Investigations on the Photo-Transformation of Retinol Acetate

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Abstract: Taking into account the advanced photosensitivity of some pharmaceutical substances, there are several special requirements for their use, storage and even their assay in pharmaceutical formulations. Despite numerous attempts made in order to characterize the intrinsic photo-transformation tendency of drugs, the problem is far to be solved satisfactorily. In this paper a model study is performed in the concrete case of retinol acetate (vitamin A), a compound with well-known photosensitivity, using a photochemical reactor of original conception. In the case of vitamin A, a single dominant photochemical process seems to be takes place, as suggested by HPLC UV-spectroscopy and chemometric processing of spectrophotometric data. An adequate interpretation of kinetic data allows to define and determine a rate constant suitable to express the intrinsic photo-instability, irrespective of some experimental factors such as initial concentration, the nature of solvent etc. The processing technique we have been used is suitable even in the case when the spectroscopic behavior of reaction product is unknown. The described strategy is based on the monomolecular pathway supposition, successfully tested in the case of vitamin A.

Keywords: Retinol acetate, vitamin A, photosensitivity, photo-transformation.

1. INTRODUCTION

Degradation over time of many active substances of pharmaceutical interest is an acute problem both in developing the industrial production processes and in their storage and use. In pharmaceutical practice, drugs with high photosensitivity are used; their modification over time may be caused by light as a contributing factor of the overall degradation. The ultraviolet solar radiation does not represent a direct issue of this work, taking into account the fact, that while storing and therapeutic using the drugs, they are rarely exposed to such radiation. But the radiation from the near ultraviolet spectral area (300 – 400 nm) may represent a real threat, especially in hospitals where natural light is replaced by radiation coming from fluorescent tubes [1, 2].

There are several studies [3-9] regarding drugs' photosensitivity and the experimental arrangement of controlled irradiation is significantly different from one laboratory to another. On the other side, studies concerning successive irradiation of the same sample are affected by a number of main errors: (i) when repetitively starting the irradiation source, the transition period, until the source reaches the stationary working regime, is difficult to control and recreate; (ii) for the continuous irradiation of the sample but with discontinuous sampling, the irradiated sample's geometry is changed during the investigation; this may result to even more incontrollable errors.

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This work aims to realize and test a photochemical study device [8, 9] meant to avoid or minimize these errors. The kinetic study of the photo-transformation process provides a general strategy for a standardized characterization of intrinsic photo-degradation tendency of drugs.

On the other side, based on the chromatographic and spectrophotometric results, this work argues that by irradiating the retinol acetate (Vitamin A) on the established conditions, a simple photochemical process takes places which generates only one dominant reaction product.

2. MATERIALS AND METHODOLOGY

The retinol acetate, provided by Sigma Company, in amorphous under-cooled state, was used as a solution in absolute ethanol without any prior purification; the purity was controlled through the infrared absorption spectrum. The absolute ethanol used as a reaction environment, provided by "Merck" (spectroscopic purity, "Uvasol" grade), was distilled before use and controlled for ultraviolet transparency. The oxalic acid, necessary for chromatographic separation, was provided from the autochthon company "Chimopar" – Bucharest. The methanol, used as well for the chromatographic separation, provided by "Merck" (HPLC grade) was purified by rectification.

The chromatographic separations were performed with a "Jasco" instrument provided with a diode array detector operating in the ultraviolet and visible range. An ultraviolet and visible spectrophotometer "Spectronic 300" was used for the kinetic study of photo-induced transformation.

A 15 W irradiation source was assembled inside the photochemical reactor to assure a continuous emission in the spectral range of 300 – 400 nm. The irradiation of sample

