different concentrations for three different days, over a period of week. Intra-day and inter-day precision was studied at 1000, 1500 and 2000 ng per band.

The accuracy of the experiment was established by over spotting drug standard solution at (80, 100 and 120%) to the pre-analyzed sample solution (1000 ng per band). The experiment was repeated in triplicate at each level.

Ruggedness and robustness

The ruggedness of the method was performed by two different analysts using same operational and environmental conditions.

For robustness study of the proposed method, various chromatographic conditions such as composition of the mobile phase (methanol:water:triethylamine in different ratios (7.5:2.5:0.5; 8:2:0.5; 8.5:1.5:0.5 *v/v*); the amount of mobile phase (10±2 mL, *i.e.*, 8, 10 and 12 mL), development distance (8±0.5, *i.e.*, 7.5, 8 and 8.5) and duration of saturation (20±5 min, *i.e.*, 15, 20 and 25 min) were varied. The time from application of LTD to the plate to development of the plate and time from development of plate to scanning were also varied (10, 20 and 30 min). The effects on the results by changing various chromatographic conditions were examined in terms of percent amount found.

The robustness and ruggedness of the method was assessed at a concentration of 1000 ng per band for six times.

Limits of detection (LOD) and quantification (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy and is used particularly for the determination of impurities and/or degradation products.

The LOQ and LOD were calculated by the use of the equations $LOD = 3.3N/B$ and $LOQ = 10N/B$, where N is the standard deviation of the peak areas of the drug ($n = 3$), taken as a measure of the noise, and B is the slope of the corresponding calibration plot. The signal to noise ratio was determined. The *LOD* was regarded as the amount for which the signal-to-noise ratio was 3:1 and *LOQ* as the amount for which the signal-to-noise ratio was 10:1. The *LOD* and *LOQ* estimated at concentration range 500–1000 ng per band.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of expected components. The specificity of the method was ascertained by analyzing standard LTD and LTD extracted from tablet formulation. The band for LTD in sample was confirmed by comparing the R_f and spectra of the band with those obtained from standard. The peak purity of LTD was assessed by comparing spectra acquired at three different positions on the band, *i.e.*, peak-start (S), peak-apex (M), and peak-end (E).

Analysis of marketed formulation

Twenty tablets were weighed and powdered. An amount of powder equivalent of 10 mg of LTD was weighed accurately and extracted with 100 mL methanol in 100 mL flask using a reciprocating shaker for 10 min (the optimal extraction time) and transferred to a 100 ml volumetric flask. After filtration through Whatman filter paper, the extract (10 µL; 1000 ng per band) was applied to an RP-HPTLC plate, followed by development and scanning as described above. The analysis was repeated six times.

Forced degradation studies

Acid- and base-induced degradation was attempted by separately adding 10 mg of LTD in 10 mL each of 0.1 M HCl and 0.01 M NaOH solutions. These solutions were kept in the dark for 8 h in order to exclude the possible degradative effect of light. The solutions (1 mL) were neutralized and diluted to 10 mL with methanol. The 10 µL of the resulting solutions (1000 ng per band) were applied to the RP--HPTLC plate and chromatograms were obtained as described above.

For oxidative degradation, 10 mg of LTD was added in 10 mL of 3% (v/v) hydrogen peroxide solution and kept in dark for 8 h. The solution (1 mL) was diluted to 10 mL with methanol and treated as described for acid and base-induced degradation.

For dry heat degradation, LTD powder was placed in an oven at 70 °C for 8 h. A solution of 10 mg LTD in 10 mL methanol was prepared from the dry heat treated sample. The solution (1 mL) was diluted to 10 mL with methanol and treated as described for acid- and base-induced degradation.

To assess the neutral degradation, a 10 mg of LTD was dissolved in 3 mL of methanol and further diluted up to 10 mL with water. From it 1 mL was diluted to 10 mL with water and analyzed as described above.

Photochemical degradation was studied by exposing 1 mg/mL solution in methanol to sunlight for 72 h. The resulting solutions (1 mL) were diluted to 10 mL with methanol and analyzed using the process described above.

RESULTS

Selection of the optimum mobile phase

The RP-HPTLC procedure was optimized to develop a stability-indicating assay method to quantify LTD in bulk and tablet formulation. The drug standard was applied on RP-HPTLC plates and chromatographed with different compositions of mobile phases.

