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Quantitative determination of piroxicam in a new formulation (piroxicam–β-cyclodextrin) by derivative UV spectrophotometric method and HPLC

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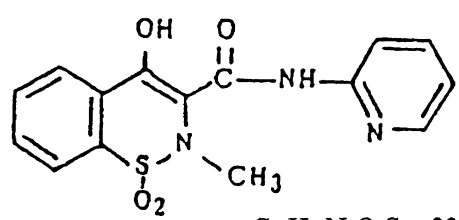
Abstract

A derivative ultraviolet (UV) spectrophotometric method for the determination of piroxicam in piroxicam–β-cyclodextrin tablets was developed. Phosphate buffer (pH 7.8, 0.1 M) and ethanol were used as a solvent system throughout the study. In this study, determination of piroxicam was conducted by using first order derivative amplitudes at 261.4 nm ($n = 4$). Standards for the calibration graph ranging from 2.40 to 20.0 μg/ml were prepared from working standard. The proposed method is accurate with 99.70% ± 0.50 recovery value and precise with coefficient of variation (CV) of 1.29. The results were compared with those obtained using a high-performance liquid chromatography (HPLC) procedure. A reversed-phase C_{18} column with aqueous phosphate buffer:methanol, 60:40, v/v, mobile phase was used. UV detector was set at 254 nm. Calibration solutions used in HPLC were ranging from 5 to 20 μg/ml. Results obtained in HPLC were comparable to those obtained by derivative UV spectrophotometric method.

Keywords: Piroxicam; β-Cyclodextrin; Derivative UV spectrophotometry; Reversed-phase HPLC

1. Introduction

Piroxicam [4-hydroxy-2-methyl-*N*-(2-pyridyl)-H-1,2-benzothiazine-3-carboxamide-1,1-di-oxide] is a non-steroidal anti-inflammatory and analgesic agent ($C_{15}H_{13}N_3O_4S = 331.35$ g/mol).



$C_{15}H_{13}N_3O_4S = 331.35$ g/mol

Several methods have been described for the piroxicam determination in biological fluids and in pharmaceuticals by chromatographic [1–7], spec-

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troscopic [8–20], and electrochemical [21,22] and capillary zone electrophoresis [23] techniques. In this study, a new pharmaceutical formulation, which contains piroxicam–β-cyclodextrin was analyzed. The new tablet form contains piroxicam complexed with β-cyclodextrin (an inert polysaccharide) in 1:2.5 molar ratio. β-Cyclodextrin is produced by enzymatic hydrolysis of starch and has a special molecular structure that may form molecular encapsulation with different drugs. While cyclodextrin is accepted as a capsule in molecular dimension, the hydrophobic part of piroxicam is settled in this capsule by physical bounds. Because of the fact that the contact side of the complex form is the hydrophilic side of β-cyclodextrin, the dextrin enhances the absorption rate of encapsulated drug and decreases the drug's contact time with mucosa in upper gastrointestinal tract. Since piroxicam–β-cyclodextrin dissolves in water much more and quickly compared with piroxicam, piroxicam in piroxicam–β-cyclodextrin complex is rapidly absorbed after oral administration. In our study, quantitative determination of piroxicam in tablets containing piroxicam–β-cyclodextrin was performed by derivative ultra violet (UV) spectrophotometric method and high-performance liquid chromatography (HPLC). Quantitative determination of piroxicam was conducted by using first order derivative spectrophotometric method at 261.4 nm ($n = 4$). Since there was no derivative spectrophotometric study about piroxicam–β-cyclodextrin in the literature and it was simple, rapid and sensitive, we decided on using this derivative method. Furthermore, quantitative determination of piroxicam was also performed with HPLC method and the results were compared with those obtained by derivative spectrophotometric method.

2. Experimental

2.1. Derivative UV spectrophotometric method

2.1.1. Apparatus

A Shimadzu UV-160 A double-beam UV–visible spectrophotometer with data processing capacity was used. UV spectra of reference and test solutions were recorded by using 1 cm quartz cells at 200–400 nm range. The first order derivative spectra were also obtained over the 200–400 nm range ($n = 4$). Spectral band width was 2 nm, and scan speed (slow mode) was set to 480 nm/min. And also response time was 0.02 s. The pH measurements were performed by using a combined pH electrode with an orion Model 720 A. And also Juan MR 18.22 type centrifuge and a sonicator were used throughout this study.

