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Rapid and simple method for detection of fenofibric acid in human serum by high-performance liquid chromatography

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A sensitive HPLC method for the determination of fenofibric acid (FA), the active form of fenofibrate in serum is discribed. FA from human serum samples was isolated by an easy one-step extraction procedure with a mixture of n-hexane and ethylacetate (90 : 10, v/v). The recovery was 84.8% of the total Fa in serum. The compound was separated isocratically on a reversed phase with acetonitrile and 0.02 M phosphoric acid (55 : 45, v/v) at a flow-rate of 1.0 ml/min. Absorbance at 287 nm was recorded for quantification. Validation presents a detection limit of 0.03 μg/ml and a quantification limit of 0.1 μg/ml (relative standard deviation at 0.1 μg/ml = 7.1%). For an extensive validation of this method we determined the serum levels of FA in one young male volunteer and examined the pharmacokinetics of standard, mikronized and slow release formulation of fenofibrate after oral intake. This method is a rapid and reliable tool for quantitative determination of fenofibric acid in pharmacokinetic investigations.

1. Introduction

Fenofibrate or isopropyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropionate is a lipid regulating drug. It is used for reducing the serum concentration of cholesterol and triglycerides. Fenofibrate is a prodrug with the active form fenofibric acid or 2[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid (FA). The fibrates are the most effective triglyceride-lowering drugs, which are important e.g. for patients with chronic renal failure and commonly seen hypertriglyceridemia [1–4]. The determination of FA can be relevant due to a spectrum of adverse effects (e.g. effects on serum levels of different enzymes or drug interactions) [1, 5, 6]. Several HPLC methods have been reported to assess FA plasma levels [7–9]. We developed a rapid and equally sensitive HPLC method for the determination of FA. For an extensive validation of this method we investigated the single-dose-pharmacokinetics of three different galenic formulations of fenofibrate in one male volunteer.

2. Investigations, results and discussion

FA was determined in human serum with an improved HPLC-UV method. We extracted the compound with a solvent mixture of n-hexane and ethylacetate. The separation was achieved on a reversed phase analytical column using a mixture of phosphoric acid and acetonitrile as mobile phase. For internal standard (I.S.) we used clofibric acid (CA). Typical chromatograms are shown in Fig. 1. The retention times of the I.S. and FA were 5.8 and 10.4 min, respectively. No interference with the analyt was observed.

The extraction recovery of FA and I.S. were determined by a comparison of the peak-heights obtained from the analysis of a spiked standard and from direct injection of known amounts of FA and I.S. The recoveries were 84.4% for FA (50 μg/ml) and 82.0% for I.S. (250 μg/ml).

Adequate linearity was achieved in the tested range of calibration. The limit of detection (LOD) was 0.03 μg/ml in the range 0.10–1.0 μg/ml and 0.50 μg/ml in the range 5.0–50.0 μg/ml (Table 1). At LOD the ratio of signal to noise was about three. The limit of quantification (LOQ) was defined as 0.1 μg/ml with a relative standard deviation (RSD) of 7.1% (Table 2). The resulting limits were found to be sufficient for pharmacokinetic studies.

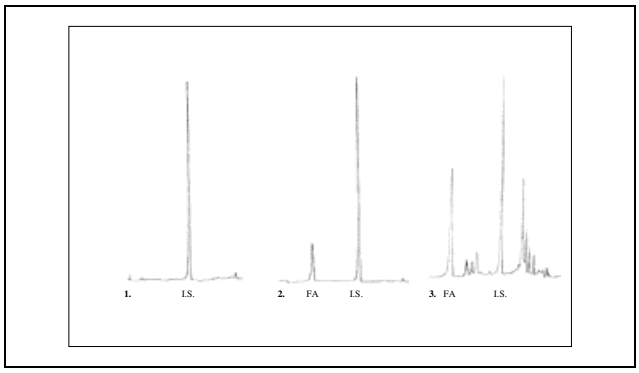

Fig. 1: Typical chromatograms show the peak of internal standard (I.S.) and fenofibric acid (FA) at 5.8 min and 10.4 min, respectively. **1** – blank; **2** – serum spiked with FA (5 μg/ml); **3** – FA in serum of the young volunteer (13.6 μg/ml)

Therefore we extended the validation and investigated the pharmacokinetics of standard, micronized and slow release formulations of fenofibrate. The serum concentration-time curves of fenofibric acid after oral intake of these formulations are shown in Fig. 2.

