Analysis of new classes of recreational drugs in sewage: synthetic cannabinoids and amphetamine-like substances

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The analysis of sewage for the residues of commonly used illicit drugs has successfully been applied as a suitable approach for estimating community illicit drug use. The drug market is increasingly dynamic with new substances continually being marketed for recreational purposes. In this study ultra-high pressure liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) was used to simultaneously and quantitatively detect the exogenous biomarkers of new classes of recreational drugs in sewage collected from three different Norwegian cities (Oslo, Bergen, Hamar). The samples were screened for the presence of khat (d-norpseudoephedrine and cathinone), mephedrone, pseudoephedrine, 7-aminoflunitrazepam, para-methoxyamphetamine (PMA), para-methoxy-N-methylamphetamine (PMMA) and a selection of urinary metabolites of synthetic cannabinoids collectively termed ´Spice´ (5-3-1-naphthoyl-1H-indol-1-yl-pentanoic acid (JWH 018 N-pentanoic acid), 1-5-hydroxypentyl-1H-indol-3-ylnaphthalen-1-yl-methanone (JWH 018 N-5-hydroxypentyl), 4-3-1-naphthoyl-1H-indol-1-yl-butanoic acid (JWH 073 N-butanoic acid), 1-4-hydroxybutyl-1H-indol-3-ylnaphthalen-1-yl-methanone (JWH 073 N-4-hydroxybutyl), 1-5-hydroxypentyl-1H-indol-3-yl4-methylnaphthalen-1-yl-methanone (JWH 122 N-5-hydroxypentyl), 1-5-fluoro-4-hydroxypentyl-1H-indol-3-ylnaphthalen-1-ylmethanone (AM2201 N-4-hydroxypentyl), and 1-5-hydroxypentyl-1H-indol-3-yl4-methoxyphenyl-methanone (RCS-4 N-5-hydroxypentyl)). Limits of detection were 1 ng/L for amphetamine like compounds and 5 ng/L for the metabolites of synthetic cannabinoids while the limits of quantification were 3 and 15 ng/L respectively. Three of the fourteen selected biomarkers (cathine, pseudoephedrine and the synthetic cannabinoid metabolite JWH-018 N-5-hydroxypentyl) were detected in sewage, alongside the illicit drugs (and/or metabolites) typically found in sewage (cocaine, benzoylecognine, methamphetamine, MDMA and THC-COOH). The khat biomarker d-norpseudoephedrine was detected in Oslo sewage at a mean

concentration of 93 ng/L that represents a daily load of 54 mg/day/1000 inhabitants. Pseudoephedrine was present at mean concentrations of between 27 and 67 ng/L representing normalised daily loads of between 10 (Hamar) and 24 mg/day/1000 inhabitants (Bergen). The daily normalised loads of JWH-018 N-5-hydroxypentyl were between 49 (Oslo) and 62 mg/day/1000 inhabitants (Hamar). For the first time this study demonstrates that sewage biomarker analysis can be applied to evaluate not only the use the traditional illicit drugs (cocaine, cannabis and amphetamines), but also the use of certain new synthetic drugs.

Keywords: sewage; legal high; khat; JWH-018; mass spectrometry, synthetic cannabinoids

Introduction

The estimation and comparison of community drug use through the analysis of selected drug biomarkers in sewage is rapidly becoming established as an effective monitoring tool. [1] The approach, akin to analysing a community urine sample, has been applied on a local [2], national, [3] and international scale [1] to take the quantitative measurements of drug biomarkers in sewage and use them to estimate drug use within a defined population. Over the past few years the approach has seen significant refinement as well as an increased awareness of its uncertainties [4,5,6,7,8,9]. This includes improvements in sample collection and the factors that affect the representativeness of a sample [4,5], the fate of biomarkers between the point of excretion and sampling [6] , the stability of biomarkers post-sampling [7,9] and those factors that most influence the estimation itself. [10, 11] The popularity of sewage analysis for estimating drug use has resulted in its wider application being proposed for example for the measurement biomarkers relating to community health [12,13] (termed BIOSCIM [13]). One obvious further application of the approach is to see whether other classes of drugs can be detected; for example so-called 'legal highs' that contain new psychoactive substances. These 'new' compounds are made to simulate the effects of common illicit drugs, sometimes by simply adding or changing a functional group on the original drug molecule. Very little is known about the use and prevalence of these drugs and their control status varies from country to country (see www.emcdda.europa.eu/drug-profiles for most recent status).