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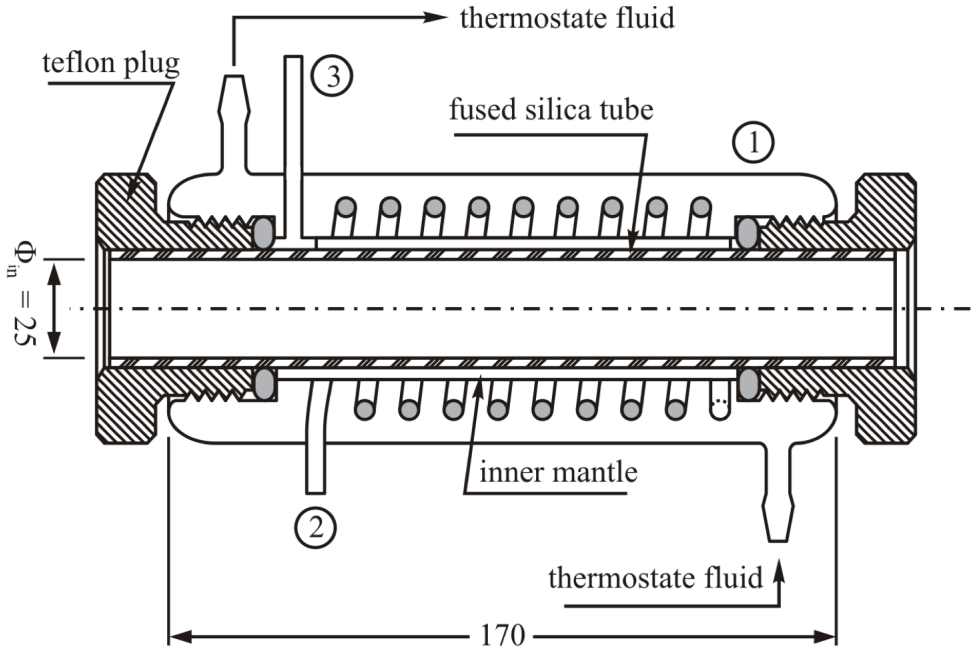

Fig. (1). Schematic representation of photochemical reactor (the dimensions are expressed in mm).

was performed with a self designed and built device, presented in Fig. (**1**).

The components of the photochemical reactor are assembled around a fused quartz tube (transparent in ultraviolet), inside of which the tubular irradiation source is installed. A fluid maintained at working temperature (30 ^{o}C) circulates through the thermostatic mantle (1) at the exterior of the reactor. The sample studied (ethanol solution) is inserted in the reactor through the tube (2); after passing through the thermostatic area, it reaches the irradiation area placed between the exterior surface of the quartz tube and the interior surface of the thermostatic mantle. The irradiation space, with a volume of 3.5 cm^3 is passed by the sample in a time interval dependent on the flow debit, controlled by a dosing pump (not shown in the figure). The irradiation time is modulated by changing the flow debit. The irradiated solution leaves the device through tube (3). While passing through the installation, a stationary state is created regarding the irradiation doze of the sample. By keeping the flow velocity at a constant level, the same level of irradiation can be kept, theoretically for unlimited time. The way the components are assembled allows a set of analysis (HPLC, UV spectrum etc.) to be realized on the solution that leaves the installation.

After irradiating an ethanol solution of retinol acetate with progressively increasing dozes, a series of HPLC assay was performed in order to follow the photoinduced transformation and to obtain information about the number of products obtained. The HPLC analysis were realized using a non-polar stationary phase (Nucleosil C_{18}, 20 cm × 4 mm) and a mobile phase containing 13% solution of oxalic acid in water (0.2 M) and 87% absolute methanol. The mobile phase passed through the column with a flow rate of 1ml/min. The chromatograms were recorded in isocratic regime at 30^{o}C. Based on the bidimensional representation, 380 nm vas chosen as the optimum wavelength for the detection. For each HPLC analysis, a volume of 20 µl of irradiated solution was injected.

The dependence of conversion on the irradiation dose was revealed by UV-spectrophotometry. The measurements were realized on the 10.46 mg/L ethanol solution of retinol acetate. The 1 cm optical path quartz cuvette was connected to the photochemical reactor's output (3). The absorption spectra, corresponding to the different irradiation dozes, were recorded between 264 - 360 nm.

3. RESULTS AND DISCUSSION

The use of the photochemical reactor with a continuous flow regime provides the following advantages:

(i). The working regime with a continuous flow through the photochemical reactor allows sampling in random moments during the experiment and at known and controlled irradiation dozes, without interrupting or modifying the stabilized working of the irradiation source.

(ii). The irradiation geometry stays constant after consecutive samplings. This is a significant advantage as opposing to the discontinuous irradiation process where the solution's volume in the irradiation space decreases as a result of consecutive samplings. Moreover, the device described allows an analytical control of the irradiation while levying the samples does not take place in a limited amount of time. Moreover, the finite time interval of sampling does not affects the precision of irradiation dose as in the usual discontinuous irradiation experiments.

