



5th EuCheMS Chemistry Congress

August 31 - September 4, 2014
Istanbul / TURKEY
WOW Convention Center Istanbul



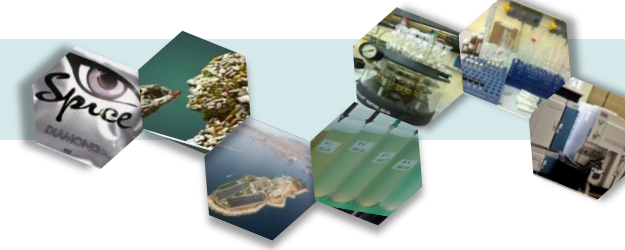
National and Kapodistrian
UNIVERSITY OF ATHENS
Faculty of Chemistry

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

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



Outline



Introduction

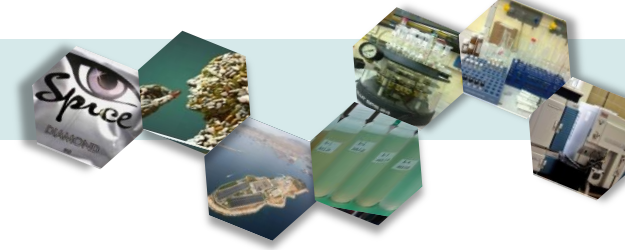
- **The New Designer Psychotropic Drugs**
- **Their occurrence in the aquatic environment**

Analytical methodology

Application in real wastewater samples

Conclusions

Introduction



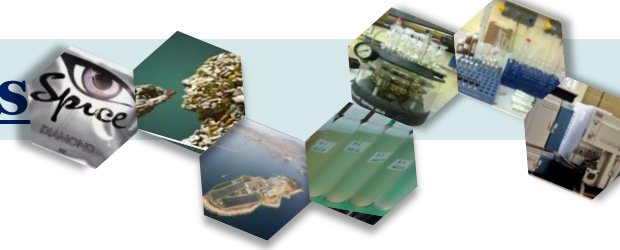
● **Designer Drugs:**

- Synthetically changed natural substances
- Completely designed molecular structures
- Psychotropic effects

● **New Designer Psychotropic Drugs:**

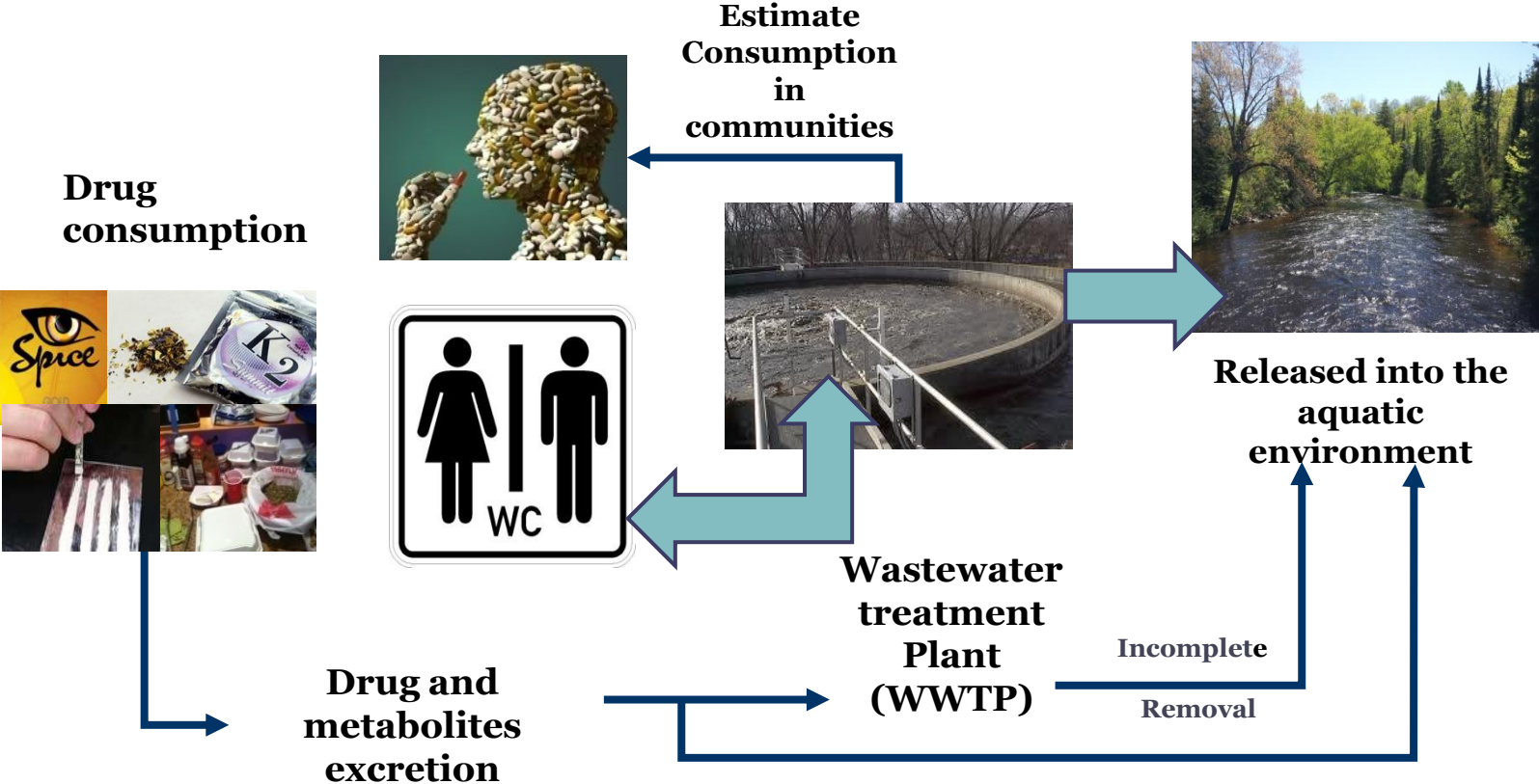
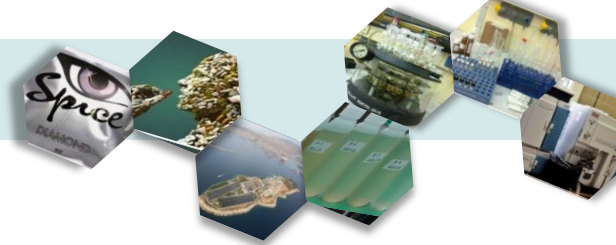
- Drug alternatives
- Optimized effects of already existing drugs
 - *Higher binding affinity with CB1, greater potency and adverse effects and longer duration of action*
- Not covered in most countries by controlled substance statutes
 - *avoid detection and legal consequences*
- “Spice” drugs (smoking mixtures, herbal mixtures), bath salts, tablets form, on the recreational drug use market

A new class of emerging contaminants

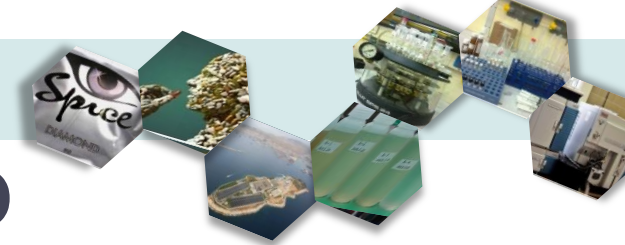


- **Not currently covered** by existing **water quality regulations**
- **Rarely investigated**
- **Newly identified** or previously unrecognized
- **Lack of environmental data**
- **Lack of analytical methods** in wastewater
- Large volume of **consumption** and **production**
- **Potential threats**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

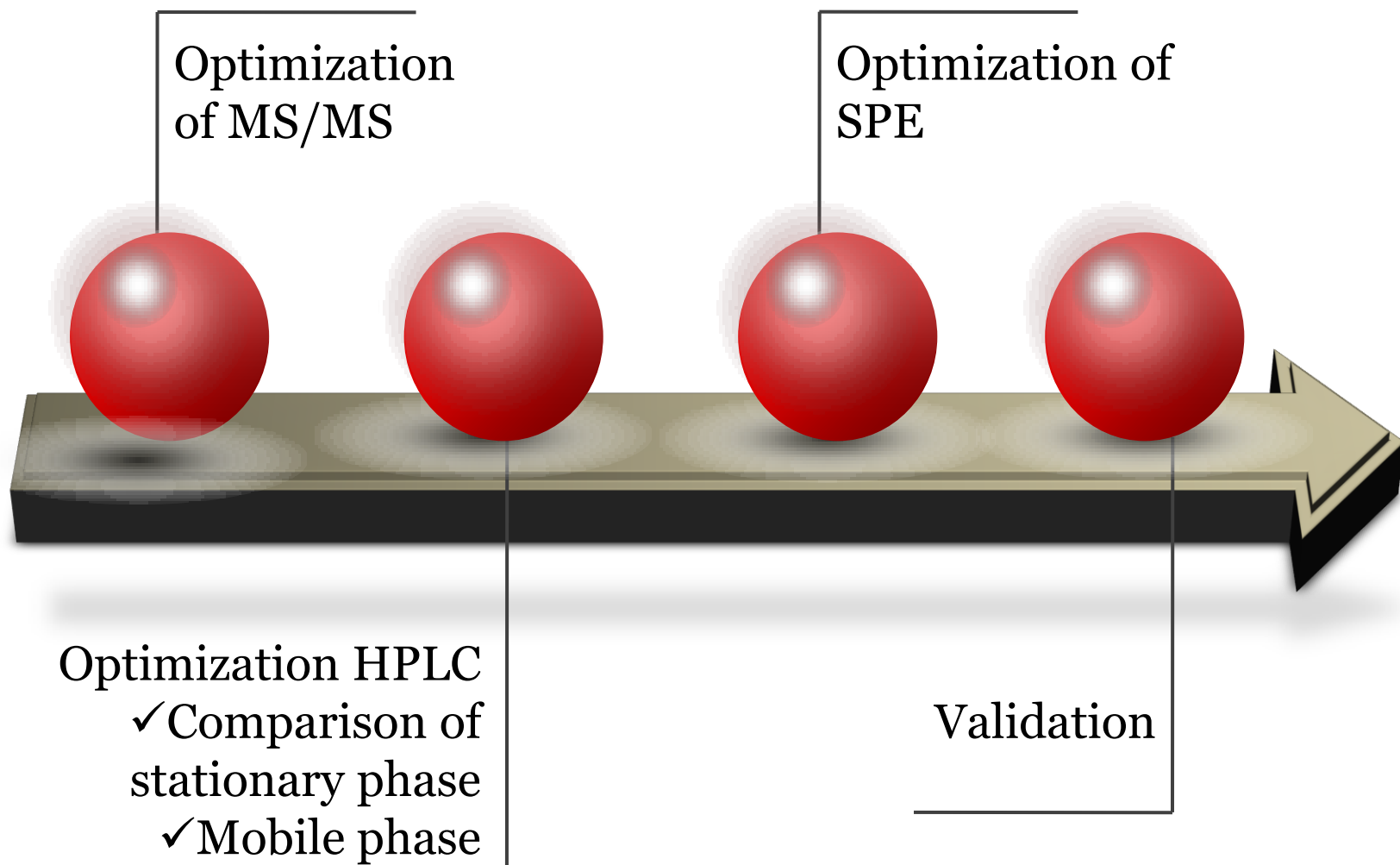
Entry to the aquatic environment



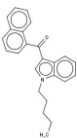
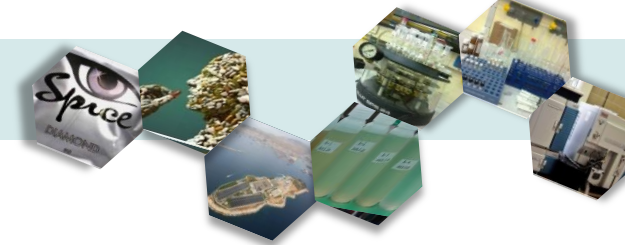
Analytical Methodology



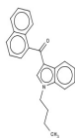
LC- (QqQ) MS/MS –ESI (+/-) Thermo Scientific Quantum Access



Target Analytes



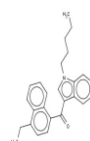
JWH-018
logP=6.51



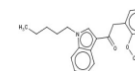
JWH-073
logP=6.07



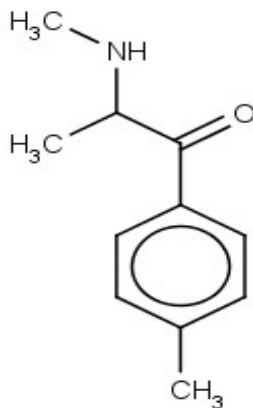
JWH-122
logP=7.03



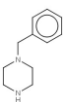
JWH-210
logP=7.47



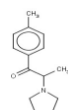
JWH-250
logP=5.30



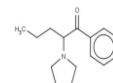
Mephedrone
logP=2.12



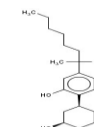
BZP
logP=1.38



MPPP
logP=2.91

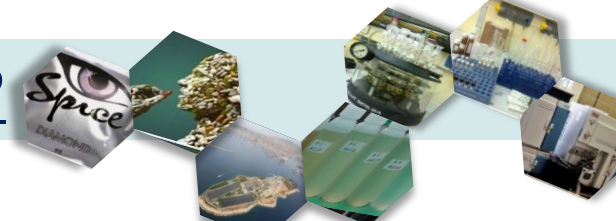


a-PVP
logP=3.36



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)

