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PHOTOCHEMICAL PROPERTIES OF SIMVASTATIN AND LOVASTATIN INDUCED BY RADIATION

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HPLC and UV spectrophotometry were developed and validated for quantitative determination of two antihiperlipoproteinemia drugs, lovastatin and simvastatin.

Analytical performance parameters such as linearity, precision, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to the ICH Q1B guidelines. Chromatography was carried out by isocratic technique on a reversed-phase C-18 column. The UV spectroscopy determinations were performed at 238 nm. The linearity of the calibration curves in the desired concentration range was good (r(2)>0.999) for both HPLC and UV methods. The relative standard deviation (RSD) for these methods was <5%. Moreover, the precision obtained with HPLC correlated well with the UV results. The methods proposed are highly sensitive and precise. Other methods used for assessment of the photostability of the substances studied were electron paramagnetic resonance (EPR) and differential scanning calorimetry (DSC).

Keywords: *DSC, EPR, HMG-CoA, HPLC, lovastatin, photodegradation, simvastatin, statins*

Introduction

Simvastatin and lovastatin are the hypolipidemic drugs belonging to the class of pharmaceuticals called statins. They are used to control hypercholesterolemia (elevated cholesterol levels) and to prevent cardiovascular disease [1–3].

Lovastatin and simvastatin are the inactive lactone, are hydrolyzed to the corresponding β-hydroxyacid form, which is antinhibitor of HMG-CoA reductase [4–8].

Lovastatin is 8-[2-(4-hydroxy-6-oxo-oxan-2--yl)ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] 2-methylbutanoate. Simvastatin, the methylated form of lovastatin, is [(1S,3R,7R,8S,8aR)-8-[2-[(2*R*,4*R*)-4-hydroxy-6-oxo-oxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl]2,2-dimethylbutanoate.

HMG-CoA reductase inhibitors are considered as relatively safe and effective cholesterol-lowering agents [5–8]. Lovastatin is a cholesterol-lowering agent isolated from a strain of *Aspergillus terreus*; Simvastatin is a synthetic derivate of a fermentation product – the methylated form of lovastatin. Lovastatin and simvastatin are used in the treatment of primary hypercholesterolemia and are effective in reducing total and LDL-cholesterol as well as plasma triglycerides and apolipoprotein B.

The 6-membered lactone ring of simvastatin and lovastatin hydrolyzes in vivo to generate mevinolinic acid, an active metabolite structurally similar to HMG-CoA (hydroxymethylglutaryl CoA). Once hydrolyzed, simvastatin competes with HMG-CoA for HMG-CoA reductase, a hepatic microsomal enzyme. Interference with the activity of this enzyme reduces the quantity of mevalonic acid, a precursor of cholesterol.

These compounds contain a naphthenyl diene bond system that is the site most susceptible to free radical oxidation.

In this study the HPLC and UV based procedures were developed and validated for the quantitative determination of two antihiperlipoproteinemia drugs. Different analytical performance parameters such as linearity, precision, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to the ICH Q2B guidelines [9]. The proposed methods are highly sensitive and precise. Other methods used for assessment of the photostability [10–14] of the substances studied were EPR and DSC. EPR spectroscopy can be used to monitor free radical formation on irradiation, identify the radical intermediates and help determine the reaction mechanism [15–26]. The DSC method is very suitable for checking the effects of irradiation as the presence of radiolysis or photolysis decrease the

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melting point and enthalpy of melting, which is manifested in the DSC curves [15–20].

Experimental

Reagents

Lovastatin – $C_{24}H_{36}O_5$ m.w.=404.54 g mol^{-1} and simvastatin – $C_{25}H_{38}O_5$ m.w.=418.57 g mol^{-1} (100% chromatographically pure) was supplied by Biofarm (Poznan, Poland). The structure of the pharmaceuticals examined is given in Figs 1.

Deionised water was obtained in the laboratory, using ionic RTV system. Methanol and acetonitrile HPLC grade (Merck) were used. All other reagents were of the highest purity commercially available.

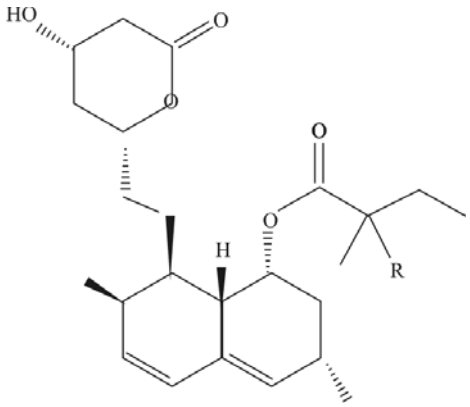

Fig. 1 Chemical structure of lovastatin where R=H (lovastatin) or R=CH_3 (simvastatin)

Methods

UV spectroscopy

All ultraviolet absorption spectra were obtained with a UV-160 spectrophotometer (Shimadzu). The spec- tra were recorded every nanometer in the range 200–400 nm, in 10 mm silica quartz cells. The optimized instrumental parameters were: scan rate 1 nm s^{-1}, time response 1 s; spectral bandwidth 1 nm. UV PC-160 package software was used to analyse the spectral data.

