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Determination of the antihyperlipidemic simvastatin by various voltammetric techniques in tablets and serum samples

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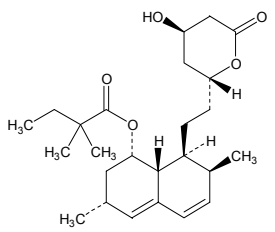
The electrochemical behavior and determination of simvastatin (SMV), a lipid-lowering drug, were studied in aqueous alcohol medium at a stationary glassy carbon electrode. Cyclic voltammetry studies showed one main, well-defined, sharp oxidation peak between pH 2 and 8. The oxidation was irreversible and exhibited a diffusion controlled mechanism. Differential pulse and square wave voltammetric methods for the quantitative determination of SMV in pharmaceutical dosage forms and spiked serum samples were developed based on the linear relationship between the peak current and the concentration. Differential pulse and square wave voltammetric techniques for the determination of SMV in 0.1 M H_2SO_4 and a constant amount of methanol (20%), which allow quantitation over the 2×10^{-6}–1×10^{-4} M range in supporting electrolyte with a detection limit of 2.71×10^{-7} M and 5.50×10^{-7} M for differential pulse and square wave voltammetric methods, respectively, are proposed. The repeatability and reproducibility of the methods were determined. Precision and accuracy were also checked. These methods were used for the determination of SMV in tablets. The standard addition method was used in biological media. No electroactive interferences from endogenous substances and excipients were found in biological fluids and pharmaceutical dosage forms, respectively.

1. Introduction

Simvastatin (SMV) is a lipid-lowering agent that is derived synthetically from a fermentation product of *Aspergillus terreus*. After oral ingestion, SMV, which is an inactive lactone, is hydrolyzed to the corresponding (beta)-hydroxyacid form. This is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol. SMV has been shown to reduce both normal and elevated LDL-C concentrations. SMV is a specific inhibitor of HMG-CoA reductase enzyme (PDR 2003; Goodman 1996).

SMV has been studied and determined by only a few analytical procedures: UV spectrophotometry (Wang 2000; Carlucci 1992a), liquid chromatography with UV detection (Carlucci 1992a, 1992b; Kim 2004), with fluorimetric detection (Ochiai 1997) and with mass spectrometry (Miao 2003; Yang 2003), and GC-MS (Takano 1990). The methods reported were influenced by interference from endogenous substances and potential loss of drug in the re-extraction procedure, involved lengthy, tedious and time-consuming plasma sample preparation and extraction processes and required sophisticated and expensive instrumentation.



Nothing has been written concerning its electrochemical oxidation behavior and analytical assay in pharmaceuticals or biological media using voltammetric techniques.

The widespread use of this compound and the need for clinical and pharmacological studies require fast and sensitive analytical techniques to determine the presence of the drug in various biological fluids. Electroanalytical techniques, especially modern pulse techniques, such as differential pulse and square wave voltammetry, have been used for the sensitive determination of a wide range of pharmaceuticals (Wang 1988; Kissinger 1996; Uslu 2002; Ozkan 2003; Uslu 2004) with the advantages that there is, in most instances, no need for derivatization, and that these methods are less sensitive to matrix effects than other analytical techniques. Electroanalytical techniques have been shown to be excellent for the determination of pharmaceutical compounds in different matrices. The selectivity of this method is normally excellent because the analyte can be readily identified by its voltammetric peak potential. The use of carbon based electrodes, especially glassy carbon electrode, for electroanalytical measurements has increased in recent years because of their applicability to the

determination of active compounds that undergo oxidation reactions, a matter of great importance in the field of clinical and pharmaceutical analysis.
The main objectives of this work is to establish the experimental conditions for investigating the oxidation behavior of SMV, and to optimize the conditions for determination of this compound in pharmaceutical dosage forms and human serum samples using cyclic, linear sweep, differential pulse (DPV) and square wave (SWV) voltammetric techniques.

