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Influence of pH on the photodegradation kinetics under UV light of climbazole solutions

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Abstract. The objective of this research was to study the effect of pH on the photodegradation kinetics of 15.8 $\mu g.ml^{-1}$ aqueous solutions of climbazole. A high-performance liquid chromatographic method for the quantification of climbazole after irradiation of aqueous solutions was carried out, using a RP-18 column with a acetonitrile - 0.05 M sodium perchlorate mobile phase. The detector was set at a wavelength of 220 nm. Calibration curve showed linear response in the interval 5 to 25 $\mu g.ml^{-1}$. Photodegradation appeared to follow first-order kinetics and was found pH-dependent. The degradation rate constant was calculated to be 10.5×10^{-3}, 9.9×10^{-3} and 16.5×10^{-3} min^{-1}, respectively at pH 5, 7 and 9.

Keywords. Climbazole – photodegradation kinetics – HPLC.

Introduction

A lot of shampoos exist on the market. The anti-dandruff shampoos have a significant place because an important percentage of the population suffer from a pytiriasic state who constitute an aesthetic harm [1-4].

Climbazole, an antifungal from the imidazole class of substances, is an anti-dandruff active.

Climbazole has a good efficiency against moulds, yeasts and dermatophytes. Its particularly good efficiency (minimal inhibition concentration 0.1-0.25 µg/ml) against *Malassezia furfur*, an anthropophilic fungus causative agent of several skin disorders, justifies its use as an anti-dandruff agent in shampoos, conditioners and hair tonics [5]. Some studies have demonstrated the efficiency of shampoos containing 0.5 %, 0.75 % or 2 % of climbazole. Besides, climbazole and ketoconazole showed similar *in vitro* activity against *Malassezia furfur* [6-8].

Climbazole (trade name Baypival®) is a safe agent; oral LD 50 determined on rats accounts for 400 mg/kg and dermal LD 50 on rats is superior to 5000 mg/kg. Dermal tolerability of Baypival® in humans has been carried out. Shampoos (based on alkylether sulphate) containing 0.5 % Baypival® produced no irritation or other adverse effects.

In this study, we tested the influence of UV irradiation on the stability of climbazole in aqueous diluted solution at a concentration of 15.8 $\mu g.ml^{-1}$. Previous study have established the thermostability of this molecule [9].

Another anti-dandruff agent, piroctone olamine, is used to formulate shampoos and it has a very low acute oral toxicity (LD 50 for the rats is 8.1 g/kg and 5 g/kg for the mouse) even for the human [10-12]. It can be interesting to compare the photostability of these two molecules. Then, the target of this study was to investigate experimental conditions on HPLC for the analysis of climbazole and to determine the photostability of this antifungal agent.

Material and methods

Chemicals

Climbazole was obtained from Bayer (batch n° 203610905). Climbazole or [1-imidazolyl-1-(4-chlorophenoxy)-3,3-dimethylbutan-2-one] (Fig. 1) is a white to slightly yellowish crystalline powder. There is a weak characteristic odour (phenolic odour). Climbazole is very little soluble in water at 20 °C. All chemicals were of analytical quality. Distilled

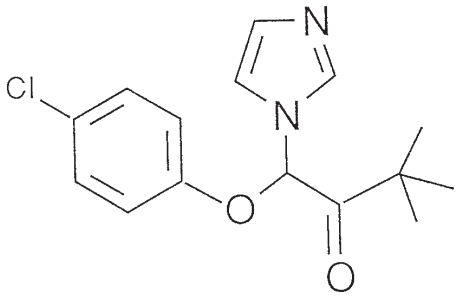

Figure 1. Climbazole chemical structure, wavelength of maximum absorption (λ_{max}) and molar extinction coefficient (ε).

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water was obtained from an Autostill 4000X (Jencons) apparatus. Demineralized, deionized water was obtained from MilliQ system (Millipore). LC quality acetonitrile was purchased from Merck. All solvents and solutions for LC analysis were filtered through a 0.45 µm Millipore filter and vacuum degassed by sonication before use.

Experimental procedure

The pH of climbazole solutions was adjusted to the desired values with a mixing $H_3BO_3/Na_2B_4O_7$ and was determined with a Metrohm Herisau pH-meter, model E300B, equipped with a Refill Ingold I3556 (pH = 0-14, T = 0-80 °C) electrode and standardized with Panreac solutions respectively at pH = 4 and pH = 10. These measures were carried out at 20 °C.

