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Investigation of an amoxicillin oxidative degradation product formed under controlled environmental conditions

Igal Gozlan,[A,B] *Adi Rotstein*[A,B] *and Dror Avisar*[A,C]

[A]The Hydro-chemistry Laboratory, Department of Geography and Environmental Studies, Tel Aviv University, Tel Aviv, 69978, Israel.

[B]The Porter School of Environmental Studies, Tel Aviv University, Tel Aviv, 69978, Israel.

[C]Corresponding author. Email: droravi@post.tau.ac.il

Environmental context. Although amoxicillin is a widely used antibiotic, it is yet to be detected in the aquatic environment. This study traces the production of the amoxicillin-S-oxide degradation product, and shows that it is consistently obtained only under sunlight irradiation. This is the first study to demonstrate the formation, under controlled environmental conditions, of this chemically stable product of amoxicillin.

Abstract. Amoxicillin (AMX) is a widely used penicillin-type antibiotic, and its presence in the environment has been widely investigated. The formation and structure of an oxidised degradation product (DP) of AMX are described in the present work. The experiments were carried out in buffer solution (pH 7.5) containing AMX at a concentration of 100 μg mL^{-1}, with and without acid and in field secondary effluent. The DP, AMX-S-oxide (sulfoxide), was consistently obtained only under sunlight irradiation and was significantly augmented by the addition of humic acid (5 mg L^{-1}) and mainly in field secondary effluent, which acts as a natural photo-sensitiser. The structure of the AMX-S-oxide DP was determined by an LC-MS technique using a mobile phase of deuterated and non-deuterated solvents. A ^{1}H NMR spectrum was obtained for the pure compound isolated by preparative HPLC. Further confirmation of the AMX-S-oxide structure was achieved by comparison of its UV spectrum with those of the two oxidation products, AMX-S-oxide and hydroxylated AMX, obtained by the ozonolysis of AMX.

Additional keywords: AMX-S-oxide, antibiotic residues, LC-MS, NMR, secondary effluent.

Introduction

The presence of pharmaceuticals, especially antibiotics, in the aquatic environment has raised increasing concern in recent years. Human and veterinary pharmaceuticals are among a group of 'emerging' contaminants.[1–3] The majority[4] of pharmaceutical compounds enter aquatic systems by the sewage treatment network after ingestion and subsequent excretion as non-metabolised parent compounds or metabolites.[5] The increasing use of pharmaceutical compounds and the formation of their metabolites have led to an increase in their presence in the environment. As has been previously reported, the elimination of these compounds by sewage treatment plants was found to be relatively low and, consequently, effluent containing antibiotic residues and their degradation products (DPs) may be released into the aquatic environment and instigate genetic selection of more harmful and antibiotic-resistant bacteria, a matter of great concern.[6]

The penicillins, β-lactam-containing antibiotics, are one of the most important groups of antibiotics in medicinal and veterinary use. Within this group, the most consumed subgroup is the aminopenicillins, which includes ampicillin, amoxicillin (AMX), epicillin and bacampicillin. These semi-synthetic penicillin-type antibiotics are distinguished from the 'conventional' penicillins by the presence of an extra amine group on the side chain. They are used in the treatment of a variety of infections, such as upper respiratory tract, urinary tract, meningitis and *Salmonella* infections, and are effective for both gram-positive and gram-negative bacteria and are therefore labelled as 'broad-spectrum penicillins'.[7]

The instability of β-lactam antibiotics in solution was observed to be a major hurdle in the development of penicillin and other useful β-lactam antibiotics. Therefore, degradation and stability studies of β-lactam antibiotics have been of paramount importance, not only in terms of their market availability, but also in order to evaluate their pharmacokinetic properties and potential for adverse reactions. It is interesting to note that the stability (or rate of degradation) of different β-lactam antibiotics in vivo and in vitro is quite different. However, the major pathways of their degradation are similar, and lead to various breakdown products in a majority of the β-lactams. The degradation of penicillins has been well studied, and it was found that various metal ions, such as mercury,[8] zinc,[9,10] cadmium,[11] cobalt[12] and copper,[13] may catalyse the degradation of the β-lactam ring. Furthermore, it was determined[14] that hydrolysis of the β-lactam ring occurred in various aqueous acidic conditions.[15]

Photolytic degradation processes carried out with an environmental sample (effluent) are caused by absorption of UV light by a chemical species (direct photolysis),[16,17] or are mediated by natural photo-sensitisers such as nitrate and humic acids (indirect photolysis).[18] Other studies reported that enhancement of the photodegradation of AMX[19] and other pharmaceuticals[20] was achieved by the presence of humic acid in the solution as a natural photo-sensitiser. An additional study

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reported on an AMX DP achieved by dye-sensitised photo-oxidation.[21]

The present study focuses on AMX, which is one of the most widely used antibiotics in both human and veterinary medicine. Amongst the known AMX DPs are diketopiperazine amoxicillin, amoxicillin penicilloic acid and amoxicillin penilloic acid (dimer and trimer),[22–24] all of which were investigated in regard to their pharmacological characteristics, with no consideration of environmental effects. Thus, the objectives of the present study were to determine the natural formation of an unidentified AMX DP (AMX-S-oxide), which was constantly obtained in this study and produced only under sunlight; and to characterise its chemical structure using various analytical techniques. In addition, an ozonation was performed in order to examine whether the AMX-S-oxide may also be formed by this process; and to verify its chemical structure. It should be mentioned that the environmental detection (e.g. secondary effluent and groundwater), of this AMX DP will be published elsewhere.