2. Investigations, results and discussion

No previous electrochemical data were available concerning the electrode behavior of SMV. Therefore, several measurements with different electrochemical techniques (cyclic, linear sweep, differential pulse and square wave voltammetry) were performed using various supporting electrolytes, buffers and different pH values in order to obtain such information. SMV was electrochemically oxidized in a broad pH range (1.5–11.06) using a glassy carbon disc electrode with one main and sharp peak or wave depending on the pH value investigated. The cyclic voltammetric behavior of SMV yielded one well-defined peak in strongly acidic media such as sulphuric acid and buffer solutions at pH <8. (Fig. 1a–c). Cyclic voltammetric measurements showed the irreversible nature of the oxidation process (Fig. 1a–d). As the pH increased, this main peak became a wave and it became an ill-defined wave above pH 9 (Fig. 1d). When scanning was started at −0.30 V in the positive direction in 0.1 M H_2SO_4, anodic oxidation of SMV did not occur until about +1.18 V. On reversing at +1.80 V no reduction wave or peak corresponding to the anodic wave was observed on the cathodic branch. It was observed that in the second cycle the SMV peak or wave decreased (Fig. 1a–d). This phenomenon may be partly attributed to the consumption of adsorbed SMV on the electrode surface.
The variation of peak intensity and peak potential with pH for a 2×10^{-4} M SMV solution were studied by cyclic, DPV and SWV techniques between pH 1.5 and 11.06. The peak potential versus pH plots with cyclic voltammetry were similar to those obtained by DPV and SWV. For this reason, only cyclic voltammetric data are given in Fig. 2a and 2b. The plot Ep versus pH showed one straight line between pH 2 and 5 (Fig. 2a), which can be expressed by the following equation in cyclic voltammetry:

$$\mathrm{Ep\,(mV)} = 1267.8 - 17.59\,\mathrm{pH} \qquad r = 0.996 \qquad (1)$$

and by the following equation in differential pulse voltammetry:

$$\mathrm{Ep\,(mV)} = 1191.2 - 15.65\,\mathrm{pH} \qquad r = 0.948 \qquad (2)$$

and by the following equation in square wave voltammetry:

$$\mathrm{Ep\,(mV)} = 1230.7 - 13.82\,\mathrm{pH} \qquad r = 0.997 \qquad (3)$$

As can be seen from Fig. 2a, the peak potential becomes nearly pH independent above pH 5.0, as the intersection of the curves is located at around pH 5.0. This can be explained by changes in protonation of acid-base functions in the molecule. The effect of solution pH on the peak enhancement is also shown in Fig. 2b. The experimental results show that the shapes of the curves were better in 0.1 M H_2SO_4. This supporting electrolyte was chosen with regard to sharp response and better peak shape for the calibration curve for pharmaceutical dosage forms and serum samples.
The effect of the potential scan rate between 5 and 1000 mVs^{-1} on the peak current and potential of SMV were evaluated. A 158 mV positive shift in the peak potential confirmed the irreversibility of the oxidation pro-

