

DEVELOPMENT OF CHROMATOGRAPHIC METHOD FOR DETERMINATION OF DRUGS REDUCING CHOLESTEROL LEVEL

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Hyperlipidemia (HLP) is a group of lipid metabolism disorders with various pathogenesis. Its characteristic feature is an increase in cholesterol level, especially the low density lipoprotein fraction (LDL) level or triglyceride level in blood. An increase in total and LDL cholesterol level is related to the increased risk of ischemic heart disease as well as cerebral, coronary and peripheral circulation disorders.

The medications against hyperlipidemia and cholesterol level reduction are 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (HMG-CoA) also known as statins and aryloxyalkylcarboxylic acid derivatives, also known as fibrates. Statins inhibit biosynthesis of endogenous cholesterol at the mevalonic acid synthesis level and reduce of total cholesterol, LDL fraction and triglycerides levels in plasma and increases HDL fraction level. A mother compound for a group of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (HMG-CoA) is lovastatin. Currently, a semi-synthetic derivatives are available (simvastatin and pravastatin sodium), as well as synthetic compounds – atorvastatin calcium, fluvastatin sodium and lately rosuvastatin calcium (1–4).

The fibrates inhibit VLDL lipoprotein synthesis in liver and accelerate catabolism by increasing lipoprotein lipase activity. Also they improve HDL level and affect the return of cholesterol. A clofibrate is a mother compound of the derivatives of

aryloxyalkylcarboxylic acids. This class of drugs include new generation drugs: bezafibrate, ciprofibrate, etofibrate, fenofibrate and gemfibrozil (1–4).

The available literature of the last several years includes several publications describing separately an identification and determination of lovastatin derivatives and derivatives of aryloxyalkylcarboxylic acids in pharmaceutical products and biological material. HPLC or UPLC methods with octadecyl packed column with UV-VIS detector (11, 13–16) and mass detector (12), the capillary electrophoresis (9) and electrochemical methods (7) are used for statins analysis.

The tests on fibrates relied mainly on the HPLC method with Symmetry ODS or Hypersil ODS columns using a spectrophotometric detector (12) or mass spectrometry (10, 12), the method of capillary electrophoresis (6) and gas chromatography with mass spectrometry detector (12).

In 2006, E. Kublin et al. have published a study on the determination of all statins by gas chromatography with FID detector (5).

In 2010, a study on determination of all drugs (fibrate group) with a single column and a uniform methodology (HPLC with Symmetry C18 column and spectrophotometric detector) has been published by the same authors (17). In the Department of Basic and Applied Pharmacy, National Medicines Institute, a determination method for all statin class drugs by HPLC with Symmetry C18 column and

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

CHEMICAL NAME	RETENTION TIME [min]
ROSUVASTATIN CALCIUM	2.4
BEZAFIBRATE	2.8
ATORVASTATIN CALCIUM	3.3
FLUVASTATIN SODIUM	3.6
CIPROFIBRATE	3.9
GEMFIBROZIL	6.1
LOVASTATIN	7.5
SIMVASTATIN	9.8
FENOFIBRATE	13.3

Table 2. Retention time R_t for the tested compounds (GC).

CHEMICAL NAME	RETENTION TIME [min]
CIPROFIBRATE	1.6
GEMFIBROZIL	2.9
FENOFIBRATE	7.1
BEZAFIBRATE	7.4
LOVASTATIN	9.6
SIMVASTATIN	9.9
ATORVASTATIN CALCIUM	11.4

Table 3. Statistical assessment of the results of simultaneous determination of active substances content in the drugs by HPLC.

