Department of Pharmacy, Xijing Hospital, Fourth Military Medical University, Shaanxi, China

Development of a LC-MS/MS method for the estimation of clinofibrate in human urine

JIAN-KANG LI, CHENTAO LU, JING YANG, FAN LI, JIE GE, SONG WEI, LI XUEQING, LIKUN DING, WEN AI-DONG

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Wen Ai-Dong, Department of Pharmacy, Xijing Hospital, Fourth Military Medical University, Changle West Street 15, Xi'an, Shaanxi 710032, China
adwen-2004@hotmail.com

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A highly sensitive and rapid liquid chromatography-tandem mass spectrometry method was developed for the determination of clinofibrate in human urine. The analyte and IS were extracted through a simple protein precipitation by the mixture of acetonitrile and 1 mol/L hydrochloric acid (95:5, v/v) and separated on an Inspire C_{18} (150 mm × 4.6 mm I.D., 5 μm particle size) column using isocratic elution with methanol and water containing 0.1 % formic acid and 10 mM ammonium acetate (90:10, v/v). Mass spectrometric detection was performed in electrospray positive ionization MRM mode. The mass transition was m/z 486.3→175.0 for clinofibrate and m/z 361.1→233.1 for IS, respectively. The flow rate was 0.6 mL/min and the column oven temperature was set at 35 °C. The total run time was 6.5 min. Good linear relationships were obtained for all analytes over the concentrations ranging of 0.1002-10.02 μg/mL ($r^2 = 0.9991$) and the limit of quantification was 0.1002 μg/mL. The extraction recovery was larger than 87.4% and intra- and inter-batch precision and accuracy with RSD were all less than 6.5%. The total amount of unchanged clinofibrate excreted in urine was less than 0.34 %. This method was successfully applied to the pharmacokinetic study of clinofibrate in human urine.

1. Introduction

Hyperlipidaemia is one of the most common forms of dyslipidemia, with abnormally elevated levels of any or all lipids and/or lipoproteins in the blood. Lipid and lipoprotein abnormalities are common in the general population, approximately 160 million people (10 % of the population) in China. Hyperlipidaemia is regarded as a modifiable risk factor for cardiovascular disease due to its influence on atherosclerosis (Hopkins et al. 2013 and Bertolotti et al. 2005).

In most cases, diet has only a limited effect and drug treatment is required. Fibrates are a class of amphipathic carboxylic acids, used for a range of metabolic disorders, mainly hyperlipidaemia. Fibrates are usually used combine with statins, but do not improve all-cause mortality and are therefore indicated only in those not tolerant to statins (Ziegler et al. 1998). Clinical benefits of clofibrate, gemfibrozil, bezafibrate and fenofibrate are limited in patients with renal failure, because these fibrates are excreted by the kidney. Some studies reported adverse effects including rhabdomyolysis and elevated serum creatine phosphokinase (CPK) due to accumulation of these drugs (Pierides et al. 1975; Kujma et al. 1977; Chan 1989).

Clinofibrate, 2,2'-(4,4'-cyclohexylidene diphenoxy)-2,2'-dimethyldibutilic acid, is a fibric acid derivative, which is safe, well tolerated and effective for all types of hyperlipidaemia. Clinofibrate decreases total cholesterol, triglycerides, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and Apo-B, but increases high density lipoprotein (HDL) and Apo-A. Unlike other fibrates, clinofibrate is mostly excreted through the bile and less than 1 % total amount of unchanged clinofibrate is excreted into human urine (Nakatani H et al. 1981 and Nishizawa Y et al. 1999).

Clinofibrate was first marketed in 1981, Japan (Sumitomo Pharmaceutical Co. Ltd), and is monographs in "The Japanese Pharmacopoeia "16 (Japanese Pharmacopoeia JP-16). Recently, clinofibrate tablets have been approved to conduct a clinical trial in China, which is done by XinTong pharmaceutical Co., Ltd. (China). So far, the excretion of clinofibrate into urine and feces was determined by TLC and GC-MS in animals, such as rat, mouse and dog (Nishizawa et al. 1993). Clinofibrate is a mixture of the meso and the racemic isomers, in ratios of almost 1/1 ∼ 3/2 (Nakazawa et al. 1979). The aim of our work was to evaluate the pharmacokinetic characteristics of clinofibrate and to determine the drug in human urine.

