

Targeted determination of new psychoactive substances, drugs of abuse, psychoactive pharmaceuticals and other biomarkers in wastewater by LC-Q-ToF- HRMS



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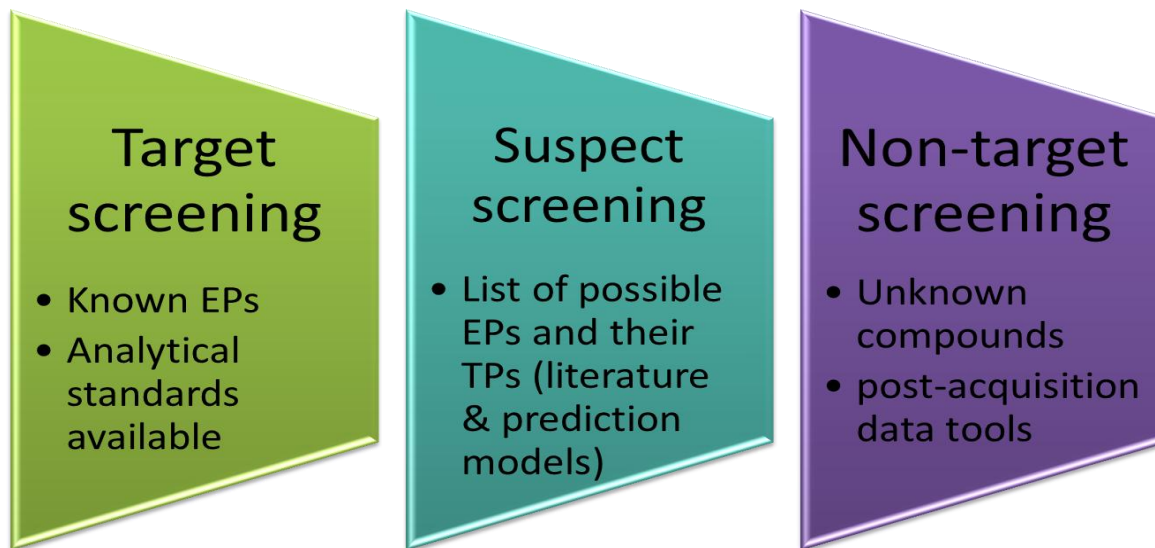
score

Testing the Waters 2015
Ascona, 10-15 October



Sign hanging in Einstein's office at Princeton
***“Not everything that counts can be counted,
and not everything that can be counted counts”***

As Daughton's paraphrases very clearly demonstrated
***“Not everything that can be measured is worth measuring,
and not everything worth measuring is measurable”***



Emerging Pollutants (EPs)

- Pesticides
- Pharmaceuticals
- Illicit drugs
- Personal care products
- Endocrine disruptive compounds (EDCs)
- Flame retardants
- Food additives
- Disinfection by-products

+

Metabolites &
Transformation Products
(TPs)



Wastewater:

Potentially tens of
thousands of substances

database of 2327 EPs

- > pesticides
- > pharmaceuticals, illicit, DoA
- > steroids & doping compounds
- > industrial chemicals, food additives, dyes
- > naturally occurring compounds (amino acids, neurotransmitters)
- > metabolites & TPs

745 NPS, DoA, PP and metabolites

Sampling

Location: WWTP of Athens, Greece

(Residential population: 3,700,000 – Mean flow rate: 766,000 m³ day⁻¹ - dewatered sludge production: 530,000 kg day⁻¹)

Period: December 2010 - March 2015

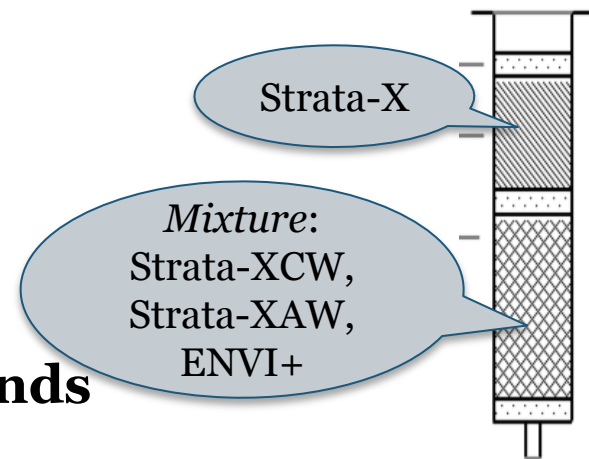
Samples: 24-h composite flow-proportional samples of influent wastewaters & effluent wastewaters (7 consecutive days)

2-h composite flow-proportional samples of influent wastewater (Thursday & Saturday, 12 samples per day, from 02:00 to 00:00)



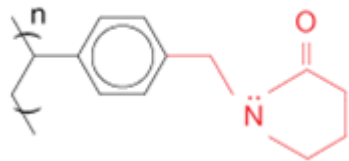
Sample Preparation

- ✓ **100 mL** wastewater (GFF filtration)
- ✓ **IS** spiking (100 ng/L)
- ✓ SPE **Mixed-bed cartridges**
- ✓ Extraction: **Neutral, Basic & Acidic Compounds**

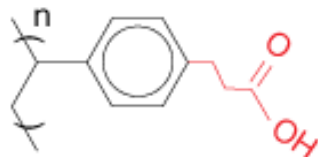


*Kern et al. *EST*
(2009) 43(18):7039

Strata-X for neutral & aromatic compounds

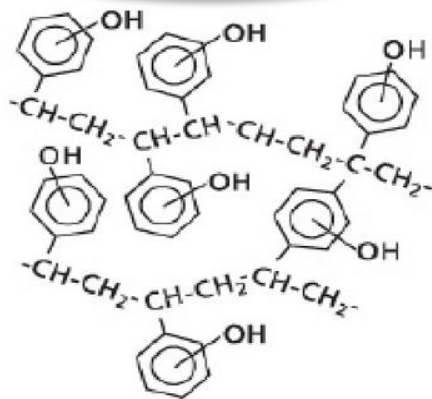


Strata-XCW for basic compounds (pKa \approx 4.5)

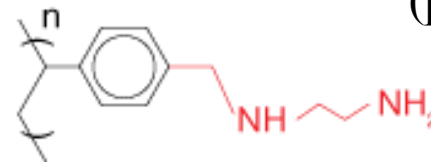


ENVI+

for polar
compounds



Strata-XAW for acidic compounds
(pKa \approx 9)



Elution step

- MeOH: ethyl acetate (1:1)
- 2% NH₃
 - 1.7% formic acid

Instrumentation

RP-UHPLC

Column: Acclaim RSLC 120 C18 2.2 μm , 2.1 \times 100 mm

Pre-column: VanGuard (Waters): Acquity UPLC BEH C18 1.7 μm , 2.1 \times 5 mm

MS



MaXis Impact
Ultra High Resolution
Time-of-Flight Mass
Spectrometer



- 20 min chromatogram
- Gradient elution program in the M.P.
- Gradient elution program in the flow rate

- Positive & Negative ESI
 - bbCID mode (MS & MS/MS simultaneously)

