



**STUDY OF DRUGS OF ABUSE CONSUMPTION
BY WASTEWATER ANALYSIS USING UHPLC-
MS/MS (QqQ). AN ATTEMPT TO UPDATE
THE METHODOLOGY**

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The logo for SCORE, featuring the word "score" in a lowercase, sans-serif font. The letter "o" is stylized with a yellow and orange gradient, resembling a sun or a drop.The logo for COST, featuring a stylized hexagonal icon to the left of the word "cost" in a lowercase, sans-serif font. Below "cost" is the text "EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY" in a smaller, uppercase, sans-serif font.The logo for Universitat Jaume I, featuring a stylized "UJI" in a colorful, blocky font (green, yellow, red) to the left of the text "UNIVERSITAT JAUME I" in a bold, uppercase, sans-serif font.The logo for Institut Universitari de Plaguicides i Aigües, featuring a stylized icon of three downward-pointing triangles above wavy lines, to the left of the text "Institut Universitari de Plaguicides i Aigües" in a bold, uppercase, sans-serif font.

Abstract

Nowadays, drug use is a reality that we must study to prevent it from getting popularized and gaining addicts. However, it is a controversial topic and it is difficult to conduct non-biased surveys. This is why Wastewater Based Epidemiology, the analysis of wastewater to obtain DOAs consumption data, is an excellent tool to obtain realistic data without consulting the population. The use of this methodology is becoming more and more popular.

The research was carried out for a city on the Mediterranean coast for two weeks, a normal week and a festive week in order to compare drug consumption. The substances studied were: MDMA, Methamphetamine, Cocaine, Benzoyllecgonine, THC-COOH, Morphine, 6-MAM, Heroin and Ketamine. In addition, a comparison study was carried out between different SPE cartridges to improve the current methodology.

For accomplishing both objectives, ultra-high performance liquid chromatography coupled to triple quadrupole with electrospray ionization (UHPLC-MS/MS (QqQ)) was used.

Higher drug intakes were found during weekends and festivities for stimulant drugs such as cocaine and MDMA. In addition, it was verified that drugs like cannabis and morphine, which are known to have relaxing effects, have a more constant consumption not only in a normal week but in a festive week. Additionally, it was proved that the use of new-generation SPE cartridges did not sustainably improve the previously used methodology.

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1. Introduction

1.1 What is the purpose of study wastewater?

Daily, thousands of products are consumed by humans that can be traced through the analysis of the wastewater. When consumed, compounds are excreted unchanged or as a mixture of metabolites in our urine and faeces, ending up in the sewage network and finally in a local wastewater treatment plant [1]. Therefore, the analysis of certain compounds in wastewater allows to monitor trends in the consumption of some product or to know if population is exposed to hazardous products to human health [1].

The concept of wastewater analysis arose in the search for contaminants due to pharmaceutical products excreted by humans with studies of surface and wastewaters [2]. Since its first application, wastewater based epidemiology (WBE) has been applied to many different fields, some of the studies that are carried out analysing wastewater are: alcohol consumption, stress factors, smoking and diabetes control or observation of diseases through marker compounds.

At the beginning of 2000s, Calamari *et al.* verified that when analysing some pharmaceutical products, these showed higher concentrations than expected which revealed some of them being consumed not only for therapeutic purposes but for recreational purposes in the North of Italy [3]. In the United States, the consumption of amphetamines was first studied in 2004 in wastewater, and in the following years the study of cocaine and its metabolites in crude water samples was also applied. At this time, the application of WBE for the monitoring of consumption of drugs of abuse (DOAs) appeared in order to establish the trends in consumption and to become an alternative to population surveys [4].

Traditionally, consumption of drugs has been established by national or local surveys. These studies are very expensive and time-consuming. Additionally, results can be affected by the answer of the interviewees because DOAs are illegal substances and not-approved by society. Consequently, as WBE can provide reliable results, almost in real time and in a non-intrusive way in defined populations, they are becoming an alternative for population surveys.

Since then, the application of WBE for the study of the use of the main illicit drugs has been used in many countries. Initially, substances such as cocaine, heroin, cannabis and amphetamines were analysed, but it was later discovered that it could also be focused on research of new psychoactive substances [1]. Between 2005 and 2010, several research groups applied their own methods to detect the use of illegal drugs, but these required working guidelines so that the results could be compared. For this reason, Sewage Analysis CORE group Europe (SCORE) was created in 2010 to implement a common protocol and to be able to compare results [2].

The number of publications related to WBE has been increasing in the last years, as can be seen in *Figure 1*.

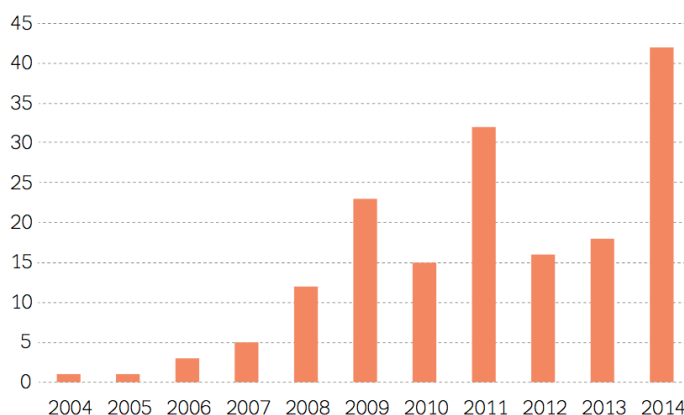


FIGURE 1. NUMBER OF PUBLICATIONS (COMBINED PUBMED SEARCH) RELATED TO WBE PER YEAR SINCE 2004 [4]

Wastewater analyses have also been used in more specific sites to study how drug use varies in different parts of the city, such as in prisons, schools, airports,

different districts within a city and also to see the different consumptions during special events, vacations or festivities .

In 2010, a 4-year investigation was carried out with the common protocol of analysis, in order to study the variation in cocaine consumption (estimated by measuring benzoylecgonine) and methamphetamine in 19 European cities for one week. The study showed differences of drug consumption between different cities in the same country. Authors proposed that this could be due to social differences or different city characteristics such as the presence of universities, holiday areas or the even the average age of the population (*Figure 2*). It was also demonstrated that cocaine consumption was higher on weekends than on weekdays, while the consumption of cannabis and methamphetamine remained constant throughout the week (*Figure 3*). The results obtained by the SCORE group were consistent with the standard monitoring data, demonstrating that WBE could be used to study and compare drug use and to detect trends in different populations [4].



