
ANALYSIS

**METHODS OF CHROMATOGRAPHIC DETERMINATION OF MEDICINES
DECREASING THE LEVEL OF CHOLESTEROL**

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Abstract: With reference to common application of HPLC to routine analytical tests on medicinal products decreasing the level of cholesterol, including three compounds from this group – fenofibrate, bezafibrate and etofibrate, we developed a new method for determining two other compounds – ciprofibrate and gemfibrozil. The developed HPLC method may be used for identification and qualitative determination of selected compounds – derivatives of aryloxyalkylcarboxylic acids as well as it may be used for simultaneous separation and determination of all compounds from the group of fibrates using one column and the same methodology. The results and statistical data indicate good sensitivity and precision. The RSD value presented is equivalent to the newly developed method of determination of ciprofibrate and gemfibrozil in the substances and medicinal products – capsules and coated tablets.

Keywords: hyperlipidemia, HPLC, derivatives of aryloxyalkylcarboxylic acids, bezafibrate, ciprofibrate, fibrate, gemfibrozil, etofibrate, clofibrate

Hyperlipidemia (HLP) is a group of disorders in the lipid balance of various pathogenesis, which demonstrate an increase in the cholesterol concentration, mostly the level of lipoprotein fractions of low density (LDL) and/or the concentration of triglycerides in blood. The increase in the total cholesterol and LDL concentration is closely connected with an increase in the risk of appearance of the cardiac ischemia and disorders in the cerebral, coronary and peripheral circulation. In May 2001, the National Cholesterol Education Program issued the third edition of the guidelines of Adult Treatment Panel (ATP III). The most important parameter of the lipid profile is the cholesterol concentration LDL – the proper level shall be below 130 mg/dL, though, the optimum concentration LDL has been assumed as below 100 mg/dL. The level for the cholesterol HDL has been also increased – the correct number shall be at the level of more than 40 mg/dL for men and more than 50 mg/dL for women and the risk factor – the concentration below 40 mg/dL. The third most important parameter is the concentration of triglycerides – the correct number shall constitute the concentration not exceeding 150 mg/dL.

The selected medicines applied in the treatment of hyperlipidemia, particularly leading to a decrease in the level of cholesterol, have been apart from statins, the derivatives of aryloxyalkylcarboxylic acids – so called fibrates.

Fibrates inhibit the synthesis of lipoproteins VLDL in the liver and they accelerate catabolism by an increase in the activity of lipoprotein lipase. They increase the removal of fractions of the cholesterol LDL from the organism and they change their structure by means of increasing their sizes and decreasing density. Moreover, the compounds from this group influence the increase of the HDL fraction and the reverse transportation of cholesterol. The specific mechanism of action of fibrates relies on their interaction with nuclear receptors, so called PPAR (peroxisome proliferator activated receptors), which are crucial transmitters of stimuli for the genes controlling the metabolism of lipids. As a result of their action, there is a decrease in the level of triglycerides by 20–50%, the increase of cholesterol HDL by 10–15% and a decrease in the LDL fraction. Apart from the significant influence on the profile of lipids and lipoproteins, this group of com-

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pounds demonstrate other mechanisms of activity that favorably influence the prevention of atherosclerosis (1, 2).

The mother compound of the group of derivatives of aryloxyalkyl carboxylic acids is clofibrate i.e., the ethyl ester of 2-(p-chlorophenoxy)-2-methylpropanoic acid introduced to health care in 1963. This group also encompasses the medicines of later generation: bezafibrate, ciprofibrate, etofibrate, fenofibrate and gemfibrozil. Chemical formulas and names are presented in Figure 1.

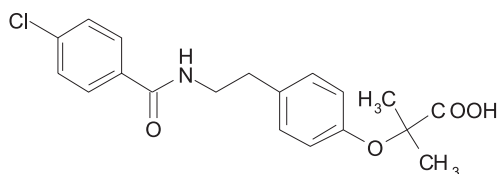
In the available literature, there are some publications discussing the identification and determination of derivatives of aryloxyalkylcarboxylic acids in pharmaceutical preparations and biological material. The tests on fibrates in substances and tablets or capsules relied mainly on the HPLC method with Symmetry ODS columns or Hypersil ODS using a spectrophotometric detector (3) or mass spectrometry (4–7), spectrofluorimetric methods (8, 9), electrochemical methods (10, 11), TLC

method (12) and the method of capillary electrophoresis (13).

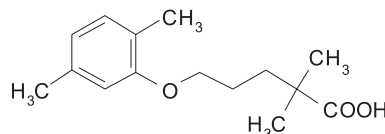
The part of the studies describes the determination of compounds from this group and their metabolites in the biological material, mainly in blood, plasma and urine. The authors applied the method of HPLC in ODS columns along with the use of the spectrofluorimetric detector (3) and the fluorescent method (9).

