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Determination of Gemfibrozil in Drug Matrix and Human Biological Fluid by Dispersive Liquid-Liquid Microextraction with High Performance Liquid Chromatography

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ABSTRACT

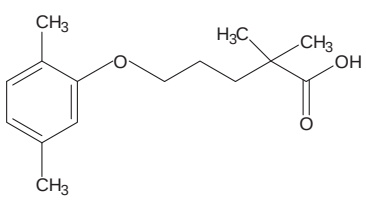
A simple and rapid dispersive liquid-liquid microextraction technique coupled with high performance liquid chromatography (HPLC) was developed for the extraction and analysis of gemfibrozil in drug matrix and biological fluids like human plasma and urine samples. In this method, carbon tetrachloride at microliter volume level and methanol were used as extraction and disperser solvents, respectively. The effects of factors including the type and volume of disperser and extraction solvents, pH and extraction time on the performance of the sample preparation were carefully evaluated. Under optimum conditions, the obtained enrichment factors were between 9.2 - 9.3 and extraction recoveries were high and ranged between 91.2 - 93.2%. The calibration curve was linear in the range of 0.1 - 100 mg/L with a detection limit of 12.3 μg/L. The relative standard deviation (RSD) for ten replicate measurements of gemfibrozil was 1.3%. The performance of the method was checked by the analysis of the gemfibrozil in presence of other drugs in water samples.

Key words: dispersive liquid-liquid microextraction, gemfibrozil, sample preparation, biological fluid, drug matrix

INTRODUCTION

Gemfibrozil 5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid (Figure 1) is a lipid- and cholesterol-modifying drug with lipophilic character and poor water solubility. It reduces triglycerides and increases cholesterol carried in high density lipoprotein (HDL) in the blood. HDL cholesterol is sometimes called ''good'' cholesterol because higher concentrations of HDL cholesterol in the blood are associated with a reduced risk of heart disease. The decrease in triglycerides is thought to be due in part to reduced release of triglycerides from fat tissue in the body[1,2]. Several methods have been developed for the determination of gemfibrozil in tablet, plasma and urine, including high performance chromatography[1,3], pressurized liquid extraction coupled to liquid chromatography-tandem mass spectrometry[4] and solid phase extraction- gas chromatography-mass spectrometry[5]. Moreover, Gemfibrozil exhibits native fluorescence[6]. This property has been used for the determination of the drug in plasma by high performance liquid chromatography (HPLC) with fluorescence detection[7] and to develop other spectrofluorimetric methods[8].

Dispersive liquid-liquid microextraction employs a water-immiscible solvent as the extractant and a water-miscible polar solvent as the disperser. Acetone, methanol and acetonitrile can be used as disperser solvents; whereas chlorinated solvents like tetrachloromethane, chlorobenzene and chloroform are useful as extractants. An appropriate mixture of an extraction solvent and a disperser solvent is rapidly injected into an aqueous sample so that a cloudy solution is formed. The analyte in the sample is then transferred to the fine droplets of the extraction solvent and phase



Gemfibrozil

Figure 1. The structures of the gemfibrozil.

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separation is performed by centrifugation. The advantages of this method include its simplicity of operation, low cost, rapidity, low consumption of organic solvents and high enrichment factors. Moreover, in this technique the large contact surface between the sample and the droplets speeds up the mass transfer processes. The technique has been applied to the determination of trace inorganic pollutants[9-11] and trace inorganic samples[12-14] dealing with miniaturized preconcentration methods.

The goal of this work is to combine dispersive liquid-liquid microextraction with high performance liquid chromatography to develop a new procedure for the determination of gemfibrozil in biological samples and drug matrix. The factors that affect on dispersive liquid-liquid microextraction extraction such as the influence of extractant and disperser solvents, as well as other parameters were considered and optimized. Finally the optimal conditions were used to extract gemfibrozil in biological and pharmaceutical samples.

MATERIALS AND METHODS

I. *Chemicals and Reagents*

Carbon tetrachloride, chloroform, dichloromethane, nitrobenzene and dichlorobenzene as extraction solvents and acetone, acetonitrile, ethanol, isopropanol and methanol as dispersive solvents, sodium chloride and other substances were purchased from Merck (Darmstadt, Germany).

