5th 2014 EuCheMS ISTANBUL Chemistry Congress

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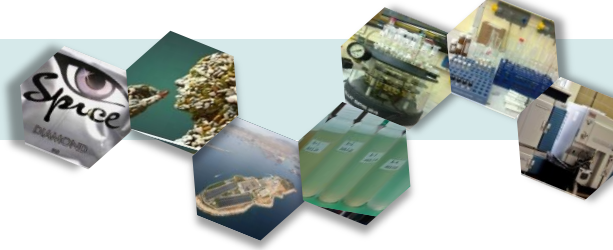
WOW Convention Center Istanbul

National and Kapodistrian UNIVERSITY OF ATHENS
Faculty of Chemistry

Simultaneous detection of New Psychoactive Substances (NPSs) in wastewater of Greece

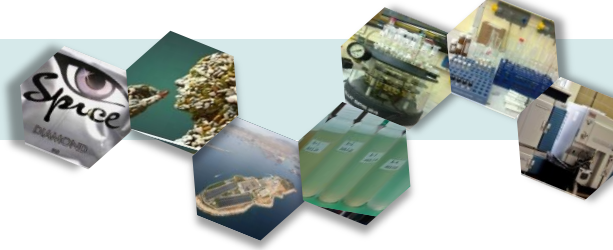
Viola Borova, **N. S. Thomaidis, C. Pistos**

Outline



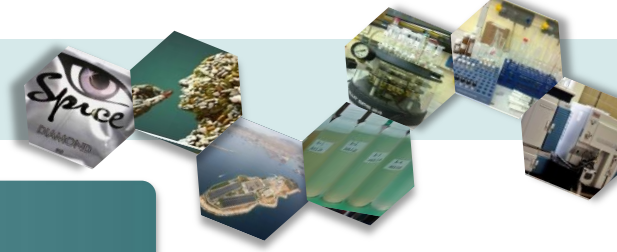
- Introduction
  - The New Designer Psychotropic Drugs
  - Their occurrence in the aquatic environment
- Analytical methodology
- Application in real wastewater samples
- Conclusions

Introduction



- ***Designer Drugs:***
  - Synthetically changed natural substances
  - Completely designed molecular structures
  - Psychotropic effects
- ***New Designer Psychotropic Drugs:***
  - Drug alternatives
  - Optimized effects of already existing drugs
    - *Higher binding affinity with CB1, greater potency and adverse effects and longer duration of action*
  - Not covered in most countries by controlled substance statutes
    - *avoid detection and legal consequences*
  - "Spice" drugs (smoking mixtures, herbal mixtures), bath salts, tablets form, on the recreational drug use market

Families of the compounds



Phenylalkylamines

- **Beta-keto** (**mephedrone**, butylone (bk-MBDB), methylone (bk-MDMA)

Piperazines

- **Phenylpiperazine** (DCPP, mCPP, MeOPP, pCPP, TFMPP)
- **Benzylpiprazine** (**BZP**, MBZP, DBZP, MDBZP)

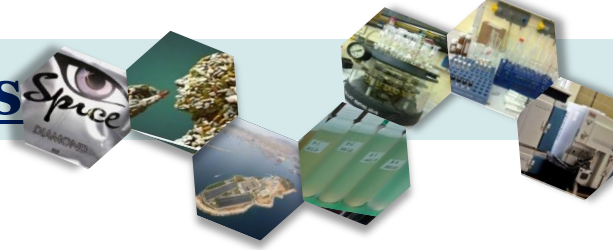
Synthetic Cannabinoids

- **JWH cannabinoids (John W. Huffman),** (e.g. **JWH 018, JWH 073** aminoalkylindoles series and more than 400 cannabinoids**)**
- CP47, 497 (From **Pfizer,** cyclohexylphenol series) (analog **CP47,497** and homologue C6,C7,C8,C9)

Pyrrolidinophenones

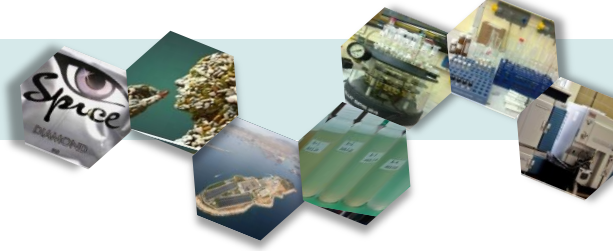
- **Derivatives (a-PVP**, **MPPP**, MPHP, MOPPP, MDPPP**)**

A new class of emerging contaminants



- **Not** currently **covered** by existing **water quality regulations**
- **Rarely investigated**
- **Newly identified** or previously unrecognized
- **Lack** of **environmental data**
- **Lack** of **analytical methods** in wastewater
- Large volume of **consumption** and **production**
- **Potential threats** to ecosystems and human (limited information)

Entry to the aquatic environment



Drug consumption

Estimate Consumption in communities

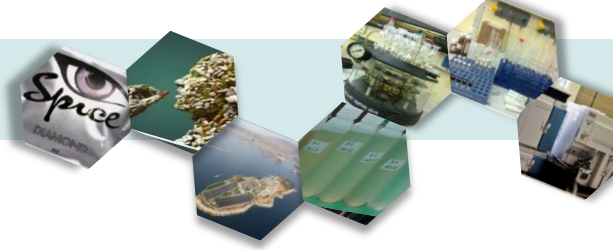
Released into the aquatic environment

Drug and metabolites excretion

Wastewater treatment Plant (WWTP)

Incomplete Removal

Analytical Methodology

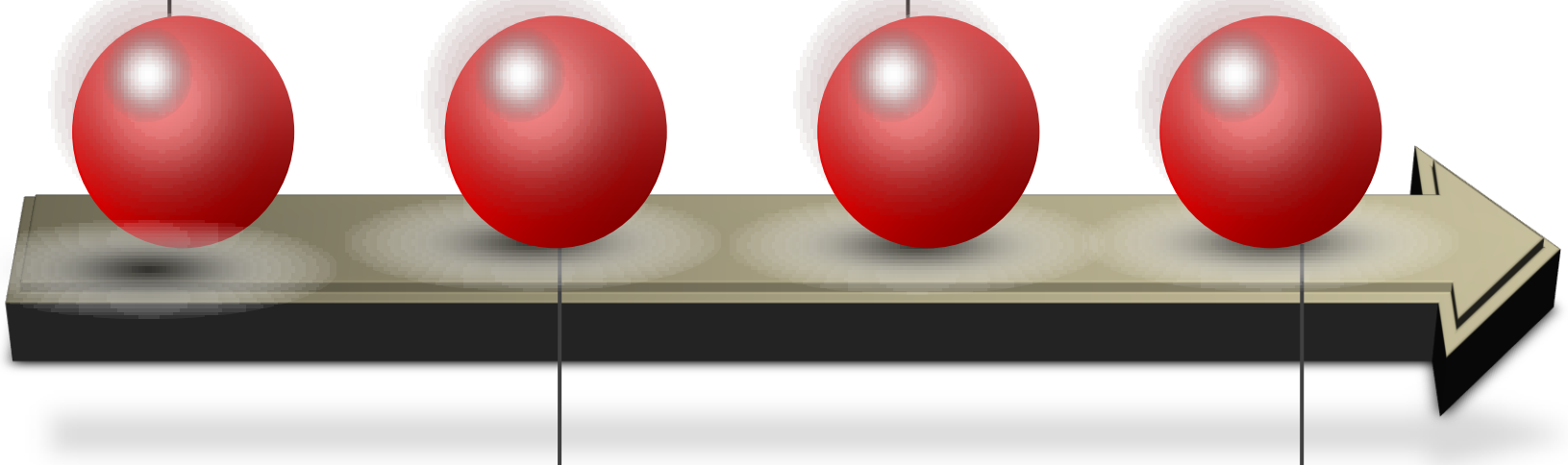


LC- (QqQ) MS/MS –ESI (+/-)

Thermo Scientific Quantum Access

Optimization of MS/MS

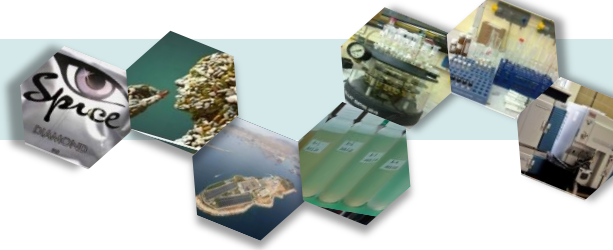
Optimization of SPE

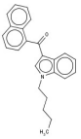


Optimization HPLC
- ✓Comparison of stationary phase
- ✓Mobile phase

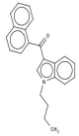
Validation

Target Analytes



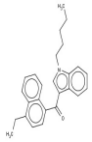


JWH-018
logP=6.51

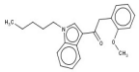


JWH-073
logP=6.07

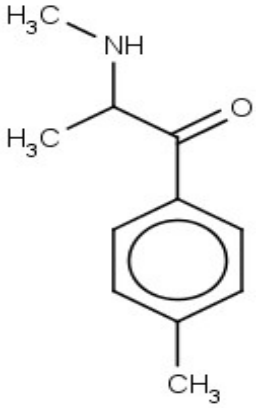
JWH-122
logP=7.03

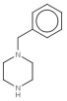


JWH-210
logP=7.47



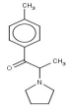
JWH-250
logP=5.30



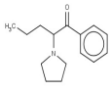


BZP
logP=1.38

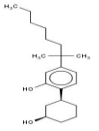
Mephedrone
logP=2.12



MPPP
logP=2.91

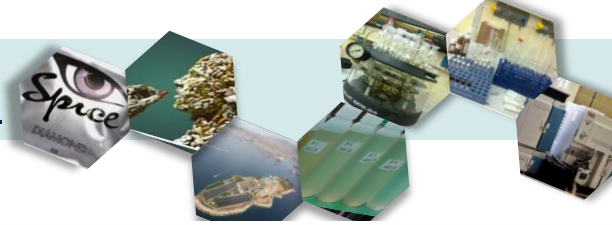


a-PVP
logP=3.36



CP47 497
logP=6.17

Why we selected these compounds???



1

Rise in notifications of NPDs usage in Europe (EMCDDA 2013)

14 per year in **2005** to **73** in **2012**

2

Occurrence in wastewater treatment plant (WWTP) (according to publications)

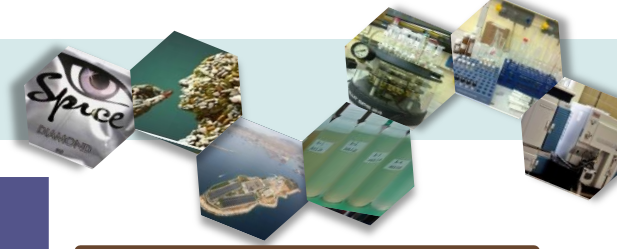
3

Cause of possible toxicological effects in the environment

4

Have been reported in Greece

Optimization of MS/MS



Compounds	IS	$[M+H]^+$	Product Ions (collision energy, V)	Tube Lens (V)	ESI
JWH-018	JWH-018 -d9	342	155 (25) 127 (44)	82	+
JWH-073	JWH-018 -d9	328	155 (25) 127 (43)	76	+
JWH-250	JWH-018 -d9	336	121 (20) 91 (35)	79	+
JWH-122	JWH-018 -d9	356	169 (25) 141 (38)	95	+
JWH-210	JWH-018 -d9	370	183 (25) 214 (24)	96	+
CP47,497	-	317	299 (24) 245 (34)	99	-
Mephedrone	Mephedrone -d3	178	160 (12) 145 (19V)	48	+
BZP	BZP- d7	177	91 (28) 65 (40)	63	+
MPPP	-	218	119 (24) 147 (18)	61	+
a-PVP	-	232	91 (27) 126 (27)	72	+

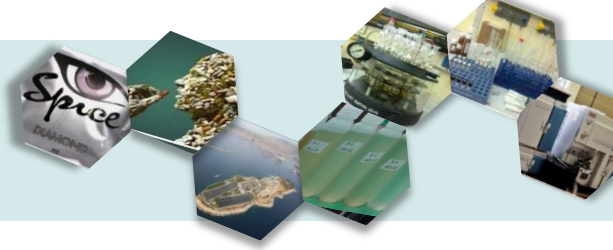
Positive Ionization

Probe:
C (0.5/1.5)
Spray Voltage:
3500 V
Sheath gas:
30 a.u.
Aux gas:
10 a.u.
Temp:
270°C

Negative Ionization

Probe:
C (0.5/1.5)
Spray Voltage:
2500 V
Sheath gas:
20 a.u.
Aux gas:
10 a.u.
Temp:
300°C

Optimization of HPLC
Comparison of Stationary phase



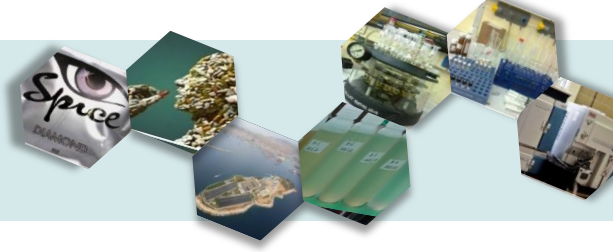
HSS XSelect T3 - C18
(100 mm x 2.1 mm, 2.5 µm, Waters)