(iii). The reactor's robust design allows the irradiation at a controlled temperature, a very important issue when investigating the kinetic aspects of the photochemical processes.

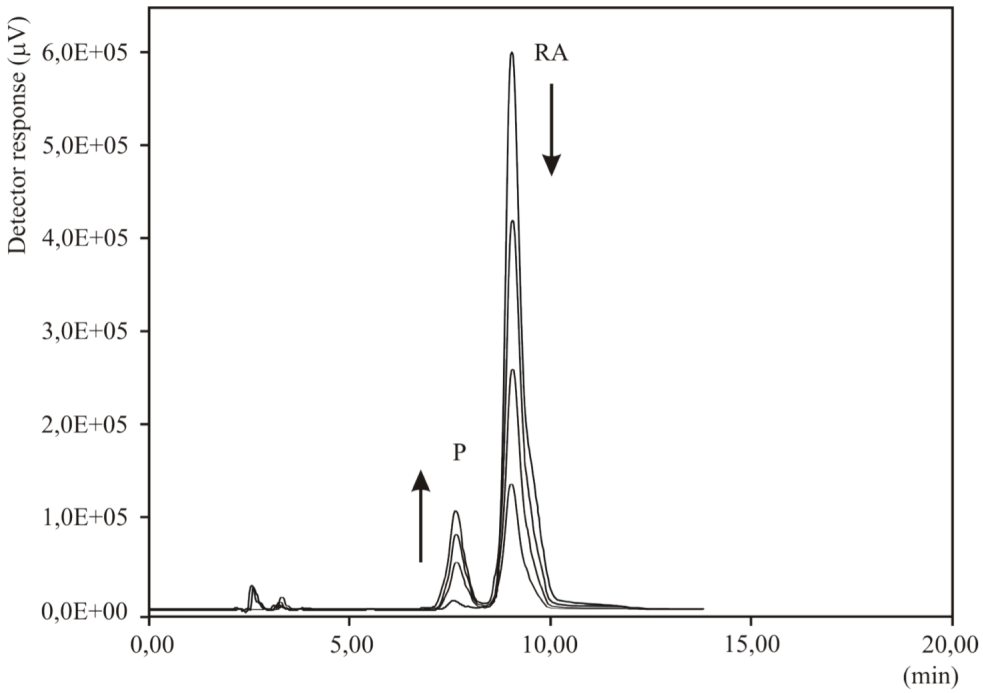

Fig. (2). HPLC monitoring of the photochemical process.

In Fig. (**2**) a few overlaid chromatograms are presented, corresponding to different irradiation dozes of the ethanol solution of retinol acetate. While the irradiation dozes increase, the peak of untransformed retinol acetate (RA) decreases while the peak of the reaction product (P) increases.

The chromatograms prove that in the given irradiation conditions only one dominant reaction product resulted. The reaction product (P) has a shorter retention time than the total-trans retinol acetate (RA). This sustains the hypothesis that the retinol acetate's photo-transformation consists mainly in a *trans-cis* isomerization at in the side chain of RA [10]. Indeed, it is plausible for a total-*trans* chain to develop a high affinity for the non polar stationary phase of the column C_{18}, compared to an isomer that with "*cis*" configuration at one of the carbon-carbon double bond.

In order to complete the argumentation regarding the formation of a single reaction product in the given reaction conditions, the UV-spectra of the reaction mixture, corresponding to progressive irradiation doses, were recorded (Fig. **3**). The overlaid spectra exhibit an isosbestic point at 289 nm.

The absorption values read at 133 wavelengths (in the spectral area 264 – 396 nm) for 17 spectra correlated with 17