The stock solution of gemfibrozil (100 mg/L) was prepared by dissolving appropriate amounts of pure gemfibrozil (from Alborz Daro Inc., Tehran, Iran) in methanol. Acetate, ammonia and phosphate buffer (0.01 M) were used to adjust the pH of the solutions in the range of 1 - 13, wherever suitable.

II. *HPLC System*

Chromatographic analysis was carried out on an Agilent HPLC, G1314B model, equipped with a UV/Vis detector. Separations were carried out on a Zorbax Extend C18 column (150 mm × 4.6 mm, 3 µm particle size) from Agilent (Wilmington, DE, USA). A mixture of methanol, water and glacial acetic acid (75 : 24 : 1) at a flow rate of 1 mL/min was used as a mobile phase in isocratic elution mode. The injection volume was 20 µL for all the samples and the detection was performed at a wavelength of 276 nm.

III. *Dispersive Liquid-Liquid Microextraction Procedure*

A 10.0 mL portion of double-distilled water was placed in a 20-mL glass tube with conical bottom and spiked with different levels of gemfibrozil. A mixture of the disperser solvent (1.0 mL of methanol) and extraction solvent (100 µL of carbon tetrachloride) was injected rapidly into the sample solution by using a 2.0-mL gastight syringe. The cloudy solution produced was centrifuged for 3 min at 4,000 rpm. After centrifuging, the dispersed fine droplets of carbon tetrachloride formed the bottom layer in the test tube (about 80 ± 5 µL). The bottom layer was completely transferred to another test tube with a conical bottom using a 100-µL HPLC syringe. After the evaporation of the solvent in water bath (50°C) the residue was dissolved in 1 mL of HPLC grade methanol and injected into the HPLC system. All experiments were performed in triplicate and the mean values of the results were reported. The gemfibrozil concentration for optimization of conditions was 2 mg/L.

IV. *The Enrichment Factor*

Dispersive liquid-liquid microextraction combined with HPLC-UV was developed for sample preparation and the determination of gemfibrozil in drug matrix, biological fluids and water samples. To obtain optimum conditions, parameters such as the type of extraction solvent, disperser solvent, pH of solution and salt additions were examined. The enrichment factor (EF) was defined as the ratio between the analyte concentration in the settled phase (C_{set}) and the initial concentration of analyte (C_0) in the aqueous sample.

$$EF = C_{set}/C_0$$

The C_{set} was obtained from direct injection of the analyte standard solution in the extraction solvent.

RESULTS AND DISCUSSION

I. *HPLC Method Validation*

The main figures of merit are given in Table 1. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. Ten replicate determinations of 0.1 mg/L of gemfibrozil solutions gave a relative standard deviation of 1.3%, indicating good reproducibility of the extraction.

II. *Selection of Extraction Solvent*

Selection of suitable organic solvents based on the requirement of a higher density than that of water, the solvent's extraction capability for selected compounds, and good

Table 1 Figures of merit of the procedure

Calibration concentration range	0.1 - 100 (mg/L)
Calibration equation	Y = 6.65x + 2.4491
Correlation coefficient	0.9991
Limit of detection	12.3(µg/L)
Limit of quantification	41(µg/L)
RSD (n =10)	1.3%
Enrichment factor	9.3

Table 2 The examined solvents and their properties

Solvent	chloroform	dichloroethane	dichloromethane	carbon tetrachloride	nitrobenzene
Density (g/mL)	1.480	1.256	1.250	1.584	1.20
Boiling point (°C)	61.2	83	39.8	76.8	210
Formula	$CHCl_3$	$C_2H_4Cl_2$	CH_2Cl_2	CCl_4	$C_6H_5NO_2$
Molecular weight (g/mol)	38.12	96.98	93.84	82.15	123.11
Enrichment factor	8.65	5.98	7.78	9.39	4.44