Experimental

Chemicals and standards

Amoxicillin trihydrate (99.8%) of analytical standard was purchased from Sigma Aldrich (Israel). AMX-S-oxide was made in situ (see below). Methanol and water (both ULC/MS grade) were purchased from Biolab (Israel). Deuterium oxide and methanol-*d* were purchased from Sigma Aldrich (Israel). Argon gas (99.999%) was purchased from Gordon Gas (Israel). Humic acid was purchased from Fluka. Samples of field secondary effluent were obtained from a waste water treatment plant in Israel (Shafdan).

Working solutions and experiments

An AMX solution, at a concentration of 100 $\mu g\,mL^{-1}$, was buffered at pH 7.5 by adding Na_2HPO_4 and H_3PO_4 in ULC/MS water. The initial, non-environmental AMX concentration (100 $\mu g\,mL^{-1}$) was chosen to be high enough for NMR analysis but sufficiently low to avoid intermolecular AMX reactions. The ionic strength was adjusted to a constant value of 0.05 M. The sealed Pyrex glass-bottled samples were kept under natural sunlight (from winter at 10–20°C to summer at 30°C, latitude: 32°, altitude: sea level), and in the shade (control) up to 7 days, during the degradation process. This time span was chosen after preliminary tests demonstrated the optimal period for the degradation process (to give the highest amount of AMX DP). Irradiation experiments were carried out during various seasons, representing different climatic conditions. An additional irradiation experiment was carried out by bubbling argon gas through an AMX solution and immediately sealing it in an ampoule. This experiment was followed by a control AMX solution without argon. A parallel irradiation experiment was carried out on an AMX solution (100 $\mu g\,mL^{-1}$, buffered at pH 7.5) with the addition of humic acid at a concentration of 5 $mg\,L^{-1}$. The chemical fate of AMX in field secondary effluent was examined separately in order to compare its degradation rate *v.* the humic acid solution. These two solutions were kept for 3, 6 and 16 days under sunlight.

Sample analysis

Liquid chromatography–mass spectrometry (LC-MS) analysis of AMX and its DPs (after exposure to sun) was performed by high performance liquid chromatography (HPLC)/UV (DAD, Agilent 1100) coupled to a mass spectrometer (Finnigan LCQ), using an RP-phenyl ACE column (250 × 2.1 mm^2, 5 μm).

The method was adapted from Lamm et al.,[25] with some modifications. The column temperature was set to 28°C, the flow rate was set to 0.5 $mL\,min^{-1}$ and the volume injection was 20 μL. UV absorption was recorded at 230 and 275 nm. The HPLC mobile phase consisted of methanol (A), and water adjusted to pH 2.5 with trifluoroacetic acid (TFA) (B). The elution gradient started with 5% A, increased to 75% over 8 or 15 min, and was then held at 75% for 2 min. The same analytical method was used for the analysis of AMX DPs obtained by the ozonation process, differing only in the gradient elution, which was converted into an isocratic condition consisting of 5% A and 95% B (for deuterated and non-deuterated solvents).

For the mass measurements, an electrospray ionisation (ESI) interface in positive mode was used. The solvent flow from the HPLC to the MS interface was split by a T connector and set to 20 $\mu L\,min^{-1}$. MS analysis was carried out using a full scan mode. Instrument control, data acquisition and evaluation were performed with *Chemstation* (for HPLC/UV) and *Xcalibur 3.2* (for MS) software. In addition, the identification of AMX-S-oxide was supported by HPLC/MS run under isocratic conditions using D_2O (95%, pH 2.5 with TFA) and MeOD (5%) as the mobile phase instead of the non-deuterated solvents. To complete the AMX-S-oxide identification, ^1H NMR (Varian, NMR system 500 MHz, model unity plus) analysis was performed.

Preparative separation of AMX-S-oxide

AMX-S-oxide was isolated from an AMX sample after irradiation by natural sunlight. The isolation process was carried out using an HP1050 HPLC system with a semi-preparative Vydac C18 column (250-mm length, 10-mm inner diameter, and 10-μm particle size). The composition of the mobile phase and the elution gradient program were the same as for the above analytical method.

Pre-column enrichment was carried out using a Rheodyne switching valve model 7000, enabling a sample loading of ~30 mL per run. Prior to loading, the sample was adjusted to pH 4 by the addition of TFA. In order to avoid degradation of the compound, the isolated fraction was immediately diluted with two volumes of water, frozen and then lyophilised.