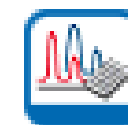
Database

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	
1	m/z (POS)	RT POS	sum formula	name	CAS	comment	comment	relativeRe	minimum	indivSigm	indivMass	Q1 1	Q1 2	Q1 3	Q1 1 min	Q1 1
2	184.0191768	3.69	C4H10NO3PS	Acephate	(30560-19-1)							142.9926				
3	142.9926277	3.69	C2H8O3PS^1+	Acephate Fragm 143	(30560-19-1)							94.9893	110.9664	124.9821		
4	270.1255331	11.34	C14H20ClNO2	Acetochlor	(34256-82-1)							224.0837				
5	224.0836682	11.41	C12H15ClNO^1+	Acetochlor Fragm 224	(34256-82-1)							148.1121	133.0886	224.0837		
6	379.03031	10.21	C14H7ClF3NO5NH4^1+	Acifluorfen (NH4)	(50594-66-6)											
7	265.0374463	11.98	C12H9ClN2O3	Aclonifen	(74070-46-5)							248.0347	218.0367	194.0475		
8	287.019391	11.98	C12H9ClN2O3Na^1+	Aclonifen (Na)	(74070-46-5)							248.0347	218.0367	194.0475		
9	181.0859207	11.26	C10H13O3^1+	Acrinathrin Fragm. 181	(101007-06-1)							181.0859	213.1121	230.1387		
10	270.1255331	11.4	C14H20ClNO2	Alachlor	(15972-60-8)							162.1277	147.1043	132.0808		
11	162.127259	11.4	C11H16N^1+	Alachlor Fragm 162	(15972-60-8)							162.1277	147.1043	132.0808		
12	238.0993183	11.4	C13H17ClNO^1+	Alachlor Fragm 238	(15972-60-8)							162.1277	147.1043	132.0808		
13	191.0848748	7.36	C7H14N2O2S	Aldicarb	(116-06-3)							89.0419				
14	208.1114239	7.36	C7H14N2O2SNH4^1+	Aldicarb (NH4)	(116-06-3)							89.0419				
15	116.0528464	7.36	C5H10NS^1+	Aldicarb Fragm 116	(116-06-3)							89.0419				
16	89.0419474	7.36	C4H9S^1+	Aldicarb Fragm 89	(116-06-3)							89.0419				

- Retention time
- Molecular Formula
- Adducts
- In-source fragments
- bbCID fragments
- Ion Ratios



TargetAnalysis



DataAnalysis

Optimization - Validation

validation dataset

61 (/195)
compounds of interest

- ❖ 195 compounds
- ❖ 10% of the compounds of the total database
 - ❖ Representative retention time
- ❖ Representative physicochemical properties
 - ❖ Representative ionization behavior
 - ❖ Compounds from every class of EPs

Screening method:

The method used to identify the non-compliant samples (true positive) with a β -error less than 5%

Optimization of the evaluation method

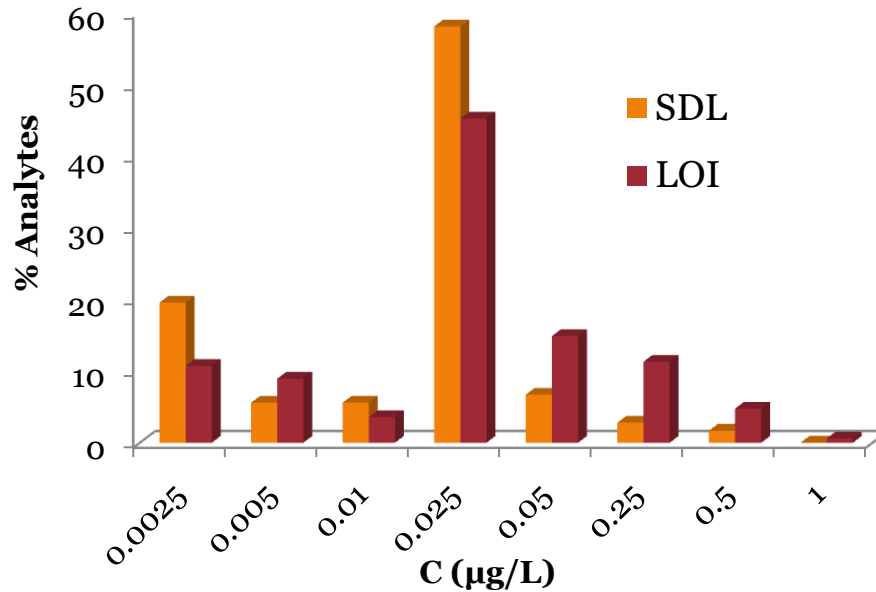
Successful Identification
Rate >95%

Area	1000 (+) / 600 (-)
Intensity	250 (+) / 150 (-)
ret. Time ΔRT (min)	0,4
accuracy (ppm)	5
mSigma threshold	200

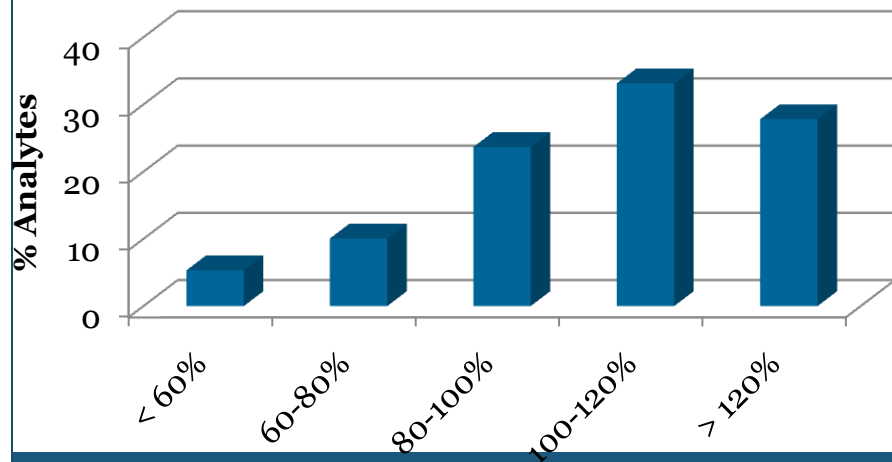
C spiked samples ($\mu\text{g/L}$)	% 'missed compounds due to Area / Intensity threshold	False Negative results (compounds)	Successful Identification Rate (%)
1	0	1	99
0.5	0	2	99
0.25	1.3	2	97
0.05	3.3	3	95
0.025	5.2	5	92

Validation Results

Screening Detection Limits (SDL) – Limits of Identification (LOI)



% Recoveries



% Matrix Effect

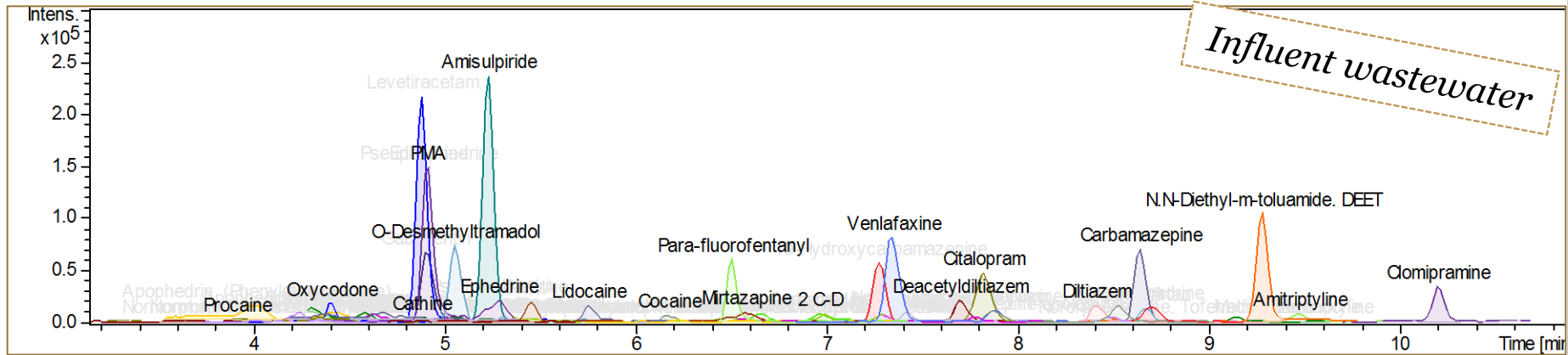
91.7 % analytes →
signal
suppression

8.3 % analytes →
signal
enhancement

% Repeatability (n=6)