There are also studies on determination of fibrates by means of gas chromatography using MS detector (7, 14, 15). All the cited methods are mainly used for testing single compounds.

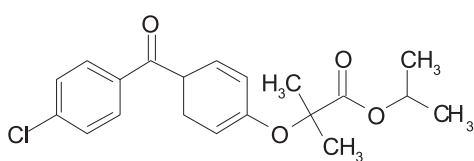
Because there is a limited number of information on the methods of identification and quantitative tests on the whole group of fibrates, particularly in pharmaceutical products and biological material, it was justified to develop a sensitive, precise, unified and easily accessible method of determination of the content and purity of the aforementioned compounds.



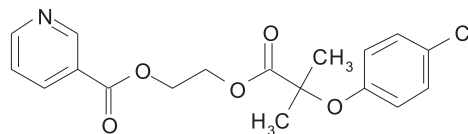
BEZAFIBRATE $C_{19}H_{20}ClNO_3$
2-(4-[2-[(4-chlorobenzoyl)amino]ethyl]-phenoxy)-2-methylpropanoic acid



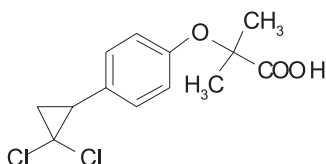
GEMFIBROZIL $C_{15}H_{22}O_3$
5-(2,5-dimethylphenoxy)-2,2-dimethyl-pentanoic acid



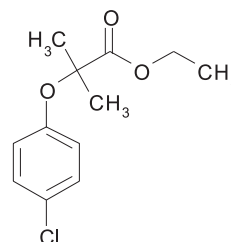
FENOFIBRATE $C_{20}H_{23}ClO_4$
propan-2-yl 2-[4-[(4-chlorophenyl)carbonyl]-phenoxy]-2-methylpropanoate



ETOFIBRATE $C_{18}H_{18}ClNO_3$
2-[(pyridin-3-yl)carbonyloxy]ethyl 2-(4-chlorophenoxy)-2-methylpropanoate



CIPROFIBRATE $C_{13}H_{14}Cl_2O_3$
2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropanoic acid



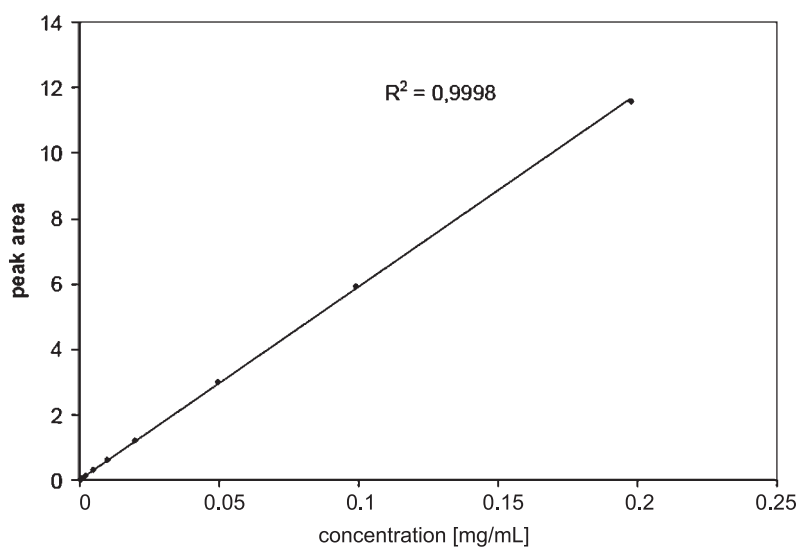
CLOFIBRATE $C_{12}H_{15}ClO_3$
ethyl 2-(4-chlorophenoxy)-2-methylpropanoate

Figure 1. Chemical formulas and names of fibrate compounds

With reference to common application of the HPLC method to routine analytical tests on medicinal products including three compounds from this group – fenofibrate, bezafibrate and etofibrate, it was decided to develop a new method for determining the two addi-

tional compounds – ciprofibrate and gemfibrozil and further, to develop another simple, sensitive and unified method of identification and quantitative determination, which might be applied to test the whole group of the derivatives of aryloxyalkylcarboxylic acids in

