

Improving wastewater-based epidemiology to estimate cannabis use: focus on the initial aspects of the analytical procedure

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31 15 pages

32 4 tables

33 6 figures

34 8 references

35 **Table SI-1.** Physico-chemical properties of some illicit drugs and metabolites.

Compound	Formula	pK _a		LogP	
		Experimental	Calculated	Experimental	Calculated
Amphetamine ¹	C9H13N	10.1	9.9	1.8	1.8
Methamphetamine ¹	C10H15N	10.1	10.4	2.1	2.2
MDMA ¹	C11H15NO2	9.4	10.3	n.a.	2.1
Cocaine ¹	C17H21NO4	8.6	8.9	2.3	2.3
Benzoyllecgonine ¹	C16H19NO4	n.a.	10.8, 3.3	-1.3	2.3
THC ²	C21H30O2	n.a.	9.3	n.a.	5.9
THC-COOH ²	C21H28O4	n.a.	4.2	n.a.	5.1

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37 ¹ Baker et al. 2011 [1]

38 ² ChemAxon software-calculated values [2]

39 n.a. not available

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41 **Table SI-2.** Full details of the analytical methodology used by each participant laboratory: sample treatment, LC conditions, MS parameters

Lab #	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9 ⁽¹⁾	Lab 10 ⁽¹⁾
ILIS	THC-COOH-d ₃	THC-COOH-d ₃	THC-COOH-d ₃	THC-COOH-d ₃	THC-COOH-d ₃	THC-COOH-d ₃	THC-COOH-d ₃	THC-COOH-d ₃	THC-COOH-d ₉	THC-COOH-d ₃
Filtering material	1.6 μm Glass fiber filter	0.2 μm RC filter (syringe)	No filtering Sample diluted 4x	No filtering. Sample centrifuged at 3000 rpm for 10 min and the supernatant used for analysis	(1) Whatman No. 41 filter paper (2) 0.2 μm PTFE syringe filter	2.7 μm Whatman, glass-fiber filter	(1) 1.6 μm glass microfiber filter GF/A (2) 0.45 μm mixed cellulose acetate & cellulose nitrate	(1) 1 μm glass fiber filter A/E (2) 0.2 μm PES membrane filter	0.2 μm Whatman PTFE syringe filter Primo 1 mL syringe	(1) 1.6 μm glass microfiber filter GF/A (2) 0.45 μm mixed cellulose acetate & cellulose nitrate filter
pH at extraction	Natural	Natural	Natural , except the acidified sample (pH 3-4)	Natural	Natural	Acid (pH=2)	Acid (pH 4.5)	Natural	n.a.	n.a.
SPE material	Oasis HLB	Phenomenex Strata-XC (3cc, 60mg)	Oasis HLB (3cc, 60mg)	Oasis HLB (3cc, 60mg)	Oasis HLB (6cc, 200 mg)	Oasis MCX (6cc, 150mg), extra clean up with Strata NH ₂ (3cc, 200mg)	Oasis MCX (6cc, 150 mg)	Oasis HLB (6cc, 150 mg)	n.a.	n.a.
SPE protocol: Conditioning	MeOH + ultrapure water	MeOH + 25mM NH ₄ CH ₃ CO ₂	MeOH + ultrapure water	MeOH + ultrapure water	MeOH + ultrapure water	MeOH + ultrapure water + 25mM H ₃ PO ₄	MeOH 5% NH ₄ OH + ultrapure water (pH 4.5)	MeOH + ultrapure water	n.a.	n.a.

SPE protocol: Sample load	50 mL sample	5 mL sample	100 mL of "sample" (25mL sample + 75mL ultrapurewater)	50ml of supernatant	100 mL of sample	125mL of sample	100 mL sample adjusted at pH 4.5	100 mL of sample	n.a.	n.a.
SPE protocol: Wash	no	85/15water/acetonitrile	no	ultrapure water	ultrapure water + 50% MeOH	ultrapure water	ultrapure water pH 4.5	ultrapure water	n.a.	n.a.
SPE protocol: Drying	yes	yes	yes	yes	yes	yes	yes	yes	n.a.	n.a.
SPE protocol: Elution	8 mL MeOH	2mL MeOH + 2 mL 85/15 ethyl acetate/isopropyl alcohol	5mL MeOH	5ml MeOH	MeOH	6mL MeOH	4 mL MeOH 5% NH4OH	8mL MeOH	n.a.	n.a.
SPE protocol: Extra clean-up	no	no	no	no	no	Conditionin: 1% HCOOH in MeOH Loading: MCX extract (MeOH) acidified with 60µL HCOOH) Additional elution: 4mL 1% HCOOH in MeOH	no	no	no	no