Like other fibrates, clinofibrate can be determined by a high-performance liquid chromatographic method (Li Jian-kang et al. 2013) and this method was applied to the pharmacokinetic study in human plasma successfully. But in human urine, the lower limit of quantification of 0.5 μg/mL was not sensitive enough the whole sample analysis process required about 13 min. For this reason, a highly sensitive, rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the determination of clinofibrate in human urine.

2. Investigations and results

2.1. Method development

For fenofibrate was the analogue of clinofibrate, (Fig. 1) which has the similar chromatographic retention behavior and physic-

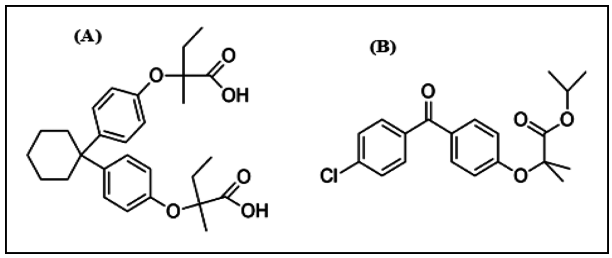

Fig. 1: Chemical structures of clinofibrate (A) and fenofibrate (B).

ochemical properties, we selected it as I.S. Ammonium acetate, formate and acetate buffers with various combinations of methanol were investigated to choose the optimal mobile phase which produced the best efficiency, sensitivity and peak shape. The results showed that methanol and water containing 0.1 % formic acid and 10 mM ammonium acetate (90:10, v/v) could achieve this purpose and finally was used as the mobile phase. Figure 2 shows that the analytes yielded major [M + NH4] + ions at m/z 486.3 for clinofibrate and m/z 361.1 for IS in the positive ion detection mode. Each of the precursor ions was subjected to collision-induced dissociation to determine the product ion. Therefore, the precursor to product mass transition was assigned as m/z 486.3→175.0 for clinofibrate and m/z 361.1→233.1 for fenofibrate, which was more sensitive and stable. In order to acquire the higher signal for clinofibrate and fenofibrate, the mass spectrometer parameters were optimized as follows: dwell time 150 ms; gas flow 12 L/min; gas temperature 350 °C; nebulizer pressure 40 psi; fragmentor voltage 150 V (clinofibrate) and 120 V (IS), collision energy 28 eV (clinofibrate) and 14 eV (IS). We used protein precipitation with the mixture of acetonitrile and 1 mol/L hydrochloric acid (95:5, v/v) as the method of sample preparation, which was rapid, convenient and inexpensive. The urine samples were precipitated with methanol or acetonitrile, but the results were unsatisfactory, only acetonitrile - 1 mol/L hydrochloric acid (95:5, v/v) gave high selectivity and recovery.

2.2. *Method validation*

2.2.1. *Selectivity*

The specificity of the method was evaluated by screening the six different batches of blank urine samples. The blank urine samples were screened for any peaks interfering at the retention time of clinofibrate and fenofibrate. Figure 3 shows the representative chromatograms of a human blank urine, a blank urine with clinofibrate or fenofibrate, and the urine samples obtained from a volunteer at 2 h after oral administration of 200 mg clinofibrate spiked with I.S. No significant interference peaks from endogenous substances were found at the retention times of clinofibrate (tR = 5.4 min) and fenofibrate (tR = 5.2 min).

2.2.2. *Linearity and LLOQ*

The calibration curve was constructed by plotting the peak area ratio (y) of the analyte to IS *versus* the plasma concentrations (x) of clinofibrate. Linear correlation was obtained in