A



B

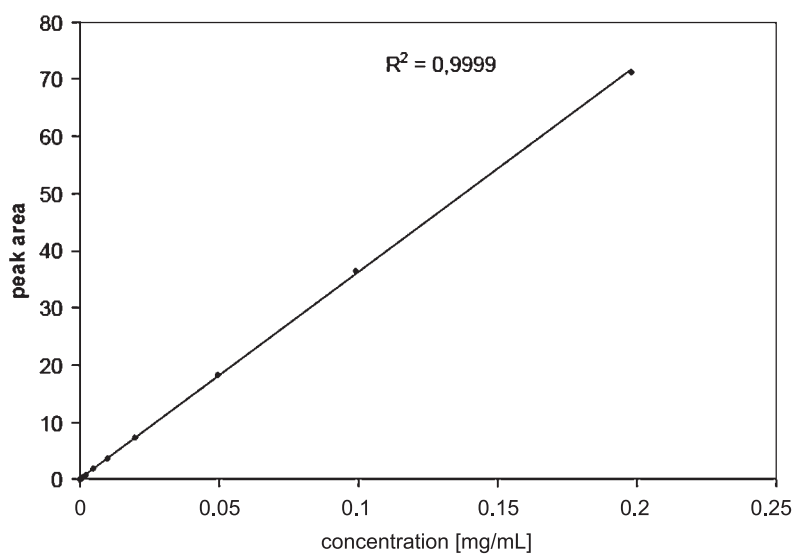


Figure 2. Regression curves for gemfibrozil (A) and ciprofibrate (B) – standard sample

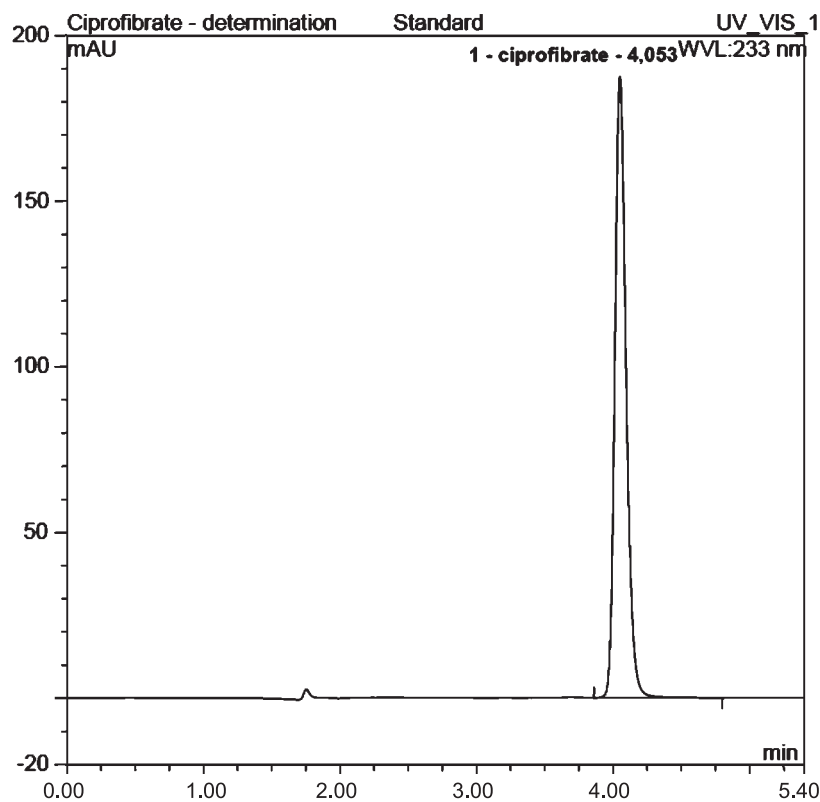


Figure 3. Chromatogram of standard sample of ciprofibrate

Table 1. Retention time (R_t) for the tested compounds

COMPOUND	R_t [min]
BEZAFIBRATE	2.9
CIPROFIBRATE	4.0
ETOFIBRATE	4.3
GEMFIBROZIL	6.3
CLOFIBRATE	7.5
FENOFIBRATE	13.6

the routine analysis and their assessment from the analytical and economical point of view.

EXPERIMENTAL

Reference materials

Bezafibrate (KRKA), etofibrate (Merz Co. GmbH), fenofibrate (Laboratoires Fournier), ciprofibrate (Sanofi Chimie), gemfibrozil (Egis Ltd.), clofibrate (ICI).

Table 2. Statistical assessment of the results of determining the contents of active substances in the substances/products.