FIGURE 2. LOADS OF METHAMPHETAMINE AND BENZOYLECGONINE (MG/DAY/1 000 POPULATION) IN THE EUROPEAN CITIES INCLUDED IN A 4-YEAR INVESTIGATION (2011-2014) [4]

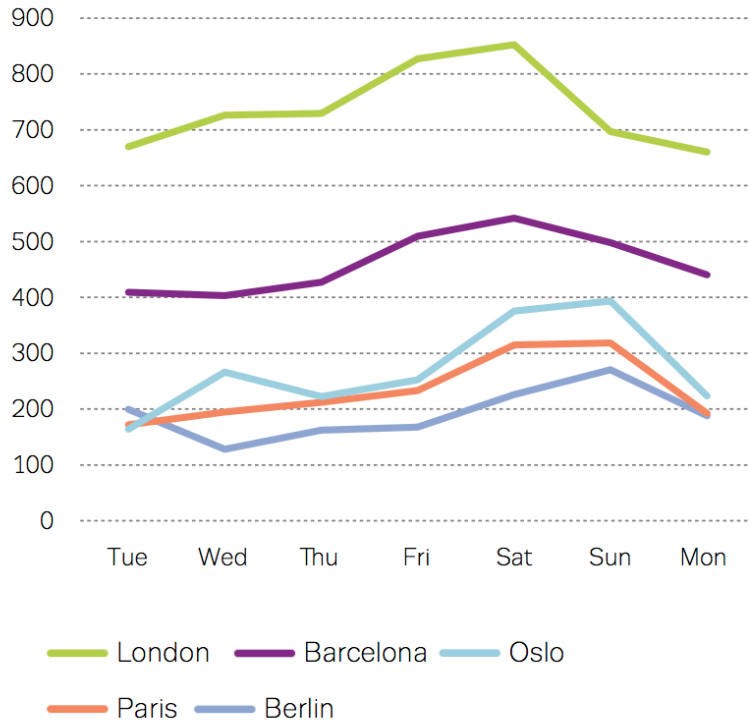


FIGURE 3. LOADS OF BENZOYLECGONINE (MG/DAY/1 000 POPULATION) IN A SELECTION OF EUROPEAN CITIES (IN 2004) [4]

For monitoring the consumption of DOAs by the analysis of wastewater is not the simple measurement of the concentration of DOAs in wastewater. Firstly, the sampling procedure is a critical point due to temporal variations in the flow entering the wastewater treatment plan depending on many factors. As samples should be representative of the assessed community, a 24h-composite sample needs to be obtained by the constant sampling of an aliquot each 15 minutes. In addition, after obtaining concentration values in extract, data should be extrapolated to doses of drugs used in a certain population in a back-calculation process as remarked in *Figure 4*.

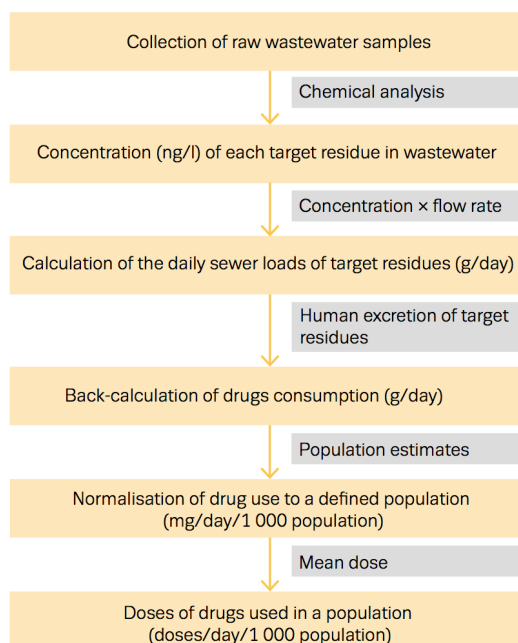


FIGURE 4. STEPS FOR WASTEWATER ANALYSIS

1.2 Analytical techniques

The analytical technique used in this project has been ultra-high performance liquid chromatography coupled to triple quadrupole with electrospray ionization (UHPLC-MS/MS (QqQ)). The main aim in this section is to describe some details of the technique employed, on the bases of chromatography and mass spectrometry.

1.2.1 UHPLC-MS/MS (QqQ)

The term chromatography can be defined as “a physical method of separation of sample components in which these components distribute themselves between two phases, one stationary (solid or a liquid supported on a solid) and the other mobile”. Chromatography encompasses a series of techniques that share its theoretical background such as gas chromatography, liquid chromatography, thin layer chromatography, ion chromatography... [5]

At the end of the last century, improvements in analytical instrumentation were important as analysis times and results were improved. Jorgensen and Lee studied the effect of diminishing the column particle size and coined that innovative technique as ultra-high performance liquid chromatography (UHPLC) [6], [7].

The reduction of the particle size (d_p) affects to a series of chromatographic parameters as can be seen in *Figure 5 (a)*. Resolution is directly proportional to number of plates (N) and these are inversely proportional to particle size. So, reduction of the particle size increases the resolution of the chromatogram. Furthermore, N is inversely proportional to the square of peak width (w). So, a diminution in particle size implies a diminution in w , which increases peak height (H) *Figure 5 (b)*.

