DRUG FORMULATIONS AND CLINICAL METHODS

Determination of Etofibrate, Fenofibrate, and Atorvastatin in Pharmaceutical Preparations and Plasma Using Differential Pulse Polarographic and Square Wave Voltammetric Techniques

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Etofibrate, fenofibrate, and atorvastatin were determined in their pharmaceutical preparations and human plasma using differential pulse polarographic and square wave voltammetric techniques by reduction at a dropping-mercury working electrode versus Ag/AgCl reference electrode. The reversibility of the electrode reactions was tested using cyclic voltammetry, and they were found to be irreversible reduction reactions. Optimum conditions such as pH, scan rate, and pulse amplitude were studied, and validation of the proposed methods was performed. The proposed methods proved to be accurate, precise, robust, and specific for determination of the 3 drugs. The relative standard deviation values were <2%, indicating that these methods are precise. Limits of detection and quantitation were in the ranges of 0.037–0.21 and 0.12–0.71 μg/mL, respectively, indicating high sensitivity.

Lipid-regulating drugs are used for the treatment of patients with hyperlipidemia. These drugs reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disorders in hyperlipidemic patients with clinical evidence of ischemic heart disease or peripheral vascular disease (1). In the present work, atorvastatin (ATOR; as an example of statins) and etofibrate (ETO) and fenofibrate (FEN; as examples of fibrates) were determined using voltammetric techniques.

A search of the literature revealed many analytical methods for the determination of these drugs. For example, ATOR was determined using high-pressure capillary electrophoresis (2) and column high-performance liquid chromatography/mass spectrometry (HPLC/MS; 3). HPLC was also utilized for determination of ATOR (4, 5), FEN (6, 7), and ETO (8). The proposed voltammetric methods proved to be sensitive, accurate, easy, and rapid.

Differential pulse polarography (DPP) plays an important role in the analysis of drugs, either in bulk or dosage forms and in biological fluids. Many drugs have been determined using DPP, including β-blockers (9), benznidazole (10), and tenoxicam (11).

An alternative and more recent voltammetric technique is square wave voltammetry (SWV). This technique offers higher sensitivity compared to other voltammetric techniques and minimizes the capacitive current contribution to the overall current (12). Piribedil (13), etodolac (14), and nifedipine (15) are examples of drugs that have been determined using SWV.

Experimental

Instrumentation

(**a**) *Voltammetry*.—Metrohm 693 VA processor and Metrohm 694 VA stand were used in both the dropping mercury electrode (DME) and the hanging mercury drop electrode (HMDE) mode. The 3-electrode system was completed by means of an Ag/AgCl (3 M KCl) reference electrode and a Pt auxiliary electrode (Brinkmann Instruments, Westbury, NY).

(**b**) *pH measurement*.—Schott Gerate pH meter Model CG 710 calibrated with standard buffers at room temperature (Schott Instruments GmbH, Mainz, Germany).

Materials and Reagents

(**a**) *ETO*.—Kindly supplied by Pharco-Pharmaceuticals, Alexandria, Egypt.

(**b**) *FEN*.—Kindly supplied by Minapharm, Cairo, Egypt.

(**c**) *ATOR*.—Kindly supplied by Pfizer, Cairo, Egypt.

(**d**) *Britton-Robinson buffer (B-R buffer)*.—Prepared from phosphoric acid, boric acid, and acetic acid, each at a concentration of 3 M.

All solvents and reagents were of analytical grade.

Preparation of Etofibrate, Fenofibrate, and Atorvastatin Stock Standard Solutions and Reagents

Stock standard solutions (1 mg/mL) of ETO, FEN, and ATOR were freshly prepared in methanol. These solutions