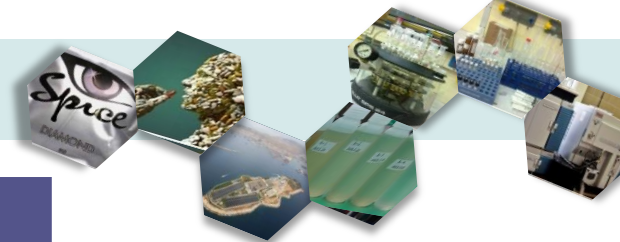
3

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

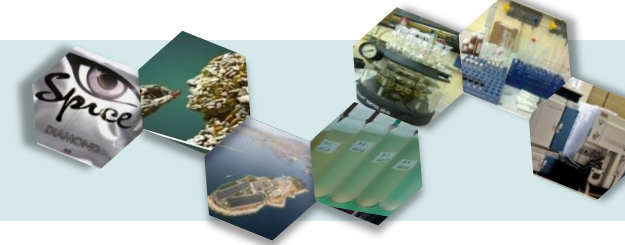
Probe:
C (0.5/1.5)
Spray Voltage:
3500 V
Sheath gas:
30 a.u.
Aux gas:
10 a.u.
Temp:
270°C

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)

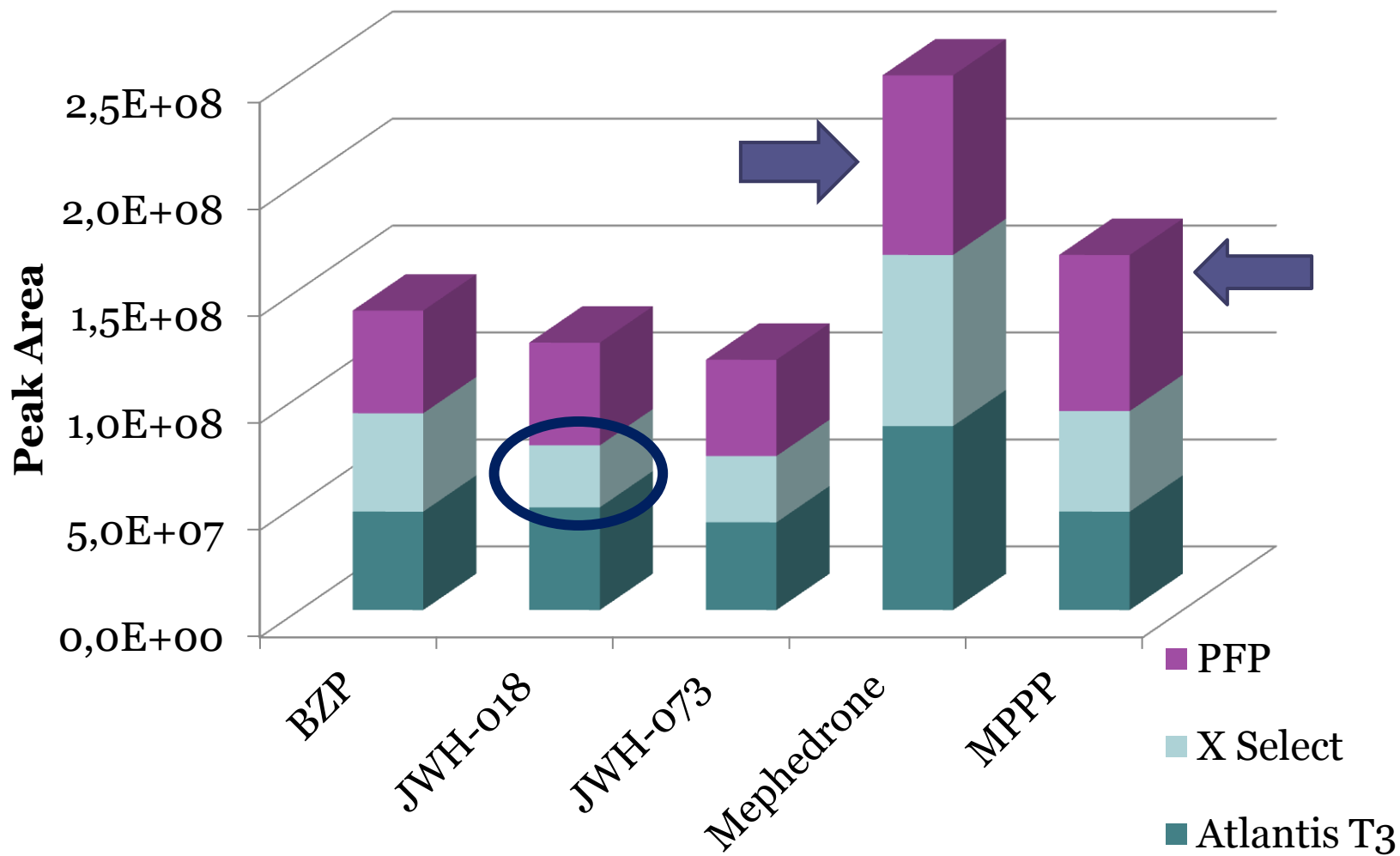
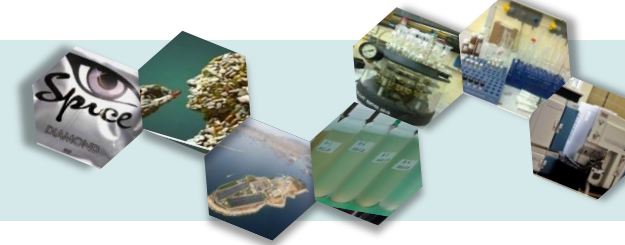
Pentafluorophenyl (PFP)

(50 mm x 2.1 mm, 1.7 μm , Phenomenex)

- ✓ Peak area & shape
- ✓ Retention time
- ✓ Resolution

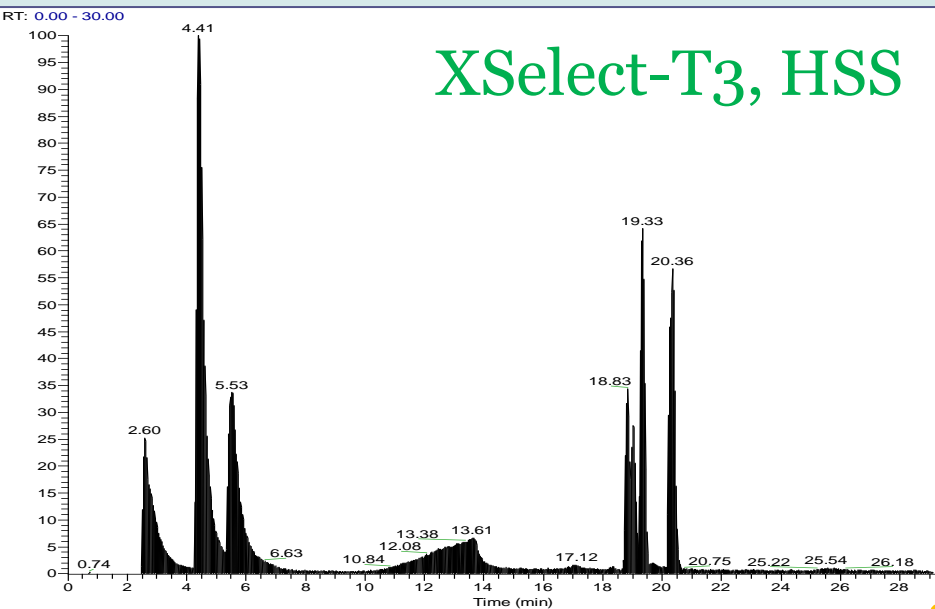
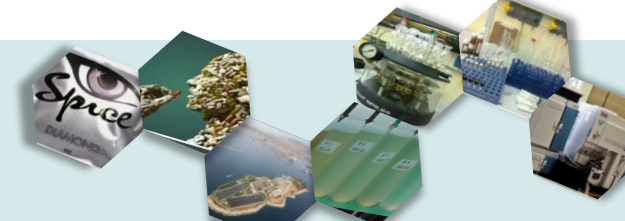
Optimization of HPLC

Comparison of Stationary phase



Optimization of HPLC

Comparison of Stationary phase

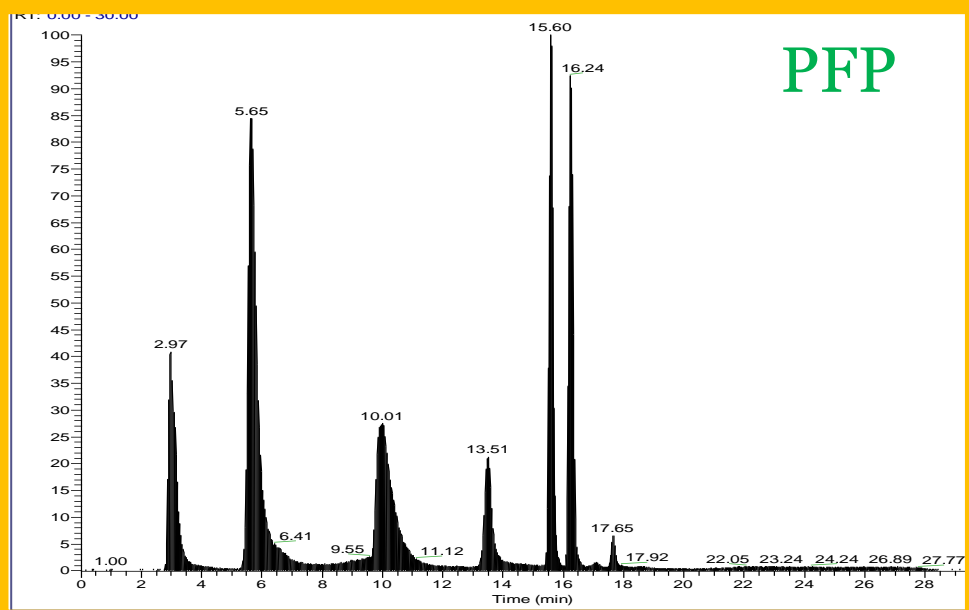
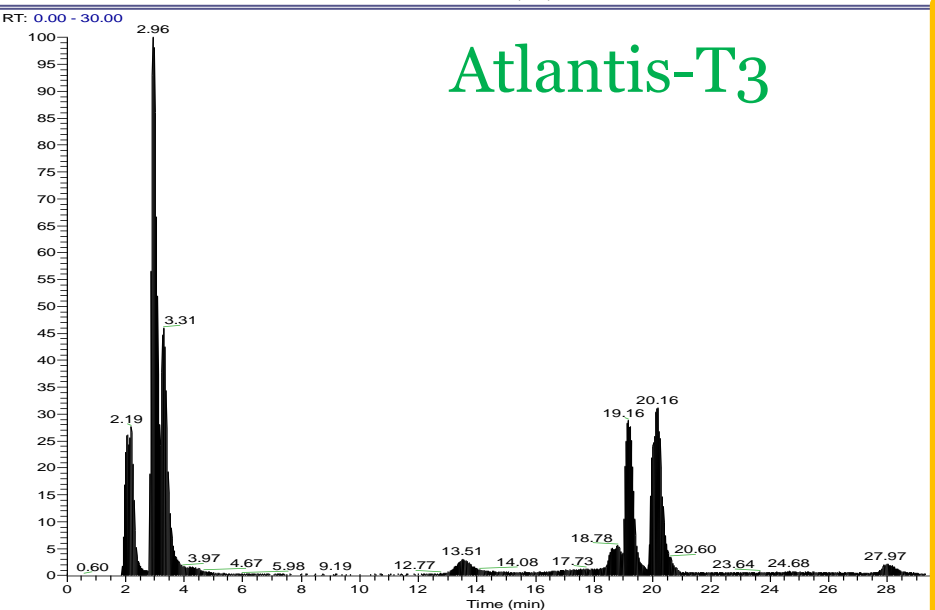


❑ **C18 columns:** presented peak asymmetry (polar compounds) and do not provide good separation.

