

National and Kapodistrian
UNIVERSITY OF ATHENS
Faculty of Chemistry

TMA-2013

**Optimization, validation, and
application of
LC-(QqQ)MS/MS for the
determination and occurrence of
new drugs of abuse in wastewater
samples**

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Constantinos Pistos and Nikolaos S. Thomaidis

What are “New Designer Drugs”

INTRODUCTION	
ANALYTICAL METHODOLOGY	
•OPTIMIZATION OF MS/MS	
•OPTIMIZATION OF HPLC	
•OPTIMIZATION OF SPE	
METHOD VALIDATION	
APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES	
CONCLUSIONS	

- **Designer Drugs:**
 - Synthetically changed natural substances
 - Completely designed molecular structures
- **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

TMA-2013

Classes of New Designer Drugs	
INTRODUCTION	
ANALYTICAL METHODOLOGY	<p>Phenylalkylamines</p> <ul style="list-style-type: none"> Beta-keto (mephedrone, butylone (bk-MBDB), methylone (bk-MDMA))
•OPTIMIZATION OF MS/MS	<p>Piperazines</p> <ul style="list-style-type: none"> Phenylpiperazine (DCPP, mCPP, MeOPP, pCPP, TFMPP) Benzylpiperazine (BZP, MBZP, DBZP, MDBZP)
•OPTIMIZATION OF HPLC	
•OPTIMIZATION OF SPE	
METHOD VALIDATION	<p>Synthetic Cannabinoids</p> <ul style="list-style-type: none"> 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)
APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES	
CONCLUSIONS	<p>Pyrrolidinophenones</p> <ul style="list-style-type: none"> Derivatives (PPP, MPPP, MPHP, MOPPP, MDPPP)

TMA-2013

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TMA-2013

New Designer Drugs... a new class of emerging organic contaminants

INTRODUCTION

ANALYTICAL METHODOLOGY

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METHOD VALIDATION

APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES

CONCLUSIONS

- 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)

The diagram illustrates the lifecycle of drug contaminants: **Drug consumption** (represented by a pill bottle) leads to **Drug and metabolites excretion** (represented by a WC icon). This waste enters a **Wastewater treatment Plant (WWTP)**. From the WWTP, there is **Uncomplete Removal**, leading to substances being **Released into the aquatic environment** (represented by a stream). A feedback loop labeled **Estimate Consumption in communities** connects the aquatic environment back to the consumption stage.

TMA-2013

ANALYTICAL METHODOLOGY

INTRODUCTION

ANALYTICAL METHODOLOGY

•OPTIMIZATION OF MS/MS

•OPTIMIZATION OF HPLC

•OPTIMIZATION OF SPE

METHOD VALIDATION

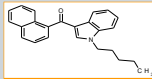
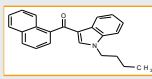
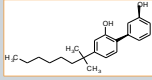
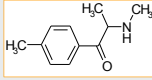
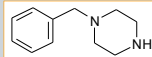
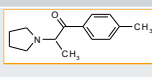
APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES

CONCLUSIONS

Thermo Scientific Quantum Access

LC- (QqQ) MS/MS -ESI

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TARGET COMPOUNDS						
INTRODUCTION						
ANALYTICAL METHODOLOGY	Compounds		M. Formula	MW	LogP	pKa
•OPTIMIZATION OF MS/MS	JWH-018 naphthalen-1-yl-(1-pentyl-1H-indol-3-yl) methanone / JWH-018 D9		C ₂₄ H ₂₃ NO	341.5	6.51	-
•OPTIMIZATION OF HPLC	JWH-073 (1-Butyl-1H-indol-3-yl)(1-naphthyl) methanone		C ₂₃ H ₂₁ NO	327.4	6.07	-
•OPTIMIZATION OF SPE	CP47,497 2-[(1R,3S)-3-hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol		C ₂₁ H ₃₄ O ₂	318.5	6.17	-
METHOD VALIDATION	Mephedrone / Mephedrone D3		C ₁₁ H ₁₅ NO	177.2	2.12	8.69
APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES	Benzylpiperazine /BZP D7		C ₁₁ H ₁₆ N ₂	176.2	1.38	9.59
CONCLUSIONS	MPPP 4'-Methyl-α-pyrrolidinopropiophenone		C ₁₄ H ₁₉ NO	217.3	2.91	-