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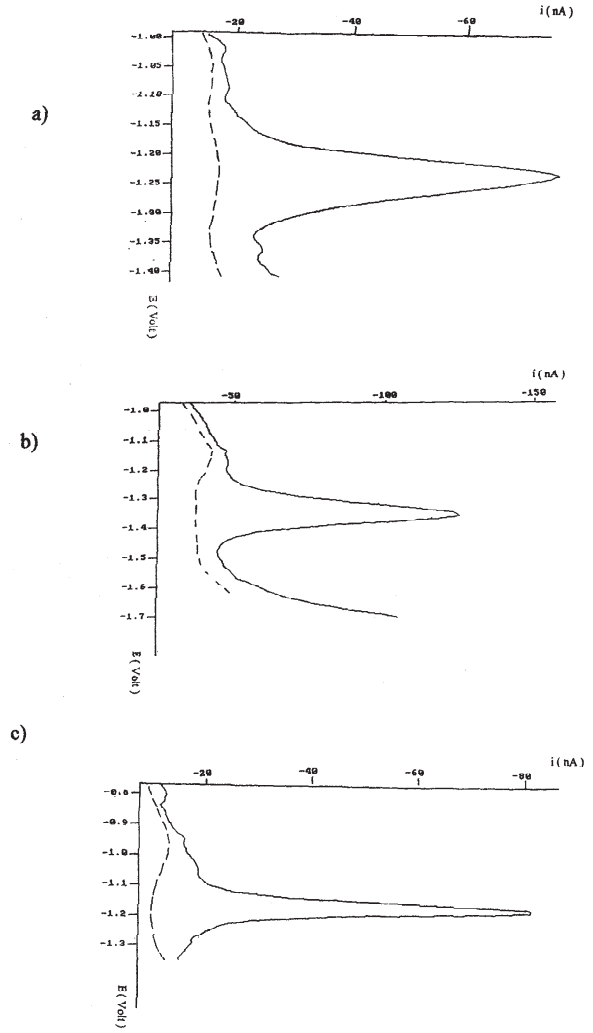

Figure 1. Differential pulse polarogram obtained from (a) 5 μg/mL etofibrate, (b) 5 μg/mL atorvastatin, (c) 1 μg/mL fenofibrate (——), and the solvent blank (- - -) after following the procedures described in the *Experimental* section.

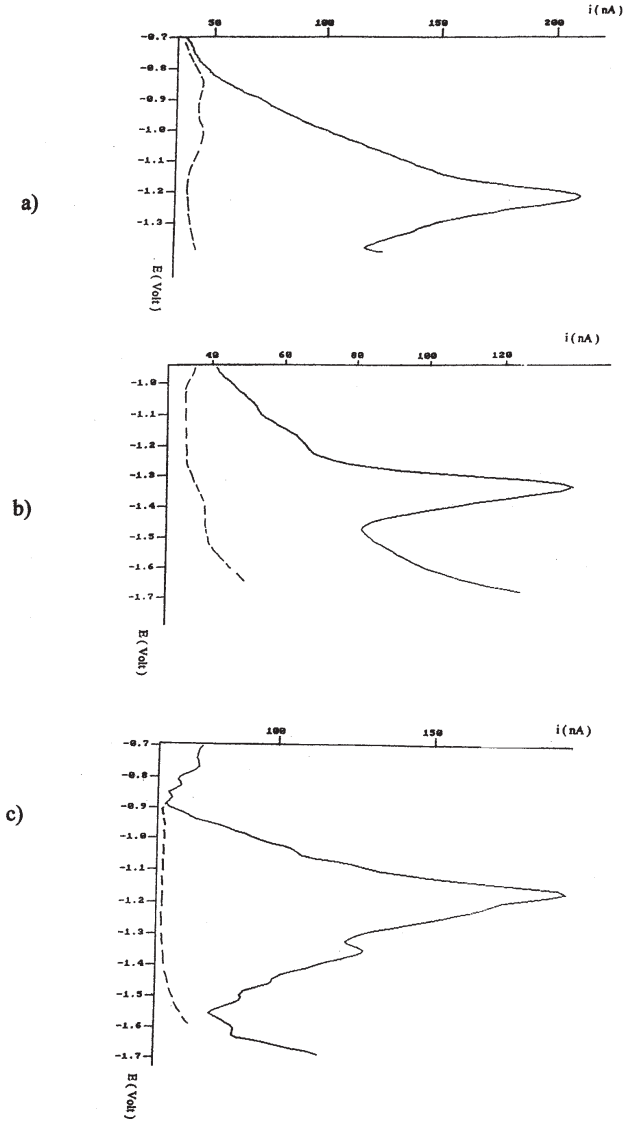

Figure 2. Square wave voltammograms obtained from (a) 2 μg/mL etofibrate, (b) 1 μg/mL atorvastatin, (c) 1 μg/mL fenofibrate (——), and the solvent blank (- - -) after following the procedures described in the *Experimental* section.

were used to prepare the dilutions that were used throughout the voltammetric investigations. Dilutions were performed in B-R buffer as the supporting electrolyte.

