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A New Fluorescent Assay for Enalapril Maleate

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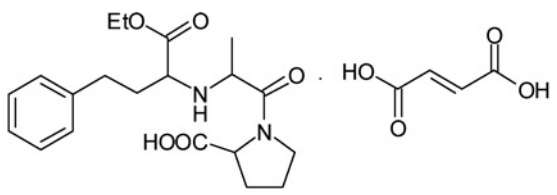
A new spectrofluorimetric method for the enalapril maleate monitoring was studied. Enalapril maleate was found to be highly photolabile. This drug was evaluated according to photodegradation assay at pH 2.5 and 6. Enalapril maleate was exposed to UVA–UVB radiations. Under these specific conditions was found as degradation product, the diketopiperazine. The modification of the fluorescent properties of enalapril maleate in solution after exposure UV-radiation and the degradation mechanisms were studied. The photodegradation was followed by the developed spectrofluorimetric assay.

KEY WORDS: Enalapril maleate; photodegradation; diketopiperazine; fluorescence.

INTRODUCTION

Enalapril (ester of phosphinic acid) is a prodrug that it is not very active by itself and must be hydrolyzed instead (Scheme 1). The final result of this process is an original active dicarboxylic acid, the enalaprilate. This conversion is carried out by using seric esterases which is fastly absorbed even in presence of food and it is widely metabolized by the liver.



Scheme. 1.

This kind of drug is an inhibitor of the converter enzyme of angiotensin (IECA) which its action consists in to block competitively the conversion enzyme, decreasing plasmatic and tissular levels of angiotensin II (AT II) and aldosterone. Consequently, it can be observed a vasodilating arteriovenous action and a noradrenaline and vasopressin plasmatic levels decreasing [1].

The Enalapril is a widely used drug in clinical practice for various treatments such as arterial hypertension in all its grades, renin-dependent hypertension and congestive cardiac insufficiency. In this last disease, this substance improves the symptoms, decreases the mortality and diminishes the frequency of patient hospitalizations. Enalapril maleate presents for oral use in tablets of 2.5, 5, 10, and 20 mg. It is usually administrated in one or two daily doses. The solution for use IV can be employed in hypertensive emergencies or when it is not feasible its oral administration [1].

On the other hand, it can be observed that Enalapril is a very photosensitive drug and it degradates in an evident way through diketopiperazine by dehydration process and enalaprilate diacid by hydrolysis [2]. Photodegradation is sensitively higher in solution. These products of degradation increase with temperature and pH solution. Many analytical procedures have been proposed for enalapril maleate degradation research. It is relevant to mention some of those products such as NRM spectroscopy [3],

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HPLC [4–6], Isothermal FT-IR Microscopic System [7], and other.

The enalapril maleate is a very powerful drug at low therapeutic doses, which implies that a loss of the active drug by photodegradation will provoke a power diminution of the compound with devastating consequences for patients who are usually treated with this kind of drug and above all, for those who received the above-mentioned substance in a cardiac emergency. In consequence, it is considered in a special way the quality control in pharmaceutical formulations which contain this drug, so that is possible to guarantee the safety and the trust in this product.

The aim of this work has been to investigate the kinetic degradation process and the modification of enalapril maleate fluorescent properties in solution after being exposed to ultraviolet light, using a spectrofluorimetric method which allows monitoring the photodegradation of the drug.

EXPERIMENTAL

Apparatus

All fluorescence measurements were made on a Shimadzu RF-5301 PC spectrofluorophotometer with excitation and emission band pass of 5 nm using 1.0-cm quartz cells.

A Beckman DU 520 UV–visible spectrometer with quartz cells of 10-mm path length for absorptiometric measurements was used.

pH values of solutions were measured with a pH-meter Orion 701-A with Ag/AgCl electrode.

The experiments were performed with a Beckman HPLC system coupled with PC-IBM compatible computer which consisted of a solvent delivery module (model Beckman Gold 126), a diode array UV/vis detector (model Beckman Gold 168) and a Gilson Rheodyne injecting valve fitted with a 20-mL sample loop.

