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Two different spectrofluorimetric methods for simultaneous determination of gemfibrozil and rosiglitazone in human plasma

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ABSTRACT

Two accurate, reliable, and highly sensitive spectrofluorimetric methods were developed for simultaneous determination of binary mixture gemfibrozil and rosiglitazone in human plasma without prior separation steps. The first method is based on synchronous fluorescence spectrometry using double scans. At $\Delta\lambda = 27$ nm, gemfibrozil yields detectable signal that is independent of the presence of rosiglitazone. Similarly, at $\Delta\lambda = 120$ nm the signal of rosiglitazone is not influenced by the presence of gemfibrozil. Signals at two wavelengths, 301 ($\Delta\lambda = 27$ nm) and 368 nm ($\Delta\lambda = 120$ nm) vary linearly with gemfibrozil and rosiglitazone concentrations over the range 100–700 ng mL^{-1} (for gemfibrozil) and 20–140 ng mL^{-1} (for rosiglitazone), respectively. The limits of detection (LOD) were 2.3 and 2.72 ng mL^{-1} for gemfibrozil and rosiglitazone, respectively. The second method is based on the technique of simultaneous equations (Vierodt's method), in which 258 nm was selected as the excitation wavelength. Two equations are constructed based on the fact that at ($\lambda_{Em_2} = 302$ nm of gemfibrozil) and ($\lambda_{Em_2} = 369$ nm of rosiglitazone) the fluorescence of the mixture is the sum of the individual fluorescence of gemfibrozil and rosiglitazone. The limits of detection (LOD) were 28.1 and 23.63 ng mL^{-1} for gemfibrozil and rosiglitazone, respectively. The proposed methods were successfully applied for the determination of the two compounds in synthetic mixtures and in human plasma with a good recovery.

1. Introduction

The thiazolidinediones (TZDs) derivatives (rosiglitazone and pioglitazone) are often referred to as insulin sensitizers, clinically these drugs decrease insulin resistance in muscle and liver, which enhance glucose utilization and decrease hepatic glucose output. The precise molecular actions of these agents remain to be clarified; however, it is known that they bind to and activate a nuclear receptor (peroxisome proliferator–activator receptor-γ [PPAR-γ]), which is expressed in many insulin-sensitive tissue [1]. Rosiglitazone has received regulatory approval for the treatment of type 2 diabetes mellitus (T2DM) in both the monotherapy and the therapy in combination with other oral anti-diabetic agents for its advantages of the therapeutic profile [2,3]. Gemfibrozil, a lipid-lowering drug, is widely used to lower high serum triglyceride concentrations in patients with T2DM. Studies have found that cytochrome P450 (CYP2C8) is primarily responsible for the metabolism of rosiglitazone in human liver, with minor contributions for CYP2C9 [4]. Gemfibrozil inhibits both CYP2C8 [5] and CYP2C9 [6]. Therefore, concomitant administration of gemfibrozil, an inhibitor of CYP2C8, and rosiglitazone for 7 days increased rosiglitazone area under the concentration–time curve (AUC) by 127% compared to the administration of rosiglitazone alone. Given the potential for dose-related adverse events with rosiglitazone, a decrease in the dose of rosiglitazone may be needed when gemfibrozil is introduced [7]. However, up to now, no spectrofluorimetric method has been reported on the simultaneous determination of the two drugs in human plasma.

Literature survey reveals several methods for the determination of rosiglitazone in pharmaceutical preparations or in biological fluids including liquid chromatography (LC) [8–14], liquid chromatography–tandem mass spectrometry (LC/MS–MS) [15,16] micellar electro-kinetic chromatography (MEKC) [8], high performance thin layer chromatography (HPTLC) [17–19] and spectrophotometry [20–22].

Several methods have also been described for the determination of gemfibrozil such as gas chromatography [23] and high performance liquid chromatography (HPLC) with ultraviolet or fluorimetric detection [24–30] have been used for the determination of gemfibrozil and its metabolites in plasma and urine samples. Near-infrared diffuse reflectance spectroscopy [31–34] has been applied to the determination of gemfibrozil in pharmaceutical

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Table 1
Comparison of the proposed methods with the previously reported HPLC method.

Parameters	The present proposed methods				The reported HPLC method [37]	
	SFS method		Vierodt's method			
	Gemfibrozil	Rosiglitazone	Gemfibrozil	Rosiglitazone	Gemfibrozil	Rosiglitazone
Excitation wavelength (nm)	$\Delta\lambda = 27$ nm	$\Delta\lambda = 120$ nm	258	258	242	250
Emission wavelength (nm)	301	368	302	369	300	370
Linearity range (ng mL^{-1})	100–700	20–140	300–2000	80–280	500–75400	5.0–751.3
Limit of detection (ng mL^{-1})	2.3	2.72	28.1	23.63	50	2
Limit of quantification (ng mL^{-1})	7	8.23	85.12	71.6	500	5
Regression equation[a]						
Slope (b)	1.005	7.03	0.41	2.56	4.75	71.95
Intercept (a)	48.52	2.34	3.39	10.68	−0.062	1.19
Correlation coefficient (r)	0.999	0.999	0.999	0.999	0.999	0.9998

[a] $F = a + bC$ where F is the fluorescence intensity and C is the concentration.

