Development and Validation of Stability-Indicating High-Performance Thin-Layer Chromatography Method for Estimation of Ciprofibrate in Bulk and in Pharmaceutical Formulation

P.S. JAIN*, H.N. JIVANI, R.N. KHATAL, AND S.J. SURANA

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

Summary. A simple, selective, precise, and stability-indicating high-performance thin-layer chromatography (HPTLC) method for the analysis of ciprofibrate both in bulk drug and pharmaceutical formulation has been developed and validated. The method employed HPTLC aluminum plates precoated with silica gel 60 RP-18 F₂₅₄ as the stationary phase. The solvent system consisted of methanol–water–triethylamine (2.8:2.2:0.2 v/v). The system was found to give compact spot for ciprofibrate (R_F value of 0.55 ± 0.02). Densitometric analysis of ciprofibrate was carried out in the absorbance mode at 232 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.998 \pm 0.0015$ with respect to peak area in the concentration range 600–1600 ng per spot. The mean values $\pm$ SD of slope and intercept were 3.38 ± 1.47 and 986.9 ± 108.78 , respectively, with respect to peak area. The method was validated for precision, recovery, and robustness. The limits of detection and quantification were 17.84 and 54.08 ng per spot, respectively. Ciprofibrate was subjected to acid and alkali hydrolysis, oxidation, and thermal degradation. The drug undergoes degradation under acidic and basic conditions. This indicates that the drug is susceptible to acid and base. The degraded product was well resolved from the pure drug with significantly different R_F value. Statistical analysis proves that the method is repeatable, selective, and accurate for the estimation of investigated drug. The proposed developed HPTLC method can be applied for the identification and quantitative determination of ciprofibrate in bulk drug and pharmaceutical formulation.

Key Words: ciprofibrate, high-performance thin-layer chromatography (HPTLC), validation, stability, degradation

Introduction

Ciprofibrate, 2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropanoic acid (Fig. 1), is used to treat “hyper-lipidaemia”, which is PPAR α 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.

Fig. 1. Chemical structure of ciprofibrate

Various methods such as the determination of benzafibrate and ciprofibrate in pharmaceutical formulations by densitometry and videodensitometry thin-layer chromatography (TLC), the determination of benzafibrate, ciprofibrate and fenofibric acid in human plasma by high-performance liquid chromatography (HPLC), 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, HPLC, TLC densitometry and capillary electrophoresis are reported for the analysis of ciprofibrate in bulk and pharmaceutical formulation individually and in combination [6–9]. To our knowledge, no articles related to the stability-indicating chromatographic determination of ciprofibrate by high-performance thin-layer chromatography (HPTLC) in pharmaceutical dosage forms have been reported in literature. The International Conference on Harmonization (ICH) guidelines entitled “Stability Testing of New Drug Substances and Products” require the stress testing to be conducted to elucidate the inherent stability characteristics of the active substance. Susceptibility to oxidation is one of the required tests. Hydrolytic and photolytic stability are also required. An ideal stability-indicating method is one that quantifies the drug and also resolves its degradation products. Unlike HPLC, consumption of mobile phase per sample basis is quite low. This saves cost per analysis and analysis time as well. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. HPTLC technique is most suited for impurity profile of drug substances and content uniformity test as per compendial specifications [10, 11]. The aim of this work is to develop an accurate, specific, repeatable, and stability-indicating method for the determination of ciprofibrate in the presence of its degradation products as per ICH guidelines [12, 13].

Experimental

Chemicals and Reagents

Ciprofibrate was supplied as a gift sample from Glenmark Pharmaceutical Ltd., Nashik, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

HPTLC Instrumentation

The samples were spotted in the form of bands of 6 mm width with a Camag microlitre syringe on precoated silica gel aluminum plates 60 RP-18 F₂₅₄ (10 × 10 cm² with 250 mm thickness, E. Merck), using a Camag Linomat 5 applicator. The plates were prewashed with methanol and activated at 60°C for 5 min prior to chromatography. The slit dimension was kept at 6.00 × 0.45 mm² (micro), and 20 mm s⁻¹ scanning speed was employed. The mobile phase consisted of methanol–water–triethylamine (TEA) (2.8:2.2:0.2, *v/v*), and 10 mL of mobile phase was used. Linear ascending development was carried out in a 10 × 10 cm² twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ± 2°C). The length of the chromatogram run was ~8 cm. Subsequent to development, the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by WinCATS software.