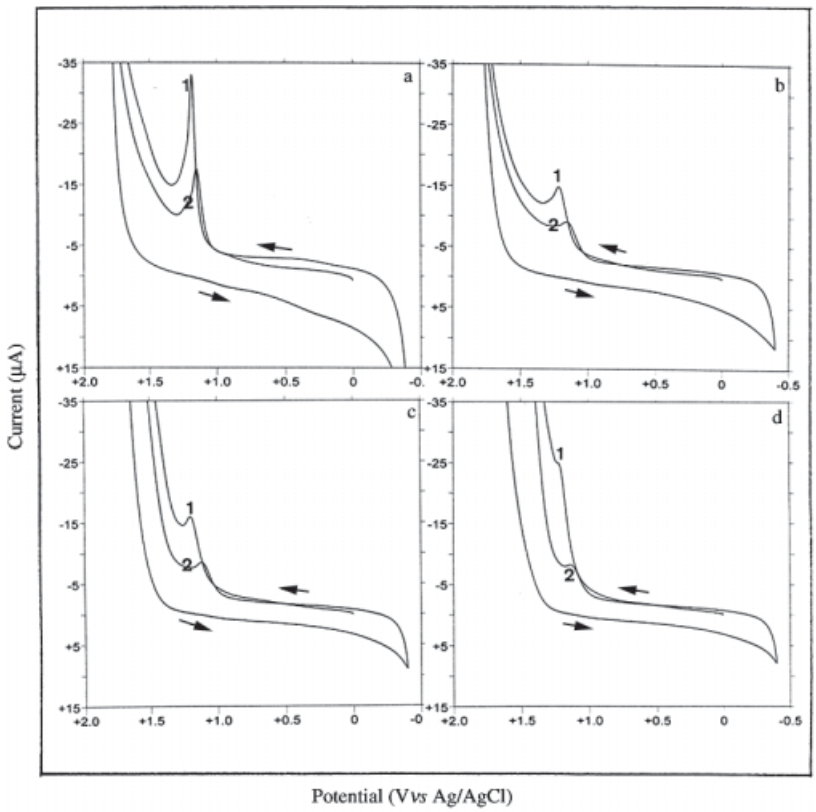


Fig. 1:
Multi sweep cyclic voltammograms of 2×10^{-4} M SMV solutions in 0.1 M H_2SO_4 (a); in Britton-Robinson buffer at pH 2.99 (b); in Britton-Robinson buffer at pH 7.03 (c);. in Britton-Robinson buffer at pH 9.07 (d); scan rate 100 mVs^{-1}; (1) first scan; (2) second scan

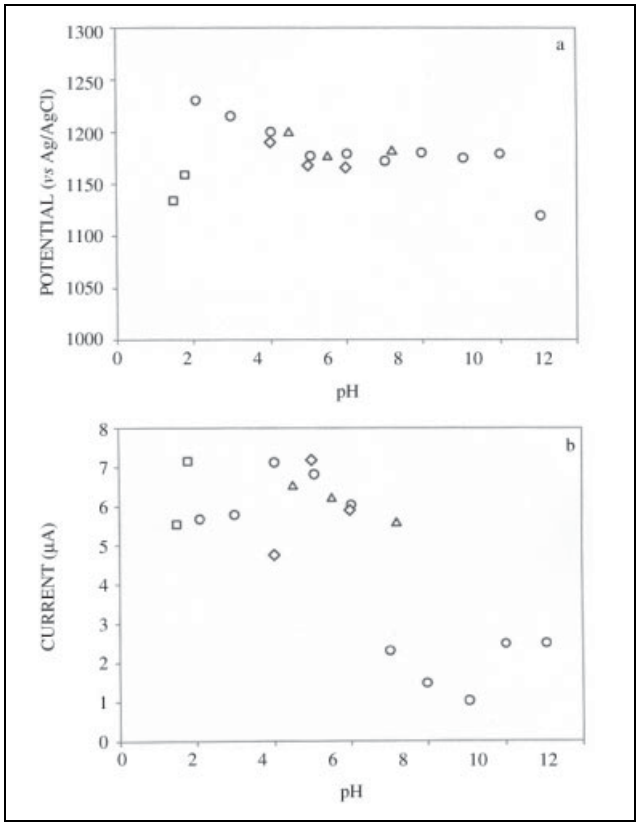


Fig. 2: Effect of pH on SMV peak potential (a) and peak current (b); SMV concentration 6×10^{-5} M. (□) 0.1 M H_2SO_4; (○) Britton–Robinson; (△) phosphate and (◇) acetate buffers

cess. Scan rate studies were carried out to assess whether the processes at the glassy carbon electrode was under diffusion or adsorption control. When the scan rate was varied from 5 to 1000 mVs^{-1} in 1×10^{-4} M solution of SMV in the presence of 20% methanol, linear dependence of peak intensity ip (μA) on the square root of the scan rate $\nu^{1/2}$ (mVs^{-1}) was found, demonstrating diffusional behavior. The equation is given below for 0.1 M H_2SO_4:

$$\text{ip}\ (\mu\text{A}) = 1.41\nu^{1/2}\ (\text{mVs}^{-1}) - 3.34$$
$$\text{r} = 0.994\ (\text{n} = 10) \qquad (4)$$

A plot of logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.64, close to the theoretical value of 0.5, that was the expected value for a process controlled by complex-diffusion (Laviron 1980). The equation obtained is:

$$\log \text{ip}\ (\mu\text{A}) = 0.64 \log \nu\ (\text{mVs}^{-1}) - 0.30$$
$$\text{r} = 0.997\ (\text{n} = 10) \qquad (5)$$

