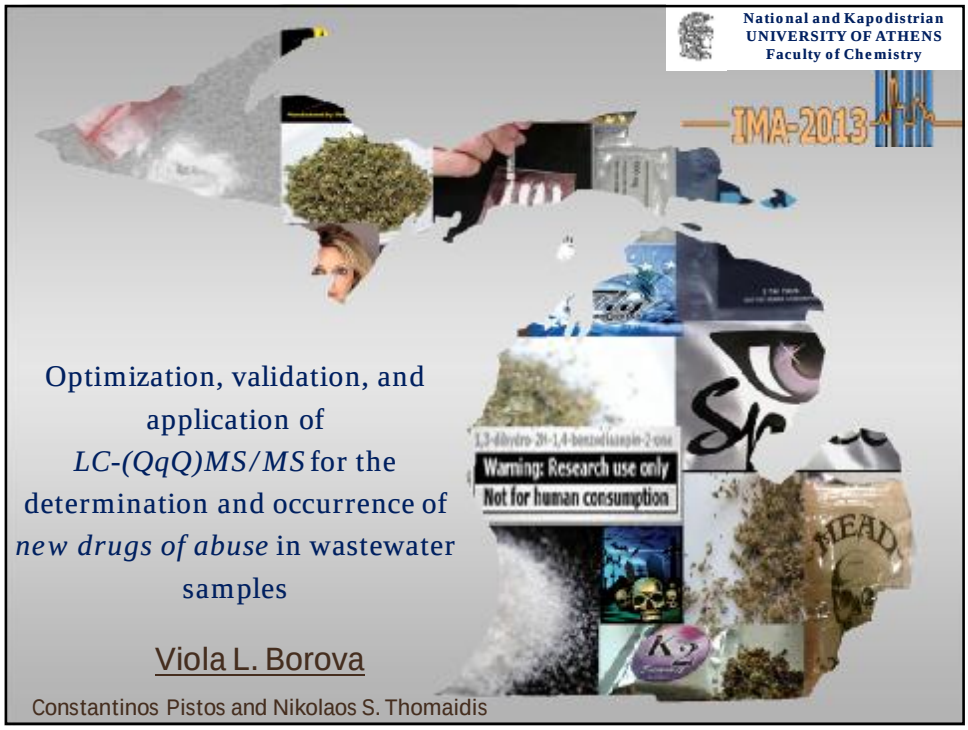
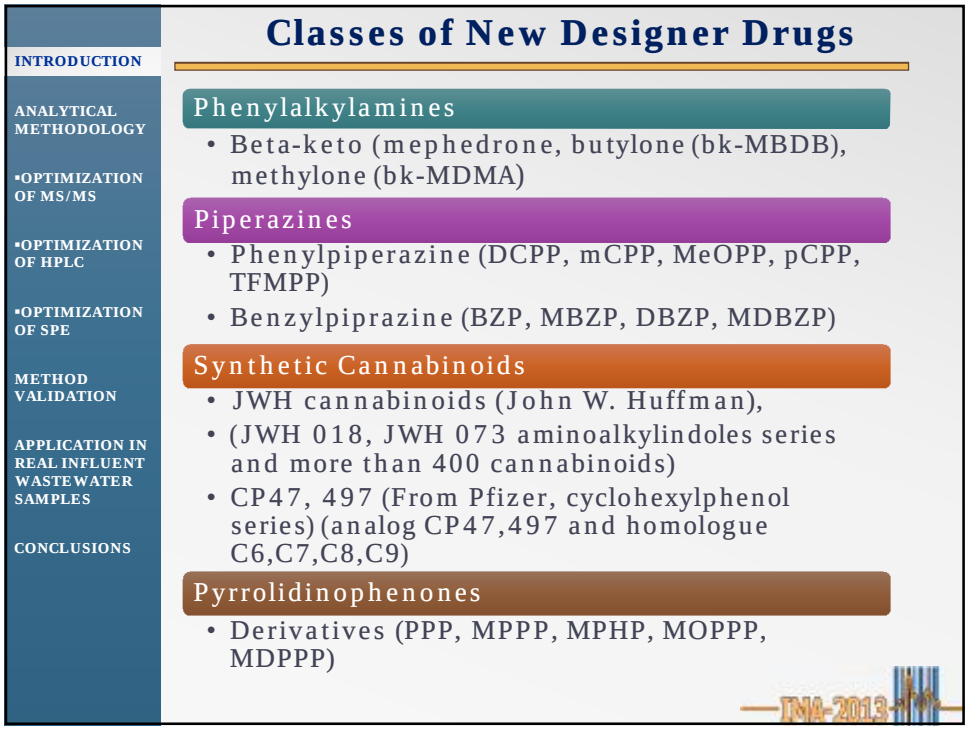


