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PAPER

Studies on photodegradation of levomepromazine and olanzapine under simulated environmental conditions

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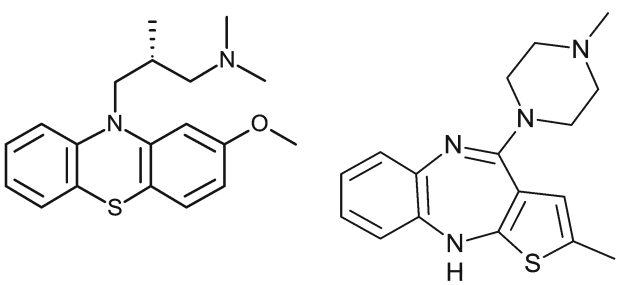
The present study discusses the influence of sunlight on the photostability of levomepromazine (LV) and olanzapine (OLA) hydrochlorides in river water. Four samples of water from different rivers were used in the research. In their course, it turned out that levomepromazine easily underwent photooxidation under simulated environmental conditions, resulting in the generation of its sulphoxide. Olanzapine, on the other hand, appeared to be more resistant to sunlight, as its photodecomposition proceeded slowly, and only one product of its decomposition was detected spectrophotometrically during the process. The photodegradation was analyzed in detail using principal component analysis (PCA) and multivariate curve resolution alternating least squares (MCR-ALS) chemometric methods, and the outcomes verified by HPLC and GC-MS analysis. It can be stated that the rates of the observed processes heavily depended on the chemical composition of the fresh water used in the experiments.

1. Introduction

Water quality is a crucial factor directly influencing the quality of human life.[1] Numerous studies have been devoted to assessing the quality of both drinking and surface water. Qualitative and quantitative results have shown that many classes of organic compounds used in everyday human activities reach the aqueous environment.[2–5] A group of these compounds which requires special attention of researchers includes pharmaceuticals.[6–10] Innumerable amounts of drugs are extensively used in human and veterinary medicine, as well as in agriculture and aquaculture. Pharmaceuticals, their metabolites and components, being excreted with urine and faeces,[11,12] enter municipal treatment systems, where they can be degraded, adsorbed into sewage sludge and eventually eluted into surface water. They diminish the activity of activated sludge in the water treatment plants[13] which results in incomplete decomposition of the organic matrix. Many of these compounds are partially or completely nonbiodegradable,[14,15] meaning they can enter surface water from the treatment plant effluents,[16,17] or reach groundwater if the sewage sludge is used as fertilizer.[18] Their concentration in surface water is estimated to be in the range from ng L^{-1} up to μg L^{-1}.[4,17,19] Needless to say, the presence of pharmaceuticals in fresh water is highly undesirable due to the adverse effects they exert on living organisms. Principally, the presence of minute but sustained amounts of antibiotics acts as a micro-vaccine, and there is evidence of increasing antibiotic resistance in bacteria.[20,21] Pharmaceuticals also have a tendency to accumulate in animal tissue,[22–24] whereas in plant organisms they have been observed to inhibit photosynthesis.[25]

Pharmaceuticals undergo biotic and abiotic transformation in an aqueous environment. In particular, sunlight is the most important abiotic factor influencing their persistence in such environments.[9] It is difficult to predict in what way sunlight may interact with dissolved organic compounds. Both quality and quantity of generated products depend on the composition of surface water, and especially on the presence of dissolved organic matter, inorganic ions, nitrite, nitrate, carbonate and iron ions.[26–28] Very often, the formed by-products are marked by a higher biological activity than their parent compounds. Therefore, the knowledge of the photolability of a given drug and the result of its phototransformation in aqueous environments is of utmost importance.

The present study is concerned with photodegradation of two psychoactive compounds: levomepromazine hydrochloride (LV) and olanzapine (OLA) (Fig. 1) in the presence of a natural matrix. Levomepromazine belongs to phenothiazine pharmaceuticals, being one of the most commonly used phenothiazine



A) Levomepromazine **B)** Olanzapine

Fig. 1 Structures of levomepromazine – LV (A) and olanzapine – OLA (B).

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derivatives and exhibiting sedative and antidepressive properties.[29] It has been observed that levomepromazine is photounstable and easily undergoes photooxidation with ensuing generation of levomepromazine sulphoxide.[30] The second of the examined compounds is used in the treatment of various types of schizophrenia.[31] Olanzapine preparations belong to the most commonly prescribed drugs by psychiatrists in Poland.[32,33] The drug is known to be a very stable compound, resistant to many stress factors, such as UV radiation, moderate acidity (0.1 mol L^{-1} HCl) or alkalinity (0.1 mol L^{-1} NaOH).[34] Insofar as the stability of the considered compounds in pharmaceuticals or in model solutions is well known, there are only limited data concerning their stability and fate in an aqueous environment.

2. Experimental methods

2.1. Materials

All the used chemicals were of reagent grade, and their further purification was not required.

Levomepromazine hydrochloride (2-methoxy-*N*,*N*′β-trimethyl-10*H*-phenothiazine-10-propanamine) was purchased from EGTO Budapest, Hungary. Olanzapine hydrochloride (2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine) was provided by Lilly (Germany). Other chemicals used in the experiments were obtained from POCh (Poland).

2.2. Irradiation systems

Two sources of radiation were employed: a solar simulator (SUNTEST CPS$^+$, ATLAS USA) equipped with a xenon lamp emitting radiation similar to sunlight in the range of 300–800 nm, and a UV lamp (Standard 16AV, Cobrabid, Poland) emitting monochromatic radiation in the range of 254 and 365 nm. All samples were irradiated by radiation with a wavelength of 365 nm which is representative of the natural solar UVA radiation.