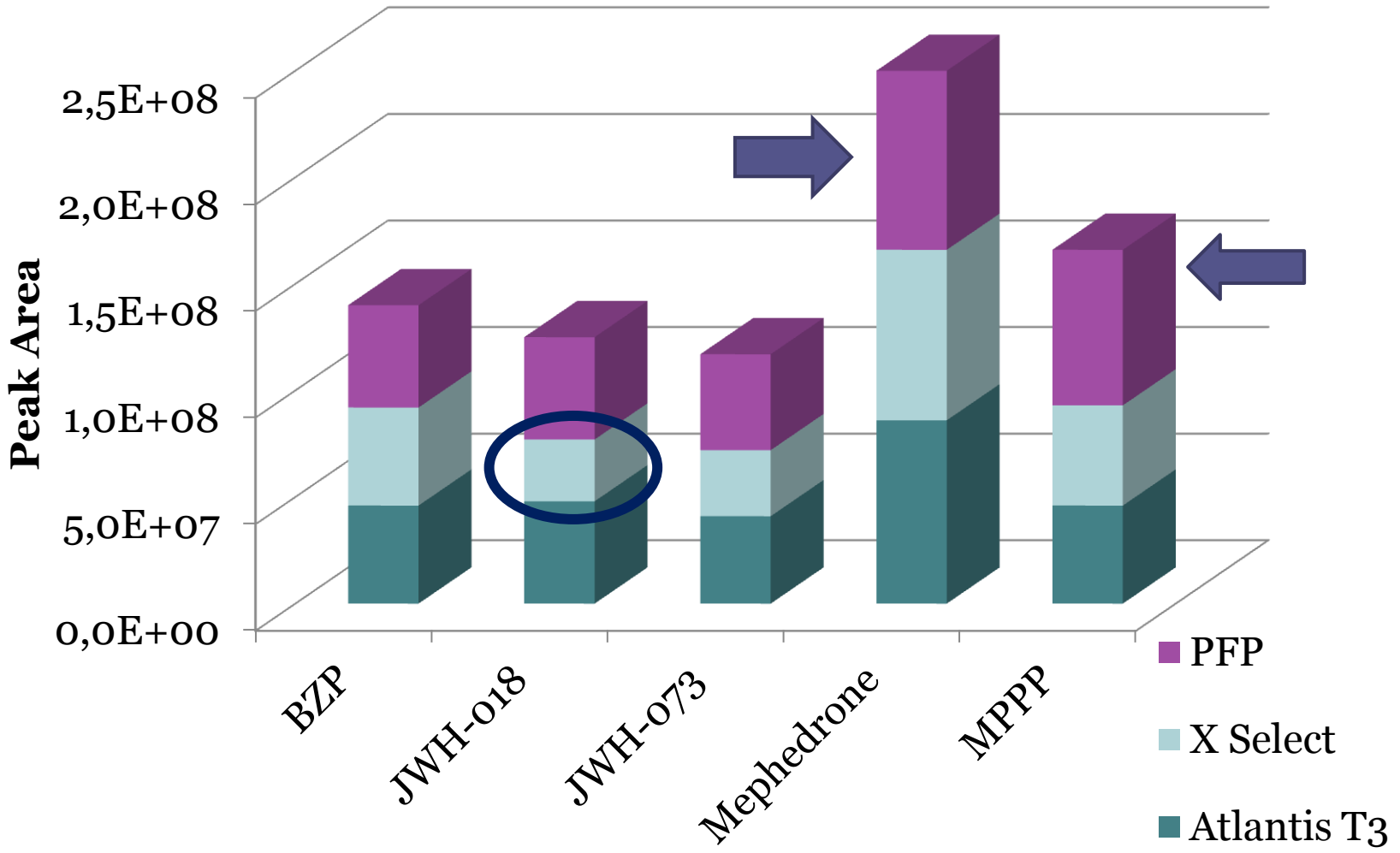
Atlantis T3 - C18
(100 mm x 2.1 mm, 3.0 µm, Waters)

Pentafluorophenyl (PFP)
(50 mm x 2.1 mm, 1.7 µm, Phenomenex)

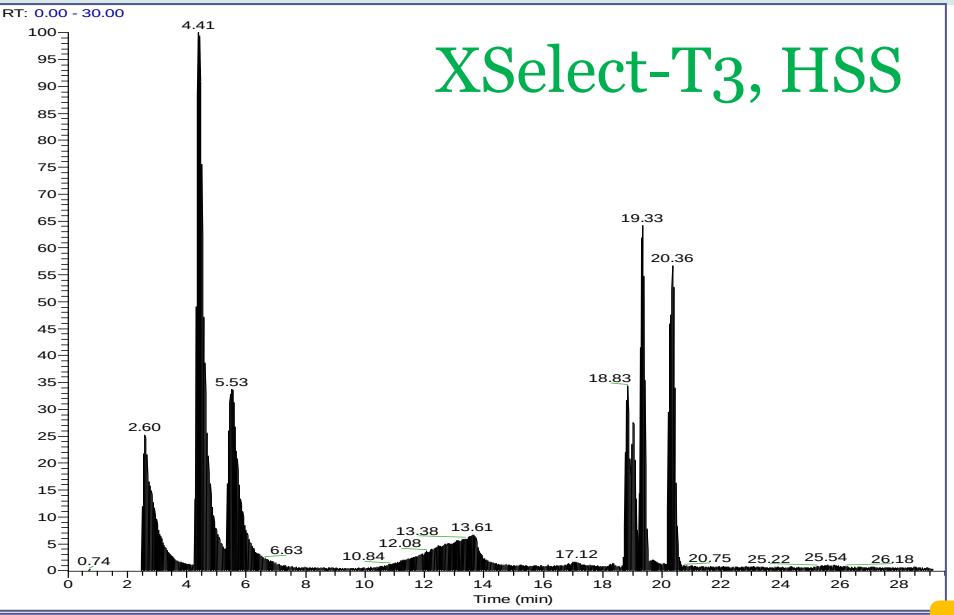
✓Peak area & shape
✓Retention time
✓Resolution

Optimization of HPLC
Comparison of Stationary phase

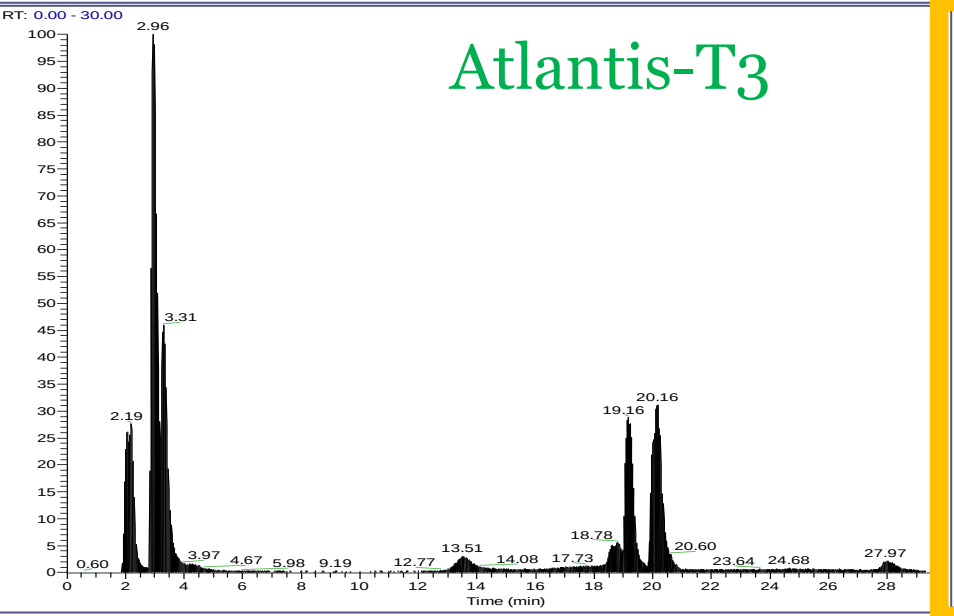


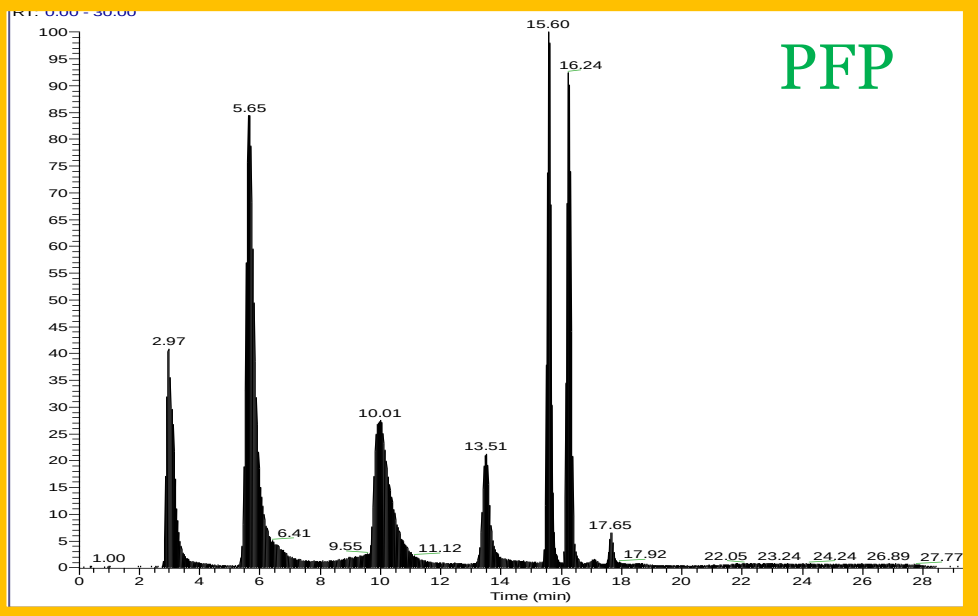


Optimization of HPLC
Comparison of Stationary phase

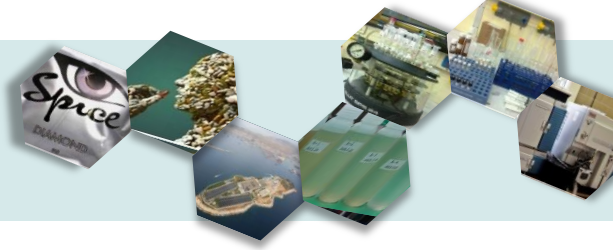


- ***C18 columns:*** presented peak asymmetry (polar compounds) and do not provide good separation.
- ***PFP:***
  1. **Higher sensitivity and resolution**
  2. More **symmetric peaks** for polar compounds containing –NH2 or –NH- groups





Optimization of HPLC
Mobile phase~ gradient programme



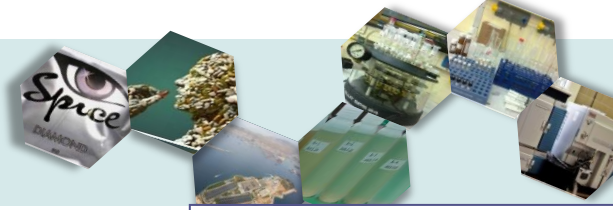
Positive Ionization mode

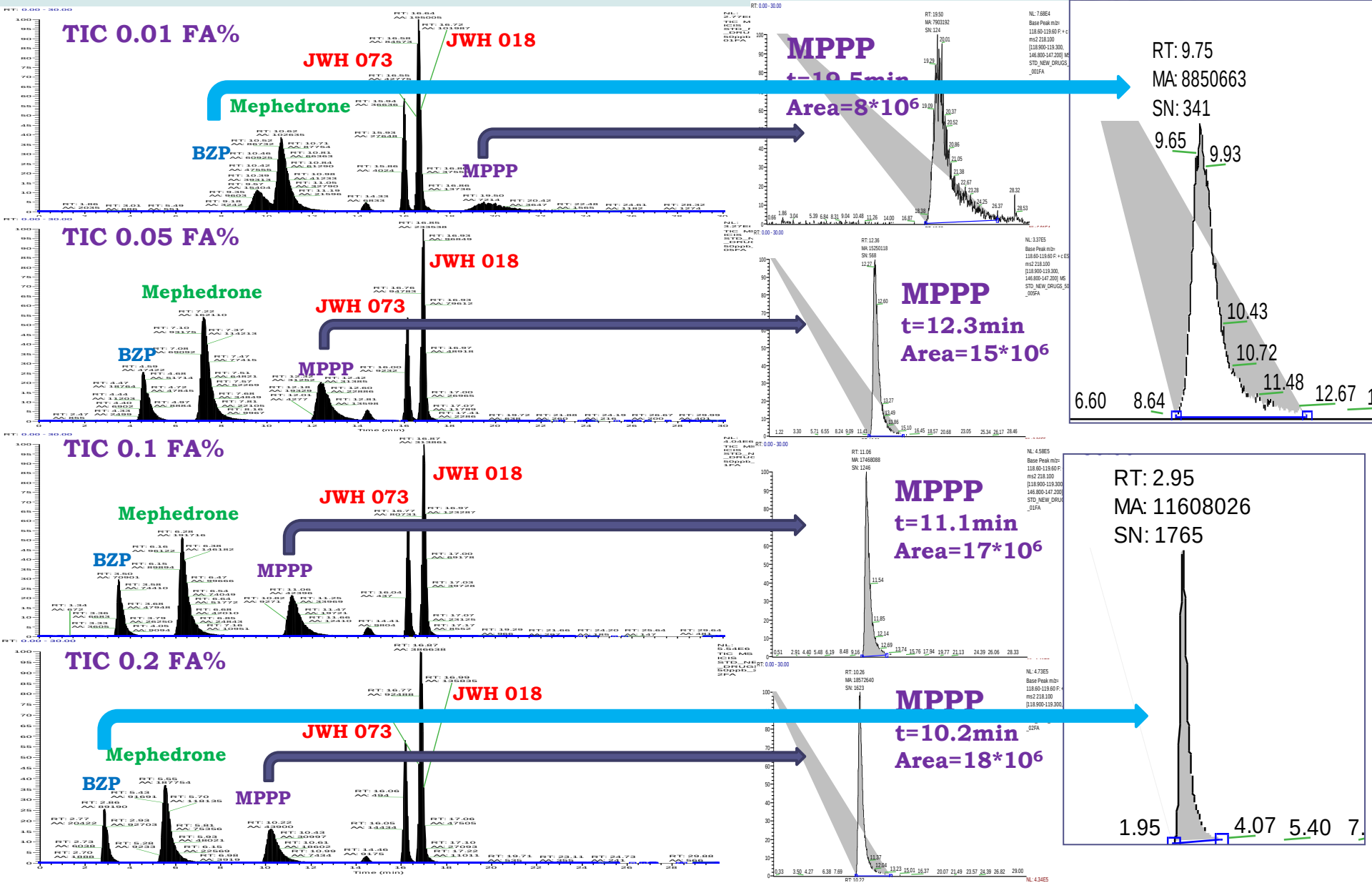
Positive (min)	H_2O (0.2% formic acid) %	MeOH %	µL/min
0.00	60	40	100
3.00	60	40	150
15.00	0	100	100
25.00	0	100	100
25.50	60	40	100
30.00	60	40	100

CP47, 497

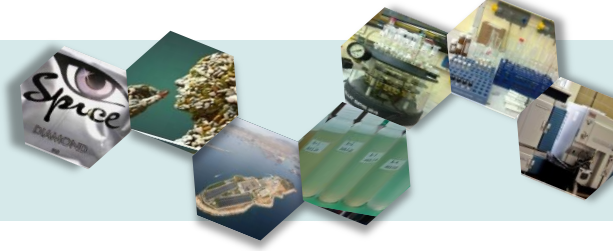
Negative (min)	MeOH %	ACN %	µL/min
0.00	90	10	100
3.00	90	10	100
8.00	90	10	100

Optimization of HPLC
Effect of the organic modifier (F.A.)



