

Validation of a spectrophotometric method to determine ciprofibrate content in tablets

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Ciprofibrate is a drug indicated in cases of hypertriglyceridemia and mixed hyperlipidemia, but no monographs are available in official compendia for the analysis of this substance in tablets. The objective of this work was to develop and validate a spectrophotometric method for routine analysis of ciprofibrate in tablets. In this study, commercial and standard ciprofibrate were used, as well as placebo in absolute ethanol, analyzed by UV spectrophotometer. All tests followed the rules of Resolution RE-899, 2003. The results showed that the developed and validated method offers low cost, easy implementation, precision and accuracy, and may be included in the routine of quality control laboratories.

Uniterms: Ciprofibrate/analysis. Spectrophotometric method/medicines analysis. Medicines/quality control.

O ciprofibrato é um fármaco indicado em casos de hipertrigliceridemia e hiperlipidemia mista, mas não há monografias em compêndios oficiais para a análise desta substância em comprimidos. O objetivo deste trabalho é desenvolver e validar um método espectrofotométrico para análise de rotina de ciprofibrato em comprimidos. Neste estudo foram empregados ciprofibrato comercial, padrão e placebo em etanol absoluto, analisadas por espectrofotometria UV. Todos os testes seguiram as regras da Resolução RE-899, 2003. Os resultados mostraram que o método desenvolvido e validado apresenta baixo custo, fácil implementação, precisão e exatidão e pode ser incluído em rotina de laboratórios de controle de qualidade.

Unitermos: Ciprofibrato/análise. Método espectrofotométrico/análise de medicamentos. Medicamentos/controle de qualidade.

INTRODUCTION

Dyslipidemia is a chronic degenerative disease characterized by abnormal levels of lipids in the blood (Molly, Brunzel, 2004) which affects a large proportion of the population in different ways according to ethnicity, socioeconomic status and other factors (Bertolami *et al.*, 1993; Eizerik, Manfroi, 2008; Souza *et al.*, 2003).

In 1998, it was found that 38% of men and 42% of women in the Brazilian population had total cholesterol levels above 200 mg/dL (DBD, 2007), and a high incidence was also found in other countries including India

and the United States of America (Davidson, Yannicelli, 2004). Dyslipidemia is one of the factors predisposing patients to a number of diseases such as atherosclerosis (Luo *et al.*, 2009) and cardiovascular problems (Lotufo, 1996).

Fibrates are a group of drugs derived from fibric acid (Xavier, 2005), referred to as drugs of choice for cases of hypertriglyceridemia as an adjuvant of statins (Schultz, 2006) and for conditions including mixed dyslipidemia in patients with type-2 diabetes mellitus (DBD, 2007; Xavier, 2005), especially for patients resistant or intolerant to statins (Abourbih, 2009).

Ciprofibrate is chemically known as 2-[4-(2,2-dichlorocyclopropyl) phenoxy]-2-methylpropanoic acid (Figure 1), having a molecular formula $C_{13}H_{14}C_{12}O_3$ and molecular weight of 289.2 (Merck Index, 2001). It is a white crystalline or lightly yellowish powder, with a melt-

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ing point of about 115.0 °C that is practically insoluble in water, freely soluble in anhydrous ethanol, and soluble in toluene (BP, 2008).

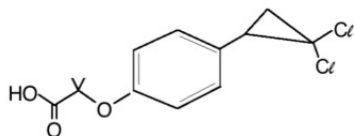


FIGURE 1 - Chemical structure of ciprofibrate.

In Brazil, ciprofibrate is marketed as Oroxadin (Sanifi-Aventis), Lipless (Biolab Sanus) and Ciprolip (Uci-Farma), all containing 100 mg per tablet in their presentation for oral administration.

According to Xavier (2005), fibrates are absorbed through the gastrointestinal tract, and glucuronidated compounds by the liver, and are excreted by the kidneys, peaking at between 2 and 8 h. Fibrates have an elimination half-life of between 2 and 80 h depending on the doses used, and act through the stimulation of nuclear receptors activated by the proliferation of alpha-peroxisomes, leading to increased production and action of lipoprotein lipase, reduction of apolipoprotein CIII; decrease in the synthesis of VLDL and increase in HDL-c levels.

