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STABILITY-INDICATING HPLC ASSAY METHOD OF LOVASTATIN

R. Al-Masri and M. A. Al-Mardini

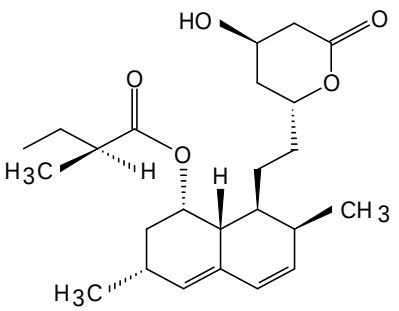
Department of Pharmaceutical Chemistry and Drugs Control, Faculty of Pharmacy, Damascus University, Damascus, Syria

لم تكن الطريقة الدستورية لتعيين اللوفاستاتين بتقنية الكروماتوغرافيا السائلة عالية الأداء HPLC قادرة على تمييز وفصل اللوفاستاتين عن نواتج تدركه، فكان الهدف من هذا البحث انتقاء طريقة HPLC حساسة، نوعية، وذات دلالة لاختبارات الثبات Stability-Indicating لتعيين اللوفاستاتين في المضغوطات. تم العمل باستخدام عمود Lichrospher C18، وطور متحرك لمزيج من: أسيتونتريل ٥٠%، وحمض الفسفور المخفف (١٠٠٠/١) ٥٠% ح/ح، وتم التحري بموجة طولها ٢٣٨ نم. أظهرت الطريقة فصلاً ما بين اللوفاستاتين ونواتج تدركه. تمت دراسة ثبات اللوفاستاتين في ظروف قاسية لإمكانية الحصول على نواتج التدرك، شملت هذه الشروط: درجات الحرارة المرتفعة، الضوء، الأكسدة، والحلمهة الحمضية القلوية. لم يتجاوز الانحراف المعياري في اليوم الواحد ٠,٠٩ ولعدة أيام ٠,٢٢. أظهرت الطريقة قيماً مقبولة من حيث الانتقائية، التكرارية، الخطية، المجال والمضبوطية.

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 (150x 3.9) 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.



Lovastatin

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.[1-3]

A sensitive, specific, and rapid determination of lovastatin was mentioned in USP. 25.[4] However, the applicability of this HPLC method on the samples containing photodegradants is still unclarified.[5-26] 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.

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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/m.[4]

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 shacked 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.[27]

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.

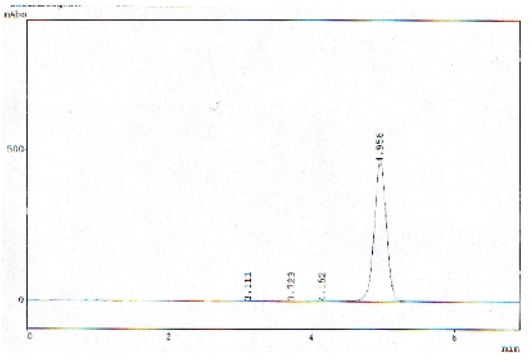

Fig. 1: HPLC chromatogram of standard solution of lovastatin.

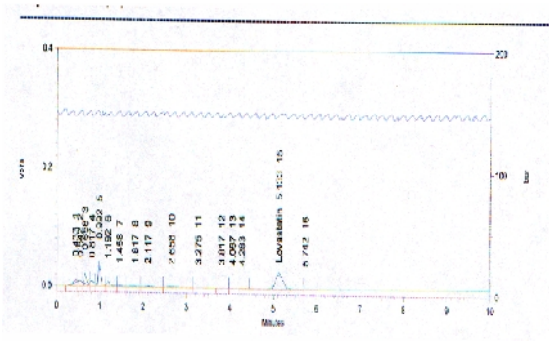

Fig. 2: HPLC chromatogram of degraded solution of lovastatin under acidic conditions.

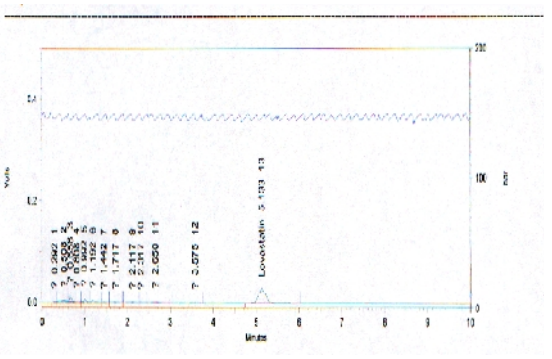

Fig. 3: HPLC chromatogram of degraded solution of lovastatin under basic conditions**.**

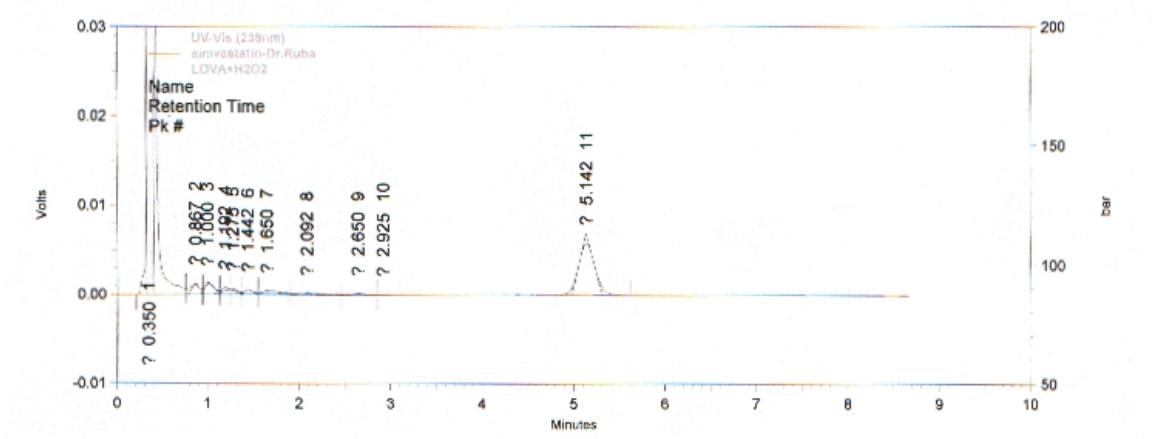

Fig. 4: HPLC chromatogram of degraded solution of lovastatin under hydrogen peroxide conditions**.**

