

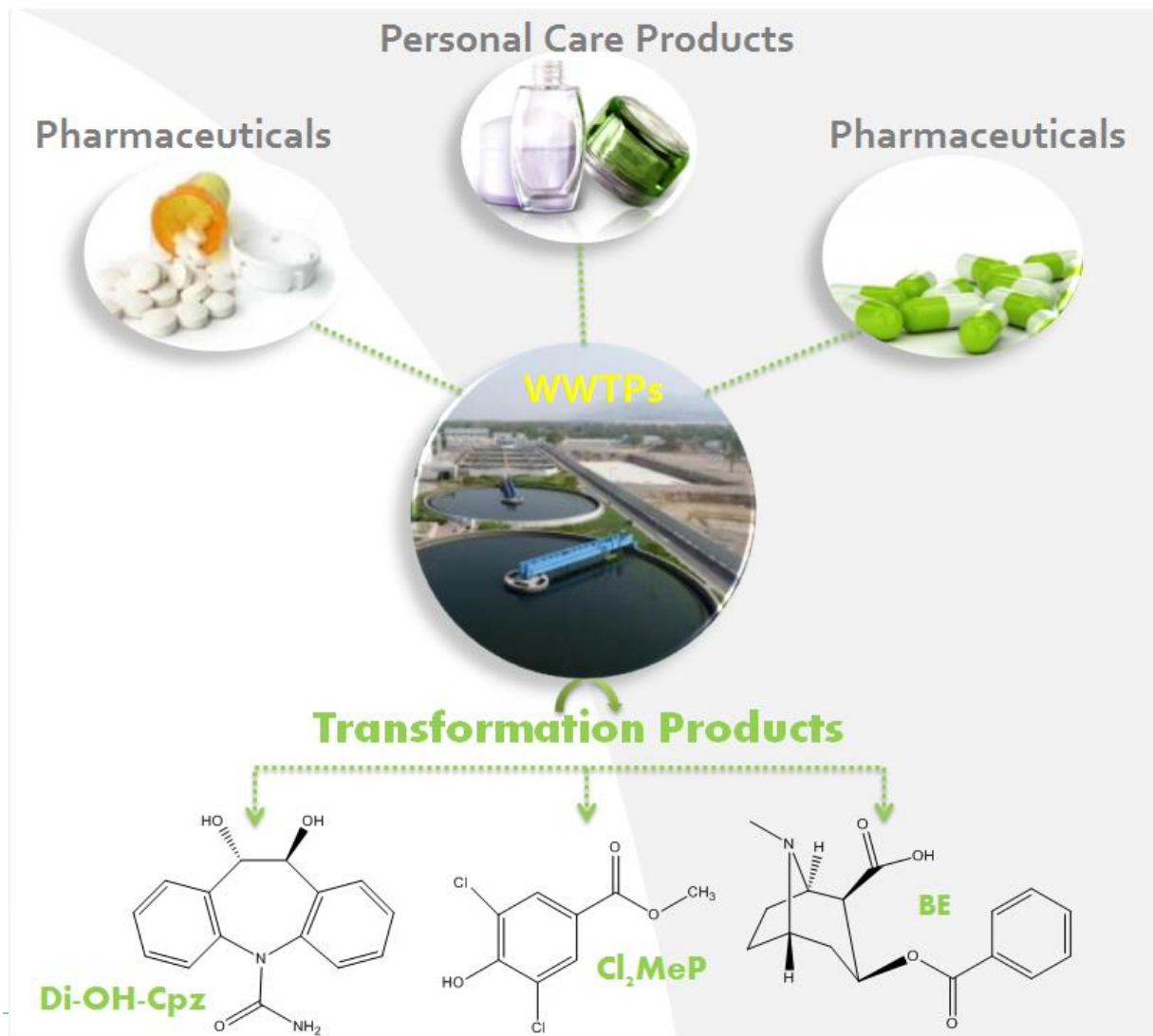
Suspect screening approach for determination of transformation products of pharmaceuticals in environmental samples using liquid chromatography high resolution mass spectrometry

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Transformation Products of Pharmaceuticals



Classification of Transformation Products of Pharmaceuticals

Biotic Processes

Biotic TPs

Human metabolites

Animal metabolites

Microbial metabolites

**in Engineered &
Natural Systems**

Abiotic Processes

Abiotic TPs

Hydrolysis

Photolysis

Chlorination

Ozonation

Advanced Oxidation

**Natural Environment &
Water-Treatment Processes**

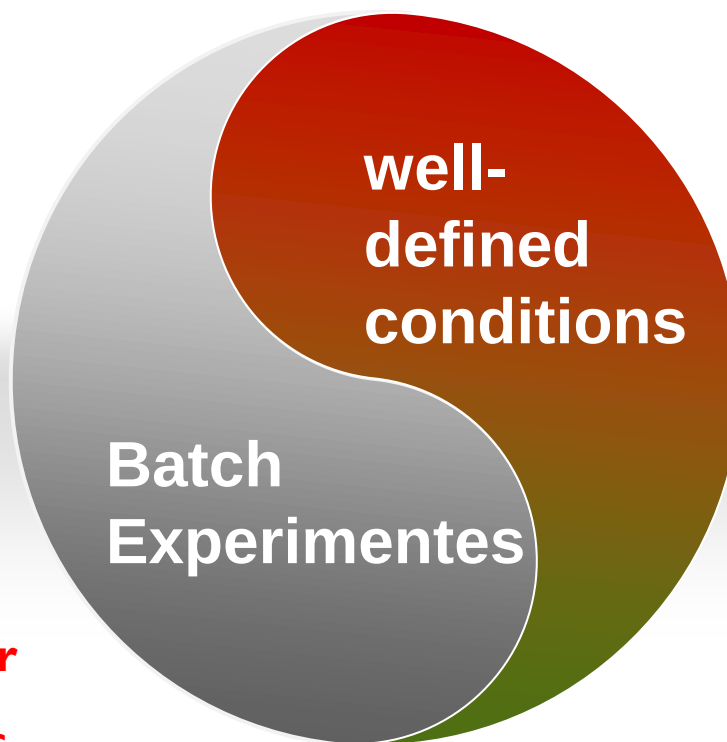
Transformation Products of Pharmaceuticals?

- Reported data show that **some TPs can be even more hazardous** than the parent compound, producing negative effects on living organisms
 - Therefore, it is important to investigate the possible presence of TPs of pharmaceuticals in environmental matrices e.g effluent wastewaters
 - However, **as many of the TPs are still unknown**, the analytical task is a challenge as not only the reported TPs must be detected and identified, **but also new/unreported TPs must be discovered**
 - Hence, there is a growing need in the field of exposure science for monitoring methods that rapidly screen environmental media **for suspect and non-target TPs.**
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Identification approaches – laboratory studies

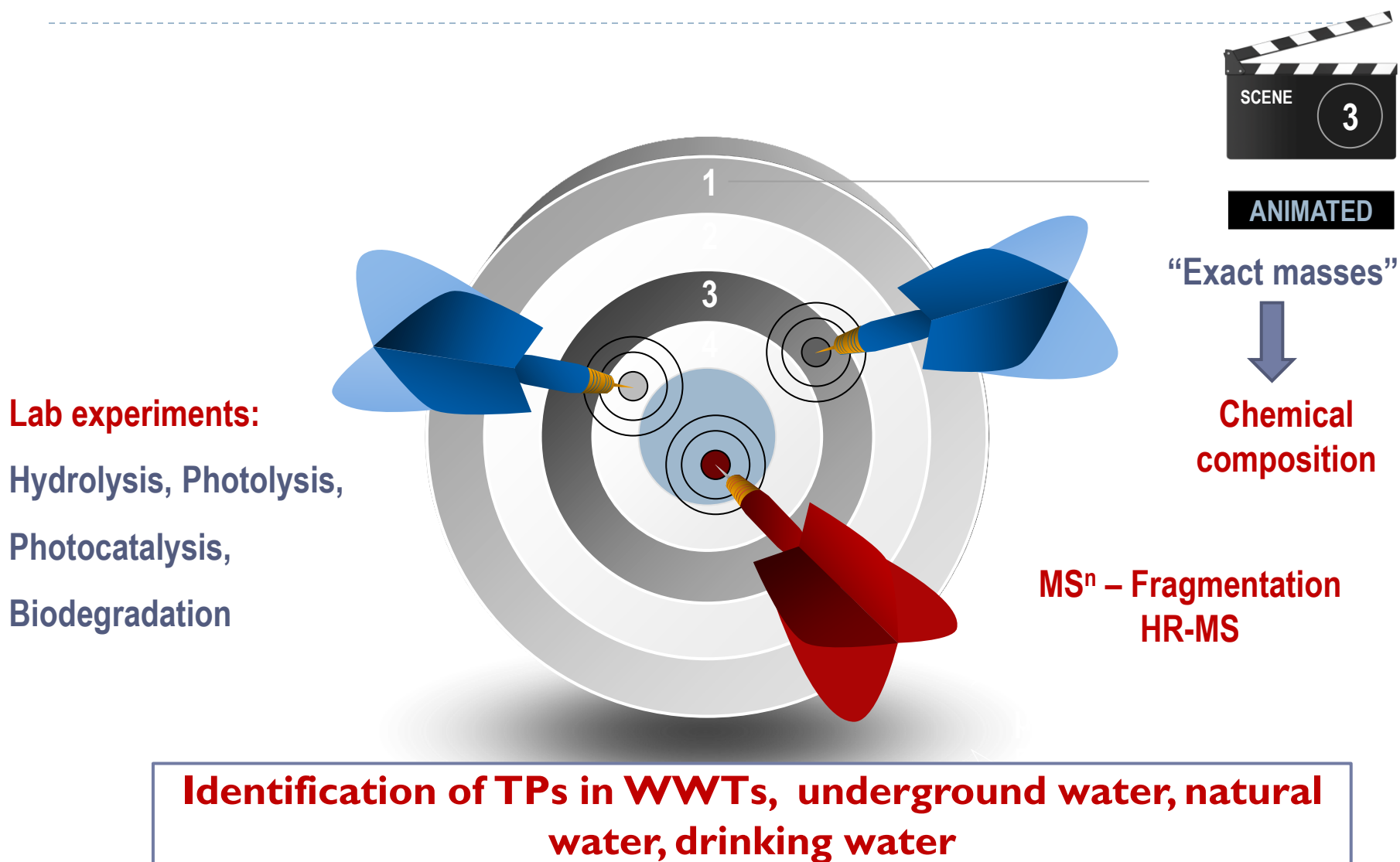
Simulation of the transformation processes in batch experiments under well-defined conditions with appropriate controls, **is a good alternative approach for the identification of TPs.**



Batch experiments can be applied **under biotic and abiotic conditions** at high concentrations of the parent Compounds.



Identification and Structure elucidation of TPs



Workflows of screening procedure of TPs

There are various workflows in the literature
for the identification of TPs,
depending on the instrumentation and
the available software

- Bletsou, A.A., Jeon, J., Hollender, J., Archontaki, E., Thomaidis, N.S. Targeted and non-targeted liquid chromatography-mass spectrometric workflows for identification of transformation products of emerging pollutants in the aquatic environment (2015) TrAC - Trends in Analytical Chemistry, 66, pp. 32-44.
- E.L. Schymanski, J. Jeon, R. Gulde, K. Fenner, M. Ruff, H.P. Singer, et al., Identifying small molecules via high resolution mass spectrometry: communicating confidence, Environ. Sci. Technol. 48 (2014) 2097–2098.
- Aurea C. Chiaia-Hernandez & Emma L. Schymanski & Praveen Kumar & Heinz P. Singer & Juliane Hollender Suspect and nontarget screening approaches to identify organic contaminant records in lake sediments, Anal Bioanal Chem, September 2014.
- C. Hug, N. Ulrich, T. Schulze, W. Brack, M. Krauss, Identification of novel micropollutants in wastewater by a combination of suspect and nontarget screening, Environ. Pollut. 184 (2014) 25–32.
- Dimitra A. Lambropoulou (Editor), Leo M. L. Nollet (Editor), Transformation Products of Emerging Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks, ISBN: 978-1-118-33959-6, 964 pages, February 2014

Workflows of screening procedure of TPs

- (a) **target analysis** (*Known TPs*), which is based on the determination of already known TPs, and identification is carried out with standard solutions;
- (b) **suspect screening** (*Known unknown TPs*), with a list of possible TPs assembled from the literature or from prediction models, and the samples are screened for those candidates; and,
- (c) **non-target screening** (*Unknown TPs - de novo identification of truly novel compounds*), with identification of novel TPs being carried out with sophisticated post-acquisition data tools and supplementary analytical techniques.

Suspect screening

- ❑ **Suspect screening** is the technique of choice for the identification of TPs, when the confirmation of the analytes with a reference standard is impossible, **but molecular formula and structure of suspected molecules can be predicted**
- ❑ In suspect screening, **an important step of the identification workflow is the prediction of possible TPs using computational (*in silico*) prediction tools.**



Data processing for screening

- ❑ **Peak detection by extracting those ions matched with entries in a database**

- Can be psuedo molecular ions and fragments

- ❑ **Recognition is based upon measurement of:**

- Accurate mass
- Isotope pattern
- Retention time (if available)
- A response threshold



Mass analyzers commonly employed

High Resolution Mass Spectrometers

time-of-flight

TOF

quadrupole
time-of-flight

Q-TOF

linear ion trap-Orbitrap
or
quadrupole-Orbitrap

OrbiTrap

**The benefits of high resolution MS in
high throughput screening of TPs**

Home made Database for TPs of pharmaceuticals

IDEA



The scope was to build a home made database
in order to provide an integrated knowledge on the presence of TPs of pharmaceuticals in influent and effluent wastewaters from Greece using LTQ-Orbitrap- MS.

Laboratory Simulated Methods

- ☐ Photolysis
- ☐ AOPs (e.g. Photocatalysis)

M



TPs

OrbiTrap

Target Compounds / Photolysis/ AOPs (32 Parent Compounds)

Analgesics-anti-inflammatory

Diclofenac
Ketoprofen
Ibuprofen
Paracetamol
Nimesulide
Naproxen
Tramadol

Antibiotics

Ciprofloxacin
Moxifloxacin
Norfloxacin
Erythromycin
Lincomycin
Sulfadiazine
Sulfamethoxazole
Trimethoprim

Phsychiatrics-antidepressants

Bupropion
Carbamazepine
Duloxetine
Fluvoxamine
Olanzapine
Risperidone
Sertraline
Venlafaxine

Beta - Blockers

Atenolol
Metoprolol
Nadolol
Pindolol
Propranolol

Cytostatics

Cytarabine
5-Flouoruracil

Antidiabetics

Metformin

Beta-agonists

Salbutamol

Database used for TPs of pharmaceuticals

A home-made database
>350 compounds



(corresponding to
metabolites/TPs)

A customized database was compiled containing :

- ☐ TPs for which reference standards were available
- ☐ compounds identified in experiments performed at laboratory under laboratory-controlled conditions

A second home-made database was also compiled, including :

theoretical exact masses of human metabolites /TPs reported in the literature



The presence of 28 TPs/human metabolites was confirmed in the samples

Photocatalysis of Venlafaxine

TPs	Rt	Elemental	Mass (m/z)		Error		D
		Formula	Theor.	Exper.	mDa	ppm	
VNF	8.72	C ₁₇ H ₂₇ N O ₂	278.2115	278.2115	0.05	-0.20	
342A	3.06	C ₁₇ H ₂₆ N O ₅	342.1911	342.1915	0.39	1.12	
342B	3.21	C ₁₇ H ₂₆ N O ₅	342.1911	342.1915	0.39	1.12	
342C	3.40	C ₁₇ H ₂₆ N O ₅	342.1911	342.1915	0.39	1.12	
342D	3.55	C ₁₇ H ₂₆ N O ₅	342.1911	342.1915	0.39	1.12	
342E	6.21	C ₁₇ H ₂₆ N O ₅	342.1911	342.1915	0.39	1.12	
342F	11.21	C ₁₇ H ₂₆ N O ₅	342.1911	342.1915	0.39	1.12	
340A	3.33	C ₁₇ H ₂₆ N O ₅	340.1754	340.1758	0.33	0.99	
340B	3.88	C ₁₇ H ₂₆ N O ₅	340.1754	340.1758	0.33	0.99	
326A	5.82	C ₁₇ H ₂₈ N O ₅	326.1950	326.1950	-1.20	-3.68	
326B	6.24	C ₁₇ H ₂₈ N O ₅	326.1950	326.1950	-1.20	-3.68	
326C	6.66	C ₁₇ H ₂₈ N O ₅	326.1950	326.1950	-1.20	-3.68	
326D	7.03	C ₁₇ H ₂₈ N O ₅	326.1950	326.1950	-1.20	-3.68	
324A	3.94	C ₁₇ H ₂₆ N O ₅	324.1805	324.1794	-1.15	-2.43	
324B	4.74	C ₁₇ H ₂₆ N O ₅	324.1805	324.1794	-1.15	-2.43	
324C	6.36	C ₁₇ H ₂₆ N O ₅	324.1805	324.1794	-1.15	-2.43	
324D	6.57	C ₁₇ H ₂₆ N O ₅	324.1805	324.1794	-1.15	-2.43	
312A	3.56	C ₁₆ H ₂₆ N O ₅	312.1805	312.1796	-0.95	-3.04	
312B	3.70	C ₁₆ H ₂₆ N O ₅	312.1805	312.1796	-0.95	-3.04	
312C	4.38	C ₁₆ H ₂₆ N O ₅	312.1805	312.1796	-0.95	-3.04	
310A*	3.18	C ₁₆ H ₂₄ N O ₅	310.1648	310.1638	-1.10	-3.54	
310B*	3.53	C ₁₆ H ₂₄ N O ₅	310.1648	310.1638	-1.10	-3.54	
310C*	7.74	C ₁₆ H ₂₄ N O ₅	310.1648	310.1638	-1.10	-3.54	
310A	5.25	C ₁₇ H ₂₈ N O ₄	310.2012	310.2007	-0.58	-1.88	
310B	5.49	C ₁₇ H ₂₈ N O ₄	310.2012	310.2007	-0.58	-1.88	
310C	6.21	C ₁₇ H ₂₈ N O ₄	310.2012	310.2007	-0.58	-1.88	
310D	6.56	C ₁₇ H ₂₈ N O ₄	310.2012	310.2007	-0.58	-1.88	
310E	7.17	C ₁₇ H ₂₈ N O ₄	310.2012	310.2007	-0.58	-1.88	
310F	8.55	C ₁₇ H ₂₈ N O ₄	310.2012	310.2007	-0.58	-1.88	
310G	8.61	C ₁₇ H ₂₈ N O ₄	310.2012	310.2007	-0.58	-1.88	
308A	5.69	C ₁₇ H ₂₈ N O ₃	308.1856	308.1846	-1.03	-3.35	
308B	5.72	C ₁₇ H ₂₈ N O ₃	308.1856	308.1846	-1.03	-3.35	
308C	5.85	C ₁₇ H ₂₈ N O ₃	308.1856	308.1846	-1.03	-3.35	
308D	6.36	C ₁₇ H ₂₈ N O ₃	308.1856	308.1846	-1.03	-3.35	
306A	5.31	C ₁₇ H ₂₄ N O ₄	306.1699	306.1693	-0.68	-2.2	
306B	6.08	C ₁₇ H ₂₄ N O ₄	306.1699	306.1693	-0.68	-2.2	
296A	2.8	C ₁₆ H ₂₆ N O ₄	296.1856	296.1855	-0.13	-0.46	
296B	7.56	C ₁₆ H ₂₆ N O ₄	296.1856	296.1855	-0.13	-0.46	

