Рекомендована д.ф.н., професором В.С.Бондарем

УДК 543.544:615.272.4:542.61

HPLC-UV METHOD OF DETERMINATION OF THE PITAVASTATIN. PART 1. CHROMATOGRAPHIC CONDITIONS. SOLID PHASE EXTRACTION. PRECISION OF RECOVERY

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The conditions of pitavastatin determination by HPLC UV method have been examined. The method of solid-phase extraction of pitavastatin has been developed. Precision of recovery of pitavastatin has been determined.

Introduction

Statins (HMG-CoA reductase inhibitors) are widely used for the treatment of hypercholesterolemia. Clinical trials in patients with and without coronary heart disease and with and without high cholesterol have demonstrated consistently that statins reduce the relative risk of major coronary events by 30% and produce a greater absolute benefit in patients with higher baseline risk [5]. The beneficial effects of statins may extend beyond their cholesterol lowering effects, to include so-called pleiotropic effects. These effects include improving endothelial function, attenuating vascular and myocardial remodelling, inhibiting vascular inflammation and oxidation, and stabilizing atherosclerotic plaques [9, 10]. Statins also exhibit important antitumor activities [7].

Currently few bioanalytical reports devoted to determination of pitavastatin are known. One report describes quantitative determination of pitavastatin in plasma by column-switching high-performance liquid chromatography with ultraviolet detection [4]. The method is complicated. Four articles are devoted to the analysis of pitavastatin in biological samples by liquid chromatography-tandem mass spectrometry [1, 2, 3, 8]. One of them described solid phase extraction procedure [1]. The method is complicated, needs to use expensive equipment. Ojha et al. proposed HPLC method with fluorescence detection for the determination of the pitavastatin in human plasma [6].

Aims of the study

The aim of this work was to develop simple, fast, accurate, reproducible, sensitive and selective analytical method for the determination of the pitavastatin by HPLC UV method.

Materials and Methods

Chemicals and reagents

Pitavastatin was purchased from Funakoshi Co., Ltd. (Tokyo, Japan), mevastatin — from Sigma-Aldrich. Chemical names of pitavastatin and mevastatin are shown in the Table 1.

Tetrabutylammonium hydroxide, glacial acetic acid and methanol (HPLC grade) were obtained from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). Solid-phase extraction cartridges Bond Elute C18, 50 mg, 1 mL (Varian, USA).

Standard solutions

Composite stock solutions (1 mg/mL) of pitavastatin and mevastatin (internal standard (IS)) were prepared by dissolving appropriate amounts of the compounds in DMSO. These stock solutions were stored at approximately 5°C.

A series of standard solutions were prepared by appropriate dilution of the above-mentioned stock solution in methanol to reach the concentration range of 5-250 ng/mL.

HPLC system

Pitavastatin was determined using a high-performance liquid chromatography (HPLC) system consisting of the pump LC-6A (Shimadzu, Kyoto, Japan) and UV-VIS detector L 4200 (Hitachi, Japan). The Line Recorder CR-6A CHROMATOPAC (Shimadzu, Kyoto, Japan) was used for data recording. The compounds were separated at 25°C on the Inertsil ODS-3 column (5 μm, 150×4.6 mm I.D.) (GL Science Co. Ltd., Tokyo, Japan) with guard column. The detection was carried out at 238 nm. For preparation of the mobile phase 290 mL of water was mixed with 10 mL of 0.5 M tetrabutylammonium hydroxide, adjusted to pH 6.0 with concentrated acetic acid and water until 310 mL, solution was mixed with 690 mL of methanol. Mixture was delivered at a flow rate of 1.0 mL/min.

Selection of Chromatographic Conditions

Influence of pH, concentration of methanol on the retention characteristics was studied.

