

Supplementary Information

The embodiment of wastewater data for the estimation of illicit drug consumption in Spain

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Text S1 - Enantiomeric analysis

Sample treatment

Wastewater samples were solid-phase extracted following a dedicated protocol employed for the quantification of drugs of abuse using Oasis MCX cartridges (González-Mariño et al., 2018). Briefly, 100 mL wastewater were spiked with 20 ng internal standard and vacuum-filtered through a 0.7 µm glass microfiber filter GF/A. Subsequently, SPE was performed using a strong cation exchange mixed-mode cartridge (Oasis MCX-150 mg, Waters Corp., Milford, MA, USA), previously conditioned with 5 mL of MeOH containing 5% of NH₃ followed by 5 mL of ultrapure water. After sample percolation, the sorbent was washed with 10 mL of ultrapure water and dried under vacuum. Analytes were recovered with 10 mL of 5% NH₃ in MeOH. The eluate was evaporated to dryness under nitrogen using both a Turbo-Vap II (Zymark, Hopkinton, MA USA) and a Mini-Vap concentrator (Sigma-Aldrich). Finally, the extract was reconstructed in 100 µL of MeOH for instrumental analysis.

Instrumentation

Instrumental analyses were performed using a Waters Acquity UPLC® H class system (Milford, MA, USA). The UPLC® system was interfaced to a triple quadrupole mass spectrometer Xevo TQD from Waters (Milford, MA, USA). The interface between the UPLC® system and the Xevo TQD mass spectrometer was an electrospray ionization (ESI) source operating in positive mode. Analyses were performed by MS/MS in Selected Reaction Monitoring (SRM) mode acquiring two precursor/ product ion transitions per analyte and one transition per internal standard (IS), with the transitions described in (González-Mariño et al., 2018).

The chromatographic separation was performed at 40 °C on a Lux AMP column (150×3 mm I.D., 3 µm particle size) from Phenomenex (Phenomenex, California, USA). Under final working conditions, a dual eluent system consisting of (A) ultrapure water with 50 mM NH₃ and (B) MeOH (LC-MS grade) was used at a flow rate of 0.4 mL/min. The gradient lasted 30 min and consisted of the following stages: 0 min (60% B), 15 min (60% B), 20 min (95% B), 25 min (95% B), 25.1 min (60% B) and 30 min (60% B). Injection volume was set at 10 µL.

Table S1: All meta-data and calculations used in this study. Please see the complete Excel file.

Table S2: Limits of detection (LOD) and limits of quantification (LOQ) in ng/L for quantitative determination of illicit drugs and new psychoactive substances in influent wastewater.

Psychoactive substance ^a	UJI ^b		USC ^c		IDAEA-CSIC ^d		UV ^e	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
Amphetamine	30	100	3	10	8.7	29	9.1	30.4
Methamphetamine	6	20	1.5	5	5.5	18.2	0.3	1.1
MDMA	3	10	2.7	9	3.3	10.9	1.7	5.5
Cocaine	1.5	5	1	3.2	0.2	0.8	2	6
Benzoylcegonine	0.6	2	1.1	3.3	0.5	1.5	2	6
THC-COOH	18	60	8	25	18	61	10	30
3,4-DiMeO- α -PVP	3	9	-	-	-	-	-	-
4-chloro- α -PPP	5	17	-	-	-	-	-	-
4-FMC	10	30	-	-	-	-	-	-
4-MEC	2	5	-	-	-	-	-	-
4-MePPP	5	18	-	-	-	-	-	-
α -PVP	13	43	-	-	-	-	-	-
bk-MDDMA	6	20	-	-	-	-	-	-
bk-DMBDP	2	6	-	-	-	-	-	-
Butylone	2	6	-	-	-	-	-	-
Ketamine	6	19	-	-	-	-	-	-
MDPV	7	21	-	-	-	-	-	-
Mephedrone	2	5	-	-	-	-	-	-
Methedrone	2	6	-	-	-	-	-	-
Methoxetamine	2	5	-	-	-	-	-	-
Methylone	1.5	4	-	-	-	-	-	-
N-ethylcathinone	14	41	-	-	-	-	-	-
PMMA	3	10	-	-	-	-	-	-