2.1.2. Reagent and solutions

Piroxicam and β-cyclodextrin were obtained from Pfizer and Sigma, respectively. Encapsulated 20 mg Cycladol® tablets (I.E. Ulugay company in Turkey) were used. The piroxicam was tested for its purity by controlling its melting point and infra red (IR) spectrum and no impurities were found. The labeled piroxicam–β-cyclodextrin molar ratio is 1:2.5. pH 7.8 buffer solution was prepared by using 0.1 M NaH_2PO_4. pH value of the solution was adjusted to desired value with 1.0 M NaOH. For the preparation of standard piroxicam stock solution (1000 μg/ml), 100 mg piroxicam was accurately weighed, dissolved in ethanol and then adjusted to 100 ml with ethanol. Standard solutions in the range of 2.40–20.0 μg/ml were prepared by appropriate dilutions of the stock solution with phosphate buffer (pH 7.8; 0.1 M) in a way that the final solution was composed of phosphate buffer:ethanol, 90:10, v/v. Additionally, for the preparation of inclusion complex of proxicam with β-cyclodextrin, 2000 μg/ml dextrin stock solution was prepared and appropriate amounts of dextrin stock solution were added to the above mentioned proxicam standard solutions. All solutions were prepared with distilled water and analytical grade chemicals supplied by Merck.

2.1.3. Procedure

2.1.3.1. Analysis of tablets. Average mass of ten tablets was determined and tablets were powdered. A definite amount of powder was transferred to a 50 ml volumetric flask and the volume was then adjusted to the mark with ethanol. The solution was sonicated in an ultrasonic sonicator for 20

min. And a portion of the solution was centrifugated at 3000 rpm for 10 min. As necessary, portion of clear centrifugate was diluted to 10 ml with phosphate buffer:ethanol, 90:10, v/v, prior to analysis. In the second part of the study, filtration procedure was performed. Tablet solution prepared as mentioned above was filtered through Schleicher and Schuell ashless blue ribbon filter paper, and then filter paper and the precipitate were washed with ethanol to filter the adsorbed piroxicam. Finally, a certain amount of filtrate was diluted to 10 ml with phosphate buffer:ethanol mixture which was prepared as 90:10 ratio prior to analysis. The piroxicam content of tablet was calculated by referring to a calibration curve obtained by using standard solutions of piroxicam ranging from 2.40 to 20.0 μg/ml. The first derivative amplitudes of these solutions at 261.4 nm ($n = 4$) were used to draw the calibration curve.

2.2. HPLC

2.2.1. Apparatus

The HPLC system consisted of a model HP series 1050 solvent delivery system with a UV–visible detector set to 254 nm. A HP ODS hypersil column (10 cm × 3.9 mm i.d., 5 μm particle size) and a HP 3396 series II integrator was used. Mobile phase filtration was performed with Erich Wiegand GmbH type N 022 AN 18 vacuum pump with All tech.47 mm, 0.45 μm filter paper. Typical operating conditions include flow rate, 1.0 ml/min; operating temperature, room temperature; and injection volume, 20 μl.

2.2.2. Reagents and solutions

The mobile phase used in HPLC was phosphate buffer (pH 7.0, 0.05 M): methanol, 60:40, v/v. After mixing, the mobile phase was degassed. Aqueous buffer solution was prepared by dissolving 7.8 g $NaH_2PO_4 \cdot 2\ H_2O$ in 1000 ml water and its pH was adjusted to 7.0 by adding 1.0 N NaOH. In order to prepare piroxicam stock solution, 50.0 mg piroxicam was accurately weighed, dissolved and diluted to 50.0 ml. Standard solutions ranging from 5 to 20 μg/ml were prepared with phosphate buffer:methanol, 60:40, v/v. All solutions were prepared with bidistilled water. Methanol was HPLC grade, Riedel-de Haen.

2.2.3. Analysis of tablets

Average mass of ten tablets was determined and tablets were powdered and accurately weighed. A definite amount of powdered tablet was transferred to a 50.0 ml volumetric flask and the volume was then adjusted to the mark with methanol. The solution was sonicated in an ultrasonic sonicator for 20 min, and the solution was centrifugated at 3000 rpm for 10 min. A clear portion of the centrifugate was diluted to 10 ml with phosphate buffer:methanol, 60:40, v/v, prior to analysis. In order to determine piroxicam content of the tablet, piroxicam standard solutions were injected and calibration curve was obtained as absorbance peak area versus concentration. Tablet solution (20 μl) was injected; detection was at 254 nm. By using calibration curve, quantitative determination of piroxicam in tablet was determined.