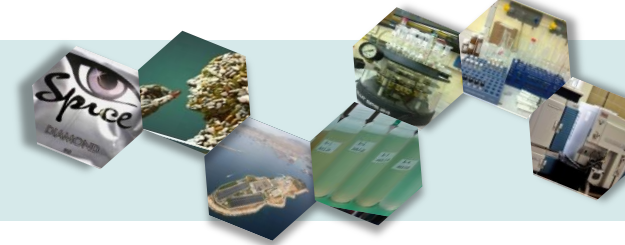
❑ **PFP:**

1. **Higher sensitivity and resolution**

2. More **symmetric peaks** for polar compounds containing $-NH_2$ or $-NH-$ groups



Optimization of HPLC Mobile phase~ gradient programme



Positive Ionization mode

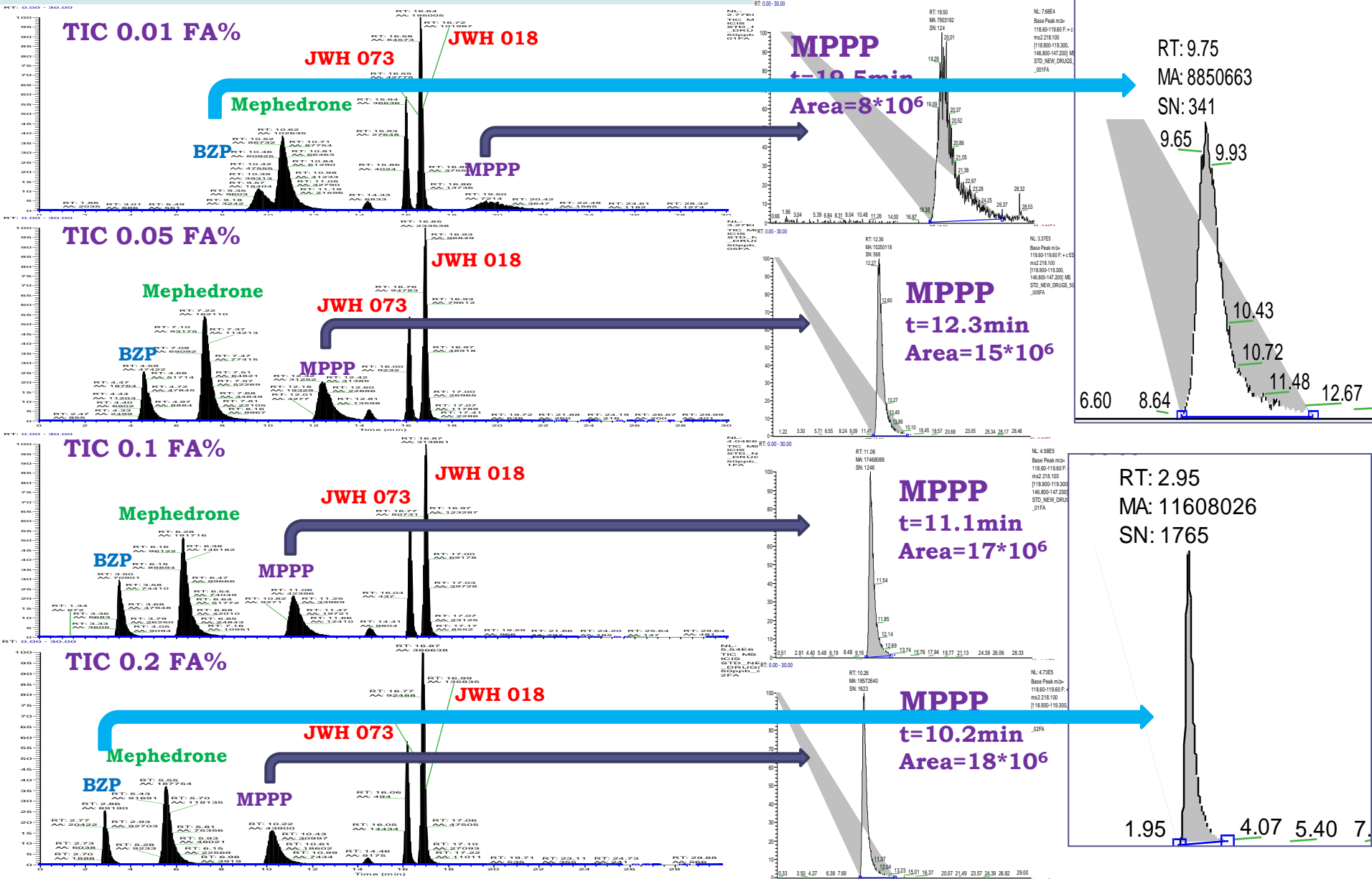
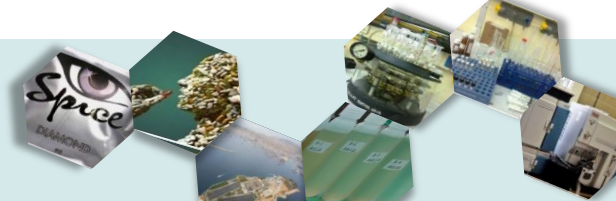
Positive (min)	H ₂ O (0.2% formic acid) %	MeOH %	μL/min
0.00	60	40	100
3.00	60	40	150
15.00	0	100	100
25.00	0	100	100
25.50	60	40	100
30.00	60	40	100

CP47, 497

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

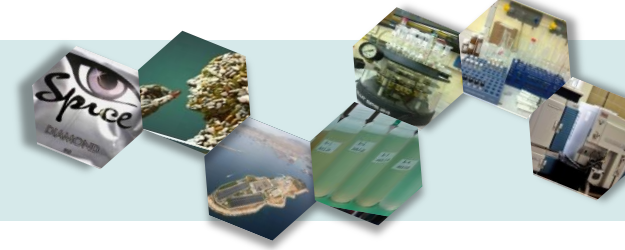
Optimization of HPLC

Effect of the organic modifier (F.A.)



Optimization of SPE

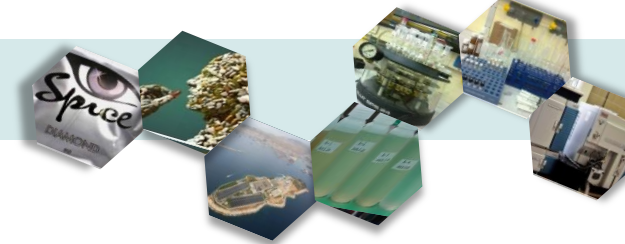
Comparison of different cartridges



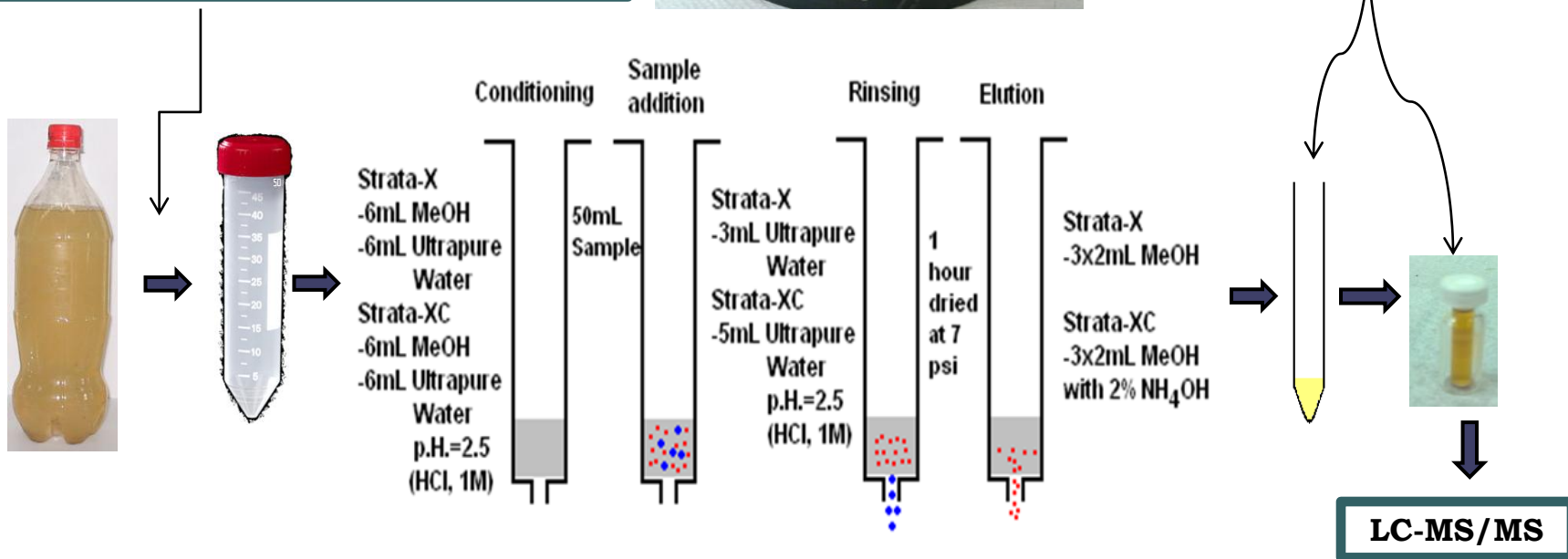
1. Strata X (Phenomenex)
2. Strata XC (Phenomenex)
3. Isolute C18 (EC) (Biotage)
4. Double SPE (C18 (EC)-
Strata XC)
5. ATOLL XC (Interchim)
6. Lichrolut EN (bottom)- C18
(top) (Merck)
7. Lichrolut EN (Merck)
8. PolyClean 2H (Interchim)
9. Lichrolute TSC (Merck)

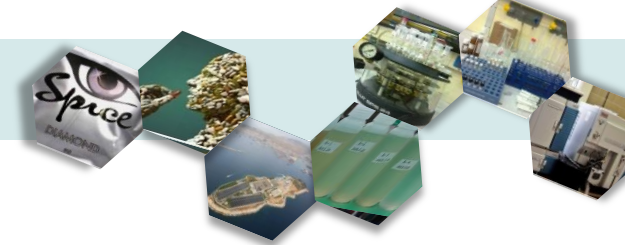
Optimization of SPE

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

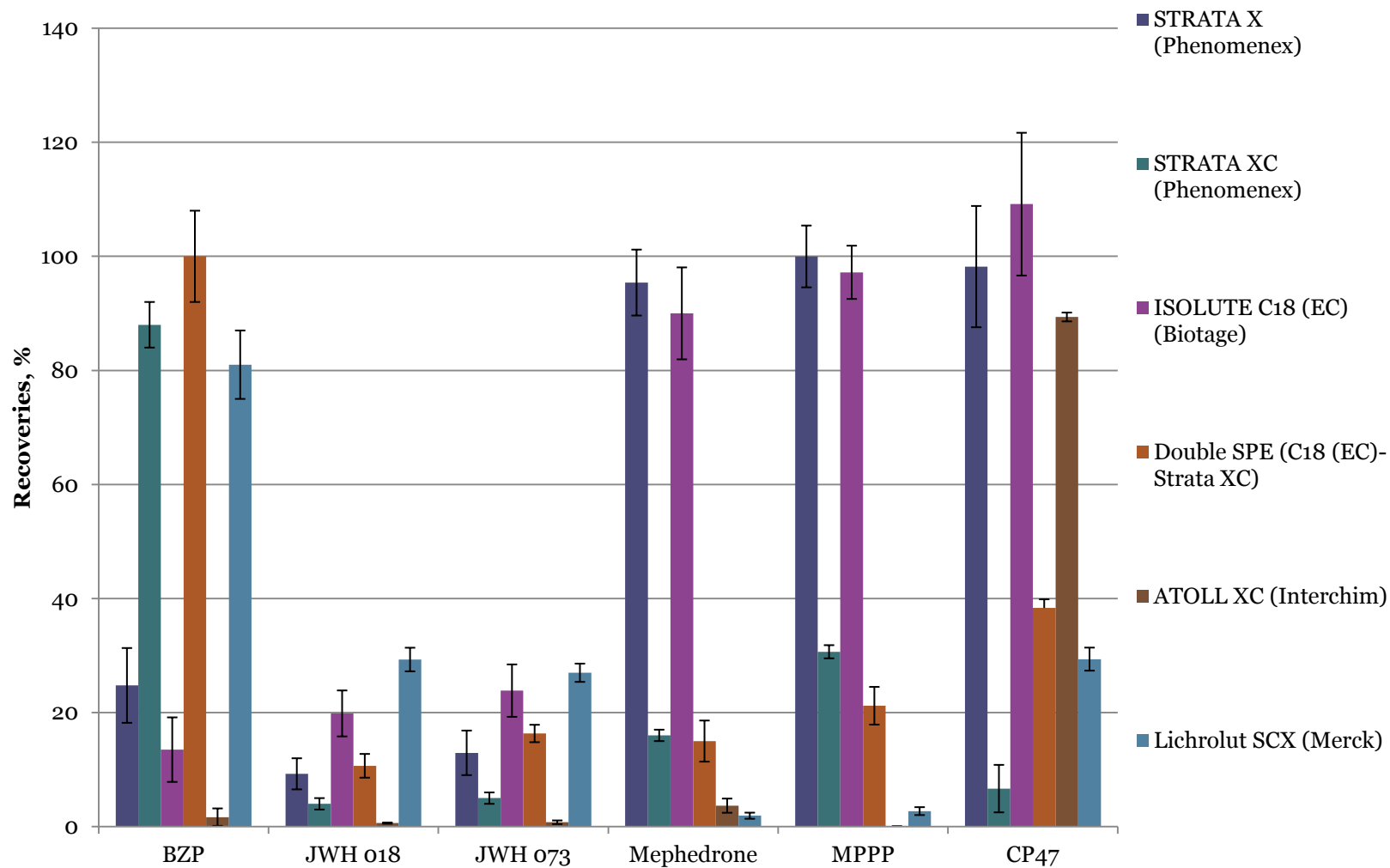


Reconstitution :
500 μ L of 60% MeOH
and 40% ultra
purified water with
0.05% v/v formic acid

