Photocatalytic (UV-A/TiO$_2$) degradation and photolytic (UV-A) degradation of steroid hormones: norethisterone and danazol in aqueous medium

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Abstract: In this paper, the photocatalytic (UV-A/TiO$_2$) degradation of two steroid hormones: norethisterone and danazol in aqueous medium has been studied using immobilized titanium dioxide as photocatalyst. The photolytic degradation (degradation without photocatalyst) of the two hormones was also investigated. UV-A irradiation was provided by black light lamp 18 W. The photocatalytic and photolytic degradation of investigated steroid hormones demonstrated first-order kinetics. The effect of H$_2$O$_2$ has been investigated. It was found that the high concentrations of H$_2$O$_2$ had an adverse effect on photocatalytic reaction, presumably due to excessive oxidant scavenging radicals and other reactive species. Results clearly demonstrate the efficiency of the photocatalytic and photolytic degradation in the elimination of norethisterone and danazol in aqueous medium.
Key Words: Norethisterone, Danazol, Photocatalytic degradation, Photolytic degradation, Steroid hormones, TiO$_2$.

1. Introduction

Endocrine disrupting compounds (EDCs) constitute an important class of emerging environmental contaminants, which pose an increasing threat to aquatic organisms, as well as to human health [1]. EDCs, which include natural estrogens, synthetic estrogens, phyto-estrogens and various industrial chemicals (i.e. pesticides, persistent, alkyl phenols, heavy metals), have the ability to interact with the endocrine system of the organisms, thus leading to a variety of developmental and reproductive disorders, as well as feminizing effects [1]. Steroid hormones have the greatest endocrine-disrupting potency among all endocrine disrupting compounds [2]. Laboratory and field studies have provided ample evidence of the effects of endocrine disruption on various species, including fish, amphibians, birds, reptiles, and mammals. Several studies also suggest a link between human exposure to EDCs and decrease in male sperm counts; increases in testicular, prostate, ovarian, and breast cancer; and reproductive malfunctions [2].

Hormones are ubiquitous in most fresh water bodies receiving effluent. They are frequently detected in North America, Europe, Japan, Brazil and China [3]. This is due to the reason that the conventional sewage treatment plants are not able to degrade residues of these chemicals, and as a result they are introduced into the aquatic environment [4]. During the past years many investigations on chemical and biological technologies have been reported for the decomposition of organic pollutants in aqueous matrices. In this context, various advanced oxidation processes (AOPs) have been successfully employed for the degradation of a wide range of

organic pollutants in water and wastewater. Among the various AOPs, heterogeneous semiconductor photocatalysis using TiO_2 as the photocatalyst has been found capable of achieving complete oxidation of the organic pollutants via hydroxyl radicals $HO^.$ and/or valence band holes h^+ generated when the semiconductor is exposed to UV irradiation [5]. TiO_2 is cheap, commercially available in various crystalline forms and particle characteristics, non-toxic and photochemically stable. Moreover, TiO_2 photocatalysis works at ambient conditions and may be induced by solar irradiation [6,7].

In this study, immobilized TiO_2 photocatalytic films were utilized for the degradation of two steroid hormones: norethisterone and danazol (Figure 1) in aqueous medium as an alternative to TiO_2 nanoparticles. This technology may be more efficient since the immobilization of the catalyst on a substrate (i.e. glass, stainless steel and ceramic) will eliminate the need for post-treatment removal of the excess turbidity caused by the nanoparticles and can also counteract the scattering of UV-light caused by the nanoparticles [8]. In addition, the photolytic degradation (degradation without photocatalyst) of the two hormones was also investigated in this study.
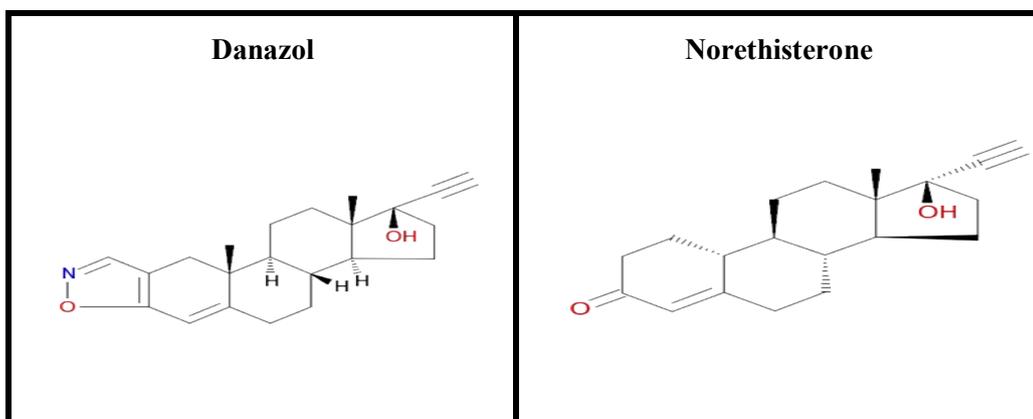
Figure 1: Chemical structures of Norethisterone and Danazol

