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Research Article

STABILITY-INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF LACIDIPINE IN TABLETS

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ABSTRACT
A stability-indicating HPLC method development and validation for the determination of Lacidipine in tablets. The determination was done for an active pharmaceutical ingredient, its pharmaceutical dosage form in the presence of degradation products impurities. The drug was subjected to stress conditions of hydrolysis (acid and base), oxidation, photolysis and thermal degradation as per International Conference on Harmonization (ICH) prescribed stress conditions to show the stability-indicating method. Degradation was observed during acid, base hydrolysis, thermal, peroxide and light stressed sample and degradant was identified by LC–MS, FTIR and 1H/13C NMR spectral analysis. The generated samples were used for forced degradation studies. In the developed HPLC method, the resolution between Lacidipine and, its related impurities (namely impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2) was found. The chromatographic separation was achieved on a phenomenex Luna C_{18}, 250 mm × 4.6 mm, 5 µm column. The LC method employed an isocratic elution, and the detection wavelength was set at 240 nm. The stress samples were assayed against a qualified reference standard and the mass balance was found to be close to 99.6 %. The developed RP-LC method was validated with respect to linearity, accuracy, precision and robustness.
KEYWORDS: Lacidipine tablets, Stability-indicating, HPLC method development and validation.

INTRODUCTION

Lacidipine is a dihidropyridine class of a drug and is widely used as a calcium channel blocker in the treatment of hypertension. Lacidipine is official in Martindales extra pharmacopeia[1]. Literature survey reveals variety of analytical methods for the determination in pharmaceutical dosage forms, as well as in biological fluids. It has long duration of action because of its high degree of lipophilicity. The active trans form is used in therapy[1, 2]. From a physico-chemical point of view, Lacidipine is slightly soluble in water, while it is more soluble in some widely used solvents as ethanol, methanol and acetone. It is very sensitive to the action of temperature and light. For this reason, it can be degraded and, after some time, it appears together with some impurities[3, 4]. Several methods have been reported for the quantitative determination of Lacidipine in tablets including spectrophotometry[5, 6]. Whereas reversed phase LC was used for the stability study of Lacidipine towards light, temperature and humidity[7].

Taking the above mentioned consideration into account, the aim of this work has been to develop a method using RP-HPLC that allows the determination at trace level not only of residual Lacidipine but also of its frequent impurities caused by temperature, acid, base, peroxide, and light.

MATERIALS AND METHODS

Chemicals

Standard of Lacidipine and its related impurities were received from Versapharm Incorporated, Warminister, PA, USA. All of the impurities and the Lacidipine standard purity are as follows: Lacidipine (98.8 %), impurity-A (97.5 %), impurity-B (98.9 %), impurity-C (94.4 %), impurity-LC1 (99.9 %) and impurity-LC2 (99.4 %). Commercially available Lacidipine tablet were purchased for this study. In addition, HPLC grade methanol and were purchased from Merck (Darmstadt, Germany). Analytical reagent grade ammonium acetate, glacial acetic acid and were purchased from Merck. Highly pure water was prepared with the millipore milli-Q Plus water purification system.

Equipment

The HPLC system used for method development, forced degradation studies and method validation consisted of a Waters-2996, photo diode array detector (PDA) with an auto sampler. The output signal was monitored and processed using Empower software. Photo stability

studies were carried out in a photo stability chamber. Thermal stability studies were carried out in a dry air oven (Thermolab, India).

Chromatographic conditions

A phenomenex Luna C_{18}, 250 mm × 4.6 mm, 5 µm column was used with a mobile phase containing an isocratic elution. The buffer was composed of ammonium acetate, with its pH adjusted to 4.5 with glacial acetic acid. Buffer and methanol in the ratio of 300:700 (v/v) was used as mobile phase. The column temperature was maintained at 40°C, sample Cooler temperature 10°C, Flow rate 1.2 mL /min. and the detection wavelength was set at 240 nm. The injection volume was 20µl.

Preparation of standard stock and standard solution

Standard stock solution was prepared by dissolving 40.0 mg Lacidipine reference standard in 100 mL of methanol to get concentration of 400µg/mL. 5ml of standard stock solution was than diluted to 50 mL with methanol to get standard solution of Lacidipine.