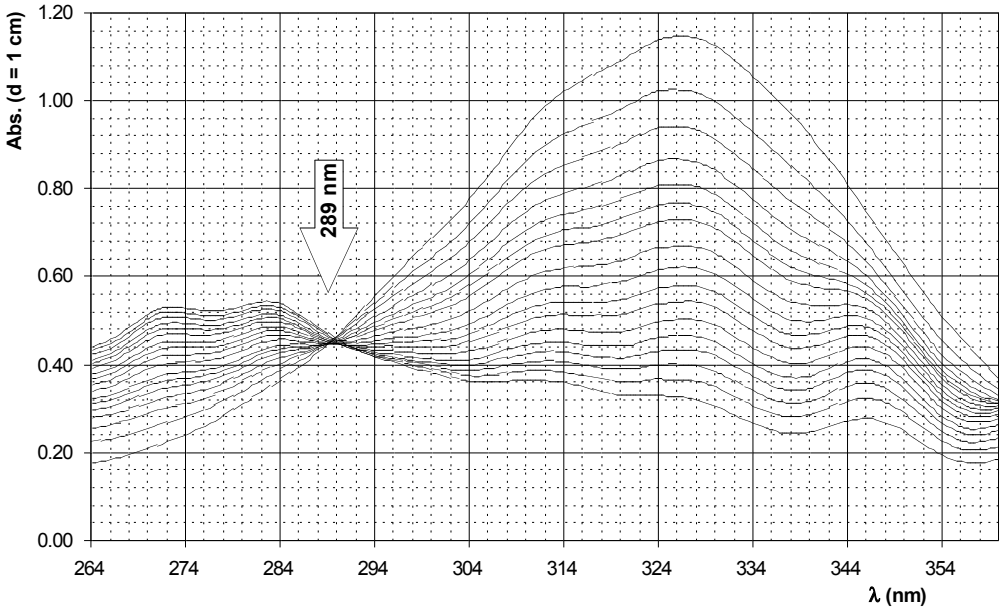

Fig. (3). UV-Spectrophotometric monitoring of photo-transformation.

values of the irradiation time (from 0 to 19.5 mins) resulted in a matrix $\boldsymbol{A}(\lambda,t)$ that contains 2261 absorption values. The matrix's rank corresponds to the number of chemical species present in the analyzed system [11-13]. To determine the rank, the matrix $\boldsymbol{A}(\lambda,t)$ is multiplied to the left with its transpose. The resulting matrix $\boldsymbol{B}(i,j)$ has the dimension 17x17.

$$\boldsymbol{B}(i,j) = \boldsymbol{A}^{T}(\lambda,t)\cdot\boldsymbol{A}(\lambda,t) \quad (1)$$

The number of eigenvalues of the matrix $\boldsymbol{B}(i,j)$, different from zero (within the limit of admitted experimental errors) is equal to the rank of the matrix and corresponds to the number of chemical species present in the chemical system during the evolution of the photoinitiated transformation. Table **1** contains the eigenvalues of matrix $\boldsymbol{B}(i,j)$ in the increasing order.

Within the limit of allowed experimental errors, only two of the seventeen eigenvalues are significantly different from zero and have detached higher values than the others (the no. 16 and no. 17 ones). This sustains the affirmation that in the irradiation conditions and at the given irradiation degree only one dominant reaction product is formed.

Table 1. Eigenvalues of Matrix *B*(i,j)

No.	Proper Value	No.	Proper Value	No.	Proper Value
1	$2{,}735\cdot10^{-5}$	7	$4{,}314\cdot10^{-5}$	13	$1{,}284\cdot10^{-4}$
2	$2{,}865\cdot10^{-5}$	8	$1{,}678\cdot10^{-5}$	14	0,010
3	$2{,}324\cdot10^{-5}$	9	$1{,}471\cdot10^{-5}$	15	0,238
4	$3{,}489\cdot10^{-5}$	10	$5{,}632\cdot10^{-5}$	16	*15,419*
5	$3{,}805\cdot10^{-5}$	11	$7{,}492\cdot10^{-5}$	17	*457,27*
6	$4{,}069\cdot10^{-5}$	12	$7{,}866\cdot10^{-5}$		

Table **2** shows the absorption values at a 327 nm wavelength (the maximum of absorption in the spectrum of the initial component, RA) and the calculated values of the function F(A(t),y).

The consideration of function F(A(t),y) is useful in investigation of the conversion dependent of the irradiation doze. Admitting the hypothesis of a "monomolecular" type process

$$RA \rightarrow P$$

the derivative of the time-dependent concentration of retinol acetate [RA] is proportional to the elapsed irradiation time.

$$-\frac{d[RA]}{dt} = k\cdot[RA] \quad (2)$$

The constant "k" in the expression 2 is similar to the velocity constant in an elementary monomolecular reaction.

The value of constant "k" represents a measure of the photoinduced transformation tendency of the investigated composite in given conditions of temperature, environment (solvent, pH etc.) and at the spectral distribution of the irradiation source. Theoretically, the constant "k" is not dependent of the initial concentration of the reactant, nor of its momentary conversion.

