P.S. JAIN
H.N. JIVANI
R.N. KHATAL
S.J. SURANA

R.C. Patel Institute of
Pharmaceutical Education and
Research, Shirpur Dist: Dhule
(M.S.), India

STABILITY-INDICATING HPLC DETERMINATION OF CIPROFIBRATE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

A novel stability-indicating high-performance liquid chromatographic assay method was developed and validated for quantitative determination of ciprofibrate in bulk drugs and in pharmaceutical dosage form in the presence of degradation products. An isocratic, reversed phase HPLC method was developed to separate the drug from the degradation products, using an Ace5-C18 (250 mm×4.6 mm, 5 µm) advanced chromatography column, and methanol and water (90:10 v/v) as a mobile phase. The detection was carried out at a wavelength of 232 nm. The ciprofibrate was subjected to stress conditions of hydrolysis (acid and base), oxidation, photolysis and thermal degradation. Degradation was observed for ciprofibrate in base, in acid and in 30% H_2O_2. The drug was found to be stable in the other stress conditions attempted. The degradation products were well resolved from the main peak. The percentage recovery of ciprofibrate was from (98.65 to 100.01%) in the pharmaceutical dosage form. The developed method was validated with respect to linearity, accuracy (recovery), precision, system suitability, specificity and robustness. The forced degradation studies prove the stability indicating power of the method.

Keywords: ciprofibrate; HPLC; validation; stability; degradation.

Ciprofibrate, chemically 2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropanoic acid (Figure 1), is used to treat hyperlipidaemia. PPARα activators have been documented to lower plasma triglycerides and cholesterol levels by decreasing elevated fibrinogen and PAI-1 levels and elevate the level of plasma HDL cholesterol [1-5].Therefore, it is beneficial in the prevention of ischemic heart disease in individuals with elevated levels of LDL cholesterol.

Various methods for the analysis of ciprofibrate in bulk and pharmaceutical formulation individually and in combination have been reported, such as: determination of benzafibrate and ciprofibrate in pharmaceutical formulations by densitometry and video--densitometry TLC, determination of cholesterol decreasing fibrates by chromatographic methods, deter-

mination of benzafibrate, ciprofibrate and fenofibric acid in human plasma by high-performance liquid chromatography, separation of fibrate-type antihyperlipidemic drugs by capillary electrophoresis, study of liquid chromatography retention of some fibrate-type antihyperlipidemic drugs on C~1~8 and CN columns, the enantiomeric resolution of ciprofibrate and related compounds by HPLC using chiral stationary phase, achiral and chiral determination of ciprofibrate and its glucuronide in human urine by capillary electrophoresis, ciprofibrate quantification in human plasma by high-performance liquid chromatography coupled with electrospray tandem mass spectrometry for pharmacokinetic studies, HPLC, TLC densitometry and capillary electrophoresis [6-13]. According to current good manufacturing practices, all drugs must be tested with a stability-indicating assay method before release. To date, no stability-indicating HPLC assay method for the determination of ciprofibrate is available in the literature. It was felt necessary to develop a stability indicating liquid chromatography (LC) method for the determination of ciprofibrate as bulk drug and pharmaceutical dosage form and separate the drugs from the

Correspondening author: P.S. Jain, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur Dist: Dhule (M.S.) 425 405, India.
E-mail: pritash79@yahoo.com

degradation products under the International Conference on Harmonization (ICH) suggested conditions (hydrolysis, oxidations, photolysis and thermal stress) [14,15]. Therefore, the aim of the present study was to develop and validate a stability-indicating HPLC assay method for ciprofibrate as bulk drug and in pharmaceutical dosage form as per ICH guidelines [15].
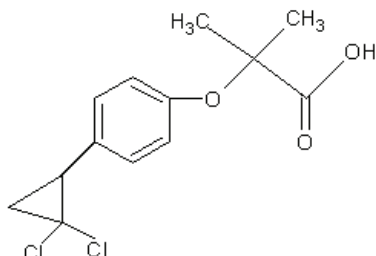

Figure 1. Chemical structure of ciprofibrate.