Preparation of sample solution

Twenty tablets were accurately weighed. An amount of powder equivalent to 25 mg of Lacidipine was accurately weighed and transferred to a 50 mL volumetric flask. Methanol (35 mL) was added and the mixture was sonicated for 20 minutes. And the solution was diluted volume with methanol. The solution was filtered through a 0.45 µm nylon filter.

Placebo preparation

Weighed accurately and transferred Placebo of each strength equivalent to 25 mg of Lacidipine in to 50 mL volumetric flask. Methanol (35 mL) was added and the mixture was sonicated for 20 minutes. And the solution was diluted volume with methanol. The solution was filtered through a 0.45 µm nylon filter.

Stress Degradation studies

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities[8]. Forced degradation studies were performed on Lacidipine to provide an indication of the stability-indicating property and specificity of the proposed method[9].

Unstressed Sample preparation

Twenty tablets were accurately weighed. An amount of powder equivalent to 25 mg of Lacidipine was accurately weighed and transferred to a 50 mL volumetric flask. Methanol (35 mL) was added and the mixture was sonicated for 20 minutes. And the solution was diluted volume with methanol. The solution was filtered through a 0.45 µm nylon filter.

Acid Hydrolysis

Twenty tablets were accurately weighed. An amount of powder equivalent to 25 mg of Lacidipine was accurately weighed and transferred to a 50 mL volumetric flask. 1N HCL (5 mL) was added and stressed in ambient temperature for 12 hrs. The above solution was then refluxed for 1hour at 80°C, then 35 mL of methanol was added and the mixture was sonicated for 20 minutes. After cooled the above solution to room temperature the solution was diluted volume with methanol. The solution was filtered through a 0.45 µm nylon filter.

Alkali Hydrolysis

Twenty tablets were accurately weighed. An amount of powder equivalent to 25 mg of Lacidipine was accurately weighed and transferred to a 50 mL volumetric flask. 1N NaoH (5 mL) was added and stressed in ambient temperature for 12 hrs. The above solution was then refluxed for 1hour at 80°C, then 35 mL of methanol was added and the mixture was sonicated for 20 minutes. After cooled the above solution to room temperature the solution was diluted volume with methanol. The solution was filtered through a 0.45 µm nylon filter.

Peroxide oxidation

Twenty tablets were accurately weighed. An amount of powder equivalent to 25 mg of Lacidipine was accurately weighed and transferred to a 50 mL volumetric flask. 30 % H_2O_2 (5 mL) was added and stressed in ambient temperature for 12 hrs. The above solution was then refluxed for 1hour at 80°C, then 35 mL of methanol was added and the mixture was sonicated for 20 minutes. After cooled the above solution to room temperature the solution was diluted volume with methanol. The solution was filtered through a 0.45 µm nylon filter.

Thermal degradation

Twenty tablets were accurately weighed. An amount of powder equivalent to 25 mg of Lacidipine was accurately weighed and stressed in hot air oven at 105°C for 24 hrs. This added into 50 mL volumetric flask, then 35 mL of methanol was added and the mixture was sonicated for 20 minutes. Then the solution was diluted volume with methanol. The solution was filtered through a 0.45 µm nylon filter.

Photo degradation (controlled)

Twenty tablets were accurately weighed. An amount of powder equivalent to 25 mg of Lacidipine was accurately weighed and wrapped in aluminium foil then stressed at 1.2 million lux hours. This sample added into a 50 mL volumetric flask, then 35 mL of methanol was added and the mixture was sonicated for 20 minutes. Then the solution was diluted volume with methanol. The solution was filtered through a 0.45 µm nylon filter.

Photo degradation (uncontrolled)

Twenty tablets were accurately weighed. An amount of powder equivalent to 25 mg of Lacidipine was accurately weighed stressed at 1.2 million lux hours. This sample added into a 50 mL volumetric flask, then 35 mL of methanol was added and the mixture was sonicated for 20 minutes. Then the solution was diluted volume with methanol. The solution was filtered through a 0.45 µm nylon filter.

Validation of the method

The proposed method was validated per ICH guidelines[10].

Stability of analyte in solution

The stability of Lacidipine in the mobile phase was assessed by injecting the standard solution (4ppm) at 0, 9, 15, 24 hours.

