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Spectrofluorimetric and micelle-enhanced spectrofluorimetric methods for the determination of gemfibrozil in pharmaceutical preparations

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Abstract

A spectrofluorimetric method for the determination of antihyperlipoproteinemic gemfibrozil was developed based on its native fluorescence. This method allows the determination of 0.10–6 μg ml^{-1} gemfibrozil in aqueous solution (without using any buffer solution) with excitation and emission wavelengths of 276 and 304 nm, respectively. Detection and quantification limits were 0.03 and 0.10 μg ml^{-1}, respectively. The fluorescence properties of gemfibrozil in micellar media were also studied. It was shown that in the presence of 0.4% Brij-35 surfactant (pH 4.0, acetic acid–acetate buffer) about 2.4-fold enhancement can be achieved in the fluorescence of this drug. Based on the obtained results, a micelle-enhanced fluorescence method was also developed that is more sensitive than aqueous fluorescence method and has lower detection limit (0.02 μg ml^{-1}). Both methods were applied satisfactorily to the determination of gemfibrozil in a commercial pharmaceutical formulation.

Keywords: Gemfibrozil; Spectrofluorimetry; Micelle-enhanced spectrofluorimetry

1. Introduction

Gemfibrozil, 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid (Fig. 1), is a fibrate hypolipidemic agent that is clinically effective in reducing serum cholesterol and triglyceride levels. It has also been demonstrated that this drug lowers the incidence of coronary heart disease in humans [1,2]. In both animals and humans it is extensively metabolized to four oxidized metabolites and an acylglucoronide conjugate [3]. More than 90% of orally administered gemfibrozil is absorbed and most of the absorbed drug is excreted in urine. Its plasma half-life is about 1.5 h and the peak blood levels are reached 1–2 h after ingestion [1].

Some reports on gemfibrozil determination have appeared in the literature. Gas chromatography [4] and high performance liquid chromatography (HPLC) with ultraviolet or fluorimetric detection [3,5–10] have been used for the determination of gemfibrozil and its metabolites in plasma and urine samples. Near-infrared diffuse reflectance

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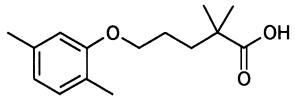

Fig. 1. Structure of gemfibrozil.

spectroscopy [11–14] has been applied to the determination of gemfibrozil in pharmaceutical preparations. Also a liquid chromatography–mass spectrometric method [15] has been reported for the determination of this drug in water and waste water samples.

In the present paper, the fluorescence characteristics of gemfibrozil in aqueous and micellar media are investigated. Based on obtained results sensitive spectrofluorimetric and micelle-enhanced spectrofluorimetric methods have been developed for the determination of gemfibrozil in pharmaceutical preparations. To the best of our knowledge, this is the first attempt to determine gemfibrozil in pharmaceutical formulations by spectrofluorimetry. The main advantages of the proposed methods compared with previously reported methods are their simplicity and lower cost. The detection limits are comparable or better than the detection limits of the most of chromatographic methods [3,4,6,7,10].

2. Experimental

2.1. Reagents

All reagent used were of analytical-reagent grade. Triply distilled water (obtained from all-glass apparatus) was used throughout.

Gemfibrozil (Dipharma, Italy) was kindly provided by Shahre Darou Laboratory. A stock standard solution of 400 μg ml^{-1} was prepared by dissolving 40.0 mg of gemfibrozil in approximately 10 ml of 0.1 M sodium hydroxide and diluting to the mark in 100 ml volumetric flask with water. Polyoxyethelene lauryl ether (Brij-35), sodium dodecyl sulfate (SDS) and *N*-cetyl-*N*,*N*,*N*-trimethylamonium bromide (CTAB), acetic acid, sodium acetate, sodium hydroxide and hydrochloric acid were purchased from Merck (Darmstadt, Germany). A 2% m/v stock solution of Brij-35 was prepared in triply distilled water.

