Study of drugs of abuse consumption patterns from on-line SPE-LCMS/MS analysis of environmental water samples

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INNOVAMED COURSE
AND
MEDITERRANEAN WORKSHOP ON
NEW TECHNOLOGIES OF RECYCLING NON CONVENTIONAL WATER IN PROTECTED CULTIVATION

28 April – 1st May 2008 Agadir, MOROCCO

Outline

1. Introduction and objectives

2. Analysis of drugs of abuse in water:

- **Method description and performance**
- **Application to real samples:**
  - *** Sewage water**
    - 1 STP from Barcelona (El Prat-Depurbaix)
    - 3 STPs from tourist locations in A.C. Valencia
  - *** Surface water (Llobregat river, Barcelona)**
- **Estimation of drug consumption**

3. Conclusions

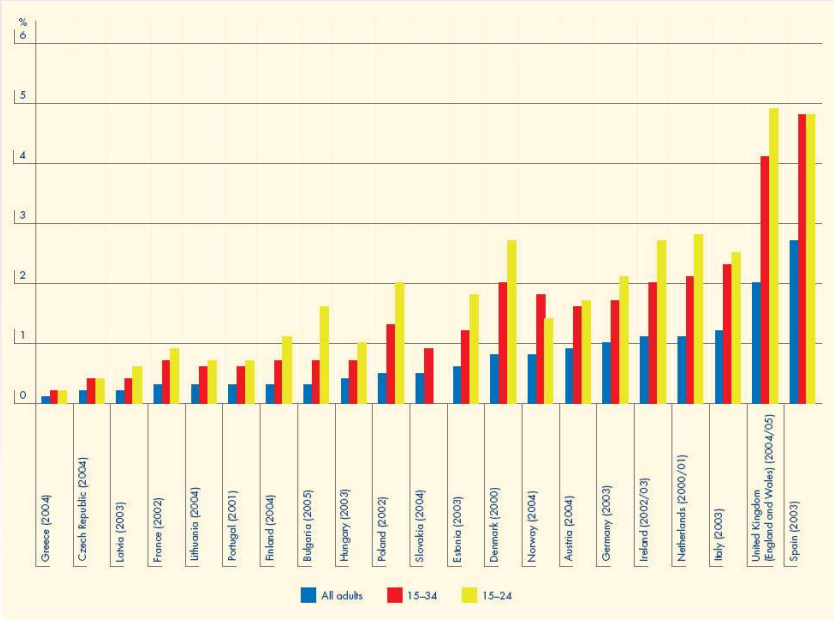
Introduction

World Drug Report 2006 of the United Nations:

-Steady increase in the worlwide consumption of drugs of abuse in the last years
-Spain: 1st in cocaine, 4th in amphetamine and MDMA consumption

-Surveys of consumption
-Medical and criminal statistics
-etc.

(costly and lengthly)
(inaccurate, subjective)
(no real-time information)



Introduction

New approach:

-Measure the concentration of drugs of abuse in sewage or surface water to estimate drug consumption

Previous works:

-Zuccato et al. (2005) Environ Health: A Global Access Science Source 4, 14 → cocaine & BE in waste and surface water from Italy.
-Castiglioni et al. (2006) Anal. Chem. 78, 8421-8429.→ 10 drugs of abuse in wastewaters from Italy and Switzerland.
-Hummel et al. (2006) Environ. Sci. Technol. 40, 7321-7328. → 20 psychoactive drugs (BE, MOR) in waters from Germany
-Huerta-Fontela et al. (2007) Anal. Chem. 79, 3821-3829. → 15 stimulatory drugs in wastewater and surface water from Spain

Analytical methodology: Off-line SPE, LC-ESI-MS/MS

Objectives

1. to develop a fully automated method based on on-line SPE-LC-MS/MS for the multi-analyte determination of 17 drugs of abuse belonging to different classes in water
2. to apply this method to the analysis of various real sewage and surface water samples
3. to obtain a first, general picture about their occurrence, patterns of consumption, and STP removal efficiency
4. to explore the possibility of air analysis for the same purpose

Target Compounds

Amphetamine-like compounds	
Amphetamine MDMA Methamphetamine *R,R, Pseudoephedrine* *1S, 2R (+) Ephedrine hydrochloride*	*Amphetamine-D_5* *MDMA-D_5* *Methamphetamine-D_{14}* *1S, 2R (+) Ephedrine D_3 hydrochloride*

Opiates	
Heroin Morphine 6-Acetylmorphine Morphine 3-ß-D-gluc. *Morphine 6-ß-D-gluc.*	*Heroin-D_9* *Morphine-D_3* *Morphine 3-ß-D-gluc.-D_3*

LSD	
LSD *Nor-LSD & nor-iso-LSD* *2-oxo-3-hydroxy LSD*	*LSD - D_3*

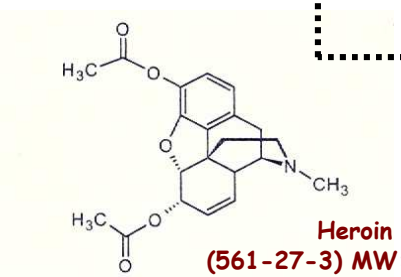
Cocainics	
Cocaine Cocaethylene Benzoylecgonine	*Cocaine - D_3* *Cocaethylene - D_3* *Benzoylecgonine - D_8*

Cannabinoids	
Δ^9-THC *11-hydroxy-THC* 11-Nor-9-Carboxy-Δ9-THC	*Δ^9-THC – D_3*

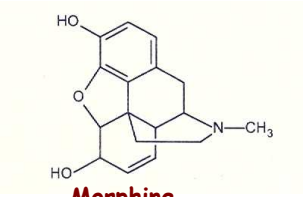
Target Compounds:

structure, CAS number and molecular weight

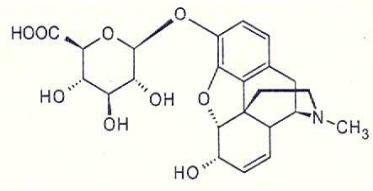
Opiates



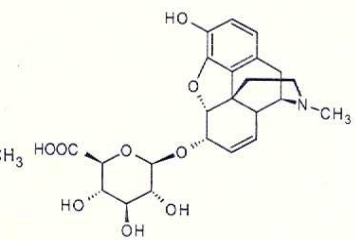
Heroin
(561-27-3) MW: 369.42



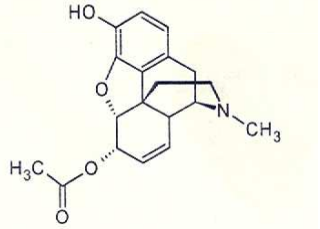
Morphine
(57-27-2) MW: 285.34



Morphine 3-β-D-glucuronide
(20290-09-9) MW: 461.67

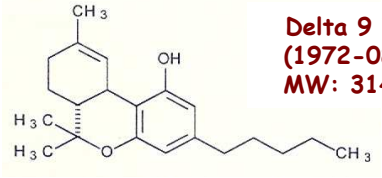


Morphine 6-β-D-glucuronide
(20290-09-9) MW:461.67

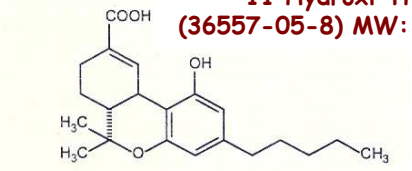


6-acetylmorphine
(2784-73-8) MW:327.38

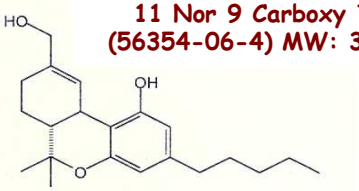
Cannabinoids



Delta 9 THC
(1972-08-3)
MW: 314.47

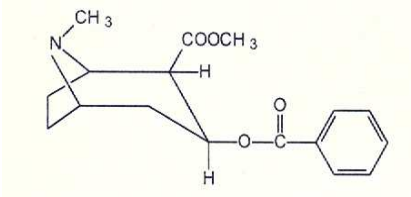


11 Hydroxi THC
(36557-05-8) MW: 330.47

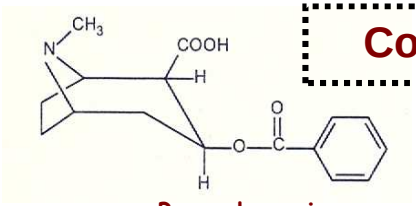


11 Nor 9 Carboxy THC
(56354-06-4) MW: 344.45

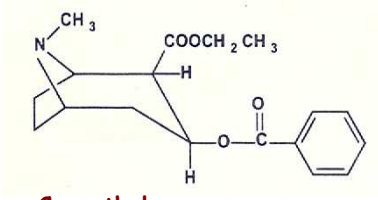
Cocainics



Cocaine
(50-36-2) MW: 303.36



Benzoylecgonine
(519-09-5) MW: 289.33

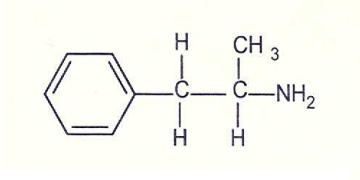


Cocaethylene
(529-38-4) MW: 317.38

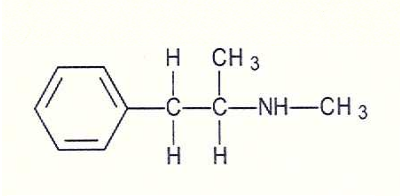
Target Compounds:

structure, CAS number and molecular weight

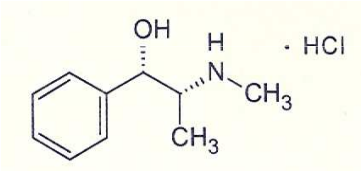
Amphetamine like compounds



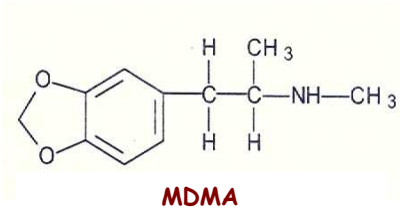
Amphetamine
(300-62-9) MW: 135.21



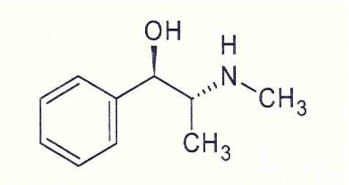
Methamphetamine
(4846-07-5) MW: 149.24



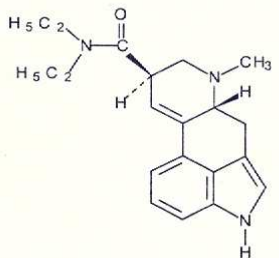
S, 2R (+) Ephedrine Hydrochloride
(24221-86-1) MW: 201.69



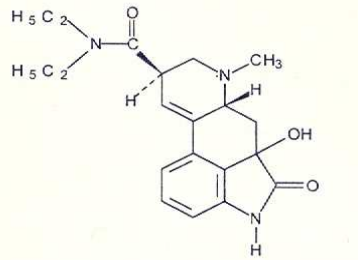
MDMA
(42542-10-9) MW: 193.25



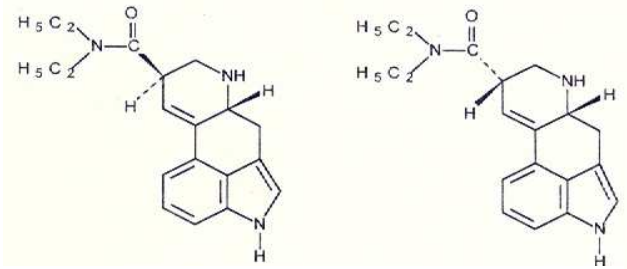
R,R, Pseudoephedrine
(321-97-1) MW: 165.24



LSD
(50-73-3) MW: 323.44



2-Oxo-3-hydroxy LSD
(N/A) MW: 355.44

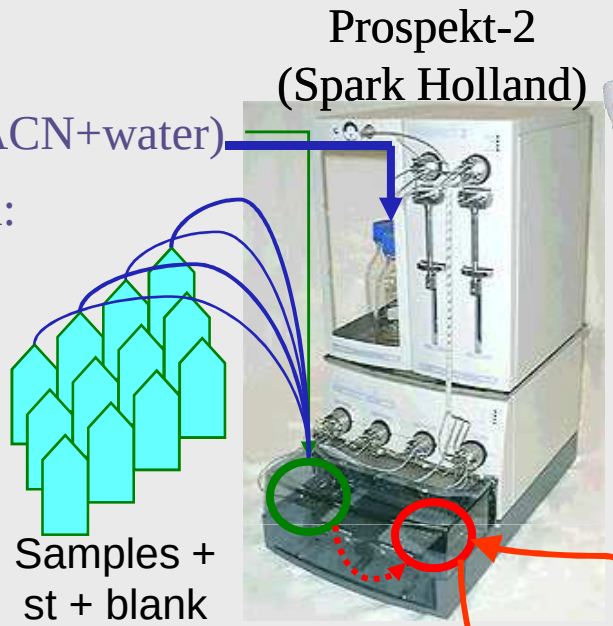


Nor-LSD & nor-iso-LSD
(35779-43-2/71953-76-9) MW: 309.41

LSD & its metabolites

Scheme of the method developed

1. Conditioning solvents (ACN+water)
2. Sample preconcentration:
   volume 5mL
   flow 1 mL/min
3. Cartridge washing
   (HPLC water)
4. Elution
   (mobile phase)



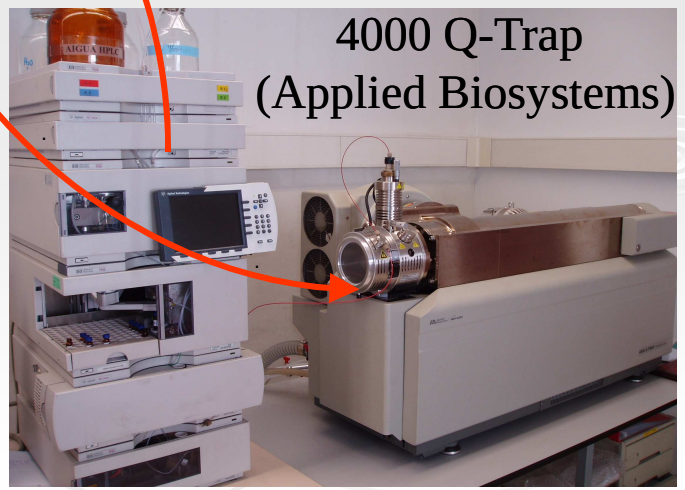
On-line SPE

Cartridge (10 × 2 mm):

- **PLRP-s** for all analytes but cannabinoids (detected in PI)
- **Oasis HLB-s** for cannabinoids (detected in NI)

+ 35 min

LC-ESI-(QqLIT)-MS/MS analysis



Column: Purospher STAR-RP-18e (125 x 2 mm, 5 µm)
Mobile phase: linear gradient ACN/water
Flow rate: 0.3 mL/min
Detection: Electrospray (PI and NI)
MRM → 2 transitions per compound (4 IPs)

Optimized MRM conditions

Target compounds	Abbrev.	Retention Time (min)	MRM transitions (m/z) Precursor ion → Product ion	DP[a] (V)	CE[b] (V)	MRM ratio (SRM1/SRM2)
Compounds analysed in Positive Ionization (PI) Mode						
1 S, 2R(+)Ephedrine Hydrochloride	EPH	9.06 ± 0.16	166.2 → 148.0	40	20	3.81 ± 0.47
			→ 133.0	30	30	
R,R, Pseudoephedrine	EPH	9.06 ± 0.16	166.2 → 148.0	40	20	3.81 ± 0.47
			→ 133.0	30	30	
1S, 2R(+) Ephedrine d3 Hydrochloride	*EPH-d_3*	9.02 ± 0.18	169.3 → 151.0	40	20	-
Amphetamine	AM	10.03 ± 0.17	136.2 → 91.0	30	20	1.68 ± 0.25
			→ 119.0	30	15	
Amphetamine d5	*AM-d_5*	9.93 ± 0.19	141.2 → 96.0	30	20	-
MDMA	MDMA	10.84 ± 0.21	194.3 → 163.0	50	20	2.41 ± 0.13
			→ 105.0	50	35	
MDMA d5	*MDMA-d_5*	10.83 ± 0.19	199.2 → 135.0	40	35	-
Methamphetamine	MA	10.82 ± 0.21	150.2 → 91.0	50	30	3.29 ± 0.42
			→ 119.0	50	20	
Methamphetamine d14	*MA-d_{14}*	10.77 ± 0.21	164.2 → 98.0	50	30	-
Benzoylecgonine	BE	7.00 ± 0.02	290.3 → 168.0	80	35	2.29 ± 0.15
			→ 77.0	70	100	
Benzoylecgonine d8	*BE-d_8*	6.94 ± 0.02	298.2 → 171.0	80	30	-
Cocaine	CO	13.73 ± 0.19	304.4 → 182.0	70	30	3.53 ± 0.43
			→ 77.0	70	90	
Cocaine d3	*CO-d_3*	13.71 ± 0.22	307.4 → 185.0	70	25	-
Cocaethylene	CE	14.52 ± 0.10	318.4 → 196.0	70	30	4.71 ± 0.66
			→ 77.0	70	95	
Cocaethylene d3	*CE-d_3*	14.51 ± 0.16	321.4 → 199.0	70	30	-
2-Oxo-3-hydroxy LSD	O-H-LSD	8.31 ± 0.02	356.4 → 237.0	50	35	2.10 ± 0.28
			→ 222.0	60	40	
nor-LSD & nor-iso-LSD	Nor-LSD	10.63 ± 0.10	310.4 → 193.0	60	40	0.37 ± 0.02
			→ 209.0	60	70	
LSD	LSD	10.52 ± 0.11	324.4 → 208.0	70	40	0.82 ± 0.05
			→ 223.0	60	40	
LSD d3	*LSD-d_3*	10.54 ± 0.13	327.4 → 226.0	60	35	-
Morphine 6-ß-D-glucuronide	M6G	-	462.5 → 286.0	80	45	-
			→ 201.0	80	65	
Morphine 3- ß -D-glucuronide	M3G	-	462.5 → 286.0	80	45	-
			→ 201.0	80	65	
Morphine 3- ß -D-glucuronide d3	MOR-d_3	-	465.2 → 289.0	80	50	-
Morphine	MOR	7.94 ± 0.06	286.3 → 152.0	90	75	1.54 ± 0.04
			→ 128.0	90	95	
Morphine d3	*MOR-d_3*	7.83 ± 0.09	289.3 → 152.0	90	75	-
6-Acetylmorphine	6ACM	9.50 ± 0.10	328.4 → 165.0	90	80	1.42 ± 0.10
			→ 152.0	90	75	
Heroin	HER	11.04 ± 0.15	370.4 → 268.0	70	50	2.50 ± 0.06
			→ 165.0	70	70	
Heroin d9	*HER-d_9*	10.98 ± 0.15	379.4 → 272.0	70	45	-
Compounds analysed in Negative Ionization (NI) Mode						
11 Nor 9 Carboxy THC	Nor-THC	12.08 ± 0.04	343.5 → 299.5	-100	-35	5.45 ± 0.45
			→ 191.2	-100	-35	
11 Hydroxy THC	OH-THC	15.49 ± 0.03	329.5 → 311.2	-70	-25	7.69 ± 0.59
			→ 268.0	-70	-35	
Delta 9 THC	THC	19.54 ± 0.05	313.5 → 245.1	-70	-40	1.13 ±0.07
			→ 191.0	-70	-40	
Delta 9 THC d3	*THC-d_3*	19.50 ± 0.02	318.4 → 196.0	-70	-40	-