Solutions of climbazole, at a concentration of 15.8 µg.ml^{-1} at various pH were enclosed in spectrophotometer tubes and exposed to the light source in the light-stability cabinet (Original Hanau, No. 7011, Original Hanau Quartzlampen GmbH). The intensity of UV-A and UV-B was measured with an Osram apparatus (Centra-UV-Meβgerät). This intensity was maintained at 6.45 and 1.47 mW.cm^{-2} for UV-A and UV-B, respectively. All tubes containing climbazole solutions were covered with aluminium foil before exposure in order to eliminate the influence of heat generated by the light within the cabinet. The analysis was carried out in triplicate and the difference between the three was less than 1 %.

The absorbance spectrum of climbazole was determined by a spectrophotometric method (Hitachi UV/visible double beam spectrophotometer, Model U 2000). Slit width was fixed at 2 nm. Solutions were recorded in 1 cm quartz cells over the 200 to 400 nm range ($\Delta\lambda$ = 2.3 nm). The scan speed was 400 nm.min^{-1}.

Initially and at different times (t) after irradiation, climbazole concentrations were determined using High Performance Liquid Chromatography. HPLC was carried out with a system consisting of a Waters Model 6000 A pump, a Waters Lambda Max model 481 LC variable-wavelength detector and a Merck D-2500 model integrator (Hitachi). Each solution was analyzed under the following conditions: column LiChrosorb RP-18 10 µm (250 × 4.6 mm i.d.) (Merck); mobile phase (pH 3.0, adjusted with perchloric acid) (40 + 60, v/v) of acetonitrile and 0.05 M sodium perchlorate; volume injected 10 µl; temperature of 20 °C; flow rate 2 ml/min.

Results and discussion

The spectrum of climbazole showed maximum at 220 nm (Fig. 2). Each solution was injected on the HPLC where the area of the peak at a retention time about 7 min were measured (Fig. 3). A linear relationship was found throughout the concentration interval analysis (5-25 µg.L^{-1}; r = 0.998). There was no significant difference between day-to-day analysis (slopes evaluation, $P < 0.05$). Detection limits

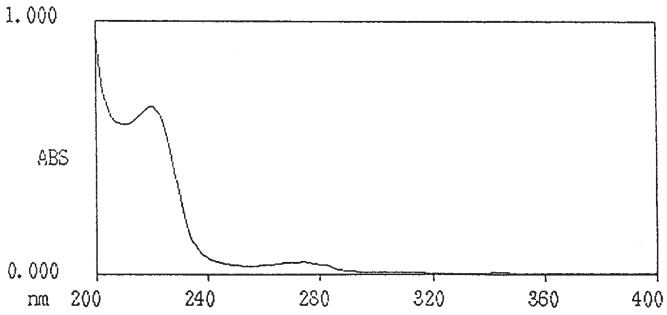

Figure 2. Absorbance spectrum obtained with the climbazole aqueous solution (15.8 µg.ml^{-1}) between 200 and 400 nm.

(LOD_s) determined as higher than three times the baseline noise level (S/N = 3) was 12.5 ng.ml^{-1}.

The order of the photodegradation reaction was determined by the least-squares method of linear adjustment and by calculating the correlation coefficients, in order to choose between the zero-order kinetics and the first-order kinetics. The degradation rate constants (k) are determined from the slope of the line of peak area *versus* time. The degradation rate constant was calculated in accordance with the determined order of the reaction.

The percentage of substance remaining was calculated. The photodegradation of climbazole in diluted aqueous

Table I. Photodegradation of aqueous solution of climbazole.

Times (min)	C/C_0 *pH = 5*	*pH = 7*	*pH = 9*
0	1.000	1.000	1.000
10	0.912	0.917	0.889
20	0.821	0.830	0.754
30	0.739	0.752	0.639
40	0.665	0.681	0.542
50	0.598	0.617	0.459
60	0.538	0.559	
70	0.485	0.506	
80	0.436	0.458	

Table II. Degradation rate constants of climbazole solutions at various pH.

pH	*Degradation rate constants* k *(min^{-1}) ± SEM*
5	$10.5 \times 10^{-3} \pm 1.1 \times 10^{-5}$
7	$9.9 \times 10^{-3} \pm 0.8 \times 10^{-5}$
9	$16.5 \times 10^{-3} \pm 1.2 \times 10^{-5}$