Precision and accuracy

System precision was evaluated by performing six consecutive injections of 4ppm Lacidipine standard solution. Method precision was determined by six repeated assays of the same lot of the tablet formulations. The accuracy of the proposed method was determined by recovery experiments using tablets from the same lot of the commercial and developed formulations. The recovery was assessed at three levels (50, 100 and 150 %).

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ concentrations were determined at signal-to-noise ratios of 3:1 and 10:1, respectively.

Linearity

Linearity test solutions for the assay and related substance method were prepared by diluting stock solution to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 150 % of the specification level.

Specificity

The specificity of method was ascertained by peak purity profiling studies. Purity of drug peak was ascertained by analyzing the spectrum at peak start, meddle, and at peak end.

Robustness

To determine the robustness of the developed method, the experimental conditions were slightly altered and the resolution between Lacidipine and impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2 was evaluated. The flow rate of the mobile phase was slightly changed by ± 10 %. The effect of pH on the resolution of the impurities was studied by varying the pH by ± 0.2 units. The effect of the column temperature on the resolution of the impurities was studied by varying the temperature by ± 5°C. The effect of organic content in mobile phase on the resolution of the impurities was studied by varying the organic content in mobile phase by ± 2 %. The effect of wavelength on the resolution of the impurities was studied by varying the wavelength by ± 2nm.

RESULTS AND DISCUSSION

Method development and optimization

The main objective of the chromatographic method was to separate impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2 from the analyte peak during stress studies. Impurities and degradation products were co-eluted by using different stationary phases, such as C_8, cyano and phenyl with various mobile phases with buffers, such as phosphate, sulfate and acetate with different pH values (3–7). A buffer was composed of ammonium acetate, with its pH adjusted to 4.5 with glacial acetic acid. Buffer and methanol in the ratio of 300:700 (v/v) was used as mobile phase, the column temperature was maintained at 40°C and the detection wavelength was set at 240 nm. The injection volume was 20 µl, flow rate of 1.50 mL/min was chosen for the test with a phenomenex Luna C_{18}, 250 mm × 4.6 mm, 5 µm column. When an impurity-spiked solution was injected, the resolution between the impurities and analyte was good. The effect of the buffer pH was also studied under the above conditions. The results are shown in Table: 1 and figure: 1.

Method validation

Precision

The percentage RSD of Lacidipine during the assay method precision study was within 1.4 % and the percentage RSD values of the area of impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2 in the related substance method precision study were within 1.5 %. The percentage RSD of the results obtained in the intermediate precision study was within 0.7 %.

Linearity

The linear calibration plot for the assay method was obtained over the tested calibration range (LOQ–150 %) and the obtained correlation coefficient was greater than 0.999. The results revealed an excellent correlation between the peak area and analyte concentration. The slope and y-intercept of the calibration curve were 49786,-827.45 respectively. The linear calibration plot for the related substance method was determined over the calibration ranges (LOQ-150 %) for impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2, a correlation coefficient of greater than 0.999 was obtained. The linearity was checked for the related substance method over the same concentration range for 3 consecutive days. These results showed an excellent correlation between the peak areas and concentrations of

impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2.

Accuracy

The percentage recovery of Lacidipine in the drug substance and product ranged from 98.9 to 100.6 and from 98.2 to 102, respectively. The percentage recoveries of impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2 in the drug substance and product ranged from 98.0 to 101.5, 98.8 to 101.1, 99.5 to 102.1, 91.9 to 103.7 and 102.5 to 104.1 respectively.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in mobile phase composition, pH, column temperature, wavelength and flow rate. The tailing factor for Lacidipine and five impurities was found to be less than 2 and the resolution for Lacidipine and five impurities was found to be good. Which demonstrated that the RP-HPLC method developed is robust.

Solution stability and mobile phase stability

The percentage RSD of the Lacidipine and five impurities was calculated for the mobile phase and solution stability experiments. The solution stability of Lacidipine and its impurities in the related substance method was carried out by leaving a spiked sample solution in a tightly capped volumetric flask at room temperature for 24 hours. The content of impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2 was determined at 6 hours intervals up to the study period. The mobile phase stability was also investigated for 24 hours by injecting the freshly prepared sample solutions for every 6 hours interval. The content of impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2 was determined in the test solutions. The prepared mobile phase remained constant during the study period. Cumulative percentage RSD was 0.7 for peak area of Lacidipine in standard. Cumulative percentage RSD was 1.4 for peak area of known impurities in sample solution.