[a] Declustering Potential, [b] Cone Voltage

Method performance

		HPLC water				Sewage water				
	Linearity r^{2a}	LOD [b] (ng/L)	LDet [c] (ng/L)	RSD [d] (%)	AR [e] (%)	LOD [b] (ng/L)	LDet [c] (ng/L)	RSD [d] (%)	AR [e] (%)	RR [f] (%)
EPH	0.9968	0.04	0.12	2.4	73	0.78	2.21	3.8	15	101
EPH-d_3(IS)					68				15	
AM	0.9990	0.07	0.20	2.3	85	0.34	0.92	12.4	15	94
AM-d_5(IS)					75				16	
MDMA	0.9994	0.05	0.14	8.2	121	1.10	2.93	9.1	27	121
MDMA-d_5(IS)					103				22	
MA	0.9979	0.03	0.08	10.4	97	0.28	0.75	2.7	20	114
MA-d_{14}(IS)					105				17	
BE	0.9941	0.01	0.02	8.2	98	0.67	5.24	2.5	8	115
BE-d_8(IS)					80				7	
CO	0.9974	0.01	0.04	8.7	85	0.18	2.40	11.7	59	173
CO-d_3(IS)					81				34	
CE	0.9945	0.00	0.04	4.2	120	0.07	0.69	6.5	52	105
CE-d_3(IS)					117				50	
O-H-LSD	0.9977	0.02	0.04	4.5	69	0.97	2.60	4.2	11	71
nor-LSD	0.9978	0.03	0.09	4.7	91	0.68	1.81	8.2	22	145
LSD	0.9975	0.01	0.02	8.2	112	0.27	0.89	3.9	17	107
LSD-d_3(IS)					96				15	
MOR	0.9974	0.04	0.10	3.4	69	1.51	5.97	2.2	14	77
MOR-d_3(IS)					60				18	
6ACM	0.9984	0.06	0.17	10	55	1.94	5.17	1.8	21	118
HER	0.9997	0.04	0.10	9.6	76	0.78	2.07	4.2	22	121
HER-d_9(IS)					67				18	
nor-THC	0.9949	0.05	0.12	1.4	93	0.43	1.13	7.0	13	266
OH-THC	0.9921	0.08	0.23	3.5	57	0.54	1.45	4.4	37	745
THC	0.9949	1.15	3.06	6.3	8	1.26	3.37	14.0	9	173
THC-d_3(IS)					8				5	

a Linearity. Calibration range 0.1-1000 ng/L (0.1-5000 ng/L for BE and CO)
b Limit of Detection of the first SRM transition
c Limit of Quantitation of the first SRM transition
d Limit of Determination: minimum concentration that can be quantified (>LOQ, SRM1) and confirmed (>LOD, SRM2)
e Repeatability. Spiking concentration: 50 ng/L (n=7)
f Calculated from the peak areas obtained in on-line anlysis of spiked (50 ng/L) water samples as percentages of the peaks areas obtained from direct chromatographic injection (5ul) of equivalent amounts of the standards in methanol.
g Relative to the associated deuterated surrogate standard.

Matrix effects - Corrected by SS

Main advantages of the method

On-line Solid Phase Extraction

- ✓ **Low sample volume requirements = 5 ml**
  - → easy sample storage (at –20ºC)
- ✓ **Minimum sample manipulation** (filtration and addition of the SS)
  - → Improved accuracy and repeatability
- ✓ **Full automation and autonomy:**
  - → 4 six-port valves = 24 solns (6 cal. solns+15 samples+1 blk+ACN+water)
  - → 2 trays x 96 cartridges each = up to 192 unattended analyses
- ✓ **High throughput:**
  - → simultaneous $SPE_{(n+1)}$ and $LC\text{-}MS\text{-}MS_{(n)}$
  - → analysis time/sample = 35 min (PI)+35 min(NI).
- ✓ **Robustness**
- ✓ **Cost and time savings:**
  - → N_2 for evaporation, eluting solvents
  - → low maintenance
  - → easy operation (no need for highly qualified staff)
  - → automatic data processing (Analyst 1.4.2) …

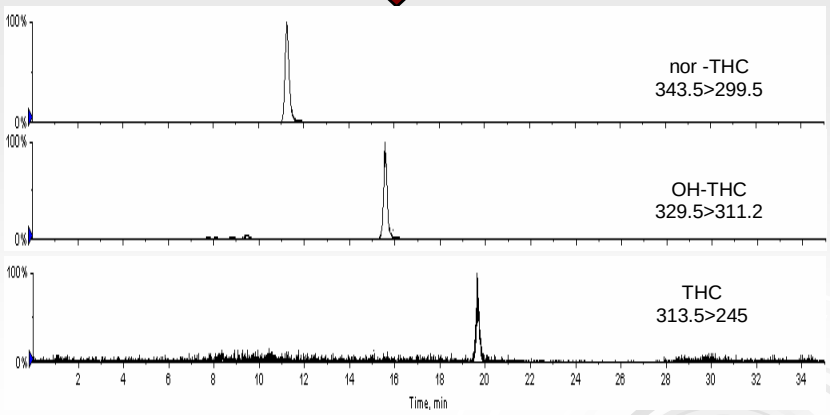
LC-MS/MS (IS quant.) → Sensitivity, selectivity, and reliability of results

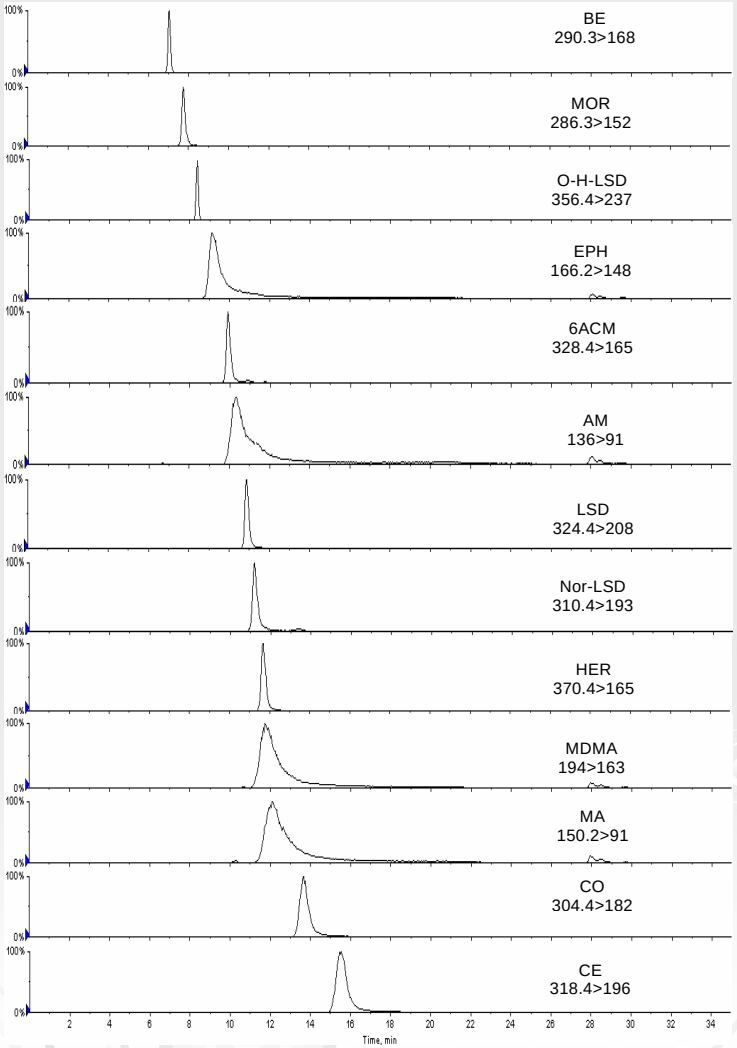
Reconstructed ion chromatograms obtained from the on-line SPE-LC-ESI--(QqLIT)MS-MS analysis of drugs of abuse