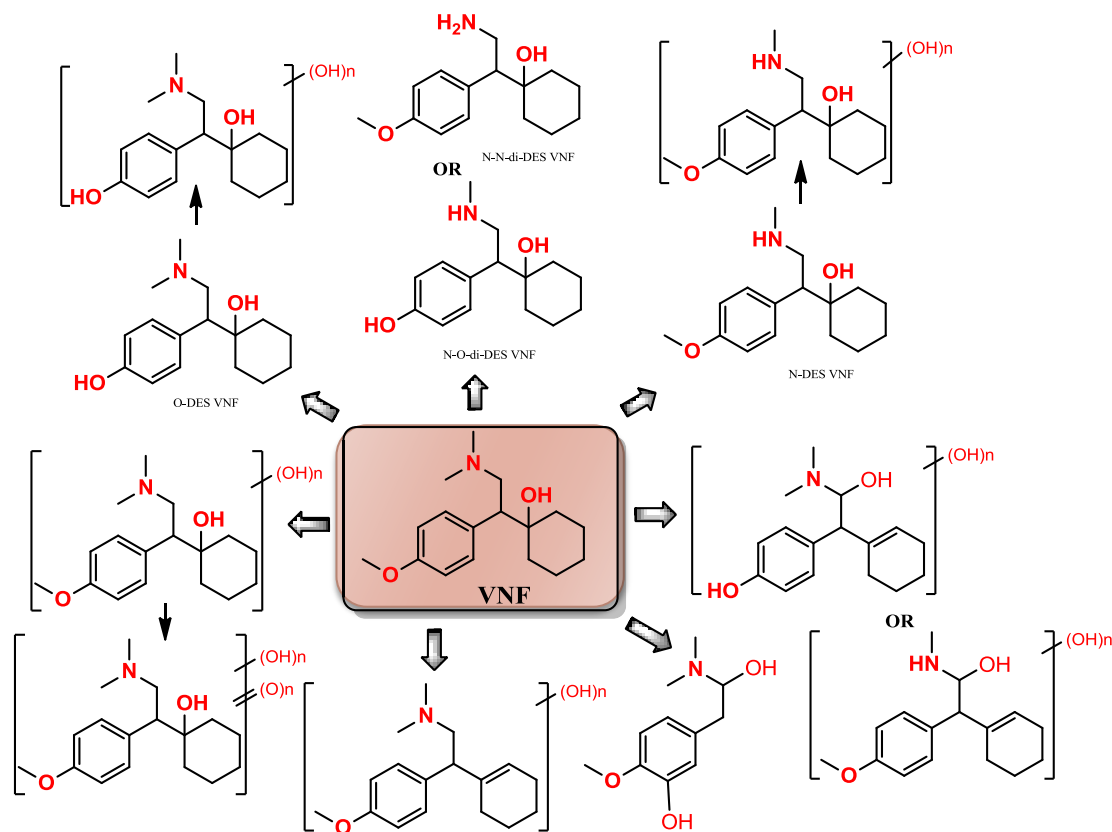
42 TPs
(OH-isomers,
demethylated,
dehydrated and
further oxidized
TPs etc)

Photocatalysis of Venlafaxine

TPs	Rt	Elemental Formula	Mass (m/z)		Error		DBE
			Theor.	Exper.	mDa	ppm	
VNF	8.72	C ₁₇ H ₂₇ N O ₂	278.2115	278.2115	0.05	-0.20	4.5
		C ₁₇ H ₂₆ N O	260.2008	260.2012	0.31	1.87	5.5
		C ₁₆ H ₂₄ N O	246.1852	246.1861	0.86	3.49	5.5
		C ₁₅ H ₁₉ O	215.1430	215.1437	0.66	3.06	6.5
		C ₈ H ₉ O	121.0647	121.0644	-0.39	-3.23	4.5
		C ₇ H ₉	93.0695	93.0698	-0.37	-4.04	3.5
294C	7.71	C ₁₇ H ₂₈ N O ₃	294.2063	294.2053	-1.07	-3.64	4.5
		C ₁₇ H ₂₆ N O ₂	276.1958	276.1951	-0.71	-2.55	5.5
		C ₁₇ H ₂₄ N O	258.1852	258.1863	1.05	4.10	6.5
		C ₁₅ H ₁₉ O ₂	231.1379	231.1372	-0.75	-3.27	6.5
		C ₁₄ H ₁₅ O	199.1111	199.1117	-0.64	-3.22	7.5
292B	6.41	C ₁₇ H ₂₆ N O ₃	292.1907	292.1899	-0.82	-2.81	5.5
		C ₁₇ H ₂₄ N O ₂	274.1801	274.1797	-0.46	-1.66	6.5
		C ₁₅ H ₁₇ O ₂	229.1223	229.1215	-0.81	-3.52	7.5
		C ₈ H ₉ O	121.0647	121.0644	-0.39	-3.23	4.5
274	7.27	C ₁₇ H ₂₄ N O ₂	274.1801	274.1793	-0.86	-3.12	6.5
		C ₁₅ H ₁₇ O ₂	229.1223	229.1216	-0.71	-3.08	7.5
		C ₁₅ H ₁₅ O	211.1117	211.1113	-0.44	-2.09	8.5
		C ₁₄ H ₁₁	179.0849	179.0855	-0.62	-3.50	9.5
		C ₁₂ H ₁₁ O	171.0804	171.0802	-0.24	-1.41	7.5
		C ₈ H ₉ O	121.0647	121.0644	-0.39	-3.23	4.5
O-DES-VNF	7.33	C ₁₆ H ₂₆ N O ₂	264.1958	264.1954	-0.40	-1.54	4.5
		C ₁₆ H ₂₄ N O	246.1852	246.1861	0.86	3.49	5.5
		C ₁₄ H ₁₇ O	201.1273	201.1270	-0.39	-1.94	6.5
		C ₁₁ H ₁₁ O	159.0822	159.0824	1.20	2.46	6.5
		C ₉ H ₉ O	133.0647	133.0644	-0.39	-2.94	5.5
		C ₈ H ₉ O	121.0647	121.0644	-0.39	-3.23	4.5
		C ₇ H ₇ O	107.0491	107.0489	-0.241	-2.25	4.5

42 TPs
(OH-isomers,
demethylated, etc)

Photocatalysis of Venlafaxine

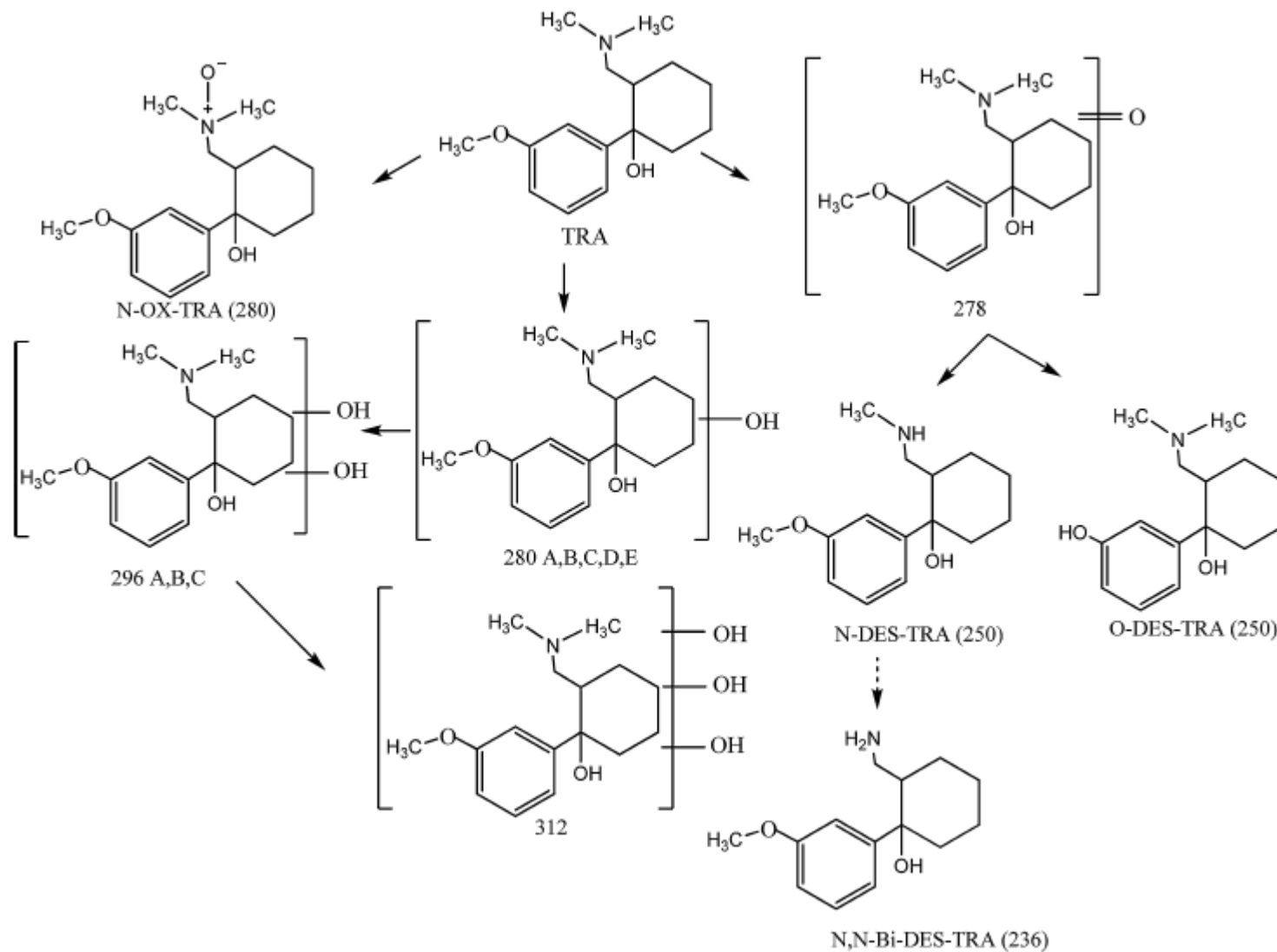


Photocatalysis of Tramadol

R _t	Molecular Formula	m/z [M+H] ⁺ / Fragments	Δ (ppm) (RDB)	MS ² fragmentation	RDB
7.30	C ₁₆ H ₂₆ NO ₂	[M+H] ⁺ 264.1949 (TRA)	-3.617 (4.5)	246.1852 C ₁₆ H ₂₄ NO (-H ₂ O)	0.565 (5.5)
5.60	C ₁₆ H ₂₆ NO ₃	[M+H] ⁺ 280.1888 (280-A)	-1.880 (4.5)	262.1803 C ₁₆ H ₂₄ NO ₂ (-H ₂ O)	0.114 (5.5)
		264.1941 C ₁₆ H ₂₆ NO ₂	-1.746 (4.5)	217.1225 C ₁₄ H ₁₇ O ₂ (-H ₂ O -C ₂ H ₇ N)	0.662 (6.5)
		250.1785 C ₁₅ H ₂₄ NO ₂	-1.626 (4.5)		
6.24	C ₁₆ H ₂₆ NO ₃	[M+H] ⁺ 280.1894 (280-B)	-1.300 (4.5)	262.1804 C ₁₆ H ₂₄ NO ₂ (-H ₂ O)	0.894 (5.5)
		264.1946 C ₁₆ H ₂₆ NO ₂	-4.715 (4.5)	217.1221 C ₁₄ H ₁₇ O ₂ (-H ₂ O -C ₂ H ₇ N)	-1.134 (6.5)
				149.0237 C ₈ H ₅ O ₃	2.748 (6.5)
6.62	C ₁₆ H ₂₆ NO ₃	[M+H] ⁺ 280.1903 (280-C)	-1.535 (4.5)	262.1804 C ₁₆ H ₂₄ NO ₂ (-H ₂ O)	0.834 (5.5)
		264.1946 C ₁₆ H ₂₆ NO ₂	-4.715 (4.5)	217.1224 C ₁₄ H ₁₇ O ₂ (-H ₂ O -C ₂ H ₇ N)	0.524 (6.5)
				149.0241 C ₈ H ₅ O ₃	5.096 (6.5)
6.84	C ₁₆ H ₂₆ NO ₃	[M+H] ⁺ 280.1904 (280-D)	-1.250 (4.5)	262.1804 C ₁₆ H ₂₄ NO ₂ (-H ₂ O)	0.834 (5.5)
		264.1946 C ₁₆ H ₂₆ NO ₂	-4.715 (4.5)	217.1224 C ₁₄ H ₁₇ O ₂ (-H ₂ O -C ₂ H ₇ N)	0.524 (6.5)
				149.0230 C ₈ H ₅ O ₃	-2.084 (6.5)
7.06	C ₁₆ H ₂₆ NO ₃	[M+H] ⁺ 280.193 (280-E)	-1.607 (4.5)	262.17996 C ₁₆ H ₂₄ NO ₂ (-H ₂ O)	-0.017 (5.5)
		264.1946 C ₁₆ H ₂₆ NO ₂	-4.715 (4.5)	217.1218 C ₁₄ H ₁₇ O ₂ (-H ₂ O -C ₂ H ₇ N)	-2.470 (5.5)
				149.0235 C ₈ H ₆ O ₃	1.070 (6.5)
5.43	C ₁₆ H ₂₄ NO ₃	[M+H] ⁺ 278.1887 (278)	-4.586 (5.5)		
4.98	C ₁₆ H ₂₆ NO ₄	[M+H] ⁺ 296.1844 (296A)	-4.101 (4.5)	278.1749 C ₁₆ H ₂₄ NO ₃ (-H ₂ O)	-0.504 (5.5)
		278.1738 C ₁₆ H ₂₄ NO ₃	-4.566 (5.5)	260.1635 C ₁₆ H ₂₂ NO ₂ (-2H ₂ O)	-3.826 (6.5)
		264.1946 C ₁₆ H ₂₆ NO ₂	-4.525 (4.5)	223.1567 C ₁₃ H ₂₁ NO ₂ (-C ₃ H ₅ O ₂)	-0.092 (4.0)
				114.0913 C ₆ H ₁₂ NO (-C ₁₀ H ₁₄ O ₃)	-0.180 (1.5)
5.89	C ₁₆ H ₂₆ NO ₄	[M+H] ⁺ 296.1844 (296B)	-4.101 (4.5)	278.1749 C ₁₆ H ₂₄ NO ₃ (-H ₂ O)	-0.504 (5.5)
		280.1894 C ₁₆ H ₂₆ NO ₃	-4.747 (4.5)	260.1635 C ₁₆ H ₂₂ NO ₂ (-2H ₂ O)	-3.826 (6.5)
		264.1945 C ₁₆ H ₂₆ NO ₂	-5.093 (4.5)	223.1567 C ₁₃ H ₂₁ NO ₂ (-C ₃ H ₅ O ₂)	-0.092 (4.0)
				114.0913 C ₆ H ₁₂ NO (-C ₁₀ H ₁₄ O ₃)	-0.180 (1.5)
7.92	C ₁₆ H ₂₆ NO ₃	[M+H] ⁺ 280.1893 (N-OX-TRA)	-5.069 (4.5)	262.1802 C ₁₆ H ₂₄ NO ₂ (-H ₂ O)	0.208 (5.5)
				201.1274 C ₁₄ H ₁₇ O (-C ₂ H ₇ NO -H ₂ O)	0.041 (6.5)
				135.0441 C ₈ H ₇ O ₂ (-C ₈ H ₁₉ NO)	0.548 (5.5)

**14 TPs
(OH-
isomers,
demethyla
ted,
oxidized
TPs etc)**

Tramadol Transformation pathways.....