Preparation of Etofibrate, Fenofibrate, and Atorvastatin Test Solutions

Twenty ATOR tablets (labeled to contain 40 mg ATOR/tablet), 20 ETO capsule contents (labeled to contain 500 mg ETO/capsule), and 20 FEN capsule contents (labeled to contain 300 mg FEN/capsule) were weighed, powdered, and mixed well, and the average weight/tablet or capsule content was determined. A quantity of the powdered tablets or capsule contents equivalent to 25 mg ATOR, ETO, or FEN was accurately weighed into a 25 mL volumetric flask, 10 mL methanol was added, and the mixture was stirred for 15 min, diluted to volume with methanol, and mixed well. The mixture was filtered, and the filtrate was used in the voltammetric procedures.

Procedures for Voltammetric Analysis

(**a**) *DPP method.*—Aliquots of 50 μL from the stock standard solutions of ATOR and ETO and a 10 μL aliquot from the stock standard solution of FEN were transferred into three 10 mL volumetric flasks using micropipets and diluted to volume with B-R buffer pH 7 for ATOR and ETO, and with B-R buffer pH 7.5 for FEN, to give final concentrations of 5 μg/mL for ATOR and ETO and 1 μg/mL for FEN. The solutions were purged with pure nitrogen for 5 min. The polarograms were recorded from 0 to –1700 mV at a DME working electrode vs an Ag/AgCl reference electrode, with

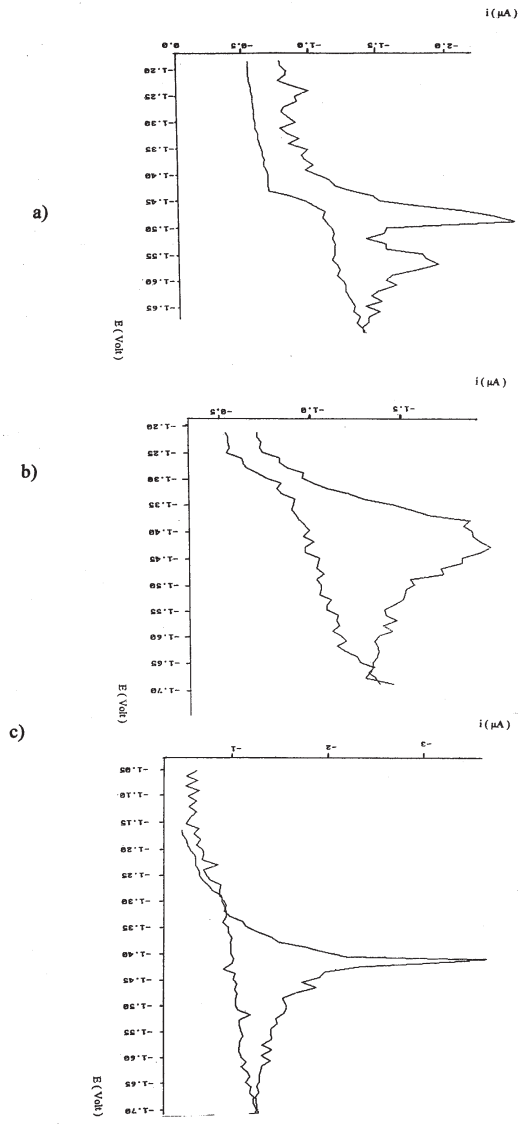

Figure 3. Cyclic voltammograms obtained from (a) 50 μg/mL etofibrate, (b) 10 μg/mL atorvastatin, and (c) 50 μg/mL fenofibrate after following the procedures described in the *Experimental* section.

