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Aerobic activated sludge transformation of vincristine and identification of the transformation products

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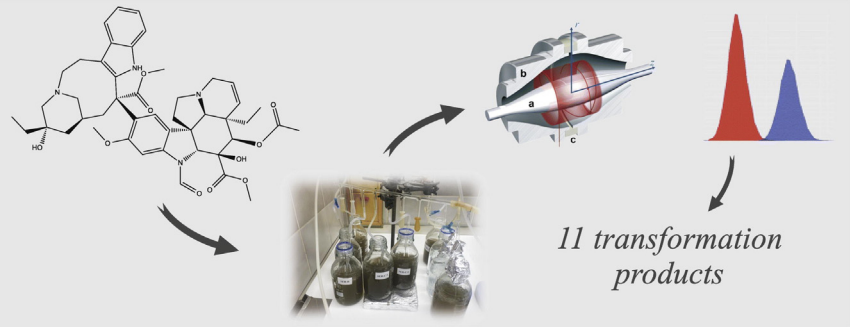
HIGHLIGHTS

- Vincristine was shown to be readily biodegradable with activated sludge.
- Eleven vincristine TPs were found and nine were tentatively identified.
- All but one of the TPs retained the typical vinca alkaloid structure.
- Non-commercial algorithms were used for detection and prediction of TPs.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aims to identify (bio)transformation products of vincristine, a plant alkaloid chemotherapy drug. A batch biotransformation experiment was set-up using activated sludge at two concentration levels with and without the addition of a carbon source. Sample analysis was performed on an ultra-high performance liquid chromatograph coupled to a high-resolution hybrid quadrupole-Orbitrap tandem mass spectrometer. To identify molecular ions of vincristine transformation products and to propose molecular and chemical structures, we performed data-dependent acquisition experiments combining full-scan mass spectrometry data with product ion spectra. In addition, the use of non-commercial detection and prediction algorithms such as MZmine 2 and EAWAG-BBD Pathway Prediction System, was proven to be proficient for screening for transformation products in complex wastewater matrix total ion chromatograms. In this study eleven vincristine transformation products were detected, nine of which were tentatively identified.

1. Introduction

Vinca alkaloids are chemotherapy agents isolated from the periwinkle plant (*Catharanthus roseus*), with the most common representatives being, vincristine (VCN), vinblastine and vinorelbine. Vincristine is used in the treatment of various neoplasms, including Hodgkin's disease, acute lymphocytic leukaemia, non-Hodgkin's lymphoma, Kaposi's sarcoma associated with AIDS, and neuroblastoma (Dorland, 2007). VCN produces its anti-cancer effects by stopping the cell from separating its chromosomes during the metaphase resulting in apoptosis. It is administered as the sulfate salt either intravenously or by infusion. There is no

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oral form. The parent compound and metabolites are mainly excreted via biliary elimination with faeces (30% in 24 h and 70% in 72 h), while approximately 10% is excreted in urine within 24 h and very little thereafter (https://pubchem.ncbi.nlm.nih.gov). Excretion of a mixture of parent compound and metabolites from the human body takes place both in the hospital and at a home. Discharges of municipal and hospital effluents typically enter the municipal sewerage system, where elimination of cytostatic drugs including VIN is often incomplete and residues eventually enter surface waters (Kosjek et al., 2013).

The literature on cycling and fate of VCN during wastewater treatment and in the environment is limited and mostly describes the development of analytical methods for the determination of multiclass cytostatics including VCN (Santana-Viera et al., 2016; Gomez-Canela et al., 2013; Negreira et al., 2013; Negreira et al., 2014). Yin et al. (2010) studied the concentration of nine cytostatics including VCN in hospital effluents in Beijing (China), but did not detect VCN (<LOD 20 ng/L), whereas Ferrando-Climent et al. (2013) did determine VCN in a hospital effluent (49.1 ng/L) and a wastewater influent (22.9 ng/L). In a follow-up study in 2014, the authors also detected the presence of VCN in a hospital effluent, a WWTP influent and effluent, and in a surface water.

So far, only four studies have investigated the degradation and water treatment of VCN (Negreira et al., 2016; Al-Ahmad and Kummerer, 2001; Franquet-Griell et al., 2017; Ferrando-Climent et al., 2015). Al-Ahmad and Kummerer (2001) found that only 30% of VCN was biodegraded in 28 days in a closed bottle test. Franquet-Griell et al. (2017) confirmed its low biodegradability, while its degradation was assigned to a competing process of hydrolysis. When exposed to UV photolysis, VCN was gradually degraded, but in the presence of H_2O_2 (1.5 mg/L) it was almost instantly removed (Franquet-Griell et al., 2017). Negreira et al. (2016), while investigating the chlorination of vinca alkaloids, found that VCN was more recalcitrant compared to other tested compounds. Although all of the studied vinca alkaloids have similar structures, VCN is the only one that has an N-formyl group in the vindoline subunit, which lowers its nucleophilicity and thus reactivity towards HOCl. In spite of its poor degradation, ten potential disinfection by-products were detected and tentative structures were proposed for seven of them (Negreira et al., 2016).