2.3. Quantitative evaluation of radiation sources

The intensity of light was determined using potassium Reinecke's salt ($K[Cr(NH_3)_2(SCN)]\cdot H_2O$), with the exposed surface area of the actinometer equal to 28.26 cm^2. Intensity of the radiation (E_s), generally defined as the fraction of the absorbed light power per unit of the surface area, was found to be equal to E_s = 17.39 (W m^{-2}) for the UV lamp. The same parameter calculated for the solar light simulator is E_s = 19.53 (W m^{-2}).

2.4. Absorbance measurements

Monitoring of the actual concentrations of the studied pharmaceuticals was carried out by means of spectrophotometry. Since the spectra of levomepromazine and of its main product overlap to a large degree, the reaction progress was monitored by measuring the increment of the sulphoxide. For this purpose, a bivariate spectrophotometric method[30] was used. The degree of olanzapine photodegradation was monitored with the absorbance at 256 nm. To this end, a calibration plot (ABS = 1.8 × 10^4 [OLA], r^2 = 0.998, where ABS – absorbance, [OLA] – concentration of OLA in mol L^{-1}) was constructed for concentrations in the range of 2.5 × 10^{-5}–10^{-4} mol L^{-1}.

All the spectrophotometric measurements were conducted with a Hitachi U-2800A spectrophotometer (Japan). The following working settings of the device were used: scan speed 1200 nm min^{-1} and spectral bandwidth 1.5 nm.

2.5. Analysis of photostability

The experiments were carried out in a 50 mL glass crystallization dish with the surface area of 28.26 cm^2 open to atmospheric air. 25 mL of a 2.0 × 10^{-5} mol L^{-1} levomepromazine solution, as well as of a 5.0 × 10^{-5} mol L^{-1} olanzapine solution were subjected to 365 nm irradiation. The spectra of the solutions were recorded every 10 min. Additionally, a corresponding solution not containing these pharmaceuticals was irradiated at the same time and used as a blank.

pH of the aqueous solutions was adjusted with 0.1 mol L^{-1} H_2SO_4 or 0.1 mol L^{-1} NaOH. Its values were measured with an Elmetron CP-501 pH-meter (produced by ELMETRON, Poland) equipped with an EPS-1 pH electrode (ELMETRON, Poland).

2.6. Separation of the studied compounds from an aqueous matrix

Because of adverse effects that the aqueous matrix has for GC analysis, the conducted GC-MS analysis was followed by isolation (*i.e.* SPE) of the studied compounds. As a result, also preconcentration of the photoproducts was achieved. The separation was carried out with a J.T. Bakers SPE-12G System (Grosgerau, Germany) connected to a KNF LAB vacuum pump (Neuberger GmbH, Germany). 3 mL solid-phase extraction C-18 columns (200 mg, 40 μm, APD, 60 Å) were purchased from J.T. Baker (Phillipsburg, NJ, USA).

(a) The isolation of levomepromazine and the products of its photodecomposition from the aqueous solution was performed using a C-18 SPE column. The C_{18} sorbent was conditioned with 10 mL of methanol and 10 mL of Milli-Q water before the extraction. Next, 25 mL of an irradiated aqueous solution of LV was let slowly through the cartridge in mild vacuum (−200 mm Hg). The retained analytes were eluted with 3 mL of a methanol–acetonitrile (8 : 2) mixture, and then the column was washed out letting through 10 mL of methanol and 10 mL of Milli-Q water.

(b) The separation of olanzapine also proceeded with the use of a C-18 separation column. For the conditioning of a cartridge, the following sequence of solvents was used: 2 mL of methanol, 2 mL of phosphoric buffer (pH 6.5) and 1 mL of Milli-Q water. A sample of 25 mL of the irradiated aqueous olanzapine solution was let (−200 mm Hg) through the column. Next, the analytes were eluted with 2 mL of methanol. Finally, the cartridge was washed out with 2.5 mL of a methanol–water mixture (2: 1).

2.7. Chromatographic analysis

a. HPLC analysis. The chromatographic system (Thermo Separation) which was used for the analysis of the irradiated solutions comprised a 3D Spectra System UV 3000, a low-gradient pump P2000, a vacuum membrane degasser SCM Thermo Separation and a Rheodyne loop injector (20 μL). ChromQuest Chromatography Data software for Windows NT was employed for the acquisition and storage of data.

Chromatographic conditions for examination of the irradiated LV solutions:

A LiChrospher100 RP-18, 125 mm × 4 mm (5 μm) column equipped with a 4 mm × 4 mm (5 μm) guard column (Merck, Germany) with the mobile phase of acetonitrile, water, acetic acid and ammonium (25%) 40 : 40 : 20 : 2 (v/v/v/v) was used for the analysis of the irradiated solutions of levomepromazine. The flow rate was set to 1 mL min^{-1}, and the detection was performed at the wavelength of 254 nm. Under these conditions, the retention time of levomepromazine was 5.30 min, and it amounted to 2.70 min in the case of LV-sulphoxide.

