



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

Aikaterini K. Psoma, Anna A. Bletsou and Nikolaos S. Thomaidis\*

National and Kapodistrian University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, Panepistimiopolis Zografou, 15771 Athens, Greece.

\*E-mail: ntho@chem.uoa.gr



## 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.

- ✓ 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.

## Experimental

Bruker Maxis Impact™

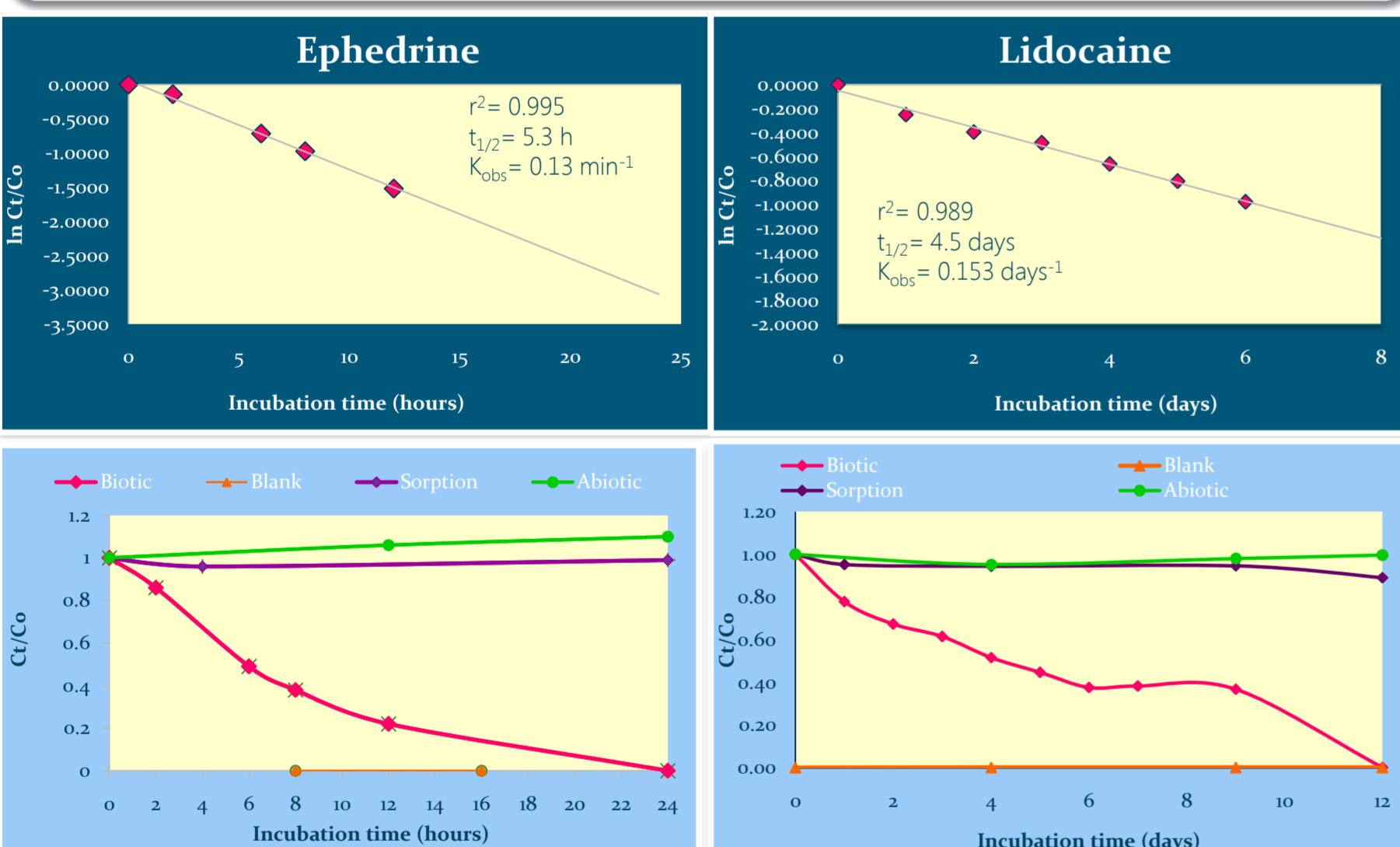


UltiMate 3000™ RSLC (Dionex) UHPLC System  
 Column: Thermo Acclaim RSLC C18, 2.2 µm 120 Å, 2.1x100 mm  