^a 3,4-dimethoxy- α -pyrrolidinopentiophenone (3,4-DiMeO- α -PVP), 4-chloro- α -pyrrolidinopropiophenone (4-chloro- α -PPP), 4-fluoromethcathinone (4-FMC), 4-methylethcathinone (4-MEC), 4-methyl- α -pyrrolidinopropiophenone (4-MePPP), α -pyrrolidinopentiophenone (α -PVP), dimethylone (bk-MDDMA), dimethylpentylone (bk-DMBDP), methylenedioxypropylone (MDPV) and *p*-methoxymethamphetamine (PMMA).

^b (Bijlsma, Beltrán, Boix, Sancho, & Hernández, 2014; Celma et al., 2019)

^c (González-Mariño et al., 2018)

^d (López-García, Mastroianni, Postigo, Barceló, & López de Alda, 2018)

^e (Andrés-Costa, Rubio-López, Morales Suárez-Varela, & Pico, 2014)

Table S3: Spearman Rank correlations between drug use and population size.

Psychoactive substance	Correlation (r)	p-value
Amphetamine	0.4396	0.1278
Methamphetamine	0.6648	0.0213
MDMA	0.6429	0.0260
Cocaine (benzoylecgonine)	0.2483	0.3898
Cannabis (THC-COOH)	0.3549	0.2189

Table S4: New psychoactive substances detected in pooled weekend samples (influent wastewater) collected in Spanish cities.

City	NPS detected
Barcelona	Mephedrone and ketamine
Bilbao	Ketamine
Castellón	-
Guadalajara	-
Lleida	-
Madrid-1	Mephedrone and ketamine
Madrid-2	Ketamine
Móstoles	Methedrone and ketamine
Palma de Mallorca	Ketamine
Reus	-
Santiago de Compostela	-
Tarragona	-
Toledo	Ketamine
Valencia-1	Ketamine
Valencia-2	Ketamine
Valencia-3	Dipentylone

Table S5: Population-normalized daily consumption estimates extrapolated to the Spanish population (mg/day/ 1000 inh.) using 2018 data of SCORE cities (Barcelona, Valencia, Santiago de Compostela and Castellón) monitored.

Day	AMP	METH	MDMA	COC	Cannabis (CF 182)	Cannabis (CF 36.4)
Tuesday	44	44	92	1984	24514	4969
Wednesday	73	46	60	1816	16895	3558
Thursday	59	54	50	1652	15201	3342
Friday	56	48	57	2209	17589	3951
Saturday	67	50	147	2862	19207	4133
Sunday	64	52	221	2784	25852	5265
Monday	51	39	138	2205	22199	4341
Average	59	48	109	2216	20208	4223
SD	10	5	63	460	4047	702

Table S6: Daily (kg/day) and annual (ton/year) consumption estimates using 2018 data of SCORE cities (Barcelona, Valencia, Santiago de Compostela and Castellón) monitored and extrapolated to total Spanish population

Day	AMP	METH	MDMA	COC	Cannabis (CF 182)	Cannabis (CF 36.4)
Tuesday	2.1	2.1	4.3	93	1143	232
Wednesday	3.4	2.1	2.8	85	788	166
Thursday	2.8	2.5	2.3	77	709	156
Friday	2.6	2.2	2.7	103	820	184
Saturday	3.1	2.3	6.8	133	896	193
Sunday	3.0	2.4	10.3	130	1206	246
Monday	2.4	1.8	6.5	103	1035	202
Average	2.8	2.2	5.1	103	943	197
SD	0.5	0.2	2.9	22	189	32
Annual	1.01	0.81	1.86	37.7	344	71.9

Table S7: Compiled information of AMP, METH, MDMA, COC and Cannabis: wastewater data (Spanish annual consumption (ton/year) estimates), prevalence (%), seizure data (kg), purity (% or mg/tablet) and price (€/gram or tablet)