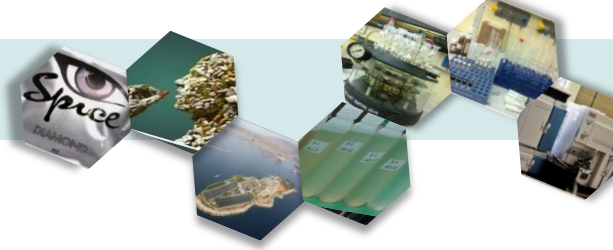


Optimization of SPE
Comparison of different cartridges



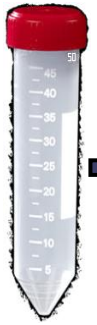
1. Strata X (Phenomenex)
2. Strata XC (Phenomenex)
3. Isolute C18 (EC) (Biotage)
4. Double SPE (C18 (EC)- Strata XC
5. ATOLL XC (Interchim)
6. Lichrolut EN (bottom)- C18 (top) (Merck)
7. Lichrolut EN (Merck)
8. PolyClean 2H (Interchim)
9. Lichrolute TSC (Merck)

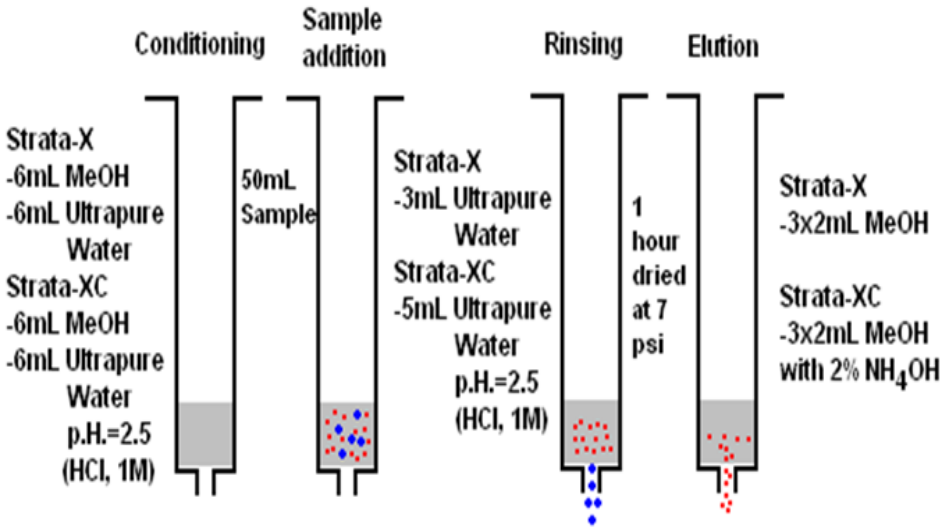
Optimization of SPE

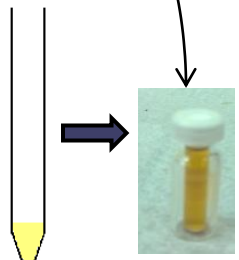


1. Wastewater samples were filtered on **Glass fiber filters**
2. 50 mL of filtrated sample were adjusted at a value of **pH 2.5** with HCl (1 M)
3. Internal deuterated standards of the compounds were added to all samples
4. SPE

Reconstitution :
500 µL of 60% MeOH and 40% ultra purified water with 0.05% v/v formic acid

