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Identification of new psychoactive substances in urban wastewater: new methods and technologies

Wastewater analysis : the rationale

Drugs

-cocaine

-cannabis

-heroin

-amphetamines

CONSUMER

Excretes residues of the drugs with urine after the intake

TREATMENT PLANT

The residues of the drugs are transported to the TP with the wastewater

DRUG RESIDUES QUANTIFICATION

Wastewater is sampled and the residues of the drugs at the STP are quantified

CONSUMPTION ESTIMATE

Amounts of the residues (g/day) are used to back-calculate drug consumption in the population served by the STP (g/day of the parent drug)

Analytical methods-Waste and surface water

Therapeutic drugs: 35 compounds + 6 deuterated standards
Illicit drugs: 27 compounds + 20 deuterated standards

(Castiglioni et al., *J Chrom A.* 2005; Castiglioni et al., *ES&T* 2006; Castiglioni *et al.*, *Anal. Chem.* 2006; Castiglioni et al., *Mass Spectrom Rev.* 2008; Zuccato et al., *Water Res.* 2008)

Filtration (1.6 and 0.45 µm)

Solid Phase Extraction (SPE)
Ultrasonic Solvent Extraction (USE)

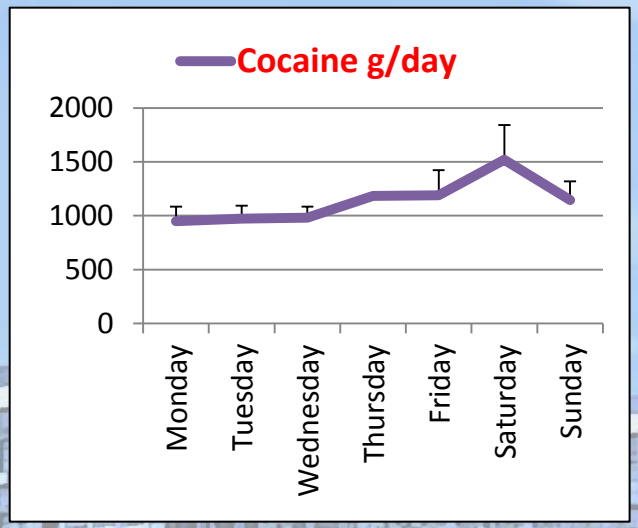
Oasis HLB and MCX (60 mg)
Lichrolut EN (200 mg)

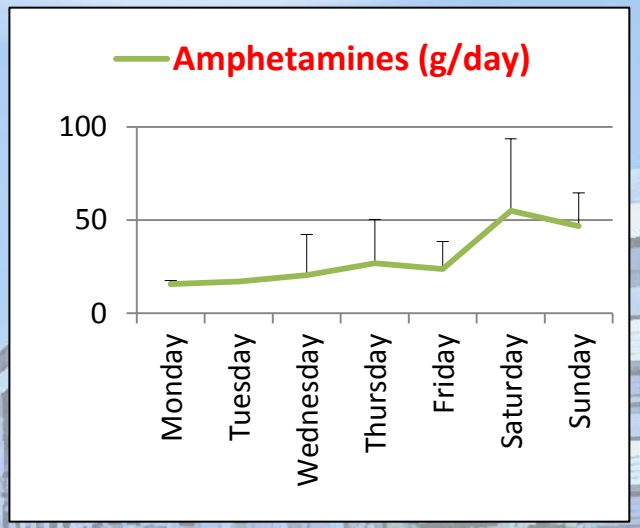
HPLC-MS/MS analysis

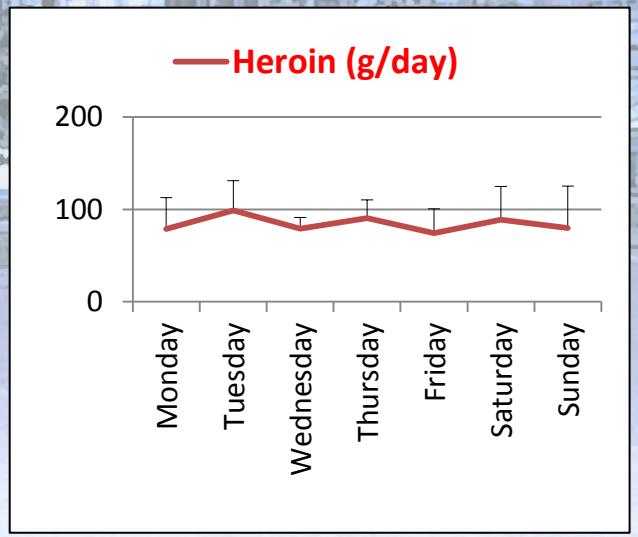
HPLC Column : C8-C18 and HILIC phases
Mass Spectrometer:
Applied Biosystem-SCIEX API 5500
triple quadrupole, turbo ion spray source
Quantification: isotope dilution

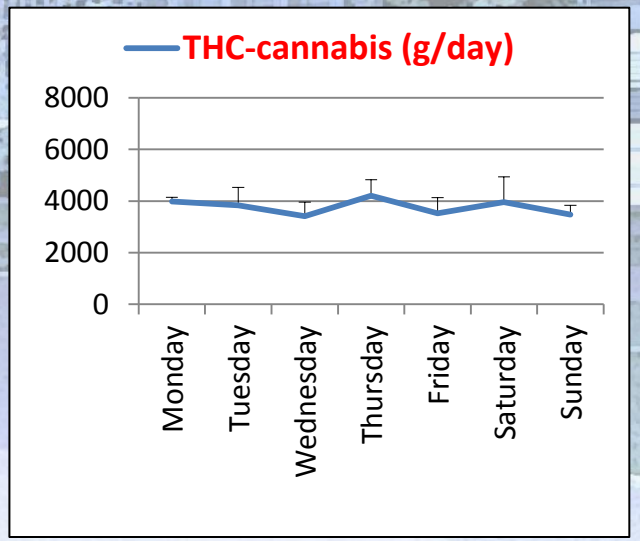
Weekly pattern of consumption – Local consumption

Drug Consumption in Milan (2006) (Zuccato et al., 2008)



