Quantitative Determination of Ciprofibrate in Tablets by Derivative UV Spectroscopy and RP-HPLC Method

P. S. JAIN*, H. N. JIVANI, R. N. KHATAL AND S. J. SURANA
Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur-425 405, India

A derivative UV spectrophotometric and a reversed phase high-performance liquid chromatographic method for the determination of ciprofibrate in tablets was developed. The first-order derivative UV spectrophotometric method was found to be accurate with 100.57±0.97 recovery and precise with a coefficient of variation of 1.44. These results were compared to those obtained by reference methods, zero-order UV spectrophotometric method and a reversed-phase high-performance liquid chromatography method. A reversed-phase C_8 column with methanol:water (90:10, v/v, pH 3.7) mobile phase was used and the detector wavelength was set at 232 nm. Calibration solutions used in HPLC were ranging from 2 to 12 μg/ml. An ANOVA test (P = 0.0226, F = 4.935) showed that the results obtained with the derivative UV spectrophotometric method were comparable to those obtained using reference methods.

Key words: Ciprofibrate, content determination, derivative UV spectrophotometry, reversed phase high-performance liquid chromatographic

Ciprofibrate, 2-(4-(2,2-dichlorocyclpropyl)phenoxy)-2-methylpropanoic acid (fig. 1), a PPARα activator, is used to treat hyperlipidaemia and was found to be beneficial in the prevention of ischemic heart disease in individuals with elevated levels of LDL cholesterol. In addition ciprofibrate is also found to modestly decrease elevated fibrinogen and plasminogen activator inhibitor-1 levels and elevate level of plasma HDL cholesterol[1-4].

Various methods have been reported for the analysis of ciprofibrate in bulk and in pharmaceutical formulations; examples of which being. A HPLC method with different column materials and mobile phase systems[5]; determination of benzafibrate, ciprofibrate and fenofibric acid in human plasma using HPLC[6]; enantiomeric resolution of ciprofibrate and related compounds by HPLC using chiral stationary phase[7]; achiral and chiral determination of ciprofibrate and its glucuronide in human urine using capillary electrophoresis[8]; and determination of benzafibrate and ciprofibrate employing densitometric and video-densitometric TLC[9].

In this communication, a first-order derivative UV spectrophotometric method at 245 nm is being reported for the determination of ciprofibrate in tablets. No spectrophotometric methods for the determination of ciprofibrate in tablets have been reported. For this reason, a derivative UV spectrophotometric method was developed which could be used in routine analysis of ciprofibrate. Furthermore, quantitative determination of ciprofibrate in tablets was also performed using a RP-HPLC method and a zero-order spectrophotometric method (as reference methods) and the results obtained by the proposed method were compared to those obtained using the reference methods.

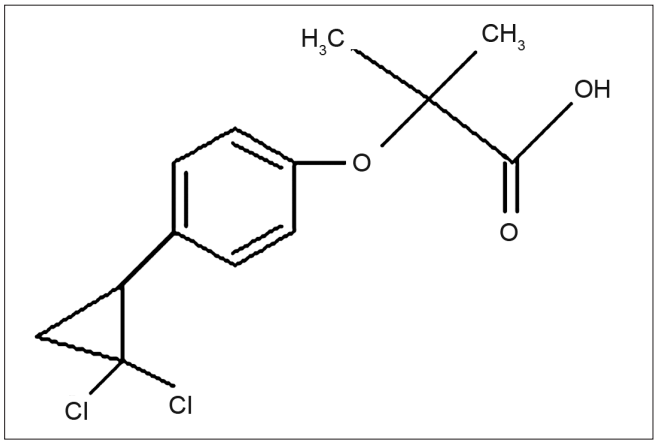

Fig. 1: Chemical structures of ciprofibrate

Spectroscopic analysis was performed on a Shimadzu 2450 (PC Series) UV/Vis double beam spectrophotometer (Software UV Probe 2.21) with a pair of 10 mm matched quartz cells, which were used to measure absorbance of resulting solutions. UV spectra of the reference and the test solutions were

***Address for correspondence**
E-mail: pritash79@yahoo.com

recorded at wavelengths with in 200-400 nm range. The first-order derivative spectra were also obtained over the 200-400 nm range (*n*=5). Spectral bandwidth used was 2 nm, and scan speed was set to 480 nm/min (slow-mode).

