Removal of organic Pollutants from wastewater using different treatment technologies

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Abstract: *Stability and removal of anti-inflammatory dexamethasone sodium phosphate (DSP), anti-anxiety drug diazepam (valium) and spironolactone (SP) from wastewater produced at Al-Quds University Campus were investigated. Kinetic studies in both pure water (abiotic degradation) and in sludge (biodegradability) at room temperature were investigated. They demonstrated that DSP underwent degradation to its hydrolytic derivative, simply named dexamethasone, in both media. The first order hydrolysis rate of DSP in activated sludge at 25°C (3.80×10^{-6} s^{-1}) was about 12-fold greater than in pure water (3.25×10^{-7} s^{-1}). Diazepam showed high chemical stability toward degradation in pure water, and underwent faster biodegradation in sludge providing two main degradation products. The degradation reactions in sludge and pure water showed first order kinetics with rate constant values of 2.6×10^{-7} s^{-1} and 9.08×10^{-8} s^{-1}, respectively. The potassium-sparing diuretic (water pill) SP underwent degradation to its hydrolytic derivative, canrenone, in both media. The first order hydrolysis rate of SP in activated sludge at 25°C (3.80×10^{-5} s^{-1}) was about 49-fold greater than in pure water (7.4×10^{-7} s^{-1}).*

The overall performance of WWTP was also assessed showing that 90% of spiked DSP and SP were removed together with its newly identified metabolites. WWTP also showed that UF and RO were relatively sufficient in removing spiked diazepam to a safe level. In order to check for different tools to be used instead of ultra-filtration membranes, the effectiveness of adsorption and filtration by micelle-clay preparation for removing DSP was ascertained in comparison with activated charcoal. Batch adsorption in aqueous suspensions of the micelle-clay composite and activated carbon was well described by Langmuir isotherms showing the best results for micelle-clay material. Besides, filtration of DSP, DZ and SP aqueous solutions by columns filled in with a mixture of sand and micelle-clay complex showed complete removal of each drug at concentration higher than sand/activated-charcoal filled filters at flow rates of 2 mL min^{-1}.

Keywords: Dexamethasone sodium phosphate, Diazepam, Spironolactone, Activated sludge, Activated charcoal, Micelle – clay complex.

1. Introduction

Persistent organic pollutants (such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs)) have for many years been investigated regarding their influence on the environment [1]-[2], and have high acute toxicity, tendencies to undergo bioaccumulation and biomagnifications. They also have a high resistance against degradation and long half-life in the environment.

Nowadays another concern is arising due to pharmaceuticals or their metabolites found in environmental samples. The presence of pharmaceutical residues in environmental bodies has increased the probability of toxicity risks for animals and humans in the last years [3]-[5]. It has been reported that the aquatic environment can become polluted with pharmaceutically active compounds (drugs) at low concentration because of the extensive consumption of pharmaceuticals in developed countries [6]-[7].

Persistence to biochemical degradation and polar structure, are indicated as mainly responsible for the incomplete removal of pharmaceuticals during conventional wastewater treatment plant [8]-[9] with efficiencies ranging between 60% and 90% for a variety of polar compounds [10]-[13]. To evaluate the efficiency of different traditional and innovative tools for the elimination of pharmaceutical residues, a series of water purification experiments were performing by using the WWTP installed at the Al-Quds University in Palestine as indicated in Figure 1 (appendix A), which includes sequential units as activated sludge (AS), ultra-filtration (UF), granular activated charcoal (GAC) and reverse osmosis (RO)[14].

Problems arising from the management of such a plant can be referred to the capability of the AS unit to favor the bio-degradation of organic pollutants as well as the fouling phenomenon affecting membrane units, which must be often replaced with high costs.

The present work reporting about the efficiency of advanced wastewater treatment technologies adopted in the Al-Quds plant for the removal of dexamethasone sodium phosphate (DSP), diazepam and spironolactone which were used as model pharmaceutical compounds due to its high solubility in water and large consumption in many Countries.

Aiming at the assessment of bacterial culture, which normally develops in the AS unit of Al-Quds WWTP, the stability of DSP, DZ and SP in pure water as well as in the activated sludge collected from the plant were investigated and it's degradation products were identified.

Finally, in order to check for different tools to be used instead of ultra-filtration membranes, the effectiveness of micelle-clay (MC) filter for removing DSP, DZ and SP were ascertained and compared to a filter filled with granular activated charcoal. Besides, the adsorption equilibrium parameters and the adsorption Langmuir coefficients were determined using both micelle-clay and fine powder activated charcoal (FAC) as adsorbent materials.

Dexamethasone sodium phosphate, 9-fluoro-11β,17-dihydroxy-16α-methyl-21-(phosphonooxy) pregna-1,4-diene-3,20-dione disodium salt (structure **1** in Figure 2, appendix A), a synthetic adrenocortical steroid, is a white or slightly yellow, crystalline powder. It is highly soluble in water and is exceedingly hygroscopic. It is widely used to treat inflammation, allergy and diseases related to adrenal cortex insufficiency. DSP is also known to reduce neointimal

hyperplasia in arteries [15]. This drug is one of the most potent corticosteroids with anti- inflammatory and immunosuppressive properties [15]-[16]. It is used for coating drug- eluting stents for local drug delivery to prevent restenosis [17]-[19] and is 5-14 times more potent than prednisolone and 25-75 times more potent than cortisone and hydrocortisone [20]. The corticosteroids cause alterations in metabolism of fats, proteins and carbohydrates, and affect a range of organs in the body including the heart, muscle and kidneys. Blood chemistry may change and there is decreased activity and shrinkage of the thymus gland, adrenal glands, spleen and lymph nodes. The liver becomes enlarged, thyroid activity decreases, and mineral is drawn away from bone. Muscle wasting occurs, and the immune system is adversely affected causing the person to be more susceptible to infections, especially of the eye [15]-[19]. DSP is one of the most water soluble of the adrenocorticoseroidal agents. It is therefore suitable for intravenous use and particularly for ophthalmic formulations [21].

Another important class of pharmaceuticals, which has received recent consideration, is known as benzodiazepines (structure **3**, Figure 3, appendix A). One of the most commonly used medicine among the members of the benzodiazepines is diazepam (structure **4**, Figure 3, appendix A) (7-chloro-l-methyl-5-phenyl-1,4-benzodiazepin-2-one). It is a long acting benzodiazepine with anticonvulsant, anxiolytic, sedative, and muscle relaxant properties [22]. It is used for the treatment of acute management of all types of seizures in both adults and children [23]. Diazepam is administered by oral, intravenous, or rectal route [24].

Most pharmaceuticals undergo biotransformation in the human body, resulting in a release of significant amounts of a variety of metabolites into the aquatic environment, which can be further transformed during the sewage treatment processes (biotic degradation) [25]. Pharmaceuticals may also be chemically degraded by abiotic processes such as hydrolysis, oxidation and photolysis. The degradation products can be also of concern because of their possible toxicity, which can sometimes be higher than that of the corresponding parent compound [26]-[27].

Diazepam (**4**) is metabolized in human liver via cytochrome P450 enzymes pathway. It has an elimination half-life of 20-100 h, and produces several pharmacologically active metabolites; the main active metabolites are obtained through desmethylation mechanism (**5**, nordiazepam, $t_{½}$ = 36-100 h), temazepam (**6**, $t_{½}$ = 8-22 h), and oxazepam (**7,** $t_{½}$ = 4-15 h) as shown in figure 4, appendix A [28]. Diazepam and their metabolites are conjugated with glucuronide and are excreted primarily in the urine. From human metabolism studies it has been shown that 30% of diazepam dose administered to patients is exerted without any change, 12% as nordiazepam (**5**), 15% as temazepam (**6**) and 32% as oxazepam (**7**);11% of the dose remaining is still unidentified [29]. The diazepam metabolism is mediated by a number of cyctochrome P450 enzymes and proceeds by both N-dealkylation and C3-hydroxylation reactions [28]-[30].

Among these pharmaceuticals Spironolactone (SP), a synthetic, yellowish, crystalline solid, is considered as one of the most widespread [31]. SP (**8**), (7α-acetylthio-3-oxo-17α-pregn-4-ene-21,17-carbolactone) [31] (Figure 5, appendix A), is a competitive aldosterone antagonist, which belongs to the steroid class of chemical compounds. SP is practically insoluble in water, soluble in alcohol, and freely soluble in benzene and in chloroform. SP is a potassium-sparing diuretic (water pill) that prevents body from absorbing too much salt and keeps potassium levels from getting too low. It has been widely used to treat inflammation, allergy and diseases related to adrenal cortex insufficiency. Spironolactone is also known to diagnose or treat a condition in which the body has too much aldosterone (hormone produced by adrenal glands to help regulate the salt and water balance in human body) [31].

SP is also used to reduce edema caused by heart, liver or kidney problems, hypertension, and hyper aldosteronism. Common side effects of SP include skin rash, headache, dizziness, and stomach pain [31]. Serious side effects of SP include hyperkalemia, altered heart beats, confusion, tremors, decreased or no urine output, shallow breathing, muscle pain or weakness, and numbness [31].

SP is rapidly and extensively metabolized in human to (7α-thiomethylspirolactone) and Canrenone [32]. Sulfur-containing products are the predominant metabolites and are thought to be primarily responsible, together with spironol, for the therapeutic effects of the drug [31]. It is extensively used in medicine, though until recently it was considered only as potassium-sparing diuretic and anti- hypertensive drug. It may also reverse aldosterone-induced cardiac fibrosis and improve morbidity and survival of patients with congestive heart failure [33]-[34].

Furthermore, it is used in neonates, infants and children with congestive heart failure secondary to congenital heart disease [35]. As with many other frequently used drugs, SP is available only as tablets, rather than in liquid dosage form suitable for pediatrics use. Over the last 25 years, many extemporaneously prepared SP containing oral liquid formulations have been reported in the literature, as well as their physical and chemical stability [36]-[42].

Pramar et al. [43] observed that the decomposition of SP consists of a series reaction (Spironolactone, Canrenone and unidentified products) or a combination of series and side reactions since some of the SP may also directly change into some unidentified products, which is probably the reason for the absence of the Canrenone in the chromatograms. Moreover, it has also been suggested that in acidic medium the lactone would hydrolyze reversibly [43]. Canrenone (CR) (**9**), (10,13-dimethylspiro [2 ,8, 9, 11, 12,14,15,16-octahydro-1H-cyclopenta [α] phenan-threne-17,5'-oxolane]-2',3-dione) (Figure 6, appendix A), a cardiovascular drug, a sort of steroid, is spironolactone's major metabolite and has been widely used as a nonselective aldosterone receptor antagonist clinically to treat heart failure, high blood pressure, edema, liver ascites, and other cardiovascular diseases [44].

Canrenone, (**9**), ($C_{22}H_{28}O_3$) a pale yellow to pale green solid used as aldosterone antagonist. The production of (11-α-hydroxy-canrenone) (**10**) by the 11-α-hydroxylation reaction can be conducted by chemical synthesis or microbial transformation (Figure 7, appendix A) [31].

The 7α-acetylthio substituent is removed completely from 80% of the administered dose yielding Canrenone as the principal non-conjugated metabolite in plasma. Canrenone is active as a Mineral corticoidant agonist in animals, and has been proposed as the principal pharmacologically active agent after administration of SP to man. Potassium canrenoate (**11**), the potassium salt of asteroid acid, is also active as an

aldosterone antagonist and has found clinical use in certain areas of the world. After administration of this drug to human both Canrenoate (**12**) (Figure 8, appendix A) and Canrenone (**9**) are found in plasma. Evidence from in vitro studies indicates that Canrenoate has a low affinity for aldosterone binding proteins, and is unlikely to contribute significantly to pharmacological activity [45]. In vitro studies have also suggested that Canrenone is the principal active metabolite of potassium canrenoate [45].