Argon experiment

The main goal of this experiment was to determine the source of the oxygen which formed the obtained AMX-S-oxide DP. In order to remove oxygen, argon was bubbled (for 10 min) into two glass ampoules (duplicate) filled with 50 mL of AMX buffer solution and then immediately flame sealed. Duplicate control samples (without argon) were prepared. Subsequently, these samples were irradiated under natural sunlight for a period of one week. The oxygen degassing efficiency, measured in different solutions using an AP64 Dissolved Oxygen Meter (Fisher Scientific), indicated a low concentration of residual oxygen (less than 3%).

Ozonolysis of AMX

Ozonolysis was performed using an ozone generator in order to explore the possibility of obtaining other AMX oxidation products from the AMX molecule. O_3 was produced in an O_3 generator (type LN 103 AT, kindly provided by Ozonia, Duebendorf, Switzerland) by regulation of the voltage (2550 mA) and gas flow (166–208 $cm^3\,min^{-1}$), with oxygen as

the substrate. AMX was dissolved in phosphate buffer (100 μg mL^{-1}) at pH 7.5. The process was conducted at room temperature over a span of 1 min.

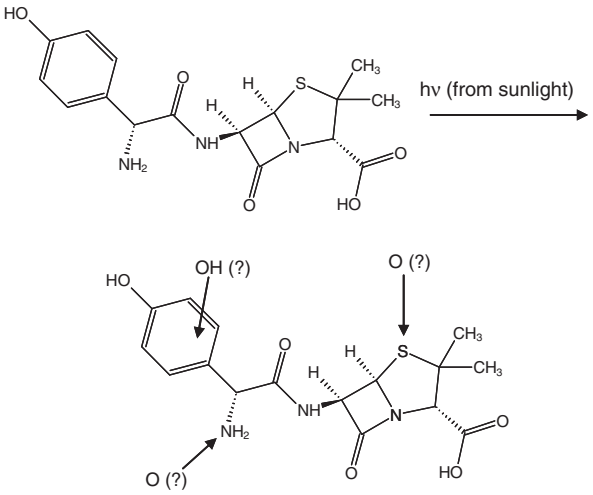
Results and discussion

The behaviour of AMX was investigated after exposure to natural sunlight compared with shadow (control), and also in response to ozonation. The sunlight irradiation experiment was also conducted in the presence of humic acid as a natural photosensitiser. The AMX solution was buffered with a phosphate solution to a pH of 7.5. An additional experiment was performed under sunlight for both AMX in humic acid solution (pH of 7.0) and field secondary effluent (pH of 6.8).

Natural sun experiments

Both AMX exposure experiments (sun and shadow) were conducted following the same procedure and under the same conditions. The two LC/UV-MS chromatograms obtained (AMX retention rime, R_t, of 6.3 min) were similar, except for an unidentified peak (R_t 5.4 min and 1.4% of the total AMX sample) which appeared only in the chromatogram of the sample exposed to sunlight (Fig. 1).

The ESI$^+$/MS spectra (Table 1) of the unknown DP and AMX, show the molecular mass of the unknown DP to be 381 ($[MH]^+ = 382$) and 365 for AMX ($[MH]^+ = 366$). Based on the difference of 16 amu between the two molecules, it was proposed that oxygen had been added to the AMX molecule. The oxygenation reaction could occur at one of the following three locations: (1) on the aromatic ring (substitution of hydrogen atom with a hydroxy group); (2) on the amine group (to obtain an N-oxide product); and (3) on the sulfur atom (to obtain an S-oxide product):



(1)

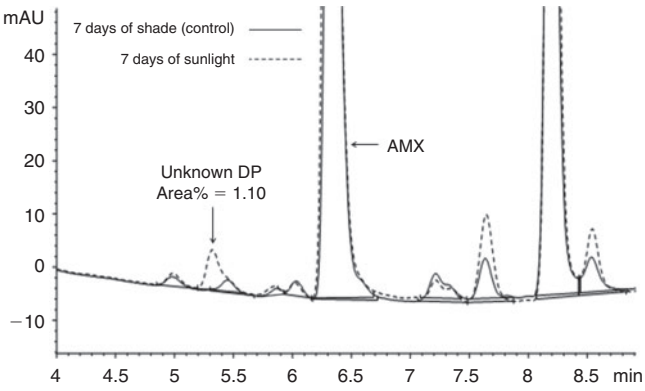


Fig. 1. High performance liquid chromatography/UV chromatograms showing amoxicillin (AMX) samples exposed to natural sunlight over 7 days under natural sun and 7 days in the shade.

In addition, the mass spectra for both compounds (Table 1) show the same fragmentation pattern: (1) the elimination of NH_3 (AMX: $m/z = 349$ and unknown DP: $m/z = 365$); (2) the elimination of CO and NH_3 (AMX: $m/z = 321$ and unknown DP: $m/z = 337$); and (3) the formation of dimers in the ESI source (AMX: $[2M + H]^+ = 731$ and unknown DP: $[2M + H]^+ = 763$). Moreover, the oxygenation of the amine group could be ruled out owing to: (1) the elimination of the NH_3 group from the unknown DP and (2) the dimer ($[2M + H]^+$) is formed in the presence of an acid group together with an amine group in the molecule.

