# WORKFLOW FOR THE IDENTIFICATION OF BIOTRANSFORMATION PRODUCTS OF AMINE-CONTAINING PSYCHOTROPIC DRUGS IN THE AQUATIC ENVIRONMENT


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## Abstract

Pharmaceuticals are continuously discarded into the aquatic system through wastewater treatment plants (WWTPs). The microbial degradation of these organic micropollutants and formation of transformation products (TPs) under aerobic conditions is the fundamental process for their elimination. It is of paramount importance to understand the microbial metabolic pathways so as to obtain knowledge of how fast micropollutants degraded and to assess the exposure to their potential TPs as they can be more polar and consequently environmentally persistent.

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 selective psychotropic drugs, containing amine moieties. Biodegradation and transformation products were identified using liquid chromatography quadrupole-time-of-flight mass spectrometry (LC-QToF-MS). A workflow for target, suspect and non-target screening was developed. Data treatment was performed by using metabolite tools accompanying Bruker's maxis impact ESI-QToF-MS and the structure elucidation of the candidate transformation products was based on accurate mass and isotopic pattern measurements by HRMS and tentative interpretation of MS/MS spectra. Finally four biotransformation products were identified for both lidocaine and ephedrine. Despite the structure similarities, different degradation constants were calculated for each compound.


## Experimental

- ✓Amber bottles seeded with activated sludge under aerobic conditions in room temperature, pH≈7.
- ✓Spiked samples with 1000 and 400 μg/L lidocaine and ephedrine, respectively.
- ✓Two control samples for abiotic and sorption losses. One blank sample.
- ✓Incubation time: 24 hours for ephedrine and 12 days for lidocaine.
- ✓Filtration through glass fiber syringe and then through 0.2 μm RC filter.

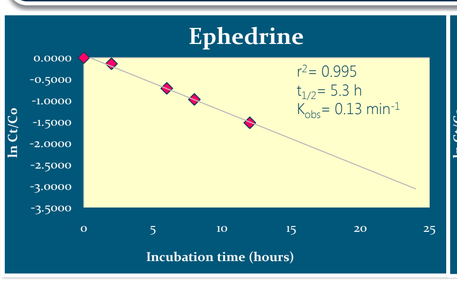
Bruker Maxis Impact™

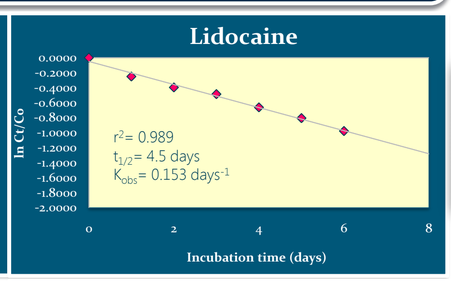
Scan: 50-1000 m/z
Spectra rate: 2Hz
Collision Energy → MS: 4eV, MS/MS: 25eV

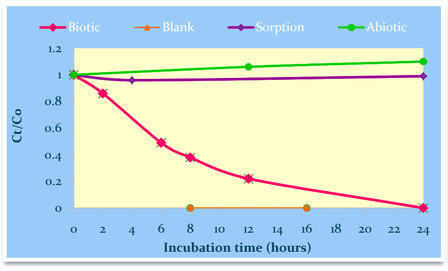
UltiMate 3000™ RSLC (Dionex) UHPLC System
Column: Thermo Acclaim RSLC C18, 2.2 μm 120 Å, 2.1x100 mm
Mobile Phase: +ESI→ MeOH:$H_2O$ (90:10)/H2O, 5 mM Amm. formate & 0.01% Formic acid. -ESI → MeOH:$H_2O$ (90:10)/H2O, 5 mM Ammonium acetate.
Flow rate: 200-480 μL/min
Inj. Volume: 5 μL
MS Mode: broad-band collision induced dissociation bbCID, (MS & MS/MS simultaneously)

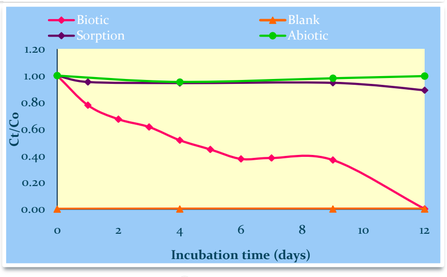
## Target screening

Kinetic experiment: Parent compound (PC) removal and determination of $t_{1/2}$ and $K_{obs.}$