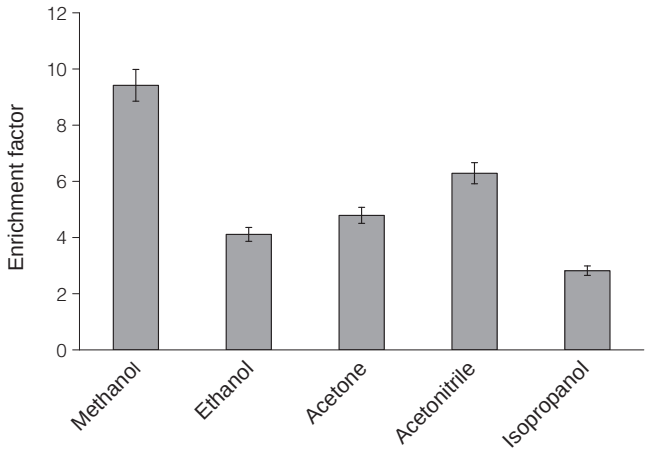

Figure 2. The effect of the type of disperser solvent on the EF of gemfibrozil. Extraction conditions: water sample volume, 10.0 mL; extraction solvent (carbon tetrachloride) 100 µL, concentration of gemfibrozil 2 mg/L.

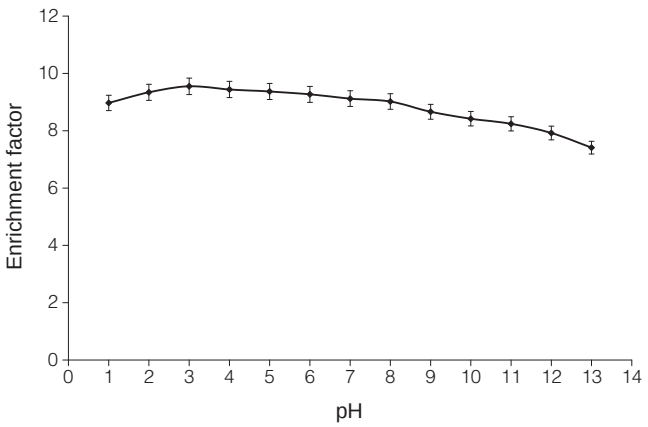

Figure 3. The effect of the pH of solution on extraction of gemfibrozil.

chromatographic behavior are vitally important in dispersive liquid-liquid microextraction. Some solvents listed in Table 1 with different properties like density, solubility and polarities were considered as extraction solvents. The results in Table 2 revealed that carbon tetrachloride has the highest enrichment factor in comparison with the other tested solvents.

III. *Selection of Disperser Solvent*

The miscibility of the disperser solvent in the extraction solvent and aqueous phase is the most important factor affecting the selection of disperser solvent in dispersive liquid-liquid microextraction. Methanol, ethanol, acetone, acetonitrile and isopropanol have this property and were selected for this purpose. A series of sample solutions were studied by using 1.00 mL of each disperser solvent containing 100 µL of carbon tetrachloride as the extraction solvent. The results are represented in Figure 2. The obtained results indicated that the EF% by using methanol as the disperser solvent was remarkably higher than those of other used solvents. Therefore, it is selected as the disperser solvent.

IV. *Effect of pH*

The degree of gemfibrozil extraction at different pH values was investigated (Figure 3). The maximum extraction was achieved at acidic pH where gemfibrozil exist mainly as a neutral molecule and tends more to organic solvent. The results in Figure 3 revealed that the highest enrichment factor can be obtained at pH 3.

V. *Effect of Disperser Solvent Volume*

The effect of the volume of the disperser solvent, methanol, was studied over the range of 0.25 - 2.5 mL. At lower volumes of methanol, the cloudy suspension of droplets was not well-formed, resulting in a decrease in enrichment factor. At higher volumes of methanol, the solubility of gemfibrozil in water increased and the extraction efficiency decreased. Figure 4 demonstrates that 1 mL of methanol is the optimum volume of the disperser solvent.

VI. *Effect of Extraction Time*

Mass transfer is a time-dependent process and one of the salient factors in most extraction procedures, especially in microextraction methods. In dispersive liquid-liquid microextraction, extraction time is defined as the time interval between injecting the mixture of disperser solvent and extraction solvent and starting to centrifuge. The effect of extraction time was studied over the range of 0.1 - 15 min, with other experimental conditions remaining constant (Figure 5). According to other literatures, [15-22] time has no influence on the EF%, because after the formation of the cloudy solution, the surface area between the extraction solvent and

the aqueous phase is very high. Hence, the transition of the analytes from the aqueous phase to the extraction solvent is fast. Subsequently, the equilibrium state is achieved quickly so the extraction time is very short. Therefore, time independence is the chief advantage of the dispersive liquid-liquid microextraction technique. In the present method, the time-consuming extraction with maximum recovery and enrichment factor takes about 2 min.