As mentioned above, the results obtained from both the UV and ESI$^+$/MS spectra clearly indicate that the oxygenation process of AMX does not change the nature of the aromatic ring. It seems that the only possible site for the oxygenation process is the thioether group ($\lambda_{max} = 275$ nm).

In order to verify these findings, the following additional experiments were performed:

1. Performing the ESI$^+$/MS experiments of AMX and the unknown DP with deuterated solvents as the mobile phase (D_2O and MeOD), in order to determine the number of exchangeable hydrogen atoms in the unknown DP molecule compared with AMX. Five such exchangeable hydrogen atoms were determined for both compounds.
2. Conducting the ozonolysis under conditions similar to those used by Andreozzi et al.,[26] which they claimed produced a phenol product.

Table 1. Mass spectrometry (positive electrospray ionisation) data for three molecules: amoxicillin (AMX), AMS-S-oxide and hydroxy-substituted AMX using both deuterated and non-deuterated solvents

Molecular ion	AMX		AMX-S-oxide (unknown DP)		Hydroxy-substituted AMX
	Non deuterated solvent	Deuterated solvent	Non deuterated solvent	Deuterated solvent	Deuterated solvent
$[MH]^+$	366	–	382	–	–
$[MD]^+$	–	372	–	389	388
$[MH–NH_3]^+$	349	–	365	–	–
$[MD–ND_3]^+$	–	352	–	368	369
$[MH–NH_3–CO]^+$	321	–	337	–	–
$[2M + H]^+$	731	–	763	–	–

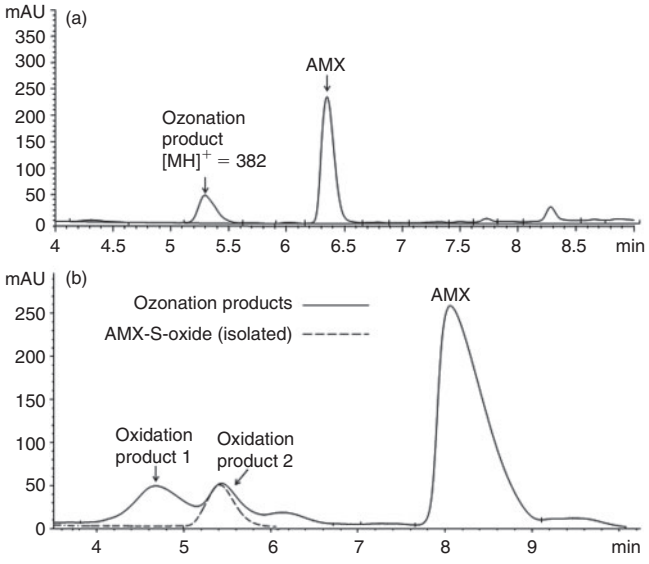


Fig. 2. Liquid chromatography/UV-mass spectrometry chromatograms of: (a) ozonation products (under solvent gradient conditions); and (b) ozonation products (under solvent isocratic conditions) *v.* the isolated amoxicillin (AMX)-S-oxide product obtained by AMX exposure to natural sunlight.

3. Isolation of the oxidised AMX, the unknown DP, by preparative HPLC, in order to obtain its ^{1}H NMR spectrum.

Experiment performed in deuterated solvents

The use of deuterated solvents as the mobile phase was initially performed for AMX in order to determine whether the exchangeable hydrogen atoms are fully replaced by the deuterium atoms. In Table 1 the AMX MS data show that the molecular ion peak $[MH]^+ = 366$ was shifted to $[MD]^+ = 372$ upon the use of deuterated solvents, whereas $[MH–NH_3]^+ = 349$ was changed to $[MD–ND_3]^+ = 352$. These results verify the existence of five exchangeable hydrogen atoms (one acidic, one amide proton, two amine protons, and one phenolic proton) in the AMX molecule.

After applying the same procedure to AMX-S-oxide, the obtained molecular ion *m/z* 382 $[MH]^+$ was shifted to *m/z* 388 $[MD]^+$, whereas *m/z* 365 $[MH–NH_3]^+$ was shifted to *m/z* 368 $[MD–ND_3]^+$ (Table 1). These results confirm the existence of only five exchangeable hydrogen atoms on both AMX and AMX-S-oxide, meaning that oxygenation did not occur on the aromatic ring. From these results it may be concluded that the oxidisation of AMX results in the formation of the S-oxide product. In order to reinforce this suggestion, the ozonolysis of AMX was carried out.

Ozonolysis

Andreozzi et al.[26] previously performed an ozonation experiment resulting in the formation of an AMX hydroxy-substituted DP obtained by the attachment of a hydroxy group to the aromatic ring. Accordingly, ozonation of AMX was carried out in our study to obtain AMX-S-oxide, or any other possible AMX oxidation DP, in order to verify the formation of AMX-S-oxide in contrast to an AMX hydroxy-substituted DP. Initial experimental results showed a molecular ion peak where the AMX-S-oxide product was originally expected to appear, with a mass of $[MH]^+ = 382$, under typical LC/UV-MS gradient conditions (Fig. 2a). LC/UV-MS separation carried out under isocratic conditions resulted in two separate peaks with $R_t = 4.6$ and 5.4 min, and a molecular ion mass of $[MH]^+ = 382$ (AMX-oxide DPs) (Fig. 2b). Furthermore, the preparatively isolated, and deliberately injected, DP (AMX-S-oxide), appeared at $R_t = 5.2$ min, matching the retention time of the oxidation product 2 presented in Fig. 2b.

