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Determination of diclofenac in pharmaceutical preparations by diffuse reflectance photometry

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Abstract

A quantitative analytical method for the determination of diclofenac in pharmaceutical preparations by diffuse reflectance in the visible region of the spectrum is presented. The color reaction is done directly in the measuring cell immediately after mixing, using small volumes of the analyte solution, of the reagent and of the buffer solutions. All reflectance measurements were carried out in a home made reflectometer equipped with a red LED as light source and a LDR as detector. The calibration curves were constructed from 1.0 to 18 mg mL^{-1} (about 3.0×10^{-3} to 5.5×10^{-2} mol L^{-1}) of sodium diclofenac or of potassium diclofenac in the analytical solution, with typical correlation coefficients equal to 0.999. The detection limit was estimated to be about 0.7 mg mL^{-1} (2×10^{-3} mol L^{-1}). The method was applied to determine diclofenac in solid and liquid pharmaceutical preparations. The R.S.D. varied from 2% to 4% depending of the sample. The results were compared with those obtained with the HPLC procedure recommended by the United States Pharmacopoeia using the statistical Student's *t*-test procedure.

Keywords: Diffuse reflectance; Diclofenac; Pharmaceutical preparations; Quantitative analysis

1. Introduction

Diclofenac, 2-[(2,6-dichlorophenyl)amino] benzene acetate, is a synthetic non steroidal compound that is more usually found as sodium or potassium salt [1] with anti-inflammatory and anti-rheumatic application.

From 1975, with the introduction of the use of diclofenac in medical treatments, quantitative analytical procedures appeared in the literature for its determination in biological materials [2], but only from 1987 more attention have been devoted to analytical procedures for pharmaceutical preparations. Several techniques have been described. For example, HPLC [3–8], reverse-phase liquid chromatography [9], fluorimetric [10–13], potentiometric [12–15], capillary electrophoresis [16,17], thermal analysis [18], AAS [19], flow methods [20–24], spectrophotometric UV [25,26], spectrophotometric visible [27–37] and gravimetric [38]. However, as far we know, there is no any reflectometric method for the determination of diclofenac related in the literature. Recently reflectometric methods were described for the determination of acetylsalicylic acid in pharmaceutical preparations [39] and of furosemide [40]. Despite the fact that analytical reflectometric methods in the visible region of the spectrum are not very common, some have been recently published [38–45]. It is usually considered that they offer low precision and accuracy when compared, for example, with transmittance procedures. However, diffuse reflectance methods can present some advantages. For example, in certain cases when they admit the presence of solids that do not absorb in the working wavelength, together with the substance which reflectance is to be measured. Such a situation in not possible in transmittance measurements. Also, good precision and accuracy can be achieved.

The aim of this work is to develop a simple, rapid to be performed, reliable, precise and accurate reflectometric method for the determination of diclofenac in pharmaceutical preparations. Contrarily to the more usual in reflectometric procedures where reflectance of a solid surface is measured,

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the present method will be based on diffuse reflectance measurements of a solid suspended in aqueous solution [41].

2. Experimental

2.1. Apparatus

A HPLC Waters 600 E, with UV–vis 484 detector and a Microsorb MV C-18 5 μm 25 cm × 4.6 mm column, was used for analytical comparative purposes. For pH measurements it was used an Analyzer model 300 with a glass electrode. A HP 8254 A spectrophotometer with the RSA HP 84 reflectance accessory, was used to obtain the reflectance spectrum of the copper(II)–diclofenac complex ($Cu(C_{14}H_{10}Cl_2NO_2)_2$) suspended in the aqueous solution. For the reflectometric measurements was used a portable reflectometer constructed in our laboratory [41] using a red LED (λ = 635 nm) as light source.

2.2. Reagents

All reagents were of analytical grade excepting diclofenac that was a pharmaceutical 99.9% certificated product. It was gently furnished by a pharmaceutical industry. This product was again analyzed in our laboratory to confirm diclofenac content [47] and, after dried in an oven at 110 °C for 3 h, it was kept in a dissecator over phosphorus pentoxide. Water was distilled in a glass apparatus and then deionized in a Milli Q Plus device.

