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Analysis of ethyl sulfate in raw wastewater for estimation of alcohol consumption and its correlation with drugs of abuse in the city of Barcelona

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ABSTRACT

The increasing, generalized consumption of alcohol, especially among young people, generates great concern in our society due to its negative consequences on public health and safety. Besides the traditional, official methods employed for estimation of alcohol consumption, the monitoring of ethyl sulfate (EtS), a urinary biomarker of alcohol ingestion, in raw wastewater has been recently proposed as an additional tool to estimate alcohol use at community level through the so-called sewage epidemiology approach.

In the presented study, a fast and reliable analytical method based on ion-pair liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been optimized and further applied to the analysis of EtS in seven 24 h composite samples collected along one week at the inlet of a large sewage treatment plant (STP) located in the Barcelona area. EtS was measured in the entire set of analysed samples, with concentrations ranging from 5.5 to 33 μg/L, which correspond to an absolute alcohol consumption of around 11,000 (Wednesday) to 25,000 (Sunday) kg/day. The average per capita absolute alcohol consumption calculated was 18 mL/day/inhabitant. Moreover, the levels of EtS measured throughout the week showed high correlation with those of some recreational illicit drugs and metabolites, namely, cocaethylene ($r^2 = 0.9391$, $n = 5$), benzoylecgonine ($r^2 = 0.9252$, $n = 7$), ecstasy ($r^2 = 0.8950$, $n = 7$), amphetamine ($r^2 = 0.8707$, $n = 7$) and cocaine ($r^2 = 0.6425$, $n = 7$), measured in the same samples. This study confirms that the analysis of EtS in raw wastewater can be a useful tool for the estimation of alcohol consumption in an anonymous, fast and economic way, and indicates that consumption of alcohol and some illicit drugs occurs often together.

1. Introduction

The harmful use of alcohol creates significant public health and safety problems throughout the world, problems that can also be very costly to communities and societies. Almost 4% of all deaths worldwide are attributed to alcohol consumption, which is also associated with many serious social issues, such as diseases and injuries [1].

Alcohol consumption can be estimated from indirect measures, such as total sales within one state or region or survey data of self-reported alcohol consumption. Total sales statistical data may not always be available, or lead to an overestimation of the alcohol per capita consumption because of international purchases of alcohol, wastage of unfinished drinks and stockpiling of alcohol beverages [2]. On the other hand, it has been demonstrated that, compared to sales figures, data obtained from self-reported alcohol consumption are prone to underestimate alcohol use [1–3].

Knowledge of the current alcohol consumption status, as well as of other licit and illicit abused substances, is critical in assessing progress in reducing the harmful use of these substances [1,4].

In recent years, monitoring the presence of psychotropic compounds in environmental matrices has been proposed as an additional tool to estimate community drug use in a fast and anonymous way [5] and it has been successfully applied already in numerous countries for collective drug abuse estimation [6–9]. In this approach, known as sewage epidemiology, the concentration of a urinary biomarker (parent drug or metabolite) measured in raw wastewater entering a STP is used to back-calculate the amount of drug used within a community or population. Levels in wastewater are normalized across the population served by the STP and the amount of wastewater treated during the sampling period (mass load), and corrected by a factor that takes into

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account the metabolic excretion data of the drug and the molar mass ratio between the consumed substance and the measured residue [10,11].

Following consumption of alcoholic beverages, ethanol undergoes different elimination pathways in the human body. A small fraction of the ingested ethanol is excreted in urine as ethyl glucuronide (EtG) and ethyl sulfate (EtS), two nonoxidative minor metabolites of ethanol which have been recently proposed, beside others, as specific and sensitive indicators of recent alcohol ingestion [12–15].

In 2011, Reid et al. published the first study dealing with the analysis of EtG and EtS in wastewater samples and the subsequent estimation of the corresponding regional alcohol consumption [16]. Due to the observed low stability of EtG in wastewater samples, EtS was deemed more appropriate as urinary biomarker for alcohol consumption estimation. The results obtained from the application of this approach in Norway in terms of community alcohol consumption were in the same scale of those derived from sales data.

More recently, Rodríguez-Álvarez et al. applied a similar approach to various samples collected in the NW of Spain finding concentrations of EtS slightly lower than those previously reported by Reid et al. and a per capita consumption of pure ethanol in the range from 9 to 24 mL/day/inhabitant [17].

In this context, the main objectives pursued within the present study were (i) to develop a fast and reliable analytical method for monitoring of EtS in untreated wastewater samples, (ii) to estimate alcohol consumption at the community level in the city of Barcelona, and (iii) to study potential correlations between the presence of EtS and some of the most common abused licit and illicit drugs. For these purposes, a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was optimized for the direct determination of EtS in wastewater and it was further applied to a series of seven 24 h composite samples collected daily from the largest STP of Barcelona where the presence of other abused substances was also investigated.

