Supplementary information

Comparison of phosphodiesterase type V inhibitors use in eight European cities through analysis of urban wastewater

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Table SI-1. WWTPs characteristics.

City		Bristol	Brussels	Castellon	Copenhagen	Milan	Oslo	Utrecht	Zurich
Residential Population		**886650**	**953987**	**180690**	**531000**	**1100000**	**580639**	**300000**	**410000**
Date of sample collection day 1	dd.mm.yyyy	16-3-2015	18-3-2015	25-3-2015	10-3-2015	10-3-2015	11-03-2015	4-3-2015	18-3-2015
Date of sample collection day 2	dd.mm.yyyy	10-3-2015	19-3-2015	26-3-2015	11-3-2015	11-3-2015	12-03-2015	5-3-2015	19-3-2015
Date of sample collection day 3	dd.mm.yyyy	11-3-2015	20-3-2015	27-3-2015	12-3-2015	12-3-2015	13-03-2015	6-3-2015	20-3-2015
Date of sample collection day 4	dd.mm.yyyy	12-3-2015	21-3-2015	28-3-2015	13-3-2015	13-3-2015	14-03-2015	7-3-2015	21-3-2015
Date of sample collection day 5	dd.mm.yyyy	13-3-2015	22-3-2015	29-3-2015	14-3-2015	14-3-2015	15-03-2015	8-3-2015	22-3-2015
Date of sample collection day 6	dd.mm.yyyy	14-3-2015	23-3-2015	30-3-2015	15-3-2015	15-3-2015	16-03-2015	9-3-2015	23-3-2015
Date of sample collection day 7	dd.mm.yyyy	15-3-2015	24-3-2015	31-3-2015	16-3-2015	16-3-2015	17-03-2015	10-3-2015	24-3-2015
Total influent day 1	m^3/24h	197493	234264	50228	148724	423110	333480	47740	157084
Total influent day 2	m^3/24h	204491	235442	49161	150936	403240	308279	45030	161005
Total influent day 3	m^3/24h	198950	234906	43728	147175	412310	277450	49530	161427
Total influent day 4	m^3/24h	197523	233096	38301	144840	402240	256766	46030	200010
Total influent day 5	m^3/24h	252682	230375	37243	145197	403020	250384	46900	243013
Total influent day 6	m^3/24h	220687	234774	37469	137793	422690	254570	45970	177167
Total influent day 7	m^3/24h	193194	359951	40476	137244	597470	252722	44580	160912
Sampling mode	-proportional	time	volume	time	volume	volume	volume	volume	volume
Sampling interval	m^3 or min	15 min	1300 m^3	15 min	2000 m^3	3800 m^3	1500 m^3	400 m^3	900 m^3
Sampling frequency day 1	min	15	8	15	19	13	6	12	8
Sampling frequency day 2	min	15	8	15	19	14	7	13	8
Sampling frequency day 3	min	15	8	15	20	13	8	12	8
Sampling frequency day 4	min	15	8	15	20	14	8	13	6
Sampling frequency day 5	min	15	8	15	20	14	9	12	5
Sampling frequency day 6	min	15	8	15	21	13	8	13	7
Sampling frequency day 7	min	15	5	15	21	9	9	13	8
Average wastewater temperature day 1	°C	n.a.	n.a.	13.1	n.a.	17.5	7.8	13.5	14.7
Average wastewater temperature day 2	°C	n.a.	n.a.	12.0	n.a.	17.6	8.0	n.a.	14.7