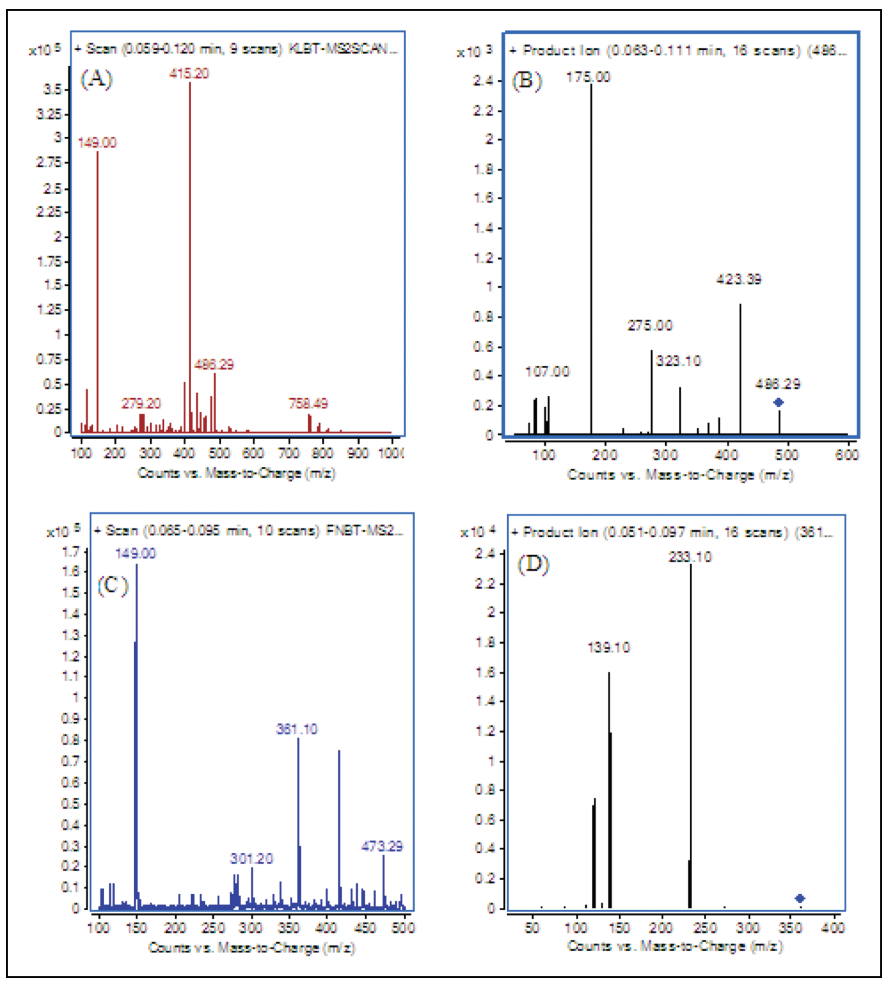

Fig. 2: Full-scan positive product ion mass spectra of (A) precursor ion spectrum of clinofibrate; (B) product ion spectrum of clinofibrate; (C) precursor ion spectrum of IS and (D) product ion spectrum of IS.