Illicit drug	Annual consumption of pure substance (ton/year) ^a	Prevalence (%) ^b	Seizure data (kg)	Purity EC (%) ^e	Purity AL (%) ⁱ	Purity PNSD (%) ^l	Price (€/gram) ⁿ
AMP	2.40 - 2.72	0.5	272 ^c	38.6 (522)	48.4 (65)	NA	26.19
METH	0.50 - 0.80	0.2		64.0 (72)	NA	NA	
MDMA	1.54 - 1.60	0.6	54.1 ^d	180 mg/tablet (424) ^f 81.2 (388) ^g	48.0 (16) ^j 98.0 (28) ^k	NA	10.29 ^o
COC	36.2	2.2	48,453 ^d	65.1 (905)	86.7 (74)	35.5-67.5 ^m	59.21
Cannabis	69.6 - 350	11	Resin 334,919 ^c	NA ^h	29.7 (2)	18.5	5.59
			Herb 34,517 ^c	NA ^h	17.1 (4)	10.1	5.22

^a WW data (2018), this study

^b Prevalence (2017) last 12 months among adults (age 15-64), (Observatorio Español de las Drogas y las Adicciones, 2019)

^c Seizure (2017), (Plan Nacional sobre Drogas & EMCDDA, 2019)

^d Seizure (2018), (Observatorio Español de las Drogas y las Adicciones, 2019)

^e Average Purity (2018), Energy Control, Unpublished. Number of samples analyzed between parentheses

^f Purity (mg/tablet) reported for tablets containing MDMA by Energy Control, Unpublished

^g Purity (%) reported for crystal MDMA by Energy Control, Unpublished

^h NA: not available

ⁱ Average Purity (2018), Ai Laket!, Unpublished. Number of samples analyzed between parentheses

^j Purity (%) reported for tablets containing MDMA by Ai Laket!, Unpublished

^k Purity (%) reported for crystal MDMA by Ai Laket!, Unpublished

^l Average Purity (2016), (Plan Nacional sobre Drogas & EMCDDA, 2018)

^m range corresponds to “small dose-related” seizures – “large kg” seizures

ⁿ Price (2018), (Observatorio Español de las Drogas y las Adicciones, 2019)