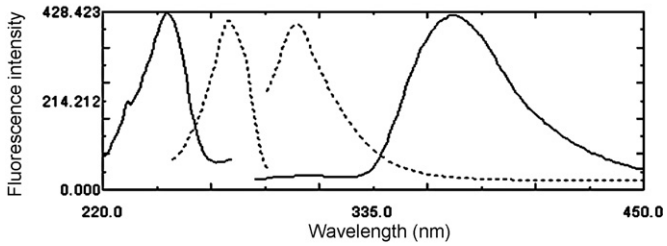

Fig. 1. Normal excitation and emission fluorescence spectra as individual component of {gemfibrozil (dashed line), [350 ng mL^{-1}; λ_{Ex} = 274 nm, λ_{Em} = 301 nm]}, and {rosiglitazone (solid line), [60 ng mL^{-1}; λ_{Ex} = 248 nm, λ_{Em} = 368 nm]}.

preparations. A liquid chromatography mass spectrometric method [35] has been reported for the determination of this drug in water and wastewater samples. Also the fluorescence characteristics of gemfibrozil in aqueous and micellar media were investigated [36]. To our knowledge, only one study has been published recently for simultaneous determination of these two drugs in plasma, based on LC [37].

Table 1 illustrates a comparison of the proposed methods with previously reported HPLC method for analysis of the mentioned drugs in plasma. The proposed methods were found to be easier than the published HPLC method [37] for the simultaneous determination of rosiglitazone and gemfibrozil in human plasma, whereas there is no need for using internal standard, gradient elution, or time programming to adjust excitation and emission wavelengths. Moreover, the proposed methods are the first spectrofluorimetric methods for the simultaneous determination of rosiglitazone and gemfibrozil in human plasma. The scientific novelty of the present work is that the methods used are simple, rapid, sensitive, and less expensive and less time consuming compared with other published LC methods.

2. Experimental

2.1. Apparatus

Fluorescence spectra and measurements were recorded using Shimadzu RF-5301 spectrofluorimeter (Kyoto, Japan); equipped with 150 W xenon arc lamp and slit widths for both monochromators were set at 5 nm. A 1 cm quartz cells were used at a high sensitivity for all measurements. Spectra were automatically obtained by Shimadzu fluorescence spectroscopy software, version 2.04. A pH-meter (Mettler-Toledo GmbH, Switzerland) was used for pH adjustment.

2.2. Materials and reagents

- Gemfibrozil standard samples were kindly provided by Pfizer, Egypt. Rosiglitazone standard samples were kindly supplied by GlaxoSmithKline, Egypt. All reagents and chemicals used were of analytical grade and all were purchased from Sigma–Aldrich (Steinheium, Germany). Glass distilled water was further purified using Milli-Q water purification system (Millipore, Bedford, MA, USA).

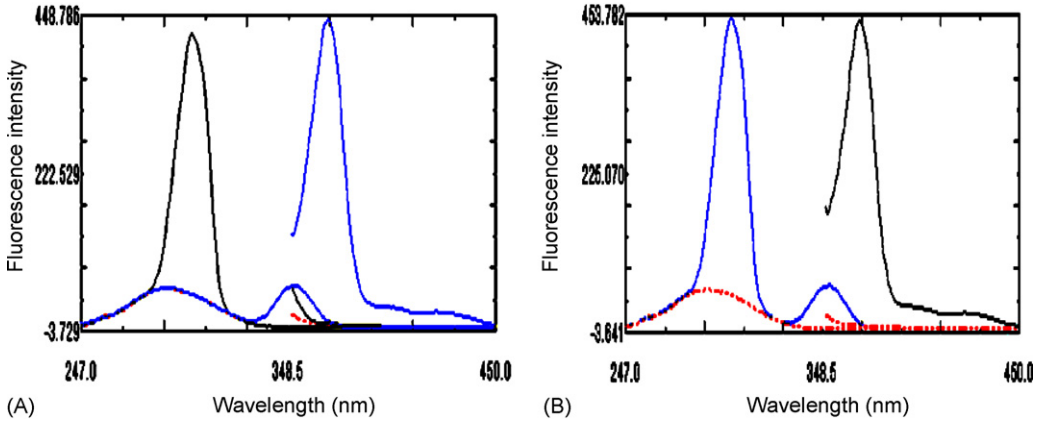

Fig. 2. Synchronous fluorescence spectra of (A) gemfibrozil (—) at both {$\Delta\lambda$ = 27 and $\Delta\lambda$ = 120 nm, (350 ng mL^{-1})}, rosiglitazone (▬) at both {$\Delta\lambda$ = 27 and $\Delta\lambda$ = 120 nm, (60 ng mL^{-1})} and blank (▬) at both {$\Delta\lambda$ = 27 and $\Delta\lambda$ = 120 nm} (B) synchronous fluorescence spectra of binary mixture gemfibrozil and rosiglitazone (350 and 60 ng mL^{-1}, respectively); and blank (▬) at both {$\Delta\lambda$ = 27 and $\Delta\lambda$ = 120 nm}.