HPLC analysis

HPLC measurements were carried out by using a Hewlett–Packard 1050 chromatograph equipped with a model 3G pump and UV detector. The data acquisition and processing were performed with the CSW software. The chromatographic column used was a Lichrosfer RP-18 (3.9·200 mm I.D, Merck), 5 μm particle size. The injector was a 20 μL Rheodyne valve. The column was kept at 25±1°C using a RT thermostat, model 105G. Analysis was accomplished using isocratic elution with a mobile phase consisting of methanol:water=40:10 (v/v). The flow rate was 1.0 mL min^{-1}. The UV detection was carried out at 238 nm.

EPR spectroscopy

The EPR experiments were carried out for non-irradiated and irradiated samples, in standard EPR quartz sample tubes from Wilmad. The measurements were performed with a Bruker EPR EMX-10 spectrometer working at 9.4 GHz (X-band) at room temperature (293 K). The sensitivity of the spectrometer is $1 \cdot 10^{10}$ spins per gram. Induction of the magnetic field was measured to an accuracy of 0.001 mT. Microwave frequency was measured to an accuracy of 0.001 GHz. The spectrometer operating conditions during the experiment were: modulation amplitude of 0.1 mT, microwave power of 2 mW and sweep width of 20 mT.

Differential scanning calorimetry (DSC)

Thermal properties of the samples were examined using a Netzsch model 204 Phoenix differential scanning calorimeter. The samples of about 5 mg were closed in aluminium crucibles with pierced lid. Before measurements, the samples were isothermally incubated at 20°C for 5 min, and the measurements were performed in the helium atmosphere in temperatures from 20 to 350°C at the heating rate of 5°C min^{-1}. The results were processed using the TAA (Netzsch) program.

Photodegradation

Photodegradation process was performed in a light cabinet, equipped with a Xenon lamp, according to the ICH Guideline for photostability testing. The apparatus was fitted up with a cooling and temperature measuring and controlling system inside the box. The system was able to closely simulate sunlight and appropriately selected spectral regions by interposition of filters. In the present study the samples were irradiated with a λ between 320–800 nm, by means of a Pyrex filter, according to the ID65 standard of ICH rules; the power was maintained to 350 W m^{-2}, at the constant temperature of 25°C. The quantum yield of the source was determined by using the Reinecke salt as a chemical actinometer; the number of quanta was $I_{SR}=2.51 \cdot 10^{16}$. The drugs in the powder form were distributed in 0.1 cm metal cell, before light exposure.

Solutions LV and SV of a concentration about 1.8×10^{-5} mol L^{-1} were transferred to a 2.8 mL quartz cuvette, perfectly stoppered and irradiated under the conditions described above.

Results and discussion

Validation procedure

The validation parameters were linearity, selectivity, precision, limit of detection and limit of quantitation.

Linearity and limit of detection and quantification

The calibration curve for LV and SV provided a reliable response from $3.3\cdot10^{-6}$ to $2.1\cdot10^{-5}$ and $5.5\cdot10^{-6}$ to $3.0\cdot10^{-5}$ mol L^{-1}. The calibration curves were constructed, obtaining the following equation for LV and SV, respectively,

	HPLC method	Spectrophotometric method
LV	$y=1.26\cdot10^7x+0.63$ ($n=8$; $r=0.999$)	$y=2.6\cdot10^4x+0.001$ ($n=8$; $r=0.999$)
SV	$y=1.40\cdot10^7x+1.60$ ($n=8$; $r=0.999$)	$y=2.5\cdot10^4x+0.007$ ($n=8$; $r=0.999$)

$\varepsilon_{\lambda=238}$ for LV=$2.5\cdot10^4$ [dm^3 mol^{-1} cm^{-1}] and
$\varepsilon_{\lambda=238}$ for SV=$2.4\cdot10^4$ [dm^3 mol^{-1} cm^{-1}]

The limit of detection (LOD) was (at a signal-to-noise (S/N_0 ratio of 3) and the limit of quantification (LOQ) was:

	HPLC method		Spectrophotometric method	
	LOD/ mol L^{-1}	(LOQ)/ mol L^{-1}	LOD [mol L^{-1}]	(LOQ) [mol L^{-1}]
LV	$4.3\cdot10^{-7}$	$1.3\cdot10^{-6}$	$4.3\cdot10^{-7}$	$1.7\cdot10^{-6}$
SV	$4.7\cdot10^{-7}$	$1.7\cdot10^{-6}$	$4.2\cdot10^{-7}$	$1.4\cdot10^{-6}$

Precision

The parameters characterising the precision of UV spectroscopy and HPLC method are given in Table 1.