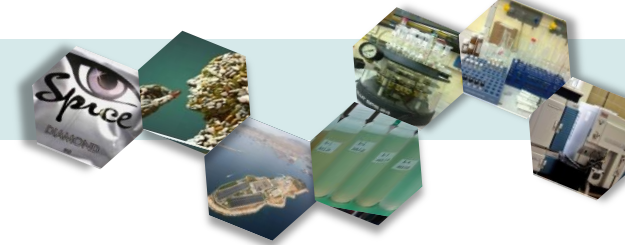




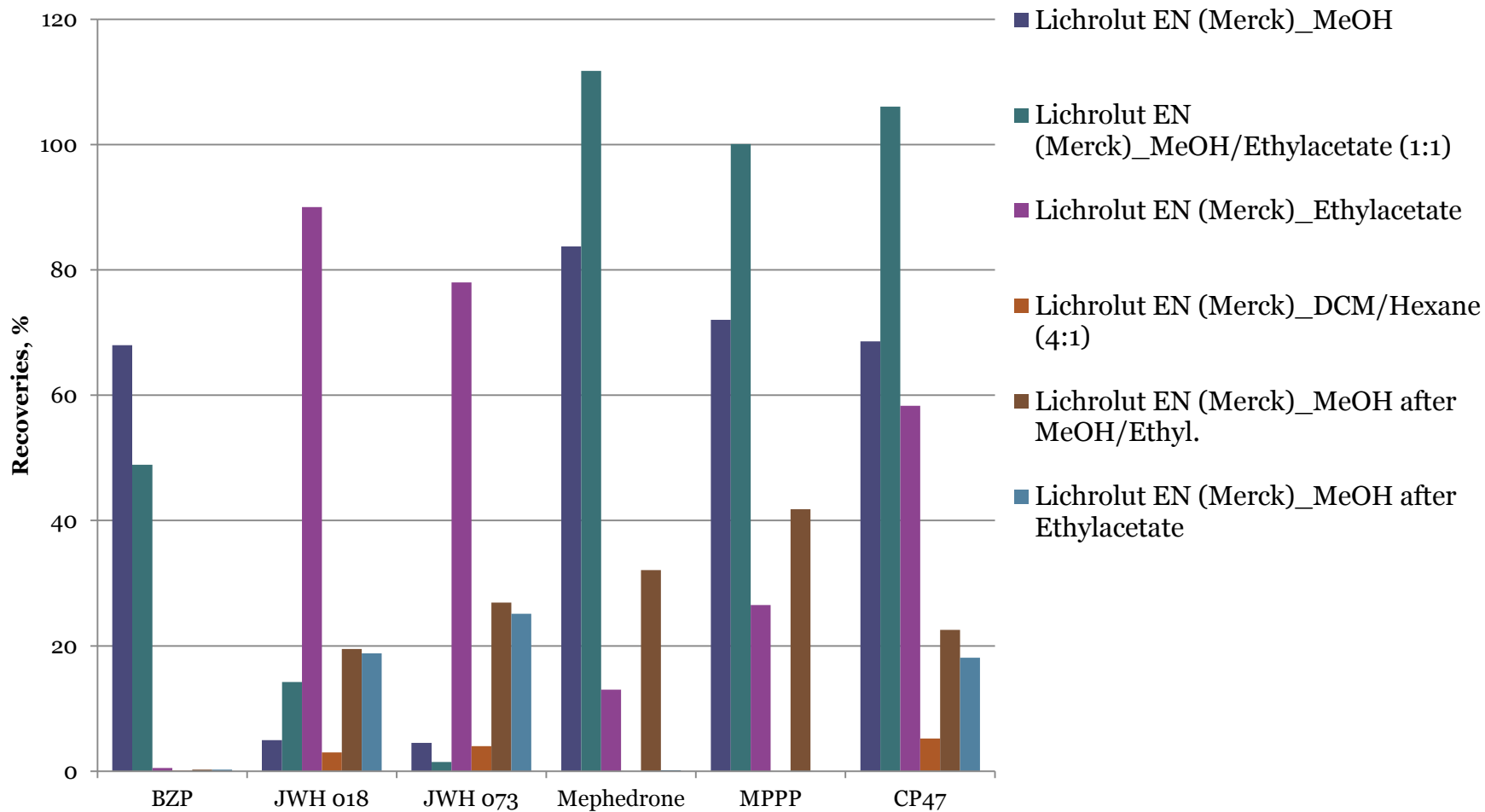
Cartridges-Recoveries, Graph 1



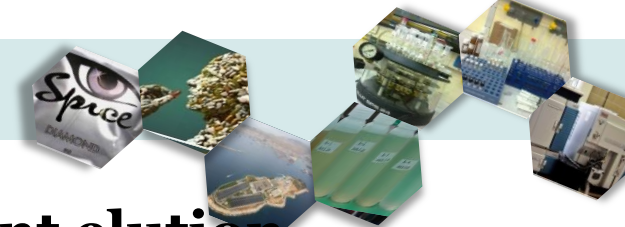
Optimization of SPE



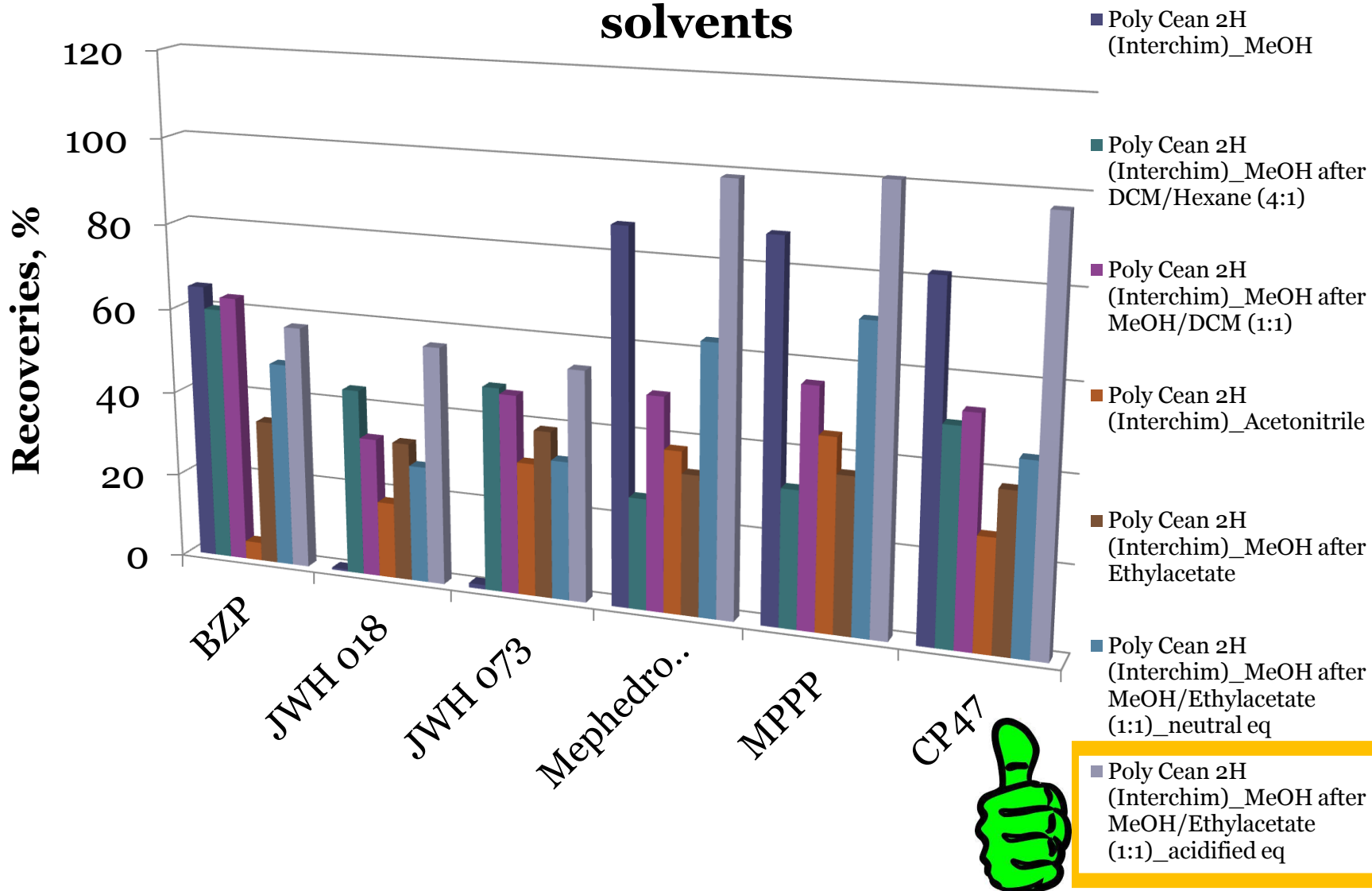
Cartridge EN - Different elution solvents



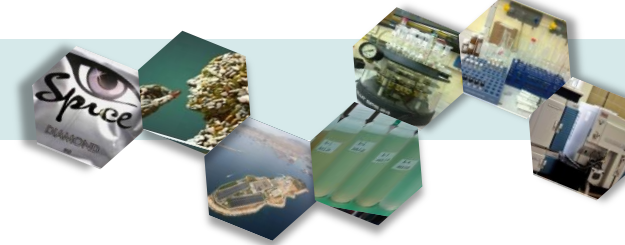
Optimization of SPE



Cartridge PolyClean 2H - Different elution solvents

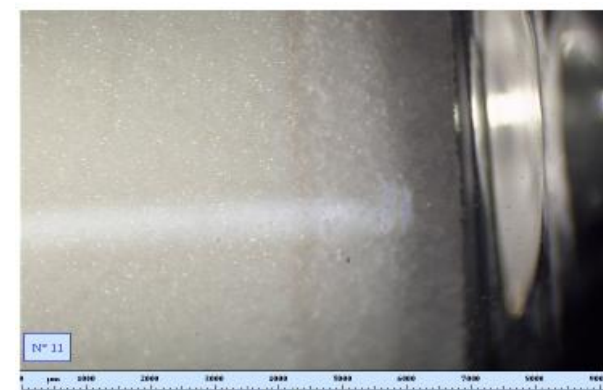


Optimization of SPE~FINAL



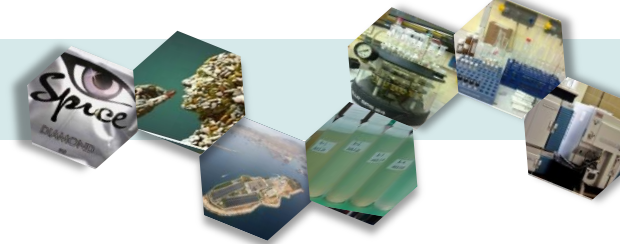
	SOLVENTS
Conditioning	6 ml methanol (under gravity)
Equilibration	6 ml H ₂ O (pH=2.5, HCl) (under gravity)
Load	50 ml sample
Wash	5 ml H ₂ O (pH=2.5, HCl)
Vacuum	30 min
Elute	4 mL (2 x 2 mL MeOH) 8 mL (4 x 2 mL) Methanol/ Ethyl acetate (50:50)

PolyClean 2H
Spherical Pure Mixed
hydrophilic / hydrophobic
Polymer
200mg (size)



PolyClean™ 2H

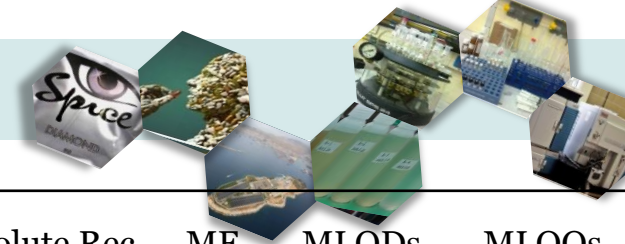
Method VALIDATION



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

	Linearity range ($\mu\text{g/L}$)	R^2	ILODs ($\mu\text{g/L}$)	ILOQs ($\mu\text{g/L}$)
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)
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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

