Anticancer Drugs Gemcitabine, Letrozole, and Tamoxifen in Municipal Wastewater and Their Photodegradation in Laboratory-Scale UV Experiments

Olga-Sofia Alitalo · Anna-Lea Rantalainen · Jukka Pellinen

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Abstract The occurrence of three anticancer drugs (gemcitabine, letrozole, tamoxifen) was studied in wastewater samples from two local wastewater treatment plants (WWTPs) in Finland. Studied pharmaceuticals were selected, as anticancer drugs are potential to cause adverse effects on organisms even at low concentrations, but they are seldom included in the analysis of emerging contaminants. The concentration of anticancer drugs was determined by liquid chromatography-triple quadrupole mass spectrometer (LC–MS/MS). Tamoxifen and letrozole were detected from influent samples ranging from 0.5 to 5.0 ng/L, respectively. Letrozole was detected from effluent samples at a concentration up to 2.4 ng/L. Letrozole has been detected in wastewater effluent only once before, at a lower concentration of 0.28 ng/L. Gemcitabine was not detected in any of the samples. UV irradiation is used in many wastewater treatment plants to disinfect the effluent. Such tertiary treatment might degrade also these potentially harmful drugs and, therefore, photodegradation of the chosen pharmaceuticals was studied in laboratory-scale experiments. Tamoxifen showed high degradation rates, 94% in spiked wastewater with UV fluence 4830 mJ/cm² and 98% in pure water with UV fluence 2520 mJ/cm², respectively. Letrozole showed the lowest degradation rates of 24% in wastewater and 34% in pure water, respectively. The degradation rate at the fluence level typical for UV disinfection stage of wastewater treatment plants was 37% for tamoxifen but only 5% for letrozole. To the best of the authors’ knowledge, this is the first report to show the effectiveness of UV irradiation to degrade letrozole.

Keywords Hormone antagonists · Cytostatics · UV radiation · Emerging contaminants

1 Introduction

The environmental fate of pharmaceuticals has been widely studied during the last decades, as they are a group of emerging contaminants potentially posing a risk to aquatic ecosystems and humans as well. Pharmaceutical residues enter the environment mainly from municipal wastewater treatment plants; other sources include pharmaceutical industry, waste disposal, and veterinary practices. Hospital effluents contain high load of pharmaceuticals that differ from those used in households. Such wastewater is usually led to municipal wastewater treatment plants without any pretreatments. As wastewater treatment plants are designed to remove oxygen-consuming organic material, nitrogen and phosphorus, their treatment processes are not efficient to remove pharmaceuticals. Consequently, some pharmaceutical residues enter the receiving water bodies with the effluents.
Mostly studied pharmaceutical groups include antibiotics, analgesics, anti-inflammatory agents, antidepressants, beta-blockers, and neuroactive compounds (Fent et al., 2006; Ternes, 1998), but anticancer drugs are usually not included. Cancer is one of the leading causes of human deaths worldwide. In Finland, over 34,000 new cancer cases were registered in 2018 (Pitkäniemi et al., 2018), yet, anticancer drugs have been overlooked in environmental studies of emerging contaminants (Jureczko & Kalka, 2020; Kosjek & Heath, 2011). As far as we know, this is the first study done in Finland on the presence of these selected anticancer drugs (Table 1) in wastewaters or the environment.

Cytostatics are one of the many groups of pharmaceuticals used in cancer treatments. Their mode of action is based on their ability to inhibit DNA and RNA synthesis, cell division, and normal cell functions. They can also disrupt certain enzyme activity in cells. Cytostatics affect unselectively in all dividing cells, which causes damage to healthy cells as well. Due to their mode of action, it can be assumed that they have adverse effects on all eukaryotic cells and, therefore, even low environmental concentrations are alarming (Johnson et al., 2008; Kovalova et al., 2009). One of the chosen cytostatics in this study is gemcitabine, which belongs to the class of antimetabolite pyrimidine analogues, and it is globally one of the most widely used cytostatic drug (Kosjek & Heath, 2011).