Chromatographic conditions for examination of the irradiated OLA solutions:

The same column (LiChrospher100 RP-18, 125 mm × 4 mm (5 μm)) with a mobile phase consisting of acetonitrile, water and ammonium (15%) (37 : 62.6 : 0.4 v/v/v) was applied in the analysis of the olanzapine solutions. Concentrated acetic acid[35] was used to adjust the pH of the mobile phase to 6.5. The flow rate was kept at 0.7 mL min^{-1}, and the wavelength of the UV detector was set to 254 nm. Overall, the retention time of olanzapine under the described chromatographic conditions was observed to be 19.96 min.

b. GC-MS analysis. GC-MS analysis was carried out using an HP 6890 gas chromatograph with an electronic pressure control device connected to a mass spectrometric detector MSD 5973 (electron impact source and quadrupole analyzer, Agilent Technologies, USA) equipped with an HP-5MS column (5% phenylmethylsiloxane) with a length of 30 m and an i.d. of 0.25 mm coated with a 0.25 μm thick film and using a split/splitless injector. The injector worked in the splitless mode at a temperature of 250 °C. Helium of 99.999% purity was used as a carrier gas at a flow rate of 1.5 mL min^{-1}. Temperature of the oven was programmed to 150 °C (1 min hold), and was increasing at a rate of 15 °C min^{-1} to 290 °C, maintaining finally the maximum temperature for 10 min. Total run time was 20 min. The MS detector worked under the following conditions: temperature of the ion source 230 °C, temperature of the quadrupole 150 °C, temperature of the transfer line 280 °C, mass range (*m*/*z*) 50–400.

2.8. Chemometric procedure

Principal component analysis (PCA)[36] and multivariate curve resolution–alternating least squares (MCR-ALS) methods were employed to determine the number of spectral forms generated during the discussed processes. For this purpose, LV and OLA solutions in surface water with the concentrations of respectively 2.0×10^{-5} mol L^{-1} and 5.0×10^{-5} mol L^{-1} were prepared. 25 mL portions of the examined solutions were subjected to irradiation in a sun-light simulator chamber. The spectra of the irradiated solutions were recorded at 10 min intervals in the range of 200–400 nm. The obtained set of spectra registered in a numerical form were standardized to molar extinction values and presented in the form of data matrix W (86 rows × 13 columns in dimension[36,37]). The obtained matrix W was then subjected to PCA and MCR-ALS numerical decomposition of spectra.[37] In the next step, the previously generated spectra matrix was subjected to PCA. Accordingly, the correlation between each pair of the spectra was calculated, and the correlation matrix was formed according to the following formula: $w_{ij} = (s_{ij} - \mu_j)/\sigma_j$, where μ_j is the mean of the jth spectrum, σ_j is the standard deviation of the jth spectrum, s_{ij} is the extinction coefficient at the ith wavelength of the jth spectrum. Subsequently, principal component decomposition of the correlation matrix was carried out, resulting in $\boldsymbol{n}$ orthogonal eigenvectors. Information describing their importance was assigned to descriptors associated with each of the eigenvectors. Their minimum number necessary to describe all variations in the data set determined the number of independent spectral species (m).[37] Afterwards, the eigenvectors were subjected to VARIMAX rotation, renormalization, and used as the first approximation of components' spectra. Respectively to the number of spectral forms present in the examined solutions, the spectra were reconstructed using multivariate curve resolution alternating least squares (MCR-ALS)[38] (Fig. 2B and 3B).

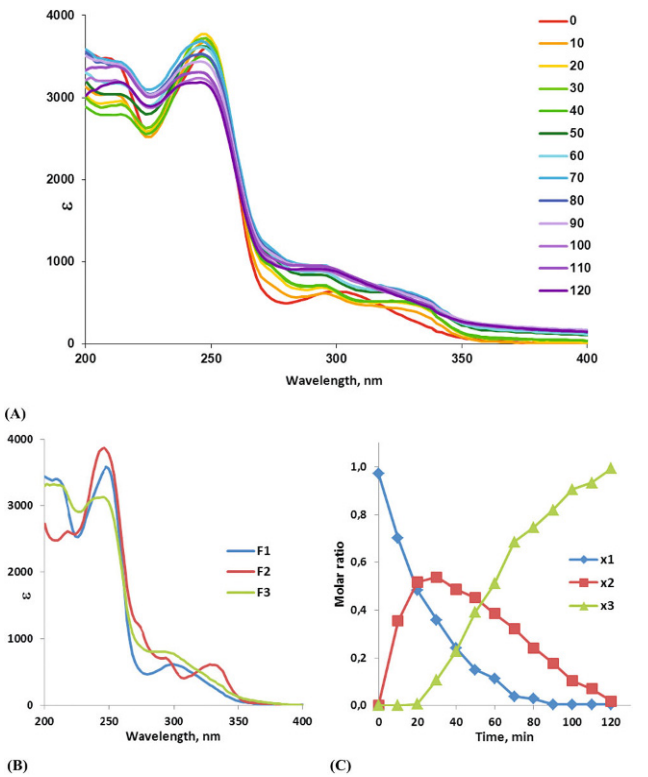

Fig. 2 Consecutive UV spectra of irradiated levomepromazine (2.0×10^{-5} mol L^{-1}) solution in river water (River 2) at 0–120 min of irradiation (A); reconstructed spectra of LV products generated in a sample of river water irradiated by simulated sunlight (B); optimum molar fraction profiles of LV and their degradation products obtained from numerical analysis (C).