2. Materials and Methods

2.1. Chemicals

Norethisterone was purchased from Chem Swiss, Switzerland, danazol was purchased from Sigma-Aldrich and both of them used as received. Hydrogen peroxide (35% w/w solution) was purchased from Merck. Purified water was used for the preparation of solutions. The titanium dioxide (Degussa P25) was mainly anatase according to the manufacturer's specifications [9]. The elementary particle in dry powder form was approximately spherical in shape and the particle size was approximately 20 nm. The specific surface area, as measured from N_2 adsorption at 77 K, was 48.3 $m^2 g^{-1}$ [10], in agreement with the manufacturer's specification [9].

2.2. Photocatalytic and Photolytic Experiments

Photocatalytic experiments with artificial irradiation were performed in a lab-scale photochemical reactor (Figure 2) where the polluted solution is recycled over a glass slide (48.0 cm ×3.8 cm) on which TiO_2 particles have been deposited and fixed. The photocatalyst activation was provided by a UV lamp emitting at 365 nm (UV-A irradiation) (Nissan Black Light 18 W, UV Ray 1.5 W). Photocatalytic experiments were carried out as follows: 1.8 L of an aqueous solution containing (1mg/L) of the steroid hormone were recycled in the photochemical reactor with a flow rate of 300 mL/min. The recycling was performed first in the dark from 60 to 120 minutes (until the adsorption-desorption equilibrium was reached) and then the lamp was turned on for 5 hours. The pH of polluted solution was adjusted at 4.1. Photolytic experiments were carried out as follows: 1.8 L of an aqueous solution containing (1mg/L) of the steroid hormone were recycled in the photochemical reactor with a flow rate of 300 mL/min without photocatalyst. The recycling was performed for 5 hours in the presence of UV-A irradiation. The pH of polluted solution was adjusted at 4.1.
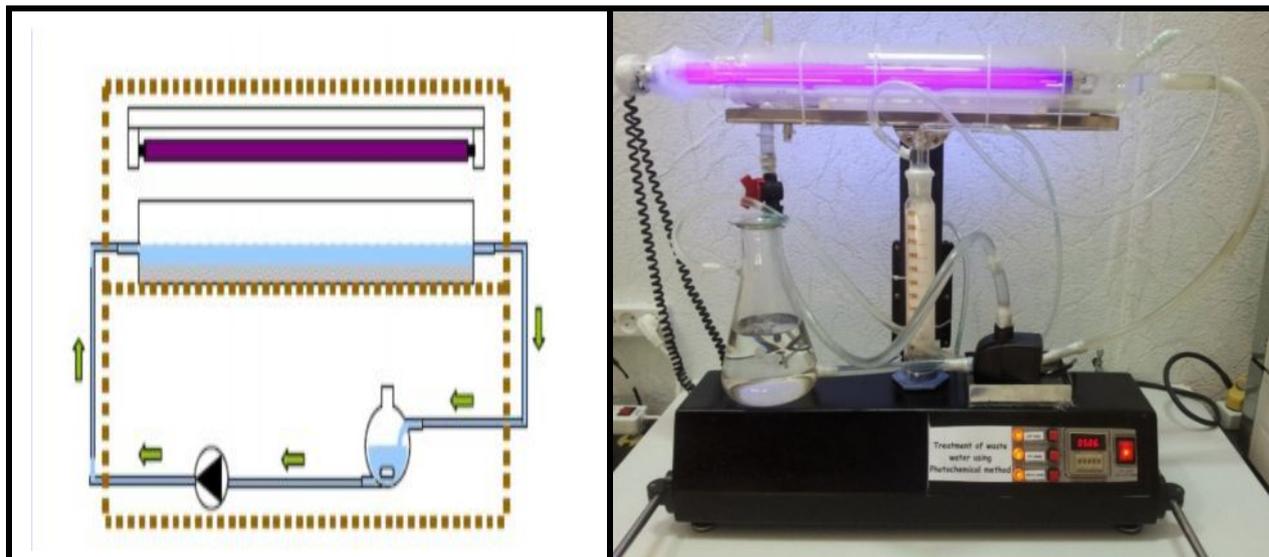
Figure 2: The lab-scale photochemical reactor