NAME OF COMPOUND/ PRODUCT	NUMBER OF TESTS n	AVERAGE FROM ALL MEASUREMENTS X (w %)	STANDARD DEVIATION S	CONFIDENCE INTERVAL $X \pm \Delta X$ $P_u = 95\%$ (w %)	RSD %
Gemfibrozil substance	6	99.59	0.17	99.59 ± 0.16	0.17
Gemfibrozil in tablets (Gemfibril)	6	103.27	0.51	103.27 ± 0.16	0.16
Ciprofibrat substance	6	99.26	0.13	99.26 ± 0.13	0.14
Ciprofibrat in capsules (Liponor)	6	103.88	0.80	103.88 ± 1.12	0.77

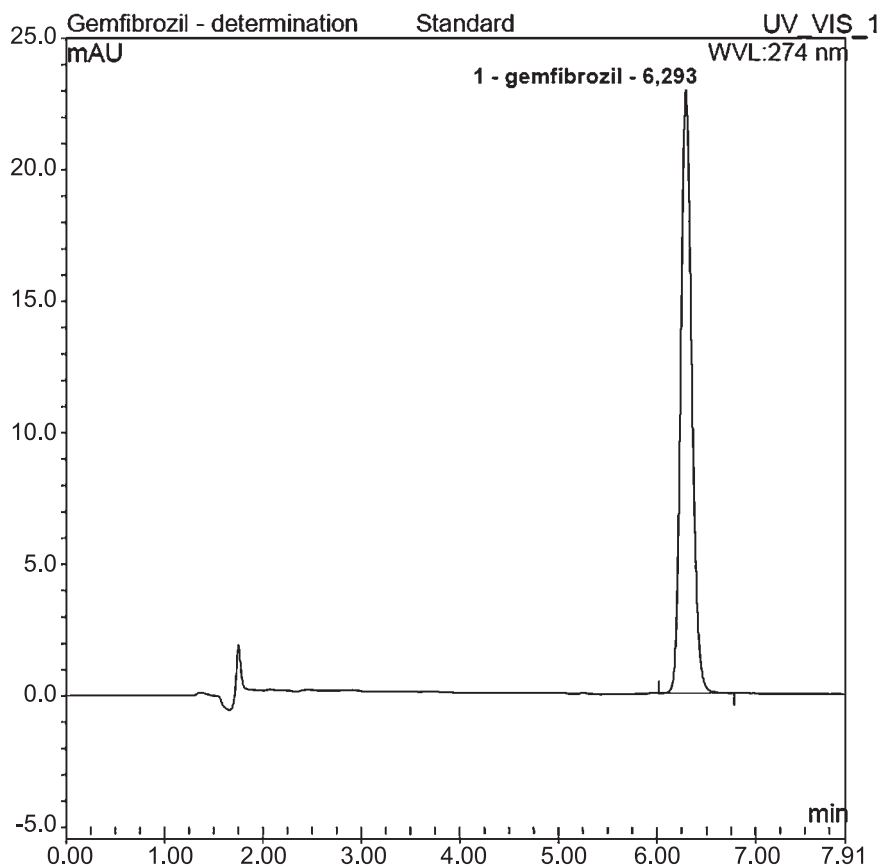


Figure 4. Chromatogram of standard sample of gemfibrozil

Tested substances and medicinal products

Ciprofibrate (Laboratories Winthrop), (gemfibrozil – Egis), Lipanor, capsules 100 mg (Sanofi Aventis), Gemfibral, coated tablets 300 mg (Polpharma S.A.).

Reagents and apparatus

Reagents of high purity for HPLC: acetonitrile, methanol (Lab-Scan), ortho-phosphoric acid 85% p.a. (AppliChem) and liquid chromatograph (Dionex) with a spectrophotometric detector.

HPLC method

Determination of ciprofibrate and gemfibrozil

After testing the series of columns, the Symmetry C18 column, 250 mm × 4.6 mm, 5 μm and a spectrophotometric detector were chosen for the test. The following determination conditions have been worked out: mobile phase: acetonitrile : water (70 : 30, v/v) at pH 2.5, column temperature: 35°C, auto sampler temperature: 25°C, mobile phase

flow rate: 1.2 mL/min, injection volume: 10 μL, detection wavelength: 233 and 274 nm for ciprofibrate and gemfibrozil, respectively.

The analyte solutions have been prepared in methanol at a concentration of 1.0 mg/mL for both ciprofibrate and gemfibrozil. The respective retention times were: 4.0 min for ciprofibrate and 6.3 min for gemfibrozil.

Standard curves

Standard solutions of ciprofibrate and gemfibrozil were prepared in methanol at selected concentrations (0.049 – 198.0 μg/mL and 0.198 – 197.9 μg/mL, respectively). Determinations have been performed under the conditions described above and standard curves were constructed (Fig. 2).