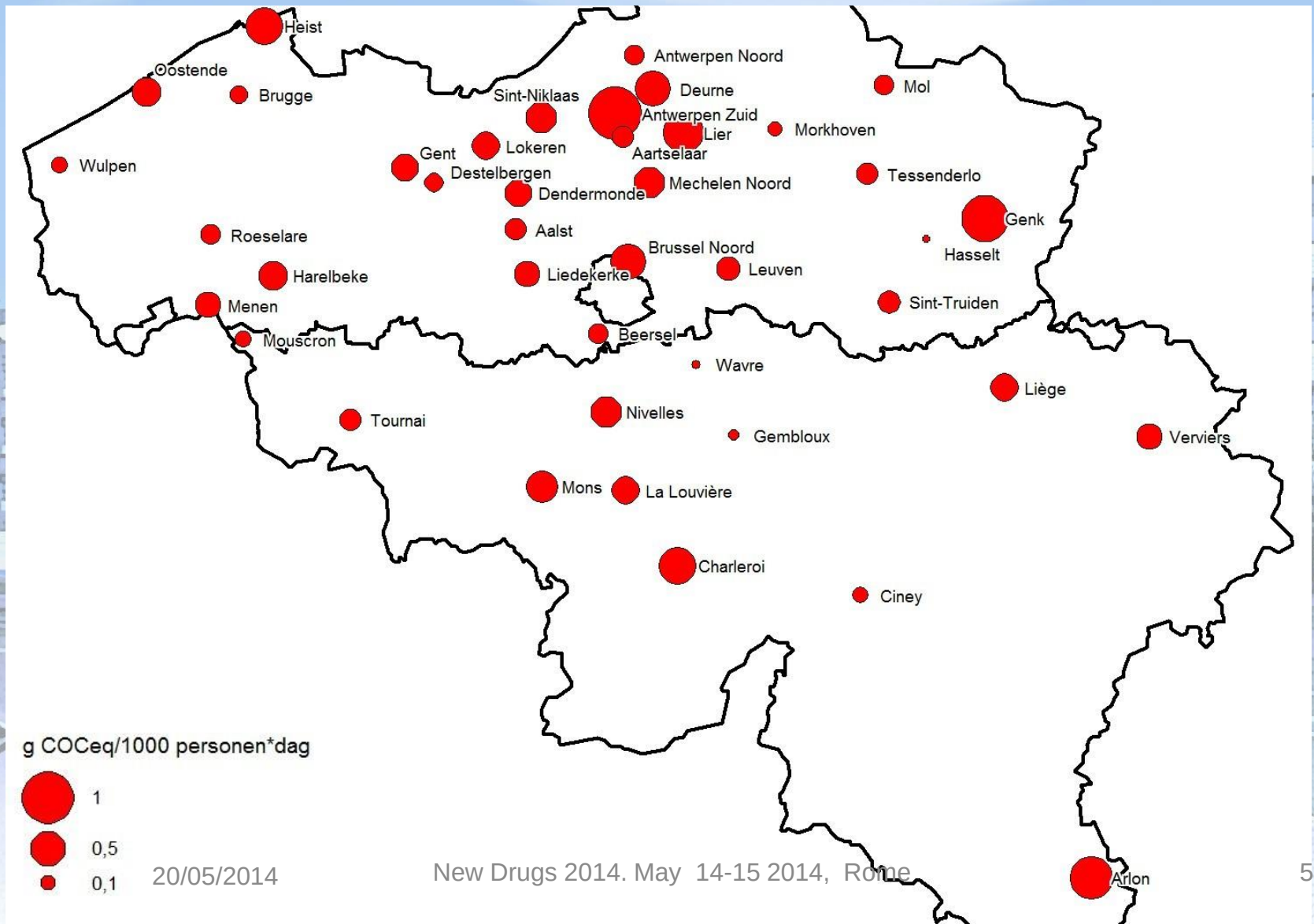






Comparison of consumption pattern in different cities in Belgium

Van Nuijs et al., 2009



Progetto Aqua Drugs

PRESIDENZA DEL CONSIGLIO DEI MINISTRI
Dipartimento Politiche Antidroga

Cocaine consumption in Italy demographic and geographic distribution

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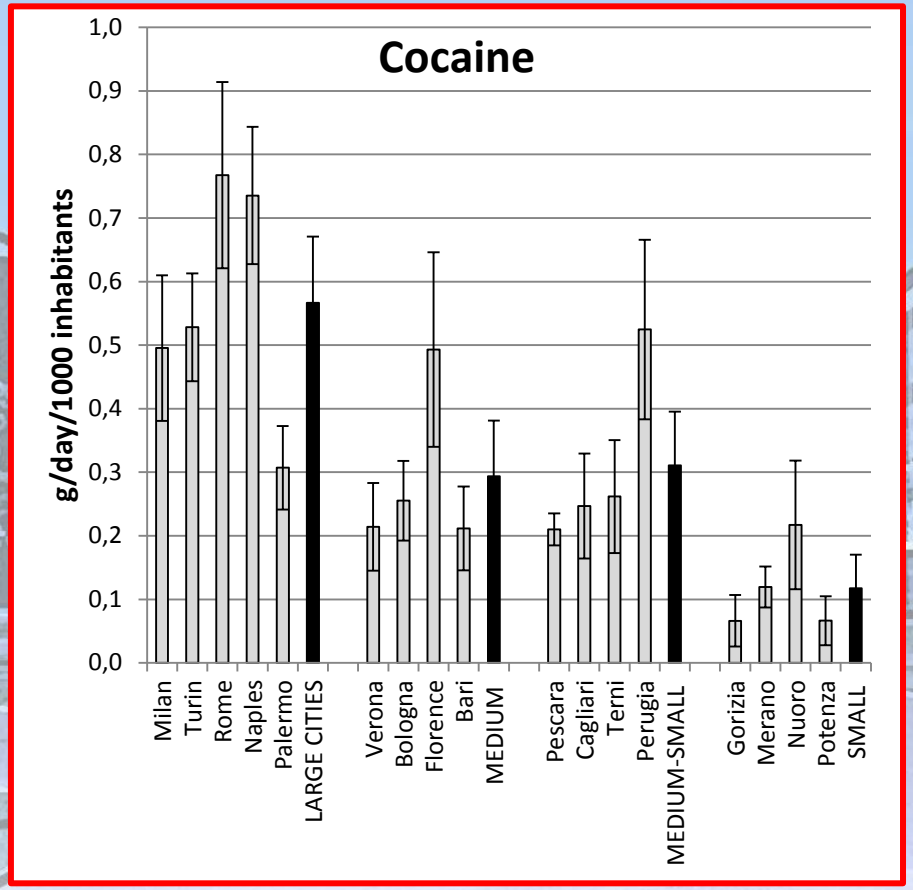
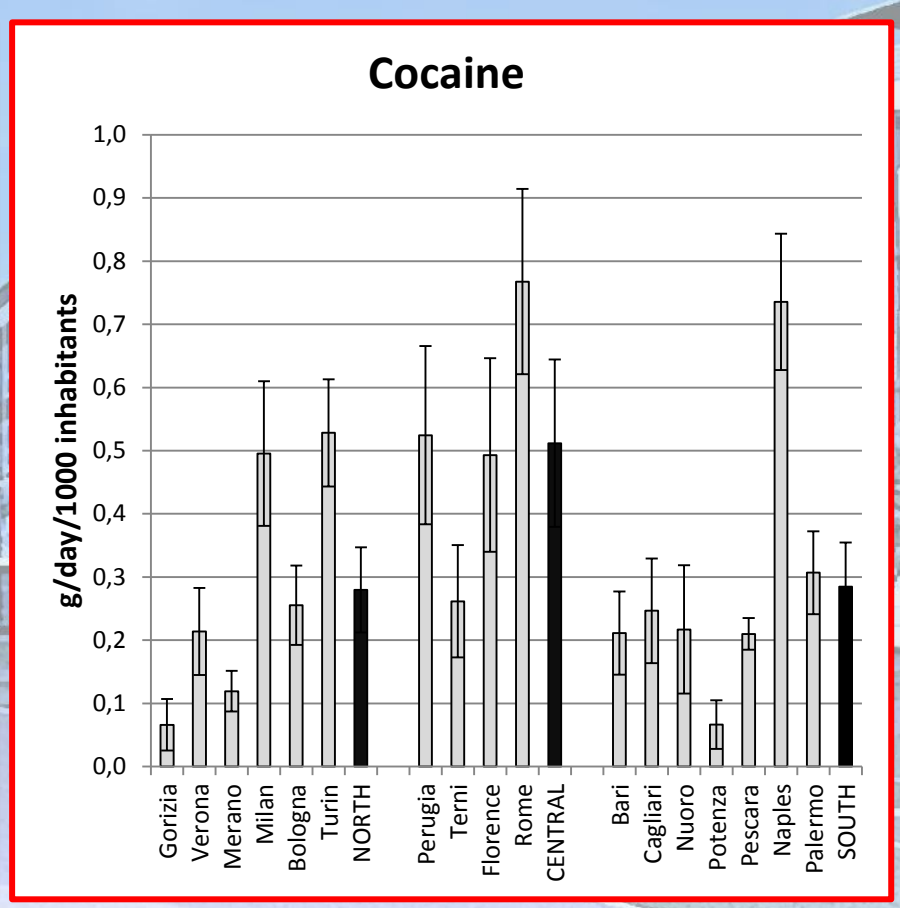
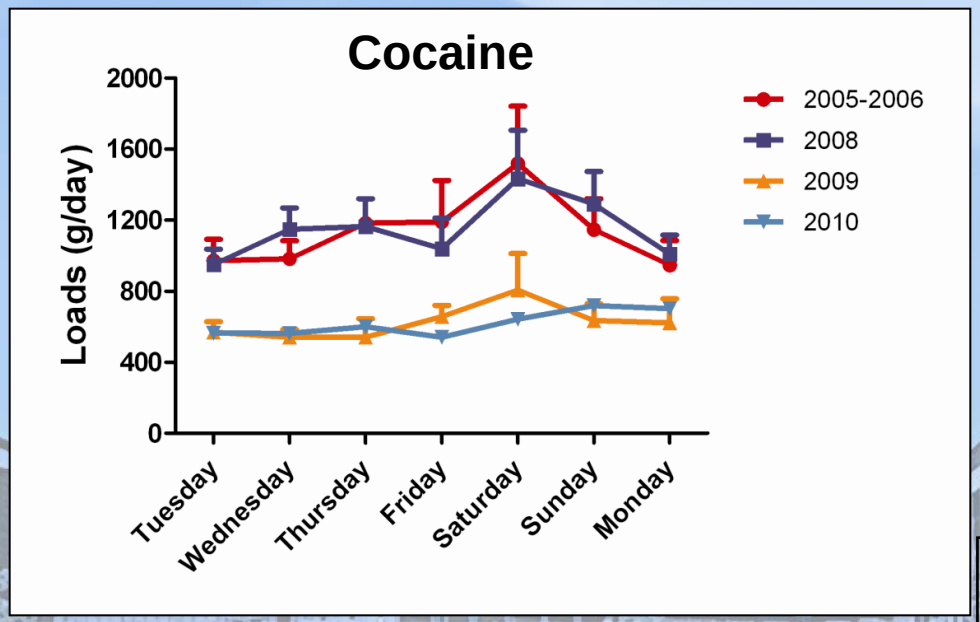


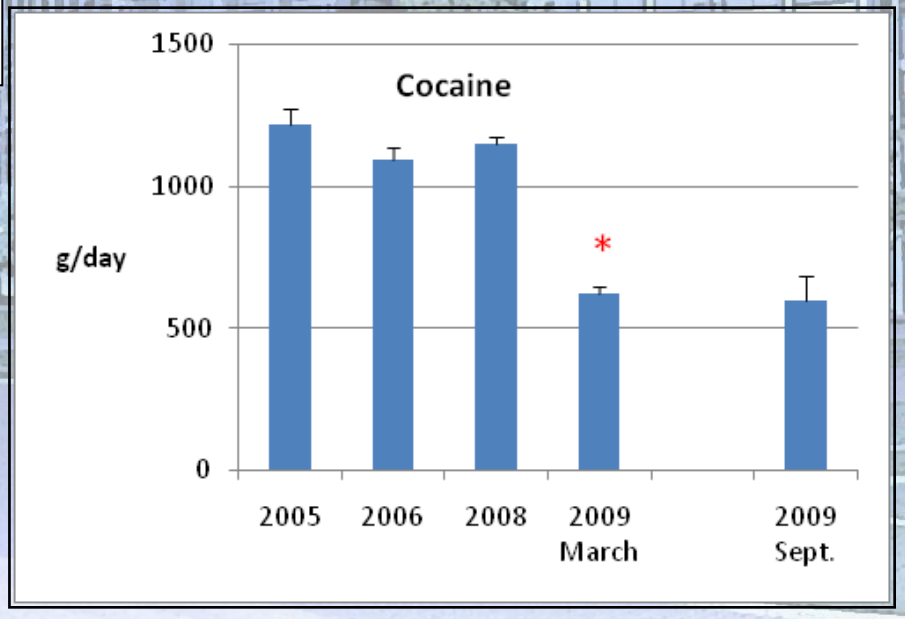


Trends in drug consumption from wastewater (Milan, 2005-2010)



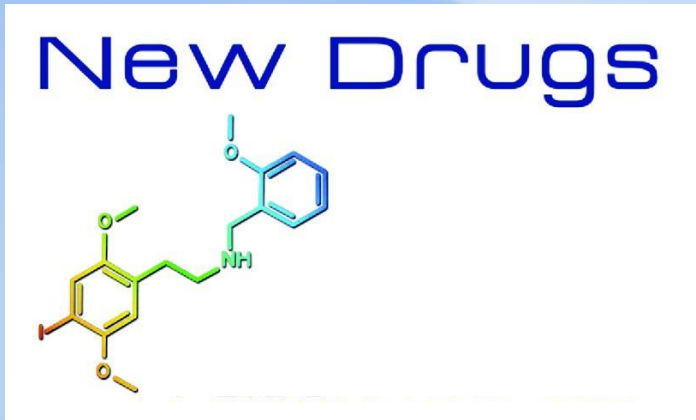
Year	Doses/1000 inhabitants
2005-2006	9.1 ± 1.9
2008	9.2 ± 1.8
2009	5.0 ± 1.1
2010	5.1 ± 0.6