Other widely used pharmaceuticals in cancer treatments are hormone antagonists, which are often used to treat breast cancer. This group can be further divided in antiestrogens and aromatase inhibitors, which effect on the function and synthesis of hormones. They also effect on the action of hormones upon their specific sites (Liu et al., 2010). As these pharmaceuticals are designed to affect hormonal functions, they may cause adverse effects on the development and reproduction of aquatic organisms. One of them is tamoxifen, which is an antiestrogen, used to treat primary and recurrent breast cancer, whereas letrozole is an aromatase inhibitor that is used to treat hormone receptor-positive metastatic breast cancer in postmenopausal women (Haynes et al., 2003). The potential risks of tamoxifen and letrozole to aquatic organisms have been studied, and the results show that these pharmaceuticals can affect the reproductive capacity, alter the vitellogenin levels of both sexes, and cause transgenerational effects in their progeny (Liao et al., 2014; Sun et al., 2007a; Sun et al., 2007b).

Considering the fact that pharmaceutical residues enter the water bodies in wastewater effluents, there is a need to develop new, effective treatment technologies. Ultraviolet (UV) irradiation is widely used to disinfect drinking water, and wastewater treatment plants use it as a tertiary treatment to improve the microbiological quality of the effluent (Kovacic et al., 2016; Hijnen et al., 2006). It is usually accomplished

| Compound       | CAS number     | Structure | pKa   | log Kow |
|----------------|----------------|-----------|-------|---------|
| Gemcitabine    | 95058-81-4     | ![Structure](image) | 3.6<sup>a</sup> | −2.01<sup>a</sup> |
| Letrozole      | 112809-51-5    | ![Structure](image) | 2.17<sup>b</sup> | 2.5<sup>b</sup> |
| Tamoxifen      | 10540-29-1     | ![Structure](image) | 8.76<sup>c</sup> | 6.3<sup>c</sup> |

<sup>a</sup>Kovalova et al. (2009), <sup>b</sup>Liu et al. (2010), <sup>c</sup>Ferrando-Climent et al. (2013)
by using low-pressure mercury lamps, which emit UV C radiation at a wavelength of 254 nm. This is an effective technique to eliminate microbes, and previous studies have shown that it is also a potential way to degrade pharmaceuticals (Pereira et al., 2007). UV irradiation is often used with peroxides and catalysts (advanced oxidation processes, AOPs), which leads to the formation of highly reactive free radicals improving the degradation of pharmaceuticals (Rodríguez-Chueca et al., 2019; Ferrando-Climent et al., 2017; Vogna et al., 2004). Different kinds of AOPs and other advanced treatment processes like membrane filtration have also been used to remove and degrade pharmaceuticals from wastewaters (Zhang et al., 2013).

The main objectives of this study were to (1) determine the concentration of abovementioned three anticancer drugs in the wastewater samples and (2) determine the extent of photodegradation of these pharmaceuticals in laboratory-scale UV treatment.

2 Materials and Methods

2.1 Chemicals and Materials

Gemcitabine and tamoxifen were purchased from Sigma-Aldrich (Steinheim, Germany). Letrozole, deuterated letrozole-[d₄], deuterated tamoxifen- [d₅], and gemcitabine-[¹³C][¹⁵N₂], were obtained from Toronto Research Chemicals (North York, Canada). Stock solutions of anticancer drugs were prepared in methanol. Acetonitrile and methanol (both UPLC-grade), and acetic and formic acid were purchased from VWR Chemicals (Fontenay-sous-Bois, France). Solid phase extraction (SPE) cartridges Oasis HLB (60 µm, 500 mg, 6 ml) were purchased from Waters (Eschborn, Germany) and Isolute ENV+(60 µm, 500 mg, 6 ml) from Biotage (Uppsala, Sweden). The UV spectra of tamoxifen and letrozole were recorded with a Shimadzu UV-2401PC recording spectrophotometer (Shimadzu Co., Japan).