TMA-2013

Method Development and Optimization of MS/MS					
INTRODUCTION					
ANALYTICAL METHODOLOGY	Compounds	[M+H] ⁺	Product Ions (collision energy)	Tube Lens (V)	ESI
•OPTIMIZATION OF MS/MS	JWH-018	342.1	155.0 (25V) 127.0 (44V)	81.8	+
•OPTIMIZATION OF HPLC	JWH-018 D9	351.1	155.0 (26V) 127.0 (45V)	80.1	+
•OPTIMIZATION OF SPE	JWH-073	328.1	154.9 (25V) 127.0 (43V)	76.3	+
METHOD VALIDATION	CP47,497	317.2	298.9 (24V) 244.7 (34V)	99.1	-
APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES	Mephedrone	178.1	160.0 (12V) 145.0 (19V)	48.1	+
CONCLUSIONS	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

TMA-2013

INTRODUCTION

ANALYTICAL METHODOLOGY

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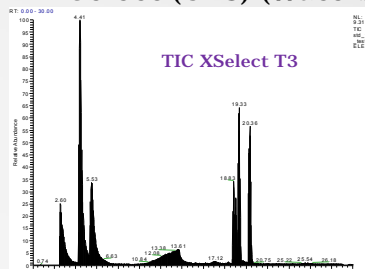
METHOD VALIDATION

APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES

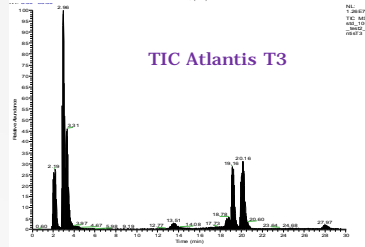
CONCLUSIONS

Comparison of stationary phases

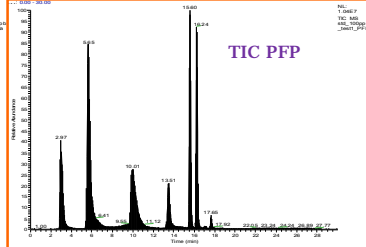
Stationary phases: Atlantis T3 (C18) (Waters), XSelect (C18) (Waters) & PFP (Phenomenex)



TIC XSelect T3




TIC Atlantis T3



TIC PFP

- ❖ Compounds with -NH₂ 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



INTRODUCTION

ANALYTICAL METHODOLOGY

•OPTIMIZATION OF MS/MS

•OPTIMIZATION OF HPLC

•OPTIMIZATION OF SPE

METHOD VALIDATION

APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES

CONCLUSIONS


Mobile Phase

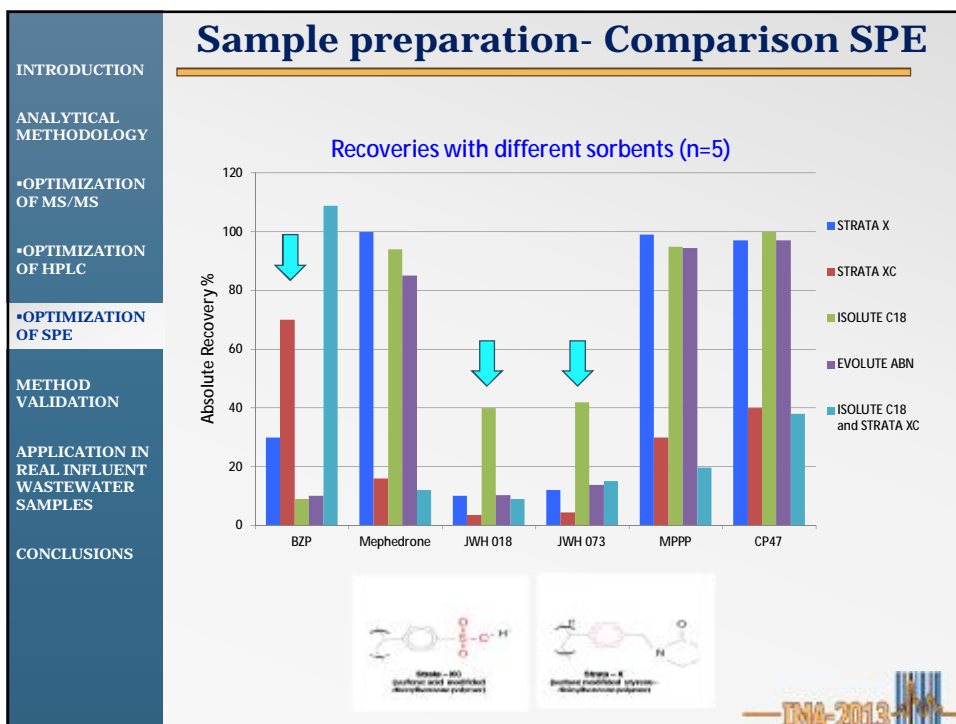
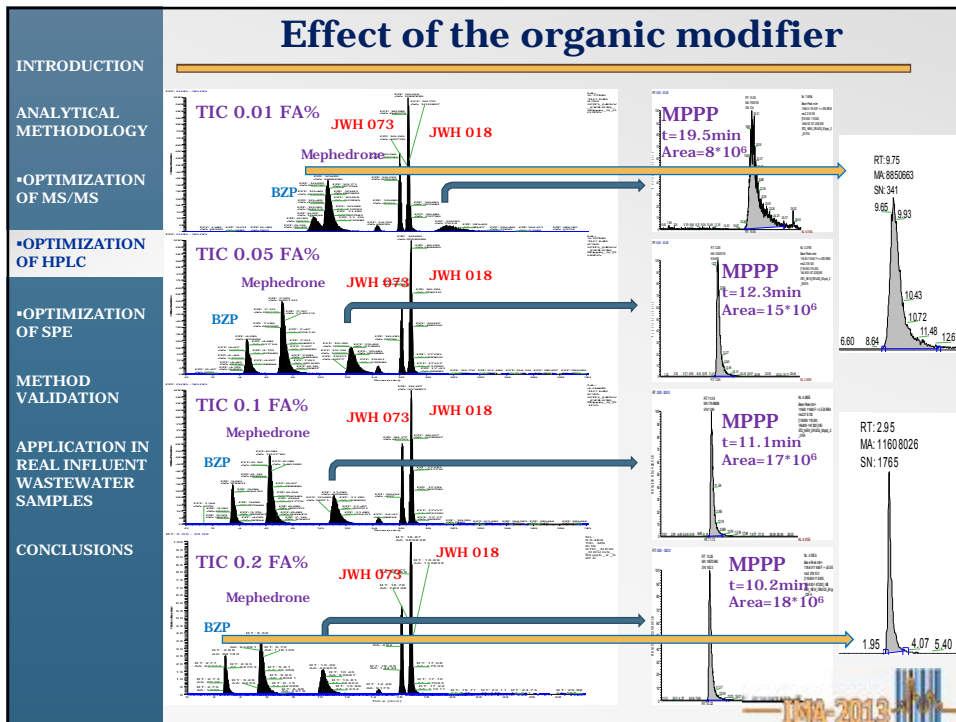
JWH 018, JWH 073,
Mephedrone, Benzylpiperazine, MPPP