The Tafel plot (log i vs. E) was obtained with a scan rate of 5 mVs^{-1} beginning from a steady-state potential in 0.1 M H_2SO_4. The ($\alpha_a n$) value of the anodic reaction corresponding to the voltammetric oxidation peak was obtained as 0.41 using the Tafel plot. The exchange current density (i_o) is 1.48×10^{-13} A/cm^2 for this system. These values together with the absence of cathodic waves in cyclic voltammetry (Fig. 1a–d) indicated the irreversibility of the oxidation reaction.

When logarithm of current (log i) for the curves obtained in 0.1 M H_2SO_4, having a SMV concentration range of 2×10^{-6}–1×10^{-4} M, was plotted against logarithm of concentration (log C), a linear relationship was obtained as follows;

$$\log \text{i}\ (\mu\text{A}) = 0.41 \log \text{C}\ (\text{M}) + 2.375 \qquad (\text{r} = 0.998) \quad (6)$$

The slope of this equation gives the reaction order. These kinetic parameters and reaction order showed that the mechanism is related to surface events and the reaction seems to be first order.

2.1. Analytical applications and validation of the proposed method

Various electrolytes, such as sulphuric acid and Britton-Robinson, acetate and phosphate buffer were examined. The best results with respect to signal enhancement and peak shape accompanied by a sharper response were obtained with 0.1 M H_2SO_4. This supporting electrolyte was chosen for the subsequent experiments. In order to develop a voltammetric procedure for determination of the drug, we selected the DPV and SWV techniques, since the peaks were sharper and better-defined at lower concentrations of SMV than those obtained by cyclic and linear sweep voltammetry with a lower background current, resulting in improved resolution. DPV and SWV are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background currents and low detection limits (Kissinger 1996; Wang 1988). Two calibration graphs were constructed with the standard solution of SMV according to the procedures described above using DPV and SWV. A linear relationship was found in the concentration range

Table 1: Regression data of the calibration lines for quantitative determination of simvastatin in 0.1 M H_2SO_4 (20% methanol) using DPV and SWV

	0.1 M H_2SO_4	
	DPV	SWV
Working electrode potential (V) (vs. Ag/AgCl)	1.095	1.12
Linearity range (M)	2×10^{-6}–1×10^{-4}	2×10^{-6}–1×10^{-4}
Number of data points	10	10
Slope (μAM^{-1})	5.41×10^{4}	4.95×10^{4}
Intercept (μA)	−0.151	0.0294
Correlation coefficient	0.999	0.999
RSD% of slope	1.80	1.55
RSD% of intercept	0.88	0.33
LOD	2.71×10^{-7}	5.50×10^{-7}
LOQ	9.02×10^{-7}	1.83×10^{-6}
Repeatability of peak potential (RSD%)	0.38	0.43
Reproducibility of peak potential (RSD%)	0.52	0.58

Table 2: Intra-day and inter-day precision of SMV standards

Theoretical concentration (M)	DPV				SWV			
	Within-day measured concentration (M)*		Between-day measured concentration (M)**		Within-day measured concentration (M)*		Between-day measured concentration (M)**	
	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%
6×10^{-6}	5.99×10^{-6}	0.33	6.00×10^{-6}	0.80	6.02×10^{-6}	0.76	6.08×10^{-6}	1.16
1×10^{-5}	9.99×10^{-6}	0.28	9.98×10^{-6}	0.92	1.00×10^{-5}	0.49	1.01×10^{-5}	1.02
6×10^{-5}	5.97×10^{-5}	0.62	5.96×10^{-5}	0.88	6.00×10^{-5}	0.51	5.99×10^{-5}	0.82

* Mean values represent five different SMV standards for each concentration
** Between-day reproducibility was determined from five different runs over a 2-weeks period

between 2×10^{-6} and 1×10^{-4} M, indicating that the response was diffusion controlled in this range. The correlation coefficient was found to be always greater than 0.999 for both methods. The characteristics of the calibration plots are summarized in Table 1.