2.3. Analytical Procedures

High performance liquid chromatography (HPLC/UV-DAD, LA Chrom ELITE, VWR Hitachi, Germany) was employed to monitor the concentrations of norethisterone and danazol during the photocatalytic and photolytic reactions at specific time intervals. Separation was achieved on Thermo Hypersil C18 column (150 mm, 4.6mm i.d., 5μm). The mobile phase consisting of 50:50 UPW:acetonitrile for the analysis of norethisterone, and 40:60 UPW:acetonitrile for the analysis of danazol, eluted isocratically at 1 mL/min and 40 °C, while the injection volume was 50 μL. The detection wavelengths were 240 and 285 nm for the analysis of norethisterone and danazol respectively. At these conditions, the limits of detection and quantitation were 6.0 and 20 μg/L for norethisterone, and 6.6 and 22 μg/L for danazol.

3. Results and Discussion

### 3.1. Results of the Photocatalytic Degradation

The results of the photocatalytic degradation of norethisterone and danazol were summarized in (Table 1). Where **A%**: is the adsorption of the steroid hormone on the surface of the photocatalyst, as percentage, **X_{300}%**: is the degradation of the steroid hormone after 5 hours (300 minutes) of the photocatalytic reaction, as percentage. These factors were calculated by the following formulas:

$$A\% = [(C_i-C_0)/C_i] \times 100$$

$$X_{300}\% = [(C_0-C_{300})/C_0] \times 100$$

In which: C_i is the initial concentration of the steroid hormone, C_0 is the equilibrium concentration of the steroid hormone when the adsorption-desorption equilibrium is reached, and C_{300} is the concentration of the steroid hormone after 5 hours (300 minutes) of the photocatalytic reaction. As clearly seen, the danazol degradation was 93.86%, while the norethisterone degradation was 53.24%. Plotting ln (C_0/C) versus time corresponds to a straight line and the slope equals the apparent first-order constant, thus implying that the degradation kinetics of these steroid hormones perfectly fit first order kinetics (Figure 3).

In further experiments, the effect of addition of various concentrations of H_2O_2 (3-25 mM) on norethisterone photocatalytic degradation was studied and the results are shown in (Table 2 and Figure 4). In general, H_2O_2 is expected to promote degradation since it may react with conduction band electrons and the superoxide radical anion to yield hydroxyl radicals and anions as follows [11]:

$$H_2O_2 + e^- \rightarrow OH^- + OH^\bullet \qquad (1)$$

$$H_2O_2 + O_2^{\bullet-} \rightarrow OH^- + OH^\bullet + O_2 \qquad (2)$$
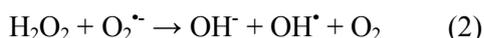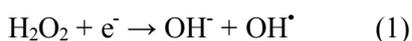
Reactions (1) and (2) enhance reactivity due to (i) the formation of additional oxidizing species and (ii) the suppression of e^-/h^+ recombination, a major cause of low TiO_2 photocatalytic quantum yield. Nonetheless, it is well documented that there is an optimum H_2O_2 concentration above which H_2O_2 acts as electron and radical scavenger, thus leading to reduced degradation [11]. This behavior is clearly demonstrated in (Table 2 and Figure 4), where the norethisterone degradation increases with increasing oxidant concentration up to 8 mM, above which scavenging effects become important.

