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Research Article

RP-HPLC method development and validation of Ciprofibrate from bulk and pharmaceutical formulation

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Abstract

A rapid, highly sensitive high performance liquid chromatographic method has been developed for the determination of Ciprofibrate (CPF) in bulk drug and in tablets. CPF was eluted from a Qualisil C_8 reversed phase column at temperature (30ºC) with a mobile phase consisting of methanol and water (90:10, v/v) at a flow rate of 1 mL/min with UV detection at 232 nm. The retention time was ~ 4.6 min and each analysis took not more than 6 min. Quantitation was achieved by measurement of peak area without using any internal standard. Calibration graph was linear from 2.0 to 12 mg/mL with limits of detection (LOD) and quantification (LOQ) being 0.24 and 0.73 mg/mL, respectively. The method was validated according to the current ICH guidelines. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 98.65– 100.01%. The % RSD value for intra-day and inter-day precision was found to be in the range of 0.29-1.56. The % RSD below 2.0 shows the high precision of proposed method. According to USP, system suitability tests are an integral part of chromatographic methods. They are used to verify the reproducibility of the chromatographic system.

Keywords: Ciprofibrate, RP-HPLC, Tablet Dosage form, Validation.

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1. Introduction

Analysis is important in every product but it is vital in medicines as it involves life. The assurance of quality is achieved through analysis of drug product. Today, absorption spectroscopy and HPLC are the most valuable analytical techniques for pharmaceuticals. They will probably remain a useful tool in the future despite further advances in analytical chemistry because of several overwhelming advantages for the solution of many problems. These advantages include speed, simplicity, specificity and sensitivity.

Ciprofibrate is a fibric acid derivative that primarily acts as an agonist of peroxisome proliferator-activated receptor alpha (PPAR-α) and is 2-[4-(2,2-dichlorocyclopropyl)phenoxy]-2-methylpropanoic acid. Ciprofibrate is a lipid-lowering medication from the fibrates class, used to treat hyperlipidemias, particularly high triglycerides and low HDL cholesterol. It works by decreasing triglyceride and VLDL cholesterol levels and increasing HDL cholesterol. It may cause muscle pain or weakness and is contraindicated during pregnancy and nursing.

The USP official method for the estimation of Ciprofibrate from tablets is by HPLC using C8 column. Various methods have been reported for estimation of clozapine in biological matrices such as plasma. Ciprofibrate having a strong chromophore shows UV absorption and hence most of these methods include the use of HPLC with UV detector. Spectrophotometric methods have also been developed for estimation of clozapine in samples of greater purity. Stability indicating methods have also been reported for its in vitro determination in gastric and intestinal fluids and pharmaceutical formulations. Most of the reported HPLC methods use the C 8 column. However, in small-scale industries, educational institutions or moderate budget analytical sections, it may be difficult to have dedicated columns for analysis. In such cases a C18 column is mostly preferred since most drugs have a considerable lipophilicity and can be analysed using this column. The purpose of this work was to develop a reproducible, robust and sensitive method for the determination of Ciprofibrate from tablets using C18column.

2. Materials and Methods

2.1 Chemicals & Reagents

Ciprofibrate (CPF) was supplied as a gift sample by Glenmark Pharmaceuticals Ltd, Nasik, India. All the chemicals used of HPLC Grade (MERCK. Chem. Ltd., Mumbai) and double distilled R.O water was used for mobile phase preparation.

2.2 Instrument

Shimadzu HPLC system with LC-10 AT VP solvent delivery system, SPD M-10AVP photo diode array detector and Class-M 10 data station were used for quantitative estimation of Ciprofibrate in pharmaceutical dosage form.

2.3 Experimental Works

Chromatographic system:

Instrument	Specifications
HPLC System Pump: Detector: Data processor: Column:	Shimadzu HPLC system LC-10 AT VP solvent delivery system SPD M-10AVP photo diode array detector Class-M 10 data station LCGC Qualisil BDS C_8 (5 µm, 250 mm X 4.6 mm i.d.)
Weighing Balance:	SHIMADZU AUX – 120
Digital pH Meter:	Systronics µ pH System 362
Ultrasonicator:	ENERTECH Electronics Pvt. Ltd.

Selection of Stationary phase

Based on reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with C_8 bonded phase i.e. LCGC Qualisil BDS C_8 (250 mm x 4.6 mm I.D.) with particle size 5 µm was selected.