3. Results and discussion

Piroxicam–β-cyclodextrin encapsulated tablet is a new formulation containing piroxicam together with β-cyclodextrin (1:2.5 molar ratio), which is a cyclic oligosaccharide, as a complex. In this study, quantitative determination of piroxicam in this complex was performed by two ways. In a derivative spectrophotometric method, being the first method, ethanol:phosphate buffer solution ratio was determined as 10:90 for the piroxicam soluble in ethanol. Tablet powder was dissolved in the chosen solvent. Zero order spectrum of the piroxicam in the chosen ethanol:phosphate buffer system and that of tablet containing piroxicam–β-cyclodextrin are shown in Figs. 1 and 2, respectively. Quantitative determination of piroxicam could have been performed by zero order UV spectrum. But, derivative spectroscopic method represents an advantage over the zero order spectroscopy in the determination of piroxicam in tablets because of the fact that piroxicam tablet solution yielded turbid solution. In addition, derivative spectrophotometry eliminates the prob-

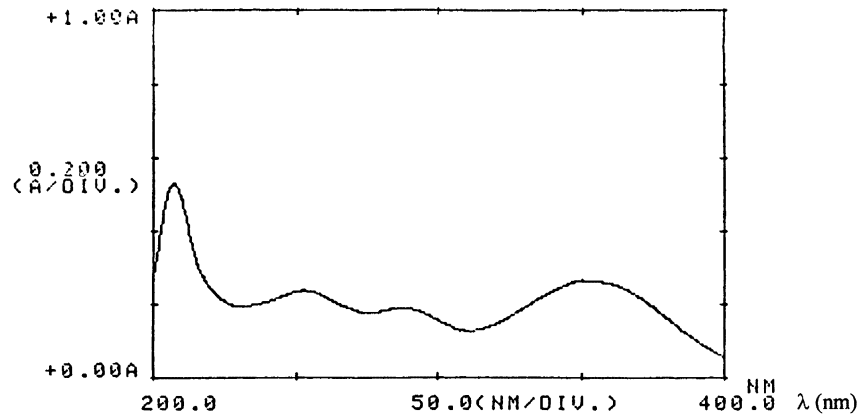

Fig. 1. UV spectrum of piroxicam (5μg/ml) in ethanol–phosphate buffer.

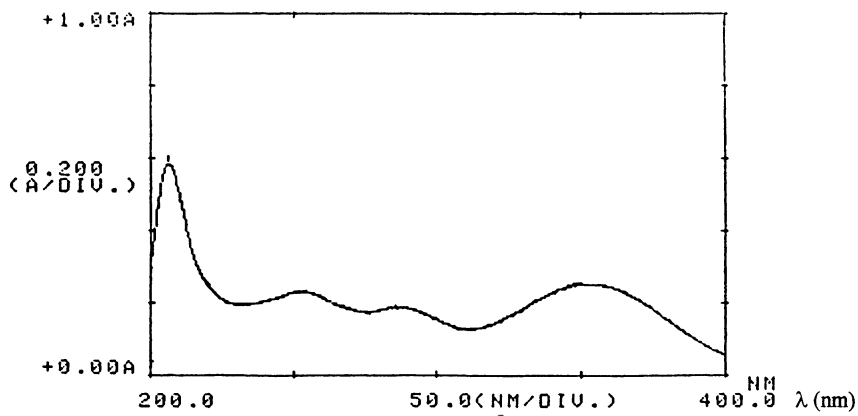

Fig. 2. UV spectrum of piroxicam–β-cyclodextrin tablet solution in ethanol–phosphate buffer.