COMPOUND/PRODUCT NAME	NO. OF SAMPLES n	TOTAL AVERAGE X (%)	STANDARD DEVIATIONS	CONFIDENCE INTERVAL $X \pm \Delta X$ $P_u = 95\%$ (%)	RSD (%)
Atorvastatin calcium – tablets (Corator)	6	100.55	0.90	100.55 ± 0.86	0.90
Fenofibrate – capsules (Lipanthyl)	6	102.47	0.83	102.47 ± 0.79	0.81
Simvastatin – tablets (Zifam)	6	95.90	0.99	95.90 ± 0.94	1,03
Fenofibrate – capsules (Lipanthyl)	6	99.48	1,51	99.48 ± 1.44	1,52

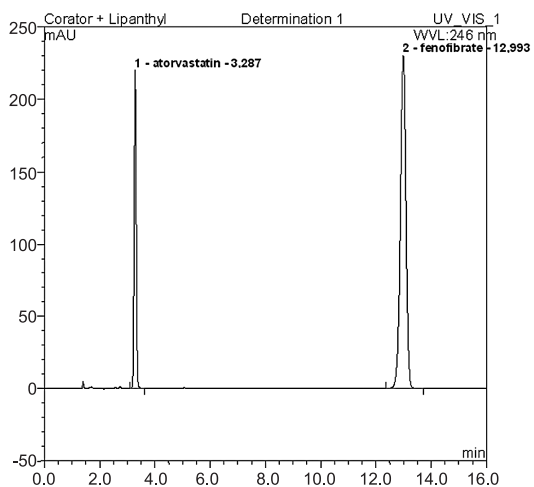


Figure 1. Atorvastatin calcium/fenofibrate solution chromatogram (at 246 nm).

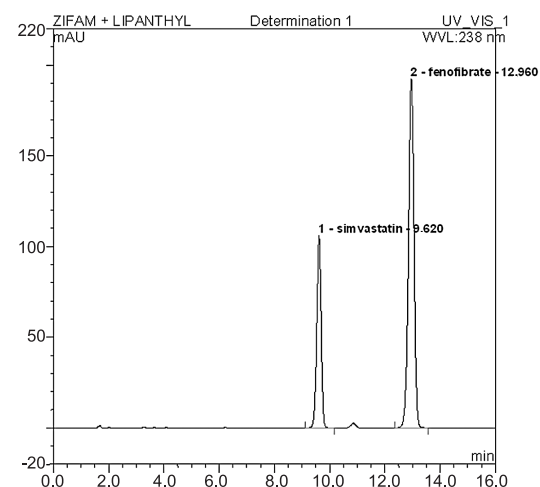


Figure 2. Simvastatin/fenofibrate solution chromatogram (at 238 nm)

spectrophotometric detector was developed and is currently in print.

The available literature of the last several years includes a single publication on simultaneous determination of cholesterol level reducing drugs (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors – HMG-CoA) i.e., statins and aryloxyalkylcarboxylic acid derivatives i.e., fibrates. The authors applied a HPLC test method with mass sensitive detector (8).

A combination of selected statin and fibrate class drugs are applied in modern treating hypocholesterolemia. The most common combination is fenofibrate (100 mg to 267 mg/day) and simvastatin (10–80 mg/day) or atorvastatin calcium (10–80 mg/day). A simultaneous determination of most commonly used drugs is presented in the study.

A purpose of this study was to develop a simple, sensitive and unified quantification method,

which could be applied to analyze the complete class of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (HMG-CoA) and aryloxyalkylcarboxylic acid derivatives in routine analysis and to assess its analytical and economical aspects.

EXPERIMENTAL

Reference materials

Rosuvastatin calcium (Zentiva), atorvastatin calcium (Biocon Ltd.), simvastatin (Ph. Eur.), lovastatin, pravastatin sodium, fluvastatin sodium fenofibrate CRS (Ph. Eur.), bezafibrate (KRKA), etofibrate (Merz Co. GmbH), ciprofibrate (Sanofi Chimie), gemfibrozil (Egis Ltd.), clofibrate (ICI).

Medical products

Corator – coated tablets 40 mg (Przedsiębiorstwo Farmaceutyczne LEK-AM), Zifam – coated tablets 20 mg (Polfa Warszawa S.A.), Lipanthyl – capsules 100 mg (Laboratoires Fourier).