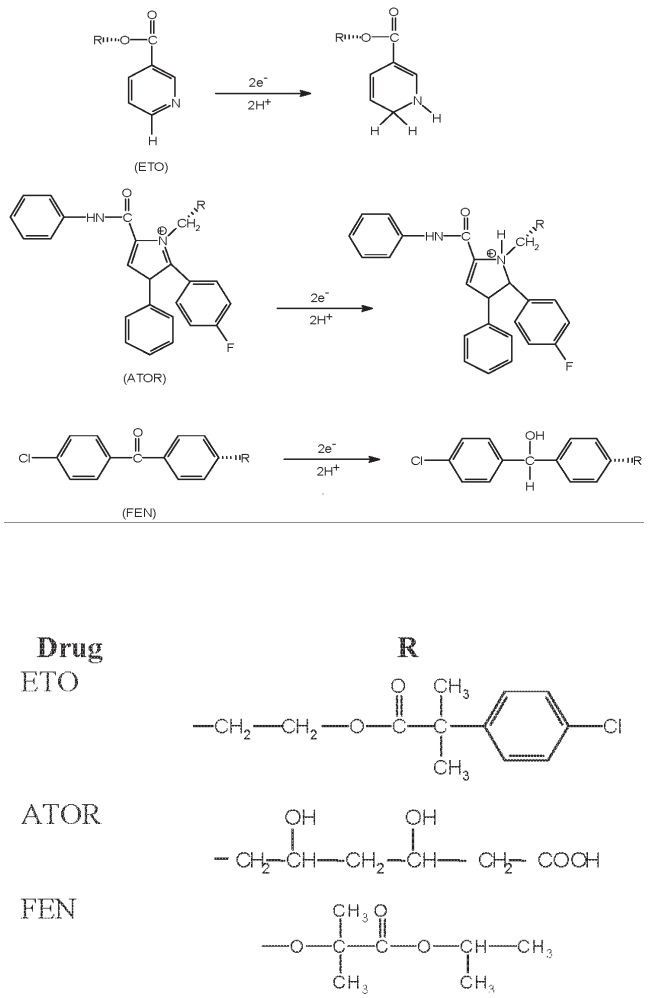

Figure 4. Proposed electrode reactions of etofibrate (ETO), atorvastatin (ATOR), and fenofibrate (FEN).

the application of a pulse amplitude (U) of –100 mV and at a scan rate (ν) of 15 mV/s for ATOR and ETO and 10 mV/s for FEN. The same procedures were repeated using 50 μL aliquots of the filtrate of ATOR tablets or ETO capsule content extract and a 10 μL aliquot of the filtrate of FEN capsule content extract; the concentrations of ATOR, ETO, and FEN in their pharmaceutical preparations were calculated from the corresponding regression equations.

(**b**) *SWV method.*—Aliquots of 50, 20, and 5 μL from the stock standard solutions of ATOR, ETO, and FEN, respectively, were transferred into three 10 mL volumetric flasks using micropipets and diluted to volume with B-R buffer pH 7 for ATOR and ETO, and with B-R buffer pH 7.5 for FEN, to give a final concentration of 5 μg/mL for ATOR, 2 μg/mL for ETO, and 0.5 μg/mL for FEN. The solutions were purged with pure nitrogen for 5 min. The polarograms were recorded from 0 to –1700 mV at a DME working electrode vs an Ag/AgCl reference electrode, with the application of a pulse amplitude of –100 mV and at a scan rate of 40 mV/s for ATOR, 50 mV/s for ETO, and 60 mV/s for FEN. The same procedures were repeated using a 50 μL aliquot of the filtrate of ATOR tablets, a 20 μL aliquot of the filtrate of ETO capsule content extract, and a 5 μL aliquot of the filtrate of FEN capsule content extract; the concentrations of ATOR, ETO, and FEN in their pharmaceutical preparations were calculated from the corresponding regression equations.

(**c**) *Cyclic voltammetry.*—A 0.1 mL aliquot from the stock standard solution of ATOR and 0.5 mL aliquots from the stock standard solutions of ETO and FEN were transferred into three 10 mL volumetric flasks using graduated pipets and diluted to volume with B-R buffer pH 7 for ATOR and ETO and B-R buffer pH 7.5 for FEN to give a final concentration of 10 μg/mL for ATOR and 50 μg/mL for ETO and FEN. The cyclic voltammograms were recorded from 0 to –1700 mV in

Table 1. Assay results obtained using the standard addition procedure for etofibrate (ETO), fenofibrate (FEN), and atorvastatin (ATOR) in their pharmaceutical preparations by the proposed differential pulse polarographic (DPP) and square wave voltammetric (SWV) methods

ETO				FEN				ATOR			
		Found, %				Found, %				Found, %	
Labeled, μg/mL	Standard added, μg/mL	DPP	SWV	Labeled, μg/mL	Standard added, μg/mL	DPP	SWV	Labeled, μg/mL	Standard added, μg/mL	DPP	SWV
2	0	98.6	99.5	1	0	99.7	99.1	5	0	99.8	100.7
2	1	97	103	1	0.5	98.6	98.2	5	2.5	100.9	98.5
2	2	98.9	101.9	1	1	101.3	101.9	5	5	101.4	101.5
2	3	99.1	100.2	1	1.5	97.7	99.5	5	7.5	99.3	99.2
2	4	97.5	100.8	1	2	98.8	101.3	5	10	98.1	99.3
Mean, %		98.2	101.1			99.2	100.0			99.9	99.8
SD		0.92	1.39			1.36	1.55			1.31	1.22
RSD, %		0.94	1.37			1.37	1.55			1.31	1.23

the cathodic branch and from –1700 to 0 mV in the anodic branch at an HMDE working electrode, with the application of a pulse amplitude of 50 mV and at a scan rate of 100 mV/s in both the cathodic and anodic branches.