2.2 Sample Collection

Wastewater samples were collected from two local wastewater treatment plants, labeled as A and B, at three separate occasions between June 2019 and January 2020. Influent and effluent samples were collected as 24-h composite samples. The WWTP A receives wastewaters from households but also from local central hospital and industry (approx. number of inhabitants 62,000, flow rate 14,936 m³/d), and therefore samples were collected twice from this WWTP, covering summer and winter seasons. The WWTP B receives wastewater mostly from households (approx. number of inhabitants 64,000, flow rate 17,845 m³/d). Treated wastewaters from both WWTPs are discharged into the same retention pond (volume 50,000 m³) located close to WWTP A. From this pond, all treated wastewater flows through the UV treatment before it is discharged to the receiving river. This UV treatment is used as a tertiary treatment to disinfect the water and secure its microbiological quality. This step is performed by the Duron UV disinfection system, which is an open channel system containing two modules of UV lamps. Modules contain 48 lamps, the power of each is 330 W, and the used wavelength is 254 nm, which is typical for UV C disinfection. UV dose is automatically adjusted with OptiDose, real-time dose control, to meet dosing requirements (typically the fluence is 40–140 mJ/cm²). The quality parameters of wastewater that is discharged to the river are BOD 5.1 mg/L, COD 52 mg/L, total phosphorus 0.22 mg/L, ammonium 1.2 mg/L, total nitrogen 14 mg/L, and suspended solids 4.0 mg/L. All samples were collected into ethanol-rinsed amber glass bottles. After sampling, samples were immediately transferred to the laboratory and stored in glass bottles at −20 °C until analysis.

2.3 Photodegradation Study in Laboratory Scale

The photodegradation of studied compounds was studied in laboratory conditions using a small UV C device UV-450L provided by Wetec Finland Corp. (Lieto, Finland). This device contains a low-pressure mercury lamp SNXIN ZW6D15W from Jiangsu Shenxing Photoelectric Medical Devices Co., Ltd. (Jiangyin New Harbour City, China) emitting UV C radiation at 254 nm. The volume of the device was 300 mL, and the thickness of the water layer was 13 mm. The fluence of the UV system was determined using iodide-iodate actinometry as described in the literature (Rahn, 1997). The irradiance value was 7 mW cm⁻² at 254 nm. The corresponding fluence values varied between 0 and 4830 mJ cm⁻² in the photodegradation experiments. The selected values covered a wide range of fluence values from levels used as tertiary treatment...
processes up to much higher levels. This way, it was possible to compare the efficiency of typically used fluence levels to the levels needed to degrade selected pharmaceuticals.

Wastewater samples used in this experiment were collected from the retention pond, which receives effluents from both local wastewater treatment plants. The experiments were done by adding 300 mL of wastewater into the UV C device, which was then turned on and the sample was treated for a predetermined time. The concentrations of these two compounds in wastewater were too low to be used as such in this photodegradation study, and therefore spiked samples were used. The concentrations of spiked compounds were selected to be high enough to follow the possible degradation rates and to ensure that the concentrations will stay above limits of quantification. The UV treatment times varied from zero to 690 s, and each irradiation point was prepared as triplicates. Samples were prepared by adding 200 ng of letrozole and tamoxifen in each sample. The same procedure was used to prepare pure water samples except that the treatment times were from zero to 360 s. After the treatment, 200 mL of treated water was transferred into a glass bottle, pH was adjusted to 2 by using HCl, 100 ng of internal standards were added to all samples, and the bottles were placed on a magnetic stirrer for 1 h. Sample pretreatment and LC–MS/MS analysis were done as described in the following sections.

2.4 Sample Pretreatment

Suitable SPE procedure for studied compounds was selected by testing two different kinds of SPE sorbents with spiked samples. Tested sorbents were Oasis HLB and Isolute ENV+. Oasis HLB is a universal sorbent for acidic, neutral, and basic compounds, made from two monomers, the hydrophilic N-vinylpyrrolidone and the lipophilic divinylbenzene. Isolute ENV+ is a hydroxylated hyper-cross-linked polystyrene sorbent, which is designed to absorb a wide range of polar compounds.