In addition, (Figure 5) shows that there is no effect of the addition of H_2O_2 on kinetics of norethisterone photocatalytic degradation, where the norethisterone photocatalytic degradation demonstrated first-order kinetics in the various concentrations of H_2O_2.

Table 1: The results of the photocatalytic degradation of norethisterone and danazol
(without any addition of H_2O_2)

Parameter	Norethisterone	Danazol
kinetics	first-order kinetics	first-order kinetics
A%	10.18	64.26
Adsorption-desorption equilibrium time	1 hour	2 hours
K_{deg} (min^{-1})	0.0025	0.0095
X_{300}%	53.24	93.86*

* according to LOQ.
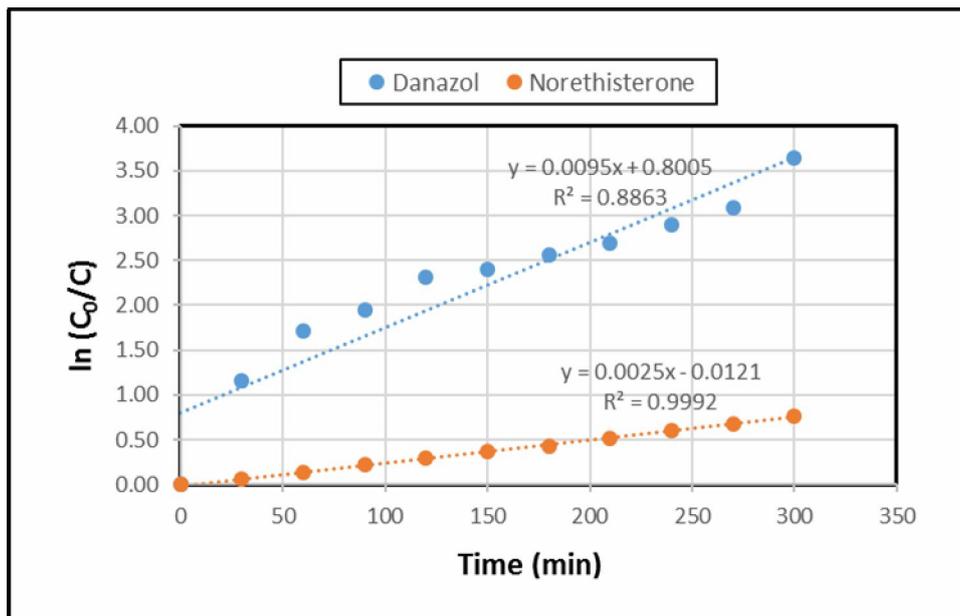
Figure 3: Kinetics of the photocatalytic degradation of norethisterone and danazol
(without any addition of H_2O_2)