In order to determine the correlation between the obtained MS peaks (Fig. 2b) to the obtained DPs (AMX-S-oxide and AMX hydroxylated substitute), their UV spectra (Fig. 3) were examined. The results indicate similarities between oxidation product 2 (Fig. 3b), the obtained AMX-S-oxide DP (Fig. 3c) and the AMX spectrum (Fig. 3a) (peak at $\lambda = 275$ nm). In addition, oxidation product 1 (Fig. 3d) demonstrates a peak shifted to $\lambda = 284$ nm, suggesting the presence of the hydroxy-substituted AMX DP with substitution on the aromatic ring.

In addition to the above described UV findings, results were obtained by LC-MS analysis using deuterated solvents as the mobile phase (Table 1). Oxidation product 2 gives the same MS spectrum as that of the AMX-S-oxide DP. Both DPs have a molecular ion mass of $[MD]^+ = 388$ (Table 1), meaning they both consist of five exchangeable hydrogen atoms. Oxidation product 1 has a molecular ion mass of $[MD]^+ = 389$ and $[MD–ND_3]^+ = 369$, indicating that the hydroxy-substituted AMX molecule has six exchangeable hydrogen atoms.

^{1}H NMR analysis

Decisive proof for the proposed AMX-S-oxide structure was obtained by comparison of the ^{1}H NMR spectra of AMX and the isolated AMX-S-oxide. A comparison of their ^{1}H NMR spectra is presented in Table 2.

A lone pair of electrons resides on the AMX sulfur atom giving it a tetrahedral molecular geometry, subsequently, the oxygen bonded sulfur turns into a chiral centre. As a result, the bonded oxygen may appear at two positions: *isomer-a*: *cis* to $H_{(4)}$ and $CH_{3(11)}$ and *trans* to $H_{(2)}$ and $CH_{3(10)}$; and *isomer-b*: *trans* to $H_{(4)}$ and $CH_{3(11)}$ and *cis* to $H_{(2)}$ and $CH_{3(10)}$. In order to determine the position of the bonded oxygen, a nuclear Overhauser enhancement (NOE) experiment was conducted. The NOE results point to *isomer-a*, because of the existence of through space interactions between: (1) $H_{(2)}$ (shift from δ 5.44 ppm in AMX to 5.25 ppm in AMX-S-oxide) and $CH_{3(10)}$ (shift from δ 1.36 to 1.20 ppm); and (2) $H_{(4)}$ (shift from δ 4.09 to 4.31 ppm) and $CH_{3(11)}$ (shift from δ 1.37 to 1.57 ppm) (Table 2; Fig. 4).

Comparing with the AMX NMR spectrum (Table 2), the chemical shift of $CH_{3(11)}$ and $H_{(4)}$ of the AMX-S-oxide, which were shifted downfield, indicate that $CH_{3(11)}$ and $H_{(4)}$ are in a *cis* position relative to the bonded oxygen. Furthermore, $CH_{3(10)}$ and $H_{(2)}$, which were shifted upfield, signify that $CH_{(10)}$ and $H_{(2)}$ are in a *trans* position relative to the bonded oxygen (Table 2; Fig. 4).

The *cis* position of the bonded oxygen of *isomer-a*, related to $CH_{3(11)}$ and $H_{(4)}$, is explained by the steric hindrance caused by the carbonyl group.

Formation of the S-oxidation degradation product

There are two possible sources for the oxygen which can attack the AMX sulfur atom to form the S-oxide product: (1) water or (2) dissolved atmospheric oxygen. The results obtained from the irradiation experiments which were carried out after degassing the oxygen by bubbling argon gas into the AMX solution were