The concern about the quality of drugs sold increases every year (La Roca *et al.*, 2007), not only in commercial terms, but also legal and ethical aspects, since the health of patients depends on the quality and effectiveness of these drugs (Linsbinski *et al.*, 2008; Ribani *et al.*, 2004). In this regard, various regulatory agencies around the world are demanding validation methods for the registration of new drugs to ensure the quality of the drugs marketed (Brasil, 2003; Grillo *et al.*, 2009; ICH, 1996; Santana *et al.*, 2007; Valentini *et al.*, 2004).

There is a great interest in developing rapid and efficient analytical methods that provide precise and accurate parameters for the quantitative analysis of drugs, important for routine analysis during quality control and development of new drug forms (Ruela *et al.*, 2009), with spectrophotometric analysis being the most likely candidate.

To date, no validated methods are available in official compendia for the analysis of ciprofibrate tablets. This fact, together with the importance of this drug in the treatment of a chronic disease that affects millions of people around the world, justified the conducting of a validation test for this drug.

The objective of this study was to develop and validate a spectrophotometric method for routine analysis of quality control of ciprofibrate tablets, to be used by industry and pharmacies.

MATERIAL AND METHODS

Equipment and reagents

The following devices were used in this study: UV / Vis spectrophotometer label Agilent model G1103A, adjusted for detection at 233nm, using a quartz cuvette with an optical path of 1.0 cm, and UV/HPLC grade methanol, labels JTBaker and Tedia.

Samples

Commercial Ciprofibrate samples of 100mg/cp and its CRS ciprofibrate placebo (Chemical Reference Substances), previously standardized with the power of 99.8%, were used.

Preparation of solutions

Standard Solution

To reach the adopted working concentration of 0.01 mg/mL, the equivalent of 50.0 mg of CRS ciprofibrate was weighed and transferred to a volumetric flask of 50.0 mL, dissolved, and this volume completed with UV/HPLC grade methanol. From this solution, 1.0 mL was transferred to a volumetric flask of 100.0 mL, completed to volume with UV/HPLC grade methanol, and then homogenized.

Sample Solution

The average weight of 20 tablets of commercial sample was determined for the ciprofibrate solution sample. Once sprayed, the equivalent of 100.0 mg of ciprofibrate (equivalent to an average weight) was weighed and transferred to volumetric flask of 100.0 mL, dissolved, and the volume completed with methanol. The solution was filtered through filter paper for the retention of insoluble particles, and 1.0 mL was then transferred to a volumetric flask of 100.0 mL, filled with methanol, and then homogenized, in order to obtain the theoretical working concentration of 0.01 mg/mL.

Method validation

The validation process was performed according to the present guidelines contained in Resolution RE-899 of 29 May 2003 (Brasil, 2003).

Specificity/selectivity

Commercial ciprofibrate sample solution, standard and placebo sample at the working concentration

(0.01 mg/mL) were determined through analysis, using UV/HPLC grade methanol as the diluent.

Linearity

A standard calibration curve was constructed by reading, in duplicate, 5 standard solutions at concentrations corresponding to 60%, 80%, 100%, 120% and 140% compared to the theoretical value of the working concentration adopted, with values 0.006 mg/mL, 0.008 mg/mL, 0.01 mg/mL, 0.012 mg/mL and 0.014 mg/mL, to verify the correspondence between absorbance and the concentrations of solutions, expressed by the correlation coefficient obtained by the least squares method.

Precision

Precision was evaluated through the repeatability and intermediate precision methods. Repeatability was determined by preparation, under the same conditions, of 6 analytical solutions of the sample at working concentration, by two different analysts. Intermediate accuracy was assessed in the same manner, on different days by different analysts. Relative standard deviation (RSD) of up to 5% was considered as acceptable, according to resolution 899/2003 (Brasil, 2003).

Accuracy

Accuracy was assessed based on the degree of recovery, as percentage of placebo solutions fortified with known amounts of CRS ciprofibrate, in triplicate, at concentrations of 80%, 100% and 120% of the working concentration.