^o Price per tablet

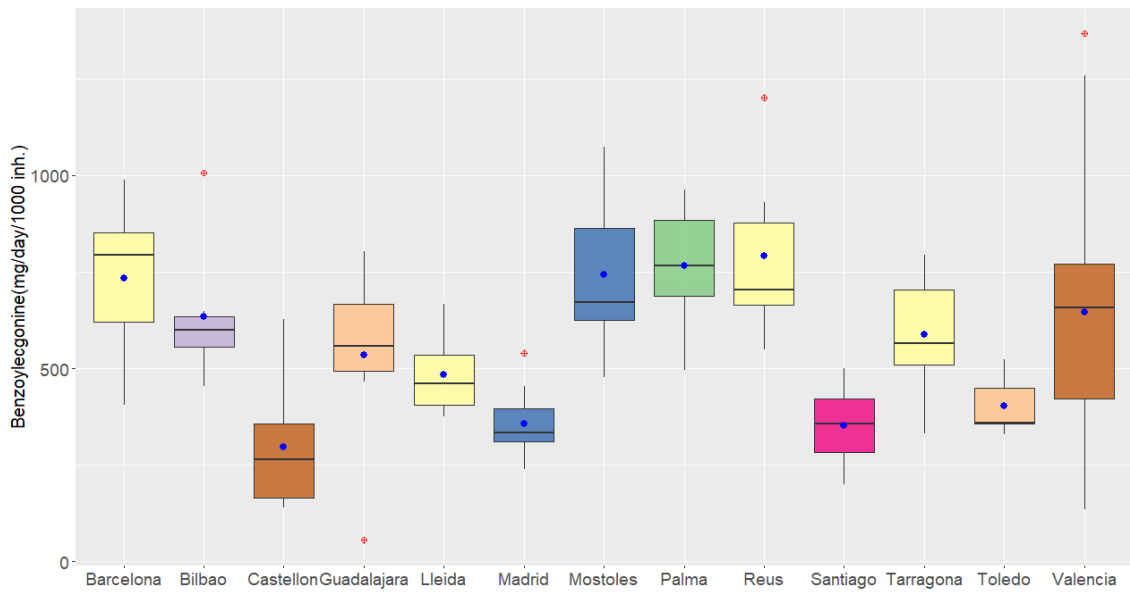


Figure S1: Population-normalized BE loads (mg/day/1000 inh.) of 2018 of all Spanish cities. Box colors indicate the region (Autonomous Community): Catalonia = yellow, Basque country = violet, Valencian Community = brown, Castile-La Mancha = orange, Community of Madrid = blue, Balearic Islands = green, Galicia = fuchsia.

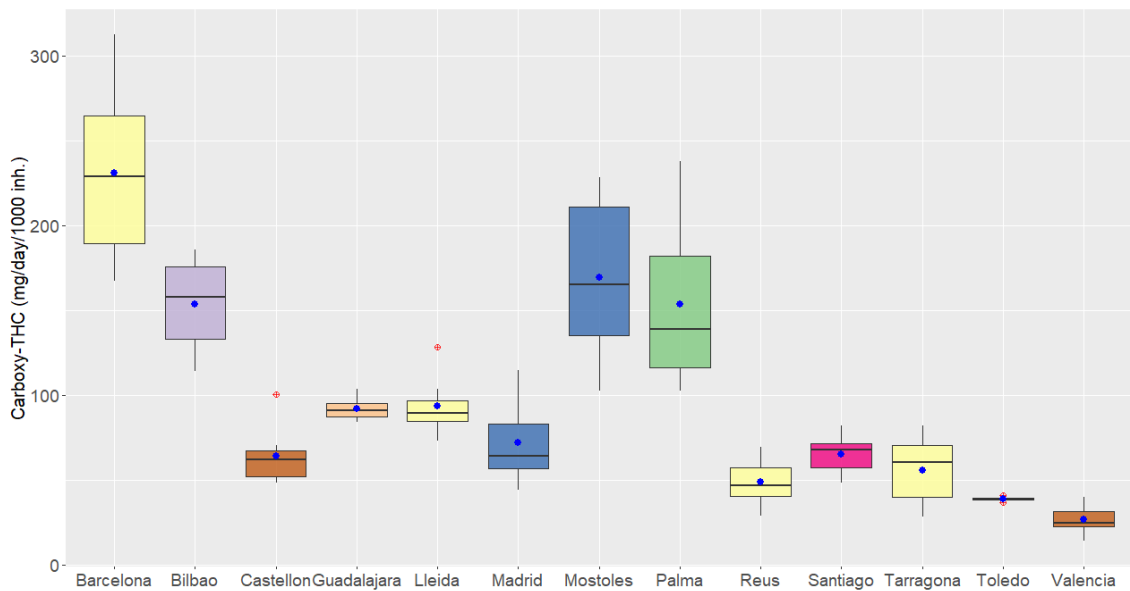


Figure S2: Population-normalized THC-COOH loads (mg/day/1000 inh.) of 2018 of all Spanish cities. Box colors indicate the region (Autonomous Community): Catalonia = yellow, Basque country = violet, Valencian Community = brown, Castile-La Mancha = orange, Community of Madrid = blue, Balearic Islands = green, Galicia = fuchsia.