EXPERIMENTAL

Materials and reagents

Ciprofibrate bulk drug (purity 99.8%) and tablet ciprofibrate (100 mg) were obtained from Glenmark Pharmaceuticals (Nasik, India). Hydrochloric acid and sodium hydroxide pellets were obtained from Rankem Laboratories India. Methanol and *o*-phosphoric acid were obtained from Merck Specialities Private Ltd. Hydrogen peroxide was obtained from Fischer Scientific, India. All chemicals used were of HPLC grade. Milli-QWater was used throughout the experiment.

Chromatographic condition

The HPLC system used was a Shimadzu system equipped with a photodiode array detector. A chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with octadecyl silane Ace5-C18 (Advanced Chromatography Technology, USA) stationary phase with particle size 5 µm were used. The instrumental setting was at a flow rate of 1 mL min^{-1}. The injection volume was 20 µL. The detection wavelength was 232 nm.

Mobile phase

The mobile phase consisted of methanol and water in the ratio 90:10 v/v. The pH 3.7 of mobile phase was adjusted with o-phosphoric acid in double distilled water. The mobile phase was premixed and filtered through a 0.45 µm nylon filter and degassed.

Preparation of standard stock solutions

All solutions were prepared on a weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard. Standard solution of ciprofibrate was prepared by dis-

solving the drugs in the diluents and diluting them to the desired concentration. Diluent A was composed of methanol and diluent B was composed of water in the ratios of 90:10 v/v. Approximately 5 mg of ciprofibrate was accurately weighed, transferred in a 50 mL volumetric flask, dissolved and diluted to 50 mL with the diluent A. From these stock solutions, 2 mL of ciprofibrate standard solution were transferred in a 10 mL volumetric flask and diluted with diluent B. This final solution contained 20 µg mL^{-1} of ciprofibrate.

Sample solution (tablets)

Ten tablets of ciprofibrate (100 mg) were finely ground using agate mortar and pestle. The ground material, which was equivalent to 10 mg of the active pharmaceutical ingredient, was extracted into diluent A in a 25 mL volumetric flask by vortex mixing followed by ultrasonication. 2 mL of the solution was diluted to 40 mL with diluent B. The solution was filtered through a 0.45 µm nylon filter and an appropriate concentration of sample (20 µg mL^{-1} assay concentration) was prepared at the time of analysis.

Procedure for forced degradation study

Stability testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light and enables recommendation of storage conditions, retest periods, and shelf life to be established. The two main aspects of drug product that play an important role in shelf-life determination are assay of the active drug and the degradation products generated during stability studies.

Preparation of sample solution

Acidic degradation. 5 mg of drug was dissolved in 5 mL of diluent A, and 50 mL of 3 M hydrochloric acid was added. The solution was kept for 1 h, after which 10 mL of solution was neutralized with 3 M sodium hydroxide. The solution was then diluted with diluent B to prepare a working solution of 20 µg mL^{-1} (pH of solution was 2.3).

Alkaline degradation. 5 mg of drug was dissolved in 5 mL of diluent A, and 50 mL of 0.5 M sodium hydroxide was added. The solution was kept for 1 h, after which 10 mL of solution was neutralized with 0.5 M hydrochloric acid. The solution was then diluted with diluent B to prepare a working solution of 20 µg mL^{-1} (pH of solution was 14).

Oxidative degradation. 5 mg of drug was dissolved in 5 mL of diluent A, and 50 mL of 30% H_2O_2

was added. The solution was kept for 4 h. The solution was then diluted with diluent B to prepare a working solution of 20 µg mL^{-1}.

Thermal degradation. 10 mg of drug was kept in the hot air oven for 48 h at 100 °C. The working solution was then prepared using diluents A and B.

Photodegradation. 10 mg of drug was exposed to the short wavelength (254 nm) and long wavelength (366 nm) UV light for 48 h. The working solution was then prepared using diluents A and B.