2.3. Solutions

Acetic acid 0.1 mol L^{-1}: 6.0 g of glacial acetic acid was dissolved in 1.0 L water.
Copper(II) acetate: 50.0 mg mL^{-1} (0.25 mol L^{-1}) was prepared by dissolving $Cu(CH_3CO_2)_2{\cdot}H_2O$ (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: to obtain the buffer solution (pH 5.3), adequate volumes of sodium acetate 2.0 mol L^{-1} and of acetic acid 2.0 mol L^{-1} solutions were mixed. A standard diclofenac solution (67 μL, 7.0 mg mL^{-1}) mixed with the copper(II) acetate solution (100 μL) and with the buffer (133 μL) was used to obtain the reflectance spectrum.

2.4. Samples

All samples were purchased in the local market.

2.5. Samples treatment

To develop this work, groups of 40 tablets of each one of the different used pharmaceutical preparations containing diclofenac were triturated, homogenized in a mortar and transfered to a flask and stocked. This material was used to develop the method.

To prepare the analytical solutions of the solid samples, a quantity of the triturated material was weighed in order to obtain a final solution with about 7.0 mg mL^{-1} of the diclofenac salt. Initially, this aliquot was treated, in a tube, with 6.0 mL of water heated to 85 °C, agitated during 1 min and maintained at that temperature for 5 min more. The solution was then centrifuged during 1 min. The supernatant was filtered directly into a 25.0 mL volumetric flask through qualitative filter paper. The remaining material in the tube was treated two times again with hot water according the procedure already described. Finally, after cooling to room temperature, water was added to complete the volume of the volumetric flask that was then agitated in order to homogenize the solution.

In the case of liquid samples the content of five ampoules was mixed and adequate samples were taken from the total volume. From this solution a volume was used in order to prepare an aqueous solutions containing about 7.0 mg mL^{-1} of the diclofenac salt.

2.6. Analytical procedure and calibration curve

The 310 μL of the analytical solution of diclofenac (or of a standard solution) was mixed with 470 μL of copper(II) acetate solution and 620 μL of the acetic acid/acetate buffer, directly in the reflectance cell, performing a total volume of 1400 μL (1.40 mL). The mixture was gently agitated with a thin glass rod. The reflectance measurements (Ω) were done directly in the reflectometer. The calibration curves were constructed plotting resistance in ohms versus the diclofenac salt content in the analytical solution from 1.0 to 18.0 mg mL^{-1} dissolving adequate quantities in 25.0 mL of water. The blank solution was simply the cell filled with the buffer and copper solution, adding water to complete 1.40 mL.

3. Results and discussion

Reflectance analytical methods, mainly in the UV–vis region, are not very popular when compared, for example, with those that use transmittance (absorbance), mainly regarding pharmaceutical analysis where very few examples were found [39–40]. This can be attributed in part to the difficulty in preparing rigorously homogeneous reflecting surfaces, which implies that is difficult to obtain reproducible analytical values. Despite this is possible to develop quantitative reflectometric methods based on solid supports for the analyte with quite good results [39–40,42–46]. Also, it is possible to work in solution, measuring the diffuse reflectance of solids there suspended [41]. This procedure was chosen for the development of the method here proposed.

In diffuse reflectance measurements, the reflectometer reads a signal of the diffused reflected radiation also called reflectance or reflection power, T_R, which is analogous to

transmittance in transmittance measurements. The optical density for reflectance measurements is $A_R = -\log T_R$, similarly to absorbance.

The reflecting power, T_R, is given by $T_R = I/I_0$, where I_0 is the intensity of the incident radiant energy and I the intensity that is reflected by the medium. T_R obviously lies within the bounds, $0 < T_R < 1$. It is proper to consider $T_R = 1$ whenever the absolute reflecting power can be measured from a standard of maximum reflectance, usually compressed $BaSO_4$ or MgO powder. However, for relative measurements, as it is the case of analytical determinations based on a calibration curve, the reflecting power can be considered equal to 1 for the reflecting material considered as the blank.

The relation between T_R and sample concentration, C, is given by the Kubelka–Munk equation, $f(T_R) = (1 - T_R)^2/2$ $T_R = \varepsilon C/S$, where ε is the molar absorptivity and S the scattering coefficient. For correct application of this equation the absolute reflectance of the reference material is needed.

For quantitative analysis many different calibrations plots can be proposed analogous to transmittance measurements, for example, in the reflectance case the correlation $A_R = kC$ can be used and, in the proposed method, it fitted well the experimental data of the calibration curve. k is a constant related to the molar absorptivity and to other characteristics of the reflecting material.