SPE protocol: Evaporation	to dryness, at 35 °C	to dryness, at 40 °C	to dryness, at 35 °C	to dryness, at 40 °C	to ~ 0,5 mL, at 40 °C.	to dryness, at 40 °C	to ~ 0,5 mL	to 250 µL, addition of 250 µL of ultrapure water, second evaporation to 250 µL.	n.a.	n.a.
SPE protocol: Reconstitution	100 µL ACN + 100 µL 5 mM NH ₄ CH ₃ CO in ultrapure water	1000 µL 5mM NH ₄ HCO ₂	100 µL MeOH + 900µL H ₂ O	1mL MeOH and diluted 1/10 with MeOH due to matrix effects	1 mL with MeOH	500 µL H ₂ O:MeOH= 8:2 with addition of 0.1% acetic acid	1 mL with MeOH	0.5 mL water:MeOH , 90:10	n.a.	n.a.
Time between samples received and analysis	Samples received on 6/9/16; stored at -20 °C until analysis on 22/9/16	Samples received on 7/9/16; stored at -20°C until analysis on 22/9/16	Samples received on 6/9/16; stored at -20°C until analysis on 16/9/16	Samples received on 6/9/16; stored at -20°C until analysis on 11/10/16	Samples received on 7/9/16; stored at 4°C until analysis on 23/9/16	Samples received on 6/9/16; stored at -20 °C until analysis on 26/9/2016.	Samples received on 7/9/16; stored at -20°C until analysis on 22/9/16	Samples received on 6/9/16; stored at -20°C until analysis on 18/11/16	n.a.	n.a.
Analytical instrument	Agilent 6410 (QqQ)	Agilent 1260 LC with a 6460 triple quad ms (QqQ)	Waters Xevo triplequad (QqQ)	Waters Xevo TQS Micro (QqQ)	Sciex Triple Quad 6500+ LC-MS/MS System (QqQ)	ThermoTSQ Quantum AM (QqQ)	Varian LC - Varian 320-MS (QqQ)	LTQ-FT-Orbitrap (Thermo Electron, Bremen, Germany)	Applied Biosystems 5500 QTrap linear ion trap triple quadrupole mass spectrometer (Sciex, Darmstadt/Germany)	Agilent LC – Agilent 6550 iFunnel Q-TOF