VII. *Salt Addition*

The salting-out effect has been used universally in different types of liquid-liquid extraction. Usually, the addition of salt decreases the solubility of analytes in the aqueous sample and enhances their partitioning into the organic phase. In this work, the effect of salt addition was considered by adding sodium chloride (NaCl; 0 - 10%, w/v) into the water sample spiked with gemfibrozil, while experimental extraction conditions were the same as those described before. It was observed the enrichment factors were increased at a level of about 7% w/v of NaCl in solution (Figure 6).

VIII. *Effect of Concentration of Gemfibrozil*

The concentration of gemfibrozil at a level of 0.1 - 100 mg/L in water sample solution was examined. The results in Figure 7 demonstrated that the extraction recoveries for all drug concentrations were more than 91%. The extraction recovery was defined as the percentage of the total drug extracted into the settled phase.

IX. *Effects of Coexisting Drug*

The possibility of extraction in the presence of other drugs was considered. This extraction was studied, using solutions containing 6 mg/L of naproxen as the interfering drug and 0 - 20 mg/L of gemfibrozil. The high recoveries of gemfibrozil in all extractions, indicating that extraction in the presence of a similar drug was possible.

X. *Application of Method*

In order to study the suitability of the proposed dispersive

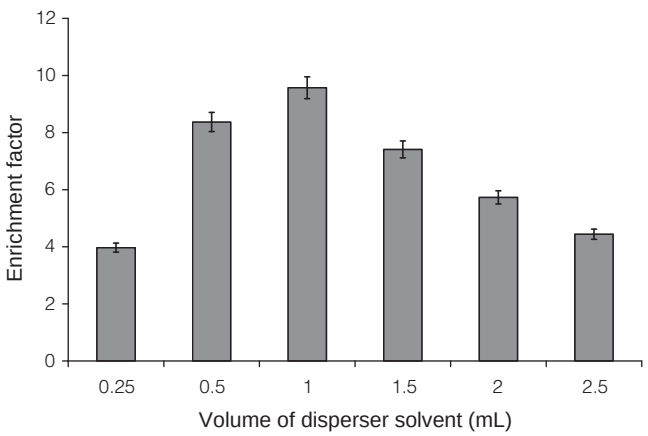

Figure 4. The effect of the extraction solvent (CCl_4) volume on the EF of gemfibrozil.

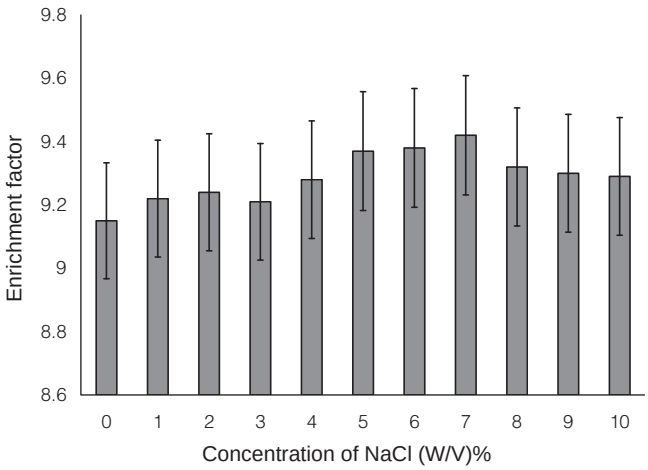

Figure 6. The effect of adding salt into the extraction solution on the EF of gemfibrozil.