2.2. Apparatus

Fluorescence spectra and intensity measurements were made on a Shimadzu RF-540 spectrofluorimeter (Kyoto, Japan) equipped with a 150 W xenon lamp, using 1.0 cm quartz cell. Slit widths of both monochromator were set at 5 nm. All measurements were performed at 25 ± 0.1 °C by the use of a thermostated cell holder and a thermostatically controlled water bath (Tokyo, Rikakika Ltd., UA-1). Absorbance measurements were carried out using a Shimadzu UV-265FW spectrophotometer (Kyoto, Japan). A Metrohm model 654 pH meter (Herisau, Switzerland) was used for pH measurements.

2.3. Calibration graphs

For gemfibrozil determination in aqueous solution, calibration solutions were prepared by suitable dilution of stock standard solution of gemfibrozil in 10.0 ml calibrated flasks, so that concentrations in the range of 0–6 μg ml^{-1} were obtained. There is no need for using buffer solution to adjust the pH. The solutions were thermostated at 25 ± 0.1 °C and fluorescence was measured at 304 nm using an excitation wavelength of 276 nm.

In micelle-enhanced spectrofluorimetric method, calibration solutions were prepared in the concentration range of 0–3 μg ml^{-1}. For each 10.0 ml calibrated flask, after addition of a suitable amount of gemfibrozil standard solution, 2 ml of stock solution of Brij-35 and 2 ml of 0.1 M acetic acid–acetate buffer (pH 4.0) were also added and diluted to the mark with water. The solutions were thermostated at 25 ± 0.1 °C and fluorescence was measured at 302 nm using an excitation wavelength of 276 nm.

2.4. Pharmaceutical preparation

Ten capsules of gemfibrozil were weighed in order to find the average mass of each capsule. Then the contents were powdered and mixed. A

portion of 10.0 mg of this powder was accurately weighed and dissolved in about 10 ml 0.1 M NaOH solution and filtered into a 100 ml volumetric flask. The residue was washed several times with water and solution was diluted to the mark. A suitable aliquot of this solution was taken for spectrofluorimetric or micelle-enhanced spectrofluorimetric determination of gemfibrozil. The recovery assay was carried out using the same procedure but adding known amount of gemfibrozil.

3. Results and discussion

3.1. Fluorescence characteristics of gemfibrozil in aqueous solution

The solubility of gemfibrozil in pure water is very low but it can be easily solubilized in the presence of sodium hydroxide.

Gemfibrozil exhibits the analytically useful, native fluorescence in aqueous solution which derived from the benzene moiety. Fig. 2 shows the fluorescence excitation and emission spectra of this drug in NaOH solution. As can be seen gemfibrizil presents two peaks at excitation wavelengths of 228 and 276 nm. This is justified in theory by the light absorption promoting an electron from the ground electronic state to the second and first excited singlet states, respectively. The fluorescence emission spectrum gives a band with a characteristic wavelength of 304 nm, corresponding to the transition from the first excited singlet state to the ground state.

The effect of irradiation with spetrofluorimeter xenon lamp ($\lambda_{ex} = 276$ nm) on the fluorescence emission of gemfibrozil ($\lambda_{em} = 304$ nm) over 1 h was studied. The results showed that fluorescence intensity was practically stable and unaffected by irradiation.

The influence of pH in the range of 2–12 on the fluorescence of gemfibrozil was studied using