									(QqQ)		
Mobile phase composition	A: Ultrapure water 5 mM ammonium acetate; B: Acetonitrile	A: Ultrapure water 5 mM ammonium acetate; B: Methanol	A: Ultrapure water 5 mM ammonium acetate + 0.01% formic acid; B: MeOH	A: Ultrapure water 5 mM ammonium acetate + 0.01% formic acid; B: MeOH	A: Ultrapure water 5 mM ammonium formate with 0.01 % formic acid; B: Acetonitrile 0.01 % formic acid	A: Ultrapure water 0.1 % acetic acid; B: MeOH 0.1 % acetic acid	A: Ultrapure water 5 mM ammonium acetate; B: MeOH 5 mM ammonium acetate	A: Ultrapure water 0.05 % formic acid; B: MeOH 0.05 % formic acid	A: Ultrapure water 5 mM ammonium formate buffer at pH 3; B: MeOH 0.5% of a 1 M ammonium formate	A: Ultrapure water 5 mM NH ₄ HCO ₂ ; B: Acetonitrile	
Ionization mode	negative	negative	positive	positive	positive	negative	negative	positive	positive	negative	
Transitions	THC-COOH Quantifier: 343>299 Qualifier: 343>245	THC-COOH Quantifier: 343>299 Qualifier: 343>245 THC-COOH-d ₃ Quantifier: 346>302 Qualifier: 346>248	THC-COOH Quantifier: 345 >193 Qualifier: 345 > 299	THC-COOH Quantifier: 345.3 >299.2 Qualifier: 345.3 > 327.3 THC-COOH-d ₃ Quantifier: 346.1>302.1	THC-COOH Quantifier: 345.2 >193.2 Qualifier: 345.2 > 299.2 THC-COOH-d ₃ Quantifier: 348.2>302.2	THC-COOH Quantifier: 343 > 245 Qualifier: 343 > 299	THC-COOH Quantifier: 343.2 > 299 Qualifier: 343.2 > 245 THC-COOH-d ₃ Quantifier: 346 > 248	THC-COOH Quantifier: 343.2 > 299 Qualifier: 343.2 > 245 THC-COOH-d ₃ Quantifier: 346.2 > 302	THC-COOH [M+H] ⁺ 345.2060 qualifiers: 345 > 327 THC-COOH-d ₃ [M+H] ⁺ 348.2249	THC-COOH Quantifier: 345.1 > 299.2 Qualifier: 345.1 > 193.1 THC-COOH-d ₉ Quantifier: 354.1 > 336.2	THC-COOH [M+H] ⁺ 343.1915 Quantifier: 299.2017 Qualifiers: 245.1547 191.1078 325,1809 THC-COOH-d ₃ [M+H] ⁺ 346.2103 Quantifier: 302.2205 Qualifiers: 248.1735 194.1266
Reference	[3]	Unpublished	[4]	Adaptation from [4]	Unpublished	[5]	Adaptation from [6]	[7]	Adaptation from [8]	Unpublished	
Instrument	6% (n=6)	2% (n=6)	7% (n=6)	3% (n=6)	1% (n=5)	5% (n=6)	4% (n=6)	2% (n=6)	10% (n=5)	8% (n=6)	

al variability³ (Intra-day, RSD (%))										
Instrument al variability³ (Inter-day, RSD (%))	11% (n=6)	3% (n=6)	7% (n=6)	3% (n=6)	2% (n=5)	7% (n=6)	5% (n=6)	4% (n=3)	6% (n=3)	7% (n=6)

43 ⁽¹⁾ Labs 9 and 10 did not participate in the interlaboratory study but provided results in preliminary experiments

44 ⁽²⁾ QqQ: triple quadrupole; LTQ-FT Orbitrap: linear ion trap-Fourier transform Orbitrap; QTOF: quadrupole-time-of-flight

45 ⁽³⁾ Instrumental variability was performed using a standard solution of 50 ng/L in solvent

46 n.a. not applicable

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48 **Table SI-3.** Loss during filtration (expressed as %) with standard deviation (n=3)

Filter material	Wastewater				Ultrapure water			
	pH = 7.5	sd (n=3)	pH = 2.5	sd (n=3)	pH = 7.5	sd (n=3)	pH = 2.5	sd (n=3)
Glass fibre + PES	27	0.1	100	0.1	-8	0,1	73	0,2
Glass fibre+ cellulose nitrate and acetate	30	0.6	82	0.1	15	0,3	90	0,1
Glass fibre (45 mm)	27	0.2	77	0.1	8	0,2	55	0,1
RC (syringe filter)	4	0.03	85	0.1	-		-	
PES syringe (syringe filter)	14	0.04	99	0.2	-		-	

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50 **Table SI-4.** Mean (m) of replicates (expressed in ng L⁻¹) and standard deviation (sd) (n=3) per sample
51 and participant laboratory in the inter-laboratory study.