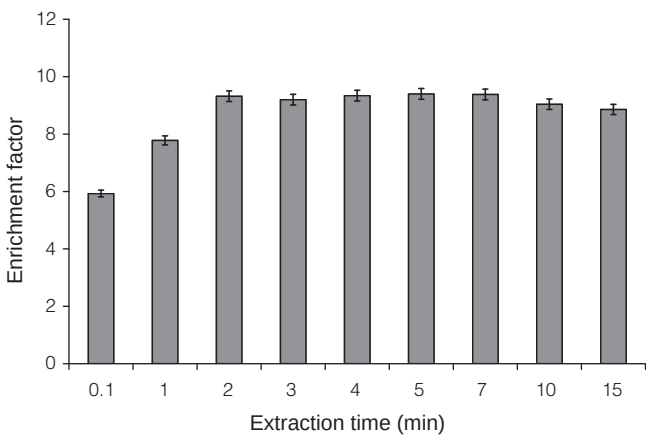

Figure 5. The effect of the extraction time on the EF of gemfibrozil.

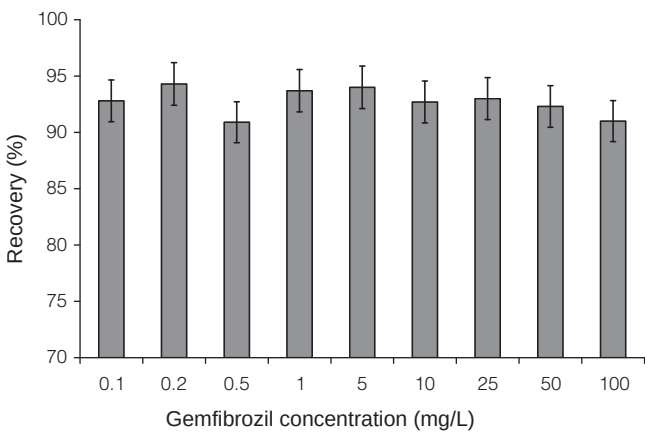

Figure 7. The effect of gemfibrozil concentration on the recovery.

Table 3 Determination of gemfibrozil in different samples

Sample	Concentration (μg/L)	Added (μg/L)	Found (μg/L)	Recovery (%)
Capsule	100.0	_____	93.2 ± 2.3	93.2
		100	92.3 ± 2.4	92.3
Urine	_____	500	461.1 ± 3.8	92.2
		1000	912.2 ± 4.1	91.2
		100	92.2 ± 2.6	92.2
Plasma	_____	500	462.0 ± 4.0	92.4
		1000	915.1 ± 4.3	91.5

For 3 experiments

liquid-liquid microextraction method for the determination of gemfibrozil in real samples, the developed technique was applied for the extraction of the drug from urine, plasma and drug matrix. Ten capsules (weighing 4.0130 ± 1 g) of gemfibrozil were extracted with methanol until a solid residue remained. The results demonstrate the applicability of the procedure for gemfibrozil determination in pharmaceutical samples with high recovery (> 93%).

Microextraction of gemfibrozil from human biological fluids like blood serum and urine were also studied. Human blood was collected from thoroughly controlled voluntary blood donors (from Tehran Clinic Hospital Medical Laboratory). Each unit was separately controlled and found negative for HBS antigen and HIV I, II and hepatitis C antibodies. No preservatives were added to the samples. Human blood was collected into EDTA-containing vacutainers and red blood cells were separated from plasma by centrifugation at 4000 × g for 30 min at room temperature, then filtered (3-μm Sartorius filter) and frozen at -20°C. Before use, the plasma was thawed for 1 h at 37°C. In order to reduce the matrix effect, the plasma was again centrifuged at 5000 ×g for 30 min at room temperature, then was diluted 1 : 5 with distilled water then spiked with gemfibrozil (100 - 1000 μg/L) before subjecting it to the recommended procedure. The extracted phase was filtered to obtain a clear solution and remove the dirty solution at the bottom of the test tubes. The experiments were performed in triplicates. The results are shown in Table 3 and indicate the suitability of the present method for the extraction and determination of gemfibrozil from plasma samples. All results in Table 3 indicate the reasonability and reliability of the proposed microextraction procedure.

CONCLUSIONS

Dispersive liquid-liquid microextraction combined with HPLC detection was applied for the determination of gemfibrozil in drug matrix and biological fluid samples. The method is rapid, simple and inexpensive. The uses of toxic organic solvents as well as time were minimized without affecting the sensitivity of the method. Although the results obtained in this study are related to the determination of gemfibrozil with HPLC, the method could be readily applied for the determination of the drug with other analytical instruments.

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