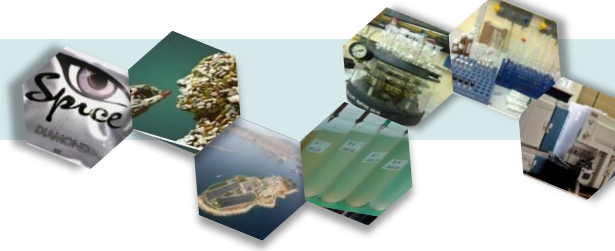




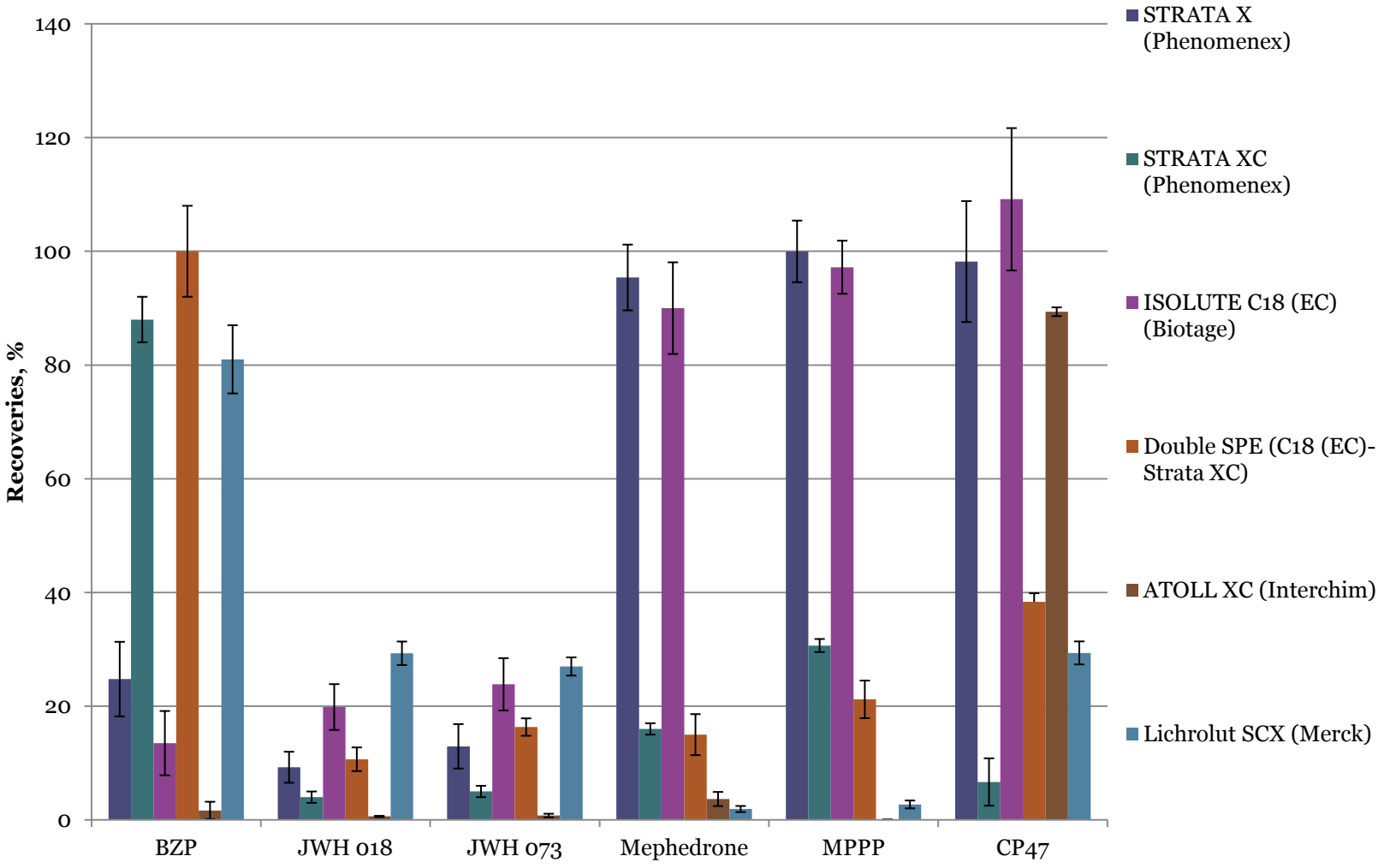


LC-MS/MS

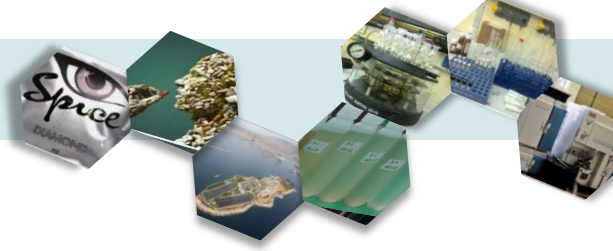
Optimization of SPE

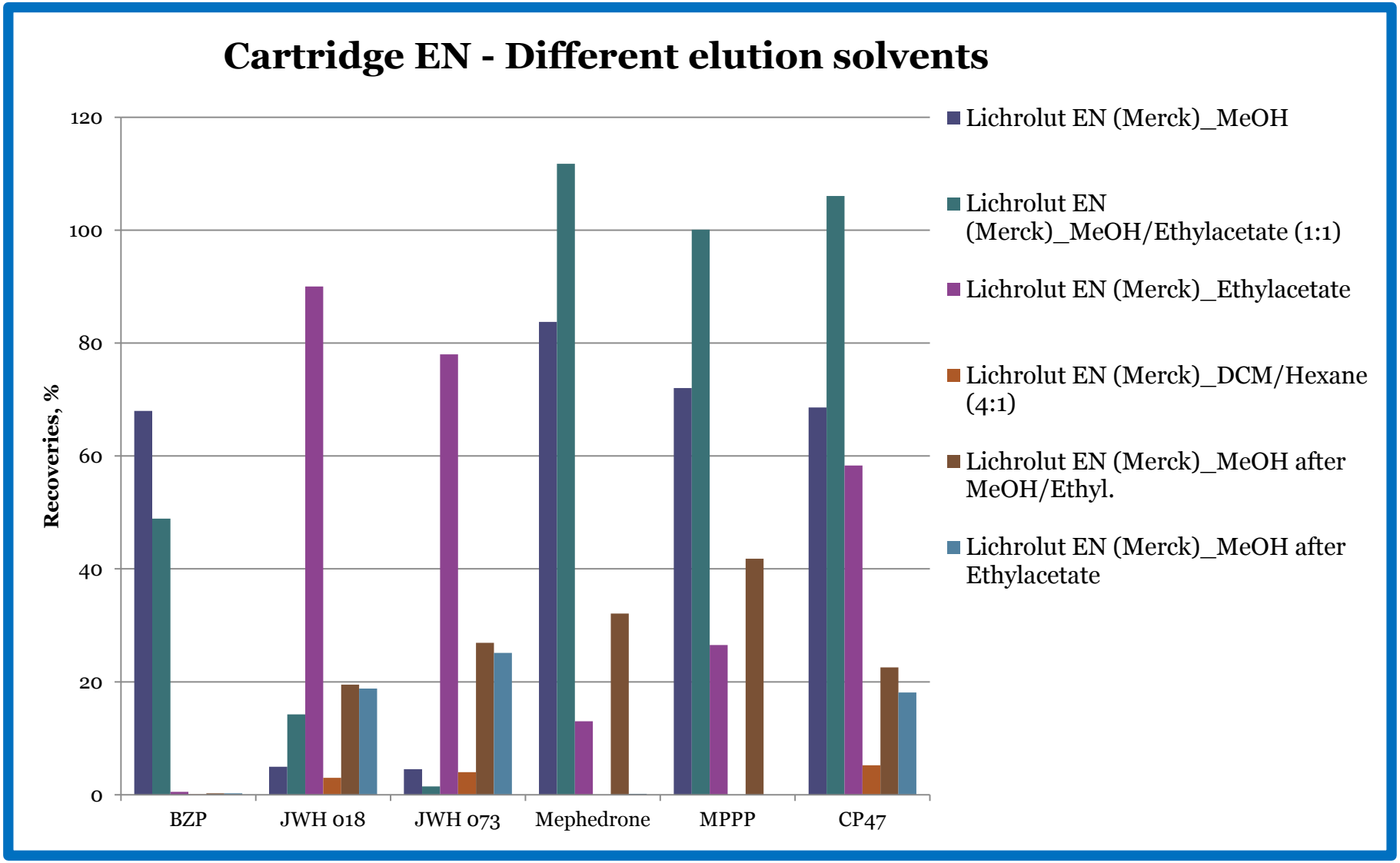


Cartridges-Recoveries, Graph 1

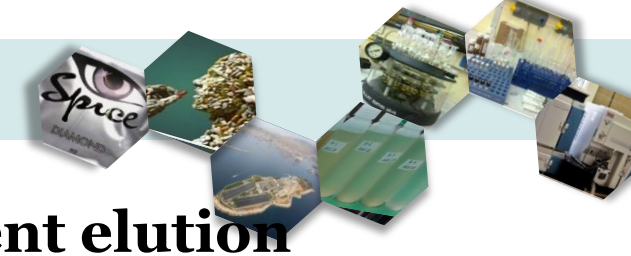


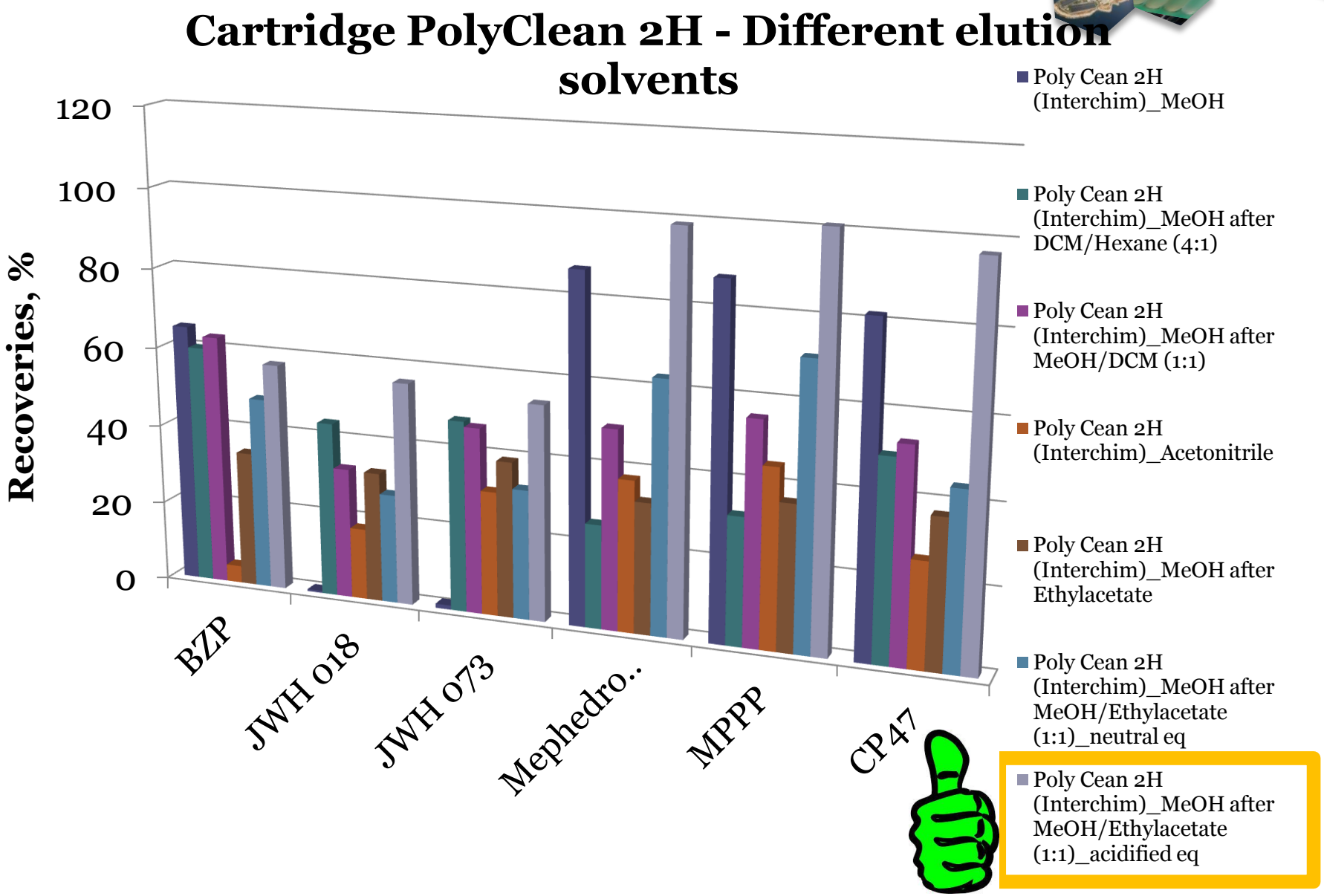
Optimization of SPE



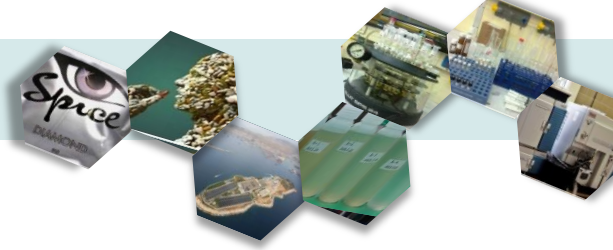


Optimization of SPE





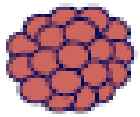
Optimization of SPE~FINAL

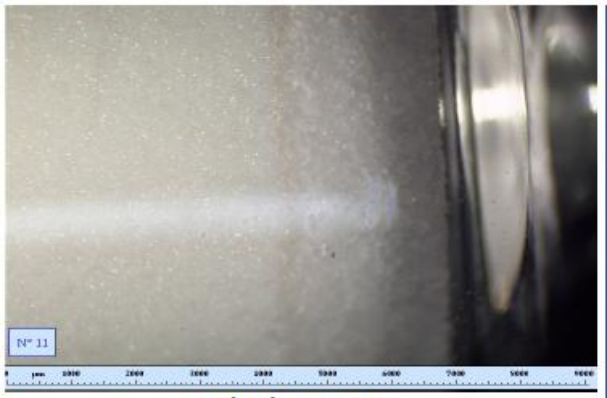


	SOLVENTS
Conditioning	6 ml methanol (under gravity)
Equilibration	6 ml H2O (pH=2.5, HCl) (under gravity)
Load	50 ml sample
Wash	5 ml H2O (pH=2.5, HCl)
Vacuum	30 min
Elute	4 mL (2 x 2 mL MeOH) 8 mL (4 x 2 mL) Methanol/ Ethyl acetate (50:50)

PolyClean 2H

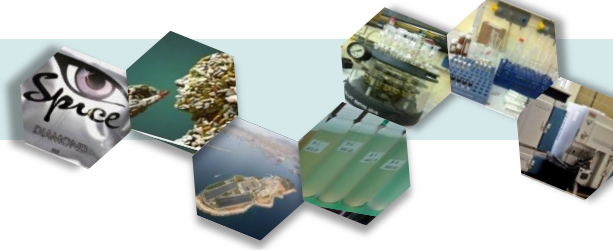
Spherical Pure Mixed hydrophilic / hydrophobic Polymer
200mg (size)





PolyClean™ 2H

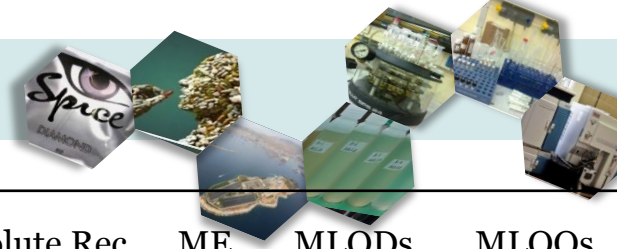
Method VALIDATION



Instrumental quality parameters of the HPLC-ESI-MS/MS method for the analysis of NPDs.

	Linearity range (µg/L)	R^2	ILODs (µg/L)	ILOQs (µg/L)
JWH-018	0.5-80	0.9994	0.20	0.62
JWH-073	0.5-80	0.9991	0.28	0.86
JWH-210	0.5-80	0.9995	0.04	0.14
JWH-122	0.5-80	0.9992	0.05	0.16
JWH-250	0.5-80	0.9996	0.23	0.71
Mephedrone	0.5-80	0.9995	0.18	0.53
BZP	0.5-80	0.9996	0.09	0.28
MPPP	0.5-80	0.9997	0.09	0.28
a-PVP	0.5-80	0.9994	0.07	0.22
CP47,497	1-80	0.9995	0.04	0.12

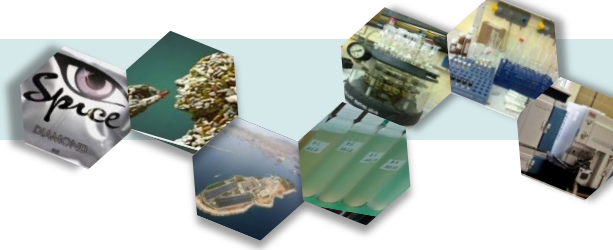
Method VALIDATION

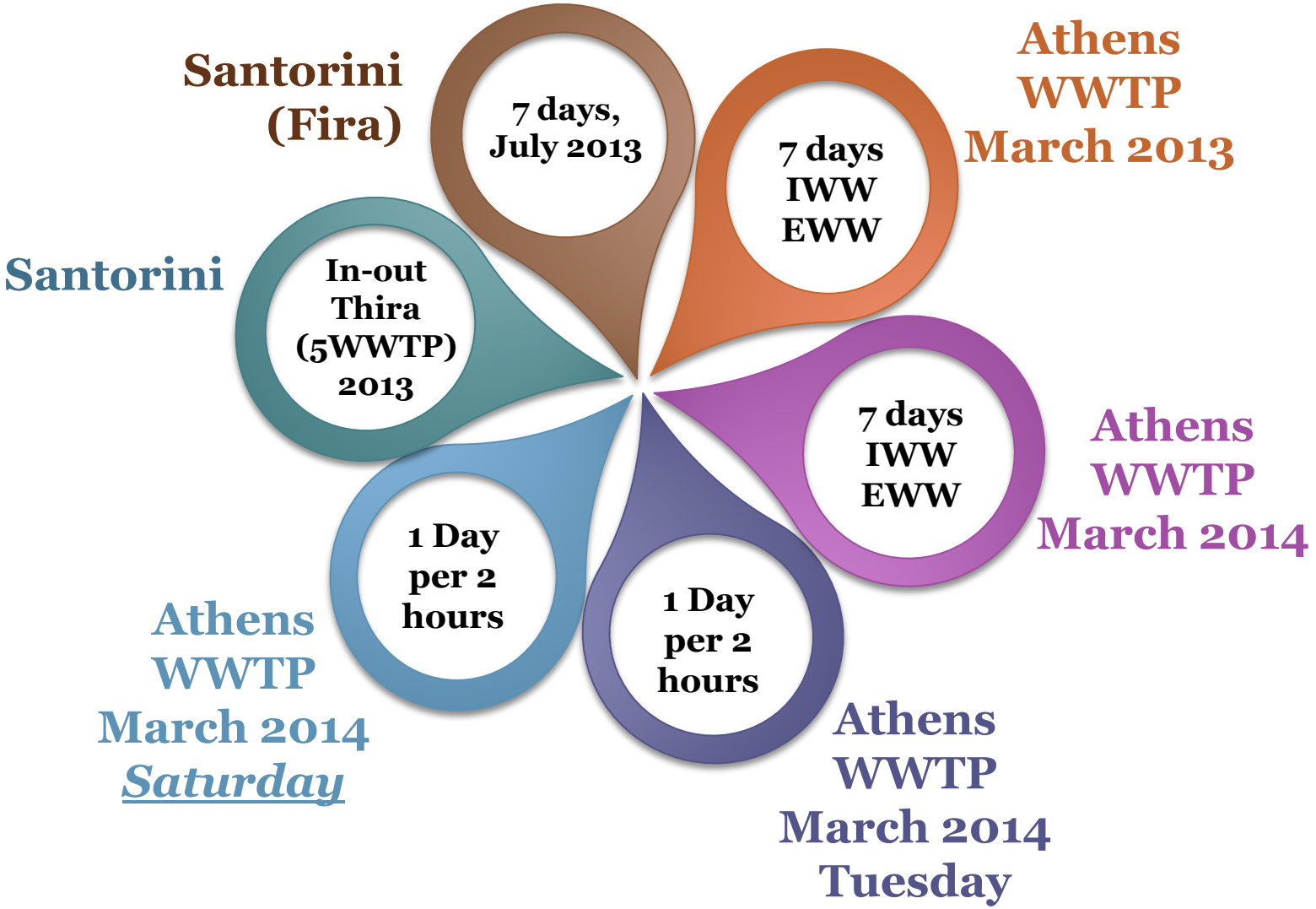


	Conc. (ng/L)	Absolute Rec. (%) ±RSD	Conc. (ng/L)	Absolute Rec. (%) ±RSD	ME (%)	MLODs (ng/L)	MLOQs (ng/L)
Influent wastewater							
JWH-018	10	65±9	800	65±10	-54	0.8	2.4
JWH-073	10	60±9	800	61±9	-61	1.4	4.1
JWH-210	10	69±9	800	73±11	-23	1.1	3.2
JWH-122	10	60±4	800	67±10	-45	1.4	4.4
JWH-250	10	54±10	800	69±9	-64	1.2	3.6
Mephedrone	10	101±1	800	95±6	-63	0.8	2.5
BZP							11.3
MPPP							1.5
a-PVP							2.3
CP47,497							111
Effluent wastew							
JWH-018							2.0
JWH-073							3.8
JWH-210							1.5
JWH-122	10	40±9	800	57±9	-50	0.3	0.9
JWH-250	10	46±6	800	61±8	-23	0.1	0.4
Mephedrone	10	102±7	800	99±6	-35	0.6	1.8
BZP	10	39±9	800	58±13	-43	4.8	14.4
MPPP	10	98±9	800	98±11	-19	0.4	1.3
a-PVP	10	98±4	800	102±8	-25	0.4	1.1
CP47,497	10	78±9	800	91±10	-20	24	74

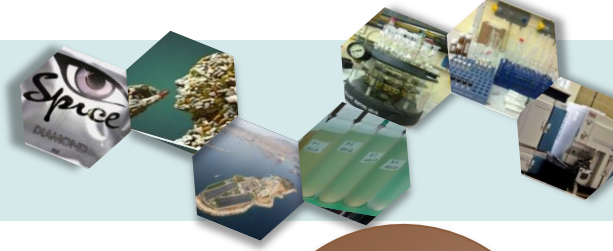
Matrix	Recovery	MLOD	MLOQ
Effluent wastewater	50 – 102 %	0.1 –24 ng/L	0.4 – 74 ng/L
Influent wastewater	61 – 103 %	0.5 – 37 ng/L	1.5 –111 ng/L

