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

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Abstract

In this work a step forward in investigating the use of prescription drugs, namely erectile dysfunction products, at European level was taken by applying the wastewater-based epidemiology approach. 24-h composite samples of untreated wastewater were collected at the entrance of eight wastewater treatment plants serving the catchment within the cities of Bristol, Brussels, Castellón, Copenhagen, Milan, Oslo, Utrecht and Zurich. A validated analytical procedure with direct injection of filtered aliquots by liquid chromatography-tandem mass spectrometry was applied. The target list included the three active pharmaceutical ingredients (sildenafil, tadalafil and vardenafil) together with (bio)transformation products and other analogues. Only sildenafil and its two human urinary metabolites desmethyl- and desethylsildenafil were detected in the samples with concentrations reaching 60 ng L−1. The concentrations were transformed into normalized measured loads and the estimated actual consumption of sildenafil was back-calculated from these loads. In addition, national prescription data from five countries was gathered in the form of the number of prescribed daily doses and transformed into predicted loads for comparison. This comparison resulted in the evidence of a different spatial trend across Europe. In Utrecht and Brussels, prescription data could only partly explain the total amount found in wastewater; whereas in Bristol, the comparison was in agreement; and in Milan and Oslo a lower amount was found in wastewater than expected from the prescription data. This study illustrates the potential of wastewater-based epidemiology to investigate the use of counterfeit medication and rogue online pharmacy sales.

Keywords: erectile dysfunction; prescription drugs; urban wastewater; LC-MS/MS; consumption; counterfeit

Highlights (3 to 5, max 85 characters):

- Wastewater-based epidemiology successfully used to track counterfeit medication
- Very sensitive LC-MS/MS method allows identification of targets at low ng L^{-1} level
- Different spatial trends in sildenafil use were found across Europe

1. Introduction

The chemical analysis of raw wastewater with advanced mass spectrometry techniques allows the determination of human urinary biomarkers when these are excreted in sufficient concentrations and remain stable in their way along the sewer system (Castiglioni et al. 2012). The finding of specific biomarkers may reveal valuable near real-time information regarding a population's lifestyle, illness, and exposure to external agents. Successful studies thus far have revealed the population's level of oxidative stress (Ryu et al. 2015), its exposure to pesticides (Rousis et al. 2017), and to phthalate plasticizers (González-Mariño et al. 2017), its consumption of legal substances such as alcohol, nicotine or caffeine (Baz-Lomba et al. 2016, Ryu et al. 2016), its use of illicit drugs (Causanilles et al. 2017, Ort et al. 2014) and other psychoactive substances (Bade et al. 2017), and its intake of certain pharmaceuticals (Causanilles et al. 2016).

The monitoring of active pharmaceutical ingredients (APIs) and their metabolites in wastewater offers an interesting value (van Nuijs et al. 2015). These substances have gone through a very detailed study and clinical trial before their final usage approval. Therefore the information regarding the absorbed dose after drug intake, the metabolic pathway and the excretion profile and rates in biological matrices is well known (Abed 2014). This information allows the selection of the appropriate target urinary biomarker in the application of wastewater-based epidemiology. Concentrations in untreated wastewater, considered a collective pooled urine sample, are then obtained as measured environmental concentration (MEC) of the unchanged product or its metabolites, which can be converted into environmental mass loads and then back-calculated into consumption estimates applying the appropriate correction factor. In addition, the number of dispensed pharmaceutical in the form of defined daily doses (DDD) or product quantities dispensed by pharmacies or doctors can be obtained (in most cases, depending of the pharmaceutical and the country). From this data the average amount of mg of the API that have been legally dispensed per day can be calculated and transformed into a predicted environmental concentration (PEC) (Carballa et al. 2008, Verlicchi et al. 2014).

The comparison between prescription data and wastewater loads may result in three different scenarios: (i) consumption estimated from wastewater load is lower than what would be expected from the dispensed data. This would represent the case of pharmaceuticals that have been less used than what it is prescribed or defined by the DDD; (ii) consumption estimated from wastewater load and the load expected from dispensed data are similar, which would represent the ideal situation, where there is no misuse; (iii) consumption estimated from wastewater load is higher than the load expected from the dispensed data. The third scenario represents the case of pharmaceuticals that are available in a counterfeit or falsified form and that can be acquired from other sources such as rogue online pharmacies or black market. This was the case observed for the phosphodiesterase type V inhibitor sildenafil, API in erectile dysfunction pharmaceuticals, in a study performed in the Netherlands in 2013 (Venhuis et al. 2014b). Results showed that only one third to one half of the consumption estimated from wastewater loads could be related to the acquisition of the drug from legal sources (Venhuis et al. 2014a).