Knowing that chronic exposure to trace amounts of cytostatic residues, including the parent compound, its metabolites and transformation products (TPs), has the potential to cause adverse effects on non-target organisms in aquatic ecosystems (Kosjek and Heath, 2011), the aim of this study is to improve our understanding of the fate and cycling of VCN residues during biological wastewater treatment. We address the susceptibility of VCN to biodegradation under specific conditions, including the presence or absence of a carbon source and with two concentrations of activated sludge (AS). In addition, a series of experiments under sludge inhibition conditions, in the absence of AS, and blanks are conducted to control over the biotic transformation of VCN. Focus is on the predicting, screening, detecting, the formation and identifying TPs formed during biotransformation experiments. For this purpose, an ultra-high performance liquid chromatograph (UHPLC) hyphenated to a hybrid quadrupole-Orbitrap mass spectrometer and MZmine software in addition to the publicly available EAWAG-BBD biodegradation pathway prediction system (EAWAG BBD/PPS) have been applied.

2. Experimental

2.1. Standards and chemicals

Standards of vincristine sulfate, and its isotopically labeled standard vincristine-d3 sulfate (VCN-d3) were obtained from Santa Cruz Biotechnology (Dallas, TX, USA) at the highest available purity (>99%). All solvents and chemicals used for chromatographic separation (methanol, water, formic acid) were of HPLC grade purity. The chemicals used for biomass inhibition (formaldehyde, FDH) or standard dissolution (dimethyl sulfoxide, DMSO) and nutrient-mineral medium (yeast and meat extract, casein peptone, CH_3COONH_4, NH_4Cl, K_2HPO_4, KH_2PO_4, $CaCO_3$, $MgCO_3$, NaCl, $FeSO_4 \times 7H_2O$) were of analytical reagent grade purity. Individual stock solutions (ca. 1000 mg/L) were prepared in DMSO and stored in the dark at −20 °C. Different working standard solutions were made by making the appropriate dilutions in DMSO (100 and 10 mg/L) and ultrapure water. Calibration standards (from 0.005 to 1 mg/L) were freshly prepared in ultrapure water on the day of the analyses.

2.2. Justification of selected experimental conditions

The aim of the study was to identify transformation products potentially present because of wastewater treatment and for fulfilling this goal, the conditions were simplified accordingly:

i. *Concentration of parent compound* is orders of magnitude higher than what might be observed in wastewater, though it is the lowest possible concentration that still allows to perform biodegradation experiments of this kind due to the following reasons:

1.) The experimental system does not allow the continuous input of the parent compound, thus its concentration needs to be high enough to be sufficient for a 9-day long experiment.

2.) To aid in the detection of transformation products. Namely, during biodegradation process the parent compound breaks down into several TPs, where most of them show considerably lower abundance as compared with VCN. Therefore, by decreasing the concentration of the parent compound down to the environmental level, the abundance of several transformation products would be insufficient for enable identification; moreover, likely it would fall below the detection limit using given analytical method.

3.) An additional (e.g. 1000-fold) preconcentration of samples is however impossible since we are strictly limited with the sample volume: from the total sample volume of 400 mL, 4-mL sub-samples were taken every day for nine consecutive days. This makes reduction of the total volume for 1% each day, which is 9% altogether, a volume reduction that is in our opinion still acceptable without significantly affect the ratio between the sludge and the liquid phase and consequently the transformation.

4.) Transformation experiments are usually performed at such concentration levels (Buth et al., 2007; Roig et al., 2014; Soufan et al., 2013; Negreira et al., 2015; Kosjek et al., 2013).

ii. *Activated sludge* is authentic and fresh, i.e. obtained on the day of the experimental set-up from a nitrification basin at a wastewater treatment plant treating 22–30 million of m^3 of wastewater per year (360,000 population units), where wastewater originates mostly from municipalities, storm water, hospitals and health centers, pharmaceutical and food industry, and waste recycling.

iii. *Concentration of activated sludge* ranges from 0.24 to 1.9 g/L (Table 1), which is lower than the typical concentration of AS in WWTP nitrification basin (2.6 to 3.7 g/L).

iv. *Wastewater* is synthetic, but mimics the actual wastewater in all wastewater quality parameters, such as COD (≈900 mg/L), nitrogen (NH_4-N ≈ 75 mg/L, NO_3-N ≈ 2.3 mg/L, NO_2-N ≈ 0.01 mg/L) and phosphorus content (Zupanc et al., 2013; Kosjek et al., 2007). The reasons for using the synthetic water are to assure the absence of the parent compound and the TPs in the primary matrix; to avoid possible AS inhibitors; to assure the comparability between biodegradation experiments; and to control metabolic and co-metabolic breakdown processes in carefully designed wastewater matrices.

v. *The duration of the experiment* is not comparable with hydraulic retention times in real WWTPs, but is adapted to long biodegradation times (by reducing AS and increasing parent compound concentration) aiming to capture as many of TPs as possible. Also, when starting with such high initial concentration of the parent compound, it actually takes longer to biodegrade, than when initial