94.5 % analytes →
<20% RSD

Results



371 compounds detected

Influent wastewaters:
338 compounds

- 61 pesticides
- 205 licit & illicit drugs
- 4 sweeteners
- 10 PFCs
- 8 Amino acids
- 47 TPs

Effluent wastewaters:
301 compounds

- 51 pesticides
- 191 licit & illicit drugs
- 4 sweeteners
- 11 PFCs
- 4 Amino acids
- 49 TPs

Results

↪ Drugs

Metformin → most abundant
Haloperidol → less abundant
Influent: 0.14 ng/L - 93 µg/L
Effluent: 0.5 ng/L - 35 µg/L

Benzoylcegonine: 0.38 µg/L
Cocaine – EME: 0.24 µg/L
THCA: 0.31 µg/L

↪ Opiates, Opioids

Influent: 5 compounds (Morphine 0.64 µg/L – EDDP 0.12 µg/L)
Effluent: 6 compounds (degradation to metabolites: Norcodeine-Hydrocodone)

↪ Stimulants- Amphetamines

AMP, MA, MDA, MDMA, MDEA
2,5-dimethoxy-4-methylphenethylamine (2C-D), Dimethoxyamphptamine,
Trimethoxyamphptamine, Ethylamphetamine, Dimethylamphetamine,
para-Methoxy-N-methylamphetamine (PMMA)
4'-Methyl- α -pyrrolidinopropiophenone (MPPP)
Cathine, Aminorex, Phendimetrazine
Mephentermine, Midodrine, Heptaminol
Bemegrade, Pemoline

↪ **Sympathomimetics:** Ephedrine, Norephedrine, Etafedrine

↪ **Antidepressants: SSRIs, SNRIs, TCAs, MAOIs (Phenelzine)**

↪ **Benzodiazepines (Oxazepam)**

↪ **Antiepileptics – Barbiturates – Anesthetics**

↪ **Antipsychotics (Clozapine)**

↪ **Pesticides**

Fluometuron, Azoxystrobin & Dimethachlor met.

Influent: 0.3 ng/L- 2.04 µg/L

Effluent: 0.2 ng/L- 13.6 µg/L

66 pesticides
in total

↪ **Sweeteners**

Influent: 0.6 µg/L sucralose - 24 µg/L cyclamate

Effluent: removal >60 %

↪ **Other chemicals**

Benzoic acid: 49 µg/L (influent) - 29 µg/L (effluent)

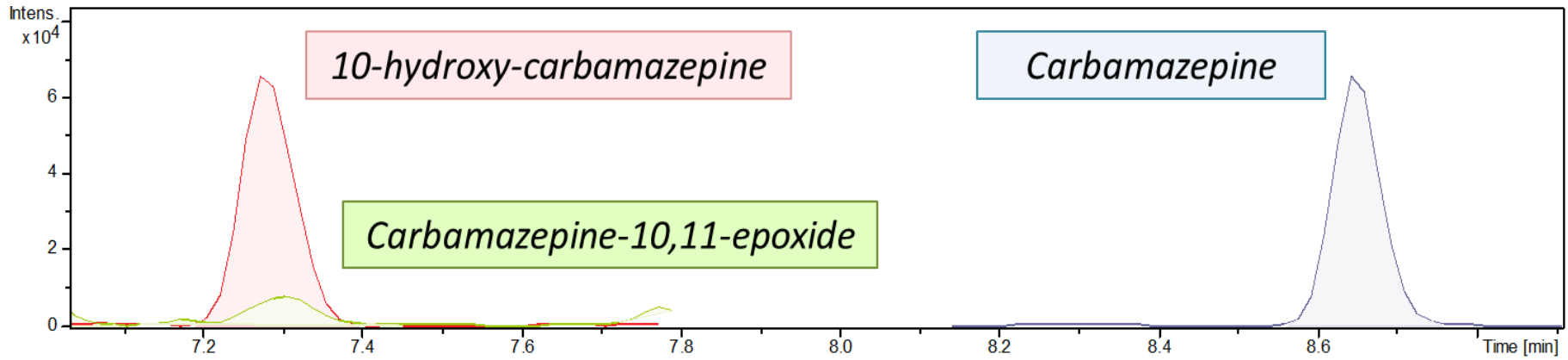
Ethyl sulfate: 10.6 µg/L (influent) – 3.3 µg/L (effluent)

↪ **Amino acids – Neurotransmitters**

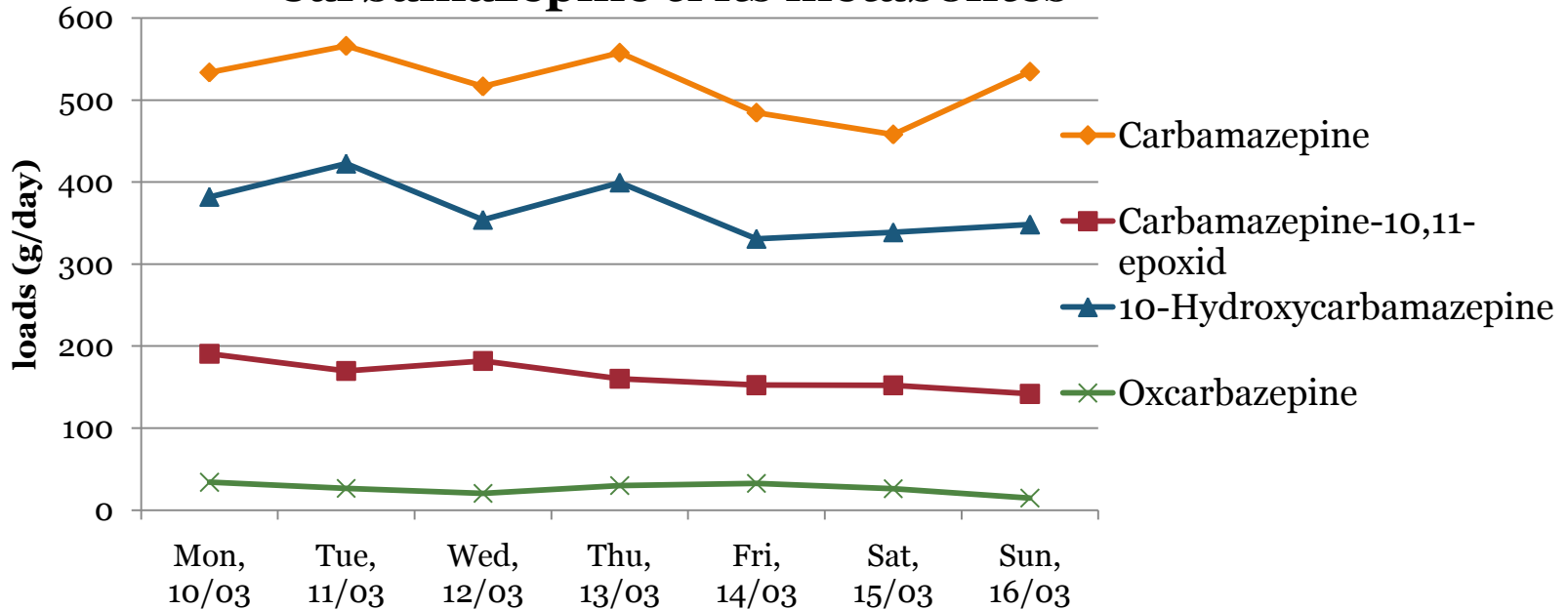
Influent: Conc. >2 µg/L (8 AAs) - Valine: most abundant (58 µg/L)

