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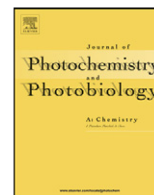

Photochemical activity of glenvastatin, a HMG-CoA reductase inhibitor

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ABSTRACT

Photochemical properties of glenvastatin (HR 780), a HMG-CoA reductase inhibitor, were studied. The drug was exposed to light in liquid state under aerobic conditions. The source of irradiation was an HBO-50 lamp. The lamp was equipped with a mercury burner and an appropriate set of glass filters. The conditions during photodegradation were the same as specified in the first version of the ICH Document.

Photoproducts formed after illumination of a methanol solution of glenvastatin were identified by several analytical techniques, including: spectrophotometry, HPLC and HPLC–MS. Quantitative evaluation of photochemical degradation of glenvastatin was made on the basis of UV–Vis spectrophotometric and HPLC. The irradiation photodecomposition of glenvastatin led preferentially to two main photoproducts being stereoisomers. The photoproducts were isolated with the help of a semipreparative HPLC method and identified by mass spectrometry. As a result of glenvastatin exposure to irradiation, the heptene chain is oxidised and its cyclisation takes place with formation of two diastereoisomers containing three condensed rings.

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1. Introduction

Stability of drugs is influenced by many factors such as temperature, moisture, oxidation as well as exposure to light [1]. Photochemical processes have severe pharmaceutical and medicinal consequences. Light can essentially change the properties of many therapeutic agents during production, storage and even in the patient's organism after their administration [2,3]. Photodegradation of drugs should not be understood only as a decomposition process as it also involves generation of free radicals or transfer of energy [4,5]. Such phenomena contribute to formation of diverse photoproducts showing various physicochemical features (solubility, viscosity, colour). The loss of pharmaceutical activity is usually unavoidable. The photoproducts can also react with endogenous substances and thus be a source of many dangerous side-effects, including phototoxicity, photoallergy or carcinogenic activity [6–9].

Until 1998 there were no well-defined directives and recommendations about photostability testing, that would guarantee proper reproducibility and credibility. In 1998 the International Conference on Harmonization (ICH) prepared a detailed guideline in order to unify photostability testing procedures [10,11]. It contains descriptions of appropriate light sources, chambers, conditions during illumination and investigation of different drug formulations [12–14].

Classification of a drug as prone to photodecomposition is only the first step. The primary tests should be followed by a detailed assay. Further analysis concerns mainly the mechanisms of photochemical reactions or isolation and identification of photoproducts. In order to improve the stability, the application of protective additives must be considered [15]. Such information is of extreme importance for determination of conditions of production process or storage [16–18]. Analysis of photochemical decomposition products is essential to establish the conditions of safe and efficient therapy.

Moore [19] have formulated some basic questions that should be answered prior to photostability testing procedure: (i) is the drug stable in a formulation or a protecting packaging, (ii) does photodegradation depend upon the irradiation wavelength (iii) are the structures of photoproducts known, (iv) what is the influence of such factors as pH, impurities and drug additives on photostability. It is also very important to establish if the drug or its metabolites accumulate in tissues that are usually exposed to light [20].

Recent assays have indicated that the group of drugs that can be potentially susceptible to light includes 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors known as statins.

Statins make a class of drugs used mainly as lipid-lowering agents. All statins consist of two specific structural components, a dihydroxyheptanoic acid unit and a ring system with lipophilic substituents. The dihydroxyheptanoic acid component is essential for

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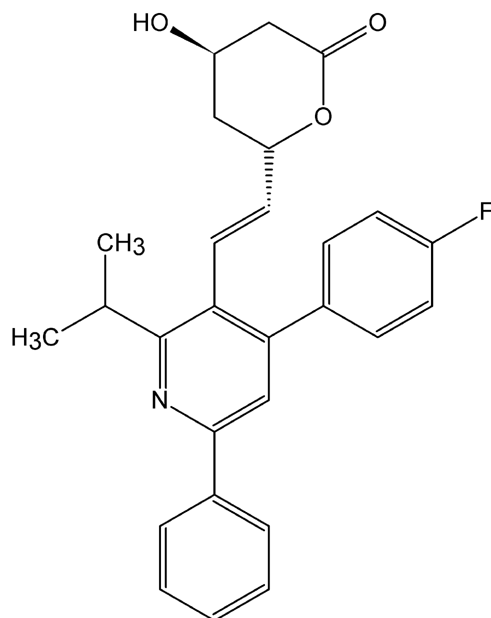


Fig. 1. The structure of glenvastatin.