All reagents used in this investigation were of analytical grade. Pharmaceutical grade ciprofibrate was obtained from Glenmark Pharmaceuticals Ltd., Mumbai, India. For the preparation of standard ciprofibrate stock solution, 10 mg ciprofibrate was accurately weighed and dissolved in methanol in a 100 ml volumetric flask and volume was made up with distilled water. Standard solutions in the range 3-18 μg/ml were prepared by appropriate dilution of the stock solution.

Twenty tablets of ciprofibrate were accurately weighted and powered and a quantity equivalent to 100 mg of ciprofibrate was transferred to a 100 ml volumetric flask, 50 ml of methanol was added and sonicated for 20 min. The resulting solution was filtered through a Whatmann filter paper no. 41 and the residue was washed thoroughly with methanol. The filtrate and the washings were combined in a 100 ml volumetric flask and diluted to the mark with methanol. Ciprofibrate content of the tablets was calculated by referring to calibration curves obtained using standard solutions of ciprofibrate in the concentration range of 3 to 18 μg/ml for both zero-order at 236 nm and for the first-order derivative method at 245 nm (n=5).

Ciprofibrate analysis was also performed on a HPLC system of Shimadzu (Japan) comprising of a LC-10AT *vp* solvent delivery system (pump), SPD M-10 A *vp* Diode array detector, CTO-10AS *vp* as a column oven and a Rheodyne injector with 20 μl loop. Class-M 10A data station was used as a data processor. Chromatographic separation was carried out on a Qualisil C_8 (5 μm, 25×4.6 mm i.d.). Prior to chromatography, the mobile phase was filtered using a 0.45 μm membrane filter and degassed by ultrasonic vibrations. All experiments were carried out at 30° and the flow rate of the mobile phase used was 1.0 ml/min. Sample injection volume used was 20 μl. All determinations were performed at 232 nm.

The mobile phase used was 90:10 (v/v) methanol:water at pH 3.7 adjusted with orthophosphoric acid. After mixing, the mobile phase was degassed and filtered. In order to prepare ciprofibrate stock solution, 10 mg ciprofibrate was accurately weighed, dissolved and diluted to 10 ml with the mobile phase. Standard solutions ranging from 2 to 12 μg/ml were prepared in the mobile phase. All solutions were prepared with doubled distilled water. All reagent used were of HPLC grade (Merck Pvt. Ltd., India)

Twenty tablets of ciprofibrate were accurately weighted and powered and the quantity equivalent to 100 mg of ciprofibrate was transferred to a 100 ml volumetric flask, 50 ml mobile phase was added and sonicated for 20 min. The solution was filtered through a Whatmann filter paper No. 41, the residue was washed thoroughly with the mobile phase; the filtrate and the washings were combined in a 100 ml volumetric flask and the volume was made up with the mobile phase. An Aliquot of this solution was diluted in the mobile phase to get the final concentration. Ciprofibrate standard solutions were injected and a calibration curve was obtained as peak area versus concentration. Tablet solution (20 μl) was injected, detection was carried out at 232 nm and the amount of ciprofibrate in tablet was calculated.

In this study, quantitative determination of ciprofibrate in tablets was performed by first-order derivative UV spectrophotometric method and the results were compared to those obtained using two reference methods, a HPLC and a zero-order UV spectrophotometric method. Derivative UV spectrophotometric method developed was to investigate if it is applicable in routine analysis. It was found to be simple, rapid and sensitive. Zero-order and first-order derivative spectra of ciprofibrate were shown in figs. 2 and 3, respectively. Regression analysis for the first-order derivative UV spectrophotometric method was carried out (Table 1) and the correlation coefficient (r^2 0.999) obtained confirmed linearity and adherence to Beer's law over the concentration range of 3-18 μg/ml. Quantitative analysis of ciprofibrate in tablets was performed using this derivative UV spectrophotometric method and the results obtained showed a good agreement with the labeled amount of ciprofibrate (Table 2). In addition, the coefficient of variation (CV) for the determination of ciprofibrate was 1.60. Closeness of the amount found to the amount taken and the low coefficient of variation value showed that the proposed method was accurate and precise. A recovery study was performed by spiking 80,100 and 120 μg/ml and the % recovery values ranged from 99.30-102.84 (Table 3). A % RSD of less than 2 indicated that this

