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Rapid spectrophotometric method for quantitative determination of simvastatin and fluvastatin in human serum and pharmaceutical formulations

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A spectrophotometric method has been developed and applied to the determination of simvastatin and fluvastatin in human serum and in tablets. Simvastatine and fluvastatin were determined by measurement of their first derivative signals at 241.6, 245.9, 249.1 nm (for simvastatin) and 259.6 nm (for fluvastatin), respectively. Calibration curves were linear and the ranges of quantification were 12.0–28.0 μg · ml^{-1} for simvastatin and 10.0–28.0 μg · ml^{-1} for fluvastatin. The procedure was successfully applied to the determination of these compounds in pharmaceutical formulations as well as in human serum with a high percentage of recovery, good accuracy and precision, and without measurable interference by the excipients.

1. Introduction

Simvastatin, [1S-[1α,3α,7β,8β(2*S**,4*S**),8aβ]]-2,-2-dimethylbutanoic acid,1,2,3,7,8,8a- hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl)ethyl]-1-naphthalenyl ester and fluvastatin, (3*R**,5*S**,6*E*)-7-[3-(*p*-fluoro-phenyl)-1-isopropylindol-2-yl]-3,5-dihydroxy-6-heptenoate, are drugs used in the treatment of hypercholesterolemia. Simvastatin undergoes extensive first-pass extraction by the liver; less than 5% of an oral dose reaches the circulation as the active drug or metabolites. Simvastatin is administered in the inactive lactone form and must be converted to the β-hydroxy acid. More than 95% of compound and the respective β-hydroxy acid are bound to plasma proteins, and less than 13% of their metabolites are excreted in urine. Fluvastatin is also administered orally in the active carboxylic acid form. Absorption is nearly complete, and hepatic extraction is extensive. More than 98% of the circulating drug is protein-bound, and less than 5% of the drug or its metabolites are excreted in urine, while more than 90% is found in the feces [1].

Published spectrophotometric methods for the determination of simvastatin are limited in number. An UV spectrophotometric determination of simvastatin in pharmaceutical dosage forms [2, 3] has been reported. Literature on simvastatin shows that high performance liquid chromatography with spectrofluorimetric detection is the major analytical method used for its determination in human serum [4–6]. Literature on fluvastatin describes UV-visible spectrophotometric methods; however, three high performance liquid chromatographic methods [7–9] and capillary electrophoresis [10] have been reported. Some of the aforementioned methods are not suitable for routine analysis in clinical laboratories. Firstly, these methods require extensive pre-treatment of the biological samples. Secondly, expensive equipment is not readily available in many control laboratories. Therefore, the aim of the present investigation was to develop a simple, economical, accurate and reproducible analytical method with improved detection ranges for the estimation of simvastatin and fluvastatin in pure form, in solid dosage forms and in human serum. The new method should not require sample pre- treatment and tedious, extraction procedures.

2. Investigations, results and discussion

In a preliminary work the influence of 0.1 N HCl, 0.1 N NaOH and methanol on the absorption spectra of simvastatin and fluvastatin were studied. From the results, it is evident that the highest absorbances were obtained with methanol for both drugs. Stability of the solutions of simvastatin and fluvastatin, used for preparing the calibration curves in the proposed method, was ascertained by measuring changes in the absorbance at their respective analytical wavelengths over a period of 8 h.

In Figs. 1 and 2 the zero (a) and first-order derivative spectra (b) for the three different concentrations of simvastatin and fluvastatin in human serum in methanol are

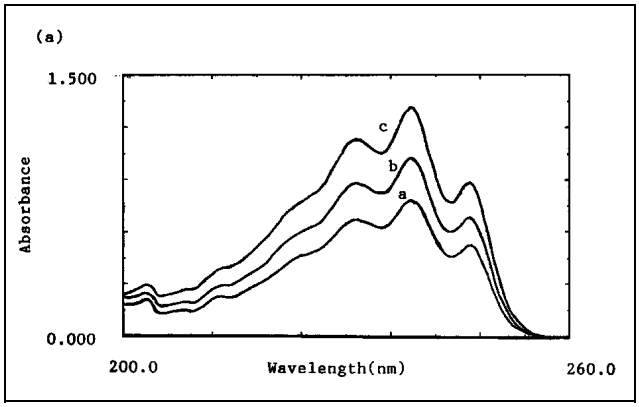

Fig. 1a: Absorption (zero-order) UV spectra of simvastatin in serum in methanol. The conentrations in derivative spectra are spectrum; a) 12.0 μg · ml^{-1}; b) 20.0 μg · ml^{-1}; and c) 28.0 μg · ml^{-1}