POSITIVE IONIZATION MODE

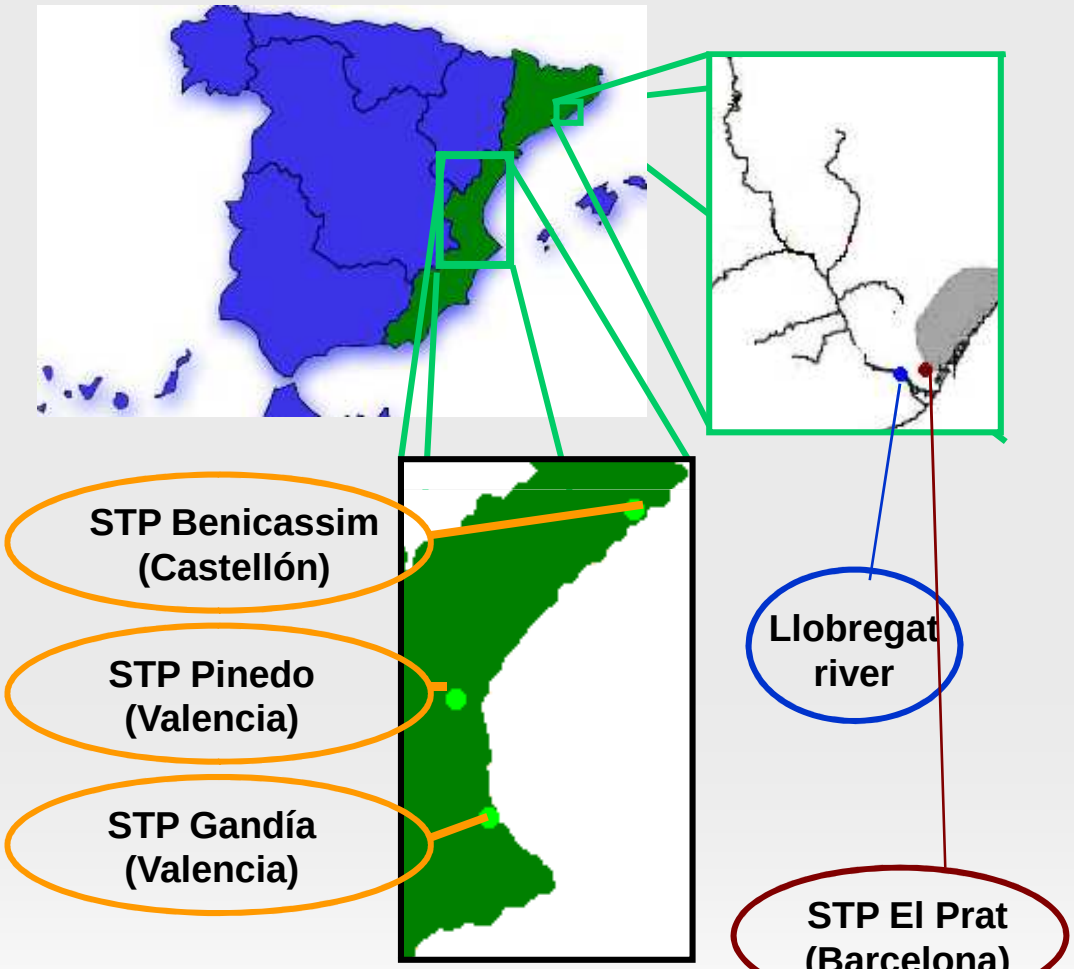
Spiked (50 ng/L) HPLC grade water

NEGATIVE IONIZATION MODE





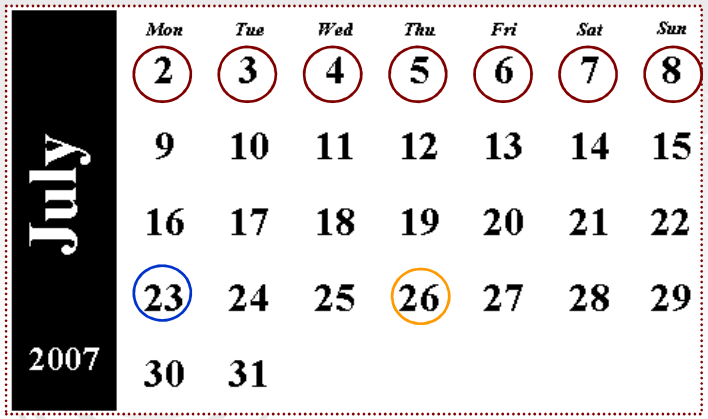
Application to real samples



24-hour integrated influent and effluent sewage water samples

Drug consumption tendencies?

Water from the Llobregat river



Criteria for positive identification

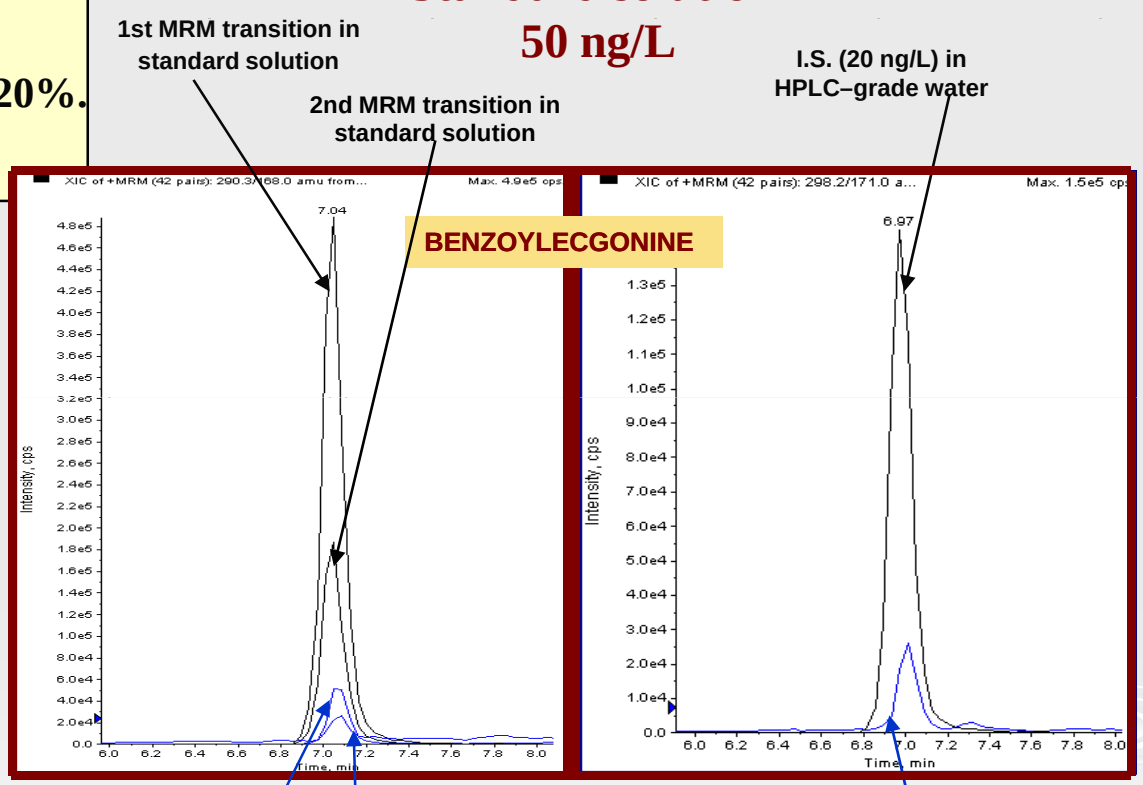
- Retention Time within 2%,
- MRM1/MRM2 ratio within ± 20%.