Table 2: Effect of H_2O_2 addition on norethisterone photocatalytic degradation

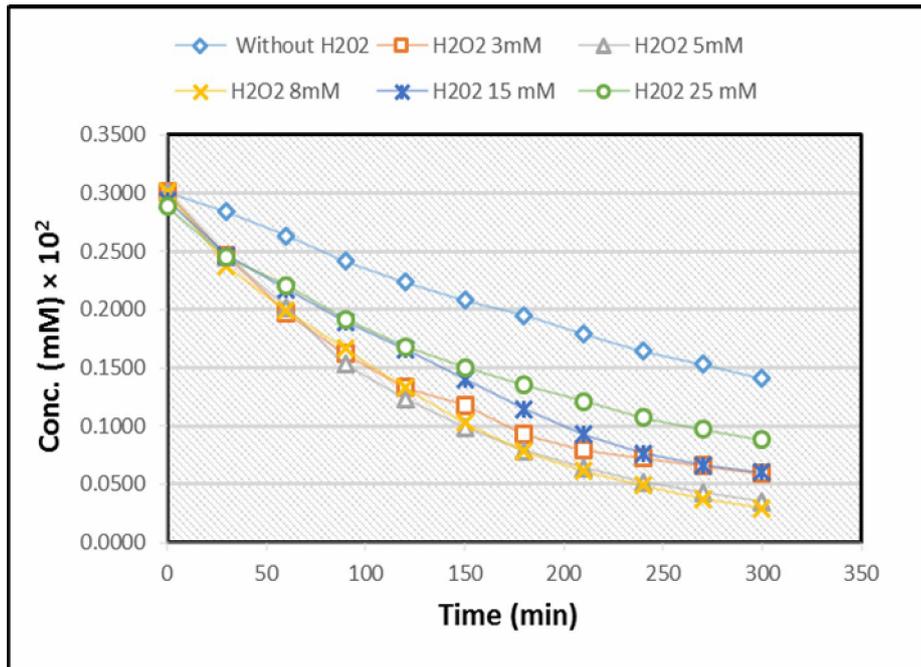| C_{H2O2} (mM)	0	3	5	8	15	25
K_{deg} (min^{-1})	0.0025	0.0055	0.0072	0.0078	0.0054	0.0039
X_{300}%	53.24	80.40	88.44	90.18	79.50	69.41
kinetics	first-order kinetics					

Figure 4: Effect of H_2O_2 addition on norethisterone photocatalytic degradation
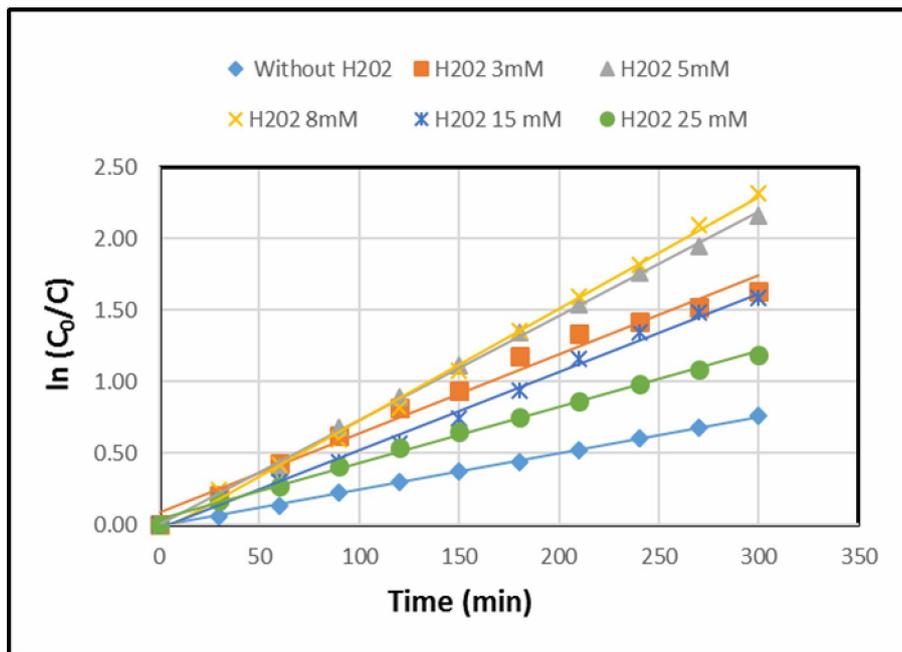

Figure 5: Kinetics of the photocatalytic degradation of norethisterone in the presence of H_2O_2

3.2. Results of the Photolytic Degradation

The results of the photolytic degradation of norethisterone and danazol were summarized in (table 3). Where $X_{300}\%$: is the degradation of the steroid hormone after 5 hours (300 minutes) of the photolytic reaction, as percentage. It was calculated by the following formula:

$$X_{300}\% = [(C_i-C_{300})/C_i] \times 100$$

In which: C_i is the initial concentration of the steroid hormone, and C_{300} is the concentration of the steroid hormone after 5 hours (300 minutes) of the photolytic reaction.