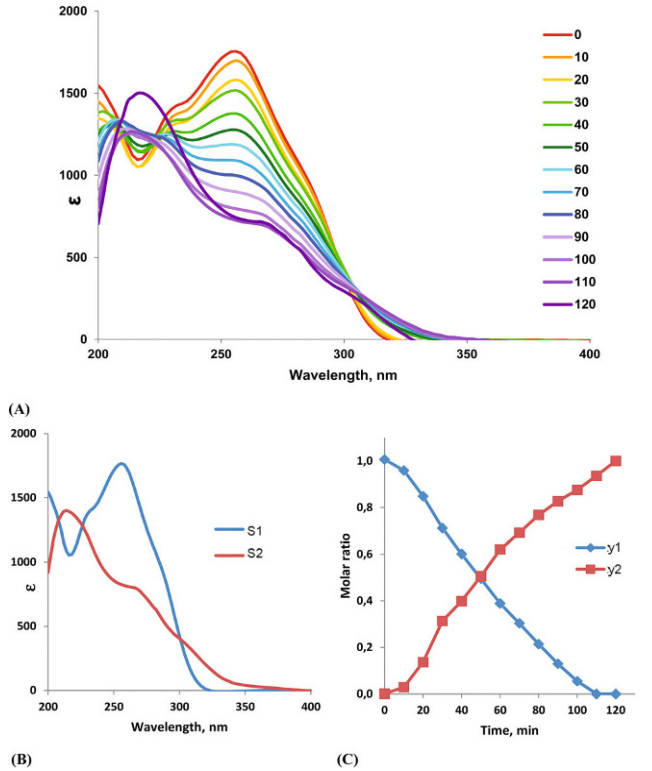

Fig. 3 Consecutive UV spectra of irradiated olanzapine (5×10^{-5} mol L^{-1}) solution in river water (River 2) at 0–120 min of irradiation (A); reconstructed spectra of OLA solutions in river water irradiated by simulated sunlight (B); optimum molar fraction profiles of OLA and their degradation products obtained from numerical analysis (C).

3. Results and discussion

3.1. Model investigation

All the kinetic studies were conducted spectrophotometrically. Since the level of pharmaceuticals in the aqueous environment was denoted in the magnitude from ng L^{-1} to μg L^{-1} [4,16,18] which was far below LOD of the UV-spectrophotometric method, photochemical analyses were realized using concentrations of 2.0×10^{-5} mol L^{-1} and 5.0×10^{-5} mol L^{-1} respectively, for levomepromazine and olanzapine. These values were consistent with the concentrations of the corresponding calibration plot and therefore ensured maximum accuracy.

First of all, the UV spectra of the studied compounds were recorded (Fig. 2 and 3). The spectrum of levomepromazine exhibited bands at 214 nm, 252 nm and a weak one at 302 nm, while olanzapine possessed only two bands: a sharp and intense one at 190 nm and a broad one at 256 nm. Next, photochemical behaviour of the studied compounds was scrutinized. Specifically, the influence of UVA radiation on the photostability of the studied compounds was verified. For this purpose, 25 mL samples of working solutions of levomepromazine and olanzapine were exposed to 365 nm radiation. The diminution of the studied pharmaceuticals was monitored spectrophotometrically by measuring their absorbance at analytical wavelengths. The observed processes had the first order kinetics. It was noticed that direct photolysis of levomepromazine under the influence of UVA, as well as of sunlight, led to the production of LV sulphoxide. Since the spectra of the parent compound and its product of photooxidation largely overlapped (Fig. 2), the course of the reaction was monitored by means of a bivariate method.[30] Progress of the reaction was estimated by measurements of the quantity of levomepromazine sulphoxide.

The obtained results clearly pointed out the differences in the photolability of the investigated compounds. It was observed that LV was promptly converted into its sulphoxide under the effect of UV irradiation. Also, the influence of medium pH on the photostability of the examined compounds was surveyed. In particular, it was noticed that low pH facilitated the photodegradation of LV. The observed reaction rates were 1.32×10^{-2} min^{-1} and 0.33×10^{-2} min^{-1} for pH 2.5 and 8.5, respectively. Thus, it can be stated that low pH intensified conversion of LV into its sulphoxide. Strictly speaking, it was recorded that 85% of the initial LV content underwent the conversion into LV-sulphoxide after 90 min of irradiation in an acidic medium. The observed decrease of the reaction rate in a basic solution could not be explained by the micellization of LV since the concentration of the examined solution was far below the CMC point for phenothiazines.[43] Analyzing the obtained data, a high rate of photolysis in the first 20 min of irradiation was noted. After this period, the rate of reaction slowed down.

As for OLA, the compound appeared to be photoresistant. No changes in its spectrum were observed during the process of irradiation. The influence of medium pH on OLA photolysis was negligible. In this case, the observed rate of reaction for pH 2 was assayed to be 8×10^{-5} min^{-1}.

3.2. Photolysis in a natural matrix

In turn, photolysis of the studied compounds under simulated environmental conditions was examined. The aim of the study was to assess persistence of LV and OLA in surface water. Natural surface water constitutes a very complicated chemical and biological system. Its diversity can have an influence on photochemical transformations of organic compounds. Photoreactions which occur in surface water depend on the intensity of light, temperature and the presence of organic matter and inorganic ions.[26–28] Therefore, it is obvious that the photochemical behaviour of a specific compound in surface water may differ significantly from analogous processes performed under laboratory conditions. In order to assess the photochemical behaviour of the studied drugs under environmental conditions, a series of their solutions containing samples of a natural matrix were exposed to radiation in a solar simulator chamber. A fresh water matrix, characterised by multiplicity and variability, is very difficult to reconstruct. In order to overcome this problem, samples of water taken from various rivers (Table 1) were used as solvents in preparation of the solutions of the examined pharmaceuticals. At the outset, some important chemical parameters for photochemical reactions were recorded, as well as the presence of the examined pharmaceuticals was tested (Table 1). It can be seen that the used surface water samples were free from any of the investigated compounds. Next, solutions of levomepromazine and olanzapine with the concentrations of