TPs	Rt	Ion Formula	Mass	m/z	m/z (Calc)	Diff (ppm)	DBE
TP1	9,518	C6 H12 N O	113,084	114,0913	114,0913	0,36	2
TP2	2,525	C7 H16 N O2	145,1103	146,1176	146,1176	-0,31	1
TP3		C9 H12 N O2	165,079	166,0863	166,0863	-0,27	5
TP4		C9 H11 Cl N O	183,0451	184,0524	184,0524	-0,17	5
TP5	11,119	C12 H14 N O2	203,0946	204,1019	204,1019	0,03	7
TP6	12,943	C11 H15 Cl N O	211,077	212,0843	212,0837	-2,99	5
TP7	12,41	C13 H19 N O2	220,1341	221,1414	221,141	-1,68	5,5
TP8	10,51	C13 H20 N O2	221,1416	222,1489	222,1489	-0,2	5
TP9	8,63	C12 H18 N O3	223,1209	224,1282	224,1281	-0,36	5
TP10	10,814	C11 H15 Cl N O2	227,0713	228,0786	228,0786	-0,08	5
TP11		C13 H17 Cl N O	237,092	238,0993	238,0993	0,08	6
TP12	10,254	C13 H20 N O3	237,1365	238,1438	238,1438	-0,13	5
	14,091	C13 H19 Cl N O	239,1077	240,115	240,115	-0,13	5
TP14	20,88	C11 H16 Cl N O3	244,0714	245,0787	245,0813	10,74	4,5
TP15	17,152	C11 H17 Cl N O3	245,0818	246,0891	246,0891	0,19	4
TP16		C13 H18 N O4	251,1157	252,123	252,123	0,14	6
TP17		C13 H20 N O4	253,1314	254,1387	254,1387	-0,06	5
TP18	12,479	C13 H17 Cl N O2	253,0869	254,0942	254,0942	0,13	6
TP19	12,202	C13 H19 Cl N O2	255,1026	256,1099	256,1099	-0,07	5
TP20	13,194	C13 H19 Cl N O2	255,1026	256,1099	256,1099	-0,07	5
TP21	13,706	C13 H19 Cl N O2	255,1026	256,1099	256,1099	-0,07	5
TP22	13,93	C13 H19 Cl N O2	255,1026	256,1099	256,1099	-0,07	5
TP23	11,759	C12 H15 Cl N O3	255,0659	256,0732	256,0735	1,17	6
TP24	11,855	C13 H17 Cl N O3	269,0815	270,0888	270,0891	1,29	6
TP25	12,474	C13 H19 Cl N O3	271,0975	272,1048	272,1048	-0,01	5
TP26	3,853	C13 H21 Cl N O3	273,1131	274,1204	274,1204	0,17	4
TP27	4,109	C13 H21 Cl N O3	273,1131	274,1204	274,1204	0,17	4
TP28	4,525	C13 H21 Cl N O3	273,1131	274,1204	274,1204	0,17	4
TP29	4,813	C13 H21 Cl N O3	273,1131	274,1204	274,1204	0,17	4
TP30	11,406	C13 H17 Cl N O4	285,0771	286,0844	286,0841	-1,19	6

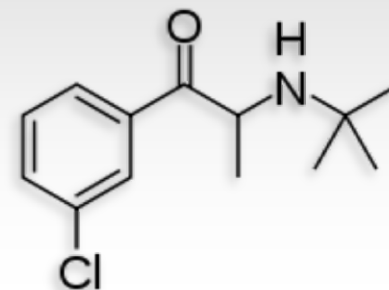
**30
TPs
(OH-
isome
rs,
dechlo
rinate
d,etc)**

BBP

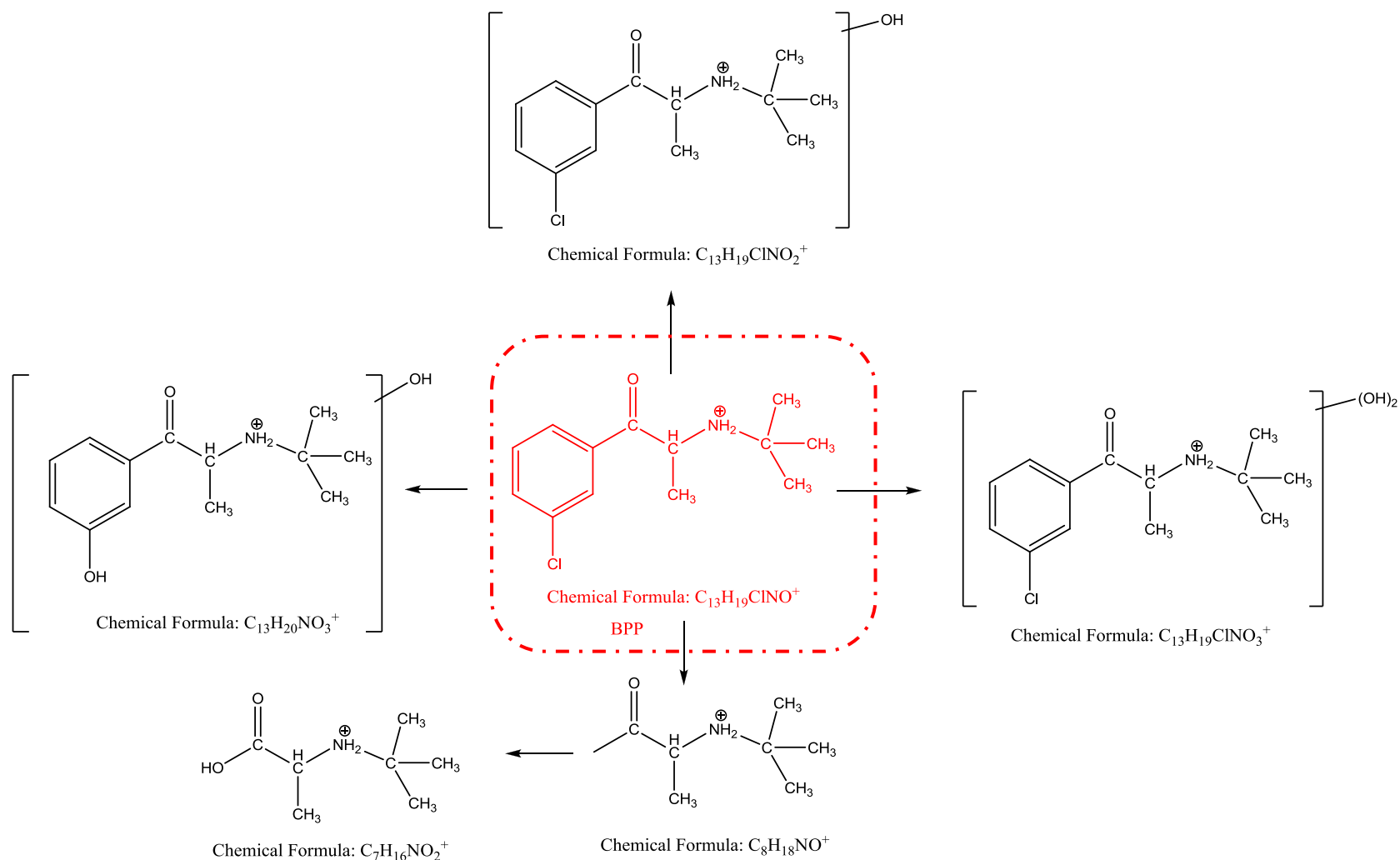


BBP – Transformation Products

- ✓ Hydroxy or keto BBP derivatives
- ✓ Ring opening derivatives
- ✓ De-chlorinated derivatives
- ✓ TPs arising from the cleavage of the amide bond



Bupropione Transformation pathways....

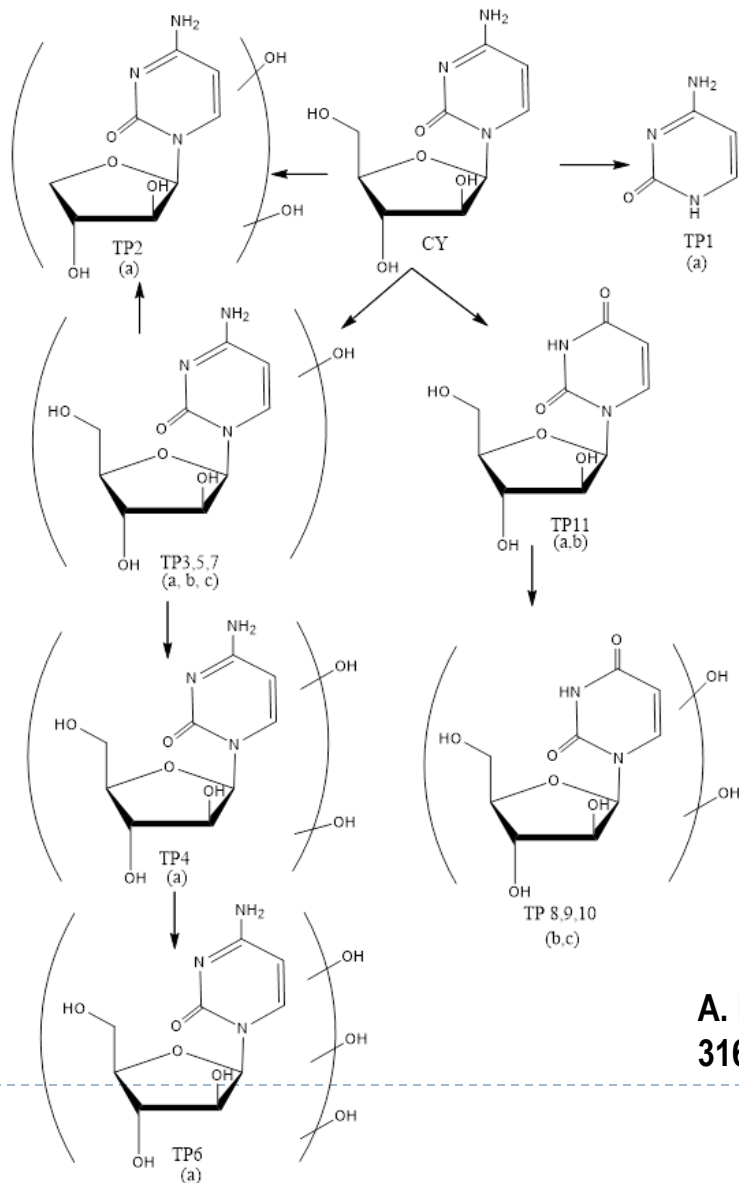


Cytarabine Transformation Products

t _R (min)	Code name	Pseudo- molecular ion formula	Theoretical m/z [m+H] ⁺	Experimental m/z [m+H] ⁺	Δ (ppm)	RDB E
1.51	CY	C ₉ H ₁₄ N ₃ O ₅	244.0928	244.0922	2.4	4.5
		C ₄ H ₆ N ₃ O	112.0505	112.0506	-0.6	3.5
0.79	TP1	C ₄ H ₆ N ₃ O	112.0505	112.0505	-0.3	3.5
		C ₄ H ₃ N ₂ O	95.0240	95.0233	-7.25	4.5
		C ₃ H ₅ N ₂	69.0447	69.0441	-9.05	2.5
0.83	TP2	C ₉ H ₁₄ N ₃ O ₈	292.0775	292.0773	0.8	4.5
		C ₉ H ₁₂ N ₃ O ₇	274.0670	274.0659	-3.9	5.5
		C ₈ H ₁₁ N ₂ O ₆	231.0612	231.0601	-4.6	4.5
0.95	TP3	C ₉ H ₁₄ N ₃ O ₆	260.0877	260.0874	0.5	1.2
		C ₉ H ₁₂ N ₃ O ₅	242.0771	242.0764	-3.08	5.5
		C ₄ H ₆ N ₃ O	112.0505	112.0500	-0.3	3.5
1.25	TP4	C ₉ H ₁₄ N ₃ O ₆	260.0877	260.0874	0.5	1.2
		C ₉ H ₁₂ N ₃ O ₅	242.0771	242.0764	-3.08	5.5
		C ₄ H ₆ N ₃ O	112.0505	112.0500	-0.3	3.5
0.75		C ₉ H ₁₄ N ₃ O ₇	276.0826	276.0820	2.3	4.5
		C ₉ H ₁₂ N ₃ O ₆	258.0721	258.0718	-1.0	5.5
0.74	TP5	C ₉ H ₁₄ N ₃ O ₈	292.0775	292.0760	5.3	4.5
		C ₉ H ₁₂ N ₃ O ₇	274.0670	274.0659	-3.9	5.5
		C ₈ H ₁₁ N ₂ O ₆	231.0612	231.0601	-4.6	4.5
0.83	TP7	C ₉ H ₁₁ N ₂ O ₈	275.0510	275.0508	-0.7	5.5
0.87	TP8	C ₉ H ₁₃ N ₂ O ₈	277.0666	277.0662	-1.6	4.5
		C ₉ H ₁₁ N ₂ O ₇	259.0561	259.0565		
0.91	TP9	C ₉ H ₁₁ N ₂ O ₈	275.0510	275.0508	-0.7	5.5
0.95	TP10	C ₉ H ₁₃ N ₂ O ₈	277.0666	277.0662	-1.6	4.5
		C ₉ H ₁₁ N ₂ O ₇	259.0561	259.0560	-0.59	5.5
1.16	TP11	C ₉ H ₁₂ N ₃ O ₇	274.0666	274.0667	-1.0	5.5
0.78	TP12	C ₉ H ₁₆ N ₃ O ₇	278.0983	278.0979	-1.4	3.5
		C ₉ H ₁₄ N ₃ O ₆	260.0877	260.0874	0.5	1.2
		C ₄ H ₆ N ₃ O	112.0505	112.0503	-2.1	3.5

12 TPs
**(OH-isomers,
etc)**

Cytarabine Transformation pathways.....

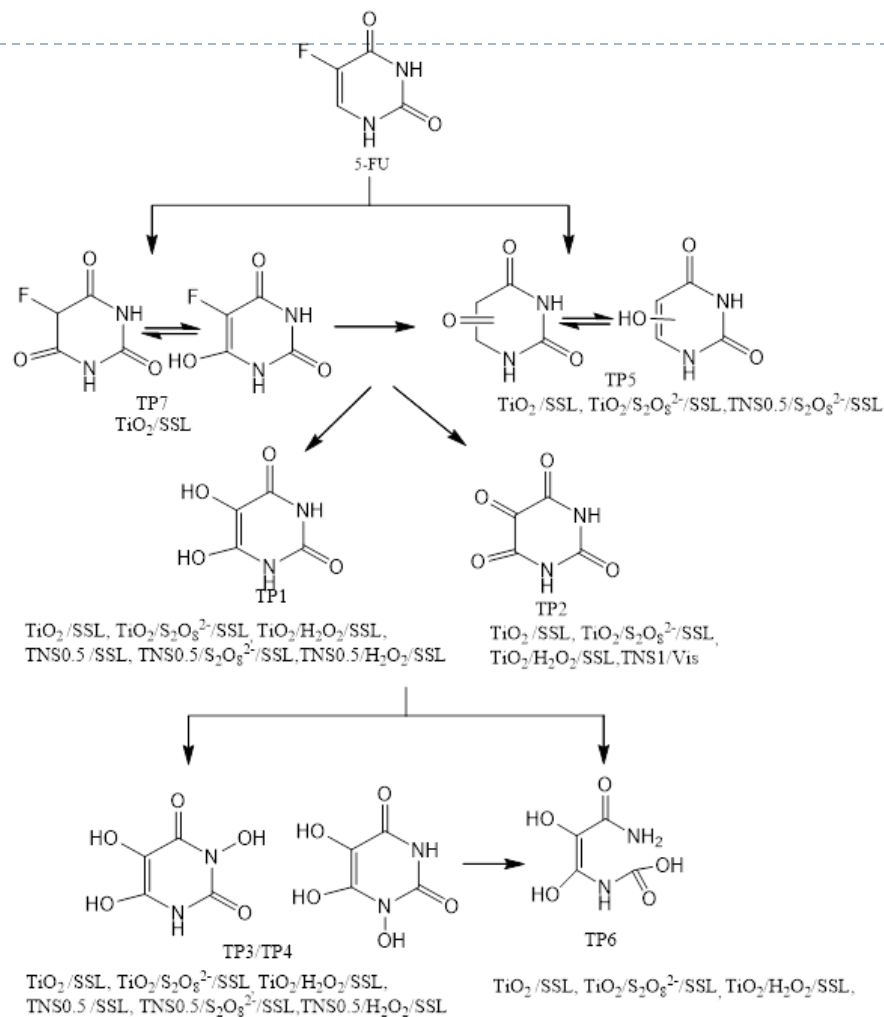


A. Koltsakidou et al. / Chemical Engineering Journal
316 (2017) 823–831

5-Fluorouracil Transformation pathways....

t_R (min)	Code name	Pseudo-molecular ion formula	Theoretical m/z $[m-H]^-$	Experimental m/z $[m-H]^-$	Δ (ppm)	RDBE
2.0	5-FU	$C_4H_2FN_2O_2$	129.0106	129.0105	0.3	4.5
1.0	TP1	$C_4H_3N_2O_4$	143.0098	143.0103	-3.0	4.5
1.2	TP2	$C_4HN_2O_4$	140.9942	140.9950	-5.6	5.5
1.1	TP3	$C_4H_3N_2O_5$	159.0047	159.0047	0.0	4.5
1.3	TP4	$C_4H_3N_2O_5$	159.0047	159.0048	-0.2	4.5
1.5	TP5*	$C_4H_3N_2O_3$	127.0149	127.0152	-2.3	4.5
t_R (min)	Code name	Pseudo-molecular ion formula	Theoretical m/z $[m+H]^-$	Experimental m/z $[m+H]^-$	Δ (ppm)	RDBE
2.0	5-FU*	$C_4H_4FN_2O_2$	131.0251	131.0254	-1.8	3.5
1.0	TP6	$C_4H_7N_2O_5$	163.0350	163.0352	-1.3	2.5
		$C_4H_5N_2$	145.0244	145.0249	-3.8	3.5
1.5	TP5*	$C_4H_5N_2O_3$	129.0295	129.0296	-0.9	3.5
2.1	TP7	$C_4H_4FN_2O_3$	147.0201	145.0198	1.7	3.5

5-Fluorouracil Transformation pathways....



**Bibliographic Research
(TPs, Human Metabolites)**

**Home Made Library
TPs (Photolysis, AOPs)**

Excel spreadsheet
Compound name, Theoretical Exact mass, t_R

Feeding masses into MS method

Sample Analysis by LC LTQ Orbitrap

Data processing

Automatic Screening

- Accuracy: Error ≤ 5 ppm
- Isotopic score $> 80\%$
- Threshold intensity > 500
- Area > 2000
- Blank subtraction

Manual Screening

- Gaussian Peak shape > 0.7
- t_R for identified TPs
- Hypothetic elution order on Theoretical LogPs

**Final
Library**

- ✓ t_R of TPs with available pure standards
- ✓ t_R of identified TPs ($t_R \pm 0.05$ min)
- ✓ MS^2 fragments

Database used for metabolites / TPs of pharmaceuticals

A broad investigation on the presence of TPs/metabolites of pharmaceuticals in effluent wastewaters from Greece using LTQ-Orbitrap- MS.