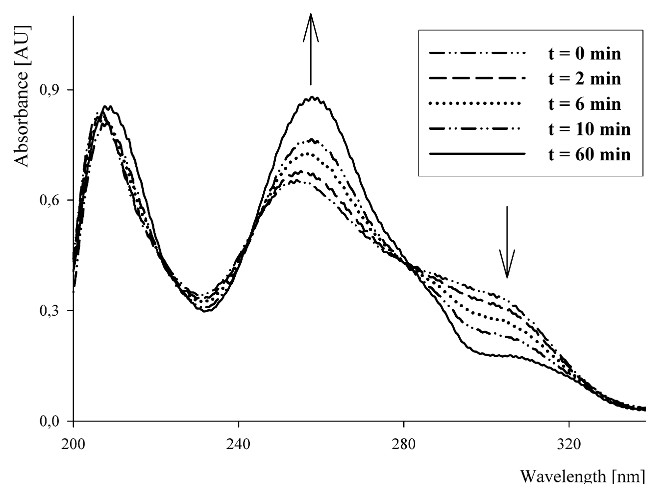


Fig. 2. Changes in the absorption spectrum of glenvastatin before and after 2, 6, 10, 60 min of irradiation.

the HMGCR-inhibiting activity. The inhibition of HMG-CoA reductase in the mevalonate pathway reduces cholesterol biosynthesis in the liver and thus causes an increase in the uptake and degradation of low-density lipoproteins (LDL). The result of such an activity is the inhibition of LDL oxidation, cholesterol accumulation and esterification with a subsequent increase in scavenger receptors expression.

High therapeutic efficiency connected with relatively rare side-effects has made statins the most potent group of drugs used in prevention and treatment of various cardiovascular diseases.

Moreover, according to the fact that the mevalonate pathway leads to formation of dolichols, ubiquinone and isoprenoids, statins exhibit salient influence on diverse inflammatory reactions, smooth muscle cell migration and proliferation, macrophage activation or platelet adhesion and aggregation [21]. Thus it is highly probable that their application can be broadened to new indications including inflammation, osteoporosis, cancer and even Alzheimer's disease prevention [22–27].

It becomes clear that the evaluation of photochemical properties of statins is obligatory in order to provide a safe and effective treatment. Photochemical testing has already been carried out for pitavastatin, fluvastatin, lovastatin, simvastatin and atorvastatin [28–32].

Therefore the aim of the presented work was to evaluate photochemical properties of glenvastatin (HR 780), one of the new generation of statins [33,34].

2. Experimental

2.1. Materials

Glenvastatin (GV) (HR 780) [(+)-(E)-6S-(2-(4-(4-fluorophenyl)-2-(1-methylethyl)-6-phenylpyridin-3-yl)ethenyl)-4R-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one] ($C_{27}H_{26}FO_3N$); M_r = 431.5 g/mol, was kindly supplied by Aventis Pharma Ltd., Germany – Fig. 1.

Methanol, ethyl acetate, acetonitrile and tetrahydrofurane (analytical quality) were obtained from *Merck* and used without further purification.

Water used in the mobile phase for HPLC was twice distilled and purified by Milipore Water Purification System Milipore Corp., USA.

