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Article

Spectrophotometric Determination of Diclofenac in Pharmaceutical Preparations

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Neste trabalho é proposto um método espectrofotométrico modificado para a determinação de diclofenaco em preparações farmacêuticas. Usa-se solução de cobre(II) como reagente, o que leva à formação de um complexo cobre(II)-diclofenaco de cor verde, com máximo de absorção em 680 nm. Foram estudadas as influências: do pH; da concentração de cobre(II); do uso de ácido acético na solução de cobre(II); da força iônica; do número de extrações com clorofórmio. Como condições ótimas, foram encontradas: pH da solução a ser extraída 5,3; 50,0 mg L^{-1} para a concentração da solução de cobre (acetato de cobre em solução de ácido acético 0,01 mol L^{-1}); três extrações com clorofórmio num volume total de 5,0 mL. Observou-se, também, que a adição de um sal para aumentar a força iônica não introduz ganho significativo na extração do complexo cobre(II)-diclofenaco. O método foi aplicado a preparações farmacêuticas comerciais. Os resultados foram comparados com aqueles obtidos com o procedimento HPLC indicado pela Farmacopéia Americana (USP) usando-se o teste ***t*** de Student, observando-se total concordância entre os resultados obtidos pelos dois métodos. Para o procedimento proposto, o desvio padrão relativo intrínseco médio observado foi de 2,3% para o diclofenaco de sódio e 2,7% para o diclofenaco de potássio. Para o caso de amostras, o desvio padrão é afetado, necessariamente, pela variação nas massas das doses individuais. O coeficiente de correlação, R, encontrado foi de 0,9984 para o sal de sódio e de 0,9993 para o sal de potássio. A faixa linear vai de 1,0 mg mL^{-1} a 25,0 mg mL^{-1} na solução de trabalho. O limite de detecção é 0,2 mg mL^{-1} e o de determinação de 0,7 mg mL^{-1}.

A modified procedure for the visible spectrophotometric determination of diclofenac, in pharmaceutical preparations using as reagent an aqueous solution of copper (II), is proposed. A green color complex is formed between copper(II) and diclofenac with a maximum light absorption at 680 nm. The influences of pH, of copper(II) concentration, of the use of acetic acid in copper(II) solution, of the ionic strength and of the number of extractions with chloroform were studied. The optimal conditions were found to be 5.3 (pH of the solution to be extracted), 50.0 mg mL^{-1} (copper (II) acetate in 0.01 mol L^{-1} acetic acid solution) and three extractions with chloroform using a total volume of 5.0 mL. It was also observed that the addition of a salt in order to increase the ionic strength does not introduce important gain in complex extraction. The method was applied to commercial pharmaceutical preparations. The results were compared with those obtained with the method recommended by the Pharmacopoeia (USP) using the statistical Student's ***t***-test procedure. Complete agreement was found between the results obtained with the two methods. The intrinsic RSD of the proposed method was about 2.3% for sodium diclofenac and 2.7% for potassium diclofenac. When applied to tablets or liquid preparations the RSD is necessarily affected by the deviation in the masses of the individual doses. The linear correlation coefficient, R, was 0.9984 for sodium diclofenac salt and 0.9993 for potassium diclofenac salt. The linear range goes from 1.0 to 25.0 mg mL^{-1} in the working solution. The detection limit is 0.2 mg mL^{-1} and the determination limit is 0.7 mg mL^{-1}.

Keywords: diclofenac, determination, pharmaceutical preparations, spectrophotometry, copper complex.

Introduction

Diclofenac, 2-[(2,6-dichlorophenyl)amino] benzene acetate (Figure 1), is a synthetic non steroidal compound that is more usually found as sodium or potassium salt.[1] It is used as anti-inflammatory and anti-rheumatic. As through oral dispensation it is rapidly absorbed by the organism and its half life is short, it is considered adequate for acute inflammatory and pain states.[2] Secondary effects occur in about 20% of the patients.[3]

From 1975, with the introduction of the use of diclofenac for the treatment of inflammations, quantitative analytical procedures appeared in the literature for its

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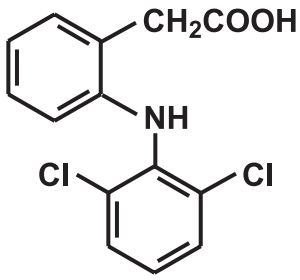

Figure 1. Structural formula of diclofenac.