High matrix effects

in sewage water

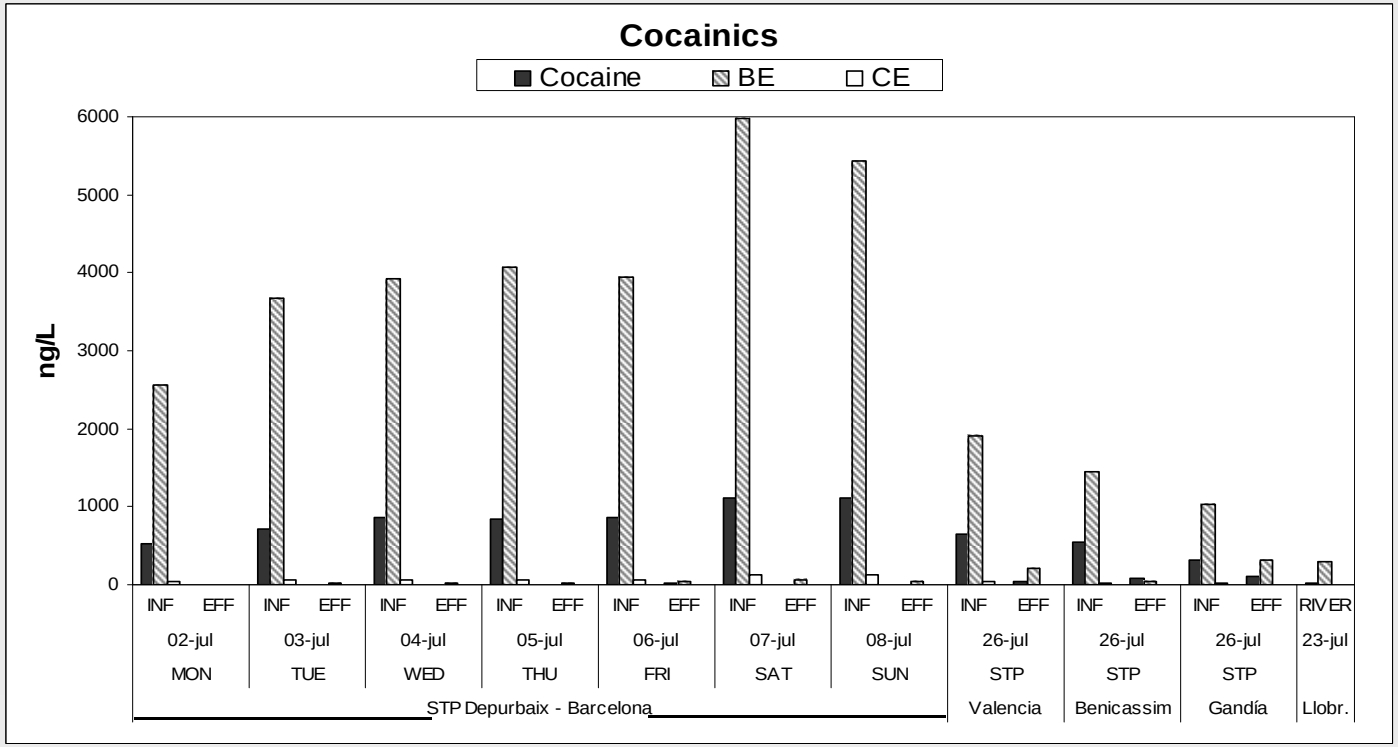
Internal standard quantitation using surrogate standards

Standard solution
50 ng/L



El Prat STP sample
Effluent 07/07
61.1 ng/L

Levels of cocainics



	INF (ng/L) Average (*St. dev*)		EFF (ng/L) Average (*St. dev*)		RIVER (ng/L)
Cocaine	753.3	(*258.6*)	26.5	(*37.2*)	16.9
BE	3395.0	(*1644.0*)	80.0	(*103.5*)	295.0
CE	63.5	(*35.7*)	2.5	(*1.9*)	4.6

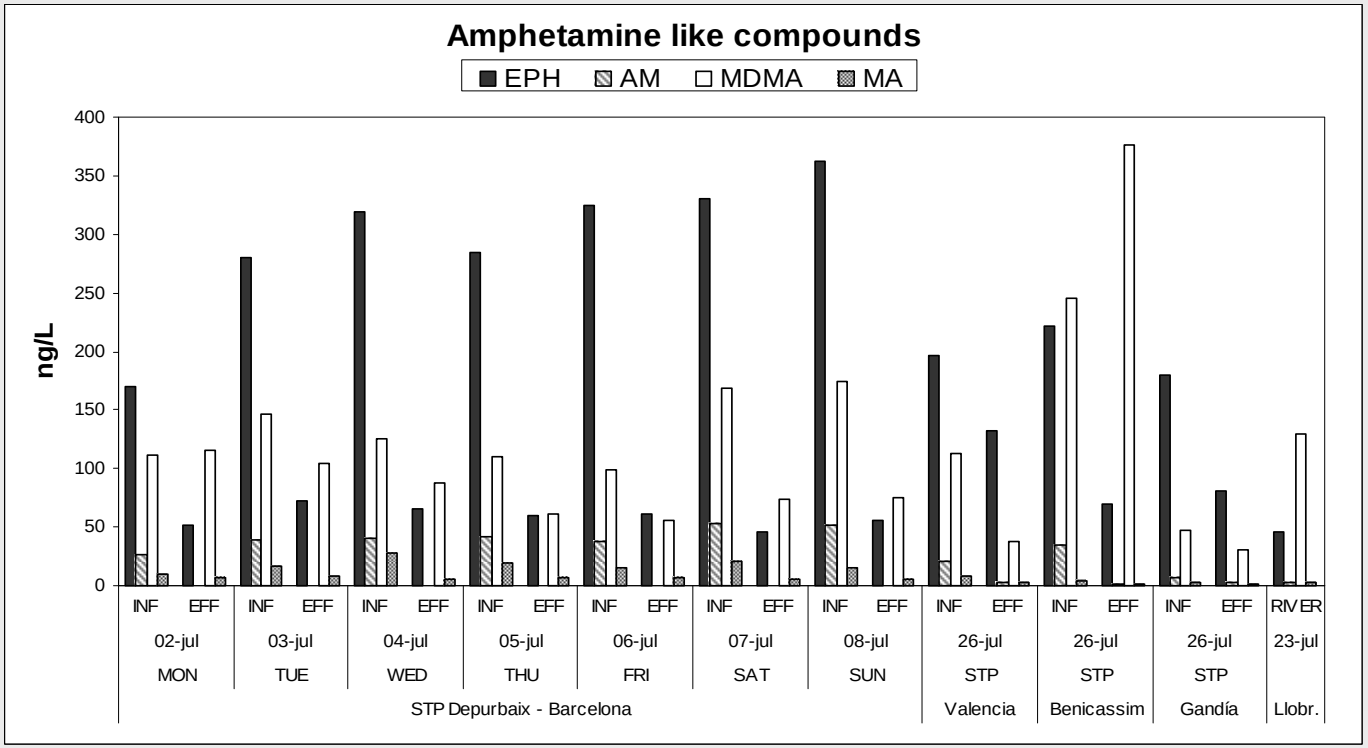
BE: good indicator of cocaine consumption

$[\ ]_{BCN} > [\ ]_{A.C.Valencia} > [\ ]_{river}$

Low day-to-day variability (only slightly higher concentrations during the weekend)

Good removal efficiency (95%)

Levels of amphetamine-like compounds



	INF (ng/L) Average (*St. dev*)		EFF (ng/L) Average (*St. dev*)		RIVER (ng/L)
EPH	267.1	(*69.6*)	69.6	(*24.5*)	45.5
AM	35.0	(*14.1*)	1.0	(*1.0*)	2.8
MDMA	134.0	(*53.3*)	101.9	(*100.0*)	129.0
MA	14.2	(*8.1*)	5.0	(*2.2*)	2.9

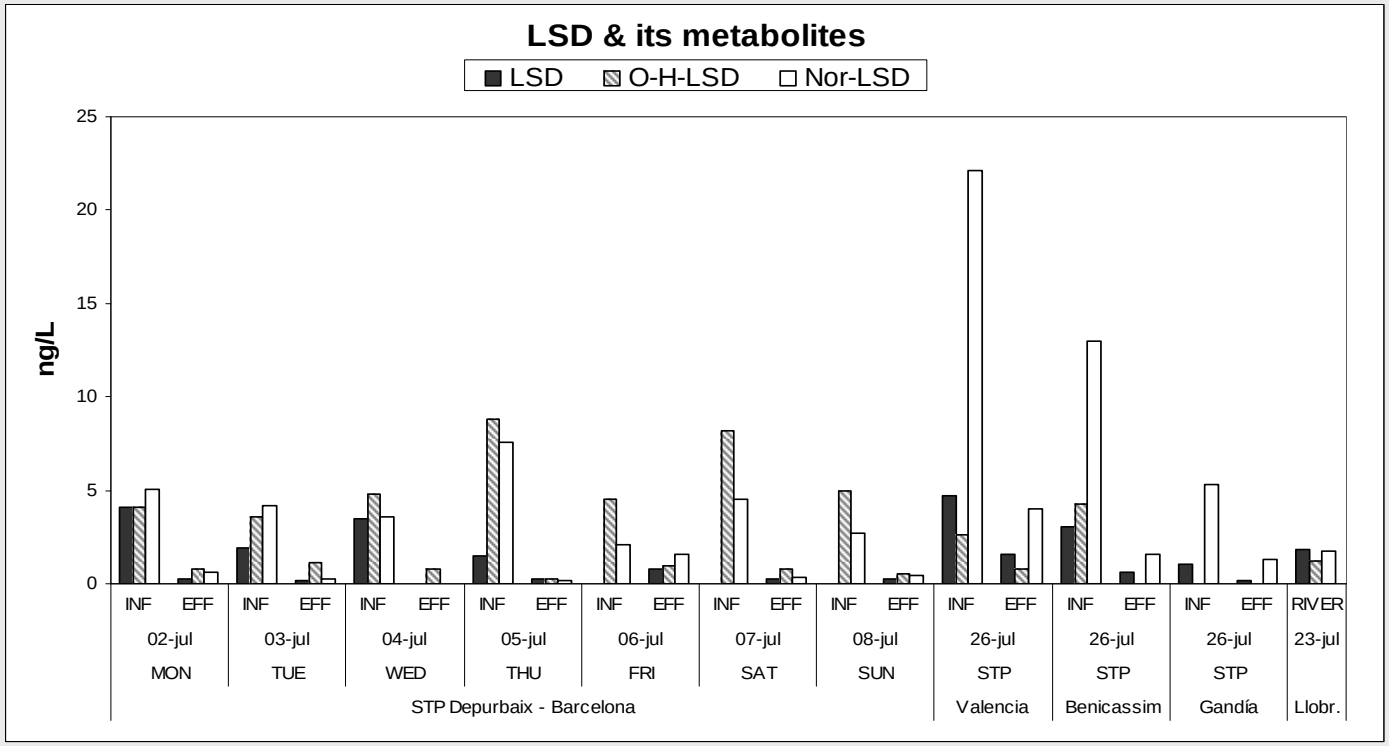
[EPH] > [MDMA] > [AM] > [MA]