However, the comparison needs to be handled with care, since other sources for discrepancy can be present. They might be related to the sewer system, with the incomplete release to the sewer system or elimination processes between the consumption point and the wastewater treatment plant, namely degradation, sorption and sedimentation (van Nuijs et al. 2015, Verlicchi et al. 2014).

Erectile dysfunction is estimated to affect 25 to 35 million men over the age of 18 in Europe, according to the European Federation of Pharmaceutical Industries and Associations (EFPIA 2017). It is a disease of increasing concern, since an aging population will result in higher prevalence. Despite the high number of men affected, it is still highly stigmatized, and users usually tend to hide their related drug use. Illegal trading with products from the internet and with counterfeit medicines is increasing (Chiang et al. 2017). However, the individuals purchasing medicines via the internet are for the most part not sufficiently aware of the risks they run in doing so (Keizers et al. 2016). Concerns about the quality of these products may arise, specially towards the possible presence of impurities that may lead to poisoning if toxic (Johnston and Holt 2014), and an increased risk of side effects or overdosing.

In this work the wastewater-based epidemiology approach was applied to assess the use of phosphodiesterase type V inhibitors in eight European cities accounting to almost 5 million inhabitant equivalents. To do so, 24-h composite wastewater samples were collected in each city for seven consecutive days and analysed by liquid chromatography coupled to tandem mass spectrometry. Measured concentrations in sample were converted into loads and back-calculated to estimate consumption with known pharmacokinetic information. In addition, available data at national level of the number of prescribed or dispensed erectile dysfunction pharmaceuticals were gathered to discuss their correlation.

2. *Materials and methods*

The analytical methodology used to perform the wastewater chemical analysis was previously validated elsewhere (Causanilles et al. 2016). The chemicals and materials section can be found in the Supplementary Information (**Section SI-1**).

2.1.Sample collection

A week-monitoring sampling campaign was performed in March 2015 in eight European cities. For seven consecutive days 24-h influent composite samples were collected at the entrance of the wastewater treatment plants (WWTPs) serving the cities of Bristol, England; Brussels, Belgium; Castellon, Spain; Copenhagen, Denmark; Milan, Italy; Oslo, Norway; Utrecht, the Netherlands; and Zurich, Switzerland. The number of inhabitants included in the total catchment area under study represented almost 5 million people in Europe. **Table SI-1** compiles detailed information about the sample collection at the different locations: date of sample collection, influent flow (m^3 $24h^{-1}$), sampling mode and frequency, average wastewater temperature (°C), pH, biological and chemical oxygen demand (BOD_5 and COD), total phosphate (P_{tot}), and nitrogen content as Kjeldahl (N_{tot}) and ammonia (NH_4-N).

2.2.Analytical methodology

All samples were collected in high density polyethylene bottles, shipped frozen to KWR in Nieuwegein (NL) and stored in the dark at –20 °C until treatment. Samples were thawed and homogenized. Then a 10 mL aliquot was spiked with deuterated analogues to act as surrogate and filtered with regenerated cellulose syringe filters. With no further pre-treatment a 100 µL aliquot of each sample was injected into the liquid chromatography coupled to triple quadruple mass spectrometer (Thermo Scientific TSQ Vantage, Thermo Electron, Bremen, Germany). Chromatographic separation was achieved with a XBridge C18 column (150 mm × 2.1 mm I.D., particle size 3.5 µm, Waters, Etten-Leur, the Netherlands) preceded by a KrudKatcher ULTRA HPLC in-line SS filter (0.5 µm × 0.1 mm I.D., Phenomenex, Torrance, USA). The mobile phase consisted of an optimized water-methanol-acetonitrile gradient at 0.3 mL min^{-1} flow. The MS system operated in selected reaction monitoring (SRM) and positive mode during data acquisition. For each compound two transitions of the molecular ion $[M+H]^+$ were monitored, one for quantification and the second for confirmation purposes. Analyte concentrations were quantified using the correspondent deuterated analogue. Specific LC-MS/MS parameters for compound identification can be found in **Table SI-2**.