The limit of detection (LOD) and the limit of quantification (LOQ) were calculated on the peak current using the following equations:

$$\mathrm{LOD} = 3\,\mathrm{s/m} \quad (7)$$

$$\mathrm{LOQ} = 10\,\mathrm{s/m} \quad (8)$$

Where s is the standard deviation of the peak currents (three runs) and m is the slope of the calibration curve. The LOD and LOQ values are also shown in Table 1.

Performing replicate analyses of the standard solutions assessed the accuracy, precision and reproducibility of the proposed methods. Three different concentrations (low, medium and high) within the calibration range were prepared in supporting electrolytes and analyzed with the relevant calibration curves to determine intra-day and inter-day variability. The intra and inter-day precision were determined as the RSD%. Results shown in Table 2 demonstrate good precision, accuracy and reproducibility.

Stability studies of SMV in supporting electrolyte indicated no significant changes in sample concentrations on storage of samples over a one-week period at 4 °C in a refrigerator.

2.2. Determination of SMV in tablets

On the basis of the above results, the proposed DPV and SWV methods were applied to the direct determination of SMV contents in marketed products using the relevant calibration straight lines without any sample extraction or filtration and after adequate dilution. The results show that the proposed methods were successfully applied to the assay of SMV in its pharmaceutical dosage forms (Table 3). The assay showed the drug content of this product to be in accordance with the labeled claim.

In order to check the accuracy and precision of the developed method and to prove the absence of interferences by excipients, recovery studies were carried out using the standard addition technique. The accuracy of the method was determined by the recovery of drug during spiked experiments. Recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulations of SMV. According to the results, excipients present in tablets do not interfere with the analysis (Table 3).

SMV was recently included in the United States Pharmacopoeia (USP 24, 2000). SMV tablets were also determined with the official procedure, which involves a HPLC method (USP 24, 2000). Table 3 compares the results of the analysis of SMV between the proposed and official methods. All methods showed similar accuracy and precision. Using Student's t-test and the F test, the calculated t and F values did not exceed the theoretical values for a significance level of 0.05. Statistical analysis of the results showed no significant difference between the performance of the official and proposed methods as regards to accuracy and precision. On the other hand, the voltammetric assay is simple, rapid and does not require time consuming sample preparation steps compared with the HPLC assay. In comparison to the HPLC method, the proposed techniques were faster and simpler. No sample treatment steps were needed for the proposed methods. It is evident that the proposed method is as sensitive as the HPLC assay.

The recovery and comparison with HPLC method results demonstrate the validity of the proposed method for the determination of SMV in tablets. These results reveal that both methods had adequate precision and accuracy and consequently can be applied to the determination of SMV in pharmaceuticals without any interference from the excipients.

Table 3: Results from commercial tablet dosage forms and mean recoveries obtained for five determinations of simvastatin in spiked Zocor tablets

	Official HPLC method (USP 24)	DPV	SWV
Labeled claim (mg)	20.0	20.0	20.0
Amount found* (mg)	19.89	19.92	19.88
RSD%	0.95	1.02	1.12
Bias%	0.55	0.40	0.60
Added (mg)	–	5.00	5.00
Found (mg)	–	4.98	4.95
Recovered*	–	99.56	99.08
RSD% of recovery	–	1.06	0.91
Bias%	–	0.40	1.00
$t_{calculated}$	$t_{theoretical}$: 2.31 (p: 0.05)	0.23	0.09
$F_{calculated}$	$F_{theoretical}$: 2.60 (p: 0.05)	0.89	0.75

* Each value is the mean of five experiments

2.3. Determination of SMV in spiked biological samples

Acetonitrile and methanol were tried as serum precipitating agents, and different amounts of acetonitrile were also tried. The best results were obtained with 0.7 mL acetonitrile. The measurements of SMV in serum samples were performed as described in section 3.