 Mobile Phase: +ESI→ MeOH:H<sub>2</sub>O (90:10)/H<sub>2</sub>O, 5 mM Amm. formate & 0.01% Formic acid. -ESI → MeOH:H<sub>2</sub>O (90:10)/H<sub>2</sub>O, 5 mM Ammonium acetate.  
 Flow rate: 200-480 µL/min  
 Inj. Volume: 5 µL  
 MS Mode: broad-band collision induced dissociation bbCID, (MS & MS/MS simultaneously)

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

## Target screening

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



## Results and Discussion

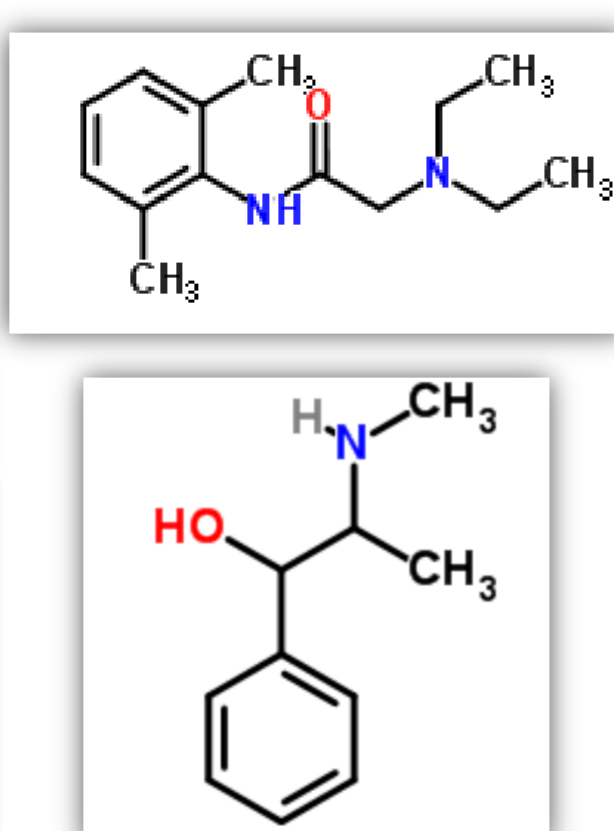


Fig 1. Structures of lidocaine and ephedrine. Lidocaine is an amide with a tertiary nitrogen and ethyl moieties. Ephedrine is a secondary amine with methyl moieties and a neighbor hydroxyl group.

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.