2.3.Calculations

Phosphodiesterase type 5 inhibitors are the API in drugs used to treat erectile dysfunction (ED). Their classification within the ATC-system (Anatomic Therapeutic Chemical) corresponds to the group of genitourinary system and sex hormones (G), urological (04B), erectile dysfunction (E). The individual codes are necessary to find the national prescription and sales data of all formulations containing them as API despite the differences in brand name. The codes of the three approved substances included in the study and their established daily defined dose (DDD) can be found in **Table 1**. Sildenafil does not only have a registration as erectile stimulant, but also for pulmonary arterial hypertension. For this indication both the DDD and the number of prescriptions is lower. In the case of Belgium only the prescription data for the application of sildenafil as vasodilator antihypertensive (VA) was available. A similar trend in the prescription data was expected compared to the neighbouring country of The Netherlands and therefore the ratio ED/VA was extrapolated to estimate the number of prescriptions of sildenafil as erectile dysfunction drug in Belgium.

The number of DDDs prescribed in the year 2015 (see **Table 1**) were multiplied by the DDD value in mg and converted into kg $year^{-1}$. The kg of each API were multiplied by the urinary excretion factor (%) and divided by the country's population and the L day^{-1} inh^{-1} estimated from the influent flow at the wastewater treatment plants included in the study (as a way of measuring the water use per inhabitant) (Carballa et al. 2008, Verlicchi et al. 2014). PECs were obtained in ng L^{-1}. The excretion factors used in the calculation were gathered from different pharmacokinetic studies. According to Muirhead and colleagues (Muirhead et al. 2002) sildenafil is not excreted unchanged in urine, however in previous work it was found to account for up to a 10% in wastewater (Causanilles et al. 2016). The excretion ratios for its human urinary metabolites desmethyl- and desthylsildenafil were 3 and 22 % respectively. In the case of tadalafil, only a minor amount is excreted unchanged in urine (Phillips et al. 2004). In the case of vardenafil, approximately 0.7 – 3 % is excreted unchanged in urine, and its major metabolite component formed by N-deethylation, up to a 5 % (EMA 2005).

The chemical analysis of the wastewater samples included in the study resulted in the MEC of each of the analytes in ng L^{-1}. The ratio PEC/MEC was calculated to evaluate the accuracy of the environmental predictions (Verlicchi et al. 2014).

The loads were obtained by multiplying the measured concentration in each sample in ng L^{-1} by the daily influent flow rate at the WWTP in m^3 $24h^{-1}$. In order to normalize the load per 1000 inhabitants, the obtained values in mg day^{-1} were divided by the population included in the catchment area. This normalization allows the direct comparison of results among the different communities included in the study. In the case of concentration values in real sample below LOQ, values were replaced by 0.5 × LOQ when at least one day in the week had a concentration value above the LOQ. Concentration values below LOD, as well as concentration values lower than LOQ when all values at that location were below LOQ, were set to 0.5 × LOD (Ort et al. 2014). The loads are expressed in mg day^{-1} 1000 inh^{-1}.

Finally, sildenafil consumption was estimated from mass loads as indicated elsewhere (Venhuis et al. 2014b). The calculation was based on the available pharmacokinetic data and the assumption that there were no elimination processes such as degradation or sorption between the consumption point to the wastewater treatment plant, or dumping of unused drugs. Earlier stability studies confirmed there was not a statistically significant decrease in concentration of the target compounds after 48h storage at 4 °C (Causanilles et al. 2016).

Statistical analysis of the data was performed using GraphPad Prism 5.

Table 1. Information of the investigated pharmaceuticals and national prescription data.

Pharmaceutical	ATC code	DDD value[a] (use)	N° DDDs in 2015				
			Belgium[1]	England[2]	Italy[3]	The Netherlands[4]	Norway[5]
Sildenafil	G04BE03	50 mg (ED) 20 mg (VA)	602,596[b] (ED) 106,648 (VA)	23,572,110 (ED) 198,800 (VA)	13,314,239 (ED+VA)	2,190,688 (ED) 387,710 (VA)	1,949,770 (ED+VA)
Tadalafil	G04BE08	10 mg (ED)	85,276	9,120,725	13,314,239	1,570,918	2,203,956
Vardenafil	G04BE09	10 mg (ED)	n.a.	1,262,350	n.a.	159,520	338,096