In order to check the applicability of the method to biological materials, recovery studies were performed in serum. Serum samples were spiked with SMV to achieve

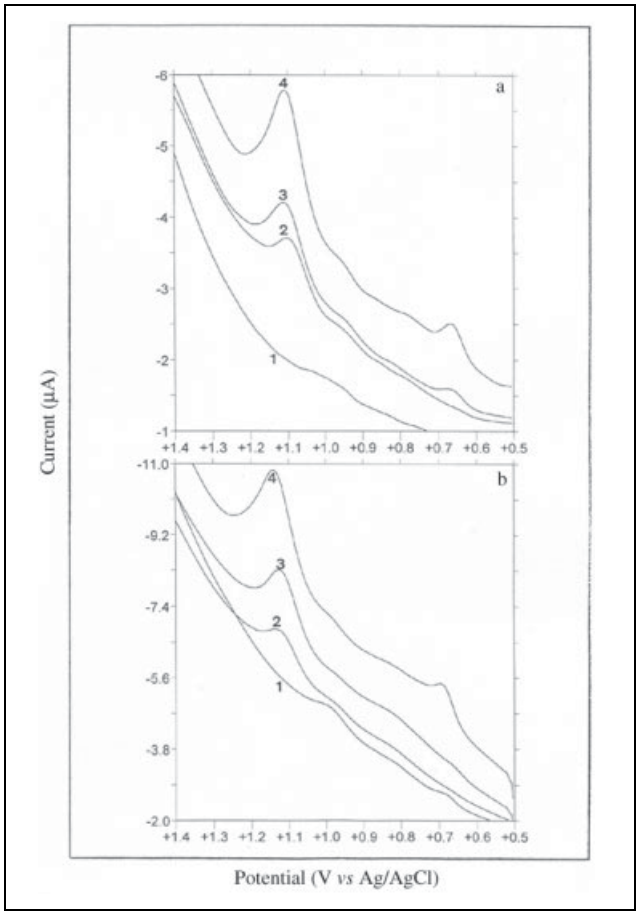


Fig. 3: Differential pulse (a) and square wave (b) voltammograms obtained for the determination of SMV in spiked human serum samples (1) blank serum extract; (2) extract containing 1×10^{-5} M; (3) extract containing 2×10^{-5} M; (4) extract containing 4×10^{-5} M

final concentrations of 1×10^{-5} M, 2×10^{-5} M and 4×10^{-5} M. The amount of SMV in human serum was calculated from relevant linear regression equations. Typical DPV and SWV curves for SMV investigated in serum are shown in Figs. 3a and 3b, respectively. The determination results and recoveries of known amounts of SMV added to the serum samples were found for both techniques to be 99.0% with relative standard deviation values between 0.57 and 1.06%. Good recoveries of SMV were achieved from serum samples with both methods. The results obtained for recovery of spiked serum samples are given in Table 4. Analysis of drugs from biological samples usually requires extensive time-consuming sample preparation, use of expensive organic solvents and other chemicals. In this study the serum proteins and endogenous substances in serum and urine samples were precipitated by the addition of acetonitrile, which was centrifuged at $5000 \times g$, and the supernatant was taken and diluted with the supporting electrolyte and directly analyzed. Using both the proposed techniques, no sample pretreatment was required, other than the precipitation and dilution steps. As can be seen in Figs. 3a and 3b, no oxidation compounds and no extra noise peaks from the presence of biological material occurred in the potential range where the analytical peak appeared. The stability of serum samples kept in a refrigerator (+4 °C) was tested by making five consecutive analyses of the sample over a period of approximately 5 h. There were no significant changes in peak currents and potentials between the first and last measurements.

3. Experimental

3.1. Apparatus

The cyclic, linear sweep, DPV and SWV studies at a stationary electrode were performed using a BAS 100 W Electrochemical analyzer. A three-electrode cell system incorporating a glassy carbon disc electrode as working electrode, a Ag/AgCl (3 M KCl) reference electrode and a platinum-wire auxiliary electrode were also used. Before each experiment the glassy carbon electrode was polished manually with alumina slurry ($\varphi = 0.01$ μm) in the presence of bi-distilled water on a smooth polishing cloth. DPV conditions were: pulse amplitude, 50 mV; pulse width 50 ms; scan rate, 20 mVs^{-1} and SWV conditions were: pulse amplitude, 25 mV; frequency, 15 Hz; potential step 4 mV.
HPLC experiments were carried out on a Waters liquid chromatograph (Model 510) equipped with a UV detector (Model 481). The chromatograms were analysed with a chromatographic workstation (Baseline 810).