For the home-made reflectance device used in this work it was shown that the resistance of the LDR is directly proportional to the reflected light and, therefore, to the concentration of the reflecting material [41].

Diclofenac forms with ions Cu^{2+} a light-green 2:1 complex ($Cu(C_{14}H_{10}Cl_2NO_2)_2$), soluble in organic solvents but insoluble in water. This property was used in the present work to develop the reflectometric method showed below. The method is based on the supposition that the diffused reflection of the light incident on the precipitated complex suspended in aqueous solution is proportional to its concentration. To guarantee the quantitative precipitation of the complex a molar ratio 20:1 (copper(II):diclofenac) was used.

The proposed method was also confronted with the HPLC procedure recommended by the United States Pharmacopoeia [48] whose obtained R.S.D. was about 4%.

Fig. 1 shows the reflectance spectrum of the copper(II)–diclofenac complex dispersed in aqueous solution. It can be easily observed that the red LED used in the experiments as light source ($\lambda = 635$ nm) is quite adequate for the proposed method.

The resistance of the light detector (LDR) in the reflectometer increases as the radiation intensity that reaches its surface decreases, leading to higher values of the resistance in ohms. Therefore, it could be expected more absorption of the red light as more complex is suspended in the aqueous solution in the reflectometric cell, allowing a correlation between concentration and resistance values.

Typical calibrations curves constructed using concentrations from 1.0 to 18.0 mg mL^{-1} of the diclofenac salt are:

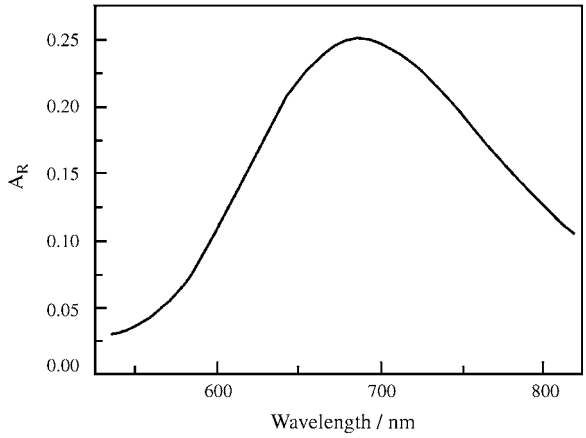

Fig. 1. Reflectance ($A_R = -\log T_R$) spectrum of the copper(II)–diclofenac complex suspended in aqueous solution.

$R = -1.1 + 20.9C_{Na}$ (R is the resistance in ohms and C_{Na} the concentration of the sodium diclofenac, in mg mL^{-1}, in the analytical solution), with correlation coefficient equal to 0.9997; $R = 7.3 + 7.7C_K$ (R is the resistance in ohms and C_K is the concentration of the potassium diclofenac, in mg mL^{-1}, in the analytical solution), with correlation coefficient equal to 0.9988. The differences in the parameters of the analytical curves of the two diclofenac salts are mainly consequence of different adjustment of the reflectometer. In the first case the detection limit is estimated to be 0.4 mg mL^{-1} (1×10^{-3} mol L^{-1}) and in the second case it is 0.9 mg mL^{-1} (3×10^{-3} mol L^{-1}). As a mean situation the detection limit can be assumed to be about 0.7 mg mL^{-1} (2×10^{-3} mol L^{-1}).

To test the intrinsic precision of the proposed method, samples of pure sodium diclofenac and of potassium diclofenac were analyzed in four different days, performing four determinations per salt each day according Table 1. It is ease to note that the value of the resistance of the detector increases with the increase of the diclofenac concentration. It is also observed that the mean of the estimate of the standard deviation, s_{Na} and s_K, can be considered constant (2 kΩ). The experiments with sodium and of potassium salts of diclofenac were done in different occasions. The reflectometer was adjusted in order to give resistance values, for potassium diclofenac analysis, in a proportion of 2:1, in relation of the resistances obtained when used sodium diclofenac ($R_{Na} \div R_K \cong 2$), corresponding to 5.0 mg mL^{-1} in both cases, in order to verify the influence of the resistance range used on the precision of the analytical results. It is observed that the R.S.D. increases with the decreasing of the resistance value. This behavior is obvious as the estimate of the standard deviation is constant. Nevertheless, the R.S.D. obtained in all cases in quite satisfactory for the analysis of diclofenac in pharmaceutical preparations.