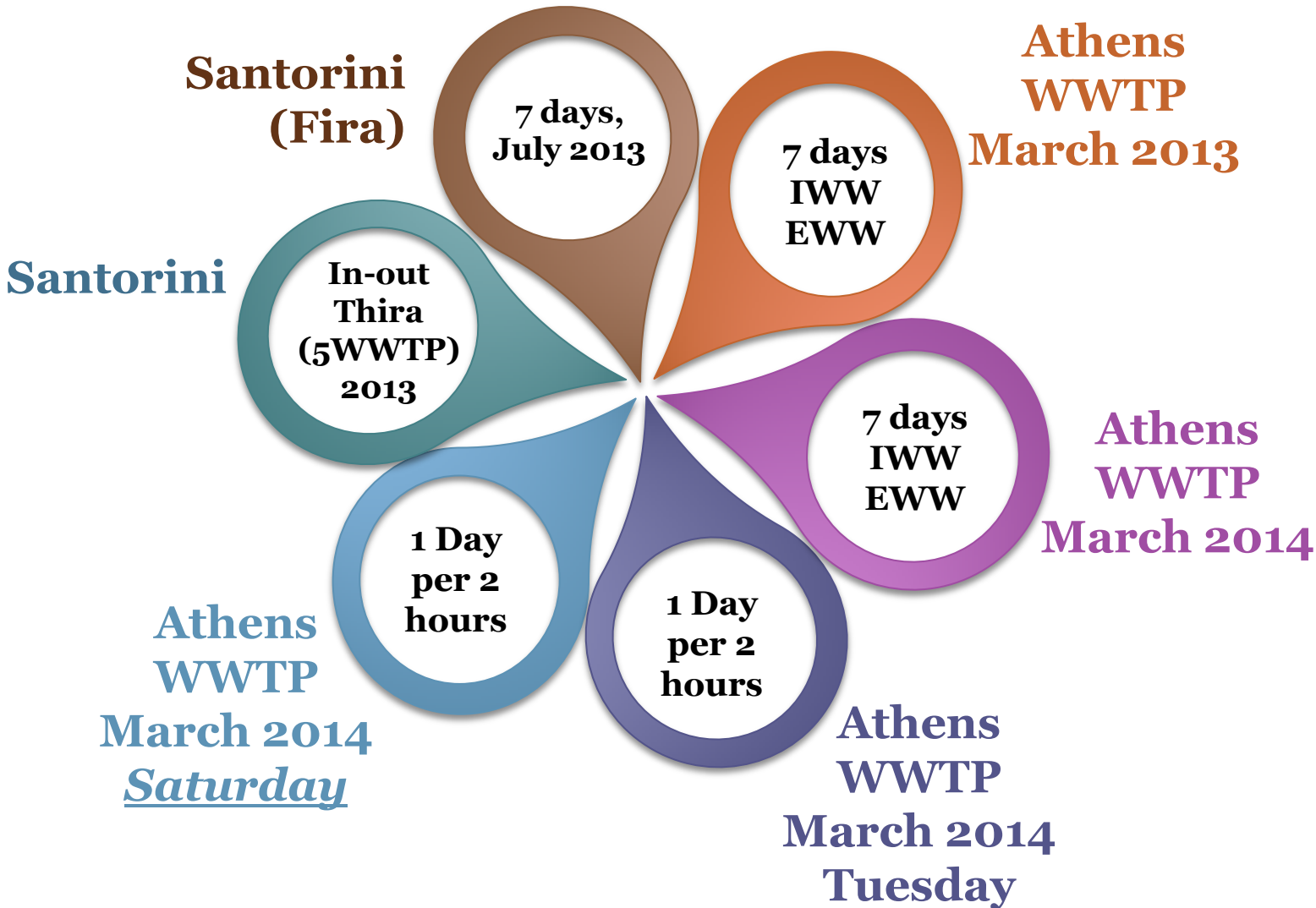
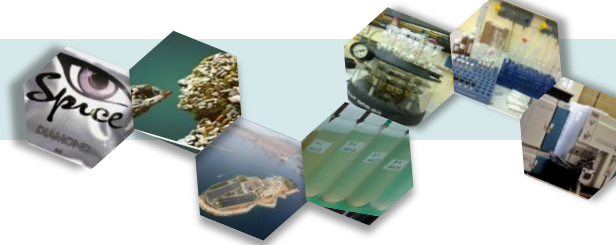
	Matrix	Recovery	MLOD	MLOQ
BZP				11.3
MPPP				1.5
a-PVP				2.3
CP47,497	Effluent wastewater	50 – 102 %	0.1 – 24 ng/L	0.4 – 74 ng/L
				111

Effluent wastewater

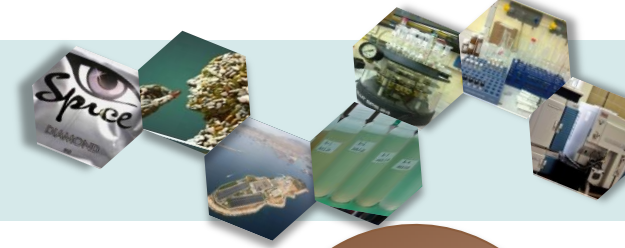
JWH-018				2.0
JWH-073				3.8
JWH-210				1.5

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

Application in real wastewater



Wastewater Samples from WWTP Santorini



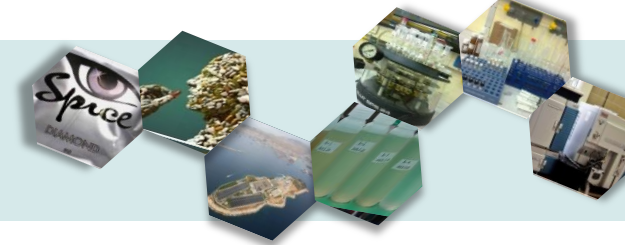
**Santorini
(Fira)
Grab samples
22/07/2013 - 28/07/2013**



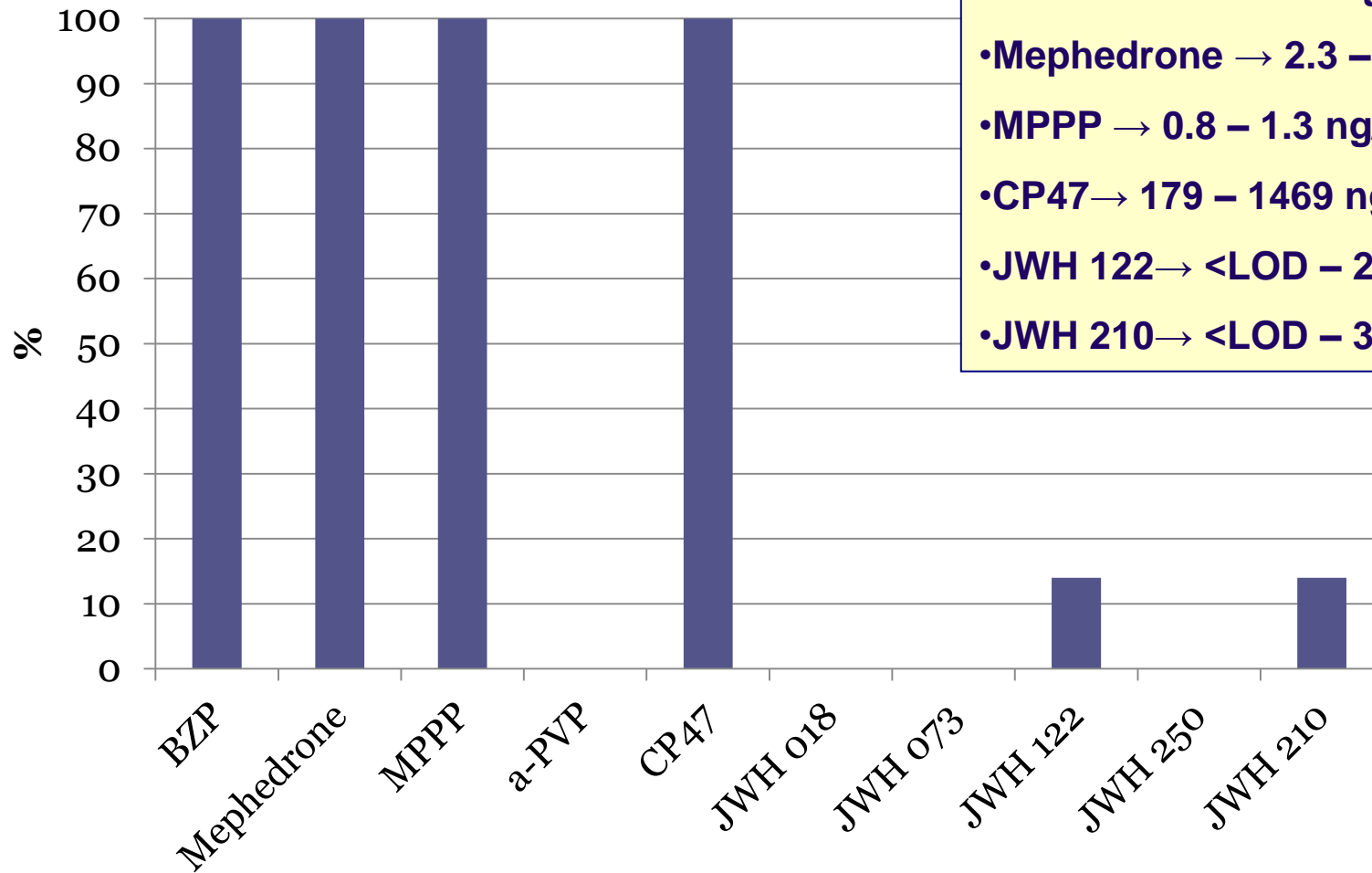
- ✓ **Population: 10500 inhabitants**
- ✓ **Flow rate: 1500 m³/day**



Wastewater Samples from WWTP Santorini



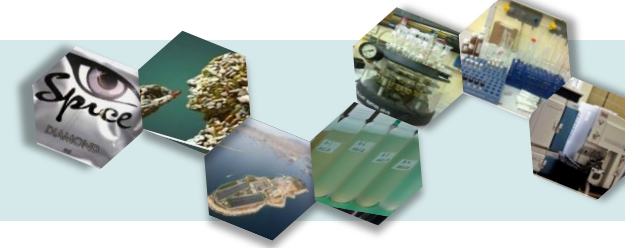
Frequency of detection



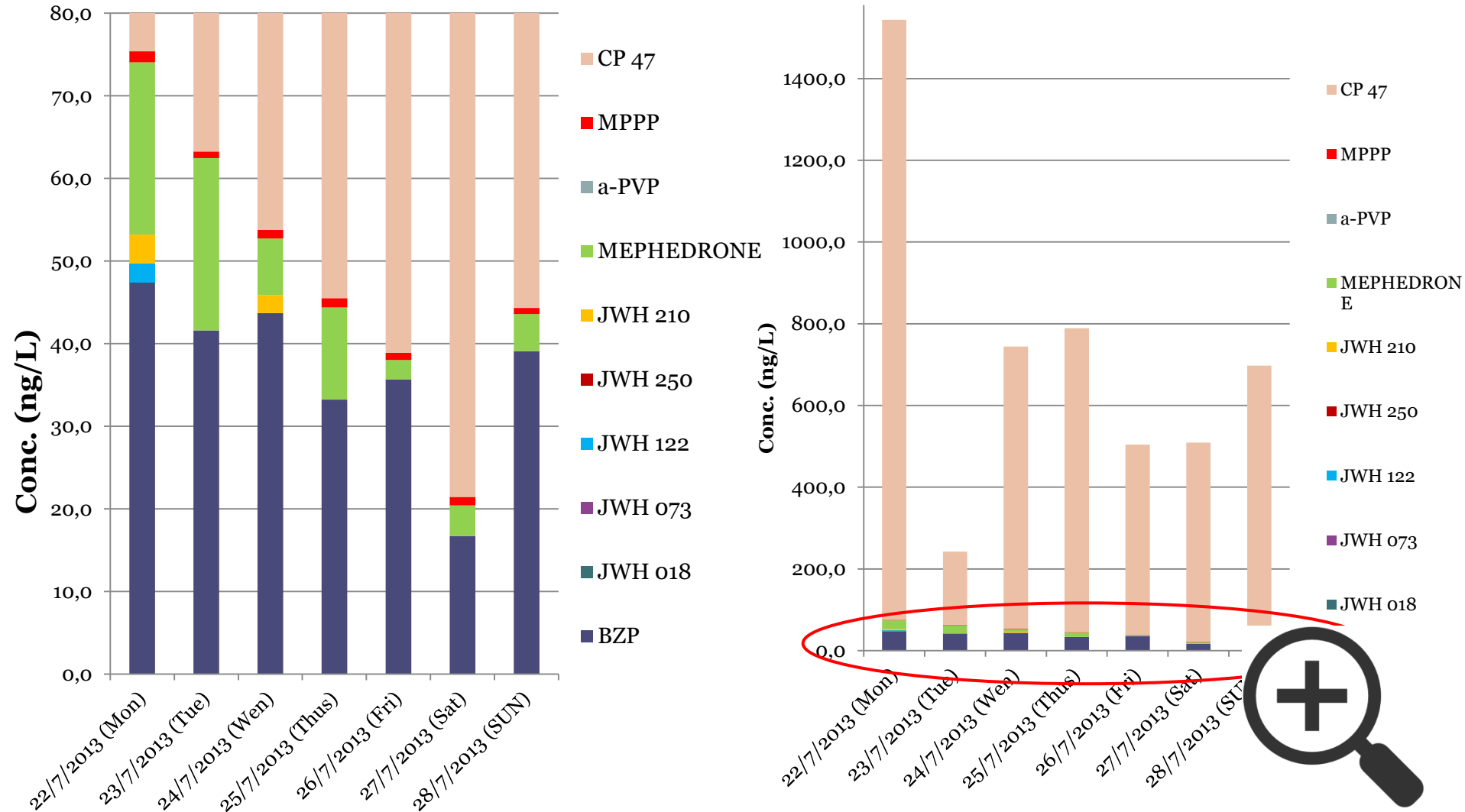
RANGES:

- 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

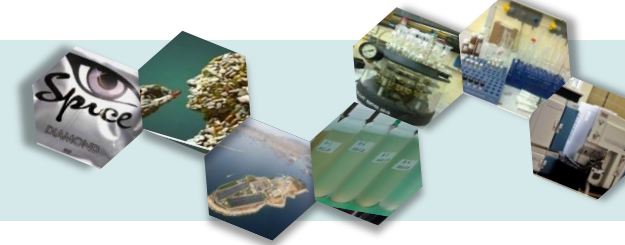
Wastewater Samples from WWTP Santorini



Daily variations of the levels of concentration in IWW



Wastewater Samples from WWTP Santorini

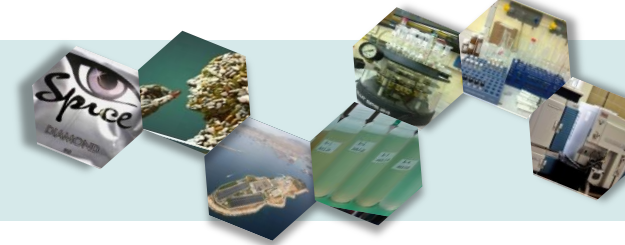


**In-out
Thira
(5WWTP)
2013**

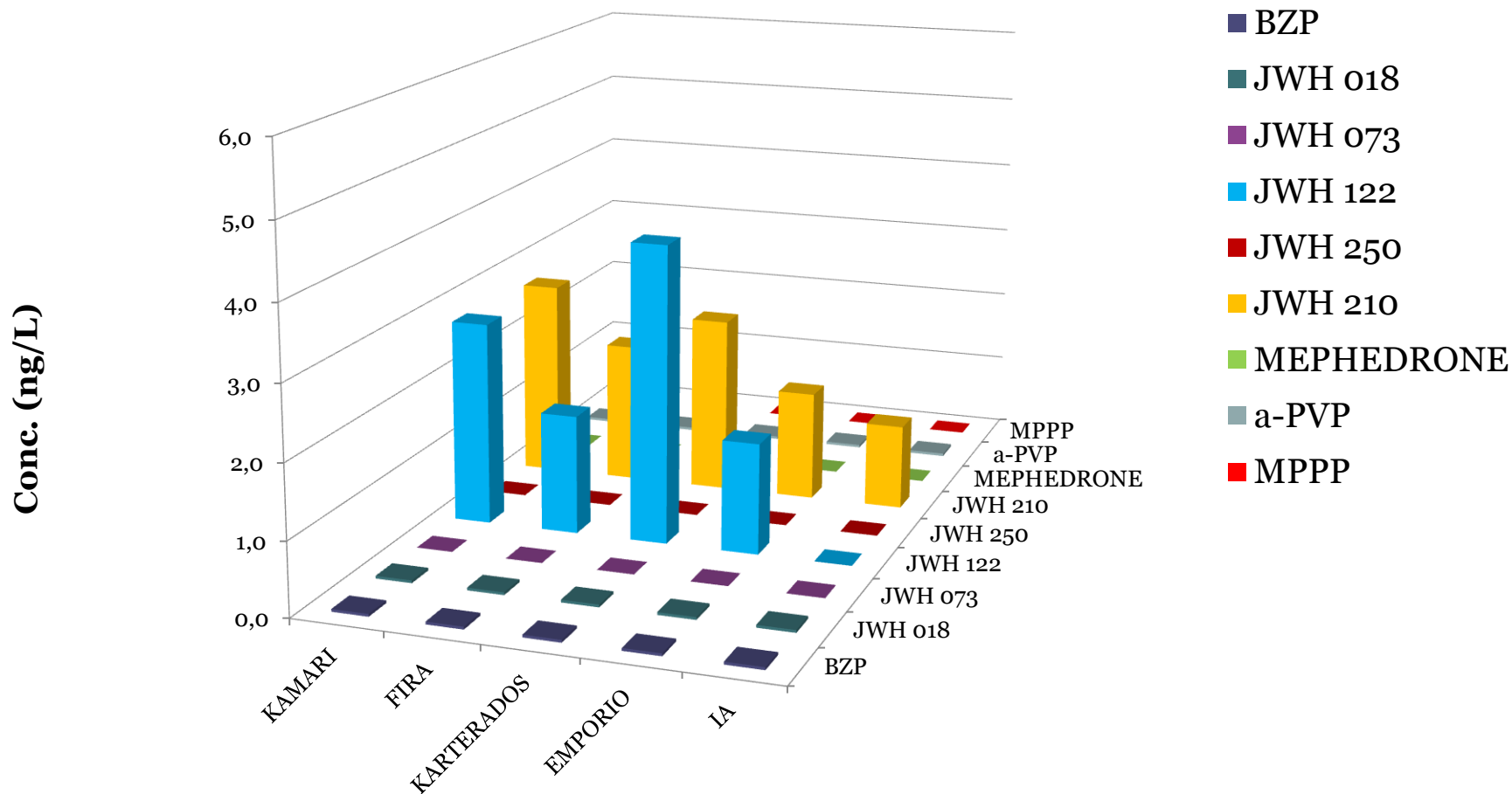
**Santorini (grab samples)
5 influent
5 effluent**

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

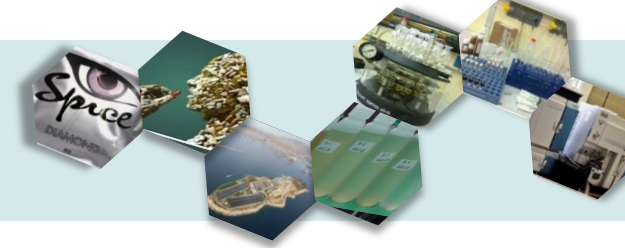
Wastewater Samples from WWTP Santorini



Effluent grab samples



Wastewater Samples from WWTP ATHENS



Two sampling campaigns in the WWTP of Athens, Greece

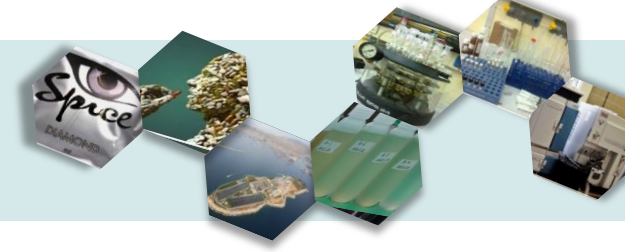
March 2013 (7 consecutive days)

March 2014 (7 consecutive days)

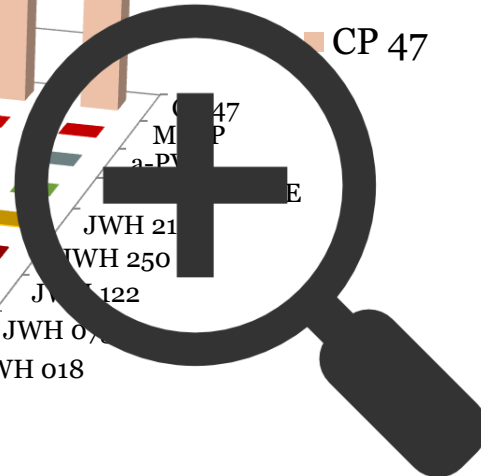
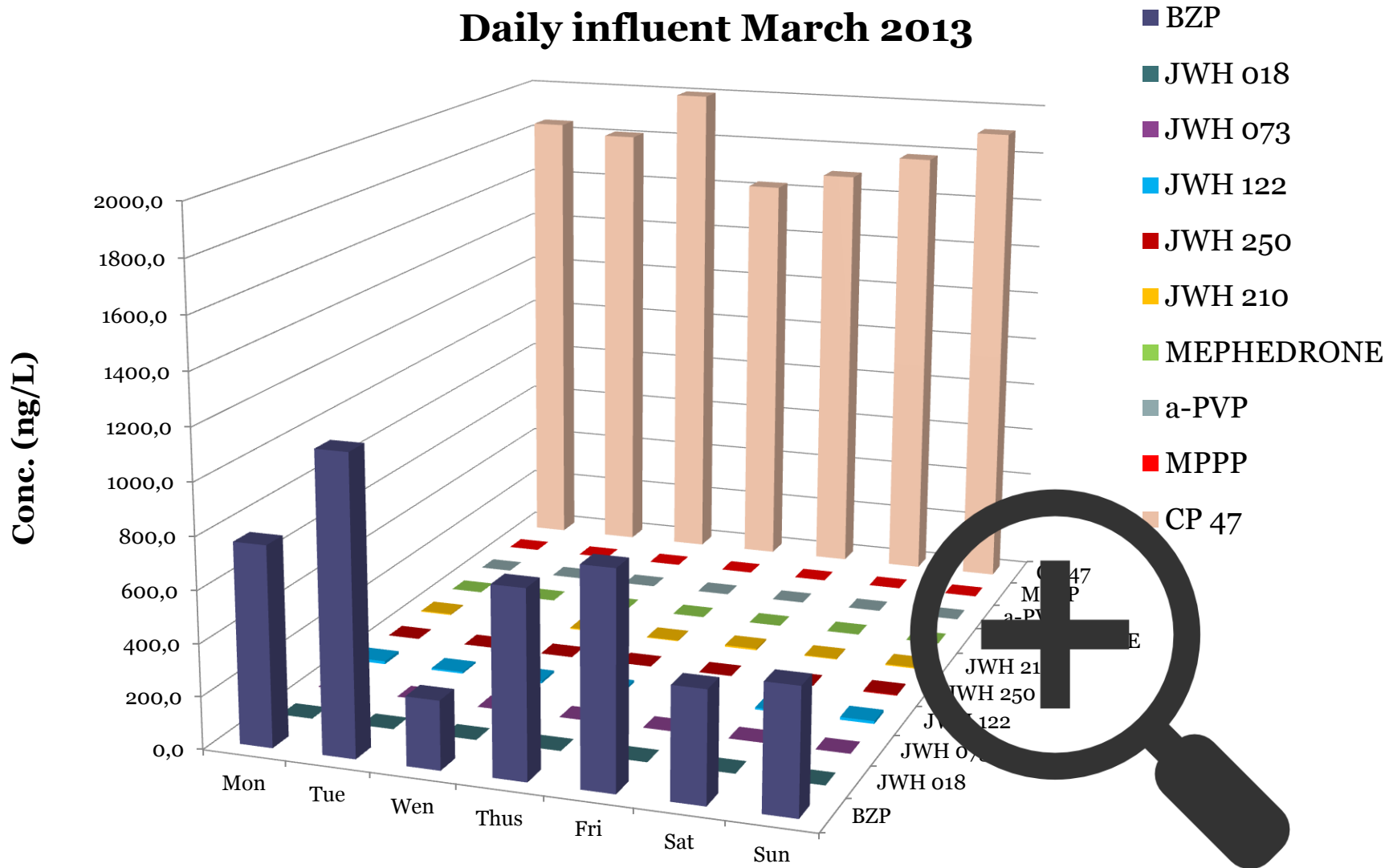
24-h composite flow-proportional samples of influents and effluents



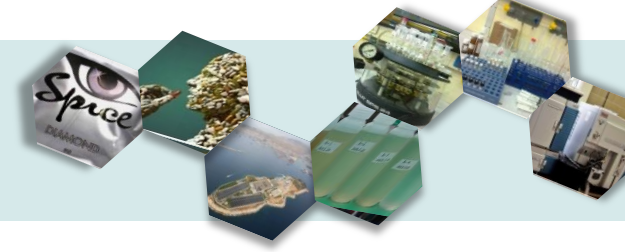
Wastewater Samples from Athens (March 2013)-influent