Table 1

Chemical names of pitavastatin and mevastatin

Name	Chemical name
Pitavastatin	Monocalcium bis{(3R, 5S, 6*E*)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3,5-dihydroxy-6-heptenoate}
Mevastatin	[(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] (2S)-2-methylbutanoate

Sample Preparation

Care was taken to keep the sunlight off. A cartridge (1 mL) was activated with 1 mL of MeOH followed by 1 mL of H_2O at vacuum 3 in Hg. 1 mL of sample (methanole solution of pitavastatin and mevastatin) was transferred to a 5-mL test tube and was diluted by 4 mL of water. After vortexing for 30 s, the sample mixture was loaded onto the prepared cartridge at vacuum 4 in Hg. The cartridge was washed with 1 mL 5% MeOH at vacuum 4 in Hg and dried at vacuum 8 in Hg. The analyte was eluted with 200 μL of MeOH. After drying up 0.5 min at vacuum 8 in Hg the eluted solution was mixed with the buffer solution (tetrabutylammonium hydroxide 16.1 mmol/L, acetic acid until pH 6.0). Then aliquot of 50 μL was injected into the chromatographic system.

Precision of Recovery

Preparation of the Quality Control solutions

1. Solution of pitavastatin (10 ng/mL), mevastatin (internal standard 50 ng/mL): 10 μL of 5 μg/mL solution of pitavastatin was mixed with the 25 μL of 10 μg/mL solution of mevastatin and 4965 μL Methanol.

2. Solution of pitavastatin (25 ng/mL), mevastatin (internal standard 50 ng/mL): 25 μL of 5 μg/mL of solution of pitavastatin was mixed with the 25 μL of 10 μg/mL solution of mevastatin and 4950 μL Methanol.

3. Solution of pitavastatin (50 ng/mL), mevastatin (internal standard 50 ng/mL : 50 μL of 5 μg/mL of solution of pitavastatin was mixed with the 25 μL of 10 μg/mL solution of mevastatin and 4925 μL Methanol.

Preparation of Standard Solutions

1. Solution of pitavastatin(50 ng/mL), mevastatin (internal standard 250 ng/mL): 20 μL of 5 μg/mL solution of pitavastatin was mixed with the 50 μL of 10 μg/mL solution of mevastatin and 1930 μL Methanol.

2. Solution of pitavastatin (125 ng/mL), mevastatin (internal standard 250 ng/mL): 50 μL of 5 μg/mL solution of pitavastatin was mixed with the 50 μL of 10 μg/mL solution of mevastatin and 1900 μL Methanol.

3. Solution of pitavastatin (250 ng/mL), mevastatin (internal standard 250 ng/mL): 100 μL of 5 μg/mL solution of pitavastatin was mixed with the 50 μL of 10 μg/mL solution of mevastatin and 1930 μL Methanol.

Preparation of solution for injection in HPLC

200 μL of each standard solution was mixed with the buffer solution (tetrabutylammonium hydroxide 16.1 mmol/L, acetic acid until pH 6.0).

Aliquot of 50 μL was injected into the chromatographic system.

Results and Discussion

Chromatographic Conditions

The satisfactory separation and retention characteristics of pitavastatin and mevastatin (internal standard) at 69% of methanol, and 31% of buffer (tetrabutylammonium hydroxide 16.1 mmol/L, acetic acid until pH 6.0) were obtained. Typical chromatogram of the 1250 ng/mL of solution of pitavastatin and solution of mevastatin 1250 ng/mL is shown in Fig.

Limit of detection and quantification

1. Without solid phase extraction

For the the pitavastatin LOD was approximately 12 ng/mL and LOQ was 40 ng/mL. For the mevastatin LOD was approximately 30 ng/mL and LOQ was 100 ng/mL.