Average wastewater temperature day 3	°C	n.a.	n.a.	16.7	n.a.	17.6	8.2	n.a.	14.7
Average wastewater temperature day 4	°C	n.a.	n.a.	n.a.	n.a.	17.7	8.1	14.1	14.1
Average wastewater temperature day 5	°C	n.a.	n.a.	n.a.	n.a.	17.5	8.2	n.a.	12.6
Average wastewater temperature day 6	°C	n.a.	n.a.	17.5	n.a.	17.2	8.5	13.0	14.2
Average wastewater temperature day 7	°C	n.a.	n.a.	19.5	n.a.	16.0	8.7	n.a.	14.7
pH in sample day 1		n.a.	n.a.	7.4	8.0	8.0	7.5	8.6	7.8
pH in sample day 2		n.a.	n.a.	6.9	8.0	7.9	n.a.	8.3	8.1
pH in sample day 3		n.a.	n.a.	7.6	8.2	7.7	n.a.	8.3	8.3
pH in sample day 4		n.a.	n.a.	n.a.	8.1	7.8	n.a.	8.0	8.0
pH in sample day 5		n.a.	n.a.	n.a.	8.0	7.7	n.a.	8.1	8.0
pH in sample day 6		n.a.	n.a.	7.5	8.0	7.7	n.a.	8.3	8.1
pH in sample day 7		n.a.	n.a.	7.7	8.1	7.6	7.4	8.1	8.0
BOD_5 day 1	mg/L	n.a.	n.a.	245	411	183	103	n.a.	n.a.
BOD_5 day 2	mg/L	n.a.	n.a.	245	377	172	n.a.	n.a.	n.a.
BOD_5 day 3	mg/L	n.a.	n.a.	250	451	179	n.a.	n.a.	n.a.
BOD_5 day 4	mg/L	n.a.	n.a.	n.a.	423	n.a.	n.a.	n.a.	n.a.
BOD_5 day 5	mg/L	n.a.	n.a.	n.a.	456	n.a.	n.a.	n.a.	n.a.
BOD_5 day 6	mg/L	n.a.	n.a.	200	434	175	n.a.	n.a.	n.a.
BOD_5 day 7	mg/L	n.a.	n.a.	360	439	102	186	n.a.	n.a.
COD day 1	mg/L	n.a.	n.a.	516	909	372	273	530	n.a.
COD day 2	mg/L	n.a.	n.a.	516	585	344	n.a.	811	n.a.
COD day 3	mg/L	n.a.	n.a.	498	664	303	n.a.	530	n.a.
COD day 4	mg/L	n.a.	n.a.	n.a.	644	298	n.a.	568	n.a.
COD day 5	mg/L	n.a.	n.a.	n.a.	755	292	n.a.	598	n.a.
COD day 6	mg/L	n.a.	n.a.	677	693	385	n.a.	648	n.a.
COD day 7	mg/L	n.a.	n.a.	807	667	226	372	524	n.a.
Ntot day 1	mg/L	n.a.	n.a.	47.5	64.4	31.0	n.a.	n.a.	n.a.
Ntot day 2	mg/L	n.a.	n.a.	n.a.	61.7	29.4	n.a.	n.a.	n.a.
Ntot day 3	mg/L	n.a.	n.a.	n.a.	57.8	29.9	n.a.	n.a.	n.a.

Ntot day 4	mg/L	n.a.	n.a.	n.a.	66.1	n.a.	n.a.	n.a.	n.a.
Ntot day 5	mg/L	n.a.	n.a.	n.a.	64.3	n.a.	n.a.	n.a.	n.a.
Ntot day 6	mg/L	n.a.	n.a.	76.0	63.2	31.6	n.a.	n.a.	n.a.
Ntot day 7	mg/L	n.a.	n.a.	n.a.	61.2	21.0	n.a.	n.a.	n.a.
Ptot day 1	mg/L	n.a.	n.a.	7.4	9.7	3.6	3.5	8.9	n.a.
Ptot day 2	mg/L	n.a.	n.a.	n.a.	8.9	3.5	3.5	9.9	n.a.
Ptot day 3	mg/L	n.a.	n.a.	n.a.	8.7	3.5	3.5	10.3	n.a.
Ptot day 4	mg/L	n.a.	n.a.	n.a.	9.0	n.a.	3.5	9.2	n.a.
Ptot day 5	mg/L	n.a.	n.a.	n.a.	10.1	n.a.	3.5	9.7	n.a.
Ptot day 6	mg/L	n.a.	n.a.	8.0	9.3	3.9	4.3	9.1	n.a.
Ptot day 7	mg/L	n.a.	n.a.	n.a.	9.5	2.4	4.3	9.7	n.a.
NH_4-N day 1	mg/L	n.a.	n.a.	n.a.	44.0	n.a.	15.9	40.7	20.9
NH_4-N day 2	mg/L	n.a.	n.a.	n.a.	41.0	n.a.	n.a.	55.8	26.6
NH_4-N day 3	mg/L	n.a.	n.a.	n.a.	41.0	n.a.	n.a.	41.9	23.0
NH_4-N day 4	mg/L	n.a.	n.a.	n.a.	45.0	n.a.	n.a.	38.7	21.1
NH_4-N day 5	mg/L	n.a.	n.a.	n.a.	44.0	n.a.	n.a.	43.3	17.8
NH_4-N day 6	mg/L	n.a.	n.a.	n.a.	42.0	n.a.	n.a.	41.1	20.8
NH_4-N day 7	mg/L	n.a.	n.a.	n.a.	41.0	n.a.	21.4	39.4	28.6