The particular solution of the differential equation (2) has the form (3), where "C" is the initial concentration (correlated with the zero doze of irradiation) of the retinol acetate.

$$\frac{[RA]}{C} = e^{-k\cdot t} \quad (3)$$

The quotient of the absorptions associated to the irradiation time "t" to initial absorption (t=0) is: (4) and (5)

$$\frac{A(\lambda,t)}{A(\lambda,0)} = \frac{\varepsilon_{AR}\cdot[RA]+\varepsilon_P\cdot[P]}{\varepsilon_{RA}\cdot C} = \frac{\varepsilon_{RA}\cdot[RA]+\varepsilon_P\cdot(C-[RA])}{\varepsilon_{RA}\cdot C} \quad (4)$$

$$\frac{A(\lambda,t)}{A(\lambda,0)} = \frac{[RA]}{C}\cdot\left(1-\frac{\varepsilon_P}{\varepsilon_{AA}}\right)+\frac{\varepsilon_P}{\varepsilon_{RA}} = \frac{[RA]}{C}\cdot(1-y)+y \quad (5)$$

The "y" parameter in (5) is the quotient of the molar absorption (ε) of the reaction product (P) to the starting material (RA). Applying the above argument to processing the kinetic data in the general case, this quotient is not known "*a priori*" because the chemical identity of the product usually is unknown. For this reason, the expression (5) is linearized according to the velocity constant "k".

Table 2. Time Evolution of Absorbance and of Function F(A(t),y)

Irradiation Time (mins)	Absorbance 327 nm d = 1 cm	Function F(A(t),y)	Irradiation Time (mins)	Absorbance 327 nm d = 1 cm	Function F(A(t),y)
0,0	1,1474	0	6,0	0,5815	-2,026170
0,5	1,0254	-0,207225	7,0	0,5454	-2,571019
1,0	0,9405	-0,381861	8,5	0,5043	-4,312121
1,5	0,8664	-0,564031	10,0	0,4669	--
2,0	0,8106	-0,727103	11,5	0,4346	--
2,5	0,7682	-0,871651	13,5	0,4011	--
3,0	0,7321	-1,013687	16,0	0,3652	--
4,0	0,6705	-1,315365	19,5	0,3265	--
5,0	0,6238	-1,625908			

$$\frac{A(\lambda,t)}{A(\lambda,0)} = e^{-k\cdot t}\cdot(1-y)+y \qquad (6)$$

$$F(A(\lambda,t)) = \ln\frac{\frac{A(\lambda,t)}{A(\lambda,0)}}{1-y} = -k\cdot t$$

If there is a proper value "y", compatible with the problem's data, for which the linear relation (6) between the function F(A(λ,t)) and the variable "t" is valid, the monomolecular assumption (2) is confirmed, and the concrete value of the constant "k" can be determined. In the particular case of the system considered, the value y = 0.4317 assure a linear relation between the function F(A(λ,t)) and the variable "t"; this relationship is presented in Fig. (**4**).

The linearity is assured by the above mentioned value of the parameter "y", in conformity with the correlation coefficient R^2 = 0.99776. The absolute value of the slope (0.32497 min^{-1}) is equal to the constant "k" that quantitatively expresses the phototransformation tendency of the retinol acetate in an environment of absolute ethanol, under the action of a source emitting in the 300 – 400 nm spectral range, at 30 ^oC.

The correlation coefficient has a maximum (the only one) for the optimum value of the parameter "y". As resulting from Fig. (**5**), the selected value for the parameter "y" is indeed the optimum one among the tested values.