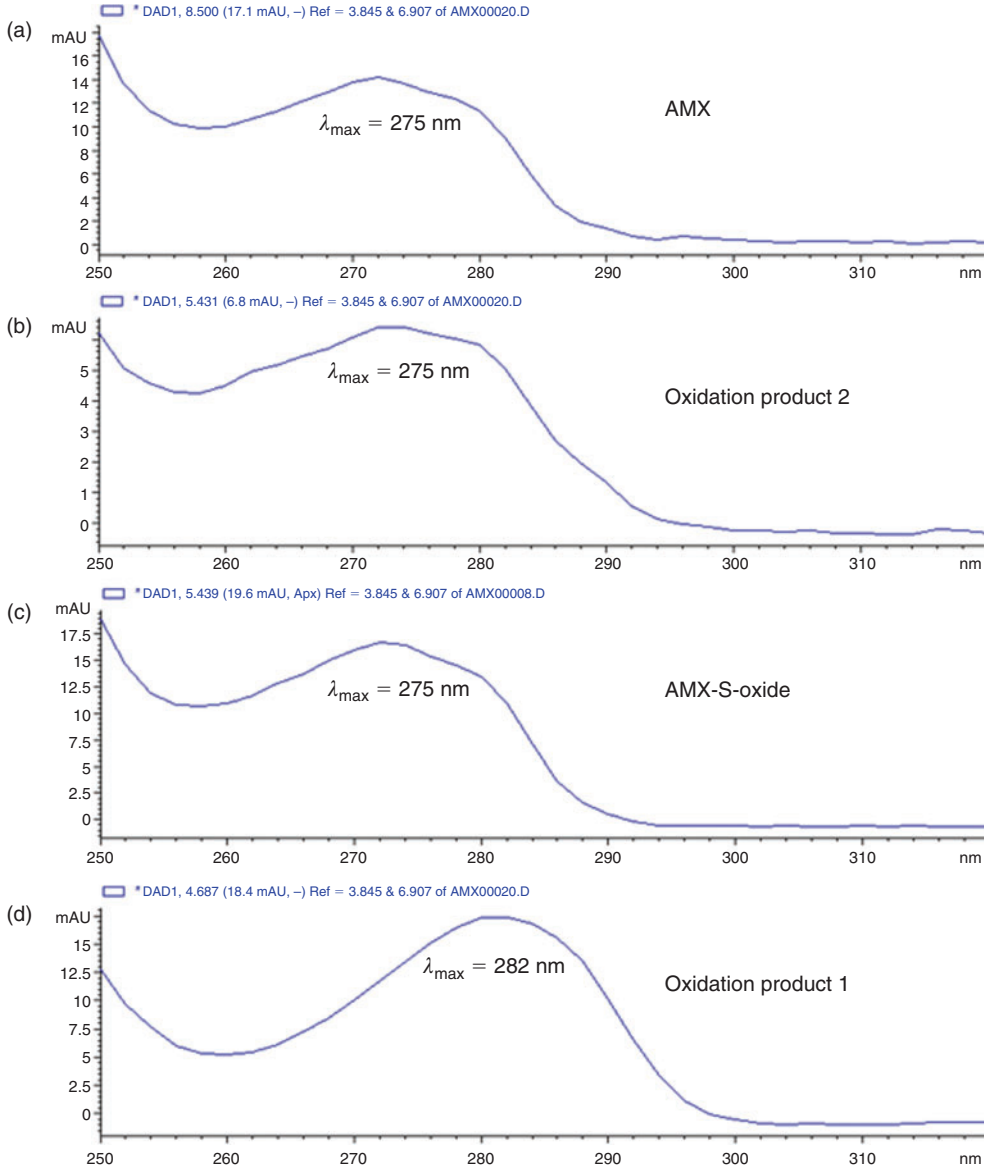
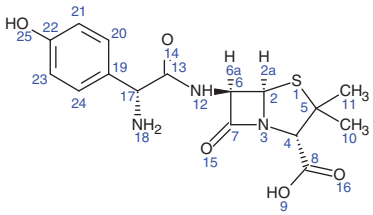
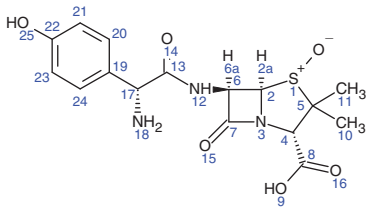


Fig. 3. UV spectra of the ozonation products: oxidation products 1 (d) and 2 (b) *v.* amoxicillin (AMX) (a) and AMX-S-oxide DP (c).

Table 2. ^{1}H NMR spectroscopic data for amoxicillin (AMX) and AMX-S-oxide

Hydrogen	AMX (chemical shift)	AMX-S-oxide (chemical shift)
		
CH_3	1.36, 1.37 (s)	1.20, 1.57 (s)
H-4	4.09 (s)	4.31 (s)
H-17	5.07 (m)	4.99 (m)
H-2	5.44 (m)	5.25 (d)
H-6	5.44 (m)	5.90 (d)
H-21,23	6.92 (d)	6.94 (d)
H-20,24	7.31 (d)	7.31 (d)

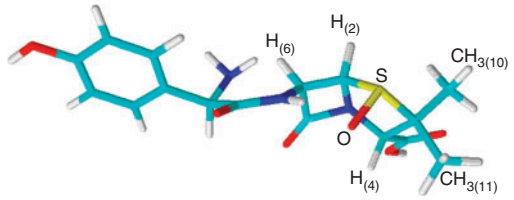


Fig. 4. 3-D molecular structure of the detected isomer of amoxicillin (AMX)-S-oxide.