n.a. not available

Table SI-2. Selected PDE5 inhibitors and LC-MS/MS parameters used for compounds identification.

	CAS number	Molecular formula	Log Kow (*)	$[M+H]^+$	Product ions (m/z)	Collision energy (V)	S-Lens	RT (min)
Sildenafil (ILIS 1)	171599-83-0	$C_{22}H_{30}N_6O_4S$	2.30	475.2	58.2 (Q)	36	118	10.5
					100.2 (q1)	28		
					283.2 (q2)	36		
Desmethylsildenafil (ILIS 2)	139755-82-1	$C_{21}H_{28}N_6O_4S$	2.09	461.1	283.1 (Q)	35	130	9.6
					311.1 (q)	29		
Desethylsildenafil (ILIS 1)	139755-91-2	$C_{20}H_{28}O_4N_6S$	1.99	449.2	283.1 (Q)	36	138	9.4
					311.1 (q)	27		
Noracetildenafil (ILIS 1)	949091-38-7	$C_{24}H_{32}N_6O_3$	n.a.	453.2	97.1 (Q)	31	148	9.2
					113.1 (q)	31		
Tadalafil (ILIS 1)	171596-29-5	$C_{22}H_{19}N_3O_4$	0.04	390.0	204.1 (Q)	57	92	13.9
					268.1 (q)	14		
Aminotadalafil (ILIS 1)	385769-84-6	$C_{21}H_{18}N_4O_4$	-1.20	391.0	204.1 (Q)	56	87	11.9
					262.1 (q)	31		
Chloropretadalafil (ILIS 1)	171489-59-1	$C_{22}H_{19}ClN_2O_5$	2.58	427.1	274.1 (Q)	31	93	16.9
					135.0 (q)	19		
N-octyl nortadalafil (ILIS 1)	1173706-35-8	$C_{29}H_{33}N_3O_4$	5.22	488.2	366.2 (Q)	17	120	17.8
					169.1 (q)	39		
Vardenafil (ILIS 1)	224789-15-5	$C_{23}H_{33}N_6O_4S$	2.79	489.3	151.1 (Q)	41	159	9.6
					312.1 (q)	39		
N-desethylvardenafil (ILIS 1)	448184-46-1	$C_{21}H_{28}N_6O_4S$	2.09	461.2	151.1 (Q)	43	143	9.6
					312.2 (q)	33		
ILIS 1 Sildenafil-d8	951385-68-5	$C_{22}H_{22}D_8N_6O_4S$	2.30	483.3	62.1 (Q)	37	126	10.5
					108.3 (q)	29		
ILIS 2: Desmethylsildenafil-d8	1185168-06-2	$C_{21}H_{20}D_8N_6O_4S$	2.09	469.2	283.1 (Q)	37	160	10.7
					311.1 (q)	30		

n.a.: not available
(*) Log Kow (KOWWIN program estimates)

