BIOTRANSFORMATION OF CITALOPRAM: INSIGHTS FROM IDENTIFICATION OF TRANSFORMATION PRODUCTS BY LC-QToF-MS

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Abstract

Biodegradation is considered to be the key process for the elimination of the majority of pharmaceuticals in the environment. During wastewater treatment or once they are disposed in the aquatic environment, pharmaceuticals may transformed to new, structurally-related compounds which are called transformation products (TPs). Since most of these compounds are unknowns, their identification is essential not only to provide a comprehensive risk assessment on micropollutants in the environment, but also to design improved removal technologies for (pseudo)persistent trace contaminants.

In this study, batch reactors seeded with activated sludge from the WWTP of Athens were set up to assess biotic, abiotic and sorption losses of a SSRI drug, citalopram. TPs were identified by reversed-phase liquid chromatography quadrupole-time-of-flight mass spectrometry (RPLC-QToF-MS). Hydrophilic interaction liquid chromatography (HILIC) was also used as a complementary, orthogonal, technique for the identified TPs, instead of NMR. A workflow for suspect and non-target screening was developed. A suspect list of possible TPs was compiled by literature and *in silico* prediction tools (EAWAG-BBD Pathway Prediction System and Bruker's Metabolite Predict). Structure elucidation of TPs was based on accurate mass and isotopic pattern measurements and interpretation of MS/MS spectra by the observed fragmentation pattern and library-spectrum match.

In total, thirteen TPs were identified. Four out of them were fully identified and confirmed by reference standards (desmethylcitalopram, citalopram amide, citalopram carboxylic acid and 3-oxo-citalopram). A probable structure based on diagnostic evidence and tentative candidates were proposed for the additional five and four TPs, respectively. Finally, a transformation pathway based on the identified compounds was presented.

EXPERIMENTAL

Activated Sludge & WW Effluent from WWTP of Athens (Psitalia)

Simulation of activated sludge process with batch reactors

Spiked activated sludge | Activated sludge only | Spiked autoclaved sludge+ WW Effluent | Spiked WW Effluent

→ Spike 2 mg/L

→ Incubation time 6 days

Column: Acclaim C18 (Dionex-Thermo Scientific)

(+) ESI MeOH & H_2O/MeOH, 90:10, both amended with Amm. Form. 5 mM, 0.01% Formic Acid
(-) ESI MeOH & H_2O/MeOH, 90:10, both amended with Amm. Acet. 5 mM

Column: ACQUITY BEH Amide (Waters)

(+) ESI H_2O & ACN/H_2O, 95:5, both amended with Amm. Form. 1 mM, 0.01% Formic Acid
(-) ESI H_2O & ACN/H_2O, 95:5, both amended with Amm. Form. 10 mM

Scan: 50-1000 m/z
Spectra rate: 2Hz
MS mode: bbCID & Auto MS with inclusion list

LC-ESI-MS/MS (QToF-MS) (Bruker MaXis Impact)