Table 1 Chemical characteristics of used waters

Parameter	River 1 53°07′N; 23°07′E	River 2 53°29′N; 22°44′E	River 3 52°20′N; 23°03′E	River 4 52°57′N; 22°57′E	Reference value	Ref.
pH	7.94	8.23	7.54	7.29	3–11	44,45
Conductivity/mS	530	560	330	460	10–4000	46
SO_4^{2-}/mg L^{-1}	15.16	77.33	116.40	14.10	10–80	47
NO_3^-/mg L^{-1}	70.00	22.84	21.88	35.58	<50	44,45,48
Cl^-/mg L^{-1}	41.40	10.70	199.00	35.50	0.4–170	49
HCO_3^-/mval L^{-1}	5.80	5.00	4.80	5.60	<14	45
Ca/mg L^{-1}	101.70	9.29	9.29	75.80	<250	50
Mg/mg L^{-1}	5.98	2.82	2.57	6.20	<150	
Fe_{diss}/mg L^{-1}	0.33	0.23	0.04	0.77	<2	51
TOC (total organic carbon)/mg L^{-1}	4.40	1.74	1.69	1.62	<40	52
$O_{2(diss)}$/mg L^{-1}	10.88	54.70	37.30	15.40	>4	53

Table 2 Kinetic parameters of photodegradation of levomepromazine and olanzapine in the presence of a natural matrix and in distilled water solutions under the influence of simulated solar light

	Levomepromazine			Olanzapine		
River	Time of observation/min	k/min^{-1}	$t_{1/2}$/min	Time of observation/min	k/min^{-1}	$t_{1/2}$/min
1	0–20	$3.07 \pm 0.1 \times 10^{-2}$	23 ± 0.7	120	$0.40 \pm 0.01 \times 10^{-2}$	173 ± 0.8
	30–120	$0.73 \pm 0.01 \times 10^{-2}$	95 ± 0.7			
2	0–20	$4.53 \pm 0.1 \times 10^{-2}$	15 ± 0.7		$1.00 \pm 0.01 \times 10^{-2}$	66.7 ± 0.7
	30–120	$0.96 \pm 0.01 \times 10^{-2}$	72 ± 0.7			
3	0–20	$3.15 \pm 0.1 \times 10^{-2}$	22 ± 0.7		$0.82 \pm 0.01 \times 10^{-2}$	84 ± 0.6
	30–120	$0.67 \pm 0.01 \times 10^{-2}$	103 ± 0.7			
4	0–20	$2.86 \pm 0.2 \times 10^{-2}$	24 ± 0.14		$1.00 \pm 0.0 \times 10^{-2}$	66 ± 0.6
	30–120	$0.53 \pm 0.01 \times 10^{-2}$	131 ± 0.7			
Model solution (pH 8.3 with carbonate buffer)	0–20	$4.64 \pm 0.1 \times 10^{-2}$	15 ± 0.7		No changes observed	
	30–120	$0.90 \pm 0.01 \times 10^{-2}$	77 ± 0.7			

2.0×10^{-5} mol L^{-1} and 5.0×10^{-5} mol L^{-1}, respectively, were subjected to irradiation in the solar simulator chamber for 120 min in the presence of a natural matrix. Changes in the concentrations of the studied drugs were monitored spectrophotometrically. Every 10 min, a sample of the irradiated solution was retrieved and its spectrum was registered against a previously irradiated sample of fresh water, which had been irradiated under the same conditions but without any pharmaceuticals, *i.e.* used as a blank. Pseudo-first order kinetics were assumed for all the studied processes. The obtained results are shown in Table 2. Kinetic profiles of the examined processes are presented in Fig. 4.

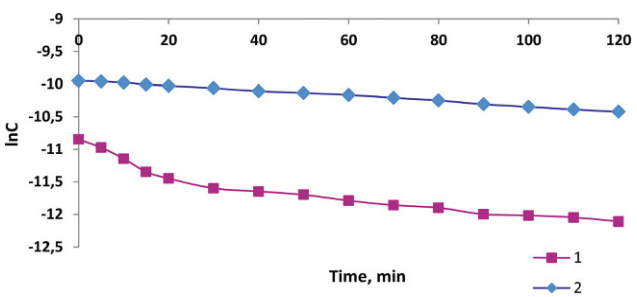

Fig. 4 Kinetic profile of LV (1) and OLA (2) solutions in river water (River 1) irradiated by simulated sunlight.

The obtained results prove (Table 2) that the chemical system created by fresh water and solar light was very efficient as far as the degradation of the examined compounds was concerned. In the case of LV, its photodegradation in river water advanced at a similar rate as in distilled water. As well as that, it also proceeded in two steps: an initial fast step and a subsequent slow one. It can be assumed that direct photolysis is the main process responsible for LV degradation. In order to explain a possible mechanism of the studied process, LV solutions (2×10^{-5} mol L^{-1}) in redistilled water with pH adjusted to 8.3 by a carbonate buffer were prepared and later subjected to simulated solar light. The rate of LV decomposition was similar to that observed in surface water, which confirms the above assumption. The process of LV decay depends on the composition of water used as a solvent.

It was noticed that the presence of a natural matrix accelerated the phototransformation of OLA (Table 2). Since the olanzapine spectrum has the main absorption band at 256 nm, it is not affected by sunlight. Its photodecomposition in a buffered laboratory solution (pH 8.3 with carbonate buffer) was not observed. However, the witnessed intensification of OLA transformation under environmental conditions can be attributed to the natural aquatic contaminants (NO_3^-, SO_4^{2-}, Fe ions and

dissolved organic matter) acting as photosensitizers.[27,28,39–41] Additionally, it was noticed that olanzapine displayed the highest stability in solutions prepared with the sample of water from river 1. This effect may be ascribed to the high level of organic carbon and the inhibitory action of dissolved organic matter[42] in this river.