2.2. Irradiation conditions

Samples were illuminated according to the recommendations in the first version of the Document ICHQ1B. A high-pressure lamp, HBO-50 was used as a source of light. The lamp was equipped with a mercury burner emitting radiation λ_{max} = 365 nm. In order to cut-off wavelengths below 365 nm a Pyrex filter was used. Solutions of GV (10^{-5} mol/L) were exposed to irradiation of 0.25 J × cm^{-2} × min^{-1}, determined with the use of a VLX-3W type radiometer with a CX-365 sensor as a physical actinometer. Methanol solutions of GV were irradiated in a cylindrical quartz cell (*Hellma*; *V* = 2.8 mL; *l* = 1 cm).

2.3. Spectrophotometric analysis

A methanol solution of GV was transferred to a cylindrical quartz cell and exposed to radiation for 52 h (full doses of radiation was 0.78 kJ × cm^{-2}). At different times the absorption spectra were recorded in the range of 200–400 nm against methanol (Fig. 2). Spectra were plotted on a Shimadzu UV-160A spectrophotometer.

2.4. High Performance Liquid Chromatography

The Agilent Technologies 1200 Series liquid chromatograph equipped with Gemini C18 250 mm × 4.6 mm (Phenomenex) analytical column (5 μm particle size) was used for the separation of GV and photoproducts thereof.

The temperature of the column was kept at 25 °C. Sample injection volume was 10 μL. The analytical HPLC gradient conditions of mobile phase are shown in Table 1.

In order to detect the photoproducts a UV–Vis diode-array detector was used. The chromatogram and the spectra are presented in Fig. 3.

Table 1
Analytical HPLC gradient conditions.

Time (min)	Methanol (%)	Acetic buffer pH = 4.0 (%)
0	70	30
25	100	0
35	100	0

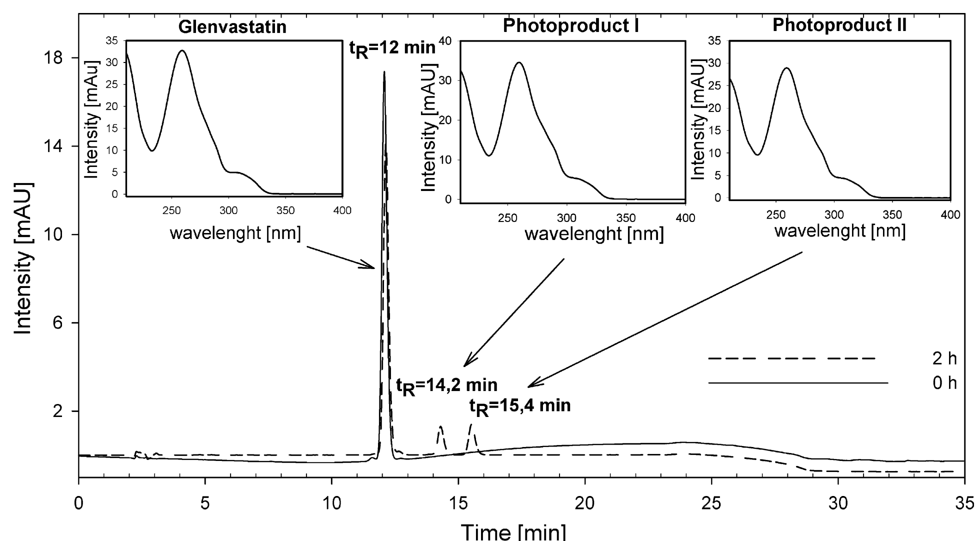


Fig. 3. Chromatogram and UV spectra of glenvastatin and its main photoproducts.

Table 2
Semipreparative HPLC gradient conditions.

Time (min)	Methanol (%)	Phosphate buffer pH = 2.5 (%)
0	60	40
5	60	40
25	90	10
35	60	40

Table 4
Kinetic parameters of photochemical decomposition of glenvastatin.