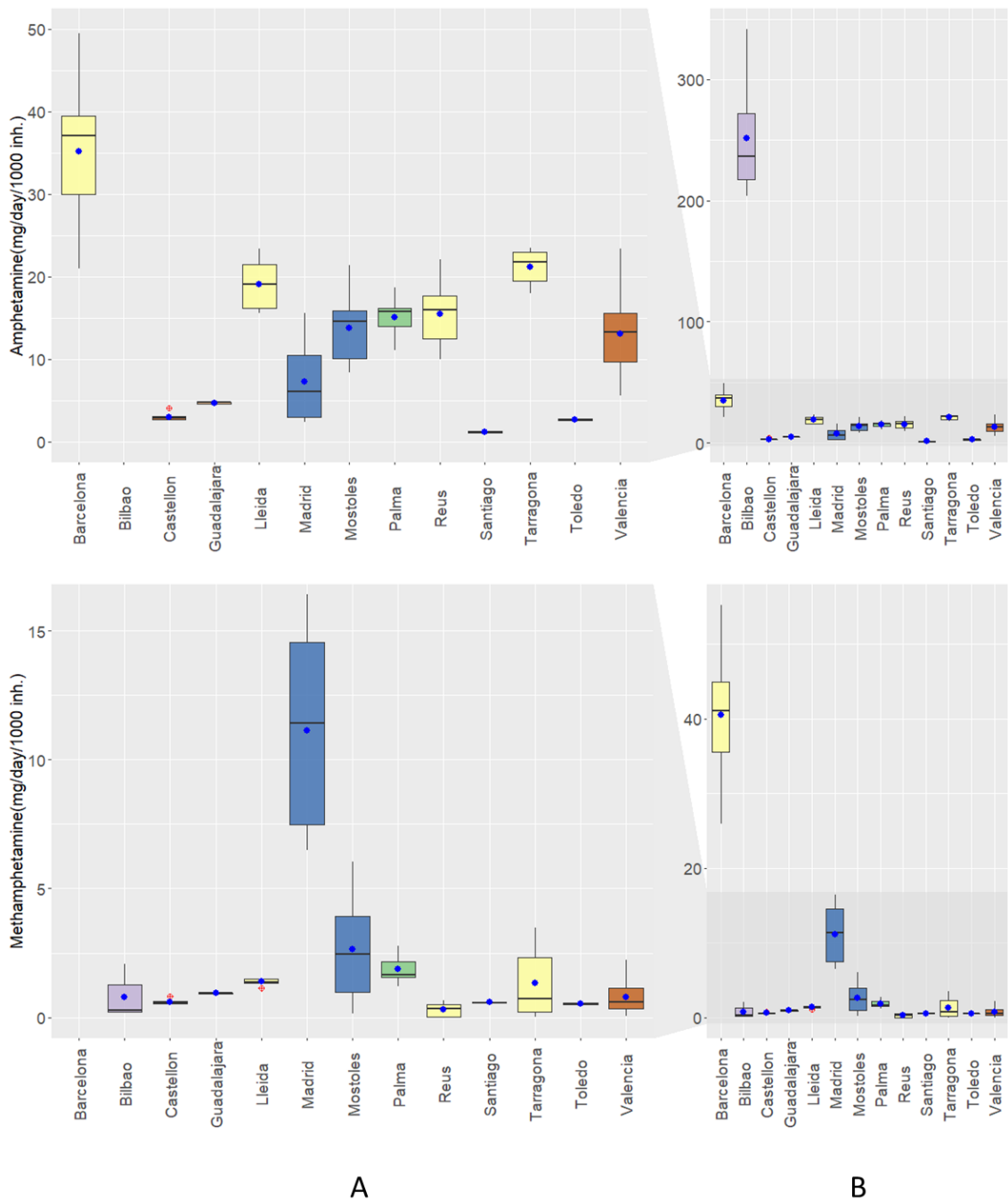


Figure S3: Population-normalized Amphetamine (Top) and Methamphetamine (Bottom) loads (mg/day/1000 inh.) of 2018 of all Spanish cities. (A). A larger scale on the right side of the figure (B) to visualize Bilbao for Amphetamine and Barcelona for Methamphetamine. Box colors indicate the region (Autonomous Community): Catalonia = yellow, Basque country = violet, Valencian Community = brown, Castile-La Mancha = orange, Community of Madrid = blue, Balearic Islands = green, Galicia = fuchsia.