As clearly seen, there is no effect of the photocatalyst on the degradation of danazol. Also the results indicated that the photolytic degradation of norethisterone reached 96.85% in the presence of 0.3 M H_2O_2

without photocatalyst. The results indicated that the photolytic degradation of danazol and norethisterone demonstrated first-order kinetics in the various concentrations of H_2O_2 without photocatalyst.

The above photolytic degradation cannot be attributed to the direct absorption of UV-A light from the substrates, because the absorption spectra of norethisterone and danazol showed no absorption in the region 350–400 nm, where the UV-A lamp predominantly emits. Therefore, the observed photolytic degradation can only be attributed to photo-oxidation of substrates from the singlet oxygen generated photochemically from the oxygen dissolved in the reaction mixture [7].

To exclude the possibility that increased degradation in the presence of H_2O_2 is due to the traditional oxidation, norethisterone was subject to photolytic experiments in the presence of 0.3 M H_2O_2, with and without the UV-A irradiation. It was clear that the absence of the UV-A irradiation led to a significant reduction of the norethisterone degradation even in the presence of high concentration of H_2O_2. Results are shown in (Table 4).

(Figures 6 and 7) show the decrease of the areas of the chromatographic peaks of norethisterone and danazol during the photolytic reaction.

Table 3: The results of the photolytic degradation of norethisterone and danazol

| Parameter	Norethisterone				Danazol
C_{H2O2} (M)	0	0.1	0.2	0.3	0
kinetics	first-order kinetics				
K_{deg} (min^{-1})	0.0008	0.0026	0.0071	0.0119	0.0126
X_{300}%	23.40	54.59	87.63	96.85	97.80*

*according to LOQ.

Table 4: Effect of the UV-A irradiation on the photolytic degradation of norethisterone