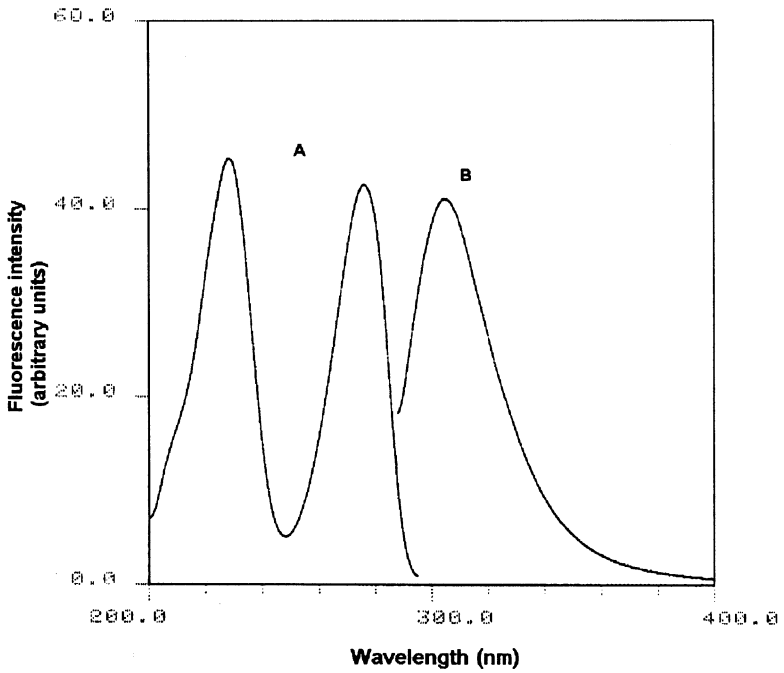

Fig. 2. Fluorescence excitation (A) ($\lambda_{em} = 304$ nm) and emission (B) ($\lambda_{ex} = 276$ nm) spectra of gemfibrozil (2 μg ml^{-1}) in 0.1 M NaOH solution.

NaOH and HCl. The results indicate that the spectral characteristics are almost independent of pH solution. No significant changes of fluorescence intensity as a function of pH were observed.

With the aim of studying the influence of ionic strength, aqueous solutions of gemfibrozil at various concentrations of NaCl were prepared. The results showed no fluorescence intensity variations for concentrations <1 M NaCl.

Another factor that affects the fluorescence intensity is temperature; the fluorescence intensity of gemfibrozil decreases when the temperature increases from 10 to 50 °C. This decrease is nearly linear with temperature coefficient of about 0.8% per °C. This effect can be explained by higher internal conversion as temperature increases, facilitating non-radiative deactivation of excited singlet state [16]. A temperature of 25 °C, close to room temperature, was selected for spectrofluorimetric determination of gemfibrozil.

3.2. Fluorescence characteristics of gemfibrozil in micellar media

It is well known that the addition of a surfactant at a concentration above its critical micellar concentration (CMC) to a given fluorophore solution increases the molar absoptivity and/or fluorescence quantum yield of fluorophore in many cases [17,18]. This fact has been used to improve the performance of spectrofluorimetric methods for the determination of various analytes [19–21].

The fluorescence properties of gemfibrozil in various micellar media were studied using anionic (SDS), cationic (CTAB) and non-ionic (Brij-35) surfactants. There was an enhancement of fluorescence intensity in the presence of SDS and Brij-35 compared with aqueous solution. Since, micelle enhancement factor for Brij-35 was higher than SDS (about 2.4 compared with about 2) and, on the other hand, the background fluorescence was lower in the case of Brij-35, thus this surfactant was chosen for further works. Fig. 3 shows the fluorescence emission spectra of gemfibrozil in the Brij-35 micellar solution. A slight blue shift in the emission maximum was observed in this media ($\lambda_{em} = 302$ nm).

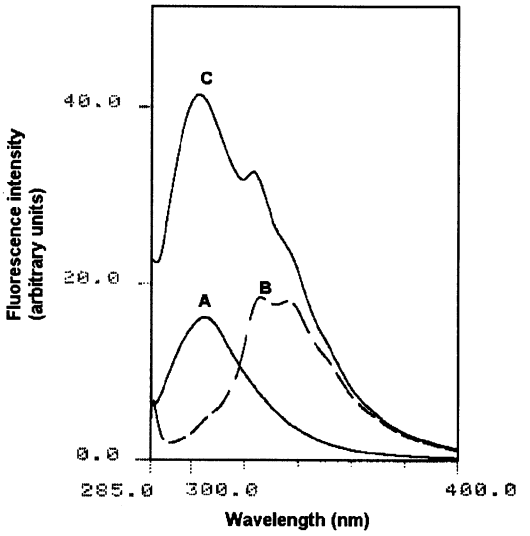

Fig. 3. Fluorescence emission spectra of, (A) 1 μg ml^{-1} gemfibrozil; (B) 0.4% Brij-35; and (C) 1 μg ml^{-1} gemfibrozil in 0.4% Brij-35 solution (pH 4.0, $\lambda_{ex} = 276$ nm).