Results of forced degradation

Peak purity test results derived from the PDA detector, confirmed that the Lacidipine peak and the degraded peaks were homogeneous and pure in all of the analyzed stress samples. Assay studies were carried out for the stress samples (at 4ppm) against a qualified reference standard of Lacidipine .The mass balance of the stressed samples was close to 99.6 %. The assay of Lacidipine was unaffected in the presence of impurity-A, impurity-B, impurity-C, impurity-LC1 and impurity-LC2 and its degradation products, confirming the stability-indicating power of the developed method.

CONCLUSION

A sensitive, specific, accurate, validated and well defined stability-indicating HPLC method for the determination of Lacidipine in the presence of degradation products and its process-related impurities was described. The behavior of Lacidipine under various stress conditions was studied. All of the degradation products and process impurities were well separated from the drug substance and drug product demonstrates the stability-indicating the method. The information presented in this study could be very useful for quality monitoring of active pharmaceutical ingredients in their, dosage forms and be used to check drug quality during stability studies.

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Table 1: CHROMATOGRAPHIC PERFORMANCE DATA

Name	RT	Area	% Area	Purity Angle	Purity threshold	Purity flag	Tailing factor	Plate count	RRT [a]
Impurity-LC 2	7.39	56648	0.26	1.509	3.057	No	1.2	5977	1
Impurity-LC 1	9.691	90714	0.41	7.522	7.743	No	1.3	6420	0.77
Impurity-B	12.539	256242	1.16	0.298	0.569	No	1.3	6610	0.69
Impurity-A	14.04	124060	0.56	0.997	1.697	No	1.2	6956	0.87
Impurity-C	15.871	158614	0.72	2.111	2.401	No	1.2	7052	0.53
Lacidipine	18.147	21488284	96.91	0.021	0.253	No	1.4	7050	0.4

[a] Relative retention time (RRT) were calculated against the retention time (RT) of Lacidipine

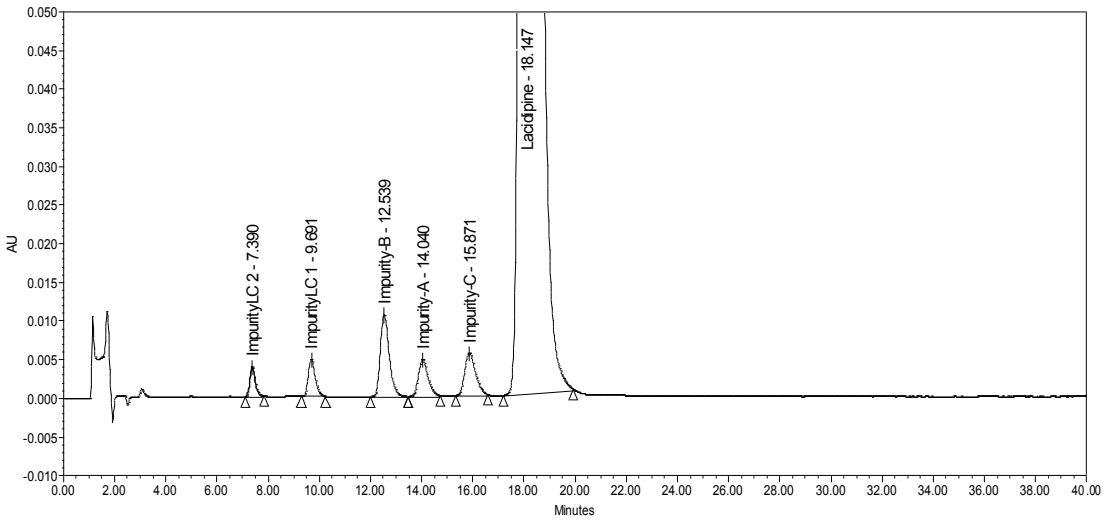

Figure 1: Typical chromatograms of Lacidipine spiked with its five impurities from method development

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