The precision of the two analytical methods applied was estimated on the basis of 8 times repeated determinations of the content of LV and SV in the methanol solutions of appropriate concentration. As follows from the results obtained the methods are sufficiently precise and can be applied for determination of LV and SV (Table 1).

Statistical analysis (test F-Snedecor and t-Student's)

Obtained results were statistically evaluated with the means of F-Snedecor and t-Student tests. The former test revealed statistically significant difference between the variances of the UV spectroscopy and HPLC results, however, verification of the zero hypothesis of Student-t test of the same mean values obtained by the two methods, proved no statistically significant differences between them.

The HPLC method

In the selected optimum experimental conditions (methanol/water), LV and SV exhibited a well-defined chromatographic peak with a retention time of 9.45±0.3 min and 11.05±0.2 min, respectively. Figures 3 and 4 show the typical chromatograms of LV and SV obtained under the optimum conditions. The chromatographic signal intensity is linearly dependent on the LV and SV concentration enabling the use of this signal for LV and SV quantification.

When LV and SV powders and solutions are exposed to radiation during 3 h, no changes in the original chromatograms are observed. Consequently, the drugs are stable under these experimental conditions of irradiation. As can be seen in Figs 2 and 3, the chromatograms of LV and SV did not reveal any new peaks.

The results obtained by the other analytical methods applied in this work (EPR, DSC) did not

Table 1 Validation of the spectrophotometric (SP) and HPLC methods for the determination of lovastatin (LV) and simvastatin (SV); precision and linearity

Parameter	Method			
	HPLC		UV spectroscopy	
	LV	SV	LV	SV
N	8	8	8	8
$\bar{x}$	$1.3495\cdot10^{-5}$	$2.5181\cdot10^{-5}$	$1.3076\cdot10^{-5}$	$2.5084\cdot10^{-5}$
S^2	$1.69343\cdot10^{-15}$	$4.4377\cdot10^{-15}$	$4.47343\cdot10^{-15}$	$6.2934\cdot10^{-15}$
S	$4.11513\cdot10^{-8}$	$6.6616\cdot10^{-8}$	$6.68837\cdot10^{-8}$	$7.9331\cdot10^{-8}$
S_y	$3.04937\cdot10^{-3}$	$2.6455\cdot10^{-3}$	$5.11499\cdot10^{-3}$	$3.1626\cdot10^{-3}$
S_x	$1.5\cdot10^{-8}$	$2.4\cdot10^{-8}$	$2.3\cdot10^{-8}$	$2.8\cdot10^{-8}$
μ	$1.3495\pm0.0036\cdot10^{-5}$	$2.5184\pm0.0055\cdot10^{-5}$	$1.3076\pm0.0055\cdot10^{-5}$	$2.5084\pm0.0066\cdot10^{-5}$
W_z/%	0.3049	0.2646	0.5115	0.3163

$\bar{x}$=mean value; S=standard deviation; S^2=variation; S_y=standard deviation of mean value; μ=confidence interval; W_z=coefficient of variation

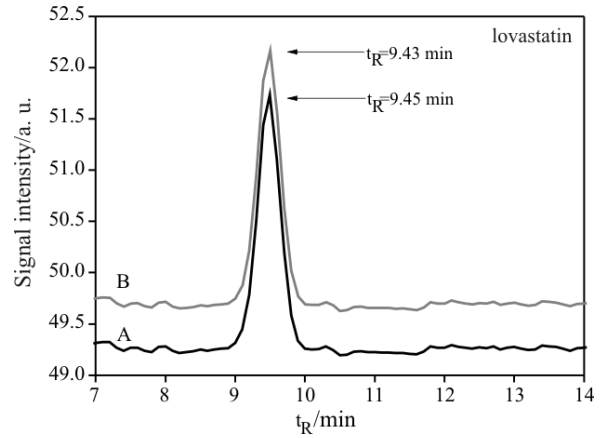

Fig. 2 HPLC chromatograms of lovastatin A – before and B – after photodegradation

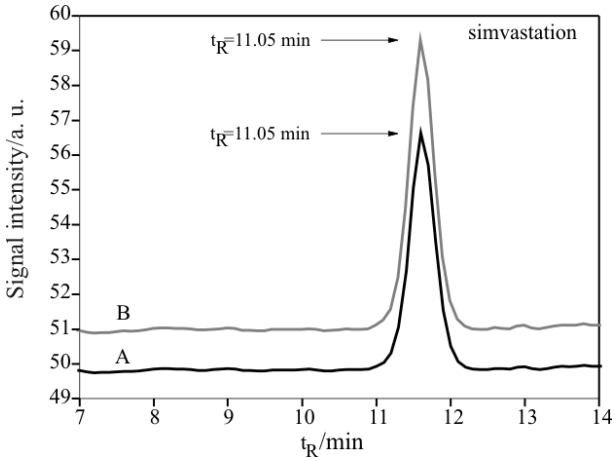

Fig. 3 HPLC chromatograms of simvastatin A – before and B – after photodegradation

show any detectable changes in the physico-chemical properties of the compounds studied.