Method	k (s^{-1})	Φ
HPLC	$1.46 \times 10^{-5} \pm 1.34 \times 10^{-6}$	$7.9 \times 10^{-4} \pm 5.4 \times 10^{-5}$

2.5. *Semi-preparative high-performance liquid chromatographic and ^{1}H NMR analysis*

The photoproducts were isolated by the semi-preparative HPLC method with the stationary phase composition as specified in Section 2.4. A portion of 100 μL of GV solution subjected earlier to irradiation in the conditions specified in Section 2.2, was injected onto the column. The rate flow of the mobile phase of 1 mL/min, while its composition and gradient are given in Table 2.

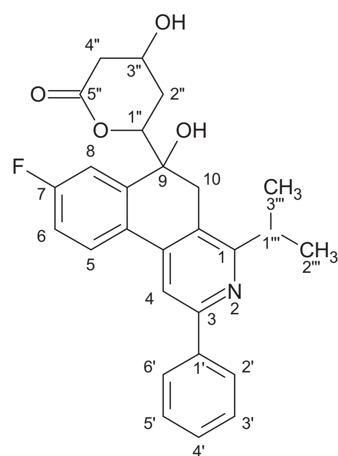
The isolated photoproducts GP-1 (t_R = 14.2 min) and GP-2 (t_R = 15.4 min) were analysed by ^{1}H NMR w CD_3OD and DMSO. The spectra were recorded using a Bruker 400 spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm) and are referred to a residual solvent peak. Coupling constants (J) are quoted in Hertz (Hz). The abbreviations s, d, t and m refer to singlet, doublet, triplet and multiplet, respectively – Table 3.

2.6. *Quantitative evaluation of photochemical changes*

The results obtained by both UV–Vis spectrophotometric and HPLC techniques were applied for quantitative evaluation of photochemical degradation of GV. Changes in the GV concentration during irradiation can be described by the following equation

$$\ln A = \ln A_0 - kt$$

Table 3
^{1}H NMR of photoproducts GP-1 and GP-2.



	GP-1 CD_3OD	GP-2 DMSO
	1H; (J)	
1	–	–
2	–	–
3	–	–
4	8.15 (s)	8.02 (s)
5	7.70 (m)	7.65; 7.73 (m)
6	7.20 (m)	7.19 (m)
7	–	–
8	7.20 (m)	7.19 (m)
9	–	–
10	–	–
1′	–	–
2′	8.25 (d; 7.2)	8.16 (d; 7.3)
3′	7.51 (t; 7.4)	7.50 (t; 7.4)
4′	7.43 (t; 7.2)	7.41 (t; 7.4)
5′	7.51 (t; 7.4)	7.50 (t; 7.4)
6′	8.25 (d; 7.2)	8.16 (d; 7.3)