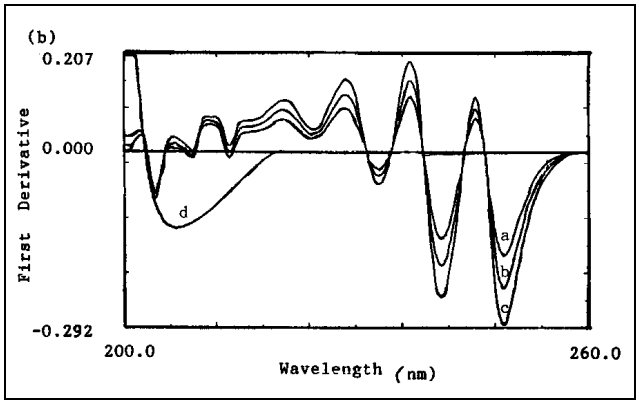

Fig. 1b: First derivative spectra of simvastatin in the serum in the presence of methanol. The concentrations in derivative spectra are spectrum a) 12.0 μg · ml^{-1}; b) 20.0 μg · ml^{-1}; c) 28.0 μg · ml^{-1} and d) blank serum with no addition of simvastatin

Table 1: Statistical analysis of calibration graph in the determination of simvastatin and fluvastatin

Parameters		Simvastatin		Fluvastatin
Wavelengths (nm)	241.6	245.9	249.1	259.6
Range ($\mu g \cdot ml^{-1}$)	12.0–28.0	12.0–28.0	12.0–28.0	10.0–28.0
Detection limits ($\mu g \cdot ml^{-1}$)		0.74		0.81
Regression equation (Y)[a]				
Slope (b)	-8.17×10^{-3}	2.92×10^{-3}	-9.87×10^{-3}	-4.17×10^{-3}
Std. dev. on slope (S_b)	5.18×10^{-5}	3.41×10^{-5}	8.23×10^{-5}	3.24×10^{-7}
Intercept (a)	8.90×10^{-3}	-5.00×10^{-4}	-7.30×10^{-3}	-6.48×10^{-3}
Std. dev. on intercept (S_a)	1.48×10^{-7}	3.72×10^{-5}	2.98×10^{-5}	8.92×10^{-6}
Std. error of estimation (S_e)	7.91×10^{-8}	1.84×10^{-5}	8.62×10^{-5}	6.73×10^{-5}
Correlation coefficient (r)	0.9998	0.9999	0.9989	0.9954
Rel. std. dev. (%)[b]	1.81	0.91	2.04	1.67
% Range of error[b] (% 95 confidence limit)	1.65	0.83	0.74	1.38

[a] $Y = a + bC$ where C is concentration in $\mu g \cdot ml^{-1}$ and Y in absorbance units. [b] Five replicate samples

shown, respectively. For both the determination of simvastatin and fluvastatin spiked to human serum and in dosage forms, the peak-zero amplitudes in the first derivative spectra at 241.6, 245.9, 249.1 nm (for simvastatin) and 259.6 nm (for fluvastatin), were used. At these wavelenghts the background absorption of the serum was also minimized.

The slopes of the calibration curves for each drug were virtually independent of the presence or absence of human serum. Therefore, the amplitudes of peak-zero in the first derivative spectra were used to measure simvastatin and fluvastatin concentrations. The linearity of the calibration graphs, the adherence of the system to Beer's law and the negligible scatter of the experimental points were validated by the values of the correlation coefficients of the regression equations and the values of the intercepts, which were close to zero (Table 1). The correlation coefficients were found to be 0.9998, 0.9999, 0.9989 (for simvastatin) and 0.9954 (for fluvastatin). A wavelength of 245.9 nm was selected for the determination of simvastatin in human serum and pharmaceutical formulations. Tables 2 and 3 show the results obtained in the analysis of serum samples. The accuracy of measurements, expressed in terms of relative errors was 2.0% or less, thus indicating negligible influence of serum proteins.