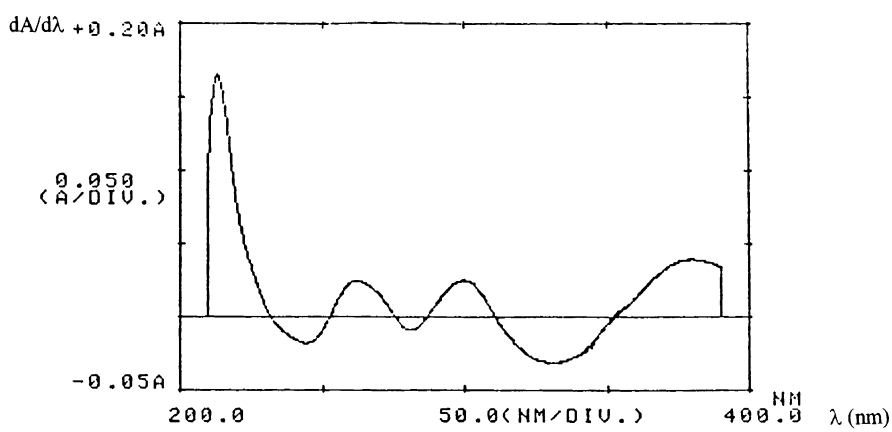

Fig. 3. First order derivative ($n = 4$) spectrum of piroxicam in ethanol–phosphate buffer.

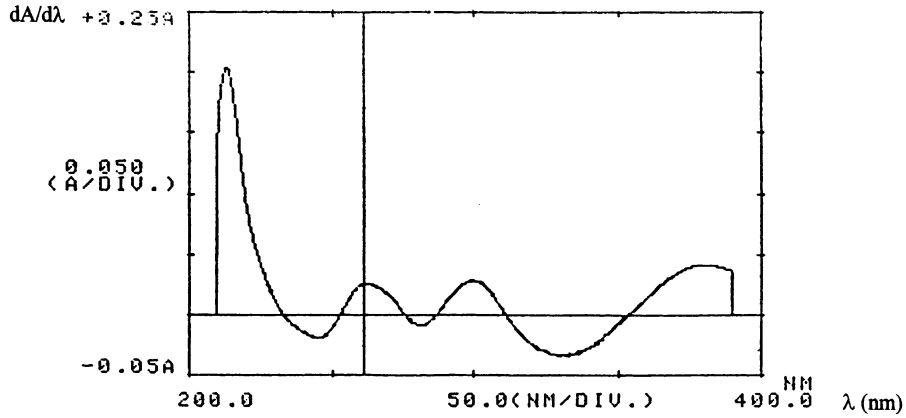

Fig. 4. First order derivative ($n = 4$) spectrum of piroxicam–β-cyclodextrin tablet solution in ethanol–phosphate buffer.

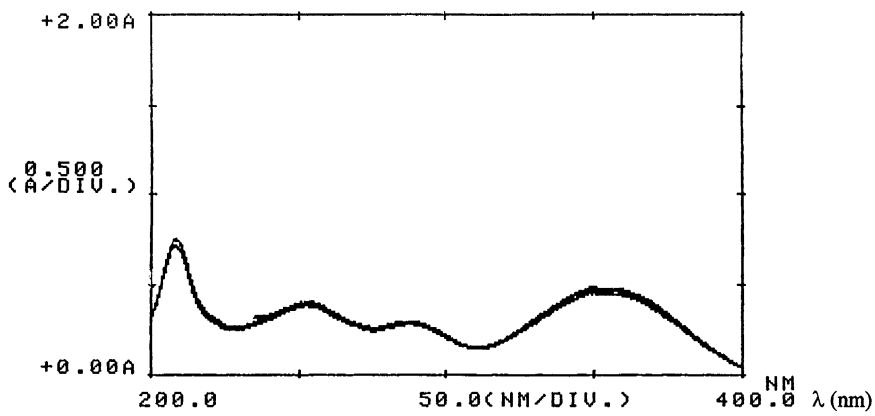

Fig. 5. Five overlapping zero order spectra of piroxicam in ethanol–phosphate buffer at various concentrations of β-cyclodextrin.