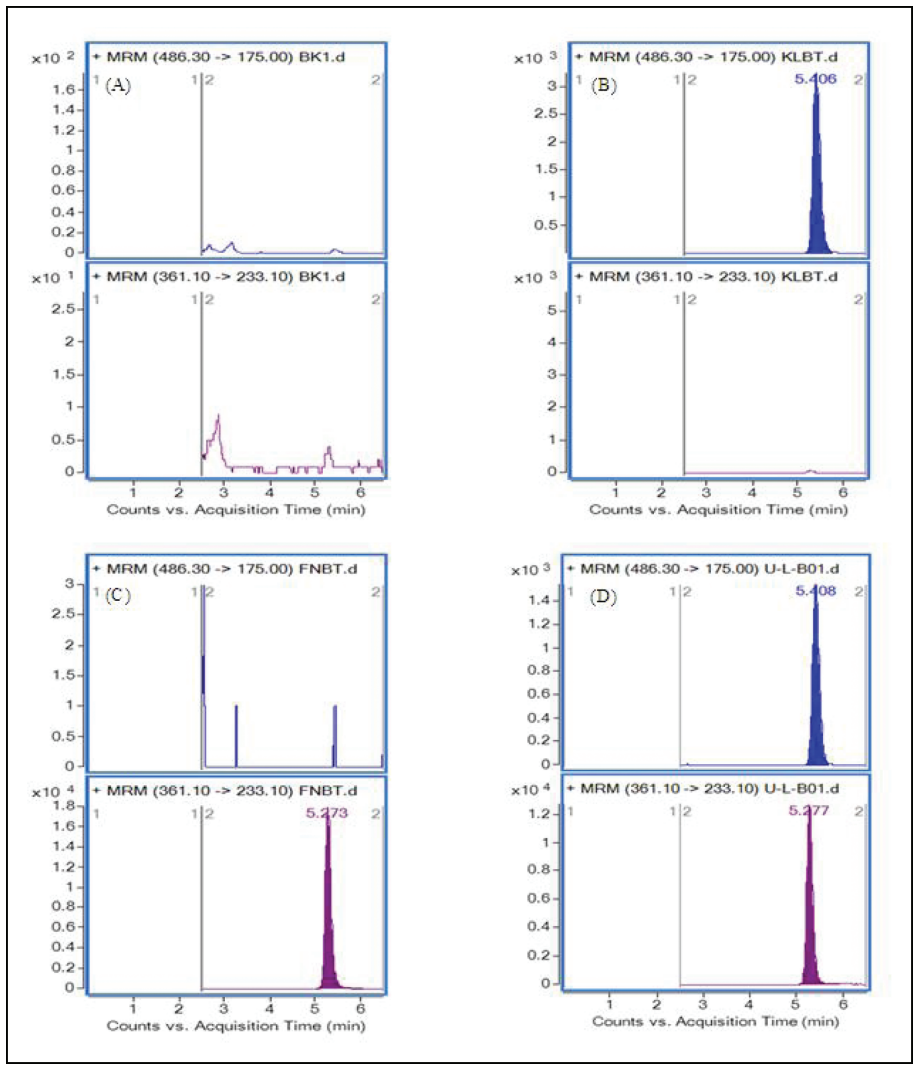

Fig. 3: Typical chromatograms of clinofibrate and IS in human urine: (A) blank urine sample; (B) a blank urine sample spiked with clinofibrate (C) a blank urine sample spiked with IS; and (D) a urine sample obtained from a volunteer at 2 h after oral administration of 200 mg clinofibrate spiked with IS.

the range of 0.1002-10.02 μg/mL and the standard curve was f = -0.01362 + 0.2151 × C, (r2 = 0.9991) for clinofibrate, where f represents the clinofibrate peak area to the IS peak area ratio and C represents the clinofibrate concentration. The calibration curves were fitted by a weighted (1/x) least-squares linear regression. The LLOQ of this method was 0.1002 μg/mL, which was sensitive enough to detective the clinofibrate in human urine. LLOQ showed that the analytical responses was at least 10 times than the background noise, and the precision and accuracy was acceptable, with 2.4 % RSD and 11.5 % RE (n = 5).

2.2.3. *Accuracy and precision*

Accuracy and precision of this method were evaluated by three QC samples (0.2004, 2.004, 8.016 μg/mL). The results both of intra-day and inter-day precision and accuracy are all shown in Table 1. The RSD range of precision was 3.5% to 6.5%, these results demonstrated that this method was accurate, precise and reproducible.

2.2.4. *Extraction recovery and matrix effect*

The extraction recovery of clinofibrate at the concentrations of 0.2004, 2.004, 8.016 μg/mL were 92.0 ± 5.3%, 93.9 ± 1.7%, 93.7 ± 4.2%, respectively. And the matrix effect at the concentrations of 0.2004, 2.004, 8.016 μg/mL were 89.1 ± 4.1%, 90.1 ± 5.7%, 87.4 ± 4.6%, respectively (Table 1). According to the result, there was no matrix effect of the analyte in this study.

2.2.5. *Stability*

The stability data of clinofibrate under various conditions are presented in Table 2. These results indicated that clinofibrate was

Table 1: Precision, accuracy, matrix effect and recovery for determination of clinofibrate in human urine

Spiked (μg/mL)	Intra-batch precision and accuracy (n = 5)			Inter-batch precision and accuracy (n = 3)			Matrix effect(n = 5)		Recovery(n = 5)	
	Measured (mean ± S.D.) (μg/mL)	RSD (%)	RE (%)	Measured (mean ± S.D.) (μg/mL)	RSD (%)	RE (%)	Mean (%)	RSD (%)	Mean (%)	RSD (%)
0.2004	0.2130 ± 0.014	6.5	6.3	0.2081 ± 0.011	5.4	3.8	92.0	5.3	89.1	4.1
2.004	2.059 ± 0.037	1.8	2.7	1.954 ± 0.13	6.5	−2.5	93.9	1.7	90.1	5.7
8.016	8.389 ± 0.19	2.2	4.7	8.216 ± 0.28	3.5	2.5	93.7	4.2	87.4	4.6

Table 2: Stability of clinofibrate in human urine (n = 3)