3.3. Chemometric and chromatographic (HPLC and GC-MS) analysis

To assess the number of intermediates generated during the photolysis, the spectra of levomepromazine and olanzapine were subjected to principal component analysis (PCA) and the multivariate curve resolution-alternating least squares (MCR-ALS) procedure. The PC-analysis of LV and OLA spectra provided information about the new spectral forms that were generated during the observed period of the irradiation. Next, spectra of the generated spectral species were reconstructed using the MCR-ALS method, and compared with those actually recorded at the time of the observation (Fig. 2B and 3B). For instance, it was noticed that the spectrum of form F_1 was identical with the spectrum of levomepromazine, while the spectral characteristic of form F_2 was the same as levomepromazine sulphoxide. Also, gradual conversion of levomepromazine into spectral form F_3 was observed. Using kinetic data, the zero-approximation of molar ratios of each spectral form was calculated (Fig. 2C and 3C). The analysis of changes in molar contribution of LV products of irradiation (Fig. 2C) suggested that its photodecomposition formed a chain of consecutive reactions. It was noticed that a mutual ratio of each of the spectral forms (LV and its products of photoreaction) depends on the type of the used natural matrix. All in all, the amount of the parent compound gradually declined, and its transformation (spectral form F_1) was almost completed after 90 min of irradiation. At the same time, two new products appeared: one unstable form (F_2) which reached its maximum at 20–30 min of irradiation, and the second stable form (F_3), which was gradually increasing in amount.

As for MCR-ALS analysis of the olanzapine solutions, it showed that during the observation period (120 min) only two spectral forms were present. The first of them was the parent compound S_1, and the second was the product of its phototransformation S_2. Similarly to the case of levomepromazine, the quantitative ratio between both forms depended on the natural matrix used.

It has to be remarked that the MCR-ALS model relies on the assumption that the analyzed solution contains 100% of the parent compound at the beginning of the observation, and 100% of the final product at the end of the process. The period of observation was, in the discussed case, 120 min, because no further changes in recorded spectra were noticed after this time. Therefore, the MCR-ALS model would require verification by other analytical methods.

The chemometric results were confirmed by HPLC and GC-MS analysis of the irradiated solutions. Specifically, it was observed that under the employed HPLC conditions, the peak of levomepromazine appeared at 5.3 min (Fig. 5). Chromatograms of irradiated LV solutions exhibited two main peaks – the first corresponding to levomepromazine at t_R 5.3 min, and the second

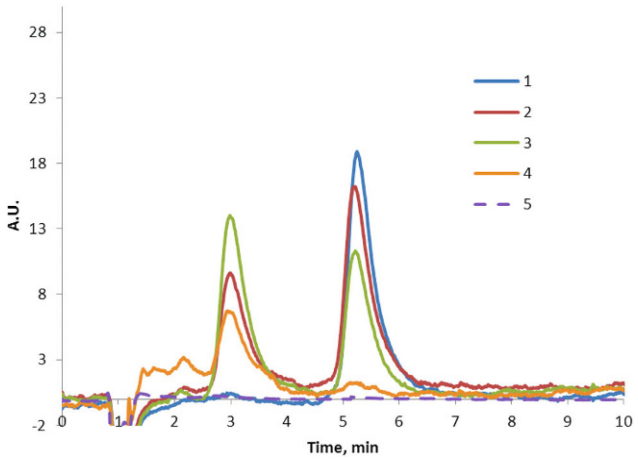

Fig. 5 Chromatograms of levomepromazine (2×10^{-5} mol L^{-1}) solution in water (River 2) irradiated by UV light. (1) Before irradiation; (2) after 60 min of irradiation; (3) after 90 min; (4) after 120 min of irradiation; (5) chromatogram of irradiated blank (river water without LV) after 120 min.

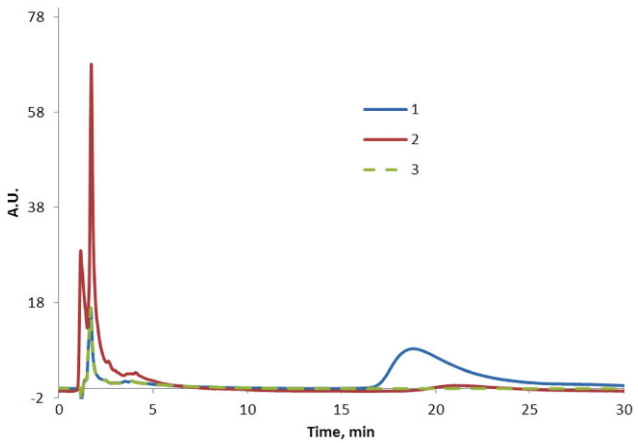

Fig. 6 Chromatograms of olanzapine (5×10^{-5} mol L^{-1}) solution in river water (River 4) irradiated by sunlight: (1) at the beginning of the experiments (0 min); (2) after 120 min; (3); chromatogram of blank (river water without OLA).

at t_R 2.7 min attributed to LV sulphoxide. Gradual changes in the height of both peaks were observed. During the irradiation, the peak related to levomepromazine was reduced, whereas the peak of its sulphoxide increased. Additionally, a small peak at the time of retention 2.0 min appeared which was gradually rising during the irradiation.

HPLC chromatograms of irradiated OLA solutions testified to the accuracy of the results of the chemometric analysis (Fig. 6). There were only two main peaks: one at 1.7 min attributed to the photoproduct of OLA and another at 19.96 min which represented the parent compound. Consecutive chromatograms of irradiated olanzapine solutions showed a gradual decrease in the height of the olanzapine peak, and an increase in the peak of its main photoproduct.