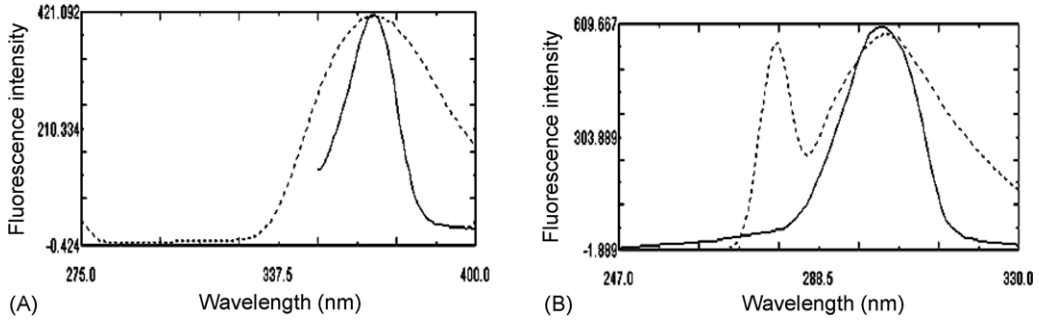

Fig. 3. Comparison of (A) synchronous fluorescence spectrum of rosiglitazone {solid line}, ([60 ng mL^{-1}], $\Delta\lambda = 120$) with its normal fluorescence emission spectrum {dashed line}, ($\lambda_{Ex} = 248$ nm). (B) Synchronous fluorescence spectrum of gemfibrozil {solid line}, ([550 ng mL^{-1}], $\Delta\lambda = 27$) with its normal fluorescence emission spectrum {dashed line}, ($\lambda_{Ex} = 274$ nm).

- *Borate buffer pH 9.0 was prepared as follow*:
  Solution A: dissolve 6.18 g of boric acid in sufficient 0.1 M potassium chloride to produce 1000 mL.
  Solution B: 0.1 M sodium hydroxide. Mix 1000 mL of solution A with 420 mL of solution B.
- *Phosphate buffer pH 7.0*: was prepared by mixing 50 mL of 0.2 M potassium dihydrogen orthophosphate with 29.63 mL of 0.2 M sodium hydroxide and diluting to 200 mL with water.

2.3. Solutions

The primary stock solution of gemfibrozil (500 μg mL^{-1}) and rosiglitazone (200 μg mL^{-1}) was prepared by dissolving appropriate amounts of the pure substances in methanol. The stock solutions of rosiglitazone and gemfibrozil were diluted in methanol to produce appropriate working solutions before using. Stock standard solutions were stable for at least 1 month at 4 °C.

2.4. Calibration curves

2.4.1. Calibration curves for SFS

Aliquots of gemfibrozil and rosiglitazone standard solutions covering the working concentration range cited in (Table 1) were transferred into a two separate series of 10 mL volumetric flasks. Two milliliters of borate buffer pH 9.0 were added and the solutions were diluted to the volume with methanol

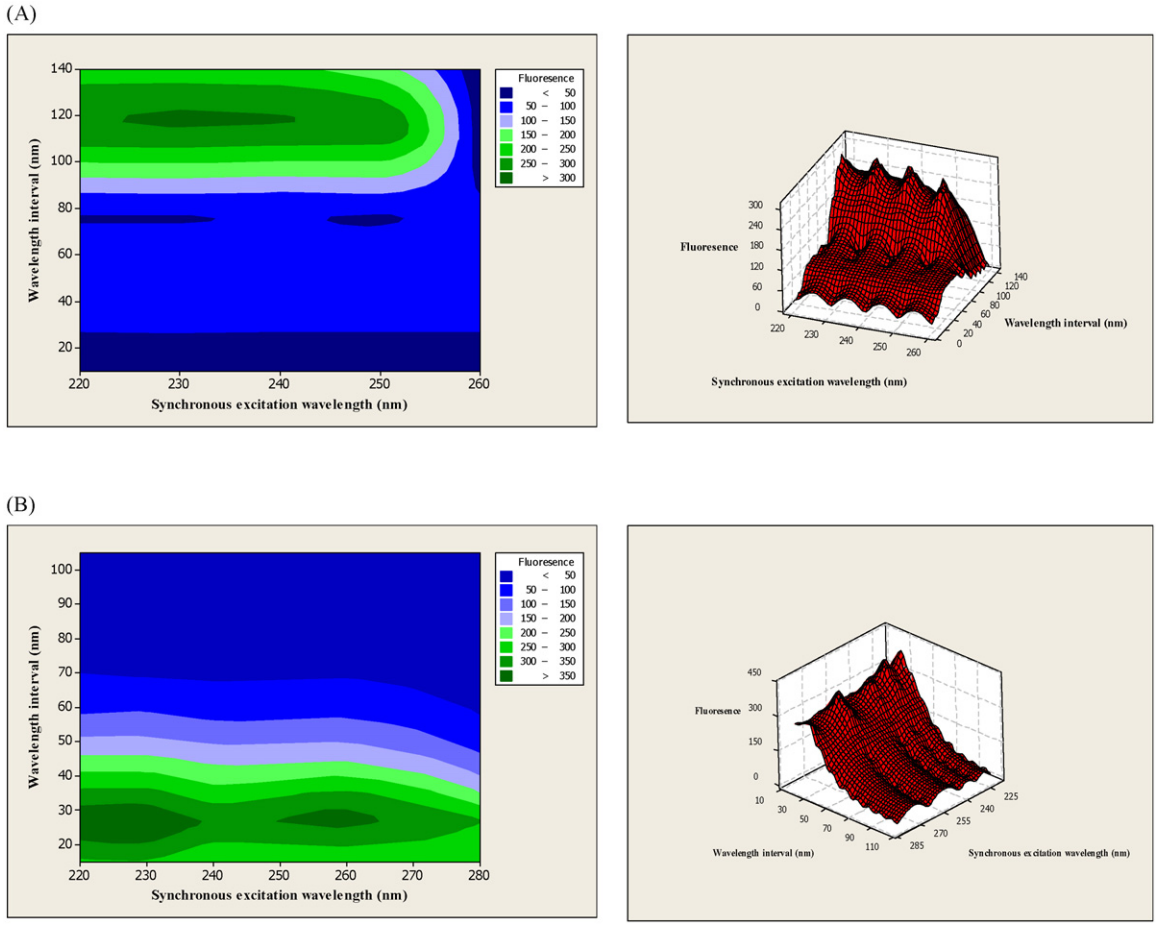

Fig. 4. Three-dimensional synchronous fluorescence spectra and their corresponding contour plots of (A) rosiglitazone (50 ng mL^{-1}) (B) gemfibrozil (350 ng mL^{-1}).