Spiked concentration of clinofibrate (ug/mL)	Measured (mean ± S.D.) (μg/mL)	RE (%)	Measured (mean ± S.D.) (μg/mL)	RE (%)	Measured (mean ± S.D.) (μg/mL)	RE (%)
	0.2004		2.004		8.016	
Short-term stability (14 h, room temperature)	0.2135 ± 0.002	6.5	2.145 ± 0.022	7.0	8.452 ± 0.058	5.4
Post-preparative stability (15 h in autosampler)	0.2016 ± 0.011	0.6	1.979 ± 0.103	−1.2	8.092 ± 0.256	0.9
Freeze and thaw stability	0.2015 ± 0.002	0.5	2.143 ± 0.054	6.9	7.896 ± 0.081	−1.5
Long-term stability (30 d, − 20°C, n = 5)	0.2038 ± 0.001	1.7	2.038 ± 0.078	1.7	8.175 ± 0.008	2.0

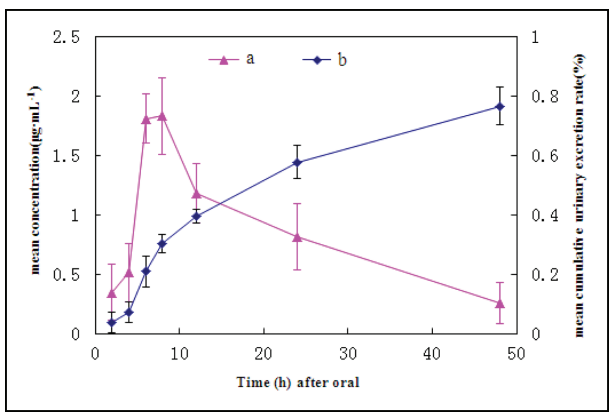

Fig. 4: Concentrations (a) and excretion rate (b) of clinofibrate in different time in urine samples after an oral of 200 mg clinofibrate.

stable in human urine at ambient temperatures for at least 14 h, after three freeze and thaw cycles, under autosampler conditions for 15 h and at -20 °C for one month.

2.3. Pharmacokinetic application

This method was successfully applied to the urinary excretion of clinofibrate in healthy volunteers. The concentration of clinofibrate in the urine samples was calculated by calibration curves according to least-squares linear regression equation. This analytical method measured the urine concentration *versus* time profiles up to 48 h for clinofibrate quantification. Figure 4 showspresented the mean cumulative urinary excretion rate-time curve (line b) and mean concentration-time curve (line a) after a single dose administration of clinofibrate in 12 healthy volunteers. The data of mean cumulative urinary excretion rate and mean concentration for 12 healthy volunteers are given in Table 3. According to the results, the total amount of unchanged clinofibrate excreted in urine was about 0.34 %.

Table 3: Mean cumulative urinary excretion rate and mean concentration for 12 healthy volunteers after an oral of 200 mg clinofibrate

Time (h)	Concentration		Cumulative urinary excretion rate	
	Mean (μg/mL)	SD	Mean(%)	SD
0–2	0.3445	0.24	0.036	0.034
2–4	0.5111	0.25	0.071	0.035
4–6	1.807	0.21	0.21	0.052
6–8	1.829	0.32	0.30	0.032
8–12	1.178	0.25	0.39	0.023
12–24	0.8122	0.28	0.58	0.056
24–48	0.2542	0.17	0.76	0.063

2.4. Conclusion

A sensitive, rapid, and selective LC-MS/MS method combined with protein precipitation for the analysis of clinofibrate in human urine has been developed and validated for the first time. The whole process of sample analysis was generally completed within 6.5 min. The method was sensitive enough for determination the concentration of clinofibrate in human urine, with a LLOQ of 0.1002 μg/mL. The total amount of unchanged clinofibrate excreted in urine was 0.34 %. This method was successfully applied to a pharmacokinetic study in human urine samples.