Reagents and apparatus

High purity HPLC reagents: acetonitrile, methanol (Rathburn), 85% orthophosphoric acid (AppliChem), Dionex liquid chromatograph with spectrophotometric detector and Agilent Technologies 6890N gas chromatogram with FID detector.

High performance liquid chromatography (HPLC)

Simultaneous quantification of atorvastatin and fenofibrate

Drugs: Corator and Lipanthyl. The determination was carried out at 246 nm for atorvastatin calcium and at 286 nm for fenofibrate.

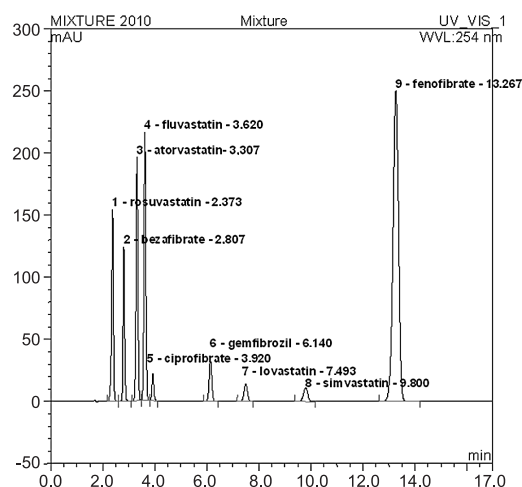


Figure 3. Chromatogram of the mixture statins + fibrates (HPLC)

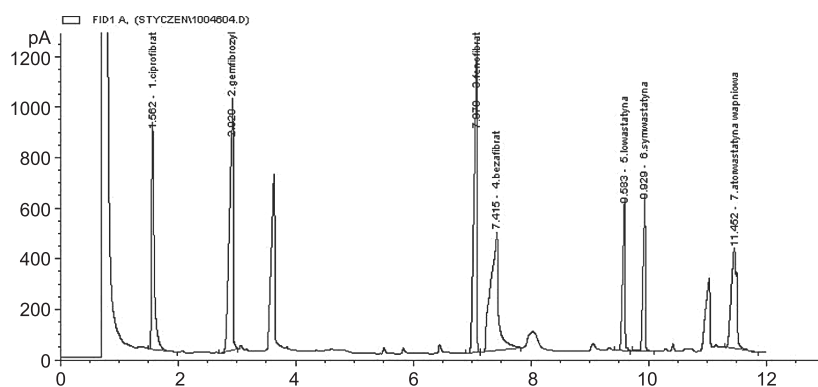


Figure 4. Chromatogram of the mixture statin/fibrate (GC)

Preparation of standard solutions: Ca. 5 mg of reference material (atorvastatin calcium) and 25 mg of reference material (fenofibrate) was transferred to a 100 mL flask, dissolved in methanol and diluted to volume with methanol.

Preparation of tested solutions: Ca. 220 mg of powdered Corator tablets (ca. 20 mg of active substance – atorvastatin calcium) and 250 mg of Lipanthy coated tablets (ca. 100 mg of active substance – fenofibrate) was transferred to a 50 mL flask, approx. 40 mL of methanol was added and shaken by mechanical means for 30 min. The solution was diluted to volume with methanol and filtered. Two and a half mL of the solution was diluted to 20 mL with methanol. Figure 1 shows an example chromatogram, Table 3 shows the results and statistical assessment.

Simultaneous quantification of simvastatin and fenofibrate

Drugs: Zifam and Lipanthy. The determination was carried out at 238 nm for simvastatin and 286 nm for fenofibrate.

Preparation of standard solutions: Ca. 5 mg of reference material (simvastatin) and 25 mg of reference material (fenofibrate) was transferred to a 100 mL flask, dissolved in methanol and diluted to volume with methanol.

Preparation of tested solutions: Ca. 210 mg of powdered Zifam tablets (approx. 20 mg of active substance – simvastatin) and 250 mg of Lipanthy capsules (approx. 100 mg of active substance – fenofibrate) was transferred to a 50 mL flask, approx. 40 mL of methanol was added and shaken by mechanical means for 30 min. The solution was diluted to volume with methanol and filtered. A volume of 2.5 mL of the solution was diluted to 20 mL with methanol. Figure 2 shows an example chromatogram, Table 3 shows the results and statistical assessment.