Actually, the GC-MS analysis provided more detailed information. It was observed that concentration of generated

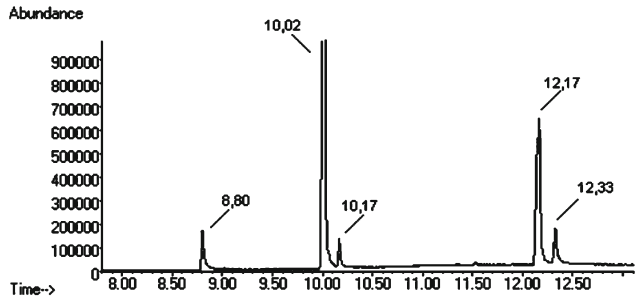

Fig. 7 GC-MS chromatogram of levomepromazine (after 120 min of irradiation); see Table 3 for details (t_R = 8.90 min – 3-methoxyphenothiazine, t_R = 10.02 min – levomepromazine, t_R = 10.17 min – methoxypromazine, t_R = 12.17 min – levomepromazine sulphoxide, t_R = 12.33 min – 10-(2-methyl-3-*N*,*N*-dimethylamine)propyl-10-oxido-10*H*-phenothiazin-2-ol).

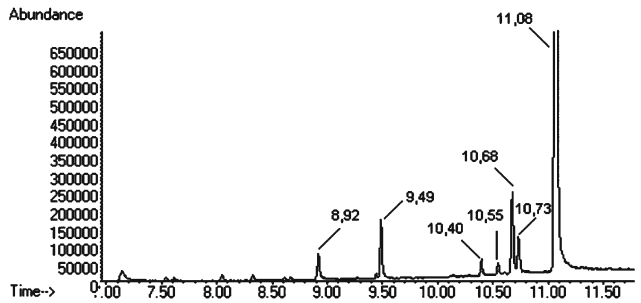

Fig. 8 GC-MS chromatogram of olanzapine (after 120 min of irradiation); see Table 3 for details (t_R = 8.92 min – 5,10-dihydro-2-methyl-4*H*-thieno[2,3-*b*][1,5]benzodiazepin-4-one, t_R = 9.49 min – *N*,*N*,2-trimethyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepin-4-amine, t_R = 10.40 min – unidentified product, t_R = 10.55 min – (3*Z*)-3-[(1-hydroxyethoxy)methylidene]-4-(4-methylpiperazin-1-yl)-1,3-dihydro-2*H*-1,5-benzodiazepine-2-thione, t_R = 10.68 min – 1-hydroxy-1-methyl-4-(2-methyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepin-4-yl)piperazin-1-ium, t_R = 10.74 min – 2-methyl-4-(4-methyl-4-oxidopiperazin-1-yl)-10*H*-thieno-[2,3-*b*][1,5]benzodiazepine, t_R = 11.08 min – olanzapine).

photoproducts and their parent compounds in the irradiated solutions was too low for direct analysis by the GC-MS system. Additionally, the presence of an aqueous matrix was a setback. That is why, in order to carry out isolation and preconcentration of the analytes, SPE extraction was employed. Examples of the recorded GC-MS chromatograms are presented in Fig. 7 and 8. It can be seen that after 120 min of irradiation in LV solutions, its derivatives, namely 10-(2-methyl-3-*N,N*-dimethylamine)-propyl-10-oxido-10*H*-phenothiazin-2-ol, 3-methoxyphenothiazine, methoxypromazine and levomepromazine sulphoxide (Table 3), could be observed. A comparison of the obtained chromatographic results with those obtained by the chemometric analysis suggests that MCR-ALS methods can only be used as an initial approximation of the number of generated products. This comes as a result of the sensitivity and selectivity of analytical techniques relying on measurements of absorbance. Strictly speaking, it is not possible to assess the number of by-products if they exhibit similar spectral characteristics, or they are present in the solution in small quantities, lower than LOD of the employed technique.

The GC-MS analysis showed that the photodecomposition of olanzapine is a somewhat more complicated process (Table 3 and Fig. 8). Principally, at least six different photoproducts were recorded. Careful scrutiny of the chromatograms proved that the products dominant at the retention time included 9.49 (*N*,*N*,2-trimethyl-10*H*-thieno[2,3-*b*][1,5]benzodiazepin-4-amine) and 10.68 (1-hydroxy-1-methyl-4-(2-methyl-10*H*-thieno[2,3-*b*][1,5]-benzodiazepin-4-yl)piperazin-1-ium).

On the whole, the conducted experiments demonstrated differences in the photolability of the studied compounds. In particular, sunlight caused direct photolysis of levomepromazine. According to the data provided in Table 3, this process advanced *via* generation of the equally photolabile levomepromazine sulphoxide. Incidentally, this compound turned out to be the main product of LV photodegradation. Additionally, it was observed that irradiation of levomepromazine leads to the generation of products with a demethylated propanamine side chain or without the propanamine side chain at all.

The achieved results indicated that photodegradation of olanzapine required the presence of photosensitizers. Its transformation involved mainly a methyl-piperazine side chain, while the benzodiazepine skeleton remained unchanged. The generated products were most probably formed in reactions with the radicals present in the aqueous matrix.