Preparation of Standard Solution and Linearity Study

An accurately weighed quantity of 10 mg ciprofibrate was transferred to 10 mL volumetric flask and dissolved in methanol, and volume was made up to mark with the same solvent to obtain concentration of 1000 ng µL⁻¹. Aliquots of standard solutions 0.6, 0.8, 1, 1.2, 1.4, and 1.6 µL of ciprofibrate were applied on TLC plates with the help of microlitre syringe, using Linomat 5 sample applicator to obtain the concentration of 600, 800, 1000, 1200, 1400, and 1600 ng per spot. The standard curves were evaluated for within-day and day-to-day reproducibility. Each experiment was repeated 6 times.

Method Validation

Precision

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (1000 ng per spot of ciprofibrate). The intra- and inter-day variation for the determination of ciprofibrate was carried out at three different concentration levels of 1000, 1200, and 1400 ng per spot.

Limit of Detection and Limit of Quantification

In order to determine the detection and quantification limits, ciprofibrate concentrations in the lower part of the linear range of the calibration curve were used. Ciprofibrate solutions of 900, 1000, 1100, 1200, 1300, and 1400 ng per spot were prepared and applied in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using equations $LOD = 3.3 \times N/B$ and $LOQ = 10 \times N/B$, where N is the standard deviation of the peak areas of the drugs ($n = 3$), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

Specificity

The specificity of the method was ascertained by analyzing the standard drug and sample. The spot for ciprofibrate in sample was confirmed by comparing the R_F values and spectra of the spot with that of the standard. The peak purity of ciprofibrate was assessed by comparing the spectra at three different levels, i.e. peak start (S), peak apex (M) and peak end (E) positions of the spot.

Ruggedness

Ruggedness of the method was performed by spotting 1000 ng of ciprofibrate by two different analysts, keeping the same experimental and environmental conditions.

Accuracy

The analysed samples were spiked with extra 80%, 100%, and 120% of the standard ciprofibrate, and the mixtures were analysed by the proposed method. At each level of the amount, six determinations were performed.

This was done to check the recovery of the drug at different levels in the formulations.

Robustness

By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different compositions of methanol-water-TEA (2.5:2.5:0.2 and 3:2:0.2, *v/v*) were tried, and chromatograms were run. The amounts of mobile phase, temperature, and relative humidity were varied in the range of $\pm 5\%$. The plates were prewashed by methanol and activated at $60 \pm 5^\circ\text{C}$ for 2, 5, and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20, and 40 min.

Application of Proposed Method to Tablet Formulation

To determine the concentration of ciprofibrate in tablets (labelled claim: 100 mg per tablet), the contents of 20 tablets were weighed, their mean weight was determined, and they were finely powdered. The powder equivalent to 10 mg of ciprofibrate was weighed. The drug from the powder was extracted with methanol. To ensure complete extraction of the drug, it was sonicated for 30 min and the volume was made up to 10 mL. The resulting solution was filtered using $0.41\ \mu\text{m}$ filter (Millifilter, Milford, MA). The above solution (1000 ng per spot) was applied on TLC plate followed by development and scanning as described in the section "HPTLC Instrumentation". The analysis was repeated in triplicate.

Forced Degradation of Ciprofibrate

Acid- and Base-Induced Degradation

Ten milligrams of ciprofibrate were separately dissolved in 10 mL of methanolic solution of 0.1 M HCl and 0.1 M NaOH. These solutions were kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. One millilitre from the above solutions was taken and neutralized and then diluted up to 10 mL with methanol. The resultant solutions were applied on TLC plate in triplicate (10 μL each, i.e. 1000 ng per spot). The chromatograms were run as described in the section "HPTLC Instrumentation".

Hydrogen Peroxide-Induced Degradation

Ten milligrams of ciprofibrate were separately dissolved in 10 mL of methanolic solution of hydrogen peroxide (6.0%, *v/v*). The solution was kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The resultant solution was applied on TLC plate in triplicate (1 μ L each, i.e. 1000 ng per spot). The chromatograms were run as described in the section "HPTLC Instrumentation".

Photochemical Degradation

The photochemical stability of the drug was also studied by exposing the stock solution to direct sunlight for 8 h. The resultant solution (1 μ L, i.e. 1000 ng per spot) was applied on a TLC plate, and chromatograms were run as described in the section "HPTLC Instrumentation".

Dry Heat Degradation Product

The powdered drug stored at 55°C for 3 h under dry heat condition showed no significant degradation. In all degradation studies, the average peak areas of ciprofibrate after application (1000 ng per spot) of three replicates were obtained.