VA: Vasodilator Antihypertensive

ED: Erectile Dysfunction

n.a.: not available

[a] defined by the WHO Collaborating Centre for Drug Statistics Methodology, www.whocc.no

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

Information source indicated with numbered superscript:

[1] National Institute for Health and Disability Insurance, www.riziv.be

[2] National Health Service, www.nhsbsa.nhs.uk

[3] Agenzia Italina del Farmaco, www.agenziafarmaco.gov.it

[4] Dutch Foundation for Pharmaceutical Statistics, www.sfk.nl

[5] The Norwegian Institute of Public Health, www.norpd.no

3. Results and discussion

3.1. Predicted and measured environmental concentrations

The obtained PECs for the unchanged API sildenafil and its two urinary metabolites desmethyl- and desethylsildenafil are presented in **Table 2** (the yearly prescribed kg are shown in **Table SI-3**). The highest predicted concentration was estimated for England, followed by The Netherlands, Norway and Italy with similar values, and the lowest was estimated for Belgium. PEC was not calculated for the API tadalafil, since the literature indicates that only a minor amount of the unchanged form was putatively identified in urine. This would result in a predicted concentration close to zero, which is below the limits of detection in wastewater for this compound. PECs for vardenafil and its metabolite, N-desethylvardenafil were calculated for the countries with prescription data available, i.e. Norway, England and The Netherlands, although their presence in the environment was estimated to be minimal, below 1 ng L^{-1} (see **Table SI-4**). This predicted value is lower than their limits of detection in wastewater.

Table 2. Predicted environmental concentrations (PECs) in wastewater influents for sildenafil and its two metabolites, expressed in ng L^{-1}.

Country	**PEC ng L^{-1}**		
	Sildenafil	**Desmethylsildenafil**	**Desethylsildenafil**
Belgium	3.0 ± 0.4	0.9 ± 0.1	7 ± 1
England	25 ± 2	8 ± 1	56 ± 5
Italy	8 ± 1	2.3 ± 0.3	17 ± 2
The Netherlands	12.2 ± 0.4	3.7 ± 0.1	27 ± 1
Norway	11 ± 1	3.3 ± 0.4	24 ± 3

Results from the week-monitoring sampling campaign are reported in **Table 3**. Environmental concentrations per city are presented as the 7-d mean with standard deviation, expressed in ng L^{-1}. Method limits of quantification, in ng L^{-1}, are included in the table. Sildenafil and its two human metabolites were quantified at different concentrations. Sildenafil was not detected in the samples collected in Castellon and Milan. In the city of Oslo only the Sunday sample was detected above the limit of quantification, and the rest were replaced by 0.5 × LOQ. Values were found in the range of 4 to 19 ng L^{-1}. Desmethylsildenafil, the less abundant sildenafil metabolite, was not quantified in the cities of Castellon, Milan, Oslo and Zurich. In the cities of Copenhagen and Utrecht 2 and 4 days were <LOQ and replaced by 0.5 × LOQ. Values

were found in the range of 14 to 36 ng L^{-1}. Desethylsildenafil, the most abundant metabolite of sildenafil, was detected and quantified in all samples. Values were found in the range of 5 to 51 ng L^{-1}. Neither the other two APIs included in the study, tadalafil and vardenafil, nor their metabolites and analogues were found above their limits of detection. The metabolite to parent concentration ratio was calculated when available. The ratio of desethylsildenafil to sildenafil ranged from 1.7 to 3.6 (6 cities, 2.8 ± 0.8). These results were in line with the range of ratios observed in the Dutch cities of Amsterdam, Eindhoven and Utrecht in the years 2013 to 2015 (Causanilles et al. 2016). The ratio of desmethylsildenafil to sildenafil ratio ranged from 0.9 to 2.3 (4 cities, 1.6 ± 0.6). These results confirm literature findings because a lower ratio is expected for desmethylsildenafil, since it is the less abundant urinary metabolite (Muirhead et al. 2002).

Table 3. Measured environmental concentrations (MECs) expressed in ng L^{-1} with standard deviation (± SD) for 7 sampling days, n=7.