New psychoactive drugs were detected in the European Union (EU) at a rate of around one per week in 2011 (49 new substances)[14] and are typically detected through the EUs Early Warning System. Typically sold online or through specialist stores the new substances are exclusively synthetic and typically dominated by synthetic cannabinoids and cathinones.

Synthetic cannabinoid (cannabinoid receptor agonists) based products, also known as ´Spice´, often contain a mixture of different compounds with new analogues being introduced as substances become restricted. Synthetic cannabinoids undergo extensive metabolism within the body, leading to very low levels of the parent compound present in urine,[15] therefore for sewage analysis the known urinary metabolites of a range of synthetic cannabinoid analogues were selected as biomarkers (JWH-018 N-Pentanoic Acid, JWH-018 N-5-hydroxypentyl (from the parent JWH-018), JWH 073 N-butanoic acid, JWH-073 N-4-hydroxybutyl (from the parent JWH-073), JWH 122 N-5-hydroxypentyl (from the parent JWH-122), AM2201 N-4-hydroxybutyl (from the parent AM2201) and RCS-4 N-5-hydroxypentyl (from the parent RCS-4) (Table 1).

Khat (*Catha edulis)* is a plant grown in Africa that is known to contain d-norpseudoephedrine (NPE) and cathinone that are released into the saliva upon chewing. [16] NPE and cathinone are very similar in structure to amphetamine with similar pharmacological effects on the central nervous system. [17] Khat chewing is a traditional practice in certain East African countries with its use in Europe being linked to immigrants from Somalia, Ethiopia, Kenya and Yemen. [17] Synthetic cathinones are the β-keto (βk) analogues of a corresponding phenethylamine. Mephedrone (4-methylmethcathinone, 4-MMC) is a synthetic cathinone that is increasingly occurring on the drug market and was therefore included in this study. [17] Para-methoxyamphetamine (PMA) and para-methoxy-N-methylamphetamine (PMMA) are synthetic derivatives of methamphetamine that are chemically analogous to MDMA (ecstasy). [17] Flunitrazepam (Rohypnol, FM2) pseudoephedrine (PEP) are pharmaceuticals that have non-medical uses that have resulted in their tight control in certain countries. [17] For example, FM2 is often used as a recreational drug while PEP is a stimulant listed on the World Anti-

Doping Agency list of prohibited substances.

The aim of this work was for the first time to develop and validate a rapid and sensitive targeted method for simultaneous quantitative determination of biomarkers specific to these drugs in raw sewage with the goal of evaluating whether sewage analysis may be a potential tool for gathering improved information on the prevalence of new illicit substances.

Experimental

Chemicals and materials

5-3-1-Naphthoyl-1H-indol-1-yl-pentanoic acid (JWH 018 N-pentanoic acid), 1-5-hydroxypentyl-1H-indol-3-ylnaphthalen-1-yl-methanone (JWH 018 N-5-hydroxypentyl), 4-3-1-naphthoyl-1H-indol-1-yl-butanoic acid (JWH 073 N-butanoic acid), 1-4-hydroxybutyl-1H-indol-3-ylnaphthalen-1-yl-methanone (JWH 073 N-4-hydroxybutyl), 1-5-hydroxypentyl-1H-indol-3-yl4-methylnaphthalen-1-yl-methanone (JWH 122 N-5-hydroxypentyl), 1-5-fluoro-4-hydroxypentyl-1H-indol-3-ylnaphthalen-1-ylmethanone (AM2201 N-4-hydroxypentyl), 1-5-hydroxypentyl-1H-indol-3-yl4-methoxyphenyl-methanone (RCS-4 N-5-hydroxypentyl), para-methoxyamphetamine (PMA), para-Methoxy-N-methylamphetamine (PMMA) were obtained from Chiron (Trondheim, Norway) in solutions of methanol or acetonitrile. Methanol solutions of 4-4MMC, NPE, benzoylethanamine (2S-cathinone), 7-aminoflunitrazepam (7-aminoFM2), mephedrone-d_3, 7 aminoflunitrazepam-d_7, Methamphetamine-d_5 and 11-Nor-9-Carboxy-delta9-THC-d_3 were obtained from Nerliens Meszansky (Norway). EP and PEP were purchased from Sigma-Aldrich (Norway). The purity of all standards was 99%.