The micelle-clay composite, which was used in this study, is positively charged, has large surface area and includes large hydrophobic domains [46]. Micelle-clay composites have already been proven useful in the removal of about 20 neutral and anionic pollutants [46]-[50].

2. Experimental

2.1 Materials and Equipments

2.1.1 Materials.

All chemicals were of analytical grade. The clay used was Wyoming Na- montmorillonite SWY-2 clay; obtained from the Source Clays Registry (Clay Mineral Society, Colombia, MO). Quartz sand (grain size 0.8—1.2 mm) was obtained from Negev industrial minerals (Israel). Octadecyltrimethylammonium (ODTMA) bromide was obtained from Sigma Aldrich. Pure DSP and its hydrolysis product dexamethasone, Diazepam and Spironolactone were obtained from Birzeit Pharmaceutical Company (Palestine) with 99% purity, and all were used as received. Fine powder activated charcoal (FAC) with particle size ≤ 60.0 µm, and granular activated charcoal (GAC) with particle size ≤ 700.0 µm were obtained from Sigma (Sigma Chemical Company, USA). The powder was used for batch adsorption experiments while the granules were used in column experiments. Magnesium sulfate anhydrous, potassium dihydrogen phosphate as well as methanol and water for analysis (HPLC grade) were purchased from Sigma Aldrich (Munich, Germany). High purity diethyl ether (> 99%) was purchased from Biolab (Israel). For sample enrichment and purification SPE 1g C-18 6 mL disposable cartridges (Waters, Milford, MA, USA) were used.

2.1.2 Equipment.

Samples were shaken using Big Bill, (Banstaed/ Themolyne, USA). The disappearance of DSP and its hydrolysis product were determined by using a high pressure liquid chromatography system model 2695 HPLC from Waters (Israel), equipped with a Waters 2996 Photodiode array. Data acquisition and control were carried out using Empower ™ software (Waters, Israel). Analytes were separated on a 4.6 mm x150 mm C18 XBridge® column (5 µm particle size) used in conjunction with a 4.6 mm, 20 µm, XBridge® C18 guard column.

HPLC conditions : mixture of 0.01M KH_2PO_4: methanol (1:1; v/v) as mobile phase for DSP; acetonitrile: water (1:1; v/v) for DZ and Acetonitrile (40:60; v/v) as mobile phase for DZ, flow rate of 1.5 mL min^{-1}; UV detection for DSP and SP at a wavelength of 254 nm and 230 nm for DZ. Acrodisc® syringe filters with GHP membrane (hydrophilic polypropylene 0.45µm porosity) from Waters were always used for all analytical filtration requirements.

The identification of drugs degradation products were performed using a liquid chromatography system coupled to a hybrid linear quadrupole ion trap (LTQ) – Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

The advanced wastewater treatment plant employed in this study is located at Al-Quds University-Palestine and was described in detail elsewhere [50]-[51]. Normally, the effluent from this plant is recycled for the irrigation of plants cropped in the field of university Campus.

2.2 Methods

2.2.1 Characterization of wastewater used.

The wastewater was characterized before the experiments according to American Public Health Association procedures [52],[27] by performing measurements listed in Table 1, appendix B.

2.2.2 Efficiency of WWTP for DSP, dexamethasone, DZ and SP removal.

The efficiency of different treatment units was ascertained by spiking separately the secondary effluent with 1.0 mg L^{-1} of either DSP or its hydrolysis product dexamethasone, DZ and SP in the activated sludge reservoir (1000 L). Samples were collected from different locations of the WWTP as depicted in figure 1, appendix A. Before analysis of DSP, 1mg of KH_2PO_4 was added to 100 mL of sample to stabilize pH, the acidic form of DSP, and the ionic strength of the solution. SPE-C18 disposable cartridges were used to pre-concentrate 10 mL of each sample by adsorption of analytes. A part (20 µL) of the methanolic solution eluted from SPE cartridge was injected into the HPLC, and analyzed using the same conditions for the determination of DSP, dexamethasone, DZ and SP. Recovery tests were performed using triplicate solutions of all substances, and values ranging from 98% to 102% were obtained.

2.2.3 Stability of DSP, DZ and SP.

Prior to perform any other experiment, stability study of DSP , DZ and SP were performed using 100 mg L^{-1} solutions in pure water, or activated sludge taken from the WWTP installed at Al-Quds University to ascertain if hydrolysis or bio-degradation reactions had taken place before the filtration stages. For this reason, Samples at specific time intervals were collected from the stability solutions (maintained under continuous orbital shaking), filtered, and analyzed by HPLC. The degradation by-products of DSP, DZ and SP were investigated using liquid chromatography/Fourier-transform ion cyclotron resonance/mass spectrometry (LC/FT-ICR/MS).

2.2.4 Micelle-clay complex preparation.

The ODMTA micelle-clay complex was prepared by mixing a smectitic clay- mineral (montmorillonite) with the cationic surfactant octadecyltrimethylammonium (as bromide salt) with a critical micelle concentration (CMC) value of 0.3 mM as described previously [50]. The obtained complex by virtue of its positive charge and hydrophobic region is capable of efficiently binding neutral and negatively charged organic molecules [47]-[51].

2.2.5 Batch adsorption experiments.

Batch adsorption experiments were carried out on DSP, DZ and SP at different concentrations. Experiments were performed in 250 mL Erlenmeyer flasks containing 200 mg of either micelle-clay complex or fine powder activated charcoal (FAC); 100 mL of each drug solutions having known initial concentration were then introduced into each flask. The flasks were shaken in an oscillating shaker for three hours at room temperature, and then the content of each flask was centrifuged (10,000 g) for 5 min and filtered using 0.45 μm filters. The equilibrium concentrations of DSP, DZ and SP were then obtained by HPLC, using the conditions reported above. The retention time of DSP was 6 min, 4.2 min for DZ and 6.9 min for SP.

2.2.6 Column filtration experiments.

Column filtering experiments were performed using 50/1 (w/w) mixtures of quartz sand and either ODTMA-clay complex, or granular activated charcoal (GAC), 20 cm layered in borosilicate columns of 25 cm length and 5 cm diameter. Each column contained 13 g of complex, or GAC. The bottom of the column was covered with 3 cm layer of quartz sand. Quartz sand was thoroughly washed by distilled water and dried at 105 °C for 24h before its use. Solutions in pure water (1 L each) containing different DSP, DZ and SP concentrations (0.01, 1, 10, and 100 mg L^{-1}) were passed through either micelle-clay or GAC columns (one column for each solution). In all cases the flow rate was 2.0 mL min^{-1}. Eluted fractions were collected in all column experiments and analyzed.

All experiments reported in sections 2.2.1- 2.2.6 were performed in three replicates and average values and standard deviations were calculated

3. Results and discussion

3.1 Calibration curves

Linearity of the proposed analytical method was verified by analyzing standard solutions in the range of 0.1 - 100 mg L^{-1} for both DSP and its hydrolysis product, for DZ and SP in pure water. The calibration curves were obtained with a determination coefficient R^2 between 0.9996- 0.9998, respectively. The repeatability of triplicate subsequent injections was ranging from 98.5% to 99.5%, depending on the sample concentration and type of analyte. The repeatability of morning/evening injections on the basis of 6-hours elapsed time was ranging from 97.6% and 98.2%, and was also affected by the concentration and type of analyte. Correction coefficients were used for experimental samples.

Calibration curves and repeatability trials were repeated preparing new calibration solutions by using wastewater taken from the activated sludge reservoir of Al-Quds WWTP. Results suffered of a major inaccuracy due to the variability of recovery percentages. Anyway, the determination coefficients of calibration curves were 0.9985 for DSP, 0.9989 for dexamethasone, 0.9996 for diazepam and 0.9999 for spironolactone. Repeatability normally was not reduced. The limit of detection, based on a signal/noise of 3, was 0.02 mg L^{-1} for DSP, 0.01 mg L^{-1} for dexamethasone, 0.03 mgL^{-1} for both DZ and SP. The limit of quantification, based on a signal/noise of 10, was 0.06 mg L^{-1} and 0.03 mg L^{-1} for DSP and its hydrolysis product, respectively, 0.08 mgL^{-1} and 0.05 mgL^{-1} for DZ and SP.

3.2 Wastewater characteristics

Table 2, appendix B summarizes chemical, physical and biological characteristics of wastewater sampled from the activated sludge reservoir of Al-Quds WWTP (figure 1, appendix A). Table 2 shows that this wastewater contains high amounts of suspended solids and organic pollution load, the relatively high values of TSS and COD may be attributed to residues of chemicals in the wastewater from laboratories, which were not well removed by the sedimentation stage and secondary biological treatment. This table also reveals that the wastewater contained high amounts of suspended solids and large populations of bacteria, which are responsible of fouling phenomena affecting ultra-filtration and reverse osmosis membranes. Moreover, high values of electrical conductivity and total dissolved solids, are typical for municipal wastewaters, and should be reduced if WWTP effluents are re-used for crop irrigation purposes.

Al-Quds activated sludge was found to contain Enterobacter and Pseudomonas species: *Escherichia coli*, *Enterobacter sakazakii*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterobacter cloacae*, *Enterobacter amnigenus*, *Enterobacter aerogenes*, *Salmonella spp.*, and *Serratia liquefaciens* [54].

Further challenge will be the isolation of strains constituting the bacterial colonies, aiming at the identification of the more active strains capable of utilizing the pharmaceutical molecules as energy source.

3.3 Efficiency of WWTP for DSP, dexamethasone, DZ and SP removal

The efficiency of WWTP at Al-Quds University for the removal of DSP, dexamethasone, DZ and SP were studied. The activated sludge reservoir (site 1 in figure 1, appendix A) was separately spiked with either DSP or dexamethasone, DZ and SP at concentration of 1.0 mg L^{-1}, which is an amount close to literature findings [55]- [56]. Samples were taken from different collecting sites of WWTP as described in section 2.2.2 and figure 1, appendix A. Analytical results of water effluent from the hollow fiber ultra-filtration membrane (UF-HF) indicated that DSP was about 63% removed at this stage, whereas about 95% of DSP was removed after passing the spiral wound (UF-SW) membrane (Table 3, appendix B). Besides, DSP was completely removed in the effluent from GAC filter. However, it should be outlined that the concentration of DSP (or dexamethasone) influent in the treatment units was diminishing along their sequence. This relationship reflected upon 100% removal by GAC filter, which influent water contained only 0.06 mg L^{-1} of DSP, or 0.07 mg L^{-1} of dexamethasone, on average, after the passage into the UF filters.

Analytical results of water effluent from the hollow fiber ultra-filtration membrane (UF-HF) indicated that the diazepam removal at this stage was about 82.1%, whereas about 90.4% of the drug was cumulatively removed after

passing the spiral wound (UF-SW) membrane (Table 4, appendix B).
Additionally, there was no complete removal of diazepam from the effluent exiting the GAC filter (93.7%); whereas, the RO unit showed 100% diazepam removal. It should be worth noting that the concentration of diazepam in the GAC effluent was 0.05mg L^{-1}, whereas, after the passage into the UF filters the concentration in the GAG influent water was just a little bit higher (0.08 mg L^{-1}).

Finally, Analytical results of water effluent from the hollow fiber ultra-filtration membrane (UF-HF) indicated that SP was about 69.9% removed at this stage, whereas about 92.8% of SP was removed after passing the spiral wound (UF-SW) membrane (Table 5, appendix B). Besides, SP was completely removed in the effluent from GAC filter. However, it should be outlined that the concentrations of SP influent in the treatment units were diminishing along their sequence. This relationship reflected upon 100% removal by GAC filter, whose influent water contained only 0.06 mg L^{-1} of SP, on average, after the passage through the UF filters.

That finding made unnecessary the use of reverse osmosis for any further purification. Nevertheless, the advanced technology adopted in the WWTP of Al-Quds University did not overcome a problem common to all plants: the production of brine, in which a large portion of the contaminants ends up being concentrated there. For this reason different methods of water filtration and purification should be experimented.