Table SI-3. Method performance: linearity, limits of detection and quantification, intraday and interday repeatability, procedural recovery and matrix effect.

	linearity	LOD	LOQ	Intraday repeatability (RSD (%) , n=7)				Interday repeatability (RSD (%), n=7, d=3)				Procedural Recovery ± RSD (%)				Matrix Effect ± RSD (%)			
	(r^2)	(ng/L)	(ng/L)	20 ng/L	50 ng/L	100 ng/L	500 ng/L	20 ng/L	50 ng/L	100 ng/L	500 ng/L	20 ng/L	50 ng/L	100 ng/L	500 ng/L	20 ng/L	50 ng/L	100 ng/L	500 ng/L
sildenafil	0.9997	1.8	6	16	10	5	5	24	9	10	9	93.1 ± 19.7	102.7 ± 10.4	100.1 ± 11.7	97.5 ± 14.7	241.9 ± 22.6	247.8 ± 15.9	82.3 ± 10.1	73.6 ± 12.2
desmethylsildenafil	0.9999	5.4	18	27	15	7	12	25	24	8	9	99.9 ± 20.2	100.4 ± 16.9	99.9 ± 12.5	90.8 ± 21.1	406.6 ± 35.0	437.1 ± 34.7	116.8 ± 12.6	82.0 ± 21.3
desethylsildenafil	0.9997	0.5	1.6	18	11	10	4	33	18	9	8	97.2 ± 22.1	100.7 ± 10.4	102.3 ± 11.4	93.2 ± 19.0	393.4 ± 30.9	549.0 ± 17.1	156.8 ± 14.7	99.8 ± 13.3
noracetil	0.9990	6	20	31	13	5	6	36	23	9	6	94.8 ± 57.7	102.7 ± 17.1	104.4 ± 13.9	99.0 ± 15.7	298.6 ± 85.3	216.1 ± 33.6	70.5 ± 51.0	46.7 ± 34.0
tadalafil	0.9998	2.3	7.5	10	11	11	7	13	13	13	11	89.3 ± 21.5	96.5 ± 7.8	96.0 ± 8.6	97.7 ± 12.7	246.6 ± 23.6	270.1 ± 14.2	84.0 ± 10.3	72.2 ± 12.5
aminotadalafil	0.9995	1.8	6	8	11	11	8	14	16	11	11	91.3 ± 16.5	100.9 ± 8.6	97.5 ± 8.8	98.2 ± 13.9	217.5 ± 15.7	251.0 ± 15.4	77.8 ± 10.8	69.1 ± 13.6
chloropretadalafil	0.9993	4	13.3	6	8	9	8	12	15	8	10	93.4 ± 15.4	87.2 ± 8.4	91.7 ± 10.2	92.4 ± 11.5	195.0 ± 20.6	243.8 ± 14.2	73.1 ± 10.2	64.9 ± 13.6
n-octylnortadalafil	0.9999	30	100	11	15	10	10	20	27	26	16	-	-	16.4 ± 20.5	27.4 ± 36.8	163.1 ± 19.3	234.0 ± 24.1	77.4 ± 18.8	75.3 ± 10.9
vardenafil	0.9998	7.2	24	17	18	9	5	22	20	14	7	92.2 ± 23.6	101.3 ± 12.2	102.1 ± 12.5	96.6 ± 12.1	320.5 ± 32.2	322.7 ± 24.9	96.5 ± 17.2	83.4 ± 12.3
n-desethylvardenafil	0.9998	9	30	26	16	9	8	37	30	15	13	95.4 ± 25.0	96.5 ± 14.4	98.9 ± 13.0	97.0 ± 16.7	607.0 ± 26.9	616.0 ± 26.0	152.1 ± 14.7	125.8 ± 13.0

Table SI-4. Amount of API prescribed in 2015, expressed in mg year^{-1}.