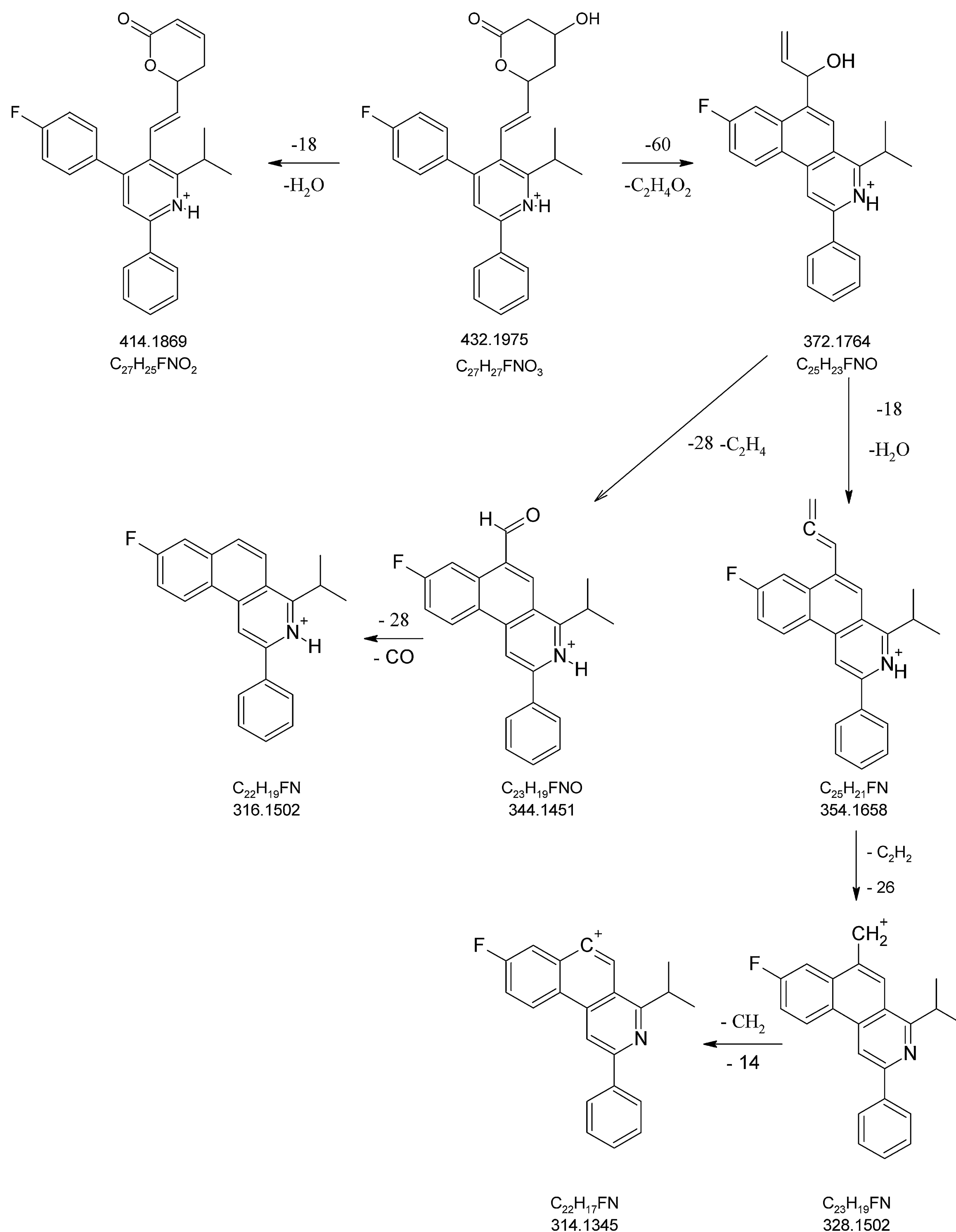


Fig. 4. The fragmentation pathway of glenvastatin (t_R = 12.0 min).

On the basis of this equation the kinetic parameters, i.e. the rate constant of photodegradation (k) and the half-life time ($t_{0.5}$) were determined.

Quantitative evaluation of photolysis included determination of quantum yield. The intensity of irradiation incident on the samples studied was determined by a physical actinometer VLX-3W type, Vilber Lourmat, with a sensor type CX-365. The measurements permitted calculation of the apparent quantum yields and by extrapolation to zero percent of substrate conversion the real quantum yield Φ was deduced.

The kinetic parameters and quantum yield of photodegradation are presented in Table 4.

2.7. HPLC–MS analysis

A methanol solution of GV was prepared and irradiated according to the conditions described in Section 2.2.

Isolation and identification of photoproducts was performed by the HPLC–MS method. The photoproducts were analysed by HPLC Agilent RR1200 SL instrument equipped with a photodiode array