Similar levels in both studied areas

Very slight increase throughout the week

Poor removal (67%)

Levels of LSD & metabolites



	INF (ng/L) Average (*St. dev*)		EFF (ng/L) Average (*St. dev*)		RIVER (ng/L)
LSD	2.8	(*1.4*)	0.5	(*0.5*)	1.8
O-H-LSD	5.1	(*2.1*)	0.6	(*0.4*)	1.2
Nor-LSD	7.0	(*6.1*)	1.1	(*1.2*)	1.7

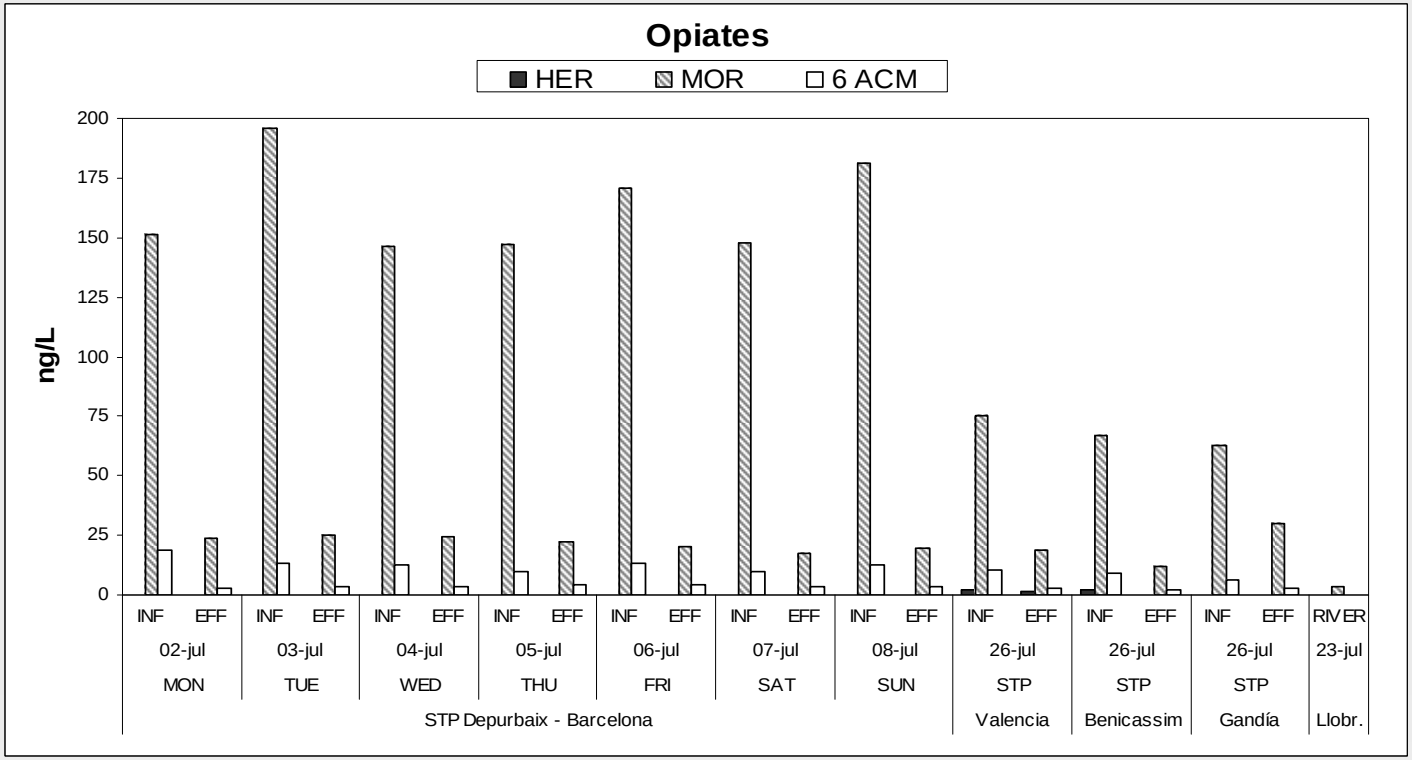
Lowest levels ($\Leftrightarrow$ lowest doses, µg *vs* mg)

[metabolites] > [LSD]

$[\ ]_{A.C.Valencia} > [\ ]_{BCN} > [\ ]_{river}$

Average removal: 72%

Levels of opiates



	INF (ng/L) Average (*St. dev*)	EFF (ng/L) Average (*St. dev*)	RIVER (ng/L)
HER	2.4 (*0.1*)	1.2 (-)	n.d.
MOR	134.4 (*48.7*)	21.3 (*5.0*)	3.25
6ACM	11.5 (*3.5*)	3.3 (*0.7*)	n.d.

n.d. = non detected

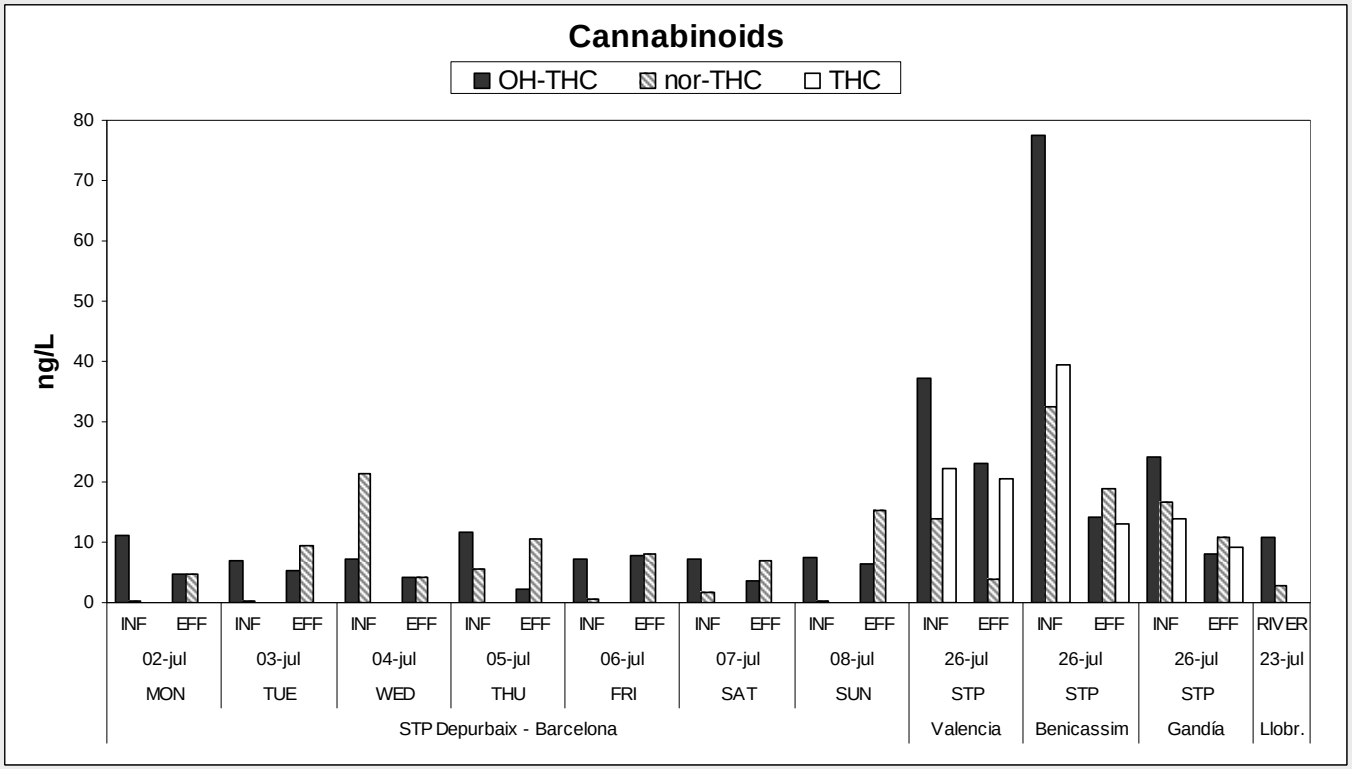
[MOR] >>> [6ACM] >>> [HER]

$[\ ]_{BCN} > [\ ]_{A.C.Valencia} > [\ ]_{river}$

No fluctuation throughout the week

Average removal = 78%

Levels of cannabinoids



	INF (ng/L) Average (*St. dev*)		EFF (ng/L) Average (*St. dev*)		RIVER (ng/L)
OH-THC	19.8	(*22.6*)	7.9	(*6.3*)	10.7
Nor-THC	0.3	(*11.3*)	9.3	(*4.9*)	2.65
THC	25.1	(*13.1*)	4.9	(*5.7*)	n.d.

n.d. = non detected

Comparatively lower levels than cocainics and amphetamine-like compounds.