What are "New Designer Drugs"

- *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 on the recreational drug use market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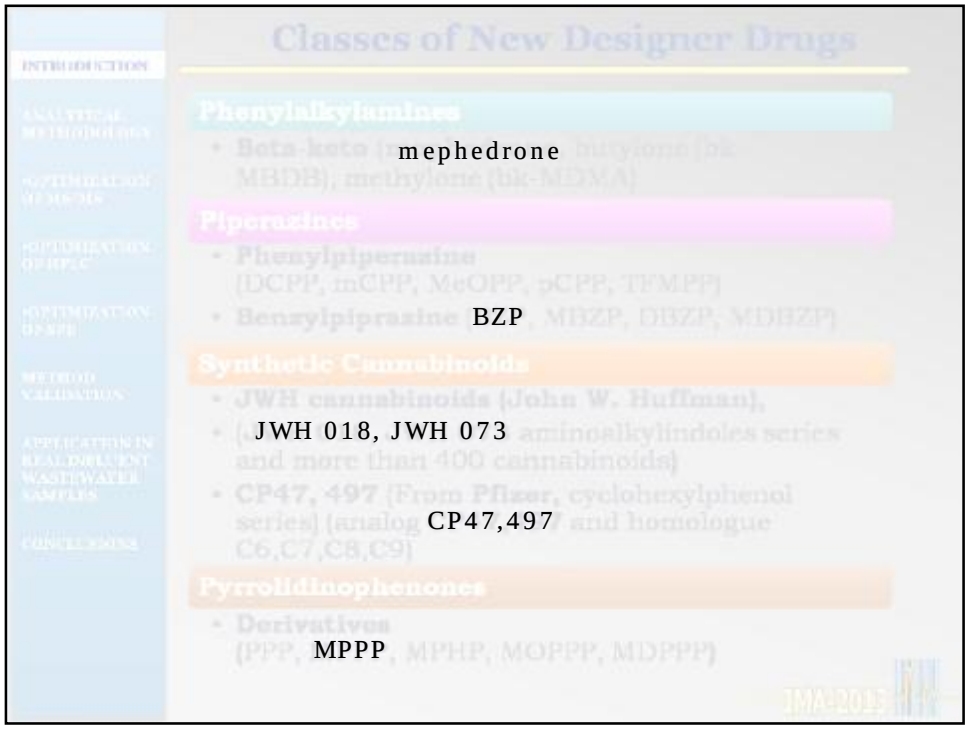
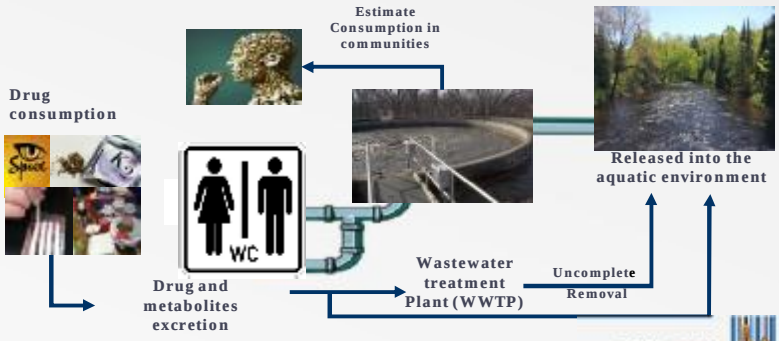
Classes of New Designer Drugs
Phenylalkylamines
• Beta-keto (mephedrone, butylone (bk-MBDB), methylone (bk-MDMA)
Piperazines
• Phenylpiperazine (DCPP, mCPP, MeOPP, pCPP, TFMPP)
• Benzylpiprazine (BZP, MBZP, DBZP, MDBZP)
Synthetic Cannabinoids
• JWH cannabinoids (John W. Huffman),
• (JWH 018, JWH 073 aminoalkylindoles series and more than 400 cannabinoids)
• CP47, 497 (From Pfizer, cyclohexylphenol series) (analog CP47,497 and homologue C6,C7,C8,C9)
Pyrrolidinophenones
• Derivatives (PPP, MPPP, MPHP, MOPPP, MDPPP)
IMA-2013


mephedrone
BZP
JWH 018, JWH 073
CP47,497
MPPP

New Designer Drugs… a new class of emerging organic contaminants

- Not currently covered by existing water quality regulations
- Not investigated or little
- 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)