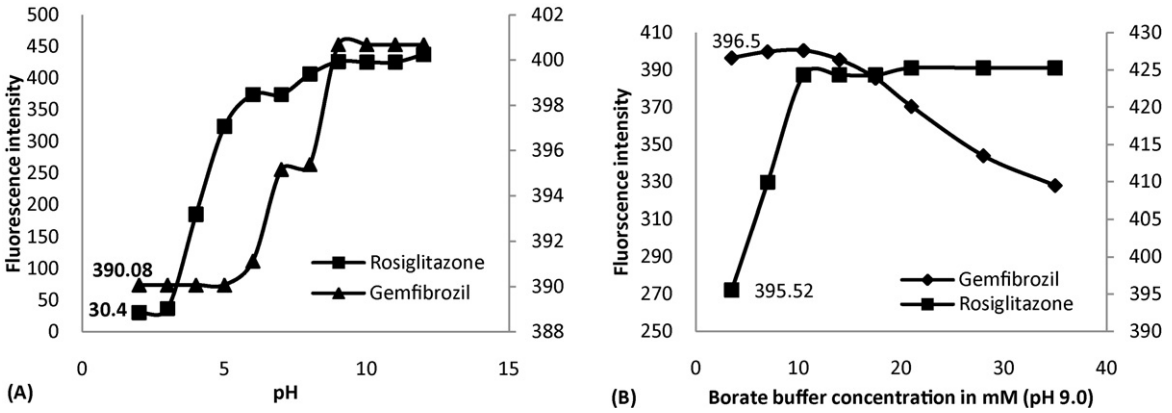

Fig. 5. (A) Effect of pH on the synchronous fluorescence intensity of gemfibrozil ([350 ng mL^{-1}], $\Delta\lambda$ = 27 nm at 302 nm); and rosiglitazone ([60 ng mL^{-1}], $\Delta\lambda$ = 120 nm at 369 nm). (B) Effect of borate buffer concentration (pH 9.0) on the synchronous fluorescence intensity of gemfibrozil ([350 ng mL^{-1}], $\Delta\lambda$ = 27 nm at 302 nm); and rosiglitazone ([60 ng mL^{-1}], $\Delta\lambda$ = 120 nm at 369 nm).

and mixed well. Synchronous spectra were obtained by scanning both monochromators simultaneously at constant wavelength differences of ($\Delta\lambda$ = 27 nm for gemfibrozil) and ($\Delta\lambda$ = 120 nm for rosiglitazone). The synchronous fluorescence intensity measurements were made at the synchronous maxima of each compound (at 301 and 368 nm for gemfibrozil and rosiglitazone, respectively).

2.4.2. Calibration curves for Vierodt's method

Aliquots of gemfibrozil and rosiglitazone standard solutions covering the working concentration range cited in (Table 1) were transferred into a two separate series of 10 mL volumetric flasks. Two milliliters of borate buffer pH 9.0 was added and the solutions were diluted to the volume with methanol and mixed well. The fluorescence intensity of gemfibrozil and rosiglitazone were measured at 302 and 369 nm, respectively, using an excitation wavelength of 258 nm.

2.5. Procedure for the synthetic mixture

Aliquot volumes of gemfibrozil and rosiglitazone standard solutions in different ratios were transferred into a series of 10 mL volumetric flasks. Two milliliters of borate buffer (pH 7.0) was added followed by dilution to volume with methanol, and mixed well. The recommended procedure under the calibration curves was then performed (Section 2.4).

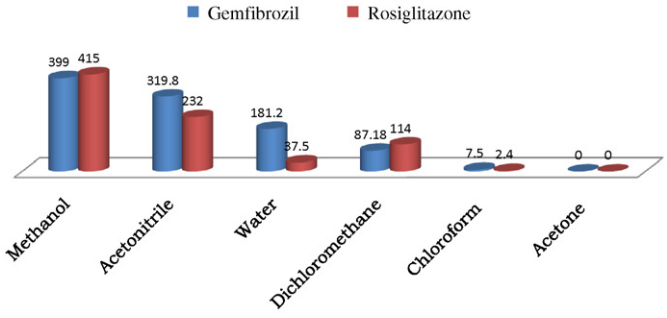

Fig. 6. Effect of solvents on the synchronous fluorescence intensity of gemfibrozil ([350 ng mL^{-1}], $\Delta\lambda$ = 27 nm at 302 nm); and rosiglitazone ([60 ng mL^{-1}], $\Delta\lambda$ = 120 nm at 369 nm).

Table 2
Application of the proposed methods to the determination of the studied drugs in pure form.