determination in biological materials,[4] but only from 1987 more attention have been devoted to analytical procedures for pharmaceutical preparations. Several techniques have been described. For example: HPLC;[5-10] reverse-phase liquid chromatography;[11] fluorimetric;[12-15] potentiometric;[14-17] capillary electrophoresis;[18,19] thermal analysis;[20] AAS;[21] flow methods;[22-26] spectrophotometric UV;[27,28] spectrophotometric visible;[29-38] gravimetric[39] and dyfuse reflectance.[40]

Among the visible spectrophotometric methods that using aqueous solution of copper (II) ions as reagents is of an elegant simplicity.[35] A light-green complex is formed in the proportion 2:1, diclofenac:copper. This method was optimized using a three variable two level factorial design. However, attempts to use this procedure, in our laboratory, for the analysis of diclofenac in pharmaceutical preparations, did not result in adequate precision and accuracy.[41] Therefore the method was experimentally re-studied in order to be adjusted to our working conditions.

Experimental

Apparatus

Spectrophotometers - An Ultrospec 200 (Pharmacia Biotech) was used to obtain spectra and a FEMTO 600 with optical glass 1.000 cm path cuvettes was used to perform analyses.

A HPLC Waters 600 E, with UV-Vis 484 detector and a Microsorb MV C-18 5 μm 25 cm × 4.6 mm column, were used for analytical comparative purposes. For pH measurements it was used an Analyzer model 300 with a glass electrode.

Reagents

All reagents were of analytical grade excepting diclofenac that was a pharmaceutical 99.9% certificated product. It was gently furnished by a pharmaceutical laboratory. This product was again analyzed in our laboratory to confirm diclofenac content.[42] Water was distilled in a glass apparatus and then deionized in a Milli Q Plus device.

Solutions

Acetic acid 0.1 mol L^{-1}: 6.0 g of glacial acetic acid were dissolved in 1.0 L water. Copper(II) acetate solutions: 40.0 mg mL^{-1} (0.20 mol L^{-1}), 50.0 mg mL^{-1} (0.25 mol L^{-1}), 60.0 mg mL^{-1} (0.30 mol L^{-1}) and 70.0 mg mL^{-1} (0.35 mol L^{-1}) were prepared by dissolving adequate masses of $Cu(CH_3CO_2)_2.H_2O$ (Molar mass = 199.65 g mol^{-1}) in water with posterior addition of 0.1 mol L^{-1} acetic acid in the proportion 9:1 v/v. Buffer solutions: to obtain the desired pH buffer solutions, adequate volumes of sodium acetate 2.0 mol L^{-1} and of acetic acid 2.0 mol L^{-1} solutions were mixed. KCl, NaCl, $NaBF_4$, KBr, solutions: were prepared 2.0 mol L^{-1} solutions. $KClO_4$ solution: was prepared a 0.1 mol L^{-1} solution. Standard diclofenac solutions: diclofenac salts of sodium and of potassium were dried at 110 °C during 3 hours and then kept in a dissecator over phosphorus pentoxide. Adequate quantities of sodium diclofenac and of potassium diclofenac were weighed and dissolved in 10.0 volumetric flasks, with water, in order to obtain solutions of concentrations 1.0 mg mL^{-1}, 5.0 mg mL^{-1}, 7.0 mg mL^{-1}, 10.0 mg mL^{-1}, 15.0 mg mL^{-1}, 20.0 mg mL^{-1} and 25.0 mg mL^{-1}. Perchloric acid solution in glacial acetic acid: 0.1 mol L^{-1} solution was prepared by dissolving 8.50 mL of concentrated (70%) perchloric acid in 500.0 mL of acetic acid and 21.0 mL of acetic anhydride. This solution was cooled in an ice bath and the volume was carefully completed to 1000 mL with glacial acetic acid. The final solution was standardized by potentiometric titration versus potassium biphthalate.[42] This solution was used to determine diclofenac content in the pharmaceutical products according to the procedure indicated by the Brazilian Pharmacopoeia.[42]

Samples

All samples were purchased in the local market.

Samples treatment

To develop this work, groups of 40 tablets of each one of the different used pharmaceutical preparations containing diclofenac were triturated and homogenized in a mortar. This material was used to develop the method. In the case of liquid samples the content of 5 ampoules was mixed and adequate samples were taken from.