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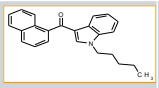
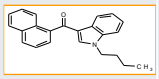
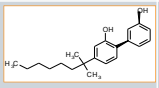
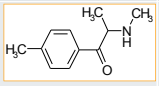
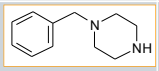
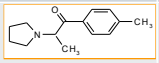
Thermo Scientific Quantum Access

LC- (QqQ) MS/MS -ESI

TARGET COMPOUNDS

Compounds		M. Formula	MW	LogP	pKa
JWH-018 naphthalen-1-yl-(1-pentyl-1H-indol-3-yl) methanone/ JWH-018 D9		$C_{24}H_{23}NO$	341.5	6.51	-
JWH-073 (1-Butyl-1H-indol-3-yl)(1-naphthyl) methanon		$C_{23}H_{21}NO$	327.4	6.07	-
CP47,497 2- [(1R,3S)-3-hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol		$C_{21}H_{34}O_2$	318.5	6.17	-
Mephedrone / Mephedrone D3		$C_{11}H_{15}NO$	177.2	2.12	8.69
Benzylpiperazine /BZP D7		$C_{11}H_{16}N_2$	176.2	1.38	9.59
MPPP 4'-Methyl-α-pyrrolidinopropiophenone		$C_{14}H_{19}NO$	217.3	2.91	-

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Method Development and Optimization of MS/MS

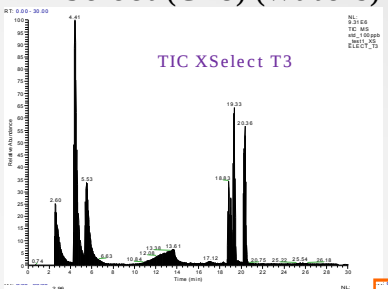
Compounds	$[M+H]^+$	Product Ions (collision energy)	Tube Lens (V)	ESI
JWH-018	342.1	155.0 (25V) 127.0 (44V)	81.8	+
JWH-018 D9	351.1	155.0 (26V) 127.0 (45V)	80.1	+
JWH-073	328.1	154.9 (25V) 127.0 (43V)	76.3	+
CP47,497	*317.2*	*298.9 (24V)* *244.7 (34V)*	*99.1*	-
Mephedrone	178.1	160.0 (12V) 145.0 (19V)	48.1	+
Mephedrone D3	181.1	163.0 (12V) 148.0 (21V)	49.0	+
BZP	177.1	91.2 (28V) 65.3 (40V)	62.5	+
BZP D7	184.1	98.2 (27V) 70.3 (40V)	66.3	+
MPPP	218.1	119.1 (24V) 146.9 (18V)	61.1	+

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

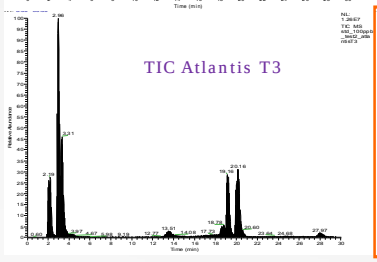
Negative Ionization
Probe: C (0.5/1.5)
Spray Voltage: 2500 V
Sheath gas: 20 a.u.
Aux gas: 10 a.u.
Temp: 300°C

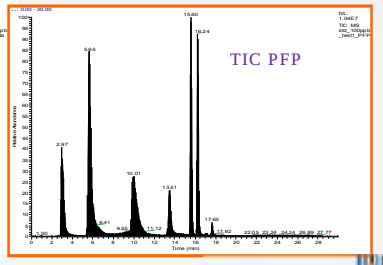
Comparison of stationary phases

- Stationary phases: Atlantis T3 (C18) (Waters), XSelect (C18) (Waters) & PFP (Phenomenex)



❖Compounds with $-NH_2$ or –NH- presented higher asymmetry (fronting) on C18 column
❖Xselect and Atlantis T3 suffer from peak shape issues and do not provide good separation of the compounds
❖PFP: excellent peak shape, good resolution and separation in short time with excellent MS sensitivity