Method	Gemfibrozil			Rosiglitazone		
	Known concentration (ng mL^{-1})	Concentration found (ng mL^{-1})	Recovery (%)	Known concentration (ng mL^{-1})	Concentration found (ng mL^{-1})	Recovery (%)
(1) SFS method						
	250	246.09	98.44	60	61.4	102.31
	350	345.91	98.83	80	79.62	99.52
	450	449.82	99.96	100	99.7	99.7
	Mean ± SD		99.07 ± 0.79			100.51 ± 1.56
	N		3			3
	V		0.62			2.44
	SD		0.79			1.56
	RSD (%)		0.46			0.9
(2) Vierodt's method						
	500	489.46	97.9	100	101.26	101.26
	1000	1005.48	100.55	160	159	99.38
	1500	1511.4	100.76	200	200.06	100.03
	Mean ± SD		99.73 ± 1.6			100.22 ± 0.96
	N		3			3
	V		2.55			0.34
	SD		1.6			0.96
	RSD (%)		0.93			0.55

Each result is the average of three separate determination.

Table 3
Validation of the proposed methods for determination of the studied drugs raw materials.

Method	Concentration taken (ng mL^{-1})		% Recovery			
	Gemfibrozil	Rosiglitazone	Gemfibrozil		Rosiglitazone	
			Intra-day (n = 3)	Inter-day (n = 9)	Intra-day (n = 3)	Inter-day (n = 9)
(1) SFS method						
	250	60	98.44	99.18	102.31	101.716
	350	80	98.83	99.14	99.52	99.64812
	450	100	99.96	99.50	99.7	99.42092
	Mean ± SD		99.08 ± 0.79	99.27 ± 0.2	100.51 ± 1.56	100.26 ± 1.26
	N		3	9	3	9
	V		0.62	0.04	2.44	1.6
	SD		0.79	0.2	1.56	1.26
	RSD (%)		0.46	0.12	0.9	0.73
(2) Vierodt's method						
	500	100	97.9	98.51	101.26	100.61
	1000	160	100.55	100.48	99.38	99.29
	1500	200	100.76	100.35	100.03	99.71
	Mean ± SD		99.73 ± 1.6	99.78 ± 1.1	100.22 ± 0.96	99.87 ± 0.67
	N		3	9	3	9
	V		2.55	1.2	0.34	0.45
	SD		1.6	1.1	0.96	0.67
	RSD (%)		0.93	0.64	0.55	0.39

2.6. Procedure for spiked human plasma

A sample of 9.0 mL of drug-free human blood was taken from healthy volunteers into a 15 mL centrifugal tube containing 1 mL of 3.8% (w/v) solution of sodium citrate and immediately centrifuges the citrated whole blood at 3500 rpm for 15 min at 4 °C. Remove the upper two thirds of the liquid and freeze it rapidly in suitable quantities in plastic tubes at a temperature of −40° or below. Half milliliter aliquots of the plasma was transferred into a 25 mL separating funnels. Aliquot volumes of gemfibrozil and rosiglitazone standard working solutions in different ratios were added. The samples were mixed well using a vortex mixer. Two milliliters of phosphate buffer pH 7.0 was added to each funnel. The contents were mixed, then extracted with 3 × 10 mL of chloroform/dichloromethane (1:1, v/v) mixture. Filter the organic layer over anhydrous sodium sulfate. Dry the extract under vacuum at 40 °C till dryness. The residue was reconstituted in 3 mL of methanol. The procedure described under Section 2.4 was followed.

Table 4
Application of the proposed methods for determination of the studied drugs in their synthetic mixtures.

Method	Concentration taken (ng mL^{-1})		Concentration found (ng mL^{-1})		Recovery (%)	
	Gemfibrozil	Rosiglitazone	Gemfibrozil	Rosiglitazone	Gemfibrozil	Rosiglitazone
SFS method						
Mix. 1	700	20	697.28	20.48	99.61	102.41
Mix. 2	600	40	596.23	39.8	99.37	99.49
Mix. 3	500	60	497.8	60.78	99.56	101.31
Mix. 4	400	80	400.11	80.48	100.03	100.6
Mix. 5	300	100	297.06	100.24	99.02	100.24
Mix. 6	200	120	198.84	121.19	99.42	100.99
	Mean ± SD				99.5 ± 0.33	100.8 ± 0.99
	N				6	6
	V				0.11	0.99
	SD				0.33	0.99
	RSD (%)				0.13	0.4
Vierodt's method						
Mix. 1	1500	80	1512.66	78.79	100.84	98.49
Mix. 2	1000	140	1022.68	139.68	102.27	99.77
Mix. 3	600	160	593.38	157.04	98.9	98.15
Mix. 4	500	200	493.33	195.15	98.67	97.57
Mix. 5	400	240	390.96	233.96	97.74	97.49
Mix. 6	300	280	291.06	272.92	97.02	97.47
	Mean ± SD				99.24 ± 1.97	98.16 ± 0.89
	N				6	6
	V				3.87	0.34
	SD				1.97	0.89
	RSD (%)				0.81	0.8

Each result is the average of three separate determination.

Table 5
Application of the proposed methods for determination of the studied drugs in spiked human plasma.

