# Suspect screening analysis of chlorinated antibiotics transformation products in water samples from tertiary treatment processes in Spain: the use of purpose-build database and (Q)SAR predictions

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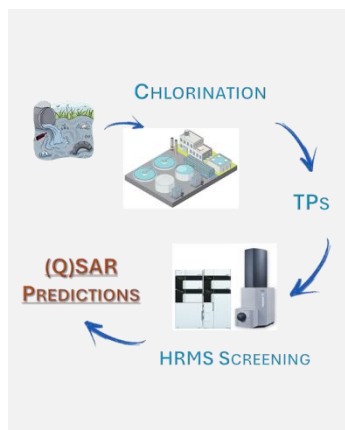
The presence of antibiotics in wastewater has been identified for a long time and the incapability of conventional wastewater treatment plants (WWTP) to remove these antibiotics is undeniable. The necessity of new approaches like tertiary treatments (e.g. chlorination) started to be studied and can remove completely many antibiotics. However, this treatment can generate transformation products (TPs). The present work constructed a purpose-built database with 104 TPs from 8 different antibiotics with data found in the literature and applied this database in the screening of 46 samples provides by 4 different WWTPs from Spain. The analysis allowed the suspect identification of 41 TPs and no antibiotic in the samples. After identifying the TPs in the samples, the toxicity of these compounds was predicted by (Q)SAR tools. The predictions results showed a lot of differences between the different endpoints evaluated, however TPs originated from CIP, generally, demonstrate to represent higher risk.

Introduction

Antibiotics were introduced in drugs industry in 1930s and now represent a huge part of the pharmaceutical industry. Antibiotics entering the aquatic environment especially by the non-efficiency of conventional WWTPs. Tertiary treatment usually is a more effective process to remove organic contaminants, e.g chlorination. Because chlorine is a very strong oxidizer, it can react to organic compounds generating different transformation products (TPs) that can represent concern, since they can be more toxic to human health and environment than the original compound [1].

The use LC-HRMS allows the separation, detection, identification, and quantification of pollutants at very low concentration levels. However, to obtain all the information to identify suspect compounds, the elaboration of purpose-build databases is necessary [2]. Besides, the evaluation of toxicity by *in silico* (Q)SAR predictions can be a very useful tool to evaluate the risk of compounds (antibiotics and their TPs) for human and environmental safety [3].

This study intends to construct a purpose-built database and perform screening analysis in real WWTP samples looking for TPs originated by chlorination treatment processes, for eight antibiotics. After that, evaluate toxicity by *in silico* (Q)SAR prediction using free available software.

Material and Methods

An extended search in literature was performed to construct a purpose-built database containing TPs generated by chlorination process from 8 antibiotics: sulfadiazine (SFD), lincomycin (LCN), ciprofloxacin (CIP), tetracycline (TCN), trimethoprim (TMP), sulfamethoxazole (SMX), sulfapyridine (SFP) and clindamicyn (CLD). 46 real samples from 4 different WWTP in Spain were analyzed by LC-HRMS after SPE sample preparation. Free available software (EPI Suite, Prometheus, QSARToolboxand VEGA Hub) were used to perform*in silico* (Q)SAR toxicity predictions for 8 different endpoints:mobility, persistence, biodegradability, STP total removal (sewage treatment plant total removal), PBT (persistence, biodegradability and toxicity), estrogen receptor binding, mutagenicity and carcinogenicity.

Results and Discussion

The purpose-built database constructed for eight antibiotics, contained 104 TPs found in the literature. Of the 104 TPs present in the purpose-built database, 47 of them (45%), presented addition of at least one chlorine atom in their structure, after chlorination process. This situation deserves attention because free chlorine is a non-selective oxidant and can produce some TPs with negatives effects for human health [4] and, sometimes, can be more toxic than the original compound [5].

A total of 46 samples from 4 different WWTP from Spain were analyzed. The screening of 8 antibiotics and 104 TPs allowed the suspect identification of 41 TPs in the analyzed samples, with 12 of them presenting chlorine in the elemental formula. One interesting fact is that no antibiotic, as active principle, was detected in any of the samples, which

is in agreement with studies that report rapid degradation of antibiotics during chlorination treatment process, however, with identification of different TPs [6]. This scenario just highlights the importance of studying the formation and evaluating the presence of TP after tertiary treatment systems, once the absence of antibiotics doesn't mean the problem was completely solved.

From the eight antibiotics evaluated, no TP was identified for SFD and LCN in analyzed samples. The antibiotics that presented a major part of the TPs identified wereCIP (11 TPs), TCN (9 TPs), TMP (7 TPs), SMX (6 TPs), SFP (5 TPs) and CLD (3 TPs). Considering the frequency of identified compounds, the most frequently detected was CIP TP 263, presented in 33 samples. These results can be considered in accordance with another studies from literature, where antibiotics can be detected in wastewater after traditional WWTP processes, however, with the use of some chlorination treatment, many antibiotics can degrade and achieve rates of 100% removal of the active principle [7].

In silico (Q)SAR predictions evaluated 8 different endpoints to try understanding the toxicity of the suspect identified TPs. Mobility evaluation showed that 14 compounds presented high mobility and represent huge risk to achieve groundwater, and, in this endpoint, the worst compound was TMP TP DAP, besides 11 TPs from CIP showed high mobility.

Considering persistence, only 3 TPs (TMP TP 444, TCN TP 477 and TCN TP 461) were classified as very persistent.

3 compounds also were predicted as non-biodegradable (TMP TP TMT, TMP TP 199 and TMP TP 343) and this result means that probably these compounds will not be degraded by biological treatment process. The STP total removal predictions showed a maximum removal for TMP TP TMP with 55,8% and SMX TP 163 with 6,5% of removal, however, all the other compounds were predicted with less than 3% of removal, in agreement with literature where antibiotics are not removed by traditional treatments process[6].

All TPs identified were predicted as non-PBT. 14 TPs were predicted to be strong binder to estrogen receptors, most of them (8 TPs) being from TCN. Mutagenic score demonstrates that 11 compounds, 2 SMX TPs and 9 CIP TPs presented high score as mutagenic compounds and the two SMX TPs results (SMX TP 110 and SMX TP 144) are based on experimental data. Carcinogenicity results showed that 16 compounds were predicted as carcinogenic with good reliability, most of them CIP TPs (9 TPs).

There is a large amount of data generated by *in silico* (Q)SAR prediction. Different endpoints generate different compounds considered most concern. The understanding of all these data is a complex task, however in this work it is possible to note that TPs originated from CIP, in general, demonstrate worst results and deserve more attention when new strategies to eliminate these TPs are studied.

Conclusions

The use of purpose-built database to evaluate the presence of 8 antibiotics and 104 TPs generated by chlorination tertiary treatment process in 4 different WWTP in Spain, demonstrated to be a very useful tool and allowed the suspect identification of 41 TPs and no original antibiotic in the analyzed samples, with 12 of them containing chlorine atoms in their structure. Besides, *in silico* (Q)SAR predictions for 8 different endpoints showed different compounds as most concern, however, in a general way, CIP TPs demonstrated worst results, considering environment and human health. These results are extremely useful to elaborate action plans to eliminate this TPs from wastewater before it returns to environment.

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