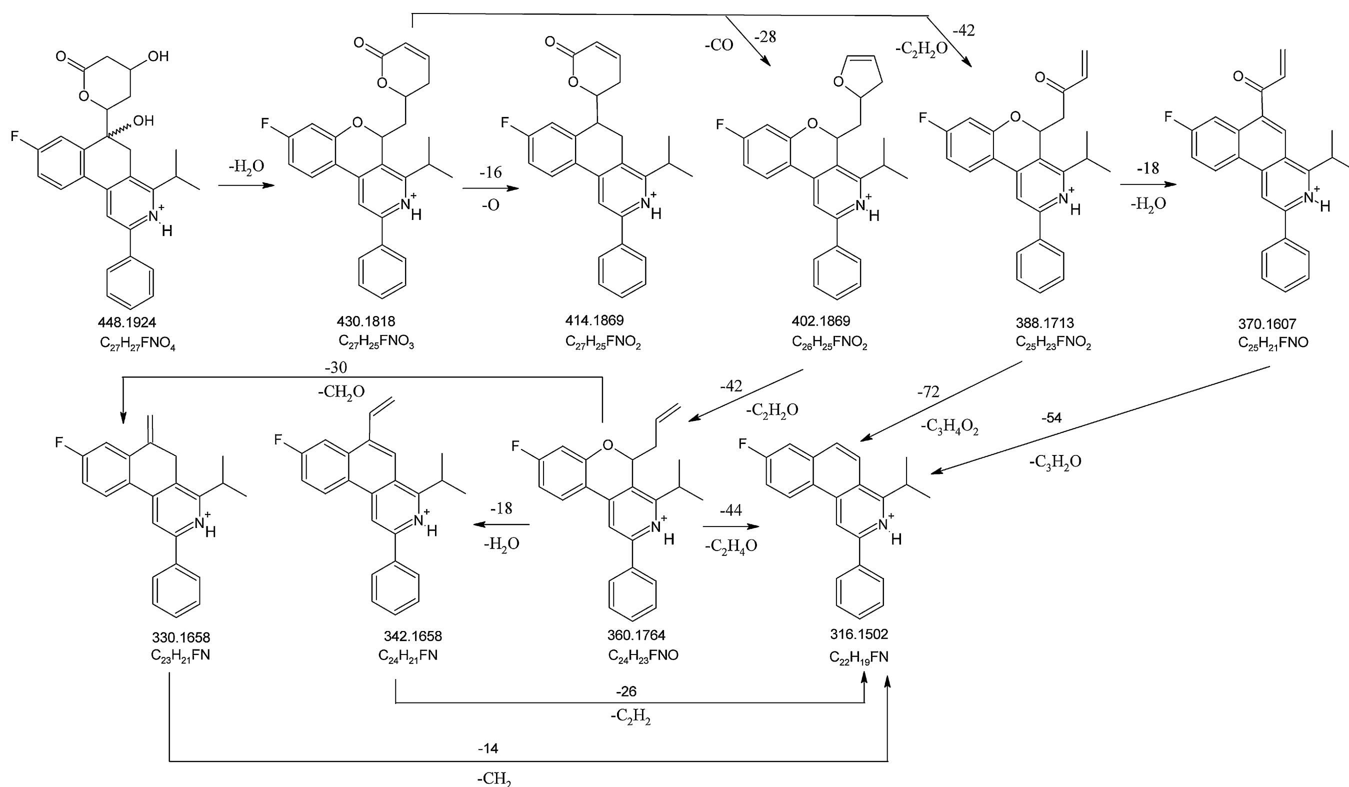


Fig. 5. The fragmentation pathway of photoproducts GP-1 and GP-2 (t_R = 14.2 min and 15.4 min).

detector and coupled to microTOF-Q spectrometer (Bruker Daltonics, Germany). An analytical column Zorbax Eclipse XDB-C18 (2 mm × 100 mm) was used with the elution gradient shown in Table 2.

The mass spectrometer used was equipped with ESI ion source operating at 4.5 kV with nebulization with N_2 at 1.6 bar at flow of 8.0 L/min and temperature 220 °C. MS/MS experiments were conducted using Ar as the collision gas and collision energy of 7 eV. The results are presented in Table 5.

3. Results and discussion

Medicaments showing photochemical reactivity in vitro can also show it in vivo, which can lead to serious side effects. It has been shown that sunlight can induce interactions between drug molecules and endogenic components thus converting the therapeutic substance into toxic photoproducts. Moreover, the interaction may involve formation of reactive forms of oxygen. Each of these processes can lead to a biological effect manifested as a phototoxic, photoallergic or photogenotoxic reaction.