Method	Concentration taken (ng mL^{-1})		Concentration found (ng mL^{-1})		% Recovery	
	Gemfibrozil	Rosiglitazone	Gemfibrozil	Rosiglitazone	Gemfibrozil	Rosiglitazone
(1) SFS method						
Mix. 1	700	20	682.53	20.82	97.50	104.10
Mix. 2	600	40	608.15	41.95	101.36	104.89
Mix. 3	500	60	516.37	60.78	103.27	101.29
	Mean ± SD				100.71 ± 2.9	103.43 ± 1.9
	N				3	3
	SD				2.9	1.9
	RSD (%)				1.68	1.06
(2) Vierodt's method						
Mix. 1	1500	80	1445.67	76.86	96.38	96.07
Mix. 2	1000	100	961.14	94.35	96.11	94.35
Mix. 3	500	160	477.47	156.69	95.49	97.93
	Mean ± SD				95.99 ± 0.45	96.11 ± 1.79
	N				3	3
	SD				0.45	1.79
	RSD (%)				0.27	1.07

Each result is the average of three separate determination.

The nominal content of the drugs was determined from previously plotted calibration graphs or using corresponding regression equations.

3. Results and discussion

The ability to analyze a binary mixture without resorting to tedious separation procedures is extremely useful for routine analysis. Therefore, two different spectrofluorimetric analytical methods were developed for simultaneous determination of binary mixture gemfibrozil and rosiglitazone in human plasma without prior separation steps. The first method is based on a synchronous fluorescence spectrometry using double scan. The second method used the technique of simultaneous equations (Vierodt's method).

3.1. Synchronous fluorescence spectroscopy (SFS)

3.1.1. Characteristics of fluorescence spectra

The normal excitation and emission spectra of gemfibrozil and rosiglitazone are shown in (Fig. 1), where gemfibrozil shows excitation maxima at 274 nm and emission maxima at 301 nm. Also rosiglitazone shows excitation maxima at 248 nm and emission maxima at 368 nm. As the emission spectra of gemfibrozil and rosiglitazone were partially overlapped, gemfibrozil and rosiglitazone cannot be determined directly by normal fluorimetric method. However, the synchronous fluorimetry can be used for determining both drugs simultaneously without separation procedure. Synchronous emission spectra of rosiglitazone were obtained by maintaining a constant interval ($\Delta\lambda$ = 120 nm) between emission and excitation wavelength at 368 and 248 nm, respectively,

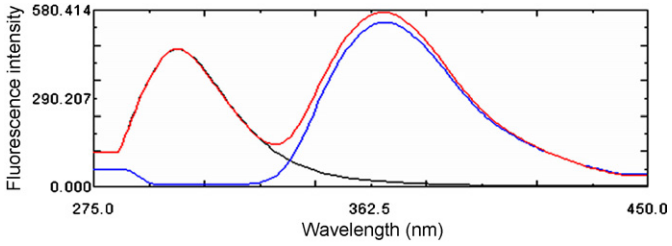

Fig. 7. Emission spectra of rosiglitazone {(▬), [200 ng mL^{-1}]}, gemfibrozil {(—), [1000 ng mL^{-1}]} and binary mixture of rosiglitazone and gemfibrozil {(▬), 200 and 1000 ng mL^{-1}, respectively} with λ_{Ex} = 258 nm for all spectra.

and maximum peak was at 368 nm as shown in (Fig. 2A). Also, synchronous emission spectra of gemfibrozil were obtained by maintaining a constant interval ($\Delta\lambda$ = 27 nm) between emission and excitation wavelength at 301 and 274 nm, respectively, and maximum peak was at 301 nm as shown in (Fig. 2A). When synchronous technique was applied, for the binary mixture gemfibrozil and rosiglitazone, using a 120 nm value for $\Delta\lambda$, only one single synchronous band at 368 nm was obtained (Fig. 2B), because the interval $\Delta\lambda$ can be found to match solely one pair of excitation and emission bands. Similarly, at $\Delta\lambda$ = 27 nm, only gemfibrozil yields a detectable signal that is independent of the presence of rosiglitazone (Fig. 2B).

3.1.2. Optimization of experimental variables

The different experimental parameters that affect the fluorescence intensity were carefully studied and optimized.

Table 6
Effect of drug added on the determination of gemfibrozil and rosiglitazone.

Drug added	Tolerance level (ng mL^{-1})			
	Gemfibrozil		Rosiglitazone	
	SFS method	Vierodt's method	SFS method	Vierodt's method
Paracetamol, betamethasone, caffeine, theophylline nifidipine	2000	2000	2000	2000
Aspirin	2000	2000	2000	1500
Ciprofloxacine	2000	2000	1800	2000
β-estradiol	5	30	1600	1000

Table 7
Statistical analysis of gemfibrozil and rosiglitazone by the proposed methods.

Items	Gemfibrozil			Rosiglitazone		
	SFS	Vierodt's method	Reported method [37]	SFS	Vierodt's method	Reported method [37]
Mean	100.71	95.99	97.97	1.03.43	96.11	99.3
N	3	3	3	3	3	3
SD	2.94	0.46	6.69	1.89	1.79	3.77
RSD (%)	1.69	0.27	3.94	1.13	1.08	2.19
F-test	0.32 (19)[a]	0.01 (19)[a]		0.4 (19)[a]	0.37 (19)[a]	
Student's *t*-test	0.64 (2.776)[a]	0.66 (2.776)[a]		0.22 (2.776)[a]	0.36 (2.776)[a]	

[a] The figures in parenthesis are the corresponding tabulated values at $P = 0.05$.

3.1.2.1. Excitation and emission slit width. The fluorescence signal in SFS has been determined largely by the excitation and emission slit widths used [38–42]. It is thought that the use of a narrow excitation slit width is beneficial to resolve the excitation structure of a compound and a broad emission slit widths is useful for high compound sensitivity (Taylor and Patterson, 1987). Therefore, throughout the study both the excitation and the emission slit widths were kept constant at 5 nm to obtain the best selectivity and good sensitivity.