A high resolution and reproducible peak was achieved using methanol:water:triethylamine 8:2:0.5 (v/v) as the mobile phase and chamber saturation period of 20 min. In this mobile phase, LTD showed a compact spot with R_f = 0.67 when the scanning was performed at 276 nm.

Calibration plots

Calibration curves were constructed by plotting peak area against drug quantity per band. Good linearity was obeyed in the concentration range of 500-3000 ng per band; calibration plot was $y = 2.248x +$ + 794.2; slope±S.D. (2.248±0.0453); intercept±S.D. (794.2±46.36) and correlation coefficient ($r^2 > 0.99$). All measurements were repeated six times.

Validation of developed stability indicating method

Precision and percent recovery study

Data obtained from precision experiments are given in Table 1, for repeatability and intra-day and inter-day precision studies. The RSD value < 2% confirms that the method is sufficiently precise. Good recoveries of LTD were obtained in the range of 97.04–98.53 (Table 2). The RSD values < 2% indicates the method is adequately accurate.

Ruggedness and robustness

The standard deviation of percent amount found was calculated for each condition and *RSD* was less than 2%. The low values of *RSD* are indicative of the robustness and ruggedness of the method. The results of the robustness study are shown in Table 3.

Limits of detection (LOD) and quantification (LOQ)

Detection limit and quantitation limit for signal-to-noise ratios of 3:1 and 10:1 were 23.51 and 71.26 ng, respectively, which indicate adequate sensitivity of the method.

Specificity

Peak purity for LTD was assessed by comparing spectra acquired at the peak-start (S), peak-apex (M) and at the peak-end (E) positions of the band, r^2(S,M) = = 0.9999 and r^2(M,E) = 0.9996. Good correlation (r^2 = = 0.9997) was also obtained between LTD standard and LTD extracted from tablets LTD (Figure 2). These correlation values indicate the ability of the method to separate and specifically detect LTD from sample solutions.

The validation data are summarized in Table 4.

Table 1. Intra-day and inter-day precision; n - number of determinations

Precision	Amount, ng per band	Amount, %	*RSD* / %
Repeatability (n = 6)	1000	100.25	1.75
Intra-day (n = 3)	1000	99.16	0.88
	1500	98.77	0.53
	2000	100.12	1.33
Inter-day (n = 3)	1000	99.51	0.85
	1500	101.12	0.76
	2000	98.42	1.86

Table 2. Results from recovery studies (n = 3, number of determinations); initial amount: 1000 ng per band

Amount added, ng per band	Recovery, %	*RSD* / %
800	98.14	0.87
1000	97.04	0.16
1200	98.53	0.90

Table 3. Results from robustness testing (n = 6, number of determinations)

Conditions	Amount found, %	RSD / %
Mobile phase composition (±0.5 mL/10mL)	99.47	1.59
Amount of mobile phase (±2 mL/10mL)	99.73	1.79
Development distance (±0.5 cm/8 cm)	99.91	0.59
Plate saturation time (±5 min/20 min)	99.79	0.74
Time from application to chromatography (±10 min/20 min)	99.59	1.35
Time from chromatography to scanning (±10 min/20 min)	100.40	0.72

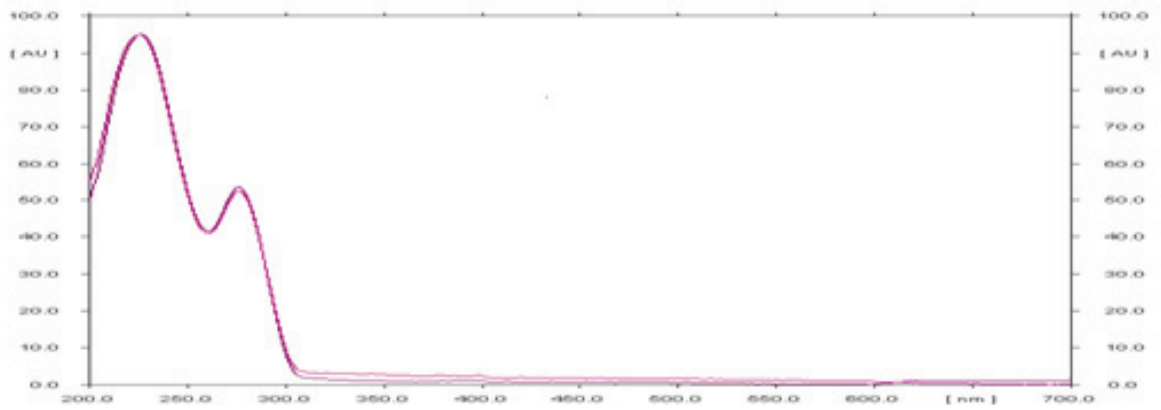

Figure 2. Peak purity spectral of LTD standard and LTD extracted from tablets scanned at peak-start (S), peak-apex (M) and at the peak-end (E) positions of the band (r^2 > 0.99).