Application to Plasma Samples

Frozen plasma was thawed at room temperature. Different aliquots from the stock standard solutions of ATOR, ETO, and FEN were transferred into 3 sets of 5 mL volumetric flasks and diluted to volume with plasma. Aliquots of 1 mL from these spiked plasma solutions were diluted to 5 mL with methanol in 3 sets of 10 mL centrifuge tubes and mixed well. The solutions were centrifuged for 10 min at 500 rpm. Aliquots (2.5 mL) of the centrifugates were transferred into sets of 5 mL volumetric flasks and diluted to volume with B-R buffer pH 7 for both ATOR and ETO and B-R buffer pH 7.5 for FEN. The procedures were completed as described above.

Results and Discussion

Development and Optimization of the Methods

DPP method.—Figure 1 shows the differential pulse polarograms of ETO (5 μg/mL solution in B-R buffer pH 7 at a scan rate of 15 mV/s and pulse amplitude of –100 mV), ATOR (5 μg/mL solution in B-R buffer pH 7 at a scan rate of 15 mV/s and pulse amplitude of –100 mV), and FEN (1 μg/mL solution in B-R buffer pH 7.5 at a scan rate of 10 mV/s and pulse amplitude of –100 mV). The voltammetric measurements were recorded for a solution of each of the 3 investigated drugs in B-R buffer system as a supporting electrolyte. The effect of the pH on the peak current (ip) and the reduction potential (Ep) was investigated over a range between 5 to 9. It was found that a pH value of 7 was optimal for both ETO and ATOR, and a pH value of 7.5 was optimal for FEN as the peak currents (ip) were the highest at these pH values. The potentials of the differential pulse peaks were shifted to more negative values upon increasing the pH of the buffer. At a pH value of 7, ETO and ATOR gave peaks with potential values of –1244 and –1354 mV, respectively, while FEN gave a peak with a potential value of –1184 mV at a pH value of 7.5. These potential values could be used as a tool for identification of the 3 drugs through calculation of the half-wave potential values ($E_{1/2}$; 16). The $E_{1/2}$ values of ETO and ATOR were –1194 and –1304 mV, respectively, at pH = 7, while the $E_{1/2}$ value for FEN was –1134 mV at pH = 7.5. The data could be used for differentiation among the 3 drugs as the peak potential of FEN at pH 7 is equal to –1200 mV and, hence, the $E_{1/2}$ value for FEN is –1150 mV at pH = 7.

The scan rate was investigated by repeating the procedure above, except that the scan rate was varied between 10 to 30 mV/s. A scan rate of 15 mV/s gave maximum responses for both ETO and ATOR, and a scan rate of 10 mV/s gave maximum response for FEN.

The pulse amplitude was investigated by repeating the procedure above, except that the pulse amplitude was varied between –10 to –100 mV. It was found that the peak current

Table 2. Determination of etofibrate (ETO), fenofibrate (FEN), and atorvastatin (ATOR) in their pharmaceutical preparations by the proposed differential pulse polarographic (DPP) and square wave voltammetric (SWV) methods and the reference methods

	Found ETO, %			Found FEN, %			Found ATOR, %		
Sample No.	DPP method	A_{max} method	SWV method	DPP method	Reference method[a]	SWV method	DPP method	Reference method[b]	SWV method
1	99.7	98.8	99.1	98.7	99.7	100.4	101.3	99.6	98.7
2	101.7	101.1	100.8	99.4	98.6	98.9	100.6	100.6	101.1
3	100.3	100	101.2	98.9	100.6	100.4	99.9	100.1	99.3
4	99.6	100	100.1	100.7	99.3	99.7	101.5	101.7	98.4
5	100.6	98.8	99.9	99.1	99.8	100.8	99.3	99.4	100.4
Mean, %	100.4	99.7	100.2	99.4	99.6	100	100.5	100.3	99.6
SD	0.85	0.97	0.82	0.79	0.73	0.75	0.93	0.92	1.14
RSD, %	0.85	0.97	0.81	0.80	0.73	0.75	0.92	0.92	1.15
t[c]	1.1	0.85		0.5	0.94		0.41	1.1	
F[c]	1.31	1.41		1.2	1.1		1.01	1.54	

[a] Reference HPLC method (ref. 14).
[b] Reference HPLC method (ref. 15).
[c] Theoretical values are t = 2.31 and F = 6.39 at the 95% confidence level (n = 5).