[OH-THC] > [nor-THC] > [THC]

$[\ ]_{A.C.Valencia} > [\ ]_{BCN} \approx [\ ]_{river}$

Poor removal = 32% with occasionally higher conc. in effluent than influent

Estimate of cocaine and MDMA consumption

Basis for calculation (from STP influent levels):

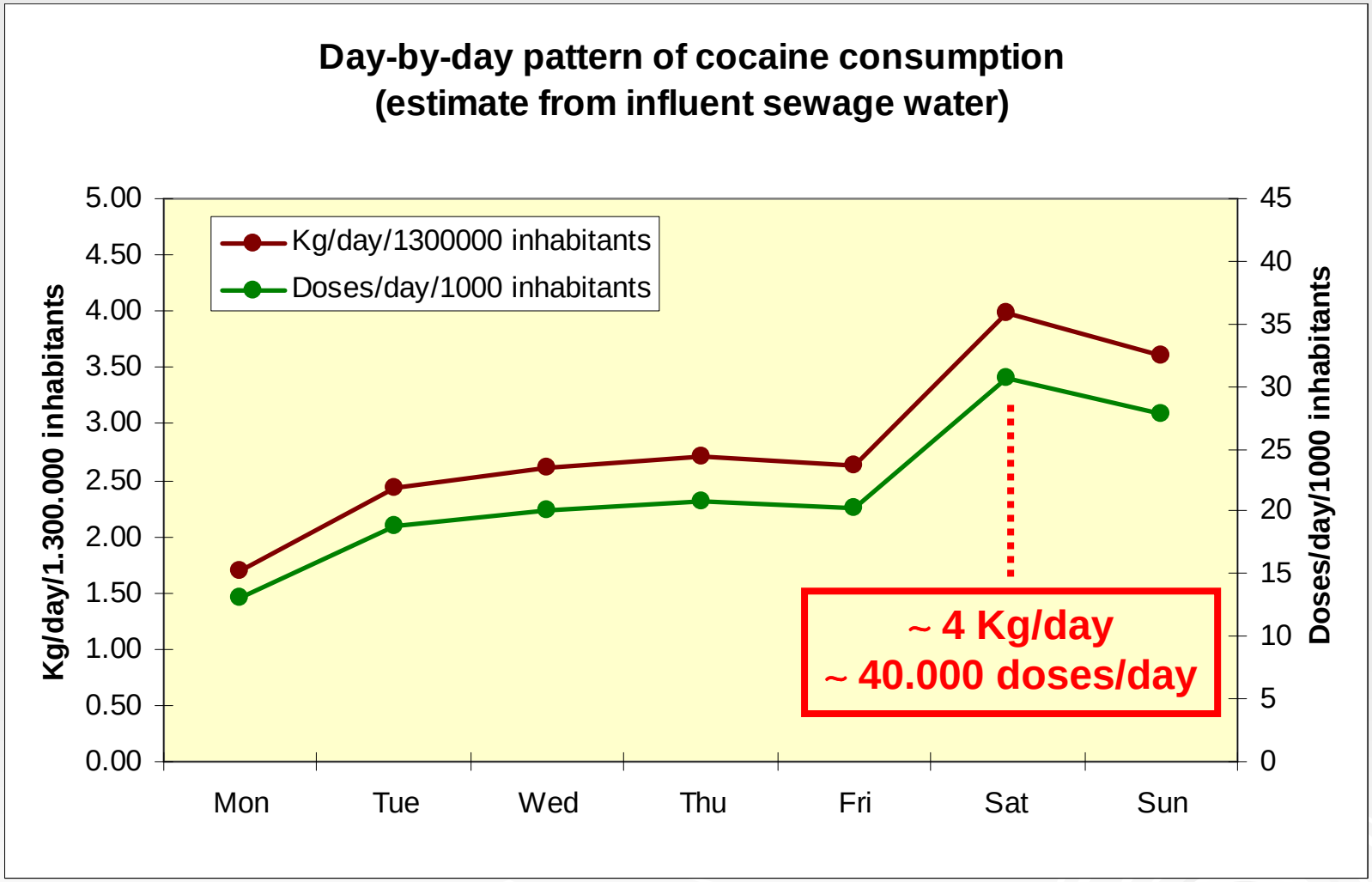
- **Volume of water treated in STP (m3/day):**
  285.000 (BCN), 112.100 (Val.), 11.420 (Ben.), 46.582 (Gan.)

- **Population served by the STP (num. Inhabitants):**
  1.300.000 (BCN), 1.000.000 (Val .), 17.267 (Ben.), 110.196 (Gan.)

- **Excretion rate:**
  45% CO excreted as BE; 65% unchanged MDMA

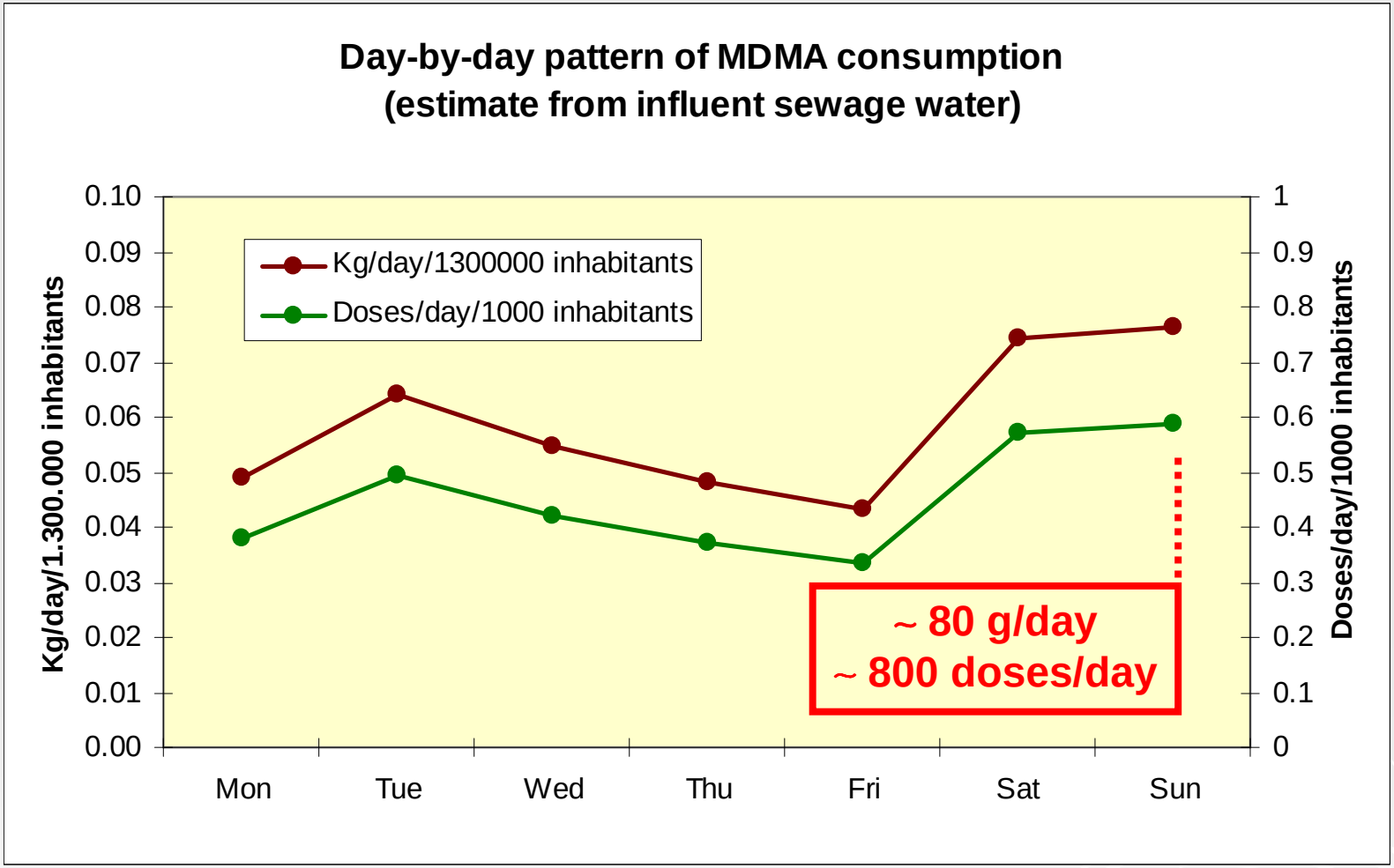
- **Dose:**
  100 mg (CO & ecstasy)

Cocaine consumption (BCN)