We developed a rapid, simple and equally reliable method to determine FA in human serum with the advantage that total time effort is considerably reduced in comparison to other methods due to an effective one-step extraction method. The method is applicable to the therapeutic concentration (5–30 μg/ml) and for pharmacokinetic studies.

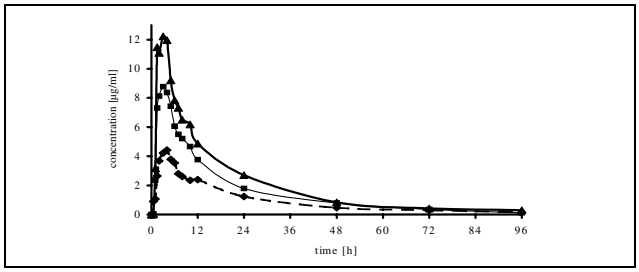

Fig. 2: Concentration of fenofibric acid in human serum of a young male volunteer after oral application of 200 mg standard (◆), of 200 mg micronized (■) and of 250 mg slow release (▲) formulation of fenofibrate

Table 1: Calibration data, limit of detection (LOD) and limit of quantification (LOQ)

Range (μg/ml)	Intercept	Slope	r	LOD (μg/ml)	LOQ (μg/ml)
5.0–50.0	−0.0514	0.0433	0.9982	0.50	2.50
0.1–1.0	0.0088	0.4349	0.9993	0.03	0.10

(n = 10)

Table 2: Precision within-day

Concentration added (μg/ml)	Mean (μg/ml)	SD (μg/ml)	RSD (%)
0.1	0.11	0.008	7.1
1.0	0.99	0.070	6.6
2.5	2.87	0.160	5.4
5.0	5.35	0.210	3.9
50.0	52.6	1.920	3.7

(n = 10)

3. Experimental

3.1. Chemicals and reagents

n-Hexane and hydrochloric acid were purchased from Merck (Darmstadt, Germany), phosphoric acid (85%) from Laborchemie Apolda (Apolda, Germany), methanol gradient grade from Merck (Darmstadt, Germany), ethylacetate and acetonitrile ultra gradient grade from Baker (Deventer, Holland), FA from Promochem (Wesel, Germany). CA (2-(4-chlorophenoxy)-2-methylpropanoic acid) was supplied by Sigma (Deisenhofen, Germany).

3.2. Instrumentation and chromatographic conditions

The HPLC system consisted of a HPLC pump Model 480 (Gynkotek, Germering, Germany) fitted with a 10 and 20 μl sample loop, respectively. The separation were achieved on a reversed phase analytical column, Superspher® 100 RP-18, 250 × 4 mm (Merck, Darmstadt, Germany). The effluent was monitored with a Knauer UV-Detector (Bad Homburg, Germany) at a wavelength of 287 nm. The mobile phase was composed of a mixture of 0.02 M phosphoric acid/acetonitrile (45 : 55, v/v). The separation was performed with this solvent mixture at a flow rate of 1 ml/min.

3.3. Calibration

Stock solutions of FA and CA (I.S.) were prepared by dissolving 5 mg of each substance in 5 ml acetonitrile and 5 ml methanol, respectively. The calibration ranges were 0.1 to 1.0 μg/ml and 5 to 50 μg/ml. The procedure of calibration was slightly modified in the range less than 1 μg/ml in sample preparation. The stock solutions of I.S. and FA were diluted by factor 0.1 and 0.02, respectively.

3.4. Extraction procedure

Serum samples, 1 ml each, were taken into glass tubes to which 250 μl of I.S. solution was added and mixed. Then the serum was acidified with 1 ml of 1 M hydrochloric acid. A volume of 5 ml of a solvent mixture made up of n-hexane/ethylacetate (90 : 10, v/v) was added. After the single extraction by end-over-end rotation for 20 min and centrifugation (5 min at 1000 × g), the supernatant from each tube were removed into a glass tube and then evaporated to dryness under vacuum. The residue was redissolved in 300 μl of acetonitrile. After vortex mixing 700 μl of water were added and a 10 μl-aliquot was injected into the chromatographic system.
A volume of 75 μl I.S.-solution instead of 250 μl was used in the calibration range less than 1 μg/ml. After evaporation the residue was dissolved in a 100 μl volume of a acetonitrile/water-mixture (3 : 7, v/v) and 20 μl were injected.

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