Compounds	LOD, ng L^{-1}	LOQ, ng L^{-1}	MEC (mean ± SD), ng L^{-1}							
			Bristol	**Brussels**	**Castellon**	**Copenhagen**	**Milan**	**Oslo**	**Utrecht**	**Zurich**
Sildenafil	2	6	12 ± 4	19 ± 3	(+)	14 ± 5	(+)	4 ± 2[a]	15 ± 4	9 ± 2
Desmethylsildenafil	5	18	26 ± 7	36 ± 2	(+)	19 ± 8[a]	(+)	(+)	14 ± 4[a]	(+)
Desethylsildenafil	1	2	28 ± 8	33 ± 5	13 ± 3	51 ± 7	5 ± 1	8 ± 4	51 ± 4	32 ± 5
Noracetildenafil	6	20	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Tadalafil	2	8	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Aminotadalafil	2	6	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Chloropretadalafil	4	13	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
N-octylnortadalafil	30	100	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Vardenafil	7	24	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
N-desethylvardenafil	9	30	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

[a] At least one value out of 7 is >LOQ; then the values <LOQ are replaced by 0.5 × LOQ

(+) below limit of quantification but above limit of detection

(-) below limit of detection

3.2. Comparison between PEC and MEC

The PEC/MEC ratio was evaluated using the arbitrary criteria given below, in order to establish the accuracy of the prediction:

- If $0.5 < PEC/MEC < 2$, then the PEC is within reasonable boundaries of a value of 1 (case (ii) explained in the introduction)
- If $PEC/MEC < 0.5$, then the PEC is relatively low, indicative of case (iii)
- If $PEC/MEC > 2$, then the PEC is relatively high, indicative of case (i)

The results are graphically presented in **Fig. 1**. The ratio for sildenafil was only satisfactory in the Netherlands (0.9 ± 0.2), for Belgium it was too low and for England, Italy and Norway, too high. The ratio for desmethylsildenafil was satisfactory in Italy (0.9 ± 0.1) and Norway (1.3 ± 0.1), although one should realize that MEC was taken as 0.5 × LOD in order to be able to calculate the ratio, because in both cities the metabolite was found in all samples at levels below the LOQ. For the remaining cities in Belgium, England and The Netherlands, the ratio was too low. The ratio for desethylsildenafil was satisfactory in the Netherlands (0.5 ± 0.1), for Belgium it was too low and for England, Italy and Norway, too high.

Two observations can be made from the evaluation of the PEC/MEC ratio. One is that in the case of Belgium, the predicted concentrations of sildenafil are much lower than the actual concentrations measured in wastewater. Although this may have been caused by unregistered use of sildenafil (case iii, see introduction), one should bear in mind that for the calculation of PEC in this case the estimation of prescribed DDDs was obtained by extrapolation from the Dutch ED/VA trend, because actual DDD data were lacking. The actual ED/VA ratio for Belgium may be different of course. One other possible reason for obtaining relatively low PECs is if during the sampling week heavy rainfall would have occurred. However, meteorological records for the city of Brussels (Ukkel station) showed that in the actual week of sampling almost no rainfall occurred.

The second observation corresponds to the three countries England, Italy and Norway, where both sildenafil and desethylsildenafil show a high PEC/MEC ratio. This translates into lower measured concentrations than what is predicted from national prescription data. This could be explained by the non-consumption of the total prescribed amount or by a higher degradation or sorption of the compounds in the local sewer systems. We currently don't have reasons to substantiate the likeliness of higher rates of in-sewer degradation in these countries.

Overall, the comparison results should be handled with care since wastewater analysis was performed only in one city per country in a limited time period (7 consecutive days), and therefore the extrapolation of results to the whole country will be biased by the specific spatial and temporal profiles of that city (versus other areas within the countries).