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

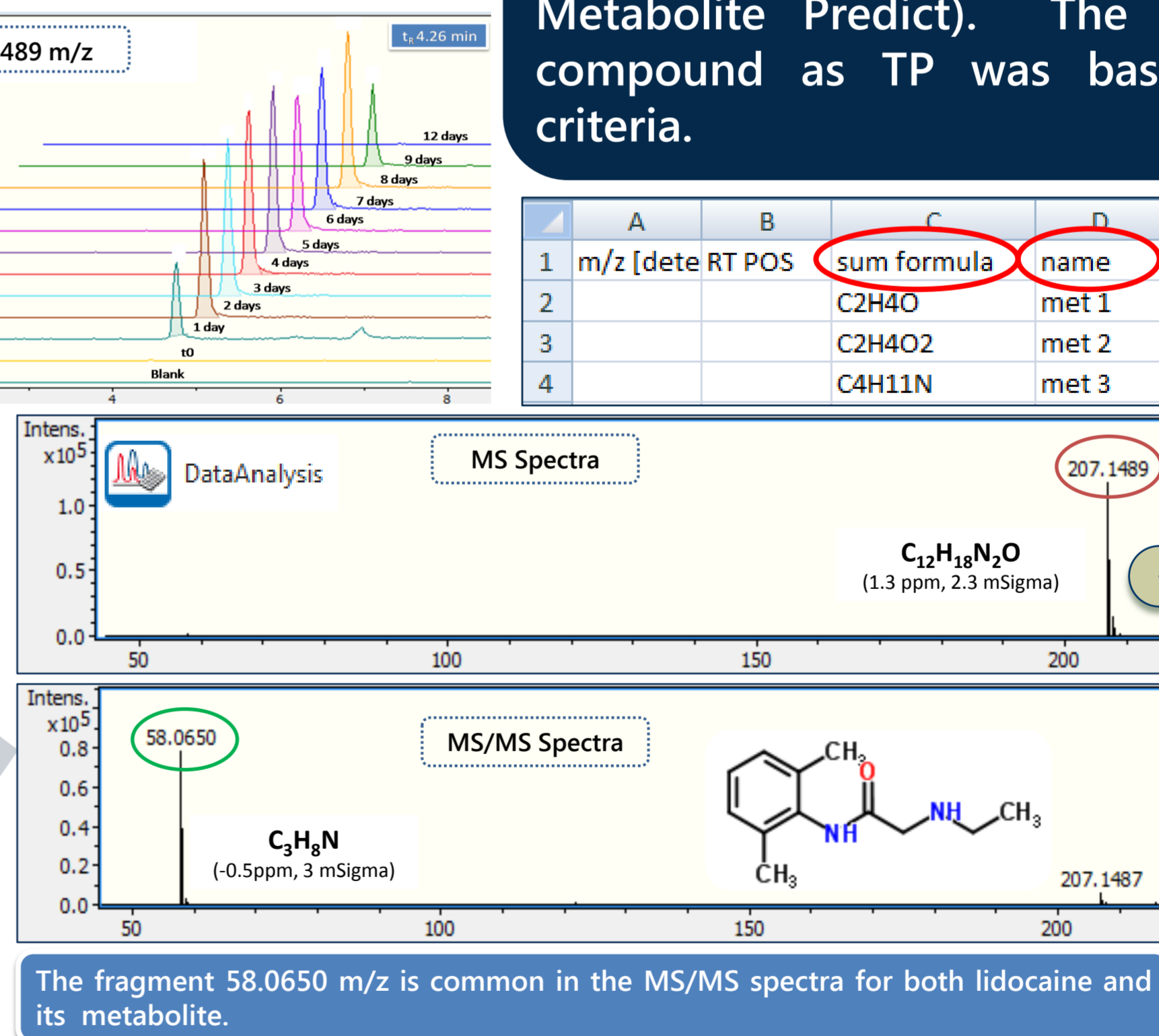
## Suspect screening

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

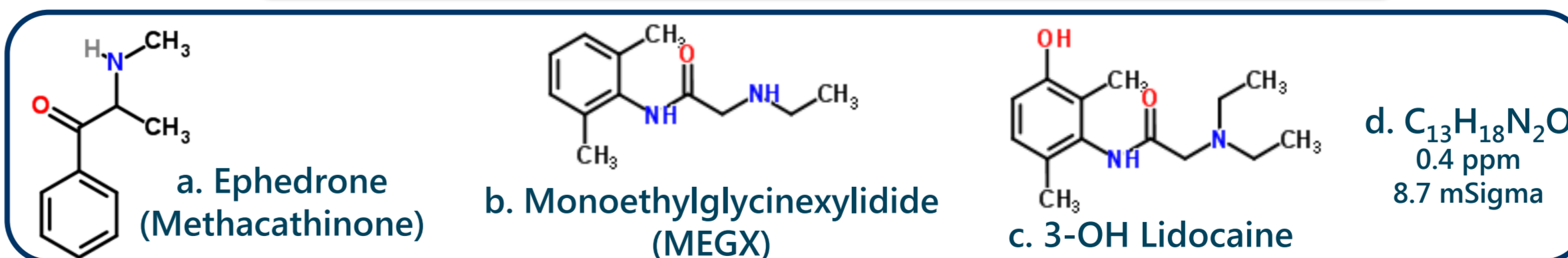


The fragment 58.0650 m/z is common in the MS/MS spectra for both lidocaine and its metabolite.



