Depression is classified as a mood disorder that manifests itself through a feeling of sadness, melancholy, and it is often accompanied by anxiety and irritability. This state of mind is characterized by slow thinking, lack of imagination, loss of memory and concentration capacity, the emergence of obsessive ideas (pessimistic and suicidal), as well as psychomotor disturbances, fatigue and restlessness. It requires special psychiatric and pharmaceutical treatment.

There are a wide variety of pharmaceutical substances used in the therapy of depression, one of the most important being the group of tricyclic antidepressants (TCAs). TCAs have been used since the early 50s to treat depression, as they were the first group of pharmaceutical substances that were synthesized for this specific purpose.

The basic structure of TCA is composed of a linear system consisting of three condensed cycles, which bind to a structure having a side chain N atom of basic character. Based on the basic structure, the classic tricyclic antidepressants can be divided into derivatives of dihydro-dibenzazepine, dibenzazepine, dibenzocycloheptadiene, dibenzoheptadiene, and dibenzothiophene.

In cases of clinical depression, the classic tricyclic compounds have antidepressive, sedative, anxiolytic or stimulant effects. Lately their effectiveness in neuropathic pain treatment has also been recognized.

TCAs and their metabolites act on a large number of receptors and their adverse effects are numerous, which is a major impediment in clinical use.

In order to induce photochemical skin reactions, the ultraviolet radiation must penetrate to the peripheral capillaries, so that the UV light reaches the absorbing molecule. After the absorption of light, the pharmaceutical substance can undergo various degradation reactions, which may include oxidation or structural rearrangement.

The photostability of pharmaceutical substances represents one of the most important aspects of drug research in the pharmaceutical field, which became an integral part of the studies that form the basis of the quality control protocol of a drug.
Due to structural particularities, the photostability of certain tricyclic antidepressants has been studied in the past (after UV irradiation) through chromatographic separation methods. The methods used were thin layer chromatography (TLC) and pressure liquid chromatography (PLC or HPLC).

For the chromatographic research of the photodegradation products of five frequently used tricyclic antidepressants, a TLC method has been developed, which allowed also a semiquantitative determination (photodensitometry) of the degradation. The best results were obtained using a mobile phase containing ethyl acetate: acetone: concentrated ammonia (80:20:5) and HPTLC GF254 silicagel plates.

As result of multiple studies, it was found that imipramine hydrochloride showed the highest stability against UV radiation, suffering a loss of only 4.31% during an irradiation period of 8 hours.

In contrast, the concentration of clomipramine hydrochloride decreased significantly at 71.56%, and after the irradiation at least nine degradation products were identified. The presence of the chlorine atom activates the dibenzazepine cycle of the clomipramine, which becomes more susceptible to external energy stimulus, in this case UV radiation, and undergoes significant decomposition. Since the chlorine atom activates the molecule, it also opens the way for the formation of several degradation products.

On the chromatogram of the amitriptyline hydrochloride very low intensity bands were observed. The amitriptyline molecule has a relatively good stability to irradiation with UV light. The degradation rate was moderate, approximately 9%.

Nortriptyline hydrochloride, similar to amitriptyline, has a relatively high stability, with a degradation rate of 9.39% after eight hours irradiation.

In UV light, doxepin hydrochloride had the most pronounced degradation, its concentration decreased by 90.67% after eight hours of irradiation. Nine degradation products were observed on the chromatoplate.

In the HPLC research, we used validated methods that allowed separation of photodegradation products from the parent substance. Based on preliminary analysis, the best separation was achieved using a phosphate buffer solution (20 mM KH₂PO₄ brought to pH −3 with phosphoric acid) and acetonitrile (ACN). The chromatographic separations were performed using a Zorbax Extend C18 column (150x3 mm, 3.5 μm), the separation temperature was 25°C with UV detection at 210 nm. The composition of the eluent was adjusted for each substance, to allow optimal separation of the degradation products and parent substances. The kinetic order was calculated for each substance, and proved to be of order 1.