Sample collection

Sewage samples (2 x 1L) were collected from the Norwegian cities of Oslo (population 557,000, mean sewer retention time 7h), Hamar (population 55,000, mean sewer retention time <3h) and Bergen (population 71,000, mean sewer retention time <3h), representing a weekend (Friday to Monday morning) in July 2012 (13^{th}-15^{th}). The samples were 3-day volume proportional composite made-up of a blended mix of inlet sewage. These were shipped to the laboratory and stored -20ºC. An additional 3 litres of sample was collected from Oslo and stored in the fridge for use in developing the method.

Sample extraction

Defrosted sewage samples (500 mL) and Milli-Q water (100 mL) were spiked with deuterated standards and the pH adjusted to 3 using acetic acid. The final concentration for each internal standard was 100 ng/L. Solid phase extraction (SPE) was performed using Oasis MCX cartridges (Waters, USA). These were conditioned using methanol (MeOH) (12 mL) followed by acidified Milli-Q water (0.1% acetic acid at pH 3; 12 mL). Samples were drawn through under vacuum and the cartridges washed using acidified water (0.1% acetic acid at pH 3; 12 mL). Cartridges were then vacuum dried for 50 minutes. The analytes were eluted using 2% ammonium hydroxide in MeOH (12 mL) and acetonitrile (6 mL), evaporated to dryness at 40 ºC under a gentle stream of nitrogen and reconstructed in a solution of acetonitrile, methanol and Milli-Q water (2 mL). This was then centrifuged at 10,000 g for 10 mins and 7µl injected into the UHPLC-MS/MS system.

Instrumentation

A Waters Acquity Ultra performance LC system coupled to a Waters Quattro Premier XE

mass spectrometer (Waters, USA) was used. Separation was performed using an Acquity UPLC BEH C8 column (1.7µm particle size). Gradient elution was performed at a constant flow rate of 0.6 mL/min, using NH_4AC 10 mM and 0.1 % acetic acid in water (Solvent A) and NH_4AC 10 mM and 0.1 % acetic acid in MeOH (Solvent B) as the mobile phase. The gradient used was as follows: Start at 0 minutes with 1 % Solvent B and 99 % Solvent A, held for 1 minute. 1-3 minutes linear rate to 35 % B, 3-5 minutes linear rate to 80 % B, 5.10-6.10 linear rate to 99 % B, 6.10-7 minutes return to initial conditions; 1 % B. Positive ionisation electrospray was used with nitrogen as the cone (flow 55 L/h) and desolvation gas (flow 800 L/h). The capillary voltage was set to 320 V, source temperature 100 ºC, desolvation temperature 450 ºC and dwell times of 0.01 s per transition were used. All data was acquired and processed using MassLynx v 4.1 software. The analyte specific MS parameters are presented in Table 2.

Quantification and method validation

Data acquisition was performed in MRM mode with the protonated molecular ion of each compound used as the precursor ion. Where possible two product ions with the greatest abundance were chosen for further confirmation (Table 2). The linearity of the method was studied by analysing standard solution in triplicate at six different concentrations. For the synthetic cannabinoids this ranged from 500 ng/mL to 15 ng/mL and for all other compounds this ranged from 50 ng/mL to around 1.6 ng/mL. Internal standard was spiked in at 100 ng/mL for ratio purposes. Linearity was assumed when the correlation coefficient (R^2) was > 0.99, based on the ratio of analyte peak area and internal standard peak area at each concentration. The limit of quantification (LOQ) was taken as the lowest concentration level for which the method was fully validated using spiked samples with satisfactory recovery (31-71%; Table

S1) and precision (Table S2). The limit of detection (LOD) was defined as the lowest concentration that the analytical process can differentiate between background levels. This was found with a signal to noise ratio of 3 to 1 with the lowest concentration that could still be detected. Precision was expressed as the repeatability of the method in terms of RSD. The % RSD value of the peak areas given determined the precision of the method when screening for each compound. Accuracy was tested by analysing the % absolute recovery from samples that had been spiked with a known amount and passed through the SPE method. All recovery experiments were performed in quintuplicate for both Milli Q and waste-water.

Analyte stability

The stability of the selected biomarkers in sewage was considered by spiking 200 ng/mL of each cannabinoid and 100 ng/mL of each of the other compounds into sewage (pH 7.2) in a 500 mL clean glass flask kept at room temperature (21˚C). An aliquot (500 µL) was taken at the following time intervals (0, 15, 30, 60, 120, 240, 360, 1440, and 2880 minutes) and injected into the UHPLC-MS. A "blank" sample was also tested alongside this, and any positive measurement was subtracted from the spiked sample.