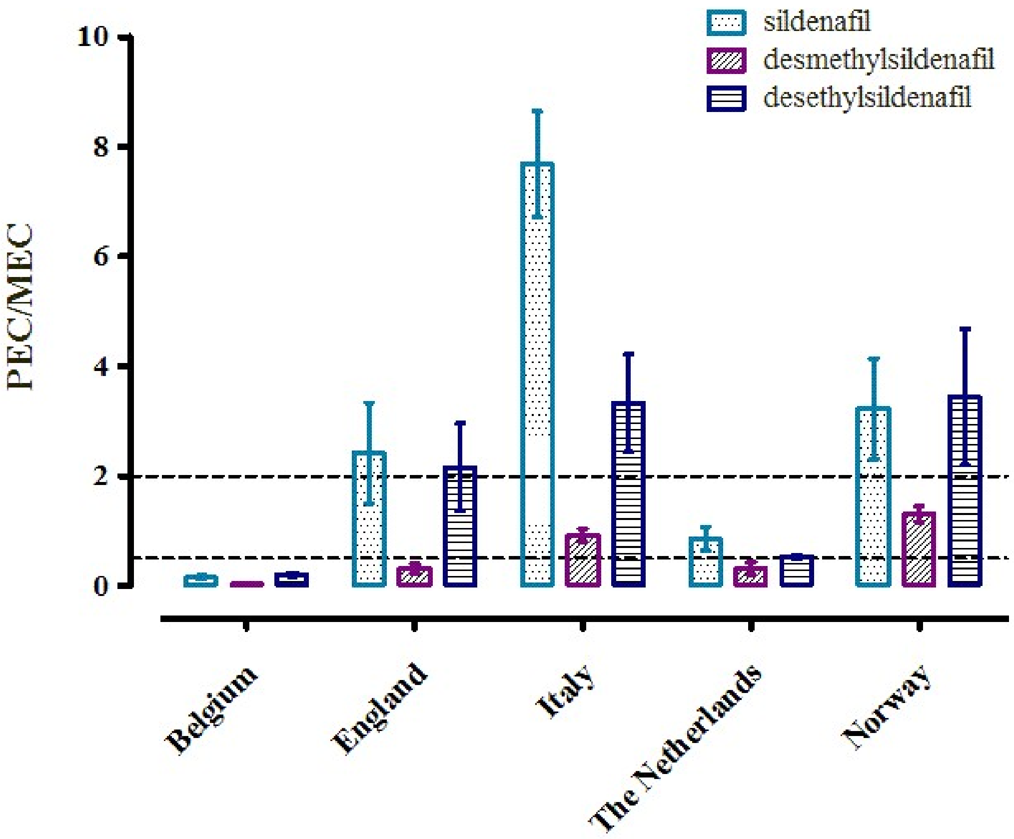


Fig. 1. Comparison of predicted and measured concentrations in influent wastewaters, expressed as the PEC/MEC ratio. Dotted lines at y = 0.5 and 2 represent the criteria limits.

For tadalafil and vardenafil the ratio PEC/MEC could not be calculated per se because of the minimal excretion ratios and non-detects in all wastewater samples. However, results of the measurements are in line with what was predicted based on prescription data because these PECs would also fall below the actual limits of detection.

3.3.Daily loads and back-calculated consumption

MEC were translated into loads in mg day^{-1} and normalized to 1000 inhabitants to allow a better comparison between the cities included in the study. The 7-day mean for each city with standard deviation is presented in **Table 4**. The highest normalized sildenafil load was found in the city of Brussels closely followed by Zurich and Copenhagen. Compared to these cities, a medium load was found in Bristol and Utrecht, and the lowest levels were observed in Milan and Castellón. For the metabolites a similar trend was found, in accordance with their excretion ratios. The daily variations are presented in **Fig. 2,** expressed as percentages of the total load. No significant increase in loads was found in weekend samples compared to weekday samples, reaffirming the use of sildenafil as needed and not with a clear recreational aim. This phenomenon known as "weekend effect" is very typical for illicit drugs such as cocaine or ecstasy (MDMA) (Causanilles et al. 2017, Salvatore et al. 2015).

Taking into account the loads for sildenafil and its two metabolites, it is possible to back-calculate into sildenafil consumption by the population connected to the studied sewer system. This estimation was done as explained elsewhere (Venhuis et al. 2014a). The estimated consumption of sildenafil in mg $week^{-1}$ $1000inh^{-1}$ back-calculated from wastewater loads arranged the cities in the following order (from higher to lower use, and including previously published results from other Dutch cities (Causanilles et al. 2016)): Amsterdam, 872; Copenhagen, 542; Brussels, 517; Zurich, 439; Eindhoven, 432; Bristol, 365; Utrecht, 292; Oslo, 145; Castellon, 100; and Milan, 87.

The comparison from the obtained sildenafil consumption from wastewater and the prescription data showed that only in the case of Brussels (where the prescription data was estimated by extrapolating the Dutch trend) and Utrecht, the estimated consumption from wastewater was higher than what could be explained by the national prescription data. In the cities of Amsterdam and Eindhoven previously published results (Causanilles et al. 2016), showed an even higher consumption, that could not be explained by national sales data, ranging up to 85% and 70%, respectively. In Bristol the predicted and measured values were in good agreement. In Milan and Oslo the estimated consumption from wastewater was lower than what could be explained by the prescription data. The final evaluation of the correlation between wastewater data and prescription data was found to be non-significant by Spearman's correlation coefficient ($\rho = -0.30$) with p-value above 0.05 ($p = 0.68$) (see **Fig. SI-1**).