The selectivity of the developed method for the estimation of the drugs in presence of various tablet and capsules excipients such as starch, lactose, talc and magnesium strearate was investigated. A placebo formulation comprising 10% starch, 40% lactose, 2% talc and 1% magnesium strearate was prepared. A 1 : 1 mixture of drug and placebo was prepared. The absorbances of the solutions were measured at 241.6, 245.9, 249.1 and 259.6 nm in the first-order derivative spectra. The results indicate that the excipients do not interfere with simvastatin and fluvastatin determinations. According to the Analytical Methods Committee [11], the detection limit (LOD) is the concentration of concentration of drug corresponding to a signal equal

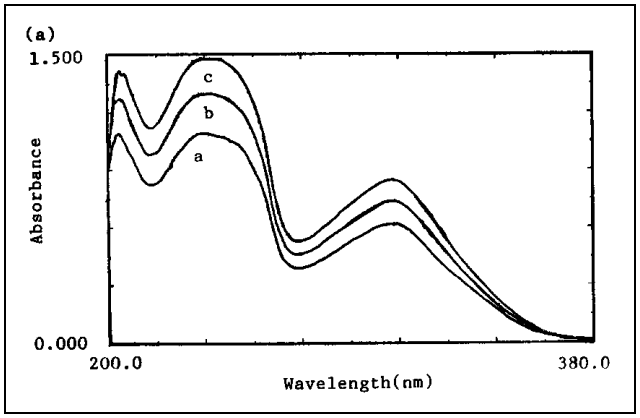

Fig. 2a: Absorption (zero-order) UV spectra of fluvastatin in serum in methanol. The concentrations in derivative spectra are spectrum; a) 10.0 $\mu g \cdot ml^{-1}$; b) 19.0 $\mu g \cdot ml^{-1}$; and c) 28.0 $\mu g \cdot ml^{-1}$

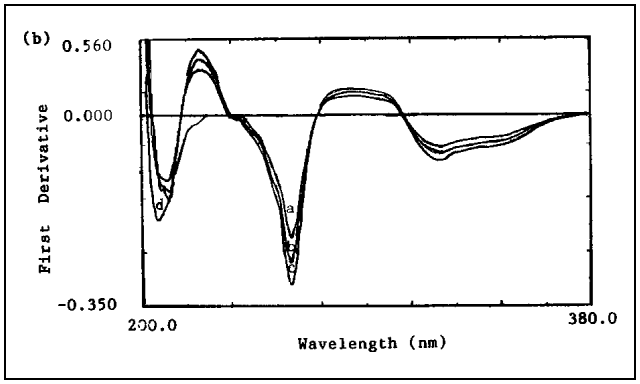

Fig. 2b: First derivative spectra of fluvastatin in the serum in the presence of methanol. The concentrations in derivative spectra are spectrum a) 10.0 $\mu g \cdot ml^{-1}$; b) 19.0 $\mu g \cdot ml^{-1}$; c) 28.0 $\mu g \cdot ml^{-1}$ and d) blank serum with no addition of fluvastatin

Table 2: Results obtained in determination of simvastatin in serum samples (n = 5)

Drug concentration ($\mu g \cdot ml^{-1}$)	Recovery (%) ± RSD[a]	RE (%)[a]
12.0	102.5 ± 0.47	1.26
16.0	97.8 ± 0.51	0.78
20.0	101.8 ± 0.42	0.99
24.0	99.6 ± 1.25	1.83
28.0	98.9 ± 1.80	2.01

[a] Relative error (RE) and relative standard deviation (RSD) in five determinations.

Table 3: Results obtained in determination of fluvastatin in serum samples (n = 5)

Drug concentration ($\mu g \cdot ml^{-1}$)	Recovery (%) ± RSD	RE (%)[a]
10.0	98.6 ± 1.03	0.84
20.0	99.4 ± 0.76	0.58
30.0	99.3 ± 1.11	0.70
40.0	100.4 ± 0.95	1.39
50.0	99.1 ± 0.67	0.63

[a] Relative error (RE) and relative standard deviation (RSD) in five determinations.