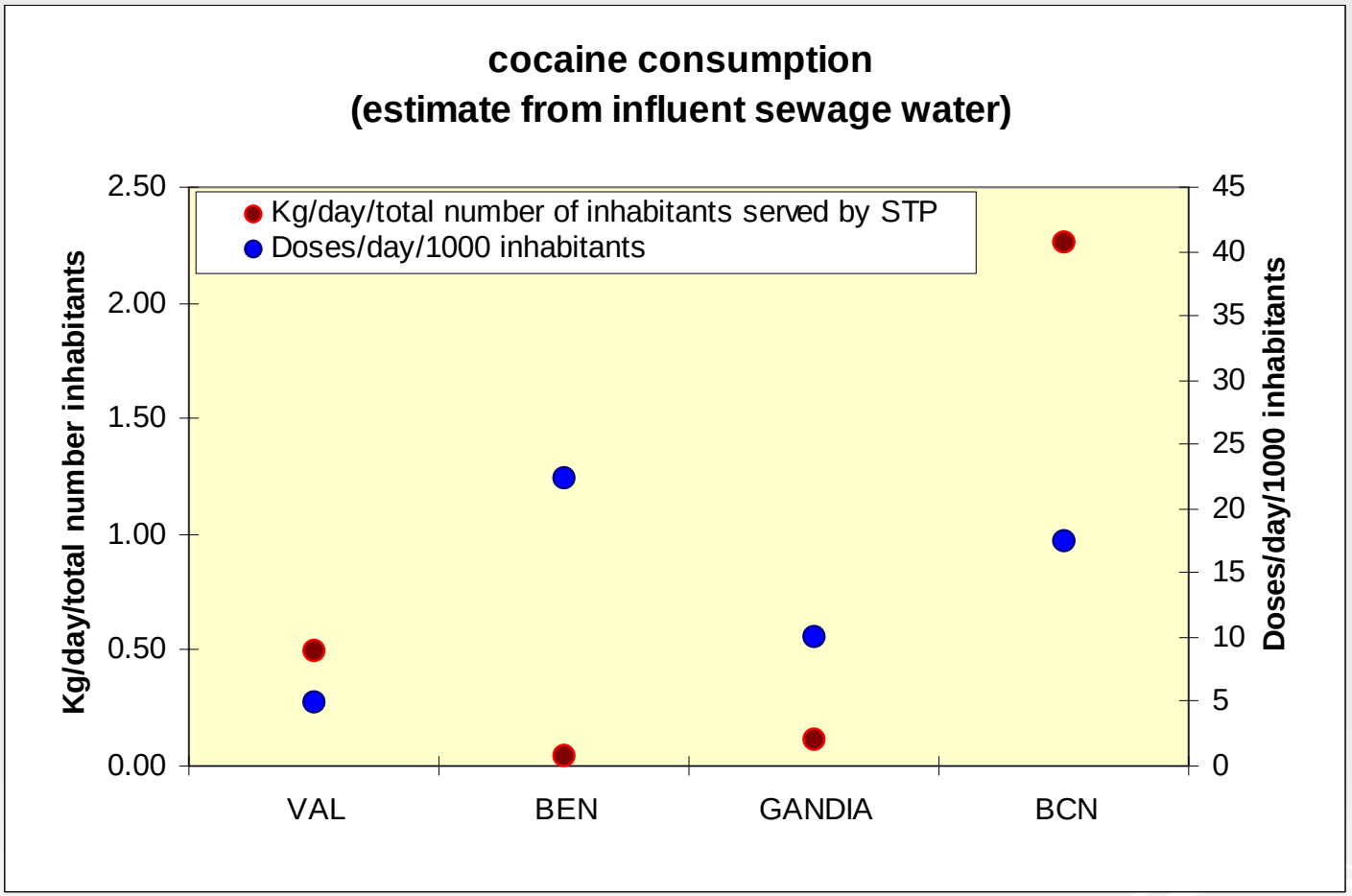
Basis for calculation: 100 mg CO/dose; 45% CO excreted as BE;
Q = 285.000 m3/day; population served by the plant = 1.300.000 hab.)

Ecstasy consumption (BCN)



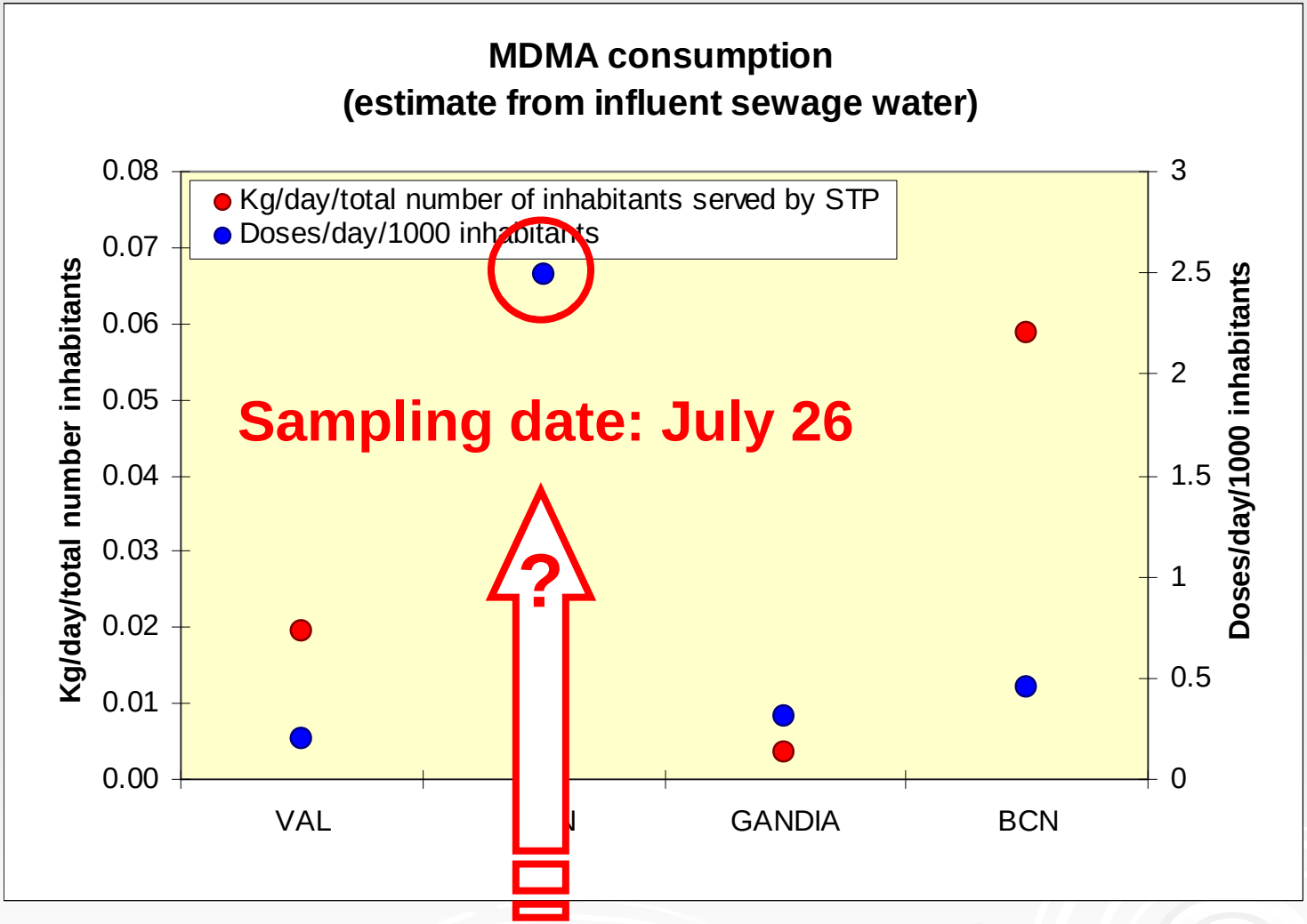
Basis for calculation: 100 mg MDMA/dose; unchanged MDMA excretion rate = 65%;
Q = 285.000 m3/day; population served by the plant = 1.300.000 hab.)

Comparative between cities: cocaine



Benicassim -Doses/day/1000 inhabitants of cocaine = 22

Comparative between cities: ecstasy



International music festival: July 19-22

Conclusions (1)

A fully automated method, based on on-line SPE-LC-ESI(QqLIT)-MS/MS analysis, has been developed for the multi-analyte determination of 17 illicit drugs of abuse in sewage and surface waters.

The application of the method to real water samples has shown:

increasing influent levels in the order LSD metabolites < cannabinoids < opiates (MOR) < amphetamine-like comp. (EPH) < cocainics (BE).

variable STP removal: from occasionally negative values for nor-THC and MDMA to 95% for cocainics (average 70 %)

very slight increasing levels along the week only for cocainics and amphetamine-like compounds

higher cocaine and MDMA consumption/inhabitant in Benicassim > BCN > Gandía > Valencia

Conclusions (2)

Drug consumption estimation from STP influent levels is pretty easy and straightforward.

The main advantages of such approach over official methods (surveys, etc.) are: real-time information, accuracy, cost-efficiency.

The analysis of drugs of abuse in air samples may also be useful to estimate drug consumption/detect areas of drug traffic.

Acknowledgments

This work has been supported by:

- the EU (project MODELKEY [GOCE 511237])

- the Spanish Ministry of Education and Science (project CEMAGUA [CGL2007-64551/HID])

Spark Holland (Emmen, The Netherlands) for the gift of SPE cartridges.

Waters (Barcelona, Spain) for the gift of SPE cartridges.

Merck (Darmstadt, Germany) for the gift of LC columns.