Effluent: significant removal rates (4 AAs), Val & GABA > 10 µg/L

Results



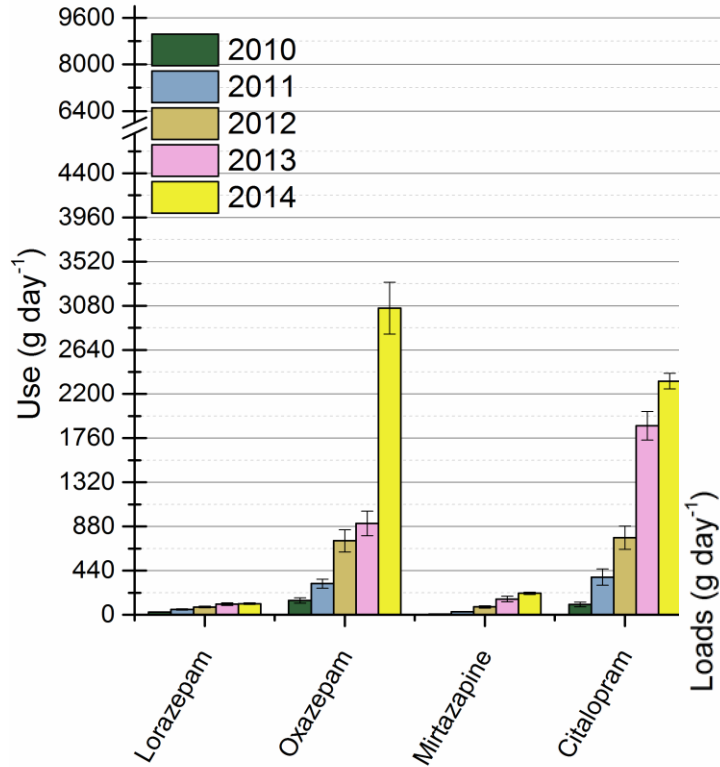
Daily Variation over a Week of Carbamazepine & its metabolites



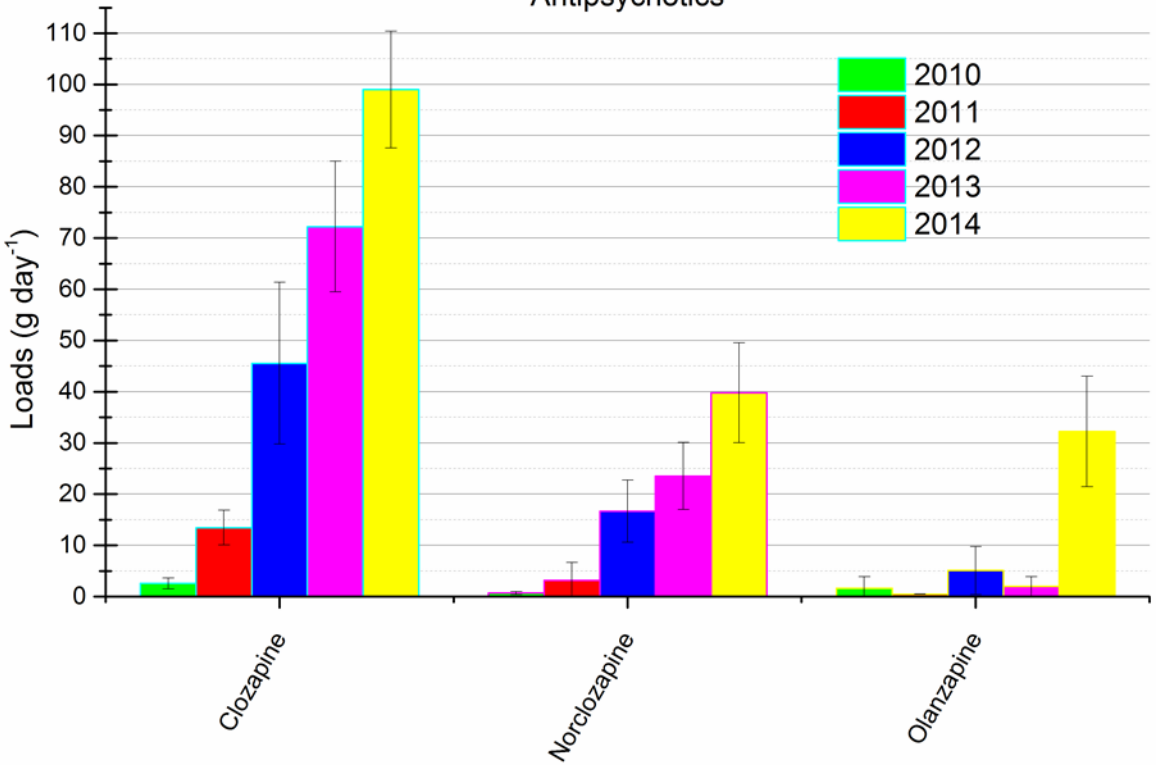
Results

Changes over the years

Benzodiazepines and Antidepressants



Antipsychotics





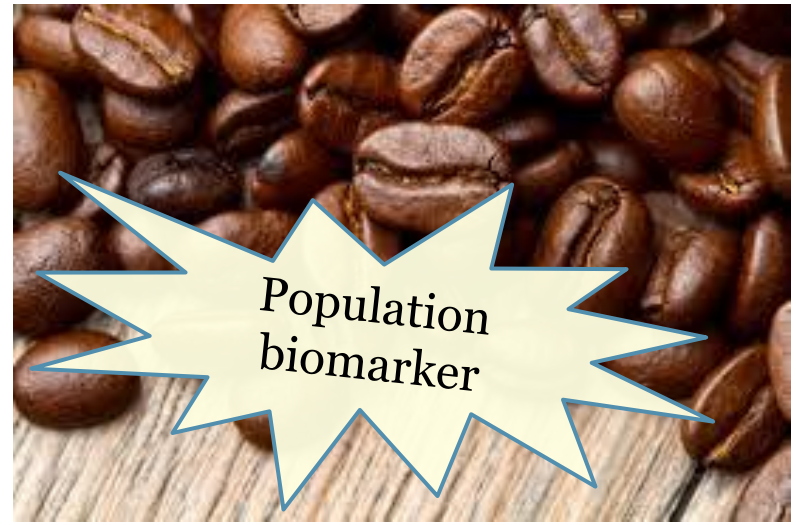


Results

Caffeine
& its metabolites

3 dimethyl-xanthines:

- Paraxanthine
- Theobromine
- Theophylline

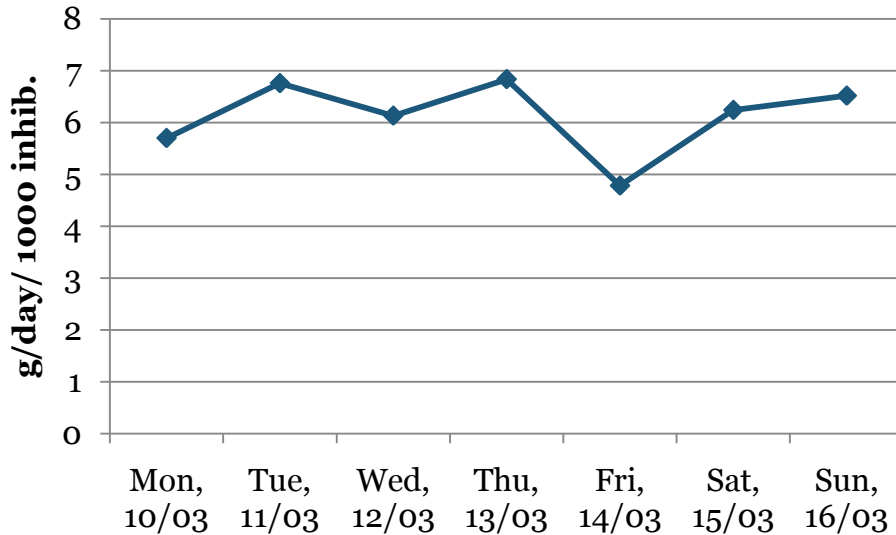


6.5 g caffeine /day/
1000 inhabitants

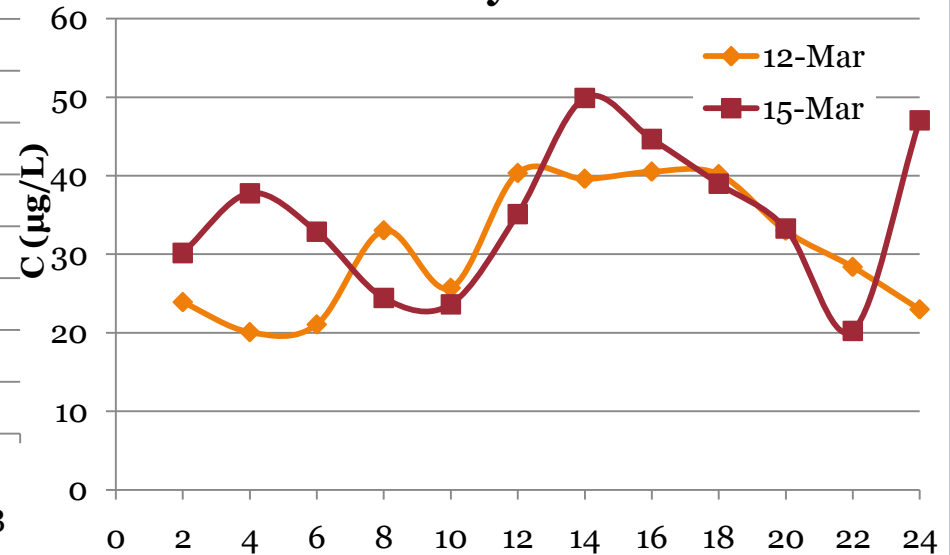
EFSA guideline:
<200 mg/day

Caffeine >
Paraxanthine > Theophylline

Intra-week trend



Intra-day trend



Results



Population biomarker

Castiglioni et al, Tob. Control, 2015

Nicotine & its metabolites

Cotinine
Hydroxy-cotinine

Daily loadings of metabolites

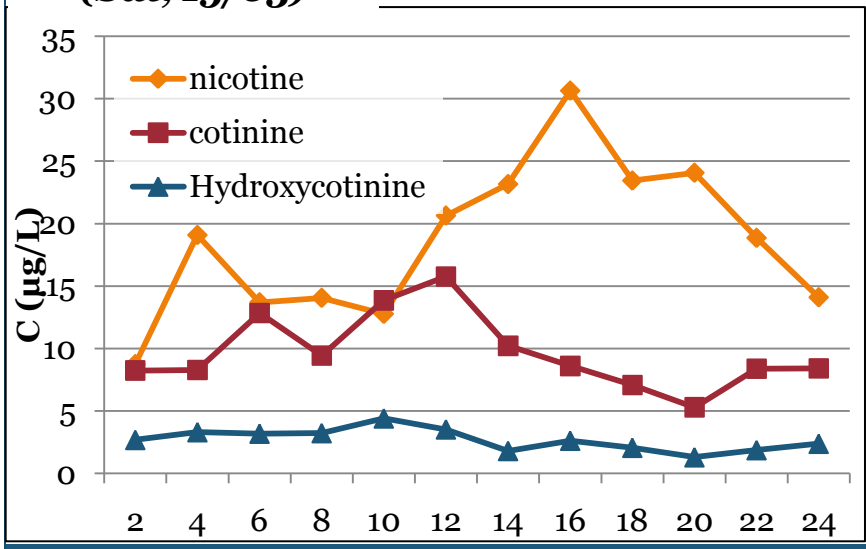
13.6 millions daily in Athens

Nicotine equivalents (excretion rates)

640,000 in Athens

Intra-day trend (Sat, 15/03)

Number of cigarettes



Number of smokers (21.4 % population, age >15)

Number of inhabitants estimated

~3.05 millions

Not completely accurate due to other sources of nicotine !

Results

Nucleosides

Components of DNA & RNA

Suspect Screening

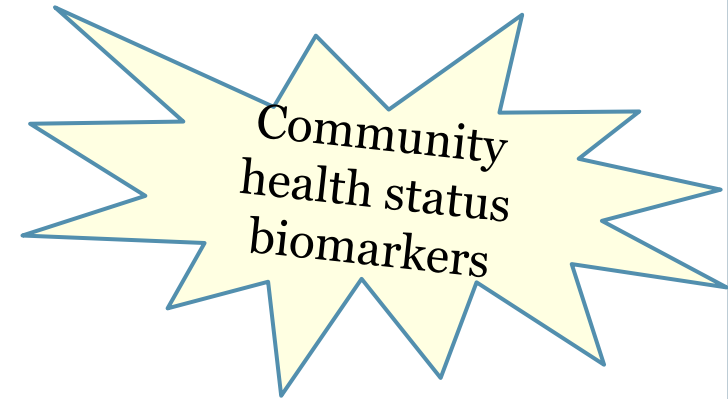
- Adenosine
- Deoxy- Adenosine
- Deoxy-Guanosine
- N-Methyl-Guanosine
- 7-Methyl-Guanosine
- Cytidine

