

Wastewater and Urine Drug Analysis by LC-MS/MS – Our Experience



SUSANA SIMÕES

NPS-EURONET LISBON MEETING 28TH SEPTEMBER

Identification and Assessment of New Psychoactive Substances: A European Network



co-funded by the European Union
HOME/2014/JDRU/AG/DRUG/7086

2013 - ...



Drug analysis in urine samples collected in an emergency department in a partnership between the Hospital de São José from the Hospital Center of Lisbon and the laboratory of chemistry and forensic chemistry of the INMLCF, IP.

Substances

JWH073 N-4-Hydroxybutyl	JWH250
JWH073 N-3-Hydroxybutyl	JWH022
JWH018 N-Pentanoic acid	THCCOOH
JWH018 N-5-Hydroxypentyl	JWH018
JWH122 N-5-Hydroxypentyl	JWH018 4-Hydroxyindole
JWH018 5-Hydroxyindole	JWH122
AM2201	JWH210
JWH073	HU210
11-OH-THC	THC

UPLC-MS/MS:

- Waters Acquity UPLC/TQ Detector
- ES+/MRM (two transitions)
- Acquity UPLC® HSS T3 (50 x 2.1 mm i.d., 1.8 µm)
- T (column) = 45°C
- Mobile phase: methanol – ammonium formate 2 mM (0.1% formic acid)
- Gradient
- Flow: 0.4 mL/min
- Total run time of 9.5 min
- Injection volume of 10 µL



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Sample preparation:

- 0.5 mL of Urine
- Enzymatic hydrolysis (β -glucuronidase)
- SPE Oasis®HLB 3cc (60 mg)
 - ✓ 2 mL of MeOH + 2 mL of H₂O
 - ✓ Sample
 - ✓ 2 mL of H₂O/ACN/NH₄OH
 - ✓ Dry for 20 min
 - ✓ Elution with 2 mL of Ethyl acetate
 - ✓ Dry for 10 min
 - ✓ Elution with 2 mL of Hexane/Ethyl acetate/Acetic acid (88:10:2, v/v/v)
- Dry under nitrogen
- Redissolve in MeOH and H₂O (1:1) and analyse

Validation:

- Selectivity
- LOD and LOQ
- Recovery
- Carryover
- Matrix Effect
- Linearity
- Intra-assay precision
- Inter-assay accuracy and precision
- Robustness

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Real Sampleo Data

- ✓ Total number of samples analysed: **79**
- ✓ Total number of positive samples for synthetic cannabinoids: **5 (6.2%)**
 - Positive samples for synthetic cannabinoids and for THC or metabolites: **3**
- ✓ Total number of positive samples for THC and/or metabolites: **30**

Sample	Gender	Age	Information	Substances
1	Male	15	Possible intoxication with mephedrone	JWH 018 N-Hydroxypentyl (0.5 ng/mL) JWH018 N-Pentanoic acid
2	Female	28	Student; Sexual assault	THCCOOH (54 ng/mL) JWH018 N-Hydroxypentyl (<0.05 ng/mL)
3	Male	51	Teacher; Possible intoxication with a plant fertilizer “Smooth Golol” obtained in a smartshop	THCCOOH (7.7 ng/mL) THC (1.1 ng/mL) JWH018 N-Hydroxypentyl (1.1 ng/mL) JWH018 N-Pentanoic acid
4	–	–	–	THCOOH (22 ng/mL) JWH018 N-Hydroxypentyl (3.9 ng/mL) JWH018 N-Pentanoic acid JWH122 N-5-Hydroxypentyl
5	–	–	–	JWH018 N-Hydroxypentyl (0.1 ng/mL) JWH018 N-Pentanoic acid