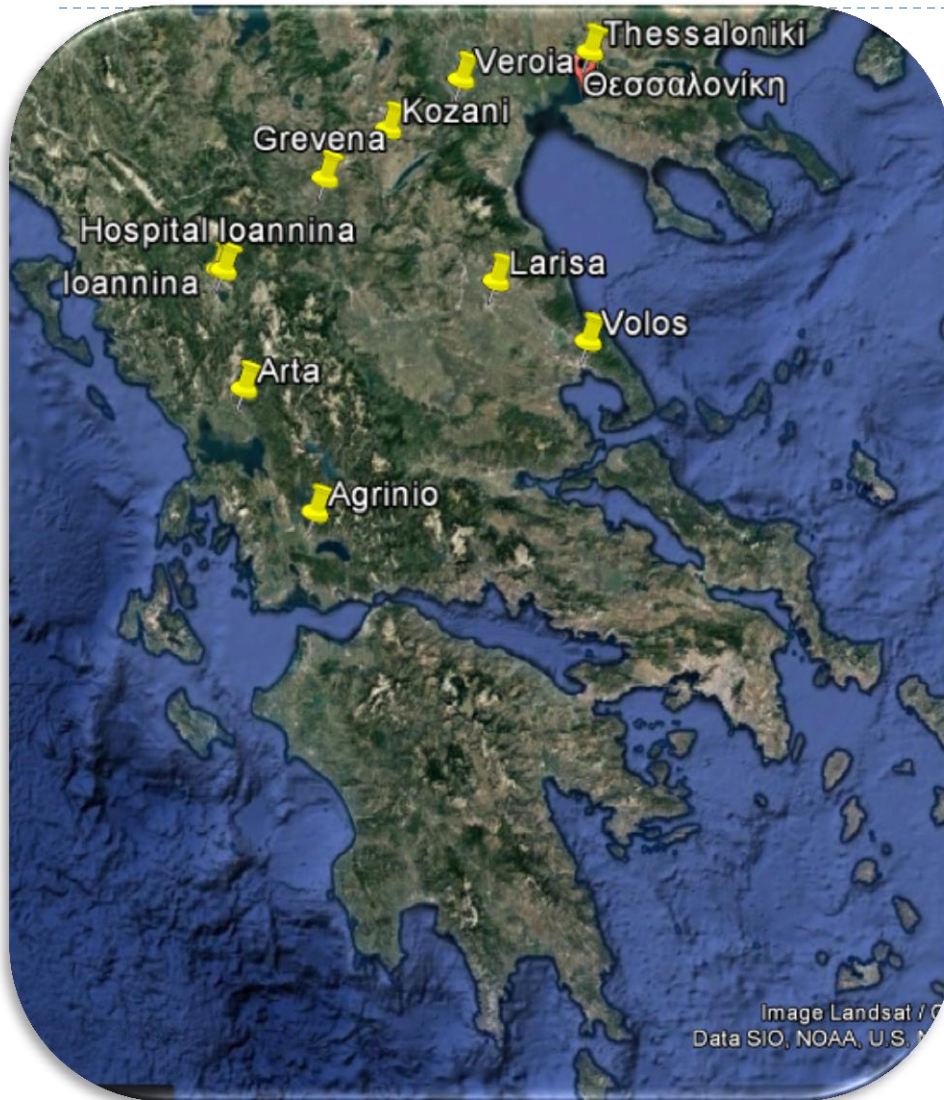
A home-made database

Up to **>350 compounds,**
including theoretical exact masses

**(corresponding to
metabolites/TPs)**



Application to real wastewater samples from Greece



Location: 8 WWTPs in Greece,
7 Municipal, 1 Hospital

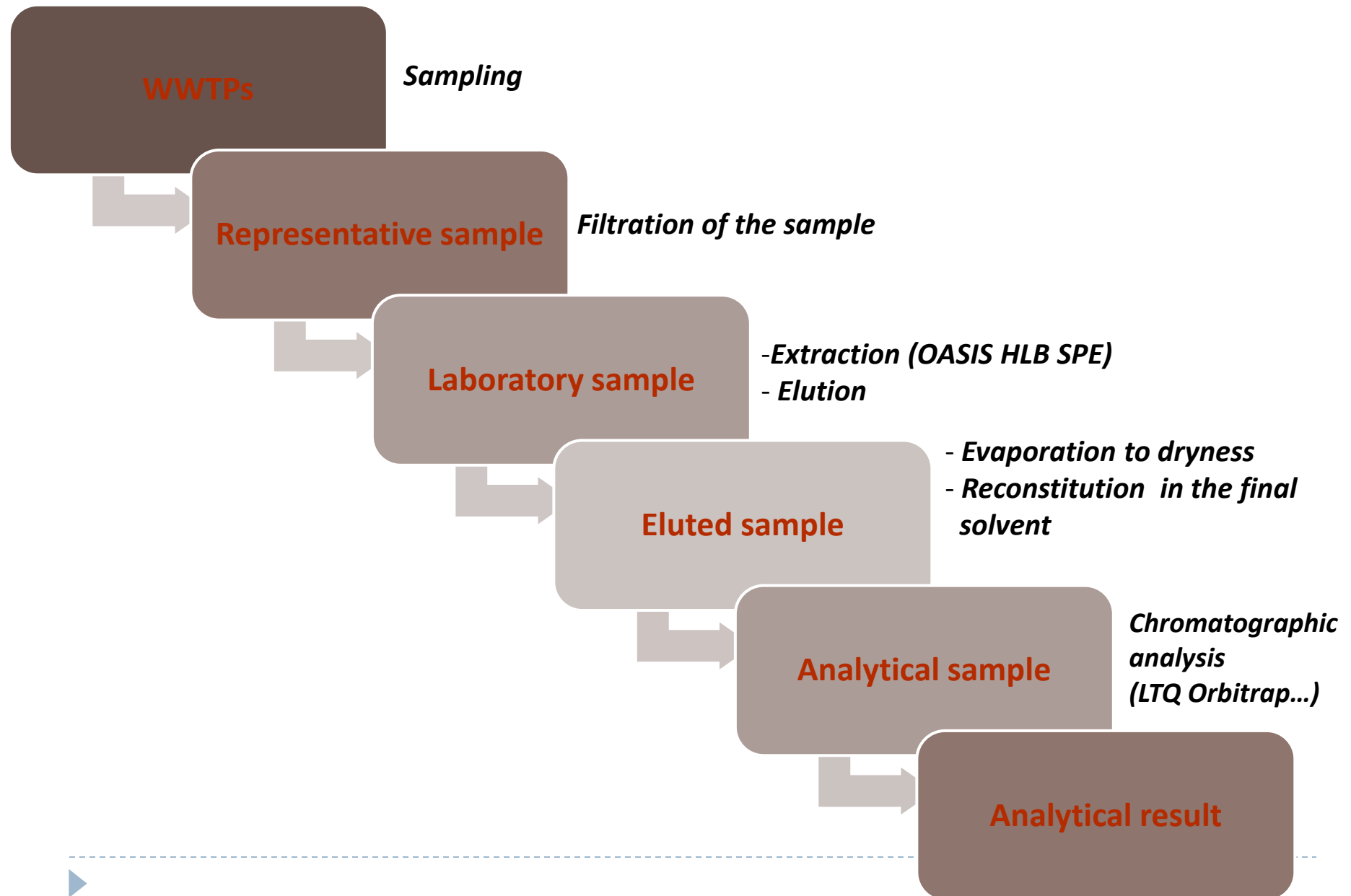
Samples: 24-h composite samples
Influents and effluents

Period : 1 year, 3 consecutive
days/month

Ch. Kosma, D. Lambropoulou, T. Albanis, *Science of The Total Environment*, Vol. 466–467, 1 2014, 421-438

Ch. Kosma, D. Lambropoulou, T. Albanis, *Water Research*, Volume 70, 1 March 2015, Pages 436-448

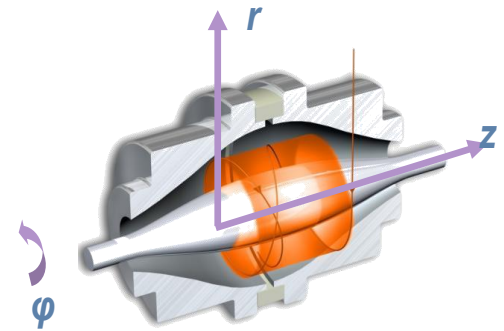
Experimental procedure



Application to real wastewater samples from Greece

- ✓ Samples analyzed in positive (PI) and negative (NI) ionization mode
- ✓ Gradient program

Orbitrap



28 transformation products were detected

TPs of Analgesics/antiinflammatory drugs

1-Hydroxy ibuprofen
2-Hydroxy ibuprofen
4-Hydroxy-diclofenac
5-Hydroxy-diclofenac
Hydroxy-ketoprofen
N-desmethyl-tramadol
O-desmethyl-tramadol

TPs of Antibiotics

Anhydro-erythromycin
Desmethyl-clarithromycin
Metabolite N-acetyl sulfamethoxazole
N-acetyl ciprofloxacin
(2 TPs of Trimethoprim - 2,4-diaminopyrimidin-5-yl)(3,4,5-trimethoxyphenyl)methanone) /2,4-diaminopyrimidine-5-carbaldehyde)

TPs of
Antidiabetics
Guanylurea

TPs of β -blockers

Hydroxy
propranolol

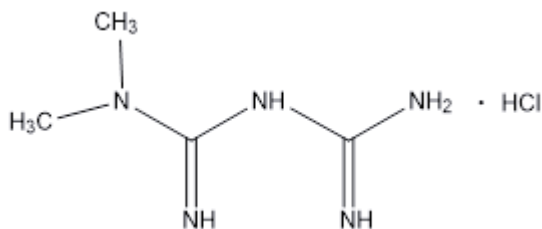
TPs of Antidepressants /psychiatrics

O-Desmethyl venlafaxine
N-Desmethyl venlafaxine
10-hydroxycarbazepine

7 TPs of Omeprazole
4 Human Metabolites
of Omeprazole

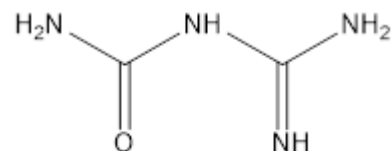
Application to real wastewater samples from Greece

The case study of Metformin



Metformin

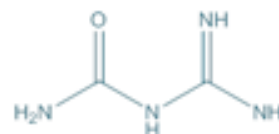
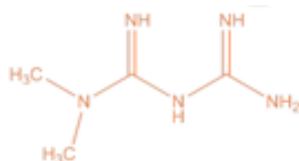
In some countries metformin **is in the top twenty list** of prescribed, produced and environmentally loaded pharmaceutically active compounds



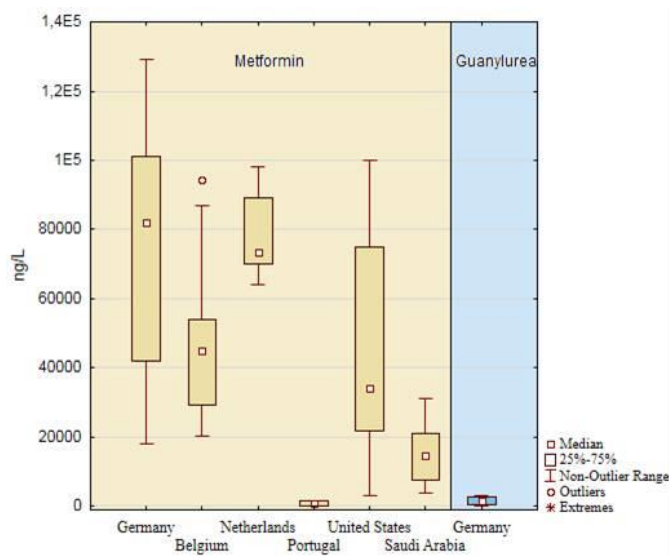
Guanyldurea

It was reported that concentrations of **metformin and guanyldurea together, account for more than half of the total load of pharmaceuticals in surface waters.**

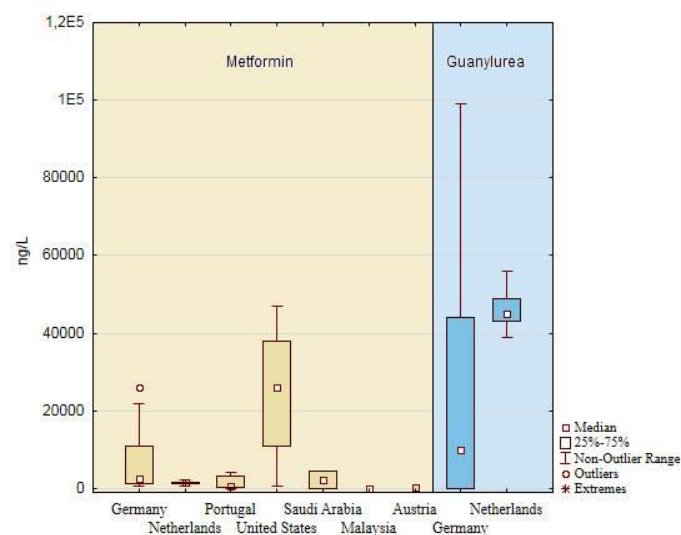
Metformin/Guanyurea in real wastewater samples from Greece



A

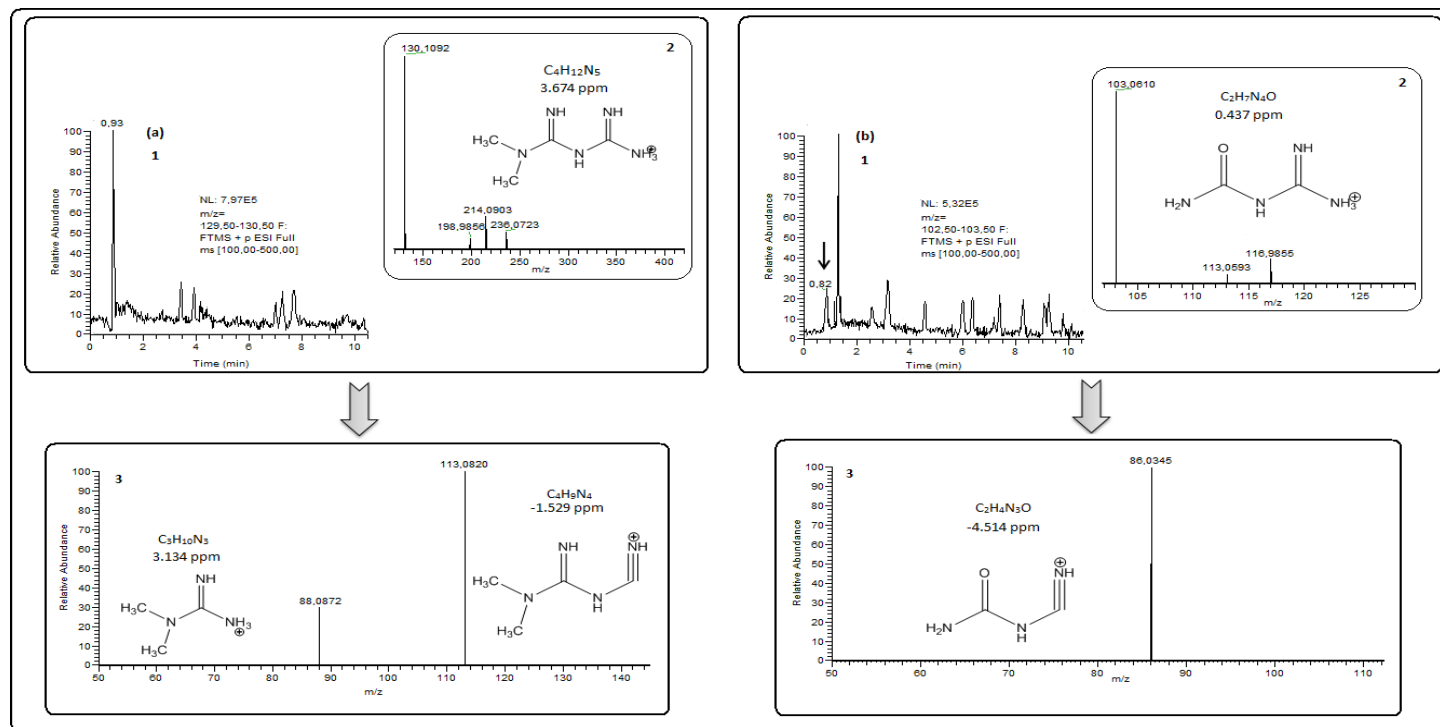


B



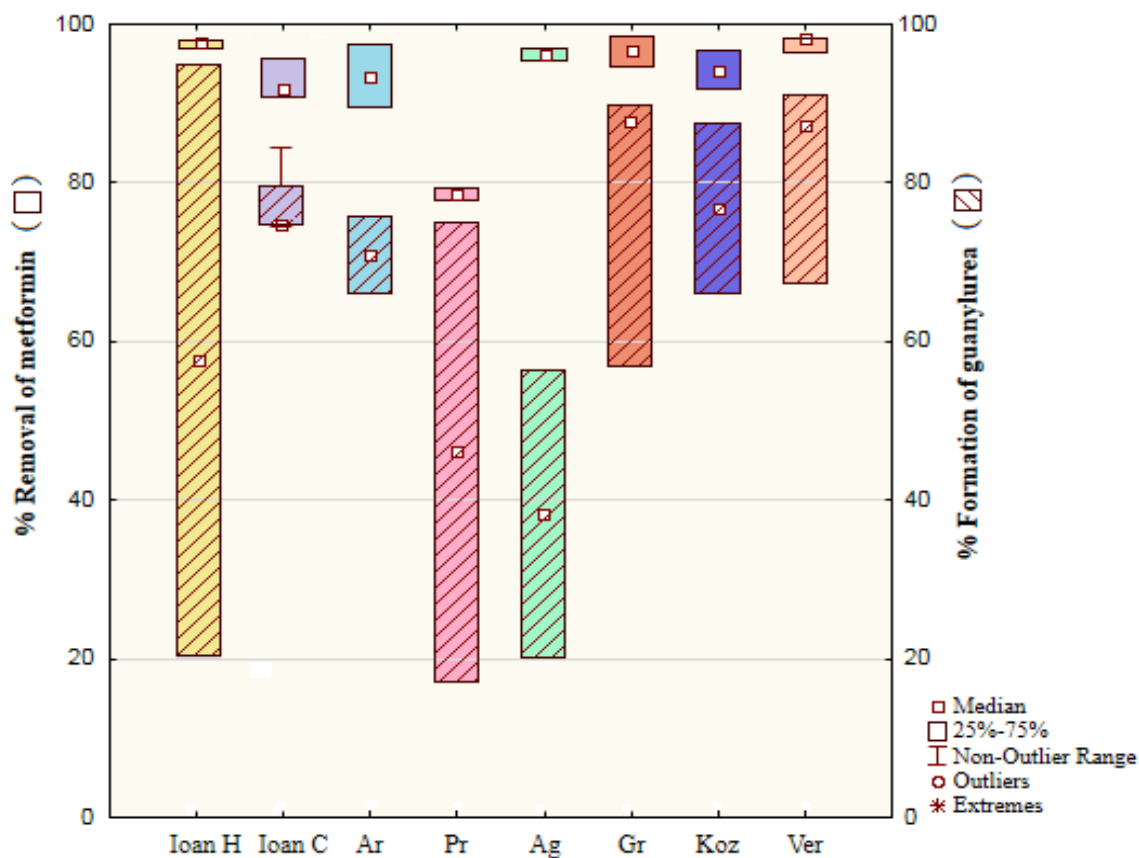
Concentration levels of metformin and guanyurea in (A) the influents and (B) the effluents of WWTPs, worldwide

Metformin/Guanylurea in real wastewater samples from Greece



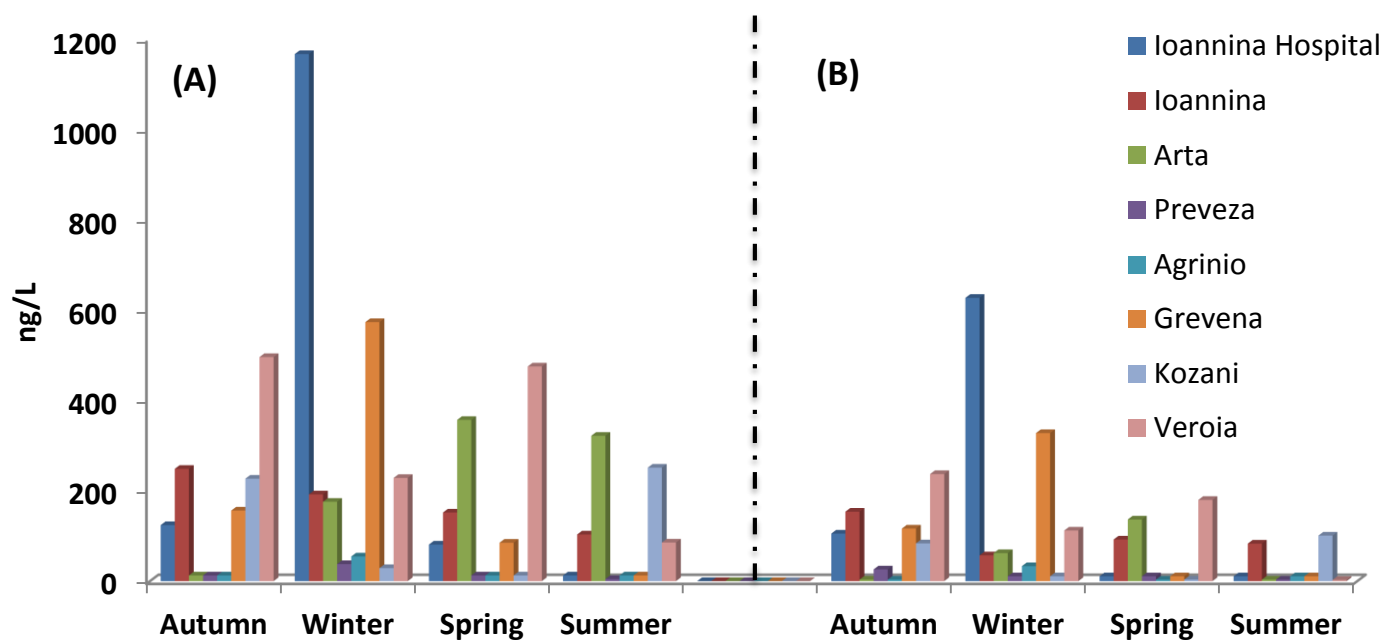
Chromatogram, (2) full scan accurate mass product ion spectrum and (3) MS/MS data obtained using Orbitrap MS targeting the corresponding ions, for (a) metformin and (b) guanylurea, respectively, found in the influent of Ioannina hospital WWTP in winter.