3.1.2.2. Selection of optimum $\Delta\lambda$. The optimum $\Delta\lambda$ value is very important in synchronous scanning. It can narrow the spectral bands, simplify emission spectra and reduce the spectral range (Fig. 3) and its analytical application of resolving a mixture of two compounds maintaining a constant interval between the emission and excitation wavelength has already been reported [43–46]. A three-dimensional total synchronous fluorescence scans were used to obtain the optimum $\Delta\lambda$ for gemfibrozil and rosiglitazone. (Fig. 4) showing the three-dimensional graphs and their corresponding contour map corresponding 350 and 50 ng mL^{-1} of gemfibrozil and rosiglitazone, respectively. Scans were recorded from 15–140 nm. Scans with $\Delta\lambda$ below 15 nm were not useful for determination of gemfibrozil or rosiglitazone. Scans with $\Delta\lambda$ above 100 nm were not useful for determination of gemfibrozil. The plots show that the maximum fluorescence intensity can be obtained at $\Delta\lambda = 27$ nm and $\Delta\lambda = 120$ nm for gemfibrozil and rosiglitazone, respectively. These values are in agreement with values which can be obtained from emission–excitation normal spectra.

3.1.2.3. Influence of pH. To regulate the pH, a solution of 0.5 M KCl, in order to maintain constant ionic strength, was used with slightly additions of HCl or NaOH to produce the desired pH. The variation of the synchronous fluorescence intensity at 301 and 368 nm for gemfibrozil and rosiglitazone, respectively, versus pH are shown in (Fig. 5A). Rosiglitazone is weakly fluorescent in acidic media and its fluorescence increased with the increase of pH up to 9.0, after which remained constant up to pH 12.0, while gemfibrozil is highly fluorescent in both acidic and basic media with slightly increasing in fluorescence with increasing in pH up to pH 9.0, then remained constant up to pH 12.0. Therefore, a pH 9.0 was selected as a working pH. And borate buffer solution of standard pH 9.0 was used to adjust the pH. Also the concentration of borate buffer (pH 9.0) on fluorescence intensity of the studied drugs was studied. Increasing the concentration of borate buffer (pH 9.0) resulted in a gradual increasing in synchronous fluorescence intensity, of rosiglitazone, up to 10 mM after which the fluorescence remained constant till 35 mM (Fig. 5B). While increasing the concentration of borate buffer (pH 9.0) resulted in slightly increasing in synchronous fluorescence intensity, of gemfibrozil, up to 10 mM after which the fluorescence decreases gradually till 35 mM (Fig. 5B). Therefore, 10 mM of borate buffer (pH 9) was chosen as the optimum buffer concentration for both gemfibrozil and rosiglitazone.

3.1.2.4. Influence of solvents. Excitation and emission spectra in different solvents were recorded. It could be observed that methanol produced the highest synchronous fluorescence intensities compared with the other solvents as shown in (Fig. 6). Thus methanol was chosen as the diluting solvent throughout the study.

3.1.2.5. Luminescence stability. The effect of time on the stability of the synchronous fluorescence intensity of the studied drugs was studied. It was found that the fluorescence intensity developed instantaneously and remained stable for more than 2 h.

3.2. Simultaneous equations (Vierodt's method)

If a sample contains two fluorescent drugs (X and Y) each of which fluorescents at the maximum emission of the other (Fig. 7), ($\lambda_{Em_1} = 302$ nm for gemfibrozil) and ($\lambda_{Em_2} = 369$ nm for rosiglitazone), it may be possible to determine both drugs by the technique of simultaneous equations (Vierodt's method) [47]. There are some requirements which must be fulfilled to achieve the quantitative determination of either component X or Y:

- The fluorescence emission spectra of X and Y should not show severe overlap.
- X and Y should be chemically inert to one another i.e. no interaction should occur between them. This has been ascertained by applying a TLC for a mixture gemfibrozil and rosiglitazone. Separation was achieved on normal phase TLC silica–gel plate using a developing solvent consists of acetone–*n*-hexane (15:20, v/v) and UV detection at 254 nm. The TLC plate shows a complete separation of gemfibrozil from rosiglitazone with significant retardation factor (R_f) values of 0.66 and 0.0, respectively.
- The emission spectrum of the mixture of (X + Y) will be simply the summation of both emission spectra X and Y. Beer-Lambert's Law should be valid for both X and Y at (λ_{Em_1} and λ_{Em_2}) over a reasonable concentration range. Therefore, the fluorescence of the mixture at any wavelength is the summation of the fluorescence values of the components at this particular wavelength. It was observed that both drugs following fluorescence additivity study with respect to theoretical and practical obtained values.

From a consideration of the excitation and fluorescence emission spectra, 258 nm was selected as the excitation wavelength to produce the desirable degree of accuracy. The maximum emission wavelength of gemfibrozil and rosiglitazone were obtained at 302 and 369 nm, respectively (Fig. 7).

Let C_X and C_Y be the concentrations of (X and Y), respectively, in the diluted sample. Two equations are constructed based upon the fact that at λ_{Em_1} and λ_{Em_2} the fluorescent of the mixture is the sum of the individual fluorescence of (X and Y).