In addition, the optimum flow F_{opt} reach maximum N increases when reducing d_p *Figure 5 (c)* and the column length decrease with particle size *Figure 5 (d)*. Swartz explained “using a flow rate three times higher due to the smaller particles and shortening the column by one third (again due to the smaller particles), the separation is completed in 1/9 the time while maintaining resolution” [8]

Finish, UHPLC provides major improvements such as higher resolution, speed and sensitivity than HPLC *Figure 6* [8].

$$\mathbf{a)} \quad R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k}{k + 1} \right); N \propto \frac{1}{dp}$$

$$\mathbf{b)} \quad N \propto \frac{1}{w^2}; H \propto \frac{1}{w}$$

$$\mathbf{c)} \quad F_{opt} \propto \frac{1}{dp}$$

$$\mathbf{d)} \quad N \propto \frac{L}{dp}$$

FIGURE 5. RESOLUTION EQUATION AND CHROMATOGRAPHY RELATIONS

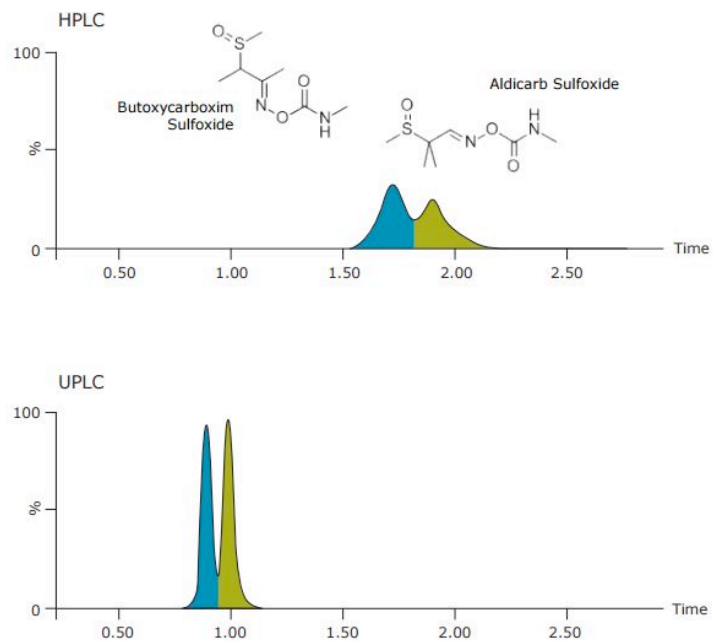


FIGURE 6. COMPARISON BETWEEN HPLC AND UPLC, DEMONSTRATING CLEAR IMPROVEMENTS IN SENSITIVITY, SELECTIVITY AND SPEED OF ANALYSIS [8]

Mass spectrometry (MS) can be defined as an analytical technique that measures the mass-to-charge ratio (m/z) of charged particles (ions). Although there are many different kinds of mass spectrometers, all of them make use of electric or magnetic fields to manipulate the motion of ions produced from an analyte of interest and determine their m/z [9]

The mass spectrometer used in the present project was composed a triple quadrupole mass analyser (QqQ) with an electrospray ionization source (ESI).

There are two problems with the use of mass spectrometry in this type of molecule and these have critically limited the application of UHPLC-MS/MS:

- The inability to ionize many of the labile and/or involatile molecules involved.
- Should ionization be possible, the lack of appropriate hardware to allow the mass analysis and efficient detection of the ions of high m/z ratio involved

Electrospray is an ionization method that conquered the two problems previously described. The liquid in which the analytes of interest are dissolved are passed through capillary at atmospheric pressure and high voltage. This liquid breaks down into highly charged droplets which are desolvated as they pass through the atmospheric-pressure region. Desolvation is assisted using usually nitrogen, being continually passed into the spraying region. From these droplets the ions of the analytes are and then pass through two pumped regions (Figure 7). Because the ionization takes place directly from the solution, the molecules do not degrade. In addition, this source of ionization compared to others, makes most of the ions are multiple charged [10].

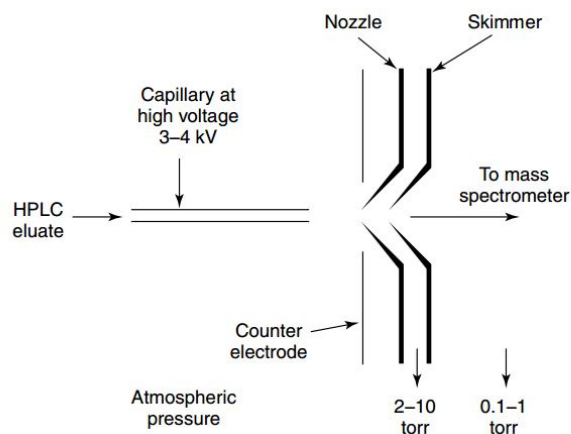


FIGURE 7. ESI SCHEME [10]

Finally, the triple quadrupole mass analyser consists of four parallel cylindrical metal rods as shown in figure 8. Opposite pairs are connected electrically and a voltage, consisting of both radiofrequency (RF) and direct-current (DC) components, is applied.

Depending upon the magnitude of these voltages it can be arranged that only ions of only certain masses are allowed to pass the whole length of the quadrupole, passing out of the quadrupole and into a detector, the other ions being deflected onto trajectories which cause them to collide with the rods and pass out of the analysing device.

The quadrupole analyser is an ideal detector for chromatography because it's fast and uses low voltages which make it tolerant of relatively high operating pressures [10].

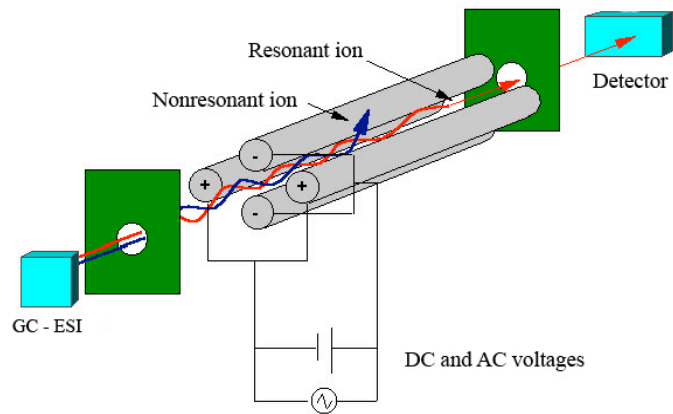


FIGURE 8. GENERAL GC-ESI-QQQ-MS/MS SCHEME [11]

2. Objectives

The 3 main objectives related to the project performed are the following:

1. Analysis of wastewater samples by means of UHPLC-MS/MS (QqQ) in order to determine the DOA consumption of a normal and festive weeks of the population of a Spanish city located in the Mediterranean Coast.
2. Verification of significant differences between the consumption of both studied weeks.
3. Comparative study of the efficiency of different cartridges for the Solid Phase Extraction (SPE) step, using Oasis® HLB (originally ones) and Oasis® HLB PRiME (cartridges which do not need pre-conditioning).