Results and Discussion

Method validation

The method was validated for both Milli Q water and raw sewage prior to its application. Analyte stability, linearity, precision, accuracy, limit of quantification (LOQ) and limit if detection (LOD) were evaluated for each analyte. The MS detector showed good linearity R^2>0.99 for all compounds selected in this method as determined by a six-point calibration curve (synthetic cannabinoids 15.6 to 500 ng/L, all other drugs 1.6 to 50 ng/L). The LOD

was estimated to be 1 ng/L (5 ng/L for cannabinoids) and LOQ was 3 ng/L (15 ng/L for cannabinoids). The mean recovery for the synthetic cannabinoids was between 31 and 71%, and 31 and 58% for the amphetamine-like drugs with RSDs of between 5 and 40% (Table S1). The stability test showed that the selected amphetamine-like substances were stable in sewage with typically between 70 and 84% remaining after 24h (Table S2). Mephedrone was however less stable with only 63% present following 24h at room temperature, suggesting that its stability should be carefully considered when storing samples and when calculating sewage loads, especially in systems with long in-sewer retention times. Some of the synthetic cannabinoid metabolites were unstable in sewage over 24h at room temperature, namely JWH 122 n-5-hydroxypentyl, AMM2201 n-4-hydroxypentyl, RCS-4 N-5-hydroxypentyl and JWH 073 N-4-hydroxybutyl (Table S2). This suggests that these synthetic cannabinoid metabolites do not make good sewage biomarkers since they will degrade between the point of excretion and sewage sample collection. JWH 018 n-pentanoic acid, JWH 073 N-butanoic acid and JWH018 N-5-hydroxypentyl were stable and are therefore suitable for consideration as sewage biomarkers.

Occurrence in sewage

Of the 14 new compounds targeted in this screening study only three were detected in the sewage samples analysed. The sample collected from Oslo tested positive for ephedrine/pseudoephedrine, JWH 018 N-5-hydroxypentyl and NPE, while PEP and JWH 018 N-5-hydroxypentyl were detected in Bergen and Hamar (Table 3). The same samples all contained COC, BE, METH and THC-COOH (Table 3). MDMA was only detected in Oslo. Mean concentrations of JWH 018-N-hydroxpentyl were between 83 and 160 ng/L, normalised to population equivalents loads of between 49 and 62 mg/day/1000 inhabitants. The presence

of JWH 018-N-hydroxpentyl, a urinary metabolite of one of the most common synthetic cannabinoids JWH 018, [18] in samples collected from all three cities suggests that its use is widespread in Norway. The samples from Hamar also indicated the presence of another synthetic cannabinoid, JWH 122 but at concentrations below the LOQ of the method. An initial report into the occurrence of synthetic cannabinoid receptor agonists identified JWH 018 as one of the most frequently occurring, however not present at the time in Norway. [14]

NPE, a sewage biomarker for khat, was only detected in Oslo that corresponded to a mean population normalised load of 54 mg/day/1000 inhabitants. The mass chromatogram for NPE contained a number of co-eluting and/or unresolved peaks (Figure SI1) that may correspond to glucuronide or sulphate metabolites of NPE or possibly phenylpropanolamine (a stereoisomer of cathine and available as the prescription medicine Rinexin). [19] The absence of the other khat biomarker cathinone is expected because only around 2% of the compound is excreted unchanged in urine [20] (the majority is excreted as NPE) and the level of use would have to be very large. NPE is also a metabolite of PEP with ~1% transformed via N-demethylation. [21] and therefore it is possible that a small amount of the NPE signal may be from the use of PEP.

PEP and ephedrine (EP) are isomers and our method does not chromatographically separate them, however this is possible by using a specific chiral column. Therefore when interpreting the PEP signal it is necessary to also include EP. PEP is not approved for use in Norway, and EP is very strictly controlled, yet both compounds were detected in all three locations that suggest widespread use of this controlled substance. Unfortunately this was not possible as part of this study. EP, a prescription only medication in Norway is rarely used and records for

2011[19] show a total annual usage of only 103 doses per thousand inhabitants in Oslo, 92 doses/1000 inhabitants in Bergen and 59 doses/1000 inhabitants in Hamar. [19] The defined daily dose (DDD) for EP is 50 mg, so prescription records would therefore suggest a consumption rate of 8-14 mg/day/1000 inhabitants in all three locations. Approximately half the initial dose of EP is excreted unchanged in urine [21,22] so sewage loads of this compound from prescription only medication will be expected to be of the order of 5-10 mg/day/1000 inhabitants. The measured load in Hamar (10.6 mg/day/1000 inhabitants) is likely therefore to be solely due to legal prescription use. Loads in Oslo (18 mg/day/1000 inhabitants) and Bergen (24 mg/day/1000 inhabitants) are however in excess of that which would be expected from prescription use, and therefore indicate the possibility of some illicit consumption. These are the first reported measurements of these drugs through sewage biomarker analysis therefore there are no data with which to compare with.