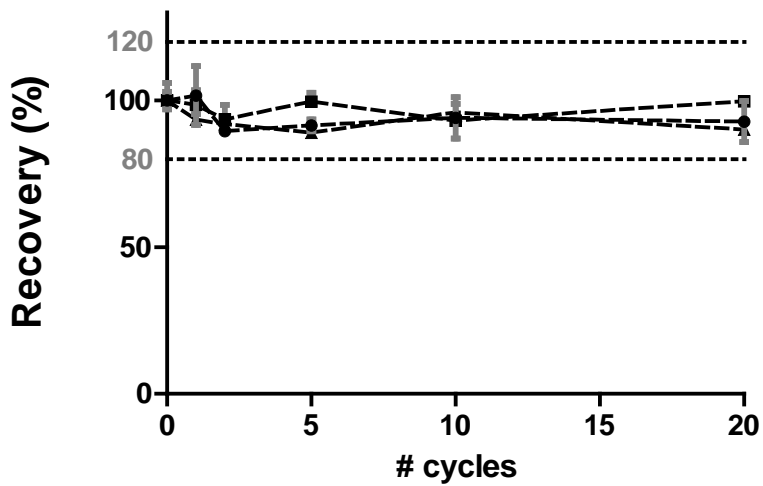
		Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8
Sample 1	m	1158	860	604	848	588	1040	602	1210
	sd	94	92	11	10	13	81	97	308
Sample 2	m	1226	983	665	727	629	1055	732	1434
	sd	15	65	22	67	20	51	137	267
Sample 3	m	1762	1580	1148	1413	975 ^a	1759	1043	2540
	sd	56	79	32	19	-	103	95	133
Sample 4	m	458	N/D	472	695	193	663	174	2532
	sd	77	-	111	74	7	82	25	390

52 N/D: non detected (below LOD)

53 ^a n=1

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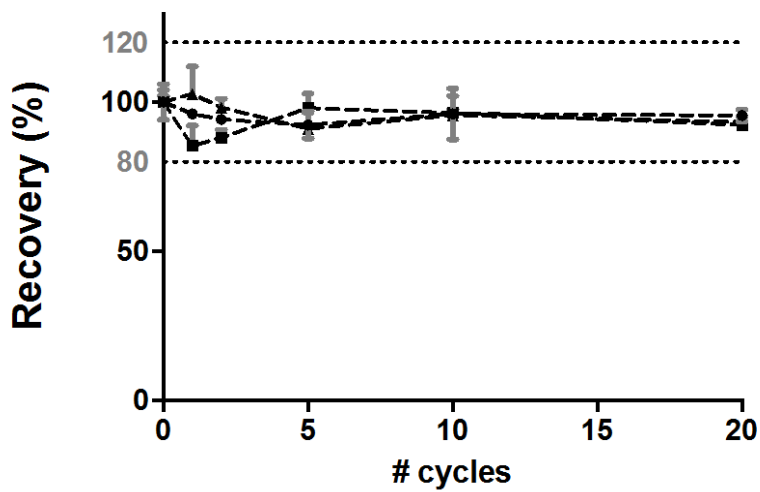
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57 **Figure SI-1.1.** Average THC-COOH recovery (in wastewater matrix) after n cycles of freezing-
 58 thawing relative to the 1st cycle. Error bars represent the standard deviation (n=3). Dotted
 59 lines at y=80 and 120%. Legend: Lab 1: circle ●; Lab 2: square ■; Lab 3: triangle ▲.