Country	Prescribed mg year^{-1}		
	Sildenafil [a]	**Tadalafil**	**Vardenafil**
Belgium	$3{,}23 \cdot 10^{7}$ [b]	$8{,}53 \cdot 10^{5}$	n.a.
England	$1{,}18 \cdot 10^{9}$	$9{,}12 \cdot 10^{7}$	$1{,}26 \cdot 10^{7}$
Italy	$6{,}66 \cdot 10^{8}$	$1{,}33 \cdot 10^{8}$	n.a.
the Netherlands	$1{,}17 \cdot 10^{8}$	$1{,}57 \cdot 10^{7}$	$1{,}60 \cdot 10^{6}$
Norway	$9{,}75 \cdot 10^{7}$	$2{,}20 \cdot 10^{7}$	$3{,}38 \cdot 10^{6}$

[a] total sildenafil

[b] Estimated from the ED/VA ratio observed in the Netherlands

n.a.: not available

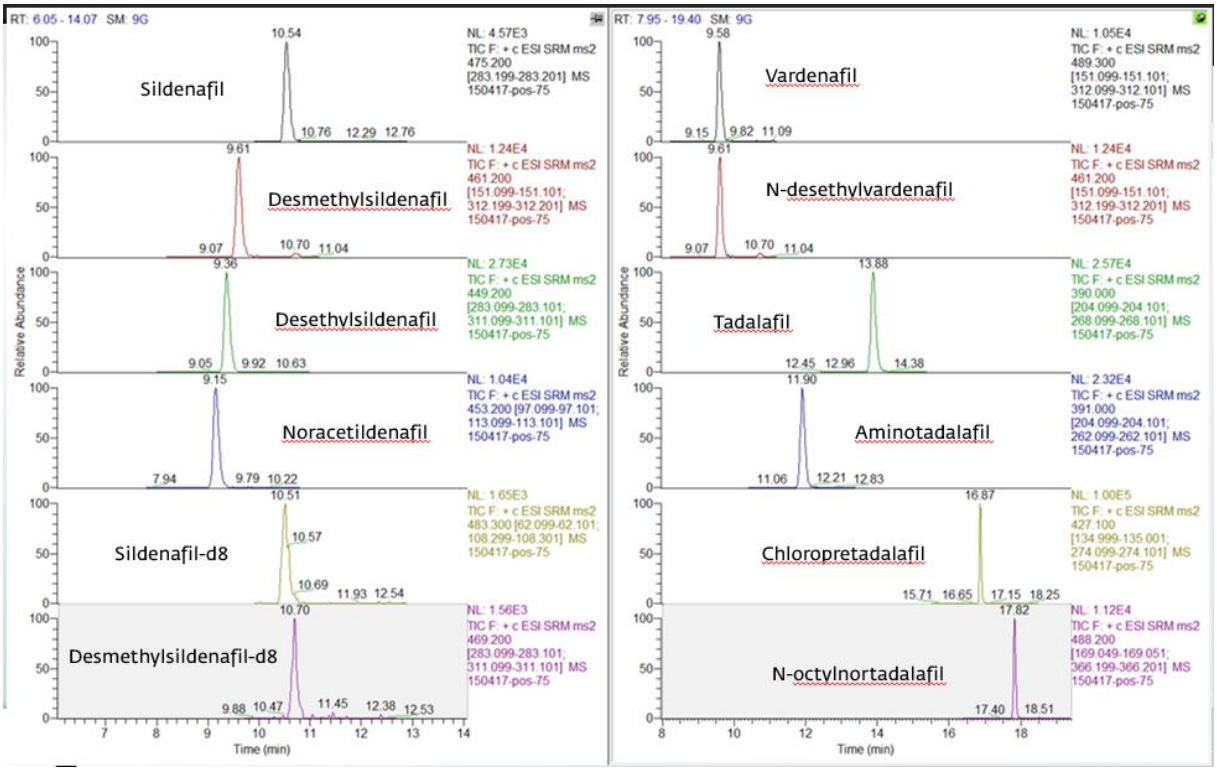

Figure SI-1. Chromatogram from a standard mixture of the selected PDE5 at 50 ng L^{-1} concentration level.