Data acquisition was performed with the System Gold Software. The absorption wavelengths were set at 254 nm for detecting drug components. A Beckman 5 mm Ultrasphere® (Phenomenex, CA) C-18 column 250 × 4.6 mm, commercially available was used in all experiments. The mobile phase consists in mixture of pH 7 monobasic sodium phosphate Buffer-Acetonitrile (90:10).

Other chromatographic conditions included 0.9 mL/min flow-rate and ambient column temperature.

The water used in all studies was ultrapure water (18 MW cm) obtained from a Barnstead Easy Pure RF compact ultrapure water system.

The used lamp to carry out photodegradation studies is a Philips GL 15 W of 30 cm of length.

NMR spectra were obtained using a Bruker AC-200 Spectrometer.

Reagents

The enalapril maleate (with a 99.6% of purity according to Analysis Certificate of Raw Material) was supplied by Bagó Lab, Argentina.

The phosphate buffer was prepared dissolving 1.4 g phosphate monobasic of sodium in 400 mL of Milli-Q water. To this preparation was added a sufficient NAOH volume (1 M) to adjust pH to 7, it was completed the final volume of 500 mL with Milli-Q water and it was filtrated.

Solutions were prepared with bidistilled water. All solvents used were HPLC grade.

Preparation of the Standard Solution

An accurate mass 24.62 mg of weighted enalapril maleate (The Molecular Weight is 492.5 g/mol). The drug was dissolved in 40 mL of bidistilled water and mixture was agitated. It was filtered to remove insoluble materials. The volume of the filtrate was then up to 50 mL.

Thus were obtained standard solutions of the concentration 492.4 μg mL^{-1}.

Working standard enalapril maleate solution was obtained by dilution with the same solvent and phosphate buffer to adjust pH 2.5 and 6 respectively to give final concentration of 1×10^{-8} M.

Five solutions of 4 ng mL^{-1} were directly exposed to an ultraviolet light at 5 cm of distance for the photodegradation study.

Construction of Calibration Graph

A set of standard solutions of Enalapril were dissolved to obtain a range of concentrations of 4–0.5 μg mL^{-1} and then were exposed in a direct way to ultraviolet light for 10 min.

Procedure for Commercial Tablets

Ten tablets were weighted and powdered. An accurately weighted mass of powder equivalent to 24.62 mg of enalapril maleato was transferred into a 50-mL volumetric flask. The drug was dissolved in 40 mL of bidistilled water and mixture was agitated. It was filtered to remove insoluble materials. The volume of the filtrate was then up to 50 mL. Working enalapril maleate solution was obtained by dilution with the same solvent and phosphate buffer to adjust pH 6 to give final concentration of 1×10^{-8} M.

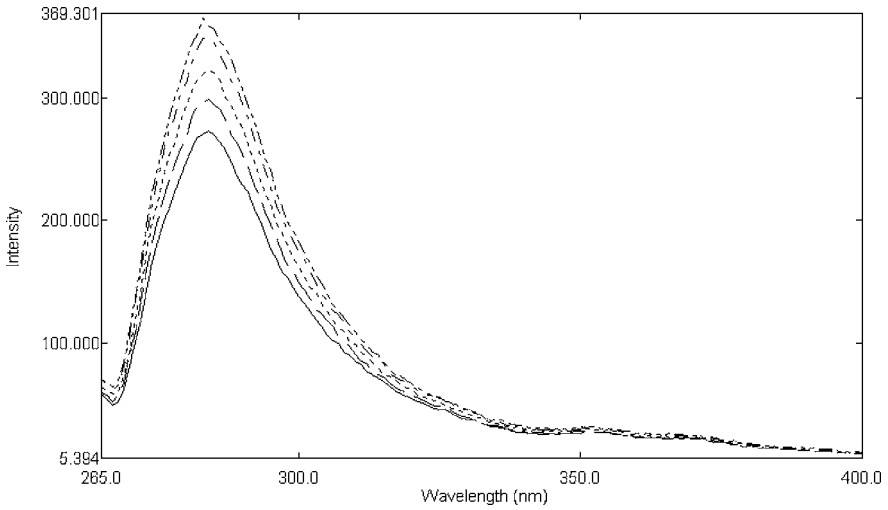


Fig. 1. Increasing of enalapril maleate fluorescence (1×10^{-8} M) in function of time exposition to direct ultraviolet light. Time exposure to UV light 5 min (——); Time exposure to UV light 10 min (— —); Time exposure to UV light 15 min (- - - - -); Time exposure to UV light 20 min (— – —); Time exposure to UV light 25 min (— – – —).