To obtain solution containing 7.0 mg mL^{-1} of the diclofenac, adequate aliquot of the triturated samples was mixed with 6.0 mL of hot water (85 °C) in an essay tube and agitated during 1 minute. The tube was kept in a water bath at 85 °C during 5 minutes. Then it was centrifuged at

2000 rpm during 1 minute and filtered through a qualitative filter paper directly in a 25.0 mL volumetric flask. The filter and the remaining solid were washed with small portions of hot water (85 °C) and the water collected in the same volumetric flask. After cooling to room temperature the volume was completed with water to the mark. This solution is called working solution. Obviously, in the case of liquid remedies this treatment is unnecessary.

2.0 mL of the working solution was mixed with 3.0 mL of the copper(II) acetate solution (50.0 mg L^{-1}), 4.0 mL of the buffer solution (acetic acid / acetate pH 5.3) and 2.0 mL of chloroform, in a 50 mL separatory funnel. The whole was agitated during 3 minutes. The organic phase, denser, was collected into a 5.0 mL volumetric flask. The procedure was repeated with about 1.8 mL of chloroform and a third time with 1.0 mL of this solvent. The volume was completed to the mark with chloroform. The absorbance was measured in the spectrophotometer at 680 nm versus chloroform as blank.

This procedure was also used to construct the analytical curve and to perform analyses.

Results and Discussion

Figure 2 shows the absorption spectrum of the copper(II) diclofenac complex, $Cu(C_{14}H_{10}Cl_2NO_2)_2$, in chloroform solution. It can be observed that the maximum absorption in the visible region is at 680 nm. Therefore all absorbance measurements were performed at this wavelength.

In order to obtain the analytical convenient absorbance measurements in the chloroform phase it was initially studied the adequate concentration of diclofenac salt in the working solution to be added in the reaction mixture. It was found that about 7.0 mg mL^{-1} leads to an absorbance value of about 0.48, in the organic phase, which is quite adequate. Therefore, solutions of all medicine samples were prepared, according to the labeled quantities, to present concentration close to this value. The analytical curve was constructed also centered in approximately this value.

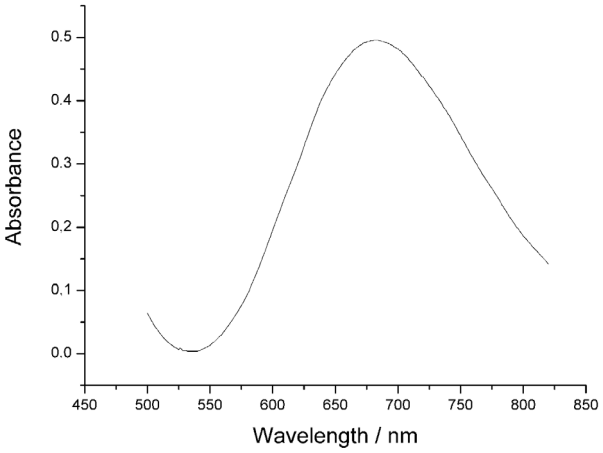

Figure 2. Spectrum of the copper(II)-diclofenac complex in chloroform solution.

An important parameter studied, which influences the extraction of the complex with chloroform, is pH. According to data previously presented in the literature, [35] where a two-level full factorial design was used, in ionic strength 0.20 mol L^{-1} (KCl) and pH 6.0 (acetic acid/acetate buffer) is the best condition for the extraction (total ionic strength was *ca.* 0.90 mol L^{-1}). Considering that this pH value is in the upper limit of the used buffer, in the present work the influence of the pH was experimentally re-studied (without salt addition to increase ionic strength) and it was found that, in the working conditions, for the extraction of the complex, the best pH is 5.3. Considering the light absorbance of the chloroform solution of the extracted copper(II)-diclofenac complex at 680 nm, and the related pH, it was observed: 0.081 at 4.6; 0.124 at 5.1; 0.476 at 5.3; 0.416 at 5.9 and 0.409 at 6.1. To perform these extractions, in the separatory funnel, 2.0 mL of a 7.0 mg mL^{-1} aqueous solution of sodium diclofenac working solution was mixed with 3.0 mL of an aqueous copper(II) acetate solution (50.0 mg mL^{-1}) and 4.0 mL of the buffer (pH 5.3). If necessary, the pH was carefully adjusted with a sodium hydroxide 0.1 mol L^{-1} solution. 2.0 mL of chloroform was added and the mixture was agitated during three minutes. A second extraction was done with about 1.8 mL and a third with 1.0 mL of the organic solvent. The organic phase was always separated in the funnel and transferred to a 5.0 mL volumetric flask. The volume was completed to the mark with chloroform. The absorbance of the organic solution was measured at 680 nm *versus* chloroform as blank.

