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Estimation of alcohol consumption during "Fallas" festivity in the wastewater of Valencia city (Spain) using ethyl sulfate as a biomarker

María Jesús Andrés-Costa [a,*], Úrsula Escrivá [a], Vicente Andreu [b], Yolanda Picó [a]

[a] Environmental and Food Safety Research Group (SAMA-UV), Desertification Research Centre CIDE (CSIC-UV-GV), Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, Burjassot, 46100 Valencia, Spain
[b] Lanscape Chemistry and Environmental Forensics Group, CIDE (CSIC-UV-GV), Carretera Moncada, Náquera, Km. 4.5, Moncada, 46113 Valencia, Spain

HIGHLIGHTS

- Direct determination of ethyl sulfate in wastewater by ion-pair LC–MS/MS
- Different ion-pairs and additives were tested and compared.
- Sewage epidemiology was applied to estimate alcohol consumption.
- The increase in the alcohol consumption during Fallas festivity is noticeable.

GRAPHICAL ABSTRACT

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ABSTRACT

Alcohol consumption has been increasing in the last years and it has become a sociological problem due its derived health and safety problems. Ethyl sulfate is a secondary metabolite of the alcohol degradation that is excreted through the urine (0.010–0.016%) after alcohol ingestion and it is quite stable in water. In this study, a new methodology to determine ethyl sulfate by ion-pair liquid chromatography-tandem mass spectrometry (LC–MS/MS) was developed. Different ion-pairs and additives were tested directly in the sample extracts or in the mobile phase. The best ion-pair was set up adding 0.5 M of tributylamine and 0.1% of formic acid to the sample. The limit of quantification was 0.3 μg L^{-1} and the intra-day and inter-day precision of the method were ≤2.8 and ≤3.0%, respectively. Good linearity ($r^2 < 0.999$) and low matrix effect (<30% corrected by using internal isotopically labelled internal standard) were achieved. The sampling campaign was from 4th to 20th March of 2014 covering the festivity of Fallas (15th to 19th March). Ethyl sulfate was determined in all influents of the 3 wastewater treatment plants (Pinedo I, Pinedo II and Quart-Benàger) belonging to Valencia and surrounding area. Ethyl sulfate concentrations ranged from 1.46 to 19.85 μg L^{-1} and alcohol consumption ranged from 1.07 to 56.11 mL day^{-1} $inhab^{-1}$, being the highest value of alcohol consumption determined during Fallas. This study presents a reliable and alternative method to traditional ones to determine alcohol consumption by population that provides real-time information of alcohol consumption.

* Corresponding author.
E-mail addresses: M.Jesus.Andres@uv.es, http://sama-uv.es (M.J. Andrés-Costa).

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1. Introduction

Alcohol is a psychoactive substance widely consumed through the world; in fact, its consumption has been increasing over time. This situation is such that today, alcohol is considered as a serious health and security problem due to the effects associated with their usual consumption. Drinking alcohol produces gastrointestinal and cardiovascular diseases as well as is one of the leading causes of accidents, from domestic to traffic related (OEDT, 2011). Common methods for determining alcohol consumption are based on general population surveys or reports of epidemiological, alcohol expenditure and sales data (WHO, 2014). However, these data are not completely reliable because surveys have important bias of the alcohol consumption. Sales data do not include illicit and informally produced alcohol or do not keep in mind the stockpiling or wastage. Thus, alternative method that helps to improve the estimation of alcohol consumption was needed.

Once the alcohol has been ingested, it is degraded to two minor metabolites, ethyl glucuronide (EtG) and ethyl sulfate (EtS), which are excreted through the urine (0.010–0.016% on molar basis) (Høiseth et al., 2008). Both are determined in clinical and forensic laboratories for the surveillance of abstinence, establishing the alcohol consumption in workplace testing and rehabilitation programmes for alcohol dependence (Thierauf et al., 2010a; Thierauf et al., 2011).

Sewage epidemiology has been successfully applied to estimate the consumption of drugs of abuse based on specific metabolites as a biomarker, which are excreted through the urine in wastewater treatment plants (WWTP) (Andres-Costa et al., 2014; Damien et al., 2014; Ort et al., 2014; Thomas et al., 2012; Vazquez-Roig et al., 2014). EtG is unstable in sewage effluent (Halter et al., 2009). Contrarily, EtS has been demonstrated to be stable in manometric respiratory test — high concentrations of bacteria and EtS — for at least 6 days and up to 28 days in closed bottle test — low EtS and bacteria density from a WWTPs effluent (Halter et al., 2009; Thierauf et al., 2008).