Quantitative determination of ciprofibrate and gemfibrozil

Preparation of standard solutions: In 100 mL flasks 5 mg reference material of ciprofibrate and gemfibrozil were dissolved and filled up with

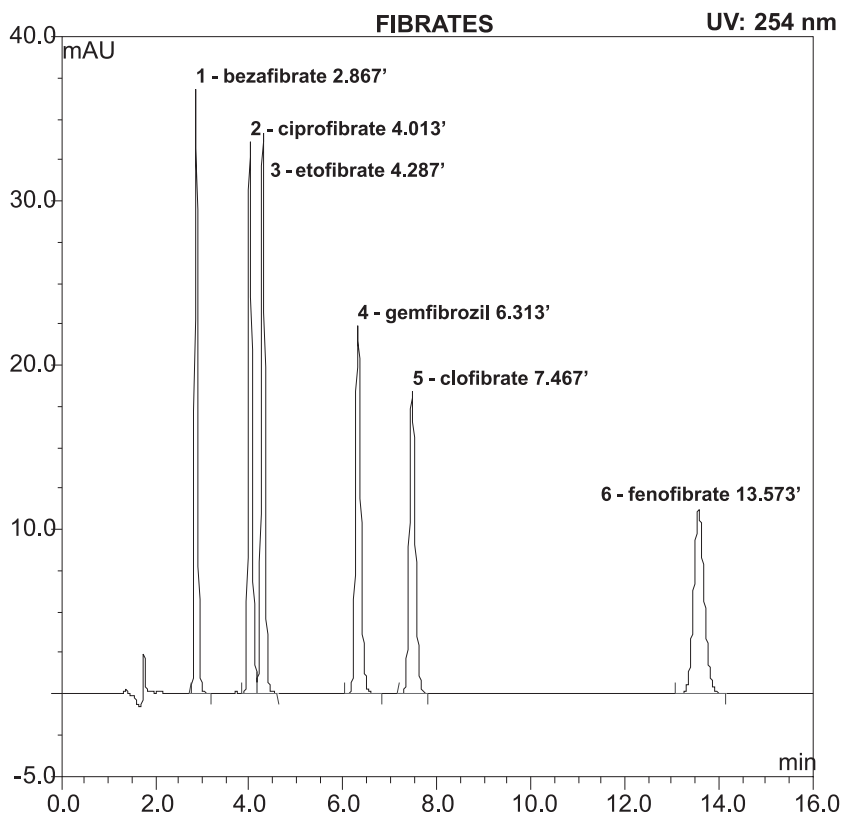


Figure 5. Chromatogram of the mixture of fibrates.

methanol yielding concentrations of 0.05 mg/mL. Preparation of tested solutions: a) To 230 mg of capsules Lapanor (equivalent to ca. 50 mg of the active substance of ciprofibrate) 40 mL of methanol was added and the mixture was shaken for 30 min. The solution was filled up to 50 mL with methanol and filtered. One mL of the solution was diluted with 20 mL of methanol and used for tests. b) To 350 mg of powdered tablet mass of Gemfibral (equivalent to ca. 250 mg of the active substance – gemfibrozil) 80 mL of methanol was added and the mixture was shaken for 30 min.. The solution was filled up to 100 mL with methanol and filtered. One mL of the solution was transferred to 50-mL flask and filled up with methanol. This solution was used for tests.

Determination of the mixture of fibrates

Simultaneous testing of six fibrates used in therapy by HPLC method under the conditions described above was accomplished for the solution

containing the following concentrations: ciprofibrate – 0.15 mg/mL, etofibrate – 0.05 mg/mL, bezafibrate – 0.015 mg/mL, fenofibrate – 0.01 mg/mL, clofibrate – 0.30 mg/mL and gemfibrozil – 0.25 mg/mL. Retention time values are presented in Table 1.

RESULTS AND DISCUSSION

The linearity of determinations within the tested ranges of concentration were found for both ciprofibrate and gembrosil (Fig. 2). The following values for determination limits (0.049 µg/mL and 0.198 µg/mL) and detection limits (0.0198 µg/mL and 0.099 µg/mL) were found for ciprofibrate and gemfibrozil, respectively. Chromatograms of standard samples of ciprofibrate and gemfibrozil are presented in Figures 3 and 4. The chromatograms of substances and medicinal products of these fibrates were identical, respectively. The results and statisti-

cal data presented in Table 2 indicate good sensitivity and precision of the method.

The chromatographic conditions established, proved to be also favorable for separation of six investigated fibrates (Fig. 5).

The developed HPLC method may be successfully used for identification and determination of fibrates – derivatives of aryloxyalkylcarboxylic acids, as substances and in medicinal products.

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Received: 04. 08. 2009