Application in real wastewater





Wastewater Samples from WWTP Santorini



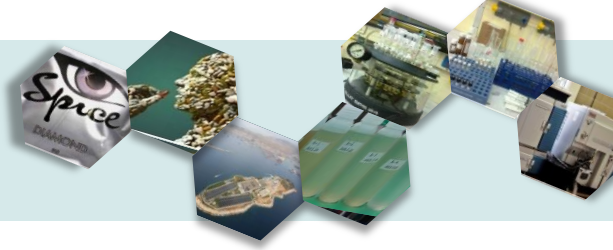
Santorini (Fira) Grab samples

22/07/2013 - 28/07/2013

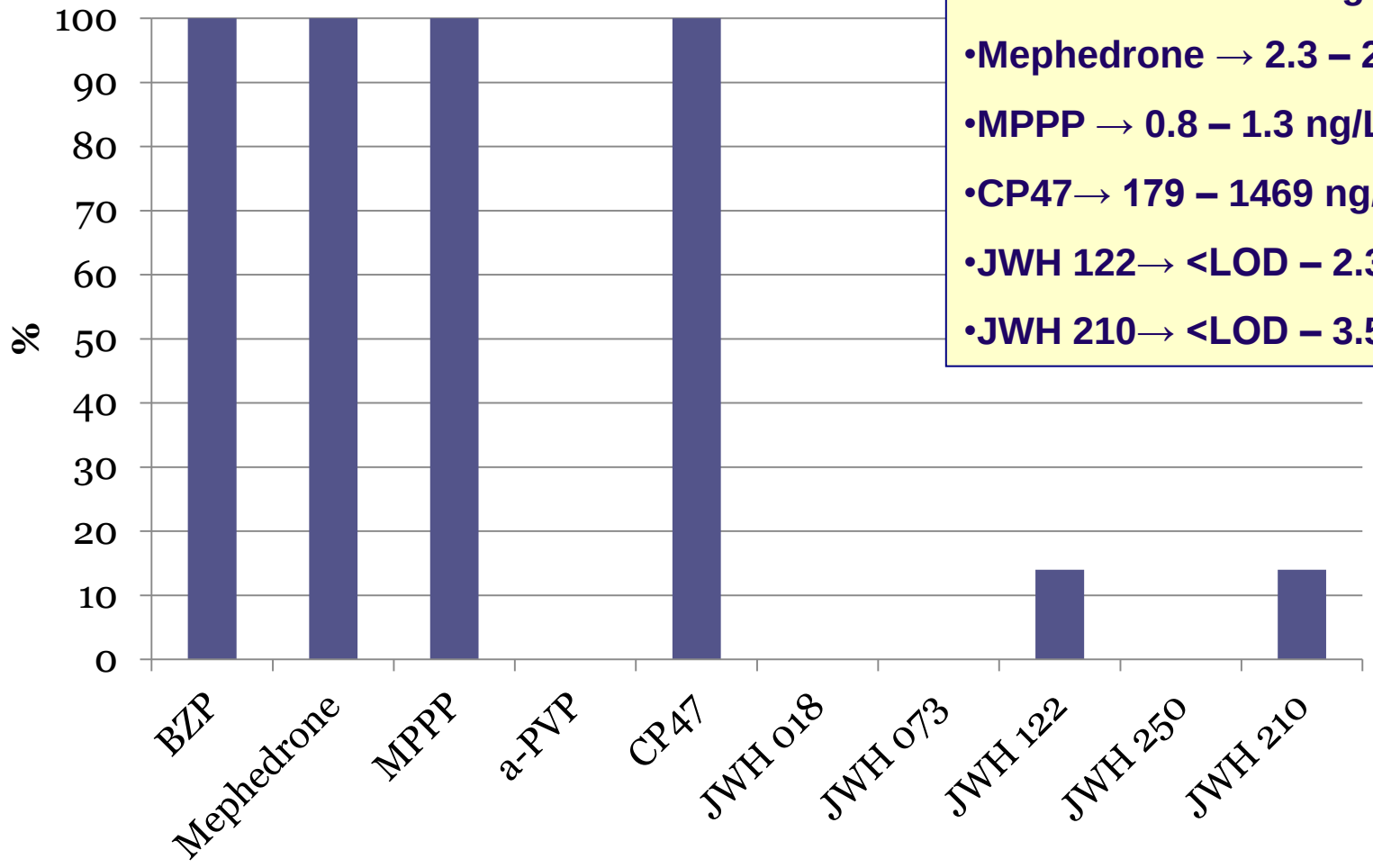
7 days, July 2013 IWW

- ✓ Population: 10500 inhabitants
- ✓ Flow rate: 1500 m^3/day

Wastewater Samples from WWTP Santorini



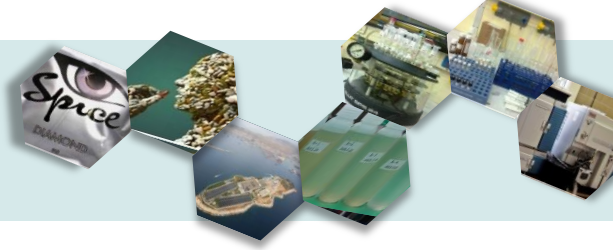
Frequency of detection



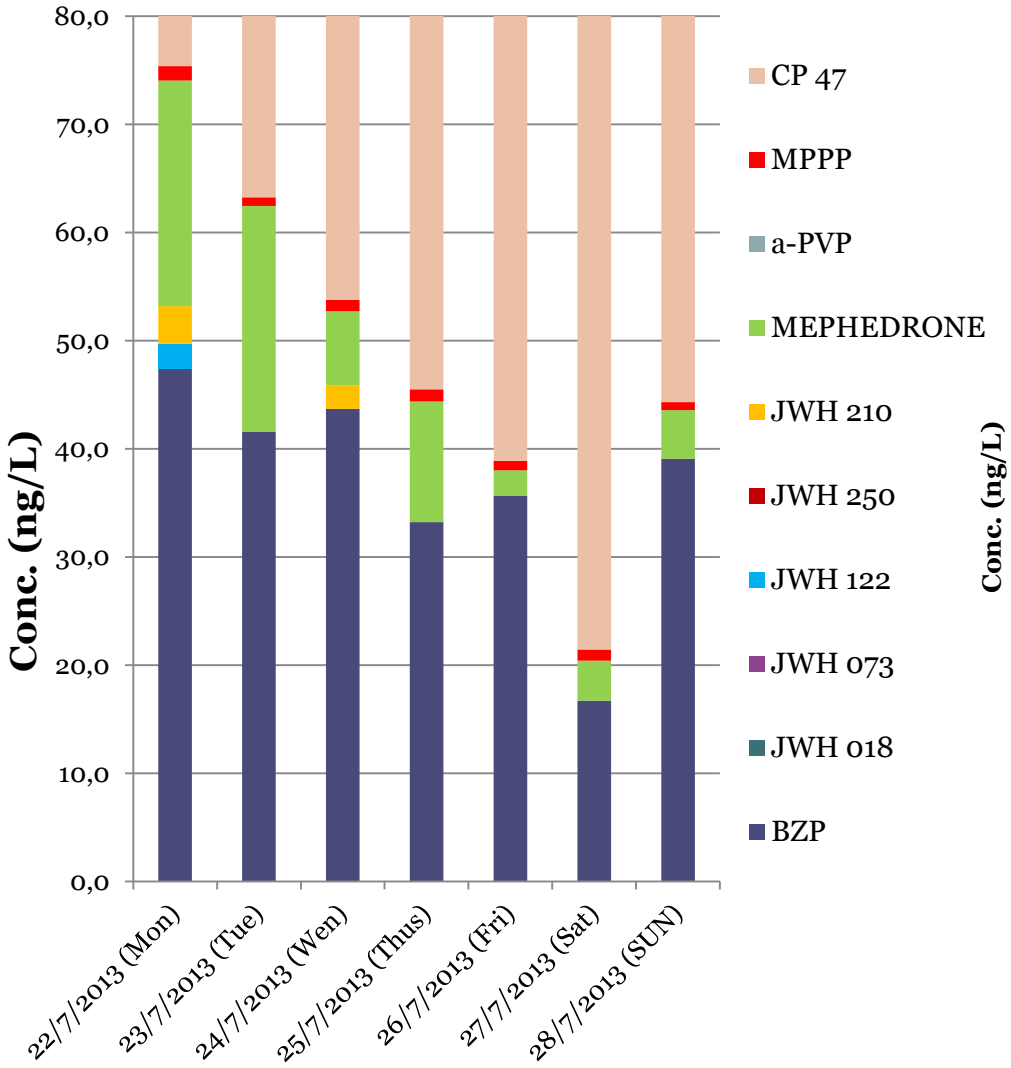
RANGES:

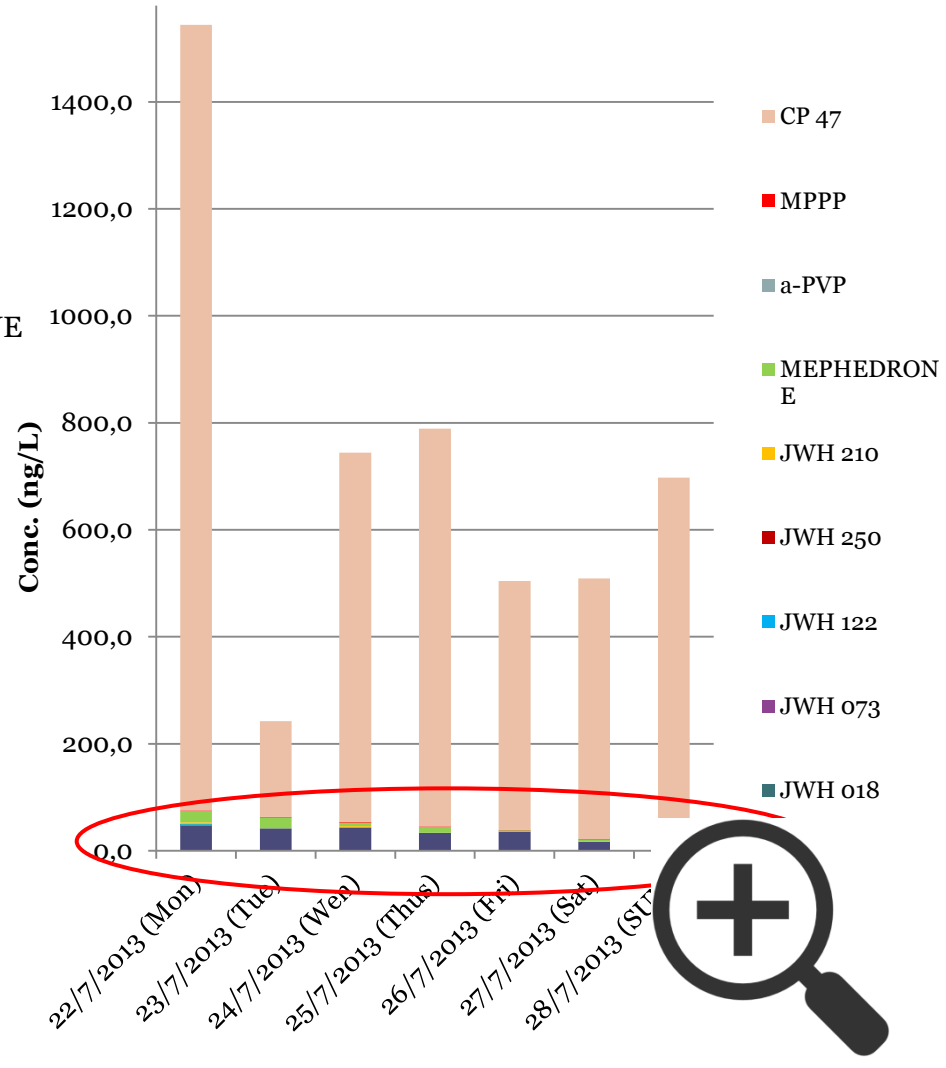
- BZP → 16.7 - 47.4 ng/L
- Mephedrone → 2.3 – 20.9 ng/L
- MPPP → 0.8 – 1.3 ng/L
- CP47→ 179 – 1469 ng/L
- JWH 122→ <LOD – 2.3 ng/L
- JWH 210→ <LOD – 3.5 ng/L

Wastewater Samples from WWTP Santorini

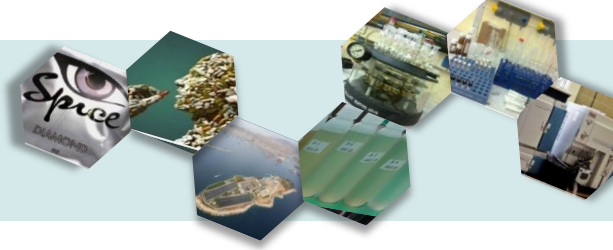


Daily variations of the levels of concentration in IWW





Wastewater Samples from WWTP Santorini

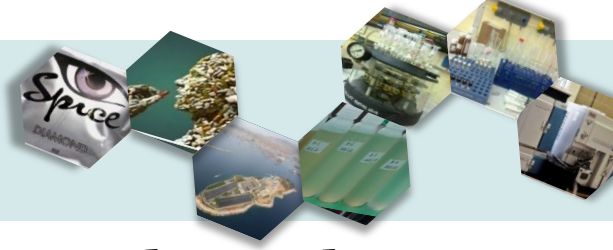


In-out Thira (5WWTP) 2013

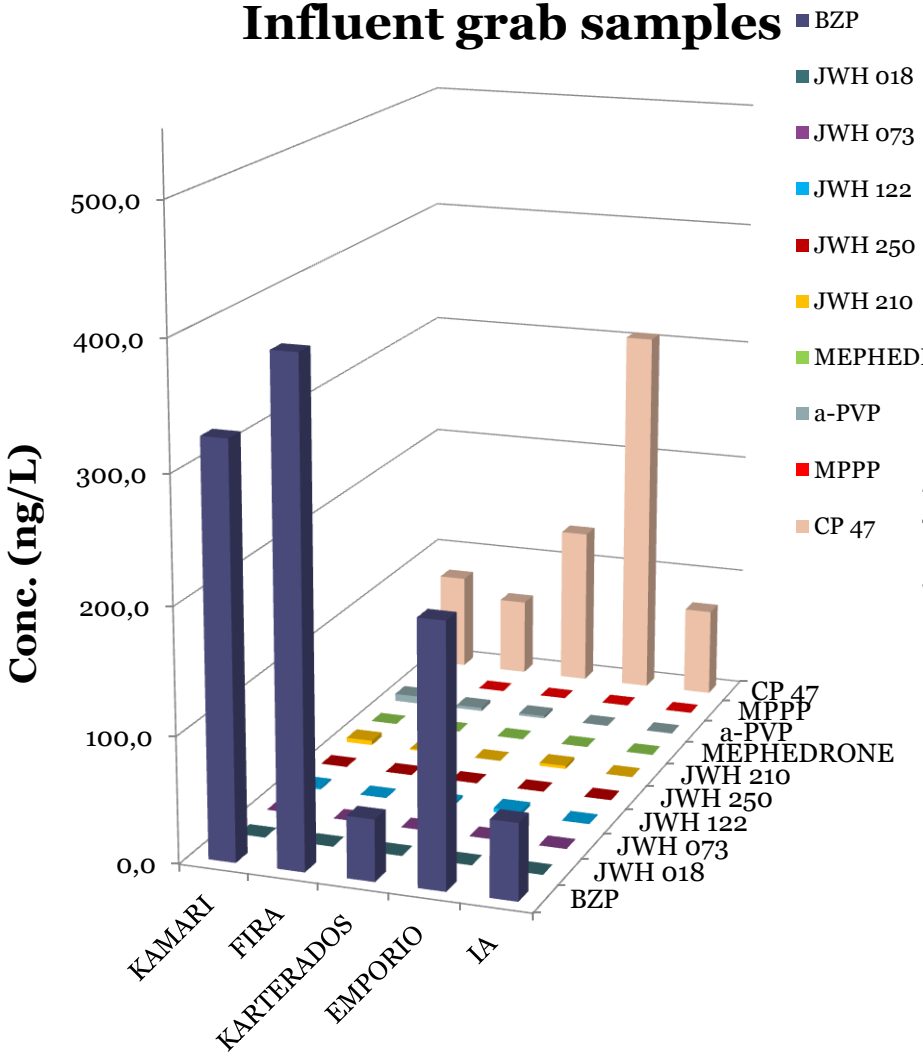
Santorini (grab samples)
5 influent
5 effluent

Area WWTP	Mean Flow (m^3/day)	Population	Primary treatment	Secondary Treatment
KAMARI	1600	15500	Gridding Desanding Sedimadation	Biological treatment Activ. Sludge
FIRA	1500	10500	same	same
KARTERADOS	900	3150	same	same
EMPORIO	600	3000	same	same
IA	700	6000	same	same