The population-normalised load for cocaine (COC) and its main metabolite BE, together with METH and THC-COOH were greatest is Oslo. This is unsurprising since Oslo is a capital city and the largest city in Norway. Estimation of COC use through the transformation of the normalised mean benzoylecognine loads using the median excretion value of 38% and a correction factor of 2.77 of BE excretion [23] shows that up to five-times as much cocaine was used in Oslo (146 mg COC/day/1000 inhabitants) compared to Hamar (33 mg COC/day/1000 inhabitants) and three-times as much as Bergen (48 mg COC/day/1000 inhabitants). When compared to previous studies performed in Oslo these data are within the same range as previously measured (100 - 900 mg COC/day/1000 inhabitants). [1] The lower level of use in less urbanized areas within a country has previously been observed [1,3] and possibly reflects the greater availability of drugs within a large urban area. The METH load relative to

population size was again higher in Oslo (117 mg METH/day/1000 inhabitants) compared to Bergen (70 mg METH/day/1000 inhabitants) and Hamar (39 mg METH/day/1000 inhabitants). When compared to other European cities there values are relatively high with only previous reports from Oslo, Helsinki, Turku (both in Finland) and Budweis (Czech Republic) being higher.[1] The comparatively high levels of METH determined in Norwegian sewage are reflected in the statistics relating to the size and frequency of seizures of METH in the Nordic region. [24] The highest measured per capita THC-COOH load was determined in Oslo (59 mg THC-COOH/day/1000 inhabitants) with lower yet similar levels estimated for Bergen and Hamar (39 and 35 mg THC-COOH/day/1000 inhabitants). The per capita THC-COOH loads are comparable with the range that has previously been reported for European cities,[1] excluding Amsterdam (14-124 mg THC-COOH/day/1000 inhabitants). MDMA is rarely detected in Oslo due to its low level of use and the concentrations measured in the samples collected from the three cities reflect this.

For the first time we have demonstrated that targeted sewage analysis can be used to evaluate the use of new synthetic drugs on a community level, however it is clear that they offer a greater challenge than for example cocaine or methamphetamine. The challenge with new synthetic drugs, and in particular synthetic cathinones and cannabinoids, is that there are numerous analogues available and new drugs are constantly being synthesised. A successful sewage based approach for determining the use of a new drug in society is dependent on the identification of the correct biomarker based on whatever pharmacokinetic data are available. Secondly the prevalence of use of the drug needs to be sufficiently high to generate a large enough concentration of the chosen biomarker in sewage that can then allow for detection and analysis. It is desirable that a selected biomarker is preferentially present in the aqueous phase and does not excessively partition onto solids. The presence of the selected biomarker(s) at a

sufficiently high concentration is also dependent on the stability of the biomarker in sewage; labile compounds will simply degrade before they reach the point of sampling. This appears to be the case for a number of synthetic cannabinoid metabolites.

The measurement of the use of synthetic cannabinoids by sewage analysis is therefore challenging since these compounds are often sold in mixtures, and it is possible that the level of one particular biomarker may never be sufficiently high to allow for analysis. One solution option is to try and get a larger more integrated sample, for example by using passive sampling techniques such as the polar organic compound integrative sampler (POCIS). [5] Since a targeted analytical approach such as the one adopted here is reliant on the availability of authentic reference standards then another option may be a non-target approach using high resolution MS. A non-target approach would however require the identification of suitable biomarkers to allow a retro-analytical approach to be used since searching for the parent drug offers no guarantee of its presence in urine and subsequently sewage. Since the majority of (recently identified) new synthetic drugs are cannabinoid or cathinone related then it may be possible to develop or apply transformation/metabolism models based upon the pharmacokinetics of known drugs to provide valuable information on what is likely to occur in sewage. Therefore it is clear that new synthetic drugs offer a number of challenges over those that have already been overcome for traditional illicit drugs such as cocaine and amphetamine but that sewage analysis is an additional tool that can be used to gauge the use and emergence of such drugs on a community level.