Positive	H ₂ O (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





Sample preparation

INTRODUCTION

ANALYTICAL METHODOLOGY

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METHOD VALIDATION

APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES

CONCLUSIONS

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

INTRODUCTION

ANALYTICAL METHODOLOGY

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METHOD VALIDATION

APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES

CONCLUSIONS

	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

INTRODUCTION

ANALYTICAL METHODOLOGY


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METHOD VALIDATION

APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES

CONCLUSIONS


Wastewater Samples from WWTP Santorini



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

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

- ✓Mediterranean climate
- ✓High anthropogenic impact



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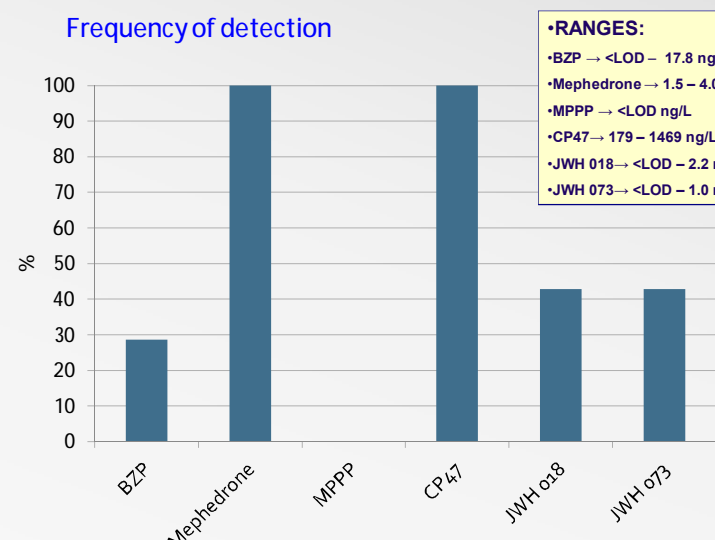
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Frequency of detection