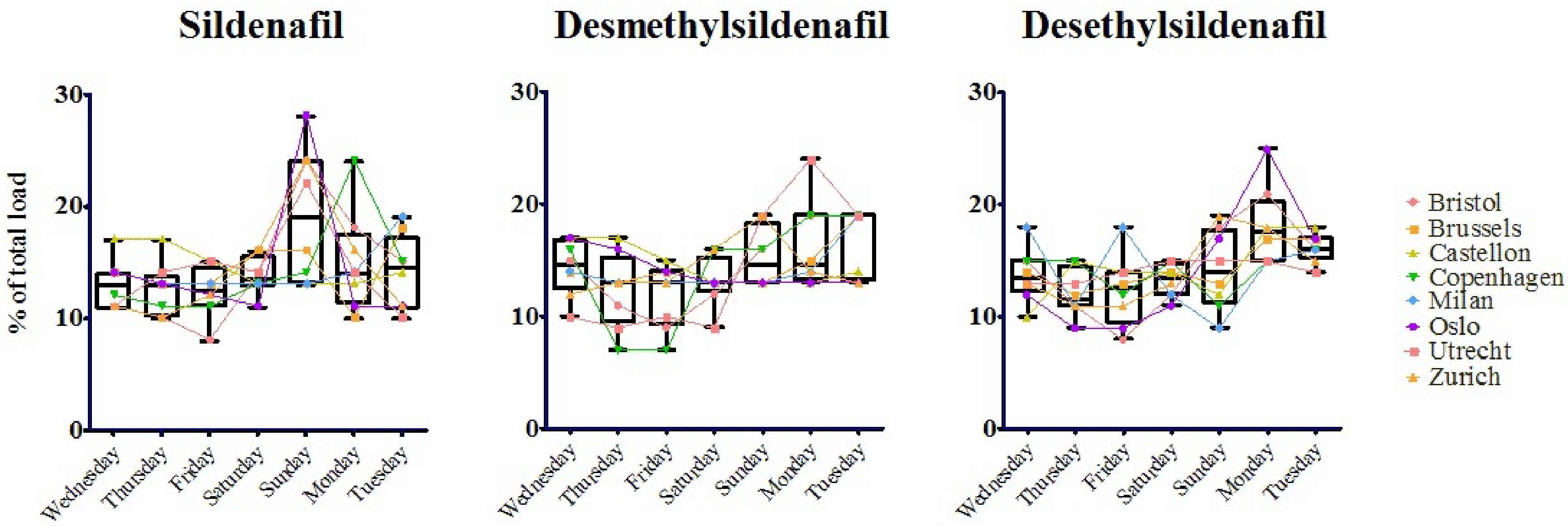
Table 4. Measured loads expressed in mg day^{-1} with standard deviation (± SD) for 7 sampling days, n=7.

	Loads (mean ± SD), mg day^{-1} 1000 inh^{-1}							
	Bristol	Brussels	Castellon	Copenhagen	Milan	Oslo	Utrecht	Zurich
Sildenafil	2.8 ± 1.1	5.1 ± 1.0	0.23 ± 0.03 [b]	3.8 ± 1.2	0.4 ± 0.1 [b]	1.7 ± 0.7 [a]	2.4 ± 0.7	4.2 ± 1.5
Desmethylsildenafil	6.2 ± 1.7	9.4 ± 1.3	0.6 ± 0.1 [b]	5.3 ± 1.9 [a]	1.0 ± 0.2 [b]	1.2 ± 0.1 [b]	2.1 ± 0.9 [a]	1.1 ± 0.2 [b]
Desethylsildenafil	6.6 ± 2.1	8.5 ± 1.2	3.0 ± 0.6	13.7 ± 1.7	2.1 ± 0.5	3.7 ± 1.5	8.0 ± 0.5	13.9 ± 3.1

[a] At least one value out of 7 is >LOQ then when <LOQ replaced by 0.5 × LOQ

[b] All values <LOQ then replaced by 0.5 × LOD

Fig.2. Daily variations expressed as the percentage of the total load, combining results for the 8 cities. The box represents the median, 25% and 75% percentile values and the error bars extend to the minimum and maximum values. The coloured lines represent each of the cities.