The influence of the volume of chloroform necessary to extract the copper(II)-diclofenac from the aqueous solutions was studied from 5.0 to 10.0 mL. It was observed that there is not an additional gain with the volume increase of the solvent. The necessary number of extractions was studied, *i.e.*, the total volume (5.0 mL) was divided in portions according to the procedure above described. As a higher number of extractions (maintaining the total volume) do not increase extraction of the complex to the organic phase, it was concluded that three extractions are enough for the transfer of the complex to the organic phase at pH 5.3. Complementary studies of the extraction with only one operation were done using 7.0 and 10.0 mL (total volume) of chloroform. In all cases incomplete extraction was observed.

The necessity of the use of acetic acid in the copper(II) solution, as originally indicated,[35] was also investigated. It was found that the use of such acid is necessary as its presence imply in higher (about 20% more) quantity of complex

transferred to the organic solution. It can be understood that this fact occurs due to the increase in the yield of the reaction of formation of the complex in the aqueous phase.

The influence of the copper(II) acetate concentration on the formation of the complex was also studied. The concentrations of 40.0 mg mL^{-1} (0.20 mol L^{-1}), 50.0 mg mL^{-1} (0.25 mol L^{-1}), 60.0 mg mL^{-1} (0.30 mol L^{-1}) and 70.0 mg mL^{-1} (0.35 mol L^{-1}) were used. It was observed an increase in the quantity of the complex formed when the concentration of the copper salt goes from 40.0 to 50.0 mg L^{-1}, remaining constant in upper concentrations. Therefore the concentration of 50.0 mg mL^{-1} was selected, which is in accordance with related results.[35]

In order to verify the contribution of the ionic strength and of the kind of ions, on the extraction of the complex with chloroform, solutions of several salts were prepared (NaCl, KCl, $KClO_4$, $NaBF_4$, KBr). 1.0 mL of the salt solution (or of pure water) was used in the reaction mixture in order to obtain a contribution of the salt (I=0.20 mol L^{-1}) to the total ionic strength (*c.a.* 1.2 mol L^{-1}) excepting in the solution containing $KClO_4$ (I=0.012) where I total was *c.a.* 1.0 mol L^{-1}. The contribution of the acetic/acetate acid buffer and of copper(II) acetate to the ionic strength was *c.a.* 1.0 mol L^{-1} (including the contribution of the acetate from the copper(II) salt). In the above cited article[35] the total ionic strength was 0.90 mol L^{-1} (the contribution of the salt was 0.20 mol L^{-1}). Therefore, as in our case the total ionic strength is about 33% higher than that of the reference,[35] it was expected a smaller contribution of the added salt to the extraction of the copper(II)-diclofenac complex.

In all cases the complex was prepared and extracted as described above and the visible spectrum registered. Figure 3 shows these spectra from 400 to 800 nm. It can be easily observed that at 680 nm there is no significant difference among the absorption of the various solutions. Therefore, it can be concluded that there is not an important contribution of the ionic strength or of the kind of ions, on the extraction of the complex due to the addition of the salt. The small changes observed on the spectra cannot be unequivocally attributed to differences in the extraction. More probably they are due to interactions of the anions with the complex leading to the formation of species like ionic pairs. Therefore, despite the small increase observed in the absorbance values with, for example, KCl, in the proposed method, in order to simplify the procedure, the ionic strength has been not increased by the addition of a salt solution.

The calibration curves are A = -0.101 + 0.087 C, with correlation coefficient R=0.9984, for sodium diclofenac and A = -0.115 + 0.086 C, with correlation coefficient R=0.9993, for potassium diclofenac. C is the diclofenac salt concentration, in mg mL^{-1} of the prepared aqueous solution.