3.4 Stability of DSP, DZ and SP in pure water and in sludge

Literature survey on the stability of DSP suggests that the drug undergoes degradation in aqueous solutions buffered at various pH values, temperature, light exposure and oxidative conditions [57]. Furthermore, DSP cleaves into four major degradation products. They were identified as dexamethasone-21-oic acid, 17-oxodexamethasone, 6ß-hydroxy dexamethasone, and 16, 17-unsaturated dexamethasone [58].

Figure 9, appendix A illustrates the HPLC chromatogram of DSP after two weeks of incubation in pure water at room temperature ($C_{(0)}$ = 100 mg L^{-1}). The peak at 6-minutes retention time is characteristic of the acidic form of DSP and the peak at 10.3-minutes can be attributed to its hydrolysis product dexamethasone.

The kinetic data of DSP hydrolysis in pure water are plotted in figure 10, appendix A (plot a) as natural logarithm of DSP concentration vs. time (days). The determination coefficient R^2 of the first order hydrolysis reaction was 0.9981, and the kinetic constant was 3.25 10^{-7} s^{-1}.
Similarly, kinetic study on the stability of DSP was conducted in the activated sludge at room temperature. The determination coefficient R^2 in this case was 0.9987 (Figure 10, plot b, appendix A), and the kinetic constant was 3.80 10^{-6} s^{-1}. The degradation half-life was diminished from 24.7 days in pure water to 2.1 days in the activated sludge where the concentration of the mother molecule was found at level of 1 mg L^{-1} after two weeks of incubation. The degradation rate in the sludge medium was about 12-fold faster than in pure water. The accelerated degradation can be attributed to bioactivity of the activated sludge.

The kinetic parameters of diazepam hydrolysis in pure water (100 mg L^{-1}) are illustrated in figure 11, appendix A as natural logarithm of diazepam concentration vs. time (days). The determination coefficient R^2 of the first order hydrolysis reaction was 0.9981, and the rate constant was 9.08 x 10^{-8} s^{-1} at pH 7.3.
Similarly, kinetics of diazepam in Al-Quds activated sludge at room temperature was studied starting from the same concentration used in pure water. The determination coefficient R^2 in this case was 0.9987 (Figure 11, appendix A), and the rate constant was 2.60 x 10^{-7} s^{-1}.The degradation half-life was diminished from 88.3 days in pure water to 30.4 days in the activated sludge, where the concentration of diazepam was found at a concentration of 14.4 mg L^{-1} after 60 days of incubation. The degradation rate in the sludge medium was about 3-fold faster than in pure water. The accelerated degradation can be attributed to the bioactivity of bacteria populations present in the activated sludge.

Kinetic studies on SP stability in pure water and sludge conditions have been undertaken, starting from an initial solution of 100 mg L^{-1}. The results showed that SP was unstable in both distilled water and Al-Quds University activated sludge, being susceptible to water hydrolysis and bacterial degradation. In both cases the degradation followed a first order rate with values of the rate constants being 7.4 × 10^{-7} s^{-1} and 3.6 × 10^{-5} s^{-1}, in pure water and in activated sludge, respectively.

The degradation half-life was diminished from 10.7 days in pure water to 0.22 days in the activated sludge where the concentration of the parent molecule was found at a level of 4.6 mg L^{-1} after one day of incubation.

Monitoring the derivative substances arisen from the degradation of DSP in the activated sludge evidenced that the first degradation product, dexamethasone, underwent further degradation to other by-products as identified–by mass spectrometrical analysis. Extracted ion chromatogram (XIC) of the 14-days biodegraded sample is shown in figure 12, appendix A. The benefit of using very selective extracted ion chromatograms by FTICR/MS, generated with a tight mass-to-charge ratio window of ±0.0010 units around each selected protonated molecule (i.e., $[M+H]^+$ ±1.0 mDa), greatly reduced the signal complexity of the total ion current trace (data not shown) allowing to completely characterize all degradation products.

In addition to dexamethasone, which was formed from hydrolysis of dexamethasone sodium phosphate, seven major biodegradation products were identified arising from dexamethasone biodegradation at retention times 8.53(**13**), 10.23(**14**), 9.68(**15**), 10.11(**16**), 8.59(**17**), 8.59(**18**) and 10.48(**19**) minutes. Based on the accurate m/z values (Table S1, in Supplementary material) and relevant literature [58], we propose the following structures for all degradation products evolved (Figure 13, appendix A):
$C_{20}H_{25}FO_3$ [17-oxodexamethasone] (**13**),
$C_{22}H_{29}FO_6$ [6'-hydroxy dexamethasone] (**14**),
$C_{20}H_{27}FO_3$ [testosterone] (**15**),

$C_{22}H_{28}O_6$ [3',4'-dihydroxy-10,13-dimethylspiro [1,2,6, 7, 8,9,12,14,15,16-deca hydro cyclopenta [α] phenanthrene-17,5'-oxolane]-2',3,11-trione] (**16**), $C_{20}H_{22}O_2$ [(8S,13S,14S,17S)-13-methyl-3-oxo-2,6,7, 8,14, 15,16,17-octahydro-1H-cyclopenta [α] phenanthren-17-yl] (**17**), $C_{20}H_{24}O_3$ [(8S,13S,14S,17R)-17-ethynyl-17-hydroxy-13-methyl-1,2,6,7,8,14,15, 16-octahydrocyclopenta [α] phenanthren-3-one] (**18**), $C_{22}H_{31}O_6F$ [6-Fluoro-11,14,17,21-tetrahydroxy-16-methylpregn-4-ene-3,20-dione (**19**).

Figure 14, appendix A describes the suggested pathway by which dexamethasone degrades to metabolites (**14**) and (**19**). The hydroxyl group can attack two different positions on the dexamethasone moiety. Figure 15, appendix A illustrates the proposed pathways for the degradation of (**14**) to the other degradation products (**13**, **16**, **17** and **18**).

The FTICR IRMPD (infrared multiphoton dissociation) MS/MS spectrum, reported in figure S1 (Supplementary material), shows that compound (**18**) gives a molecular peak at m/z 313 and three fragments with m/z 295 $[M-H_2O+H]^+$, 277 $[M-2H_2O+H]^+$, and 267 $[M-H_2O-CO+H]^+$, due to the loss of H_2O and CO. In figure 15, Appendix A, the first way (A-B) leads from compound (**14**) to compound (**13**) by the loss of H_2O, formaldehyde and CO moieties, then to compound (**18**) through the loss of HF. The second way (C-D) leads from compound (**14**) to compound (**16**) by the loss of HF molecule, and successively to compound (**18**) with the loss of water, formaldehyde and CO groups.

At our knowledge no papers have been published on biodegradation of DSP or dexamethasone in wastewater. However, there are some *in vitro* and *in vivo* studies in rat and human livers on DSP metabolism to 17-oxodexamethasone and 6-hydroxy dexamethasone, which are two of main derivative substances we report in figure 13 as number (**13**) and (**14**), and side chain cleaved metabolites [58] having a structure different from derivatives identified in our work.

Monitoring the substances arising from the degradation of SP in the activated sludge indicated that SP underwent degradation to two by-products, as identified by mass spectrometrical analysis. Extracted ion chromatogram (XIC) of the 16-days biodegraded sample is shown in figure 16, appendix A. The benefit of using very selective extracted ion chromatograms by FTICR/MS, generated with a tight mass-to-charge ratio window of ±0.0010 units around each selected protonated molecule (i.e., $[M+H]^+$ ±1.0 mDa), greatly reduced the signal complexity of the total ion current trace (data not shown) allowing to completely characterize all degradation products.

Degradation products of SP in wastewater solution were identified through LC/MS analysis, which showed that SP can degrade to two metabolites, Canrenone ($C_{22}H_{28}O_3$) (**9**) (Figure 6, appendix A) with high percent and to other metabolites, e.g., Canrenoic acid (**20**) (Figure 17, Appendix A) which has the chemical formula ($C_{22}H_{30}O_4$) with less amount; the analysis showed that SP was still present in solution but at very low concentration. All metabolites were identified with an error lower than 2 ppm.

There is no predominant metabolite containing sulfur which is thought to be primarily responsible together with spironolactone for the therapeutic effects of the drug [31]. LCMS analysis results agree with the conclusions of Pramar et al.[38] who described Spironolactone decomposition to Canrenone by a series of reaction [Spironolactone → Canrenone→ unknown products]. Spironolactone is extensively metabolized in humans, and ≈79% of the spironolactone oral dose is converted to canrenone, its major biologically active metabolite [38]. Canrenone undergoes hydrolysis of its γ-lactone ring to canrenoic acid (CA), which is water soluble. Thus, after equilibrium, similar plasma concentrations of CA and canrenone are reached [45]. LCMS analysis showed no presence of metabolites containing potassium or sodium; this disagrees with other evidence from invitro studies,[45] which suggested that Canrenone is the principal active metabolite of potassium canrenoate.

3.5 Adsorption isotherms

The adsorption of DSP, DZ and SP at several initial concentrations on micelle-clay complex and activated charcoal were investigated. Equilibrium relationships between adsorbent and adsorbate can be described by Langmuir adsorption isotherm [50], represented by equation (1):

$$C_e/Q_e=1/(k.Q_{max})+C_e/Q_{max} \quad (1)$$

where C_e (mg L^{-1}) is the equilibrium concentration of the drug in the solution, Q_e (mg g^{-1}) is the equilibrium mass of adsorbed drug per gram of complex or activated charcoal, k (L mg^{-1}) is the Langmuir binding constant, and Q_{max} (mg g^{-1}) is the maximum mass of drug removed per gram of complex.

DSP Data fitted well the Langmuir equation giving R^2 = 0.9953 for activated charcoal and 0.9997 for the micelle-clay (Figure 18, appendix A). The calculated Langmuir constants k and Q_{max} are presented in table 6, Appendix B. The values of k and Q_{max} parameters for the adsorption isotherm obtained using micelle-clay complex were larger than those concerning activated charcoal, suggesting the former as the best adsorbent for DSP removal. In particular, the Langmuir binding constant <k> for micelle-clay complex was about 15 fold greater compared to the activated charcoal, and the value of Q_{max} was nearly 6 fold higher for the former.

Diazepam data fitted well the Langmuir equation giving R^2 = 0.9945 for activated charcoal, and 0.9970 for the micelle-clay (Table 7, appendix B). The calculated Langmuir constants k and Q_{max} are presented in Table 7, Appendix B. The values of k and Q_{max} parameters for the adsorption isotherm obtained using micelle-clay complex were larger than activated charcoal, suggesting the former to be a more efficient adsorbent for diazepam removal. In particular, the Langmuir binding constant <k> for micelle-clay complex was about 1.4 fold greater than activated charcoal, and the value of Q_{max} was nearly 1.1 fold higher for the former.

The data fitted well the Langmuir equation for SP giving R^2 = 0.964 for activated charcoal and 0.935 for the micelle-clay. The calculated Langmuir constants k and Q_{max} are

presented in Table 8, appendix B. The values of k and Q_{max} parameters for the adsorption isotherm obtained using the micelle-clay complex were 1.2- and 1.7- fold larger than the corresponding values deduced for activated charcoal. The analysis of the Langmuir equation yields that a deduction of an overestimate for the value of Q_{max} would yield an underestimate in the value of k and vice versa. The presentation of the Langmuir equation in another form as in [47] emphasizes the fact that the quantity which governs the adsorption is the product k* Q_{max}. Hence we added in Table 8, appendix B, this quantity, whose values are 58.7 and 28.6 Lg^{-1}, for the adsorption of SP by the micelle-clay, or activated charcoal, respectively, which emphasizes the former as the better adsorbent for SP removal.