2.4.1 Solid-Phase Extraction (SPE) of Tamoxifen and Letrozole

Tamoxifen and letrozole were extracted from wastewater using Oasis HLB 500-mg cartridges. First, the pH of wastewater was adjusted to pH 2 by using HCl. Then, wastewater samples were filtered through a 1.6-μm GF/A glass fiber filter (Whatman, Dassel, Germany). Three replicates of each sample (sample volume 200 mL) were prepared, and 50 ng of labeled internal standards deuterated letrozole-[d₄] and deuterated tamoxifen-[d₅] was added in each sample. The samples were mixed for an hour before loading on the cartridge. The cartridges were preconditioned with 6 mL of methanol and a 6-mL ultrapure water. The sample bottles were connected to the cartridges using Teflon tubes, and the samples were loaded through the cartridges at a flow rate below 3 mL/min with help of a vacuum pump. After the extraction, the sample bottles and cartridges were washed with 6 mL of water, following 6 mL of methanol–water (5:95, v/v), and both of the rinses were transferred to the cartridges. The cartridges were dried under vacuum until they were completely dry. The analytes were eluted with 9 mL of methanol and evaporated to dryness under a gentle nitrogen stream. The samples were redissolved in 1 mL of methanol and filtered through a 0.45 μm GHP Acrodisc syringe filter prior to analysis. For validation of the method, analytes were spiked into ultrapure water and wastewater samples (sample volume 200 mL, three replicates) in concentrations of 250 and 1000 ng/L. To calculate the relative recovery, internal standards were added into samples shortly before loading to cartridge and in case of absolute recovery, internal standards were added right before the analysis.

In the first tests, the absolute recovery of letrozole was 97%, but for tamoxifen, it was 58%, which was lower than the acceptable range (the target recovery being 70–120%). During the second tests, the elution step was done with two different solvents. First, the cartridges were eluted with 9 mL of ethyl acetate and then with 9 mL of methanol in new test tubes. Ethyl acetate was used as it is less polar than methanol. After the evaporation, both eluates were analyzed separately, and the results were summed. This way, a slight improvement in recovery values was achieved. For the final tests to improve the absolute recovery of tamoxifen, pH of the samples was adjusted to 2 prior the loading to the cartridges. According to Liu et al. (2010), pH 2 is optimal for tamoxifen. With these modifications, the absolute recovery of both compounds was in acceptable range (Table 3) and similar to the ones reported previously (Liu et al., 2010).
2.4.2 Solid-Phase Extraction (SPE) of Gemcitabine

Gemcitabine was not retained in the HLB sorbent, and Gómez-Canela et al. (2014) have previously published a similar finding. Compared to other studied compounds, gemcitabine is the most polar as its logP value is $-1.4$ whereas for the others, it varies between 2.5 and 6.3. Therefore, another SPE cartridge was needed and based on the study of Kovalova et al. (2009), Isolute ENV$^+$ was used. Wastewater samples were filtered and prepared as mentioned in Sect. 2.4.1. Cartridges were preconditioned with 6 mL of methanol and 6 mL of 10-mM ammonium acetate buffer. Samples were loaded into the cartridges via Teflon tubes at a flow rate of 3 mL/min using a vacuum pump. After the extraction, cartridges were washed with 6 mL of water, then dried completely with the vacuum pump, eluted with 9 mL of methanol, and evaporated to dryness. Samples were redissolved in 1 mL of acetonitrile:methanol (75:25, v/v) and filtered through a 0.45-µm GHP syringe filter. The method validation was carried out in the same way as for tamoxifen and letrozole, as well as the determination of relative and absolute recoveries. These SPE tests gave favorable results, and the relative and absolute recovery values were 91–110%.

2.5 Liquid Chromatography Triple Quadrupole Mass Spectrometer (LC–MS/MS)

The analysis of the anticancer compounds was carried out by using ACQUITY$^\text{TM}$ UPLC$^\text{TM}$ liquid chromatography system (Waters Corp., Milford, MA, USA) coupled to a Micromass Quattro Premier (Waters Corp., Milford, MA, USA) triple quadrupole mass spectrometer with electrospray ionization (ESI).