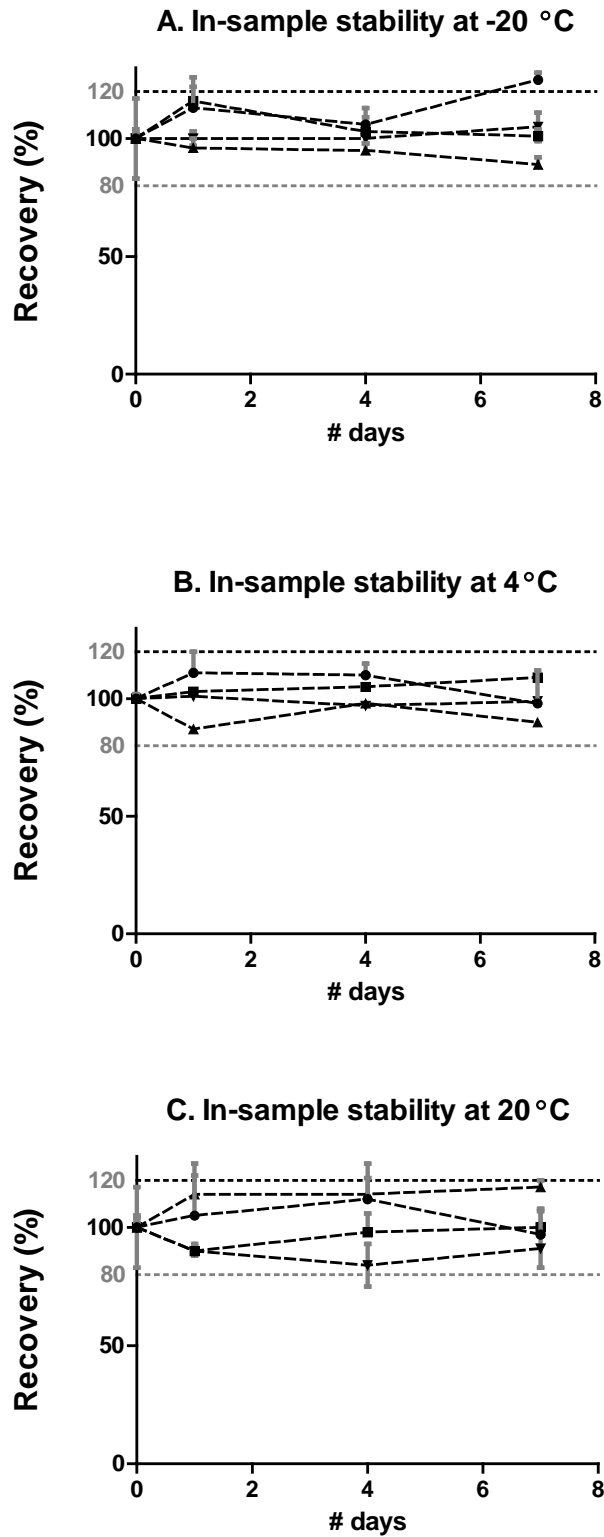
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62 **Figure SI-1.2.** Average THC-COOH recovery (in ultrapure water matrix) after n cycles of
 63 freezing-thawing relative to the 1st cycle. Error bars represent the standard deviation (n=3).
 64 Dotted lines at y=80 and 120%. Legend: Lab 1: circle ●; Lab 2: square ■; Lab 3: triangle ▲.