Robustness

The variation among solvent manufacturers was evaluated with sample and standard solutions at working concentration (0.01 mg/mL), with two different solvent brands (A and B), concomitant with the stability time of sample and standard solutions, by making periodic readings at 0, 2, 4, 6, 8, 10, 12, 15, 20 and 24 h of standard and sample solutions at working concentration (0.01 mg/mL).

RESULTS

Specificity/selectivity

The method used was specific and selective for ciprofibrate through comparative analysis of placebo,

standard and samples, prepared under the same conditions. There was no significant interference from other components (placebo) at a wavelength of 233nm (Figure 2).

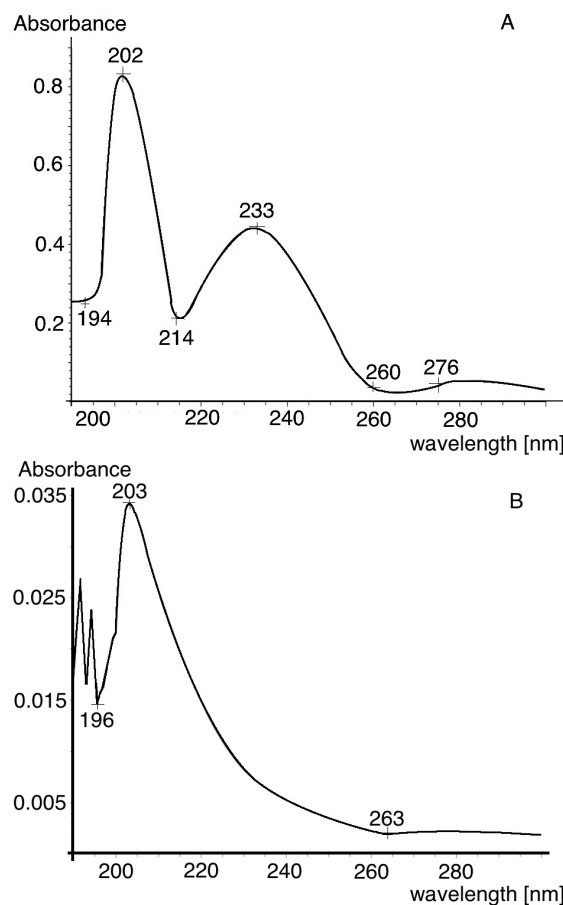


FIGURE 2 - UV spectra of standard solution (0.01 mg/mL) of ciprofibrate (A) and placebo solution (B) in absolute methanol.

Linearity

The correlation coefficient (r) of the calibration curve was 0.99, according to RE 899/2003 (Brasil, 2003) minimum acceptable criterion (Figure 3).

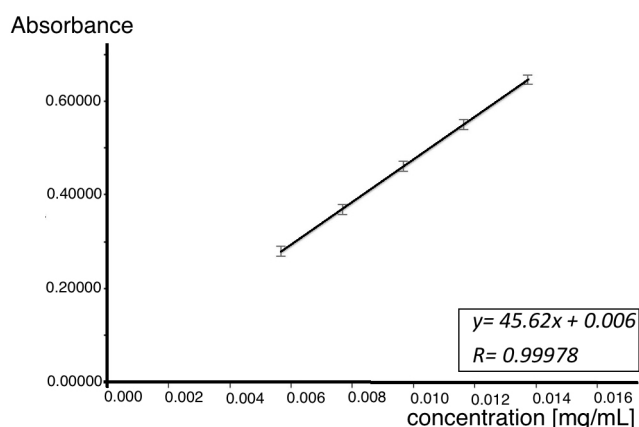
Precision

Considering data obtained from the six analytical solutions prepared, the method demonstrated repeatability with a relative standard deviation (RSD%) of up to 2.09% among samples prepared on the same day under the same conditions, within the maximum permitted by applicable law of 5% RE 899/2003 (Brasil, 2003). The method also showed intermediate precision with RSD% of 1.86% among results of six analytical solutions prepared by two different analysts on different days.