3. Discussion

A LC-MS/MS method was established for the quantification of clinofibrate in urine and successfully applied to determine the urinary excretion of clinofibrate in healthy volunteers. Although we have established a high-performance liquid chromatographic method for determination of clinofibrate in plasma (Li Jian-kang et al. 2013), the concentration of clinofibrate in urine was not high enough to be determined. The HPLC method also presented the disadvantage of a time-consuming chromatographic analysis (t = 13 min) (Li Jian-kang et al. 2013), while the whole sample analysis process of the method described here was about 6.5 min. Although it is known that less than 1 % total amount of unchanged clinofibrate is excreted into human urine, no suitable method has been reported for the determination of clinofibrate in urine. For the concentration of clinofibrate in urine was rather low, we established a LC-MS/MS method which was sensitive enough to detective the clinofibrate in human urine. The validation parameters selectivity, linearity and LLOQ, accuracy and precision, recovery, matrix effect and stability were all evaluated according to the FDA guideline for validation of bio-analytical methods for the reliable application of the method to pharmacokinetic assays.The method is simple, inexpensive, selective and applicable. In addition, a published GC method was mainly used for the preparation process of active pharmaceutical ingredient and the quality control of clinofibrate (Ai and li 2009).
According to the results of a pharmacokinetic study in human urine, the total amount of unchanged clinofibrate excreted in urine was about 0.34 %, which demonstrated that clinofibrate is not excreted by the kidney, unlike other fibrates. The concentration of clinofibrate changed similar in plasma and urine, the elimination half-life ($t_{1/2}$) corresponded with the pharmacokinetics in human plasma. Almost all fibrates are eliminated into the urine as glucuronides, so the total amount of unchanged clinofibrate excreted in urine was extremely low.
Climical benefits of clofibrate, gemfibrozil, bezafibrate and fenofibrate are limited in patients with renal failure, because these fibrates are excreted from the kidney, and some studies reported the occurrence of adverse effects including rhabdomyolysis and elevated serum creatine phosphokinase (CPK) due to accumulation of the drugs (Pierides et al. 1975; Kujma et al. 1977; Chan 1989). So perhaps clinofibrate will be more effec-

tive in patients with continuous ambulatory peritoneal dialysis (CAPD) or renal failure.

4. Experimental

4.1. Materials

Clinofibrate (99.5 % purity) was obtained from XinTong pharmaceutical Co., Ltd. and the internal standard (IS), fenofibrate (100 % purity) (Fig. 1) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol of HPLC-grade was purchased from Fisher Scientific (Pittsburgh, PA, USA). Ammonium acetate, formic acid and hydrochloric acid were all analytical grade from Tianjin Tianli Chemical Reagent Co., Ltd (Tianjin, China). Purified water from a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the experiment.

4.2. Instrumentation

Analyses were performed on an Agilent 1200 series (Agilent Technologies, USA) liquid chromatographic system an Agilent 6410 Triple Quadrupole mass spectrometer equipped with an electrospray ionization source (Agilent Technologies, USA). The analytical column employed was a DIKMA Inspire C_{18} (150 mm × 4.6 mm I.D., 5 μm particle size) column. All data were acquired employing Agilent 6410 Quantitative Analysis version analyst data processing software.

4.3. Chromatographic conditions

The mobile phase consisted of methanol and water containing 0.1 % formic acid and 10 mM ammonium acetate (90:10, v/v). The flow rate was 0.6 mL/min and the column oven temperature was set at 35 °C. The total run time was 6.5 min. The sample injection volume was 5.0 μL. Mass spectrometric detection was performed on a Series 6410 Triple Quad LC–MS/MS (Agilent Technologies, USA) in electrospray positive ionization using multiple reaction monitoring (MRM). The mass transition was m/z 486.3→175.0 for clinofibrate, and m/z 361.1→233.1 for IS, respectively (Fig. 2). The other working parameters of the mass spectrometer were as follows: dwell time 150 ms; gas flow 12 L/min; gas temperature 350 °C; nebulizer pressure 40 psi; fragmentor voltage 150 V (clinofibrate) and 120 V (IS); collision energy 28 eV (clinofibrate) and 14 eV (IS).

4.4. Preparation of the stock and standard solutions

Stock solution of clinofibrate was prepared in methanol at a concentration of 1.002 mg/mL. Working solutions were diluted with methanol to obtain clinofibrate concentrations of 50.1, 20.04, 10.02, 5.01, 2.004 and 1.002 μg/mL. The IS stock solution was prepared by dissolving 10.12 mg fenofibrate in 100 mL acetonitrile to give a nominal concentration of 0.1012 mg/mL, and then was diluted with acetonitrile at a concentration of 2.024 μg/mL as IS working solution. All stock solutions were kept at -20 °C and brought to room temperature before use.