Specificity

Specificity is the ability of the method to unequivocally assess the analyte in the presence of components, which may be expected to be present. Typically, these might include degradation products, matrix, etc. [15]. The specificity study of the developed HPLC method for ciprofibrate was carried out in the presence of its degradation products. Stress studies were performed for ciprofibrate bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress conditions exposing it to acid (3 M hydrochloric acid), alkali (0.5 M NaOH), hydrogen peroxide (30%), heat (100 °C) and UV light (254 and 366 nm wavelength) to evaluate the ability of the proposed method to separate ciprofibrate from its degradation products. For the light and heat studies, the study period was 48 h, whereas the study period was 1 h for acid and base, and 4 h for oxidetion studies. Peak purity testing for ciprofibrate was done by using a PDA detector in stress samples.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The primary target in developing this stability indicating HPLC method is to achieve the resolution between ciprofibrate and its degradation products. To achieve the separation of degradation products we used a stationary phase C-18 and combination of mobile phase methanol and water (90:10 v/v). The separation of the degradation product and ciprofibrate was achieved on Ace5 octadecyl silane C-18 stationary phase and methanol and water (90:10 v/v) as a mobile phase. The tailing factor obtained was less than two and retention time was about 3.3 min for the main peak, and less than 4 min for the degradation products, which would reduce the total run time and ultimately increase the productivity thus reducing the cost of analysis per sample. Forced degradation study showed the method is highly specific and entire degradation products were well resolved from the main peak. The developed method was found to be specific and the method was validated as per international guidelines.

Result of forced degradation experiments

Degradation was not observed for ciprofibrate samples during stress conditions like heat, UV and light, but was observed in base, acid and oxidation studies. Ciprofibrate was degraded in acid (Figure 2), base (Figure 3) and by oxidation (Figure 4) and formed polar impurities. The observed degradation of ciprofibrate was 7.54% in acidic conditions, 4.88% in basic condition after 1 h, and 58.70% after 4 h under
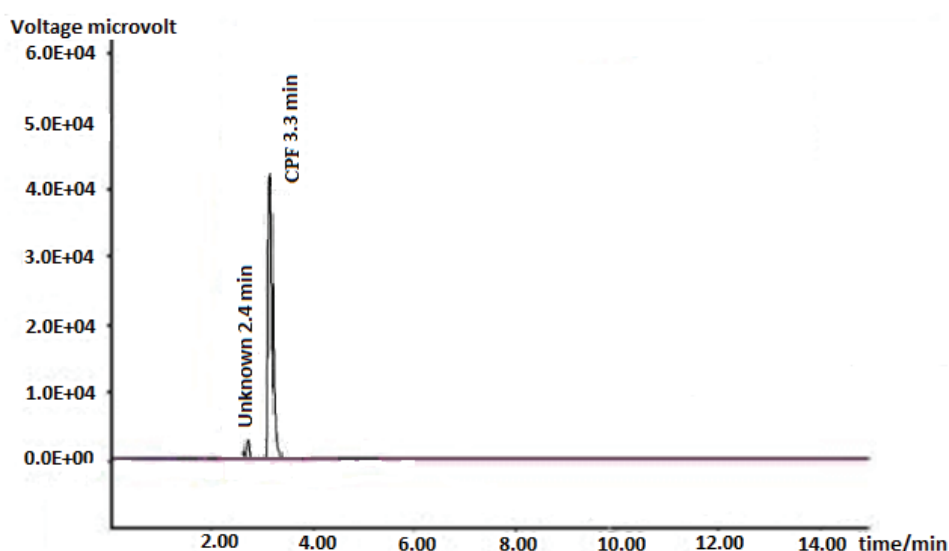