Metformin/Guanylurea in real wastewater samples from Greece

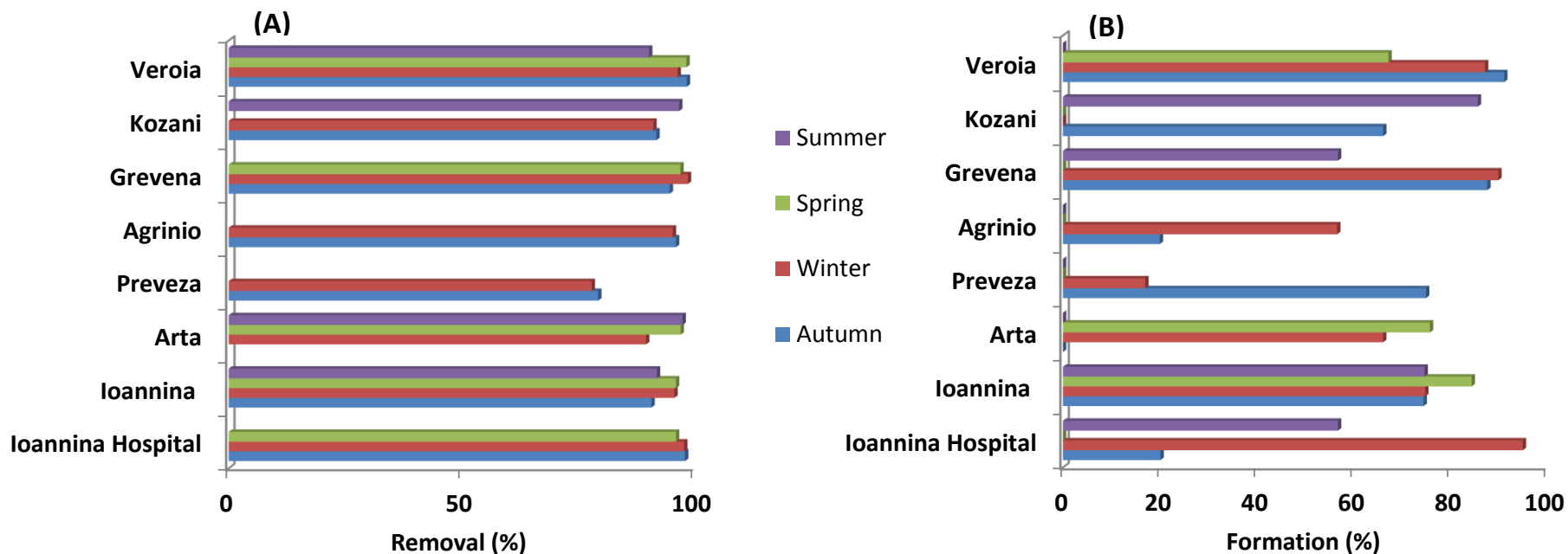


- Box and Whisker graphs showing the removal efficiencies (%) of metformin and the formation of guanylurea (%), in the eight WWTPs (Ioan C: Ioannina City, Ioan H: Ioannina Hospital, Ar: Arta, Pr: Preveza, Ag: Agrinio, Gr: Grevena, Koz: Kozani, Ver: Veroia).

Seasonal occurrence of (A) metformin in the influents and (B) guanylurea in the effluents



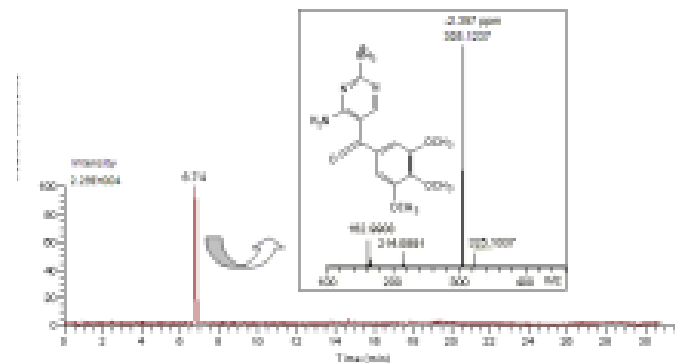
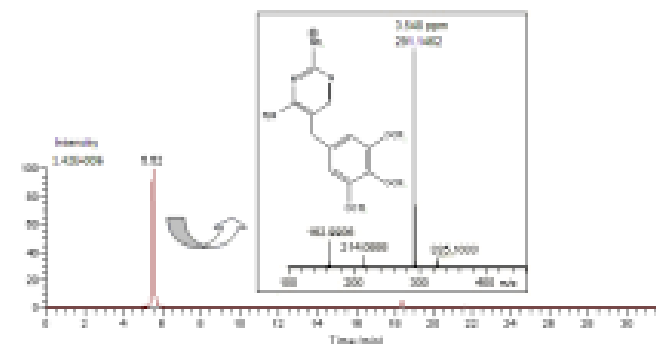
Metformin/Guanylurea in real wastewater samples from Greece



Seasonal removal efficiency (%) of metformin and (B) Seasonal formation (%) of guanylurea

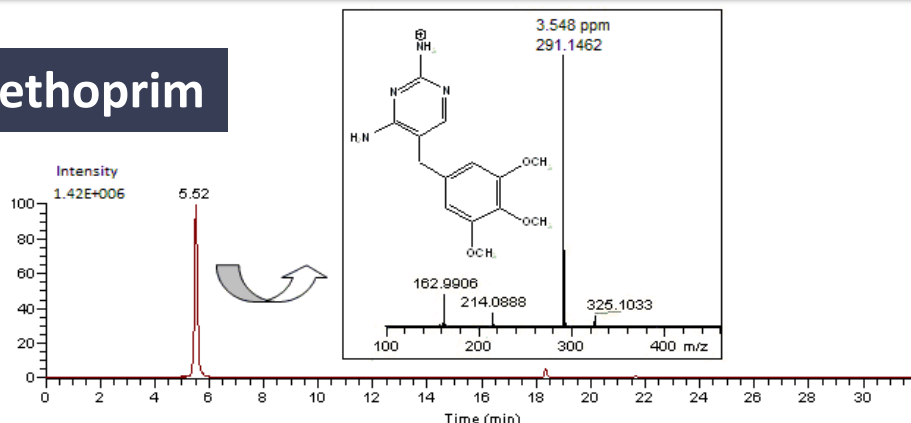
Application to real wastewater samples from Greece

The case study of Trimethoprim

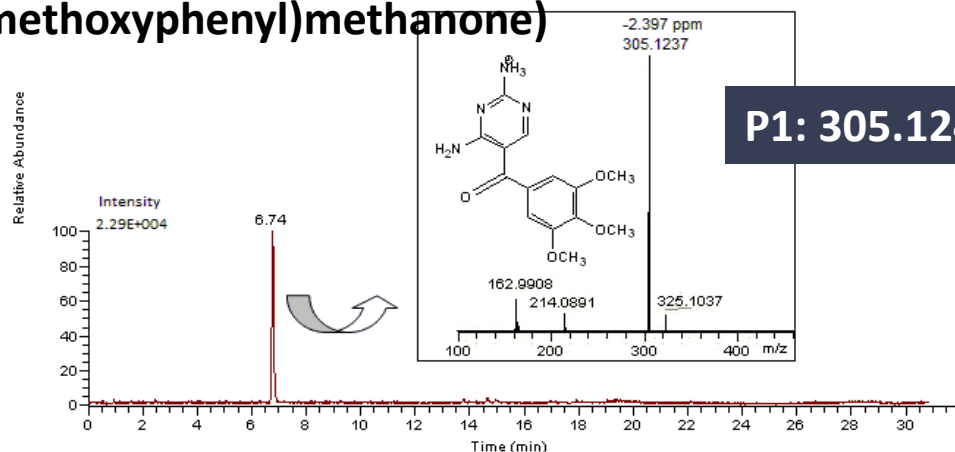


Transformation products (TPs) of Trimethoprim in WWTPs

Trimethoprim



2,4-diaminopyrimidin-5-yl(3,4,5-trimethoxyphenyl)methanone



P1: 305.1244 m/z

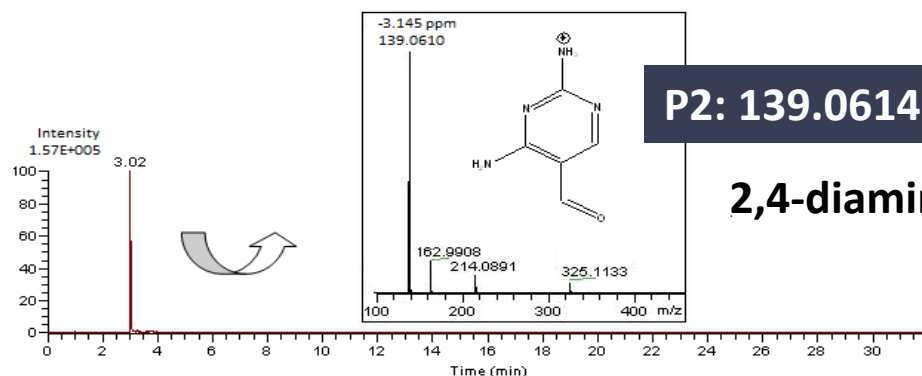
LTQ Orbitrap MS

Suspect screening
analysis



WWTP Effluent

(Ioannina – Veroia in Summer)

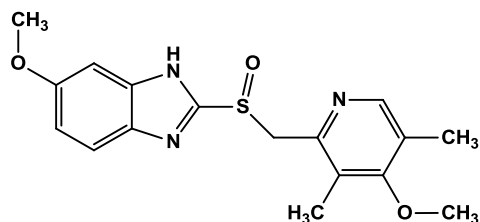


P2: 139.0614 m/z

2,4-diaminopyrimidine-5-carbaldehyde

Application to real wastewater samples from Greece

The case study of Omeprazole



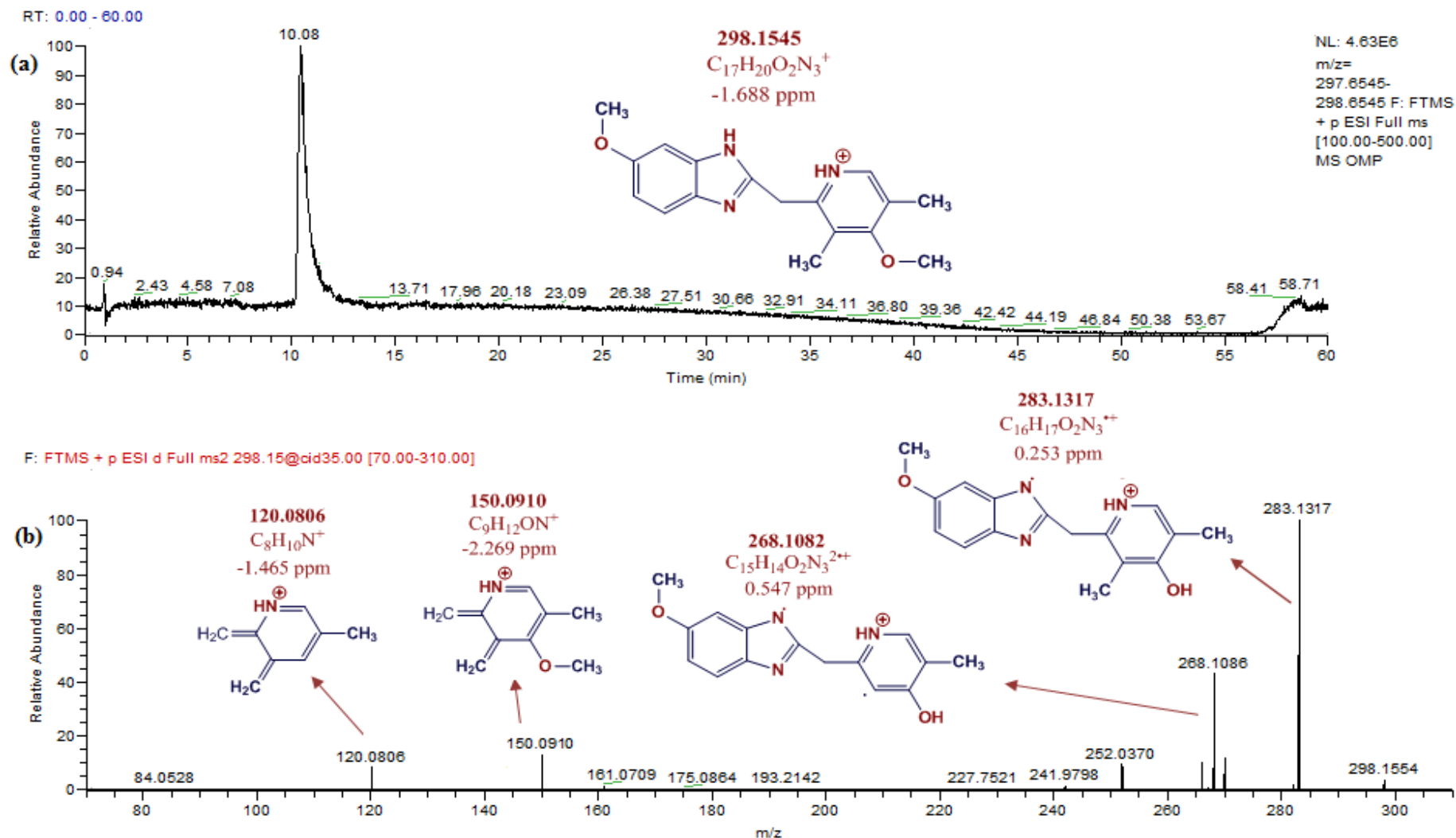
Omeprazole belongs **in the top twenty** prescribed and produced pharmaceuticals worldwide

It is **the first of the “proton pump inhibitors”** widely used in the treatment of dyspepsia, peptic ulcer disease etc

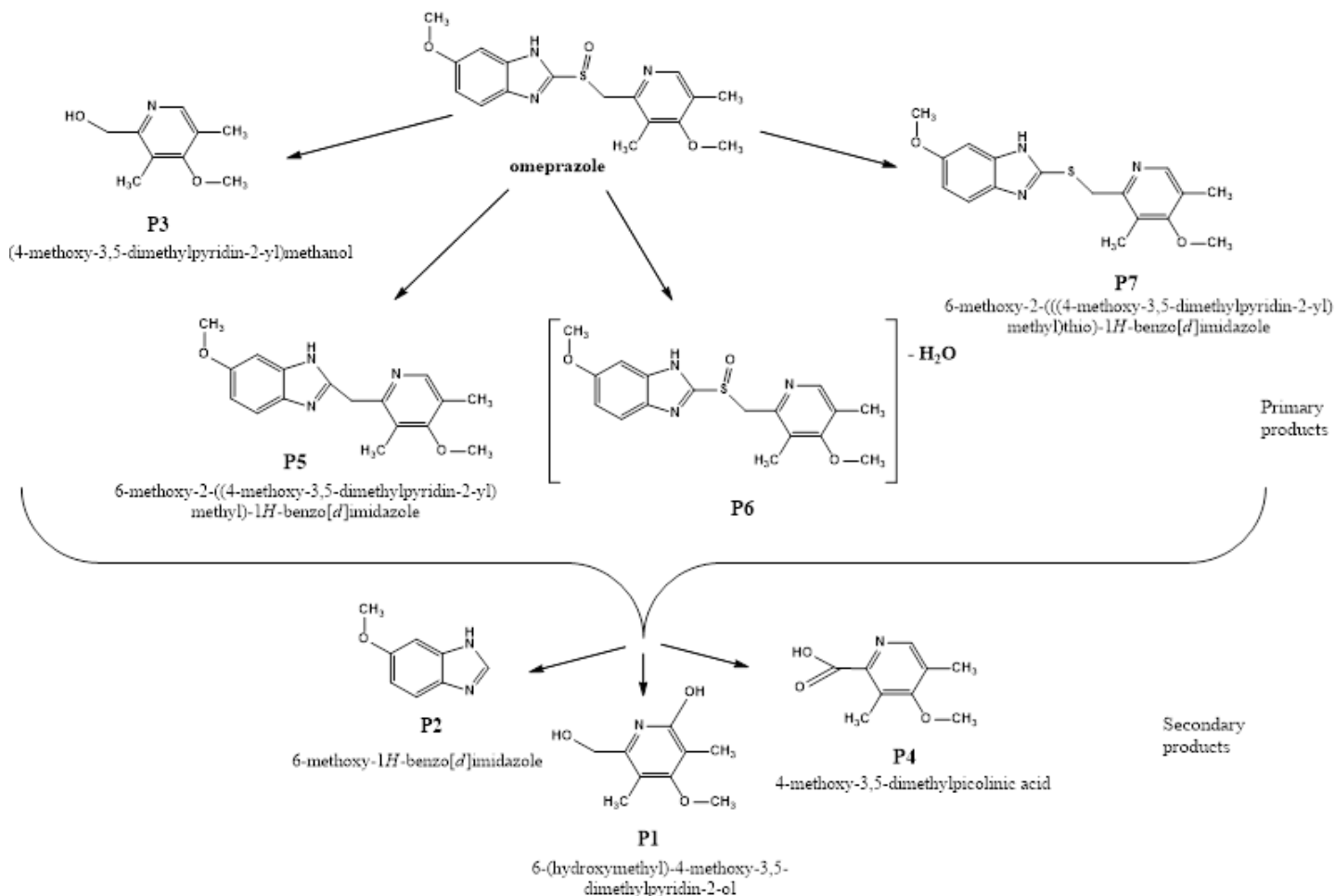
Environmental Fate

It can undergo various natural biotic and abiotic transformations (i.e. biodegradation, hydrolysis, photolysis etc.).