4.5. Sample preparation

30 μL I.S. (2.024 μg/mL) was added to 200 μL urine samples in a 1.5 mL tube, the mixture was vortexed for 30 s. Clinofibrate and IS were extracted with 600 μL acetonitrile and 1 mol/L hydrochloric acid (95:5, v/v) by vortexing for 3 min. After centrifuged at 16,000 × g for 10 min at room temperature, the supernatant was transferred into the test tube. 20 μL aliquot of the supernatant fraction was injected into the LC-MS/MS system for analysis.

4.6. Method validation

Validation of method was done according to the FDA guideline for validation of bioanalytical methods (US Food and Drug Administration, Center for Drug Evaluationand Research, 2001) by evaluating the validation parameters: selectivity, linearity and lower limit of quantification (LLOQ), accuracy and precision, recovery, matrix effect and stability.

4.6.1. Selectivity

The chromatograms of drug-free human urine from six healthy donors were investigated for endogenous interferences. Each blank urine sample was tested using the precipitation procedure to ensure that whether endogenous peaks co-eluted with clinofibrate or IS.

4.6.2. Linearity and LLOQ

Linearity was assessed at seven-point (0.1002, 0.2004, 0.501, 1.002, 2.004, 5.01, 10.02 μg/mL) in the range of 0.1002-10.02 μg/mL by spiking an appropriate amount of the standard solutions in 200 μL blank human urine. The standard curves were run on three different days and fitted by a weighted (1/x) least-squares linear regression. The calibration curves were constructed by plotting the peak area ratio of the analyte to IS. The standard deviations of each back-calculated standard concentration were evaluated and the acceptance criterion of correlation coefficient for each calibration curve was 0.99 or better. LLOQ was defined as the lowest concentration of the standard curve that could be measured with acceptable accuracy and precision which the relative standard deviation (RSD) (n = 6) and relative error (RE) were within ± 20 %.

4.6.3. Accuracy and precision

The accuracy and precision of this method were evaluated by quality control (QC) samples which were prepared in blank human urine at the nominal concentrations of 0.2004, 2.004, 8.016 μg/mL. The accuracy was evaluated through that the three QC samples in quintuplicate. The precision of the procedures was evaluated by repeatability (intra-day) and intermediate precision (inter-day). The intra-day precision was evaluated the three QC samples in quintuplicate during the same day and the inter-day precision was evaluated through that the three QC samples in three consecutive days. The precision was calculated by RSD and the accuracy was expressed as RE. The precision and accuracy were considered acceptable within ± 15%.

4.6.4. Extraction recovery

Recovery presents the extraction efficiency of a method, which was performed at three QC levels (five samples each). The recoveries were evaluated by comparing peak areas of analytes in spiked urine samples with those of samples to which the analytes had been added after extraction.

4.6.5. Matrix effect

The matrix effect was the combined effect of all components of the sample other than the analyte on the measurement of the quantity. It was evaluated by comparing the peak area of the analytes dissolved in the blank urine sample's precipitated solution with that of the analytes dissolved in the mobile phase. Three different concentration levels (0.2004, 2.004, 8.016 μg/mL) were evaluated in quintuplicate at each level. The matrix effect was accepted for the peak area ratio which was between 85 % and 115 %.

4.6.6. Stability

Stability of clinofibrate in urine was evaluated by the QC samples at three different concentrations under different conditions. It was evaluated by investigating short-term (14 h at room temperature), long-term (1 month, − 20 °C), freeze–thaw (three freeze–thaw cycles) and post-preparative (15 h in autosampler) stability.
QC samples were prepared at three different concentrations of 0.2004, 2.004, 8.016 μg/mL (twenty-one samples each concentration). Three samples of each concentration were subjected to short-term room temperature condition for 14 h and to post-preparative stability of the processed samples after 15 h on the first day, respectively. Nine samples of each concentration were subjected to three freeze–thaw stability studies in the next three days. The rest of six samples of each concentration were subjected to long-term storage conditions (−20 °C) on the 15th and 30th day.