Cocaine consumption fell 45% in 2009 ($p<0.05$, Tukey-Kramer HSD test)
Zuccato et al., 2011



Zuccato et al., 2011

Identification of New Drugs in wastewater



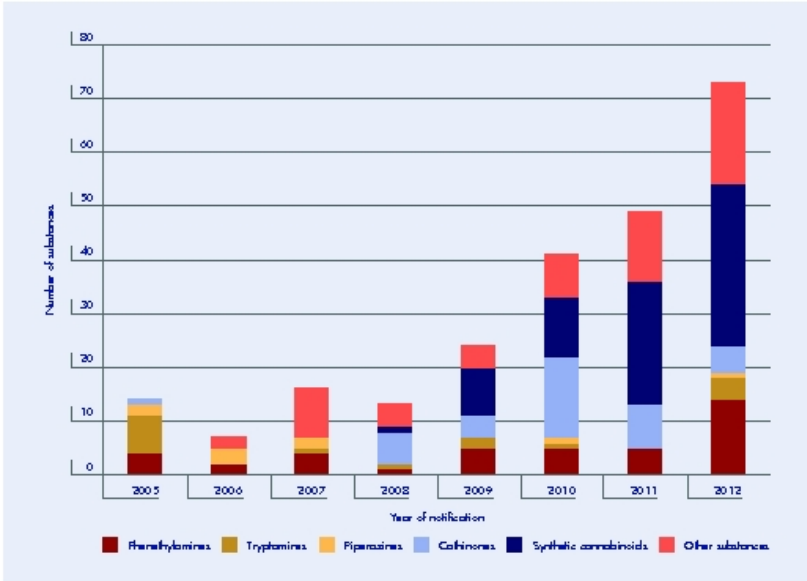
Main characteristics

- Hundreds of different substances
- Similar structures
- Limited use – low levels in wastewater
- Human metabolism unknown
- High toxic effects

EMCDDA–Europol 2012 Annual Report on the implementation of Council Decision 2005/387/JHA (New drugs in Europe, 2012)

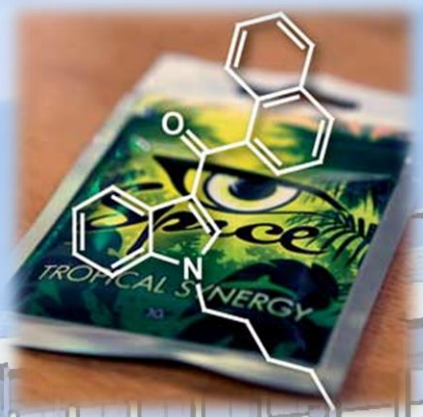
Early Warning Systems (EWS) were established to identify these substances in the market in a short time

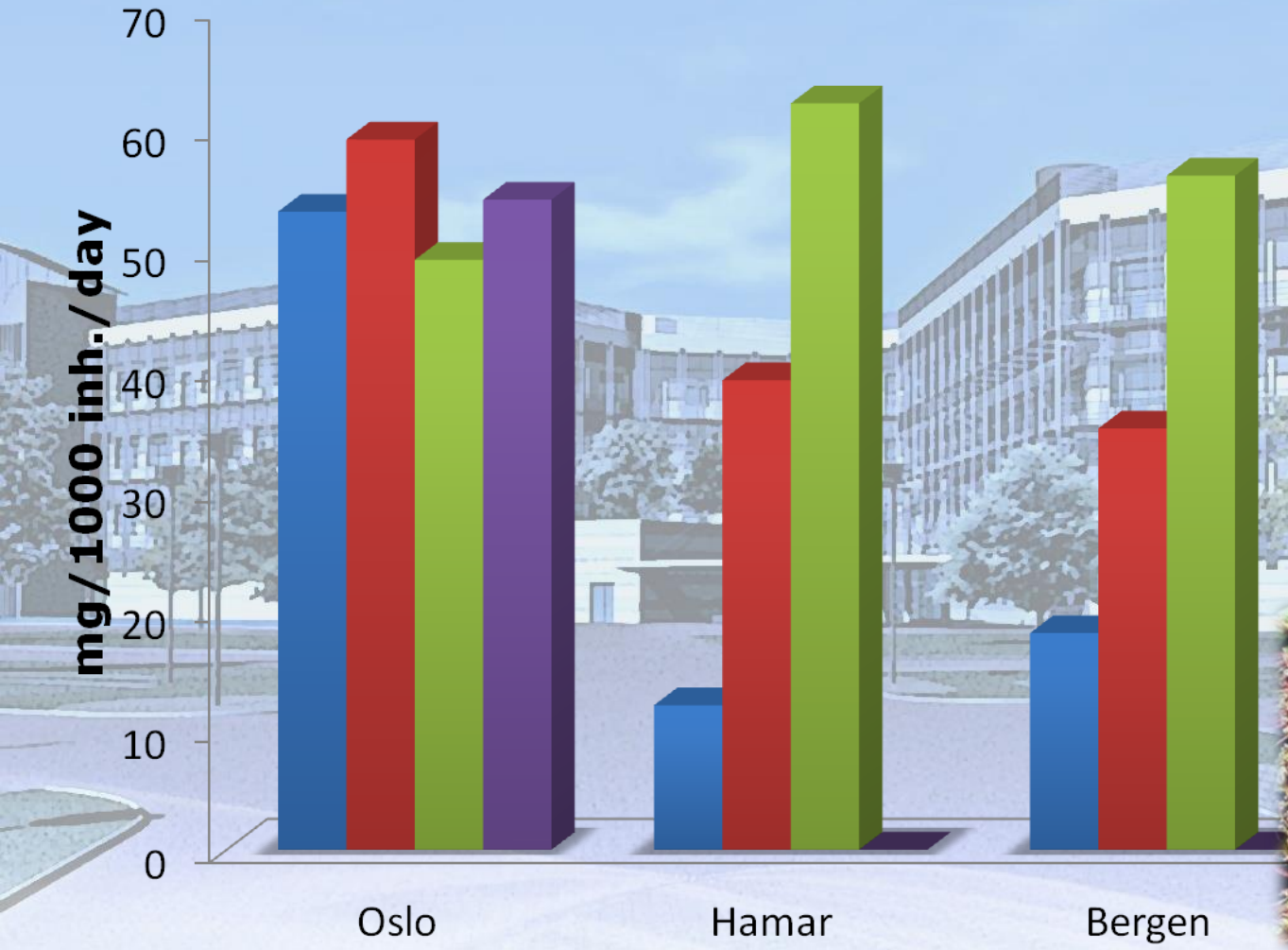
Number of new psychoactive substances notified to the European Early warning system, 2005–2012



Source: EMCDDA/EWS.

New psychoactive substances





Reid et al. 2013. *Drug Anal Test*. DOI: 10.1002/dta.1461

Collaboration Thermo-Mario Negri

Claudia Martins
Frans Schoutsen

Sara Castiglioni
Renzo Bagnati
Ettore Zuccato

Development of a screening workflow for the determination of illicit drugs in wastewater

Screening workflow development based on the combination of different MS experiments and the use of spectral library confirmation criteria

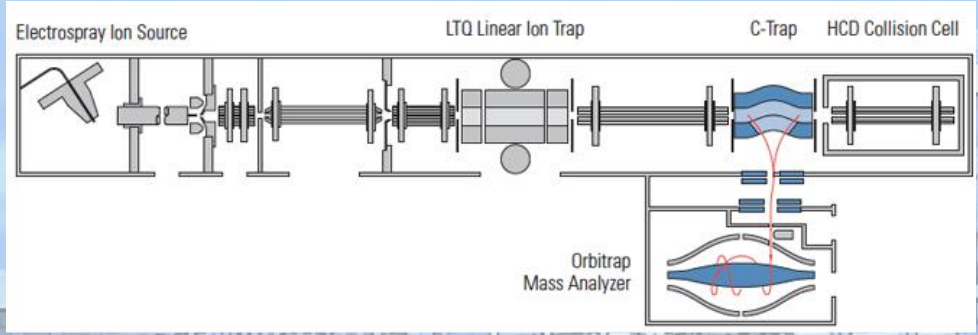
Collaboration Aims

- Develop state of the art workflows related to determine **illicit drugs** in **environmental matrices** with **Thermo hardware/software** suites
- Develop methods to identify **new drugs of abuse**
- **Software evaluation** – screening workflows
- Publish **case studies** and present results at scientific conferences

Collaboration Thermo-Mario Negri

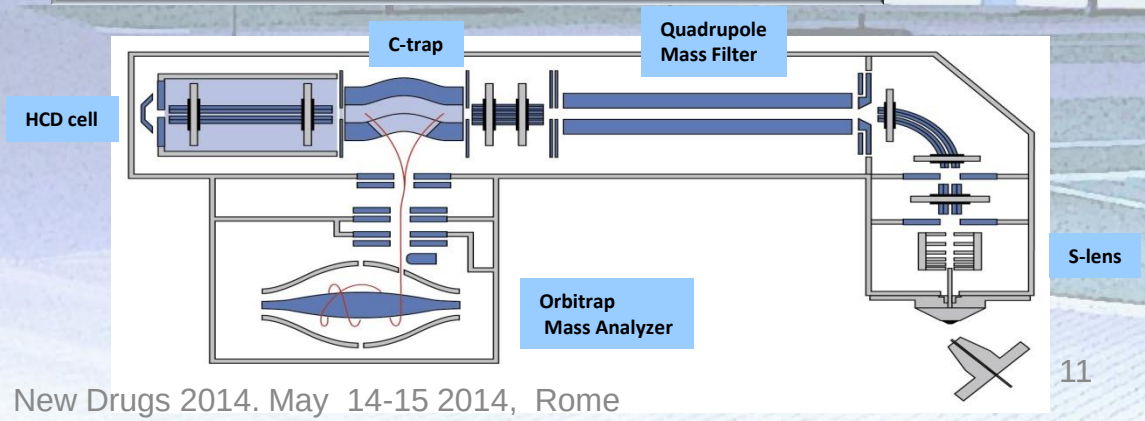
Thermo Scientific LTQ Orbitrap XL

Nanospray source with capillary pumps
Column Zorbax SB-C18 0.5x150mm
Flow 10 µL/min