3. Materials and methods

3.1 Reagents and chemicals

The DOA (Drugs Of Abuse) selected for this research are 11 drugs and/or main metabolites: amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA, or ecstasy), cocaine, benzoylecgonine (BE), 11-nor-9-carboxy- Δ 9-tetrahydrocannabinol (THC-COOH), heroin, morphine, 6-monoacetylmorphine (6-MAM), codeine and ketamine.

Reference standards of these compounds were purchased from Cerilliant (Round Rock, TX, USA) and the National Measurement Institute (Pymble, Australia) as solutions in methanol, acetonitrile or as salt. Isotopically labelled analogues used for quantification were: amphetamine-d₆, methamphetamine-d₅ (MDMA-d₅), cocaine-d₃, benzoylecgonine-d₃ (BE-d₃), THC-COOH- d₃, heroin-d₉, morphine-d₃, 6-MAM-d₆, codeine-d₆ and ketamine-d₄. All ILIS (isotopically labelled internal standards) were purchased from Cerilliant as solutions in methanol or acetonitrile.

HPLC-grade methanol (MeOH), HPLC-grade acetonitrile (ACN), ammonium acetate, formic acid (>98 %) and primary secondary amine (PSA, 40–60 μ m) sorbent were acquired from Scharlau (Barcelona, Spain). HPLC-grade water was obtained by purifying demineralised water in a Milli-Q plus system from Millipore (Bedford, MA, USA).

Standard stock solutions of each compound were prepared at 100 mg/L in MeOH or ACN. Intermediate solutions (10 mg/L) were prepared by diluting the stock solution ten times with MeOH. Mixed working solutions containing all analytes were prepared from intermediate solutions by appropriate dilution with Milli-Q water, and were used for preparation of the calibration standards, internal quality controls and also for spiking samples in the validation study.

Individual stock solutions of isotope-labelled standards were prepared in MeOH or ACN at a concentration of 10 mg/L. A mixed standard working solution at 100 μ g/L was prepared in water and was used as surrogate internal standard. All standard solutions were stored in amber glass bottles at –20 °C. SPE cartridges

used were Oasis HLB 3 cm³ (60 mg) and Oasis HLB Prime (60 mg) and were purchased from Manchester, UK.

3.2 Samples

In total, 13 urban influent wastewater (IWW) samples were taken from a WWTP of a city sited in the Mediterranean Coast. The WWTP investigated is equipped with conventional activated sludge secondary treatment and tertiary nitrogen and phosphate removal. The estimated residence time of the water in the WWTP is 24 h. The 24 h composite samples (flow dependent, starting and finishing time (9:00 am to 9:00 am)) were collected over March/April 2017. All 13 samples were collected in polyethylene high density bottles and directly transported to the laboratory. Upon reception in the laboratory, samples were fortified with a mixed surrogate ILIS, filtered and immediately stored in the dark at -20 °C until analysis.

3.3 Instrumentation

A Waters Acquity UHPLC system (Milford, MA, USA) was interfaced to a triple quadrupole mass spectrometer (Waters Micromass, Manchester, UK) equipped with T-Wave devices and an electrospray ionization interface (ESI) operated in positive-ion mode.

Chromatographic separation was carried out using an Acquity UPLC BEH C₁₈ column, 1.7 μm, 50 mm × 2.1 mm (i.d.) (Waters) at a flow rate of 0.3 mL/min. The temperature remains constant at 40°C and the sample manager at 5°C. Mobile phase is constituted of water with 5mM ammonium acetate and 0.01% formic acid (solvent A) and MeOH (solvent B). The percentage of MeOH changed linearly as follows: 0 min, 10%; 3 min, 90%; 3.5 min, 90%; 3.6 min, 10%; 6 min, 10%, equilibration of the column.

Cone gas was dry nitrogen. Cone gas and desolvation gas flows were set to 250 and 1200 L/h, respectively. For the MS/MS mode, collision gas was argon 99.995% (Praxair, Madrid, Spain) with a pressure of 4x10⁻³ mbar in the collision cell

(0.15mL/min). Capillary voltage, source temperature and desolvation temperature were set at 3.0 kV, 150°C and 650 °C, respectively. Dwell times of 0.01 s/transition were selected.

All data were acquired and processed using MassLynx v4.1 software (Waters, Manchester, UK).

3.4 Analytical Procedure

Two analytical procedures have been described depending which SPE cartridge has been used.

3.4.1 Oasis® HLB

Previous to solid-phase extraction, and 100 mL four-fold diluted influent wastewater (IWW) samples were spiked with a mixed surrogate ILIS and centrifugated. The final concentration in sample for each ILIS was 20 ng/L. SPE was performed using Oasis HLB cartridges that were conditioned by washing and rinsing with 6 mL of MeOH and 6 mL of Milli-Q water. Samples were percolated through the cartridges by gravity (flow rate around 3 mL/min), and vacuum dried for proximately 15 min. Analytes were eluted using 5 mL of MeOH. The extracts were evaporated to dryness at 35 °C under a gentle stream of nitrogen and reconstructed in 1 mL MeOH:water (10:90, v/v).

Analyses were performed by injecting 3 µL of the final extract in the UHPLC-MS/MS system *Figure 9* [12].