Table 4. Summary of validation data; n - number of determinations

Parameter	Value
Linear range, ng per band (*n* = 6)	500-3000
Correlation coefficient	0.997
Limit of detection, ng	23.51
Limit of quantification, ng	71.26
Recovery, % (*n* = 9)	97.04 - 98.53
Precision, % of *RSD*	
Repeatability (*n* = 6)	1.75
Intra-day (*n* = 3)	0.88-1.33
Inter-day (*n* = 3)	0.85-1.86
Ruggedness, % of *RSD*	
Analyst - I (*n* = 6)	1.19
Analyst - II (*n* = 6)	1.59
Robustness	Robust
Specificity	Specific

Analysis of marketed formulation

The results obtained for the amount of LTD in tablets as against the label claims were in good agreement. The amount of drug estimated using this method was found to be 98.34±1.23, demonstrated that there was no interference from the excipients usually present in tablet formulation. It may therefore be inferred that degradation of LTD did not occur in the formulations analyzed by the method described above. The good performance of the method indicated the suitability of this method for routine analysis of LTD in tablets dosage forms.

Forced degradation studies

The chromatograms obtained from samples exposure to acidic, alkaline, oxidative and dry heat degradation depicted well-separated bands of pure LTD and some additional peaks at different R_f values. No major degradation product was seen when sample were subjected to neutral and photochemical degradation. The percent of degradation products with their R_f values is listed in Table 5. Peak purity of these LTD peaks during stability studies was also examined by comparing the respective spectra obtained from RP--HPTLC/densitometry at the peak-start (S), peak-apex (M), and peak-end (E). The values of r(S,M) and r(M,E) were all > 0.99 with standard spectra LTD peak, indicating the purity of peaks of the remaining LTD. The results of forced degradation are shown in (Figure 3).

CONCLUSIONS

This densitometric RP-HPTLC method is quite simple, accurate, highly precise, reproducible, rapid, sensitive and specific. It was successfully used to study the stability of LTD under different stress degradation conditions. Statistical analysis proved that the method is repeatable and selective for the anal-

Table 5. Results from degradation studies

Conditions	Time, h	R_f of degradation product	Degradation product content, %
Acid, 1 M HCl, room temperature	8	0.42 and 0.53	12.75 and 16.71
Base, 0.01 M NaOH, room temperature	8	0.48	14.28
H_2O_2, 3 mass%, room temperature	8	0.61	6.25
Dry heat (70±2 °C)	8	0.84 and 0.97	17.53 and 35.24
Neutral degradation	8	Not detected	-
Photo degradation	72	Not detected	-