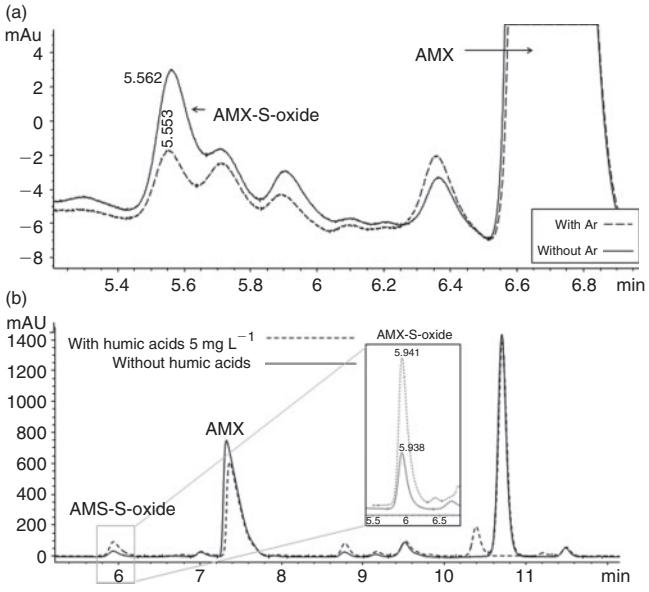


Fig. 5. High performance liquid chromatography/UV chromatograms of: (a) sunlight irradiated amoxicillin (AMX) solution with and without argon degassing, and (b) sunlight irradiated AMX solution with and without the addition of humic acids.

compared with the irradiation experiments of the AMX solution without degassing (Fig. 5a). Fig. 5a clearly demonstrates that the HPLC peak related to AMX-S-oxide (degassed) is dramatically reduced (~60% less) than the peak obtained without degassing. The appearance of AMX-S-oxide in the degassed solution, expressed by its reduced HPLC peak (Fig. 5a), is a result of residual (trace) oxygen measured (DO probe). It can therefore be suggested that the oxygen for the sulfur atom oxidation process originates in the dissolved atmospheric oxygen naturally found in aqueous solution (direct photolysis) as shown[19]:

$$\text{Direct photolysis: AMX (sulfide)} \xrightarrow[O_2]{h\nu(\text{sunlight})} \text{AMX-S-oxide (sulfoxide)}$$

An experiment was conducted with a buffer solution (pH 7.5) that contained humic acids (5 mg L^{-1}) as a natural photo-sensitiser, to examine whether its presence would enhance the production of AMX-S-oxide (indirect photolysis) as shown by the following[19]:

$$\text{Indirect photolysis: Sens (humic acids)} \xrightarrow[O_2]{h\nu(\text{sunlight})} \text{Sens}^*$$

$$\text{Sens}^* + O_2 \longrightarrow O_2{}^* + \text{Sens}$$

$$\text{AMX (sulfide)} \xrightarrow[O_2]{} \text{AMX-S-oxide (sulfoxide)}$$

Based on this experiment, the results (Fig. 5b) show that the AMX-S-oxide production was more than three-fold (4.2%) in the irradiated solution containing humic acids, indicating that the humic acid indeed acts as a natural photo-sensitiser, providing a significant augmentation of the AMX-S-oxide DP by the dominant indirect process.

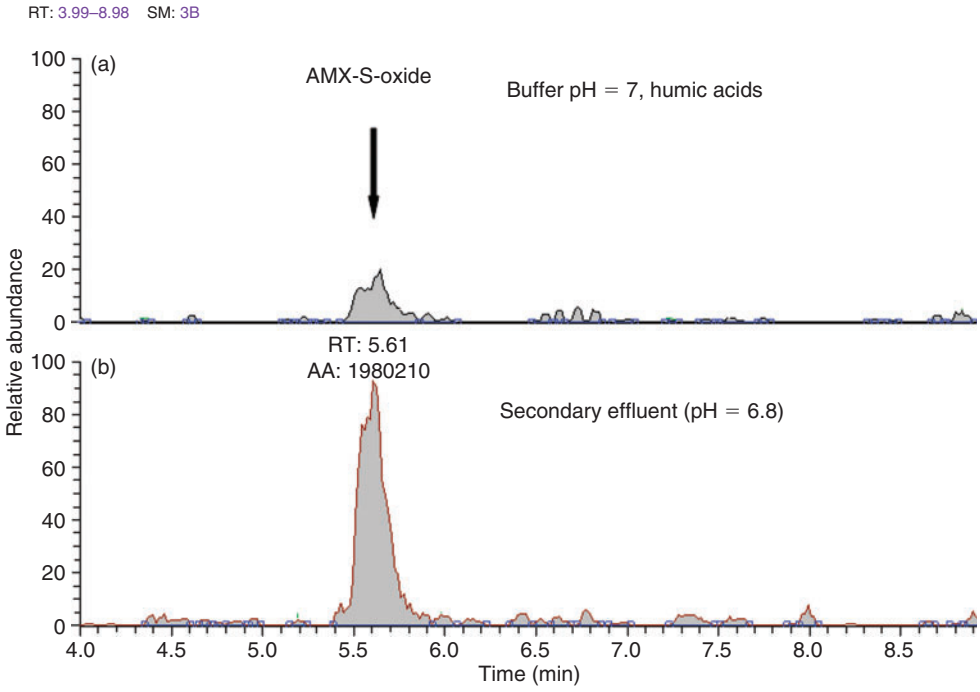


Fig. 6. Liquid chromatography/mass spectrometry chromatograms (trace of $m/z = 382$) of: (a) sunlight irradiated AMX solution with Buffer phosphate pH = 7 and 5 mg L^{-1} humic acids; (b) sunlight irradiated AMX in field secondary effluents (pH = 6.8) after 3 days of exposure.