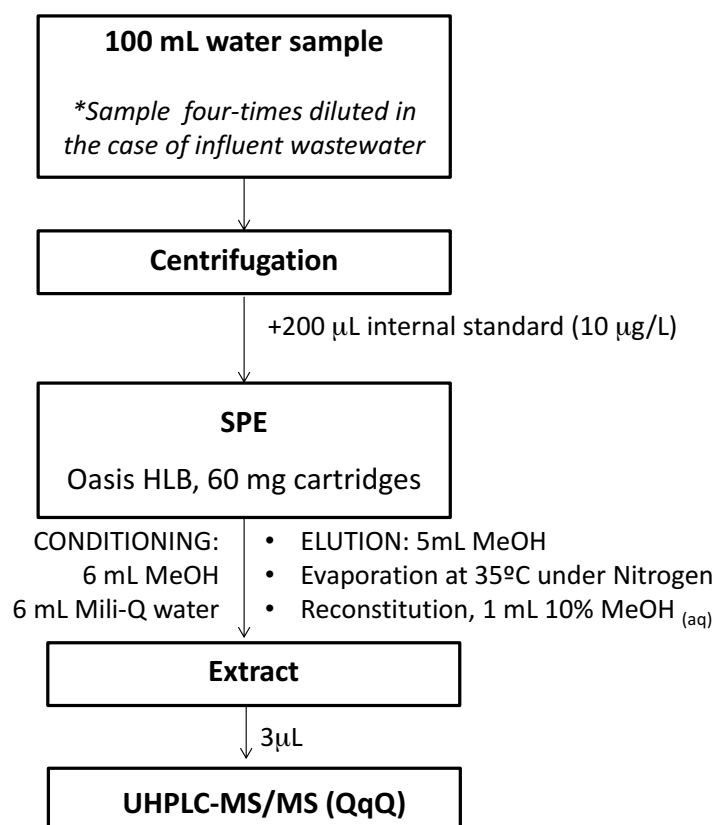


FIGURE 9. FLUX DIAGRAM FOR BOTH ANALYTICAL PROCEDURE [12]

3.4.2 Oasis® HLB PRiME

The procedure followed when Oasis® HLB PRiME cartridges were used, was the same described in section 2.4.1 without any conditioning step .

4. Results and discussion

4.1 Requirements for identification

The requirements for identification are based on document “*Analytical quality control and method validation procedures for pesticides residues analysis in food and feed (SANTE 2015)*” of the European Commission for Health and Food Safety, following this guideline and extrapolating that for the analysis of DOAs, criteria for discriminating between detected or identified compounds were established considering retention time, peak shape, q_i/Q ratios and additional product ions [13].

- The minimum acceptable retention time deviation for the analytes under examination should be at least twice the retention time corresponding to the void volume of the column. The retention time of the analyte in the extract should correspond to that of the calibration standard with a tolerance of ± 0.2 min.
- Extracted ion chromatograms of sample extracts should have peaks of similar retention time, peak shape and response ratio to those obtained from calibration standards analysed at comparable concentrations in the same batch. Chromatographic peaks from different selective ions for the analyte must fully overlap. Where an ion chromatogram shows evidence of significant chromatographic interference, it must not be relied upon for identification.
- The relative intensities or ratios of selective ions, expressed as a ratio relative to the most intense ion used for identification, should correspond to those of the calibration standard solutions within the linear range of the MS detector when measured under the same conditions. Matrix-matched calibration solutions may need to be used. The ion ratio should not deviate more than 30% (relative).
- For a higher degree of confidence in identification, further evidence may be gained from additional mass spectrometric information. In this research, three additional product ions (in MS/MS).

An example of cocaine identification is shown in *Figure 10*. The retention time, peak shape and ion q_i/Q ratio of standard complies with above requirements.

COCAINE

Standard 10 ppb

090417 HLB

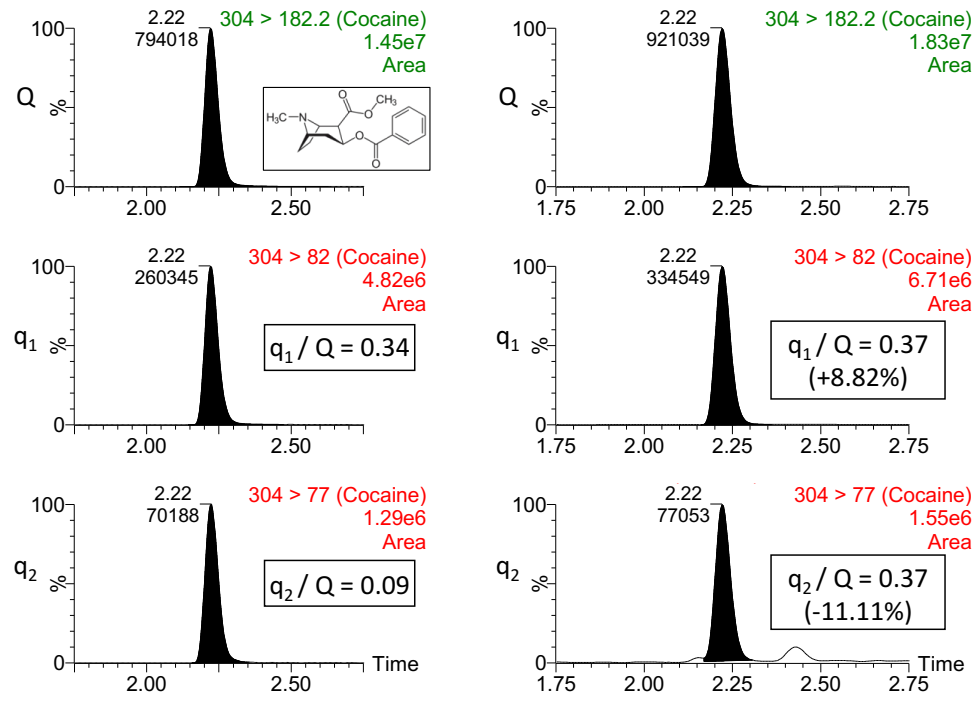


FIGURE 10. IDENTIFICATION FOR COCAINE

4.2 The case of Cocaine

As an illustrative example and for the simplification of the reading of that memory, an example of the whole calculation process for one model is shown in this section. Identification, quantification and back calculation for cocaine will be highlighted.

The type of samples used in this study were really complex matrices and, therefore, analytes could suffer from strong matrix effect. One solution for that analytical challenge is the application of Surrogate Method with Isotopically Labelled Internal Standard. Surrogates are organic compounds that are similar in chemical structure to the analytes of interest. Spiked into samples prior to sample preparation and analysis, they are used for the correction of any matrix interference or mistake during sample preparation. In order for this approach to be profitable, the surrogates must behave in the same manner as the corresponding target analytes that are native to the matrices of interest. Therefore, the most appropriate compounds are the isotopically labelled analogues of the compounds of interest.

A calibration curve was obtained for each compound with the relation between area and internal standard area (ISArea) in front of concentration of standard. *Figure 11* shows the calibration curve for the case of cocaine.