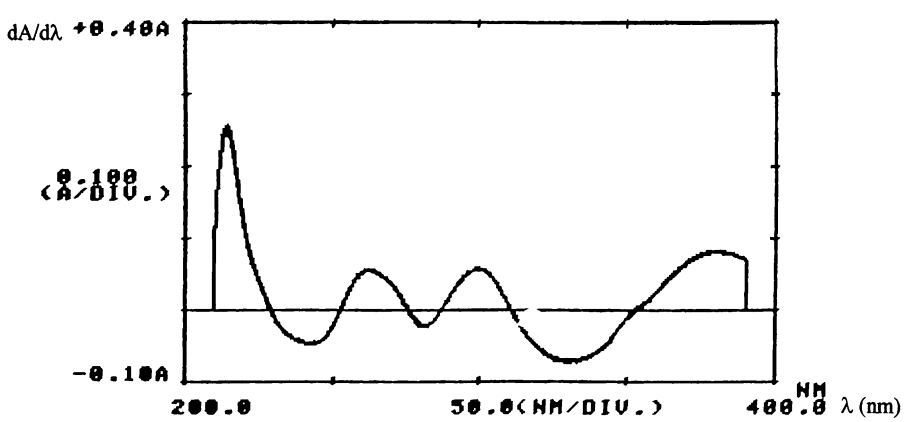

Fig. 6. Five overlapping first order derivative spectra of piroxicam in ethanol–phosphate buffer at various concentrations of β-cyclodextrin.

Table 1
Statistical analysis for the calibration curve of piroxicam[a]

	Analytical wavelength (nm)	Linearity range (μg/ml)	Regression equation	Correlation coefficient
First order derivative ($n=4$)	261.4	2.40–20.0	$Y=5.2\times10^{-3}\ C-4.04\times10^{-4}$ S.D. of slope($n=3$) $=1.21\times10^{-4}$ S.D. of intercept($n=3$) $=1.88\times10^{-3}$	$r=0.9986$
HPLC	254	5.0–20.0	$Y=11447.6\ C+2715$ S.D. of slope($n=3$) $=57.66$ S.D. of intercept($n=3$) $=32.79$	$r=0.9996$

[a] C is the concentration of the analyte (μg/ml).

Table 2
Results obtained in the determination of piroxicam in encapsulated tablets using first order derivative spectrophotometry[a]

Sample number	Amount found (mg per tablet)	Amount labeled (20 mg per tablet)
1	19.84	Mean = 19.54
2	19.76	S.D. = 0.25
3	19.31	CV = 1.29
4	19.29	Confidence limits = 19.22–19.85
5	19.48	$P=0.05$

[a] S.D., standard deviation; CV, coefficient of variation.

Table 3
Recovery analysis of piroxicam in piroxicam–β-cyclodextrin dosage form

Amount labeled (mg)	Added (mg)	Recovered (mg)[a]	Recovery (%)	S.D.
20	10	9.97	99.70	0.50

[a] This is the mean of three experiments.

lems caused by scattering effect of colloidal particles that may remain after centrifuge. So, it was decided to conduct the study by derivative spectrophotometric method.

First order derivative spectrum of piroxicam in this ethanol:phosphate buffer system and that of piroxicam tablet in the same solvent system are shown in Figs. 3 and 4, respectively. At 261.4 nm, a linear relation was observed between piroxicam concentration and derivative absorbance values. The effect of β-cyclodextrin on the molar absorptivity (ε) and λ_{max} of piroxicam was also investigated. For this reason, zero order and first order derivative spectra of piroxicam solutions containing various concentrations of β-cyclodextrin were obtained. Five overlapping zero order spectra and five first order spectra of piroxicam are shown in Figs. 5 and 6, respectively. As it can be seen from these figures, presence of various amounts of β-cyclodextrin did not cause any spectral interference. Regression analysis was carried out on the slope, intercept and correlation coefficient (Table 1). The linearity of the calibration graph and the adherence of the system to Beer's Law were validated by the high value of the correlation coefficient of the regression equation and by the value of the intercepts on the ordinate. The experimental intercept was not significantly different from theoretical zero value because when we conducted Student's t-test we found $t_{calculated}$ as 3.40 ($n=3$, $P=0.05$) while $t_{theoretical}=4.30$. Quantitative determination of piroxicam in tablets was performed using derivative spectrophotometric method and the results were in good agreement with the labeled amount of the piroxicam (Table 2). Recovery studies on the proposed method were performed by analyzing spiking sample of the powdered tablets with appropriate amounts of the stock solution of the piroxicam. The results of the recovery studies are shown in Table 3. Table 4 represents the results obtained for intra-day and inter-day variability studies of the piroxicam sam-

Table 4
Intra-day and inter-day precision of piroxicam standards

Theoretical concentration (μg/ml)	Intra-day measured concentration (μg/ml)[a]		Inter-day measured concentration (μg/ml)[b]	
	Mean	R.S.D.%	Mean	R.S.D.%
8	8.26	1.47	8.21	1.04
15	15.1	0.94	15.19	0.94

[a] Mean values represent five different piroxicam standards for each concentration.
[b] Inter-day mean values were determined from five different runs over 1 week period.

