STABILITY-INDICATING HPLC ASSAY METHOD OF LOVASTATIN

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An HPLC assay method for determining of lovastatin in the presence of its degradation products was validated under acidic, basic, hydrogen peroxide, high temperature, and photo-irradiated conditions. The HPLC system consisted of a Lichrospher 100 RP-18 (5µm) column, and a guard column of Lichro CART (150x3.9) using a mobile phase of acetonitrile-phosphoric acid (0.1%) (50:50, v/v) with UV detection at 238 nm. The results indicate that the established assay method is suitable for stability measurements of lovastatin. From the stress treatments, lovastatin was determined to be sensitive to the light, acidic, and basic medium.

INTRODUCTION

Lovastatin: (1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2S)-2-methylbutanoate.

It represents the first of a new class of cholesterol-lowering agents, the HMG-CoA reductase inhibitors, which are indicated for the treatment of primary hypercholesterolaemia. Lovastatin was also the first HMG-CoA reductase inhibitor acknowledged to slow coronary atherosclerosis. It was approved by FDA for the treatment of hypercholesterolaemia in August 1987.

A sensitive, specific, and rapid determination of lovastatin was mentioned in USP. However, the applicability of this HPLC method on the samples containing photodegradants is still unclarified. It is therefore desirable to study its stability-indicating nature which may enable simultaneous detection of acid-induced, base-induced, and photo-induced degradants of lovastatin.
MATERIALS AND METHODS

Chemicals
Lovastatin standard was from World Health Organization (WHO). Acetonitrile, phosphoric acid, potassium phosphate monobase, and water for HPLC were from Merck (Darmstadt, Germany).

HPLC Apparatus and Assay Conditions
A SHIMADZU LC-10 AD liquid chromatograph equipped with a SPD-10AV Shimadzu UV-visible detector, a CTO-10A SHIMADZU column oven, a SIL-10 AD SHIMADZU auto injector, and a Merck Lichrospher 100 RP-18 (5 µm) (150 x 3.9 mm i.d.) column equipped with a guard column of Lichro CART (4 x 125) were used with a mobile phase of acetonitrile-phosphoric acid (0.1%) (50:50,v/v). The UV detector was set at 238 nm and a flow rate 3.0 ml/min.

Stress Treatment of Lovastatin in Acidic, Basic, hydrogen peroxide, high temperature, or photo-irradiated conditions.
Buffer was prepared by dissolving an amount of 1.3609 g of monobasic potassium phosphate in a 1000-mL volumetric flask with distilled water and diluting to volume with it.

Dilution solution was prepared using acetonitrile and potassium phosphate buffer 0.01M in the ratio of 40:60 v/v, pH was adjusted to 4 with phosphoric acid.

An amount of 3 mg of lovastatin was accurately weighed and placed in a 100-mL volumetric flask. A concentration of 0.03 mg/mL solution was prepared as a stock solution by adding the dilution solution to the marked volume.

Ten milliliters were taken from the stock solution and placed in a 100-mL volumetric flask (this procedure was repeated five times) and each one was treated as follows to make each solution with 3 µg/mL concentration:
1- 20 mL of 0.5 N HCl was added to the first flask then placed in boiling water for 60 minutes.
2- 20 mL of 0.5 N NaOH was added to the second flask then placed in boiling water for 60 minutes.
3- 10 mL of 10% H_2O_2 was added to the third flask then shaken thoroughly and let in room temperature for 30 minutes.
4- The fourth flask was incubated at 60° temperature for 7 days.
5- The fifth flask was irradiated under a Hanovia 200-W high-pressure mercury lamp for 7 days. The distance of the light source to the sample was maintained at 25 cm.

The acidic solution was neutralized with 20 mL of 0.5 N NaOH, while the basic solution was neutralized with 20 mL of 0.5N HCl, then the five samples were diluted with distilled water to the mark. The samples were then subjected to HPLC analysis. Each of the above 5 stress treatments was tested in triplicates.

RESULTS AND DISCUSSION

Degradation of Lovastatin
The chromatograms of lovastatin degraded in acidic, basic, hydrogen peroxide, high temperature, and photo-irradiated conditions are shown in Figures 1,2,3,4,5, and 6. After stress treatment under acidic, basic, and hydrogen peroxide, the amounts of lovastatin remained were 15.5%, 14.9% and 17.3%, respectively whereas under Hg lamp irradiation and 60° temperature, they were 55.4% and 57.7% respectively. The results clearly show that lovastatin is more labile to photo-irradiation than to high temperature treatment.

Fig. 1: HPLC chromatogram of standard solution of lovastatin.
Fig. 2: HPLC chromatogram of degraded solution of lovastatin under acidic conditions.

Fig. 3: HPLC chromatogram of degraded solution of lovastatin under basic conditions.

Fig. 4: HPLC chromatogram of degraded solution of lovastatin under hydrogen peroxide conditions.

Fig. 5: HPLC chromatogram of photodegraded solution of lovastatin by a high-pressure mercury lamp for 7 days.
Validation of HPLC Method

A quantitation method must selectively separate the parent drug from its potential impurities and degradants. Our established method satisfies the system suitability criteria, peak integrity, and resolution between the parent drug and degradants. The results clearly indicate that the established assay method has good selectivity and specificity for quantitation and stability measurements of lovastatin.

The linearity of the calibration curve was checked over the range of 1 to 3 µg/mL in a diluted solution. The calibration curve was constructed by plotting the lovastatin response area ratio vs. concentration. The calibration curve for lovastatin is rectilinear in the concentration range studied. The related coefficient of the linear regression analysis is \( r^2 = 0.9997 \). The results of linear regression give the equation \( y = 0.9981x + 0.0058 \).

The intraday (Table 1) and interday (Table 2) standard deviations (S.D.) of six replicate determination for six consecutive days at the usual working concentrations of 1.0 to 3.0 µg/mL were among 0.007 and 0.220 with CV between 0.234% and 1.698% for the former; 0.019 to 0.098 with CV between 1.269% and 3.821% for the latter. The accuracy of the method as referred by recovery tests at five concentrations (1.5, 2.25, 3, 3.75, and 4.5 µg/mL), was determined to be 99.47%, 97.87%, 99.8%, 101.1% and 98.13%, respectively, indicating good accuracy for the assay method. Clearly, the assay method is reliable and applicable for stability assessment of lovastatin degraded under photo-irradiated condition.

**Table 1: Intraday analytical precision and accuracy for lovastatin (n= 6).**

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<tr>
<th>Conc. (µg/mL)</th>
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**Table 2: Interday analytical precision and accuracy for lovastatin (n= 6).**

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REFERENCES