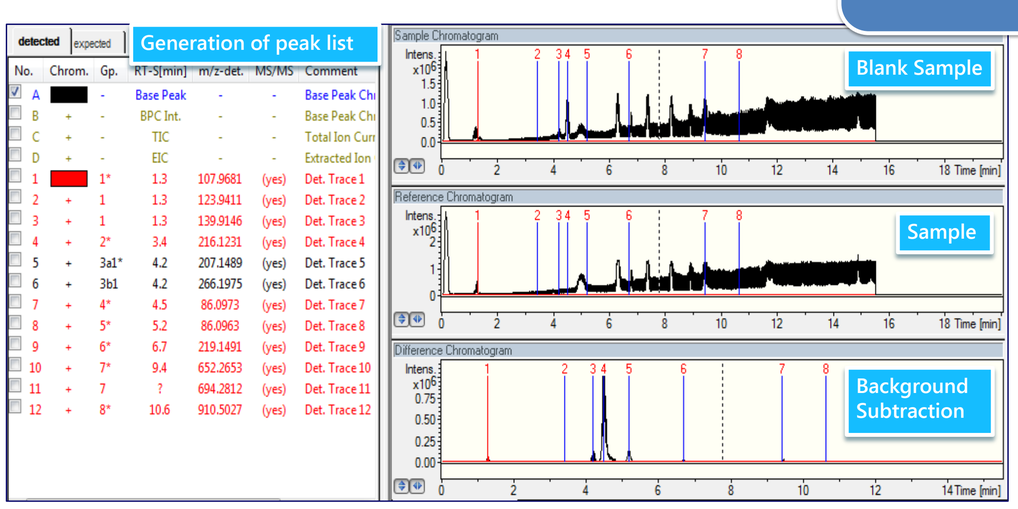




## Non-target screening

- ✓Determination of TPs with no prior knowledge.
- ✓Subtraction of the background (blank sample) with Bruker's MetaboliteDetect 2.0.
- ✓Generation of a peak list of unknown.
- ✓All the time interval samples were checked for identification of a time trend and absence in
- ✓the blank.

Parameters
Ratio: 5, Δtime: 0.1 min, Δmass: 0.05 m/z
Mass Spectrum Parameters
Intensity threshold: 30%
Max. No. Peaks: 10




## Results and Discussion

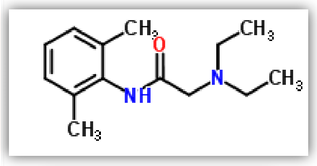
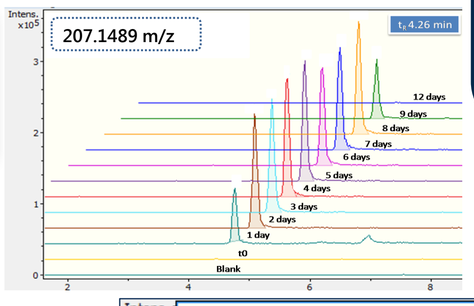
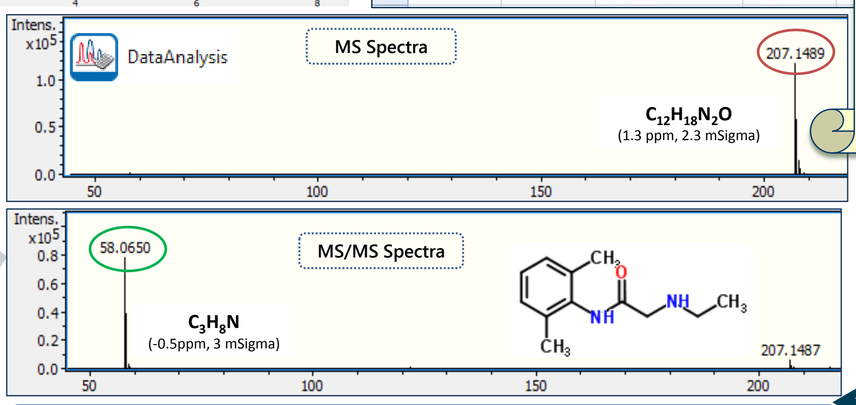




Fig 1. Structures of lidocaine and ephedrine. Lidocaine is an amide with a tertiary nitrogen and ethyl moieties. Ephedrine is a secondary amine with methyl moieties and a neighbor hydroxyl group.




Afterwards, the detected m/z which showed a time-trend, were treated and annotated as potential TPs only in case that the aforementioned criteria were met.




The fragment 58.0650 m/z is common in the MS/MS spectra for both lidocaine and its metabolite.

## Suspect screening

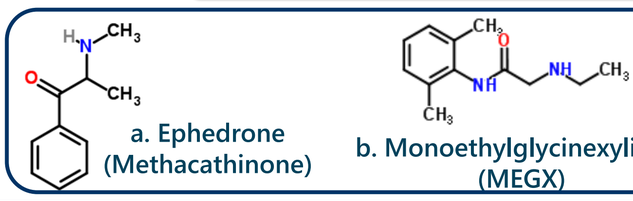
Determination of transformation products (TPs): In-house databases were compiled for both PCs from literature and prediction models (UM-PPS & Metabolite Predict). The characterization of a compound as TP was based on the following criteria.

| | A | B | C | D |
|---|---|---|---|---|
| 1 | m/z [dete | RT POS | sum formula | name |
| 2 | | | C2H4O | met 1 |
| 3 | | | C2H4O2 | met 2 |
| 4 | | | C4H11N | met 3 |

**Criteria**
- deltaRT ≤ 0.05 min
- Accuracy: Error ≤ 5 ppm
- Isotopic fit: ≤ 20 mSigma
- Intensity > 1000 (+ESI) / 500 (-ESI)
- Occurrence of a time trend

✓Matched peaks from both MS & MS/MS spectra send to MetFrag to identify probable structures
✓Consider Rt & common fragments with the PC

MetFrag



a. Ephedrone (Methacathinone)
b. Monoethylglycinexylidide (MEGX)
c. 3-OH Lidocaine
d. $C_{13}H_{18}N_2O$ 0.4 ppm 8.7 mSigma

Fig 2. Identified metabolites of ephedrine (a) and lidocaine (b,c and d).

## Conclusions

- ▪HR-MS & MS/MS data in a single run with resolution ≥30,000.
- ▪In a single workflow target, suspect and non-target screening was performed for the identification and structure elucidation of biotransformation products.
- ▪Bruker's Metabolomic tools allow metabolites to be rapidly identified.
- ▪Three TPs and one TP were found for lidocaine and ephedrine, respectively.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