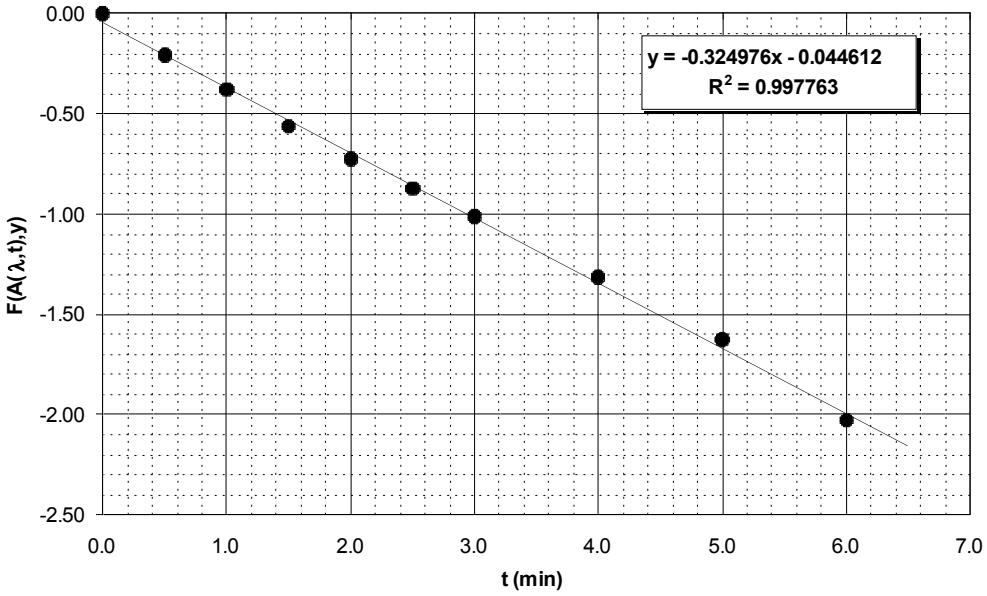

Fig. (4). The linear relationship F(A(t),y) *vs* time of irradiation.

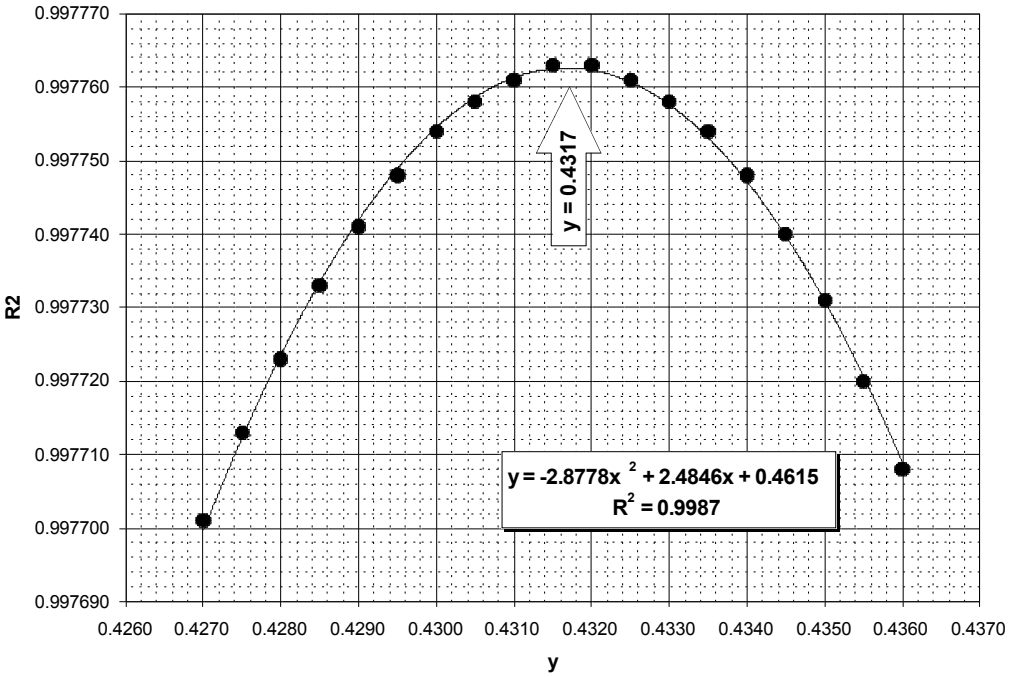

Fig. (5). Selection of the optimal value for parameter "y".

4. CONCLUSIONS

The characterization of photodegradation tendency of drugs imposes a strict standardization of an important number of experimental details. The experiments described in the literature, meant to express quantitatively the intrinsic photosensitivity of composites with pharmaceutical interest, usually provide results not comparable, due to the variety of the realization conditions. Investigating the photosensitivity using a self designed reactor with continuous flow, most of the usual errors may be avoided or minimized. Following the phototransformation process of retinol acetate in a continuous flow regime and interpreting the results according to a monomolecular kinetic model – confirmed "*a posteriori*" – provides the determination of a proper measure ("k") to express the photosensitivity quantitatively. The described technique can be generalized for characterization of other photosensitive substances as well.

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