RESULTS & DISCUSSION

KINETIC EXPERIMENT
Degradation of citalopram

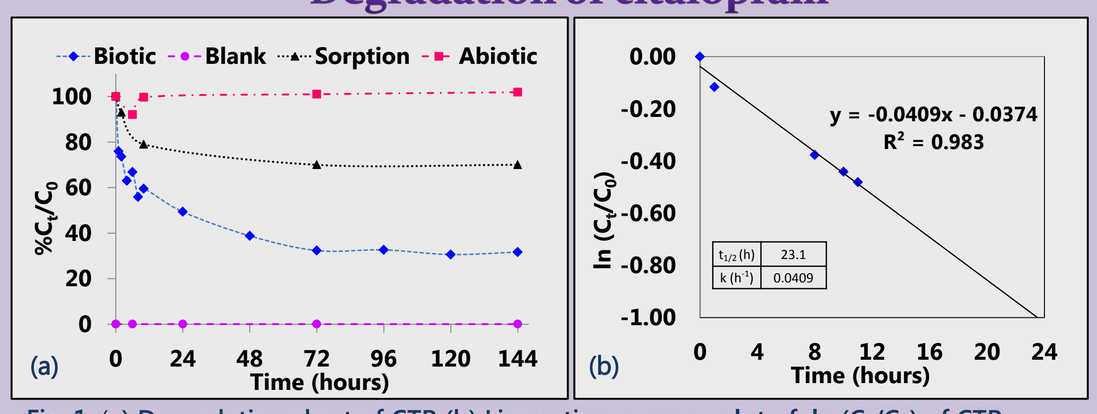
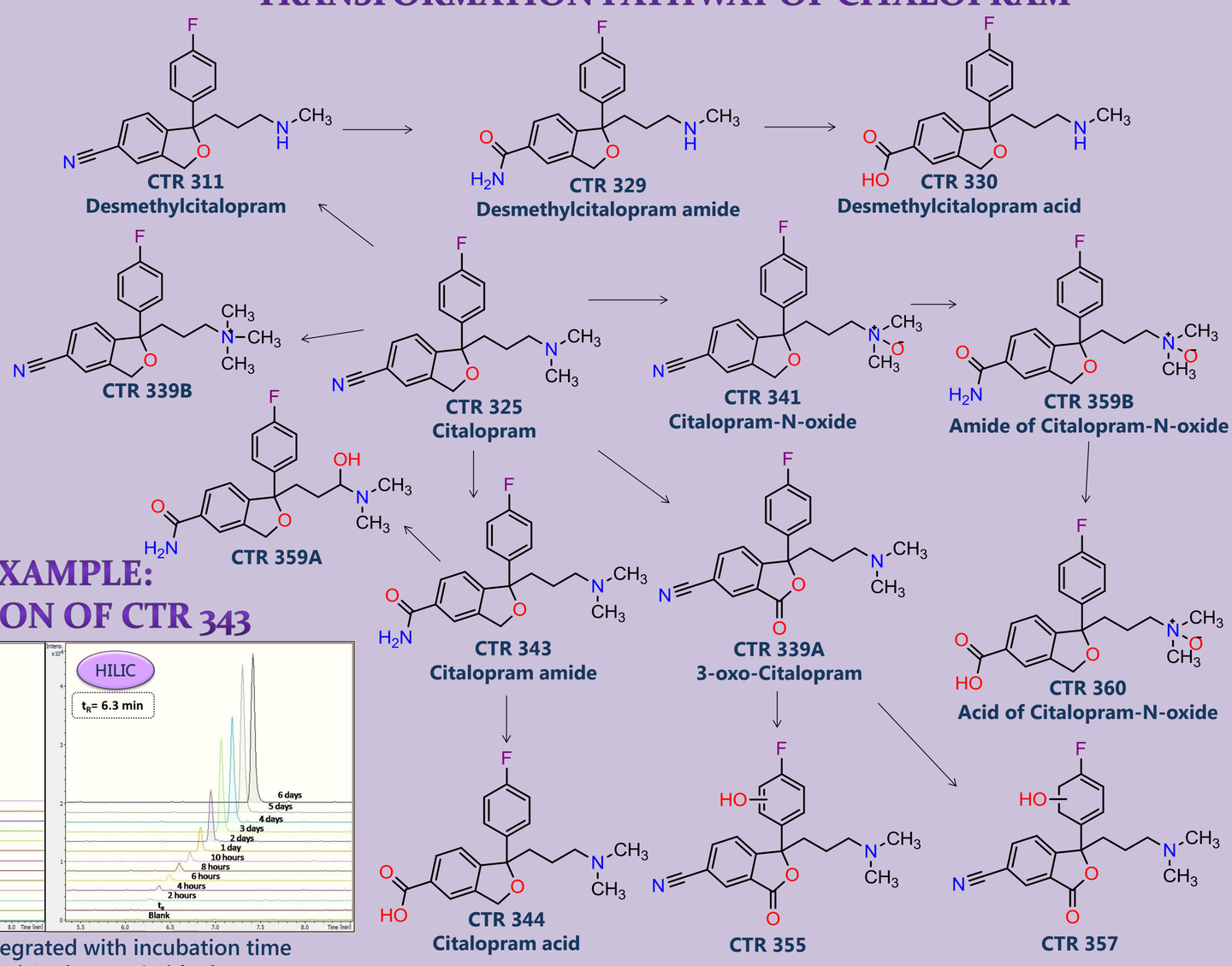


Fig. 1. (a) Degradation chart of CTR (b) Linear time-course plot of ln (C_t/C_0) of CTR.

TRANSFORMATION PATHWAY OF CITALOPRAM



SUSPECT SCREENING

Compilation of suspect list of TPs: EAWAG-BBD Pathway Prediction System + literature + Metabolite Predict (Bruker)

Screening all the time interval **chromatograms** in RP & HILIC (+ESI/-ESI)

Evaluation of tentative candidates: meet the set criteria, absence in blank & time trend

Acquisition of MS/MS spectra in RP & HILIC: Interpretation of the fragmentation pathway of TPs

Confirmation if possible with reference standard (RT & MS/MS spectra)

Criteria
Accuracy: 5 mDa
Isotopic fit: 1000 mSigma
Intensity: 500 (+)/200 (-)
Area: 2000 (+)/800 (-)

EXAMPLE: IDENTIFICATION OF CTR 343

Fig. 3. XICs of CTR 343 integrated with incubation time in RP and HILIC (Time trend & absence in blank).