2. After solid phase extraction

For the the pitavastatin LOD was approximately 3 ng/mL and LOQ was 10 ng/mL. For the mevastatin

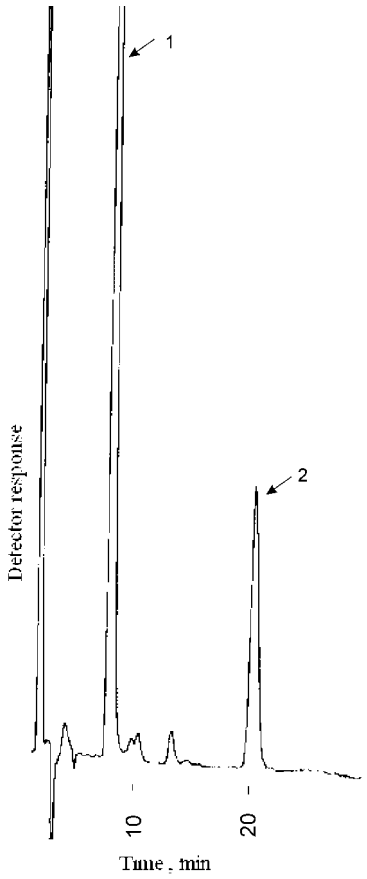

Fig. Typical chromatogram of standard solutions of pitavastatin (1) and mevastatin (2).

Table 2

Recovery data for pitavastatin and IS (mevastatin) (according to peak area)

Nominal concentration (ng/mL)	$\overline{X}$	CV%	$\overline{X}\pm\Delta\overline{X}$
Pitavastatin, 10	122,5	11,94%	122,5±18,2
Mevastatin, 50	102,3	2,691%	102,3±3,4
Pitavastatin, 25	105,9	2,846%	105,9±3,7
Mevastatin, 50	101,9	5,008%	101,9±6,3
Pitavastatin, 50	108,4	9,913%	108,4±13,4
Mevastatin, 50	102,3	11,12%	102,3±14,1

Table 3

Recovery data for pitavastatin and IS (mevastatin) (according to peak heght)

Nominal concentration (ng/mL)	X	CV%	$X\pm\Delta X$
Pitavastatin, 10	132,3	11,46%	132,3±18,8
Mevastatin, 50	102,3	2,691%	102,3±3,4
Pitavastatin, 25	114,6	3,957%	114,6±5,6
Mevastatin, 50	105,3	6,747%	105,3±8,8
Pitavastatin, 50	120,4	8,622%	120,4±12,9
Mevastatin, 50	103,7	7,537%	103,7±9,7

LOD was approximately 8 ng/mL and LOQ was 25 ng/mL.

Precision of Recovery

The data of determination of presicion of recovery are shown in the Table 2 and 3.

CONCLUSIONS

1. Conditions of determination of pitavastatin by HPLC UV method were examined.

2. Precision of recovery of the pitavastatin was determined.

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УДК 543.544:615.272.4:542.61

ВЭЖХ-УФ МЕТОД ОПРЕДЕЛЕНИЯ ПИТАВАСТАТИНА. ЧАСТЬ 1. ХРОМАТОГРАФИЧЕСКИЕ УСЛОВИЯ. ТВЕРДОФАЗНАЯ ЭКСТРАКЦИЯ. СХОДИМОСТЬ СТЕПЕНИ ИЗВЛЕЧЕНИЯ

Б.Варинский, С.Накагава, С.Ямато

Изучены условия определения питавастатина с помощью ВЭЖХ-УФ метода. Разработана процедура твердофазной экстракции питавастатина. Определена сходимость степени извлечения питавастатина.

УДК 543.544:615.272.4:542.61

ВЕРХ-УФ МЕТОД ВИЗНАЧЕННЯ ПІТАВАСТАТИНУ. ЧАСТИНА 1. ХРОМАТОГРАФІЧНІ УМОВИ. ТВЕРДОФАЗНА ЕКСТРАКЦІЯ. ЗБІЖНІСТЬ СТУПЕНЯ ВИТЯЖКИ

Б.Варинський, С.Накагава, С.Ямато

Вивчені умови визначення пітавастатину за допомогою ВЕРХ-УФ методу. Розроблена процедура твердофазної екстракції пітавастатину. Визначена збіжність ступеня витяжки пітавастатину.