3.6 Filtration results

DSP, DZ and SP solutions were passed separately through filters which included the micelle-clay complex or activated charcoal mixed with excess sand at 1:50 ratios (w/w). Results (Table 9, appendix B) indicate a significant advantage of the micelle-clay filter in removing DSP compared to the drug amount removed by activated charcoal. The efficiency of filter filled with activated charcoal and sand was acceptable only for the lowest DSP concentration while the micelle clay system was able to retain the drug also at the higher concentration experimented. This finding was not surprising, since parameters obtained for adsorption isotherms have clearly shown that the micelle-clay complex was more efficient than activated charcoal in adsorbing DSP from water.

Diazepam solutions were passed through filters which included the micelle-clay complex or activated charcoal mixed with excess sand at 1:50 ratio (w/w). The results shown in Table 10, appendix B indicate a significant advantage of the micelle-clay filter in removing diazepam compared to the amount removed by the activated charcoal. The efficiency of filter filled with activated charcoal and sand was adequate only for the lowest diazepam concentration, while the micelle clay system was able to remove the drug also at the higher concentration experimented. This result is not surprising, since the parameters obtained for adsorption isotherms of the two adsorbents clearly showed that the micelle-clay complex was more efficient than activated charcoal in adsorbing and removing diazepam from water.

The results in Table 11 (Appendix B) demonstrate removal of SP by filtration of 1 L of its several solutions (100, 10, 1.0, 0.01 mg L^{-1}) through a filter which included mixtures of micelle-clay or activated charcoal with excess sand. Complete removal was observed for SP concentrations of 10 mg L^{-1} or less by both filters. In the case of the higher concentration of 100 mg L^{-1} the emerging concentration of SP through the activated charcoal filter was almost two-fold larger than through the micelle-clay filter. This result is in accord with the results of adsorption in suspension, indicating the higher efficiency of the micelle-clay complex to remove this pharmaceutical from water.

Previously reported experiments demonstrated the poor capability of activated carbon filters towards removing of anionic and certain neutral pollutants [47-49].

Polubesova et al. [47] found very efficient removal of three anionic pollutants (imazaquin, sulfentrazone, sulfosulfuron) and 4 neutral pollutants (alachlor, acetochlor, chlorotoluron and bromacil) by micelle –clay complexes in aqueous dispersions. In another study [48] column filters filled with a mixture of quartz sand and micelle–clay complex provided very efficient result for the retention of tetracycline and sulfonamide pharmaceuticals from wastewater.

Zadaka et al. [49] tested column filters with either a mixture of quartz sand and organic micelle – montmorillonite or zeolite; both filters were capable to well remove ethylene di-bromide, anionic pollutants as sulfosulfuron, imazaquin and sulfentrazone, and neutral compounds as bromacil and chlorotoluron from aqueous environments; in contrast a filter filled with the same weight of activated carbon and sand removed only partially these pollutants.

In a recent paper, Karaman et al. [50] showed that micelle-clay filters are more efficient towards removal of diclofenac potassium from wastewater than activated carbon. Moreover, Khamis et al. [27] concluded that the incorporation of micelle-clay filters in sewage treatment systems with loose tertiary capability can be a promising technology. More recently, Khalaf et al. [51] suggested that the integration of clay-micelle complex filters in existing WWTPs may be helpful for improving removal efficiency of recalcitrant residues of non steroid anti inflammatory drugs (NSAIDs).

It can be argued that in addition to DSP, DZ and SP residues wastewater usually includes other recalcitrant organic pollutants. In such cases GAC filters can be used as first stage tertiary process to remove the majority of neutral pollutants, and additional micelle-clay filters can be adopted as second stage to eliminate anionic pollutants, and neutral compounds not retained by GAC filters.

4. Conclusions

The kinetic study revealed that DSP and SP were unstable in pure water and in sludge. The degradation products were identified by LC-MS and LC/MS/MS techniques, and those occurred in the sewage sludge were found to include not only the already known metabolites 17-oxodexamethasone and 6'-hydroxy dexamethasone for DSP and Canrenone as known metabolites for SP , but also many others derivatives not previously investigated.

Diazepam was found to undergo not-complete degradation both in water and sludge. Hence, further removal of this pharmaceutical from WWTP effluents can be a required issue. The WWTP of Al-Quds University showed that the advanced treatment technologies installed were effective for the complete removing of few quantities of both DSP and its hydrolysis product and also SP from 1 mg L^{-1} spiked wastewater.

The filtration performed using the mixture sand/micelle-clay complex was able to retain very high concentrations of DSP, DZ and SP from aqueous solutions. The large effectiveness and removal capacity of the micelle-clay complex are due to the high adsorption affinity towards the anionic DSP by the relatively large number of positively charged and hydrophobic sites of the micelle-clay complex based on ODTMA. The results indicate that integration of

micelle clay complex is very promising in achieving complete elimination of this drug from WWTP effluents.

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Appendix A

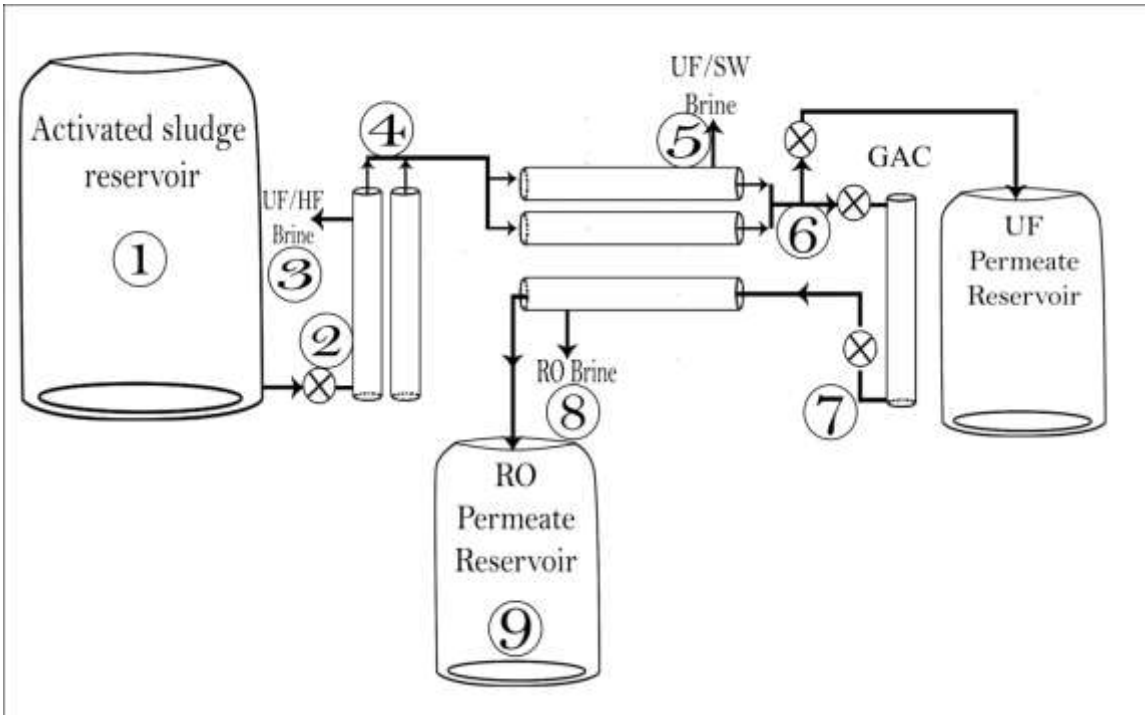


Figure 1: Flow diagram schematizing the WWTP at Al-Quds University. Sampling sites are indicated by numbers. UF/HF, hollow fiber ultrafiltration membrane; UF/SW, spiral wound ultrafiltration membrane; RO, reverse osmosis; GAC, granular activated charcoal filter.

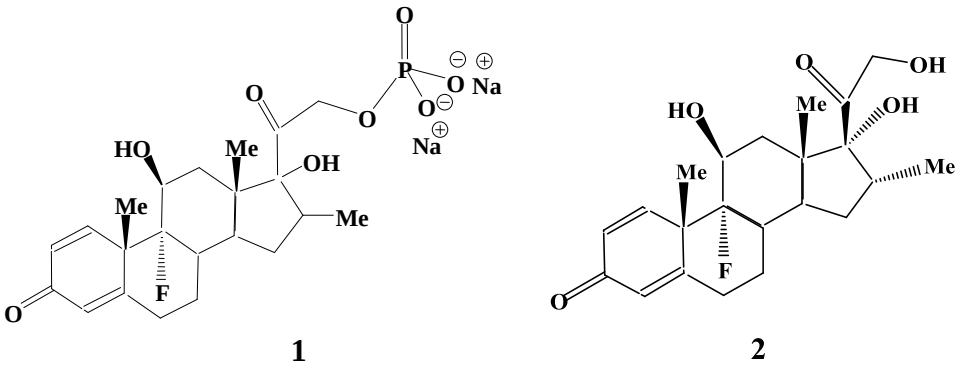


Figure 2: Structures of DSP (**1**) and its hydrolysis product dexamethasone (**2**).

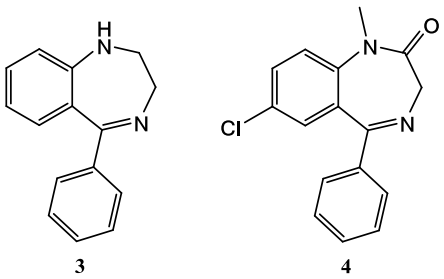


Figure 3: benzodiazepines basic structure (**3**), and diazepam (**4**) chemical structure.

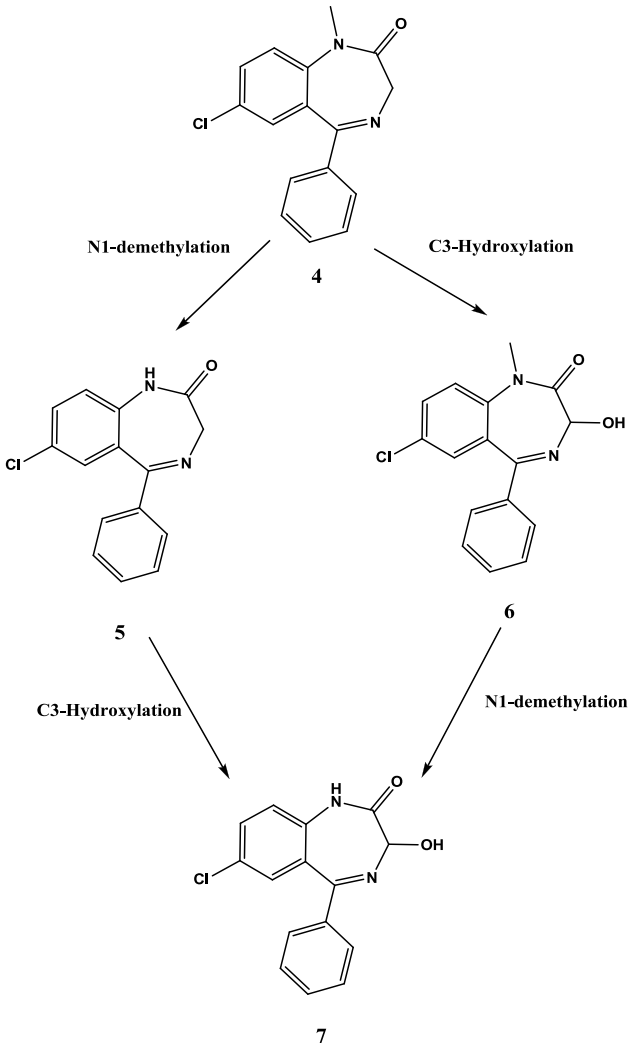


Figure 4: Metabolism pathways for diazepam (**4**) in humans.