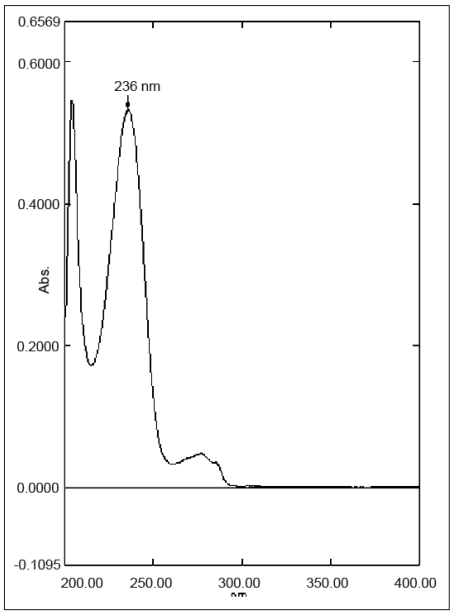

Fig. 2: Zero Order UV Spectrum of ciprofibrate

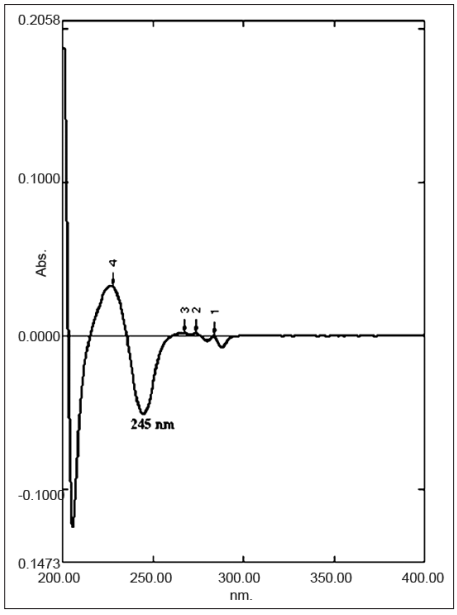

Fig. 3: First Order Derivative Spectrum of ciprofibrate

TABLE 1: STATISTICAL ANALYSIS OF THE CALIBRATION CURVE OF CIPROFIBRATE

Method	Analytical wavelength (nm)	Linearity range (μg/ml)	Regression equation	Correlation coefficient (r^2)
Zero order*	236	3-18	y=0.051C+0.039	0.999
First order*	245	3-18	y=0.004 C+0.001	0.999
RP-HPLC*	232	2-12	y=56747 C+151566	0.998

C is the concentration of analyte in μg/ml. *n=5

TABLE 2: DETERMINATION OF CIPROFIBRATE IN TABLETS

Method	Amount taken (μg/ml)	Amount found (μg/ml)	% Amount found*	SD	CV
Zero order[a]	9	9.13	101.48	1.56	1.54
First order[a]	9	8.98	99.82	1.60	1.60
RP-HPLC[a]	9	8.91	99.06	0.69	0.70

*Average of six experiments, [a]n=5, SD is standard deviation and CV is coefficient of variation

TABLE 3: RECOVERY ANALYSIS OF CIPROFIBRATE IN TABLETS

Method	Amount taken (μg/ml)	Amount added (μg/ml)	Amount recovered (μg/ml)	% Recovery	SD
Zero order[a]	9	80	7.2	99.30	0.78
		100	9	99.66	0.49
		120	10.2	102.84	1.66
First order[a]	9	80	7.2	7.26	100.83
		100	9	9.19	102.11
		120	10.2	10.32	101.17
RP-HPLC[a]	6	4.8	4.78	99.58	1.89
		6	5.98	99.66	1.32
		7.2	7.17	99.58	1.12