HMG-CoA inhibitors have also been found to be potentially unstable when exposed to light. Bearing in mind high popularity of these lipid-lowering agents it is quite obvious that detailed photochemical data should be elaborated prior to the introduction of new drugs from this series to medical treatment.

For these reasons, the first step of our work was a qualitative evaluation of photodegradation of GV. Irradiation with light was found to cause explicit changes in the shape of the absorption spectrum. Fig. 2 shows that a red-shift (batochromic) effect was observed for bands near 206 and 254 nm. Simultaneously, the bands in the range of 243–283 nm were blue-shifted. Three isoabsorption points were observed (223, 243 and 283 nm).

As mentioned before, the photodegradation process was also evaluated by a HPLC technique. Optimization of the analysis conditions enabled a separation of GV from the light induced compounds. Two explicit photoproducts, eluting at 14.2 and 15.4 min, were generated as a result of GV illumination (Fig. 3). Their concentration was found to grow proportionally to the time of exposition. Results of our measurements permitted a quantitative estimation of the photodegradation process as well as the calculation of kinetic parameters and quantum efficiency ($\Phi = 10^{-4}$).

At the next stage of the study, the products were identified by HPLC–MS, using a gradient of the mobile phase. The need to use such conditions followed from considerable differences in polarity of the compounds formed upon irradiation. As the N atom of the GV pyridine ring can be easily protonated, electrospray ionisation (ESI) in the positive ion mode was applied in the mass spectrometer. The $[M+H]^+$ ion at m/z 432.2020 (432.1970 calculated for $C_{27}H_{27}FO_3N^+$) was observed and submitted to fragmentation in the MS/MS (Table 5). The proposed fragmentation pathways of glenvastatin are shown in Fig. 4.

The mass spectra of the photoproducts characterised by the retention times of 14.2 and 15.4 min, respectively, showed the molecular ions at the same m/z $[M+H]^+ = 448.1845$, which suggested formation of isomeric compounds, i.e. diastereoisomers. As

Table 5
HPLC–MS gradient conditions.

Sample	Retention time t_R (min)	Mass of $[M+H]^+$		λ_{max} (nm)
		Calculated (Da)	Measured (Da)	
GV	12.0	432.1970	432.2020	244
Photoproducts				
GP-1	14.2	448.1919	448.1909	260
GP-2	15.4	448.1919	448.1935	260