Thermo Scientific Q Exactive

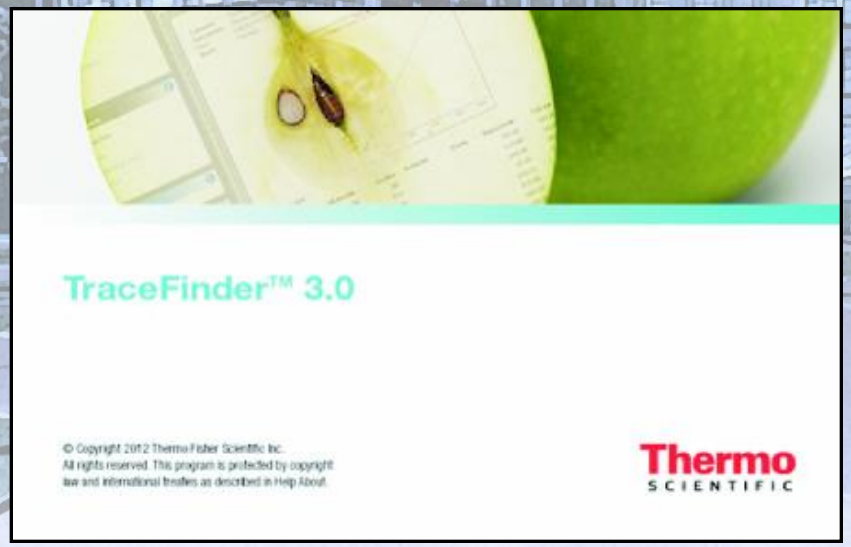
ESI source
Column Zorbax SB-C18 **2.1x150mm**
Flow 200 µL/min



Collaboration Thermo-Mario Negri

1. **Screening** of Illicit Drug in complex matrices

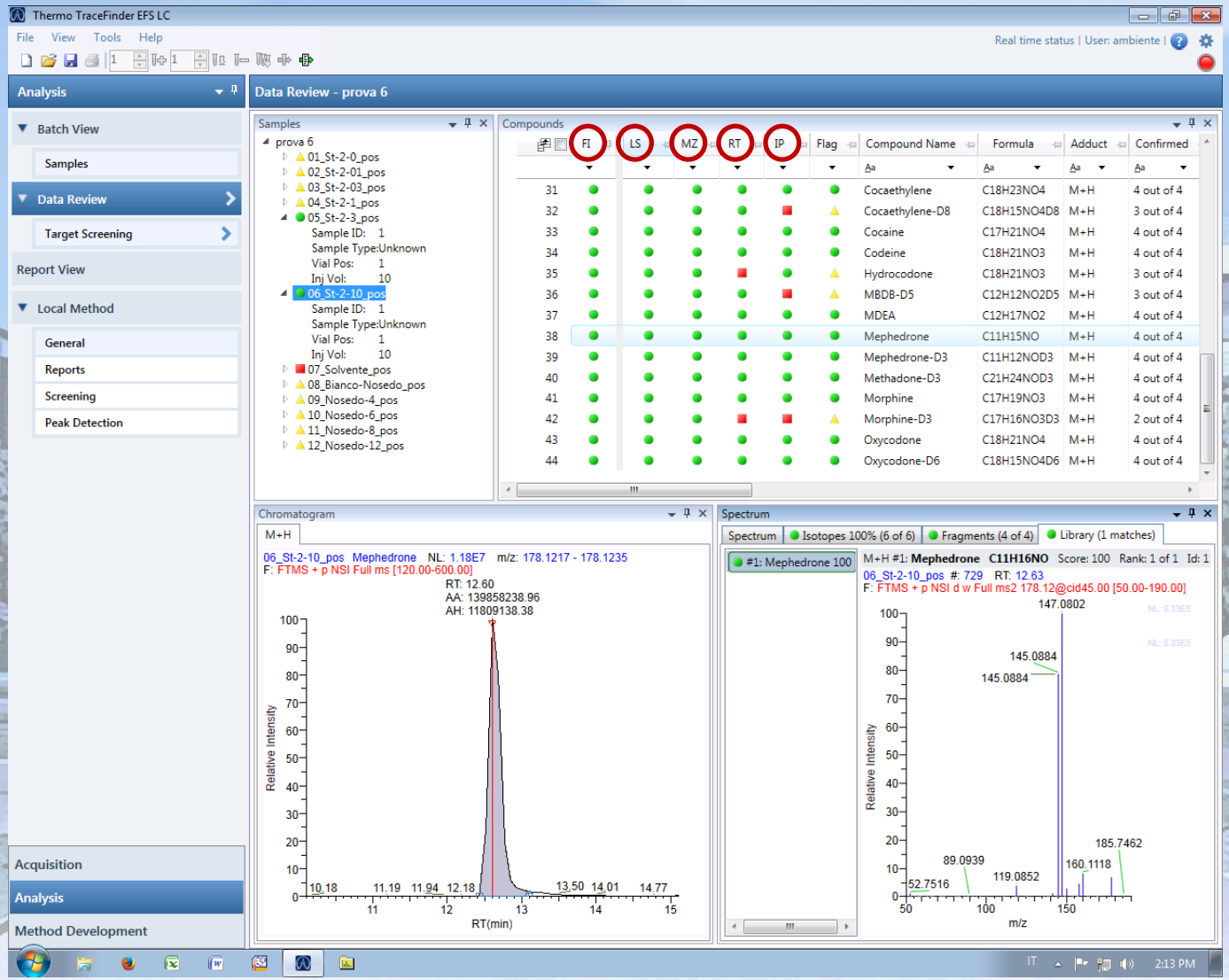
2. Identification and confirmation of **Illicit Drugs and New Drugs** in urban wastewater



TraceFinder application

Software able to do a **rapid screening** of compounds **and identify** then according to different parameters.

Trace Finder screening mode



m/z = mass/charge

RT = Retention time

FI = Fragment Ions

IP = Isotopic Pattern

LS = Library Search (Thermo Library Toxicology HCD 30-70-110 eV)

Screening of therapeutic pharmaceuticals and Illicit Drugs

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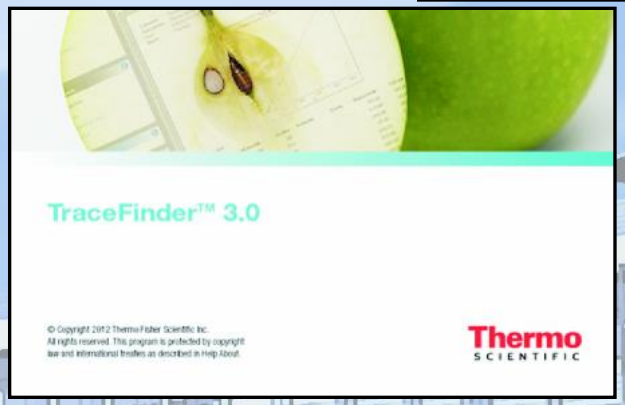
Screening of 290 compounds



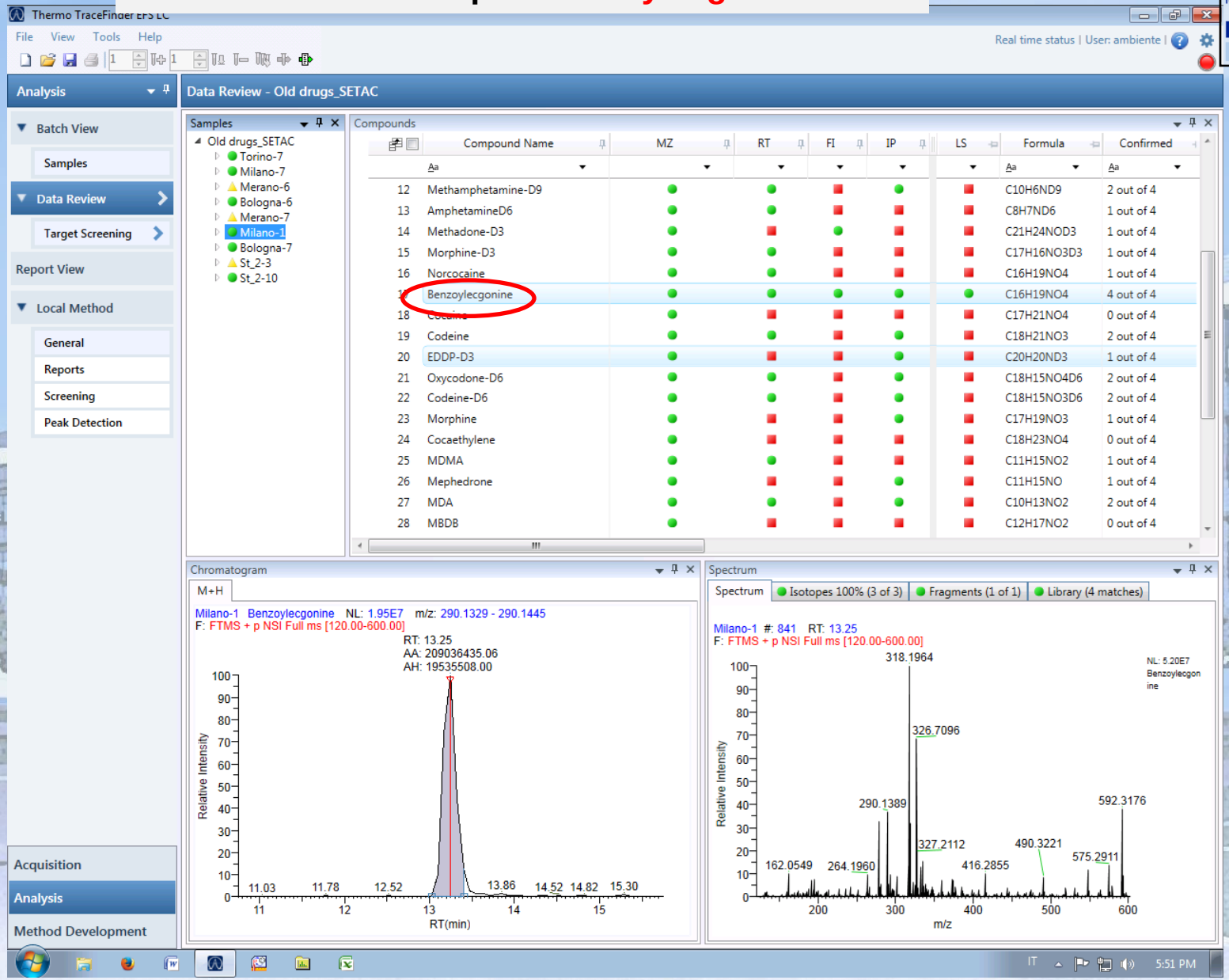
TraceFinder Compound database (drugs and pharmaceuticals)