TABLE 4: PRECISION STUDIES (INTRA-DAY AND INTER-DAY) FOR DERIVATIVE SPECTROSCOPY AND RP-HPLC

Drug	Conc. (μg/ml)	Intraday amount found* (μg/ml)		Interday amount found* (μg/ml)	
		Mean±SD	%RSD	Mean±SD	%RSD
CPF	9	99.16±0.0006	1.69	100.63±0.0006	1.61
	12	103.83±0.0008	1.20	109.88±0.0009	1.84
	15	102.11±0.0007	1.09	101.80±0.00056	0.89
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

*Mean of three estimations at each level

method is highly precise (Table 4). High Recovery values, low % RSD and low standard deviations established the suitability of the proposed method for accurate and precise determination of ciprofibrate in tablets. Ciprofibrate was also analysed using a modified reversed-phase high-performance liquid chromatographic method. Typical chromatogram obtained for standard ciprofibrate is shown in fig. 4. Ciprofibrate gave a well-shaped, symmetrical single peak that was well separated from the solvent front indicating no additional extractions or separations were required. High correlation coefficient value (Table 1) and low standard deviation (Table 2) proved that HPLC method was precise and accurate. In addition,

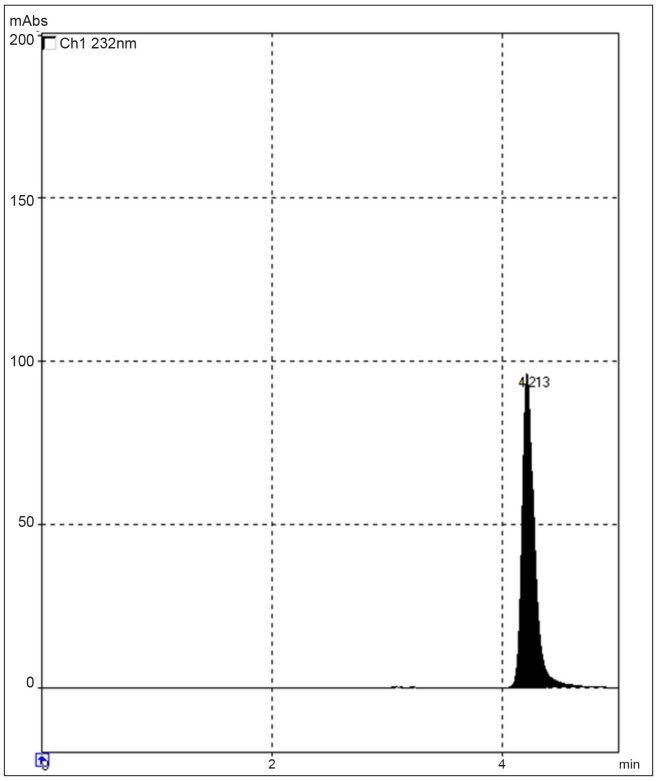

Fig. 4: Typical HPLC chromatogram of ciprofibrate The chromatogram was developed in mobile phase consisting of 90:10 methanol:water, pH 3.7 at 232 nm and a flow rate of 1.0 ml/min.

TABLE 5: COMPARISON OF THE THREE METHODS FOR THE DETERMINATION OF CIPROFIBRATE IN TABLETS, ANOVA TEST

Amount taken (9 µg/ml)	Zero order	First order	RP-HPLC
Amount found*	9.13	8.98	8.91
CV	1.54	1.60	0.70

*Average of six experiments, ANOVA, $P = 0.0226$; $F = 4.935$

relatively high recovery value, 99.58-99.68% (Table 3), was obtained. Furthermore, the results obtained with HPLC were in good agreement with those obtained using the first order derivative UV spectrophotometric method. Finally, results obtained using all three methods for the determination of ciprofibrate in tablets were subjected to ANOVA test (Table 5) which indicated that there were no significant differences between results.

Thus a simple, sensitive and reproducible derivative UV spectrophotometric method for quantitative determination of ciprofibrate was developed and this method is suitable for the determination of ciprofibrate in tablets and could be used in a quality control laboratory for routine sample analysis. It can be concluded that the proposed first-order derivative UV spectrophotometric and HPLC method is suitable for the analysis of ciprofibrate in commercial tablets.

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