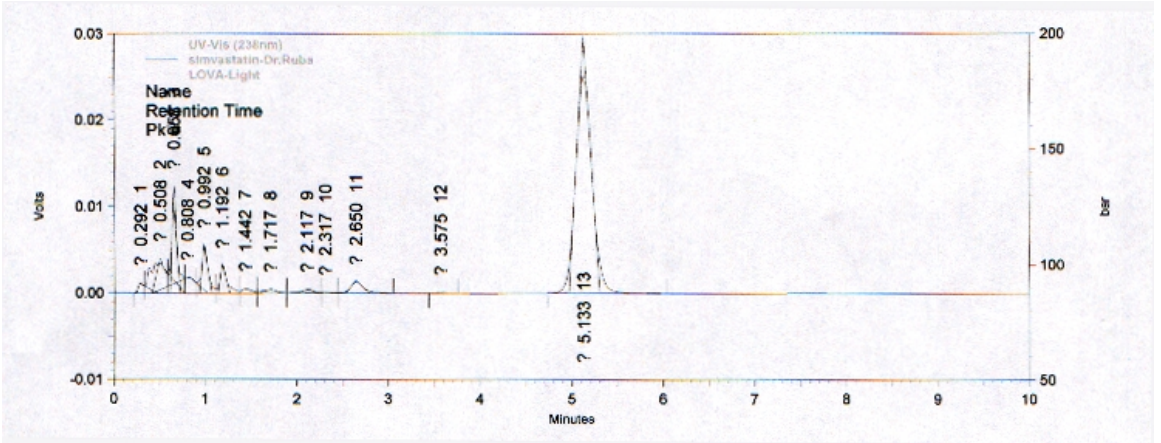

Fig. 5: HPLC chromatogram of photodegraded solution of lovastatin by a high-pressure mercury lamp for 7 days

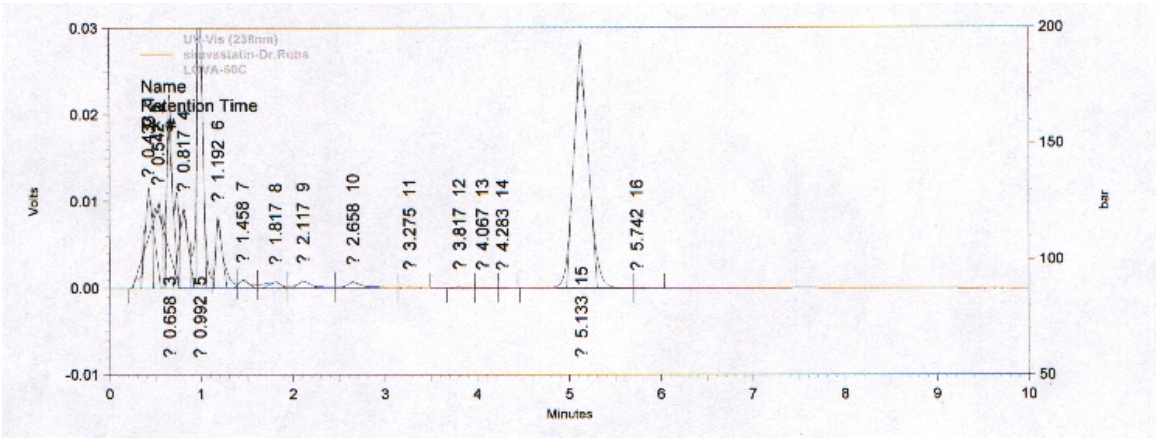

Fig. 6: HPLC chromatogram of degraded solution of lovastatin at 60° temperature for 7 days.

Validation of HPLC Method

A quantitation method must selectively separate the parent drug from its potential impurities and degradants.[28-37] 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).

Conc. (µg/mL)	1	1.5	2	2.5	3
Y1	1.002	1.472	2.014	2.503	2.997
Y2	0.982	1.480	2.025	2.510	2.986
Y3	1.009	1.529	2.011	2.480	2.991
Y4	0.995	1.492	2.013	2.495	3.005
Y5	0.988	1.475	2.042	2.486	2.989
Y6	1.031	1.505	2.009	2.479	2.993
Mean	1.001	1.492	2.022	2.492	2.994
SD	0.017	0.022	0.013	0.012	0.007
CV (%)	1.698	1.475	0.643	0.482	0.234

Table 2: Interday analytical precision and accuracy for lovastatin (n= 6).

Conc. (µg/mL)	1	1.5	2	2.5	3
Y1	1.002	1.472	2.014	2.503	2.997
Y2	0.968	1.498	1.981	2.575	3.101
Y3	0.982	1.525	2.170	2.498	3.043
Y4	1.015	1.489	1.915	2.539	2.981
Y5	0.979	1.486	1.993	2.517	2.909
Y6	1.024	1.514	2.019	2.479	2.825
Mean	0.995	1.497	2.015	2.519	2.976
SD	0.020	0.019	0.077	0.034	0.098
CV (%)	2.010	1.269	3.821	1.349	3.293

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