Conclusion

A method has been successfully developed and applied to screen for the presence of new

classes of illicit drugs, including synthetic cannabinoids and amphetamine like compounds, in sewage. Our initial study suggests that JWH 018 N-5-hydroxypentyl and JWH 073 N-butanoic acid are suitable sewage biomarkers for their respective parent synthetic cannabinoids, whilst NPE has the potential to be a sewage biomarker for khat. Further work is needed for a number of the other compounds evaluated, however it is clear that the following proposed sewage biomarkers are not suitable due to being rapidly transformed in sewage; JWH 122 n-5-hydroxypentyl, AM2201 n-4-hydroxylpentyl, RCS-4 N-5-hydroxypentyl, JWH 073 N-4-hydroxybutyl. Evaluation of the technique at three Norwegian cities showed that the synthetic cannabinoid JWH 018 and ephedrine/pseudoephedrine are used in all three cities, along with the traditional drugs such as COC, METH and THC-COOH. The use of khat was only detected in Oslo.

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References

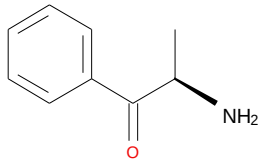
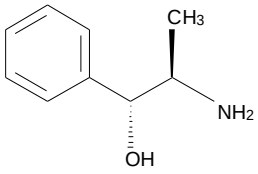
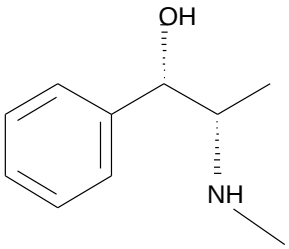
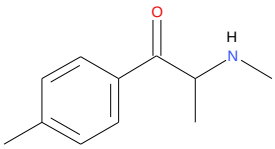
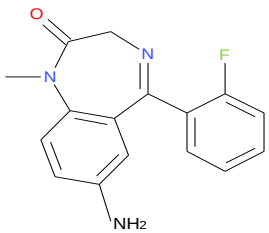
[1] K. V. Thomas, L. Bijlsma, S. Castiglioni, A. Covaci, E. Emke, R. Grabic, F. Hernández, S. Karolak, B. Kasprzyk-Hordern, R. H. Lindberg, M. Lopez de Alda, A. Meierjohann, C. Ort, Y. Pico, J. B. Quintana, M. Reid, J. Rieckermann, S. Terzic, A. L. N. van Nuijs, P. de Voogt. Comparing illicit drug use in 19 European cities through sewage analysis. *Sci.. Tot.. Environ.* **2012** *432*, 432.

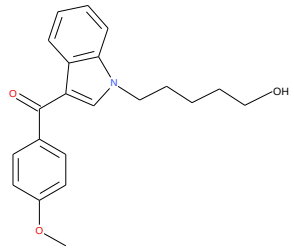
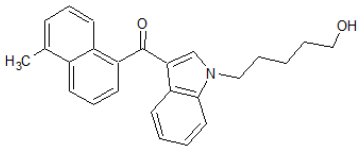
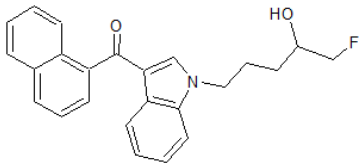
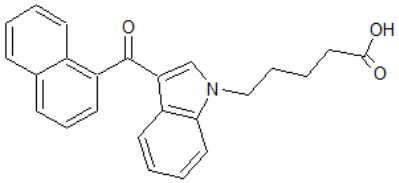
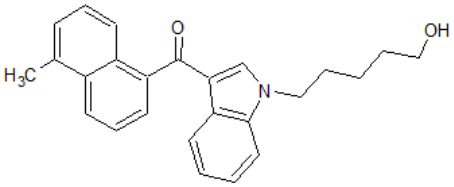
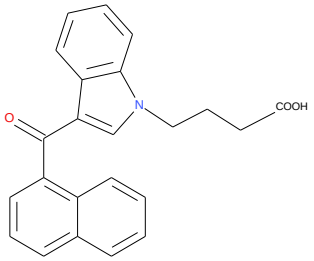
[2] E. Zuccato, C. Chiabrando, S. Castiglioni, D. Calamari, R. Bagnati, S. Schiarea, R. Fanelli. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. *Environ. Health Global Access Sci. Source*. **2005** *4*,14.