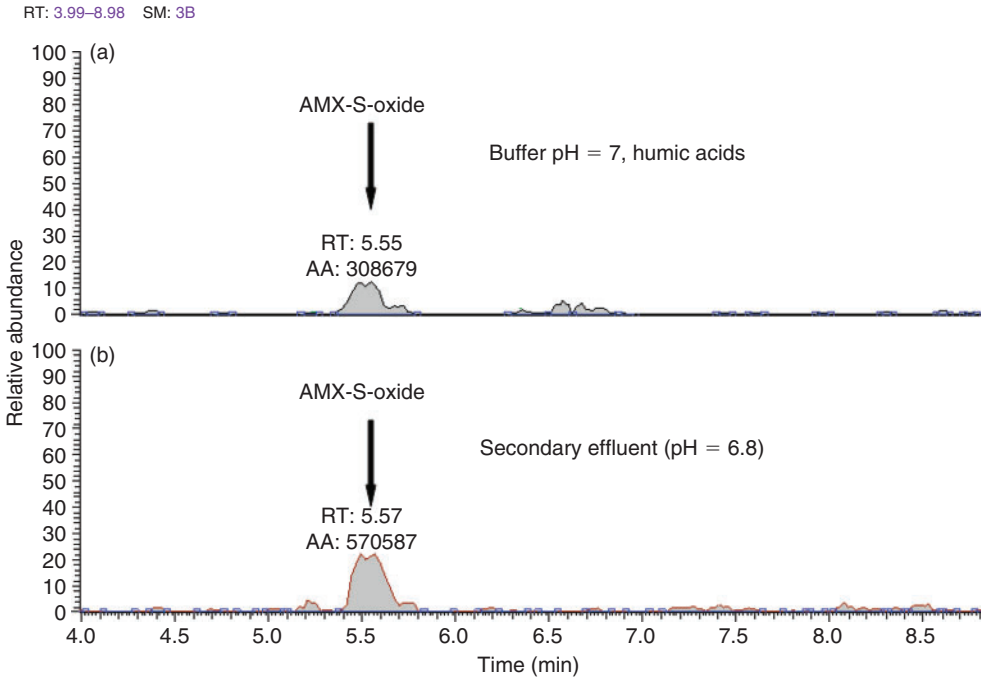


Fig. 7. Liquid chromatography/mass spectrometry chromatograms (trace of $m/z = 382$) of: (a) sunlight irradiated amoxicillin (AMX) solution with phosphate buffer at pH = 7 and 5 mg L^{-1} humic acids; (b) sunlight irradiated AMX in field secondary effluents (pH = 6.8) after 6 days of exposure.

Obtaining the AMX-S-oxide DP in field secondary effluent

An experiment was performed under sunlight for AMX (100 μg mL^{-1}) in humic acid (5 mg L^{-1}, phosphate buffer solution at pH 7.0) solution compared with field secondary effluent (pH 6.8). This experiment examined the AMX-S-oxide formation rate in both solutions. The results indicate that after 3 days the formation of AMX-S-oxide is accelerated in the secondary effluent compared with the humic acid solution by 4-fold (Fig. 6) and by 1.7-fold after 6 days (Fig. 7). This acceleration can be explained by the comparatively high occurrence of sensitisers, which may degrade the AMX to AMX-S-oxide by an indirect process.[19] Moreover, both results demonstrate a decrease in AMX-S-oxide amount throughout the time span of days (3–6 days), up to 0 in secondary effluent solution, after 16 days. This reduction may be explained by relatively high metal concentrations measured in the secondary effluent ($Ca^{2+} = 76$; $Fe^{2+} = 77$; $Sr^{2+} = 639$ μg L^{-1}), which may catalyse to form a metal chelate.[14]

Summary and conclusions

To the best of our knowledge, the present study is the first to produce and identify the AMX-S-oxide DP under controlled environmental conditions.

In environmental conditions, the AMX-S-oxide is produced under sunlight irradiation merely as an indirect photolysis process. In addition, a significant augmentation of the AMX-S-oxide production was obtained in the presence of natural photo-sensitisers (humic acids), which activate the oxygen to oxidise the AMX, forming the AMX-S-oxide DP.

The formation of the AMX-S-oxide DP is accelerated in the secondary effluent, probably because of the presence of natural photo-sensitisers that exist in the solution. The possible presence of this compound in aquatic environments (e.g. wastewater, rivers and groundwater) is of great concern, because the AMX-S-oxide β-lactam ring is still active and may lead to the development of resistant bacteria and even cause other possible health hazards to human and wild and domestic animals.

Furthermore, the fact that this product is also obtained under an ozonation process, should be taken into consideration when using this technique to remove antibiotic residues. Thus, it is recommended that the identification of DPs should be an integral part of any study that may be used for the removal of antibiotic residues from water.

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