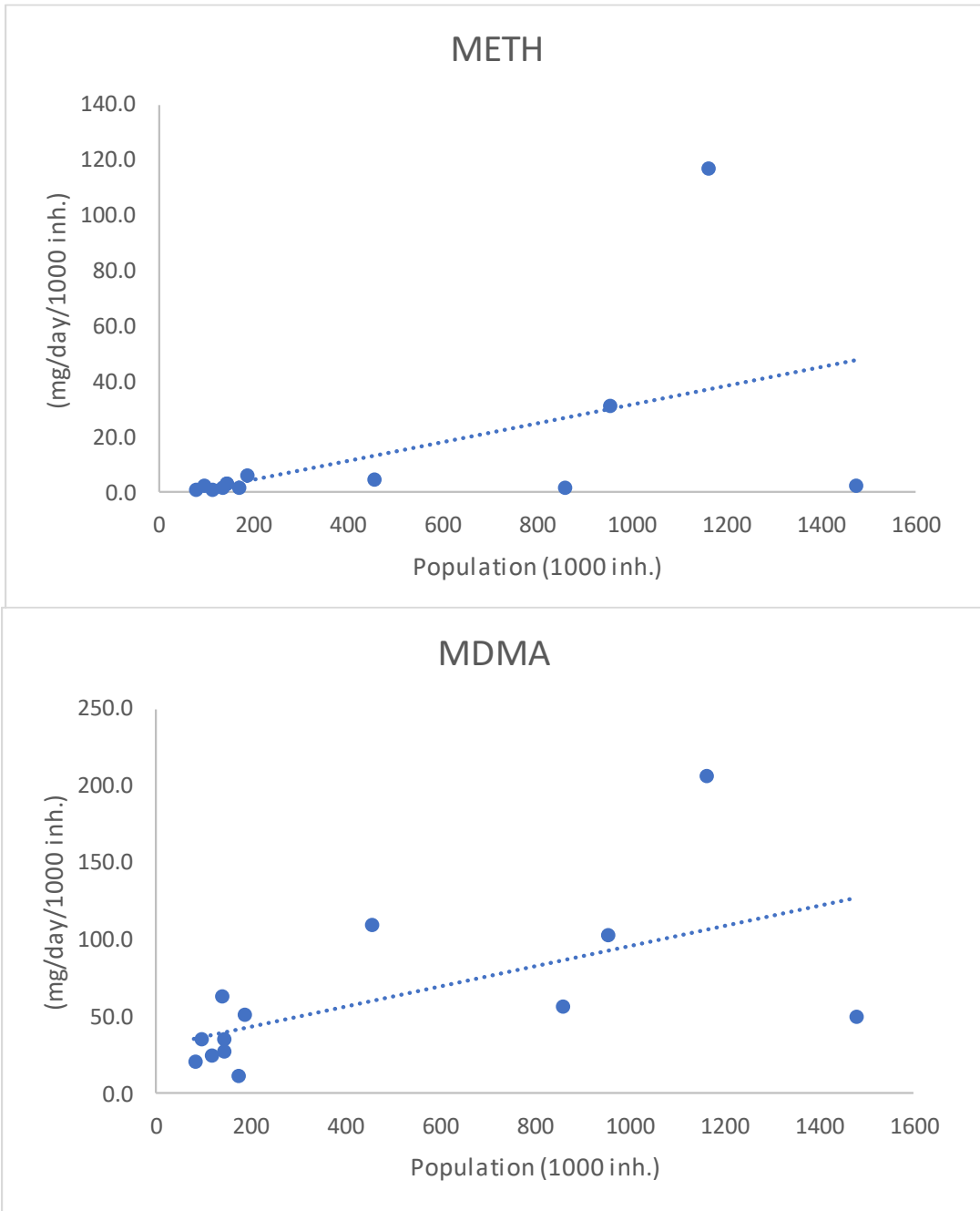


Figure S4: Representation of the population of the different cities analyzed vs. the estimated consumption of METH and MDMA. See Table S3 for correlation significance data.