4.7. Pharmacokinetic study

The clinical protocol and associated informed consent statements were reviewed and approved by the committee on human rights related to human experimentation at Xijing Hospital and the volunteers were gave written informed consent to participate in the study after explanation about the experimental procedure.
Twelve healthy volunteers (males and females, aged 27-37 y, body weight 56.5-64 kg) were enrolled in this study. We selected volunteers who had no cardiovascular, hepatic, gastrointestinal, nervous, no history of hematologic or any acute or chronic diseases or drug allergy, not undergoing any pharmacological treatment and physical examination and laboratory tests should be normal. The volunteers were required to refrain from caffeine, smoking, alcohol and strenuous exercise during the study and under direct medical supervision at the study site. Urine samples were collected over the intervals of 0-2, 2-4, 4-6, 6-8, 8-12, 12-24 and 24-48 h after administration of a single dose of 200 mg clinofibrate in tablets.
Pharmacokinetic parameters were calculated from the urine concentration–time data. Excretion was calculated as the excretion (μg) = C*V. C was the urine concentration of the certain intervals after

administered a single dose of 200 mg clinofibrate tablets and V was the corresponding volume of the certain intervals. Cumulative urinary excretion was calculated as the total excretion of all intervals. Cumulative urinary excretion rate was calculated as cumulative urinary excretion/oral dose *100%

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References

Ai, Li (2009) Method for detecting clinofibrate and its intermediate by gas chromatography, Faming Zhuanli Shenqing Gongkai Shuomingshu, Patent CN 101609076 A 20091223.

Bertolotti M, Maurantonio M, Gabbi C, Anzivino C, Carulli N (2005) Review article: hyperlipidaemia and cardiovascular risk. Aliment Pharmacol Ther 22: 28–30.

Chan Mk (1989) Gemfibrozil improves abnormalities of lipid metabolism in patients on continuous ambulatory peritoneal dialysis: The role of postheparin lipases in the metabolism of high-density lipoprotein subfractions. Metabolism 38: 939–945.

Guidance for Industry: Bioanalytical Method Validation, U.S. Department of Health and Human Services, Food and Drug Administration Centre for Drug Evaluation and Research (CDER), Centre for Veterinary Medicine (CVM), May 2001.

Hopkins AL, Lamm MG, Funk J, Ritenbaugh C (2013) Hibiscus sabdariffa L. in the treatment of hypertension and hyperlipidemia: A comprehensive review of animal and human studies. Fitoterapia 85: 84–94.

Japanese Pharmacopoeia Editing Committee. Japanese Pharmacopoeia JP-16 [M]. Japan's health ministry. Tokyo. JP XVI (2011) 654–655.

Kujma Y, Sasaoka T, Kanayama M, Kubota S (1977) Untoward effects of clofibrate in hemodialyzed patients. N Engl J Med 296: 515.

Li Jian-Kang, Song Ying, Wang Lei, Chen Minchun, Song Wei, Li Xueqing, Sun Xiao-Li, Li Guangqing, Jia Yan-Yan, Wen Ai-Dong (2013) High-performance liquid chromatographic method for determination of clinofibrate and its application to a pharmacokinetic study in healthy volunteers. J Pharm Biomed Anal 76: 152–156.

Nakatani H, Nakagome T. Yokota M, Miyawaki H, Aono S, Sakai S (1981) Agatsume K, Hanma N. Lipoclin®. Sumitomo Kagaku 2:37–52.

Nakazawa, H, Kanamaru Y, Murano A (1979) Determination of optical isomers of clinofibrate by high-performance liquid chromatography. Chem Pharm Bull 27: 1694–1696.

Nishizawa Y, Shoji T, Tabata T, Inoue T, Morii H (1999) Effects of lipid-lowering drugs on intermediate-density lipoprotein in uremic patients. Kidney Int Suppl 71:134–136.

Pierides Am, Alvarez-Ude F, Kerr DNS (1975) Clofibrate-induced muscle damage in patients with chronic renal failure. Lancet 2: 1279–1282.

Nishizawa Y, Shoji T, Nishitani H, Yamakawa M, Konishi T, Kawasaki K, Morii H (1993) Hypertriglyceridemia and lowered apolipoprotein C-II/C-III ratio in uremia: Effect of a fibric acid, clinofibrate. Kidney Int 44: 1352–1359.

Ziegler O, Guerci B, Drouin P (1998) The "second atherogenic phenotype" or the role of insulin resistance in vascular risk. Arch Mal Coeur Vaiss 5: 33–39.