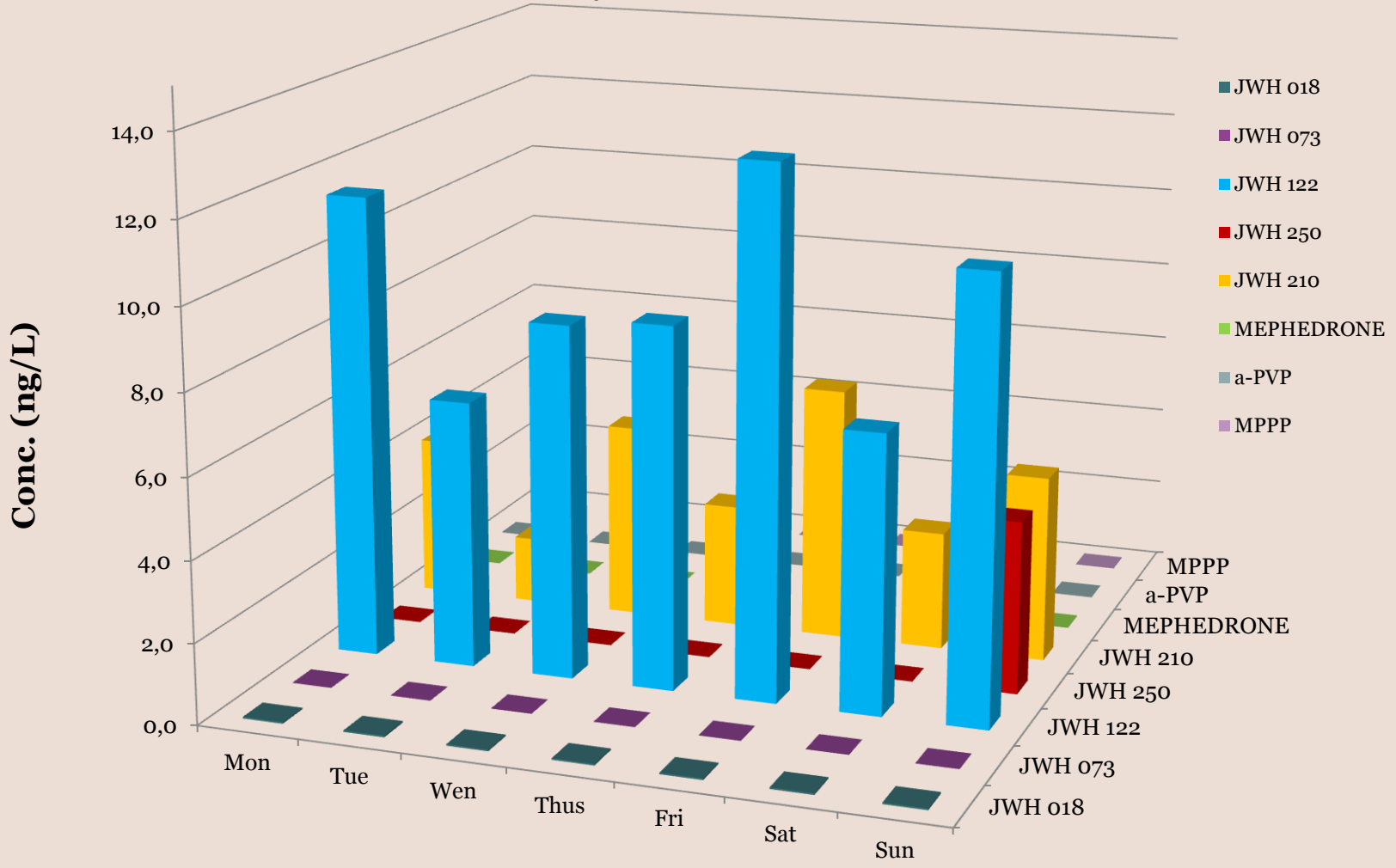
Daily influent March 2013



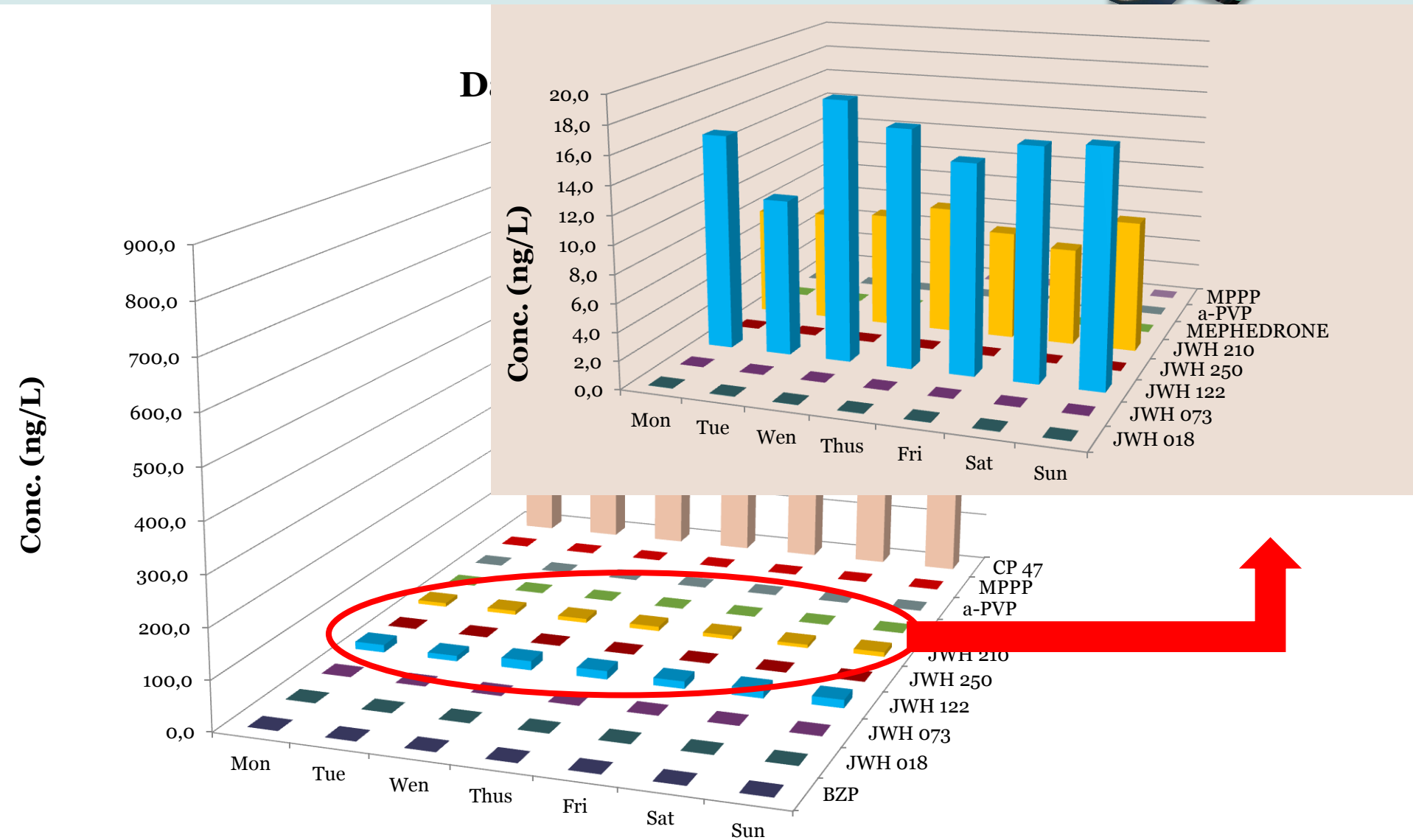
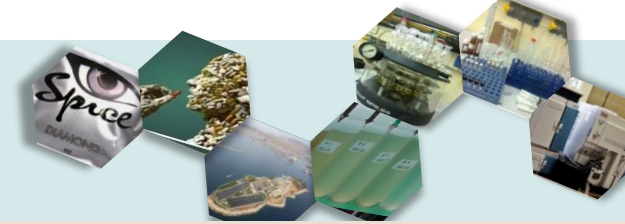
Wastewater Samples from Athens (March 2013)-influent



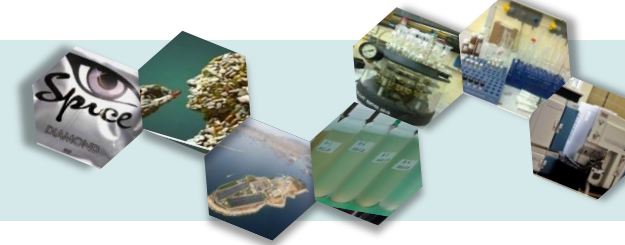
Daily influent March 2013



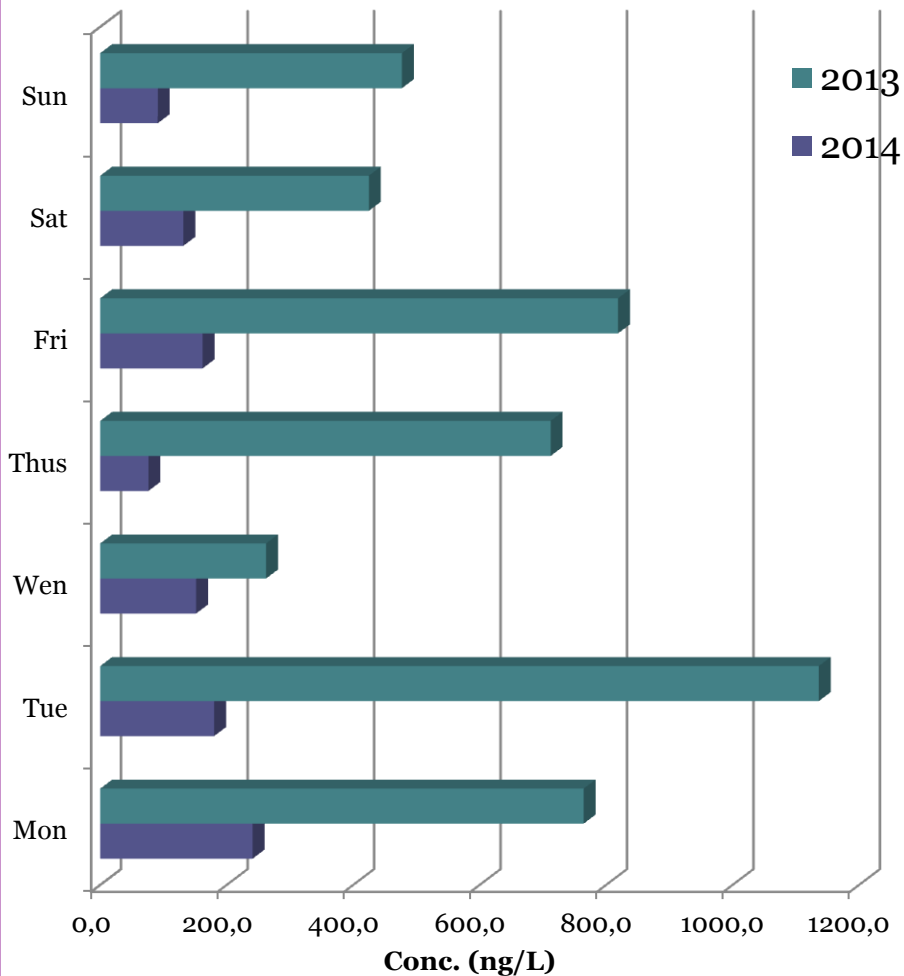
Wastewater Samples from Athens (March 2013)-effluent



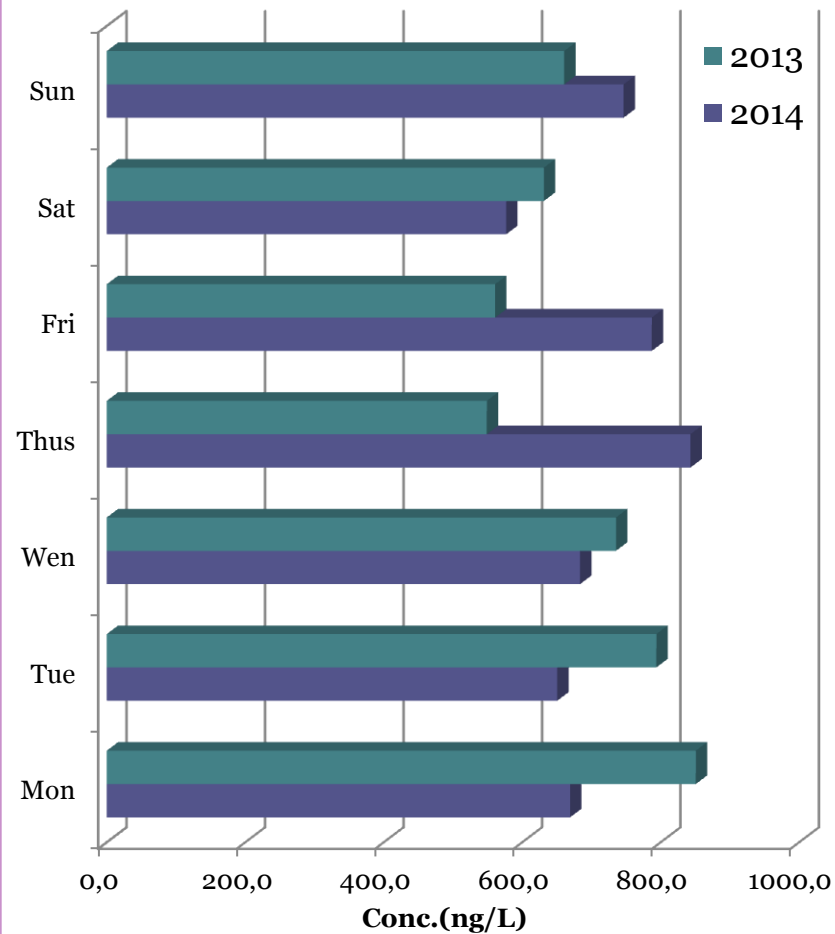
Wastewater Samples from Comparison 2013-2014 (trends)