(ip) was greatly affected by pulse amplitude (U). Plots were linear up to an amplitude of –100 mV for the 3 drugs.

SWV method.—Figure 2 shows the square wave voltammograms of ETO (2 μg/mL solution in B-R buffer pH 7 at a scan rate of 50 mV/s and pulse amplitude of 50 mV); ATOR (1 μg/mL solution in B-R buffer pH 7 at a scan rate of 40 mV/s and pulse amplitude of 50 mV); and FEN (1 μg/mL solution in B-R buffer pH 7.5 at a scan rate of 60 mV/s and pulse amplitude of 50 mV). The voltammetric measurements were recorded for a solution of each of the 3 investigated drugs in B-R buffer system as a supporting electrolyte. The effect of the pH on the peak current (ip) and the reduction potential (Ep) was investigated over a range of 5 to 9. It was found that a pH value of 7 was optimal for both ETO and ATOR, and a pH value of 7.5 was optimal for FEN. The differential peak current (Δi) values were highest at such pH values. The potentials of the square wave peaks were shifted to more negative values upon increasing the pH value of the buffer.

Table 3. Regression and statistical parameters for the relationship between the peak current (ip) or the differential peak current (Δi) of etofibrate (ETO), fenofibrate (FEN), and atorvastatin (ATOR) and their corresponding concentrations by the proposed differential pulse polarographic (DPP) and square wave voltammetric (SWV) methods

	DPP method			SWV method		
Parameter	ETO	FEN	ATOR	ETO	FEN	ATOR
Linearity range, μg/mL	2–10	0.5–2.5	3–15	0.5–2.5	0.2–1	3–15
Intercept (a)	–1.81	0.09	0.14	1.26	–0.28	0.12
Slope (b)	158.95	898.6	251.7	1166.8	1931	280.5
Correlation coefficient (r)	0.9998	0.9998	0.9998	0.9999	0.9995	0.9998
$S_{y/x}$[a]	1.07	1.5	1.5	1.31	2.3	1.87
S_a[b]	1.124	1.6	1.6	1.37	2.4	1.96
S_b[c]	1.69	9.9	2.4	8.3	36.7	2.96
LOD, μg/mL	0.19	0.05	0.2	0.04	0.037	0.21
LOQ, μg/mL	0.64	0.17	0.68	0.13	0.12	0.71

[a] $S_{y/x}$ = Standard error of estimate.
[b] S_a = Standard deviation of intercept.
[c] S_b = Standard deviation of slope.

Table 4. Effect of slight change of the pH of the buffer on the position of the peak potential (Ep) and its response (ip or Δi) for etofibrate (ETO), fenofibrate (FEN), and atorvastatin (ATOR) by the proposed differential pulse polarographic (DPP) and square wave voltammetric (SWV) methods

		Method			
		DPP		SWV	
Drug	pH	Ep, –mV	ip, –nA	Ep, –mV	Δi, nA
ETO[a]	6.9	1242	79	1218	122
	7	1244	77.5	1220	121
	7.1	1244	78.8	1221	125
FEN[b]	7.4	1184	90	1180	93
	7.5	1184	93	1180	93
	7.6	1185	91	1180	90
ATOR[c]	6.9	1354	247	1354	280
	7	1354	251	1355	284
	7.1	1355	248	1355	281

[a] Concentration of ETO = 5 μg/mL in the DPP method and 1 μg/mL in the SWV method.

[b] Concentration of FEN = 1 μg/mL in the DPP method and 0.5 μg/mL in the SWV method.

[c] Concentration of ATOR = 10 μg/mL in both the DPP and SWV methods.

The scan rate was investigated by repeating the procedure above except that the scan rate was varied between 10 to 60 mV/s. It was found that the scan rate (ν) had no effect on the differential peak current (Δi). The response was almost constant over the range of 10–60 mV/s for the 3 drugs.