Thermo library (Library Toxicology HCD 30-70-110 eV)

Results obtained for the analysis of **2 urban wastewater samples:**

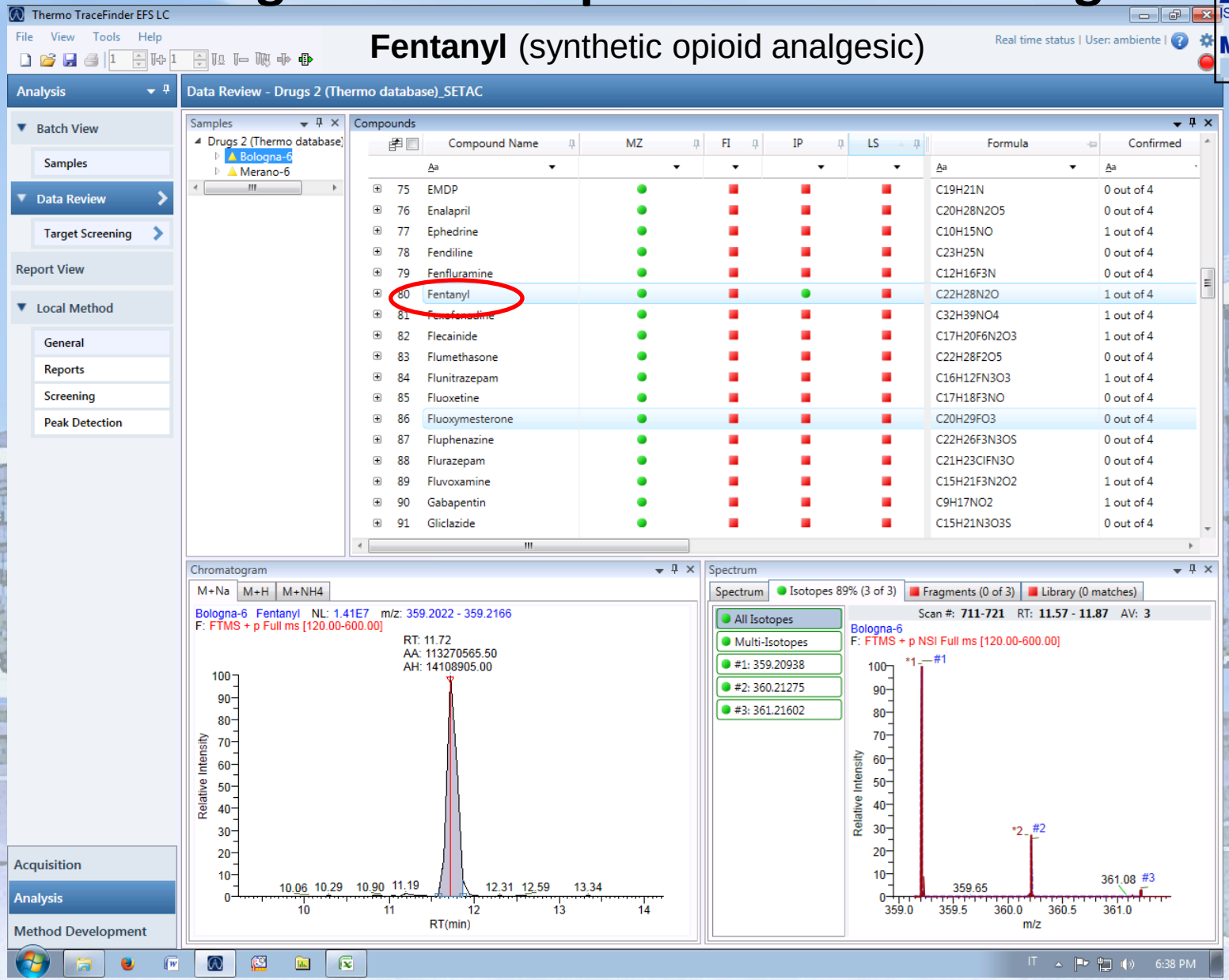
- ✓ **Bologna**: **85** suspected compounds
- ✓ **Merano**: **83** suspected compounds

Real wastewater sample: Benzoylecgonine



Screening of of Therapeutic and Illicit Drugs

Fentanyl (synthetic opioid analgesic)

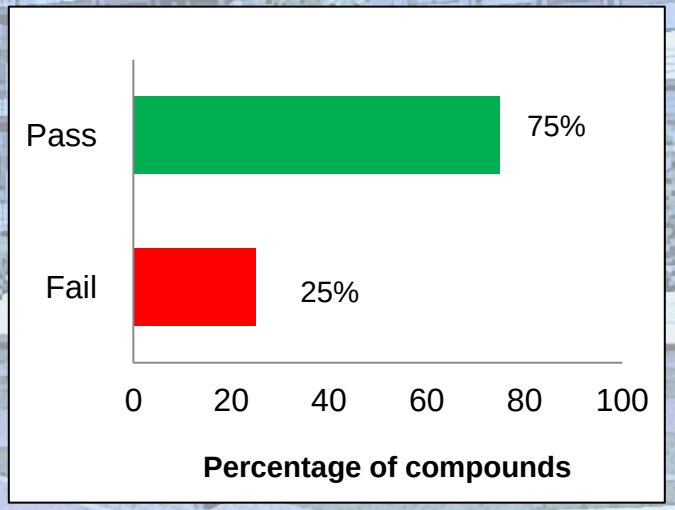


Identification of Illicit Drugs in Raw Wastewater

Screening of 24 illicit selected drugs

a) In-house Compound database
b) Empirical spectral library built with our own standards to have better match

	RT	Isotopes	Fragments	Library (%)
Benzoylecgonine				80
Norbenzoylecgonine				85
Cocaine				77
Norcocaine				82
Cocaethylene				91
Mephedrone				90
Morphine				
6-Acetyl Morphine				
Morphine 3-beta-D-Glucuronide				
Morphine 6-beta-D-Glucuronide				
Codeine				100
6-Acetylcodeine				100
Hydrocodone				90
Oxycodone				100
Amphetamine				
Metamphetamine				
3,4 MDA				70
3,4 MDMA				76
MDEA				87
MBDB				40
Ketamine				100
Norketamine				100
Methadone				79
EDDP				81



Identification of New Drugs and Metabolites

Main groups:

- Synthetic cannabinoids
- Phenethylamines
- Tryptamines
- Cathinones
- Piperazines
- Other miscellaneous substances

Selected list:

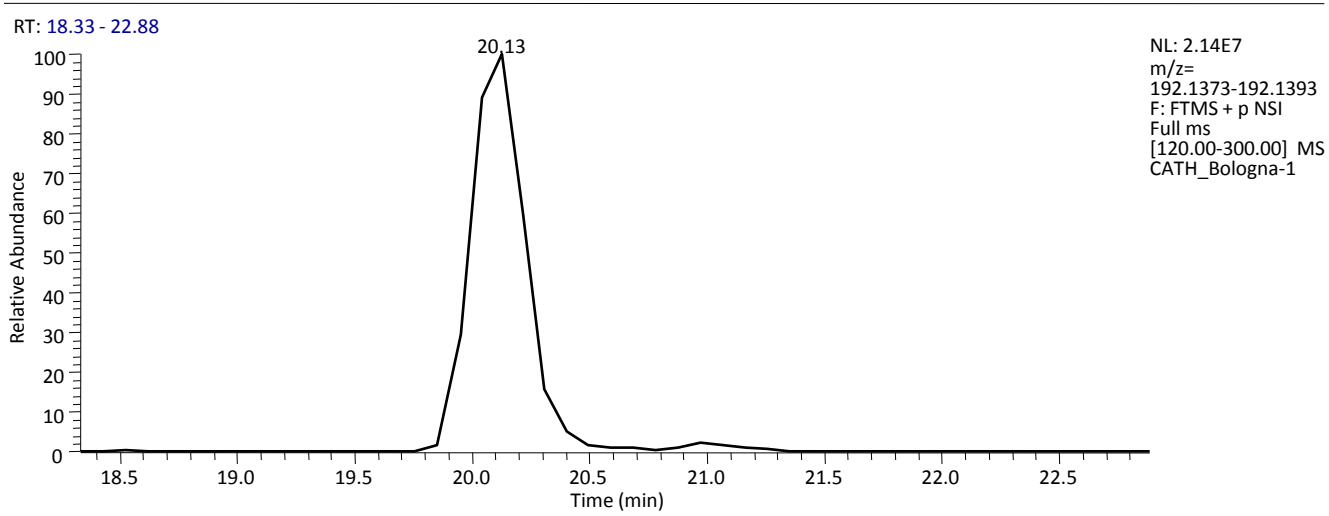
- ***50 compounds*** among 230 substances found in the market
- **Compound Database**
- **Data - Dependent Analysis**
- Comparison of fragments with different libraries
- Analyses with **LTQ Orbitrap and QExactive**

Issues to be solved :