[3] A. L. N. van Nuijs, B. Pecceu, L. Theunis, N. Dubois, C. Charlier, P. G. Jorens, L. Bervoets, R. Blust, H. Meulemans, H. Neels, A. Covaci. Can cocaine use be evaluated through analysis of wastewater? A nation-wide approach conducted in Belgium. *Addiction*. **2009** *104*,734.
[4] C. Ort, M. G. Lawrence, J. Rieckermann, A. Joss. Sampling for Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs in Wastewater Systems: Are Your Conclusions Valid? A Critical Review. *Environ. Sci. Technol.* **2010** *44*, 6024.
[5] C. Harman, M. Reid, K. V. Thomas. In Situ Calibration of a Passive Sampling Device for Selected Illicit Drugs and Their Metabolites in Wastewater, And Subsequent Year-Long Assessment of Community Drug Usage. *Environ. Sci. Technol*. **2011** *45*, 5676.
[6] B.G. Plósz, M.J. Reid, M. Borup‡, K.H. Langford, K.V. Thomas. Biotransformation kinetics and sorption of cocaine and its metabolites and the factors influencing their estimation in wastewater. *Wat. Res*. In press.
[7] D. R. Baker, B. Kasprzyk-Hordern. Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography–mass spectrometry. *J. Chromatogra. A*. **2011**, *1218*, 8036–8059.
[8] F. Y. Lai, C. Ort, C. Gartner, S. Carter, J. Prichard, P. Kirkbride, R. Bruno, W. Hall, G. Eaglesham, J. F. Mueller. Refining the estimation of illicit drug consumptions from wastewater analysis: Co-analysis of prescription pharmaceuticals and uncertainty assessment. *Wat. Res.* **2011**, *45*, 4437.
[9] A. L. N. van Nuijs, K. Abdellati, L. Bervoets, R. Blust, P. G. Jorens, H. Neels, A. Covaci. The stability of illicit drugs and metabolites in wastewater, an important issue for sewage epidemiology?. *J. Haz. Mat.* **2012**, 239-240, 19.
[10] U. Khan, J. A. Nicell. Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. *Environ. Int.* **2011**, *37*, 1236.
[11] U. Khan, J. A. Nicell. Sewer epidemiology mass balances for assessing the illicit use of methamphetamine, amphetamine and tetrahydrocannabinol. *Sci. Tot. Environ.* **2012**, *421–422*, 144.
[12] K.V. Thomas, M. Read. What Else Can the Analysis of Sewage for Urinary Biomarkers Reveal About Communities?. *Environ. Sci. Technol.* **2011**, *45*, 7611.
[13] C. G. Daughton. Using biomarkers in sewage to monitor community-wide human health: Isoprostanes as conceptual prototype. *Sci. Tot. Environ.* **2012,** *424*, 16.
[14] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 'EMCDDA–Europol 2011 Annual Report on the implementation of Council Decision 2005/387/JHA'. Apr-2012.
[15] B. Logan, M. McMullin, R. Middleberg, 'Technical Bulletin: Identification of Primary JWH-018 and JWH-073 Metabolites in Human Urine'. http://toxwiki.wikispaces.com/file/view/JWH_metabolites_Technical_Bulletin_Final_v1.1.pdf.
[16] G. Cox. Adverse effects of khat: a review. *Adv. Psychiatr. Treat.* **2003**, *9*, 456.
[17] European Monitoring Centre for Drugs and Drug Addicition (EMCDDA) drug profiles. http://www.emcdda.europa.eu/drug-profiles
[18] N. Uchiyama, R. Kikura-Hanajiri, J. Ogata, Y. Goda. Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic. Sci. Int.* **2010**, 198, 31.
[19] Norwegian Prescription Database. http://www.norpd.no/
[20] S. W. Toennes, G. F. Kauert. Excretion and Detection of Cathinone, Cathine, and Phenylpropanolamine in Urine after Kath Chewing.*Clin. Chem.* **2002**, *48*, 1715.

[21] N. Chester, D. R. Mottram, T. Reilly, M. Powell. Elimination of ephedrines in urine following multiple dosing: the consequences for athletes, in relation to doping control. *Br. J. Clin. Pharmacol.* **2004**, *57*, 62.
[22] Y. L. Tseng, M.-H. Shieh, F.-H. Kuo. Metabolites of ephedrines in human urine after administration of a single therapeutic dose. *Forensic. Sci. Int.* **2006**, *157*,149.
[23] S. Castiglioni, R. Bagnati, M. Melis, D. Panawennage, P. Chiarelli, R. Fanelli, E. Zuccato. Identification of cocaine and its metabolites in urban wastewater and comparison with the human excretion profile in urine. *Wat. Res.* **2011**, *45*, 5141.
[24] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), '2011 Annual report on the state of the drugs problem in Europe', Lisbon, Portugal, Nov. 2011.