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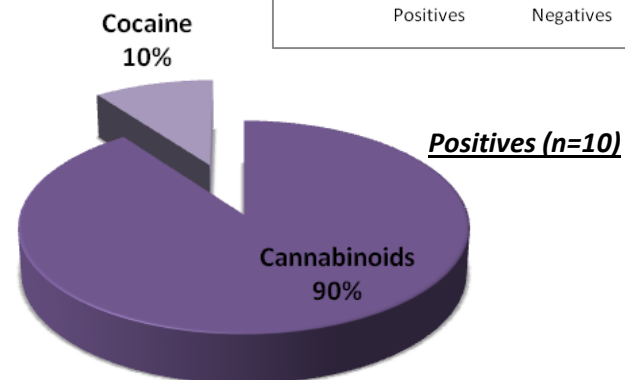
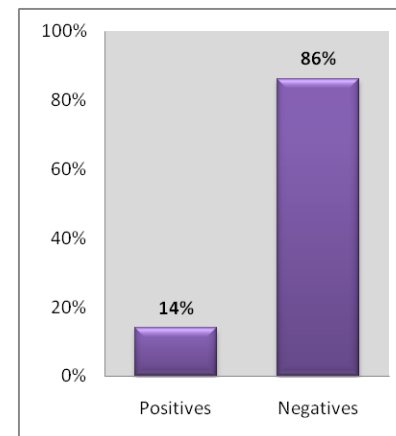
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Results				
Sample	Immunoassay		GC-MS / UPLC-MS-MS	
2	Negative		Positive	Methadone
3	Negative		Positive	Methadone
5	Positive	Cannabinoids	Positive	THC-COOH; JWH-018
7	Positive	Cannabinoids	Positive	Morphine; THC-COOH
10	Negative		Positive	THC-COOH
11	Negative		Positive	JWH-018
13	Negative		Positive	Methadone; THC-COOH
18	Positive	Cannabinoids	Positive	THC-COOH
20	Negative		Positive	Methadone
22	Negative		Positive	Methadone
23	Negative		Positive	Methadone; THC-COOH
24	Positive	Cannabinoids	Positive	THC-COOH
27	Positive	Cannabinoids	Positive	Morphine; THC-COOH
30	Negative		Positive	Methadone
34	Positive	Cannabinoids	Positive	THC; 11-OH-THC; THC-COOH
35	Negative		Positive	Morphine; Benzoylcegonine
36	Negative		Positive	THC-COOH
37	Negative		Positive	THC-COOH
38	Positive	Cannabinoids	Positive	THC-COOH
39	Negative		Positive	mCPP
40	Positive	Cannabinoids	Positive	THC-COOH
42	Negative		Positive	Methadone
44	Negative		Positive	Methadone; THC-COOH
46	Positive	Cocaine	Positive	THC-COOH
48	Negative		Positive	Methadone
51	Positive	Cannabinoids	Positive	THC-COOH
53	Negative		Positive	Methadone
55	Negative		Positive	Methadone; THC-COOH
56	Negative		Positive	Methadone; Morphine; THC-COOH
57	Negative		Positive	Methadone
60	Negative		Positive	Cocaine; Benzoylcegonine; THC-COOH
61	Negative		Positive	Tramadol
62	Negative		Positive	Methadone; THC-COOH
65	Negative		Positive	Methadone; MDA; THC-COOH
66	Negative		Positive	Methadone
67	Negative		Positive	THC-COOH
68	Negative		Positive	Tramadol
69	Negative		Positive	THC-COOH
70	Negative		Positive	THC; THC-COOH
71	Negative		Positive	THC; 11-OH-THC; THC-COOH
73	Negative		Positive	THC-COOH

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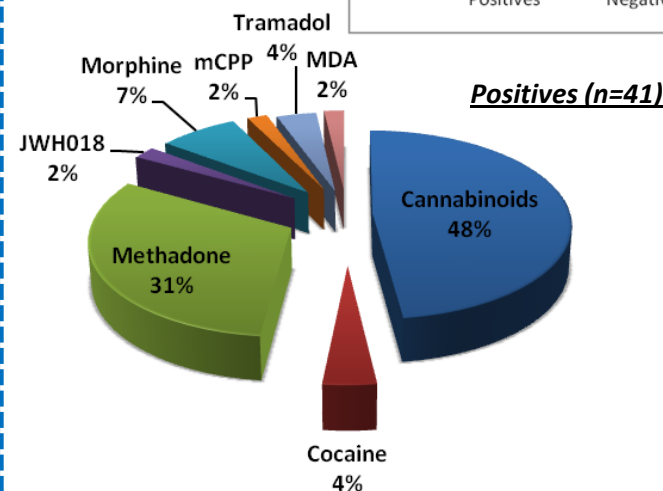
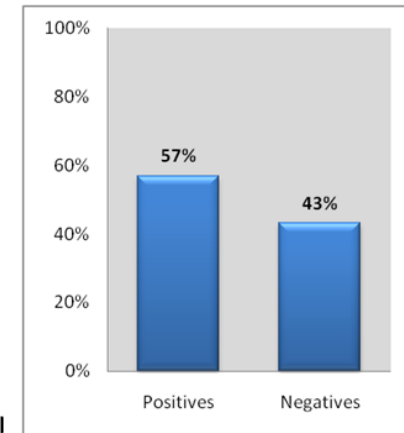
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38	Positive Cannabinoids	Positive THC-COOH
39	Negative	Positive mCPP
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42	Negative	Positive Methadone
44	Negative	Positive Methadone; THC-COOH
46	Positive Cocaine	Positive THC-COOH
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2014 - ...



Participation in the SCORE project in collaboration with the University of Pharmacy of Lisbon. This project involves the wastewater analysis in over 40 European cities (21 countries) to explore the drug-taking habits of those who live in them.

Substances	
Morphine	Ketamine
Cotinine	Benzoylcegonine
6-MAM	Cocaine
MDA	Cocaethylene
MDMA	EDDP
Amphetamine	Methadone
Methamphetamine	THCCOOH
Mephedrone	

UPLC-MS/MS:

- Waters Acquity UPLC/TQ Detector
- ES+/MRM (Two transitions)
- Acquity UPLC® HSS T3 (100 x 2.1 mm i.d., 1.8 µm)
- T (column) = 45 °C
- Mobile phase: methanol – ammonium formate 2 mM (0.1% formic acid)
- Gradient
- Flow: 0.4 mL/min
- Total run time of 9.0 min
- Injection volume of 10 µL

Identification and Assessment of New Psychoactive Substances: A European Network

The logo for SCORE (Sewage Analysis CORE group Europe) features the word "score" in a bold, lowercase, sans-serif font. The letter "o" is replaced by a stylized yellow and orange circular graphic.