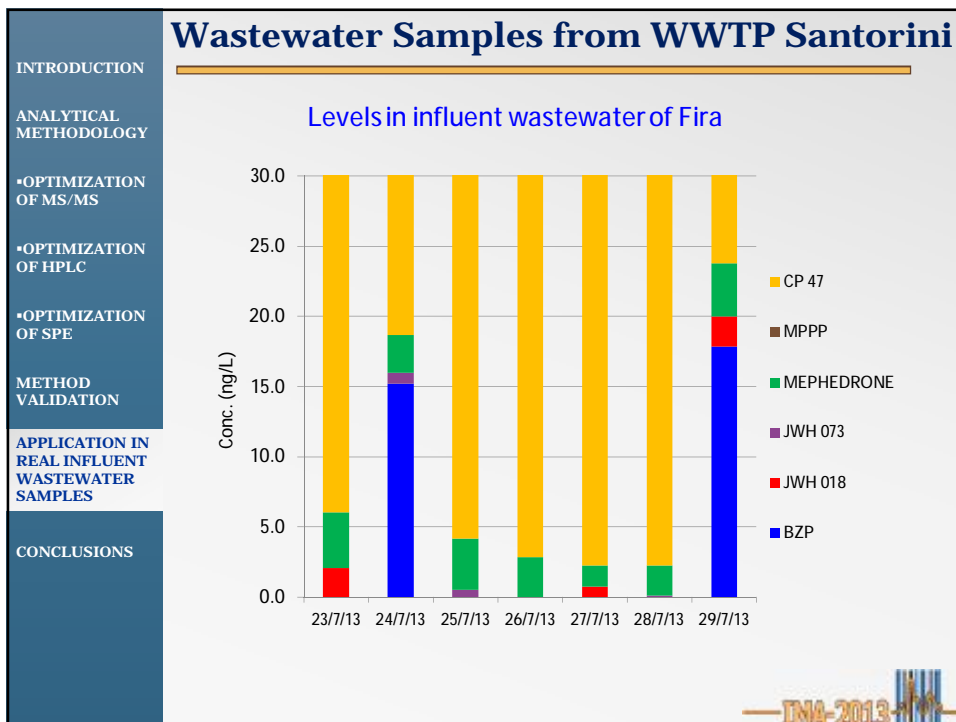
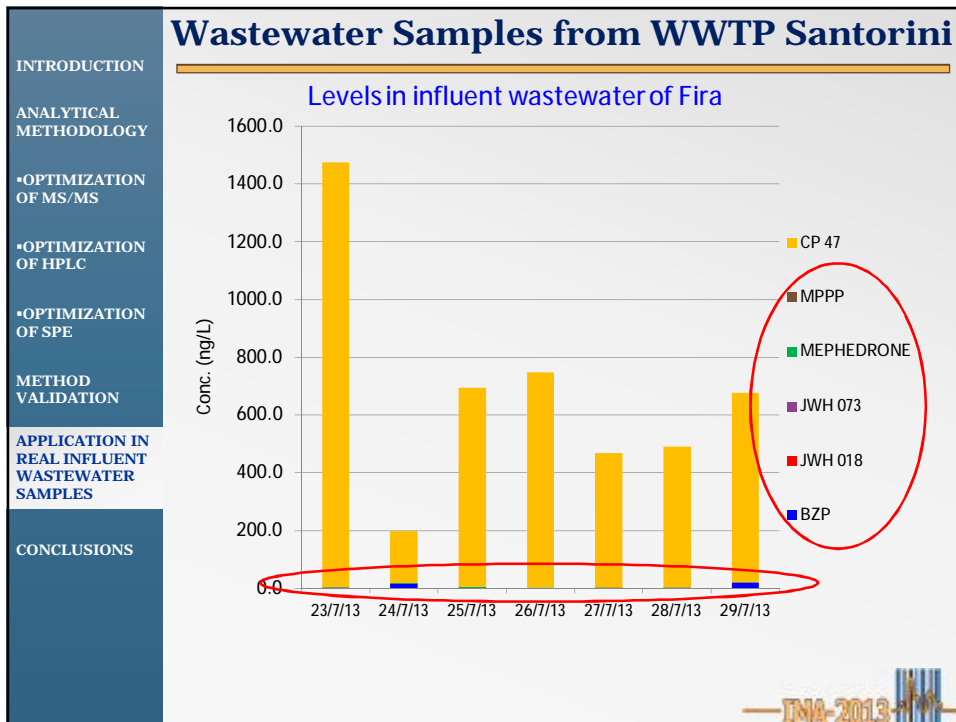



Substance	Frequency of detection (%)
BZP	28
Mephedrone	100
MPPP	0
CP47	100
JWH 018	42
JWH 073	42


•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





INTRODUCTION ANALYTICAL METHODOLOGY •OPTIMIZATION OF MS/MS •OPTIMIZATION OF HPLC •OPTIMIZATION OF SPE METHOD VALIDATION APPLICATION IN REAL INFLUENT WASTEWATER SAMPLES CONCLUSIONS	<h2 style="color: #1f4e79;">CONCLUSIONS</h2> <hr style="border: 1px solid #1f4e79;"/> <ul style="list-style-type: none"> ✓ 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 <div style="text-align: right; margin-top: 20px;">  </div>
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THANK YOU VERY MUCH!!!

Questions?

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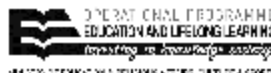


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