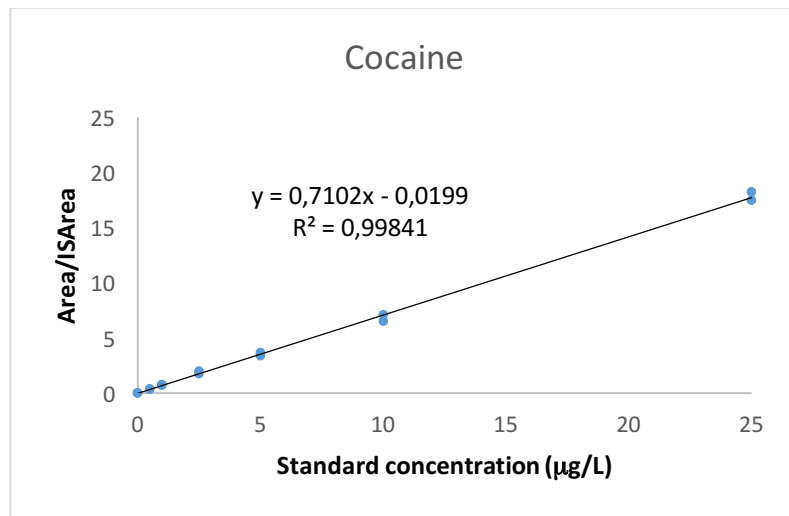


FIGURE 11. CALIBRATION CURVE FOR COCAINE

From this equation, the concentration of cocaine could be obtained. But, these results need back-calculation because the concentrations obtained are for the extract and not for WW. To calculate the drug load in mg/day, first, a concentration factor should be applied for calculation of real concentration in wastewater (Figure 12). After that, three correction factors are used to obtain the final drug load in sewage(mg/day) (Equation 1).

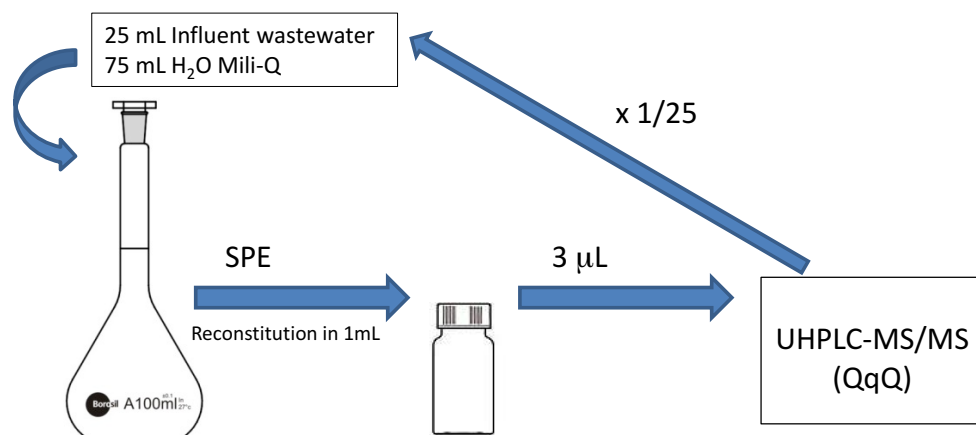


FIGURE 12. SCHEME FOR BACK-CALCULATION


$$\text{Daily sewer loads (mg/day)} = \frac{\text{Extract concentration (ng/l)}}{25} \cdot \frac{1 \text{ mg}}{10^6 \text{ ng}} \cdot \frac{10^3 \text{ l}}{1 \text{ m}^3} \cdot \frac{\text{Flow rate (m}^3\text{)}}{\text{day}}$$

EQUATION 1. CONVERSION FACTOR FOR DAILY SEWER LOADS

A back-calculation example for cocaine is show in *Table 1*.

TABLE 1. EXAMPLE OF CALCULATIONS FOR A SAMPLE OF COCAINE

| Day | Conc (µg/L) in extract | Conc (ppt) in WW | Daily sewer loads (mg/day) |
|----------|------------------------|------------------|----------------------------|
| 09/04/17 | 18708.73 | 748.35 | 25.12 |


 1:25

4.3 Consumption of DOAs in a normal week

The results of DOAs consumption in a normal week at a city of Mediterranean Coast obtained were consistent with which was expected (see *Table 2*). In general, concentrations were higher on Saturday and Sunday because drugs of abuse are usually more consumed during weekends and, consequently, DOAs and metabolites are excreted on the same night or Sunday. This behaviour is in accordance with that reported by EMCDDA in European Drug Report 2016 [2].

Heroin, its major metabolite 6-monoacetylmorphine (6-MAM) and ketamine were not found in the samples analysed, including the weekend samples, and therefore reported as below the limit of detection (LOD). In general these drugs are known to be less popular DOA. Besides heroin is not considered as a so-called “party drug”, which means that this drugs is normally consumed on a regular basis without increased consumption during the weekend.

MDMA is a substance that produces euphoria, so normally its consumption is limited to parties or festivals at weekends. Small concentrations found on Monday

and Tuesday could be due to people still excreting this compound and not due to daily consumption.

Morphine is commonly used in hospitals as an analgesic to treat strong pain as well as less used for recreational purposes. Therefore, we cannot ensure that the obtained concentration corresponds to non-therapeutic intake.

Finally, the most consumed drugs were the most popular ones as it was expected. Cocaine, its major metabolite Benzoyllecgonine and the main metabolite for cannabis (THC-COOH) were found in high concentration in all samples analysed.

A summary of results found in all samples for all DOAs analysed can be observed in *Table 2 and Figure 13*.