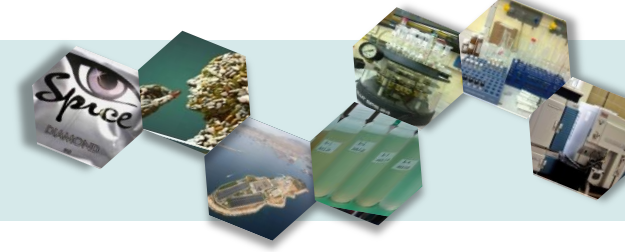
BZP trends



CP47,497 trends



Wastewater Samples from WWTP of Athens

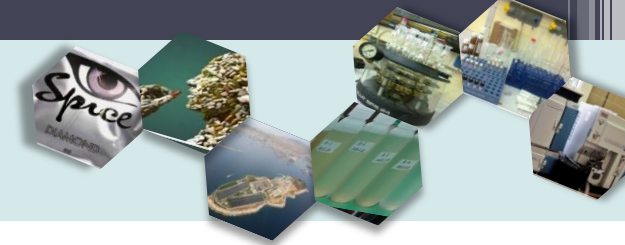


**Athens
WWTP
March 2014
Tuesday**

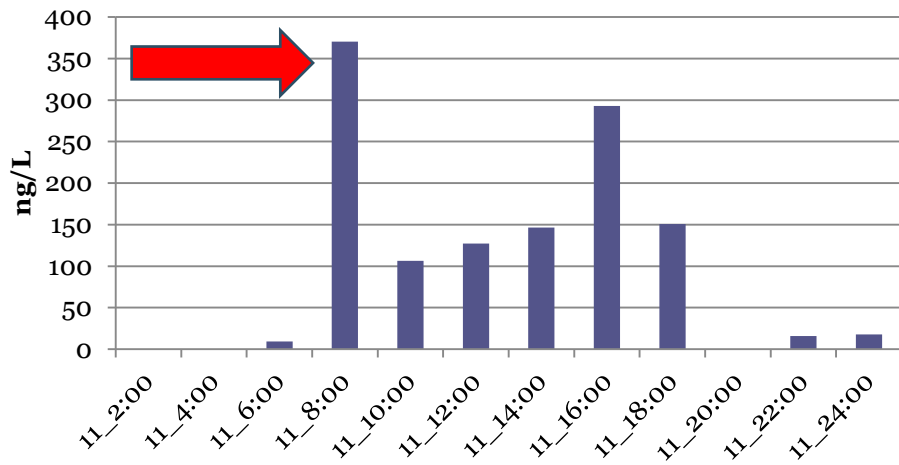


**Athens
WWTP
March 2014
Saturday**

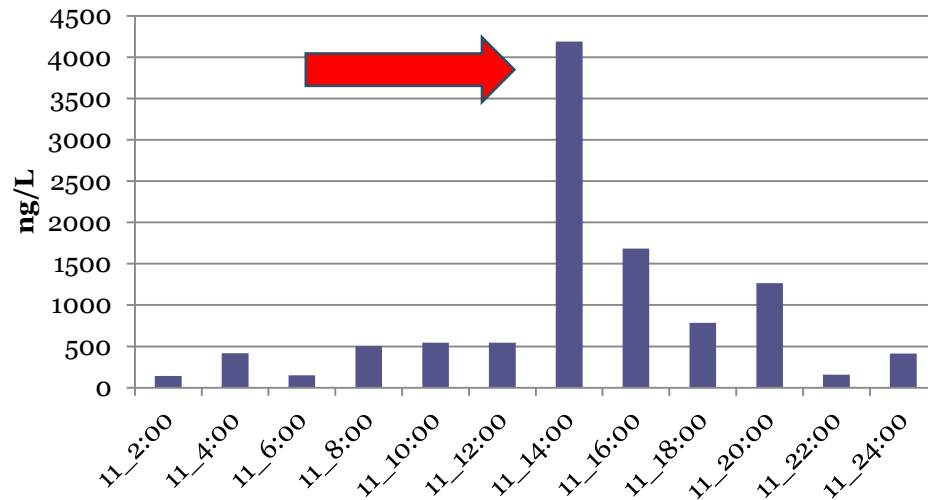
Wastewater Samples from WWTP of Athens



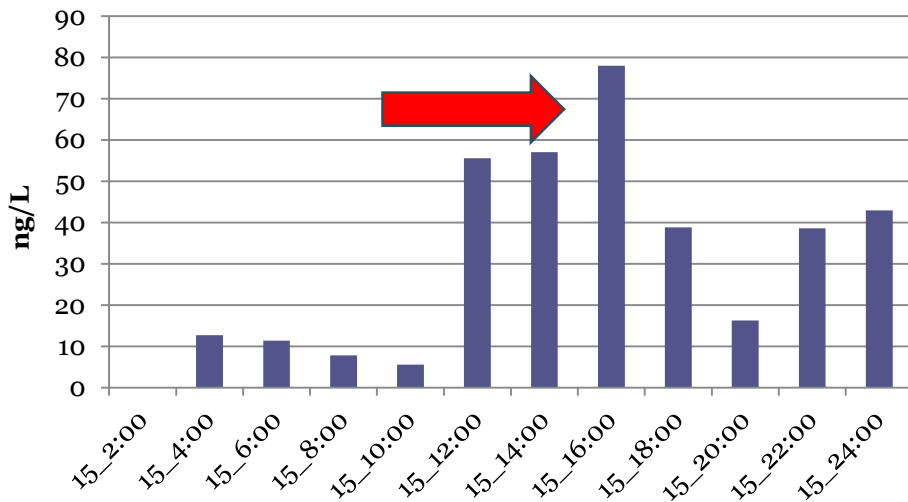
BZP on Tuesday



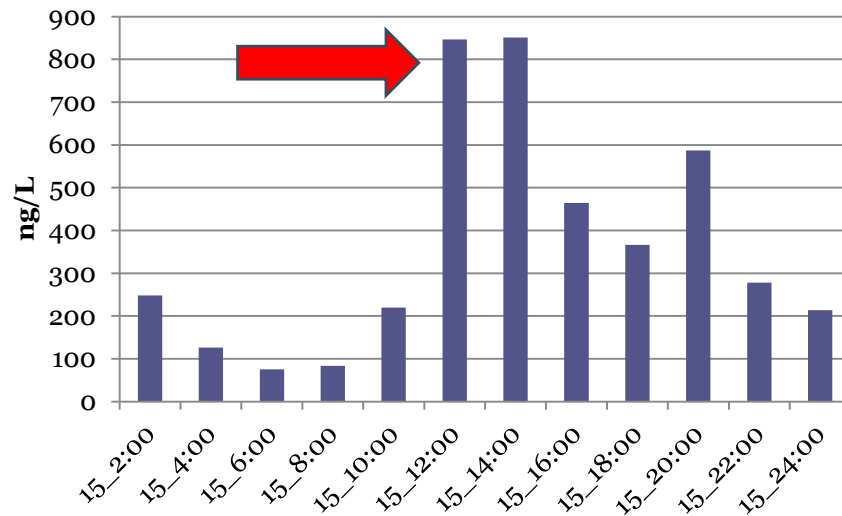
CP47, 497 on Tuesday



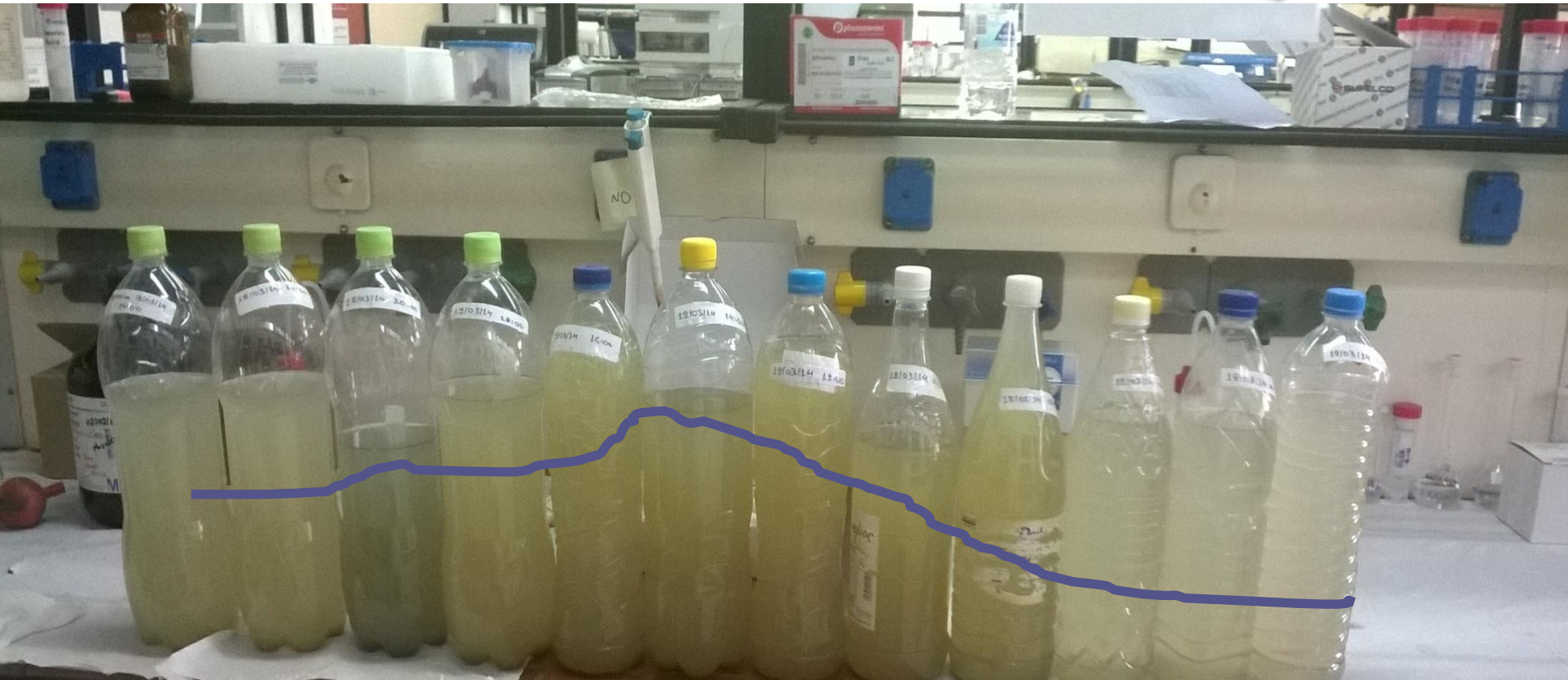
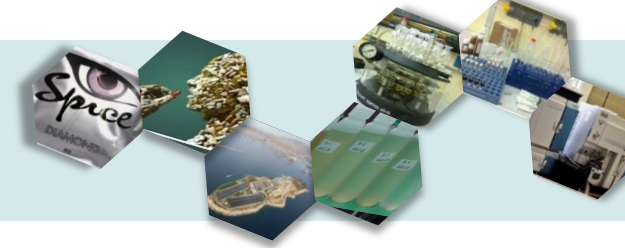
BZP on Saturday



CP47, 497 on Saturday



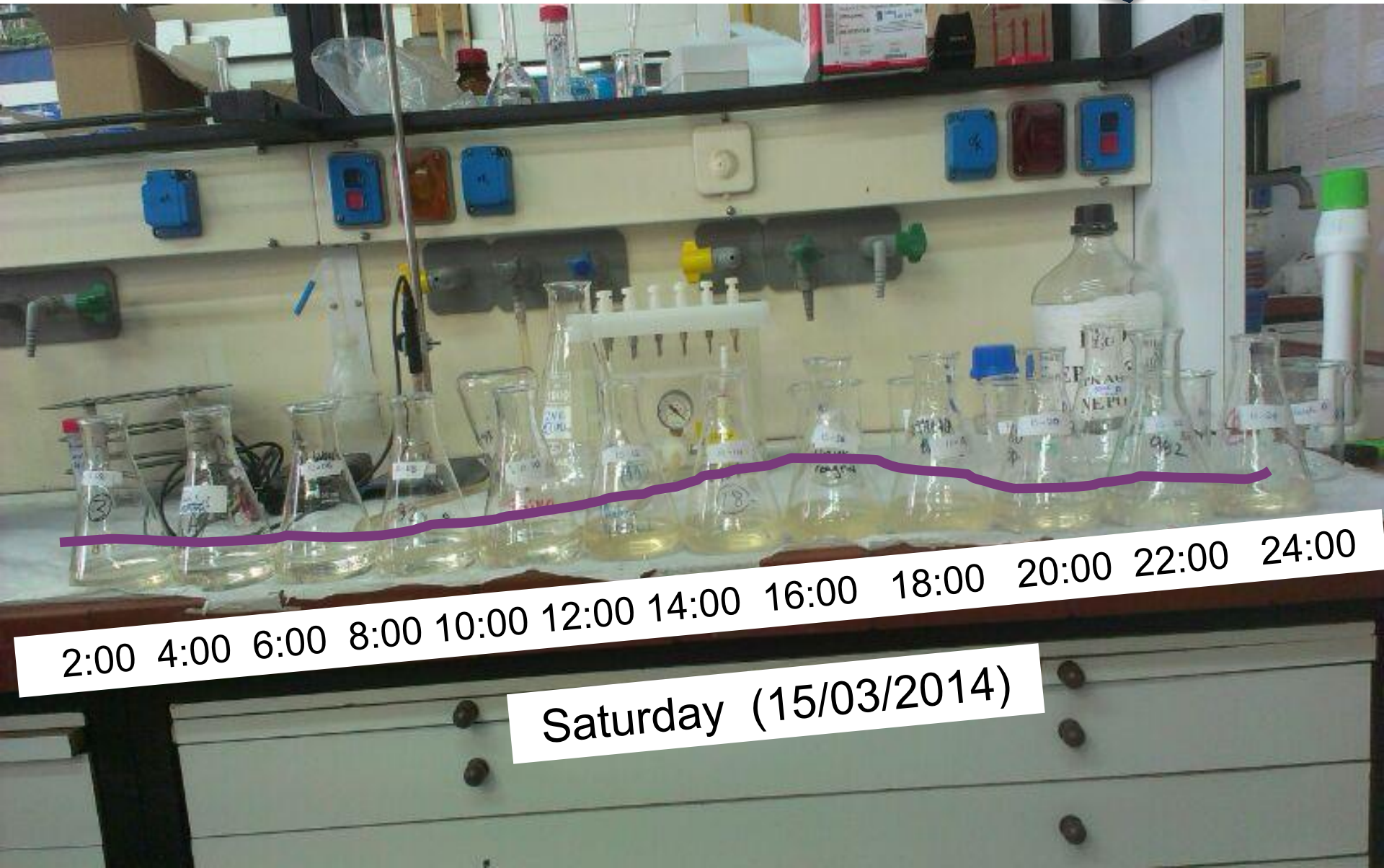
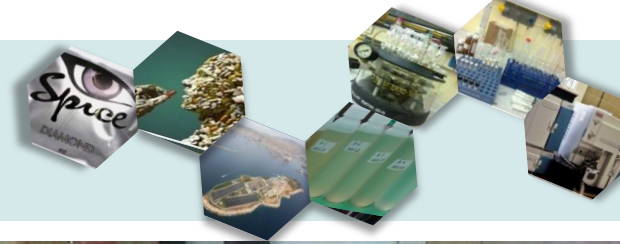
Wastewater Samples from WWTP of Athens



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



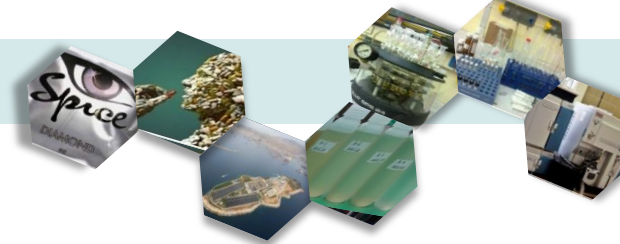
2:00 4:00 6:00 8:00 10:00 12:00 14:00 16:00 18:00 20:00 22:00 24:00

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.



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



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MANAGING AUTHORITY

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EUROPEAN SOCIAL FUND



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