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving 10 mg of CPF in 10 ml methanol that gives concentration of 1000 µg/ml solution of CPF. From this stock solution appropriate dilution were prepared.

Optimization of Chromatographic Parameters

Optimization in HPLC is the process of finding a set of conditions that adequately separate and enable the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

Optimization of mobile phase strength

The mobile phase was chosen after several trials with Methanol and water in various proportions. A mobile phase consisted of Methanol: water (90:10, v/v), pH 3.7(pH adjusted with orthophospharic acid) was selected to achieve symmetrical peak and Sensitivity. The effects of flow rates in the ranges of 0.5 to 1.5 ml/min were examined. A flow rate of 1 ml /min gave reasonable retention time; using reverse phase C_8 column, the retention times of CPF was observed 4.2 min. The total time of analysis was less than 7 min.

Table 1: Optimization of Mobile Phase Strength

Sr No.	Mobile phase composition [Methanol: Water, v/v]	t_R of CPF [min.]
1	**60:40**	**3.23**
2	**70:30**	**3.45**
3	**80:20**	**3.82**

Optimization of the mobile phase

Optimization of mobile phase was performed based on resolution, asymmetry factor and peak area obtained. Mobile phase was prepared using various combinations of polar and non-polar solvents. The pH of the mobile phase was varied from 5.8 to 8.3 using various ratios of 0.05 M phosphate buffer. The buffer was prepared by dissolving 2.04 g of potassium dihydrogen phosphate in 300 ml water and filtered using membrane filter. This solution was then mixed with required quantity of acetonitrile and the solution was sonicated for 20 mins before use.

Table 2: Optimized program for the assay

Parameters	Condition
Stationary Phase	**Lachrom** C_{18}(150 x 4.6mm, 5µm)
Flow Rate	1.0 mL/min
Detection	270nm
Pump Mode	Isocratic
Injection Volume	20 µl
Run Time	5min
Column Temperature	25˚C
Thermostat	25˚C
Retention Time	About 3.5 min

Optimization of Detection Wavelength

PDA detector was selected, as it is reliable and easy to set at the correct wavelength. A fixed concentration of analyte was analyzed at different wavelengths. As per the response of analyte, 232 nm was selected. UV-spectra of CPF are shown in **Fig.1.**

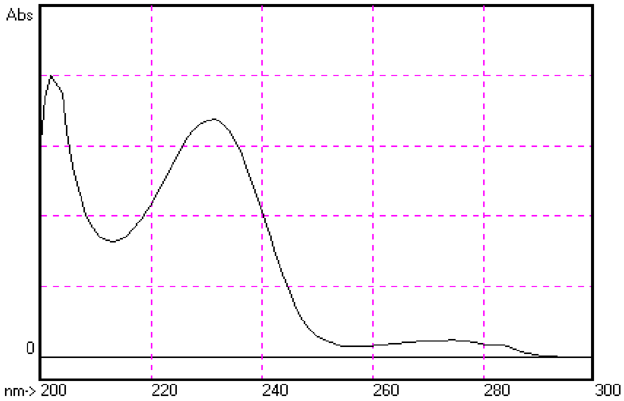

Fig. 1: UV-Spectra of CPF

Linearity Studies

From stock solution aliquots of 20, 40, 60, 80, 100 and 120 µl were taken in 10 ml volumetric flasks and diluted up to the mark with the mobile phase. such that the final concentration of CPF in the range 2 – 12 µg/ml. Volume of 20 µL of each sample was injected with the help of Hamilton Syringe. All measurements were repeated six times for each concentration and calibration curve was constructed by plotting the peak area *vs* the drug concentration.