Analytical methods have been described for the determination of EtS in biological matrices such as plasma, serum, vitreous humour, placental and foetal tissues, and meconium (Kummer et al., 2013; Morini et al., 2010; Morini et al., 2007; Thierauf et al., 2010a; Thierauf et al., 2011; Thierauf et al., 2008; Thierauf et al., 2010b) either by gas chromatography–mass spectrometry, liquid chromatography–tandem mass spectrometry (LC–MS/MS), liquid chromatography with pulsed electrochemical detection, capillary zone electrophoresis, or immunochemical test. Otherwise, few bioanalytical methods are available for the determination of EtS in wastewater where a lower detection limit is required (Mastroianni et al., 2014; Reid et al., 2011; Rodríguez-Álvarez et al., 2014). These methods are based on LC–MS/MS exploiting ionic exchange mechanisms because EtS is poorly retained on conventional C_8 and C_{18} reverse phase chromatographic columns (Reid et al., 2011). Methods reported showed that different ion-pairs can be used and can be added to the mobile phase or to the sample. The first method reported used an ion-pair reagent dihexylammonium acetate (DHAAc) into the mobile phase (Reid et al., 2011). More recently, a similar approach based on dibutylammonium acetate (DBAAc) as ion-pair reagent added into the mobile phase was proposed (Mastroianni et al., 2014). Alternatively, method based on tetrabutylammonium bromide (TBAB) ion-pair added to the sample achieves a determination of EtS in wastewater directly (after filtration, internal standard and ion-pair addition) by LC–quadrupole time-of-flight (QqTOF)–MS. This method permits the use of stronger non-volatile amines, such as TBAB, as the amount entering the MS is reduced in comparison to its introduction in the eluent. However, TBAB entering into the MS system also can have an important impact on analyte signal (Rodríguez-Álvarez et al., 2015; Rodríguez-Álvarez et al., 2014).

In this context, the aim of the present study is to develop a simple, fast and reliable method to determine EtS by ion-pair LC–MS/MS. Thus, different ion-pairs have been tested and were added to the sample or mobile phase in order to select the best option. The developed method was applied to calculate the alcohol consumption through the analysis of the influents of 3 WWTPs, Pinedo I, Pinedo II and Quart-Benàger. These WWTPs treat the wastewater of Valencia and its surrounding area. The sampling period was from 4th to 20th March of 2014 including the big festivity of Fallas. That festivity is in honour of Saint Joseph that takes place every year in Valencia from 15th to 19th March. As in many other festivities, heavy drinking is an important part of Fallas fun, and Valencia is plenty of drinking stalls and bars all over the city.

2. Materials and methods

2.1. Chemicals

EtS sodium salt and EtS-d5 sodium salt were obtained from Sigma-Aldrich (Madrid, Spain) as solutions in methanol at a concentration of 1 mg mL^{-1}. Stock standard solutions were prepared at 1 μg mL^{-1} by appropriate dilution of the commercial standards in methanol and were stored in the dark at −20 °C. Working solutions were prepared by diluting stock solution in methanol as on daily basis and stored at 4 °C. EtS-d5 was maintained at a final concentration of 25 μg L^{-1} into the standard calibration solutions and samples.

Tetrabutylammonium chloride (TBAC), diethylamine (DEA), tributylammonium (TBAmm), dihexylamine (DHA) and isopropylamine (IPA) were from Sigma-Aldrich and tributylamine (TBA) from Merk (Schuchardt, Germany). Other reagents and solvents were formic acid (FA) from Amresco-inc (Solon, Ohio, USA) and ammonium formate (AmF) and acetic acid (AcA) from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany). DHAAc was prepared from equimolar volumes of DHA and AcA. Methanol was purchased from Panreac (Madrid, Spain) and ultrapure water obtained from a Milli-Q water purification system.

2.2. Sample collection and treatment

Wastewater samples were collected from influents and effluents of Pinedo I, Pinedo II and Quart-Benàger, that treat about 1,500,000 people and a flow of 355,233 m^3 day^{-1} (EPSAR, 2014). Fig. 1 shows the technical characteristics and the location of each WWTP (more information of each WWTP have been presented in Tables S1, S2 and S3 in Supplementary information). The sampling was conducted from 4th to 20th March of 2014.

Wastewater samples were provided by WWTP operators and were arranged in 1 L polypropylene bottles, previously rinsed with ultrapure water and wastewater samples prior to the wastewater collection. The samples were transported back to the laboratory in a dark and iced cool box. Once at the laboratory, aliquots of 15 mL of wastewater samples were frozen at −20 °C until analysis. Then the samples were thawed and prepared in 2 mL amber vials appropriately. The best ion-pair was set up adding 0.5 M of TBA and 0.1% of FA to the sample.

2.3. Liquid chromatography–mass spectrometry (LC–MS/MS)

Chromatographic separation of EtS was performed using an Agilent Technologies 1260 Infinity Ultra High-Performance Liquid Chromatograph (UHPLC). The column was Kinetex C18 (1.7 μm, 100 Å, 50 × 2.10 mm) and it was maintained at temperature of 30 °C and a constant flow rate of 0.2 mL min^{-1}. The isocratic mobile phase was 90% eluent A (FA 0.1% in Milli-Q water) and 10% eluent B (FA 0.1% in methanol). The injected volume of sample was 5 μL. Methanol–water (10: 90 v/v) both with (1) 7 mM DHAAc; (2) 10 mM DEA, 10 mM AmF and 10 mM AcA; and (3) 10 mM TBA, 10 mM AmF and 10 mM AcA.

The UHPLC was coupled to an Agilent Technologies 6410 triple quadrupole mass spectrometer with an electrospray Turbo V ionization source working in negative ionization (ESI^-) mode, 300 °C gas temperature, 11 L min^{-1} gas flow and 25 psi nebulizer. Quantifier and qualifier