Figure 2. Chromatogram of ciprofibrate in acid degradation (acid degraded product (2.4 min) and ciprofibrate (3.3 min)). Concentration of ciprofibrate injected was 20 µg mL^{-1}.
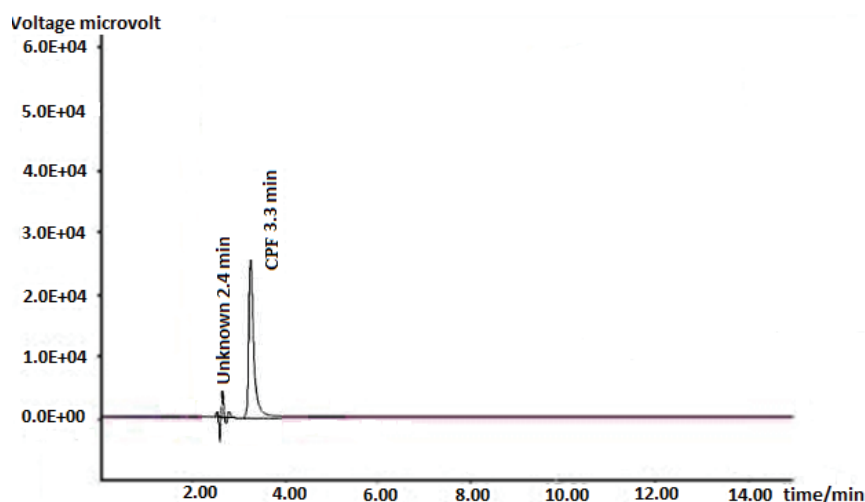
Figure 3. Chromatogram of ciprofibrate in base degradation (base degraded product (2.4 min) and ciprofibrate(3.3 min)).
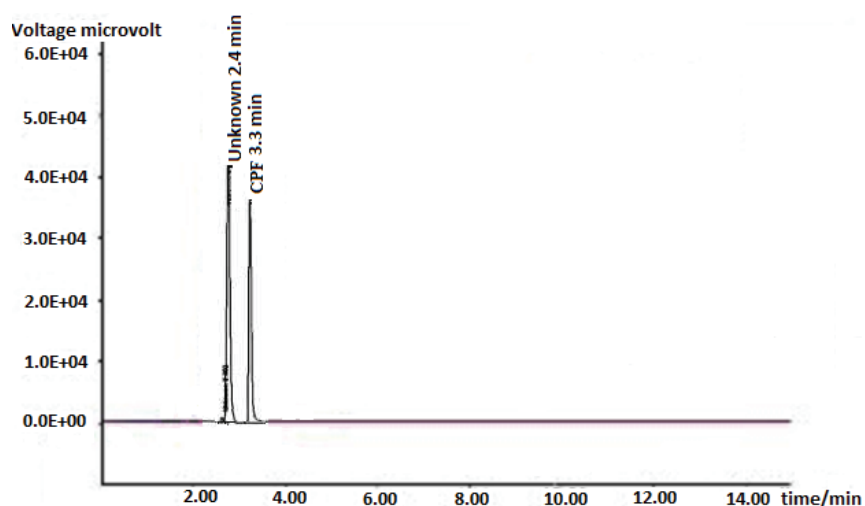

Figure 4. Chromatogram of ciprofibrate in oxidative degradation (oxidative degraded product (2.4 min) and ciprofibrate (3.3 min)).

oxidation conditions. Peak purity results greater than 990 indicate that the ciprofibrate peak is homogeneous in all stress conditions tested. The unaffected assay of ciprofibrate in the tablets confirms the stability indicating power of the method (Table 1).

Determination of active ingredients in tablets

The contents of drug in tablets were determined by the proposed method using the calibration curve.

Method validation

Precision

Method precision (intra-day precision) was evaluated by carrying out six independent assays of ciprofibrate test samples against a reference standard. The percentage of *RSD* of six assay values obtained was calculated. The intermediate precision (inter-day precision) of the method was also evaluated using

Table 1. Summary of forced degradation results

Stress conditions	Time, h	Assay of active substance	Degradation, %	Peak purity[a]
Acid hydrolysis (3 mol L^{-1} HCl)	1	90.24	7.54	999
Base hydrolysis (0.5 mol L^{-1} NaOH)	1	88.69	4.88	999
Oxidation (30% H$_2$O$_2$)	4	40.08	58.70	999
Thermal (100 °C)	48	99.88	No degradation	999
Photo	48	99.65	No degradation	999

[a]Peak purity values in the range of 990-1000 indicate the homogenous peak [16]

two different analysts, different HPLC systems and different days in the same laboratory (Table 2).