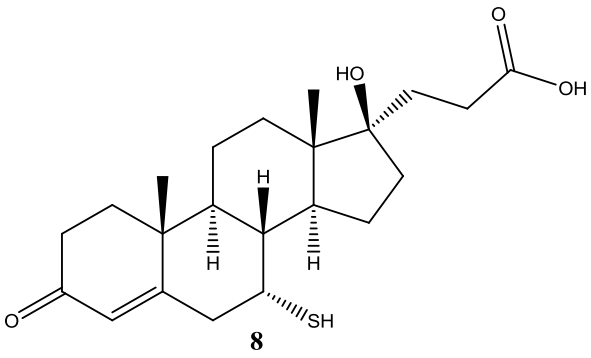


Figure 5: Chemical structure of Spironolactone (**8**)

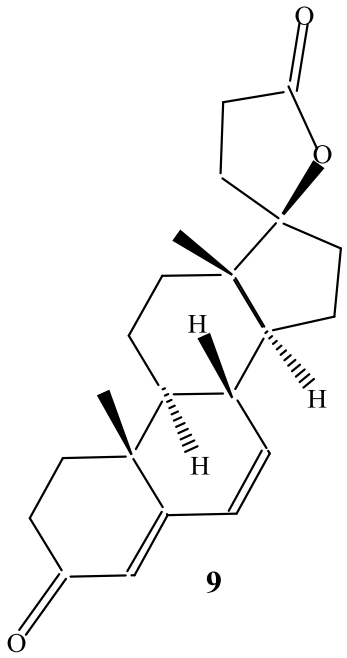


Figure 6: Chemical structure of Canrenone (CR) (**9**)

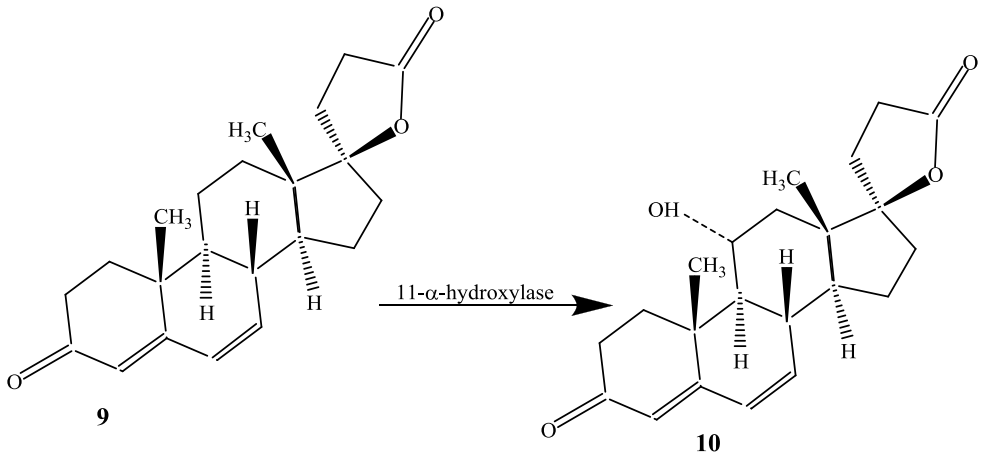


Figure 7: 11- α-hydroxylation of Canrenone by microbial transformation

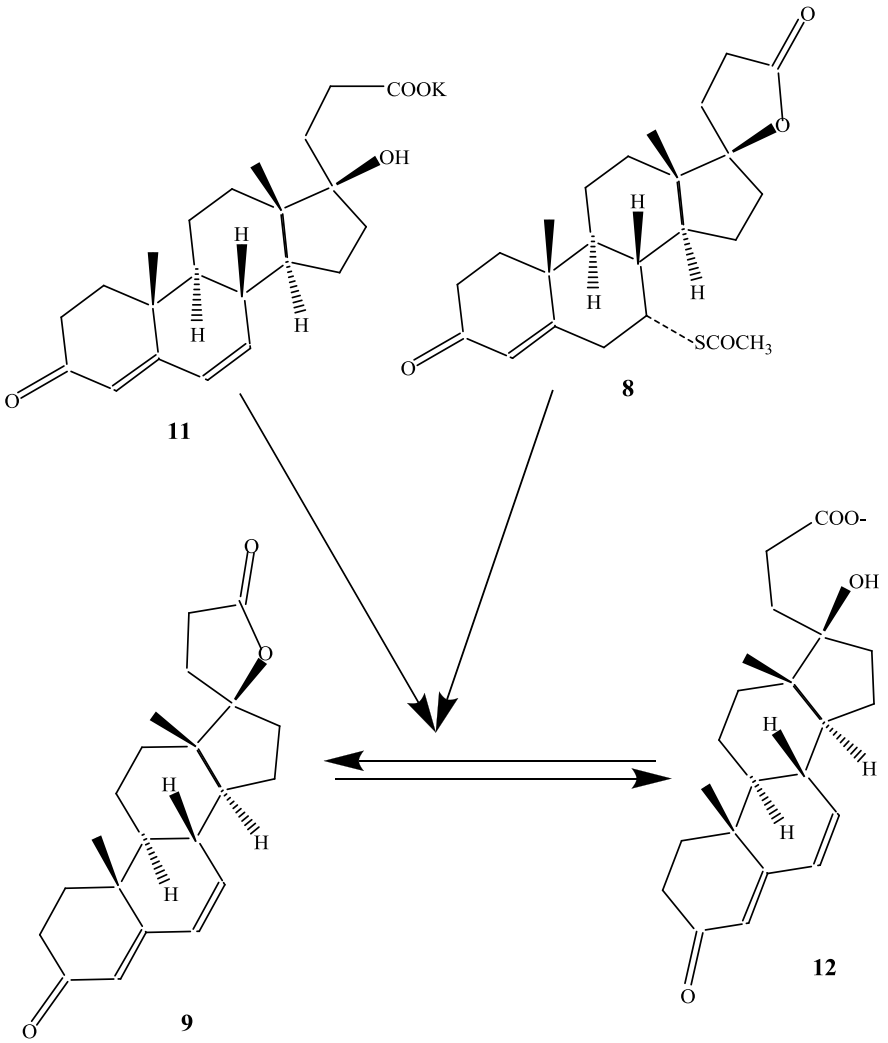
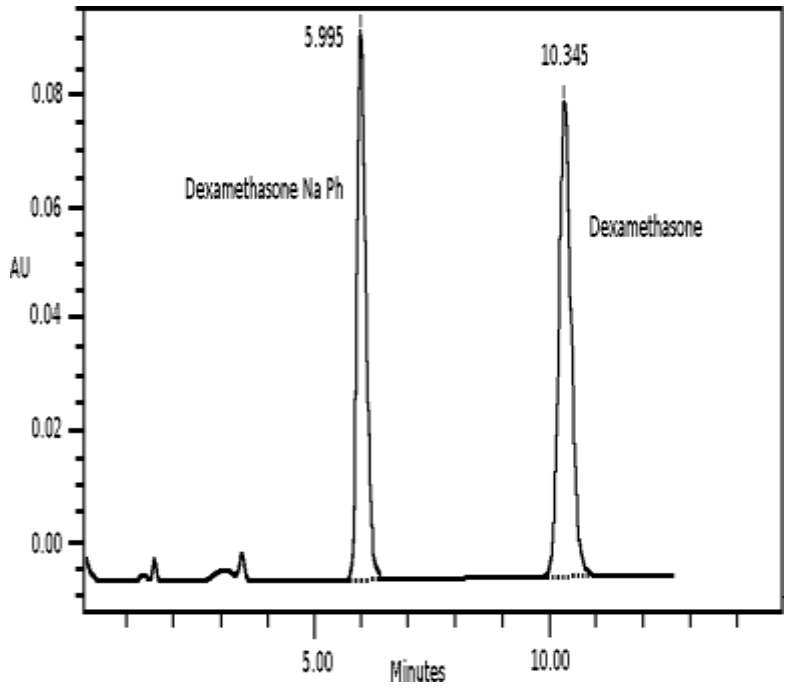


Figure 8: Structures of Spironolactone (**8**), Canrenone (**9**), Potassium canrenoate (**11**), and canrenoate (**12**)

Figure 9: Chromatogram showing the appearance of an intense peak attributable to dexamethasone, the hydrolysis product of



dexamethasone sodium phosphate risen after two weeks of incubation in pure water.

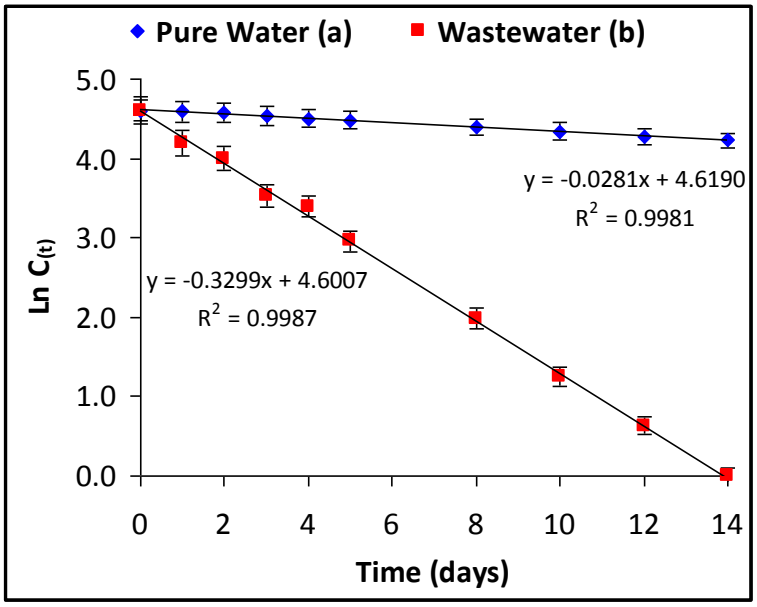


Figure 10: Kinetics of DSP degradation in pure water (plot a) and activated sludge (plot b). Data are reported as natural logarithm of concentrations ($C_{(t)}$) *vs.* time. Initial concentration ($C_{(0)}$) = 100 mg L^{-1}. Plotted values are the means of three replicates; bars represent the standard deviations calculated for each average value.

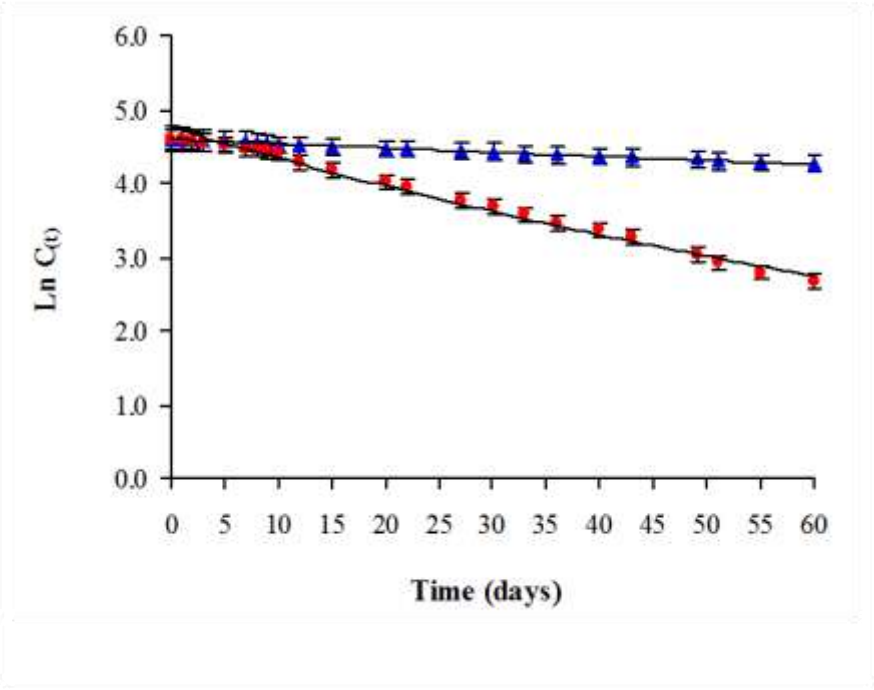


Figure 11: Plot of Ln of diazepam vs. time in pure water (▲) (SD± 0.12) and in sludge (●) (SD ± 0.17). T = 25 °C, pH 7.3.