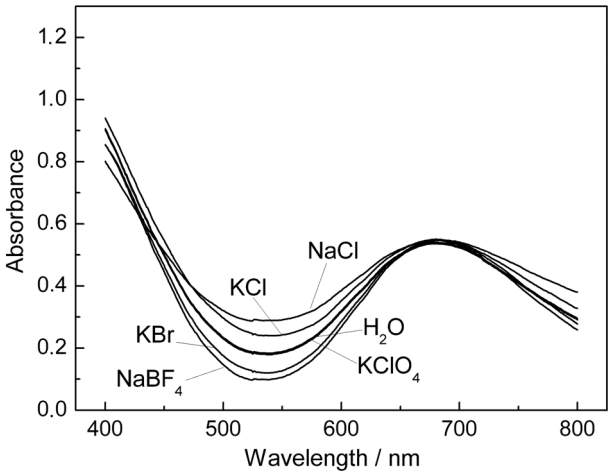

Figure 3. Spectra of the copper-diclofenac complex in chloroform solution, extracted from aqueous solutions containing different dissolved salts, in pH 5.3 (acetic acid / acetate buffer).

It is interesting to observe that the linear coefficients of the calibrations curves are negatives. This fact can be understood as an incomplete formation of the copper(II)-diclofenac complex. In presence of relatively high concentration of copper(II) ions, small concentrations of diclofenac would not quantitatively form $Cu(C_{14}H_{10}Cl_2NO_2)_2$ and, therefore, the drug would be not completely extracted by the organic phase.

In Table 1 are shown the results obtained in the study of the precision of the method. Three different concentrations were used, 5.0 mg mL^{-1}, 10.0 mg mL^{-1} and 15.0 mg mL^{-1} and five determinations were done with each one, every day during five days. The observed mean intrinsic relative standard deviations for the sodium salt and for the potassium salt are very similar, *i.e.*, 2.3% and 2.7% respectively.

Table 2 shows the results obtained for the determination of sodium diclofenac and of potassium diclofenac in solid (tablets) and liquid preparations (ampoules), using the proposed method and that indicated in USP (HPLC).[43]

The statistical Student's *t*-test was applied to compare the results obtained by the two analytical methods.[44] Considering that for the degree of freedom (ν) 4 the tabulated *t* value is 2.78 (a=0.05), no significant differences were observed between the results obtained by the two methods at 95% confidence level.

To verify the specificity of the method,[45] *i.e.*, the analyte response in the presence of all other components of the pharmaceutical preparation, some aliquots of the samples were submitted to stress conditions: base (0.1 mol L^{-1} NaOH) and oxidant (3% H_2O_2 m/v). The aliquots were directly dissolved in NaOH or H_2O_2 solutions in place of water and the procedure done according to the explained above in samples treatment. In both cases no interference was observed.

Considering the low cost, the precision, the accuracy and the reliability of the method it can be recommended

Table 1. Study of the precision of the method, intra essay (mean of 5 determinations done in the same day) and inter essay (determinations done in 5 different days)

C [a]/ mg mL^{-1}	A_{Na} [b]	SD[c]	RSD/%	A_K	SD	RSD [d]/%
5.0	0.305	0.007	2.4	0.282	0.009	3.4
10.0	0.789	0.019	2.3	0.731	0.019	2.6
15.0	1.342	0.031	2.3	1.248	0.027	2.1
Mean RSD / %			2.3			2.7

[a] C is the diclofenac salt concentration in mg mL^{-1} in the working solution; [b] A_{Na} and A_K are the absorbances, at 680 nm, of the copper diclofenac complex extracted in chloroform, respectively from the sodium and potassium salts solutions; [c] SD is the estimate of the standard deviation; [d] RSD is the relative (percent) estimate of the standard deviation.

Table 2. Comparison of the determination of sodium salt and potassium salt diclofenac in pharmaceutical preparations using the proposed spectrophotometric method and HPLC according the United States Pharmacopoeia[43]

	Sodium diclofenac m ± SD [a]/ mg			Potassium diclofenac m ± SD [a]/ mg			Nominal
Sample	Spectr. method	USP[43] method	*t*	Spectr. method	USP[43] method	*t*	value [b]/ mg
Tablet	46 ± 2	48 ± 2	1.0	48 ± 2	48 ± 2	0.0	50
Ampoule	74 ± 3	76 ± 3	0.7	73 ± 3	74 ± 3	0.3	75

[a] is the mass of the diclofenac salt found in the tablet or in the liquid pharmaceutical preparation packed in ampoule (injectable); *t* is the Student's parameter for 95% confidence level; N is the number of determinations, *i.e.*, N=5 (*t*=2.78) for the spectrophotometric method and N=3 (t=4.30) for the USP[43] method; SD is the estimate of the standard deviation that is affected by the weight of each individual tablet which it was observed to vary about ± 3.6%; [b] The nominal value per tablet or per ampoule.

for the determination of diclofenac sodium or potassium salts in pharmaceutical preparations.

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