4. Conclusion

The presented study deals with photochemical behaviour of two psychoactive compounds in model solutions and in the presence of a natural matrix. It turned out that the examined compounds exhibited different photolability. Levomepromazine easily underwent oxidation under the influence of both UV radiation and sunlight. These processes led to the generation of levomepromazine sulphoxide. The same experiments performed on aqueous solutions of olanzapine proved its stability and resistance to UV radiation. The presence of a natural matrix acted as a natural photosensitiser, and for both compounds there was an enhancement in the rate of photodegradation in surface water. Moreover, the composition of the natural matrix had an influence on the ratios of decomposition and products generation. It was noticed that two main spectral forms were generated during LV irradiation by sunlight, the primary product being LV sulphoxide. This conclusion was confirmed by chemometric and chromatographic (HPLC and GC-MS) analyses. As for the enhancements of OLA photodecomposition in the presence of a natural matrix, it can be speculated that the main role in this process is played by reactions with reactive species such as NO_3^-, SO_4^{2-} and Fe ions as well as with dissolved organic matter.

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Table 3 Results of GC-MS analysis of irradiated LV and OLA solutions

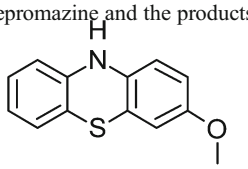
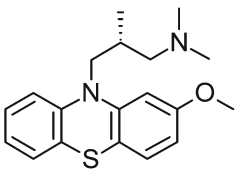
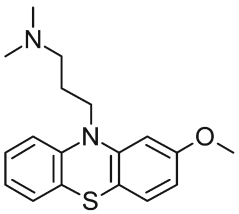
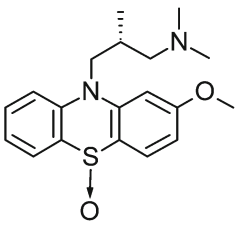
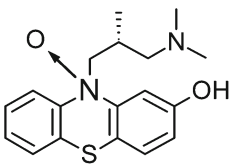
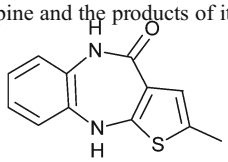
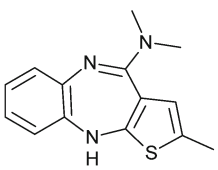
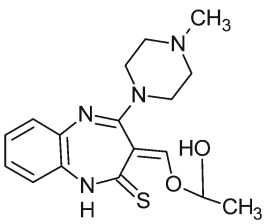
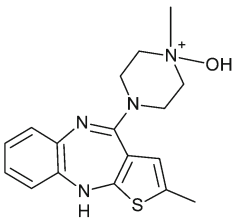
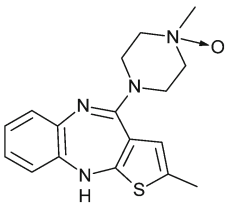
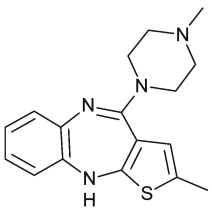
t_R	Structure	M (g mol^{-1})	$I_R \pm$ SD exp ($n = 4$)	NIST library match (%)	m/z	Photodegradation product
Levomepromazine and the products of its photodegradation.						
8.80		229, 298	2384	82	229, 186, 214, 186, 230, 186	3-Methoxyphenothiazine
10.02		328, 472	2618	99	58, 328, 228, 185, 228, 282	Levomepromazine
10.17		314, 445	2648	96	314, 229, 242, 228, 185, 210	Methoxypromazine
12.17		344, 487	3042	99	58, 242, 328, 229, 228, 185	Levomepromazine sulfoxide
12.33		330, 460	3068	—[a]	242, 228, 229, 312, 314, 185	10-(2-Methyl-3-*N,N*-dimethylamine)propyl-10-oxido-10*H*-phenothiazin-2-ol
Olanzapine and the products of its photodegradation.						
8.92		230, 286	2406	—[a]	189, 135, 232, 134, 55, 106	5,10-Dihydro-2-methyl-4*H*-thieno[2,3-*b*][1,5]-benzodiazepine-4-one
9.49		257, 354	2514	—[a]	70, 83, 188, 201, 131, 200	*N*,*N*,2-Trimethyl-10*H*-thieno[2,3-*b*][1,5]-benzodiazepine-4-amine
10.40	—	—	2694	—[a]	314, 229, 242, 185, 210, 86	NN
10.55		346, 447	2726	—[a]	70, 83, 217, 175, 204, 56	(3*Z*)-3-[(1-Hydroxyethoxy)methylidene]-4-(4-methylpiperazin-1-yl)-1,3-dihydro-2*H*-1,5-benzodiazepine-2-thione

Table 3 *(Contd.)*

t_R	Structure	M (g mol^{-1})	$I_R \pm$ SD exp ($n = 4$)	NIST library match (%)	m/z	Photodegradation product
10.68		329, 439	2753	—[a]	70, 98, 143, 269, 212, 83	1-Hydroxy-1-methyl-4-(2-methyl-10*H*-thieno-[2,3-*b*][1,5]benzodiazepine-4-yl)piperazin-1-ium
10.74		328, 432	2766	—[a]	70, 83, 56, 228, 271, 285	2-Methyl-4-(4-methyl-4-oxidopiperazin-1-yl)-10*H*-thieno[2,3-*b*][1,5]benzodiazepine
11.08		312, 433	2838	99	198, 213, 229, 242, 312	2-Methyl-4-(4-methylpiperazin-1-yl)-10*H*-thieno-[2,3-*b*][1,5]benzodiazepine (olanzapine)

t_r – retention time. M – molar mass. I_R – retention index. m/z – mass-to-charge ratio of most intensive peaks. NN – unidentified compound.[a] Compound not included in the NIST library, identified by the comparison of the measured spectrum with literature data.[54–56]

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