In the beginning of the study, Waters ACQUITY$^\text{TM}$ UPLC$^\text{TM}$ HSS T3 and Waters X-Bridge$^\text{TM}$ Phenyl columns were tested to perform the chromatographic separation of gemcitabine. Rabii et al. (2014) and Gómez-Canela et al. (2014) have reported methods using either PFP (pentafluorophenyl) or C18 columns, but according to our experience, gemcitabine did not retain or had a retention time less than a minute in this kind of columns. Next tests were performed by Waters BEH Amide column, which is a hydrophilic interaction chromatography (HILIC) column utilizing chemically stable, trifunctionally bonded amide phase. It is designed to retain polar compounds that are too polar to retain by reversed-phase chromatography, and a previous method published by Kovalova et al. (2009) showed successful results of retaining gemcitabine and other similar compounds by ZIC-HILIC column. This column was selected for the final analysis, as it showed good retention of gemcitabine. The same column was tested for the separation of tamoxifen and letrozole, but the retention time of these compounds was not constant. Waters ACQUITY$^\text{TM}$ UPLC$^\text{TM}$ HSS T3 was selected for those two compounds.

The separation of tamoxifen and letrozole was thus performed by Waters ACQUITY$^\text{TM}$ UPLC$^\text{TM}$ HSS T3 column (2.1×50 mm, 1.8 µm, 100 Å). Column temperature was 30 °C and injection volume 5 µL. The mobile phase composition consisted of binary mixtures of 0.1% formic acid in ultrapure water (A) and acetonitrile (B). Gradient elution started at 5% of eluent B, increased to 60% B in 3 min, then increased to 100% B in 1.5 min, and then kept for 2.5 min. Column was re-equilibrated for 3 min between runs, and the total flow rate was 0.3 mL/min. Chromatographic separation of gemcitabine was performed by Waters ACQUITY$^\text{TM}$ UPLC$^\text{TM}$ BEH Amide column (2.1×100 mm, 1.7 µm, 130 Å). Column temperature was 30 °C and injection volume 5 µL. The mobile phase A consisted of 10-mM ammonium acetate buffer containing 0.04% acetic acid and B was acetonitrile. Elution was done by using the following gradient: 0–4 min 95% B, 4–5.5 min 50% B, 5.5–8.5 min 50% B, and 8.6–14 min 95% B. The flow rate was 0.3 mL/min.

The mass spectrometer was operated in positive mode (ESI+) under the following conditions: source temperature 120 °C, desolvation temperature 300 °C, capillary voltage 3.0 kV, the nitrogen gas flow rates of the desolvation and nebulizing gas were 700 and 50 L/h, respectively. Argon was used as the collision gas and the pressure of the chamber was maintained at 3.5×10$^{-3}$ bar. Data acquisition was performed in the multiple reaction monitoring (MRM) mode. The selection of the specific MRM conditions for each of the studied pharmaceuticals was performed by injecting individual standard solution directly into the source. Two MRM transitions per compound were acquired, and the most intense one was used for quantification, while the other one was used for identification and confirmation. Cone voltage was the parameter influencing the intensity the most, and
it was optimized for each compound, as well as the optimum collision energy (CE). The used parameters are shown in Table 2.

### 2.6 Method Validation and Quality Assurance

Internal standard calibration was used to correct for MS responses and to ensure exact quantification performance. The concentration of the internal standards was in all cases 20 µg/L for tamoxifen and letrozole and 5 µg/L for gemcitabine. The validation data are shown in Table 3. The linearity of the method was evaluated with standards at six different concentrations, from 2.5 to 30 µg/L for tamoxifen and letrozole and from 0.5 to 10 µg/L for gemcitabine. Correlation coefficients ($R^2$) above 0.99 were obtained for all compounds over the concentration range studied. The coefficient of variation (CV %) was calculated as the ratio of standard deviation to the mean from the spiked samples. The method’s limits of detection (MLD) and quantification (MLQ) were determined as the minimum detectable amount of each analyte with a signal-to-noise of 3 and 10, respectively. These were calculated from final, concentrated wastewater samples. MLD and MQL for tamoxifen and letrozole were 0.1 and 0.5 ng/L and 0.5 to 1.7 ng/L, respectively. The values obtained for tamoxifen and letrozole are comparable to those in previously published method by Liu et al. (2010), but slightly lower than in the study performed by Rabii et al. (2014) and Jones-Lepp et al. (2015). In the case of gemcitabine, only instrumental detection (IDL) and quantification limits (IQL) were determined, as it wasn’t detected from the final wastewater samples.