After, these samples were exposed to ultraviolet light for 10 min.

The nominal content of the tablets was calculated either from a previously plotted calibration graph or using the regression equation.

PHOTODEGRADATION STUDIES

All the samples were exposed to UV light abovementioned for a total time period of 60 min at ambient temperature carrying out spectrofluorimetric and spectrophotometric measures each 5 min.

The enalapril maleate degradation produces an increasing of fluorescent signal. The kinetic study reveals a reaction of first order with reference to drug concentration. The velocity constant of degradation reaction (k) is obtained according to the following equation:

$$\ln F/F_o = kt$$

F and F_o is fluorescent signal at 284 nm ($\lambda_{exc} = 260$ nm) of time t and $t = 0$ respectively.

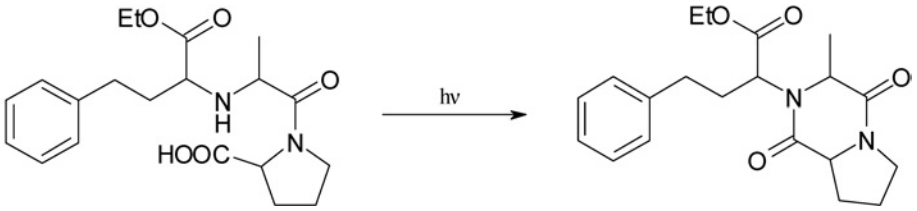
RESULTS AND DISCUSSIONS

The photodegradation of enalapril maleate originates an increasing of fluorescence intensity due to the formation of diketopiperazinic derivated (Fig. 1). This substance is obtained by intramolecular cyclization of enalapril maleate (Scheme 2). This product of degradation can also be found in solid phase.

Photodegradation is an autocatalytic reaction with kinetics of first order with reference to drug concentration and data of linear regression corresponding to fluorescent signal increase in function of time are shown in Table I and in Fig. 2.

Data obtained using the spectrophotometer UV–Vis show that do not exist significant differences after drug exposition at ultraviolet light. However, it can be observed a notable increasing of fluorescent emission of signal at 284 nm after being excited at 260 nm. It is evident the difference between the two methodologies at the moment to monitoring the degradation process of enalapril maleate.

This increase of fluorescent signal is due to folded conformation that is adopted by diketopiperazine in



Scheme. 2.

Table I. The Linear Regression Data Corresponding to the Increasing of Fluorescent Signal (Expressed as log F) in Function of Time

($n = 5$)	
In F_o	2.5048
Error	0.00741
k	0.00726
Error	0.00079243
R	0.9883
SD	0.0886
T_{90} (min)	14.46

solution or in solid phase. This last one is highly favored according to energy matter due to interaction between aromatic ring and diketopiperazinic ring. These results were obtained through calculation of molecular modeling [3] (Scheme 3).

Interaction between both rings originates an inflexibility system increasing; therefore, it can be observed an energy decreasing through rotational and vibrational relaxation and an increasing of efficiency quantum fluorescence.

The loss of this active drug for the most of the medicaments represents the principal source of instability. If the concentration of the active principle is less than 90% it can be considered that quality of this product is not acceptable. Therefore, it was calculated starting from kinetics equation, the t_{90}, that is, the necessary time to obtain a 10% of the active principle decomposition (Table I).