2. Materials and methods

2.1. Reagent and materials

Ethyl sulfate sodium salt and ethyl-d5 sulfate sodium salt were obtained from Cerilliant (*Round Rock*, *TX*, *USA*) as solutions in methanol (MeOH) at a concentration of 1 mg/mL (as ethyl sulfate).

Water and methanol solvents, both of HPLC grade, and acetic acid (98% purity) were purchased from Merck (*Darmstadt*, *Germany*). Dibutylamine (>99.5% purity) and ammonium acetate (≥98%) for mobile phase modification were purchased from Sigma Aldrich (*Steinheim*, *Germany*).

2.2. Standard solutions

Stock standard solutions were prepared at different concentrations by appropriate dilution of the commercial standards in methanol and were stored in the dark at −20 °C.

Working standard solutions for construction of the calibration curve and the recovery and repeatability tests were freshly prepared at varying concentrations by dilution of the stock standard solutions (100 μL) in HPLC water (10 mL). The concentration of ethyl-d5 sulfate (EtS-d5), used as surrogate standard, was maintained at 25 μg/L in both the standard calibration solutions and the samples.

2.3. Samples and sample preparation

Samples were taken on consecutive days along 1 week at the inlet to a STP located in Barcelona (Spain), which processes sewage water from a population census of approximately 1,157,000 people living in the city and its metropolitan area [18].

Composite samples (24 h, from 9 am to 9 am) were collected by means of an Isco 6712c portable automatic sampler (*Teledyne ISCO*, *Lincoln*, *USA*) programmed to sample 50 mL every 10 min up to a total sample volume of ca. 7.2 L. Samples were kept refrigerated during collection and transport to the laboratory and once in the lab the samples were manually shaken for homogenisation and an aliquot of approximately 500 mL of each sample was transfer to an amber-polyethylene terephthalate (PET) flask and stored at −20 °C until analysis. Prior to analysis, the samples were defrosted and shaken and an aliquot of 10 mL of each sample was spiked with the surrogate standard at a concentration of 25 μg/L. One mL of this solution was transferred to a 1.5 mL microcentrifuge tube and was centrifuged at 10000 rpm for 10 minutes at a temperature of 4 °C (*Eppendorf 5810R*, *Hamburg*, *Germany*). Then the supernatant formed was transferred to a glass vial for subsequent LC–MS/MS determination.

2.4. LC–ESI–MS/MS

LC–MS/MS analysis was performed with a HPLC Symbiosis™ Pico System (*Spark Holland*, *Emmen*, *The Netherlands*) equipped with a 100 μL sample loop. Chromatographic separation was achieved with a Purospher STAR RP-18 end-capped column (125 mm × 2.0 mm, particle size 5 μm) preceded by a guard column of the same packing material (*Merck*, *Darmstad*, *Germany*), and a binary mobile phase consisting of MeOH and water both containing 5 mM of dibutylammonium acetate (DBAA) (ion-pair reagent). During the chromatographic analysis, the proportion of the organic solvent was increased as follows: 0 min–5%, 6 min–90%, 8 min–100%. Pure organic conditions were held for 1 min to clean the column. Return to initial conditions was done in 2 more min, and maintained for 2 min for column equilibration before the next injection. The flow rate was kept at 0.3 mL/min throughout the run, and the sample volume injected was 20 μL.

The HPLC system was coupled to an Applied Biosystems 4000QTRAP hybrid quadrupole-linear ion trap (QqLIT) mass spectrometer equipped with a Turbo Ion Spray source (*Applied Biosystems -Sciex*, *Foster City*, *CA*, *U.S.A.*) operating in negative ion mode (ESI−). Detection was performed in the selected reaction monitoring (SRM) mode acquiring two characteristic SRM transitions for each of the target compound, EtS, and its deuterated analogue in order to obtain sufficient identification points for compound confirmation. In both cases the deprotonated molecular ion ($[M–H]^-$) and the two most abundant fragment ions were selected as precursor ion and product ions, respectively. Data were acquired and evaluated via Analyst (software version 1.4.2).

Experimental MS/MS conditions were optimized by continuous infusion of a 500 μg/L methanol solution of the analyte via a syringe pump. The selected fragment ions and optimal values for declustering potential (DP), collision energy (CE) and collision exit potential (CXP) for both the analyte and the surrogate standard are shown in Table 1. Optimization of other parameters influencing the ESI source, such as temperature and ion transfer heater voltage, was performed by on column injection (20 μL) of a standard solution of the compound in water at 100 μg/L. The optimum selected conditions were as follows: source temperature, 500 °C; ion transfer heater voltage, −4000 V; nebulizer gas 1 (GS1) and gas 2 (GS2), both 50 psi; curtain gas (CUR), 30 psi, collision gas (CAD), low; and channel electron multiplier (CEM), 2200 V.