Mobile Phase

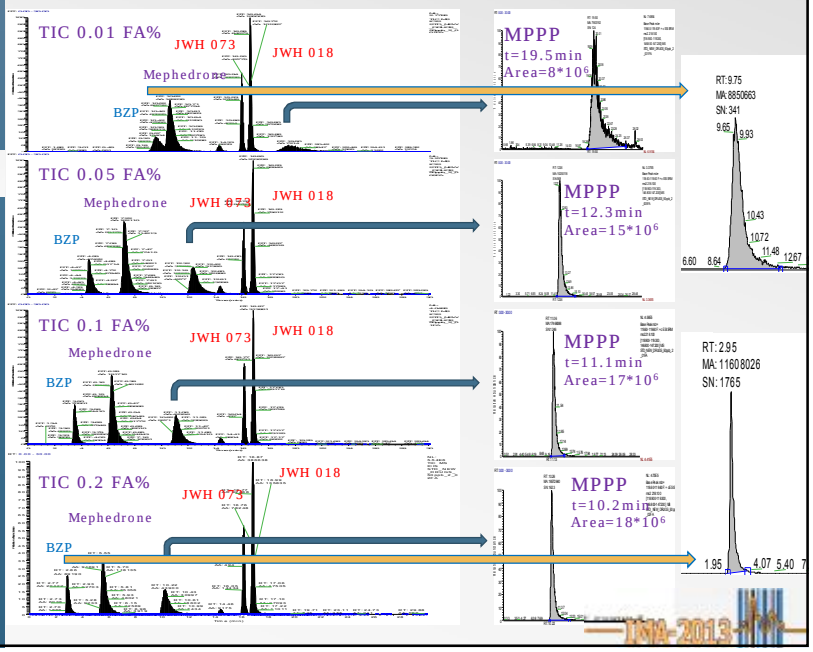
JWH 018, JWH 073,
Mephedrone, Benzylpiperazine, MPPP

Positive	H_2O (0.2% formic acid)	MeOH	µL/min
0.00	60	40	100
3.00	60	40	150
15.00	0.0	100	100
25.00	0.0	100	100
25.50	60	40	100
30.00	60	40	100

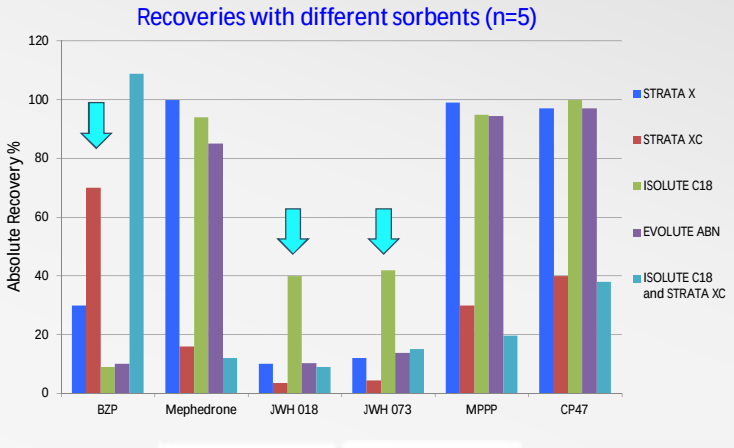
CP47, 497

Negative	MeOH	ACN	µL/min
0.00	90	10	100
3.00	90	10	100
15.00	90	10	100

Effect of the organic modifier



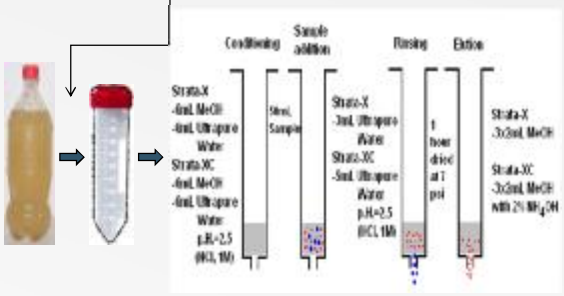
Sample preparation- Comparison SPE



Sample preparation

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 (STRATA-X/ISOLUTE C18, STATA-XC)

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



LC-MS/MS

Validation for wastewater samples

	Linear range µg/L	Corr. Coefficient R^2	method LOD [ng/L]	Absolute Recovery % (n=5)	RSD% (n=5)
BZP	1 – 100	0.9993	4.8	29.0	14.8
Mephedrone	1 – 100	0.9994	0.3	100	3.7
MPPP	1 – 100	0.9994	0.6	99.0	4.2
CP47,497	1 – 100	0.9991	37	97.0	15.2
JWH 018	1 – 100	0.9991	1.4	40.0	8.3
JWH 073	1 – 100	0.9992	0.8	42.0	15.3

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Wastewater Samples from WWTP Santorini