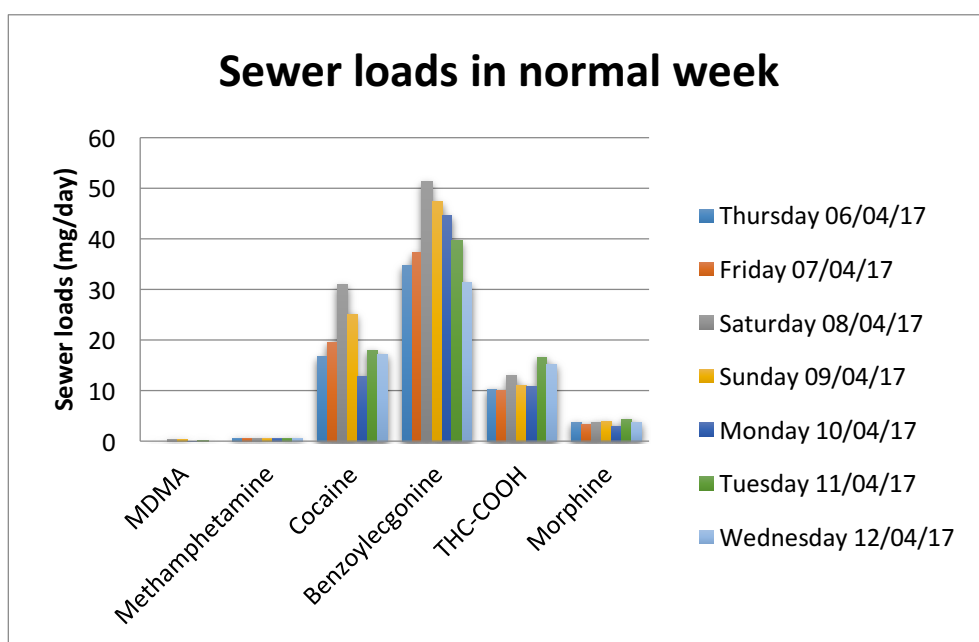


FIGURE 13. SEWER LOADS IN NORMAL WEEK

TABLE 2. DAILY SEWER LOADS FOR COCAINE IN NORMAL WEEK

| Day | Concentration (mg/day) | | | | | | | | |
|-------------------------------|------------------------|-----------------|---------|-----------------|----------|----------|-------|--------|----------|
| | MDMA | Methamphetamine | Cocaine | Benzoylcegonine | THC-COOH | Morphine | 6-MAM | Heroin | Ketamine |
| Thursday 06/04/17 | <LOD | 0.73 | 16.9 | 34.7 | 10.4 | 3.8 | <LOD | <LOD | <LOD |
| Friday 07/04/17 | <LOD | 0.74 | 19.6 | 37.3 | 10.3 | 3.6 | <LOD | <LOD | <LOD |
| Saturday 08/04/17 | 0.51 | 0.78 | 31.0 | 51.3 | 13.0 | 3.9 | <LOD | <LOD | <LOD |
| Sunday 09/04/17 | 0.49 | 0.71 | 25.1 | 47.3 | 11.2 | 4.1 | <LOD | <LOD | <LOD |
| Monday 10/04/17 | 0.17 | 0.71 | 13.0 | 44.7 | 10.9 | 3.2 | <LOD | <LOD | <LOD |
| Tuesday 11/04/17 | 0.22 | 0.75 | 18.1 | 39.6 | 16.7 | 4.5 | <LOD | <LOD | <LOD |
| Wednesday 12/04/17 | <LOD | 0.76 | 17.3 | 31.4 | 15.3 | 3.8 | <LOD | <LOD | <LOD |

<LOD : Below limit of detection (< 3 S/N)

4.4 Consumption of DOAs in a festive week

The results of DOAs consumption in a festive week at a city of Mediterranean Coast obtained were consistent with which was expected as some DOAs were more consumed during festivities.

Firstly, the cocaine consumption and its major metabolite benzoylecgonine and MDMA were higher than normal week. This could be due to the fact that people intakes more stimulant drugs during parties than in a common week. Otherwise, the concentration of THC-COOH and morphine were similar to those concentrations found in April samples. A possible explanation could be that people often consume that relaxing drugs not during festivities but whichever day.

Additionally, the consumption of methamphetamine plays second fiddle, giving rise to cocaine and MDMA higher intakes. Heroin and ketamine are kept below LOD like in normal week, as expected because there are not party drugs. A comparison of DOAs consumption in normal and festivity Sundays can be seen in *Figure 14* and the results in *Table 3*.

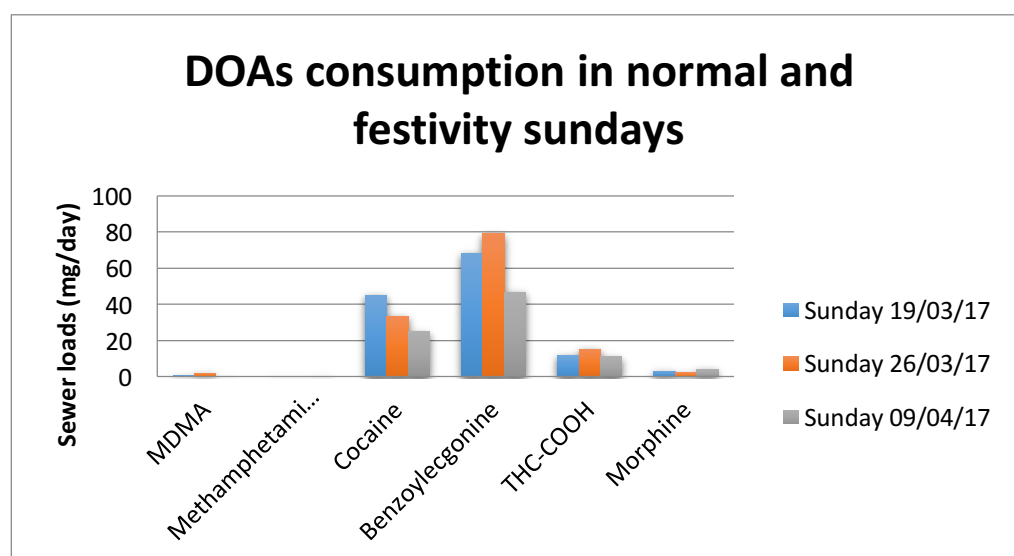


FIGURE 14. COMPARISON OF DOAs CONSUMPTION IN NORMAL AND FESTIVITY SUNDAYS

TABLE 3. DAILY SEWER LOADS FOR COCAINE IN FESTIVE WEEK

| Concentration (mg/day) | | | | | | | | | |
|-------------------------------|-------------|------------------------|----------------|------------------------|-----------------|-----------------|--------------|---------------|-----------------|
| Day | MDMA | Methamphetamine | Cocaine | Benzoylcegonine | THC-COOH | Morphine | 6-MAM | Heroin | Ketamine |
| Saturday 18/03/17 | 0.09 | <LOD | 31.3 | 39.3 | 8.2 | 3.7 | <LOD | <LOD | <LOD |
| Sunday 19/03/17 | 0.97 | <LOD | 45.5 | 68.3 | 12.0 | 3.6 | <LOD | <LOD | <LOD |
| Monday 20/03/17 | 1.82 | <LOD | 30.7 | 56.4 | 8.0 | 2.5 | <LOD | <LOD | <LOD |
| Saturday 25/03/17 | 0.43 | <LOD | 18.1 | 37.0 | 9.0 | 2.5 | <LOD | <LOD | <LOD |
| Sunday 26/03/17 | 2.04 | <LOD | 33.6 | 79.3 | 15.6 | 2.6 | <LOD | <LOD | <LOD |
| Monday 27/03/17 | 2.43 | <LOD | 36.0 | 74.0 | 11.5 | 2.7 | <LOD | <LOD | <LOD |