Accuracy (recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80, 100 and 120% of the label claim of the tablet (100 mg of ciprofibrate). The recovery samples were prepared in the aforementioned procedure, and then 5 mL of ciprofibrate solution were transferred into a 50 mL volumetric flask and diluted to volume with diluent B. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for ciprofibrate ranged from 98.65 to 100.01%. The average recoveries of three levels for ciprofibrate were 99.5% (Table 3).

Linearity

The linearity of the response of the drug was verified at seven concentration levels, ranging from 10 to 150% of the targeted level (20 µg mL^{-1}). Concentration standard solutions containing 2-30 µg mL^{-1}

of ciprofibrate in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by the plotting peak area *versus* the concentration data and were treated by least-squares linear regression analysis. The equation of the calibration curve for ciprofibrate obtained was $y = 56747x + 15156$, the calibration graphs were found to be linear in the aforementioned concentrations. The coefficient of determination was 0.998 (Table 4).

Limit of detection and limit of quantification (LOD and LOQ)

The limit of detection (*LOD*) and limit of quantification (*LOQ*) were determined by calibration curve method.[11] Specific calibration curve was constructed using samples containing the analytes in the range of *LOD* and *LOQ*. The *LOD* and *LOQ* for ciprofibrate in *LC* were 0.24 and 0.73 µg mL^{-1}, respectively. *LOD* and *LOQ* were calculated by using the following equations:

$$LOD = 3.3\,Sa/b$$

$$LOQ = 10\,Sa/b$$

Table 2. Result of precision of test method

Sample number	Assay of ciprofibrate as % of labeled amount	
	Analyst 1 (intra-day precision)	Analyst 2 (inter-day precision)
1	99.97	99.75
2	99.87	99.40
3	99.98	99.23
4	99.56	99.31
5	99.45	99.43
6	99.68	99.13
Mean	99.75	99.45
RSD	0.12	0.101

Table 3. Results of recovery tests of ciprofibrate

Level of addition, %	Amount added, µg	Recovery, %	Average recovery, %
80	4.8	98.65	99.5
100	6	100.01	
120	7.2	99.84	

Table 4. Linearity study of ciprofibrate

Ser. no.	Concentration, µg/ml	Mean peak area ± *SD* (*n* = 6)	*RSD* / %
1	2	275833±3445.87	1.24
2	4	365974±4217.05	1.15
3	6	491417±5656.12	1.15
4	8	604837±5995.67	0.99
5	10	718773±10348.2	1.43
6	12	835922±5788.57	0.69

where *Sa* is the standard deviation of the calibration curve and *b* is the slope of the calibration curve. Precision at limit of quantification and limit of detection was checked by analyzing six test solutions prepared at *LOQ* and *LOD* levels and calculating the percentage *RSD* of area.

Robustness

In order to determine the robustness of the developed method, experimental conditions were purposely altered and the resolution between ciprofibrate and acid degradation products was evaluated. The flow rate of the mobile phase was 1.0 mL min^{-1}. To study the effect of flow rate on the resolution, it was changed by 0.2 units from 0.8 to 1.2 mL min^{-1}. The effect of percent organic strength on resolution was studied by varying methanol from -10 to +10%. The resolution in the robustness study was not less than 3.5 in all conditions. The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48 and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The *RSD* for the assay results determined up to 72 h for ciprofibrate was 0.53%. The assay values were within 1.5% after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

CONCLUSIONS

The developed method is stability-indicating and can be used for assessing the stability of ciprofibrate bulk drugs and pharmaceutical dosage form. The developed method is specific, selective, robust, rugged and precise. This method can be conveniently used for assessing stability assay of selected substances and dissolution of tablets containing ciprofibrate in quality control laboratory. The study showed that the drug was stable for thermal and photo degradation conditions, whereas moderately degraded in acid (7.54%) and base (4.88%) conditions but highly degraded in the oxidative (58.70%) conditions.