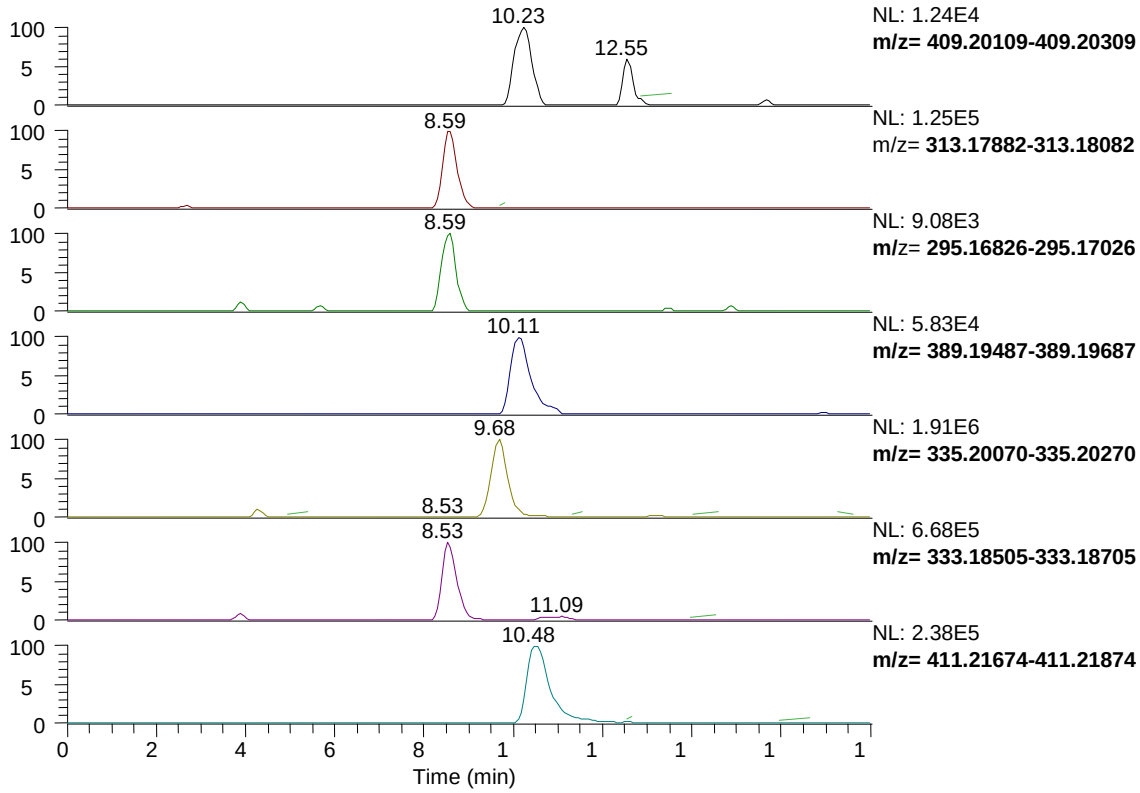
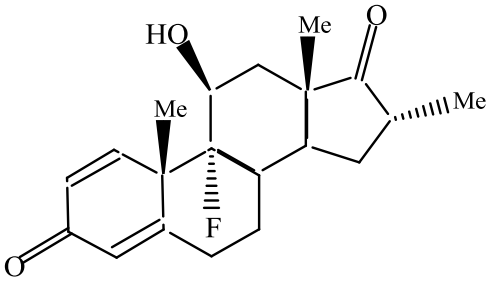


Figure 12: Extracted ion chromatograms (XICs) acquired by LC/ESI-FTICRMS in positive ion mode for the aqueous sample collected after two weeks of biodegradation from the activated sludge spiked with 100 mg L^{-1} of DSP. The ions monitored are displayed in each trace and correspond to the most abundant protonated moieties $[M+H]^+$, using a restricted window of ±0.0010 m/z unit centered around each selected ion (Table S1 in Supplementary material).



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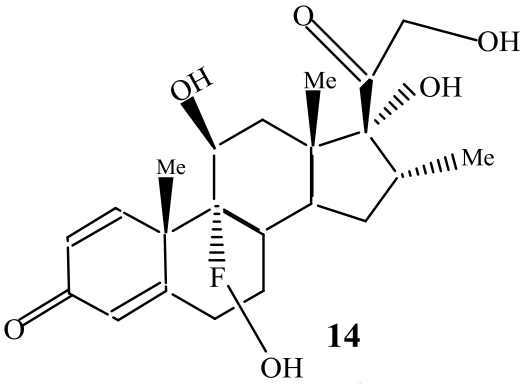

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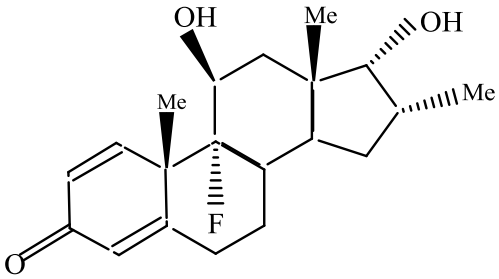

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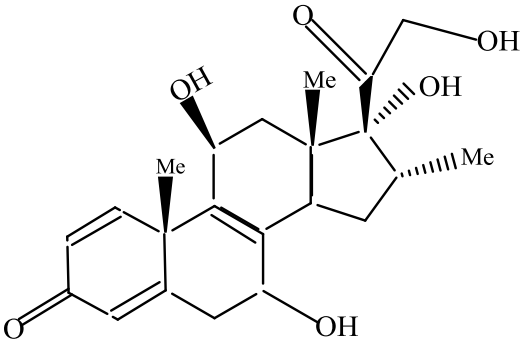

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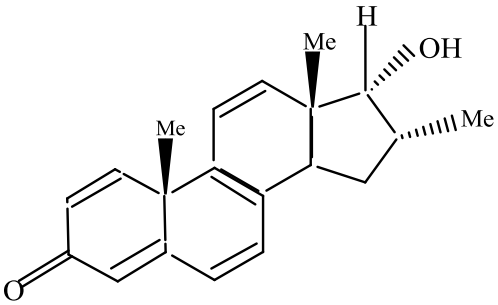

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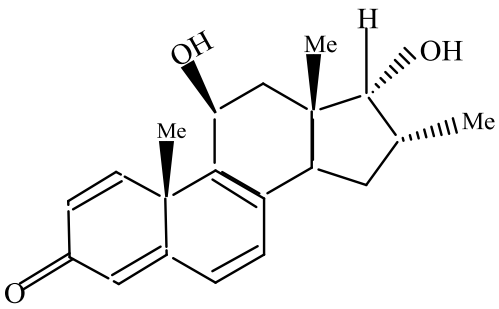

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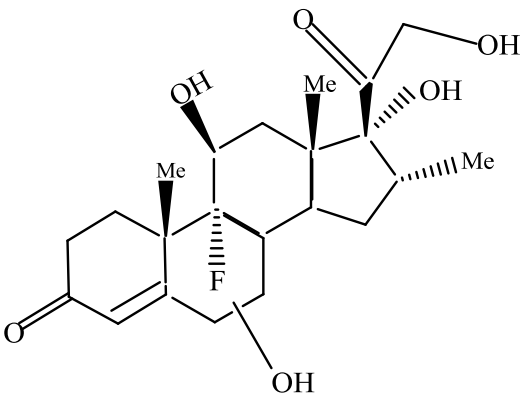


19

Figure 13: Chemical structures of dexamethasone biodegradation products. 17-oxodexamethasone $C_{20}H_{25}FO_3$, exact MW 332.17822 (**13**); 6-hydroxy dexamethasone $C_{22}H_{29}FO_6$, exact MW 408.19427(**14**); testosterone $C_{20}H_{27}FO_3$, exact MW 334.19387(**15**); 3',4'-dihydroxy-10,13-dimethylspiro[1,2,6,7,8,9,12,14,15,16-deca hydro cyclo penta [a] phenanthrene-17,5'-oxolane]-2',3,11-trione $C_{22}H_{28}O_6$, exact MW 388.18804(**16**); [(8S,13S,14S,17S)-13-methyl-3-oxo-2,6,7,8,14,15,16,17-octahydro-1H- clopenta[a]phenanthren-17-yl] acetate $C_{20}H_{24}O_3$, exact MW 312.17200 (**17**); (8S,13S,14S,17R)-17-ethynyl-17-hydroxy-13-methyl-1,2,6,7,8,14,15,16-octahydrocyclopenta[a]phenanthren-3-one $C_{20}H_{22}O_2$, exact MW 294.16143(**18**); [6-Fluoro-11,14,17,21-tetrahydroxy-16-methylpregn-4-ene-3,20-dione $C_{22}H_{31}O_6F$, exact MW 410.20992 (**19**).

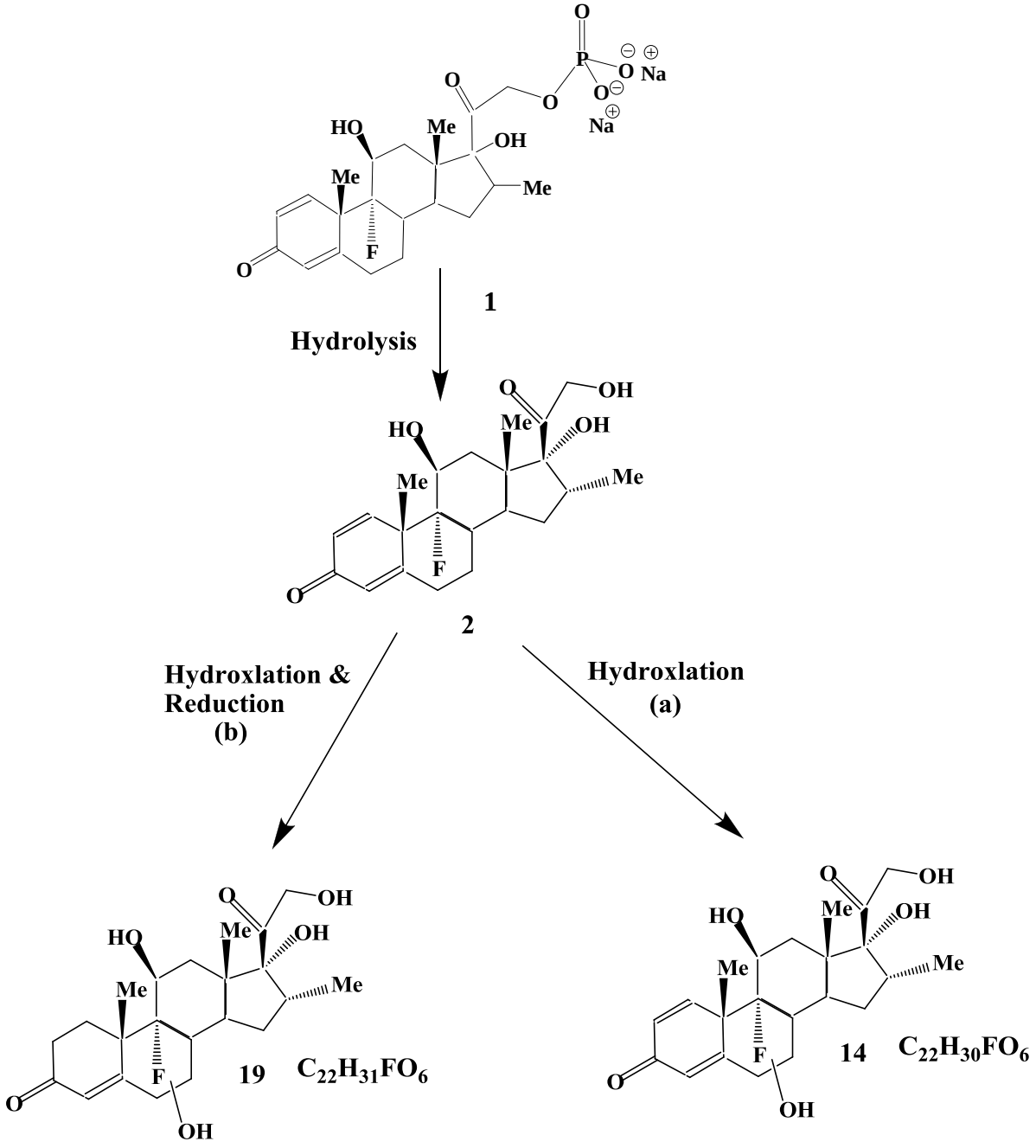


Figure 14: Proposed transformation pathway for the biodegradation of dexamethasone sodium phosphate (**1**).