Table 4: Results obtained in determination of simvastatin in pharmaceutical dosage forms

Pharmaceutical	Amount of label claim (mg/film tablet)	Mean recovery ± SD % (n = 5)
Batch no 1	20.0	101.5 ± 1.25
Batch no 2	20.0	99.8 ± 0.62
Batch no 3	20.0	100.4 ± 1.68

Table 5: Results obtained in determination of fluvastatin in pharmaceutical dosage forms

Pharmaceutical	Amount of label claim (mg/capsules)	Mean recovery ± SD % (n = 5)
Batch no 1	40.0	98.5 ± 0.98
Batch no 2	40.0	100.1 ± 1.36
Batch no 3	40.0	99.4 ± 1.21

to the blank mean (Y_b) plus three times the standard deviation of the blank (S_b). Eleven blank measurements gave an average signal blank $Y_b = 0.35$ and a standard deviation $S_b = 0.13$ for the simvastatin blank, $Y_b = 0.09$ and a standard deviation $S_b = 0.24$ for the fluvastatin blank. Thus, the analytical signal corresponding to LOD is $Y_b + 3S_b$. This value is transformed in to LOD through the equations of the calibration lines, giving 0.74 $\mu g \cdot ml^{-1}$ for simvastatin and 0.81 $\mu g \cdot ml^{-1}$ for fluvastatin (Table 1). From the calibration curves it is also possible to estimate the quantitation limits as the concentration corresponding to the ratio between three times the S.D. of the intercept and the slope of the calibration line [12]. This leads to a quantitation limit of 1.48 $\mu g \cdot ml^{-1}$ for simvastatin and 1.03 $\mu g \cdot ml^{-1}$ for fluvastatin.

The developed method was applied to the recovery of simvastatin and fluvastatin in three batches of commercial formulations. The results presented in Tables 4 and 5 are in good agreement with the declared content. All data represent the average of five determinations. The low relative standard deviations indicate very good reproducibility of the measurement.

The proposed procedure was successfully applied to the determination of the studied compounds in human serum and pharmaceutical dosage forms. The first derivative spectrophotometric method is a new, simple and accurate procedure requiring inexpensive reagents that can be used for rapid and reliable clinical studies of simvastatin and fluvastatin.

3. Experimental

3.1. Apparatus

A double beam Shimadzu 1601 spectrophotometer model with a fixed slit width (2 nm) connected to an IBM-PC computer was used for all the absorbance measurements and data analysis.

3.2. Reagents and materials

Simvastatin substance and Zocor® film tablets (20.0 mg of simvastatin) were from Merck Sharp and Dohme, and fluvastatin substance and Lescol® capsules (40.0 mg of fluvastatin) were from Novartis Pharm.Ind., Turkey. Methanol was of analytical reagent grade (Merck).

3.3. Calibration graph and procedure for tablets and capsules

Stock solutions of 1.0 $\mu g \cdot ml^{-1}$ of simvastatin were prepared in methanol, respectively. The concentration range were 12.0–28.0 $\mu g \cdot ml^{-1}$ for simvastatin and 10.0–28.0 $\mu g \cdot ml^{-1}$ for fluvastatin, respectively. The first-order derivative spectra were recorded against methanol as a reagent blank. Fresh stock standard solutions were prepared daily.

Twenty film tablets or the contents of 20 capsules were accurately weighed and powdered in a mortar. An accurately weighed amount equivalent to one film tablet or the content of one capsule was dissolved in methanol in a 100 ml calibrated flask. After 30 min of mechanical shaking, the solution was filtered in a 100 ml calibrated flask through Whatman No 42 filter paper. The residue was washed three times with 10 ml of solvent and then the volume was completed to 100 ml with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrates and diluting in methanol.

3.4. Calibration graph and procedure for serum samples

A serum pool was used for calibration, consisting of sera from 5 different persons. Blood was centrifuged and the serum samples were frozen until assayed. The calibration set was designed with 5 diluted serum pool samples. The known amounts of simvastatin and fluvastatin and ethanol (2 ml, 96 wt%) were added to drug free human serum. Thus, addition of ethanol prevents simvastatin and fluvastatin binding to proteins and coagulate serum proteins. The mixtures were vortexed for 10 min. After deproteinization and centrifugation of samples for 20 min at 5000 rpm, supernatants (1 ml) were separated. The concentration range thus covered were 12.0–28.0 $\mu g \cdot ml^{-1}$ for simvastatin and 10.0–28.0 $\mu g \cdot ml^{-1}$ for fluvastatin. The first-order derivative spectra of the prepared solutions were taken against the serum blank, prepared as described above but without addition of the drugs. No anticoagulant was used in this study.

3.5. Recovery studies in human serum samples

Five different concentrations of simvastatin and fluvastatin were added to human serum, giving concentrations of 12.0–28.0 $\mu g \cdot ml^{-1}$ and 10.0–28.0 $\mu g \cdot ml^{-1}$, respectively. These serum samples were treated in the same manner as for the calibration curve.

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