Solvent blanks were used to monitor possible carryover between runs. Spiked samples, as well as wastewater samples, were prepared in triplicates. Procedural blanks were used to trace possible contaminations.

### Table 2

| Compound          | Precursor ion (m/z) | Product ion (m/z) | Cone voltage (CV) | Collision energy (CE) |
|-------------------|---------------------|------------------|-------------------|------------------------|
| Gemcitabine       | 264.0               | 112.0            | 30                | 25                     |
| Letrozole         | 286.0               | 217.0            | 18                | 15                     |
| Tamoxifen         | 372.1               | 72.1             | 35                | 25                     |
| Gemcitabine$^{[13C][15N2]}$ | 267.0               | 115.0            | 30                | 25                     |
| Letrozole$^{[d4]}$ | 290.0               | 221.0            | 18                | 12                     |
| Tamoxifen$^{[d5]}$ | 377.2               | 72.1             | 35                | 25                     |

### Table 3

| Compound    | Linearity (µg/L) (standard solutions) | $R^2$ | MLD (ng/L) | MLQ (ng/L) | RE% | CV% |
|-------------|---------------------------------------|-------|------------|------------|-----|-----|
| Gemcitabine | 0.5–10.0                              | 0.9996| 9.4$^{[IDL]}$| 31*        | 106±5.2 | 5.0 |
| Letrozole   | 2.5–30.0                              | 0.9992| 0.5        | 1.7        | 96.7±0.8 | 1.0 |
| Tamoxifen   | 2.5–30.0                              | 0.9998| 0.1        | 0.5        | 99.1±10.8 | 11  |

$R^2$, correlation coefficients; MLD, methods limit of detection; MLQ, methods limit of quantification; RE %, absolute recoveries (mean±standard deviation, n=3); and CV %, coefficient of variation of target compounds from the wastewater effluent samples spiked at 0.25 µg/L. Note that wastewater samples were concentrated 200-fold during processing. *Only instrumental limit of detection and limit of quantification were determined for gemcitabine.
3 Results and Discussion

3.1 Concentrations of Pharmaceutical in Wastewater Samples

The concentration of the anticancer drugs was determined in influent and effluent samples collected from two local WWTPs at three separate occasions between April 2019 and January 2020. Table 4 summarizes the levels of analyzed compounds. Letrozole was detected in all influent and effluent samples at the low ng/L range. The highest concentration of letrozole (5 ng/L) was detected in influent samples collected from WWTP B in November 2019. The concentration in effluent samples was between 2.3 and 2.4 ng/L, which indicates that this compound is only partially removed during the wastewater treatment processes. Our results are higher compared to the previous study of Liu et al., 2010, where the concentration of letrozole in influent and effluent samples ranged from 0.28 to 0.8 ng/L and 0.27 to 0.6 ng/L, respectively. However, their results show similar behavior of letrozole in wastewater treatment processes. Literature review shows that there are not many studies reporting the presence of letrozole in wastewater samples. Jones-Lepp et al. (2015) detected letrozole in one out of 23 effluent samples, but the concentration was below LOD. In their study, the LOD was 15 ng/L, which is higher compared to the present study (0.5 ng/L). According to our knowledge, there are no other previous studies reporting the presence of letrozole in wastewater influents and effluents in addition to those two mentioned above.

Tamoxifen was detected only in the influent samples collected in November 2019 at of 0.5 ng/L. In other influent and effluent samples, the concentrations were below LOQ. In previous studies, tamoxifen has been found in wastewater influents and effluents, as well as from surface waters at varying concentrations, and it is well known for being nonbiodegradable during wastewater treatment processes (Ferrando-Climent et al., 2014, 2017). Roberts and Thomas (2006) reported effluent concentrations up to 396 ng/L and in surface waters up to 212 ng/L. Other results show concentrations in influents and effluents between 3.5 and 179 ng/L and 0.28 to 113.5 ng/L, respectively (Ashton et al., 2004; Liu et al., 2010; Negreira et al., 2013, 2014). Tamoxifen is a highly lipophilic compound (log $K_{ow}$ = 6.3, Ferrando-Climent et al., 2013), so it is likely that it is adsorbed onto particles and transported to sludge during the wastewater treatment processes. Any remaining particles were removed during the sample pretreatment, which could be one explanation for the low levels of tamoxifen in wastewater samples. Additionally, tamoxifen is extensively metabolized in the human body, which leads to formation of multiple metabolites, for example, N-desmethyl-4-hydroxytamoxifen, endoxifen, and 4-hydroxytamoxifen (Tré-Hardy et al., 2016). These metabolites are considered to be most therapeutically active, up to 100 times more potent than the parent compound, and therefore of great interest (Santana-Viera et al., 2016).