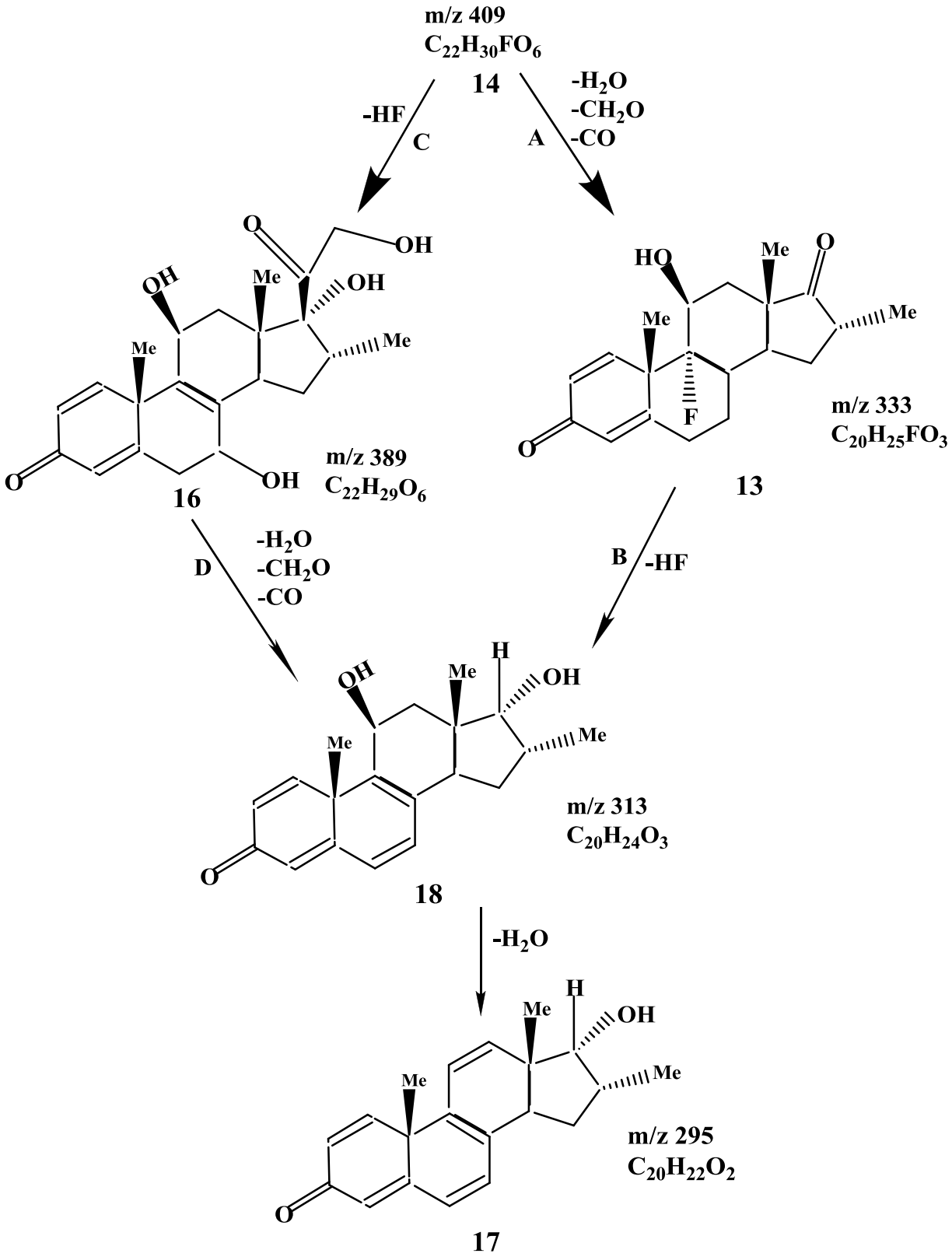


Figure 15: Proposed transformation pathways for dexamethasone (**14**).

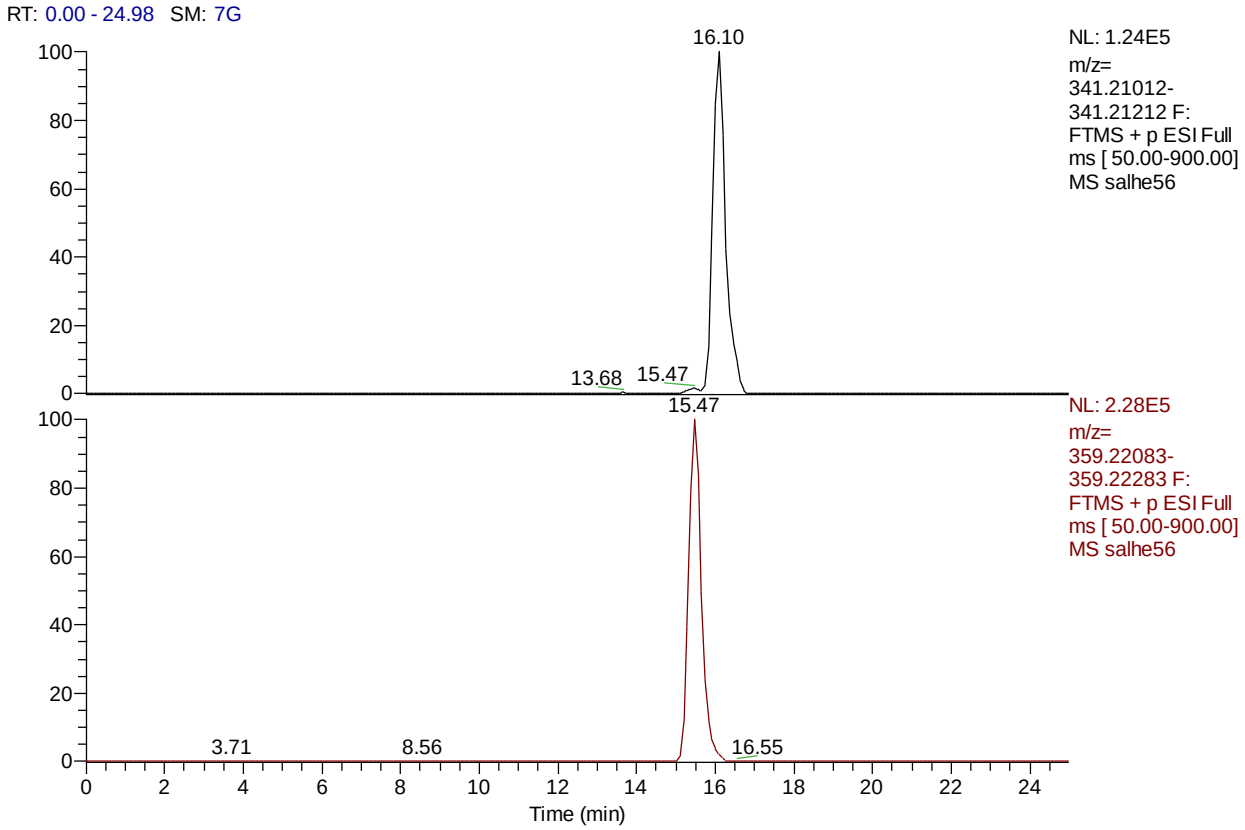
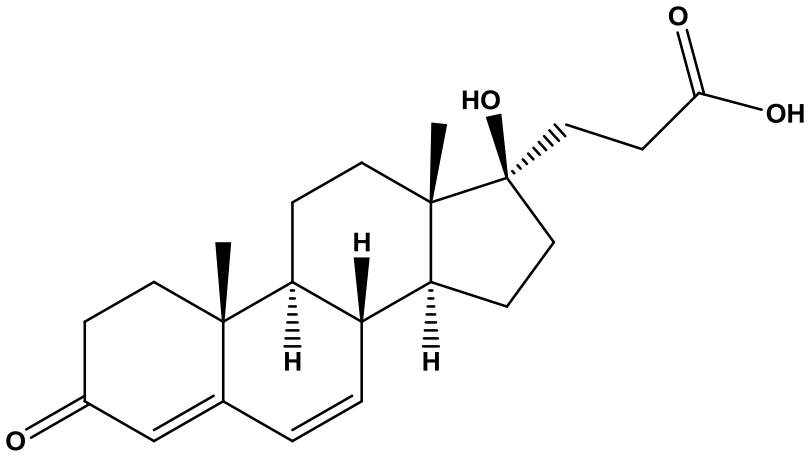


Figure 16: Extracted ion chromatograms (XICs) by LC/ESI-FTICRMS acquired in positive ion mode of SP solution after one month of biodegradation. The ions monitored are displayed in each trace and correspond to the most abundant molecules, $[M+H]^+$, using a restricted window of ±0.0010 m/z unit centered around each selected ion.



20
Canrenoic acid

Figure 17: Chemical structure for canrenoic acid (**20**)

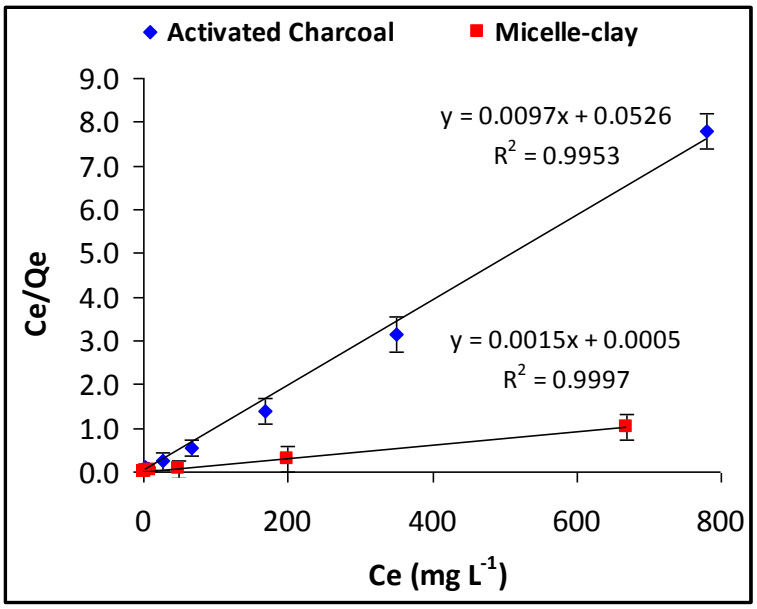


Figure 18: Langmuir isotherms for the adsorption of DSP by micelle-clay complex (■) and by activated charcoal (◆). Dosage of adsorbent (0.2 g). Data reported are means of three replicates. Bars represents standard deviations of means.

Appendix B

Table 1: Methods used and wastewater quality parameters measured in the Al-Quds WWTP.