Omeprazole/ Metabolites/TPs in real wastewater samples from Greece

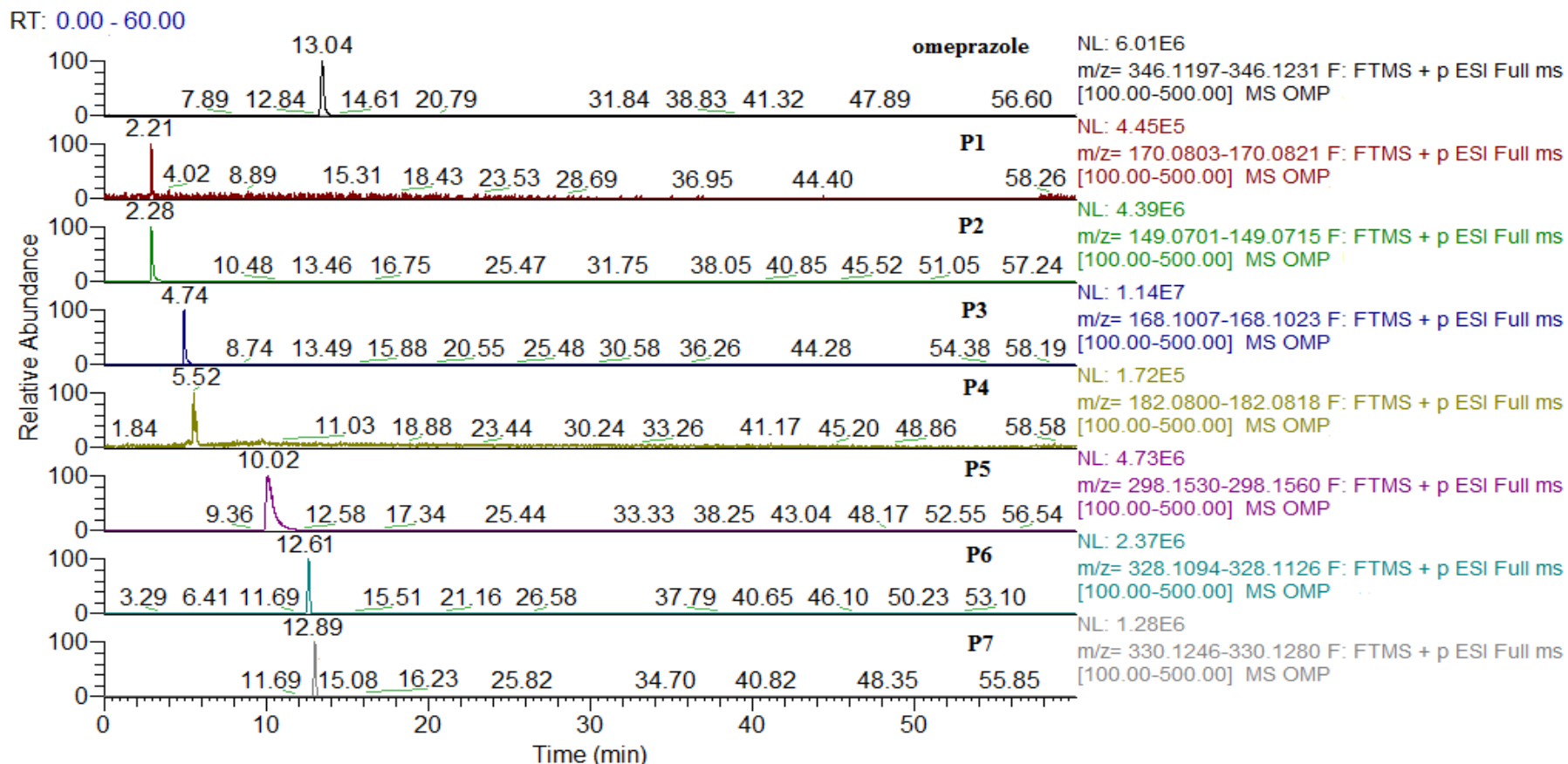


Omeprazole/ Metabolites/TPs in real wastewater samples from Greece



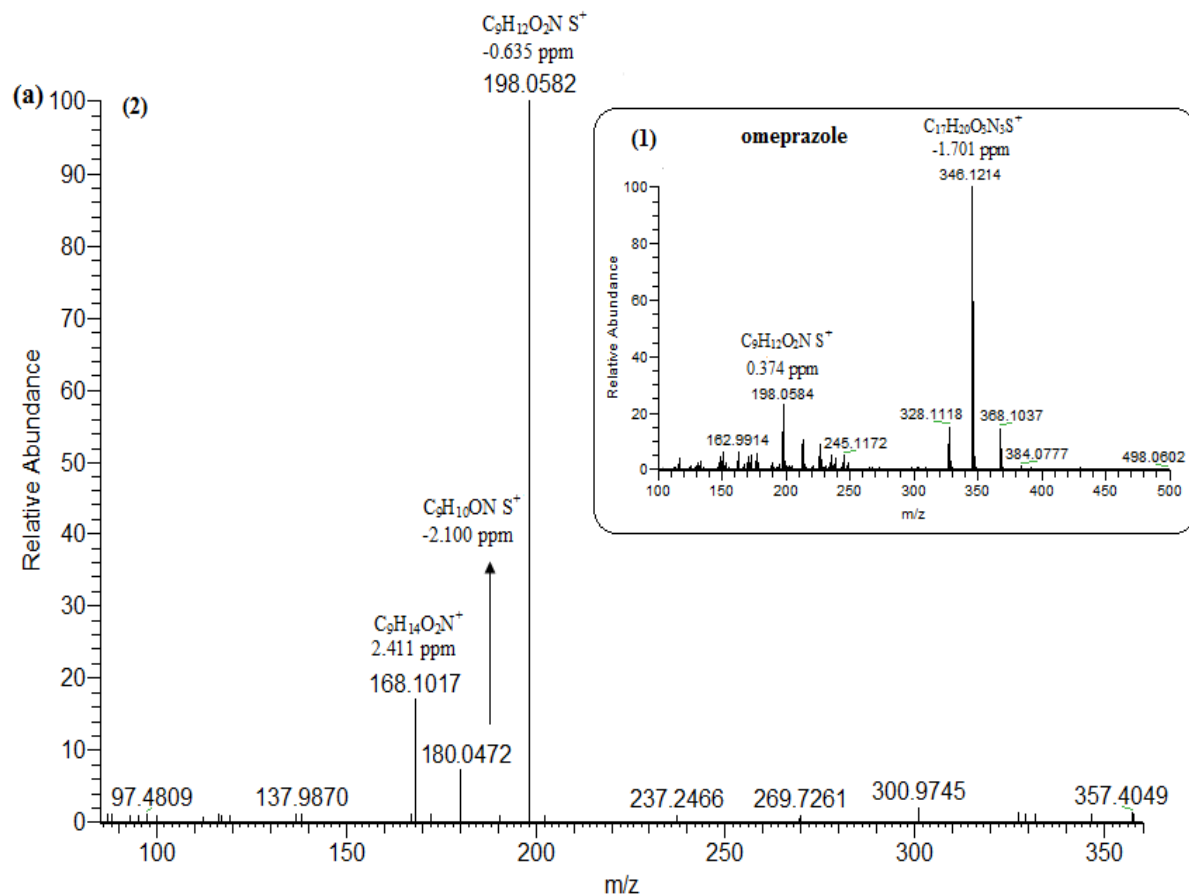
Proposed photolysis transformation pathways of omeprazole in natural waters

Omeprazole/ Metabolites/TPs in real wastewater samples from Greece

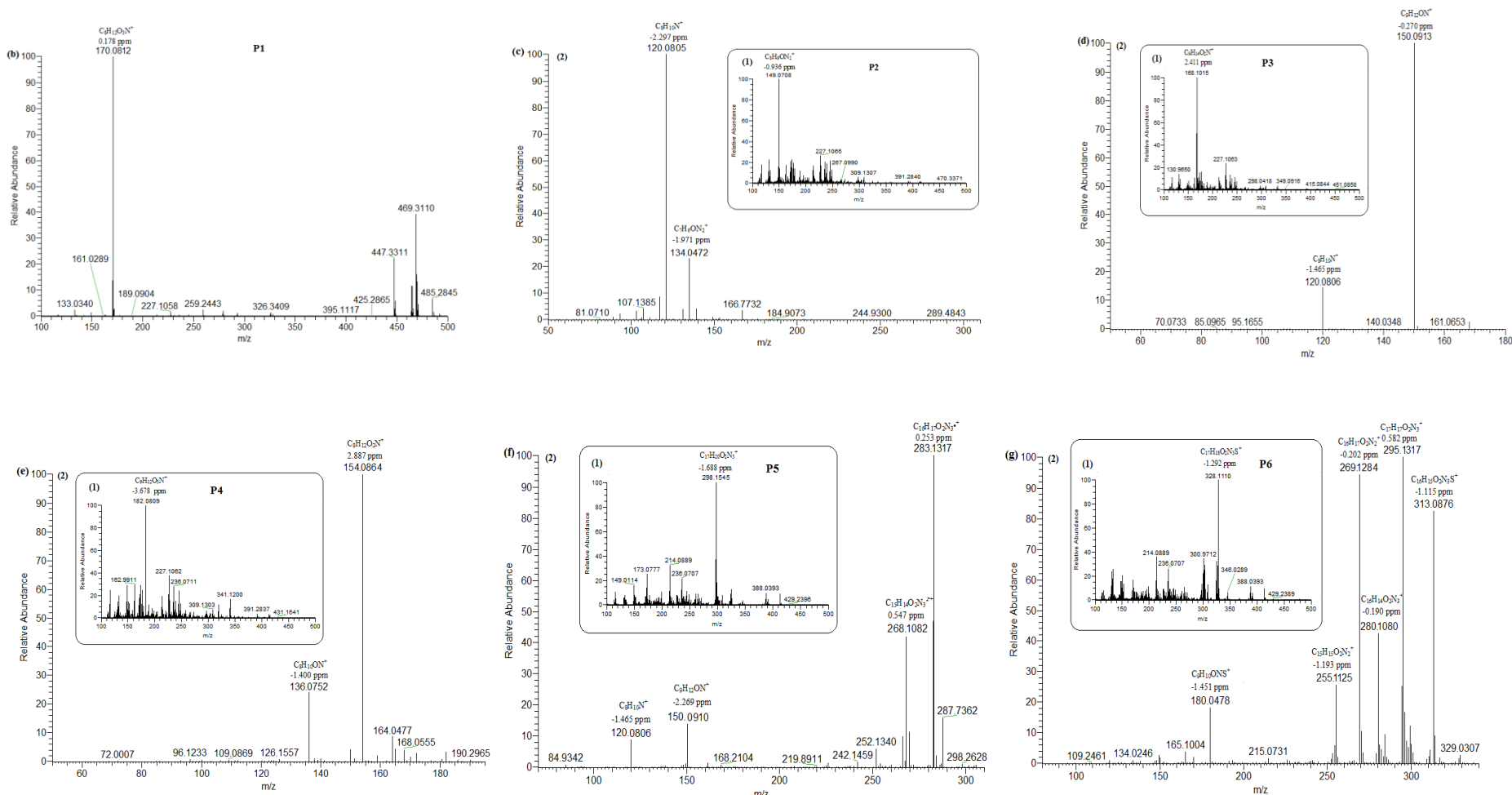


Extracted ion chromatogram (XIC) of omeprazole and its TPs (P1, P2, P3, P4, P5, P6 and P7) obtained after photolysis under natural irradiation, in distilled water

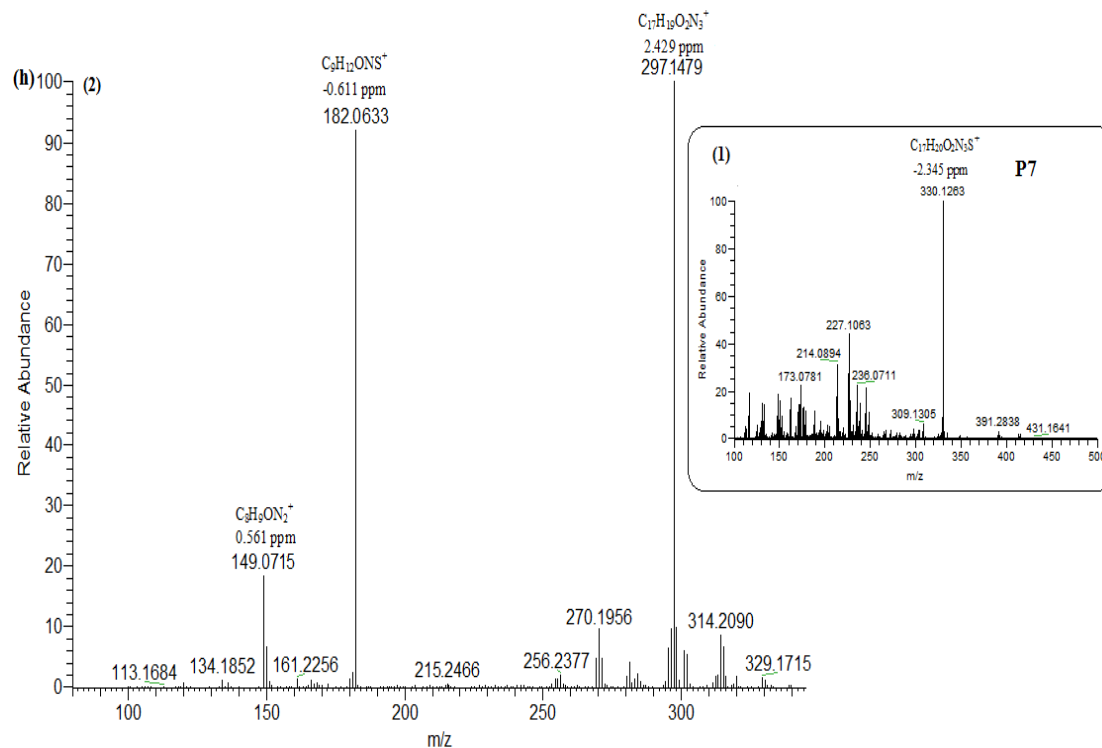
Omeprazole/ Metabolites/TPs in real wastewater samples from Greece



(1) Full scan accurate mass product ion spectrum and (2) MS/MS data obtained using Orbitrap MS targeting the corresponding ions, for (a) omeprazole and its TPs



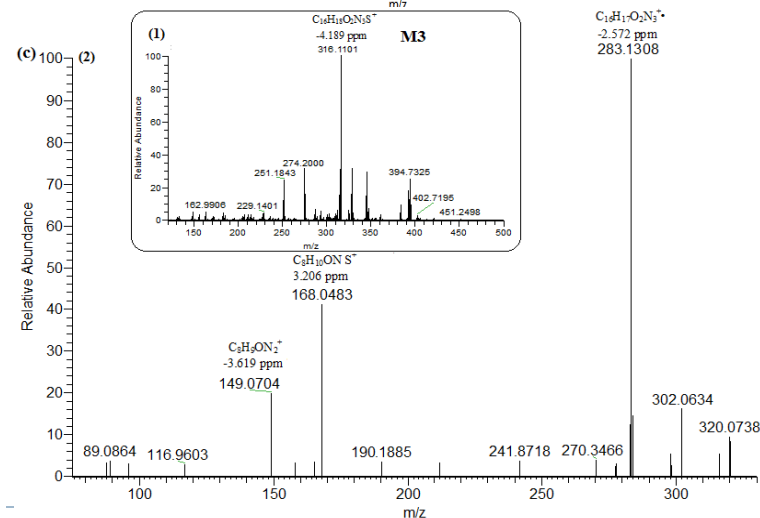
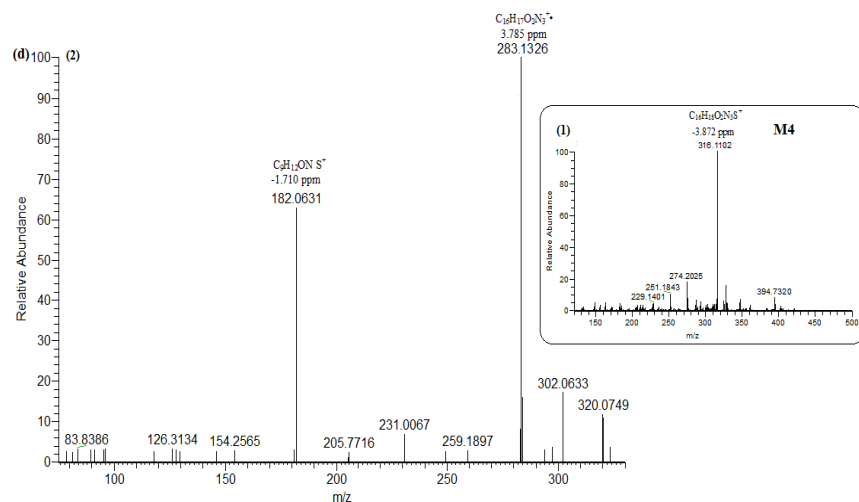
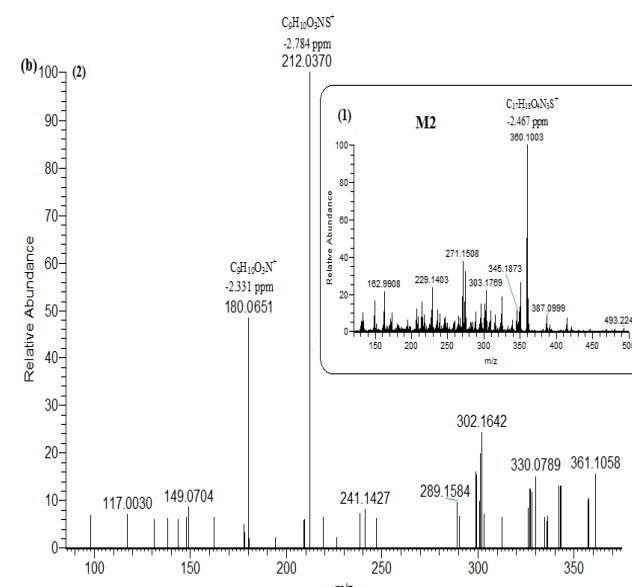
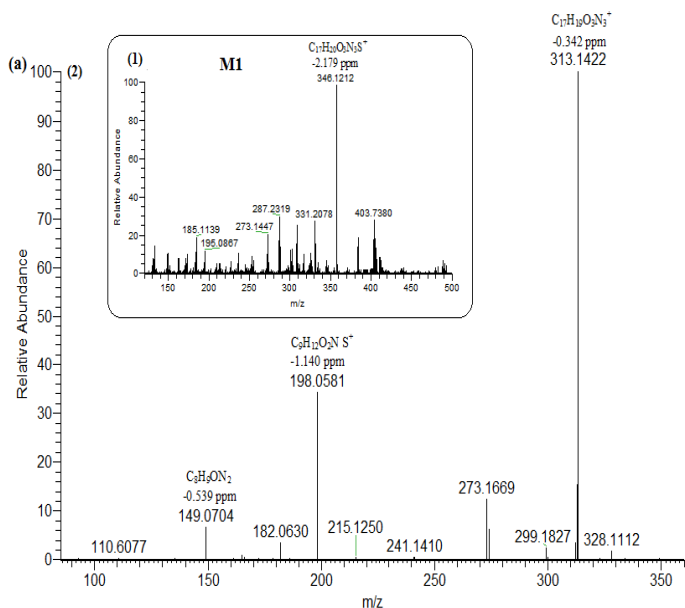
(1) Full scan accurate mass product ion spectrum and (2) MS/MS data obtained using Orbitrap MS targeting the corresponding ions, for (a) omeprazole and its TPs