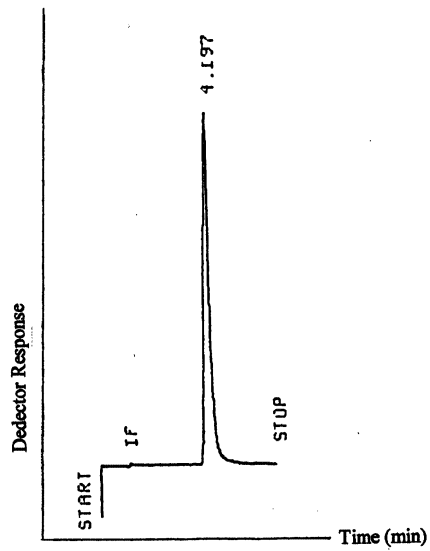

Fig. 7. HPLC chromatogram of the piroxicam (10 μg/ml) in methanol–phosphate buffer.

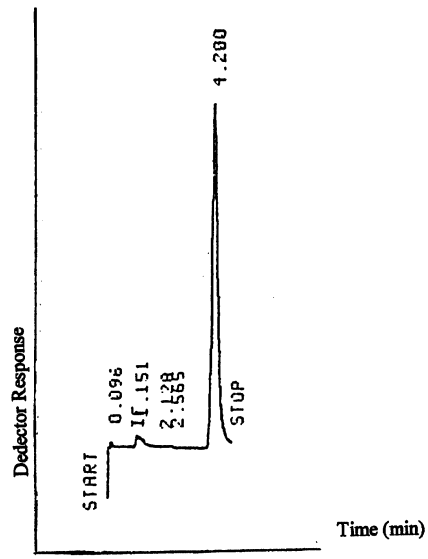

Fig. 8. HPLC chromatogram of the piroxicam–β-cyclodextrin tablet solution in methanol–phosphate buffer.

ples. The results show the accuracy and reproducibility of the assay, which was tested within day and between days.

Quantitative determination of piroxicam in tablets was also conducted by HPLC method. Figs. 7 and 8 show typical chromatograms obtained from the analysis of standard piroxicam solution and piroxicam–β-cyclodextrin containing tablet solution, respectively. As shown in these figures, standard piroxicam and tablet solutions were eluted, forming well shaped, symmetrical single peaks and well separated from the solvent front. The HPLC method used was a new modified method. It was seen that results obtained with HPLC method was in good agreement with those obtained by derivative spectrophotometric method (Table 5). Results

Table 5
Results obtained in the determination of piroxicam in encapsulated tablets using HPLC method

Sample number	Amount found (mg per tablet)	Amount labeled (20 mg per tablet)
1	19.83	Mean = 19.57
2	19.51	S.D. = 0.16
3	19.40	CV = 0.82
4	19.58	Confidence limits = 19.37–19.77
5	19.51	$P = 0.05$

of both methods were compared by Student's t-test and fisher test. When precisions of the both methods were compared with f- (fisher) test, there was no significant difference between the S.D. of the two methods. In addition, as far as Student's t-test results concerned, there did not exist any significant

Table 6
Comparison of the two methods

Amount labeled (20 mg per tablet)	Derivative UV method	HPLC method
Amount found (mg per tablet), average values ($n = 5$)	19.54	19.57
CV	1.29	0.82
$t_{Calculated} = 0.235$	$t_{Theoretical} = 2.31$ ($P = 0.05$)	
$f_{Calculated} = 2.49$	$f_{Theoretical} = 6.39$ ($P = 0.05$)	

difference between two methods with respect to mean values (Table 6).

It can be concluded that derivative spectrophotometric method has the advantage of being simpler, more rapid and practical without any interference from ingredients present in encapsulated tablets. Furthermore, recovery studies performed by derivative method on the synthetic mixtures show the accuracy, which is $99.70\% \pm 0.50$, of the proposed method.

Thus, the proposed derivative spectrophotometric method represents a good analytical alternative for the determination of piroxicam.

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