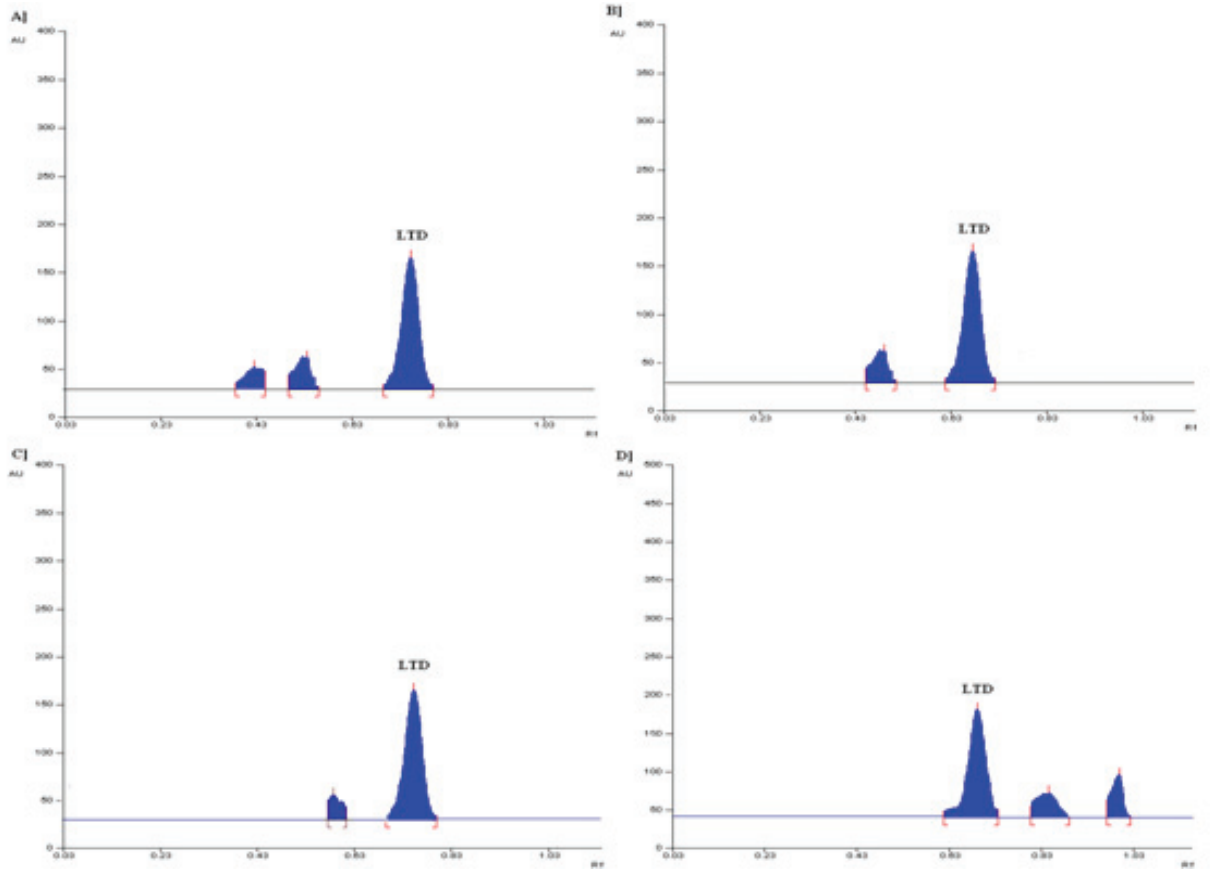

Figure 3. Typical RP-HPTLC densitogram after: A) acid hydrolysis; B) alkaline hydrolysis; C) oxidative degradation; D) dry heat degradation.

ysis of LTD as a bulk drug and in pharmaceutical formulations, without interference from excipients. It can also be utilized successfully for determination of the chemical stability and shelf life of LTD in tablets formulations, because it has stability-indicating properties.

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NAUČNI RAD

ODREĐIVANJE LAFUTIDINA U FARMACEUTSKOJ SUPSTANCI I TABLETAMA I ISPITIVANJE STABILNOSTI REVERSNO-FAZNOM TANKOSLOJNOM HROMATOGRAFIJOM VISOKE REZOLUCIJE SA DENZITOMETRIJOM

Lafutidin (LTD) je histamin antagonist H2 receptora i anti-ulcer sa mukoznom zaštitnom aktivnošću. U radu je predložena osetljiva, selektivna, reversno-fazna tankoslojna hromatografska metoda visoke rezolucije sa denzitometrijom za određivanje LTD u prisustvu degradacionih produkata koji nastaju suvim zagrevanjem, oksidacijom i kiselim i baznim tretiranjem. Da bi validirali stabilnost predloženih analitičkih metoda urađeno je stres testiranje LTD u skladu sa ICH uputstvom. Stres testiranje je urađeno ispitivanjem kisele, bazne, oksidativne i degradacije suvim zagrevanjem. LTD je pokazao stabilnost prema neutralnoj i fotodegradaciji. Hromatografija je izvedena na pločama sa silika aluminijumovim gelom 60 RP-18 F254 S sa mobilnom fazom metanol:voda:trietanolamin 8:2:0.5 (v/v). Denzitometrijsko skeniranje je izvedeno na 276 nm. Sistem daje jasne trake za LTD (Rf 0.67±0.02). Linearna regresiona analiza podataka za kalibracione krive pokazuje dobru lineranu zavisnost sa $r^2 > 0.99$ za opseg koncentracije od 500 do 3000 ng po traci. Limit detekcije i limit kvantifikacije iznose 23,51 i 71,26 ng. Razvijena analitička metoda se može rutinski koristiti za analizu LTD u farmacetskoj supstanci i farmaceutskim preparatima.

Ključne reči: lafutidin; RP-HPTLC; validacija; stabilnost.