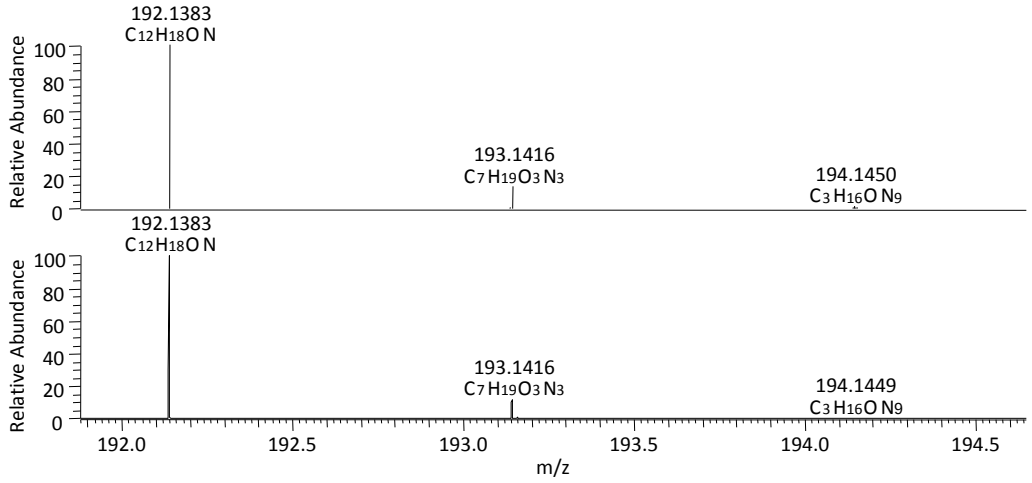
- No or few standards available
- Unknown retention times
- Fragmentation can vary according to different libraries and instruments
- High sensitivity required

Identification of New Drugs and Metabolites

m/z 192.1383: potential 4-Methylethcatinone or Pentedrone – LTQ-Orbitrap XL



Extracted Ion Chromatogram



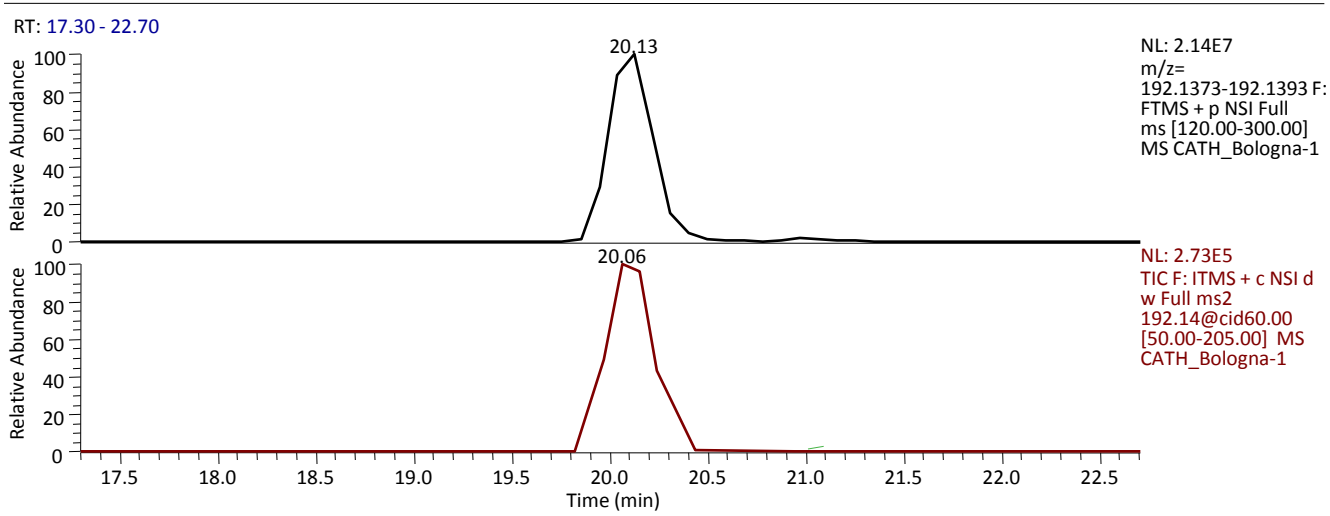
Theoretical MS spectrum for a compound with the same empirical formula

Experimental MS spectrum

✓ **Perfect match**

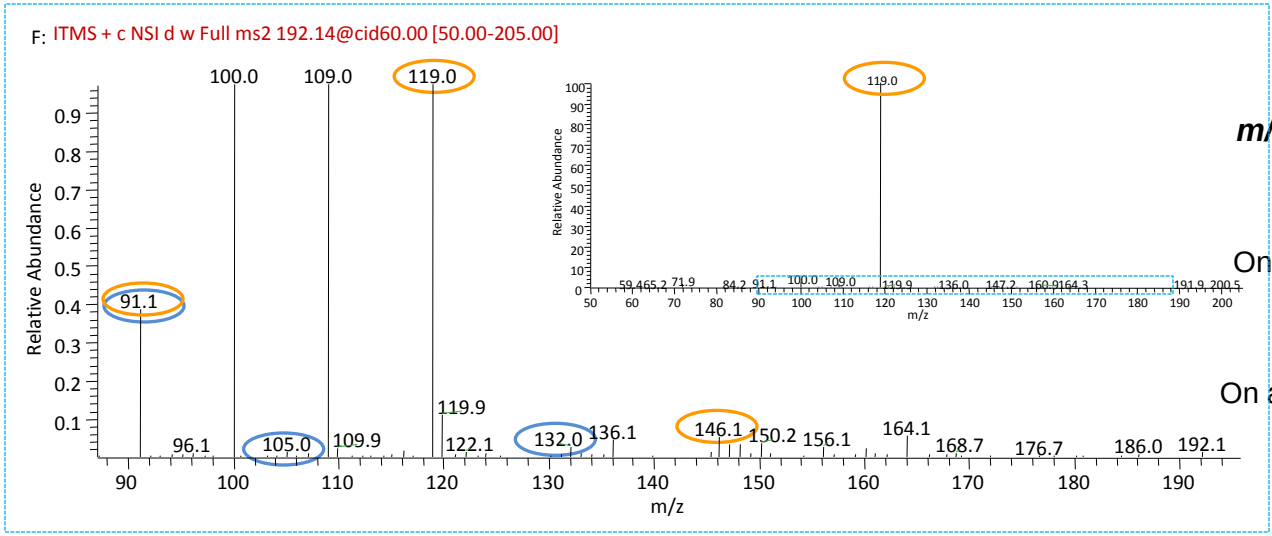
Identification of New Drugs and Metabolites

m/z 192.1383: potential 4-MEC or Pentedrone – LTQ-Orbitrap XL



Extracted Ion Chromatogram

Total Ion Chromatogram (MS/MS)



MS/MS spectrum

***m/z* fragments already recorded for:**

Pentedrone

On a QTOF (METLIN library)

On a Q Exactive™ (ABC 405 (2013) 9437)

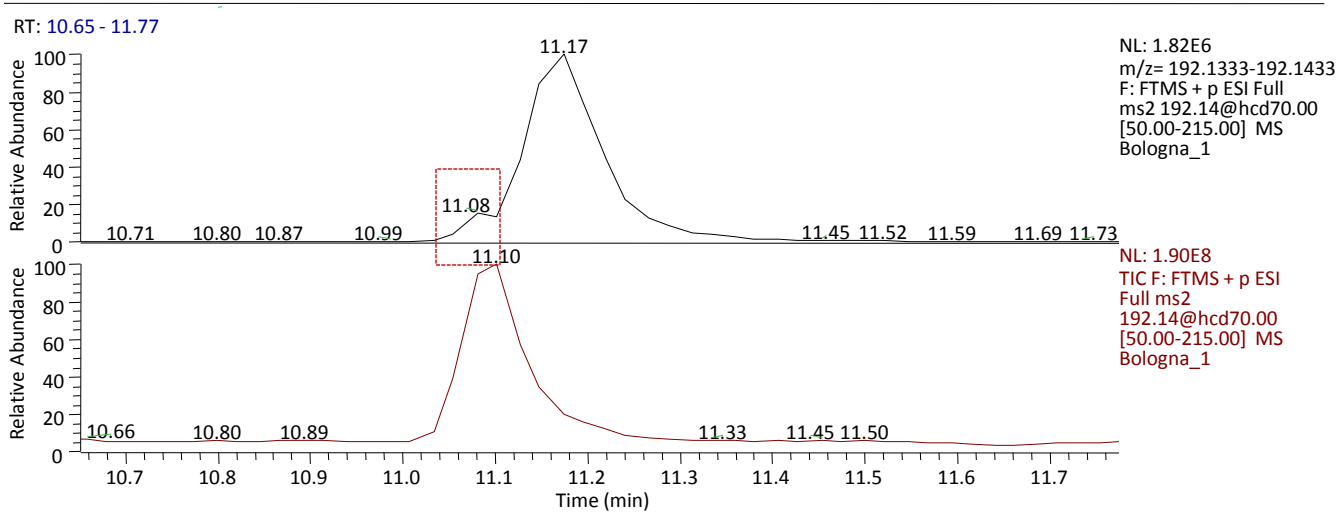
4-MEC

On a QTOF (METLIN library)

On a LTQ-Orbitrap (ForSciInt 234 (2014) 50)

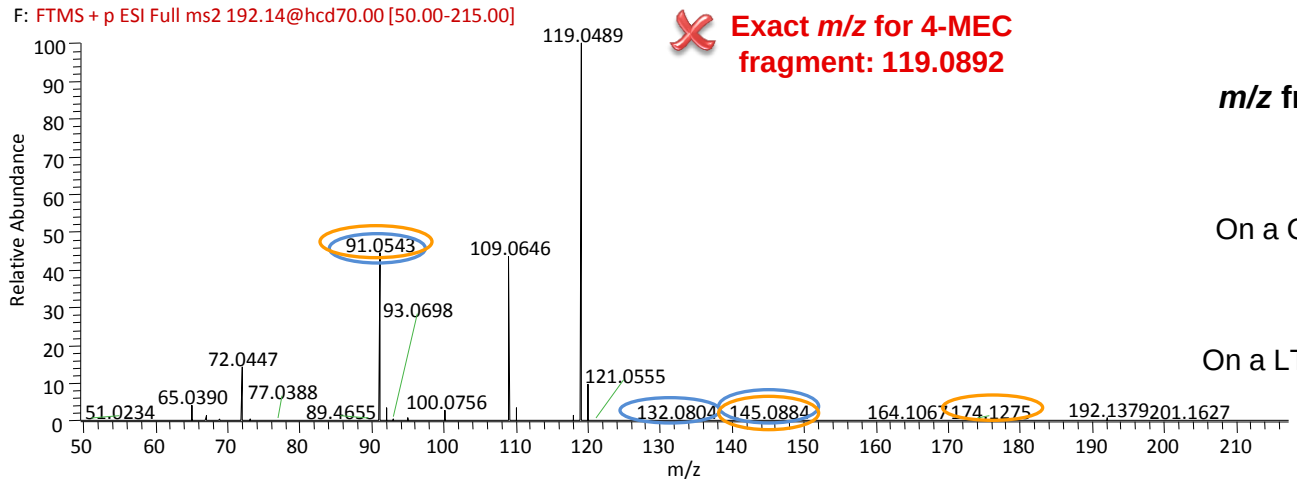
Identification of New Drugs and Metabolites

m/z 192.1383: potential 4-MEC or Pentedrone – Q Exactive™



Extracted Ion Chromatogram

Total Ion Chromatogram (MS/MS)