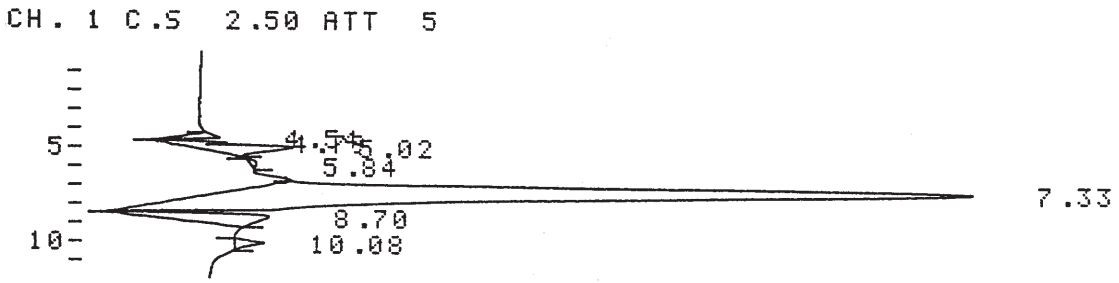

Figure 3. HPLC chromatogram obtained from the irradiated climbazole aqueous solution (15.8 µg.ml^{-1}).

solution (Fig. 4) follows apparent first-order kinetics and is described by the following equation:

$$C/C_0 = e^{-k_a t} \quad \textbf{(Eq. 1)}$$

where C and C_0 are the concentrations of climbazole at time t and initially and k_a is the apparent first-order degradation rate constant [13-15]. Equation 1 gives us the value of the degradation rate constant, which is equal to 9.9×10^{-3} min^{-1} at pH 7.

The photodegradation of climbazole (15.8 µg.ml^{-1}) in buffer solution at various pH was then studied. The chromatograms obtained during photolysis demonstrate a gradual decrease in the area of the peak. The degradation rate constant was calculated from the slope of the line of area of the peak *versus* time. The percentage of climbazole remaining was calculated at various pH (Tab. I). Whatever pH, the photodegradation of climbazole in diluted buffer solution follows apparent first-order kinetics (Fig. 4) and is described by the following equation:

$$C/C_0 = e^{-k_b t} \quad \textbf{(Eq. 2)}$$

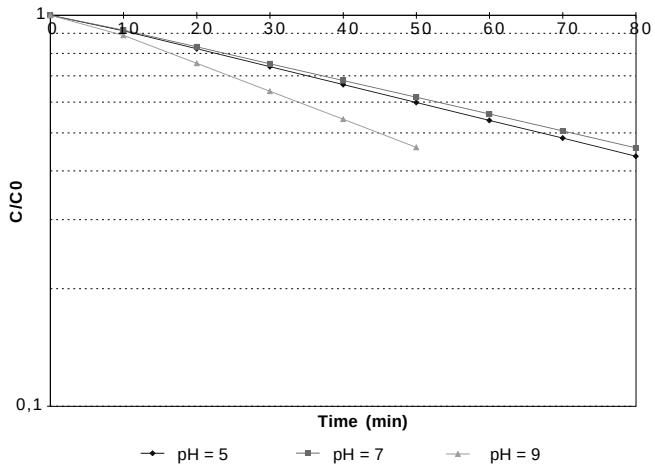

Figure 4. Kinetic diagram for the photodegradation during irradiation of the climbazole aqueous solution (15.8 µg.ml^{-1}) at various pH (n = 3).

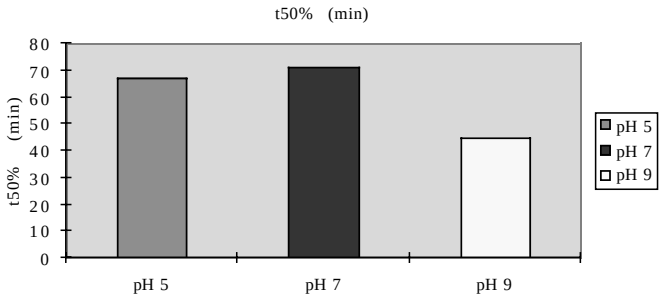

Figure 5. Values of shelf-life as function of pH for climbazole (15.8 µg.ml^{-1}).

where C and C_0 are the concentrations of climbazole at time t and initially and k_b is the apparent first-order degradation rate constant. The HPLC analysis demonstrates the gradual decrease of the climbazole concentration after irradiation (Tab. I). At various pH, we have note a variation of the values of rate constant k_b (see Tab. II). The pH of the solution have an influence on the photostability of climbazole. This conclusion has already been reached for many organic molecules [16], piroctone olamine for example [17]. The effect of pH on the climbazole shelf-life is significant between pH 7 and pH 9 (Fig. 5). The percentage decrease in the stability of climbazole by the addition of buffer was found to be about 67 % between pH 7 and pH 9.

The present study has completed climbazole stability knowledge. This molecule appeared to be very photodegradable. Before we have demonstrate that this antidandruff molecule is relatively thermostable [9]. We have established that the best pH storage conditions correspond to the antidandruff formulations at neutral pH.

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