TABLE I - Results of recovery percentage of CRS ciprofibrate in placebo at 80, 100 and 120%, in triplicate at wavelength of 233 nm

	80%		100%		120%	
	Absorbance	Conc. (%)	Absorbance	Conc. (%)	Absorbance	Conc. (%)
Average	0.3718	80.68	0.4605	99.92	0.5501	119.37
S	0.00211	0.46	0.00307	0.67	0.00438	0.95
R.S.D.%	0.56884	0.57	0.66628	0.67	0.79536	0.80
% Recovery	-----	100.84	-----	99.92	-----	99.47

* where S is the standard deviation, and RSD% is the relative standard deviation in %

**FIGURE 3** - Calibration of CRS ciprofibrate through absorption spectrophotometry in the UV region at 233nm.

Accuracy

The results for accuracy, at concentrations of 80%, 100% and 120%, showed a mean recovery percentage of between 99.47% and 100.84% (Table I), compared to the ideal recovery of 98.0 to 102.0%.

TABLE II - Results for robustness test using ciprofibrate standard solution (0.01 mg/mL), varying the absolute methanol supplier

Label	Absorbance	% Variation
Reagent 1 - Methanol - A	0.45816	0.00000
Reagent 2 - Methanol - B	0.45860	0.09610

TABLE III - Results for robustness test using ciprofibrate sample solution (0.01 mg/mL), varying the absolute methanol supplier

Label	Absorbance	% Variation
Reagent 1 - Methanol - A	0.45639	0.00000
Reagent 2 - Methanol - B	0.45400	-0.52259

Robustness

For the same sample, both standard solution and ciprofibrate sample solutions analyzed using different solvent brands (methanol), A and B, revealed significant variations in the robustness test results (Table II and Table III).

The stability of analytical standard and sample solutions after 2, 4, 6, 8, 10, 12, 15, 20 and 24 hours from the initial analysis was also evaluated. The ciprofibrate sample and standard solutions seemed to be stable for 24 hours after the initial analysis, and a maximum variation of 1.78% in 6 hours was found (Table IV and Table V).

Statics analysis of linearity

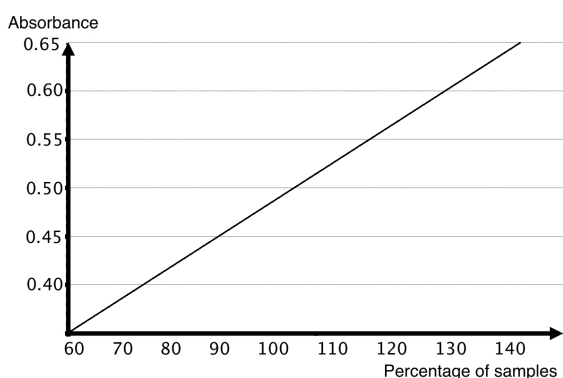
The correlation coefficient (r) for linearity was according to RE 899/2003 (Brasil, 2003), established at 0.99 as a minimum criterion. Therefore, statistical analysis of linearity can be done to verify the robustness of this figure (Figure 4, Table VI). From the statistical data seen in Table VI, it is possible to affirm that these data present strong linearity (Figure 4, Table VI).

TABLE IV - Results for stability test for ciprofibrate standard solution (0.01 mg/mL), varying solution preparation time

Analysis Timetable (hours after preparation)	Absorbance	Variation (%)
Initial Analysis	0.45947	0.00
2 hours	0.45836	-0.24
4 hours	0.45885	-0.13
6 hours	0.46277	0.72
8 hours	0.45741	-0.45
10 hours	0.46142	0.42
12 hours	0.45531	-0.91
15 hours	0.45411	-1.17
20 hours	0.45874	-0.16
24 hours	0.46061	0.25

TABLE V - Results for stability test for ciprofibrate solution sample (0.01 mg/mL), varying solution preparation time

Analysis Timetable (hours after preparation)	Absorbance	Variation (%)
Initial Analysis	0.45608	0.00
2 hours	0.45623	0.03
4 hours	0.46051	0.97
6 hours	0.46418	1.78
8 hours	0.45604	-0.01
10 hours	0.45385	-0.49
12 hours	0.45589	-0.04
15 hours	0.46024	0.91
20 hours	0.45433	-0.38
24 hours	0.45656	0.11

**FIGURE 4** – Linear regression graph of percentage of samples and absorbance.

DISCUSSION

Methods to determine the content of drugs should never fail because, according to Honda and Magalhães (2001), possible failures may lead to erroneous decisions subsequently being taken based on these results.