The occurrence of gemcitabine has been previously studied in Europe and Canada from hospital effluents and from municipal wastewater treatment plants and from surface water (Gómez-Canela et al., 2014; Kovalova et al., 2009; Martín et al., 2011, 2014; Rabii et al., 2014). In our study, gemcitabine was not detected in any of the influent or effluent samples. Other researchers have reported similar results (Gómez-Canela et al., 2014; Rabii et al., 2014), whereas Martín et al. (2014) detected effluent concentrations up to 76 ng/L. Martín et al. (2011) detected gemcitabine also from river water samples (Guadalquivir River, Spain) at a concentration of

| Table 4 Concentrations (mean ± standard deviation, $n=3$) of anticancer drugs in influent and effluent samples from two local wastewater treatment plants at three separate sampling times between June 2019 and January 2020 |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Compounds                      | WWTP A          | WWTP B          | WWTP B          | WWTP B          | WWTP B          | WWTP B          |
|                                | Concentration (ng/L) | Concentration (ng/L) | Concentration (ng/L) | Concentration (ng/L) | Concentration (ng/L) | Concentration (ng/L) |
|                                | June 2019       | January 2020    | November 2019    | June 2019       | January 2020    | November 2019    |
|                                | Influent | Effluent | Influent | Effluent | Influent | Effluent | Influent | Effluent | Influent | Effluent |
| Gemcitabine                    | <LOD      | <LOD      | <LOD      | <LOD      | <LOD     | <LOD     | <LOD     | <LOD     | <LOD     | <LOD     |
| Letrozole                      | 3.5 ± 1.0   | 2.4 ± 0.2  | 4.1 ± 0.2 | 2.3 ± 0.1 | 5.0 ± 0.6 | 2.4 ± 0.1 | 5.0 ± 0.6 | 2.4 ± 0.1 | 5.0 ± 0.6 | 2.4 ± 0.1 |
| Tamoxifen                      | <LOQ       | <LOQ      | <LOQ       | <LOQ       | 0.5 ± 0.05 | <LOQ     | <LOQ      | <LOQ      | 0.5 ± 0.05 | <LOQ     |
2.4 ng/L. According to Kovalova et al. (2009), gemcitabine was detected in hospital wastewater only in samples collected on the days when it was administered to patients. This could explain its absence in wastewater samples in this present study, as the sampling days were randomly chosen.

3.2 Photodegradation in Laboratory-Scale UV Experiment

Controlled UV experiments were carried out in a laboratory scale to discover the effectiveness of UV irradiation on the degradation of the other studied compounds, except gemcitabine, as it was not detected in any wastewater samples. Such UV treatment could be useful as a tertiary process at wastewater treatment plants or at hospitals and other welfare institutions to remove harmful compounds. In wastewater samples, tamoxifen showed the highest degradation rate as the percentual changes with the highest UV fluence (4830 mJ/cm²) was 94% (Fig. 1a). Spiked pure water samples gave similar results, as tamoxifen gave the highest degradation rate of 98% with the highest UV fluence (2520 mJ/cm²) (Fig. 1b). The UV fluences for pure water samples were lower, as the degradation was assumed to happen more efficiently compared to wastewater samples. Wastewater characteristics, such as turbidity, suspended solids, and UV absorbing inorganic and organic compounds may affect the irradiation efficiency (Antonelli et al., 2008).