	With UV-A irradiation	Without UV-A irradiation
C_{H2O2} (M)	0.3	0.3
X_{300}%	96.85	14.12
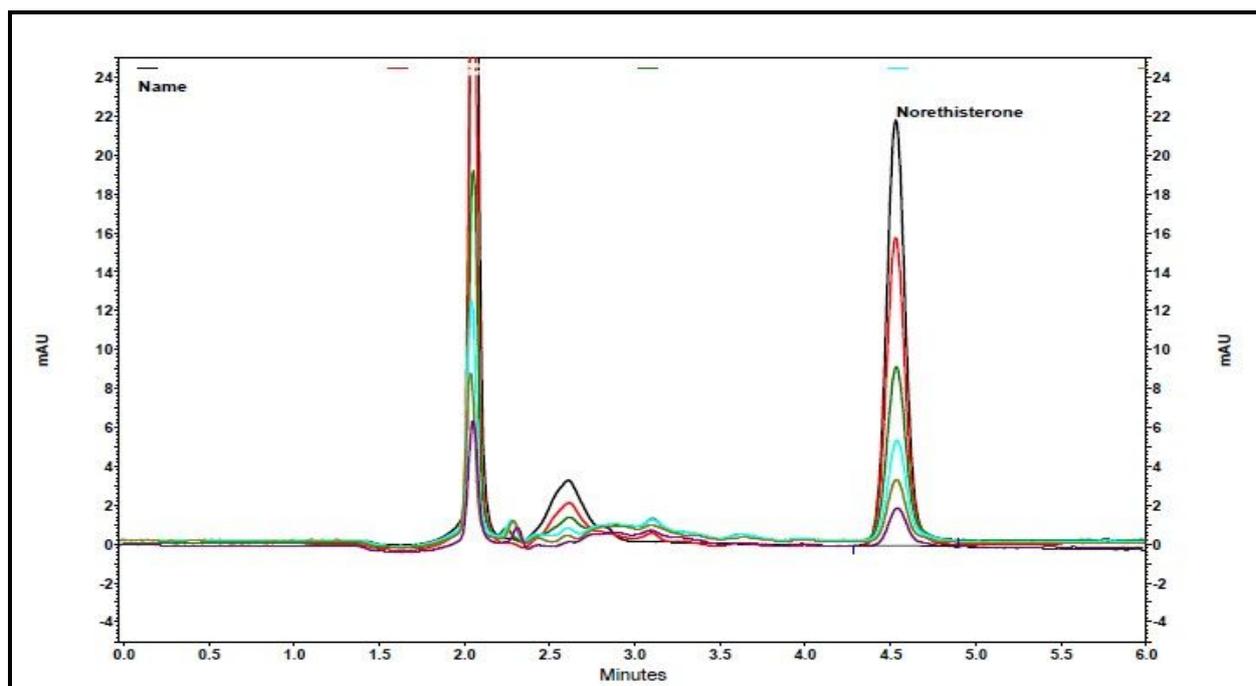
Figure 6: The decrease of the areas of the chromatographic peak of norethisterone
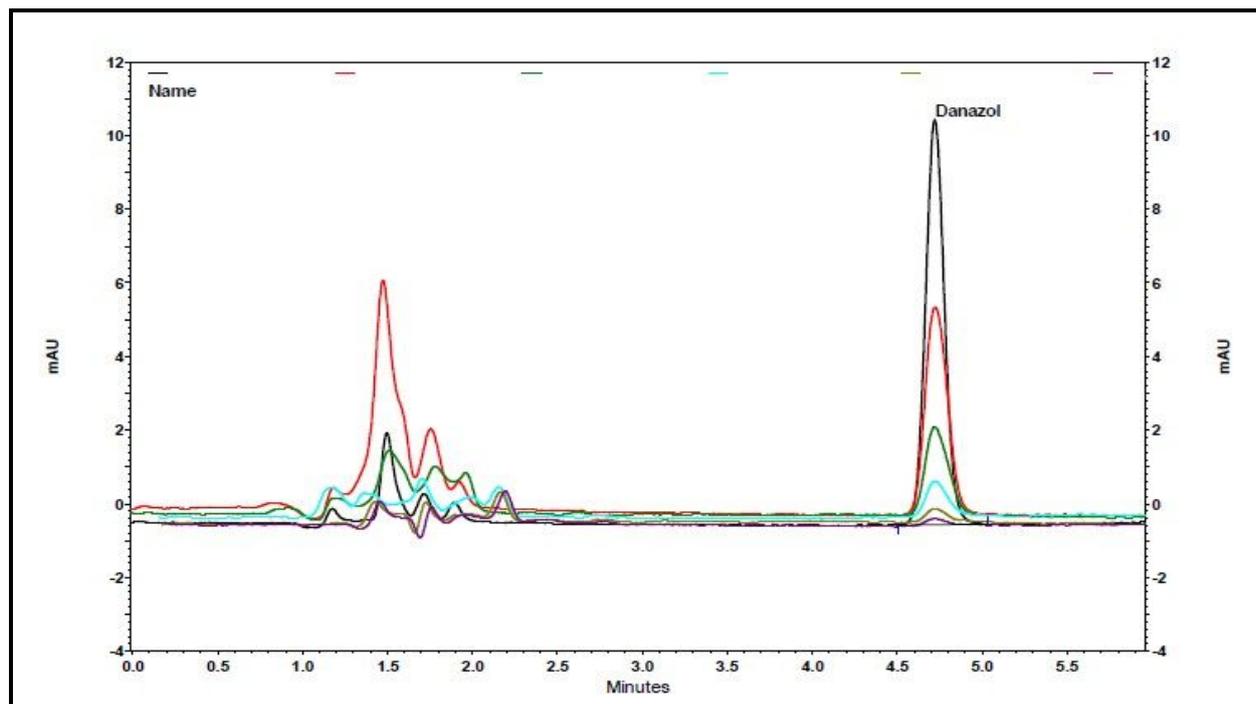
Figure 7: The decrease of the areas of the chromatographic peak of danazol

4. Conclusions

Degradation of norethisterone and danazol has been successfully conducted by photocatalytic process using immobilized TiO_2. The technique is promising as immobilization of the photocatalyst avoids the problem of recovery of the nanoparticles. In addition, the photolytic degradation of norethisterone and danazol has been studied. It has been found that the photolytic degradation was an effective technology for the elimination of norethisterone and danazol in aqueous medium.

5. Acknowledgements

6. References

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