REFERENCES

[1] Martindale, The Complete drug reference, 35th ed., Council of Royal Pharmaceutical Society of Great Britain (2007) 1118

[2] S. Budavari, The Merck Index, 14th ed., Merck and Co. Inc., White House station, NJ, USA (1996) 386

[3] Goodman and Gilmans, The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill Publisher, New York (2003) 971

[4] British Pharmacopoeia, The Stationary Office Medicinal and Pharmaceutical Substances, Vol-I (2005) 106-108

[5] E.G.B. Bighetti, P.R. Patricio, A.C. Casquero, J.A. Berti, H.C.F. Oliveira, Lipids Health Dis. **50**(8) (2009) 1-5

[6] G. Misztal, L. Komsta, J. Planar Chromatogr. **18**(3) (2005) 188-193

[7] E. Kublin, B. Kaczmarska-Graczyk, E. Malanowicz, A. P. Mazurek, Acta Pol. Pharmaceut. **67**(5) (2010) 455-461

[8] L.D. Masnatta, L.A. Cuniberti, R.H. Rey, J. Werba. J. Chromatogr., B. **687**(2) (1996) 437-442

[9] L. Komsta, G. Misztal, E. Majchrzak, A. Hauzer. J. Pharm. Biomed. Anal. **41**(2) (2006) 408-414

[10] G. Misztal, L. Komsta. J AOAC Int. **88**(2) (2005) 479-489

[11] N.H. Anderson, D. Johnston, P.R. Vojvodic, J. Pharm. Biomed. Anal. **10**(7) (1992) 501-505

[12] H. Huttemann, G. Blaschke. J. Chromatogr., B **729**(1-2) (1999) 33-41

[13] S. Singh, M. Bakshi, J. Pharm. Biomed. Anal. **28** (2002) 1011-1040

[14] S. Baboota, S. Faiyaz, A. Ahuja, J. Ali, S. Shafiq, S. Ahmed. Acta Chromatogr. **18** (2007) 116-129

[15] F.D. Mendes, L.S. Chend, A. Borgesd, T. Babadopulosd, J. O. Ilhad, K. M. Alkharfye, G.D. Mendesb, G. De Nuccie, J. Chromatogr., B **879**(24) (2011) 2361-2368

[16] V.K. Ahirrao, D.R. Sangale, V.S. Sonekar, V.V. Thorat, R. P. MArathe, R.B. Nawale, R.P. Pawar. Int. J. Ind. Chem. **2**(2) (2011) 69-77.

P.S. JAIN
H.N. JIVANI
R.N. KHATAL
S.J. SURANA

R.C. Patel Institute of Pharmaceutical
Education and Research, Shirpur Dist:
Dhule (M.S.), India

NAUČNI RAD

PRIMENA HPLC METODE ZA ODREĐIVANJE STABILNOSTI CIPROFIBRATA U FARMACEUTSKOJ SUPSTANCI I FARMACEUTSKIM PREPARATIMA

Razvijena je i validirana nova HPLC metoda za kvantitativno određivanje stabilnosti cip-rofibrata u farmaceutskoj supstanci i farmaceutskim preparatima u prisustvu degrada-cionih proizvoda. Za razdvajanje i određivanje aktivne supstance i degradacionih proiz-voda korišćena je RP-HPLC metoda, sa kolonom Ace5-C18 (250 mm×4.6 mm, 5 μm) i mobilnom fazom metanol/voda u odnosu 90:10 v/v; komponente su detektovane na 232 nm. Ciprofibrat je podrvgnut kiseloj i alkalnoj hidrolizi, oksidaciji u 30% H_2O_2, fotolizi i degradaciji pri zagrevanju. Degradacioni proizvodi se dobro razdvajaju od glavnog pika aktivne susptance. Recovery vrednosti za farmaceutske formulacije su od 98.65 do 100.01%. Razvijena metoda je validirana na linearnost, tačnost (recovery), preciznost, proveru pogodnosti sistema, specifičnost i robustnost.

Ključne reči: ciprofibrat; HPLC; validacija; stabilnost; degradacija.