Tamoxifen has been previously reported to be degraded by UV irradiation used as a tertiary disinfection treatment at a wastewater treatment plant (Roberts & Thomas, 2006). They reported a drop of over 60% in the concentrations measured before and after the treatment. The fluence levels typically used in water disinfection are from 40 to 140 mJ/cm² (Pereira et al., 2007). According to our results, irradiation with a comparable UV fluence of 84 mJ/cm² (irradiation for 12 s) decreased the tamoxifen concentration by 37%. Thus, a typical UV disinfection process is not efficient enough to remove all tamoxifen from wastewater.

Ferrando-Climent et al. (2017) have previously published a study on the effectiveness of UV irradiation to degrade tamoxifen in laboratory conditions. Our results are in accordance with theirs but the comparison of results is challenging, as our experimental parameters were not the same, and Ferrando-Climent et al. (2017) do not present the used UV fluences in their study. They reached almost complete degradation after 240 min, and in our study, 94% degradation was achieved with the highest UV fluence, which took 11.5 min. Although these results show high degradation rates, it is important to notice that instead of total mineralization, by-products of tamoxifen may be formed and these may have higher toxicity compared to the parent compound (Ferrando-Climent et al., 2017).

Letrozole showed low degradation rates during the UV treatment in both wastewater and pure water samples. In wastewater, the concentration decreased by 24% with the highest UV fluence (Fig. 2a). At the fluence level typical for UV disinfection at wastewater treatment plants, just about 5% of letrozole was degraded. The concentration was expected to decrease more in pure water samples and with the highest UV fluence it decreased by 34% (Fig. 2b). In the case of letrozole, no previous publications on its photodegradation by UV C irradiation was found, and according to our findings, it seems to be quite resistant. The maxima of UV absorption of letrozole are at 205 and 240 nm, and the absorption decreases strongly at higher wavelengths. At 254 nm, the measured absorption of a 10-mg/L solution of letrozole in methanol was just 0.103 as compared to the absorption maximum at 240 nm which

![Fig. 1 The photodegradation of tamoxifen in wastewater (a) and in pure water (b) samples. All analyses were done in triplicates](image-url)
was 1.38 (Fig. 3). Therefore, the photodegradation was low when irradiated at 254 nm. Tamoxifen had absorption maxima at 205, 237, and 269 nm, and it absorbs at 254 much more than letrozole (the absorbance of a 10-mg/L solution in methanol was 0.316 at 254 nm, Fig. 3). This explains at least partly why tamoxifen was more degraded than letrozole in the UV experiments.

To improve the degradation of letrozole, an application of advanced oxidation technique (e.g., UV/hydrogen peroxide, UV/ozone, or Fenton’s process) should be studied, as it is shown that UV irradiation alone, even with high fluence levels, is not efficient enough.

4 Conclusions

To the best of our knowledge, this is the first study done in Finland, presenting the presence of anticancer drugs tamoxifen and letrozole in wastewater influents and

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**Fig. 2** The photodegradation of letrozole in wastewater (a) and in pure water (b) samples. All analyses were done in triplicates.

**Fig. 3** The absorption spectra of tamoxifen and letrozole in methanol (10 mg/L)
effluents. Tamoxifen has been previously detected from samples around the world, but only two previous publications were found to present the incidence of letrozole in wastewater samples. Letrozole has been detected in effluents only once before, at a much lower level compared to our results. Laboratory-scale UV experiments showed high degradation rates for tamoxifen, but the effectiveness on letrozole remained low. This seems to be due to the different absorption coefficients of these two compounds at 254 nm. To the best of authors’ knowledge, this is the first time when the photodegradation of letrozole under UV treatment has been studied. These results indicate the need to improve this treatment, e.g., by using advanced oxidation techniques.

The concentration of detected anticancer drugs in effluents was low, but due to the lack of research, we do not know much about their environmental risk. The negative effects of low levels of anticancer drugs cannot be ruled out, as chronic toxicity data is missing. The real impact of these pharmaceuticals in aquatic ecosystems might be underestimated, as chronic and synergistic effects have not been widely studied. In addition, the degradation products may have adverse effects on aquatic organisms (Ferrando-Climent et al., 2017), and therefore more research is needed. In this study, we only analyzed water samples, so further investigations of sludge and sediment samples could provide important knowledge of the fate of these pharmaceuticals.

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Data Availability Statement The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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