<LOD : Below limit of detection (< 3 S/N)

4.5 Comparison cartridges HLB vs PRiME

In an attempt to optimize the methodology, a comparison was made between the utilisation of two different types of commercially available cartridges, Oasis® HLB and Oasis® PRiME. The recently developed PRiME cartridges do not need preconditioning phase and, therefore, the analysis time is expected to be lower as well as the consumption of solvents.

The study was performed by analysing spiked Milli-Q water samples at low, medium and high (2.5, 10 and 20 µg/L) concentration levels in order to compare the calculated concentration with the one theoretically applied. The comparison was based on the recovery factor, which is defined as the ratio between the concentration calculated in the extract and the real concentration (*Equation 2*).

$$\text{Recovery (\%)} = \frac{\text{Calculated extract concentration}}{\text{Real concentracion}} \cdot 100$$

EQUATION 2. RECOVERY FACTOR

The results are shown in *Table 4*.

TABLE 4. RECOVERIES (%) FOR DOAs EXTRACTION IN WATER SPIKEAD AT LOW, MEDIUM AND HIGH CONCENTRATION (2.5, 10 AND 20 µg/L) AFTER APPLICATION OF SPE USING BOTH CARTRIDGES

| Compound | Recovery (%) | | | | | | | | | | | |
|------------|--------------|-------|-----------------|-------|---------|-------|------------------|-------|----------|-------|----------|-------|
| | MDMA | | Methamphetamine | | Cocaine | | Benzoyllecgonine | | THC-COOH | | Morphine | |
| | HLB | PRiME | HLB | PRiME | HLB | PRiME | HLB | PRiME | HLB | PRiME | HLB | PRiME |
| QCL | 111* | 38 | 108* | 69 | 88 | 72 | 86 | 11 | 97 | 91 | 94 | 98 |
| QCM | 102* | 46 | 98 | 65 | 83 | 70 | 97 | 44 | 101* | 90 | 91 | 85 |
| QCH | 101* | 52 | 98 | 66 | 82 | 76 | 88 | 49 | 98 | 93 | 91 | 90 |

*RECOVERY ABOVE 100% CAN BE DUE TO POSSIBLE MATRIX INFERENCES

The results for PRiME cartridges were not favourable. For example, in the case of MDMA, the HLB cartridge has twice the recovery rate of the PRiME cartridge. These percentages are lower for the three levels of concentration as it can be seen in *Figure 15*.

In addition, PRiME cartridges were slower and time consuming in the extraction procedure. Additionally, their cost is considerably higher than it for HLB.

Finally, these results lead to the conclusion that PRiME cartridges did not optimise the methodology. Consequently, the update of procedure was not recommended.

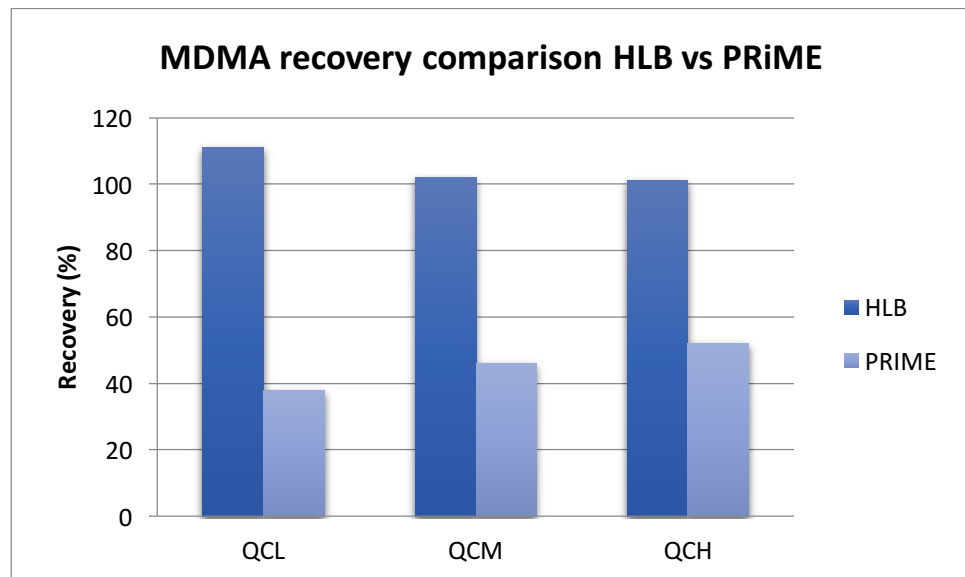


FIGURE 15. MDMA RECOVERY COMPARISON BETWEEN HLB AND PRiME

5. Conclusion

The results have been as expected. It can be stated that:

- Consumption of MDMA and cocaine are higher at weekends than on weekdays while methamphetamine, cannabis (THC-COOH) and morphine remain constant.
- In festive weeks the consumption of stimulant drugs increases, as in the case of MDMA and cocaine, but the consumption of drugs like methamphetamine, cannabis (THC-COOH) and morphine remain constant.
- Intake of methamphetamine, cannabis (THC-COOH) and morphine are constant in their use regardless of the period of time.
- The consumption of ketamine and heroin could not be detected. So their consumption seem to be low.
- The use of Oasis® PRiME cartridges does not improve the methodology regarding Oasis® HLB cartridges.

6. Future work

Regarding this study we recommend continue this strategies to complete the research:

- Increase the sampling period in order to be more representative
- Include new drugs in research in order to study whether the population consumes usual drugs or try new ones.
- Compare results with other water treatment plants on the Mediterranean coast and Spain to check if there are significant differences in consumption.

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