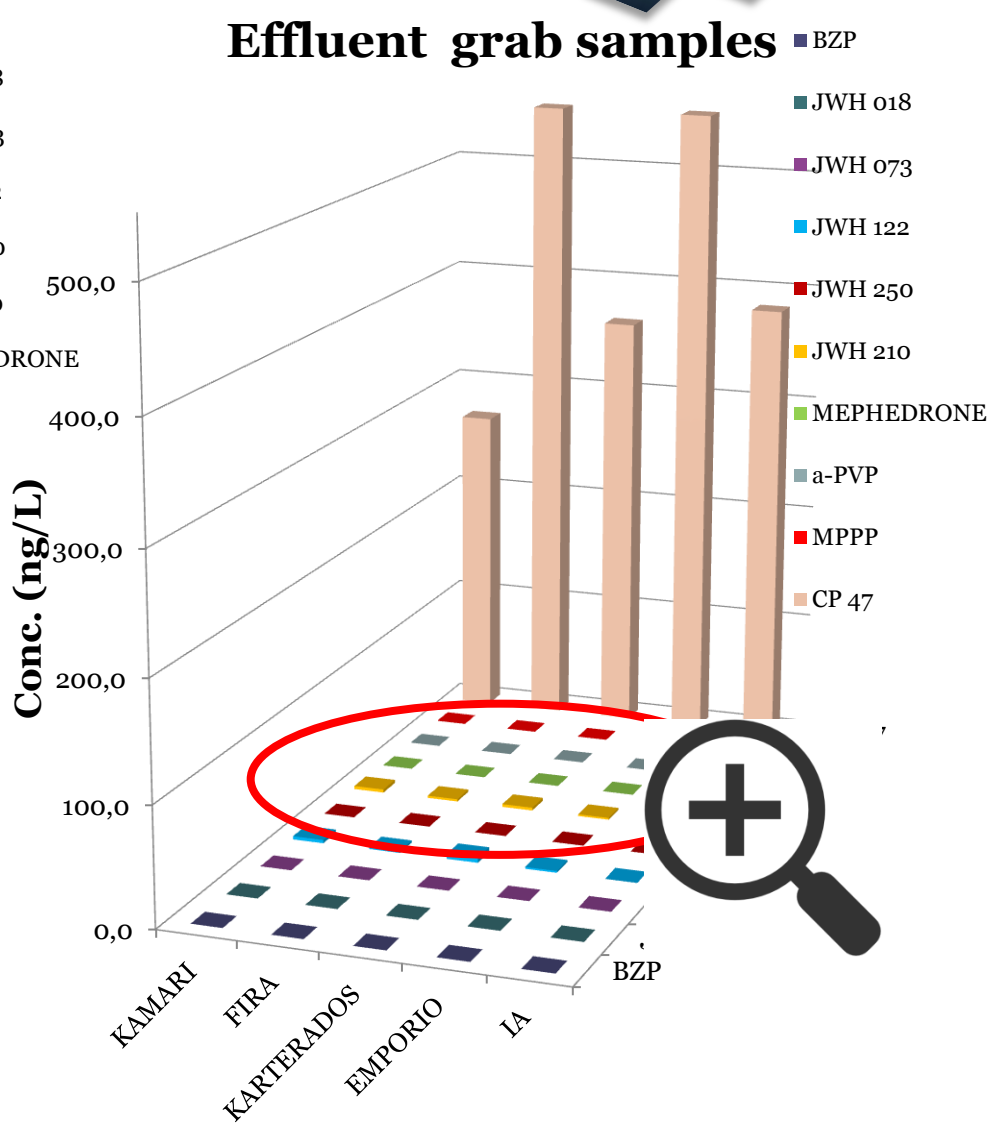
Wastewater Samples from WWTP Santorini



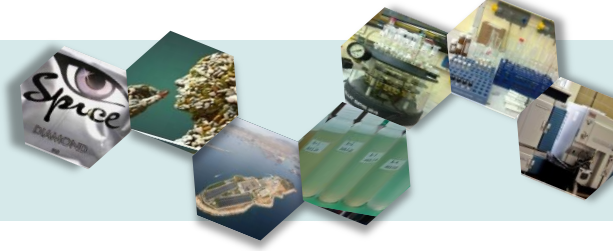
Influent grab samples

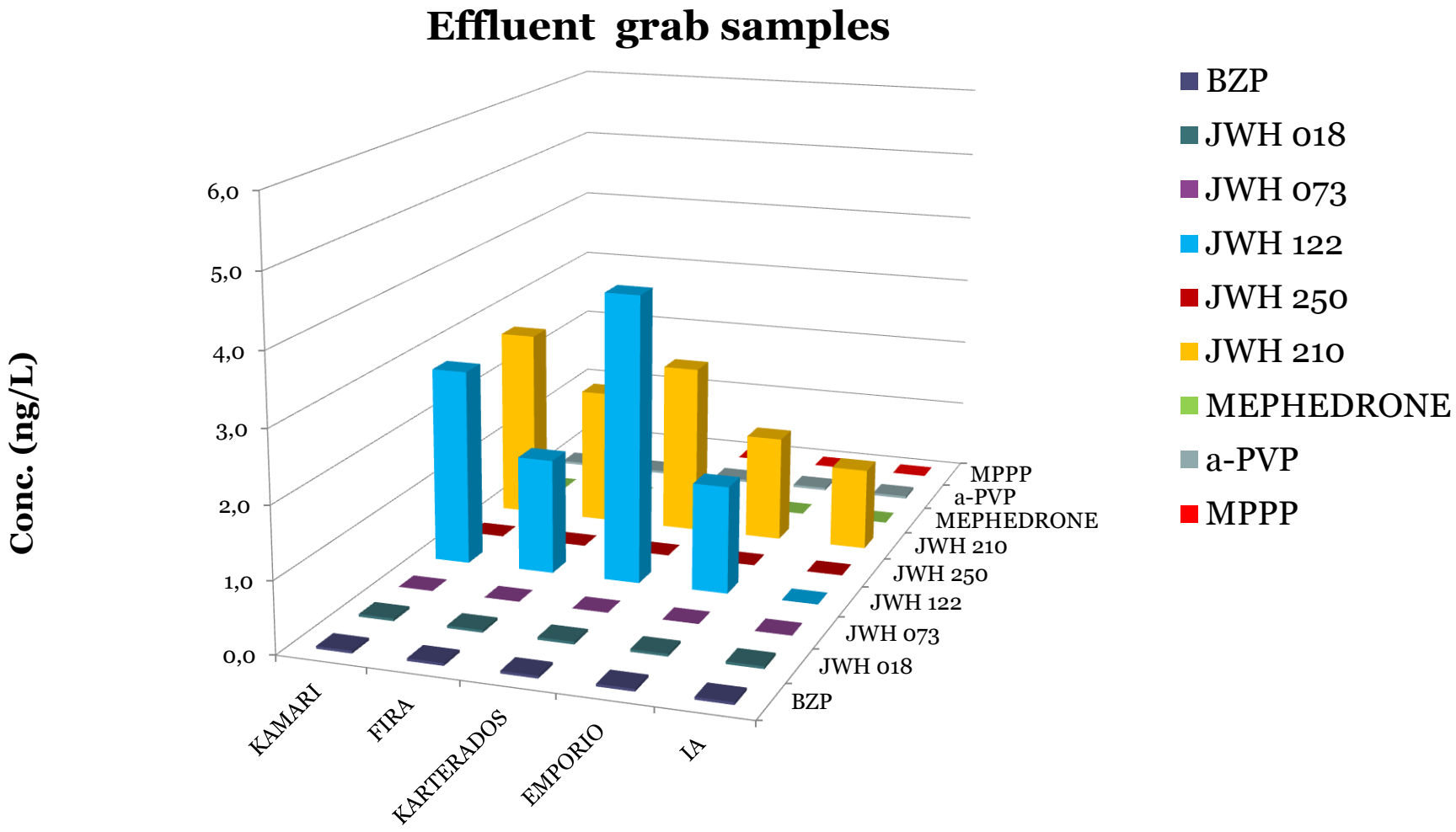


Effluent grab samples

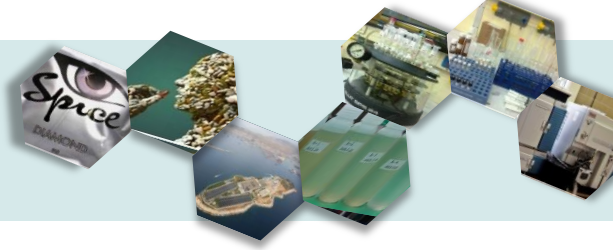


Wastewater Samples from WWTP Santorini





Wastewater Samples from WWTP ATHENS



Two sampling campaigns in the WWTP of Athens, Greece

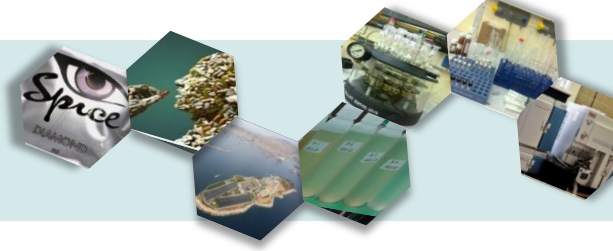
March 2013 (7 consecutive days)
March 2014 (7 consecutive days)

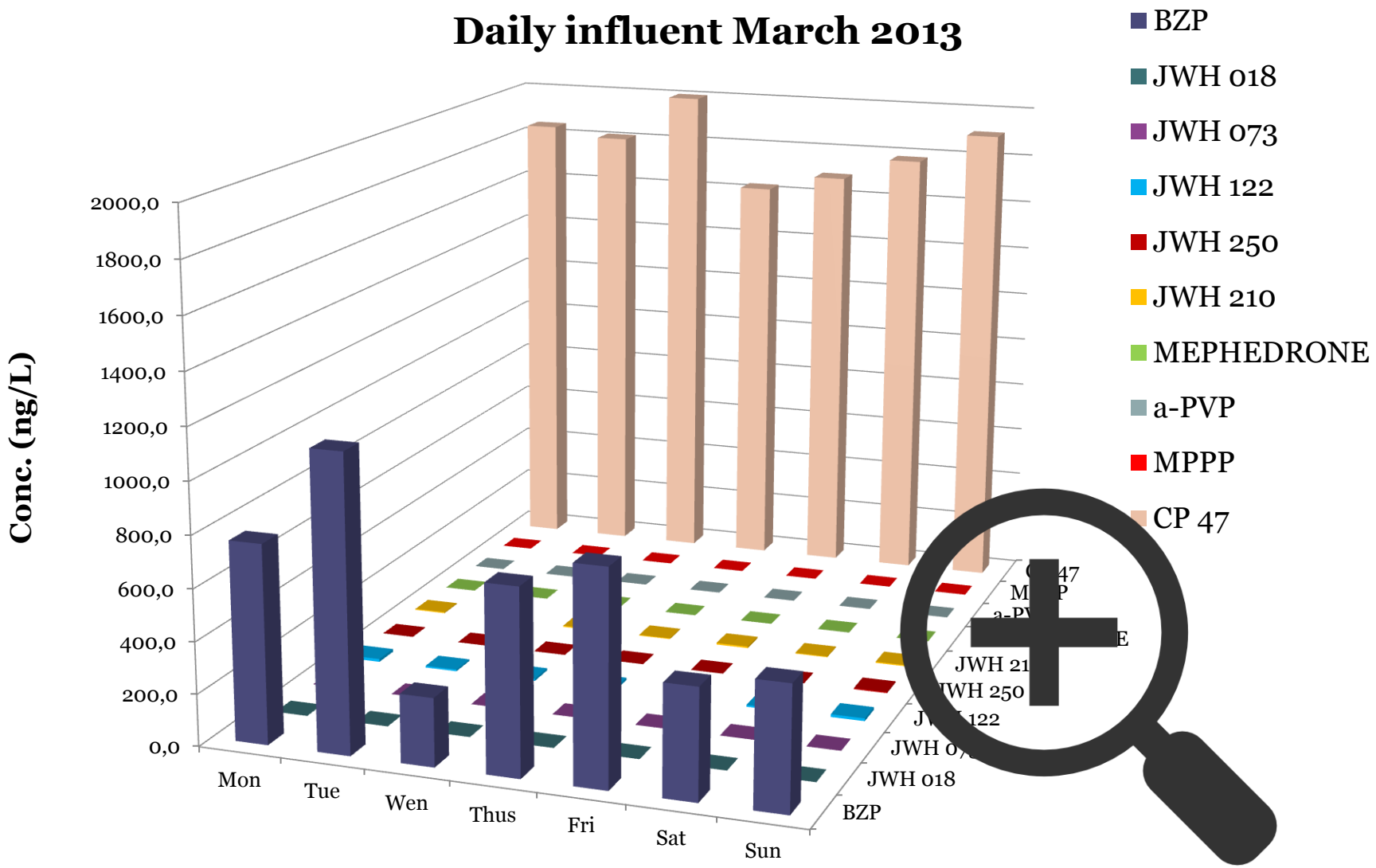
24-h composite flow-proportional samples of influents and effluents

7 days IWW EWW 2013

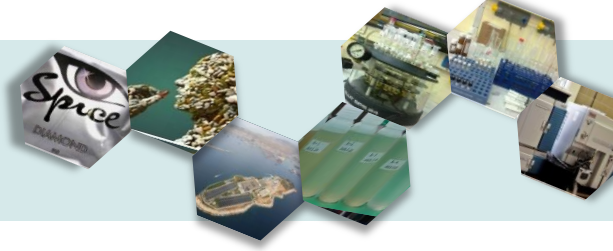
7 days IWW EWW 2014

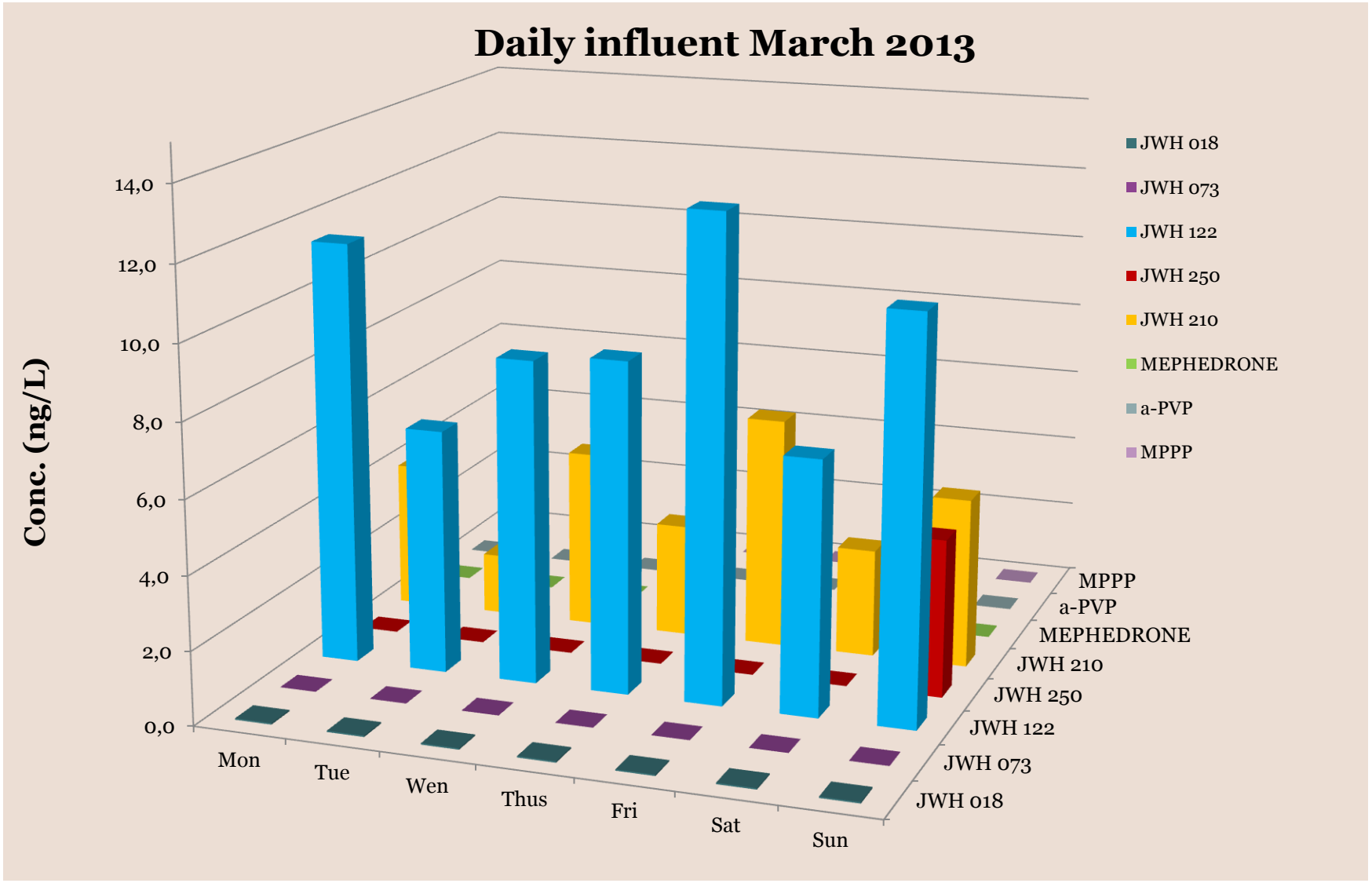
Wastewater Samples from Athens (March 2013)-influent