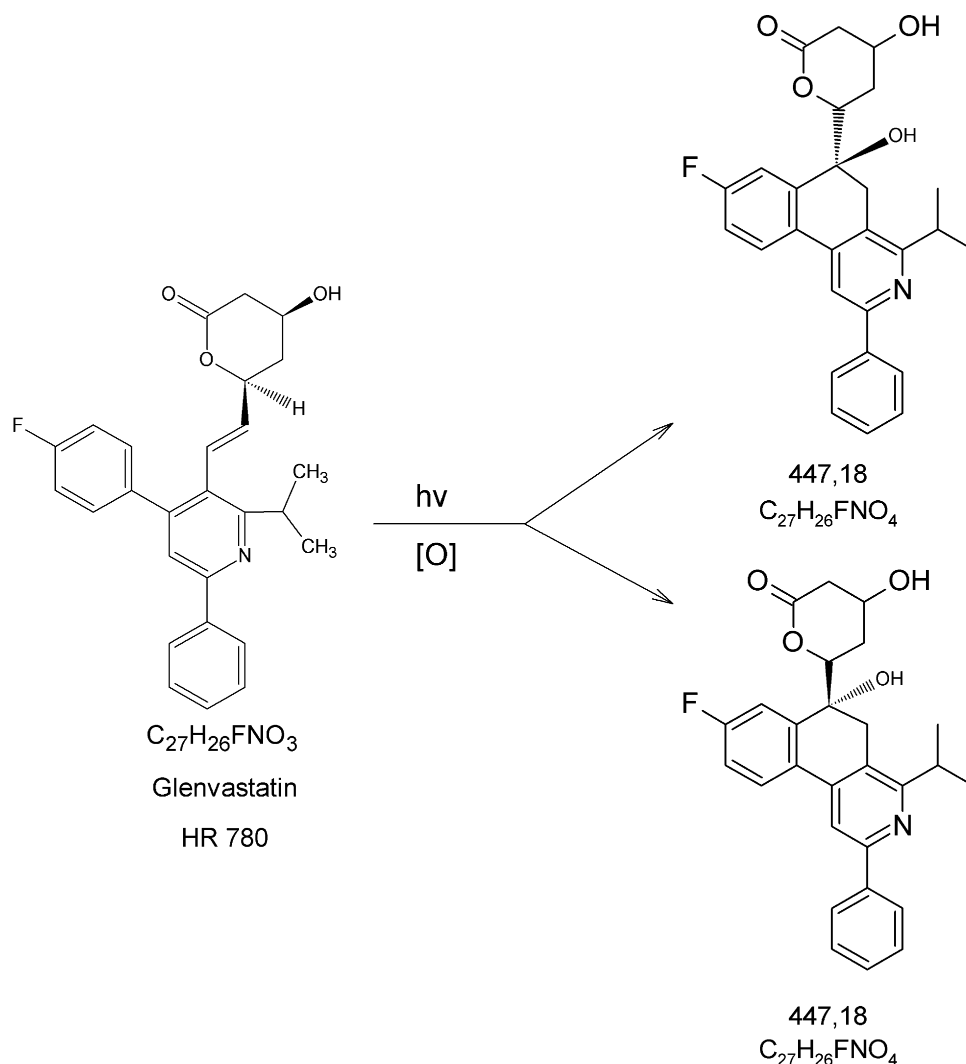


Fig. 6. Proposed chemical structure of the products of glenvastatin photodegradation.

a result of GV exposure to irradiation, the double bond in heptene chain is oxidised and then it undergoes a process of photocyclisation. The cyclisation leads to formation of compounds composed of three condensed rings.

Together with generation of a new ring, the photoproduct molecule gets another chiral centre following from the presence of an asymmetric carbon atom. In comparison with GV, having two centres of symmetry, the photoproduct has an additional third asymmetric carbon atom and has a possibility of diastereoisomers generation.

Literature provides examples of similar interpretations of the photodegradation pathway for other therapeutic substances from the group of statins. For example, the process of oxidation of the double bond and cyclisation of the heptene chain in the molecule of cerivastatin have been reported by Krol et al. [35]. The possibility of cyclisation upon irradiation of atorvastatin has been indicated by Cermola et al. [32]. The same authors have also reported that exposure of atorvastatin to UV irradiation induced photocyclisation leading to formation of a derivative of phenanthrene. The occurrence of photocyclisation accompanied by generation of isomeric diastereoisomers has been pointed out by Suzumura et al., who analysed phototransformation of fluvastatin in different conditions [36].

The proposed pathway of degradation of GV photoproducts are given in Fig. 5.

4. Conclusion

Analysis of the results obtained by mass spectrometry allowed us to conclude that the products of photochemical decomposition of glenvastatin are two diastereoisomers:

- (S) 6-(8-fluoro-6-hydroxy-2-phenyl-4-(propan-2-yl)-5,6-dihydrobenzo[*f*]isoquinolin-6-yl)-4-hydroxytetrahydro-2*H*-pyran-2-one *(GP-1)*.
- (R) 6-(8-fluoro-6-hydroxy-2-phenyl-4-(propan-2-yl)-5,6-dihydrobenzo[*f*]isoquinolin-6-yl)-4-hydroxytetrahydro-2*H*-pyran-2-one *(GP-2)*.

Analysis of the results of our study together against the hitherto knowledge on the photochemistry of statins has permitted us to propose the following scheme of photochemical decomposition of glenvastatin (Fig. 6).

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