The pulse amplitude was investigated by repeating the procedure above, except that the pulse amplitude was varied between 10 to 50 mV. It was found that a pulse amplitude (U) of 50 mV gave maximum responses for the 3 drugs.

The electrode reactions.—The investigated drugs show DPP peaks at –1244, –1354, and –1184 mV (Figure 1) and SWV peaks at –1220, –1355, and –1180 mV (Figure 2) for ETO, ATOR, and FEN, respectively. The reduction reactions of the 3 drugs were irreversible, as indicated by their cyclic voltammograms. The 3 cyclic voltammograms exhibited single cathodic waves at –1490, –1410, and –1450 mV for ETO, FEN, and ATOR, respectively, without the appearance of any waves in the anodic branch (Figure 3). On increasing the scan rate, peak potential (Ep) values were shifted to more negative values. This finding was considered as further proof that strengthens the irreversibility of the 3 electrode reactions.

Based on these findings, the schemes shown in Figure 4 are postulated for the electrode reactions of ETO, ATOR, and FEN. The reduction peaks of ETO and ATOR may be due to the azomethine group. The reduction peak of FEN may be due to the carbonyl group present in its structure.

It has been reported that drugs containing an azomethine group in their structures, such as doxazosin (17) and

Table 5. Application of the proposed differential pulse polarographic (DPP) and square wave voltammetric (SWV) methods for determination of etofibrate (ETO), fenofibrate (FEN), and atorvastatin (ATOR) in their pharmaceutical preparations in 3 different batches

	Found ± SD[b], %					
	DPP method			SWV method		
Batch No.[a]	ETO	FEN	ATOR	ETO	FEN	ATOR
B_1	98.2 ± 0.40	101.6 ± 0.70	98.6 ± 0.58	101.1 ± 0.85	98.2 ± 0.45	100.5 ± 0.75
B_2	100.9 ± 0.48	100.2 ± 0.33	100.3 ± 0.61	99.1 ± 0.63	99.3 ± 0.75	99.9 ± 0.66
B_3	98.7 ± 0.55	98.7 ± 0.91	99.7 ± 0.83	99.4 ± 0.59	98.5 ± 0.88	99.5 ± 0.96

[a] B_1–B_3 refer to 3 different batch numbers: 957, 963, and 983 for ETO; 022867, 033744, and 034855 for FEN; and 04670, 04753, and 04951 for ATOR.

[b] Mean ± SD, n = 5.

Table 6. Determination of etofibrate (ETO), fenofibrate (FEN), and atorvastatin (ATOR) in human plasma by the proposed differential pulse polarographic (DPP) and square wave voltammetric (SWV) methods

	DPP method		SWV method	
Drug	μg Spiked/mL plasma	Recovery ± SD[a], %	μg Spiked/mL plasma	Recovery ± SD[a], %
ETO	30	98.7 ± 1.19	5	101.4 ± 0.70
	60	100.6 ± 0.58	10	100.8 ± 0.55
	90	97.6 ± 0.72	20	99.5 ± 0.64
FEN	5	99.7 ± 0.61	4	98.1 ± 0.50
	10	100.4 ± 0.85	6	100.4 ± 0.86
	20	100.1 ± 0.52	8	99.7 ± 0.87
ATOR	30	99.8 ± 0.49	30	99.4 ± 0.96
	60	99.4 ± 0.69	60	98.6 ± 0.51
	90	99.6 ± 0.91	90	99.1 ± 0.62

[a] Mean ± SD of 5 determinations.

loratadine (18), produce reduction peaks at –1330 and –1200 mV, respectively, under experimental conditions similar to those applied for ETO and ATOR. Drugs containing carbonyl group in their structures, such as ketorolac (19) and benazepril (20), produce reduction peaks at –960 and –1280 mV, respectively, under experimental conditions similar to those applied for FEN. The electrode reactions of these drugs were reported to be irreversible. This strengthens the suggested postulations that the reduction of ETO and ATOR is due to the azomethine group, while that of FEN is due to the carbonyl group.

Validation

Validation was performed according to the U.S. Pharmacopeia (USP; 16) validation standards.