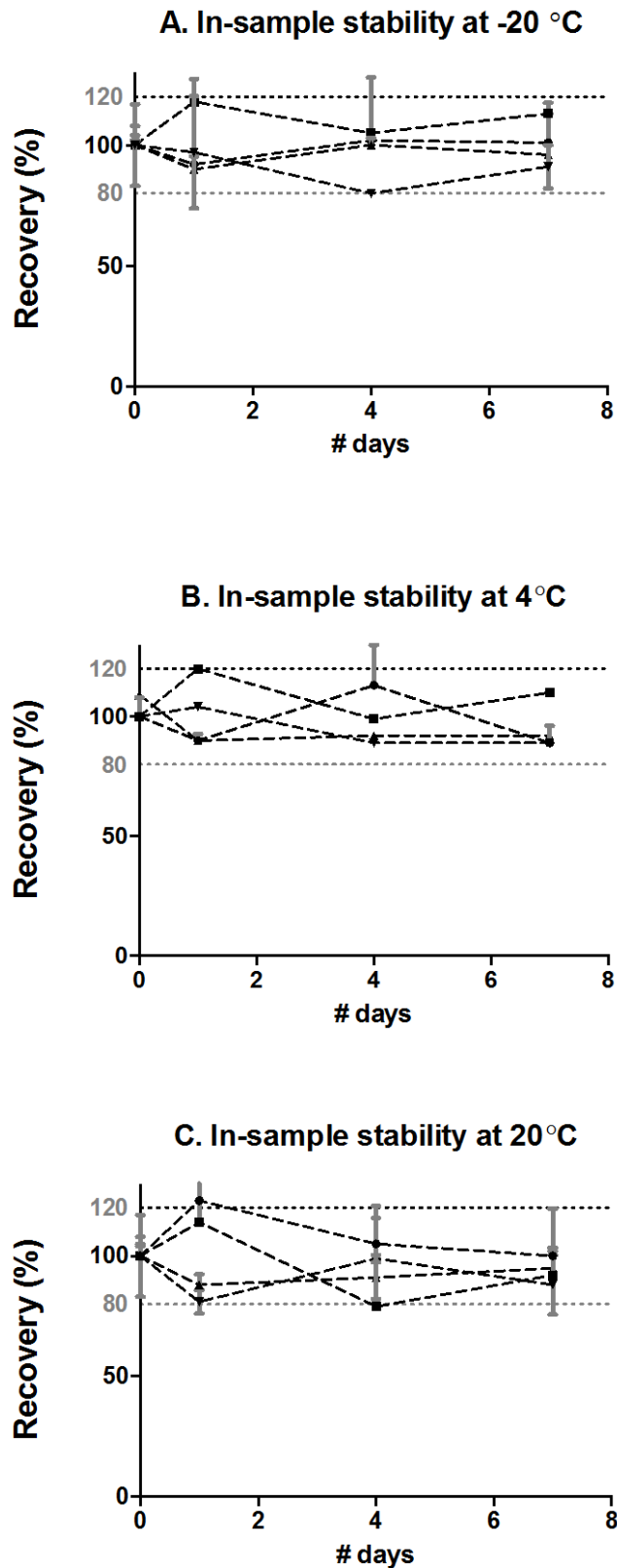
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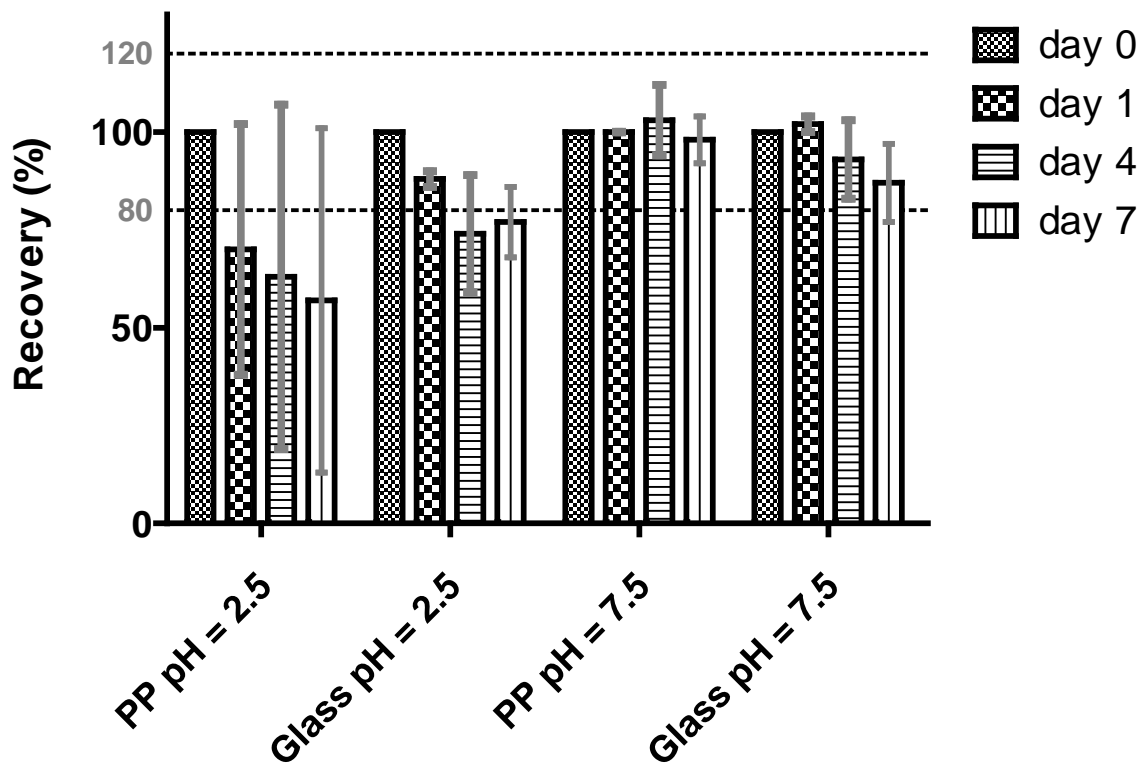
67 **Figure SI-2.1.** Stability of THC-COOH in wastewater stored at different temperatures. Data are
 68 expressed as recovery relative to day 0. A at -20°C, B at 4°C, C at 20°C. Lab 1: circle ●; Lab 2: square
 69 ■; Lab 3: triangle ▲; Lab 4: triangle upside down ▼