The remainder enalapril maleate concentration after induced photodegradation process, was calculated theo-

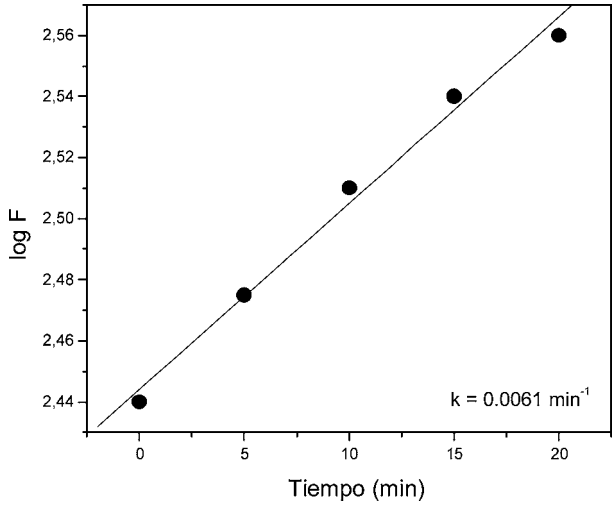
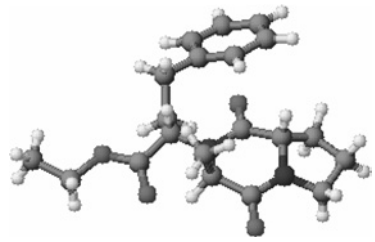


Fig. 2. Representation of the equation $F/F_o = kt$ corresponding to the determination of the order of reaction of the enalapril maleate photodegradation.



Scheme. 3.

retically according to the following integrate equation

$$\log C = \log C_o - kt/2.303$$

After 10 min of direct ultraviolet light exposition was calculated that 93% of the active principle must be remaining unaltered.

The relative fluorescence intensity was measured at $\lambda_{emi} = 284$ nm with $\lambda_{exc} = 260$ nm and plotted against the concentration of enalapril maleate to obtain the standard calibration graph.

Thus, it was calculated the remainder enalapril maleate concentration after photodegradation using the adequate calibration curve of the solution pattern and the analyzed pharmaceutical formulations (Table II)

The calibration graph is described for the following equation, $y = 56712 + 10107x$, where y is the fluorescence intensity and x is the drug concentration (Fig. 3).

The value of 56712 corresponds to the fluorescence intensity of enalapril maleate without previous exposure to UV light.

The degradation product was extracted from standard solution with ethyl acetate.

After extracting, the solvent was evaporated at reduce pressure and low temperature.

This extract was used to carry out spectrocospic studies (NMR ^{1}H y NMR ^{13}C) which allowed to characterize and identify enalapril maleate degradation product.

NMR spectra for degradation product of enalapril maleate showed a disappearance of the peak at 11 ppm indicating the loss of the carboxyl group.

The peak observed at 1.5 ppm was not present in the spectrum of the enalapril maleate this peak may be assigned to a methyl group.

^{13}C NMR for the degradation product indicated the presence of 20 unequivalent carbon atoms.

VALIDATION

Linearity and Range

Linearity and range of the method were performed by analyzing 12 different concentrations ($n = 5$) of de

Table II. Enalapril Maleate (%) Found in Pharmaceutical Formulations Using the Proposed Method

Number of samples	Enalapril Maleate found after induced photodegradation		
	Lotrial 5® (Roemmers) Enalapril maleate 5 mg	Renitec® (MSD) Enalapril maleate 5 mg	Glioten 10® (BAGÒ) Enalapril maleate 10 mg
1	91.6	92.7	92.5
2	92.6	93.4	91.1
3	90.9	90.8	94.3
4	90.6	91.5	92.2
5	94.1	93.4	91.6
$X \pm$ SD	91.96 ± 1.42	92.36 ± 1.17	92.34 ± 1.22

standard solution contained 0.0005–0.7 μg mL^{-1}. The calibration curve was plotted using relative fluorescence intensity versus concentration of the standard solutions.

Calibration curve was found to be linear over the concentration range 0.004–0.5 μg mL^{-1}.

Repeatability and Reproducibility

The mean of the relative fluorescence intensity of five separate samples solutions of the commercial tablets of the enalapril maleate of the same batch number gave relative standard deviation minor than 0.0157.

This level of precision is suitable for the routine quality control analysis pharmaceutical dosage forms.