Results and Discussion

Development of Optimum Mobile Phase

TLC procedure was optimized with a view to developing a stability-indicating assay method. Initially, methanol-water (3:2, *v/v*) gave good resolution with an R_F value of 0.55 for ciprofibrate, but typical peak nature was missing. Finally, the mobile phase consisting of methanol-water-TEA (2.8:2.2:0.2, *v/v*) gave a sharp and well-defined peak at R_F value of 0.55 (Fig. 2). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature.

Fig. 2. Chromatogram of standard ciprofibrate (R_F : 0.55), UV detection at 232 nm, mobile phase – methanol–water–triethylamine (2.8:2.2:0.2 v/v)

Calibration Curve

The linear regression data for the calibration curves showed good linear relationship over the concentration range 600–1600 ng per spot. Linear regression equation was found to be $Y = 3.953X + 986.9$ ($r^2 = 0.998$).

Validation of Method

Precision

The precision of the developed HPTLC method was expressed in terms of percent relative standard deviation (%RSD). The results, presented in Table I, revealed high precision of the method.

Table I. Intra-day and inter-day precision of HPTLC method

Drugs	Conc. (ng mL ⁻¹)	Intra-day		Inter-day	
		Amount found (%) ^a	%RSD	Amount found (%) ^a	%RSD
CPF	1000	96.68	1.17	98.46	1.22
	1200	103.94	0.84	101.23	0.72
	1400	99.10	0.31	99.23	0.94

^aMean of three estimations

LOD and LOQ

Detection limit and quantification limit were calculated by the method described in the section “Limit of Detection and Limit of Quantification”. The LOQ and LOD were found to be 54.08 and 17.84 ng, respectively. This indicates the adequate sensitivity of the method.

Recovery Studies

The proposed method when used for extraction and subsequent estimation of ciprofibrate from the pharmaceutical dosage formed after over spotting with 80%, 100%, and 120% of additional drug afforded good recovery of ciprofibrate. The amounts of drug added and determined and the percentage recovery are listed in Table II.

Table II. Recovery study

Drug	Label claim (mg per tablet)	Amount of standard drug added (%) ^a	Drug recov- ered	%RSD
CPF	100	0	98.40	1.02
		80	97.39	1.80
		100	103.84	0.98
		120	103.72	1.33

^aMean of three estimations at each level

Specificity

The peak purity of ciprofibrate was assessed by comparing the spectra at peak start, peak apex, and peak end positions of the spot, i.e. r^2 (S , M) = 0.9998 and r^2 (M , E) = 0.9988. Good correlation (r^2 = 0.9989) was also obtained between standard and sample spectra of ciprofibrate (Fig. 3).

Fig. 3. Typical overlaid spectra of standard drug and drug extracted from tablet

Robustness of the Method

The standard deviation of peak areas was calculated for each parameter, and %RSD was found to be <2%. The low values of %RSD values, shown in Table III, indicated the robustness of the method.

Table III. Robustness of the method

Parameter	SD of peak area	%RSD
Mobile phase composition	44.52	0.90
Mobile phase volume	39.82	0.81
Development distance	32.37	0.65
Activation of TLC plate	39.84	0.81
Duration of saturation	29.79	0.70
Time from spotting to chromatography	21.16	0.50
Time from chromatography to scanning	23.34	0.43

$n = 6$

Analysis of the Marketed Formulation

A single spot at R_F 0.55 was observed in the chromatogram of the drug samples extracted from tablets. There was no interference from the excipients commonly present in the tablet. The % drug content and %RSD were calculated. The low %RSD value indicated the suitability of this method for the routine analysis of ciprofibrate in pharmaceutical dosage forms (Table IV).

Table IV. Summary of validation parameters

Parameter data	CPF
Linearity range (ng per spot)	600–1600
Correlation coefficient	0.998
Limit of detection (ng per spot)	17.84
Limit of quantification (ng per spot)	54.08
Recovery ($n = 6$)	100.83
Ruggedness (%RSD)	
Analyst-I ($n = 6$)	1.13
Analyst-II ($n = 6$)	1.63
Precision (%RSD)	
Repeatability of application ($n = 6$)	0.96–1.29
Inter-day ($n = 6$)	0.56–1.50
Intra-day ($n = 6$)	0.21–1.62
Robustness	Robust
Specificity	Specific

Forced Degradation

The chromatogram of the acid-degraded samples for ciprofibrate showed additional peak at R_F value of 0.10 and 0.44 (Fig. 4), and base-degraded drug shows at 0.03, 0.15, and 0.17 (Fig. 5), respectively. The spot of the degraded product was well resolved from the ciprofibrate spot. In both cases, the concentration of the drug was changing from the initial concentration, indicating that ciprofibrate undergoes degradation under acidic and basic conditions. This indicates that the drug is susceptible to acid-base hydrolysis. The chromatograms of hydrogen peroxide, photo-degraded, and dry heat sam-

ples of ciprofibrate showed only the spots of the pure drug (Figs 6–8). The lower R_F values of degraded components indicated that they were less polar than the analyte itself. The results are listed in Table V.