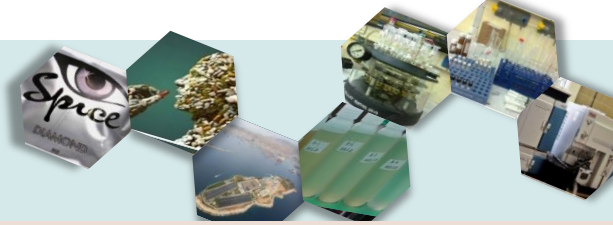


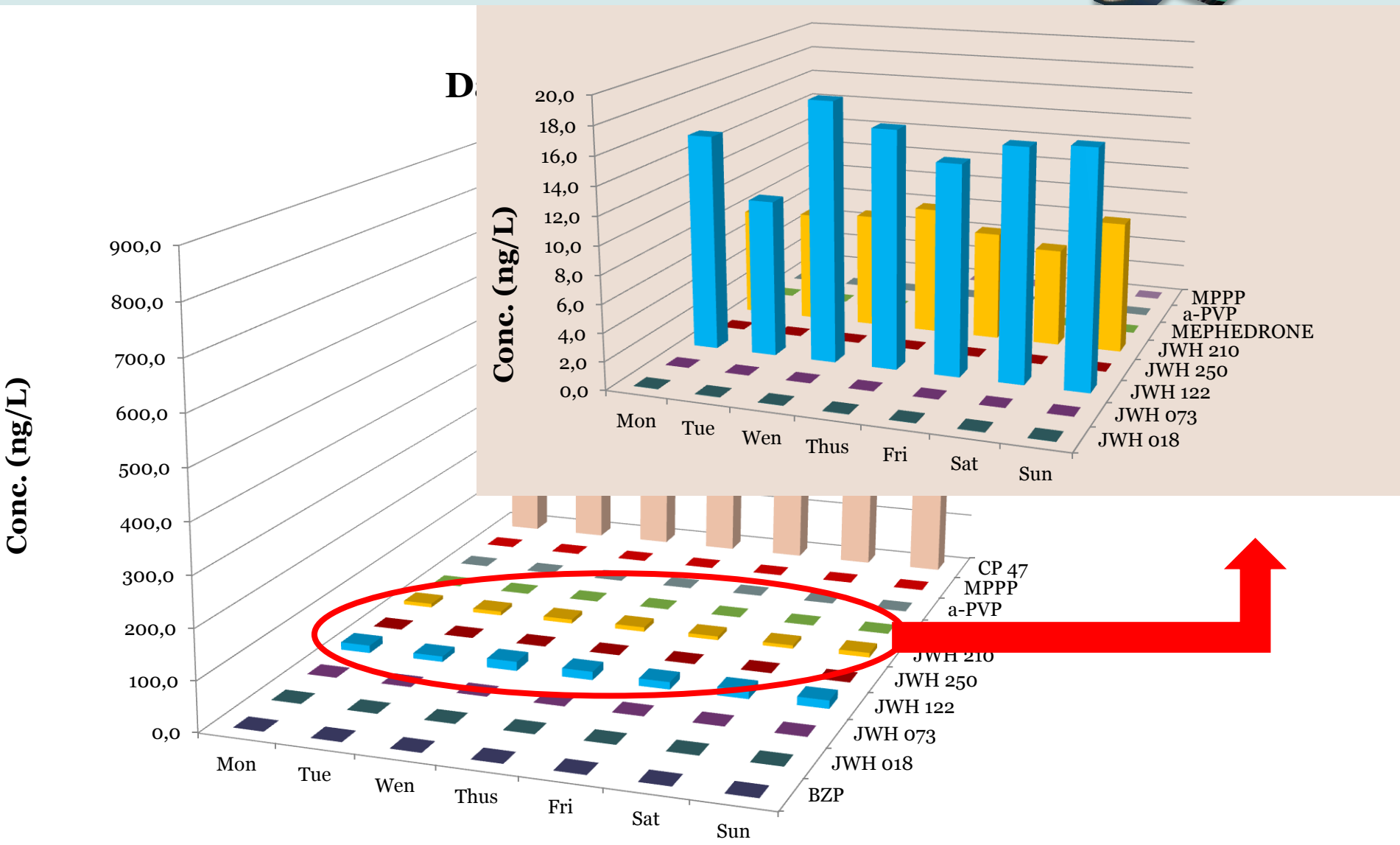
Wastewater Samples from Athens (March 2013)-influent