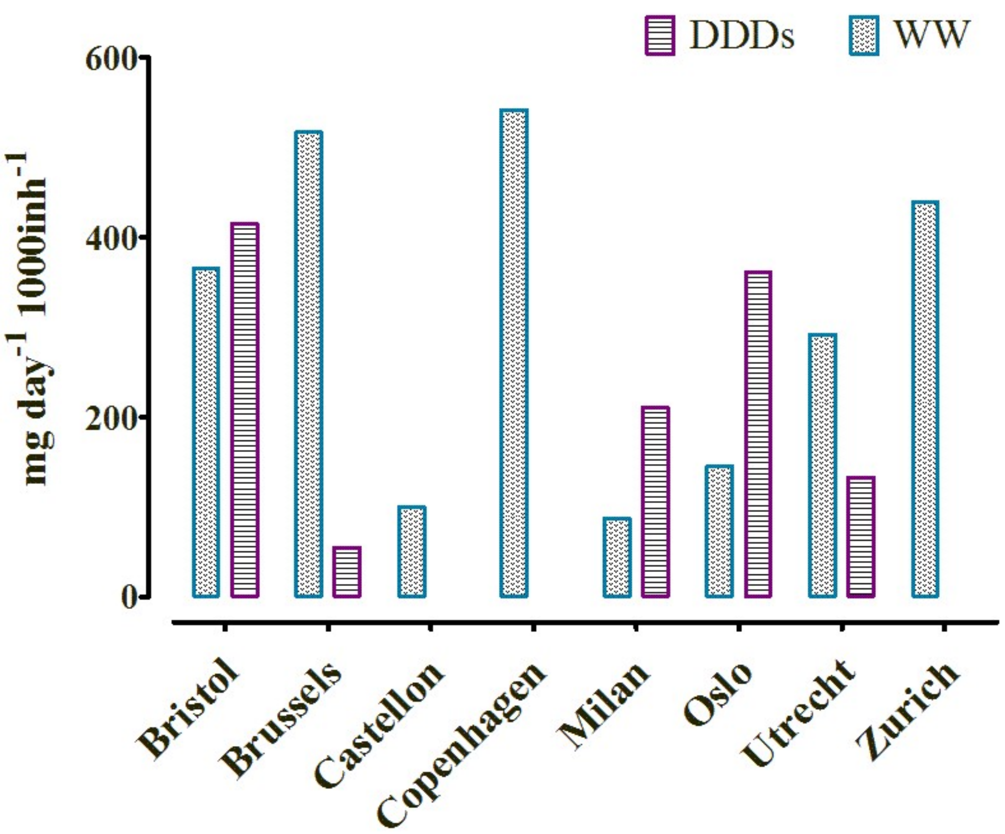


Fig. 3. Comparison of the estimated consumption of sildenafil from wastewater loads (WW, blue coloured bars) and prescription data (DDDs, red coloured bars). For Spain, Denmark and Switzerland no prescription data was available.

4. Conclusions

The present study is the first to compare the use of the erectile dysfunction products in different European cities through chemical analysis of wastewater. The analysis of influents revealed the presence of sildenafil and its two human metabolites in all cities sampled with average loads varying between 0.2 and 14 mg day−1 1000 inh−1. None of the other ED products analysed were observed in concentrations above the method detection limits. While it is known that sildenafil is available in products from illegal sources such as internet shops, the results of the present study show that consumption beyond prescribed doses is not common across Europe. Despite the limitations related to the assessment of both predicted and measured loads, it seems that the populations in Utrecht (and also in other cities in The Netherlands) and in Brussels might be more inclined towards the use of products from illegal sources or rogue online pharmacies than in the other three European cities included in the study for which prescription data were available (Bristol, Milan and Oslo). After this first study illustrating the potential of wastewater-based epidemiology in this field, further research will allow to improve the application of this approach for investigating the use of rogue pharmacies and counterfeit medication.

Author's contribution

AC and DRC performed wastewater analysis. AC drafted the manuscript with significant contributions from PdV. RB, JABL, SC, EC, EGL, FH, BKH, JK, AKM, AvN, CO, BGP, PR, NIR YR and KT organised the collection of the wastewater samplers and provided relevant data for WBE calculations and national prescription data. All authors read and approved the final manuscript.

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