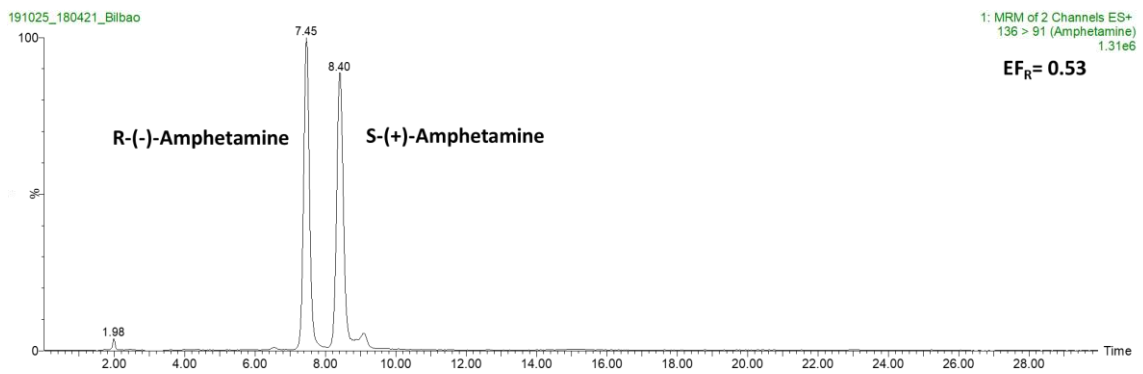


Figure S5: Chromatogram of enantiomeric amphetamine in a wastewater sample collected from Bilbao of April 24th, 2018.

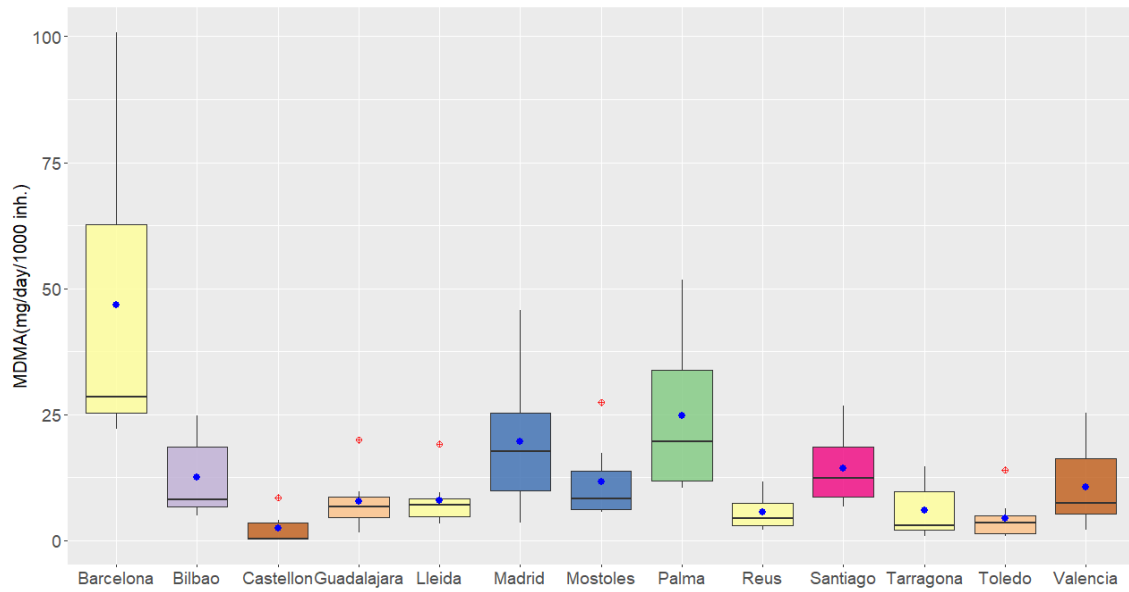


Figure S6: Population-normalized MDMA loads (mg/day/1000 inh.) of 2018 of all Spanish cities. Box colors indicate the region (Autonomous Community): Catalonia = yellow, Basque country = violet, Valencian Community = brown, Castile-La Mancha = orange, Community of Madrid = blue, Balearic Islands = green, Galicia = fuchsia.

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