Exact *m/z* for 4-MEC fragment: 119.0892

MS/MS spectrum

***m/z* fragments already recorded for:**

Pentedrone

On a QTOF (METLIN library)

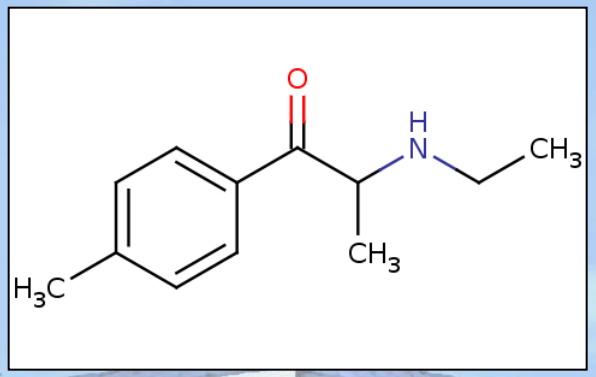
On a Q Exactive™ (ABC 405 (2013) 9437)

4-MEC

On a QTOF (METLIN library)

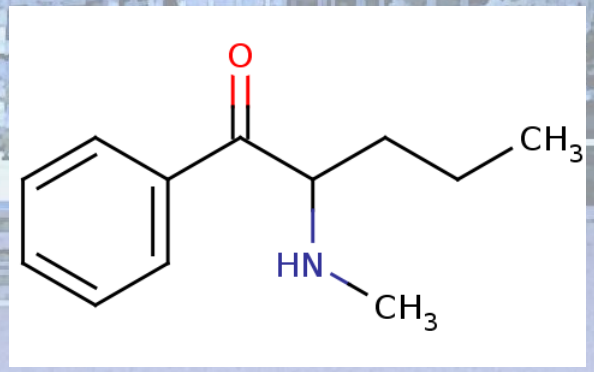
On a LTQ-Orbitrap (ForSciInt 234 (2014) 50)

Identification of New Drugs and Metabolites



4-Methylethcathinone (4-MEC)

C12H18NO 192,1383

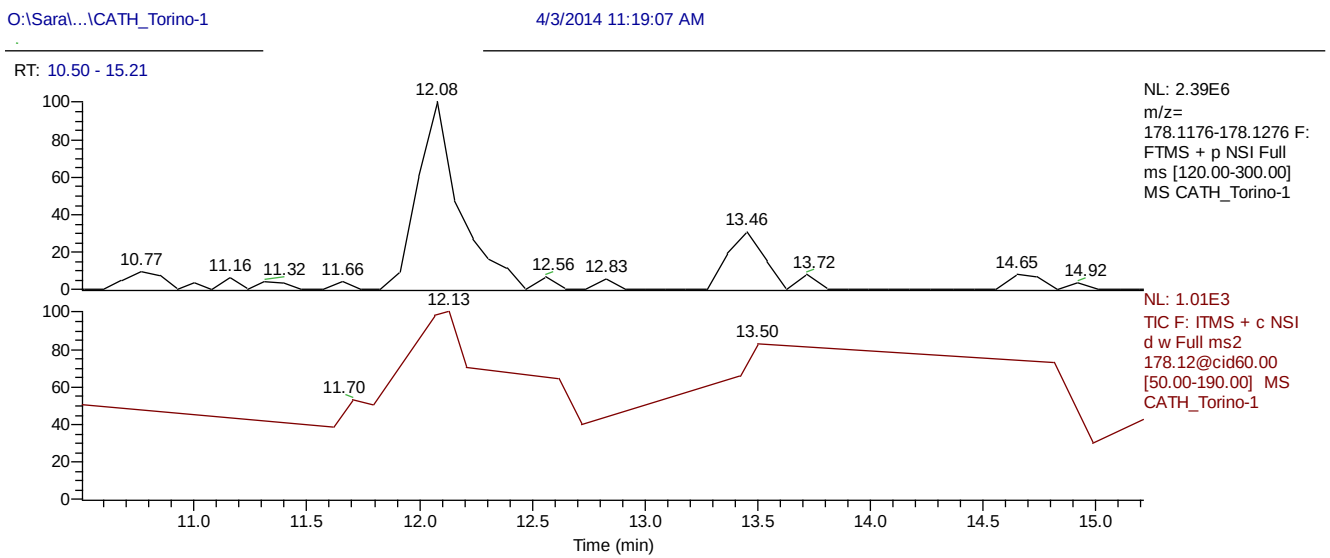


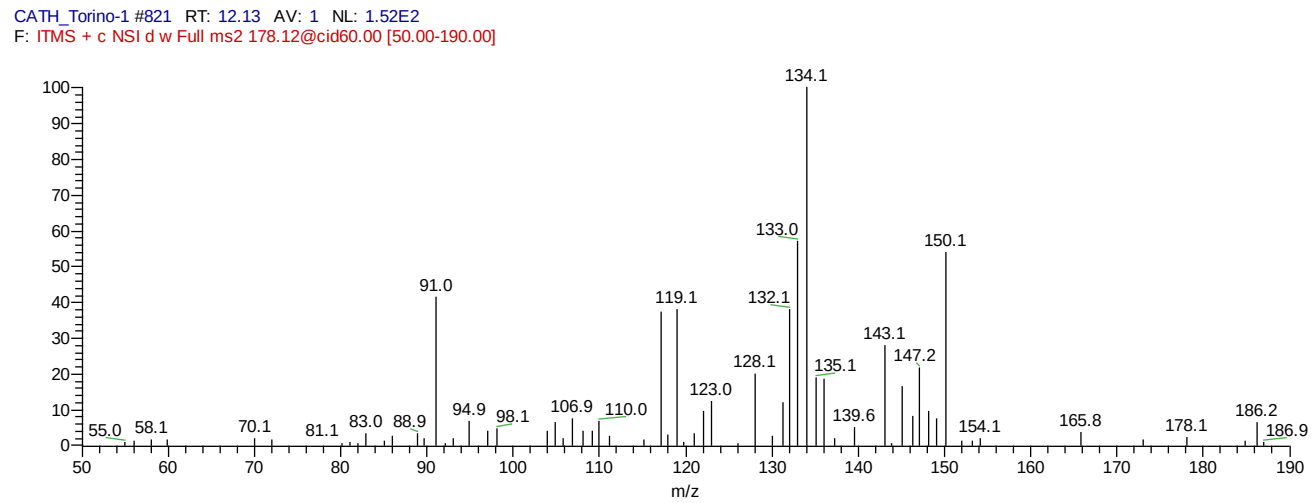
Pentedrone

C12H18NO 192,1383

Identification of New Drugs and Metabolites

m/z 178.1226: potential different cathinones – LTQ-Orbitrap XL





Extracted Ion Chromatogram

Total Ion Chromatogram (MS/MS)

MS/MS spectrum

Some fragments (91, 119, 150) match some *m/z* fragments already recorded for some of the cathinones