Amino acids

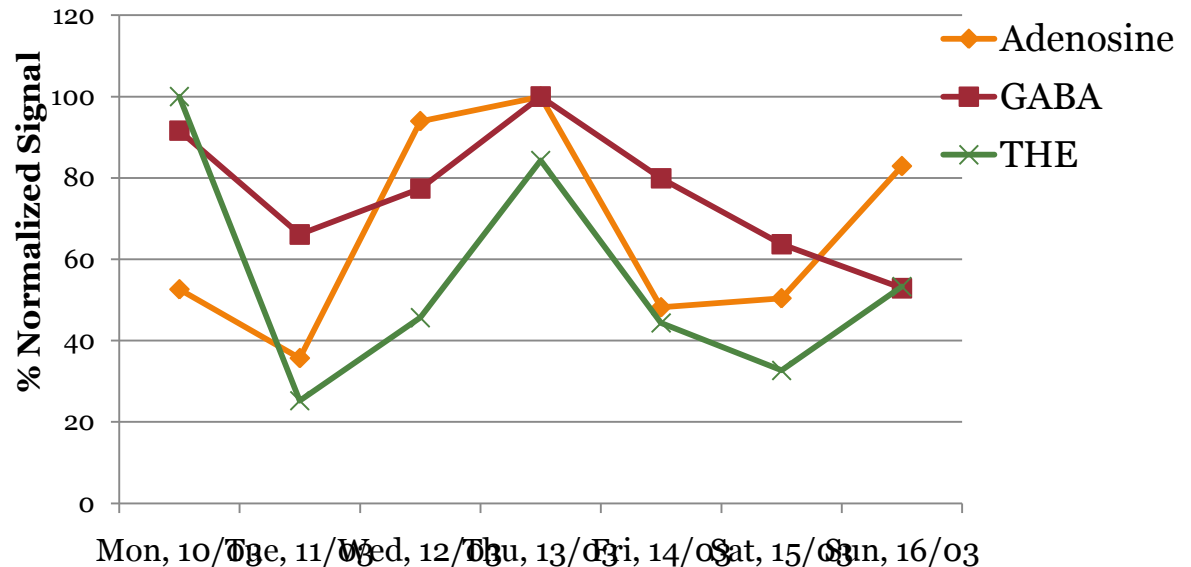
Neurotransmitters

Steroids

THE, THF



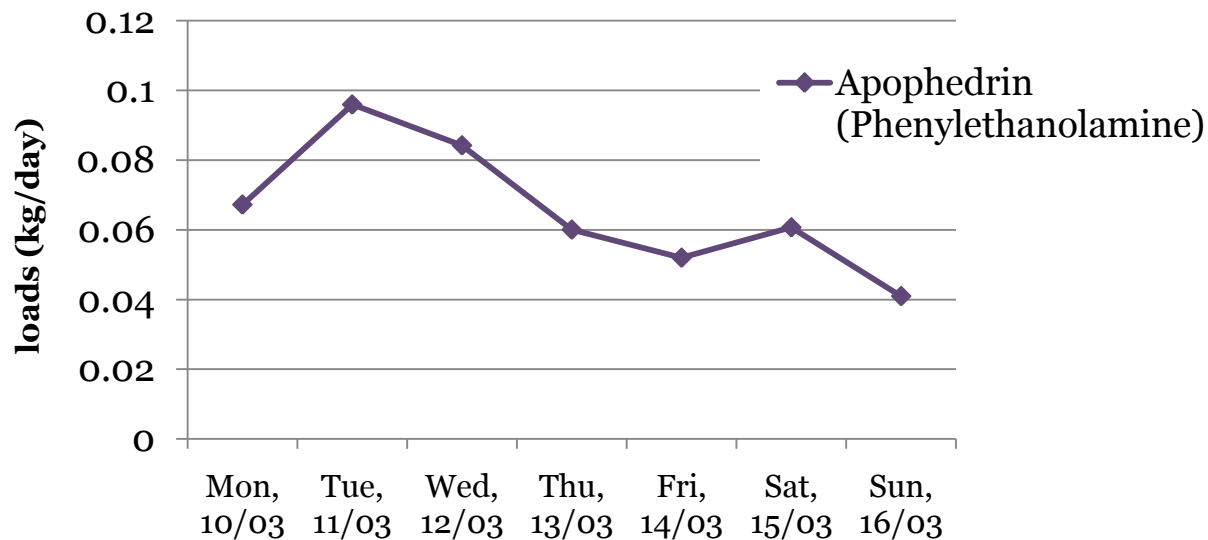
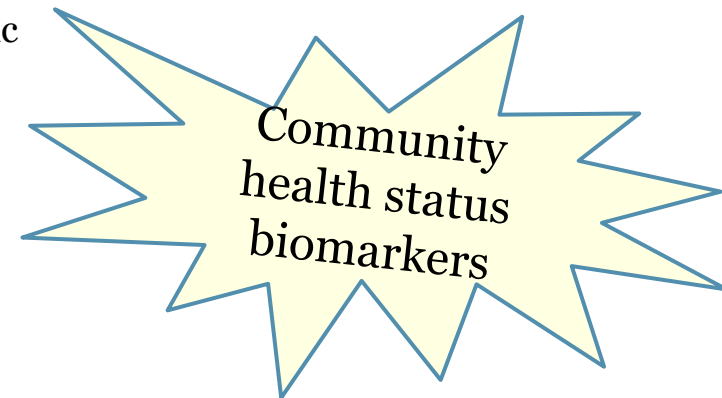
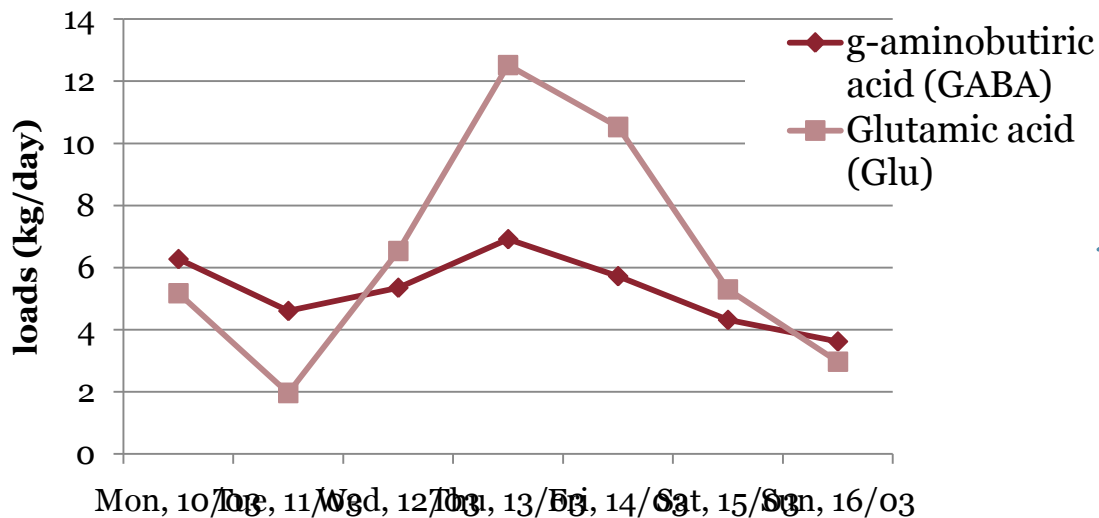
health stressors &
human health endpoints