At λ_{Em_1}

$$F_1 = \alpha_1 b C_X + \beta_1 b C_Y \quad (1)$$

At λ_{Em_2}

$$F_2 = \alpha_2 b C_X + \beta_2 b C_Y \quad (2)$$

For measurements in 1 cm cells, $b = 1$

Rearrange Eq. (2)

$$C_Y = \frac{F_2 - (\alpha_2 C_X)}{\beta_2}$$

Substituting for C_Y in Eq. (1) and rearranging gives

$$C_X = \frac{F_1\beta_2 - F_2\beta_1}{\alpha_1\beta_2 - \alpha_2\beta_1} = \frac{2700109 \times F_1 - 22552.1 \times F_2}{417044 \times 2700109 - 8541.6 \times 22552.1} \quad (3)$$

and

$$C_Y = \frac{F_2\alpha_1 - F_1\alpha_2}{\alpha_1\beta_2 - \alpha_2\beta_1} = \frac{417044 \times F_2 - 8541.6 \times F_1}{417044 \times 2700109 - 8541.6 \times 22552.1} \quad (4)$$

where F_1 and F_2 are the fluorescence of the mixture at 302 and 369 nm, respectively. C_X and C_Y are the concentrations of gemfibrozil and rosiglitazone in g L^{-1}, respectively.

$$\alpha_1 = \frac{F_{X1} - F_{Blank1}}{\text{Standard Conc. of X}}; \quad \alpha_2 = \frac{F_{X2} - F_{Blank2}}{\text{Standard Conc. of X}};$$

$$\beta_1 = \frac{F_{Y1} - F_{Blank1}}{\text{Standard Conc. of Y}} \text{ and } \beta_2 = \frac{F_{Y2} - F_{Blank}}{\text{Standard Conc. of Y}}$$

3.3. Validation of the methods

3.3.1. Linearity, DL, and QL

Regression characteristics and system suitability parameters of the proposed methods are summarized in (Table 1). The detection limit and the quantification limit were calculated using the following equation [48].

$$\text{DL; QL} = \frac{(F \times \text{SD})}{b}$$

where F: factor of 3.3 and 10 for DL and QL, respectively. SD: standard deviation of the ordinate intercept and b: slope of the regression line.

3.3.2. Precision and accuracy

Precision was evaluated at three different concentrations for each drug, within the same day to obtain repeatability (intra-day precision) and over three different days to obtain intermediate precision (inter-day precision), both expressed as RSD % values. Accuracy was recorded as percent recovery ± standard deviation. Precision and accuracy results of the validation are summarized in (Tables 2 and 3).

3.4. Analysis of synthetic mixture of gemfibrozil and rosiglitazone

To test the proposed methods, a set of synthetic mixtures containing the two analytes in variable proportions were prepared and analyzed as described under (Section 2.4). The concentrations of both drugs in the synthetic mixture were calculated according to the linear regression equation of the calibration graphs. The results indicate high accuracy of the proposed methods as shown in (Table 4).

3.5. Analysis of spiked human plasma

The high sensitivity of the proposed methods allowed the determination of gemfibrozil and rosiglitazone in spiked human plasma. Rosiglitazone has an absolute oral bioavailability of 99% and, a peak plasma concentration of about 887.07 ng mL^{-1} is attained 1 h after a single 8 mg dose [49]. Gemfibrozil is rapidly and completely absorbed following oral administration, and a peak plasma concentration of about 20 μg mL^{-1} is attained 1–2 h after a single 600 mg dose [50]. These values lie above the working concentration range of the proposed methods. Thus they could be determined by the proposed methods. Table 5 shows the results obtained by the proposed methods. As can be seen the percent recovery values by SFS method were between 97.5% and 103.27% for gemfibrozil and between 101.29% and 104.89% for rosiglitazone. Also, the percent recovery values by Vierodt's method were between 95.49% and 96.38% for gemfibrozil and between 94.35% and 97.98% for rosiglitazone. The results indicate that the proposed methods are so sensitive enough to determine the studied drugs in spiked human plasma with satisfactory accuracy and precision.

3.6. Interference studies

Under the optimal conditions, the influence of possible concomitant administrated drugs on the determination of rosiglitazone and gemfibrozil are investigated. A 2000 ng mL^{-1} level of each interfering species was tested. If the interference occurred, the ratio was decreased gradually till the interference ceased. The tolerance level was defined as an error not exceeding ±5% in the determination of the analyte. The results were summarized in (Table 6). Most added drugs are tolerated in relatively high concentration. Only β-estradiol causes interference with gemfibrozil, in the same time β-estradiol is tolerated in high concentration with rosiglitazone.

3.7. Statistical analysis of the results in comparison with the reported methods

The results of the analysis of the drugs were compared statistically by the student's t-test and the variance ratio F-test with those obtained by the reported methods [37]. The student's t-values at 95% confidence level did not exceed the theoretical values, indicating that there was no significant difference between the proposed methods and the reported methods. It was also noticed that the variance ratio F-values calculated for $p = 0.05$ did not exceed the theoretical values, indicating that there was no significant difference between the precision of the proposed methods and the reported methods. The results are given in (Table 7).

4. Conclusion

The present study proposes simple, inexpensive, precise, accurate and highly sensitive methods for the simultaneous determination of binary mixture gemfibrozil and rosiglitazone in human plasma without prior separation steps. Unlike the gas chromatography and HPLC procedures, the instrument is simple and is not of high cost. The reagents utilized in the proposed method are cheaper and readily available besides they are being less time consuming.

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