Parameter measured	**Instrument used for analysis**	**Method of analysis**	**Reference**
pH	pH-meter 3320, Jenway	SM#4500-H+(B) (on site)	Direct measurement as manufacturer procedure APHA 1995, 19th ed. [52]
Conductivity	Conductivity meter 4320, Jenway	2520-B	
Total Coliforms and Fecal Coliforms	Membrane filter method	9222-B 9221-E	APHA 1995, 19th ed.
Orthophosphate	Automated ascorbic acid reduction	SM# 4500-PF	APHA 1995, 19th ed.
COD BOD_5	Hach COD reactor DO meter – Oxy 197	5210-B 5220-D	APHA 1995, 19th ed.
NH_4^+	Nesselarization method	4500A-NH_3	APHA 1995, 19th ed.
Total bacterial count	Pour plate method after serial dilutions	9215D	APHA 2005, 21th ed. [53]
Solids	Gravimetric methods	2540B 2540C 2540D	APHA 2005, 21th ed.

Table 2: Physical, chemical and biological parameters of wastewater to be treated

.

Parameters	Results	Units	Parameters	Results	Units
pH	7.32 ±0.01	------	TSS	3668 ±6	mg L^{-1}
Conductivity	2000 ±8	µSm cm^{-1}	BOD	915 ±5	mg L^{-1}
Temperature	15.5 ±0.2	°C	COD	1936 ±6	mg L^{-1}
Turbidity	4960 ±7	NTU	NH_4-N	59.5 ±0.1	mg L^{-1}
DO	0.40 ±0.01	mg L^{-1}	PO_4-P	14.3 ±0.1	mg L^{-1}
TS	4218 ±8	mg L^{-1}	FC (*E. coli*)	$2.9 \times 10^5 \pm 0.3 \times 10^5$	cfu/100mL
TDS	618 ± 4	mg L^{-1}	TC	$6.5 \times 10^6 \pm 1.3 \times 10^6$	cfu/100mL
Settable solids	240 ± 3	mg L^{-1}	TAC	$2.6 \times 10^7 \pm 1.3 \times 10^7$	cfu/100mL

[a]DO, dissolved oxygen; TS, total solid; TDS, total dissolved solids; TSS, total suspended solids; BOD, biological oxygen demand; COD, chemical oxygen demand; FC, fecal coliforms; TC, total coliforms; TAC, total aerobic count.

Table 3: Removal of DSP and dexamethasone (Hydrolysis Product – HP) from wastewater by different treatment units in Al-Quds WWTP; average values of three replicates.

Sample- description		Sampling site as in figure 1	Concentration of DSP and dexamethasone (HP) mg L^{-1}			
			Means ± S.D.		Remaining %	
			DSP	HP	DSP	HP
AS spiked amount		1	1.0	1.0		
UF-HF	influent	2	0.83 ± 0.05	0.81 ± 0.02		
	brine produced	3	0.46 ± 0.04	0.43 ± 0.04		
	effluent	4	0.31 ± 0.03	0.24 ± 0.02	37.3	29.6
UF-SW	brine produced	5	0.19 ± 0.02	0.17 ± 0.02		
	effluent	6	0.06 ± 0.03	0.07 ± 0.04	5.3	8.6
GAC effluent		7	b.l.d.	b.l.d.	≈0.0	≈0.0

b.l.d. = below the limit of detection

Table 4: Removal of diazepam from wastewater by different treatment units in Al-Quds WWTP; average values of three replicates.

Sample description		Sampling point as in figure 1	Concentration of DZ mg L^{-1}	
			Means ± S.D.	Removal %
The initial concentration of Diazepam in storage tank		1	0.98 ± 0.10	
UF-HF	influent	2	0.84 ± 0.05	
	brine produced	3	0.57 ± 0.03	
	effluent	4	0.15 ± 0.02	82.1
UF-SW	brine	5	0.15 ± 0.04	
	effluent	6	0.08 ± 0.01	90.4
GAC effluent		7	0.05 ± 0.02	93.7
RO	brine	8	0.07 ± 0.01	
	effluent	9	b.l.d.	≈ 100.0

b.l.d. = below the limit of detection

Table 5. Removal of SP from wastewater by different treatment units in Al-Quds WWTP; average values of three replicates.

Sample description		Sampling site	Concentration of SP mg L^{-1}	
			Means ± S.D.	Removal %
The initial concentration of SP in storage tank (after addition of SP)		1	1.1 ± 0.06	
UF-HF	influent	2	0.83 ± 0.02	
	brine produced	3	0.49 ± 0.05	
	effluent	4	0.25 ± 0.01	69.9
UF-SW	brine	5	0.22 ± 0.02	
	effluent	6	0.06 ± 0.04	92.8
GAC effluent		7	b.l.d.	≈ 100.0

b.l.d. = below the limit of detection

Table 6: Langmuir adsorption parameters (k and Q_{max}) and determination coefficients (R^2) obtained from the adsorption of DSP on the micelle-clay complex and activated charcoal.

Adsorbent	**k** (L mg^{-1})	**Q_{max}** (mg g^{-1})	**R^2**
Micelle-clay complex	2.795	652.1	0.9997
Activated charcoal	0.184	103.4	0.9953
Ratio micelle-clay/charcoal	15.2	6.3	-

Table 7: Langmuir adsorption parameters (k and Q_{max}) and determination coefficients (R^2) obtained from the adsorption of diazepam on the micelle-clay complex and activated charcoal.

Adsorbent	**k (L mg^{-1})**	**Q_{max} (mg g^{-1})**	**R^2**
Micelle-clay complex	5.3±0.2	31.2 ± 1.7	0.997
Activated charcoal	3.8 ±0.3	28.9 ± 1.5	0.994
Ratio micelle-clay/charcoal	1.4	1.1	-

Table 3: Langmuir adsorption parameters (k and Q_{max}) and determination coefficients (R^2) obtained from the adsorption of SP on the micelle-clay complex and activated charcoal.

Adsorbent	**k** (L mg^{-1})	**Q_{max}** (mg g^{-1})	k* Q_{max} (L g^{-1})	**R^2**
Micelle-clay complex	3.3± 0.3	17.8± 2.5	58.7±1.5	0.935
Activated charcoal	2.7± 0.3	10.6± 2	28.6±1.1	0.964

Table 9: Removal of DSP by filtration of 1L of pure water solutions (100, 10, 1.0, 0.01 mg L^{-1}) through laboratory filters, which included either MC or GAC mixed with excess sand at 1:50 (w/w) ratio; means of three replicates.

Initial concentration (mg L^{-1})	**Column type** [a]	**Average eluted concentration (mg L^{-1})**	**±SD**
100	MC	b.l.d.	-
100	GAC	64.3	1.2
10	MC	b.l.d.	-
10	GAC	3.1	0.5
1.0	MC	b.l.d.	-
1.0	GAC	0.17	0.08
0.01	MC	b.l.d.	-
0.01	GAC	b.l.d.	-

[a] Flow rate, 2 mL min^{-1}; temperature, 25 ^{0}C; b.l.d., below the detection limit of the analytical method used.

Table 10: Removal of diazepam by filtration of 1L of water solutions diazepam (100, 10, 1.0, 0.01 mg L^{-1}) through laboratory filters consisting of either micelle-clay (MC) or GAC mixed with excess sand at 1:50 (w/w) ratio; means of three replicates.

Initial concentration (mg L^{-1})	Column type[a]	Average eluted concentration (mg L^{-1})	±SD
100	MC	0.7	0.1
100	GAC	1.95	0.5
10	MC	b.l.d.	-
10	GAC	1.4	0.9
1.0	MC	b.l.d.	-
1.0	GAC	0.1	0.02
0.01	MC	b.l.d.	-
0.01	GAC	b.l.d.	-

[a]Flow rate, 2 mL min^{-1}; temperature, 25 ^{0}C; b.l.d., below the detection limit of the analytical method used.

Table 11: Removal of SP by filtration of 1L of water solutions through laboratory filters, which included either MC or GAC mixed with excess sand at 1:50 (w/w) ratio; means of three replicates. [a]

Initial concentration (mg L^{-1})	Column type [a]	Average eluted concentration (mg L^{-1})	±SD
100	MC	13.5	3.5
100	GAC	24	4.2
10	MC	b.l.d.	-
10	GAC	b.l.d.	-
1.0	MC	b.l.d.	-
1.0	GAC	b.l.d.	-
0.01	MC	b.l.d.	-
0.01	GAC	b.l.d.	-

[a]Flow rate, 2 mL min^{-1}; temperature, 25^{0}C; b.l.d., below the detection limit of the analytical method used.

Supplementary Material

Table S1: Compounds occurring in DSP solution (activated sludge) after two weeks of biodegradation, identified as $[M+H]^+$ ions by LC–ESI-FTICR/MS.

Accurate m/z	Formula of identified ions $[M+H]^+$	Retention time (min)	Error (ppm)
411.21808	$C_{22}H_{31}FO_6$ (**9**)	10.42	0.80
409.20232	$C_{22}H_{30}FO_6$ (**4**)	10.23	0.50
389.19595	$C_{22}H_{29}O_6$ (**6**)	10.11	0.20
335.20163	$C_{20}H_{27}O_3F$ (**5**)	9.68	-0.20
333.18619	$C_{20}H_{26}O_3F$ (**3**)	8.56	0.40
313.17987	$C_{20}H_{25}O_3$ (**8**)	8.59	0.15
295.16928	$C_{20}H_{23}O_2$ (**7**)	8.59	0.08

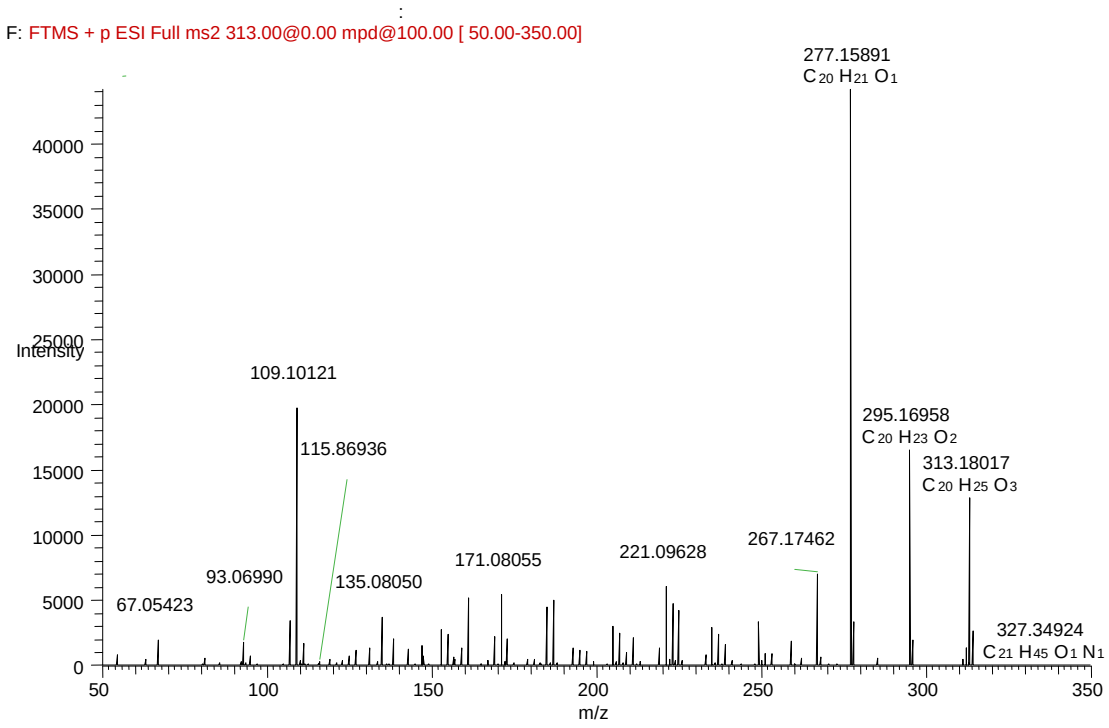


Figure S1: Positive ion electrospray - IRMPD mass spectrum of degradation moiety peak at m/z 313.