Table 2 shows the diclofenac (sodium and potassium salts) content in tablets and in ampoules (solution), using aliquots

Table 1
Study of the intra day and inter day precision of the reflectometric method proposed for sodium and for diclofenac salts

Day	C (mg mL^{-1})	Intra day					
		R_{Na} (kΩ)	s_{Na} (kΩ)	R.S.D. (%)	R_K (kΩ)	s_K (kΩ)	R.S.D. (%)
First	5.0	92	2	2.2	47	1	2.1
Second		96	2	2.1	49	2	4.1
Third		99	2	2.0	46	2	4.3
Fourth		93	2	2.2	46	2	4.3
Mean inter day		95	2	2.1	47	2	3.7
First	10.0	202	1	0.5	91	2	2.2
Second		202	2	1.0	87	2	2.3
Third		202	1	0.5	91	2	2.2
Fourth		202	2	1.0	90	3	3.3
Mean inter day		202	2	0.8	90	2	2.5
First	15.0	310	3	1.0	120	2	1.7
Second		311	2	0.6	117	1	0.9
Third		313	2	0.6	114	2	1.8
Fourth		312	2	0.6	116	4	3.4
Mean inter day		312	2	0.7	117	2	2.0
Mean R.S.D. (%)			1.2			2.7	

C is the Concentration of the diclofenac salt, of sodium or of potassium; R_{Na} and R_K, mean resistance in kΩ ($N = 5$ measurements) for sodium diclofenac and potassium diclofenac, respectively; s_{Na} and s_K, estimate of the standard deviation for sodium diclofenac and for potassium diclofenac, respectively; R.S.D., estimate of the relative standard deviation.

Table 2
Determination of sodium diclofenac and of potassium diclofenac in tablets and in solutions

Samples	Na^+-diclofenac content ±S.D.			K^+-diclofenac content ±S.D.			Nominal content (mg)
	Reflectometric (mg)	HPLC (mg)	t calc.	Reflectometric (mg)	HPLC (mg)	t calc.	
Tablets	48 ± 1[a]	48 ± 2[a]	0.0	50 ± 2[b]	48 ± 2[b]	1.2	50
Ampoules	74 ± 1[c]	76 ± 3[c]	1.2	71 ± 2[c]	74 ± 3[c]	1.5	75

Comparison of the results obtained with the proposed reflectometric method with those obtained using the method recommended by the United States Pharmacopoeia [48] (HPLC), using the statistical Student's t-test [49]. Number of determinations: $N = 5$ for the reflectometric method and $N = 3$ for the HPLC method. Confidence level 95%. The tabulated critical t-value is 2.57.

[a] Sample also contains: polyvinylpyrrolidone, lactose, microcrystalline cellulose, corn starch, sodium starch glycolate, silicon dioxide, magnesium stearate, metacrilic acid, polyethyleneglycol, titanium dioxide, talc, 6-hydroxy-5-((4-sulfophenyl)azo)-2-naphthalenesulfonic acid disodium salt (FD and C Yellow No. 6), Sicovit Brown 75 E 172.

[b] Sample also contains: lactose, corn starch, microcrystalline cellulose, silicon dioxide, magnesium stearate, titanium dioxide, polyethyleneglycol, blue dye, red dye, yellow dye, sodium starch glycolate, sodium croscarmelose, opadry clear, polyvinylpyrrolidone.

[c] Samples also contain: mannitol, sodium metabisulfite, benzyl alcohol, propylene glycol, sodium hydroxide and water.

with about the same nominal content per tablet or ampoule, compared with the results obtained with the reference HPLC method. The statistical Student's t-test was applied to compare the results obtained by the two analytical methods [49]. Considering that for the degree of freedom (ν) 4 the tabulated t-value is 2.78 ($\alpha = 0.05$), no significant differences were observed between the results obtained by the two methods at 95% confidence level. In Table 2 are also described the excipients of the related four preparations. In all cases agreement was observed, between the limits of the S.D. of the proposed method.

4. Conclusion

According to the results above presented, the reflectometric method here proposed is reliable and of very simple procedure. Also, the precision and the accuracy are quite satisfactory for the quantitative analysis of diclofenac in pharmaceutical preparations. Therefore, it can be recommended for the quantitative analysis of diclofenac in pharmaceutical preparations.

Also, the method here described clearly shows that diffuse reflectance photometry in the visible region of the spectrum, for quantitative analysis, is quite feasible even when the measured form of the analyte is a solid suspended in water.

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