3.2. Reagents

Simvastatin (SMV) and its pharmaceutical dosage forms were kindly provided by Merck Sharp Dohme Pharm. Ind. (Istanbul, Turkey).
SMV stock solutions were prepared daily by direct dissolution in methanol. The solutions under voltammetric investigation were prepared by dilution of the stock solution in the presence of methanol (20%). 0.1 M H_2SO_4, 0.2 M phosphate buffer at pH 4.5–7.21, 0.04 M Britton–Robinson buffer at pH 2.06–11.04 and 1 M acetate buffer at pH 3.5–5.7 were used for the supporting electrolytes.
All solutions were protected from light and were used within 24 h in order to avoid decomposition.
Standard solutions were prepared by dilution of the stock solution with the selected supporting electrolyte to give solutions containing SMV in the concentration range of 2×10^{-6}–1×10^{-4} M. The calibration curve for DPV and SWV analysis was constructed by plotting the peak current against the SMV concentration.
The ruggedness and precision were checked on different days, within day ($n = 5$), and between days ($n = 5$) for three different concentrations. Relative standard deviations were calculated to check the ruggedness and precision of the method (Riley 1996; Swartz 1997).
The precision and accuracy of analytical methods are described in a quantitative fashion by the use of relative errors (Bias%). One example of relative error is the accuracy, which describes the deviation from the expected results.
All solutions were protected from light and were used within 24 h to avoid decomposition. However, current – potential curves of sample solutions recorded 72 h after preparation did not show any appreciable change in assay values.

3.3. Tablet assay procedure and recovery experiments from tablets

Ten tablets of Zocor each containing 20 mg of SMV were crushed in a glass mortar. An adequate amount of this powder corresponding to a stock solution of ca. 1×10^{-3} M was accurately weighed and transferred into a

Table 4: Application of the proposed method to the determination of simvastatin in spiked human serum using DPV and SWV modes

Technique	Simvastatin added (M)	n	Simvastatin found (M)	Average recovery (%)	RSD%	Bias%
DPV	1×10^{-5}	3	1.00×10^{-5}	100.00	0.74	0.00
	2×10^{-5}	3	1.96×10^{-5}	98.20	1.06	2.00
	4×10^{-5}	3	3.98×10^{-5}	99.50	0.57	0.01
SWV	1×10^{-5}	3	1.003×10^{-5}	100.30	0.99	−0.30
	2×10^{-5}	3	1.99×10^{-5}	99.30	1.04	0.50
	4×10^{-5}	3	3.98×10^{-5}	99.60	0.58	0.50

50 ml calibrated flask and completed to the volume with methanol. The contents of the flask were sonicated for 10 min to effect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte. Voltammograms were recorded as for pure SMV.
The amount of SMV per tablet was calculated using the linear regression equation obtained from the calibration curve of pure SMV.

3.4. Recovery experiments from tablets

To study the accuracy, reproducibility and precision of the proposed methods, and to check for possible interferences from common excipients such as cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, iron oxides, lactose, magnesium stearate, starch, talc, titanium dioxide and other ingredients (butylated hydroxyanisole as preservative) used in tablet dosage forms, recovery experiments were carried out. For this procedure, the known amounts of the pure drug were added to the previously analyzed tablet formulation of SMV. In order to find whether the excipients show any interference with the analysis, known amounts of pure drug were added to the different pre-analyzed formulations of SMV and the mixtures were analyzed by the proposed methods.

3.5. Recovery studies in spiked human serum samples

Serum samples, obtained from healthy individuals (after obtaining their written consent), were stored frozen until assay. After gentle thawing, aliquots of serum were spiked with SMV dissolved in methanol to achieve a final concentration of 10^{-3} M and treated with 700 µl acetonitrile as serum protein precipitating agent, then the volume was completed to 2 ml with the same serum sample. The tubes were vortexed for 5 min at 1500 rpm and then centrifuged for 10 min at 5000 × g to get rid of protein residues. The supernatant was taken carefully. Appropriate volumes of this solution were analyzed in a voltammetric cell containing the selected supporting electrolyte. Voltammograms were recorded as for pure SMV.

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