Table 1. Structural and molecular details of the selected drugs

Drug	Target biomarker(s)	Chemical structure
Khat	Cathinone	
	Norpseudoephedrine (NPE)	
Pseudoephedrine (PEP)	PEP	
Mephedrone (4-methylmethcathinone, 4-MMC)	Mephedrone	
Flunitrazepam	7-aminoflunitrazepam (7-aminoFM2)	

Synthetic cannabinoids	RCS-4 N-5-hydroxypentyl	
	JWH 122 N-5-hydroxypentyl	
	AM 2201 N-4-hydroxypentyl	
	JWH-018 N-pentanoic acid	
	JWH 018 N-5-hydroxypentyl	
	JWH 073 N-butanoic acid	

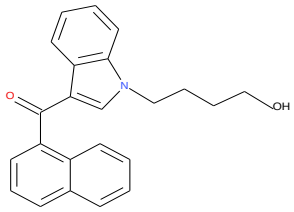
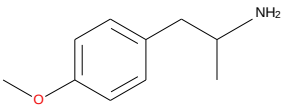
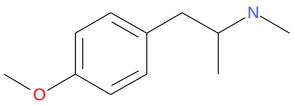
	JWH 073 N-4- hydroxybutyl	
***para*-Methoxyamphetamine (PMA)**	PMA	
***para*-Methoxy-*N*-methylamphetamine (PMMA)**	PMMA	

Table 2 Compound parameters, showing transitions for reporter and qualifier ions.

Compound	**t_r (min)**	**Precursor ion (*m/z*) $[M+H]^+$**	**Cone voltage (V)**	**CE[a] (eV)**	**Product Ion (*m/z*)**
Mephedrone	2.06	178	25	20	145
				12	160
7-aminoflunitrazepam	2.9	284	30	25	135
Norpseduoephedrine	1.27	152	25	15	117
				10	134
Cathinone	1.28	150	20	15	132
Pseudoephedrine	1.46	166	20	15	117
					148
PMA	1.81	166	15	10	149
				15	121
PMMA	1.9	180	20	15	120
				10	149
JWH-018 N-pentanoic acid	3.87	372	40	25	155
JWH 018 N-(5	3.97	358	35	20	155
hydroxypentyl)				35	144
JWH 122 N-(5	4.23	372	40	25	169
hydroxypentyl)					230
AM 2201 N-(4-	3.98	376	45	25	155

hydroxypentyl)					
RCS-4 N-(5-hydroxypentyl)	3.85	338	35	20	134
JWH 073-N-butanoic acid	4.08	358	40	20	155
JWH 073 N-(4-hydroxybutyl)	3.95	344	40	20	127
				20	155
7-aminoflunitrazepam-d_7	2.87	291	45	35	138
Mephedrone-d_3					
Methamphetamine-d_5	2.05	181	25	20	147
THC-11-oic acid-d_3	1.74	155	25	15	92
	4.73	348	35	30	196

[a] CE : collision energy.

Table 3 Mean concentration of selected drug biomarkers in sewage collected from Oslo, Hamar and Bergen (Norway) in July 2012.

Biomarker	**Oslo**		**Hamar**		**Bergen**	
	Mean concentration (ng/L)	**Normalised Load (mg/day/1000 inhabitants)[a]**	**Mean concentration (ng/L)**	**Normalised Load (mg/day/1000 inhabitants)[a]**	**Mean concentration (ng/L)**	**Normalised Load (mg/day/1000 inhabitants)[a]**
JWH 018 N-5-hydroxypentyl	83.4	48.9	157	62.0	160	56.1
Norpseuoephedrine	92.5	54.3	<3	0.6[e]	<3	0.5[e]
Pseudoephedrine	30.8	18.1	26.8	10.6	67.0	23.5
Cocaine[b]	25	14.7 23-34[c]	2.5	1.0	15	5.3
Benzoylecgonine[b]	90	52.8 52-105[c]	30	11.8	50	17.5

Methamphetamine[b]	200	117.3 245-383[c]	100	39.39	200	70.04
THC-COOH[b]	100[d]	58.7 n/a[c]	100[d]	39.4	100[d]	35.0
MDMA[b]	10[d]	5.9 n/a[c]	n/a	-	n/a	-

[a] Sewer catchment population data provided by STW operators based upon census data.

[b] estimated concentrations and normalised loads

[c] range of previous loads measured in Oslo [16,18]

[d] Semi-quantitative results based on single-point calibration curves derived from a spike of deuterated MDMA and THC-COOH into each sewage sample

[e] Calculated from a concentration of LOQ/2.

n/a: data not available.