Sampling
23/07/2013- 29/07/2013

✓Population: 10500 inhabitants

✓Flow rate: 1500 m^3/day

✓Mediterranean climate

✓High anthropogenic impact

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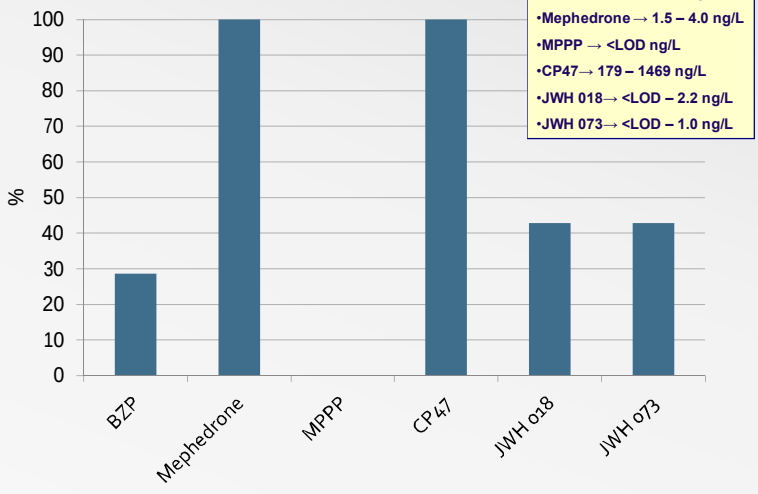
Frequency of detection



▪RANGES:
- ▪BZP → <LOD – 17.8 ng/L
- ▪Mephedrone → 1.5 – 4.0 ng/L
- ▪MPPP → <LOD ng/L
- ▪CP47→ 179 – 1469 ng/L
- ▪JWH 018→ <LOD – 2.2 ng/L
- ▪JWH 073→ <LOD – 1.0 ng/L

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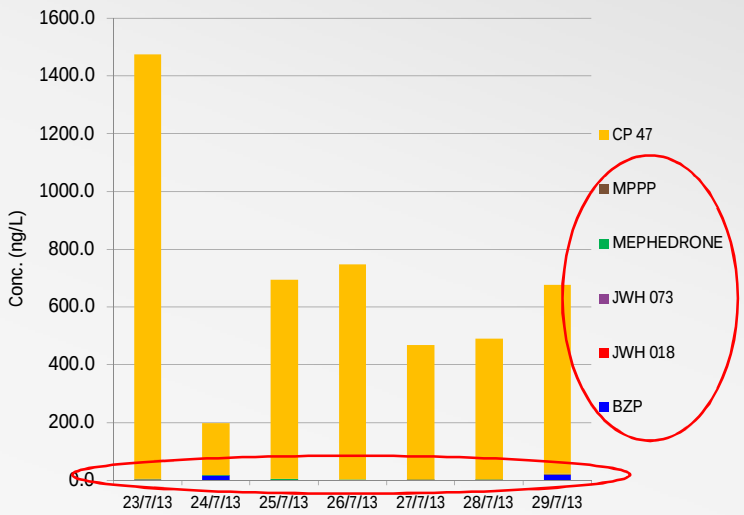
Wastewater Samples from WWTP Santorini

Levels in influent wastewater of Fira



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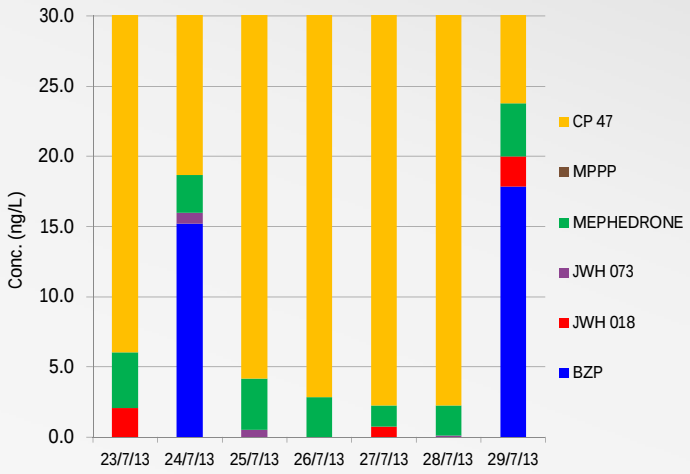

CONCLUSIONS

- ✓ 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
- ✓5 out of the 6 compounds were detected at least in one day
- ✓CP47 was detected for the first time in influent wastewater
- ✓Not any special trend among the days was observed for mephedrone and CP47

Future Perspectives

- ✓Integration into one multi-analyte method which provides the elution of all the compounds with one cartridge
- ✓Identification of their transformation products
- ✓Application of the method in the WWTP of Athens

THANK YOU VERY MUCH!!!

Questions?

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TrAMS

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

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