The influence of pH on the micelle-enhanced fluorescence was studied. The results are shown in Fig. 4. As can be seen, the fluorescence intensity of gemfibrozil in micellar media reaches a maximum plateau below the pH 6, hence pH 4.0 was selected for further measurements in micellar media. This pH was adjusted by using sodium acetate–acetic acid buffer. The concentration of this buffer up to 0.5 M did not affect the fluorescence intensity.

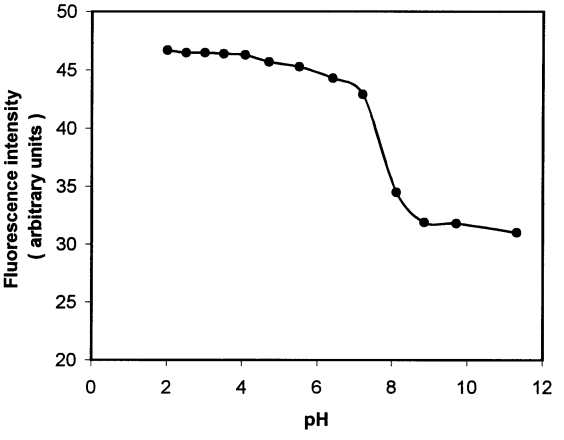

Fig. 4. Influence of pH on the fluorescence intensity of 1 μg ml^{-1} gemfibrozil in 0.4% Brij-35 solution ($\lambda_{ex} = 276$ nm and $\lambda_{em} = 302$ nm).

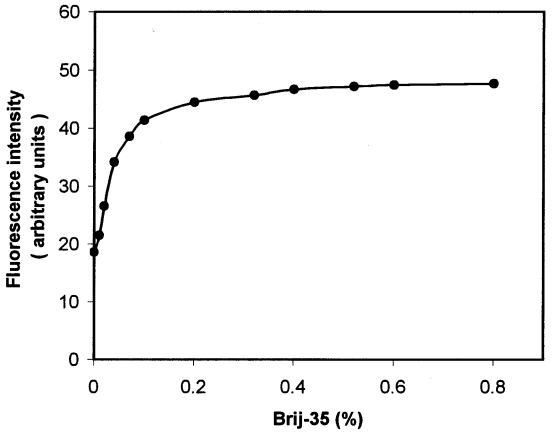

Fig. 5. Influence of Brij-35 concentration on the fluorescence intensity of 1 μg ml^{-1} gemfibrozil (pH 4.0, $\lambda_{ex} = 276$ nm and $\lambda_{em} = 302$ nm).

Fig. 5 shows the effect of Brij-35 concentration on the fluorescence of gemfibrozil. The fluorescence intensity increases with the increase of Brij-35 concentration and reaches a stable level at 0.3% Brij-35. Further additions of this surfactant provoked no increment of fluorescence intensity; thus a 0.4% concentration was chosen as suitable concentration.

In order to establish whether the sensitization process of gemfibrozil fluorescence is due only to an increase in quantum yield or whether it is affected by an increase in absorption at the excitation wavelength, the molar absorptivity of gemfibrozil in the presence of Brij-35 was determined at 274 nm. The $\varepsilon_{micellar}/\varepsilon_{water}$ ratio is approximately 1.1, which indicates that the increase in sensitivity is mainly due to an increase in the fluorescence quantum yield of gemfibrozil (by protection of the lowest excited singlet state in the micellar microenvironment from non-radiative processes).

3.3. Analytical figures of merit

For both spectrofluorimetric and micelle-enhanced spectrofluorimetric methods, a series of ten standard solutions (three replicate for each) of gemfibrozil were measured by following the procedures under Experimental. The equation for calibration graph in all cases is $F = h + mC$, where F is the fluorescence intensity (in arbitrary units) and C is the concentration of gemfibrozil expressed in μg ml^{-1}. Table 1 summarizes the results obtained from a statistical analysis of the data. The calibration graphs were found to be linear in the ranges of 0.10–6 and 0.05–3 μg ml^{-1} in aqueous and micellar media, respectively. The detection and quantification limits were calculated as $3/m[s_b^2 + s_h^2 + (h/m)^2\, s_m^2]^{1/2}$ and $10/m[s_b^2 + s_h^2 + (h/m)^2\, s_m^2]^{1/2}$ where s_b, s_h and s_m are the standard deviation of the blank, intercept and slope, respectively [22]. The slope of the calibration graph (m) is the calibration sensitivity according to IUPAC definition. As can be appreciated, the analytical parameters, specially the sensitivity, significantly improve in the presence of Brij-35 surfactant.