Table V. Degradation of ciprofibrate

S. no.	Sample exposure condition	Number of degradation product	Ciprofibrate remained (ng per 1000 ng) ($\pm$ SD, $n = 3$)	SE	Recovery (%)
1	0.1 N HCl, 8 h, RT	2 (0.10, 0.44)	638.53	2.45	12.96
2	0.5 N NaOH, 8 h, RT	3 (0.03, 0.15, 0.17)	4737.7	3.04	96.54
3	10% H ₂ O ₂ , 8 h, RT	0	4893.3	1.76	99.71
4	Heat, 3 h, RT	0	4876.16	1.74	99.37

Fig. 4. HPTLC chromatogram of acid-treated (0.1 N HCl, 8 h, RT) ciprofibrate: peak 1 (impurity) (R_F : 0.10); peak 2 (impurity) (R_F : 0.44); peak 3 (ciprofibrate) (R_F : 0.55)

Fig. 5. HPTLC chromatogram of base-treated (0.5 N NaOH, 8 h, RT) ciprofibrate: peak 1 (impurity) (R_F : 0.03); peak 2 (impurity) (R_F : 0.15); peak 3 (ciprofibrate) (R_F : 0.17); peak 4 (ciprofibrate) (R_F : 0.56)

Fig. 6. HPTLC chromatogram of hydrogen peroxide-treated (20% w/v, 8 h, RT) ciprofibrate: peak 1 (ciprofibrate) (R_F : 0.55)

Fig. 7. HPTLC chromatogram of photo-degraded (24 h) ciprofibrate: peak 1 (ciprofibrate) (R_F : 0.55)

Fig. 8. HPTLC chromatogram of heat-treated (8 h, RT) ciprofibrate: peak 1 (ciprofibrate) (R_F : 0.55)

Conclusion

The developed HPTLC method was precise, specific, accurate, and stability-indicating and validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of ciprofibrate as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities. As the method separates the drug from its degradation products, it can be employed as a stability-indicating one.

References

- [1] Martindale: The Complete Drug Reference, 35th edn, Council of Royal Pharmaceutical Society of Great Britain, 2007, pp. 1118-1119
- [2] S. Budavari, The Merck Index, 14th edn, Merck and Co., White House Station, New Jersey, 1996, p. 386
- [3] Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 10th edn, McGraw-Hill, New York, 2003-2004, pp. 971-1002
- [4] British Pharmacopoeia, Medicinal and Pharmaceutical Substances (A-I), The Stationery Office, London, 2005, vol. 1, pp. 106-108
- [5] E.G.B. Bighetti, P.R. Patricio, A.C. Casquero, J.A. Berti, and H.C.F. Oliveira, *Lipids Health Dis.*, **50**(8), 1-9 (2009)
- [6] G. Misztal and L. Komsta, *J. Planar Chromatogr.*, **18**(3), 188-193 (2005)
- [7] L.D. Masnatta, L. A. Cuniberti, R.H. Rey, and J. Werba, *J. Chromatogr. B*, **687**(2), 437-442 (1996)
- [8] N.H. Anderson, D. Johnston, and P.R. Vojvodic, *J. Pharm. Biomed. Anal.*, **10**(7), 501-505 (1992)
- [9] H. Huttemann and G. Blaschke, *J. Chromatogr. B*, **729**(1-2), 33-41 (1999)
- [10] P.D. Sethi, *High Performance Thin Layer Chromatography: Quantitative Analysis of Pharmaceutical Formulations*, CBS, New Delhi, 1996, pp. 162-165
- [11] M. Bakshi and S. Singh, *J. Pharm. Biomed. Anal.*, **28**, 1011-1040 (2002)
- [12] ICH, Q2A text on validation of analytical procedures, International Conference on Harmonization, Geneva, October 1994
- [13] ICH, Q3B validation of analytical procedures: methodology, International Conference on Harmonization, Geneva, November 1996

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