Sewage Analysis CORE
group Europe (SCORE)



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Samples:

SCORE 2016 Inter-laboratory Calibration Protocol

Wastewater collected from two STP (7 consecutive days)

Sample preparation (Method 1):

- Filtration (Whatman GF/C 0.45 µm Glass microfiber filters)
- SPE Oasis®MCX 3cc (60 mg)
 - ✓ 3 mL of MeOH + 3 mL of H₂O + 3 mL of HCL 0.01 N (pH 2)
 - ✓ Sample (50 mL; adjusted to pH 2 with HCL + 30 µL of the internal standard mixture)
 - ✓ Dry for 15 min
 - ✓ Elution with 3 mL of MeOH + 3 mL of MeOH/NH₄OH (98:2, v/v)
- Dry under nitrogen
- Redissolve in MeOH and H₂O (1:1) and analyse

Sample preparation (Method 2):

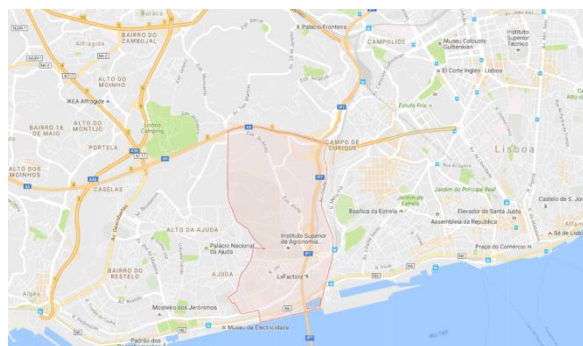
- Filtration (Whatman GF/C 0.45 µm Glass microfiber filters)
- SPE Oasis®HLB 3cc (60 mg)
 - ✓ 2 mL of MeOH + 2 mL of H₂O
 - ✓ Sample (50 mL; adjusted to pH 7 with HCL + 30 µL of the IS mixture)
 - ✓ 2 mL of H₂O/ACN/NH₄OH
 - ✓ Dry for 20 min
 - ✓ Elution with 2 mL of Ethyl acetate
 - ✓ Dry for 10 min
 - ✓ Elution with 2 mL of Hexane/Ethyl acetate/Acetic acid (88:10:2, v/v/v)
- Dry under nitrogen
- Redissolve in MeOH and H₂O (1:1) and analyse

Identification and Assessment of New Psychoactive Substances: A European Network



score

Sewage Analysis CORE
group Europe (SCORE)



Results

STP (Alcântara)

Calibration curve : 10 – 4000 ng/mL

Substance

Concentration (ng/L)

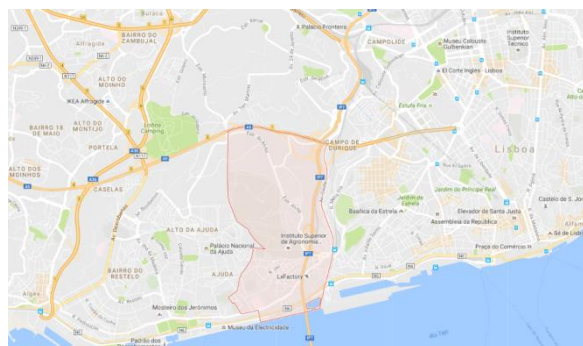
	A1	A2	A3	A4	A5	A6	A7	LDQ (ng/L)
Morphine	101	126	109	81	78	93	124	10
Cotinine	1152	1351	1151	869	830	984	1378	1
6-MAM	< 10 (4,8)	< 10 (4,2)	—	< 10 (4,0)	< 10 (4,6)	< 10 (4,4)	—	2
MDA	—	—	—	—	—	—	—	10
MDMA	36	34	31	22	75	117	67	1
Amphetamine	10	11	< 10 (9,2)	< 10 (7,9)	< 10 (8,2)	< 10 (9,2)	< 10 (9,1)	5
Methamphetamine	< 10 (7,0)	< 10 (7,6)	< 10 (8,2)	< 10 (6,4)	< 10 (6,4)	< 10 (6,9)	< 10 (7,2)	1
Mephedrone	—	—	—	—	—	11	—	5
Ketamine	< 10 (9,4)	< 10 (4,4)	—	< 10 (6,8)	< 10 (2,8)	—	—	1
Benzoylcegonine	441	492	447	380	521	654	641	1
Cocaine	124	121	80	63	83	126	72	1
Cocaethylene	< 10 (1,1)	< 10 (3,2)	< 10 (3,5)	< 10 (1,0)	< 10 (3,7)	12	< 10 (2,8)	1
EDDP	76	82	77	55	58	97	88	1
Methadone	28	33	31	22	23	35	36	1
THCCOOH	165	196	150	101	109	111	172	5

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score

Sewage Analysis CORE
group Europe (SCORE)



Results

STP (Alcântara)

Calibration curve : 10 – 4000 ng/mL

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THE END

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