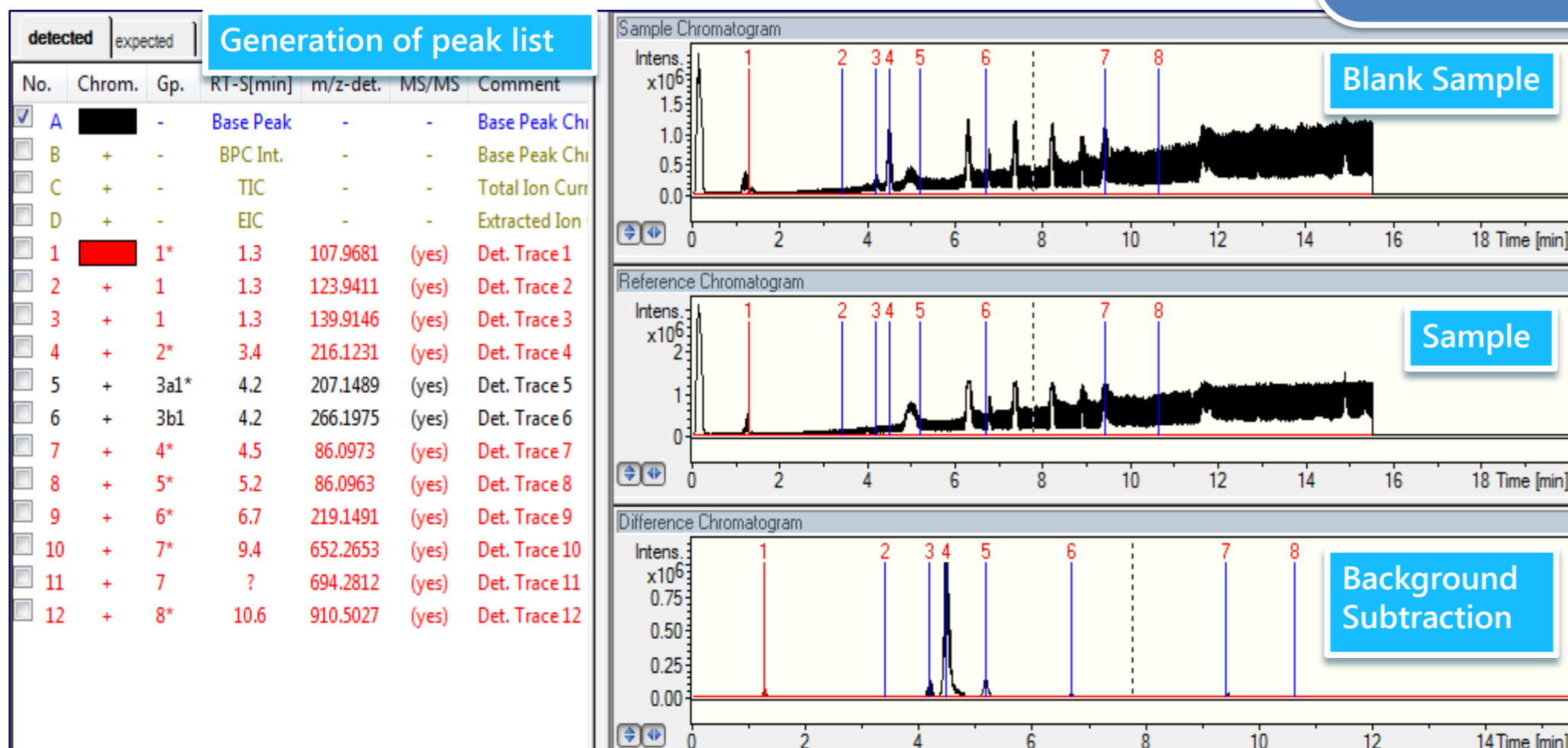
## Non-target screening

- ✓ 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.



## 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.



**Acknowledgments**  
 This research has been co-financed by the European Union and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – ARISTEA 624 (TREMPEPOL project).