Specificity.—The specificity of the proposed voltammetric methods was investigated by applying the voltammetric procedures to the standard solutions of ETO, FEN, and ATOR and methanolic solutions of the coformulated adjuvants commonly added to tablets or used as capsule fillers. The obtained voltammograms from the solutions containing the coformulated adjuvants were almost identical to those of the blank, while the voltammograms obtained from the solutions containing either the pure drugs or mixtures of the drugs with the coformulated adjuvants were almost identical in the position of the peak potential and its amplitude (Figures 1 and 2). These findings prove that the suggested methods are specific for determination of the investigated drugs without interference from the coformulated adjuvants.

Accuracy.—The accuracy of the proposed voltammetric methods was assessed by a standard addition procedure. Known concentrations of the reference standard drugs ETO, FEN, and ATOR were added to their appropriate pharmaceutical preparations. The percentage recovery of each drug was calculated after following the previously described procedures. The results (Table 1) indicate that the proposed DPP and SWV methods are accurate for the determination of the investigated drugs without interference from the coformulated adjuvants.

To prove further that the proposed DPP and SWV methods are accurate, the investigated drugs were determined in their pharmaceutical preparations using the proposed methods and 2 published reference methods (21; Merck Sharp and Dohme Research Laboratories, Rahway, NJ, personal communication, 2004). The results (Table 2) show that the calculated *t*- and *F*-values are less than the theoretical values. Consequently, the proposed methods are as accurate as the reference methods.

Linearity.—Under the optimal experimental conditions for each of the investigated drugs, a linear relationship existed between either the peak current (ip) or the differential peak current (Δi) of each drug and its corresponding concentration. The regression equation data, correlation coefficient (r), and other statistical parameters are listed (Table 3).

Range.—The methods were applicable for determination of concentrations as low as 40% and as high as 200%, and as low as 25% and as high as 125% of the working concentration of ETO using the DPP and SWV methods, respectively. The methods were applicable for determination of concentrations as low as 50% and as high as 250%, and as low as 40% and as high as 200% of the working concentration of FEN using the DPP and SWV methods, respectively. The methods were applicable for determination of concentrations as low as 60% and as high as 300% of the working concentration of ATOR using both the DPP and SWV methods. This indicates that the proposed voltammetric methods could be adopted as methods for determination of the investigated drugs in their pharmaceutical preparations, to study the content uniformity of tablets and capsules, and to follow their dissolution rates.

Precision.—The precision of the proposed voltammetric methods measured as relative standard deviation (RSD) was tested by repeating the proposed procedures 9 times using the working concentration of each of the investigated drugs. The RSD values for such determinations were 0.53, 0.61, and 0.43% using the DPP method and 0.44, 0.54, and 0.39% using

the SWV method for ETO, FEN, and ATOR, respectively. These RSD values are <2% (16), indicating that the proposed methods are precise.

Robustness.—The robustness of the proposed voltammetric methods was investigated by studying the effect of slight variation of the optimal pH on the position of peak potential (Ep) and its response (ip or Δi). The data (Table 4) shows that a slight change in pH value (±0.1) did not affect the position of peak potential (Ep) or its response (ip or Δi). This observation indicates that the methods are robust.

Limits of detection and quantitation.—The limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to the USP (16). The values (Table 3) indicate that the proposed methods are sensitive for detection and determination of the investigated drugs.

Applications

Application to pharmaceutical preparations.—The proposed voltammetric methods were applied for the determination of ETO, FEN, and ATOR in their pharmaceutical preparations in 3 different batches. The data are reported as mean percentage found with the standard deviation (SD) values (Table 5).

Application to plasma samples.—The proposed voltammetric methods have been applied for the determination of ETO, FEN, and ATOR in plasma. It has been reported that these drugs undergo rapid absorption and metabolism to their active metabolite by plasma and tissue esterases (22, 23). FEN has a half-life of about 20 h, and its typical peak concentration is 5–15 μg/mL (24). Deproteination using methanol was performed to avoid possible interferences that may arise from the components of plasma. Moreover, such deproteination avoids the lengthy, tedious extraction of very small concentrations of drugs that usually results in negative error as large as 30% (25). The specificity of such a procedure for determination of the investigated drugs without interference from methanol–deproteinated plasma fluid was tested by applying the voltammetric procedures to blank methanol–deproteinated plasma. The voltammogram was almost identical to that obtained with the buffer, indicating complete elimination of any interfering reducible species if present. The mean recoveries and the SD values (Table 6) indicate that the proposed methods are both accurate and precise for determination of the investigated drugs in plasma. This strengthens the suggestion for the application of the proposed methods to study the bioavailability of the investigated drugs.

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