The selectivity and specific features of the proposed method is the primary principle of a high-accuracy procedure, as demonstrated for the method in this study, since no significant interference was observed from other components (placebo) at a wavelength of 233 nm (Figure 2).

In Figure 2a, for $\lambda = 233$ nm, the absorbance is approximately 0.45 AU, with coordinates located at the peak of a typical curve obtained in spectroscopy, while in Figure 2b, for $\lambda = 233$ nm, the absorbance is less than 0.01, with coordinates located on a decreasing curve after the methanol absorbance peak. Based on these results, it was confirmed that the method to determine ciprofibrate content in tablets via spectrophotometer is both specific and selective.

The linearity of a method must be obtained by the correlation coefficient (r) of the calibration curve. According to RE 899/2003 (Brasil, 2003), the minimum acceptable criterion is 0.99, a level verified by statistical analysis (Figure 4, Table VI). According to Pimentel and Barros Neto (1996), the least squares method is widely used to verify the proximity of points obtained in relation to the standard line, since it provides unbiased results with minimum variance.

Figure 3 shows the value of $R = 0.99978$ for the equation $y = 45.62x + 0006$ in the calibration curve of the CRS ciprofibrate through absorption spectrophotometry in the

TABLE VI – Regression analysis of percentage of samples and absorbance

Regression Statistics					
R					0.9995
R-square					0.999
R-square adjusted					0.9989
S					0.0043
Absorbance=0.0059 + 0.0046 x samples (%)					
ANOVA	d.f.	SS	MS	F	p-level
Regression	1	0.3329	0.3329	17.973.5309	0
Residual	18	0.0003	0.0000		
Total	19	0.3332			
	Coefficients	Standard error	LCL	UCL	State
Intercept	0.0059	0.0035	-0.0015	0.0133	1.6645
Samples (%)	0.0046	0.0000	0.0045	0.0046	134.0654
T (5%)	2.1009				

UV region at 233nm. According to Braga and Poppi (2004) and to findings of the present analysis, it can be concluded that the linear model efficiently fits the data obtained.

The degree of accuracy of a method is demonstrated by the correlation among measurements made for the same homogeneous sample under repeatability conditions, prepared on the same day under the same conditions, and by different analysts (Braga, Poppi, 2004).

For the spectrophotometric determination method used in this work, the repeatability assessment had a maximum standard deviation of 2.09% for samples (6) prepared on the same day. This value lies within the limits allowed under current legislation (Brasil, 2003), stipulating that this value should not exceed 5%, while intermediate precision evaluation should have a maximum standard deviation of 1.86% for samples (6) analyzed by different analysts. Therefore, the method to determine ciprofibrate content is highly accurate under the conditions of this experiment.

Moreover, a method must be exact. The RE 899/2003 (Brasil, 2003) stipulates that the ideal recovery degree for concentrations of 80%, 100% and 120% should range from a minimum of 98.0% to a maximum of 102.0%. The data in Table I show a recovery degree for these concentrations of between a minimum of 99.47% and maximum of 100.84% (Table I). In statistical terms, the data obtained in the present study are consistent and within the limits required by law.

In statistical terms, the least squares estimator is the minimum linear variance of the response variable (Pimentel, Barros Neto, 1996), and therefore the values obtained for the detection and quantification method were within the parameters of safety for the linearity of data.

The results obtained by quantification of the analytical solution of the same sample, analyzed with different brands (methanol), A and B, revealed significant variations in the results, indicating high and satisfactory robustness of the method for small variations (Table II and Table III).

Regarding the stability of standard and sample analytical solutions, a maximum variation of 1.78% was detected in 6 hours, thus not exceeding the maximum acceptable limit adopted of 2% according to RE 899/2003 (Brasil, 2003) (Table IV and Table 5).

CONCLUSIONS

The method to determine ciprofibrate content in tablets through spectrophotometry in the ultraviolet region was validated according to resolution 899/2003. The results showed the method to be simple, inexpensive, easy to apply, precise and accurate for the determination of

ciprofibrate. Moreover, the method provides the reliability required for an analytical method, and also the practicality required for the routine of quality control laboratories.

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