Same trend over the week

Results

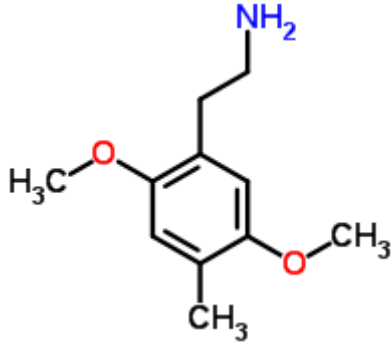
Daily Variations over a Week



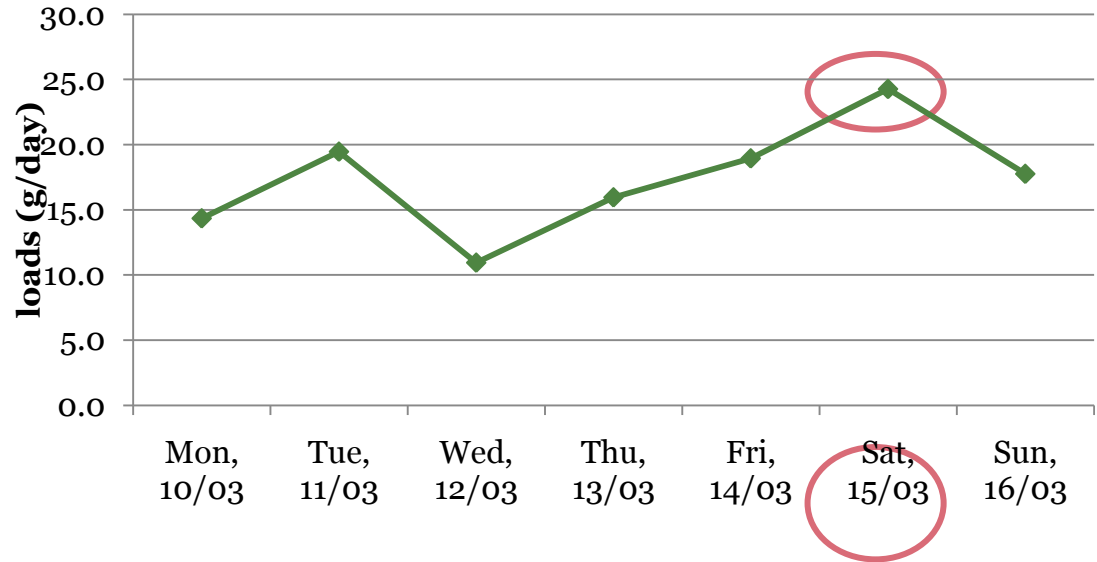
Results

2 C-D

2-(2,5-dimethoxy-4-methylphenyl)ethanamine

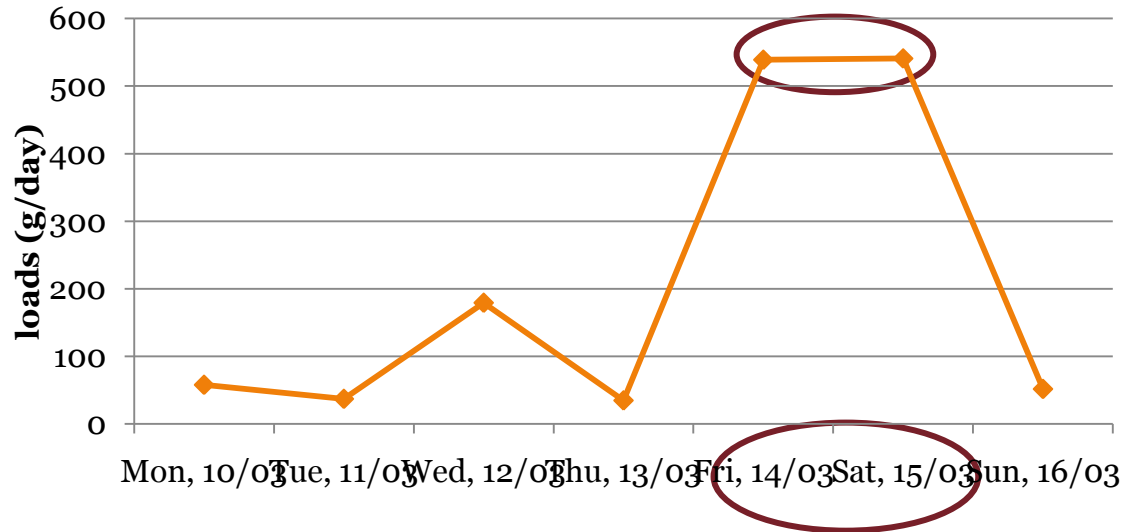
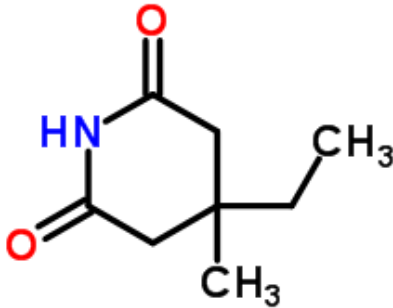


Daily Variation over a Week



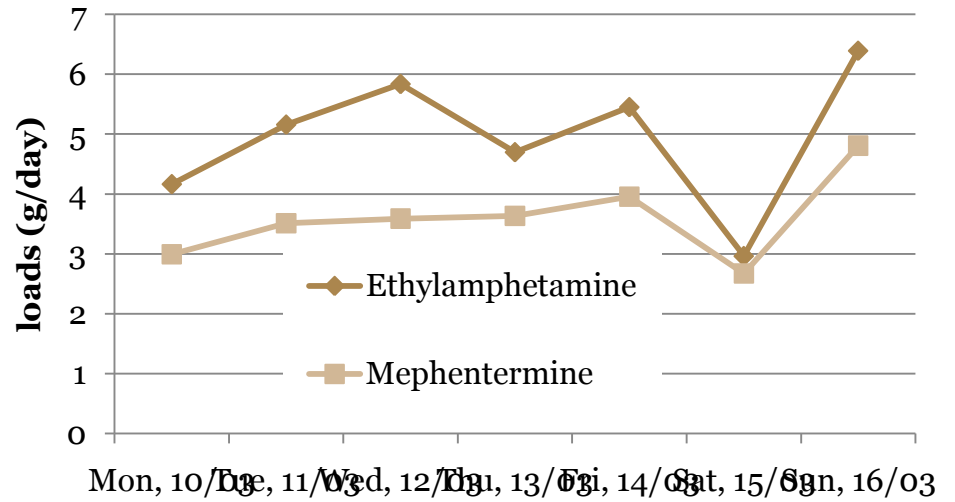
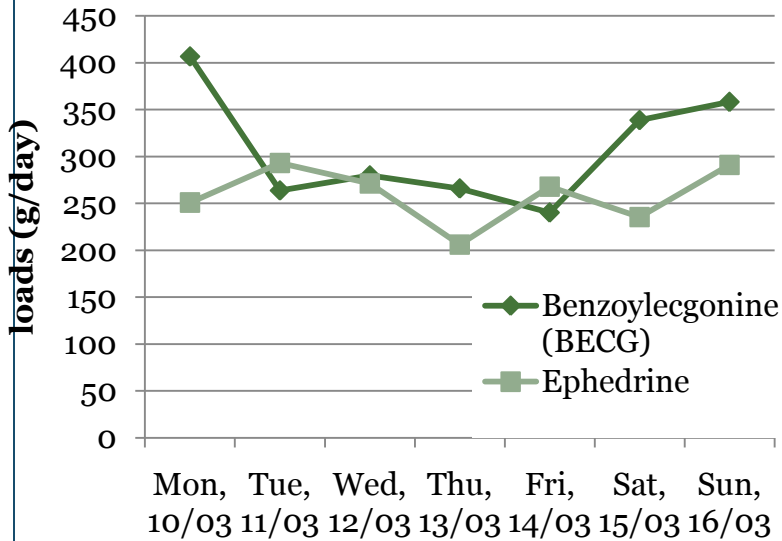
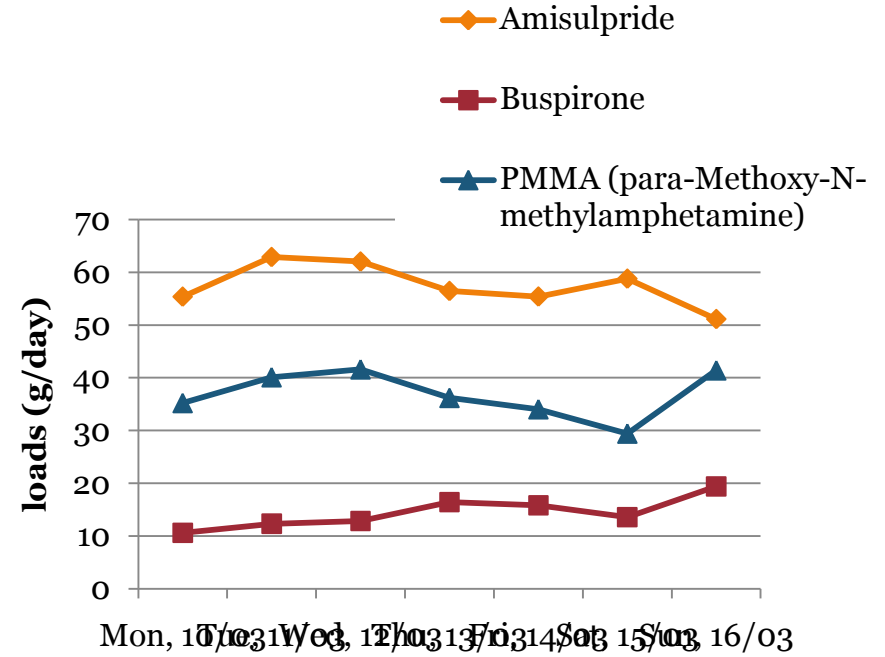
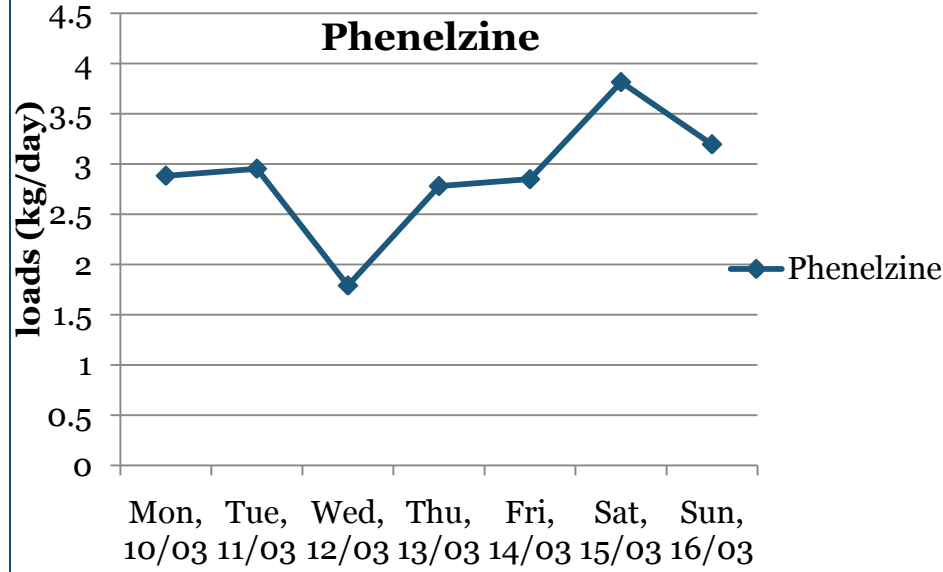
Bemegride