In the case of imipramine hydrochloride, an isocratic separation method was applied, with a mobile phase containing 20 mM KH₂PO₄ and 5 mM of triethylamine, brought to pH - 3 with H₃PO₄ (solution A - 72%) and acetonitrile (solution B - 28%). The imipramine hydrochloride solution was found to be relatively stable during a 4 hour irradiation, the concentration decreased by 10.4%. Photodegradation resulted in several degradation products, out of which one product is dominant. The substance half-life was 1504 minutes (approximately 25 hours).

In the case of clomipramine hydrochloride, a gradient separation method was applied. The mobile phase contained 20 mM KH₂PO₄ solution and 5 mM triethylamine, brought to pH - 3 with H₃PO₄ (solution A) and acetonitrile (ACN) (solution B). The clomipramine hydrochloride concentration decreased by 75.8% after four hours of irradiation in UV light, which resulted in many degradation products. The calculated half-life was about 120 minutes.
For the HPLC analysis of the amitriptyline hydrochloride an isocratic separation method was used. The mobile phase contained 20 mM KH$_2$PO$_4$ solution and 5 mM triethylamine brought to pH - 3 with H$_3$PO$_4$ (solution A - 68%) and acetonitrile (solution B - 68%). After four hours of irradiation, the amitriptyline hydrochloride solution concentration decreased to 80.2% and at least five degradation products were observable. The substance half-life was 752 minutes (approximately 12 hours and 30 minutes).

In the case of the nortriptyline hydrochloride, the same isocratic separation method was used as in the case of the amitriptyline hydrochloride. The concentration of the irradiated solution decreased by 25.65% and the method resulted in six degradation products. The substance half-life was 601.8 minutes (approximately 10 hours).

In order to separate the doxepin hydrochloride from its photodegradation products, the used method was an isocratic separation, followed by washing of the column to remove degradation products. The mobile phase consisted of 20 mM KH$_2$PO$_4$ solution and 5 mM triethylamine brought to pH - 3 with H$_3$PO$_4$ (solution A), acetonitrile (solution B) and 50% tetrahydrofuran solution. Triethylamine and tetrahydrofuran were added to increase the separation capacity of the method. After four hours of irradiation, the concentration of doxepin hydrochloride decreased to 16.76%. Degradation products with polar and nonpolar character were observed. The substance half-life was 91.18 minutes (approximately 1 hour 30 minutes).

The difference between the TLC and HPLC research results can be explained taking into account the higher accuracy and sensitivity of the HPLC method. The difference between the order of degradation for amitriptyline and nortriptyline hydrochloride is explained by the fact that the HPLC method also resulted in the separation of degradation products, which migrated with the parent substance when the TLC method was used.

In order establish the phototoxicity on erythrocytes of the five TCAs, five mixtures were prepared: saline solution with erythrocytes, saline solution with irradiated erythrocytes, 100 μg/ml TCA solution with erythrocytes, 100 μg/ml TCA solution with irradiated erythrocytes, and 100 μg/ml solution photodegraded mixed with erythrocytes. To demonstrate the statistically significant differences, the DUNN non-parametric test was used.

The DUNN test showed no significant difference between the lysis of erythrocytes in saline solution and the solutions of the studied antidepressant substances and the TCA solutions with a concentration of 100 μg/ml, which were previously irradiated in UV light (Q values being below the critical values of 2.7996).

Irradiation of erythrocytes with UV light leads to hemolysis in saline solutions as well as the antidepressant substances studied, and the values obtained indicate a significant difference to irradiated solutions.

With regard to lysis of erythrocytes in the presence of saline solution and the studied substances, there was a statistically significant difference between the two levels of hemolysis. Lysis in the presence of the studied antidepressants indicated a higher significance level compared to the one in saline solution.

A difference between the effects of the studied antidepressant substances over the lysis of erythrocytes in the presence of UV light can be observed, lysis taking place in the following descending order: clomipramine hydrochloride, amitriptyline hydrochloride, nortriptyline hydrochloride, imipramine hydrochloride and doxepin hydrochloride.

The study demonstrates the phototoxic property of the studied substances under in vitro conditions on biological systems.
In conclusion, the methods developed and optimized by thin layer chromatography, liquid chromatography under pressure and the research of phototoxicity on erythrocytes can be successfully used in the analysis of TCA, having practical applications in the separation, purity control and stability research of these substances.