	Name	ChemSpider/PubChem ID	Formula $[M+H]^+$	m/z $[M+H]^+$	Formula	m/z	Formula	m/z	Formula	m/z	Formula	m/z
SYNTHETIC CANNABINOIDS	JWH-081	8722599	C25H26NO2	372,1958	C12H9O2	185,0597	C14H16NO	214,1226	C11H9O	157,0648	C9H6NO	144,0444
	JWH-018	8558143	C24H24NO	342,1852	C11H7O	155,0491	C14H16NO	214,1226	C9H6NO	144,0444	C10H7	127,0542
	JWH-250	23256117	C22H26NO2	336,1958	C8H9O	121,0648	C7H7	91,0542	C10H10N	144,0808	C14H16NO	214,1226
	JWH-073	8647081	C23H23NO	328,1696	C11H7O	155,0491	C13H14NO	200,1070	C9H6NO	144,0444	C10H7	127,0542
	JWH-122	24623066	C25H26NO	356,2009	C12H9O	169,0648	C14H16NO	214,1226	C9H6NO	144,0444	C11H9	141,0699
	AM-694	8064843	C20H20FINO	436,0568	C7H4IO	230,9301	C20H20NFO	309,1523	C9H6NO	144,0444		
	JWH-210	24617616	C26H28NO	370,2165	C14H16NO	214,1226	C13H11O	183,0804	C12H9	153,0699	C9H6NO	144,0444
	RCS-4	29341522	C21H24NO2	322,1802	C14H16NO	214,1226	C7H7O	107,0491	C8H7O2	135,0441	C9H6NO	144,0444
	JWH-073 methyl derivative		C24H24NO	342,1852	C14H16NO	214,1226	C11H9	141,0699	C12H9O	169,0648	C9H6NO	144,0444
	JWH-019	24598813	C25H26NO	356,2009	C15H18NO	228,1383	C11H7O	155,0491	C9H6NO	144,0444	C10H7	127,0542
	AM-2201	24751884	C24H23FNO	360,1758	C14H15NFO	232,1132	C10H7	127,0542	C11H7O	155,0491		
	APINAKA (AKB48)	28189076	C23H32N3O	366,2540	C10H15	135,1168	C8H5N2O	145,0396	C13H15N2O	215,1179	C8H11	107,0855
	AKB-48F		C23H31FN3O	384,2446	C10H15	135,1168	C13H14N2OF	233,1085	C8H5N2O	145,0396		
	MAM-2201	CID 66570720	C25H25FNO	374,1915	C14H15FNO	232,1132	C12H9O	169,0648				
	RCS-4 (C4)	29341521	C20H22NO2	308,1645	C13H14NO	200,1070	C8O2H7	135,0441	C9H6NO	144,0444	C7H7O	107,0491
	STS-135	CID 66570720	C24H32FN2O	383,2493	C14H15FNO	232,1132	C10H15	135,1168				
	5FUR-144	28537382	C21H29FNO	330,2228	C14H15FNO	232,1132	C9H6NO	144,0444	C8H13O	125,0961	C7H13	97,1012
	5F-PB-22	29341631	C23H22FN2O2	377,1660	C14H15FNO	232,1132	C9H6NO	144,0444	C8H6N	116,0495	C5H10F	89,0761
	JWH 018 N-5-hydroxypentyl		C24H24NO2	358,1802	C11H7O	155,0491	C14H16NO2	230,1176	C10H7	127,0542	C9H6NO	144,0444
	JWH 018 N-pentanoic acid		C24H22NO3	372,1594	C11H7O	155,0491						
	JWH 073 N-butanoic acid		C23H20NO3	358,1438	C11H7O	155,0491						
	JWH 073 N-4-hydroxybutyl		C23H22NO2	344,1645	C10H7	127,0542						
	JWH 122 N-5-hydroxypentyl		C25H26NO2	372,1958	C12H10O	169,06479	C14H16NO2	230,1176				
	AM2201 N-4-hydroxypentyl		C24H23FNO2	376,1707	C11H7O	155,0491						
	RCS-4 N-5-hydroxypentyl		C21H24NO3	338,1751		134						
	PB-22 metabolite (ester hydrolysis+oxidation at 4'-pentyl chain)		C14H18NO3	248,1283		130	C9H6NO	144,0444		158		174
	PB-22 metabolite (ester hydrolysis+oxidation at indole)		C14H18NO3	248,1283		116		134		148		160
CATHINONES + KETAMINES	Mephedrone	21485694	C11H16NO	178,1226	C10H11O	147,0802		145,0884		160,1118		119,0852
	4-Methylethcathinone (4-MEC)	25630091	C12H18NO	192,1383		174,1274		146,0956		145,0886		119,0852
	Methylone (bk-MDMA)	21106350	C11H14NO3	208,0975		190,0869		160,0763	C9H10N	132,0813		
	Methylenedioxypyrovalerone (MDPV)	16788110	C16H22NO3	276,1594	C8H16N	126,1277	C8H7O2	135,0441	C11H11O2	175,0755	C12H13O3	205,0859
	Butylone (bk-MBDB)	21073070	C12H16NO3	222,1125		204,1083	C11H11O3	191,0703		174,0913		146,0963
	β-Ethyl-methcathinone (Pentedrone)	26286729	C12H18NO	192,1383		132,0808		145,0903		161,0980		174,1304
	3-Fluoromethcathinone (3-FMC)	24958236	C10H13FNO	182,0976		164,0911		129,0815		103,0575		149,0676
	3-Methylmethcathinone (3-MMC)	9341753 (HCl)	C11H16NO	178,1226		119,0849		160,1115		145,088	C7H7	91,0542
	Ethylcathinone	403504	C11H16NO	178,1226		160,1125		132,0809		131,073	C8H9	105,0701
	α-Pyrrolidinopentiophenone (α-PVP)	9324063	C15H22NO	232,1696	C12H15NO	189,1144	C7H5O	105,0335	C8H16N	126,1277	C11H13O	161,0961
	Buphedrone	26286946	C11H16NO	178,1226		160,1117	C10H11O	147,0804	C9H10N	132,0804		119,0851
	Pyrovalerone	13733	C16H24NO	246,1852	C13H17NO	203,1305	C8H9	105,0699	C8H16N	126,1277	C12H15O	175,1117
	4-Fluoromethcathinone (Flephedrone, 4-FMC)	21477355	C10H13FNO	182,0976		123,0601		149,0631		164,087		
	Ketamine	3689	C13H17ClNO	238,0993		207,0572		163,0308		179,0621		125,015
	Methoxetamine	24721792	C15H22NO2	248,1645	C13H15O2	203,1067	C12H15O	175,1117	C11H11O	159,0804	C8H9O	121,0648
	2-MeO-Ketamine	27470964	C14H20NO2	234,1489	C13H15O2	203,1067	C12H15O	175,1117	C11H11O	159,0804	C8H9O	121,0648
	N-Ethylketamine		C14H19ClNO	252,1150	C12H12ClO	207,0571	C11H12ClO	195,0571				
PHENETHYLAMINES + OTHERS	25I-NBOMe	427392	C18H23INO3	428,0717	C8H9O	121,0648				272,1425		
	5-(2-aminopropyl)benzofuran (5-APB)	8012953	C11H14NO	176,1070		159,0798		131,0534		115,0538		103,0541
	6-APDB	29341766 (HCl)	C11H16NO	178,1226		161,0964	C9H9O	133,0648	C8H9	105,0699	C7H7	91,0542
	4-Fluoroamphetamine (4-FA)	9592	C9H13FN	154,1027		137,0755	C7H6F	109,0448				
	2C-B (MFT)	88978	C10H15BrNO2	260,0281	C9H11BrO2	229,9937	C8H9BrO2	215,9780				
	2C-E	21106222	C12H20NO2	210,1489	C11H16O2	180,1145	C10H13O2	165,0910				
	5-APDB	167143	C11H16NO	178,1226		161,0966	C9H9O	133,0648		105		91
	5-MAPB	29763705 (HCl)	C12H16NO	190,1226	C9H7O	131,0491	C11H11O	159,0804		164,9543		108,9046
	p-Methoxymethamphetamine (PMMA)	171194 (HCl)	C11H18NO	180,1383	C8H9O	121,0648						
	p-Methoxyamphetamine (PMA)	29417	C10H16NO	166,1226	C8H9O	121,0648						
	2C-C-NBOMe	24583389	C18H23ClNO3	336,1361	C8H9O	121,0648		199,0518		214,0632		91,0543
	4-Metamphetamine (4-MA)	CID 199116	C10H16N	150,1277	C9H11N	133,0886	C8H9	105,0699				
	25H-NBOMe	26599681 (HCl)	C18H24NO3	302,1751	C8H9O	121,0648	C7H7	91,0542				
	CPP	1314	C10H14ClN2	197,0840	C8H9ClN	154,0418	C8H9N	119,0730	C6H4Cl	110,9996		
	α-Methyltryptamine (AMT)	8930	C11H15N2	175,1230	C9H8N	130,0651	C7H5N	103,0417				
	Fentanyl	3228	C22H29N2O	337,2274	C13H18N	188,1434	C8H9	105,0701				
	Diphenidine		C19H24N	266,1903	C14H13	181,1012	C5H12N	86,0964	C8H7	103,0542	C8H8N	118,06513

Standards arrived on March 2014 (New drugs)

Alerta compounds, selected

Compounds already detected in Italy, selected

Metabolites included in study by Reid et al (Drug Test. Analysis 2013) and Huestis et al (ABC 2014) Only JWH 018 N5-hydroxypentyl found in real samples

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	Name	Selected NPS List	ChemSpider/PubChem ID	Formula $[M+H]^+$	Theo. *m/z* $[M+H]^+$
CATHINONES	Methcathinone		1519	C10H14NO	164,1070
	N,N-Dimethylcathinone (Metamfepramone)		64889	C11H16NO	178,1226
	Ethcathinone		403504	C11H16NO	178,1226
	Methedrone (bk-PMMA)		187475	C11H16NO2	194,1176
	4-Fluoromethcathinone (Flephedrone, 4-FMC)	Yes	21477355	C10H13FNO	182,0976
	3,4-Dimethylmethcathinone (3,4-DMMC)		25630192	C12H18NO	192,1383
	4-Methylethcathinone (4-MEC)	Yes	25630091	C12H18NO	192,1383
	Buphedrone	Yes	26286946	C11H16NO	178,1226
	β-Ethyl-methcathinone (Pentedrone)	Yes	26286729	C12H18NO	192,1383
	Methylone (bk-MDMA)	Yes	21106350	C11H14NO3	208,0968
	Ethylone		21106271	C12H16NO3	222,1125
	Butylone (bk-MBDB)	Yes	21073070	C12H16NO3	222,1125
	Pentylone (bk-MBDP)		29786041	C13H18NO3	236,1281
	1-Naphyrone		-	C19H24NO	282,1852
	Naphyrone (NRG-1, O-2482, naphthylpyrovalerone)		9418039	C19H24NO	282,1852
SYNTHETIC CANNABINOIDS	JWH-007		8536309	C25H26NO	356,2009
	JWH-016		3224428	C24H24NO	342,1852
	JWH-019	Yes	24598813	C25H26NO	356,2009
	JWH-081	Yes	8722599	C25H26NO2	372,1958
	JWH-098		CID 45272116	C26H28NO2	386,2115
	JWH-122	Yes	24623066	C25H26NO	356,2009
	JWH-147		23277882	C27H28NO	382,2165
	JWH-203		23256082	C21H23ClNO	340,1463
	JWH-210	Yes	24617616	C26H28NO	370,2165
	JWH-251		9791472	C22H26NO	320,2009
	JWH-302		9668546	C22H26NO2	336,1958
	JWH-307		13178178	C26H25FNO	386,1915
	JWH-398		28647395	C24H23ClNO	376,1463
	AM-694	Yes	8064843	C20H20FINO	436,0568
	AM-2201	Yes	24751884	C24H23FNO	360,1758
	RCS-4	Yes	24769418	C21H24NO2	322,1802
	RCS-8		24751863	C25H30NO2	376,2271
	CB-13		7975182	C26H25O2	369,1849
	Pravadoline (WIN 48,098)		50942	C23H27N2O3	379,2016
	AM-2233		8401830	C22H24IN2O	459,0928
	Methylenedioxypyrovalerone (MDPV)	Yes	16788110	C16H22NO3	276,1594

Standards arrived on March 2014 (New drugs)

Conclusions

- **The application of new integrated methods of analysis and the availability of analytical standard can implement the identification of the residues of new PS in wastewater.**
- **Wastewater analysis can contribute to study the use of new PS in a population.**
- **The identification of new PS in wastewater is indicative of a significant use in the population.**
- **The identification of the same molecule in different daily samples of wastewater is indicative of a continuous use of a given PS in the population.**
- **Wastewater analysis can help to discriminate the PS which became of current use in the population.**

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