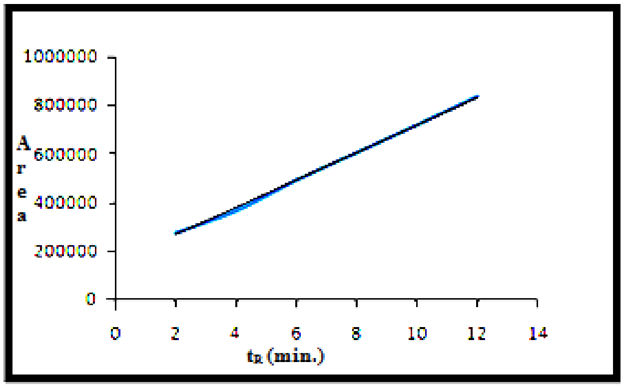

Fig. 2: Linearity curve of CPF

Y= 56747 X +151566

Correlation Coefficient = 0.998

Application of the proposed method to bulk sample

Accurately weighed quantity 10 mg (CPF) was transferred to 10 ml volumetric flask and volume was adjusted to mark with the mobile phase. The solution was further diluted to get concentration 8 µg/ml was subjected to proposed method and amount of CPF was determined. The procedure was repeated for six times; results are shown in **Table 3** and chromatogram of laboratory mixture showed in **Fig. 3.**

Table 3: Analysis of laboratory mixture

Component	Amount taken in [µg/mL]	Amount found [µg/mL] Mean ± S.D.	Amount found (%) Mean ± S.D.
CPF	**8**	**8.14 ± 0.12**	**101.78 ± 1.58**
	%R.S.D.	**1.58**	**1.55**

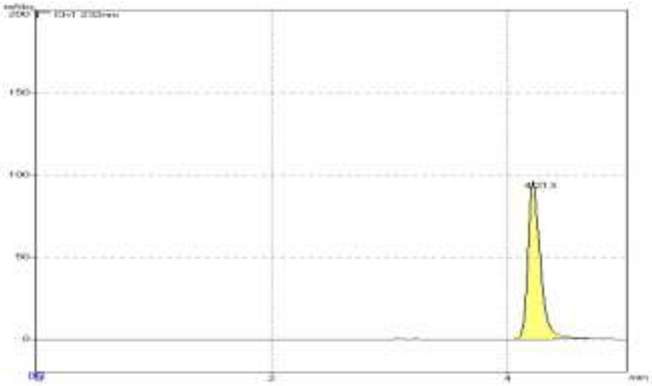

Fig. 3: Chromatogram standard

Application of proposed method to tablet formulations

To determine the content of CPF in conventional tablets (Label claim 100 mg CPF per tablet); the twenty tablets were weighed, their mean weight determined and they were finely powered and powder equivalent 10 mg CPF was transferred into a 100 mL volumetric flask diluted to 100 mL with methanol, sonicated for 20 min. The resulting solution was filtered, using 0.45 µm filter (Millifilter, Milford, MA). Excipients were separated by filtration. The solution was further diluted to get concentration 8 µg/mL was subjected to proposed method and amount of CPF was determined. The assay procedure was repeated for six times; results are shown in **Table 5** and chromatogram of tablet solution in **Fig. 4**.

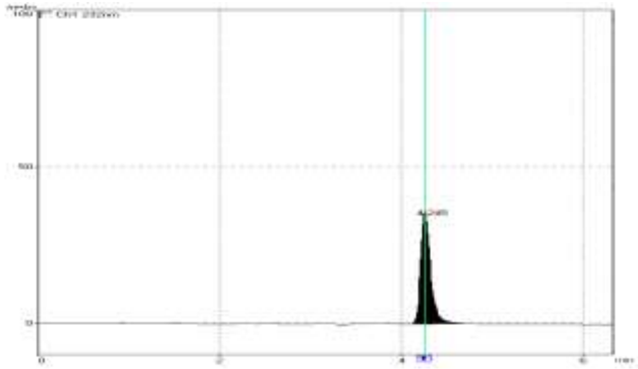

Fig. 4: Chromatogram of Tablet Solution

Analysis of tablet formulation

Average wt = 0.250 gm

Component	Amount taken in [µg/mL]	Amount found [µg] Mean ± S.D.	% Amount found Mean ± S.D.
CPF	**8**	**8.14 ± 0.12**	**101.78 ± 1.58**
	%R.S.D.	**1.55**	**1.55**

***Average of six determinations**

%Amount found was found to be 101.78% with %RSD less than 2, so it was concluded that this method was specific for determination of ciprofibrate from pharmaceutical formulation.