NON-TARGET SCREENING

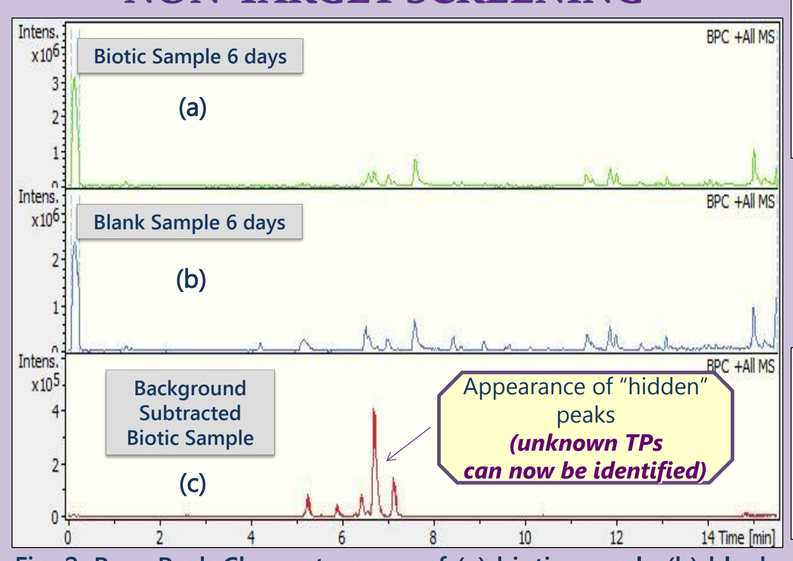


Fig. 2. Base Peak Chromatograms of (a) biotic sample (b) blank sample (c) biotic sample after background subtraction.

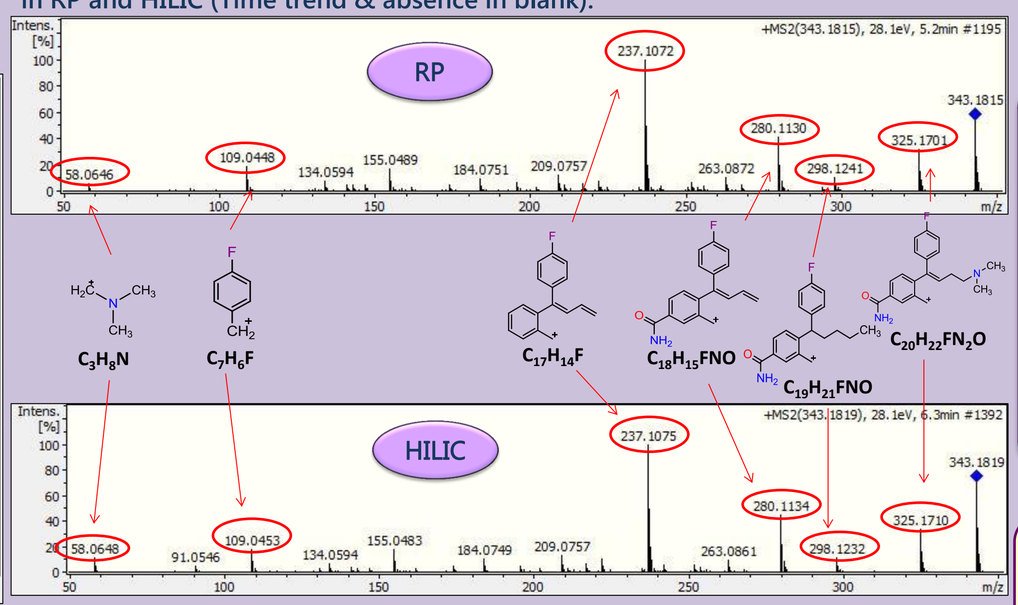


Fig. 4. MS/MS spectra in RP and HILIC and proposed fragments of CTR 343, $[M+H]^+$: $C_{20}H_{24}FN_2O_2$.

CONCLUSIONS

- Suspect and non-target screening was performed for the identification and structure elucidation of TPs.
- HILIC was used complementary to RP (identical MS/MS spectra of TPs in RP and HILIC).
- 13 TPs were identified in total.

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