Identification of a mixture of aryloxyalkylcarboxylic acid derivatives (fibrates) and 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins)

A mixture of the following substances in methanol was used for HPLC: atorvastatin calcium – 49.2 µg/mL, lovastatin – 49.0 µg/mL, simvastatin – 48.2 µg/mL, fluvastatin sodium – 50.2 µg/mL, rosuvastatin calcium – 47.5 µg/mL, fenofibrate – 206.3 µg/mL, ciprofibrate – 95.5 µg/mL, bezafibrate – 48.2 µg/mL and gemfibrozil – 330.8 µg/mL. The following assay conditions were used: Symmetry C18, 250 × 4.6 mm, 5 µm column;

mobile phase: ACN : water (70:30, v/v), at pH = 2.5; column temperature: 35°C, autosampler temperature: 15°C, mobile phase flow rate: 1.2 mL/min, injection volume: 10 µL, wavelength: 238 nm.

All tested compounds were separated (Table 1 shows the retention times). Separation factor between two adjacent peaks (fluvastatin and ciprofibrate – peak 4 and 5, Fig. 3, is 2.30. Fig. 3 shows an example chromatogram.

A mixture of the following substances with methanol was used (GC method): atorvastatin calcium – 5.94 mg/mL, lovastatin – 1.56 mg/mL, simvastatin – 1.48 mg/mL, fenofibrate – 1.42 mg/mL, ciprofibrate – 5.96 mg/mL, bezafibrate – 4.88 mg/mL and gemfibrozil – 1.34 mg/mL.

The following identification conditions were developed: column HP – 1; 30 m × 0.25 mm × 0.25 µm; detector temperature: 320°C, injection chamber temperature: 300°C, column temperature: program – initial temperature 190°C for 1 min; increment 10°C/1 min to final temperature 280°C, 2 min, gas flow: 2.9 mL/min, injection volume: 1.0 µL, split: 10:1.

All tested compounds were separated. Table 2 shows the retention times. Separation factor between two adjacent peaks (fenofibrate and bezafibrate – peak 3 and 4, Fig. 4) is 2.08. Figure 4 shows an example chromatogram.

DISCUSSION

In conjunction with a small number of reports on identification methods and simultaneous quantification of statins and fibrates, especially for drugs and biological material, a sensitive, accurate, unified and easily available method for determination of content and purity is required. The quoted methods are usually used for the analysis of single 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor (HMG-CoA) class and aryloxyalkylcarboxylic acid derivative class compounds (maximum 2–3 compounds).

For HPLC, a Symmetry C18 250 × 4.6 mm, 5 µm column was used for the qualitative and quantitative assay of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (HMG-CoA, statins) and aryloxyalkylcarboxylic acid derivatives (fibrates) with the mobile phase: ACN : water (70:30, v/v), at pH = 2.5.

A new GC method for identification of analyzed fibrates and statins was developed. Column HP – 1; 30 m × 0.25 mm × 0.25 µm at initial temperature 190°C for 1 min; increment 10°C/1 min to final temp. 280°C, 2 min was used.

A simultaneous quantification of commonly used drugs by HPLC method was carried out due to the common combination of selected fibrates (fenofibrate) and statins (simvastatin and atorvastatin calcium) in modern treating hypocholesterolemia.

The results and statistical data presented in Table 3 show high sensitivity and precision of a method. RSD values are valid for the developed method of simultaneous determination of fenofibrate/simvastatin and fenofibrate/atorvastatin calcium in tablets and capsules.

CONCLUSIONS

The presented HPLC method can be used for simultaneous separation and determination of all analyzed 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (HMG-CoA, statins) and aryloxyalkylcarboxylic acid derivatives (fibrates) with a single column and a single run. The GC method allows for fast identification of analyzed drugs from both classes of compounds.

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