EPR measurements

EPR spectroscopy can be used to monitor free radical formation in the process of photodegradation, identify the radical intermediates and help determine the reaction mechanism. EPR measurements of the samples LV and SV were made before and after irradiation. No presence of free radicals as potential products of photodegradation was detected (Fig. 4).

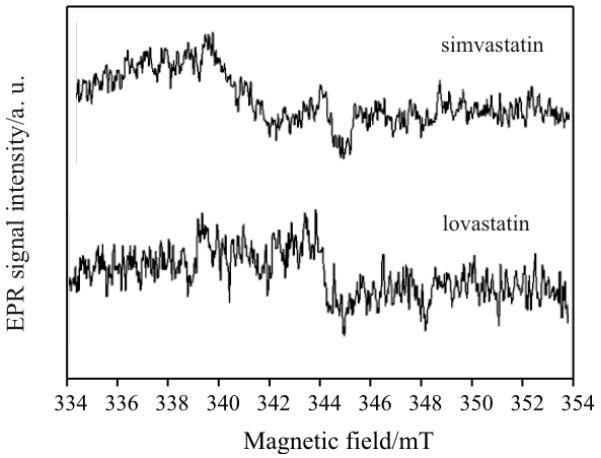

Fig. 4 EPR spectra of simvastatin (SV) and lovastatin (LV) after photodegradation

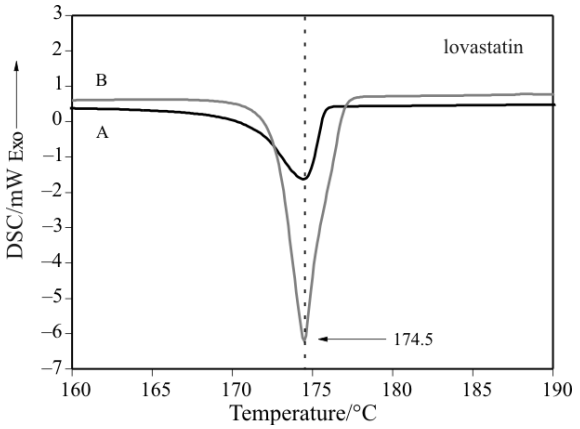

Fig. 5 DSC curves of lovastatin A – before and B – after photodegradation

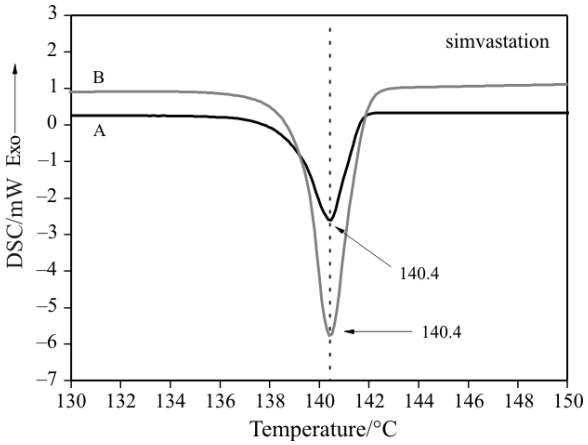

Fig. 6 DSC curves of simvastatin A – before and B – after photodegradation

DSC measurements

At the next stage of the study the samples LV and SV before and after irradiation were studied by the DSC

method to look for possible changes in their melting points, which would indicate the formation of the photolysis products. The DSC results of the compounds studied revealed no changes indicating their high photostability (Figs 5 and 6).

Conclusions

The validation process HPLC and UV spectroscopy showed that the applied methods were linear (the parameters of regression were as shown in Table 1). HPLC and UV spectroscopy methods were of good precision reflected by the low coefficient of variation values, 1.08%. Statistical analysis (test F-Snedecora and t-Student's) of the determination of LV and SV in the liquid phase did not show any significant differences between the HPLC and UV spectroscopy methods. The presented investigations may be applied for routine determinations of LV and SV and its stability. The other analytical methods used: EPR and DSC, have proved very helpful in checking the formation of photolysis products and can be used as supplementary methods in the process of validation described in the paper.

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