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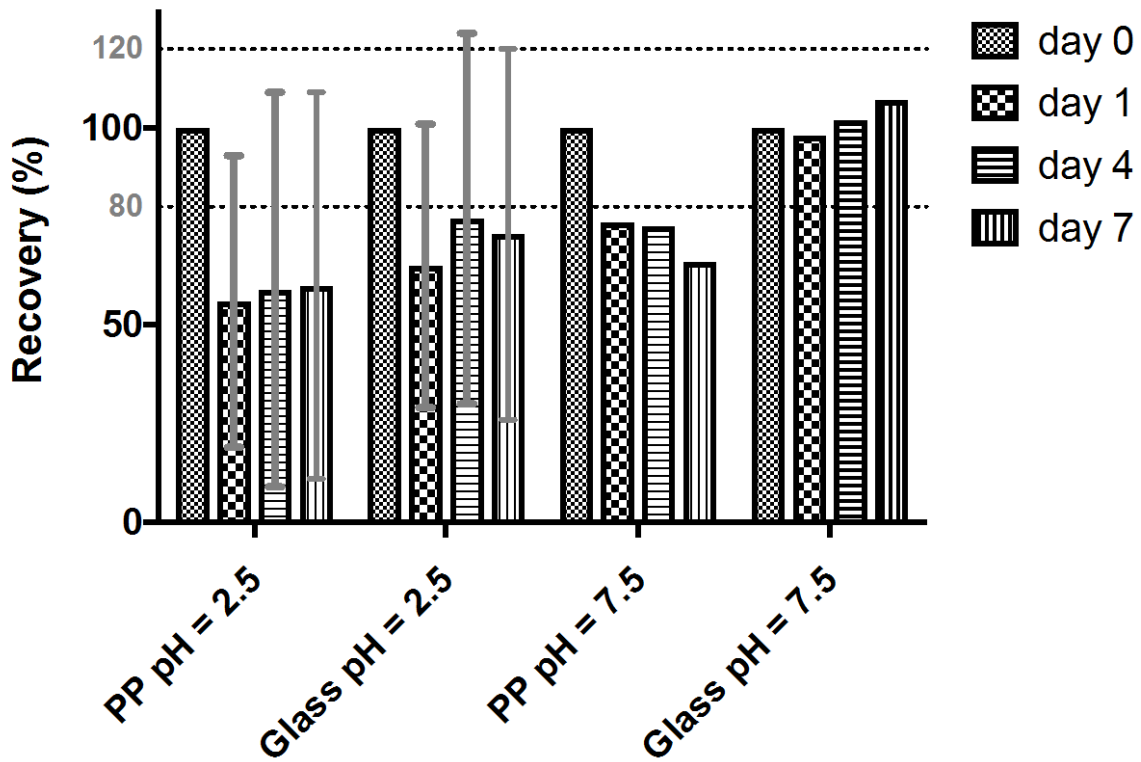
72 **Figure SI-2.2.** Stability of THC-COOH in ultrapure water stored at different temperatures. Data are
 73 expressed as recovery relative to day 0. A at -20°C, B at 4°C, C at 20°C. Lab 1: circle ●; Lab 2: square
 74 ■; Lab 3: triangle ▲; Lab 4: triangle upside down ▼



76

77 **Figure SI-3.1.** Influence of pH on sorption to polypropylene or glass container walls of THC-
 78 COOH spiked in wastewater. Data collected during a period of 7 days and expressed as
 79 recovery relative to day 0.

80



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82 **Figure SI-3.2.** Influence of pH on sorption to polypropylene or glass container walls of THC-
 83 COOH spiked in ultrapure water. Data collected during a period of 7 days and expressed as
 84 recovery relative to day 0.

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