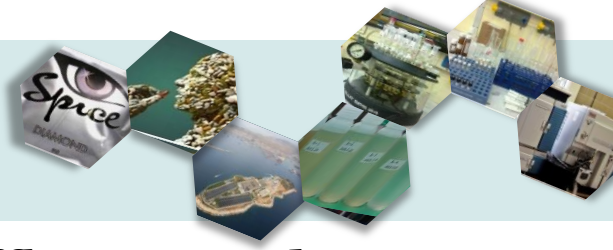


Wastewater Samples from Athens (March 2013)-effluent

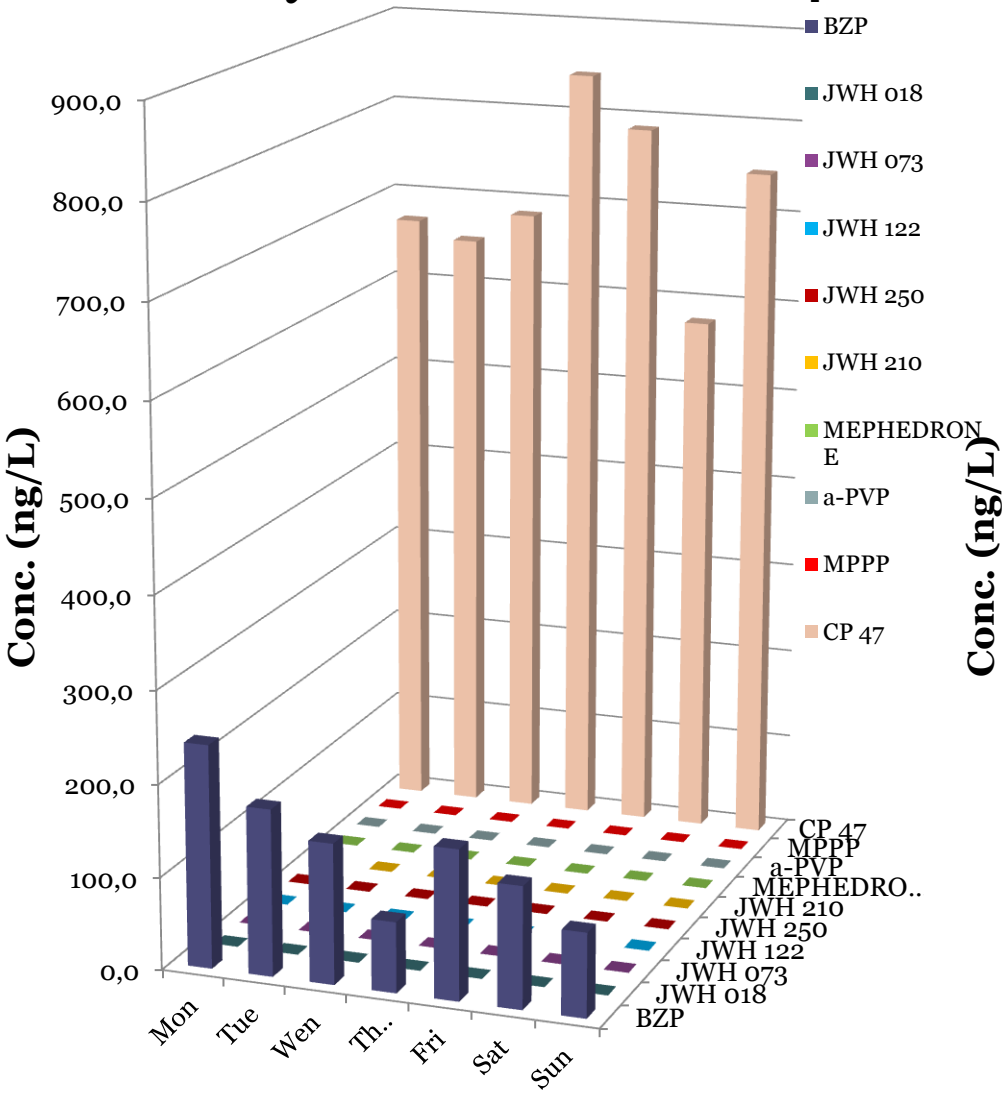




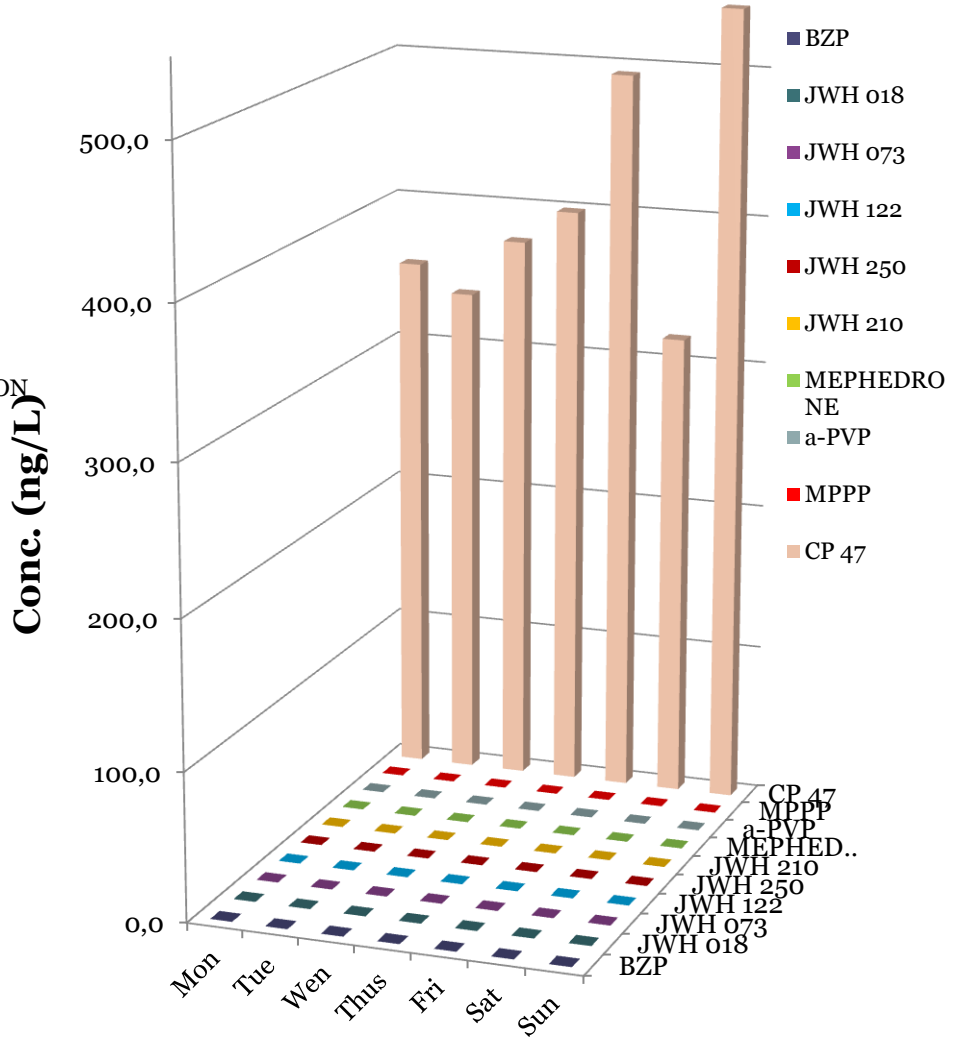
Wastewater Samples from WWTP of Athens



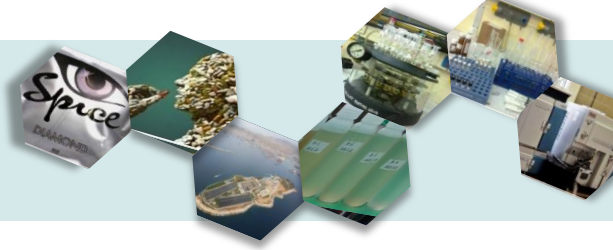
Daily influent March 2014

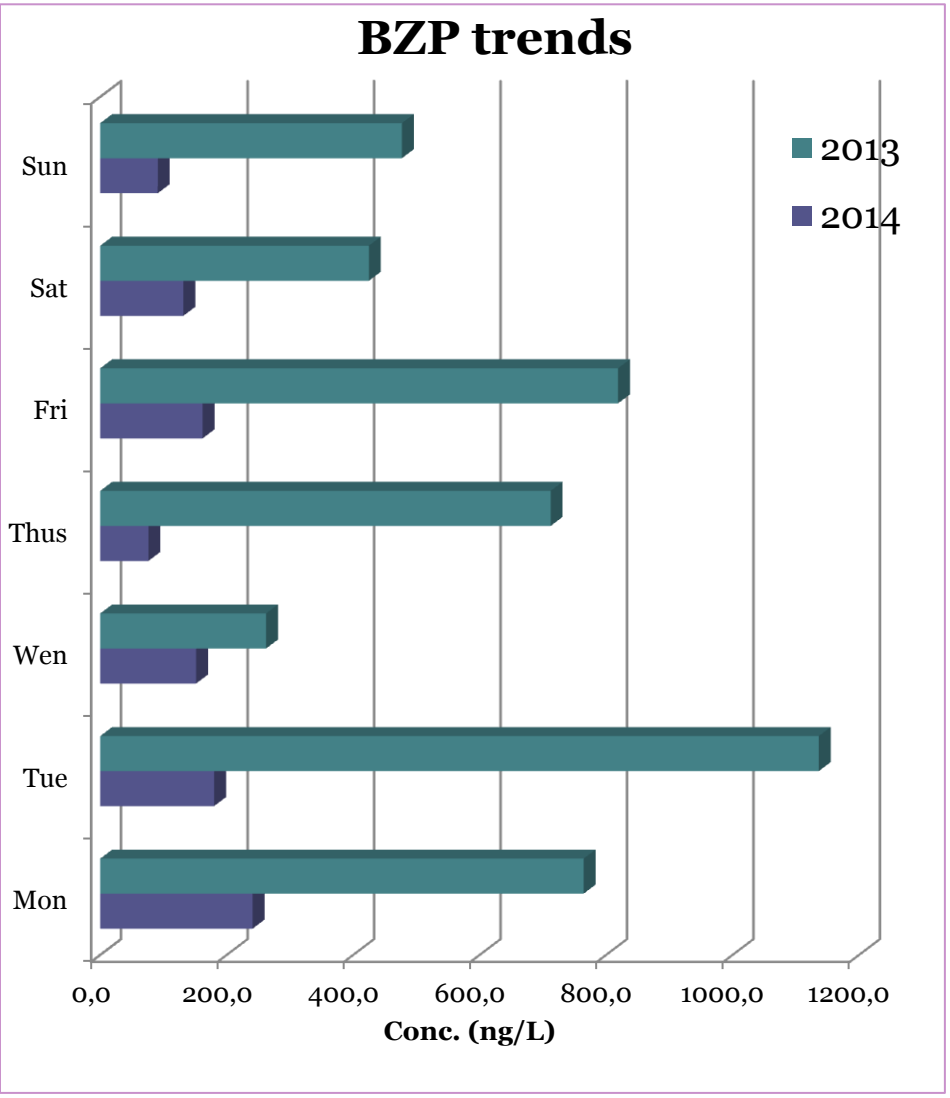


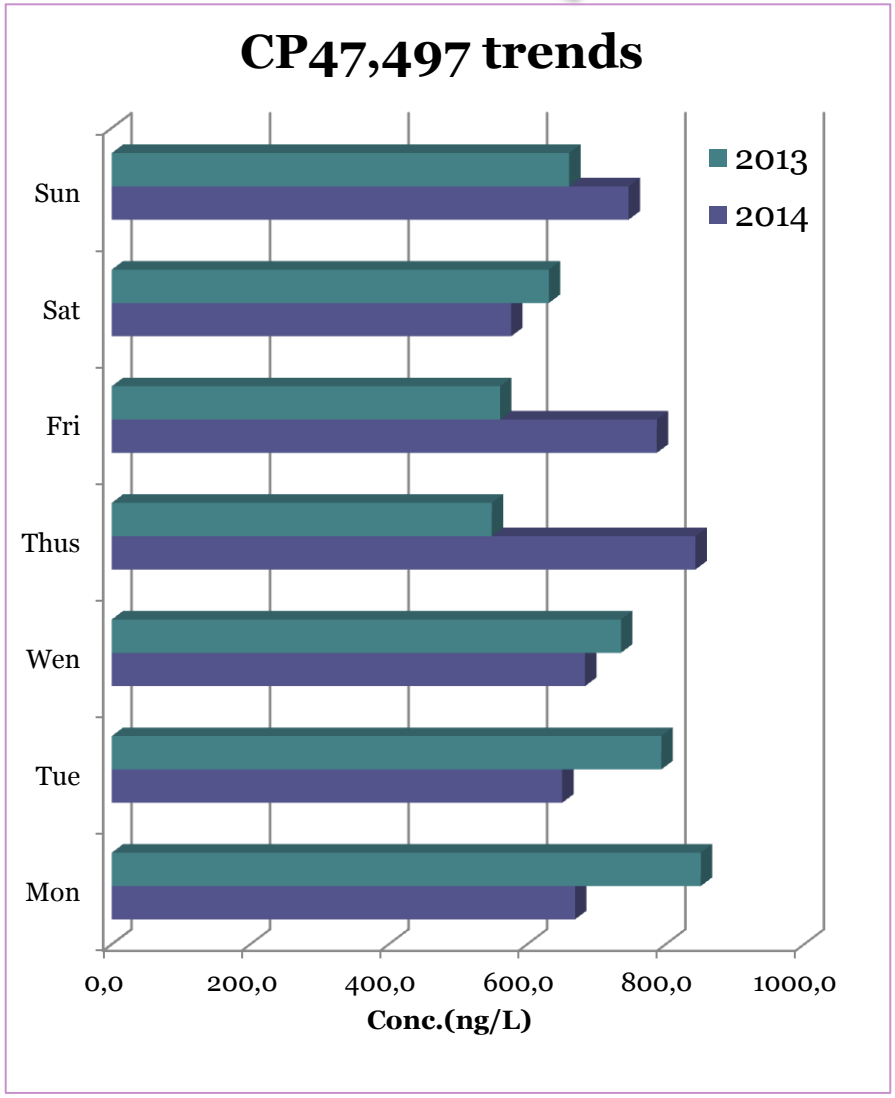
Daily effluent March 2014



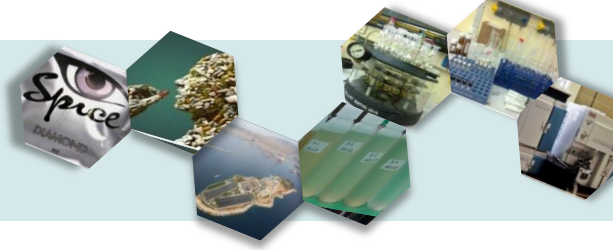
Wastewater Samples from Comparison 2013-2014 (trends)







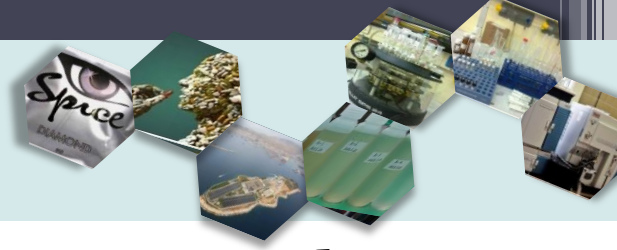
Wastewater Samples from WWTP of Athens



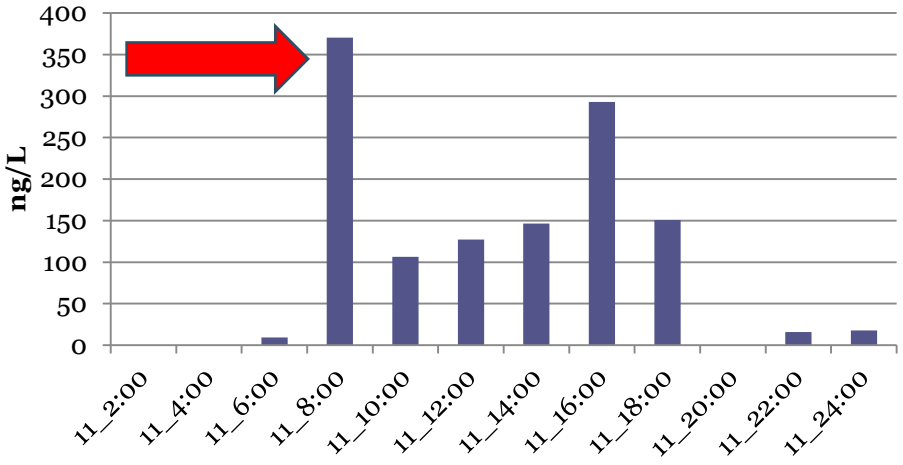
Athens
WWTP
March 2014
Tuesday

Athens
WWTP
March 2014
Saturday

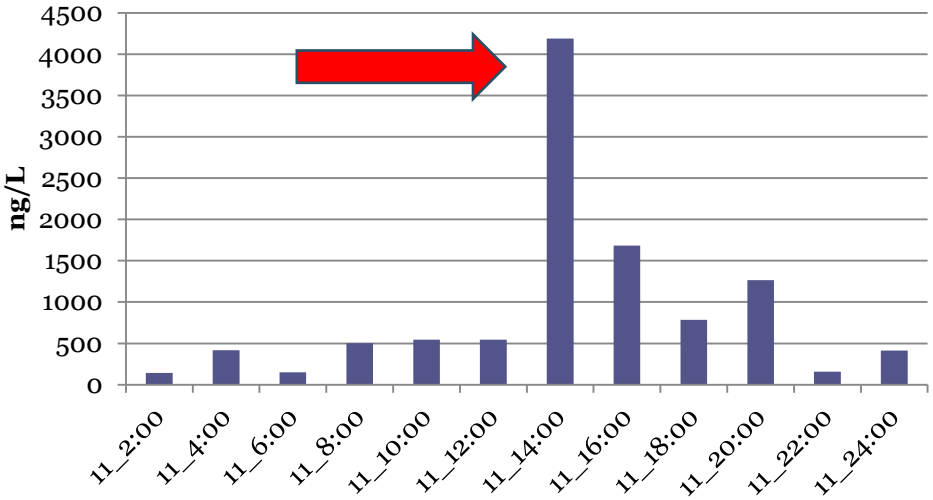
Wastewater Samples from WWTP of Athens



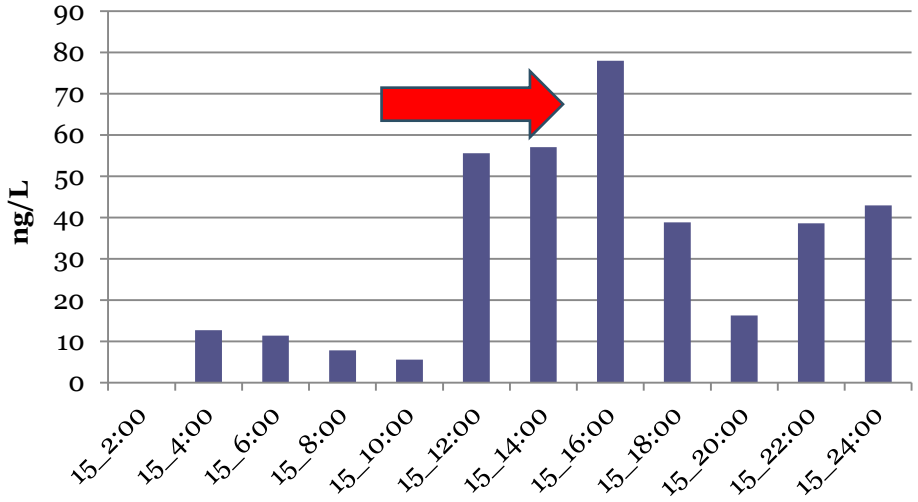
BZP on Tuesday



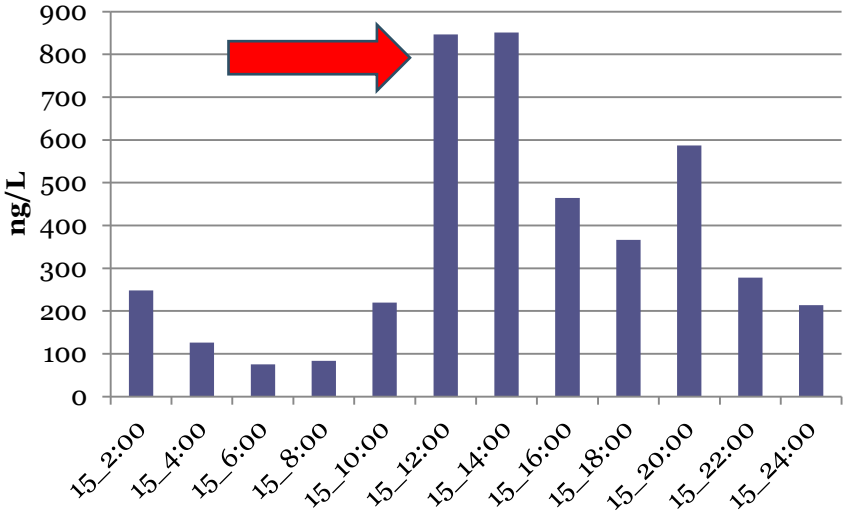
CP47, 497 on Tuesday



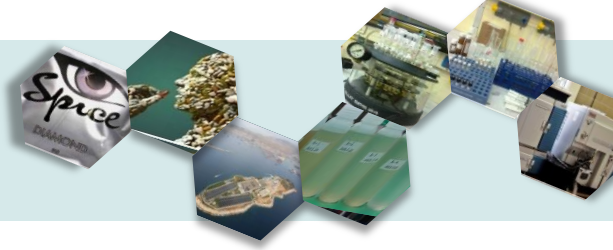
BZP on Saturday



CP47, 497 on Saturday



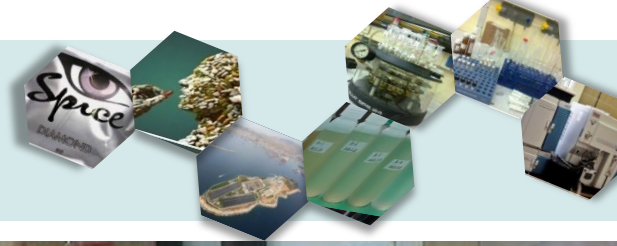
Wastewater Samples from WWTP of Athens



24:00 22:00 20:00 18:00 16:00 14:00 12:00 10:00 8:00 6:00 4:00 2:00

Tuesday (11/03/2014)

Wastewater Samples from WWTP of Athens



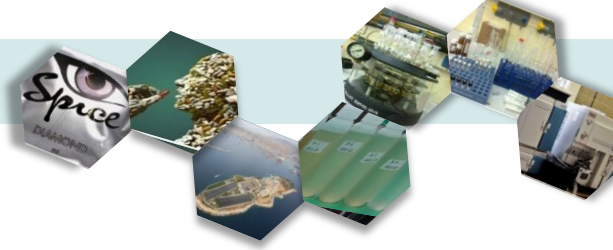
2:00 4:00 6:00 8:00 10:00 12:00 14:00 16:00 18:00 20:00 22:00 24:00

Saturday (15/03/2014)

CONCLUSIONS

- ✓ Development and validation of a novel method for new designer drugs in wastewater by LC-MS/MS
- ✓ Investigation of their occurrence in a WWTP in Santorini Island
- ✓ CP47, mppp and JWH 122/210 were detected for the first time in influent wastewater
- ✓ 6 out of the 10 compounds were detected at least in one day, whereas mephedrone, bzp,cp47 and mppp were detected all days in Santorini
- ✓ Not any special trend among the days
- ✓ The most ubiquitous compounds in influents of all WWTPs in both Santorini and Athens were CP47 and BZP. BZP was removed completely, whereas CP47 was partially removed.
- ✓ JWH compounds were detected at slightly higher concentrations in effluents.

OUTLINE



- THANK YOU VERY MUCH FOR YOUR ATTENTION!!!
- Questions???

European Union
European Social Fund

OPERATIONAL PROGRAMME
EDUCATION AND LIFELONG LEARNING
investing in knowledge society
MINISTRY OF EDUCATION & RELIGIOUS AFFAIRS
MANAGING AUTHORITY

NSRF 2007-2013
programme for development
EUROPEAN SOCIAL FUND

Co- financed by Greece and the European Union

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TrAMS
Trace Analysis and Mass Spectrometry Group

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ntho@chem.uoa.gr