Omeprazole/ Human Metabolites in real wastewater samples from Greece

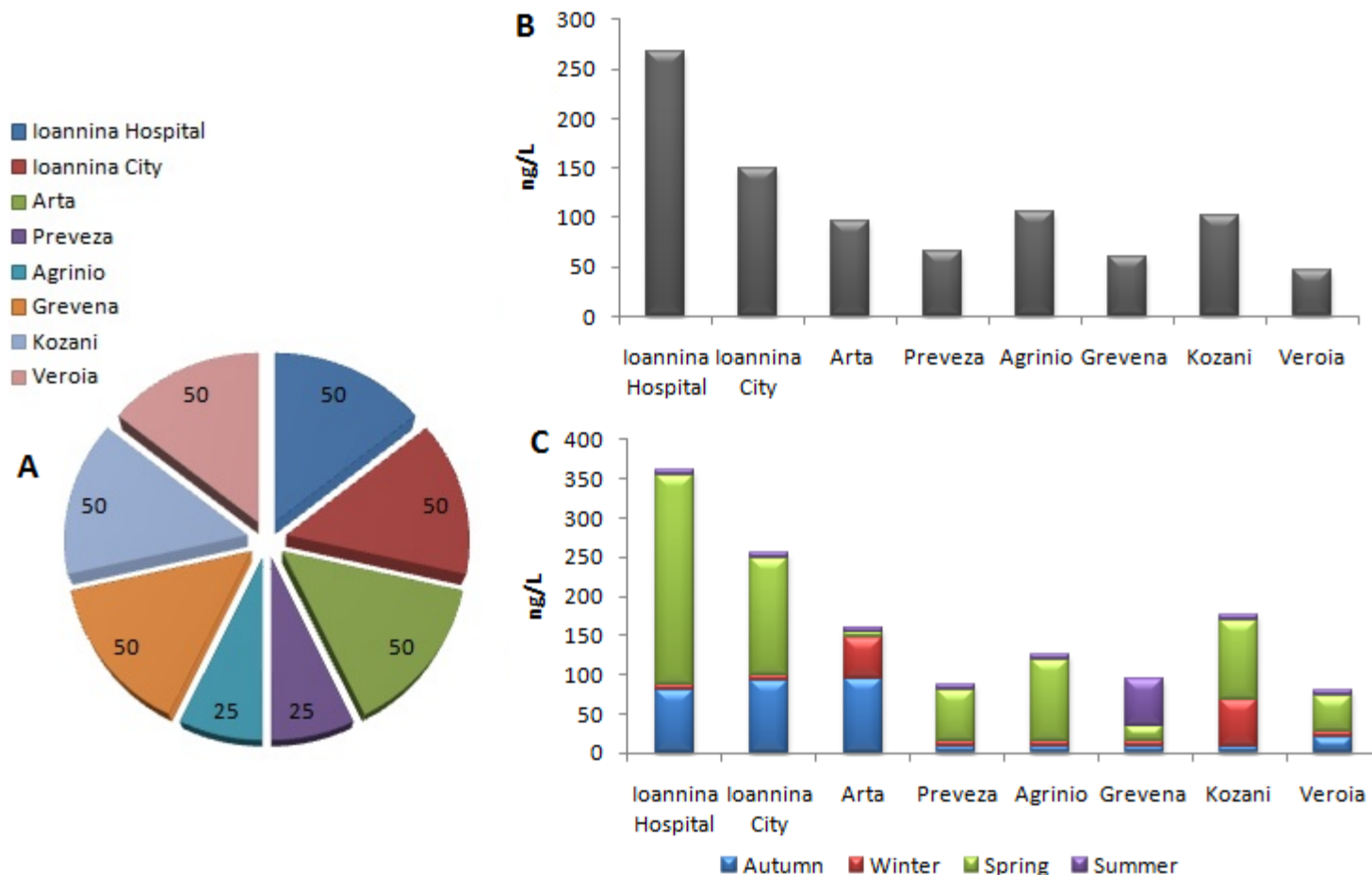
			Mass			
Comp ound	RT (min)	Elemental composition	Experimental	Theoretical	RDB	Error (ppm)
M1	17.40	C ₁₇ H ₂₀ O ₃ N ₃ S ⁺	346.1212	346.1220	9.5	-2.179
		C ₁₇ H ₁₉ O ₃ N ₃ ⁺	313.1422	313.1421	10	-0.342
		C ₉ H ₁₂ O ₂ N S ⁺	198.0581	198.0583	4.5	-1.140
		C ₈ H ₉ ON ₂	149.0704	149.0709	5.5	-0.539
M2	12.01	C ₁₇ H ₁₈ O ₄ N ₃ S ⁺	360.1003	360.1013	10.5	-2.467
		C ₉ H ₁₀ O ₃ NS ⁺	212.0370	212.0376	5.5	-2.784
		C ₉ H ₁₀ O ₃ N ⁺	180.0651	180.0655	5.5	-2.331
M3	11.36	C ₁₆ H ₁₈ O ₂ N ₃ S ⁺	316.1101	316.1114	9.5	-4.189
		C ₁₆ H ₁₇ O ₂ N ₃ ^{•+}	283.1308	283.1315	10.0	-2.572
		C ₈ H ₁₀ ON S ⁺	168.0483	168.0478	4.5	3.206
		C ₈ H ₉ ON ₂ ⁺	149.0704	149.0709	5.5	-3.619
M4	9.03	C ₁₆ H ₁₈ O ₂ N ₃ S ⁺	316.1102	316.1114	9.5	-3.872
		C ₁₆ H ₁₇ O ₂ N ₃ ^{•+}	283.1326	283.1315	10.0	3.785
		C ₉ H ₁₂ ON S ⁺	182.0631	182.0634	4.5	-1.710

(1) Full scan accurate mass product ion spectrum and (2) MS/MS data obtained using Orbitrap MS targeting the corresponding ions, for the metabolites



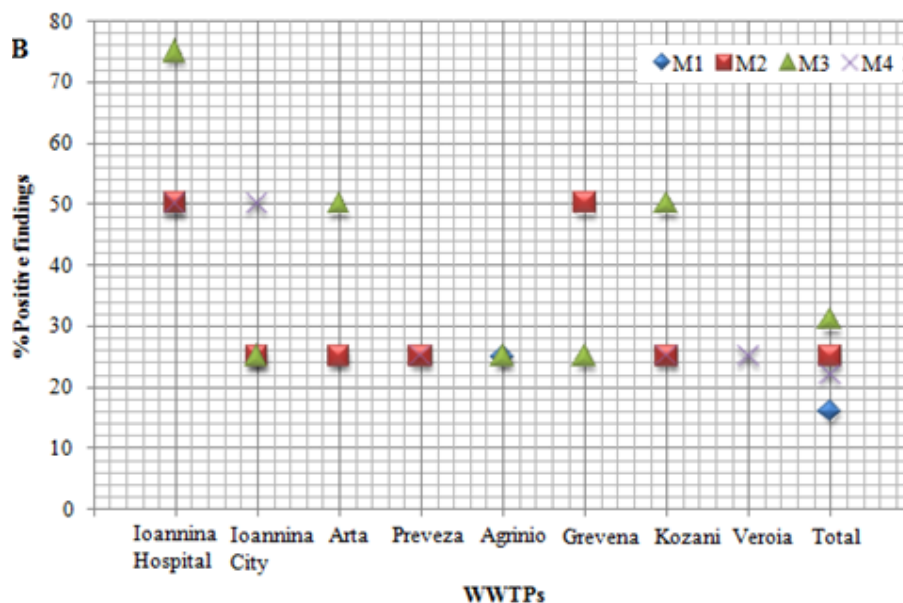
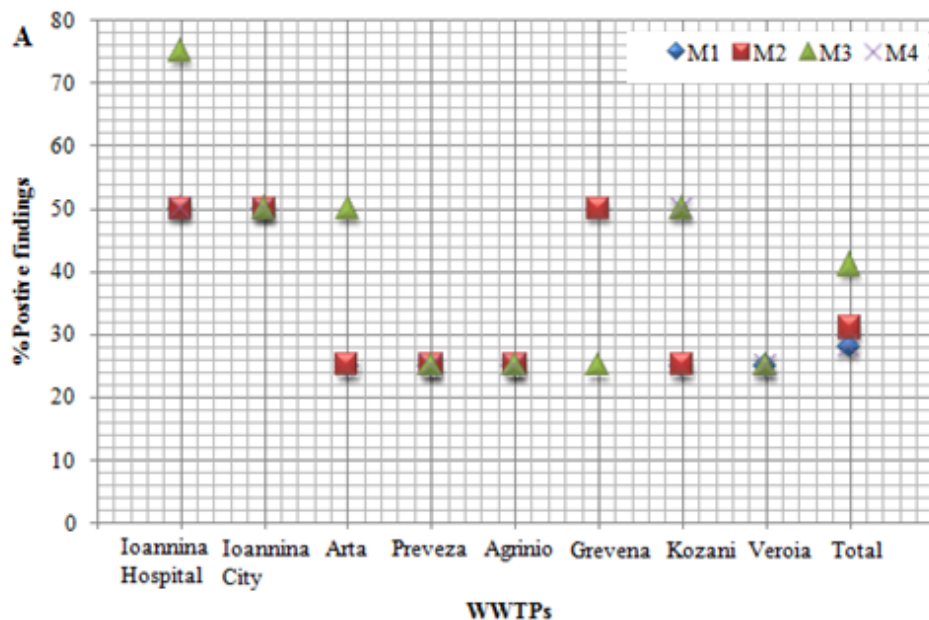
Omeprazole/ Metabolites/TPs in real wastewater samples from Greece

Fig. 4. A: Positive findings (%); B: Concentration ranges (ng/L) and C: Seasonal variation of omeprazole found in the influents of the eight WWTPs.



Omeprazole/ Metabolites/TPs in real wastewater samples from Greece

Positive findings of omeprazole metabolites in the (A) influents and (B) effluents of the eight WWTPs



28 transformation products were detected

TPs of Analgesics/antiinflammatory drugs

1-Hydroxy ibuprofen
2-Hydroxy ibuprofen
4-Hydroxy-diclofenac
5-Hydroxy-diclofenac
Hydroxy-ketoprofen

N-desmethyl-tramadol

O-desmethyl-tramadol

TPs of Antibiotics

Anhydro-erythromycin

Desmethyl-clarithromycin

Metabolite N-acetyl sulfamethoxazole

N-acetyl ciprofloxacin

(2 TP of Trimethoprim - 2,4-diaminopyrimidin-5-yl)(3,4,5-trimethoxyphenyl)methanone)
/2,4-diaminopyrimidine-5-carbaldehyde)

TPs of Antidiabetics

Guanylurea

TPs of β - blockers

Hydroxy
propranolol

TPs of Antidepressants /psychiatrics

O-Desmethyl venlafaxine

N-Desmethyl venlafaxine

10-hydroxycarbazepine

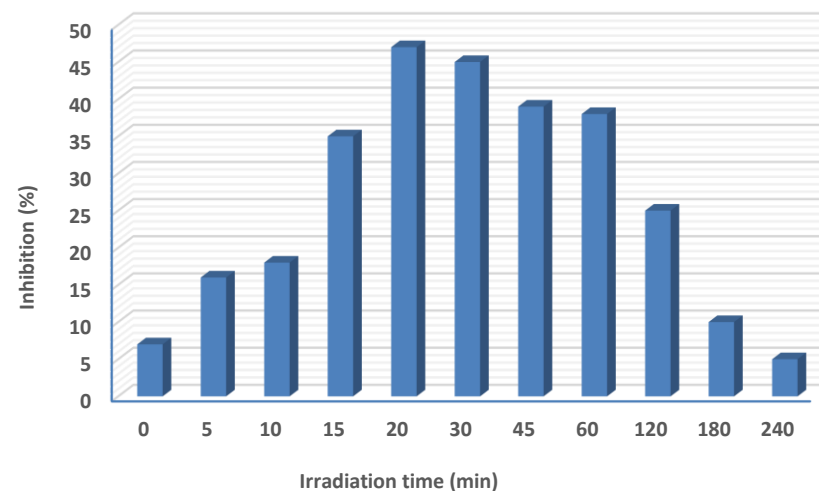
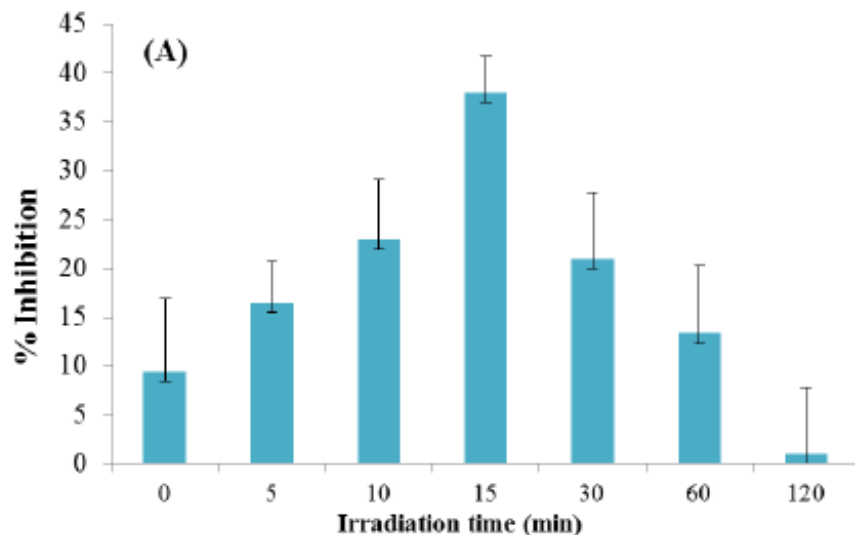
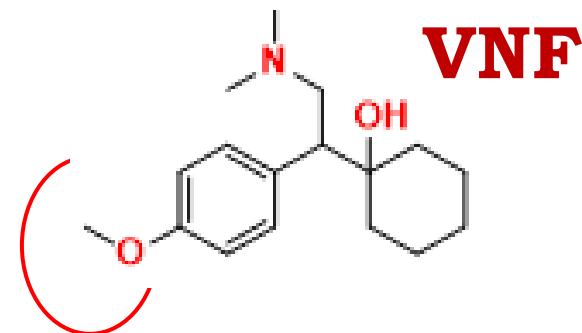
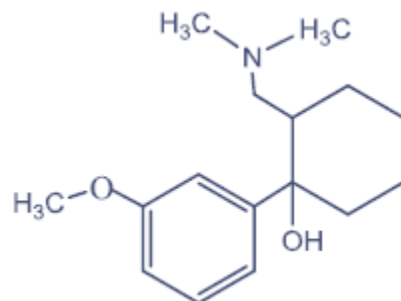
7 TPs of Omeprazole

4 Human Metabolites
of Omeprazole



Prioritization of TPs

Tramadol

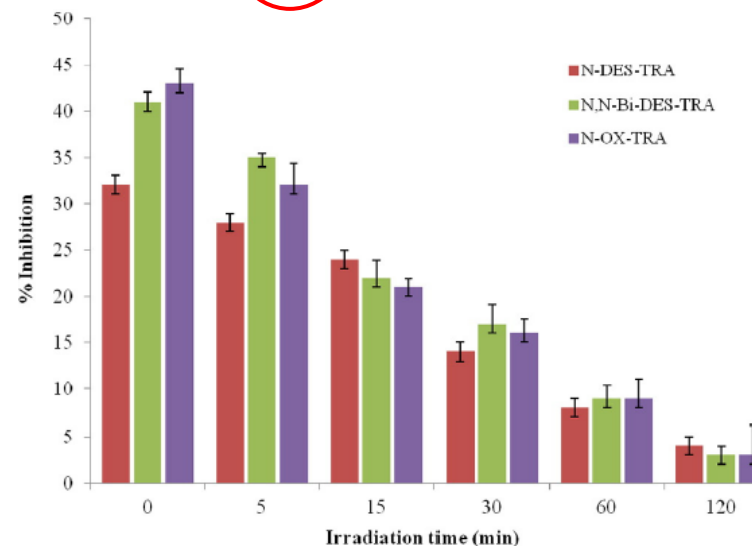
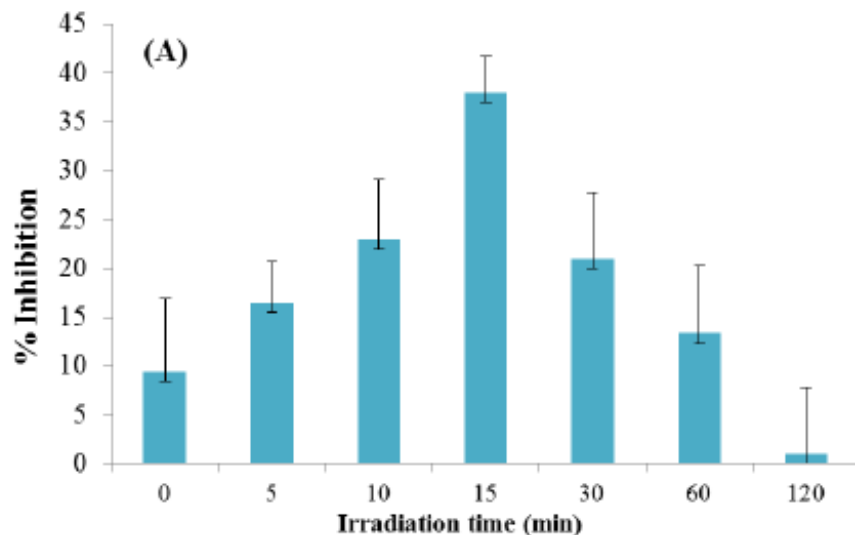
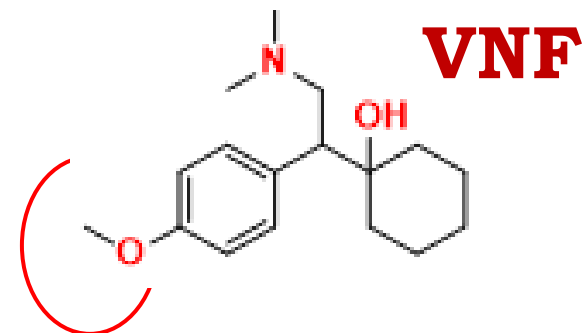
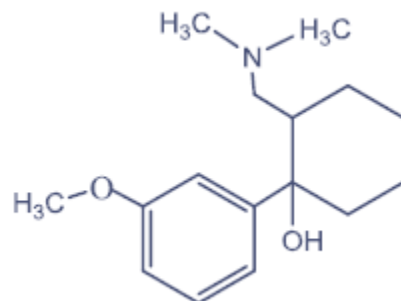