Supporting measure in treating depressant overdose



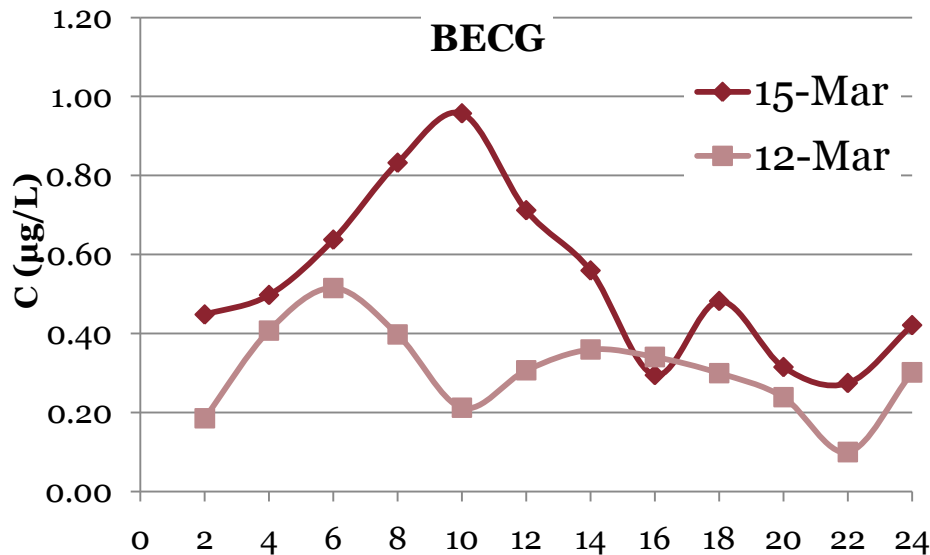
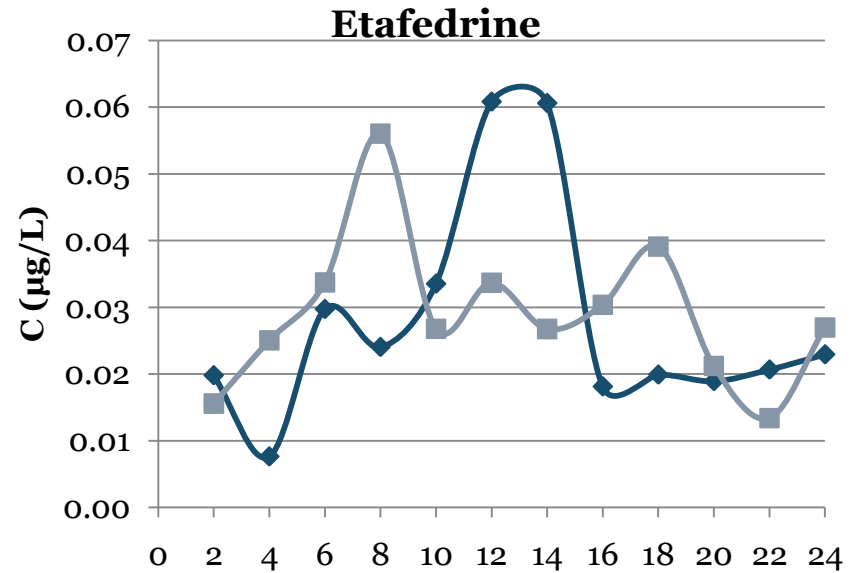
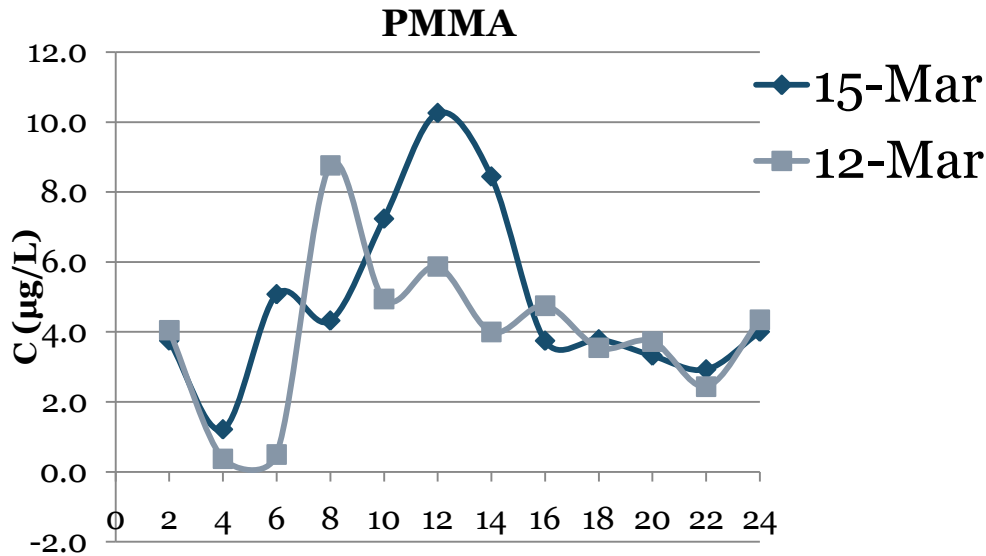
Results

Daily Variations over a Week



Results

Intra-Day Variation on the Weekend (15/03) & on Weekday (12/03)



✓ Same trend (same peak) during the same days
✓ Different peak between the days

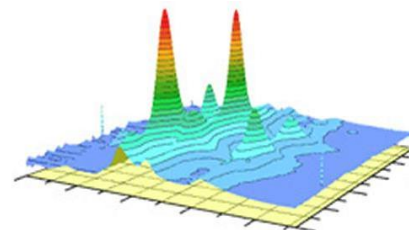
Conclusions

- ❖ Wide-scope target screening methods are useful: The wider, the better
- ❖ *In-house* database with 2327 compounds,
including 745 NPS, DoA, PP & metabolites
- ❖ Possibility for biomarkers search in one method after *statistical analysis*
- ❖ Retrospective analysis - Continuously increasing database
- ❖ Possibility for Suspect & Non-target Screening

Thank you for your attention!

Acknowledgments

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score

<http://trams.chem.uoa.gr/>

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