In order to study the precision of the aqueous fluorescence method, a series of nine solutions of 1

Table 1
Analytical parameters for gemfibrozil in aqueous and micellar solutions

	Aqueous solution	Brij-35 micellar solution
Linear range (μg ml^{-1})	0.10–6	0.05–3
Intercept on the ordinate (h)	3.68	7.63
Standard deviation of intercept (s_h)	0.31	0.59
Slope (m)	36.31	85.14
Standard deviation of slope (s_m)	0.10	0.40
Number of points (n)	10	10
Correlation coefficient (r)	0.9999	0.9999
Detection limit (μg ml^{-1})	0.03	0.02
Quantification limit (μg ml^{-1})	0.10	0.07

Table 2
Determination of gemfibrozil in a pharmaceutical preparation (300 mg capsules)

	Aqueous fluorescence method	Micelle-enhanced fluorescence method
Found $\pm s$ (mg per capsule)[a]	296 $\pm$ 6	297 $\pm$ 6
Recovery $\pm s$ (%)[b]	99 $\pm$ 2	99 $\pm$ 2

[a] Standard deviation (average of five determinations).
[b] Recovery is calculated from the content reported by the laboratory.

Table 3
Recovery of gemfibrozil added to a pharmaceutical preparation

Amount added (μg ml^{-1})[a]	Amount found (μg ml^{-1})[b]	
	Aqueous fluorescence method	Micelle-enhanced fluorescence method
0.160	0.17±0.01, (104±5)	0.16±0.01, (100±4)
0.400	0.40±0.02, (101±4)	0.39±0.02, (99±2)
0.740	0.76±0.01, (102±2)	0.73±0.01, (99±1)
1.00	0.99±0.03, (99±3)	1.02±0.01, (102±1)
1.50	1.58±0.03, (105±2)	1.55±0.03, (103±2)
2.50	2.53±0.02, (101±1)	2.55±0.04, (102±2)
5.20	5.19±0.09, (100±2)	–

[a] The given values are concentrations in the final solutions.
[b] Average of three determinations±S.D.

μg ml^{-1} gemfibrozil were measured on the same day. By applying the IUPAC definition, the relative error is 0.6%. The relative standard deviation for nine replicate analysis was 1.09%.

The precision of micelle-enhanced fluorescence method was also studied by measuring a series of nine solution of 0.6 μg ml^{-1} gemfibrozil on the same day. The relative error and the relative standard deviation were 1.66 and 2.06%, respectively.

3.4. Application

Gemfibrozil was satisfactorily determined in the only one pharmaceutical product commercialized in Iran (Gemfibrozil capsules with a nominal content of 300 mg) by using both spectrofluorimetric and micelle-enhanced spectrofluorimetric methods. According to spectral characteristics of fluorescence spectra obtained for this preparation there is no interference from the excipients. Table 2 shows the results for five replicate analyses. Statistical analysis of the assay results showed satisfactory precision of the both proposed methods with no significant differences between certified and experimental results.

Recovery experiments on pharmaceutical preparations spiked with different amounts of gemfibrozil were also carried out (Table 3). As can be seen, the obtained recoveries are between 99 and 105% and RSDs are between 1 and 5%.

4. Conclusions

Direct spectrofluorimetry is a simple, accurate, fast and precise procedure to determine gemfibrozil in aqueous solution. The method was applied successfully to the determination of active constituent of commercial pharmaceuticals.

The use of Brij-35 micellar system provides a simple means to enhance the fluorescence from gemfibrozil. This phenomenon can be explained by protection of lowest excited state of fluorophore in micellar microenvironment from non-radiative or possible quenching processes that normally readily occur in bulk aqueous solutions. The addition of 0.4% Brij-35 gives about 2.4-fold increase in sensitivity and improve the limit of detection without further sample manipulation.

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