Validation of Proposed Method

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

Accuracy

It was done by recovery study using standard addition method at 80, 100 and 120 % level; known amount of standard CPF was added to preanalyzed sample (6 µg/ml of CPF) and subjected them to the proposed HPLC method. Results are shown in **(Table 6).**

Table 6: Results of Recovery Studies*

Drug	Initial amount of drug [µg/mL]	Excess drug added to the analyte [%]	Amount added [µg/mL]	% Recovery [n = 3]	% R.S.D.
CPF	6	0	0	100.01	1.61
	6	80	4.8	98.78	1.46
	6	100	6	99.84	1.32
	6	120	7.2	98.65	1.49

***Average of six determinations**

High recovery (98.65-100.01%) and %R.S.D. confirmed that proposed method accurate for determination of ciprofibrate in different pharmaceutical formulation.

Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Repeatability

It was measured by multiple injections of a homogenous sample of 6 µg/ml of CPF that indicates the performance of the HPLC instrument under chromatographic conditions. Results are shown in **Table 7.**

Table 7: Results of Repeatability study

Drug	Concentration (µg/ml)	Amt found* Mean ± S.D.
CPF	**6**	**100.53 ± 1.06**
	%R.S.D.	**1.06**

***Average of six determinations**

8.2.2. Intra – day and Inter – day Precision

Intra – day precision was determined by analyzing, the three different concentrations 4 µg/ml, 6 µg/ml and 8 µg/ml of CPF, for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three different days, over a period of one week. These result shows reproducibility of the assay. The % R.S.D. values are showed in **(Table 8)** i.e. less than 2 and low standard deviation, so that indicate this method precise for the determination of ciprofibrate.

Table 8: Results of precision studies (Intra-day and Inter-day)

Drug	Conc. (µg/mL)	Intraday Amount found(µg/mL)		Interday Amount found(µg/mL)	
		Mean ± S.D.	%R.S.D. n=3	Mean ± S.D.	%R.S.D. n=3
CPF	**4**	**4.19 ± 0.029**	**0.69**	**4.13 ± 0.064**	**1.56**
	6	**5.91 ± 0.039**	**0.65**	**6.03 ± 0.072**	**1.20**
	8	**8.34 ± 0.043**	**0.52**	**8.35 ± 0.024**	**0.29**

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The DL and QL were calculated using Eqs. (1) and (2), respectively. LOD and LOQ were found to be 0.24 and 0.73 for CPF, respectively.

DL = (S.D. / α) × 3.3……(1)

QL = (S.D. / α) × 10…….(2)

Where, S.D. was the standard deviation of curve and α was the slope of curve.

Specificity and Selectivity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method was quite selective. There was no other interfering peak around the retention time of CPF; also, the base line did not show any significant noise.

Ruggedness

From stock solution, sample solution of CPF (6 µg/ml) was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times. The results are shown in **(Table 9).**

Table 9: Results of Ruggedness study

Analyst	%Amount found of CPF (Mean ± S.D.)	%R.S.D. [n=3]
I	99.47 ± 0.95	0.95
II	100.35 ± 1.30	1.30

System Suitability Test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing. Results are shown in **(Table 11).**

Table 10: System Suitability Test

System Suitability Parameters	Proposed Method
Retention Time (t_R)	4.20
Theoretical Plate (N)	6590
Tailing Factor (T)	1.16

AS shown in table, no. of high theoretical plate, peak symmetry ($N \geq 1$) and proper retention time indicates that proposed method was suitable for determination CPF.

Robustness

To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate, changing the pH of the solution, changing column oven temperature, using 6 µg/ml solution of CPF. Results are shown in **(Table 11)** shows that proposed method was robust.

4. Conclusion

A rapid, highly sensitive high performance liquid chromatographic method has been developed for the determination of ciprofibrate (CPF) in bulk drug and in tablets. CPF was eluted from a Qualisil C_8 reversed phase column at temperature (30ºC) with a mobile phase consisting of methanol and water (90:10, v/v) at a flow rate of 1 mL/min with UV detection at 232 nm. The retention time was ~ 4.6 min and each analysis took not more than 6 min. Quantitation was achieved by measurement of peak area without using any internal standard. Calibration graph was linear from 2.0 to 12 mg/mL with limits of detection (LOD) and quantification (LOQ) being 0.24 and 0.73 mg/mL, respectively. The method was validated according to the current ICH guidelines. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 98.65– 100.01%. The % RSD value for intra-day and inter-day precision was found to be in the range of 0.29-1.56. The % RSD below 2.0 shows the high precision of proposed method. According to USP, system suitability tests are an integral part of chromatographic methods. They are used to verify the reproducibility of the chromatographic system.

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