Inhibition of *Vibrio fischeri* bioluminescence as a function of photocatalytic treatment of TRA, and VNF



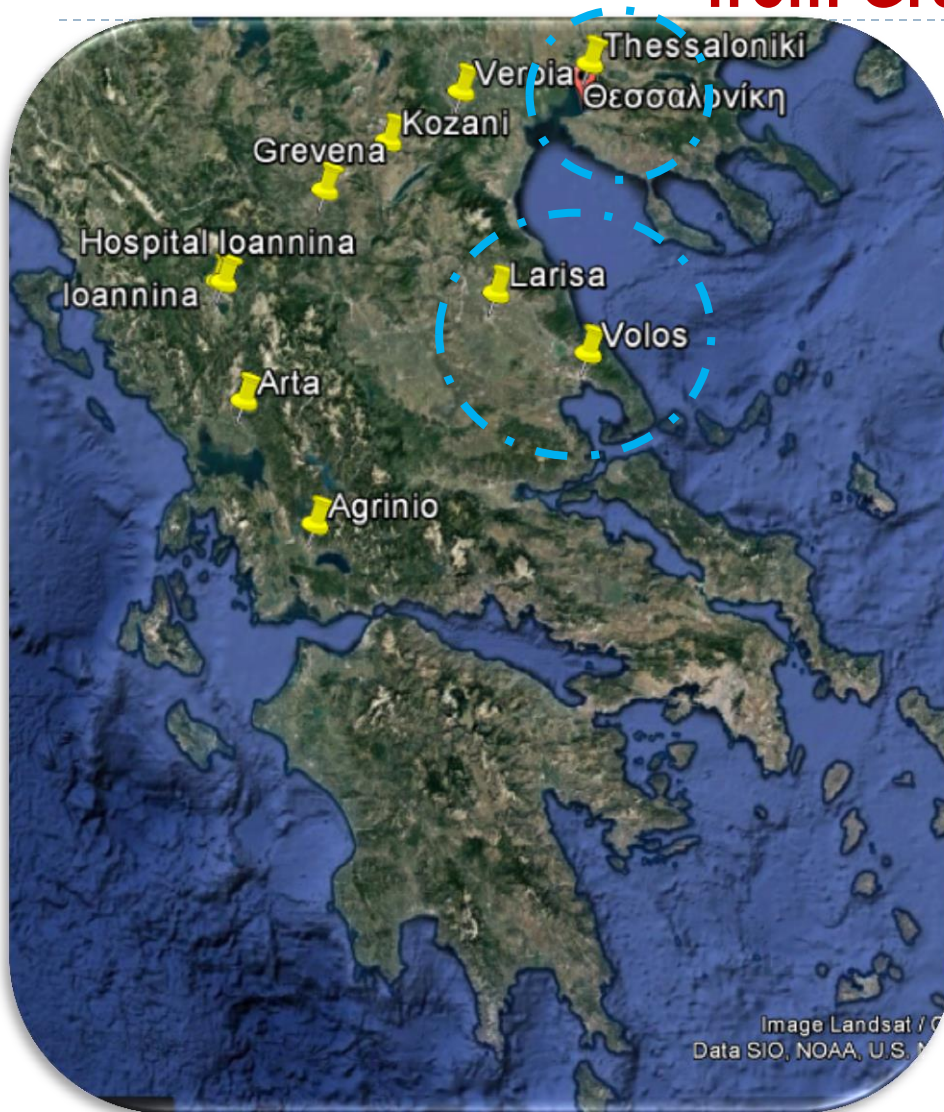
Prioritization of TPs

Tramadol



Inhibition of *Vibrio fischeri* bioluminescence as a function of photocatalytic treatment of TRA, N-DES-TRA, N,N-Bi-DES-TRA, N-OX-TRA

Application to real wastewater samples from Greece



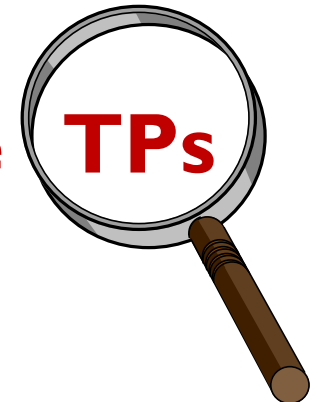
Location: 4 WWTPs in Greece,
4 Municipal

Samples: 24-h composite samples
Influents and effluents

Period : 2 year, 3 consecutive
days/month

More TPs

The work is in
progress....



Databases used for metabolites / TPs of pharmaceuticals

**Transformation
Products
LSM, Literature**

**Human Metabolites
Literature**

**High Resolution Mass Spectral Libraries for MS/MS data
(MassBank, MetFrag, MZmine etc)**

High Quality Mass Spectral Library

**In silico fragmentation for computer
assisted identification of metabolite
mass spectra**

**Open-source software for mass-
spectrometry data processing**



The use of Databases



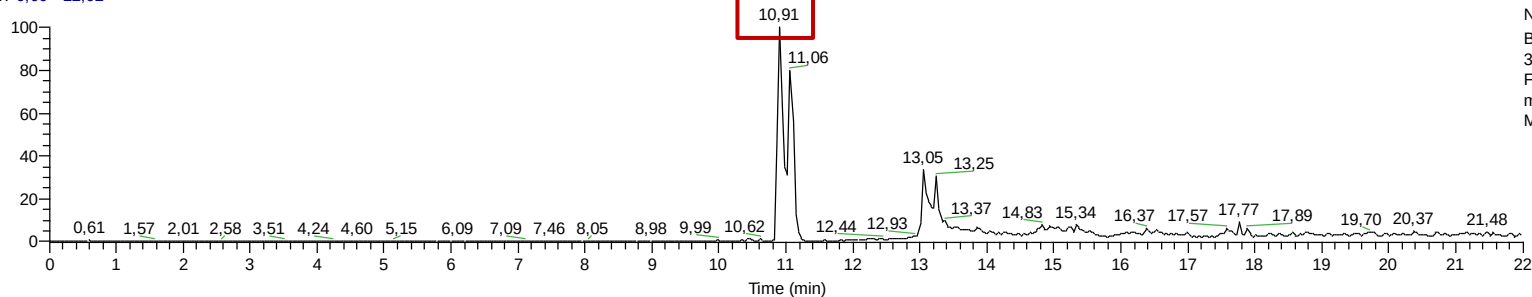
Open-source software for mass-spectrometry data processing

fluticasone 17beta-carboxylic acid, Rt: 13,05

G:\Orbi\20170117\NU_S_1^^

4/2/2017 3:42:57 μ m

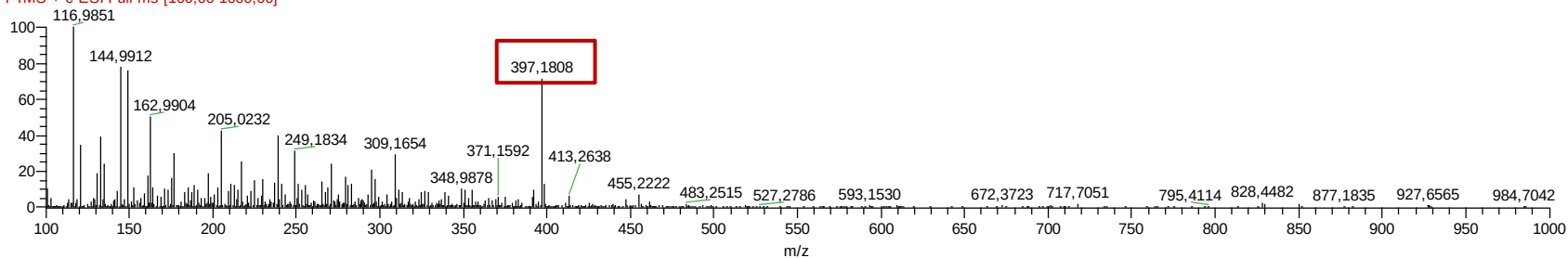
RT: 0,00 - 22,02



NL: 3,24E6
Base Peak m/z=
396,6809-397,6809 F:
FTMS + c ESI Full
ms [100,00-1000,00]
MS NU_S_106

NU_S_106 #972 RT: 13,05 AV: 1 NL: 1,52E6

F: FTMS + c ESI Full ms [100,00-1000,00]

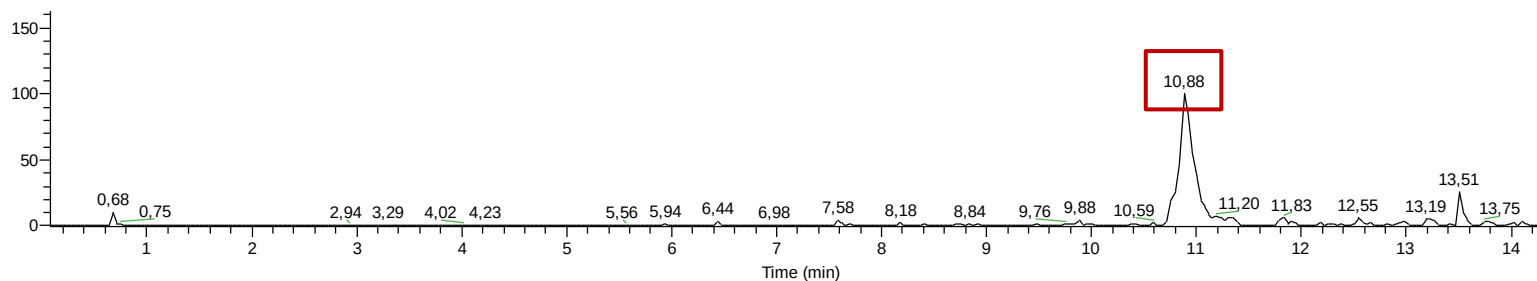


14-hydroxycclarithromycin, Rt: 10,88

C:\Users\...\Orbi\20170117\... 100

4/2/2017 9:32:33 µµ

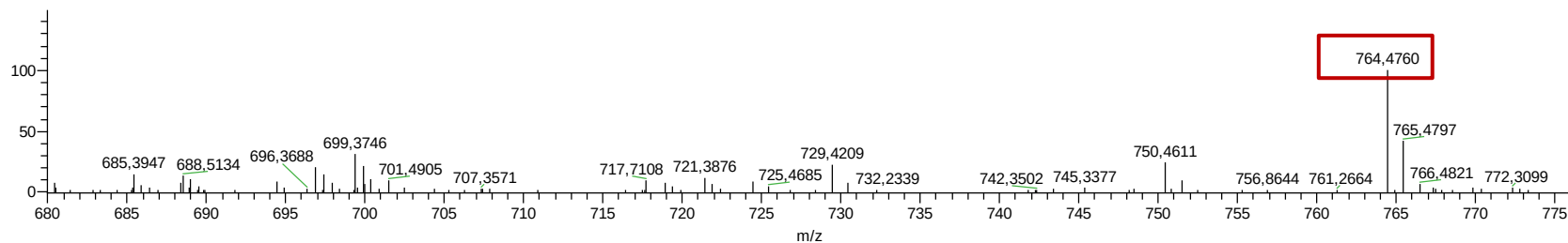
RT: 0,07 - 14,38



NL: 6,90E5
m/z=
763,9762-764,9762
F: FTMS + c ESI
Full ms
[100,00-1000,00]
MS NU_S_103

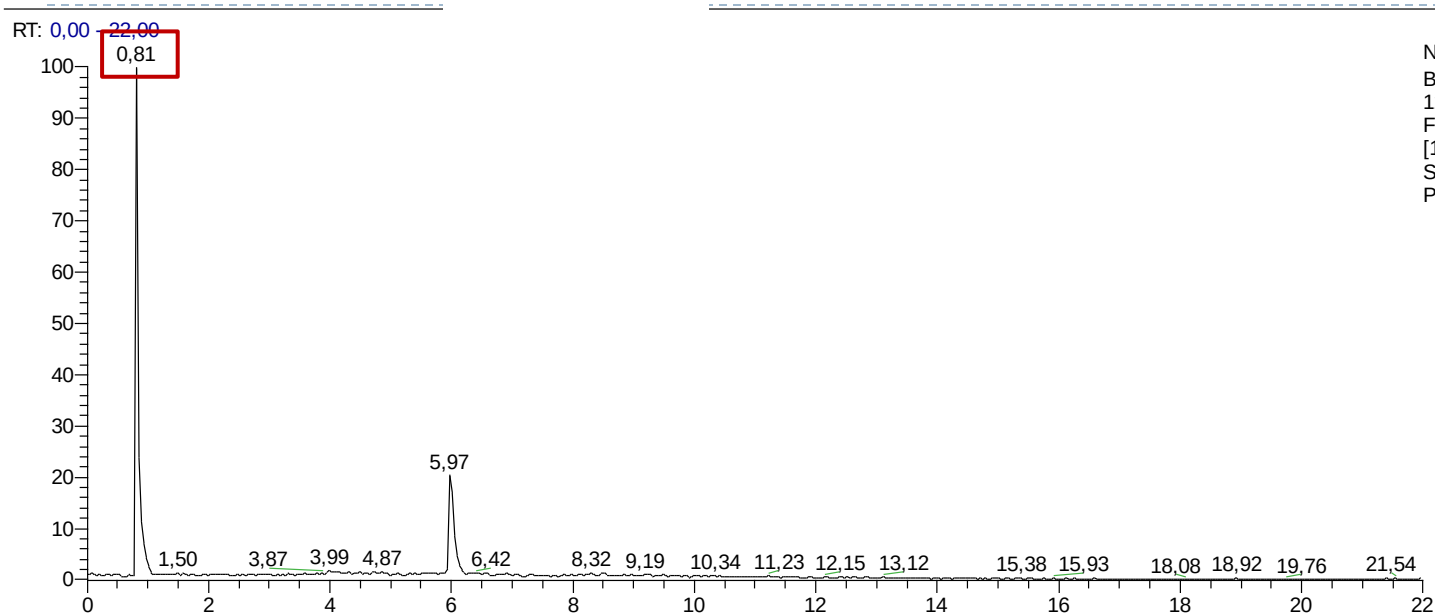
NU_S_103 #825 RT: 10,88 AV: 1 NL: 6,80E5

F: FTMS + c ESI Full ms [100,00-1000,00]



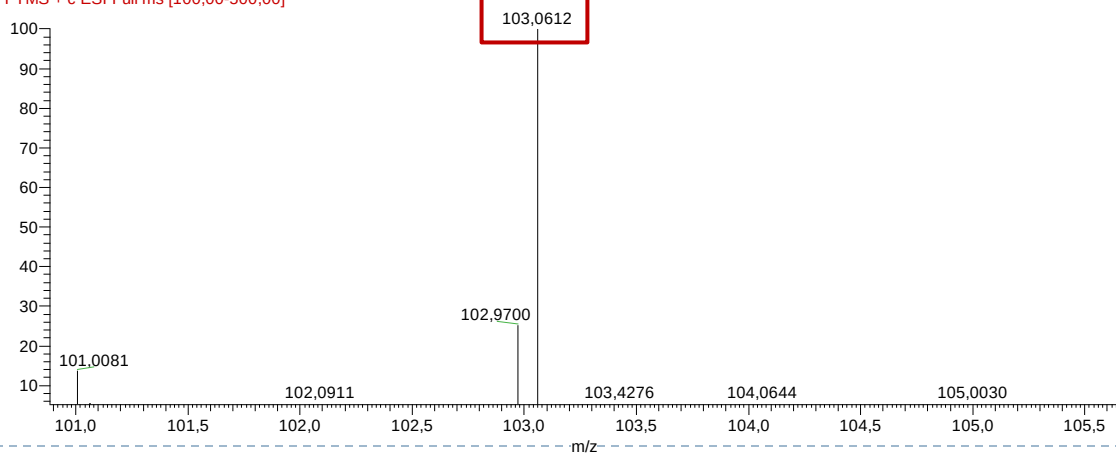
Quanylurea, Rt: 0,81

7/12/2016 6:51:49 $\mu\mu$

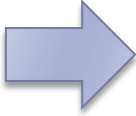


NL: 1,72E6
Base Peak m/z=
129,5000-130,5000 F:
FTMS + c ESI Full ms
[100,00-500,00] MS
STD_WATER_250ppb_P
PCPs147

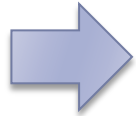
STD_WATER Time (min) PCPs147 #55 RT: 0,73 AV: 1 NI: 7 28F5
F: FTMS + c ESI Full ms [100,00-500,00]



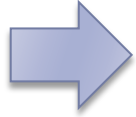
To conclude



The application of rapid screening methods for the identification and detection of unknown compounds by LC–HRMS is still under development



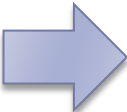
Suspect screening is a promising tool for the tentative detection of emerging contaminants for which pure standards are not available, such as transformation products



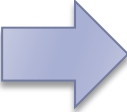
Open source and commercial software such as Mass Frontier and Mass Fragmenter are available to predict mass spectral fragments using different fragmentation rules, but they need a lot of improvement.



To conclude



From recent published data it is evident that the presence of TPs in the aquatic environment is not negligible and that TPs clearly contribute to the environmental and human health risk of Pharmaceuticals



A tentative method to prioritize TPs is very important, especially due to the large number of TPs that may form through various environmental transformation processes



conclusions....

Research in the field of TPs of PPCPs is ongoing

&

a great progress is anticipated in the near future!!



Acknowledgments....

The Unit of Environmental, Organic and Biochemical high resolution analysis-ORBITRAP-LC-MS of the University of Ioannina for providing access to the facilities

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Advanced Microextraction Approaches Based on Novel nano- Polymers to Measure Pharmaceuticals, Personal Care Products (PPCPs) and their Transformation Products (TPs) in the Aquatic Environment



Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης



Thank you for your attention!



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