Stability of Standard and Synthetic Enalapril Maleate Solutions

Stability of enalapril maleate in standard solutions and in matrix used for tablets was investigated. After storage of 72 h, the variations of enalapril maleate concentration were in all cases less than 1%. This indicates that enalapril maleate solutions (standard or synthetic matrices) are stable within 72 h (previously without fluorescent light exposition) and can be used without having any significant effects on the results.

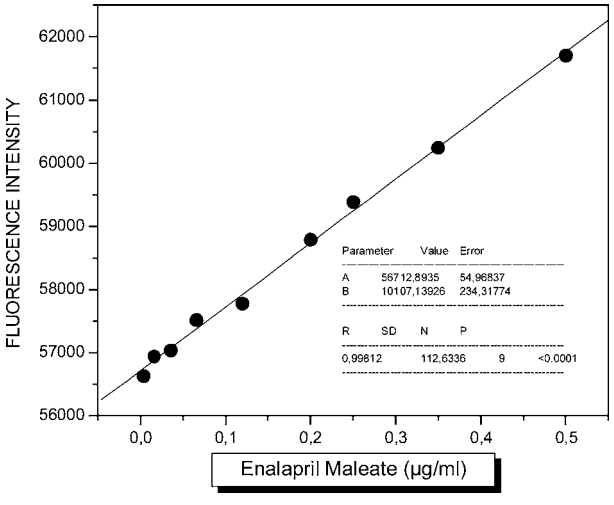


Fig. 3. Calibration curve to the determination of enalapril maleate concentration and its degradation product in pharmaceutical formulations.

Selectivity

The selectivity of the method was investigated by observing interferences between enalapril maleate, degradation products and excipients.

Reference Method

Aiming at the evaluation of the accuracy of the results obtained with developed procedure, enalapril maleate bulk drug was analyzed by HPLC according to the *United States Pharmacopoeia*. Since all of the pharmaceutical preparations analyzed containing enalapril maleate, the reference procedure used was the chromatographic liquid method with UV detection referred in the *United States Pharmacopoeia* for the tablets containing this drug (Table III).

Preliminary HPLC runs for enalapril maleate form, after being exposed at ultraviolet light, showed two chromatographic peaks. The first chromatographic peak eluting at 3.1 min is enalapril maleate. The second chromatographic peak eluting at 3.6 min was attributed of the degradation product mentioned.

The peak intensity of the degradation product increases in a proportional way compared with the exposition time at the ultraviolet lamp. Consequently, it is possible to observe a diminution of the peak of enalapril maleate.

The spectrophotometric detector was set to 260 nm since enalapril maleate and its degradation product show a maximum absorbance at this wavelength.

Table III. Enalapril Maleate (%) Found in Pharmaceutical Formulations Using HPLC

	Enalapril Maleate found after induced photodegradation		
Number of samples	Lotrial 5® (Roemmers) Enalapril maleate 5 mg	Renitec® (MSD) Enalapril maleate 5 mg	Glioten 10® (BAGÒ Enalapril maleate 10 mg
1	93.7	92.8	89.6
2	93	92.1	93.2
3	91.1	89.4	91.8
4	90.6	90	94.85
5	90.3	91.9	91.9
$X \pm$ SD	91.74 ± 1.52	91.24 ± 1.46	92.27 ± 1.94

CONCLUSION

The work reported here demonstrates that enalapril maleate fluorescence can be an effective way for determinate the accurate concentration of the drug and the degradation product after induced degradation. The aim here was to test the applicability of the method, specifically in relation to some commercially available pharmaceutical preparations. These samples were treated and analyzed as described in the *Procedure of Commercial Tablets* section.

The proposed method in this work has been satisfactorily applied to pharmaceutical preparations and describes a simple, fast, economical, precise, sensitive and selective technique for monitoring photodegradation of enalapril maleate in pharmaceutical formulations. The assays were carried out as described under the procedure for pharmaceuticals and they were good agreement with values for the nominal contents of enalapril maleate calculated experimental and theoretically.

The results obtained do not differ of those obtained through official methods and are summarized in Table III. This situation suggests a well precision of the proposed method.

ACKNOWLEDGMENT

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